ORGANIC LETTERS

2011
Vol. 13, No. 6
1390–1393

# Probing the Role of the Mycosamine C2′-OH on the Activity of Amphotericin B

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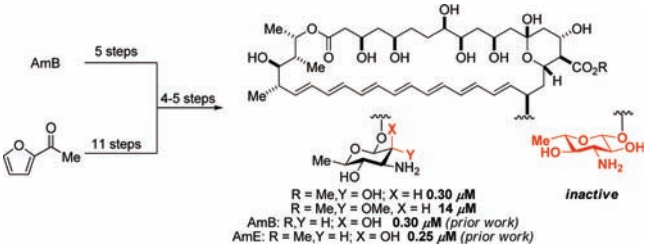
**Received January 11, 2011**

## ABSTRACT




**A synthetic route to a mycosamine donor was designed and provided access to a set of AmB derivatives targeted to probe the effect of the C2′-OH. It was determined that the configuration of the C2′-position is inconsequential but that *O*-methylation of this alcohol was deleterious to its mode of action. Additionally, the analog incorporating a mycosamine derivative from the enantiomeric series was devoid of activity.**

The drug of choice for hospital patients with systemic fungal infections is amphotericin B (AmB, **1**, Figure 1). Yet, the full details concerning its mode of action remain elusive despite efforts over four decades.[1] Indeed, there is a plenum of questions unanswered concerning the remarkable ability of this small molecule to undergo self-assembly and recognition in lipid bilayers. This becomes even more significant given the fact that AmB has been found to have activity in treating Alzheimers, Creutzfeldt−Jakob, and HIV infections, wherein it has been suggested that AmB/protein interactions may be relevant. A number of derivatives and closely related structures have been isolated,[2] biosynthesized,[3] and accessed by total synthesis.[4,5] These have been used to probe the role of the various functional groups in the mode of action, including the hydroxyls along the C1−C12 spine, the polyene,[6] and carboxylic acid.[7] Carbohydrates appended to natural products are increasingly being recognized as critical to their activity.[8] Yet, despite the fact that mycosamine is a highly conserved moiety in the family of polyene macrolide antibiotics, no studies have been directed at understanding its effect on yeast at a resolution that focuses on the individual functional groups. Herein we document an initial investigation of the role of the mycosamine C2′-OH on the fungicidal activity of AmB. In the course of our studies, we have

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10.1021/ol2000765 
**Published on Web 02/15/2011**

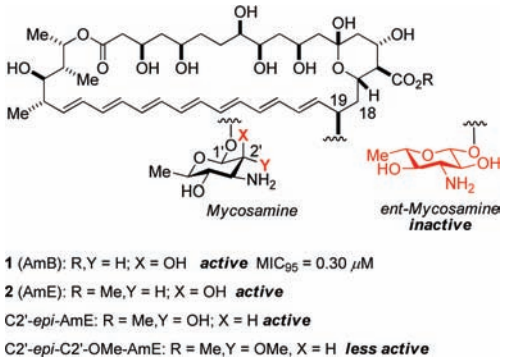


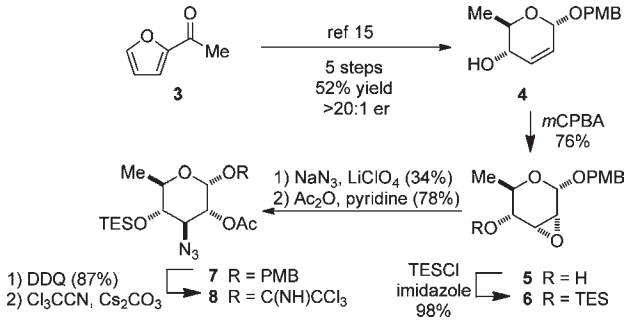

**Figure 1.** Structure of amphotericin B and synthetic analogs.

observed that the relative configuration at C2′ is not a determinant for activity, and yet the corresponding C2′-methyl ether analog is 50-fold less effective (Figure 1). Interestingly, the preparation of AmB that incorporates the *ent*-C2′-*epi*-mycosamine displays 100-fold decreased activity in yeast. These results implicate a key role for mycosamine in AmB wherein the aminosugar is not merely a polar headgroup and, importantly, whose role is more significant than merely forming a hydrogen bond to sterols residing nearby.

The early literature of AmB describes simple derivatives at the C-16-$CO_2H$ and mycosamine-$NH_2$ that provide some indication of their importance.[1] For example, AmB methyl ester (AmE, **2**) is equipotent to AmB in yeast (AmB = 0.30 $\mu$M and AmE = 0.25 $\mu$M), but mycosamine *N*-acetamides are inactive, leading to the suggestion that a basic amine at C3′ is necessary.[9] Caffrey has determined that the aglycone of AmB is inactive, thus underscoring the relevance of the aminosugar itself.[7] Moreover, we have shown that amphotericin B incorporating *N,N*-bis(aminopropylene) derived mycosamines display enhanced fungicidal activity and possess an improved therapeutic window.[10]

An interaction involving mycosamine C2′ and ergosterol hydroxyl groups was suggested some time ago to be critical in enabling the sterol to stabilize the putative barrel-stave pore in fungal membranes.[11] More recently, studies have been conducted with conformationally rigidified analogs in which short hydrophobic tethers linked the mycosamine amine with the C-16 carboxylic acid.[12] It is well worth noting that the calculated conformation for the three structures prepared and analyzed did not correlate to the most stable conformation relating mycosamine and the macrocycle, discussed in detail below. Despite the

**Scheme 1.** Synthesis of Mycosamine Donors




intriguing aspects of this model the role of C2′-OH has not been experimentally explicitly tested with derivatives that do not extensively alter the mycosamine or its relationship to the macrocycle. We therefore designed analogues that would directly probe the putative H-bonding interaction between mycosamine and ergosterol and further map out subtleties of the mycosamine subunit on the activity of the polyene macrolide antibiotic AmB. In approaching the problem, we decided to take a course of action that minimized the perturbations of the mycosamine itself. Thus it was our intention to maintain the overall polar and structural characteristics of the parent sugar.

We have described an improved glycosidation method for the AmB aglycone in the context of a synthesis of C-35 deoxyAmB.[13,14] The approach relies on the use of the C2′-mycosamine epimer as a donor because of exquisite anomeric control that ensues in the coupling reaction. Thus, the route enables rapid access to C2′-derivatives and provides a point of entry for studies at this locus. Consequently, our investigation was designed to develop a general approach to the mycosamine platform (Scheme 1). The synthesis commences with the conversion of 2-acetylfuran **3** to allylic alcohol **4** in five steps following the route developed by O'Doherty.[15] This resulting alcohol was engaged in a directed epoxidation reaction and subsequently protected to afford epoxide **5**. The synthesis of the mycosamine donor analog was completed by epoxide opening with azide, acylation of the resultant alcohol, and generation of the corresponding trichloroacetimidate (**8**), whose configuration was established by comparison to previously synthesized donors.[4] The synthesis route is shorter than any previously described[14a] and, more importantly, allows for facile manipulation of different groups of the sugar.

A semisynthetic route that commences with the natural product was relied upon to access a suitably protected aglycone (Scheme 2).[7b] AmB was subjected to amine

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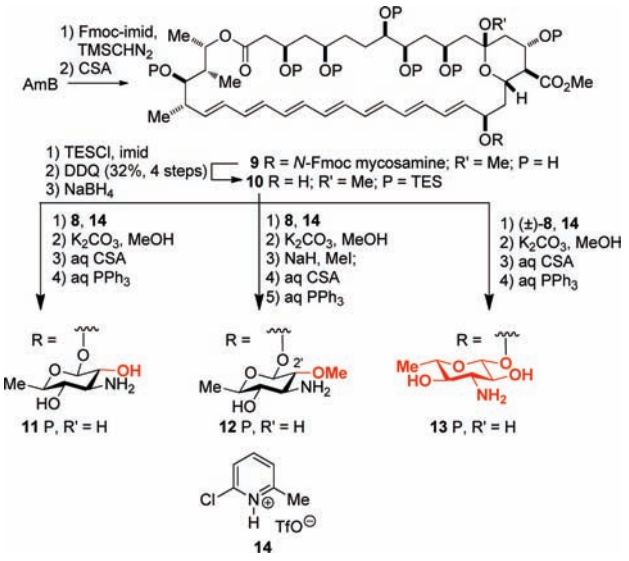
**Scheme 2.** Divergent Synthesis of AmB Analogs




protection (Fmoc); esterification ($C_{16}$-$CO_2H$ → $C_{16}$-$CO_2Me$); and methyl ketal formation to yield **9**. Subsequent silylation of the nine alcohols, oxidative cleavage of the sugar, and diastereoselective C=O reduction complete the synthesis of alcohol **10**.

Mycosamine donor **8** was coupled to aglycone **10** to produce a mixture of the glycosylated product (Scheme 2), along with an orthoester, which is a well-known coproduct of the glycosidation of the hindered secondary alcohol. These are conveniently separated by chromatography on silica gel, following alkaline cleavage of the C2′-acetate. The glycosylated products were subjected to global deprotection to provide AmB analog **11** (C2′-*epi*-AmE), incorporating the C2′-epimer of the mycosamine. Alternatively, the C2′-OH was subjected to *O*-methylation after acetate cleavage, which following deprotection provided **12** (C2′-*epi*-C2′-OMe-AmE). Coupling of the aglycone to (±)-**8** provided access to **13** (C2′-*epi*-*ent*-mycosamine-AmE), which is the C2′-*epi* isomer derived from the unnatural *ent*-mycosamine.

With analogs **11**−**13** in hand, their growth inhibition activity against *Saccharomyces cerevisiae* (BY4741) was assayed (Figure 2). The C2′-epimer (**11**) displayed activity equivalent to AmE **2** (0.30 $\mu$M for **11** and 0.25 $\mu$M for AmE). To further understand analog **11**, it was subjected to a liposome assay which we have utilized to measure potassium efflux. In the assay **11** was indistinguishable from AmE (**2**) in the presence of cholesterol and ergosterol (see Supporting Information). Thus, the configuration at C2′ is not a major determinant for activity in either yeast or liposomes. This result is significant because it questions the models in which the C2′-alcohol forms a hydrogen bond with the ergosterol that is critical for channel formation

(16) The lack of importance of the C2′-OH configuration is consistent with reports in which AmB has been observed to form channels in sterol-free membranes, a fact that would itself cast doubt on the necessity for H-bonding as a key stabilizing feature.

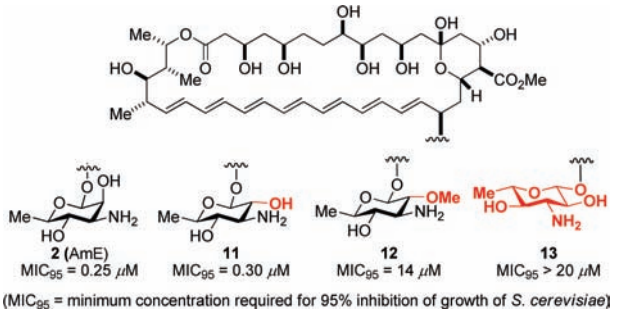



**Figure 2.** Biological activity of the AmB analogs.

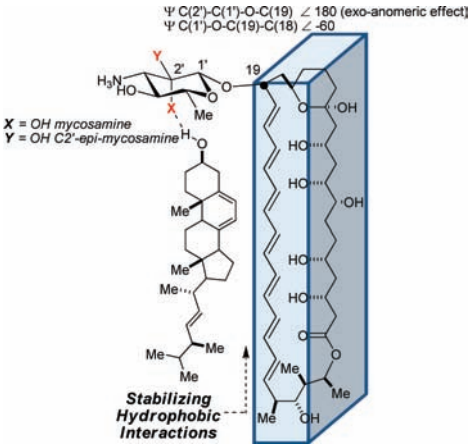



**Figure 3.** Models for the association of ergosterol with AmB.

(Figure 3).[11,16] It is well worth noting that the only experimental study examining this hypothesis relied on covalently tethered, conformationally restricted derivatives of AmB whose activities in yeast and liposomes failed to correlate altogether.[12] Additionally, in these models the calculated minima for the conformationally restricted analogs do not correspond to the low energy minima calculated by Borowski.[11] In the low energy conformer, the C(1′)−O−C(19)−C(18) dihedral of −60° minimizes the unfavorable steric interactions between the sugar and polyene backbone, and the C(2′)−C(1′)−O-C(19) dihedral subtends an angle of 180° as a consequence of the exo-anomeric effect (Figure 3).

Analysis of the model in light of the results with C2′-*epi*-mycosamine is revealing. The structure illustrated for mycosamine in Figure 3 corresponds to the lowest energy conformer. In the model that is commonly invoked, the axially disposed C2′-OH (X in Figure 3) is engaged in H-bonding to the hydroxyl group of ergosterol. This interaction is proposed to bring the steroid in proximity to the macrolide heptaene, thereby stabilizing the integrity of the channel. The fact that the C2′-*epi* diastereomer (Y = OH in Figure 3) displays similar activity cannot be reconciled with this model, because the corresponding interaction in the

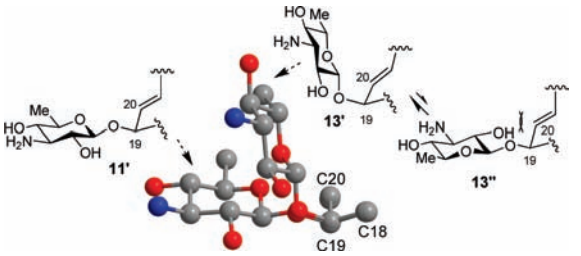



**Figure 4.** Modeling of C2′-*epi*-analogs **11** and **13** overlaying C18−20.

C2′-*epi* series would necessarily move the steroid substantively further away.

We next proceeded to examine the C2′-methyl ether derivative (**12**). It was found to be approximately 50-fold less active than **2** and **11**. The methyl ether group precludes the possibility for the C2′-position to act as a hydrogen-bond donor altogether. This observation is intriguing in light of the results indicating the insignificance of the configuration at C2′. We subsequently examined the analog incorporating the mycosamine derivative from the enantiomeric series. Analog **13** was essentially inactive ($MIC_{95} > 20\,\mu M$). This is interesting because the equatorial disposed substituents of the mycosamine in analogs **11** and **13** occupy similar spatial dispositions relative to the pyran ring (Figure 2). However, further scrutiny reveals significant differences in the conformational profile of the two diastereomers. Analysis of models of **11** and **13** suggests a key difference with respect to the spatial disposition of the enantiomeric mycosamine derivative relative to the macrocycle (Figure 4 and Supporting Information). In the current study, the lack of activity of **13** when contrasted to **11** is consistent with conformer **11′** as critical for activity. Analysis of a conformer of **13** that maps onto **11′** (see **13″** in Figure 4) reveals that it suffers from destabilizing double gauche pentane interactions between C2′-OH and C-20 of the macrocycle, in addition to positioning of the polar amine into the lipophilic membrane of the putatively formed pore.

In summary, a synthetic route to mycosamine donor **8** provided access to a set of mycosamine derivatives targeted to probe the effect of C2′-OH on the biological properties of AmB. A key observation in this study is that the configuration of the C2′-OH is inconsequential to the activity of the polyene macrolide in yeast and liposomes; however, O-methylation of this alcohol was significantly deleterious. Additionally, the analog incorporating a mycosamine derivative from the enantiomeric series was devoid of activity. Collectively, the results underscore the fact that the mycosamine subunit is not merely a polar group and that the theorized lowest energy conformation by Borowski is critical for full expression of the biological activity in yeast. The active conformation is one which incorporates an exoanomeric stabilization and minimization of steric effects between the mycosamine and the polyene backbone. Further structure−activity relationships involving the mycosamine are currently under investigation. In general any attempts at exploring structure−activity relationships ultimately must contend with the prospect that the fundamental structure has been altered; in a broader sense the work we have delineated underscores the possibilities in utilizing stereochemical probes to gain insight into the biological mode of action with relatively minimal structural perturbation.

**Acknowledgment.** This research was funded by a grant from the Swiss National Science Foundation. Support in the form of an ETH Fellowship (M.P.C.) is gratefully acknowledged.

**Supporting Information Available.** Experimental procedures and characterization for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.