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# Significant Improvement of Antifungal Activity of Polyene Macrolides by Bisalkylation of the Mycosamine

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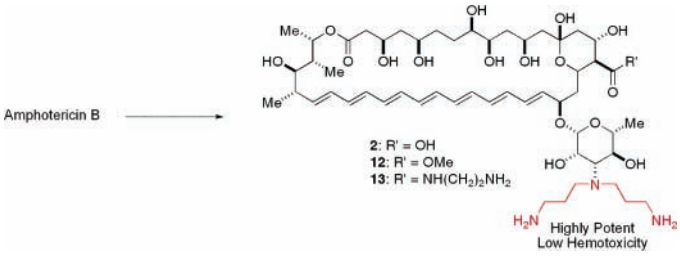
## ABSTRACT




**New derivatives of Amphotericin B (AmB) were synthesized through a double reductive alkylation of the mycosamine. These derivatives of AmB displayed superior antifungal activity against *Saccharomyces cerevisiae* wild-type strain and especially in the case of an AmB-resistant *Candida albicans* strain. Moreover, these compounds are potential drug candidates because of significantly reduced hemotoxicity compared to AmB. Furthermore, the same mycosamine modification can be applied to other polyene macrolides such as Nystatin and Pimaricin to improve their antifungal activity.**

The polyene macrolide antibiotics comprise a class of natural products that enjoy wide use: Amphotericin B (AmB, **1**) is clinically prescribed as an antifungal agent; Nystatin (**3**) is employed against oral and skin infections; and Pimaricin (**5**) is used as a food additive, as a veterinary medicament, and for the treatment of corneal infections (Figure 1). Attempts to generate more active low molecular weight analogues through structural modifications have so far eluded the field. Herein, we describe potent derivatives of AmB (**1**), which are more active than AmB (**1**) against a *Saccharomyces cerevisiae* wild type and against AmB-resistant *Candida albicans* strains. Moreover, these exhibit lower hemotoxicity when compared to AmB (**1**). Specifically, the bis(aminopropylene) mycosamine derivative (**2**) is remarkable because of its high antifungal activity and minimal hemotoxicity. The ability of the bis(aminopropylene) moiety to lead to increased antifungal activity is structurally unique because other closely related side chains, such as aminoethylene, were less effective. Its effect, however, is general, as the attachment of aminopropylene side chains to Nystatin (**3**) and Pimaricin (**5**) also leads to marked improvement of their antifungal activity (Figure 1).

Our investigations initially focused largely on AmB (**1**) because it is the most effective antifungal agent available for the treatment of systemic mycoses in humans.[1] Although its use can lead to severe side effects, these can be managed through delivery of the drug via special formulations (Fungizone or AmBisome).[2] Nonetheless, AmB (**1**) remains the drug of choice for invasive aspergillosis, candidemia (in particular, fluconazole-resistant *Candida*), mucormycosis, fusariosis, and cryptococcosis meningitis. A recent study of trends in the epidemiology of fungal infections in the United States revealed that 10−15% of all hospital-acquired blood-

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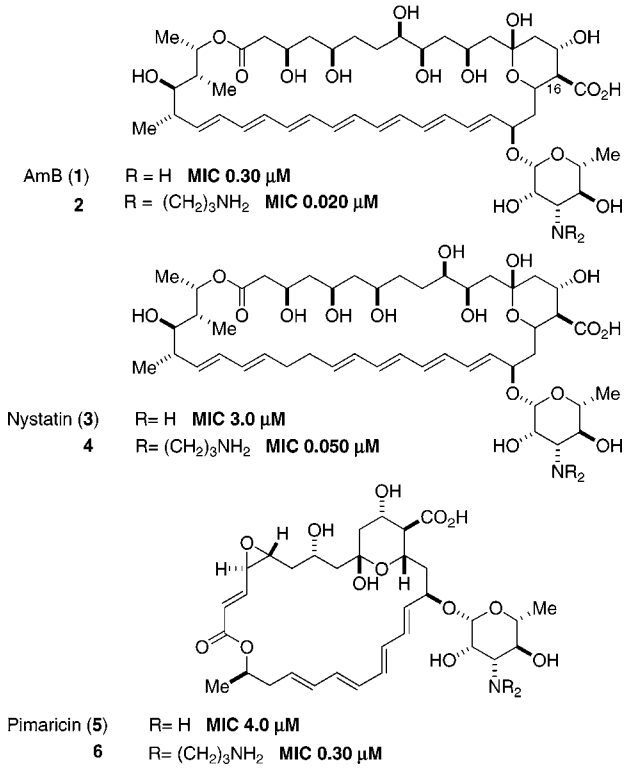
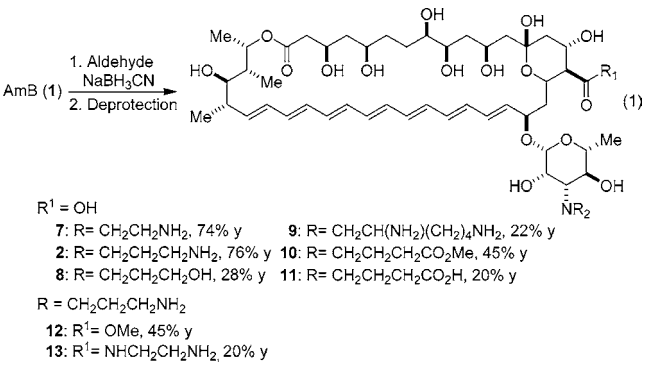



**Figure 1.** Minimal inhibitory concentration (MIC) against *S. cerevisiae* for AmB (**1**), Nystatin (**3**), Pimaricin (**5**), and their corresponding bis(aminopropylene) derivatives **2**, **4**, and **6**.

stream infections are caused by nosocomial candidemia.[3] It is generally believed that the presumed mode of action of AmB (**1**) involving the formation of channels traversing the membrane would make resistance unlikely. However, resistant strains have been isolated and may eventually become more prevalent.[4] Thus, there is a pressing need for the identification and development of more active, less toxic analogues.

The amines in the mycosamine appendage of AmB (**1**) and the C-16 carboxylate have been previously recognized as convenient sites for chemoselective elaboration. Notably, the primary amine has been functionalized in several ways, including acetylation, aminoacylation, glycosylation, alkylation, and conjugation to poly(ethylene glycol).[5] However, modifications of the amine have yielded analogues displaying diminished activity, leading to the conclusion that the $NH_2$ group in native AmB (**1**) tolerates little alteration. To a much more limited extent, the carboxylic acid has been exploited to access dimers[6] and functionalized carbon nanotube derivatives.[7]

In yeast biology, certain membrane uptake systems have been proposed to interact specifically with exogenous polyamines, leading to their internalization.[8] Consequently, we became interested in the generation and study of AmB (**1**) polyamine chimeras with the expectation of synthesizing analogues displaying increased activity. The reactivity of the mycosamine provides the opportunity to readily introduce a diverse set of side chains onto the natural product.[9] The synthetic modifications of AmB (**1**) commence with its double reductive alkylation reaction with a range of aldehydes (eq 1). This is conveniently carried out on native AmB (**1**) to afford several derivatives in 20−76% yield with no attempt at optimization in this initial discovery phase. C-16 ester and amide derivatives were synthesized because the ester derivatives of AmB (**1**) bearing a free mycosamine $NH_2$ group had been previously shown to display reduced hemotoxicity albeit at the expense of antifungal activity.[5a,10]




The antifungal activities of compounds **7**−**13** were determined by measurement of minimal inhibitory concentrations (MIC) required to completely inhibit growth of *S. cerevisiae* (Table 1).[11] The incorporation of aminoethylene side chains on the mycosamine led to derivatives displaying no beneficial effect; thus, the potency of **7** (0.25 $\mu$M) was similar to that of AmB (**1**) (0.30 $\mu$M). Interestingly, aminopropylene derivative **2** was highly active with a MIC value of 0.020 $\mu$M, which constitutes a 15-fold increase over AmB (**1**). Simply increasing the number of amines did not lead to enhanced activity: for **9**, the MIC was only 0.080 $\mu$M. We next aimed to determine whether the bis(aminopropylene)

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(11) See Supporting Information for details.

**Table 1.** MIC Values for Dialkylated Derivatives of AmB[a]

| entry | $R^1$ | mycosamine derivative ($NR_2$) | compound | MIC (μM) |
|---|---|---|---|---|
| **1** | OH | $-NH_2$ | **1 (AmB)** | 0.30 |
| **2** | OH | $-N(CH_2CH_2CH_2NH_2)_2$ | **7** | 0.25 |
| **3** | OH | $-N(CH_2CH_2NH_2)_2$ | **2** | 0.020 |
| **4** | OH | $-N(CH_2CH_2OH)_2$ | **8** | 0.50 |
| **5** | OH | $-N(CH_2CH_2CH_2NH_2)_2$, $NH_2$ | **9** | 0.080 |
| **6** | OH | $-N(CH_2CO_2Me)_2$ | **10** | 2.0 |
| **7** | OH | $-N(CH_2CO_2H)_2$ | **11** | >10 |
| **8** | OMe | $-N(CH_2CH_2NH_2)_2$ | **12** | 0.10 |
| **9** | $-N(H)CH_2CH_2NH_2$ | $-N(CH_2CH_2NH_2)_2$ | **13** | 0.040 |

[a] Assayed with *S. cerevisiae* wild type (BY4741) following the NCCLS protocol; see Supporting Information for details.

moiety was unique in its ability to confer high activity by examining a collection of other derivatives with polar side chains.

Diol **8** (MIC 0.50 μM), diester **10** (MIC 2.0 μM), and diacid **11** (MIC >10 μM) were significantly less active than native AmB (**1**). These observations, when taken together with those of derivatives **2** and **7**−**9**, underscore the unique aspects of the aminopropylene moiety. In previous work, the conversion of AmB to C-16 ester and amide derivatives was shown to be detrimental to activity, albeit these were shown to display lower toxicity.[10] We wondered whether the high potency conferred by incorporation of bis(aminopropylene) side chains would be sufficient to overcome the loss in antifungal activity obtained upon modification of C-16. In analogy to **2**, compounds **12** and **13** were also more active against *S. cerevisiae* than AmB (**1**): MIC of 0.10 and 0.040 μM, respectively.

The most active compounds identified in our study were screened against an AmB-resistant strain, namely, *C. albicans* (DSY1764) (Table 2).[11] AmB (**1**) has a MIC >50 μM against this strain; it is well worth noting that such high concentrations of AmB would be lethal for the host undergoing treatment. In the assay, **2** (MIC 1.0 μM) displayed a dramatic increase in activity over AmB (**1**) (MIC >50 μM). Compounds **12** and **13** also exhibited significant inhibitory activity against this resistant strain with a MIC value of 4.0 μM. We then investigated the toxicity of these derivatives toward human erythrocytes because hemotoxicity is a major problem associated with AmB (**1**). In this respect, an $EHB_{50}$ value can be measured employing a commonly used assay.[12] AmB derivative **2** ($EHB_{50}$ 10 μM) was 2.5 times less toxic than

**Table 2.** Antifungal Activity against an AmB-Resistant Strain and Hemotoxicity

| compound | MIC BY4741 (μM)[a] | MIC DYS1764 (μM)[a] | $EH_{50}$ (μM)[b] |
|---|---|---|---|
| **1** | 0.30 | >50 | 4.0 |
| **2** | 0.020 | 1.0 | 10 |
| **12** | 0.10 | 4.0 | 50 |
| **13** | 0.040 | 4.0 | 30 |

[a] Assayed following the NCCLS protocol; see Supporting Information for details. [b] Determined according to the procedure in ref 12. $EH_{50}$: concentration causing 50% hemoglobin release in human erythrocytes.

AmB (**1**) ($EHB_{50}$ 4.0 μM), whereas **12** displayed even less toxicity ($EHB_{50}$ 50 μM). Significantly, **13** not only is a highly active antifungal agent but also displays lower toxicity ($EHB_{50}$ 30 μM).

We next sought to examine whether the notable increase in activity against *S. cerevisiae* that arises from incorporation of bis(aminopropylene) side chains on AmB (**1**) could also be generalized and extended to other polyene macrolides that are in clinical use (Figure 1). The bis(aminopropylene) derivative of Pimaricin **6** (MIC 0.30 μM) was more active than native Pimaricin (**5**) (MIC 4.0 μM). Significantly, when compared to Nystatin (**3**) (MIC 3.0 μM), the bis(aminopropylene) derivative of Nystatin **4** (MIC 0.050 μM) is 60 times more active.

In conclusion, we have found that alkylation of the mycosamine moiety of AmB (**1**) with two aminopropylene groups leads to congeners with significantly improved antifungal activity, even against an AmB-resistant strain. This observed increase in biological activity allows rapid access to C-16 carboxylate derivatives which possess very low hemotoxicity while retaining high potency. The key role played by the bis(aminopropylene) mycosamine can be extended to other polyene macrolides, leading to a significant increase in antifungal activity. The broader implications of bis(aminopropylene) conjugates with natural products in enhancing their interaction with yeast and the molecular basis of this effect are the subjects of ongoing investigations, which will be reported in due course.

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**Supporting Information Available:** Experimental procedures, compound characterization data, and $^1H$ and $^{13}C$ spectra of all the products. This material is available free of charge via the Internet at http://pubs.acs.org.

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