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A Lipophilic Polyamino-Bolaamphiphile Designed to Dissipate pH Gradients Across a Bilayer Membrane: Synthesis and Proton Transport

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Abstract A linear, bolaamphiphilic octaamine **5** containing eight adamantyl groups and two sulfonates as polar head groups was efficiently prepared from spermine. The compound is designed to span a bilayer membrane and dissipate a pH gradient by proton transfer along the backbone. Results from proton transport experiments using liposomes were consistent with design. Copyright © 1996 Elsevier Science Ltd

Efficient ion transport across phospholipid membranes is an essential process in organisms that requires mediation by carrier transport (i.e., monensin) or transmembrane protein channels.[1] Recently we have shown that dissipation of proton gradients between the cytosol and acidic vesicles such as lysosomes with membrane-active proton carriers may have therapeutic benefits against multi-drug resistance in cancer cells and viral uncoating.[2] Artificial ion channels have become a popular target for bioorganic chemists. Most are designed to transport metal ions or protons through a polyether framework that is extended through the membrane by polar head groups.[3] Groves and co-workers have constructed a proton-selective channel using four identical heptapeptide units attached to a tetraphenylporphyrin.[4] Others use amphiphiles that are proposed to self-associate within the membrane.[5] Finally, Menger has shown that ion flux can be promoted simply by attaching alkyl groups of various sizes to the hydrophobic portion of the lipid, presumably creating reversible defects in the bilayer.[6] As part of our interest in selective proton transport through biological membranes we have prepared a linear, hydrophobic octaamine **5**, where each nitrogen along the chain is substituted with an adamantylmethyl group. The molecule is terminated on each end by a propylsulfonate head group, and is designed to be 48 Å long when fully extended and thus able to span a typical bilayer membrane (figure 1).[7] Hydrogen ion flux is envisioned to occur by proton transfer along the chain, aided initially perhaps by the anionic head group, and by the built-in flexibility of the chain that should allow the amino nitrogens to approach each other adequately.

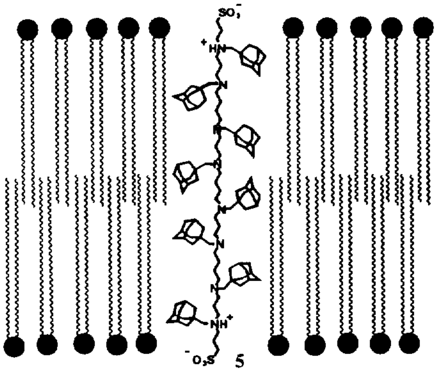
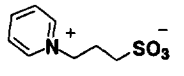


Figure 1

The backbone of the molecule was provided by two spermine units linked by a four-carbon spacer. Spermine was efficiently acylated with adamantanecarbonyl chloride in a two-phase CH_2Cl_2/10M NaCl system to give the tetraamide **1** (see scheme).[8] All four amide carbonyls were reduced by treatment with BH_3•THF (at reflux overnight) and then methanol (at reflux, repeated three times). The tetraamine **2** was easily purified on alumina (grade II-III), eluting with ethyl acetate/hexane. We wanted to monoalkylate **2** with propanesultone on one of the secondary amines, and expected that the desired product would have to be purified from a statistical mixture. We anticipated this to be more selective in the absence of external base since protonation of the product following the first alkylation might inhibit subsequent alkylation. In practice, however, the reaction was very sluggish and had to be carried out at higher temperatures (i.e., chloroform at reflux), a result of which was significant bis-alkylation. In contrast, alkylation of **2** with ten equivalents of propanesultone in CH_2Cl_2/pyridine at room temperature overnight provided only the desired monosulfonate **3** in good yield.[9] Perhaps the presence of pyridine provides the acyclic, activated intermediate **6**, leading to more facile reaction. However, the selectivity remains surprising and we have no good explanation for it.



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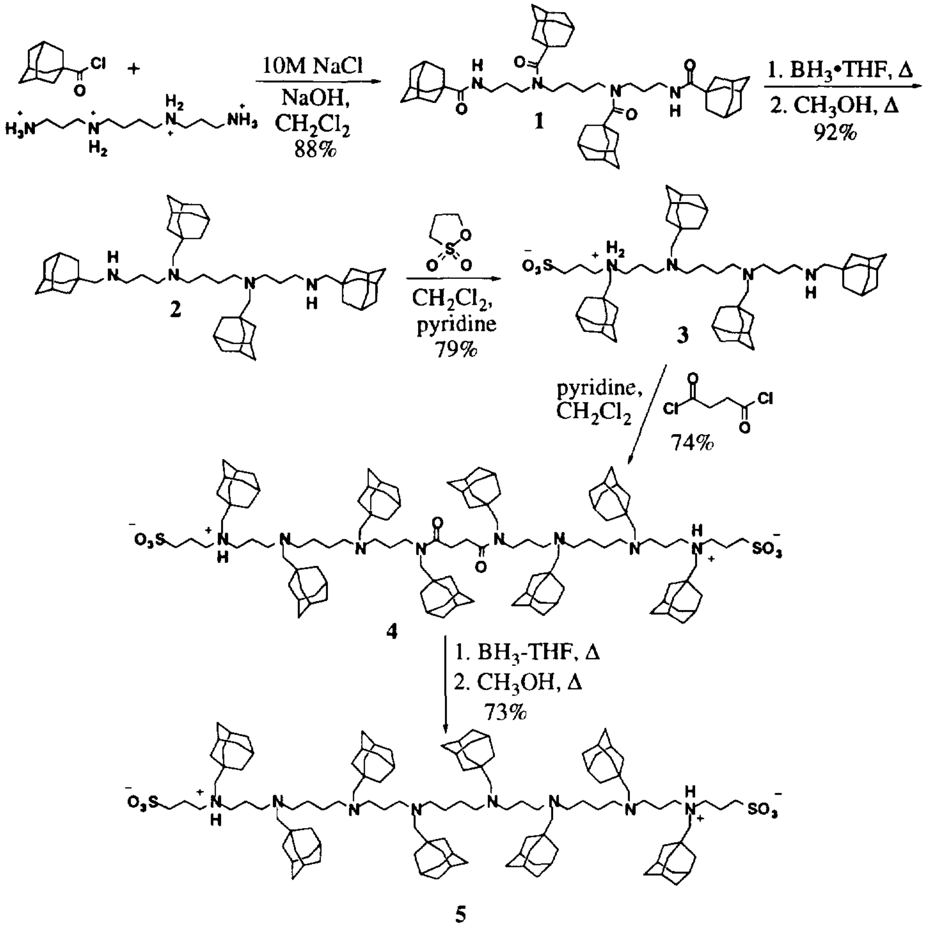
The coupling of two equivalents of **3** using succinyl chloride was clean and efficient considering the steric bulk surrounding the nucleophiles. In contrast, attempted reaction of **3** with 0.5 equiv. of 1,4-diiodobutane to give **5** directly led to slow formation of an intractable mixture. Reduction of the two amide groups in **4** proceeded as with **1** except that longer reaction times were required for both steps, presumably because of their crowded environment. Purification of the bis-zwitterion **5** was carried out on neutral alumina (5:1-3:1CH_2Cl_2/CH_3OH).

Scheme



The proton NMR of 5 in $CDCl_3$ at low concentrations (figure 2a) contains a very broad signal, extending from δ 2-3, representing all of the methylene groups next to nitrogen. This suggested to us that the two acidic protons are not localized on the two endmost amino nitrogens as shown but instead are being shared among the nitrogens along the chain as would be desirable in a functional 'proton wire'. Broadening of the corresponding carbon signals, especially for those next to nitrogen is also observed in the ^{13}C-NMR. In contrast, the proton spectrum of the bis-sodium salt (figure 2b), prepared by deprotonation with two equiv. of NaOH in methanol, is much sharper and shows the expected postion of the NCH_2 groups (δ 2.35 and 1.97).[10] Likewise, all of the carbon signals resolve into single, sharp peaks.

Figure 2.

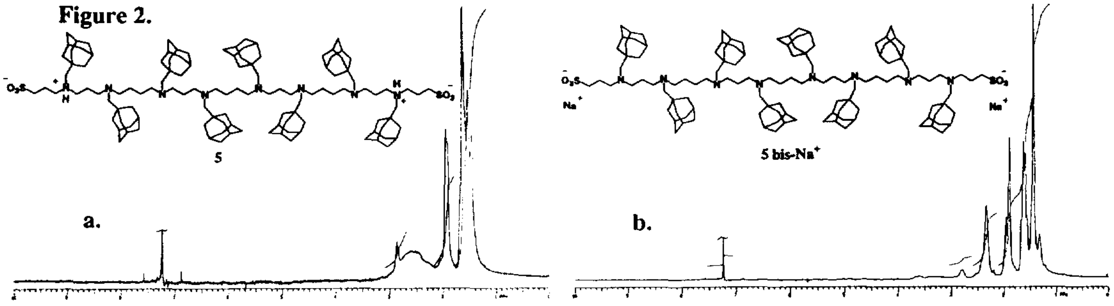
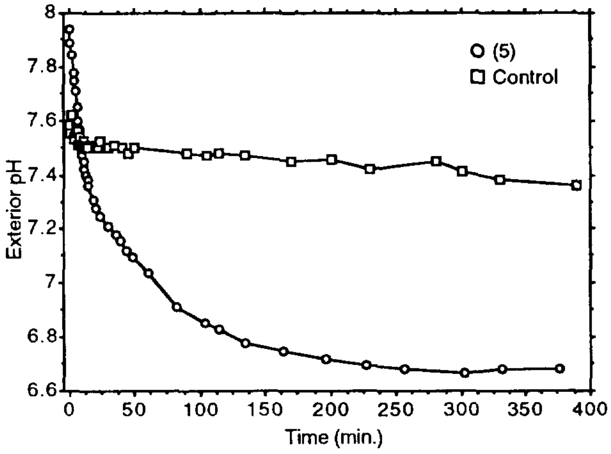


Figure 3. Effect of (5) on Proton Transport from Aspartate-Buffered Liposome Interior (pH 4) to Unbuffered Exterior at 22°C.



An experiment was performed to determine whether 5 could incorporate itself into phosphatidylcholine liposomes and act as a proton transporter. Liposomes were prepared by the detergent depletion method of Tanford[11] in a 0.04M aspartate buffer (with 0.22M NaCl, pH 4.0). Under a nitrogen atmosphere the external pH was raised by addition of 0.1M NaOH/0.22M NaCl to between pH 7.5-8 in which range the exterior loses nearly all buffering capacity. A methanol solution (4 μL) of 5 was added (ca. 1 molecule of 5/1600 molecules of lipid) and the change in pH over time was monitored using a solid state pH electrode (AccuFet).[12]

The results are shown in figure 3. The exterior pH dropped steadily over 2 h almost immediately upon addition of 5. At the end of the experiment the liposomes were sonicated for 10 min and the final pH measured was 6.56. A control batch of liposomes, to which was added only an equivalent amount of methanol, showed, in agreement with Tanford,[12] only a very slow leakage of proton equivalents after a quick initial pH drop, most likely due to lysis of a certain fraction of fragile, defective liposomes. Although the results of this experiment are consistent with proton transport by **5** acting as designed, we cannot rule out the possibility that bilayer membrane defects caused by other kinds of interactions with **5** are the cause.

The octahydrochloride of **5** could be dissolved in aqueous solution as a complex with eight equivalents of hydroxypropylated cyclodextrin. The proton NMR in D_2O was consistent with encapsulation of each adamantyl group by one cyclodextrin.[13]

References and Notes

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7. Kobuke, Y.; Yamanishi, M.; Hamachi, I.; Kagawa, H. and Ogoshi, H. *J. Chem. Soc., Chem. Comm.* **1991**, 895-897.
8. For **1**: ^{1}H-NMR δ ($CDCl_3$) 1.50 (4H, brm), 1.71 (24H, brs), 1.84 (12H, brs), 1.91 (12H, brs), 1.99 (16H, brm), 3.11 (4H, q), 3.38 (8H, q), 6.51 (2H, br). MS (FAB) 851.5 $(MH)^+$, 873.6 $(M+Na)^+$, 889.6 $(M+K)^+$. Anal. calc. for $C_{54}H_{82}N_4O_4 \cdot H_2O$: C-74.61, H-9.74, N-6.44. Found: C-74.26, H-9.59, N-6.27. For **2** ^{1}H-NMR δ ($CDCl_3$) 1.31 (4H, brm), 1.44 (12H, s), 1.49 (12H, s), 1.62 (28H, m), 2.91 (12H, m), 2.95 (4H, s), 2.20 (4H, s), 2.31 (4H, brt), 2.38 (4H, t), 2.53 (4H, t). MS (DCI). 795 $(MH)^+$, 659 $(M-C_{10}H_{15})^+$. Anal. calc. for $C_{54}H_{90}N_4$: C-81.55, H-11.41, N-7.04. Found: C-81.03, H-11.09, N-6.93. For **3**: ^{1}H-NMR δ ($CDCl_3$) 2.89 (12H, m), 1.90 and 1.92 (each 2H, s), 2.02 (4H, brs), 2.18 (2H, m), 2.41 (10H, m), 2.57 (2H, brt), 2.66 (2H, s), 2.89 (2H, ABq), 3.19 (2H, t). MS (FAB) 918 $(MH)^+$. Anal. calc. for $C_{57}H_{96}N_4O_3S \cdot CH_2Cl_2$: C-71.10, H-10.07, N-5.75. Found: C-70.94, H-9.80, N-5.67. For **4**: ^{1}H-NMR δ ($CDCl_3$)1.60 (96H, m), 1.98 (32H, brm), very broad signals for the backbone from 1.1-3.2 except for amide NCH_2 and $^{-}O_3SCH_2$ multiplets at 2.69 and 3.00, and $COCH_2$ at 3.40; MS (FAB) 1918 $(MH)^+$. Anal. calc. for $C_{118}H_{194}N_8O_8S_2 \cdot 10H_2O$: C-67.58, H-10.28, N-5.34. Found: C-67.55, H-10.48, N-5.23. For **5**: ^{1}H-NMR δ ($CDCl_3$) Figure 2. MS (FAB) 1889 $(MH)^+$; HRMS calc. for $C_{118}H_{199}N_8O_6S_2$: 1888.4954. Found: 1888.4977.
9. Selective mono-alkylation (91% yield) of a primary diamine with one equiv. of propanesultone in polar, aprotic solvents was described in a Japanese patent (**79 36, 214**). However, we found these conditions to be unacceptably slow, perhaps because our nucleophile is a hindered secondary amine.
10. The signal at δ 3.8-3.9 in both proton spectra represents the methylene group adjacent to sulfur.
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