12.7 Glycoside vs. Aglycon: The Role of Glycosidic Residue in Biological Activity

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Abstract

A large number of biologically active compounds are glycosides. Sometimes the glycosidic residue is crucial for their activity, in other cases glycosylation only improves pharmacokinetic parameters. Recent developments in molecular glycobiology brought better understanding of aglycon vs. glycoside activities, and made possible the development of new, more active or more effective glycodrugs based on these findings – a very illustrative recent example is vancomycin. The new enzymatic methodology "glycorandomization" enabled preparation of glycoside libraries and opened up paths to the preparation of optimized or entirely novel glycoside antibiotics. This chapter deals with an array of glycosidic compounds currently used in medicine but also covers the biological activity of some glycosidic metabolites of known drugs. The chapter discusses glycosides of vitamins, polyphenolic glycosides (flavonoids), alkaloid glycosides, glycosides of antibiotics, glycopeptides, cardiac glycosides, steroid and terpenoid glycosides etc. The physiological role of the glycosyl moiety and structure-activity relations (SAR) in the glycosidic moiety (-ies) are also discussed.

Keywords

Natural products; Glycosides; Structure-activity relations; Glycorandomization; Flavonoid glycosides; Vitamin glycosides; Antibiotics; Alkaloid glycosides; Steroid glycosides; DNA interactions

Abbreviations

ATP adenosine triphosphate
CNS central nervous system
DNA deoxyribonucleic acid
GABA gamma amino butyric acid
HBA heavenly blue anthocyanidin
i.v. intra venous
PBMC peripheral blood mononuclear cells
p.o. per oral
MRSA methicilline-resistant *Staphylococcus aureus*
NK natural killer
RA retinoic acid
RAG retinoic acid glucuronide
RNA ribonucleic acid

1 Introduction

Many biologically active compounds are glycosides. Glycosides comprise several important classes of compounds such as antibiotics, hormones, sweeteners, alkaloids, flavonoids, etc. Sometimes the glycosidic residue is crucial for their activity; in other cases it only improves pharmacokinetic parameters. Owing to recent developments in molecular glycobiology a greater understanding has been gained of aglycon vs. glycoside activity and – based on these findings – it has become possible to develop new, more active or more effective glycodrugs.

Nevertheless, it is nearly impossible to define a general pattern of biological activities for the glycosides compared to the respective aglycons. Some well-selected examples can illustrate general trends and show the effects and potential applications of the compounds carrying the glycosidic moiety in relation to the respective aglycons.

It is generally accepted that glycosides are more water-soluble than the respective aglycons. Attaching the glycosidic moiety to a molecule increases its hydrophilicity. This effect influences pharmacokinetic properties of the respective compounds, e. g., circulation, elimination and concentration in the body fluids.

Modified hydrophilicity, however, influences mainly the membrane transport. Some compounds enter the cells just because of their "solubility" in the membrane components. Glycosylation can, in some cases, restrict or inhibit cell uptake of the particular compounds. Glycosylation can strongly influence transport through such important barriers as the hemato-encephalitic barrier and block the entrance of many compounds into brain tissue. Contrary to this, some glucosides can be transported actively into the brain tissue using the glucose-transport system. Another important barrier, in which glycosylation plays a crucial role is the placental barrier. Here entry of many glucuronides to fetal tissue is blocked, thus preventing intoxication by metabolites of xenobiotics. On the other hand, some glycosidic moieties can interact with receptors or lectins on the cell surfaces followed by their active uptake. A good example is the high affinity of β-galactosides to hepatocytes due to galectin-C occurring in high concentration on their surface.

Carbohydrates and glycosides recently emerged as a novel class of nucleic acid-binding compounds. Detailed study of the factors affecting the site-selectivity of some recently discovered antitumor antibiotics has shed new light on the role that oligosaccharides may play in nucleic acid recognition.

An important aspect for prediction of the respective glycoconjugate activities is also their susceptibility towards glycosidic cleavage at various sites of application. In the stomach and in the intestine most of the glycosides are hydrolyzed, either by the action of the acidic environment (stomach) or by the action of glycosidases (small intestine). There are, however, glycosides that are not hydrolyzed easily – e. g. α-galactosides – and such compounds are either unable to pass the hemato-intestinal barrier or they penetrate unhydrolyzed. Nonresorbed glycoconjugates can be cleaved later in the colon or metabolized by the action of the intestinal microflora. Glycosidases are present also in other body fluids, e. g. blood serum contains lysozyme that cleaves effectively β-*N*-acetylhexosaminides.

There exist, however, glycosides with specific individual biological activity that cannot be simply derived from the respective activity of the aglycon. The final activity is then given by the overall molecular structure.

Comparison of biological activities of aglycon and its respective glycoside can indicate some structure-effect correlations and also demonstrate the advantage (or uselessness) of introducing glycosyl moieties into pharmacologically interesting molecules.

Exploitation of the SAR data of the glycosides (most often glycosidic antibiotics) enabled synthetic modification and optimization of their glycon part. Recently, however, the new brilliant methodology "glycorandomization", based on the enzymatic modifications of glycosides, enabled the preparation of glycoside libraries. This method employing natural and mutant glycosyltransferases with wobbling specificities opened up new paths to the preparation of optimized or entirely novel glycoside antibiotics.

This chapter deals with "small molecules" – glycosides – aiming at the description of the function of the glycon part in the biological activities of the respective compounds. New, recently developed methods, leading to the bio-functional optimization of this moiety are also described.

2 Polyphenol Glycosides

Most of the polyphenols are produced by plants. Phenolic OH groups are generally good targets for biological glycosylations and many phenolic compounds occur virtually only in their glycosylated forms.

Glycosides of polyphenolic compounds, e. g. those of flavonoids, constitute a large group and novel findings increase their number extremely quickly. The list of flavone and flavonol glycosides known up to 1986 contains about 900 entries [1]. In the survey of Harborne and Williams [2] covering discoveries in anthocyanins and other flavonoids from January 1995 to December 1997 over 160 new glycosides of flavonoids are reported.

Many of them served as important components of traditional medicines. It is very interesting from the point-of-view of structure-effect correlations that minute alterations in their structures, e. g. positional changes of OH groups, bring about dramatic changes in their biological effects. Naturally, in the view of such minor changes, glycosylation of these compounds

sometimes completely changes their activity. For instance, quercetin (3,3′,4′,5,7-pentahydroxyflavon, 16) is now considered to be an extremely dangerous toxic and mutagenic compound (even though it was used previously as a colorant). However, its various glycosides [e. g., quercitrin ([17]), hyperin, spiraeosid, rutin (see also ❯ *Sect. 6.1.4* in this chapter)] occur frequently in plant material sometimes used as food, and many are considered to be beneficial to human health.

2.1 Anthocyanins

Anthocyanins are the most important group of water-soluble plant pigments visible to human eyes. Glycosylation of anthocyanins has many effects – physico-chemical effects such as antioxidant stabilization and also color stabilization or "deepening" caused by "sandwich stacking" controlled by the glycosyl substitution. This effect is well-known in "heavenly blue anthocyanidin" (HBA, **1**) (❯ *Scheme 1*) from *Ipomoea tricolor* (Convolvulaceae). HBA carries complex glycosyl-ester moieties attached to C-3 of peonidin (aglycon) containing ester-bound residues of caffeyl acid (❯ *Fig. 1*).

The whole structure then folds into a sandwich structure, in which aromatic residues of caffeic acid interact with the aromatic system of the aglycon peonidin [3]. This stacking results in the bathochromic shift of the complex. Moreover, this and similar glycosyl-acyl conjugates of the anthocyanidines stabilize these pigments at various pH within the plant vacuoles in the range of 4–6 against hydration and tautomerization resulting in profound changes of electron spectral properties (loss of color). Stability of color is often crucial for the biology of the plants, e. g. pollination by insects.

Anthocyanidins may also be important factors – with other flavonoids – in the resistance of plants to insect attack. Thus, the complete cyanidin 3-β-glucoside (**2**)(❯ *Scheme 2*) and not only the aglycon itself was shown to protect cotton leaves against the feeding of tobacco budworm [1].

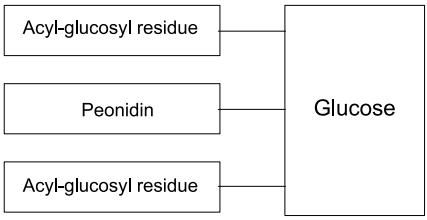
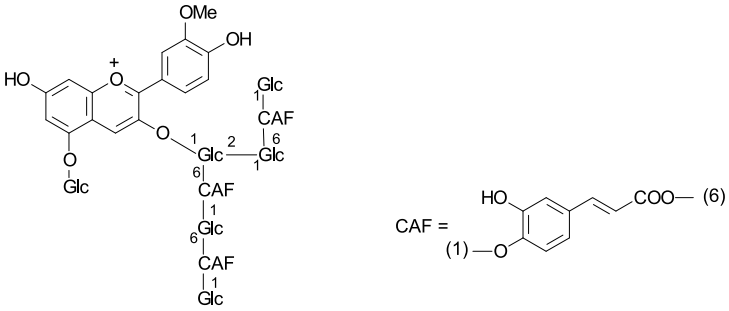


Figure 1
Model of the sandwich stacking

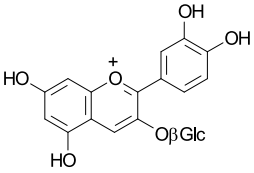
2.2 Flavonoid and Isoflavonoid Glycosides

The raison d'être for the proliferation of flavone and flavonol glycosides in nature continues to intrigue plant scientists. The ability of UV-B radiation to damage DNA, RNA and proteins as



1 (HBA) peonidin acyl-glycoside

Scheme 1



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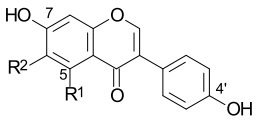
Scheme 2

well as to impair processes like photosynthesis is well known. Constituents of leaf epidermis such as polyphenols and their glucosides provide a means of absorbing the damaging radiation. An example of this has been demonstrated in the case of the needles of *Pinus silvestris* (pine tree). Two main compounds, isoquercitrin 3′,6′-di-*p*-coumarate and kaempferol 3-β-glucoside, have been found to be induced in seedlings under simulated global radiation. The concentration of the acylated kaempferol 3-β-glucoside reached 2.4 µmol/g fresh wt. [4].

In the case of plant pollens, it has long been recognized that flavonol glycosides are widely present and apparently contribute to the yellow color in the pollen. It is interesting that the two glycosides most frequently encountered are the 3-sophorosides of kaempferol and quercetin, and, furthermore, if these are not present, closely related 2′-*O*-glycosides of a flavonol 3-β-glucoside are normally encountered. Further examples are two methylated herbacetin 3-sophorosides reported in *Ranunculus*, *Raphanus* and *Klea* pollens [1,5]. The function of flavonoid glycosides in pollen is still uncertain in most plants, but in the case of *Petunia hybrida*, there is rather good evidence that it has a critical role in subsequent pollen tube growth, once the pollination has occurred. Structure-activity relationships have been explored in the case of *Petunia*, and a kaempferol diglycoside does appear to be the most active constituent [6].

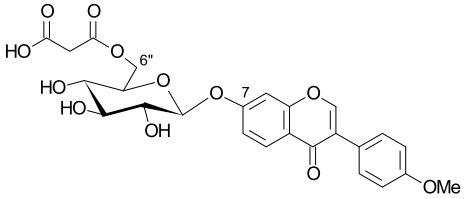
Glycosides and malonyl glycosides of isoflavones, and some other flavonoids often serve as their storage and transport forms. The most-studied systems for isoflavonoid glycoside metabolism are white lupine (*Lupinus albus*) root and cell cultures containing a range of mono- and diglucosides of genistein (**4**), 2′-hydroxygenistein and their 6- or 3′-prenyl derivatives [7], and soybean seeds and seedlings, and chickpea cell suspension cultures.



3 R^1 = H, R^2 = H

4 R^1 = OH, R^2 = H

5 R^1 = H, R^2 = OMe



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Scheme 3

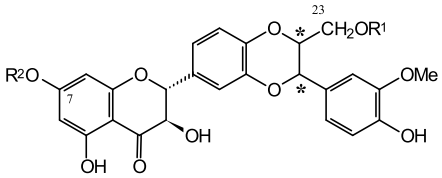
Features of the accumulation and metabolism of these compounds differ somewhat in the different species. In soybean seed hypocotyls, the 7-*O*-*β*-glucosides, 7-*O*-*β*-glucoside-6″-*O*-malonates, and 7-*O*-*β*-glucoside-6″-*O*-acetates of the isoflavones daidzenin (**3**), genistenin (**4**), and glycitein (**5**) occur (*Scheme 3*). All of them have been shown to increase during seed development in the pod to maximum levels between 45 and 60 days after flowering [8]. Three days after germination, the metabolism of the young leaf shifts from isoflavonoid to flavonoid accumulation [9], although low levels of isoflavone glycosides remain [10].

A similar pattern of isoflavonoids and their glycosides is observed in alfalfa where formonetin 7-*O*-*β*-glucoside-6″-*O*-malonate (**6**) accumulates in roots along with other isoflavonoid constituents [11].

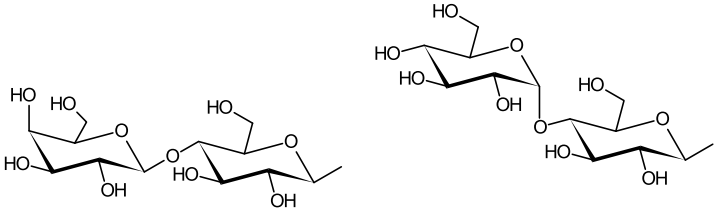
Infection of soybean with pathogenic fungus *Phytophthora sojae* leads to dramatic changes in isoflavone glycoside profiles and distribution. In the leaves, the nonglycosylated pterocarpan glyceolin accumulates to high levels only in the hypersensitive lesion formed in a resistant interaction, whereas the glucosides and malonyl glucosides of **3**, **4** and **5** accumulate in a broad area around the lesion [10]. In cotyledons the already constitutive pools of isoflavone glycosides are rapidly mobilized and, in the case of **3** the aglycon can be used for phytoalexin synthesis [12].

Some flavonoid glycosides are prepared synthetically or by biotransformations, usually for pharmaceutical purposes. Silybin (**7**) is a flavonolignan that is extracted from seeds of milk thistle (*Silybum marianum*) and it is used extensively as a potent hepatoprotectant and an antidote in mushroom poisoning. The major drawback of this compound is its low water solubility (about 0.43 g/L). Therefore, its glycosylation was accomplished by biological [13] and chemical methods [14].

Biotransformation using plant cell cultures afforded silybin 7-*β*-glucoside (**8**) [13] and chemical glycosylations gave silybin glycosides at C-23 (*β*-glucoside, *β*-galactoside, *β*-maltoside and *β*-lactoside **9**–**12**). The solubility of silybin glycosides was improved considerably compared to the aglycon (**9**, 13.0; **10**, 1.7; **11**, 3.8 and **12**, 5.6 g/L)(*Scheme 4*). Biological tests



7 R^1 = H, R^2 = H (silybin)
8 R^1 = H, R^2 = βGlc
9 R^1 = βGlc, R^2 = H
10 R^1 = βGal, R^2 = H
11 R^1 = βLac, R^2 = H
12 R^1 = βMalt, R^2 = H

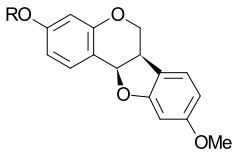


β-Lactosyl (β-Lac) β-Maltosyl (β-Malt)

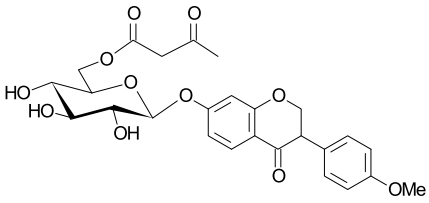
Scheme 4

showed that the above silybin glycosides have considerably higher antioxidative activity, the β-glucoside being the best that is ca. 10-times better than aglycon itself. Silybin monoglycosides show also better hepatoprotective activity than the aglycon. Similar effects were observed also in the tests with membrane lipoperoxidation of the mitochondrial membranes, in which silybin glycosides, mainly β-glucoside and β-galactoside, were found to be ca. 50–70% better antilipoperoxidants. The cytotoxicity at higher concentrations of silybin towards hepatocytes at conc. over 0.1 mM can be lowered by its glycosylation – in this case especially the β-maltoside and β-lactoside display substantially lower toxicity at high concentrations. Silybin β-galactoside was found to have better hepatoprotective activities in vivo presumably due to β-galactosyl residue causing higher affinity towards hepatocytes.

Flavonoids occurring virtually in all plant-derived food have a strong effect on the mammalian biology, mainly on immunity, inflammation and cancer [15]. A well described case of an immunomodulative effect for flavonoid glycoside is plantagoside (5,7,3′,4′,5′-pentahydroxyflavanone 3′-β-*O*-glucoside) that inhibits proliferative response of Balb/c spleen cells to the T-cell mitogen concanavalin A. On the contrary it has no effect on the mitogenic activity of lipopolysaccharides or phytohaemaglutinin thus demonstrating that the later two mitogens probably utilize activation pathways that are insensitive to this particular glyco-flavonoid. The fact that plantagoside is an α-mannosidase inhibitor [16] is of interest as well. Glycosides of isoflavonoids also have important functions in the communication between leguminous plants and nitrogen-fixing bacteria. Some of them are able to trigger *Nod* genes responsible for for-



13 (-)-medicarpin R = H
14 R = βGlc



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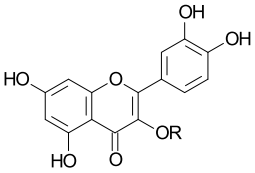
Scheme 5



16 quercetin R = H
17 quercitrin R = βGlc

Scheme 6

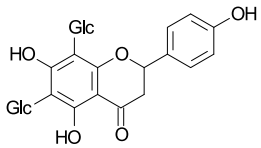
mation of lipochitooligosaccharide signal molecules, which in turn induce root hair curling and the cortical cell division that characterize the early development of the nodule.

Root exudates from alfalfa inoculated with *Rhizobium melioti* contain the pterocarpan medicarpin (**13**) and its β-glucoside (**14**), as well as formonetin 7-*O*-β-glucoside-6″-*O*-malonate (**15**) (Scheme 5). Levels of **14** are increased when plants are grown under low nitrogen conditions [17]. Formonetin and its 7-*O*-glucoside do not possess *Nod* gene-inducing activity for the alfalfa symbiont. Surprisingly, however, **15** can induce *Rhizobium nod* genes via interactions with both the NodD1 and NodD1-recognition proteins [18]. The Nod factors synthesized as a result of *Nod* gene activation are active on alfalfa roots at concentrations of around 10^{-9} M.

Flavonoids and their glycosides mediate also communication between plants and insects, serving like "pollination factors". This effect is caused mainly by the final color of the anthocyanin complex and is not related to the extent of glycosylation. Flavonoid glycosides also serve as feeding stimulants, e. g. isoquercitrin (quercetin 3-*O*-β-glucoside, **17**)(Scheme 6) that serves as a "biting factor" in feeding of *Bombyx mori* (silkworm) on the *Morus alba* leaves [19].

There are further examples of feeding stimulants from the group of flavonoid glycosides such as, e. g. kaempferol 3-*O*-β-xyloside and some others.

These compounds can be also effective antifeedants and here the glycosylation pattern is of crucial importance. Rutin [91] is a feeding stimulant to the tobacco hornworm, *Manduca sexta*,



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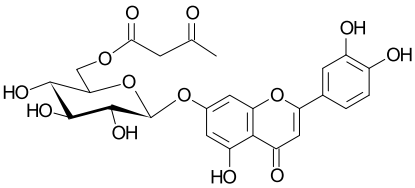

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Scheme 7

and quercetin 3-rhamnoside (less one glucose in comparison to rutin) acts as an antifeedant to the same species [20].

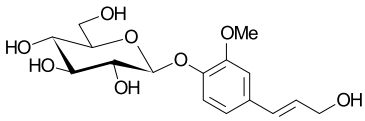
The larvae of most butterfly and moth species are monophagous or oligophagous, i. e. they feed from only one or a few (usually closely related) host plants. Caterpillars are much less mobile than winged adults and are unlikely to find the right food if the eggs, from which they hatch, have been laid on the wrong plant species. Thus, the oviposition choice of an adult female is crucial to larval survival. Most butterflies use visual and/or olfactory cues for the oviposition. The female will lay eggs only when she detects specific oviposition stimulants on the plant. As oviposition stimulants usually a complex cocktail is involved and flavonoid glycosides are an important part of this cocktail.

The first flavonoid glycoside that was found to induce ovipositional response in a butterfly was vicenin-2 (apigenin 6,8-di-*C*-glucoside, **18**) in the *Citrus*-feeding swallowtail *Papilio xuthus* [21]. Later more flavonoid glycosides were identified to be oviposition stimulants (naringenin 7-*O*-β-rutinoside, hesperetin 7-*O*-β-rutinoside and rutin) [22]. Another documented oviposition inducing flavonoid glycoside is 7-(6″-malonyl-β-D-glucopyranosyl)-luteolin (**19**) present in carrot (*Daucus carota*) that attracts *Papilio polyxenes* (Black swallowtail). The malonyl group in the flavonoid glycoside seems to be crucial, since the unmalonylated luteolin 7-glucoside and the corresponding glucuronide (also present in the carrot leaves) has little or no effect [23] (*Scheme 7*).

Flavonoid glycosides after intake by the insects are also sequestrated without hydrolysis and can be found in the insect tissues.

2.3 Lignins and Lignans

Coniferin (**20**) is one of the most abundant lignan glycosides occurring mostly in coniferous trees and some other plants. It is the dominant transport and storage form of coniferyl alcohol, which in turn constitutes the main building block of lignins.



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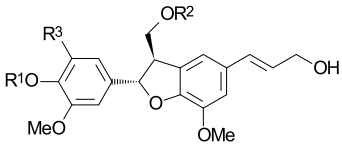
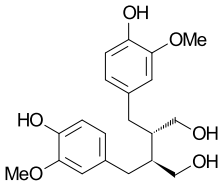


21 R^1 = βGlc R^2 = R^3 = H

22 R^1 = H R^2 = βGlc R^3 = H

23 R^1 = H R^2 = βGlc R^3 = OMe



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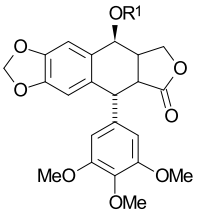
Scheme 8

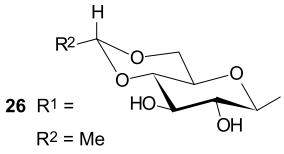
β-Glucosides of dehydroconiferyl alcohols (**21**–**23**) (*Scheme 8*) and the respective 2,3-epimers are growth factors isolated from transformed *Vinca rosea* tumor cells [24]. These compounds are not fragments of cell walls, but are produced directly from coniferyl alcohol [25]. Nonglycosylated derivatives do not have the cytokinine activity.

One of the most abundant lignans, for example in flaxseed, is di-β-glucoside of (+)-secoisolariciresinol (**24**) (both phenolic OH glycosylated). This compound is in vivo deglycosylated by intestinal microflora and by further demethylation and oxidation converted into enterolactone. These compounds called "mammalian lactones" enter the enterohepatic circulation, and there exists good evidence that their presence lovers incidence of the hormone-related cnacers [26]. They also lower the incidence of colon tumors. The above-mentioned diglycoside of **24** was also shown to act as an effective radical scavenger [27].

One of the most prominent lignans in terms of pharmacological application, in which glycosylation plays a crucial role, is podophylotoxin (**25**). This compound is the main active principle of podophyllin, a resin obtained from rhizomes of *Podophyllum peltatum*. Early pharmacological results with *Podophylum* lignans were disappointing. In spite of its antitumor promise direct administration of **25** was compromised by severe gastrointestinal toxicity. However, derivatized glycosides showed very promising activity in vitro and in vivo [28] and two of



25 podophylotoxin R^1 = H



26 R^1 =
R^2 = Me

27 R^2 =

Scheme 9

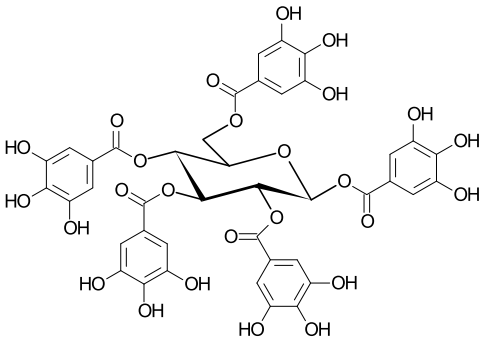
them, the ethylidene derivative etoposide (**26**) and the 4,6-alkylidene derivative of thiophencarbaldehyde, teniposide (**27**), were developed as anticancer drugs. In contrast to a classical spindle poison **25**, which causes arrest of the cells in the metaphase, attaching sugar moieties prevented tubulin interaction and thus microtubular assembly was not inhibited [29,30]. Etoposide (**26**) has proven to be useful in the treatment of patients with small-cell lung cancer, Kaposi's sarcoma, lymphoma and leukemia while teniposide (**27**) is mainly used to treat acute lymphatic leukemia, neuroblastoma and brain tumors in children (*Scheme 9*).

2.4 Gallotannins

Gallotannins (e. g., 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose, **28** (*Scheme 10*)) are abundant compounds of glycosidic character in plant material. As plant preparations these compounds had been used from ancient times as traditional medicine in wound healing, as an astringent and as an antidote in many intoxications (precipitation of the noxa) and the compounds also act as an effective antioxidant. These effects, however, cannot be attributed to the glycosidic moiety in the center of the molecule but mostly to the polyphenolic nature of the galloyl residues.

3 Glycosidic Antibiotics

Antibiotics form a large group of compounds of a vast structural diversity that are able to suppress or inhibit growth of one type of cells (microbial, fungal, tumor, genetically altered, etc.) and do not or to a lesser extent affect the growth of host cells. This definition is only very broad as it cannot embrace all the subtle effects of the various types of antibiotics.



28

Scheme 10

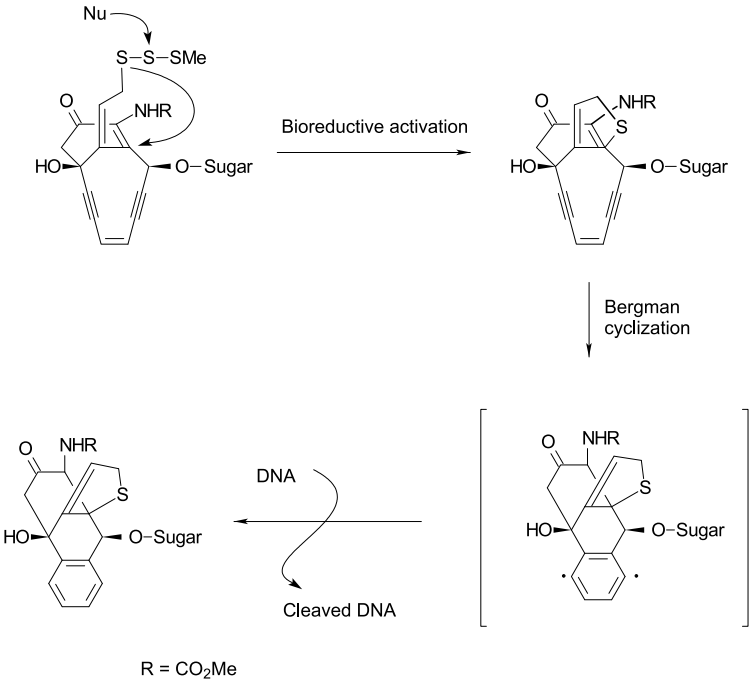
As in virtually all other groups of biologically active compounds, also in the group of antibiotics sugars play a very important role [31]. Occurrence of quite uncommon deoxy- and aminosugars is another typical feature of a large group of glycosidic antibiotics.
In this section we would like to focus on recent findings or typical cases.

3.1 Enediyne Antibiotics

The enediyne antibiotics [32] are extremely potent antitumor agents with a unique bicyclo[7.3.1]enediyne substructure. This group includes mainly calicheamycins [33], esperamycins [34], and dynemycins. Neocarzinostatin [35] and others (e. g., maduropeptin and kedarcidin) are also classified as belonging to this family. At room temperature in the presence of DNA, the core system of an enedyine antibiotic undergoes an interesting reaction yielding diradicals on sp^2 carbon that causes DNA strand breakage (*Scheme 11*). During studies of the structures of calicheamycin γ^1 and esperamycin A_1, it became apparent that enedyines could be triggered to aromatize via cleavage of the trisulfide with formation of a diradical intermediate as the biologically active species.

3.1.1 Calicheamycin

Calicheamycin γ^1 (**29**) was isolated from the cultivation broth of *Micromonospora echinospora* enriched with iodide. Compound **29** binds to the minor groove of DNA showing a high degree of base pair sequence-specificity, and a high degree of double strand to single strand cleavage of DNA. This occurs mostly at the sites 5′-TCCT/AGGA and 5′-TCTC/GAGA. The double stranded scissions suggest that **29** binds in the minor groove with the diradical positioned in such a way as to allow hydrogen abstraction from each strand [36]. The degree of specificity has been attributed to the carbohydrate moiety of the molecule, with the oligosaccharidic moiety aligned towards the 3′-end of the DNA. Studies have shown that the aglycon of **29** binds to DNA with lower affinity and in a nonsequence-specific manner [37]. Carbohydrates of calicheamycin γ^1 are lipophilic, which makes them favorable to interact with DNA.

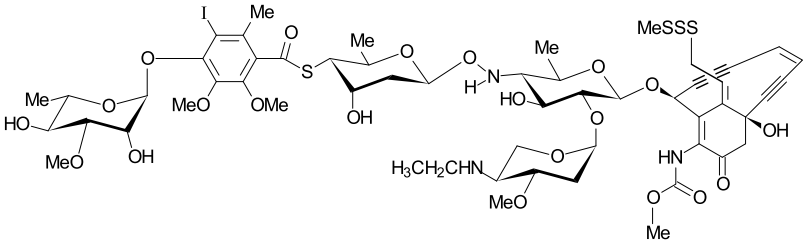


Scheme 11

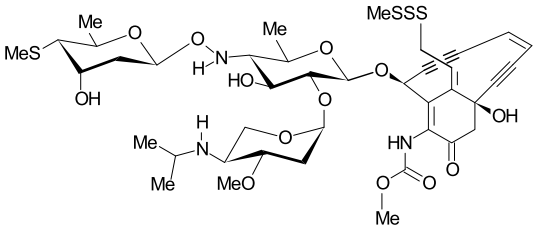
There is evidence that the iodine atom of the aromatic ring interacts with the C-2 amino groups of one or both guanines in TCCT:AGGA duplex DNA [38]. The binding energy of the oligosaccharide domain of **29** to the above DNA sequence within the duplex DNA is 31.6 kJ/mol. The sulfur atom of the third glycosidic moiety forms a hydrogen bond with an exposed amino proton from 3′-guanine in a drug-DNA complex [39]. This sugar was shown to be positioned edgewise in the minor groove allowing the aromatic ring to be placed between the minor groove with its iodine and methyl group positioned deep inside the minor groove.

Esperamycin C (**30**) can be considered to be an analogue of calicheamycine void of the rhamnosyl moiety and the aromatic part. Compound **30** causes double strand DNA cleavage but with considerably lower sequence selectivity. The aromatic and rhamnose moieties of **29** seem to determine the sequence specificity and the monodirectional mode of binding but they have little or no effect on orientating the aglycon within the DNA. NMR studies and molecular modeling demonstrated that also other sugar moieties in **29** specifically interact with DNA, e. g. the second carbohydrate moiety within the sugar-phosphate of DNA. The hydroxylamine glycosidic linkage causes two linked sugars to adopt an unusual eclipsed conformation giving the oligosaccharides the correct shape and rigidity to allow selective binding in the minor groove of DNA [38] (*Scheme 12*).

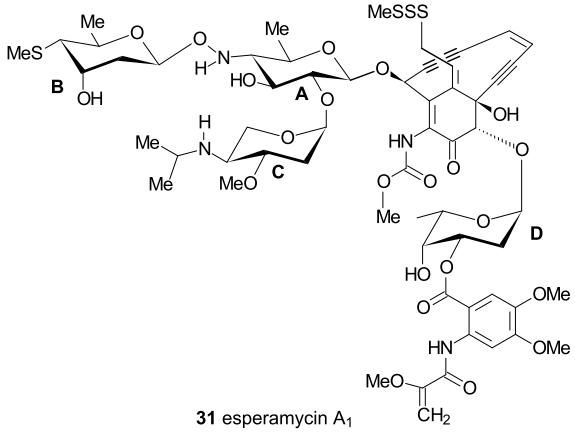
The monomer and the "head-to-head" dimer of the oligosaccharide segment of **29** have been shown to inhibit DNA transcription factors [39]. The dimeric glycoside was a more effective inhibitor than the corresponding respective monomer. The inhibitory effect has been attribut-



29 calicheamycin γ^1



30 esperamycin C



31 esperamycin A_1

Scheme 12

ed to the conformational changes in DNA caused by interactions with the sugar and this in turn interferes with the transcription factor DNA recognition. The base sequence selectivity of the calicheamycin carbohydrate to DNA may open up new possibilities for the chemical control of genetic information. It can, however, be stated that obviously deoxy- and substituted saccharides should be employed to enhance hydrophobic interactions.

3.1.2 Esperamycin

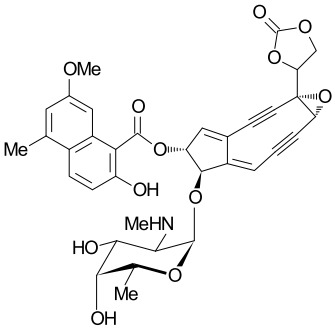
Esperamycins (esperamycin C **30**, esperamycin A_1 **31** and others), isolated from *Actinomadura verrucosospora*, display very strong antitumor activity due to DNA cleavage effected by the aglycon [40]. Esperamycin A_1 causes mostly single strand DNA cleavage and is far less sequence-specific than **29** having a similar aglycon.
Structure analysis of the esperamycin A_1–d(CGGATCCG) duplex showed that the drug bound in the minor groove with the methoxyanthranilate moiety intercalates at the (G2-G3):(6′-C7′) segment [41]. 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 to the binding energy of **31**.
Esperamycin C causes double strand lesions involving deoxyribose hydrogen abstraction from the 4′- and 5′-positions, whereas esperamycin A_1 causes lesions from the 1′- and 5′-positions. This change to the 1′-position for esperamycin 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 into the minor groove. The OH groups of the trisaccharide A-B-C are positioned close to potential hydrogen bond acceptors, and favorable van der Waals interactions thus help to stabilize the complex [42].

3.1.3 Neocarzinostatin

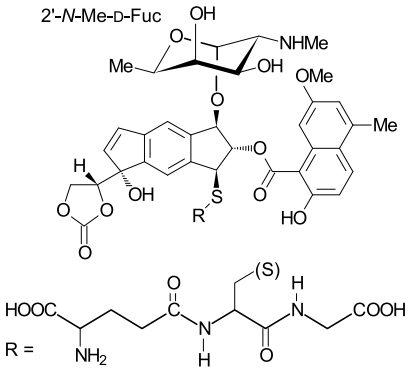
Neocarzinostatin, isolated from *Streptomyces carzinostaticus*, consists of a mixture of chromophore (**32**) and a protein [43]. This compound having potent antitumor and antibacterial activities induces single strand and double strand scissions in a ration 5:1, respectively, with a preference for T and A residues. The sequence specificity differs from other enedyines partly because of the different glycosylation pattern of the molecule.
The 2′-*N*-methyl-D-fucosamine, naphthoate and tetrahydroindacene of the postactivated-neocarzinostatin (rearranged complex with oligopeptide, **33**) (*Scheme 13*) 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 selectivity of binding/cleavage, one of these sites being the N-2 of the 2′-*N*-methyl-D-fucosamine [44].

3.2 Anthracyclines

Anthracyclines represent a relatively large group of natural, semisynthetic and synthetic compounds [45]. Some compounds of this type are used in cancer treatment, several promising candidates are in clinical testing. Anthracyclines have also good antibacterial activity, however, due to their toxicity they are not used clinically in the treatment of infectious diseases. At present daunomycin (**34**) is used for the treatment of leukemia and adriamycin (**35**) is used for treatment of some solid tumors. These two compounds were studied in great detail. Modifications on both the aglycon and saccharidic parts have been performed. Changes of the saccharidic moiety were achieved by substitution of existing groups, e. g. *N*-alkylation, *N*-acylation, *O*-alkylation, formation of tetrahydropyranyl derivatives etc.



32 neocarzinostatin



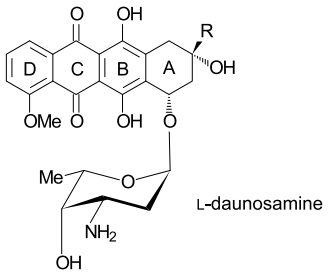
33 post-activated neocarzinostatin

Scheme 13

3.2.1 Daunomycin

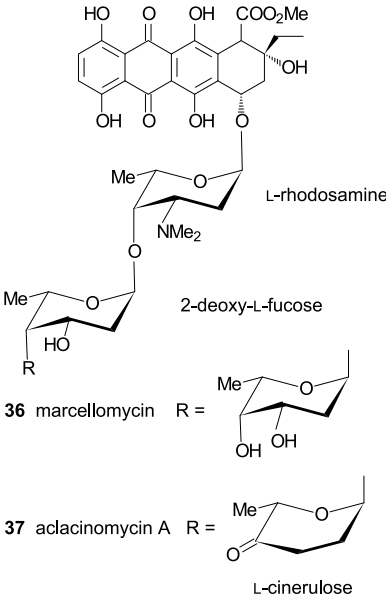
The anticancer activity of daunomycin (**34**) (*Scheme 14*) is attributed to the intercalation of the drug with DNA. This pattern of effect is common to all anthracyclines [46]. Two daunomycin molecules intercalate at each of the two C:G sites at either site of the duplex d(CGTACG). This binding results 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 [47].

Rings B-D of the aglycon 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 show hydrogen bonds to adjacent bases. The amino and hydroxy groups of the sugar face out of the minor groove, and it has been suggested that they may interact with polymerases, which could prevent or retard the action of these enzymes.



34 daunomycin R = COMe

35 adriamycin R = $COCH_2OH$



Scheme 14

It was observed that anthracyclines containing several glycosyl moieties have, in general, less side effects. For example, marcellomycin (**36**) and aclacinomycin A (**37**) show lower cardiotoxicity than daunomycin.

This is also the reason for the many attempts to introduce one or more additional carbohydrate moieties. Reaction of daunomycinone with diols providing its 7-*O*-hydroxyalkyl derivatives enabled attachment of the sugars in a different manner leading to derivatives with lower toxicity [48].

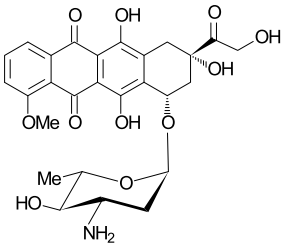
Nogalamycine, which contains two sugars (one attached to the D-ring and the second attached at C-7 of the A-ring), binds to DNA in a slightly different manner [31].

3.2.2 Glycosyl Derivatives of Anthracyclines

In natural anthracyclines the sugars are linked by α-glycosidic linkages, the corresponding β-glycosides were found to be inactive [49]. L-arabino analogues of daunomycin and doxorubicin, 4′-epidaunomycin and 4′-epidoxorubicin (**38**), were shown to have similar activity to the natural anthracyclines, the latter (**38**) also has a lower cardiotoxicity and is now used clinically [50] (*Scheme 15*).

Neutral synthetic sugar derivatives, for example SM-5887 (**39**), display good antitumor activity with reduction in local tissue toxicity and cardiotoxicity compared to, for example, adriamycin (**35**) [51].

Drug resistance in the anthracycline cancerostatics has been addressed by the study of the influence of the amino group of daunosamine. It seems that this group is recognized by the P-gp multidrug transporter (P-glycoprotein is an ATP-dependent transmembrane drug exporter). Comparative studies of doxorubicin and hydroxyrubicin (**40**) in drug-resistant tumor cells demonstrated that **40** partially or completely circumvents P-gp-mediated drug resistance due to its decreased transport by P-gp compared with doxorubicin [52].



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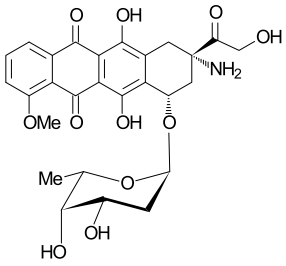

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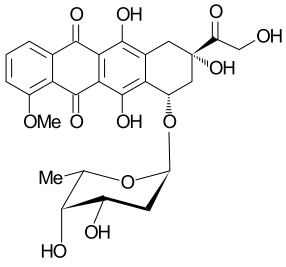

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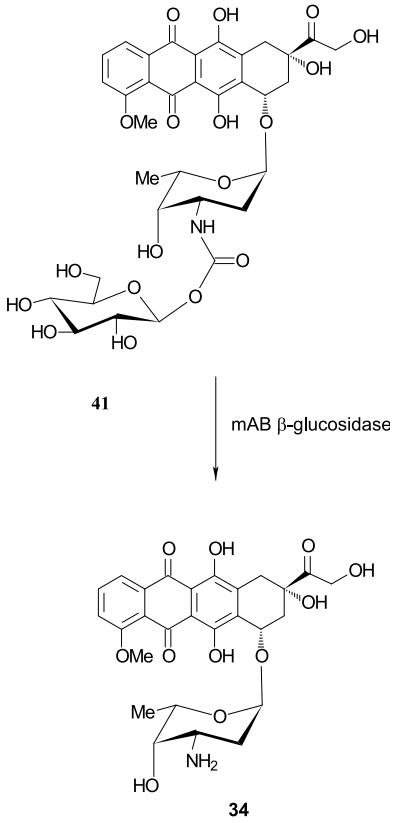
Scheme 15



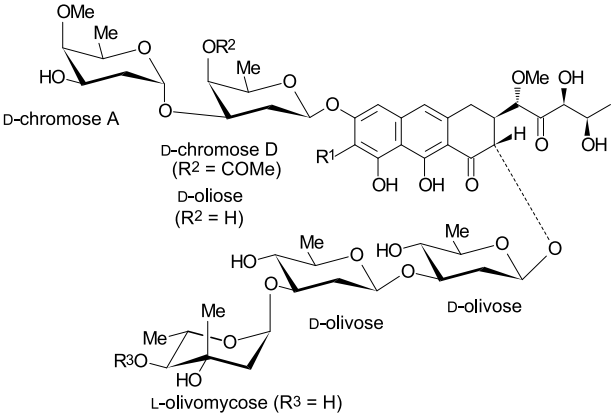
Scheme 16

There have been also attempts to prepare targeted drugs based on anthracyclines employing the recognition of some specific sugars by receptors on the tumor cells. Thus, a β-glucoside derivative of adriamycin (**41**) was used in an antibody-directed enzyme-prodrug therapy (*Scheme 16*) [53].

3.2.3 Aureolic Acids

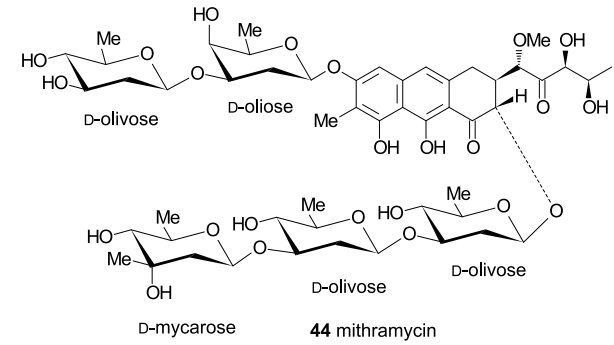
Members of the aureolic acid group include chromomycin A_3 (**42**), olivomycin A (**43**) and mithramycin (plicamycin) (**44**). The aglycon of chromomycin A_3 is identical to that of mithramycin, but differs from that of olivomycin A by a methyl group. Conversely, the sugar components for chromomycin A_3 and olivomycin are nearly identical, while those in mithramycin are different (*Scheme 17*).

These drugs require a divalent metal ion, preferably an Mg^{2+} ion and a guanine-containing target for activity. They were identified to be inhibitors of DNA-dependent RNA polymerase. It was found that **42** lacking some of the sugar moieties was less active [54] and the aglycon does not bind to DNA at all [55].



42 chromomycin A_3 R^1 = Me, R^2 = R^3 = COMe

43 olivomycin A R^1 = H, R^2 = COMe, R^3 = $COCHMe_2$



44 mithramycin

Scheme 17

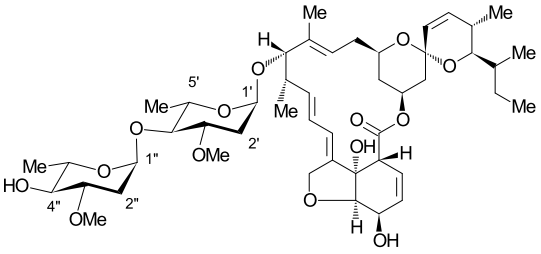
The mode of action of these antibiotics is different from other anthracyclines despite their aglycon similarity. Aureolic acids do not intercalate into DNA but they bind to CG rich regions. NMR studies demonstrated that **42** forms a symmetrical dimer with self complementary d(TTGGCCAA) with the hydrophobic edge of the chromophore located next to the GC:CC site [56]. 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 are directed to the phosphate backbone. The trisaccharide part olivose-olivose-olivomycose is essential for stabilization of the drug-Mg^{2+} complex that binds effectively to DNA, removal of chromose A has no effect on the complex formation [57].

The action of mithramycin (**44**) is virtually the same, however, it is less specific for the respective DNA sequence, which can be explained by the additional hydrogen-binding potential of D-mycarose [58].

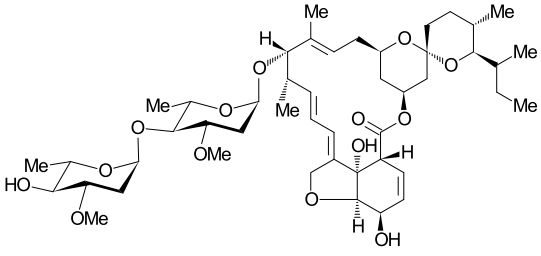
3.3 Avermectins

This class of macrolide antibiotic has mostly antiparasitic activity. Avermectin B_{1a} (**45**) and ivermectin (**46**) (Scheme 18) are used mostly in veterinary medicine, however, some semisynthetic derivatives are also used for treatment of onchocerciasis in humans [59]. The action of avermectin is believed to stimulate specific chloride ion transport systems increasing the membrane permeability to Cl^- ions via GABA (γ-butyrate) receptors and non-GABA receptors [60].

The aglycon of avermectin has poor antiparasitic activity. Positions 4′ and 4″ of the oleandrose moieties were modified partly because of feasibility. The 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 [61]. One of the derivatives, 4″-*epi*-acetamido-4″-deoxyavermectin B_1, is currently under development as a novel avermectin endectoside [61]. Besides, also 2″-α-fluoro (*ax.*) and 2″-β-fluoro (*eq.*) derivatives of avermectins were prepared to strengthen the glycosidic bond. These derivatives have interesting activities in some bioassays compared to the parental compounds [62].



45 avermectin B_{1a}



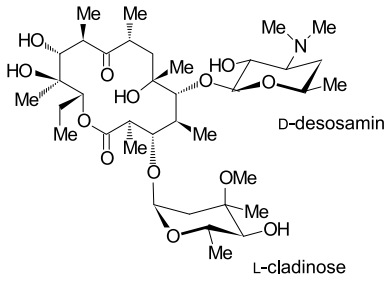
46 ivermectin

Scheme 18

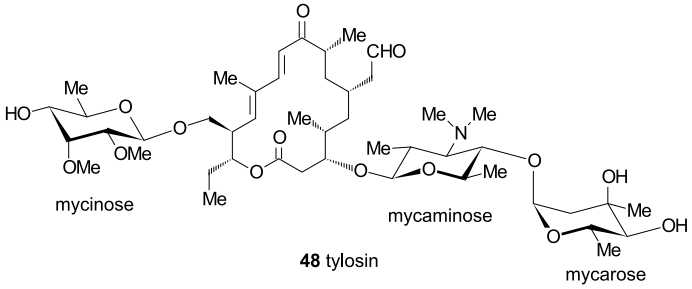
3.4 Macrolide Antibiotics

Macrolide antibiotics containing a large lactone ring (hence the title macrolide) consisting of 14 atoms are biosynthetically related to avermectins being also polyketides. The first member of this group erythromycin A (**47**) was isolated in 1952 [63] and it soon became an important antibiotic widely used in clinical medicine against Gram-positive 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 (**48**) (*Scheme 19*), a commercially important antibacterial agent used in veterinary medicine. The 12-membered ring macrolides have not been used clinically. Most of the macrolides contain deoxy- and aminosugars. Although it was established [64,65] that the sugar moieties are absolutely essential for the microbial activity of these compounds it is difficult to judge their role in the activity as the exact mode of action is unclear.

The mode of action of macrolide antibiotics involves the inhibition of protein synthesis of specific binding to the 50S ribosomal subunit but without a specific target at the 23S ribosomal subunit and various proteins [66]. Nevertheless, the exact interaction of the macrolide and the ribosome unit is still not fully understood. In principle, the macrolide antibiotic should inhibit also mammalian mitochondrial protein synthesis but they are unable to penetrate the mitochondrial membrane.



47 erythromycin A



48 tylosin

Scheme 19

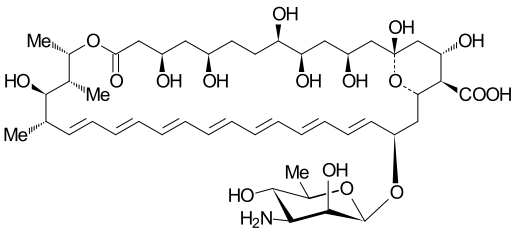
Sugar moieties have been linked with many pharmacological properties displayed by the macrolide antibiotics [67]. Basicity of the nitrogen in aminosugars improves the active transport of the compounds into the cell, however, its protonation/deprotonation does not affect the uptake. It was shown [68] that the more basic macrolides are the more effective ones. Esterification of the glycosidic position 2′ improves pharmacological and pharmacokinetic properties of the resulting drug lowering unwanted gastrointestinal side effects [69].

3.5 Polyenes

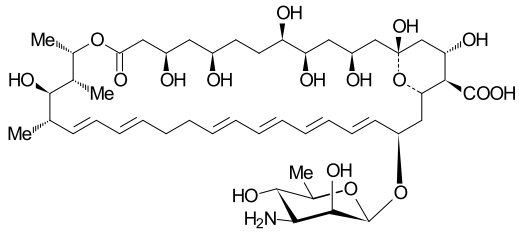
Polyene macrolides consist of a macrocyclic lactone, a polyene chromophore, and a polyhydroxylated chain which often bears glycosidic residues. These antibiotics, e. g., amphotericin B (**49**) and nystatin A_1 (**50**) (› *Scheme 20*), are used as potent antifungal antibiotics although they cause side effects such as nephrotoxicity, hemolytic anemia and electrolyte abnormalities [70].

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 leach the cellular ions [71].

Structure-activity studies on amphotericin B (**49**) have shown the importance of the basic nitrogen of D-mycosamine for the activity [72]. The polyene is orientated with the polar head at the



49 amphotericin B



50 nystatin A_1

Scheme 20

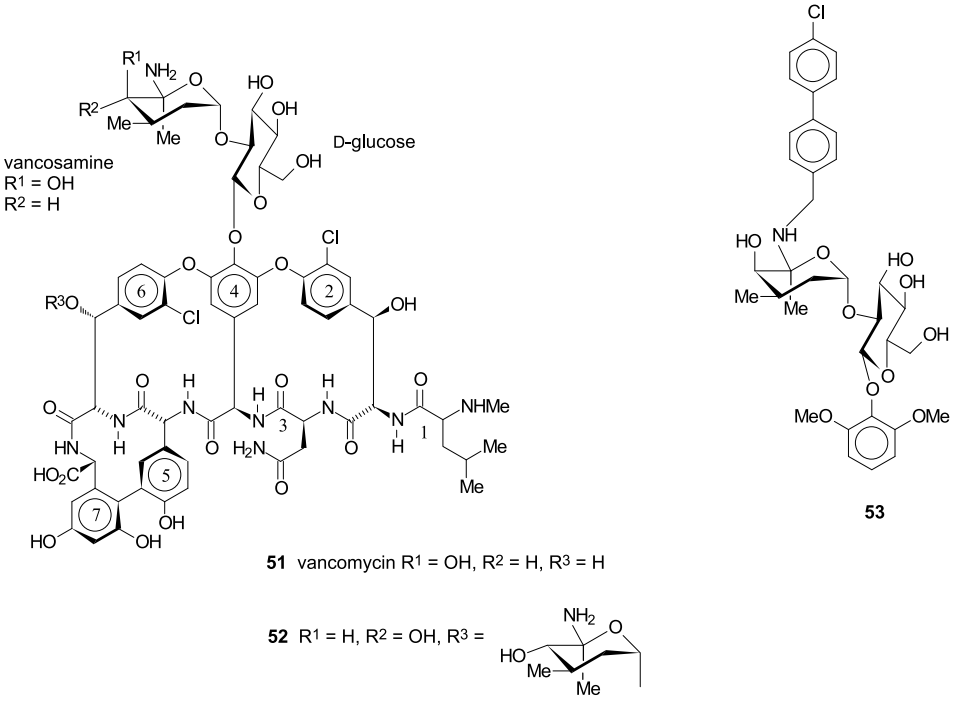
membrane–water interface and it has been suggested that the basic amino group of the sugar and the carbonyl group form a "cage-like" hydrogen-bonded structure with sterol containing a 3-β-hydroxy group and water.
N-Glycosylation of D-mycosamine in the polyenes leads to antibiotics with reduced toxicity and similar activity to the parent compound but increased water solubility and, therefore, better bioavailability [73].

3.6 Glycopeptides

This group of antibiotics is made up of complex polypeptide aglycones, which are abundantly glycosylated with mono-, di- and tetrasaccharides [74]. To date about 100 members of this group are known, such as vancomycin, teicoplanin, bleomycin, ristocetin etc. Some of them are very important, as they are often *ultimum refugium* – the last possible treatment for multiply resistant bacterial infections. Recent discoveries demonstrated that glycosidic residues play crucial roles in their activity and this opens up a large area for developing new glycosidic antibiotics, as shown in the case of 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 multiply resistant (even vancomycin-resistant) bacteria.

3.6.1 Vancomycin

Vancomycin (**51**) (❯ *Scheme 21*) [75] 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. At present its importance has increased considerably as it is one of the few antibiotics effective against nosokomial infections, e. g., multiply resistant bacteria – a typical example is methiciline-resistant *Staphylococcus aureus* (MRSA), and together with gentamycin it is an antibiotic of last resort.
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 [76], 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 the resistance to **51**.
Because of the ultimate importance of vancomycin in the treatment of often fatal infections caused by multiply resistant bacteria, extensive efforts have been directed toward the discovery of vancomycin derivatives with activity against the drug-resistant bacteria [77]. As a result of these efforts, several derivatives such as **52** (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 the 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 [75]. It was also demonstrated that the conformations of vancomycin and its aglycon differ in their alignment of the amide protons



Scheme 21

that participate in the hydrogen-binding network with cell-wall precursors [78]. Alkylation of the 3-amino group on the disaccharide at amino acid residue 4 further enhances the activity, probably by serving as a hydrophobic anchor to the cell membrane [79].

Another target for modification of vancomycin is the vancosamine moiety. Recently, it was found that *N*-alkylation with *n*-decyl or 4-chlorobiphenylyl groups results in an antibiotic acting by a different mechanism than vancomycin itself [80] and, therefore, exhibits activity against vancomycin-resistant microorganisms. Sugar-altered vancomycin interferes with the polymerization of a glycopeptide monomer and disrupts its ability to form cell-wall material in the first place. Thus, altered vancomycin may disrupt cell-wall formation at multiple targets of the glycopeptide monomer, straight-chain polymer, and cross-link reinforcement.

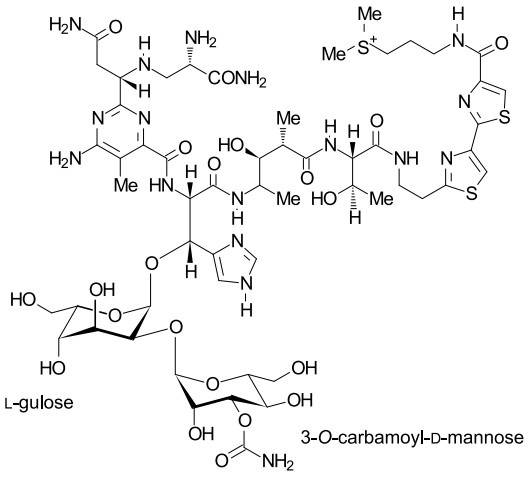
In fact, the altered disaccharide derivative itself (**53**) has strong antibacterial activity, even if not attached to the rest of the large vancomycin molecule [80]. This compound, however, acts by a different mechanism than vancomycin. It inhibits the transglycosylation step that precedes the transpeptidase step blocked by vancomycin itself. This finding explains a complex mechanism of action of the derivative **52**.

These findings offer possibilities to use sugar chemistry to make glycopeptide antibiotics such as vancomycin more potent against bacteria, for example, if vancosamine was replaced by daunosamine (3-desmethyl-vancosamine). Although this derivative differs from **51** only in one methyl group at the terminal sugar, it shows some notable differences in activity [81]. A general methodology for selective glycosylation of the vancomycin aglycon has been developed

by Kahne's group [82]. It is quite likely that a wide-ranging investigation of different sugars will lead to more significant improvements across a range of bacterial strains. This case and analogous approaches are discussed in detail in ❯ *Sect. 8* of this chapter.

3.6.2 Bleomycin

Bleomycin A_2 (**54**) (❯ *Scheme 22*) has antimicrobial activity against both Gram-positive and Gram-negative bacteria but it is clinically often used as an antitumor agent. The mode of action of bleomycin is DNA strand scission occurring in the presence of Fe^{2+} and molecular oxygen [74]. It was demonstrated that the sugars do not contribute to the binding affinity or the DNA cleavage selectivity, although the presence of the sugar enhances the DNA cleavage efficiency by 2–5 times [78]. The sugar moieties are suggested to contribute to the binding of O_2 and activation and protection of the reactive iron-oxo or perferryl intermediate to activated bleomycin [83].

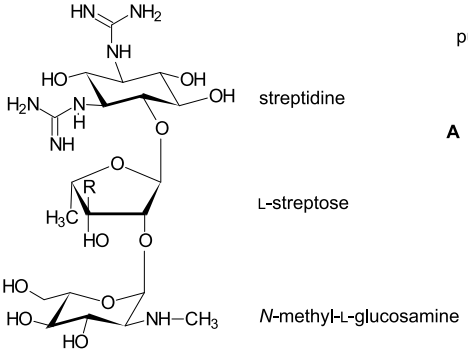


54 bleomycin A_2

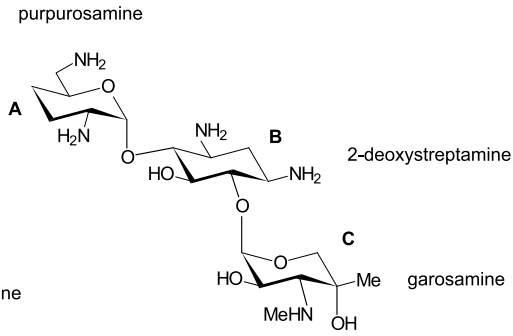
Scheme 22

3.7 Aminocyclitol Antibiotics

The term "aminoglycoside" refers to the structural aspects. Most of the aminoglycoside antibiotics contain aminosugar(s) and an aminocyclitol or a cyclitol moiety; therefore, this group is also called "aminocyclitol antibiotics." A review on structural aspects, synthesis and chemical modifications of these compounds was published by Ikeda and Umezawa in 1999 [210].
Streptomycin (**55**), dihydrostreptomycin (**56**) and gentamycin (**57**) (❯ *Scheme 23*) are typical representatives of a large group of the aminocyclitol antibiotics. They interact with ribosomal RNA causing a decrease in translational accuracy and inhibit translocation of the ribosome [84]. 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.



55 streptomycin R = CHO
56 dihydrostreptomycin R = CH_2OH



57 gentamycin A

Scheme 23

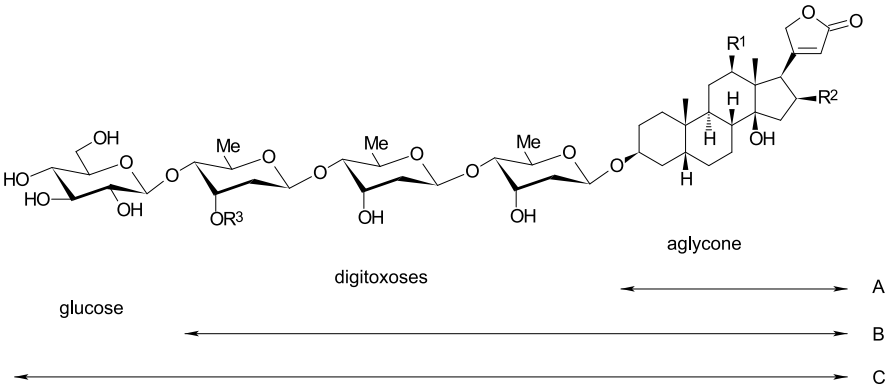
A comparison of various aminoglycosidic antibiotics suggests chemical groups that are essential for their function [85]. Both amino groups of the B ring (see e. g. in **57**), which are positively charged at physiological pH, contribute to RNA binding as hydrogen donors.
Recent investigation of the binding mechanism of gentamycin C1A (**57**) has shown that it binds in the major groove of the RNA. Rings A and B of gentamycin direct specific RNA-drug interactions. Ring C of gentamycin, which distinguishes this subclass of aminoglycosides, also directs specific RNA interactions with conserved base pairs [86].
Resistance to this type of antibiotic is usually mediated by acetylation, adenylation and phosphorylation mostly in rings A and B causing thus lowered or abolished antibiotic affinity to the RNA [87].

4 Steroid and Terpenoid Glycosides

This group involves a considerable number of physiologically active compounds whose activity is largely dependent on the complete structure, including the glycosidic moiety. A number of these compounds have detergent properties, e. g., saponins, and such physicochemical properties are partly responsible for their toxicity, e. g. the hemolytic activity of some saponins is caused by the damage of the erythrocyte membrane.

4.1 Cardiac Glycosides

Cardiac glycosides consist of a five-ring cardenolide aglycon, called a genin, with a number of attached monosaccharides, which often include deoxysugars. These steroidal compounds are usually isolated from plant material (digitoxin, strophantidine), but they have been dis-



Structure part	Description	R^1	R^2	R^3
Part A (aglycon)	Digitoxigenin	H	H	–
	Gitoxigenin	H	H	–
	Digoxigenin	OH	H	–
Part B (trioside)	Digitoxin	H	H	H
	Gitoxin	H	OH	H
	Digoxin	OH	H	H
Part C (tetraoside)	Purpurea glycoside A	H	H	H
	Purpurea glycoside B	H	OH	H
		OH	H	H
Acetylated tetraosides	Lanatosid A	H	H	CH_3CO
	Lanatosid B	H	OH	CH_3CO
	Lanatosid C	OH	H	CH_3CO

Figure 2

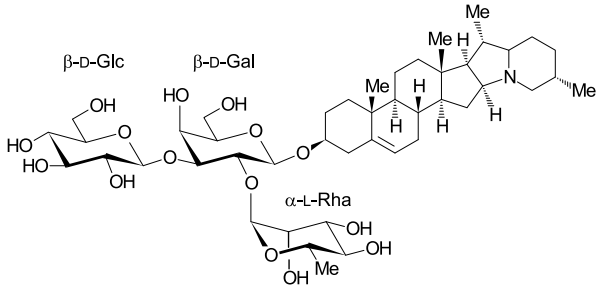
covered in higher mammals as adrenal cortical hormones (ouabain) [88]. Possibly using the same binding site as the natural hormone, cardiac glycosides inhibit Na^+/K^+-ATPases [89,90], resulting in an ionotropic activity that has proven to be useful in the treatment of various heart conditions, e. g., atrial tachyarrythmias, and it is used to produce a positive ionotropic effect in congestive heart failure [91]. This activity is enhanced several-fold due to the presence of the sugars in these compounds [92,93]. The sugar moiety is thought to be important in uptake, distribution and binding stability [94]. Cleavage of the sugar moieties forms the genin (*Fig. 2*) which retains a diminished affinity while the sugar moiety alone has no activity [94,95]. Altering the structure of the sugar(s) has a dramatic influence on the activity of the cardiac glycoside. Cardiac glycosides having $\beta(1\rightarrow4)$ sugar linkages have stronger activities than those with $\beta(1\rightarrow6)$ and $\beta(1\rightarrow2)$ linkages [96,97], α-linkages further diminish the activity [98]. Sugars having 4′-OH axial groups (e. g., galactose) diminish the binding, 3′-OH axial and 2′-OH equatorial groups appear to contribute to the binding [99]. Also, the addition of a 6′-OH group slightly diminishes the activity [99].

Therefore, existing natural digitoxose/glucose containing cardenolides seem to be optimal despite extensive structure-effect studies targeted to its optimization.

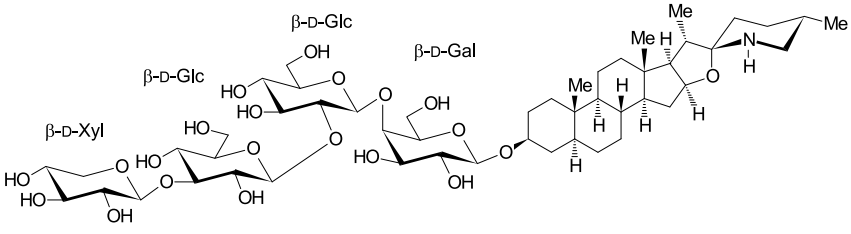
Some studies have revealed that the saccharides lengthen the half-life of the activity by preventing the host from modifying the genin and neutralizing its inhibitory activity [100]. The sugar proximate to the genin has the strangest effect on binding and activity of the drug [101]. Even though the structure of the cardiac glycoside binding site has been extensively studied [102], its detailed nature remains unknown, namely the aminoacid residues which interact with the sugar moiety. Nevertheless, a three-step model has been proposed to account for the interaction between a cardiac glycoside and its receptor. The binding event can proceed via reversible binding of the steroid core with the receptor, followed by a conformational change that exposes a sugar-binding motif, which then binds to the saccharides and hinders dissociation [103].

4.2 Saponins

Originally, the name "saponin" implied a group of soap-like natural surfactants that form long-lasting bubbles on shaking an aqueous solution. An aglycone of saponins is designated as sapogenin or sapogenol (triterpene sapogenins and steroidal sapogenins). Saponins having one sugar moiety are called monodesmosides, and those having two sugar moieties at different positions are called bisdesmosides. Saponins often occur in higher plants, which are frequently used for human and animal consumption, therefore, their physiological activities are of utmost



58 α-solanine



59 α-tomatine

Scheme 24

importance [104]. They can be found in larger amounts in, e. g., soybeans, peas, beans, oats, potatoes, tomatoes, tea, liquorice, ginseng, and in forage as, e. g., alfalfa, lupine, sunflowers, guar etc. Some of the saponins contain nitrogen and these are sometimes included in the alkaloids, e. g., solasodine, α-solanine (**58**) or α-tomatine (**59**) (› *Scheme 24*) and they are rather toxic acting mostly as acetylcholine esterase inhibitors. Hydrolysis (cleavage of glycoside) of, e. g., α-solanin results in overall loss of its toxicity.

Recently, a series of 20 steroidal glycosides from *Solanum* (e. g., glycosides of diosgenin, solasodine, solasonine, etc.) was examined for their anticarcinogenic activities and the following conclusions were drawn: As regarding the sugar linkage, the glycosides possessing a terminal α-L-rhamnosyl moiety (β-chacotriose) are the most effective; as for the aglycon, the glycosides carrying a spirostanol aglycon showed the strongest activity, and even the aglycon without the sugar had strong activity [105]. Generally, saponins due to their large structural diversity have many biological effects. As a raison d'être of saponins in plants its defensive role against microbial pathogens and some predators is often reported. Many saponins were also tested for their antibiotic and anticancer activities, however, no detailed data for the function of their glycosidic moieties were given, therefore, it is beyond of the scope of this review.

4.2.1 Ginsengosides

Ginseng saponins, e. g., dammarane-type saponins are extracted from the root of *Panax ginseng*. They have been used in various formulations in oriental countries for more than 5000 years especially as a tonic [106]. Ginsengosides are highly glycosylated and their activity often differs depending on the number of glycosyl units attached. They can be interconverted by trimming by glycosidases and this is a way by which some more scarce ginsengosides are produced [107].

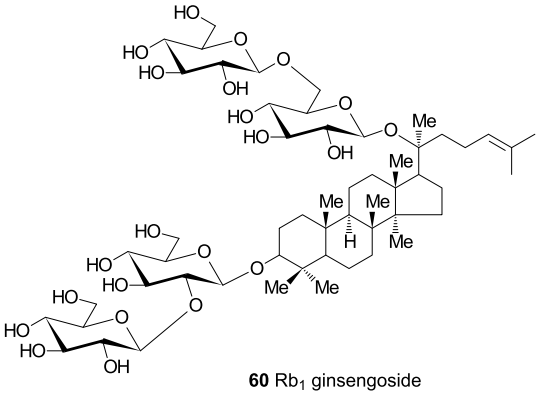
Ginsengoside Rb_1 (**60**) is a representative of the saponins derived from 20(*S*)-protopanaxadiol. It exhibits central nervous system-depressant and antipsychotic activity, protection against stress ulcer, increase of gastrointestinal motility and weak anti-inflammatory action. Rg_1 (**61**) – the major saponin of 20(*S*)-protopanaxatriol – shows weak CNS-stimulant action, antifatigue activity and blood pressure activity [108,109] (› *Scheme 25*).

Some other saponins have been reported to have potentiation activity on the nerve growth factor [110], stimulation of the pituitary-adrenocortical system [111], etc.

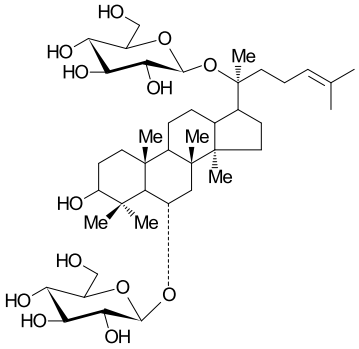
4.3 "Sweet Glycosides"

4.3.1 Osladin

Osladin (**62**) is a steroidal glycoside that is about 500-times sweeter than sucrose. It was isolated by the Czech chemists Jizba and Herout in 1967 [112] from the rhizomes of European fern *Polypodium vulgare* known for its very sweet taste. Its structure has been recently revised [113] by total synthesis. During the synthesis it was shown that minute changes in the structure result in total loss of the sweet taste. Thus, this is a typical glycoside whose overall structure – including the glycosidic part – is crucial for the respective activity.



60 Rb_1 ginsengoside



61 Rg_1 ginsengoside

Scheme 25

4.3.2 Steviol Glycosides

Leaves of *Stevia rebaudiana* (Compositae) are a source of several sweet glycosides of steviol (**63**) [114]. The major glycoside, stevioside (**64**), is used in oriental countries as a food sweetener and the second major glycoside named rebaudioside (**65**), which is sweeter and more delicious than stevioside, is utilized in beverages.

Stevioside (**64**) and rubusoside (**66**) taste somehow bitter, and show aftertaste. To improve the sweetness and the taste, modifications of sugar moieties of both the glycosides were performed by enzymatic glycosylations and/or enzymatic trimming. Cyclomaltodextrin-glucanotransferase (CGTase) efficiently catalyzes transfer of the α-glucosyl moiety (one or more) from starch onto the 4-OH of a glucosyl moiety. Stevioside was treated with this system and it resulted in a complex mixture of mono-, di-, tri-, and polyglucosylated derivatives on both existing glucose moieties of the original compound. Significant improvements of the quality of

taste were observed for most of the glucosylated products, especially for S1a and S2a, which were mono- and di- glucosylated at the 13-sophorosyl moiety of stevioside [115]. A remarkable enhancement of the intensity of sweetness was also observed for both these products, while glucosylation at the 19-*O*-glucosyl moiety (ester bound) resulted in a decrease of the intensity of sweetness (◉ *Table 1*).

Rubusoside was also transglucosylated by the same enzyme system and a large number of products were obtained. Strong enhancement of the sweetness intensity was observed for the products, which were di- or tri-glucosylated at the 13-*O*-glucosyl moiety. Tetraglucosylation at the 13-*O*-glucosyl moiety as well as glucosylation at the 19-*O*-glucosyl moiety led to a decrease in sweetness [116]. Therefore, after transglucosylation the products are "trimmed" enzymatically using β-amylase which releases maltose from the nonreducing end of the α-glucoside. By this treatment maltotriose- and higher α1-4-glucosyl chains were trimmed into monoglucosides and maltosides, which resulted in further improvement of sweetness. The commercial product of transglucosylated stevioside always undergoes this treatment [117].

Replacement of the 19-*O*-glucosyl group by a β-galactosyl group led to worsening of the taste [118]. Significant improvement of the taste quality was achieved by enzymatic transfructosylation at the 19-*O*-Glc moiety of both stevioside and rubusoside [119]. The β-fructofuranosyl moiety is, however, unstable and prone to hydrolysis.

This case is a practical demonstration of the advantage of a glycosidic moiety modification for optimization of physiological properties of the compound(s).

4.3.3 Glycosides of Glycyrrhetic Acid

The major sweet principle of licorice root (*Glycyrrhiza glabra*), glycyrrhizin (**67**) (◉ *Scheme 28*) (content ~ 4%), has been used as a sweetener and flavor enhancer in foods, tobacco products and also in medicine as an anti-inflammatory agent.

A structure-sweetness relationship study has shown that the monoglucuronide of glycyrrhetic acid is about 5-times sweeter than **67** and ca. 1000-times sweeter than sucrose [120]. Replacement of the second glucuronic acid by, e. g., xylose or glucose resulted also in an improvement of the taste and enhancement of sweetness [120].

The monoglucuronide of **67** is now commercially produced using a β-glucuronidase from yeast *Cryptococcus magnus* MG-27 that cleaves selectively only one GlcA moiety [121]. This compound has also better pharmacological properties such as, e. g., an inhibitory effect against skin carcinogenesis and pulmonary tumorigenesis (in mice) [122].

4.4 Glycosides as Aroma Precursors

Glycosides serve often as aroma precursors. It is known that many plants after crushing develop strong aroma. Many of these cases can be attributed to the hydrolysis of glycosides as released aglycons are more volatile than the respective glycosides.

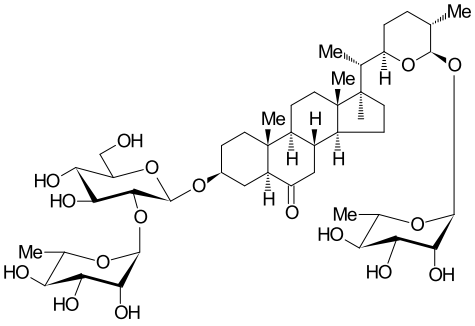
Saliva contains α-glucosidase (ptyalin) and, therefore, experiments with α-glucosides of some aromatic alcohols were performed to enable slow liberation of the fragrances while chewing the food or a chewing gum. However, these approaches have not been very successful up to now.

Table 1

Sweetness quality and intensity of various glucosyl derivatives of stevioside

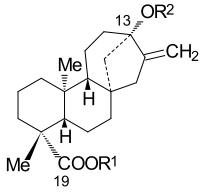
Compound	19-*O*-glycosyl (R1)	13-*O*-glycosyl (R2)	RS	QT
Stevioside (**64**)	-Glc	-Glc-Glc	160	0
Rebaudioside (**65**)	-Glc	-Glc-Glc Glc	210	+2
Rubusoside (**66**)	-Glc	-Glc	134	-2
S1a	-Glc	-Glc-Glc-α-Glc	180	+4
S2a	-Glc	-Glc-Glc-α-Glc-α-Glc	205	+4
S3a	-Glc	-Glc-Glc-α-Glc-α-Glc-α-Glc	117	+3
S1b	-Glc-α-Glc	-Glc-Glc	133	+2
S2b	-Glc-α-Glc	-Glc-Glc-α-Glc	136	+1
S3b	-Glc-α-Glc	-Glc-Glc-α-Glc-α-Glc	146	0
S2c	-Glc-α-Glc-α-Glc	-Glc-Glc	136	0
S3c	-Glc-α-Glc-α-Glc	-Glc-Glc-α-Glc	150	+1
S3d	-Glc-α-Glc-α-Glc-α-Glc	-Glc-Glc	121	+3

RS – relative sweetness to sucrose; QT – quality of taste, stevioside: 0, + better, - worse



62 osladin

Scheme 26



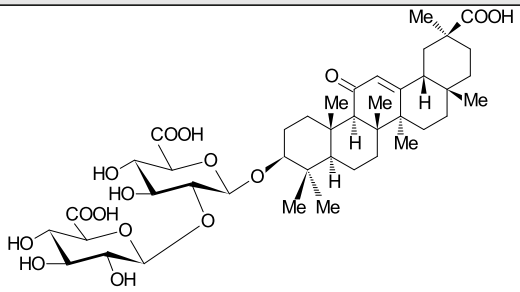
63 steviol R^1 = H, R^2 = H

64 stevioside R^1 = β-D-Glcp, R^2 = Glc(β1→2)Glcβ

65 rebaudioside-A R^1 = β-D-Glcp, R^2 = Glc(β1→2)Glcβ
3
↑
Glcβ1

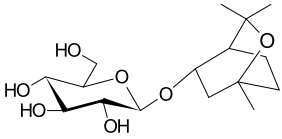
66 Rubusoside R^1 = β-D-Glcp, R^2 = β-D-Glcp

Scheme 27

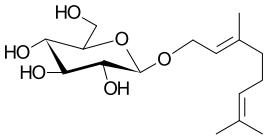


67 glycyrrhizin

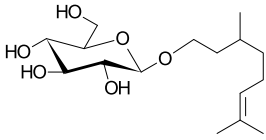
Scheme 28



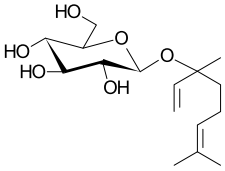
68 1,8-epoxy-*p*-menthan-yl β-D-glucopyranoside



69 geranyl β-D-glucopyranoside



70 citronellyl β-D-glucopyranoside



71 linalyl β-D-glucopyranoside

Scheme 29

Another possibility is to use glycosides of some fragrances to be liberated from tobacco products (by pyrolysis). Since the respective glycosides are not volatile this would limit loss of aroma during storage.

4.4.1 Terpenoid Glycosides from Ginger

A typical example for glycosidic aroma precursors are the terpenoid glycosides discovered recently in fresh young ginger [123].

Linalool and geraniol contribute the most to the fresh ginger aroma. From fresh ginger β-glucopyranosides of 5-hydroxyborneol, 1,8-epoxy-*p*-menthan-3-ol (**68**), 2-heptanol, geraniol (**69**), nerol, citronellol (**70**), (*R*)-linalool (**71**), and some others were isolated. Enzymatic hydrolysis of these compounds with acetone powder prepared from ginger (cold acetone protein precipitate that usually contains intact enzymes from the respective tissue) liberated from the above glycosides the respective fragrance aglycons. This demonstrates that some fragrance components are stored in their glycosidic form and they are released during processing of the ginger.

5 Alkaloid Glycosides

Although alkaloids are mostly produced by plants where glycosylation occurs quite often there are only a few examples of natural alkaloid glycosides, and their biological activities have not been studied to a large extent. There are, however, many examples of alkaloid glycosides prepared artificially for a specific (pharmacological) reason.

5.1 Glycosides of Ergot Alkaloids

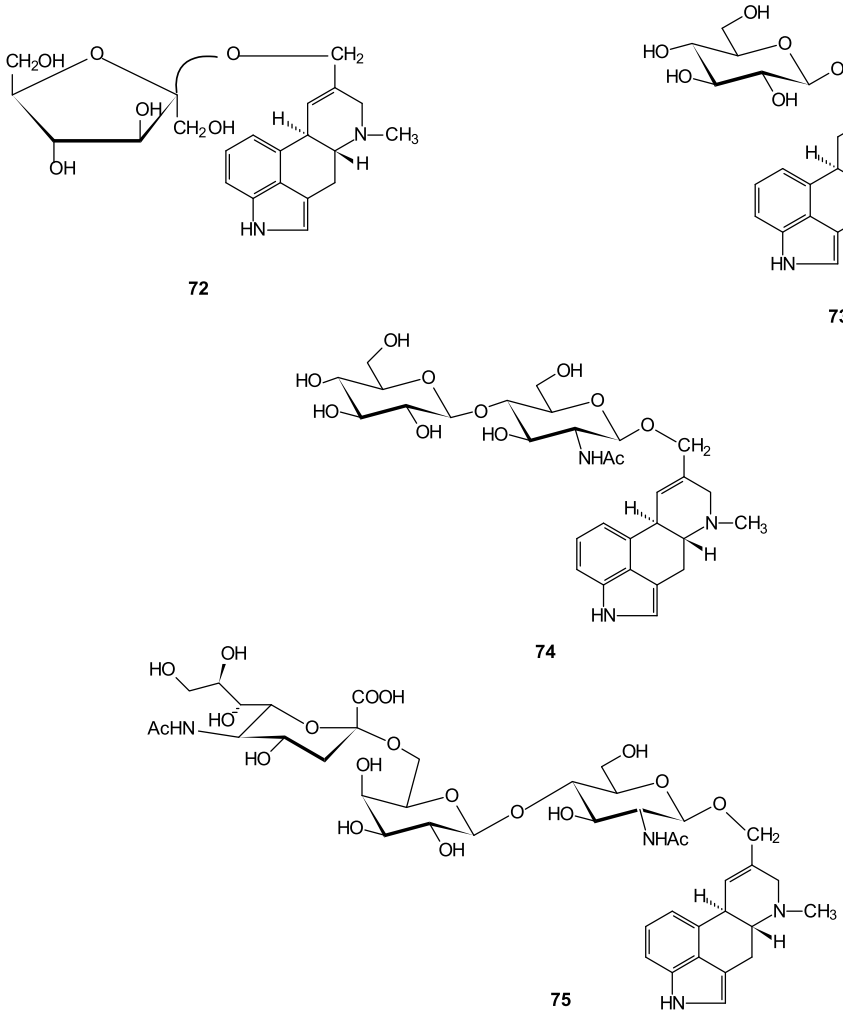
Ergot alkaloids that are produced by the parasitic fungus *Claviceps purpurea* (Ergot) [124] cover a large field of therapeutic uses as drugs of high potency in the treatment of uterine atonia, postpartum bleeding, migraine, orthostatic circulatory disturbances, senile cerebral insufficiency, hypertension, hyperprolactinemia, acromegaly, and parkinsonism [125]. Recently, new therapeutic uses have emerged, such as, for example, against schizophrenia, applications based on newly discovered antibacterial and cytostatic effects, immunomodulatory and hypolipemic activity [126,127,128,129]. Glycosides of EA were isolated as naturally occurring products, and recently a number were prepared by chemical and enzymatic methods. Their promising physiological effects stimulate future research in this field. The first natural EA glycoside, elymoclavine-*O*-β-D-fructofuranoside (**72**) (*Scheme 30*), was isolated from a saprophytic culture of *Claviceps* sp. strain SD-58 by Floss et al. [130]. This glycoside was formed from elymoclavine produced by the microorganism by the action of enzyme invertase present in the fungal mycelium.

Recently, a large series of glycosides of ergot alkaloids was prepared using both chemical and enzymatic methods [131,132,133,134]. Preliminary results obtained indicate that some of these derivatives could have very interesting activities compared to their aglycons.

Some glycosides of 9,10-dihydrolysergol and elymoclavine were tested for their inhibitory activity to prolactin secretion [135,136]. Only 9,10-dihydrolysegol-*O*-β-D-glucopyranoside (**73**) exhibited significantly ($p < 0.001$) higher inhibitory activity.

More systematic studies were performed on the immunomodulatory activity of these new alkaloid glycosides. A large panel of the glycosides, e. g., of elymoclavine and 9,10-dihydrolysergol was tested for their stimulatory activity on cytotoxic lymphocytes. These lymphocytes form the effector arm of cell-mediated immune responses to infection and tumors.

The effect of the alkaloid glycosides was tested on natural killer (NK) cell mediated cytotoxicity of the human resting and activated peripheral blood mononuclear cells (PBMC) against MOLT4 T lymphoma cells (resistant to lysis by fresh PBMC cells and sensitive to activated cells) [128,134,137]. All compounds were tested in a concentration range from 10^{-6} to 10^{-15} M. The maximum immunomodulatory effect was obtained at 10^{-10} M. These effects are mediated by cell surface receptors. After addition of free saccharides to the effector-target cell mixture, the cytotoxic activity of resting fresh lymphocytes has been enhanced in all cases. The most potent stimulation was observed by addition of glucose. The glycosylation of elymoclavine does not influence its cytotoxic activity, however in the case of DH-lysergol its cytotoxic-potentiating activity strongly increased especially in the case of the β-glucoside

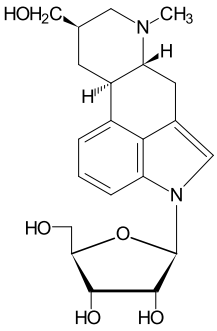


Scheme 30

(**73**) and the β-lactoside (**74**). The lytic capacity of activated killer cells was not influenced by any preparation tested.

The effects of elymoclavine and DH-lysergol glycosides were tested also with the cytotoxicity of resting PBMC cells against the NK-sensitive cell tumor line K562 and against the NK-resistant RAJI tumor cell line [128,134,137]. Stimulation of NK cells against the sensitive K 562 was best with the DH-lysergol itself, its glycosylation lowered the stimulatory effects. Galactosylation of elymoclavine potentiated stimulation activity compared to the aglycon.

Interesting results were obtained in stimulation of NK cells against the resistant tumor cell line RAJI. The attachment of β-Glc and mainly Neu5Acα(2→6)Galβ(1→4)GlcNAcβ-*O*-



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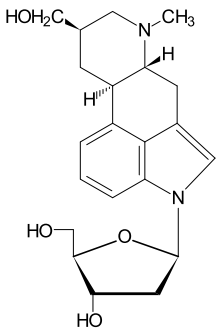


77

Scheme 31

glycosidic moieties to elymoclavine (**75**) had a strong stimulatory effect [134,137]. β-Galactosylation of DH-lysergol also potentiated its effects.

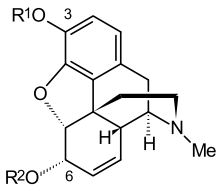
These and some other glycosides were further tested for their immunomodulatory activity on mouse splenocyte models (Balb/c and athymic nude Nu/Nu mice). Here mostly elymoclavine β-galactoside and lactoside had the highest activity [128].

A large panel of ergot alkaloid glycosides and their aglycons was tested for antiviral activities including anti-HIV activity against the replication of HIV-1(III_B) and HIV-2(ROD) in acutely infected MT-4 cells, for their activity in persistently infected HUT-78/III_B cells and for broad spectrum antiviral activity in E_6SM cells cultures against *Herpes simplex* virus-1 (KOS), *Herpes simplex* virus-2 (G), *Vaccinia* virus, *Vesicular stomatitis* virus, *Herpes simplex* virus-1 TK^- B2006 and *Herpes simplex* virus-1 TK^- VMW1837, in HeLa cell cultures against *Vesicular stomatitis* virus, *Coxsackie* virus B4 and *Respiratory syncytial* virus and in Vero cell cultures against *Parainfluenza*-3 virus, *Reovirus*, *Sindbis* virus, *Coxsackie* virus B4 and *Punta Toro* virus [134,138,139]. It was found, however, that virtually all alkaloids and their glycosides tested have cytotoxic concentration below the threshold of their antiviral activity. In some alkaloids, e. g., dihydrolysergol, their cytotoxity was considerably lowered by *N*-ribosylation (**76**) and deoxyribosylation (**77**) (*Scheme 31*). Testing of cytostatic activity of mainly *N*-glycosides of ergot alkaloid (ribosides and deoxyribosides) will be necessary.

Some selected β-galactosides of ergot alkaloids and respective aglycons (elymoclavine and chanoclavine) were tested for their capability to influence basal and forskoline stimulated adenylate-cyclase in ciliary protrusion (*recessus cilliaris*) of the rabbit eye. No significant effects were found. However, both ergot alkaloidgalactosides strongly diminished intraocular pressure in rabbits and their effect was considerably higher than in the respective aglycons [134]. This suggests their potential anti-glaucoma activity.

5.2 Morphine Glucuronides

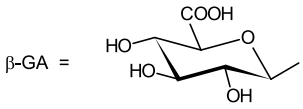
Morphine (**78**) is an important analgesic with a long history of usage. This drug has, however, unwanted side effects. Recently it was found that one glycosidic metabolite – morphine-β-6-



78 (morphine) $R^1 = H$, $R^2 = H$

79 $R^1 = H$, $R^2 = \beta$-GA

80 $R^1 = \beta$-GA, $R^2 = H$



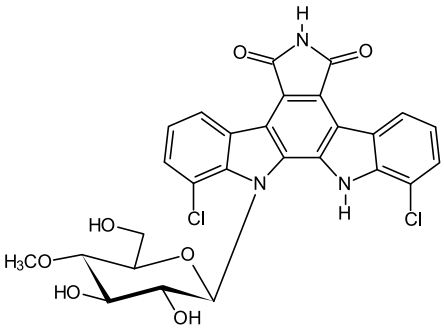
Scheme 32

glucuronide (**79**) – has quite interesting biological effects that are rather different from those of the parental drug.

Morphine has two nucleophilic sites that may be glucuronylated, a 3-hydroxy group on an aromatic ring and an alcoholic 6-hydroxy group.

Conjugation of the 3-hydroxy group occurs in many mammalian species, while glucuronylation at the 6 position appears to be unique to man [140]. This metabolite accumulates in blood of humans with chronic dosing to values greater than morphine [141]. Although the analgesic potency of **79** has been acknowledged for more than two decades, the potential clinical use of this glycoside has only recently been recognized [142]. Morphine β-6-glucuronide (**79**), but not the morphine β-3-glucuronide (**80**) (*Scheme 32*), binds to μ_1 and μ_2 receptors with affinities similar to morphine in mouse brain [140,141]. On the basis of the pharmacodynamic action the 6-glucuronide (**79**) is more than three-times more active than an equimolar subcutaneous dose of morphine in mice [140]. Recent clinical studies in cancer patients given **79** indicated that useful analgesic effects are achieved with an absence of nausea and vomiting [143] that is often caused by morphine itself. Interestingly, **79** present in plasma is distributed into the cerebral spinal fluid, but only one tenth compared to morphine [142]. The potency of morphine β-6-glucuronide is in part explained by an unexpectedly high lipophilicity of the folded form of the molecule. Studies [143] using force-field and quantum mechanical calculations indicate that the glucuronide conjugates of morphine can exist in conformational equilibrium between the extended and folded form of the molecule. The extended conformer, which predominates in aqueous media, is highly hydrophilic because it efficiently exposes polar groups of the molecule to the surrounding aqueous milieu. On the other hand, the folded conformers mask part of these polar groups and are much more lipophilic. The folded forms likely predominate in media of low polarity such as biological membranes [143].

Contrary to its 6-isomer (**79**) morphine-β-3-glucuronide is not an analgesic but it is a potent μ-opioid receptor antagonist. The 3-glucuronide also resembles morphine in that it cau-



81 rebeccamycin

Scheme 33

ses allodynia, a condition in which an ordinarily innocuous stimulus is perceived as painful [144].

5.3 Rebeccamycin

Rebeccamycin **81** (*Scheme 33*) is an interesting alkaloid-type antibiotic that is produced by *Streptomycetes* [145]. Rebeccamycin inhibits the growth of human lung adenocarcinoma cells and produces single strand breaks in the DNA of these cells. A related antibiotic without chlorine, staurosporin, produced by *Streptomyces staurosporeus*, was reported to have antifungal and hypotensive activity [143]. In both structures the 4-*O*-methyl-β-glucopyranosyl moiety forms an *N*-glycosidic bond. A certain analogy with the nucleosides can be traced which may be responsible for its antineoplastic activity.

6 Glycosides of Vitamins

Both hydrophilic and lipophilic glycosides of vitamins often occur in nature and some of them were prepared by chemical and biochemical methods. Recently, an outbreak of papers [146,147,148,149,150,151] was observed dealing with their chemistry and biology, and a comprehensive review on the biological significance of various vitamin glycosides was published [152].

Glycosylated vitamins have an advantage over the respective aglycons in their better solubility in water (especially the lipophilic ones), stability against UV-light, heat and oxidation, reduction in bitter taste and odor (e. g., thiamin), and resistance to enzymatic actions. Some of the vitamin glycoconjugates have altered or improved pharmacokinetic properties. For the synthesis and biosynthesis of vitamin glycosides the presence of a free OH group is mandatory.

We will discuss the vitamin glycosides at length not only because of the quite active research in this area but also for the reason that many vitamin glycosides occur in natural sources as vitamers or they are formed as important and physiologically active metabolites in organism.

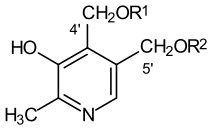
6.1 Water-Soluble Vitamins

6.1.1 Pyridoxine

Extensive research on biological activities has been performed on 5′-*O*-(*β*-D-glucopyranosyl)-pyridoxine **83** (*Scheme 34*), because it is a major form of pyridoxine (**82**, vitamin B_6) in plant-derived foods. Experiments in vivo and in vitro have indicated the lowering of uptake of vitamin B_6 caused by its *β*-glucoside **83** [153,154,155,156]. The presence of pyridoxine *β*-glucoside reduces the bioavailability of pyridoxine usually from plant material by 75–80% [157]. This compound (**83**) is cleaved partly only in the small intestine, but not in the liver [148]. A highly selective pyridoxine-*β*-D-glucosidase was found in the jejunal pig mucosa [149].
Less work was, however, devoted to *α*-glucosides, *β*-glucosides, and *β*-fructosides of pyridoxine, which occur less commonly in nature as a result of transglycosylations.
Recently, it was demonstrated that pyridoxine *α*-glucosides (**84**, **85**) serve nutritionally as well as pyridoxine in terms of transport across everted intestinal sacs, metabolic conversion to active form by liver or kidney homogenate, and the appearance of B_6-derivatives (pyridoxine, pyridoxal, and pyridoxal phosphate) in the blood of B_6-deficient rats after oral administration, while **83** serves very poorly as a B_6 nutrient [157]. Moreover, it has been reported that the 5′-*O*-(*α*-D-glucopyranosyl)-pyridoxine (**84**) does not inhibit the uptake of pyridoxine and is readily converted to pyridoxine in freshly isolated liver cells, while **83** competitively inhibits the uptake of pyridoxine into liver cells [158]. It was found that the 5′-glucoside (**84**) is cleaved in the liver about 5–6-times faster than its regioisomer 4′-*O*-(*α*-D-glucopyranosyl)-pyridoxine (**85**). These facts support the excellent nutritional efficiency of pyridoxine-*α*-glucosides, especially the 5′-isomer. Pyridoxine *α*-glucoside (**84**) has been observed to be much more stable against UV light irradiation and heating than pyridoxine itself [159].
The case of pyridoxine glycosides clearly demonstrates that type of glycosylation strongly modulates the biological activity. In addition, the type of glycosidic linkage (*α*- vs. *β*-) and the site of attachment at quasi-identical carbons also has a strong influence on the final biological activity of the drug.

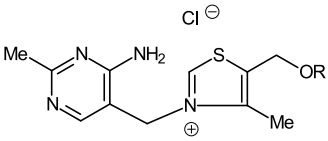
6.1.2 Thiamin

Thiamin (**86**, vitamin B_1) (*Scheme 35*) occurs in nature free and in phosphorylated form. Its glycosides have not been identified in natural material. The artificial glycosylation of



82 (pyridoxine) $R^1 = R^2 = H$
83 $R^1 = H$, $R^2 = \beta$-Glc*p*
84 $R^1 = H$, $R^2 = \alpha$-Glc*p*
85 $R^1 = \alpha$-Glc*p*, $R^2 = H$

Scheme 34



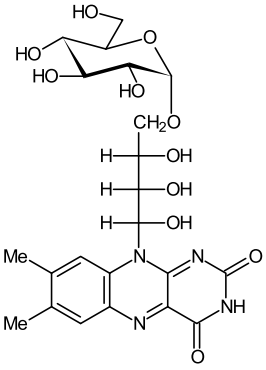
86 (thiamin) R = H
87 R = β-Gal*p*
88 R = α-Glc*p*
89 R^1 = β-Glc*p*NAc

Scheme 35

thiamin was motivated mostly by needs to remove unpleasant taste and odor of the compound and to increase its stability against UV light. *O*-*β*-Galactoside (**87**) [160], *O*-*α*-glucoside (**88**) [161], and *0*-*β*-*N*-acetylglucosaminide (**89**) [162] of thiamin were prepared by enzymatic synthesis using glycosidases. Biological activities were tested only with the *α*-glucoside. This compound, similarly to other glycosides does not have the specific thiamin odor and strong tongue-pricking taste, in contrast it is mildly sweet. *O*-*α*-Glucosylthiamine has 73% activity of a molar equivalent of thiamin hydrochloride, when its use by thiamin-deficient male rats on semisynthetic diet was examined in terms of its effect on the growth curve, food intake, liver weight, and hepatic thiamin content [161]. Pig liver *α*-glucosidase completely hydrolyzed **88** to glucose and thiamin thus some of the thiamin glycosides can be useful as food additives or in medicine.

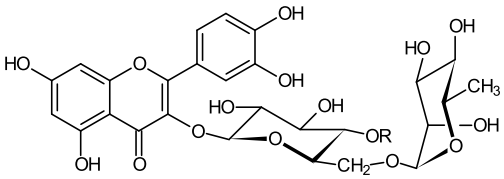
6.1.3 Riboflavin

Riboflavin (vitamin B_2) occurs in nature also in the form of the glycoside, e. g., as riboflavin 5′-*α*-D-glucoside in the liver (**90**) (*Scheme 36*). It is formed from free riboflavin by liver *α*-transglucosylase employing maltose as a donor for an *α*-glucosyl unit [163]. This glycoside is nearly bio-equivalent to riboflavin [164]; it enters the isolated hepatocytes more slowly than free riboflavin and it is hydrolyzed to the free vitamin upon entry.



90

Scheme 36



91 (rutin) R = H
92 R = α-Glcp

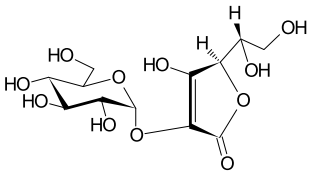
Scheme 37

6.1.4 Rutin

Rutin (**91**, vitamin P) (*Scheme 37*) is one of the most widespread quercetin glycosides (quercetin-3-*O*-rutinoside). Formally, it should be classified as a flavonoid but because of its activities (essential as a capillary protectant) it is included in the vitamin group. Rutin is a glycoside itself, composed of quercetin and the disaccharide rutinose [6-*O*-(α-L-rhamnopyranosyl)-D-glucose]. Its water solubility is rather low (ca. 0.1 g/l) and, therefore, by enzymatic glycosylation 4^{G}-α-D-glucopyranosyl-rutin (**92**) was prepared [165]. Its solubility was considerably increased (30000-times higher than rutin). Its improved biological activity has been predicted based on the finding that this glucoside is hydrolyzed by pig liver α-glucosidase [165].

6.1.5 Ascorbic Acid

Ascorbic acid (vitamin C) is involved in many biological processes, such as collagen synthesis, antioxidation, intestinal absorption of iron, and metabolism of some aminoacids. Search for and preparation of its glycosides was motivated mostly by the need for more stable compounds (resistant to oxidation) having the same or better bioavailability. The first glycoside of ascorbic acid was prepared by Yamamoto [166] and its structure was presumed to be 6-*O*-α-glucopyranosyl-L-ascorbic acid, and it was postulated that this glycoconjugate is formed in vivo by α-glucosidases. Its sensitivity to oxidation was the same as ascorbic acid and its biological activity was inferior to it. Later, another glycoside, 2-*O*-α-glucopyranosyl-L-ascorbic acid (**93**) (*Scheme 38*), was synthesized by an enzymatic regioselective transglucosylation [167]. This glycoside was found to show similar bioavailability as the aglycon in vivo [168] and in vitro [169]. This compound was, however, considerably more stable towards oxidative stress and UV irradiation. In fact, the glycoside **93** itself has no reducing power because of the substitution at the 2-OH group that is involved in redox reactions of ascorbic acid. Oral administration of **93** (guinea pigs) resulted in a remarkable increase of ascorbate in various tissues as well as in plasma [170]. Ascorbic acid was released from **93** at the mucosal side, and it was actively taken up across the intestinal membrane into the serosal side, whereas **93** itself permeated but poorly. Hydrolysis of **93** was mediated by maltase that could be inhibited by castanospermine. Ascorbate was transported by an active uptake system.
Ascorbate, because of its in vivo inhibitory action on melanin synthesis is also used as a skin-whitening agent in cosmetics (e. g., in Japan) [171]. Glycoside **93** was tested in vitro and in



93

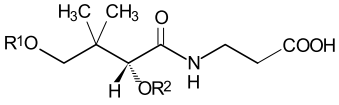
Scheme 38

vivo in humans and compared with ascorbate and ascorbic acid 2-phosphate that is conventionally used for these purposes. It was found that after percutaneous application of **93** the level of ascorbate sustained for a longer period than in the case of phosphate. Also the melanin synthesis (in B18 melanoma cells) was better inhibited by **93** than by ascorbic acid 2-phosphate. From these in vivo and in vitro results it was concluded that by using **93** the level of ascorbate sustains for a longer period. The compound has also UV protective activity against UV-induced damage of human skin keratocytes and fibroblasts.

6.1.6 Pantothenate

Pantothenic acid (**94**) (*Scheme 39*) (denoted as vitamin B_3 or B_5 – sometimes confused with nicotinamide) is widely distributed in animals, plants, and microorganisms as a component of CoA and thus plays an important role in many metabolic pathways. 4′-*O*-(*β*-glucopyranosyl)-D(*R*)-pantothenic acid (**95**) was isolated from tomato juice [172,173] as a growth factor of malo-lactic fermentation bacteria, responsible for the fermentative formation of malic and lactic acids in various food processes, such as for example, wine making. The structure of compound **95** was confirmed by the synthesis and also other glycosides, e. g., 2′-*O*-*β*-glucopyranoside (**96**) and respective glycosides from L and DL forms of **94** were prepared [173]. From comparative studies it follows that the 4′-glucoside was about 50-times more effective than the respective 2′-glucoside or the aglycon (all in D form), the L-form being ineffective. It was suggested that the activity of (**95**) was due to a specific membrane transport of malo-lactic fermentative bacteria. This case also demonstrates the significance of regioisomers of glycosides for their physiological activities.

Following these studies, D-pantothenic acid *β*-glucopyranoside (**95**) was prepared by action of various *β*-glucosidases [174]. Also *α*-glucoside of pantotheic acid was isolated, however, the



94 (D-pantothenic acid) $R^1 = H,\ R^2 = H$
95 $R^1 = \beta\text{-Glc}p,\ R^2 = H$
96 $R^1 = H,\ R^2 = \beta\text{-Glc}p$

Scheme 39

site of the glucosyl attachment was not exactly determined (proposed 4′- position) [175]. Studies of pantothenate metabolism in dogs have demonstrated in vivo glucosylation [176]. Following administration of an oral dose of pantothenate (3 mg/kg), about 60% of pantothenate was excreted in the form of its glucoside **95**.

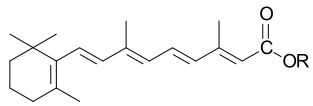
6.1.7 Nicotinamide

Glycosides of this vitamin (sometimes denoted as vitamin B_3) were not described, however, there exists an evidence that bound niacin from wheat bran (termed niacytin) has a single nicotinic acid moiety at least partially linked to an aromatic amine with glucose, xylose, and arabinose in a 6:3:1 molar ratio per molecule, with approximately three cinnamic acid esters [152,177,178]. It seems that these glycosidic complexes limit the bioavailability of the nicotinamide and for its liberation they must be treated, e. g., by soaking corn in a lime solution, traditionally performed in Central America during production of tortillas [179].

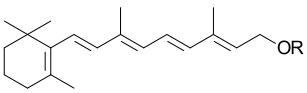
6.2 Lipophilic Vitamins

6.2.1 Retinol

Vitamin A has long been recognized as an essential nutrient in mammals for growth, vision, reproduction, cell differentiation, and the integrity of the immune system [180]. Different metabolic forms of vitamin A show different activities: 11-*cis* retinal is the major ligand for the opsins in vision, 9-*cis* and all-*trans* retinoic acid (**97**, RA) (*Scheme 40*) are active in cell differentiation and in embryogenesis, and retinol and its esters primarily serve as transport and storage forms of the vitamin [180]. In addition to fulfilling their physiological functions, vitamin A and its derivatives and analogs show therapeutic utility in several types of cancer and skin disorders [180]. After the important observation that retinoic acid reduced the onset and number of chemically induced papillomas in mice [181], many analogs, now termed retinoids,



97 (all-*trans*-retinoic acid) R = H
100 (retinoyl β-glucuronide) R = β-glucuronyl



98 (all-*trans*-retinol) R = H
99 (retinol β-glucuronide) R = β-glucuronyl

Scheme 40

were synthesized and tested for their efficacy [182]. The major problem that arose in using retinoids therapeutically was their toxicity [180].

Besides synthetic derivatives useful in therapy there exists a natural derivative of retinol (**98**), and retinoic acid(s) e. g., all-*trans*-retinyl β-glucuronide (**99**) [183] and all-*trans*-retinoyl β-glucuronide (**100**) [184]. These vitamin A glycosides were first identified as billiary metabolites of vitamin A, and they are now prepared also chemically for their favorable biological effects [185,186].

After administration of all-*trans*-, 13-*cis*- or 9-*cis*-retinoic acid, the respective retinoyl glucuronides (RAG) have been identified as the major metabolite in most mammal models including the human system. Like RA, RAG is biologically active in promoting the growth of vitamin A-deficient rats [187] and in the induction of differentiation of, e. g., HL-60 cells [188], but unlike RA, RAG is less cytotoxic and teratogenic. Like RA, RAG is also effective in the topical treatment of human acne [189], but unlike RA, it does not produce any side effects associated with RA therapy [189].

All-*trans*-RAG was investigated (in mice) for its teratogenicity because of its lower placental transfer compared to free RA [190]. Surprisingly, it was found to be a more potent teratogen than RA itself because intravenous or subcutaneous application of RAG was followed by its fast hydrolysis causing high levels of RA. Pharmacokinetic studies nevertheless confirmed lower transplacental transfer of RAG compared to RA [190].

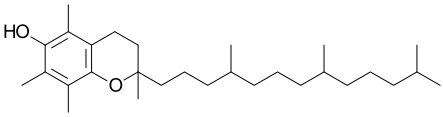
All-*trans*-retinyl β-glucuronide (**99**), another vitamin A glucuronide metabolite was prepared synthetically [185] and it was found to be equally effective in its growth-promoting activity as its aglycon [185,191]. The glucuronide, although converted to retinol in vivo, might also act physiologically in an intact form.

The mechanism of action for retinoid glucuronides is not established [192]. A novel action of the conjugates is suggested, because the glucuronides do not bind to cellular retinoic acid-binding proteins or to nuclear receptors for retinoic acid, although they are active prior to their hydrolytic cleavage.

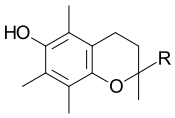
There exist other glycoconjugates of retinoic acid that have potential physiological application. Retinyl phosphate is mannosylated by rat liver microsomes using GDP-mannose in a way analogous to formation of mannophosphoryldolichol, however, the latter reaction is reversible compared to the former one [193]. Probably due to this irreversible reaction retinol is no longer available for its physiological functions in the organism. Besides retinoyl glucuronide, the corresponding galacturonide is formed in the organism at about 10–30% of the rate of the glucuronide formation [194]. Other mostly synthetic glycoconjugates such as retinoyl β-glucose, retinoyl adenosine, and retinoyl sucrose [195] show usually lower activities than RAG [196].

6.2.2 Tocopherol

Tocopherol (vitamin E) (**101**) (*Scheme 41*) is considered to be one of the most prominent antioxidants and radical scavengers in the organism, and it plays also an important role in fertility. It is generally accepted that it is incorporated into biological membranes and is especially effective as a lipid-peroxyl radical scavenger. Glycosylation of tocopherol is motivated mostly by the needs to increase its solubility. Therefore, an attachment of glucosyl unit(s) would be especially useful. Attachment of β-glucosyl, β-maltosyl, and β-oligomaltosyl units via the 6-OH group of tocopherol was achieved [197]. The biological applicability of such a compound



101 α-tocopherol



102 R = COOH
103 R = CH_2OH
104 R = CH_2O-α-Glc*p*

Scheme 41

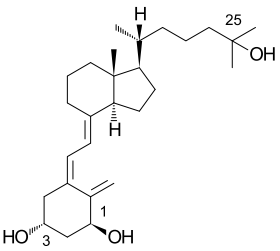
is, however, dependent on its deglycosylation in vivo because the free 6-OH group is essential for the redox properties of tocopherol.

Another probably more feasible strategy is based on the partly water soluble tocopherol derivative Trolox (**102**). The solubility of this compound in water is quite low, therefore enzymatic glycosylation of the corresponding alcohol (**103**) using α-glucosidase from *Saccharomyces* sp. was attempted [198]. The glycosylated product having the structure **104** is well water-soluble (> 1 g/mL) and its radical scavenging activity measured using the 1,1-diphenyl-2-picrylhydrazyl radical is nearly the same as that of α-tocopherol, Trolox, and ascorbic acid. Kinetic studies of the inhibition of the radical chain reaction of methyl linoleate in solution demonstrated that the peroxyl radical scavenging activity was not changed by the replacement of the phytyl side chain of tocopherol to the glucosyl group [199]. Its effectiveness was even higher than that of ascorbic acid when a liposomal suspension was exposed to a lipid-soluble radical generator 2,2′-azobis(2,4-dimethyl-valeronitrile) (AMVN). Also, formation of cholesteryl ester hydroperoxides in human plasma exposed to radical generators was considerably retarded by using compound **104**.

This is probably a good example for a positive influence of glycosylation by transforming a compound active almost solely in the lipid pool into the water pool of the organism. There is, however, one concern – glycosides bearing highly lipophilic moieties can act as nonionic detergents thus causing negative effects like hemolysis, etc. These potential problems must be addressed by thorough in vivo tests.

6.2.3 Calcitriol

Calcitriol (1α,25-dihydroxycholecalciferol, vitamin D_3, **105**) (*Scheme 42*) is involved in calcium metabolism. Glycosides of this compound occur in plants [200,201] and also as metabolites in mammal organisms. β-Glucopyranosides of **105** linked to the C-1, C-3, or C-25 hydroxyfunctions were tested in vivo (rats) and compared with the aglycon. These glycosides occur as potential sources of vitamin D in plants, e. g., *Solanum malacoxylon* [200] where



105

Scheme 42

they cause pathogenic calcifications in cattle grazing on this pasture. It was concluded that all three glycosides were equipotent but they were less active than the parental compound. The least active was the 3-β-glucopyranoside [150]. Their activity is probably a result of their hydrolysis [150,202] after administration *i.v.* or *p.o.*

Biological activity of the three β-glucopyranosides of **105** was studied in chicks and Japanese quails. While the 1- and the 3-glucoside showed no or only little effect on serum calcium, bone weight, calcium binding protein or calcium deposition in the egg shell, the 25-glucoside was found to be more than half as active as the aglycon **105**. The bioactivity of this glucoside parallels a higher binding constant to the intestinal calcitriol receptor compared to those of the two glucosides [203]. β-Glucuronides are the most prominent mammal metabolites of **105** [204].

7 Carbohydrate – Nucleic Acid Interactions

Traditionally, the glycan chains of the 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 the calichenamycin antibiotics partially determine the selectivity of the process and even the calichenamycin aryltetrasaccharide (without possessing the enediyne ring) [39] binds well to the DNA.

Carbohydrate minor groove DNA [205,206] binders are neutral neoglycoconjugates, whose sugar residues are deoxygenated, thus showing a delicate balance between hydrophylic and hydrophobic domains (*Fig. 3*). 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. Often, 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 suggest that rather the complementary shapes, and hence specific van der Waals contacts between the carbohydrates and minor groove, may determine the site-selectivity displayed by many carbohydrate-containing minor groove binders [207]. Additionally, the relatively rigid carbohydrates may take advantage of the sequence-dependent flexibility of double stranded DNA to bind their target

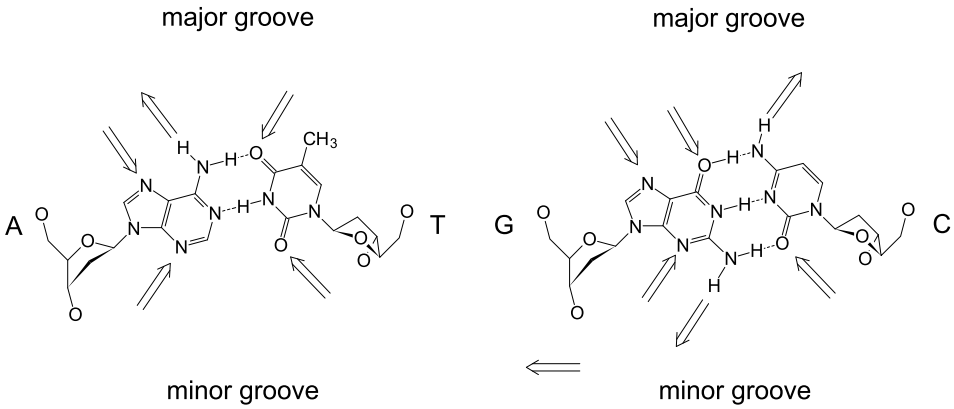
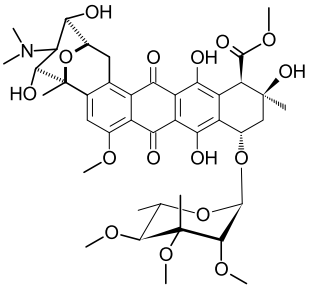


Figure 3
Substitution pattern of H-bonding donor and acceptor groups for adenine-thymine and guanine-cytosine base pairs in the major and minor groove

sites [208]. Furthermore, the hydrophobicity of DNA-binding carbohydrates may differentiate these 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 be also effective DNA major groove binders. So far, there is only a limited number of examples of glycosides that have been found to interact with the DNA major groove [209]: neocarzinostatin (**32**, DNA cleavage agent); altromycin B (alkylating agent; nogalamycin **106** (*Scheme 43*), respinomycin (tandem-intercalative-groove binding ligands); neomycin-Hoechst33258 conjugate (dual-groove binding ligand).
Dual groove binding natural products such as nogalamycin have shown enhanced binding affinity over 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.



106 nogalamycin

Scheme 43

8 Glycorandomization

The isolation of several sugar biosynthesis gene clusters and glycosyltransferases from different antibiotic-producing organisms, and the 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 that can allow one to alter the types of sugar transferred to the different aglycons or, less frequently, to change the position of its attachment [211,212].

Recently, two complementary glycorandomization strategies have been described, namely, neoglycorandomization, a chemical approach based on a one-step sugar ligation reaction that does not require any prior sugar protection or activation, and chemoenzymatic glycorandomization, a biocatalytic approach that relies on the substrate promiscuity of enzymes to activate and attach sugars to natural products.

These "glycorandomization" approaches (occasionally referred to as "glycodiversification") are expected to foster our understanding of the role of sugars in a variety of glycoconjugates and the exploitation of these critical attachments. Neoglycorandomization that was developed by J. S. Thorson and coworkers relies on a broad variety of reducing monosaccharides without protection/deprotection methodology [212]. Neoglycorandomization is based on the selective formation of glycosidic bonds between reducing sugars and secondary alkoxyamine-containing aglycons [213] to form a library of "neoglycosides".

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 [112] secondary alkoxyamines react to form closed-ring neoglycosides. Presumably, such secondary alkoxyamines react with reducing sugars to form an intermediate oxo-imminium species, which then undergoes ring closure [113].

Chemoenzymatic glycorandomization [215,216] employs the inherent or engineered substrate promiscuity of anomeric kinases and nucleotidylyltransferases, and it attempts to provide activation pathways for the synthesis of nucleotide diphosphosugar donor libraries. In chemoenzymatic glycorandomization these activated sugar libraries, in turn, serve as substrates for inherently promiscuous natural product glycosyltransferases, thereby providing 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 three years a variety of examples has appeared supporting this robust methodology: Four glycosyltransferases from two distinct natural product biosynthetic pathways – calicheamicin and vancomycin – readily catalyzed reversible reactions, allowing sugars and aglycons to be exchanged. More than 70 differentially glycosylated calicheamicin and vancomycin variants were prepared. This study [217] suggests the reversibility of glycosyltransferase-catalyzed reactions and may be general and useful for generating exotic nucleotide sugars, establishing in vitro glycosyltransferase activity in complex systems. A monoglycosylated vancomycin library was prepared by the use of flexible glycosyltransferases with nucleotide diphosphosugar libraries [218].

High-level expression of three macrolide glycosyltransferases established a synthetic "tool kit" with such plasticity that 12 modified macrolide antibiotics (oleandromycin and erythromycin) have been readily created [219].

It is quite obvious that more interesting examples will follow bringing this method close to practical exploitation. Here, the basic knowledge of the role of carbohydrates in the biological activity of glycosides can be capitalized upon.

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