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BIOMIMETIC ION TRANSPORT: SYNTHESIS AND ACTIVITY OF AN AMPHOTERICIN MIMIC

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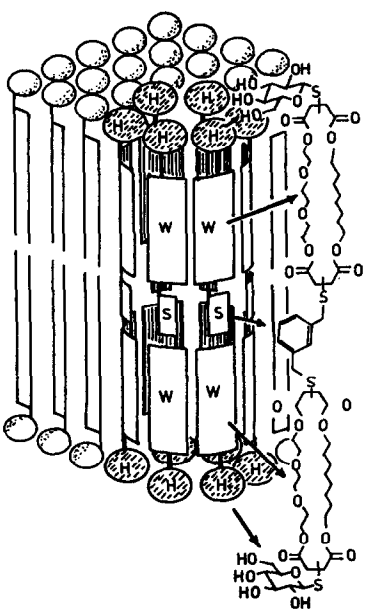
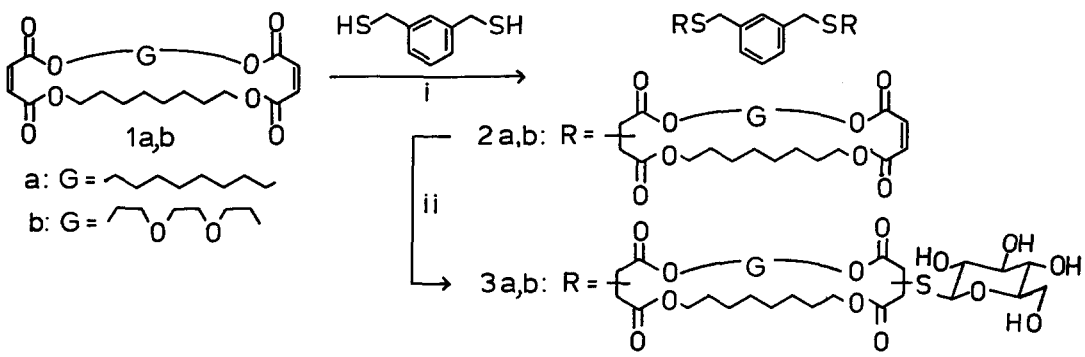
Abstract: A bolaform amphiphile derived from two macrocyclic tetra ester units was prepared as a mimic of the pore-forming antibiotic amphotericin. The mimic mediates collapse of cation and pH gradients across vesicle bilayer membranes.

Biomimetic ion transport systems seek to mimic the essential functional features of natural membrane transport processes[1]. The structural complexity of naturally occurring transport proteins precludes direct mimicry, but simpler pore-forming natural products such as gramicidin[2] or amphotericin[3], offer challenging models for synthetic and mechanistic mimicry. Few artificial pore-formers have been reported: channel-type structures reported by Tabushi[4], Nolte[5] and ourselves[6] loosely mimic the gramicidin channel while Furhop[7] and Kunitake[8] have reported pores formed by aggregation or phase separation within a membrane. This latter behavior is akin to the mode of action of amphotericin and other polyene antibiotics,whereby an aggregate structure offers a hydrophilic core region for ion transport[3]. As a strategy for mimicry it offers the potential for creation of structures large enough to span a membrane using the self-assembly of small molecules, readily available from synthesis.

Our design proposal for an amphotericin mimic is sketched at left. The pore-former is composed of two macrocyclic tetraester units which could incorporate a variety of polar and/or hydrogen bonding groups to drive the self-assembly process. For ease of synthesis a spacer unit links two identical units at the bilayer midplane. The polar head groups at the bilayer surfaces, serve to hold the structure in the membrane, as do hydrophobic contacts between the spacer unit , the tetra ester units and the membrane lipids. The sketch illustrates a well ordered "pore" composed of six units: this is certainly excessively simplistic. Rather the sketch serves as an architectural model to define design parameters such as overall length and functionality required. It implies neither stoichiometry, nor mechanism of ion transport.





Synthesis of pore-formers. Conditions: i) dropwise addition of 1.05 equiv. of dithiol to diene in isopropanol containing excess piperidine/ 2hr./ rt.; ii) citrate buffer (30:70 water:isopropanol), pH 8 maintained by addition of NaOH / 8 hr./ 50 °C.

The synthesis is outlined above. The macrocycles **1a,b** were prepared as previously[6]. Initial attempts using 1,3-propane dithiol as spacer were not successful, but meta-xylylene dithiol reacted readily to give the dimer dienes **2a,b** in 50% yield after chromatographic purification[9] Compound **1b** was obtained as a statistical mixture of regioisomers. The ^{1}H and ^{13}C NMR spectra were complex, but could be completely assigned by comparison with related thiol adducts of the macrocycles. Further reaction of the dimer dienes **2a,b** with sodium β-D-glucose-1-mercaptide at pH8 in aqueous isopropanol gave **3a,b** in quantitative yield. Both **3a** and **3b** are insoluble in water, and could be isolated from the buffer salts by removal of the organic solvent and repeated water washes. Again the ^{1}H and ^{13}C NMR spectra are complex, due to both regio and diastero-isomerism. The principal features of the spectra (succinate units, sugar groups, aromatic unit, ester carbonyl and ether OCH_2 units) were completely assigned, but the number of isomers precluded assignment of all the resolved resonances due to the polymethylene and polyethylene oxide units[9].

The transport of cations across vesicle bilayer membranes was determined by a pH-stat method[10,11]. Vesicles were prepared and purified in a choline sulfate/Bis-tris buffer at pH 6.60, diluted in unbuffered choline sulfate and brought to pH 7.5 with choline base. As illustrated in the Figure, addition of the proton carrier FCCP, and the pore former has little effect on the slow rate of proton efflux due to the collapse of the pH gradient. Upon addition of metal ion, a trans-membrane cation gradient is established, which collapses via electroneutral cation/proton antiport[10]. The proton efflux can then be determined by addition of choline base to maintain the external pH. The efflux rate eventually slows to the background rate, whereupon addition of Triton X100 provokes complete vesicle lysis and releases any entrapped buffer. As previously observed for amphotericin and gramicidin[10], and for our gramicidin channel mimic[12], the synthetic materials do not release the total entrapped proton titer. This result implies that the pore-former does not move between vesicles, or cannot act on the entire population of vesicles.

Figure: Sample pH-stat experiment for the transport of K^+ by **3b**. The points are every tenth experimental point collected, the line is calculated from the rate constant and the initial volume.

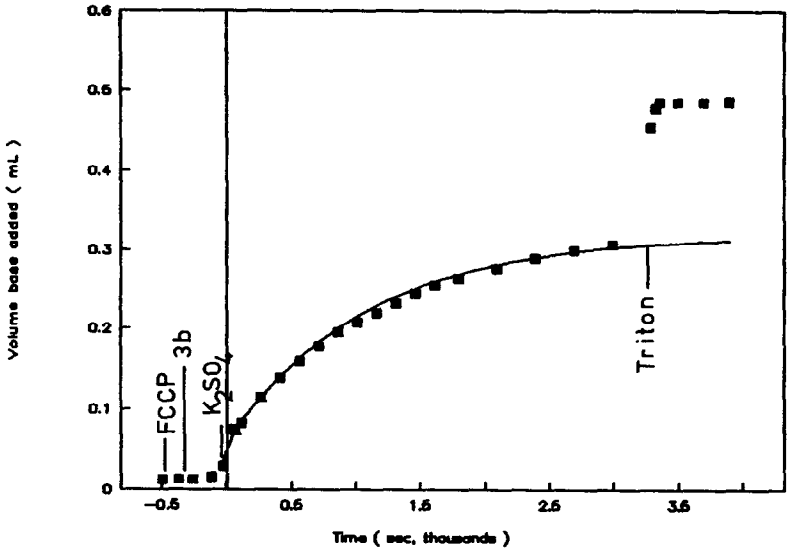


Table: Transport rate constant and extent of transport of cations by **3a,b** and amphotericin at 25 °C, determined by pH-stat[a]

Pore-former	Concentration (x 10^9 mol)	Cation (4mM)	k x 10^3 (sec^{-1})	Extent (%)
3a	148	Na^+	0.72	49
3a	148	K^+	0.92	54
3b	130	Na^+	0.51	53
3b	130	K^+	0.98	62
amphotericin	6.5	K^+	1.75	57

a) Conditions as described[11]. Variability between duplicates ±10%

The approach to the steady-state plateau is apparently a first-order process and a first-order rate constant can be determined. The Table gives results for the transport of Na^+ and K^+ by the two mimics in comparison with amphotericin. The concentrations were adjusted to give similar pH-stat behaviors (rate, extent of transport). By this criterion, the synthetic mimics are about a factor of 20 less active than amphotericin, although the concentration dependence of the activity has not been determined for either **3a,b** or amphotericin in this vesicle system. Although **3b** bears more polar functionality than **3a**, there is at best a modest difference in activity between the two mimics. Neither compound has the self-hydrogen bonding capability of amphotericin, and enhanced activity could be expected for a mimic incorporating hydroxyl, amido or like functional groups. The modest cation selectivity of the rates and extent of transport, are similar to the behavior of our gramicidin mimics[6] which apparently act via a channel mechanism[12]. Together with the plateau behavior, this provides preliminary evidence of the formation of some type of pore structure as envisaged in the design sketch. A detailed examination of the mechanism of action of these materials is in progress.

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11) Vesicles were prepared form egg phosphatidyl choline, cholesterol and phosphatidic acid (8:1:1) by reverse evaporation, sized through 3μm and 0.4μm cellulose acetate filters and isolated by chromatography on Sephadex G10. The lipid content was 3.3 mg/mL. Vesicles were predominantly unilamellar (125 nm diameter) with some multilamellar structures (10-15%). The pH-stat method described in the text utilized a vesicle sample containing 2.4 μmol lipid. FCCP (carbonyl cyanide 4-(trifluro methoxy)phenyl hydrazone,4 nmol,a proton carrier[10]) and the pore-former were added as solutions in methanol. Amphotericin (Sigma) was added as a solution in DMSO. Transport was initiated by addition of metal sulfates. Controls establish: no transport without the addition of transporter, no effect of methanol alone, no detergent effect of the transporter on the vesicles, vesicles are intact at the end of the pH-stat experiment.

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