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Differential Interaction of an AmB Analogue and Ergosterol in Enantiomeric Membranes

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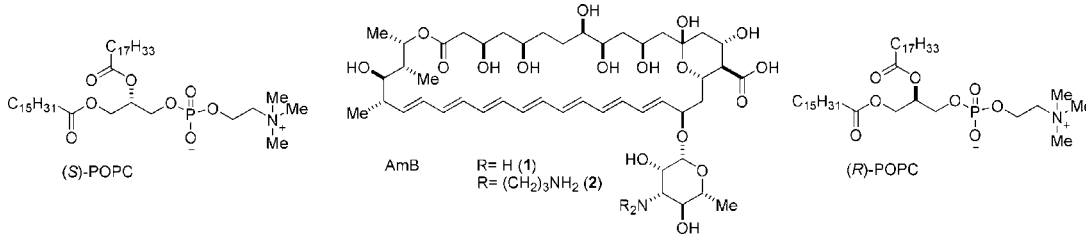
ABSTRACT



An amphotericin B derivative exhibits differential interaction with vesicles formed from either natural (*R*)-or unnatural (*S*)-POPC and ergosterol. This implicates a close association between the polyene macrolide and the phospholipid bilayer and may account for its increased antifungal activity.

Amphotericin B (AmB, **1**) is a commonly used antifungal agent for numerous systemic fungal infections.[1] It was originally isolated from fermented cultures of *Streptomyces nodosus*.[2] In the widely accepted model for its mode of action, AmB forms ion channels in cell membranes that lead to efflux of potassium ions (K^+) and other small electrolytes.[3] It has been suggested that the channels are preferentially stabilized by ergosterol over cholesterol, accounting for the selective action against fungal membranes.[4] In addition to its current clinical use as an antifungal agent, there have been reports that AmB may have therapeutic utility in treatment of HIV, Creutzfeld Jakob, and other diseases with implications of other possible modes of action.[5] Thus, it is increasingly important to understand in greater detail the role of chemistry, biology, and biochemistry of AmB.[6]

Herein we disclose an unexpected result in which the most active anlogue of AmB (**2**, Figure 1) reported to date leads to K^+-efflux in liposomes that is sensitive to the configuration of the phospholipid which constitutes the liposome, namely (*R*)- versus (*S*)-POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine). Although previous studies have examined the use of *ent*-cholesterol[7] on K^+-efflux, there have been no studies on the effect the configuration of the phospholipid has on channel formation. We believe the observations from such a study could shed light on the underlying reasons for the increased activity of AmB derivative **2**.

In landmark studies, Rychnovsky examined conductance in liposomes induced by AmB in the presence of cholesterol

(1) (a) Gallis, H. A.; Drew, R. H.; Pickard, W. W. *Rev. Infect. Dis.* **1990**, *12*, 308–329. (b) Hann, I. M.; Prentice, H. G. *Int. J. Antimicrob. Agents* **2001**, *17*, 161–169. (c) Lemke, A.; Kiderlen, A. F.; Kayser, O. *Appl. Microbiol. Biotechnol.* **2005**, *68*, 151–162.

(2) (a) Sternberg, T. H.; Wright, E. T.; Oura, M. A. *Antibiot. Annu.* **1955**, *3*, 566–573. (b) Gold, W.; Stout, H. A.; Pagano, J. F.; Donovick, R. *Antibiot. Annu.* **1955**, *3*, 579–586. (c) Vandeputte, J.; Wachtel, J. L.; Stiller, E. T. *Antibiot. Annu.* **1955**, *3*, 587–591.

(3) For a recent review, see: Cereghetti, D. M.; Carreira, E. M. *Synthesis* **2006**, 914–942.

(4) (a) Kotler-Brajtburg, J.; Price, H. D.; Medoff, G.; Schlessinger, D.; Kobayashi, G. S. *Antimicrob. Agents Chemother.* **1974**, *5*, 377–382. (b) Herve, M.; Debouzy, J. C.; Borowski, E.; Cybulska, B.; Garybobo, C. M. *Biochim. Biophys. Acta* **1989**, *980*, 261–272.

(5) Hartsel, S.; Bolard, J. *Trends Pharmacol. Sci.* **1996**, *12*, 445–449.

(6) (a) Szpilman, A. M.; Manthorpe, J. M.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 4339–4342. (b) Szpilman, A. M.; Cereghetti, D. M.; Manthorpe, J. M.; Wurtz, N. R.; Carreira, E. M. *Chem.—Eur. J.* **2009**, *15*, 7117–7128.

(7) Mickus, D. E.; Levitt, D. G.; Rychnovsky, S. D. *J. Am. Chem. Soc.* **1992**, *114*, 359–360.

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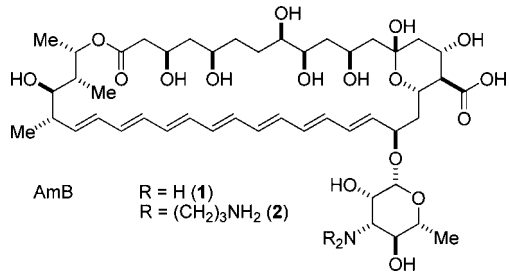


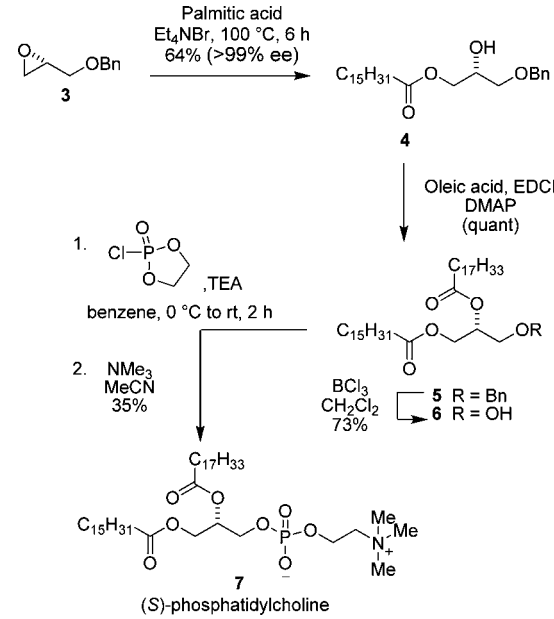
Figure 1. Structures of AmB (**1**) and *N*,*N*-bis(3-aminopropyl) AmB (**2**).

and *ent*-cholesterol.[8] Interestingly, no conductance was observed for liposomes incorporating *ent*-cholesterol at concentrations in which the corresponding vesicles including natural cholesterol displayed ion leakage. These results are consistent with direct interaction between cholesterol and AmB channels, as suggested by the models for channel formation wherein sterol participation in stabilization of the channel is critical.[9] The study leads to an intriguing question of whether cholesterol may also be altering the overall macroscopic properties of the lipid membrane in addition to interacting with the channel formed by AmB. These experiments are landmark, as they initiated a new approach involving the use of enantiomeric lipids as probes for membrane function.[10]

There are numerous studies that document the effect of various sterols on the membrane and susbsequent activity of AmB.[11,12] However, it is also important to note that AmB is active with sterol-free phospholipid membranes, leading to electrolyte efflux.[13] Consequently, studies of the role of the specific role of membrane components, specifically phospholipids, would warrant investigation.[14] Thus, we embarked on a study involving the effect of phospholipid stereochemistry on channel properties as assayed by K^+-efflux.

Recently, we documented a novel analogue of AmB, namely, *N*,*N*-bis(3-aminopropyl) AmB derivative (**2**), which possesses notable activity in a variety of fungal assays with the added benefit of displaying increased therapeutic index.[15] Thus, *N*,*N*-bis(3-aminopropyl) AmB displays MIC (minimal inhibitory concentration) = 0.020 μM compared to AmB with MIC = 0.30 μM in yeast assays involving *Saccharomyces cerevisiae*. Morever, **2** elicits reduced hemotoxicity (EH_{50} = 10 μM for **2** vs 4.0 μM for AmB) and displays fast K^+-efflux above 1.0 μM, similar to AmB. This represents one of a very select few analogues of AmB that have been prepared with increased activity.[16] However, we have not been able to discern the underlying reason for its enhanced activity.

Scheme 1. Synthesis of (*S*)-POPC



As shown in Scheme 1, optically pure (*S*)-POPC was synthesized in six steps from commercially available racemic benzyl ether of 2,3-epoxy-1-propanol. Hydrolytic kinetic resolution (HKR)[17] with [(*R*,*R*)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexandiaminato(2−)]cobalt(III) acetate complex gave an (*S*)-benzyl glycidyl ether (**3**) in optically pure form (>99%ee).[18] Palmitic acid was shown to participate in opening of (*S*)-benzyl glycidol epoxide (**3**) in the presence of tetraethylammonium bromide (Et_4NBr) to provide 1-*O*-benzyl-3-*O*-palmitoyl-*sn*-glycerol (**4**) in 64% yield.[19] The secondary hydroxyl group of 1-*O*-benzyl-3-*O*-palmitoyl-*sn*-glycerol (**4**) was efficiently acylated with oleic acid in quantitative yield using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and a stochiometric amount of *N*,*N*-dimethyl-4-aminopyridine (DMAP).[20]

The *O*-benzyl protecting group in (**5**) was removed by the action of boron trichloride (BCl_3) in dry dichloromethane (CH_2Cl_2) at −78 °C, affording alcohol **6** in 73% yield.

(8) Belani, J. D.; Rychnovsky, S. D. *J. Org. Chem.* **2008**, *73*, 2768–2773.
(9) Matsumori, N.; Sawada, Y.; Murata, M. *J. Am. Chem. Soc.* **2006**, *128*, 11977–11984.
(10) Biellmann, J. F. *Chem. Rev.* **2003**, *103*, 2019–2033.
(11) (a) De Kruijff, B.; Demel, R. A. *Biochim. Biophys. Acta* **1980**, *599*, 484–492. (b) Brutyan, R. A.; McPhie, P. *J. Gen. Physiol.* **1996**, *107*, 69–78.
(12) Zumbuehl, A.; Stano, P.; Heer, D.; Walde, P.; Carreira, E. M. *Org. Lett.* **2004**, *6*, 3683–3686.
(13) Backes, B. J.; Rychnovsky, S. D. *Anal. Biochem.* **1992**, *205*, 96–99.
(14) (a) Barwicz, J.; Tancrede, P. *Chem. Phys. Lipids* **1997**, *85*, 145–155. (b) H[1]c-Wydro, K.; Kapusta, J.; Jagoda, A.; Wydro, P.; Dynarowicz-Łatka, P. *Chem. Phys. Lipids* **2007**, *150*, 125–135.
(15) Paquet, V.; Carreira, E. M. *Org. Lett.* **2006**, *8*, 1807–1809.
(16) Paquet, V.; Volmer, A. A.; Carreira, E. M. *Chem.—Eur. J.* **2008**, *14*, 2465–2481.
(17) (a) Tokunaga, M.; Larrow, J. F.; Kakiuchi, F.; Jacobsen, E. N. *Science* **1997**, *277*, 936–938. (b) Furrow, M. E.; Schaus, S. E.; Jacobsen, E. N. *J. Org. Chem.* **1998**, *63*, 6776–6777.
(18) Schaus, S. E.; Brandes, B. D.; Larrow, J. F.; Tokunaga, M.; Hansen, K. B.; Gould, A. E.; Furrow, M. E.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2002**, *124*, 1307–1315.
(19) Lok, C. M. *Chem. Phys. Lipids* **1978**, *22*, 323–337.
(20) Dyer, B. S.; Jones, J. D.; Ainge, G. D.; Denis, M.; Larsen, D. S.; Painter, G. F. *J. Org. Chem.* **2007**, *72*, 3282–3288.

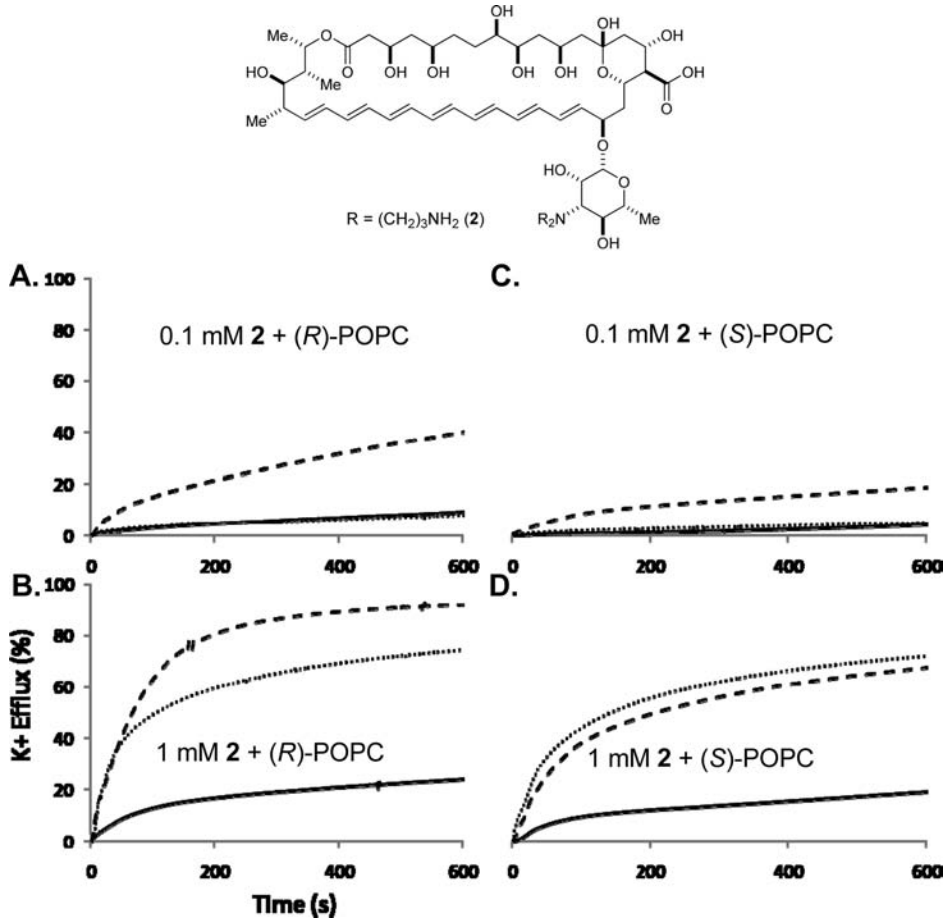


Figure 2. K^+ release from LUV after external addition of *N*,*N*-bis(3-aminopropyl) AmB (**2**): (*R*)-POPC or (*S*)-POPC (—), with 13% of ergosterol (- - -) and with 30 mol % of cholesterol (▪▪▪): (A) **2** (0.1 μM) with (*R*)-POPC; (B) **2** (1 μM) with (*R*)-POPC; (C) **2** (0.1 μM) with (*S*)-POPC; (D) **2** (1 μM) with (*S*)-POPC.

Installation of the choline headgroup was effected by treatment of **6** with 2-chloro-2-oxo-1,3,2-dioxaphospholane to give a glycerophosphoryl intermediate, which was then subjected to a ring-opening substitution reaction by exposure to excess anhydrous NMe_3 to provide (*S*)-POPC (**7**).[21]

The large unilamellar vesicles (LUV) containing either (*R*)-POPC or (*S*)-POPC were prepared according to literature procedures[22] (see the Supporting Information) in the presence of ergosterol (13%), cholesterol (30%) or absent sterol altogether. The induced potassium ion (K^+) efflux was measured by K^+ selective electrodes in the enantiomeric LUVs at low (0.1 μM) as well as high (1 μM) concentrations of AmB or *N*,*N*-bis(3-aminopropyl) AmB. The parent polyene macrolide AmB (0.1 or 1 μM) did not show any marked differenece in K^+-efflux in (*S*)-POPC versus (*R*)-POPC membranes and in the presence or absence of ergosterol or cholesterol.

In contrast to the results with AmB, for the active bis(aminopropylene) derivative **2** marked differences were observed in the K^+-efflux experiments involving liposomes prepared from (*R*)-POPC or (*S*)-POPC (Figure 2). Comparison of the efflux data at both concentrations tested reveals differences between the two enantiomeric liposomes: at 0.1 μM, Figure 2 A versus C and at 1 μM, Figure 2 B versus D. The difference is especially marked for liposomes incorporating ergosterol (- - -). In this respect, the liposomes including cholesterol (▪▪▪) and liposomes with only POPC (—) display no discernible difference: compare A versus B and C versus D. The data at the higher concentration is particularly telling (Figure 2 B vs D). At 1 μM, *N*,*N*-bis(3-aminopropyl) AmB (**2**) in (*R*)-POPC liposomes with ergosterol displayed full K^+ leakage within 10 min, whereas the cholesterol-containing liposomes plateau at about 70% efflux. By contrast, in (*S*)-POPC liposomes efflux proceeds to about 60% in both ergosterol and cholesterol containing membranes over the course of 10 min. Thus, interestingly, at this higher

(21) Menger, F. M.; Chen, X. Y.; Brocchini, S.; Hopkins, H. P.; Hamilton, D. *J. Am. Chem. Soc.* **1993**, *115*, 6600–6608.

(22) (a) Zumbuehl, A.; Martin, S. E.; Hussey, S. L.; Sohrmann, M.; Clark, D. D.; Peter, M.; Peterson, B. R.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2004**, *43*, 5181–5185. (b) Paquet, V.; Zumbuehl, A.; Carreira, E. M. *Bioconjugate Chem.* **2006**, *17*, 1460–1463. (c) Szpilman, A. M.; Cereghetti, D. M.; Wurtz, N. R.; Manthorpe, J. M.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 4335–4338. (d) Zumbuehl, A.; Stano, P.; Sohrmann, M.; Dietiker, R.; Peter, M.; Carreira, E. M. *ChemBioChem* **2009**, *10*, 1617–1620.

concentration the efflux in liposomes constituted from (*S*)-POPC in ergosterol containing liposomes substantially diminished while that of cholesterol containing LUVs remains effectively unchanged.

It has been previously reported by Covey[23] that cholesterol enantioselectively interacts with egg yolk sphingomyelin (SPM), and this specific interaction influences the physical properties of the membrane. Thus, in the experiment, SPM monolayers containing 20−40 mol % of either cholesterol or *ent*-cholesterol exhibited different compression behavior, and this was suggested to be indicative of diastereomeric sterol−lipid interactions occurring in these monolayers. However, it should be noted that in a later publication no significant enantiospecific interactions were observed (differential scanning calorimetry, X-ray diffraction, and neutral buoyant-density experiments) in a lipid bilayer model system consisting of enantiomeric cholesterol and egg sphingomyelin.[24]

The results of our investigation indicates that enantiospecific interactions can be observed in a more complex three-bodied situation involving the phospholipid ((*R*)- or (*S*)-POPC), steroid (cholesterol or ergosterol) and a natural product analogue (polyene macrolide antibiotic derivative). Importantly, it suggests a role for the phospholipid component of the membrane in the differential stabilization of the putative channels formed by the polyene antibiotic in combination with ergosterol. Although there have been models that focus on the interaction of sterols with the polyene macrolide channel, the role of the phospholipid itself is not considerably well investigated.

In the case at hand, there are two plausible explanations for the observed differences between (*S*)-POPC and (*R*)-POPC liposomes and potassium efflux with **2**. First, it may very well be that the phospholipid itself plays a role in stabilization of the channels. The fact that **2** is more active and that it incorporates a greater number of protonated amines may in fact enhance the interaction between the polyene macrolide and the phospholipid. Alternatively, it may be that a stronger interaction for ergosterol with (*S*)-POPC membranes over (*R*)-POPC would make it less available to or less mobile to interact and stabilize channel formation. The differentiation between these two modes of action provides stimulus for further investigation.

In summary, we document an unusual effect in enantiomeric liposomes with a novel, potent AmB analog. In liposomes constituted from (*R*)-POPC and ergosterol the observed efflux of K^+ is greater than that observed in the corresonding liposomes generated from the enantiomeric (*S*)-POPC and ergosterol. Interestingly, the effect is not observed for AmB and, consequently, may be indicative of the observed enhanced activity of **2** (MIC = 0.020 μM) compared to AmB (MIC = 0.30 μM) in yeast. In a broader sense the experiments delineated herein underscore the possibilities for the investigation of biologically relevant processes with additional stereochemical probes beyond steroids, such as enantiomeric phospholipids.

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Supporting Information Available: Experimental procedures and characterization data of all new compounds; experimental details involving K^+-efflux studies with liposomes. This material is available free of charge via the Internet at http://pubs.acs.org.

OL1003516

(23) (a) Lalitha, S.; Kumar, A. S.; Covey, D. F. *Chem. Commun.* **2001**, 1192–1193. (b) Lalitha, S.; Kumar, A. S.; Stine, K. J.; Covey, D. F. *J. Supramol. Chem.* **2001**, *1*, 53–61.

(24) (a) Mannock, A.; McIntosh, T. J.; Jiang, X.; Covey, D. F.; McElhaney, R. N. *Biophys. J.* **2003**, *84*, 1038–1046. We thank a reviewer for pointing this out.