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Preface

More than 15 years have passed since publication of the last monograph on anthracycline antibiotics, the ACS Symposium Series 574, edited by W. Priebe. However, anthracycline antibiotics continue to be one of the most applied antitumor agents, mostly in combination therapy. In addition, a number of exciting new developments such as prodrug development or new synthetic, semi-synthetic, or biosynthetic derivatives have emerged in spite of a certain decrease in synthetic activity. With this background in mind, I accepted the invitation of Prof. J. Thiem to edit an updated collection of reviews on anthracycline antibiotics. In fact, this task turned out to be an exciting endeavor and instead of the initially planned single volume, the numerous contributions from many experts in this exciting field had to be collected into two volumes. The last decade has provided a much greater amount of new information then initially anticipated and these volumes represent a condensed review of this data derived from journals representing quite different fields.

The first volume is dedicated to biological occurrence and biosynthesis as well as the synthesis and chemistry of anthracyclines. Since the pioneering review of H. Brockmann on naturally occurring anthracyclines in 1963, no systematic overview has appeared and this volume will provide a review of the latest information. This topic is closely related to biosynthesis and the intriguing progress in biotechnology to produce biosynthetic anthracycline variants is presented. The part of the volume covering synthesis comprises an updated overview on asymmetric synthesis, combinatorial synthesis using the Diels–Alder reaction, synthesis of fluorinated anthracyclines, the sugar moieties, non-natural glycosyl anthraquinones as DNA binding and photocleaving agents, and finally of anthracyclines and fredericamycin A via strong base-induced cycloaddition reaction.

The second volume is devoted to mode of action, clinical aspects, and new drugs. At this point I would like to thank F. M. Arcamone for his invaluable help in selecting the topics and authors of this second volume. Knowledge of the molecular mechanisms of anthracycline activity is of prime importance, also for clinical application, and therefore this is the first contribution of the second volume. The most severe side effect of anthracyclines and many other anticancer drugs is cardiotoxicity, and this has to be given prime importance. Future attempts at reducing this and other side effects include the

development of less toxic prodrugs. Therefore, four reviews within this volume are dedicated to this topic: Daunomycin–TFO conjugates for downregulation of gene expression, acid-sensitive prodrugs of doxorubicin, anthracycline–formaldehyde conjugates and their targeted prodrugs, and doxorubicin conjugates for selective delivery to tumors. Last but not least, two chapters are devoted to the recent development of new and hopefully even better anthracycline anticancer drugs: Sabarubicin and nemorubicin. Clinical development of these compounds is approaching and will hopefully give encouraging results.

The two volumes on anthracyclines cover a large area from biotechnology to synthesis and clinical application. Thus, although the chemical aspects dominate, the books will be of value to a broader spectrum of readers looking for recent information on this most important class of antitumor antibiotics.

It has been a great pleasure to work with the competent team of Springer, in particular Dr. Marion Hertel and Birgit Kollmar-Thoni. They have my thanks in addition to all of the authors for their (mostly) timely contributions.

Paderborn, January 2008 Karsten Krohn

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Naturally Occurring Anthracyclines

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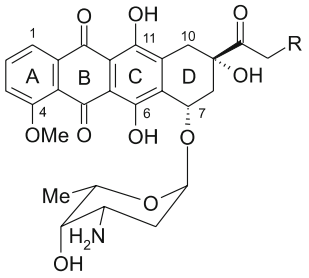
Abstract The present article gives an overview of the natural occurring anthracyclines and anthracyclinones reported from microorganisms. A general description, discussion of their physicochemical properties, including NMR increments, and their structural classification are reported. In addition to a compilation of their sugar moieties, an exhaustive list of naturally occurring anthracyclines and anthracyclinones has been added.

Keywords Anthracyclines · Microbial metabolites · Natural products · Streptomycetes

1 Introduction

The anthracycline antibiotics form a group of red aromatic microbial polyketides. Most have been isolated from bacteria of the order *Streptomycetales*, about one quarter was found in so-called rare actinomycetes [1–3]. Their diversity is based on structural differences in the aglycone and on a wide array of attached sugar residues.

The high scientific and industrial interest in anthracyclines arose from their remarkable pharmaceutical properties. Their significant antitumor activity makes them commercially valuable, and they have been some of the most intensively studied natural products over the past 50 years.



1a: R = H
1b: R = OH

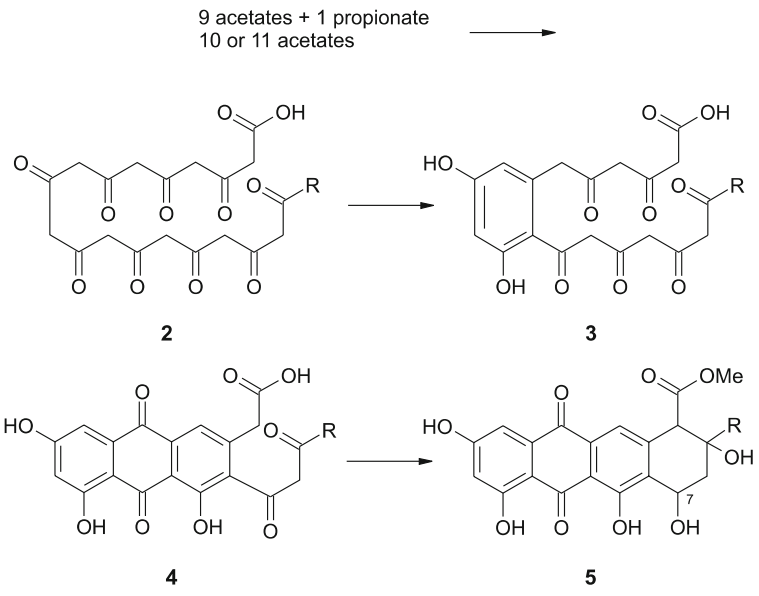
Daunomycin (**1a**) [4, 5] and doxorubicin (**1b**) [6] are clinically especially useful as antineoplastic agents with a broad spectrum of activity; they are also active against certain solid tumors that are normally resistant to most other modes of chemotherapy [7, 8]. The clinical use of these drugs, however, is hampered by a number of undesired side effects, the most serious being the dose-related cardiotoxicity. Therefore there is a great interest in related natural or synthetic compounds having improved therapeutic indices [7–9]. Many efforts have been made to modify the anthracycline molecule with the objective of developing analogs with a wider spectrum of activity and reduced toxicity [8]. As anthracyclines are easily detectable, due to their color and bioactivity, the number of natural members has increased dramatically since a first review in 1963 [1].

2
General Properties of Anthracyclines

2.1
Basic Aglycone Structures of Anthracyclines

Hans Brockmann, a pioneer of research on antibiotics, published the isolation and structure elucidation of the first anthracyclines. According to his definition [1], anthracyclines are red to orange natural or synthetic dyes with a skeleton of 7,8,9,10-tetrahydro-tetracene-5,12-quinone and mono- to tetrasaccharide moieties attached, usually to ring D of the aglycone. In this review, the numbering system proposed by Brockmann (see structure **1**) will be used.

Biosynthetically, anthracyclinones are linear annellated deca- or undecaketides, which differ in the number of hydroxy groups in the anthraquinone part, the substitution of ring D as well as the stereochemistry, and in the more or less complex glycoside pattern. According to the folding of the polyketide chain **2**, firstly an anthraquinone derivative **4** is formed via **3** [10], which then cyclizes resulting in quinones of D-ring type 1 (**5**) with a carboxy group in position 10. By decarboxylation of the latter, anthracyclines of type 2 (**6**) arise. Additionally, variations at C-9 are found, depending on whether an acetate or propionate-derived "starter" side chain at C-9 is attached in an oxygenated or reduced form.



As the benzylic hydroxy group in position 7 is removed easily, and further hydroxylation and/or oxidation in other positions can occur, at least 15 D-ring modifications (**5–19**) can be distinguished (Fig. 1), which gave rise to nearly 500 natural anthracyclines isolated to date. However, as the nitrogen-free sugar residues are split off easily already under weakly acidic conditions during the isolation process, not all of them may be truly natural.

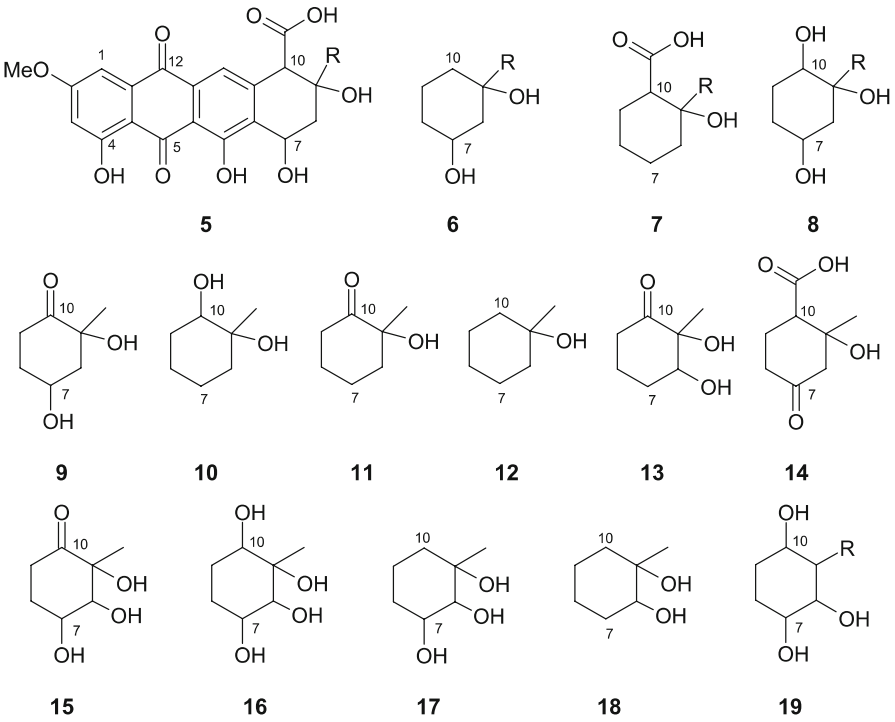


Fig. 1 D-Ring types of anthracyclines

The absolute configuration in ring D was investigated as early as 1961 [11–13], mainly by comparison of their circular dichroism (CD) curves: Most anthracyclinones known at that time were (7*S*,9*R*,10*R*)-configured; a few (e.g. α-isorhodomycinone, 7-*epi*-**41c**) had the 7*R* configuration. Later, other stereoisomers were also isolated.

2.2 Isolation and Structure Elucidation

The polarity of anthracyclines may vary over a wide range. While the aglycones are usually stable and rather unpolar, the acid-sensitive highly glycosy-

lated derivatives are very polar and readily soluble even in water; those with amino sugars form salts with acids.

For the isolation and purification of anthracyclines and anthracyclinones, the culture broth is sometimes acidified or basidified, depending on the pK_a value, followed by filtration and extraction with organic solvents. The lipophilic anthracyclinones are mostly concentrated in the mycelium and must be extracted with acetone and acetone/dichloromethane mixtures. The more polar members of the glycosides are present mainly in the culture filtrate and can be recovered by repeated extraction with ethyl acetate or – more conveniently – by adsorption resins like XAD-7 or Diaion HP-20. To isolate residual parts from the mycelium, acetone under addition of acetic acid may be required. Under these conditions, however, some anthracyclines are already cleaved, and this may be the reason that only very few glycosides with more than four sugar units in a chain have been described in the literature.

Analytical monitoring is done by thin-layer chromatography (TLC) on silica gel using organic solvent systems, sometimes in combination with small amounts of acetic acid, ammonia, or water [14]. The preparative separation of the individual quinones is a simple chromatographic exercise, if the strain produces only a few components. In this case, chromatography on silica gel with a dichloromethane/methanol gradient of increasing polarity is sufficiently effective. Chloroform instead of dichloromethane should be avoided, as HCl is formed easily under influence of light, which transforms sensitive anthracyclines quickly into complex mixtures of cleavage products. For the separation of glycosides with differing chain lengths, gel filtration, usually with methanol on Sephadex LH-20, is the method of choice. The separation of more complex mixtures of glycosides requires multistep procedures, including counter current chromatography or the use of heavy metal chelates [15]. However, this procedure usually results in poor reproducibility and low yields.

The structure elucidation of the first anthracyclines was done without modern spectroscopic methods, mostly by chemical degradations and by comparison with synthetic model compounds. The molecular weight of glycosides was estimated from UV data or, e.g., ebullioscopic measurements. The sugar residues were split off by hydrolysis, and the aglycones further degraded by aromatization, periodic acid reaction, oxidation etc. The position of hydroxy groups was determined by UV data of the pyroborate reaction, of acetates, and by synthesis of the respective tetracenequinone. Thomson has discussed these analytical procedures in detail [16, 17].

As many physical and chemical properties are closely related within the anthracyclines, especially with respect to the spectroscopic properties, these topics will be discussed in common, before selected examples of the individual groups are described.

2.3 UV Spectra

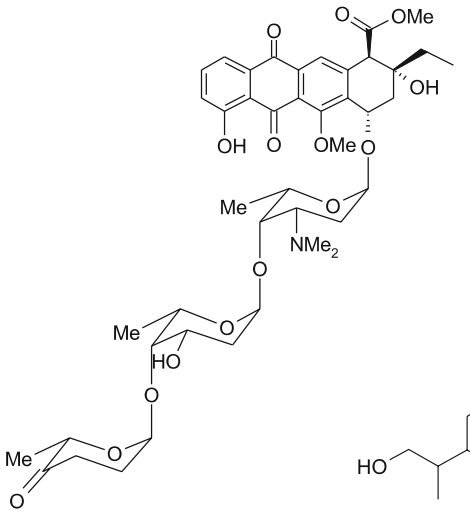
All anthracyclines show the typical behavior of *peri*-hydroxyquinones and give, also on TLC, deep blue to purple color reactions with diluted sodium hydroxide solution. Their UV spectroscopic properties are mainly influenced by the substitution pattern of the anthraquinone unit and have been reviewed by Brockmann [1] and Thomson [16, 17]. Hydroxy groups in the *peri*-positions 1,4,6, and 11 cause a strong bathochromic shift of the anthraquinone absorption; substituents in other positions change the spectra only weakly. Since the structure of ring D has only a moderate influence, anthracyclines and anthracyclinones are fairly well represented by the UV data of the corresponding *peri*-hydroxyanthraquinones.

One *peri*-tetrahydroxyanthracycline and four trihydroxyanthracyclines are expected. Out of the latter, mainly the 4,6,11- and 1,4,6-oxygenated patterns were found, which cannot be distinguished easily by their UV data. In accordance with their biosynthetic origin, of the six possible *peri*-dihydroxyanthracycline derivatives, the majority are 4,6-dihydroxylated (152 of 172); however, of the four expected monohydroxyquinone patterns, only six 6-hydroxyanthracyclinone derivatives have been described.

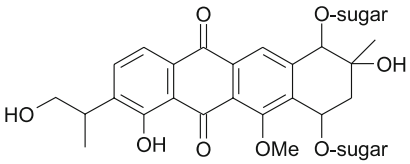
Table 1 UV data (MeOH) of the six chromophore types found in natural anthracyclines; the numbering system of anthracyclines is used

Position of OH groups[a]	λ_{max} (nm)	OH groups	λ_{max} (nm)
1 (0); 4 (0); 11 (0); 6 (6)	254 ± 5, (284 ± 5)[b], 330 ± 5, 405 ± 5[c]	4,6 (152); 1,11 (1)	228 ± 1, 258 ± 1, 288 ± 2, 431 ± 2
1,4 (2); 6,11 (3)	235 ± 1, 258 ± 1, 293 ± 2, 483 ± 12	1,4,6 (68); 1,4,11 (9); 1,6,11 (6); 4,6,11 (196)	235 ± 1, 255 ± 1, 293 ± 3, (470 ± 2), (479 ± 3), 494 ± 3, (515 ± 2), 529 ± 2, (583 ± 6)
1,6 (6); 4,11 (10)	(230 ± 2), 258 ± 3, (292 ± 2), (425 ± 6), 439 ± 4	1,4,6,11 (32)	237 ± 3, 291 ± 6, (493 ± 2), 522 ± 2, 548 ± 3, (562 ± 2), (605 ± 5)

[a] Values in brackets = number of natural derivatives
[b] Maxima in brackets may be missing or appear as shoulders in a number of compounds
[c] Value estimated from 1-hydroxyanthraquinones



20



21

Altogether, only six out of the 15 types of *peri*-hydroxyanthraquinone chromophores have been found in nature (Table 1). Most data were recorded in methanol. In ethanol, the maxima at longest wavelength show a bathochromic shift of 5–20 nm, if data are available. In chloroform, the long-wavelength maxima are ~5 nm bathochromically shifted, and a better resolution may be observed. With the exception of aclacinomycin A 6-methyl ether (**20**) and the incompletely defined fragilomycin A (**21**, sugar part unknown), C-4 is the only position where *peri*-methoxy groups are found. Monomethylation in this position does not change the spectra significantly with respect to the phenolic parent compounds.

2.4 NMR Spectra

Most anthracyclines are sufficiently soluble in $CDCl_3$ for the measurement of ^1H and even ^{13}C NMR spectra. In comparison with DMSO or other solvents of higher polarity, chloroform has the advantage of giving clearer and sharper signals, and also the offset signals of *peri*-hydroxy groups are usually well resolved. The OH-shifts in DMSO and in $CDCl_3$, however, are very similar.

All anthracyclines possess one or more chelated OH groups and show therefore at least one signal in the offset region. As for anthraquinones [18], these *peri*-OH shifts are strongly dependent of the substituent pattern of the chromophore, however, are scarcely influenced by substituents in positions 8 and 9 ($\Delta\delta < \pm 0.03$). Hydroxy groups or sugar residues both in C-7α or C-10β

positions usually contribute by not more than $\Delta\delta\pm0.15$; the very strong influence of 7β/C-10α substituents on the shift of the adjacent 4- or 11-hydroxy group was explained by stronger steric interactions [19, 20]. In most cases, the *peri*-OH shifts can be predicted from increments with a surprising accuracy (Table 2), and the quality could be further increased by elimination of wrongly assigned α/β-assignments in the literature. It should also be noted that the OH ^{1}H shifts show a statistically significant inverse correlation with the respective *ipso* C shift ($1/\delta_{13C}$). This fact can be used for simple shift assignments, if long-range correlations between the OH group and the *ipso*-C atom are not available. Predicted OH-shifts can also be used for the determination of the relative configuration at C-7/C-10.

By far the most naturally occurring anthracyclines are monooxygenated in ring A (~300). With the exception of celastramycin B (**22a**) and the struc-

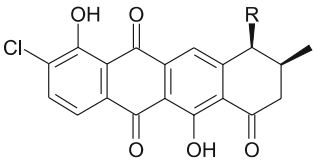
Table 2 Increments for the prediction of *peri*-hydroxy ^{1}H NMR shifts of anthracyclines in $CDCl_3$

Substituent cases/mult. r standard deviat.	1-OH 19/0.996 ±0.02	4-OH 67/0.980 ±0.07	6-OH 105/0.990 ±0.08	11-OH 60/0.987 ±0.06
Base value	13.340	12.644	13.046	12.987
1-OH	0 [a]	+ 0.742	– 0.304	– 0.207
2-OMe	–	+ 0.116	+ 0.215	– 0.106
3-OMe	–	–	– 0.072	+ 0.084
4-OH	–	0 [a]	– 0.568	+0.157
4-OMe	– 0.003	–	+ 0.264	– 0.005
6-OH	– 0.320	– 0.497	0 [a]	+ 0.489
6-OMe	–	+ 0.286	–	+ 0.437
7-αOH	– 0.040	– 0.120	+ 0.145	– 0.130
7-βOH	–	– 0.120	+ 0.410	– 0.151
7-αOMe	–	– 0.103	+ 0.087	–
7-βOMe	–	– 0.088	+ 0.047	–
7-αO-sugar	– 0.058	– 0.044	– 0.073	+ 0.099
7-βO-sugar	+ 0.345	–	+ 0.756	– 0.663
10-CO	–	– 0.084	– 0.015	–
10-COOMe	–	– 0.048	+ 0.074	– 0.239
10-βOH	–	– 0.081	– 0.146	+ 0.144
10-αOH	–	– 0.099	– 0.191	+ 0.536
10-βO-sugar	+ 0.108	– 0.109	+ 0.885	– 0.862
11-OH	– 0.660	+ 0.154	+ 0.437	0 [a]

Experimental shift values were taken from Vigevani et al. [19, 20] and from our own measurements

[a] *ipso*-OH

turally unique quinocycline-type compounds (e.g., quinocycline B, **26a**), a hydroxy or methoxy group is positioned at C-4, which gives rise to a typical aromatic ABC system, consisting of two doublets and a triplet. While the upfield doublet of 3-H next to oxygen is usually broadened, the doublet of 1-H often also shows a *meta* coupling. Oxygen in 1,4-position (~100 compounds) causes a singlet or a narrow AB signal for 2,3-H near δ 7.3. Only about ten compounds are unsubstituted in ring A and show AA′BB′ signals for the aromatic protons (Table 3).



22a: R = OH
22b: R = H

Only two natural anthracyclines with protons in 6,11-positions were described; about 20 are monohydroxylated at C-11, and about 200 are 6-monohydroxylated or 6,11-bishydroxylated. In the monohydroxyquinones, the remaining *peri*-H singlet of 6-H is usually in the range of δ 7.60–7.85 in 7-*O*-glycosides (at δ 8.00–8.05 in the aglycones), and that of 11-H between δ 7.65 and 7.78.

The signals of the annellated cyclohexane ring are usually complex and overlapping with the sugar signals, if present. Clearly separated, however, are the signals of 10-CH-COOMe and of the benzylic protons 7-CH-OR and 10-CH-OR, both in the same region as the anomeric sugar signals (see Table 2). Vigevani et al. have discussed further data extensively [19, 20].

The ^{13}C NMR data are of high diagnostic value, as the quinone-type can be derived easily from the sp^2 region of the spectrum: Non-chelated quinone CO signals appear in the region of δ 180±3, and one chelated OH group induces a shift of the chelated CO to δ 186; doubly chelated carbonyls appear at δ 190–192, while the non-chelated ones remains at δ 180. At least the same number of oxymethine signals are visible at δ 162±5, counting for the OH and additional methoxy groups. The 10-carbomethoxy signal of anthracyclines such as **23** appears usually at δ 170–172. Further CO signals at lower field are indicative for non-conjugated (δ 210) or conjugated (δ 195–200) keto sugars, or for carbonyl groups at C-7 or C-10.

The acetal signals in the glycosides are well separated and appear at δ 100±5. The C-1 signal of the fused sugar deoxyfucose-cinerulose A (dFuc=cinA) is found, however, at an unusually high field (δ 90), together with the anomeric C signal of C-9 glycosides, such as in cytorhodin X (Fo 296) or Y (Fo 303).

Table 3 ^{13}C NMR Shifts (δ values) of selected anthracyclines in $CDCl_3$

C	Fo 303	Fo 173	Fo 177	**1a**	Fo 82	**38h**	Fo 459	**38i**	*epi*-**26a**	**36c**	Fo 427
1	119.5	157.9	157.9	118.5	120.1	120.6	120.5	118.5	161.6	108.1	122.6
2	137.0	129.8	129.7	135.8	137.3	135.3	138.3	135.8	124.8	166.6	137.1
2-OMe	–	–	–	–	–	–	–	–	–	56.4	–
3	124.3	130.1	130.1	119.8	124.8	124.1	125.8	119.9	137.3	106.6	141.5
3-Me	–	–	–	–	–	–	–	–	–	–	16.3
4	162.6	158.5	158.4	161.2	162.5	157.9	163.5	161.2	119.0	164.7	159.1
4a	116.3	112.4	112.4	120.9	115.8	122.6	116.9	121.0	132.9	110.0	125.0
5	191.0	190.6	186.8	186.6[a]	192.6	186.8	191.8	186.7[a]	187.5	189.5	186.7
5a	110.4	115.9	114.9	111.4[a]	114.7	115.5	112.5	111.4[a]	114.6	118.4	110.6
6	156.3	162.3	162.2	156.6[a]	162.0	156.5	157.6	156.5[a]	158.1	161.2	157.6
6a	138.3	132.9	132.2	134.6[a]	132.7	135.3	137.0	134.5[a]	133.7	132.9	135.7
7	20.9	70.7	66.5	69.7	69.9	73.7	72.6	69.9	43.5	71.9	74.7
8	24.7	33.8	32.6	34.9[a]	34.7	43.6	35.3[a]	36.3[a]	34.3	85.9	72.8
8-OMe	–	–	–	–	–	–	–	–	–	59.7	–
9	77.4	71.7	71.6	76.9	71.0	67.7	71.5	76.9	83.1	76.1	70.0
10	69.7	57.1	57.2	33.3[a]	56.7	38.2	53.2	34.9[a]	76.4	198.4	33.6
10a	140.2	142.4	142.3	134.5[a]	141.7	135.3	137.2	134.4[a]	144.3	135.3	137.6
11	158.3	120.4	120.4	155.9[a]	121.0	158.0	157.9	155.9[a]	120.0	115.3	154.5
11a	110.2	131.5	132.9	111.2	131.2	115.5	112.2	111.3[a]	131.5	134.6	110.1
12	185.6	185.8	183.9	186.9[a]	181.1	183.9	187.2	187.0	187.0	180.0	186.1
12a	133.9	112.6	112.4	135.5	132.7	136.0	134.4	135.6	115.8	133.1	133.0
13	26.5	32.2	32.1	212.0	51.0	29.2	33.7[a]	212.4	22.5	23.3	26.6
14	7.0	6.7	6.7	24.8	207.0	–	7.3	24.9	112.8	–	–
15	–	171.3	171.3	–	32.5	–	173.6	–	126.7	–	–
15a	–	–	–	–	–	–	–	–	142.4	–	–

Table 3 (continued)

C	Fo 303	Fo 173	Fo 177	**1a**	Fo 82	**38h**	Fo 459	**38i**	*epi*-**26a**	**36c**	Fo 427
16	–	–	–	–	–	–	–	–	21.6	–	–
17	–		–	–	–	–	–	–	52.4	–	–
18a									169.4		
COO*Me*	–	52.6	52.6	–	52.7	–	–	–	–	–	–
4-OMe	–	–	–	56.7	–	–	–	56.7	–	–	60.7
1′	79.7	101.6	100.5	101.1	101.4	104.7	101.6	101.2	95.7	100.7	103.9
2′	29.8	29.3	29.7	32.6	29.2	67.1	29.5	33.4	32.8	80.9 d	66.4
2′-OMe	–	–	–	–	–	–	–	–	–	59.9	60.0
3′-OMe	–	–	–	–	–	56.6	–	–	–	–	–
3′	61.4	61.5	54.7	46.4	61.6	81.7	48.6	45.9	70.1	70.2	80.5
4′	74.4	74.1	77.4	67.4	74.2	72.0	67.9	82.0	80.7	82.3	65.2
*CO*Me	–	–	–	–	–	–	–	–	210.8	–	–
CO*Me*	–	–	–	–	–	–	–	–	26.7	–	–
4′-OMe	–	–	–	–	–	–	–	–	–	58.5	–
5′	68.0	66.6	86.1	70.8	66.2	69.8	68.0	64.2	66.1	68.7	63.8
6′	18.1	17.0	18.1	17.0	16.7	18.0	17.0	17.1	14.6	17.8	–
NMe_2	43.4	43.2	40.8	–	43.2	–	–	–	–	–	–
1″	99.5	99.3	99.0	106.7	99.1	99.4			–	–	–
2″	34.3	34.4	27.0	45.9	33.0	71.3			–	–	–
3″	65.6	65.6	67.9	75.9	65.9	71.3	Sugar		–	–	–
4″	83.7	83.9	86.2	23.4	71.5	72.2	at C-4		–	–	–
5″	66.8	68.3	66.7	68.0	68.5	70.8			–	–	–
6″	17.0	20.0	16.2	66.9	18.0	17.9			–	–	–
7″	–	–	–	18.1	–	–	–	–	–	–	–
1‴	100.3	95.7	91.4	–	–	–	–	–	–	–	–
2‴	24.0	142.3	78.0	–	–	–	–	–	–	–	–

Table 3 (continued)

C	Fo 303	Fo 173	Fo 177	**1a**	Fo 82	**38h**	Fo 459	**38i**	*epi*-**26a**	**36c**	Fo 427
3‴	25.5	127.2	39.6	–	–	–	–	–	–	–	–
4‴	67.3	195.8	209.0	–	–	–	–	–	–	–	–
5‴	68.4	77.9	74.6	–	–	–	–	–	–	–	–
6‴	17.0	15.2	16.0	–	–	–	–	–	–	–	–
1⁗	90.9			–	–	–	–	–	–	–	–
2⁗	29.8			–	–	–	–	–	–	–	–
3⁗	59.5			–	–	–	–	–	–	–	–
4⁗	65.9	Sugar		–	–	–	–	–	–	–	–
5⁗	66.4	at C-9		–	–	–	–	–	–	–	–
6⁗	16.5			–	–	–	–	–	–	–	–
NMe_2	42.0			–	–	–	–	–	–	–	–

Bold numbers concern structures in the text, Fo numbers relate to the entries in Table 5

[a] Interchangeable assignments

2.5 Mass Spectra

In contrast to the anthracyclinones [21, 22], the more polar glycosides usually do not give reliable mass spectra on electron impact ionization (EI-MS). In cases such as cinerubin A (**23**), where aromatization can take place easily, the sugar residues are split off completely, and the bisanhydro-aglycones are formed. In the spectrum of **23**, the signal of η-pyrromycinone (**24**) is detected ($m/z = 392$).

In contrast, fast atom bombardment (FAB), field desorption (FD), and especially electrospray ionization (ESI) usually do not give fragmentation. These are very powerful techniques in structure elucidation or dereplication of anthracyclines, especially in combination with HPLC and MS/MS fragmentation [23] or with high resolution (HRMS).

Both (–)- and (+)-ionization modes deliver molecular ions of sufficiently high intensity for MS/MS. In the positive mode, the $[M + H]^+$, $[M + Na]^+$ and sometimes also $[2M + Na]^+$ quasimolecular ions are visible; (–)-ESI delivers $[M–H]^-$ and $[2M–2H + Na]^-$ ions. In MS/MS measurements, again the high

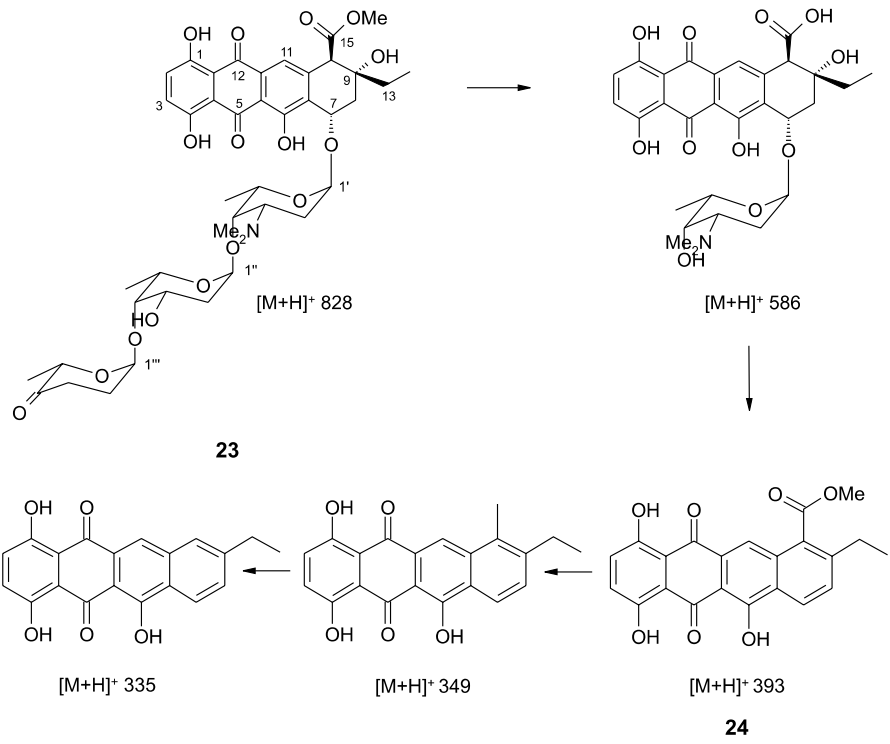


Fig. 2 (+)-ESI MS/MS fragmentation of cinerubin A (**23**)

sensitivity of the glycoside linkage is reflected: In most cases, the sugar chain is cleaved behind rhodosamine, which is finally lost simultaneously with two molecules of water, if aromatization is favored; again the tetracenequinone **24** is formed from **23** (Fig. 2).

On the glycoside cleavage, the terminal sugar is split off as an oxonium ion, and the bridging oxygen forms an OH group at the remaining glycoside. This again needs protonation to be detectable as $[M + H]^+$. Auramycin C (Fo 81) gives a signal at $m/z = 686$ for $[M + H]^+$ on (+)-ESI MS. On MS/MS, a first fragment with $m/z = 556$ is observed, which is equivalent to auramycin D (**29c**). The mass difference of $\Delta m/z = 130$ points to 2-deoxyfucose or 2-deoxyrhamnose. Other mass differences between the pseudomolecular mass of the parent ion and the $[M–sugar + H]^+$ ions are listed in Table 4.

2.6 The Sugar Moiety in Anthracyclines

According to the literature, only one C-glycosidic anthracycline (caeseorhodomycin, Fo 455 in Table 5) has been described. All other anthracyclines are *O*-glycosides with sugar attachments mostly at the benzylic positions at C-7, C-10, or both. Two anthracyclines, the cytorhodins X (Fo 296) and Y (Fo 303), are tertiary glycosides at C-9. In four anthracyclines, a sugar residue is connected with a phenolic OH in position 1 or 4. Compounds of this type may be precursors of a unique class of *C*,*O*-glycosides, the nogalamycins and some 20 related derivatives (Table 6).

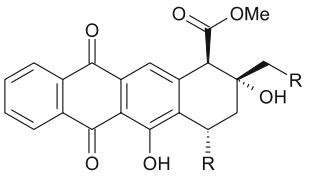
The sugars are usually obtained by mild acid hydrolysis or alcoholysis of the anthracyclines followed by separation using DOWEX 50×12 (H^+) with ammonia, or by chromatography on paper and silica gel [24–26]. They are identified by means of optical rotation, melting point, color reactions, and by NMR and mass spectra. At least 60 different sugars have been described in anthracyclines so far (Table 4). More than 340 anthracyclines are connected directly with one or two amino sugars at C-7 and/or C-10. In more than 200 cases, this is L-rhodosamine, about 60 glycosides contain L-daunosamine (didemethylrhodosamine); monodemethylrhodosamine-glycosides are rare. Remarkably, there is never found a neutral sugar between an aminosugar and the aglycone.

In the following text, abbreviation of the sugars (see Table 4) will be used to simplify the structure description of the respective glycosides. As all anthracyclinone glycosides contain only sugars in the 1,4-connection, the sugar chain, e.g. in aclacinomycin A 6-methyl ether (**20**), 2,3,6-trideoxy-4-*O*-[2,6-dideoxy-4-*O*-[(2*R*-*trans*)-tetrahydro-6-methyl-5-oxo-2*H*-pyran-2-yl]-α-L-lyxo-hexopyranosyl]-3-(dimethylamino)-α-L-lyxo-hexopyranose, can be abbreviated as Roa-dFuc-Rho. The first sugar in the descriptor (here Roa) is always the one directly connected with the chromophore via oxygen.

3
Selected Anthraquinones

3.1
6-Hydroxyanthracyclines

Anthracyclines without substituents in ring A are rare and not more than ten are known. 4-Deoxyauramycinone (**25a**) was isolated from *Streptomyces galilaeus* in 1983 [27, 28] and re-isolated from a marine-derived *Streptomyces* strain [29]. Antibiotic and antitumor activities were reported, although the biological activities of the aglycones are usually negligible.



	R^1	R^2
25a	OH	H
25b	Dau-dFuc-CinA	Me
25c	Dau-dFuc=CinA	Me
25d	H	COMe
25e	Dau	$COCH_2OH$

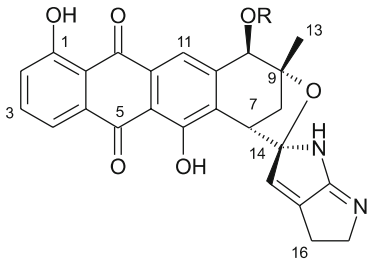
Natural glycosides of **25a** are not known, however, the closely related 9-ethyl derivatives 4-deoxyaclacinomycin A (**25b**) and B (**25c**) [27, 28] have been obtained from another strain of *S. galilaeus*; their antibiotic and cytotoxic properties are much stronger, as expected. Besides deoxyauramycinone (**25a**) and others having a C_2 fragment at the 9-position, 4-deoxysulfurmycinone (**25d**) and 4-demethoxy-11-deoxyadriamycin (**25e**) are examples of anthracyclines with a C_3-chain C-9. **25e** exhibited a higher cytotoxicity than doxorubicin (**1b**).

3.2
Anthracyclines with the Skeleton of Dihydroxyanthraquinones

Dihydroxylated anthracyclines can be classified into six groups, with hydroxyls in 1,4, 1,6, 1,11, 4,6 (aklavinones), 4,11 (citromycinones), and 6,11 positions. Only two compounds have been reported possessing 1,4-dihydroxy groups: rhodirubin G (Fo 7) and ruticulomycin B (Fo 8); the only 1,11-dihydroxylated quinone (Fo 18) has to be further confirmed.

3.3
1,6-Dihydroxyanthracyclinones and Their Glycosides

The group of 1,6-dihydroxylated anthracyclines is also small and consists of only eight members, all with remarkable structures: the previously mentioned celastramycin B (**22a**), three further derivatives of the same [30], and four quinocylines. Quinoclines were isolated from *Streptomyces aureofaciens* [31], and recently from *Streptomyces violaceusniger* strain HAL64 isolated from an Egyptian soil sample [32]. They possess antituberculosis activity [33] and are structurally conspicuous by an unusual pyrrolopyrrole moiety coupled via C-7/O-9 by a spiro bond to the anthracyclinone. Among these compounds, quinocycline B (**26a**, also known as kosinostatin), is reported to inhibit the growth of gram-positive bacteria *Staphylococcus aureus* 209P JC-1 and *Bacillus subtilis* ATCC 6633 with MIC values of 0.039 $\mu g\,mL^{-1}$. It also exhibited cytotoxicity against a panel of 39 human cancer cell lines [34–37]. Isoquinocycline B [35–37] is an epimer of quinocycline B (**26a**) with opposite configuration at the spiro center; the respective aglycones are known as TPU 0025BG (Fo 13) and TPU 0025AG (Fo 14). Other compounds such as P-371ε PA (**26b**) [38, 39] and isoquinocycline A (P-371γ PA, **26c**) [16, 17, 40] exhibited activity against mycobacteria; however, the structure of **26b** seems to be uncertain.

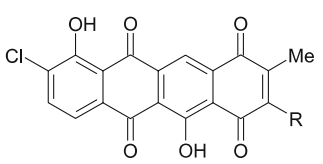


	R
26a	4CdHex1
26b	4CdHex2
26c	4CHex

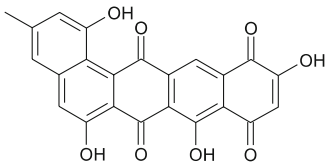
The isomerization of quinocycline B (**26a**) to isoquinomycin B (2′-*epi*-**26a**) starts with the protonation of the heterocycle, followed by ring opening of the pyrrolopyrrole moiety, rotation, and recyclization [34].

Celastramycin B (**22a**) has been isolated together with celastramycin A (which is a halogenated acylpyrrole, and not an anthracycline) from endophytic *Streptomyces setonii* and *S. sampsonii* growing on wood of different *Celastraceae* plants [41]. It was the only halogen-containing anthracycline;

however, **22a** was recently re-isolated from a taxonomically undetermined streptomycete, together with 10-deoxycelastramycin B (**22b**) and two new anthracycline-derived quinones, celastramycin C (**27a**) and its adduct of methylsulfide, celastramycin D (**27b**) [30].



27a: R = H
27b: R = SMe



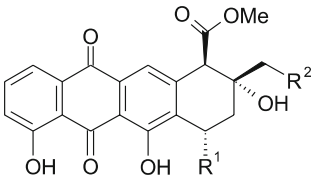
28

A single diquinone, BE-45985X (**28**), has been isolated previously from nature [42]. Diquinones usually have a higher oxidation potential than simple quinones, so that rapid reduction by substrate components occurs. The stability, especially in the case of **27b**, is certainly due to the sulfur substituent, which lowers the oxidation potential. The new celastramycins exhibited moderate antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*.

3.4
4,6-Dihydroxyanthracyclines and Anthracyclinones

4,6-Dihydroxylated anthracyclines and anthracyclinones, the aklavinones, represent the second largest subgroup of anthracyclinones (about 120 of 500). The 4-hydroxy group exists in free form in about 100 compounds, and as methyl ether in about 20. The majority of 4,6-dihydroxylated compounds has been isolated from strains of *S. galilaeus* like the aglycone aklavinone (**29a**). The configuration in ring D of the latter has been determined by X-ray analysis as 7*S*,9*R*,10*R*. The stereoisomers aklavinone I and aklavinone II have 7*R*,9*S*,10*R* and 7*R*,9*R*, 10*R* configuration, respectively. Mono-, di- and triglycoside derivatives of aklavinone are known. Aklavin (**29b**), first isolated from a taxonomically undetermined streptomycete obtained from soil samples in Aklavik, Canada [43], and its 9-demethyl derivative auramycin D (**29c**), both possess the amino sugar rhodosamine. They are the building blocks of further di- and triglycosides of this series, the latter being better also termed as aclacinomycin derivatives.

The isolation and structure elucidation of aclacinomycin A (**29d**) was the key trigger in the determination of the sugar residue of aklavin. In spite of some difference in the substituents, such as the presence of 11-hydroxy, 4- and 6-methoxy or 9-isopropyl groups (instead of ethyl at C-9), the sequence of the sugar moieties in trisaccharide aclacinomycins are in most cases Roa-dFuc; the third sugar varies from cinerulose A to rednosamine. Exceptions are aclacinomycin K (**29e**) and L (**29f**), where the sugars linked to the

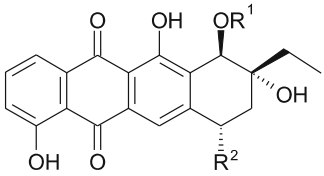


	R^1	R^2
29a	H	Me
29b	Roa	Me
29c	Roa	H
29d	Roa-dFuc-CinA	Me
29e	Dau-dFuc-CinA	Me
29f	NDau-dFuc-CinA	Me

aglycone are daunosamine and methyldaunosamine, respectively. All these compounds showed remarkable cytotoxicity against human cancer cell lines, compared to their counterparts MA-144 U5 (Fo 57) and MA-144 U6 (Fo 60) lacking the amino sugar.

3.5
4,11-Dihydroxyanthracyclinones

Only ten compounds have been described in the literature as belonging to the group of 4,11-dihydroxylated anthracyclines (citromycinones). The general feature here is the presence of a hydroxy group instead of a carbomethoxy substituent in the 10-position.

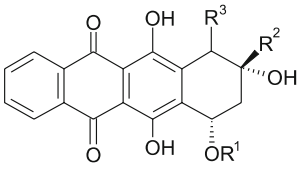


	R^1	R^2
30a	H	OH
30b	H	H
30c	H	O-Roa
30d	Roa	O-Roa
30e	Roa	OH
30f	Roa-dFuc-CinA	Roa-dFuc=CinA
30g	Roa-dFuc-CinA	OH

Various glycosylated derivatives of α-citromycinone (**30a**) and γ-citromycinone (**30b**) have been reported, such as yellamycin A (**30c**), B (**30d**), and C (**30e**) isolated from *Streptomyces violaceus* A262. They show an in vitro cytotoxicity and inhibition of DNA and RNA syntheses in cultures of murine L2110 leukemia cells. Yellamycin A was also described as possessing higher cytotoxicity than doxorubicin [44]. Higher glycosylated compounds like cosmocarcin B (**30f**) and C (**30g**) have also been reported.

3.6
6,11-Dihydroxyanthracyclines

Only three 6,11-dihydroxyanthracyclines have been reported, all of them are monoglycosylated. Although no 6,11-dihydroxylated aglycones have been isolated, this class of compound would have as aglycone the 4-deoxycarminomycinone (**38e**). The antineoplastic idarubicin (4-demethoxydaunorubicin, **31a**) [45], the anticancer agents F 840020 (**31b**) [46] and medorubicin (4-demethoxyadriamycin, **31c**) were isolated from *Streptomyces peucetius* var. *aureus*.



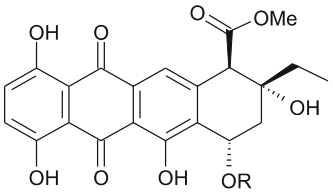
	R^1	R^2	R^3
31a	Dau	COMe	H
31b	Dau	$COCH_2OH$	H
31c	dFuc	CH_2OH	OH

3.7
1,4,6-Trihydroxyanthracyclines

Trihydroxylated anthracycline and anthracyclinones can be divided into four majors classes, based on the position of the OH groups: 1,4,6-trihydroxyanthracyclinones belong in majority to the pyrromycinone type; the 1,4,11- and the 4,6,11-trihydroxyquinones are usually summarized as rhodomycinones.

Anthracyclines with free hydroxy groups in the 1,6,11-position are unknown so far; they are related, however, to the unusual anthracyclines of the nogalarol and nogarol families, which differ strongly from the previous groups and are described separately in "Unusual Anthracyclines" (Sect. 3.12).

Three types of pyrromycinones have been described, derivatives of ε-pyrromycinone (**32a**), ε1-pyrromycinone, and ζ-pyrromycinone (see Table 5). They differ in the structure of the C-9 residue and/or in the oxidation state at C-7. Pyrromycin (**32b**) isolated from *Streptomyces* sp. DOA 1205 was the first glycoside of this type [47]. The red orange crystalline cinerubin A (**32c** = **22**) and B (**32d**) are further well known examples from this group, isolated from *Streptomyces cinereoruber*; they exhibit various antibacterial activities [48]. In all compounds cited above, rhodosamine (Roa) is directly linked to pyrromycinone. There are, however, also nitrogen-free examples such as ciclamycin A (**32e**) [49] or cinerubin X (**32f**) [50] with neutral sugar moieties in the first position.

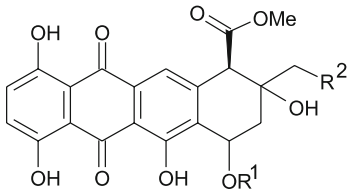


	R
32a	H
32b	Roa
32c	Roa-dFuc-CinA
32d	Roa-dFuc=CinA
32e	Rho-dFuc-CinA
32f	dFuc-dFuc-CinA
32g	dFucC-dFuc=CinA
32h	dFucC-dFuc-CinB

The carbamates Carb-A (**32g**) and Carb-B (**32h**) [51] have been isolated from marine *Streptomyces* spp. isolates B9054 and B8904, respectively. Both contain the unusual glycocarbaminate fragment, which is typical for the bleomycin group but rare in other antibiotics. The only related anthracyclines are the monoglycosides rubomycin F (Fo 400) and H (Fo 399) [52].

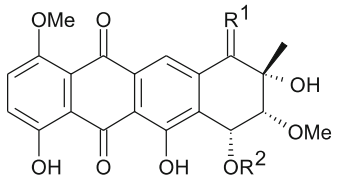
Four monorhodosaminosides are known in this group, 1-hydroxyauramycin T (**33a**), 1-hydroxysulfurmycin T (**33b**) and the diastereomeric (7*S**,9*S**,10*R**)-(**33c**) and (7*R**,9*R**, 10*R**)-pyrromycins (**33d**), which showed the expected cytotoxicity against a P-388 cell line [53]. These compounds differ in their stereochemistry from the "normal" (7*S*,9*R*,10*R*) configuration of pyrromycin described by Brockmann et al. [47].

Nocardicyclin A (**34a**) and B (**34b**) isolated from *Nocardia pseudobrasiliensis* IFM 0624 (JCM 9894) indicated a particularity of having a keto functionality at C-10 and a methoxy group at C-1. They exhibited cytotoxic activity



	R^1	R^2
33a	α-Roa	α-Me
33b	α-Roa	α-CH_2-COMe
33c	α-Roa	α-Et
33d	β-Roa	β-Et

against leukemia L1210 cells and inhibited gram-positive and gram-negative bacteria [54]. From another *Nocardia* sp., the antimycobacterial nothramicin (**34c**) was reported, the reduced form of the 10-ketone **34a.**

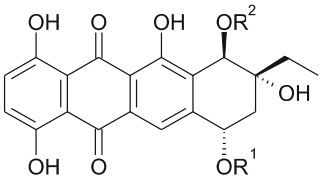


	R^1	R^2
34a	O	B [a]
34b	O	AB
34c	H, OH	B

[a] Brasiliose, *O*-acetylbrasiliose; see Table 4

3.8
1,4,11- and 1,6,11-Trihydroxyanthracyclines

Two aglycones were reported in the small group of 1,4,11-trihydroxylated anthracyclines, α2-rhodomycinone (**35a**) and δ-rhodomycinone (Fo 214). Glycosides such as alldimycin-A (**35b**), B (**35c**), C (**35d**), and violamycin-B2 (**35e**) isolated from *Streptomyces violaceus* A262, as well as 1-hydroxy-serirubicin (**35f**) isolated from *Streptomyces cyaneus* MG344-hF49 all have the α2-rhodomycinone aglycone **35a.**

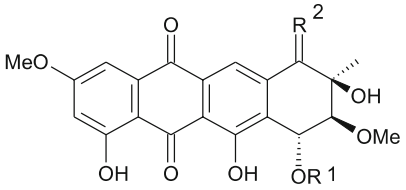


	R^1	R^2
35a	H	H
35b	Roa	H
35c	Roa	Roa
35d	H	Roa
35e	Roa-dFuc-Rho	H
35f	Roa-dFuc=CinA	Roa-dFuc=CinA

1,6,11-Trihydroxyanthracyclines have not been isolated from natural sources so far. They may be, however, precursors of the unique nogarol group, which is described in Sect. 3.12.

3.9
2,4,6-Trihydroxyanthracyclines

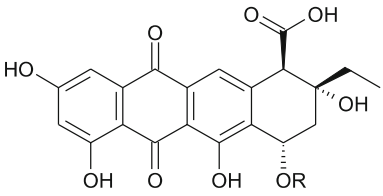
The small group of 2,4,6-trioxyanthracyclines is represented by the steffimycinone (**36a**) derivatives and the 2-hydroxyaklavinone (**37a**) family. All steffimycinones possess a carbonyl group at C-10 and a (9*S*)-methyl group with a stereochemistry opposite to that of "normal" anthracycline and anthracyclinone antibiotics. Another characteristic feature of this family is the presence of 2,8-dimethoxy groups. To date, only the aglycone steffimycinone (**36a**)



	R^1	R^2
36a	H	O
36b	mRha	O
36c	2,4-mRha	O
36d	2,4-mRha	H, H

and monoglycosylated derivatives such as steffimycin A (**36b**), B (**36c**), and C (**36d**) have been isolated [55].

In contrast to the steffimycinone derivatives, the 2-hydroxyaklavinones have (9*R*)-ethyl groups at C-9 position. Compounds of the aclacinomycin group, such as 2-hydroxyaclacinomycin-A (**37b**), B (**37c**), and N (**37d**), were reported from *S. galilaeus* KE 303 (FERM-P 4808) [56, 57].

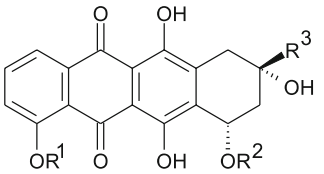


	R
37a	H
37b	Roa-dFuc-CinA
37c	Roa-dFuc=CinA
37d	Roa-dFuc-Rho

3.10 4,6,11-Trihydroxyanthracyclines

4,6,11-Trihydroxylated quinones form the largest group within the anthracyclines (~195). It contains the most important antibiotics used in anticancer therapy; daunomycinone (**38a**), feudomycinones A (**38b**), C (**38c**), adriamycinone (**38d**), and carminomycinone (**38e**) are their aglycones. They were obtained as high-melting red powders from mutants of *Streptomyces coeruleorubidus* ME130-A4 and differ only by the alkyl or acetonyl group at position C-9 [58]. 4-Demethyl-feudomycinone C (**38f**), also known as komodoquinone B, was isolated from a marine *Streptomyces* sp. SK3 and belongs to the rare anthracyclines possessing a C-9-methyl substituent. Its absolute configuration was determined as (7*S*,9*S*)-*syn* by application of the modified Mosher method [59]. Komodoquinone B possesses no biological activity. However, its glycoside with the rare sugar *epi*-nogalamine, komodoquinone A (**38g**) exhibits neuritogenic activity on neuro 2A cells [59]. The sugar at 4-*O* is also described as a C-4 epimer of mycaminose. Mutactimycin PR (**38h**) has been isolated from *Saccharothrix* sp. SA 103-I and indicated activity against *Bacillus subtilis* [38, 39].

Daunomycin (**1a**) and doxorubicin (**1b**) (adriamycin, 14-hydroxydaunomycin) are the best known metabolites from this group. They were isolated

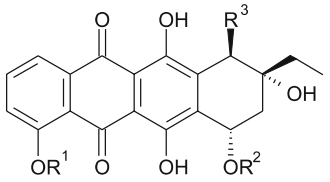


	R^1	R^2	R^3
38a	Me	H	COMe
38b	Me	H	Et
38c	Me	H	Me
38d	Me	H	$COCH_2OH$
38e	H	H	COMe
38f	H	H	Me
38g	eNgm	H	Me
38h	Rha	3-mRha	Me
38i	Me	DauA1	COMe
38j	Me	DauA2	COMe
38k	Me	NfDau	COMe
38l	Me	NfDau	CH(OH)Me

for the first time in 1964 [60] and 1967 [61] by Farmitalia (now Pfizer) from a mutant strain of *Streptomyces peuceticus*, and approved by the FDA in 1974 for commercial use. Daunomycin (**1a**) was initially reported to exhibit strong activity against a variety of experimental tumors. Nowadays it is mostly used against acute leukemia. In contrast to most antitumor drugs, **1b** displayed remarkable activity against a broad range of tumors and is also less toxic [62]. To date it is one of the most successful anticancer drugs.

Baumycins are daunomycin analogs containing monoglycoside moieties. They were first isolated from *Streptomyces* sp. ME130-A4, which was finally classified as *S. coeruleorubidus*. Beside baumycin C1 (**38k**) and C2 (**38l**), a characteristic feature of all other baumycins is the presence of an acetal side chain at C-4 of daunosamine, e.g., in baumycin A1 (**38i**) or B1 (**38j**) [63]. Strong antimicrobial activities of baumycin A1, C1, and C2 against gram-positive bacteria have been reported. In addition, **38i** also showed strong inhibition of growth, DNA, and RNA synthesis of cultured L1210 cells [63–65].

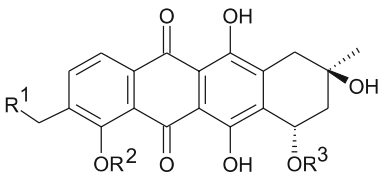
Anthracyclines glycosylated at the 4-position are very rarely mentioned in the literature, and 4-*O*-(β-D-glucopyranosyl)-ε-rhodomycinone (**39a**) isolated from a mutant of *Actinomadura roseoviolacea* was the first one of this series. It exhibited no antibacterial or cytotoxic activity [66]. However, its glucuronic acid derivative histomodulin (**39b**), isolated from *Streptomyces ruber* JCM3131, was reported as an up-regulator of MHC class-I molecules in B16/BL6 cells [67].



	R^1	R^2	R^3
39a	Glc	OH	COOMe
39b	GlcA	OH	COOMe
39c	H	Dau	COOH
39d	H	Dau	αOH
39e	H	Dau	βOH
39f	H	H	OH

In the group of trihydroxyanthracyclines, four of the five natural free 10-carboxylic acids are found; one of them is carborubicin (**39c**). Semisynthetically, the free acids can be easily decarboxylated and simultaneously hydroxylated under inversion of configuration by UV irradiation in the presence of iodine [68]. In a typical experiment, carborubicin (**39c**) was irradiated at 350 nm in acetone/water at pH 5.2 for 1 h to deliver the rhodomycinone-glycoside 10-*epi*-oxaunomycin (D-788-7, **39d**, α-configuration at C-10) in a yield of 34%. In ammonia/acetone, decarboxylation occurs under retention, and oxaunomycin (**39e**) is formed [69].

About 60 glycosides of β-rhodomycinone (**39f**) and other stereoisomers with sugar residues at C-7, C-10, or in both positions have been reported (see Table 5). C-substitution in ring A is scarcely observed in anthracyclines; exceptions are mutactimycin A (**40a**) and D (**40b**) from a *Strepto-*



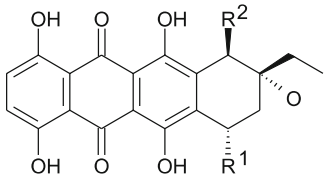
	R^1	R^2	R^3
40a	H	Me	3-mMan
40b	CH_2OH	Me	3-mMan
40c	H	Me	3,4-dRha
40d	H	H	Rha
40e	H	H	Rha

myces sp. 1245 mutant and *Streptomyces* sp. 1254. Mutactimycin A (**40a**) possesses an IC_{50} value of 6.25–12.5 μg mL^{-1} against *Herpes simplex virus-1*, and IC_{50} 6.25–12.5 μg mL^{-1} for *Herpes simplex virus-2*. They also indicated activity against influenza virus A3 (H3N2), with an IC_{50} of 6.25–12.5 μg mL^{-1}, and coxsackie virus B6, (IC_{50} 6.25 μg mL^{-1}) [70–72]. SO-075-R1 (**40c**) isolated from *Nocardia brasiliensis* exhibited activity against gram-positive bacteria [73, 74]. Two further *C*-substituted mutactimycins, 3′-*O*,4-*O*-didemethylmutactimycin (**40d**) and 3′-*O*-demethylmutactimycin (**40e**) have been isolated from the terrestrial *Streptomyces* sp. GW60/1571 [75]. All these compounds have a 7,9-*anti* configuration of the oxygen functions, compared to the usually observed 7,9-*syn* orientation.

3.11
Anthracyclines with the Skeleton of 1,4,6,11-Tetrahydroxyanthraquinone

Due to historical reasons, 1,4,6,11-tetrahydroxyanthracyclines were named isorhodomycinones. They can be classified in two subgroups: into those with a carbomethoxy group at C-10, as in ε-isorhodomycinone (**41a**) and ζ-isorhodomycinone (**41b**), and into those where the carbomethoxy group at C-10 is replaced by a hydroxyl group, as in α-, β- (**41c**) or γ-isorhodomycinone (**41d**). α-Isorhodomycinone differs from β-isorhodomycinone (**41c**) by the opposite stereochemistry at C-7.

Glycosides of both subgroups have been isolated. Examples are the antibacterial 11-hydroxycinerubin A (**41e**) from a *Streptomyces galilaeus*



	R^1	R^2
41a	OH	COOMe
41b	H	COOMe
41c	OH	OH
41d	H	OH
41e	O-Roa-dFuc-CinA	COOMe
41f	O-Roa-dFuc-Rho	COOMe
41g	O-Roa-dFuc-CinA	OH
41h	O-Dau	O-Dau
41i	O-Roa-Rho	O-Roa-Rho-Rho
41j	O-Roa-Rho-Rho	O-Roa-Rho-Rho

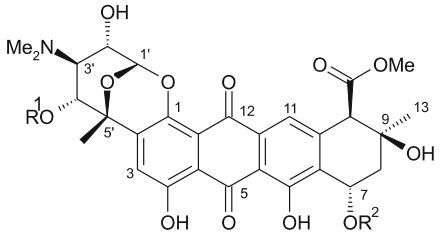
MA 144-M1 mutant KE 303 (FERM-P 4808) [76, 77], or violamycin-B5 (**41f**) reported from *Streptomyces violaceus* with activity against gram-positive, gram-negative bacteria, and *Mycoplasma* sp. [78]. The number of glycosides of the α,β-rhodomycinone type, where a hydroxy group replaced the carbomethoxy group, is higher. An example is β-isorhodomycinone RDC (**41g**), also isolated from *Streptomyces galilaeus* MA 144-M1 mutant KE 303 (FERM-P 4808).

Other glycosides such as isorhodomycin A (**41h**), β-isorhodomycin-*S*-1A (**41i**) isolated from *Streptomyces purpurascens* [79], and the antitumor antibiotic obelmycin-B (**41j**) from *Streptomyces violaceus* A262 [81] are listed in Table 5.

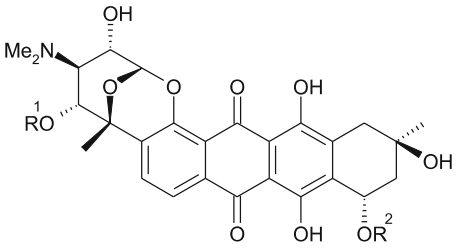
3.12 Unusual Anthracyclines

The nogalarol and nogarol derivatives represent a group amongst the anthracyclines that is unique in many respects. They are derived from 1,4,6- and 1,6,11-trihydroxyanthracyclines, where the latter, however, have not been found in nature so far. An amino sugar – nogalamine or *epi*-nogalamine – is attached at C-1 and C-2 as a *C*,*O*-glycoside. As a normal *O*-glycoside, this sugar is only found in a single example, komodoquinone A (**38g**). A unique neutral sugar (nogalose) is sometimes present at 7-position, other metabolites contain nitro or nitroso sugars. The nogalarols have a 10-β-carboxymethyl group (type **42**), while nogarols are the respective decarboxylation products (**43**). Nogalarol (**42a**) and 7-*con*-*O*-methylnogarol (**43a**) are the simplest members of these groups.

Arugomycin (**42c**) and viriplanin D (**42d**) (both are connected with 4′-*epi*-nogalamine) from *Streptomyces violaceochromogenes* [82] and *Ampullariella*



	R^1	R^2
42a	H	H
42b	H	Nog
42c	dFuc-Dec-Dig	Dig-dFuc-Dig-FDig
42d	dFuc-Dec-dFuc	dFuc-dFuc-Dec-MDig

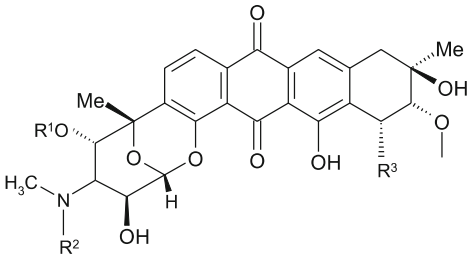


	R^1	R^2
43a	H	Me
43b	H	Roa
43c	Dec-dDec-SDig	Roa
43d	Dec-Dec-SDig	Roa

regularis [83, 84] indicated antiviral and antitumor activity, but were too toxic for clinical use. With a molecular mass of 1708 amu, **42d** is at present the most complex anthracycline of all.

The related nogarols, such as 301-A (**43b**), 301-B (**43c**) and decilorubicin (**43d**) [85, 86], have been isolated from *Streptomyces virginiae*, var. *nanpingenesis* 301. They are 1,6,11-trihydroxyanthracycline derivatives.

While nogalamine is 1,2-connected with the chromophore in **42** and **43**, the five respinomycins A_1, A_2, B, C, and D (**44a–e**) from *Streptomyces xanthocidicus* and *Streptomyces* sp. RK-483 [87, 88] are 4,6-dihydroxyanthracycline



	R^1	R^2	R^3
44a	Avi-Vin	Me	O-Vin
44b	Avi-Vin	Me	H
44c	H	H	O-Vin
44d	H	Me	O-Vin
44e	Dec-Vin	Me	O-Vin

derivatives and surprise by a 3,4-attached nogalamine and additional methoxy groups at C-8; stereochemical details are still unknown. The respinomycins influence the induction of terminal differentiation of human leukemia K-562 cells and inhibit gram-positive and gram-negative bacteria. Altogether, more than 20 anthracycline-*C*,*O*-glycosides have been reported; they are listed in Table 6.

4 Conclusions

Since the successful application of daunorubicin (daunomycin, **1a**) and especially doxorubicin (adriamycin, **1b**) as anticancer drugs, international research activities have led to the discovery of an enormous number of new natural and especially synthetic anthracyclines. The natural derivatives came from a fairly low number of streptomycetes and rare actinomycetes; the majority were, however, isolated from mutants, especially from *S. galilaeus* (see e.g. [89]) many others were obtained by fermentative transformation of related precursors, e.g. by microbial glycosylation of anthracyclinones [90]. Progress in genetic techniques will certainly further increase their number.

Beginning in the early 1960s, when the first results on anthracyclines were published, the number of research contributions published every year has increased almost in a linear manner and totals at present about 16 300 (63 000 if a substructure search is used) articles (Fig. 3). This reflects clearly the still very high scientific and practical relevance of this topic in basic research and especially in medicine. It can be predicted that this development will certainly continue into the next decade.

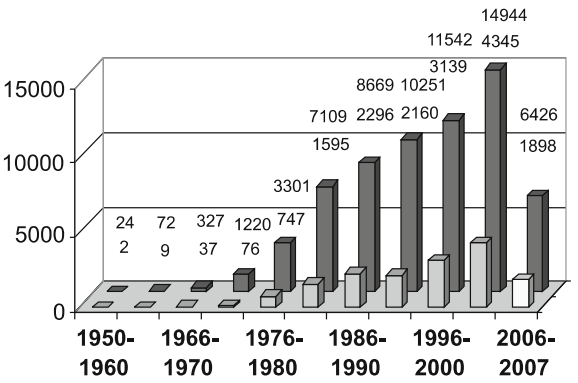


Fig. 3 Number of articles concerning anthracyclines published in 5-year periods. Values in the *first row* were obtained with a fact search for "anthracyclines"; the columns in the *second row* (*upper values*) were derived from a substructure search in Chemical Abstracts

Table 4 Sugars present in anthracyclines, with color reactions and mass differences of the glycosides on MS/MS [91]

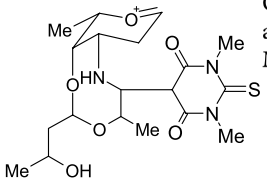
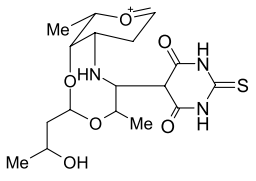
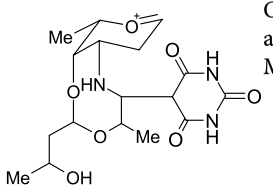
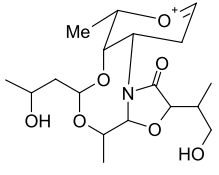
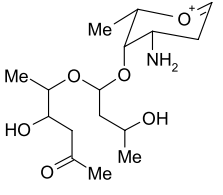
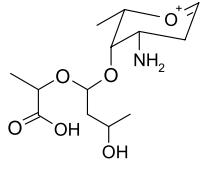
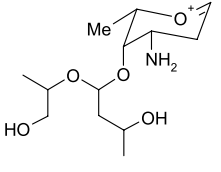
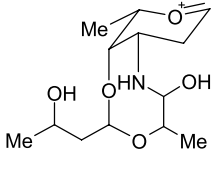
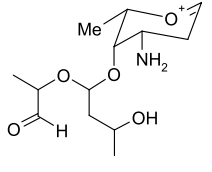
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	Cyclic daunosamine acetal 3; (C3Dau) M–427; $n = 1$		Cyclic daunosamine acetal 6; (C6Dau) M–399; $n = 1$		Cyclic daunosamine acetal 4; (C4Dau) M–383; $n = 1$
	Cyclic daunosamine acetal 5; (C5Dau) M–371; $n = 1$		Daunosamine acetal 4; (DauA4) M–331; $n = 1$		Daunosamine acetal 2; (DauA2) M–289; $n = 2$
	Daunosamine acetal 1; (DauA1) M–275; $n = 8$		Cyclic daunosamine acetal 1; (C1Dau) M–273; $n = 2$		Daunosamine acetal 3; (DauA3) M–273; $n = 1$

Table 4 (continued)

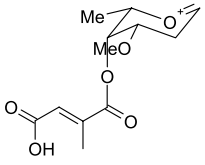
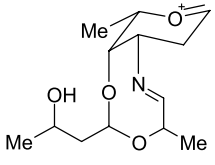
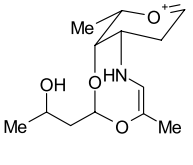
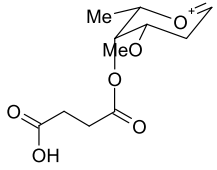
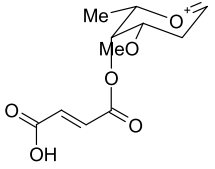
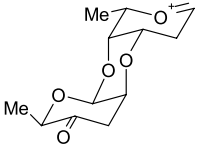
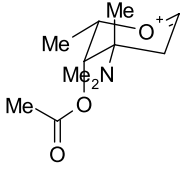
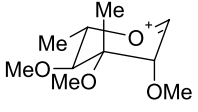
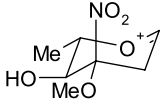
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	4-*O*-Mesaconoyl-L-diginose; (MDig) M–256; $n = 2$		Cyclic daunosamine acetal 2; (C2Dau) M–255; $n = 1$		Cyclic daunosamine acetal 7; (C7Dau) M–255; $n = 1$
	4-*O*-Succinyl-L-diginose; (SDig) M–244; $n = 4$		4-*O*-Fumaryl-L-diginose; (FDig) M–242; $n = 1$		L-Deoxyfucose-L-cinerulose A; (dFuc=CinA) M–240; $n = 41$
	4-*O*-Acetyl-brasiliose (AB) M–213; $n = 1$		Nogalose; (Nog) M–202; $n = 2$		Sugar 1; (S1) M–189; $n = 1$

Table 4 (continued)

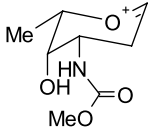
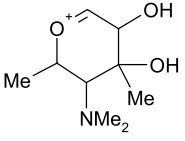
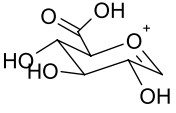
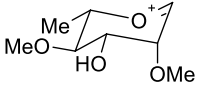
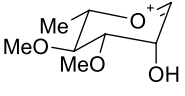
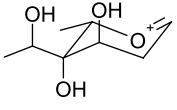
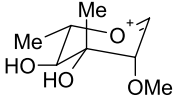
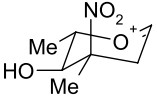
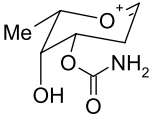
Structures	Name; (abbrev.) R_f [a], color [b], $\Delta m/z$; frequency	Structures	Name; (abbrev.) R_f [a], color [b], $\Delta m/z$; frequency	Structures	Name; (abbrev.) R_f [a], color [b], $\Delta m/z$; frequency
	Daunosamine carbamat; (DauC1) M–187; $n = 1$		Sugar 2; (S2) M–187; $n = 5$		Glucuronic acid; (GlcA) M–176; $n = 1$
	2,4-Di-*O*-methyl-rhamnose (2,4-dRha) M–174; $n = 9$		3,4-Di-*O*-methyl-rhamnose; (3,4-dRha) M–174; $n = 1$		2,6-Dideoxy-4-C-[1′-hydroxyethyl]-L-xylo-hexopyranose; (4CHex) M–174; $n = 1$
	L-Vinelose; (Vin) M–174; $n = 5$		L-Decilonitrose; (Dec) M–173; $n = 8$		Deoxyfucose carbamat; (dFucC) M–173; $n = 2$

Table 4 (continued)

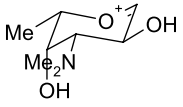
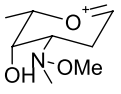
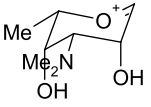
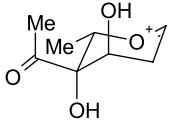
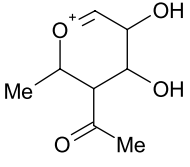
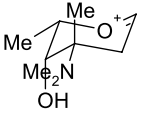
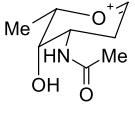
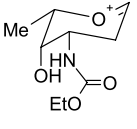
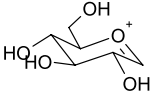
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	4-*epi*-Nogalamine (eNgm) M–173; $n = 8$		Kadaminose; (Kad) M–173; $n = 2$		Nogalamine; (Ngm) M–173; $n = 22$
	4-*C*-Acetyl-2,6-dideoxy-L-xylo-hexopyranoside; (4CdHex1) M–172; $n = 2$		4-*C*-Acetyl-4,6-dideoxy-L-xylo-hexopyranoside; (4CdHex2) M–172; $n = 2$		Brasiliose; (B) M–171; $n = 2$
	*N*-Acetyl-daunosamine; (NaDau) M–171; $n = 1$		Daunosamine carbamat 2; (DauC2) M–169; $n = 1$		D-Glucose; (Glc) M–162; $n = 1$

Table 4 (continued)

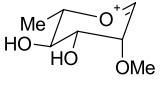
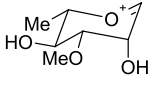
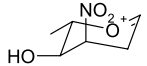
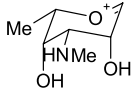
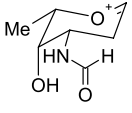
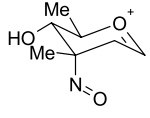
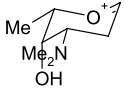
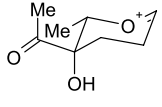
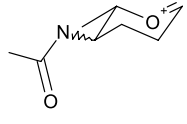
Structures	Name; (abbrev.) R_f [a], color [b], $\Delta m/z$; frequency	Structures	Name; (abbrev.) R_f [a], color [b], $\Delta m/z$; frequency	Structures	Name; (abbrev.) R_f [a], color [b], $\Delta m/z$; frequency
	2-*O*-Methyl-rhamnose; (2mRha) M–160; $n = 7$		3-*O*-Methyl-rhamnose; (3mRha) M–160; $n = 5$		3-Demethyl decilonitrose; (dDec) M–159; $n = 1$
	*N*-Demethyl nogalamine; (dNgm) M–159; $n = 2$		*N*-Formyl-daunosamine; (NfDau) M–157; $n = 4$		Nitroso sugar; (NS) M–157; $n = 1$
	L-Rhodosamine; (Roa) M–157; $n = 233$		4-*C*-Acetyl-2,3,6-dideoxy-L-xylo-hexopyranoside; (4CdHex3) M–156; $n = 1$		Acetamido-demethylforos-aminose; (AdmF) M–155; $n = 3$

Table 4 (continued)

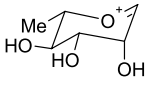
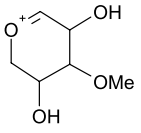
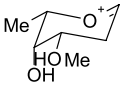
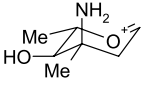
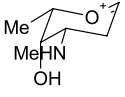
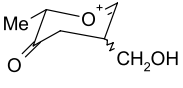
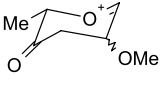
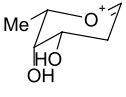
Structures	Name; (abbrev.) R_f [a], color[b], $\Delta m/z$; frequency	Structures	Name; (abbrev.) R_f [a], color[b], $\Delta m/z$; frequency	Structures	Name; (abbrev.) R_f [a], color[b], $\Delta m/z$; frequency
	Rhamnose; (Rha) M–146; $n = 5$		Sugar 3; (S3) M–146; $n = 1$		L-Diginose; (Dig) M–144; $n = 2$
	L-Avidinoamine; (Avi) M–143; $n = 3$		5-Demethyl- rhodosamine; (dmRoa) M–143; $n = 1$		*N*-Methyl- daunosamine; (NDau) M–143; $n = 10$
	2-Hydroxymethyl- cinerulose A; (HmCinA) M–142; $n = 1$		Methoxy-L- cinerulose A; (MCinA) M–142; $n = 3$		2-Deoxy-L- Fucose; (dFuc) 0.51-0.56, grayish blue M–130; $n = 156$

Table 4 (continued)

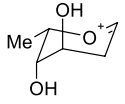
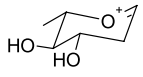
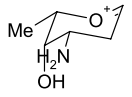
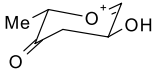
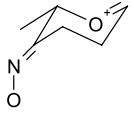
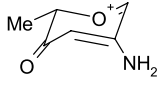
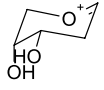
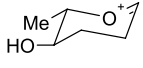
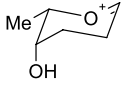
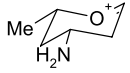
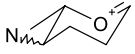
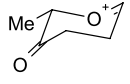
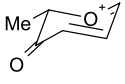
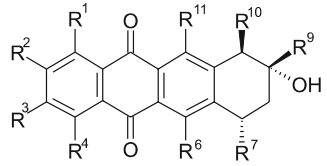
Structures	Name; (abbrev.) R_f [a], color[b], $\Delta m/z$; frequency	Structures	Name; (abbrev.) R_f [a], color[b], $\Delta m/z$; frequency	Structures	Name; (abbrev.) R_f [a], color[b], $\Delta m/z$; frequency
	2,6-Dideoxygulose; (dGul) M–130; $n = 1$		2-Deoxyrhamnose; (dRha) M–130; $n = 2$		L-Daunosamine; (Dau) 0.24, sky blue M–129; $n = 49$
	L-CineruloseB; (CinB) M–128; $n = 1$		Demethyl- forosaminose oxime; (dmFO) M–127; $n = 1$		L-Rednosamine; (Rdm) M–125; $n = 4$
	5-Demethyl- deoxyfucose; (dmdFuc) M–116; $n = 1$		L-Amicetose; (Ami) 0.67–0.74, yellowish brown M–114; $n = 9$		L-Rhodinose; (R, Rho) 0.64–0.71 M–114; $n = 66$

Table 4 (continued)

Structures	Name; (abbrev.) R_f [a], color[b], $\Delta m/z$; frequency	Structures	Name; (abbrev.) R_f [a], color[b], $\Delta m/z$; frequency	Structures	Name; (abbrev.) R_f [a], color[b], $\Delta m/z$; frequency
	4-Deoxydaunosamine; (dDau) M–113; $n = 1$		Demethyl-forosaminose; (dmF) M–113; $n = 1$		D-Cinerulose A; (D-CinA) M–112; $n = 7$
	L-Cinerulose A; (L-CinA) 0.75–0.82, greenish blue M–112; $n = 49$		Aculose; (Acu) M–110; $n = 14$		

Abbreviations as used in Tables 5 and 6. Residues are sorted according to their MS fragment weight
n indicates the frequency of their occurrence in glycosides with at least one molecule of the respective sugar
[a] BuOH-AcOH-H2O (4:1:1)
[b] Visualization was carried out with anisaldehyde

Table 5 List of natural anthracyclines, according to AntiBase [92]. All sugars are connected with the aglycone as *O*-glycosides via oxygen



Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
	6-Hydroxy-anthracyclines												
1	4-Demethoxy-11-deoxyadriamycin	H	H	H	H	OH	Dau	H	H	$COCH_2OH$	H	H	[91]
2	4-Demethoxy-11-deoxydaunomycin	H	H	H	H	OH	Dau	H	H	COMe	H	H	[91]
3	4-Deoxyaclacinomycin A	H	H	H	H	OH	Roa-dFuc-CinA[a]	H	H	Et	COOMe	H	[93]
4	4-Deoxyaclacinomycin B	H	H	H	H	OH	Roa-dFuc=CinA	H	H	Et	COOMe	H	[93]
5	4-Deoxyauramycinone	H	H	H	H	OH	OH	H	H	Me	COOMe	H	[27, 28]
6	4-Deoxysulfurmycinone	H	H	H	H	OH	OH	H	H	CH_2COMe	COOMe	H	[27, 28]
	1,4-Dihydroxyanthracyclines												
7	Rhodirubin G	OH	H	H	OH	H	Roa-dFuc-D-CinA	H	H	Et	COOMe	H	[94, 95]
8	Ruticulomycin B	OH	H	H	OH	H	OH	H	H	αEt	COOMe	H	[96]
	1,6-Dihydroxyanthracyclines and quinocyclines												
9	10-Deoxycelastramycin B	OH	Cl	H	H	OH	CO	–	H	Me/H, no OH	H	H	[30]
10	Celastramycin B	OH	Cl	H	H	OH	CO	–	H	Me/H, no OH	OH	H	[41]
11	Celastramycin C	OH	Cl	H	H	OH	CO	–	H	Me/H, no OH	CO	H	[30]
12	Celastramycin D	OH	Cl	H	H	OH	CO	–	SMe	Me/H, no OH	CO	H	[30]
13	TPU 0025BG	OH	H	H	H	OH	see text	H	H	Me	OH	H	[97]
14	TPU 0025AG	OH	H	H	H	OH	see text	H	H	Me	OH	H	[97]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
15	Quinocycline B; kosinostatin	OH	H	H	H	OH	see text	H	H	Me	4-CdHex2	H	[32]
16	Isoquinocycline A; PA 371g	OH	H	H	H	OH	see text	H	H	Me	4-CHex	H	[33, 98]
17	Isoquinocycline B; TPU 0025B	OH	H	H	H	OH	see text	H	H	Me	4-CdHex2	H	[97, 99–101]
	1,11-Dihydroxyanthracyclines												
18	Tentative structure	OH	H	H	H	H	CO	–	H	Me	H	OH	(Zhang Q, Laatsch H unpublished results)
	4,6-Dihydroxyanthracyclines												
19	Boshracin D	H	H	H	OH	OH	H	OH	H	Me	H	H	[102]
20	10-Decarbomethoxy-aclavinone II	H	H	H	OH	OH	H	OH	H	αEt	H	H	[103]
21	7-*epi*-10-Decarbomethoxy-aclacinomycin A	H	H	H	OH	OH	H	Roa-dFuc-CinA	H	Et	H	H	[91]
22	11-Deoxy-13-dihydrocarmino-mycinone	H	H	H	OH	OH	OH	H	H	CHOHMe	H	H	[16, 17]
23	11-Deoxy-14-hydroxycarmino-mycinone	H	H	H	OH	OH	OH	H	H	$COCH_2OH$	H	H	[16, 17]
24	11-Deoxycarminomycinone	H	H	H	OH	OH	OH	H	H	COMe	H	H	[16, 17]
25	13-Deoxo-11-deoxycarmino-mycinone	H	H	H	OH	OH	OH	H	H	Et	H	H	[16, 17]
27	4-*O*-Demethyl-11,13-dideoxy-daunorubicin	H	H	H	OH	OH	Dau	H	H	Et	H	H	[104]
28	11-Deoxycarminomycin I	H	H	H	OH	OH	Dau	H	H	COMe	H	H	[104]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
29	4-*O*-Demethyl-11-deoxydoxo-rubicin	H	H	H	OH	OH	Dau	H	H	$COCH_2OH$	H	H	[104]
30	11-Deoxy-13-hydroxycarminomycin	H	H	H	OH	OH	Dau	H	H	CHOHMe	H	H	[104]
31	11-Deoxyrubeomycin A	H	H	H	OH	OH	DauA1	H	H	COMe	H	H	[105]
32	Decarboxymethylaclacinomycin T	H	H	H	OH	OH	Roa	H	H	Et	H	H	[106]
33	10-Decarbomethoxyaclacino-mycin A; CG2; G-2	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	H	H	[76, 77]
34	β1-Citromycinone	H	H	H	OH	OH	H	H	H	Et	OH	H	[107]
35	No trivial name	H	H	H	OH	OH	OH	H	H	αMe	OH	H	[108, 109]
36	11-Deoxy-β-rhodomycinone	H	H	H	OH	OH	OH	H	H	Et	OH	H	[110]
37	10-*epi*-11-Deoxyoxaunomycin	H	H	H	OH	OH	Dau	H	H	Et	αOH	H	[111]
38	11-Deoxyoxaunomycin	H	H	H	OH	OH	Dau	H	H	Et	OH	H	[111]
39	Unnamed	H	H	H	OH	OH	$dFuc_2$	H	H	Me	OH	H	[112]
40	Decarboxymethylaclacinomycin T	H	H	H	OH	OH	NDau	H	H	Et	OH	H	[113]
41	11-Deoxy-rhodomycin B	H	H	H	OH	OH	Roa	H	H	Et	OH	H	[114]
42	Unnamed	H	H	H	OH	OH	Roa	H	H	Me	OH	H	[112]
43	Unnamed	H	H	H	OH	OH	Roa-dFuc	H	H	Et	OH	H	[112]
44	Unnamed	H	H	H	OH	OH	Roa-dFuc	H	H	Me	OH	H	[112]
45	11-Deoxy-betaclamycin B	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	OH	H	[114]
46	Unnamed	H	H	H	OH	OH	Roa-$dFuc_2$	H	H	Et	OH	H	[112]
47	11-Deoxy-betaclamycin A	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	OH	H	[114]
48	YL-01641P-B2	H	H	H	OH	OH	2,4-dRha	H	H	Me	S2	H	[115]
49	D-788-3	H	H	H	OH	OH	Dau	H	H	Et	COOH	H	[116]
50	Aklaviketone	H	H	H	OH	OH	CO	H	H	Et	COOMe	H	[117, 118]
51	7-*O*-Daunosaminylaklavinone	H	H	H	OH	OH	Dau	H	H	Et	COOMe	H	[119]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
52	Sulfurmycin D; anthracycline 14A9	H	H	H	OH	OH	Dau	H	H	CH_2COMe	COOMe	H	[120]
53	Aclacinomycin K	H	H	H	OH	OH	Dau-dFuc-CinA	H	H	Et	COOMe	H	[48]
54	Glycoside 1	H	H	H	OH	OH	$dFuc_2$	H	H	Me	COOMe	H	[108]
55	MA-144-U9	H	H	H	OH	OH	$dFuc_2$	H	H	Et	COOMe	H	[14]
56	MA-144-U7	H	H	H	OH	OH	$dFuc_2$-Ami	H	H	Et	COOMe	H	[14]
57	MA-144-U5	H	H	H	OH	OH	$dFuc_2$-CinA	H	H	Et	COOMe	H	[14]
58	Unnamed	H	H	H	OH	OH	$dFuc_3$	H	H	Me	COOMe	H	[112]
59	MA-144-U8	H	H	H	OH	OH	dFuc-Rho	H	H	Et	COOMe	H	[14]
60	MA-144-U6	H	H	H	OH	OH	dFuc-Rho_2	H	H	Et	COOMe	H	[14]
61	Unnamed	H	H	H	OH	OH	dRha	H	H	Et	COOMe	H	[109]
62	7-Deoxyauramycinone	H	H	H	OH	OH	H	H	H	Me	COOMe	H	[16, 17]
63	7-Deoxysulfurmycinone	H	H	H	OH	OH	H	H	H	CH_2COMe	COOMe	H	[16, 17]
64	Galirubinone D	H	H	H	OH	OH	H	H	H	Et	COOMe	H	[16, 17, 117, 118]
65	(7*R*,9*R*,10*R*)-Aklavinone II	H	H	H	OH	OH	H	OH	H	Et	COOMe	H	[121, 122]
66	Aklavinone I	H	H	H	OH	OH	H	OH	H	αEt	COOMe	H	[121, 122]
67	*N*-Demethylaklavine	H	H	H	OH	OH	NDau	H	H	Et	COOMe	H	[94]
68	Aclacinomycin L	H	H	H	OH	OH	NDau-dFuc-CinA	H	H	Et	COOMe	H	[48, 94]
69	13-Methylaklavinone	H	H	H	OH	OH	OH	H	H	*i*Pr	COOMe	H	[16, 17]
70	Aklavinone	H	H	H	OH	OH	OH	H	H	Et	COOMe	H	[16, 17, 121, 122]
71	Auramycinone	H	H	H	OH	OH	OH	H	H	Me	COOMe	H	[18, 27, 28]
72	Nogalamycinone	H	H	H	OH	OH	OH	H	H	αMe	COOMe	H	[123]
73	Sulfurmycinone	H	H	H	OH	OH	OH	H	H	CH_2COMe	COOMe	H	[16, 17, 27, 28]
74	Unnamed	H	H	H	OH	OH	Rho_2	H	H	Et	COOMe	H	[106]
75	Unnamed	H	H	H	OH	OH	Rho-dFuc	H	H	Et	COOMe	H	[56, 57]
76	13-Methyl-aclacinomycin T	H	H	H	OH	OH	Roa	H	H	*i*Pr	COOMe	H	[91]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
77	Aclacinomycin T	H	H	H	OH	OH	Roa	H	H	Et	COOMe	H	[94]
78	Auramycin D	H	H	H	OH	OH	Roa	H	H	Me	COOMe	H	[120]
79	13-Methyl-aclacinomycin S	H	H	H	OH	OH	Roa-dFuc	H	H	*i*Pr	COOMe	H	[91]
80	Aclacinomycin S	H	H	H	OH	OH	Roa-dFuc	H	H	Et	COOMe	H	[48, 94]
81	Auramycin C	H	H	H	OH	OH	Roa-dFuc	H	H	Me	COOMe	H	[120]
82	Sulfurmycin C	H	H	H	OH	OH	Roa-dFuc	H	H	CH_2COMe	COOMe	H	[120]
83	Aclacinomycin B; galirubin B	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	COOMe	H	[94]
84	Aclacinomycin X; siwenmycin	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	COOMe	H	[124–126]
85	Auramycin B	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Me	COOMe	H	[127]
86	Sulfurmycin B	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	CH_2COMe	COOMe	H	[127]
87	MA-144-U1	H	H	H	OH	OH	Roa-$dFuc_2$	H	H	Et	COOMe	H	[14]
88	Aclacinomycin Y	H	H	H	OH	OH	Roa-dFuc-Acu	H	H	Et	COOMe	H	[128, 129]
90	Sulfurmycin Y	H	H	H	OH	OH	Roa-dFuc-Acu	H	H	CH_2COMe	COOMe	H	[130]
91	13-Methylaclacinomycin M	H	H	H	OH	OH	Roa-dFuc-Ami	H	H	*i*Pr	COOMe	H	[14]
92	Auramycin E	H	H	H	OH	OH	Roa-dFuc-Ami	H	H	Me	COOMe	H	[120]
93	Marcellomycin M1; aclacinomycin M	H	H	H	OH	OH	Roa-dFuc-Ami	H	H	Et	COOMe	H	[48, 94]
94	Sulfurmycin E	H	H	H	OH	OH	Roa-dFuc-Ami	H	H	CH_2COMe	COOMe	H	[131]
95	Aclacinomycin-A oxime	H	H	H	OH	OH	Roa-dFuc-dmFO	H	H	Et	COOMe	H	[91]
96	13-Methylaclacinomycin A	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	*i*Pr	COOMe	H	[132]
97	5C-*epi*-Aclacinomycin A	H	H	H	OH	OH	Roa-dFuc-D-CinA	H	H	Et	COOMe	H	[94]
98	Aclacinomycin G	H	H	H	OH	OH	Roa-dFuc-D-CinA	H	H	Et	COOMe	H	[48]
99	Aclacinomycin A; aclarubicin	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	COOMe	H	[94]
100	Auramycin A	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Me	COOMe	H	[127]
101	Sulfurmycin A	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	CH_2COMe	COOMe	H	[127]
102	4‴-(*R*)-Amino-aclacinomycin A	H	H	H	OH	OH	Roa-dFuc-dmF	H	H	Et	COOMe	H	[91]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
103	Aclacinomycin X	H	H	H	OH	OH	Roa-dFuc-Rdm	H	H	Et	COOMe	H	[133, 134]
104	4‴-(*R*)-*N*-Acetamido-aclacinomycin A	H	H	H	OH	OH	Roa-dFuc-AdmF	H	H	Et	COOMe	H	[91]
105	13-Methylaclacinomycin N	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	*i*Pr	COOMe	H	[14]
106	Aclacinomycin N; marcellomycin M1	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	COOMe	H	[48, 94]
107	Auramycin F	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	Me	COOMe	H	[120]
108	Sulfurmycin F	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	CH_2COMe	COOMe	H	[120]
109	4‴-(*S*)-Amino-aclacinomycin A	H	H	H	OH	OH	Roa-dFuc-AdmF	H	H	Et	COOMe	H	[91]
110	4‴-(*S*)-*N*-Acetamido-aclacinomycin A	H	H	H	OH	OH	Roa-dFuc-AdmF	H	H	Et	COOMe	H	[91]
111	Auramycin H; 3″-deoxyauramycin C	H	H	H	OH	OH	Roa-Rho	H	H	Me	COOMe	H	[120]
112	Sulfurmycin H	H	H	H	OH	OH	Roa-Rho	H	H	CH_2COMe	COOMe	H	[120]
113	Auramycin G	H	H	H	OH	OH	Roa-Rho_2	H	H	Me	COOMe	H	[120]
114	Sulfurmycin G	H	H	H	OH	OH	Roa-Rho_2	H	H	CH_2COMe	COOMe	H	[131]
115	Micromonomycin	H	H	H	OH	OH	4CdHex3	H	H	Me	COOMe	H	[135]
116	(9*R*,10*S*)-7-Deoxyaklavinone	H	H	H	OH	OH	H	H	H	Et	αCOOMe	H	[16, 17, 136]
117	Resomycin A	H	H	H	OH	OH	H	H	H	Me	αCOOMe	H	[137–139]
118	10-*epi*-Aclacinomycin A	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	αCOOMe	H	[91]
119	Aranciamycin	H	H	H	OH	OH	2-mRha	H	βOMe	Me	CO	H	[99–101]
120	Aranciamycinone	H	H	H	OH	OH	OH	H	βOMe	Me	CO	H	[140]
121	SM-173-B	H	H	H	OH	OH	H	H	βOMe	aMe	CO	H	[141]
122	11-Deoxy-13-deoxodaunomycin	H	H	H	OMe	OH	Dau	H	H	Et	H	H	[142–144]
123	11-Deoxy-13-dihydrodaunomycinone	H	H	H	OMe	OH	OH	H	H	CHOHMe	H	H	[16, 17]
124	11-Deoxy-13-dihydrodaunorubicin	H	H	H	OMe	OH	Dau	H	H	CHOHMe	H	H	[145–147]
125	11-Deoxyadriamycinone	H	H	H	OMe	OH	OH	H	H	$COCH_2OH$	H	H	[16, 17]
126	11-Deoxydaunomycinone	H	H	H	OMe	OH	OH	H	H	COMe	H	H	[16, 17]
127	11-Deoxydaunorubicin	H	H	H	OMe	OH	Dau	H	H	COMe	H	H	[142–144]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
128	11-Deoxydoxorubicin	H	H	H	OMe	OH	Dau	H	H	$COCH_2OH$	H	H	[142–144]
129	13-Deoxo-11-deoxydaunomycinone	H	H	H	OMe	OH	OH	H	H	Et	H	H	[16, 17]
130	6,10-Dideoxy-13-dihydrodauno-mycinone	H	H	H	OMe	OH	H	H	H	CHOHMe	H	H	[145–147]
131	Mutactimycin B	H	H	H	OMe	OH	3-mRha	H	H	αMe	H	H	[148]
132	4-*O*-Methyl-aclacinomycin A	H	H	H	OMe	OH	Roa-dFuc-CinA	H	H	Et	COOMe	H	[91]
133	4-*O*-Methyl-aclacinomycin M	H	H	H	OMe	OH	Roa-dFuc-Ami	H	H	Et	COOMe	H	[91]
134	4-*O*-Methyl-aclacinomycin N	H	H	H	OMe	OH	Roa-dFuc-Rho	H	H	Et	COOMe	H	[91]
135	4-*O*-Methyl-aclacinomycin S	H	H	H	OMe	OH	Roa-dFuc	H	H	Et	COOMe	H	[91]
136	4-*O*-Methyl-aclacinomycin T	H	H	H	OMe	OH	Roa	H	H	Et	COOMe	H	[91]
137	Aclacinomycin A-7-methylether	H	H	H	OMe	OH	Roa-dFuc-CinA	H	H	Et	COOMe	H	[76, 77]
138	D-788-16	H	H	H	OMe	OH	Dau	H	H	Et	COOMe	H	[149]
139	YL-01641P-C1	H	H	H	OMe	OH	2,4-dRha	H	H	Me/OAc	S2	H	[115]
140	Aclacinomycin A 6-methyl ether	H	H	H	OH	OMe	Roa-dFuc-CinA	H	H	Et	COOMe	H	[130]
141	Fragilomycin A	H	H	R[a]	OH	OMe	Sugar	H	H	Me	OH	H	[150]
	4,11-Dihydroxyanthracyclines												
142	CG12	H	H	H	OH	H	H	H	H	Et	Roa-dFuc-CinA	OH	[82]
143	TMF 518D	H	H	H	OH	H	H	H	H	Et	Roa-dFuc=CinA	OH	[151]
144	Cosmocarcin C	H	H	H	OH	H	OH	H	H	Et	Roa-dFuc-D-CinA	OH	[151]
145	γ-Citromycinone	H	H	H	OH	H	OH	H	H	Et	H	OH	[16, 17, 107]
146	α-Citromycinone	H	H	H	OH	H	OH	H	H	Et	OH	OH	[152]
147	Yellamycin C	H	H	H	OH	H	OH	H	H	Et	Roa	OH	[80]
148	Yellamycin A	H	H	H	OH	H	Roa	H	H	Et	OH	OH	[80]
149	Yellamycin B	H	H	H	OH	H	Roa	H	H	Et	Roa	OH	[80]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
150	Cosmocarcin B	H	H	H	OH	H	Roa-dFuc=CinA	H	H	Et	Roa-dFuc-CinA	OH	[151]
151	Cosmocarcin A; serirubicin	H	H	H	OH	H	Roa-dFuc=CinA	H	H	Et	Roa-dFuc=CinA	OH	[107, 151]
	6,11-Dihydroxyanthracyclines												
152	Idarubicin	H	H	H	H	OH	Dau	H	H	COMe	H	OH	[45]
153	Medorubicin	H	H	H	H	OH	Dau	H	H	$COCH_2OH$	H	OH	[153, 154]
154	F 840020	H	H	H	H	OH	dFuc	H	H	CH_2OH	OH	OH	[46]
	1,4,6-Trihydroxyanthracyclines												
155	DC 44A	OH	H	H	OH	OH	H	H	H	CH_2COMe	COOMe	H	[16,17, 155,156]
156	(9*R*,10*R*)-ζ-Pyrromycinonic acid	OH	H	H	OH	OH	H	H	H	Et	COOH	H	[157]
157	Galirubinone C; ζ-pyrromycinone	OH	H	H	OH	OH	H	H	H	Et	COOMe	H	[16, 17, 157]
158	DC 44B	OH	H	H	OH	OH	H	H	H	Me	COOMe	H	[16,17, 155,156]
159	Cinerubin C	OH	H	H	OH	OH	H	Rho_2	H	Et	COOMe	H	[158]
160	Cinerubin D	OH	H	H	OH	OH	H	Rho-dFuc	H	Et	COOMe	H	[158]
161	(7*R**9*R**10*R**)-Pyrromycin	OH	H	H	OH	OH	H	Roa	H	Et	COOMe	H	[53]
162	1-Hydroxy-11-deoxy-carminomycin II	OH	H	H	OH	OH	DauA1	H	H	COMe	H	H	[103]
163	Musettamycin	OH	H	H	OH	OH	H	Roa-dFuc	H	Et	COOMe	H	[159–161]
164	1-Hydroxyauramycinone; ε1-pyrromycinone	OH	H	H	OH	OH	OH	H	H	Me	COOMe	H	[155, 156]
165	Bohemic acid	OH	H	H	OH	OH	OH	H	H	Et	COOH	H	[162]
166	ε-Pyrromycinone	OH	H	H	OH	OH	OH	H	H	Et	COOMe	H	[16, 17, 155, 156]
167	ε1-Hydroxysulfurmycinone	OH	H	H	OH	OH	OH	H	H	CH_2COMe	COOMe	H	[155, 156]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
168	YL-01641P-B1	OH	H	H	OH	OH	2,4-dRha	H	H	Me/OCOMe	S5	H	[115]
169	Carbamat A	OH	H	H	OH	OH	CdFuc-dFuc-CinA	H	H	Et	COOMe	H	[51]
170	Carbamat B	OH	H	H	OH	OH	CdFuc-dFuc-CinB	H	H	Et	COOMe	H	[51]
171	Cyclamycin A	OH	H	H	OH	OH	$dFuc_2$-CinA	H	H	Et	COOMe	H	[49]
172	Ciclamycin 4	OH	H	H	OH	OH	dFuc-Rho_2	H	H	Et	COOMe	H	[49]
173	Pyrraculomycin	OH	H	H	OH	OH	Roa-dFuc-Acu	H	H	Et	COOMe	H	[163, 164]
174	Kadamamycin B	OH	H	H	OH	OH	Kad-dFuc=CinA	H	H	Me	COOMe	H	[29]
175	Kadamamycin A	OH	H	H	OH	OH	Kad-Rho-Acu	H	H	Me	COOMe	H	[29]
176	Schaunardimycin	OH	H	H	OH	OH	NDau-dFuc	H	H	Et	COOMe	H	[165]
177	Cinerubin M	OH	H	H	OH	OH	NDau-dFuc=CinA	H	H	Et	COOMe	H	[51]
178	Alcindoromycin	OH	H	H	OH	OH	NDau-$dFuc_2$	H	H	Et	COOMe	H	[166]
179	Cinerubin K	OH	H	H	OH	OH	Rho_3	H	H	Et	COOMe	H	[102]
180	1-Hydroxyaklavin; ciclamycin 6; pyrromycin	OH	H	H	OH	OH	Roa	H	H	Et	COOMe	H	[47]
181	1-Hydroxysulfurmycin T	OH	H	H	OH	OH	Roa	H	H	αCH_2COMe	COOMe	H	[53]
182	(7*S**9*S**10*R**)-Pyrromycin	OH	H	H	OH	OH	Roa	H	H	αEt	COOMe	H	[53]
183	1-Hydroxyauramycin T	OH	H	H	OH	OH	Roa	H	H	αMe	COOMe	H	[53]
184	Collinemycin; 10-*epi*-musettamycin	OH	H	H	OH	OH	Roa-dFuc	H	H	Et	αCOOMe	H	[166]
185	Cinerubin Y	OH	H	H	OH	OH	Rho-dFuc=CinA	H	H	Et	COOMe	H	[167]
186	Taurimycin B	OH	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	COOMe	H	[168]
187	1-Hydroxyauramycin B	OH	H	H	OH	OH	Roa-dFuc=CinA	H	H	Me	COOMe	H	[27, 28]
188	Spartanamicin A; ciclamycin B	OH	H	H	OH	OH	Roa-dFuc=DCinA	H	H	Et	COOMe	H	[169, 170]
189	Mimimycin; 10-*epi*-marcellomycin	OH	H	H	OH	OH	Roa-$dFuc_2$	H	H	Et	αCOOMe	H	[14, 166]
190	Marcellomycin; rhodirubin E	OH	H	H	OH	OH	Roa-$dFuc_2$	H	H	Et	COOMe	H	[159–161]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
191	Dihydrocinerubin-A	OH	H	H	OH	OH	Roa-dFuc-Ami	H	H	Et	COOMe	H	[94]
192	β-Pyrromycin RDC	OH	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	OH	H	[76, 77]
193	MA-144-G2	OH	H	H	OH	OH	Roa-dFuc-D-CinA	H	H	Et	COOMe	H	[94]
194	Spartanamicin B; cinerubin A	OH	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	COOMe	H	[169, 170]
195	Taurimycin A	OH	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	COOMe	H	[168]
196	1-Hydroxyauramycin A	OH	H	H	OH	OH	Roa-dFuc-CinA	H	H	Me	COOMe	H	[27, 28]
197	Rudolfomycin; rudolphomycin	OH	H	H	OH	OH	Roa-dFuc-Rdm	H	H	Et	COOMe	H	[166]
198	Rhodirubin A; trypanomycin A2	OH	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	COOMe	H	[94]
199	Rhodirubin B; marcellomycin M2	OH	H	H	OH	OH	Roa-Rho_2	H	H	Et	COOMe	H	[91, 171]
200	Cinerubin R	OH	H	H	OH	OH	Roa-Rho-Acu	H	H	Et	COOMe	H	[164, 172]
201	Nocardicyclin A	OMe	H	H	OH	OH	B	H	αOMe	Me	CO	H	[54]
202	Nocardicyclin B	OMe	H	H	OH	OH	AB	H	αOMe	Me	CO	H	[54]
203	Nothramicin	OMe	H	H	OH	OH	B	H	OMe	Me	OH	H	[54]
204	YL-01641P-A1	OH	H	H	OMe	OH	2,4-dRha	H	H	Me/OAc	S2	H	[115]
205	YL-01641P-A2	OH	H	H	OMe	OH	2,4-dRha	H	H	Me	S2	H	[115]
	1,4,11-Trihydroxyanthracyclines												
206	α2-Rhodomycinone	OH	H	H	OH	H	OH	H	H	Et	OH	OH	[16, 17, 173]
207	Alldimycin C; alpharubicin I	OH	H	H	OH	H	OH	H	H	Et	Roa	OH	[80]
208	α2-Rhodomycinone RDC	OH	H	H	OH	H	OH	H	H	Et	Roa-dFuc-CinA	OH	[90]
209	1-Hydroxyserirubicin	OH	H	H	OH	H	Roa-dFuc=CinA	H	H	Et	Roa-dFuc=CinA	OH	[107]
210	Alldimycin A; violamycin A2	OH	H	H	OH	H	Roa	H	H	Et	OH	OH	[80]
211	Alldimycin B; α2-rhodomycin-Roa_2	OH	H	H	OH	H	Roa	H	H	Et	Roa	OH	[80]
212	α2-Rhodomycin A	OH	H	H	OH	H	Roa-dFuc-CinA	H	H	Et	OH	OH	[91]
213	Violamycin B2	OH	H	H	OH	H	Roa-dFuc-Rho	H	H	Et	OH	OH	[78, 214, 215]
214	δ-Rhodomycinone	OH	H	H	OH	H	OH	H	H	Et	COOMe	OH	[12, 13, 16, 17, 152]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
	1,6,11-Trihydroxyanthracyclines: see Table 6												
	2,4,6-Trioxyanthracyclines												
215	2-Demethylsteffimycin D	H	OH	H	OH	OH	2-mRha	H	OMe	Me	H	H	[174]
216	2,2′,8-Demethoxysteffimycin C	H	OH	H	OH	OH	Rha	H	H	αMe	H	H	[175]
217	2-Hydroxy-7-deoxyaklavinone	H	OH	H	OH	OH	H	H	H	Et	COOMe	H	[16, 17]
218	4-Deoxy-9-hydroxyaklavinone	H	OH	H	OH	OH	H	H	H	Et	COOMe	H	[16, 17, 121, 122, 176, 177]
219	2-Hydroxyaklavinone	H	OH	H	OH	OH	OH	H	H	Et	COOMe	H	[16, 17, 121, 122, 176, 177]
220	2-Hydroxyaclacinomycin T	H	OH	H	OH	OH	Roa	H	H	Et	COOMe	H	[91]
221	2-Hydroxyaclacinomycin S	H	OH	H	OH	OH	Roa-dFuc	H	H	Et	COOMe	H	[91]
222	2-Hydroxyaclacinomycin B	H	OH	H	OH	OH	Roa-dFuc=CinA	H	H	Et	COOMe	H	[178]
223	2-Hydroxyaclacinomycin M	H	OH	H	OH	OH	Roa-dFuc-Ami	H	H	Et	COOMe	H	[91]
224	2-Hydroxyaclacinomycin A	H	OH	H	OH	OH	Roa-dFuc-CinA	H	H	Et	COOMe	H	[56, 57]
225	2-Hydroxyaclacinomycin N	H	OH	H	OH	OH	Roa-dFuc-Rho	H	H	Et	COOMe	H	[179]
226	Steffimycin C	H	OMe	H	OH	OH	2,4-dRha	H	βOMe	αMe	H	H	[55]
227	Steffimycin E	H	OMe	H	OH	OH	2,4-dRha	H	H	αMe	H	H	[175]
228	8-Demethoxy-steffimycin D	H	OMe	H	OH	OH	2-mRha	H	H	αMe	H	H	[175]
229	8,2′-Demethoxy-steffimycin C	H	OMe	H	OH	OH	Rha	H	H	αMe	H	H	[175]
230	Steffimycin D	H	OMe	H	OH	OH	2-mRha	H	OMe	Me	H	H	[174]
231	10-Dihydrosteffimycin B	H	OMe	H	OH	OH	2,4-dRha	H	βOMe	αMe	OH	H	[180, 181]
232	10-Dihydrosteffimycin	H	OMe	H	OH	OH	2-mRha	H	βOMe	αMe	OH	H	[180, 181]
233	Steffimycinol	H	OMe	H	OH	OH	OH	H	OMe	Me	OH	H	[182, 183]
234	Steffimycin B	H	OMe	H	OH	OH	2,4-dRha	H	βOMe	αMe	CO	H	[35–37, 184]
235	8-Demethoxy-steffimycin A	H	OMe	H	OH	OH	2-mRha	H	H	αMe	CO	H	[175]
236	Steffimycin A	H	OMe	H	OH	OH	2-mRha	H	βOMe	αMe	CO	H	[184–186]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
237	7-Deoxy-8-demethoxysteffimycinone	H	OMe	H	OH	OH	H	H	H	Me	CO	H	[188, 189]
238	7-Deoxy-steffimycinone	H	OMe	H	OH	OH	H	H	OMe	Me	CO	H	[182, 183, 190]
239	Steffimycinone	H	OMe	H	OH	OH	OH	H	OMe	Me	CO	H	[16, 17]
	4,6,11-Trioxyanthracyclines												
240	Komodoquinone A	H	H	H	eNgm	OH	OH	H	H	Me	H	OH	[191]
241	10-Desoxy-γ-rhodomycinon	H	H	H	OH	OH	H	H	H	Et	H	OH	[12, 13, 16, 17]
242	Trisarubicinol	H	H	H	OH	OH	H	Roa-dFuc-CinA	H	CHOHMe	H	OH	[192]
243	10-Deoxy-β-rhodomycinone	H	H	H	OH	OH	OH	H	H	Et	H	OH	[193]
244	13-Dihydrocarminomycinone	H	H	H	OH	OH	OH	H	H	CHOHMe	H	OH	[16, 17]
245	Carminomycinone	H	H	H	OH	OH	OH	H	H	COMe	H	OH	[104]
246	Komodoquinone B	H	H	H	OH	OH	OH	H	H	Me	H	OH	[191]
247	13-Deoxocarminomycin	H	H	H	OH	OH	Dau	H	H	Et	H	OH	[194]
248	Carminomycin I	H	H	H	OH	OH	Dau	H	H	COMe	H	OH	[24–26]
249	Carminomycinol	H	H	H	OH	OH	Dau	H	H	CHOHMe	H	OH	[194]
250	Carminomycin II; rubeomycin A	H	H	H	OH	OH	DauA1	H	H	COMe	H	OH	[24–26]
251	Carminomycin III; rubeomycin A1	H	H	H	OH	OH	DauA1	H	H	COMe	H	OH	[195, 196]
252	Rubeomycin-B	H	H	H	OH	OH	DauA1	H	H	CHOHMe	H	OH	[197–199]
253	Rubeomycin B1	H	H	H	OH	OH	DauA1	H	H	CHOHMe	H	OH	[198, 199]
254	Barminomycin Ia	H	H	H	OH	OH	DauA3	H	H	COMe	H	OH	[200, 202, 203]
255	SN-706	H	H	H	OH	OH	DauA4	H	H	COMe	H	OH	[201]
256	Barminomycin Ic	H	H	H	OH	OH	C1Dau	H	H	COMe	H	OH	[202, 203]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
257	Barminomycin Ib	H	H	H	OH	OH	C2Dau	H	H	COMe	H	OH	[202, 203]
258	SN-07 dehydratation product 2	H	H	H	OH	OH	C7Dau	H	H	COMe	H	OH	[204]
259	*N*-Formyl-13-dihydro-carminomycin I	H	H	H	OH	OH	NfDau	H	H	CHOHMe	H	OH	[104]
260	Kesarirhodin A	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	H	OH	[205]
261	Kesarirhodin B	H	H	H	OH	OH	Roa-Rho_2	H	H	Et	H	OH	[205]
262	Oxaunomycin	H	H	H	OH	OH	Dau	H	H	Et	OH	OH	[194, 206, 207]
263	R20X10		H	H	OH	OH	Dau	H	H	Me	OH	OH	[208]
264	R20X2	H	H	H	OH	OH	Dau	H	H	Et	OH	OH	[209]
265	α-Rhodomycinone	H	H	H	OH	OH	H	OH	H	Et	OH	OH	[16, 17, 193]
266	β1-Rhodomycinone	H	H	H	OH	OH	H	H	H	Me	OH	OH	[12, 13, 16, 17]
267	γ-Rhodomycinone; iremycinone	H	H	H	OH	OH	H	H	H	Et	OH	OH	[16, 17, 72, 193]
268	α1-Rhodomycinone	H	H	H	OH	OH	OH	H	H	Me	OH	OH	[210]
269	β-Rhodomycinone	H	H	H	OH	OH	OH	H	H	Et	OH	OH	[16, 17, 72, 193]
270	BA-32-C	H	H	H	OH	OH	Roa	H	H	Et	OH	OH	[211]
271	Rhodomycin B; β-rhodomycin I	H	H	H	OH	OH	Roa	H	H	Et	OH	OH	[78, 193, 212]
272	Betaclamycin S	H	H	H	OH	OH	Roa-dFuc	H	H	Et	OH	OH	[133, 134, 212, 230]
273	Betaclamycin B	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	OH	OH	[213]
274	Unnamed	H	H	H	OH	OH	Roa-$dFuc_2$	H	H	Et	OH	OH	[112]
275	β-Rhodomycin RDC; betaclamycin A	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	OH	OH	[76, 77]
276	CG-21-C	H	H	H	OH	OH	Roa-dFuc-Rdm	H	H	Et	OH	OH	[133, 134]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
277	Betaclamycin M	H	H	H	OH	OH	Roa-dFuc-Ami	H	H	Et	OH	OH	[212]
278	Violamycin B6; betaclamycin N	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	OH	OH	[78, 212, 214, 215]
279	10-*epi*-Oxaunomycin	H	H	H	OH	OH	Dau	H	H	Et	αOH	OH	[111]
280	10-*epi*-γ-rhodomycinone	H	H	H	OH	OH	H	H	H	Et	αOH	OH	[12, 13]
281	β-Rhodomycin S-3	H	H	H	OH	OH	Roa-dFuc	H	H	Et	Roa-Rho	OH	[79]
282	A 447D	H	H	H	OH	OH	Roa-Rho_2	H	H	Et	Roa-Rho-CinA	OH	[216]
283	Ditrisarubicin D	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	Roa-Rho-CinA	OH	[94]
284	Ditrisarubicin I	H	H	H	OH	OH	Roa-Rho-D-CinA	H	H	Et	Roa-Rho-CinA	OH	[72]
285	2‴-Methoxy-2‴,3‴-dihydroditrisarubicin H	H	H	H	OH	OH	Roa-Rho-MCinA	H	H	Et	Roa-Rho-Acu	OH	[72]
286	Cytorhodin P	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	Roa-Rho-Acu	OH	[217, 218]
287	Cytorhodin TF	H	H	H	OH	OH	Roa	H	H	Et	Roa-Rho-Acu	OH	[219]
288	Ditrisarubicin C	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	Roa-Rho-Acu	OH	[72, 220]
289	Ditrisarubicin H; cytorhodin F	H	H	H	OH	OH	Roa-Rho-Acu	H	H	Et	Roa-Rho-Acu	OH	[72]
290	2*C*,3*C*-Didehydro-4*C*-deoxy-1-hydroxy-cytorhodin A	H	H	H	OH	OH	Roa-Rho-Acu	H	H	Et	Roa-Rho_2	OH	[221]
291	Cosmomycin A; rhodilunancin A	H	H	H	OH	OH	H	H	H	Et	Roa-Rho_2	OH	[72,217,218, 222,223]
292	Cosmomycin C; cytorhodin O	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	Roa-Rho_2	OH	[217, 218, 224]
293	Cytorhodin A	H	H	H	OH	OH	Roa-Rho_2	H	H	Et	Roa-Rho_2	OH	[216]
294	Cytorhodin B	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	Roa-Rho_2	OH	[217, 218, 225]
295	Cytorhodin V	H	H	H	OH	OH	Roa-Rho_2	H	H	Et	Roa-Rho_2	OH	[217, 218]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
296	Cytorhodin X	H	H	H	OH	OH	H	H	H	Et/Roa	Roa-Rho_2	OH	[72, 226]
297	Ditrisarubicin F	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	Roa-Rho_2	OH	[95]
298	β-Rhodomycin S-1B	H	H	H	OH	OH	Roa-Rho	H	H	Et	Roa-Rho_2	OH	[79]
299	Cytorhodin L	H	H	H	OH	OH	H	H	H	Et	Roa-Rho	OH	[217, 218]
300	Cosmomycin B	H	H	H	OH	OH	H	H	H	Et	Roa-dFuc-Rho	OH	[222, 223]
301	Cosmomycin D	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	Roa-dFuc-Rho	OH	[216, 227, 228]
302	Cytorhodin N	H	H	H	OH	OH	Roa-Rho_2	H	H	Et	Roa-dFuc-Rho	OH	[217, 218]
303	Cytorhodin Y	H	H	H	OH	OH	H	H	H	Et/Roa	Roa-dFuc-Rho	OH	[72, 229]
304	Ditrisarubicin G	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	Roa-dFuc-Rho	OH	[94]
305	Rhodomycin X	H	H	H	OH	OH	OH	H	H	Et	Roa-dFuc-Rho	OH	[230]
306	2‴‴‴-Methoxy-2‴‴‴,3‴‴‴ dihydroditrisarubicin C	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	Roa-dFuc-MCinA	OH	[72]
307	A 447D′	H	H	H	OH	OH	H	H	H	Et	Roa-dFuc-CinA	OH	[216]
308	Ditrisarubicin E	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	Roa-dFuc-CinA	OH	[94]
309	Ditrisarubicin A	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	Roa-dFuc-CinA	OH	[220]
310	γ-Rhodomycin RDC	H	H	H	OH	OH	H	H	H	Et	Roa-dFuc-D-CinA	OH	[76, 77]
311	SS-288A	H	H	H	OH	OH	Roa-$dFuc_2$	H	H	Et	Roa-$dFuc_2$	OH	[231]
312	Andhramycin A	H	H	H	OH	OH	H	H	H	Et	Roa-$dFuc_2$	OH	(Rao VL, Laatsch H, unpublished results)
313	2‴-Methoxy-2‴,3‴-dihydroditrisarubicin CR	H	H	H	OH	OH	Roa-Rho-MCinA	H	H	Et	Roa-dFuc=CinA	OH	[72]
314	Cytorhodin I	H	H	H	OH	OH	H	Roa-dFuc-CinA	H	Et	Roa-dFuc=CinA	OH	[217, 218]
315	Cytorhodin T	H	H	H	OH	OH	Roa	H	H	Et	Roa-dFuc=CinA	OH	[94]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
316	Ditrisarubicin B/7de-dF-CB	H	H	H	OH	OH	Roa	H	H	Et	Roa-dFuc=CinA	OH	[94]
317	Ditrisarubicin B; cytorhodin C	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	Roa-dFuc=CinA	OH	[72, 107, 220]
318	Ditrisarubicin CR	H	H	H	OH	OH	Roa-Rho-Acu	H	H	Et	Roa-dFuc=CinA	OH	[229]
319	No trivial name	H	H	H	OH	OH	Roa-dFuc	H	H	Et	Roa-dFuc=CinA	OH	[94]
320	Ditrisarubicin F	H	H	H	OH	OH	Roa-Rho-dFuc	H	H	Et	Roa-dFuc=CinA	OH	[94]
321	Ditrisarubicin GR	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	Roa-dFuc=CinA	OH	[94]
322	Ditrisarubicin GR/de-Ro	H	H	H	OH	OH	Roa-dFuc	H	H	Et	Roa-dFuc=CinA	OH	[94]
323	Cytorhodin M	H	H	H	OH	OH	OH	H	H	Et	Roa-dFuc	OH	[217, 218]
324	Ditrisarubicin G/de-Ro	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	Roa-dFuc	OH	[95]
325	β-Rhodomycin S4	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	Roa-dFuc	OH	[79]
326	γ-Rhodomycin-Roa_2-Rod	H	H	H	OH	OH	H	H	H	Et	Roa_2-Rho	OH	[232, 233]
327	Roseorubicin A	H	H	H	OH	OH	H	H	H	Et	Roa_2-dFuc-Rho_2	OH	[91]
328	γ-Rhodomycin IV	H	H	H	OH	OH	OH	H	H	Et	Roa_2-dFuc-Rho	OH	[234, 235]
329	γ-Rhodomycin III	H	H	H	OH	OH	H	H	H	Et	Roa_2-dFuc	OH	[234, 235]
330	Roseorubicin B	H	H	H	OH	OH	H	H	H	Et	Roa_2	OH	[91, 234, 235]
331	γ-Rhodomycin II	H	H	H	OH	OH	OH	H	H	Et	Roa_2	OH	[232, 236]
332	10-*O*-Rhodosaminyl-β-rhodomycinone	H	H	H	OH	OH	OH	H	H	Et	Roa	OH	[237]
333	BA-32-B	H	H	H	OH	OH	NDau	H	H	Et	Roa	OH	[211]
334	BA-32-D	H	H	H	OH	OH	OH	H	H	Et	Roa	OH	[211]
335	Cytorhodin S; rhodorubicin	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	Roa	OH	[94]
336	Cytorhodin SD	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	Roa	OH	[219]
337	Cytorhodin SE	H	H	H	OH	OH	Roa-Rho-Acu	H	H	Et	Roa	OH	[219]
338	G0041-3c	H	H	H	OH	OH	dmRoa-dmdFuc-HmCinA	H	H	Et	Roa	OH	[40]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
339	Iremycin; γ-rhodomycin I	H	H	H	OH	OH	H	H	H	Et	Roa	OH	[238, 239]
340	Rhodomycin A; β-rhodomycin II	H	H	H	OH	OH	Roa	H	H	Et	Roa	OH	[232, 233]
341	β-Rhodomycin IV	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	Roa	OH	[232, 233, 240]
342	β-Rhodomycin-Roa_2.deofuc.rod	H	H	H	OH	OH	Roa-Rho-dFuc	H	H	Et	Roa	OH	[232, 236]
343	β-Rhodomycin-Roa_2-Rod_3	H	H	H	OH	OH	Roa-Rod_3	H	H	Et	Roa	OH	[232, 233, 240]
344	β-Rhodomycin S2	H	H	H	OH	OH	Roa-dFuc-Rho_3	H	H	Et	Roa	OH	[79]
345	β-Rhodomycin V	H	H	H	OH	OH	Roa-dFuc-Rho_2	H	H	Et	Roa	OH	[240]
346	BA-32-A	H	H	H	OH	OH	Roa	H	H	Et	NDau	OH	[211]
347	R20X4	H	H	H	OH	OH	OH	H	H	Et	Dau	OH	[241]
348	ζ-Rhodomycinone	H	H	H	OH	OH	H	H	H	Et	COOMe	OH	[12, 13, 16, 17]
349	Maggiemycin	H	H	H	OH	OH	CO	H	H	Et	COOMe	OH	[242–244]
350	(7*S*,9*R*,10*R*)-ζ-Rhodomycinone	H	H	H	OH	OH	OH	H	H	Et	COOMe	OH	[16, 17, 242–244]
351	Anthracycline 14-A10	H	H	H	OH	OH	Dau	H	H	Me	COOMe	OH	[194]
352	D-788-15	H	H	H	OH	OH	Dau	H	H	Me	COOMe	OH	[194]
353	D-788-9	H	H	H	OH	OH	Dau	H	H	CH_2COMe	COOMe	OH	[194]
354	Rhodomycin D	H	H	H	OH	OH	Dau	H	H	Et	COOMe	OH	[194, 206, 207, 245]
355	Anthracycline 14-A12	H	H	H	OH	OH	Dau	H	H	Me	COOH	OH	[194]
356	Andhramycin B	H	H	H	OH	OH	$dFuc_2$-Rho	H	H	Et	COOMe	OH	(Rao VL, Laatsch H, unpublished results)
357	Andhramycin C	H	H	H	OH	OH	$dFuc_3$	H	H	Et	COOMe	OH	(Rao VL, Laatsch H, unpublished results)
358	Andhramycin D	H	H	H	OH	OH	dFuc-Rho_2	H	H	Et	COOMe	OH	(Rao VL, Laatsch H, unpublished results)
359	Andhramycin E	H	H	H	OH	OH	Rho-dFuc-Rho	H	H	Et	COOMe	OH	(Rao VL, Laatsch H, unpublished results)

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
360	Epelmycin D; violamycin A4	H	H	H	OH	OH	Roa	H	H	Et	COOMe	OH	[78, 80, 214, 215, 246]
361	Epelmycin C	H	H	H	OH	OH	Roa-dFuc	H	H	Et	COOMe	OH	[80, 246]
362	11-Hydroxy-aclacinomycin B	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	COOMe	OH	[247]
363	11-Hydroxyauramycin B	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Me	COOMe	OH	[27, 28]
364	11-Hydroxysulfurmycin B	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	CH_2COMe	COOMe	OH	[27, 28]
365	Epelmycin B	H	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	COOMe	OH	[80, 246]
366	Unnamed	H	H	H	OH	OH	Roa-$dFuc_2$	H	H	Et	COOMe	OH	[112]
367	11-Hydroxyaclacinomycin A; epelmycin E	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	COOMe	OH	[76, 77]
368	11-Hydroxyauramycin A	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	Me	COOMe	OH	[27, 28]
369	11-Hydroxysulfurmycin A	H	H	H	OH	OH	Roa-dFuc-CinA	H	H	CH_2COMe	COOMe	OH	[27, 28]
370	11-Hydroxy-aclacinomycin X	H	H	H	OH	OH	Roa-dFuc-Rdm	H	H	Et	COOMe	OH	[247]
371	Violamycin B4	H	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	COOMe	OH	[78, 214, 215]
372	Epelmycin A	H	H	H	OH	OH	Roa-Rho-CinA	H	H	Et	COOMe	OH	[246]
373	ε-Rhodomycinone-7-Rod_3; cytorhodin J	H	H	H	OH	OH	Rho_3	H	H	Et	COOMe	OH	[217, 218, 248]
374	3′-*O*,4-*O*-Didemethyl-mutactimycin	H	H	MeOH		OH	Rha	H	H	αMe	H	OH	[72, 75]
375	4-*O*-(β-D-Glucopyranosyl)-ε-rhodomycinone	H	H	H	Glc	OH	OH	H	H	Et	COOMe	OH	[66]
376	7-Deoxydaunomycinone	H	H	H	OMe	OH	H	H	H	COMe	H	OH	[249]
377	7-Deoxydihydrodaunorubicinone	H	H	H	OMe	OH	H	H	H	COHOHMe	H	OH	[16, 17]
378	7-Deoxy-feudomycinone C	H	H	H	OMe	OH	H	H	H	Me	H	OH	(Asolkar R, Laatsch H, unpublished results)
379	Adriamycinone	H	H	H	OMe	OH	OH	H	H	$COCH_2OH$	H	OH	[16, 17, 250]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
380	Daunomycinol; leukaemomycinone D	H	H	H	OMe	OH	OH	H	H	CHOHMe	H	OH	[89]
381	Daunomycinone	H	H	H	OMe	OH	OH	H	H	COMe	H	OH	[16, 17, 153, 154]
382	Dihydrofeudomycinone B	H	H	H	OMe	OH	OH	H	H	$CH_2CHOHMe$	H	OH	[58]
383	Feudomycinone A	H	H	H	OMe	OH	OH	H	H	Et	H	OH	[58]
384	Feudomycinone B	H	H	H	OMe	OH	OH	H	H	CH_2COMe	H	OH	[58]
385	Feudomycinone C	H	H	H	OMe	OH	OH	H	H	Me	H	OH	[58, 91]
386	Rubomycin B; leucaemomycin B1	H	H	H	OMe	OH	OH	H	H	COMe	H	OH	[251–253]
387	Adriamycin; doxorubicin	H	H	H	OMe	OH	Dau	H	H	$COCH_2OH$	H	OH	[61, 252]
388	Daunomycin	H	H	H	OMe	OH	Dau	H	H	COMe	H	OH	[61, 254, 255, 267]
389	Daunorubicinol	H	H	H	OMe	OH	Dau	H	H	CHOHMe	H	OH	[256, 257]
390	Detorubicin	H	H	H	OMe	OH	Dau	H	H	R^b	H	OH	[258]
391	Feudomycin A	H	H	H	OMe	OH	Dau	H	H	Et	H	OH	[58]
392	Feudomycin B	H	H	H	OMe	OH	Dau	H	H	CH_2COMe	H	OH	[58]
393	Feudomycin C	H	H	H	OMe	OH	Dau	H	H	Me	H	OH	[58, 91]
394	Leukaemomycin B2	H	H	H	OMe	OH	Dau_2	H	H	COMe	H	OH	[89]
395	Baumycin A1	H	H	H	OMe	OH	DauA1	H	H	COMe	H	OH	[64, 65]
396	Baumycin A2	H	H	H	OMe	OH	DauA1	H	H	COMe	H	OH	[64, 65]
397	Baumycin B1	H	H	H	OMe	OH	DauA2	H	H	COMe	H	OH	[64, 65]
398	Baumycin B2	H	H	H	OMe	OH	DauA2	H	H	COMe	H	OH	[64, 65]
399	Rubomycin H	H	H	H	OMe	OH	DauC1	H	H	COMe	H	OH	[52]
400	Rubomycin F	H	H	H	OMe	OH	DauC2	H	H	COMe	H	OH	[52]
401	TAN-1120 I	H	H	H	OMe	OH	C1Dau	H	H	COMe	H	OH	[259]
402	FCE 21424	H	H	H	OMe	OH	C4Dau	H	H	COMe	H	OH	[260]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
403	FCE 24366	H	H	H	OMe	OH	C3Dau	H	H	COMe	H	OH	[260]
404	FCE 24367	H	H	H	OMe	OH	C6Dau	H	H	COMe	H	OH	[260]
405	IT-62-B	H	H	H	OMe	OH	C5Dau	H	H	COMe	H	OH	[261–263]
406	Esorubicin	H	H	H	OMe	OH	dDau	H	H	$COCH_2OH$	H	OH	[153, 154]
407	Rubidomycin B; rubomycin B1	H	H	H	OMe	OH	Dau-Rho	H	H	COMe	H	OH	[264, 265]
408	Rubomycin M	H	H	H	OMe	OH	Dau-dFuc	H	H	COMe	H	OH	[266]
409	Rubomycin N	H	H	H	OMe	OH	Dau-dGul	H	H	COMe	H	OH	[266]
410	Daunomycinone K	H	H	H	OMe	OH	Dau-dFuc-CinA	H	H	COMe	H	OH	[91]
411	Daunomycinone L	H	H	H	OMe	OH	NDau-dFuc-CinA	H	H	COMe	H	OH	[91]
412	Baumycin C2	H	H	H	OMe	OH	NfDau	H	H	CHOHMe	H	OH	[64, 65]
413	*N*-Acetyldaunomycin; F-7-A	H	H	H	OMe	OH	NaDau	H	H	COMe	H	OH	[64, 65, 267]
414	Mutactimycin C	H	H	H	OMe	OH	3-mRha	H	H	αMe	H	OH	[148]
415	*N*,*N*-Dimethyladriamycin	H	H	H	OMe	OH	Roa	H	H	$COCH_2OH_2O$	H	OH	[91]
416	*N*,*N*-Dimethyldaunomycin	H	H	H	OMe	OH	Roa	H	H	COMe	H	OH	[91]
417	Daunomycinone S	H	H	H	OMe	OH	Roa-dFuc	H	H	COMe	H	OH	[91]
418	Daunomycinone A	H	H	H	OMe	OH	Roa-dFuc-CinA	H	H	COMe	H	OH	[91]
419	Baumycin C1	H	H	H	OMe	OH	NfDau	H	H	COMe	H	OH	[64, 65]
420	Mutactimycin PR	H	H	H	Rha	OH	3-mRha	H	H	αMe	H	OH	[38, 39]
421	Feudomycinone D	H	H	H	OMe	OH	OH	H	H	Me	OH	OH	[58]
422	Feudomycin D	H	H	H	OMe	OH	Dau	H	H	Me	OH	OH	[91]
423	Unnamed	H	H	H	OMe	OH	H	H	H	CHOHMe	OH	OH	[268]
424	4-*O*-Methylepelmycin D	H	H	H	OMe	OH	Roa	H	H	Et	COOMe	OH	[269]
425	D-788-5	H	H	H	OMe	OH	Dau	H	H	Et	COOMe	OH	[270]
426	Histomodulin	H	H	H	GlcA	OH	OH	H	H	Et	COOMe	OH	[67]
427	XF-1	H	H	Me	OMe	OH	S3	H	H	Me	H	OH	[271]

Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
428	3′-*O*-Demethylmutactimycin	H	H	Me	OMe	OH	Rha	H	H	αMe	H	OH	[72, 75]
429	SO-075-R1	H	H	Me	OMe	OH	3,4-dRha	H	H	αMe	H	OH	[73, 74]
430	Mutactimycin A	H	H	Me	OMe	OH	3-mRha	H	H	αMe	H	OH	[70–72, 148]
431	Mutactimycin D	H	H	CH_2OH	OMe	OH	3-mRha	H	H	αMe	H	OH	[148]
	1,4,6,11-Tetrahydroxyanthracyclines												
432	1-Hydroxy-13-dihydro-daunomycin	OH	H	H	OMe	OH	Dau	H	H	CHOHMe	H	OH	[272]
433	*N*-Formyl-1-hydroxy-13-dihydrodaunomycin	OH	H	H	OMe	OH	NfDau	H	H	CHOHMe	H	OH	[272]
434	α-Isorhodomycinone	OH	H	H	OH	OH	H	OH	H	Et	OH	OH	[12, 13]
435	γ-Isorhodomycinone	OH	H	H	OH	OH	H	H	H	Et	OH	OH	[16, 17, 273]
436	β-Isorhodomycinone	OH	H	H	OH	OH	OH	H	H	Et	OH	OH	[16, 17, 232, 236]
437	Obelmycin A	OH	H	H	OH	OH	Roa	H	H	Et	OH	OH	[81]
438	Violamycin A1; isorhodomycin B	OH	H	H	OH	OH	Roa	H	H	Et	OH	OH	[78, 214, 215]
439	β-Isorhodo-mycinone RDC	OH	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	OH	OH	[90]
440	Violamycin B1	OH	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	OH	OH	[78, 214, 215]
441	Obelmycin G	OH	H	H	OH	OH	H	H	H	Et	Roa	OH	[81]
442	Obelmycin H	OH	H	H	OH	OH	OH	H	H	Et	Roa	OH	[237]
443	Isorhodomycin A	OH	H	H	OH	OH	Roa	H	H	Et	Roa	OH	[33]
444	Cytorhodin D	OH	H	H	OH	OH	Roa-dFuc=CinA	H	H	Et	Roa-dFuc=CinA	OH	[221]
445	SS-288B	OH	H	H	OH	OH	Roa-$dFuc_2$	H	H	Et	Roa-$dFuc_2$	OH	[231]

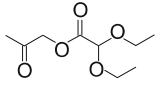
Table 5 (continued)

Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8	R^9	R^{10}	R^{11}	Refs.
446	2*F*,3*F*-Didehydro-4*C*,4*F*-dideoxy-4*C*,4*F*-dioxo-cytorhodin A	OH	H	H	OH	OH	Roa-dFuc-CinA/	H	H	Et	Roa-dFuc-Acu	OH	[221]
447	Obelmycin F	OH	H	H	OH	OH	OH	H	H	Et	Roa-dFuc-Rho	OH	[81]
448	Obelmycin D	OH	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	Roa-dFuc-Rho	OH	[81]
449	1,3E-Dihydroxy-cytorhodrin A	OH	H	H	OH	OH	Roa-Rho_2	H	H	Et	Roa-dFuc-Rho	OH	[221]
450	Obelmycin E	OH	H	H	OH	OH	OH	H	H	Et	Roa-Rho_2	OH	[81]
451	4*C*-Deoxy-1,3B-dihydroxy-4*C*-oxo-cytorhodin A	OH	H	H	OH	OH	Roa-dFuc-CinA	H	H	Et	Roa-Rho_2	OH	[221]
452	Obelmycin C	OH	H	H	OH	OH	Roa-dFuc-Rho	H	H	Et	Roa-Rho_2	OH	[81]
453	β-Isorhodomycin S-1A	OH	H	H	OH	OH	Roa-Rho	H	H	Et	Roa-Rho_2	OH	[79]
454	Obelmycin B	OH	H	H	OH	OH	Roa-Rho_2	H	H	Et	Roa-Rho_2	OH	[81]
455	Caeseorhodomycin	Roa	H	H	OH	OH	OH	H	H	Et	OH	OH	[274, 275]
456	ζ-Isorhodomycinone	OH	H	H	OH	OH	H	H	H	Et	COOMe	OH	[12, 13, 16, 17]
457	Cinerubin B; ryemycin B1	OH	H	H	OH	OH	H	Roa-dFuc=CinA	H	Et	COOMe	OH	[169]
458	ζ-Isorhodomycinone	OH	H	H	OH	OH	OH	H	H	Et	COOMe	OH	[12, 13, 16, 17]
459	D-788-1; carbolbicin	OH	H	H	OH	OH	Dau	H	H	Et	COOH	OH	[104, 194]
460	Cinerubin X	OH	H	H	OH	OH	Rho-dFuc-CinA	H	H	Et	COOMe	OH	[50, 167]
461	Violamycin A3	OH	H	H	OH	OH	Roa	H	H	Et	COOMe	OH	[78, 214, 215]
462	Violamycin A5	OH	H	H	OH	OH	Roa	H	H	Et	COOMe	OH	[78, 214, 215]

Table 5 (continued)

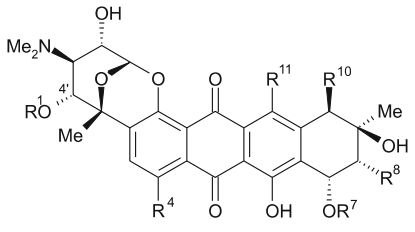
Fo no	Name	R^1	R^2	R^3	R^4	R^6	$R^7\alpha$	$R^7\beta$	R^8R^9	R^{10}	R^{11}	Refs.
463	11-Hydroxycinerubin A; ε-isorhodomycin RD	OH	H	H	OH	OH	Roa-dFuc-CinA	H	H Et	COOMe	OH	[76, 77]
464	11-Hydroxysulfurmycin A	OH	H	H	OH	OH	Roa-dFuc-CinA	H	H CH_2COMe	COOMe	OH	[27, 28]
465	11-Hydroxysulfurmycin B	OH	H	H	OH	OH	Roa-dFuc=CinA	H	H CH_2COMe	COOMe	OH	[27, 28]
466	Violamycin B3	OH	H	H	OH	OH	Roa-dFuc-Rho	H	H Et	COOMe	OH	[78, 214, 215]
467	Violamycin B5	OH	H	H	OH	OH	Roa-dFuc-Rho	H	H Et	COOMe	OH	[78, 214, 215]

[a] R = –CHMe–CH_2OH

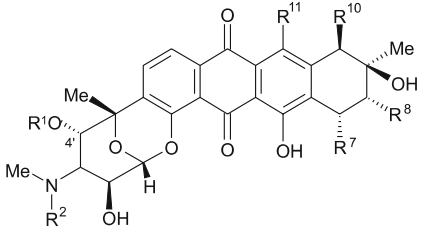
[b] R = 

Cin A = L-CinA, if not stated otherwise

Table 6 Anthracyclines of the nogalarol and nogarol type; the numbering system of anthracyclines is used



A



B

Fo no.	Name	Type	R^1	R^2	R^4	$R^7\alpha$	R^8	R^{10}	R^{11}	Refs.
468	301-C	A	S1-Dec-SDig	–	H	Roa	H	H	OH	[276]
469	301-A1	A	H	–	H	Roa	H	H	OH	[276]
470	Nogalarol	A	H	–	OH	OH	H	COOMe	H	[282]
471	Nogalamycin	A	H	–	OH	Nog	H	COOMe	H	[277, 278]
472	Nogamycin	A	H	–	OH	Nog	H	H	H	[91]
473	7-*con*-*C*-Methylnogarol	A	H	–	OH	OMe	H	H	H	[91]
474	7-*C*-Methylnogalarol	A	H	–	OH	OMe	H	COOMe	H	[277, 278, 280]
475	7-*C*-Methylviriplanol	A[a]	H	–	OH	OMe	H	COOMe	H	[277, 278, 280]
476	AG-2	A	dFuc-Dec-Dig	–	OH	Dig	H	COOMe	H	[281]
477	Arugomycin	A	dFuc-Dec-Dig	–	OH	Dig-dFuc-Dig-FDig	H	COOMe	H	[82]
478	Cororubicin	A	dFuc-Dec-dFuc	–	OH	H	H	COOMe	H	[87, 88]
479	301-B	A	Dec2-SDig	–	H	Roa	H	H	OH	[276]
480	Decilorubicin	A	Dec2-SDig	–	OH	Roa	H	H	OH	[85, 86]
481	Arugorol; 4′-*epi*-nogalarol	A[a]	H	–	OH	OH	H	COOMe	H	[282]
482	Viriplanol	A[a]	H	–	OH	OH	H	COOMe	H	[83, 84]

Table 6 (continued)

Fo no.	Name	Type	R^1	R^2	R^4	$R^7\alpha$	R^8	R^{10}	R^{11}	Refs.
483	Viriplanin A	A [a]	dFuc-NS-dFuc	–	OH	$dFuc_2$-NS-MDig	H	COOMe	H	[83, 84]
484	Viriplanin D	A [a]	dFuc-Dec-dFuc	–	OH	$dFuc_2$-Dec-MDig	H	COOMe	H	[83, 84]
485	Avidinorubicin	A [a]	Avi2-SDig	–	H	Roa	H	H	OH	[283]
486	Respinomycin B	B [a]	H	H	–	Dig	OMe	H	H	[87, 88]
487	Respinomycin C	B [a]	H	Me	–	Vin	OMe	H	H	[87, 88]
488	Respinomycin A1	B [a]	Avi-Vin	Me	–	Vin	OMe	H	H	[87, 88]
489	Respinomycin A2	B [a]	Avi-Vin	Me	–	H	OMe	H	H	[87, 88]
490	Respinomycin D	B [a]	Dec-Vin	Me	–	Vin	OMe	H	H	[87, 88]

The C-glycosidic sugar is nogalamine (Ngm), *epi*-nogalamine (eNgm), or *N*-demethyl-nogalamine (dNgm)

[a] 4′-*epi*-Nogalamine instead of nogalamine

References

1. Brockmann H (1963) Anthracyclinone und Anthracycline. Fortschr Chem Org Naturst 21:121–182
2. Furlan RLA, Watt SJ, Garrido LM, Amarante MGP, Nur-e-Alam M, Rohr J, Brana A, Mendez C, Salas JA, Sheil MM, Beck JL, Padilla G (2004) J Antibiot 57:647
3. Behal V (2000) Adv App Microbiol 47:113
4. Sasaki T, Tsukada Y, Deutsch HF, Hirai H (1984) Cancer Chemother Pharmacol 13:75
5. Yang JY, Luo HY, Lin QY, Liu ZM, Yan LN, Lin P, Zhang J, Lei S (2002) World J Gastroenterol 8:44
6. Gewirtz DA (1999) Biochem Pharmacol 57:727
7. Di Marco A, Gaetani M, Orezzi P, Scarpinato BM, Silvestrini R, Soldati M, Dasdia T, Valentini L (1964) Nature 201:706
8. Taatjes DJ, Gaudiano G, Resing K, Koch TH (1996) J Med Chem 39:4135
9. Smith TH, Fujiwara AN, Lee WW, Wu HY, Henry DW (1977) J Org Chem 42:3653
10. Hautala A, Torkkell S, Räty K, Kunnari T, Kantola J, Mäntsälä P, Hakala J, Ylihonko K (2003) J Antibiot 56:143
11. Brockmann H Jr, Legrand M (1963) Tetrahedron 19:395
12. Brockmann H, Brockmann H Jr, Niemeyer J (1968) Tetrahedron Lett 45:4719
13. Brockmann H, Boldt P (1960) Naturwissenschaften 47:134
14. Yoshimoto A, Matsuzawa Y, Oki T, Takeuchi T, Umezawa H (1981) J Antibiot 34:951
15. Strauss D (1983) Type-specific separation of anthraquinone derivatives. DD 200 671 A1 (Chem Abstr Chem Abstr 100:6205)
16. Thomson RH (1971) Naturally occurring quinones, vol II, 2nd edn. Academic, New York
17. Thomson RH (1987) Naturally occurring quinones, vol III, 3nd edn. Academic, New York
18. Schripsema J, Dagnino D (1996) Phytochemistry 42:177
19. Ballabio M, Vigevani A (1990) Chim Oggi 8:33
20. Vigevani A, Ballabio M, Gandini E, Penco S (1985) Magn Resonance Chem 23:344
21. Brockmann H Jr, Budzikiewicz H, Djerassi C, Brockmann H, Niemeyer J (1965) Chem Ber 98:1260
22. Reed RI, Reid WK (1963) Tetrahedron 19:1817
23. Hirayama K, Akashi S, Toshihiko A, Issei H, Yuzuru E, Hajumu M, Hiroshiro S, Asao M (1987) Biomed Environm Mass Spectrom 4:305
24. Brazhnikova MG, Zbarskii VB, Ponomarenko VL, Potapova NP (1974) J Antibiot 27:254
25. Kende AS, Tsay YG, Mills JE (1976) J Am Chem Soc 98:1967
26. Pettit GR, Einck JJ, Herald CL, Ode RH, von Dreele RB, Brown P, Brazhnikova MG, Gauze GF (1975) J Am Chem Soc 97:7387
27. Fujiwara A, Tazoe M, Hoshino T, Sekine Y, Masuda S, Nomura S (1981) J Antibiot 34:912
28. Hoshino T, Setoguchi Y, Fujiwara A (1984) J Antibiot 37:1469
29. Maskey RP (2001) Neuartige Wirkstoffe aus marinen Streptomyceten: Sagunamycine, Parimycin, Himalomycin, Gottingamycin, Dhanyabadomycin, Akashine und stark cytotoxische Trioxacarcine mit hoher Anti-Malaria Aktivität. PhD thesis, University of Göttingen
30. Fotso-Fondja Yao CB (2008) Aqabamycins, rare nitro maleimides and other novel metabolites from microorganisms; generation and application of an HPLC-UV-ESI MS/MS database. PhD thesis, University of Göttingen

31. Webb JS, Broschard RW, Cosulich DB, Mowat JH, Lancaster JE (1962) J Am Chem Soc 84:3183
32. El-Naggar MY (2007) J Microbiol 45:262 (Korea)
33. Celmer WD, Murai K, Rao KV, Tanner FW, Marsh WS (1957–1958) The quinocycline complex, I isolation and characterisation. In: Antibiotics annual. Medical Encyclopedia, New York, pp 484–492
34. Yasuhiro I, Hiroko H, Toshikazu O, Furumai T (2002) J Antibiot 55:134
35. Furumai T, Igarashi Y, Higuchi H, Saito N, Oki T (2002) J Antibiot 55:128
36. Igarashi Y, Futamata K, Fujita T, Sekine A, Senda H, Naoki H, Furumai T (2003) J Antibiot 56:107
37. Igarashi Y, Iwashita T, Fujita T, Naoki H, Yamakawa T, Yoshida R, Furumai T (2003) J Antibiot 56:705
38. Zitouni A, Boudjella H, Mathieu F, Sabaou N, Lebrihi A (2004) J Antibiot 57:367
39. Zitouni A, Mathieu F, Coppel Y, Pont F, Sabaou N, Lebrihi A (2004) J Antibiot 57:373
40. Xiao C, Si S, Zhang Y, Otani T, Yoshida K (2001) Zhongguo Kangshengsu Zazhi 26:85
41. Pullen C, Schmitz P, Meurer K, von Bamberg D, Lohmann S, de Castro Franca S, Groth I, Schlegel B, Mollmann U, Gollmick F, Gräfe U, Leistner E (2002) Planta Med 216:162
42. Torikoshi K, Hirano A, Sugiura Y, Nakajima S, Ojiri K, Suda H (1999) BE-45985 derivatives, their manufacture, Streptomyces species producing them, and antitumor agents. Jpn Kokai Tokkyo Koho. JP Patent 11 021 263 (Chem Abstr 130:191872)
43. Strelitz F, Flon H, Weiss U, Asheshov N (1956) J Bact 72:9094
44. Johdo O, Tone H, Okamoto R, Yoshimoto A, Naganawa H, Sawa T, Takeuchi T (1991) J Antibiot 44:1155
45. Broadhurst MJ, Hassall CH, Thomas GJ (1982) J Chem Soc Perkin Trans 1, p 2249
46. Monneret C, Florent JC, Bennani F, Sedlacek HH, Kraemer HP (1988) Actual Chim Therapeut 15:253
47. Brockmann H, Lenk W (1959) Chem Ber 92:1904
48. Oki T, Kitamura I, Matsuzawa Y, Shibamoto N, Ogasawara T, Yoshimoto A, Inui T, Naganawa H, Takeuchi T, Umezawa H (1979) J Antibiot 32:801
49. Bieber LW, Da Silva Filho AA, De Mello JF, De Lima OG, Do Nascimento MS, Veith HJ, Von der Saal W (1987) J Antibiot 40:1335
50. Nakagawa M, Furihata K, Furihata K, Adachi K, Seto H, Otake N (1986) J Antibiot 39:1178
51. Shaaban M (2004) Bioactive secondary metabolites from marine and terrestrial bacteria: isoquinolinequinones, bacterial compounds with a novel pharmacophor. PhD thesis, University of Göttingen
52. Fomicheva EV, Fedorova GB, Potapova NP, Katrukha GS (1992) J Antibiot 45:1185
53. Phipps RK, Blunt JW, Cole ALJ, Munro MHG (2004) ARKIVOC, p 94
54. Tanaka Y, Gräfe U, Yazawa K, Mikami Y, Ritzau M (1997) J Antibiot 50:822
55. Brodasky TF, Mizsak S, Hoffstetter JR (1985) J Antibiot 38:849
56. Oki T, Yoshimoto A, Matsuzawa Y, Takeuchi T, Umezawa H (1981) J Antibiot 34:916
57. Matsuzawa Y, Yoshimoto A, Shibamoto N, Tobe H, Oki T, Naganawa H, Takeuchi T, Umezawa H (1981) J Antibiot 34:959
58. Oki T, Matsuzawa Y, Kiyoshima K, Yoshimoto A, Naganawa H, Takeuchi T, Umezawa H (1981) J Antibiot 34:783
59. Itoh T, Kinoshita M, Wei H, Kobayashi M (2003) Chem Pharm Bull 51:1402
60. Arcamone F, Cassinelli G, Orezzi P, Franceschi G, Mondelli R (1964) J Am Chem Soc 86:5335

61. Arcamone F, Cassinelli G, Fantini G, Grein A, Orezzi P, Pol C, Spalla C (1969) Biotechnol Bioeng 11:1101
62. Bonadonna G, Monfardini S (1969) Lancet 1(7599):837
63. Takahashi Y, Naganawa H, Takeuchi T, Umezawa H, Komiyama T, Oki T, Inui T (1977) J Antibiot 30:622
64. Komiyama T, Matsuzawa Y, Oki T, Inui T, Takahashi Y, Naganawa H, Takeuchi T, Umezawa H (1977) J Antibiot 30:619
65. Oki T, Yoshimoto A, Kouno K, Inui T, Takeuchi T, Umezawa H (1981) Anthracycline glycosides. EP Patent 26 849 (Chem Abstr 95:40852)
66. Nakagawa M, Kawai H, Hayakawa Y, Seto H, Otake N (1985) J Antibiot 38:1622
67. Komatsu Y, Takahashi O, Hayashi H (1998) J Antibiot 51:85
68. Yoshimoto A, Jodo O, Watanabe Y, Okamoto R, Takeuchi T (1990) Preparation of antibiotic D788-7 from carborubicin as antitumor agent. EP Patent 355 843 (Chem Abstr 113:59778)
69. Umezawa H, Odagawa A, Kataoka S, Nakajima S (1988) Preparation of anthracycline compound. EP Patent 251 327 (Chem Abstr 110:24243)
70. Tao P, Wang S, Zhang G, Cai S, Li H, Jin W (1990) Zhongguo Kangshengsu Zazhi 15:170
71. Jin W, Chen J, Zhang Y, Li H, Tao P (1990) Zhongguo Kangshengsu Zazhi 15:399
72. Speitling M (1998) Vergleich der metabolischen Kapazität mariner und terristrischer Mikroorganismen – Isolierung und Strukturaufklärung von Branimycin, Bromalterochromid A/B und weiteren Stoffwechselprodukten. PhD thesis, University of Göttingen
73. Maeda A, Yazawa K, Mikami Y, Ishibashi M, Kobayashi J (1992) J Antibiot 45:1848
74. Mikami Y, Yazawa K, Ohashi S, Maeda A, Akao M, Ishibashi M, Kobayashi J, Yamazaki C (1992) J Antibiot 45:995
75. Speitling M, Nattewan P, Yazawa K, Mikami Y, Grün-Wollny I, Ritzau M, Laatsch H, Gräfe U (1998) J Antibiot 51:693
76. Oki T, Yoshimoto A, Matsuzawa Y, Takeuchi T, Umezawa H (1980) J Antibiot 33: 1331
77. Matsuzawa Y, Yoshimoto A, Oki T, Naganawa H, Takeuchi T, Umezawa H (1980) J Antibiot 33:1341
78. Seeber C, Fleck W (1974) Z Allg Mikrobiol 14:503
79. Brockmann H, Greve H (1975) Tetrahedron Lett 16:831
80. Yoshimoto A, Jodo O, Watanabe Y, Ishikura T, Osanawa H, Sawa T, Takeuchi T, Umezawa H (1988) Novel anthracyline antibiotics Y267-1, Y262-3, and Y262-3 manufacture with Streptomyces violacens. Jpn Kokai Tokkyo Koho JP Patent 63 066 194 (Chem Abstr 110:113191)
81. Johdo O, Watanabe Y, Ishikura T, Yoshimoto A (1991) J Antibiot 41:1130
82. Kawai H, Hayakawa Y, Nakagawa M, Furihata K, Seto H, Otake N (1987) J Antibiot 40:1273
83. Huetter K, Baader E, Frobel K, Zeeck A, Bauer K, Gau W, Kurz J, Schroeder T, Wuensche C, Karl W, Wendisch D (1986) J Antibiot 39:1193
84. Kind R, Huetter K, Zeeck A, Schmidt-Baese K, Egert E (1989) J Antibiot 42:7
85. Nishimura Y, Ishii K, Kondo S (1990) J Antibiot 43:54
86. Ishii K, Kondo S, Nishimura Y, Hamada M, Takeuchi T, Umezawa H (1983) J Antibiot 36:451
87. Ubukata M, Osada H, Kudo T, Isono K (1993) J Antibiot 46:936
88. Ubukata M, Uzawa J, Osada H, Isono K (1993) J Antibiot 46:942
89. Blumauerova M, Kralovcova E, Mateju J, Jizba J, Vanek Z (1979) Folia Microbiol 24:117 (Prague, Czech Republic)

90. Yoshimoto A, Johdo O, Takatsuki Y, Ishikura T, Sawa T, Takeuchi T, Umezawa H (1984) J Antibiot 37:935
91. Matsuzawa Y, Oki T, Takeuchi T, Umezawa H (1981) J Antibiot 34:1596
92. Laatsch H (2008) AntiBase, a data base for rapid structural determination of microbial natural products, and annual updates. Wiley-VCH, Weinheim. See also http://www.gwdg.de/~ucoc/laatsch/, last visited 24 March 2008
93. Fujiwara A, Hoshino T (1983) Optically active anthracycline glycosides, 4-deoxyaclacinomycins A and B and pharmaceutical preparations. EP Patent 83 019 (Chem Abstr 99:120690)
94. Oki T, Shibamoto N, Matsuzawa Y, Ogasawara T, Yoshimoto A, Kitamura I, Inui T, Naganawa H, Takeuchi T, Umezawa H (1977) J Antibiot 30:683
95. Umezawa H, Takeuchi T, Hamada M, Naganawa H, Sawa T, Uchida T, Imoto M (1986) Anthracycline derivatives and their use as medicaments. EP Patent 167 935 (Chem Abstr 104:184852)
96. Mitscher LA, McCrae W, Andres WW, Lowery JA, Bohonos N (1964) J Pharm Sci 53:1139
97. Tamotsu K, Yasuhiro I, Shunichi O (2002) Isoquinocycline antibiotics manufacture with Micromonospora. Jpn Kokai Tokkyo Koho JP Patent 2 002 212 187 (Chem Abstr 137:139486)
98. Tulinsky A (1964) J Am Chem Soc 86:5368
99. Cosulich DB, Mowat JH, Broschard RW, Patrick JB, Meyer WE (1963) Tetrahedron Lett 63:453
100. Igarashi Y, Higuchi H, Oki T, Furumai T (2002) J Antibiot 55:134
101. Igarashi Y, Higuchi H, Oki T, Furumai T (2003) J Antibiot 56:C1
102. Shaaban KA (2008) Metabolites from marine and terrestrial bacteria. PhD thesis, University of Göttingen
103. Tanaka H, Yoshioka T, Shimauchi Y, Matsuzawa Y, Oki T, Inui T (1980) J Antibiot 33:1323
104. Fujii S, Kubo K, Johdo O, Yoshimoto A, Ishikura T, Naganawa H, Sawa T, Takeuchi T, Umezawa H (1986) J Antibiot 39:473
105. Otake N, Munetsuka Y, Munetsuka J, Toda Y (1986) Antitumor agent 11-deoxyrubeomycin A manufacture and use. Jpn Kokai Tokkyo Koho JP Patent 61 152 692 (Chem Abstr 106:17032)
106. Wang Y, Niemi J, Mäntsälä P (2002) FEMS Microbiol Lett 208:117
107. Uchida T, Imoto M, Masuda T, Yoshimoto H, Imamura K, Sawa T, Naganawa H, Takeuchi T, Umezawa H (1985) J Antibiot 38:795
108. Kunnari TJ, Ylihonko KPJ, Klika KD, Mäntsälä PI, Hakala JML (2000) J Org Chem 65:2851
109. Ylihonko K, Torkkell S, Palmu K, Hakala J (2000) Gene cluster involved in nogalamycin biosynthesis and its use in production of hybrid antibiotics. WO Patent 2 000 024 775 (Chem Abstr 132:333446)
110. Rho Y, Kang HS, Sin HS (1993) Yakhak Hoechi 37:555
111. Johdo O, Nishida H, Okamoto R, Yoshimoto A, Takeuchi T (1995) J Antibiot 48:1153
112. Ylihonko K, Hakala J, Kunnari T (1999) Hybrid anthracyclines for cancer therapy from genetically engineered Streptomyces galilaeus. WO Patent 9 958 544 (Chem Abstr 131:332095)
113. Yoshimoto A, Johdo O (1996) Anthracycline antibiotics manufacture with Streptomyces. Jpn Kokai Tokkyo Koho JP Patent 08 113 587 (Chem Abstr 125:112917)
114. Niemi J, Ylihonko K, Hakala J, Paerssinen R, Kopio A, Mäntsälä P (1994) Microbiology 140:1351

115. Morioka M, Sasaki T, Suzuki K, Nagai K, Yamaguchi H, Nohara C, Tokunaga T, Hiramoto M, Takebayashi Y (1990) Antitumor anthracyclines from Nocardia. EP Patent 387 830 (Chem Abstr 114:227481)
116. Yoshimoto A, Johdo O, Fujii S, Kubo K, Nishida H, Okamoto R, Takeuchi T (1992) J Antibiot 45:1255
117. Eckardt K, Schumann G, Tresselt D, Ihn W (1988) J Antibiot 41:788
118. Tresselt D, Sedmera P, Eckardt K (1990) Pharmazie 45:775
119. Yoshimoto A, Toshikazu O, Oki O, Takeuchi T, Umzawa H (1980) J Antibiot 33:1158
120. Hoshino T, Tazoe M, Nomura S, Fujiwara A (1982) J Antibiot 35:1271
121. Arora SK (1985) J Antibiot 38:1788
122. Tresselt D, Eckardt K, Tax J (1975) Tetrahedron 31:613
123. Torkkell S, Kunnari T, Palmu K, Hakala J, Mäntsälä P, Ylihonko K (2000) Antimicrob Agents Chemother 44:396
124. Chen Y, Du R, Hao Y, Li M (1996) Zhongguo Kangshengsu Zazhi 21:168–179
125. Oki T, Matsuzawa Y, Yoshimoto A, Numata K, Kitamura I, Hori S, Takamatsu A, Umezawa H, Ishizuka M, Naganawa H, Suda H, Hamada M, Takeuchi T (1975) J Antibiot 28:830
126. Oki T, Matsuzawa Y, Yoshimoto A, Numata K, Kitamura I, Hori S, Takamatsu A, Umezawa H, Ishizuka M, Naganawa H, Suda H, Hamada M, Takeuchi T (1979) J Antibiot 32:791
127. Cassinelli G, Giovanni R, Ruggieri D, Arcamone F, Grein A, Merli S, Spalla C, Casazza AM, di Marco A, Pratesi G (1982) J Antibiot 35:164
128. Yoshimoto A, Ogasawara T, Kitamura I, Oki T, Inui T, Takeuchi T, Umezawa H (1979) J Antibiot 32:472
129. Ihn W, Wetzstein H, Graefe U, Eckardt K, Dornberger K, Fleck W, Tresselt D (1988) Manufacture of aclacinomycin Y with a novel Streptomyces strain. DD Patent 259 207 (Chem Abstr 112:53723)
130. Hoshino T, Sekine Y, Fujiwara Y (1983) J Antibiot 36:1458
131. Fujiwara A, Hoshino T, Tazoe M (1982) Tetracyclic compounds and pharmaceutical preparations containing them. Fr Demande. FR Patent 2 492 386 (Chem Abstr 98:3537)
132. Katsuyoshi S, Hirofumi F, Shunro M, Toshikazu O (1981) J Antibiot 34:770
133. Johdo O, Yoshioka T, Naganawa H, Takeuchi T, Yoshimoto A (1996) J Antibiot 49:669
134. Kim HS, Kim YH, Yoo OJ, Lee JJ (1996) Biosc Biotechnol Biochem 60:906
135. Yang S-W, Chan T-M, Terracciano J, Patel R, Loebenberg D, Chen G, Patel M, Gullo V, Pramanik B, Chu MA (2004) J Antibiot 57:601
136. Jizba JV, Sedmera P, Vokoun J, Blumauerova M, Vanek Z (1981) Coll Czech Chem Comm 46:2129
137. Uno H, Naruta Y, Maruyama K (1984) Tetrahedron 40:4725
138. Asahi Chemical Industry Co Ltd Japan (1985) Aklavinone derivatives. Jpn Kokai Tokkyo Koho JP Patent 60 058 944 (Chem Abstr 103:71082)
139. Maskey RP, Grün-Wollny I, Laatsch H (2003) J Antibiot 56:795
140. Kende AS, Johnson S (1985) J Org Chem 50:727
141. Fujiwara M, Fujiwara A, Hoshino T, Tazoe M, Sekine Y (1978) Antibiotic SM-173B. DE Patent 2 804 495 (Chem Abstr 89:195401)
142. Grein A, Merli S, Celestino S (1980) J Antibiot 33:1462
143. Cassinelli G, Di Matteo F, Forenza S, Ripamonti MC, Arcamone GRF, Di Marco A, Casazza AM, Soranzo CL Pratesi G (1980) J Antibiot 33:1468
144. Arcamone F, Cassinelli J, DiMatteo F, Forenza S, Ripamonti MC, Rivola G, Vigevani A, Clardy J, McGabe T (1980) J Am Chem Soc 102:1462

145. Kelly RT, Ananthasubramanian L, Borah K, Gillard JW, Goerner RN Jr, King PF, Lyding JM, Tsang W-G, Vaya J (1984) Tetrahedron 40:4569
146. Kraus GA, Taschner MJ (1977) Tetrahedron Lett 52:4575
147. Braun M (1984) Tetrahedron 40:4585
148. Zhang Y, Jin W (1991) Isolation and structure determination of minor components of mutactimycins, a group of new anthracycline antibiotics. Zhongguo Kangshengsu Zazhi 16:157 [Chem Abstr 116:55211]
149. Yoshimoto A, Jodo O, Watanabe Y, Ishikura T, Osanawa H, Sawa T, Takeuchi T, Umezawa H (1988) Novel anthracycline antibiotic D788-16 manufacture with Streptomyces. Jpn Kokai Tokkyo Koho JP Patent 63 066 193 (Chem Abstr 110:22306)
150. Nash CH, Shearer MC, Snader KM, Valenta JR, Cooper D (1981) Anthracycline antibiotics by *Streptosporangium fragilis* Shearer sp. nov. ATCC 31519. US Patent 4 293 546 (Chem Abstr 96:50693)
151. Tsuji T, Takesawa M, Kida T, Morioka H, Eto Y, Shibai H, Horino K (1986) Manufacture of the anthracyclines TMF518C and TMF518D. Jpn Kokai Tokkyo Koho JP Patent 61 005 090 (Chem Abstr 111:113762)
152. Brockmann H, Niemeyer J (1968) Chem Ber 101:1341
153. Terajima A, Kimura Y, Suzuki M, Abe R, Matsumoto M (1986) 4-Demethoxyadriamycin. Jpn Kokai Tokkyo Koho JP Patent 61 078 797 (Chem Abstr 105:227232)
154. Arcamone F, Bernardi L, Patelli B, Giardino P, di Marco A, Casazza AM, Soranzo C, Pratesi G (1978) Experientia 34:1255
155. Bakina EV, Yartseva IV, Povarov LS, Aleksandrova LG, Tulemisova KA, Mamonova LP, Preobrazhenskaya MN (1989) Bioorg Khim 15:1423
156. Bakina EV, Yartseva IV, Povarov LS, Aleksandrova LG, Tulemisova KA, Mamonova LP, Preobrazhenskaya MN (1981) Antibiotics DC-44-A and DC-44-B. Jpn Kokai Tokkyo Koho JP Patent 56 051 435 (Chem Abstr 95:148696)
157. Brockmann H, Lenk W (1960) Naturwissenschaften 47:135
158. Balk W (1995) Neue Naturstoffe aus dem Screening mariner Actinomyceten – Isolierung und Strukturaufklärung der Lagumycine und des Oceamycins. PhD thesis, University of Göttingen
159. Nettleton DE Jr, Bradner WT, Bush JA, Coon AB, Moseley JE, Myllymaki RW, O'Herron FA, Schreiber RH, Vulcano AL (1977) J Antibiot 30:525
160. Akihiro Y, Toshikazu O, Taiji I (1978) J Antibiot 31:92
161. Bradner WT, Misiek M (1977) J Antibiot 30:519
162. Nettleton DE Jr, Balitz DM, Doyle TW, Bradner WT, Johnson DL, O'Herron FA, Schreiber RH, Coon AB, Moseley JE, Myllymaki RW (1980) J Nat Prod 43:242
163. Tresselt D, Ihn W, Schlegel B, Fleck W (1987) Z Chemie 27:444
164. Huth F (1999) Einsatz der Neutronenaktivierungsanalyse und der Elektrospray-Massenspektrometrie im Screening nach Organohalogenverbindungen – Strukturaufklärung cyclischer Peptide durch MS-Methoden. PhD thesis, University of Göttingen
165. Nettleton DE Jr, Doyle TW, Grossman JH, Bradner WT, Moseley JE, Bray SW (1984) J Nat Prod 47:698
166. Doyle TW, Nettleton DE, Grulich RE, Balitz DM, Johnson DL, Vilcano AL (1979) J Am Chem Soc 101:7041
167. Nakagawa M, Okazaki T, Furihata K, Seto H, Otake N (1987) Agric Biol Chem 51:3431
168. Frolova VI, Kuzovkov AD, Rozynov BV, Taig MM, Ivanitskaya LP, Terent'eva TG (1982) Antibiotiki 27:326 (Moscow)
169. Ettlinger L, Gräumann E, Hütter R, Keller-Schierlein W, Kradolfer F, Neipp L, Prelog V, Reusser P, Zähner H (1959) Chem Ber 92:1867

170. Nair MG, Mishra SK, Putnam AR, Pandey RC (1992) J Antibiot 45:1738
171. Iwao KI, Norio S, Toshikazu O, Taiji I, Hiroshi N, Masaaki I, Toru M, Tomio T, Umezawa H (1977) J Antibiot 30:616
172. Nakata M, Saito M, Inouye Y, Nakamura S, Hayakawa Y, Seto H (1992) J Antibiot 45:1599
173. Kendee AS, Tsay YG (1977) J Chem Soc Chem Commun, p 140
174. Suzukake TK, Moriya Y, Yamazaki K, Hori M, Hosokawa N, Sawa T, Iinuma H, Naganawa H, Imada C, Hamada M (1990) J Antibiot 43:1489
175. Kunnari T, Tuikkanen J, Hautala A, Hakala J, Ylihonko K, Mäntsälä K (1997) J Antibiot 50:496
176. Bauman JG, Hawley RC, Rapoport H (1985) J Org Chem 50:1569
177. Rawlings BJ (1999) Nat Prod Rep 16:425
178. Rongsheng D, Shuquan Z, Yongle C, Ning C, Qijian S, Xiaoqing C (1983) Kangshengsu 8:44
179. Yoshimoto A, Tobe H, Ishikura, T, Takeuchi T, Umezawa H (1983) Anthracycline antibiotics. EP Patent 72 974 (Chem Abstr 98:214137)
180. Wiley PF, Elrod DW, Slavicek JM, Marshall VP (1980) J Antibiot 33:819
181. Elrod DW, Marshall VP, Wiley PF (1980) Dihydrosteffimycin compounds. US Patent 4 209 611 (Chem Abstr 93:184275)
182. Wiley PF, Marshall VP (1975) J Antibiot 28:838
183. Marshall VP, Elrod DW, Koert JM, Reisender EA, Wiley PF (1978) Steffimycinol. US Patent 4 077 844 (Chem Abstr 89:4583)
184. Kelly RC, Schletter I, Koert J, MacKellar FA, Wiley PF (1977) J Org Chem 42:3591
185. Brodasky TF, Reusser F (1974) J Antibiot 27:809
186. Wiley PF, Koert JM, Elrod DW, Reisender EA, Marshall VP (1977) J Antibiot 30:649
187. Wiley PF, Elrod DW, Marshall VP (1978) J Org Chem 43:3457
188. Laboratoires Hoechst SA, Fr (1986) Steffimycinone analogs. Jpn Kokai Tokkyo Koho JP Patent 61 047 439 (Chem Abstr 105:152829)
189. Gesson JP, Jacquesy JC, Renoux B (1983) Tetrahedron Lett 24:2761
190. Kelly RC (1976) Steffimycinone, 7-deoxysteffimycinone and derivatives. US Patent 3 976 667 (Chem Abstr 86:5220)
191. Itoh T, Kinoshita M, Aoki S, Kobayashi M (2003) J Nat Prod 66:1373
192. Yoshimoto A, Matsuzawa Y, Matsushita Y, Toshikazu O, Tomio T, Umezawa H (1981) J Antibiot 34:1492
193. Prikrylova V, Podojil M, Sedmera P, Vokoun J, Vanek Z, Brazhnikova MG, Kudinova MK (1980) Collect Czech Chem Commun 45:1991
194. Yoshimoto A, Fujii S, Johdo O, Kubo K, Nishida H, Okamoto R (1993) J Antibiot 46:56
195. Barskii VB, Potapova NP, Brazhnikova MG, Rozynov BV, Sibel'dina LA, Sepetov NF (1980) Antibiotiki 25:488 (Moscow)
196. Povarov LS, Gol'dberg LE, Bazhanov VS, Shepelevtseva NG Vertogradova TP, Averbukh LA (1981) Antibiotiki 26:620 (Moscow)
197. Ogawa Y, Sugi H, Fujikawa N, Mori H (1981) J Antibiot 34:938
198. Ogawa Y, Mori H, Yamada N, Kon K (1984) J Antibiot 37:44
199. Mori H, Tamai K, Ogawa Y, Sugi H, Fujikawa N (1981) Anthracycline antibiotic and a pharmaceutical composition containing it. EP Patent 25 713 (Chem Abstr 95:113370)
200. Hirofumi N, Mitsunobu H, Yoshinori Y, Katsuhiko A, Katsuichi S (1988) J Antibiot 41:404
201. Koyama T, Nakayama M, Tamura K, Shimada S, Kumazawa E, Miyata N (1987) Manufacture of novel anthracycline antibiotics with Actinomadura. Jpn Kokai Tokkyo Koho JP Patent 62 289 195 (Chem Abstr 108:203292)

202. Kimura K-I, Nakayama S, Miyata N, Kawanishi G (1989) J Antibiot 42:127
203. Kimura K, Nakayama M, Koyama T, Shimada S, Miyata N (1989) Stable barminomycin I-DNA conjugates, novel anthracycline compounds. Jpn Kokai Tokkyo Koho JP Patent 01 040 497 (Chem Abstr 111:152155)
204. Kimura KI, Nakayama S, Miyata N, Takeshita Y, Kawanishi G (1988) J Antibiot 41: 411
205. Fehlhaber HW, Kraemer HP, Franco CM, Mathew MT, Desikan KR, Ganguli BN (1986) Anthracycline antibiotics. DE Patent 3 446 052 (Chem Abstr 105:170623)
206. Yoshimoto A, Fujii S, Kubo K, Ishikura T, Sawa T, Takeuchi T, Umezawa H (1986) Anthracycline antibiotics. EP Patent 171 677 (Chem Abstr 105:96048)
207. Yoshimoto A, Fujii S, Osamu J, Katsuro K, Tomoyuki I, Hiroshi N, Tsutomu S, Takeuchi T, Umezawa H (1986) J Antibiot 39:902
208. Otake N, Odakawa A, Nakajima S (1986) Manufacture of antitumor agent R20X10 by *Actinomadura roseoviolacea*. Jpn Kokai Tokkyo Koho JP Patent 61 152 693 (Chem Abstr 106:17031)
209. Otake N, Odakawa A, Nakajima S (1986) Manufacture of anthracycline compound R20X2 and its use. Jpn Kokai Tokkyo Koho JP Patent 61 076 498 (Chem Abstr 106:3844)
210. Krohn K, Priyono W (1984) Tetrahedron 40:4609
211. Arai, Y, Takeda Y, Sato T, Watanabe N, Kamimura M (1987) Antitumor antibiotics BA32 and their manufacture with Streptomyces. Jpn Kokai Tokkyo Koho JP Patent 62 207 293 (Chem Abstr 108:220355)
212. Yoshimoto A, Matsuzawa Y, Ishikura T, Sawa T, Takeuchi T, Umezawa T (1984) J Antibiot 37:920
213. Yoshimoto A, Johdo O, Watanabe Y, Nishida H, Okamoto R, Takeuchi T (1992) J Antibiot 45:1005
214. Fleck W, Strauss D, Koch W, Prauser H (1974) Z Allg Mikrobiol 14:551
215. Fleck W, Strauss D, Koch W, Prauser H (1975) Antibiotiki 20:966 (Moscow)
216. Shimosaka A, Hayakawa Y, Nakagawa M, Furihata K, Seto H, Otake N (1987) J Antibiot 40:116
217. Aretz W, Berscheid HG, Huber G, Fehlhaber HW, Kraemer HP, Sedlacek HH, Ganguli BN, Sood RS, Gandhi J, Reddy GC (1985) Microbiological production of anthracycline derivatives, and their cytostatic use. DE Patent 3 323 025 (Chem Abstr 102:147547)
218. Aretz W, Berscheid HG, Huber G, Fehlhaber HW, Kraemer HP, Sedlacek HH, Ganguli BN, Sood RS, Gandhi J, Reddy GC (1990) Microbiological production of anthracycline derivatives, and their cytostatic use. EP Patent 0 131 181
219. Berscheid HG, Boettger D, Kraemer HP (1987) Preparation of anthracycline tetrasaccharides as neoplasm inhibitors. EP Patent 207 463 (Chem Abstr 107:176411)
220. Uchida T, Imoto M, Masuda T, Imamura K, Hatori Y, Sawa T, Naganawa H, Hamada M, Takeuchi T, Umezawa H (1983) J Antibiot 36:1080
221. Aretz W, Berscheid HG, Fehlhaber HW, Boettger D, Kraemer HP (1986) 1-Hydroxycytorhodins, their microbiological preparation and their use as cytostatic agents. EP Patent 167 954 (Chem Abstr 104:147150)
222. Li M, Chenn YL (1986) J Antibiot 39:430
223. Morioka H, Etoh Y, Horino I, Takezawa M, Ando T, Hirayama K, Kano H, Shibai H (1985) Agric Biol Chem 49:1951
224. Ando T, Hirayama K, Murai A, Tsuji T, Morioka H, Takesawa M, Shibai H, Horino K, Eto Y (1985) Antitumor agent cosmomycin c manufacture from Streptomyces. Jpn Kokai Tokkyo Koho JP Patent 60 248 614 (Chem Abstr 104:166917)

225. Aretz W, Berscheid HG, Fehlhaber, HW, Huber G, Kraemer HP (1985) Microbiological production of anthracycline derivatives. DE Patent 3 415 544 (Chem Abstr 104:107912)
226. Hedtmann U, Fehlhaber HW, Sukatsch DA, Weber M, Hoffmann D, Kraemer HP (1992) J Antibiot 45:1373
227. Ando T, Hirayama K, Takahashi R, Horino I, Etoh Y, Morioka H, Shibai H, Murai A (1985) Agr Biol Chem 49:259
228. Morioka H, Etoh Y, Horino I, Takezawa M, Ando T, Hirayama K, Kano H, Shibai H (1985) Agr Biol Chem 49:1951
229. Speitling M, Gruen-Wollny I, Fiebig HH, Laatsch H (2001) Rhodomycinone-derivatives. WO Patent 2 001 096 352 (Chem Abstr 136:36486)
230. Biedermann E, Braeuniger H (1972) Pharmazie 27:782
231. Saito S, Katsuda Y, Johdo O, Yoshimoto A (1995) Biosci Biotechnol Biochem 59:135
232. Brockmann H, Waehneldt T, Niemeyer J (1969) Tetrahedron Lett 10:415
233. Brockmann H, Scheffer B, Stein C (1973) Tetrahedron Lett 14:3699
234. Matsuzawa Y, Yoshimoto A, Oki T, Inui T, Takeuchi T, Umezawa H (1979) J Antibiot 32:420
235. Brockmann H, Wähneldt H (1963) Naturwissenschaften 50:43
236. Brockmann H, Niemeyer J, Rode W (1965) Chem Ber 98:3145
237. Johdo O, Yoshioka T, Takeuchi T, Yoshimoto A (1997) J Antibiot 50:522
238. Schlegel B, Ihn W, Fleck WF (1980) Z Allg Mikrobiol 20:531
239. Podojil M, Blumauerova M, Prikrylova V, Vanek Z, Gauze GF, Maksimova TS (1980) Folia Microbiologica 25:464 (Prague)
240. Brockmann H, Scheer M (1971) β-Rhodomycin V from Streptomyces purpurascens. DE Patent 2 012 808 (Chem Abstr 76:44690)
241. Otaka N, Odakawa A, Nakajima S, Yoneshima N (1986) Antitumor agent R20X4 manufacture with Actinomadura roseoviolacea. Jpn Kokai Tokkyo Koho JP Patent 61 137 893 (Chem Abstr 106:194766)
242. Connors NC, Bartel PL, Strohl WR (1990) J Gen Microbiol 136:1887
243. Brockmann H, Franck B (1955) Chem Ber 88:1792
244. Brockmann H, Brockmann H Jr (1961) Chem Ber 94:2681
245. Olano C, Lomovskaya N, Fonstein L, Roll JT, Hutchinson CR (1999) Chem Biol 6:845
246. Johdo O, Watanabe Y, Ishikura T, Yoshimoto A (1991) J Antibiot 44:1121
247. Kim HS, Hong YS, Kim YH, Yoo OL, Lee JJ (1996) J Antibiot 49:355
248. Reddy GCS, Sahai R, Fehlhaber HW, Ganguli BN (1985) J Antibiot 38:1423
249. Ishizumi K, Ohashi N, Tanno N (1987) J Org Chem 52:4477
250. Swenton JS, Raynolds PW (1978) J Am Chem Soc 100:6188
251. Brazhnikova MG, Konstantinova NV, Mezentsev AS, Pomazkova VA (1968) Antibiotiki 13:781 (Moscow)
252. Arcamone F, Franceschi G, Penco S, Selva A (1969) Tetrahedron Lett 10:1007
253. Toshikazu O, Yukio T, Hiroyasu T, Akihiro Y, Tomio T, Umezawa H (1981) J Antibiot 34:1229
254. Arcamone F, Franceschi G, Orezzi P, Penco S, Mondelli R (1968) Tetrahedron Lett 9:3349
255. Paulick RC, Casey ML, Whitlock HW (1976) J Am Chem Soc 98:3370
256. Bachur NR (1971) J Pharm Exp Therap 177:573
257. Arcamone F, Cassinelli G, Penco S, Tognoli L (2970) Antibiotic from Streptomyces fermentations. DE Patent 1 923 885 (Chem Abstr 72:131086)
258. Smith RG (1982) Anal Chem 54:2006

259. Kanamaru T, Nozaki Y, Muroi M (1990) Isolation of antitumor substances TAN-1120 from the culture broth of *Streptomyces triangulatus angiostaticus*. EP Patent 376 177 (Chem Abstr 114:41033)
260. Cassinelli G, Arlandini E, Ballabio M, Bordoni T, Geroni C, Giuliani F, Grein A, Merli S, Rivola G (1990) J Antibiot 43:19
261. Tsuchiya KS, Ishii T, Ikeno S, Kunimoto S, Kawauchi T, Otani T, Hori M (1997) J Antibiot 50:853
262. Kawauchi T, Sasaki T, Yoshida KI, Matsumoto H, Chen RX, Huang MY, Otani T (1997) J Antibiot 50:297
263. Kawauchi T, Sasaki T, Matsumoto H, Otani T, Chen RX, Huang MY, Yoshida KI (1996) IT-62-B substance and medicinal composition containing the same. WO Patent 9 602 659 (Chem Abstr 124:315172)
264. Pokras LS, Snezhnova LP, Brazhnikova MG, Grigor'eva VM (1978) Antibiotiki 23:788 (Moscow)
265. Julou L, Ducrot R, Fournel J, Ganter P, Maral R, Populaire P, Koenig F, Myon J, Pascal S, Pasquet J (1967) Arzneimittel-Forsch 17:948
266. Zbarskii VB, Lazhko EI, Potapova NP, Fomicheva EV, Saburova TP (1991) Bioorg Khim 17:1698
267. Arcamone F, Cassinelli G, Franceschi G, Orezzi P, Mondelli R (1968) Tetrahedron Lett 30:3353
268. Johdo O, Nishimura M, Tone H, Okamoto R (1993) Preparation of anthracycline compounds as antitumor agents. Jpn Kokai Tokkyo Koho JP Patent 05 039 242 (Chem Abstr 119:93691)
269. Miyamoto Y, Ohta S, Johdo O, Nagamatsu Y, Yoshimoto A (2000) J Antibiot 53:828
270. Yoshimoto A, Kubo K, Fujii S, Ishikura T, Sawa T, Takeuchi T, Umezawa H (1985) Manufacture of novel anthracyline antibiotics with Streptomyces D788 mutant. Jpn Kokai Tokkyo Koho JP Patent 60 185 797 (Chem Abstr 105:5172)
271. Yan X, Wang S, Wu J (1993) Chin Chem Lett 4:787
272. Yoshimoto A, Matsuzawa Y, Oki T, Naganawa H, Takeuchi T, Umezawa H (1980) J Antibiot 33:1150
273. Engel DW, Zechmeister K, Hoppe W (1972) Tetrahedron Lett 14:1323
274. Mansour FA, El-Sayed SA, El-Moughith AA, El-Sherbiny SA (1997) Egypt J Microbiol 32:69
275. El-Shirbiny SA, Abdel HF (1998) Egypt J Microbiol 33:469
276. Kiyo K, Sho S, Marunaka T, Miyake Y, Minami Y (1987) Antibiotic compounds 301 manufacture with *Streptomyces virginiae*. Jpn Kokai Tokkyo Koho JP Patent 62 030 793 (Chem Abstr 107:5739)
277. Bhuyan BK, Kelly RB, Smith RM (1965) Nogalamycin and its production. US Patent 3 183 157 (Chem Abstr 63:20125)
278. Bhuyan BK, Dietz A (1965) Antimicrob Agents Chemother 836
279. Wiley PF, MacKellar FA, Caron EL, Kelly RB (1968) Tetrahedron Lett 9:663
280. Wiley PF, Kelly RB, Caron EL, Wiley VH, Johnson JH, MacKellar FA, Mizsak SA (1977) J Am Chem Soc 99:542
281. Kirin Brewery Co., Ltd, Japan (1985) The anthracycline compound AG-2 and its antitumor activity. Jpn Kokai Tokkyo Koho JP Patent 60 025 999 (Chem Abstr 104:33023)
282. Kawai H, Hayakawa Y, Nakagawa M, Furihata K, Shimazu A, Seto H, Otake N (1987) J Antibiot 40:1266
283. Aoki M, Shirai H, Nakayama N, Itezone Y, Mori M, Satoh T, Ohshima S, Watanabe J, Yokose K (1991) J Antibiot 44:635

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Biosynthetic Anthracycline Variants

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Abstract In addition to synthetic and semisynthetic methods, new anthracycline structures have been generated by biosynthetic methods: genetic engineering of *Streptomyces* production strains, bioconversion and chemoenzymatic synthesis. In this review, we discuss the set of molecules potentially producible by biosynthetic methods and which structures have so far been realized. Biosynthetic variation in the anthracycline molecule manifests itself either as structure changes in the tetracyclic aglycone, or as variation in glycosylation. Understanding the biosynthetic sequence and knowledge of the substrate specificities of the enzymes participating in it enable rational generation of new anthracycline diversity. Future possibilities include protein engineering of the biosynthetic enzymes to improve the production of new structural combinations.

Keywords Anthracycline · Combinatorial biosynthesis · Polyketide · Streptomyces

Abbreviations

Acu	Aculose
Acv	Aklavinone
CinA	Cinerulose A
CoA	Coenzyme A
dF	2-Deoxy-L-fucose
DN	Daunosamine
Dnm	Daunomycinone
dTDP	Deoxythymidine diphosphate
Rho	Rhodinose
RN	Rhodosamine

1 Introduction

Natural anthracyclines are tetracyclic anthraquinone derivatives, produced by strains of *Streptomyces* and related bacteria. Among them are some of the best cytostatic agents in current use. In the quest to improve the pharmaceutical properties of these compounds numerous (>2000 [1]) structural variants of the anthracycline molecule have been produced and tested for antitumor activity. In addition to semisynthetic (and in a few cases fully synthetic) molecules, these variants have been produced by biosynthetic means, the subject of this review. While the most interesting method is arguably pathway manipulation of the producer organisms by means of recombinant DNA methods, we will also discuss biosynthetic methods using bioconversion and purified enzymes. All three are based ultimately on biosynthetic enzymes, whether those synthesized in the producing or bioconverting living organism in vivo, or as purified enzymes in the chemist's reaction vessel in vitro. The success and failure of the biosynthetic methods therefore rests heavily on the properties of the enzymes involved.

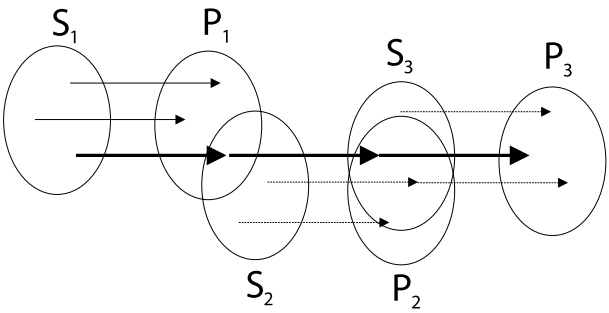


Fig. 1 Emergence of specificity from a chain of unspecific enzymatic reactions. S_{1-3}: substrate range of enzymes 1–3. P_{1-3}: product ranges of the enzymes. *Bold line*: productive sequence leading to final product

Antibiotic biosynthetic enzymes are generally believed to possess low substrate specificity. This can be rationalized from an evolutionary point of view: reduced substrate specificity increases the probability of a chain of enzymes to yield a product, even when the enzymes form new combinations (Fig. 1). When the intermediates are structurally distinct from the intermediates of primary metabolism, there is little need for stringent selectivity (and thus little evolutionary pressure to increase it) as no competing intermediates are present. Since the evolutionary advantage in antibiotic biosynthesis originates from the diversity of the compounds produced and is rather indifferent to the quantity of production, many pathways have converged such that a long chain of enzymes with broad substrate specificity yields a specific product.

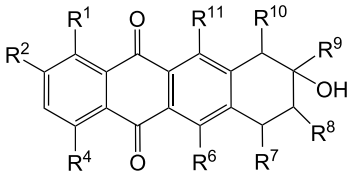
1.1
Benefits and Limitations of Biosynthetic Methods

Anthracyclines are large and chemically labile molecules, which makes their synthetic modification challenging. In biosynthetic methods, reactions are performed mostly in neutral aqueous solutions at moderate temperatures. Enzymatic reactions are highly specific for the position as well as the stereoisomer modified; likewise, chiral products are exclusively of one stereoisomer. Particularly in vivo, in living organisms, enzymatic reactions can be combined to perform several modifications in one process, or, indeed, to build the entire molecule from simple precursors. One of the strengths of biosynthetic methods is also their main weakness: only modifications for which an enzyme exists can be performed.

2
Possibilities for Biosynthetic Modification

To map the set of anthracyclines that can potentially be produced by biosynthetic means, we start with the substituents actually present in natural anthracyclines (Struct. 1) [2]:

R^1 = H, OH or nogalamine.
R^2 = H, OH, OCH_3 or nogalamine
R^4 = OH, OCH_3
R^6 = H, OH
R^7 = H, OH, O, glycosylation
R^8 = H, OCH_3
R^9 = CH_3, C_2H_5, CH_2COCH_3, $COHCH_3$, $COCH_3$, $COCH_2OH$
R^{10} = $COOCH_3$, COOH, H, OH, O, glycosylation
R^{11} = H, OH.

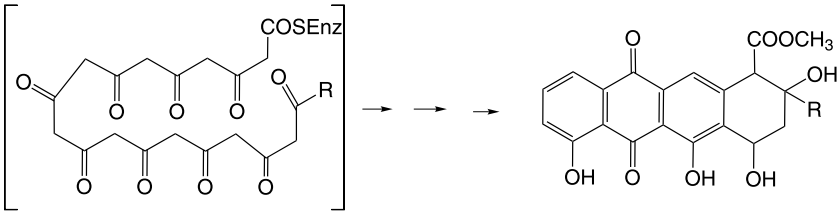


Structure 1 Substituent sites in natural anthracyclines

In the following sections, we will review the reactions responsible for each of these structural variations (where known), and what opportunities these present for the generation of new molecules.

2.1 Variation of the Polyketide Starter Unit

The anthracycline core is assembled from acetyl units in the form of malonyl-CoA by an iterative, type II polyketide synthase (Metsä-Ketelä et al. "Anthracycline Biosynthesis: Genes, Enzymes and Mechanisms", this volume) followed by aromatase, cyclase and tailoring enzymes, which produce the tetracyclic ring structure from the unstable polyketide chain as shown in Scheme 1.



Scheme 1 Cyclization pattern of the anthracycline aglycone

In the biosynthesis of most anthracyclines, the first polyketide unit is a propionate instead of acetate; two enzymes, a ketosynthase [3] and a thioesterase [4], cause the addition of a propionyl unit from propionyl-CoA as the first unit of the polyketide chain. This results in R^9 being an ethyl group.

Nogalamycin biosynthesis is exceptional as the starter unit determining enzymes are absent, and instead polyketide biosynthesis starts with acetyl-CoA, resulting in methyl as the R^9 group. When the defective PKS of a *Streptomyces galilaeus* mutant was complemented with a plasmid containing the *S. nogalater* PKS [5, 6], auramycinone glycosides with $R^9 = CH_3$ were produced.

Other variants of R^9, with the exception of CH_2COCH_3 seen in sulfurmycins, result from post-polyketide modification by DoxA (see below). Sulfurmycins are closely related to aclacinomycins and are produced as minor components by some strains of *S. galilaeus* [7]. The CH_2COCH_3 side chain

can be rationalized biosynthetically as either having an acetoacetyl instead of propionyl starter unit, or having an acetyl starter and a polyketide chain consisting of 11 acetates.

2.2 Aglycone Modification

The substituents present on the aglycone are of two kinds; those that arise directly from the polyketide cyclization process, and those that are added (or removed) by specific enzymes known as "tailoring" enzymes. The tailoring steps appear to be the ones most suitable for biosynthetic variation. The abbreviations for enzymes/genes mentioned in this review are listed in Table 1.

Table 1 Enzyme/gene abbreviations used in the text

Abbreviation	Name	Refs.
AclR	Putative anthracyclinone 1-hydroxylase	[8]
AknK	Glycosyl transferase (second and third sugar)	[9]
AknM	TDP-4-keto-6-deoxyhexose reductase (4*S*)	[10]
AknP	dTDP-hexose 3-dehydratase	[10]
AknQ	3-Ketoreductase	[10]
AknS	Glycosyl transferase (first sugar)	[11]
AknT	AknS accessory protein	[12]
AknY	dTDP-glucose 1-synthase	[10]
AknZ	Aminotransferase	[10]
AvrE	TDP-4-keto-6-deoxyhexose reductase (4*R*)	[13]
DauK	4-O-Methyltransferase	[14]
DauP	Anthracycline esterase	[14]
DnmV	TDP-4-keto-6-deoxyhexose reductase (4*S*)	[13]
DnrK	4-O-Methyltransferase	[15]
DnrV	Putative accessory protein for DoxA	[16]
DoxA	Multifunctional anthracycline oxidase	[16]
DpsE	2-Ketoreductase	[17]
EryBIV	TDP-4-keto-6-deoxyhexose reductase (4*R*)	[13]
RdmB	Anthracycline 10-hydroxylase	[18]
RdmC	Anthracycline esterase	[19]
RdmE	Aklavinone-11-hydroxylase	[20]
SnoaL	Cyclase	[21]
SnoaL2	Putative anthracyclinone 1-hydroxylase	[21]
SnogC	TDP-4-keto-6-deoxyhexose reductase (4*R*)	[22]
StfE	Anthracycline 10-oxidase	[23]
StfG	Glycosyltransferase	[23]
StfMI	2-O-Methyltransferase	[23]
StfPI	Anthracycline 10-hydroxylase	[23]
StfX	Cyclase	[23]

R^1 is initially hydrogen. A hydroxyl is present in, among others, cinerubins [24], isorhodomycins [24] and also before glycosylation in nogalamycin [21]. Hybrid molecules with R^1 =OH have been generated by expressing *snoaL2* from nogalamycin biosynthesis [21] or *aclR* from cinerubin biosynthesis in an aklavinone-producing *S. galilaeus* mutant [8]; in both cases 1-hydroxyaklavinone (cinerubinone) was produced.

Originally R^2 would be a polyketide-derived hydroxyl group, but in the overwhelming majority of anthracyclines it is removed in connection with cyclization as the consequence of the action of a specific ketoreductase (DpsE [17] and homologs). The natural anthracycline steffimycin [23] has an OCH_3 substituent in this position and accordingly, a ketoreductase gene is not present in the steffimycin biosynthetic cluster. Furthermore, the authors propose that an O-methyltransferase, StfMI, methylates the hydroxyl thus remaining in the molecule. The authors were also able to produce a 2-hydroxylanthracycline aglycone by expressing a partial set of steffimycin biosynthetic genes in a heterologous host. In an interesting reversal, the 2-methoxy substituent could be removed from the steffimycin molecule by introducing into the producing strain (*S. steffisburgensis*) the missing ketoreductase gene (together with a gene encoding aromatase, the following step) from nogalamycin biosynthesis [25].

The cyclization process produces a hydroxyl as R^4. Among natural anthracyclines, the hydroxyl is either free or O-methylated. As the *O*-methyl substituent is one of the distinguishing features of the highly successful daunorubicin–doxorubicin group of anthracyclines, the enzyme, DnrK, catalyzing this methylation reaction was one of the earliest biosynthetic enzymes subjected to enzymological research [14, 26, 27]. In this context it should be noted that although DnrK modifies the aglycone, it requires a glycoside substrate, and thus acts in the biosynthetic chain after glycosylation.

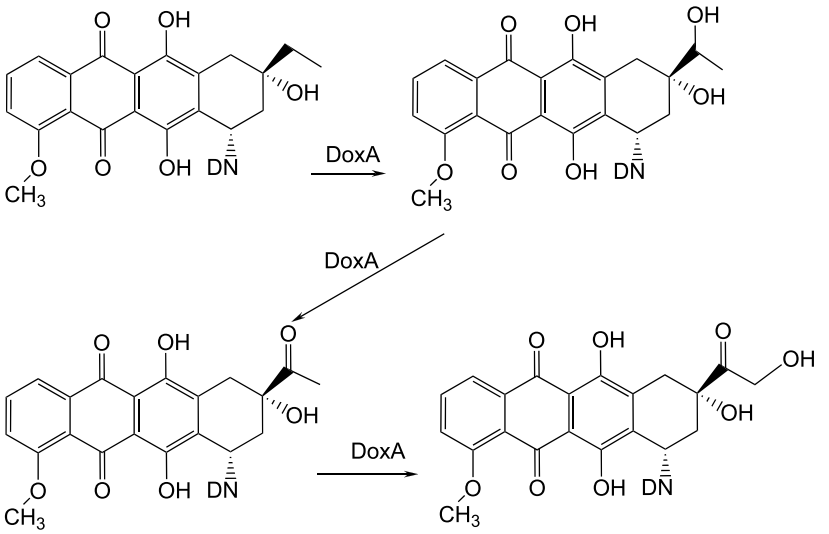
DnrK, or its close homolog DauK, appears to have quite low substrate specificity; rhodomycin D, 13-deoxycarminomycin, 13-dihydrocarminomycin and carminomycin were all efficiently methylated [14] by in-vivo bioconversion. A new anthracycline, 4-*O*-methylepelmycin, was produced by expressing the *dnrK* gene in the epelmycin (ε-rhodomycinone–rhodosamine) producer *S. violaceus* [28]. When the three-dimensional structure of a DnrK–product complex was determined [15], it became apparent that only monoglycosylated substrates fit into the active site, explaining the failure to produce 4-O-methylated aclacinomycin A (a triglycoside) derivatives by similarly expressing the *dnrK* gene in an aclacinomycin A producer.

The default substituent at R^6 is hydroxyl. Among natural anthracyclines, apparently only α-2-citromycinone [29], a minor component in *S. purpurascens*, lacks this substituent. From a biosynthetic point of view, this is difficult to understand and it is doubtful whether this phenomenon can be used in pathway engineering. Nevertheless, a new anthracycline glycoside was produced by microbial glycosylation (see below) of α-2-citromycinone [30].

R^7 is the primary site of glycosylation of the anthracycline molecule. Initially produced as carbonyl, the substituent is reduced to a 7*S*-hydroxyl by a specific ketoreductase as a prerequisite for glycosylation. If this enzyme is missing through mutation or genetic engineering, a carbonyl remains at this site. Such unglycosylated molecules have been considered uninteresting; however, recently maggiemycin [31] with R^7 = O was shown to inhibit integrin binding to collagen.

R^8 is initially hydrogen; in steffimycin this position is modified with a $-OCH_3$ moiety. Although the steffimycin biosynthetic cluster has been cloned and sequenced [23], the genes responsible for this modification have not been unequivocally identified, and this modification has not been used in combinatorial biosynthesis.

The length of the carbon chain at R^9 is determined initially by the polyketide starter. In the most important anthracyclines, daunorubicin and doxorubicin, the ethyl moiety present is oxidized by the cytochrome P_{450} enzyme DoxA in three steps (Scheme 2).



Scheme 2 Reactions catalyzed by DoxA in doxorubicin biosynthesis

DoxA has been extensively studied [16, 32] as it is the enzyme that introduces key substituents of daunorubicin and doxorubicin. In particular, the 14-hydroxyl substituent of doxorubicin has been of great concern. In this respect it has been quite disappointing that the last reaction seems to be a minor side reaction for DoxA; its reaction rate in vitro is about 1/100 of the preceding reactions. Nevertheless, overexpression of *doxA* together with the linked *dnrV* in genetically engineered *S. peucetius* mutants yielded strains

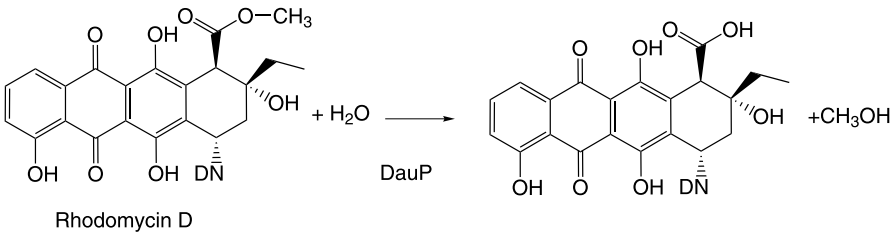
that produced predominantly doxorubicin [16]. Further development, in all likelihood, should yield strains suitable for industrial production.

For the production of new structures, the usefulness of DoxA has been limited. Only, when R^9 was completely missing, was a new product obtained [33] – and in that case DoxA surprisingly hydroxylated carbon-10, showing that it is more of a regioselective than a specific hydroxylating enzyme. DoxA is apparently not active with substrates containing R^9 = $-COOCH_3$ and glycosylation = rhodosamine; both restrictions effectively prevent application of DoxA for the aclacinomycin-like anthracyclines.

In addition to the alkyl or modified alkyl side chain, carbon-9 has a hydroxyl substituent, which originates from the polyketide cyclization process, producing a chiral center. In the majority of anthracyclines carbon-9 has the *R* configuration, but in nogalamycin the *S* configuration is present. Torkkell and coworkers [21] were able to produce the 9-epimer of aklavinone by expressing the fourth ring cyclase, SnoaL, from the nogalamycin biosynthetic cluster in a *S. galilaeus* mutant.

R^{10} is initially $-COOCH_3$, except in the biosynthesis of steffimycin. The SnoaL-like cyclases [34, 35] catalyze an intramolecular aldol condensation, and require that the substrate is an ester. In steffimycin biosynthesis an unrelated cyclase StfX [23] uses a carboxylic acid as the substrate and, simultaneously with the cyclization, the carboxyl group is presumed to be lost as CO_2.

In the daunorubicin–doxorubicin group of anthracyclines, R^{10} is removed by tailoring reactions after glycosylation. DauP [14] produces the free 10-carboxylic acid from the methyl ester (Scheme 3).

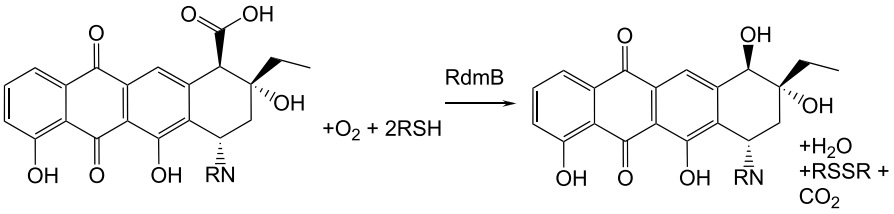


Scheme 3 Ester hydrolysis by DauP

The latter is unstable, and decarboxylates to yield a decarboxymethyl anthracycline. Dickens and coworkers [14] describe the 10-carboxylic acid produced from rhodomycin D as reasonably stable. On the other hand, the 10-carboxylic acid produced from aclacinomycin A was quite unstable [18]. In daunorubicin biosynthesis DnrK, the next step in the biosynthetic chain, is believed to enhance the decarboxylation reaction [14]. The enzymatic properties and structure of the closely related anthracycline 10-esterase RdmC from the rhodomycin biosynthetic cluster have been investigated [19, 36]. RdmC is a α/β hydrolase with a classical Ser-His-Asp catalytic triad, and it uses

a glycosylated substrate. Both mono- and triglycosides could be effectively hydrolyzed.

In β-rhodomycinone and related anthracyclines, R^{10} is replaced by OH (see Sect. 4.1). RdmB from *S. purpurascens* is a DnrK homolog. However, it is not a methylase like DnrK, but a hydroxylase, which replaces the carboxyl group remaining after the RdmC reaction with a hydroxyl (Scheme 4).



Scheme 4 Unusual hydroxylation sequence catalyzed by RdmB

Unlike DnrK, RdmB is able to use both monoglycosylated and triglycosylated substrates, enabling the production of 10-hydroxylated molecules from both.

Steffimycin has a carbonyl substituent at C-10 [23]. As noted above, in steffimycin biosynthesis R^{10} is completely removed in connection with cyclization; therefore this substituent is added by tailoring enzymes apparently after glycosylation. The authors implicate the cytochrome P450 enzyme StfPI

Table 2 Pathway engineering of the anthracycline aglycone

Host	Gene(s) used	Substituent(s) changed	To:	Refs.
S. galilaeus	*rdmC, rdmB* (*S. purpurascens*)	R^{10}	H, OH	[49]
S. galilaeus	*rdmE*	R^{11}	OH	[49]
S. galilaeus H028	*S. nogalater* polyketide synthase	R^{9}	CH_3	[5]
S. galilaeus H038	*rdmC, rdmB*	R^{10}	H, OH	[50]
S. steffisburgensis	*snoaD, snoaE* (*S. nogalater*)	R^{2}	H	[25]
S. peucetius M18	*snoaL*	R^{9}	*S*-Configuration	[22]
S. galilaeus	*snoaL2*	R^{1}	OH	[22]
S. violaceus	*dnrK* (*S. peucetius*)	R^{4}	OCH_3	[28]
S. galilaeus	*dnrF*	R^{11}	OH	[51]
S. galilaeus	*dnrF*	R^{11}	OH	[52]
S. albus	Partial steffimycin biosynthetic cluster	R^{2}	OH	[23]
S. steffisburgensis	Disruption of *stfG*	R^{8}, R^{10}	H	[23]
S. galilaeus H039	*aclR*	R^{1}	OH	[8]

Table 3 Glycosylation of aglycones by *S. galilaeus* KE303 [43]

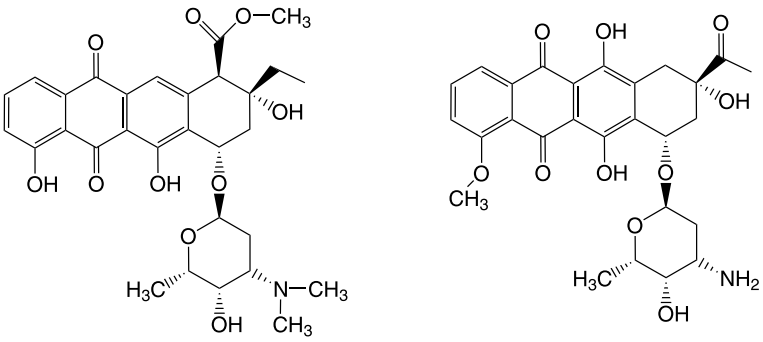
		R^1	R^2	R^4	R^7	R^8	R^9	R^{10}	R^{11}
Glycosylated	Aklavinone	H	H	OH	OH	H	CH_2CH_3	$COOCH_3$	H
	10-Decarbomethoxyaklavinone	H	H	OH	OH	H	CH_2CH_3	H	H
	4-*O*-Methylaklavinone	H	H	OCH_3	OH	H	CH_2CH_3	$COOCH_3$	H
	ε-Pyrromycinone	OH	H	OH	OH	H	CH_2CH_3	$COOCH_3$	H
	ε-Rhodomycinone	H	H	OH	OH	H	CH_2CH_3	$COOCH_3$	OH
	ε-Isorhodomycinone	OH	H	OH	OH	H	CH_2CH_3	$COOCH_3$	OH
	β-Rhodomycinone	H	H	OH	OH	H	CH_2CH_3	OH	OH
	γ-Rhodomycinone	H	H	OH	H	H	CH_2CH_3	OH	OH
	β-Pyrromycinone	OH	H	OH	OH	H	CH_2CH_3	OH	H
Not glycosylated	7-Deoxyaklavinone	H	H	OH	H	H	CH_2CH_3	$COOCH_3$	H
	Daunomycinone	H	H	OCH_3	OH	H	$COCH_3$	H	OH
	Dihydrodaunomycinone	H	H	OCH_3	OH	H	$COHCH_3$	H	OH
	7-Deoxydihydrodaunomycinone	H	H	OCH_3	H	H	$COHCH_3$	H	OH
	13-Deoxydaunomycinone	H	H	OCH_3	OH	H	CH_2CH_3	H	OH
	Carminomycinone	H	H	OH	OH	H	$COCH_3$	H	OH
	Adriamycinone	H	H	OCH_3	OH	H	$COCH_2OH$	H	OH
	Steffimycinone	H	OCH_3	OH	OH	OCH_3	CH_3	=O	H

in hydroxylating the 10-position and the AknOx-like [37] flavoprotein StfE in oxidizing it to carbonyl.

R^{11} is initially hydrogen. Aklavinone 11-hydroxylase is a FAD hydroxylase, which hydroxylates the 11-position of an aglycone substrate. This enzyme has been described both from *S. peucetius* (RdmE) and *S. purpurascens* (DnrF) [20, 38, 39], and has been used to produce ε-rhodomycinone glycosides (see below) by expressing it in aklavinone glycoside-producing *S. galilaeus*.

2.3 Variation of Anthracycline Glycosylation

Biologically active anthracyclines are glycosides, and superficially the function of the sugar portion of the molecule is to render the molecule more water-soluble and provide polar and/or charged groups for interaction with a target molecule. The glycoside components of anthracyclines typically contain certain deoxysugars and aminodeoxysugars. Overall, the most common glycosylation component is either rhodosamine (RN) or daunosamine (DN) attached at the hydroxyl at carbon-7 as seen in aclacinomycin T and daunorubicin (Scheme 5).



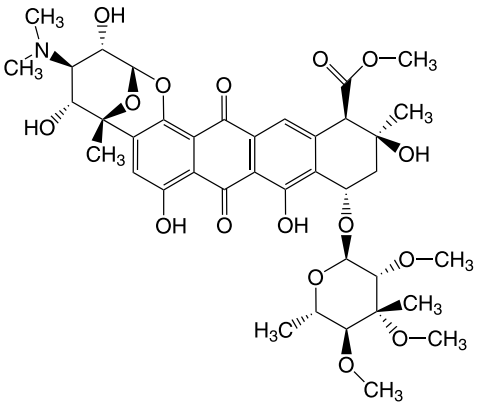
Aclacinomycin T
rhodosaminyl-aklavinone
(Acv-RN)

Daunorubicin
daunosaminyl-daunorubicinone
(Dnm-DN)

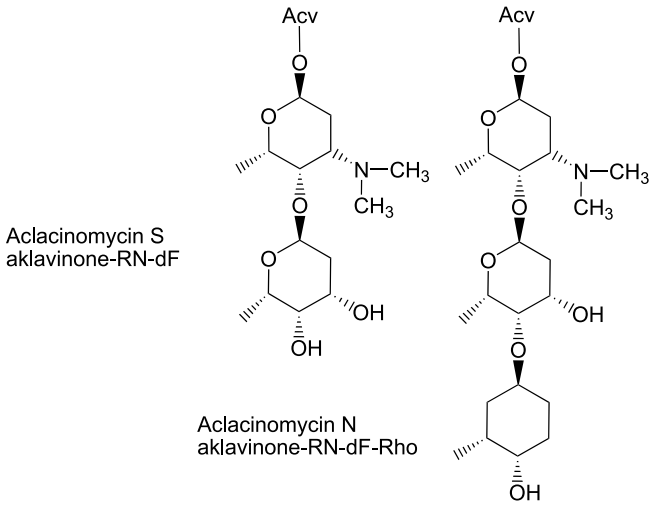
Scheme 5 Aclacinomycin T and daunorubicin, two representative anthracycline monoglycosides

An alternative position for the amino sugar (nogalamine) is seen in nogalamycin (Struct. 2).

Neutral sugars can be attached to carbon 4′ of the amino sugar (Struct. 3). The most common second sugar in RN glycosides is 2-deoxy-L-fucose (dF) (aclacinomycin S). In glycosides, where the first sugar is RN, the main product is usually a triglycoside. The third sugar is initially L-rhodinose (Rho) (aclacinomycin N).



Structure 2 Nogalamycin

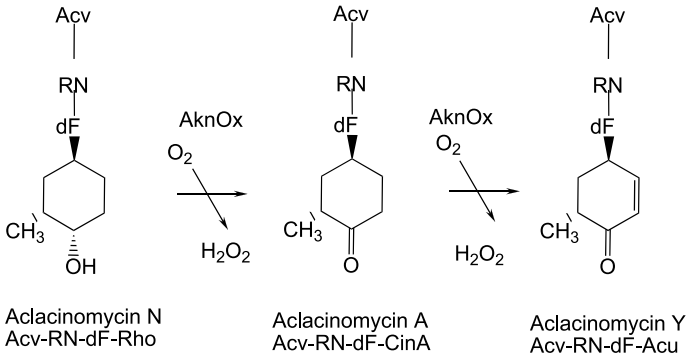


Structure 3 Aklavinone di- and triglycosides aclacinomycin S and N

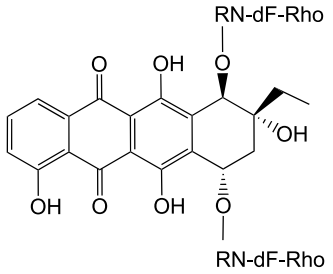
Subsequently, the attached third sugar is modified by an interesting flavoprotein oxidoreductase [37, 40], which oxidizes the Rho moiety first to L-cinerulose A (CinA) and then to L-aculose (Acu) (Scheme 6).

In anthracyclines, where the aglycone is β-rhodomycinone ($R^7 = R^{10} =$ – OH) a glycoside chain can be attached either to carbon-7, or carbon-10 or both, such as in cosmomycin D (Struct. 4).

Anthracyclines with daunosamine glycosylation may also initially have a second sugar attached to carbon 4′; however, this sugar is further modified to yield baumycins [41], products in which this sugar appears to have been oxidatively cleaved. The hypothetical initial glycoside component has not been identified.



Scheme 6 Terminal sugar oxidations catalyzed by AknOx

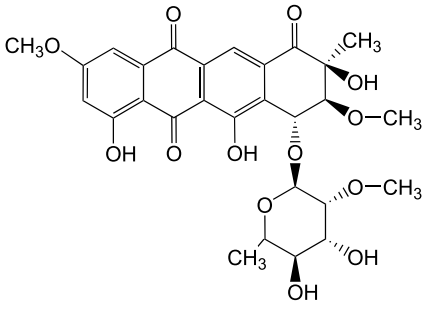


Structure 4 Cosmomycin D

Steffimycin (Struct. 5) is unusual in that the glycosylation at carbon-7 is a neutral deoxysugar, 2-*O*-methyl rhamnose.

Glycosylation is catalyzed by glycosyl transferases, which use dTDP-sugar substrates. Separate transferases usually add each component. In terms of generating novel glycosides, the transferases are obviously key enzymes.

Early on, bioconversion experiments indicated low substrate specificity for the glycosyl transferases as various aglycones could be glycosylated



Structure 5 Steffimycin

by aglycone-nonproducing mutants with both daunorubicin-like [42] and aclacinomycin-like [43] sugars (see below). Studies on the two glycosyl transferases in aclacinomycin biosynthesis [9, 11, 12] provided important information on the logic of the glycosylation. One of the transferases, AknS, was found to be responsible for attaching the first sugar on the aglycone; for full activity an accessory protein, AknT, was needed in the reaction. This enzyme attached a rhodosaminyl moiety from dTDP-RN as the first sugar. Deoxy-TDP-daunosamine was a very poor substrate, but dTDP-2-deoxyfucose yielded an anthracycline glycoside with dF as the first sugar.

The other transferase, AknK, was found to add deoxyfucose as the second sugar; no glycosylation of the aglycone was observed. On the other hand, AknK was able to use dTDP-daunosamine as a substrate, producing novel glycosides with daunosamine as the second sugar. Likewise, AknK was able to add a third dF unit to a diglycoside.

It is instructive to combine these observations with data derived from *S. galilaeus* mutants with unusual glycosylation. Ylihonko and coworkers [44] produced a series of glycosylation mutants, for most of which the mutated gene was subsequently identified by complementation and sequencing [10]. In all investigated mutants, the gene defect was in the dTDP–sugar biosynthetic pathway, not in the glycosyl transferases.

One of the mutants, H075, produces anthracyclines with the glycosylation RN-dF-dF. In this strain, a nonsense mutation inactivates dTDP-hexose 3-dehydratase *aknP*, abolishing production of dTDP-rhodinose. As in vitro [9], AknK now adds dF-dF instead of dF-Rho as second and third sugar. Apparently, as long as sugar nucleotides were present, the glycosyl transferases added them on the aglycone; if production of both neutral sugars was abolished (mutant H038; mutation in dTDP-hexose 3-ketoreductase *aknQ*) glycosylation was RN alone. If production of rhodosamine was abolished (H054, aminotransferase *aknZ*), glycosylation dF-Rho-CinA, dF-Rho-Rho and similar combinations of neutral sugars were observed. Only if production of dTDP sugars was entirely prevented (H063, dTDP-glucose 1-synthase *aknY*) was the aglycone, aklavinone, produced alone [10].

The broad substrate specificity is also illustrated in cases where new dTDP sugars have been produced in anthracycline production strains by genetic engineering. The important doxorubicin analog epirubicin, previously produced by semisynthetic means, could be produced by artificially modifying the dTDP-amino sugar production pathway in *S. peucetius* [13] (see below).

3
Genetic Engineering of Production Strains

From the very start of recombinant DNA technology of antibiotic producers [45], the aim, in addition to increased yields of known antibiotics, has

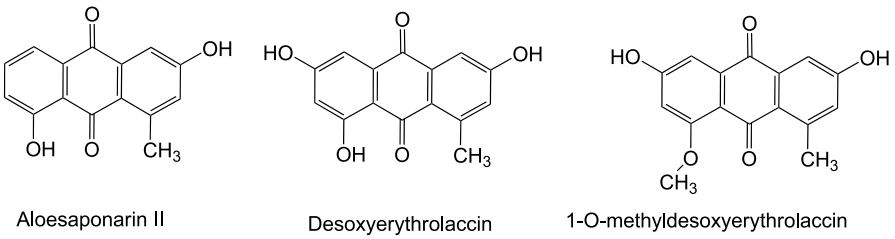
been the generation of new antibiotic structures. Genes originally participating in the production of more than one antibiotic are combined in a single producing organism by genetic engineering. The term "hybrid antibiotics" originally used has been replaced by "combinatorial biosynthesis"; however, it is useful to divide the production of new structures by genetic engineering into "pathway engineering" and "combinatorial biosynthesis" [46].

3.1 Pathway Engineering

Pathway engineering is "targeted modification of a biosynthetic pathway to generate a single or limited number of modified structures" [46]. Frequently, new molecules have been generated in experiments that have sought to clarify the function of a new gene; thus the new product has not always been determined beforehand.

3.1.1 Pathway Engineering of the Aglycone

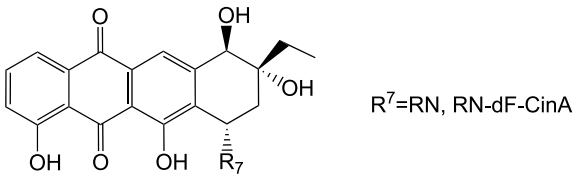
Bartel and coworkers [47] were the first to produce new structures involving anthracycline biosynthetic genes. In this case, an anthracycline producer (*S. galilaeus*) was used as the cloning host, and the new genes introduced originated from the actinorhodin biosynthetic pathway [48]. The new products (Scheme 7) were tricyclic aromatic polyketides aloesaponarin II, desoxyerythrolaccin and 1-*O*-methyldesoxyerythrolaccin.



Scheme 7 Hybrid anthraquinones produced by combining actinorhodin and aclacinomycin biosynthetic genes

Table 2 lists publications that describe the alteration of the structure of the aglycone by pathway engineering.

The first hybrid anthracyclines were produced by introducing parts of the rhodomycin biosynthetic gene cluster from *S. purpurascens* into *S. galilaeus* [49, 53]. The transgenic strain produced glycosides of aklavinone, β-rhodomycinone, ε-rhodomycinone, 10-decarbomethoxyaklavinone and, as novel products, glycosides of 11-deoxy-β-rhodomycinone (Struct. 6).



Structure 6 11-Deoxy-β-rhodomycin hybrids

Subsequently, it was found that production of the hybrids was caused by the genes *rdmE*, *rdmC* and *rdmB* in the construct used [53]. Superficially it is surprising that several aglycones were observed; if the enzymes corresponding to the above genes converted their respective substrates at 100% efficiency, only β-rhodomycinone glycosides would be produced. However, in pathway manipulation it is to be expected that there is competition between the original biosynthetic pathway and the "detour" or "fork" created by the added biosynthetic enzymes (Fig. 2).

The role of RdmB remained enigmatic for several years [36, 54], and only after recent biochemical and structural studies [18] did it became clear that the enzyme is not a methylase but a substrate-assisted hydroxylase. The enzyme replaces the carboxyl group remaining after the RdmC reaction with a hydroxyl by using *S*-adenosylmethionine as a charged group polarizing the polycyclic aromatic core of the molecule.

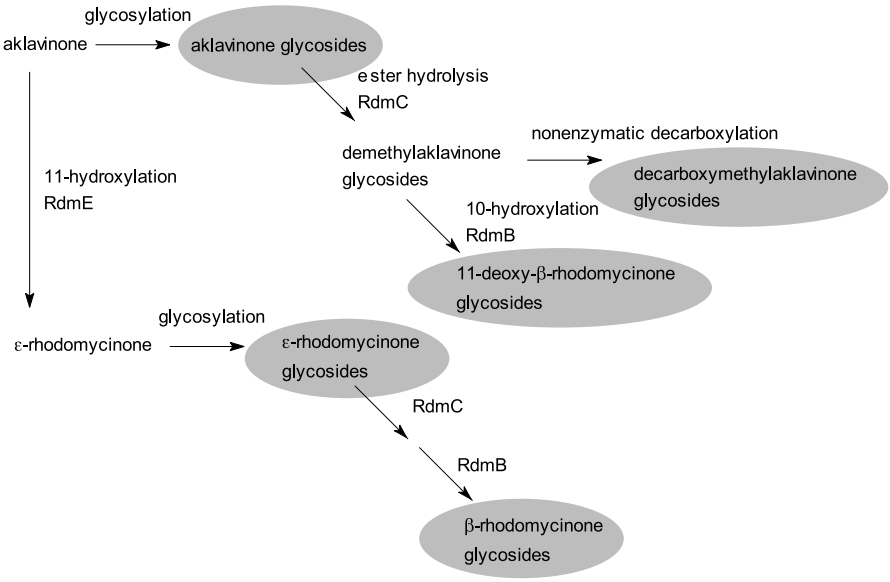
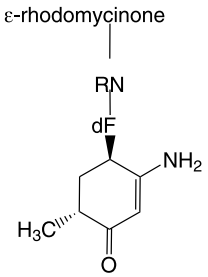


Fig. 2 Pathway manipulation of aclarubicin biosynthesis with rhodomycin biosynthetic genes *rdmE*, *rdmC* and *rdmB*

The acetate starter unit characteristic of nogalamycin biosynthesis yielded the next hybrids with the production of auramycinone glycosides, as already described [5].

The 11-hydroxylase genes (*rdmE*, *dnrF*) have been used by several investigators. The 11-hydroxylated form of aklavinone, ε-rhodomycinone, has been the aglycone in all of these experiments, but there have been differences in glycosylation. Hwang and coworkers [51] produced ε-rhodomycin A (ε-rhodomycinone-RN-dF-CinA, which was previously known from bioconversion experiments (see below) and also from a *S. violaceus* mutant, where it had been named epelmycin E [55]). Kim et al. [52] found a new glycoside, 11-hydroxyaclacinomycin X, in which the terminal sugar is the unusual amino sugar rednose (Struct. 7).



Structure 7 11-Hydroxyaclacinomycin X

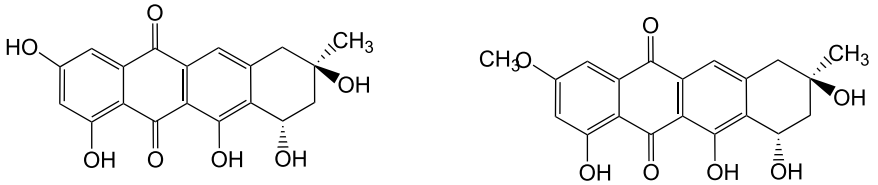
The 4-O-methyltransferase gene *dnrK* was used to produce 4-*O*-methylepelmycin D (4-*O*-methyl-ε-rhodomycinone-RN) in *S. violaceus* [28]. Failure to produce methylated aclacinomycin A or 1-deoxyobelmycin (a β-rhodomycinone glycoside) in the appropriate production strains may be due to the monoglycoside specificity of DnrK [15].

Removal of the 2-*O*-methyl moiety from steffimycin by introduction of the *S. nogalater* ketoreductase and aromatase in *S. steffisburgensis* has already been described [25]. Interestingly, this change was associated with two other alterations with respect to the parent compound; both the 8-*O*-methyl moiety and the 2′-methylation were missing. This suggests that the enzymes causing these modifications require the presence of the 2-O-methylation in their substrate.

In several publications only an aglycone was produced for various reasons. When the (9*S*) cyclase *snoaL* was introduced in the *S. peucetius* mutant M18, 9-*epi*-aklavinone was obtained [22]. The host is a ε-rhodomycinone producer and, although uncharacterized, likely to have a defect in amino sugar biosynthesis or glycosyl transferase (see argumentation above for *S. galilaeus* mutants). The lack of 11-hydroxylation, on the other hand, is likely to be due to stereospecificity of DnrF for the (9*R*) epimer, as observed for the homologous 11-hydroxylase RdmE [35].

Anthracyclines with 1-hydroxylation were obtained as aglycones after introduction of *snoaL2* [22] or *aclR* [8] in the *S. galilaeus* mutants H039 or H063. H039 is an aklavinone and neutral glycoside-producing strain; H063 is an aklavinone producer. Transformation of the *aclR* construct was also attempted into wild-type *S. galilaeus*, but the clones obtained either did not grow properly on media inducing anthracycline production, or lost the 1-hydroxylation (which can be detected on the basis of color). One possible explanation for this phenomenon is specificity of the self-resistance mechanism in the host; if it cannot provide protection from 1-hydroxylated aminoglycosides, they cannot be produced due to self-toxicity.

Gullon and coworkers [23] produced two steffimycin-related aglycones (Scheme 8).



Scheme 8 New steffimycin-type structures produced by Gullon and coworkers [23]

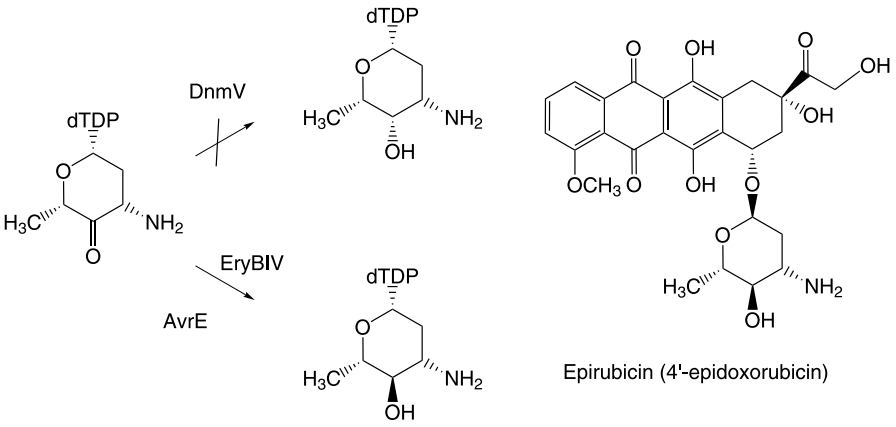
The first structure was generated by expressing in *S. albus* the genes required for the aglycone biosynthesis, except the 2-O-methylase; the second by disrupting the glycosyltransferase gene *stfG* in *S. steffisburgensis*. The lack of 8- and 10-modifications demonstrates that the tailoring enzymes introducing them require a glycosylated substrate.

3.1.2 Engineering Glycosylation

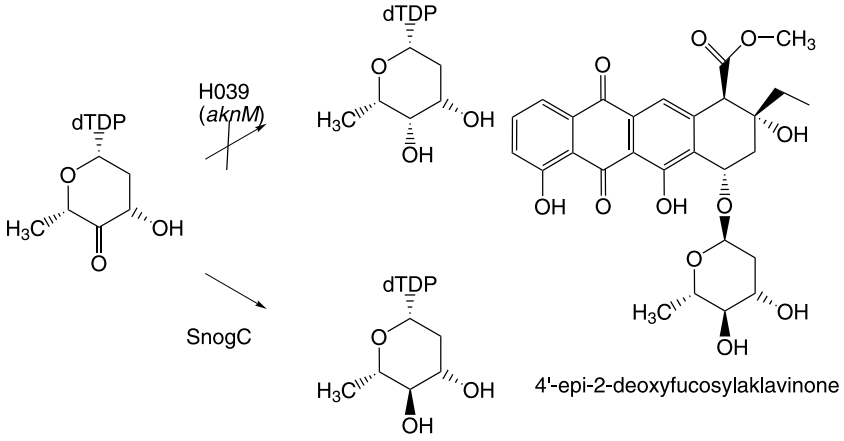
An instance of pathway manipulation with potentially high commercial significance was the production of epirubicin in *S. peucetius* [13]. The (4*S*)-TDP-4-keto-6-deoxyhexose reductase gene, *dnmV*, was disrupted by insertional inactivation, and the gene for a homologous reductase from erythromycin (EryBIV) or avermectin (AvrE) biosynthesis, with different product stereochemistry (4*R*), was introduced to the production strain (Scheme 9).

A similar reversal of stereochemistry [22] was achieved with the TDP-4-keto-6-deoxyhexose reductase gene from *S. nogalater*, *snogC*, which caused the 4′-ketoreductase mutant *S. galilaeus* H039 to produce an aklavinone glycoside with 4′-epi-2-deoxyfucose glycosylation (Scheme 10).

Novel glycosylation was produced in an interesting way using the biosynthetic genes of aranciamycins, which are essentially 2-demethoxysteffimycins [56]. A single cosmid clone containing apparently all the biosynthetic genes of aranciamycin caused the production of the aglycone in *S. albus*.



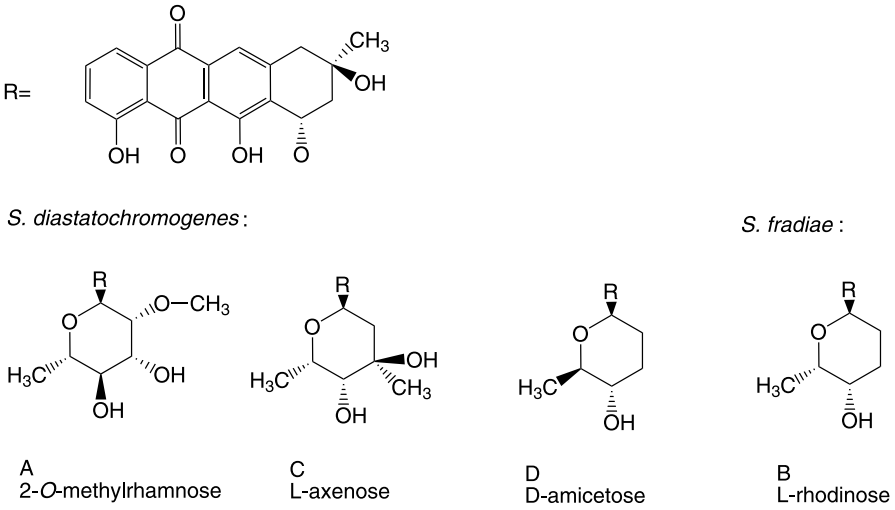
Scheme 9 Production of epirubicin by pathway engineering



Scheme 10 Alteration of sugar stereochemistry by pathway engineering

However, when the clone was transferred into *S. fradiae* A0 or *S. diastatochromogenes* Tü6028, new glycosides were produced (Scheme 11).

In the work described in this publication the whole aglycone biosynthetic cluster, including the glycosyltransferase, was used. Thus it was shown that the glycosyltransferase from aranciamycin biosynthesis was able to add various sugars available in the heterologous strains. Certain structural variations were seen: in the original aranciamycin molecule R^{10} = O and R^{8} = OCH_3, like in the steffimycin molecule. These post-glycosylation modifications were absent in the hybrid molecules.



Scheme 11 New aranciamycin-like glycosides produced with aranciamycin biosynthetic genes

3.2 Combinatorial Biosynthesis

Combinatorial biosynthesis "uses sets of genes from different biosynthetic pathways in different combinations to generate libraries of hybrid structures" [46]. In the field of anthracyclines, the research effort of the company Galilaeus OY (Finland) is perhaps the best example of such library construction [57, 58]. "Gene cassettes" are plasmids, in which biosynthetic genes are arranged as an artificial operon. A strong, constitutive promoter such as the *ermE* promoter [59] assures effective transcription of the genes. Between each gene unique restriction sites have been introduced; thus it is possible to remove each gene from the construct at will. The modification cassette of Galilaeus OY contained the nogalamycin polyketide synthase genes, which introduce $R^9 = CH_3$ and the modifying genes *rdmE*, *rdmC* and *rdmB*. With this cassette in the *S. galilaeus* mutant H075 described previously, producing RN-dF-dF glycosylation, a library of 60 compounds could be produced.

4 Biotransformation (Bioconversion) Experiments

Living organisms can take up externally supplied chemicals and metabolize them to other chemicals. In biotechnology, such biotransformation or bioconversion processes have long been used, e.g., for hydroxylation of steroids [60].

4.1 Blocked Mutants of Production Strains

It is possible to use mutants of antibiotic-producing microorganisms for bioconversion of related molecules. A blocked mutant is required, with a mutation in the gene of an enzyme acting early in the biosynthesis of the antibiotic. It is essential, however, that the expression of enzymes for the later biosynthetic steps is unaffected.

Oki et al. [43] isolated an aclacinomycin-nonproducing mutant of *S. galilaeus*, which was found to glycosylate added aklavinone to aclacinomycin A. Seventeen different aglycones were tested for glycosylation by this mutant, and nine of them yielded glycoside products (Table 3).

Table 3 allows some conclusions concerning the aglycone specificity of the glycosyl transferase(s). The enzymes appear to be insensitive to the 1- and 11-hydroxyls; on the other hand no aglycones with DoxA-modified R^9 have been hydroxylated. Considerable variation is tolerated at R^{10}. The non-glycosylation of 13-deoxydaunomycinone is surprising, because all of the substitutions distinguishing it from aklavinone – $R^4 = OCH_3$, R^{10} = H and R^{11} = OH – are individually tolerated.

In the work of Blumauerova and coworkers [42] glycosylation of daunomycinone was achieved with mutants of *S. coeruleorubidus*, a daunomycin producer, indicating different substrate specificity in this strain.

4.2 Bioconversion with Genetically Engineered Strains

Particularly in earlier work the functions of cloned genes have often been demonstrated by expressing them in a heterologous strain (frequently, the popular *Streptomyces* cloning host *S. lividans*), and observing the bioconversion reactions catalyzed. Thus, Dickens et al. [61] first identified DoxA by its ability to convert daunorubicin to doxorubicin in transgenic *S. lividans*. Subsequently, the functions of DauP and DauK, as well as the fact that DoxA actually catalyzes the three last reactions in doxorubicin biosynthesis, were demonstrated by bioconversion of rhodomycin D (ε-rhodomycinone-DN) to doxorubicin [14]. In a similar manner, the functions of RdmC and RdmB were confirmed by bioconversion [54]. Olano and coworkers [62] demonstrated that their recombinant DNA constructs contained all the genes necessary for dTDP-daunosamine biosynthesis and glycosyl transfer by bioconverting ε-rhodomycinone (Table 1) to rhodomycin D using *S. lividans*. Up to 85% bioconversion rates were obtained.

An interesting example of recent biotransformation experiments is the use of DoxA [33] to introduce a 10-hydroxyl substituent to the anthracycline core of desacetyladriamycin. In the examples above, no new products (i.e. never observed before) were produced, and the products could in principle be pro-

duced by pathway engineering. Desacetyladriamycin, on the other hand, is a semisynthetic product, and could not be produced by recombinant DNA methods alone (unless, of course, a hitherto-unknown deacetylating enzyme could be found).

5
Chemoenzymatic Synthesis

Antibiotic biosynthetic enzymes can be efficiently produced and purified utilizing recombinant DNA technology and affinity tags. In research applications, anthracycline compounds have been produced using pure enzymes and pure anthracyclines in vitro; for instance, for use as a ligand in crystallization [18], 11-deoxy-β-rhodomycin A was produced with pure RdmC and RdmB from commercial aclacinomycin A. Such chemoenzymatic synthesis is not necessarily cost-effective when a larger amount of the substance is needed. However, initial tests could be performed with chemoenzymatically synthesized compounds, before an effort was made to produce a substance by pathway engineering.

Chemoenzymatic synthesis can also be more efficient than bioconversion. When DoxA was used to produce 10-hydroxyldesacetyladriamycin [33], bioconversion only gave a 10% yield, whereas with pure enzyme complete conversion was achieved. However, to convert 1 mg of substrate, 3 mg of enzyme was used; such proportions are not feasible in industrial production scale.

6
Future Possibilities: Engineering of Biosynthetic Enzymes

What is clear from the above is that the low specificity of antibiotic biosynthetic enzymes does not mean that "anything goes". There are structural features by which the enzyme recognizes its substrate, and the enzyme does not tolerate changes in these. For instance, the apparent intolerance of DoxA to rhodosamine glycosylation [33] excludes a large group of anthracyclines from modification of the 9-side chain.

As more and more structures of antibiotic biosynthetic enzymes become available [63], engineering the specificity of the enzymes becomes a possibility. Site-directed mutagenesis experiments, made to verify the reaction mechanisms of enzymes, have already yielded mutants with potential for application.

Thus, an active site mutation removed from AknOx the ability to catalyze the second reaction in Scheme 6 [37]; if this mutation were to be introduced in the production strain in place of the wild-type gene, a strain producing only aclacinomycin A and not aclacinomycin Y should result.

7 Concluding Remarks

Biosynthetic modification of anthracycline biosynthesis has produced numerous new molecules, and its potential appears to be by no means exhausted. The research effort also reveals several instances of possible pitfalls, which may also be of interest when modifying other classes of antibiotic molecules:

Enzyme specificity. The substrate range of the introduced enzyme must include the intermediates available in the host; but also the altered molecule must be a substrate for the host's downstream biosynthetic enzymes (as illustrated by the steffimycin hybrids).

Self-resistance. When the structure of the antibiotic is changed, the self-resistance mechanism of the host may no longer be able to provide protection from the new molecule (e.g., 1-hydroxyl aminoglycosides).

Biosynthetic forks. Unless the enzyme for the corresponding step in the host is inactivated, competition for the substrate ensues between the introduced and native enzymes, leading to multiple products (e.g., *rdmE/C/B* hybrids).

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References

1. Arcamone F, Cassinelli G (1998) Curr Med Chem 5:391
2. Fujiwara A, Hoshino T (1986) Crit Rev Biotechnol 3:133
3. Bao W, Sheldon PJ, Wendt-Pienkowski E, Hutchinson CR (1999) J Bacteriol 181:4690
4. Tang Y, Koppisch AT, Khosla C (2004) Biochemistry 43:9546
5. Ylihonko K, Hakala J, Kunnari T, Mäntsälä P (1996) Microbiology 142:1965
6. Kunnari TJ, Ylihonko KP, Klika KD, Mäntsälä PI, Hakala JM (2000) J Org Chem 65:2851
7. Fujiwara A, Hoshino T, Tazoe M, Fujiwara M (1982) J Antibiot 35:164
8. Beinker P, Lohkamp B, Peltonen T, Niemi J, Mantsala P, Schneider G (2006) J Mol Biol 359:728
9. Lu W, Leimkuhler C, Oberthür M, Kahne D, Walsh CT (2004) Biochemistry 43:4548
10. Räty K, Hautala A, Torkkell S, Kantola J, Mäntsälä P, Hakala J, Ylihonko K (2002) Microbiology 148:3375
11. Leimkuhler C, Fridman M, Lupoli T, Walker S, Walsh CT, Kahne D (2007) J Am Chem Soc 129:10546
12. Lu W, Leimkuhler C, Gatto GJ, Kruger RG, Oberthür M, Kahne D, Walsh CT (2005) Chem Biol 12:527
13. Madduri K, Kennedy J, Rivola G, Inventi-Solari A, Filippini S, Zanuso G, Colombo AL, Gewain KM, Occi JL, MacNeil DJ, Hutchinson CR (1998) Nat Biotechnol 16:69
14. Dickens ML, Priestley ND, Strohl WR (1997) J Bacteriol 179:2641
15. Jansson A, Koskiniemi H, Mäntsälä P, Niemi J, Schneider GH (2004) J Biol Chem 279:41149

16. Lomovskaya N, Otten SL, Doi-Katayama Y, Fonstein L, Liu XC, Takatsu T, Inventi-Solari A, Filippini S, Torti F, Colombo AL, Hutchinson CR (1999) J Bacteriol 181:305
17. Grimm A, Madduri K, Ali A, Hutchinson CR (1994) Gene 151:1
18. Jansson A, Koskiniemi H, Erola A, Wang J, Mäntsälä P, Schneider G, Niemi J (2005) J Biol Chem 280:3636
19. Jansson A, Niemi J, Mäntsälä P, Schneider G (2003) J Biol Chem 278:39006
20. Niemi J, Wang Y, Airas K, Ylihonko K, Hakala J, Mäntsälä P (1999) Biochim Biophys Acta 1430:57
21. Torkkell S, Kunnari T, Palmu K, Hakala J, Mäntsälä P, Ylihonko K (2000) Antimicrob Agents Chemother 44:396
22. Torkkell S, Kunnari T, Palmu K, Mäntsälä P, Hakala J, Ylihonko K (2001) Mol Genet Genomics 266:276
23. Gullón S, Olano C, Abdelfattah MS, Braña AF, Rohr J, Mendéz C, Salas JA (2006) Appl Environ Microbiol 72:4172
24. Brockmann H (1963) Fortschr Chem Org Naturstoffe 21:121
25. Kunnari T, Tuikkanen J, Hautala A, Hakala J, Ylihonko K, Mantsala P (1997) J Antibiot 50:496
26. Connors NC, Strohl WR (1993) J Gen Microbiol 139:1353
27. Madduri K, Torti F, Colombo AL, Hutchinson CR (1993) J Bacteriol 175:3900
28. Miyamoto Y, Ohta S, Johdo O, Nagamatsu Y, Yoshimoto A (2000) J Antibiot 53:828
29. Brockmann H, Niemeyer J (1968) Chem Ber 101:1341
30. Yoshimoto A, Johdo O, Takatsuki Y, Ishikura T, Sawa T, Takeuchi T, Umezawa H (1984) J Antibiot 37:935
31. Käpylä J, Pentikäinen OT, Nyrönen T, Nissinen L, Lassander S, Jokinen J, Lahti M, Marjamäki A, Johnson MS, Heino J (2007) J Med Chem 50:2742
32. Walczak RJ, Dickens ML, Priestley ND, Strohl WR (1999) J Bacteriol 181:298
33. Walczak RJ, Hines JW, Strohl WR, Priestley ND (2001) Org Lett 3:2277
34. Sultana A, Kallio P, Jansson A, Wang J-S, Niemi J, Mäntsälä P, Schneider G (2004) EMBO J 23:1911
35. Kallio P, Sultana A, Niemi J, Mäntsälä P, Schneider G (2006) J Mol Biol 357:210
36. Wang Y, Niemi J, Mäntsälä P (2002) FEMS Microbiol Lett 208:117
37. Alexeev I, Sultana A, Mäntsälä P, Niemi J, Schneider G (2007) Proc Natl Acad Sci USA 104:6170
38. Hong YS, Hwang CK, Hong SK, Kim YH, Lee JJ (1994) J Bacteriol 176:7096
39. Filippini S, Solinas MM, Breme U, Schlüter MB, Gabellini D, Biamonti G, Colombo AL, Garofano L (1995) Microbiology 141:1007
40. Yoshimoto A, Ogasawara T, Kitamura I, Oki T, Inui T, Takeuchi T, Umezawa H (1979) J Antibiot 32:472
41. Takahashi Y, Naganawa H, Takeuchi T, Umezawa H, Komiyana T, Oki T, Iui T (1977) J Antibiot 30:622
42. Blumauerova M, Kralovcova E, Mateju J, Jizba J, Vanek Z (1979) Folia Microbiol 24:117
43. Oki T, Yoshimoto A, Matsuzawa Y, Takeuchi T, Umezawa H (1980) J Antibiot 33: 1331
44. Ylihonko K, Hakala J, Niemi J, Lundell J, Mäntsälä P (1994) Microbiology 140:1359
45. Hopwood DA, Chater KF (1980) Philos Trans R Soc Lond B Biol Sci 290:313
46. Floss HG (2006) J Biotechnol 124:242
47. Bartel PL, Zhu CB, Lampel JS, Dosch DC, Connors NC, Strohl WR, Beale JM Jr, Floss HG (1990) J Bacteriol 172:4816
48. Malpartida F, Hopwood DA (1984) Nature 309:462

49. Niemi J, Ylihonko K, Hakala J, Kopio A, Pärssinen A, Mäntsälä P (1994) Microbiology 140:1351
50. Kunnari T, Niemi J, Ylihonko K, Mäntsälä P, Hakala J (1997) Bioorg Med Chem Lett 7:725
51. Hwang CK, Kim HS, Hong YS, Kim YH, Hong SK, Kim SJ, Lee JJ (1995) Antimicrob Agents Chemother 39:1616
52. Kim HS, Hong YS, Kim YH, Yoo OJ, Lee JJ (1996) J Antibiot 49:355
53. Niemi J, Mäntsälä P (1995) J Bacteriol 177:2942
54. Wang Y, Niemi J, Airas K, Ylihonko K, Hakala J, Mäntsälä P (2000) Biochim Biophys Acta 1480:191
55. Johdo O, Watanabe Y, Ishikura T, Yoshimoto A, Naganawa H, Sawa T, Takeuchi T (1991) J Antibiot 44:1121
56. Luzhetskyy A, Mayer A, Hoffmann J, Pelzer S, Holzenkämper M, Schmitt B, Wohlert SE, Vente A, Bechthold A (2007) Chem Bio Chem 8:599
57. Kantola J, Kunnari T, Mäntsälä P, Ylihonko K (2003) Comb Chem High Throughput Screen 6:501
58. Ylihonko K, Hakala J, Kunnari T (2003) EU Patent 1 000 078 B1
59. Bibb MJ, Janssen GR, Ward JM (1985) Gene 38:215
60. Sedlaczek L (1988) Crit Rev Biotechnol 7:187
61. Dickens ML, Ye J, Strohl WR (1996) J Bacteriol 178:3389
62. Olano C, Lomovskaya N, Fonstein L, Roll JT, Hutchinson CR (1999) Chem Biol 6:845
63. Schneider G (2005) Curr Opin Struct Biol 15:629

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Anthracycline Biosynthesis: Genes, Enzymes and Mechanisms

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Abstract Anthracyclines are an important family of tetracyclic aromatic polyketides produced via a type II iterative polyketide synthase pathway. The polyaromatic aglycones are modified by a variety of tailoring enzymes, including glycosylation with deoxy- and aminodeoxysugars. Several of these compounds are presently in clinical use as anticancer drugs. During the last few years important advances in the molecular genetics, structural biology and enzymology of the biosynthetic pathways of anthracyclines have been made. Insights into the mechanistic features of the enzymes involved are expected to have implications for the production of novel compounds with improved therapeutic profiles by combinatorial biosynthesis.

Keywords Anthracycline · Biosynthesis · Enzymology · Polyketide · Streptomyces

Abbreviations

AAME	Aklanonic acid methyl ester
ACP	Acyl carrier protein
ARO	Aromatase
CLF	Chain length factor
CYC	Cyclase
dTDP	Thymidine diphosphate
KR	Ketoreductase
KS	Ketoacyl synthase
MCAT	Malonyl-CoA:ACP malonyltransferase
minPKS	Minimal polyketide synthase
NAME	Nogalonic acid methyl ester
PKS	Polyketide synthase
PLP	Pyridoxal-5-phosphate
SAM	*S*-Adenosyl-L-methionine

1 Introduction

The anthracycline family of polyketide antibiotics has been one of the most intensely studied classes of microbial natural products. Anthracyclines belong to the type II aromatic polyketides, which have been the subject of several recent reviews [1–7]. Their antimicrobial activity was observed as early as 1939 [8], but structures of the first anthracyclines, the rhodomycins, were elucidated only in 1950 [9]. The term anthracyclinones was introduced later to describe the "yellow-red or red, optically active dyes" that in chemical terms are derivatives of 7,8,9,10-tetrahydro-5,12-naphtacenoquinones [10]. The potent cytotoxic activity of the anthracycline cinerubicin was discovered in 1959 [11], but it was found to be too toxic for development as an antitumor agent. The most prominent candidate for clinical trials in the early days was daunorubicin [12, 13], which displayed excellent antitumor activity and it became the first anthracycline to be approved for clinical use. Daunorubicin was, however, soon discovered to be cardiotoxic [14], but the success of the compound greatly enhanced the interest of pharmaceutical companies in anthracyclines. Eventually, random mutagenesis of the daunorubicin-producing strain *S. peucetius* at Farmitalia using *N*-nitroso-*N*-methyl urethane yielded the novel compound doxorubicin [15] with improved pharmacological properties [16]. Doxorubicin was approved as an anticancer drug in 1974 and since then it has been one of the most widely used cancer chemoterapeutic agents worldwide.

Despite their usefulness, both daunorubicin and doxorubicin have several acute and long-term side effects. The most severe of these is cumulative cardiotoxicity [14], since it limits the dosage that can be prescribed. In addition, the emergence of multi-drug resistance in cancer cells upon long-term treatment poses serious problems in the clinic. Therefore it is not surpris-

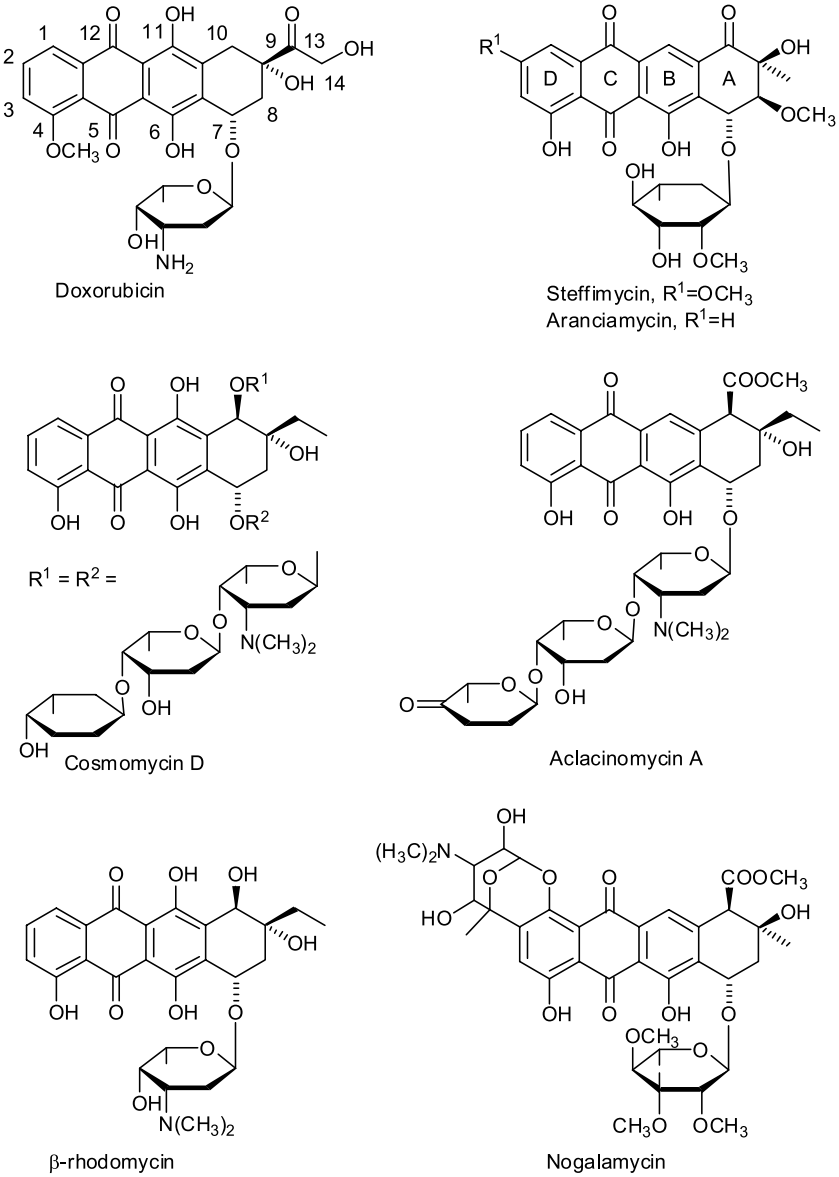
ing that even after the discovery of doxorubicin the search for anthracyclines with enhanced bioactive properties and reduced side effects continued in large screening programs, which led to the isolation of hundreds of naturally occurring compounds. Soil-dwelling Gram-positive actinobacteria, especially members of the genus *Streptomyces*, became the main source of novel anthracycline analogues. Chemical synthesis and semisynthetic approaches (discussed in more detail in Vol. 228 "Synthesis and Chemistry") were also adapted early on for generation of diversity among these molecules. These approaches have led to the isolation and synthesis of more than 2000 different anthracycline analogues. The successful advances were, however, hindered by the large costs of the numerous synthetic steps that were required due to the high complexity of the anthracycline molecules, including multiple chiral centres. More recently, the advent of recombinant DNA technology and the expanding molecular genetic toolbox for manipulation of *Streptomyces* has facilitated the study of the biosynthesis of these complex molecules. These methodological advances have led to significant progress in our understanding of how the diversity of anthracyclines is generated from common intermediates of cellular metabolism. One of the main incentives for these studies has been the prospect of generation of novel compounds via pathway engineering, which is the topic of Vol. 282: Biological Ocurrence and Biosynthesis; Niemi et al. "Biosynthetic Anthracycline Variants".

During the past decade, studies on the biosynthesis of anthracyclines and other aromatic polyketides have progressively expanded from molecular genetics towards mechanistic enzymology. This shift in focus of the research field has been partly due to the interest in the exciting chemistry that the biosynthetic enzymes are capable of catalysing, but also due to the existing problems with pathway engineering. Rational design rules for aromatic type II polyketides, which were devised in the 1990s [17, 18], have been hampered in many instances where promising gene combinations either have been non-functional [19, 20], resulted in unexpected alterations in the compounds produced [21] or, as in the case of type I polyketides, led to drastically decreased yields [22]. Therefore it has become evident that detailed knowledge of the biosynthetic enzymes is essential in order to understand the limitations and possibilities of the biosynthetic combinatorial approaches. The structure/function studies also open up other promising applications (like directed evolution and site-directed mutagenesis) for expansion of natural product diversity by changing the substrate specificities of the biosynthetic enzymes.

2
Structures of Anthracyclines

The structures of the anthracyclines, for which molecular genetic studies have been carried out and which are discussed in more detail in this chap-

ter, are presented in Scheme 1. All of the compounds are anthracyclinone glycosides composed of the characteristic tetracyclic 7,8,9,10-tetrahydro-5,12-naphtacenoquinone aglycone chromophore [10] and one or more deoxysugar moieties attached at C-7 via O-glycosylation. The diversity of these



Scheme 1 Structures of anthracyclines doxorubicin, nogalamycin, aclacinomycin A, β-rhodomycin, cosmomycin D, steffimycin and aranciamycin

compounds is generated by differences in the chemical modification of the aglycone moiety and the composition, number and point of attachment of additional deoxysugar residues.

Variations in the substitution profiles result from modifications of a few selected key positions (Scheme 1). The largest repertoire of substituents can be seen at C-9, where the side chains differ in length, possible modifications and stereochemistry. Aclacinomycin A, cosmomycin D and β-rhodomycin carry an ethyl moiety as substituent at carbon atom C-9 with 9*R* stereochemistry [9, 23, 24], whereas nogalamycin and steffimycin contain a methyl group. The stereochemistry of the carbon atom C-9 (9*S*) is also different in nogalamycin and steffimycin [25, 26]. Doxorubicin is the most modified compound at this position as it contains an ethyl side chain, which has an oxo moiety at C-13 and a hydroxyl group at C-14 [15]. Another highly variant position is C-10, which contains functional groups ranging from their total absence in doxorubicin to the carboxymethyl group seen in aclacinomycin and nogalamycin. Cosmomycin D and β-rhodomycin are hydroxylated at that position, whereas in steffimycin the group is further oxidized to a keto group. Other dissimilarities in the aglycone tailoring patterns include O-methylation of the hydroxyl group at C-4, as seen in doxorubicin, and C-11 hydroxylation in the cases of doxorubicin, cosmomycin and β-rhodomycin. In addition, nogalamycin biosynthesis proceeds via hydroxylation of C-1, and steffimycin has methoxy groups at the unusual positions C-2 and C-8. Aranciamycin is identical to steffimycin except that it lacks the methoxy group at C-2.

More structural diversity is generated by the various glycosylation patterns, which are imperative for the biological activity of anthracyclines. Doxorubicin, steffimycin, nogalamycin and β-rhodomycin contain single sugar residues at position 7, which are L-daunosamine, 2′-*O*-methyl-L-rhamnose, L-nogalose and L-rhodosamine, respectively. Aclacinomycin A has three sugar residues, L-rhodosamine, L-2-deoxyfucose and L-cinerulose, attached at the same position, whereas cosmomycin D contains two sets of matching trisaccharides at positions 7 and 10. These trisaccharide chains are identical to those of aclacinomycin N and similar to aclacinomycin A, except for the third deoxysugar, which is L-rhodinose in cosmomycin D. A unique feature in nogalamycin is the attachment of L-nogalamine; the deoxysugar residue is connected to the aglycone through two bonds, O-glycosylation at C-1 and via a carbon–carbon bond between C-2 and C-5′. All of the compounds, with the exception of steffimycin and the related aranciamycin, contain aminosugar residues.

3
Genetics of Anthracycline Biosynthesis

The genomes of *Streptomyces* bacteria are very large by bacterial standards. Pulsed-field gel electrophoresis studies have revealed that the genomes of

many *Streptomyces* species are over 8 Mb [27–29], almost twice the size of the *Escherichia coli* genome. There is also surprising variability between different species; the genomes of *S. clavurigerus* ATCC 27064 [29] and *S. ambofaciens* ATCC15154 [27] were found to be only 6.5 Mb in length, whereas the genome of *S. coelicolor* A3(2) is over 8.6 Mb [30]. Other unusual features are that the genomes of these bacteria have been shown to be linear [28], contain telomeres with terminal inverted repeats [29] and hold proteins covalently bound to the telomers [31]. The distribution of different types of genes revealed a central core of essential genes (cell division, DNA replication etc.) and a pair of chromosome arms coding for genes with non-essential functions (secondary metabolite biosynthesis, secreted hydrolytic enzymes etc.) [30]. The regions near the chromosome arms also contain many transposase genes, some of which were found adjacent to secondary metabolite clusters. This indicates that these genes could present a means for both horizontal transfer and internal rearrangement of secondary metabolite gene clusters, as well as being a model for the evolution of different biosynthetic pathways [32].

No genome sequences are currently available from anthracycline-producing *Streptomyces* species, but the genomes of both *S. coelicolor* and *S. avermitilis* were found to harbour over 20 gene clusters that are related to secondary metabolism [30, 32, 33]. The composition of the different kinds of gene clusters were highly dissimilar in the two strains, which in part explains the huge diversity and number of different bioactive compounds that have been isolated from *Streptomyces* species. Gene clusters for the biosynthesis of aromatic polyketides, to which anthracyclines are classified, were relatively scarce: the genome of *S. coelicolor* encoded genes for actinorhodin [34] and spore pigment synthesis [35], whereas the genome of *S. avermitilis* harbours two aromatic polyketide gene clusters of unknown function in addition to a spore pigment gene cluster [32]. However, the abundance of anthracycline biosynthesis genes could be seen in a DNA-fingerprinting study of 87 *Streptomyces* isolates, where 9% of the identified aromatic polyketide antibiotic gene clusters could be classified as anthracycline type [36].

Genes responsible for the biosynthesis of both the anthracycline aglycones and deoxysugar moieties have been found clustered on contiguous DNA fragments, which has tremendously facilitated the molecular genetic analyses. An exception to this rule is the steffimycin gene cluster, which does not harbour genes responsible for L-rhamnose biosynthesis [37]. The absence of L-rhamnose biosynthetic genes appears to be a common phenomenon in secondary metabolite gene clusters, possibly because of the general availability of this sugar that is also required for cell wall synthesis. In total, anthracycline biosynthetic genes have been cloned and sequenced to a varying extent from 11 different *Streptomyces* species. The biosynthesis of the clinical agents daunomycin and doxorubicin has been extensively studied in the daunomycin producers *S. peucetius* ATCC 29050 [38] and *Streptomyces* sp. C5 [39], from which the entire and about 65%, respectively, of the gene clusters have

been sequenced. Daunomycin biosynthesis genes have also been cloned from *S. griseus* IMET JA3933 [40]. Other compounds for which complete sequence data exist are nogalamycin produced by *S. nogalater* ATCC 21451 [41] and steffimycin produced by *S. steffisburgensis* NRRL 3193 [37], while the gene cluster for the steffimycin-related aranciamycin produced by *S. echinatus* has recently been partially sequenced [42]. The genes responsible for aclacinomycin production have also been characterized from two strains, *S. galilaeus* 3AR-33 [43] and *S. galilaeus* ATCC 31615 [44, 45], leading to the discovery of the majority of biosynthetic genes. Partial sequence data exist for rhodomycin biosynthesis from the producing strains *S. violaceus* SC-7 [46] and *S. purpurascens* ATCC 25489 [47], as well as for the related compound cosmomycin from *S. olindensis* DAUFPE 5622 [48].

The anthracycline biosynthesis gene clusters have been cloned using a variety of techniques, which have been compiled in Table 1. Most commonly, λ-phage gene libraries and heterologous hybridization probes derived from the actinorhodin pathway have been utilized. This approach was used in the isolation of the daunomycin gene cluster from *S. peucetius* C5 [39] and the aclacinomycin gene cluster from *S. galilaeus* 3AR-33 [49]. The doxorubicin biosynthetic genes were identified from a genomic cosmid library of *S. peucetius* ATCC 29050 using heterologous hybridization probes derived from actinorhodin (*actI*) and tetracenomycin PKS regions and selecting against daunomycin resistance of cloned fragments in *S. lividans* TK24 [50, 51]. The approach was followed by in vivo and in vitro functional studies of several genes and gene products, respectively, and by sequencing of the entire gene cluster spanning approximately 32 kbp. The polyketide synthase (PKS) regions of the nogalamycin and aclacinomycin gene cluster were located from partial plasmid gene libraries using the *act* probe, followed by cloning of larger segments from λ-phage gene libraries using the isolated homologous regions as probes [41, 45, 52, 53]. The nogalamycin gene cluster (34 kbp) was originally cloned in smaller segments in plasmids, but at present the whole gene cluster exists also in a single cosmid [53]. Heterologous hybridization probes derived from the aclacinomycin cluster were used in the characterization of the rhodomycin (13 kbp) gene cluster from *S. purpurascens* ATCC 25489 [52] λ-phage genomic library, whereas aclacinomycin deoxysugar genes (15 kbp) were located using a homologous hybridization probe generated by amplification of a part of the 4,6-dehydratase gene with degenerate primers [44]. More recently clusters of genes responsible for synthesis of steffimycin (25 kbp) and cosmomycin (14 kbp) have been isolated from cosmid genomic libraries using homologous hybridization probes [37, 48] whereas an *act* probe was used for cloning the aranciamycin (36 kbp) cluster [42]. These studies have produced a wealth of sequence information and led to the identification of a library of over 100 genes responsible for the biosynthesis of these metabolites. Table 2 summarizes the biosynthetic genes identified so far from these pathways. As can be seen from the table, the clus-

Table 1 Comparison of cloning methods of anthracycline biosynthesis gene clusters

Gene cluster	Strain	Genomic library	Hybridization probe		Refs.
			Heterologous	Homologous	
Aclacinomycin	*S. galilaeus* 3AR-33	λ-Phage library	*actI* and *actIII*		[43, 49]
Aclacinomycin deoxysugar	*S. galilaeus* ATCC 31615	λ-Phage library		4,6-Dehydratase PCR product	[44]
Aclacinomycin PKS region	*S. galilaeus* ATCC 31615	λ-phage library	*ACT*		[45, 52]
Aranciamycin	*S. echinatus*	Cosmid	*act*		[42]
Cosmomycin	*S. olindensis* DAUFPE 5622	Cosmid	*mtm*	Ketosynthase PCR product	[48]
Daunomycin	*S. peucetius* ATCC 29050	Cosmid	*act* and *tcm*		[50, 51]
Daunomycin	*S. peucetius* C5	λ-Phage library	*act*		[39]
Nogalamycin	*S. nogalater* ATCC 21451	λ-Phage library	*act*		[41, 53]
Rhodomycin	*S. purpurascens* ATCC 25489	λ-Phage library	*acm*		[52]
Steffimycin	*S. steffisburgensis* NRRL 3193	Cosmid		PKS region	[37]

act actinorhodin, *mtm* mithramycin, *tcm* tetracenomycin, *acm* aclacinomycin

ters contain many homologous genes that perform similar functions in these biosynthetic pathways.

4
Biosynthetic Enzymes

4.1
From Starter Units to Aklanonic Acid

The biosynthesis of the polyketide core is initiated by a sequence of reactions catalysed by the minimal PKS (minPKS), the enzyme complex that catalyses repeated Claisen condensations between acyl thioesters to build the initial polyketide chain [2, 3, 72–75]. The minPKS consists of four different subunits; an acyl carrier protein (ACP) carrying the growing polyketide chain, malonyl-CoA:ACP malonyltransferase (MCAT) as well as the heterodimeric core of the complex of the homologous ketosynthase (KS) and the chain length factor (CLF) subunits. While the KS subunits catalyse the chain initiation and chain elongation reactions, the CLF subunits play a decisive role in length of the polyketide chain [75, 76]. This enzyme complex is conserved in pathways leading to aromatic polyketides (Table 2) and shares common ancestry with bacterial fatty acid synthase machineries [77].

In the first step of the synthesis of the ketoacyl chain, ACP is malonylated, most likely by MCAT, whereby the malonate moiety is attached to ACP through the phosphopantetheine arm [78, 79]. The necessity for a separate MCAT in polyketide biosynthesis has been questioned since it was demonstrated that in vitro polyketide ACPs harbour surprising intrinsic self-acylation activity [80, 81], which fatty acid ACPs do not possess [82]. The significance of this activity in vivo at physiologically relevant concentrations of malonyl-CoA is, however, not clear.

Encounter of malonyl-ACP with the KS-CLF heterodimer leads to initiation of polyketide chain synthesis (Scheme 2, step **1**). Decarboxylation of the malonyl group followed by transfer of the resulting acetyl moiety to the active site of the KS subunit leads to priming of the synthase. Elongation of the polyketide chain by a number of extension cycles with malonyl units proceeds until the full-length poly-β-ketoacyl chain is synthesized. The catalytically active cysteine residue required for Claisen-type condensation reactions is conserved in the KS subunit, whereas in the CLF subunit the cysteine residue acting as a nucleophile is replaced by a glutamine side chain, rendering it incapable of catalysing polyketide synthesis. The crystal structure of the actinorhodin KS-CLF enzyme provided a number of novel insights into the enzymatic machinery of polyketide chain biosynthesis [83]. The putative active site pocket in CLF is filled by large side chains, making the potential nucleophile Ser347 inaccessible. This argues against an enzymatic function of

Table 2 Anthracycline biosynthesis genes thus far identified

Category	Gene function[a]	Anthracycline gene clusters									
		Dnr/Dau	Refs.	Acl/Akn	Refs.	Sno	Refs.	Rdm/Rho/Cos	Refs.	Stf/Ara	Refs.
Polyketide	**1** minPKS, KS	*dpsA*	[38, 39]	*aknB*	[45]	*snoa1*	[41]	*cosB*	[48]	*stfP/ara-orf11*	[37, 42]
	minPKS, AT	*dpsB*	[38, 39]	*aknC*	[45]	*snoa2*	[41]	*cosC*	[48]	*stfK/ara-orf10*	[37, 42]
	minPKS, ACP	*dpsG*	[38, 39]	*aknD*	[45]	*snoa3*	[41]			*stfS/ara-orf9*	[37, 42]
	Propionate starter unit, KSIII KSIII	*dpsC*	[38, 39]	*aknE2*	[45]			*cosE*	[48]		
	Propionate starter unit, AT	*dpsD*	[38, 39]	*aknF*	[45]			*cosF*	[48]		
	2 KR, keto-reductase	*dpsE*	[38, 39]	*aknA*	[45]	*snoaD*	[41]	*rdmJ* #		*ara-orf7*	[42]
	3 ARO, aromatase	*dpsF*	[38, 39]	*aknE1*	[45]	*snoaE*	[41]	*rdmK* #		*stfQ/ara-orf6*	[37, 42]
	4 CYC, cyclase	*dpsY*	[55]	*aknW*	[44]	*snoaM*	[41]			*stfY/ara-orf12*	[37, 42]
	5 OXY, oxygenase	*dnr/dauG*	[38, 39]	*aknX*	[43, 45]	*snoaB*	[41]	*cosX*	[48]	*stfOI/ara-orf16*	[37, 42]
Post-polyketide	**6** MET, methylase	*dnr/dauC*	[57, 58]	*aknG*	[45]	*snoaC*	[41]				
	7 CYC, cyclase	*dnr/dauD*	[57, 58]	*aknH*	[45, 70]	*snoaL*	[53]	*rdmA**	[47]		
	14 CYC, cyclase									*stfX/ara-orf8*	[37, 42]
	8 C-7 KR, keto-reductase	*dnr/dauE*	[38, 59]	*aknU*	[44]	*snoaF*	[53]	*rdmL**, #		*stfT/ara-orf23*	[37, 42]

Table 2 (continued)

Category	G	ene function[a]	Anthracycline gene clusters									
			Dnr/Dau	Refs.	Acl/Akn	Refs.	Sno	Refs.	Rdm/Rho/Cos	Refs.	Stf/Ara	Refs.
Aglycone modifications	**9**	C-11 hydroxylase	*dnr/dauF*	[60]					*rdmE*	[47]		
	11	EST, esterase	*dnr/dauP*	[57, 58, 61]					*rdmC*	[47]		
	12	MET, methylase	*dnr/dauK*	[61, 62]								
	13	P450 oxygenase	*doxA*	[54, 61]								
	15	O-Methyl-transferase									*stfMI,MII/ara-orf19*	[37, 42]
	16	C-10 oxidation, P450									*stfP1/ara-orf5*	[37, 42]
		C-10 oxidation, dehydrogenase									*stfE/ara-orf22*	[37, 42]
	17	C-8 hydroxylase									*stfOII*	[37]
	18	C-1 hydroxylase			*aclR*	[43]	*snoaL2*	[53]				
	19	C-10 hydroxylase							*rdmB*	[47]		
		13-Ketoreductase hydrodaunomycin	*dnr/dauU*	[54]								
Sugar biosynthesis or transfer	**10**	Glycosyltransferase	*dnmS, dnr/dauH*	[63, 64]	*aknS, K*	[44, 45]	*snogD, gE, gZ*	[53]	*rdmH* [#]*/rhoG/cosK,G*	[46, 48]	*stfG*	[37]
		P450-like, GTF activation	*dnmQ*	[64]	*aknT*	[44]	*snogN*	[53]	*rdmG* [#]*/rhoF/cosT*	[46, 48]	*stfPII*	[37]
	20	TDP-glucose-synthase	*dnmL*	[65]	*akn/aclY*	[43, 44]	*snogJ*	[53]				
	21	4,6-Dehydratase	*dnmM*	[65]	*aknR*	[44]	*snogK*	[53]	*rhoH/cosH*	[46, 48]		
	22	2,3-Dehydratase	*dnmT*	[63]	*aclN*	[43]	*snogH*	[53]				
	23	3-Ketoreductase			*aknQ*	[44]			*rdmF*	[47]		

Table 2 (continued)

Category	Gene function[a]		Anthracycline gene clusters							
		Dnr/Dau	Refs.	Acl/Akn	Refs.	Sno	Refs.	Rdm/Rho/Cos	Refs.	Stf/Ara	Refs.
	27 4-Ketoreductase	*dnmV*	[56]	*aknM*/aclM*	[45]	*snogG , gC*	[53]	*cosM*	[48]		
	25 3-Dehydratase			*aknP*	[44]			*rdmI* [#]			
	26 3,5-Epimerase	*dnmU*	[56]	*akn/aclL*	[43, 45]	*snogF*	[53]	*cosL*	[48]		
	24 Aminotransferase	*dnmJ*	[66]	*akn/aclZ*	[43, 44]	*snogI*	[53]				
	28 Aminomethylase			*aknX2, aclP*	[43, 44]	*snogA,gX*	[53]	*rdmD*	[47]		
	29 *C*-Methyltransferase					*snogG2*	[53]				
	O-Methyltransferase					*snogY,gL, gM*	[41, 53]			*stfMII/ ara-orf24*	[37, 42]
	31 Oxidoreductase			*aclO, aknOx*	[43, 71]						
Regulation		*dnr/dauI*	[66]	*aknO*	[44]					*stfRI/ ara-orf15*	[37, 42]
				akn/aclI	[43, 45]	*snorA*	[41]				
				aclS	[43]			*cosS*	[48]		
		dnr/dauO	[67]								
		dnr/dauN	[67]							*stfRII/ ara-orf13*	[37, 42]
										stfRIII	[37]
										ara-orf14	[42]
Resistance		*drrA*	[68]	*acrV*	[43]			*rhoI/cosI*	[46, 48]		
		drrB	[68]	*acrW*	[43]						
		drrC	[69]			*snorO*	[53]				
		drrD [#]									
										sfrA	[37]

Table 2 (continued)

Category	Gene function[a]	Anthracycline gene clusters									
		Dnr/Dau	Refs.	Acl/Akn	Refs.	Sno	Refs.	Rdm/Rho/Cos	Refs.	Stf/Ara	Refs.
										sfrB/ ara-orf20	[37, 42]
Unknown	Putative oxidoreductase	*dpsH (dnmW)*	[54, 59]	*aknV*	[44]	*snoO*	[53]				
	Putative hydroxylase			*aclQ*	[43]	*snoW*	[53]				

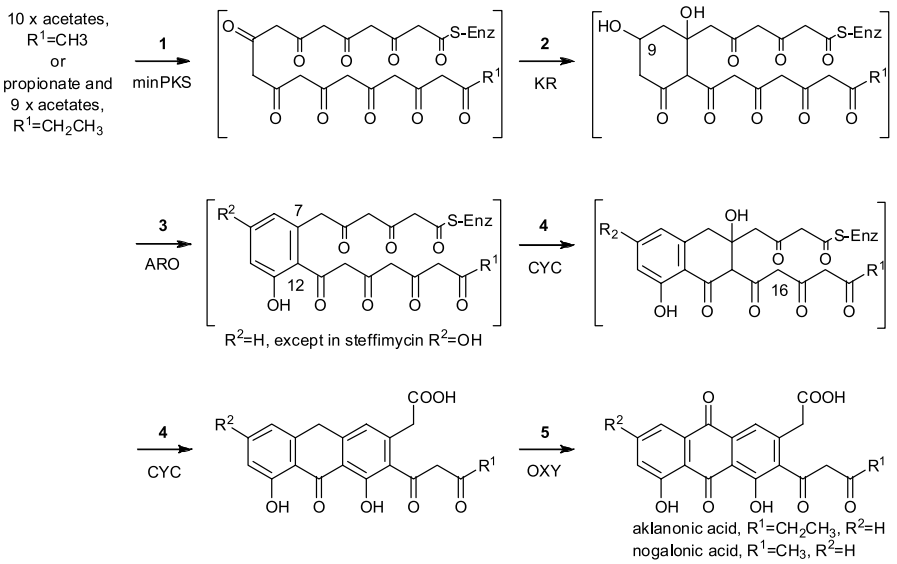
Homologous genes performing similar steps are presented in the same row

[a] Numbers in bold refer to Schemes 2 to 11 where the biosynthetic pathways are presented schematically

* Incomplete sequence

Unpublished results

Abbreviations used for genes derived from different gene clusters: *dau/dnr* daunorubicin, *sno* nogalamycin, *acl/akn* aclacinomycin, *rdm/rho* rhodomycin, *cos* cosmomycin, *stf* steffimycin, and *ara* aranciamycin. In addition, several genes have been discovered of unknown function for which no homologous equivalents have been found from other anthracycline gene clusters: *dnr/dauV* [54], *dnr/dauX* [55], *dnmZ* [56], *snoT, snoN, snoK* [53], *aknN* [44, 45], *aclJ* [43], *cosY* [48], *stfC/ara-orf17, stfD/ara-orf18, stfF, stfOII, ara-orf1, ara-orf2* and *ara-orf3* [37, 42]



Scheme 2 Model for polyketide assembly. Putative early biosynthetic steps to the first stable pathway intermediates, aklanonic acid and nogalonic acid. Structures within *brackets* indicate hypothetical intermediates. The numbering of the reaction steps corresponds to the numbering in Table 2, see text for discussion. Note that the carbon numbering before the polyketides are cyclized is different to the numbering of the final anthracycline end products

this subunit in catalysing chain initiation, as had been proposed earlier [84]. A narrow amphipathic tunnel at the dimer interface harbours the growing polyketide chain in an extended conformation and prevents aberrant cyclization events. The structure of the PKS core complex further suggests that the first cyclization of the polyketide occurs within this tunnel.

An intriguing feature of ACP is its obvious ability to interact with a variety of different, unrelated enzymes of this pathway. First, apo-ACP must be converted to the active form by attachment of the phosphopantetheine prosthetic group by a holo-ACP synthase [85, 86]. Second, a malonyl group has to be transferred from malonyl-CoA to ACP by MCAT. Finally, ACP must interact with the KS-CLF heterodimer and possibly also with other enzymes further down the pathway. The three-dimensional structures of ACPs from the frenolicin [87], oxytetracycline [88] and *act* [89] pathways have been determined by NMR, but no complexes of holo-ACP molecules with enzymes from polyketide biosynthetic pathways are known. Crystallographic studies of the complex of holo-ACP with holo-acyl carrier protein synthase involved in fatty acid biosynthesis have revealed the importance of helix II of ACP in protein–protein interactions [86]. Computational studies suggested that the same conserved recognition helix is involved in binding of holo-ACP by sev-

eral target enzymes [90], recently confirmed by the crystal structure of the yeast fatty acid synthase complex [91]. Whether or not this recognition helix is used also for interactions with the various ACP binding partners during polyketide biosynthesis remains, however, to be established.

The biosynthesis of anthracyclines that carry an ethyl side chain at C-9 initiates from a longer propionate starter unit. Functional studies with the daunorubicin gene clusters indicated that gene products DpsC and DpsD, which again have common ancestry with enzymes initiating fatty acid biosynthesis in bacteria, determine the use of alternative starter units [92, 93], although DpsD was shown to be nonessential for propionyl priming [93]. Similar studies from the aclacinomycin pathway have confirmed these observations [45]. DpsC is homologous to FabH ketosynthase III and condenses propionyl-CoA and malonyl-ACP to form a β-ketoester [94]. Crystal structures of priming ketosynthases from polyketide and fatty acid biosynthesis pathways have emphasized the importance in catalysis of a conserved active site cysteine residue, whose nucleophilicity is enhanced by a proximal histidine. The architecture of the acyl group binding pocket has been shown to determine substrate specificity of these enzymes [95–97]. The role of gene products homologous to acyltransferases, like DpsD, has been more difficult to define. Originally it was thought that these enzymes transfer the β-ketoester formed by the priming ketosynthases to the minPKS complex, leading to the incorporation of non-acetate starter units [94, 98]. A recent study from the R1128 pathway has surprisingly shown that the homologous ZhuC priming acyltransferase acts in effect as a thioesterase that selectively hydrolyses acetyl-ACPs, which led to the proposal that ZhuC functions as a gatekeeper enzyme that purges incorrect acetate primer units that would otherwise dominate polyketide chain initiation [99].

The end product of the minPKS complex is an exceedingly unstable poly-β-keto intermediate, which rapidly undergoes various cyclization events generating a variety of differently folded polyketides unless the next biosynthetic enzymes are present. It has been thought that many enzymes, like various cyclases (CYC) and aromatases (ARO), act in a concerted fashion guiding the intermediates to correct folding patterns, which eventually result in the formation of stable polyaromatic intermediates [1, 7]. The lack of stable substrates has severely complicated mechanistic in vitro structure/function studies, and hence very little is known about their biochemistry. Most of the acquired knowledge originates from molecular genetic work and deductions made from analysis of compounds produced by blocked mutants.

The majority of the anthracyclines belong to the class of reduced polyketides, meaning that after formation of the polyketide chain the keto group at C-9 (numbered from the enzyme-bound carboxy termini) is reduced after cyclization of the first ring between C-7 and C-12 by a ketoreductase (Scheme 2, step **2**). The ketoreduction occurs in all anthracycline biosynthetic pathways apart from steffimycin, where the lack of this enzymatic step leads

to a hydroxyl group at the C-2 carbon atom (Scheme 1) [37]. A gene encoding a ketoreductase (KR) was present in the aranciamycin cluster, explaining the slight difference seen in the end product to steffimycin [42]. Complementation studies utilizing KR-deficient mutants have mapped the reaction to gene products belonging to the short chain alcohol reductase/dehydrogenase superfamily of enzymes [41, 49, 100], which use NAD(P)H as a cofactor leading to reduction of the keto group and generation of a secondary alcohol in a stereospecic manner [101, 102]. The crystal structure of the actinorhodin KR revealed the typical fold of short-chain dehydrogenases and that the active site contains the characteristic catalytic Ser–Tyr–Lys triad (Fig. 1) [101, 102]. The correct regiochemistry of the first ring cyclization event is most likely determined by the combination of the minimal PKS complex and the KR; in the *act* KS-CLF structure a full length octaketide fits into the substrate binding tunnel only by bending in such a way that the C-7 is positioned adjacent to C-12, thus favouring C7–C12 cyclization, as observed in actinorhodin. A water molecule capable of donating a proton to the C-7 oxygen atom is suitably located in the crystal structure [83]. However, misfolded polyketide intermediates have been observed in the absence of downstream enzymes such as KR, indicating that the regiospecificity of the cyclization of the first ring is not only controlled by the components of the minPKS [103, 104].

Molecular genetic studies have indicated that the *dpsF* and *snoaE* gene products are responsible for first ring aromatization (Scheme 2, step **3**)

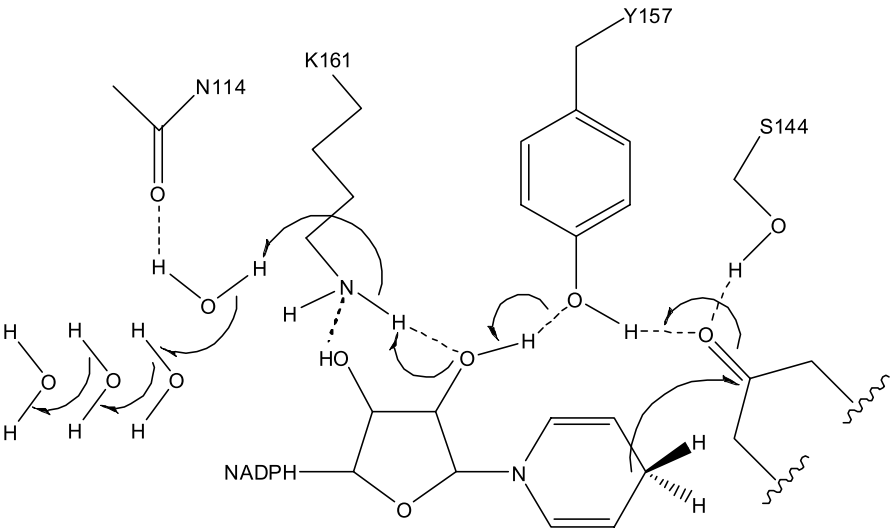


Fig. 1 Schematic view of the catalytic triad and the proton relay network involving several water molecules in the active site of actinorhodin ketoreductase, as proposed by Korman et al. [102]

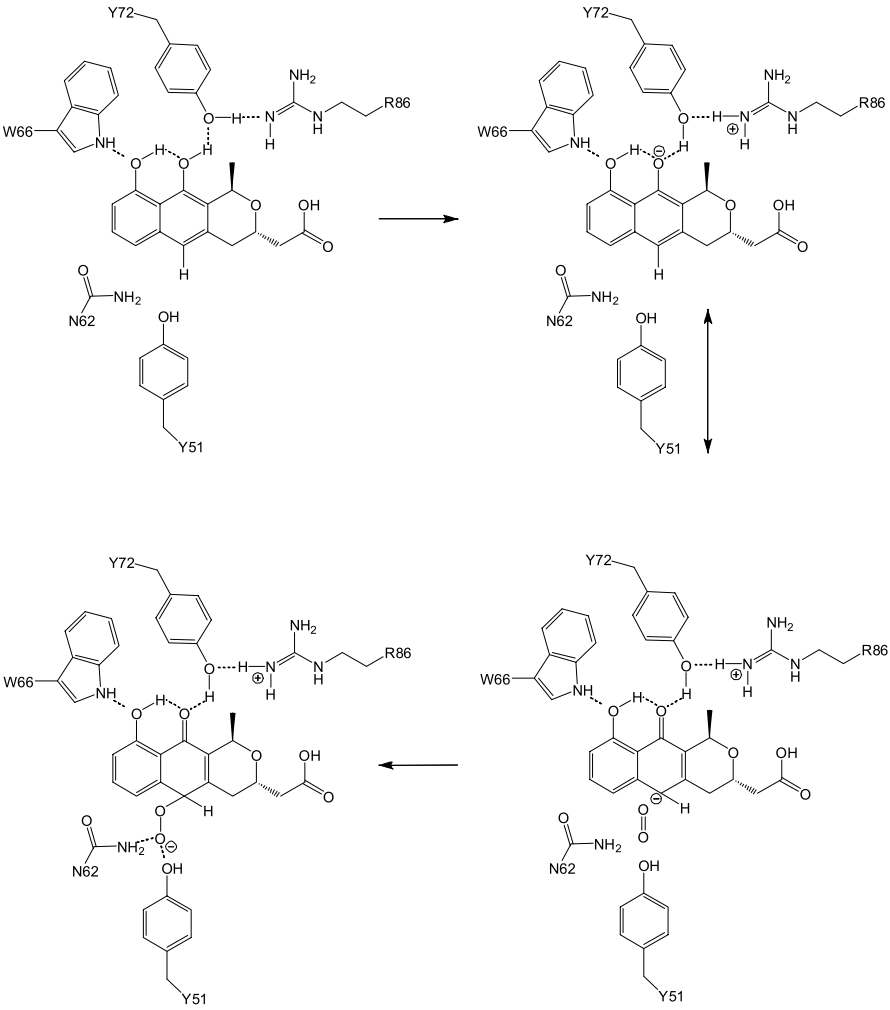


Fig. 2 Putative catalytic mechanism of ActVA-Orf6 as proposed by Sciara et al. [109] Proton transfer from the substrate to Tyr72 results in an anionic form, which reacts with molecular oxygen. The peroxy intermediate is stabilized by hydrogen bonds to Asn62 and Tyr51. The reaction is completed by protonation and cleavage of the peroxy intermediate. It is not quite clear, however, why Arg86 would be deprotonated at the beginning of the catalytic cycle

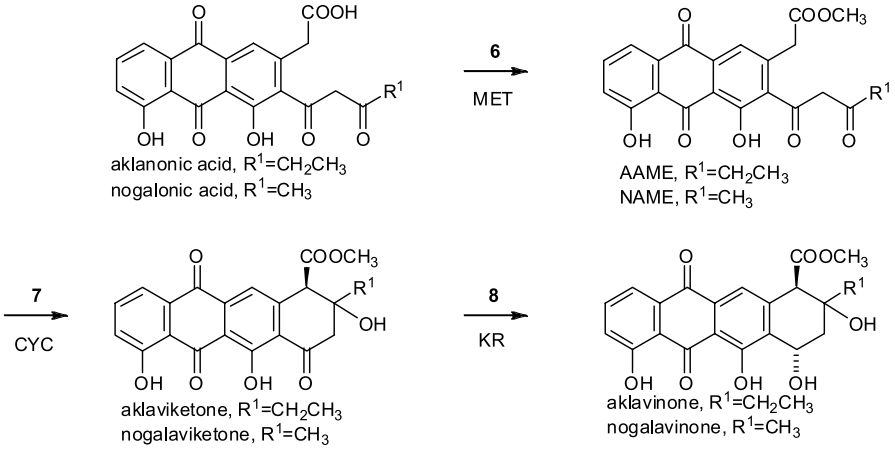
[38, 70, 105], followed by second and third ring cyclization steps (Scheme 2, step **4**). In anthracycline biosynthesis, genes responsible for these subsequent cyclizations have been mapped to *snoaM*, *dpsY* and their homologues [55, 106]. No intermediates with only two closed rings have been isolated, which indicates that these cyclases most likely catalyse formation of both the second and the third rings [106]. A common denominator of many

of these polyketide cyclases is a conserved motif HXGTHXDXPXH, which is hence thought to form a part of the active site, but no key residues required for catalysis have been identified so far. However, since the cyclization reactions can also occur non-enzymatically, it is conceivable that the enzymes simply act as "chaperones" and determine the regiospecificity of the reaction by the architecture of the active site cavities.

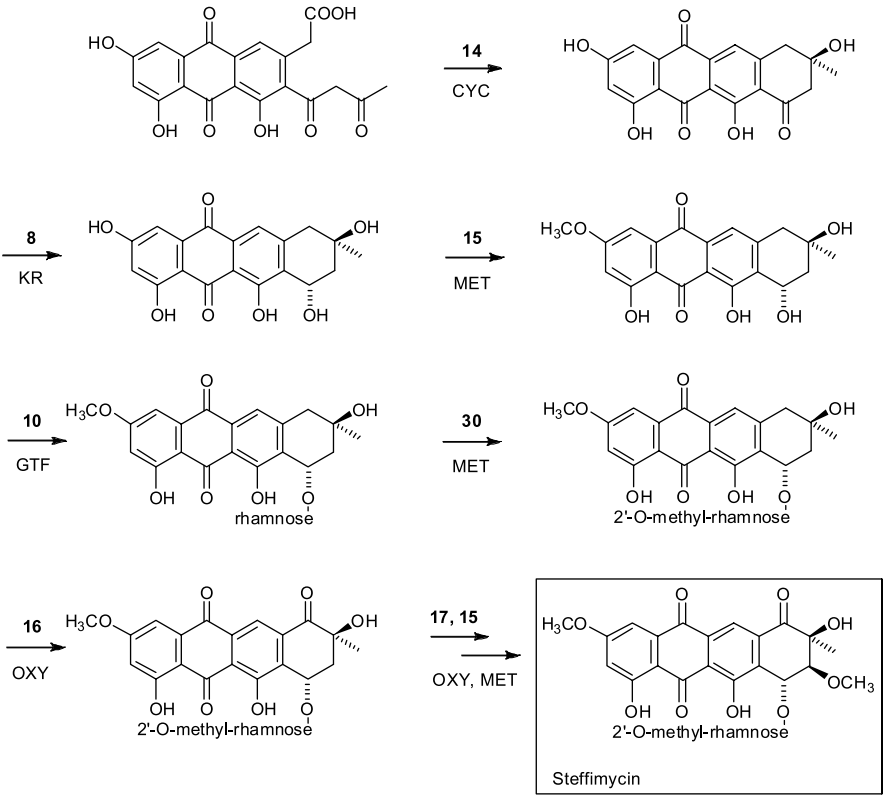
The final step leading to the formation of the first stable pathway intermediates, aclanonic acid and nogalonic acid, is oxidation at C-12 (Scheme 2, step **5**). Small monooxygenases like SnoaB and Dnr/DauG have been identified as catalysing this step in vivo [38, 39, 41]. The in vitro studies with the related enzymes AknX from the aclacinomycin pathway [43] and ActVA-orf6 from the *act* pathway [107] suggested that they do not require the assistance of any prosthetic group, metal ions or cofactors normally used for activation of molecular oxygen and that they are members of the structurally and mechanistically diverse family of cofactorless oxygenases [108]. The crystal structure of the first representative of this class of enzymes, ActVA-orf6, revealed a ferrodoxin-like fold not previously associated with oxygenase function [109]. A key feature of the reaction is the activation of oxygen by the substrate itself, in a mechanism reminiscent of the reaction of FAD/FMN with oxygen in many flavoenzymes (Fig. 2). Related mechanisms of oxygen activation have been identified for DpgC, a cofactorless dioxygenase in vancomycin biosynthesis [110], and for RdmB, a SAM-dependent hydroxylase in the biosynthesis of rhodomycin (see below) [111].

4.2 From Aklanonic Acid to Aglycones

After formation of nogalonic acid, the acetate-primed equivalent of aklanonic acid, the biosynthetic pathway of steffimycin deviates from that of other anthracyclines. Most pathways proceed through methylation of the carboxy group (Scheme 3, step **6**) and stereospecific cyclization (Scheme 3, step **7**), but in steffimycin biosynthesis nogalonic acid undergoes direct decarboxylative cyclization of the fourth ring (Scheme 4, step **14**) in a manner analogous to cyclization events in angucycline biosynthesis [19, 112]. Expression studies indicated that *stfX*, which does not contain any known conserved sequence motifs, is solely responsible for the generation of 2-*O*-demethyl-8-demethoxy-10-deoxysteffimycinone [37]. Curiously, genes similar to *stfX* (like *snoO* and *dpsH*) are present in all anthracycline gene clusters (Table 2), but the functional assignment of the corresponding proteins has been exceedingly elusive. Over the years, *dpsH* (the name *dnmW* is also in use) has been proposed to be involved in both cyclization [54] and deoxysugar biosynthesis [113], whereas *actVI-orfA* from the *act* pathway has been suggested to encode a protein stabilizing the multi-component PKS complex [114], although recently it was identified as a transcriptional regulator [115].



Scheme 3 Common biosynthetic steps from aklanonic acid and nogalonic acid to aklavinone and nogalavinone, respectively



Scheme 4 Proposed biosynthetic pathway for the formation of steffimycin, as presented by Gullón et al. [37]

On pathways leading to the common anthracycline intermediates aklavinone and nogalavinone, methylation of the carboxyl group of aklanonic acid has been demonstrated in bioconversion studies with *dnrC*, which is a S-adenosyl-L-methionine (SAM)-dependent methyl transferase, resulting in the formation of aklanonic acid methyl ester (Scheme 3, step **6**) [57, 58]. The enantiospecific cyclizations of the fourth ring are catalysed by a group of ester cyclases [58, 116–119] (like AknH and SnoaL) that determine the 9*R* configuration found in aclacinomycin and the 9*S* stereochemistry of nogalamycin, respectively (Scheme 3, step **7**). Crystal structures of SnoaL and AknH revealed a novel mechanism of enzymatic aldol condensation [118, 119], which does not employ Schiff-base formation or metal

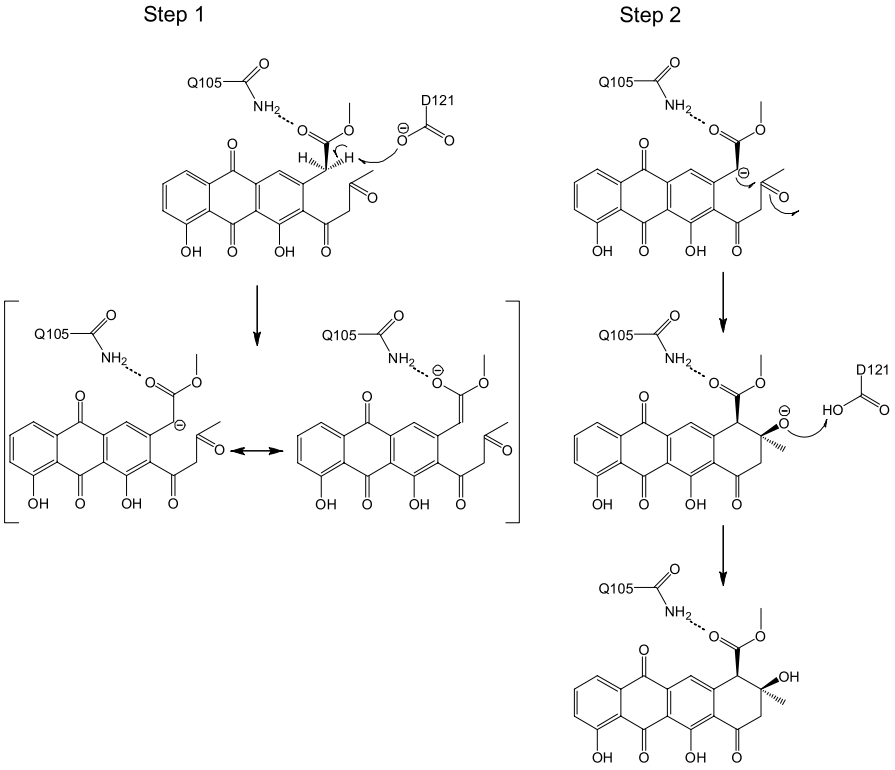
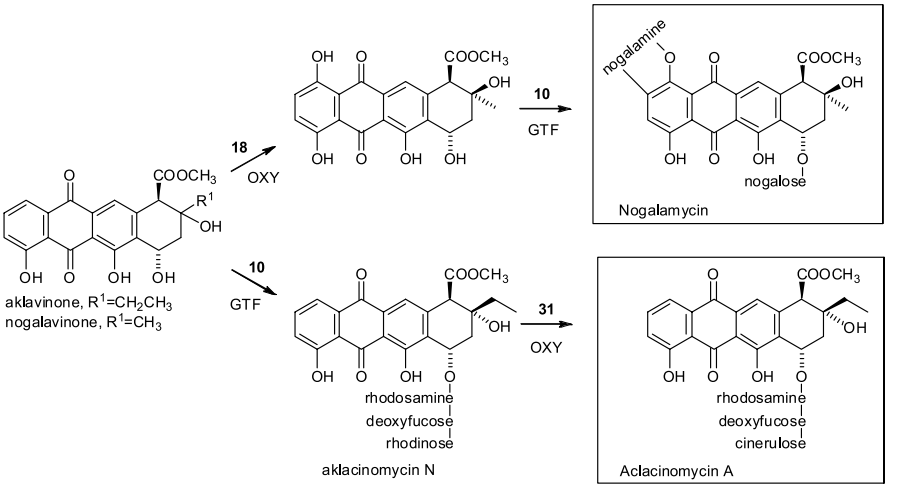


Fig. 3 Mechanistic proposal for the intramolecular aldol condensation catalysed by SnoaL. *Step 1*: Generation of the carbanion/enol(ate) intermediate by proton abstraction at C-10 by the conserved residue Asp121. The negative charge of the enolate is stabilized by resonance over the aromatic ring system of the polyketide. *Step 2*: The carbanion attacks the C-9 carbonyl carbon atom resulting in ring closure. The negative charge at the carbonyl carbon developing in the transition state is stabilized by a hydrogen bond to the side chain of Asp121. The same residue also transfers a proton to the oxygen atom at C-9 of the aglycone moiety

ions as in class I or class II aldolases, respectively (Fig. 3). Comparison of the crystal structures of SnoaL and AknH enabled the identification of two key residues that are involved in the determination of the 9*R* stereochemistry in AknH [119]. Finally, all anthracycline gene clusters encode enzymes for ketoreduction of C-7, which is an essential step before attachment of deoxysugar moieties is possible [59, 70]. The reduction of the C-7 keto group is carried out by an NAD(P)H dependent ketoreductase, for instance SnoaF/AknU/DnrE/DauE [38, 44, 53, 59] that produces the aklavinone/nogalamycinone intermediates with 7*S* configuration at carbon atom C-7 (Scheme 3, step **8**). Sequence analysis suggests that these enzymes belong to the family of short-chain dehydrogenases, although detailed structural and biochemical characterization is still missing.

Aklavinone and nogalavinone are key intermediates on the biosynthetic pathways, from which the aclacinomycin and nogalamycin pathways branch toward the end products. Aklavinone is the aglycone moiety of aclacinomycin and hence the biosynthesis continues with the attachment of deoxysugar moieties (Scheme 5, step **10**), whereas C-1 hydroxylation of nogalavinone results in the formation of the aglycone moiety of nogalamycin (Scheme 5, step **18**). The exact timing of the hydroxylation reaction in the pathway is uncertain, but in vivo studies identified *snoaL2* as a necessary (and sufficient) gene for formation of 1-hydroxylated products [53]. The determination of crystal structures of SnoaL2 and AclR, which is responsible for generation of 1-hydroxyaclacinomycins, revealed that the fold of the two enzymes is similar to that of the polyketide cyclases SnoaL and AknH, as predicted by the moderate degree of sequence similarity between these enzymes [120]. The



Scheme 5 Overview of the reactions leading to the anthracycline end-products nogalamycin and aclacinomycin A from nogalavinone and aklavinone, respectively

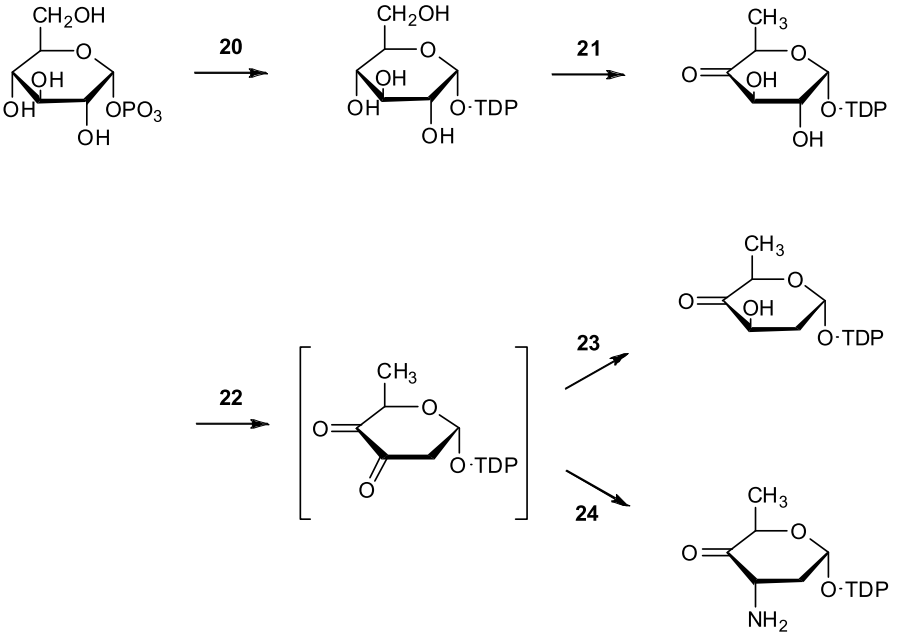
lack of functional and structural similarities to flavin or metal-dependent hydroxylases indicates that SnoaL2 and AclR might be other examples of cofactorless oxygenases, like AknX and ActVI-orf6. Enzymatic activity could not be detected in vitro using nogalonic acid methyl ester or nogalaviketone as substrates, suggesting that the 1-hydroxylation reaction might actually occur earlier in the biosynthetic pathway, possibly even before formation of the polyaromatic carbon skeletons [120].

4.3 From Glucose to Deoxysugars

The biochemical analysis of the underlying enzymatic steps leading to the biosynthesis of the deoxysugar moieties attached to many natural products has been hampered by the difficulties in obtaining activated carbohydrate substrates for enzymatic studies. The limited availability of these substrates is due to the complicated chemical synthesis of these multi-chiral compounds and the inherent instability of many of the pathway intermediates. Nevertheless, during the last decade considerable progress has been made in this field [121, 122]. For instance, the complete pathways for dTDP-L-epivancosamine [123] and dTDP-L-mycarose [124] biosynthesis have been reconstructed in vitro by the use of six and seven enzymes and the common pathway intermediates dTDP-D-glucose and glucose-1-phosphate, respectively. Crystallographic studies of some of the enzymes from these pathways [125, 126] as well as from the related L-rhamnose pathway [127–130] have provided detailed mechanistic insights into the structural basis of substrate recognition and catalysis. So far, most of the data for the biosynthesis of deoxysugars specific for anthracyclines are derived from molecular genetic complementation studies.

4.3.1 Early Common Steps from Glucose-1-phosphate to 2-Deoxysugars

The biosynthesis of the deoxysugar compounds is initiated by the activation of glucose-1-phosphate to dTDP-D-glucose (Scheme 6, step **20**), followed by conversion to dTDP-4-keto-6-deoxy-D-glucose (Scheme 6, step **21**), reactions that are common in all antibiotic 6-deoxyhexose pathways [131, 132] and the formation of the important cell wall component L-rhamnose in prokaryotes [133]. The thymidyl transfer reaction has been studied extensively in L-rhamnose pathways in several bacterial species, and kinetic and structural data suggest an ordered sequential reaction mechanism [127, 128]. The conversion of dTDP-glucose to dTDP-4-keto-6-deoxy-D-glucose is catalysed by 4,6-dehydratase, a member of the short-chain-dehydrogenase family. These enzymes are involved in the biosynthetic pathways of deoxysugars that decorate the polyketide core of anthracyclines and macrolide



Scheme 6 Putative early common steps in the formation of deoxysugar and aminodeoxysugars in anthracycline biosynthesis. Glucose-3-phosphate is converted to dTDP-4-keto-2,6-deoxy-D-glucose and dTDP-3-amino-2,3,6-deoxy-4-keto-D-glucose via an instable 3,4-diketointermediate

antibiotics. The structure of the 4,6-dehydratase from *Streptomyces venezuelae* has been determined to high resolution [125], and mechanistic conclusions are in line with previous proposals derived from structures of the homologous dTDP-glucose 4,6-dehydratases from *Salmonella enterica* and *Streptococcus suis* [129]. Catalysis by these enzymes involves a complicated set of proton transfer steps from and to the substrate, elimination of the C-6 hydroxyl group, and hydride transfer from the substrate to NAD^+ and back (Fig. 4).

From the common dTDP-4-keto-6-deoxy-D-glucose intermediate, different combinations of reactions such as elimination, reduction, isomerization, epimerization, amino- and methyl group transfer yield the various 2,6-dideoxy- and 2,3,6-trideoxyhexoses seen in anthracyclines [131, 132]. The next step on pathways towards several deoxysugars found in anthracyclines is removal of the C-2 hydroxyl group, resulting in the formation of an unstable 3,4-diketo intermediate (Scheme 6, step **22**). This step has been extensively studied using the 2,3 -dehydratases TylX3 [134], Gra Orf27 and Tü99 Orf10 [135], respectively, from the tylosin, granaticin and oleandomycin pathways. The notable instability of the 3,4-diketo intermediate suggests that the 2,3-dehydratases must act in concert with the

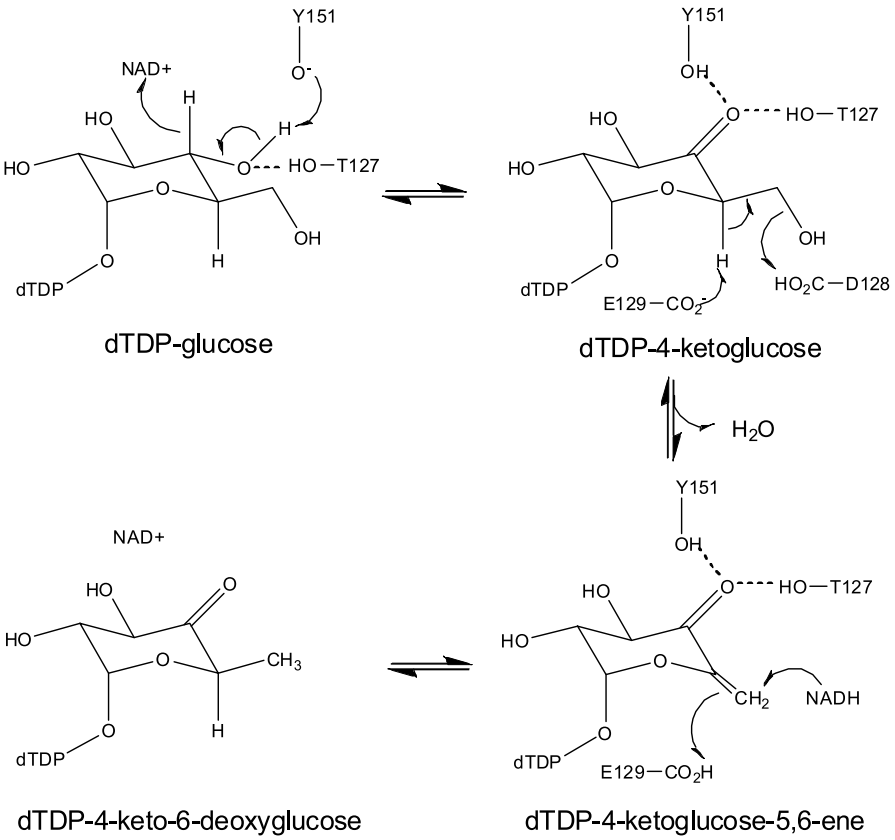
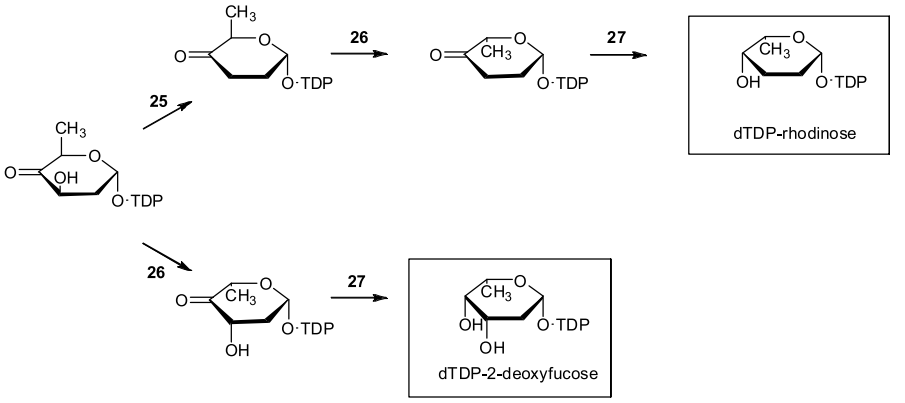


Fig. 4 Reaction mechanism of dTDP-glucose 4,6-dehydratase as proposed by Allard et al. [125]. The first half of the reaction involves abstraction of a proton from the 4′ hydroxyl group, and hydride transfer from C-4 to NAD^+. In the next step, a proton is removed from the C-5 atom of the sugar, and the C-6 hydroxyl group is protonated and eliminated as water. This yields the 4-keto-5,6-ene intermediate. The product is obtained after hydride transfer from NADH to carbon atom C-6 and protonation of carbon atom C-5

next biosynthetic enzymes. On pathways leading to dTDP-L-rhodinose and dTDP-L-2-deoxyfucose, the reaction proceeds by 3-ketoreduction (Scheme 6, step **23**) as has been demonstrated for the homologous enzymes TylC1 [134], Gra Orf 26 and Tü99 Orf11 [135]. In dTDP-L-daunosamine and dTDP-L-rhodosamine biosynthesis, the unstable diketo-sugar is converted into a more stable amino derivative (Scheme 6, step **24**). EvaB from the dTDP-L-epivancosamine pathway, which is homologous to the putative aminotransferases from the anthracycline pathways (Table 2), has been shown to catalyse the transamination reaction in a coupled reaction with EvaA, the 2,3-dehydratase, in the presence of pyridoxal-5-phosphate (PLP) and L-glutamate as a cosubstrate [123].

4.3.2
Pathways Leading to dTDP-L-Rhodinose and dTDP-L-2-Deoxyfucose

On the pathway towards the trideoxysugar dTDP-L-rhodinose, the next biosynthetic step is elimination of the C-3 hydroxyl group, which is mechanistically more complex than C-2 hydroxyl removal due to the close proximity of the 4-keto group (Scheme 7, step **25**) [132]. Complementation studies of blocked pathway mutants have indicated that the gene product of *aknP* functions in this part of the pathway in aclacinomycin biosynthesis [45]. This enzyme is homologous to the [2Fe-2S] iron sulphur cluster containing enzyme SpnQ from the spinosyn pathway in D-forosamine biosynthesis, which removes the C-3 hydroxyl group of a 2,6-dideoxysugar using pyridoxamine-5-phosphate as cofactor and external electron donors like ferredoxin/ferredoxin reductase in a complex redox reaction cascade [136]. Details of the subsequent C-5 epimerization step have been uncovered by mechanistic studies from related pathways, especially from L-rhamnose biosynthesis, which have further highlighted the importance of the 4-keto group. Formation of an enolate anion intermediate at C-4 facilitates proton abstraction and epimerization of either C-3 and C-5, like in the case of the C-5 epimerase EvaD [126], or of both as in L-rhamnose biosynthesis by the 3,5-epimerase RmlC [130, 137]. In the dTDP-L-rhodinose pathway, the epimerization is most likely catalysed by AknL [45] (Scheme 7, step **26**). The final step on this pathway is stereospecific reduction of C-4 by AknM using NADPH as the reducing agent [45] (Scheme 7, step **27**).



Scheme 7 Proposed biosynthetic pathways of the aclacinomycin deoxysugars dTDP-rhodinose and dTDP-2-deoxyfucose

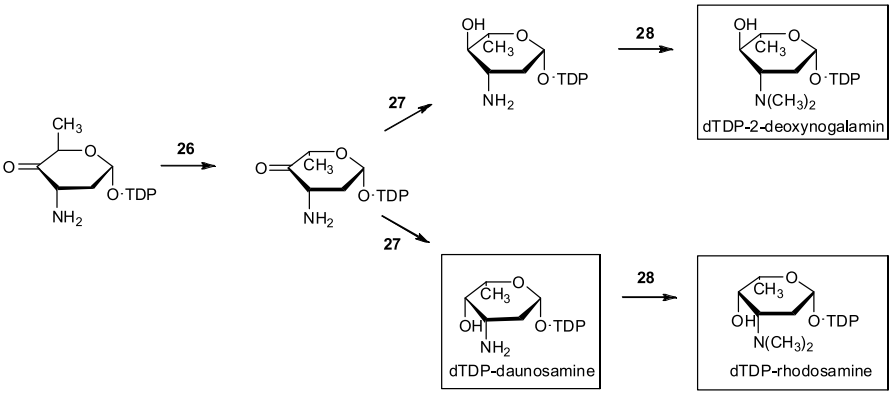
Curiously, *aknL* and *aknM* are the only genes found from the aclacinomycin gene cluster that bear resemblance to 3,5-epimerases and 4-ketoreductases, respectively, and as such they could formally be involved in the biosynthesis of all

carbohydrate residues of aclacinomycin. This would require broad substrate specificity since, for instance, the 4-ketoreduction is likely to be the final step on all of the pathways leading to the various sugars. Indeed, mutation of a single amino acid in the active site of the 5-epimerase EvaD resulted in an increase in 3,5-epimerase activity, demonstrating the delicate balance of factors that determine substrate specificity in these enzymes [126]. Alternatively, the missing deoxysugar biosynthesis genes might reside in the unsequenced region of the aclacinomycin gene cluster and are yet to be discovered.

The biosynthesis of dTDP-L-2-deoxyfucose is highly similar to that of dTDP-L-rhodinose. Since C-3 deoxygenation is not necessary on this pathway, direct 3,5-epimerization (Scheme 7, step **26**) and 4-ketoreduction (Scheme 7, step **27**) of the common dTDP-4-keto-2,6-deoxyglucose intermediate are sufficient for formation of dTDP-L-2-deoxyfucose [45]. If AknL and AknM catalyse these steps, the difference in substrates compared to the dTDP-L-rhodinose pathway would hence be in the retained 3-hydroxy group.

4.3.3 Formation of dTDP-L-Daunosamine and dTDP-L-Rhodosamine

Gene inactivation studies in *S. peucetius* have identified the involvement of *dnmJ, dnmU* and *dnmV* in the biosynthesis of dTDP-L-daunosamine [56, 66]. Based on sequence similarities to known proteins, and in particular to the enzymes found in the dTDP-L-epivancosamine pathway, it is possible to deduce the functions and reaction order of these gene products [123]. After formation of the labile 3,4-diketone intermediate, DnmJ directs biosynthesis towards aminosugars by regio- and stereospecific transamination to produce a 2,3,6-trideoxy skeleton with a C-3 amino substituent [66] (Scheme 6, step **24**). The biosynthesis is completed by DnmU-catalysed 3,5-epimerization



Scheme 8 Biosynthetic steps towards the formation of anthracycline aminosugars dTDP-2-deoxynogalamin, dTDP-daunosamine and dTDP-rhodosamine

(Scheme 8, step **26**) and reduction of the C-4 keto group (Scheme 8, step **27**, lower arrow) by DnmV in a manner analogous to dTDP-L-rhodinose and dTDP-L-2-deoxyfucose biosynthesis [56].

The biosynthetic pathway of the third carbohydrate moiety of aclacinomycin, dTDP-L-rhodosamine, is very similar to the pathway proposed for dTDP-L-daunosamine. The gene *aknZ* has been identified as the transaminase in complementation studies [45], whereas the gene products AknL and AknM, as discussed above, might be responsible for the 3,5-epimerization and 4-ketoreduction steps. The final step in dTDP-L-rhodosamine biosynthesis is dimethylation of the 3-amino group (Scheme 8, step **28**). This is most likely catalysed by AknX2/RdmD, which have significant sequence similarity to DesVI [138], an N-methyltransferase from the dTDP-L-desosamine pathway, and TylM1 from the dTDP-L-mycaminose pathway [139], which have been shown to sequentially attach methyl groups to aminosugars in a metal ion independent manner [139].

4.3.4 Biosynthesis of dTDP-L-Nogalose and dTDP-L-2-Deoxynogalamine

Some unresolved issues remain concerning the biosynthesis of the deoxysugar moieties of nogalamycin. After formation of the common pathway intermediate dTDP-4-keto-6-deoxy-D-glucose, nogalose biosynthesis requires C-3 methylation (Scheme 9, step **29**) in addition to epimerization (Scheme 9, step **26**) and 4-ketoreduction (Scheme 9, step **27**). The C-methylation putatively catalysed by *snogG2* is likely to lead to inversion of the configuration of C-3, as demonstrated with EvaC from the dTDP-L-epivancosamine pathway [123]. This suggests that the epimerase *snogF* possibly catalyses 5-epimerization and not 3,5-epimerization as has been proposed [53]. The gene *snogC* has been identified as the 4-ketoreductase in nogalose biosynthesis [53].



Scheme 9 Possible biosynthetic pathway of dTDP-nogalose as proposed by Torkkell et al. [53]. The pathway deviates from deoxysugar biosynthetic routes after formation of the common intermediate dTDP-4-keto-6-deoxy-D-glucose

The nogalamycin gene cluster harbours only one epimerase gene *snogF* [140], although the biosynthesis of both dTDP-L-nogalose and dTDP-L-nogalamine require this enzymatic activity. This indicates that SnogF either accepts different intermediates from the pathways or that the 5-epimerization proceeds unconventionally early using a common substrate. More experi-

mental work is also required for clarification of the strereochemistry of nogalamine biosynthesis. It has been proposed that the final intermediate before attachment to the aglycone is the 3-epimer of dTDP-rhodosamine [53]. In the final product nogalamycin, however, the 3-demethylamino group has the same configuration as in rhodosamine, whereas the 4-hydroxyl has the opposite stereochemistry. The gene *snogG* has been identified as the 4-ketoreductase in nogalamine biosynthesis [140] (Scheme 8, step **27**).

The nogalamycin cluster contains six putative methyl transferase genes, of which three (*snogY, snogL* and *snogM*) are suggested to be responsible for O-methylation of nogalose (Table 2, step **30**), whereas two genes (*snogA* and *snogX*) are homologous to aminosugar N-methylases [53] (Scheme 8, step **28**). It is unclear whether these methyl transfer reactions take place before or after attachment of the carbohydrate moieties to the aglocone.

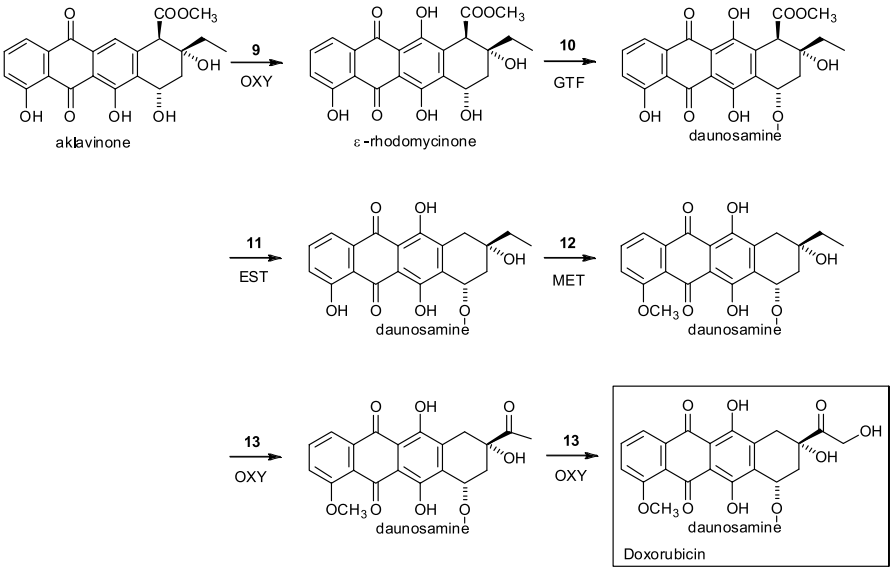
4.4 Tailoring Reactions

The polyketide scaffold is chemically modified by the action of a number of tailoring enzymes that will give rise to the broad diversity of the anthracyclines. Most of the tailoring reactions occur after the aromatic polyketide core has been synthesized, but in several cases tailoring reactions can occur in the earlier stages of polyketide biosynthesis. For instance, the enzymes SnoaB [41] and AknX [43] from the nogalamycin and aclacinomycin biosynthetic pathways catalyse the oxidation of ring C before the formation of the tetracyclic ring system is completed. These enzymes show sequence homology to the well-characterized cofactorless monooxygenase Act-Orf6 from the biosynthesis pathway of actinorhodin (see above) [109]. Other examples are the methyltransferases SnoaC/AknG/DnrC/DauC [41, 45, 57, 58] that convert the tricyclic nogalonic and aklanonic acids to their corresponding methyl esters, which is the prerequisite for the closure of the fourth ring by the cyclases SnoaL/AknH/DauD/DnrD [116–119].

4.4.1 Glycosylation

The arguably most important chemical modification of the aglycone core is glycosylation at one or several positions (Scheme 1), because it is required for the antimicrobial and antitumor activity of the anthracyclines [141]. The carbohydrate moieties are usually attached at position C-7 and/or C-10 of the aglycone core by a variety of glycosyltransferases. An interesting feature in nogalamycin biosynthesis is the attachment of the nogalamine sugar residue, since it is connected to the aglycone from two carbons; C-1′ of nogalamine is attached to the oxygen at C-1 and C-5′ is connected to C-2 by a carbon–carbon bond (Scheme 1).

Molecular genetic studies have been conducted to elucidate the functions of the two glycosyltransferases found from the daunomycin gene cluster. Heterologous expression studies in *S. lividans* identified *dnsS* as the gene responsible for attachment of daunosamine to ε-rhodomycin (Scheme 10, step **10**) [113], while *dau/dnrH* is postulated to participate in baumycin biosynthesis, since inactivation of this gene resulted in 8.5-fold increase in daunorubin production [63]. Biosynthetic studies from the cosmomycin pathway suggested that dTDP-L-rhodosamine is attached sequentially to the hydroxyl groups at C-7 and C-10 by *cosG*, followed by transfer of dTDP-L-2-deoxyfucose to both chains as second carbohydrate moieties by *cosK* [48].



Scheme 10 Late tailoring steps proposed for the conversion of aklavinone to doxorubicin

Sequence comparisons have shown that most glycosyltransferases from the biosynthetic pathways of aromatic polyketides belong to the GT-1 family as defined by the CAZy classification system [142]. Difficulties in obtaining soluble, active enzymes and limited availability of the NDP-deoxysugar substrates have hampered biochemical and structural analysis of glycosyltransferases from anthracycline biosynthetic pathways. First biochemical studies have been carried out with glycosyltransferases AknK [143] and AknS [144] from the biosynthetic pathway of aclacinomycin (Table 2). AknS transfers the first deoxysugar, L-rhodosamine to the C-7 hydroxyl group of the aclacinomycin aglycone. In studies using recombinant AknS, the enzyme exhibited rather low activity towards its substrates aklavinone and dTDP-rhodosamine, but the activity was surprisingly increased 200-fold by the addition of AknT [145]. The mechanism of rate enhancement by AknT has been

proposed to derive from interactions with AknS that lead to stabilization of the transition state. Furthermore, it has been shown that recombinant AknK catalyses the addition of the second sugar, L-2-deoxyfucose to the carbohydrate chain [144]. Both enzymes showed some degree of substrate flexibility that allowed the biosynthesis of new anthracycline glycosides, suggesting that these enzymes may be useful for the chemoenzymatic synthesis of anthracycline variants.

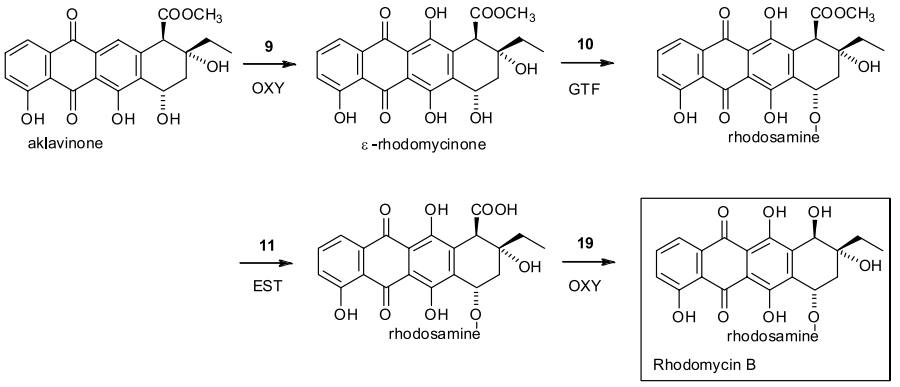
The crystal structures of two related macrolide glycosyltransferases OleI and OleD, which inactivate endogenous antibiotic oleandomycin by glycosylation, have recently been published [146]. In these enzymes that belong to the GT-B fold type of glycosyltransferases, the substrates interact with the enzyme primarily through hydrophobic interactions and van-der-Waals forces, as observed for many tailoring enzymes in anthracycline biosynthesis [6]. The glycosyltransferase UrdGT2 catalyses the formation of an unusual glycosidic C–C bond between the C9 carbon atom of the aglycone core and D-olivose in urdamycin biosynthesis [147]. The three-dimensional structure of the enzyme revealed that it is a member of the GT-B fold glycosyltransferases [148]. Modelling studies suggest a mechanism whereby the carbon atom C-9 of the substrate is activated by a buried aspartic acid side chain. The mechanistic proposal further requires a catalytic base involved in deprotonation of carbon atom C-9. In the crystal structures presently available there is no potential candidate in the proximity of the modelled substrate, and the catalytic base thus remains elusive. A more detailed view of this mechanism will require structures of enzyme–substrate and enzyme–product complexes.

4.4.2 Methyl Transfer

Methyl transfer reactions play a significant part in the modifications of aromatic polyketides, both of the polyketide core [61, 62] as well as of several of the sugar moieties [44, 53]. In *Streptomyces*, more than 20 amino acid sequences have been found that may represent enzymes involved in methyl transfer reactions in the biosynthesis of aromatic polyketides [149]. One of these enzymes, the *S*-adenosyl-L-methionine-dependent DnrK, is involved in the methylation of the C-4 hydroxyl group in daunorubicin/doxorubicin biosynthesis (Scheme 10, step **12**). The subunit of the homo-dimeric enzyme displays a fold typical for small-molecule methyltransferases. The structure of the ternary complex with bound products *S*-adenosyl-L-homocysteine and 4-methoxy-ε-rhodomycin provided insights into the structural basis of substrate recognition and catalysis [149]. The position and orientation of the substrates suggest an S_N2 mechanism for methyl transfer, and mutagenesis experiments show that there is no catalytic base in the vicinity of the substrate. Rate enhancement is thus most likely due to orientational and proximity effects [149].

4.4.3 Methylester Hydrolysis

The methylesterase RdmC [150] and its homologues DnrP from *S. peucetic-cus* [58] and DauP from *Streptomyces* sp. strain C5 [57] catalyse hydrolysis of the carboxymethyl ester at the C-15 position of the aklavinone core in the biosynthesis of rhodomycin (Scheme 11, step **11**) and daunorubicin/doxorubicin (Scheme 10, step **11**), respectively. Biochemical and structural data from the rhodomycin pathway have shown that RdmC prefers monoglycosylated substrates [151, 152]. The crystal structures of RdmC with the product analogues 10-decarboxymethylaclacinomycin T and 10-decarboxymethylaclacinomycin A, respectively, revealed that these enzymes belong to the family of α/β hydrolases, and contain the catalytic Ser–His–Asp triad [152]. The binding of the ligands is dominated by hydrophobic interactions, and specificity is controlled mainly by the shape of the binding pocket, rather than through specific hydrogen bonds, a feature that is common to many enzymes involved in anthracycline tailoring [6].



Scheme 11 Putative biosynthetic pathway of rhodomycin B from aklavinone

The free 10-carboxylic acid produced after methylester hydrolysis has been described as unstable. Bioconversion experiments using rhodomycin D as a substrate have indicated that when the DauP reaction was coupled to the following 4-*O*-methyl transfer reaction catalysed by DauK (Scheme 10, step **12**), the main products had gone through 10-decarboxylation [61]. Studies with purified RdmC confirmed these observations because a reaction using aclacinomycin A as a substrate also led to 10-decarbolylation [152]. It is interesting to note, however, that the next biosynthetic enzyme RdmB (Scheme 11, step **19**) on the rhodomycin pathway requires a 10-carboxylated substrate [111].

4.4.4 Oxygenation/Hydroxylation

A major cause of the large structural diversity of the anthracyclines is the spectrum of oxidative tailoring reactions that is seen in the biosynthesis of these metabolites. It is noteworthy that a variety of mechanistically different oxygenases have been found in anthracycline biosynthesis, including flavoenzymes, cytochrome P450-type mono-oxygenases, a novel class of SAM-dependent hydroxylases as well as members of the family of cofactorless oxygenases.

Flavin-Dependent Hydroxylases

Flavine-dependent oxygenases are particularly common in biosynthetic pathways leading to aromatic polyketides [7] and are thus also found in anthracycline biosynthesis. Aklavinone is converted to another important pathway intermediate ε-rhodomycinone by DnrF (Scheme 10, step **9**) and RdmE (Scheme 11, step **9**) [47, 60, 153]. These enzymes belong to a well-known family of FAD-dependent monooxygenases, which use molecular oxygen for hydroxylation of aromatic substrates [151, 154]. Aklavinone 11-hydroxylases have been cloned from the *dau/dnr* [153] and the *rdm* [154] gene clusters. Recombinant RdmE from *S. purpurascens* is a FAD-dependent hydroxylase that catalyses the hydroxylation of the C-11 of aklavinone in the biosynthesis of rhodomycin. There is at present no three-dimensional structure of a flavin-dependent hydroxylase from anthracycline biosynthetic pathways known, but a crystal structure analysis [155] of two homologous enzymes from angucycline biosynthetic pathways, PgaE and CabE, revealed that these hydroxylases belong to the family of pHBH flavoenzymes, with closest structural similarity to phenolhydroxylase [156].

A peculiar sugar modification occurs in the biosynthesis of the aclacinomycins. These anthracyclines contain a trisaccharide moiety attached to the aklavinone scaffold at the C-7 position (Scheme 1). The first two carbohydrates in the aclacinomycins are rhodosamine and 2-deoxyfucose, but they differ structurally in their third sugar component, which is rhodinose in AclN, cinerulose A in AclA, L-aculose in AclY and cinerulose B in AclB [157]. The conversion of rhodinose to L-aculose is catalysed in a two-step process by the FAD-dependent enzyme aclacinomycin oxidoreductase [71] (Scheme 5, step **31**). The three-dimensional structure of this oxidase revealed that the cofactor FAD is bound via two covalent bonds to the enzyme. Crystal structure and functional data further established a mechanism where the two different reactions are catalysed in the same active site of the enzyme but by different active site residues [71].

Cytochrome-Dependent Mono-oxygenases

The final steps of daunorubicin/doxorubicin biosynthesis involve the sequential oxidation of the C-13 position to a hydroxyl and keto functional group, and hydroxylation of position C-14 (Scheme 10, step **13**). These reactions are catalysed by the cytochrome P450-dependent oxygenase DoxA, and are dependent on molecular oxygen and NADPH [158]. The enzyme has a rather relaxed substrate specificity and can also accept anthracycline analogues as substrate, resulting in novel anthracycline metabolites [159]. There is presently no three-dimensional structure of a P450 mono-oxygenase from anthracycline biosynthetic pathways known, but a crystal structure of the related cytochrome P450 monooxygenase CYP154C1 involved in macrolide polyketide tailoring reaction in *S. coelicolor* A3(2) has been determined to 1.9 Å resolution [160]. Furthermore, the crystal structures of the cytochrome P450 StaP have been determined in the ligand-free and ligand-bound forms [161]. The analysis reveals that the enzyme adopts a more ordered state upon binding of the ligand and provides structural insights into staurosporine synthesis and diversity of P450 chemistry.

S-Adenosyl-L-methionine-Dependent Hydroxylases

After hydrolysis of the carboxylmethyl ester at the C-15 position of aklavinone, the next step in the synthesis of rhodomycin is the hydroxylation at position C-10, catalysed by RdmB [151] (Scheme 11, step **19**). Similar to RdmC, this enzyme has a preference for monoglycosylated substrates [111, 151]. RdmB is homologous to small-molecule O-methyltransferases, with 52% sequence identity to DnrK. It displays the methyltransferase fold and binds SAM [162]. Surprisingly, RdmB does not catalyse methyl transfer, but a regiospecific SAM-dependent hydroxylation reaction. The biochemical analysis of the reaction mechanism revealed a novel, previously undescribed function for SAM as a cofactor in enzymatic hydroxylation reactions (Fig. 5) [111]. RdmB shows some mechanistic similarities to the mono-oxygenase Act-Orf6 in that in both mechanisms the aromatic ring system of the substrate is involved in the activation of molecular oxygen in a fashion similar to flavoenzymes. DnrK and RdmB are particularly illustrative examples of divergent evolution in an enzyme fold family, leading toward new function [111].

5 Outlook

In spite of the large advances that have been made in the molecular biology, structural biology and enzymology of anthracycline biosynthesis in the last few years there are quite a few remaining questions that have not yet

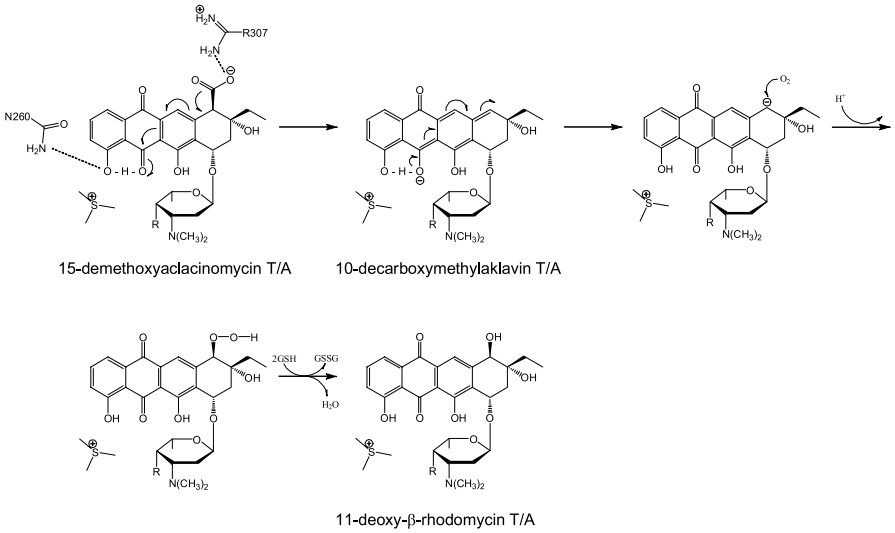


Fig. 5 Catalytic mechanism for the SAM-dependent hydroxylation reaction catalysed by RdmB. Decarboxylation of the substrate results in a carbanion intermediate, which is stabilized by resonance and the positive charge of SAM. The carbanion reacts with molecular oxygen in a similar manner as flavins, yielding a peroxy intermediate that is subsequently reduced to the hydroxyl group by a thiol reagent

been solved. One of the key issues is the role of the minPKS in chain folding and cyclization, i.e. is the first ring formed when still bound to the PKS or subsequently by another cyclase. Another feature of the biosynthesis of the polyketide skeleton that is not yet understood is the timing and choreography of the chain folding and the cyclization steps. Furthermore, there are several early tailoring reactions that most likely occur when the polyketide chain is still bound to ACP, and that are little understood. The biochemical studies of these early events in anthracycline biosynthesis are particularly challenging and will require considerable efforts due to the fact that the substrates most likely are ACP bound, and are also unstable due to the inherent reactivity of the polyketide chains.

A second area that is presently little characterized is the enzymology of the biosynthesis of the deoxysugars in anthracyclines, and in particular the glycosyl transferases involved in sugar transfer. Several of these enzymes have unique features, as for instance the as yet unidentified enzyme in nogalomycin biosynthesis that catalyses formation of the C-glycosidic bond at position C-2 of the aglycone core. The glycosyltransferases are also of biocatalytic interest because they offer a chemoenzymatic route to generate diversity in anthracyclines.

Finally the application of the mechanistic knowledge of anthracycline biosynthetic enzymes for combinatorial biosynthesis is timely. The redesign

of biosynthetic enzymes with respect to chemistry and substrate specificity, based on structural and functional knowledge, or by molecular evolution approaches offers the possibility to expand the diversity of these metabolites by using novel, non-natural enzymes and not only combinations of enzymes from already existing pathways. While this approach holds great promises for the future its general applicability remains to be established.

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References

1. Hopwood DA (1997) Chem Rev 97:2465
2. Dickens ML, Rajgarhia VB, Woo AJ, Priestley ND (1997) In: Strohl WR (ed) Biotechnology of antibiotics: anthracyclines, 2nd edn. Marcel Dekker, New York, p 577
3. Hutchinson CR (1997) Chem Rev 97:2525
4. Hutchinson CR, Colombo AL (1999) J Ind Microbiol Biotechnol 23:647
5. Niemi J, Kantola J, Ylihonko K, Mäntsälä P (2002) In: Ferro F, Martin JM (eds) Microbial secondary metabolites: biosynthesis, genetics and regulation. Research Signpost, Kerala, India, p 122
6. Schneider G (2005) Curr Opin Struct Biol 15:629
7. Hertweck C, Luzhetskyy A, Rebets Y, Bechthold A (2007) Nat Prod Rep 24:162
8. Krassilnikov NA, Koreniako AJ (1939) Microbiologiya 8:673
9. Brockmann H, Bauer K (1950) Naturwissenschaften 38:492
10. Brockmann H, Brockmann H Jr (1963) Chem Ber 96:1771
11. Ettlinger H, Gäumann E, Hutter R, Keller-Schierlein W, Kradolfer F, Neipp L, Prelog V, Reusser P, Zahner H (1959) Chem Ber 92:1867
12. DiMarco A, Gaetani M, Orezzi P, Scarpinato BM, Slivestrini R, Soldati M, Dasdia T, Valentini L (1964) Nature 201:706
13. Dubost M, Ganter P, Maral R, Ninet L, Pinnert S, Prud'homme J, Werner GH (1963) CR Acad Sci 257:1813
14. Shan K, Lincoff AM, Young JB (1996) Ann Intern Med 125:47
15. Arcamone F, Cassinelli G, Fantini G, Grein A, Orezzi P, Pol C, Spalla C (1969) Biotechnol Bioeng 11:1101
16. Blum R, Carter S (1974) Ann Intern Med 80:249
17. McDaniel R, Ebert-Khosla S, Hopwood DA, Khosla C (1995) Nature 375:549
18. Khosla C, Zawada RJ (1996) Trends Biotechnol 14:335
19. Kulowski K, Wendt-Pienkowski E, Han L, Yang K, Vining LC, Hutchinson CR (1999) J Am Chem Soc 121:1786
20. Wohlert SE, Wendt-Pienkowski E, Bao W, Hutchinson CR (2001) J Nat Prod 64:1077
21. Palmu K, Ishida K, Mäntsälä P, Hertweck C, Metsä-Ketelä M (2007) Chembiochem 8:1577
22. McDaniel R, Thamchaipenet A, Gustafsson C, Fu H, Betlach M, Ashley G (1999) Proc Natl Acad Sci USA 96:1846
23. Oki T, Matsuzawa Y, Yoshimoto A, Numata K, Kitamura I, Hori S, Takamatsu A, Umezawa H, Ishizuka M, Naganawa H, Suda H, Hamada M, Takeuchi T (1975) J Antibiot 28:830

24. Furlan RLA, Watt SJ, Garrido LM, Amarante-Mendez GP, Nur-e-Alam M, Rohr J, Braña A, Mendez C, Salas JA, Sheil MM, Becj JL, Padilla G (2004) J Antibiot 57:647
25. Arora SK (1983) J Am Chem Soc 105:1328
26. Arora SK (1985) J Biomol Struct Dyn 3:377
27. Leblond P, Francou FX, Simonet JM, Decaris B (1990) FEMS Microbiol Lett 60:79
28. Leblond P, Redenbach M, Cullum J (1993) J Bacteriol 175:3422
29. Huang CH, Lin YS, Yang YL, Huang SW, Chen CW (1998) Mol Microbiol 28:905
30. Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, Bateman A, Brown S, Chandra G, Chen CW, Collins M, Cronin A, Fraser A, Goble A, Hidalgo J, Hornsby T, Howarth S, Huang CH, Kieser T, Larke L, Murphy L, Oliver K, O'Neil S, Rabbinowitsch E, Rajandream MA, Rutherford K, Rutter S, Seeger K, Saunders D, Sharp S, Squares R, Squares S, Taylor K, Warren T, Wietzorrek A, Woodward J, Barrell BG, Parkhill J, Hopwood DA (2002) Nature 417:141
31. Yang CC, Huang CH, Li CY, Tsay YG, Lee SC, Chen CW (2002) Mol Microbiol 43: 297
32. Omura S, Ikeda H, Ishikawa J, Hanamoto A, Takahashi C, Shinose M, Takahashi Y, Horikawa H, Nakazawa H, Osonoe T, Kikuchi H, Shiba T, Sakaki Y, Hattori M (2001) Proc Natl Acad Sci USA 98:12215
33. Ikeda H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T, Sakaki Y, Hattori M, Omura S (2003) Nat Biotechnol 21:526
34. Fernandez-Moreno MA, Martinez E, Boto L, Hopwood DA, Malpartida F (1992) J Biol Chem 267:19278
35. Davis NK, Chater KF (1990) Mol Microbiol 4:1679
36. Metsä-Ketelä M, Halo L, Munukka E, Hakala J, Mäntsälä P, Ylihonko K (2002) Appl Environ Microbiol 68:4472
37. Gullón S, Olano C, Abdelfattah MS, Braña AF, Rohr J, Mendéz C, Salas JA (2006) Appl Environ Microbiol 72:4172
38. Grimm A, Madduri K, Ali A, Hutchinson CR (1994) Gene 151:1
39. Ye J, Dickens ML, Plater R, Li Y, Lawrence J, Strohl WR (1994) J Bacteriol 176:6270
40. Krugel H, Hanel F, Fiedler G (1993) Mol Gen Genet 241:193
41. Ylihonko K, Tuikkanen J, Jussila S, Cong L, Mäntsälä P (1996) Mol Gen Genet 251:113
42. Luzhetskyy A, Mayer A, Hoffmann J, Pelzer S, Holzenkämper M, Schmitt B, Wohlert SE, Vente A, Bechthold A (2007) Chembiochem 8:599
43. Chung JY, Fujii I, Harada S, Sankawa U, Ebizuka Y (2002) J Bacteriol 184:6115
44. Räty K, Kunnari T, Hakala J, Mäntsälä P, Ylihonko K (2000) Mol Gen Genet 264:164
45. Räty K, Kantola J, Hautala A, Hakala J, Ylihonko K, Mäntsälä P (2002) Gene 293:115
46. Miyamoto Y, Johdo O, Nagamatsu Y, Yoshimoto A (2002) FEMS Microbiol Lett 206:163
47. Niemi J, Mantsälä P (1995) J Bacteriol 177:2942
48. Garrido LM, Lombó F, Baig I, Nur-E-Alam M, Furlan RL, Borda CC, Braña A, Mendéz C, Salas JA, Rohr J, Padilla G (2006) Appl Microbiol Biotechnol 73:122
49. Tsukamoto N, Fujii I, Ebizuka Y, Sankawa U (1992) J Antibiot 45:1286
50. Stutzman-Engwall KJ, Hutchinson CR (1989) Proc Natl Acad Sci USA 86:3135
51. Otten SL, Stutzman-Engwall KJ, Hutchinson CR (1990) J Bacteriol 172:3427
52. Niemi J, Ylihonko K, Hakala J, Pärssinen R, Kopio A, Mäntsälä P (1994) Microbiology 140:1351
53. Torkkell S, Kunnari T, Palmu K, Mäntsälä P, Hakala J, Ylihonko K (2001) Mol Genet Genomics 266:276

54. Lomovskaya N, Otten SL, Doi-Katayama Y, Fonstein L, Liu XC, Takatsu T, Inventi-Solari A, Filippini S, Torti F, Colombo AL, Hutchinson CR (1999) J Bacteriol 181: 305
55. Lomovskaya N, Doi-Katayama Y, Filippini S, Nastro C, Fonstein L, Gallo M, Colombo AL, Hutchinson CR (1998) J Bacteriol 180:2379
56. Otten SL, Gallo MA, Madduri K, Liu X, Hutchinson CR (1997) J Bacteriol 179:4446
57. Dickens ML, Ye J, Strohl WR (1995) J Bacteriol 177:536
58. Madduri K, Hutchinson CR (1995) J Bacteriol 177:3879
59. Dickens ML, Ye J, Strohl WR (1996) J Bacteriol 178:3384
60. Filippini S, Solinas MM, Breme U, Schluter MB, Gabellini D, Biamonti G, Colombo AL, Garofano L (1995) Microbiology 141:1007
61. Dickens ML, Priestley ND, Strohl WR (1997) J Bacteriol 179:2641
62. Madduri K, Torti F, Colombo AL, Hutchinson CR (1993) J Bacteriol 175:3900
63. Scotti C, Hutchinson CR (1996) J Bacteriol 178:7316
64. Otten SL, Liu X, Ferguson J, Hutchinson CR (1995) J Bacteriol 177:6688
65. Gallo MA, Ward J, Hutchinson CR (1996) Microbiology 142:269
66. Madduri K, Hutchinson CR (1995) J Bacteriol 177:1208
67. Otten SL, Ferguson J, Hutchinson CR (1995) J Bacteriol 177:1216
68. Kaur P (1997) J Bacteriol 179:569
69. Lomovskaya N, Hong SK, Kim SU, Fonstein L, Furuya K, Hutchinson RC (1996) J Bacteriol 178:3238
70. Kantola J, Kunnari T, Hautala A, Hakala J, Ylihonko K, Mäntsälä P (2000) Microbiology 146:155
71. Alexeev I, Sultana A, Mäntsälä P, Niemi J, Schneider G (2007) Proc Natl Acad Sci USA 104:6170
72. Dreier J, Khosla C (2000) Biochemistry 39:2088
73. Katz L, Donadio S (1993) Annu Rev Microbiol 47:875
74. Khosla C, Gokhale RS, Jacobsen JR, Cane DE (1999) Annu Rev Biochem 68:219
75. McDaniel R, Ebert-Khosla S, Hopwood DA, Khosla C (1993) Science 262:1546
76. Tang Y, Tsai SC, Khosla C (2003) J Am Chem Soc 125:12708
77. Jenke-Kodama H, Sandmann A, Müller R, Dittmann E (2005) Mol Biol Evol 22:2027
78. Florova G, Kazanina G, Reynolds KA (2002) Biochemistry 41:10462
79. Keatinge-Clay AT, Shelat AA, Savage DF, Tsai SC, Miercke LJ, O'Connell JD 3rd, Khosla C, Stroud RM (2003) Structure 11:147
80. Hitchman TS, Crosby J, Byrom KJ, Cox RJ, Simpson TJ (1998) Chem Biol 5:35
81. Arthur CJ, Szafranska A, Evans SE, Findlow SC, Burston SG, Owen P, Clark-Lewis I, Simpson TJ, Crosby J, Crump MP (2005) Biochemistry 44:15414
82. Arthur CJ, Szafranska AE, Long J, Mills J, Cox RJ, Findlow SC, Simpson TJ, Crump MP, Crosby J (2006) Chem Biol 13:587
83. Keatinge-Clay AT, Maltby DA, Medzihradszky KF, Khosla C, Stroud RM (2004) Nat Struct Mol Biol 11:888
84. Bisang C, Long PF, Cortes J, Westcott J, Crosby J, Matharu AL, Cox RJ, Simpson TJ, Staunton J, Leadlay PF (1999) Nature 401:502
85. Lambalot RH, Gehring AM, Flugel RS, Zuber P, LaCelle M, Marahiel MA, Reid R, Khosla C, Walsh CT (1996) Chem Biol 3:923
86. Parris KD, Lin L, Tam A, Mathew R, Hixon J, Stahl M, Fritz CC, Seehra J, Somers WS (2000) Structure 8:883
87. Li Q, Khosla C, Puglisi JD, Liu CW (2003) Biochemistry 42:4648
88. Findlow SC, Winsor C, Simpson TJ, Crosby J, Crump MP (2003) Biochemistry 42:8423

89. Crump MP, Crosby J, Dempsey CE, Parkinson JA, Murray M, Hopwood DA, Simpson TJ (1997) Biochemistry 36:6000
90. Zhang YM, Marrakchi H, White SW, Rock CO (2003) J Lipid Res 44:1
91. Leibundgut M, Jenni S, Frick C, Ban N (2007) Science 316:288
92. Bao W, Sheldon PJ, Wendt-Pienkowski E, Hutchinson CR (1999) J Bacteriol 181:4690
93. Rajgarhia VB, Priestley ND, Strohl WR (2001) Metab Eng 3:49
94. Bao W, Sheldon PJ, Hutchinson CR (1999) Biochemistry 38:9752
95. Davies C, Heath RJ, White SW, Rock CO (2000) Structure 8:185
96. Scarsdale JN, Kazanina G, He X, Reynolds KS, Wright HT (2001) J Biol Chem 276:20616
97. Pan H, Tsai S, Meadows ES, Miercke LJ, Keatinge-Clay AT, O'Connell J, Khosla C, Stroud RM (2002) Structure 10:1559
98. Meadows ES, Khosla C (2001) Biochemistry 40:14855
99. Tang Y, Koppisch AT, Khosla C (2004) Biochemistry 43:9546
100. Bartel PL, Zhu CB, Lampel JS, Dosch DC, Connors NC, Strohl WR, Beale JM Jr, Floss HG (1990) J Bacteriol 172:4816
101. Hadfield AT, Limpkin C, Teartasin W, Simpson TJ, Crosby J, Crump MP (2004) Structure 12:1865
102. Korman TP, Hill JA, Vu TN, Tsai SC (2004) Biochemistry 43:14529
103. Kantola J, Blanco G, Hautala A, Kunnari T, Hakala J, Mendez C, Ylihonko K, Mäntsälä P, Salas J (1997) Chem Biol 4:751
104. Kunnari T, Ylihonko K, Hautala A, Klika KD, Mäntsälä P, Hakala J (1999) Bioorg Med Chem Lett 9:2639
105. Rajgarhia VB, Strohl WR (1997) J Bacteriol 179:2690
106. Hautala A, Torkkell S, Räty K, Kunnari T, Kantola J, Mäntsälä P, Hakala J, Ylihonko K (2003) J Antibiot 56:143
107. Kendrew SG, Hopwood DA, Marsh EN (1997) J Bacteriol 179:4305
108. Fetzner S (2002) Appl Microbiol Biotechnol 60:243
109. Sciara G, Kendrew SG, Miele AE, Marsh NG, Federici L, Malatesta F, Schimperna G, Savino C, Vallone B (2003) EMBO J 22:205
110. Widboom PF, Fielding EN, Liu Y, Bruner SD (2007) Nature 447:342–345
111. Jansson A, Koskiniemi H, Erola A, Wang J, Mäntsälä P, Schneider G, Niemi J (2005) J Biol Chem 280:3636
112. Metsä-Ketelä M, Palmu K, Kunnari T, Ylihonko K, Mäntsälä P (2003) Antimicrob Agents Chemother 27:1291
113. Olano C, Lomovskaya N, Fonstein L, Roll JT, Hutchinson CR (1999) Chem Biol 6:845
114. Taguchi T, Itou K, Ebizuka Y, Malpartida F, Hopwood DA, Surti CM, Booker-Milburn KI, Stephenson GR, Ichinose K (2000) J Antibiot 53:144
115. Taguchi T, Okamoto S, Lezhava A, Li A, Ochi K, Ebizuka Y, Ichinose K (2007) FEMS Microbiol Lett 269:234
116. Kendrew SG, Katayama K, Deutsch E, Madduri K, Hutchinson CR (1999) Biochemistry 38:4794
117. Torkkell S, Kunnari T, Palmu K, Hakala J, Mäntsälä P, Ylihonko K (2000) Antimicrob Agents Chemother 44:396
118. Sultana A, Kallio P, Jansson A, Wang JS, Niemi J, Mäntsälä P, Schneider G (2004) EMBO J 23:1911
119. Kallio P, Sultana A, Niemi J, Mäntsälä P, Schneider G (2006) J Mol Biol 357:210
120. Beinker P, Lohkamp B, Peltonen T, Niemi J, Mäntsälä P, Schneider G (2006) J Mol Biol 359:728
121. Trefner A, Salas JA, Bechthold A (1999) Nat Prod Rep 16:283

122. Rodriguez L, Aguirrezabalaga I, Allende N, Brana AF, Mendez C, Salas JA (2002) Chem Biol 9:721
123. Chen H, Thomas MG, Hubbard BK, Losey HC, Walsh CT, Burkart MD (2000) Proc Natl Acad Sci USA 97:11942
124. Takahashi H, Liu YN, Liu HW (2006) J Am Chem Soc 128:1432
125. Allard STM, Cleland WW, Holden HM (2004) J Biol Chem 279:2211
126. Merkel AB, Major LL, Errey JC, Burkar MD, Field RA, Walsh CT, Naismith JH (2004) J Biol Chem 279:32684
127. Blankenfeldt W, Asuncion M, Lam JS, Naismith JS (2000) EMBO J 19:6652
128. Zuccotti S, Zanardi D, Rosano C, Sturla L, Tonetti M, Bolognesi M (2001) J Mol Biol 313:831
129. Allard STM, Beis K, Giraud MF, Hegeman AD, Gross JW, Wilmouth RC, Whitfield C, Graninger M, Messner P, Allen AG, Maskell DJ, Naismith JH (2002) Structure 10:81
130. Dong C, Major LL, Allen A, Blankenfeldt W, Maskell D, Naismith JH (2003) Structure 11:715
131. Salas JA, Mendéz C (2005) J Mol Microbiol Biotechnol 9:77
132. Thibodeaux CJ, Melançon CE, Liu HW (2007) Nature 446:1008
133. Giraud MF, Naismith JH (2000) Curr Opin Struct Biol 10:687
134. Chen H, Agnihotri G, Guo Z, Que NLS, Chen XH, Liu HW (1999) J Am Chem Soc 121:8124
135. Draeger G, Park SH, Floss HG (1999) J Am Chem Soc 121:2611
136. Hong L, Zhao Z, Liu HW (2006) J Am Chem Soc 128:14262
137. Dong C, Major LL, Srikannathasan V, Errey JC, Giraud MF, Lam JS, Graninger M, Messner P, McNeil MR, Field RA, Whitfield C, Naismith JH (2007) J Mol Biol 365:146
138. Chang CW, Liu HW (2002) Bioorg Med Chem Lett 12:1493
139. Chen H, Yamase H, Murakami K, Chang CW, Zhao L, Zhao Z, Liu HW (2002) Biochemistry 41:9165
140. Torkkell S, Ylihonko K, Hakala J, Skurnik M, Mäntsälä P (1997) Mol Gen Genet 256:203
141. Fujii I, Ebizuka Y (1997) Chem Rev 97:2511
142. Campbell JA, Davies GJ, Bulone V, Henrissat B (1997) Biochem J 326:929
143. Lu W, Leimkuhler C, Oberthür M, Kahne D, Walsh CT (2004) Biochemistry 43:4548
144. Lu W, Leimkuhler C, Gatto GJ, Kruger RG, Oberthür M, Kahne D, Walsh CT (2005) Chem Biol 12:527
145. Leimkuhler C, Fridman M, Lupoli T, Walker S, Walsh CT, Kahne D (2007) J Am Chem Soc 129:10546
146. Bolam DN, Roberts S, Proctor MR, Turkenburg JP, Dodson EJ, Martinez-Fleites C, Yang M, Davis BG, Davies GJ, Gilbert HJ (2007) Proc Natl Acad Sci USA 104:5336
147. Kunzel E, Faust B, Oelkers C, Weissbach U, Bearden DW, Weitnauer G, Westrich G, Bechthold A, Rohr J (1999) J Am Chem Soc 121:11058
148. Mittler M, Bechthold A, Schulz GE (2007) J Mol Biol 372:67
149. Jansson A, Koskiniemi H, Mäntsälä P, Niemi J, Schneider G (2004) J Biol Chem 279:41149
150. Wang Y, Niemi J, Airas K, Ylihonko Y, Hakala K, Mäntsälä P (2000) Biochem Biophys Acta 1430:57
151. Wang Y, Niemi J, Mäntsälä P (2002) FEMS Microbiol Lett 208:117
152. Jansson A, Niemi J, Mäntsälä P, Schneider G (2003) J Biol Chem 278:39006
153. Hong YS, Hwang CK, Hong SK, Kim YH, Lee JJ (1994) J Bacteriol 176:7096
154. Niemi J, Wang Y, Airas K, Ylihonko K, Hakala J, Mäntsälä P (1999) Biochim Biophys Acta 1430:57

155. Koskiniemi H, Metsä-Ketelä M, Dobritzsch D, Kallio P, Korhonen H, Mäntsälä P, Schneider G, Niemi J (2007) J Mol Biol 372:633
156. Enroth C, Neujahr H, Schneider G, Lindqvist Y (1998) Structure 6:605
157. Ylihonko K, Hakala J, Niemi J, Lundell J, Mäntsälä P (1994) Microbiology 140:1359
158. Walczak RJ, Dickens MD, Priestley ND, Strohl WR (1999) J Bacteriol 181:298
159. Walczak RJ, Hines JV, Strohl WR, Priestley ND (2001) Org Lett 3:2277
160. Podust LM, Kim Y, Arase M, Neely BA, Beck BJ, Bach H, Sherman DH, Lamb DC, Kelly SL, Waterman MR (2003) J Biol Chem 278:12214
161. Makino M, Sugimoto S, Shiro Y, Asamizu S, Onaka H, Nagano S (2007) Proc Natl Acad Sci USA 104:11591
162. Jansson A, Niemi J, Lindqvist Y, Mäntsälä P, Schneider G (2003) J Mol Biol 334:269

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Synthesis of Enantiomerically Pure Anthracyclinones

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Abstract The anthracycline antibiotics are among the most important clinical drugs used in the treatment of human cancer. The search for new agents with improved therapeutic efficacy and reduced cardiotoxicity stimulated considerable efforts in the synthesis of new analogues. Since the biological activity of anthracyclines depends on their natural absolute configuration, various strategies for the synthesis of enantiomerically pure anthracyclinones (aglycones) have been developed. They comprise: resolution of racemic intermediate, incorporation of a chiral fragment derived from natural and non-natural chiral pools, asymmetric synthesis with the use of a chiral auxiliary or a chiral reagent, and enantioselective catalysis. Synthetic advances towards enantiopure anthracyclinones reported over the last 17 years are reviewed.

Keywords Anthracyclinones · Anthracycline antibiotics · Diastereoselective synthesis · Enantioselective catalysis

Abbreviations

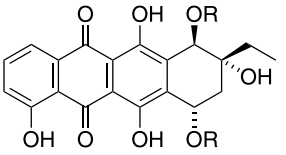
Ac	Acetyl
acac	Acetylacetonate

AIBN	2,2-Azobis(2-methylpropionitrile)
BINAP	2,2′-Bis(diphenylphosphino)-1,1′-binaphthyl
BINOL	1,1′-Bi(2-naphthol)
BIPHEP	(Biphenyl-2,2′-diyl)bis(diphenylphosphine)
Bn	Benzyl
Bu	Butyl
^{t}Bu, *t*-Bu	*tertiary*-Butyl
BuLi	Butyllithium
CAN	Cerium ammonium nitrate
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	Dicyanodichloroquinone
de	Diastereoisomeric excess
DEAD	Diethyl azodicarboxylate
DET	Diethyl tartrate
DIBAL(-H)	Diisobutylaluminium hydride
DIPEA	*N*,*N*-Diisopropylethylamine
DIPT	Diisopropyl tartrate
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMPU(33)	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO	Dimethyl sulfoxide
DMT	Dess–Martin periodinane
DMTMPS	Dimethyl(1,1,2-trimethylpropyl)silyl
ee	Enantiomeric excess
Et	Ethyl
h	Hour
HMPT	Hexamethylphosphoric triamide
IBX	Iodoxybenzoic acid
KHMDS, LHMDS	Potassium (lithium) hexamethyldisilazane
LAH	Lithium aluminum hydride
LDA	Lithium diethylamide
M	Molar
MCPBA	*m*-Chloroperbenzoic acid
Me	Methyl
Ms	Methanesulfonyl
MS	Molecular sieves
NBS	*N*-Bromosuccinimide
NCS	*N*-Chlorosuccinimide
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
Ph	Phenyl
PHT	Pyrrolidinone hydrotribromide
PPA	Polyphosphoric acid
PPTS	Pyridinium *p*-toluenesulfonate
Pr, *i*-Pr	Propyl, isopropyl
py	Pyridine
rt	Room temperature
TBA	Tetrabutylammonium

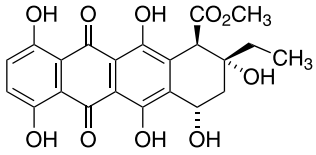
TES	Triethylsilyl
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TMEDA	Tetramethylethylenediamine
TMP	Tetramethylpiperidine
TMS	Trimethylsilyl
TPFPFeCl	[5,10,15,20-Tetrakis(pentafluorophenyl)-21*H*,23*H*-porphine]iron(III) chloride

1 Introduction

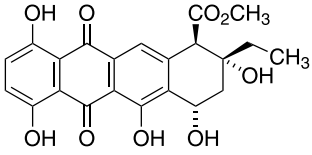
The anthracyclines are pigmented glycosidic antibiotics produced by different strains of *Streptomyces*. The first of these naturally occurring compounds, β-rhodomycine (**1a**), was isolated by Brockmann and Bauer in 1950 from *Streptomyces purpurescens* [1]. In the 1950s other rhodomycins were obtained and subsequently their structures were elucidated, mainly by Brockmann [2] and Ollis [3]. The aglycone moieties of these compounds were named anthracyclinones and belong to the various types of rhodomycinones. Examples of representative structures are shown in Fig. 1.



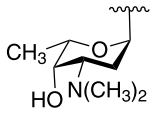
1a β-rodomycine R = rhodosaminyl
1b β-rhodomycinone R = H



2 ε-isorhodomycinone



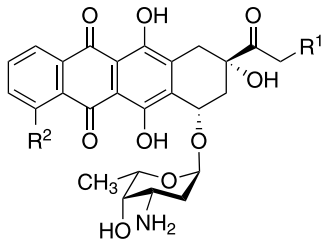
3 ε-pyrromycinone



rhodosaminyl

Fig. 1

The anthracyclines attracted considerable attention after the independent discovery of daunorubicin (**4**) by groups in Farmitalia [4] and in Rhone Poulenc [5], and later after the discovery of doxorubicin (**5**) [6].



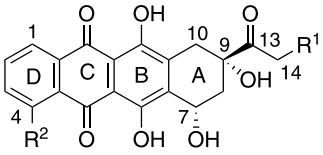
4 Daunorubicin $R^1 = H, R^2 = OCH_3$

5 Doxorubicin $R^1 = OH, R^2 = OCH_3$

6 Idarubicin $R^1 = R^2 = H$

Fig. 2

Both daunorubicin (**4**) and doxorubicin (**5**) were shown to be endowed with remarkably high levels of antitumor activity. Doxorubicin (**5**) was developed into an anticancer drug widely used for treatment of human solid tumors and leukaemias [7, 8]. The therapeutic efficacy of doxorubicin (**5**) is hampered by toxic side effects, of which the most serious are dose-related cardiotoxicity and myeloma suppression [9]. To overcome these limitations much effort was expended in the synthesis of analogues of natural anthracyclines in the hope of obtaining improved pharmacological properties. A successful example was idarubicin (**6**) (Fig. 2), a drug approved in 1990 in the USA for treatment of acute non-lymphocytic and lymphoblastic leukemia and possessing superior therapeutic efficacy and reduced cardiotoxicity relative to daunorubicin (**4**) [10, 11]. The synthesis of modified anthracycline aglycones, the anthracyclinones (Fig. 3), was the focus of synthetic work in many laboratories. In the 1970s and 1980s efficient methods for the synthesis of the tetracyclic moiety of the anthracyclinones were developed. They comprise various synthetic strategies based on construction of the tetracyclic system through Friedel–Crafts, carbanion cyclization, Marschalk and Diels–Alder reactions. Methods for the construction of the linear tetracyclic skeleton and the control of the regiochemical orientation of substituents in rings A and D and the relative configuration of labile functionalities in ring A were successfully developed. The results are summarized in several excellent review articles [12–15]. The biological activity of glycosidic anthracyclines depends crucially on the natural absolute configuration of ring A [16, 17]. Therefore the synthesis of enantiomerically pure aglycones with the desired absolute configuration became not only an academic exercise but also a challenge of practical importance. Whereas at the beginning of synthetic studies



7 Daunorubicinone $R^1 = H, R^2 = OCH_3$

8 Doxorubicinone $R^1 = OH, R^2 = OCH_3$

9 Idarubicinone $R^1 = R^2 = H$

Fig. 3

on anthracyclinones most publications were concerned with methodologies leading to racemic products, in the 1980s the problem of enantioselectivity became the principal focus. The efforts of many research groups are summarized in the excellent, comprehensive review by Krohn and Ekkundi [18], the first solely devoted to the synthesis of enantiomerically pure anthracyclinones. Their article was published in 1989 and covered the literature up to early 1988. This review provides an update of significant advances since that time.

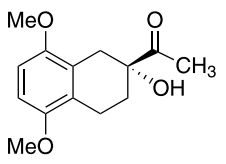
In this review, the generally accepted ring designations and numbering system, shown in Fig. 3, will be followed. The system will also be applied in the case of precursors of the tetracyclic anthracyclinone moiety, where numbering of the atoms will correspond to their position in the target molecule.

2
Resolution

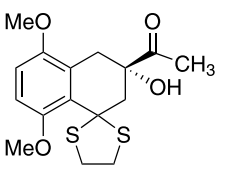
Early syntheses of enantiopure anthracyclines depended on glycosidation of the racemic anthracyclinone and separation of the diastereoisomeric products [19]. This approach entailed the loss of at least half of the valuable material during the last stage of the synthesis. The resolution of a racemic intermediate at an early stage of the synthesis is obviously much more expedient. It has been carried out with tetracyclic precursors of the aglycone or more often with AB building blocks. This line of research attracted much interest in the 1980s and resolution was a key step in several synthetic approaches to natural or modified aglycones. In more recent literature, due to the development of enantioselective syntheses, there are few examples of the use of resolution as a route to enantiopure aglycones.

2.1
Resolution of the AB Building Blocks

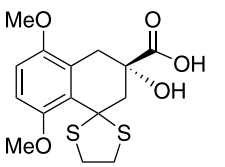
In the 1980s, three important AB building blocks were obtained by fractional crystallization of their diastereoisomeric derivatives (Fig. 4):



(*R*)-**10**



(*S*)-**11**

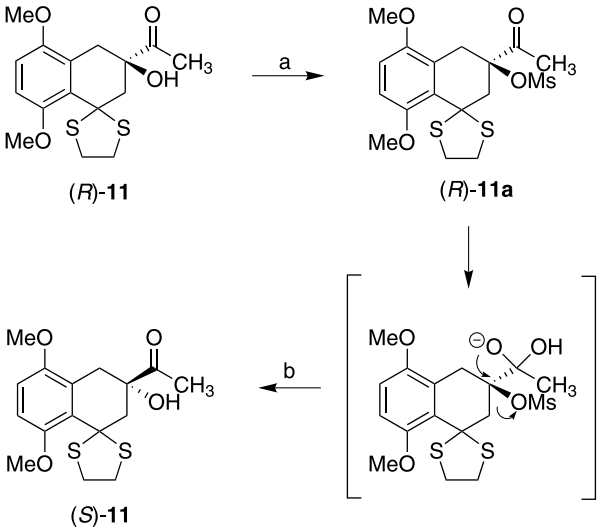


(*S*)-**12**

Fig. 4

- Acid (*S*)-**12** was separated as its brucine salt [20, 21] and subsequently converted into ketone (S)-**11**. Hydroxyketones (*R*)-**10** [22] and (*S*)-**11** [23] were obtained by separation of the mixture of diastereoisomeric imines obtained from *rac*-**10** or *rac*-**11** and (–)-(*S*)-phenylethylamine.
- Hydroxyketone (*R*)-**10** was used as a chiral substrate in the synthesis of daunorubicinone **7** and its analogue idarubicinone (4-demethoxydaunomycinone) **9** in the route developed by Wong et al. [24] in which the oxygen function at C-7 was introduced at a late stage of the synthesis.
- Hydroxyketone (*S*)-**11**, developed by Broadhurst et al. [20], has the advantage of already possessing functionality at C-7. It was used by Swenton et al. [23] in the synthesis of enantiopure aglycones, notably idarubicinone (**9**), the aglycone of idarubicin (**6**), a synthetic anthracycline important in cancer chemotherapy.

Recently, the advantage of using building block (*S*)-**11** was enhanced by the following findings. Firstly, recrystallization of the diastereoisomeric imines obtained from *rac*-**11** and (–)-(*S*)-phenylethylamine from THF raised the yield of (*S*)-**11** to 40% (from 25%) [25]. Secondly, a method of inversion of configuration at C-7 in ketone (*S*)-**11**, without loss of enantiomeric purity, was developed. Thus, the waste material from the separation of the diastereoisomeric imines could be utilized. Hydrolysis of the imines remaining after the separation of the desired (*S*,*S*)-diasteroisomer, afforded a mixture of ketones (*R*)-**11** and (*S*)-**11** in the ratio 70 : 30. Trituration of this mix-

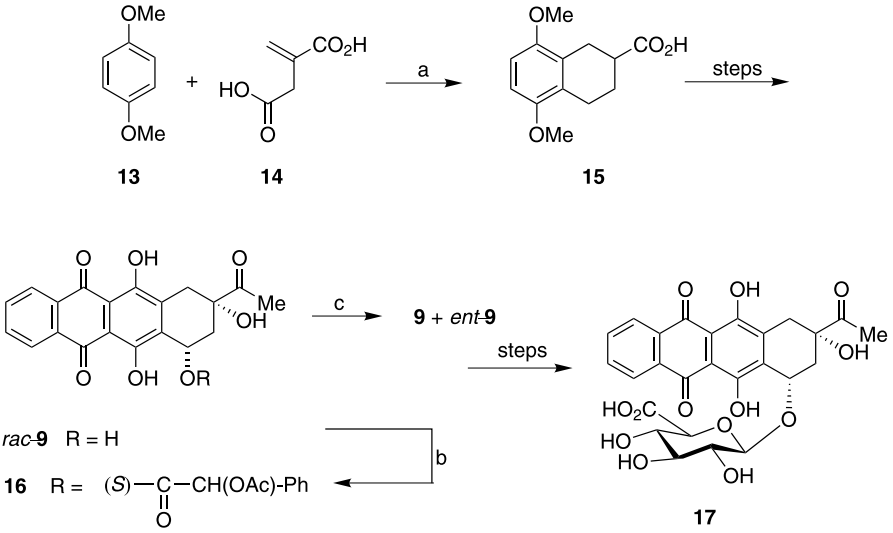


Scheme 1 Reagents and conditions: *a* MsCl, *N*,*N*,*N*′,*N*′-tetramethylhexane-1,6-diamine, DCM, 0 °C → rt, 4 h. *b* 20% $NaOH_{aq}$, Bu_4NHSO_4, DCM, rt, 2 h. Yield was 39% over two steps, 98.8% *ee*

ture with hot ethyl acetate and one recrystallization from the same solvent gave hydroxyketone (*R*)-**11** of 98% *ee* (chiral HPLC). Reaction of (*R*)-**11** with methanesulfonyl chloride in the presence of *N*,*N*,*N*′,*N*′-tetramethylhexane-1,6-diamine [26] gave methanesulfonate (*R*)-**11a**. This, without purification, was treated with sodium hydroxide in the biphasic system H_2O–CH_2Cl_2, in the presence of tetrabutylammonium hydrogensulfate, to give (*S*)-**11** in 40% yield and 99.5% *ee* according to chiral HPLC [27], presumably via the intramolecular substitution [28] shown in Scheme 1. Thus a marked improvement of the resolution of *rac*-**11** was achieved.

2.2 Resolution of Tetracyclic Precursors

In an attempt to improve drug efficacy, Rho et al. synthesized several idarubicin (**6**) analogues [29]. The total synthesis of idarubicin-7-β-D-glucuronide (**20**) involved a resolution of racemic idarubicinone (*rac*-**9**) obtained in the following manner [30]. Cyclization of 1,4-dimethoxybenzene (**13**) with 2-methylenesuccinic acid (**14**) in PPA afforded, after reduction of C-7 keto group, acid **15**, which was transformed by known methodology into racemic idarubicinone (*rac*-**9**). Esterification of *rac*-**9** with (*S*)-*O*-acetylmandelic acid and chromatographic separation of the resulting diastereoisomers **16** followed by hydrolysis afforded enantiopure idarubicinone **9** and *ent*-**9** (in Scheme 2 only one enantiomer or diastereoisomer is depicted).

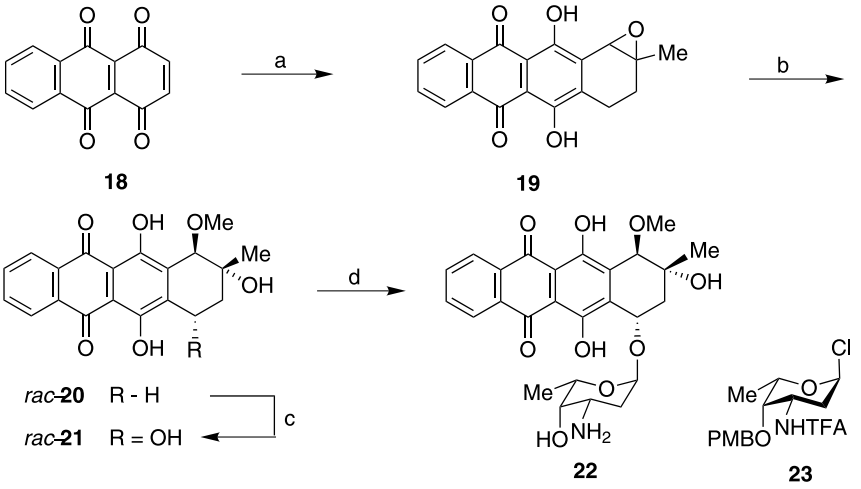


Scheme 2 Reagents and conditions: *a* (i) PPA, 100 °C, 10 min; (ii) $HSiEt_3$, TFA, 0 °C, 2 h. *b* (i) (+)-(*S*)-*O*-Acetylmandelic amide, DCC, DMAP, DCM, rt, 15 h, 87%. *c* (i) Separation of diastereoisomers by chromatography; (ii) 0.1 M NaOH

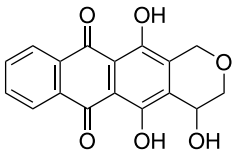
Reaction of both enantiomers, **9** and *ent*-**9** with methyl 2,3,4-tri-*O*-acetyl-D-glucopyranosyluronate bromide in the presence of $ZnBr_2$ in dichloromethane and subsequent removal of protecting groups yielded glucuronide **17** and its diasteroisomer, respectively.

Enantiopure anthracyclines can be obtained by the glycosidation of racemic aglycones and separation of the resulting diastereoisomers. This approach was employed in the synthesis of potential antitumor glycosides, structurally related to the anthracyclines [31]. A series of racemic aglycones was obtained as shown in Scheme 3. Diels–Alder addition of isoprene to anthracene-1,4,9,10-tetraone (**18**) led directly to a tetracyclic olefin. This afforded epoxide **19** after tautomerization upon exposure to sodium acetate, migration of the double bond by treatment with concentrated sulfuric acid, and epoxidation. The oxirane ring in **19** was opened with various nucleophiles (SEt, OAc, SOEt, SO_2Et, OMe). For example, reaction with methanol in the presence of PTSA gave 7-deoxyaglycone **20**. Oxidation of **20** with NBS in aqueous carbon tetrachloride in the presence of AIBN [32] stereoselectively introduced a hydroxyl group at C-7, yielding aglycone **21**. Glycosidation of *rac*-**21** with L-daunopyranosyl chloride **23** [19] gave a mixture of diastereoisomeric products, which afforded glycoside **22** after chromatographic separation and removal of the protecting groups (Scheme 3 depicts one enantiomer).

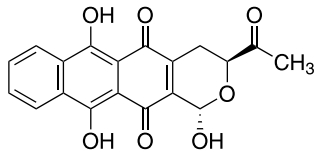
Resolution was also employed as a last step in the total synthesis of enantiomerically pure anthracycline oxaanalogues. Glycosidation of racemic agly-



Scheme 3 Reagents and conditions: *a* (i) Isoprene, 50 °C, PhH, 2 h; (ii) NaOAc, reflux, 2 min, 34% over two steps; (iii) conc. H_2SO_4, 0 °C, 2 min, 81%; (iv) MCPBA, $NaHCO_3$, DCM, 25 °C, 3 h, 99%. *b* MeOH, *p*-TsOH, reflux, 60%. *c* NBS, CCl_4, H_2O, 60 °C, 0.75 h, 51%. *d* (i) **23**, CF_3CO_2Ag, DMF–DCM, MS, rt, 1.5 h; (ii) chromatographic separation, 75%; (iii) Et_3N–H_2O-MeOH, rt, 10 min, 80%; (iv) 0.1 M NaOH, THF, 0 °C, 3 h, 66%



24



rac-**25**

Fig. 5

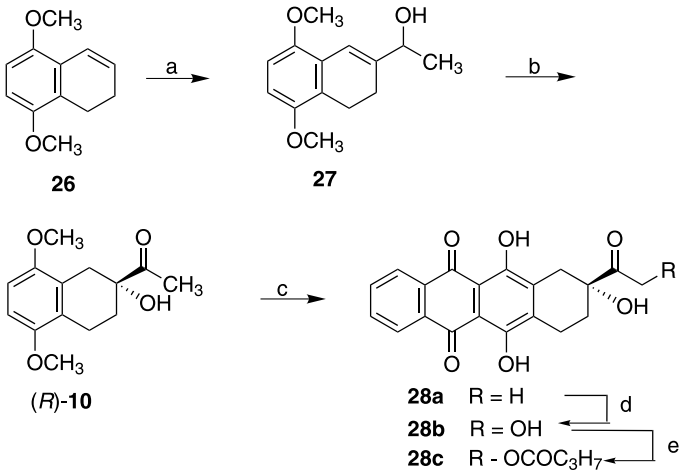
cones **24** [33] and **25** [34] (Fig. 5) and separation of the diastereoisomeric glycosides yielded enantiopure oxaanalogues of the anthracyclines.

3
Stereoselective Oxidation

Oxidative and reductive functionalization of achiral or racemic intermediates have been extensively used to produce enantiopure bicyclic and tetracyclic building blocks of anthracyclinones. These approaches included enantioselective reactions and kinetic resolution in Sharpless epoxidation, catalytic (OsO_4) dihydroxylation, stoichiometric epoxidation with the molybdenum(VI) oxodiperoxo complex with chiral ligands, as well as reduction of carbonyl groups with LAH modified by chiral additives. In spite of enormous progress in methods for enantioselective reduction of carbonyl groups [35, 36] these have not been utilized in the more recent anthracyclinone syntheses, with the exception of Baker's yeast reduction. Since the pioneering work of Wong [24, 37] and Arcamone [22] enantioselective catalytic epoxidation and dihydroxylation, as well as stoichiometric epoxidation and hydroxylation, have been utilized mainly for the preparation of hydroxyketone (*R*)-**10**, an important AB building block for the anthracyclinones.

3.1
Catalytic Epoxidation and Dihydroxylation

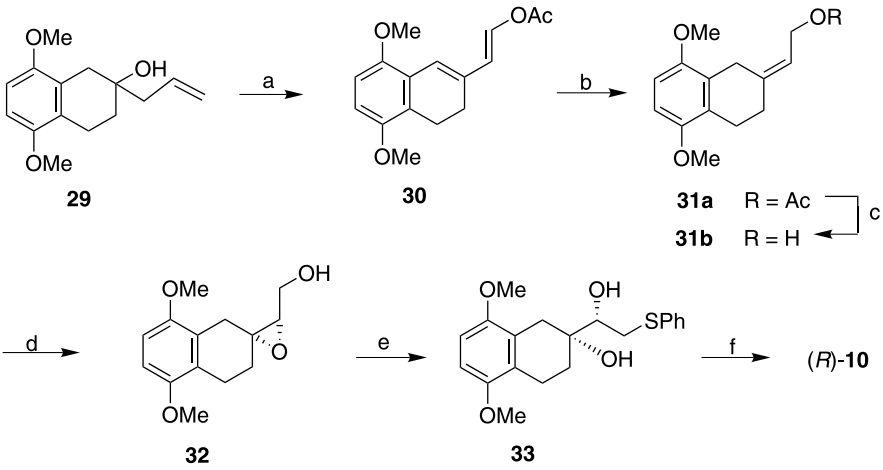
Rho et al. [38] used AB building block (*R*)-**10** [22] in the synthesis of 4-demethoxy-7-deoxydoxorubicine pentanoic acid ester (**28c**). (*R*)-**10** was obtained by the kinetic resolution of allylic alcohol **27** prepared in three steps (Scheme 4) from olefin **26** [39]. Sharpless epoxidation [40] of **27**, reductive cleavage of the oxirane ring with LAH, followed by oxidation with the Fetizon reagent [41] furnished hydroxyketone (*R*)-**10**. Friedel–Crafts acylation of (*R*)-**10** with phthaloyl dichloride [42] gave tetracyclic **28a**. Subsequent bromination of **28a**, followed by hydrolysis, led to aglycone **28b**, which was acylated to give the target ester **28c**. The hydroxyketone (*R*)-**10** was also obtained by a more efficient route involving enantioselective epoxidation or dihydroxylation.



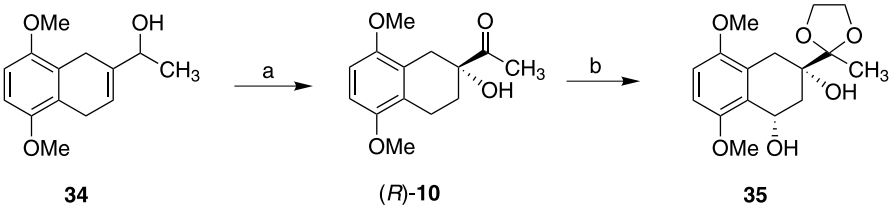
Scheme 4 Reagents and conditions: *a* (i) $POCl_3$, DMF, 0 °C, then reflux 2–3 h; (ii) MeMgBr, THF, 10 °C. 1 h, 66%. *b* (i) (+)-DIPT, $Ti(O-i-Pr)_4$, *t*-BuOOH, DCM, –70 →0 °C; (ii) LAH, THF, rt, 42% over two steps; (iii) Ag_2CO_3 on celite, PhH, reflux, 30 min, 78%. *c* Phthaloyl dichloride, $AlCl_3$, $PhNO_2$, 80–100 °C. *d* (i) PHT, THF, rt, 24 h; (ii) 1% NaOH, acetone, reflux 10 min, 69% over two steps. *e* $BuCO_2H$, DCC, DMAP, DMF, rt, 6 h, 90%

The success of the enantioselective Sharpless epoxidation to a large extent depends on the configuration of the double bond of an available substrate. Shibasaki et al. developed an efficient method of stereospecific synthesis of exocyclic double bonds using 1,4-hydrogenation of conjugated dienes catalyzed by an arene-$Cr(CO)_3$ complex [43]. Allylic alcohol **31b** [44, 45], a substrate for Sharpless epoxidation, was obtained using this procedure. Alcohol **29** [46] was regio- and stereoselectively transformed into the dienyl acetate **30** in 60% overall yield. The crucial step of the synthesis, 1,4-hydrogenation of **30**, was performed using a naphthalene·$Cr(CO)_3$ complex to afford stereochemically homogenous (*Z*)-allylic acetate **31a** in 91% yield. Hydrolysis of **31a** and Sharpless epoxidation of the resulting allylic alcohol **31b** gave epoxide **32** in 97% yield and 93% *ee*. Opening of the oxirane ring in **32** with benzenethiol and further transformations of the resulting sulphide **33** (shown in Scheme 5) afforded enantiopure AB building block (*R*)-**10** in 49% overall yield from **30**.

Kinetic resolution of another allylic alcohol in the Sharpless epoxidation was also employed in the synthesis of (*R*)-**10** and fully oxygenated AB building block **35** (Scheme 6) [47]. Epoxidation of alcohol **34** [48] followed by LAH reduction and then oxidation with the Fetizon reagent [41, 49] furnished hydroxyketone (*R*)-**10**. Ketalization of the carbonyl group and subsequent stereoselective benzylic hydroxylation, using iodosobenzene in the presence of [5,10,15,20-tetrakis(pentafluorophenyl)-21*H*,23*H*-porphine]iron(III) chlo-



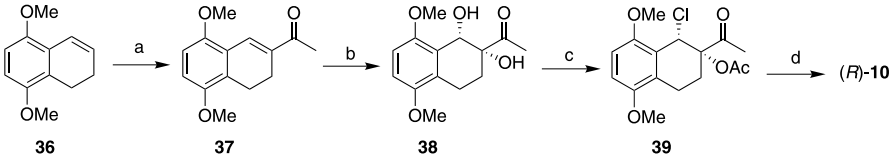
Scheme 5 Reagents and conditions: *a* (i) Ac_2O, py, DMAP, DCM, 12 h; (ii) OsO_4-Et_2O, $NaIO_4$aq, rt, 6.5 h; (iii) Ac_2O, Et_3N, DMAP, 80 °C, 50 min, 60% from **29**. *b* Naphthalene-$Cr(CO)_3$, acetone, H_2, 140 kg/cm^2, 45 °C, 13 h, 87%. *c* K_2CO_3, MeOH, rt, 1 h, 100%. *d* (+)-DET, Ti(*O* – *i* – Pr)$_4$, *t*-BuOOH, DCM, –42 °C, 2.5 h, 97%, 93.2% *ee*. *e* PhSH, 0.5 M NaOH, *t*-BuOH, 85–90 °C, 3 h, 76%. *f* (i) Raney-Ni (W-2), EtOH, reflux, 4.5 h, 90%; (ii) SO_3-py, DMSO, NEt_3, rt, 75 min, 84%



Scheme 6 Reagents and conditions: *a* (i) (–)-DET, Ti(*O* – *i*Pr)$_4$, *t*-BuOOH, DCM, –78 → 0 °C; (ii) LAH, THF, 0 °C, 43% over two steps; (iii) Ag_2CO_3, PhH, reflux, 80%. *b* (i) $(CH_2OH)_2$, *p*-TsOH, PhH, reflux, 96%; (ii) PhIO, TPFPFeCl, CH_2Cl_2, rt, 1 h, 60%

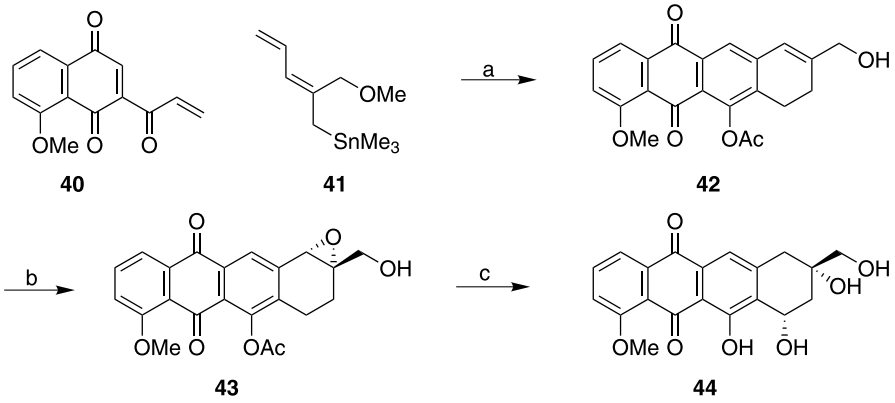
ride (TPTPFeCl) [50], afforded enantiopure AB segment **35** in 16% overall yield.

An Italian group [51, 52] showed that an alternative method, the Sharpless enantioselective dihydroxylation [53], was an efficient procedure for the preparation of AB block (*R*)-**10**. Thus enone **37**, obtained by acylation (AcCl-$AlCl_3$) of dihydronaphthalene **36** [54], on treatment with the enriched (1% $K_2OsO_4 \cdot 2H_2O$) AD-mix-α [55] gave diol **38** in 71% yield and 98% *ee* (Scheme 7). Reaction of **38** with trimethylorthoacetate and subsequent treatment of the intermediate cyclic orthoester with trimethylsilyl chloride afforded chloroacetate **39**. Reduction of **39** with tributyltin hydride, followed by hydrolysis, furnished enantiopure (*R*)-**10** in 52% overall yield (from **37**).



Scheme 7 Reagents and conditions: *a* AcCl, $AlCl_3$, DCM, 0 °C, 30 min, 70%. *b* AD-mix-α, 1% $K_2OsO_2(OH)_4$, H_2O-*t*-BuOH, $MeSO_2NH_2$, rt, 96 h, 71%, 98% *ee*. *c* (i) $MeC(OMe)_3$, PPTS, DCM, rt, 24 h; (ii) TBDMSCl, DCM, rt, 1 h, 99%. *d* (i) Bu_3SnH, AIBN, PhH, rt, 24 h; (ii) Amberlyst IRA-400(OH), MeOH, rt, 18 h, 73%

Preparation of enantiopure intermediates by the use of oxidation reactions was not limited to the synthesis of AB segment (*R*)-**10**. The synthesis of the modified daunomycinone, 9-deacetyl-11-deoxy-9-hydroxymethyldaunomycinone **44** by Naruta et al. provides an example of enantioselective epoxidation at the stage of a tetracyclic intermediate (Scheme 8) [56]. Tetracyclic quinone **42**, with the required stereochemistry of the C-4 and C-9 substituents and a protected phenolic hydroxyl group, was prepared in a tandem Michael–Diels–Alder addition of pentadienyltin with acryoylquinone **40** mediated by Lewis acid [57], followed by demethylation, acetylation, and selective hydrolysis. Sharpless enantioselective epoxidation [58] of **42** yielded epoxide **43** in 80% yield and 96% *ee*. Further transformations of **43** by known procedures, indicated in Scheme 8, furnished the target anthracyclinone **44** in 36% yield from **42**.

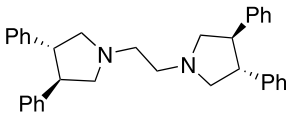


Scheme 8 Reagents and conditions: *a* (i) $(i\text{-PrO})_3TiCl$; (ii) O_2, 69%; (iii) $HClO_4$, Ac_2O-DCM, 0 °C, 5 min; (iv) H_2SO_4, MeOH-acetone, reflux 4 h, 51%. *b* $(i-PrO)_4Ti$, (+)-DET, *t*-BuOOH, MS 4A, DCM, −20 °C, 15 h, 80%, 96% ee. *c* (i) $Na_2S_2O_4$, $NaOH_{aq}$, 0 °C, 1 h; (ii) Ac_2O, py, DCM, rt, 12 h; (iii) Br_2, CCl_4, light, rt, 1 h; (iv) NaOH, H_2O, 0 °C, 1 h, 45%

Enantioselective dihydroxylations [59] and epoxidations [60] of various other tetracyclic systems related to anthracyclinones have also been studied.

3.2
Stoichiometric Epoxidation and Hydroxylation

Tomioka et al. [61, 62] demonstrated a highly enantioselective dihydroxylation of olefins using stoichiometric amounts of an osmium tetroxide complex with chiral diamine **45** (Fig. 6).



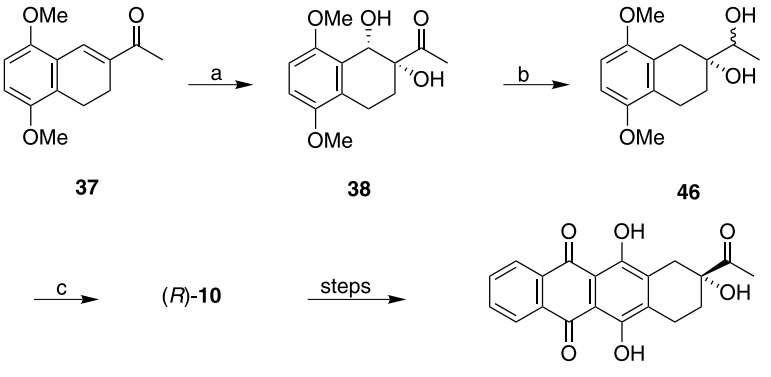
45

Fig. 6

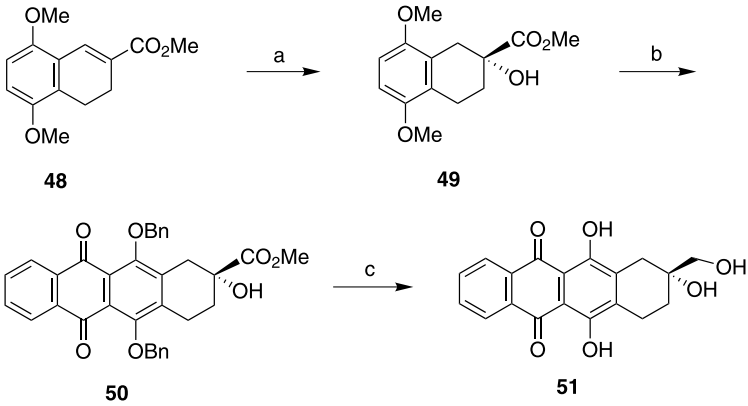
They utilized this reagent in the synthesis of two AB building blocks: (*R*)-**10** and **49**.

Reacting ketone **37** with 0.01 M osmium tetroxide complex with diamine **45** in THF (1.1 eq) at –110 °C, followed by reductive hydrolysis of the resulting osmate, provided diol **38** in 95% yield and high (82–85%) enantiomeric excess (Scheme 9), albeit lower than that achieved with the same substrate using α-AD-mix [55] (98% *ee* [51, 52]). Chromatography of the reaction mixture allowed the recovery of 95% diamine **45**. Reduction of the benzylic hydroxyl group and the carbonyl groups in **38**, followed by oxidation of **46**, yielded (*R*)-**10** in 65% overall yield. Hydroxyketone (*R*)-**10** was transformed by known procedures into 7-deoxyidarubicinone (**47**) (Scheme 9).

Similarly, oxidation–reduction of **48** gave hydroxyester **49** (Scheme 10). Annelation of **49** using phthalic dichloride, with concomitant demethylation



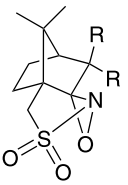
Scheme 9 Reagents and conditions: *a* (i) **45**, OsO_4, THF, –78 °C, then –110 °C, **37**, 6 h; (ii) $NaHSO_3$, H_2O, reflux, 14 h, 96%. *b* $HSiEt_3$, TFA, –20 °C, 12 h, 78%. *c* SO_3-py, NEt_3, DMSO, 87%



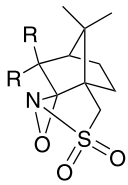
Scheme 10 Reagents and conditions: *a* (i) **45**, OsO_4, THF, −78 °C, then −110 °C, **37**, 6 h; (ii) $NaHSO_3$, H_2O, reflux, 14 h, 95%. *b* (i) $Ph(COCl)_2$, $AlCl_3$, 100 °C, 30 min; (ii) NaH, BnBr, TBAI, THF, 5 h. *c* (i) 3% KOH, THF, H_2O, 1 h; (ii) 1 M B_2H_6, THF, 0 °C, 4 h; (iii) Pd/C, H_2, dioxane-EtOH, rt, 61%

followed by benzylation, provided tetracyclic intermediate **50** that, after reduction of the ester function and removal of the benzyl groups, afforded anthracyclinone **51**.

Detailed studies by Davis et al. [63] demonstrated that enolate oxidation, using (camphorsulfonyl)oxaziridine (1*S*)-(+)-**52** or (1*R*)-(−)-**52** (Fig. 7) as a chiral oxidizing agent, was highly enantioselective. However, the yields and *ee*s of the hydroxylated products are strongly dependent on the structure of the substrate and oxaziridine as well as the reaction conditions (enolate counter-ion, solvent, temperature) [64].



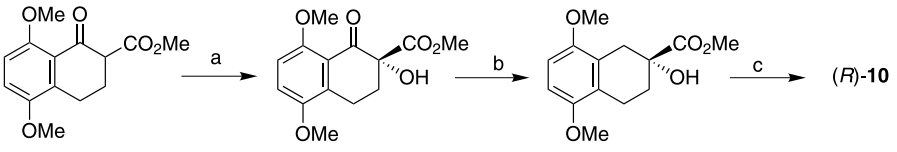
(1*S*)-(+)-**52a** R = H
(1*S*)-(+)-**52b** R = Cl
(1*S*)-(+)-**52c** R = OMe



(1*R*)-(−)-**52a** R = H
(1*R*)-(−)-**52b** R = Cl
(1*R*)-(−)-**52c** R = OMe

Fig. 7

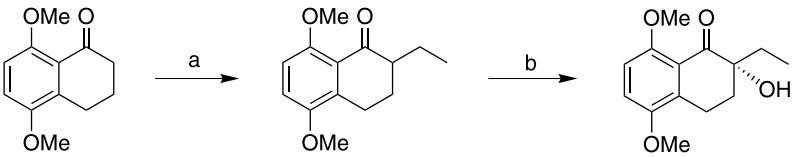
For the synthesis of AB-block (*R*)-**10**, the laevorotatory oxaziridine (1*R*)-(−)-**52** was required and the best results were achieved with (1*R*)-(−)-**52c** (Scheme 11) [65, 66]. The potassium enolate, obtained from the readily available ketoester **53** [67] by treatment with potassium bis(trimethylsilyl)amide (KHMDS), was reacted with oxaziridine (1*R*)-(−)-**52c** at -78 °C to give the hy-



Scheme 11 Reagents and conditions: *a* (i) KHDMS, THF, −78 °C, 30 min; (ii) (1*R*)-(−)-**52c**, THF, −78 °C, 5 h, 70%. *b* $HSiEt_3$, AcOH, 0 °C, 2 h, 97%. *c* (i) $CH_3S(O)CH_2Na$, 0 °C; (ii) Al/Hg, 66% yield, > 95% *ee*

droxylation product **54** (> 95% *ee*). Reduction of the carbonyl group in **54** furnished hydroxyester **49** that, in two steps (reaction with dimsyl sodium, and subsequent reduction with Al(Hg) [68]), afforded AB block (*R*)-**10** in 45% overall yield and > 95% *ee*.

The same type of chiral oxidizing reagent was used in the synthesis of γ-rhodomycinone, α-citromycinone [69] and aklavinone [70]. Enantioselective oxidation was applied in the synthesis of the known hydroxyketone **58** (Scheme 12), which was used previously as a key enantiopure intermediate in the synthesis of (−)-γ-rhodomycinone [71]. In this case the required sense of chirality was induced using the dextrorotatory oxaziridine (1*S*)-(+)-**52**, and the highest enantiomeric excess was obtained using (1*S*)-(+)-**52c** (Fig. 7). The latter was prepared from the imine **55** [72] (Fig. 8) by refluxing with trimethyl orthoformate and subsequent oxidation with 3-chloroperbenzoic acid in 91% yield [73].



Scheme 12 Reagents and conditions: *a* (i) 1M NaHMDS, THF, 0 °C, 30 min; (ii) EtI, −78 °C→ rt, 12 h 60%. *b* (i) 1 M LDA, THF, −78 °C, 30 min; (ii) (1*S*)-(+)-**52c**., THF; −78 °C→ rt, 66%, 94% *ee*

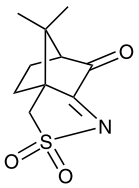


Fig. 8

Ketone **57** was obtained by reacting tetralone **56** [74] with excess iodoethane. Oxidation of the lithium enolate of **57** with oxaziridine (1*R*)-(–)-**53c** gave the target hydroxyketone **58** in 94% *ee* and 66% isolated yield.

4 Chiral Auxiliaries

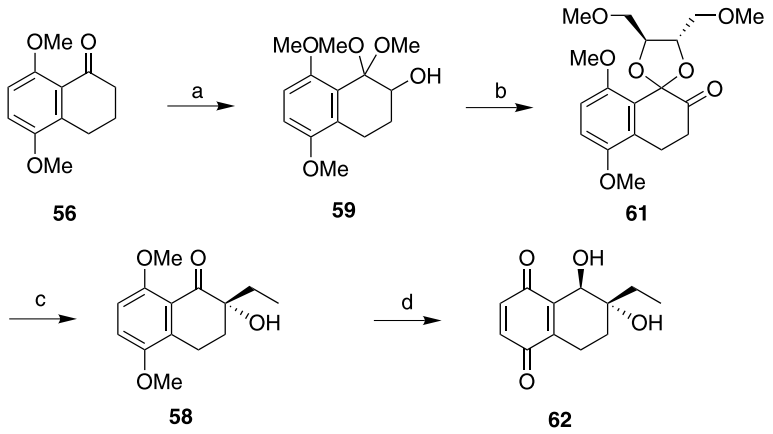
The attachment of a chiral auxiliary to a substrate is a well-established strategy for achieving a diastereoselective transformation, a key step on the route to enantiomerically pure product [75]. In the field of anthracyclinone chemistry, several chiral auxiliaries have been used and diastereodifferentiation has been realized in various reactions: the aldol condensation of (*R*,*R*)-butane-2,3-diol acetals, alkylation of α-ketoacetals of (2*S*,3*S*)-1,4-dimethoxybutane-2,3-diol and oxazolines derived from chiral aminoalcohols, bromolactonization of (*S*)-proline derivatives or (2*R*,3*R*)-tartaric acid amides, and Diels–Alder reactions with a chiral auxiliary located in the diene or dienophile. In general, good diastereoselectivities were achieved, yielding target compounds of high enantiomeric excess. These results have been comprehensively discussed by Krohn and Ekkundi [18]. More recently the previously used (2*S*,3*S*)-1,4-dimethoxybutane-2,3-diol and new chiral auxiliaries have been utilized for the synthesis of enantiomerically pure AB building blocks.

In the enantioselective synthesis of anthracyclinone AB building blocks Japanese workers continue to successfully exploit their methodology based on the addition of Grignard reagents to the α-carbonyl group of the ketals obtained from tetralone and (2*S*,3*S*)-1,4-dimethoxybutane-2,3-diol (**60**) [76]. This highly diastereoselective reaction was applied in the synthesis of (–)-7-deoxydaunomycinone [77, 78]. Later it was extended to the synthesis of AB segment **58**, an intermediate in the first enantioselective synthesis of (–)-γ-rhodomycinone (**65**) [71, 79].

On treatment with phenyliodine(III) diacetate and potassium hydroxide in methanol [80], tetralone **56** gave the hydroxyketone acetal **59** (Scheme 13). Trans-ketalization of **59** with (2*S*,3*S*)-1,4-dimethoxybutane-2,3-diol (**60**), followed by oxidation with pyridinium dichromate (PDC) [80], yielded oxoketal **61** [76].

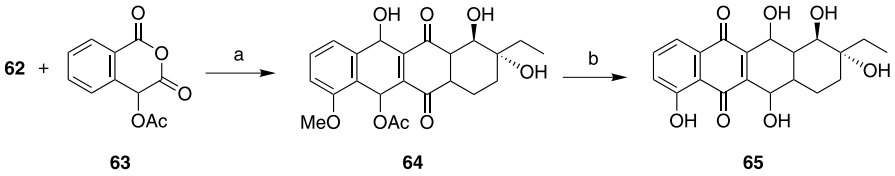
Reaction of **61** with ethylmagnesium chloride in THF at –78 °C was fully stereoselective yielding a single product that furnished hydroxyketone **58** after hydrolysis. Reduction of **58** with potassium borohydride gave a mixture of diols in the ratio 15 : 2, from which a major isomer was isolated by column chromatography and oxidized with ceric ammonium nitrate (CAN) to quinone **62** in 40% overall yield (Scheme 13).

The tetracyclic moiety of the aglycone was assembled by the base-induced coupling of quinone **62** with 4-acetoxy-5-methoxyhomophthalic anhydride (**63**) [82]. Thus, anhydride **63** on treatment with sodium hydride and reaction



Scheme 13 Reagents and conditions. *a* $PhI(OAc)_2$, KOH, MeOH, 0 °C, 3 h. *b* (i) **60**, *p*-TsOH, DCM, 10 min, rt, 72%; (ii) PDC, MS 3A, Ac_2O, DCM, rt, 2 h, 90%. *c* (i) EtMgCl, THF, –78 °C, 5 h, 93%, 100% *ee*; (ii) conc. HCl-THF (1 : 3), 50 °C, 1 h, 92%. *d* (i) KBH_4, MeOH, –78 °C; 30 min, then 0 °C overnight; (ii) CAN, H_2O-MeCN, 0 °C, 1 h, 87%

with quinone **62** at 0 °C gave a 55% yield of tetracyclic adduct **64** as the sole product. The remarkably high regioselectivity of the coupling reaction was demonstrated by carrying out the analogous reaction with the 8-methoxy isomer of **63**, which also led to a single product isomeric with **64** and with the methoxy group at C-1 (Scheme 14).



Scheme 14 Reagents and conditions. *a* NaH, THF, 0 °C, 30 min, 55%. *b* (i) TFA-H_2O (2 : 1), 50–55 °C, 2 h, 93%; (ii) $AlCl_3$, PhH, rt, 2.5 h, 66%

The previously noted regioselectivity [77, 78] of the coupling reaction between homophthalic anhydride and quinones depends on the presence of the bromine substituent in the quinone moiety. In the absence of the bromine, the extremely high regioselectivity is attributed to the strong intramolecular hydrogen bonding (Fig. 9) that polarizes the quinone double bond and directs the regiochemistry of addition.

Deacetylation of adduct **64** and a shift of the quinone moiety to ring C was accomplished by treatment with aqueous trifluoroacetic acid, followed by demethylation with aluminum chloride to give the target aglycone, (–)-γ-rhodomycinone (**65**), in 34% yield from **62**.

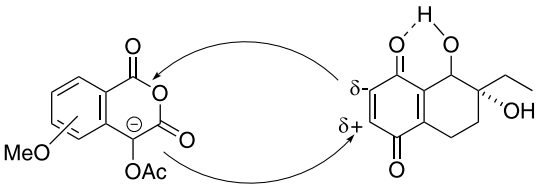
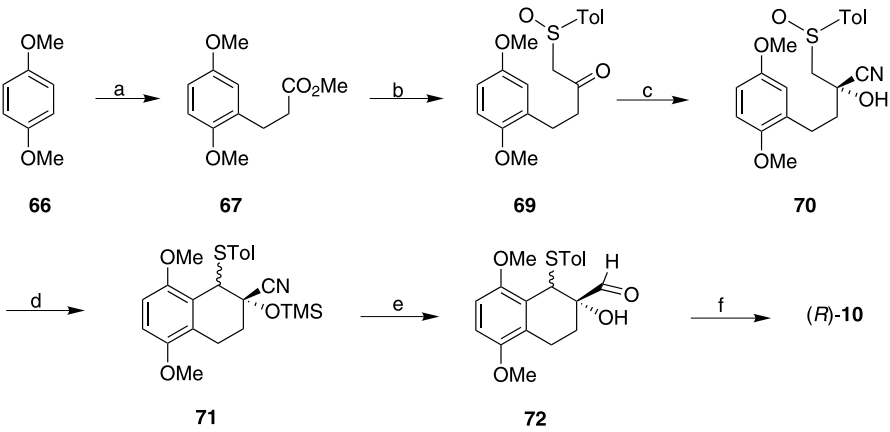


Fig. 9

Ruano and coworkers, relying on their extensive studies of the use of chiral sulfoxides in enantioselective synthesis [83–85], introduced the sulfinyl group as a new chiral auxiliary for the synthesis of enantiopure AB building blocks [86–88]. In their approach, the sulfur substituent served two purposes: to induce the required configuration at C-9 and to facilitate the cyclization reaction completing the construction of the AB segment.

The required ketosulfoxide **69** (Scheme 15) was obtained in two steps in a one-pot procedure from 1,4-dimethoxybenzene (**66**). *Ortho* lithiation of **66**, lithium–copper exchange, and addition to methyl acrylate gave ester **67**. Reaction of the anion obtained from (*R*)-methyl(*p*-tolyl)sulfoxide (**68**) [89] by treatment with LDA, with ester **67** afforded ketone **69**. Diastereoselective addition of diethylaluminum cyanide to the carbonyl group of **69** [90] furnished sulfinylcyanohydrin **70** as the sole product. The (*S*) configuration of the new stereogenic center was inferred from the previous studies of acyclic β-ketosulfoxides [91, 92].

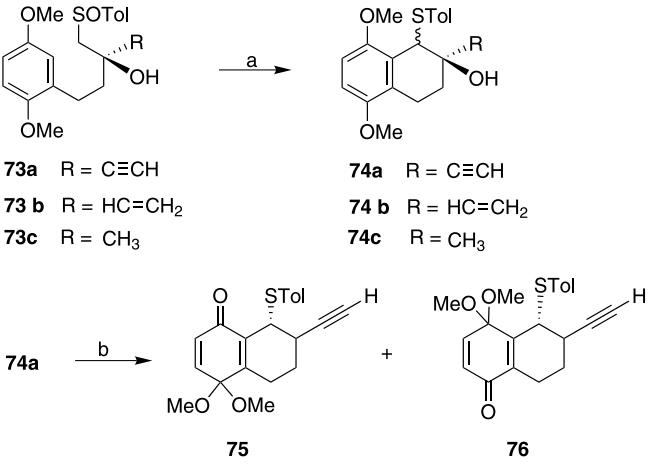


Scheme 15 Reagents and conditions: *a* (i) BuLi, THF, rt, 1 h; (ii) CuI, THF, 0 °C, 1 h; (iii) TMSCl, $CH_2 = CH - CO_2Me$, –78 °C→ rt, 2 h, 70%. *b* **68**, LDA, THF, –78 °C, 90 min, 80%. *c* (i) Et_2AlCN, THF, Ph-Me, 5 min; (ii) conc. HCl-MeOH (1 : 1), –78 °C, 15 min, 97%. *d* (i) TMSOTf, DIPEA, DCM, 0 °C, 30 min, 97 °C. *e* DIBAL-H, Ph-Me, rt, 1 h, then 5% H_2SO_4; *f* (i) MeMgBr, THF, 0 °C, 4 h, 68%; (ii) Raney-Ni, EtOH, rt, 90 min, 90%; (iii) SO_3·py, Et_3N, DMSO, 56%

The intramolecular capture of the thionium cation, generated by treatment of sulfoxide **70** with trimethylsilyl trifluoromethanesulfonate (TMSOTf) and *N*,*N*-diisopropylethylamine (DIPEA) [93], furnished cyanohydrin **71** as a 9 : 1 epimeric mixture at C-10, with a protected tertiary hydroxyl group. DIBAL reduction of the cyano group in **71**, followed by hydrolysis, yielded aldehyde **72**. The addition of methylmagnesium bromide to **72**, then Raney-Ni reduction of the tolylsulfanyl group and subsequent oxidation of the secondary hydroxyl group [94] furnished the AB segment (*R*)-**10**.

Other AB building blocks were also synthesized from aldehyde **72** by transformation of the formyl group into hydroxymethyl, ethyl, and methyl groups.

Reaction of the key intermediate, ketosulfoxide **69**, with ethenylmagnesium bromide, vinylmagnesium bromide, and trimethylaluminum in the presence of zinc bromide afforded the respective tertiary alcohols **73** in good yields (Scheme 16); however, with much lower diastereoselectivity (70–80% *de*) than in the case of cyanohydrin **70**.

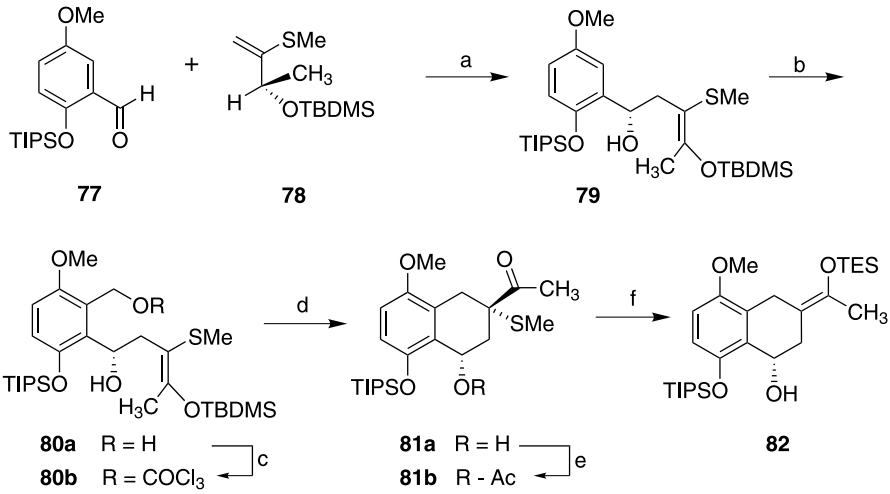


Scheme 16 Reagents and conditions. *a* (i) TMSOTf, DIPEA, DCM, 0 °C → rt, 30 min; (ii) $NaHCO_3$, H_2O. *b* (i) KOH, MeOH, 0 °C, Pt/Cu, 100 mA, 85%; (ii) AcOH, acetone, 30 °C, 2 h, 37%

Analogous cyclization of **73** under Pummerer conditions yielded the corresponding tetrahydronaphthol derivatives **74**. Their utility as precursors of the AB building blocks was demonstrated by their conversion into monoketals to ensure a regioselective annelation of the CD segment. For example, anodic oxidation of **74a** at 0 °C and subsequent partial hydrolysis afforded a mixture of monoketals **75** and **76** in the ratio 3 : 1, from which the major isomer was separated by chromatography in 37% yield [88].

An elegant route to AB building blocks with 14-CH_3 and 14-CH_2OH groups has been developed by Kuwajima and coworkers [95]. In their ap-

proach, the decisive chirality transfer occurred in the carbonyl-ene reaction [96]. Reaction of 5-methoxy-2-(triisopropylsilyloxy)benzaldehyde (**77**) with (*S*)-3-(*tert*-butyldimethylsilyloxy)-2-methylthiobut-1-ene (**78**) in the presence of dimethylaluminum chloride [97] gave the ene adduct **79** in 95% yield and 99% *ee* (measured on a degradation product) (Scheme 17).

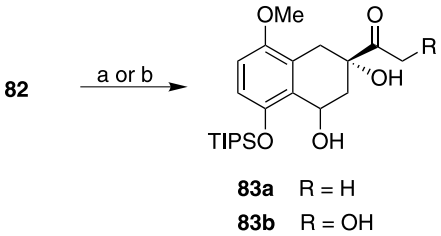


Scheme 17 Reagents and conditions: *a* Me_2AlCl, Ph-Me, –78 °C, 95%, 99% *ee*. *b* BuLi, hexane, 0 °C, then $(CH_2O)_n$, rt, 78%. *c* $(CCl_3)_2CO$, Me_2NEt, MeCN-DCM, 0 °C. *d* Me_2AlCl, DCM, –78 °C. *e* Ac_2O, py, DMAP, rt, 97%. *f* (i) Zn, TESCl, Et_2Zn, THF, reflux, 86%; (ii) DIBAL, DCM, –78 °C, 99%

Regioselective lithiation of **79** and subsequent treatment with paraformaldehyde furnished hydroxymethyl derivative **80a**. To enable ring closure, the primary hydroxy group was converted into a good leaving group. Reaction of **80a** with hexachloroacetone and ethyldimethylamine [98] gave trichloroacetate **80b** (Scheme 17). Treatment of **80b** with dimethylaluminum chloride at –70 °C facilitated cyclization, resulting in the formation of bicyclic ketone **81a** as a single stereoisomer. The (7*S*,9*S*)-configuration of **81a** was established by X-ray crystallographic analysis. After acetylation of the hydroxy group in **81a**, the resulting acetate **81b** was converted into silyl ether **82** in 85% overall yield by heating with activated zinc and triethylsilyl chloride in the presence of diethylzinc, followed by the removal of acetyl group with DIBAL.

Two AB building blocks have been obtained by oxidation of **82** with *tert*-butylhydroperoxide and a vanadium catalyst (Scheme 18). In a toluene at 0 °C, the reaction gave ketodiol **83a** in 69% yield, whereas in dichloromethane it furnished ketotriol **83b** in 37% yield.

Enantioselective syntheses of anthracyclinone tetracyclic intermediates or AB building blocks have also been carried out by Diels–Alder reactions uti-



Scheme 18 Reagents and conditions: *a* *t*-BuOOH, $VO(acac)_2$, MS 4A, Ph-Me, 0 °C, 69%. *b* *t*-BuOOH, $VO(acac)_2$, MS 4A, DCM, 0 °C, 37%

lizing chiral auxiliaries located in either the dienophile, e.g. [99, 100], or the diene, e.g. [101–103]. However, these approaches are the subject of the chapter by Pierre Vogel in this volume.

5
Chiral Pool

The incorporation of chiral molecular fragments, obtained from natural sources (the chiral pool) into target molecules is a firmly established, useful methodology in the syntheses of enantiomerically pure compounds, in particular complex natural products [104]. In the early days of anthracyclinone chemistry, chiral building blocks derived from sugars, amino acids, and hydroxy acids have been utilized [18]. More recently, only sugars have been used. Now approaches have appeared based on the chiral substrates arising from biosynthetic (microbial and enzymatic) transformations. Quinic acid (**84**) represents a novel source of chirality of natural origin and is used in the synthesis of an A-ring building block [105].

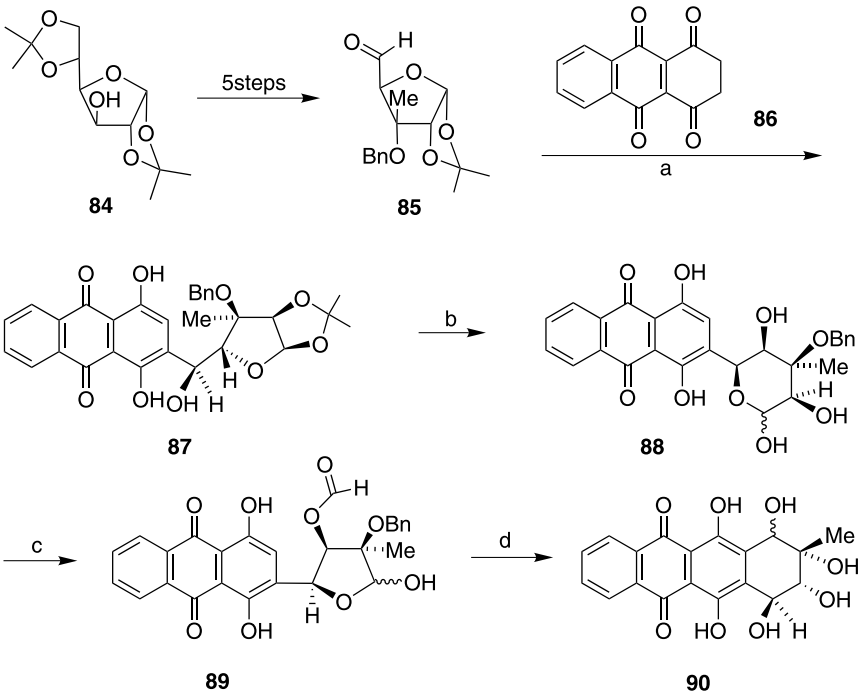
Much of the work carried out with carbohydrates is an extension of the strategies initiated in the earlier period. The use of chiral fragments derived from D-glucose, α-D-isosaccharino-1,4-lactone and D-glucosaccharinolactone, continues in the synthesis of enantiopure AB building blocks as well as for annelation to leucoquinizarine, resulting in an anthracyclinone tetracyclic moiety with defined ring A stereochemistry.

5.1
Sugars

The possibilities of attaching a sugar unit by a modified Marshalk reaction [106] were explored by Shaw et al. [107] as the first step on the route to the anthracyclinones fully substituted in ring A. The occurrence of the natural anthracyclines aranciamycin and steffimycin [7], fully substituted with oxygen functions and a methyl group at C-9 in ring A, was an incentive to use the

above method to obtain a series of this type of anthracyclinone, e.g., **90** [108] (Scheme 18).

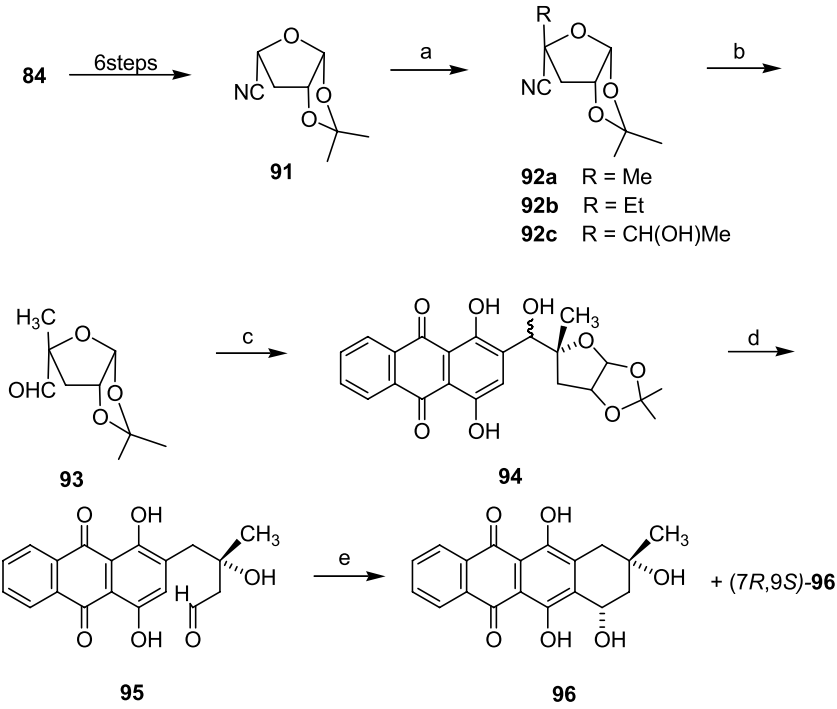
The sugar aldehyde **85**, obtained in five steps from 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose (**84**), was used to introduce a methyl substituent into the aglycone at C-9 [109] (Scheme 19). Base-catalyzed condensation of the furanose **85** with leucoquinizarine (**86**) followed by aerial oxidation gave derivative **87** with the (7*S*) configuration. Heating **87** with aqueous acetic acid removed the isopropylidene group and produced the pyranone derivative **88**. Periodate oxidation of **88**, followed by cyclization of the resulting hemiacetal **89** with sodium dithionite and subsequent removal of the benzyl group with boron trichloride, furnished the target anthracyclinone **90** in 16% overall yield.



Scheme 19 Reagents and conditions: *a* NaOH, MeOH-THF, –10 °C, 1 h then air, 1.5 h, 37%. *b* AcOH-H_2O (7 : 3), reflux, 2.5 h, 99%. *c* $NaIO_4$, AcOH-H_2O (7 : 3), rt, 5.5 h, 98%. *d* (i) 7% NaOH, 0 °C, 1.5 h then $Na_2S_2O_4$, 1.5 h then air 2 h; (ii) BCl_3, $CHCl_3$, –78 °C, 46% over two steps

An Italian group [110] obtained another useful anthracyclinone ring A precursor, the alkylated sugar cyanide **92**, from 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose (**84**). Cyanide **91** was prepared in six steps from the readily available furanose **84** [111]. Alkylation of the carbanion generated from

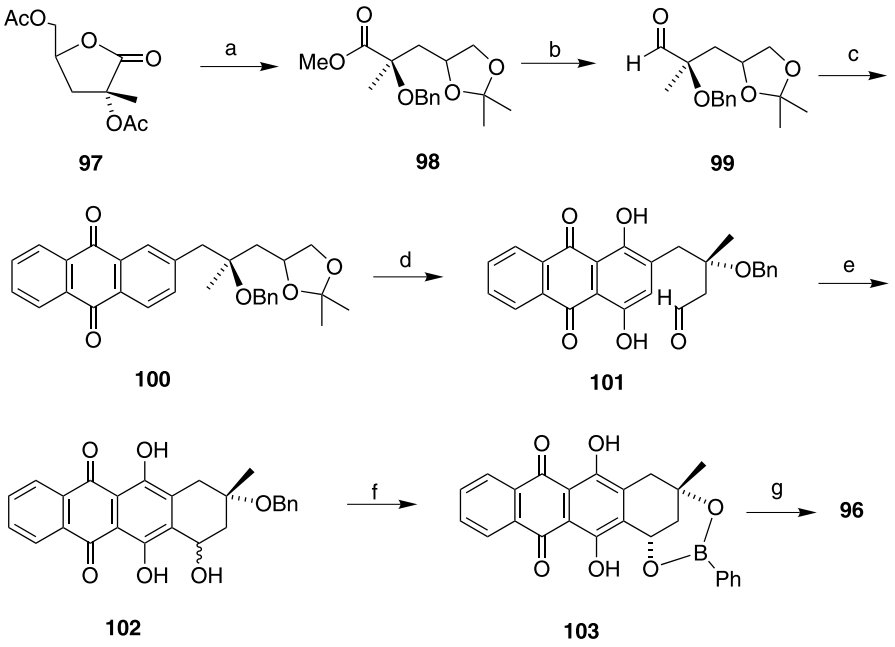
91with LDA or KHMDS, proceeded stereoselectively from the less hindered β-face of the furanose ring, yielding the branched sugars **92**. The configuration of **92a** was confirmed by X-ray analysis. The utility of the alkylated sugars **92** was demonstrated by the synthesis of 4-demethoxyfeudomycin C (**96**) (Scheme 20).



Scheme 20 Reagents and conditions: *a* KHMDS, THF, −78 °C, electrophile, 84% (**92a**), 80% (**92b**), 55% (**92c**). *b* Raney-Ni, AcOH-H_2O. *c* **86**, DMF, rt, air, 74%. *d* (i) $Na_2S_2O_4$, DMF-H_2O, 85%; (ii) AcOH-H_2O (4 : 1), reflux, 90%; (iii) $NaIO_4$, AcOH-H_2O (1 : 1), 80%. *e* 7% NaOH, $Na_2S_2O_4$, THF-H_2O then air, 82%

Reductive hydrolysis with Raney nickel [112] yielded aldehyde **93**, which was reacted with leucoquinizarine (**86**) with aeration to give the epimeric mixture **94**. Reduction of the benzylic hydroxy group in **94** with sodium dithionite followed by hydrolysis of the isopropylidene protection and subsequent periodate oxidation furnished aldehyde **95**. Sequential treatment of **95** with sodium dithionite in 7% NaOH and air oxidation provided a 4 : 1 C-7 epimeric mixture of anthracyclinones. The major diastereoisomer, 4-demethoxyfeudomycinone C (**96**) was isolated by column chromatography.

Another synthesis of 4-demethoxyfeudomycinone C (**96**) [113] used furanone **97** prepared from isosaccharinic acid [114]. Methanolysis of **97** followed by reaction with 2,2-dimethoxypropane gave ester **98** (Scheme 21). Protection



Scheme 21 Reagents and conditions: *a* (i) MeOH, rt, 12 h, 82%; (ii) $Me_2C(OMe)_2$, MeOH, Amberlyst 15, rt, 48 h, 62%; (iii) NaH, DMF, –20 °C, then BnBr, –20 °C, 4 h, 82%. *b* DIBAL-H, Ph-Me, –78 °C, 3 h, 75%. *c* **86**, AcOH, piperidine, *i*-PrOH, 3 h, 75%. *d* (i) AcOH (9 : 1), rt, 20 h; (ii) $NaIO_4$, CH_2Cl_2, rt, 20 h, 78% over two steps. *e* $Na_2S_2O_4$, $NaOH_{aq}$, MeOH-THF, –20 °C→ rt, then aeration, 67%. *f* (i) BBr_3, DCM, –78 °C, 5 h, 62%; (ii) $PhB(OH)_2$, *p*-TsOH, Ph-Me, rt, 48 h, 50%. *g* CH_3-$C(CH_3)(OH)$-CH_2-$CH(OH)$-CH_3

of the hydroxy group in **98** followed by DIBAL-H reduction gave aldehyde **99** in 31% overall yield. Coupling of **99** with leucoquinizarin (**86**) according to the Lewis procedure [115] yielded adduct **100**.

Removal of the isopropylidene group of **100** with aqueous acetic acid and subsequent periodate cleavage of the resulting diol led to aldehyde **101**. The intramolecular Marschalk reaction [106], unlike the analogous cyclizations catalyzed by Lewis acid [116], proceeded with low stereoselectivity yielding **102** as a mixture of the epimers at C-7 in the ratio 6 : 4. Deprotection of the benzyl group of **102** allowed separation of the (7*S*,9*S*)-diastereoisomer (*cis*-diol) as the phenylboronic ester **103**. Cleavage of the boronate with 2-methyl-2,4-pentanediol gave 4-demethoxyfeudomycinone C (**96**) in 12% yield from **99**.

Monneret and coworkers continue to exploit α-D-isosaccharino-1,4-lactone (**107**) (Fig. 10), available by degradation of lactose with calcium hydroxide, [117], as a source of chiral fragments (e.g., **108** and **109**) for incorporation into AB-ring segments or tetracyclic anthracyclinone moieties.

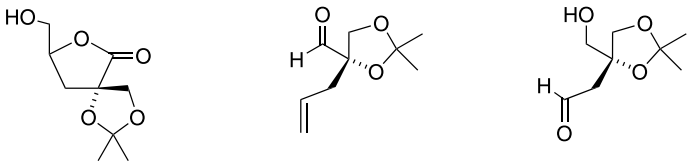
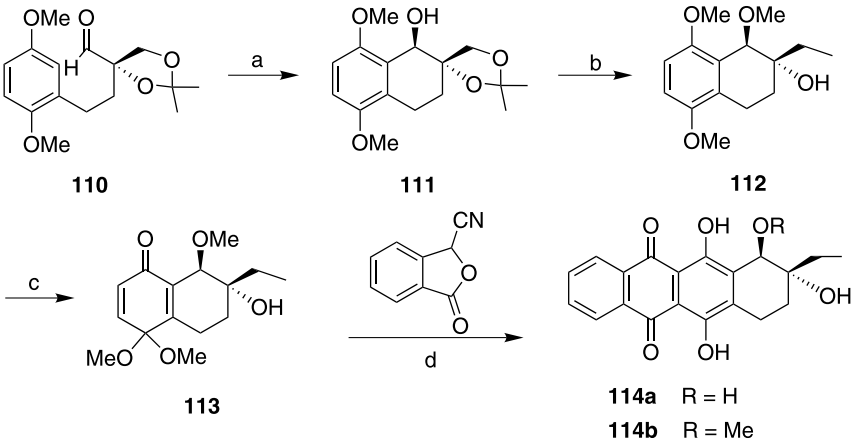


Fig. 10

In studies towards the synthesis of 4-deoxy-γ-rhodomycinone (**114a**), the steric course of the cyclization of aldehyde **110** was reexamined [118]. Aldehyde **110** was obtained as before, via condensation of aldehyde **109** with leucoquinizarine (**86**) [116] (Scheme 22). Reaction of **110** with tin tetrachloride gave tetralin **111**, with the (*R*) configuration at C-10, as the major product (epimeric ratio 5 : 1). The observed stereoselectivity was attributed to the prevalence of the complex (Fig. 11) with the Lewis acid chelated by the



Scheme 22 Reagents and conditions: *a* $SnCl_4$, DCM, −78 °C, 2 h, 85%. *b* (i) MeI, DMSO, KOH; (ii) AcOH-H_2O (4 : 1), 80% for two steps; (iii) MsCl, py, 65%; (iv) Me_2CuLi, Et_2O, 0 °C, 76%. *c* (i) Anodic oxidation: 1% KOH, MeOH, Pt, 1.3 V, 2 h; (ii) AcOH-H_2O, 66%. *d* (i) LDA, HMPT, THF, −78 °C→ rt, 2 h, 50%; (ii) BCl_3, DCM, −78 °C, 98%

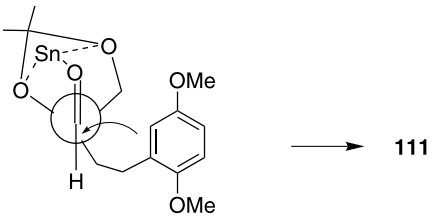
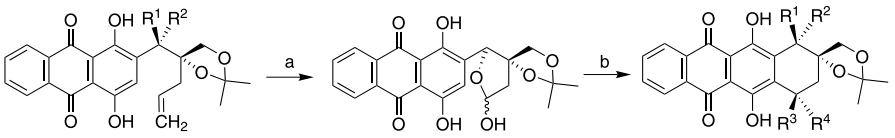


Fig. 11

α- and β-alkoxy oxygen atoms [119]. Protection of the C-10 hydroxy group in **111**, followed by hydrolysis and subsequent one carbon elongation of the C-9 substituent (mesylation and treatment with Me_2CuLi) yielded tetralin **112**.

Conversion of **112** into hemiketal **113** and its regioisomer, by known procedures [23, 120], followed by annelation of the mixture with the anion of 3-cyano-1(3*H*)-isobenzofuranone (**115**) and then deprotection of the phenolic hydroxy groups, furnished *O*-methyl-4-deoxy-γ-rhodomycinone (**114b**) in 11% overall yield. Cleavage of the *O*-methyl group was not achieved.

A French group [121] examined the steric course of the intramolecular Marschalk reaction. Condensation of leucoquinizarine (**86**) and aldehyde **109** using DBU in THF [122] yielded a mixture (3 : 1) of carbinols **116a** and **116b**, which were separated by chromatography (Scheme 23). The configuration assignment of the epimers **116a** and **116b** is based on the comparison of their CD spectra with those of analogous compounds of known stereochemistry. Oxidation (OsO_4-$NaIO_4$) of the methylene group in **116a** produced an aldehyde as its hemiacetal **117**. Intramolecular cyclization of **117**, under Marschalk conditions [123] afforded anthracyclinone (7*S*,10*S*)-**118a** as the sole product. When **116b** was used as a starting material, the same sequence of reactions gave anthracyclinone (7*R*,10*R*)-**118b**, with high stereoselectivity, demonstrating that the diastereoselectivity of the cyclization is governed by the configuration of the hydroxy group at C-10.



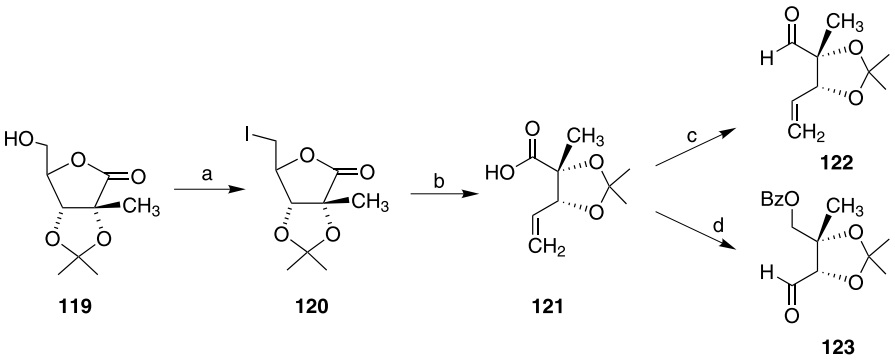
116a R^1 = H, R^2 = OH
116b R^1 = OH, R^2 = H

117

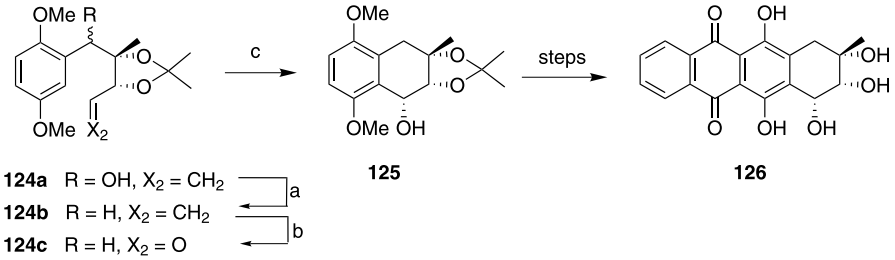
118a $R^1 = R^3$ = H, $R^2 = R^4$ = OH
118b $R^1 = R^3$ = OH, $R^2 = R^4$ = H

Scheme 23 Reagents and conditions: *a* OsO_4-$NaIO_4$, 100%. *b* NaOH-$Na_2S_2O_4$, THF-MeOH (1 : 1), –20 °C, 5 min, 81%

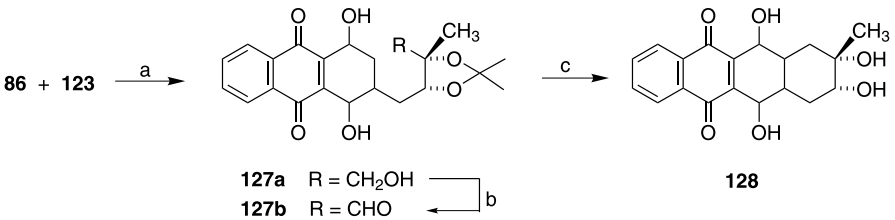
Monneret and coworkers [124] utilized yet another sugar compound as a source of chiral substrates. The isopropylidene derivative of α-D-glucosaccharino-1,4-lactone (**119**), readily available by alkaline treatment of fructose [125], was transformed into iodide **120**. Fragmentation of **120** with Zn/acetic acid according to the Vasella procedure [126], gave a high yield of acid **121**, which was transformed in three steps (esterification, reduction, oxidation) into aldehyde **122**, and in four steps (esterification, reduction, benzoylation, double bond scission) into aldehyde **123** (Scheme 24). Aldehydes **122** and **123** were used, respectively, for the construction of an AB building block (Scheme 25) and as a ring A segment of a tetracyclic anthracyclinone moiety (Scheme 26).



Scheme 24 Reagents and conditions: *a* (i) *p*-TsCl, py; (ii) NaI, EtCOEt,; (iii) reflux 3 h, 95%. *b* Zn, AcOH, THF, H_2O, rt, 3 h, 95%. *c* (i) CH_2N_2, Et_2O, 100%; (ii) LAH, THF, 0 °C, rt, 98%; (iii) DMSO, TFAA, DCM, −60 °C→ rt then NEt_3, 80%. *d* (i) CH_2N_2, Et_2O, 100%; (ii) LAH, THF, 0 °C, rt, 98%; (iii) BzCl, py, rt, 1 h, 95%; (iv) OsO_4, $NaIO_4$, Et_2O-H_2O-*t*-BuOH, 0 °C, 24 h, 80%



Scheme 25 Reagents and conditions: *a* CS_2, NaH, THF, 0 °C, 1.5 h, then Bu_3SnH, AIBN, Ph-Me, reflux, 2.5 h, 60%. *b* OsO_4, $NaIO_4$, Et_2O-*t*-BuOH-H_2O, rt, 48 h, 80%. *c* $SnCl_4$, DCM, −78 °C, 2 h, 44%



Scheme 26 *a* DBU, DMF, $Na_2S_2O_4$, 80 °C, 0.5 h, then 1 M NaOH, 1 h, 60%. *b* NCS, DMSO, −25 °C, Ph-Me, 2 h, then NEt_3, rt, 12 h, 90%. *c* (i) $Na_2S_2O_4$, KOH, H_2O-THF-MeOH, 0 °C, 0.5 h, air, 60%; (ii) AcOH-H_2O (8 : 2), 90 °C, 3 h

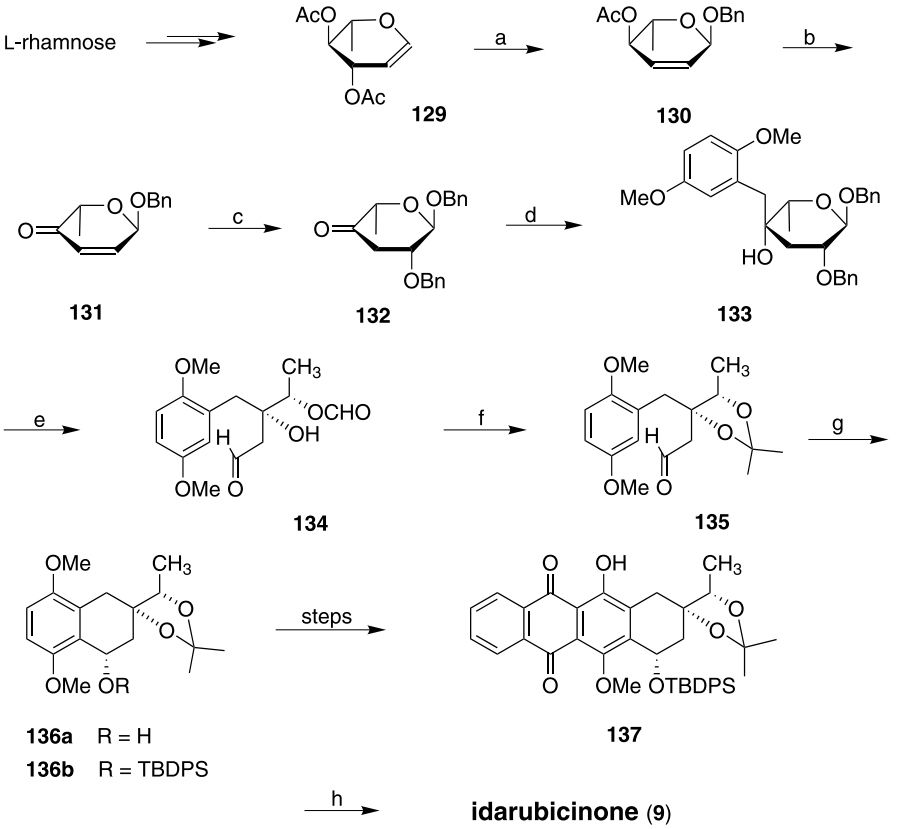
Coupling of aldehyde **122** with lithiated 1,4-dimethoxybenzene afforded adduct **124a** (Scheme 25). Radical deoxygenation [127] of methyl dithiocarbonate derivatives of **124a** with Bu_3SnH led to **124b**, and subsequent oxidation with the OsO_4-$NaIO_4$ reagent [128] furnished aldehyde **124c** in 50%

overall yield. Ring closure catalyzed by tin tetrachloride at –70 °C furnished the AB building block **125**. Its (7*R*)-configuration was assigned on the basis of CD evidence. Coupling of **125** with cyanophthalide **115** by known procedures [23, 129] led to the anthracyclinone **126**.

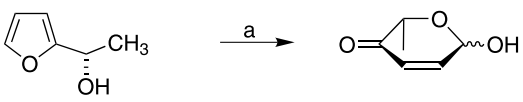
In the synthesis of 7-deoxyanthracyclinone **128**, an A + BCD route was followed [124]. The condensation of aldehyde **123** with leucoquinizarine (**86**) and subsequent one-pot deoxygenation of the benzylic position and deprotection of the primary hydroxy group furnished anthraquinone **127a** (Scheme 26). Oxidation of the hydroxymethyl group using the Corey procedure [130] provided aldehyde **127b**. An intramolecular Marschalk reaction [106, 123], followed by benzylic deoxygenation and acidic hydrolysis, gave the target anthracyclinone **128**.

In our laboratory we have developed a novel approach to the synthesis of an enantiopure AB segment of the anthracyclinones based on a new type of chiral substrate, enone **131** [131, 132]. Di-*O*-acetyl-L-rhamnal (**129**), obtained from L-rhamnose, was converted into benzyl glycoside **130** [133] by the Ferrier procedure [134]. Hydrolysis and oxidation of the C-4 (carbohydrate numbering) hydroxyl group in **130** gave enone **131** in 67% overall yield (Scheme 27). It is worthy of note that uloside **131** is also readily available from a non-sugar chiral pool. Enzymatic resolution of racemic 1-(2-furyl)ethanol yielded the (*S*)-enantiomer **138** in 98% *ee* [135]. This was transformed in two steps, by known procedures, into ulose **139** (Scheme 28) [136, 137] from which uloside **131** was readily obtained.

Treatment of uloside **131** with benzyl alcohol in the presence of potassium carbonate gave ketone **132**. The presence of the benzyloxy substituent at C-2 eliminated the possibility of 1,4-addition (which prevailed in the case of **131**) and ensured the required steric course of nucleophilic addition to the carbonyl group. Indeed, reaction of 2,5-dimethoxybenzyllithium with ketone **132** resulted in the formation of a single adduct **133** (63%). The expected configuration of the new stereogenic center was established by X-ray analysis. Removal of the *O*-benzyl substituents in **133** by catalytic hydrogenation and subsequent oxidative scission of the diol provided aldehyde **134**. Previously, it was observed that an aldehyde analogous to **134** formed a cyclic hemiacetal that failed to give a carbocyclic product on treatment with tin tetrachloride [124, 138, 139]. To circumvent this problem, aldehyde **134** was converted into acetonide **135**, with both hydroxyl groups protected. Reaction of **135** with tin tetrachloride at –70 °C [140] gave tetralin **136a** as a single product with the (7*S*) configuration, confirmed by X-ray analysis, in 17% overall yield. Annelation of the CD-segment to the chiral AB block **136b** was carried out by the Kraus addition cyclization method [141], following the Swenton procedure [23, 120, 123, 142], to yield the tetracyclic adduct **137**. Hydrolysis of the isopropylidene group, oxidation of C-13 hydroxy group with IBX [143], and subsequent removal of the remaining protecting groups afforded idarubicinone (**9**) in 73% yield from **137**.

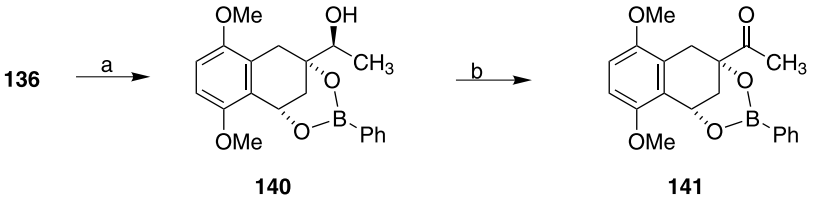


Scheme 27 Reagents and conditions: *a* BF_3, BnOH, DCM, 5–10 °C, 1 h, 89%. *b* (i) MeONa, MeOH, rt, 3 h; (ii) PDC, DMF, 77%. *c* BnOH, K_2CO_3, rt, 4 h, 63%. *d* 2,5-Dimethoxybenzyl-ethyl ether, Li, THF-Et_2O, –20 °C, 1 h then **132**, Ph-Me, –70 °C, 3 h, 63%. *e* (i) H_2, 5% Pd/C, EtOAc-EtOH (2 : 1), 4 h, 96%; (ii) $NaIO_4$, H_2O-MeOH (2 : 3), rt, 2 h, 85%. *f* (i) $NaBH_4$, H_2O-PrOH (1 : 1) -THF, 1 h, 100%; (ii) $(Me)_2C(OMe)_2$, *p*-TsOH, acetone, rt, 3 h then PPTS, acetone-H_2O, 2 h, 90%; (iii) IBX, DMSO, rt, 3 h, 86%. *g* (i) $SnCl_4$, DCM, –70 °C; (ii) TBDPS, imidazole, DMF, 70 °C. *h* (i) Ac_2O, py, DMAP, 45–50 °C, 3 h, 98%; (ii) AcOH-H_2O (4 : 1), THF, 80 °C, 5 h, 100%; (iii) IBX, DMSO, rt, 18 h, 87%; (iv) BCl_3, DCM, –70 °C, 1 h, 100%; (v) 3% HCl, MeOH, rt, 48 h, 86%



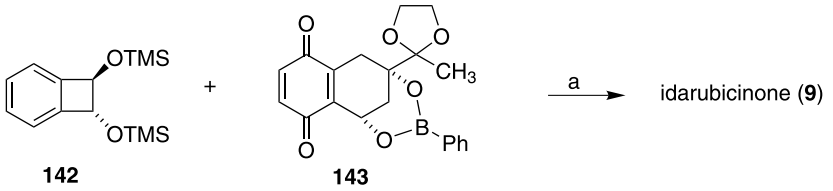
Scheme 28 Reagents and conditions: *a* (i) MeOH, Br_2, –40 °C; (ii) H_2O-AcOH, rt, 1 h, 85% over two steps

In the above approach, the oxidation of the C-13 hydroxy group was carried out on the tetracyclic moiety in the third-to-last step of the synthetic route. An alternative procedure allows for the introduction of the required carbonyl group at C-13 at an earlier stage of the preparation of the AB segment. Treatment of the isopropylidene derivative **136** with phenylboronic acid in the presence of PTSA gave phenylboronate **140** (Scheme 29), which was oxidized with pyridinium chlorochromate to give ketone **141** in 70% overall yield. The product was identical with that obtained previously by Krohn by a different route and used in a synthesis of idarubicinone (**9**) [144, 145].



Scheme 29 Reagents and conditions: *a* $PhB(OH)_2$, *p*-TsOH, Ph-Me, 40–50 °C, 2 h. *b* PCC, DCM, MS 3A, 40 °C, 1 h, 70% over two steps

The utility of **141** was emphasized by a recent synthesis of idarubicinone (**9**) [146] (Scheme 30). Cycloaddition of quinone **143**, prepared from **141** by ketalization and cerium ammonium nitrate (CAN) oxidation [20], with a novel precursor of *o*-quinone dimethide [146], *trans*-1,2-bis(trimethylsilyloxy)benzocyclobutane (**142**), and subsequent removal of protecting groups gave idarubicinone (**9**) in 65% overall yield.

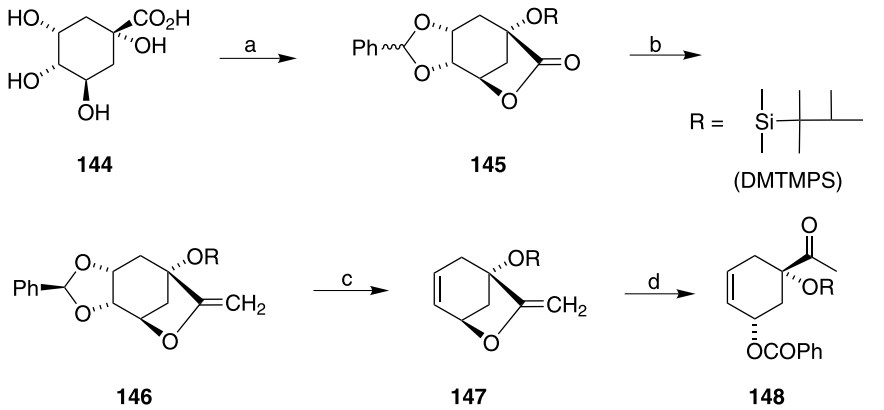


Scheme 30 Reagents and conditions: *a* (i) 50 °C, 2 h, then DDQ, THF, rt, 10 h; (ii) H_2O_2, NaOH, THF; (iii) *p*-TsOH, acetone, 65% over three steps

5.2
(–)-Quinic Acid

Extensive studies, pioneered by Vollhardt [147–149], on the cobalt-mediated [2 + 2 + 2] cycloaddition of alkynes to other unsaturated substrates amply demonstrated its versatility in the rapid construction of polycyclic linear and angular systems. This novel approach in anthracyclinone chemistry re-

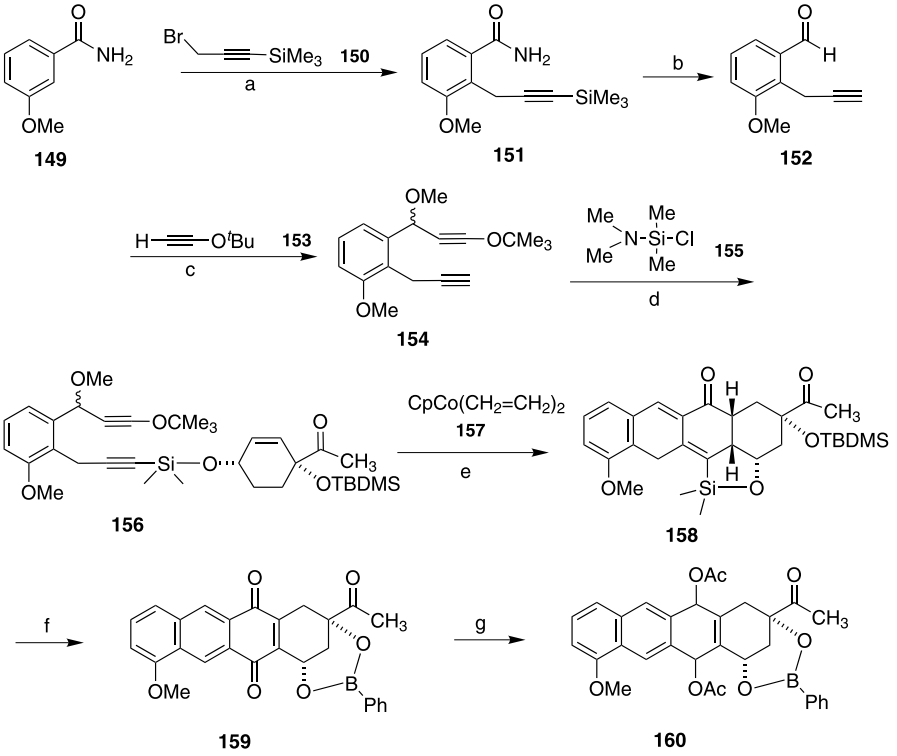
quired an enantiopure A-ring precursor. In a new approach to the synthesis of daunomycinone (**7**), Vollhardt developed an efficient synthesis of ketone **148** using (–)-quinic acid (**144**) as a natural chiral precursor [105]. Dihydroxyketone **148** (unprotected hydroxy groups) had been prepared previously but its synthesis, also from (–)-quinic acid (**144**), involved 12 steps and gave the final product in 4% overall yield [150, 151]. The improved, six-step synthesis is depicted in Scheme 31.



Scheme 31 Reagents and conditions: *a* (i) PhCHO, *p*-TsOH, Ph-H, reflux, 12 h, 64%; (ii) NaH, THF, reflux, 30 min, then DMTMPSiCl, reflux, 2 h, 88%. *b* Zn, TMEDA, $TiCl_4$, CH_2Br_2, THF-DCM, rt, 2 h, 74%. *c* BuLi, THF, –25 *to* –10 °C, 4.5 h, 53%. *d* (i) SiO_2, $(CO_2H)_2$, rt, 30 min, 92%; (ii) DEAD, PPh_3, $PhCO_2H$, Et_2O, rt, 2.5 h, 83%

Reaction of (–)-quinic acid (**144**) with benzaldehyde in the presence of PTSA [152, 153] and subsequent protection of the tertiary hydroxyl group with dimethyl(1,1,2-trimethylpropyl)silyl chloride [154] gave an epimeric mixture of lactones **145**. Separation of the **145** epimers and methylenation of the major diastereoisomer with dibromomethane-Zn-$TiCl_4$-TMEDA reagent [155] provided enol ether **146**, which afforded lactone **147** on deprotonation followed by retro [4 + 2]cycloaddition [156] of the resulting anion. Mild hydrolysis of the enol ether [157] **147** followed by the inversion of configuration of the allylic hydroxy group in the Mitsunobu reaction [158] gave the requisite dihydroxyketone **148** in 17% overall yield. This unsaturated ketone **148** provided a starting point for the implementation of the [2 + 2 + 2]cycloaddition methodology in the synthesis of enantiopure anthracyclinones.

The attachment of **148** to the appropriate diyne **155** led to an intramolecular reaction, which ensured the required regiochemistry of the substituents of rings A and D [159] (Scheme 32). Diyne **154** was obtained in four steps from 3-methoxybenzamide (**149**) (Scheme 32). *Ortho* lithiation of **149** and subsequent transmetalation with $ZnCl_2$-CuCN [160] followed by the reac-



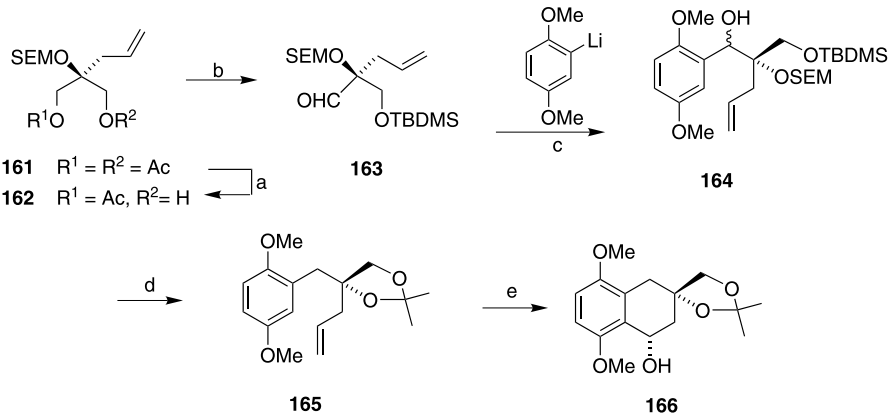
Scheme 32 Reagents and conditions: *a* *sec*-BuLi, TMEDA, $ZnCl_2$, CuCN, LiCl, THF, 78%. *b* (i) DIBAL-H, BuLi, THF, 0 °C then rt, 5 h, 62%; (ii) NH_4F, $TBAHSO_4$, H_2O-DCM, rt, 10 h, 100%. *c* BuLi, THF, −78 °C, 4 h then MeI, DMSO, −30 °C, 12 h, 89%. *d* LHDMS, THF, −78 °C then **148**, 91%. *e* −78 °C→ rt then $FeCl_3 \cdot 6H_2O$, MeCN, −30 °C, 40%. *f* (i) DDQ, H_2O, $CHCl_3$, reflux, 4 h, 79%; (ii) HF·py, THF, 0 °C → rt, 60%; (iii) $PhB(OH)_2$, *p*-TsOH, MS 4A, Ph-Me, rt, 20 h, 100%. *g* H_2-Pd/C, Ac_2O-py, 90 min, 68%

tion with 3-bromotrimethylsilylpropyne (**150**) gave amide **151**. Reduction of **151** with the ate complex from DIBAL-H and BuLi [161], and removal of the trimethylsilyl substituent by treatment with ammonium fluoride, provided aldehyde **152**. Sequential deprotonation of *tert*-butoxyethyne (**153**) with BuLi, addition to the aldehyde **152**, and quenching of the resulting anion with an excess of methyl iodide furnished diyne **154** in 43% overall yield. Reaction of diyne **154** with (dimethylamino)dimethylsilyl chloride (**155**) and hydroxyketone **148** in the presence of freshly prepared LHMDS gave intermediate **156**. The synthesis of **156** with the chiral A-ring segment attached through a silicone tether, set the stage for the intramolecular [2 + 2 + 2] cycloaddition. Treatment of **156** with a 20% molar excess of cyclopentadienecobaltbis ethene [$CpCO(CH_2 = CH_2)_2$] (**157**) followed by decomposition of the resulting complex with ferric chloride at −30 °C in acetonitrile solution, yielded ketone **158**. Oxidation of **158** with DDQ and subsequent removal of the TBDMS group

with the HF in pyridine, followed by esterification with phenylboronic acid, gave boronate **159** in five steps and 17% yield from diyne **154**. Catalytic hydrogenation of **159** in an acetic anhydride-pyridine mixture gave diacetate **160**, an advanced intermediate that was previously [20] transformed in two steps into daunomycinone (**7**).

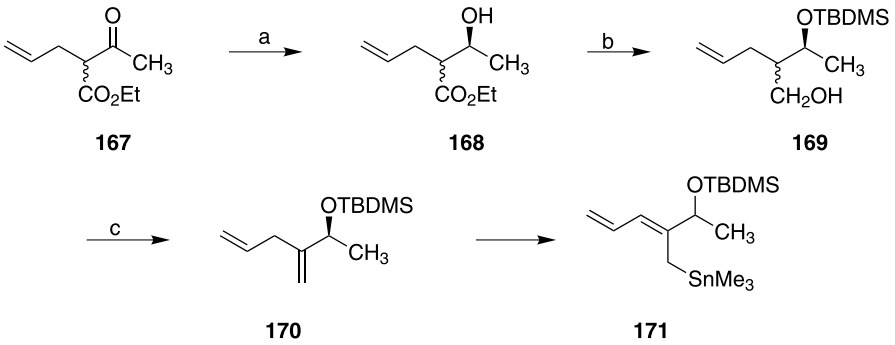
5.3 Non-natural Chiral Substrates

Enantiomerically pure precursors used in the synthesis of anthracyclinones are not only derived from the natural chiral pool. In one approach desymmetrization of a glycerol derivative was used (Scheme 33). Enzymatic hydrolysis of the prochiral diacetate **161** using lipase LP gave monoacetate **162** in 87% *ee* [162]. To achieve the requisite absolute configuration at the stereogenic center, the free hydroxyl group in **162** was protected with TBDMS, the acetate was removed by methanolysis, and subsequent oxidation with PDC furnished aldehyde **163** [163]. Reaction of aldehyde **163** with 2,5-dimethoxyphenyllithium followed by deoxygenation of adduct **164** by the Barton procedure [127], deprotection of both hydroxyl groups with TBAF [164], and reprotection with an isopropylidene group led to acetonide **165**, previously prepared from α-D-saccharinolactone (**119**) and used in the synthesis of AB segment **166** [116]. Following the known procedure, **165** was converted into **166**, in 25% overall yield from **162** and 87% *ee*. The enantiomeric purity of **167** was raised to 98% *ee* by recrystallization from a mixture of hexane–dichloromethane.



Scheme 33 Reagents and conditions: *a* Lipase LT, 0.06 M buffer-DMF (1 : 1), 0 °C. *b* (i) TBDMSCl, imidazole, DMF; (ii) K_2CO_3, MeOH; (iii) PDC, MS 3A, DCM, 88%. *c* (i) THF, –78 °C. *d* (i) NaH, CS_2, MeI, THF; (ii) Bu_3SnH, AIBN, Ph-Me; (iii) TBAF, MS 4A, DMPU; (iv) $(Me)_2C(OMe)_2$, *p*-TsOH, acetone, 53%. *e* (i) OsO_4-$NaIO_4$, dioxane-water (1 : 1); (ii) $SnCl_4$, DCM, –78 °C, 55%

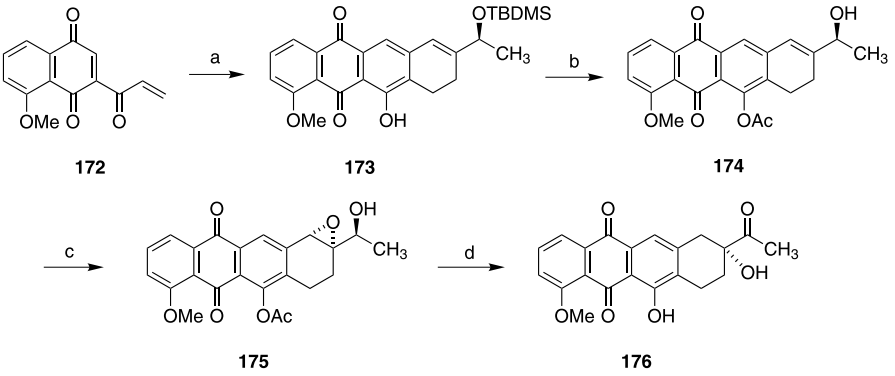
Naruta and coworkers developed a method for the synthesis of an anthracyclinone tetracyclic moiety based on a Lewis acid-mediated tandem Michael–Diels–Alder reaction of acryloyl quinone with pentadienyltins [165]. Subsequent enantioselective Sharpless epoxidation led to an enantiopure intermediate that was converted into 11-deoxydaunomycinone [166]. In more recent work, they obtained enantiomerically pure 7,11-deoxydaunomycinone (**176**) and its analogues by the above strategy using the enantiopure pentadienyltin derivative **171** [167], which was obtained in six steps from racemic ester **167** [168] (Scheme 34).



Scheme 34 Reagents and conditions: *a* Baker's yeast, sucrose, H_2O, 30–35 °C, 60%. *b* (i) TBDMSCl, imidazole, DMF, 0–40 °C, 12 h; (ii) DIBAL, hexane, 0 °C, 5 h, 91%. *c* (i) *p*-TsCl, py, DCM, 0 °C → –20 °C, 12 h; (ii) NaI, DMF, 55 °C, 5 h then DBU, 85 °C, 3 h, 60%. *d* *t*-BuOK, BuLi, hexane, then Me_3SnCl in THF, 80%

Baker's yeast reduction [169] of **167** was highly diastereoselective and gave hydroxyester **168** with the (*S*) configuration of the carbinol center. Protection of the hydroxyl group with TBDMS followed by reduction of the ester furnished alcohol **169**. Diene **170** was obtained from **169** by the Wolff procedure [170], comprising sequential tosylation, iodination, and dehydroiodination. Treatment of diene **170** with a *t*-BuOK-BuLi mixture [171], and trapping of the resulting anion with trimethyltin chloride, afforded the requisite pentadienyltin derivative **171** in ≥ 95% *ee* and 36% overall yield.

The tandem Michael–Diels–Alder addition of **171** to aryloylquinone **172** (catalyzed by $(PrO)_3TiCl$), after aerial oxidation [172] furnished the tetracyclic intermediate **173** (Scheme 35). Acetylation of the phenolic hydroxyl and removal of the silyl substituent with hydrofluoric acid led to allylic acohol **174** without significant racemization. Diastereoselective epoxidation of **174** with $VO(acac)_2$ [173] gave epoxy alcohol **175** as the only isolated product. Oxidation of the hydroxyl group in **175** with PDC [174], followed by reductive opening of the oxirane ring with sodium dithionate and concomitant hydrolysis of acetate, furnished enantiomerically pure (*R*)-7,11-dideoxydaunomycinone (accompanied by the dehydration product) in 21% overall yield.



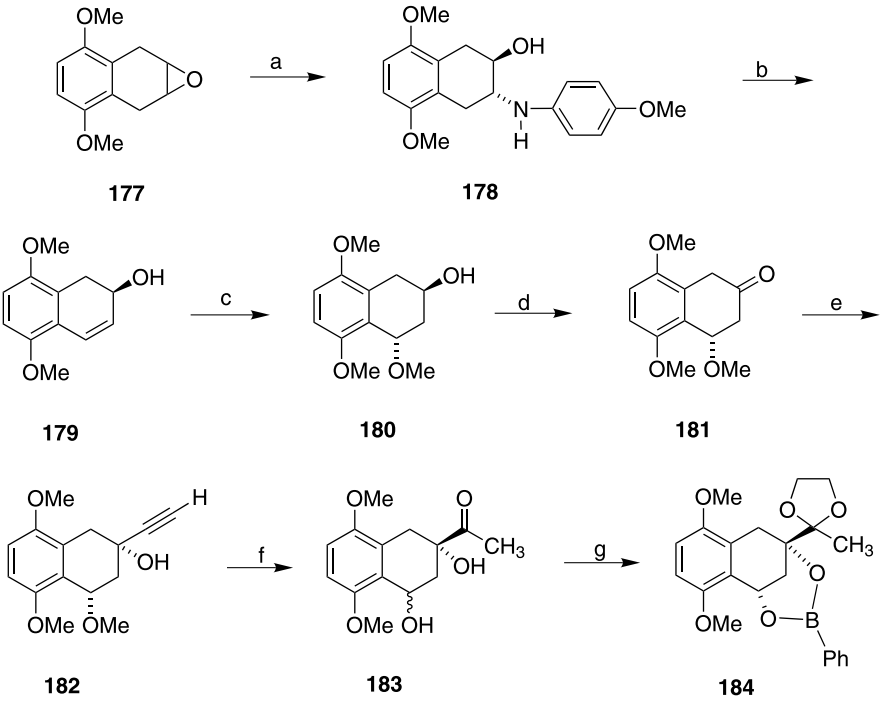
Scheme 35 Reagents and conditions: *a* (i) **171**, $(PrO)_3TiCl$, CH_2Cl_2, –78 °C, 15 min; (ii) DMF, air, 100 °C, 3 h, 62%. *b* (i) Ac_2O, Et_3N, DMAP, DCM, rt, 12 h; (ii) HF, MeCN, 0 °C, 1 h, 90%, 95% *ee*. *c* $VO(acac)_2$, *t*-BuOOH, DCM, –20 °C, 12 h, 90%. *d* (i) PDC, TFA-py, DCM, rt, 2 d, 90%; (ii) $Na_2S_2O_4$, NaOH, H_2O, 0 °C, 4 h, 46%

Chiral precursors have been also used for the preparation of enantiopure dienes utilized in the construction of an AB segment [175] and a tetracyclic moiety [176] of the anthracyclinones.

6 Enantioselective Catalysis

In spite of the tremendous progress in catalytic asymmetric synthesis [36, 177] its use in the syntheses of anthracyclinones is limited, apart from Baker's yeast reduction and Sharpless epoxidation and dihydroxylation. In the recent anthracyclinone literature two contributions have appeared on the application of catalytic enantioselective synthesis. Both are based on the desymmetrization of a *meso* compound, but in entirely different chemical contexts.

The synthesis of the enantiopure AB segment **184** [68] by Shibasaki et al. [178] relied on the enantioselective opening of the oxirane ring of the readily available *meso*-epoxide **177** with *p*-anisidine [179, 180], followed by two steps with 1,3-chirality transfer. After extensive experimentation the best enantiomeric excess was obtained with a catalyst prepared from $Pr(O\text{-}i\text{-}Pr)_3$–(*R*)-BINAP in the ratio 1 : 1.5 with three equivalents of triphenylphosphine oxide as an additive. Thus, reaction of **177** with *p*-anisidine in the presence of 10 mol % of the chiral catalyst provided *trans*-aminoalcohol **178** in 80% yield with a moderate enantiomeric excess (65% *ee*), which was raised to 95% *ee* by one recrystallization in 40% yield (Scheme 36). Methylation of **178** and Hofmann degradation of the resulting quaternary salt with butyllithium at –78 °C gave allylic alcohol **179**. Oxymercuration of alcohol **179** with $Hg(OAc)_2$ in methanol, followed by sodium borohydride reduction [181]



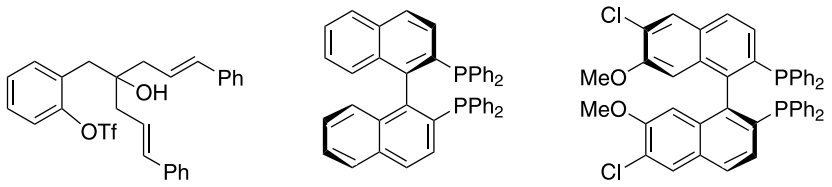
Scheme 36 Reagents and conditions: *a* (*R*)-BINOL, $Pr(O\text{-}i-Pr)_3$, $Ph_3P=O$, *p*-anisidine, Ph-Me, 50 °C, 17 h, 80%; 65% *ee*, recrystallization 40%, 95% *ee*. *b* (i) MeI, K_2CO_3, MeOH, reflux 24 h; (ii) BuLi, THF, –78 °C, 1 h, 52%, 90% *ee*. *c* (i) $Hg(OAc)_2$, MeOH, rt; (ii) $NaBH_4$, NaOH, H_2O, 50 °C, 74%. *d* DMP, DCM, 0 °C → rt, 1.5 h, 95%, 90% *ee*. *e* HC≡CMgBr, $CeCl_3$, THF, –78 → –30 °C, 1 h, then **181**, 2 h, 76%. *f* 2% H_2SO_4, $HgSO_4$, acetone, rt, 40 h. *g* (i) $(CH_2OH)_2$, $MgSO_4$, *p*-TsOH, 60 °C, 24 h; (ii) $PhB(OH)_2$, *p*-TsOH, Ph-Me, rt, 12 h, 70% from **182**

proceeded with full regio- and diastereoselectivity [182] yielding ether **180**. Dess–Martin periodinate oxidation of the hydroxyl group in **180** furnished ketone **181** and set the stage for the second 1,3-chirality transfer. Addition of an ethenyl group to the ketone **181** with the aid of the cerium reagent prepared from ethinylmagnesium bromide and cerium(III) chloride [183], gave propargyl alcohol **182** with high (10 : 1) diastereoselectivity. Hydration of the ethinyl group with 2% sulfuric acid in the presence of mercuric sulfate and concomitant substitution of the methoxy group afforded hydroxyketone **183**, as a C-7 epimeric mixture (1 : 1).

Ketalization of **183** and subsequent treatment with phenylboronic acid gave the known AB building block **184**, with the required configuration at C-7, in 15% yield from **177**.

The other approach to an enantiopure AB segment based on the desymmetrization of a *meso*-compound is related to the catalytic enantioselective Heck reaction [184]. Oestreich et al. [185] developed a highly (92–94% *ee*)

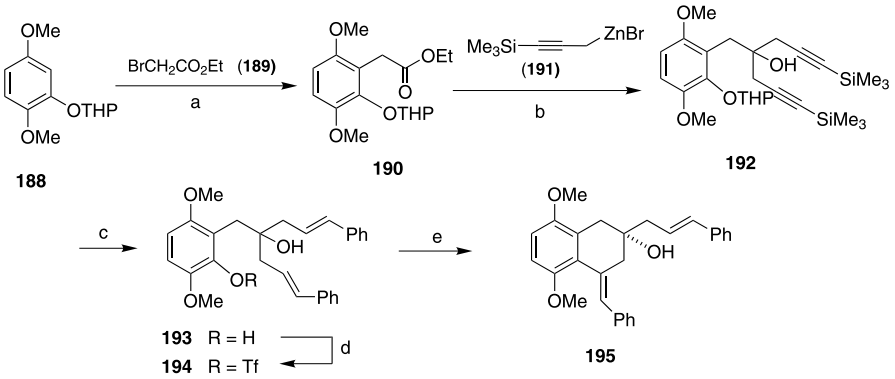
enantioselective desymmetrizing Heck cyclization of the novel prochiral substrate **185**, using (*R*)-BINAP (**187**) or (*S*)-Cl-MeOH-BIPHEP (**188**) as chiral ligands (Fig. 12).



185 **186** **187**

Fig. 12

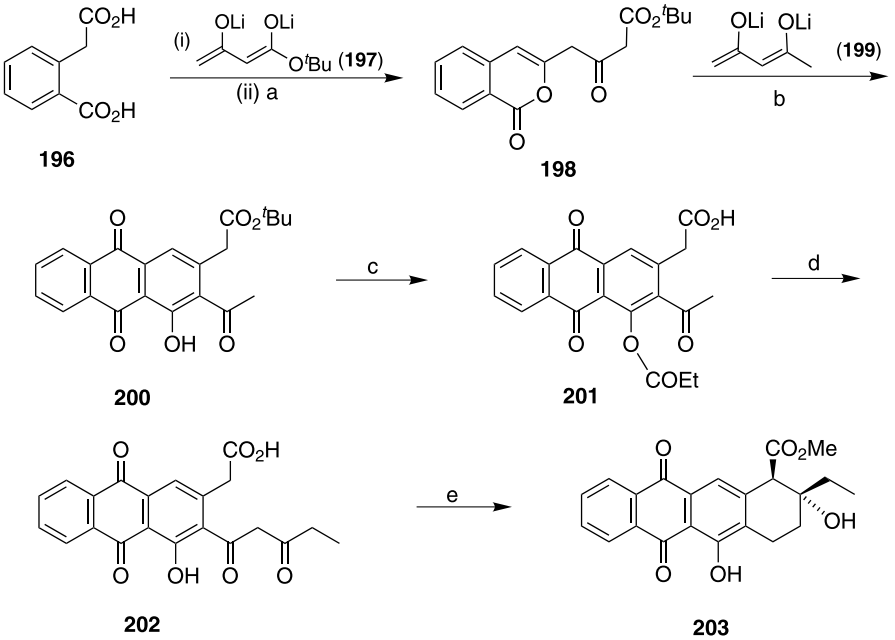
The efficient differentiation of the enantiotopic substituents in **185** was attributed to the coordination of the transition metal to the hydroxyl group in the catalytic cycle [186]. Moreover, the location of the OH group in the cyclization product corresponds to the 9-OH group in the AB fragment of an anthracyclinone. The requisite precursor for the enantioselective cyclization, with methoxy groups in ring B, was synthesized as follows (Scheme 37). *Ortho* lithiation of tetrahydropyranyl ether **188** [187] followed by transmetalation with copper(I) iodide and substitution of the intermediate cuprate with ethyl bromoacetate (**189**) [188] gave ester **190**. Bis-propargylation of **190** using 3-(trimethylsilyl)propargylzinc bromide (**191**) [189] furnished diyne **192**. Desilylation of **192** with TBAF followed by Sonogashira cross-coupling with iodobenzene [190] and subsequent lithium aluminum hydride reduction



Scheme 37 Reagents and conditions: *a* BuLi, –78 °C, CuI, 0 → –78 °C then **189**, –78 °C→ rt, THF. *b* **191**, THF, rt. *c* (i) TBAF·3H_2O, THF, rt; (ii) $(Ph_3P)_2PdCl_2$, CuI, Et_3N, PhI, THF, rt; (iii) LAH, THF, 0 °C → rt. *d* $PhNTf_2$, $CsCO_3$, DMF, rt. *e* $Pd(OAc)_2$, **187**, TMP, 100 °C, 35%; 58% *ee*

of the triple bonds provided bishomoallylic alcohol **193**, with high diastereoselectivity (*E*,*E* : *E*,*Z* : *Z*,*Z* = 96 : 4 : 0). Treatment of **193** with $PhNTf_2$ and cesium carbonate afforded the cyclization precursor **194**. However, the crucial desymmetrization step in the Heck cyclization met with only moderate success. Reaction of **194** in the presence of (*R*)-BINAP **186** gave only a modest yield (35%) of **195** with moderate enantioselectivity (58% *ee*) in contrast to the excellent results (85%, 92% *ee*) achieved with the model compound **185**. The use of another chiral ligand **187** in the case of **194** gave no reaction. Apparently, tuning of the catalytic system is required. Nevertheless the possibility of a novel approach to the construction of enantiopure AB precursors by catalytic asymmetric synthesis has been demonstrated.

Studies on the biomimetic synthesis of quinone antibiotics [191] led Krohn to yet another type of enantioselective transformation, namely microbial conversion of an achiral substrate into enantiomerically pure anthracyclinones. An adroit synthesis of the microbial transformation substrate, 4-deoxyaklanonic acid (**202**) [192] (Scheme 38), started from homophthalic ester **196** [193] with addition of dilithiated *tert*-butyl acetoacetate (**197**) and subsequent cyclization to isocoumarine **198** [194]. Reaction of **198** with the dilithium salt **199** gave anthrone **200** [195]. Aeration of **200** in the pres-



Scheme 38 Reagents and conditions: *a* (ii) Ac_2O, 5 °C, 16 h, 57%. *b* –78 °C→ 0 °C, 1 h then AcOH, 45 °C, 1 h, 72%. *c* (i) $CuBr_2$, O_2; (ii) EtCOCl, DMAP; (iii) TFA, 70%. *d* LDA, THF, –40 °C, 70%. *e* LDA, THF, –40 °C, 70%. *f* *Streptomyces galilaeus*, S727, 24 h, 56%

ence of copper bromide, followed by acylation with propionyl chloride and subsequent hydrolysis of the *tert*-butyl group, afforded acid **201**, the substrate for the Baker–Venkataraman rearrangement [196, 197]. Treatment of **201** with LDA at –70 °C provided 4-deoxyaklanonic acid (**202**) in 20% overall yield [192].

Incubation of **202** with the mutant strain S727 (obtained by treating *Streptomyces galilaeus* with the mutagen *N*-methyl-*N'*-nitro-*N'*-nitrosoguanidine [198]) for 24 h gave, as a major product, enantiomerically pure (9*R*, 10*R*)-4,7-dideoxyaklavinone **203** in 56% yield [192]. 4-Deoxyaklavinone II [199] was isolated as a side product. Thus, the microbial conversion of an achiral unnatural precursor into enantiopure anthracyclinones was accomplished, indicating the possibility of using other mutants and precursors to produce a variety of anthracyclinones or anthracyclines.

7 Conclusions

The synthesis of enantiomerically pure anthracyclinones continued to attract considerable attention in the review period, though the number of contributions that appeared in the literature diminished in comparison to those of the 1980s. Most of the approaches depended on the synthesis of the enantiopure key intermediates, the AB building blocks. Chirality was introduced by use of strategies developed earlier. However, resolution of racemic precursors and asymmetric synthesis aided by chiral auxiliaries were employed less often in favor of utilizing chiral building blocks derived from natural and unnatural sources, chiral reagents, and catalytic enantioselective synthesis. Moreover, use of the natural chiral pool was limited to sugars and (–)-quinic acid, a novel source of chirality in anthracyclinone synthesis. Recent advances, including the use of L-rhamnose and (–)-quinic acid, [2 + 2 + 2] cyclizations, enantioselective Heck cyclizations, and enantioselective microbial transformations in the construction of tetracyclic moieties and AB segments, indicate the ongoing interest in the field of enantiopure anthracyclinone synthesis (strategies and methods). More progress will be made in the future.

References

1. Brockmann H, Bauer K (1950) Naturwissenschaften 37:492
2. Brockmann H (1963) Fortsch Chem Org Naturst 21:121
3. Ollis WD, Sutherland IO (1961) In: Ollis WD (ed) Chemistry of natural phenolic compounds. Pergamon, London, p 212
4. Grein A, Spalla C, DiMarco A, Canevazzi G (1963) Giorn Microbiol 11:109
5. Dubost M, Ganter P, Maval R, Ninet L, Pinneret S, Preud'Homme J, Werner GH (1963) CR Acad Sci 257:1813

6. Arcamone F, Cassinelli G, Fantini G, Grein A, Orezzi P, Pol C, Spala C (1969) Biotechnol Bioeng 11:1101
7. Arcamone F (1981) Doxorubicin anticancer antibiotics. Academic, New York
8. Grever R, Chabner BA (1997) Cancer drug discovery and development, vol I, 5th edn. Lipincott-Raven, New York
9. Brown JR, Imam SH (1984) Prog Med Chem 21:169
10. Hollingshead LM, Faulds D (1991) Drugs 42:690
11. Cersosimo RJ (1992) Clin Pharm 11:152
12. Krohn K (1989) Building blocks for the total synthesis of anthracyclinones. In: Hertz W, Tamm Ch (eds) Progress in the chemistry of natural products, vol 55. Springer, Berlin Heidelberg New York, p 37
13. Krohn K (1990) Synthesis of anthracyclinones by electrophilic and nucleophilic addition to anthraquinones. Tetrahedron 46:291
14. Thomas GJ (1990) Synthesis of anthracyclines related to daunomycine. In: Lukacs G, Ohno M (eds) Recent progress in the chemical synthesis of antibiotics. Springer, Berlin Heidelberg New York
15. Wheeler DMS, Wheeler MM (1994) Stereoselective synthesis of doxorubicine and related compounds. In: Atta-ur-Rahman (ed) Studies in natural products chemistry, vol 14. Elsevier, Amsterdam, p 3
16. Broadhurst MJ, Hassal CH, Thomas GJ, Lederer F (1983) EP 0 104 654 A2
17. Penco S, Angelucci F, Vigevani A, Arlandini E, Arcamone F (1977) J Antibiot 30: 764
18. Krohn K, Ekkundi VS (1989) Total synthesis of enantiomerically pure anthracyclinones. In: Atta-ur-Rahman (ed) Natural products chemistry, vol 4. Elsevier, Amsterdam, p 317
19. Arcamone F, Bernardi, Patelli B, Giardino P, DiMarco A, Casazza A, Soranzo C, Pratesi G (1978) Experientia 34:1255
20. Broadhurst MJ, Hassall CM, Thomas GJ (1982) J Chem Soc Perkin Trans I 2249
21. Broadhurst MJ, Hassall CM, Thomas GJ (1984) Tetrahedron 40:4649
22. Arcamone F, Bernardi N, Patelli B, DiMarco A (1976) German Patent 2601785
23. Swenton JS, Frescos JN, Morrow GW, Sercel AD (1984) Tetrahedron 40:4625
24. Wong CM, Popien D, Schwenk R, Te Raa J (1971) Can J Chem 49:2712
25. Kaczmarek Ł, Szelejewski W (2004) PL 188391 B1
26. Yoshida Y, Shimonishi K, Sakakuran Y, Okada S, Aso N, Tanabe Y (1999) Synthesis 9:1633
27. Szechner B, Achmatowicz O, Bodalski R, Koszuk J (2006) Przem Chem 85(5):333
28. Achmatowicz O, Sadownik A, Bielski R (1985) Pol J Chem 59:533
29. Rho YS, Park S, Kim WJ, Kim G, Yoo DJ, Kang HS, Chung SR (2002) Synth Commun 32:1961
30. Rho YS, Park J, Kim G, Kim H, Sin H, Suh PW, Yoo DJ (2004) Synth Commun 34:1703
31. Confalone PN, Pizzolato G (1990) J Org Chem 55:5520
32. Confalone PN, Pizzolato G (1981) J Am Chem Soc 103:4251
33. Van Dufat-Trinh H, Sequin E, Tillequin F, Monneret C, Koch M (1989) Chem Pharm Bull 37:3294
34. Lavallee JF, Rej R, Courchesne M, Nguyen D, Attardo G (1993) Tetrahedron Lett 34:3519
35. Lin GQ, Li YM, Chan ASC (2001) Priciples and applications of asymmetric synthesis. Wiley-Interscience, New York, p 355
36. Halpern J, Trost BM (2004) Proc Natl Acad Sci USA 101:5347
37. Wong CM, Popien D, Ho TL (1973) Can J Chem 51:466

38. Rho YS, Ko HK, Kim WJ, Yoo DJ, Kang HS (2000) Bull Korean Chem Soc 21:774
39. Hauser FM, Prasanna S (1980) Synthesis, p 621
40. Martin VS, Woodward SS, Katsuki T, Yamada Y, Ikeda M, Sharpless KB (1981) J Am Chem Soc 103:6237
41. Fetizon M, Golfier M (1968) CR Acad Sci 267:900
42. Rho YS, Ko HK, Sin H, Yoo DJ (1999) Bull Korean Chem Soc 20:1517
43. Sodeoka M, Ogawa Y, Kirio Y, Shibasaki M (1991) Chem Pharm Bull 39:309
44. Sodeoka M, Imori T, Shibasaki M (1985) Tetrahedron Lett 26:6497
45. Sodeoka M, Imori T, Shibasaki M (1991) Chem Pharm Bull 39:323
46. Hiyama T, Sawahata M, Kusano Y (1985) Chem Lett, p 611
47. Holland HL, Viski P (1991) J Org Chem 56:5226
48. Kondo S, Dubashi S, Matsumoto M (1976) Chem Lett, p 1077
49. Rao AVR, Yadav JS, Reddy BK, Mehendale AR (1984) Tetrahedron 40:4643
50. Watanabe Y, Ishimura Y (1989) J Am Chem Soc 111:410
51. Badalassi F, Crotti P, DiBugno C, D'Arata F, Favero L, Ramacciotti A (2001) Tetrahedron: Asymmetry 12:3155
52. DiBugno C, D'Arata F, Ramacciotti A, Crotti P (2005) US Patent 6921841 B2
53. Sharpless KB, Amberg W, Bennani YL, Crispino GA, Hartung J, Jeong KS, Kwong HL, Morikawa K, Wang ZM, Xu D, Zhang XL (1992) J Org Chem 57:2768
54. Rama Rao AV, Yadav JS, Reddy BK, Mehendale AR (1984) Tetrahedron 40:4643
55. Cornforth JW (1994) Aldrichimica Acta 27:71
56. Naruta Y, Nishigaichi Y, Maruyama K (1989) Tetrahedron Lett 30:3319
57. Naruta Y, Nishigaichi Y, Maruyama K (1988) J Org Chem 53:1192
58. Gao Y, Hanson RM, Klunder JM, Ko SY, Masamune H, Sharpless KB (1987) J Am Chem Soc 109:5765
59. Combie RC, Clark RB, Rustenhoven JJ, Rutledge PS (1999) Aust J Chem 52:781
60. Tolstikov GA, Shul'ts EE, Vafina GF, Spirinkhin LV (1992) J Org Chem 28:192
61. Tomioka K, Nakajima M, Koga K (1987) J Am Chem Soc 109:6213
62. Tomioka K, Nakajima M, Titaka Y, Koga K (1988) Tetrahedron Lett 29:573
63. Davies FA, Chen BC (1992) Chem Rev 92:919
64. Davies FA, Sheppard AC, Chen BC, Haque MS (1990) J Am Chem Soc 112:6679
65. Davies FA, Kumar A, Chen BC (1991) Tetrahedron Lett 37:867
66. Davies FA, Clark C, Kumar A, Chen BC (1994) J Org Chem 59:1184
67. Johnson DW, Mander LN (1978) Aust J Chem 31:1561
68. Broadhurst MJ, Hassal CM, Thomas GJ (1982) J Chem Soc Perkin Trans I, p 2239
69. Davies FA, Kumar A, Chen BC (1991) J Org Chem 56:1143
70. Davies FA, Kumar A (1991) Tetrahedron Lett 32:7871
71. Fujioka H, Yamamoto H, Kondo H, Annoura H, Kita Y (1989) J Chem Soc Chem Commun, p 1509
72. Glashal C, Hermann R (1988) J Chem Soc Perkin Trans I, p 1753
73. Davies FA, Weismiller ME (1990) J Org Chem 55:3715
74. Moore JA, Rahm M (1961) J Org Chem 26:1109
75. Eliel EL, Wielen SM, Mander LN (1994) Stereochemistry of organic compounds. Wiley, New York, p 868
76. Fujioka H, Kondo H, Annoura H, Yamamoto H, Ko T, Kita Y, Tamura Y, Aoe K (1989) Chem Pharm Bull 37:1488
77. Tamura Y, Annoura H, Yamamoto H, Kondo H, Kita Y, Fujioka H (1987) Tetrahedron Lett 28:5709
78. Fujioka H, Yamamoto H, Annoura H, Miyazaki M, Kita Y (1990) Chem Pharm Bull 38:1872

79. Fujioka H, Yamamoto H, Annoura H, Maeda H, Kita Y (1992) Chem Pharm Bull 40:31
80. Moriarty RM, How KC (1984) Tetrahedron Lett 25:691
81. Czarnecki S, Georgoulis S, Stevens CL, Vijayakumaran K (1986) Synth Commun 16:11
82. Tamura Y, Sasho M, Akai S, Kishimoto H, Sekihachi J, Kita Y (1986) Tetrahedron Lett 27:195
83. Garcia Ruano JL, Carretero JC, Carrefio MC, Cabrejas LMM, Urbano A (1966) Pure Appl Chem 68:925
84. Bueno AB, Carrefio MC, Garcia Ruano JL, Arrayds R, Zarzuelo MM (1997) J Org Chem 62:2139
85. Adrio J, Carretero JC, Garcia Ruano JL, Martin Cabrejas LM (1997) Tetrahedron: Asymmetry 8:1623
86. Garcia Ruano JL, Garcia Paredes C, Hamdouchi C (1999) Tetrahedron: Asymmetry 10:2925
87. Garcia Ruano JL, Garcia Paredes C (2000) Tetrahedron Lett 41:261
88. Garcia Ruano LG, Garcia Paredes C, Alemán J (2005) Arkivoc 9:253
89. Solladié G, Hutt J, Girardin A (1987) Synthesis, p 173
90. Garcia Ruano JL, Martin-Castro AM, Rodriguez JH (1992) J Org Chem 57:7235
91. Escribano A, Garcia Ruano JL, Martin-Castro AM, Rodriguez JH (1994) Tetrahedron 50:7567
92. Garcia Ruano JL, Martin-Castro AM, Rodriguez JH, Flamarique ACR (1997) Tetrahedron: Asymmetry 8:3503
93. Craig D, Daniels K, MacKenzie AR (1992) Tetrahedron 48:7803
94. Nakajima M, Tamioka K, Koga K (1993) Tetrahedron 49:10807
95. Adachi A, Masuya K, Tamino K, Kuwajima I (1993) J Org Chem 58:4189
96. Mikami K, Shimizu M (1992) Chem Rev 92:1021
97. Tanio K, Shoda H, Nakamura T, Kuwajima I (1992) Tetrahedron Lett 33:1337
98. Panetta CA, Casanova TG (1979) J Org Chem 35:2423
99. Hauser FM, Tommasi RA (1991) J Org Chem 56:5758
100. Dienes Z, Antonsson T, Vogel P (1993) Tetrahedron Lett 34:1013
101. Flock M, Nieger M, Breitmaier E (1993) Liebigs Ann Chem, p 451
102. Theurillat-Moritz V, Guidi A, Vogel P (1997) Tetrahedron: Asymmetry 8:3497
103. Bourghli LMS, Stoodley RJ (2004) Bioorg Med Chem 12:2863
104. Hanessian S (1983) Total synthesis of natural products: the chiron approach. Pergamon, Oxford, England
105. Johnson BM, Vollhardt KPC (1990) Synlett, p 209
106. Marshalk C, Koenig F, Ourousoff N (1936) Bull Soc Chem Fr 3:1545
107. Mills SK, Mincher DJ, Shaw G (1988) J Chem Soc Chem Commun, p 399
108. Ali Z, Qureshi S, Shaw G (1990) J Chem Soc Perkin Trans 1, p 2627
109. Funabashi M, Yamazaki S, Yoshimura J (1976) Carbohydr Res 44:275
110. Cousson A, Le Gouadec G, Monneret C, Florent JC (1993) J Chem Soc Chem Commun, p 388
111. Weidmann H, Schwarz H (1972) Monatsh Chem 103:218
112. Albrecht HP, Repke DB, Moffat JG (1973) J Org Chem 38:1836
113. Bertounesque E, Florent JC, Monneret C (1991) Synthesis, p 270
114. Bock K, Castilla IM, Lundt I, Pedersen C (1987) Acta Chem Scand B41:13
115. Lewis CE (1970) J Org Chem 35:2938
116. Genot A, Florent JC, Monneret C (1987) J Org Chem 52:1057
117. Whistler RL, BeMiller JN (1963) Methods in carbohydrate chemistry, vol II. Academic, New York, p 477

118. Genot A, Florent JC, Monneret C (1989) Tetrahedron Lett 30:711
119. Suzuki K, Yuki Y, Mukaiyama T (1981) Chem Lett, p 1529
120. Henton DR, McCreery RA, Swenton JS (1980) J Org Chem 45:369
121. Bertounesque E, Florent JC, Monneret C (1990) Tetrahedron Lett 31:7153
122. Quereshi S, Shaw G, Burgess GE (1985) J Chem Soc Perkin Trans I, p 1557
123. Marschalk C (1939) Bull Soc Chim Fr, p 655
124. Deguin B, Florent JC, Monneret C (1991) J Org Chem 56:405
125. Whistler RL, BeMiller JN (1963) Methods in carbohydrate chemistry, vol II. Academic, New York
126. Bernet B, Vasella A (1984) Helv Chim Acta 67:1328
127. Barton DHR, McCombie SW (1975) J Chem Soc Perkin Trans I, p 1574
128. Pappo R, Allen Jr DS, Lemieux RU, Johnson WS (1956) J Org Chem 21:478
129. Freskos JN, Morrow GW, Swenton JS (1985) J Org Chem 50:805
130. Corey EJ, Kim CV (1972) J Am Chem Soc 94:7586
131. Achmatowicz O, Szechner B (2003) J Org Chem 68:2398
132. Szechner B, Achmatowicz O, Maurin JK (2005) Tetrahedron 61:1981
133. Banaszek A, Pakulski Z, Zamojski A (1995) Carbohydr Res 75:335
134. Ferrier RJ, Prasad N (1969) J Chem Soc C, p 575
135. Kamińska J, Góra J, Sikora M, Achmatowicz O, Szechner B (1997) PL 183014
136. Achmatowicz O, Bukowski P, Szechner B, Zwierzchowska Z, Zamojski A (1971) Tetrahedron 27:1973
137. Szechner B, Achmatowicz O, Badowska-Rosłonek K (1999) Pol J Chem 73:1133
138. Florent JC, Ughetto MJ, Monneret C (1977) J Chem Soc 52:1051
139. Krohn K, Heins H (1989) Carbohydr Res 191:253
140. Deguin B, Florent JC, Monneret C (1991) J Org Chem 56:411
141. Kraus GA, Sugimoti H (1978) Tetrahedron Lett 19:2263
142. Chenard BL, Dolson MG, Sercel AD, Swenton JS (1984) J Org Chem 49:328
143. Frigerio M, Santagostino M, Sputore S, Palmisano G (1995) J Org Chem 60:7272
144. Krohn K, Rieger H (1987) Liebigs Ann Chem, p 515
145. Krohn K, Rieger H, Broser E, Schiess P, Chen S, Strubin T (1988) Liebigs Ann Chem, p 943
146. Allen JG, Hentemann MF, Danishefsky SJ (2000) J Am Chem Soc 122:571
147. Vollhardt KPC (1984) Angew Chem Int Ed Engl 23:539
148. Schore NE (1988) Chem Rev 88:1081
149. Gandon V, Aubert C, Malacria M (2006) Chem Commun, p 2209
150. Dyong I, Hermann R, Mattes R (1980) Chem Ber 113:1931
151. Dyong I, Hermann (1981) Tetrahedron Lett 22:422
152. Lesuisse D, Berchtold GA (1985) J Org Chem 50:888
153. White JD, Cammack JH, Sakuma K (1989) J Am Chem Soc 111:8970
154. Oertle K, Wetter H (1985) Tetrahedron Lett 26:5511
155. Okazoe T, Takai K, Oshima K, Utimoto K (1987) J Org Chem 52:4410
156. Hines JN, Peagram MJ, Thomas EJ, Whitham GH (1973) J Chem Soc Perkin Trans I, p 2332
157. Huet F, Lechevallier A, Pellet M, Conia JM (1978) Synthesis, p 63
158. Mitsunobu O (1981) Synthesis, p 1
159. Kreye P, Groth U, Eckenberg P (1998) DE 19708496 A1
160. Knochel P, Singer RD (1993) Chem Rev 93:2117
161. Kim S, Ahn KH (1984) J Org Chem 49:1717
162. Watanabe N, Sugai T, Ohta H (1992) Chem Lett 21:657
163. Watanabe N, Ohta H (1992) Chem Lett 21:861

164. Lipshutz BH, Miller TA (1989) Tetrahedron Lett 30:7149
165. Naruta Y, Nagai N, Arita Y, Maruyama K (1983) Chem Lett, p 1685
166. Naruta Y, Nishigaichi Y, Maruyama K (1988) J Org Chem 53:1192
167. Naruta Y, Nishigaichi Y, Maruyama K (1989) J Chem Soc Perkin Trans I, p 831
168. Frater G (1979) Helv Chim Acta 62:2825
169. Csuk R, Glänzer B (1991) Chem Rev 91:49
170. Wolff S, Huecas ME, Agosta WC (1982) J Org Chem 47:4358
171. Schlosser M (1967) J Organomet Chem 8:9
172. Hauser FM, Mal D (1983) J Am Chem Soc 105:5688
173. Sharpless KB, Verhoeven TR (1979) Aldrichim Acta 12:63
174. Corey EJ, Schmidt G (1979) Tetrahedron Lett 20:399
175. Hatakeyama S, Sugawara K, Takano S (1991) J Chem Soc Chem Commun, p 1533
176. Amati F, Lombardi P, Rossi C (1996) WO Patent 98/01410
177. Iwas O (ed) (2000) Catalytic asymmetric synthesis, 2nd edn. Wiley, New York
178. Sekine A, Ohshima T, Shibasaki M (2002) Tetrahedron 58:75
179. Hanek EP, Cimarusti CM, Sundeen JE (1975) US Patent 3930022
180. Arvidsson LE, Hacksell U, Johansson Nilsson JLG, Linberg P, Sanchez D, Wikstroem H, Swensson K, Hjorth S, Carlsson AJ (1984) J Med Chem 27:45
181. Roubaud V, LeMoigne F, Mercier A, Tordo P (1996) Synth Commun 26:1507
182. Senda Y, Takayanagi S, Sudo T, Itoh H (2001) J Chem Soc Perkin Trans I, p 270
183. Hottop T, Gutke H-J, Murahashi SI (2001) Tetrahedron Lett 42:3343
184. Shibasaki M, Vogel EM, Ohshima T (2004) Adv Synth Catal 343:1533
185. Oestreich M, Sempere-Culler F, Machotta AB (2005) Angew Chem Int Ed Engl 44:149
186. Oestreich M (2005) Eur J Org Chem, p 783
187. Snieckus V (1990) Chem Rev 90:879
188. Velkov J, Mincheva Z, Bary J, Boireau G, Fujier C (1997) Synth Commun 27:375
189. Oestreich M, Sempere-Culler F (2004) J Chem Soc Chem Commun, p 692
190. Sonogashira K, Tohda Y, Hagihara N (1975) Tetrahedron Lett 16:4467
191. Krohn K (2002) Eur J Org Chem, p 1351
192. Krohn K, Roemer E, Top M, Wagner C (1993) Angew Chem Int Ed Engl 32:1151
193. Fieser LF, Fryt M (1940) J Am Chem Soc 62:3489
194. Harris TM, Harris CH, Oster TA, Brown Jr LE, Lee YC (1988) J Am Chem Soc 110:6180
195. Yamaguchi M, Hasche K, Higashi H, Uchida M, Irie A, Minami T (1990) J Org Chem 55:1611
196. Cheema VS, Gulanti KC, Venkataraman K (1932) J Chem Soc, p 933
197. Baker W (1933) J Chem Soc, p 1381
198. Schumann G, Stengel C, Eckhardt K, Ihn W (1986) J Basic Microbiol 26:249
199. Krohn K, Klimars M, Köhle HJ, Ebeling E (1984) Tetrahedron 40:3677

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Combinatorial Synthesis of Linearly Condensed Polycyclic Compounds, Including Anthracyclinones, Through Tandem Diels–Alder Additions

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Abstract Double exocyclic 1,3-dienes such as 2,3,5,6-tetramethylidene-7-oxabicyclo-[2.2.1]heptane and its 1-substituted derivatives undergo two successive Diels–Alder additions with large reactivity difference between the addition of the first equivalent (k_1) and the second equivalent (k_2) of dienophile. This allows one to prepare, through parallel synthesis, a large number of linearly condensed polycyclic systems containing three annulated six-membered rings, including naphthacenyl systems and anthracyclinones. The large k_1/k_2 rate constant ratio is a consequence of the Dimroth principle, the first cycloaddition being significantly more exothermic then the second one. Control of regio- and stereoselectivity of the two successive cycloadditions is possible by 1-substitution of the 2,3,5,6-tetramethylidene-7-oxabicyclo[2.2.1]heptane, for instance by a 1-(dimethoxymethyl) group, or by stereoselective disubstitution of the double diene

by arenesulfenyl substituents. Enantiomerically pure anthracyclinones and analogues are obtained using enantiomerically pure dienophiles such as 3-oxo-but-2-en-2-yl esters. The chemistry so-developed has allowed the preparation of enantiomerically pure 6-((aminoalkoxy)oxy)methyl-6,7-dideoxyidarubicinones that are DNA intercalators and inhibitors of topoisomerase II-induced DNA strained religation.

Keywords Anthracyclines · Desymmetrization · Diels–Alder reactions · DNA strand religation · Furans · 2,3,5,6-Tetramethylidene-7-oxabicyclo[2.2.1]heptane · Topoisomerase inhibitors

Abbreviations

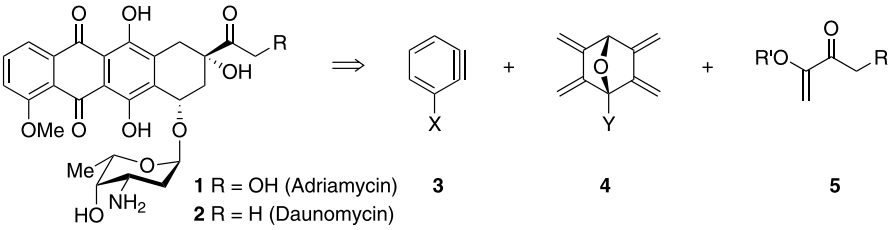
$\Delta H^{\ddagger}$	Activation enthalpy
ΔH_r	Heat of reaction
Ac	Acetyl
CD	Circular dichorism
DBN	1,5-Diazabicyclo[4.3.0]non-5-ene
DDQ	2,3-Dichloro-5,6-dicyanobenzoquinone
DME	1,2-Dimethoxyethane
[2.2.1]hericene	2,3,5,6,7-Pentamethylidenebicyclo[2.2.1]heptane
[2.2.2]hericene	2,3,5,6,7,8-Hexamethylidenebicyclo[2.2.2]octane
HMPA	Hexamethylphosphortriamide
HOMO	Highest occupied molecular orbital
IE	Ionization energy
LUMO	Lowest unoccupied molecular orbital
NBS	*N*-Bromosuccinimide
PCC	Pyridinium chlorochromate
Pd/C	Palladium metal on charcoal
PES	Photoelectron spectroscopy
PhH	Benzene
PMO	Perturbation of molecular orbitals
[4]radialene	1,2,3,4-Tetramethylidenecyclobutane
RADO(Et)-OH	(1*R*,5*R*,8*R*)-3-Ethyl-2-oxo-3-aza-6,9-dioxabicyclo[3.2.1]nonane-7-carboxylic acid
SADO(Et)-OH	(1*S*,5*S*,8*S*)-3-Ethyl-2-oxo-3-aza-6,9-dioxabicyclo[3.2.1]nonane-7-carboxylic acid
STO	Standard orbital
t-BuOH	*tert*-Butanol
t-BuOK	Potassium *tert*-butoxide
TCNE	Tetracyanoethylene
THF	Tetrahydrofuran

1 Introduction

Since their discovery in 1963 [1, 2] and their use as antineoplastic agents against human tumors [3–5] it has become clear that natural Adriamycin (Doxorubicin: **1**) and Daunomycin (Daunorubicin: **2**) would see their ther-

apeutic indexes improved upon structural modifications, both at the aglycone (antracyclinone [6–31]) and sugar (L-daunosamine [32–43]) parts. Cardiotoxicity represents a major factor that limits the use of **1** and **2** [44–47], although adjuvant can reduce their toxicity [48–50] and widen their therapeutic applications [51, 52]. Search for anthracycline analogues that overcome resistance build-up [53–59] and that have oral bioavailability [60–62] have been priority issues.

The most investigated pathways for the preparation of anthracyclinones [63–93] imply the coupling of two synthetic intermediates through Friedel–Crafts double acylation [12, 94], Fries rearrangement [95], Marshall reaction [96, 97], Claisen rearrangement [98], Diels–Alder cycloaddition [99, 100], Michael addition [101, 102], or via metalation of arenes [103]. The glycosidation of the anthracyclinones is generally accomplished with an adequately protected sugar under modified Koening–Knorr conditions [104, 105]. We have developed a combinatorial approach to the synthesis of linearly condensed six-membered ring systems, a method based on the three component condensations via two successive Diels–Alder additions (Scheme 1). The method disclosed in 1979 [106] (for a first synthesis of (±)-1-methoxydaunomycinone, applying our combinatorial approach, see [107]) has been applied to generate various anthracyclinones of biological interest, including enantiomerically pure idarubicinone and a new series of DNA intercalators of the type 6-(aminoalkyloxy)methyl-6,7-dideoxyidarubicinones that are mimics of anthracyclines.



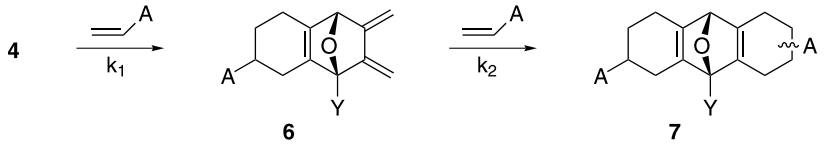
Scheme 1 Retro-synthetic analysis for the convergent and combinatorial synthesis of anthracyclinones and analoguous linearly condensed polycyclic compounds

2
Combinatorial Approach to the Synthesis of Anthracyclinones

When we embarked on the synthesis of anthracyclinones, our goal was to develop a strategy that would allow one to prepare not only the aglycones of natural anthracyclines but also a large variety of modified derivatives. Our methodology had thus to be highly versatile, meaning short and tolerant as far as the nature and the number of substituents that could be introduced

onto the anthracenyl system. It appeared to us that a doubly convergent [108] approach such as that outlined in Scheme 1 would realize a short and efficient way to prepare a large variety of anthracyclinones. In that strategy, two rings of the anthracyclinones are generated by two successive Diels–Alder additions (tandem cycloadditions) onto tetraenes of type **4** with two different dienophiles (e.g. **3**, **5**). The Diels–Alder addition [109] is probably the most versatile method for generating six-membered rings since it tolerates the use of a great variety of dienophiles and dienes [110–123].

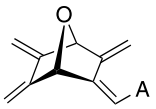
Thus, with the possibility of varying the substituents of the two dienophiles and those of tetraenes **4** our synthetic approach was expected to be a highly flexible one and amenable to the generation of a large number of linearly condensed six-membered ring systems in parallel syntheses or as libraries of compounds. The scope of this methodology depended on two conditions. The first one implied that tetraenes **4** would have to add to a given dienophile (leading to monoadducts **6**) with a rate constant k_1 larger than that (k_2) of the addition of second equivalent of the same dienophile to generate the bisadducts **7** (Scheme 2). Our goal was to avoid the formation of **7** during the reactions of one equivalent of the first set of dienophiles and to be able to use the corresponding monoadducts **6** directly with a second set of dienophiles, generating a collection of bis-adducts **7**. In practice, this goal requires a rate constant ratio $k_1/k_2 > 50$. If both types of dienophiles are not symmetrical, one also wishes to be able to control the regioselectivity, and the stereoselectivity of the two successive Diels–Alder additions. This is the second condition for useful synthetic strategies that should lead to the natural anthracyclines and analogues as well as to a library of useful anthracenyl systems.



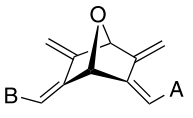
Scheme 2 The necessity of $k_1/k_2 \gg 2$

The first condition ($k_1/k_2 > 50$) can be satisfied, in principle, by substituting tetraenes **4** (see, e.g., 2-[(*E*)-methoxymethylidene]-3,5,6-trimethylidene-7-oxabicyclo[2.2.1]heptane, 2-[(*Z*)-chloromethylidene]- and 2-[(*E*)-chloromethylidene-3,5,6-trimethylidene-7-oxabicyclo[2.2.1]heptane) in a way that one of the two diene moieties [124] becomes more reactive than the other one (e.g., tetraenes of type **8**, **9**).

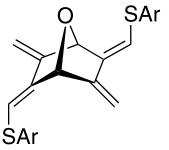
The first and the second conditions could be satisfied by introducing two different substituents A and B (tetraenes **9**), both capable of controlling the regioselectivity of the two successive cycloadditions, but A and B should be different enough to make one of the substituted diene units



8



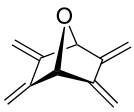
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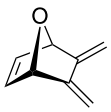


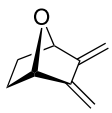
10 Ar = 2-$NO_2C_6H_4$

Structure 1

in **9** more reactive than the other one. Tetraenes **8** and **9** are complicated molecules, in the sense that they will require a relatively large number of synthetic steps as one has to introduce two different dienes onto the 7-oxabicyclo[2.2.1]heptane skeleton. With symmetrical tetraenes such as **4** the four methylidene groups can be attached to the bicyclic framework at the same time, thus reducing the number of synthetic steps for the preparation of these tetraenes compared with the preparations of the non-symmetrical derivatives **8** and **9**. We have found that the first condition is met for all the dienophiles tested. For instance, in the case of the Diels–Alder addition of **11** to ethylenetetracarbonitrile (TCNE), $k_1/k_2 = 376$ at 25 °C, with $\Delta H^{\ddagger}$ $(k_1) = 14.8 \pm 0.7$ kcal mol^{-1}, $\Delta S^{\ddagger}$ $(k_1) = -26.4 \pm 2.3$ e.u. and $\Delta H^{\ddagger}$ $(k_2) = 17 \pm 1.5$ kcal mol^{-1}, $\Delta S^{\ddagger}(k_2) = -30 \pm 4$ e.u. [125–127]. The possible reasons for the observed reactivity difference between tetraenes **4** and their monoadducts of type **6** will be discussed briefly below. The second condition (regioselectivity, stereoselectivity) has been realized with 1-substituted 2,3,5,6-tetremethylidene-7-oxabicyclo[2.2.1]heptanes (see below) and by the doubly substituted tetraene **10** (Ar = 2-NO_2-C_6H_4), the latter being derived in two steps from the readily available 2,3,5,6-tetramethylidene-7-oxanorbornane (**11**), as we shall see.







	11	**12**	**13**
HOMO energies:	-7.09	-7.28	-7.21 eV (STO 3G)
IE's:	8.60	8.87	8.74 eV (PES)

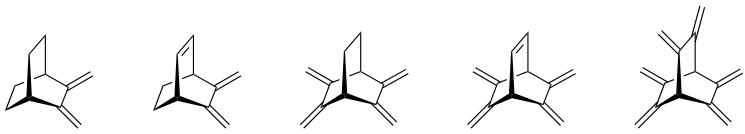
Structure 2

3
Kinetic Desymmetrization: Consequence of the Dimroth Principle

At the beginning of our studies, using simple perturbation molecular orbital (PMO) theory [128–130], we predicted that tetraenes **4** should be more reactive than the corresponding monoadducts **6** toward electron-poor

dienophiles. We reasoned that the Diels–Alder reactivity between **4** and **6** should be governed mostly by the difference between their ionization energies (IEs, hypothesis 1) [131–141]. We assumed that the geometry of the *s-cis*-butadiene unit does not change significantly between **4** and **6** (hypothesis II) [142, 143] and that the exothermicities of the two successive cycloadditions are nearly the same (hypothesis III) and therefore, do not affect the rate of the reactions as a consequence of the Dimroth [144] or Bell–Evans–Polanyi (BEP) principle [145–147]. Although the same number of sp^2 carbon atoms are present in the bicyclic skeletons of tetraene monoadducts and bisadducts, hypothesis III cannot be maintained, as we shall see.

If a significant overlap exists between the π-functions (through-space interactions [148–151]) and/or if there is a through-bond (hyperconjugation) mechanism that makes these functions "feel" each other [148, 149], PMO theory predicts that the HOMOs in trienes of type **6** must be somewhat stabilized compared with those in the corresponding tetraenes of type **4**. The LUMO localized on the endocyclic double bond in **6** can interact with the HOMO localized on the *s-cis*-butadiene moiety and thus stabilize the latter molecular orbital. For reasons of symmetry, such a stabilizing interaction is not possible between the LUMO and HOMO localized on the exocyclic diene units of the corresponding tetraenes. Qualitative quantum calculations on **11**, **12**, and **13** as well as gas phase PES are in apparent agreement with the PMO arguments presented above [127].



	14	**15**	**16**	**17**	**18**
HOMO energies:	-6.76	-6.79	-6.73	-6.85	eV (STO 3G)
IE's:	8.37	8.36	8.38	-8.36	8.38 eV (PES)

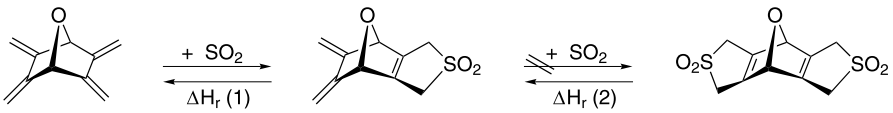
Structure 3

Other calculations and PES data on the relative bicyclo[2.2.2]octane derivatives **14–18** suggested, however, that the possible homoconjugative interaction predicted in triene **12** (and of monoadducts **6**) should not be overemphasized. In fact, it may not exist all as Roth and coworkers [152] have found that the heat of hydrogenation of the endocyclic double bond in 3,4-dimethylidenecyclobutene (31.6 kcal mol^{-1}) is not lower (due to lack of conjugative stabilization or aromaticity) than that of cyclobutene (30.7 kcal mol^{-1}). Furthermore, the PES spectra of 1,2-dimethylidenecyclobutane (IE: 8.66 eV) [153], 3,4-dimethylidenecyclobutene (IE: 8.80 eV) [154] and [4]radialene (IE: 8.35 eV) [155] do not show any significant conjugation effect between the endocyclic double bond and the exocyclic *s-cis*-butadiene moieties in 3,4-dimethylidenecyclobutene.

Quantum calculations and force-field calculations [126] on our exocyclic tetraenes **11**, **16**, **17**, and [2.2.2]hericene (**18**) and their Diels–Alder adducts (or model compounds of them) suggested that the *s-cis*-butadiene moieties in these systems are all planar and that the 1,4-distance between the extremities of the dienes does not vary between a tetraene and its monoadducts. Radiocrystallography of crystalline derivative [150, 151], as well as variable temperature circular dichroism (CD) data for enantiomerically pure (+)-(7*R*)-7-deutero-2,3,5,6-tetramethylidenebicyclo[2.2.2]octane and (+)-(7*S*)-7-methyl-2,3,5,6-tetramethylidenebicyclo[2.2.2]octane confirmed these predictions.

The absence of temperature and solvent dependence for the CD spectra of these tetraenes demonstrated that these systems do not equilibrate between conformers that imply twisting of the bicyclic skeletons and of the exocyclic diene moieties [156], as is expected to be the case also for the more rigid 7-oxabicyclo[2.2.1]heptane derivatives **11–13**.

Hints that a change in exothermicity between the two successive cycloadditions **4** → **6** → **7** is in fact responsible for the relatively large rate constant ratio k_1/k_2 (Scheme 2) came with the observation that this ratio was close to unity for the two successive Diels–Alder additions of tetraene **16**, whereas it was much larger than unity for pentaene **17**. In the case of [2.2.2]hericene (**18**), which undergoes three successive Diels–Alder additions, the two first additions have nearly the same rate constants (same $\Delta H^{\ddagger}$), whereas the third addition, which generates a barrelene system as in the case of the double Diels–Alder additions of pentaene **17**, is much slower for a given dienophile (higher $\Delta H^{\ddagger}$ value). The cycloadditions generating a barrelene system are less exothermic (by about 8–10 kcal mol^{-1}) due to the electronic strain of barrelene [157–161]. This was confirmed by measuring the heat and entropy of reaction for the cheletropic additions of sulfur dioxide to **16**, **17**, and **18**. Whereas **16** generates the corresponding bis-sulfolene, **17** adds only one equivalent of SO_2. Similarly, **18** adds successively two equivalents of SO_2 with similar heats of reaction but refuses to generate a tris-sulfolene [126] because of insufficient exothermicity due to the barrelene effect. With 2,3,5,6-tetramethylidene-7-oxabicyclo[2.2.1]heptane (**11**), only the corresponding monosulfolene **19** is formed between – 10 and 30 °C. Under forcing conditions (high concentration, long reaction time) no trace of the bis-sulfolene **20** can be detected [162].



11 |ΔH$_r$(1)|>|ΔH$_r$(2)| **19** **20**

Structure 4

The larger strain of 7-oxabicyclo[2.2.1]hepta-2,5-dienes such as **7** and **20** compared with that of 5,6-dimethylidene-7-oxabicyclo[2.2.1]hept-2-enes

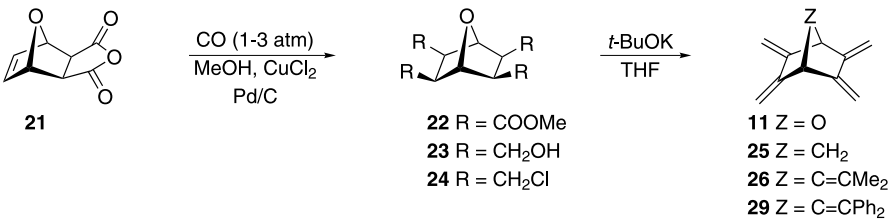


Fig. 1 Representation of the geometrical features of bicyclo[2.2.1]hept-2-ene (e.g., **6**) and bicyclo[2.2.1]hepta-2,5-ene (e.g., **7**)

such as **6** and **19** cannot be attributed to a change in the number of sp^2-hybridized carbon centers. It arises from repulsive σ, π interactions between the endocyclic double bonds and the 7-oxa bridge, these being more severe in the bis-adducts (**7**, **20**) than in the corresponding monoadducts (**6**, **19**), as suggested by crystalline molecular structures [163] (X-ray diffraction studies) of monoadducts of type **6** (Fig. 1). These showed, as for other bicyclo[2.2.1]heptenes [163–166], non-planar endocyclic double bonds, the electrons of which repel the 7-oxa bridge [167, 168].

4
Synthesis of 2,3,5,6-Tetramethylidene-7-oxabicyclo[2.2.1]heptanes

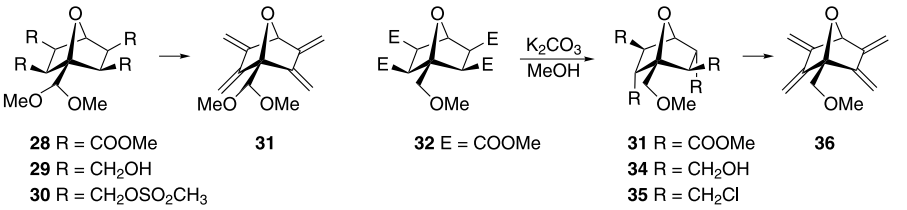
An efficient synthesis of **11** was developed based on the double methoxycarbonylation of the inexpensive Diels–Alder adduct of furan to maleic anhydride [169]. Under a CO pressure of 1–3 atm and the presence of $CuCl_2$ (oxidant) and a catalytical amount of Pd on charcoal in MeOH, adduct **21** furnishes the all-*exo* tetraester **22**, the esterification of the anhydride being complete due to HCl formation. Reduction with $LiAlH_4$ gives the corresponding tetrol **23**, which is chlorinated with $SOCl_2$/pyridine into **24**. Treatment with *t*-BuOH/THF (0–20 °C) gives **11** (64% overall yield based on **21**). The method has also been successful in the syntheses of 2,3,5,6-



Scheme 3 Syntheses of 2,3,5,6-tetramethylidenebicyclo[2.2.1]heptanes

tetramethylidinebicyclo[2.2.1]heptane (**25**) [125], 7,7-dimethyl[2.2.1]hericene (**26**), and 7,7-diphenyl[2.2.1]hericene (**27**) [170].

A similar method was applied to the synthesis of tetraene **31** starting with the Diels–Alder adduct of maleic anhydride to the dimethyl acetal of furfural. Instead of generating a tetrachloride from tetrol **29**, mesylate **30** is made on treatment with methanesulfonyl chloride and pyridine (Scheme 4). The tetramesylate **30** eliminates four equivalents of MsOH in the presence of *t*-BuOK, yielding **31** (60%). Lower yields are obtained through the tetrachloride route. In the case of the synthesis of **36**, it was found that the tetraester **32** has to be isomerized before the reduction with $LiAlH_4$ for a successful conversion into the corresponding tetraene **36**. On treatment of the all-*exo* tetraester **32** with anhydrous K_2CO_3 in absolute MeOH, the all-*trans* isomer **33** is obtained after 45 h at 20 °C (90% yield). Reduction with $LiAlH_4$ produces tetrol **34**, which is then chlorinated ($SOCl_2$/pyridine) to **35** (80%); quadruple elimination of HCl (*t*-BuOH/THF) affords **36** (69%).



28 R = COOMe
29 R = CH_2OH
30 R = $CH_2OSO_2CH_3$

31

32 E = COOMe

31 R = COOMe
34 R = CH_2OH
35 R = CH_2Cl

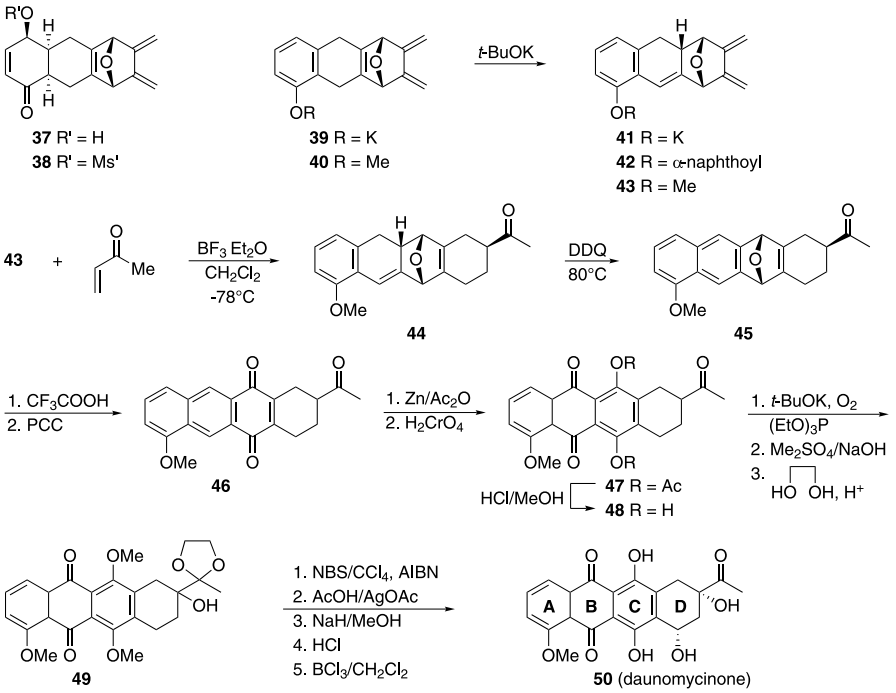
36

Scheme 4 Syntheses of 1-substituted 2,3,5,6-tetramethylidene-7-oxabicyclo[2.2.1]heptanes staring from furfural and furfuryl alcohol

5
Synthesis of Daunomycinone: Remote Control of the Diels–Alder Regioselectivity

The monoadduct of tetraene **11** and benzoquinone [107] can be reduced selectively into alcohol **37** (94%), the structure of which has been established by X-ray radiocrystallography [163]. The corresponding mesylate **38** eliminates one equivalent of methanesulfonic acid in the presence of two equivalents of *t*-BuOH/THF giving a potassium phenolate **39** that is reacted with MeI to give the corresponding anisole **40** (99%). When an excess of base is used (4.3 equivalents of *t*-BuOK), the phenolate intermediate **39** is isomerized regioselectively (7 : 3) into the vinylphenolate **41**.

As mentioned above, the 7-oxanorbornene moiety of **39** (Scheme 5) is more strained than the 5-methylidene-7-oxanorbornane system **41**. The regioselectivity of this process can be attributed to the formation of mixed aggregates involving *t*-BuOK and phenolate **41** [171, 172], as it was found that under similar conditions anisole **40** was isomerized into a 1 : 1 mixture of the



Scheme 5 Synthesis of (±)-daunomycinone

two possible regioisomeric vinylanisoles. Quenching **41** (contaminated with its regioisomer) with α-naphthoyl chloride gives the corresponding naphthoate **42** that is purified. It is then treated with K_2CO_3/MeOH followed by quenching with MeI, yielding **43**.

Diels–Alder cycloadditions to **40** with methyl vinyl ketone and methyl acrylate coordinated to strong Lewis acids such as $BF_3{\cdot}Et_2O$ or $TiCl_4$ were not regioselective. The PMO theory [73–175] has been very successful in predicting the regioselectivity of Diels–Alder additions of non-symmetrical dienes to dienophiles such as methyl vinyl ketone, which is given by the shape (eigenvectors) of the diene HOMO and dienophile LUMO. In the case of 2,3,5-trimethylidenenorbornane (**51**), a model compound for the non-symmetrical diene **43**, all quantum calculation techniques predicted similar 2p eigenvectors for the two methylene units of the exocyclic *s-cis*-butadiene moiety [176] (Fig. 2), leading to the prediction that the Diels–Alder cycloadditions of **51** and **43** to electron-poor dienophiles such as methyl vinyl ketone or methyl acrylate should not be regioselective, which is not the case [177, 178], fortunately.

Woodward and Katz [179], and later Firestone [180, 181], represented Diels–Alder transition states as diradicals resulting from the condensation of the two less-substituted centers of the diene and dienophile. In order to interpret the energy of concert in these cycloadditions, Epiotis [182] and

Dewar [147, 183, 184] include zwitterionic forms together with the diradicals (model of the diradicaloids (Fig. 2), which considers the fact that most Diels–Alder additions, although being concerted, form the two new σ bonds in a non-synchronous fashion in their transition states). Applied to **51** (and **43**), this model predicts a *para* regioselectivity (**52**) for the Diels–Alder addition of methyl vinyl ketone due to favorable homoconjugative participation of the non-conjugated alkene moiety [185–189], as shown with the zwitterionic form **52′**.

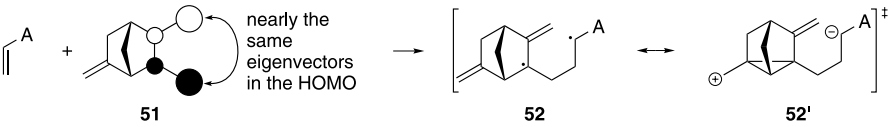
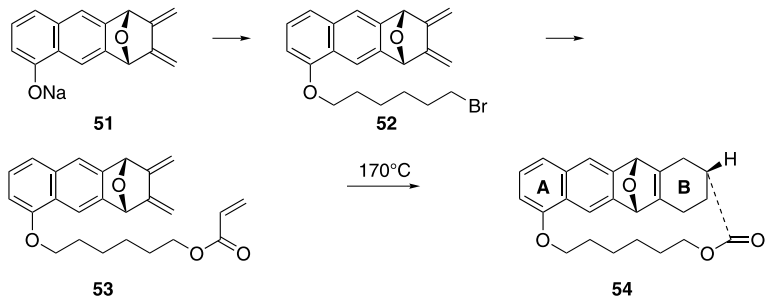


Fig. 2 Interpretation of the Diels–Alder regioselectivity of 2,3,5-trimethylidenebicyclo-[2.2.1]heptane applying the diradicaloid model for the transition state (*A* = electron-withdrawing substituent)

Good *para* regioselectivity (9 : 1) and stereoselectivity (β vs. $\alpha > 98 : 2$) were observed for the Diels–Alder addition of **43** to methyl vinyl ketone coordinated to $BF_3 \cdot Et_2O$ [185–189] in CH_2Cl_2 (–78 °C). This afforded adduct **44** (80% yield), which was oxidized into **45** on treatment with DDQ. Acidic rearrangement of the 7-oxanorbornadiene moiety [190] of **45** gave a mixture of naphthacenols that were oxidized into **46** with PCC. Reduction with Zn in Ac_2O/Et_3N followed by Jones oxidation gave **47** [100]. Treatment of **47** with HCl/MeOH gave **48**, which was oxidized at position C(9) with *t*-BuOK and O_2, then methylated with Me_2SO_4/NaOH. Acetylation of the methyl ketone with ethyleneglycol afforded **49**. Following the procedure of Broadhurst and Hassall [191], oxidation at C(7) used NBS bromation was followed by acetolysis. After deprotection, daunomycinone (±)-**50** was obtained.

Control of the regio- and stereoselectivity in the construction of ring A of the anthracyclinones was achieved, also relying on the intramolecular Diels–Alder addition [118, 192, 193]. Condensation of the sodium naph-



Scheme 6 Macrocycles by intramolecular Diels–Alder addition

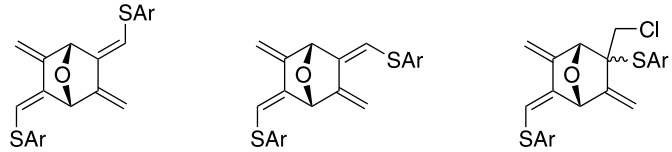
thoate **51** (derived from **39** through DDQ oxidation) with 1,6-dibromohexane (THF/HMPA, 0 °C) afforded bromide **52** (75%), which reacted with acrylic acid in the presence of DBN **53** to provide **53** (Scheme 6). Diluted solutions of **53** (1.6×10^{-3} M) were heated to 170 °C yielding the product of macrocyclization **54** [194].

The length of the tether is crucial for a successful macrocycle formation. When **51** was attached to acrylic acid through a three carbon linker, no intramolecular cycloaddition was observed to compete with the thermal polymerization of the diene (for other examples of macrocyclic formation through intramolecular Diels–Alder addition, see e.g., [195–200]).

6
Regio- and Stereoselective Tandem Diels–Alder Additions

The two successive Diels–Alder additions (tandem cycloadditions) of the C_{2V}-symmetrical tetraene **11** are not regioselective, i.e., mixtures of all the possible regioisomeric bis-adducts are obtained when utilizing non-symmetrical dienophiles. There is no control of the monoadduct on the regioselectivity of the second cycloaddition. As we have seen with the synthesis of daunomycinone, control of the substitution pattern of the two remote rings A and D of the anthracyclinones can be achieved provided a number of adequate manipulations are carried out with the benzoquinone monoadduct of **11**. A more efficient and more versatile way to control the regioselectivity of the tandem cycloadditions is through appropriate and stereoselective double substitutions of tetraene **11**.

Double addition of 2-nitrobenzenesulfenyl chloride (2.25 molar equivalents, AcOH, 8–9%, LiCl, 20 °C) to **11** gives a mixture of bis-adducts from which the major isomer precipitates (70%). This unstable bis-adduct undergoes double elimination of HCl on treatment with *t*-BuOK in THF (– 78 to 20 °C), yielding the C_2-symmetrical disubstituted tetraene **10** (80%). The bis-adducts left in the mother-liquor from the sulfenylation give a mixture of **10** (major), **55**, and **56** when treated with *t*-BuOK in THF (Struct. 5). The high regioselectivity in the sulfenylation of **11** can be due either to kinetic control in the addition, or to preferential stabilization of one bis-adduct (through



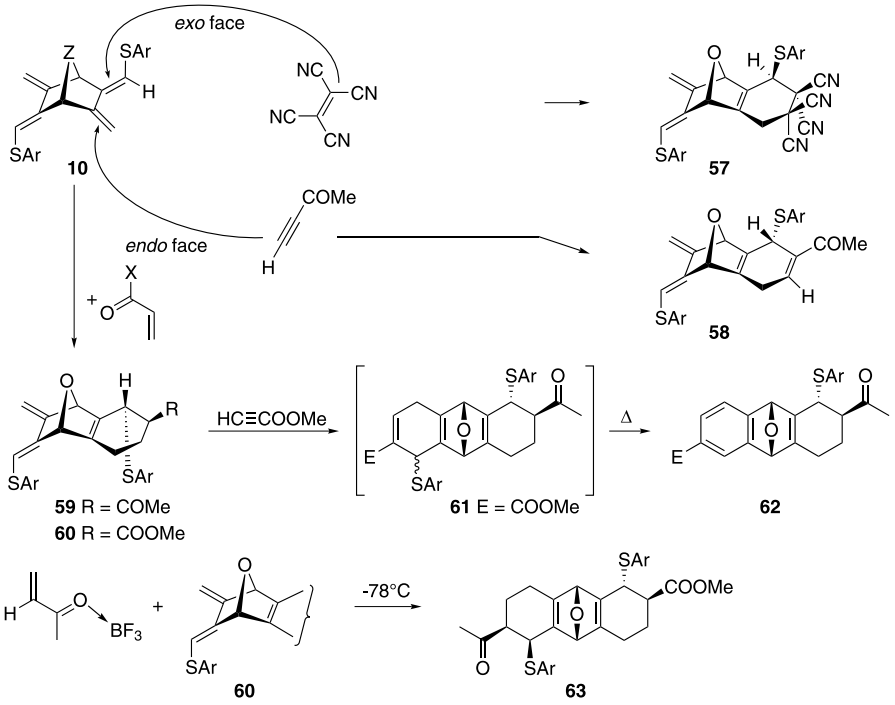
10 Ar = 2-$NO_2C_6H_4$ **55** **56**

Structure 5

crystallization). It is interesting to note that the double HCl elimination is also highly stereoselective.

The monoaddition of bulky dienophiles such as ethylenetetracarbonitirle (TCNE) to **10** are generally *exo* face selective (e.g., giving adduct **57**). However, less bulky dienophiles such as butynone or methyl vinyl ketone prefer, for stereoelectronic reasons, to add onto the *endo* face of **10** giving monoadducts **58** and **59**, respectively [201].

Interestingly, the cycloaddition of **10** and methyl vinyl ketone is also highly stereoselective with respect to the acetyl side chain in **59**. Analogous double diastereoselectivity (*endo* face attack, Alder *endo* rule [139, 202–204]) is observed for the cycloaddition of **10** to methyl acrylate (Scheme 7). As expected [179–184, 205], the Diels–Alder additions of **10** are *ortho* regioselective.



Scheme 7 Regio- and stereoselective tandem Diels–Alder additions

As in the case of tetraene **11** (and **17**, **25–27**, **31**, **36**), the formation of bis-adducts of **10** is found to be at least 50 times slower than the first dienophile addition, thus allowing the one-pot construction of a collection of bis-adducts with two series of different dienophiles. For example, when **59** is heated with methyl propyonate (PhH, 80 °C, 72 h), adduct **61** is formed, which eliminates one equivalent of 2-nitrobenzenesulfenic acid under the conditions of its for-

mation, yielding **62** (70%). The addition of methyl vinyl ketone coordinated to $BF_3{\cdot}Et_2O$ (CH_2Cl_2, –70 °C) to **60** gives **63** as major adduct (70%). It is interesting to note that, in contrast to the thermal addition of methyl vinyl ketone and methyl acrylate that are *endo* selective, when coordinated to a Lewis-acid, the more bulky dienophiles prefer the *exo* face of the dienes grafted to 7-oxanorbornane systems [201].

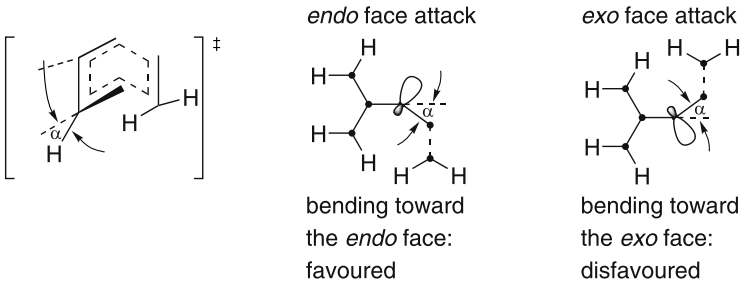


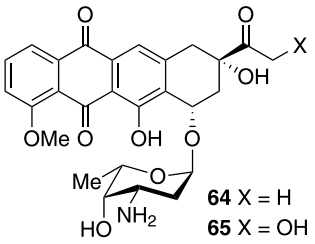
Fig. 3 Houk's model for the transition state of the synchronous Diels–Adler addition and its application to the cycloadditions of 2,3-dimethylidenebicyclo[2.2.1]heptane

Houk's model (Fig. 3 [206, 207]) assumes that the factors that are responsible for the non-planarity of the norbornene double bond intervene in the transition states of the cycloadditions of *s-cis*-butadiene moieties grafted at C(2), C(3) of norbornane and 7-oxanorbornane systems. The tighter the transition states, the higher the *endo* face selectivity. The less synchronous the cycloaddition (e.g., with non-symmetrical dienophiles coordinated to Lewis acids), the lower the *endo* face preference, steric factors competing in favor of the *exo* mode of addition. This theory is verified for a large number of cycloadditions including those we reported for deuterated 2,3-dimethylidenebicyclo[2.2.1]heptane [208].

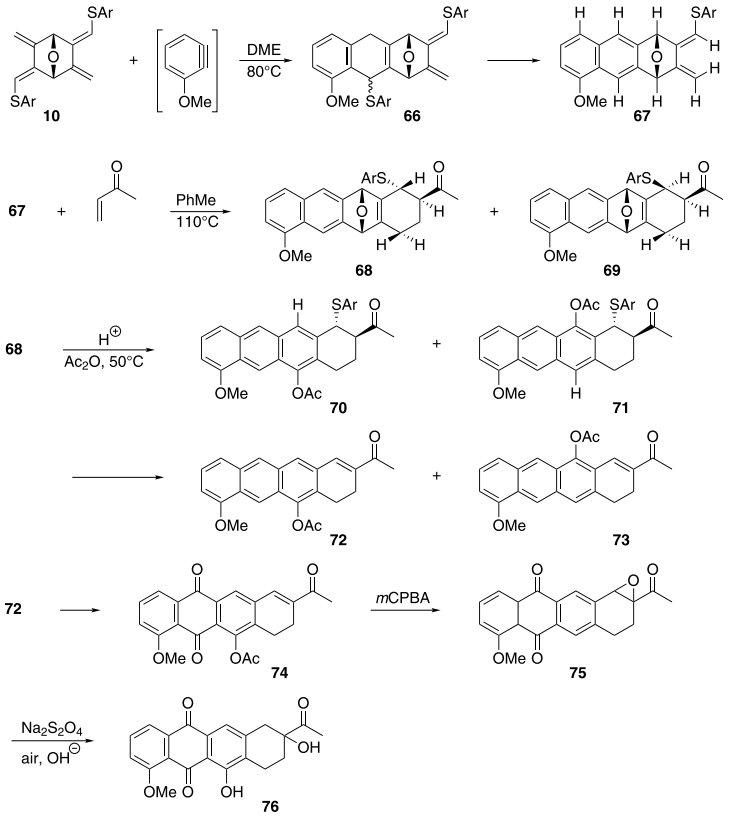
7
Synthesis of 11-Deoxydaunomycinone

Arcamone and coworkers [209] isolated from *Micromonospora peucetica* the 11-deoxydaunomycin (**64**) and 11-deoxyadramycin (**65**) (Struct. 6), which were shown to possess significant antitumor activity. Several efficient syntheses of the aglycones of these anthracyclines have been reported [210–234]. In our approach we start with the doubly substituted tetraene **10**.

The deamination of 2-amino-6-methoxybenzoic acid (pentyl nitrite, dimethoxyethane (DME), 85 °C) in the presence of tetraene **10** gives a mixture of the adduct **66** and its product of elimination. On treating this mixture with K_2CO_3 in boiling acetone, **67** is the sole product isolated in 46% yield.



Structure 6



Scheme 8 Synthesis of (±)-11-deoxydaunomycinone

The reaction of the 2,3-didehydroanisole (assumed to be generated during the deamination reaction) with **10** is highly regioselective since no trace of any regioisomer other than **67** could be observed. Furthermore, the reaction **66** or/and **67** with 2,3-didehydroanisole is much slower than with **10** as no trace

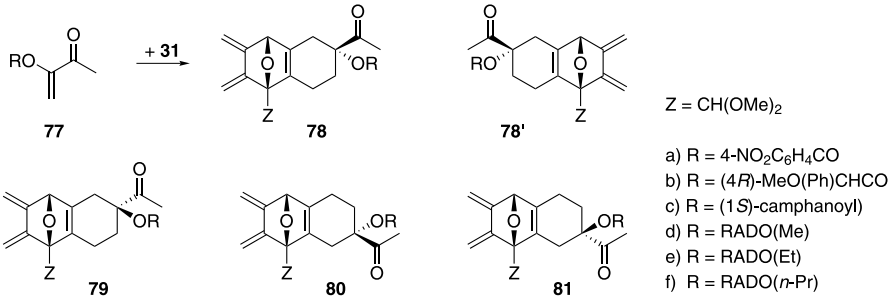
of the corresponding bis-adduct could be detected. This is in contrast with the reaction of didehydrobenzene with tetraene **11**, which was shown to give the corresponding mono- and bis-adducts competitively [235]. The modest yield of the transformation **10** → **67** is attributed to a certain lability of tetraene **10** and diene **67** under the reaction conditions.

The addition of methyl vinyl ketone (MVK) to **67** in toluene (110 °C) gives a 9 : 1 mixture of the adducts **68** and **69** (88%) (Scheme 8). The major isomer **68** is isolated in pure form by recrystallization from CH_2Cl_2/Et_2O 4 : 1. No trace of any other isomeric adduct could be detected, thus demonstrating the completely regioselective nature and the high stereoselectivity of the Diels–Alder reaction **67** + MVK. On treating, **68** in Ac_2O with a small amount of CF_3COOH (80 °C, 1 h), a 4 : 1 mixture of the tetrahydronaphthacene derivatives **70** and **71** is obtained. The crude mixture is then treated with *meta*-chloroperbenzoic acid to afford a mixture of **72** and **73** from which the major isomer **72** is obtained in pure form (65% based on **68**) by crystallization form CH_2Cl_2/Et_2O 5 : 1. Jones' oxidation of **72** in acetone (0 °C, N_2 atmosphere, 1 h) gives the anthraquinone **74** in 64% yield. The latter is then epoxidized to **75** (42%) at 20 °C. The reduction of the epoxide **75** with $Na_2S_2O_4$ under alkaline conditions yields 7,11-dideoxydaunomycinone **76**, which is then converted into (±)-11-deoxydaunomycinone applying known techniques [210–234].

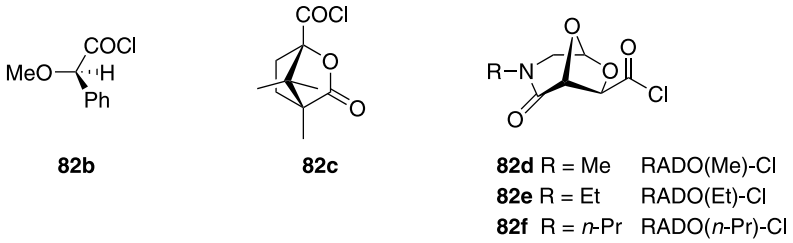
8
Desymmetrization with Enantiomerically Pure Dienophiles

The cycloadditions of tetraene **31** to 1-acetylvinyl esters of type **77** are expected to generate eight diastereomeric monoadducts of type **78–81** (Struct. 7). There are 16 modes of addition corresponding to the *para* (→ **78** + **79**) and *meta* (→ **80** + **81**) orientation, to the "Alder" or "*anti*-Alder" rule and whether the *exo* and *endo* face of the bicyclic exocyclic diene is involved. Under thermal conditions the Diels–Alder addition of methyl vinyl ketone to **31** is not stereoselective and gives a mixture of all possible monoadducts [236]. However, when methyl vinyl ketone is coordinated to a strong Lewis acid some selectivity is observed, the best results being obtained with $EtAlCl_2$. It is found also that the regioselectivity (product ratio) depends on the solvent. A single monoadduct (**78a**) is obtained for the cycloaddition of **31** to 1-acetylvinyl *p*-nitrobenzoate [237, 238] precomplexed to $BF_3 \cdot Et_2O$ [239]. For all these Diels–Alder additions the formation of the bis-adducts of **15** are at least 100 times slower than the additions of the first equivalent of the dienophile **77**.

Condensation of diacetyl with the optically pure acyl chlorides **82b–f** (Struct. 8) in toluene (pyr., 0 °C) gives the corresponding dienophiles **77b–f**. They add slowly and with low stereoselectivity to tetraene **31** on heating. A mixture of eight monoadducts are obtained in all cases. However, in the



Structure 7



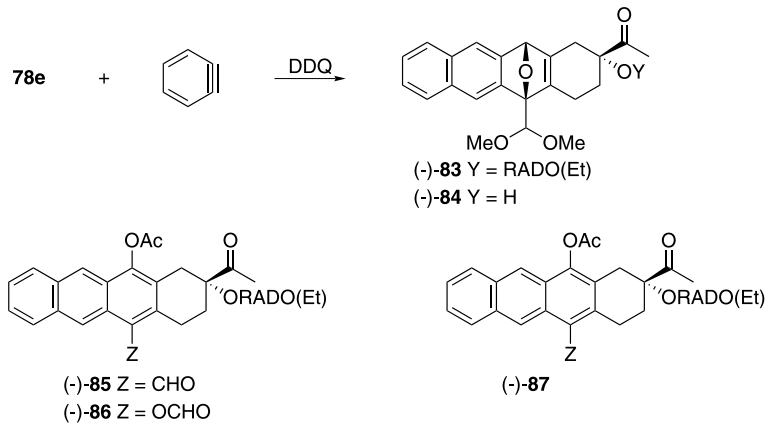
82b **82c**

82d R = Me RADO(Me)-Cl
82e R = Et RADO(Et)-Cl
82f R = *n*-Pr RADO(*n*-Pr)-Cl

Structure 8

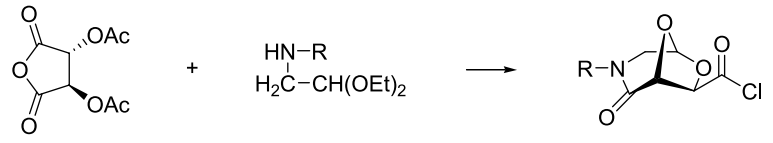
presence of a large excess of $BF_3{\cdot}Et_2O$, **31** adds to **77b–f** giving the corresponding monoadducts **78b–f** and **78b–f** with less than 10% of the other stereomeric adducts, thus confirming the high regio- and stereoselectivity of the Lewis-acid-promoted Diels–Alder additions. The best diastereoselectivity is observed with **77e** (product ratio **78e/78′e** 87 : 13) in CH_2Cl_2. Changing the solvent from pure CH_2Cl_2 to $CH_2Cl_2/MeNO_2$ 6 : 1 (–78 °C) leads to a higher yield but with lower diastereoselectivity (83 : 17). In CH_2Cl_2/hexane 5 : 1 (–55 °C) and CH_2Cl_2/CF_2Cl_2 2 : 1 (–78 °C) the yields and diastereoselectivity are not improved (51% (81 : 19) and 20% (82 : 18), respectively). Surprisingly, in pure propionitrile the yield climbs to 90% but with lower and reversed diastereoselectivity (43 : 57). The 87 : 13 mixture of **78e** and **78′e** reacts with didehydrobenzene (generated through nitrosation of anthranilic acid with isopentyl nitrite in 1,2-dimethoxyethane (DME/$CHCl_3$). After treatment with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ in DME, 55 °C) the naphthacenyl methyl ketone (–)-**83** is saponified with 1 M NaOH/EtOH to give (–)-**84** (Scheme 9).

Treatment of crude (–)-**83** with $Me_3SiOSO_2CF_3$ in CH_2Cl_2 (0 °C), followed by acetylation (Ac_2O/pyridine, 25 °C) and recrystallization from EtOAc furnishes the diastereomerically pure naphthacenyl methyl ketone (–)-**85** (55%). Baeyer–Villiger oxidation with SeO_2 and H_2O_2 leads to (–)-**86** (94%), which upon oxidation (4 N Jones-reagent, acetone, 0–20 °C) and saponification (1 N NaOH/THF, 20 °C) affords (–)-**87** in 80% yield, a known precursor of (–)-7-deoxyidarubicinone [240, 241].



Scheme 9 Synthesis of enantiomerically pure naphthacene derivatives

The chiral auxiliaries **82d–f** (RADO(R)-Cl) are obtained readily from (*R*,*R*)-tartaric acid. Treatment of di-*O*-acetyl-(*R*,*R*)-tartaric anhydride ((*R*,*R*)-**88**) with alkylaminoacetaldehyde diethyl acetate **89** in CH_2Cl_2 affords, after treatment with MeOH/$SOCl_2$, the corresponding methyl esters RADO(R)-OMe. Acidic hydrolysis gives the corresponding carboxylic acids RADO(R)-OH, which are converted into the corresponding acyl chlorides **82d–f** (RADO(R)-Cl) with $SOCl_2$. The acronym RADO(R) stands for 3-aza-6,8-dioxabicyclo[3.2.1]octane derived from (*R*,*R*)-tartaric acid. Using (*S*,*S*)-tartaric acid, the corresponding SADO(R)-X derivatives are prepared with the same ease. Esters derived from our chiral auxiliaries RADO(R) and SADO(R) are particularly useful because they can be treated under relatively strong acidic conditions (Lewis acids) without epimerization or decomposition. Their saponification under alkaline media allows one to recover the corresponding carboxylic salts in the aqueous phase of the work-up extracts without epimerization or/and hydrolysis. If deprotonation at C(7) occurs, the *exo* carboxylic moiety is formed upon deprotonation because the carboxylic derivatives are more stable in their *exo* than *endo* relative configuration [242]. The same chiral auxiliaries can be used to generate enantiomerically pure 7-oxabicyclo[2.2.1]hept-5-en-2-yl derivatives ("naked sugars of the first generation") that have been used to prepare enantiomerically pure sugars such

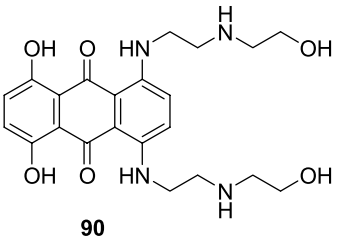


Structure 9

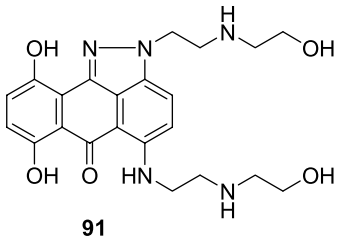
as L-daunosamine [243] and 2-deoxy-L-fucose [244] sugars that are found in anthracyclines).

9
Synthesis of Enantiomerically Pure 6-[[(Aminoalkyl)oxy]methyl]-6,7-dideoxyidarubicinones

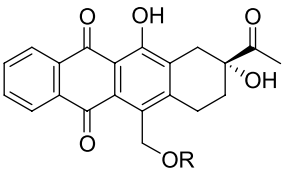
Simple 1,4-bis[(aminoalkyl)amino]-9,10-anthraquinones [245] such as mitoxantrone **90** [246] and iosoxantrone **91** [247, 248, 250] have useful anticancer properties. These observations together with the fact that polyamines are able to inhibit cancer cell growth [251–254] led us to conceive a new series of anthracycline analogues (e.g., (–)-**92**–(–)-**96**) bearing aminoalkyl chains through a benzyl ether link at C(6).



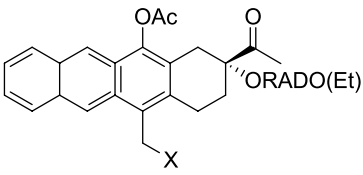
90



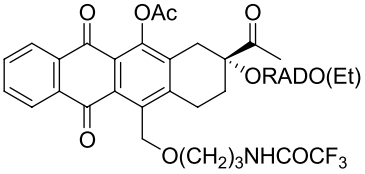
91



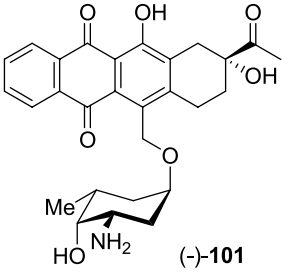
(-)-**92** R = $(CH_2)_3NH_2 \cdot HCl$
(-)-**93** R = $(CH_2)_4NH_2 \cdot HCl$
(-)-**94** R = $(CH_2)_5NH_2 \cdot HCl$
(-)-**95** R = $(CH_2)_2NH(CH_2)_3NH_2 \cdot (HCl)_2$
(-)-**96** R = $(CH_2)_3NH(CH_2)_3NH_2 \cdot (HCl)_2$



(-)-**97** X = OH
(-)-**98** X = Br
(-)-**99** X = $O(CH_2)_3NHCOCF_3$



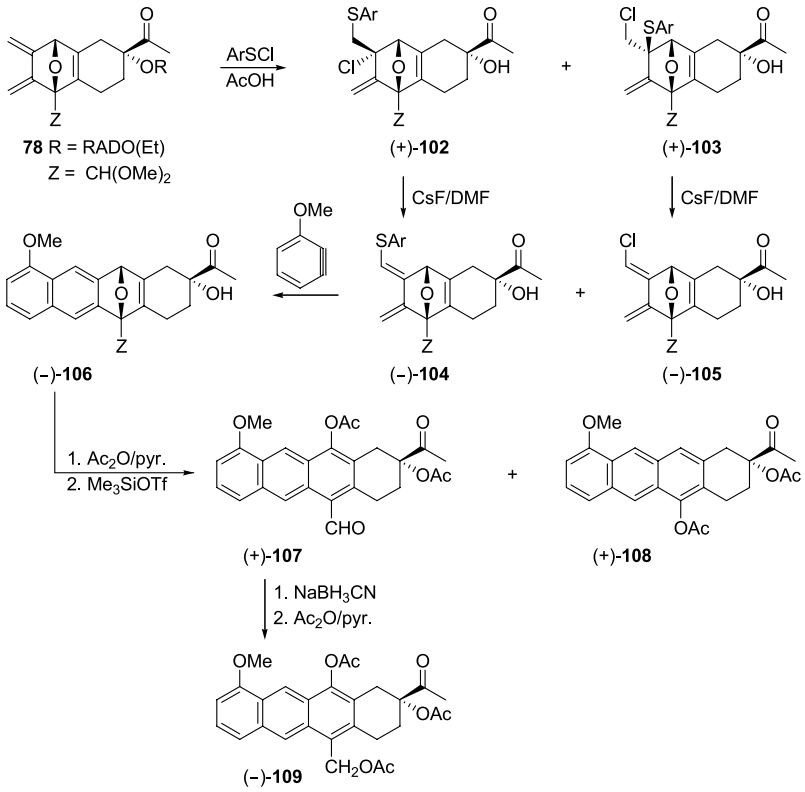
(-)-**100**



(-)-**101**

Structure 10

The reduction of aldehyde (–)-**85** (Scheme 9) with $NaBH_3CN$ in AcOH/MeOH/$CHCl_3$ (20 °C) is selective (no reduction of the methyl ketone moiety) and affords benzyl alcohol (–)-**97** (93%), which can be converted into bromide (–)-**98** (97%) on treatment with AcBr in $CHCl_3$. Nucleophilic displacement of (–)-**98** with 3-(trifluoroacetamido)propanol (AgOTf, THF, 20 °C) gives (–)-**99** (87%), the Jones oxidation of which leads to naphthacequinone (–)-**100** (85%). Treatment with LiOH (THF/H_2O, 0 °C) cleaves the RADO(Et) and acetate esters. Without isolating the corresponding alcohol, the mixture is then treated with aqueous 0.02 M NaOH (25 °C) and finally with aqueous HCl to furnish (–)-**92** (62%). The same method has been applied to the synthesis of (–)-**93**–(–)-**96**. We found that (–)-**92**, (–)-**93**, and (–)-**94** are intercalators of calf thymus DNA ($Kb = 1.1 \times 10^{-5}$ M^{-1} for (–)-**92**) and are inhibitors of topoisomeriase II-induced DNA strand religation [255]. Glycosidation of alcohol (–)-**97** with 2,3,6-trideoxy-4-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamide)-(α-L-lyxo-hexopyranosyl *p*-nitrobenzoate (Me_3SiOTf/THF, –25 °C) gives a 2 : 1 mixture of the α- and β-glycosides. Jones oxidation of the α-anomer, followed



Scheme 10 Synthesis of enantiomerically pure 1-methoxy-4,6-dideoxy-6-hydroxymethyl-daunomycinone precursors

by ester cleavage with $LiOH/H_2O/THF$ and hydrolysis of the trifluoroacetamido moiety with aqueous 0.02 M NaOH, followed by acidificaton with 0.1 M HCl provides (–)-**101** (23% overall yield based on (–)-**97**), which does not intercalate calf thymus DNA. Thus the simpler systems (–)-**92**–(–)-**94** seem to be better mimics of natural anthracyclines than (–)-**101**.

Analogues of compounds (–)-**92**–(–)-**96** with a 1-methoxy substitutent at the D-ring can be prepared in the following way (Scheme 10) [256]. Addition of 2-nitrobenzenesulfenyl chloride in AcOH to the enantiomerically pure adduct **78** gives a mixture of products from which saponification with 1 N NaOH in $MeOH/H_2O$ furnishes alcohols (+)-**102**; in DMF (80 °C) in the presence of CsF it gives the (*Z*)-thiodiene (–)-**104** (78%) and its (*E*)-isomer (15%). Under similar conditions (+)-**103** affords the (*Z*)-chlorodiene (–)-**105**. The two dienes (–)-**104** and (–)-**105** add to 2,3-didehydroanisole giving the expected major product (+)-**106** (70%). Acetylation of the tertiary alcohol (–)-**106** followed by treatment with $Me_3SiOSO_2CF_3$ affords (–)-**109** (59%) and (+)-**108** (17%). Selective reduction of aldehyde (+)-**107** with $NaBH_3CN$ in $MeOH/CHCl_3/AcOH$ gives the benzylic alcohol, which was acetylated into (–)-**109**.

10 Conclusion

Furan and 2-substituted furans can be converted readily into 2,3,5,6-tetramethylidene-7-oxabicyclo[2.2.1]heptane and 1-substituted derivatives, respectively. These double dienes react with a large number of dienophiles giving the corresponding monoadducts. The additions of the same dienophiles to the *s-cis*-butadiene moieties of the monoadducts occur much more slowly because the formation of the bis-adducts (7-oxabicyclo[2.2.1]hepta-2,5-dienes) is less exothermic than the formation of the corresponding monoadducts (5,6-dimethylidene-7-oxabicyclo[2.2.1]hept-2-enes). Thanks to the Dimroth principle, the 2,3,5,6-tetramethylidene-7-oxabicyclo[2.2.1]heptanes can be combined with two different dienophiles in two successive cycloadditions without protection and deprotection steps. This allows one to generate large libraries of linearly condensed six-membered ring systems, including antracycline precursors. Using enantiomerically pure 1-acetylvinyl esters as dienophiles, optically active cycloadducts can be obtained, and some of them have been converted into enantiomerically pure anthracyclines and analogues. The chiral auxiliaries used in this work (RADO(Et), SADO(Et)) are derived from the abundant (*R*,*R*)- and (*S*,*S*)-tartaric acids.

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References

1. Grein A, Spalla C, Canevazzi G, Dimarco A (1963) Giorn Microbiol 11:109
2. Dubost M, Pinnert S, Maral R, Ganter P, Preudhomme J, Werner GH, Ninet L (1963) CR Acad Sci Paris 257:1813
3. Arcarmone F (1981) Doxorubicin: anticancer antibiotics. Academic, New York, p 369
4. Neidle S (1977) Nature 268:195
5. Lown JW (1993) Chem Soc Rev 22:165
6. Maral R, Ponsinet G, Jolles G (1972) CR Acad Sci Ser D 275:301
7. Zunino F, Gambetta R, Dimarco A, Luoni G, Zaccara A (1976) Biochem Biophys Res Commun 69:744
8. Arcamone F, Bernardi L, Giardino P, Patelli B, Dimarco A, Casazza AM, Pratesi G, Reggiani P (1976) Cancer Treat Rep 60:829
9. Gabbay EJ, Grier D, Fingerle RE, Reimer R, Levy R, Pearce SW, Wilson WD (1976) Biochemistry 15:2062
10. Penco S, Angelucci F, Vigevani A, Arlandini E, Arcamone F (1977) J Antibiot 30:764
11. Dimarco A, Casazza AM, Giuliani F, Pratesi G, Arcamone F, Bernardi L, Franchi G, Giardino P, Patelli B, Penco S (1978) Cancer Treat Rep 62:375
12. Arcamone F, Bernardi L, Patelli B, Giardino P, Dimarco A, Casazza AM, Soranzo C, Pratesi G (1978) Experientia 34:1255
13. Smith TH, Fujiwara AN, Henry DW (1978) J Med Chem 21:280
14. Ducep J, Farge D, Ponsinet G, Reisdorf D (1978) US Patent 4075328
15. Bernardi L, Masi P, Sapini O, Suarato A, Arcamone F (1979) Farmaco Ed Sci 34:884
16. Lown JW, Chen HH, Plambeck JA, Acton EM (1979) Biochem Pharmacol 28:2563
17. Plumbridge TW, Brown JR (1979) Biochem Biophys Acta 563:181
18. Arlandini E, Vigevani A, Arcamone F (1980) Farmaco Ed Sci 35:65
19. Acton EM, Tong GL (1981) J Med Chem 24:669
20. Yoshimoto A, Fujii S, Johdo O, Kubo K, Ishikura T, Naganawa H, Sawa T, Takeuchi T, Umezawa H (1986) J Antibiot 39:902
21. Swenton J, Morrow G, Priebe W (1987) US Patent 4697005
22. Ishizumi K, Ohashi N, Tanno N (1987) J Org Chem 52:4477
23. Caruso M, Suaroto A, Angelucci F, Arcamone F (1988) Eur Pat Appl EP 288268
24. Matsuda F, Matsumoto T, Ohsaki M, Terashima S (1989) Tetrahedron Lett 30:4259
25. Adams N, Blake C, Broadhurst MJ, Bushnell DJ, Hassall CH, Hartmann HR, Keech E, Stratton AR, Thomas GJ (1990) J Med Chem 33:2380
26. Giolitti A, Guidi A, Pasqui F, Pestellini V, Arcamone F (1991) Can Pat Appl CA 2029715
27. Barr KE, Russell RA, Warrener RN, Collins JG (1993) FEBS Lett 322:173
28. Canfarini F, Giolitti A, Guidi A, Pasqui F, Pestellini V, Arcamone F (1993) Tetrahedron Lett 34:4697
29. Lown JW (1993) Chem Soc Rev 22:165
30. Priebe W (1995) Curr Pharm Des 1:51
31. Priebe W (1995) Anthracycline antibiotics: new analogs, methods of delivery, and mechanisms of action. Am Chem Soc, Washington DC, p 332
32. Israel M, Modest EJ, Frei E (1975) Cancer Res 35:1365

33. Arcamone F, Penco S, Redaelli S, Hanessian S (1976) J Med Chem 19:1424
34. Penco S, Franchi G, Arcamone F (1978) DE 751395
35. Cassinelli G, Ruggieri D, Arcamone F (1979) J Med Chem 22:122
36. Horton D, Priebe W, Varela O (1984) J Antibiot 37:1635
37. Cassinelli G, Ballabio M, Arcamone F, Casazza AM, Podesta A (1985) J Antibiot 38:856
38. Florent JC, Genot A, Monneret C (1989) J Antibiot 42:1823
39. Kunimoto S, Komuro K, Nosaka C, Tsuchiya T, Fukatsu S, Takeuchi T (1990) J Antibiot 43:556
40. Priebe W, Neamati N, Perez-Soler R (1992) J Antibiot 45:386
41. Florent JC, Gaudel G, Monneret C, Hoffmann D, Kraemer HP (1993) J Med Chem 36:1364
42. Zhang G, Fang L, Wang PG, Sun D (2006) PIXXD2 WO 2006/124720 A1 2006/1123
43. Zhang G, Fang L, Zhu L, Zhong Y, Wang PG, Sun D (2006) J Med Chem 49:1792
44. Al-Ismail SAD, Parry DH, Whittaker JA (1977) Brit Med J 1:815
45. Mcguire WP (1978) Cancer Treat Rep 62:855
46. Villani F, Meazza R, Materazzo C (2006) Anticancer Res 26:797
47. Menna P, Minotti G, Salvatorelli E (2007) Cell Biol Toxicol 23:49
48. Wiseman LR, Spencer CM (1998) Drugs 56:385
49. Cvetkokic RS, Scott LJ (2005) Drugs 65:1005
50. Hasinoff B (2006) Future Oncol 2:15
51. Kaufman PA (1999) Semin Oncol 26:39
52. Danesi R, Conte PF, Del Tacca M (1999) Clin Pharmacokinet 37:195
53. Bakker M, Van der Graaf W, Groen H, De Vries E (1995) Curr Pharm Des 1:133
54. Lenaz L, Page JA (1976) Cancer Treat Rev 3:111
55. Von Hoff DD, Layard M, Rozencweig M, Muggia FM (1977) Cancer Treat Rep 61:1411
56. Beck WT (1987) Biochem Pharmacol 36:2879
57. Keizer HG, Pinedo HM, Schuurhuis GJ, Joenje H (1990) Pharmacol Ther 47:219
58. Mazue G, Iatropoulos M, Imondi A, Castellino S, Brughera M, Podesta A, Dellatorre P, Moneta D (1995) Int J Oncol 7:713
59. Ravdin PM (1995) Eur J Cancer 31A:S11
60. Cersosimo RJ (1992) Clin Pharm 11:152
61. Goebel M (1993) Ann Hematol 66:33
62. Buckley MM, Lamb HM (1997) Drugs Aging 11:61
63. Krohn K (1990) Tetrahedron 46:291
64. Krohn K (1986) Angew Chem Int Ed Engl 25:790
65. Krohn K (1989) Prog Chem Org Nat Prod 55:37
66. Broadhurst MJ, Hassall CH, Thomas GJ (1985) Chem Ind 106
67. Kawasaki M, Matsuda F, Terashima S (1988) Tetrahedron Lett 29:791
68. Cameron DW, Feutrill GI, Griffiths PG (1988) Tetrahedron Lett 29:4629
69. Adams N, Blake C, Broadhurst MJ, Bushnell DJ, Hassall CH, Hartmann HR, Keech E, Stratton AR, Thomas GJ (1990) J Med Chem 33:2375
70. Farquhar D, Newman RA, Zuckerman JE, Andersson BS (1991) J Med Chem 34:561
71. Bertounesque E, Florent JC, Monneret C (1991) Synthesis, p 270
72. Cherif A, Farquhar D (1992) J Med Chem 35:3208
73. Laugraud S, Guingant A, Dangelo J (1992) Tetrahedron Lett 33:1289
74. Giolitti A, Guidi A, Pasqui F, Pestellini V, Arcamone FM (1992) Tetrahedron Lett 33:1637
75. Deguin B, Bertounesque E, Gaudel G, Florent JC, Monneret C (1992) Tetrahedron 48:4885
76. Terashima S (1992) Synlett, p 691

77. Cousson A, Legouadec G, Monneret C, Florent JC (1993) J Chem Soc, Chem Commun, p 388
78. Kita Y, Maeda H, Takahashi F, Fukui S (1993) J Chem Soc, Chem Commun, p 410
79. Florent JC, Gaudel G, Monneret C, Hoffmann D, Kraemer HP (1993) J Med Chem 36:1364
80. Lavallee JF, Rej R, Courchesne M, Nguyen D, Attardo G (1993) Tetrahedron Lett 34:3519
81. Welch SC, Levine JA, Arimilli MN (1993) Synth Commun 23:131
82. Krohn K, Roemer E, Top M, Wagner C (1993) Angew Chem Int Ed Engl 32:1151
83. Farina F, Paredes MC, Valderrama JA (1993) Tetrahedron 49:10715
84. Canfarini F, Giolitti A, Guidi A, Pasqui F, Pestellini V, Arcamone F (1993) Tetrahedron Lett 34:4697
85. Cameron DW, Griffiths PG (1993) Tetrahedron Lett 34:4685
86. Monneret C, Florent JC (1994) Synlett, p 305
87. Guidi A, Canfarini F, Giolitti A, Pasqui F, Pestellini F, Pestellini V, Arcamone F (1994) Pure Appl Chem 66:2319
88. Tanaka H, Yoshioka T (1994) Chem Lett 953
89. Gesson JP, Jacquesy JC, Renoux B (1994) Tetrahedron 50:1165
90. Guidi A, Arcamone F (1996) Tetrahedron Lett 37:1123
91. Takagi Y, Nakai K, Tsuchiya T, Takeuchi T (1996) J Med Chem 39:1582
92. Asenjo P, Farina F, Martin MV, Paredes MC, Soto JJ (1997) Tetrahedron 53:1823
93. TheurillatMoritz V, Guidi A, Vogel P (1997) Tetrahedron: Asymmetry 8:3497
94. Wong CM, Popien D, Schwenk R, Teraa J (1971) Can J Chem 49:2712
95. Kende AS, Belletire J, Bentley TJ, Hume E, Airey J (1975) J Am Chem Soc 97:4425
96. Suzuki F, Trenbeath S, Gleim RD, Sih CJ (1978) J Org Chem 43:4159
97. Morris MJ, Brown JR (1978) Tetrahedron Lett 19:2937
98. Hauser FM, Hewawasam P (1988) J Org Chem 53:4515
99. Gupta RC, Harland PA, Stoodley RJ (1983) J Chem Soc, Chem Commun, p 754
100. Tamariz J, Vogel P (1984) Tetrahedron 40:4549
101. Kende AS, Rizzi J, Riemer J (1979) Tetrahedron Lett 20:1201
102. Chenard BL, Dolson MG, Sercel AD, Swenton JS (1984) J Org Chem 49:318
103. Watanabe M, Maenosono H, Furukawa S (1983) Chem Pharm Bull 31:2662
104. Acton EM, Fujiwara AN, Henry DW (1974) J Med Chem 17:659
105. Kimura Y, Suzuki M, Matsumoto T, Abe R, Terashima S (1986) Bull Chem Soc Jpn 59:423
106. Vogel P, Carrupt PA (1979) CH Patent 4060/100
107. Carrupt PA, Vogel P (1979) Tetrahedron Lett 20:4533
108. Bertz SH (1982) J Am Chem Soc 104:5801
109. Diels O, Alder K (1928) Liebigs Ann Chem 460:98
110. Wollweber H (1970) Houben-Weyl, Methoden der Organischen Chemie, 4th edn. Thieme, Stuttgart
111. Wollweber H (1972) Diels–Alder Reaktion. Thieme, Stuttgart
112. Carruthers W (1978) Some modern methods of organic synthesis, 2nd edn. Cambridge University Press, Cambridge
113. Oppolzer W (1978) Synthesis, p 793
114. Brieger G, Bennett JN (1980) Chem Rev 80:63
115. Petrzilka M, Grayson JI (1981) Synthesis, p 753
116. Weinreb SM, Staib RR (1982) Tetrahedron 38:3087
117. Welzel P (1983) Nachr Chem Tech Lab 31:979
118. Fallis AG (1984) Can J Chem 62:183

119. Oppolzer W (1984) Angew Chem Int Ed Engl 23:876
120. Matsumoto K, Sera A (1985) Synthesis, p 999
121. Carruthers W (1990) Cycloaddition reactions in organic synthesis. Pergamon, Oxford
122. Fringuelli F, Taticchi A (1990) Dienes in the Diels–Alder reaction. Wiley, New York, p 348
123. Boger DL, Weinreb SM (1987) Hetero Diels–Alder methodology in organic synthesis. Academic, San Diego, p 366
124. Mahaim C, Vogel P (1982) Helv Chim Acta 65:866
125. Pilet O, Vogel P (1981) Helv Chim Acta 64:2563
126. Pilet O, Birbaum JL, Vogel P (1983) Helv Chim Acta 66:19
127. Vogel P (1989) In: Thummel R (ed) Advances in theoretically interesting molecules. JAI, Greenwich, CT, p 201
128. Dewar MJS, Dougherty R (1975) The PMO theory of organic chemistry. Plenum, New York
129. Whangbo MH, Schlegel HB, Wolfe S (1977) J Am Chem Soc 99:1296
130. Borden W (1975) Modern molecular orbital theory for organic chemists. Prentice-Hall, Englewood Cliffs, NJ
131. Fukui K (1966) Bull Chem Soc Jpn 39:498
132. Klopman G (1968) J Am Chem Soc 90:223
133. Salem L (1968) J Am Chem Soc 90:543
134. Salem L (1968) J Am Chem Soc 90:553
135. Fukui K (1971) Acc Chem Res 4:57
136. Sustmann R, Schubert R (1972) Angew Chem Int Ed 11:840
137. Epiotis N (1978) Theory of organic reactions. Springer, Berlin Heidelberg New York
138. Epiotis ND (1979) Pure Appl Chem 51:203
139. Sauer J, Sustmann R (1980) Angew Chem Int Ed Engl 19:779
140. Rucker C, Lang D, Sauer J, Friege H, Sustmann R (1980) Chem Ber Rec 113:1663
141. Houk KN (1977) Pericyclic reactions. Academic, New York
142. Scharf HD, Plum H, Fleischhauer J, Schleker W (1979) Chem Ber Rec 112:862
143. Sustmann R, Bohm M, Sauer J (1979) Chem Ber Rec 112:883
144. Dimroth O (1933) Angew Chem 46:571
145. Evans MG, Polanyi M (1938) Trans Faraday Soc 34:0011
146. Bell R (1936) Proc R Soc Lond A 154:414
147. Dewar MJS (1984) J Am Chem Soc 106:209
148. Hoffmann R (1971) Acc Chem Res 4:1
149. Hoffmann R, Imamura A, Hehre WJ (1968) J Am Chem Soc 90:1499
150. Gleiter R (1974) Angew Chem 86:770
151. Heilbronner E, Schmelzer A (1975) Helv Chim Acta 58:936
152. Roth WR, Lennartz HW, Vogel E, Leiendecker M, Oda M (1986) Chem Ber Rec 119:837
153. Hemmersbach P, Klessinger M, Bruckmann P (1978) J Am Chem Soc 100:6344
154. Heilbronner E, Gleiter R, Hopf H, Hornung V, de Meijere A (1971) Helv Chim Acta 54:783
155. Bally T, Buser U, Haselbach E (1978) Helv Chim Acta 61:38
156. Gabioud R, Vogel P (1984) Tetrahedron Lett 25:1729
157. Turner RB, Meador WR, Winkler RE (1957) J Am Chem Soc 79:4116
158. Turner RB (1964) J Am Chem Soc 86:3586
159. Wong SWS, Westrum EF (1971) J Am Chem Soc 93:5317
160. Schulman JM, Miller MA, Disch RL (1989) Pure Appl Chem 61:2103

161. Schmitz LR, Allinger NL, Flurchick KM (1988) J Comput Chem 9:281
162. Roulet JM, Vogel P, Wiesemann F, Pinkerton AA (1995) Tetrahedron 51:1685
163. Pinkerton AA, Schwarzenbach D, Stibbard JHA, Carrupt PA, Vogel P (1981) J Am Chem Soc 103:2095
164. Pinkerton AA, Schwarzenbach D, Birbaum JL, Carrupt PA, Schwager L, Vogel P (1984) Helv Chim Acta 67:1136
165. Ermer O, Bell P, Mason SA (1989) Angew Chem Int Ed Engl 28:1239
166. Houk KN, Rondan NG, Brown FK, Jorgensen WL, Madura JD, Spellmeyer DC (1983) J Am Chem Soc 105:5980
167. Carrupt PA, Vogel P (1985) J Mol Struct-Theochem 25:9
168. Bartlett PD, Roof AAM, Winter WJ (1981) J Am Chem Soc 103:6520
169. Mahaim C, Carrupt PA, Hagenbuch JP, Florey A, Vogel P (1980) Helv Chim Acta 63:1149
170. De Picciotto L, Carrupt PA, Vogel P (1982) J Org Chem 47:3796
171. Msayib KJ, Watt CIF (1992) Chem Soc Rev 21:237
172. Juaristi E, Beck AK, Hansen J, Matt T, Mukhopadhyay T, Simson M, Seebach D (1993) Synthesis, p 1271
173. Sustmann R, Binsch G (1971) Mol Phys 20:9
174. Sustmann R (1974) Pure Appl Chem 40:569
175. Houk KN (1975) Acc Chem Res 8:361
176. Vogel P (1983) Methods in stereochemical analysis. In: Watson W (ed) Stereochemistry and reactivity of systems containing π electrons. Verlag Chemie International, FL, p 147
177. Burnier G, Schwager L, Vogel P (1986) Helv Chim Acta 69:1310
178. Avenati M, Carrupt PA, Quarroz D, Vogel P (1982) Helv Chim Acta 65:188
179. Woodward RB, Katz TJ (1959) Tetrahedron 5:70
180. Firestone RA (1968) J Org Chem 33:2285
181. Firestone RA (1977) Tetrahedron 33:3009
182. Epiotis ND (1978) Theory of organic reactions, Chap 6. Springer, Berlin Heidelberg, New York
183. Dewar MJS, Pierini AB (1984) J Am Chem Soc 106:203
184. Dewar MJS, Olivella S, Stewart JJP (1986) J Am Chem Soc 108:5771
185. Wassermann A (1942) J Chem Soc, p 623
186. Rodgman A, Wright GF (1953) J Org Chem 18:465
187. Yates P, Eaton P (1960) J Am Chem Soc 82:4436
188. Olah GA, Meidar D, Fung AP (1979) Synthesis, p 270
189. Fringuelli F, Pizzo F, Taticchi A, Halls TDJ, Wenkert E (1982) J Org Chem 47:5056
190. Sonney JM, Vogel P, Burger U (1980) Helv Chim Acta 63:1016
191. Broadhurst MJ, Hassall CH (1982) J Chem Soc, Perkin Trans, p 12227
192. Oppolzer W (1977) Angew Chem Int Ed Engl 16:10
193. Smith MB (1990) Org Prep Proced Int 22:315
194. Tamariz J, Vogel P (1984) Angew Chem Int Ed Engl 23:74
195. Corey EJ, Petrzilka M (1975) Tetrahedron Lett 16:2537
196. Bailey SJ, Thomas EJ, Turner WB, Jarvis JAJ (1978) J Chem Soc, Chem Commun, p 474
197. Stork G, Nakahara Y, Nakahara Y, Greenlee WJ (1978) J Am Chem Soc 100:7775
198. Bailey SJ, Thomas EJ, Vather SM, Wallis J (1983) J Chem Soc, Perkin Trans, p 1851
199. Stork G, Nakamura E (1983) J Am Chem Soc 105:5510
200. Thomas EJ (1991) Acc Chem Res 24:229
201. Tornare JM, Vogel P (1984) J Org Chem 49:2510

202. Alder K, Stein G (1937) Angew Chem 50:510
203. Hoffmann R, Woodward RB (1968) Acc Chem Res 1:17
204. Gleiter R, Bohm MC (1983) Pure Appl Chem 55:237
205. Cohen T, Ruffner RJ, Shull DW, Daniewski WM, Ottenbrite RM, Alston PV (1978) J Org Chem 43:4052
206. Brown FK, Houk KN (1985) J Am Chem Soc 107:1971
207. Brown FK, Houk KN (1984) Tetrahedron Lett 25:4609
208. Carrupt PA, Berchier F, Vogel P (1985) Helv Chim Acta 68:1716
209. Arcamone F, Cassinelli G, Dimatteo F, Forenza S, Ripamonti MC, Rivola G, Vigevani A, Clardy J, Mccabe T (1980) J Am Chem Soc 102:1462
210. Gesson JP, Jacquesy JC, Mondon M (1980) Tetrahedron Lett 21:3351
211. Bauman JG, Barber RB, Gless RD, Rapoport H (1980) Tetrahedron Lett 21:4777
212. Kende AS, Boettger SD (1981) J Org Chem 46:2799
213. Krohn K (1981) Angew Chem Int Ed Engl 20:576
214. Alexander J, Flynn DL, Mitscher LA, Veysoglu T (1981) Tetrahedron Lett 22:3711
215. Kende AS, Rizzi JP (1981) Tetrahedron Lett 22:1779
216. Yadav J, Corey P, Hsu CT, Perlman K, Sih CJ (1981) Tetrahedron Lett 22:811
217. Kimball SD, Walt DR, Johnson F (1981) J Am Chem Soc 103:1561
218. Gesson JP, Mondon M (1982) J Chem Soc, Chem Commun, p 421
219. Rao AVR, Deshpande VH, Reddy NL (1982) Tetrahedron Lett 23:775
220. Rao AVR, Mehendale AR, Reddy KB (1982) Tetrahedron Lett 23:2415
221. Jung ME, Node M, Pfluger RW, Lyster MA, Lowe JA (1982) J Org Chem 47:1150
222. Sekizaki H, Jung M, Mcnamara JM, Kishi Y (1982) J Am Chem Soc 104:7372
223. Gesson JP, Jacquesy JC, Renoux B (1983) Tetrahedron Lett 24:2757
224. Rao AVR, Reddy KB, Mehendale AR (1983) J Chem Soc, Chem Commun, p 564
225. Gesson JP, Jacquesy JC, Mondon M (1983) New J Chem 7:205
226. Hauser FM, Mal D (1983) J Am Chem Soc 105:5688
227. Hauser FM, Prasanna S, Combs DW (1983) J Org Chem 48:1328
228. Vedejs E, Miller WH, Pribish JR (1983) J Org Chem 48:3611
229. Gesson JP, Jacquesy JC, Renoux B (1984) Tetrahedron 40:4743
230. Jung ME, Lowe JA, Lyster MA, Node M, Pfluger RW, Brown RW (1984) Tetrahedron 40:4751
231. Hauser FM, Baghdanov VM (1984) Tetrahedron 40:4719
232. Tamura Y, Akai S, Sasho M, Kita Y (1984) Tetrahedron Lett 25:1167
233. Uemura M, Minami T, Hayashi Y (1984) J Chem Soc, Chem Commun, p 1193
234. Parker KA, Tallman EA (1984) Tetrahedron 40:4781
235. Bessiere Y, Vogel P (1980) Helv Chim Acta 63:232
236. Metral JL, Lauterwein J, Vogel P (1986) Helv Chim Acta 69:1287
237. Tamariz J, Vogel P (1981) Helv Chim Acta 64:188
238. Aguilar R, Reyes A, Tamariz J, Birbaum JL (1987) Tetrahedron Lett 28:865
239. Antonsson T, Vogel P (1990) Tetrahedron Lett 31:89
240. Tamoto K, Sugimori M, Terashima S (1984) Tetrahedron 40:4617
241. Suzuki M, Kimura Y, Terashima S (1985) Chem Lett, p 367
242. Reymond JL, Vogel P (1990) Tetrahedron: Asymmetry 1:729
243. Warm A, Vogel P (1986) J Org Chem 51:5348
244. Durgnat JM, Warm A, Vogel P (1992) Synth Commun 22:1883
245. Murdock KC, Child RG, Fabio PF, Angier RB, Wallace RE, Durr FE, Citarella RV (1979) J Med Chem 22:1024
246. Showalter HDH, Johnson JL, Hoftiezer JM, Turner WR, Werbel LM, Leopold WR, Shillis JL, Jackson RC, Elslager EF (1987) J Med Chem 30:121

247. Showalter HDH, Johnson JL, Werbel LM, Leopold WR, Jackson RC, Elslager EF (1984) J Med Chem 27:253
248. Leopold WR, Nelson JM, Plowman J, Jackson RC (1985) Cancer Res 45:5532
249. Showalter HDH, Johnson JL, Hoftiezer JM (1986) J Heterocycl Chem 23:1491
250. Zhang LH, Meier WE, Watson EJ, Gibson EP (1994) Tetrahedron Lett 35:3675
251. Tabor CW, Tabor H (1984) Ann Rev Biochem 53:749
252. Porter CW, Cavanaugh PF, Stolowich N, Ganis B, Kelly E, Bergeron RJ (1985) Cancer Res 45:2050
253. Bergeron RJ, Mcmanis JS, Liu CZ, Feng Y, Weimar WR, Luchetta GR, Wu QH, Ortizocasio J, Vinson JRT, Kramer D, Porter C (1994) J Med Chem 37:3464
254. Bergeron RJ, Mcmanis JS, Weimar WR, Schreier KM, Gao FL, Wu QH, Ortizocasio J, Luchetta GR, Porter C, Vinson JRT (1995) J Med Chem 38:2278
255. Dienes Z, Vogel P (1996) J Org Chem 61:6958
256. Mosimann H, Dienes Z, Vogel P (1995) Tetrahedron 51:6495

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Fluorinated Anthracyclines

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Abstract The introduction of fluorine into biologically active molecules today represents one of the classic approaches used in medicinal chemistry in the search for both increased potency and a more favorable metabolic pathway.

This chapter aims to offer an updated overview of the efforts made by several research groups in the field of fluorinated anthracyclines. We also give, along with the structures, brief descriptions of the syntheses and current status of development.

Keywords Anthracyclines · Fluoroanthracyclines · Fluorinated antitumor agents

Abbreviations

P388	Lymphocytic leukemia cell line
P388/DX	Lymphocytic leukemia cell line resistant to doxorubicin
LoVo	Human colon adenocarcinoma cell line
LoVo/DX	Human colon adenocarcinoma cell line resistant to doxorubicin
MCF-7	Human breast adenocarcinoma cell line
MCF-7/DX	Human breast adenocarcinoma cell line resistant to doxorubicin
HeLa	Human cervical cancer cell line
L1210	Mouse lymphocytic leukemia cell line
K562	Human erythroleukemia cell line
HMV-1	Human melanoma cell line
KB	Human epidermoid carcinoma cell
MKN-1	Gastric cancer cell line
PC-14	Lung adenocarcinoma cell line
T24	Human Bladder cell line
KB-A-1	Human epidermoid carcinoma cell line resistant to doxorubicin
KB-3-1	Human epidermoid carcinoma cell line
MDR	Multidrug Resistance
A, T, C, G	Adenine, Timine, Citosine, Guanine
DAST	Diethylaminosulfur trifluoride
DAUNO	Daunorubicin
IDA	Idarubicin
DOXO	Doxorubicin
EPI	Epidaunorubicin

1 Introduction

Organic structures with fluorine atoms are not common in nature. Fluorine has quite unique properties and is often considered a sort of "magic element", whose incorporation is able to bring about improvements and important modifications to the biological activity of a molecule. However, it is not possible to formulate a general rule about the influence of fluorine on activity except in each single case immediately following a comparison between a fluorinated compound and the corresponding nonfluorinated analogue.

Therefore, starting in the 1980s, medicinal chemists discovered that the selective introduction of fluorine into biologically active molecules exercised an influence on activity: there are numerous reports of compounds incorporating fluorine as either a bioisosteric replacement for hydrogen or an isoelectronic replacement for the hydroxyl group [1, 2].

The practice of introducing fluorine atoms into molecules of biological interest, such as steroids, carbohydrates, antitumor agents, and other biologi-

cally important molecules, occupies an increasing position in recent scientific literature [3]. Currently, around a fifth of all pharmaceutical products on the market contain at least one fluorine moiety [1, 2].

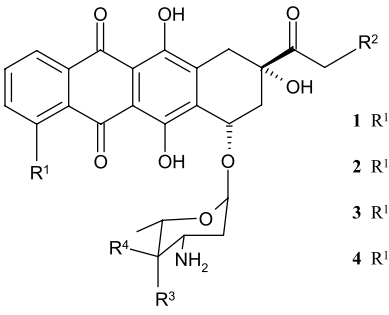
This growing interest is due to the particular effects that fluorine can exert on the properties of a compound without altering its steric bulk [4, 5]. Electron withdrawal by fluorine results in a strong polarization of the C-F bond and the pronounced electronic effects, associated with poor steric demand, can have implications for reactions at adjacent centers, i.e., for drug-target interactions [3].

Traditionally, elemental fluorine and hydrogen fluoride were used as fluorinating agents. However, it was soon evident that fluorine was extremely reactive and too unselective to be synthetically useful. Over the years, many other fluorinating agents were developed, making the fluorination step a safe and reliable process. Through modifications of techniques, direct fluorination of the simplest hydrocarbons is, in some instances, possible.

Therefore, it is not surprising that the "fascination of fluorine" entered the field of anthracycline glycoside synthesis. Remarkable chemical interest consequently arose on the introduction of fluorine into the anthracycline molecule with the aim of altering the biorelevance of functional groups, thus providing new compounds whose DNA-drug interaction and antitumor properties could be compared with those exhibited by the clinically used anthracyclines (**1**–**4**) (Fig. 1).

Through both total and/or semi-synthetic approaches, various research groups explored the introduction of fluorine atoms into different positions of the anthracycline scaffold, as summarized in Fig. 2.

Nevertheless, there are no current examples of fluorinated anthracyclines being included in clinical trials or better, in clinical practice, and the few compounds reaching advanced development all failed to enter the marketing phase [6]. As a consequence, there is reduced interest in recent years by the scientific community in this class of fluorinated compounds. A few years ago, one of the authors published an overall review on this subject [7]. Sub-



1 R^1=OMe; R^2=OH; R^3=OH; R^4=H DOXORUBICIN (DOXO)

2 R^1=OMe; R^2=H; R^3=OH; R^4=H DAUNORUBICIN (DAUNO)

3 R^1=OMe; R^2=OH; R^3=H; R^4=OH EPIRUBICIN (EPI)

4 R^1=H; R^2=H; R^3=OH; R^4=H IDARUBICIN (IDA)

Fig. 1 Anthracyclines in clinical practice

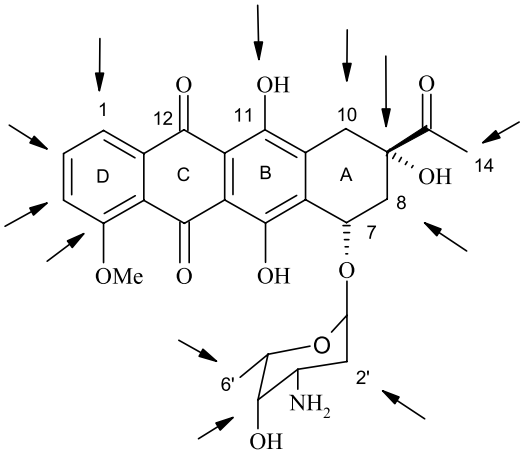


Fig. 2 Fluorine insertion on anthracycline scaffold

sequently, only one synthetic work was published. In this paper we describe a brief collection of compounds with anthracycline structures with fluorine atoms introduced in the aglycone and/or sugar moiety. Together with the chemical structures, we provide synthetic information and, if available, a succinct explanation of the biological activity.

The introduction of the strongly electron-withdrawing fluorine atom into different positions on the aglycones and on the sugar moiety allowed a study of the stability and effect of an increase in lipophilicity, thus enabling elucidation of a more precise relationship between the structure and antitumor activity of these compounds.

Generally, fluorinated anthracyclines are obtained via total synthesis of the aglycone or sugar moieties to introduce the fluorine into the specific position.

We will begin with an overview of fluorinated anthracyclines and go on to describe the compounds reported in the scientific literature and claimed in patents, sketching, at the same time, their total synthesis. Subsequently, we will propose an analysis of the opportunity to introduce the fluorine atom into this class of antitumor drugs to increase biological activity.

2
Overview on Fluorinated Anthracyclines

From a historical point of view, the first introduction of fluorine atoms was performed on the D-ring of the aglycone and on C-6′ of the sugar moiety, as a trifluoromethyl derivative. Subsequently, Terajima et al. described the introduction of a fluorine atom at C-14 of the aglycone and later Arcamone et al.

described the C-8 position on the A-ring as a very promising position for the introduction of fluorine. In fact, this position permitted a study of the influence of the stereochemistry at C-8 on biological activity and the effect on the stability of the glycosidic bond under physiological conditions by the introduction of the polar carbon-fluorine bond. The same effect was shown by the introduction of fluorine on the sugar moiety at C-2′.

There are hundreds of bibliographic references on fluorine-containing anthracyclines, especially during the early 1980s and the late 1990s, showing the great interest devoted to these compounds by the scientific community. Some derivatives were introduced in clinical trials and one (ME2303) (**58**), with F on C-2′ of the sugar residue, reached Phase II clinical trials.

3
Fluorine on D-Ring

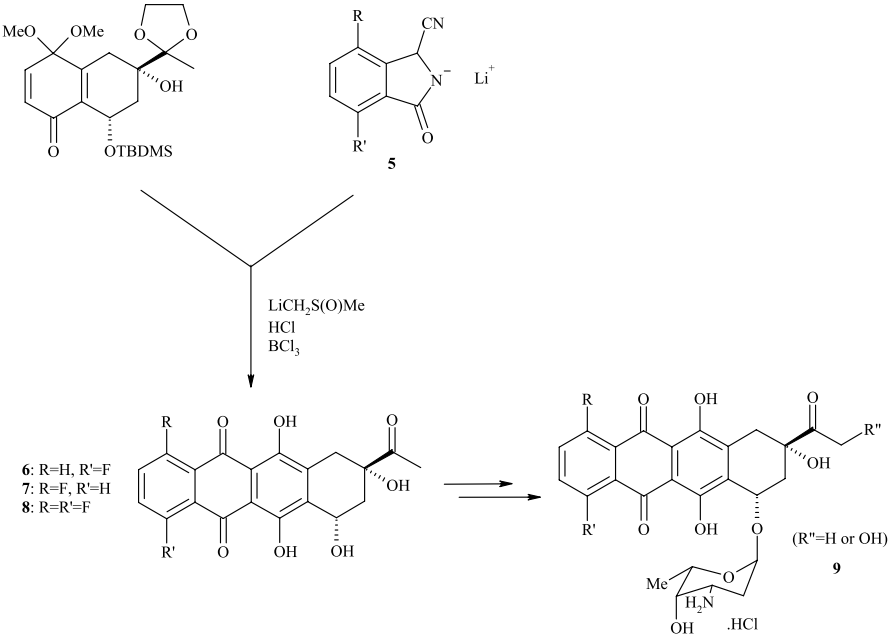
Between the end of the 1980s and the beginning of the 1990s, various international research groups were interested in investigating the effect of fluorine on the D-ring of the anthracyclines. Therefore, several derivatives, bearing mono- and bis-fluoro substitutions at all four possible positions of this ring, were prepared. However, the introduction of fluorine in place of hydrogen in the D-ring appears to have a minimal effect in vitro on biological activity, as measured by the P388 screen. When these compounds were tested in vivo against P388 and P388/DX murine leukemia in comparison with DOXO, almost all these fluoro derivatives were more potent on sensitive and resistant leukemia cells.

The 1- and 4-fluoro derivatives showed efficacy and activity similar to those of IDA, a derivative 5–8 times more potent than the naturally occurring DAUNO. However, the 1,4-difluoro derivative, while showing good activity and diminished toxicity, is substantially less potent than IDA.

These compounds were also tested in vitro against LoVo (human colon adenocarcinoma) and LoVo/DX cells in comparison with DOXO. The results showed that these derivatives were less potent than the natural drug on the sensitive tumor. While they were more potent on DOXO-resistant cells. Nevertheless, these compounds aroused only academic interest [8].

3.1
1-Fluoroanthracyclines and 1,4-Difluoroanthracyclines

The derivatives containing fluorine atoms at positions 1 and/or 4 were prepared from aglycones obtained by the synthesis outlined in [9, 10]. A cyanophtalide anion (**5**), obtained by different routes, was a strategic intermediate in obtaining the fluoroanthracyclinones **6–8** (Scheme 1).



Scheme 1 Synthesis of 1,4 fluoro-anthracyclines

Anthracyclinones fluorinated on the D-ring were used to obtain the different anthracyclines **9** and also derivatives modified on the sugar moiety, such as morpholinyl-**10** [11] or 4′-epi-4′amino-anthracyclines **11** (Fig. 3) [12].

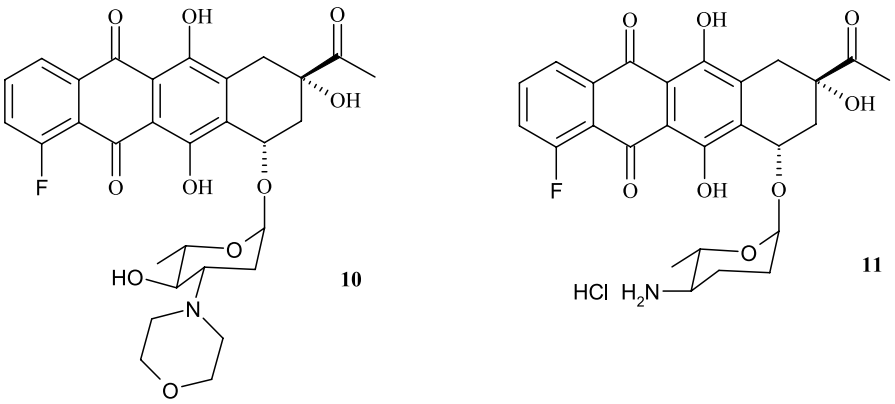
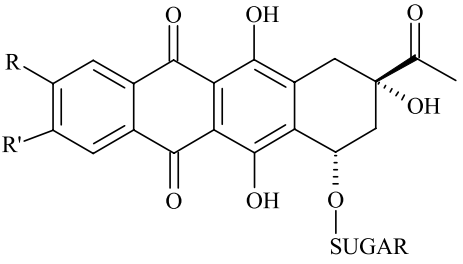


Fig. 3 Example of 4-fluoro-anthracycline derivatives

3.2
2-Fluoro- and 3-Fluoroanthracyclines [13]

The 2-fluoro anthracycline derivative **12** was synthesized by Warrener and Collins in the early 1990s [14] to explore, via ^{19}F-NMR experiments, its distribution between two different oligonucleotides in response to changes in parameters such as NaCl concentrations and pH.

Later on, they explored, using the same NMR technique, how a fluorine atom in position 3 affected the intercalation of IDA derivative **13** into the DNA major groove using a hexanucleotide duplex as an intercalation model (Fig. 4) [15].



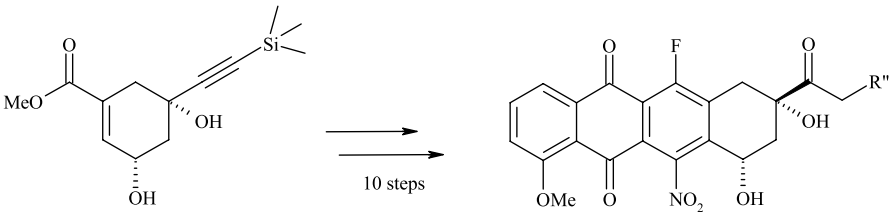
12: R=F, R'=H
13: R=H, R'=F

Fig. 4 General structure of 2,3-fluoro-anthracyclines

4
Fluorine on B-Ring

4.1
11-Fluoroanthracyclines

The synthesis of the 11-fluoroanthracycline **14** was described fifteen years ago at a meeting in Montreal (ICOS 1992) [16]. It was obtained after several steps,



14

Scheme 2 Synthesis of 11-fluoro-anthracyclinone

starting from the unsaturated diol [17]. This compound was also substituted in position 6, as an NO_2 derivative (Scheme 2).

5
Fluorine on A-Ring

X-ray analysis and theoretical calculations clearly indicate that the A-ring is a fundamental structural element in interactions between anthracyclines and DNA. Therefore, even the slightest modification on this ring can deeply influence biological response, and only a few examples of these derivatives are reported in the literature. Since fluorine is a well-known bioisoster of hydrogen, it was worth trying an H – F substitution on this part of the molecule hoping of not significantly altering the activity.

In the 1990s, Arcamone et al. started a program aimed at extensive modification of the A-ring in antitumor anthracyclines. The program yielded anthracyclines bearing a fluorine substitution at position 8 or 10. Such a substitution could alter the hydrogen bonding capability of the C-9 alcoholic function through modulation of its electronic environment with minimal changes at the steric bulk of the A-ring. This notion stemmed from the observation that intranuclear DNA-bound drug accumulation is responsible for cytotoxicity and that all anthracyclines, once the clinical stage is reached, belong to the group showing the highest affinity for DNA. In addition, the strong change in electronic density induced by fluorine on the glycosidic linkage in C-7 could stabilize the drug.

These new derivatives showed cytotoxic proprieties in vitro and antitumor activity in animal models comparable to those shown by derivatives without fluorine.

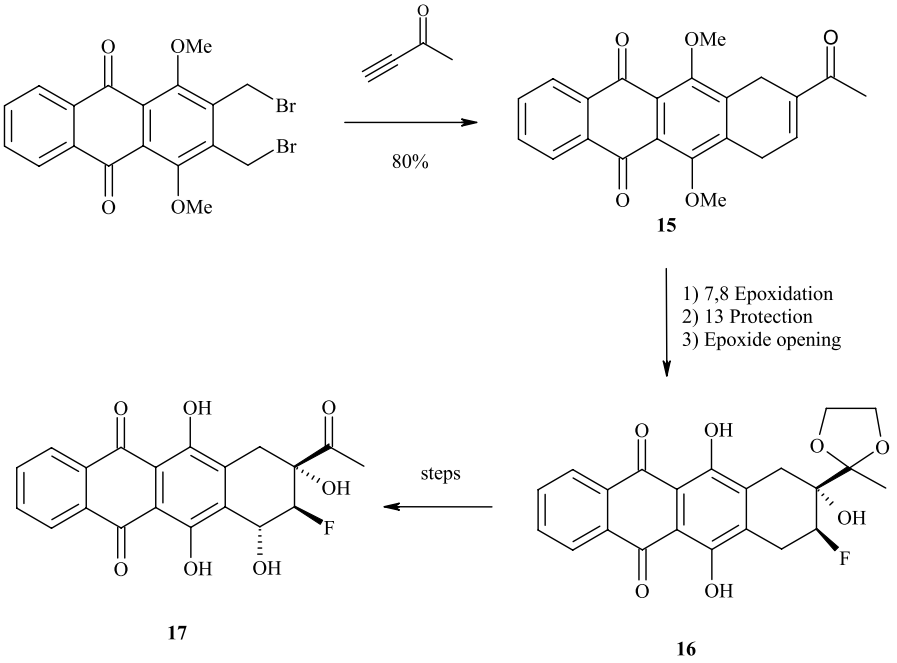
The antiproliferative activity of 10-fluoroanthracyclines, in different subpanels of tumor cell lines in vitro, was less potent than that exhibited by the DOXO, although these 10-fluoro-derivatives showed a resistance index (RI) value lower than the reference compound.

Conversely, the antiproliferative activity of the two 8-fluoroanthracycline analogues was markedly affected by the stereochemistry of the fluorine substituent. The (8*S*)-8-fluoro-IDA derivative showed a cytotoxicity comparable to that of DOXO and much lower than that of the structurally related IDA [18]. By contrast, the (8*R*)-8-fluoro-IDA derivative showed a cytotoxic activity higher than that of DOXO and comparable to that of IDA [19].

In conclusion, fluorine substitution on the A-ring of antitumor anthracyclines was studied. All four possible monosubstitution topologies at positions 8 and 10 were synthesized, showing that the new fluoroanthracyclines retain bioactivity. Nevertheless, the four derivatives show different activity and RI values in different subpanels of tumor cell lines in vitro.

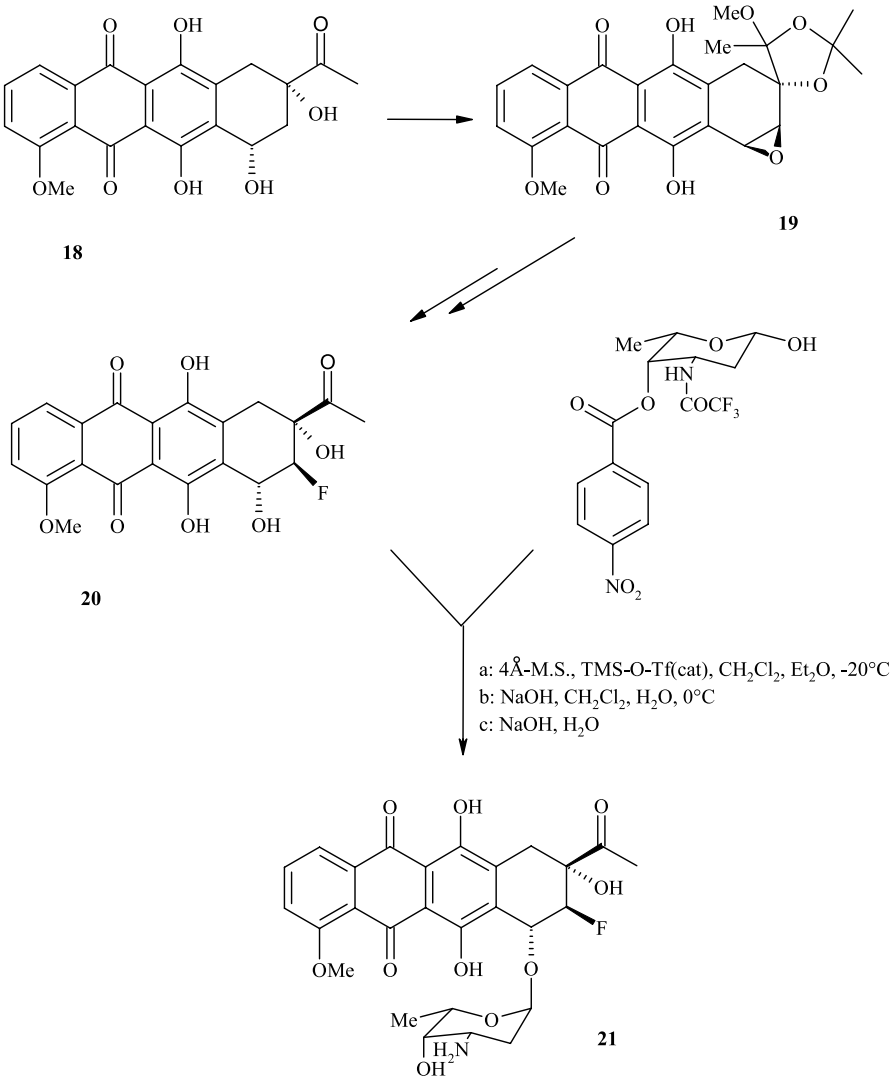
5.1
8-Fluoroanthracyclines

DAUNO analogues possessing a fluorine atom at position C-8 of the A-ring were synthesized with the aim of comparing their DNA-drug interaction and antitumor properties with those of the clinically useful anthracyclines DOXO and IDA [18, 20–23]. This was done in an attempt to better understand the role of the cyclohexane ring (A-ring) in the biochemical and pharmacological properties of anthracyclines. Different approaches for the synthesis of (8*S*)-8-fluoro-anthracyclines were reported either by total synthesis (Scheme 3) or by the available natural aglycone **18** (Scheme 4).



Scheme 3 Synthesis of 8-(*S*)-fluoro-daunomycinone

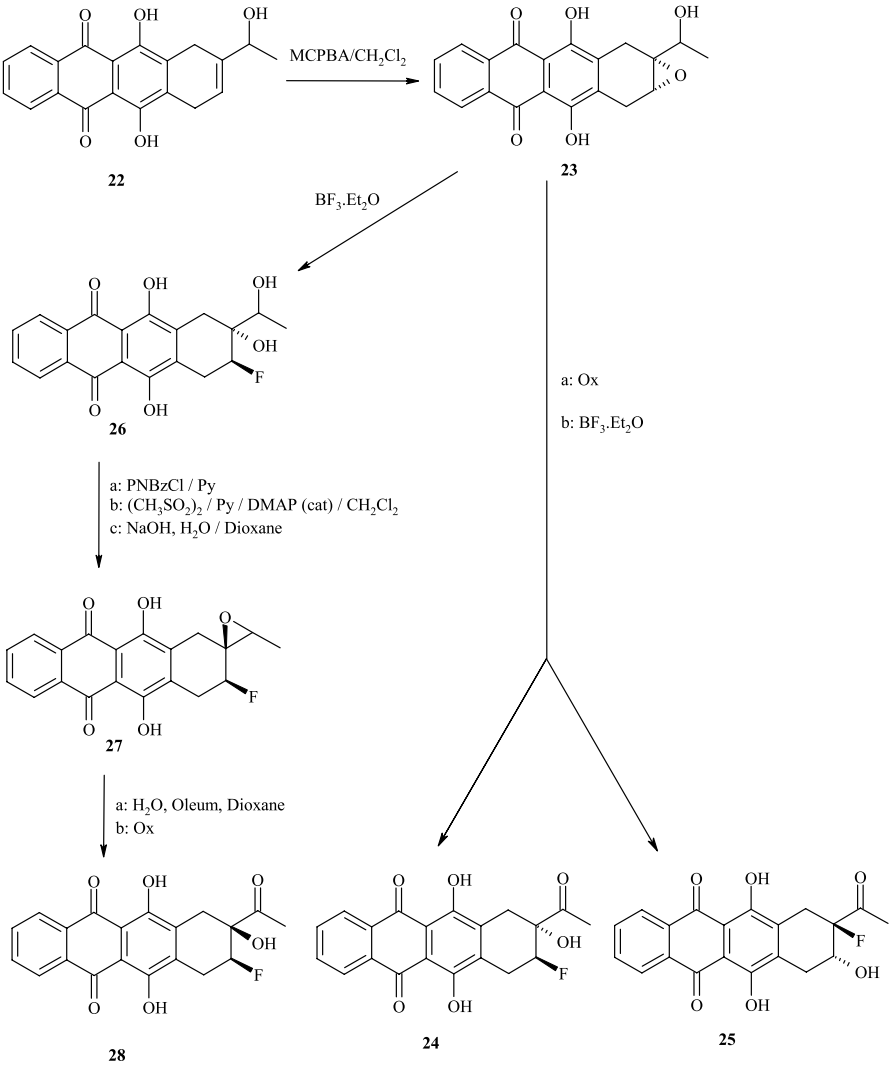
Moreover, the (8*S*)- and (8*R*)-8-fluoro-IDA were prepared by divergent routes, starting from the common intermediate **22**, obtained via the Diels–Alder cyclization between quinazarin diquinone, and 2-(1-hydroxyethyl)-1,3-butadiene [19]. The synthesis of the (8*S*)-fluoroepimer proceeded through epoxidation of the C(8)–C(9) olefinic bond of the common intermediate, oxidation and oxirane **23** ring opening by $BF_3 \cdot Et_2O$ to give *trans*-1,2-fluorohydrines **24** and **25**. The same oxirane **23**, via inversion of the C-9 hydroxyl (by opening the epoxy **27**), gives the *cis*-1,2-fluorohydrin **28** (Scheme 5).



Scheme 4 Synthesis of 8-(*S*)-fluoro-daunomycin

The desired aglycones may be obtained by hydroxylation in C-7. It is noteworthy that all these synthetic approaches lead to enantiomeric mixtures; an asymmetric epoxidation of the quinazarinic intermediate **22** could be used to obtain the desired enantiomer.

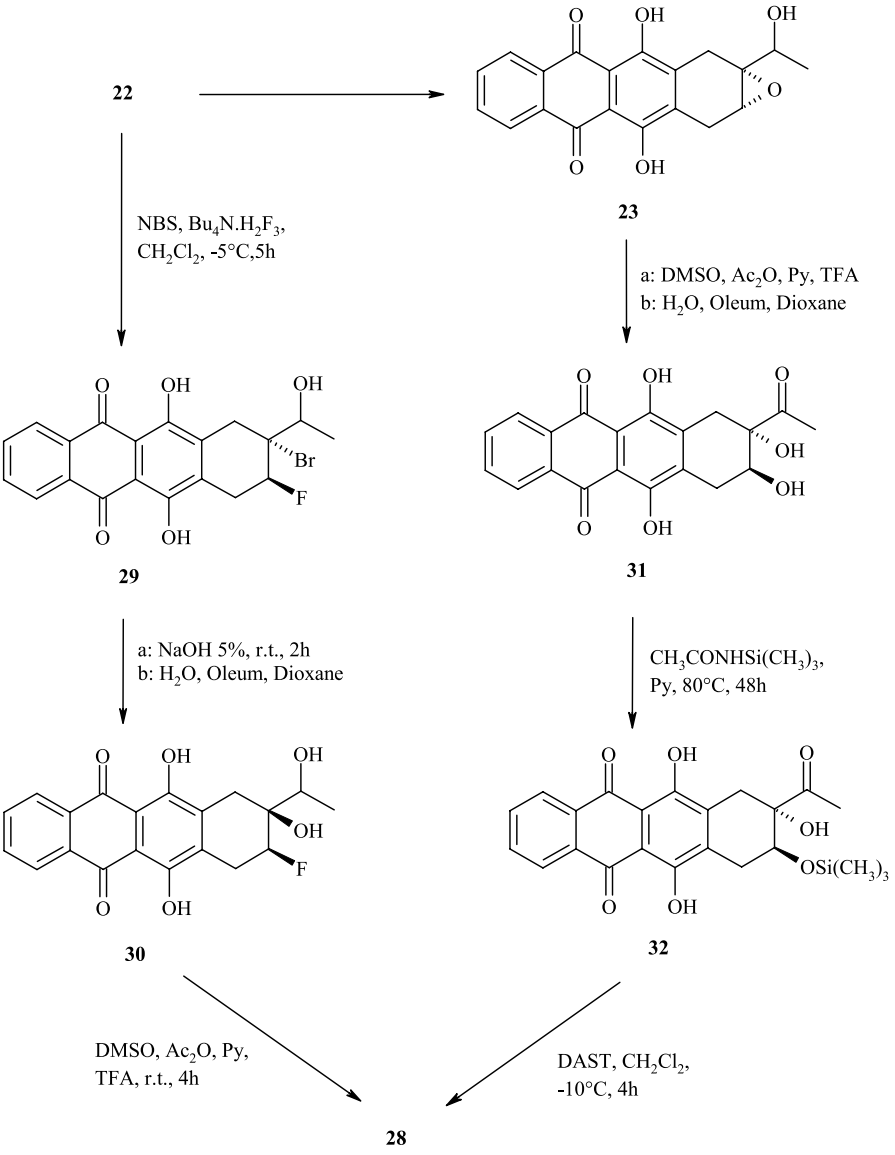
Conversely (Scheme 6), another approach for the synthesis of (8*R*)-fluoroepimer **28** involved the fluorobromination of the C(8)–C(9) olefinic bond of common intermediate **22** and formation of the C(9)–C(13) epoxide **27**. This compound, after regioselective hydrolysis and oxidation of the re-



Scheme 5 Synthesis of 8–9 fluorohydrines

sulting fluorodiol to the epimeric fluoro-hydroxyketone **28**, similarly gave the desired fluoroaglycone, and hence the corresponding glycoside. An alternative approach used the ketodiol **31**, which gave the (8*R*)-fluoroepimer **28** after silylation of the C-8 hydroxyl and following fluorination with DAST [24].

As above described in Scheme 5, compound **28** was obviously obtained as a mixture of enantiomers that, after hydroxylation in C-7 and glycosylation, gave the two separable diastereomers.



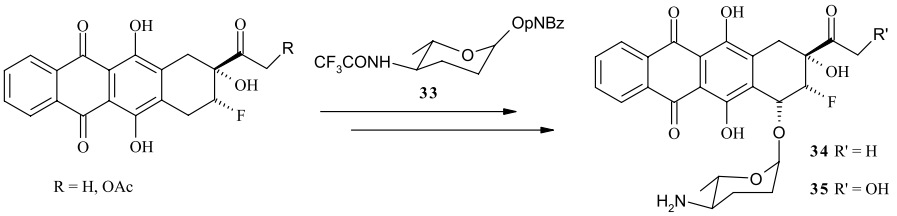
Scheme 6 Alternative approaches to synthesis of 8–9 *cis*-fluorohydrine

The cytotoxic properties of the two (8*S*)- and (8*R*)-fluoroanthracycline analogues were markedly affected by the stereochemistry of the fluorine substituent [19, 20]. The most promising compound of the series was MEN11079 [6].

These results showed that the stereochemistry of the substituent at position 8 influenced the half-chair conformation [25, 26]. In the (8*R*)-

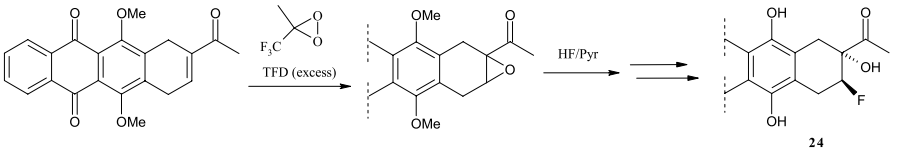
fluoroepimer, the A-ring retained the "α half-chair" conformation, which is the most stable for natural compounds (i.e., DAUNO and DOXO), while the (8*S*)-fluoroepimer preferred the "β half-chair" conformation [27, 28].

The (8*S*)-8-fluoroaglycone and its (8*R*)-epimer were used to obtain new anthracyclines where daunosamine was substituted with 4-aminosugar **33**, prepared starting from rhamnose [29] (Scheme 7). Antitumor activity of the two fluoro-epimers **34**, **35** (8*S*)- and (8*R*)-8-fluoroaglycone was evaluated in vitro against MCF-7 and LoVo and their corresponding DOXO-resistant cell lines (MCF-7/DX, LoVo/DX). These derivatives exhibited good activity (10 to 100 times stronger than DOXO) against DOXO-resistant lines.



Scheme 7 Synthesis of 8-(*R*)-fluoro-anthracycline derivatives

More recently, a particularly efficient new approach to the 8-fluoroaglycone of IDA was reported by Curci et al., in which the key epoxidation step was performed in high yield with methyl trifluoromethyl dioxirane (TFD) (Scheme 8) [30].



Scheme 8 Synthesis of 8-(*S*)-fluoro-aglycone via TFD

5.2 9-Fluoroanthracyclines

The hydroxyl group in C-9 is very important for the antitumor activity of anthracyclines, and many derivatives have been synthesized confirming this. The only compound with an interesting activity was amrubicin (SM-5887), a 9-aminoanthracycline that in clinical trials showed almost the same antitumor activity and less cystic irritability as compared to DOXO and EPI [31, 32]. When this hydroxyl group was substituted with a fluorine atom, the correspondent derivative showed lower activity as an antitumor agent than the reference compounds (personal unpublished data). Therefore, only chemical references are present in the literature on these derivatives.

The earlier work in 1993, when Cambie et al. synthesized the C-9 fluorine analogue of anthraquinones, showed that treatment of the anthraquinonyl chloroallyl acetal with boron trifluoride etherate at room temperature gave the 9-chloro-9-fluoro derivative with a low yield (4%) [33, 34].

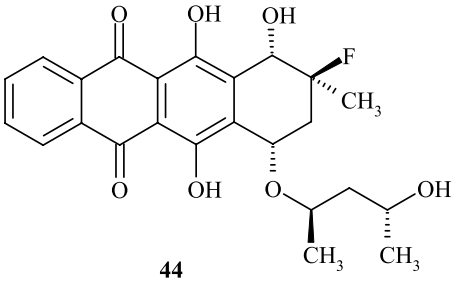
One of the authors (GG), during a study involving fluorination of anthracyclinone substrates using $BF_3 \cdot Et_20$ as fluorinating agent to open the 8,9-epoxide intermediate **23**, obtained two different regioisomeric forms, the 8-fluoro and the 9-fluoro compounds **37** and **36** which gave the two isomers, 7-9-*trans* **38** and 7-9-*cis* **39** in a few steps. After glycosidation, the mixture could be separated into the single enantiomers, **40**–**43** (Scheme 9).

At that time, there was interest in obtaining the 8-(*R*)-fluoro derivative, hence, work on the other regioisomer was dropped. The same happened to the other fluorinated aglycones such as the 8,9-difluoro, 8,13-difluoro and 9,13-difluoro derivatives, which were obtained using an intermediate of the synthesis of *cis*-1,2-fluorohydrin **28** [35].

In 1993, Cambie et al. published the synthesis of the 9-chloro-9-fluoro derivative [33]. Subsequently, they continued to study the introduction of fluorine at C-9 into an anthracyclinone and later, in 1996, finished the synthesis of 9-fluoro-9-methylanthracyclinone **44** (Fig. 5). The 9-fluoro derivatives were achieved by treating *ortho*-methallyl-substituted anthraquinonyl homochiral dioxanes with boron trifluoride etherate. In this case, the yields of the fluoro derivatives were better than in the earlier work [36].

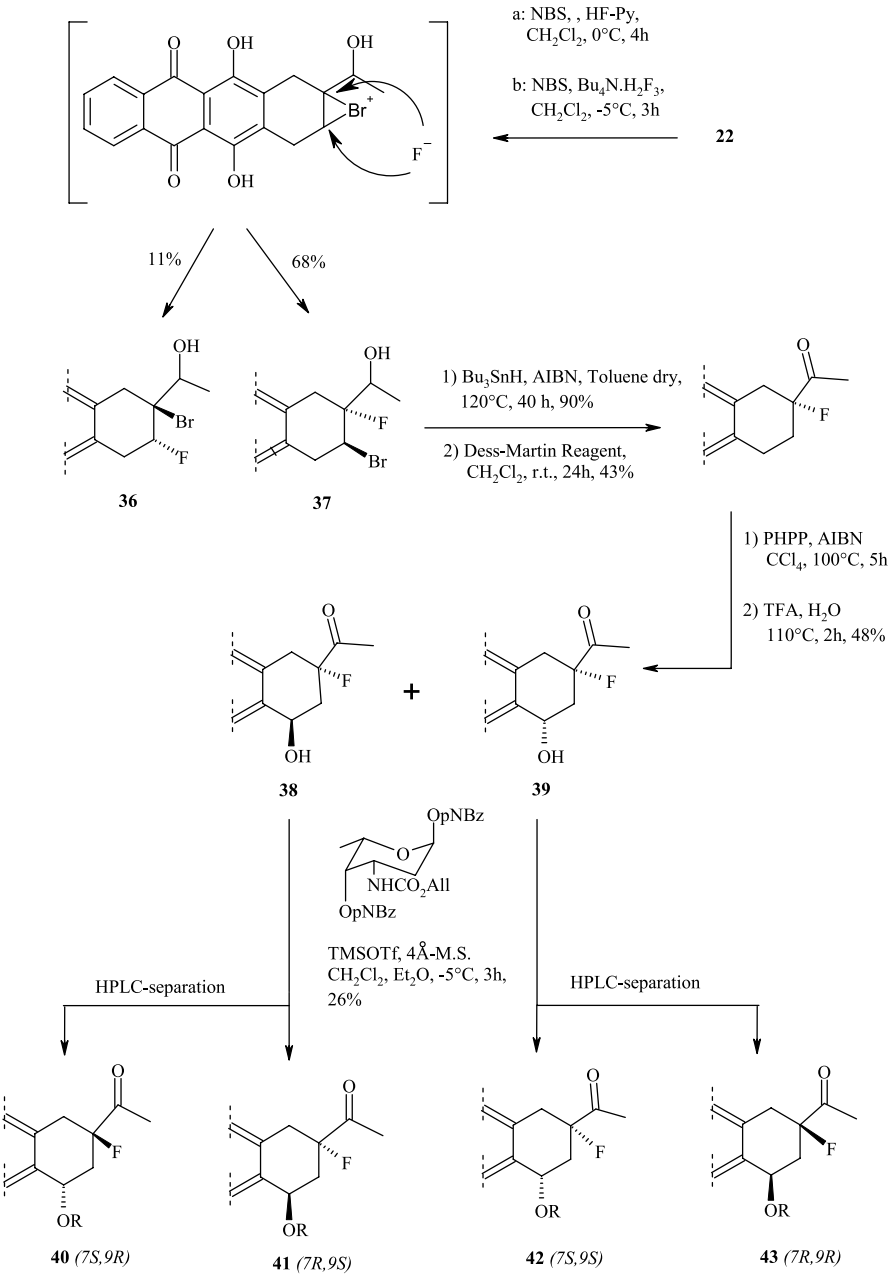
It is important to note in experiments directed towards synthesis of anthracyclinones that the fluoro-substituted tetracycle **45** exists in the expected half-chair conformation, with the bulky C-7 side in a pseudo-equatorial position. In contrast, the tetracycle **46** (6-OMe), exists in a significantly perturbed half-chair conformation with a pseudo-axial side chain (Scheme 10). This information is in accord with the influence on conformation of the A-ring by a fluorine atom in position 8 or 10.

Despite all these studies, the first synthesis of a natural anthracycline modified at position 9 with the introduction of a fluoro atom was shown in

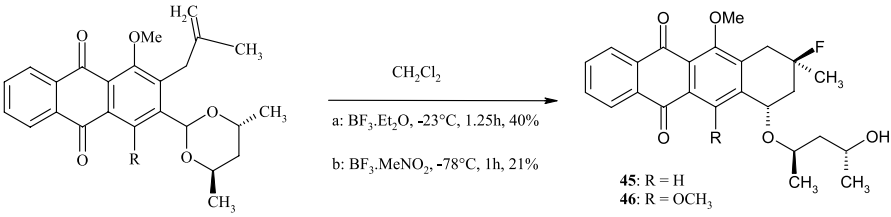


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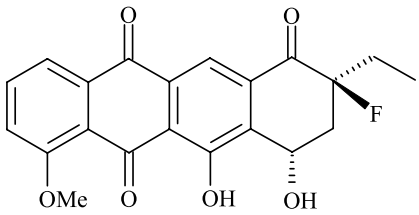
Fig. 5 9-Fluoro-anthracycline derivative



Scheme 9 Synthesis of 9-fluoro-Anthracyclines via a fluoro-bromination approach



Scheme 10 Synthesis of 9-fluoro-anthracycline derivative



47

Fig. 6 9-Fluoro-aglycone

1997, as previously described [37]. The synthesis of a new 9-fluoro anthracycline, aglycone **47**, was described in the literature (Fig. 6) [38].

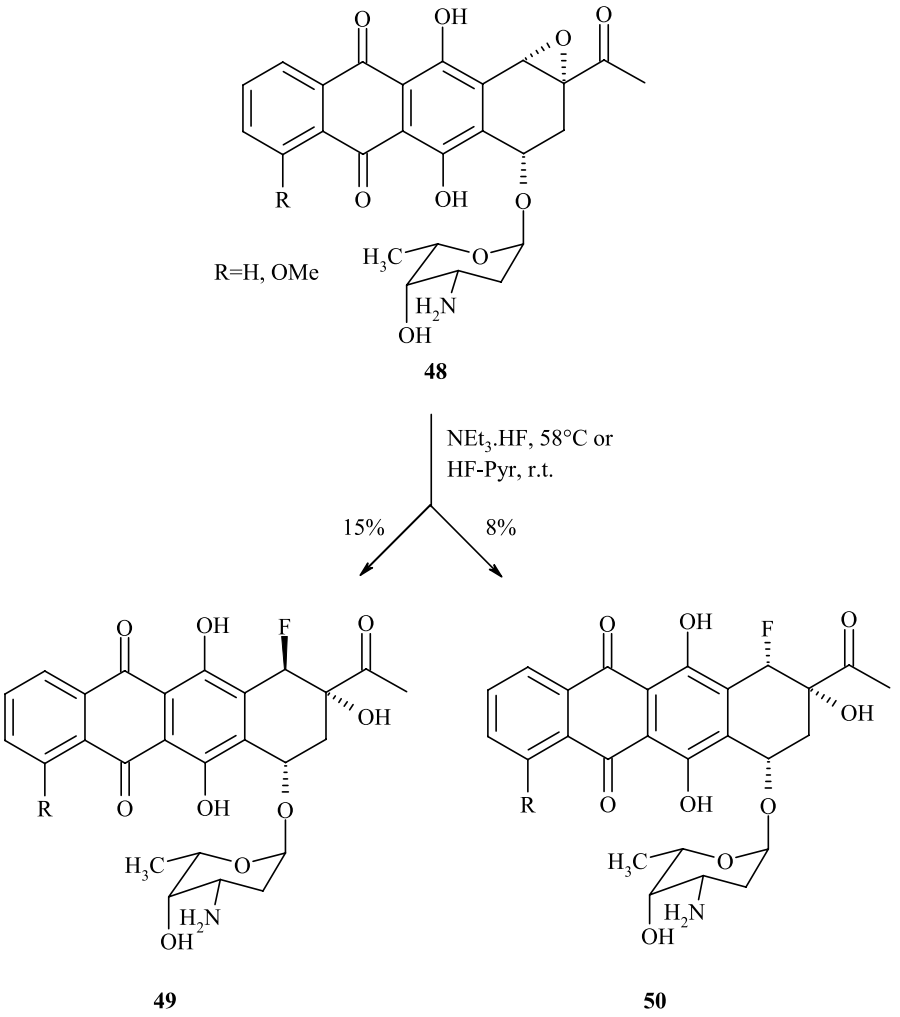
5.3
10-Fluoroanthracyclines [27, 39]

For the same purpose as with the 8-fluoro derivatives, the 10-fluoro derivatives were synthesized to obtain molecules with higher affinity for typical anthracycline intercalation sites 5′-(A,T)CG-3′ or 5′-(A,T)GC-3′ in double stranded DNA. The (10*R*)-(**49**) and (10*S*)-10-fluoro anthracyclines (**50**) were obtained starting from a 9–10 oxirane **48** followed by opening with DAST [27] or with Olah's reagent (HF-Pyr) at room temperature (Scheme 11) [26].

6
Derivatives on the C-9 Side Chain

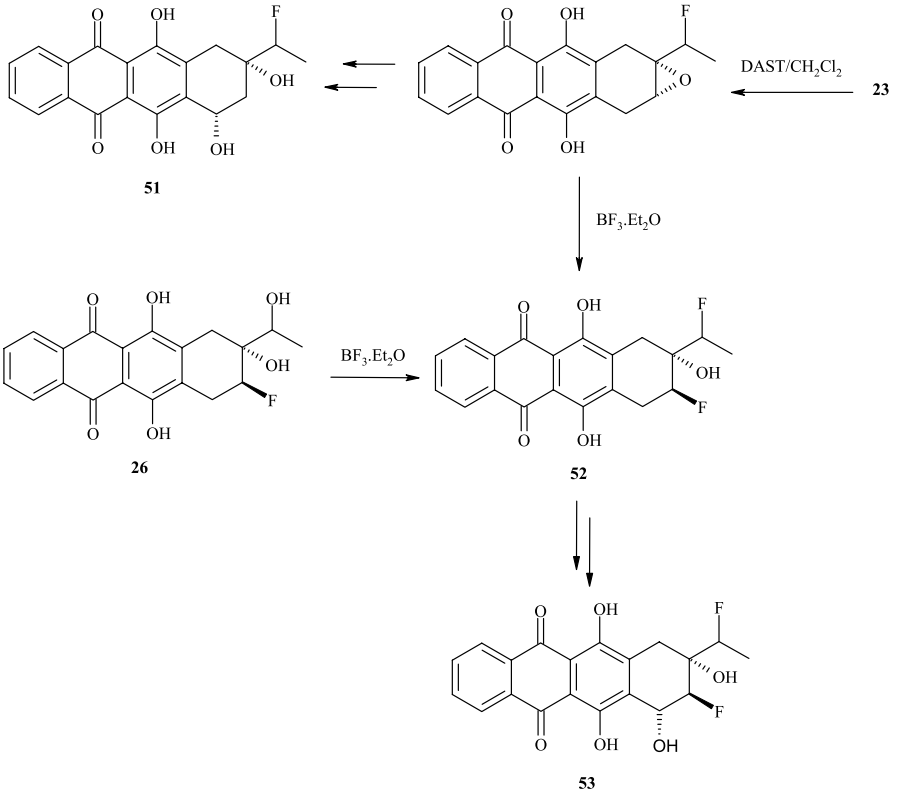
6.1
13-Fluoroanthracyclines

Natural anthracyclines such as doxorubicin present a carbonyl group on C-13. This is very important for anthracycline toxicity studies, because this group, after biological reduction in vivo, gives the 13-hydroxy derivative that proves to be more cardiotoxic [40].



Scheme 11 Synthesis of 11-fluoro-anthracycline derivatives

Until now, no further work has been published on 13-fluoroanthracycline derivatives, except the synthesis of the 13-fluoroaglycone **51** that was obtained from the intermediate 8,9-epoxide **23** by reaction with DAST, followed by oxirane opening. Moreover, the 8,13-difluoro aglycone **53** could be prepared from 13-fluorinated analogue of compound **23** or from the 8-fluoro intermediate **26** by reaction with $BF_3 \cdot Et_2O$ (Scheme 12) [36, 41].



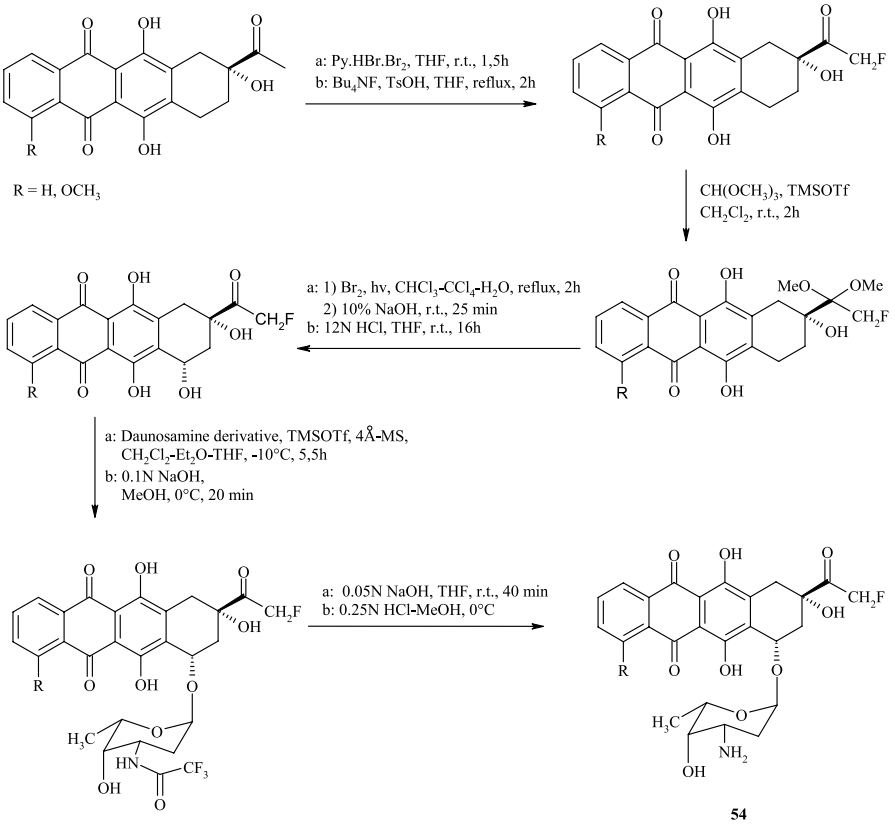
Scheme 12 Synthesis of 8,13-difluoro-aglycone

6.2
Fluorine on C-14

There are two different classes of 14-fluoroanthracyclines, the 14-fluoro- and the 14,14-difluoroanthracyclines, which have a fluoroacetyl or difluoroacetyl group as the C-9 side chain, synthesized and studied by Terashima et al. Both showed excellent in vitro cytotoxicity ($IC_{50} = 5 \times 10^{-3} - 1 \times 10^{-4}$ μg/mL) and in vivo antitumor activity ($T/C = 0.3–10$ mg/kg) for different derivatives against P388 murine leukemia [42].

6.2.1
14-Fluoroanthracyclines

These derivatives were obtained by total synthesis of aglycone. The 14-fluoro-IDA/DAUNO **54** was obtained in several steps by 7-deoxy-idarubicinone/daunorubicinone (Scheme 13) [42–47].

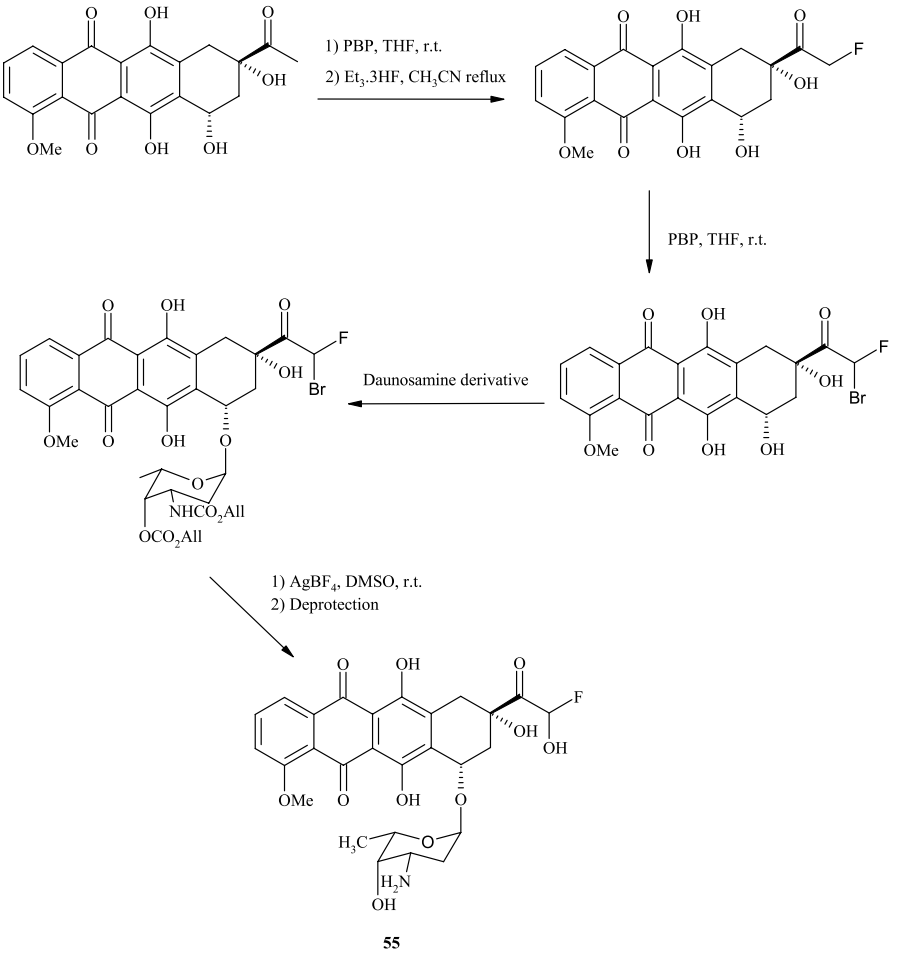


Scheme 13 Synthesis of 14-fluoro-aglycone

Subsequently, Animati et al. obtained the derivative **55** [48] (Scheme 14). This 14-fluoro-DOXO, a geminal fluorohydrine, was obtained by a C-14 bromination of the 14-fluoroaglycone, following glycosidation with the sugar and final hydrolysis of the bromine. In vitro experiments showed this compound to be ten times less active than doxorubicin.

6.2.2 14,14-Difluoroanthracycline

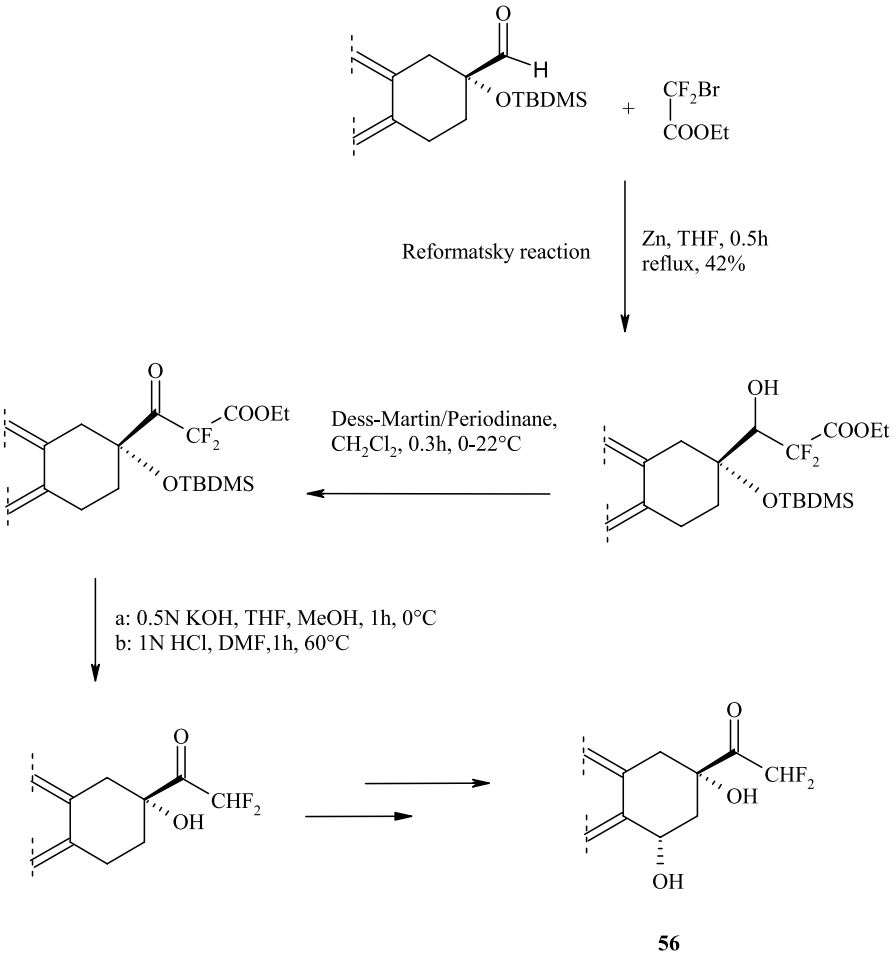
The 14,14-difluoroanthracyclinone **56** was obtained by employing a Reformatsky reaction as a key step (Scheme 15) [49–51].



Scheme 14 Synthesis of 14-fluoro-doxorubicin

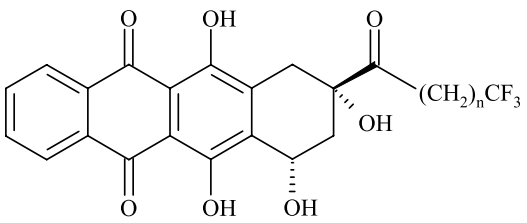
6.2.3 Fluorinated Esters of Anthracyclines

These esters (*p*-trifluoromethyl- and *p*-fluoro-benzoate) are prodrugs of DOXO [52] and have been used in cellular uptake studies. Despite the low cytotoxicity with respect to the parent anthracyclines, these esters may find application for slow release. In fact, this possibility has already been explored. Some 14-(ω, ω, ω-trifluoromethyl)-alkyl-anthracycline derivatives **57** (Fig. 7) with satisfactory carcinostatic properties have also been patented [53].



56

Scheme 15 Synthesis of 14,14-difluoro-aglycone



57

Fig. 7 14-(ω-trifluoroalkyl)-derivative

7
Fluorine on Sugar Moiety

The literature reports several examples of substitution of the natural sugar moiety of anthracyclines (daunosamine) with different modified sugars. One of these modifications was the use of a fluorinated sugar.

In 1984, Horton and coworkers synthesized 3′-deamino-3′hydroxy-DOXO [54], which had fairly good antitumor activity with less toxicity, although it has no amino group. This indicates that the C-3′ amino group is not essential for its activity. Another factor was the instability of anthracyclines in acidic conditions, giving inactive aglycones. This problem, however, was solved by introducing a strongly electron-withdrawing fluorine at the C-2′-position. These 2′-fluoro derivatives were stable against acids and exhibited stronger antitumor activity with less toxicity in comparison to that of DOXO. Additionally, these compounds were found to be absorbed more rapidly and accumulated more readily into tumor cells than DOXO [55].

The same stabilizing effect was achieved by introducing an electron-withdrawing group into position C-5′. A trifluoromethyl group was chosen to strengthen the glycosidic bond by decreasing the electron density of the glycosidic oxygen similarly as with a fluoro atom at C-2′. Moreover, the lipophilicity increased by incorporation of the trifluoromethyl group as compared to the methyl group [76].

7.1
2′-Fluoroanthracyclines

Among the 2′-fluoroanthracycline derivatives, compound **58** (Fig. 8, ME2303 or FAD104), possessing an impressive antitumor activity in a variety of tumor models, was extensively studied. According to the information that the replacement of the 3′-amino group of DOXO with a hydroxyl group reduces toxicity and increases antitumor activity of the compound, ME2303, which has a hydroxyl group at the 3′ position and a fluorine atom at the 2′ position, showed lower toxicity. In animal tumor models, **58** showed stronger chemotherapeutic effects than DOXO. ME2303 increased the survival time of L1210- and P388-bearing mice over a wide dosage range without weight loss and many mice were cured. The compound also showed strong tumor-inhibiting activity against colon adenocarcinomas 26 and 38, Lewis lung carcinoma, B16 melanoma and murine multidrug-resistant tumor cells. The antitumor effects of ME2303 on human tumor xenografts in nude mice showed effects against several gastric, breast, and lung xenografts, and the effects were higher than those of DOXO.

Furthermore, ME2303 was the most effective anthracycline derivative in mice, bearing established liver metastases of Lewis lung carcinoma. This compound was investigated in pharmacokinetic studies: the tissue distribution of

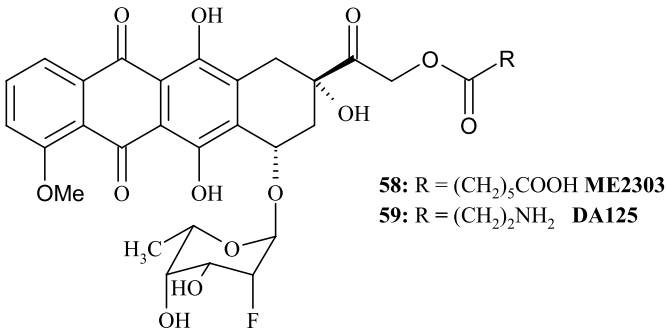


Fig. 8 2′-Fluoro-anthracylcine derivatives ME2303 and DA125

ME2303 and its metabolites showed no significant differences between normal and hepatic-metastases-bearing mice. ME2303 was rapidly metabolized to its metabolites M1 (esterolysis in C-14) and M2 (reduced at C-13 to a hydroxy derivative). In the plasma and liver, the active metabolite, M1, was maintained for a long period, similar to DOXO. These results may indicate a possible low toxicity of ME2303 [57, 58].

In preclinical studies ME2303 showed interesting characteristics on multidrug-resistant tumor cells. In fact, it alone proved effective against drug-resistant human and murine tumor cells [59–76].

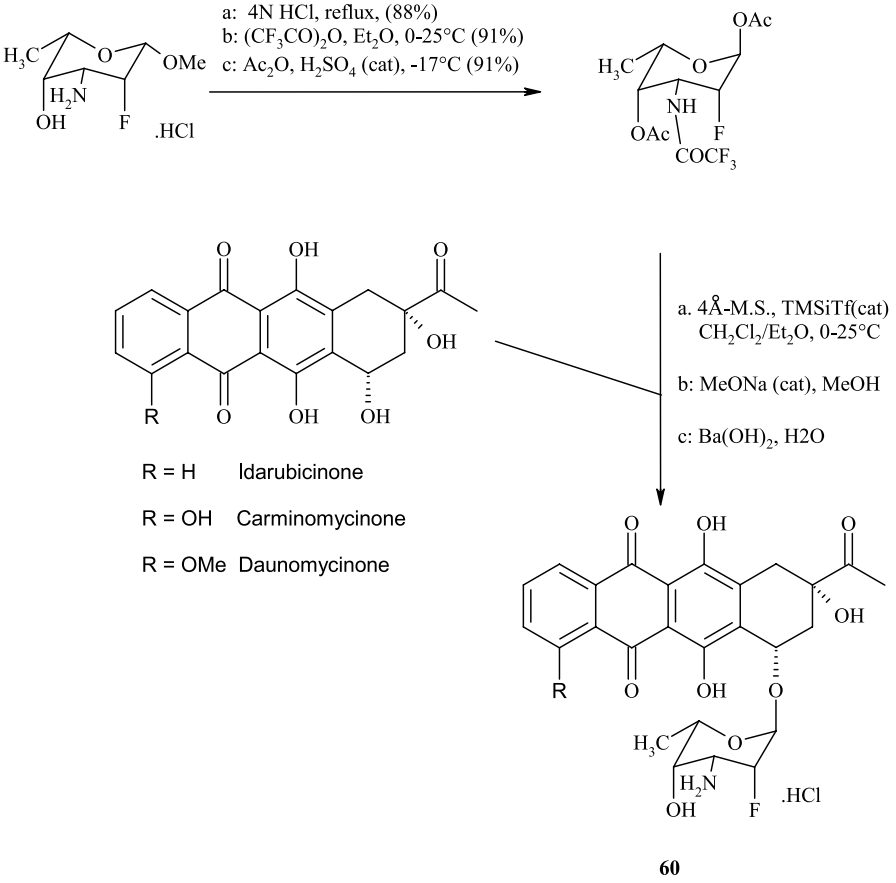
Compound **59** (Fig. 8, DA-125), another 2′-fluoroanthracycline derivative, a prodrug of FT-ADM (Dong-A-Pharm. Co), with a β-alanin as protective group on C-14, was well tolerated in clinical studies, showing no cardiotoxicity, alopecia or abnormalities in liver or kidney function [77]. DA-125 showed embryotoxic effects [78].

Other 2′-fluoro derivatives have been claimed in different patents [63, 79] (Scheme 16). Generally, the cytotoxicity of the carminomycin, (4-hydroxy-IDA), and that of the 2′-fluoro derivative against a number of tumor cell lines are equal in vitro. Both compounds show the same activity in vivo in tests against murine P-388 leukemia. Acute toxicity of the fluorine compounds appears to be lower than carminomycin. Carminomycin is another natural glycoside within the family of the anthracyclines.

7.2
3′-Fluoroalkylaminoanthracyclines and 3′-Fluoroanthracyclines

Some anthracyclines bearing a fluorine atom on the chain binding the amino group in position 3′ were described by Yoneshima et al. [80]. In 1988, they reported the synthesis of 3′-fluoroalkyl-, 3′-fluoroaryl- and 3′-fluoroaralkylaminoanthracyclines.

In 1998, Tsuchiya et al. [81] obtained some DOXO- and DAUNO-type anthracyclines **61**, bearing a fluorine atom in positions 2′ and 3′. These deriva-

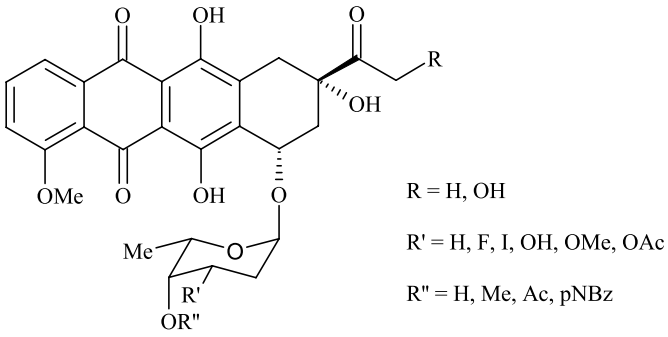


Scheme 16 Synthesis of 2′-fluoro-anthracycline derivatives

tives were prepared by coupling suitable protected glycosyl bromides with the aglycones DAUNO or DOXO. Among the compounds prepared, the 2′-fluoro showed higher antitumor activity than 2′,3′-difluoro derivatives.

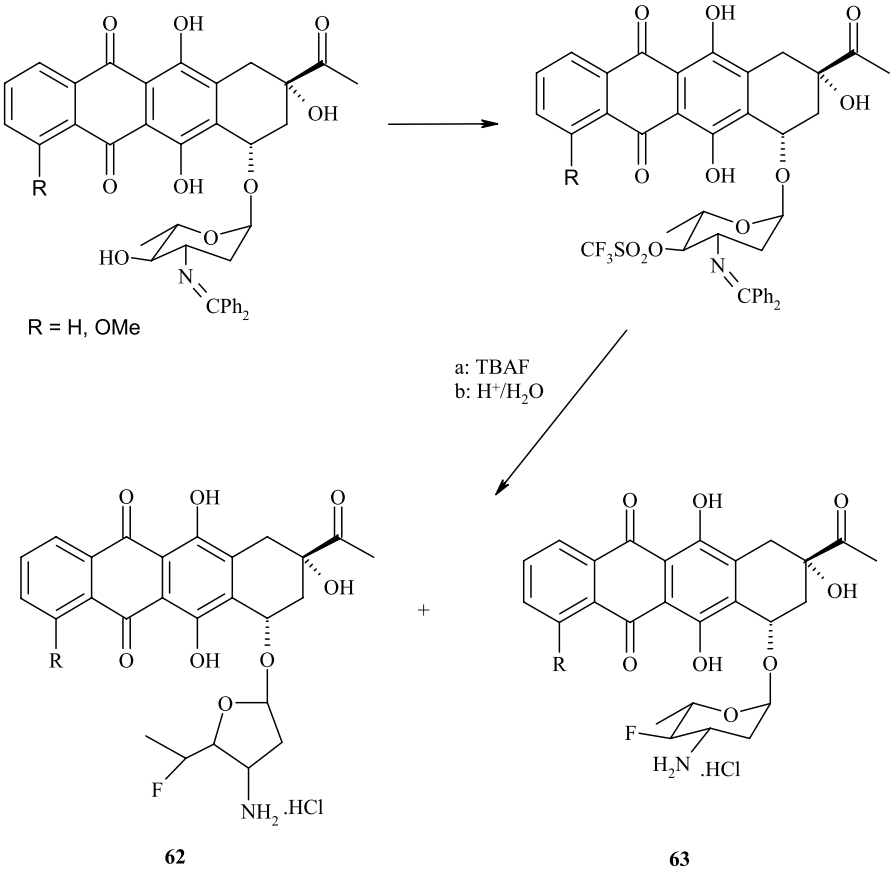
7.3 4′-Fluoroanthracyclines

4′-Fluoroanthracyclines were obtained in the early 1980s in Farmitalia Carlo Erba, using 4′-epi-DAUNO or its 4-demethoxy analogue as the starting material, which, after protection of the amino group on the sugar moiety as benzophenone Schiff base, were reacted with trifluoromethanesulfonic anhydride to obtain the corresponding 4′-epi-4′-*O*-trifluoromethanesulfonates. From these, after treatment with *n*-tetrabutyl ammonium fluoride and a subsequent mild acidic hydrolysis of the *N*-protecting group, the DAUNO deriva-



61

Fig. 9 3′-Deamino-anthracycline derivatives



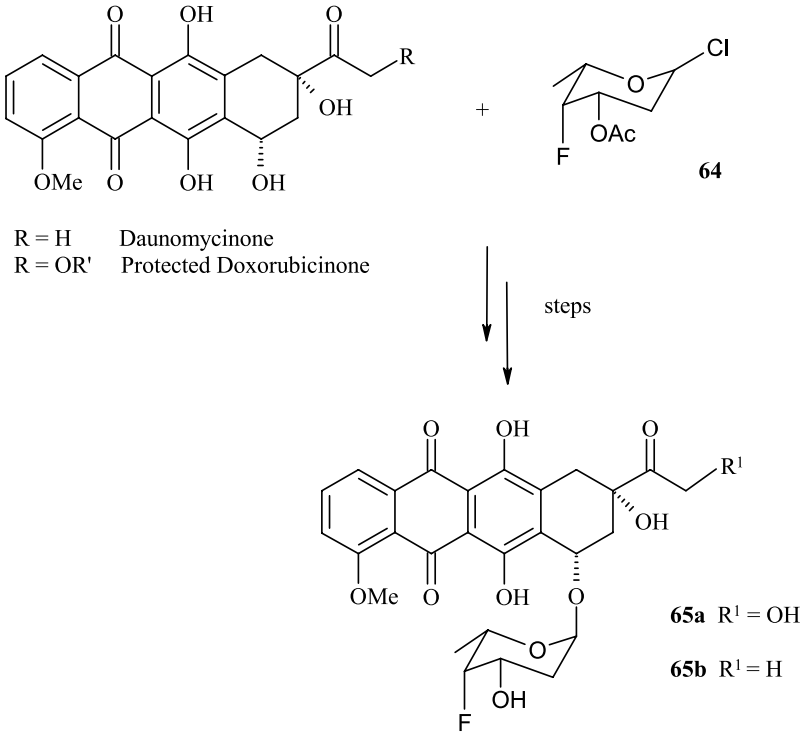
62 **63**

Scheme 17 Synthesis of 4′/5′-fluoro-anthracycline derivatives

tives **62** and **63** were obtained as a mixture of free bases (Scheme 17). After chromatographic separation, the two compounds were isolated and then transformed into their DOXO analogues via 14-bromo derivatives and subsequent hydrolysis with aqueous sodium formate [82, 83].

4′-Deoxy-4′-fluoro-DOXO was the object of studies, together with several other derivatives, for determination of the influence of lipophilicity on the cytotoxicity of anthracyclines in LoVo and LoVo/Dx human cell lines. The lipophilic character of selected anthracyclines was measured by means of reverse-phase HPLC, under appropriate experimental conditions. The results obtained in these in vitro models indicate that lipophilicity plays a role in anthracycline activity, influencing drug availability at the site of action. The introduction of a fluorine atom in the sugar moiety structure increases the lipophilicity and cytotoxicity of the drug [84].

Another 4′-fluoro-DAUNO/DOXO derivative, **65** (Scheme 18), was synthesized in the early 1990s in Dong-A Pharm. Co. as a 3′-deamino-3′-hydroxy derivative. The glycosides were obtained by the coupling of 2,4-dideoxy-3-acetyl-4-fluoro-alpha-L-fucopyranosyl chloride **64** with dauno-rubicinone

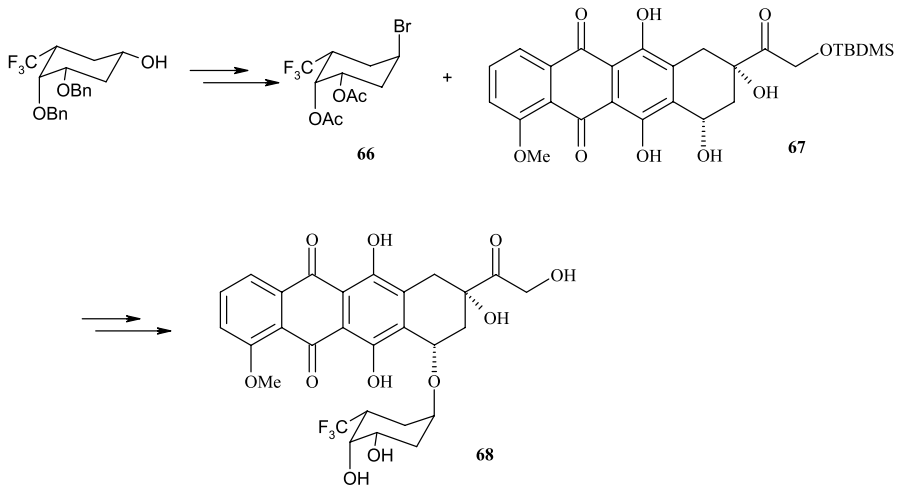


Scheme 18 Synthesis of 3′-deamino-3′-hydroxy-4′-fluoro-derivatives of DAUNO and DOXO

or 14-hydroxy-protected doxorubicinone and deprotection of the protected glycosides. When administered i.p., these drugs showed significant antitumor activity for L1210 murine leukemia at a wide range of doses (T/C = 605%) [85].

7.4 6′-Fluoroanthracyclines

The 5′-(trifluoromethyl)-DOXO **68** was obtained by coupling a 2,6-dideoxy-6,6,6-trifluoro sugar (**66**) with the protected doxorubicinone derivative **67** (Scheme 19). Antitumor activity of the compound was evaluated in vivo, by growth inhibition assay, against human and murine tumor cell lines. Against six human cell lines (K562, HMV-1, KB, MKN-1, PC-14 and T24) the trifluoro-DOXO derivative exhibited comparable or slightly weaker activity than natural DOXO, although an activity three times stronger than DOXO against DOXO-resistant (P388/DX) murine leukemia.



Scheme 19 Synthesis of 6′-fluoro-DOXO

The higher activity of trifluoro-derivative vs DOXO may be reasonably ascribed to the presence of a trifluoromethyl group at C-5′, whose contribution to its stability, as well as to an increased lipophilicity, may enhance the cellular uptake of this analogue, also facilitating organ penetration [63, 86, 87].

Derivatives with both fluorines on C-2′ and C-6′ (**69**) (Fig. 10) were claimed [88] where the daunosamine was substituted by a taropyranosyl moiety. These anthracyclines showed low toxicity and higher activity than current carcinostatic agents.

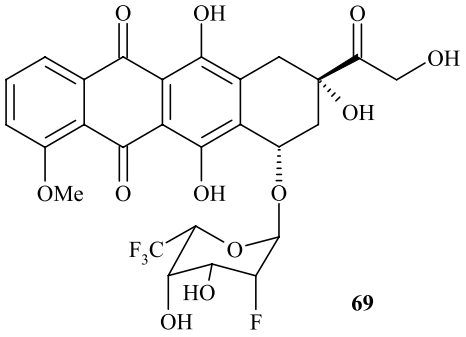


Fig. 10 2′,6′-Fluoro-3′-deamino-3′-hydroxy-DOXO

8
Disaccharide Fluoroanthracyclines

Disaccharide anthracyclines were synthesized, in which the daunosamine moiety is separated from the aglycone by either a rhamnose or fucose residue. Fluorine was reported on the fucose moiety (**70**) [89] and on the aglycone at C-8 (**71**) [20] (Fig. 11). Both examples indicate two important considerations: first, anthracyclines, with an activity comparable to that of DOXO, possess the daunosamine moiety not directly linked to the aglycone; second, the fluorine does not lower the activity of these compounds but allows the synthesis of new derivatives with a particularly high activity in inhibiting the growth of cultured human tumor cells.

Derivatives of MEN10755, an anthracycline analogue currently under evaluation in Phase II clinical trials [90–93], with fluorine on C-8, were synthesized (Scheme 20) and the cytotoxic and antitumor properties of disaccharide 8-fluoro-anthracyclines were markedly affected by the stereochemistry of the

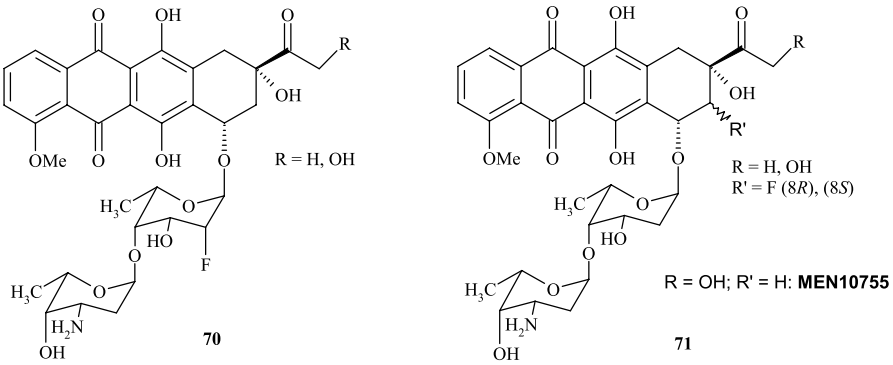
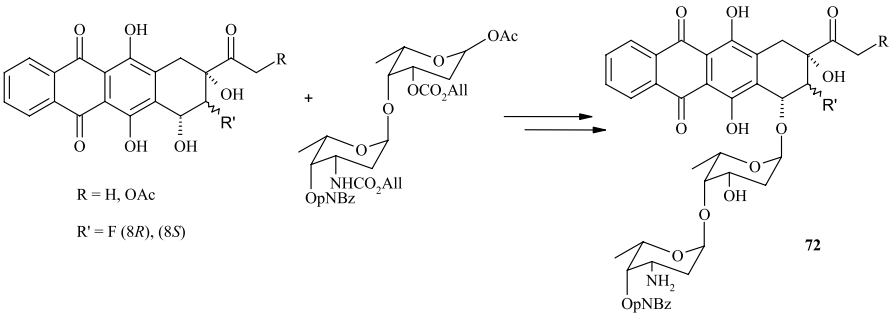


Fig. 11 Fluorinated disaccharide anthracyclines

fluorine substituent in position C-8 of the A-ring, as well as in the above-mentioned natural monosaccharides (see Sect. 5). Cytotoxicity of the (8*S*)-fluoroepimer was comparable to that of DOXO, whereas cytotoxicity potency of the (8*R*)-fluoroepimer was similar to that of IDA.

Moreover, reduced cross-resistance was shown for the (8*S*)-fluoroepimer, which exhibited an ability to overcome resistance comparable to IDA. In contrast, the (8*R*)-fluoroepimer proved able to almost completely overcome resistance in all cell systems examined. The DOXO-derivatives had an activity 3–4 times stronger than the DAUNO derivatives against DOXO-resistant lines.



Scheme 20 Synthesis of fluorinated disaccharide anthracyclines

9
Fluorine-Incorporating Prodrugs of Anthracyclines

A good cancer chemotherapeutic prodrug should be poorly active until metabolized by a tumor-specific enzyme. Several human cancers are known to possess intense enzymatic activity. This raises the possibility that tumor enzymes could be a target for tumor-specific prodrug activation to maintain a high drug concentration at the tumor site and a relatively low systemic concentration of the drug, resulting in decreased systemic toxicity.

There are reports of a large family of anthracycline prodrugs. Many of them possess fluorinated groups that give different properties from the parent compounds [52]. Figure 12 shows two examples: compound **73** was synthesized as prodrug [94], exhibiting reduced cytotoxicity against L1210 cells (1/20 of parent compound) but after hydrolysis, the drug was activated, representing a new targeting strategy in cancer chemotherapy. Compound **74** was less potent than the parent compounds when tested on the sensitive L1210 cells or on the KB-3-1 cells, but when it was tested on the KB-A-1 cells (350 times more resistant to DOXO than the sensitive KB-3-1 line), **74** was more potent [95]. In this case, the prodrug was able to overcome MDR, confirming the observation on the amino group in the sugar por-

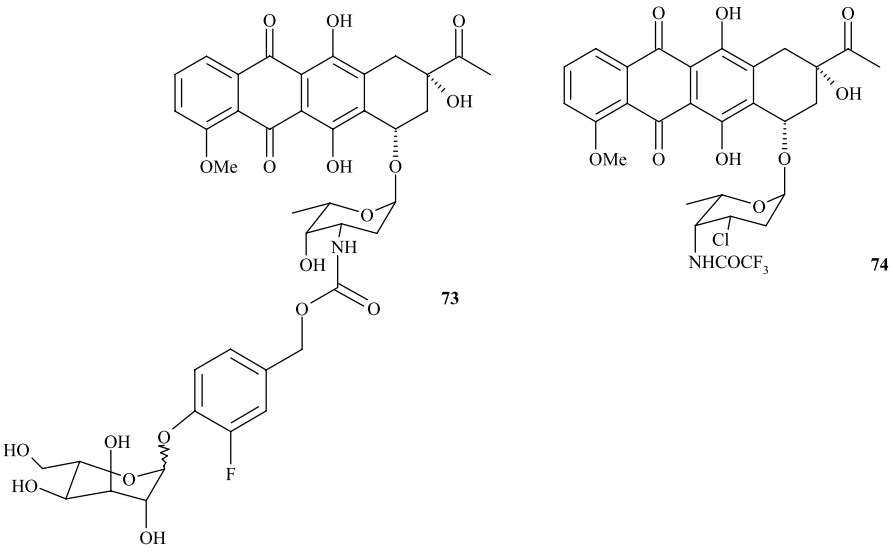


Fig. 12 Fluorinated prodrugs of anthracyclines

tion of anthracyclines as a key function for substrate recognition by the P-glycoprotein [96, 97].

10 Conclusions

The natural anthracycline glycosides and closely related compounds represent a fundamental resource for a therapeutic approach against a wide variety of hematological malignancies and solid tumors. However, their clinical effectiveness, well established for different compounds of the series and widely applied in medical practice, shows remarkable limitations because of the development of resistance and the natural refractoriness of several cancers. These drawbacks, together with the considerable toxicity of anthracyclines, induced the researchers to propose analogous molecules with improved characteristics.

In fact, more than 2000 anthracycline analogues are available. The scientific literature describes their pharmacodynamic and pharmacokinetic properties, pointing up appreciable knowledge of this class of antitumor agents. Among these, a large number of fluorinated derivatives found application in clinical practice, Iressa possibly being the best known example, but an overall analysis see about a fifth of all pharmaceutical products on the market containing at least one fluorine moiety [98].

Many studies have been performed on fluorinated anthracyclines and their comparison with non-fluorinated analogues allow a better and deeper knowledge of the biological mechanisms involved in the activity of this class of drugs. We now know the influence of this small electrophilic atom, when introduced on different positions of the aglycone and sugar moiety.

The glycosidic bond is stabilized with the introduction of a strong electron-withdrawing atom such as fluorine in position C-8 of the aglycone and in position C-2′ of the sugar moiety. The absolute configuration of fluorinated C-8 has a strong influence on the natural half-chair conformation of the A-ring, thereby affecting and differentiating the activity pattern of the two enantiomers. Fluorination on C-2′ has a dual effect of stabilizing the glycosidic bond and influencing the C-3′ aminic group, a key function for substrate recognition by the P-glycoprotein.

Although many synthesized fluorinated derivatives are able to overcome MDR and reduce cardiotoxicity, two of the main drawbacks of the anthracyclines, no such analogue, to the best of our knowledge, is in an advanced clinical phase and not one has reached the market.

References

1. Kirk KL (2006) J Fluor Chem 127:1013
2. Swinson J (2007) Chem Today 25:46
3. Hertel LW, Ternansky RJ (1993) In Stud Org Chem (Amsterdam) 48:23
4. Welch JT (1991) Selective Fluorination in Organic and Bioorganic Chemistry; ACS Symposium Series 456, Washington DC
5. Seebach D (1990) Angew Chem Int Ed Engl 29:1320
6. Biscardi M, Caporale R, Pagliai G, Leoni F, Bernabei P, Santini V, Ciolli S, Grossi A (2003) Leuk Res 27:1125
7. Giannini G (2002) Curr Med Chem 9:687. Ibidem (2004) Medicinal Chemistry Review-Online 1:47
8. Morrow GW, Swenton JS, Filippi JA, Wolgemuth RL (1987) J Org Chem 52:713
9. Swenton JS, Morrow GW, Priebe W (1987) US Patent 4 697 005
10. Swenton JS, Horton D, Priebe W, Morrow GW (1987) US Patent 4 663 445
11. Suarato A, Angelucci F, Bargiotti A, Grandi M, Pezzoni G (1990) EU Patent 0 387 661
12. Angelucci F, Faiardo D, Stefanelli S, Suarato A (1990) EU Patent 0 381 989
13. Irvine RW, Kinloch SA, McCormick AS, Russell RA, Warrener RN (1988) Tetrahedron 44:4591
14. Hammer BC, Russell RA, Warrener RN, Collins JG (1989) FEBS Lett 244:227
15. Barr KE, Russell RA, Warrener RN, Collins JG (1993) FEBS Lett 322:173
16. Fu X, Pechacek JT, Wheeler DMS, Wheeler MM (1992) ICOS-92 MT-25. Montrèal, Canada
17. Chamberlain T, Fu X, Pechacek JT, Peng X, Wheeler DMS, Wheeler MM (1991) Tetrahedron Lett 32:1707
18. Animati F, Lombardi P, Arcamone F (1991) EU Patent 457215
19. Lombardi P, Animati F, Cipollone A, Giannini G, Monteagudo E, Arcamone F (1995) Acta Biochim Pol 42:433

20. Animati F, Arcamone F, Giannini G, Lombardi P, Monteagudo E (1995) WO Patent 9604292
21. Mankhetkorn S, Dubru F, Hesschenbrouck J, Fiallo M, Garnier-Suillerot A (1996) Mol Pharm 49:532
22. Giolitti A, Guidi A, Pasqui F, Pestellini V, Arcamone F (1992) Tetrahedron Lett 33:1637, Ibidem EU Patent 436474
23. Canfarini F, Giolitti A, Guidi A, Pasqui F, Pestellini V, Arcamone F (1993) Tetrahedron Lett 34:4697
24. Giannini G, Animati F, Arcamone F, Lombardi P, Rossi C (1997) 10th ESOC-n.061 Basel, Switzerland
25. Guidi A, Canfarini F, Giolitti A, Pasqui F, Pestellini V, Arcamone F (1995) ACS Symp Ser (Anthracycline Antibiotics) 574:47
26. Guidi A, Canfarini F, Giolitti A, Pasqui F, Pestellini V, Arcamone F (1994) Pure Appl Chem 66:2319
27. Pasqui F, Canfarini F, Giolitti A, Guidi A, Pestellini V, Arcamone F (1996) Tetrahedron 52:185
28. Animati F, Arcamone F, Bigioni M, Capranico G, Caserini C, De Cesare M, Lombardi P, Pratesi G, Salvatore C, Supino R, Zunino F (1996) Mol Pharmacol 50:603
29. Giannini G, Salvatore C, Animati F, Lombardi P, Arcamone F (1996) 25th National Medicinal Chemistry Symposium. Ann Arbor (Michigan), USA
30. D'Accolti L, Fusco C, Rella MR, Curci R (2003) Synth Commun 33:3009
31. Tsushima T (1995) 19th Int. Congress of Chemotherapy Montreal. Canada
32. Nakamura S, Ikegami H, Negoro S, Fukuoka M, Sugiura T, Ariyoshi Y (1995) 19th Int. Congress of Chemotherapy. Montreal, Canada
33. Cambie RC, Holroyd SE, Rutledge PS, Woodgate PD (1993) Aust J Chem 46:703
34. Rutledge PS (1992) ICOS-92. Montrèal, Canada
35. Giannini G (1996) Gazzetta Chimica Italiana 126:771
36. Cambie RC, Higgs KC, Rustenhoven JJ, Rutledge PS (1996) Aust J Chem 49:751
37. Giannini G (1997) Gazzetta Chimica Italiana 127:545
38. Rho YS, Park S, Kim SY, Cho I, Lee C, Kang HS, Cheong C (1998) Bull Korean Chem Soc 19:74
39. Arcamone F (1994) Gene 149:57
40. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L (2004) Pharmacol Rev 56:185
41. Del Nero S, Gandolfi C, Lombardi P, Arcamone F (1981) Chem Ind 810
42. Matsumoto T, Ohsaki M, Yamada K, Matsuda F, Terashima S (1988) Chem Pharm Bull 36:3793
43. Matsumoto T, Ohsaki M, Matsuda F, Terashima S (1987) Tetrahedron Lett 28:4419
44. Matsuda F, Matsumoto T, Ohsaki M, Terashima S (1989) Tennen Yuki Kagobutsu Toronkai Koen Yoshishu 31:444
45. Terashima S, Matsuda F, Matsumoto T, Suzuki M, Ohsaki M, Yamada K (1988) WO Patent 8 805 780
46. Terajima A, Matsuda F, Matsumoto M, Osaki M, Yamada K (1988) JP Patent 01 093 580
47. Terajima A, Matsuda F, Matsumoto M, Suzuki M, Osaki M, Yamada K (1988) JP Patent 63 188 674
48. Berettoni M, Cipollone A, Olivieri L, Palomba D, Arcamone FM, Maggi CA, Animati F (2000) 13th ICOS-13. Warsaw, Poland. Ibidem (2002) Tetrahedron Lett 43:2867
49. Matsuda F, Matsumoto T, Ohsaki M, Terashima S (1991) Bull Chem Soc Jpn 64:2983
50. Matsuda F, Matsumoto T, Ohsaki M, Terashima S (1989) Tetrahedron Lett 30:4259

51. Terajima A, Matsuda F, Matsumoto M, Futamura M, Yamada K (1990) JP Patent 02 215 792
52. Tonkin KC, Brownlee RTC, Zunino F, Phillips DR (1990) Invest New Drugs 8:1
53. Terajima A, Suzuki M, Matsuda F, Matsumoto M, Osaki M, Yamada K (1986) JP Patent 000 301 330
54. Horton D, Priebe W, Varela O (1984) J Antibiot 37:853
55. Kunimoto S, Komuro K, Nosaka C, Tsuchiya T, Fukatsu S, Takeuki T (1990) J Antibiot 43:556
56. Takagi Y, Nakai K, Tsuchiya T, Takeuchi T (1996) J Med Chem 39:1582
57. Iigo M, Nishikata K, Nakajima Y, Hoshi A, Kadosawa H, Nakajima S (1990) Anticancer Drugs 1:77
58. Iigo M, Hoshi A, Kadosawa H, Fujigaki M (1991) Jpn J Cancer Res 82:1317
59. Tsuruo T, Yusa K, Sudo Y, Takamori R, Sugimoto Y (1989) Cancer Res 49:5537
60. Baer HH, Hernandez Mateo F (1990) Can J Chem 68:2055
61. Bols M (1991) Carbohydr Res 222:141
62. Baer BH, Hernandez Mateo F (1988) Carbohydr Res 184:151
63. Takeuchi T, Umezawa S, Tsuchiya O, Takagi Y, Sohtome H (1997) EU Patent 0 761 678
64. Takeuchi T, Umezawa S, Tsuchiya O, Umezawa K, Takagi Y (1989) JP Patent 01 203 397
65. Priebe W, Neamati N, Perez-Soler R (1992) J Antibiot 45:386
66. Rhee SW, Ryan KJ, Tracy M, Kelson AB, Clizbe LA, Chang MH, Park JS, Roh JK, Kong JY, Yang JG, Kim WB, Ok KD (1997) J Lab Comp Radiopharm 39:773
67. Tamura Y, Kita Y, Maeda H, Fujii Y (1992) JP Patent 0429089
68. Nishiyama M, Horichi N, Mazouzi Z, Bungo M, Fourcade A, Trincal G, Lampidis T, Mathe JG, Saijo N, Tapiero H (1991) J Cell Pharm 2:104
69. Tsuruo T, Sato S, Yusa K (1989) Jpn J Cancer Res 80:686
70. Huxham IM, Barlow AL, Mairs R, Gaze MN, Workman P (1993) Cell Biol Int 17:685
71. Kunimoto S, Komuro K, Nosaka C, Tsuchiya T, Fukatsu S, Takeuchi T (1990) J Antibiot 43:556
72. Tanno N, Fujita I, Miyauchi H, Ishizumi K, Yanagi Y, Morisada S (1990) JP Patent 02 178 298
73. Umezawa H, Umezawa S, Tsucha O, Takeuchi T, Takagi Y (1987) JP Patent 62 145 097
74. Umezawa H, Umezawa S, Tsuchiya O, Takeuchi T, Takagi Y, Yoneda T, Fukatsu S (1988) JP Patent 63 141 992
75. Umezawa K, Takeuchi T, Komuro K, Nosaka C, Kunimoto S, Tsuchiya T, Takagi Y, Umezawa S, Umezawa H (1988) Drugs Exp Clin Res 14:429
76. Hammer BC, Russell RA, Warrener RN, Collins JG (1989) FEBS Lett 244:227
77. Roh JK, Rha SY, Lee CI, Lee KH, Park JO, Cho JY (1996) 87th Ann Meet Am Assoc Cancer Res
78. Chung MK, Kim JC, Roh JK (1994) Teratology 49(5):385
79. Takeuchi T, Umezawa S, Tsuchia T, Takagi Y (1997) WO Patent 9 700 880
80. Yoneshima N, Matsumura Y, Yasuda A, Uchida K (1988) JP Patent 63 101 393
81. Takagi Y, Kobayashi N, Chang MS, Lim GJ, Tsuchiya T (1998) Carbohydr Res 307:217
82. Suarato A, Penco S, Caruso M (1985) GB Patent 2 159 518
83. Suarato A, Penco S, Giuliani F, Arcamone F (1985) DE Patent 3 428 945
84. Facchetti I, Grandi M, Cucchi P, Geroni C, Penco S, Vigevani A (1991) Anticancer Drug Design 6:385
85. Kwang-Dae O, Jeong-Bae P, Moon-Sung K, Dong-Yoon J, Junnick Y (1996) Yakhak Hoechi 40:117
86. Takagi Y, Nakai K, Tsuchiya T, Takeuchi T (1996) J Med Chem 39:1582

87. Dziewiszek K, Grynkiewicz G, Perezsoler R, Priebe W (1993) Abstr of papers of the 205th ACS
88. Takeuchi T, Umezawa S, Tsuchia T, Takagi Y (1995) WO Patent 9 524 412
89. Takeuchi T, Umezawa S, Tsuchiya O, Takagi Y (1994) JP Patent 06 256 377
90. Arcamone F, Animati F, Berettoni M, Bigioni M, Capranico G, Pratesi G (1997) J Natl Cancer Inst 89:1217
91. Animati F, Lombardi P, Arcamone F, Cipollone A (1995) WO Patent 9 509 173
92. Caponigro F, Willemse P, Sorio R, Floquet A, van Belle S, Demol J, Tambaro R, Comandini A, Capriati A, Adank S, Wanders J (2005) Invest New Drugs 23:85
93. Fiedler W, Tchen N, Bloch J, Fargeot P, Sorio R, Vermorken JB, Collette L, Lacombe D, Twelves C (2006) Eur J Cancer 42:200
94. Niculescu-Duvaz I, Springer CJ (1995) Curr Med Chem 2:607
95. Aligiannis N, Pouli N, Marakos P, Skaltsounis L, Leonce S, Pierre A, Atassi G (1996) Bioorg Med Chem Lett 6:2473
96. Priebe W, Van NT, Burke TG, Perezsoler R (1993) Anticancer Drug Des 4:37
97. Mankhetkorn S, Dubru F, Hesschenbrouck J, Fiallo M, Garnier-Suillerot A (1996) Mol Pharm 49:532
98. Isanabor C, O'Hagan D (2006) J Fluor Chem 127:303

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Synthesis of the Sugar Moieties

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Abstract Biological activity of the anthracycline antibiotics, which have found wide application in clinical oncology, is strongly related to their glycosidic structure. Modification or switch of the saccharide moiety became an important line of new drug discovery and study of their mechanism of action. Natural glycons (sugar moieties) of the anthracycline antibiotics belong to the 2,6-dideoxypyranose family and their principal representative, daunosamine, is 3-amino-2,3,6-trideoxy-L-*lyxo*-pyranose. Some newer chemical syntheses of this sugar, from a chiral pool as well as from achiral starting materials, are presented and their capability for scale-up and process development are commented upon. Rational sugar structural modifications, which are either useful for synthetic purposes or offer advantages in experimental therapy of cancer, are discussed from the chemical point of view.

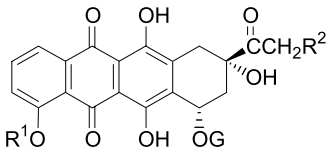
Keywords Anthracyclines · Antibiotics · Daunosamine · Glycosylation · Synthesis

Abbreviations

Ac	Acetyl
AD-mix-α,β	Reagents for Sharpless asymmetric dihydroxylation, Aldrich
AIBN	2,2-Azobis(2-methylpropionitrile)
All	Allyl
aq	Aqueous
Bn	Benzyl
Boc	Tertiary butoxycarbonyl
Bu	Butyl
t-Bu	Tertiary butyl
Bz	Benzoyl
Dau	Daunomycin
DCM	Dichloromethane
DDQ	Dicyanodichloroquinone
DEAD	Diethyl azodicarboxylate
DIBAL-H	Diisobutylaluminiumhydride
DIPT	Diisopropyltartrate
DME	Dimethoxyethane
DMF	Dimethylformamide
DMP	2,2-Dimethoxypropane
DNA	Deoxyribonucleic acid
Dox	Doxorubicin (adriamycin)
DPPA	Diphenylphosphoryl azide
Et	Ethyl
HMDS	Hexamethyldisilazane
HNL	Hydroxynitrile lyase
Im	Imidazole
i-Pr	Isopropyl
MCPBA	Metachloroperbenzoic acid
Me	Methyl
Ms	Methanesulfonyl
NIS	*N*-Iodosuccinimide
Ph	Phenyl
Py	Pyridine
rt	Room temperature
TBAF	Tetrabutylammonium fluoride
TBDMS	Tertiary-butyldimethylsilyl
TBS	(1,1-Dimethylethyl)dimethylsilyl
TEA	Triethylamine
TES	Triethylsilyl
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
Tf	Trifluoromethanesulfonyl
TMSCl	Trimethylsilyl chloride
TPHB	Triphenylphosphine hydrobromide
Ts	*p*-Toluenesulfonyl

1 Introduction

The need for new chemical entities, regardless of whether their origin is natural or synthetic, which can become better drugs (i.e., safer and more efficacious) than those used at present, is obvious to manufacturers, customers, and regulators [1, 2]. In modern times, more often than not new generations of medicines surfaced from the depository of Nature, revealing a new type of chemical structure to be turned into a group of valuable drugs by synthetic chemical transformations. Such was the case of the anticancer anthracycline antibiotics [3–5], which are pigments of microbial origin with unique glycosidic structure. They are now widely applied clinically, and also offer continuous inspiration for the design of new analogs. There is one particular compound of the class, doxorubicin (Dox, Fig. 1), that, although being far from ideal in its pharmacological characteristics, nevertheless became one of the most frequently applied anticancer drugs in numerous combination therapy schemes characteristic of contemporary oncological therapy. Doxorubicin and some other members of the group were exploited as leads for structure-based drug design, which gave birth to thousands of new synthetic analogs. We can still hope to discover the proverbial "better doxorubicin", as a yet unknown secondary metabolite [6], but it seems that active combinatorial synthetic or biotechnological approaches, aimed at new compounds capable of specific target ligand interactions, offer more chances for success [7]. Anthracyclines, discovered as dye-like metabolites of *Streptomyces* species in the early 1960s [8], gained wide acceptance as clinically useful DNA intercalators and topoisomerase inhibitors [9–12] but their side effects, including myelosupression and cardiotoxicity, were recognized as dose-limiting and potentially life-threatening. This, together with the occurrence of multidrug resistance (MDR) evoked by application of anthracycline antibiotics,



1 $R^1 = CH_3$ $R^2 = H$ Daunomycinone
2 $R^1 = CH_3$ $R^2 = OH$ Adriamycinone
3 $R^1 = H$ $R^2 = H$ Carminomycinone
G = H; indicates glycosylation site
G = α-L-daunosaminyl for the corresponding antibiotics:
Daunomycin (Dau), Doxorubicin (Dox) and Carminomycin (Car)

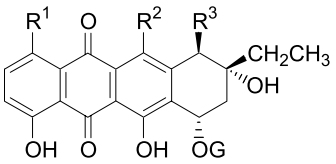
Fig. 1 Structures of aglycones of daunomycin group antibiotics

initiated many searches for new analogs, which started as chemistry-inspired derivatization programs. It soon turned out that the most interesting natural antibiotics, daunorubicin (Dau) and doxorubicin (Dox), are somewhat difficult as objects for chemical modifications because of their glycosidic structure and inherent susceptibility towards degradation under stressing (acidic, basic, oxidative, etc.) conditions. Moreover, most of the reasonably doable synthetic transformations resulted in loss of biological activity.

It is now obvious that many of the desirable radical structural modifications, both in aglycone as well as in the sugar part, have to be carried out by disconnection of the glycosidic bond and reassembly. Hence there is a need for specific glycosylating donors, the naturally occurring deoxyhexoses and their congeners [5, 13]. Recent progress in glycobiology has brought molecular recognition of carbohydrates to the forefront of medicinal chemistry [14], and the role of sugar moieties in various drugs of natural origin became of particular interest in the drug discovery field [15–18]. While it is well realized that biopolymer recognition requires cooperation of many weak interactions, at the drug design level sugars have recently gained considerable attention, not only as biologically competent oligomers, but also as pharmacophore structure-modifying monosaccharide building blocs. Thus, antibiotic sugar moieties can be conceived as reagents of wide synthetic application. Since, at the same time, their commercial availability is quite low, progress in synthetic methods is well worth discussing. The book *Synthetic aspects of aminodeoxy sugars of antibiotics* by I.F. Pelyvas, C. Monneret and P. Herczegh [19], published in 1988, with its meticulous discussion of details, has provided an invaluable landmark in this respect. A decade later, L.A. Otsomaa and A.M.P. Koskinen published a book chapter "Synthesis of 6-deoxyamino sugars" [20] that systematically covered advances in the same area. The present account is intended as a commentary on the rather extensive list of new achievements in the synthesis of antibiotic sugar moieties that have appeared in the original chemical literature during the last 10 years or so.

2
Sugars Naturally Occurring in Anthracycline Antibiotics

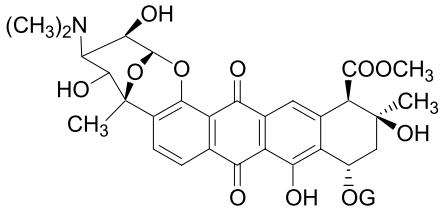
The anthracycline antibiotic group, well recognized for its therapeutic potential in oncology [11, 21, 22], accommodates some hundred secondary metabolites of *Streptomyces* genus [23], which are classified according to the aglycone type (Figs. 1–3) as daunomycin derivatives, rhodomycins, and nogalomycins. All aglycones are acetogenins of common biogenetic origin [24], which are post-translationally modified by the action of glycosyltransferases [25–27]. Naturally occurring anthracycline antibiotics are *O*-glycosides with a mono-, di-, or trisaccharide glycon component from the 2,6-dideoxy- or 2,3,6-trideoxypyranoside family (Fig. 4). Besides easy to spot



4	R^1 = H	R^2 = H	R^3 = $COOCH_3$	Aklavinone
5	R^1 = OH	R^2 = H	R^3 = $COOCH_3$	ε-Pyrromycinone
6	R^1 = OH	R^2 = H	R^3 = OH	β-Pyrromycinone
7	R^1 = H	R^2 = OH	R^3 = $COOCH_3$	ε-Rhodomycinone
8	R^1 = H	R^2 = OH	R^3 = OH	β-Rhodomycinone
9	R^1 = OH	R^2 = OH	R^3 = $COOCH_3$	ε-Isorhodomycinone
10	R^1 = H	R^2 = OH	R^3 = OH	γ-Rhodomycinone

G = H; indicates glycosylation site

Fig. 2 Aglycones of rhodomycin family antibiotics



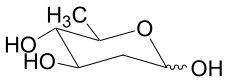
G = nogalose

11 Nogalamycin

Fig. 3 Structure of nogalomycin

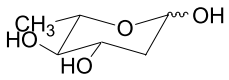
structural differences, the presented aglycone categories are characterized by distinctly different glycosylation patterns. Type one, daunomycinones, typically bear only the L-daunosamine moiety. Rhodomycinones and akavinones are linked to the 1–3 monosaccharide unit, composed of rhodosamine, 2-deoxyfucose, and cinerulose A. Nogalomycin has an aminosugar moiety built into the aglycone skeleton and, additionally, a C-3 branched 6-deoxy monosaccharide (nogalose) linked through a *O*-glycosidic bond. Apparently both parts, the aglycone and the sugar moiety, are essential for biological activity, which is pleiotropic.

Anthracyclines, like daunomycin and doxorubicin, are known to interact with nucleic acids, particularly with DNA, as well as with a variety of proteins, including albumins, ABC cassette transporters like Pgp, cytochromes, topoisomerases, and other enzymes. Focus on the anthracycline–DNA interactions in current research stems from recognition of the role of DNA

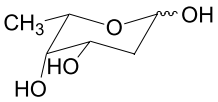


12 (D - *arabino*)

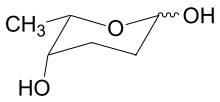
Olivose



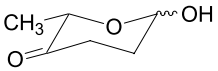
13 (L - *arabino*)



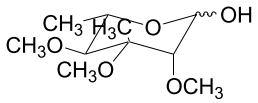
14 2-Deoxyfucose
(L - *lyxo*)



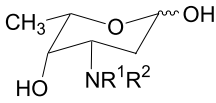
15 Rhodinose
(L - *erythro*)



16 Cinerulose A
(L - *glycero*)



17 Nogalose
(L - *manno*)



(L - *lyxo*)

18 $R^1=R^2=H$ Daunosamine

19 $R^1=H\ R^2=CH_3$ Desmethylrhodosamine

20 $R^1=R^2=CH_3$ Rhodosamine

Fig. 4 Structures of the sugar moieties of anthracycline antibiotics

as the principal target of this group of antitumor antibiotics. Various experimental techniques (X-ray, microcalorimetry, multinuclear NMR spectroscopy, DNA foot-printing etc.) have been applied to study the constitution of anthracycline–DNA complexes, as well as their kinetics and dynamics. It has been established that the main mode of intermolecular interaction consists of sequence-specific non-covalent binding resulting from aglycone intercalation, assisted by a groove binding of the sugar moiety. The energy of such binding, estimated in various experiments for naturally occurring anthracyclines, amounts to ca. 10 kcal, and ca. 20% of this value is attributed to the glycon part of the ligand [28–30].

Metabolic activation of anthracyclines can also lead to covalent DNA binding, resulting from interactions of free radicals or formaldehyde ami-

nal formation [31, 32]. Results of such adduct studies performed on synthetic analogs containing sugars other then daunosamine allowed formulation of the structural requirements, which obviously must be different for targeting DNA and/or topoisomerase II. It turned out that the presence of the C-3′ basic center (like the NH_2 group of Dau) is not necessary for anticancer activity. This is exemplified by synthetic annamycin, a new drug candidate in advanced clinical trials, which has 2,6-dideoxy-2-iodo-L-*talo*-pyranose in place of L-daunosamine [33–35]. Although in this case the binding energy with DNA is lower than for daunomycin, annamycin (being more lipophilic and having much lower affinity to Pgp efflux pump) offers some advantage in treatment of MDR leukemias, particularly when applied in liposomal formulation [36, 37].

In parallel to measurements of anthracycline–DNA interaction energies, there has been some effort to calculate corresponding values with the help of molecular modeling [38]. Recently, a computational model for anthracycline binding to DNA, based on 65 doxorubicin analogs and correlated with biological data, has been put forward [39]. Both experimental and theoretical results indicate that the tolerance for structural variations in the glycon part of anthracyclines, securing sustenance of antitumor activity, is fairly high, especially for 2,3,6-trideoxyhexoses. For hexopyranoses bearing substituents at all stereogenic centers, the α-L-*manno*- and α-L-*talo*- 6-deoxy configurations (having an axial C-2′ substituent) seem to be particularly promising in terms of antitumor activity. Preferred C-3′ substitution comprises an equatorial nitrogen substituent of variable constitution and basicity, while C-4′ substitution is optional, both in terms of structure and/or configuration. Foreseeing further development of this group of antitumor agents, both natural and modified glycons will be sought for combinatorial synthetic/biogenetic approaches to novel anthracyclines. It can be assumed that glycosylation will remain the critical step in such enterprises and that both chemical and enzymatic methods for sugar conjugation will be pursued. This means that a wide variety of mono- and oligosaccharides, both in protected and non-protected forms, can be considered as potential glycosylating reagents. It should be pointed out that there are practically no commercial sources of the sugars occurring as anthracycline antibiotic glycons. Thus, simple and efficient methods for their preparation remain in high demand. More comments concerning the role of sugar moieties in the design of new antitumor therapeutics are presented in Sect. 6.

3
Synthetic Approaches to 6-Deoxypyranoses

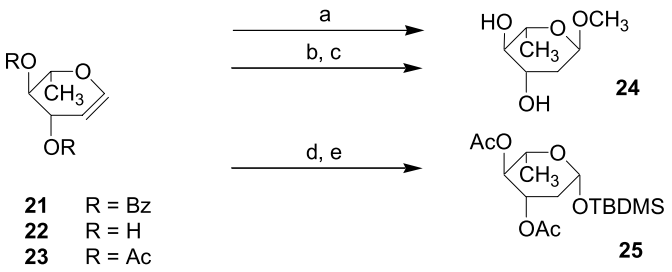
In contrast to an almost unlimited pool of possible synthetic glycons, the group of natural sugar constituents of anthracycline antibiotics is quite

limited, as seen from Fig. 4. Although all presented monosaccharides stem from the same general biogenetic pathway [40], deoxyhexoses pictured in the upper part of the figure are distinctly different, in terms of the synthetic effort needed for their preparation, from the last three sugars, which are 3-amino-monosaccharides of L-*lyxo*-configuration and require an altogether different preparative approach for proper functionality. In Sect. 3.1, olivose (**12**, **13**) is singled out for illustration of typical synthetic approaches to 2,6-dideoxyhexopyranoses. Isomeric 2-deoxyfucose (**14**), which can be obtained by analogous procedures, was much less frequently chosen as synthetic target, judging from the chemical literature. Cinerulose A (**16**), (and rhodinose **15**) can be very readily obtained from L-rhamnose via Ferrier rearrangement of acylated rhamnal, and also from 2-furyl ethanol, via Achmatowicz rearrangement [41]. Synthesis of nogalose represents some specific problems connected with stereoselective C-3 branching and exhaustive *O*-methylation, successfully resolved with use of known synthetic transformations [42, 43]. L-Daunosamine (**18**), an extremely popular synthetic target, is very often linked in the synthetic chemical literature with two other close structural analogs, L-acosamine and L-ristosamine. The latter two aminosugars have not been encountered among the natural anthracycline antibiotics (acosamine was isolated from the antibiotic actinoidin, while ristosamine is a component of some vancomycin group antibiotics), but they were repeatedly used for preparation of new semisynthetic anthracyclines [5]. Besides, acosamine is often prepared as an intermediate in daunosamine synthesis, which involves inversion of the hydroxy group configuration at C-4 (C-4′ of the antibiotic).

3.1
Syntheses of Olivose and Olivosides

Preparation of deoxyhexopyranoses and their glycosides from common sugars is a relatively easy task, considering well-developed synthetic carbohydrate chemistry documented in numerous monographs and manuals [44–46]. For example, L-olivose and its derivatives can be obtained in a couple of simple steps from readily available L-rhamnal derivatives. Thus, treatment of 3,4-di-*O*-benzoyl-L-rhamnal (**21**) with methanol in the presence of acidic resin resulted in formation of methyl L-olivoside (**24**) in a single step [47]. Similarly, reaction of rhamnal diacetate (**23**) with aqueous hydrobromic acid in THF gave olivose diacetate, which was isolated as a single anomer (**25**) after silylation [48]. Methoxymercuration of deprotected rhamnal (**22**), followed by reduction and hydrolysis, afforded methyl α-L-olivoside **24** in 50% yield, as depicted in Scheme 1 [49].

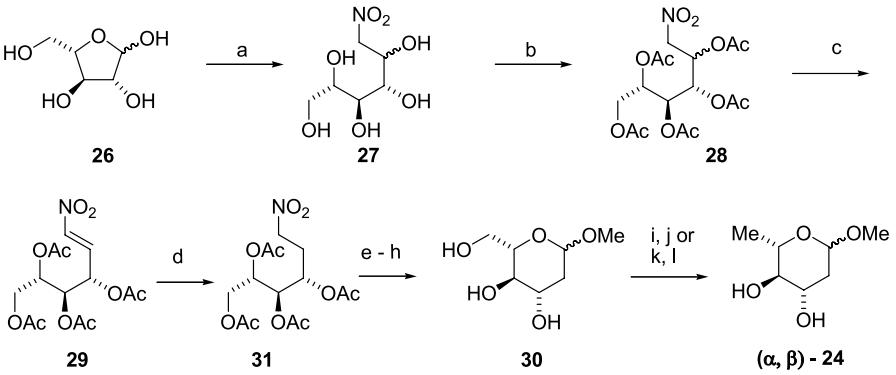
It might be presumed that by simple analogy, antibiotic monosaccharide 2-deoxy-L-fucose (**14**) could be best prepared from the corresponding hex-1-enitol (L-fucal), but scarce availability of the latter favors other routes. Total asymmetric synthesis from furan was elaborated by Vogel [50]. Alternative



Scheme 1 Reagents: *a* MeOH/H^+; *b* $Hg(OAc)_2$/MeOH; *c* KBH_4/OH^-; *d* HBr/THF; *e* TBDMSCl/Im/DMF

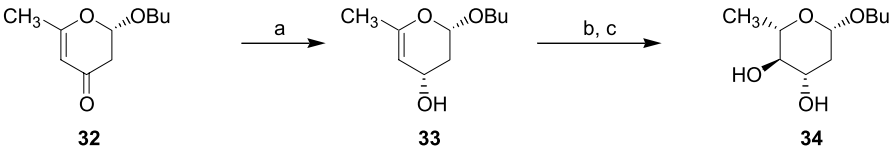
synthesis from D-galactose requires eight steps for conversion, yielding 25% overall yield of the 2,6-dideoxypyranose [51].

A different approach to methyl olivosides (**24**), starting from L-arabinose, is much more laborious and involves chain extension through the Henry reaction (Scheme 2). The 2-deoxy function is introduced by elimination/hydrogenation and 6-deoxygenation is achieved either via tosylation/LAH reduction or iodination/Raney nickel reductive dehalogenation of the primary hydroxyl group [52].



Scheme 2 Reagents: *a* $MeNO_2$, MeONa, MeOH; *b* BF_3Et_2O, Ac_2O; *c* $NaHCO_3$, toluene, heat; *d* H_2, Pd/C, EtOAc; *e* $Ba(OH)_2 \cdot 8H_2O$; *f* H_2SO_4; *g* $BaCO_3$; *h* $AG_5\text{-}X_4(H^+)$, MeOH; *i* TsCl, Py; *j* $LiAlH_4$; *k* NIS, PPh_3, DMF; *l* Raney Ni, MeOH

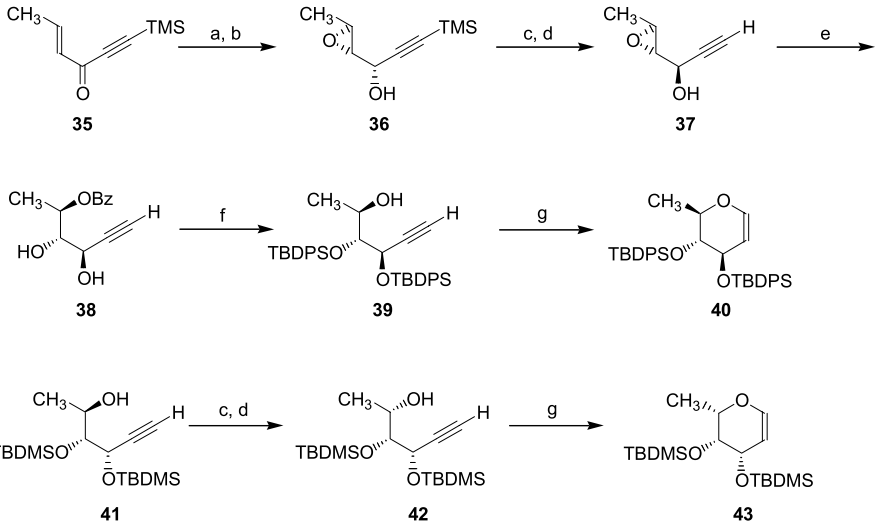
Acetylketene [4 + 2] cycloaddition with vinyl *n*-butyl ether gave the useful dihydropyranone intermediate (**32**), from which various 2,6-dideoxy monosaccharides were obtained. The key steps in synthesis of racemic butyl olivoside (**34**) consisted of stereoselective 1,2-reduction of conjugated ketone with diisobutylaluminum hydride, followed by standard hyroboration/oxidation procedure (Scheme 3) [53].



32 **33** **34**

Scheme 3 Reagents: *a* *t*-Bu_2AlH; *b* BH_3/SMe_2; *c* $NaBO_3$

Another synthesis of D-olivose derivative exploited a tungsten carbonyl catalyzed alkynyl alcohol cycloisomerization reaction [54, 55]. This useful method allows for synthesis of variety of glycals from an achiral precursor (**35**), which after enantioselective reduction and Sharpless' epoxidation afforded intermediates **39** and **42** (by Mitsunobu inversion), precursors of "unnatural" D-rhamnal (**40**) and valuable L-fucal (**43**) derivatives, respectively (Scheme 4).



35 **36** **37**

38 **39** **40**

41 **42** **43**

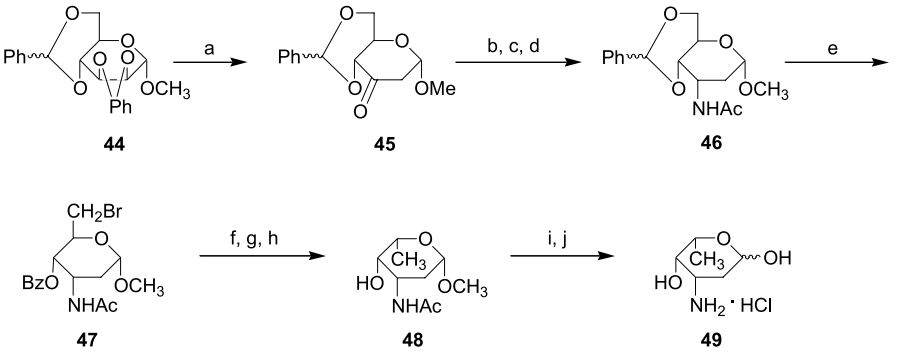
Scheme 4 Reagents: *a* (*R*)-2-methyl-CBS-oksazaborolidine/BH_3-SMe_2/THF; *b* Ti(*O*-*i*-Pr)$_4$/L-(+)-DIPT/$PhCMe_2OOH$/DCM; *c* Ph_3P/DEAD/PhCOOH; *d* KCN/MeOH; *e* Ti(*O*-*i*-Pr)$_4$/$PhCO_2H$; *f* TBDMSCl/Im/DMF; *g* $W(CO)_6/h\nu Et_3N$/THF

Methyl L-olivoside (**24**) is an important compound, because it has been repeatedly used as a key intermediate for synthesis of other deoxypyranosides, e.g., L-oleandroside, L-kedarosaminide, L-ristosaminide, and L-daunosaminide [20]. The examples of its syntheses presented above could be easily multiplied by application of known principles of acyclic stereoselection, e.g., asymmetric hydroboration, epoxidation, dihydroxylation, etc. Considering recent advances in enantioselective synthesis based on asymmetric

catalysis [56], there are almost endless possibilities for designing syntheses of simple deoxy monosaccharides bearing three consecutive chirality centers assembled into L-*lyxo* arrangement, starting from prochiral or achiral substrates. Chiral pool syntheses using starting materials other than carbohydrates (aminoacids, hydroxyacids) should also be taken into account. Some of these possibilities are presented in connection with the more demanding problem of L-daunosamine synthesis. However, it seems that in terms of cost/efficiency, a reductionist approach utilizing cheap sugar raw materials for selective chemical deoxygenations has no match until new selective biotechnological procedures, utilizing genetic engineering, are developed. The argument, often repeated in the older literature, that L-rhamnose is an expensive starting material, does not apply any longer and commercial availability of glycals, including 3,4-di-*O*-acetyl-L-rhamnal **23**, make synthetic accessibility of simple L-deoxypyranosides even easier.

4
Syntheses of Daunosamine and Related Glycosylating Agents

3-Amino-2,3,6-trideoxy-L-*lyxo*-hexopyranose (**18**, L-daunosamine), is the sugar moiety of several clinically important anthracycline antitumor antibiotics, such as daunomycin, doxorubicin, carminomycin, idarubicin, and valrubicin. A practical way to prepare L-daunosamine synthons comprises solvolytic cleavage of the glycosidic bond (directly or after partial protection) in the natural anthracycline antibiotic daunomycin, which is produced on a large scale by fermentation. Since daunomycin is not freely available, its sugar moiety became a popular target of total or partial synthetic approaches and numerous ways for preparation of **18** have been designed. The first synthesis of *N*-acetyl methyl L-daunosaminide used L-olivoside (**24**) as the substrate and the amino group was introduced by C-3 *O*-sulfonate ester exchange in reaction with sodium azide [49]. Reviews mentioned in the "Introduction" and more recent papers on the subject quote well over 70 successful synthetic efforts aiming at daunosamine [57, 58], but this wave of innovation subsided at the turn of the century. Many synthetic transformations known from general organic chemistry were successfully applied to the preparation of daunosamine from monosaccharide precursors, via introduction of a nitrogen functionality at C-3 in the key step. These include: nucleophilic displacement of sulfonate esters with azide anion, oxirane ring opening with ammonia or amines, oxyamination of 2,3-unsaturated pyranosides, reaction of "sugar dialdehydes" (generated in periodate cleavage of cyclic vicinal diols) with nitromethane, reduction of 3-oximino compounds derived from corresponding uloses, halogen promoted cyclization of allylic iminoesters, etc. [58].



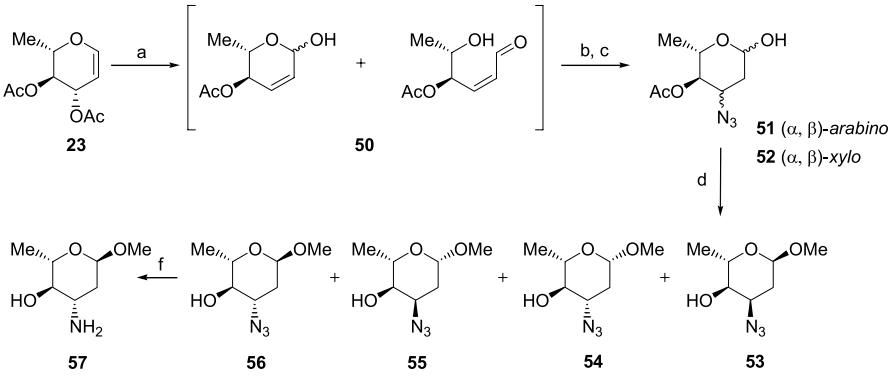
Scheme 5 Reagents: *a* BuLi; *b* H_2NOH; *c* $LiAlH_4$; *d* Ac_2O; *e* NBS; *f* AgF; *g* $NaOCH_3$; *h* H_2/Pd; *i* $Ba(OH)_2$; *j* HCl(aq)

It seems obvious that readily available natural hexoses should be considered first as raw materials of choice for preparation of daunosamine. However, when starting from cheap and easily accessible D-sugars, problems encountered on the way to target antibiotic sugars include: the need for switch of configuration to the L-series, getting rid of excess oxygen functionality (C-2), and amination of the C-3 position with proper stereochemistry. Despite these difficulties, Horton's synthesis of daunosamine from D-mannose, based on some novel acetal transformations, afforded a quite reasonable yield of the product and considerable potential for scale-up, becoming a reference preparation that deserves to be remembered in the context of newer achievements. Intermediate isomeric oximes formed from ulose **45** were isolated without recourse to chromatographic separation and the desired D-*ribo* compound **46** was transformed into L-*lyxo* 3-aminomonosaccharide **47** with high selectivity (Scheme 5). Daunosamine hydrochloride **49** was obtained from starting D-mannose in nine steps with overall yield of 40% [59].

4.1
Syntheses from L-Rhamnal and its Derivatives. Introduction of 3-Amino Functionality by Michael Addition of Hydrazoic Acid

Interestingly, certain synthetic approaches that did not look very elegant and promising at first sight, because of inherent lack of stereospecificity, were the subjects of considerable effort towards yield improvement and optimization of reaction conditions in several separate laboratories. An addition of azides to the "rhamnal hydrolyzate" described below in some detail, is a good example of such a situation.

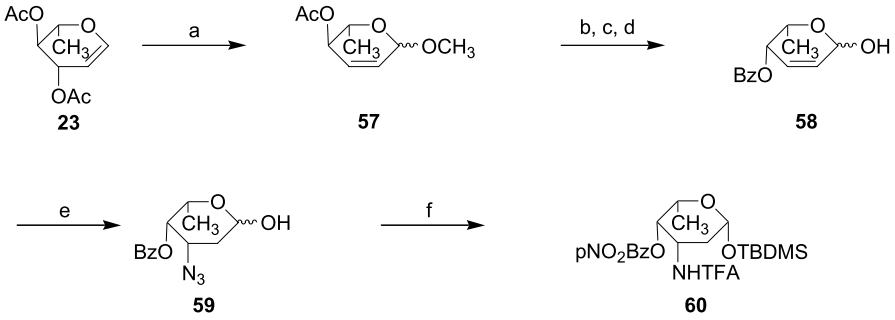
This reaction was first described over 20 years ago by Florent and Monneret [60, 61], who have subsequently found many followers. The reaction



Scheme 6 Reagents: *a* H_2O, heat; *b* NaN_3, H_2O, AcOH; *c* Ac_2O/Py, CH_2Cl_2; *d* MeOH, K10 montmorillonite; *e* NaOMe, MeOH; *f* H_2, Pd/C, EtOH, TEA

proved only slightly stereoselective, but the preferred L-*arabino*-azide **51** prevailed, with ca. 2 : 1 ratio over the C-3 epimer (**52**) (Scheme 6). Nevertheless, the reaction mixture consisted of four diastereomeric 3-azidohexoses (as evidenced by formation of 3-azido-pyranosides **53–56**) and although some separation was possible by selective crystallization, further synthesis from this point looked inevitably inefficient. The reaction was further exploited only after the incidental finding that silylation of the anomeric group of formed azidohexoses with *t*-butyldimethylsilyl chloride resulted in a chromatographically separable mixture of only two products with β-anomeric configuration [62]. Since it turned out that silylation of 2-deoxyhexapiranoses is generally β-stereoselective [48], more attention was given to derivatives obtained in such transformations. Apart from their crucial role in facilitating notoriously difficult separation of anomeric mixtures, they were found to be very useful synthetic intermediates capable of reacting directly as glycosyl donors and also as stable precursors of other glycosylating reagents, including glycosyl halides, glycals and thioglycosides [48].

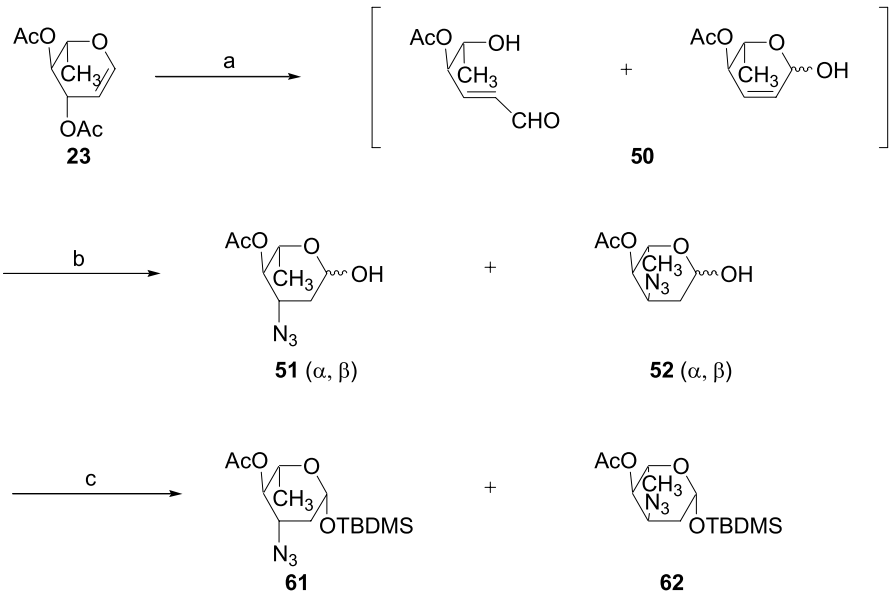
Two different preparative procedures for synthesis of daunosamine glycosyl donors, based on anomeric silyl derivatives, have evolved from the laboratories of Laatsch and Priebe, both apparently aiming at a large scale. In the first, L-rhamnal **23** was subjected to Ferrier rearrangement with methanol, then unsaturated glycosides **57** were deacetylated and two methods of invertion of configuration at C-4 were tried, both aiming at 4-*O*-benzoate **58**, believed to give better stereoselection at the HN_3 addition step. Indeed, after hemiacetal hydrolysis, compound **58** afforded only the required L-*lyxo*-azide (**59**), which was converted into 1-β-*O*-silyl derivative and subsequently into the appropriate glycosylating donor **60** (Scheme 7) [63]. This approach is, at least formally, equal to using 3,4-di-*O*-acetyl-L-fucal as a starting material, but there are two arguments against choosing this particular substrate: (i) the high price of L-fucose, and (ii) some inefficiency at the azide addition step,



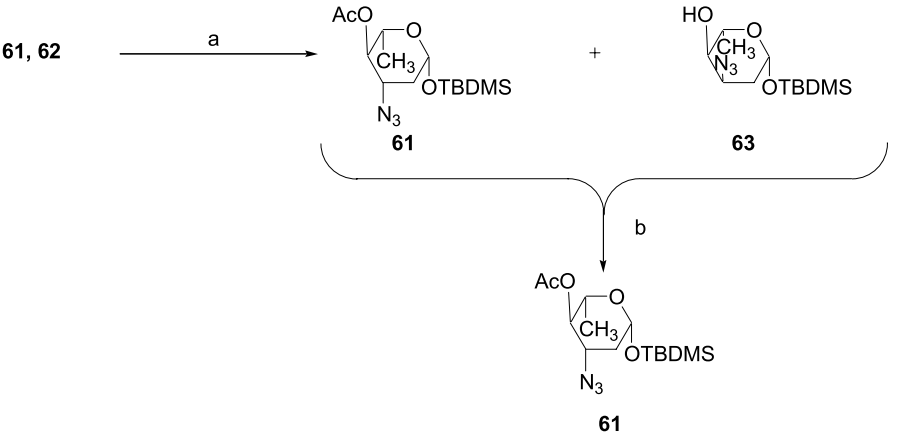
Scheme 7 Reagents: *a* MeOH/$ZnCl_2$; *b* MeONa/MeOH; *c* BzOH/DEAD/TPP; *d* HCl(aq); *e* NaN_3/AcOH; *f* *p*-NO_2BzCl/Py; *g* H_2/Pd; *h* F_3CCO_2Et; *i* TBDMSCl/Im

i.e., the corresponding hex-2-enose affords mainly the azido precursor of the 3-epimer of daunosamine in the HN_3 addition step.

In the second procedure, a reaction mixture resulting from addition of hydrazoic acid to the rhamnal hydrolyzate **50** was silylated to give L-*arabino* and L-*xylo* products (**61**, **62**), practically inseparable by column chromatography (Scheme 8). The mixture was then subjected to controlled saponification, which allowed diastereoisomeric separation by simple silica gel filtration, because the still-esterified desired component (**61**) retained its chromatographic mobility, while deprotected azido alcohol (**63**), treated as impurity, was absorbed more strongly and retained [64] on the gel pad (Scheme 9).

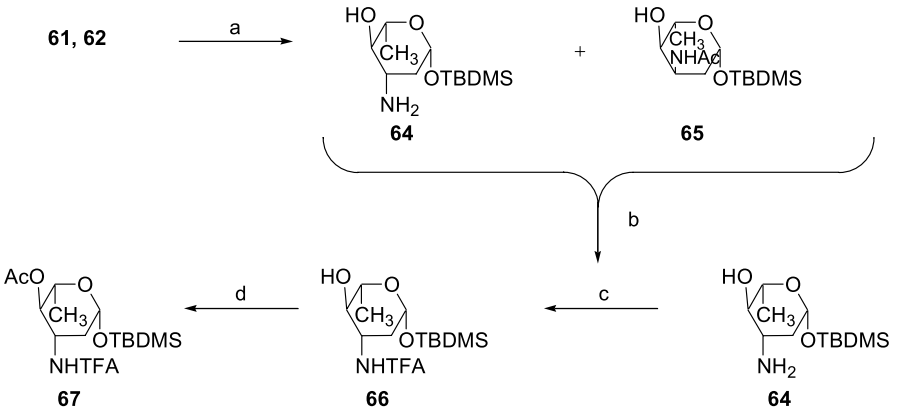


Scheme 8 Reagents: *a* H_2O, 70 °C; *b* NaN_3/AcOH; *c* TBDMSCl/Im/DMF



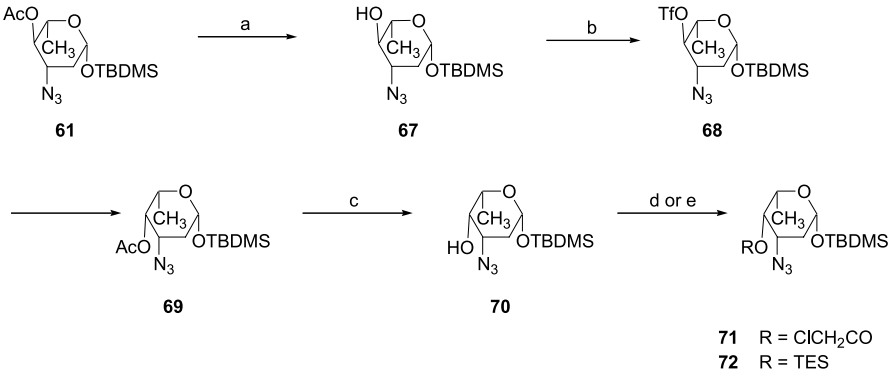
Scheme 9 Reagents: *a* 1N MeONa, MeOH; *b* silicagel filtration

In another variant, the mixture of azides (**61**, **62**) was subjected to catalytic hydrogenation in methanol, during which process *O*-acetyl groups were removed by solvolysis. In case of the *xylo*-isomer, the 4-*O*-acetyl group took part in an intramolecular O- to N-transfer, resulting in formation of neutral acetamide **65**, while the *arabino*-isomer remained in form of the protonable base **64**, which allowed separation of the diastereoisomers by simple, pH-controlled extraction (Scheme 10) [65].



Scheme 10 Reagents: *a* H_2, Pd/C, MeOH; *b* separation by extraction; *c* CF_3COOEt, Et_3N, CH_2Cl_2; *d* AcCl, Py, CH_2Cl_2

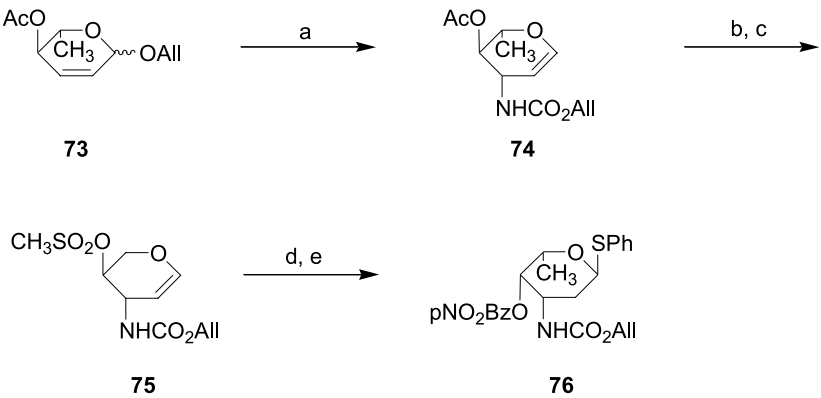
The preferred glycosylating synthon of L-*lyxo* configuration was the azide **69**, obtained by nucleophilic displacement of 4-*O*-triflate with tetrabutylammonium acetate, as shown in Scheme 11. Chloroacetyl ester **71** and tri-



Scheme 11 Reagents: *a* K_2CO_3/MeOH; *b* Tf_2O/TEA; *c* Bu_4NOAc/DMF; *d* $ClCH_2COCl$/Py; *e* Et_3SiCl/Im/DMF

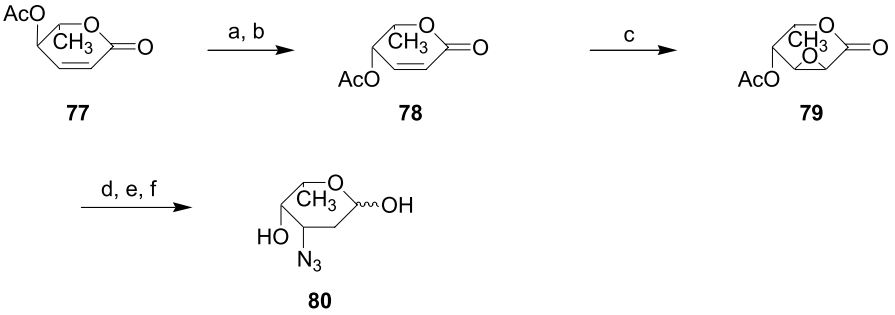
etylsilyl eter **72** were the reagents of choice, selected for the special cases when standard deacetylation conditions caused decomposition of the final antibiotic [65].

Another method for synthesis of useful derivatives of L-acosamine and L-daunosamine from rhamnal **23** was based on addition of chlorosulfonyl isocyanate to 2,3-unsaturated pyranosides **73** [66, 67]. Since the anomeric alkyl substituent in this reaction ends up as the carbamate ester, allyl glycoside securing easy deprotection was chosen for elaborating this reaction into large laboratory scale technical process. Method development for acosaminal derivative **74** involved multiparameter optimization of the isocyanate addition step by exploration of the reaction response surface. The final daunosaminyl glycosylation synthon **76** was obtained in overall 35% yield (Scheme 12) [68].



Scheme 12 Reagents: *a* $ClSO_2NCO$/DME; *b* K_2CO_3/MeOH; *c* CH_3SO_2Cl/Py; *d* PhSH/TPHB/THF; *e* *p*-NO_2BzOCs/DMF

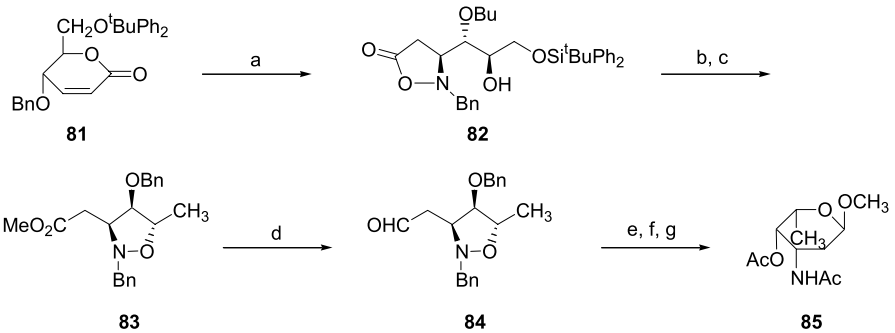
2,3-Unsaturated glycosides and particularly their corresponding lactones offer some other possibilities as substrates for 3-aminohexoses, as illustrated in Scheme 13.



Scheme 13 Reagents: *a* K_2CO_3/MeOH; *b* AcOH/Ph_3P/DEAD; *c* MCPBA; *d* $NaBH_4$/$(PhSe)_2$/AcOH/*i*-PrOH; *e* DPPA/Ph_3P/DEAD; *f* DIBAL-H

Lactone substrate **77** was obtained from **57** in one step in 90% yield and its C-4 epimeric analog was prepared by standard inversion procedure. Epoxidation was achieved by action of sodium hypochlorite in pyridine, while conversion to 3-azide required reduction of the epoxide with phenylseleno(triisopropyloxy)borate, followed by treatment with diphenylphosphorylazide under Mitsunobu reaction conditions [69]. The acetylated mixture of thioglycosides, obtained from **80**, afforded good yield of the desired α-linked antibiotic upon reaction with daunomycinone. All four 2,6-dideoxy-3-azido-L-hexopyranoses were obtained from rhamnal via 4-epimeric lactones.

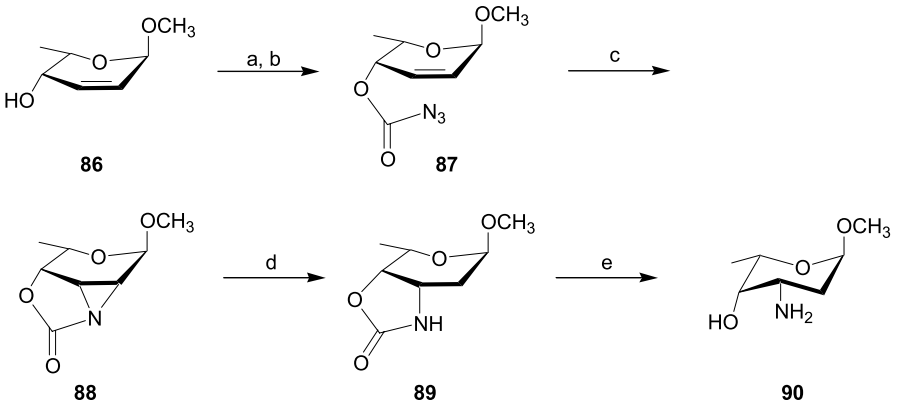
Lactone **81** reacted with *N*-benzylhydroxylamine affording isoxazolidin-5-one **82**, which easily rearranged to isoxazolidine ester **83** (Scheme 14) [70]. The latter was subjected to some standard transformations to give aldehyde **84**, which afforded *N*-acetyl daunosaminide **85** after treatment with acidi-



Scheme 14 Reagents: *a* BnNHOH; *b* MsCl/Py; *c* MeOH; *d* DIBAL-H; *e* H_2/$Pd(OH)_2$; *f* MeOH/HCl; *g* Ac_2O/Py

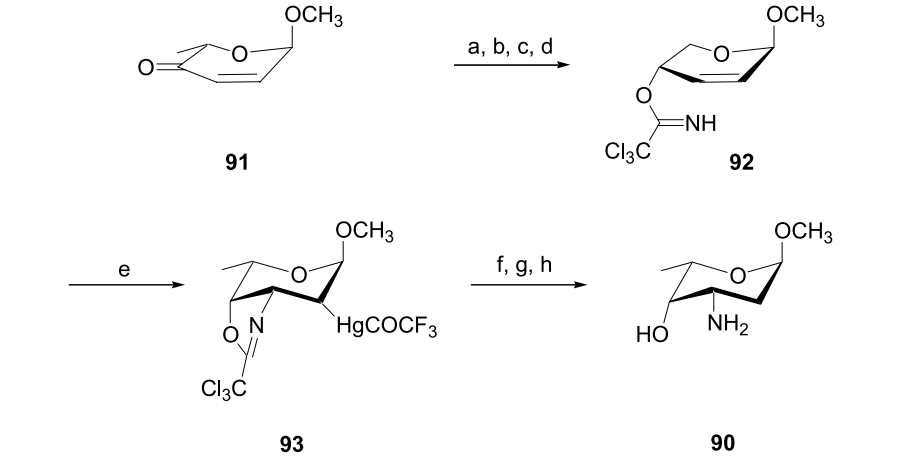
fied methanol followed by acetylation. An analogous procedure was applied for the synthesis of methyl acosaminide, starting from the C-4 epimeric lactone [71].

Glycosides resulting from Ferrier rearrangement of glycals have all the necessary features for stereoselective placement of nitrogen functionality at C-3. Thus, known 2,3-unsaturated methyl glycoside of L-*threo*-configuration **86** (which can be obtained from L-rhamnal via rearrangement followed by inversion of configuration at C-4; altogether, six steps from rhamnose) was converted to azidoester **87**, a source of photochemically induced nitrene, which added across the double bond with formation of aziridine **88** (Scheme 15). The latter was transformed into methyl α-L-daunosaminide (**90**) via cyclic carbonate **89** [72].



Scheme 15 Reagents: *a* *N*,*N*′-Carbonyldiimidazole, pyridine, benzene, rt, 2 h; *b* NaN_3, HOAc, HCl, DMF, rt; *c* 254 nm light, CH_2Cl_2, rt, 1 h; *d* Pd/C, H_2, EtOH/EtOAc 5 : 1, rt, 3 h; *e* $Ba(OH)_2 \cdot 8H_2O$ (70 equiv), H_2O, 125 °C, 1 h, then CO_2(s), rt

Interestingly, 2,3-unsaturated 6-deoxypyranosides of L-*erythro*- and L-*threo*-configuration are also available from a non-carbohydrate precursors, namely (*S*)-2-furylmethylcarbinol, via Achmatowicz rearrangement [41]. Enantiomers of 1-(2-furyl)ethanol can be obtained, among others, from D-glucal [73] or by biocatalytic resolution of the racemate [74], as well as by reduction of acetylfuran in the presence of Noyori catalyst [75]. The rearrangement represents reversal of the well known degradation of sugars under acidic conditions (leading from monosaccharides to furan derivatives). Using this route, Polish authors have achieved efficient, enantioselective synthesis of **90** (Scheme 16). Just as in case of rhamnal rearrangement described above, inversion of configuration at C-4 was necessary for construction of an intramolecular nitrogen donor, trichloromethylimidate ester **92**. Cyclization was effected by treatment of **92** with mercuric trifluoroacetate. Sequential reduction and hydrolysis afforded the desired daunosaminide [76]. These

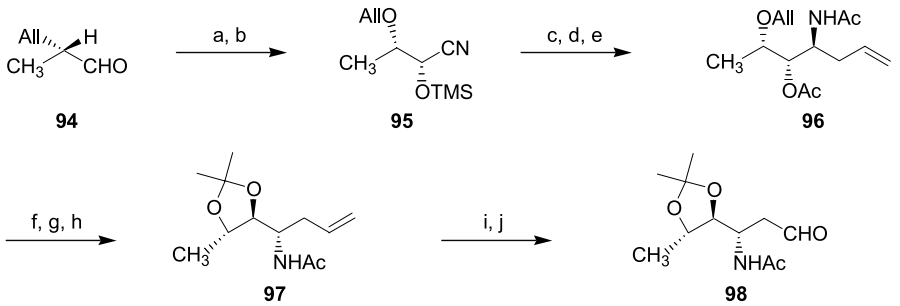


Scheme 16 Reagents: *a* $NaBH_4/CeCl_3$/MeOH; *b* DEAD/Ph_3P; *c* Na/MeOH; *d* NaH/Cl_3CCN; *e* CF_3CO_2Hg; *f* $NaBH_4$; *g* $Ba(OH)_2/H_2O$; *h* MeOH/HCl

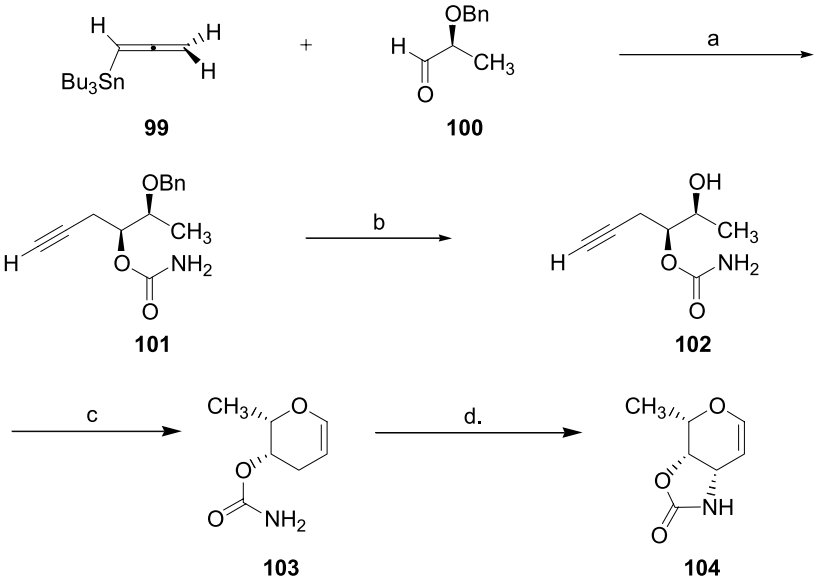
stereofacial transformations, which afford cyclic precursors of daunosamine and are also suitable for preparation of ristosamine from a C-4 epimeric precursor, are also mentioned in the reviews quoted in Sect. 1 [57, 58].

4.2 Syntheses from Non-carbohydrate Chiral Precursors

Aminoacid (D-threonine, L-aspartic) and hydroxyacid (L-tartaric, L-lactic) derivatives were repeatedly employed in the past as chiral substrates in the synthesis of daunosamine. More recently, protected L-lactaldehyde was used for the same purpose. Stereospecific synthesis of intermediate diol **95** was achieved with help of the enzyme (*R*)-hydroxynitrile lyase, which



Scheme 17 Reagents: *a* HCN/(*R*)-HNL/*i*-Pr_2O; *b* TMSCl/Py; *c* AllMgBr; *d* $NaBH_4$; *e* A_2O/Py; *f* Pd; *g* Na/MeOH; *h* $Me_2C(OMe)_2/H^+$; *i* O_3/MeOH; *j* Me_2S



Scheme 18 Reagents: *a* $MgBr_2 \cdot OEt_2$, $Cl_3CC(O)NCO$; K_2CO_3, MeOH; *b* DDQ; *c* 10% $W(CO)_6$, Et_3N, hν 350 nm; *d* 10% $Rh_2(OAc)_4$, $PhI(OAc)_2$, MgO

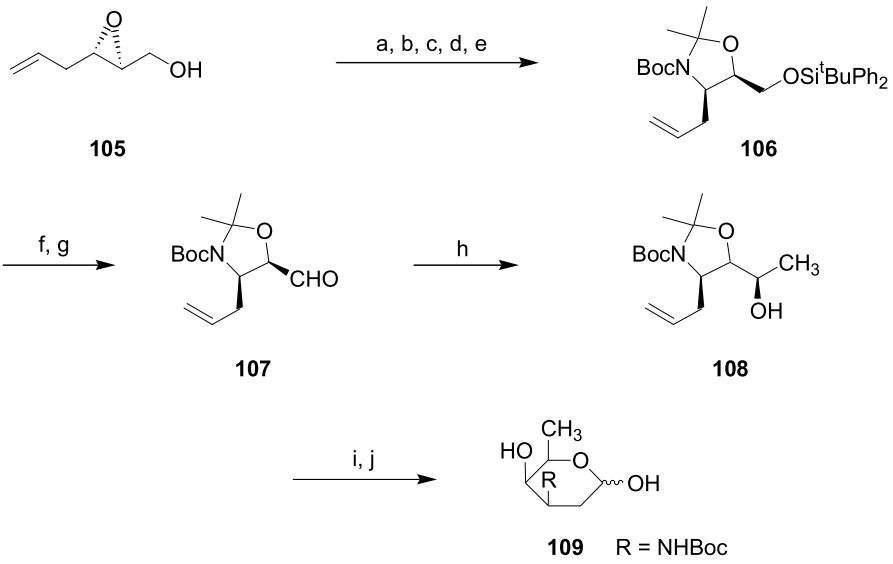
efficiently catalyzed addition of hydrogen cyanide to the substrate. Diastereoselective addition of Grignard reagent to the nitrile group, followed by a change of protecting groups and ozonolysis, completed the synthesis of **98** (Scheme 17) [77].

Preparation of an useful synthon, the cyclic carbamate of L-daunosaminal (**104**), was achieved by sequential catalytic transformations of a key intermediate. Suitably protected propargyl diol **101**, which was obtained by diastereoselective addition of allenyl stannate **99** to *O*-benzyl (*S*)-lactyl aldehyde **100**, was transformed as depicted in Scheme 18. Cycloisomerization to 3-deoxyglycal **103** was achieved by irradiation in the presence of tungsten hexacarbonyl, and subsequent nitrene insertion was catalyzed by rhodium acetate. Overall yield of the bicyclic daunosaminal (**104**) derivative from lactic acid derivative amounted to 44% [78].

4.3 Enantioselective Syntheses

General approaches to synthesis of monosaccharides, both: racemic and homochiral, from non-carbohydrate sources have been repeatedly discussed in chemical literature [79–81]. Many established methods for functionalization of prochiral molecules are now available for execution in enantioselective manner, with easy access to a new generation of commercial catalysts.

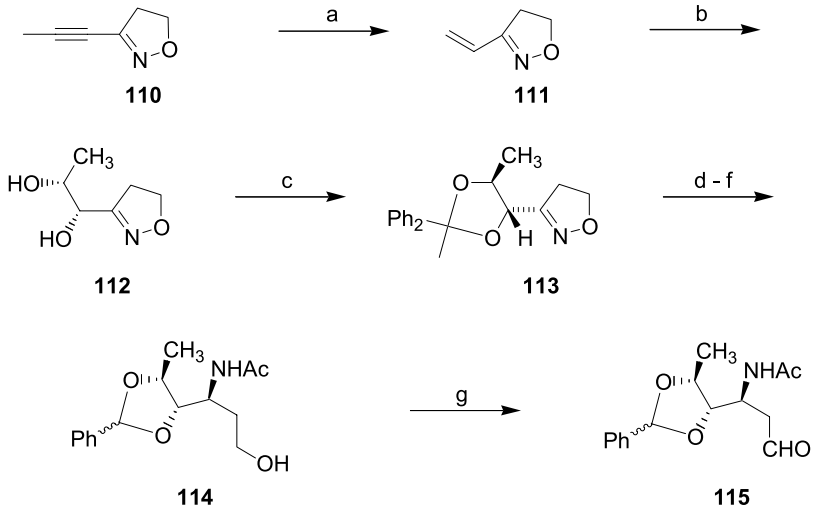
All four diastereomeric 3-amino-2,3,6-trideoxyhexoses have been obtained from (2*E*)-2,5-hexadien-1-ol via Sharpless catalytic epoxidation, followed by regioselective oxirane ring opening with azide anion. After few more standard transformations unsaturated oxazolidine-aldehyde **107** was obtained, which was subjected to stepwise chain extension to form the carbonyl end and chain shortening from the unsaturated end (Scheme 19). Methyl lithium reagents could be added to the carbonyl group in a stereoselective manner affording either *cis-syn* or *cis-anti* product in great excess, depending on the reaction conditions. Ozonolysis of the first (**108**) led to D-daunosamine derivative **109**, while the second was converted to L-ristosamine [82].



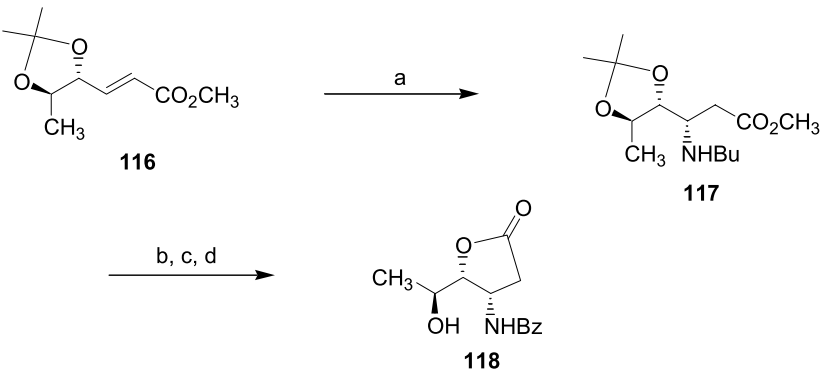
Scheme 19 Reagents: *a* Ti(O–i– Pr)$_2$(N$_3$)$_2$; *b* Ph$_3$P/THF; *c* Boc$_2$O; *d* TBDMSCl/Im/DMF; *e* DMP/H$^+$; *f* Bu$_4$NF/THF; *g* (CO)$_2$Cl$_2$/DMSO; *h* O$_3$/Me$_2$S; *i* HClaq

4,5-Dihydroisoxazole derivatives are recognized as protected γ-amino-alcohols, which can be employed for preparation of aminosugars. Synthesis of protected L-daunosamine based on this motif, is presented in the Scheme 20 [83].

Another achiral olefinic precursor, methyl sorbate, was chosen to exercise formal synthesis of L-daunosamine through enzymatic chiral induction of the C-4, C-5 centers and the diastereoselective conjugated addition of benzylamine to the resulting α,β-unsaturated ester. Thus, intermediate acetonide (**116**) gave upon benzylamine addition compound **117**, which after deprotection-acylation underwent lactonization to **118**, completing formal synthesis, since the compound was earlier converted to NTFA-daunosamine (Scheme 21) [84, 85].



Scheme 20 Reagents: *a* H_2/Lindlar catalyst/quinoline; *b* AD mix-α, $PhSO_2NH_2$, *t*-BuOH/H_2O; *c* PhCHO/$ZnCl_2$; *d* $LiBH_4/Et_2O$; *e* $HOCH_2CH_2NH_2/C_6H_6$; *f* $Ac_2O/NaHCO_3$/MeOH aq; *g* Swern oxidation

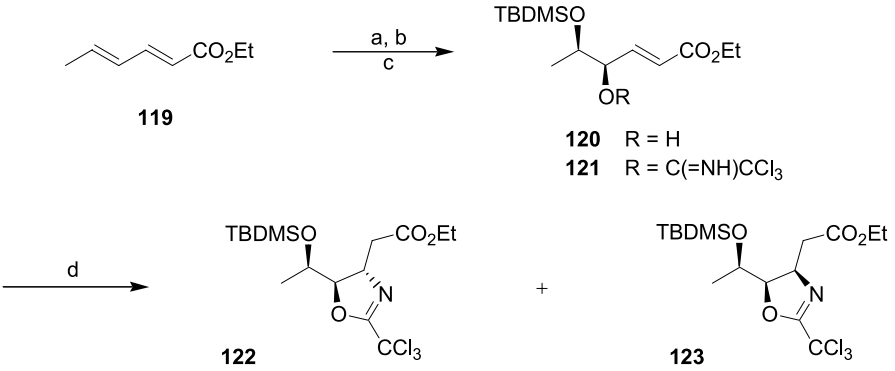


Scheme 21 Reagents: *a* $PhCH_2NH_2$; *b* $H_2/Pd(OH)_2/C$; *c* PhCOCl/Py; *d* 80% AcOH/reflux

Analogous substrate {*t*-butyl (*EE*)-hexa-2,4-dienoate} was shown to undergo a highly diastereoselective conjugate addition with chiral lithium amides (e.g., *N*-benzyl-α-methylbenzylamide). Subsequent transformations of intermediate unsaturated aminoester, like iodolactonization or dihydroxylation, gave the expected daunosamine derivatives, although with very limited effectiveness [86].

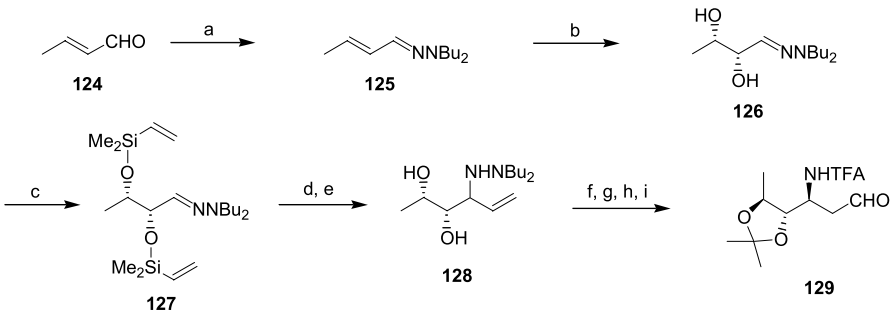
Commercially available ethyl sorbate was easily converted into chiral diol by Sharpless asymmetric dihydroxylation and by subsequent selective silylation into allyl alcohol **120**. This intermediate was transformed into

trichloroiminoester **121** and then subjected to cyclization affording oxazolines (**122** and **123**) (Scheme 22). Both steps presented some unexpected difficulties such as migration of the protecting group during reaction with trichloroacetonitrile, and low diastereoselection of cyclization, which turned out to be remarkably base-selective [87].



Scheme 22 Reagents: *a* AD-mix-β; *b* TBDMSCl/Im/Py; *c* Cl_3CCN/DBU; *d* *t*-BuOK or DBU

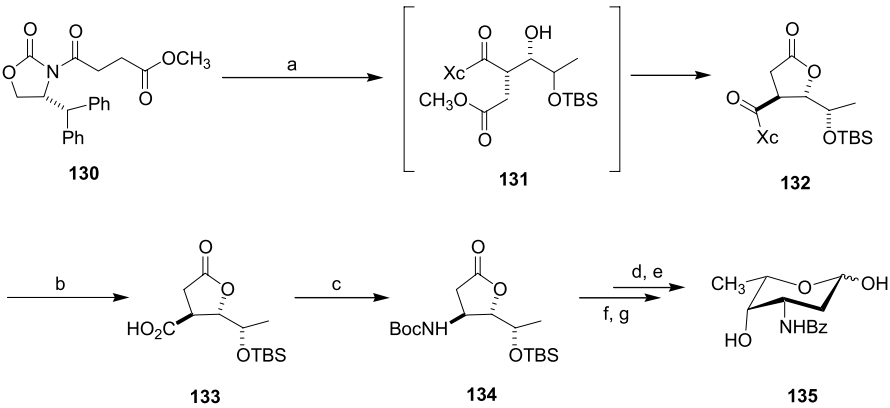
A new approach to construction of 3-aminosugar moieties by stereospecific intramolecular addition of a carbon-free radical to hydrazone **125** derived from crotonaldehyde was recently demonstrated by Friestad. This synthesis, comprising asymmetric dihydroxylation, PhS radical-induced C = N bond alkylation (*C*-vinylation) and subsequent Wacker oxidation [88] of terminal olefin **128**, which afforded L-daunosaminide derivative **129**, in overall 32% yield, is outlined in Scheme 23 [89].



Scheme 23 Reagents: *a* H_2NNBu_2; *b* AD-mix-α/$MeSO_2NH_2$; *c* $Me_2Si(C=CH_2)Cl/Et_3N$/DMAP; *d* AIBN/PhSH; *e* TBAF/THF; *f* DMP/PPTS; *g* BuLi/TFAA; *h* SmI_2; *i* $O_2/PdCl_2/CuCl$

4.4
Synthesis Utilizing a Chiral Auxiliary

Boron-mediated asymmetric aldol condensation methodology developed by Evans [90] served as an inspiration for preparation of daunosamine starting from chiral oxazolidinones. It appeared that the choice of chiral auxiliary is quite important for the stereochemical outcome of planned reactions [91]. A successful series of reactions started from *N*-succinoylation of (*R*)-3-(1-oxo-3-carbomethoxypropyl)-4-diphenylmethyl)oxazolidin-2-one as a novel chiral auxiliary. The chain extension was achieved in aldol condensation with protected lactaldehyde and the key intermediate **132** was converted into the target aminosugar **135**, via Curtius rearrangement of carboxylic acid azide, and reduction of lactone to lactol, as depicted in Scheme 24 [58]. Unexpectedly, boron catalysts were rather ineffective in the aldol condensation step and had to be replaced with more reactive lithium enolates (which proved to be non-Evans *syn* selective).



Scheme 24 Reagents: *a* LiHMDS/THF/TBS-lactaldehyde; *b* $LiOH/H_2O_2$; *c* $DPPA/Et_3N/t$-BuOH; *d* TBAF/THF; *e* DIBAL/THF; *f* MeOH/HCl; *g* BzCl/Py; Xc = chiral auxiliary group: (*R*)-4-(diphenylmethyl)oxazolidin-2-one

5
Syntheses of the Antibiotic Sugar Analogs

Since Brockman's discovery of toxic pigments produced by *Streptomyces* in the early 1960s [8], thousands of new daunomycin derivatives have been obtained, either by synthesis involving glycosidic bond reassembly or by directed biogenesis. Early discussions on structure–activity relationships are presented in a fundamental account elaborated by Arcamone [3, 92]. Over the years, as the methods for evaluation of drug candidates became more so-

phisticated, a wealth of new data has accumulated, giving rise to revival of thorough discussion concerning the role of the sugar moiety in the biological activity of anthracyclines [93–95]. The main conclusion of investigations carried out over many years by Italian scientists affiliated with Menarini pharmaceutical company can be summarized in the statement that anthracyclinones do not need to be connected to daunosamine in order to exert desirable biological activity. Compound MEN-10775, presently known as Sabarubicin (discussed in detail in another chapter by Arcamone in this volume) [96], provided experimental proof that combination of a synthetic aglycone with an artificial disaccharide unit can bring about advantageous pharmacological and therapeutical effects in oncological clinics. It is noteworthy that both monosaccharide moieties in sabarubicin belong to the α-L-2,6-dideoxypyranose category. Prior to publication of these results, some novel ideas concerning structure–activity relationships of anthracyclines have been advanced by Priebe, who stressed the role of C-3′ center basicity and demonstrated that exchange of daunosamine C-3 amino function for the hydroxyl group does not abolish anticancer activity [33, 97, 98]. Typical structural modifications of L-2,6-dideoxyhexoses, carried out with the intention of replacing daunosamine, include halogenation. Substitution at C-2 is usually achieved through halogen or pseudohalogen (e.g., NIS) addition to a selected glycal. It turned out that anthracyclines containing sugars with an axial halogen substituent are more active in cytotoxicity tests [99, 100] than their equatorial counterparts. Thus, 2-fluoro-2,6-dideoxy-L-*talo*-hexopiranose is considered a prospective replacement for daunosamine [101, 102]. Halogenation at C-4 position could be easily achieved via sulfonyl ester nucleophilic substitution with a chosen halogen anion, as exemplified for esorubicin (4′-iodo-4′deoxydaunoruicin) [103, 104]. 6,6,6-Trifluorosubstituted L-daunosamine and L-acosamine were obtained by chiral auxiliary strategy, via hetero Diels–Alder reaction, employing ethyl vinyl ether as dienophile [105]. Recently, daunorubicinone was stereoselectively glycosylated with a variety of 2,6-deoxy and 2,3,6-trideoxy-L-pyranosyl donors to produce a small library of new anthracycline antibiotics, some of which exhibited significant antitumor activity against colon cancer cell line SW620 [106]. This was followed by a set of eight synthetic "disaccharide daunorubicins" with a 1–4 link between daunosamine and the second deoxypyranose moiety [107].

Although 3-azido 2,3,6-trideoxyhexopiranose derivatives were used for glycosylation of anthracyclinones many years ago [108, 109] there was not much interest in the biological activity of the products. Their conversion to antibiotics seemed problematic from a synthetic point of view because of functional incompatibility of aglycones and sugar protecting groups with the reducing reagents typically applied for azide to amine conversion. At present, the Staudinger method is successfully used for azide to amine transformation in complex systems. Consequently the azidosugars, primarily considered only as early intermediates in 3-amino monosaccharide synthesis, recently ad-

vanced to the group of favorite glycosyl donors [64, 110–113]. Azido analogs of Dau and Dox were found to be biologically active but not substrates for Pgp, which allows them to attain higher intracellular concentrations than for corresponding 3′-amino analogs. Therefore, they became valuable probes for mechanistic studies and also anticancer drug leads in their own right.

5.1 Exemplary Chemical Transformations of Daunomycin-Type Antibiotics Resulting in Modifications of the Sugar Moiety

Among the transformations involving the sugar moiety of intact anthracycline antibiotics that are relatively easy to perform and high yielding, selective N-acylations, taking advantage of high nucleophilicity of daunosamine free amino group, have to be mentioned in the first place. Various substituted benzamides of anthracycline antibiotics were obtained, equipped with a marker and a linker function (e.g., radioactive iodine atom and aromatic azido group, the precursor of reactive nitrene intermediate), for a study of their macromolecular interactions, for example via photoaffinity labeling [114]. Doxorubicin carbamates were designed as ADEPT prodrugs, capable of tumor-targeted release of the parent antibiotic, following β-glucuronidase-induced 1,6-elimination of the aromatic carbamide fragment [115]. Along the same concept, *N*-sulfamide and *N*-phosphamide derivatives of Dau were obtained, counting on biodeprotection by sulfatases and phosphatases, respectively [116]. Anthracycline antibiotic prodrugs comprise a large group of semisynthetic derivatives, designed for targeted delivery and site-selective release, according to various practical and hypothetical principles of bio/chemoconjugation and biodegredation. A comprehensive review of the field, citing 330 references was published recently [117]. It is likely that anthracyclines are the most explored class of medicines, for which new ways of passive and active drug targeting was sought via high and low molecular weight prodrug design. Tumor targeting cytotoxic conjugates remain a dynamically evolving area [118]. Constructs ca. 70-fold more potent than Dox were obtained by dipeptide-based monoclonal antibody conjugates able to release potent 2-pyrrolidino-Dox at the target [119].

Particularly facile is trifluoroacetylation of daunomycin, which can be achieved by action of ethyl trifluoroacetate in the presence of triethylamine or other basic catalyst. The product of this reaction has considerably reduced cytotoxicity, but it can be more easily accumulated in a cell because it is no longer a substrate for the Pgp efflux pump. Its lipophilic esters (products of acylation at C-14 primary hydroxyl group) became experimental antitumor drugs [120, 121] and valrubicin (*N*-trifluoroacetyl-14-*O*-valeroyldoxorubicin) was registered for use in bladder cancer treatment. A new method for preparation of the drug, which consists of efficient two-step chemoenzymatic transformation of Dox was disclosed recently [122].

The possibility of practical synthesis of azides from amines, which emerged about a decade ago [123], recently attracted considerable attention [107, 112] and was successfully applied for the syntheses of mono- and disaccharide analogs of Dau bearing the azidopyranose moiety.

A variety of Dau and Dox derivatives were prepared that incorporated the C-3′ amino group into a heterocyclic ring. Daunomycin can be easily N-alkylated through reductive amination using a variety of carbonyl reagents. Application of dialdehydes in this reaction resulted in formation of pyrroline, piperidine, or morpholine derivatives of Dau, some of which were found to display extraordinary high potency in cytotoxicity tests. For example, the 2-cyanomorfolino derivative of Dox (first reported by Acton [124] as a side product of reductive alkylation in the presence of cyanoborohydride) was found to exhibit cytotoxicity a few orders of magnitude higher than the parent antibiotic. The compound, obtained from Dox in reaction with dialdehyde prepared by periodate oxidation of methyl β-L-arabinopyranoside, made it to advanced clinical studies under the name Nemorubicin [125]. The 3′-(2-pyrroline) analog of Dox, 500–1000 times more potent in cytotoxicity test than Dox [126], could be obtained in high yield without using protecting groups by action of 4-iodobutyraldehyde applied in large excess [127]. The idea of using acylal/aminal-type derivatives for construction of prodrugs and antibody conjugates that can release highly toxic anthracycline antibiotic upon biological activation (e.g., deprotection of *N*-acyl derivative with peptidase, which triggers intramolecular cyclization of acylal with formation of pyrroline) is apparently pursued in several projects [128, 129]. Several more conventional peptide conjugates at 3′-*N*-, which release Dox and *N*-L-leucyl Dox, are in various stages of preclinical evaluations [130, 131]. The newest generation of prodrugs from this family is designed in such a way that it comprises in one molecule a tumor-specific recognition site (e.g., monoclonal antibody) and a tumor-selective bioactivation site (e.g., glucuronidase-cleavable N-protecting carbamate group [132, 133].

A group of amidine derivatives of DAU was obtained in reaction with appropriate *N*-formyl cyclic amines. They exerted cytotoxic and antiproliferative activity by inhibition of topoisomerase II and not by covalent binding to DNA [134, 135].

Structural changes introduced at position C-4′ of Dau/Dox scored very high success rate. Epimerization at C-4′ of Dox, which leads to clinically useful therapeutic epirubicin [136, 137], can be realized on the intact antibiotic, starting from Dau, or using L-*arabino* aminosugar glycosyl donor (an acosamine derivative) for coupling [138, 139]. Since the efficiency of chemical processes for epirubicin synthesis are not quite satisfactory, alternative solutions are being sought. It was demonstrated that a genetically engineered strain of *Streptomyces peucetius* is capable of producing epirubicin directly by fermentation [140]. 4′-Iodo-4′-deoxy- and 4′-deoxy-Dox (esorubicin), once promising drug candidates, were not developed beyond Phase II of clinical

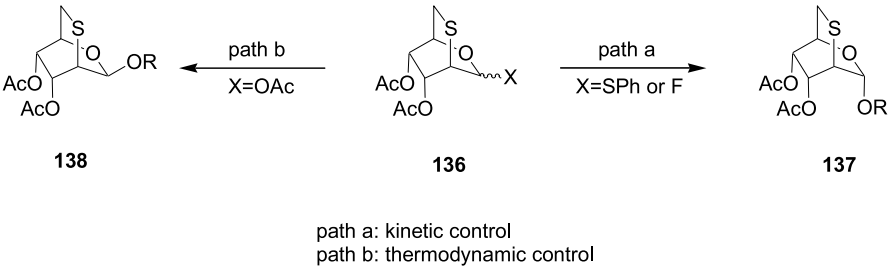
trials [5]. The tetrahydropyranyl derivative of Dox (pirarubicin), first obtained in Japan as an intended prodrug [141], was subsequently developed into a successful drug in its own right. A novel semisynthetic anthracycline-4′-*O*-benzyl Dox derivative (WP744) is considerably more lipophilic than Dox, crosses the blood–brain barrier, and shows good activity against neuroblastoma [142]. It was also found to overcome resistance mediated by Pgp and MDR proteins [143].

Another type of potentially useful transformation of anthracyclines, leading to enhanced selectivity and efficacy, is also based on N-alkylation and involves covalent linking of two molecules of antibiotic with a dialkylating agent. This line of research, aiming at rationally designed bis-intercalators, was seemingly inspired by discovery of anthracycline–formaldehyde–DNA adducts formed in biological material [144]. Daunomycin-based bis-intercalator WP631, prepared by application of *p*-xylene linker, has indeed shown large binding free energy ($-15.3\ \mathrm{kcal\,mol^{-1}}$) in interaction with DNA samples [29, 145]. Currently, the idea of poly-intercalating DNA agents is developing by design of a great structural variety of linkers, and by using new methods like click chemistry [146, 147].

5.2 Problems and Solutions Connected with Synthesis of 2-Deoxypyranose Conjugates

Although chemical glycosylation of complex substrates, including saccharides, remains a great challenge for practical synthetic chemists, considerable progress has been made in the design of new, more efficient and more selective methods for an anomeric bond formation, covering nearly all classes of glycosides and glycoconjugates [147, 148].

Synthetic methodology for assemblage of 2-deoxyglycosidic bonds (α and β) have been a subject of many studies, since a variety of biologically important natural products have this particular structural feature. However, securing stereocontrol in the glycosidation step is rather difficult in the absence of a factor directing the incoming nucleophile, such as a bulky or orbitally interacting C-2 substituent. Earlier achievements along the line have been summarized in some review articles [149–151]. The main approaches to 2-deoxypyranoses and their glycosides utilize glycals as the substrates, exploiting various electrophilic addition modes, which have been systematically covered in reviews devoted to these cyclic vinyl ethers of sugar origin [152–154]. There are many variants of electrophilic addition modes, widely exploited in 2-deoxy glycoside synthesis from glycals, e.g., (i) protic acid-catalyzed α-selective addition of an acceptor, usually catalyzed by TPPH [155]; (ii) NIS or phenylsuphanyl chloride-catalyzed addition, which usually leads to a mixture of 2-deoxy-2-substituted glycosides [155–158]. Interestingly, new glycal chemistry of general interest is constantly evolving. Recently a new method for the synthesis of 2-deoxy pyranoside deriva-



Scheme 25 Reagents: *a* NBS/MeCN for X = SPh, $SuCl_2/Et_2O$ for X = F; *b* Tf_2O or TMSOTf for X = OAc

tives based on catalytic rhenium-oxo complex addition, which accommodates a wide variety of nucleophiles, was developed [159].

It seems that thioglycosides (and their higher *S*-oxidation state analogs) are currently firmly established as leading alternatives to glycals in the role of glycosyl donors for 2-deoxyglycosidic bond assembly. Although α-2-deoxypiranosides are easier to obtain than their anomeric counterparts (because their formation is favored thermodynamically), achieving complete stereospecificity in combination with good chemical yield is very difficult. Among many activators used for promoting anomeric exchange of thioglycosyl substituents, $AgPF_6$ was recently singled out as a powerful and stereoselective reagent [107, 160], affording good yields in glycosylation of daunomycinone, as well as N-protected daunomycin. A novel strategy for the stereocontrolled glycosylation of 2,6-dideoxypyranosides was forwarded by Toshima [161]. 2,6-Anhydro-2,6-dideoxy-2,6-dithio hexopyranoses served as convenient glycosyl donors, whose activity could be tuned by choice of anomeric leaving group (e.g., thiophenyl or fluoride), bridge sulfur atom oxidation state, and glycosidation reaction conditions, resulting in remarkable α or β stereoselectivity.

2-Deoxy-1-phospates and phosphonates attract more and more attention as glycosyl donors, while chemical and enzymatic approaches to glycosylation tend to merge into amalgamated technologies [162–164]. Chemoenzymatic synthesis of deoxythymidine diphosphates of D- and L-olivose was performed on multimilligram scale and their deoxyhexose donor capabilities towards various glycosyltransferases were demonstrated, thus opening some new opportunities in combinatorial synthesis of deoxyhexose conjugates [165].

6 Conclusions

Biologically active natural products containing glycosidic structures (antibiotics, terpenoids, flavonoids, lignans, glycolipids, etc.) continue to attract

attention as lead compounds for drug discovery [15–18, 166–168]. Their structures are intuitively recognized as hybridic and objects resulting from recombination of their elements are often considered as preferred targets for initial SAR studies. Switching the natural sugars is one of the first approaches in the lead validation of a glycosidic drug candidate of natural origin. Other approaches, involving unnatural, or synthetically modified sugar structures are usually methodically more difficult, both on the theoretical and practical level. However, recent progress in the chemical synthesis of glycosides from multifunctional alcohols and phenols has considerably influenced not only natural products chemistry, but also de novo design of synthetic drugs. The sugar moiety is perceived as a desirable functional element favorably influencing pharmacokinetic and/or pharmacodynamic properties of the recognized pharmacophores [13, 17, 18]. It has been repeatedly demonstrated that in principle all kind of sugar derivatives needed for design of new lead compounds can be delivered by chemical synthesis in the form of viable glycosidic synthons, which can be further conjugated even with very complex aglycones, with the required stereoselectivity.

Prototype anthracycline antibiotics, isolated from natural sources, have undergone very extensive biochemical, pharmacological, and clinical studies, which revealed a multitude of molecular mechanisms for their biological action, both therapeutically useful and detrimental for oncological patients. Although there is not enough theoretical ground for fully rational drug design in this group of compounds, there is no doubt that the sugar moiety plays a very important role in determining biological activity. Fine tuning of activity by structural modification of a glycon is possible over a wide range of defined molecular interactions, such as DNA sequence recognition and complexation, and affinity to protein targets such as ATP-dependent efflux pumps, cytochromes or topoisomerases [169]. It can be concluded that α-L-2,6-dideoxypyranose (with 3-amino, 3-hydroxy, 3-C-branching and 3-deoxy functions considered as a viable option) [170] remains the favorite glucon structural motif among newly obtained anthracyclines. Some of these have made it into advanced preclinical studies and a limited number to clinical evaluation. Among the features that were brought up as particularly important for overall antitumor efficacy, a sugar C-3′ basicity and an antibiotic lipophilicity (which can be enhanced by modifying either aglycone or sugar) were subjects of some detailed discussions. While the "normal" basicity characteristic for unsubstituted daunosamine favors interactions with DNA, it is also responsible in large part for affinity to Pgp protein. This pumps protonated organic bases out of cytosol, preventing accumulation of an antibiotic to a concentration needed to exert its antitumor action. In terms of lipophilicity, apparently a very delicate balance between logP and other molecular indices is needed in order to achieve a positive new trait, such as an ability to penetrate the blood–brain barrier, required for treatment of certain CNS malignancies. Relative stability of the glycosidic bond is also recognized as a pos-

sible determinant of clinical efficacy. It has been pointed out that placement of properly configured (axial) halogen atom at C-2′ exerts multiple desirable effects, reducing basicity, enhancing lipophilicity, and increasing stability of the glycosidic bond towards chemical or enzymatic hydrolysis [13, 33, 98].

Syntheses of anthracycline antibiotic sugar moieties, natural and modified, has attained a high level of sophistication and efficiency from the point of view of academic research standards. However, the compounds that are the subjects of the synthetic activities outlined in this review have not made it to the catalogues of commercially available reagents, and the new methods have seldom shown signs of development into industrially useful processes. This undoubtedly hampers further development of new analogs and drug candidates from the anthracycline antibiotics category. It is conceivable that the present trend to use anthracyclines as prodrugs and targeted conjugates will continue and therefore the therapeutic construct formation is likely to utilize the complete antibiotic molecule, rather than the glycosylating synthon. Nevertheless, chances for a new, potent drug in a small molecular weight region should not be overlooked, as shown by examples of Dox-cyanomorpholine derivatives or, more recently, WP744 [35]. In the authors opinion, the last decade brought some significant advances in the synthesis of structurally variable deoxysugar synthons. As examples of new, powerful approaches not covered in earlier reviews on anthracycline antibiotic aminosugars, we would like to mention: Lewis-acid-catalyzed-diene-aldehyde-condensation, particularly in its enantioselective version [171], and tungsten carbonyl-catalyzed alkynyl alcohol cycloisomerization reactions [172], both well suited for preparation of glycal-type synthons. While, at present, discovery of new anthracycline antitumor antibiotics is pursued in parallel by biosynthetic and chemical methods [173, 174], future success is likely to emerge from combination of the two. Knowledge of the biosynthetic pathways of secondary metabolites has matured to the point where genetic intervention can be applied to generate mutants where the synthesis of a particular metabolite precursor is either enhanced or prevented, to obtain hitherto unreported structures.

References

1. Erhardt PW (2002) Pure Appl Chem 74:703
2. Goodnow RA Jr, Gillespie P (2007) Progr Med Chem 45:1
3. Arcamone F (1981) Doxorubicin anticancer antibiotics. Academic, New York
4. Strauss DG (1987) Pharmazie 42:289
5. Monneret C (2001) Eur J Med Chem 36:483
6. Weiss RB (1992) Semin Oncol 19:670
7. Berdy J (2005) J Antibiot 58:1
8. Brockmann H (1963) Fortsch Chem Org Naturst 21:121
9. Chaires JB (1998) Curr Opin Struct Biol 8:314
10. Gewirtz DA (1999) Biochem Pharmacol 57:727

11. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L (2004) Pharmacol Rev 56:185
12. Nadas J, Sun D (2006) Exp Opin Drug Disc 1:549
13. Priebe W (ed) (1995) Anthracycline antibiotics: new analogs, methods of delivery and mechanisms of action. American Chemical Society, Washington DC
14. Taylor ME, Drickamer K (2003) Introduction to glycobiology. Oxford University Press, Oxford, UK
15. Witczak ZJ, Nieforth KA (eds) (1997) Carbohydrates in drug design. Dekker, New York
16. Ernst B, Hart GW, Sinay P (eds) (2000) Carbohydrates in chemistry and biology. Wiley, Weinheim
17. Wong CH (ed) (2003) Carbohydrate-based drug discovery. Wiley, Weinheim
18. Schreiber SL, Kapoor TM, Wess G (eds) (2007) Chemical biology; from small molecules to systems biology and drug design. Wiley, Weinheim
19. Pelyvas IF, Monneret C, Herczegh P (1988) Synthetic aspects of aminodeoxy sugars of antibiotics. Springer, Berlin
20. Otsomaa LA, Koskinen AMP (1998) Synthesis of 6-deoxyamino sugars. In: Herz W, Kirby GW, Moore RE, Steglich W, Tamm C (eds) Progress in the chemistry of organic natural products, vol 74. Springer, Wien, p 197
21. Booser DJ, Hortobagyi GN (1994) Drugs 47:223
22. Rabbani A, Finn RM, Ausio J (2005) Bioessays 27:50
23. Strohl WR, Dickens ML, Rajgharia VB, Woo AJ, Priestley ND (1997) In: Strohl WR (ed) Biotechnology of antibiotics. Dekker, New York, p 577
24. Thomas R (2001) Chem Bio Chem 2:612
25. Trefzer A, Salas JA, Bechthold A (1999) Nat Prod Rep 16:283
26. Fischbach MA, Walsh CT (2006) Chem Rev 106:3468
27. Salas JA, Mendez C (2005) J Mol Microbiol Biotechnol 9:77
28. Portugal J, Cashman DJ, Trent JO, Ferrer-Miralles N, Przewloka T, Fokt I, Priebe W, Chaires JB (2005) J Med Chem 48:8209
29. Chaires JB, Leng F, Przewloka T, Fokt I, Ling IH, Perez-Soler R, Priebe W (1997) J Med Chem 40:261
30. Chaires JB, Satyanarayana S, Suh D, Fokt I, Przewloka T, Priebe W (1996) Biochemistry 35:2047
31. Leng F, Savkur R, Fokt I, Przewloka T, Priebe W, Chaires JB (1996) J Am Chem Soc 118:4731
32. Zeman SM, Phillips DR, Crothers DM (1998) Proc Natl Acad Sci USA 95:11561
33. Priebe W, Perez-Soler R (1993) Pharmacol Ther 60:215
34. Priebe WA, Perez-Soler R (1997) Carbohydr Drug Des 1997:551
35. Trevino AV, Woynarowska BA, Herman TS, Priebe W, Woynarowski JM (2004) Mol Cancer Ther 3:1403
36. Zou Y, Ling YH, Reddy S, Priebe W, Perez-Soler R (1995) Int J Cancer 61:666
37. Drummond DC, Meyer O, Hong K, Kirpotin BD, Padahadjopulos D (1999) Pharmacol Rev 51:691
38. Rudnicki WR, Kurzepa M, Szczepanik T, Priebe W, Lesyng B (2000) Acta Biochim Pol 47:1
39. Cashman DJ, Kellogg GE (2004) J Med Chem 47:1360
40. Trefzer A, Salas JA, Bechthold A (1999) Nat Prod Rep 16:283
41. Achmatowicz O, Bukowski P, Szechner B, Zwierzchowska Z, Zamojski A (1971) Tetrahedron 27:1973
42. Parker KA, Meschwitz SM (1988) Carbohydr Res 172:319
43. Toth A, Remenyik J, Bajza I, Liptak A (2003) Arkivoc 2003:28

44. Hanessian S (ed) (1997) Preparative carbohydrate chemistry. Dekker, New York
45. Osborne HMI (ed) (2003) Carbohydrates; best synthetic methods. Academic, New York
46. Levy DE, Fugedi P (eds) (2006) The organic chemistry of sugars. CRC, Boca Raton, FL
47. Hadfield AF, Cunningham L, Sartorelli AC (1979) Carbohydr Res 72:93
48. Priebe W, Grynkiewicz G, Neamati N (1991) Tetrahedron Lett 19:2079
49. Marsh JP Jr, Mosher CW, Acton EM, Goodman L (1967) J Chem Soc Chem Commun 1967:973
50. Durgant JM, Warm A, Vogel P (1992) Synth Commun 22:1883
51. Shafer CM, Molinski TF (1998) Carbohydr Res 310:223
52. Grethe G, Mitt T, Williams TH, Uskokovic MR (1983) J Org Chem 48:5309
53. Coleman RS, Fraser JR (1993) J Org Chem 58:385
54. McDonald FE, Subba Reddy K, Diaz Y (2000) J Am Chem Soc 122:4304
55. McDonald FE, Subba Reddy K (2001) J Organomet Chem 617–618:446
56. Jacobsen EN, Pfaltz A, Yamamoto H (eds) (1999) Comprehensive asymmetric catalysis. Springer, Berlin
57. Hauser FM, Ellenberger SR (1986) Chem Rev 86:35
58. Sibi MP, Lu J, Edwards J (1997) J Org Chem 62:5864
59. Horton D, Weckerle W (1975) Carbohydr Res 44:227
60. Florent JC, Monneret C (1987) J Chem Soc Chem Commun, p 1171
61. Abacci B, Florent JC, Monneret C (1989) Bull Soc Chim France 667
62. Kolar C, Kneissl G (1990) Angew Chem Int Ed Engl 102:827
63. Renneberg B, Li YM, Laatsch H, Fiebig HH (2000) Carbohydr Res 329:861
64. Grynkiewicz G, Fokt I, Skibicki P, Przewloka T, Szeja W, Priebe W (2005) Polish J Chem 79:335
65. Fokt I, Grynkiewicz G, Przewloka T, Priebe W (2005) Polish J Chem 79:348
66. Hall RH, Jordan A (1973) J Chem Soc Perkin I 1973:38
67. Hall RH, Jordan A (1975) J Chem Soc Perkin I 1975:626
68. Pucko W, Les A, Ramza J, Zagrodzka J, Cieplucha G, Cichy B, Szelejewski W (2006) Ind Eng Chem Res 45:2962
69. Zhang G, Shi L, Liu Q, Liu X, Li L, Wang J (2007) Tetrahedron Lett 48:3413
70. Jurczak M, Socha D, Chmielewski M (1996) Tetrahedron 52:1411
71. Socha D, Jurczak M, Chmielewski M (1997) Tetrahedron 53:739
72. Mendlik MT, Tao P, Hadad CM, Coleman RS, Lowary TL (2006) J Org Chem 71:8
73. Hauser FM, Ellenberger SR, Ellenberger WP (1988) Tetrahedron Lett 29:4939
74. Kaminska JE, Smigielski K, Lobodzinska D, Gora J (2000) Tetrahedron: Asymmetry 11:1211
75. Zhou M, O'Doherty GA (2007) J Org Chem 72:2485
76. Szechner B, Achmatowicz O, Badowska-Roslonek K (1999) Polish J Chem 73:1133
77. Effenberger F, Roos J (2000) Tetrahedron: Asymmetry 11:1085
78. Parker KA, Chang W (2005) Org Lett 7:1785
79. Zamojski A, Banaszek A, Grynkiewicz G (1982) Adv Carbohydr Chem Biochem 40:1
80. Hudlicky T, Entwistle DA, Pitzer KK, Thorpe AJ (1996) Chem Rev 96:1195
81. Vogel P (2000) Curr Org Chem 4:455
82. Ginesta X, Pasto M, Pericas MA, Riera A (2003) Org Lett 5:3001
83. Wade PA, D'Ambrosio SG, Rao JA, Shah-Patel S, Cole DT, Murray JK Jr, Carrol PJ (1997) J Org Chem 62:3671
84. Nagumo S, Umezawa I, Akiyama J, Akita H (1995) Chem Pharm Bull 43:171
85. Fronza G, Fuganti C, Graselli P (1980) J Chem Soc Chem Commun 1980:442

86. Davies SG, Smyth GD, Chipindale AM (1999) J Chem Soc Perkin I 1999:3089
87. Matsushima Y, Kino J (2006) Tetrahedron Lett 47:8777
88. Takacs JM, Jiang XT (2003) Curr Org Chem 7:369
89. Friestad GK, Jing T, Mathies AK (2007) Org Lett 9:777
90. Evans DA, Nelson JV, Taber TR (1981) J Am Chem Soc 103:3099
91. Sibi MP (1999) Aldichim Acta 32:93
92. Arcamone F (1998) Biochemie 80:201
93. Arcamone F, Animati F, Bigioni M, Capranico G, Caserini C, Cipollone A, De Cesare M, Ettore A, Guano F, Manzini S, Monteagudo E, Pratesi G, Salvatore C, Supino R, Zunino F (1999) Biochem Pharmacol 57:1133
94. Zunino F, Pratesi G, Perego P (2001) Biochem Pharmacol 61:933
95. Cipollone A, Berettoni M, Bigioni M, Binaschi M, Cermele C, Monteagudo E, Olivieri L, Palomba D, Animati F, Goso C, Maggi CA (2002) Bioorg Med Chem 10:1459
96. Arcamone F (2008) In: Krohn K (ed) Anthracyclines: chemistry and biology. Springer, Berlin (in press)
97. Priebe W, Van NT, Burke TG, Perez-Soler R (1993) Anticancer Drugs 4:37
98. Priebe W (1995) Curr Pharm Des 1:51
99. Horton D, Priebe W, Varela O (1985) Carbohydr Res 144:305
100. Gao Y, Priebe W, Wang HJ (1996) Eur J Biochem 240:331
101. Deal ST, Horton D (1999) Carbohydr Res 315:187
102. Horton D, Khare A (2006) Carbohydr Res 341:2631
103. Arcamone F (1985) Cancer Res 45:5995
104. St-Denis Y, Lavallee JF, Nguyen D, Attardo G (1994) Synlett 1994:272
105. Hayman CM, Larsen DS, Simpson J, Bailey KB, Gill GS (2006) Org Biomol Chem 4:2794
106. Zhu L, Cao X, Chen W, Zhang G, Sun D, Wang PG (2005) Bioorg Med Chem 13:6381
107. Zhang G, Fang L, Zhu L, Aimiuwu JE, Shen J, Cheng H, Muller MT, Lee GE, Sun D, Wang PG (2005) J Med Chem 48:5269
108. Sztaricskai F, Menyhart M, Bognar R (1982) Carbohydr Res 100:C14
109. Menyhart M, Kover K, Sztaricskay F (1990) J Carbohydr Chem 9:253
110. Oberthur M, Leimkuhler C, Kahne D (2004) Org Lett 6:2873
111. Fang L, Zhang G, Zheng X, Zhu L, Xiao JJ, Szakacs G, Nadas J, Chan KK, Wang PG, Sun D (2006) J Med Chem 49:932
112. Zhang G, Fang L, Zhu L, Zhong Y, Wang PG, Sun D (2006) J Med Chem 49:1792
113. Zhang G, Shi L, Liu Q, Liu X, Li L, Wang J (2007) Tetrahedron Lett 48:3413
114. Averbuch SD, Glover CJ, Felsted RL (1986) Cancer Res 46:6120
115. Haisma HJ, van Muijen M, Pinedo HM, Boven E (1994) Cell Biophys 24/25:185
116. Leenders RGG, Scheeren JW, Houba PHJ, Boven E, Haisma HJ (1995) Bioorg Med Chem Lett 24:2975
117. Kratz F, Warnecke A, Schmid B, Chung DE, Gitzel M (2006) Curr Med Chem 13:477
118. Chen J, Jaracz S, Zhao X, Chen S, Ojima I (2005) Bioorg Med Chem 13:5043
119. Jeffrey SC, Nguyen MT, Andreyka JB, Meyer DL, Doronina SO, Senter PD (2006) Bioorg Med Chem Lett 16:358
120. Israel M, Modest E, Frei E (1975) Cancer Res 35:1365
121. Israel M, Potti PG, Seshadri R (1985) J Med Chem 28:1223
122. Cotterill IC, Rich JO (2005) Org Process Res Dev 9:818
123. Alper PB, Hung SC, Wong CH (1996) Tetrahedron Lett 37:6029
124. Acton EM, Tong GL, Mosher CW, Wolgemuth RL (1984) J Med Chem 27:638
125. Anonymus (1997) Drugs Fut 22:1319

126. Castex C, Merida P, Blanc E, Clair P, Rees A, Temsamani J (2004) Anticancer Drugs 15:609
127. Nagy A, Armatis P, Schally AV (1996) Proc Natl Acad Sci USA 93:2464
128. Nagy A, Schally AV (2005) Biol Reprod 73:851
129. Jeffrey SC, Nguyen MT, Andreyka JB, Meyer DL, Doronina SO, Senter PD (2006) Bioorg Med Chem Lett 16:358
130. Denmeade SR, Nagy A, Gao J, Lilja H, Schally AV, Isaacs JT (1998) Cancer Res 58:2537
131. Nagy A, Plonowski A, Schally AV (2000) Proc Natl Acad Sci USA 97:829
132. Haisma HJ, Brakenfoff RH, van den Muelen-Muileman I, Pinedo HM, Boven E (1998) Cancer Immunol Immunother 45:266
133. Platel D, Bonoron-Adele S, Dix RK, Robert J (1999) Br J Cancer 81:24
134. Pawlowska J, Tarasiuk J, Borowski E, Wasowska M, Oszczapowicz I, Wolf CR (2000) Acta Biochim Pol 47:141
135. Ciesielska E, Studzian K, Wasowska M, Oszczapowicz I, Szmigiero L (2005) Cell Biol Toxicol 21:139
136. Cersosimo RJ, Hong WK (1986) J Clin Oncol 4:425
137. Coukell AJ, Faulds D (1997) Drugs 53:453
138. Arcamone F, Penco S, Vigevani A, Redaelli S, Franchi G, Di Marco A, Casazza AM, Dasida T, Formelli F, Necco A, Soranzo C (1975) J Med Chem 18:703
139. Kimura Y, Suzuki M, Matsumoto M, Abe R, Terashima S (1986) Bull Chem Soc Jpn 59:423
140. Madduri K, Kennedy J, Rivola G, Inventi-Solari A, Filippini S, Zanuso G, Colombo AL, Gewain KM, Occi JL, MacNeil DJ, Hutchison CR (1998) Nat Biotechnol 16:69
141. Umezawa H, Takahashi Y, Kinoshita M, Naganawa H, Matsuda T, Ishizuka M, Tatsuta K, Takeuchi T (1979) J Antibiot 32:1082
142. Inge TH, Harris NL, Wu J Azizhan RG, Priebe W (2004) J Surg Res 121:187
143. Brooks T, O'Loughlin K, Minderman H, Bundy B, Ford L, Vredenburg M, Bernacki R, Priebe W, Baer M (2007) Invest New Drugs 25:115
144. Taatjes DJ, Gaudiano G, Koch TH (1997) Chem Res Toxicol 10:953
145. Fox KR, Webster R, Phelps RJ, Fokt I, Priebe W (2004) Eur J Biochem 271:3556
146. Priebe W, Fokt I, Przewloka T, Chaires JB, Portugal J, Trent JO (2001) Methods Enzymol 340:529
147. Zhang G, Fang L, Zhu L, Sun D, Wang PG (2006) Bioorg Med Chem 14:426
148. Schreiber SL, Kapoor TM, Wess G (eds) (2007) Chemical biology; from small molecules to systems biology and drug design. Wiley-VCH, Weinheim
149. Thiem J, Klaffke W (1990) Top Curr Chem 154:285
150. Marzabadi CH, Franck RW (2000) Tetrahedron 56:8385
151. Veyrieres A (2000) Special problems in glycosylation reactions: 2-deoxy sugars. In: Beat E, Hart GW, Sinay P (eds) Carbohydrates in chemistry and biology, vol 1. Wiley-VCH, Weinheim, p 367
152. Priebe W, Grynkiewicz G (2001) Formation and reactions of glycal derivatives. In: Fraser-Reid B, Tatsuta K, Thiem J (eds) Glycoscience chemistry and chemical biology, vol 1. Springer, Berlin, p 749
153. Ferrier RJ, Hoberg JO (2003) Adv Carbohydr Chem Biochem 58:55
154. Ferrier RJ, Zubkov OA (2003) Org Reac 62:569
155. Bollit V, Mioskowski C, Lee SG, Falck JR (1990) J Org Chem 55:5812
156. Thiem J, Gerken M (1985) J Org Chem 50:954
157. Roush WR, Hartz RA, Gustin DJ (1999) J Am Chem Soc 121:1990

158. Johnston BD, Pinto BM (2000) J Org Chem 65:4607
159. Sherry BD, Loy RN, Toste D (2004) J Am Chem Soc 126:4510
160. Lear MJ, Yoshimura F, Hirama MA (2001) Angew Chem Int Ed 40:946
161. Toshima K (2006) Carbohydr Res 341:1282
162. Niggemann J, Lindhorst TK, Walfort M, Laupichler L, Sajus H, Thiem J (1993) Carbohydr Res 246:173
163. Oberthur M, Leimkuhler C, Kahne D (2004) Org Lett 6:2873
164. Fu X, Albermann C, Jiang J, Liao J, Zhang C, Thorson JS (2003) Nat Biotechnol 21:1467
165. Amann S, Drager G, Rupprath C, Kirschning A, Elling L (2001) Carbohydr Res 335:23
166. Weymouth-Wilson AC (1997) Nat Prod Rep 14:99
167. Kren V, Martinkova L (2001) Curr Med Chem 8:1313
168. Barkley A, Arya P (2001) Chem Eur J 7:555
169. Bailly C, Qu X, Graves DE, Prudhomme M, Chaires JB (1999) Chem Biol 6:277
170. Grisebach H, Schmid R (2003) Angew Chem Int Ed 11:159
171. Danishefsky S, Bilodeau T (1996) Angew Chem Int Ed 35:1380
172. McDonald FE, Zhu HYH (1998) J Am Chem Soc 120:4246
173. Hoffmeister D, Drager G, Ichinose K, Rohr J, Bethold A (2003) J Am Chem Soc 125:4678
174. Remsing LL, Gonzales AM, Nur-e-Alam M, Fernandez-Lozano MJ, Brana AF, Rix U, Oliveira MA, Mendez C, Salas JA, Rohr J (2003) J Am Chem Soc 125:5745

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Nonnatural Glycosyl Anthraquinones as DNA Binding and Photocleaving Agents

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Abstract Several artificial anthraquinone–carbohydrate hybrids were designed and synthesized. A selected number of hybrids did bind to DNA via intercalation in a sequence-selective manner, leading to inhibition of DNA cleavage by DNase I, whereas other hybrids cleaved DNA with base selectivity upon photoirradiation. This hybrid system was found to be very important for both DNA binding and photocleaving.

Keywords Anthraquinone · Carbohydrate · DNA · Intercalation · Photocleavage

Abbreviations

Bz	Benzoyl
CAN	Ceric ammonium nitrate
DNA	Deoxyribonucleic acid
MS	Molecular sieve
rt	Room temperature
Tf	Trifluoromethanesulfonyl
THF	Tetrahydrofuran
TMS	Trimethylsilyl

1 Introduction

Novel DNA binding or cleaving molecules, particularly those with high efficiency and sequence specificity, are very interesting from the chemical and

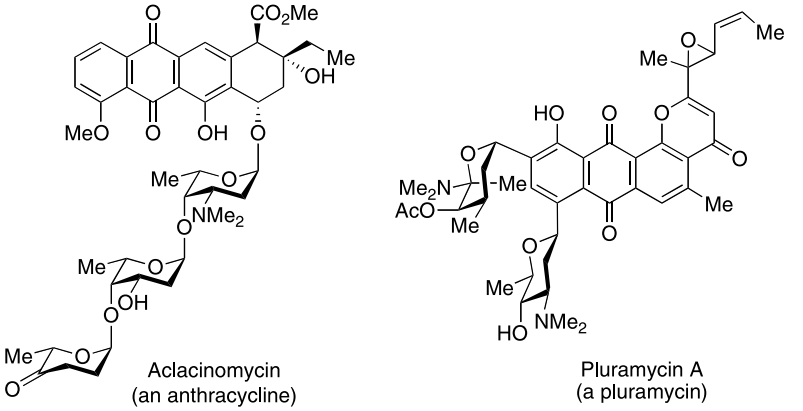


Fig. 1 Representative naturally occurring DNA binding antitumor antibiotics constructed from aromatic and carbohydrate domains

biological standpoint and offer considerable potential in medicine [1, 2]. The design and synthesis of artificial molecules that exhibit such interactions with DNA is therefore an important goal in contemporary chemistry. Certain clinically important antitumor antibiotics, such as anthracyclines [3, 4] and pluramycins [5], which strongly bind to DNA in a sequence-selective manner, have been found in nature (Fig. 1) [6]. Although these various agents have been regarded as belonging to distinct classes of antibiotics from a chemical standpoint, they are commonly found to contain aromatic and carbohydrate domains. In their DNA binding modes, the aromatic moieties function as DNA intercalators, whereas the carbohydrate residues bind to the DNA minor groove. Even enediyne antibiotics, which effectively cleave DNA via cycloaromatization reactions, can be viewed as aromatic–carbohydrate hybrids [7, 8]. These facts concerning DNA binding compounds of natural origin led us to consider the possibility of creating artificial DNA binding or cleaving molecules that consist of aromatic and carbohydrate domains [9–17]. In this study, anthraquinone, which is the key structure in anthracycline antibiotics, was selected as the DNA intercalating and photocleaving aromatic [9–11]. This chapter describes the molecular design, chemical synthesis, DNA binding, and DNA photocleaving properties, as well as the cytotoxic profiles, of several novel and artificial anthraquinone–carbohydrate hybrids.

2
Design, Synthesis, and Evaluation of Nonnatural Glycosyl Anthraquinones as DNA Binders

In our approach to DNA binding hybrids, the anthraquinone molecule was selected as the aromatic building block because it is the aromatic skeleton

present in many DNA binding antitumor antibiotics such as the anthracyclines. Certain 2,6-dideoxy sugars seemed appropriate as the carbohydrate component since these sugars are also found in many naturally occurring DNA binding antitumor antibiotics, and the hydrophobicity of 2,6-dideoxy sugars appeared advantageous for binding to the DNA minor groove [18–20]. Based on these considerations, we designed several artificial glycosyl anthraquinones constructed from anthraquinone and a 2,6-dideoxy sugar (Fig. 2) [9].

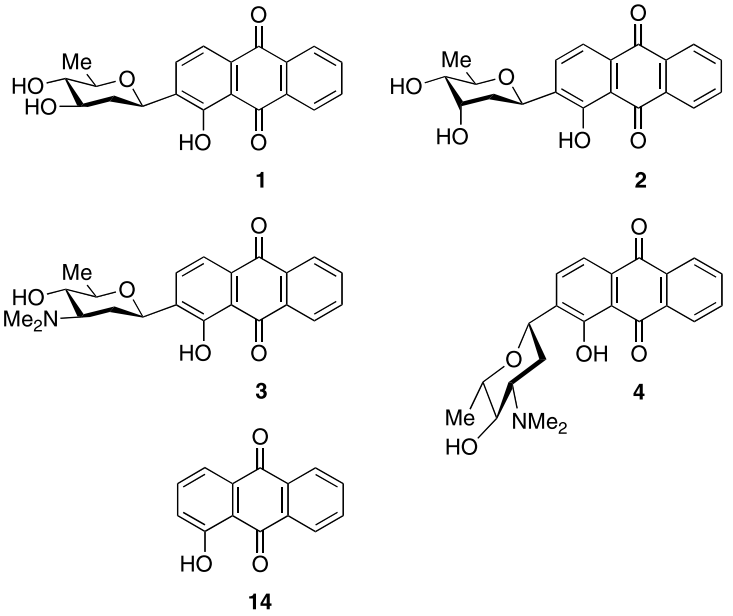
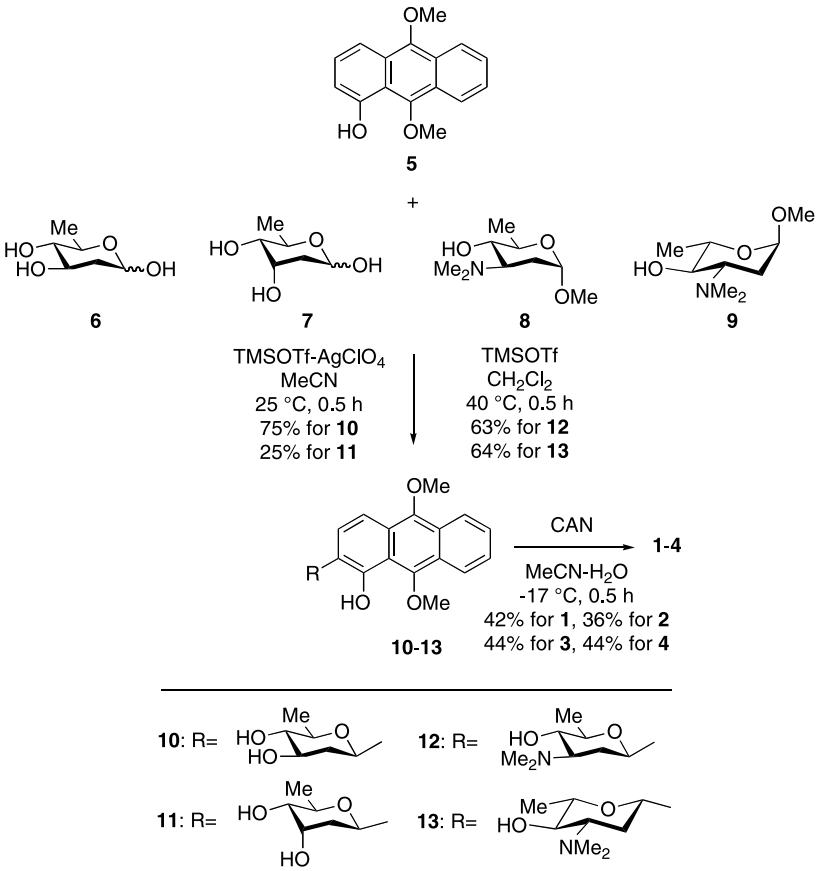


Fig. 2 Artificial anthraquinone-carbohydrate hybrids **1–4** and 1-hydroxyanthraquinone (**14**)

The designed anthraquinone–carbohydrate hybrids **1–4** were prepared by a short synthetic route via a strategy recently developed in our laboratory, the aryl C-glycosylation reaction using an unprotected sugar (Scheme 1) [21]. C-glycosylation of 9,10-dimethoxy-1-hydroxyanthracene (**5**) and the unprotected 2,6-dideoxy sugars, D-olivose (**6**) and D-digitoxose (**7**), in the presence of a catalytic amount of TMSOTf–$AgClO_4$ in MeCN, proceeded at room temperature (rt) to afford the unprotected aryl β-*C*-glycosides **10** and **11**, respectively, in a regio- and stereoselective fashion. In the case of the amino sugar **8** and its enantiomer **9**, C-glycosylation with **5** using TMSOTf in CH_2Cl_2 at 40 °C provided the regio- and stereoselective unprotected aryl β-*C*-glycoside **12** and its enantiomer **13**, respectively. Subsequent oxidation of aryl β-*C*-glycosides **10–13** using ceric ammonium nitrate (CAN) in MeCN–H_2O at –17 °C furnished the desired anthraquinone–carbohydrate hybrids **1–4**.

The DNA binding profiles of hybrids **1-4** were first assayed by the DNase I footprinting method using double-stranded (ds) M13mp18 DNA singly labeled



Scheme 1 Synthesis of **1**–**4**

at the 5′ end with ^{32}P [22]. In the case of hybrids **1** and **2**, both of which have a neutral sugar, no significant change in the cleavage pattern was observed compared to that of the chemical compound-free (control) lane. In contrast, hybrids **3** and **4**, which possess an amino sugar, produced relative cleavage patterns substantially different from the control, and cleavage in the 5′-TGC regions by DNase I was significantly reduced, as shown in Fig. 3. These results convincingly demonstrate that hybrids **3** and **4** bind to DNA in a sequence-selective fashion. Interestingly, it was also found that hybrid **3**, which possesses a D-amino sugar, binds DNA more strongly than hybrid **4,** which has the corresponding L-amino sugar. Furthermore, the DNase I footprinting assay showed that the components of hybrid **3** (1-hydroxyanthraquinone **14** and the amino sugar **8**) do not bind DNA. These results clearly indicate that the hybrid structure constructed from the anthraquinone and the amino sugar is required for DNA binding.

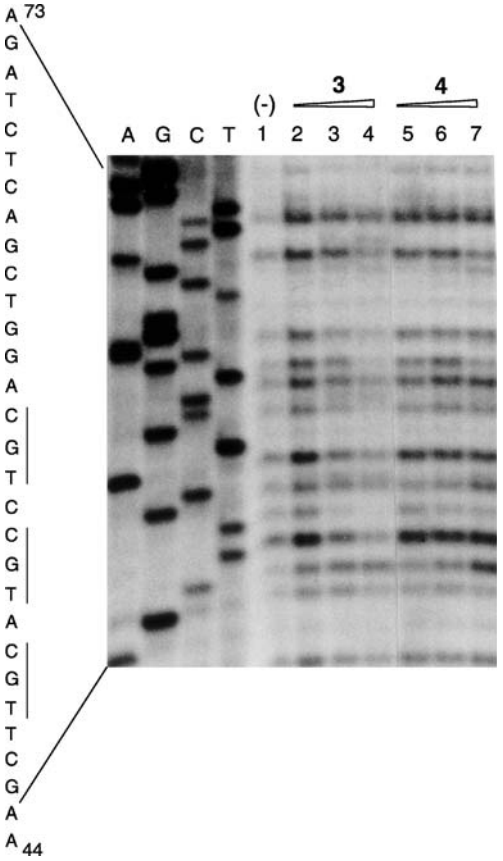


Fig. 3 DNase I footprinting of hybrids **3** and **4** bound to the 5′ end of labeled M13mp18 ds DNA. DNase I digestion was carried out for 1 min at rt. The samples were assayed by electrophoresis using 15% polyacrylamide/8M urea slab gels (bases 46–73 are shown). *Lanes A*, *G*, *C*, and *T*: Sanger A, G, C, and T reactions, respectively; *lane 1*: control in the absence of added chemical compound; *lanes 2–7*: **3** (20), **3** (200), **3** (2000), **4** (20), **4** (200), and **4** (2000 μM), respectively

The binding of hybrids **3** and **4** to DNA via intercalation was confirmed by the DNA unwinding assay using linearized pBR322 DNA and T4 DNA ligase [23, 24]. In this assay, the DNA intercalator first unwinds linearized DNA, which changes the twist of the duplex helix. Circularization of the chemical compound–DNA complex by T4 ligase freezes the linking number of the unwound DNA. Upon removal of the chemical compound, the twist reverts to normal while the linking number remains constant, resulting in negative superhelicity of the DNA. The agarose gel depicted in Fig. 4 shows the products of the DNA unwinding assay obtained with hybrids **3** and **4**. Control DNA molecules appear as a lightly positively supercoiled population of topoisomers (lane 2).

Upon ligation in the presence of an increasing concentration of intercalator, the product molecules become increasingly negatively supercoiled compared to the control. The topoisomers initially appear more relaxed and then progressively become more negatively supercoiled. Under this assay condition, hybrids **3** and **4** converted the topoisomers to more relaxed forms at low concentration, and to a completely negative supercoiled form at high concentration. Furthermore, it was found that DNA intercalation by **3** was about fivefold stronger than intercalation by **4**, demonstrating that DNA intercalation is clearly dependent on the configuration of the sugar moiety of the hybrids.

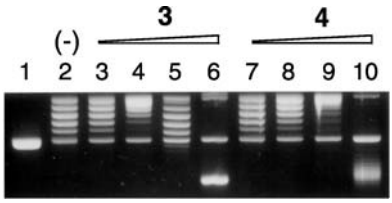


Fig. 4 Effects of hybrids **3** and **4** on the DNA unwinding assay with T4 ligase. Unwinding measurements were conducted as described in [23, 24]. In this assay, linearized plasmid DNA was incubated with T4 DNA ligase in the presence of the hybrids at various concentrations. Hybrid-induced DNA unwinding was detected on an agarose gel lacking ethidium bromide. *Lane 1*: linear DNA; *lane 2*: control in the absence of added chemical compound; *lanes 3–10*: **3** (0.4), **3** (2.0), **3** (10), **3** (50), **4** (0.4), **4** (2.0), **4** (10), and **4** (50 μM), respectively

The cytotoxicity of hybrids **3** and **4** was examined by exposing HeLa S3 cells to each hybrid for 72 h [25]. The IC_{50} values of **3** and **4** were 9 and 58 μM, respectively. The sixfold higher toxicity of hybrid **3** compared to hybrid **4** must be connected to the observation that **3** intercalates DNA about fivefold more strongly than does hybrid **4**. These results indicate that the cytotoxic activity of these anthraquinone–carbohydrate hybrids correlates with their ability to bind DNA.

3
Design, Synthesis, and Evaluation of Nonnatural Glycosyl Anthraquinones as DNA Photocleavers

3.1
First Generation

The development of photochemical DNA cleaving agents, which selectively cleave DNA upon irradiation with a specific wavelength under mild conditions and without any additives such as metals or reducing agents, has attracted much attention in chemistry, biology, and medicine [26]. Furthermore, photodynamic therapy using photosensitizing drugs has re-

cently emerged as a promising modality against cancer and associated diseases [27–29]. Our approach to creating novel photochemical DNA cleaving molecules focuses on the anthraquinone molecule. Previously, Schuster et al. demonstrated that suitably substituted anthraquinones have DNA intercalating and DNA photocleaving properties [30, 31]. Based on these findings, we designed the novel artificial anthraquinone–carbohydrate hybrids **15** and **16**, consisting of anthraquinone and a 2,6-dideoxy amino sugar (Fig. 5) [10].

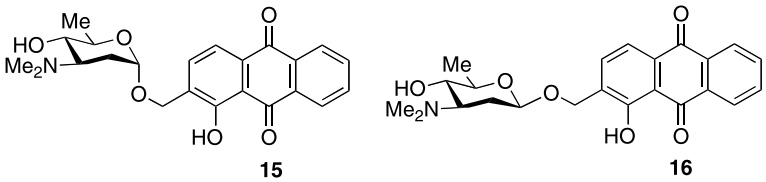
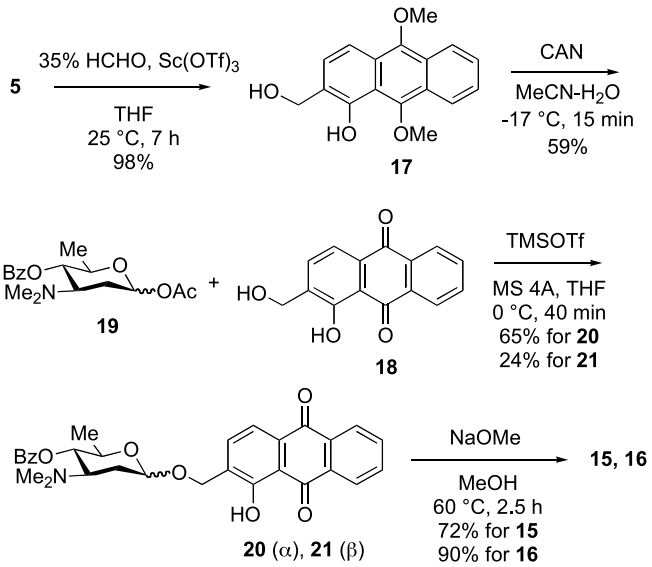


Fig. 5 Artificial anthraquinone–carbohydrate hybrids **15** and **16**

Hybrids **15** and **16** were synthesized by a concise synthetic strategy via the effective glycosylation of the 1-OAc sugar **19** with the anthraquinone derivative **18**. Compound **18** was easily obtained from **5** via regioselective hydroxymethylation by Kobayashi's method using 35% HCHO(aq.) and $Sc(OTf)_3$ [32], and subsequent oxidation of the resultant diol **17** using CAN (Scheme 2). Glycosylation of the 1-OAc sugar **19** with **18** using TMSOTf in the presence of MS 4A in THF gave both α-glycoside **20** and β-glycoside **21** in 89% yield in a ratio of 2.7 : 1. After their separation by column chromato-



Scheme 2 Synthesis of **15** and **16**

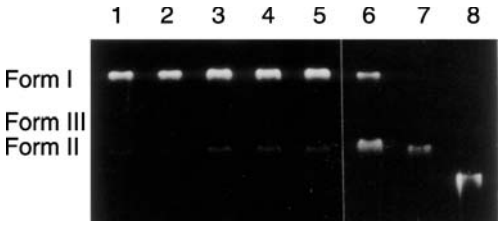


Fig. 6 Photocleavage of supercoiled ΦX174 DNA. ΦX174 DNA (50 μM per base pair) was incubated with various compounds in 20% acetonitrile in Tris–HCl buffer (pH 7.5, 50 mM) at 25 °C for 2 h under a UV lamp (365 nm, 15 W) placed 10 cm from the sample, then analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain). *Lane 1*: DNA alone; *lane 2*: DNA following UV irradiation; *lanes 3–8*: compounds **14**, **18**, **8**, **3**, **15**, and **16** (1000 μM), respectively, following UV irradiation. Form I: covalently closed supercoiled DNA; Form II: open circular DNA; and Form III: linear DNA

graphy, the deprotection of the benzoyl groups in **20** and **21** using NaOMe afforded the designed hybrids **15** and **16** in 72 and 90% yields, respectively.

Photoinduced DNA cleavage by the anthraquinone–carbohydrate hybrids **3**, **15**, and **16**, along with the components of these hybrids (**8**, **14**, and **18**), was assayed using supercoiled ΦX174 DNA at pH 7.5 under aerobic conditions. The dimethylamino groups of the carbohydrate moieties in **3**, **8**, **15**, and **16** were protonated under these conditions. Figure 6 clearly shows that the anthraquinone–carbohydrate hybrids **3**, **15**, and **16** (1000 μM) cleaved DNA following irradiation with long wavelength UV light (365 nm, 15 W), while **8**, **14**, and **18** showed no DNA cleaving activity under the same conditions. These results demonstrate the importance of the hybrid structure constructed from anthraquinone and the 2,6-dideoxy amino sugar for DNA cleavage. These results also strongly suggest that the 2,6-dideoxy amino sugar binds to the DNA groove and significantly enhances the intercalating ability of the anthraquinone. It was confirmed that DNA is not cleaved by **3**, **15**, and **16** in the absence of light. Hybrid **15** cleaves DNA more effectively than does **3**. Interestingly, hybrid **16** forms a complex with DNA after DNA cleavage, as shown by the lower mobility of the DNA compared to Form II DNA. Extraction of hybrid **16** with chloroform confirmed that the lower mobility DNA was transformed into Form II DNA following the removal of **16**. These results clearly demonstrate that DNA cleavage and binding are dependent on the structure of the sugar moiety in the hybrid.

3.2 Second Generation

To further improve DNA cleavage by anthraquinone–carbohydrate hybrids, we designed novel anthraquinone–carbohydrate hybrids **22** and **23**, which are anomeric to each other (Fig. 7) [11]. These new hybrids lack the aromatic hy-

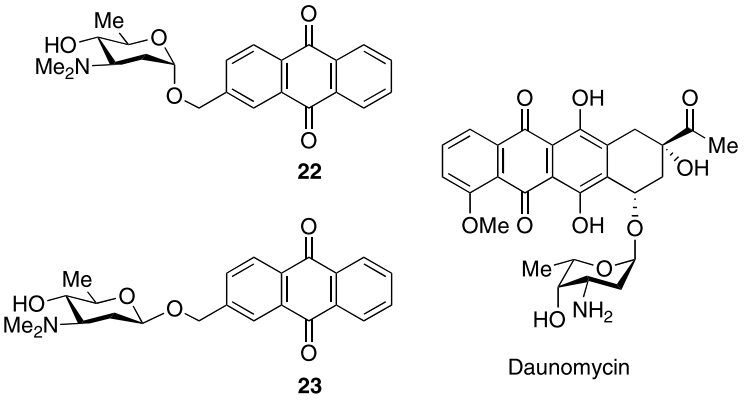
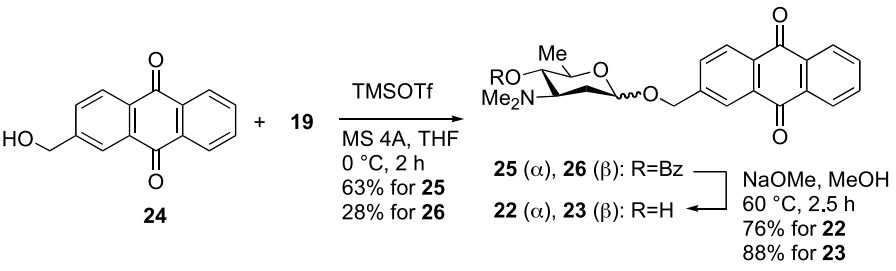


Fig. 7 Artificial anthraquinone–carbohydrate hybrids **22** and **23,** and daunomycin

droxy group present in hybrids **15** and **16**. We anticipated that the hydroxy group in **15** and **16**, which forms a hydrogen bond with one of the quinone carbonyl oxygens, could inhibit photoexcitation of the anthraquinone. Therefore, the newly designed anthraquinone–carbohydrate hybrids **22** and **23** should have stronger DNA photocleaving activities than **15** or **16**.

Hybrids **22** and **23** were synthesized via the effective glycosylation of commercially available 2-hydroxymethylanthraquinone (**24**) and the 1-OAc sugar **19** (Scheme 3). The glycosylation of **24** and **19** using TMSOTf as an activator in the presence of MS 4A in THF at 0 °C for 2 h provided α-glycoside **25** and β-glycoside **26** in 91% yield in a ratio of 2.3 : 1. After the separation and isolation of each anomer by column chromatography, deprotection of the benzoyl group in **25** and **26** using NaOMe in MeOH at 60 °C for 2.5 h afforded the desired hybrids **22** and **23** in 76 and 88% yields, respectively.



Scheme 3 Synthesis of **22** and **23**

The photoinduced DNA cleaving activities of hybrids **22** and **23**, along with reference compound **24**, were assayed using 10–0.1 μM supercoiled ΦX174 DNA. Figure 8 clearly shows that hybrids **22** and **23** very effectively cleave DNA upon photoirradiation with long wavelength UV light (365 nm,

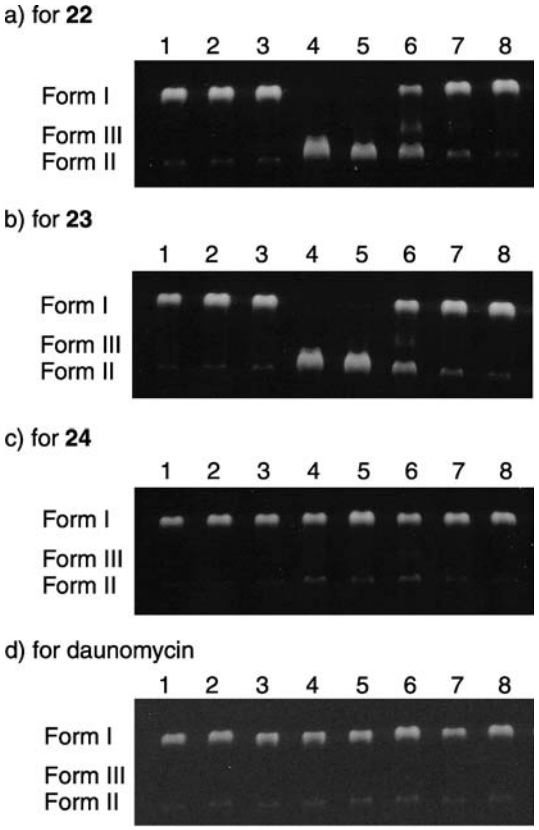


Fig. 8 Photocleavage of supercoiled ΦX174 DNA. ΦX174 DNA (50 μM per base pair) was incubated with each compound in 20% acetonitrile in Tris–HCl buffer (pH 7.5, 50 mM) at 25 °C for 2 h under a UV lamp (365 nm, 15 W) placed 10 cm from the sample, then analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain). **a**, **b**, **c** , and **d** for compounds **22**, **23**, **24**, and daunomycin, respectively. *Lane 1*: DNA alone; *lane 2*: DNA following UV irradiation; *lane 3*: DNA+compound (10 μM) without UV irradiation; *lanes 4–8*: compound (10 μM), compound (3 μM), compound (1 μM), compound (0.3 μM), and compound (0.1 μM), respectively, following UV irradiation. Form I: covalently closed supercoiled DNA; Form II: open circular DNA; and Form III: linear DNA

15 W) (lanes a and b in Fig. 8), while **24** shows little DNA cleaving activity under the same conditions (lane c in Fig. 8). It was confirmed that no DNA cleavage by **22**–**24** occurred in the absence of light (lane 3 in Fig. 8). Thus, UV light functions as a trigger to initiate DNA cleavage by these anthraquinone derivatives. Surprisingly, the DNA cleaving abilities of **22** and **23** were found to be about 300 times higher than those of **15** and **16**: **22** and **23** cleaved DNA at concentrations over 0.3 μM, and cleaved DNA with 100% efficiency at concentrations over 3 μM. These results verify the importance of the absence of the hydroxy group at the C-1 position of the anthraquinone moiety

for DNA cleavage. Furthermore, it was confirmed that the hybrid structure constructed from the anthraquinone and the deoxyamino sugar was very effective for DNA cleavage. In addition, DNA cleaving ability was dependent on the configuration of the anomeric position of the hybrid, with the α-anomer **22** having higher activity than the β-anomer **23**.

At this stage, our attention turned to comparing these designed compounds with natural products in terms of their efficacy as DNA photocleavers.

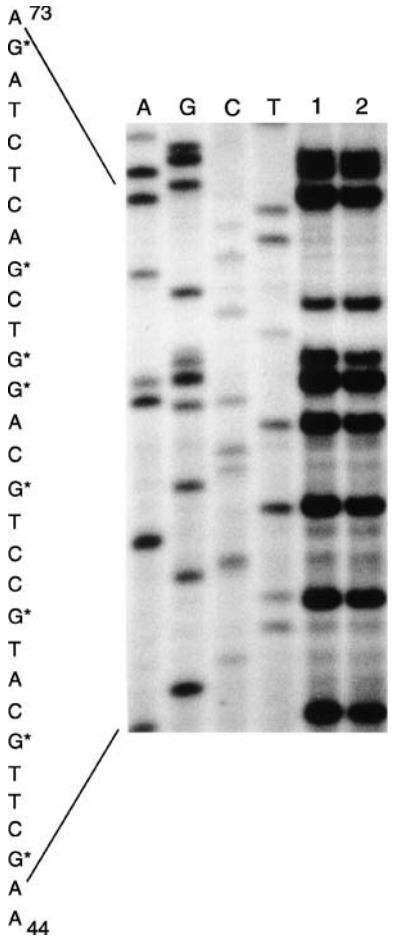


Fig. 9 Autoradiogram of a 12% polyacrylamide/8 M urea slab gel following electrophoresis for sequence analysis (bases 46–73 are shown). The 5′-end-labeled M13mp18 DNA at the primer site was cleaved by each compound at pH 7.5 and 25 °C for 2 h during irradiation from a UV lamp (365 nm, 15 W) placed 10 cm from the sample. *Lanes A*, *G*, *C*, and *T*: Sanger A, G, C, and T reactions, respectively; *lanes 1* and *2*: **3** and **4** (10 μM), respectively, with UV irradiation. DNA in *lanes 1* and *2* was treated with hot piperidine prior to gel electrophoresis

The DNA photocleaving abilities of these hybrids were much higher than that of the natural anthraquinone antibiotic, daunomycin (lanes a and b vs lane d in Fig. 8). These results demonstrate that artificial anthraquinone–carbohydrate hybrids are superior to this natural anthraquinone product as a DNA photocleaving agent.

The DNA cleavage site-specificity of hybrids **22** and **23** was analyzed according to the Sanger protocol [33]. Since the Sanger sequencing reactions result in base incorporation, cleavage at nucleotide *N* (sequencing) represents a cleavage site by the agent or the Maxam–Gilbert reaction at $N + 1$. The results shown in Fig. 9 clearly indicate that hybrids **22** and **23** selectively cleave DNA at the guanine site, and site-selective DNA cleavage is enhanced upon treatment with hot piperidine. Since the free radical scavenger dimethyl sulfoxide did not inhibit DNA cleavage, while the singlet oxygen scavenger 2,2,6,6-tetramethylpiperidine significantly prevented this reaction, it is very likely that oxidation of guanine by singlet oxygen (generated from the photoexcited anthraquinone and O_2) is the initial step in the photoinduced destruction of the guanine base [26].

The cytotoxicity of DNA cleaving hybrids **15**, **16**, **22**, and **23** was next examined using HeLa S3 cancer cells exposed to each agent for 72 h with or without 1 h of photoirradiation (Fig. 10). It was confirmed that when the HeLa S3 cells were exposed to 10 μM **15**, **16**, **22**, or **23** without photoirradiation, most of the cells survived. In addition, when HeLa S3 cells were treated with 10 μM **15** or **16** combined with photoirradiation, 70–80% of the cells survived. In drastic contrast, similar treatment with **22** or **23** killed all the cells. These results indicate that the cytotoxic activities of **22** and **23** in the presence of UV light are significantly higher than those of **15** and **16**, and that DNA cleavage initiated by photoirradiation strongly affects the cytotoxicity of the hybrids. Furthermore, these results also demonstrate that the viability of can-

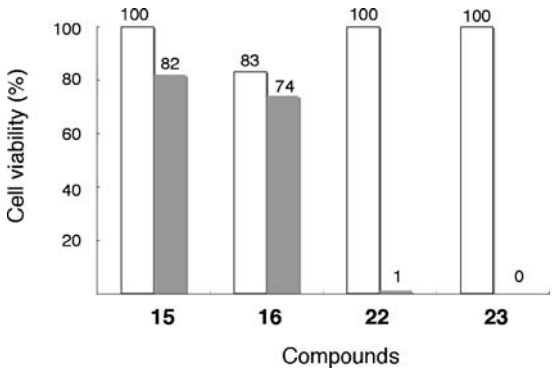


Fig. 10 Viability of HeLa S3 cells. HeLa S3 cancer cells exposed to **15**, **16**, **22**, or **23** (10 μM) for 72 h with or without 1 h of photoirradiation. *Gray* and *white bars* represent cell viability of HeLa S3 cells with and without photoirradiation, respectively

cer cells can be controlled by treatment with appropriate amounts of these novel anthraquinone–carbohydrate hybrids.

4 Concluding Remarks

In summary, the present work describes both the molecular design and chemical synthesis of novel anthraquinone–carbohydrate hybrids, and their DNA binding and photocleaving profiles and cytotoxic activities. The results show that even a simple designed molecule can strongly bind DNA in a sequence-selective fashion. In addition, it was found that some of these designed molecules are capable of photocleaving DNA base-selectively. The described chemistry and biological evaluations provide significant information about the molecular design of photoreactive anthraquinone–carbohydrate hybrid systems that bind and cleave DNA, and exhibit significant cytotoxicity towards HeLa cells.

Acknowledgements This research was supported in part by the 21st Century COE Program "Keio Life-Conjugated Chemistry" of the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT).

References

1. Demeunynck M, Bailly C, Wilson WD (eds) (2003) DNA and RNA binders, vols 1 and 2. Wiley-VCH, Weinheim
2. Bashkin JK (ed) (1998) Chem Rev 98:937
3. Arcamone F (1981) In: Stevens G (ed) Doxorubicin anticancer antibiotics. Medicinal chemistry series of monographs, vol 17. Academic, New York
4. Lown JW (ed) (1988) Anthracycline and anthracenedione based anticancer agents. Elsevier, Amsterdam
5. Hansen MR, Hurley LH (1996) Acc Chem Res 29:249
6. Bycroft BW (ed) (1988) Dictionary of antibiotics and related substances. Chapman & Hall, London
7. Nicolaou KC, Smith AL (1992) Acc Chem Res 25:497
8. Maier ME (1995) Synlett 13
9. Toshima K, Ouchi H, Okazaki Y, Kano T, Moriguchi M, Asai A, Matsumura S (1997) Angew Chem Int Ed Engl 36:2748
10. Toshima K, Maeda Y, Ouchi H, Asai A, Matsumura S (2000) Bioorg Med Chem Lett 10:2163
11. Toshima K, Nakajima Y, Maeda Y, Matsumura S (2004) Lett Org Chem 1:31
12. Toshima K, Hasegawa M, Shimizu J, Matsumura S (2004) ARKIVOC 28
13. Toshima K, Takano R, Ozawa T, Matsumura S (2002) Chem Commun 212
14. Toshima K, Takano R, Ozawa T, Ariga A, Shima Y, Umezawa K, Matsumura S (2003) Tetrahedron 59:7057

15. Toshima K, Takano R, Maeda Y, Suzuki M, Asai A, Matsumura S (1999) Angew Chem Int Ed 38:3733
16. Toshima K, Okuno Y, Nakajima Y, Matsumura S (2002) Bioorg Med Chem Lett 12:671
17. Toshima K, Takai S, Maeda Y, Takano R, Matsumura S (2000) Angew Chem Int Ed 39:3656
18. Walker S, Valentine KG, Kahne D (1990) J Am Chem Soc 112:6428
19. Ding W, Ellestad GA (1991) J Am Chem Soc 113:6617
20. Uesugi M, Sugiura Y (1993) Biochemistry 32:4622
21. Toshima K, Matsuo G, Nakata M (1994) J Chem Soc Chem Commun 997
22. Kingston RE (1995) DNA–protein interactions. In: Ausubel F, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds) Short protocols in molecular biology, 3rd edn. Wiley, Canada, p 12-1
23. Camilloni G, Seta FD, Negri R, Ficca AG, Mauro ED (1986) EMBO J 5:763
24. Yamashita Y, Kawada S, Fujii N, Nakano H (1991) Biochemistry 30:5838
25. Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, Currens MJ, Seniff D, Boyd MR (1988) Cancer Res 48:4827
26. Armitage B (1998) Chem Rev 98:1171
27. Morgan AR (1995) Curr Med Chem 2:604
28. Bonnett R (1995) Chem Soc Rev 19
29. Henderson RW, Dougherty TJ (eds) (1992) Photodynamic therapy: basic principles and clinical applications. Marcel Dekker, New York
30. Koch T, Ropp JD, Sligar SG, Schuster GB (1993) Photochem Photobiol 58:554
31. Armitage B, Yu C, Devadoss C, Schuster GB (1994) J Am Chem Soc 116:9847
32. Kobayashi S, Hachiya I (1994) J Org Chem 59:3590
33. Sanger F, Nicklen S, Coulsen AR (1977) Proc Natl Acad Sci USA 74:5463

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Syntheses of Anthracyclines and Fredericamycin A via Strong Base-Induced Cycloaddition Reaction of Homophthalic Anhydrides

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Abstract Strong base-induced cycloaddition reactions of homophthalic anhydrides with quinone derivatives afford *peri*-hydroxyl aromatic compounds in a regioselective manner. The reactions have been applied to the syntheses of biologically important natural products. This review focuses on the following topics: (i) preparation of homophthalic anhydrides from homophthalic acids using (trimethylsilyl)ethoxyacetylene, (ii) strong base-induced cycloaddition of homophthalic anhydrides with the carbon–carbon double bond and the reaction mechanism, especially addressing the regiochemistry of the reaction, and (iii) application of the reaction to the efficient asymmetric syntheses of anthracyclines and fredericamycin A.

Keywords Anthracycline · Fredericamycin A · Homophthalic anhydride · Strong base-induced cycloaddition

Abbreviations

Ac	Acetyl
acac	Actylacetonate
AIBN	2,2′-Azobisisobutyronitrile
DEAD	Diethyl azodicarboxylate
Cp	(–)-Camphanyl
LDA	Lithium diisopropylamide
m-CPBA	*m*-Chloroperbenzoic acid

Me	Methyl
Ph	Phenyl
TMS	Trimethylsilyl
Tf	Trifluoromethanesulfonyl
$LiN(TMS)_2$	Lithium bis(trimethylsilyl)amide

1 Introduction

In this chapter, we describe the syntheses of anthracyclines (daunomycin is shown as a representative compound in Fig. 1) and fredericamycin A based on the cycloaddition reactions of homophthalic anhydrides and dienophiles, which produce *peri*-hydroxyl aromatic compounds.

Homophthalic anhydrides are very useful molecules in organic synthesis. Their 1,3-positions are electron-poor. They then react with nucleophiles at these positions. On the other hand, their 4-position is electron-rich, and electrophiles can react at the 4-position. Before our first use of homophthalic anhydrides in the cycloaddition reaction with a C – C multiple bond, many studies on their reactions with the C = O [1] or C = N [2] double bond and their applications to the syntheses of natural products and heterocyclic compounds [3–8] had been reported. However, no study on the cycloaddition of homophthalic anhydride with a C – C multiple bond, such as an olefin and acetylene, had been reported before our work.

We now present the chemistry of homophthalic anhydrides, mainly addressing the strong base-induced [4 + 2]-cycloaddition reaction with the C – C double bond, especially in quinone and dienophiles, and its application to the syntheses of anthracyclines and fredericamycin A.

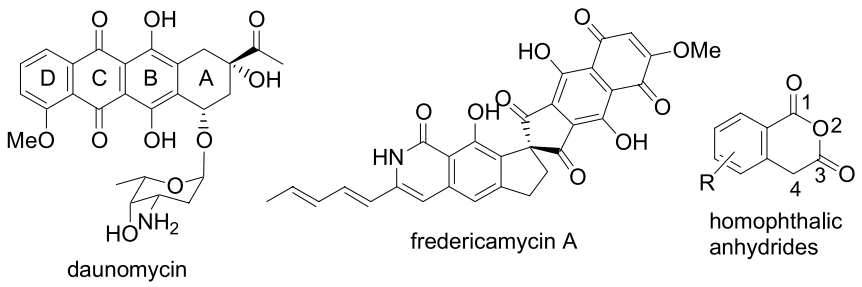


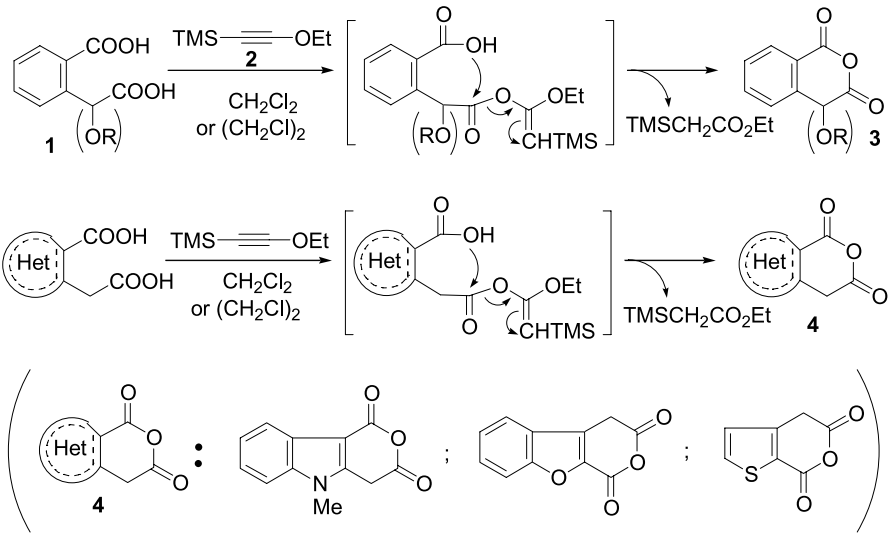
Fig. 1 Structures of daunomycin, fredericamycin A, and homophthalic anhydrides

2
Chemistry of Homophthalic Anhydride

2.1
Preparation

Homophthalic anhydrides can be prepared by the dehydration of homophthalic acid with acid chloride, acid anhydride, phosgene, thionyl chloride, benzene sulfonyl chloride, ketene, phosphorous pentoxide, dicyclohexylcarbodiimide, or *N*,*N*-carbonyldiimidazole. However, these methods are not always effective for the acid-sensitive and/or unreactive homophthalic acid derivatives. We developed a very mild and efficient method for obtaining homophthalic anhydrides using (trimethylsilyl)ethoxyacetylene [9] (Scheme 1). Thus the treatment of homophthalic acid **1** with (trimethylsilyl)ethoxyacetylene **2** in inert solvents such as methylene chloride, 1,2-dichloroethane, and acetonitrile gave the homophthalic anhydride **3** in a quantitative yield accompanied by ethyl trimethylsilyl acetate as the only side product. The high purity homophthalic anhydride **3** could be obtained for the next cycloaddition reaction just by evaporation to remove the reaction solvent and the formed ethyl trimethylsilyl acetate. The method is quite useful, and was also applicable to the syntheses of hetero-homophthalic anhydrides **4**.

The reaction proceeds under neutral conditions, and no aqueous work-up is necessary. This condition is available for acid-labile dicarboxylic acids, such



Scheme 1 Preparation of homophthalic anhydrides

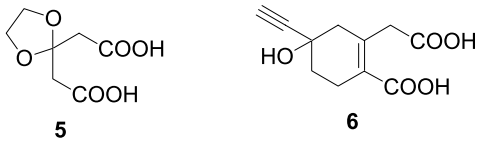
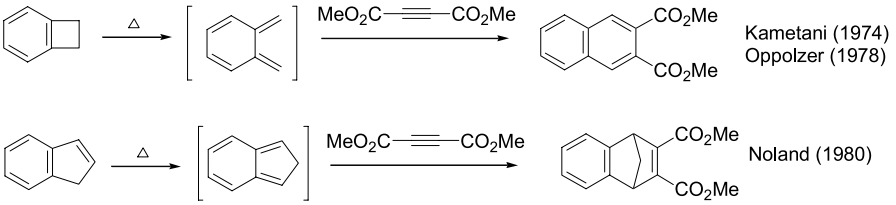


Fig. 2 Acid-labile dicarboxylic acids

as acetal dicarboxylic acid **5** and *tert*-hydroxyl dicarboxylic acid **6** (Fig. 2). The yields of their anhydrides were almost quantitative.

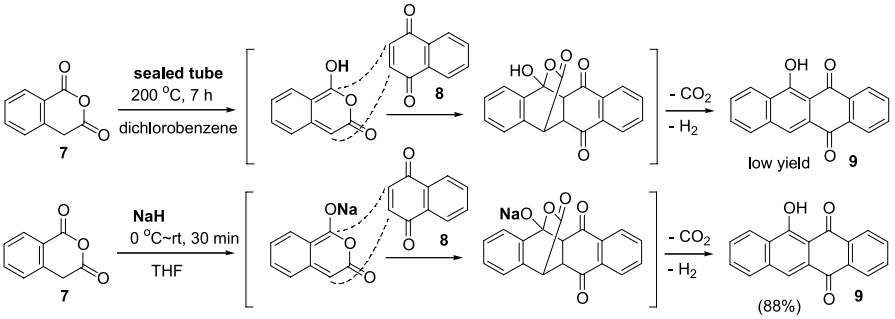
2.2
Reaction of Homophthalic Anhydride

Kametani et al. [10] and Oppolzer et al. [11] reported the Diels–Alder reaction of benzocyclobutane by heating. In this reaction, the benzocyclobutanes produced *o*-quinodimethane intermediates, which readily reacted with acetylenic compounds to afford cycloadduct aromatic compounds. Furthermore, Noland et al. reported the Diels–Alder reaction of the *o*-quinodimethane intermediate obtained by heating indene [12].



Scheme 2 Diels–Alder reaction via *o*-quinodimethane intermediate

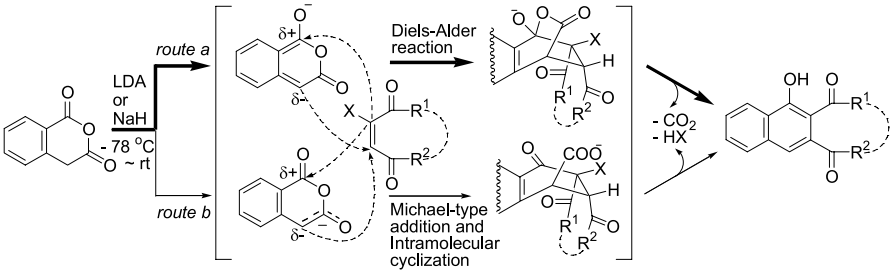
Based on these results, we postulated the formation of *o*-quinodimethanes from homophthalic anhydrides. At this time, the proper dienophile exists in the reaction mixture, and the cycloaddition reaction would produce the *peri*-hydroxyl aromatic compound by decarboxylation of the first adduct. We first heated the mixture of homophthalic anhydride **7** and benzoquinone **8**. Although heating the mixture did not give the cycloadduct product at all, a long reaction time in a sealed tube afforded the coupled product **9** in low yield. The reactivity of the homophthalic anhydrides was significantly enhanced by treatment with base, such as LDA or NaH. Thus the reaction in the presence of NaH proceeded at low temperature with a short reaction time to give the coupled product **9** in high yield (Scheme 3) [13–15].



Scheme 3 Reaction of homophthalic anhydride **7** and benzoquinone **8** under different conditions

2.3
Reaction Mechanism of Cycloaddition

As a reaction mechanism for the strong base-induced cycloaddition reaction of homophthalic anhydrides and dienophiles, two routes are possible (Scheme 4). One is *route a* via the Diels–Alder reaction, decarboxylation and de-HX. The other is *route b* via Michael-type addition, intramolecular cyclization, decarboxylation and de-HX. Several studies showed that *route a* was the more preferable. After cycloaddition of the homophthalic anhydrides with dienophiles, the reaction is always accompanied by decarboxylation and aromatization. The stereochemistry of the cycloaddition reaction of the homophthalic anhydride is not considered.



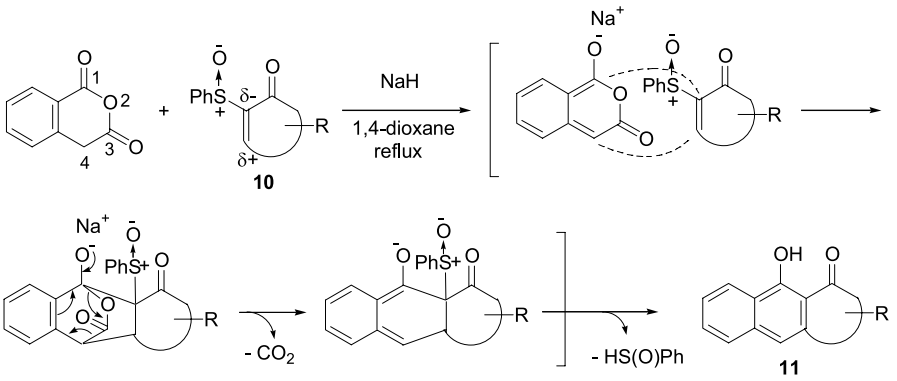
Scheme 4 Two plausible reaction mechanisms for cycloaddition

Regiochemistry

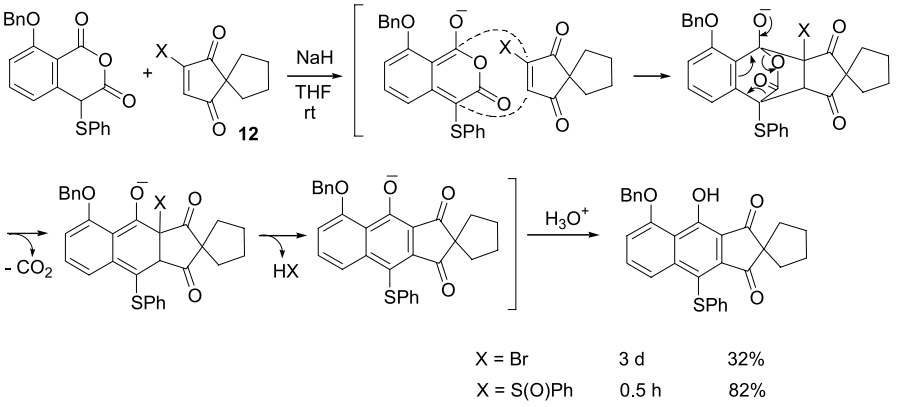
The regiochemistry of the strong base-induced [4 + 2]-cycloaddition reaction can be controlled by the electronic state of the dienophiles. For the α,β-unsaturated carbonyl derivatives **10**, such as cycloalkenones, the β-carbon is more electron-deficient, and the more electron-rich C4-carbon of the homophthalic anhydride is connected to the β-carbon of the enone. The α-

sulfinyl substituent significantly enhanced the regioselectivity, and single regio-isomers **11** were obtained. In these cases, the sulfinyl group not only promotes the cycloaddition reaction, but also undergoes in situ elimination under these conditions to afford the *peri*-hydroxy aromatic compounds in a single step (Scheme 5) [13–15]. For the reactions of the 2-ene-1,3-dione compounds **12**, the regiochemistry must be controlled by substituent X. Although a halogen substituent can control the regiochemistry, the reactivity is not very high. On the other hand, the sulfinyl group also proved its utility in this case (Scheme 6) [16, 17].

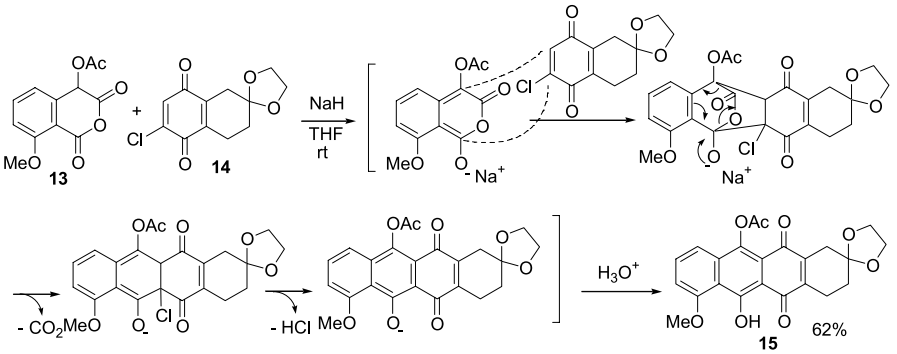
For the quinone derivatives, the olefinic electron-withdrawing group controls the regiochemistry. For example, the reaction of 4-acetoxy homophthalic anhydride **13** and a chloroquinone compound **14** produced a single regioisomer **15** in high yield (Scheme 7) [18, 19].



Scheme 5 Reaction of homophthalic anhydride and enone

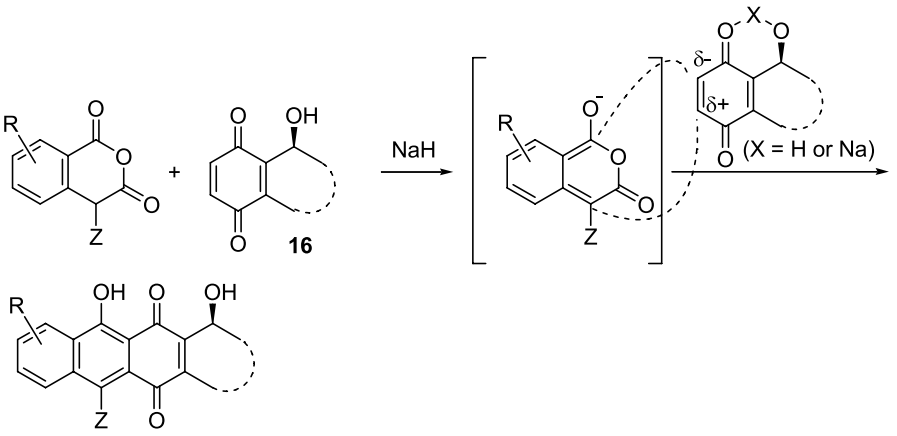


Scheme 6 Reaction of homophthalic anhydride and 2-ene-1,3-dione



Scheme 7 Reaction of 4-acetoxy homophthalic anhydride **13** and chloroquinone **14**

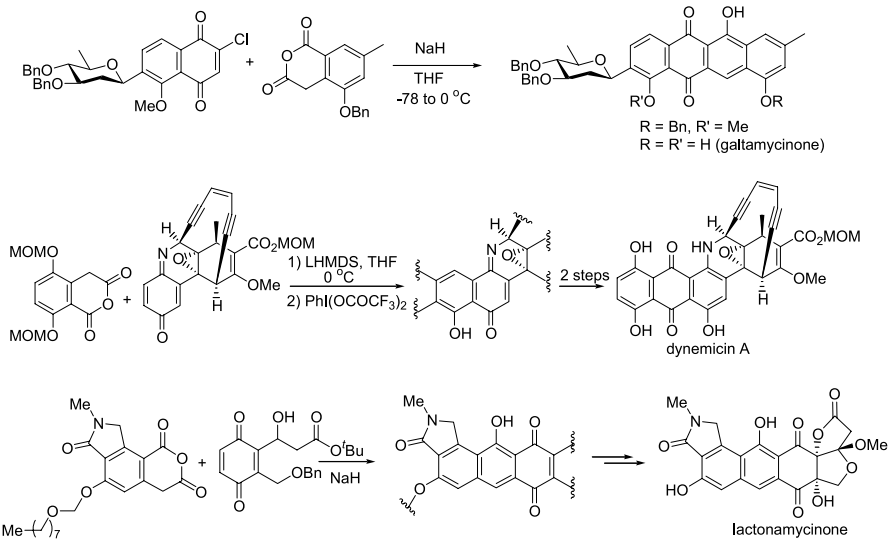
The presence of the *peri*-hydroxyl group can also control the regiochemistry of the coupling reaction. That is, the chelation between one of the two carbonyl groups of the quinone unit **16** and the hydroxyl group differentiates the electronic states of the two olefinic carbons. The cycloaddition reaction then proceeds in a regioselective manner (Scheme 8). This reactivity was used in the regioselective syntheses of γ-rhodomycinone [20, 21] and lactonamycin [22, 23].



Scheme 8 Reaction of quinone having *peri*-hydroxyl group

3
Application to Biologically Active Compounds

The cycloaddition reaction of the homophthalic anhydride with quinone derivatives followed by aromatization is a very useful protocol for produc-



Scheme 9 Syntheses of galtamycinone, dynemicin A, and lactonamycinone

ing linear polycyclic aromatic compounds. The reaction was then applied to the syntheses of bioactive polycyclic aromatic compounds. Although many synthetic studies of *peri*-hydroxy natural compounds, including the total syntheses of galtamycinone [24], dynemicin A [25], and lactonamycin [22, 23], have already been reported (Scheme 9), a brief review of the syntheses of anthracycline antibiotics and the asymmetric synthesis of fredericamycin A is described in this chapter.

3.1 Anthracycline Antibiotics

Anthracycline antibiotics are some of the most studied anticancer compounds. Anthracycline antibiotics, such as daunomycin, oxaunomycin, etc., are clinically used anticancer antibiotics. They are composed of two parts, an aglycon unit and sugar moiety. Figure 3 shows the structures of daunomycin and oxaunomycin. For the aglycon part, all anthracycline antibiotics contain a linear four-cyclic structure with A-, B-, C-, and D-rings, in which only the A-ring has asymmetric centers.

The most popular chemical synthetic method for the aglycon skeletons of the anthracyclines is the cycloaddition reaction of two units, the unit corresponding to the AB-ring unit and the unit corresponding to the CD-ring unit. The cycloaddition reaction using a homophthalic anhydride is one of the most popular cycloaddition reactions for the anthracyclines. For example, Scheme 10 shows our synthetic route to (±)-daunomycin, focusing on the

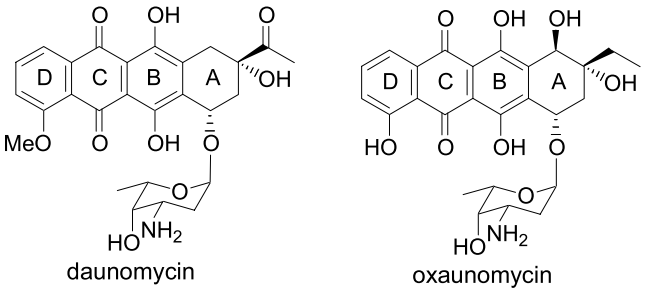
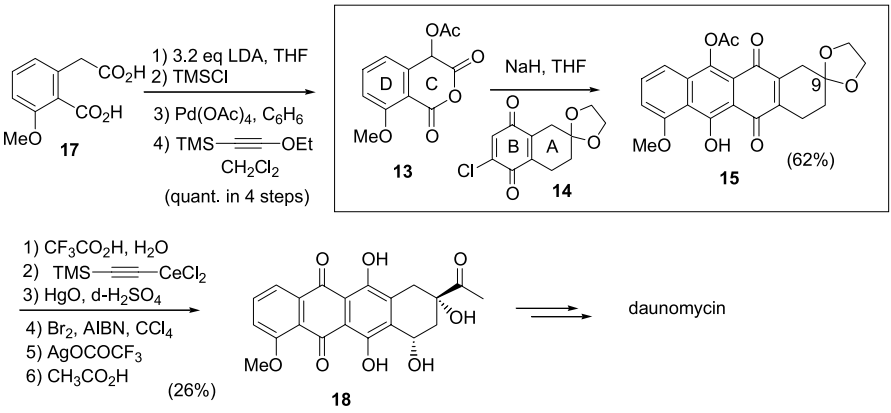


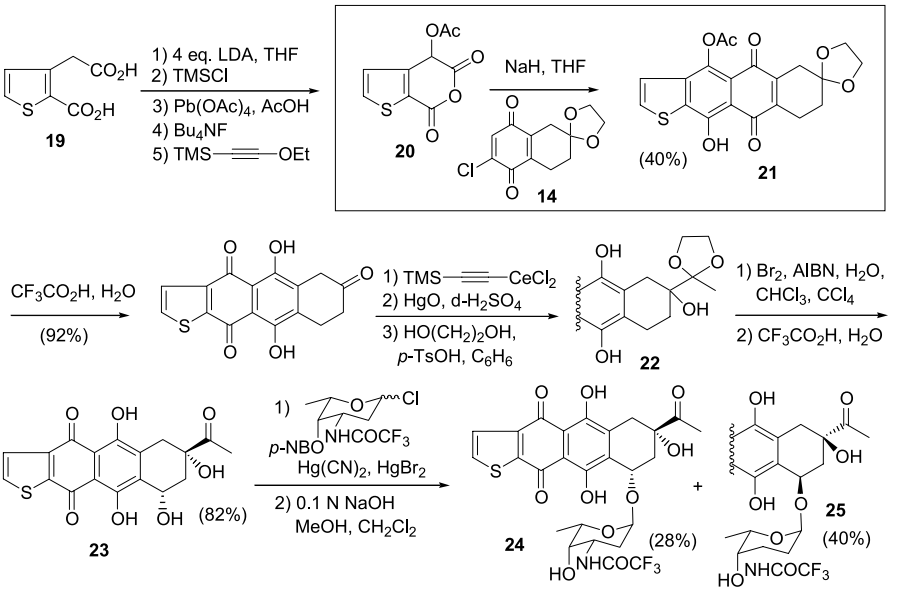
Fig. 3 Structures of daunomycin and oxaunomycin



Scheme 10 Synthesis of daunomycin

cycloaddition of the 4-acetoxy-8-methoxy homophthalic anhydride **13** and chloronaphthoquinone acetal **14** for the synthesis of the tetracyclic anthracycline skeleton **15**. Thus, the introduction of the 4-acetoxy group to the methoxy dicarboxylic acid **17** and successive treatment with (trimethylsilyl)ethoxyacetylene produced **13**. The coupling reaction proceeded in a regioselective manner, as the presence of the chlorine atom in the quinine moiety **14** produced **15** in a 62% yield as the sole product. The acetal group at the C9-position was hydrolyzed to give the ketone function. Introduction of the acetylene unit was done by nucleophilic attack of the trimethylsilyletynyl cerium chloride on the C9-ketone. Transformation of the acetylene unit to an acetyl function and introduction of the C7-hydroxyl group by bromination was followed by hydrolysis to produce the daunomycinone **18**, which was transformed into daunomycin by glycosidation of the C7-hydroxyl group [18, 19].

This protocol for the synthesis of anthracycline antibiotics was very efficient and useful, and was used for various kinds of anthracycline antibiotics, such as 11-deoxydaunomycin [26–28] and heteroanthracyclines [29–36]. D-

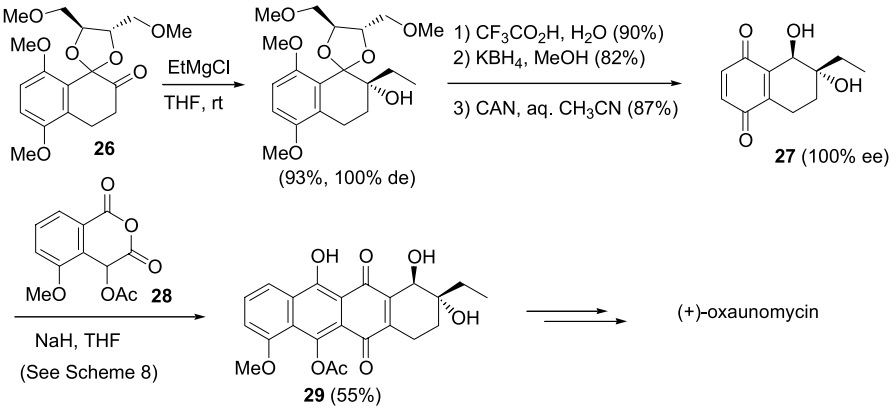


Scheme 11 Synthesis of D-ring thiophene anthracyclines

ring heteroanthracyclines, having thiophene, indole, pyridine, or pyradine as a D-ring, were synthesized. Scheme 11 shows our synthetic work-up of the D-ring thiophene anthracyclines. Thus, the thiophene dicarboxylic acid **19** was converted into the acetoxythiophene anhydride **20**. The coupling reaction of **20** and **14** using NaH gave the D-ring thiophene anthracyclinone skeleton **21** as a single product, which was transformed into the hydroxyacetal compound **22**. Introduction of the C7-hydroxyl group by bromination followed by hydrolysis gave the racemic dihydroxy compound **23**. Glycosidation of the C7-hydroxyl group with the optically pure L-daunosamine derivative gave two diastereomeric D-ring thiophene anthracyclines, **24** and **25**.

The method was also applied to the syntheses of optically active daunomycinone [37, 38] and oxaunomycin [20, 21, 39, 40] using optically active quinone units. Asymmetric synthesis of (+)-oxaunomycin is shown in Scheme 12. Optically pure quinone **27** was synthesized by EtMgCl attack to α-keto acetal **26** followed by acid hydrolysis, KBH_4-reduction of the ketone, and CAN-oxidation. The coupling reaction of **27** with homophthalic anhydride **28** afforded single regio-isomer **29** with retention of chirality. Several further steps converted **29** to optically pure (+)-oxaunomycin.

For our anthracycline synthesis, the anionic cycloaddition reaction of the homophthalic anhydrides and quinone dienophiles, leading to the aglycone skeletons of anthracyclines, plays a key role. Table 1 shows the results of the cycloaddition reactions of the homophthalic anhydrides with quinone



Scheme 12 Asymmetric synthesis of oxaunomycin

Table 1 Cycloaddition reactions of homophthalic anhydrides with quinone dienophiles

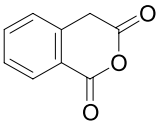
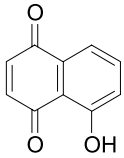
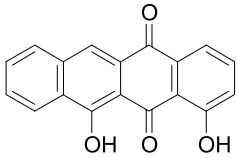
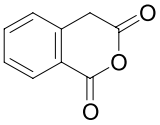
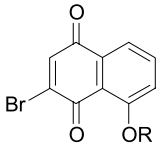
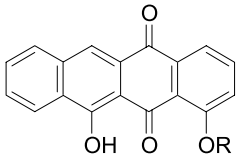
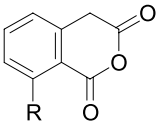
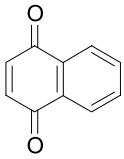
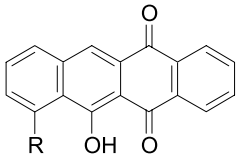
Homophthalic anhydride	Quinone	Reaction condition	Product (yield)	Refs.
		Heat	 (49%)	[41]
	 R=H, Me	Heat	 R=H (41%) R=Me (39%)	[42]
 R=H, OMe		LDA	 R=H (76%) R=OMe (68%)	[14]

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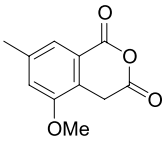
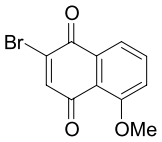
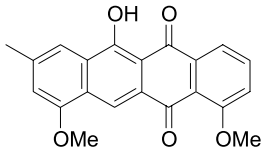
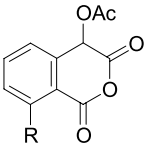
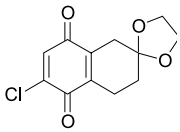
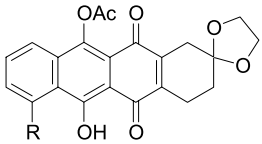
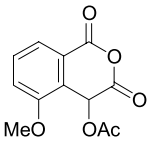
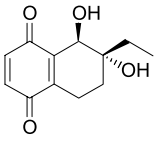
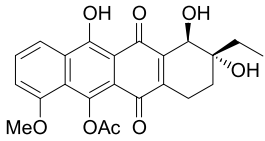
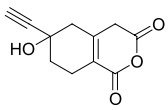
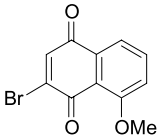
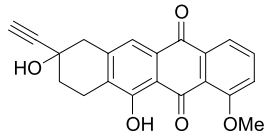
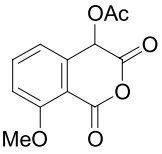
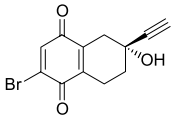
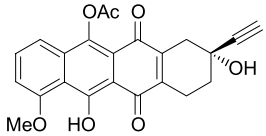
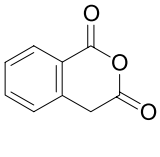
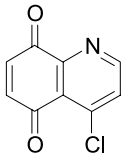
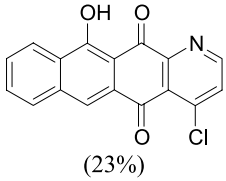
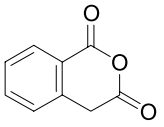
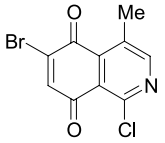
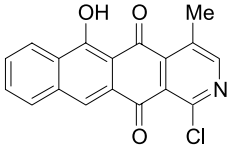
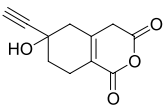
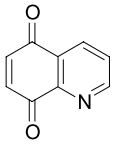
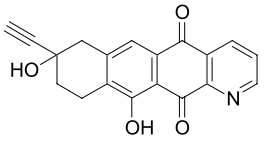
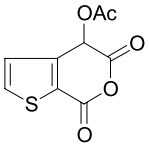
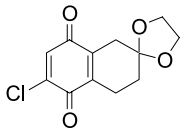
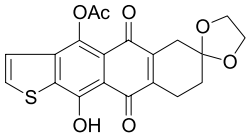
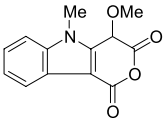
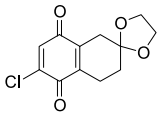
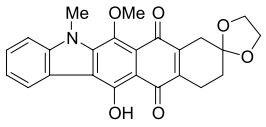
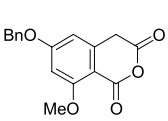
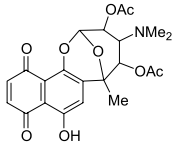
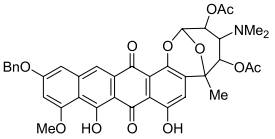
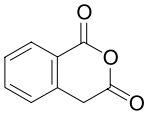
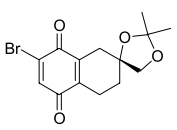
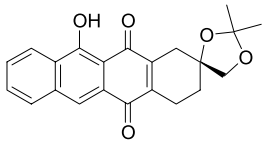
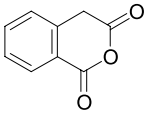
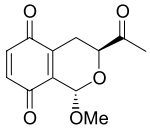
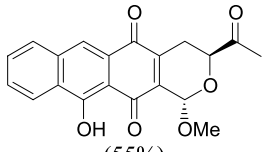
Homophthalic anhydride	Quinone	Reaction condition	Product (yield)	Refs.
		NaH	 (87%)	[15]
 R=H, OMe		NaH	 R=H (75%) R=OMe (62%)	[18, 19]
		NaH	 (55%)	[20, 21]
		NaH	 (42%)	[28]
		NaH	 (70%)	[37, 38]
		NaH	 (23%)	[43]

Table 1 (continued)

Homophthalic anhydride	Quinone	Reaction condition	Product (yield)	Refs.
		NaH	 (77%)	[44]
		NaH	 (77%)	[45]
		NaH	 (30%)	[46]
		NaH	 (57%)	[47]
		NaH	 (100%)	[48, 49]
		LDA	 (78%)	[50]
		LDA	 (55%)	[51]

dienophiles, taken from reports on natural and unnatural anthracycline syntheses.

3.2 Fredericamycin A

Fredericamycin A (Fig. 4) was isolated by Professor Pandey in 1981 and its structure was determined in 1982 [52–54]. Fredericamycin A has strong in vitro and in vivo cytotoxicities against several tumor models, such as P388 leukemia, B16 melanoma, and CD8F mammary carcinoma, and does not show any mutagenicity in the Ames test [55–59]. It has only one asymmetric center, i.e., a chiral spiro carbon center in the CD-ring system, which is based on the methoxy group at the farthest position on the A-ring. X-ray analysis cannot be used to identify it. Therefore, its absolute configuration still remained unknown when we started the project involving asymmetric synthesis of fredericamycin A [60, 61], although five racemic syntheses [62–66] and one asymmetric synthesis of the optically active fredericamycin A using a chiral HPLC separation during the final stage of the synthesis [67] had been achieved at that time.

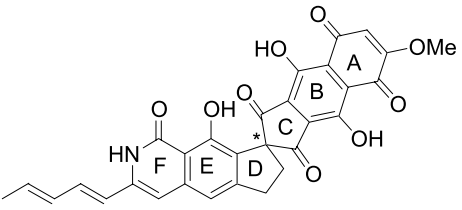
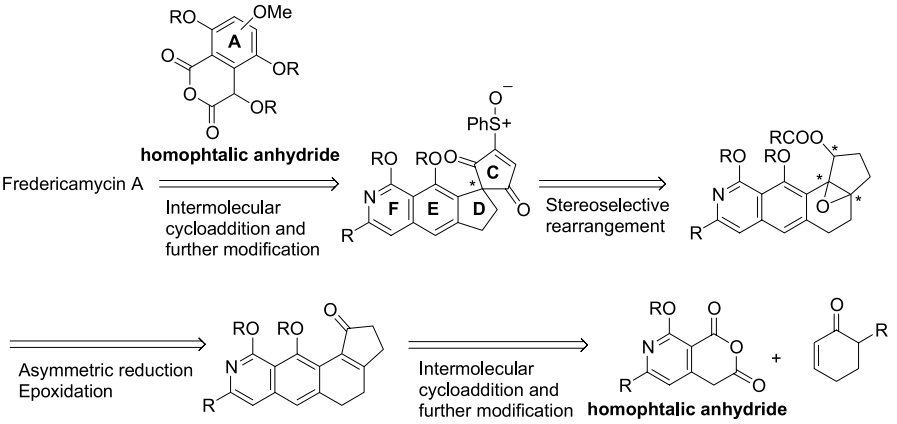
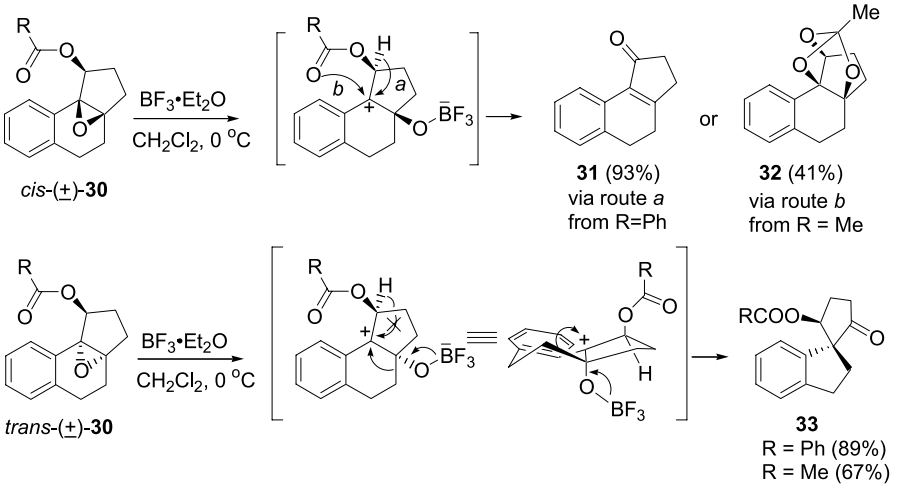


Fig. 4 Structure of fredericamycin A

Scheme 13 shows the retrosynthetic analysis. The strong base-induced intermolecular [4 + 2]-cycloaddition of an optically active CDEF-ring unit and a suitably functionalized AB-ring unit would afford the optically active fredericamycin A. The determination of the absolute stereochemistry of the spiro center is usually difficult. The development of a new methodology for constructing the chiral spiro center with an unambiguous stereochemistry is then the most important issue. To construct the spiro center of fredericamycin A with an apparent absolute configuration, the stereospecific rearrangement of the optically active epoxy acylate with an unambiguous absolute configuration was planned. This must be prepared from the asymmetric reduction of the corresponding enone followed by stereoselective epoxidation of the resulting allyl alcohol. During the synthesis, the cycloaddition reaction of the homophthalic anhydride was used twice.



Scheme 13 Retrosynthetic analysis of fredericamycin A

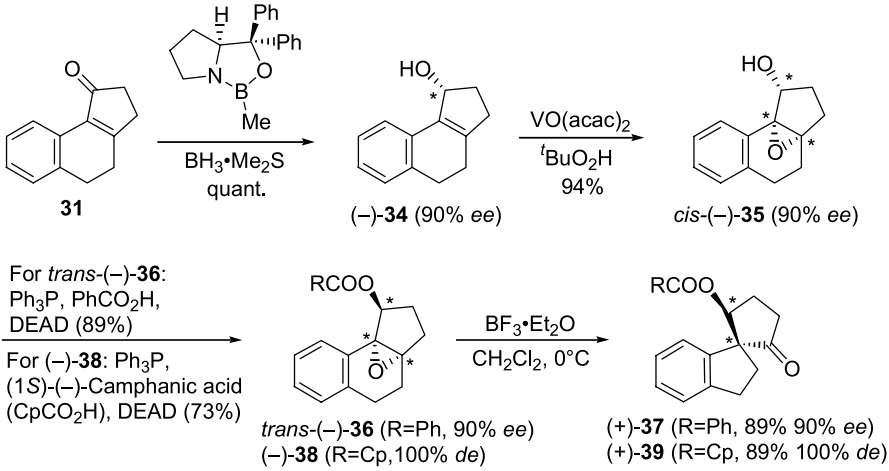


Scheme 14 Reaction of *cis*- and *trans*-epoxy acylates with $BF_3{\cdot}Et_2O$

Scheme 14 shows the results of the reactions of the model epoxy acylates with $BF_3{\cdot}Et_2O$. As the epoxy alcohol derivatives, epoxy acylates were chosen because the acyloxy group would suppress the rearrangement of the substituent on the carbon attached to the acyloxy group because of its electron-withdrawing nature [68–71]. To examine the reactions of the epoxy acylates, the reactions of the racemic tricyclic *cis*- and *trans*-2,3-epoxy acylates (*cis*-(±)-**30** and *trans*-(±)-**30**) were initially investigated. The treatment of *cis*-(±)-**30** with 1 equiv. of $BF_3{\cdot}Et_2O$ in CH_2Cl_2 at 0 °C afforded some undesired products, the enone **31** (93%) from *cis*-(±)-**30** (R = Ph) and the orthoester **32** (41%) from *cis*-(±)-**30** (R = Me). On the other hand, the treatment

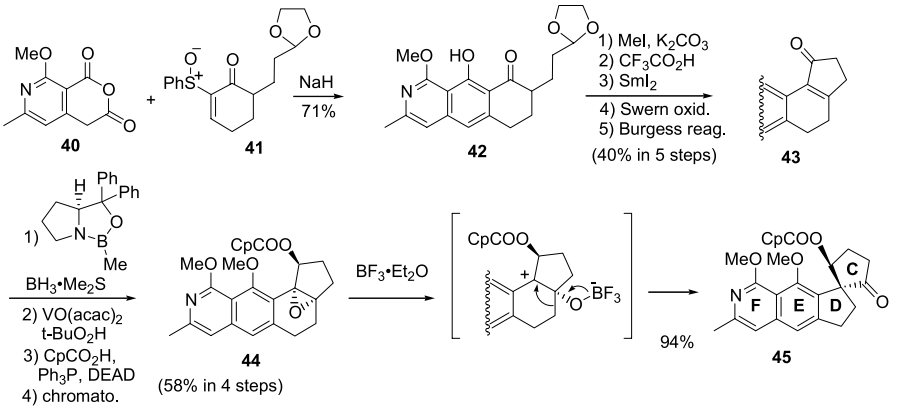
of *trans*-(±)-**30** with $BF_3{\cdot}Et_2O$ under similar conditions gave the desired spiro products (±)-**33** in good yields.

The encouraging result of the *trans*-epoxy acylates with the chiral spiro compounds was applied to the optically active system (Scheme 15). Asymmetric reduction of the enone **31** by Corey's method [72] afforded the allyl alcohol (–)-**34** (90% ee). Epoxidation of (–)-**34** by the stereoselective Sharpless epoxidation [73] afforded the *cis*-epoxy alcohol, *cis*-(–)-**35**, as the sole product. The Mitsunobu reaction [74] of *cis*-(–)-**35** with benzoic acid gave the *trans*-epoxy benzoate, *trans*-(–)-**36**, (90% ee) in 89% yield. Treatment of *trans*-(–)-**36** with $BF_3{\cdot}Et_2O$ afforded the optically active spiro compound (+)-**37** in 89% yield with retention of the optical purity (90% ee). This means that the rearrangement occurs stereospecifically. The optically pure epoxy camphanate (–)-**38** could be obtained after one recrystallization of the crude (–)-**38** (90% de), which was obtained by the Mitsunobu reaction of *cis*-(–)-**35** with D-camphanic acid. The optically pure spiro compound (+)-**39** (100% de) was obtained from the optically pure (–)-**38** in 89% yield.



Scheme 15 Synthesis and rearrangement of optically active epoxy acylates

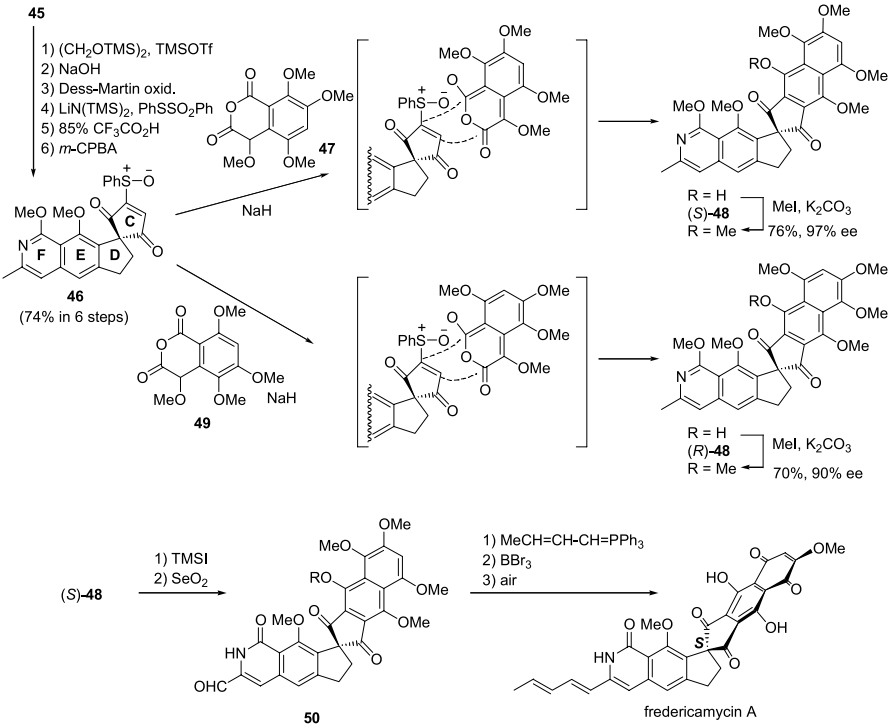
Since a new stereoselective synthesis of the chiral, non-racemic spiro[cyclopentane-1,1′-indan]-2,5-dione system found in fredericamycin A was achieved, this methodology was next studied for the synthesis of the optically active CDEF-ring unit (Scheme 16). The strong base-induced cycloaddition of the homophthalic anhydride **40** with α-sulfinylenone **41** afforded the tricyclic keto acetal **42**, which was transformed into the tetracyclic enone **43** by methylation of the phenolic hydroxyl function, deacetalization, pinacol coupling of the ketone and aldehyde, Swern oxidation of the secondary alcohol, and dehydration using Burgess reagent. The procedure from the tetracyclic enone to the epoxy camphanate is the same as stated above. Although Corey's asym-



Scheme 16 Synthesis of optically active CDEF-ring unit

metric reduction of **43** gave the (*R*)-alcohol with a 74% ee, the optically pure (–)-**44** (≥ 99% de) was obtained by SiO_2-column chromatography purification of the mixture of diastereomers obtained by the Sharpless epoxidation followed by the Mitsunobu reaction with (–)-camphanic acid (> 98% ee). The rearrangement reaction of (–)-**44** (≥ 99% de) with $BF_3 \cdot Et_2O$ proceeded at 0 °C to give the optically pure spiro compound (+)-**45** (≥ 99% de).

The complete synthesis of fredericamycin A from the optically pure spiro compound (+)-**45** is shown in Scheme 17. The transformation of (+)-**45** to the vinyl sulfoxide **46** was achieved along with retention of the chiral integrity. (+)-**45** was acetalized to prevent the easy racemization of the spiro center by the retro-aldol and aldol reaction during alkaline hydrolysis. Since a sulfinyl group was estimated to be a powerful directing and activating substituent on the dienophile [16, 17], the diketovinylsulfide was oxidized by mCPBA to afford the optically active CDEF-ring unit **46**. The [4 + 2]-cycloaddition of **46** with 4,5,7,8-tetramethoxyhomophthalic anhydride **47** followed by methylation afforded the hexacyclic product (*S*)-**48** (76%, 97% ee). On the other hand, its enantiomer (*R*)-**48** (71%, 94% ee) was obtained using the 4,5,6,8-tetramethoxyhomophthalic anhydride **49** in place of **47**. Both enantiomers were converted into fredericamycin A and its enantiomer. In Scheme 17, only the process from (*S*)-**48** to fredericamycin A is shown. Thus, the selective demethylation of the methyl ether on the F-ring of (*S*)-**48** followed by oxidation with SeO_2 produced (*S*)-**50**. The Wittig reaction of (*S*)-**50** with *trans*-2-butenyl triphenylphosphonium bromide gave a mixture of (*E*,*E*)- and (*E*,*Z*)-side chain isomers. Deprotection of the mixture and subsequent autoxidation afforded a 5:1 mixture of fredericamycin A and its (*E*,*Z*)-side chain isomer. Purification by HPLC column chromatography gave the pure natural fredericamycin A.



Scheme 17 Completion of fredericamycin A synthesis

4 Conclusion

The cycloaddition reaction of homophthalic anhydrides with dienophiles provides a very useful methodology for synthesizing polycyclic aromatic compounds. The reaction under basic conditions (such as with NaH) proceeds under mild conditions, low temperature, and short reaction time. Many functional groups can survive these reactions. Furthermore, the degree of regiochemistry in the reaction is very high. It was successfully applied to the syntheses of anthracycline antibiotics and optically active fredericamycin A. Especially, a high regioselectivity for the anionic cycloaddition reaction of homophthalic anhydrides and dienophiles was essential to determine the absolute configuration of the natural fredericamycin A.

References

1. Haimova MA, Mollow NM, Ivanova SC, Dimitrova AI, Ognyanov VI (1977) Tetrahedron 33:331
2. Haimova MA, Ognyanov VI, Mollov NM (198) Syntrhesis, p 845
3. Cushman M, Gentry J, Dekow FW (1977) J Org Chem 42:1111
4. Cushman M, Dekow FW (1978) Tetrahedron 34:1435
5. Cushman M, Dekow FW (1979) J Org Chem 44:407
6. de Silva SO, Ahmad I, Snieckus V (1979) Can J Chem 57:598
7. Cushman M, Choong TC, Valko JT, Koleck MP (1980) J Org Chem 45:5067
8. Iwasa K, Gupta YP, Cushman M (1981) Tetrahedron Lett 22:2333
9. Kita Y, Akai S, Ajimura N, Yoshigi M, Tsugoshi T, Yasuda H, Kita Y (1986) J Org Chem 51:4150
10. Kametani T, Takahashi T, Honda T, Ogasawara K, Fukumoto K (1974) J Org Chem 39:447
11. Oppolzer W (1978) Synthesis, p 793
12. Noland WE, Kameswaran V, Landucci L (1980) J Org Chem 45:4564
13. Tamura Y, Wada A, Sasho M, Fukunaga K, Maeda H, Kita Y (1982) J Org Chem 47:4376
14. Tamura Y, Sasho M, Nakagawa K, Tsugoshi T, Kita Y (1984) J Org Chem 49:473
15. Tamura Y, Fukata F, Sasho M, Tsugoshi T, Kita Y (1985) J Org Chem 50:2273
16. Iio K, Ramesh NG, Okajima A, Higuchi K, Fujioka H, Akai S, Kita Y (2000) J Org Chem 65:89
17. Kita Y, Iio K, Okajima A, Takeda Y, Kawaguchi K, Whelan VA, Akai S (1998) Synlett, p 292
18. Tamura Y, Sasho M, Akai S, Kishimoto H, Sekihachi J, Kita Y (1986) Tetrahedron Lett 27:195
19. Tamura Y, Sasho M, Akai S, Kishimoto H, Sekihachi J, Kita Y (1987) Chem Pharm Bull 35:1405
20. Fujioka H, Yamamoto H, Kondo H, Annoura H, Kita Y (1989) J Chem Soc Chem Commun, p 1509
21. Fujioka H, Yamamoto H, Annoura H, Maeda H, Kita Y (1992) Chem Pharm Bull 40:32
22. Cox CD, Siu T, Danishefsky SJ (2003) Angew Chem Int Ed 42:5625
23. Siu T, Cox CD, Danishefsky SJ (2003) Angew Chem Int Ed 42:5629
24. Matsumoto T, Yamaguchi H, Suzuki K(1996) Synlett, p 433
25. Shair MD, Yoon TY, Mosny KK, Chou TC, Danishefsky SJ (1996) J Am Chem Soc 118:9509
26. Tamura Y, Sasho M, Ohe H, Akai S, Kita Y (1985) Tetrahedron Lett 26:1549
27. Tamura Y, Akai S, Kishimoto H, Sasho M, Kirihara M, Kita Y (1987) Tetrahedron Lett 28:4583
28. Tamura Y, Akai S, Kishimoto H, Kirihara M, Sasho M, Kita Y (1987) Chem Pharm Bull 35:1405
29. Kita Y, Maeda H, Omori K, Okuno T, Tamura Y (1993) Synlett, p 273
30. Kita Y, Maeda H, Takahashi F, Fukui S (1993) J Chem Soc Chem Commun, p 419
31. Kita Y, Maeda H, Takahashi F, Fukui S (1993) J Chem Soc Perkin Trans 1, p 2639
32. Kita Y, Maeda H, Omori K, Okuno T, Tamura Y (1993) J Chem Soc Perkin Trans 1, p 2999
33. Tamura Y, Kirihara M, Sasho M, Akai S, Sekihachi J, Okunaka R, Kita Y (1987) J Chem Soc Chem Commun, p 1474
34. Kirihara M, Kita Y (1997) Heterocycles 46:705

35. Tamura Y, Kirihara M, Sekihachi J, Okunaka R, Mohri S, Tsugoshi M, Akai S, Sasho M, Kita Y (1987) Tetrahedron Lett 28:3971
36. Kita Y, Kirihara M, Sekihachi J, Okunaka R, Sasho M, Mohri S, Honda T, Akai S, Tamura Y, Shimooka K (1990) Chem Pharm Bull 38:1836
37. Tamura Y, Annoura H, Yamamoto H, Kondo H, Kita Y, Fujioka H (1987) Tetrahedron Lett 28:5709
38. Fujioka H, Yamamoto H, Annoura H, Miyazaki M, Kita Y (1990) Chem Pharm Bull 38:1872
39. Kita Y, Maeda H, Kirihara M, Fujii Y, Nakajima T, Yamamoto H, Fujioka H (1990) Tetrahedron Lett 31:7173
40. Kita Y, Maeda H, Kirihara M, Fujii Y, Nakajima T, Yamamoto H, Tamura Y, Fujioka H (1992) Chem Pharm Bull 40:61
41. Tamura Y, Wada A, Sasho M, Kita Y (1981) Tetrahedron Lett 22:4283
42. Tamura Y, Wada A, Sasho M, Kita Y (1983) Chem Pharm Bull 31:2691
43. Croisy-Delcey M, Huel C, Bisagni E (1993) J Heterocyclic Chem 30:55
44. Croisy-Delcey M, Bisagni E, Huel C, Zilberfarb D, Croisy A (1991) J Heterocyclic Chem 28:65
45. Kita Y, Kirihara M, Fujii Y, Okunaka R, Akai S, Maeda H, Tamura Y, Shimooka K, Ohishi H, Ishida T (1991) Chem Pharm Bull 39:857
46. Kita Y, Kirihara M, Sekihachi J, Okunaka R, Sasho M, Mohri S, Honda T, Akai S, Tamura Y, Shimooka K (1990) Chem Pharm Bull 38:1836
47. Kita Y, Kirihara M, Sasho M, Fujii Y, Sekihachi J, Okunaka R, Tamura Y, Shimooka K (1990) Chem Pharm Bull 38:585
48. Matsuda F, Kawasaki M, Ohsaki M, Yamada K, Terashima S (1988) Chem Lett, p 653
49. Matsuda F, Kawasaki M, Ohsaki M, Yamada K, Terashima S (1988) Tetrahedron 44:5745
50. Izawa T, Wang Z-G, Nishimura Y, Kondo S, Umezawa H (1987) Chem Lett, p 1655
51. Lavallée J-F, Rej R, Courchesne M, Nguyen D, Attardo G (1993) Tetrahedron Lett 34:3519
52. Pandey RC, Toussaint MW, Stroshane RM, Kalita CC, Aszalos AA, Garretson AL, Wei TT, Byrne KM, Geoghegan RF Jr, White RJ (1981) J Antibiot 34:1389
53. Misra R, Pandey RC, Silverton JV (1982) J Am Chem Soc 104:4478
54. Misra R, Pandey RC, Hilton BD, Roller PP, Silverton JV (1987) J Antibiot 40:786
55. Warnick-Pickle DJ, Byrne KM, Pandey RC, White RJ (1981) J Antibiot 34:1402
56. Hilton BD, Misra R, Zweier JL (1986) Biochemistry 25:5533
57. Misra R (1988) J Antibiot 41:976
58. Latham MD, King CK, Gorycki P, Macdonald TL, Ross WE (1989) Cancer Chemother Pharmacol 24:167
59. Dalai NS, Shi X (1989) Biochemistry 28:748
60. Kita Y, Higuchi K, Yoshida Y, Iio K, Kitagaki S, Ueda K, Akai S, Fujioka H (1999) Angew Chem Int Ed 38:683
61. Kita Y, Higuchi K, Yoshida Y, Iio K, Kitagaki S, Ueda K, Akai S, Fujioka H (2001) J Am Chem Soc 123:3214
62. Kelly TR, Bell SH, Ohashi N, Armstrong-Chong RJ (1988) J Am Chem Soc 110:6471
63. Clive DLJ, Tao Y, Khodaborus A, Wu Y–J, Angoh AG, Bennett SM, Boddy CN, Bordeleau L, Kellner D, Kleiner G, Middleton DS, Nichols CJ, Richardson SR, Vernon PG (1994) J Am Chem Soc 116:11275
64. RamaRao AV, Singh AK, Rao BV, Reddy KM (1994) Heterocycles 37:893
65. Saint-Jalmes L, Lila C, Xu JZ, Moreau L, Pfeiffer B, Eck G, Pelsez L, Rolando C, Julia M (1993) Bull Soc Chim Fr 130:447

66. Wendt JA, Gauvreau PJ, Bach RD (1994) J Am Chem Soc 116:9921
67. Boger DL, Hüter O, Mbiya K, Zhang M (1995) J Am Chem Soc 117:11839
68. Fujioka H, Kitagaki S, Imai R, Kondo M, Okamoto S, Yoshida Y, Akai S, Kita Y (1995) Tetrahedron Lett 36:3219
69. Kita Y, Kitagaki S, Yoshida Y, Mihara S, Fang D-F, Fujioka H (1997) Tetrahedron Lett 38:1061
70. Kita Y, Kitagaki S, Yoshida Y, Mihara S, Fang D-F, Kondo M, Okamoto S, Imai R, Akai S, Fujioka H (1997) J Org Chem 62:4991
71. Kita Y, Yoshida Y, Kitagaki S, Mihara S, Fang D-F, Furukawa A, Higuchi K, Fujioka H (1999) Tetrahedron 55:4979
72. Corey EJ, Bakshi RK, Shibata S, Chen CP, Singh VK (1987) J Am Chem Soc 109:7925
73. Sharpless KB, Michaelson RC (1973) J Am Chem Soc 95:6136
74. Mitsunobu O, Yamada M (1967) Bull Chem Soc Jpn 40:2380

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