Russian Chemical Reviews **80** (2) 103 – 126 (2011) © 2011 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2011v080n02ABEH4145

Chemical modification of antifungal polyene macrolide antibiotics

S E Solovieva, E N Olsufyeva, M N Preobrazhenskaya

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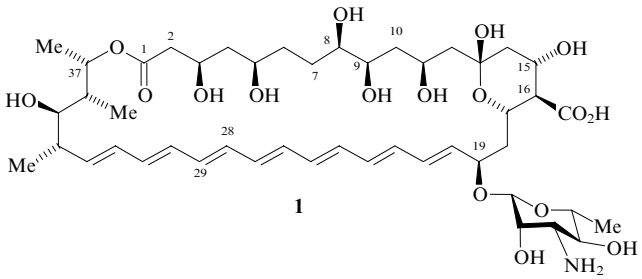
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Abstract. The review summarizes advances in the methods for the synthesis of polyene antibiotics (amphotericin B, partricin A, *etc.*) and investigations of the structure – activity relationship made in the last 15 years. State-of-the-art approaches based on the combination of the chemical synthesis and genetic engineering are considered. Emphasis is given to the design of semisynthetic antifungal agents against chemotherapy-resistant pathogens having the highest therapeutic indices. Recent results of research on the mechanisms of action of polyenes are outlined. The bibliography includes 62 references.

I. Introduction

In the last 20 years, the number of patients with fungal infections (mycoses), both superficial and invasive (systemic), greatly increased. This is associated primarily with a considerable increase in the number of immunocompromised patients.[1, 2] Invasive mycoses are particularly dangerous. For example, invasive candidiasis is characterized by severe clinical presentation and a high mortality rate (30% – 70%); the mortality due to invasive aspergillosis is higher than 50%.[1] The frequency of invasive mycoses in oncology/hematology clinics is high (50%), and the mortality due to mycoses in children with leukemia is 29% – 39% even after intensive antifungal therapy.[3]

The armamentarium of drugs for the treatment of systemic fungal infections is limited. Polyene macrolide antibiotics have been used for this purpose for more than 50 years. Among these agents, amphotericin B (AmB, **1**) is of most importance due to its high efficacy and a broad spectrum of activity.



1

For some time, the successful use of azole antifungal agents has relegated polyene macrolides to the background; however, the rapid development of azole resistance in microorganisms [1, 2] gave impetus to the search for new drugs.[2, 4]

An important advantage of polyenes over other types of antifungal agents is the relatively low frequency of resistance to these drugs. Nowadays, AmB is the only polyene macrolide approved for use in the treatment of systemic fungal infections. However, the use of AmB is essentially limited because of pronounced nephrotoxicity, hematotoxicity, very low solubility in water and suboptimal pharmacokinetics, when therapeutic doses of the drug are close to the maximum tolerated doses.[1, 2]

The necessity of the search for new polyene antifungal agents is associated with the need in new promising drugs without acute side effects for the treatment of invasive mycoses. The design of these drugs is performed primarily by chemical modifications of natural antibiotics. In the last 15 years the development of new reagents and methods resulted in the synthesis of the previously unknown semisynthetic derivatives based on natural polyenes. The influence of various structural fragments of antibiotics on the antifungal activity was studied in *in vitro* and *in vivo* model experiments. In some cases, new-generation drugs were introduced into clinical practice.

The mechanism of action of polyenes, which could help in the search for active structures, is not fully understood.

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Received 19 February 2010
Uspekhi Khimii **80** (2) 115 – 138 (2011); translated by T N Safonova

Detailed studies of the mechanism and research on the synthesis of new derivatives are underway. With the advent of genetic engineering, it becomes possible to design biosynthetic analogues of polyene antibiotics.[4] The latter can be used by themselves or as the starting compounds in the synthesis of new antifungal agents with improved pharmacological properties.

In the past decade, polyene antibiotics were considered in a number of reviews. However, these reviews either provide only a general overview of the topic (see, for example, the monograph [5]) or are focused on the total synthesis of compound **1** (see, for example, Ref. 6).

The present review summarizes advances in the search for the previously unknown analogues of AmB and other related polyenes by the chemical design made in the last 15 years. Emphasis is given to the state-of the art in the design of new-generation antifungal drugs based on the combination of the chemical synthesis and genetic engineering methods.

II. Natural and genetically engineered polyene macrolide antibiotics

1. Natural antifungal polyenes

In addition to amphotericin B, the polyene macrolide antibiotics nystatin A_1 (**2**) and partricin A (**3**) are most often used for the chemical modification.

Nystatin A_1 is active mainly against the fungi *Candida* and is used both topically and orally. The gastrointestinal absorption of nystatin is insignificant and the drug acts only locally. Amphotericin B has a broader spectrum of antifungal activity and is used mainly intravenously for the treatment of acute systemic mycoses. This compound is used as the complex with sodium deoxycholate (the drug Fungizone® manufactured by Bristol Myers Squibb, USA) and in the liposomal form (the drug AmBisome®, Astellas Pharma, USA).

The main structural unit of this class of antibiotics is the macrocyclic lactone consisting of the polyol region, the internal cyclic hemiketal with the carboxy group and the polyene moiety to which the amino sugar mycosamine (3-amino-3,6-dideoxy-D-mannose) is attached. Due to the presence of the carboxy group of the aglycone and of 3′-amino group of the carbohydrate residue, the molecules are amphoteric. Compounds **1** and **2** contain the 38-membered lactone ring but they differ in the arrangement of hydroxy groups. The aglycone of AmB contains the linear *trans*-heptaene system, whereas the C(28)–C(29) bond in nystatin A_1 is single and, consequently, compound **2** belongs to tetraene polyenes 4 + 2.

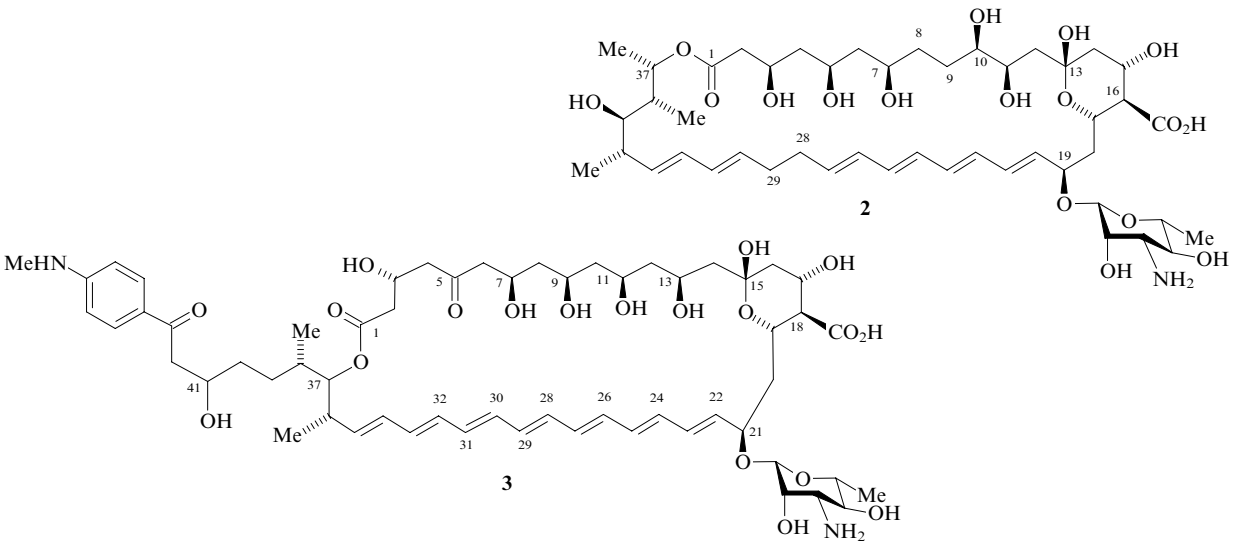
Partricin A is identical to one of the components of the antibiotic aureofacin, *viz*., gedamycin A, and belongs to heptaenes. In the early study,[7] the C(28)–C(31) moiety in the antibiotic structure was described in the *trans* configuration, whereas this moiety in other derivatives of this compound described more recently (see below) has the *cis* configuration. Under particular conditions, the diene moiety in heptaene antibiotics can be reversibly transformed from the *trans* configuration into the *cis* configuration. Thus, the energy of the transformation of one diene group in AmB from the *trans* configuration into the *cis* configuration is relatively low (16.4 kcal mol^{-1}).[8]

The 38-membered lactone ring of compound **3** contains the additional *p*-methylaminobenzoylalkyl substituent at the C(37) atom. The structures of the polyhydroxylated moiety of antibiotics **1** and **3** are also different. The C(1)–C(12) moiety in amphotericin contains 12 carbon atoms and 5 hydroxy groups, whereas this moiety in partricin A is two carbon atoms longer [C(1)–C(14)] and contains, in addition to five hydroxy groups, the keto group at the C(5) atom. The carbonyl group at the C(13 or 15) atom and the hydroxy group at the C(17 or 19) atom in antibiotics **1**–**3** form the internal cyclic hemiketal adopting a chair conformation.

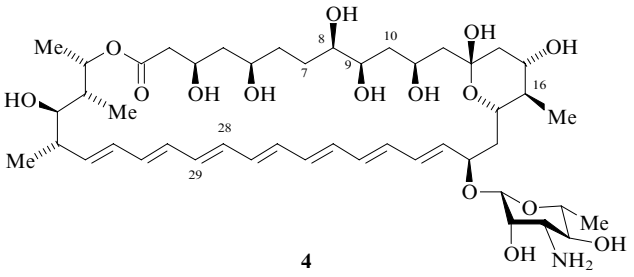
2. Genetically engineered analogues of polyenes

In recent years, the design of analogues of polyene antibiotics by means of genetic engineering has become a promising approach to the search for new macrolide derivatives with improved pharmacological properties.[4,9–12] Genetic engineering methods are based on the data obtained by the complete sequencing of gene clusters responsible for the biosynthesis of antibiotics by producer strains.

The main steps of the biosynthesis of such polyene macrolides as amphotericin B and nystatin A_1 are as follows: the formation of the 38-membered macrolactone ring whose assembly occurs with the participation of six polyketide synthases (PKS), the oxidation of the methyl



group at position 16 catalyzed by methyl oxidase, the transferase-catalyzed biosynthesis and binding of mycosamine followed by the hydroxylation of position C(8) or C(10) catalyzed by hydroxylase. The biosynthetic pathway of antibiotics can be altered with the use of the genetic material of the starting strains cloned into a specially constructed vector for the replacement of fragments of genes encoding enzymes of the biosynthesis. This replacement produces mutant strains with an altered biosynthetic pathway giving target derivatives of antibiotics. For example, the genetic construction of the mutant strain of the bacterium *Streptomyces nodosus*, where the oxidation of the C(16)-methyl group to the carboxy group catalyzed by the corresponding oxidase is switched off, was used for the synthesis of 16-decarboxy-16-methylamphotericin B (**4**).[12] Compound **4** was prepared also by the multi-step chemical synthesis (see below).[13]

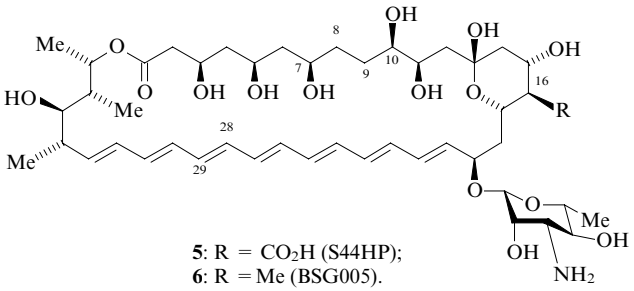


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The biosynthetic pathway of nystatin A_1 was determined based on the cloning and analysis of gene clusters. The genetic manipulations with the producer of compound **2**, *viz*., the bacterium *Streptomyces noursei* ATCC 11455, afforded a series of new genetically engineered polyene macrolides.[9,10] The biosynthetic pathway of nystatin A_1 differs from that of AmB in that in the former case one of PKS genes contains a domain with the keto reductase activity and, in addition, enoyl reductase. The work of this enzyme results in the formation of the C(28)–C(29) single bond, whereas the biosynthesis of AmB is not accompanied by the reduction of the double bond in this position.

The recombinant strain of *S. noursei* with the genetically altered PKS NysC, which produced the heptaene analogue of nystatin A_1, 28,29-didehydronystatin A_1 (S44HP, **5**), was constructed by the genetic engineering method.[10]

Compound **5** showed the 2–10 times higher antifungal activity compared to the starting compound **2** in most of *in vitro* trials and is characterized by a wider range between the maximum tolerable dose (MTD) and the median lethal dose (LD_{50}) in experiments in animals compared to antibiotic **1**.[14]



5: R = CO_2H (S44HP);
6: R = Me (BSG005).

The presence of the additional C(28)–C(29) double bond in the polyene moiety of the nystatin A_1 molecule results in an increase in the antifungal activity against most of the strains under study, which can be attributed to an increase in the efficiency of the interaction of the antibiotic molecule with the target of microorganisms (see Section II.3).

The importance of the configuration of the linear heptaene hydrophobic moiety in the antibiotic molecule for the interaction with the target was shown by an example of new genetically engineered analogues of nystatin A_1 containing an additional hydroxy group in the polyene moiety of the molecule, *viz*., 31-hydroxynystatin and 33-hydroxynystatin. The solubility of these derivatives in water is 2000 times higher than that of compound **2**; however, these derivatives lack antifungal activity.[9] Consequently, the introduction of the hydroxy group into the polyene moiety of the molecule leads to a change in the configuration of the linear hydrophobic moiety of the antibiotic molecule (see below for the mechanism of action).

The structure of the polyol moiety of the antibiotic molecules also influences its activity. The synthesis of derivatives of polyenes **5**[14] and **1**,[11,12] which differ in the number and arrangement of hydroxy groups in the aglycone moiety [C(5)–C(10)] of the molecule, and compounds, in which the hydroxy groups at the C(5) and C(7) atoms were replaced by carbonyl groups, was reported. It was shown that the introduction of the keto group instead of the hydroxy group generally leads to a decrease in the activity and toxicity of the antibiotic (see Section II.3).

The inactivation of the gene responsible for the work of oxidase catalyzing the transformation of the C(16)-methyl group into the C(16)-carboxy group in the recombinant producer strain of compound **5** (*S. noursei*) led to the formation of highly active 16-decarboxy-16-methyl-28,29-didehydronystatin A_1 (BSG005, **6**). Compound **6** retains high antifungal activity (comparable to that of S44HP) but it is essentially less toxic than compounds **1** and **5**.[15]

Genetic engineering methods were used mainly for the modification of the polyene and hydroxylated parts of the macrolide ring and for the specific modification of the carboxy group. The functional groups (the carboxy group and/or the amino group at position 3′ of mycosamine) were transformed mainly by chemical methods.

The genetically engineered antibiotics S44HP and BSG005 are the most promising compounds for further investigations with the aim of applying in medical practice. In addition, these antibiotics can be used as the starting compounds for the synthesis of new derivatives.

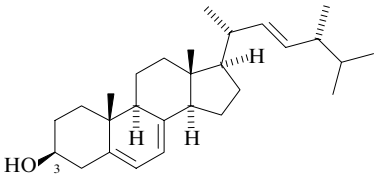
3. Mechanism of action of polyene macrolides

The mechanism of action of polyene macrolides is associated with their ability to interact with cytoplasmic sterol-containing membranes and form pores (channels) in the membranes through which ions are pumped out of cells, resulting in their death.[4,16–18]

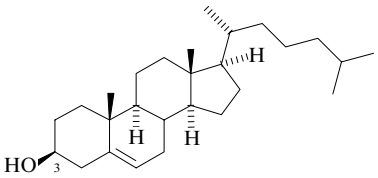
Experiments showed that polyene macrolides interact with liposomes of various fungi and mammalian cells, and sterols enhance this interaction. However, it remains unclear whether antibiotic molecules form complexes with sterols or only indirectly influence the formation of channels. In theory, the antibiotic can interact with a lipid bilayer membrane and change its structure in such a way that the formation of channels in the membrane is facilitated both in the presence and in the absence of sterol.

It was repeatedly mentioned that microorganisms containing sterols in the lipid coat are more sensitive to polyene

antibiotics than microorganisms in which sterols are absent.[17] The investigation of the dynamics of the binding of antibiotics to membranes in the presence of sterols also confirmed the possibility of the direct interaction of antibiotics with sterols.[17] This fact has been recently confirmed by ^{2}H NMR spectroscopy by an example of the interaction of AmB with ergosterol in a lipid bilayer membrane.[16]



Ergosterol



Cholesterol

The selectivity of the action of polyenes against fungal pathogens is determined by their stronger binding to ergosterol incorporated into a fungal membrane compared to cholesterol, which is present in human and animal cell membranes. The ergosterol molecule contains two more double bonds than the cholesterol molecule, due to which it is more planar and more rigid than the cholesterol molecule.

Model experiments showed that AmB causes a fourfold increase in the flow of K^+ ions through the channel across the ergosterol-containing liposome compared to the cholesterol-containing liposome and an approximately 4.5-fold increase in the flow compared to the sterol-free liposome.[17]

Therefore, undesired toxic side effects in the treatment of fungal infections with polyene macrolides are due to the relatively low selectivity of the action of polyenes on ergosterol-containing pathogenic fungi compared to their action on cholesterol-containing mammalian cells. The resistance of microorganisms to polyenes is developed very slowly.[1] A decreases in the sensitivity of fungi to polyene antibiotics is associated primarily with a decrease in the ergosterol content in cytoplasmic membranes.[4, 17]

The 'barrel stave' model is the commonly accepted molecular model for ion channels. This model was proposed in 1974 independently by Japanese[18] and US[19] researchers. Eight AmB molecules and eight sterol molecules form a pore with an inner diameter of $\sim$8 Å (Fig. 1).

Polyene antibiotic molecules are amphiphilic, *i.e.*, they have affinity for both organic and aqueous phases and can be conventionally divided into the hydrophobic and hydrophilic parts. The linear polyene region of AmB is attached to phospholipid acyl chains by hydrophobic interactions to form a stable complex of the antibiotic with a cell membrane, and the polyol moiety forms the hydrophilic pore in the membrane. The planar sterol molecule whose size matches the size of the hydrophobic part of the polyene is embedded in this complex thus increasing the strength of the wall of the ion pore. It was shown that for the interaction with the antibiotic to occur it is necessary that the C(3) atom bound to the hydroxy group in the sterol

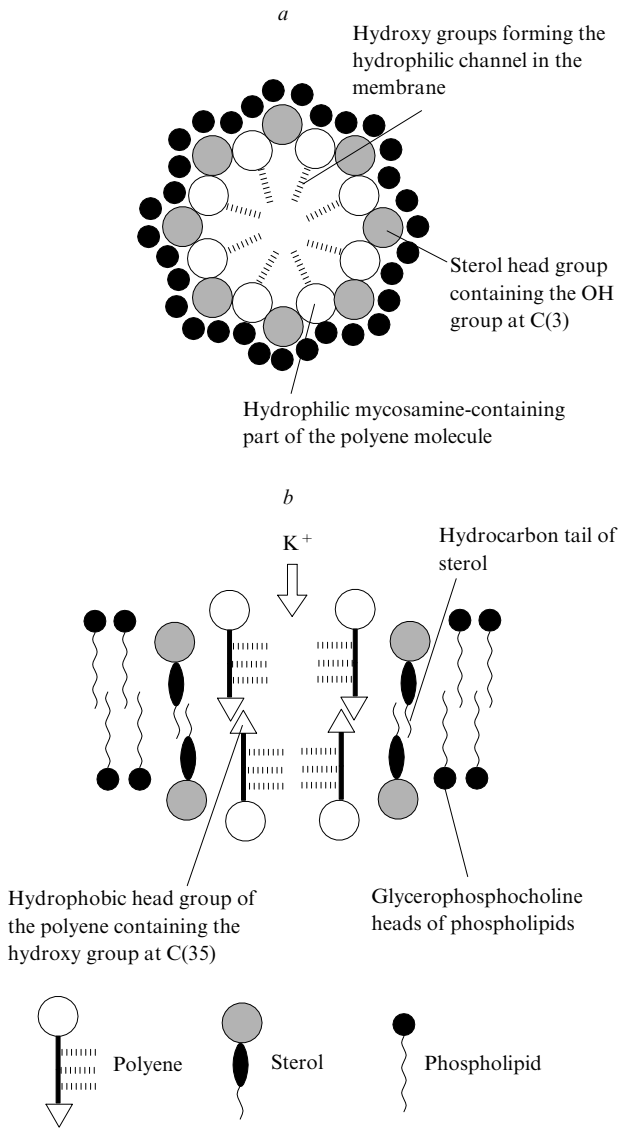


Figure 1. The 'barrel stave' model for the formation of an ion channel by a polyene macrolide in a phospholipid membrane in the presence of sterol: (*a*) a top view; (*b*) a side view.

molecule has the absolute *S* configuration. For the formation of the ion channel across the membrane, two such pores should be formed on the opposite sides of the membrane. The wall of the channel consists of AmB dimers. Two molecules **1** are extended along the channel in such a way that there is a hydrogen bond between the hydroxy groups at the C(35) atoms located in the head group of each AmB molecule. The dimeric antibiotic–sterol complex is embedded in the hydrophobic interior of the lipid bilayer, whereas the amino carbohydrate moiety is exposed to the aqueous medium surrounding the membrane (see Fig. 1).[17–20]

The amino group of the mycosamine moiety capable of being protonated plays a great role in the formation of the complex of AmB with a target.[20] The presence of the carboxy group at the C(16) atom, which can bear a negative charge, is not a necessary condition.[12, 15] It was shown that the presence of neutral groups or substituents containing

the amino group at position 16 does not lead to the loss of activity; moreover, it results in that the antibiotic molecule has certain advantages over natural polyenes.

Cell membranes with a high cholesterol content (erythrocytes, liver cells, adrenal cells, nervous system cells, *etc.*) are particularly sensitive to polyenes. The low solubility of the antibiotic in water resulting in the problems with the elimination of the drug from the body is also an adverse factor. Apparently, this problem can be solved by introducing additional hydrophilic groups into the molecule.[17]

In the design of new drugs, it is particularly important to find an optimal combination of the high antifungal activity and the low toxic side effect. The main tool for the selection of promising compounds is still based on *in vitro* tests of their action using panels of test fungal cultures followed by investigations of the most active derivatives in animal experiments.

III. Semisynthetic derivatives of polyene macrolide antibiotics: synthesis and properties

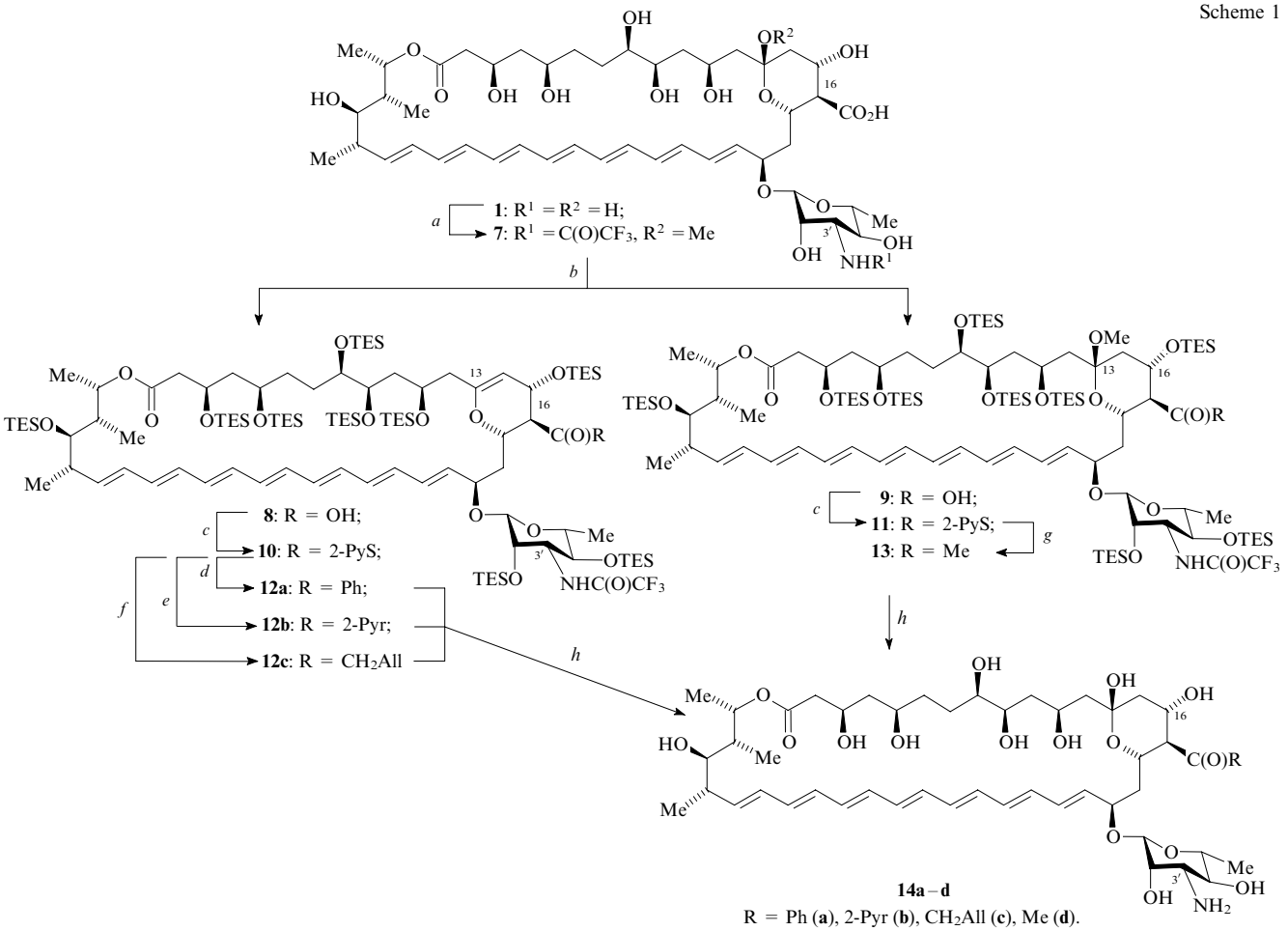
The chemical modification of polyene macrolides is seriously complicated by the fact that these compounds are labile in acidic and alkaline media, undergo aggregation (dimerization), are easily oxidized by atmospheric oxygen, and are prone to light-induced isomerization of the *trans*-diene moieties to the *cis* form. The most important functionalization pathways of the natural polyenes AmB (**1**) and nystatin A_1 (**2**) were developed in the 1980 – 1990s and were described in the reviews by Shenin *et al.*[21,22] First semisynthetic derivatives of partricin (**3**) were described in the publication[7] (see below). However, new publications devoted to the synthesis and chemical modifications of new polyene structures and investigations of the mechanism of their action appeared in the last 15 years. Moreover, certain important aspects were not considered in-depth in the earlier reviews. In some cases, semisynthetic polyenes synthesized previously were used for the repeated functionalization with the aim to study the mechanisms of action or to investigate the biological properties in more detail.

1. Functionalization of amphotericin B at the internal hemiketal ring

The synthesis of a series of 16-decarboxy-16-acyl analogues of AmB was reported[23] (Scheme 1).

In the first step, the 3′-amino group was protected by treating compound **1** with ethyl trifluoroacetate, and the hemiacetal hydroxy group in position 13 was transformed into the methoxy group by the reaction with methanol and pyridinium *p*-toluenesulfonate (PPTS), resulting in the for-

Scheme 1



(*a*) 1) CF_3CO_2Et, Pr^iNEt_2, DMF; 2) MeOH, PPTS, THF; (*b*) TESOTf, 2,6-lutidine, CH_2Cl_2 or THF; (*c*) 2-PySC(O)Cl, Et_3N, Et_2O; (*d*) 1) PhMgBr, THF; 2) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2; (*e*) 2-PyrMgBr, THF; (*f*) 1) $AllCH_2MgBr$, THF; 2) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2; (*g*) MeMgBr, THF; (*h*) 1) HF – PyH, THF, MeOH; 2) PPTS, THF, H_2O; 3) NH_3, THF, MeOH; Py is pyridyl, Pyr is pyrrolyl.

mation of the corresponding intermediate **7**. The subsequent exhaustive silylation of the secondary hydroxy groups with triethylsilyl trifluoromethanesulfonate (TESOTf) afforded the elimination product of methanol **8** and 13-O-methyl derivative **9** suitable for the subsequent modification. The ratio of the products depends on the solvent used. Thus, the reaction in dichloromethane gave a mixture of compounds **8** and **9** in 21% and 38% yields, respectively, whereas the reaction in tetrahydrofuran gave compound **9** as the major product (in 61% yield). The subsequent activation of the carboxy group at the C(16) atom by 2-pyridyl thiochloroformate afforded the corresponding 2-pyridylthio analogues **10** and **11**, which reacted with Grignard reagents to form the corresponding protected keto derivatives **12a–c** and **13**. After the deprotection, a series of keto derivatives of AmB, *viz*., compounds **14a–d**, were obtained (see Scheme 1).

Keto analogues **14a–d** exhibit a relatively high antifungal activity and a relatively low nephrotoxicity.

A series of AmB derivatives, which do not contain the carboxyl group at the C(16) atom, was synthesized. Scheme 2 shows the pathway of the synthesis of 16-decarboxy-16-methylamphotericin B (**4**), which has been earlier prepared by the genetic engineering method,[12] starting from protected AmB (compound **15**).[13] The latter was initially transformed into intermediate thio ether **16** followed by the reduction with $LiBH_4$ to form 16-decarboxy-16-hydroxymethyl derivative **17**. The replacement of the hydroxy group by iodine in compound **17** in the reaction with the I_2-PPh_3 mixture afforded the iodomethyl derivative, which was desilylated. The subsequent reduction of the iodomethyl group in compound **18** to the methyl group with $NaBH_4$ gave protected 16-decarboxy-16-methyl derivative **19**. The successive elimination of the protecting groups in compound **19** resulted in the formation of target product **4**.

The similar scheme of the synthesis of 16-decarboxy-16-hydroxymethylamphotericin B (**20**) but without the use of triethylsilyl protecting groups was described in the study[24] (Scheme 3). Initially, compound **1** was transformed into N-Fmoc derivative **21** followed by the esterification of the carboxy group at position 16 with diazomethane or iodomethane to form doubly substituted analogue **22**. The transformation of the hydroxy group at the C(13) atom into the methoxy group was performed with the use of MeOH in the presence of PPTS. The key 13-O-methyl derivative **23** was reduced with an excess of $NaBH_4$ in methanol to form 16-hydroxymethyl derivative **24**. The hydrolysis of acetal **24** through intermediate *N*-Fmoc-hemiketal **25** afforded the target 16-decarboxy-16-hydroxymethylamphotericin (**20**).

Compound **20** exhibits insignificant nephrotoxicity and retains the antifungal activity comparable to that of the starting antibiotic.

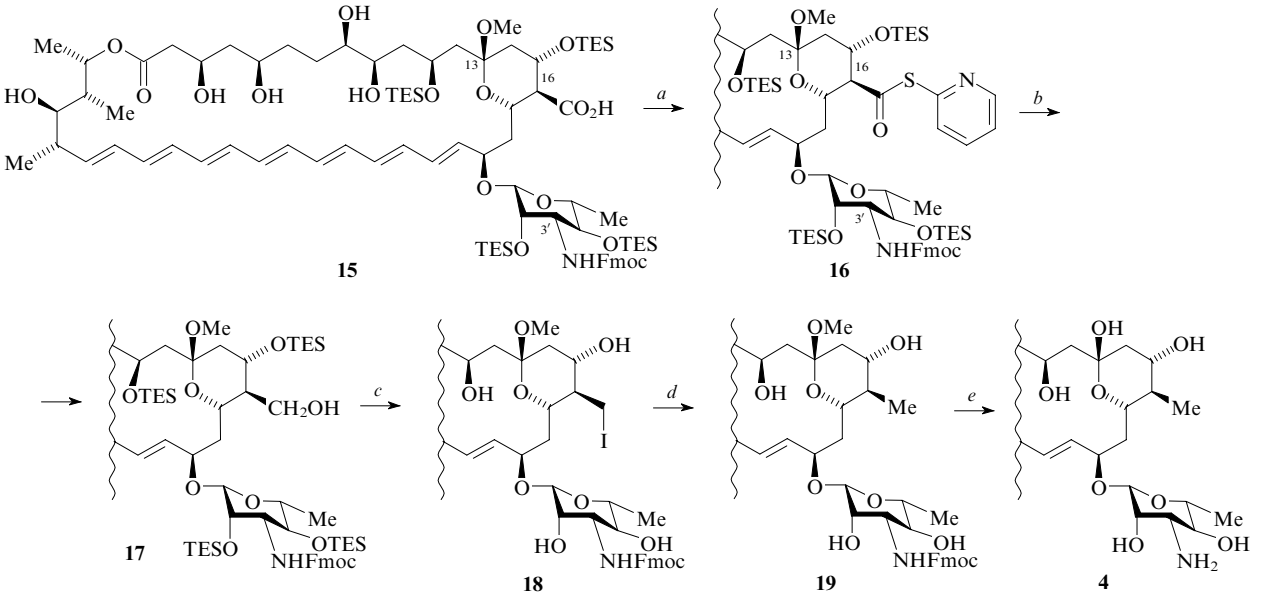
Per-*O*-triethylsilyl-*N*-Fmoc-16-decarboxy-16-hydroxymethylamphotericin B (**17**) proved to be a convenient starting compound for the synthesis of C(16)-carbaldehyde **26**, and the latter was used for the synthesis of C(16)-carbaldoximes **27a**,**b** and C(16)-vinyl analogues of AmB **28a–d** (Scheme 4).[25]

The Swern oxidation of the hydroxymethyl group at position 16 of compound **17** to the aldehyde group afforded fully protected C(16)-formyl analogue **26**. The reaction of compound **26** with hydroxylamine or methoxyamine followed by the removal of all protecting groups gave the corresponding C(16)-carbaldoximes **27a**,**b**. C(16)-Vinyl analogues **28a–d** were synthesized from compound **26** in the reactions with the corresponding Wittig reagents (phosphoranes).

The i*n vitro* biological evaluation of oximes **27a**,**b** and vinyl analogues **28a–d** showed that these compounds exhibit antifungal activity comparable to that of AmB; however, they have pronounced hemolytic activity.

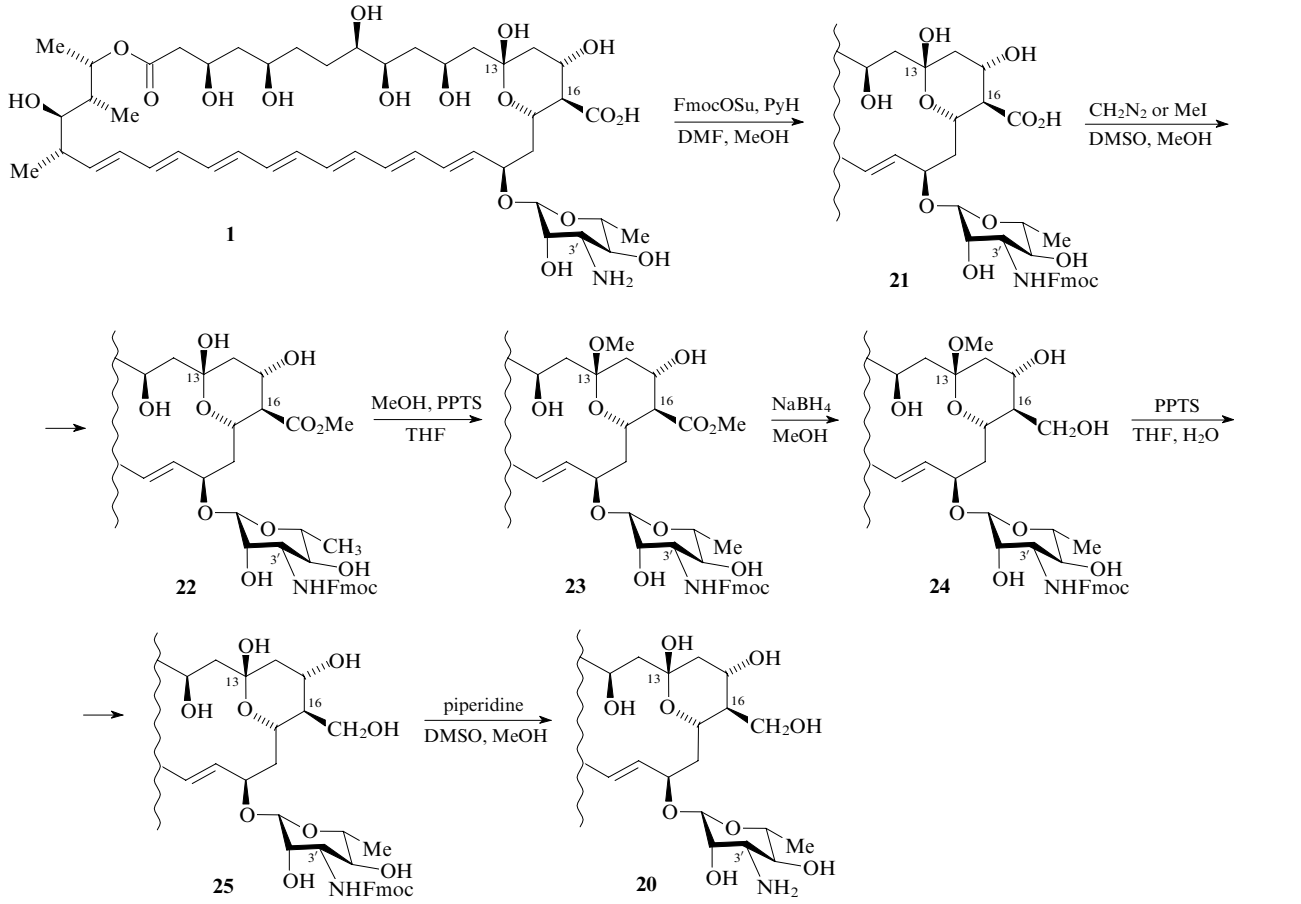
C(13)-Alkoxy derivatives **29** were synthesized by the reaction of N-protected AmB allyl ester **30** with the corre-

Scheme 2



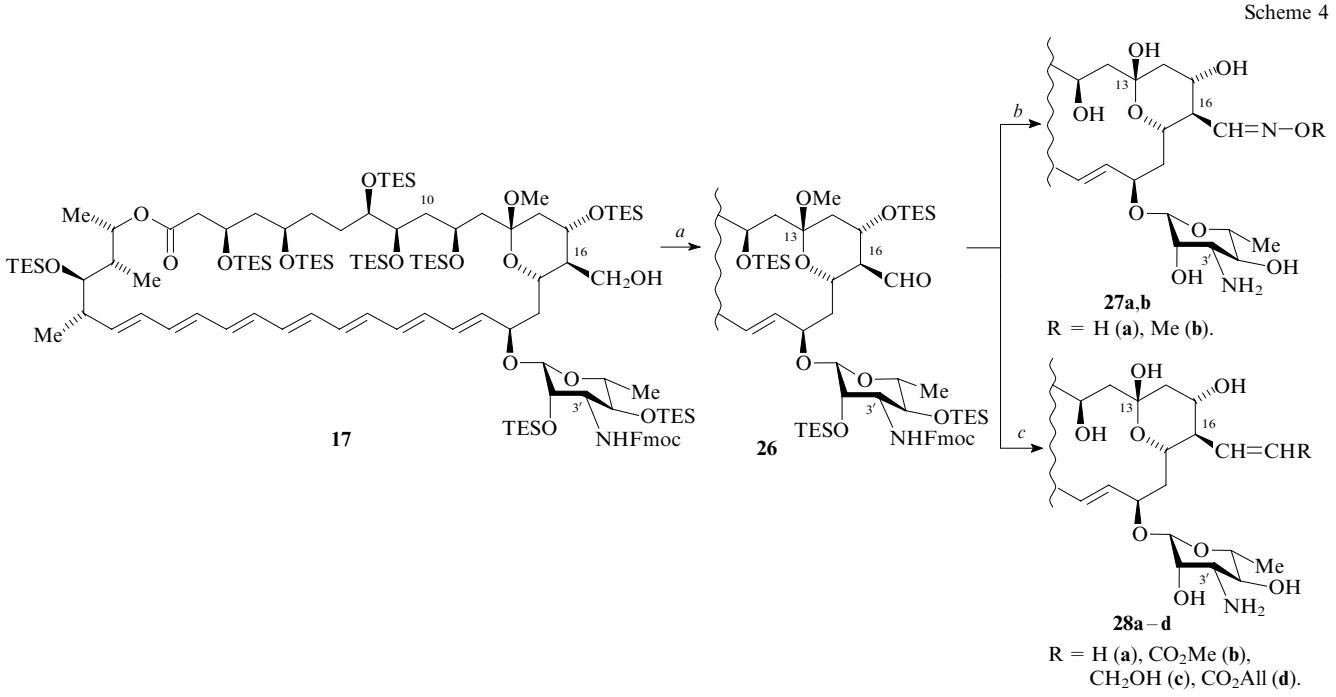
(*a*) 2-PySC(O)Cl, Et_3N, Et_2O, 0 °C, 30 min; (*b*) $LiBH_4$, Et_2O, 23 °C, 2 h; (*c*) 1) I_2, PPh_3, imidazole, THF, 0 °C, 6 h; 2) HF–PyH, THF–PyH (5 : 3), 0 → 23 °C, 6.5 h; (*d*) $NaBH_4$, DMSO, 23 °C, 8 h; (*e*) 1) CSA, THF–H_2O (2 : 1), 23 °C, 30 min; 2) piperidine, DMSO–MeOH (4 : 1), 23 °C, 3 h; 3) HPLC; Fmoc is 9-fluorenylmethoxycarbonyl, CSA is camphorsulfonic acid.

Scheme 3



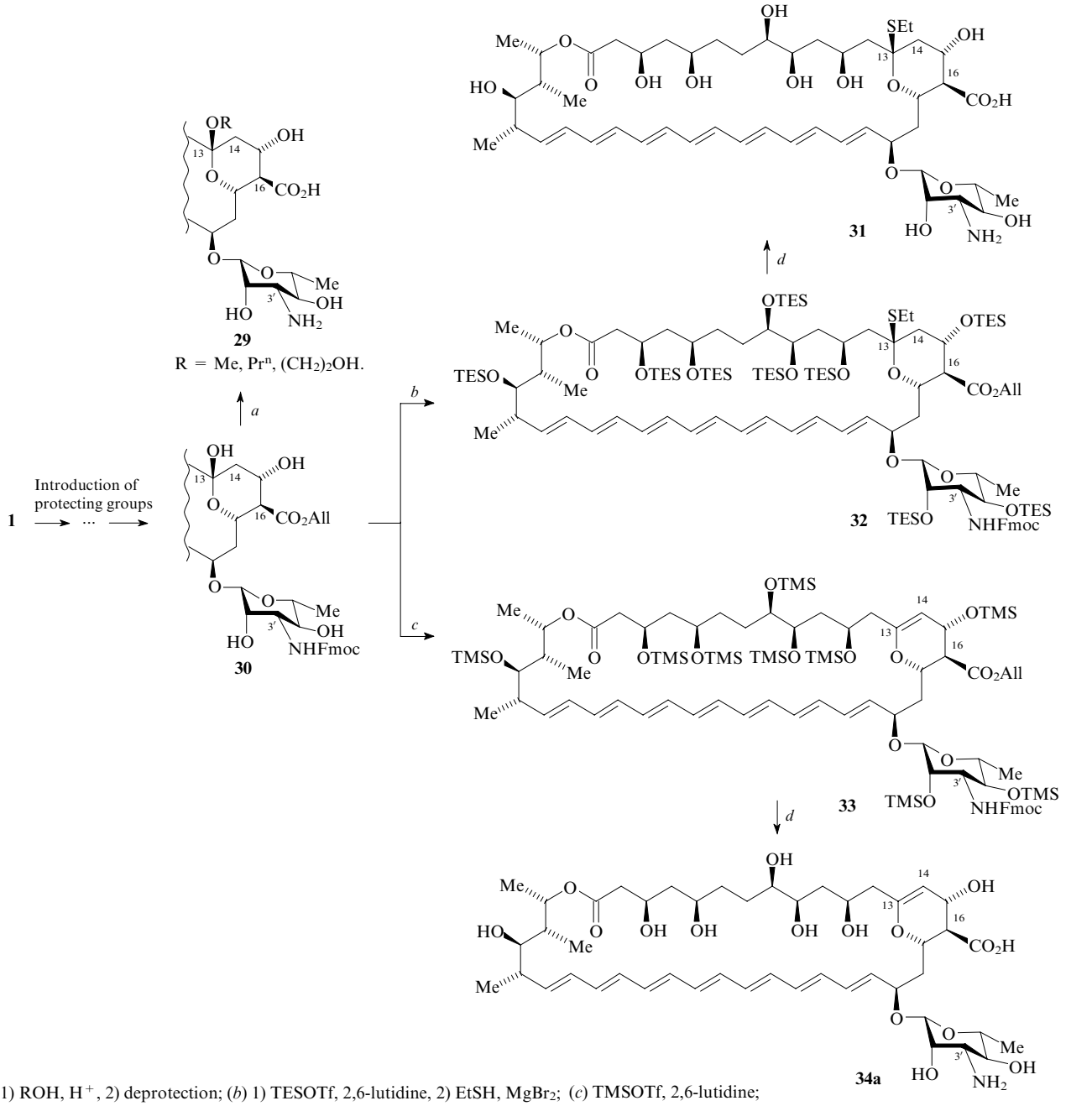
FmocOSu is *N*-(9-fluorenylmethoxycarbonyloxy)succinimide.

Scheme 4



(*a*) Swern oxidation; (*b*) 1) $NH_2OR \cdot HCl$ (R = H, Me), 2) deprotection; (*c*) 1) Wittig reaction, 2) deprotection.

Scheme 5



(*a*) 1) ROH, H^+, 2) deprotection; (*b*) 1) TESOTf, 2,6-lutidine, 2) EtSH, $MgBr_2$; (*c*) TMSOTf, 2,6-lutidine; (*d*) HF – PyH or piperidine, or pyrrolidine – $(Ph_2P)_4Pd$.

sponding alcohol in an acidic medium followed by the deprotection (Scheme 5).[26]

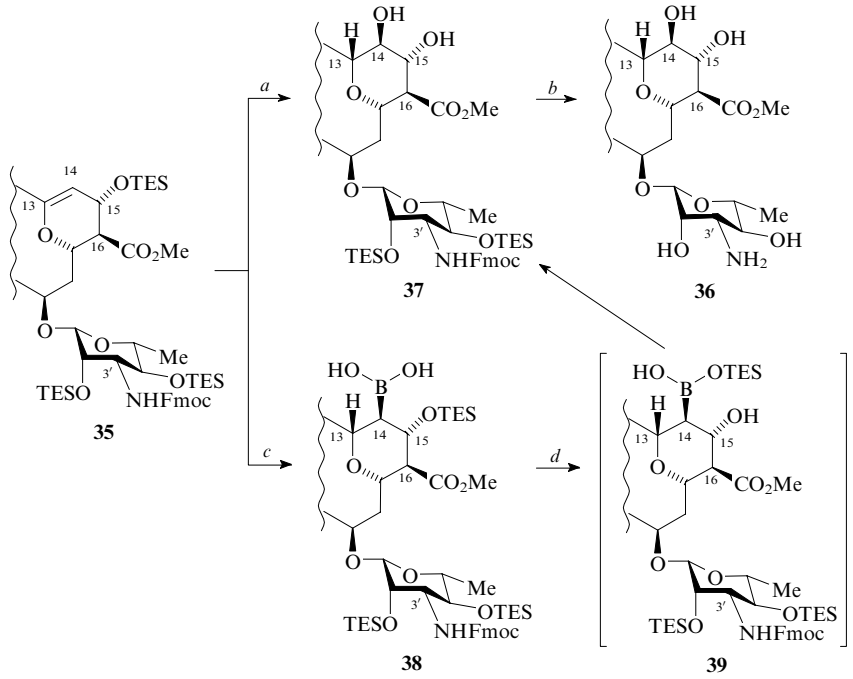
Compounds **29** proved to be much less toxic than the starting antibiotic **1** and are easily soluble in water ($\geqslant$30 mg ml^{-1} for R = OMe). However, the antifungal activity of these compounds was two times lower than that of AmB.

To prevent *in vivo* hydrolysis of the C(13)-alkoxy group to the hydroxy group catalyzed by hydrolytic enzymes, more stable hemithioacetal **31** was synthesized (see Scheme 5). For this purpose, all hydroxy groups in intermediate compound **30** were subjected to the silylation with TESOTf, and the hemiketal hydroxy group was replaced by the ethylthioacetal group in the reaction with an excess of ethanethiol in the presence of solid $MgBr_2$. The subsequent deprotection of fully protected C(13)-ethylthio derivative **32** afforded target compound **31** in 74% yield. However, contrary to expectations, the replacement of the C(13)-alkoxy group by the thioethyl group was accompanied by a substantial decrease in the activity.[27]

In the case of the trimethylsilyl protection of hydroxy groups [with the use of trimethylsilyl trifluoromethanesulfonate (TMSOTf) instead of the triethylsilyl derivative], the dehydration at positions 13 and 14 was the major process. The deprotection of dehydration product **33** afforded low-toxic 13-deoxy-13,14-didehydroamphotericin B (**34a**) (see Scheme 5) having weak antifungal activity.[26]

13-Deoxy-14(*S*)-hydroxyamphotericin B methyl ester (**36**) was synthesized starting from fully protected per-*O*-triethylsilyl-*N*-Fmoc-13-deoxy-13,14-didehydroamphoteri-

Scheme 6



(*a*) 1) BH_3, THF; 2) $NaBO_3 \cdot 4H_2O$, KOH, THF, H_2O; (*b*) deprotection; (*c*) 1) BH_3, THF; 2) H_2O; (*d*) $NaBO_3 \cdot 4H_2O$, KOH, THF, H_2O.

cin B methyl ester (**35**) (Scheme 6).[28] The transformation involved the stereospecific hydroboration of compound **35**, the oxidation by sodium peroxoborate in an alkaline medium and the deprotection. However, the total yield of protected intermediate **37** was only ~20% although all subsequent deblocking reactions gave products in quatitative yields.

To determine whether the hydroboration or oxidation is the rate-limiting step, the hydroboration product was subjected to hydrolysis. As a result, the corresponding boronic acid derivative **38** was prepared in 17% yield. Although the reaction conditions were varied, only compounds **37** and **38** were isolated. The low yield of the hydroboration product was attributed to the competitive reaction of the *in situ* formed intermediate borane **39** (or alkylboranes) with the polyene moiety of the antibiotic molecule. The oxidation of compound **38** by sodium peroxoborate in an alkaline medium afforded the product in 80% yield and was accompanied by the migration of the triethylsilyl protecting group from position 15 to the boronic acid moiety at the C(14) atom.

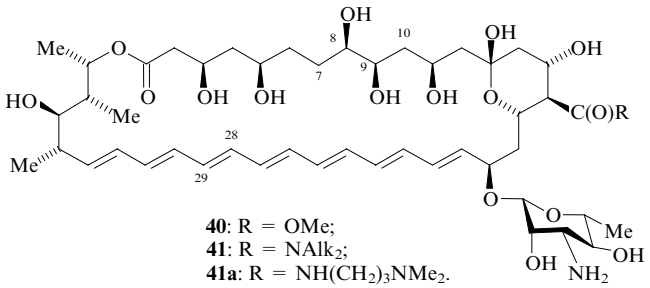
The antifungal activity of compound **36** is lower than that of AmB, but the modification resulted in the improvement of hemolytic properties.

Therefore, the functionalization of the C(13) position, as well as of the C(14) position, did not lead to a substantial improvement of the antifungal activity of the antibiotic although derivatives showing the activity comparable to that of AmB (**1**) and having lower toxicity were obtained in some cases.

It should also be taken into account that the design of new drugs by the multi-step synthesis is often not economically efficient. Hence, modifications of the carboxy and amino groups, which are generally performed in one to three steps, are of most interest for the synthesis of new derivatives of polyene antibiotics.

2. Functionalization of polyenes at the carboxy group

The esterification of the carboxyl group of compound **1** with diazoalkanes affords esters retaining the antifungal activity in high yields.[29] Among these derivatives, AmB methyl ester (**40**) is worthy of note.

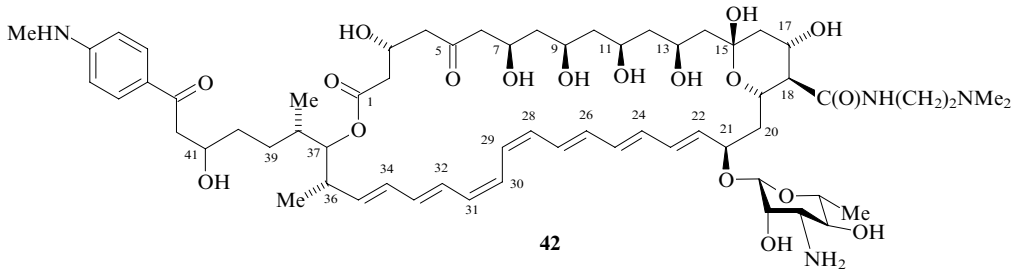


40: R = OMe;
41: R = $NAlk_2$;
41a: R = $NH(CH_2)_3NMe_2$.

Amphotericin B methyl ester hydrochloride is easily soluble in water and is less toxic in animal experiments when administered both intravenously and intraperitoneally than the starting antibiotic. However, clinical trials showed that this compound has neurotoxic effects.[21] Compound **40** was used in various subsequent transformations.

A series of aliphatic amides of AmB (**41**) was synthesized. The amides contain both the linear and branched alkyl chains consisting of 1 to 18 carbon atoms and functional substituents at the end of the alkyl group. In early studies, amides **41** have been synthesized by the azide method with the use of diphenylphosphoryl azide (DPPA) in the presence of triethylamine.[30]

It appeared that the antifungal activity of amides **41** decreases with increasing length of the alkyl substituent. Amides containing short alkyl substituents showed higher activity compared to amphotericin B in *in vitro* experiments and similar toxicity. In addition, these derivatives are poorly soluble in water. The introduction of additional



42

functional groups, for example, of amino, carboxy or dialkylamino groups, leads to a decrease in the antifungal activity. All amides **41** exhibited lower hemolytic activity than the starting AmB. Among these amides, 3-(*N*,*N*-dimethylamino)propyl amide (**41a**) is worthy of note. This compound suppresses the development of the multidrug resistance in fungi.[31]

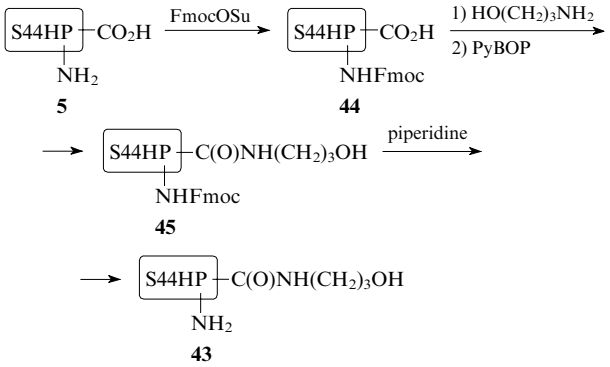
Compounds containing the carboxamide group at position 18 were synthesized from the antibiotic partricin A with the use of DPPA.[32] The *in vitro* experiments showed that derivatives containing short alkyl substituents at the amide nitrogen atom are more active than AmB but they have similar toxicity and are poorly soluble in water. Analogous compounds containing the additional amino group in the amide substituent exhibit high activity and can form water-soluble salts, but they have a relatively high toxicity. 2-(*N*,*N*-Dimethylamino)ethyl amide **42** is the most active amide of partricin A, its activity being comparable to that of amphotericin B.[33]

State-of-the-art reagents, in particular, benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP), are presently used for the amidation, resulting in the synthesis of amides in higher yields and with a higher purity. The synthesis of a series of amides of the genetically engineered polyene antibiotic S44HP (**5**) was reported.[34] Carboxamides contained various alkyl-type substituents with functional polar groups, including amino acid residues.

In some cases, an alternative procedure was used for the preparation of hydrophilic derivatives (for example, compounds **43**) because the purification of these compounds is a laborious process. Initially, the 3′-amino group of the mycosamine moiety of S44HP (**5**) was blocked by Fmoc with the use of FmocOSu. The resulting *N*-Fmoc-S44HP (**44**) was subjected to the amidation with amine hydrochloride in the presence of PyBOP. After the deprotection of compound **45**, the corresponding amide **43** was obtained with a high purity (Scheme 7).[34]

The antifungal activity of all the amides synthesized from S44HP was studied in *in vitro* experiments against yeast cultures (*Candida albicans* and *Cryptococcus humicolus*) and imperfect fungi (moulds) (*Aspergillus niger* and *Fusarium oxysporum*). 2-(*N*,*N*-Dimethylamino)ethyl amide **46a** and 3-(*N*,*N*-dimethylamino)propyl amide **46b** proved to be the most active compounds [the minimum inhibitory concentration (MIC) 1–2 μg ml^{-1}] compared to amphotericin B and starting compound **5** (as in the case of amide **41a**).

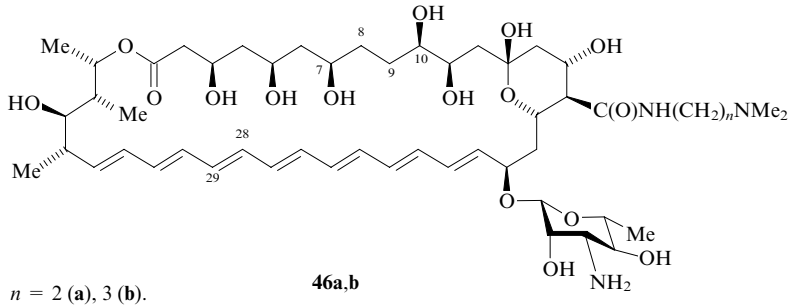
Scheme 7



The frame encloses the macrolide; the reacting groups are drawn outside the frame.

Derivative **46b** is much less toxic (LD_{50} = 16.4 mg kg^{-1}) than AmB and S44HP (2.8 and 2.2 mg kg^{-1}, respectively) and is more effective in protecting animals from mortality in a mouse model of experimental Candida sepsis.[34]

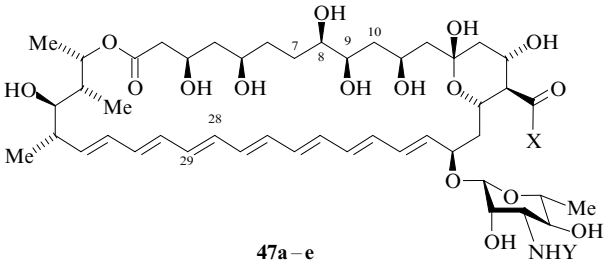
The chemotherapeutic activity of compound **46a** (in the form of the water-soluble L-glutamate salt) in experiments in immunocompromised mice as a result of the preadministration with cyclophosphamide proved to be substantially higher than the activity of AmB. The high efficacy of this agent in a model of Candida sepsis in animals with an artificially weakened immune system may be of interest for the treatment of immunocompromised patients.[35] Compound **46a** is one of the promising analogues of S44HP recommended for further investigation.



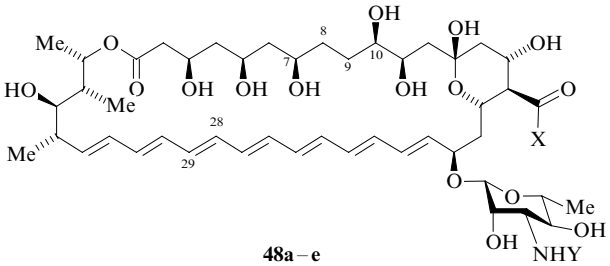
n = 2 (**a**), 3 (**b**).

46a,**b**

The study of the same-type reactions at the functional groups [the C(16)-carboxy group and the 3′-amino group] of polyene antibiotics, which differ in the arrangement of hydroxy groups, revealed the role of the polyol moiety in the demonstration of antifungal activity. Selected derivatives of the antibiotics AmB and S44HP containing identical substituents are given below. The authors compared four pairs of the synthesized carboxamides (**47a–d**, **48a–d**) and one pair of carbohydrate-containing N-alkyl derivatives (**47e**, **48e**).[34, 36]

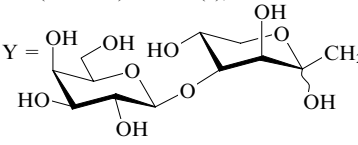


47a–e



48a–e

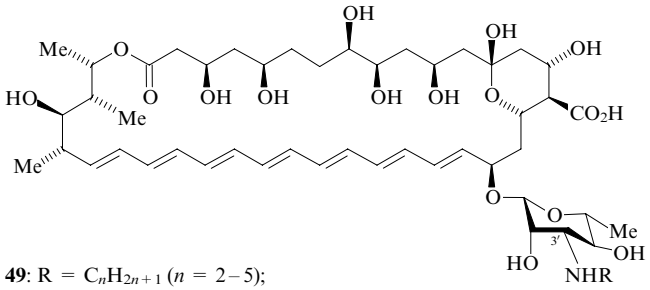
Y = H: X = $NH(CH_2)_2OH$ (**a**), NHMe (**b**), $NHCMe(CH_2OH)_2$ (**c**), (D,L)-$NHCH(CH_2OH)CO_2Me$ (**d**);

X = OH, Y =  (**e**).

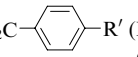
It appeared that similar type derivatives of AmB and S44HP have very similar *in vitro* antifungal activities. Thus, MIC for the strains of *C. albicans*, *C. humicolus* and *A. niger* is 2–4 μg ml^{-1}, and MIC for the strain of *F. oxysporum* is 4–8 μg ml^{-1}; however, these values are slightly lower than those of the corresponding starting antibiotics. Consequently, the differences in the structure of the polyol moiety of molecules **1** and **5** have no effect on the antifungal activity of these compounds in *in vitro* experiments. There are preliminary data that analogues of S44HP characterized by another arrangement of hydroxy groups in the polyol moiety exhibit a lower antifungal activity than compound **1** or **5**. In these cases, the additional modification of the carboxy group does not enhance the antifungal activity.[37]

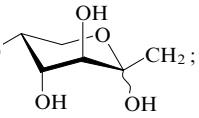
3. Functionalization of polyenes at the amino group of the mycosamine moiety

The synthesis of derivatives at the 3′-amino group of the mycosamine moiety is an important trend of chemical modifications of polyenes. N-Alkyl derivatives of AmB (**49**) containing the alkyl chain (with a length from C_2 to C_5) as the substituent were synthesized by reductive alkylation. The activity and toxicity of the derivatives decrease with increasing length of the hydrocarbon chain.[21]



49: R = C_nH_{2n+1} (n = 2–5);

50: R = H_2C––R′ (R′ = H, Cl, Br, I, OH, NO_2, OMe, OEt, NMe_2, Me, Et);

51: R = ;

52: R = $CH(HP(O)OH)C_6H_4R'$-4 (R′ = H, OH, OMe, NO_2, NMe_2);

53: R = C(O)R′ (R′ = Me, Et , *etc.*).

N-Benzyl derivatives of AmB (**50**) were synthesized by a similar procedure and were tested for the antifungal activity.[38]

For these compounds, the acute toxicity and the antifungal activity against six strains of the yeast-like fungi *Candida*, as well as against causative agents of blastomycosis, aspergillosis, mould mycosis, trichomycosis, and some other pathogens, were determined. It was found that analogues **50** are three times less toxic than the starting antibiotic **1**. Compounds containing halogen atoms (chlorine, bromine or iodine) in the *para* position of the phenyl ring exhibit the highest activity comparable to the activity of AmB. Compounds containing alkyl substituents in the *para* position of the phenyl ring or containing the unsubstituted ring show the lowest activity.

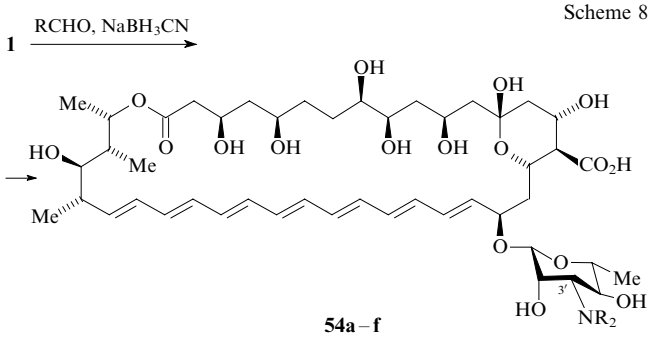
The reactions of polyenes with various mono- and disaccharides in DMF at 37 °C afford 1-deoxy-1-ulose derivatives in quantitative yields as a result of the Amadori rearrangement. These products retain the antifungal activity against *Saccharomyces cerevisiae*, have low toxicity in mice with candidiasis and exhibit low hemolytic activity.[20] The product of the reaction of AmB with glucose (**51**) shows the highest activity compared to other related derivatives.

Derivatives **52** were synthesized by the reactions of compound **1** with aromatic aldehydes and hypophosphorous acid. These compounds have low toxicity and exhibit high antifungal activity comparable to that of the starting antibiotic. In addition, these compounds exhibit pronounced antiviral activity against the influenza A and B viruses, the smallpox virus, and the Rous sarcoma retrovirus.[39]

The acylation of AmB at position 3′ afforded N-acyl derivatives **53**, whose activity is substantially lower than that of the starting compound **1**. The importance of the amino function of the mycosamine moiety for the antifungal activity was confirmed by the study of aminoacyl analogues of AmB at the 3′-amino group. In particular, the aminoacylation with amino acids by the anhydride method gave a series of *N*-aminoacyl derivatives of AmB, which exhibit activity comparable to that of the starting antibiotic.[40]

The preparation of N,N-bisalkyl derivatives containing various hydrophilic functional groups is one of the new trends in the synthesis of AmB analogues. The reductive

Scheme 8



R = $(CH_2)_2NH_2$ (**a**), $(CH_2)_3NH_2$ (**b**), $(CH_2)_3OH$ (**c**), $CH_2CH(NH_2)(CH_2)_4NH_2$ (**d**), $(CH_2)_3CO_2Me$ (**e**), $(CH_2)_3CO_2H$ (**f**).

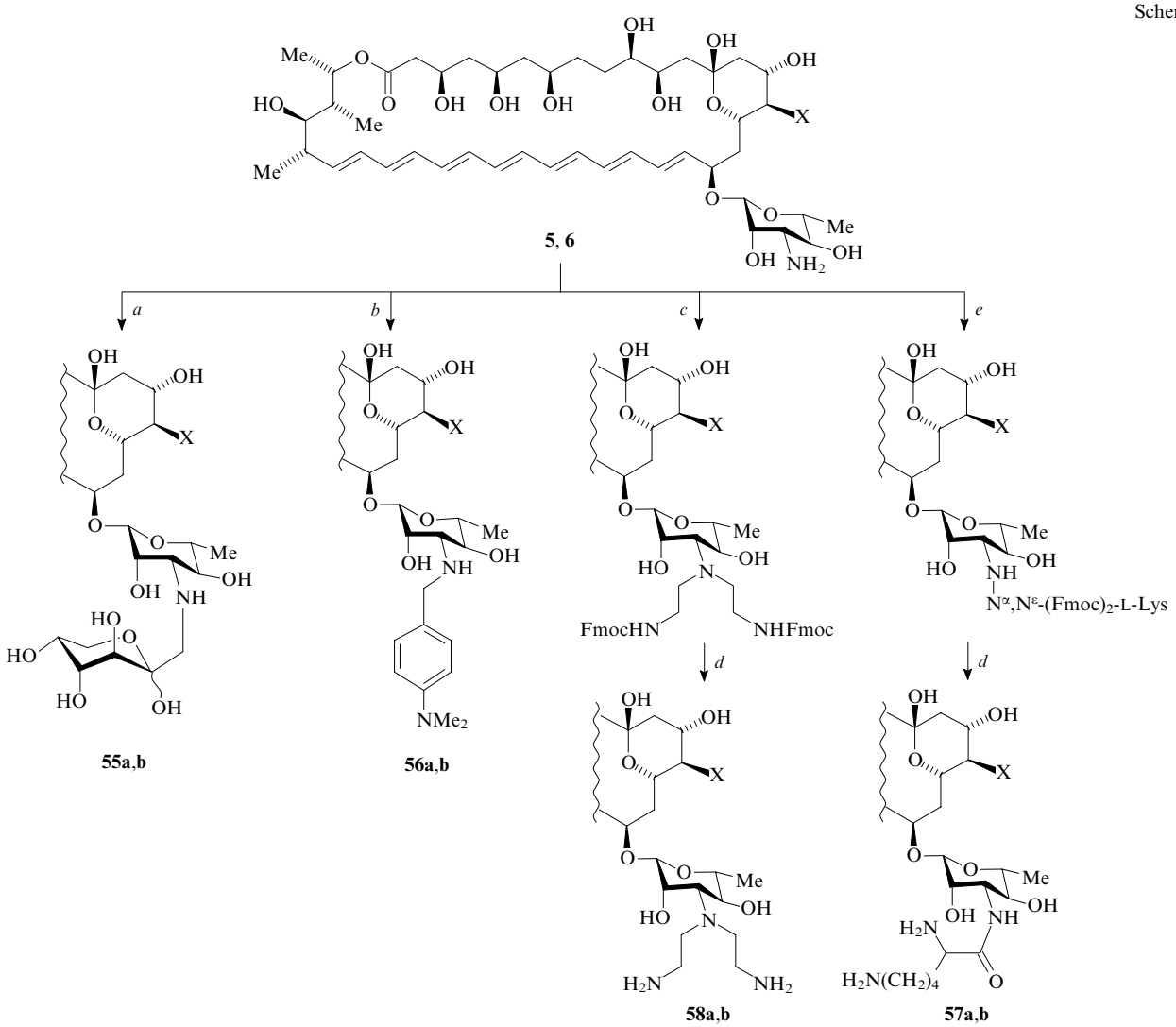
alkylation of AmB with the corresponding aldehydes in the presence of $NaBH_3CN$ gave derivatives **54a–f** (Scheme 8).[41] In the case of compounds **54a**,**b**,**d**, the amino groups were protected with the use of the Fmoc group, which was removed in the last step.

Bis(3-aminopropyl) derivative **54b** proved to be most active. The antifungal activity of **54b** (MIC = 0.020 μmol litre^{-1}) is higher than the activity of AmB (0.3 μmol litre^{-1}) against the wild strain of *Saccharomyces cerevisiae*, as well as against the strain of *Candida albicans* resistant to compound **1**. Disubstituted analogues were synthesized based on derivative **54b** (see Section III.4).[41]

Semisynthetic derivatives at the 3′-amino group of the mycosamine moiety of the genetically engineered polyenes S44HP[34,35] and BSG005[42] were documented (Scheme 9). These antibiotics differ only in the nature of the substituent at the C(16) atom. Consequently, investigations of the structure–activity relationship for derivatives containing identical substituents at the 3′-amino group of the mycosamine moiety provide an insight into the effect of the substituent at the C(16) atom. The synthesis of these compounds involves the Amadori rearrangement, the reductive N-alkylation and the N,N-bis-alkylation, as well as the N-aminoacylation.

It appeared that the antifungal activity of S44HP derivatives in *in vitro* experiments differs from the activity of the related BSG005 derivatives in spite of the fact that the starting antibiotics **5** and **6** exhibit similar activities (Table 1).[42] N-D-Fructopyranosyl derivatives **55a**,**b** show approximately equal activity against four strains under

Scheme 9



X = CO_2H (**a**), Me (**b**); (*a*) D-glucose; (*b*) 4-$Me_2NC_6H_4CHO$, $NaBH_3CN$; (*c*) $FmocNH(CH_2)_2CHO$, $NaBH_3CN$; (*d*) piperidine, DMSO; (*e*) N^{α},N^{ε}-$(Fmoc)_2$-L-LysOSu, Et_3N, pH 7, DMSO.

Table 1. Activities (MIC, μg ml^{-1}) of the starting antibiotics (**1**, **5**, **6**) and derivatives of S44HP (**55a**–**58a**) and BSG005 (**55b**–**58b**).

Compound	Strains			
	Candida albicans ATCC 14053	*Cryptococcus humicolus* ATCC 9949	*Aspergillus niger* ATCC 16404	*Fusarium oxysporum* VKM F-140
1	1	1	1	4
5	1	1	1	4
6	1	1	2	2
55a	1	1	2	8
55b	2	2	2	8
56a	2	2	4	>16
56b	16	>16	>16	>16
57a	8	8	16	>16
57b	1	1	1	2
58a	1	1	1	1
58b	4	8	8	8

examination. Compound **56a** containing the amino group in the aromatic ring proved to be less active than compounds **55a**,**b**; related derivative **56b** was completely inactive. *N*-L-Lysine derivative **57b** is the most active compound in this series (MIC = 1–2 μg kg^{-1}), whereas related compound **57a** exhibits relatively low activity (MIC = 8–16 μg ml^{-1}). The reverse situation was observed in the case of N-alkyl derivatives. Thus, the N,N-bisalkyl derivative of BSG005 (**58b**) is much less active (MIC = 4–8 μg ml^{-1}) than the S44HP analogue (**58a**) (MIC = 1–2 μg ml^{-1}).

These results suggest that the activity of the antibiotic depends not only on the structure of the substituents at the amino group but also on the nature of the substituent at the C(16) atom. Therefore, similar transformations of antibiotics having similar antifungal activities can give different results.

4. Double functionalization of polyenes

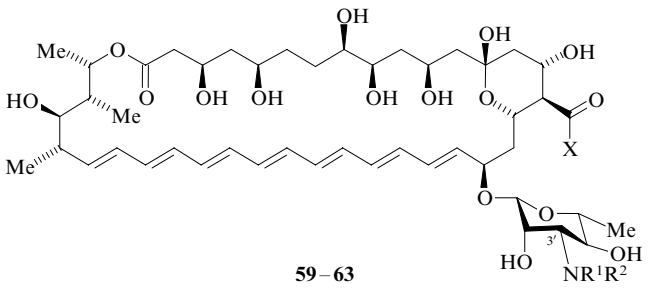
In some cases, the modification of polyene antibiotics at two positions leads to the improvement of biological properties. In most cases, methyl esters are prepared by the functionalization of the carboxy group at position 16 because this transformation leads to a decrease in the hemolytic activity.[43] Moreover, the additional esterification of the C(16)-carboxy group of N-acyl derivatives does not lead to changes in their activity. For example, *N*-(3-piperidinopropionyl)amphotericin B methyl ester (**59**) exhibits high antifungal activity and has low toxicity.[44]

The secondary modification of the C(16)-carboxy group in compound **54b** was performed. The resulting methyl ester **60** and aminoethyl amide **61** show the relatively high antifungal activity compared to compound **1**. However, this transformation does not lead to the improvement of the activity of the starting monosubstituted antibiotic **54b**. The minimum inhibitory concentrations (in μmol litre^{-1}) for these compounds against two strains are given below:

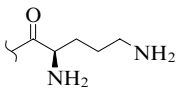
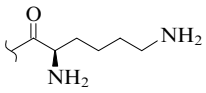
Compound	*C. albicans* (BY4741)	*S. cerevisiae* (DYS1764)
1	0.30	>50
54b	0.020	1.0
60	0.10	4.0
61	0.040	4.0

Methyl esters of *N*-methyl-*N*-(1-deoxy-2-ketosyl) derivatives of polyene macrolides were synthesized. Among these compounds, the water-soluble salt, *N*-methyl-*N*-(1-deoxy-D-fructopyranos-1-yl)amphotericin B methyl ester L-aspartate (**62**), is worthy of note. This salt was synthesized by methylation of derivative **51** with diazomethane. Although the activity of compound **62** is somewhat lower than that of the starting antibiotic [IC_{50} (see †) against *C. albicans* is 0.15 μg ml^{-1} *versus* 0.07 μg ml^{-1} for AmB], the hemolytic activity against human erythrocytes is substantially lower. In addition, the acute toxicity of compound **62** in experiments in mice was higher than 400 μg kg^{-1}, which is substantially higher than the corresponding dose for AmB (LD_{50} = 6 μg kg^{-1}).[45] Compound **62** is active also against the resistant strain of *Saccharomyces cerevisiae*.[46]

N-Aminoacyl analogues of AmB esters (**63**) containing an additional amino group were synthesized with the use of various α- and β-amino acids. The condensation was carried out by the activated ester method with the use of N-Fmoc-protected amino acids. The deprotection was carried out with an equimolar amount of 1,5-diazabicyclo[4.3.0]non-5-ene (DBN).[47] Among N-aminoacyl derivatives of amino acids of AmB, *N*-D-ornityl- (**63a**) and *N*-D-lysylamphotericin B (**63b**) methyl esters show the *in vitro* and *in vivo* antifungal activity comparable to that of compound **1**.

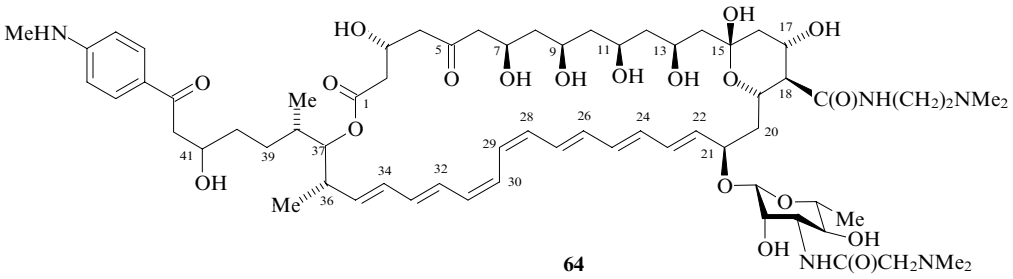


59–**63**

Compound	R^1	R^2	X
59	$C(O)(CH_2)_2N(CH_2)_5$	H	OMe
60	$(CH_2)_3NH_2$	H	OMe
61	$(CH_2)_3NH_2$	H	$NH(CH_2)_2NH_2$
62	1-deoxy-D-fructopyranos-1-yl	Me	OMe
63	α-(or β)-amino acid residue	H	OMe, $CH_2C{\equiv}CH$, $CH_2C{\equiv}CPh$
63a		H	OMe
63b		H	OMe

The double chemical modification of partricin A (**3**) afforded a series of derivatives at the C(19)-carboxy group and at the 3′-amino group of mycosamine. The amidation of the carboxy group was carried out in the presence of DPPA, and the amino group was modified with the use of activated amino acid esters.[32] The antifungal activity of the resulting compounds is lower than that of monosubstituted analogues, but these compounds are more active than AmB

† IC_{50} is the concentration of the agent necessary for 50% inhibition of the growth of test organisms.



64

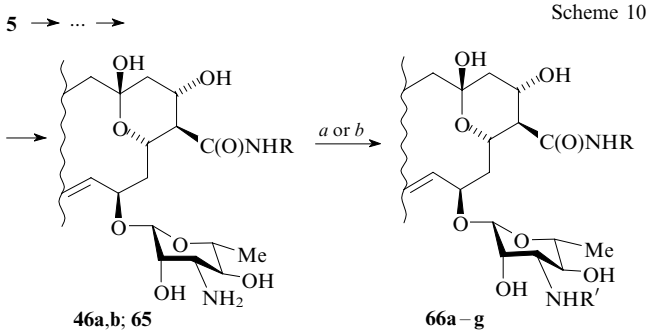
and can form water-soluble salts. The 3′-*N*,*N*-dimethylaminoacetyl derivative of partricin A *N*-dimethylaminoethyl amide (**64**) was studied in more detail.

Water-soluble salts of compound **64**, *viz*., diaspartate (SPA-s-753) and diascorbate (SPK-843), were studied in 116 strains of the fungi *Candida*, *Cryptococcus* and *Saccharomyces*. The salt SPK-843 exhibits higher antifungal activity against all these strains than compound **1**.[48] The detailed study of water-soluble salts of derivative **64** in *in vivo* experiments showed that the toxicity of these compounds is substantially lower and their therapeutic indices are enhanced compared to the corresponding parameters of AmB. The semisynthetic water-soluble salt SPK-843 is currently under clinical trials.[49]

Doubly modified derivatives of the genetically engineered polyene macrolide antibiotic S44HP (**5**) protected both at the C(16)-carboxy group and the 3′-amino group of mycosamine were synthesized.[35] The functionalization was carried out with the use of two synthetic approaches. The choice of the synthetic routes was determined by the problems with the isolation of the target products and/or intermediates. The chromatographic purification of the products in the form of hydrophobic Fmoc-protected compounds proved to be the technique of choice.

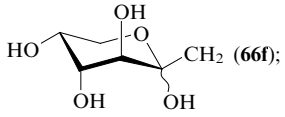
Most of the derivatives were synthesized from carboxamides, which were transformed into the corresponding 3′-N-acyl or 3′-N-alkyl compounds (Scheme 10).

Scheme 10

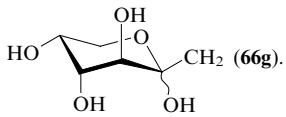


46a,**b**; **65** **66a**–**g**

(*a*) 1) PyBOP, $Me_2NCH_2CO_2H$ or N^{α},N^{ε}-$(Fmoc)_2$-L-Lys;
(*b*) D-glucose or 4-$Me_2NC_6H_4CHO$, $NaBH_3CN$;
R = $(CH_2)_2NMe_2$ (**46a**): R′ = $C(O)CH_2NMe_2$ (**66a**),
$CH_2C_6H_4NMe_2$-4 (**66d**),



(**66f**);
R = $(CH_2)_3NMe_2$ (**46b**): R′ = $C(O)CH(NH_2)(CH_2)_4NH_2$ (**66c**);
R = $(CH_2)_3OH$ (**65**): R′ = $C(O)CH_2NMe_2$ (**66b**), $CH_2C_6H_4NMe_2$-4 (**66e**),

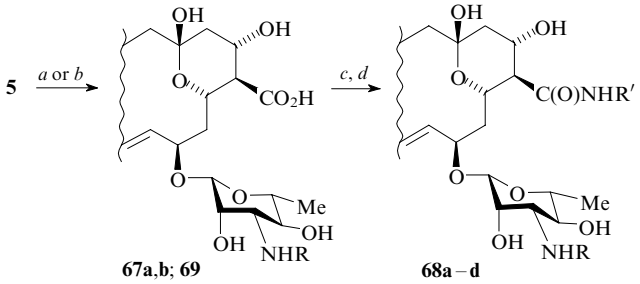


(**66g**).

The synthesis of carboxamides **46a**,**b** and **65** involved the following three steps: the protection of the 3′-amino group, the amidation of the C(16)-carboxy group followed by the chromatographic purification and the deprotection. Then the 3′-amino group of the mycosamine moiety in carboxamides **46a**,**b** and **65** was acylated with *N*,*N*-dimethylglycine or N^{α},N^{ε}-$(Fmoc)_2$-L-lysine to obtain finally compounds **66a**–**c**. In addition, the alkylation of amides **46a** and **65** with sugars (Amadori rearrangement) or *p*-dimethylaminobenzaldehyde in the presence of $NaBH_3CN$ afforded derivatives **66d**–**g**.

Some doubly substituted derivatives of S44HP were synthesized from 3′-N-acyl derivatives **67a**,**b** (Scheme 11).[35]

Scheme 11



67a,**b**; **69** **68a**–**d**

d: **67a**: R = $C(O)C_6H_4CH_2NHFmoc$-4; **67b**: R = $C(O)CH(NHFmoc)(CH_2)_4NHFmoc$; **69**: R = $C(O)C_6H_4CH_2NH_2$-4.

R = $C(O)C_6H_4CH_2NH_2$-4: R′ = $(CH_2)_2NMe_2$ (**68a**), $(CH_2)_3OH$ (**68b**);
R = $C(O)CH(NH_2)(CH_2)_4NH_2$: R′ = $(CH_2)_2NMe_2$ (**68c**),
$(CH_2)_3OH$ (**68d**); (*a*) 4-FmocNHCH$_2$C$_6$H$_4$CO$_2$H, PyBOP, Et_3N;
(*b*) N^{α},N^{e}-$(Fmoc)_2$-L-Lys, DCC, HOBt, Pr^{i}_2NEt; (*c*) $R'NH_2 \cdot HCl$, PyBOP, Et_3N; (*d*) piperidine, DMSO, MeOH; DCC is dicyclohexylcarbodiimide, HOBT is 1-hydroxybenzotriazole.

The amino group of antibiotic **5** was initially acylated with N^{α},N^{ε}-$(Fmoc)_2$-L-lysine or *N*-Fmoc-*p*-aminomethylbenzoic acid. Then the C(16)-carboxy group was subjected to the amidation with the corresponding amines followed by the chromatographic purification and the deprotection to prepare disubstituted analogues of S44HP, *viz*., compounds **68a**–**d**.[35]

The study of the antifungal activity of the resulting compounds showed that the antifungal activities of amide **46a** and its N-derivative **66f** in *in vitro* tests using four cultures of microorganisms (the strains are listed in Table 1) are higher than those of the starting compounds **1** and **5**. In addition, the *in vivo* experiments showed that derivatives **46a** and **66f** are 10 times less toxic than AmB. The study of the antifungal activity in a mouse model of disseminated candidiasis also showed high efficacy of these derivatives compared to compound **1**.[35] For these compounds, the ratio of the effective dose to the maximum tolerated dose is 2% and 6%, respectively, whereas this ratio for AmB is 62%. It

should be noted that the introduction of additional substituents into the amino group at position 3′ of amides **46a**,**b** and **65** or the amidation of the C(16)-carboxy group in N-aminomethylbenzoyl derivative **69** did not lead to significant changes in the activity. By contrast, the double modification of *N*-L-lysyl-S44HP (**57a**) (MIC ≈ 8–16 μg ml^{-1}) results in a substantial improvement of the activity of its derivatives **68c**–**d** against all four strains of the fungi under study (MIC ≈ 1–4 μg ml^{-1}). Double modified derivative **66f** exhibits higher *in vivo* activity in a mouse model of Candida sepsis than that of AmB but it is not higher than the activity of the starting monosubstituted amide **46a**.[35]

5. Fluorine-containing analogues of amphotericin B

The introduction of the fluorine atom into the antibiotic molecules makes it possible to estimate the interaction of the antibiotic with a target at the molecular level using the special NMR technique, the rotational-echo double resonance (REDOR), in the solid state. For this purpose, AmB derivatives containing ^{19}F atoms in the hemiketal ring [at the C(14) atom] (**70**, **71**),[50] in the substituent at the amino group of the mycosamine moiety (**72**),[50] and in the polyene moiety of the molecules (position 28) (**73**)[51] were synthesized.

The fluorine atom was introduced at position 14 with the use of the electrophilic fluorinating agent Selectfluor® [1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate)] (Scheme 12).

It should be noted that the fluorination of 13-deoxy-13,14-didehydro derivative **34b** afforded almost exclusively product **70** containing the F atom in the equatorial position. N-Fluorobenzyl derivative **72** was synthesized by the reductive N-alkylation with *p*-fluorobenzaldehyde and $NaBH_3CN$. The reaction was carried out at room temperature during two days.

The introduction of the fluorine atom into the polyene moiety of the antibiotic molecule was the most difficult problem. The strategy of the introduction of the fluorine atom at position 28 is based on the combination of the degradation of the natural antibiotic and the chemical synthesis.[51] Scheme 13 presents the retrosynthetic analysis of compound **73**, *viz*., fragments **74** and **75**.

The total synthesis of AmB performed by several research teams was considered in detail in the review.[6]

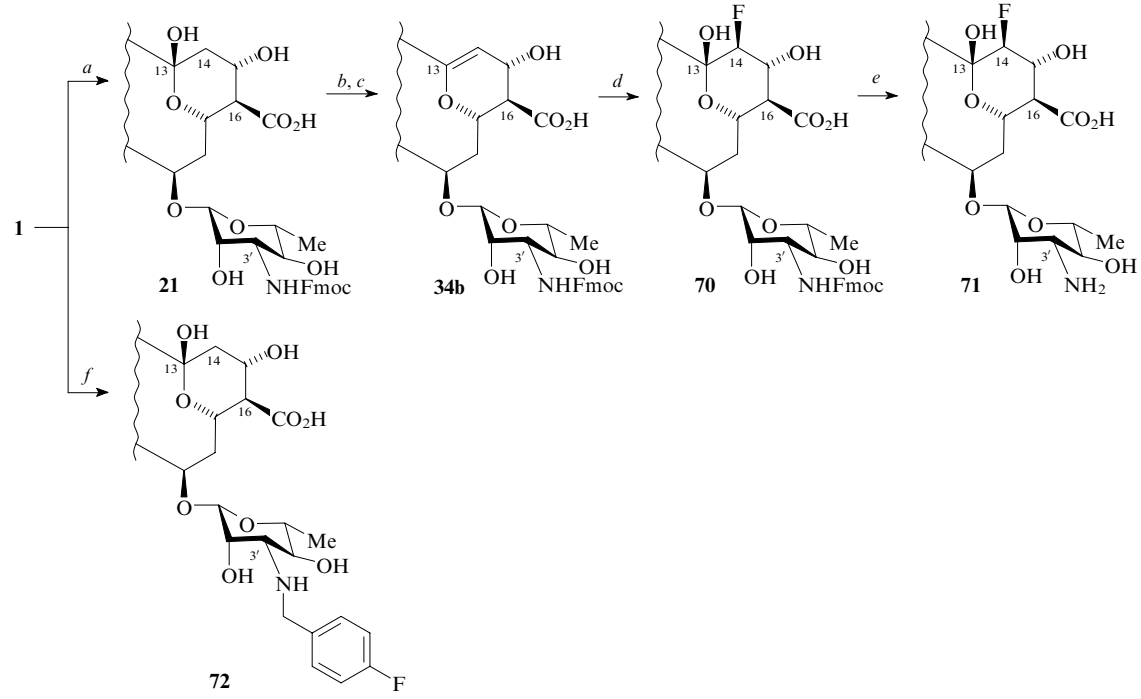
The C(1)–C(21) fragment (**74**) was prepared by the degradation of AmB (Scheme 14).

Initially, all reactive groups in AmB were successively blocked (step *a*). Then the polyene moiety was cut out by the exhaustive ozonolysis to form dialdehyde. The subsequent condensation of compound **76** with triiodomethane afforded the corresponding bis(iodovinyl) derivative **77**. The selective saponification of the ester group with LiOH gave the target C(1)–C(21) fragment **74**.

The heptaene region of the molecule [C(22)–C(37) fragment (**75**)] was constructed in the 12-step synthesis involving the Stille and Horner–Wadsworth–Emmons reactions (Scheme 15).

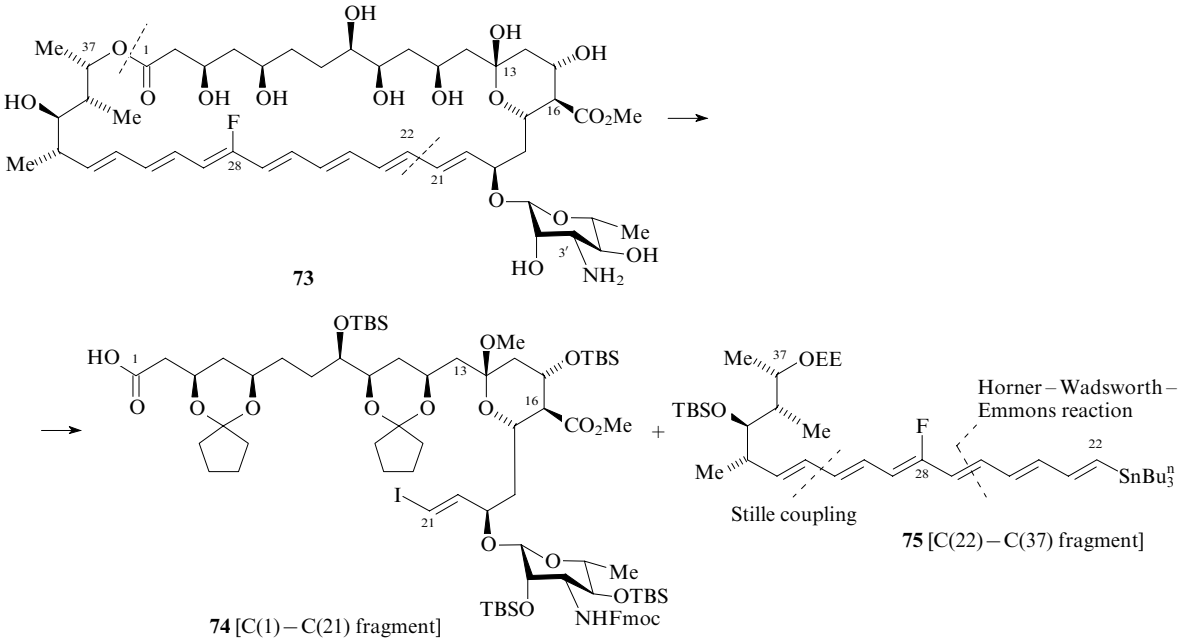
Hydroxy-δ-lactone **78** was used as the starting compound, in which the hydroxy group was protected (step *a*, compound **79**). The lactone ring cleavage giving Weinreb amide (**80**) (step *b*) followed by the reduction of protected amide **81** with diisobutylaluminium hydride (DIBAL) and the treatment of the resulting aldehyde with the $TMSCHN_2$–Bu^nLi system (step *d*) gave alkyne **82**. The hydrostannylation in the presence of the palladium complex and the replacement of tributylstannyl by the iodine atom (step *e*) afforded iodine-containing derivative **83**. Under these reaction conditions, the ethoxyethyl (EE) protecting

Scheme 12

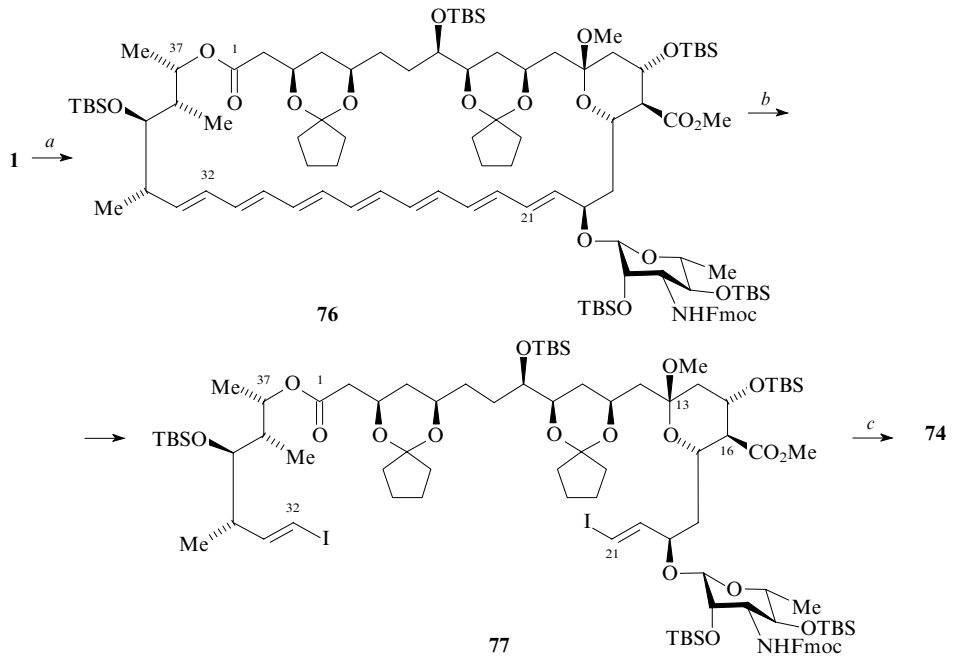


(*a*) FmocOSu, DMF, PyH; (*b*) TMSOTf, 2,6-lutidine, CH_2Cl_2; (*c*) HF–PyH, THF; (*d*) Selectfluor®, DMF–H_2O; (*e*) piperidine, DMSO–MeOH (4:1); (*f*) 4-FC_6H_4CHO, $NaBH_3CN$, DMF–MeOH (10:7).

Scheme 13



Scheme 14



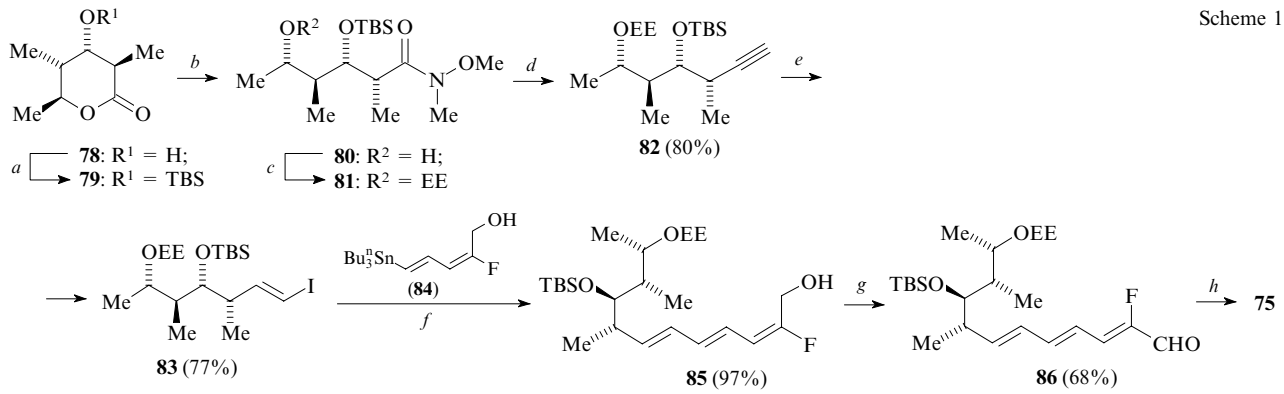
(*a*) 1) FmocOSu, PyH, DMF, 20 °C, 14.5 h; 2) MeI, Na_2CO_3, DMF, 20 °C, 3.5 h (yield in two steps is 79%); 3) cyclopentanone dimethyl acetal, CSA, MeOH, 30 min; 4) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 50 min (57% yield); (*b*) 1) O_3, −78 °C; 2) PPh_3, 20 °C, 14 h (57% yield); 3) $CrCl_2$, CHI_3, THF, 20 °C, 18 h (65% yield); (*c*) 1) LiOH, THF, H_2O, MeOH, 20 °C, 24 h; 2) FmocOSu, PyH, DMF, 20 °C, 11 h (50% yield); TBS is tri-n-butylsilyl.

group was partially removed, thus requiring the repeated treatment of the reaction mixture with ethyl vinyl ether. The fluorine-containing moiety was introduced into the molecule with the use of fluorinated alkadienylstannane **84**. The Stille condensation of compounds **83** and **84** (step *f*) afforded the *E* isomer of fluorinated compound **85**. The subsequent oxidation of compound **85** with the Dess – Martin reagent, the isomerization induced by diphenyl diselenide and radiation with a tungsten lamp (step *g*) gave fluorinated aldehyde **86** exclusively as the *Z* isomer. The subsequent Horner – Wadsworth – Emmons condensation of aldehyde **86** with stannyl phosphonate **87** afforded the target fluorinated polyene moiety C(22) – C(37) (**75**).

The condensation of the C(1)-C(21) (**74**) and C(22) – C(37) (**75**) moieties was carried out according to Scheme 16.

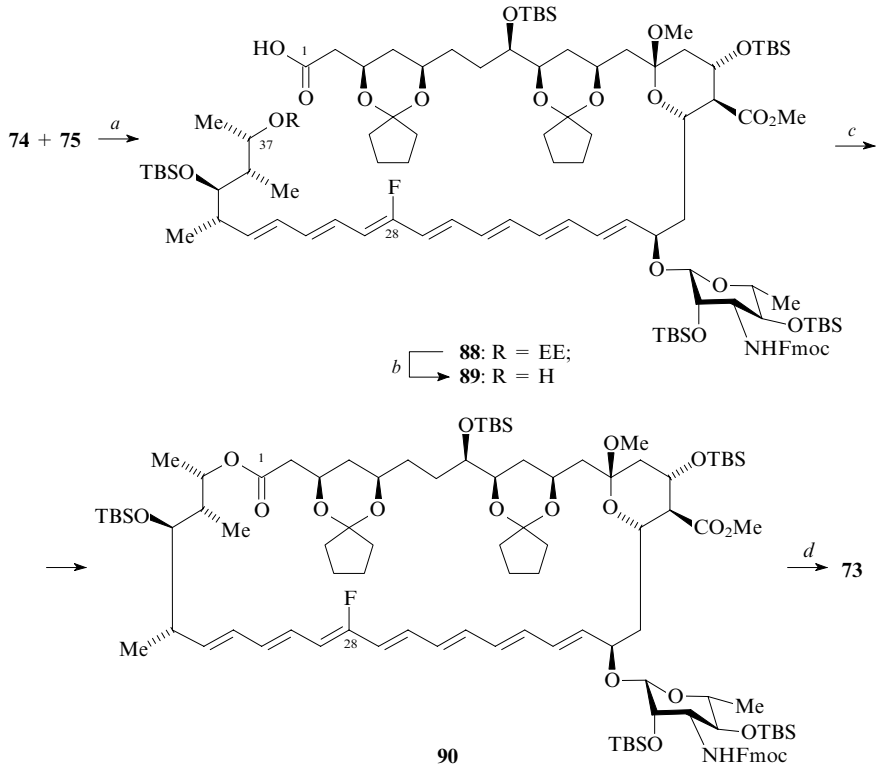
The reaction of iodide **74** with stannane **75** in the presence of the palladium complex (step *a*) gave heptaene **88** in 70% yield. The subsequent cyclization of compound **89** was performed according to the Shiina method (step *c*). Fluorinated macrolactone **90** was synthesized in 69% yield.

Scheme 15



(*a*) TBSOTf, 2,6-lutidine, CH_2Cl_2, −45 °C, 40 min (80% yield); (*b*) MeONHMe·HCl, Me_3Al, CH_2Cl_2, 20 °C, 6 h; (*c*) CH_2=CHOEt, PPTS, 20 °C, 2.5 h (90% yield); (*d*)1) DIBAL, THF, −78 °C, 50 min; 2) $TMSCHN_2$, Bu^nLi, THF, −78→0 °C; (*e*) 1) Bu^n_3SnH, CH_2Cl_2, $PdCl_2(PPh_3)_2$, THF, 0 °C, 20 min; 2) I_2, THF, 0 °C, 10 min; 3) CH_2=CHOEt, PPTS, CH_2Cl_2, 20 °C, 15 min; (*f*) $PdCl_2(MeCN)_2$, DMF, 20 °C, 1 h; (*g*) 1) Dess–Martin reagent, CH_2Cl_2, 20 °C, 30 min; 2) PhSeSePh, *hv*, CH_2Cl_2, 20 °C, 35 min; (*h*) $(MeO)_2P(O)CH_2(CH$=$CH)_2SnBu^n_3$ (**87**), LiHMDS, THF, −78 °C (84% yield); LiHMDS is lithium hexamethyldisilazide.

Scheme 16



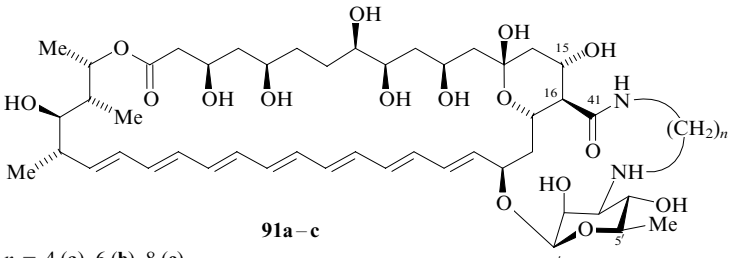
(*a*) $Pd_2(dba)_3 \cdot CHCl_3$, $AsPh_3$, Pr^i_2NEt, THF, 3 h; (*b*) PPTS, cyclopentanone dimethyl acetal, MeOH, 20 °C, 1.5 h; (*c*) 2-methyl-6-nitrobenzoic anhydride, DMAP, CH_2Cl_2, 20 °C, 1.5 h; (*d*) 1) HF–PyH, PyH, MeOH, 50 °C, 27 h (82% yield); 2) piperidine, MeOH, 20 °C, 3 h (79% yield); 3) HCl, MeOH, 0 °C, 34 h; 4) HCl, H_2O, Bu^tOH, 20 °C, 1 h (53% yield); dba is dibenzylideneacetone, DMAP is 4-dimethylaminopyridine.

The final successive deprotection (step *d*) afforded the C(28)-fluorinated analogue of AmB (**73**).

The biological properties of the fluorine-containing analogues were studied. Fluorine derivative **72** exhibits the antifungal activity against *Aspergillus niger* and has membrane-permeation properties almost identical to those of AmB. Compound **71** almost do not exhibit antifungal activity. The activity of compound **73** against *Aspergillus niger* is also similar to that of AmB. The NMR spectroscopic study showed that fluorine derivatives **71** and **72** form ion channels like compound **1**. Fluorine derivatives **71**–**73** appeared to be convenient for studying the mechanisms of the formation of ion channels.

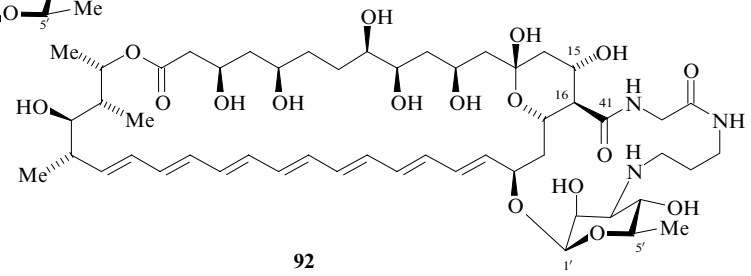
6. Cyclic derivatives of amphotericin B

Amphotericin B (**1**) contains two rigid moieties, *viz*., the macrolide ring connected to the mycosamine moiety by a glycosidic bond capable of rotating. The relative arrangement of these moieties is responsible for the possibility of AmB–AmB and AmB–ergosterol interactions, in which



91a–c

n = 4 (**a**), 6 (**b**), 8 (**c**).



92

intermolecular and intramolecular hydrogen bonds play a key role. To determine the relationship between the orientation of the amino sugar in the polyene antibiotic molecule and the antifungal activity, AmB derivatives containing different intramolecular spacers between the carboxy and amino groups were synthesized.[18, 52]

Compounds **91a–c** contain oligomethylene chains of different lengths (C_4, C_6 or C_8) as spacers. It was found that the interactions of these derivatives with fungal membrane ergosterol are different. Compound **91b** has a higher affinity for ergosterol than other derivatives and AmB by itself.[52] However, the antifungal activity of **91b** against *Aspergillus niger* is four times lower than that of compound **1**.

The authors suggested that a weakening of the activity is associated with a decrease in the permeability through the fungal cell wall due to the relatively high hydrophobicity of the introduced spacer. To increase the hydrophilicity, the oligomethylene chain was replaced by the glycine-containing spacer to form derivative **92**, in which the total number of carbon atoms was retained.[20] The synthesis of compound **92** is shown in Scheme 17.

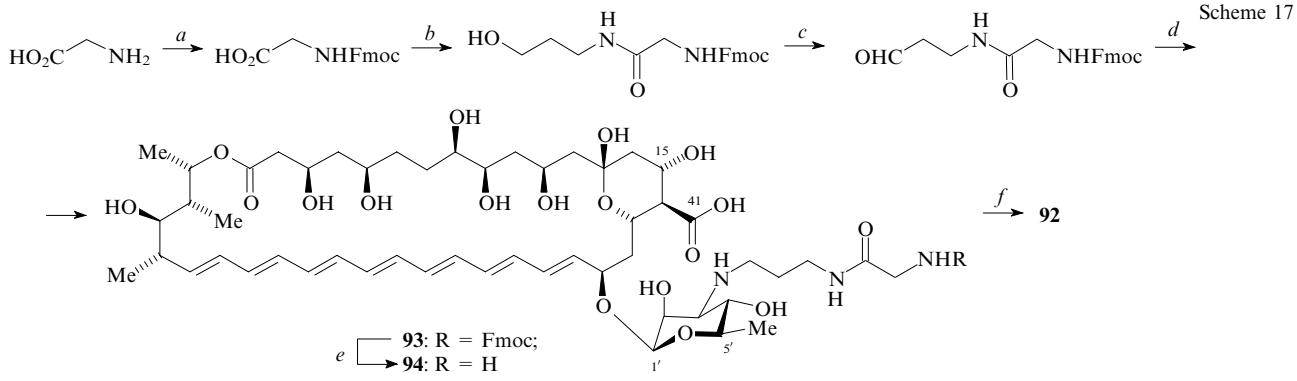
The condensation of N-Fmoc-glycine (step *a*) with 3-aminopropanol was performed in the presence of PyBOP (step *b*). The oxidation of the primary hydroxy group to the aldehyde group was carried out according to the Dess–Martin method (step *c*). The subsequent reductive alkylation of the amino group of the mycosamine moiety with the aldehyde that is formed in step *c* gave protected amine **93** (step *d*). The deprotection giving compound **94** (step *e*) followed by the intramolecular amidation of the carboxy group with PyBOP (step *f*) afforded derivative **92**.

The antifungal activity of compound **92** against *Aspergillus niger* is twice as high as that of hexamethylene analogue **91b**. Studies by UV and CD spectroscopy showed that compound **91b** forms mainly insoluble aggregates in a sodium phosphate buffer, whereas compound **92**, like AmB, forms water-soluble oligomers. Derivative **92** containing isotope-labelled [1-^{13}C,^{15}N]glycine in the spacer was synthesized. The study by the REDOR technique showed that water-soluble oligomers of the antibiotic form aggregates immobilized in ergosterol-containing liposomes.[18]

7. Amphotericin B dimers

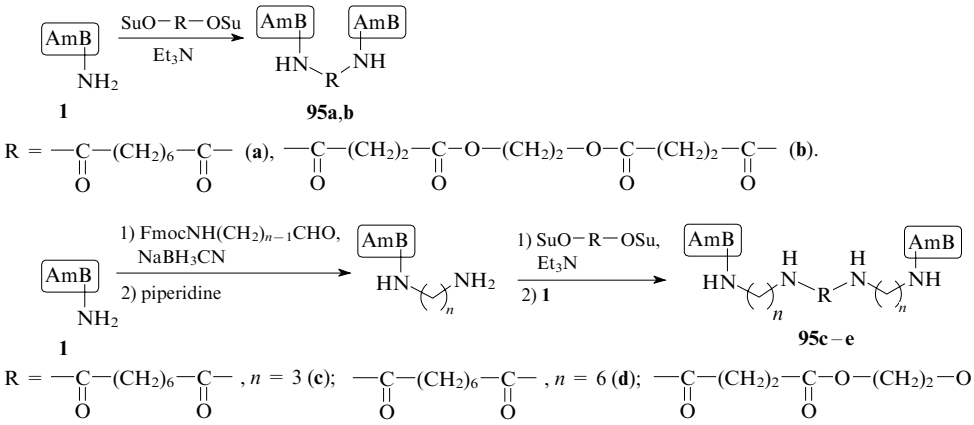
The channel formed by amphotericin B in a membrane is in the equilibrium state, *i.e.*, the structure of the channel is unstable and the channel can undergo dissociation.[17] It was suggested that the stability of the channel would be increased in AmB dimers containing spacers between the amino groups of the antibiotic (N,N′ dimers) [53] and between the carboxy and amino groups (C,N dimers).[54] N,N′ Dimers **95a**,**b** were synthesized with the use of *N*-hydroxysuccinimide esters of dicarboxylic acids according to Scheme 18. These dimers contain the oligomethylene spacer between the amide bonds and do not exhibit antifungal activity in *in vitro* experiments. The NMR spectroscopic data also showed that compounds **95a**,**b** do not form ion channels in a model membrane.

Scheme 17



(*a*) FmocOSu, PyH, MeOH; (*b*) 3-aminopropanol, PyBOP, HOBt, Pr^i_2NEt, DMF; (*c*) Dess–Martin reagent, CH_2Cl_2; (*d*) **1**, $NaBH_3CN$, DMF–MeOH; (*e*) piperidine, DMF–MeOH; (*f*) PyBOP, HOBt, Pr^i_2NEt, DMF.

Scheme 18

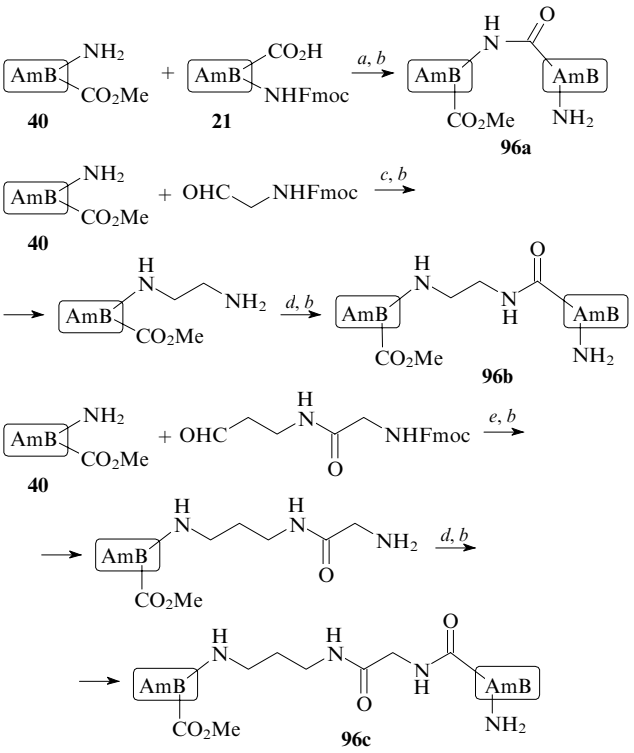


As mentioned above, the amino group in the antibiotic molecule plays an important role in the demonstration of the antifungal activity of polyenes. Dimers **95c–e** were synthesized to preserve the amino function. In these compounds, the AmB molecules are connected by a similar spacer but with the use of the 3′-N-amidoalkyl bond.

Studies in model membrane systems with the use of ergosterol-containing liposomes showed that dimers **95c–e** can form ion channels similarly to AmB, dimer **95d** being better in this respect.

Another type of dimers (C,N-dimers) were synthesized according to Scheme 19.[54] For this purpose, the carbonyl group of one AmB molecule was linked to the amino group of another AmB molecule. In all three cases, PyBOP was used as the condensing agent for the formation of the amide bond. Dimer **96a** was synthesized by the direct condensation of AmB methyl ester (**40**) with *N*-Fmoc-AmB (**21**). Dimers **96b** and **96c** contain a spacer between two antibiotic molecules. These compounds were synthesized with the use of the corresponding *N*-Fmoc-amino aldehydes, which were attached to the amino group of AmB methyl ester (**40**) by reductive alkylation. The subsequent condensation with *N*-Fmoc-AmB (**21**) followed by the Fmoc deprotection afforded dimers **96b**,**c**.

Scheme 19



(*a*) PyBOP, HOBt, Pr^i_2NEt, DMF; (*b*) piperidine, DMSO–MeOH; (*c*) **1**, $NaBH_3CN$, DMF–MeOH; (*d*) **21**, PyBOP, HOBt, Pr^i_2EtN, DMF; (*e*) $NaBH(OAc)_2$, DMF, Pr^iOH.

Dimers containing short spacers (**96a**,**b**) showed the complete absence of antifungal activity against the strain of *Aspergillus niger* in *in vitro* experiments, which was confirmed by the investigation of the channel-forming ability of these dimers compared to AmB. However, dimer **96c** containing the longer spacer between the antibiotic molecules exhibited antifungal activity comparable to that of compound **1** and it formed ion channels in a model experiment similarly to AmB.

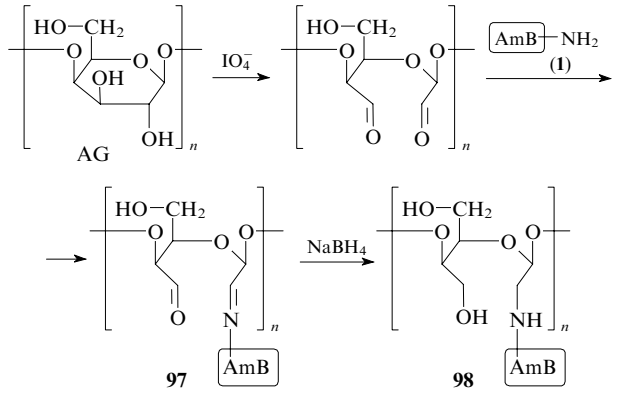
IV. Conjugates of amphotericin B with macromolecules

One of promising modifications of polyene antibiotics is based on the synthesis of conjugates with various macromolecules. This approach was used for the improvement of the therapeutic indices of many antibiotics.[55] The formation of conjugates of moderately soluble agents, such as AmB, with water-soluble polymer carriers (proteins, polypeptides, polysaccharides, synthetic polymers) can lead to changes in the physical properties of the starting compound, including its stability and solubility. As a result, the targeted delivery can be performed and the levels of drug accumulation in injured tissues can be increased, thus improving the therapeutic index and decreasing the toxicity.

1. Conjugates of amphotericin B with polysaccharides

Conjugates of AmB with arabinogalactan (AG), which is the highly branched polysaccharide of plant origin, were synthesized. The backbone of this polysaccharide consists of galactose units, and the side chains consist of galactose and arabinose units, single arabinose units and uronic acids, primarily, glucuronic acid. Due to high solubility in water, the biocompatibility, the biodegradation and the ease of the formation of conjugates with various mono- and bifunctional reagents, AG can be used as a drug carrier in the body.[55]

Scheme 20



Arabinogalactan was activated by the periodate cleavage. Scheme 20 shows the transformations of the backbone of arabinogalactan, (1→4)-β-Gal. To remove an excess of periodate after the oxidative cleavage, activated arabinogalactan was purified on an ion-exchange resin. The reaction of the purified oxidized polysaccharide with compound **1** led to the formation of the C═N bond between the aldehyde groups of oxidized AG and the 3′-amino group of the mycosamine moiety of AmB. The imine bond in conjugate **97** was reduced to the more stable amine bond with the use of $NaBH_4$. The intact aldehyde groups of AG were reduced to alcohol groups. All reactions were carried out in an aqueous medium, which is a considerable advantage of this method.[56, 57]

Conjugates **97** and **98** proved to be sufficiently stable in the solid state at room temperature. Conjugates of AmB with arabinogalactan containing different percentages of the antibiotic were synthesized in different buffer systems at various pH of the reaction mixture. Under optimal conjugation conditions (0.1 M borate buffer, pH 11, room temperature, 48 h), conjugate was obtained in a yield of >90%.[57] Conjugate **98** containing ~20 mass % of the antibiotic holds the most promise. The solubility of **98** in water is >1000 mg ml^{-1}, whereas AmB is almost insoluble in water (<0.1 mg ml^{-1}). Conjugate **98** retains high antifungal activity against *Candida albicans* and *Cryptococcus neoformans* in *in vitro* experiments (MIC = 0.12–0.25 μg ml^{-1}) but is much less toxic in test on animals (MTD = 50 mg kg^{-1} *versus* 4 mg kg^{-1} for the complex of AmB with sodium deoxycholate).

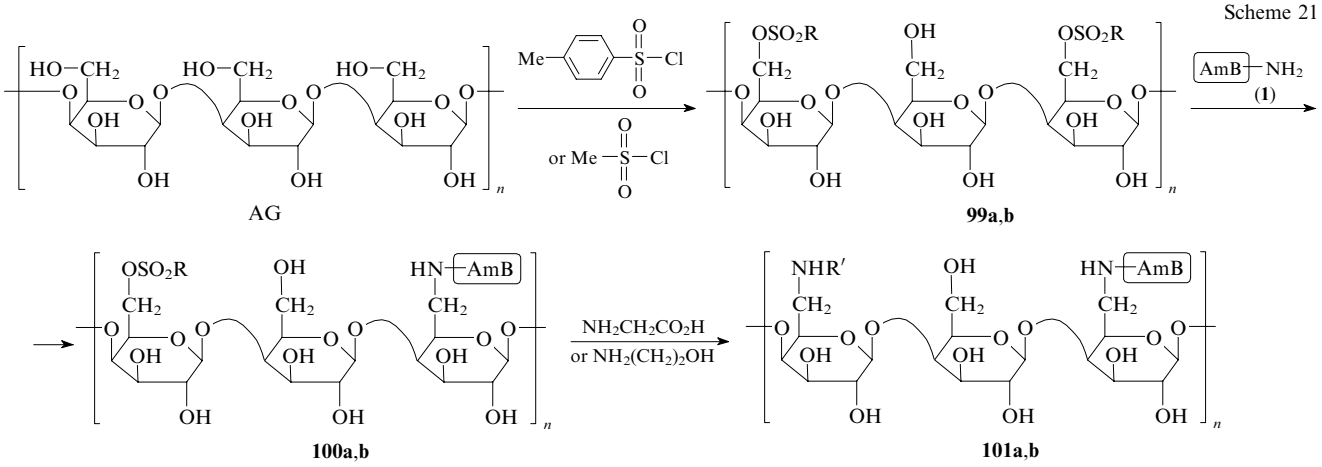
Such a considerable decrease in the toxicity was achieved due to the purification of the conjugate by gel permeation chromatography on Sephadex, which led to the removal of AmB, which remained in the free state and can be adsorbed on the conjugate to form non-covalent bonds. The *in vivo* experiments showed that the therapeutic efficacy of conjugate **98** is similar to that of the liposomal form of AmB (drug AmBisome) and is higher than that of the complex of AmB with sodium deoxycholate (drug Fungizone).

The imine conjugate of AmB with dextran was synthesized according to a similar procedure.[58] To reduce the toxicity of the conjugate, the intact aldehyde groups of AG were blocked by the reaction with ethanolamine. As a result, the acute toxicity of the agent in the cell line RAW 264.7 was decreased by 100%.[58]

However, in spite of the high efficacy of the conjugates against pathogenic fungi, the oxidation of the polysaccharide chain with periodate can lead to the partial destruction of the polymer, the change in its natural structure and biodistribution. Hence, a method was developed for the synthesis of conjugates of AmB with undegraded polysaccharides.[59] The method is based on the direct replacement of the hydroxy groups of polysaccharides by amino-containing AmB.

In the first step, the hydroxy groups were activated by the reaction of arabinogalactan with tosyl or mesyl chloride. Scheme 21 shows the fragment of the arabinogalactan backbone (1→4)-β-Gal. The subsequent condensation of the resulting AG tosylate (**99a**) or AG mesylate (**99b**) with AmB afforded the corresponding conjugates **100a**,**b**. The intact tosylate and mesylate groups in the conjugates were replaced by glycine or ethanolamine (compounds **101a**,**b**). The percentage of the antibiotic in the conjugates was ~20 mass %. Conjugates **101a**,**b** are water-soluble and are more stable in aqueous solutions than compound **1**. These conjugates are highly active against *Leishmania major* (LD_{50} ≈ 0.1 μg ml^{-1}) and *C. albicans* (MIC ≈ 1 μg ml^{-1}). Experiments in BALB/c mice revealed a decrease in the toxicity of the conjugates (MTD = 10–30 mg kg^{-1}) compared to the toxicity of the complex of AmB with sodium deoxycholate (MTD = 1 mg kg^{-1}). The main drawback of this method of the conjugation compared to the reductive

Scheme 21



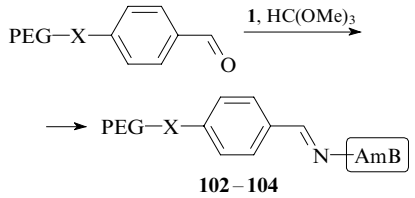
R = 4-MeC_6H_4 (**a**), Me (**b**); R′ = CH_2CO_2H (**a**), $(CH_2)_2OH$ (**b**).

amination is that it requires toxic tosylate or mesylate and organic solvents and they should be thoroughly removed after the reaction.

2. Conjugates of amphotericin B with polyethylene glycol

Various conjugates of compound **1** with polyethylene glycol (PEG) prepared for the targeted delivery of drugs to regions of injured organs and tissues were documented.[60] Since the pH level in the focus of the infection is lower than that in normal tissues, the conjugates were prepared by the formation of pH-sensitive bonds between the drug and the carrier. The imine bond between the 3′-amino group of the mycosamine moiety of AmB and the aldehyde group of PEG derivatives often serves as this bond. It is stable at physiological pH (7.4 – 7.6) but is easily hydrolyzed at lower pH. Conjugates **102** – **104**, which differ in the nature of the spacer between the antibiotic molecule and the polymer, were synthesized starting from compound **1** and PEG (Scheme 22).

Scheme 22



102 – **104**

X = O (**102**), NHC(O) (**103**), OC(O) (**104a** – **c**);
M_{PEG} /kDa: 5 (**a**), 10 (**b**), 20 (**c**).

As can be seen from Scheme 22, the 1,4-disubstituted benzene ring serves as a spacer, which is linked from one side to AmB by the imine bond and from another side to the polymer by different types of bonds (ether, ester or amide bonds). In addition, the molecular weight of the polymer in compounds **104** was varied.[60]

The behaviour of the conjugates in a buffer system, in the blood plasma and serum was studied. All the conjugates proved to be relatively stable in a phosphate buffer in physiological conditions (pH 7.4, 37 °C). A decrease in the pH of the buffer to 5.5 led to the release of free AmB as a result of the hydrolysis of the Schiff base. The rate of acid hydrolysis depends on the nature of the substituents in the benzene ring and is independent of the molecular weight of PEG. Conjugates **104a** – **c** containing the ester bond between the polymer and the spacer were subjected to enzymatic cleavage in the human blood plasma/serum; the rate of cleavage depends on the molecular weight of the polymer. 4-Carboxybenzylideneaminoamphotericin B (**105**) was formed in the presence of hydrolases, and then it was decomposed to 4-formylbenzoic acid and free compound **1** (Scheme 23).

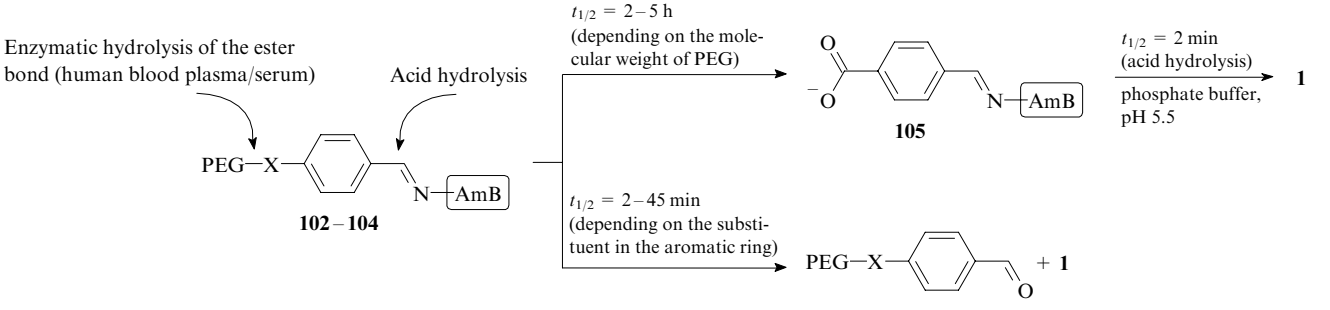
The investigation of the acute toxicity of conjugates **104a**,**b** in mice showed that these compounds have a considerable advantage over the free antibiotic. The LD_{50} values are 40.5 and 20.7 mg kg^{-1} for compounds **104a** and **104b**, respectively, which is indicative of a 6 – 11-fold decrease in the toxicity of the agent. The investigations confirmed that these conjugates can be used as prolonged-action prodrugs (depot forms) for the targeted delivery of the active substance to the focus of the pathological process.

The PEG-based block copolymer poly(ethylene glycol)-*b*-poly(L-lysine) (PEG-*b*-PLL, **106**) is rather extensively used for the drug delivery (Scheme 24).

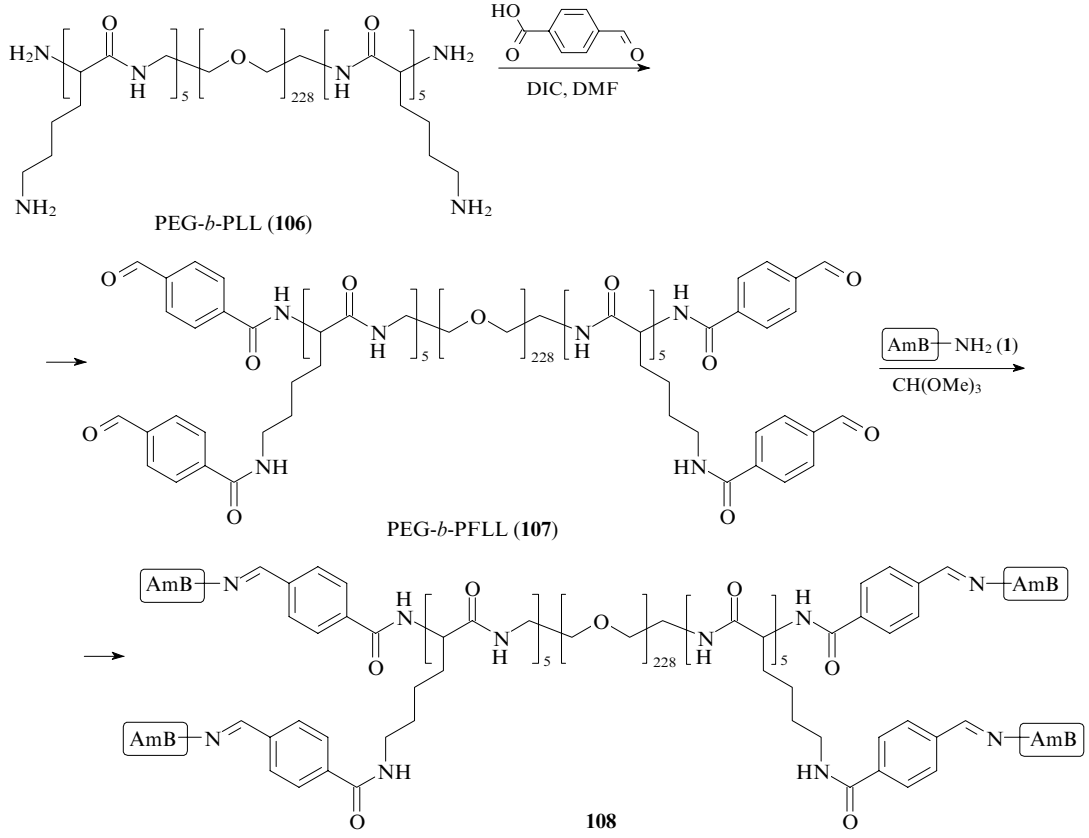
It is the completely biocompatible non-toxic polymer, which can bind a large number of drug molecules (for example, of AmB). The block copolymer PEG-*b*-PLL (**106**) was synthesized by the polycondensation of protected L-lysine (N^{ε}-benzyloxycarbonyl-L-lysine) with α,ω-diaminopoly(ethylene glycol). After the removal of the benzyloxycarbonyl protecting groups, a polymer with the molecular weight of 13 200 containing free amino groups was formed.[61] The synthesis of the conjugate from compound **1** and PEG-*b*-PLL (**106**) is shown in Scheme 24. The activation of PEG-*b*-PLL was performed with 4-formylbenzoic acid in the presence of diisopropylcarbodiimide (DIC). Then the condensation of the polymer PEG-*b*-PFLL (**107**) with compound **1** was performed. The aldehyde groups of PEG-*b*-PFLL react with the primary 3′-amino group of the mycosamine moiety of the polyene to form a Schiff base. Conjugate **108** contains 12 antibiotic molecules linked to one polymer molecule by pH-sensitive imine bonds. It was found that conjugate **108** is stable in the blood plasma and in a phosphate buffer at pH 7.4; however, the release of AmB was observed at pH 5.5 with a half-life of 2 min. In *in vivo* experiments, conjugate **108** exhibited relatively low toxicity (LD_{50} = 45 mg kg^{-1}). Hence, this conjugate is considered as a promising agent for further investigations.

Nowadays, the enhancement of the drug selectivity is an important problem. One of approaches to the solution of this problem is based on the results of investigations of the nature of pathogens. It was found that many fungal families, such as *Aspegillus*, *Candida* and *Trichosporon*, contain specific β-glucosidases in the enzyme composition. In the human body, β-glucosidases are present only in certain types of bacteria of intestinal microflora. This fact was used for the preparation of conjugates capable of performing targeted delivery of antibiotics to the focus of the infection. The synthesis of the conjugate of AmB with

Scheme 23



Scheme 24

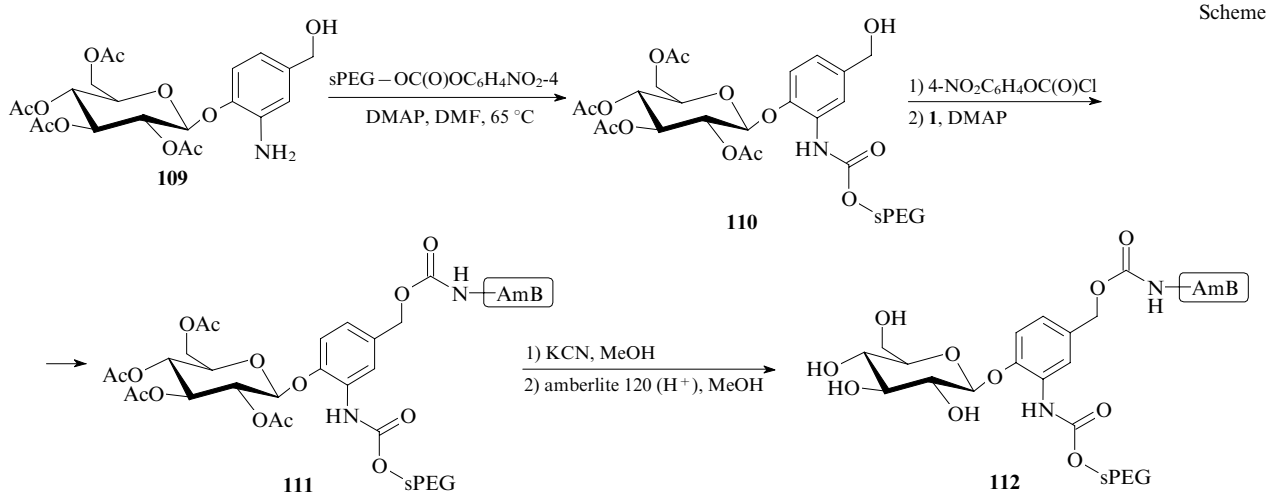


branched PEG, *viz*., pentaerythritol poly(ethylene glycol) ether (sPEG), with the molecular weight of 20 000 containing the hydrolase-sensitive β-glycosidic bond in the spacer was documented (Scheme 25). The structure of the conjugate contains also two types of carbamate bonds: the stable carbamate bond that links the polymer to a spacer and the relatively labile carbamate bond between the 3′-amino group of the mycosamine moiety of the antibiotic molecule and the spacer. Glucopyranoside **109** was used as the spacer. This compounds was synthesized staring from tetra-*O*-acetyl-α-D-glucopyranosyl bromide and 4-hydroxymethyl-2-nitrophenol.[62] Then the condensation of glucopyranoside **109** with modified sPEG was performed. Polymer **110** was bound to AmB by means of a labile carbamate bond with the use of 4-nitrophenyl chloroformate to form the corresponding conjugate **111**.

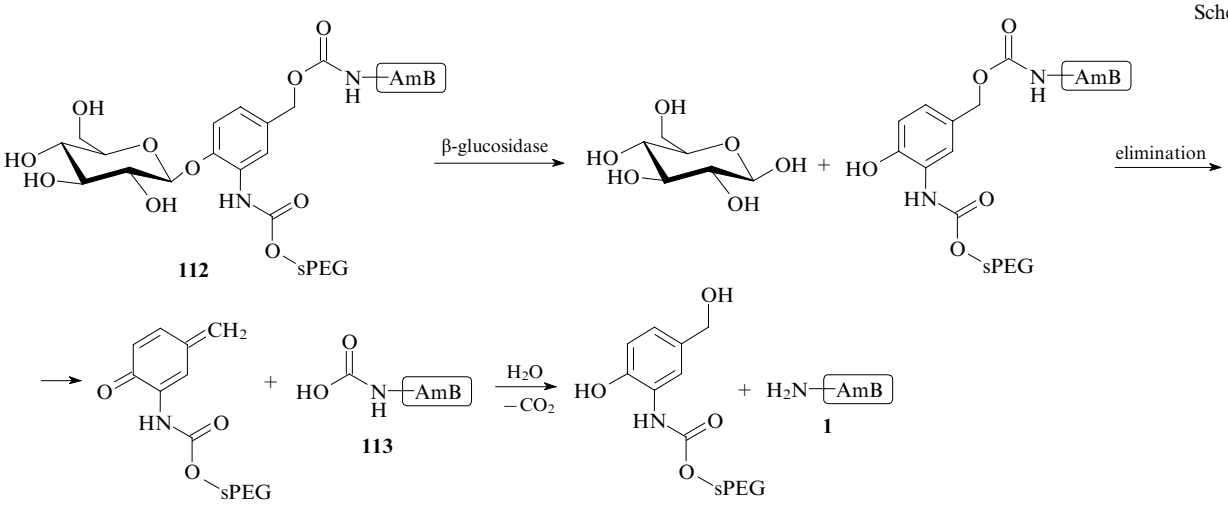
Due to instability of amphotericin B, the acetyl protecting groups were removed from the glucose residue with the use of catalytic amounts of potassium cyanide, which was then removed from the reaction mixture with an ion-exchange resin. Conjugate **112** is highly soluble in water. It was found that the antibiotic to polymer molar ratio is 4 : 1, *i.e*., one sPEG molecule is linked to four AmB molecules.

Based on the preliminary *in vitro* data on the stability of conjugate **112** in a buffer solution in the presence of β-glucosidases, the probable mechanism of action was

Scheme 25



Scheme 26



suggested. After the migration to the focus of the infection, conjugate **112** undergoes the glycosidic bond cleavage in the spacer catalyzed by β-glucosidase to elimiante glucose (Scheme 26).

The subsequent elimination affords carboxamido-AmB (**113**), which is easily decarboxylated to give free antibiotic **1**. However, more detailed preclinical trials are required before the use of conjugate **112** in medical practice.

Therefore, the nature of the polymer carrier, the type of the bond between the carrier and the active substance and the structure of the spacer between the polymer and the active substance play a considerable role in the preparation of conjugates of polyene antibiotics.

V. Conclusion

In recent years, previously unknown polyene macrolides were prepared by the chemical and genetic engineering design. The *in vitro* and *in vivo* analysis of the structure–activity relationship for new polyenes not only provides detailed insight into the mechanisms of action of these compounds but also makes it possible to design more effective and less toxic new-generation antifungal agents.

The results of *in vitro* studies of this relationship help in choosing the most active compounds and in understanding which structural elements of antibiotics are responsible for the binding to a target and which elements are not involved in the binding. Investigations of the antifungal activity and toxicity of the leading compounds in animals can reveal agents having the highest efficacy and lowest toxicity. Experiments in immunocompromised animals are of particular importance because they are most similar to the conditions of the future clinical use of polyene antibiotics for the treatment of systemic fungal diseases.

We thank Professor S B Zotchev (Department of Biotechnology, Norwegian University for Science and Technology, Trondheim, Norway) for valuable comments.

This review has been written with the financial support of Federal Programme 'Leading Scientific Schools' (Project NSh-5290-2010.4) and the Russian Foundation for Basic Research (Project No. 10-03-00210-a).

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