NMR (DMSO-d_6/D_2O) δ 150.06, 135.25, 135.21, 125.31, 69.44, 54.28.

(*S*)-Naphthylethyltrimethylammonium iodide–(−)-G17: prisms from toluene/acetonitrile; ^1H NMR (D_2O) δ 8.17 (1 H), 7.58, 7.49 (2 t, 1 H each), 7.88 (d, 1 H), 7.92 (d, 1 H), 7.51 (t, 1 H), 7.72 (d, 1 H), 5.54 (q, 1 H), 2.91 (s, 9 H), 1.74 (d, 3 H); ^{13}C NMR ($CDCl_3$) δ 132.27, 131.25, 130.37, 128.12, 127.78, 127.35, 126.96, 125.43, 123.97, 123.00, 66.92, 51.45, 16.54; $[\alpha]_D$ −47° (*c* 0.16, borate-*d*).

(*R*)-Naphthylethyltrimethylammonium iodide–(+)-G17: prisms from toluene/acetonitrile; ^1H NMR (D_2O) δ 8.17 (d, 1 H), 7.57, 7.49 (2 t, 1 H each), 7.87 (d, 1 H), 7.92 (d, 1 H), 7.50 (t, 1 H), 7.71 (d, 1 H), 5.54 (q, 1 H), 2.91 (s, 9 H), 1.74 (d, 3 H); ^{13}C NMR ($CDCl_3$) δ 133.42, 131.88, 131.07, 128.32, 127.79, 127.71, 126.12, 124.46, 123.63, 123.04, 67.57, 52.05, 17.10; $[\alpha]_D$ +44° (*c* 0.11, borate-*d*).

Phenyltrimethylammonium tetrafluoroborate–G11: crystallized from CH_3CN/Et_2O; ^1H NMR (borate-*d*) δ 7.83 (br d, 2 H), 7.64 (m, 3 H), 3.65 (s, 9 H). Anal. Calcd: C, 48.47; H, 6.33; N, 6.27. Found: C, 48.41; H, 6.53; N, 6.27.

(4-*tert*-Butylphenyl)trimethylammonium tetrafluoroborate–G12: crystallized from CH_3CN/Et_2O; ^1H NMR (borate-*d*) δ 7.75 (d, 2 H, *J* = 7.0), 7.70 (d, 2 H, *J* = 7.0), 3.63 (s, 9 H), 1.34 (s, 9 H). Anal. Calcd: C, 55.93; H, 7.94; N, 5.02. Found: C, 55.61; H, 7.94; N, 4.99.

1,4-Bis(trimethylammonium)benzene bis(tetrafluoroborate)–G13: crystallized from CH_3CN; ^1H NMR (borate-*d*) δ 8.14 (s, 4 H, 3.71 (s, 18 H). Anal. Calcd: C, 39.17; H, 6.14; N, 7.61. Found: C, 39.07; H, 6.14; N, 7.34.

Cyclohexyltrimethylammonium iodide–G18: plates from aqueous acetone; ^1H NMR (CD_3CN) δ 3.18 (tt, 1 H, *J* = 3, 12), 2.92 (s, 9 H), 2.08 (dd, 2 H, *J* = 2, 12), 1.82 (dd, 2 H, *J* = 2, 13), 1.54 (dt, 1 H, *J* = 2, 14), 1.36 (dq, 2 H, *J* = 2, 14), 1.22 (tq, 2 H, *J* = 2, 14), 1.03 (qt, 1 H, *J* = 2, 14); ^{13}C NMR (borate-*d*, external TSP-d_4 at 0.00 ppm) δ 56.77, 33.10 (t, *J* = 4), 8.28, 7.27, 6.61.

1-Naphthyltrimethylammoniummethyl Iodide–G25. The product was formed in 82% yield and was recrystallized from CH_3CN: ^1H NMR (borate-*d*) δ 8.28 (d, 1 H, *J* = 10.6), 8.17 (d, 1 H, *J* = 10.6), 8.09 (d, 1 H, *J* = 10.6), 7.74 (mt, 1 H), 7.67 (t, 2 H, *J* = 10.6), 5.06 (s, 2 H), 3.16 (s, 9 H); ^{13}C NMR (DMSO/D_2O) δ 135.1, 133.3, 130.7, 129.3, 128.1, 126.8, 124.8, 124.6, 66.9, 54.6.

Adamantyltrimethylammonium iodide (ATMA)–G10: needles from CH_3CN; ^1H NMR (borate-*d*, external TSP-d_4 at 0.00 ppm) δ 2.99 (s, 9 H), (s, 6 H, 2.07), 2.31 (s, 3 H), 1.70 (AB, 6 H, *J* = 14, Δν = 31.8 Hz); ^{13}C NMR ($CDCl_3$) δ 73.16, 48.85, 35.29, 35.14, 30.21.

***N*-Methylisoquinolinium iodide–G9**: needles from acetone/water; ^1H NMR (CD_3CN) δ 9.95 (s, 1 H), 8.76–8.34 (m's, 6 H), 5.10 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 150.25, 137.30, 137.08, 135.32, 131.42, 130.78, 127.10, 126.10, 48.97.

(−)-Bornyltrimethylammonium iodide bornyl-TMA–G21: ^1H NMR (borate-*d*) δ 3.61 (dd, 1 H), 3.05 (dd, 9 H), 2.21, 1.77, 1.71, 1.46, 1.21 (m's, 7 H), 1.01, 0.90, 0.86 (3s, 3 H each); ^{13}C NMR ($CDCl_3$) δ 82.01, 54.7 (br), 52.35, 51.60, 43.17, 31.58, 28.47, 27.89, 19.89, 19.83, 19.60, 17.48; $[\alpha]_D$ −8.0° (*c* 0.075, in borate-*d*).

(*S*)-(*cis*)-Myrtanyl-TMA–G22: plates from acetonitrile; ^1H NMR ($CDCl_3$) δ 3.78 (dd, 1 H), 3.56 (br d, 1 H), 3.22 (s, 9 H), 2.59, 2.43, 1.86, 1.10 (m's, 11 H), 1.86, 0.94 (s, 3 H each); ^{13}C NMR ($CDCl_3$) δ 76.88, 53.94, 53.82, 47.74, 40.39, 38.30, 35.77, 27.40, 25.75, 23.82, 23.39; $[\alpha]_D$ +23° (*c* 0.10, borate-*d*).

(1*S*,2*R*)-(1-Methyl-2-phenyl-2-hydroxy)ethyltrimethylammonium iodide [(−)-dimethylephedrine]–G23: ^1H NMR (D_2O) δ 7.45 (d, 2 H), 7.39 (t, 2 H), 7.31 (t, 1 H), 5.62 (d, 1 H), 4.43 (d, 1 H), 3.60 (q, 1 H), 3.24 (s, 9 H), 1.16 (dt, 1:1:1, 3 H); ^{13}C NMR (CD_3CN at 1.3 ppm) δ 141.47, 128.80, 128.19, 126.29, 75.22, 69.39, 53.23 (1:1:1, t, *J* = 4.4), 7.65; $[\alpha]_D$ −22° (*c* 0.12, borate-*d*).

(*R*)-α-Trimethylammoniumethyl-3,4-dimethoxybenzyl alcohol iodide salt (tetramethylepinephrine)–G24: needles from acetonitrile; cesium carbonate was used instead of K_2CO_3 for the alkylation reaction; ^1H NMR (borate-*d*, external TSP-d_4 at 0.00 ppm) δ 7.09 (m, 3 H), 5.36 (d, 1 H), 3.96 (s, 3 H), 3.88 (s, 3 H), 3.69 + 3.51 (2 dd, 2 H), 3.29 (s, 9 H); ^{13}C NMR (CD_3CN at 1.3 ppm) δ 141.24, 132.93, 118.63, 114.65, 111.83, 110.26, 71.04, 68.45, 53.66, 56.11, 54.97; $[\alpha]_D$ +33° (*c* 0.15, borate-*d*).

Acknowledgment. We thank the National Institutes of Health and the Office of Naval Research for financial support of this work and Dave Wheeler for substantial help with various NMR experiments.

A Macrocyclic Tetraether Bolaamphiphile and an Oligoamino α,ω-Dicarboxylate Combine To Form Monolayered, Porous Vesicle Membranes, Which Are Reversibly Sealed by EDTA and Other Bulky Anions

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Abstract: The hydrophobic tetraether macrocycle 1,20-disulfonyl-4,17,23,36-tetraoxacyclooctatriacontane is obtained in the gram scale from 2,2-dithioethanol and 1,12-dodecanediol. Oxidation or methylation of the sulfur atoms leads to bolaamphiphiles which vesiculate on ultrasonication. These amphiphiles are simple analogues of the membrane constituents of *archaebacteria*. The vesicles are acid stable and entrap metal ions (Li^+, Fe^{2+}) as well as fluorescent dyes (pyranine, calcein). The dipotassium salt of 2,19-dimethyl-3,6,9,12,15,18-hexaazaeicosanedicarboxylate introduces pores for metal ions into the membrane, but not for the organic dyes. The cationic pores could be closed with water-soluble bulky anions such as camphorsulfonic acid, taurine, and EDTA. The EDTA stopper was extracted from the pore by an excess of Fe(II) ions. Excess EDTA reclosed the pore. This cycle could be repeated several times.

Water-insoluble amphiphiles with head groups on both ends of a hydrophobic chain have been named "bolaamphiphiles". The self-organization of bolaamphiphiles in aqueous media may produce planar or spherical monolayered membranes.[1,2] The thickness of the hydrophobic membranes is identical with the length of the bipolar amphiphiles and may be as thin as 1.5 nm.[3] Within the hydrophobic membrane, guest amphiphiles of similar lengths and containing a hydrophobic edge ("edge amphiphiles") self-organize to form "domains". The core of these domains is

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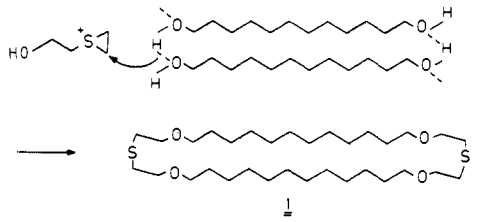
Scheme I



hydrophilic and functions as a "pore" for the diffusion of salts through the membrane.[3] These domains serve as simple models for membrane proteins, biological receptors, and ion channels.[4] A major function, which models should realize, is the reversible closing and opening of the ion channel by water-soluble substances (chemical signals, transmitters).

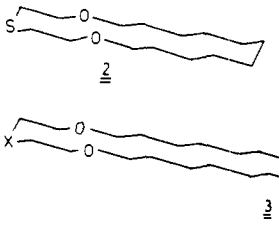
Electroneutral[5] and electronegative[3] monolayered vesicle membranes have already been perforated with edge amphiphiles, i.e., negatively charged monensin pyromellitate. Lithium salts diffuse through these pores, and it has been shown conclusively that the pyromellitate has no carrier function.[5] When the bolaamphiphile 1,12-bis(trimethylammonio)dodecyl dichloride was also dissolved within the membrane, the pore remained closed.[5] However, it was not possible to close the monensin pyromellitate pore reversibly by addition of ammonium salts to the bulk aqueous phase. Binding constants between cationic stoppers and the anionic pores were too low. In search of a pore with high affinity to water-soluble counterions, we synthesized a pentaethylenehexamine with two carboxylate end groups. This highly charged oligocationic pore could bind various organic anions tightly enough to prevent metal ion transport.

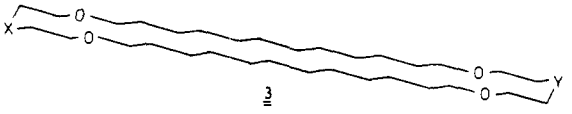
After useful pore stopper combinations were found, the membrane-forming bolaamphiphiles had to be modified because the oligoamine edge would have attacked the ester bonds of the original macrolides. Bolaamphiphiles with a macrocyclic tetraether core were thus developed in loose analogy to the strategy of *archaebacteria*.[6] We report here for the first time on vesicles that can be reversibly opened and closed by chemical "transmitters" in the bulk aqueous phase.

Results

Syntheses and Structures. Attempts to produce macrocyclic ethers by reduction of macrolides,[7,8] base-catalyzed condensation, or substitution reactions[8] failed. Only thermal reaction between 2,2-thiodiethanol and α,ω-diols[9] in the presence of *p*-toluenesulfonic acid gave the desired macrocycles in reproducible yields of ~50%. This success is explainable by (i) the thermal formation of reactive *S*-(2-hydroxyethyl)thiiranium ion and (ii) by tight hydrogen bridges between diol molecules in the melt (Scheme I). The reactive intermediate allows mild reaction conditions; the diol aggregate resembles the final macrocycle.

Ether macrocycles were easily separated from open-chain oligomers containing polar hydroxyl groups. It was more difficult to differentiate the diether macrocycle **2** from the tetraether **1**. We isolated only one nonpolar macrocycle from the reaction mixture, namely, the tetraether **1**. The arguments in favor of structure **1** are as follows: (i) the molecular weight was determined by vapor pressure osmometry as being 600 ± 50, (ii) the mono-S-methylated sulfonium salt **3d** shows one *S*-methyl group per two $(CH_2)_{12}$ chains in the ^1H NMR spectrum, and (iii) mass



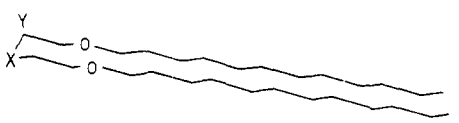


a X = Y = SO_2
b X = Y = SO
c X = Y = S^+–CH_3 ClO_4^-
d X = S Y = S^+–CH_3 ClO_4^-

spectra give a molecular peak at *m/e* 576 for a compound that is pure as judged by analytical HPLC.

The conversion of the apolar thioether **1** to bipolar bolaamphiphiles was achieved by methylation or oxidation of the sulfur atoms. As expected, the trialkylsulfonium salt, sulfone, and sulfoxide, all produced stable vesicle membranes (see below) and constitute the first examples of nonhydrolyzable synthetic monolayer lipid membranes. The sulfonium salts **3c** and **d** and the sulfoxide **3b** contain anisotropies at the sulfur atoms. These are detected in the ^1H NMR signal of the methylene groups neighboring the sulfur atoms, which appear as double triplets. In the mass spectra of the macrocyclic ethers **3a–d** strong peaks at half the molecular mass ±1 H or ±2 H are to be found. Similar peaks at half the mass were also described for archaebacterial tetraethers,[10] synthetic poly(ethylene glycol) macrocycles,[11] and macrolides.[12]

An attempt was made to extrude the sulfur atoms from the tetraether macrocycles, the standard method being the Ramberg–Bäcklund reaction. This starts with an α-chlorinated sulfone and goes on with an α'-deprotonation and cyclization to an episulfone. An olefin is produced by sulfur dioxide elimination.[13] The sequence was first studied with the open-chain compound **4a**.



a X = S Y=H
b X = SO_2 Y=H
c X = SO_2 Y=Cl
d X = SO Y = H
e X = SO Y = Cl

Oxidation and chlorination yielded chloro sulfone **4c** and chloro sulfoxide **4e**. Treatment with a strong base, however, leads to quantitative elimination of the long-chain alcohol. Several other variations of the Ramberg–Bäcklund reaction, e.g., treatment of the open-chain sulfone with potassium *tert*-butoxide in carbon tetrachloride,[14] always ended with the same results. The cyclic tetraether sulfone **3a** gave only 1,12-dodecanediol as a product. It appears that the alkoxide groups neighboring the carbanion are better leaving groups than the chlorine atom, which is separated by an electronegative sulfone group from the carbanion. This is another example of facile β-elimination of an ether group next to an electron-withdrawing group.[15,16]

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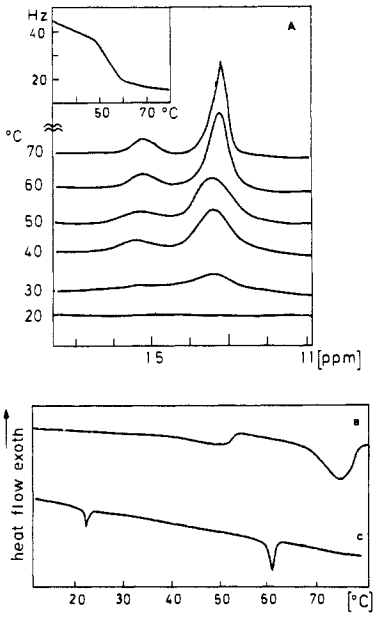
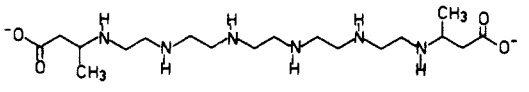


Figure 1. (A) Temperature dependence of ^1H NMR signal widths of disulfone **3a** vesicles in D_2O (in hertz), (B) thermograms of monolayer membrane vesicles made from disulfone **3a**, and (C) bilayer membrane vesicles from open-chain sulfone **4a**.

The oligoamine **5**, with carboxylate end groups, which proved its function as a versatile membrane pore (see below), was prepared from commercial pentaethylenehexamine and methyl acetoacetate followed by reduction with sodium cyanoborohydride in acidic medium.



5

Vesicles, Pores, and Stoppers. Vesicles were obtained by ultrasonication of 10^{-3} M dispersions in water or 0.03 M lithium chloride solutions of the disulfone (**3a**), the disulfoxide (**3b**), and the disulfonium salt (**3c**). Within a few hours the slightly opaque solutions turned white, and after 1 day, precipates were observed. Electron micrographs revealed aggregated vesicles (diameters $\leq$ 200 nm) even in freshly sonicated dispersions. It was not clear from the electron micrographs or the transport experiments described in this paper whether multilayered vesicles were present or not. The vesicles were characterized by entrapment of metal ions (Li^+, Fe^{2+}) and water-soluble fluorescence dyes (pyranine,[17] calcein[18]). Entrapment volumes between 0.02 and 0.5% of the total water volume were found. This corresponded well to calculated values for vesicles with average diameters of 100 nm and amphiphile concentrations between 10^{-4} and 10^{-3} M.

Monolayer vesicles from the disulfone **3a** gave broad ^1H NMR signals at 20 °C. Above 50 °C, the membrane "melted" and sharp proton signals were observed. To our knowledge this is the first example of a monolayer vesicle membrane that produces a gel phase. The crystallinity of the membrane can be traced back to the fact that it is composed of a single compound. Analogous membranes without a gel phase, which have been reported earlier,[3] always consisted of mixtures of diastereomers. Bilayer vesicle membranes made from the open-chain analogue **4a** showed a phase transition at 20 °C which is typical for any bilayer made from amphiphiles with two C_{12} chains and a neutral head group.[19]

Table I. Lithium Ion Release Mediated by Oligoamine **5**

	lithium ions (ppb) in vesicle fractn, vesicles made from	
5, mol %	**3a**	lecithin
0	180	120
0.001	60	
0.01	0	
0.1	0	
1	0	100
10	0	

Table II. Lithium Ion Release in Dependence of Concentration of the "Stopper" **6** in the Eluent

6 in eluent, mol L^{-1}	lithium ions in vesicle fractn, ppb
10^{-3}	180
10^{-4}	120
10^{-5}	50
10^{-6}	0
0	0

Differential scanning calorimetry thermograms of the vesicular solutions confirmed the NMR results (Figure 1). Endothermic peaks were found at 52 °C (ΔH = 25 kJ/mol) for the macrocyclic disulfone **3a** and at 22 °C (ΔH = 11 kJ/mol) for its open-chain analogue **4b**. The peak for the monolayer lipid membrane (MLM) was much broader than for the bilayer membrane (BLM), which indicates lack of cooperativity in the MLM melting. We assume that the incomplete space filling of the curved vesicle membrane by the symmetric bolaamphiphile is responsible for this effect. In the BLM the packing can be tighter, because its outer layer contains more molecules than the inner layer. The same broadening of the DSC peak is observed at the melting point of the MLM (76 °C) as compared to the BLM (61 °C).

Sonication of bolaamphiphiles **3a**, **b**, and **d** at 60 °C and pH 12 or 2 produced the same vesicular solutions as in neutral water. No hydrolysis products could be detected by TLC. The amphiphiles were chemically stable even at more extreme pH values, but in more acidic or basic solutions, fast aggregation and precipitation of vesicles were observed.

The hexamine **5** was then added in concentrations of 10^{-3}, 10^{-2}, 10^{-1} or 1.0 mol % in respect to the tetraether sulfone **3a**. Fluorescent dyes (pyranine, calcein) and metal ions were also added before the sonication procedure. The quantities of entrapped fluorescent dyes were found to be independent of the added hexamine. Eventual pores made from **5** are therefore impermeable for both dyes. Lithium ions, on the other hand, were only found in vesicular fractions containing less then 10^{-3} mol % of **5**. A concentration of 10 molecules of tetramine **5** per 10^5 molecules of **3a** was sufficient to release all lithium ions within the time needed to perform gel chromatography (10 min). This result implies that $\sim$10 molecules of hexamine **5** was sufficient to produce a hole in a vesicle membrane. This is the same order of magnitude as observed for anionic pores made from monensin pyromellitate.[3] Control experiments with bilayer membrane vesicles made from the lecithin analogue DPPC showed no lithium ion release in the presence of **5** (Table I). The pores are nonspecific for metal salts. Iron(II) sulfate is also released from the vesicles within 10 min.

The cationic pores made from **5** could be sealed with various anionic stopper molecules, which were added to the bulk aqueous solution and to the eluent in gel chromatography. We successfully applied D-camphorsulfonic acid (**6**), EDTA (**7**), taurine (**8**), *N*-trimethyl-γ-aminobutyric acid (**9**), and *N*-trimethyl-ϵ-aminocaproic acid (**10**) in concentrations above 10^{-3} M to reach quantitative retainment of lithium ions during gel chromatography (Table II). These stopper molecules were always added *after* ultrasonication.

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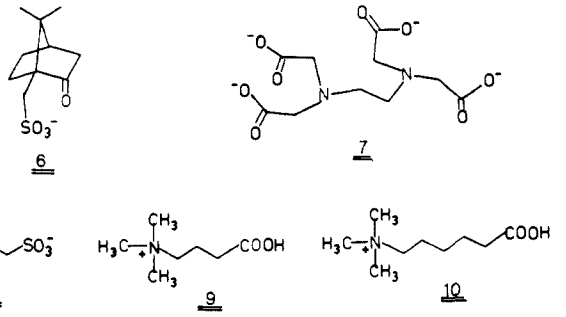

It was also possible to seal the vesicle pores without addition of stopper molecules to the eluent in gel chromatography. For this purpose the sulfonate **6** (10^{-3} M) was added *before* sonication. The entrapped stopper irreversibly sealed pores made from 0.1 mol % of **5**.

The most intriguing application of these stoppers is the reversible sealing of the pores by EDTA (**7**) at pH 7. Sulfone **3a** vesicles with 0.1 mol % of **5** and entrapped calcein were mixed with a few microliters of 10^{-2} M $FeSO_4$ solution. The calcein fluorescence was almost quantitatively quenched. This indicated that the iron(II) ions had passed the sealed pore, which had been impermeable for entrapped lithium ions. We assume that the iron ions formed a neutral metal complex with the EDTA and thereby pulled it away from the positively charged pore. Excess iron ions could then pass the pore and bind to the calcein. If an excess of EDTA was now added, it would chelate the iron ions and also withdraw them from the calcein, which has a much lower affinity to iron. The fluorescence reappeared and reached the original value. With more iron(II) being added, fluorescence would eventually disappear again to be restored by more EDTA (Figure 2). It was also demonstrated that the calcein always remained within the perforated membrane: (i) Calcein was only found in the vesicle fraction in gel chromatography, and (ii) when one of the nonchelating stoppers was added in excess, e.g., taurine (**8**), addition of EDTA could not stimulate calcein fluorescence. If, on the other hand, the vesicles were destroyed by detergents or solvents, EDTA would immediately remove the iron ions from the dye and fluorescence would reappear.

Summary and Outlook

Cationic domains of "edge amphiphiles" function in neutral monolayer vesicle membranes as selective pores and receptors (Figure 3). Inorganic ions pass through the pores, whereas large organic anions plug the hole. Chelating anions can be removed by neutralization of the negative charges with complexing metal ions. Neither the anionic stoppers nor the water-soluble dyes diffuse through the pores, even at high concentrations of the pore forming compound **5**.

The detection of ion flows by fluorescence measurements is amenable to fast kinetic measurements. Since calcein interacts with a wide variety of bivalent metal ions, the binding characteristics of the positively charged water channels can be studied in detail. Stopped-flow experiments in the millisecond range are currently undertaken.[21]

Experimental Section

Methods. Elemental Microanalyses were performed by the Microlabor des Instituts für Organische Chemie der Freien Universität Berlin.

Vapor Pressure Osmometry. A Knauer osmometer was used. Thioether **1** (34.8 and 38.4 mg) was dissolved in 10 g of toluene and T was measured in scale units (23 and 25). Comparisons with the reference compound 1,6,19,24-tetraoxa-3,21-cyclohexatriacontadiene-2,5,20,23-tetrone[3] (MW 564.8; 73.7, 30.1 and 102.1 mg gave T values of 8, 18, and 60 scale units) yielded a molecular weight of 600 ± 50.

Vesicle Preparations. The amphiphile (0.01 mmol) was dissolved in a few drops of chloroform. The solvent was removed in a steam of nitrogen. A 10-mL volume of water or 10^{-3} M phosphate buffer (pH 10) was added and the mixture sonicated at 60 °C and 20 W. After 30 min, the slight turbidity of the solution remained constant. The concentration of amphiphiles was 1×10^{-3} M. Precipitates formed within 24 h. The lifetime of the vesicular suspension (8–24 h) did not change when the basic pore of **5** was enclosed in the membrane.

Acid and Base Stability of Vesicles. Bolaamphiphiles **3a,b**, and **d** were sonicated for 60 min at 60 °C in 0.01 M hydrochloric acid or sodium hydroxide. The amphiphile was then extracted with 2 mL of chloroform and examined by TLC (silica gel; chloroform/methanol 8:2) together with authentic 1,12-dodecanediol. TLC spots were visualized with 2,7-dichlorofluorescein.

Perforation and Resealing of Vesicle Membranes. (i) Entrapment of Lithium Chloride in 3a Vesicles. A 64-mg sample of amphiphile **3a** was dissolved in 100 mL of dichloromethane; 5 mL of this solution was evaporated in a steam of nitrogen. A 10-mL aliquot of 0.03 M LiCl and 10 μL of 0.1 M NaOH were added, and the mixture was sonicated as described above. A 1-mL aliquot of the freshly prepared vesicle solution was then gel filtrated on Sephacryl S 1000 (1 × 20 cm) with 0.03 M sodium chloride containing sodium hydroxide (pH 10) as eluent. The filtrate was collected in 2-mL portions which were analyzed for lithium ions by atom absorption spectroscopy. The vesicle fraction (4–6 mL) was found to contain 160–180 ppb of lithium ions. This was calculated from comparisons with standard solutions, which were obtained by dilution of a 10 ppm lithium carbonate stock solution. Aliquots (250 μL, 500 μL, 1 mL, and 2.5 mL) of the solutions was brought to 100 mL each with water to yield 25, 50, 100, and 250 ppb standards.

(ii) Lithium Chloride Release in the Presence of Pores Made of 5. Portions (5 mL) of a solution of 64 mg of **3a** in 100 mL of dichloromethane were mixed with various volumes of methanolic solutions of the pore-forming oligoamine **5**. A sample (500, 50, or 5 μL) of a 10^{-3} M solution of **5** and 50 or 5 μL of a 10^{-5} M solution were used. The molar ratios **3a**:**5** correspond then to 10, 10^2, 10^3, 10^4, and 10^5. If one assumes an average vesicle diameter of 10^3 Å and a molecular surface of 30 Å^2 for **3a** (from electron microscopy and the calculated surface area of the cross section of two oligomethylene chains), then each vesicle averaged 10^4, 10^3, 10^2, 10, and 1 molecules of **5**. The vesicles containing **5** were prepared and gel filtrated as described under (i). Results of atomic absorption measurements are summarized in Table I.

(iii) Sealing of the Pores. Vesicles were prepared in the presence of 0.03 M lithium chloride and the pore-forming amphiphile **5** by sonication as described above. The following stopper molecules were, however, added after sonication: 10 μL of a 10^{-3} M solution of one of the sulfonates or carboxylates **6**–**10** to 1 mL of the vesicular solution, which was then gel filtrated. No efflux of lithium ions took place when the eluent contained 10^{-3} M of any of these stopper molecules. In the case of 6 (camphorsulfonic acid), the stopper solution was also diluted. The results of AAS measurements are summarized in Table II.

A 10-μL aliquot of a 10^{-3} M solution of stopper molecule **6** was also added to 10 mL of a vesicular solution of **3a** (5×10^{-4} M) containing 3 10^{-5} M LiCl as well the oligoamine **5** (5×10^{-7} M). This mixture was sonicated and gel filtrated. Only entrapped stopper molecules remained in the vesicular fraction. No lithium chloride was released from these vesicles as shown by AAS, although the stopper concentration was low (see text and compare with Table II).

(iv) Entrapment of Calcein. A 2.235-mg aliquot of a mixture of 10 g of KCl and 100 mg of calcein was dissolved in 10 mL of tridistilled water. This solution was sonicated with a lipid film made from amphiphile **3a** and 0.1 mol % of the oligoamine **5**. The vesicular solution was gel filtrated as described above. The pH was kept at 10.0. The filtrate was collected in 2-mL portions which were analyzed for entrapped calcein by fluorescence spectroscopy (λ_{exc} = 468 nm; λ_{em} = 517 nm). Fluorescence was found in the vesicular fraction (4–6 mL) as well as in the later fractions (12–16 mL). The fluorescence of the vesicle fraction was between 0.02 and 0.05% of that of the later fractions containing the free dye.

(v) Detection of the Opening of the Pores by Quenching of the Entrapped Fluorescence Dye with Iron Ions. A 10-μL aliquot of a 10^{-3} M EDTA (**8**) solution was added to a 1-mL probe of a vesicle solution prepared as described under (iv). A mixture of 0.03 M sodium chloride/10^{-3} M EDTA was used as eluent for gel filtration. Fluorescence was found in the vesicle fraction in the presence of opened pores. A 10-μL aliquot of 10^{-2} M iron(II) sulfate was then added to the vesicle fraction. The fluorescence was quenched quantitatively. When the iron sulfate solution was added to solutions of vesicles without pores, no quenching of the calcein fluorescence occurred. The quenching of calcein fluorescence depended on the presence of the vesicles. In plain aqueous solutions, lower pH values and higher iron(II) concentrations were necessary. The quenching process also became much faster when Triton X-100 was added, which obviously solubilized calcein aggregates.

(vi) Resealing of the Pores. When 100 μL of 10^{-3} M EDTA solution was added to the bulk of the above vesicle fraction, the fluorescence reached its original intensity.

(21) Dencher, N. A.; Burghaus, P. A.; Grzesiek, S. *Methods Enzymol.* **1986**, *127*, 746–760.

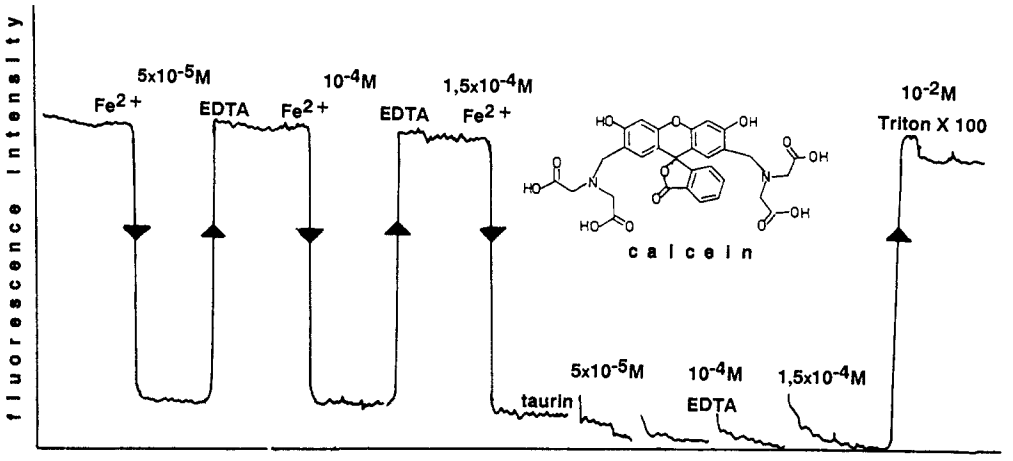

Figure 2. Fluorescence quenching of vesicle-entrapped calcein by Fe^{2+} ions. A taurine stopper (5×10^{-5} mol L^{-1}) on the basic pore prevents removal of iron ions by EDTA.

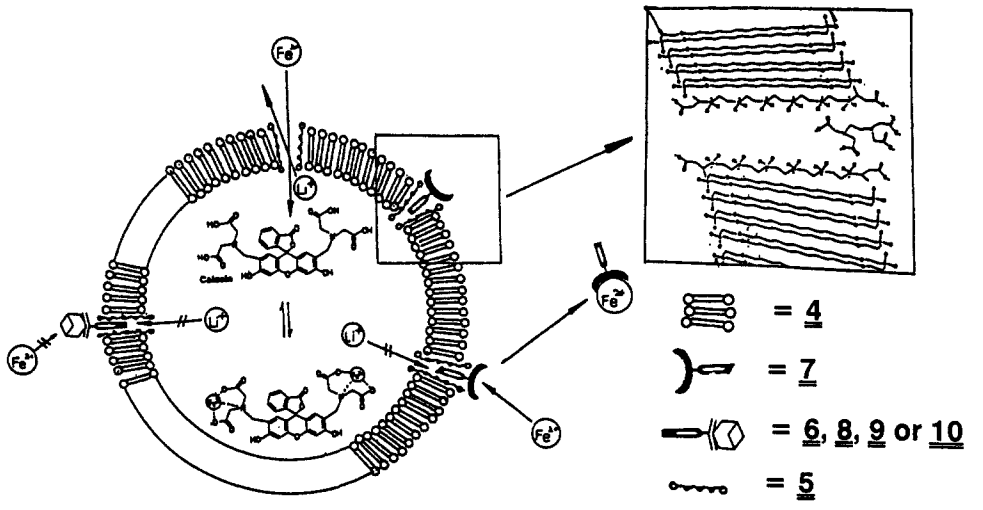

Figure 3. Model of the monolayered vesicle membrane with an encapsulated metal ion indicator and various anionic stoppers on cationic pores.

(vii) Alternating Opening and Resealing of the Pore. Renewed addition of 10 μL of 10^{-2} M iron sulfate to the same probe quenched the fluorescence to ~90%. Results of several repetitive experiments are reproduced in Figure 3.

(viii) Irreversible Blocking of the Pore with 7. When 100 μL of a 10^{-3} M solution of taurine(**7**) was added to 2 mL of the above probe containing an excess of iron(II) ions (the pores were open), the fluorescence remained quenched. The sulfonate stopper did not remove iron ions. Addition of a great excess of EDTA did not stimulate fluorescence. Addition of Triton X-100 dissolved the vesicles and the fluorescence reached its original value (see Figure 3).

Entrapment of Dyes. A 10^{-4} M pyranine solution in 10^{-3} M phosphate buffer (pH 10) was sonicated with bolaamphiphiles as described above. A 1-mL aliquot of the vesicle solution was then chromatographed on Sephacryl S 1000 (1.0 × 20 cm) with the same buffer as eluent. Pyranine entrapped in vesicles appeared in the 4–6 mL fraction and was measured without liberation into the media (fluorescence, λ_{exc} = 460 nm; λ_{em} = 510 nm). The relative fluorescence intensity of the vesicle fraction was related to the starting volume of gel chromatography and also compared with standard solutions. Up to 0.5% of the total pyranine was entrapped in the vesicles. The values for calcein entrapment were between 0.2 and 0.4%. It was shown for both pyranine and calcein that in the range between 10^{-5} and 10^{-10} mol/L the fluorescence concentration dependencies were linear.

^{1}H NMR Spectra of Vesicles. A Bruker WH 270-MHz instrument was used. Vesicle dispersions (10^{-3} M) of **3a** or **4c** were measured at eight temperatures. See Figure 1.

Differential Scanning Calorimetry. A Perkin-Elmer DSC-2 calorimeter was used.

Materials. All solvents and reagents were of analytical grade.

Attempts To Reduce Macrocyclic Tetraesters (Macrolides) to a Macrocyclic Ether. A 564-mg aliquot (1 mmol) of 1,6,19,24-tetraoxa-3,21-cyclohexatriacontadiene-2,5,20,23-tetrone[3] in 70 mL of absolute tetrahydrofuran containing 50 mL of boron trifluoride etherate and 1 g of lithium chloride was mixed with 1 g of sodium borohydride and stirred for 1 h at room temperature and 2 h with refluxing. Preparative TLC (silica gel, $CHCl_3$) of the reaction mixture gave 1,12-dodecandiol as the only major product. Apolar products at the solvent front, corresponding to cyclic tetraethers, were not detected.

Milder conditions such as reduced reaction times and change of catalysts and reducing reagents gave either cleavage of the ring or starting materials. Several fruitless attempts to produce macrocyclic tetraether by acid- or base-catalyzed condensation or substitution reactions are described in ref 8.

1,20-Dithia-4,17,23,36-tetraoxacyclooctatriacontane (1). A mixture of 12 g (0.1 mol) of 2,2-thiodiethanol and 20 g (0.1 mol) of 1,12-dodecanediol was heated with 1 g of *p*-toluenesulfonic acid to 110 °C for 4 h. After being cooled, the reaction mixture was dissolved in a few milliliters of chloroform and flash chromatographed (ICN silica gel 32–63, 3 × 30 cm, $CHCl_3$). The main fraction was collected and recrystallized from 2-propanol: yield 17 g (61%) of colorless and odorless platelets; mp 69 °C; R_f 0.4 (silica gel, $CHCl_3$). A 0.5-g sample of this product was dissolved in 2 mL of $CHCl_3$; 200 μL of this solution was applied to a 7 μm Si Knauer HPLC column (16 × 250 mm). Elution was with 20 mL/min $CHCl_3$ at 60 bar and detection with a Knauer differential refractometer. Only **1** could be detected: IR (KBr) no bands in the hydroxyl region; MS (80 eV, 150 °C) *m/e* 576 (M^+, 12), 473 (22), 289 (100), 289.5 (4); ^{1}H NMR (DMSO-d_6, 270 MHz) 1.28 (s, 40 H), 1.56 (m, 8 H, OCH_2CH_2) 2.76 (t, 8 H, SCH_2), 3.44 (t, 8 H, OCH_2), 3.60 (t, 8 H, CH_2O) ppm. Anal. Calcd for $C_{32}H_{64}O_4S_2$ (577.3): C, 66.61; H, 11.18; S, 11.11. Found: C, 66.22; H, 10.91; S, 11.01.

1,20-Dithia-1,1,20,20-tetraoxo-4,17,23,36-tetraoxacyclooctatriacontane (3a). A 1.14-g aliquot (2.0 mmol) of the thioether **1** was dissolved in 10 mL of glacial acetic acid, mixed with 0.36 mL of hydrogen peroxide (30%), and kept at 90 °C for 16 h. The solvent was removed, and 50 mL of ethanol was added. The product crystallized overnight at 4 °C: yield 1.10 g (85%) of white platelets, mp 75 °C, R_f 0.4 (silica gel, $CHCl_3/CH_3OH$ 95:5); MS (80 eV, 350 °C) *m/e* 640 (M^+, 11), 562 (49), 548 (53), 319 (100); ^{1}H NMR (DMSO-d_6 270 MHz) identical with **1**, except 3.84 (t, 8 H, SO_2CH_2) ppm. Anal. Calcd for $C_{32}H_{64}O_8S_2$

(641.0): C, 59.96; H, 10.07; S, 10.01. Found: C, 59.57; H, 10.02; S, 10.13.

1,20-Dithia-1,20-dioxo-4,17,23,36-tetraoxacyclooctatriacontane (3b). A 1-g aliquot (173 mmol) of the thioether **1** in 10 mL of dichloromethane was stirred at 0 °C with 0.6 g (3.48 mmol) of *m*-chloroperbenzoic acid for 1 h. Another 50 mL of dichloromethane was added, *m*-chlorobenzoic acid was removed, and residual acid reagent was extracted with 30 mL of 1 M sodium hydroxide. The organic layer was neutralized and washed with water, and the solvent was removed. Crystallization from ethanol yielded 0.8 g (76%) of the sulfoxide **3b**: mp 72 °C; R_f 0.6 (silica gel, $CHCl_3/CH_3OH$ 95:5); MS (80 eV, 200 °C) *m/e* 608 (M^+, 7), 548 (26), 473 (23), 299 (100); ^1H NMR ($CDCl_3$) similar to **1**, except for 3.86 (dt, 8 H, $SOCH_2$, anisotropy center) ppm. Anal. Calcd for $C_{32}H_{64}O_6S_2$ (609.0): C, 63.11; H, 10.59; S, 10.53. Found: C, 62.92; H, 10.52; S, 10.42.

1-Thionia-20-thia-1-methyl-4,17,23,36-tetraoxacyclooctatriacontane Perchlorate (3d). To 1.44 g (2,5 mmol) of molten **1** was added 90 μL (0.5 mmol) of dimethyl sulfate and the resultant mixture stirred for 30 min at 90 °C. The melt was dissolved in 3 mL of a $LiClO_4$-saturated solution in chloroform/methanol (8:2) and applied to a silica gel column (1 × 25 cm, ICN Silica Woelm, $CHCl_3/CH_3OH$ 8:2); R_f 0.2; ^1H NMR (CD_3OD) as with **1**, except 273 (t, 4 H, SCH_2) 3.0 (s, 3 H, SCH_3), 3.45 (t, 4 H, OCH_2), 353 (t, 4 H, CH_2O), 3.60 (t, 4 H, CH_2O), 3.76 (m, 4 H, SCH_2), 3.9 (t, 4 H, CH_2) ppm. Anal. Calcd for $C_{33}H_{67}O_4S_2ClO_4$ (691.47): C, 57.32; H, 9.77. Found: C, 56.94; H, 9.65.

1,20-Dithionia-1,20-dimethyl-4,17,23,36-tetraoxacyclooctatriacontane Diperchlorate (3c). A 0.570-g aliquot (1 mmol) of the thioether **1** was mixed with 0.18 g (1 mmol) dimethyl sulfate and kept at 90 °C for 1.5 h. The mixture was dissolved in 20 mL of methanol. The product was precipitated by the addition of 2 mL of saturated, methanolic lithium perchlorate solution and recrystallized from methanol: yield 540 mg (62%) of white powder; mp 95 °C; R_f 0.1 (silica gel, $CHCl_3/CH_3OH$ 95:5), MS (80 eV, 190 °C) *m/e* 471 (5), 302 (100); ^1H NMR (DMSO-d_6; 270 MHz) as with **1**, except 3.34 (t, 8 H, OCH_2), 3.54 (t, 8 H, OCH_2), 3.08 (s, 6 H, SCH_3), 3.91 (m, 8 H, SCH_2) ppm. Anal. Calcd for $C_{34}H_{70}O_4S_2 \cdot 2ClO_4$ 805.95): C, 50.67; H, 8.75; S, 8.10. Found: C, 50.36; H, 8.86; S, 7.89.

13,19-Dioxa-hentriacontyl 16-Sulfone (4b). First the corresponding sulfide **4a** was prepared by heating 20.5 g (0.11 mol) of *n*-dodecanol with 5.0 mL (0.10 mol) of 2,2-dithioethanol and 0.6 g of *p*-toluenesulfonic acid to 130 °C and the resultant mixture was filtered. The residue was recrystallized several times from ethanol until a melting point of 42 °C was reached; yield 20 g (80%) of **4a**.[9]

A 4.56-g aliquot (0.0.1 mol) of **4a** was dissolved in 75 mL of acetic acid and mixed with 5 mL (0.04 mol) of 30% hydrogen peroxide. The mixture was kept at 90 °C for 12 h. The solvent was then removed and the residue recrystallized from 2-propanol/methanol (1:1): yield 2.15 g (44%); R_f 0.3 (silica gel, $CHCl_3/CH_3OH$ 95:5); mp 65 °C; MS (70 eV, 120 °C) *m/e* M^+ (490, 3), 398 ($M - CH_2CH_2SO_2$, 5), 323 ($M - SO_2(CH_2CH_2O)_2CH_3$, 100); ^1H NMR ($CDCl_3$) 270 MHz) 0.90 (t, 6 H, CH_3) 1.3 (s, 36 H, CH_2), 1.6 (m, 4 H, CH_2CH_2O), 3.33; 3.46 (2 t, 8 H, OCH_2), 3.86 (t, 4 H, SO_2CH_2) ppm. Anal. Calcd for $C_{28}H_{58}O_4$ (490.8): C, 68.51; H, 11.91; S, 6.53. Found: C, 67.83; H, 11.71; S, 6.48.

17-Chloro-13,19-dioxahentriacontyl 16-Sulfone (4c) and 16-Sulfoxide (4e). A 45.6-g aliquot (0.1 mol) of the sulfide **4a** was dissolved in 500 mL of dichloromethane and oxidized to the sulfoxide **4d** with 17.5 g (1 mol) of *m*-chlorobenzoic acid as described for **3b**; yield 36.5 g (77%) of **4d**, mp 63 °C. To 20 g (42 mmol) of sulfoxide **4d** in 300 mL of dichloromethane containing 7.2 mL of pyridine was added dropwise 44 mL of a 1 M sulfuryl chloride solution in dichloromethane within 30 min at 0 °C. The solution was then extracted with 1 M HCl (2 × 100 mL) and water (3 × 100 mL). The organic phase was removed and the residue redissolved in ethanol and precipitated with ether. **4e**: yield 15 g (70%); mp 24 °C; MS (100 °C, 80 eV) *m/e* 508 (M^+, 16), 491 (100); ^1H NMR ($CDCl_3$) as with **4**, except 4.76 (dd, 1 H, CHClSO) ppm. Anal. Calcd for $C_{28}H_{57}ClO_3S$ (509.3): C, 66.04; H, 11.29; Cl, 6.96. Found: C, 66.11; H, 11.10; Cl, 6.84.

A 10-g aliquot of the above chlorosulfoxide in 150 mL of acetic acid containing 7 mL of 30% hydrogen peroxide was oxidized as described for **3c**. **4c**: yield 13 g (83%); mp 39–42 °C; ^1H NMR as with **4**, except ($CDCl_3$, 270 MHz) 5.04 (dd, 1 H, $CHClSO_2$) ppm; MS (104 °C, 80 eV) *m/e* 524 (M^+, 7), 489 (M − Cl 26), 459 (M − SO_2H, 100). Anal. Calcd for $C_{28}H_{57}ClO_4S$ (525.3): C, 64.03; H, 10.94; S, 6.10. Found: C, 64.10; H, 10.98; S, 6.20.

Attempts To Extrude Sulfur Dioxide from the Above Chlorosulfone and Sulfones 3a and 4c. (i) A 0.5-g aliquot of the chlorosulfone **4c** was dissolved in 20 mL of tetrahydrofuran, and the resultant mixture was stirred with 1 g of potassium *tert*-butoxide at 0 °C. Water was added and the resultant mixture extracted with pentane. Only *n*-dodecanol was isolated by chromatography. It was characterized by comparative TLC with authentic material and a ^1H NMR spectrum. (ii) The same procedure as above was used but with sodium methoxide instead of the butoxide. Identical results were obtained. (iii) The same procedure as above, but with sodium hydroxide, was used giving the same results. (iv) A 0.5-g aliquot of the sulfone **4b** in 40 mL of tetrahydrofuran was refluxed for 12 h in carbon tetrachloride containing 0.5 g of potassium *tert*-butoxide. Again, only *n*-dodecanol was isolated and characterized. (v) The same procedure with macrocyclic tetraether **3a** gave 1,12-dodecanediol as the only major product.

Dipotassium 2,19-Dimethyl-3,6,9,12,15,18-hexaazaeicosane-1,20-dicarboxylate (5). A 50-g aliquot (0.430 mol) of methyl acetoacetate in 50 mL of dichloromethane was dropped into 50 g (0.215 mol) of pentaethylenehexamine within 60 min at room temperature and under nitrogen. The mixture was stirred for 4 h and solidified slowly to a gel. Excess acetoacetate and solvent were removed in vacuo. The residue was redissolved in 100 mL of dichloromethane and precipitated with 300 mL of diethyl ether. The yellow crystals were filtered and washed with cold ether. From the mother liquors more white flakes precipitated after addition of more ether and standing at 5 °C. A total of ~50 g of white crystals was collected. A 30-g aliquot of this resulting diimide raw material (0.07 mol) was dissolved in 50 mL of methanol and acidified with 120 mL of 2 M methanolic HCl. Sodium cyanoborohydride (9 g, 0.143 mol) was added in small portions, and the pH was kept between 4.7 and 4.9 by the addition of more methanolic HCl. Inorganic precipitates were filtered, and the solvent was removed from the filtrate. The residue was extracted twice with 60 mL of methanol, the solution was cooled to 5 °C and filtered, and the filtrate was again evaporated. A total of 18 g (0.04 mol, 60%) of the dimethyl ester of **5** was obtained as a yellow resin. A 20-g portion of such a resin was redissolved in 10 mL of methanol, and 5 mL of aqueous 25% hydrochloride was added. The precipitate was filtered and dried in vacuo, mp 224 °C. Anal. Calcd for $C_{20}H_{50}Cl_6N_6O_4$ (651.38): C, 36.88; H, 7.74; Cl, 32.66; N, 12.90. Found: C, 36.38; H, 7.67; Cl, 32.51; 32.72; N, 12.80; 12.90.

The dipotassium salt was obtained from the dimethyl ester by refluxing 5 g of the above hydrochloride in 80 mL of 1 M methanolic potassium hydroxide for 2 h. The mixture was filtered, the solvent evaporated, and the oily residue dried under vacuum. It was then redissolved in absolute methanol and filtered and the solvent was again removed. The oily residue was redissolved in 2-propanol and again filtered from eventual white solids. After evaporation of the solvent, 3.37 g (92%) of a colorless oil was obtained, which was the dipotassium salt **4**: MS (FAB) *m/e* 461 (100, $M^+ - 2K^+$); ^1H NMR and IR spectra were as expected. Anal. Calcd for $C_{18}H_{38}K_2N_6O_4$ (480.74): C, 44.97; H, 7.97; N, 17.48. Found: C, 45.26; H, 8.03; N, 15.72. The hexamine **5** was also precipitated from methanolic solution with aqueous perchloric acid (60%). A white, slimy precipitate was obtained. Anal. Calcd for $C_{18}H_{40}Cl_6N_6O_{28}$ (1001.28): C, 21.59; H, 4.03; N, 8.39. Found: C, 21.61; H, 4.27; N, 7.81.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 312 Gerichtete Membranprozesse), the FNK of the Freie Universität Berlin, and the Fonds der Chemischen Industrie.