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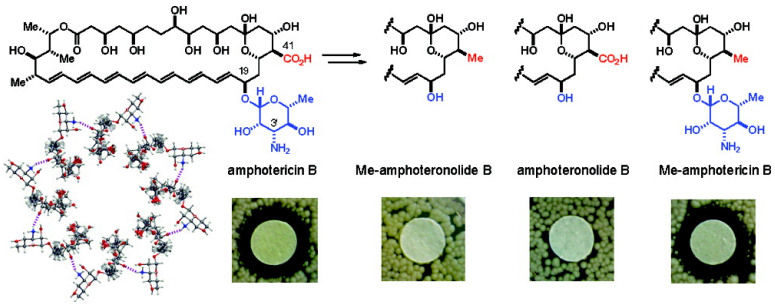

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A Post-PKS Oxidation of the Amphotericin B Skeleton Predicted to be Critical for Channel Formation Is Not Required for Potent Antifungal Activity

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The leading model for the antifungal action of amphotericin B (AmB, **1**) involves its self-assembly into a membrane-spanning ion channel.[1] This natural product thus represents a potential prototype for small molecules with the capacity to perform ion channel-like functions in living systems. Efforts to harness this potential and/or improve the notoriously poor therapeutic index of this clinically vital antimycotic[2] would benefit from a molecular-level understanding of this channel activity.

Although the evidence that AmB can self-assemble in lipid membranes to form discrete ion conducting channels is strong,[1,3] the molecular architecture of this channel assemblage and its role in antifungal activity remain poorly understood.[4] Despite this, the leading "barrel-stave" model[5] is an often cited textbook classic (Figure 1A).[6] Extensive computer modeling studies predict that this complex is stabilized by a ring of salt bridges[7a] and/or hydrogen bonds[7b,c] at the channel periphery between oppositely charged C(41) carboxylate and C(3′) ammonium ions. Conspicuously, these two functional groups are installed biosynthetically as post-polyketide synthase (PKS) modifications of the macrolide skeleton, that is, a P450-mediated oxidation of the C(41) methyl group and a glycosyl transferase-mediated attachment of mycosamine to the C(19) alcohol (Figure 1B).[8,9]

Many interesting studies have probed the action of AmB via covalent modification of the C(41) carboxylic acid and/or C(3′) amine.[4e,10] However, the self-assembly of small molecules can be exquisitely sensitive to steric effects,[11] and this phenomenon may complicate this experimental approach. We herein report an alternative strategy that involves synthetically deleting chemical groups appended to the macrolide skeleton and determining the functional consequences.[12] This approach has led to the striking observation that, contrary to the current channel model (Figure 1A), *oxidation at C(41) is not required for potent antifungal activity*.

The synthetic manipulation of AmB is challenging because of its sensitivity to light, oxygen, and acid as well as its minimal solubility in most organic solvents and water. Nevertheless, we ultimately developed a flexible degradative pathway that enables the synthetic reversal of either[13] or both of the two post-PKS modifications predicted to be critical for self-assembly of the AmB channel (Scheme 1). Synthesis of the novel MeAmdeB **2** (Figure 1B) commenced with the conversion of AmB into the suitably protected and more soluble nonasilylated *N*-Fmoc methyl ketal **7**.[13b] The C(41) carboxylic acid was then selectively reduced to the corresponding primary alcohol via the intermediacy of a 2-pyridinethiol ester.[14] Subsequent iodination with PPh_3/I_2[15] yielded the desired C(41) iodomethyl derivative **8**. Oxidative deglycosylation of this intermediate using DDQ[16] in the presence of $CaCO_3$ smoothly generated enone **9**. Reduction of the C(19) ketone with $NaBH_4$/MeOH resulted in a ~2:1 mixture of diastereomers, while use of the (*S*)-CBS oxazaborolidine catalyst[17] provided the desired 19-*R* isomer in a synthetically useful 6:1 dr (Supporting Information). A subsequent reductive cleavage of the C(41) iodide was achieved with $NaBH_4$[18] in DMPU to yield advanced intermediate **10**. Global desilylation with HF/pyridine, hydrolysis of the methyl ketal, and preparative high performance liquid chromatography (HPLC) yielded diastereomerically pure MeAmdeB **2**. The funneling of intermediates **7** and **8** into modified versions of this flexible pathway enabled the preparation of the remaining two

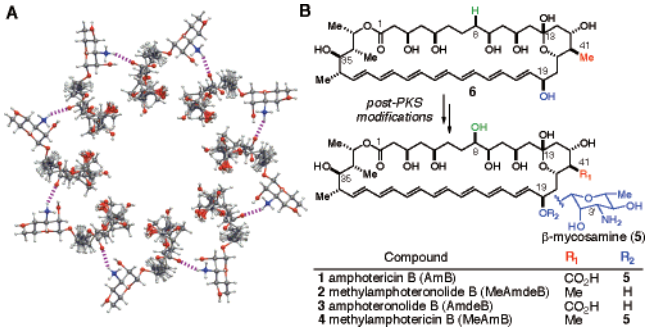
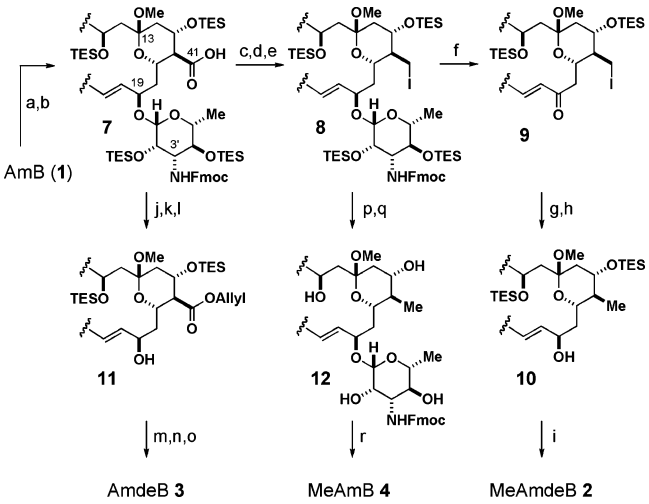


Figure 1. (A) A bird's eye view of the current "barrel stave" model for the AmB channel. Salt bridges and/or hydrogen bonds (dashed lines) between oppositely charged C(41) carboxylate and C(3′) ammonium ions are predicted to be critical for channel stabilization. (B) These two functional groups are installed as post-PKS modifications of the macrolide skeleton.

Scheme 1 [a]



[a] Reagents and conditions: (a) (i) Fmoc-succinimide, pyridine, DMF/MeOH 2:1, 23 °C, 12 h; (ii) CSA, THF/MeOH 1:1, 0 °C, 1 h, 90% (two steps); (b) TESOTf, 2,6-lutidine, hexanes, 0 °C, 3 h, 96%; (c) 2-thiopyridyl chloroformate, Et_3N, Et_2O, 0 °C, 30 min, 91%; (d) $LiBH_4$, Et_2O, 23 °C, 2 h, 88%; (e) I_2, PPh_3, imidazole, THF, 0 °C, 1 h, 78%; (f) DDQ, $CaCO_3$, THF, 23 °C, 10 min, 67%; (g) (*S*)-CBS oxazaborolidine, $Me_2S{\cdot}BH_3$, CH_2Cl_2, −10 °C, 30 min, 6:1 dr, 79%; (h) $NaBH_4$, DMPU, 23 °C, 6 h, 78%; (i) (i) HF/pyridine, THF/pyridine 3:2, 0 °C, 6 h; (ii) $AcOH/H_2O$/THF 1:1:2, 23 °C, 30 min; HPLC, 38% (two steps); (j) allyl bromide, *i*-Pr_2NEt, DMF, 23 °C, 8 h, 86%; (k) DDQ, $CaCO_3$, THF, 23 °C, 20 min, 65%; (l) $NaBH_4$, THF/MeOH 3:1, 0 °C, 30 min, >20:1 dr., 77%; (m) HF/pyridine, THF/pyridine 5:3, 0 → 23 °C, 6 h, 56%; (n) CSA, THF/H_2O 2:1, 23 °C, 5 h; HPLC, 81%; (o) $Pd(PPh_3)_4$, thiosalicylic acid, THF, 23 °C, 13 h; HPLC, 50%; (p) HF/pyridine, THF/pyridine 5:3, 0 → 23 °C, 6.5 h, 73%; (q) $NaBH_4$, DMSO, 23 °C, 8 h, 58%; (r) (i) CSA, THF/H_2O 2:1, 23 °C, 30 min; (ii) piperidine, DMSO/MeOH 4:1, 23 °C, 3 h; HPLC, 56% (two steps).

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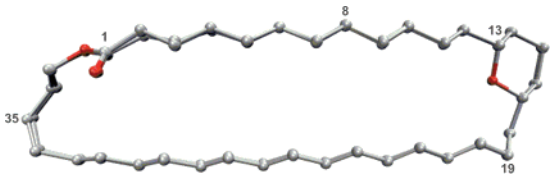

Figure 2. Superposition of the ground-state conformation of the macrolactone skeletons of compounds **1**−**4** (or their more soluble analogues; see Supporting Information for details).

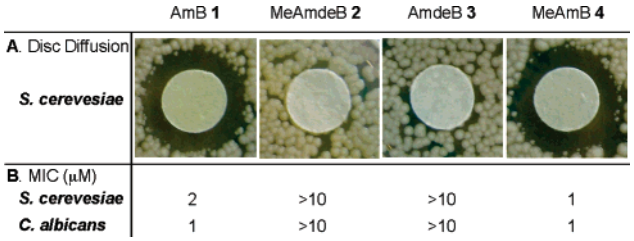

Figure 3. (A) Disc diffusion assay with *S. cerevisiae* (40 μg of compound per disc). Similar results were achieved with *C. albicans* (Supporting Information). (B) Broth dilution assays; values represent the average of three experiments.

targeted derivatives AmdeB **3**[13a] and MeAmB **4**[13b,c] (Scheme 1, Figure 1B).

Because the AmB framework is known to be quite rigid,[19] we postulated that the ground-state conformation would be unchanged by these appendage deletions, thereby further facilitating the interpretation of structure/function data generated with this approach. To confirm this hypothesis, we determined the ground-state conformation of compounds **1**−**4** (or more soluble analogues; see Supporting Information for details) using Monte Carlo methods constrained by extensive NOESY and phase-sensitive COSY NMR data processed using amplitude-constrained multiplet evaluation.[20] As shown in Figure 2, the conformation of the macrolide skeleton was unaltered by these appendage deletions (root-mean-square deviation for all four compounds = 0.081 Å).

The impact of deleting these functional groups on antifungal activity against *Saccharomyces cerevisiae* was qualitatively evaluated using a disc diffusion assay.[21] As shown in Figure 3A, derivatives **2** and **3**, both of which lack the mycosamine appendage, were completely inactive.[22] *In stark contrast, and counter to the current channel model, MeAmB* ***4*** *was found to be roughly equipotent to the natural product.* This striking result was confirmed quantitatively in a broth dilution assay[23] (MIC: AmB = 2 μM, MeAmB = 1 μM) (Figure 3B). Similar results were observed in both assays with clinically relevant *Candida albicans* (Figure 3). Clearly, post-PKS oxidation of the AmB macrolide at C(41) is not required for potent antifungal activity.

These findings stand in strong contradiction with the current model for the mechanism of action of AmB (Figure 1A). There are at least two possible explanations: oxidation at C(41) may not be required for channel formation and/or channel formation may not be required for antifungal activity.[4] An extensive series of biophysical studies with compounds **1**−**4** are planned to distinguish between these possibilities. In preliminary studies using pyranine-impregnated liposomes,[24] MeAmB demonstrates membrane-permeabilizing activity similar to that of AmB (Supporting Information).

These results also demonstrate that the deletion of appended functional groups represents a powerful approach for probing the still poorly understood activity of AmB. The general application of this strategy to systematically dissect the structure/function relationships that underlie this potentially prototypical channel-forming small molecule is currently underway in our laboratories.

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Supporting Information Available: Detailed synthesis, NMR, and assay procedures, spectral data, and spectra; full citations for refs 4e, 7b, 8a,b, 10a,b,e, 13a−d, 15, 18, 19a, and 24. This material is available free of charge via the Internet at http://pubs.acs.org.

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