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Transmembrane Ion Conductance by an Acyclic Bolaamphiphile

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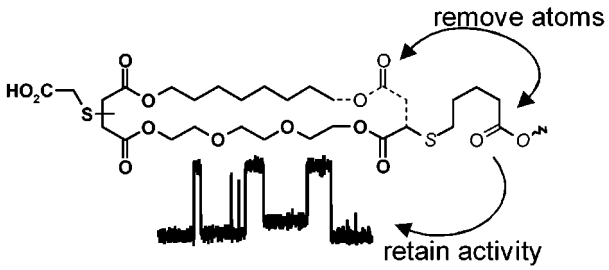
ABSTRACT



An acyclic bolaamphiphile forms ion channels in vesicles and planar bilayer membranes that are closely similar to the channels formed by related bismacrocyclic compounds. Thus the function of these ion channels does not depend on synthetically difficult macrocyclic subunits.

Most known examples of synthetic ion channels have macrocyclic or polycyclic structural components.[1,2] Such components are claimed (by us and others) to be a useful building-block motif to facilitate synthesis,[3] to provide portals for cation entry and transfer through the bilayer,[4] and/or to provide rigidity to limit the conformations open to the transporter.[5] All of these factors are supposed to promote the entry of the transporter into the bilayer and to provide the structural integrity of the transmembrane pore. This is plausible to the extent that synthetic channels of high activity have been designed using these principles. A few contrary examples of flexible acyclic materials apparently form some type of ion-conducting structure in bilayer membranes without the supposed benefit of macrocyclic or rigid polycyclic components.[6,7] Apart from the scientific question of whether the design assertions are sound, there is a considerable practical issue at stake: flexible acyclic compounds would be easier to make than macrocycles bearing the same structural elements.

We have reported the ion-transporting activity of a series of bismacrocyclic bolaamphiphiles such as **1**.[8] Such compounds readily insert into bilayers and open cation-selective channels, and they can be modified to produce voltage-gated pores.[9] Counterbalancing these desirable functions is a tedious and inelegant synthesis. Is the macrocycle synthesis

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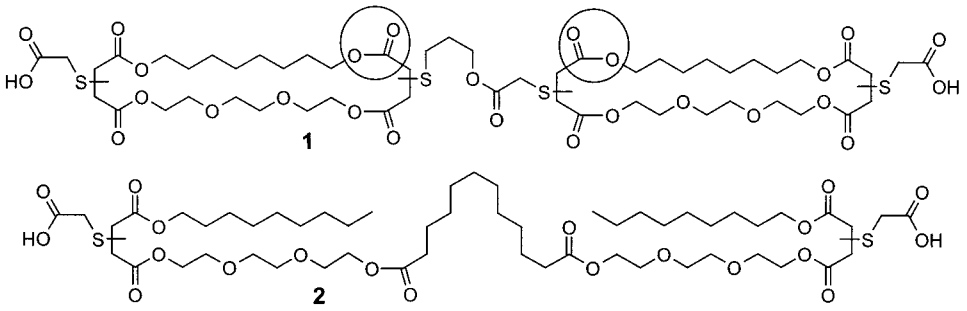
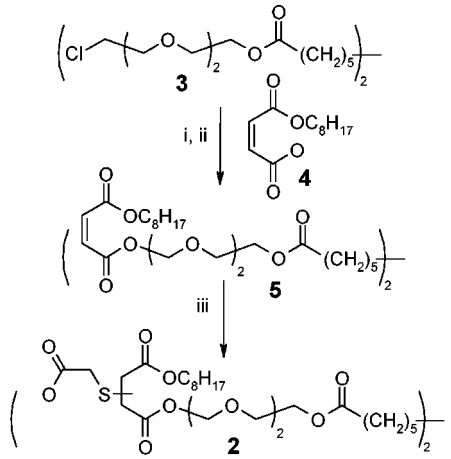


worth the effort? Are the macrocyclic components of **1** essential to its function? A compound to test this question is **2**, conceptually derived from **1** by (1) deletion of the two esters (circled) to open the macrocyclic rings, and (2) replacement of the linking thioether and ester units by methylenes. Compound **2** has the same number of atoms between the terminal carboxylic acids as the regioisomer of **1** where the thioethers are located α to the triethylene glycol esters.

The synthesis follows directly from the structure (Scheme 1). Dodecanedioyl dichloride reacted with the monochloride

Scheme 1. Synthesis of **2**[a]



[a] (i) NaI (10 equiv), acetone, reflux, 6 h, quant 92% conversion to iodide; (ii) mixture from (i) (2 equiv), **4** (2 equiv), Me_4NOH (2 equiv), DMSO, 60 °C, 16 h, 90%; (iii) mercaptoacetic acid (2 equiv per alkene), tetramethyl piperidine (2 equiv per alkene), THF, rt, 4 d, quant.

of triethylene glycol to give the diester **3** in 56% yield following chromatography. The dichloride was treated with NaI in acetone to generate a mixture containing predominantly the diiodide. The monooctyl ester of maleic acid (**4**) was readily prepared, and the carboxylate of **4** was alkylated with the chloro/iodo mixture from **3** to give the diene tetraester **5** in 90% overall yield. Michael addition of mercaptoacetate to the diene gave the target **2**. The unoptimized overall yield for the entire process is 50%. This is stark contrast to the synthesis of **1** in a yield below 0.1%.[8]

The transport activity of **2** was assessed in both vesicles and planar bilayers. The vesicle experiments used the pH-stat method used previously with **1** and related compounds.[10] Vesicles were prepared by sonication of an ether solution of phosphatidyl choline, phosphatic acid, and cholesterol (8:1:1) mixed with a pH 6.5 buffer, followed by addition of unbuffered choline sulfate solution and evaporation of the ethereal solvent. Vesicles were sized by multiple extrusion though a 0.4 μm Nucelopore filter to a monodisperse population of unilamellar vesicles that were 180 nm in diameter.

Compound **2** can induce collapse of the transmembrane pH gradient via a proton-cation exchange process. There is essentially no selectivity in rate between cesium, sodium, or potassium sulfates. Initial rate experiments as a function of the added concentration of **2** give an apparent kinetic order of 2.1 ± 0.3, consistent with the aggregates previously proposed for **1**. The specific activity of **2** is about 10-fold lower than that of **1** under similar conditions (24 vs 2.7 (± 0.5) × 10^{-10} mol H^+ sec^{-1} at a concentration of 21 μM normalized by the apparent kinetic order).[8] Compound **2** bears some structural similarity to membrane-disrupting bolaamphipiles reported previously.[7] Consequently we investigated the ability of **2** to release vesicle-entrapped carboxyfluorescein via large membrane defects. In the concentration range of the pH-stat experiments (10−80 μM of **2**) there is no detectable carboxyfluorescein release. Higher concentrations (above 0.2 mM of **2**) do result in membrane disruption with an R_{50} (half-maximum release concentration) of 0.3 mM.

Planar bilayer experiments used the voltage-clamp experiment described previously.[8,9] Bilayers were formed from diphytanoyl phosphatidyl choline, either by the "painting" technique or by a "dipping" technique in which a lipid-in-decane droplet in the hole of the cell barrier is thinned by briefly lowering then raising the electrolyte level on the *cis* side of the cell. Compound **2** was added as a methanol solution to the *cis* side of the cell. Incorporation was inconsistent by this technique; only half of the attempts produced single-channel ion currents. This "success rate" is comparable to some linear peptides, such as alamethicin, that act via oriented aggregates in the bilayer membrane, as a result of the complexity of the insertion, orientation, and aggregation pathway. Activity, when observed, was the same on a day-to-day basis.

Typical data are given in Figure 1. Step conductance changes of 1−3 s duration are observed at both positive and

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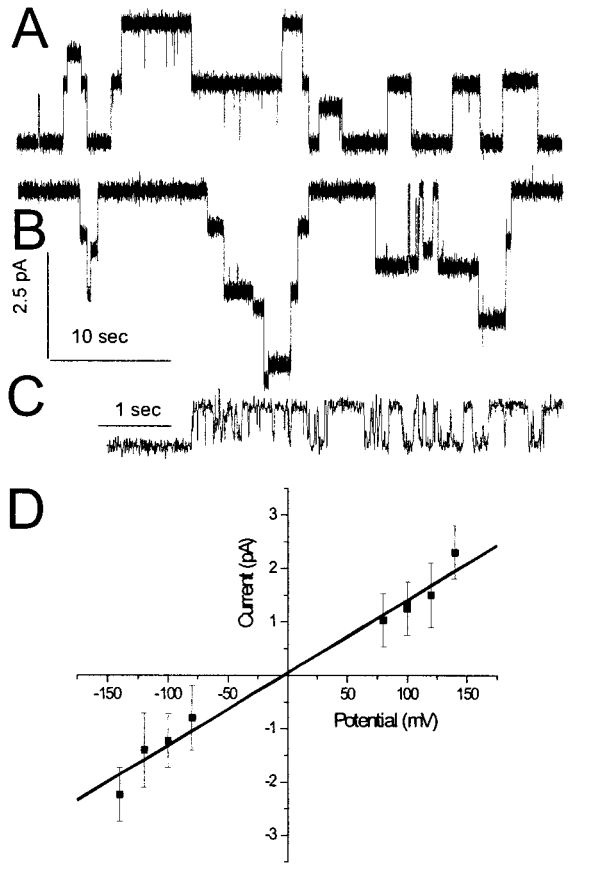

Figure 1. Single channel recordings of **2** in a diphytanoyl phosphatidyl choline bilayer: (A) +100 mV applied potential; (B) −100 mV applied potential; (C) expansion of a trace at −100 mV showing rapid opening/closing events; (D) current−voltage relationship.

negative applied potentials. Some nonuniformity of the step heights is evident in Figure 1A,B, but over a 2 min period these differences are insignificant in the all-points histograms generated. The sole result is a broadening of the distributions and an increased uncertainty in the mean current as reflected in the error bars plotted in Figure 1D. A linear current−voltage relationship is likely ($r^2 > 0.98$) with a specific conductance of 13.7 ± 0.7 pS. Under the same conditions the specific conductance of **1** is 15.0 pS.[8] Figure 1C shows a flickering behavior of rapid opening−closing events that was observed for short periods of time in about 5% of the successful incorporations. The magnitude of these openings is similar to the smaller step conductance changes shown as part of Figure 1A,B, but as noted these are not statistically significant in a larger sample. This type of flickering has not been observed with **1**, although it is occasionally observed in some related derivatives.[8]

The principal conclusion is that macrocyclic structures are not essential for the functioning of **1**. The macrocycles may assist insertion into the bilayers, and this may be the origin of the reduced activity of **2** relative to **1** in vesicles, but the channels once formed are apparently similar between the two compounds. By analogy, the activity of **2** may derive from a membrane-spanning aggregate of a few molecules that stabilizes an aqueous defect in the bilayer. Further mechanistic insights will be derived from a more extensive series of acyclic compounds based on **2**.

Acknowledgment. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

Supporting Information Available: Synthetic details for **2**−**5**, ^{1}H and ^{13}C NMR spectra for **2**, **3**, and **5**, and membrane methods and data. This material is available free of charge via the Internet at http://pubs.acs.org.

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