Chemical Modification and Biological Evaluation of New Semisynthetic Derivatives of 28,29-Didehydronystatin A_1 (S44HP), a Genetically Engineered Antifungal Polyene Macrolide Antibiotic

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Twenty-three new derivatives of the heptaene nystatin analogue 28,29-didehydronystatin A_1 (**1**) (S44HP) were obtained by chemical modification of C16 carboxyl and amino groups of mycosamine. These derivatives comprised 15 carboxamides, 4 *N*-alkyl derivatives, 3 N-derivatives containing additional N-linked monosaccharide or disaccharide moiety (products of Amadori rearrangement), and 1 *N*-aminoacyl derivative. The derivatives have been tested in vitro against yeasts *Candida albicans, Cryptococcus humicolus*, and filamentous fungi (molds) *Aspergillus niger* and *Fusarum oxysporum,* as well as for hemolytic activity against human erythrocytes. Structure−activity relationships for the compounds obtained are discussed. The most active and least hemolytic derivative 3-(*N*,*N*-dimethylamino)propylamide of S44HP (**6**) was tested for acute toxicity and antifungal activity in animal model. Whereas amphotericin B and S44HP were active in vivo at doses close to the maximal tolerated dose, **6** was considerably less toxic and more active compared to these two antibiotics.

Introduction

Systemic fungal infections represent a serious threat to immunocompromised patients, and the arsenal of available antifungal drugs to treat such infections is severely limited. Polyene macrolide antibiotics have been used as antifungal agents for over 50 years because of their efficacy and broad spectrum of antifungal activity. Amphotericin B (AmB[a]) (Figure 1a) has been shown to have excellent efficacy against a number of fungal pathogens and until now is the only polyene macrolide approved for treatment of systemic fungal infections.[1] However, its use is limited by considerable nephrotoxicity and very poor water solubility. The latter and suboptimal pharmacokinetics undermine the therapeutic value of this antibiotic, prompting the search for alternative antifungals.

Several approaches have been used to address the problems in medical use of polyene macrolides mentioned above. A number of polyene macrolide analogues, both semisynthetic [2−4] and genetically engineered,[5−8] have been generated over the past 20 years in an attempt to reduce toxicity and improve solubility. Numerous AmB derivatives have been obtained by chemical modifications of C16 carboxyl (methyl ester and amides)[4,9−11] or amino group (*N*-acyl- and *N*-alkyl groups).[4,10−13] Biological testing of these derivatives has contributed greatly to our understanding of the structure−activity relationship of polyene macrolides. It has been demonstrated that modification of the C16 carboxy group of AmB leading to charge suppression reduces toxicity to mammalian cells while having little effect on antifungal activity.[14−16] It has also been shown that specific modifications of mycosamine amino group with two amino-

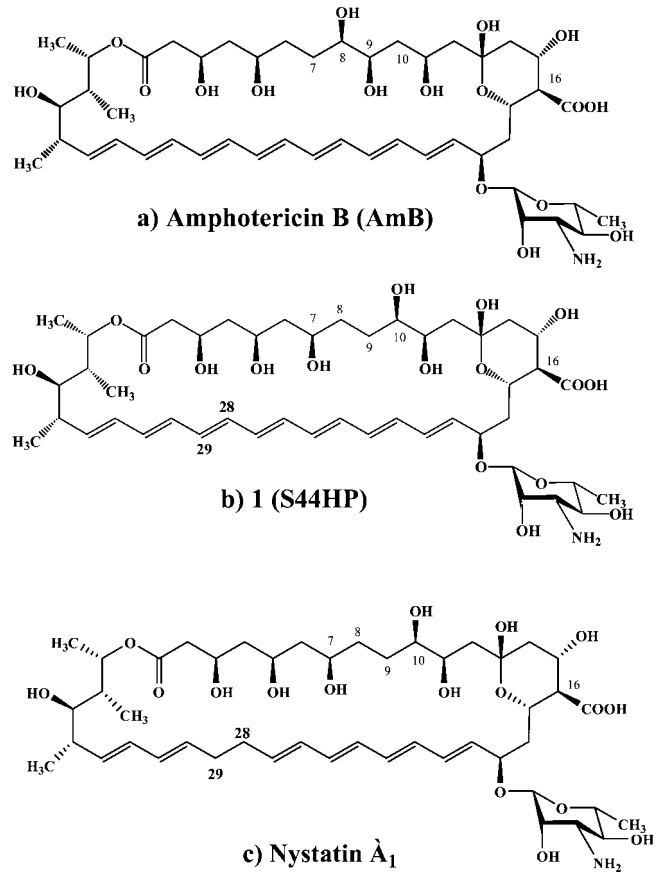

Figure 1. Chemical structures of amphotericin B (AmB) (a), **1** (S44HP) (b), and nystatin A_1 (c).

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[a] Abbreviations: AmB, amphotericin B; CFU, colony forming units; APT, attached proton test; MIC, minimal inhibitory concentration of antibiotic (μg/mL); PyBOP, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; DIPEA, diisopropylethylamine; DMSO, dimethyl sulfoxide; Fmoc, 9-fluorenylmethoxycarbonyl; MTD, maximal tolerated dose.

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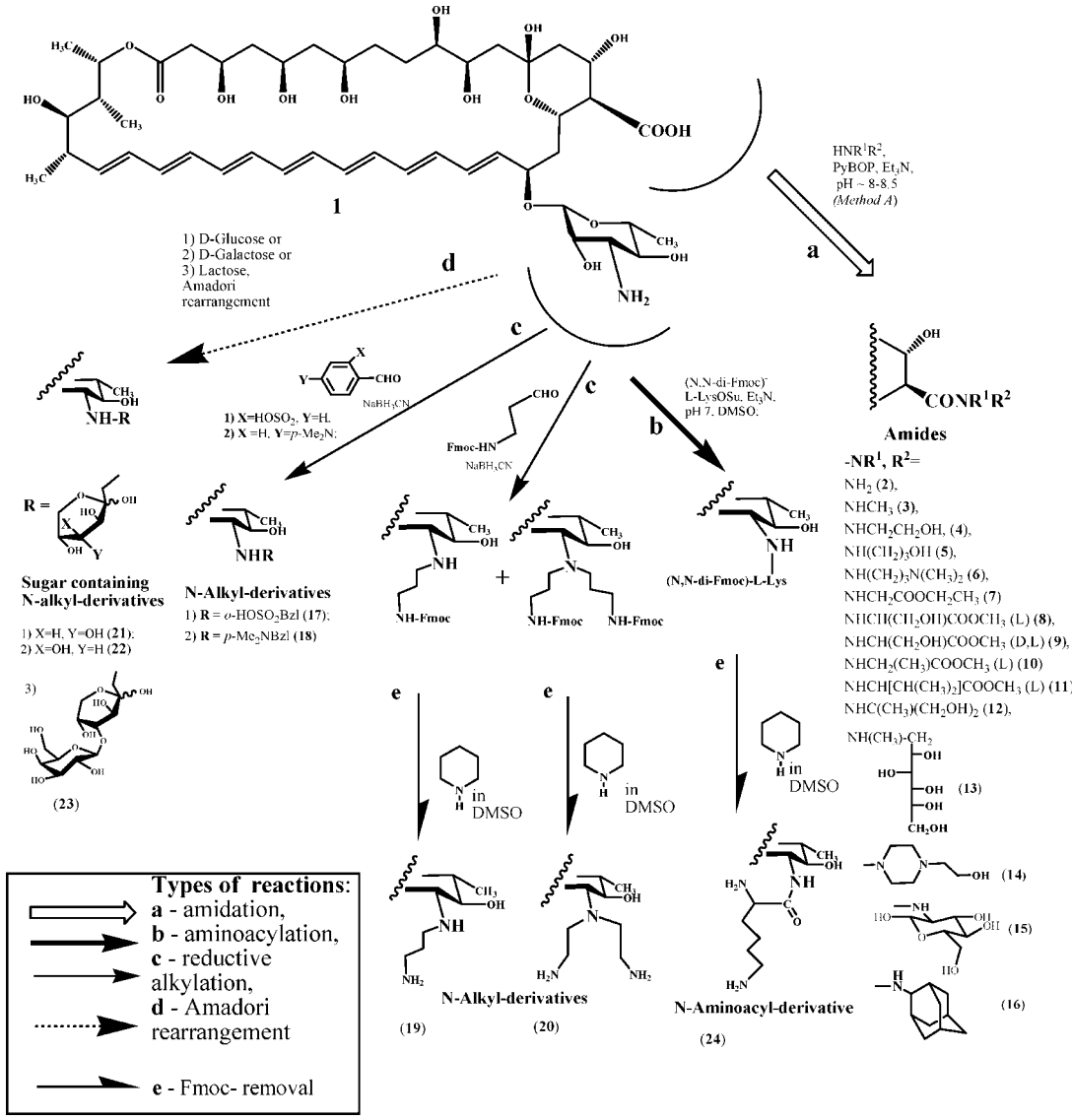
Scheme 1. Synthesis of the S44HP Derivatives **2**−**24**



propyl chains result in highly active derivatives with low hemolytic activity.[13,17] Despite these promising results, no new AmB-based antifungal agent has appeared on the market except for lipid and liposomal formulations.[1] The latter fact suggests that the search for novel polyene macrolide antifungals for human use is still a challenging task.

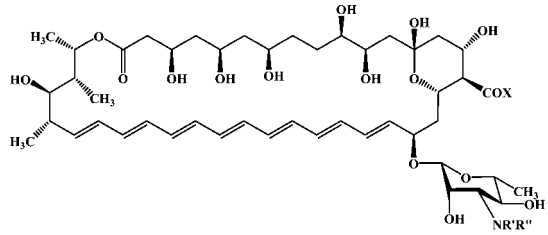
A heptaene nystatin analogue, 28,29-didehydronystatin A_1 (**1**) (S44HP) (Figure 1b), has been generated via genetic manipulation of the nystatin polyketide synthase in the production of bacterium *Streptomyces noursei* and was shown to have antifungal activity considerably higher than that of nystatin A_1 (Figure 1c) and equal to that of AmB.[7,18] **1** is structurally very similar to AmB but differs by the positions of hydroxyl groups in the fragment C7−C10 of the polyol region (Figure 1b and Figure 1a). There are two hydroxyl groups in positions C7 and C10 on **1** (S44HP), while these groups are in positions C8 and C9 on AmB. Although a lower maximal tolerated dose (MTD) value has been demonstrated for **1** in the mouse model compared to AmB, the former antibiotic appeared to have a considerably wider therapeutic window (MTD/LD_{50} dose interval).[18] The latter suggested that **1** may be used as a scaffold for the development of new polyene macrolides for human therapy. In the present work we report preparation of novel S44HP analogues by chemical modifications and their biological investigation.

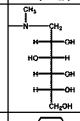
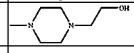
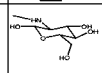
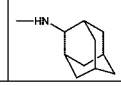
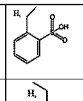
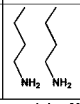
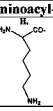
Results and Discussion

Chemistry. Chemical modification of polyene macrolide antibiotics meets serious problems that stem from the high molecular weight of these compounds and their poor solubility in common organic solvents, as well as high chemo- and photosensitivities and formation of aggregates.[19] On the basis of the data for AmB, the exocyclic carboxyl and the amino groups of mycosamine seem to be particularly important for selective toxicity and activity.[20−22] Therefore, modification of **1** (S44HP) was carried out in the following directions (Scheme 1): (a) amidation[9] to give compounds **2**−**16**, (b) reductive alkylation[13] of the amino group to yield **17**−**20**, (c) Amadori rearrangement[4,12] of *N*-glycosylderivatives to give ketose derivatives **21**−**23**, and (d) N-aminoacylation[10] to produce **24** (Scheme 1 and Table 1).

Amides **2**−**16** were obtained starting from **1** and the appropriate amine hydrochloride (ammonia, methylamine, 2-hydroxyethylamine, 3-hydroxypropylamine, 3-(*N*,*N*-dimethylamino)propylamine, glycine ethyl ester, L-serine methyl ester, D, L-serine methyl ester, L-alanine methyl ester, L-valine methyl

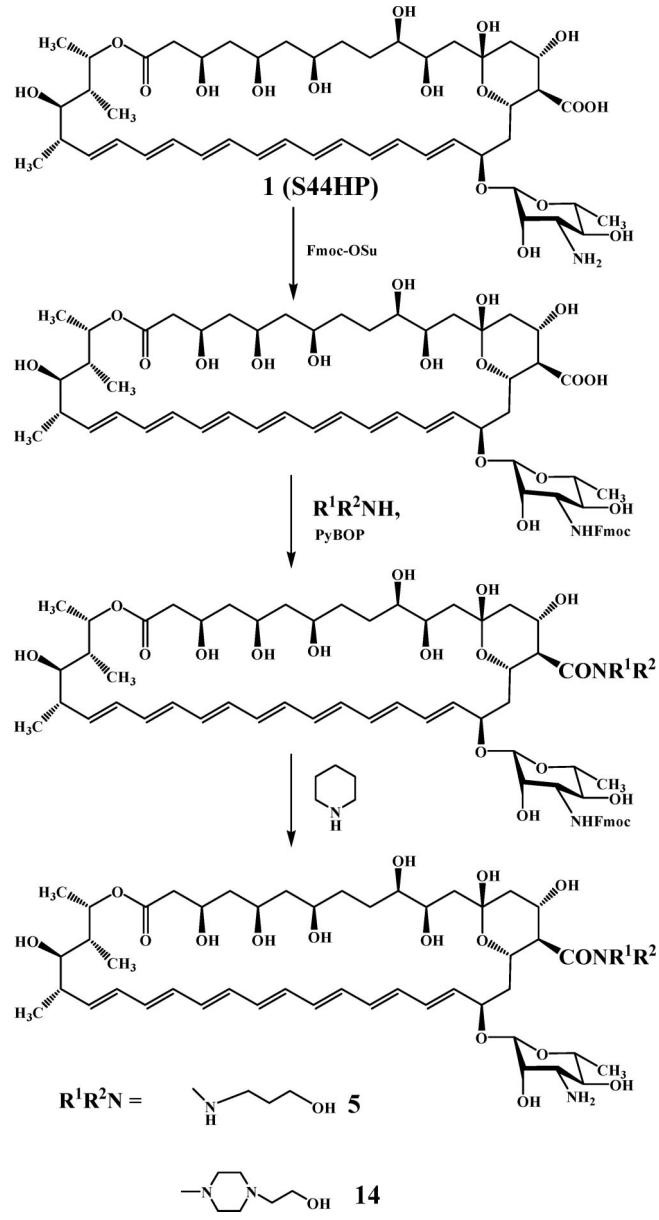
Table 1. Physicochemical Properties of Semisynthetic Derivatives of Antibiotic S44HP[a]



Compound	X	R', R''	HPLC (Rt)*	Molecular formula	MS, calc.	MS#, found: $[M+Na]^+$
Amides						
2	NH_2	H, H	11.52	$C_{47}H_{78}N_2O_{17}$	922.50	923.07##
3	$NHCH_3$	H, H	11.60	$C_{48}H_{76}N_2O_{16}$	936.52	959.69
4	$NHCH_2CH_2OH$	H, H	10.71	$C_{49}H_{78}N_2O_{17}$	966.15	989.70
5	$NH(CH_2)_3OH$	H, H	10.86	$C_{50}H_{80}N_2O_{17}$	980.55	981.55##
6	$NH(CH_2)_3N(CH_3)_2$	H, H	7.65	$C_{52}H_{84}N_3O_{16}$	1007.59	1008.60##
7	$NHCH_2COOEt$	H, H	14.33	$C_{52}H_{80}N_2O_{16}$	1008.54	1032.09
8	$NHCH(CH_2OH)COOCH_3$ (L)	H, H	11.55	$C_{51}H_{80}N_2O_{19}$	1024.54	1025.57##
9	$NHCH(CH_2OH)COOCH_3$ (DL)	H, H	12.66, 13.22	$C_{51}H_{80}N_2O_{19}$	1024.54	1025.48##
10	$NHCH(CH_3)COOCH_3$ (L)	H, H	13.47	$C_{51}H_{80}N_2O_{18}$	1008.54	1031.99
11	$NHCH[CH(CH_3)_2]COOCH_3$ (L)	H, H	16.83	$C_{48}H_{78}N_2O_{16}$	1036.57	1058.15
12	$NHC(CH_3)(CH_2OH)_2$	H, H	20.08	$C_{51}H_{82}N_2O_{18}$	1010.56	1011.57##
13		H, H	9.31	$C_{54}H_{88}N_2O_{21}$	1100.59	1124.05
14		H, H	7.43	$C_{53}H_{88}N_2O_{17}$	1035.59	1059.16
15		H, H	9.35	$C_{55}H_{84}N_2O_{21}$	1084.56	1085.48##
16		H, H	20.40	$C_{57}H_{88}N_2O_{16}$	1056.61	1079.79
N-Alkyl-derivatives						
17	OH		16.15	$C_{54}H_{79}NO_{20}S$	1093.49	1117.09
18	OH		14.35	$C_{56}H_{84}N_2O_{18}$	1056.58	1080.01
19	OH		7.40	$C_{53}H_{87}N_3O_{17}$	1037.60	1038.64##
20	OH		7.70	$C_{55}H_{89}N_3O_{18}$	1051.58	1052.65##
Sugar containing N-alkyl-derivatives						
21	OH		11.54	$C_{53}H_{83}NO_{22}$	1085.54	1109.15
22	OH		13.98**	$C_{53}H_{83}NO_{22}$	1085.54	1109.00
23	OH		11.64	$C_{59}H_{93}NO_{27}$	1247.59	1271.12
N-Aminoacyl-derivatives						
24	OH		7.70	$C_{53}H_{89}N_3O_{18}$	1051.58	1052.65##

[a] (*) HPLC was carried out on a Shimadzu HPLC instrument of the LC 10 series on a Kromasil 100-C18 column (4 mm × 250 mm, particle size 6 μm) at an injection volume of 20 μL and a wavelength 408 nm. System comprised 0.01 M H_3PO_4 at pH 2.6 and acetonitrile. The proportion of acetonitrile varied from 30% to 70% for 30 min with a flow rate of 1.0 mL/min. (**) HPLC isocratic system, 35% MeCN. (#) Mass spectral data were obtained on MALDI TOF Bruker BIFLEX III instrument. (##) $[M + H]^{+1}$. (###) $[M + K]^{+1}$.

Scheme 2. Synthesis of the S44HP Derivatives **5** and **14**



ester, 2-amino-2-methylpropan-1,3-diol, 1-desoxy-1-(*N*-methylamino)-D-glucitol, *N*-(2-hydroxyethyl)piperazine, D-glucosamine, or 2-aminoadamantane) in the presence of PyBOP as the condensing reagent at pH ≈ 8−8.5 (method A).

Amides **5** and **14** were obtained starting from **1** in three steps (method B) (Scheme 2), via *N*-Fmoc derivatives that helped to overcome problems with purification of these compounds. The amino group of **1** was first blocked by 9-fluorenylmethoxycarbonyl (Fmoc) group using 9-Fmoc-*N*-oxysuccinimide ester (Fmoc-OSu). The resulting *N*-Fmoc-S44HP was purified by column chromatography up to 95% purity (as shown by HPLC). *N*-Fmoc-carboxamides were obtained by condensation of *N*-Fmoc-S44HP with the corresponding 3-hydroxypropylamine or *N*-(2-hydroxyethyl)piperazine hydrochlorides using PyBOP at pH ≈ 7−7.5. *N*-Fmoc derivatives of **5** and **14** were purified by column chromatography on silica gel. Deprotection with piperidine in DMSO gave compound **5** or **14**, correspondingly, with >95% purity.

The reaction of the 2-sulfobenzaldehyde or 4-(*N*,*N*-dimethylamino)benzaldehyde with **1** in the presence of $NaBH_3CN$

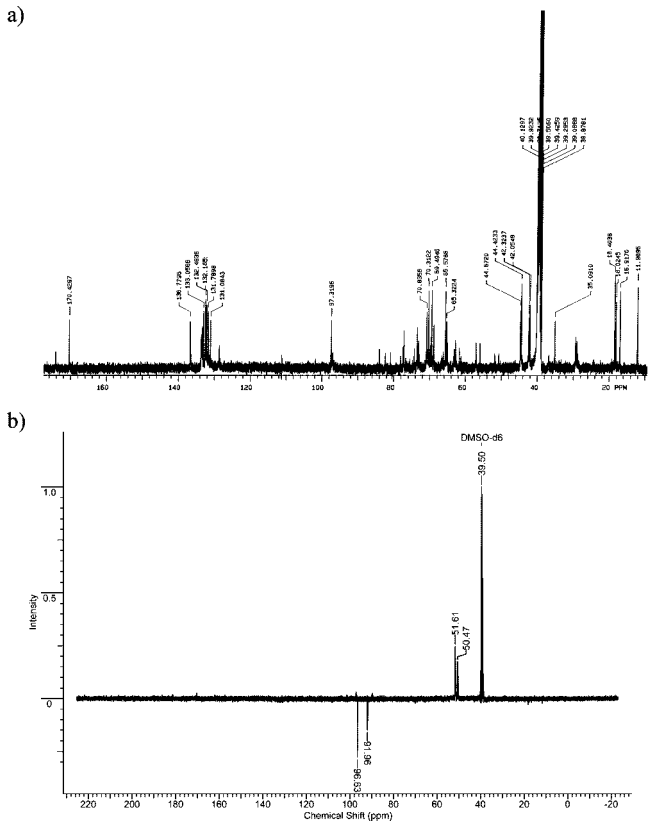


Figure 2. NMR spectra for **21**: (a) ^{13}C NMR, DMSO-d_6, 35 °C; (b) attached proton test (APT) and undecoupled ^{13}C spectrum for the artificial mixture of 1-^{13}C labeled 1-desoxy-D-fructos-1-yl derivative of S44HP (1-^{13}C-**21**) (signals for $^{13}CH_2$-1 group of 1-desoxy-D-fructos-1-yl moiety at 51.61 and 50.47 ppm) and 1-^{13}C- labeled D-glucose (signals for ^{13}CH-1 group, 96.63 and 91.96 ppm) (structure for **21**; see Figure 3c−e). Scheme 1 represents one variant of the structure, α,β-pyranose (Figure 3d).

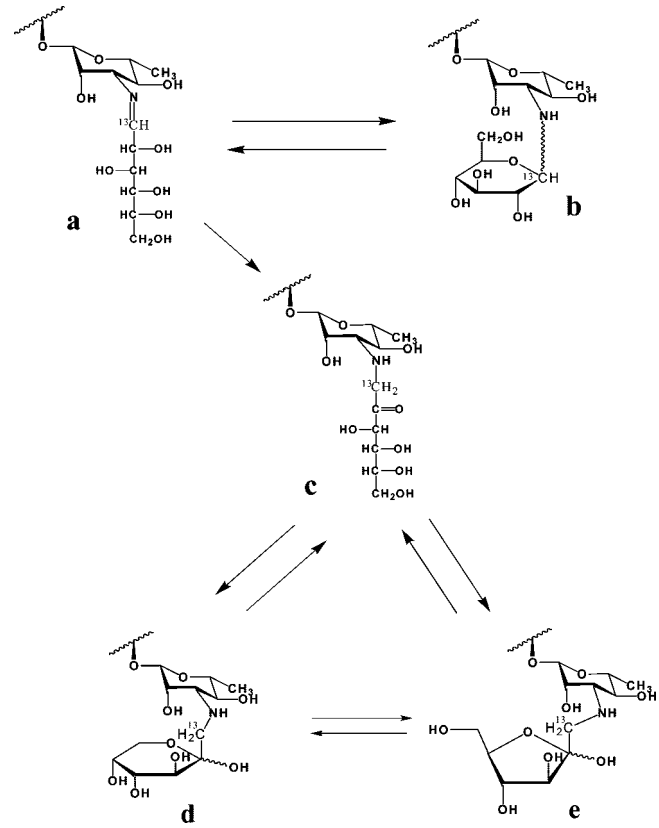


Figure 3. Possible structures of the *N*-alkyl sugar containing ^{13}C-labeled derivative of S44HP obtained by Amadori rearrangement (**a**−**e**). The structure ^{13}C-labeled *N*-(1-desoxy-D-fructos-1-yl) S44HP (1-^{13}C **21**) is the equilibrium of **c**, **d**, and **e** structures.

yielded the corresponding *N*-2-sulfobenzyl- (**17**) or *N*-(4-*N*,*N*-dimethylamino)benzyl derivative of S44HP (**18**).

The reaction of **1** with *N*-Fmoc-3-aminopropanal in the presence of $NaBH_3CN$ gave N-monosubstituted and N,N-disubstituted intermediate derivatives, which were separated by flash column chromatography. The removal of the Fmoc groups resulted in *N*-(3-aminopropyl)- (**19**) or *N*,*N*-di-(3-aminopropyl) derivatives of S44HP (**20**).

Compound **1** was reacted with D-glucose, D-galactose, or lactose in an Amadori rearrangement[4,12] to produce the *N*-(1-D-desoxyfructos-1-yl)- (**21**), *N*-(1-D-desoxytagatos-1-yl)- (**22**), or *N*-[(β-D-(galactopyranosyl-1→4)-*O*-1-desoxy-D-fructos-1-yl] derivative of S44HP (**23**), respectively, which was purified by column chromatography on Sephadex G-25.

The reaction of N^{α},N^{ε}-di-Fmoc-L-lysine *N*-oxysuccinimide ester with **1** in the presence of Et_3N yielded an intermediate di-Fmoc derivative, which was separated by flash chromatography. The removal of the Fmoc group with piperidine in DMSO resulted in *N*-L-lisyl-S44HP (**24**).

New derivatives of antibiotic S44HP were characterized by chromatography (TLC and HPLC) methods and by mass spectrometry (Table 1). The structure of Amadori rearrangement product **21** was confirmed by ^1H and ^{13}C NMR spectra and by NMR study of ^{13}C-labeled glucose derivative. The ^{13}C NMR spectra of **21** (unlabeled) and the artificial mixture of ^{13}C-**21** and 1-^{13}C-D-glucose are shown in parts a and b of Figure 2, respectively. The sample of the compound (^{13}C-**21**), containing the 1-$^{13}CH_2$ group of the 1-desoxy-D-fructos-1-yl moiety was prepared starting from **1** and 1-^{13}C-D-glucose. The interaction with a monosaccharide may lead to the structures of five types (Figure 3): Shiff-base derivative (**a**), *N*-glycoside derivative (**b**), N-linear sugar ketose derivative (**c**), and the cyclic α,β-pyranose (**d**) and/or α,β-furanose (**e**) forms. The ^{13}C NMR spectrum of ^{13}C-**21** contained ^{13}C signals at 51.61 and 50.47 ppm, which were determined as $^{13}CH_2$ group using the attached proton test (APT), showing that they correspond to two predominant tautomeric (anomeric) forms of 1-substituted 1-desoxyketose derivative **21**. For 1-^{13}C-D-glucose in an undecoupled ^{13}C spectrum, signals at 96.63 and 91.96 ppm were determined as $^{13}C_1H$ group (two anomeric or tautomeric structures). These data, also supported by the ^1H NMR spectrum, confirmed that **21** has the structure of the Amadori rearrangement product, *N*-(1-desoxy-D-fructos-1-yl) S44HP, which exists as an equilibrium mixture of structures **c**, **d**, and/or **e** (Figure 3).

Biological Evaluation. Antifungal activity of S44HP derivatives was tested in comparison with AmB and the parent compound against two strains of yeasts *Candida albicans* and *Cryptococcus humicolus* and two strains of filamentous fungi *Aspergillus niger* and *Fusarium oxysporum*, using the broth microdilution method as described in NCCLS documents M27-A,[23] and M38-A as well as by the special method developed and modified with usage of sensitive strain *C. albicans* ATCC 10231 (Tables 2 and 3).[7,23,24] In all experiments, in vitro microdilution technique in 96-microwell plates were applied. Amides **12**, **13**, and **16**, *N*-alkyl derivative **17**, and *N*-aminoacyl derivative *N*-lysyl S44HP (**24**) were shown to have low activity against all test organisms (MIC = 4 to >16 μg/mL). Amides **3**, **10**, **11**, **15** and *N*-alkyl derivatives

Table 2. In Vitro Antifungal and Hemolytic Activities for Polyene Macrolides

	MIC,[a] μg/mL test organism				
compd	*Candida albicans* ATCC 14053	*Cryptococcus humicolus* ATCC 9949	*Aspergillus niger* ATCC 16404	*Fusarium oxysporum* VKM F-140	hemolytic activity,[b] % (5 μg/mL)
AmB (AmB)[c]	1 (2)	1 (2)	1 (2)	4 (4)	30−39
1 (**1**)[c]	1 (2)	1 (2)	1 (2)	4 (4)	41−64
			Amides		
2	2	2	4	4	8−9
3	2	1	2	8	5−6
4	2	4	4	4	3−4
5	1	1	2	2	4−5
6	1	1	2	2	2−3
7	1	1	2	4	38−40
8[c]	(2)	(2)	(2)	(4)	14−16
9[c]	(2)	(2)	(4)	(4)	4−5
10	2	2	4	8	nd
11	2	2	4	8	nd
12	4	4	4	16	nd
13	4	4	4	>16	nd
14	1	2	2	4	2−3
15	1	1	2	8	3−4
16	4	4	4	8	nd
			N-Alkyl Derivatives		
17	4	4	4	>16	nd
18	2	2	4	>16	nd
19[c]	(2)	(4)	(4)	(4)	45−76
20	1	1	1	1	15−24
			Sugar Containing *N*-Alkyl Derivatives		
21	1	1	2	8	5−6
22	1	1	2	8	8−10
23	2	2	2	8	4−5
			N-Aminoacyl Derivatives		
24	8	8	16	>16	nd

[a] MICs are measured as the lowest concentration of agents that prevents any visible growth. The results of the experiments were definitely reproducible. In cases of full coincidence of the data obtained the MIC is represented as a single number. [b] Hemolytic activity based on mean data from two blood samples taken from two donors. nd = not detected. [c] Compounds **8**, **9**, and **19** were investigated in comparison with AmB, S44HP in separate experiments (data are given in parentheses).

Table 3. In Vitro Antifungal Activity Study on *Candida albicans* ATCC 10231 for the Semisynthetic Derivatives **5**−**7**, **14**, **20** Compared to AmB and **1** (S44HP)

compd	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
AmB	0.11 ± 0.02	0.36 ± 0.10
1	0.12 ± 0.02	0.40 ± 0.08
5	0.09 ± 0.03	0.27 ± 0.04
6	0.08 ± 0.03	0.30 ± 0.04
7	0.15 ± 0.01	0.36 ± 0.03
14	0.13 ± 0.04	0.52 ± 0.13
20	0.04 ± 0.02	0.13 ± 0.02

18, **21**, **22**, and **23** were similar in activity or even more active that the parent antibiotic **1** against *Candida albicans*, *Cryptococcus humicolus*, and *Aspergillus niger* (MIC = 1−4 μg/mL) but had low activity against *Fusarium oxysporum* (MIC = 8 to >16 μg/mL). Amide **4** had moderate antifungal activity against all four test organisms (MIC = 4−8 μg/mL). Amides **2**, **5**−**9**, **14** and *N*-alkyl derivatives **19** and **20** were similar in activity or even more active than the parent antibiotic for all four strains of tested microorganisms (MIC = 1−4 μg/mL).

Results obtained for *C. albicans* ATCC 10231 in a more sensitive test, where MIC_{50} and MIC_{100} values were measured (Table 3), showed a significant difference between compounds **6** and **20**, showing improved antifungal activity compared with **1** (S44HP). The results shown in Tables 2 and 3 confirm that a number of S44HP analogues have improved antifungal activity compared to both AmB and **1**.

Table 4. Acute Toxicity for Compound **6** on Mice (v/v) Compared to AmB and **1** (S44HP)

compd	LD_{50} (mg/kg)	MTD (mg/kg)
AmB	2.8 (1.74−2.69)	2.01 (1.73−2.14)
1	2.2 (1.62−2.97)	0.64 (0.47−0.86)
6	16.4 (13.9−18.9)	11.9 (10.3−13.6)

On the basis of the AmB ion-channel model where the proximity of neighboring molecules is affected by interaction between carboxyl and amino groups, it was hypothesized that inter- and intramolecular hydrogen bonds play critical roles in AmB−AmB and AmB−sterol interactions.[22,25,26] Our data demonstrate that the conversion of the free C16 carboxy group on S44HP to an amide does not always lead to the loss or decrease of antifungal activity, and this is in accordance with results obtained by previous investigators for AmB amides.[2,6,9] However, the presence of a polar group (OH, NH_2, or COOH) on the amide moiety appears to be important for activity. Amides of L-alanine or L-valine methyl esters (**10**, **11**) were considerably less active than amide of glycine ethyl ester (**7**) or amides of L or DL-serine methyl esters (**8**, **9**) which retain good activity comparable with that of the parent antibiotic. The presence of hydroxyl group on the amides of L or DL-serine (**8**, **9**) also did not lead to serious decrease of activity. Derivatives with bulky substituents on amide moiety (**16**), even containing several hydroxyl groups (**12** or **13**), had lower antifungal activity. The size of a substituent containing a polar group seems important: amide of ethanolamine (**4**) or 2-amino-2-methylpropan-1,3-diol (**12**) was less active than amide of propanol-3-amine (**5**). Among the derivatives of S44HP substituted at the amino group, derivatives containing an aromatic substituent (**17**, **18**) were the least active. The size of the substituent carrying a polar group also appears to be important, as the product of Amadori rearrangement obtained from disaccharide was less active (**23**) than the monosaccharide derivatives (**21**, **22**). It shall be noted that the mono-*N*-(3-aminopropyl)-S44HP (**19**) was less active than the disubstituted derivative (**20**), suggesting that the presence of polar groups and the size of the substituent introduced at carboxyl or aminogroup are important for the demonstration of antifungal activity of S44HP polyene macrolide.

Hemolysis assays for the novel polyene macrolide derivatives were performed by monitoring their ability to cause lysis of erythrocytes in defibrinated human blood under buffered conditions (Table 2). Hemolytic activities of amide **7** and monoalkyl derivative **19** (38−76%, concentration 5 μg/mL) were close to that of the parent antibiotic. Amides **2**−**6**, **8**, **9**, **14**, **15** and *N*-alkyl derivatives **20**−**23** showed considerably lower hemolytic activity (2−24%, concentration 5 μg/mL), compared with the activity of **1** and AmB (30−64%, concentration 5 μg/mL).

On the basis of these tests, we selected S44HP derivative **6**, exhibiting the highest activity against four fungal strains and lower hemolytic activity compared to AmB and **1** (S44HP) for in vivo studies. This analogue, along with AmB and **1**, was tested for acute toxicity and was shown to be several times less toxic than AmB and **1** (Table 4). Lower toxicity of **6** permitted us to study the efficacy of this compound in the mouse model infected with *Candida albicans* (strain ATCC 14053) (Figure 4). Almost no colonies in kidney tissue were observed when the compound was

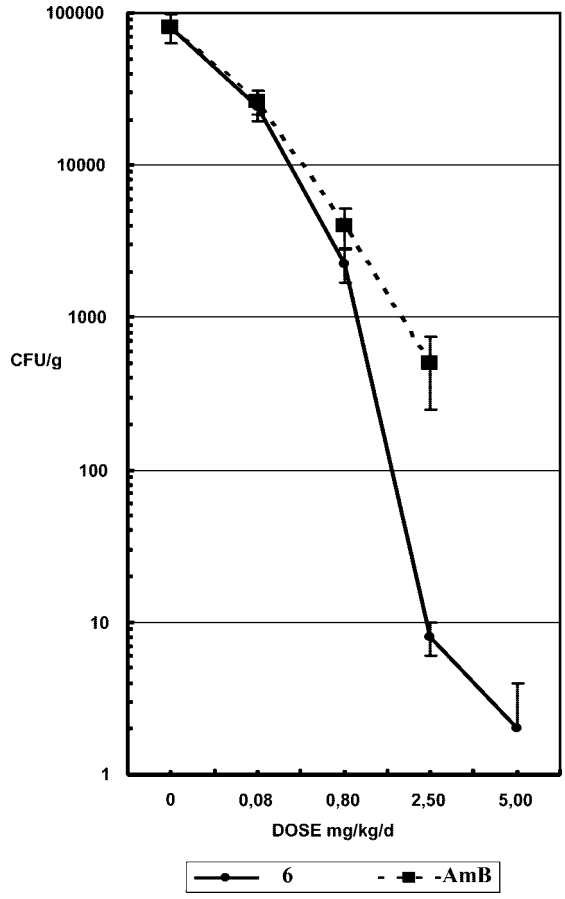


Figure 4. Efficacy of **6** in comparison with AmB after treatment for 4 days daily. CFU/g is the number of colony forming units on 1 g of kidney tissue.

administered in the once-a-day dose of 5 mg/kg over 4 days. This effect was not observed with the highest tolerated doses of AmB or **1**. These data strongly suggest that the new S44HP derivative **6** may become a lead compound for further development of safer polyene macrolides to be used for treatment of systemic fungal infections.

Conclusion

The amides, *N*-aminoacyl-, *N*,*N*-dialkyl-, *N*-monoalkyl derivatives, and the products of Amadori rearrangement reaction for genetically engineered heptaene nystatin analogue S44HP were prepared. Testing of these analogues in vitro expanded our understanding of the structure−activity relationship of polyene macrolides and allowed selection of the most active and least hemolytic compound **6**. In vivo experiments confirmed superiority of this S44HP derivative over AmB, the only polyene macrolide currently used for treatment of systemic fungal infections. This analogue might therefore represent a promising lead compound for the optimization and further development of safe and efficient antifungal drugs for human therapy. It is important to emphasize that while in vitro antifungal activities of novel semisynthetic derivatives were comparable to those of AmB and **1** (S44HP), the in vivo studies clearly suggested superior pharmacological properties of the novel derivative **6** over AmB. The data obtained in this study also imply that the combination of biosynthetic engineering and chemical modification is a powerful approach to generation of new drug leads.

Experimental Section

Materials and Chemicals. Polyene macrolide **1** (S44HP) was produced and purified as described in ref 27.

Chemistry. Amides 2−16 (Method A). To a mixture of **1** (18 mg, 0.02 mmol) and 0.06 mmol of the appropriate amine hydrochloride dissolved in 0.3 mL of DMSO was added, portionwise, Et_3N to adjust to pH 8 and afterward during 15 min 0.03 mmol of PyBOP reagent. The reaction mixture was stirred at room temperature for 1 h. Subsequent addition of diethyl ether (∼3 mL) to the reaction mixture led to an oily residue, which was shaken successively with diethyl ether (3 mL × 2). After addition of 5 mL of acetone to this oil, a yellow precipitate of amide was formed. The precipitate was filtrated, washed with acetone, and then dried in vacuo. The yields of all amides were 70−90%. All samples were obtained with purity of more than 94% by HPLC.

Crude **6** (88% of purity by HPLC data and containing salts) was additionally purified by column chromatography on Sephadex G-25 (Pharmacia Fine Chemicals AB, Uppsala, Sweden). Column was performed using water. Purity of the fraction was controlled by HPLC, resulting in target amide **6** with a purity of >95% by HPLC.

The physicochemical data for these compounds are summarized in Table 1.

Amides 5 and 14 (Method B). To a solution of **1** (500 mg, 0.54 mmol) in 6 mL of the mixture DMFA−MeOH (5:1) and 0.06 mL of pyridine was added 290 mg (0.73 mmol) FmocOSu portionwise. The mixture was stirred for 5 h and was poured into an excess of ether. The residue was filtered, washed by ether, and dried in vacuo. The obtained crude *N*-Fmoc S44HP (purity 81% by HPLC) was purified by column chromatography on silica gel in a solvent system of $CHCl_3$−MeOH−H_2O−NH_4OH (260:120:14:1) to yield 170 mg (40%) of *N*-Fmoc-S44HP of 95% purity. To a stirred solution of 80 mg (0.07 mmol) of *N*-Fmoc-S44HP in 1 mL of DMSO (0.21 mmol) of the corresponding 3-hydroxypropylamine or 4-*N*-(2-hydroxyethyl)piperazine were added hydrochlorides. Then 55 mg (0.105 mmol) PyBOP was added portionwise to the reaction mixture during 15 min; the pH of reaction mixture was kept at 7−7.5 by adding Et_3N. The reaction mixture was stirred at room temperature for 1 h. Subsequent addition of diethyl ether (5 mL) to the reaction mixture led to an oily residue, which was shaken successively with diethyl ether (5 mL). Addition of acetone (10 mL) led to the yellow precipitate, which was filtrated off, washed with acetone, and dried. Both derivatives were purified by column chromatography on silica gel in $CHCl_3$−MeOH−NH_4OH, 160:40:1 (for *N*-Fmoc- **5**), and $CHCl_3$−MeOH−H_2O−HCOOH, 260:120:14:1 (for N-Fmoc- **14**) in yields of ∼30−35%. The Fmoc group from the obtained derivatives was removed using piperidine (0.1 mL) in 3 mL of DMSO/MeOH, 3:1, for 1 h at room temperature. The resulting amides **5** and **14** were precipitated by diethyl ether with purity of more than 95% by HPLC. The analytical data for **5** and **14** are summarized in Table 1.

***N*-Alkyl Derivatives 17 and 18.** To a solution of 2-sulfobenzaldehyde or 4-*N*,*N*-dimethylaminobenzaldehyde (0.065 mmol) and **1** (20 mg, 0.22 mmol) in DMF (2 mL) was added $NaBH_3CN$ (4.1 mg, 0.065 mmol). The reaction mixture was kept at 37 °C for 20 h. Addition of diethyl ether (10 mL) led to an oily residue, which was shaken successively with diethyl ether (10 mL × 2). Yellow precipitate was formed after addition of acetone (10 mL). The precipitate was filtered off, washed with diethyl ester, and dried. The obtained solid was purified by flash chromatography ($CHCl_3$−MeOH−H_2O−HCOOH, 130:60:10:1). Fractions containing the desired compound were collected, the solution was concentrated down, and the addition of diethyl ester gave a yellow precipitate that was filtered off, washed with diethyl ester, and dried in vacuo. Yields were 40−45%. The purity was more than 94% by HPLC. The analytical data for **17** and **18** are summarized in Table 1.

***N*-Alkyl Derivatives 19 and 20.** To a solution of *N*-Fmoc-3-aminopropanal (160 mg, 0.054 mmol) (obtained from 3-aminopropanol by (1) protection of the amino group with FmocOSu, (2) oxidation hydroxy group with Dess−Martine periodinane[13] and **1** (100 mg, 0.011 mmol) in DMF (3 mL) was added $NaBH_3CN$ (34 mg, 0.054 mmol). The reaction mixture was kept at 37 °C for 20 h. Boron anions were removed from the reaction mixture by treating with boron-specific resin Amberlit IRA-743. The yellow precipitate was filtered off and purified by flash chromatography on Merck silica gel for column chromatography (0.040−0.063 mm) in gradient system $CHCl_3$−MeOH−HCOOH (300:100:1) → $CHCl_3$−MeOH−H_2O−HCOOH (130:60:10:1) to yield *N*-[*N*-Fmoc-3-aminopropyl]-S44HP and *N*,*N*-di-[*N*-Fmoc-3-aminopropyl]-S44HP as yellow solid. To a solution of *N*-[*N*-Fmoc-3-aminopropyl]-S44HP or *N*,*N*-di-[*N*-Fmoc-3-aminopropyl]-S44HP in DMSO (3 mL) was added piperidine (0.1 mL). After 2 h at room temperature, diethyl ether (∼7 mL) was added and an oily residue formed, which was shaken successively with diethyl ether (7 mL × 2). Addition of acetone (10 mL) gave a yellow precipitate which was filtered off, washed with diethyl ester, and dried in vacuo. The yield for *N*-(3-aminopropyl)-S44HP 10 mg (12%) and for *N*,*N*-di-(3-aminopropyl)-S44HP was 17 mg (20%). The purity was more than 95% by HPLC. The analytical data for **19** and **20** are summarized in Table 1.

***N*-Ketosyl Derivatives (Products of Amadori Rearrangement) 21−23.** Appropriate monosaccharide (D-glucose or D-galactose) or disaccharide (lactose) (0.086 mmol) was added to a solution of **1** (40 mg, 0.043 mmol) in DMF (2 mL). The reaction mixture was kept at 37 °C for 20 h, and then the solution was added dropwise to diethyl ether (50 mL). The resulting precipitate was filtered off, washed with diethyl ester, and dried. The obtained yellow precipitate was purified by flash chromatography ($CHCl_3$−MeOH−H_2O−HCOOH, 130:60:10:1). Fractions containing the desired compound were collected, and the solution was concentrated. The addition of diethyl ester then gave a yellow precipitate which was filtered off, washed with diethyl ester, and dried in vacuo. All samples of **21**, **22**, and **23** were obtained in yields of 40−45% (Table 1).

***N*-Aminoacyl Derivative 24.** N^{α},N^{ε}-Di-(9-fluorenylmethoxycarbonyl)-L-lysine *N*-oxysuccinimide ester (221 mg, 0.33 mmol) and Et_3N (15.3 μL, 0.11 mmol) were added to a solution of **1** (100 mg, 0.11 mmol) in dry DMF (2 mL). The reaction mixture was kept at 37 °C for 1 h, and then H_2O (5 mL) was added. The mixture was extracted with *n*-BuOH (3 × 5 mL). Organic fractions were combined and washed with 0.01 N HCl (1 × 5 mL) and H_2O (3 × 5 mL). The solution was concentrated. The addition of diethyl ester gave yellow precipitate, which was filtered off, washed with diethyl ester, and purified by flash chromatography with $CHCl_3$ → $CHCl_3$−MeOH (13:3), followed by $CHCl_3$−MeOH−H_2O−HCOOH conc (1300:400:50:1). Fractions containing the desired compound were collected, the solution was concentrated down, and the addition of diethyl ester gave a yellow precipitate which was filtered off, washed with diethyl ester, and dried in vacuo. Piperidine (0.1 mL) was added to a solution of the isolated yellow solid (30 mg) in DMSO (3 mL). After 2 h at room temperature, diethyl ether (∼7 mL) was added and the oily residue formed was shaken successively with diethyl ether (7 mL × 2). Addition of acetone (10 mL) gave yellow precipitate **24** which was filtered off, washed with diethyl ester, and dried in vacuo. Yield was 25 mg (22%). The purity was more than 96% by HPLC. The analytical data for **24** are summarized in Table 1.

Biology. Determination of in Vitro Antifungal (1) and Hemolytic Activities. Determination of antifungal activity (MIC values) was done as described previously.[7,18,21] Antifungal susceptibility testing of yeasts and filamentous fungi (molds) was done by using the broth microdilution method described in NCCLS documents M27-A[23] and M38-A,[24] respectively (Tables 2 and 3).

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Supporting Information Available: Chemistry (general part); HPLC tracings for the most active compounds **5**−**7**, **14**, **20**; ^1H NMR results for compound **21**; experimental details for in vitro, acute toxicity, and mouse efficacy studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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