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Synthesis and Biological Studies of 35-Deoxy Amphotericin B Methyl Ester**

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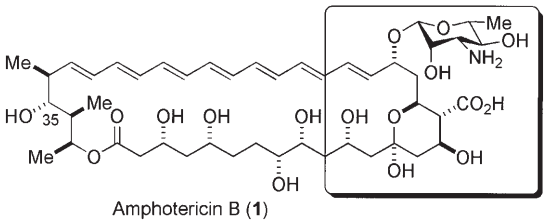
In the preceding communication we disclosed the synthesis of 35-deoxy amphotericin B aglycone in its protected form.[1] Herein, we describe the key glycosidation that completes the assembly of 35-deoxy amphotericin B methyl ester. Additionally, we present our findings concerning its activity in potassium-efflux assays and in fungi. We discuss the significance of our observations to the mechanism of action of the clinically important antifungal agent amphotericin B (**1**).



Amphotericin B (**1**)

In the most widely accepted mechanism, commonly referred to as the barrel-stave model, amphotericin B is suggested to form ion channels in the membrane. These ion channels are believed to be generated through a self-assembly process involving 4–12 molecules of the polyene macrolide stabilized by an equal number of sterols.[1,2] Of particular importance for the present work is the discrepancy between the overall length of amphotericin B (about 21 Å)[3] and the average width of the phospholipid membrane (about 43 Å). Two models have been postulated to reconcile this discrepancy, involving single- and double-barrel channels (Figure 1).[1,2] In the first of these, the bilayer narrows to accommodate the ion channel as it spans the membrane (Figure 1a). According to the second hypothesis, two barrel units link up in a tail-to-tail fashion, accommodating to the width of the membrane (Figure 1b). The hydroxy group on C35 is suggested to play a pivotal role in the stabilization of the double-barrel construct.[4]

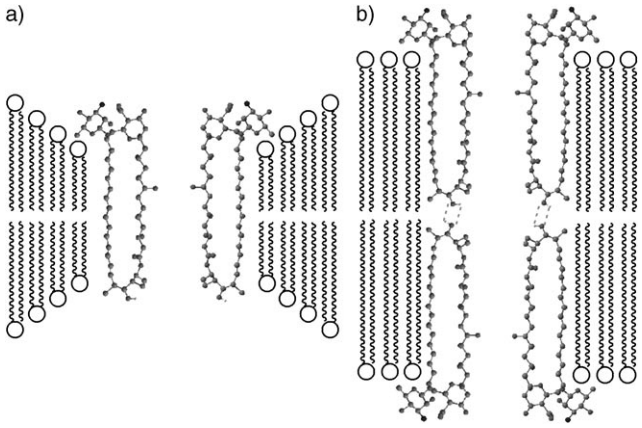


Figure 1. Side views of the proposed single- (a) and double-barrel (b) ion channels. H-bonds are indicated with dotted lines.

Amphotericin B incorporates mycosamine and hemiketal units that characterize its family of macrolide antibiotics.[5] Mycosamine is a 3,6-dideoxy-3-aza-mannose that is attached as the β anomer to the amphotericin B aglycone. The introduction of β-mannoside residues is widely acknowledged to be one of the most challenging problems in carbohydrate chemistry.[6] In the synthesis of mycosamine macrolides, the problem is augmented by the acute acid-sensitivity of the aglycone **2** and the obstruction of the hydroxy acceptor by its position in a groove. To ensure formation of the β anomer, the glycosidic coupling has been carried out following the method of Schmidt with the C2′-epi-mycosamine donor bearing ester groups capable of lending anchimeric assistance. Unfortunately, this approach has been shown to provide the orthoester **6a** as a major byproduct of the reaction and proceeds to low conversion.[7] In our hands, the reaction of **2a**[7b] with 3 equiv of **3a** and 30 mol% of PPTS afforded 4% of the desired glycoside **5a**, 12% of orthoester **6a**, and 75% of recovered aglycone **2a** (Scheme 1, Table 1, entry 1). A number of other methods were also examined without success.[8] Orthoesters can be rearranged under acidic conditions to afford the corresponding glycoside.[9] However, the resilience of the orthoester and the acid sensitivity of the unsaturated alcohol combined to impair our efforts in this direction. For example, the use of a wide range of acids returned starting orthoester (e.g. PPTS) or resulted in destruction of the material (e.g. BF_3).

In the Schmidt glycosidation reaction, glycoside formation results from attack of aglycone at C1′ on intermediate **4**, while attack at the acetoxonium carbon leads to orthoester formation. We hypothesized that modifying the steric and electronic properties of the participating ester might tune the

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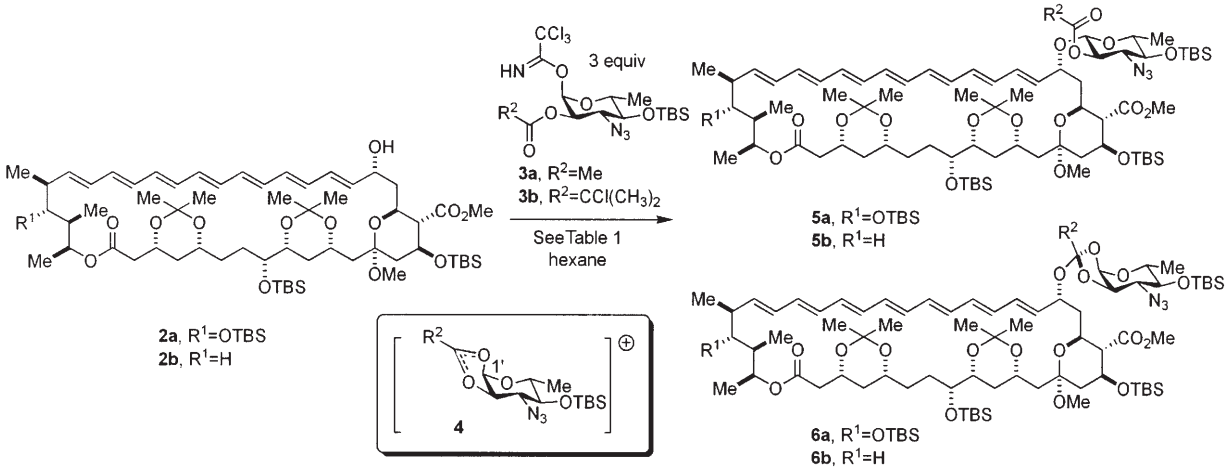


Scheme 1. Glycosidation of amphoteronolide and 35-deoxy amphoteronolide (see Table 1 for combinations and conditions).

Table 1: Introduction of the mycosamine moiety (Scheme 1).

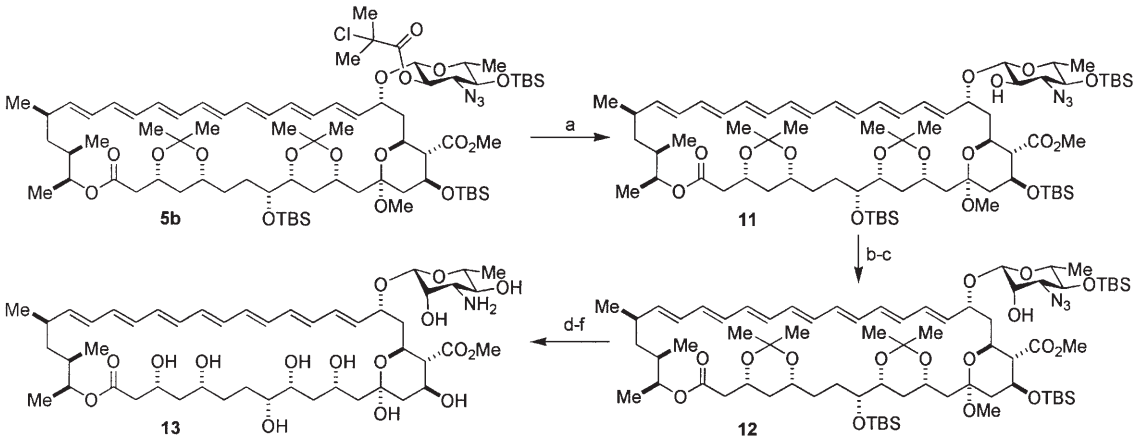
Acceptor	Donor	Activator	[2]	5	6	2
2a	**3a**	PPTS[a]	7 mM	4%	12%	75%
2a	**3b**	PPTS[a]	20 mM	27%	11%	57%
2a	**3b**	CMPT[b]	20 mM	43%	23%	–
2b	**3b**	CMPT[c]	20 mM	45%	30%	–

[a] 30 mol%. [b] 50 mol% CMPT and 100 mol% 2-chloro-6-methyl-pyridine. [c] 10 mol% CMPT and 20 mol% 2-chloro-6-methyl-pyridine. CMPT = 2-chloro-6-methyl-pyridinium triflate; PPTS = pyridinium *para*-toluenesulfonate.

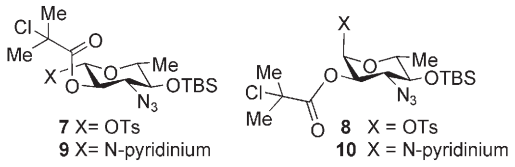
chemoselectivity in favour of glycoside formation (i.e. of R^2 in **3**/**4**, Scheme 1).[10] This proved to be the case. Thus, the use of the auxiliary 2-chloroisobutyrate furnished a 2.5:1 mixture of glycoside **5a** and orthoester **6a**. Importantly, a range of other esters of mycosamine donor **3** failed to improve the yield of the glycoside, giving predominantly orthoester instead.[10] The benzoate afforded a 1:1 mixture of glycoside and orthoester, but this ester could not be subsequently hydrolyzed without deleterious effects on the sensitive macrolactone. We speculate that glycosidation is favoured for the 2-chloroisobutyrate ester because of a fine balance of factors: The presence of the electronegative chloride substituent may lead to greater charge localization on C1′ in **4**; the steric bulk of the 2-chloroisobutyrate is expected to disfavour attack that leads to orthoester. Additionally, the chloride also enables facile subsequent hydrolysis of the ester (Scheme 2).

Although the studies outlined above resulted in an increase in formation of glycoside **5a**, conversion remained low. Full conversion could be achieved by use of additional PPTS, but the yields were unsatisfactory. NMR experiments provided clues for the poor conversion: Activation of the trichloroacetimidate **3b** by one equivalent of PPTS led to rapid formation of the β tosylate **7**, which on standing is converted into the more stable α anomer **8**. The mixture proved unreactive in the glycosidation of amphoteronolide **2a**.[11] We surmised that replacement of the tosylate with the less nucleophilic triflate counterion could be productive.

To our surprise, activation of **3b** with one equivalent of pyridinium triflate led to formation of a mixture of the two anomeric pyridinium salts (**9** and **10**). These species were also unreactive towards the aglycone. After extensive experimen-



Scheme 2. a) K_2CO_3, MeOH; 83% yield; b) $(CF_3CO)_2O$, DMSO, $(Me_2N)_2CO$, Et_3N, CH_2Cl_2/Et_2O, −78 °C to RT; c) $NaBH_4$, MeOH; 82% yield over two steps (brsm); d) HF/pyridine, MeOH, 40 °C; 72%; e) CSA, MeCN/water; 37% (six cycles); f) Bu_3P, THF/MeOH/water; 67%. brsm = based on recovered starting material; CSA = camphor sulfonic acid.



tation we identified 2-chloro-6-methyl-pyridinium triflate as uniquely efficient for the glycosidation. Thus, reaction of protected 35-deoxy amphoteronolide **2b** with donor **3b** in the presence of 10 mol% 2-chloro-6-methyl-pyridinium triflate and 20 mol% 2-chloro-6-methyl-pyridine afforded the desired glycoside in 45% yield accompanied by 30% of the orthoester (Table 1, entry 4). Importantly, glycosidation of the natural amphoteronolide **2a** gave similar yields (entry 3).

With the successful assembly of glycoside **5b**, we could attend to the inversion of the C2′ stereogenic center and removal of the protecting groups. The 2-chloroisobutyrate ester was hydrolyzed under mildly basic conditions (Scheme 2). The configuration at C2′ was inverted by an oxidation–reduction sequence as described earlier.[7]

The TBS groups were removed from **12** by the action of HF/pyridine in methanol over 12 h. In contrast, for the analogous intermediate in the synthesis of amphotericin B, desilylation required 48 h.[7b] The acetonide groups were hydrolyzed under acidic conditions. Azide reduction with 1,3-propanedithiol/triethylamine was very slow (50% conversion after 24 h) and led to a complex mixture.[7b,12] Fortunately, the azide could be reduced under neutral conditions using tributylphosphine to cleanly afford 35-deoxy amphotericin B methyl ester.

At this point, the stage was set to determine the consequence of deleting the 35-hydroxy group on the biological profile. We measured and compared the activity of 35-deoxy amphotericin B methyl ester (**13**) and amphotericin B methyl ester against *Saccharomyces cerevisiae* and *Candida albicans* (Table 2). Strikingly, for both strains, **13** was more than an order of magnitude less active.

Table 2: Antifungal activity of amphotericin B methyl ester (AME) and 35-deoxy amphotericin B methyl ester (**13**).[a]

Entry	Compound	*Saccharomyces cerevisiae* BY4741 [μM]	*Candida albicans* CAF2-1 [μM]
1	AME	0.25	0.1
2	**13**	4.6	2.6

[a] Measured according to the National Committee on Clinical Laboratory Standards protocol. See reference [13] for experimental details.

We proceeded to examine the ability of 35-deoxy amphotericin B methyl ester (**13**) to cause K-efflux from large unilamellar vesicles (LUV), as monitored by using potassium-ion selective electrodes.[14,15] We utilized LUV with a membrane made up of POPC with or without ergosterol as a component (see Figure 2 and the Supporting Information).[16] 35-Deoxy amphotericin B methyl ester (**13**) at 1 μM concentration showed severely diminished ability to induce the leakage of K^+ compared to amphotericin B methyl ester.

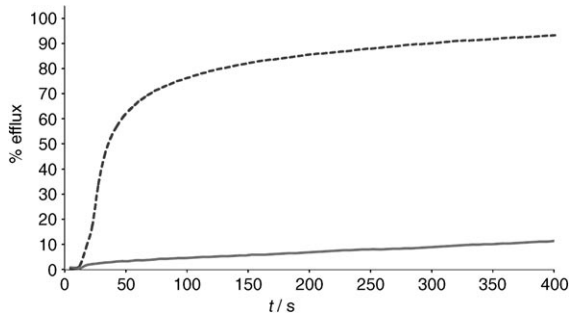


Figure 2. K^+ efflux from LUV induced by **13** (——) and amphotericin B methyl ester (-----) measured by potentiometry. The substrate was added externally (as a DMSO solution) to afford a final concentration of 1 μM. LUV with a diameter of 100 nm and containing 13% ergosterol and 87% POPC in their membranes were utilized.[15] LUV = large unilamellar vesicle, POPC = 1-palmitoyl-2-oleoyl-*sn*-glycero-phosphocholine.

Efflux all but ceased at 0.1 μM or in pure POPC-LUV while at 10 μM a weak efflux could be detected.

We have presented a strategy for the total synthesis of amphotericin B analogs, which addresses the necessary glycosidation and subsequent elaboration steps. Specifically, we disclose its application to the synthesis of 35-deoxy amphotericin B methyl ester (**13**). We also present data that for the first time experimentally confirms the importance of the hydroxy group at C35 of amphotericin B in its role as a fungicide and its ability to cause electrolyte efflux in liposomes. Collectively, these observations are consistent with the involvement of double-barrel ion channels in the membrane for the activity of amphotericin B and its derivatives. The approach we have described herein sets the stage for additional investigations involving the study of conjugates to various carbohydrates and other small molecules. Further work to elucidate details of the mechanism of action using fully synthetic analogs prepared according to the presented strategy is underway.

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[16] 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 the Supporting Information for additional measurements.

