DOI: 10.1002/chem.200900231

Synthesis and Biophysical Studies on 35-Deoxy Amphotericin B Methyl Ester

Alex M. Szpilman, Damiano M. Cereghetti, Jeffrey M. Manthorpe, Nicholas R. Wurtz, and Erick M. Carreira*[a]

Abstract: The use of molecular editing in the elucidation of the mechanism of action of amphotericin B is presented. A modular strategy for the synthesis of amphotericin B and its designed analogues is developed, which relies on an efficient gram-scale synthesis of various subunits of amphotericin B. A novel method for the coupling of the mycosamine to the aglycone was identified. The implementation of the approach has enabled the preparation of 35-deoxy amphotericin B methyl ester. Investigation of the antifungal activity and efflux-inducing ability of this amphotericin B congener provided new clues to the role of the 35-hydroxy group and is consistent with the involvement of double barrel ion channels in causing electrolyte efflux.

Keywords: amphotericin B · antifungal agents · natural products · structure–activity relationship · total synthesis

Introduction

Amphotericin B (**1**), a prominent member of the mycosamine family that also includes nystatin (**2**), candidin (**3**) and rimocidin (**4**), remains the treatment of last resort for systemic fungal infections.[1,2] Since amphotericin B (**1**) entered the clinic in 1958, its use has been relegated to closely monitored treatment in hospitals, due to a number of debilitating side effects.[3] Its use has been extended to treat multi-drug resistant types of the *Leshmania* parasite.[4] Leshmaniasis is at present one of the most common parasite infections worldwide, second only to malaria. As the first member of the mycosamine family to have its structure fully elucidated,[5] amphotericin B (**1**) has attracted wide attention from synthetic groups. Numerous synthetic approaches have been reported since the early 1980s.[6] However, to date only one total synthesis has been accomplished.[7] In addition, syntheses of the aglycones of candidin (**3**) and rimocidin (**4**) have been documented (Figure 1).[8]

The ability of amphotericin B (**1**) to cause efflux of intracellular electrolytes was observed early on and linked to its fungicidal activity.[9] Further studies led to the suggestion by

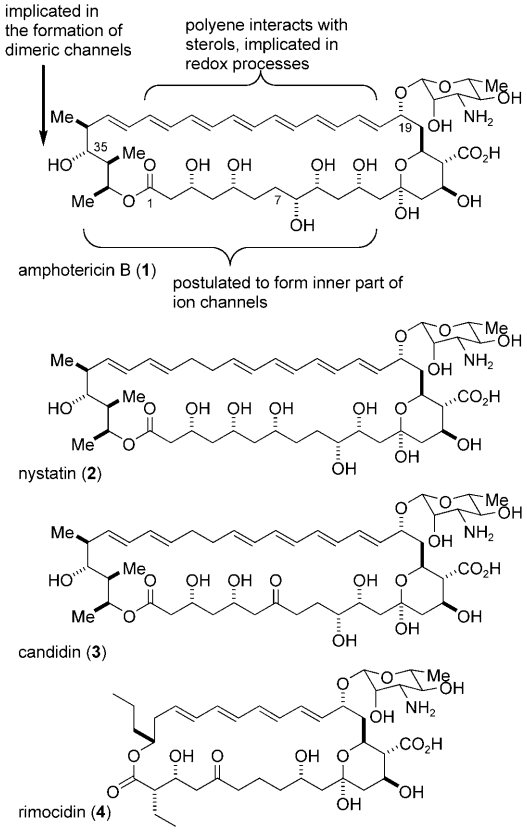


Figure 1. Structure of amphotericin B (**1**) and related mycosamine polyketides.

[a] Dr. A. M. Szpilman, Dr. D. M. Cereghetti, Dr. J. M. Manthorpe, Dr. N. R. Wurtz, Prof. Dr. E. M. Carreira
Laboratorium für Organische Chemie der ETH-Zürich
HCI H 335, Wolfgang Pauli Strasse 10
8093 Zurich (Switzerland)
E-mail: carreira@org.chem.ethz.ch

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200900231.

Finkelstein et al. that electrolyte efflux could be accounted for by the formation of barrel-like transmembrane ion channels.[10] These constructs are believed to arise from 4–12 molecules of amphotericin B (**1**) and are constituted in the fungal membrane through a process of self-assembly. This model is commonly referred to as the barrel-stave model.[1b]

The relatively strong electrolyte efflux observed for ergosterol containing membranes in comparison to cholesterol containing membranes was proposed to derive from the active participation of sterols in the self-assembly process and/or from the incorporation of the sterols as an integral part of the ion channel construct.[11] An alternative mechanism of action has also been proposed. According to this hypothesis the polyene moiety participates in redox processes that upset the intracellular redox balance.[12] In spite of numerous studies a full understanding of the molecular mechanism of action of amphotericin B (**1**) remains elusive.[1b] It could very well be that multiple fungicidal mechanisms are operative.

An intriguing discussion surrounding the barrel-stave model, relates to the discrepancy between the overall length of amphotericin B (**1**) and that of the typical fungal cell membrane (21 and 40 Å, respectively).[1b,5] This incongruity has been addressed in models that suggest constriction of the cell membrane around the site of the ion channel (Figure 2a) or the formation of ion channels consisting of two AmB subunits oriented in a tail-to-tail fashion (Figure 2b).[1b] Computational studies have pinpointed specific structural elements of amphotericin B (**1**) as potential "hot spots" of importance for antifungal activity (Figure 1).[13] The C35-hydroxyl unit has been singled out and hypothesized to be essential for the formation and stabilization of these dimeric ion channels through hydrogen bonding. Baginski et al. noted that: "…Concerning the interactions between AmB molecules in the channel, we suggest eliminating these hydroxyl groups or substituting related (e.g., methoxy or methyl) groups. However, we are aware that selective modification of the hydroxyl groups involves a very difficult chemical synthesis task. Other AmB hydroxyl groups are very similar; probably only through total synthesis from fragments can AmB derivatives be prepared with modified hydroxyl groups in certain positions."[13]

Recently the use of amphotericin B analogues as probes for various aspects in the barrel-stave model has emerged as a powerful tool for the study of its mechanism of action and associated toxicity.[1b] Access to these analogues has been achieved through semisynthesis as well as through genetic modification of the amphotericin B producing polyketide synthase of *Streptomyces nodosus*.[14–17] In line with the remarks of Baginski et al., we proposed that a fully synthetic access to amphotericin B analogues would be a powerful complement to these methods as they would in principal render any structural part of the amphotericin B backbone amenable to modification.

In this paper, we deliver a full account of our synthetic preparation of 35-deoxy amphotericin B methyl ester and biophysical studies that revealed the significance of the C35-hydroxy group for biological activity.[18] We also disclose alternative routes to the C1–C13 and C33–C37 moieties.

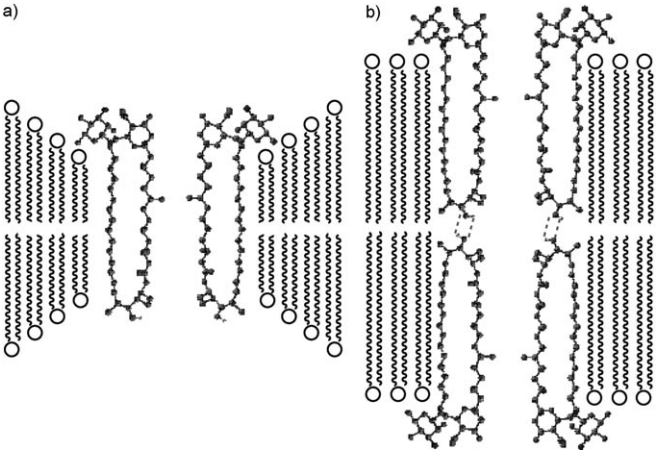
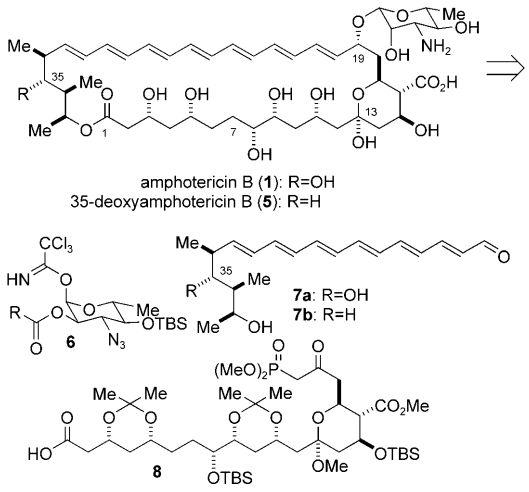


Figure 2. Barrel-stave models for ion channel formation in the fungal membrane.

Our synthetic strategy is outlined in Scheme 1. The structure of amphotericin B (**1**) may be divided into three principal components: the mycosamine sugar **6** as well as the C1–C20 and C21–C37 moieties that collectively comprise the macrocycle.[19] Our approach is predicated on a strategy in which each of these is prepared in a synthetically efficient and flexible manner, thus enabling a practical strategy for the molecular editing of amphotericin B (**1**).[20]



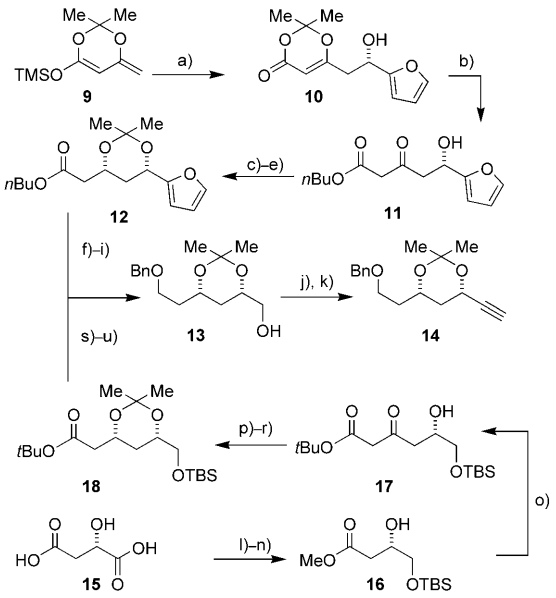
Scheme 1. Retrosynthetic analysis of amphotericin B (**1**).

Results and Discussion

Taking note of the inherent latent elements of symmetry in the C1–C13 moiety we pursued a strategy that would allow access to both the C1–C7 and C8–C13 subunits using identical tactics.[7] To this end, we developed two complementary syntheses of the C1–C7 and C8–C13 fragments: One based on catalytic asymmetric reactions developed in our labs and

one based on starting materials from the chiral pool (Scheme 2). The former involves the copper-tol-BINAP (tol-BINAP = 2,2′-bis(di-*p*-tolylphosphino)-1,1′-binaphthyl) catalyzed enantioselective aldol addition of bis-enol ether **9** to commercially available furylaldehyde.[21] In order to facilitate the gram-scale preparation of aldol adduct **10**, we investigated the direct use of commercially available solvents. Fortuitously, the reaction could be successfully scaled to provide more than 160 g (0.67 mol, 99% yield) of aldol product in a single batch, using only 2 mol% of the in situ formed catalyst and HPLC grade THF (<0.02% water). Refluxing the acetonide enol ether **10** in butanol converted it into the keto *n*-butyl ester **11** in 92% yield. At this point the 1,3-*syn* diol motif was introduced by application of the boron-mediated Prasad reduction.[22] The hydroxyl ketone was treated with in situ formed or premade methoxydiethylboron to form a complex which was reduced with sodium borohydride (3 equiv) at −78°C. The resulting boron ester was hydrolyzed using hydrogen peroxide in water/THF leading to liberation of the free *syn*-diol. The unpurified diol was protected as the acetonide using pyridinium *p*-toluenesulfonate (PPTS; 0.9 mol%) as the catalyst and 2,3-dimethoxypropane as a co-solvent with DMF. Subsequently, the carboxylic ester was converted into a primary alcohol by the action of 2 equivalents of $LiAlH_4$ at 0°C (68% yield).

The synthetic plan required the C1 alcohol to be protected. This was achieved by deprotonating it with potassium hydride (1.55 equiv) and alkylation by benzyl bromide (1.04 equiv) in the presence of 12 mol% tetrabutylammonium iodide as a catalyst. The furan was cleaved oxidatively by ozone and the resulting ozonide was reduced in situ by triphenylphosphine (1.5 equiv). Finally, the revealed carboxylic acid was converted into a methyl ester by addition of diazomethane to the reaction vessel. The yield for this three-step-one-pot transformation was 66%. The methyl ester was dissolved in THF and reduced at 0°C to a primary alcohol using solid lithium aluminium hydride (1 equiv). Unfortunately, the product proved difficult to extract from the heterogeneous reaction mixture. Addition of sodium sulfate decahydrate, followed by filtration, concentration at reduced pressure and flash chromatography led to the isolation of alcohol **13** in only 39% yield (see below). Subsequent oxidation of the alcohol to an aldehyde can be achieved by reaction with 1.2 equivalents of the Dess–Martin periodinane in the presence of 10 equivalents of pyridine in dichloromethane[23] in 80% yield. However, for large-scale applications, the aldehyde was best prepared by oxidation of the alcohol in dichloromethane by the action of buffered bleach (1 equiv, phosphate buffer pH 8.6)) and TEMPO (1 mol%)/potassium bromide (10 mol%) as catalysts at 0°C.[24] This method produces the aldehyde in quantitative yield and also has the advantage that the air-sensitive aldehyde can be used directly without need for time consuming chromatographic purification. Furthermore, this protocol obviates the need to produce the Dess–Martin reagent on large-scale. Exposure of the unpurified aldehyde to a slight excess (1.3 equiv) of the Ohira reagent under mild basic conditions

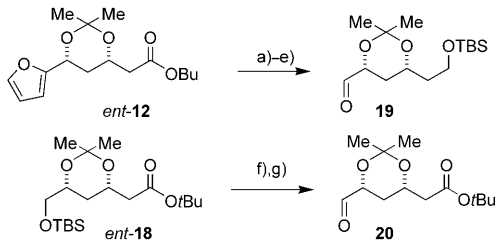


Scheme 2. a) 2 mol% CuF_2, 2.2 mol% (*R*)-tol-BINAP, 4 mol% TBAT, 1 equiv furylaldehyde, THF, −78°C, 99% yield, 94% *ee*; b) *n*BuOH, 110°C, 92%; c) 1.1 equiv Et_2BOMe, 3 equiv $NaBH_4$, MeOH, THF, −78°C; d) H_2O_2 water/THF; e) 0.9 mol% PPTS, $(MeO)_2CMe_2$, DMF, 72% over 3 steps; f) 2 equiv $LiAlH_4$, THF, 0°C, 68%; g) 1.55 equiv KH, 1.05 equiv BnBr, 12 mol% $(nBu)_4NI$, THF/DMF 10:1, 95%; h) O_3 CH_2Cl_2/MeOH 1:1, then 1.5 equiv Ph_3P, then CH_2N_2, 66%; i) $LiAlH_4$, THF, 0°C, 39%; j) 1 mol% TEMPO, 10 mol% KBr, NaOCl, pH 8.6 buffer, CH_2Cl_2, 0–7°C; k) $EtO_2CC(N_2)P(O)(OEt)_2$, K_2CO_3, MeOH, 51% over 3 steps; l) $SOCl_2$, MeOH; m) $BH_3{\cdot}SMe_2$, cat. $NaBH_4$, THF, 0°C; n) TBSCl, imidazole, CH_2Cl_2, 0 to 23°C; o) LDA, *tert*-butyl acetate, THF, −78 to −10°C, 55% over 4 steps; p) Bu_3B, $NaBH_4$, MeOH, THF −78°C; q) H_2O_2, water/THF; r) 1 mol% PPTS, 1 equiv 2-methoxypropene, CH_2Cl_2, −35°C to RT, 69% over 3 steps, d.r. 15:1; s) $LiAlH_4$, THF, −10°C; t) 1.2 equiv NaH, 2 equiv BnBr, 1 equiv $(C_4H_9)_4NI$, THF/DMF 10:1, 88% over 2 steps; u) excess HF/pyridine, THF, 0°C. TEMPO = tetramethylpiperidoxyl; TBAT = tetrabutylammonium triphenyldifluorosilicate; tol-BINAP = 2,2′-bis(di-*p*-tolylphosphino)-1,1′-binaphthyl.

(2 equiv of K_2CO_3/methanol) afforded the desired alkyne **14** in 51% yield over three steps.[25]

Alkyne **14** can also be accessed starting from commercially available, inexpensive (*S*)-malic acid (**15**). Esterification as the dimethyl ester was effected using two equivalents of thionyl chloride in methanol. The ester function proximal to the hydroxy group could then be reduced selectively using borane and 10 mol% sodium borohydride as a catalyst.[26] The C6-primary alcohol of the resulting diol was then protected chemoselectively by TBS-Cl and two equivalents of imidazole in dry dichloromethane. Earlier syntheses of acetal protected *syn*-diol **18** had relied on the use of sturdy but heavy TBDPS[27a] (M_W = 239) or TIPS[27b] (M_W = 157) protecting groups. A synthesis of a large natural product such as amphotericin B (**1**) must ultimately contend with the issue of mass efficiency: introduction of a unduly heavy protecting group early on demands that larger quantities of starting materials be carried through the early steps only to be subject to large weight loss later in the synthesis. For this reason we chose to rely on the lighter TBS (M_W = 115) group. For comparison, the molecular weight of methyl-3,4-

dihydroxy butanoate is a mere 134 g mol^{-1}. As a consequence of this decision, laborious operations such as large-scale chromatographic separations were made considerably easier, but, as a corollary, we had to overcome a rather curious problem in a subsequent acetonide protection step. Prasad reduction of **17**, this time using methoxydibutylboron but otherwise identical conditions as above, followed by treatment of the crude diol product with 10 mol% TsOH in 2,2-dimethoxypropane,[27] led to formation of a complex mixture of products in which the TBS group had undergone migration. Exposure of the diol to PPTS (10 mol%) and excess 2-methoxypropene afforded the bis(methoxypropane) acetal in poor yield. Only by using a combination of low temperature (−35 °C to room temperature), low PPTS loading (1 mol%) and one equivalent of 2-methoxypropane in dichloromethane could TBS migration be avoided and high yield ensured. Under these conditions the acetonide **18** was isolated in 70% yield (two steps). At this point the C1 ester group was reduced to the primary alcohol by lithium aluminum hydride in THF at −10 °C. Upon workup the crude alcohol was dried by distillation as an azeotrope with toluene and immediately subjected to the following step. This entailed benzylation of the alcohol by benzyl bromide (2 equiv) and tetrabutylammonium iodide after deprotonation by sodium hydride. The benzyl ether was isolated after purification in a yield of 88% (2 steps). Key to obtaining this result was the use of a commercial solution of lithium aluminium hydride in the reduction step rather than the solid form. As discussed above, the C8–C13 fragment **19**/**20** could be accessed using similar methods but starting from either *ent*-**12** or (*R*)-malic acid diethyl ester (Scheme 3).



Scheme 3. a) $LiAlH_4$, THF, 0 °C; b) 1.5 equiv TBSCl, 1.5 equiv imidazole, CH_2Cl_2, 0 to 23 °C, 84% over 2 steps; c) O_3, CH_2Cl_2/MeOH 1:1, then 1.5 equiv Ph_3P, then CH_2N_2; d) $LiAlH_4$, THF, 0 °C; e) 1.2 equiv DMP, 10 equiv pyridine, CH_2Cl_2, 80% yield; f) HF/pyridine, THF, 0 °C, 99%; g) 1 mol% TEMPO, 10 mol% KBr, NaOCl, pH 8.6 buffer, CH_2Cl_2, 0–7 °C, quantitative. DMP = Dess–Martin periodinane.

Initially, coupling of the C1–C7 and C8–C13 fragments was attempted by lithiation of alkyne **14** with 1 equiv of butyllithium and subsequent addition to aldehyde **20**. This reaction proceeded at a surprisingly low rate, ultimately requiring 14 h to reach completion. In addition, the propargylic alcohol **22** was formed with the undesired *S* epimer as the major product as shown by Mosher's ester analysis.[28,29] Indeed this stereochemical result was expected based on a polar Felkin–Anh model and is in line with the observations of Masamune and Hanessian.[6a,7e]

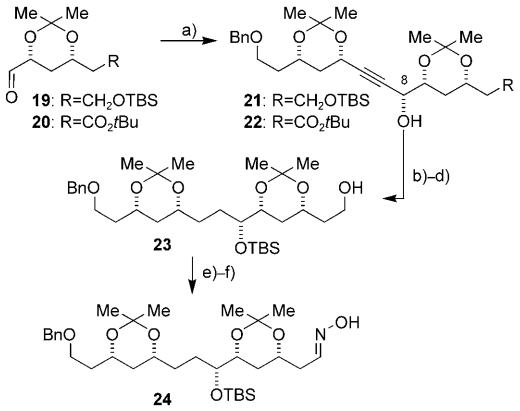
We recognized this outcome as an opportunity to test the asymmetric addition of zinc acetylides to aldehydes, disclosed from our laboratory.[30] In the situation at hand, the chiral system is faced with a challenging mismatched scenario, as evidenced from the result involving the organolithium reagent. In the experiment, coupling of **14** and **20** using 1.1 equivalents of zinc triflate, 1.2 equivalents of *N*-methylephedrine (NME) and 1.3 equivalents of triethylamine at room temperature in toluene lead to the formation of the desired *R* epimer **22** as the major stereoisomer in excellent yield and in only 2.5 h (Table 1, entry 2). Similarly, union of **14** and **19** under these conditions affords the desired *R* propargylic alcohol **21** (entry 3).

Table 1. Addition of alkyne **14** to aldehydes **19** and **20**.

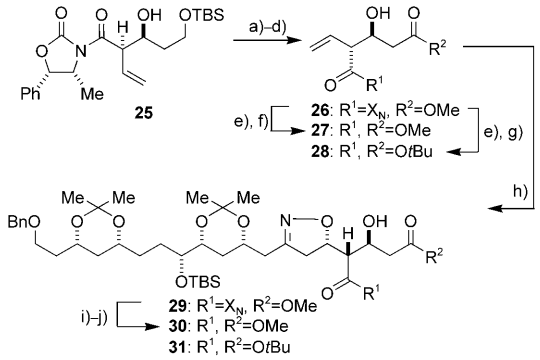
Entry	Aldehyde	Conditions	Yield [%] of **21** or **22**	d.r. C8-*R*/*S*
1	**20**	*n*BuLi, THF −78 °C	64 (**22**)	1:4
2	**20**	1.1 equiv $Zn(OTf)_2$, 1.2 equiv (−)-NME,[a] 1.3 equiv Et_3N, toluene	98 (**22**)	16:1
3	**19**	1.1 equiv $Zn(OTf)_2$, 1.2 equiv (−)-NME,[a] 1.3 equiv Et_3N, toluene	83 (**21**)	16:1

[a] NME = *N*-Methylephedrine.

Alkyne **22** underwent chemoselective reduction using H_2, 10 w/w Pd/C as the catalyst in the presence of $NaHCO_3$ (Scheme 4) in quantitative yield with no need for purification. Next, the free C8-alcohol function was protected by the action of excess TBS-Cl/imidazole in DMF. The TBS ether **23** was isolated in quantitative yield after flash chromatography. Subsequently, the ester functionality was reduced to an alcohol using lithium aluminium hydride (solution) and without purification oxidized to an aldehyde. This oxidation can be carried out with high yield using either the Dess–Martin periodinane or the TEMPO/bleach protocols described above. However, as discussed above TEMPO/bleach oxidation proved superior for large-scale work as the unpurified aldehyde could be used directly in the subsequent step. Accordingly, treatment of the crude aldehyde with hydroxylamine hydrochloride (3 equiv) in pyridine yielded the desired oxime **24** (84% over two steps). Only two chromatographic purifications were needed in the course of this five-step sequence, which greatly facilitated the preparation of over 40 g of **24**. In an alternative sequence, the alcohol function in **21** was protected as the TBS ether using TBSOTf, 2,6-lutidine as the base at 0 °C in dichloromethane (90%) yield. Hydrogenation of the alkyne was carried out as described above in 90% yield. Attempts to chemoselectively cleave the primary TBS group using CSA as the catalyst in methanol led to a complex mixture of mono TBS-protected and unprotected alcohol products. However, a yield of 89% of the desired alcohol **23** can be achieved by carefully monitoring the reaction of the bis-TBS-ether with excess HF/pyridine at 0 °C. Thus, the two routes intersected at compound **23**.



Scheme 4. a) See Table 1; R = COO*t*Bu: b) H_2, 3% w/w 10% Pd/C, $NaHCO_3$, MeOH; c) 2 equiv TBSCl, 4.2 equiv imidazole, DMF, 40°C; d) $LiAlH_4$, THF, 0°C 97% over three steps; R = CH_2OTBS; b) 1.1 equiv TBSOTf, 1.3 equiv 2,6-lutidine, CH_2Cl_2, 0°C, 90%; c) H_2, 10% w/w 10% Pd/C, $NaHCO_3$, MeOH, 90%; d) HF/pyridine, THF, 0°C, 89%; e) 1 mol% TEMPO, 10 mol% KBr, NaOCl, pH 8.6 buffer, CH_2Cl_2, 0°C; f) 3 equiv $HONH_2$·HCl, pyridine, 84% over 2 steps.



Scheme 5. a) HF/pyridine, THF, 0°C; b) 1.5 mol% TEMPO, 10 mol% KBr, NaOCl, pH 8.6 buffer/CH_2Cl_2, 0°C; c) 10 equiv $NaClO_2$, *t*BuOH/2-methyl-2-butene/2 M NaH_2PO_4, 0°C; d) $TMSCHN_2$, MeOH/EtOAc, 63% over 4 steps; e) 1.3 equiv LiOOH, water/THF; f) $TMSCHN_2$, MeOH/EtOAc, 63% over 2 steps; g) *tert*-butyltrichloroacetimidate, hexane, 15% over 2 steps; h) see Table 2; i) 1.3 equiv LiOOH, water/dioxane; j) $TMSCHN_2$, MeOH/EtOAc, 67% over 2 steps. X_N = Evans' norephedrine derived auxiliary.

A nitrile oxide cycloaddition was applied as a means to append the C14–C19 fragment, in the form of alkene dipolarophiles **26**, **27** and **28** to oxime **24** (Scheme 5, Table 2). The homo-allylic alcohols **26**–**27** could be conveniently prepared from the known Evans crotonate aldol product **25**.[31] First, the TBS-protecting group was removed using excess HF/pyridine in THF at 0°C. The free primary alcohol group was oxidized to the aldehyde using the standard TEMPO/bleach protocol[24] and then to the acid oxidation state using the Pinnick modification of the Lindgren oxidation ($NaClO_2$, excess 2-methylbutene in *tert*-butanol).[32] The carboxylic acid was methylated using TMS-diazomethane. This step was conveniently carried out by concentrating the combined extracts (ethyl acetate) of the oxidation reaction and using this solution, with the addition of about 25% methanol (by volume), as the reaction medium for the alkylation reaction. The acid was titrated with TMS-diazomethane until a faint yellow color persisted. The methyl ester **26** was purified by flash chromatography and isolated in 63% yield (4 steps). Alternative coupling partners **27** and **28** were fashioned by the extrusion of the Evans' auxiliary using one equivalent of lithium peroxide in water/dioxane.[33] Alkylation of the free carboxylic acid function could then be achieved by TMS-diazomethane to form the dimethyl ester **27** in 63% yield (2 steps) or by *tert*-butyl trichloroacetimidate in hexane with a trace of acetic acid to afford the bis-*tert*-butyl ester **28** in 30% yield (2 steps).

We have recently reported a stereospecific synthesis of broad scope of isoxazolines via cycloaddition of magnesium alkoxides of chiral allylic and homoallylic alcohols to in situ formed nitrile oxides.[34] To our disappointment, no trace of product could be isolated when the union of **24** and **26** was attempted under the conditions shown in Table 2, entry 1. Presumably, the rate of cycloaddition is outstripped by the competing dimerization of the reactive nitrile oxide. An alternative procedure involves stannylation of the oxime bis-

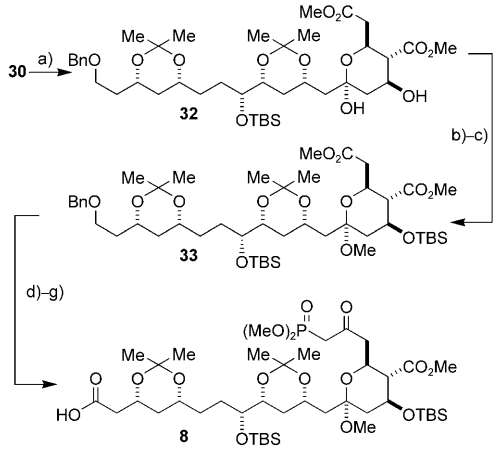
Table 2. Synthesis of isoxazolines **29**–**31** (see Scheme 5).

Entry	Alkene	Conditions	Yield [%]	d.r.
1	**26**	**24**, *i*PrOH, *i*PrMgCl, *t*BuOCl, −78°C to RT	0	–
2	**26**	**24**, 0.55 equiv $(Bu_3Sn)_2O$, *t*BuOCl, −30°C → RT	95 (**29**)	88:12
3	**27**	**24**, 0.55 equiv $(Bu_3Sn)_2O$, *t*BuOCl, −40 C → RT	81 (**30**)	72:28
4	**28**	**24**, 0.55 equiv $(Bu_3Sn)_2O$, *t*BuOCl, −40 C → RT	50 (**31**)	67:33

tributyltinoxide (0.55 equivalents) in dichloromethane prior to oxidation by *tert*-butyl hyperchlorite (1 equiv) at −30°C. Upon slow warming to room temperature the incipient nitrile oxide undergoes cycloaddition to alkene **26** to form the desired isoxazoline **29** in excellent yield and stereoselectivity (95%, 88:12).[35] The tributyltin nitrile oxide adduct likely acts as a reservoir that slowly releases the free nitrile oxide, thus minimizing the unproductive dimerization side reaction.

The significance of the nature of the C16-carbonyl substituent for the stereochemical outcome of the dipolar cycloaddition reaction was examined. The reaction of the dimethyl ester **27** with **24** under the conditions described above led to the formation of **29** but at the cost of a drop in yield and stereoselectivity (entry 3). Similarly, reaction of **24** with the di-*tert*-butyl ester **28** led to formation of the product **31** in moderate yield and stereoselectivity (entry 4). Thus, the role of the Evans' auxiliary in the reaction is not simply that of a bulky substituent. Stereoelectronic factors appear to be involved in determining the stereoselectivity of the reaction. Treatment of **29** with LiOOH led to efficient extrusion of the Evans' auxiliary.[33] The resulting free carboxylic acid function was protected as the methyl ester by TMS-diazomethane as discussed above. Subsequent purification by flash chromatography afforded the methyl ester **30** in 67% yield (over 2 steps).

Finally, the synthesis of the C1–C20 moiety of the aglycone was completed by the sequence shown in Scheme 6. Reduction of the N−O bond by excess Raney-Nickel in the presence of water led to formation of a hydroxyketone that immediately cyclized to form hemiacetal **32**. Unfortunately, the product was isolated in only 34% yield. Better results were achieved using molybdenum hexacarbonyl complex (1.6 equiv) in refluxing wet acetonitrile for 4 h.[36] The yield under these conditions was highly dependent on the workup procedure. Thus, when the crude tar-like product formed on removal of the reaction solvent was submitted directly to column chromatography the product was formed in only 55% yield. In contrast, suspension of the crude product with silica gel in ethyl acetate/hexane and filtration of the suspension through a short silica gel plug afforded the pure hemiacetal reproducibly in 80–86% yield. Thus, physical contact with silica gel was essential for hydrolyzing the putative molybdenum product complex. However, simple evaporation to dryness of the crude product on silica gel prior to flash chromatography lead to a yield of only 28%.[37]



Scheme 6. a) 1.6 equiv $[Mo(CO)_6]$, MeCN/water, 80°C, 86%; b) 20 mol% 2-chloropyridine·CSA, $(MeO)_2CMe_2$, MeOH; c) 1.1 equiv TBSOTf, 10 equiv 2,6-lutidine, CH_2Cl_2, 75% over 2 steps; d) 1.8 equiv $(MeO)_2P(O)CH_2Li$, THF, −35–0°C, 71% based on recovered starting material; e) 10% w/w 10% $Pd(OH)_2/C$, H_2, EtOAc; f) DMP, pyridine, CH_2Cl_2; g) 2 equiv $NaClO_2$, *t*BuOH, 2-methyl-2-butene, 2M NaH_2PO_4, 99% (over 3 steps).

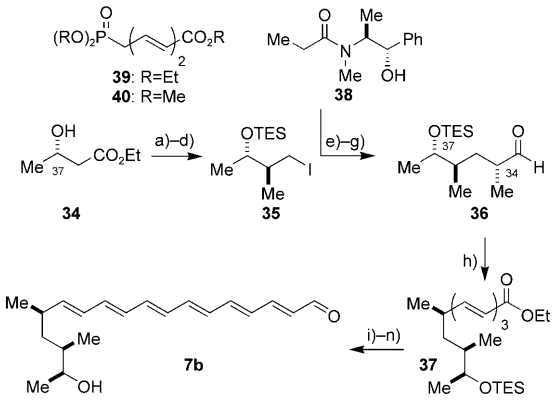
Hemi-acetal **32** was converted into the corresponding methyl ketal and the remaining free hydroxyl group was silylated using 1.1 equiv TBSOTf/2,6-lutidine (10 equiv) in dichloromethane at 0°C to afford **33** (Scheme 6). Curiously, methyl-ketal formation did not proceed using PPTS (0.1–1 equiv) as the acid catalyst and acetic acid trimethylorthoester as the dehydrating reagent and cosolvent with methanol.[7e] In contrast, the use of CSA (10 mol%) as the catalyst led to the formation of a mixture of mono-acetonide products. The need for a strongly acidic catalyst and conditions that would minimize loss of acetonide protection groups was reconciled by using chloro-pyridinum camphor sulphonate ($pK_a \approx 0.8$) in a methanol/2,2-dimethoxy propane 1:30 mixture. Using these conditions a yield of 75% was achieved for the methyl ketal formation–silylation sequence.

The keto-phosphonate function, an important handle for the subsequent macrocyclization, was introduced by selective addition of $(MeO)_2P(O)CH_2Li$ (1.8 equiv) to the less sterically hindered methyl ester in **33** at 0°C in THF. Optimal yields were achieved by running this reaction to approximately 70% conversion. Selectivity and thus overall yield dropped if the reaction was allowed to proceed to higher conversion. Subsequently, the benzyl ether was cleaved under reductive conditions. The resulting free primary alcohol was converted into an aldehyde by Dess–Martin periodinane[23] and finally to carboxylic acid **8** by a Pinnick/Lindgren oxidation.[32]

The completion of C1–C20 fragment **8** entails a formal total synthesis of amphotericin B (**1**).[7] Consequently, it is important to take stock of the route. A total of 28 steps were required to synthesize **8** from either bis-enol ether **9** or malic acid **15**. In both cases an overall yield of 4% was achieved, making these sequences the shortest and highest yielding synthesis of **8** to date. Indeed more than 8 grams of **8** have been prepared to date. Importantly, we had fulfilled the prerequisite of our synthetic strategy of having access to all the subunits of amphotericin B through efficient and scalable syntheses (see above).[19] This, set the stage for the synthesis of the first target, 35-deoxy amphotericin B (**5**).

The goal, to prepare 35-deoxy amphotericin B, dictated that we first access the C21–C37 moiety of the amphotericin B aglycone in which the C35-hydroxy function would be absent, namely **7b** (Scheme 1). At the outset, a synthetic approach to **7b** was by no means obvious as the exclusion of the C35-hydroxyl function in the target precluded the use of standard aldol methodology for its construction. We choose (*S*)-3-hydroxybutanoic acid ethyl ester **34** as the starting point and to rely on the use of substrate control in setting the stereogenic center at C36. This was done by a Fratér–Seebach alkylation.[38] The enolate of **34** was formed in situ using 1 equiv LDA, then 1.25 equiv methyl iodide in THF/HMPA was added. The reaction was performed at −78°C and proceeded with excellent stereocontrol (>95:5 d.r.) and in 92% yield (Scheme 7). Reduction of the ester group with lithium aluminum hydride in Et_2O at 0°C afforded a diol in 84% yield. This diol could be selectively iodinated at the primary alcohol position through an Appel reaction which employed 1.5 equiv triphenylphosphine, 1.4 equiv of iodine and 3.1 equiv of pyridine. The secondary alcohol was then protected as the TES ether in 93% yield by triethylsilyl triflate (1.04 equiv) and 2,6-lutidine (1.3 equiv) as the base in dichloromethane (0°C).

The powerful method developed by Myers,[39] which involves alkylation of the enolate of **38**, was used to set the stereogenic center at C34 of fragment **7b**. The enolate was formed using LDA as the base in the presence of lithium chloride at −78°C. The iodide **35** was then added at 0°C. The reaction mixture was worked up by addition to a biphasic system consisting of hexane and aqeous HCl. The hexane



Scheme 7. a) 2.1 equiv LDA, 1.25 equiv MeI, HMPA/THF 1:10, −78 °C, 92 %, d.r. 95:5; b) 2 equiv $LiAlH_4$, Et_2O, 0 °C, 84 %; c) 1.5 equiv Ph_3P, 1.4 equiv I_2, 3.1 equiv pyridine, THF, 81 %; d) 1.04 equiv TESOTf, 1.3 equiv 2,6-lutidine, CH_2Cl_2, 0 °C, 93 %; e) 2.1 equiv **38**, 4.3 equiv LDA, LiCl, THF, −78 to 0 °C, 78 %, brsm, d.r. 95:5; f) 3.9 equiv LDA, 3.9 equiv $BH_3{\cdot}NH_3$, THF, 0 °C to RT 97 %; g) 1 mol % TEMPO, 10 mol % KBr, NaOCl, pH 8.6 buffer/CH_2Cl_2, 0 °C, 98 %; h) 1.25 equiv **39**, 1.2 equiv LDA, THF, −78 to 0 °C, 78 %; i) 5 equiv DIBAL, CH_2Cl_2, −78 °C; j) 10 equiv MnO_2, CH_2Cl_2; k) 1.4 equiv **40**, 1.2 equiv LDA, THF, −78 to 0 °C, 55 % over 3 steps; l) HF/pyridine, THF, 0 °C; m) 6.3 equiv DIBAL, CH_2Cl_2, −78 °C; n) 10 equiv MnO_2, CH_2Cl_2, 62 % over 3 steps. brsm = based on recovered starting material.

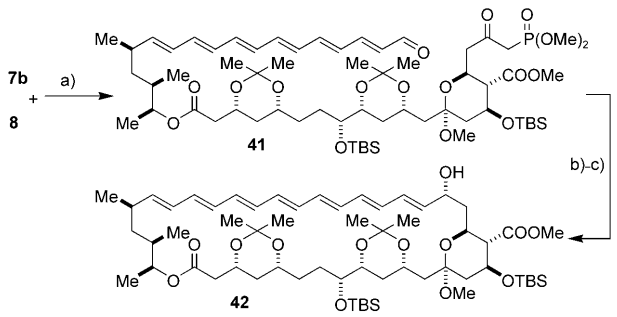
cosolvent served to protect the sensitive TES ether from the HCl while allowing slow quenching of the base. Excess **38** was crystallized out from a toluene solution of the crude product. The product was isolated after chromatography in 60 % yield along with 23 % recovered iodide. Reductive cleavage of the Myers' auxiliary using lithium amidoborohydride (LAB, 4 equiv) in THF reagent proceeded efficiently in 97 % yield even in the presence of the labile TES group. Again special attention had to be given to the acidic workup, which was carried out in a similar fashion to the alkylation step.

The alcohol was then oxidized to aldehyde **36** using the TEMPO/bleach protocol[24] and the resulting aldehyde was subjected to olefination using Horner–Wadsworth–Emmons reagent **39**, LDA in THF at −78 °C. The product triene **37** was obtained in 78 % yield. The ester functionality was reduced to the primary alcohol using excess DIBAL in dichloromethane at −78 °C. The unpurified alcohol was dissolved in dichloromethane and then oxidized by MnO_2 (10 equiv) to the aldehyde. Purification of the product was performed by simply filtering off the excess reagent and subjecting it to a Horner–Wadsworth–Emmons reaction this time with phosphonate **40**. Accordingly, using 1.4 equivalents of **40**, 1.2 equivalents of LDA as the base and THF solvent, the desired hexane product was obtained in 55 % (over three steps). All that remained was to adjust the oxidation stage of C20 to the aldehyde stage and liberate the protected C37 alcohol. The latter step was tackled first using excess HF/pyridine in THF at 0 °C. The crude product was subjected to DIBAL reduction (6.3 equiv) in dichloromethane and oxidation by MnO_2 (10 equiv) in the manner already described. The overall yield of the target aldehyde **7** for this three-step procedure was 62 %.

Compound **7b** was characterized by a strong yellow-orange hue identical to that of the corresponding fragment **7a** of amphotericin B.[7] In contrast to **7a**, **7b** proved insoluble in all solvents examined with the exception of dichloromethane. This proved a serious impediment in optimizing the subsequent coupling with carboxylic acid **8**. Even more problematic, **7b** was found to be unreactive towards a large variety of activated esters of **8**. For example, the use of DCC/DMAP[7c] or PYBOP, afforded none of the desired ester. Esterification using standard two stage Yamaguchi conditions[40] (i.e., 1.2 equiv of the Yamaguchi reagent and 1.2 equiv of triethylamine in THF followed by exchange of the solvent for dichloromethane and addition of **7b**) afforded the ester **41** in only 12 % yield. Exchanging strongly basic triethylamine for the milder 2,6-lutidine increased the yield somewhat although with poor reproducibility. Careful analysis of the by-products led to identification of the β-elimination product of **8** by HRMS. This implied that the activated esters of **8** in the presence of strong bases would undergo elimination of acetone at a rate greater than reaction with **7b**. Based on this observation a successful one pot protocol was developed, that avoided the use of strong bases such as DMAP (pK_a 9.2). Accordingly, a mixture of acid **8** with 4.6 equivalents of pyridine (pK_a 5.2) was added over 10 h to a stirred mixture of Yamaguchi's reagent (2.5 equiv), **7b** (3 equiv) and an additional 0.5 equivalent of pyridine. Under these conditions ester **41** was produced in a reproducible yield of 48–52 %. The Shiina reagent[41] was also examined, but did not offer any immediate advantage. Due to the instability of ester **41** it was used immediately after purification on silica gel. Excess alcohol **7b** could be recovered and recycled.

Horner–Wadsworth–Emmons (HWE) macrocyclization was carried out using six equivalents of potassium carbonate/[18]crown-6 (12 equiv) in toluene at 60 °C as described for the synthesis of natural amphotericin B aglycone.[7] Under these conditions, the red-colored unsaturated ketone is produced in a yield of 48–55 %. A legion of other conditions were examined, for example, LiCl/DBU,[42] $Ba(OH)_2$[43] or $Li(OCH(CF_3)_2)$,[44] but unfortunately these methods afforded the product in lower yields (12–30 %). Reversing the order of steps, that is, coupling the two fragments through a HWE reaction, employing and closing the macrocycle by a macrolactonization reaction, also did not offer any advantages in terms of yield. Reduction of the ketone with sodum borohydride (10 equiv) to the unsaturated alcohol **42** (Scheme 8) proceeded in 73 % yield with excellent stereoselectivity and was accompanied by a gratifying, readily observable color change from bright red to pale yellow.[7a]

A final obstacle remained: the forging of the bond between the aglycone and the mycosamine appendage. The challenge of forming the glycosidic bond is partially a product of the poor nucleophilicity of the C19 alcohol which is both electronically deactivated and sterically hindered by virtue of its position inside the concave grove of the agly-



Scheme 8. a) 2.5 equiv 2,4,6-trichlorobenzoylchloride, 5.1 equiv pyridine, 3 equiv **7b**; CH_2Cl_2, 0°C, 48%; b) 6 equiv K_2CO_3, 12 equiv [18]crown-6, toluene, 60°C, 52%; c) 10 equiv $NaBH_4$, MeOH, 0°C, 73%.

cone. It is further compounded by the fact that mycosamine has a mannoside configuration and is attached as the thermodynamically less favored β-anomer. Indeed, the introduction of β-mannoside residues remains one of the greatest general challenges to carbohydrate chemistry.[45]

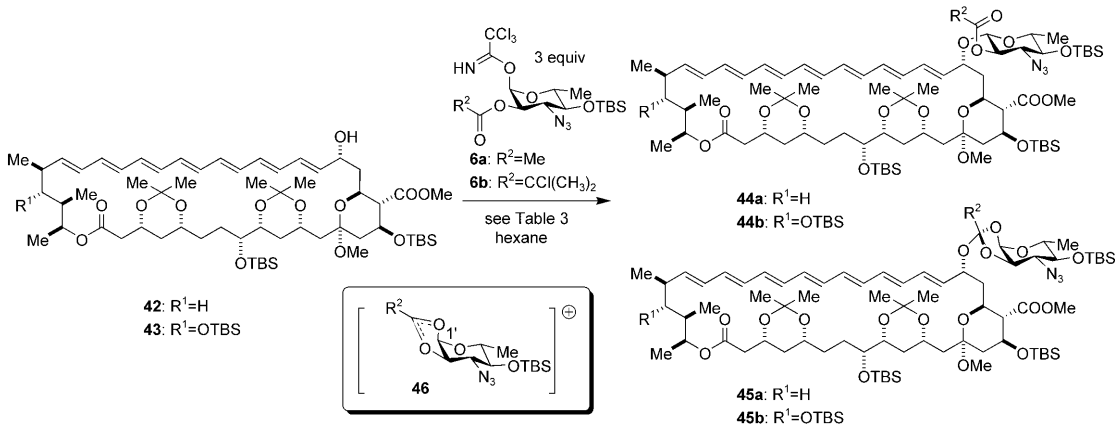
We brought a number of the powerful and widely applicable glycosidation methods that have appeared in the literature in the last two decades to bear on this problem with little to show for our efforts.[46] While the lack of success was distressing, it led us to re-examine the union of amphoteronolide **43** and trichloroacetimidate **6a** (Scheme 9, Table 3).[7d] This method employed 30 mol% PPTS (pK_a 5.2) as a mild-acid activator of the trichloroacetimidate and hexane as the solvent. The use of hexane inhibits formation of trichloroacetamide sugar adducts, a common problem, since trichloroacetamide is highly insoluble in hexane and precipitates from the reaction mixture. This protocol stood out by its ability to successfully afford glycoside **44b**, albeit in very low yield of 4%. Additionally, the reaction was marred by low conversion (75% of the starting alcohol **43** was recovered from the reaction) and formation of significant amounts (12%) of the orthoester **45b** as a side-product (Table 3, entry 1). Rearrangement[47] of orthoester **45b** to glycoside **44b** was attempted using a variety of acids ranging from BF_3 to PPTS. However, the inherent acid sensitivity of the aglycone conspired with the stability of the orthoester functionality to foil these efforts. Weak acids such as PPTS (0.3–2 equivalents) in $CDCl_3$ were unable to induce rearrangement while stronger acids such as methanesulfonic (pK_a −2.6) or triflic acid (pK_a −14) led to decomposition of the orthoester. The latter was usually accompanied by a transient blue color, possibly indicative of the formation of a polyene stabilized carbenium ion by the breaking of the C19–O bond.

Table 3. Introduction of the mycosamine moiety (Scheme 9).

Acceptor	Donor	Activator	Aglycone [mM]	**44** [%]	**45** [%]	**42** [%]
43	**6a**	PPTS[a]	7[7d]	4	12	75
43	**6b**	PPTS[a]	20	27	11	57
43	**6b**	CMPT[b]	20	43	23	–
42	**6b**	CMPT[c]	20	45	30	–

[a] 30 mol% [b] 50 mol% CMPT and 100 mol% 2-chloro-6-methylpyridine. [c] 10 mol% CMPT and 20 mol% 2-chloro-6-methylpyridine. CMPT = 2-chloro-6-methylpyridinium triflate.

Careful examination of the problem including mechanistic considerations pointed us in a productive direction. In the Schmidt reaction, the first step is the extrusion of trichloroacetamide by the action of a Lewis acid with concomitant formation of an oxocarbenium ion. When a C2′ ester group capable of providing anchimeric assistance is present, an acetoxonium ion (e.g., **46**) is formed. In the normal manifold attack of the aglycone on C1′ of the pyran ring leads to the formation of the glycoside (e.g., **44b**). However, it is well known that in the case of sterically hindered or electronically deactivated alcohols, such as amphotericin B aglycone **43**, attack may take place at the sterically less encumbered acetoxonium carbon leading to formation of orthoesters. We postulated that by increasing the spatial demand of the ester substituent R in **6** it would be possible to inhibit orthoester formation in favor of glycoside.[48] This approach was not without some potential problems, because of the need for subsequent hydrolysis of the directing ester group in the presence of the macrocyclic lactone. Accordingly, we turned our attention to esters possessing significant steric bulk



Scheme 9. Glycosidation of amphoteronolide **43** and 35-deoxy amphoteronolide **42** with trichloroacetimidates **6** (see Table 3).

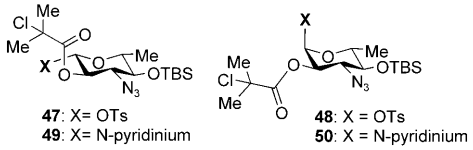
while being electronically disposed for hydrolysis under mild conditions (Table 3).

Accordingly we screened a range of esters as ancillary steering groups in the glycosidation reaction still using 30 mol% of PPTS as the activator and hexane as the solvent. The determination of yields was complicated by the fact that often the orthoester and glycoside products would elute together. However, the glycoside to orthoester ratio could be determined from the crude NMR since the characteristic H1′ signal of the glycoside typically at around 4.5 ppm could be clearly distinguished from the orthoester H1′ signal that commonly appeared around 5.6 ppm. The ability to cleave the steering group under mild conditions after glycosidation was a chief parameter in selection of esters for examination.

Dominant formation of orthoester **45b** resulted in the case of R^2 being $ClCH_2$- or CH_3CH_2-. A benzoyl ester in **6** affords a 1:1 mixture of orthoester **45** (R = Bz) and glycoside **44** (R = Bz). In contrast reaction of the more bulky **6b** (R = $Cl(CH_3)_2C$- with 30 mol% PPTS in the presence of **43** afforded the desired glycoside **44b** as the major product (entry 2). An additional finding of critical importance was that the 2-chloro-2-methylpropanoic (CMP) ester could be cleaved under mild basic condition (2 equiv K_2CO_3 in methanol) in 69% yield.[29]

Glycoside donor **6b** proved less reactive than **6a** towards aglycone **43**. This necessitated performing the glycosidation reaction at higher concentration to achieve conversion.[7d] In order to address the issue of low conversion, the activation of **6b** by PPTS was studied by NMR. Within two minutes of addition of 1 equivalent of PPTS to trichloroacetimidate **6b** in $CDCl_3$ the formation of β-tosylate **47** was observed. In the course of a few hours, **46** slowly converted into the more stable α-anomer **48**. While glucotosylates are able to glycosidate simple alcohols,[49] exposure of **47**/**48** to **43** did not lead to formation of product. As tosylate appeared to inhibit conversion, we surmised that substituting the tosylate for the less nucleophilic triflate as the acid counter anion should increase the reactivity of the activator/glycoside donor couple.

To our consternation reaction of **6b** with pyridinium triflate (30 mol%) and **43** only proceeded to about 30% conversion. NMR studies revealed the source of inhibition to be pyridine. Thus, one equivalent of pyridinium triflate was able to fully activate **6b** within 50 min leading to formation of a mixture of the epimeric pyridinium species **49**/**50**. The

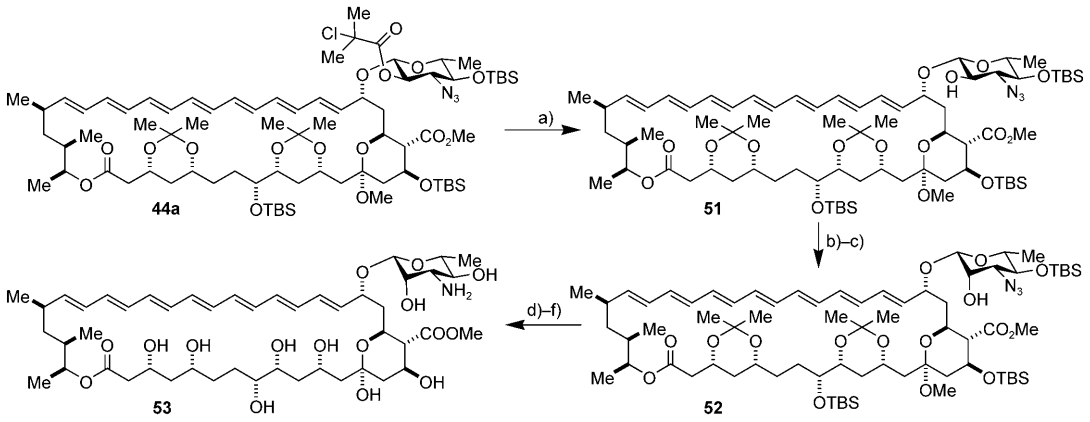


longer time required for activation using pyridinium triflate compared to PPTS was traced to the lower solubility of pyridinium triflate in $CDCl_3$. The triflate salts **49**/**50** proved highly unstable, decomposing slowly over the course of 24 h. Furthermore these species were unreactive towards aglycone **43**.

Accordingly, we investigated the use of sterically hindered pyridinium triflates as catalysts for the reaction. Extensive experimentation led to the identification of 2-chloro-6-methylpyridinium triflate (pK_a 2.8) as a highly efficient catalyst for the reaction. In contrast, 2,6-lutidinum triflate (pK_a 6.9) was not acidic enough to catalyze the reaction. As expected based on a previous experience with the activation of orthoesters, attempts to use triflic acid (10 mol%) led to decomposition of the aglycone.

Exposure of **6b** and **43** to 10 mol% of 2-chloro-6-methylpyridinum triflate in the presence of 20 mol% of 2-chloro-6-methylpyridine led to rapid and complete conversion of **43** into glycoside **44b** (43%) and orthoester **45b** (23%). Importantly, glycosidation of **42** under identical conditions afforded the desired glycoside **33b** in 45% yield (Table 3, entry 4). The CMP ester could be hydrolyzed under mild conditions (K_2CO_3, methanol/THF) to afford the C2′ alcohol **51** in 83% within 2 h (Scheme 10).

The stereogenic center at C2′ of **51** was now inverted by oxidation to the ketone and immediate reduction with



Scheme 10. a) 2 equiv K_2CO_3, MeOH/THF, 83% yield; b) 3 equiv $(CF_3CO)_2O$, 6 equiv DMSO, 6 equiv $(Me_2N)_2CO$, 6 equiv Et_3N, CH_2Cl_2/Et_2O, −78 °C to RT; c) 2 equiv $NaBH_4$, MeOH, 82% yield over 2 steps based on recovered **50**; d) excess HF·4py, MeOH, 40 °C, 72%; e) 20 mol% CSA, MeCN/water, 37% (6 cycles); f) 1.2 equiv Bu_3P, THF/MeOH/water, 67%.

$NaBH_4$ to afford protected 35-deoxy amphotericin B **52** (Scheme 10).[7d] The three TBS protecting groups were removed by the action of HF·4py complex in methanol. Removal of the acetonide groups proved problematic due to the acid sensitive nature of the unsaturated glycoside function. Best yields were obtained by performing the deacetalization using 20 mol% CSA in acetonitrile/water. The initially formed mixture of mono-acetonides was isolated by flash chromatography and resubmitted to the same conditions several times. Unfortunately, the overall yield of deprotected compound remained low. Importantly sufficient material was produced for completion of the synthesis. Thus, Staudinger reduction of the C3′ azide afforded 35-deoxy amphotericin B methyl ester (**53**). Reduction using propane dithiol/triethylamine (10 equivalents) proceeded in low yields and formation of by products. Attempts to hydrolyze the methyl ester to give free 35-deoxy amphotericin B were unsuccessful.[50] Ultimately, this was of no consequence as the biological profile of amphotericin B methyl ester (AME) is comparable to that of amphotericin B (**1**). Thus, the importance of the 35-hydroxy function for biological activity could be evaluated by direct comparison of the properties of methyl ester **53** to those of AME.

The antifungal activity of 35-deoxy amphotericin B methyl ester **53** and AME was evaluated against *Saccharomyces cerevisiae* (Table 4). Strikingly, **53** is more than 18-fold less active than AME. Further evaluation was done using the CAF2-1 strain of *Candida albicans*. Against this strain, **53** is less than 26-fold active compared to the parent AME. These results illustrate the importance of the 35-hydroxy group for activity. To further examine its role in the formation of ion channels, we studied the ability of 35-deoxy amphotericin B methyl ester (**53**) to cause K^+ efflux from large unilamellar vesicles (LUV), as monitored using potassium ion selective electrodes.[17,51] We utilized LUV with a membrane composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) with or without ergosterol as a component (Figure 3 and Supporting Information).[7] At 1 μM concentration 35-deoxy amphotericin B methyl ester (**53**) showed severely diminished ability to induce the leakage of K^+ compared with amphotericin B methyl ester. At the concentration of 0.1 μM or in pure POPC LUV no efflux could be observed. Only at exceedingly high concentrations (10 μM) could a weak efflux be detected (see Supporting Information).

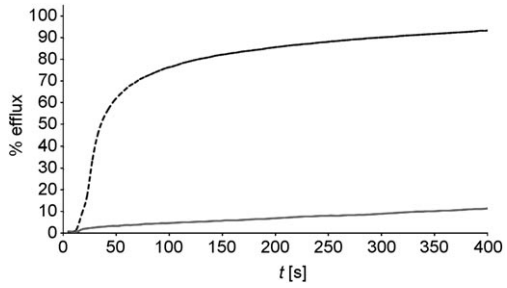


Figure 3. KCl efflux from LUV induced by **53** (——) and amphotericin B methyl ester (-----) measured via potentiometry. The substrate was added externally (as a DMSO solution) to afford a final concentration of 1 μM. LUV with a diameter of 100 nm and containing 13% ergosterol and 87% POPC in their membranes were utilized. LUV = large unilamellar vesicle, POPC = 1-palmitoyl-2-oleoyl-*sn*-glycero-phosphocholine.

Table 4. Antifungal activity (MIC) of amphotericin B methyl ester (AME) and 35-deoxy amphotericin B methyl ester.

Entry	Compound	*S. cerevisiae* BY4741 [μM]	*C. albicans* CAF2-1 [μM]
1	AME	0.25	0.10
2	**53**	4.60	2.60

[a] Measured according to the NCCLS protocol. See ref. [51] for experimental details.

Collectively these results underscore the pivotal role of the C35-hydroxyl group for the biological mechanism of action as well as lend support for ion channel formation as a necessary condition for the fungicidal effect of amphotericin B (**1**).[52,53] Importantly, it provides the first direct experimental support for the formation of tail-to-tail dimeric ion channels. Furthermore, the hydroxyl group is unlikely to play a role in oxidative processes related to the polyene function.[1b] Hence, the fact that the absence of this specific hydroxy group is of importance, argues against the oxidative damage mechanism.

Conclusion

In summary, we have demonstrated the molecular editing of amphotericin B (**1**) through diverted total synthesis.[20] An important feature of this stratagem was the development of an efficient synthetic strategy that allowed access all the moieties of amphotericin B (**1**) on a multi-gram scale. Using the synthetic strategy described we have studied the importance of the C35-hydroxy group of amphotericin B (**1**). Our results reveal the pivotal role of this group and are consistent with the formation of dimeric ion channels and their importance for the mechanism of action of amphotericin B (**1**). Furthermore, we have developed a glycosidation method that give improved yields of glycoside over orthoesters. Recent studies in our labs show the generality of this approach.[54]

Acknowledgements

This research was supported by ETH. Generous postdoctoral scholarships were provided by the Carlsberg foundation (A.M.S.), Fonds NATEQ (J.M.M.) and NSF (N.R.W.). We thank Professor Samuel Danishefsky for a stimulating discussion.

[1] a) *Macrolide Antibiotics, Chemistry, Biology and Practice* (Ed.: S. Omura), Academic Press, New York, **1984**; b) for a recent review see: D. M. Cereghetti, E. M. Carreira, *Synthesis* **2006**, 914–942.
[2] T. H. Sternberg, E. T. Wright, M. Oura, *Antibiot. Annu.* **1956**, 566–573.

[3] a) H. A. Gallis, R. H. Drew, W. W. Pickard, *Rev. Infect. Dis.* **1990**, *12*, 308–329; b) I. M. Hann, H. G. Prentice, *Int. J. Antimicrob. Agents* **2001**, *17*, 161–169.
[4] A. Lemke, A. F. Kiderlen, O. Kayser, *Appl. Microbiol. Biotechnol.* **2005**, *68*, 151–162.
[5] P. Ganis, G. Avitabile, W. Mechlin, C. P. Schaffner, *J. Am. Chem. Soc.* **1971**, *93*, 4560–4564.
[6] Selected synthetic approaches towards amphotericin B: a) S. Hanessian, S. P. Sahoo, M. Botta, *Tetrahedron Lett.* **1987**, *28*, 1143–1146; b) T. Berkenbusch, R. Brueckner, *Chem. Eur. J.* **2004**, *10*, 1545–1557; c) C. Bonini, L. Chiummiento, A. Martuscelli, L. Viggiani, *Tetrahedron Lett.* **2004**, *45*, 2177–2179; d) K. Karisalmi, K. Rissanen, A. M. P. Koskinen, *Org. Biomol. Chem.* **2003**, *1*, 3193–3196; e) K. Karisalmi, A. M. P. Koskinen, M. Nissinen, K. Rissanen, *Tetrahedron* **2003**, *59*, 1421–1427; f) G. Solladié, N. Wilb, C. Bauder, *Eur. J. Org. Chem.* **1999**, 3021–3026; g) G. Solladie, J. Hutt, *Tetrahedron Lett.* **1987**, *28*, 797–800; h) G. J. McGarvey, J. M. Williams, R. N. Hiner, Y. Matsubara, T. Oh, *J. Am. Chem. Soc.* **1986**, *108*, 4943–4952; i) J. M. Williams, G. J. McGarvey, *Tetrahedron Lett.* **1985**, *26*, 4891–4894.
[7] See: a) K. C. Nicolaou, T. K. Chakraborty, Y. Ogawa, R. A. Daines, N. S. Simpkins, G. T. Furst, *J. Am. Chem. Soc.* **1988**, *110*, 4660–4672; b) K. C. Nicolaou, R. A. Daines, J. Uenishi, W. S. Li, D. P. Papahatjis, T. K. Chakraborty, *J. Am. Chem. Soc.* **1988**, *110*, 4672–4685; c) K. C. Nicolaou, R. A. Daines, T. K. Chakraborty, Y. Ogawa, *J. Am. Chem. Soc.* **1988**, *110*, 4685–4696; d) K. C. Nicolaou, R. A. Daines, Y. Ogawa, T. K. Chakraborty, *J. Am. Chem. Soc.* **1988**, *110*, 4696–4705; e) for another seminal contribution see the synthesis of the aglycone: R. M. Kennedy, A. Abiko, T. Takemasa, H. Okumoto, S. Masamune, *Tetrahedron Lett.* **1988**, *29*, 451–454.
[8] For the synthesis of the candidin aglycon, see: I. Kadota, Y. Hu, G. K. Packard, S. D. Rychnovsky, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11992–11995; for the synthesis of the rimocidin aglycon, see: G. K. Packard, Y. Hu, A. Vescovi, S. D. Rychnovsky, *Angew. Chem.* **2004**, *116*, 2882–2886; *Angew. Chem. Int. Ed.* **2004**, *43*, 2822–2826.
[9] For a leading review, see: S. C. Hartsel, C. Hatch, W. Ayenew, *J. Liposome Res.* **1993**, *3*, 377–408.
[10] a) A. Finkelstein, R. Holz in *Membranes* (Ed.: G. Eisenman), Marcel Dekker, New York, **1973**, pp. 377–408; b) A. Marty, A. Finkelstein, *J. Gen. Physiol.* **1975**, *65*, 515–526; c) R. W. Holz, *Ann. N. Y. Acad. Sci.* **1974**, *235*, 469–479.
[11] a) B. De Kruijff, W. J. Gerritsen, A. Oerlemans, R. A. Demel, L. L. M. Van Deenen, *Biochim. Biophys. Acta Biomembr.* **1974**, *339*, 30–43; b) B. De Kruijff, W. J. Gerritsen, A. Oerlemans, P. W. M. Van Dijck, R. A. Demel, L. L. M. Van Deenen, *Biochim. Biophys. Acta Biomembr.* **1974**, *339*, 44–56; c) B. De Kruijff, R. A. Demel, *Biochim. Biophys. Acta Biomembr.* **1974**, *339*, 57–70; d) A. W. Norman, *Biochim. Biophys. Acta Biomembr.* **1972**, *290*, 1–14.
[12] a) J. Brajtburg, S. Elberg, D. R. Schwartz, A. Vertut-Croquin, D. Schlessinger, G. S. Kobayashi, G. Medoff, *Antimicrob. Agents Chemother.* **1985**, *27*, 172–176; b) M. L. Sokol-Anderson, J. Brajtburg, G. Medoff, *J. Infect. Dis.* **1986**, *154*, 76–83.
[13] M. Baginski, H. Resat, J. A. Mccammon, *Mol. Pharmacol.* **1997**, *52*, 560–570.
[14] a) For the preparation of an analogue of amphotericin B lacking the carboxylic acid group, see: M. Carmody, B. Murphy, B. Byrne, P. Power, D. Rai, B. Rawlings, P. Caffrey, *J. Biol. Chem.* **2005**, *280*, 34420–34426; b) A semisynthetic approach to this same molecule has also recently been reported: D. S. Palacios, T. M. Anderson, M. D. Burke, *J. Am. Chem. Soc.* **2007**, *129*, 13804–13805.
[15] a) B. N. Rogers, M. E. Selsted, S. D. Rychnovsky, *Bioorg. Med. Chem.* **1997**, *5*, 3177–3182; b) For a fluorinated polyene analogue whose biological profile has not been determined, see: H. Tsuchikawa, N. Matsushita, N. Matsumori, M. Murata, T. Oishi, *Tetrahedron Lett.* **2006**, *47*, 6187–6191.
[16] V. Paquet, E. M. Carreira, *Org. Lett.* **2006**, *8*, 1807–1809, and references therein.
[17] a) A. Zumbuehl, P. Stano, M. Sohrmann, T. Vigassy, E. Pretsch, M. Peter, P. Walde, D. Jeannerat E. M. Carreira, *Angew. Chem.* **2004**, *116*, 5293–5297; *Angew. Chem. Int. Ed.* **2004**, *43*, 5181–5185; b) A. Zumbuehl, P. Stano, M. Sohrmann, M. Peter, P. Walde, E. M. Carreira, *Org. Biomol. Chem.* **2007**, *5*, 1339–1342; c) V. Paquet, A. Zumbuehl, E. M. Carreira, *Bioconjugate Chem.* **2006**, *17*, 1460–1463; d) A. Zumbuehl, P. Stano, D. Heer, P. Walde, E. M. Carreira, *Org. Lett.* **2004**, *6*, 3683–3686.
[18] For a preliminary report, see: a) A. M. Szpilman, D. M. Cereghetti, N. R. Wurtz, J. M. Manthorpe, E. M. Carreira, *Angew. Chem.* **2008**, *120*, 4407–4410; *Angew. Chem. Int. Ed.* **2008**, *47*, 4335–4338; b) A. M. Szpilman, J. M. Manthorpe, E. M. Carreira, *Angew. Chem.* **2008**, *120*, 4411–4414; *Angew. Chem. Int. Ed.* **2008**, *47*, 4339–4341.
[19] We have previously disclosed full accounts of our gram scale syntheses of the C33–C38 fragment: J. Tholander, E. M. Carreira, *Helv. Chim. Acta* **2001**, *84*, 613–622; and the mycosamine subunit: J. M. Manthorpe, A. M. Szpilman, E. M. Carreira, *Synthesis* **2005**, 3380–3388.
[20] Professor Danishefsky suggested that diverted total synthesis could be used as way to perform "molecular editing" of undesired or unnecessary structural features of natural products: R. M. Wilson, S. J. Danishefsky, *J. Org. Chem.* **2006**, *71*, 8329–8351.
[21] a) R. A. Singer, E. M. Carreira, *J. Am. Chem. Soc.* **1995**, *117*, 12360–12361; b) E. M. Carreira, R. A. Singer, *Drug Discovery Today* **1996**, *1*, 145–150; c) J. Krüger, E. M. Carreira, *J. Am. Chem. Soc.* **1998**, *120*, 837–838; d) J. Krüger, E. M. Carreira, *Tetrahedron Lett.* **1998**, *39*, 7013–7016; e) Y. Kim, R. A. Singer, E. M. Carreira, *Angew. Chem.* **1998**, *110*, 1261–1263; f) B. L. Pagenkopf, J. Krüger, A. Stojanovic, E. M. Carreira, *Angew. Chem.* **1998**, *110*, 3312–3314; *Angew. Chem. Int. Ed.* **1998**, *37*, 3124–3126; g) R. Singer, J. Brock, E. M. Carreira, *Helv. Chim. Acta* **2003**, *86*, 1040–1044.
[22] K.-M. Chen, G. E. Hardtmann, K. Prasad, O. Repic, M. J. Shapiro, *Tetrahedron Lett.* **1987**, *28*, 155–158.
[23] D. B. Dess, J. C. Martin, *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.
[24] P. L. Anelli, C. Biffi, F. Montanari, S. Quici, *J. Org. Chem.* **1987**, *52*, 2559–2562.
[25] S. Ohira, *Synth. Commun.* **1989**, *19*, 561–564.
[26] a) S. Saito, T. Hasegawa, M. Inaba, R. Nishida, T. Fujii, S. Nomizu, T. Moriwake, *Chem. Lett.* **1984**, 1389–1392; b) S. Saito, T. Ishikawa, A. Kuroda, K. Koga, T. Moriwake, *Tetrahedron* **1992**, *48*, 4067–4086.
[27] a) G. Wess, K. Kesseler, E. Baader, W. Bartmann, G. Beck, A. Bergmann, H. Jendralla, K. Bock, G. Holzstein, H. Kleine, M. Schnierer, *Tetrahedron Lett.* **1990**, *31*, 2545–2548; b) J. L. Hubbs, C. H. Heathcock, *J. Am. Chem. Soc.* **2003**, *125*, 12836–12843.
[28] a) J. A. Dale, H. S. Mosher, *J. Am. Chem. Soc.* **1973**, *95*, 512–519; b) G. R. Sullivan, J. A. Dale, H. S. Mosher, *J. Org. Chem.* **1973**, *38*, 2143–2147; c) I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
[29] See the Supporting Information.
[30] a) D. E. Frantz, R. Fässler, E. M. Carreira, *J. Am. Chem. Soc.* **2000**, *122*, 1806–1807; b) N. K. Anand, E. M. Carreira, *J. Am. Chem. Soc.* **2001**, *123*, 9687–9688; c) D. Boyall, F. Lopez, H. Sasaki, D. Frantz, E. M. Carreira, *Org. Lett.* **2000**, *2*, 4233–4236; d) H. Sasaki, D. Boyall, E. M. Carreira, *Helv. Chim. Acta* **2001**, *84*, 964–971; e) R. Sans-Diez, B. Adger, E. M. Carreira, *Tetrahedron* **2002**, *58*, 8341–8344; f) For an application in total synthesis, see: A. Fettes, E. M. Carreira, *Angew. Chem.* **2002**, *114*, 4272–4275; *Angew. Chem. Int. Ed.* **2002**, *41*, 4098–4101; g) S. Reber, T. F. Knoepfel, E. M. Carreira, *Tetrahedron* **2003**, *59*, 6813–6817; h) F. Kleinbeck, E. M. Carreira, *Angew. Chem.* **2009**, *121*, 586–589; *Angew. Chem. Int. Ed.* **2009**, *48*, 578–581.
[31] D. A. Evans, E. B. Sjogren, J. Bartroli, R. L. Dow, *Tetrahedron Lett.* **1986**, *27*, 4957–4960.
[32] a) B. O. Lindgren, T. Nilsson, *Acta Chem. Scand.* **1973**, *27*, 888–890; b) B. S. Bal, W. E. Childers, H. W. Pinnick, *Tetrahedron* **1981**, *37*, 2091–2096.
[33] D. A. Evans, T. C. Britton, J. A. Ellman, *Tetrahedron Lett.* **1987**, *28*, 6141–6144.
[34] a) J. W. Bode, N. Frafel, D. Muri, E. M. Carreira, *Angew. Chem.* **2001**, *113*, 2128–2131; *Angew. Chem. Int. Ed.* **2001**, *40*, 2082–2085;

b) L. Fader, E. M. Carreira, *Org. Lett.* **2004**, *6*, 2485–2488; c) N. Lohse-Fraefel, E. M. Carreira, *Org. Lett.* **2005**, *7*, 2011–2014; d) N. Becker, E. M. Carreira, *Org. Lett.* **2007**, *9*, 3857–3860; For applications in total synthesis, see: e) D. Muri, N. Lohse, E. M. Carreira, *Angew. Chem.* **2005**, *117*, 4104–4106; *Angew. Chem. Int. Ed.* **2005**, *44*, 4036–4038. F. Kleinbeck, E. M. Carreira, *Angew. Chem.* **2008**, *120*, 596–598; *Angew. Chem. Int. Ed.* **2008**, *47*, 586–589.

[35] This method was reported by the McGarvey group in their synthetic studies towards amphotericin B (**1**) using a different homoallylic acohol. See: a) G. J. McGarvey, J. A. Mathys, K. J. Wilson, *J. Org. Chem.* **1996**, *61*, 5704–5705; b) G. J. McGarvey, J. A. Mathys, K. J. Wilson, *Tetrahedron Lett.* **2000**, *41*, 6011–6015; NMR analysis of hemiketal **32** and **33** indicated that the expected *anti* product was formed.

[36] P. G. Baraldi, A. Barco, S. Benneti, S. Manfredini, D. Simoni, *Synthesis* **1987**, 276–278.

[37] A. Guarna, A. Guidi, A. Goti, A. Brandi, F. De Sarlo, *Synthesis* **1989**, 175–178.

[38] a) G. Fráter, *Helv. Chim. Acta* **1979**, *62*, 2825–2828; b) D. Seebach, D. Wasmuth, *Helv. Chim. Acta* **1980**, *63*, 197–200.

[39] A. G. Myers, B. H. Yang, H. Chen, L. McKinstry, D. J. Kopecky, J. L. Gleason, *J. Am. Chem. Soc.* **1997**, *119*, 6496–6511.

[40] J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993.

[41] I. Shiina, R. Ibuka, M. Kubota, *Chem. Lett.* **2002**, 286–287.

[42] M. A. Blanchette, W. Choy, J. T. Davis, A. P. Essenfeld, S. Masamune, W. Roush, T. Sakai, *Tetrahedron Lett.* **1984**, *25*, 2183–2186.

[43] I. Paterson, K.-S. Yeung, J. B. Smaill, *Synlett* **1993**, 774–776.

[44] L. K. Blasdel, A. G. Myers, *Org. Lett.* **2005**, *7*, 4281–4283.

[45] a) A. V. Demchenko, *Synlett* **2003**, 1225–1240; b) E. S. H. El Ashry, N. Rashed, E. S. I. Ibrahim, *Curr. Org. Synth.* **2005**, *2*, 175–213.

[46] a) R. L. Halcomb, S. J. Danishefsky, *J. Am. Chem. Soc.* **1989**, *111*, 6661–6666; b) D. Kahne, S. Walker, Y. Cheng, D. Van Engen, *J. Am. Chem. Soc.* **1989**, *111*, 6881–6882; c) G. K. Packard, S. D. Rychnovsky, *Org. Lett.* **2001**, *3*, 3393–3396.

[47] a) N. K. Kochetkov, A. Y. Khorlin, A. F. Bochkov, *Tetrahedron* **1967**, *23*, 693–707; b) For a recent review, see: F. Kong, *Carbohydr. Res.* **2007**, *342*, 345–373.

[48] a) A related approach was recently published, see: H. Yu, D. L. Williams H. E. Ensley, *Tetrahedron Lett.* **2005**, *46*, 3417–3421; See also: b) G. Desmares, D. Lefebvre, G. Renevret, C. Le Drian, *Helv. Chim. Acta* **2001**, *84*, 880–889.

[49] Glycoside tosylates have been reported to be capable of glycosidating simple alcohols: R. Eby, C. Schuerch, *Carbohydr. Res.* **1974**, *34*, 79–90.

[50] Treating **53** with LiOH led to formation of a complex mixture of products. A complex mixture of byproducts is also formed during azide reduction with basic triethylamine and 1,3-propanedithiol. The base sensitivity of amphotericin B derivatives is highly dependant on structure. Chemical evidence in support of this supposition is the fact that while saponification of amphotericin B methyl ester (AME) with LiOH proceeds cleanly, hydrolysis of the acetonide protected amphotericin B methyl ester leads to epimerization, presumably at C34.

[51] V. Paquet, A. A. Volmer, E. M. Carreira, *Chem. Eur. J.* **2008**, *14*, 2465–2481.

[52] a) P. van Hoogevest, B. de Kruijff, *Biochim. Biophys. Acta Biomembr.* **1978**, *511*, 397–407; b) D. S. Orlov, T. Nguyen and R. I. Lehrer, *J. Microbiol. Methods* **2002**, *49*, 325–328.

[53] The width of the phospholipid bilayer of these LUVs is 37 Å and thus any formation of single barrel ion channels should be precluded. See supporting information for additional measurements.

[54] A. M. Szpilman, E. M. Carreira, *Org. Lett.* **2009**, *11*, 1305–1307.

Received: January 27, 2009
Published online: June 19, 2009

