REVIEW ARTICLE

Sweet antibiotics – the role of glycosidic residues in antibiotic and antitumor activity and their randomization

Vladimír Křen & Tomáš Řezanka

Centre of Biocatalysis and Biotransformation, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Correspondence: Vladimír Křen, Centre of Biocatalysis and Biotransformation, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-142 20, Prague 4, Czech Republic. Tel.: 420296 442510; fax: 420296 442509; e-mail: kren@biomed.cas.cz

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Abstract

A large number of antibiotics are glycosides. In numerous cases the glycosidic residues are crucial to their activity; sometimes, glycosylation only improves their pharmacokinetic parameters. Recent developments in molecular glycobiology have improved our understanding of aglycone vs. glycoside activities and made it possible to develop new, more active or more effective glycodrugs based on these findings – a very illustrative recent example is vancomycin. The majority of attention has been devoted to glycosidic antibiotics including their past, present, and probably future position in antimicrobial therapy. The role of the glycosidic residue in the biological activity of glycosidic antibiotics, and the attendant targeting and antibiotic selectivity mediated by glycone and aglycone in antibiotics some antitumor agents is discussed here in detail. Chemical and enzymatic modifications of aglycones in antibiotics, including their synthesis, are demonstrated on various examples, with particular emphasis on the role of specific and mutant glycosyltransferases and glycorandomization in the preparation of these compounds. The last section of this review describes and explains the interactions of the glycone moiety of the antibiotics with DNA and especially the design and structure–activity relationship of glycosidic antibiotics, including their classification based on their aglycone and glycosidic moiety. The new enzymatic methodology 'glycorandomization' enabled the preparation of glycoside libraries and opened up new ways to prepare optimized or entirely novel glycoside antibiotics.

Introduction

In the 'ancient' years of antibiotics, the carbohydrate moieties in glycosidic antibiotics were mainly believed to modify their pharmacokinetic parameters, such as solubility, transport, and bioavailability, and also modulate their toxicity. Numerous experiments aimed at modifying these parameters generated large libraries of glycosides that could only be deconvoluted by extensive testing. Most of these pioneering experiments were carried out by chemical synthesis and quite often using common carbohydrates such as glucose, galactose, etc. In the last two decades, complementary enzymatic and chemo-enzymatic approaches have been used (Křen & Thiem, 1997; Bojarová-Fialová & Křen, 2007).

Later, when more data accumulated, the role of the glycosidic residue in numerous antibiotics became clearer (Křen & Martínková, 2001). This knowledge was also driven by the explosive development of glycomics and molecular studies of carbohydrate–protein interactions, as well as later studies of carbohydrate–DNA and carbohydrate–RNA interactions.

Although a significant amount of this knowledge can be generalized, all molecules must always be considered to be unique and all assumptions must always be verified by experiments. Because it is hard to define a uniform standard for structure–activity relationships (SAR), minimal inhibitory concentrations (MICs) and some *in vitro* activities are in most cases mentioned as biomarkers of these modifications. (Abbreviations are shown in Table 1). If necessary, other improvements to the biological profile are also included, such as selectivity, binding affinity, substrate of the bacterial enzyme, off-target activities, etc.

Exploitation of the SAR data of glycosides (glycosidic antibiotics) enabled synthetic modification and optimization of their glycone part. Recently, however, a brilliant new methodology, 'glycorandomization', based on enzymatic

Table 1. List of abbreviations

ADEPT	Antibody-directed enzyme prodrug therapy
AveBI	Avermectin glycosyltransferase
CalG1	Glycosyl transferase from *Micromonospora echinospora*
DNR	Daunorubicin (+ derivatives)
DOX	Doxorubicine
DRI	Drug resistance index
GABA	γ-Aminobutyrate
Glc-1-P-TT	α-D-Glucopyranosyl-1-phosphate thymidyltransferase
GT	Glycosyl transferase
GtfD	Glycosyltransferase gene GtfD from *Amycolatopsis orientalis*
GtfE	Glycosyltransferase gene GtfE from *Amycolatopsis orientalis*
HeLa	Cervical cancer cell line
HIV	Human immunodeficiency virus
IC_{50}	Half maximal (50%) inhibitory concentration (IC) of a substance
IDA	Idarubicin
IVG	*In vitro* glycorandomization
K562	Leukemia cell line (doxorubicine sensitive)
K562/Dox	Leukemia cell line (doxorubicine resistant)
LC–MS	Liquid chromatography–mass spectrometry
MBC	Minimum bactericidal concentrations
MFC	Minimum fungicidal concentrations
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass spectrometry
NDP	Nucleotide diphosphate
NMR	Nuclear magnetic resonance
PMT	Prodrug monotherapy
SAR	Structure–activity relationship
SgcA1	(TDP)-glucose synthetase
SW620	Human Caucasian colon adenocarcinoma cell line

modifications of glycosides, made it possible to build up glycoside libraries. This method, using natural and mutant glycosyltransferases with wobbling specificities, opened up new ways to prepare optimized or entirely novel glycoside antibiotics.

This review aims to describe the role of glycone in antibiotic activity, its modulation and targeting and its potential in creating new, better 'tuned' antibiotics. Besides 'classical' examples such as the streptomycin group, modern antibiotics such as glycopeptides and enediynes are also discussed in terms of their glycone/aglycone activity. All reports on derivatizations that do not involve the sugar moieties were omitted on purpose.

Numerous papers including those using new enzymatic glycorandomization approaches often only describe a 'proof of concept' based on mass-spectrometric characterization of the derivatives. There is also a plethora of synthetic papers with interesting working hypotheses on the glycosylation/glycorandomization of antibiotics in which, however, biological tests are completely absent. Such papers do not provide any SAR information and we tried to avoid those papers in this review.

At any rate, we hope that new glyco-derivatives and methodologies will bring some inspiration to pharmacists and pharmaceutical companies and thus help to combat the re-emergence of the worst diseases, for example, deadly infections.

Aminocyclitol antibiotics

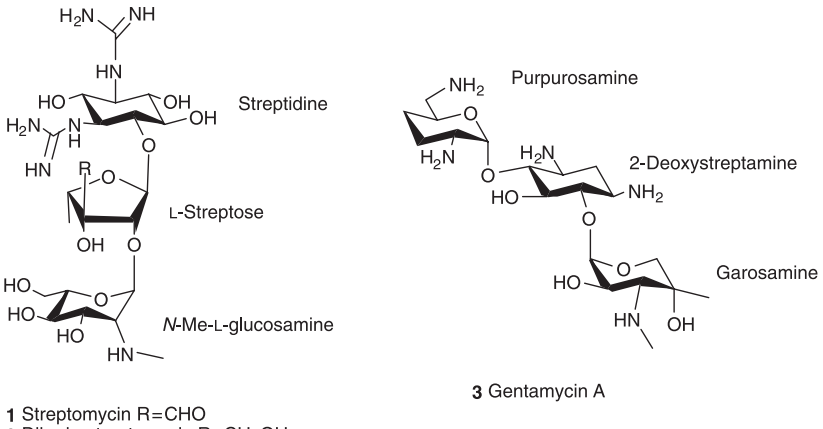
Aminocyclitol antibiotics contain aminosugar(s) and an aminocyclitol or a cyclitol moiety. This group consists entirely of moieties derived from carbohydrate metabolism, i.e. the molecules can be better characterized as 'oligosaccharides' rather than 'glycoconjugates'.

Streptomycin was discovered in Selman Waksman's laboratory in 1944 and neomycin in 1949; subsequently, kanamycin was reported by Hamao Umezawa in 1957, and gentamycin in 1963 by Marvin Weinstein's group at Schering Plough (Lemieux & Wolfrom, 1948; Ikeda & Umezawa, 1999). These antibiotics were mainly isolated from cultures of Actinomycetes and bacteria such as *Streptomyces*, *Nocardia*, *Micromonospora*, and *Bacillus*. In recent years, many related compounds have been isolated by fermentation or semisynthetic approaches. Aminoglycosides have long been used as very efficient drugs against G^+ and G^- bacteria as well as mycobacterial infections. Their widespread use over recent decades has been significantly compromised by their oto- and nephrotoxicity and the rapid emergence of bacterial resistance (Magnet & Blanchard, 2005).

Streptomycin (**1**), dihydrostreptomycin (**2**), and gentamycin C1A (**3**) – currently in clinical use – are typical representatives of the large group of aminocyclitol antibiotics. They interact with rRNA by affecting the fidelity of protein synthesis and inhibiting translocation of the ribosome (Davies & Davis, 1968). These antibiotics bind to a conserved sequence of rRNA that is near the site of codon–anticodon recognition in the aminoacyl-tRNA site of the 30S subunit.

It is well known that the most prevalent mode of aminoglycoside resistance is enzyme-catalyzed modification. Over 50 different types of aminoglycoside-modifying enzymes, found in resistant bacteria, are known to be responsible for the inactivation of aminoglycosides. These aminoglycoside-modifying enzymes, which are grouped into three classes, including aminoglycoside phosphotransferases, aminoglycoside acetyltransferases, and aminoglycoside nucleotidyltransferases, introduce chemical groups (mostly acetylation, adenylation, and phosphorylation) almost exclusively into the neamine core (rings I and II) of the aminoglycosides, including neomycin and kanamycin, thus causing a lowered or abolished antibiotic affinity to the RNA (Davies, 1994).

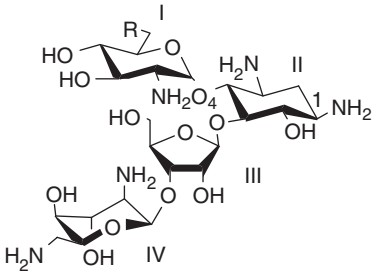
Unfortunately, the clinical application of aminoglycosides has been curtailed by their toxicity and a rapid increase in microbial resistance. It is, therefore, highly desirable to design new modified aminoglycosides that will overcome



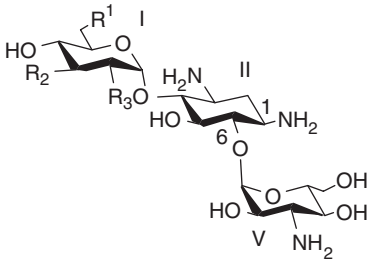
1 Streptomycin R=CHO
2 Dihydrostreptomycin R=CH_2OH

3 Gentamycin A

Fig. 1. Structures of streptomycin and gentamycin.



	R
4 Neomycin B	NH_2
5 Paramomycin	OH



	R^1	R^2	R^3
6 Kanamycin A	NH_2	OH	OH
7 Kanamycin B	NH_2	OH	NH_2
8 Kanamycin C	OH	OH	NH_2
9 TobramycinNH	NH_2	H	NH_2

Fig. 2. Typical structures of aminoglycoside antibiotics.

the adverse properties of naturally occurring aminoglycosides. Besides antimicrobial activity, aminoglycosides were also reported to be potential antiviral (HIV) agents, which makes them far more attractive.

In order to search for novel substances that act on RNA targets, several strategies are usually applied. One effort is mimicking the pharmacophore of a naturally occurring aminoglycoside and using a combinatorial approach to construct its respective derivatives. In the second approach, the pharmacophore is dimerically bound to the same or other aminoglycosides. The third strategy involves the addition of other moieties to the aminoglycoside pharmacophore to make twin drugs or conjugates. The fourth strategy is the rational design of aminoglycoside derivatives based on structural information or computer-aided analyses of the aminoglycoside–RNA complex. Generally, all of the above approaches utilize specific carbohydrate (vs. RNA) interactions.

A comparison of various aminoglycosidic antibiotics suggests the chemical groups that are essential to their function (Shaw *et al.*, 1993). Both amino groups of the 2-deoxystreptamine moiety (see e.g. in **3**, Fig. 1), which are positively charged at physiological pH, contribute to RNA binding as hydrogen donors.

A recent investigation of the binding mechanism of gentamycin C1A (**3**) showed that it binds in the major groove of the RNA. Rings I and II (this pseudodisaccharide is also called neamine) of gentamycin direct specific RNA–drug interactions. Ring III of gentamycin, which distinguishes this subclass of aminoglycosides, also directs specific RNA interactions with conserved base pairs (Yoshizawa *et al.*, 1998).

In terms of chemical structure, most aminoglycoside antibiotics can be regarded as the derivatives of neomycin or kanamycin (Fig. 2). However, cyclohexane ring II can mimic a glucopyranosyl structure and can behave as a 'glycomimetic'.

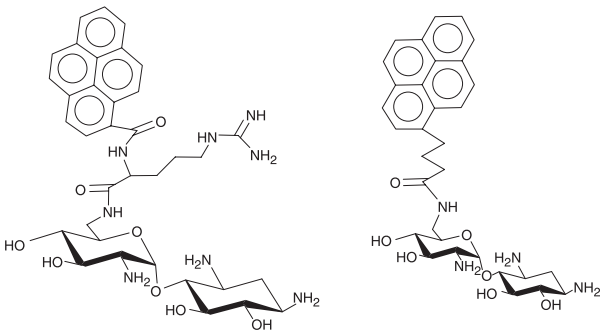
Particular modifications can be analyzed relative to the respective moiety in the tetracyclic structure (rings I–IV).

Greenberg *et al.* (1999) synthesized a series of compounds with amino groups at various positions on ring I. These compounds were analyzed for their binding to models of the *Escherichia coli* 16S A-site rRNA gene and for their

antibacterial activity. Analysis of a large range of compounds clearly demonstrated that the 2′,6′-diamino derivative of neamine was the most effective and specific A-site binder, while the mono amino compounds exhibited little activity. Based on these results, it was concluded that neamine was the most promising pseudodisaccharide core.

Another example is the parallel synthesis of a library of novel aminoglycoside analogs based on 2-amino-2-deoxy-D-glucose (ring I) and D-ribose scaffolds that is analogous to the above example (Rosenbohm *et al.*, 2001). The 2-amino group was left unreacted to mimic natural aminoglycosides and the 6-position was modified with different amines by various methods. Most of the new derivatives of this aminoglycoside moiety have a β-configuration, which is more synthetically accessible due to the neighboring group participation of the 2-NH_2 as a protecting group, whereas natural neamine is linked with an α-glycosidic bond. All these synthetic compounds were tested for their potential antibacterial, antifungal, and antiviral properties by *in vitro* standard methods (Moazed *et al.*, 1986). The final results showed MIC, minimum bactericidal concentrations (MBC), and minimum fungicidal concentrations (MFC) higher than 100 μg mL^{-1}, which is rather poor. This indirectly confirms the importance of the respective α-linkage in the basic neamine structure (Rosenbohm *et al.*, 2001).

Expecting that the compounds containing an aromatic ring are capable of intercalating with the major groove or stacking with the base of polynucleotides, Hamasaki *et al.* (2000) combined neamine with anthracene, acridine, and similar aromatic rings [e.g. pyrene (**11**)], and their binding affinities with RNA were examined (Fig. 3). The binding assay was evaluated by utilizing fluorescent resonance energy transfer with the intrinsically flexible Tat49–57 peptide (Matsumoto *et al.*, 2000). The binding affinities of these pyrene-substituted (the length of the linker was not critical) neamines are approximately two orders of magnitude higher than those of native neamine. These results suggest that the pyrene unit may intercalate with the groove or stack with the base in the RNA and, therefore, enhance the binding affinity. As the binding regions of both the Tat and the Rev proteins are arginine-rich peptide sequences (Litovchick *et al.*, 2001), the conjugation of neamine with arginine should enhance the binding affinity of the compound towards the RNA. A combination of both approaches [pyrene+arginine moiety conjugation (**10**)] created a ligand that binds to the respective RNA with an affinity *c.* 1000 times higher than the native neamine – this new molecule has multiple intermolecular interactions including electrostatic, hydrogen bonding, and aromatic stacking with the target RNA (Fig. 3).



10 11

Fig. 3. Neamine derivatives containing aromatic rings.

To circumvent the microbial resistance that occurred with aminoglycoside, Haddad *et al.* (1999) designed a self-regenerating aminoglycoside **12,** making the presence of aminoglycoside phosphotransferases irrelevant. Generally, these enzymes act on aminoglycosides to yield resistance via transferring the γ-phosphoryl group of ATP to the 30-hydroxyl of aminoglycosides (Fig. 4). The designed

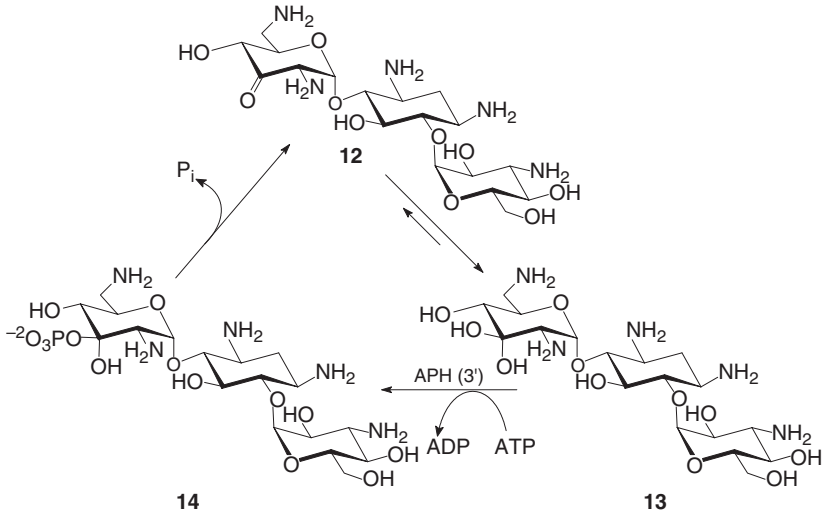


Fig. 4. Self-regeneration of aminoglycoside.

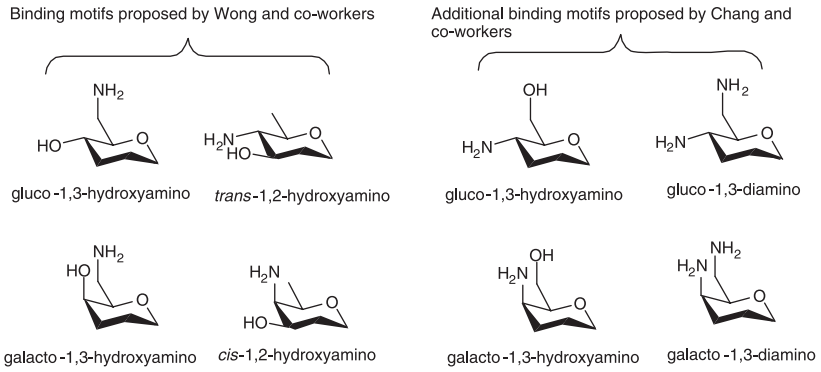


Fig. 5. Proposed binding motifs of aminosugars based on ring V in kanamycine B analogs (see also Fig. 2)

compound **12**, which mainly exists in the stable hydrated form **13**, undergoes enzymatic phosphorylation at the 3′-hydroxyl of ring I to produce **14**, but chemically the structure of the phosphorylated molecule would be unstable, leading to the elimination of phosphate and regeneration of the original antibiotic. The group evaluated the effectiveness of the modified aminoglycoside in killing both susceptible and resistant *E. coli*. When resistant *E. coli* was treated with kanamycin A, the MIC increased 500–1000-fold. With the regenerating aminoglycoside **12**, the MIC increased only fourfold. However, the MIC for the nonresistant *E. coli* was higher for the regenerating aminoglycoside **12** than for kanamycin A. The results demonstrated that this strategy is perfectly viable for lowering the MIC for resistant organisms.

The ubiquitousness and central location of 2-deoxystreptamin in aminoglycoside structures suggests that they play a pivotal role in biological activity. The presence of a bivalent structure of 2-deoxystreptamine alone, that is without accompanying aminosugars, exhibited low-micromolar binding to RNA hairpin loops (Liu *et al.*, 2004). Owing to its importance in maintaining biological activity as a whole, significant effort has been expended on ring II-based modifications and reconstructions. One major work was a series of substitutions of this ring with a broad array of substituents, aiming at improving binding to particular RNA moieties, as for example, polyamino, amino alcohol, or aromatic substituents (Greenberg *et al.*, 1999). Based on computer modeling studies (Jin *et al.*, 2000), which indicated that if ring I of neomycin B (**4**) (Fig. 2) was replaced with a benzyl group, the resulting compound would retain the conformation of neomycin B, and together with the consideration that heterocyclic rings in many cases comprise the very core of the active moiety or pharmacophore (Fink *et al.*, 1999; Ding *et al.*, 2001, 2003), 4-heterocyclic 2-deoxystreptamine was synthesized.

Haddad *et al.* (2002) took into account the steric and electronic contributions to interactions between RNA and aminoglycosides to make a random search of 273 000 compounds from the Cambridge structural database and the National Cancer Institute 3-D database for ribosomal aminoglycoside-binding pockets. A short series of compounds was synthesized, with the expectation that they would bind to the A-site of bacterial rRNA. Indeed, all synthetic compounds were found to bind to the target RNA comparably to the parent antibiotic neamine, with dissociation constants in the lower micromolar range. The synthetic compounds were evaluated for antibacterial activity against a set of important pathogenic bacteria. These designed antibiotics exhibited considerably enhanced antibacterial activities against pathogens, including organisms that hyperexpressed resistance enzymes to aminoglycosides.

A large series of modifications have been carried out to ring V of kanamycin B (Fig. 2). On the basis of commonly observed structural features of unusual sugars on aminoglycosides, Wong and colleagues (Hendrix *et al.*, 1997; Wong *et al.*, 1998) have proposed several binding motifs. Following these binding motifs, Chang and colleagues (Li *et al.*, 2004) proposed four new natural and nonnatural-binding motifs, including *gluco*-/*galacto*-1,3-diamino and variants of *gluco*-/*galacto*-1,3-hydroxylamino substructures (Fig. 5). Based on this proposal and combined with previous work on pyranmycin, they prepared a library of novel aminosugars with natural and nonnatural-binding motifs using an efficient divergent synthetic strategy starting from a common starting material (Elchert *et al.*, 2004).

Another work on ring II, reported by Chang, was the synthesis and antibacterial evaluation of a library of kanamycin B analogs (Li *et al.*, 2004), in which the 4,5-substituted 2-deoxystreptamine (neomycin B scaffold) was changed to a 4,6-substituted 2-deoxystreptamine (kanamycin B scaffold). The kanamycin analogs were tested against *E. coli* and *Staphylococcus aureus* using kanamycin B as the control, and binding toward the target site of 16S rRNA gene was evaluated by molecular modeling. Based on these evaluation results, the SAR of ring III is summarized in Fig. 6.

Supramolecular structure **15**, carrying two types of RNA binders, for example, neomycin B binding to the RNA stem and a chloramphenicol as a loop-binding component (Lee *et al.*, 2004), was designed and synthesized (Fig. 7). The

design was based on the hypothesis that hetero-aminoglycoside conjugates, which possess both anchoring groups (stem-affinity) and sequence-recognizing groups (loop-affinity), may have expanded regions of interaction with RNA. The results of foot-printing and mutation studies demonstrated that this conjugate displayed enhanced, site-selective binding to several RNA targets.

Significantly, it was observed that nonspecific RNA binders, such as neomycin B and a chloramphenicol, could be transformed into specific-binding agents by their incorporation into conjugates.

Recently, new supramolecular concepts leading to selectivity increases and the application of multivalency approaches have become a popular and often exploited area of glycodiversification of various biologically active compounds. Novel methodologies such as 'click chemistry' based on the Huisgen reaction and many others were developed and used. This approach was also used for derivatives based on aminoglycosidic antibiotics.

Wong and colleagues (Sucheck *et al.*, 2000) have also reported the synthesis of dimeric neamines. Using surface plasmon resonance and an immobilized 27-mer RNA construct, they found that neamine binds the oligonucleotide with a 2 : 1 stoichiometry and a K_d of *c.* 10 mM at each binding site. Thus, a library of neamine dimers was constructed using various amide- and amine-linkers (Fig. 8). Certain dimeric compounds were found to possess binding affinities improved 10-fold compared with neamine. Meanwhile, some of the dimers possessed a significantly increased antibiotic activity against *E. coli* compared with neamine. Additionally, these molecules were observed to be poor substrates for some aminoglycoside-modifying enzymes responsible for 6′- and 3′-*N*-acetylation and *O*-phosporylation. This concept was subsequently used by numerous authors, often with substantial success (Zhou *et al.*, 2007).

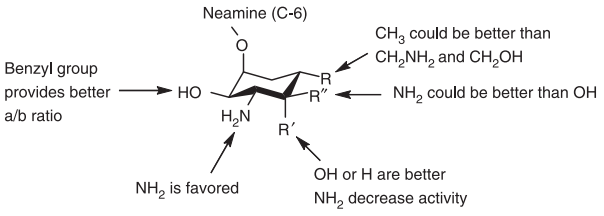


Fig. 6. Summary of SAR of ring V of kanamycin B analogs.

Anthracyclines

Anthracyclines represent a relatively large group of natural, semisynthetic, and synthetic compounds. Most of them are composed of a tetracyclic aromatic polyketide planar scaffold coupled with L-deoxysugars (Luzhetskyy *et al.*, 2007). The main molecular target that determines the clinical activity of these compounds is the inactivation of DNA topoisomerase II (Wang, 1996; Arcamone & Gassinelli, 1998). This topic has been reviewed extensively (Sperker *et al.*, 1997) and therefore only the most promising and recent results will be discussed here. Most of their structures and additional information are also given in a recent paper (Kratz *et al.*, 2006).

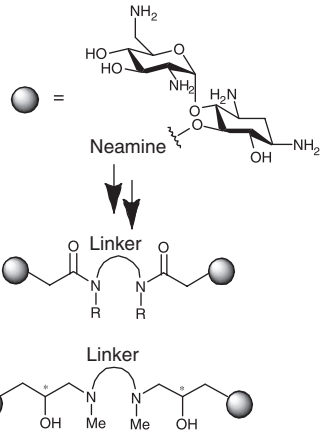


Fig. 8. Neamine dimers with various amide- and amine-linkers.

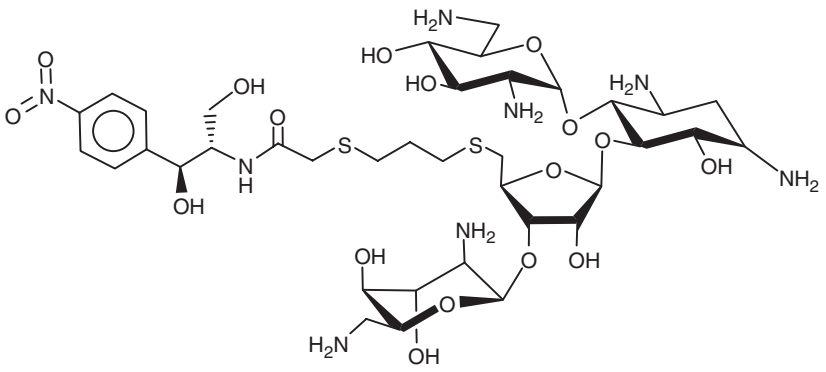


Fig. 7. Structures of heterodimeric conjugate of neomycin B and a chloramphenicol.

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The five major anthracyclines in clinical use are doxorubicin, daunorubicin, epirubicin, adriamycin, and idarubicin. These compounds are approved for the treatment of breast and small cell lung cancers, acute myeloid leukemia, pancreatic and gastric cancers, various sarcomas, and some others (Arcamone *et al.*, 1997; Kratz *et al.*, 2006). The clinical application of anthracyclines is limited by their dose-related side effects including bone marrow toxicity, gastrointestinal disorders, stomatitis, alopecia, acute and cumulative cardiotoxicity as well as extravasation (Gianni *et al.*, 2003). Both the antitumor potency and the toxicological profile of the anthracyclines are powerful stimuli for an industrial search for more effective and less toxic anthracycline analogs; thus, *c.* 2000 derivatives have been prepared over the past 20 years. Numerous modifications were carried out in the deoxysugar unit of anthracyclines because this portion of the molecule appeared to be positioned in the DNA minor groove adjacent to the intercalation site, and it plays an important role in forming and stabilizing interactions in the ternary DNA–topoisomerase II–drug complex through specific direct molecular contact. Chemical and configurational changes in the sugar moiety of the anthracycline molecules were reported to attenuate their cytotoxic activity (Minotti *et al.*, 2004). However, chemical synthesis and modification of these anthracycline compounds, especially their deoxysugar-containing parts, is not a trivial operation (Gildersleeve *et al.*, 1999). Combinatorial biosynthesis is an alternative technology for accessing otherwise unavailable structures through the combinatorial application of genetic engineering, that is, new biosynthetic pathways are built from the large and growing collection of genes for natural product biosynthesis (Floss, 2006).

Typical examples are daunomycin (**16**) or adriamycin (**17**), whose anticancer activities are attributed to the intercalation of the drug with DNA (Wang *et al.*, 1987). Two daunomycin molecules intercalate at each of the two C : G sites at either of the sites of the duplex d(CGTACG). This binding results in an increase in base pair separation from 3.4 to 6.8 Å as the molecule associates, which in turn leads to an unwinding of the helix and the formation of a noncovalent complex with the DNA. This results in the inhibition of DNA replication and RNA transcription (Moore *et al.*, 1989).

Rings B–D of the aglycone are intercalated with the DNA, leaving ring A and the aminosugar as anchoring units. The cationic aminosugar and the hydroxy group of ring A fill the minor groove, which displaces water and ions from the groove. The aminosugar does not form hydrogen bonds with the adjacent bases. The amino and hydroxy groups of the sugar face away from the minor groove, and it has been suggested that they may interact with polymerases, which could prevent or retard the action of these enzymes.

It was observed that anthracyclines containing several glycosyl moieties have, in general, less side effects. For example, marcellomycin (**18**) and aclacinomycin A (**19**) exhibit a lower cardiotoxicity than daunomycin.

This is also the reason why many attempts to introduce another and/or more carbohydrate moieties have been made. The reaction of daunomycinone with diols, producing its 7-*O*-hydroxyalkyl (Fig. 9) derivatives, enabled the

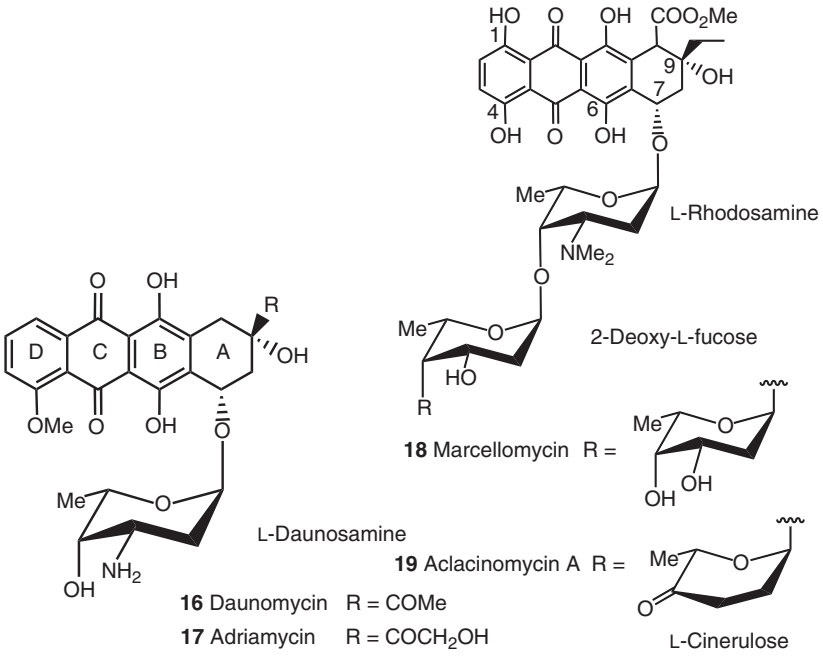


Fig. 9. Structures of typical anthracyclines.

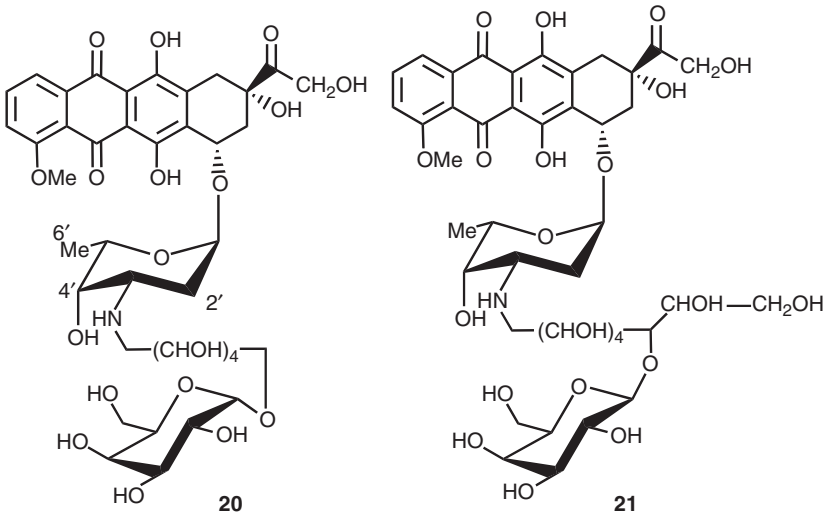


Fig. 10. Glycosyl derivatives of daunomycin.

sugars to be attached in a different manner, leading to derivatives with a lower toxicity (Jizba *et al.*, 1998).

Nogalamycine, which contains two sugars (one attached to the D-ring and the other attached at C-7 of the A-ring), binds to DNA in a slightly different manner (Weymouth-Wilson, 1997).

The prodrugs, in which glucuronic acid, galactose, and glucose are linked directly or through a self-immolative spacer to the 3′-amino position of the anthracycline, are characterized by good water solubility, a considerable reduction in cytotoxicity compared with the free anthracycline, and the possibility of being activated at the tumor site, either by elevated levels of endogenous enzymes [prodrug monotherapy (PMT)] or by enzymes (β-glucuronidase, α- and β-galactosidase, and β-glycosidase) of nonhuman origin, which are delivered by antibody-directed enzyme prodrug therapy (ADEPT). PMT utilizes the increased activity of endogenous β-glucuronidase that occurs due to lysosomal liberation in inflammatory cell infiltration sites and necrotic areas of solid tumors (Murdter *et al.*, 1997). Considerable β-glucuronidase activity is also observed in a variety of tissues, especially in the liver, spleen, and intestinal microorganisms, where nontarget-oriented prodrug activation takes place (Bosslet *et al.*, 1994). Further problems with PMT lie in the fact that the β-glucuronide prodrug, with its relatively high polarity, has to pass through the cell membrane to be lysosomally activated by β-glucuronidase. For this reason, ADEPT approaches could have advantages. Human β-glucuronidase, however, is not an optimal enzyme for enzyme prodrug therapy, because it exhibits only 10% of its activity at neutral pH and, in addition, needs to be glycosylated to exert its activity at all (Sperker *et al.*, 1997). In terms of the potential practical use of their antitumor effects, it should be noted that none of the 30-plus compounds tested had IC_{50} values lower than the standards epirubicin, doxorubicin, and daunorubicin.

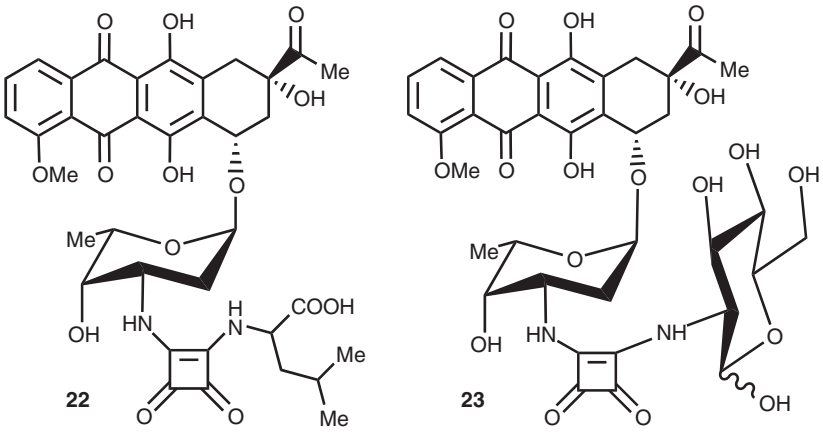
In the first part of this survey, we summarized modifications of the 3′-amino group in daunosamine. Owing to the nonsystematic preparation of these derivatives (Olsufyeva *et al.*, 2003), complete SAR are unknown. Glycosylation at this position (**20** and **21**) diminishes their inhibitory activity towards murine leukemia L1210/0 by approximately two orders of magnitude compared with doxorubicin (Fig. 10).

Further modifications of the amino group of daunosamine via squaric acid (Sztaricskai *et al.*, 2005) diminished their activity against human leukemia (HL-60) (**22**, $IC_{50} = 0.18\,\mu M$ and **23**, $IC_{50} = 0.22\,\mu M$) compared with daunosamine itself ($IC_{50} = 0.12\,\mu M$) (Fig. 11).

Replacing the 3′ amino group with an azide enables further conjugation using so-called 'click-chemistry' (Huisgen reaction) with ethinyl group-substituted ligands to form a peptide-isosteric triazole linker (Fang *et al.*, 2006). The ensuing derivatives (**24–27**) had a lower cytotoxicity in both drug-sensitive (K562) and drug-resistant leukemia cells (K562/Dox), with a 25-fold lower drug resistance index than daunorubicin (Fig. 12).

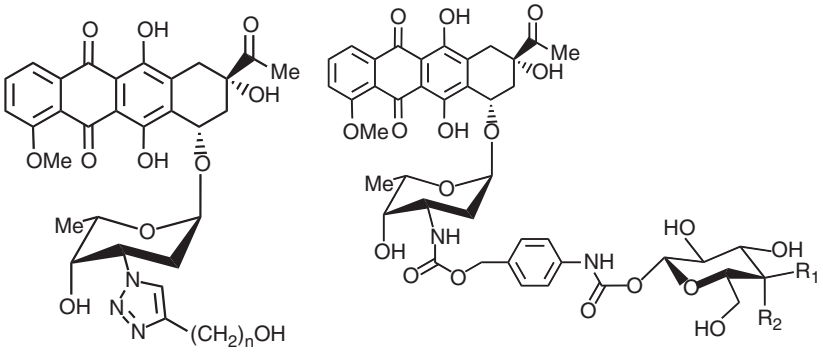
One of the possible applications of targeted drugs is an enzyme–prodrug therapy, i.e., introduction of the given glycoside with an enzyme directly to the target site where the active substance is then released by enzymatic hydrolysis. The treatment of a human ovarian cancer cell line with human β-glycosidase ($0.5\,U\,mL^{-1}$) and 0.5 mM galactoside (**28**) or a glucoside (**29**) prodrug of daunorubicin resulted in 86% and 81% cell growth inhibition, respectively, while the prodrugs alone inhibited growth by only 19% and 1% (de Graaf *et al.*, 2003) (Fig. 12).

Another large series of studies deals with modifying the saccharide moiety of anthracyclines, introducing mainly



22 **23**

Fig. 11. Modifications of the amino group of daunosamine via squaric acid.



24 (*n*=1), **25** (*n*=2), **26** (*n*=3), **27** (*n*=4).

28 DNR-G1A3: R_1=H, R_2=OH
29 DNR-GsA3: R_1=OH, R_2=H

Fig. 12. Modifications of the glycone part of anthracyclines (DNR = Daunorubicin + derivatives).

rare, unusual saccharides. The sugar moiety is an essential component of anthracycline antibiotics for their topoisomerase poisoning activity and antitumor efficacy (Zunino *et al.*, 2001). Because the sugar interacts with the minor groove, modifications in this moiety could enhance the recognition potential of the drug at the substrate level. Based on this hypothesis, novel anthracyclines were designed – disaccharides lacking the amino group in the first (aglycone-linked) sugar. The 3′-amino group in the first sugar was replaced with a hydroxyl group, and the second sugar residue was bound to the first sugar via a (1 → 4) linkage. The cytotoxic and antitumor activities of disaccharide analogs of idarubicin were critically dependent on the optimal (axial) orientation of the second sugar residue. Although the configurational requirements of the sugar moiety for optimal drug activity support a critical role for the external (non-intercalating) drug domains in the interaction of anthracyclines with the DNA–topoisomerase (ternary complex), the antitumor efficacy of disaccharide analogs is not fully explained by effects mediated by the nuclear enzyme target. The development of this novel disaccharide series may provide insights into a rational synthesis of anthracycline analogs with improved pharmacological profiles.

2,6-Dideoxy sugars are a better choice for generating biologically active daunorubicin analogs than 6-deoxysugars, 2,3,6-trideoxysugars, or 2,3,4,6-tetradeoxysugars (Zhu *et al.*, 2005). Aglycone without a sugar moiety has only a 70–100-fold lower activity against cancer cells than daunorubicin derivatives with various uncommon sugars. In compounds **30–35**, carrying various sugars, the 4′-OH of the sugar is an important determinant for their activity, while the axial-3′-substituent in the sugar interferes with the binding of daunorubicins to DNA (Fig. 13).

The disaccharide analogs (**36–41**), where the first (inner) sugar in the carbohydrate chain was a 3-azido-2,3,6-trideoxy-L-*lyxo*-α-hexopyranose and the second (outer) sugar was from a series of uncommon sugars, were synthesized by (Zhang *et al.*, 2006c) (Fig. 14). Their cytotoxicities were examined in drug-sensitive leukemia cells K562 and doxorubicin-resistant K562/Dox cells. In drug-sensitive cells, compounds **36–41** were found to be active against leukemia K562 cells with an IC_{50} in the nanomolar range (200–1100 nM), while compounds **37–40** with 2,6-dideoxy sugars showed a higher activity than compounds **36** and **41** with 2,3,6-trideoxy sugars. In doxorubicin-resistant K562/Dox cells, compounds **36**, **38**, and **40** exhibited much higher

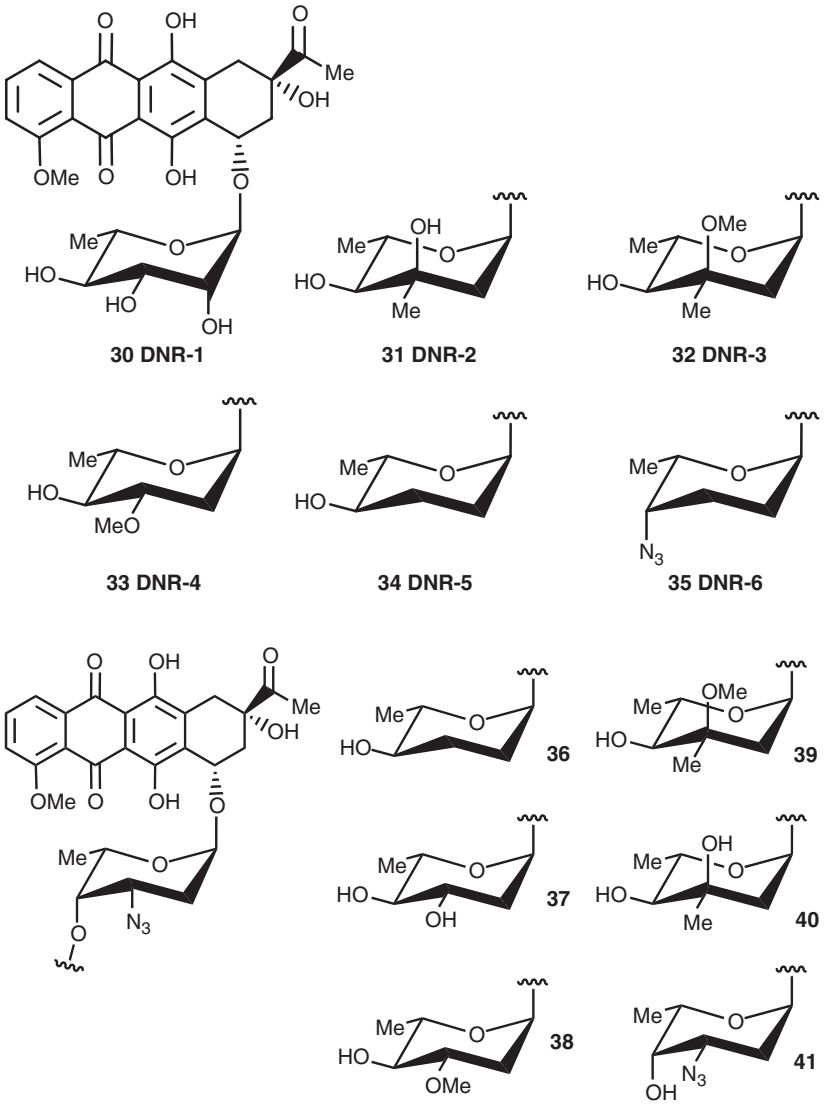


Fig. 13. 2,6-Dideoxy sugar derivatives of daunorubicin.

Fig. 14. Disaccharide analogs of daunorubicin.

Table 2. Cytotoxicity (IC_{50}) and drug resistance index (DRI) of disaccharide daunorubicins and DNR compounds

	Compounds						
	36	**37**	**38**	**39**	**40**	**41**	DNR
IC_{50} in K562 (μM)	0.787	0.287	0.278	0.225	0.448	1.132	0.015
IC_{50} in K562/Dox (μM)	1.926	5.437	0.289	12.070	1.628	6.388	> 5
DRI	2.45	18.94	1.04	53.64	3.63	5.64	> 333

activities (with an IC_{50} between 0.29 and 2.0 μM) than daunorubicin (with $IC_{50} > 5$ μM). Compound **38** emerged as the most active compound, exhibiting an activity at least 17-fold higher against drug-resistant cells than the parent compound DNR. SAR studies showed that the substitution and orientation of the 3-OH group in the second sugar significantly influences its activity against drug-resistant leukemia (Table 2).

In both leukemia K562 and colon cancer SW620 cell lines, compounds with various terminal 2,6-dideoxy sugars (compounds **42**, **44**, **46**, and **49**) (Fig. 15) exhibited anticancer activities 30–60-fold higher than compounds with 2-deoxy- or 6-deoxy sugar (compounds **47** and **48**) (Zhang *et al.*, 2005). Compounds with an α-linkage between two sugar units (compound **44**) exhibited a 35-fold higher anticancer activity than compounds with a β-linkage (compound **45**) (Table 3). In addition, the anticancer activities of these compounds correlated with their ability to target topoisomerase II-mediated genomic DNA damage *in vivo*. Compounds **42** and **44** with 2,6-dideoxy sugars produced a more covalent topo–DNA complex than compounds with 2-deoxy sugar (**47**) and 6-deoxy sugar (**48**). Compounds with an α-configuration of terminal 2,6-dideoxy sugar

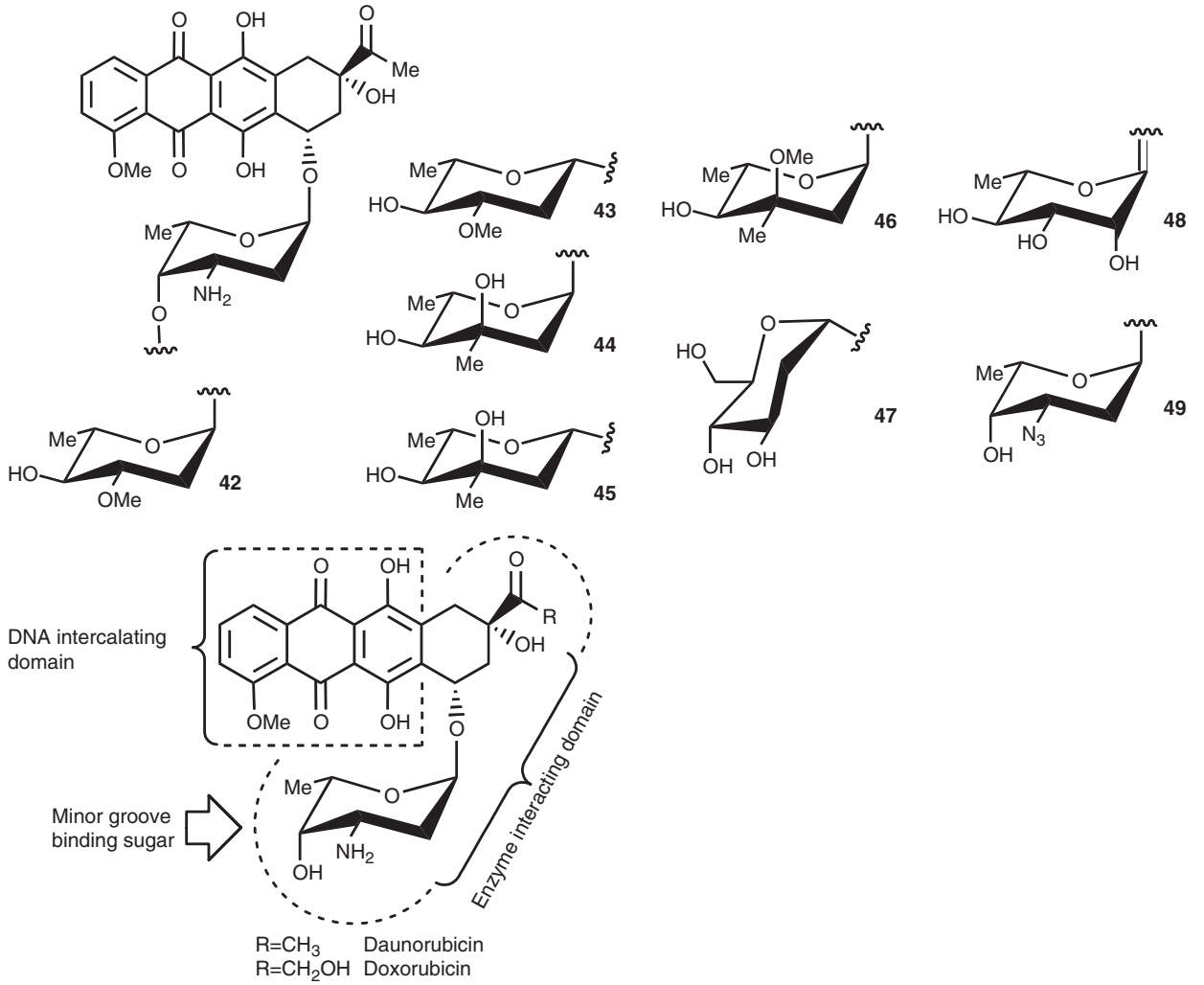


Fig. 15. Major moieties of daunorubicin participating in DNA binding.

Table 3. Anticancer activity (IC_{50}) of synthesized compounds in two cancer cell lines compounds

	Compounds								
	42	**43**	**44**	**45**	**46**	**47**	**48**	**49**	DNR
IC_{50} in K562 (nM)	39.5	45.8	44.3	1531.6	21.0	1378.3	>4000	31.3	15.6
IC_{50} in SW620 (nM)	18.2	38.9	21.7	>1000	11.4	>1000	>1000	60.3	8.6

(compounds **42** and **44**) exhibited higher topoisomerase II poisoning than their counterparts with the β-configuration (compounds **43** and **45**). These results indicate that the sugar moieties in daunorubicin play a significant role in its anticancer activity and topoisomerase II inhibition. Daunorubicin derivatives possessing an α-linked 2,6-dideoxy-type saccharide in the terminal position (typically **42** and **44**) exhibited the best anticancer activity.

With the exception of the trisaccharide analog **51**, for which other biological events, such as cellular uptake, must be involved in order to explain its lack of cytotoxicity, the disaccharide derivatives **50**, **52**, and **53** (Figs 16 and 17) showed a cytotoxic potency similar to that of the reference compound (Cipollone *et al.*, 2002). On the other hand, the ability to poison topoisomerase II was different for these compounds. Epimerization of the C-4″ position (**50**) or introduction of a further carbohydrate residue (**51**) did not affect their capacity to induce DNA breaks, while removal of the hydroxyl group at the C-3′ position of compound **52** seemed to influence the stability of the ternary complex, as

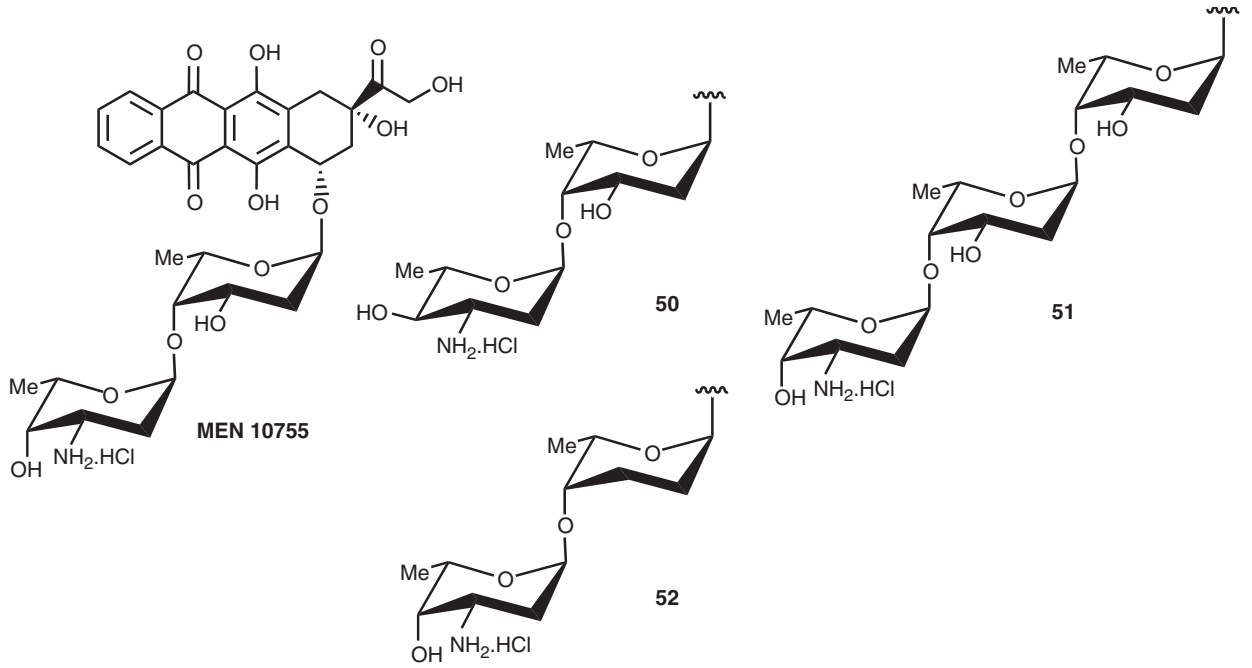


Fig. 16. Di- and trisaccharide derivatives of daunorubicin.

the intensity of the topoisomerase II-mediated DNA cleavage appeared to be slightly reduced. These results seem to indicate that no direct correlation exists between the extent of enzyme-induced DNA cleavage and cytotoxicity in this class of oligosaccharide anthracyclines.

Structure–activity studies in experimental solid tumors showed that replacing daunosamine with a disaccharide moiety dramatically reduced the cytotoxic potency of the drugs in the 4-methoxy series. In contrast, in the 4-demethoxy series, the C-4 axial configuration (but not the equatorial configuration) conferred a cytotoxic potency and antitumor activity several times higher than that of daunorubicin (DNR) or idarubicin (IDA) and virtually comparable with that of doxorubicine (DOX) (Arcamone *et al.*, 1999). The configuration also influenced the ability of disaccharide anthracyclines to stimulate topoisomerase II-mediated DNA cleavage. Only glycosides with an axial orientation proved to be active, but methoxy-substituted analogs were significantly less effective than demethoxy analogs. On balance, it became apparent that the axial orientation only governed the optimal interactions of disaccharide anthracyclines with the DNA–topoisomerase II complex in the absence of the methoxy group (Arcamone *et al.*, 1999).

Members of the aureolic acid group include chromomycin A_3 (**53**), olivomycin A (**54**), and mithramycin (plicamycin) (**55**) (Fig. 17). The aglycone of chromomycin A_3 is identical to that of mithramycin, but differs from that of olivomycin A by a methyl group. Conversely, the sugar components for chromomycin A_3 and olivomycin are nearly identical, while those in mithramycin are different.

These drugs require a divalent metal ion, preferably an Mg^{2+} ion, and a guanine-containing target to be active. They were identified to be inhibitors of DNA-dependent RNA polymerase. It was found that compound **42** (Fig. 15), lacking some of the sugar moieties, was less active (Haysaka & Inoue, 1969) and their aglycone does not bind to DNA at all (Kaziro & Kamiyama, 1967).

The mode of action of these antibiotics is different from other anthracyclines, despite their aglycone similarity. Aureolic acids do not intercalate into DNA but they bind to CG-rich regions. Nuclear magnetic resonance (NMR) studies demonstrated that **53** forms a symmetrical dimer with self-complementary d(TTGGCCAA) with the hydrophobic edge of the chromophore located next to the GC : CC site (Gao & Patel, 1989). The sugars in the olivose–olivose–olivomycose trisaccharide extend towards the 3′-direction of the octanucleotide in the minor groove. The chromose disaccharide and the hydrophilic side chain point towards the phosphate backbone. The trisaccharide olivose–olivose–olivomycose part is essential for stabilizing the drug–Mg^{2+} complex that binds effectively to DNA; removal of the terminal sugar D-chromose A (e.g. from **53** or **54**) has no effect on complex formation (Silva & Kahne, 1993).

The action of mithramycin (**55**) is virtually the same; however, it is less specific for the DNA sequences flanking the central GC : GC step due to the presence of D-mycarose (compared with L-olivomycose in **53** or **54**) (Sastry & Patel, 1993).

A recent review (Salas & Méndez, 2005) provides fundamental insights into the SAR of glycosylated antracyclines,

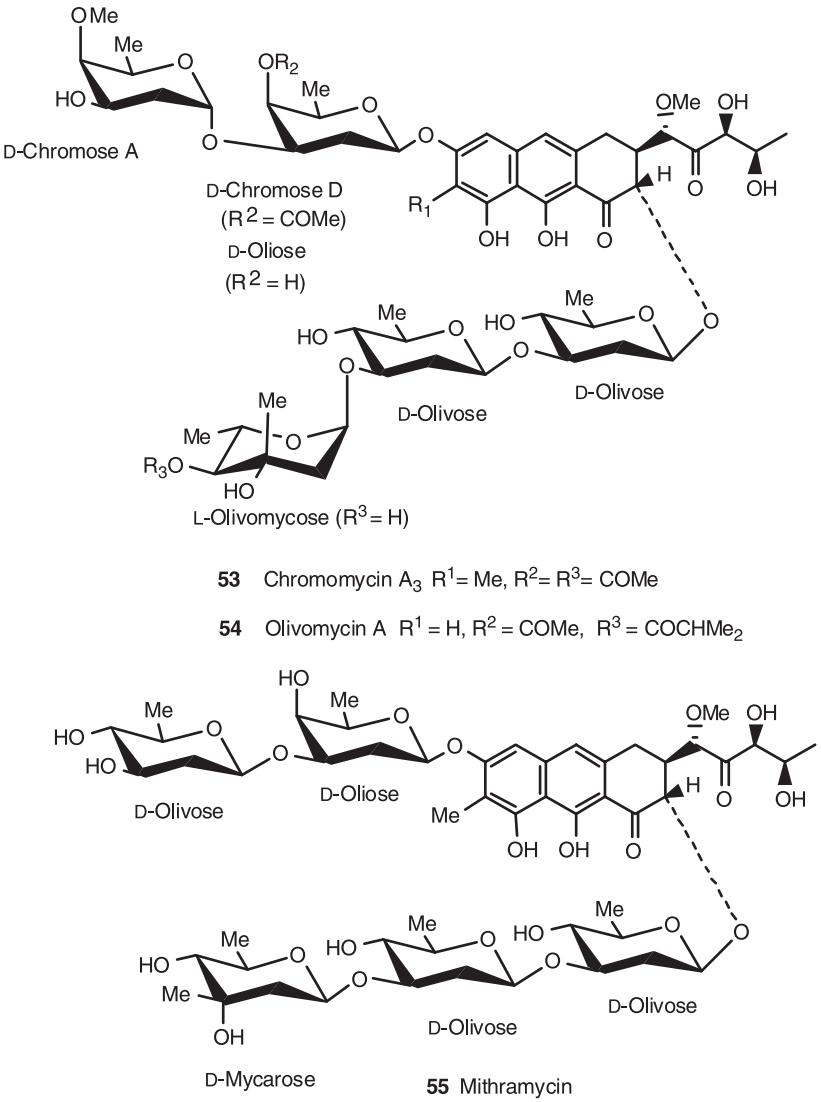


Fig. 17. Structures of the aureolic acid-group anthracyclines.

describing glycosylated compounds, in which the appended sugars contribute to specific interactions with their biological target. Most of these sugars are 6-deoxyhexoses, of which more than 70 different forms have been identified, and an increasing number of gene clusters involved in 6-deoxyhexose biosynthesis are being characterized from antibiotic-producing actinomycetes. Novel glycosylated compounds have been generated by modifying natural deoxysugar biosynthesis pathways in the producer organisms, and/or the simultaneous expression in these strains of selected deoxysugar biosynthesis genes from other strains. Nonproducing strains endowed with the capacity to synthesize novel deoxysugars through the expression of engineered deoxysugar biosynthesis clusters can also be used as alternative hosts. The transfer of these deoxysugars to a multiplicity of aglycones relies on the existence of glycosyltransferases with an inherent degree of 'relaxed substrate specificity'. This same review (Salas & Méndez, 2005) describes the glycorandomization of antracyclines with deoxysugars, using the detailed knowledge of deoxysugar pathways from actinomycetes.

Glycopeptides

This group of antibiotics is made up of complex polypeptide aglycones, which are abundantly glycosylated with mono-, di-, and tetrasaccharides (Stubbe & Kozarich, 1987). Approximately 100 members of this group have been discovered so far, such as vancomycin, teicoplanin, bleomycin, ristocetin, etc. Some of them are very important, as they are often *ultimum refugium* – the last possible treatment of multiple-resistant bacteria infections – typically methicillin-resistant *S. aureus* (MRSA). Recent discoveries demonstrated that glycosidic residues play crucial roles in their activity and this

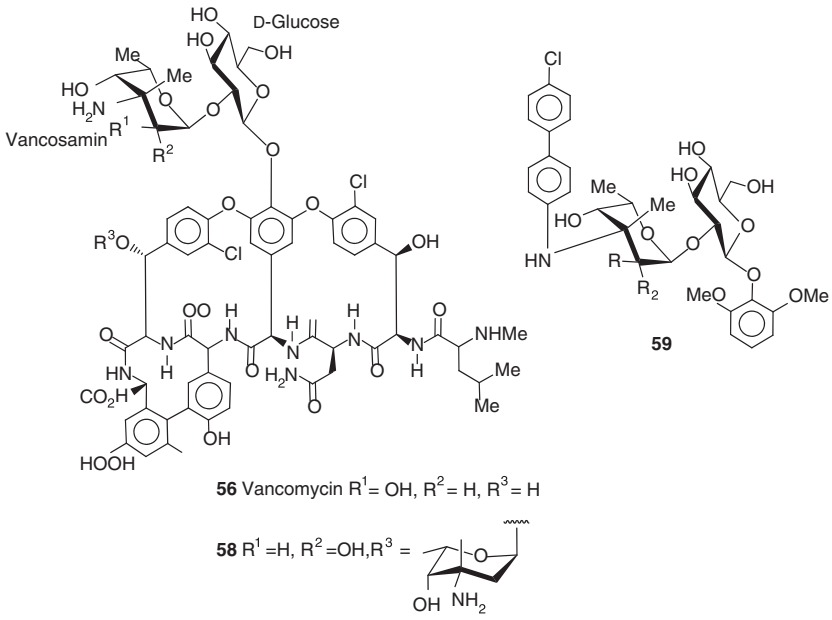


Fig. 18. Structure of vancomycin.

opens up a large field for developing new glycosidic antibiotics, as was shown with vancomycin.

The story of the involvement of glycosyl moieties in vancomycin activity is probably one of the most illustrative and also very topical, as glycosyl derivatives of vancomycin quite recently proved to be very effective against multiple-resistant (even vancomycin-resistant) bacteria.

Vancomycin group

Vancomycin (**56**) (Fig. 18) (Williams, 1996) is a glycopeptide antibiotic that is widely used in the treatment of Gram-positive bacterial infections. It was discovered in a soil sample from the jungles of Borneo during a research program carried out by the pharmaceutical company Eli Lilly in the mid-1950s. *Streptomyces orientalis* (later reclassified as *Nocardia orientalis* and finally *Amycolatopsis orientalis*) were used to produce this antibiotic. It was first used in clinics in 1959, and today teicoplanin (**57**) (Fig. 19) is also being used clinically. Its importance has increased considerably recently, as it is one of the few antibiotics effective against nosocomial infections, for example, multiple-resistant bacteria – a typical example is MRSA, and together with gentamycin it is a last-resort antibiotic. Unfortunately, resistants against vancomycin and teicoplanin (Mayville *et al.*, 1999; Sieradzki *et al.*, 1999) have also developed and therefore its further modification, including glycorandomization, is of utmost importance.

Glycopeptide antibiotics are normally produced as a mixture of compounds by a microbial producer. Teicoplanin A_2-2 differs from other glycopeptides in its *N*-acylation of the glucosamine moiety by the C_{11}-side chain. This structural feature has been used recently in the structural variation of other glycopeptides (Griffith *et al.*, 2007) – *vide infra*.

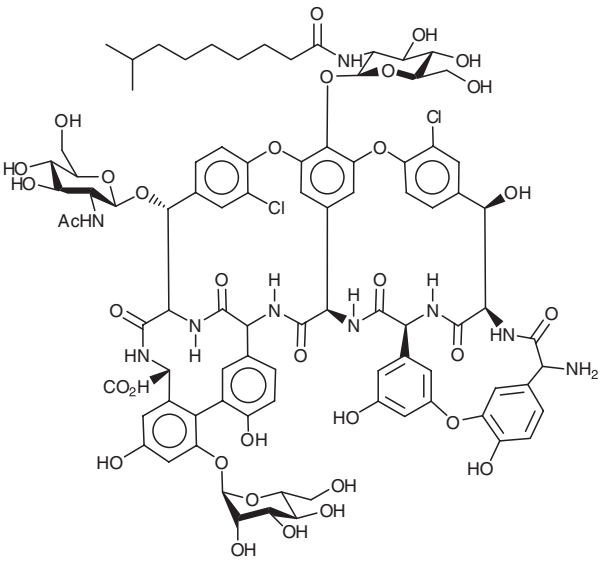


Fig. 19. Structure of teicoplanin.

Vancomycin consists of a core heptapeptide with attached saccharide moieties, one of which is the deoxyaminosugar vancosamine. Vancomycin exhibits its antibacterial activity by binding bacterial cell wall mucopeptide precursors terminating in the sequence L-Lys–D-Ala–D-Ala (Perkins, 1969), thereby impeding further processing of these intermediates into peptidoglycans. Five hydrogen bonds account for this binding specificity, and the disruption of one of these

hydrogen bonds by the replacement of the terminal alanine with lactate (D-Ala–D-Lac) in the mucopeptide precursor is the molecular basis for resistance to **56**. Bacterial cell wall biosynthesis proceeds in two steps at the outer edge of the cell wall, starting with the disaccharide moiety attached to the peptide being transported from the cytoplasm out of the cell. The disaccharide moiety and peptide are linked together by transglycosidase.

Because of the ultimate importance of vancomycin in the treatment of often fatal infections caused by multiple-resistant bacteria, extensive efforts have been directed towards finding vancomycin derivatives with activity against drug-resistant bacteria (Nagarajan, 1993). As a result of these efforts, several derivatives such as **58** (LY264826) have been found to be up to 500 times more effective than vancomycin itself. The most notable difference is the presence of another aminosugar (R^3) attached to amino acid 6. This extra sugar possibly facilitates the dimerization of the antibiotic and/or anchors the antibiotic to the cell membrane, both of which have been shown to be important for vancomycin activity (Williams, 1996). It was also demonstrated that the conformations of vancomycin and its aglycone differ in their alignment of the amide protons that participate in the hydrogen-binding network with cellwall precursors (Boger *et al.*, 1995). Alkylation of the 3-amino group on the disaccharide at amino acid residue 4 further enhances their activity, probably by serving as a hydrophobic anchor to the cell membrane (Nicas *et al.*, 1996).

Another target in the modification of vancomycin is the vancosamine moiety. It was recently found that *N*-alkylation with *n*-decyl or 4-chlorobiphenyl groups results in an antibiotic that acts via a different mechanism than vancomycin itself (Ge *et al.*, 1999) and, therefore, exhibits activity against vancomycin-resistant microorganisms. Sugar-altered vancomycin interferes with the polymerization of a glycopeptide monomer to form cell wall material in the first place. Thus, altered vancomycin may disrupt cell wall formation at multiple levels by inhibiting the formation of the glycopeptide monomer, straight-chain polymer, and cross-link reinforcement.

In fact, the altered disaccharide derivative itself (**59**) has strong antibacterial activity, even if not attached to the rest of the large vancomycin molecule (Ge *et al.*, 1999). This compound, however, acts via a different mechanism than vancomycin. It inhibits the transglycosylation step that precedes the transpeptidase step blocked by vancomycin itself. This finding explains the complex mechanism of action of derivative **58**.

These findings offer the possibility of using sugar chemistry to make glycopeptide antibiotics such as vancomycin more potent against bacteria, for example, if vancosamine was replaced with daunosamine (3-desmethyl-vancosamine). Although this derivative only differs from **57** in one methyl group on the terminal sugar, it shows some notable differences in activity (Ge *et al.*, 1998). A general methodology for selective glycosylation of the vancomycin aglycone has been developed by Kahne's group (Thompson *et al.*, 1999). It is quite likely that a wide-ranging investigation with various sugars will lead to more significant improvements across a range of bacterial strains.

Structural inspiration based on a combination of structural features from the teicoplanin and vancomycin molecules led to the design of glyconeopeptides active against vancomycin-resistant *Enterococci* (Griffith *et al.*, 2007). The neoglycosylation of a methoxyamine-linked vancomycin aglycone with all possible *N*′-decanoylglucopyranose and *N*′-biphenoylglucopyranose regioisomers resulted in a set of liponeoglycopeptide variants in good yields. Antibacterial assays using a panel of vancomycin-resistant *Enterococci faecalis* and *Enterococcus faecium* clinical isolates revealed glucose C3′ or C4′ as the optimal position for neoglycopeptide lipidation.

Another strategy leading to vancomycin derivatives that are active against VRE is the modification of the macrocyclic-binding pocket (Jia *et al.*, 2006a, b). The basic structural features of these biaryl ether-containing macrocycles are: (1) deletion of the carboxyl group of vancomycin's central amino acid (amino acid D); (2) elongation of the *N*-terminal; and (3) presence of lipidated aminoglucose on the D-ring. Glycosylation is also crucial to this derivative's activity.

As in, for example, calicheamicin antibiotics, neoglycorandomization, pioneered by J. Thorson, has also been used with vancomycin [see the synthesis of compounds **60–63** (Griffith *et al.*, 2007)]. This further expanded its potential to fight vancomycin-resistant strains (Fig. 20). Unfortunately, antibacterial testing (G^+ strains *Bacillus subtilis*, *S. aureus,* and *E. faecium*) revealed that vancomycin itself is superior to the new preparations, which have *c.* 20–25% of the activity of the parent compound. This finding is consistent with the observation that all previously tested monoglycosylated vancomycin derivatives are uniformly less active.

The glycorandomization of vancomycin using glycosyltransferases with nucleosides from natural and modifed carbohydrates has been accomplished by Fu *et al.* (2003). Most of the products were, however, only obtained in sufficient amounts for MS (HPLC-MS) structure confirmation but not enough for biological tests (Fig. 21).

Bleomycin

Bleomycin A_2 (**63**) exhibits antimicrobial activity against both Gram-positive and Gram-negative bacteria, but is clinically often used as an antitumor agent. Blenoxane is a marketed chemotherapeutic drug composed primarily of bleomycin A_2 (**63**) and B_2 (**64**) (Fig. 22). The mode of action of bleomycin is DNA-strand scission occurring in the presence of Fe^{2+} and

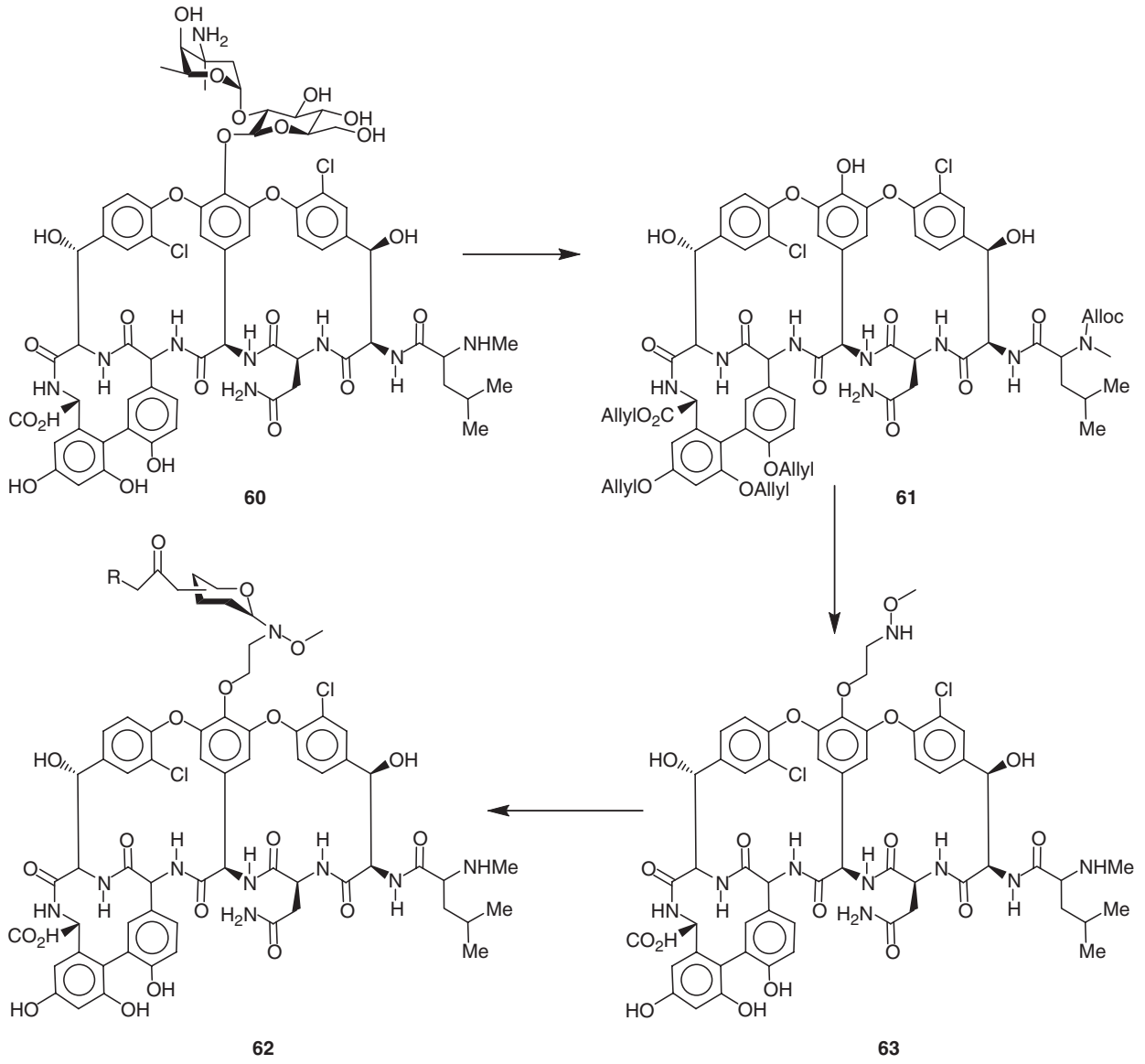


Fig. 20. Vancomycin neoglycorandomization (R in the formula 62 is a long aliphatic chain, e.g. tetradecyl, hexadecyl, hexadecenyl, etc.) (Griffith *et al.*, 2007).

molecular oxygen (Stubbe & Kozarich, 1987). It was demonstrated that the sugars neither contribute to binding affinity nor DNA cleavage selectivity, although the presence of the sugar enhances the DNA cleavage efficiency by a factor of 2–5 (Boger *et al.*, 1994). It is suggested that the sugar moieties contribute to the binding of O_2 and the activation and protection (as a metal ligand) of the reactive iron-oxo or perferryl intermediate to activated bleomycin (Grdadolnik *et al.*, 1998). Besides this, the carbohydrate moiety may be involved in cell surface recognition by bleomycin (Natrajan & Hecht, 1993). It was shown recently that bleomycines affect the cleavage of a variety of RNA substrates (Magliozzo *et al.*, 1989; Hecht, 1994). Hecht's group (Ma *et al.*, 2007) demonstrated recently that deglycosylated bleomycin (library of 108 derivatives) was found to mediate concentration-dependent plasmid DNA relaxation to some extent, and a number exhibited double-strand cleavage. Further, some analogs having modified linker and metal-binding domains mediated a modified sequence-selective cleavage, and a few were found to cleave a $tRNA_3^{Lys}$ transcript both in the presence and in the absence of a metal cofactor.

Enediyne antibiotics

Enediyne antibiotics (Nicolaou & Dai, 1991; Rezanka *et al.*, 2006) are extremely potent antitumor agents with a unique

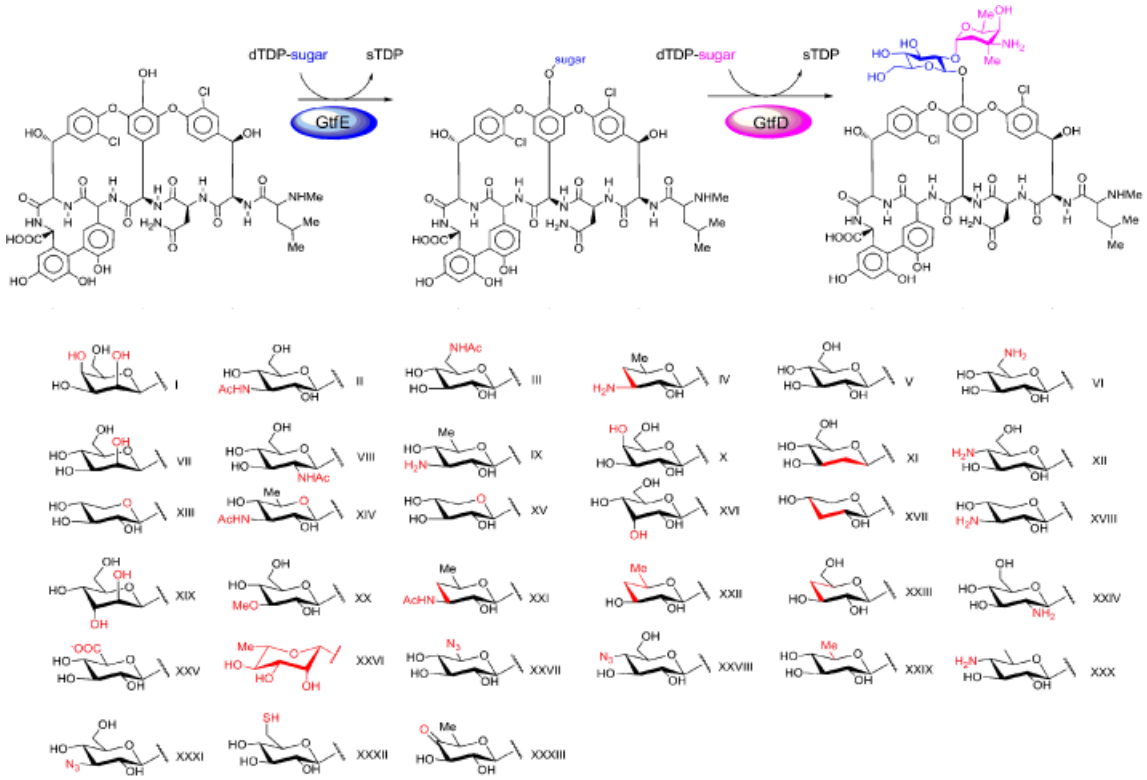


Fig. 21. Culminating steps of vancomycin biosynthesis and a phase I vancomycin IVG library. (a) Final stages of vancomycin biosynthesis catalyzed by GtfE (glucosyltransferase; green) and GtfD (vancosaminyltransferase; magenta). (b) Variants from stage I IVG of the vancomycin aglycone, where numbered compounds represent products and (–) indicates no conversion. Compounds highlighted in the box represent previously reported products. Changes from the GtfE wild-type substrate (glucose) are highlighted in red (Fu *et al.*, 2003). Names of sugars: I = D-talose; II = 3-deoxy-3-acetamido-D-glucose; III = 6-deoxy-6-acetamido-D-glucose; IV = 3, 4, 6-trideoxy-3-amino-D-*xylo*-hexopyranose; V = D-glucose; VI = 6-deoxy-6-amino-D-glucose; VII = D-mannose; VIII = 2-deoxy-2-acetamido-D-glucose; IX = 3,6-dideoxy-3-amino-D-glucose; X = D-galactose; XI = 2-deoxy-D-glucose; XII = 4-deoxy-4-amino-D-glucose; XIII = D-xylopyranose; XIV = 3,6-dideoxy-3-acetamido-D-glucose; XV = D-idose; XVI = D-allose; XVII = 3-deoxy-D-glucose; XVIII = 3-deoxy-3-amino-D-glucose; XIX = D-altrose; XX = 3-*O*-methyl-D-glucose; XXI = 3, 4, 6-trideoxy-3-acetamido-D-glucose; XXII = 4,6-dideoxy-D-*xylo*-hexopyranose; XXIII = 4-deoxy-D-glucose; XXIV = 2-deoxy-2-amino-D-glucose; XXV = D-glucuronic acid; XXVI = L-rhamnose; XXVII = 6-deoxy-6-azido-D-glucose; XXVIII = 4-deoxy-4-azido-D-glucose; XXIX = D-quinovose; XXX = 4,6-dideoxy-4-amino-D-glucose; XXXI = 3-deoxy-3-azido-D-glucose; XXXII = 6-deoxy-6-thio-D-glucose; XXXIII = 6-deoxy-D-xylohexopyranos-4-ulose. Adapted by permission from Macmillan Publishers Ltd. *Nat Biotechnol* **21**: 1467–1469 © 2003 (Fu *et al.*, 2003).

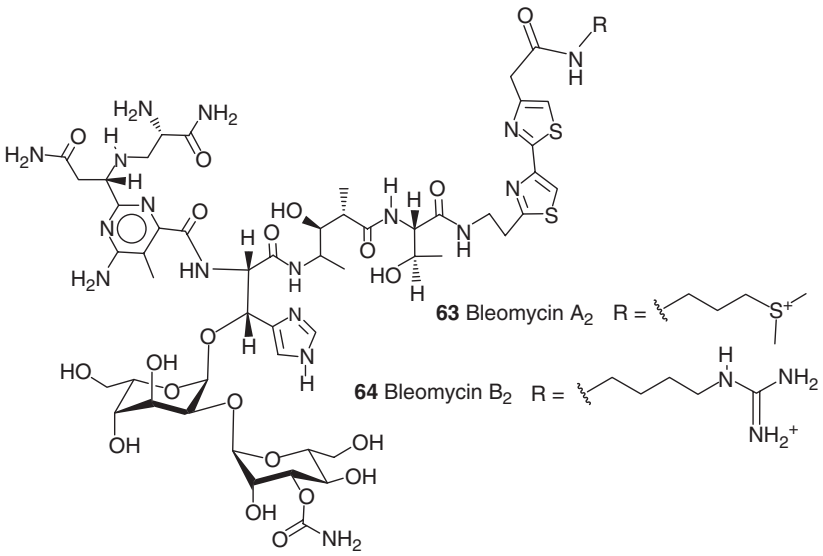


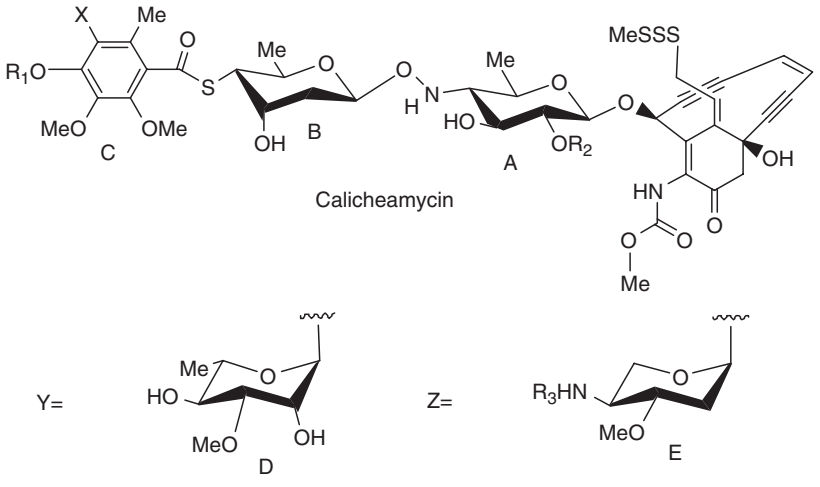
Fig. 22. Structures of bleomycins.

bicyclo[7.3.1]enediyne substructure. This group includes mainly calicheamicins (**65–71**) (Fig. 23) (Lee *et al.*, 1987) from *Micromonospora echinospora* spp. *calichensis*, which were isolated from caliche soils in Texas, esperamicins (**72–78**) (Fig. 24) (Golik *et al.*, 1987) from *Actinomadura verrucosospora* isolated from a soil sample collected in Argentina and dynemicins (Smith & Nicolaou, 1996). Iodine-containing calicheamicins (e.g. calicheamicin γ_1 (**70**)) were obtained after the addition of iodide to the fermentation broth (Lee *et al.*, 1989; Maiese *et al.*, 1989). Neocarzinostatin (**79**) (Fig. 25) (Edo *et al.*, 1985) and others (e.g. maduropeptin and kedarcidin) are also classified into this family.

At room temperature, in the presence of DNA, the core system of an enediyne antibiotic undergoes an interesting reaction, yielding a diradical on the sp^2 carbons, which causes DNA strand breakage (Fig. 26). During studies of the structures of calicheamicin γ_1 and esperamicin A1, it became apparent that enediynes could be triggered to aromatize via cleavage of the trisulfide, forming a diradical intermediate as the biologically active species.

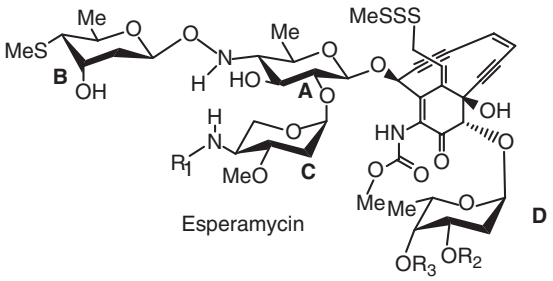
The degree of specificity of binding to the minor groove has been attributed to the carbohydrate moiety of the molecule, with the tail (appendage) of the oligosaccharide aligned towards the 3′-end of the DNA. The sugar moeity of calicheamicin γ_1^I (**70**) has some unusual structural features, for example, the iodine-substituted aromatic ring C, the thio sugar B, and the hydroxylamine glycosidic link between sugars A and B. The carbohydrates in calicheamicin are liphophilic in nature, which makes them favorable to interact with DNA, which along with its other features, gives the molecule the required chemical architecture to bind in a sequence-selective manner to DNA. The binding energy of the oligosaccharide domain of calicheamicin (Nicolaou *et al.*, 1992) to TCCT : AGGA sites within duplex DNA was shown to be 31.6 kJ mol^{-1}.

The sulfur atom of sugar group B forms hydrogen bonds (Fig. 23) with an exposed amino proton from 3′-guanine in the drug–DNA complex (Ikemoto *et al.*, 1995). This sugar is positioned edgewise in the minor groove, allowing the aromatic ring C to be placed between the edges of the minor groove with its iodine and methyl group positioned deep within the minor groove. These results suggest that the aromatic and rhamnose fragments of calicheamicin determine the sequence specificity and the monodirectional mode of binding but have little or no effect on orientating the aglycone within the DNA. The removal of the rhamnose sugar D of calicheamicin reduces its cutting efficiency but its sequence specificity is unchanged. This sugar, in NMR studies (Paloma *et al.*, 1994) of the drug–DNA complex, plunges less deeply into the minor groove and interacts with hydroxy groups of the phosphate backbone. In the same studies, sugar group E was found to straddle the DNA sugar–phosphate backbone with the amino group interacting with the backbone phosphates. Molecular modeling of



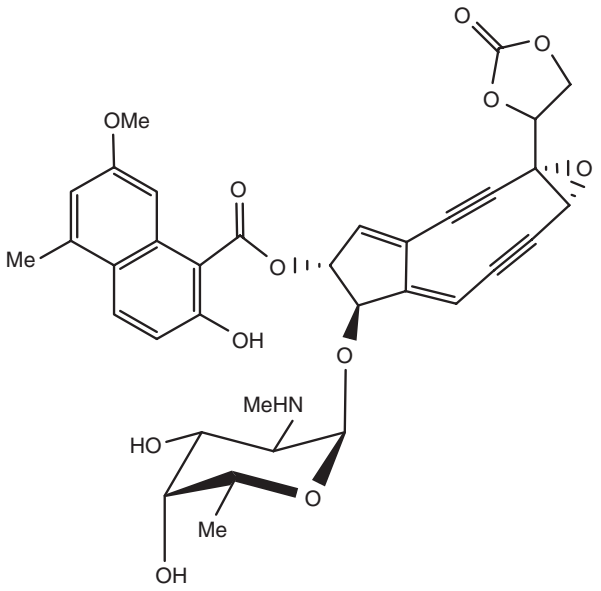
No.	Calicheamicin	X	R_1	R_2	R_3
65	β_1^{Br}	Br	Y	Z	i-Pr
66	γ_1^{Br}	Br	Y	Z	Et
67	α_2^{I}	I	H	Z	Et
68	α_3^{I}	I	Y	H	–
69	β_1^{Ir}	I	Y	Z	i-Pr
70	γ_1^{I}	I	Y	Z	Et
71	δ_1^{I}	I	Y	Z	Me

Fig. 23. Structures of calicheamicins.



No.	Esperamicin	n	R_1	R_2	R_3
72	A_1	3	i-Pr	W	H
73	A_{1b}	3	Et	W	H
74	A_{1c}	3	Me	W	H
75	P	4	i-Pr	W	H
76	A_2	3	i-Pr	H	AC
77	A_{2b}	3	Et	H	AC
78	A_{2c}	3	Me	H	AC

Fig. 24. Structures of esperamicins.



79 Neocarzinostatin

Fig. 25. Structure of neocarzinostatin.

the hydroxylamine glycosidic bond of calicheamicin γ_1^I has shown that the A and B sugars adopt an unusual eclipsed conformation due to this atypical three-bond glycosidic linkage (Walker *et al.*, 1994) (Fig. 23). This conformation-induced shape of the drug was shown to be complementary to the form of the minor groove. Hydroxylamine glycosidic linkage is thought to give the oligosaccharides the correct shape and rigidity to allow selective binding in the minor groove of DNA.

Further, the monomer and the head-to-head dimer of the oligosaccharide segment of calicheamicin (Fig. 23) have been shown to inhibit DNA transcription factors (Ho *et al.*, 1994; Liu *et al.*, 1996). The DNA targets contained 5′-TCCT/AGGA and 5′-TCTC/GAGA sequences (the sugar targets), which were within, or adjacent to, the binding sites of transcription factors. The inhibitory concentration was found to be in the micromolar range, with the dimeric carbohydrate being much more effective than the monomer. This inhibitory effect has tentatively been attributed to the conformational change in DNA as the sugar interacts with it; this then interferes with the transcription factor's DNA recognition. This interference with the transcription factor by the carbohydrates also displays itself in the inhibition of transcriptional stimulation (Liu *et al.*, 1996).

Structure analysis of the esperamicin A_1 – d(CGGA TCCG) duplex showed that the drug (Fig. 24) bound in the minor groove with the methoxyanthranilate moiety intercalates at the (G2–G3) : (6′–C7′) segment (Kumar *et al.*, 1997). It was demonstrated that the isolated deoxyfucose anthranilate group did not interact with DNA; however, this moiety was shown to contribute 4–8 kJ mol^{-1} to its binding energy.

Esperamicin C (**80**) (Fig. 27) causes double-strand lesions involving deoxyribose hydrogen abstraction from the 4′- and 5′-positions, whereas esperamicin A_1 causes lesions from the 1′- and 5′-positions. This change to the 1′-position for esperamicin A_1 is believed to be due to deoxyfucose anthranilate, which intercalates in the DNA. The A–B sugars of the A–B–C trisaccharide are positioned deep inside the minor groove. The OH groups of the trisaccharide A–B–C are positioned close to potential hydrogen bond acceptors, and favorable van der Waal's interactions thus help to stabilize the complex (Lu *et al.*, 1994).

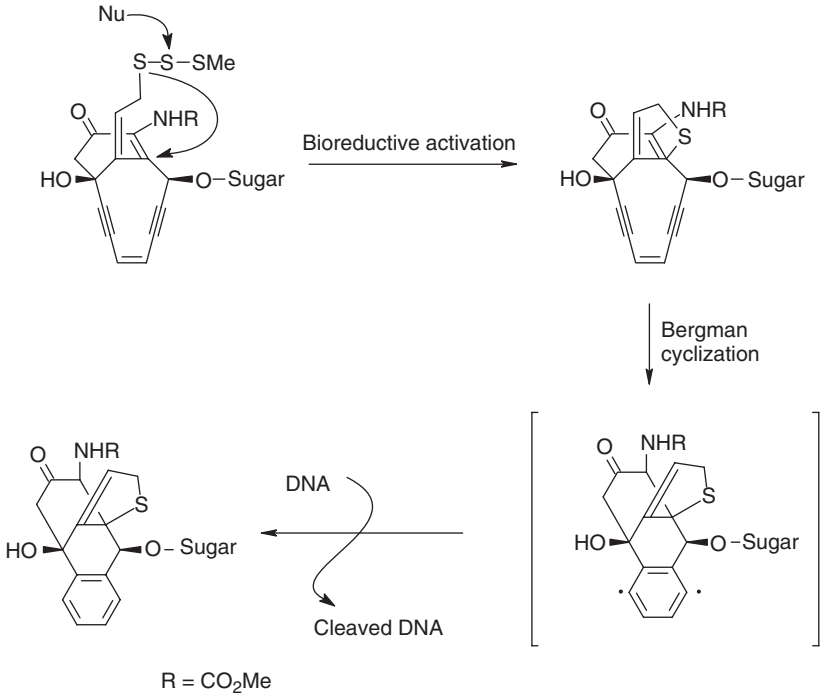


Fig. 26. Mechanism of activation and DNA cleavage by enediynes.

Neocarzinostatin (**79**) (Fig. 25) has been shown to induce single- and double-strand DNA scission with a single-stranded : double-stranded ratio of 5 : 1 and a preference for T and A residues (T > A ≫ C > G) (Stassinopoulos & Goldberg, 1995). The 2′-*N*-methyl-D-fucosamine, naphthoate, and tetrahydroindacene of postactivated-neocarzinostatin (rearranged complex with oligopeptide) were shown to have the correct structure to bind to specific sequences of DNA such as AGC sites. Specific sites are suggested for chemical modification that may alter the selectivity of binding/cleavage, one of these sites being the N-2 of 2′-*N*-methyl-D-fucosamine (Gao *et al.*, 1995). The sequence specificity of neocarzinostatin differs from calicheamicin and esperamicin, which may be partly due to the reduction in glycosylation compared with the latter compounds. There are certain similarities between the way esperamicin A and neocarzinostatin cleave, whereby lesions occur from the 1′-position of the deoxyribose.

This activity of calicheamicin has sparked considerable interest in the pharmaceutical industry, culminating in the calicheamicin–antibody conjugate MyloTarg (Giles *et al.*, 2003) to treat acute myelogenous leukemia. Additionally, similar strategies have been used in phase I trials to treat breast cancer. A massive program to examine calicheamicin conjugated to alternative delivery systems has also been undertaken recently. The biological activity and molecular architecture of calicheamicin has also prompted a search for potentially useful analogs.

A sequence analysis of the biosynthetic gene cluster for the enediyne antitumor antibiotic C-1027 from *Streptomyces*

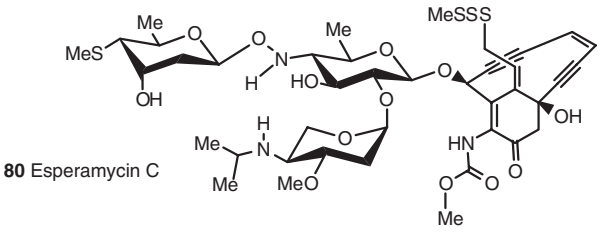


Fig. 27. Structure of esperamicin C.

globisporus suggested that the *sgcA1* gene encodes an α-D-glucopyranosyl-1-phosphate thymidyltransferase (Glc-1-P-TT), catalyzing the first step in the biosynthesis of the 4-deoxy-4-(dimethylamino)-5,5-dimethyl-D-ribopyranose moiety by activating α-D-glucopyranosyl-1-phosphate (Glc-1-P) into deoxythymidine diphosphate-α-D-glucose (dTDP-Glc). A report on the overexpression of *sgcA1* in *E. coli*, purification of the overproduced SgcA1 to homogenetity, biochemical and kinetic characterization of the purified SgcA1 as a Glc-1-P-TT, and yield improvement for C-1027 production by the overexpression of *sgcA1* and its flanking gene in *S. globisporus* (Murrell *et al.*, 2004) forms a basis for product yield improvement via a combinatorial biosynthesis method.

The toxicity of the enediyne compounds, including calicheamicin, centers on the problem of directing the compound to only cleave the DNA of interest, such as tumor cell DNA, and not the DNA of the host. The enediyne ring structure induces DNA damage, which is frequently a double-stranded cleavage. This type of DNA damage is usually nonrepairable

and most often lethal; however, many of these compounds are too toxic to be used in clinical studies. The enediynes are also potential anti-infective agents. For DNA synthesis inhibition, the IC_{50} was found to be 39 fmol mL^{-1}, 12 pmol mL^{-1} for RNA synthesis inhibition and 193 fmol mL^{-1} for protein synthesis inhibition. Calicheamicin has a *c.* 4000-fold higher antineoplastic activity than one of the best currently used cancerostatics–adriamycine.

Naturally occurring enediyne cytostatics turned out to be highly toxic in animal experiments; considerable effort was spent on the synthesis of more selective derivatives to achieve high biological activity towards tumor cells in combination with a minimum toxicity towards normal cells (Nicolaou, 1993, 2003; Nicolaou & Smith, 1993).

The glycosyltransferases involved in the biosynthesis of enediyne calicheamicin catalyze reversible, bidirectional reactions (Zhang *et al.*, 2006a). Specifically, two glycosyltransferase were tested (CalG1 and CalG4, Fig. 28), and were found to catalyze three new reactions: (1) synthesis of exotic NDP (nucleotide diphosphate)–sugars from glycosylated natural products, (2) exchange of native natural product glycosides with exogenous carbohydrates supplied as NDP–sugars, and (3) transfer of a sugar from one natural product backbone to a distinct natural-product scaffold. The calG1 gene was amplified from the genomic DNA of *Micromonospora echinospora* and overexpressed in *E. coli*; then the recombinant CalG1 was purified to homogeneity. Incubation of the aglycone with the surrogate substrates [thymidine diphosphate (TDP)–sugars] in the presence of CalG1 led to the formation of new products. Unfortunately, none of their biological activities were determined. Melancon *et al.* (2006) published a study very similar to that of Zhang *et al.* (2006a), again without any tests of the biological activity of any of the more than 70 synthesized compounds. Authors usually publish only MS (or LC-MS) spectra of new compounds (libraries), which demonstrates that the respective new compounds were never isolated in their pure state to enable a detailed structural study and respective biological tests. Such publications may be considered to be more 'proof-of-concept' work than the preparation of new antibiotics.

Three new derivatives of enediyne antibiotics, shishijimicins A–C (**81–83**) (Fig. 29), have been isolated from the thin encrusting orange ascidian *Didemnum proliferum* collected in southern Japan (Oku *et al.*, 2003). They encompass a novel

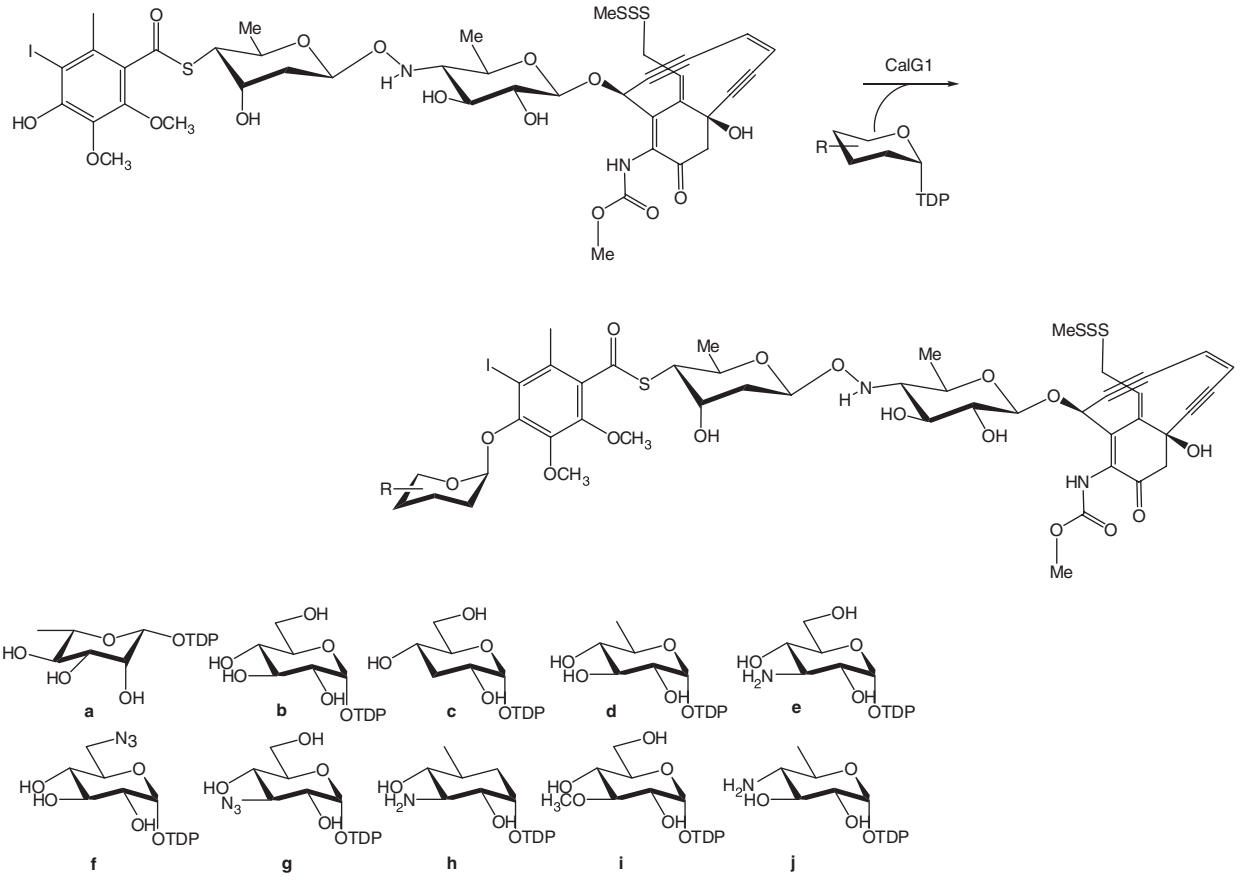
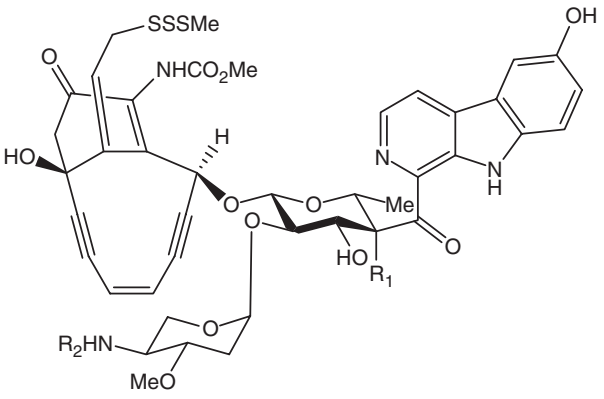


Fig. 28. *In vitro* CalG1-catalyzed reactions. The CalG1-catalyzed transfer of unnatural sugars to the acceptor (e.g. **68–71**). The TDP–sugars were **a–j** (Zhang *et al.*, 2006b).



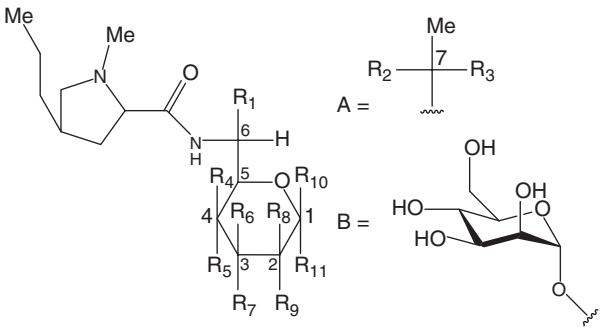
Shishijimicins	R_1	R_2
81	SMe	i-Pr
82	H	i-Pr
83	SMe	Et

Fig. 29. Structures of shishijimicins.

sugar component, which is a conjugation product of a hexose and a β-carboline, attached to the calicheamicinone aglycone. Shishijimicins showed extremely potent cytotoxicity against HeLa cells with IC_{50} values of 1.8–6.9 pM. Shishijimicins (**81–83**) (Oku *et al.*, 2003) are highly cytotoxic, as is namenamicin. However, shishijimicin A was almost 10 times more active than namenamicin in the three cell lines tested. It is highly likely that shishijimicins cleave DNA in a manner similar to other enediyne antibiotics, including namenamicin. The sequence specificity of calicheamicin γ_1^I was reported to be ascribed to the presence of the aryl-substituted saccharides. Because β-carboline not only intercalates DNA, but also the β-carboline unit in shishijimicin A occupies a position similar to that of the aryl group in calicheamicin γ_1^I, it may exhibit a pattern of DNA sequence recognition different from those of other enediynes.

Lincosamides

Lincosamides (Fig. 30) constitute a relatively small group of antibiotics with chemical structures consisting of amino acid and sugar moieties (Spizek *et al.*, 2004c; Spizek & Rezanka, 2004a, b Rezanka *et al.*, 2007). Natural lincosamides are produced by several *Streptomyces* species, mainly by *Streptomyces lincolnensis*, *Streptomyces roseolus*, *Streptomyces celestis*, and by *Micromonospora halophytica*. Their mechanism of action is the inhibition of protein synthesis in sensitive microorganisms. Lincosamides have an unusual antimicrobial spectrum, only being active against Gram-positive bacteria but also strongly active against anaerobic bacteria and some protozoa. The major route of resistance to lincosamides is modification of the 23S rRNA gene in the 50S ribosomal subunit, similar to resistance against macrolides and streptogramin B.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}
84	A	OH	H	OH	H	OH	H	H	OH	H	SMe
85	A	OH	H	OH	H	OH	H	H	H	H	SMe
86	A	OH	H	OH	H	OH	H	H	H	SMe	H
87	A	OH	H	H	OH	OH	H	H	OH	H	SMe
88	A	OH	H	OH	H	H	OH	OH	H	H	SMe
89	A	OMe	H	OH	H	OH	H	H	OH	SMe	H
90	H	–	–	OH	H	OH	H	H	OH	SMe	H
91	A	OMe	H	OH	H	OH	H	H	OH	H	SMe
92	H	–	–	OH	H	OH	H	H	OH	H	SMe
93	A	H	OMe	OH	H	OH	H	H	OH	H	SMe
94	A	H	Cl	OH	H	OH	H	H	H	H	SMe
95	A	H	OH	OH	H	OH	H	H	OH	H	SMe
96	A	H	OEt	OH	H	OH	H	H	OH	H	SMe
97	A	H	O-iPr	OH	H	OH	H	H	OH	H	SMe
98	A	H	OCH_2CH_2OH	OH	H	OH	H	H	OH	H	SMe
99	A	H	OCH_2CH_2OMe	OH	H	OH	H	H	OH	H	SMe
100	A	OH	H	OH	H	OH	H	H	OH	H	S(O)Me
101	A	OH	H	OH	H	OH	H	H	OH	H	S(O)Me
102	A	OH	H	OH	H	OH	H	H	OH	H	SO_2Me
103	A	B	H	OH	H	OH	H	H	OH	H	SMe
104	A	OH	H	OH	H	NMe_2	H	H	OH	H	SMe

Fig. 30. Structures of lincosamides.

Lincomycin (**84**) (Fig. 30) belongs to a class of antibiotics characterized by an alkyl 6-amino-6,8-dideoxy-l-thio-D-*erythro*-α-D-galacto-octapyranoside linked with a proline moiety via an amide linkage. This structure has great potential for chemical and microbiological modification. Numerous semi-synthetic derivatives of lincomycin have been prepared. The chemical modification (Birkenmeyer, 1981, 1982a, b; Argoudelis & Stroman, 1984; Patt *et al.*, 1984) of lincomycin derivatives consisted mainly of the substitution of hydrogen atoms bound to a heteroatom (e.g. O, N or S). Substitutions were performed primarily in positions 1–4 and 7.

Bannister (1972a, b, 1973, 1974) described a total of 15 derivatives of lincomycin. The first paper describes two 2-deoxy anomers (**85, 86**) having only *c.* 1% of the activity of lincomycin. The β-anomer had only marginal activity. These results demonstrate the considerable influence of the environment of the methylthio moiety on the antibacterial activity of the antibiotic, and may imply the requirement of a *cis*-configuration of the 1-methylthio- and 2-hydroxy groups for effective binding to the target site.

Both analogs of lincomycin possessing a modified glycosidic moiety (i.e. *ido*- and *gluco*-) (**87, 88**) were virtually devoid of antibacterial activity, exhibiting minimum inhibitory concentrations $> 200\,\mu g\,mL^{-1}$. Hence, because even the single configuration change of the 4-hydroxy group, yielding the D-*gluco*-analog, lowers its activity more, it modifies the 1-position, which is critical for maintaining activity.

The β-anomeric glycosides (**89** and **90**) were found to have $< 0.01\%$ of the activity of lincomycin in the *in vitro* tests. 7-*O*-Methyl-lincomycin (**91**) shows the same spectrum of antibacterial activity as lincomycin, and 1.3–1.8 times the *in vitro* activity (standard curve assay against *Sarcina lutea*); *in vivo* in the mouse, it is equivalent to lincomycin in terms of protection against *S. aureus* upon subcutaneous injection.

6-De-(l-hydroxyethyl)-lincomycin (**92**) again shows the same spectrum of activity as lincomycin, but only 1% of the activity *in vitro*. While this activity is low, it may indicate that the function of the side chain is involved in cellwall permeation or an increase in binding at an active site.

The (7*S*)-7-methoxy-analog (**93**) gives 3.5 times the response of lincomycin against *S. lutea* in a standard curve agar diffusion assay; a broth dilution assay shows a two- to fourfold enhancement in activity against a variety of G^+ bacteria with no broadening of the spectrum of lincomycin. In a mouse infected experimentally with a lethal challenge of *S. aureus*, this analog exhibited a CD_{50} indistinguishable from that of (7*S*)-7-chloro-7-deoxy-lincomycin (**94**) (Table 4).

The tabulated results show that, while replacement of the 7-hydroxy group of 7-epi-lincomycin (**95**) with a methoxy group results in a significant enhancement of antibacterial activity (**93**), replacement with larger alkoxy and substituted alkoxy groups results in a smaller enhancement [with the ethoxy- (**96**) and isopropoxy-substituents (**97**)] or in decreased activity [with the 2-hydroxyethoxy- (**98**) and 2-methoxyethoxy-substituents (**99**)].

Other modifications concerned the C_l-bound sulfur atom. Sulfur was oxidized to sulfoxides (**100, 101**) and further to sulfone (**102**). None of the oxidation products, when tested on a set of 18 microorganisms, exceeded lincomycin in activity (Sztaricskai *et al.*, 1997).

Enzymatic modification of C_7 by jack bean α-mannosidase gave a low yield (2.4%) of 7-*O*-α-D-mannopyranosyl-lincomycin (**103**) (Weignerova *et al.*, 2001). The biological activity of this compound has not yet been published.

Hanessian & Kothakonda (2005) have synthesized 3-*N*,*N*-dimethylamino-3-deoxy lincomycin (**104**) in eight steps as a structure-based inspired hybrid between the methyl thiolincosaminide portion of lincomycin and the desosamine unit of erythromycin. The biological activity was disappointingly low; it displayed any inhibition of the growth of *E. coli* or *S. aureus* (MIC $> 10\,\mu M$). Against the same strains, lincomycin exhibited an MIC > 10 and $2.5\,\mu g\,mL^{-1}$, respectively. This lack of activity may be related to inefficient cell penetration, efflux, or none of the recognition at the ribosomal level that is required for effective inhibition of protein biosynthesis.

Although hundreds of lincomycin derivatives were prepared, among them derivatives produced purely by chemical synthesis, clindamycin (see structure **94**, i.e. 7-epichlorolincomycin) is the only drug that has been successfully used in clinical practice. Detailed synthetic procedures leading to a number of lincomycin derivatives and their biological activities were described by Magerlein (1971). It is also obvious that in this case – in contrast to many other examples presented in this review – glycorandomization has an apparently low potential to improve/modify the antibiotic activity of the lincomycin group.

Table 4. Standard curve agar diffusion assay of analogs vs. *Sarcina lutea*

7(*S*) -Substituent	Formula	Relative activity (**84** = 1)
OMe	93	3.5
OH	95	0.5
OEt	96	1.1
OiPr	97	0.7
OCH_2CH_2OH	98	0.3
OCH_2CH_2OMe	99	0.3

Avermectins

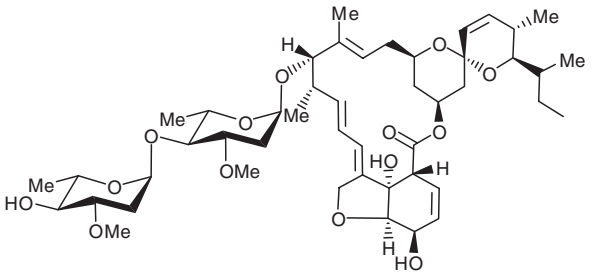
Avermectins are a family of 16-membered macrocyclic lactones that have a disaccharide on C-13 of the macrocycle and are isolated as natural fermentation products of *Streptomyces avermitilis*. They possess a broad spectrum of activity against helminthes and arthropods with relatively low toxicity in both humans and animals and are currently in use in both human and veterinary medicine. Avermectin B_{1a} (**105**) and C_{22}–C_{23}-reduced ivermectin (**106**) (Fig. 31) are mostly used in veterinary medicine; however, some semisynthetic derivatives are also used in the treatment of onchocerciasis, stongyloidiasis, and lymphatic filariasis in humans (Greene *et al.*, 1989). The action of avermectin is believed to stimulate specific chloride ion transport systems, increasing membrane permeability to Cl^- ions via GABA (γ-butyrate) receptors and non-GABA receptors (Martin & Pennington, 1989).

The aglycone of avermectin has poor antiparasitic activity. Positions 4′ and 4″ of oleandrose moieties were modified partly for feasibility reasons. Synthesis of the 4″-amino-4″-deoxyoleandrose derivative of avermectin B_1 and ivermectin was based on the observation that most macrolide antibiotics contained a basic amino group (Mrozik *et al.*, 1995). One of the derivatives, 4″-*epi*-acetamido-4″-deoxyavermectin B_1, is currently under development as a novel avermectin endectoside (Mrozik *et al.*, 1995). Besides this, the 2″-α-fluoro and 2″-β-fluoro derivatives of avermectins were also prepared to strengthen the glycosidic bond. In some bioassays, these derivatives have interesting activities compared with their parental compounds (Bliard *et al.*, 1987).

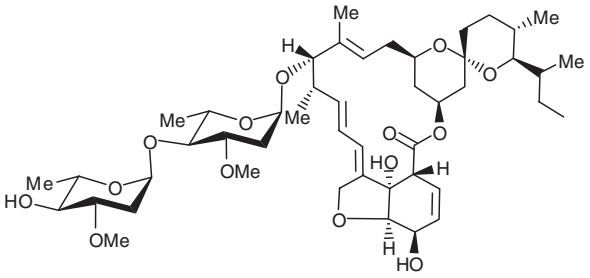
A series of avermectin derivatives modified at the 4′-C position of the carbohydrate moiety [e.g. variation of the second carbohydrate with semisynthetic sugars e.g. 4′-fucosyl avermectin (Wei *et al.*, 2004)] was designed and synthesized. Some of the new synthetic compounds showed excellent

in vivo bioactivity against cabbage larvae when compared with commercially available avermectin B1a (Wei *et al.*, 2005).

Finally, a proof of concept of the neoglycorandomization of ivermectin has been accomplished by Thorson's group using the reaction reversibility of the avermectin glycosyltransferase AveBI (Zhang *et al.*, 2006b). In this case, however, the respective products were not produced in sufficient amounts for biological tests.



105 Avermectin B_{1a}



106 Ivermectin

Fig. 31. Structures of avermectins.

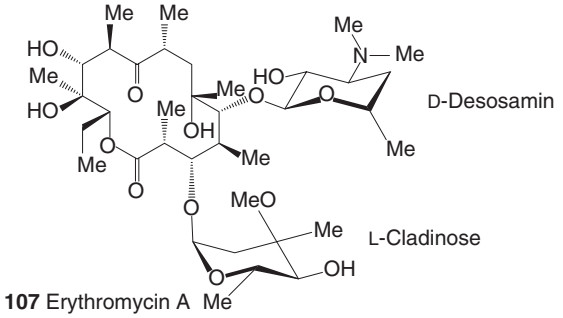
Macrolide antibiotics

Macrolide antibiotics containing a large lactone ring consisting of 14 atoms are biosynthetically related to avermectins. The first member of this group, erythromycin A (**107**) (Fig. 32), was isolated in 1952 (McGuire *et al.*, 1952) and soon became an important antibiotic widely used in clinical medicine against G^+ bacteria. It constitutes the main treatment for many pulmonary infections, such as Legionnaire's disease. Other families of macrolides are known, containing 12- or 16-membered macrolide rings. A representative of the latter family is tylosin (**108**), a commercially important antibacterial agent used in veterinary medicine.

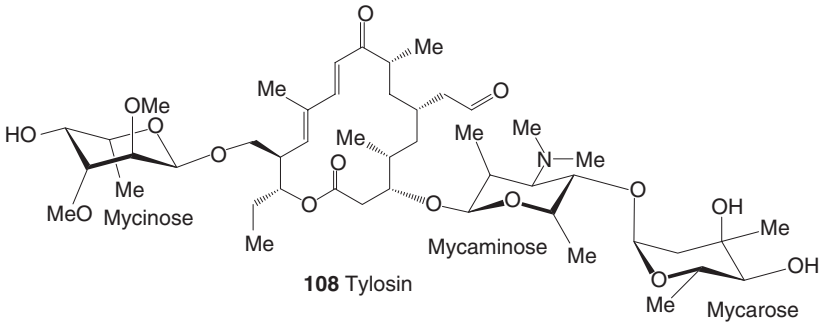
Most of the macrolides contain deoxy- and aminosugars. Although it was established (Flynn *et al.*, 1955; Jones & Rowley, 1968) that the sugar moieties are absolutely essential for the microbial activity of these compounds, it is difficult to perceive their role in the activity as their exact mode of action is unclear.

The mode of action of macrolide antibiotics involves the inhibition of protein synthesis via specific binding to the 50S ribosomal subunit (Boger *et al.*, 1995). Nevertheless, the exact interaction of the macrolide and the ribosome unit is still not fully understood. In principle, macrolide antibiotics should also inhibit mammalian mitochondrial protein synthesis, but they are unable to penetrate the mitochondrial membrane.

Sugar moieties have been linked with many pharmacological properties displayed by macrolide antibiotics



107 Erythromycin A



108 Tylosin

Fig. 32. Structures of macrolide antibiotics.

(Williams & Sefton, 1993). The basicity of the nitrogen in aminosugars improves the active transport of the compounds into the cell, however; its protonation/deprotonation does not affect uptake. It was shown that the more basic macrolides are the more effective ones. Esterification of glycosidic position 2′ improves the pharmacological and pharmacokinetic properties of the resulting drug, reducing unwanted gastrointestinal side effects (Kirst & Sides, 1989).

By modifying the desosamine sugar on the C-30 amino group, it was possible to differentiate between its antibiotic and anti-inflammatory action. Such compounds are completely devoid of antimicrobial effects but their anti-inflammatory properties are enhanced. These results strongly suggest that macrolides could potentially be used as a new class of anti-inflammatory agents (Mereu *et al*., 2006).

Cao & Zhang (2007) recently published a review summarizing the synthesis pathway of glycosyl linked to macrolide antibiotics, the structural features of their glycosyltransferase, and glycosyl biofunction of macrolide antibiotics. They also reported progress in modifying glycosyl to synthesize antibiotics with novel structures by genetic engineering.

The rather interesting effect of glycosylation acting as a resistance mechanism of pathogenic *Nocardia* to the macrolide antibiotics chalcomycin and tylosin has been demonstrated by Morisaki *et al*. (2001). They showed that most of the *Nocardia* species examined were highly resistant to both antibiotics. *Nocardia asteroides* IFM 0339 converted these macrolides into inactive metabolites by glycosylation at 2′-OH or glycosylation and reduction of the 20-formyl group. This clearly demonstrates the negative effect of glycosylation on their antibiotic activity.

Polyenes

Polyene macrolides consist of a macrocyclic lactone, a polyene chromophore, and a polyhydroxylated chain, which often bears glycosidic residues. These antibiotics, for example, amphotericin B (**109**) and nystatin A_1 (**110**) (Fig. 33), are used as potent antifungal antibiotics although they cause side effects such as nephrotoxicity, hemolytic anemia, and electrolyte abnormalities (Brajtburg *et al*., 1990).

These compounds interact with ergosterol, contained in cell membranes of lower eukaryotes (fungi, yeasts), forming pores in their membranes. These channels cause a shunt in the membrane potential and leak of cellular ions (Hartsel *et al*., 1991).

Structure–activity studies on amphotericin B (**109**) have shown the importance of the basic nitrogen of D-mycosamine to their activity (Herve *et al*., 1989). This polyene is oriented with its polar head at the membrane–water interface and it has been suggested that the basic amino group of the sugar and the carbonyl group forms a 'cage'-like hydrogen bonded structure with sterol (containing a 3-β-hydroxy group) and water.

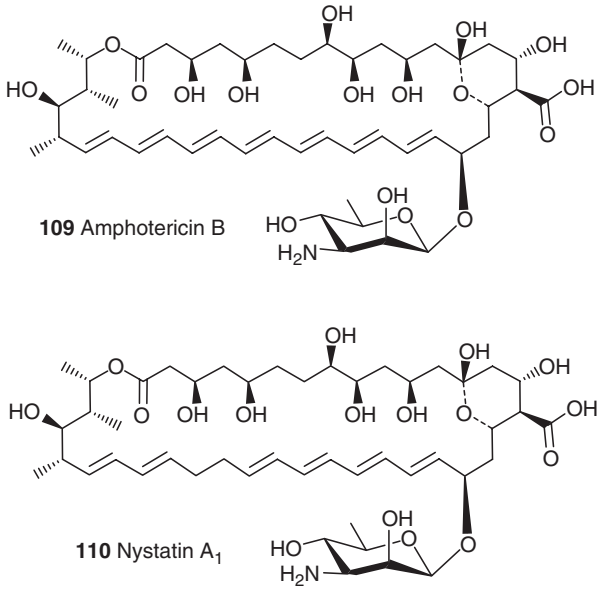


Fig. 33. Structures of polyene antibiotics.

Amphotericin B derivatives lacking the mycosamine sugar that contains a C(3′) ammonium ion are completely inactive against *Saccharomyces cerevisiae* and *Candida albicans* (Palacios *et al*., 2007).

N-Glycosylation of D-mycosamine in polyenes leads to antibiotics with a reduced toxicity and activity similar to the parent compound but increased water solubility and, therefore, better bioavailability (Cheron *et al*., 1988).

Derivatives of amphotericin B prepared by double-reductive alkylation of the mycosamine displayed superior antifungal activity against the *S. cerevisiae* wild-type strain and especially with an AmB-resistant *C. albicans* strain. Moreover, these compounds are potential drug candidates because of their significantly reduced hemotoxicity compared with AmB. Furthermore, the same mycosamine modification can be applied to other polyene macrolides such as nystatin and Pimaricin to improve their antifungal activity (Paquet & Carreira, 2006).

An excellent review published by Thirsk & Whiting (2002) describes a large array of polyenes and their respective derivatives including their biogenesis, total synthesis, and how their various modifications alter their biological activities compared with the parent molecules.

Carbohydrate–nucleic acid interactions

Traditionally, the glycan chains of DNA-binding antibiotics have been viewed as molecular elements that control the pharmacokinetics of a drug, such as absorption, distribution, metabolism, and excretion. This notion changed a few years ago with the finding that the carbohydrate residues present in calicheamycin antibiotics partially determine the

selectivity of the process and even calicheamycin aryltetrasaccharide (which does not possess the enediyne ring) (Ho *et al.*, 1994) binds well to DNA (Fig. 34).

Carbohydrate minor groove DNA (Hunziker, 1996; Dervan, 2001) binders are neutral neoglycoconjugates, whose sugar residues are deoxygenated, thus exhibiting a delicate balance between the hydrophylic and the hydrophobic domains. There is rarely more than a single positively charged ammonium group in the respective carbohydrate and many of the sugars are of the 2,6-deoxyhexopyranose type. In many cases, the remaining hydroxy groups are further alkylated or acylated. Thus, the recognition process relies on just a few H-bonding interactions and is mostly driven by the hydrophobicity of the DNA-binding saccharides. This suggests that it is rather the complementary shapes, and hence specific van der Waals contacts between the carbohydrates and minor groove, which may determine the site selectivity displayed by many carbohydrate-containing minor groove binders (Hunziker, 1996). Additionally, the relatively rigid carbohydrates may take advantage of the sequence-dependent flexibility of double-stranded DNA to bind their target sites (Martin *et al.*, 2005). Furthermore, the hydrophobicity of DNA-binding carbohydrates may differentiate them from the hydrophilic and often negatively charged cell-surface saccharides, which are involved in protein recognition and may enhance the cellular uptake of the respective drugs.

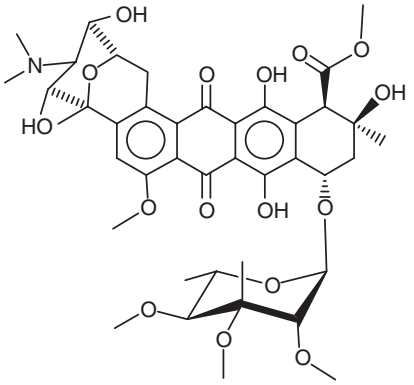
Quite recently, evidence has accumulated that some carbohydrates can also be effective DNA major groove binders. So far, there are only a limited number of examples of glycosides that have been found to interact with the DNA major groove (Willis & Arya, 2006): neocarzinostatin (DNA-cleavage agent), altromycin B (alkylating agent), nogalamycin (**109**) (Fig. 35), and respinomycin (tandem-intercalative-groove-binding ligands) and the neomycin–Hoechst33258 conjugate (dual-groove-binding ligand).

Natural dual-groove-binding products such as nogalamycin have displayed an enhanced binding affinity over their parent single carbohydrate (single-groove binding) compounds (e.g. daunomycin **34**). Therefore, the application of synthetic chemistry and/or glycorandomization can result in novel ligands capable of dual-groove recognition and increased binding affinity.

Glycorandomization

The isolation of several sugar biosynthesis gene clusters and glycosyltransferases from different antibiotic-producing organisms, and increasing knowledge about these biosynthetic pathways opened up the possibility of generating novel bioactive compounds through combinatorial biosynthesis. Recent advances in this area indicate that antibiotic glycosyltransferases show some substrate flexibility, which can allow the types of sugar transferred to the different aglycones to be altered or, less frequently, to change the position of its attachment (Mendez & Salas, 2001; Langenhan *et al.*, 2005).

Recently, two complementary glycorandomization strategies have been described, namely, (1) neoglycorandomization, a chemical approach based on a one-step sugar ligation reaction that does not require any prior sugar protection or activation (Chisolm & Van Vranken, 2000; Langenhan *et al.*, 2005), and (2) chemoenzymatic glycorandomization, a biocatalytic approach that relies on the substrate promiscuity of enzymes to activate and attach sugars to natural products (Daines *et al.*, 2004; Griffith *et al.*, 2005).



109 Nogalamycin

Fig. 35. Structure of nogalamycin.

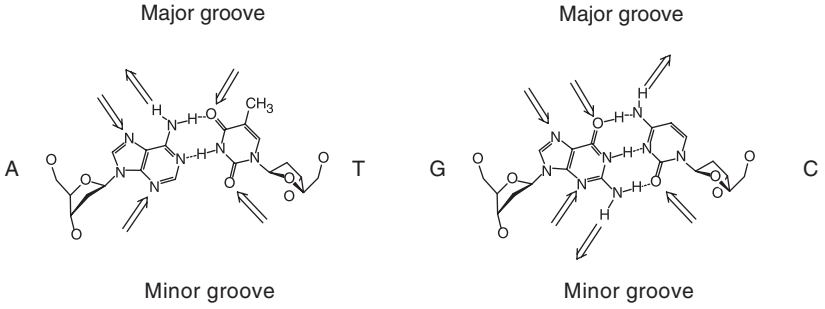


Fig. 34. Substitution pattern of H-bonding donor and acceptor groups for adenine–thymine and guanine–cytosine base pairs in the major and minor groove.

These 'glycorandomization' approaches (occasionally referred to as 'glycodiversification') are expected to enhance our understanding of the role of sugars in a variety of glycoconjungates and enable the exploitation of these critical attachments.

Neoglycorandomization is based on the selective formation of glycosidic bonds between reducing sugars and secondary alkoxyamine-containing aglycones to form a library of 'neoglycosides' (Chisolm & Van Vranken, 2000).

Sugars and acceptors containing secondary alkoxyamines are reacted with reducing sugars to generate oligosaccharide and glycopeptide mimics. Unlike primary alkoxyamines, which provide open-chain oxime isomers, secondary alkoxyamines react to form closed-ring neoglycosides. Presumably, such secondary alkoxyamines react with reducing sugars to form an intermediate oxy-imminium species, which then undergoes ring closure (Peri & Nicotra, 2004). A good example of neoglycorandomization using a synthetic approach has been published by Thorson's group (Griffith *et al.*, 2007) (Fig. 20), where the *O*-glycosidic bond has been replaced by the 'mimic' methoxamin linkage.

In chemoenzymatic glycorandomization, these activated sugar libraries, in turn, serve as substrates for inherently promiscuous natural product glycosyltransferases, thereby providing a rapid chemoenzymatic means to glycodiversity. Such multienzyme, one-pot reactions offer an attractive alternative to the extensive synthetic manipulation typically required for chemical glycosylation strategies.

In the last 3 years, a variety of examples have appeared supporting this robust methodology: four glycosyltransferases from two distinct natural biosynthetic pathways – calicheamicin and vancomycin – readily catalyzed reversible reactions, allowing sugars and aglycones to be exchanged. More than 70 differentially glycosylated calicheamicin (Fig. 28) and vancomycin variants were prepared (Fig. 21). This study (Zhang *et al.*, 2006a) suggests that the reversibility of glycosyltransferase-catalyzed reactions may be general and useful for generating exotic nucleotide sugars, establishing *in vitro* glycosyltransferase activity in complex systems. A monoglycosylated vancomycin library was prepared using flexible glycosyltransferases with a nucleotide diphosphosugar library (Fu *et al.*, 2003).

High-level expression of three macrolide glycosyltransferases created a synthetic 'tool kit' with such plasticity that 12 modified macrolide antibiotics (oleandromycin and erythromycin) have been created relatively easily (Yang *et al.*, 2005).

Another, more advanced methodology uses a combination of chemoenzymatic synthesis (substrate modifications) together with *in vivo* pathway engineering as demonstrated in the excellent recent review by Thibodeaux & Liu (2007) on macrolide glycodiversification.

Promiscuity, or better proficiency, can be further extended by respective glycosyltransferase engineering as documented recently in the study by Williams *et al.* (2008a) dealing with directed evolution of oleandomycin glycosyl transferase (OleD). Directed evolution of this enzyme altered, via a single or a few combined mutations, enzyme specifity towards a whole range of new acceptors of medicinal relevance, as for example, novobiocin (Williams *et al.*, 2008b).

Successful implementation of these sugar-engineering studies relies on our understanding of the substrate flexibilities of the enzymes involved in sugar synthesis. Studies elucidating the mechanisms and biochemical properties of sugar biosynthetic enzymes are of paramount significance.

On the other hand, mainly synthetic methodologies have been developed so far, which has led to a better understanding of the activity mechanisms through specific SAR studies. This has enabled optimization of existing antibiotics as well as the construction of new ones. Most of these papers often demonstrate an extension of the methodologies and the products are either not isolated (structure characterization by the LC-MS methods) or isolated in minute amounts that are insufficient for detailed biological studies. Therefore, further scale-up will be required.

It is quite obvious that further interesting discoveries lie ahead, bringing this method closer to practical exploitation. In this way, basic knowledge of the role of carbohydrates in the biological activity of glycosides can be capitalized on.

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References

Arcamone F & Gassinelli G (1998) Biosynthetic anthracyclines. *Curr Med Chem* **5**: 391–419.

Arcamone F, Animati F, Capranico G, Lombardi P, Pratesi G, Manzini S, Supino R & Zunino F (1997) New developments in antitumor anthracyclines. *Pharmacol Ther* **76**: 117–124.

Arcamone F, Animati F, Bigioni M *et al.* (1999) Configurational requirements of the sugar moiety for the pharmacological activity of anthracycline disaccharides. *Biochem Pharmacol* **57**: 1133–1139.

Argoudelis AD & Stroman DW (1984) Process of producing lincomycin nucleotides. U.S. Patent 4,464,466.

Bannister B (1972a) Modifications of lincomycin involving carbohydrate portion. 1. 2-*O*-methyl and 2-deoxy-analogues. *J Chem Soc Perkin Trans* **1**: 3025–3030.

Bannister B (1972b) Modifications of lincomycin involving carbohydrate portion. 2. Analogs with D-gluco-stereochemistry and D-ido-stereochemistry. *J Chem Soc Perkin Trans* **1**: 3031–3036.

Bannister B (1973) Modifications of lincomycin involving carbohydrate portion. 3. 7-*O*-methyl and 6-de-(1-hydroxyethyl) analogs. *J Chem Soc Perkin Trans* **1**: 1676–1682.

Bannister B (1974) Modifications of lincomycin involving carbohydrate portion. 4. (7*S*)-7-alkoxy-7-deoxy-analogues. *J Chem Soc Perkin Trans* **1**: 360–369.

Birkenmeyer RD (1981) Lincomycin compounds. Novel useful analogs of the well known antibiotics lincomycin and clindamycin. U.S. Patent 4,278,789.

Birkenmeyer RD (1982a) Lincomycin compounds. Novel useful analogs of the well known antibiotics lincomycin and clindamycin. U.S. Patent 4,309,533.

Birkenmeyer RD (1982b) Lincomycin compounds. Novel useful analogs of the well known antibiotics lincomycin and clindamycin. U.S. Patent 4,310,660.

Bliard C, Escribano FC, Lukacs G, Olesker A & Sarda P (1987) Synthesis of C-2″β- and C-2″α-fluoro avermectin B_{1a}. *J Chem Soc Chem Commun* **5**: 368–370.

Boger D, Colletti SL, Teramoto S, Ramsey TM & Zhou J (1995) Synthesis of key analogs of bleomycin A2 that permit a systematic evaluation of the linker region: identification of an exceptionally prominent role for the L-threonine substituent. *Bioorg Med Chem* **3**: 1281–1295.

Boger DL, Honda T, Menezes RF & Colletti SL (1994) Total synthesis of bleomycin A2 and related agents. 3. Synthesis and comparative evaluation of deglycobleomycin A2, epideglycobleomycin A2, deglycobleomycin A1, and desacetamido-, descarboxamido-, desmethyl-, and desimidazolyldeglycobleomycin A2. *J Am Chem Soc* **116**: 5631–5646.

Bojarová-Fialová P & Křen V (2007) Enzymatic approaches to *O*-glycoside introduction: glycosidases. *Comprehensive Glycoscience* (Kamerling JP, ed), pp. 453–487. Elsevier, Oxford.

Bosslet K, Czech J & Hoffmann D (1994) Tumor-selective prodrug activation by fusion protein-mediated catalysis. *Cancer Res* **54**: 2151–2159.

Brajtburg J, Powderly WG, Kobayashi GS & Medoff G (1990) Amphotericin-B – current understanding of mechanisms of action. *Antimicrob Agents Chemother* **34**: 183–188.

Cao MC & Zhang BC (2007) Glycosyl synthesis and biofunction of macrolide antibiotics. *Chinese J Antibiot* **32**: 140–145.

Cheron M, Cybulska B, Mazerski J, Grzybowska J, Czerwinski A & Borowski E (1988) Quantitative structure activity relationships in amphotericin-B derivatives. *Biochem Pharmacol* **37**: 827–836.

Chisolm JD & Van Vranken DL (2000) Regiocontrolled synthesis of the antitumor antibiotic AT2433-A1. *J Org Chem* **65**: 7541–7553.

Cipollone A, Berettoni M, Bigioni M *et al.* (2002) Novel anthracycline oligosaccharides: influence of chemical modifications of the carbohydrate moiety on biological activity. *Bioorg Med Chem* **10**: 1459–1470.

Daines AM, Maltman BA & Flitsch SL (2004) Synthesis and modifications of carbohydrates, using biotransformations. *Curr Opin Chem Biol* **8**: 106–113.

Davies J (1994) Inactivation of antibiotics and the dissemination of resistance genes. *Science* **264**: 375–382.

Davies J & Davis BD (1968) Misreading of ribonucleic acid code words induced by aminoglycoside antibiotics. The effect of drug concentration. *J Biol Chem* **243**: 3312–3316.

de Graaf M, Pinedo HM, Quadir R, Haisma HJ & Boven E (2003) Cytosolic β-glycosidases for activation of glycoside prodrugs of daunorubicin. *Biochem Pharmacol* **65**: 1875–1881.

Dervan PB (2001) Molecular recognition of DNA by small molecules. *Bioorg Med Chem* **9**: 2215–2235.

Ding YL, Hofstadler SA, Swayze EE & Griffey RH (2001) An efficient synthesis of mimetics of neamine for RNA recognition. *Org Lett* **3**: 1621–1623.

Ding YL, Hofstadler SA, Swayze EE & Griffey RH (2003) Efficient synthesis of heterocyclic 2-deoxysteptamine derivatives as RNA binding ligands. *Chem Lett* **32**: 908–909.

Edo K, Mizugaki M, Koide Y, Seto H, Furihata K, Ohtake N & Ishida N (1985) The structure of neocarzinostatin chromophore possessing a novel bicyclo-[7,3,0]dodecadiyne system. *Tetrahedron Lett* **26**: 331–334.

Elchert B, Li J, Wang J, Hui Y, Rai R, Ptak R, Ward P, Takemoto JY, Bensaci M & Chang C-WT (2004) Application of the synthetic aminosugars for glycodiversification: synthesis and antimicrobial studies of pyranmycin. *J Org Chem* **69**: 1513–1523.

Fang L, Zhang G, Li C *et al.* (2006) Discovery of a daunorubicin analogue that exhibits potent antitumor activity and overcomes P-gp-mediated drug resistance. *J Med Chem* **49**: 932–941.

Fink BE, Mortensen DS, Stauffer SR, Aron ZD & Katzenellenbogen JA (1999) Novel structural templates for estrogen-receptor ligands and prospects for combinatorial synthesis of estrogens. *Chem Biol* **6**: 205–219.

Floss H (2006) Combinatorial biosynthesis – potential and problems. *J Biotechnol* **124**: 242–257.

Flynn EH, Murphy HW & McMahon RE (1955) Erythromycin. II. Des-*N*-methylerythromycin and *N*-methyl-C14-erythromycin. *J Am Chem Soc* **77**: 3104–3106.

Fu X, Albermann C, Jiang J, Liao J, Zhang C & Thorson JS (2003) Antibiotic optimization via *in vitro* glycorandomization. *Nat Biotechnol* **21**: 1467–1469.

Gao X & Patel DJ (1989) Solution structure of the chromomycin–DNA complex. *Biochemistry* **28**: 751–762.

Gao X, Stassinopoulos A, Rice JW & Goldberg IH (1995) Structural basis for the sequence-specific DNA strand cleavage by the enediyne neocarzinostatin chromophore. Structure of the post activated chromophore–DNA complex. *Biochemistry* **34**: 40–49.

Ge M, Thompson C & Kahne D (1998) Reconstruction of vancomycin by chemical glycosylation of the pseudoaglycone. *J Am Chem Soc* **120**: 11014–11015.

Ge M, Chen Z, Onishi HR, Kohler J, Silver LL, Kerns R, Fukuzawa S, Thompson C & Kahne D (1999) Vancomycin derivatives that inhibit peptidoglycan biosynthesis without binding D-Ala-D-Ala. *Science* **284**: 507–511.

Gianni L, Grasselli G, Cresta S, Locatelli A, Vigano L & Minotti G (2003) New developments in antitumor anthracyclines. *Cancer Chemother Biol Response Modif* **21**: 29–40.

Gildersleeve J, Smith A, Sakurai K, Raghavan S & Kahne D (1999) Scavenging byproducts in the sulfoxide glycosylation reaction: application to the synthesis of ciclamycin. *J Am Chem Soc* **121**: 6176–6182.

Giles F, Estey E & O'Brien S (2003) Gemtuzumab ozogamicin in the treatment of acute myeloid leukemia. *Cancer* **98**: 2095–2104.

Golik J, Clardy J, Dubay G, Groenewold G, Kawaguchi H, Konishi M, Krishnan B, Ohkuma H, Saitoh K & Doyle TW (1987) Esperamicins, a novel class of potent antitumor antibiotics. 2. Structure of esperamicin X. *J Am Chem Soc* **109**: 3461–3462.

Grdadolnik SG, Pristovsek P & Mierke DF (1998) Vancomycin: conformational consequences of the sugar substituent. *J Med Chem* **41**: 2090–2099.

Greenberg WA, Priestley ES, Sears PS, Alper PB, Rosenbohm C, Hendrix M, Hung SC & Wong C-H (1999) Design and synthesis of new aminoglycoside antibiotics containing neamine as an optimal core structure: correlation of antibiotic activity with *in vitro* inhibition of translation. *J Am Chem Soc* **121**: 6527–6541.

Greene BM, Brown KR & Taylor HR (1989) Use of Ivermectins in humans. *Ivermectin and Abamectin* (Campbell WC, ed), pp. 311–327. Springer Verlag, New York.

Griffith BR, Langenhan JM & Thorson JS (2005) 'Sweetening' natural products *via* glycorandomization. *Curr Opin Biotechnol* **16**: 622–630.

Griffith BR, Krepel C, Fu X, Blanchard S, Ahmed A, Edmiston CE & Thorson JS (2007) Model for antibiotic optimization *via* neoglycosylation: synthesis of liponeoglycopeptides active against VRE. *J Am Chem Soc* **129**: 8150–8155.

Haddad J, Vakulenko S & Mobashery S (1999) An antibiotic cloaked by its own resistance enzyme. *J Am Chem Soc* **121**: 11922–11923.

Haddad J, Kotra LP, Llano-Sotelo B, Kim C, Azucena EF, Liu MZ, Vakulenko SB, Chow CS & Mobashery S (2002) Design of novel antibiotics that bind to the ribosomal acyltransfer site. *J Am Chem Soc* **124**: 3229–3237.

Hamasaki K, Woo M-C & Ueno A (2000) An aminoglycoside antibiotic, neamine, and its aromatic ring-substituted derivatives as potential inhibitors for HIV-1 RRE-Rev. *Tetrahedron Lett* **41**: 8327–8332.

Hanessian S & Kothakonda KK (2005) 3-*N*,*N*-dimethylamino-3-deoxy lincomycin: a structure-based hybrid between lincomycin and the desosamine unit of erythromycin. *Bioorg Med Chem* **13**: 5283–5288.

Hartsel SC, Benz SK, Peterson RP & Whyte BS (1991) Potassium-selective amphotericin-B channels are predominant in vesicles regardless of sidedness. *Biochemistry* **30**: 77–82.

Haysaka T & Inoue Y (1969) Chromomycin A3 studies in aqueous solutions. Spectrophotometric evidence for aggregation and interaction with herring sperm deoxyribonucleic acid. *Biochemistry* **8**: 2342–2347.

Hecht SM (1994) RNA degradation by bleomycin, a naturally occurring bioconjugate. *Bioconjugate Chem* **5**: 513–526.

Hendrix M, Alper PB, Priestley ES & Wong C-H (1997) Hydroxyamines as a new motif for the molecular recognition of phosphodiesters: implications for aminoglycoside-RNA interactions. *Angew Chem Int Ed* **36**: 95–98.

Herve M, Debouzy JC, Borowski E, Cybulska B & Garybobo CM (1989) The role of the carboxyl and amino-groups of polyene macrolides in their interactions with sterols and their selective toxicity – A P-31-NMR study. *Biochim Biophys Acta* **980**: 261–272.

Ho SN, Boyer SH, Schreiber SL, Danishefsky SJ & Crabtree GR (1994) Specific inhibition of formation of transcription complexes by a calicheamicin oligosaccharide: a paradigm for the development of transcriptional antagonists. *Proc Natl Acad Sci USA* **91**: 9203–9207.

Hunziker J (1996) Nucleic acid ligands based on carbohydrates. *Chimia* **50**: 248–256.

Ikeda D & Umezawa S (1999) Aminoglycoside antibiotics. *Naturally Occurring Glycosides* (Ikan R, ed), pp. 1–42. John Wiley & Sons Ltd., Chichester, NY.

Ikemoto N, Kumar RA, Ling TT, Ellestad GA, Danishefsky SJ & Patel DJ (1995) Calicheamicin-DNA complexes: warhead alignment and saccharide recognition of the minor groove. *Proc Natl Acad Sci USA* **92**: 10506–10510.

Jia Y, Bois-Choussy M, Malabarba A, Brunati C & Zhu J (2006a) Importance of the structure of vancomycin binding pocket in designing compounds active against vancomycin-resistant enterococci (VRE). *J Antibiot* **59**: 543–552.

Jia Y, Ma N, Liu Z, Bois-Choussy M, Gonzalez-Zamora E, Malabarba A, Brunati C & Zhu J (2006b) Design and synthesis of simple macrocycles active against vancomycin-resistant enterococci (VRE). *Chem Eur J* **12**: 5334–5351.

Jin E, Katritch V, Olson WK, Kharatisvili M, Abagyan R & Pilch DS (2000) Aminoglycoside binding in the major groove of duplex RNA: the thermodynamic and electrostatic forces that govern recognition. *J Mol Biol* **298**: 95–110.

Jizba JV, Sedmera P, Přikrylová V, Vokoun J, Mikulík K & Vaněk Z (1998) New semisynthetic anthracycline glycosides. *Collect Czech Chem Commun* **54**: 1104–1117.

Jones PH & Rowley EK (1968) Chemical modifications of erythromycin antibiotics. I. 3′-De(dimethylamino)erythromycin A and B. *J Org Chem* **33**: 665–670.

Kaziro Y & Kamiyama MJ (1967) Mechanism of action of Chromomycin A3: II. Inhibition of RNA polymerase reaction. *J Biochem (Tokyo)* **62**: 424–429.

Kirst HA & Sides GD (1989) New directions for macrolide antibiotics: structural modifications and *in vitro* activity. *Antimicrob Agents Chemother* **33**: 1413–1418.

Kratz F, Warnecke A, Schmid B, Chung D & Gitzel M (2006) Prodrugs of anthracyclines in cancer chemotherapy. *Curr Med Chem* **13**: 477–523.

Křen V & Martínková L (2001) Glycosides in medicine: "the role of glycosidic residue in biological activity". *Curr Med Chem Rev* **8**: 1313–1338.

Křen V & Thiem J (1997) Glycosylation employing bio-systems: from enzymes to whole cells. *Chem Soc Rev* **26**: 463–474.

Kumar RA, Ikemoto N & Patel DJ (1997) Solution structure of the esperamicin A1-DNA complex. *J Mol Biol* **265**: 173–186.

Langenhan JM, Griffith BR & Thorson JS (2005) Neoglycorandomization and chemoenzymatic glycorandomization: two complementary tools for natural product diversification. *J Nat Prod* **68**: 1696–1711.

Lee J, Kwon M, Lee KH, Jeong S, Hyun S, Shin KJ & Yu J (2004) An approach to enhance specificity against RNA targets using heteroconjugates of aminoglycosides and chloramphenicol (or linezolid). *J Am Chem Soc* **126**: 1956–1957.

Lee MD, Dunne TS, Chang CC, Ellestad GA, Siegel MM, Morton GO, McGahren WJ & Borders DB (1987) Calichemicins, a novel family of antitumor antibiotics. 1. Chemistry and partial structure of calichemicin γ_1^I. *J Am Chem Soc* **109**: 3464–3466.

Lee MD, Manning JK, Williams DR, Kuck N, Testa RT & Borders DB (1989) Calicheamicins, a novel family of antitumor antibiotics. 3. Isolation, purification and characterization of calicheamicins β_1^{Br}, γ_1^{Br}, α_2^I, α_3^I, β_1^I, γ_1^I and δ_1^I. *J Antibiot* **42**: 1070–1087.

Lemieux RU & Wolfrom ML (1948) The chemistry of streptomycin. *Adv Carbohydr Chem* **3**: 337–384.

Li J, Wang J, Czyryca PJ, Chang H, Orsak TW, Evanson R & Chang CWT (2004) Application of glycodiversification: expedient synthesis and antibacterial evaluation of a library of kanamycin B analogues. *Org Lett* **6**: 1381–1384.

Litovchick A, Lapidot A, Eisenstein M, Kalinkovich A & Borkow G (2001) Neomycin B-arginine conjugate, a novel HIV-1 Tat antagonist: synthesis and anti-HIV activities. *Biochemistry* **40**: 15612–15623.

Liu C, Smith BM, Ajito K, Komatsu H, Gomez-Paloma L, Li T, Theodorakis EA, Nicolaou KC & Vogy PK (1996) Sequence-selective carbohydrate–DNA interaction: dimeric and monomeric forms of the calicheamicin oligosaccharide interfere with transcription factor function. *Proc Natl Acad Sci USA* **93**: 940–944.

Liu X, Thomas JR & Hergenrother PJ (2004) Deoxystreptamine dimers bind to RNA hairpin loops. *J Am Chem Soc* **126**: 9196–9197.

Lu L, Golik J, Harrison R & Dedon P (1994) The deoxyfucose-anthranilate of esperamicin a1 confers intercalative DNA-binding and causes a switch in the chemistry of bi stranded DNA lesions. *J Am Chem Soc* **116**: 9733–9738.

Luzhetskyy A, Mayer A, Hoffmann J, Pelzer S, Holzenkämper M, Schmitt B, Wohlert S-E, Vente A & Bechthold A (2007) Cloning and heterologous expression of the aranciamycin biosynthetic gene cluster revealed a new flexible glycosyltransferase. *Chem Bio Chem* **8**: 599–602.

Ma Q, Xu Z, Schroeder BR, Sun W, Wei F, Hashimoto S, Konishi K, Leitheiser CJ & Hecht SM (2007) Biochemical evaluation of a 108-member deglycobleomycin library: viability of a selection strategy for identifying bleomycin analogues with altered properties. *J Am Chem Soc* **129**: 12439–12452.

Magerlein BJ (1971) Modification of lincomycin. *Adv Appl Microbiol* **14**: 185–229.

Magliozzo RS, Piesach J & Ciriolo MR (1989) Transfer-RNA is cleaved by activated bleomycin. *Mol Pharmacol* **35**: 428–432.

Magnet S & Blanchard JS (2005) Molecular insights into aminoglycoside action and resistance. *Chem Rev* **105**: 477–497.

Maiese WM, Lechevalier MP, Lechevalier HA, Korshalla J, Kuck NA, Fantini A, Wildey MJ, Thomas J & Greenstein M (1989) Calicheamicins, a novel family of antitumor antibiotics: taxonomy, fermentation and biological properties. *J Antibiot* **42**: 558–563.

Martin JN, Muñoz EM, Schwergold C, Souard F, Asensio JL, Jimenez-Barbero J, Cañada J & Vicent C (2005) Carbohydrate-based DNA ligands: sugar-oligoamides as a tool to study carbohydrate-nucleic acid interactions. *J Am Chem Soc* **127**: 9518–9533.

Martin RJ & Pennington AJ (1989) A patch-clamp study of effects of dihydroavermectin on *Ascaris* muscle. *Br J Pharmacol* **98**: 747–756.

Matsumoto C, Hamasaki K, Mihara H & Ueno A (2000) A high-throughput screening utilizing intramolecular fluorescence resonance energy transfer for the discovery of the molecules that bind HIV-1 TAR RNA specifically. *Bioorg Med Chem Lett* **10**: 1857–1861.

Mayville P, Novick RP, Ji G, Beavis R, Yang H, Goger M & Muir TW (1999) Structure-activity analysis of synthetic autoinducing thiolactone peptides from *Staphylococcus aureus* responsible for virulence. *Proc Nat Acad Sci USA* **96**: 1218–1223.

McGuire JM, Bunch RL, Anderson RC, Boaz HE, Flynn EH, Powell M & Smith JW (1952) Ilotycin, a new antibiotic. *Schweiz Med Wochenschr* **82**: 1064–1065.

Melancon CE, Thibodeaux CJ & Liu HW (2006) Glyco-stripping and glyco-swapping. *ACS Chem Biol* **1**: 499–504.

Mendez C & Salas JA (2001) Altering the glycosylation pattern of bioactive compounds. *Trends Biotech* **19**: 449–456.

Mereu A, Moriggi E, Napoletano M, Regazzoni C, Manfredini S, Mercurio TP & Pellacini F (2006) Design, synthesis and *in vivo* activity of 9-(*S*)-dihydroerythromycin derivatives as potent anti-inflammatory agents. *Bioorg Med Chem Lett* **16**: 5801–5804.

Minotti G, Menna P, Salvatorelli E, Cairo G & Gianni L (2004) Anthracyclines: molecular advances and pharmacologie developments in antitumor activity and cardiotoxicity. *Pharmacol Rev* **56**: 185–229.

Moazed D, Stern S & Noller HF (1986) Rapid chemical probing of conformation in 16S ribosomal RNA and 30S ribosomal subunits using primer extension. *J Mol Biol* **187**: 399–416.

Moore MH, Hunter WN, d'Estaintot BL & Kennard O (1989) DNA – drug interactions. The crystal structure of d(CGATCG) complexed with daunomycin. *J Mol Biol* **206**: 693–705.

Morisaki N, Hashimoto Y, Furihata K, Yazawa K, Tamura M & Mikami Y (2001) Glycosylative inactivation of chalcomycin

and tylosin by a clinically isolated *Nocardia asteroides* strain. *J Antibiot* **54**: 157–165.

Mrozik H, Eskola P, Arison BH *et al.* (1995) 4″-Deoxy-4″-aminoavermectins with potent broad spectrum antiparasitic activities. *Bioorg Med Chem Lett* **5**: 2435–2440.

Murdter TE, Sperker B, Kivisto KT *et al.* (1997) Enhanced uptake of doxorubicin into bronchial carcinoma: β-glucuronidase mediates release of doxorubicin from a glucuronide prodrug (HMR 1826) at the tumor site. *Cancer Res* **57**: 2440–2445.

Murrell JM, Liu W & Shen B (2004) Biochemical characterization of the SgcA1 α-D-glucopyranosyl-1-phosphate thymidylyltransferase from the enediyne antitumor antibiotic C-1027 biosynthetic pathway and over expression of *sgcA1* in *Streptomyces globisporus* to improve C-1027 production. *J Nat Prod* **67**: 206–213.

Nagarajan R (1993) Structure–activity relationships of vancomycin-type glycopeptide antibiotics. *J Antibiot* **46**: 1181–1195.

Natrajan A & Hecht SM (1993) Bleomycin: mechanism of polynucleotide recognition and oxidative degradation. *Molecular Aspects of Anticancer Drug–DNA Interactions* (Neidle S & Waring M, eds), pp. 197–242. MacMillan, London.

Nicas TI, Mullen DL, Flokowitsch JE, Preston DA, Snyder NJ, Zweifel MJ, Wilkie SC, Rodriquez MJ, Thompson RC & Cooper RDG (1996) Semisynthetic glycopeptide antibiotics derived from LY264826 active against vancomycin-resistant enterococci. *Antimicrob Agents Chemother* **40**: 2194–2199.

Nicolaou KC (1993) The battle of calicheamicin γ_1^I. *Angew Chem Int Ed Engl* **32**: 1377–1385.

Nicolaou KC (2003) Perspectives in total synthesis: a personal account. *Tetrahedron* **59**: 6683–6738.

Nicolaou KC & Dai WM (1991) Chemistry and biology of the enediyne anticancer antibiotics. *Angew Chem Int Ed Engl* **30**: 1387–1416.

Nicolaou KC & Smith AL (1993) Progress towards the total synthesis of the enediyne anticancer antibiotics. *Pure Appl Chem* **65**: 1271–1280.

Nicolaou KC, Tsay SC, Suzuki T & Joyce GF (1992) DNA–carbohydrate interactions. Specific binding of the calicheamicin γ_1^I oligosaccharide with duplex DNA. *J Am Chem Soc* **114**: 7555–7557.

Oku N, Matsunaga S & Fusetani N (2003) Shishijimicins A–C, novel enediyne antitumor antibiotics from the ascidian *Didemnum proliferum*. *J Amer Chem Soc* **125**: 2044–2045.

Olsufyeva EN, Tevyashova AN, Trestchalin ID, Preobrazhenskaya MN, Platt D & Klyosov A (2003) Synthesis and antitumor activity of new D-galactose-containing derivatives of doxorubicin. *Carbohydr Res* **338**: 1359–1367.

Palacios DS, Anderson TM & Burke MD (2007) A Post-PKS oxidation of the amphotericin B skeleton predicted to be critical for channel formation is not required for potent antifungal activity. *J Amer Chem Soc* **129**: 13804–13805.

Paloma LG, Smith JA, Chazin WJ & Nicolaou KC (1994) Interaction of calicheamicin with duplex DNA: role of the oligosaccharide domain and identification of multiple binding modes. *J Am Chem Soc* **116**: 3697–3708.

Paquet V & Carreira EM (2006) Significant improvement of antifungal activity of polyene macrolides by bisalkylation of the mycosamine. *Org Lett* **8**: 1807–1809.

Patt TE, Argoudells AD & Marshall VP (1984) Process for preparing lincomycin and clindamycin ribonucleotides. U.S. Patent 4,430,495.

Peri F & Nicotra F (2004) Chemoselective ligation in glycochemistry. *Chem Commun* 623–627.

Perkins HR (1969) Specificity of combination between mucopeptide precursors and vancomycin or ristocetin. *Biochem J* **111**: 195–205.

Rezanka T, Sobotka M, Spizek J & Sigler K (2006) Pharmacologically active sulfur-containing compounds. *Anti-Infect Agents Med Chem* **5**: 187–224.

Rezanka T, Spizek J & Sigler K (2007) Medicinal use of lincosamides and microbial resistance to them. *Anti-Infect Agents Med Chem* **6**: 133–144.

Rosenbohm C, Berghe DV, Vlietinck A & Wengel J (2001) Parallel synthesis of a small library of novel aminoglycoside analogs based on 2-amino-2-deoxy-D-glucose and D-ribose scaffolds. *Tetrahedron* **57**: 6277–6287.

Salas JA & Méndez C (2005) Biosynthesis pathways for deoxysugars in antibiotic-producing actinomycetes: isolation, characterization and generation of novel glycosylated derivatives. *J Mol Microbiol Biotechnol* **9**: 77–85.

Sastry M & Patel DJ (1993) Solution structure of the mithramycin dimer-DNA complex. *Biochemistry* **32**: 6588–6604.

Shaw KJ, Rather PN, Hare RS & Miller GH (1993) Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev* **57**: 138–163.

Sieradzki K, Roberts RB, Haber SW & Tomasz A (1999) The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. *New England J Med* **340**: 517–523.

Silva DJ & Kahne DE (1993) Studies of the 2:1 chromomycin A3-Mg^{2+} complex in methanol: role of the carbohydrates in complex formation. *J Am Chem Soc* **115**: 7962–7970.

Smith AL & Nicolaou KC (1996) The enediyne antibiotics. *J Med Chem* **39**: 2103–2117.

Sperker B, Backman JT & Kroemer HK (1997) The role of β-glucuronidase in drug disposition and drug targeting in humans. *Clin Pharmacokinet* **33**: 18–31.

Spizek J & Rezanka T (2004a) Lincomycin, cultivation of producing strains and biosynthesis. *Appl Microbiol Biotechnol* **63**: 510–519.

Spizek J & Rezanka T (2004b) Lincomycin, clindamycin and their applications. *Appl Microbiol Biotechnol* **64**: 455–464.

Spizek J, Novotna J & Rezanka T (2004c) Lincosamides, chemical structure, biosynthesis, mechanism of action, resistance and applications. *Adv Appl Microbiol* **56**: 121–154.

Stassinopoulos A & Goldberg IH (1995) Binding and cleavage characteristics of the complexes formed between the

neocarzinostatin chromophore and single site containing oligonucleotides. *Bioorg Med Chem* **3**: 713–721.

Stubbe J & Kozarich JW (1987) Mechanisms of bleomycin-induced DNA degradation. *Chem Rev* **87**: 1107–1136.

Sucheck SJ, Wong AL, Koeller KM, Boehr DD, Draker K, Sears P, Wright GD & Wong C-H (2000) Design of bifunctional antibiotics that target bacterial rRNA and inhibit resistance-causing enzymes. *J Am Chem Soc* **122**: 5230–5231.

Sztaricskai F, Dinya Z, Batta G, Mocsari A, Hollosi M, Majer Z, Masuma R & Omura S (1997) Chemical synthesis and structural study of lincomycin sulfoxides and a sulfone. *J Antibiot* **50**: 866–873.

Sztaricskai F, Sum A, Roth E, Pelyvás IF, Sándor S, Gyla Batta G, Herczegh P, Reményi J, Miklán Z & Hudec F (2005) A new class of semisynthetic anthracycline glycoside antibiotics incorporating a squaric acid moiety. *J Antibiot* **58**: 704–714.

Thibodeaux CJ & Liu HW (2007) Manipulating nature's sugar biosynthetic machineries for glycodiversification of macrolides: recent advandes and future prospects. *Pure Appl Chem* **79**: 785–799.

Thirsk C & Whiting A (2002) Polyene natural products. *J Chem Soc Perkin Tr* **1**: 999–1023.

Thompson C, Ge M & Kahne D (1999) Synthesis of vancomycin from the aglycone. *J Am Chem Soc* **121**: 1237–1244.

Walker S, Gange D, Gupta V & Kahne D (1994) Analysis of hydroxylamine glycosidic linkages: structural consequences of the NO bond in calicheamicin. *J Am Chem Soc* **116**: 3197–3206.

Wang HJ, Ughetto G, Quiqley GJ & Rich A (1987) Interactions between an anthracycline antibiotic and DNA: molecular structure of daunomycin complexed to d(CpGpTpApCpG) at 1.2-Å resolution. *Biochemistry* **26**: 1152–1163.

Wang J (1996) DNA topoisomerases. *Annu Rev Biochem* **65**: 635–692.

Wei G, Du Y & Linhardt RJ (2004) New potent insecticidal agent: 4′-fucosyl avermectin derivative. *Tetrahedron Lett* **45**: 6895–6898.

Wei L, Wei G, Zhang H, Wang PG & Du Y (2005) Synthesis of new, potent avermectin-like insecticidal agents. *Carbohydr Res* **340**: 1583–1590.

Weignerova L, Spizek J, Najmanova L & Kren V (2001) Enzymatic glycosylation of lincomycin. *Biosci Biotechnol Biochem* **65**: 1897–1899.

Weymouth-Wilson AC (1997) The role of carbohydrates in biologically active natural products. *Nat Prod Rep* **14**: 99–110.

Williams DH (1996) The glycopeptide story – How to kill the deadly 'Superbugs'. *Nat Prod Rep* **13**: 469–477.

Williams GJ, Zhang C & Thorson JS (2008a) Expanding the promiscuity of a natural-product glycosyltransferase by directed evolution. *Nat Chem Biol* **3**: 657–662.

Williams GJ, Goff RD, Zhang C & Thorson JS (2008b) Optimizing glycosyltransferase specificity *via* "hot spot" saturation mutagenesis presents a catalyst for novobiocin glycorandomization. *Chem Biol* **15**: 393–401.

Williams JD & Sefton AM (1993) Comparison of macrolide antibiotics. *J Antimicrob Chemother* **31**: 11–26.

Willis B & Arya DP (2006) Major groove recognition of DNA by carbohydrates. *Curr Org Chem* **10**: 663–673.

Wong CH, Hendrix M, Manning DD, Rosenbohm C & Greenberg WA (1998) A library approach to the discovery of small molecules that recognize RNA: use of a 1,3-hydroxyamine motif as core. *J Am Chem Soc* **120**: 8319–8327.

Yang M, Proctor MR, Bolam DN, Errey JC, Field RA, Gilbert HJ & Davis BG (2005) Probing the breadth of macrolide glycosyltransferases: *in vitro* remodeling of a polyketide antibiotic creates active bacterial uptake and enhances potency. *J Am Chem Soc* **127**: 9336–9337.

Yoshizawa S, Fourmy D & Puglisi JD (1998) Structural origins of gentamicin antibiotic action. *EMBO J* **17**: 6437–6448.

Zhang C, Griffith BR, Fu Q, Albermann C, Fu X, Lee I-K, Li L & Thorson JS (2006a) Exploiting the reversibility of natural product glycosyltransferase-catalyzed reactions. *Science* **313**: 1291–1294.

Zhang CC, Albermann X, Fu JS & Thorson JS (2006b) The *in vitro* characterization of the iterative avermectin glycosyltransferase AveBI reveals reaction reversibility and sugar nucleotide flexibility. *J Am Chem Soc* **128**: 14224–14225.

Zhang G, Fang L, Zhu L, Aimiuwu JE, Shen J, Cheng H, Muller MT, Lee GE, Sun D & Wang D (2005) Syntheses and biological activities of disaccharide daunorubicins. *J Med Chem* **48**: 5269–5278.

Zhang G, Fang L, Zhu L, Zhong Y, Wang PG & Sun D (2006c) Syntheses and biological activities of 3′-azido disaccharide analogues of daunorubicin against drug-resistant leukemia. *J Med Chem* **49**: 1792–1799.

Zhou J, Wang G, Zhang LH & Ye XS (2007) Modifications of aminoglycoside antibiotics targeting RNA. *Med Res Rev* **27**: 279–316.

Zhu L, Cao X, Chen W, Zhang G, Sun D & Wang PG (2005) Syntheses and biological activities of daunorubicin analogs with uncommon sugars. *Bioorg Med Chem* **13**: 6381–6387.

Zunino F, Pratesi G & Pereno P (2001) Role of the sugar moiety in the pharmacological activity of anthracyclines: development of a novel series of disaccharide analogs. *Biochem Pharmacol* **61**: 933–938.