Synthetic Models for Transmembrane Channels: Structural Variations That Alter Cation Flux

Oscar Murillo, Shigeru Watanabe,† Akio Nakano,‡ and George W. Gokel*

Contribution from the Bioorganic Chemistry Program and Department of Molecular Biology and Pharmacology, Washington University School of Medicine, Campus Box 8103, 660 South Euclid Avenue, St. Louis, Missouri 63110

Received December 12, 1994®

Abstract: Twelve novel bis- or tris(macrocyclic) compounds have been designed as models for cation-conducting channels that function in phospholipid bilayer vesicle membranes. In general, the channel model systems have the structure "sidearm–crown–spacer–crown–spacer–crown–sidearm", although certain features have been altered from compound to compound to assess the structure–activity relationship. Two additional compounds have been prepared exclusively as controls. The ionophores have been incorporated into the membranes either by warming the compound with the preformed vesicle or by incorporation during vesicle formation. The two methods gave identical results within experimental error. Cation flux was assessed by two different analytical methods. In one case, the fluorescent dye pyranine was encapsulated within vesicles containing ionophore. Proton transport was then monitored by changes in dye fluorescence with time following an acid pulse. Ionophoretic activity for most of the compounds was studied using a dynamic NMR method in which the flux rate of $^{23}Na^+$ through the bilayer was monitored. All NMR studies were done in conjunction with gramicidin as a control to minimize experimental variations from run to run. Several of the synthetic ionophores showed cation conduction of as much as 40% of the activity of gramicidin. Apparently, small structural changes significantly altered flux rates and two known carriers closely related to the channel formers failed to exhibit measurable transport under comparable conditions.

Introduction

Conduction of cations and molecules across membranes is a paradoxical process in the sense that the membrane is required to be an impermeable structural barrier but certain species must traverse it or the organism will expire. In addition, conveyance of too much or too little of a substance may be fatal. Although certain naturally-occurring compounds, such as valinomycin, transport cations by a carrier mechanism, conduction is by far the dominant mechanism for transmembrane migration. To date, no clear understanding of the chemical details inherent to cation channel operation has resulted from much ambitious and excellent study.[1,2] One reason for this dilemma is that the low molecular weight, experimentally amenable, channel-forming systems such as gramicidin[3] and amphotericin B[4] are not necessarily models for most natural channels. The bulk of known channel-forming structures involve transmembrane proteins comprised of putative α-helixes[5] that (unlike gramicidin) contain no intra-protein axial tube or conduit through which cations can flow. A process of self-assembly or aggregation of the transmembrane segments appears to form the requisite conduit. At the present time, it is not known with certainty in most cases which protein segments span the membrane nor how those segments are anchored in the membrane and/or align to form the required conduit. It is not known what functional groups serve as cation relays, and the mechanism(s) that foster(s) selectivity also remain obscure.

We report here an effort to obtain rudimentary chemical information on these mechanistic issues using structurally simple, synthetic model compounds which both insert in membranes and foster cation flux.

Results and Discussion

Shorthand Designations for Structures. Because the compounds discussed here have cumbersome names even using the semisystematic "crown" designations, we use two versions of a structural shorthand used previously.[6] By this shorthand, 18-crown-6 is represented as <18> and aza-18-crown-6 is <N18> or <18N>. *N,N'*-Bis(dodecyl)-4,13-diaza-18-crown-6 can be represented as $C_{12}H_{25}$<N18N>$C_{12}H_{25}$, although this structure may be further reduced to C_{12}<N18N>C_{12} to conserve space. The presence of a hydrogen on the unsubstituted, macroring nitrogen atom of a monosubstituted diaza-18-crown-6 is understood and may be represented either by R<N18N>H or R<N18N> depending upon which is more appropriate to the context. In addition, "M" and "E" are used to designate CH_2 and CH_2CH_2, respectively.

† Current address: Department of Chemistry, Faculty of Science, Kochi University, Akebono-cho, Kochi 780, Japan.

‡ Current address: Department of Food and Technology, Faculty of Engineering, Toa University, Ichinomiya-Gakuencho 2-1, Shimonoseki, Yamaguchi 751, Japan.

® Abstract published in *Advance ACS Abstracts*, July 1, 1995.

(1) Stein, in his 1990 monograph *Channels, Carriers, and Pumps*, stated that "[W]e know a good deal about transport kinetics and about what regulates transport through channels, carriers, and pumps. But we do not have, even in one instance, a clear understanding of how these molecules function." Stein, W. D. *Channels, Carriers, and Pumps*; Academic Press: New York, 1990; p xi.

(2) Hille has written thus "[Hodgkin and Huxley in 1952] reluctantly concluded that still more needed to be known before a unique mechanism could be proven. Indeed, this conclusion is unfortunately still valid." Hille, B. *Ionic Channels of Excitable Membranes*, 2nd ed.; Sinauer Associates: Sunderland, MA, 1992; p 44.

(3) (a) Urry, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 672. (b) Urry, D. W.; Goodall, M. C.; Glickson, J. D.; Phyers, D. F. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1907.

(4) (a) Keim, G. R., Jr.; Poutsiaka, J. W.; Kirpan, J.; Keysser, C. H. *Science* **1973**, *179*, 584. (b) Bolard, J. *Biochim. Biophys. Acta* **1986**, *864*, 257.

(5) Questions about the certainty with which it is known that the transmembrane segments are actually helical have recently been raised: Hucho, F.; Görne-Tschelnokow, U.; Strecker, A. β-Structure in the membrane-spanning part of the nicotinic acetylcholine receptor (or how helical are transmembrane helices?) *Trends Biochem. Sci.* **1994**, *19*, 383.

(6) Hernandez, J. C.; Trafton, J. E.; Gokel, G. W. *Tetrahedron Lett.* **1991**, 6269–6272.

0002-7863/95/1517-7665$09.00/0

Design Criteria. Since the mechanism(s) by which channels function is unknown, modeling the chemistry presents a challenge. Much is known about various channels' cation and molecular selectivities, interferences, anion effects, etc., but what transpires within the channel itself remains obscure. In such channel model compounds as gramicidin, the amide carbonyl groups are believed to be the "relay" elements that serve to pass a cation from one side of the membrane to the other. In amphotericin B, the polyol chain presumably serves this function.[7] In protein channels, the relays are limited to the amide carbonyl groups and to the amino acid side chains. If the transmembrane segments of transport proteins are, as found for bacteriorhodopsin,[8] α-helical, the availability of carbonyl groups in the way they function in gramicidin[9] is doubtful because of conformational restrictions. The transport function must presumably be served by the amino acid side chains which encompass a wide variety of donors.

The essential amino acids can be grouped according to the chemical character of their side chains. Those having aliphatic side chains (A, G, I, L, V) are presumably nonfunctional in the (Lewis basic) relay context unless their carbonyl groups are involved. Like the polypeptide chain, asparagine (N) and glutamine (Q) have amide donor groups. The sulfur functional groups of C (−SH) and M ($-SCH_3$) should be weak donors whereas aspartic and glutamic acids (D, E) should be powerful donors since they are ionized at physiologic pH (isoelectric pH 2.77, and 3.24, respectively). The hydroxyl function is less readily ionized and available in S, T, and Y than in D or E. In the tyrosine case, ionization occurs at lower pH than in serine or threonine because the hydroxyl is phenolic. Amine donor groups are present in H, K, W, and R, the latter being guanidinium. Finally, aromatic residues that may potentially function as donors are found in F, H, W, and Y.

Several issues were considered in the design of our channel model system.[10] First, the length of the channel model had to correspond to the appropriate membrane dimensions. A natural phospholipid bilayer is generally regarded as requiring a transmembrane span of approximately 30 Å. Solid state structures for gramicidin dimer channels exhibit extended lengths of 26 and 31 Å which correspond to this value.[11]

Second, it was surmised that head groups would be required at either end of the channel former to anchor the structure in an extended conformation and to afford a cation an entry point into the bilayer. Crown ethers were selected to suit this purpose, although at the outset of this project, no example of an uncomplexed crown serving as an amphiphile head group had been reported. Extensive work has been undertaken in our laboratory to demonstrate that amphiphilic crown ethers can aggregate into stable vesicles[12] and that bola-amphiphilic crowns (crown−hydrocarbon chain−crown) can form monolayer amphisomes.[13]

Third, the question of relay groups was daunting. Since crowns were to be used as entry points and anchors, it was decided to use a macrocycle also as the central relay unit. This also had the aesthetic advantage that the functional channel conformation could be envisioned as a "tunnel" as often assumed for natural systems. This assumption is bolstered by the "semisynthetic" channel prepared by Schrieber in which two gramicidin pentadecapeptides are fixed by covalent linkage to tartaric acid.[14] A number of cleverly-devised *de novo* channel model systems have been designed, synthesized, and assessed for cation flux.[15] In most but not all of these, ether oxygen atoms play a central role as relays. This is also true of our own approach to channel function which provides a membrane-spanning system in which crown ethers constitute the entry, exit, and central relay points.

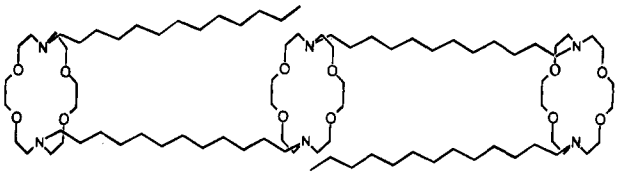


Figure 1. Structure of compound **1** shown in the hypothetical "tunnel" conformation.

Compounds Prepared. A family of 10 bis- or tris-(macrocyclic) compounds was prepared in the expectation that they would function to varying degrees as channel formers. It was anticipated that the structural variations would provide information about the chemical requirements for cation transport by a noncarrier mechanism. The synthetic channel structures are identified in Table 1 along with several relatives not expected to function as channels. All of the channel formers have the basic structure shown schematically in Figure 2, to which Table 1 is referred.

The first compound to be prepared was designed to incorporate three crown ethers in the structure. The two distal macrocycles would serve as entry points for cations and serve as head groups for the bola-amphiphilic structure. The central crown, a diaza-18-crown-6 macrocycle like the others, would serve as a central relay unit. The latter would reside within the bilayer and serve to transiently capture and relay the cation on its transmembrane journey. The spacers and head groups constitute two "side chains" that make each distal crown formally an amphiphile. It was thought that such a design would foster membrane stability. As noted above, the ability of crowns to function as amphiphile head groups in crown-based amphiphiles that form membranes was unknown although the evidence confirming this possibility is now overwhelming.[12,13]

(7) Aumercier, M.; Le Goffic, F. Mechanism of action of the macrolide and streptogramin antibiotics. In *Macrolides*; Bryskier, A. J., Butzler, J.-P., Neu, H. C., Tulkens, P. M., Eds.; Arnette Blackwell: Oxford, U.K., 1993; p 115.

(8) Henderson, R.; Baldwin, J. M.; Ceska, T. A.; Zemlin, F.; Beckmann, E.; Downing, K. H. *J. Mol. Biol.* **1990**, *213*, 899.

(9) Roux, B.; Karplus, M. *J. Am. Chem. Soc.* **1993**, *115*, 3250.

(10) Nakano, A.; Xie, Q.; Mallen, J. V.; Echegoyen, L.; Gokel, G. W. *J. Am. Chem. Soc.* **1990**, *112*, 1287−1289.

(11) (a) Wallace, B. A.; Ravikumar, K. *Science* **1989**, *241*, 182. (b) Langs, D. A. *Science* **1989**, *241*, 188.

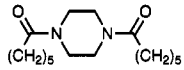
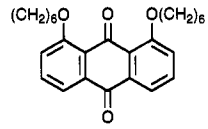
(12) (a) Echegoyen, L. E.; Hernandez, J. C.; Kaifer, A.; Gokel, G. W.; Echegoyen, L. *J. Chem. Soc., Chem. Commun.* **1988**, 836−837. (b) Gokel, G. W. Steroidal Lariat Ethers. Number 4,783,528, November 8, 1988. (c) Wang, K.; Berger, D.; Watanabe, S.; Hernandez, J. C.; Gokel, G. W. *J. Org. Chem.* **1995**, submitted.

(13) (a) Muñoz, S.; Mallén, J. V.; Nakano, A.; Chen, Z.; Echegoyen, L.; Gay, I.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1992**, 520−522. (b) Muñoz, S.; Mallén, J.; Nakano, A.; Chen, Z.; Gay, I.; Echegoyen, L.; Gokel, G. W. *J. Am. Chem. Soc.* **1993**, *115*, 1705.

(14) Stankovic, C.; Heinemann, S.; Delfino, J.; Sigworth, F.; Schrieber, S. *Science* **1989**, *244*, 813.

(15) (a) Tabushi, I.; Kuroda, Y.; Yokota, K. *Tetrahedron Lett.* **1982**, 4601. (b) Neevel, J. G.; Nolte, R. J. M. *Tetrahedron Lett.* **1984**, 2263. (c) Kragten, U. F.; Roks, M. F.; Nolte, R. J. M. *J. Chem. Soc., Chem. Commun.* **1985**, 1275. (d) Fuhrhop, J.-H.; Liman, U.; David, H. H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 339. (e) Jullien, L.; Lehn, J.-M. *Tetrahedron Lett.* **1988**, 3803. (f) Carmichael, V. E.; Dutton, P. J.; Fyles, T. M.; James, T. D.; Swan, J. A.; Zojaji, M. *J. Am. Chem. Soc.* **1989**, *111*, 767. (g) Fyles, T. M.; Kaye, K. C.; James, T. D.; Smiley, D. W. M. *Tetrahedron Lett.* **1990**, 1233. (h) Menger, F. M.; Davis, D. S.; Persichetti, R. A.; Lee, J.-J. *J. Am. Chem. Soc.* **1990**, *112*, 2451. (I) Kobuke, Y.; Ueda, K.; Sokabe, M. *J. Am. Chem. Soc.* **1992**, *114*, 7618. (j) Pregel, M. J.; Jullien, L.; Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1637. (k) Canceill, J.; Jullien, L.; Lacombe, L.; Lehn, J.-M. *Helv. Chim. Acta* **1992**, *75*, 791. (l) Jullien, L.; Lehn, J.-M. *J. Inclusion Phenom.* **1992**, *12*, 55. (m) Ghadiri, M. R.; Granja, J. R.; Buehler, L. K. *Nature* **1994**, *369*, 301. (n) Khazanovich, N.; Granja, J. R.; McRee, D. E.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994**, *116*, 6011. (o) Stadler, R.; Dedek, P.; Yamashita, K.; Regen, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 6677.

Table 1. Synthetic Model Compounds to Assess Bilayer Cation Flux[a]

no.	sidearms	spacers	central unit	distal crowns
1	$C_{12}H_{25}$	$C_{12}H_{24}$	<N18N>	<N18N>
2	$C_{12}H_{25}$	EOEOEOE	<N18N>	<N18N>
3	$C_{12}H_{25}$		<N18N>	<N18N>
4	$C_{12}H_{25}$		<N18N>	<N18N>
5	$C_{12}H_{25}$	$C_{12}H_{24}$	<N15N>	<N18N>
6	$C_{12}H_{25}$	$C_{12}H_{24}$	OEOEOEO	<N18N>
7	$C_6H_5CH_2$	$C_{12}H_{24}$	<N18N>	<N18N>
8	H	$C_{12}H_{24}$	<N18N>	<N18N>
9	-	$C_{12}H_{24}$	<N18N>	<N18>
10	CH_2CH_2O-3-cholestanyl	$C_{12}H_{24}$	<N18N>	<N18N>
11	CH_2COO-3-cholestanyl	$C_{12}H_{24}$	<N18N>	<N18N>
12	$C_{12}H_{25}$		<N18N>	
13	$C_6H_5CH_2$		<N18N>	
14	$C_{12}H_{25}$	$C_{12}H_{24}$ (1)	<N18N>	<N18N> (1)

[a] See Figure 2 for position information.

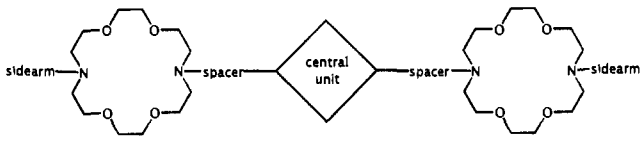


Figure 2. Generalized structure showing the functional elements for the channel compounds.

It was also thought, perhaps naively, that the central macroring would function as a tunnel-like structure through which the cation would pass. This notion was based upon the fact that known transmembrane proteins such as bacteriorhodopsin,[8] rhodopsin,[16] shaker channel,[17] or the channel of *Torpedo californica*[18] appear to form tunnel-like channels by insertion of multiple 20-odd amino acid segments in the membrane adjacent to each other. This appears to form a "tunnel" through which cations pass, but the mechanism by which transport occurs in all of these cases remains obscure.

In compound **2**, the alkyl spacers were replaced by ethyleneoxy spacers. It was recognized that this would increase polarity near the bilayer's midplane, but it was thought that there would be an advantage in the larger number of donors spanning a greater distance within the membrane that would lower the energy for cation transfer from one donor to the next and thus enhance flux rate.

Compounds **3** and **4** were designed to mimic gating properties known to be possessed by natural channel formers. In **3**, a pair of amide carbonyl groups were placed at the midpoint of each spacer. This structural arrangement was intended to mimic the presence of amide carbonyl groups found in such natural, transmembrane ionophores as gramicidin. It is currently unknown how many (if any) carbonyl groups participate in cation relay in transmembrane protein channels, but this phenomenon is well-documented for gramicidin.[9] Compound **4** was designed to serve as a potential-gated channel model. Changes in potential across membranes are known to affect cation flux, and the ability of anthraquinone to accept one or two electrons to form a water-stable anion suggested this residue as a possible flux regulator.[19] In the above structures, the potential regulators were incorporated as spacer groups.

Compounds **5** and **6**, in concert with **1**, were designed and prepared to directly assess the "requirement" for the tunnel-like conformation. To be sure, in the cases of natural channel formers where structural information is known with certainty, multiple peptide segments align to form a conduit. It seemed quite possible that the structures prepared for the present study could align themselves into pores, as transmembrane proteins are believed to do, and that passage of a cation through a macrocyclic ring would not be required. Thus compounds **1**, **5**, and **6** are identical except that the 18-membered crown ring of **1** is reduced to a 15-membered ring in **5** and replaced by an open-chained ethyleneoxy unit in **6**.

Structures **7**–**9** were designed to probe variations in sidearm structure and, indeed, whether or not they are necessary to foster cation flux. If the tris(macrocyclic) compounds are extended within the bilayer as we presume them to be from the bola-amphiphile studies, then the primary function of the dodecyl side chain in **1** is probably to serve as a second chain rendering the distal crown a head group for a two-tailed amphiphile. Compounds **1**, **7**, **8**, and **9** permit us to examine the effect of changing the sidearms from aliphatic to aromatic, removing the nitrogen-attached sidearms, and then removing the nitrogen itself.

Compounds **10** and **11** have steroidal sidearms attached. It is well-known that steroids such as cholesterol are organizing and stabilizing elements in many membranes. Indeed, rat liver plasma membrane contains approximately 25% of cholesterol within its structure.[20] As a rigidifying element of membranes, it was thought that the steroidal sidearms would organize the channel former and stabilize it within the membrane. Our finding in earlier studies that the steroidal lariat ethers possessed mobility similar to frozen lecithin vesicles seemed to confirm this.[21]

(16) (a) Nathans, J. *Biochemistry* **1992**, *31*, 4923. (b) Schertler, G. F. X.; Villa, C.; Henderson, R. *Nature* **1993**, *362*, 170. (c) Kaushal, S.; Ridge, K. D.; Khorana, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4024. (d) Davidson, F. F.; Loewen, P. C.; Khorana, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4029. (b) Kaushal, S.; Khorana, H. G. *Biochemistry* **1994**, *33*, 6121.

(17) Tempel, B. L.; Papazian, D. M.; Schwarz, T. L.; Jan, Y. N.; Jan, L. Y. *Science* **1987**, 237, 770.

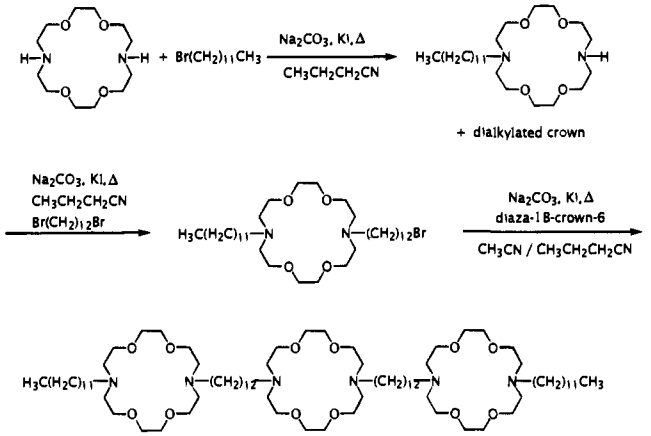
(18) Noda, M.; Takahashi, H.; Tanabe, T.; Toyosato, M.; Furutani, Y.; Hirose, T.; Asai, M.; Inayama, S.; Miyata, T.; Numa, S. *Nature* **1982**, *299*, 793.

(19) (a) Echegoyen, L. E.; Yoo, H. K.; Gatto, V. J.; Gokel, G. W.; Echegoyen, L. *J. Am. Chem. Soc.* **1989**, *111*, 2440–2443.

(20) Sim, E. *Membrane Biochemistry*; Chapman and Hall: London, 1982; p 26.

(21) Fasoli, H.; Echegoyen, L. E.; Hernandez, J. C.; Gokel, G. W.; Echegoyen, L. *J. Chem. Soc., Chem. Commun.* **1989**, 578–580.

Scheme 1



Finally, compounds **12**−**14** were prepared to be controls for the other structures studied. Compound **12** is bis(dodecyl)diaza-18-crown-6 and represents the potential carrier molecule that is actually a fragment of **1**. Dibenzyldiaza-18-crown-6 (**13**) has two benzyl sidearms that are similar to those in **7**, but **13** is expected to be a carrier rather than a conductor. Finally, compound **14** represents approximately two-thirds of **1** but the absence of a spacer and macroring should prevent it from spanning the bilayer.

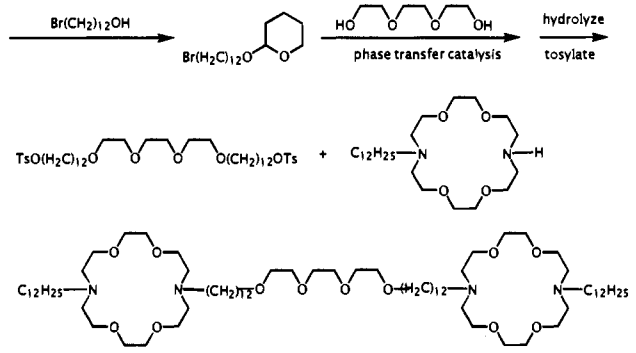
Synthetic Access. The channel model structures prepared for this study are generally symmetrical structures. From the synthetic perspective, this is both an advantage and a problem. Consider, for example, the central ring of **1**. It is attached to two identical sidearms, each of which contains another diaza-18-crown-6 macrocycle. Each of the two distal crowns is unsymmetrically substituted although the substituents are very similar. The strategy we devised for the preparation of the various channel model compounds deals with the asymmetry issue early in the synthesis where the yield sacrifice is more tolerable. The approach is illustrated in Scheme 1.

Diaza-18-crown-6 was prepared as previously reported.[22] Reaction with a substoichiometric (80%) amount of 1-bromododecane, Na_2CO_3, and KI (catalytic amount) in refluxing butyronitrile for 4 d gave *N*-dodecyl-4,13-diaza-18-crown-6 [C_{12}<N18N>H] in 27% yield along with 14% of *N,N'*-bis(dodecyl)-4,13-diaza-18-crown-6. The monosubstituted diaza crown was treated with 1,12-dibromododecane (≈3 equiv) under conditions essentially similar to those described above except that the reflux time was reduced (1.5 h). The bromododecyl crown $CH_3(CH_2)_{11}$<N18N>$(CH_2)_{12}$Br [≡C_{12}<N18N>C_{12}Br] was obtained as a pale, yellow oil in 66% yield. The two precursors, diaza-18-crown-6 (H<N18N>H), C_{12}<N18N>C_{12}Br (2 equiv), Na_2CO_3 (20 equiv), and KI (0.3 equiv) were heated in 2/3 v/v $CH_3CN/CH_3CH_2CH_2CN$ for 4 d. Channel model **1** was obtained as a white, crystalline solid (mp 61−63 °C) in 23% yield.

A strategy similar to that employed for the preparation of **1** was used to prepare C_{12}<N18N>EOEOEOE<N18N>EOEOEOE<N18N>C_{12} (**2** (E=CH_2CH_2)). The synthesis was accomplished by treating C_{12}<N18N>H with I−EOEOEOE−I in the presence of Na_2CO_3. This afforded C_{12}<N18N>EOEOEOE−I. Reaction between 2 equiv of the latter and 4,13-diaza-18-crown-6 gave the desired structure which was isolated as the oily tetrahydrate. Infrared and combustion analyses both clearly showed the presence of water which could not be removed by extended vacuum drying. This observation suggests that water may also reside within this and other channels when inserted into a lipid bilayer.

(22) Gatto, V. J.; Miller, S. R.; Gokel, G. W. *Org. Synth.* **1989**, *68*, 227.

Scheme 2



Compounds **3** and **4** were synthesized in a fashion related to that described above except that in each case an intermediate of the type $X(CH_2)_n-Y-(CH_2)_nX$ was prepared first. In the case of **3**, 2 equiv of 6-bromohexanoyl chloride was allowed to react with piperazine to give the bis(amide) dibromide. For anthraquinone channel **4**, 1,6-diiodohexane and 1,8-dihydroxyanthraquinone were allowed to react to afford the bis(ether) bis(iodide). In each case, the intermediate was then allowed to react with 4,13-diaza-18-crown-6 in the presence of Cs_2CO_3 to give $X(CH_2)_n-Y-(CH_2)_n$<N18N>$(CH_2)_n-Y-(CH_2)_nX$. Further reaction in either system with C_{12}<N18N>H gave **3** or **4**.

The analog of compound **1**, *i.e.* **5**, in which the central 18-membered ring is replaced by an 15-atom cycle was prepared essentially as was the former. Thus C_{12}<N18N>C_{12}Br was heated at reflux with Na_2CO_3 and KI in butyronitrile with 4,10-diaza-15-crown-5 to afford **5** in 21% yield.

We anticipated that **6**, an open-chained analog of **1** and **5**, would be available by an analogous approach. In fact, the preparation of this compound proved extremely arduous. Ultimately, **6** was obtained by the sequence shown in Scheme 2. 12-Bromododecanol was protected as its THP derivative. Reaction with tris(ethylene glycol) under phase transfer catalytic conditions gave $THPOC_{12}OEOEOEOC_{12}OTHP$. Hydrolysis of the THP protecting group using TsOH in aqueous methanol at 60 °C gave the corresponding diol contaminated by the monodeprotected product. The diol was converted into its ditosylate which was then treated with C_{12}<N18N>H (Na_2CO_3, CH_3CN, Δ) to afford C_{12}<N18N>$C_{12}OEOEOEOC_{12}$<N18N>C_{12} (**6**) in 1% overall yield.

Compound **8** was obtained by hydrogenolysis of **7**. The latter was prepared as follows. 12-Bromododecanoic acid was converted into the acid chloride by using thionyl chloride. Reaction between the acid chloride (2 equiv) and H<N18N>H in the presence of Et_3N and (dimethylamino)pyridine gave $Br(CH_2)_{11}CO$<N18N>$CO(CH_2)_{11}Br$. The carbonyl groups were reduced without bromine loss using BH_3·THF. *N*-Benzyl-4,13-diaza-18-crown-6 was prepared (33%, 18% dibenzyl) by treating diaza-18-crown-6 with 1 equiv of benzyl bromide. Reaction between BrC_{12}<N18N>C_{12}Br and $PhCH_2$<N18N>H gave $PhCH_2$<N18N>C_{12}<N18N>C_{12}<N18N>CH_2Ph (**7**). Hydrogenation in ethanol using 10% Pd/C afforded H<N18N>C_{12}<N18N>C_{12}<N18N>H (**8**) in 75% yield after recrystallization from EtOAc (mp = 63−65 °C).

Compound **9**, the analog of **8** was prepared from aza-18-crown-6 by reaction with 1,12-dibromododecane (Na_2CO_3, KI, PrCN). The product, <18N>C_{12}Br (2 equiv) was treated with H<18N>H to give <18N>C_{12}<N18N>C_{12}<N18>.

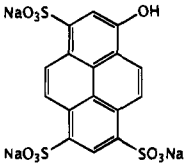
Steroidal sidearms were introduced in compounds **10** and **11**. Most of the effort involved in the preparation of **10** was expended to obtain the sidearm precursor. 3-Cholestanone was protected as its ethylene glycol ketal in 85% yield. Ring

opening was effected using BH_3·THF. This procedure afforded 51% of 3-(2-hydroxyethoxy)cholestane and 18% of the 5-isomer. The former was tosylated and then allowed to react with **8** in the presence of Na_2CO_3 and THF. Compound **10** was obtained as a white solid, mp 71−72 °C.

Although compounds **10** and **11** are quite closely related in structure, the successful synthetic approaches were quite different for the two compounds. Cholestanol was treated with chloroacetyl chloride to give the chloroacetyl ester as a crystalline, white solid (mp 184−185 °C) in 78% yield. Treatment of the latter with 4,13-diaza-18−crown-6 gave 27% of the monosubstituted crown and 20% of the known[23] disubstituted derivative. Condensation of cholOCOCH$_2$<N18N>H with Br(CH$_2$)$_{12}$Br afforded cholOCOCH$_2$<N18N>(CH$_2$)$_{12}$Br which, when treated with diaza-18-crown-6, afforded cholOCOCH$_2$<N18N>C$_{12}$<N18N>C$_{12}$<N18N>CH$_2$COOchol (**11**), in 20% yield.

Compounds **12**[24] and **13**[25] were previously prepared. Compound **14**, was obtained as a byproduct from the reaction between C$_{12}$<N18N> and BrC$_{12}$Br in 2% yield.

Assessment of Cation Flux by Fluorescence Methods. Kano and Fendler[26] showed some years ago that proton flux through a bilayer could be assessed by using the pH sensitivity of pyranine dye's fluorescence spectrum. The dye trapped



within vesicles is isolated from the medium surrounding the vesicles. When a proton conductor is present in the bilayer, proton flux may be assessed by rapidly lowering the external pH (an acid pulse). As the protons flow into the vesicle, the pH of the medium surrounding pyranine lowers and the fluorescence spectrum corresponding alters. If the change is monitored continuously, a rate constant may be calculated. We have used a modification of this method as prescribed by Menger.[15h,27]

Vesicles were prepared from distearoyl-D,L-α-phosphatidylcholine (DSPC) and sodium dicetyl phosphate (SDP) as described in the literature cited above. In a typical experiment, a thin film of the mixture was obtained by evaporation of a chloroform solution containing approximately equimolar solutions of the two. The thin film was dispersed into buffer containing pyranine and vesicles were then formed by sonication. The resulting solution was purified by passing it through a Sephadex G50 column. Additional details are recorded in the Experimental Section.

A group of four potential channel-forming compounds (**1**−**4**) was studied along with gramicidin D, *N*,*N*′-didodecyl-4,13-diaza-18-crown-6 (**12**), and Triton X-100. The pyranine/fluorescence method was used to determine the relative efficacies of proton transport. This procedure was used in part because others have employed variations in proton flux rates to evaluate channel performance and in part because we wished to compare

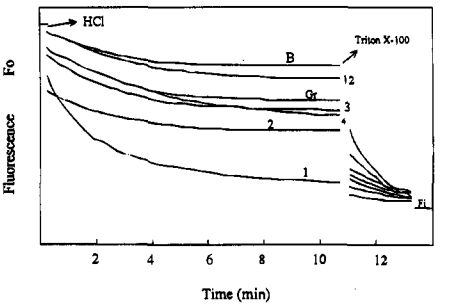


Figure 3. Fluorescence data for compounds **1**, **2**, **3**, **4**, **12**, gramicidin, and Triton X-100. B represents blank and is identical to the line for Triton X-100.

proton and sodium flux of identical compounds in one or more experimental systems.

The fluorescence spectra for **1**, **2**, **3**, **4**, **12**, Triton X-100 and gramicidin D, obtained for each compound at a concentration of $1.50 \pm 0.08 \times 10^{-5}$ M, are shown in Figure 3. The top line corresponds to the natural proton leakage through the vesicular membrane. The presence of 15 μM Triton X-100, a non-ionic detergent, did not alter the flux rate in any measurable way. It is especially important to note this because Triton X-100 is regarded as a powerful detergent and it is always possible that the proton flux could be detergent-induced leakage (see below). Indeed, addition of 0.1 M Triton X-100 after approximately 10 min lysed the vesicles, showing that the aggregates had remained intact throughout the experiment.

In order to test whether a simple carrier mechanism might operate in this system, we conducted an identical experiment using the known alkali metal carrier *N*,*N*′-bis(dodecyl)diaza-18-crown-6. Although limited data are available for crown-mediated transport through lipid bilayers, we recently prepared a family of lariat ether structures having systematically varied Lewis base donicities and overall hydrophobicities.[6] These compounds were not studied as proton carriers, but they were shown to be effective Na^+ carriers in lipid bilayers.[28] The efficacy of the crown carrier in this system is far lower than for **1** and inferior by some margin to all of the presumed channel formers.

Based upon the fluorescence results, the proton transport rate observed for **1** exceeds those of all the other compounds tested. The second best is **2**, which contains ethyleneoxy spacer chains. According to our original design concepts, compound **2** was expected to be a more effective cation conductor than **1** because relay atoms are present throughout the length of the presumed channel conformation. That it is significantly less effective than **1** was surprising to us. The two channel formers which were designed to have a potential gating mechanism (**3** and **4**) were, like gramicidin D, poor proton transporters. We concluded from this series of experiments that all of the synthetic (**1**−**4**) and the bacterial (gramicidin D) channel formers were, to a greater or lesser extent, effective proton conductors. All were superior to a structurally-related synthetic carrier, a detergent, and to the control.

It is interesting to note that only **1** appeared to be a good proton conductor. In contrast, the three compounds having polar residues in the channel's interior (**2**−**4**) were all only modest proton conductors. This behavior might be easier to rationalize if compounds **3** and **4** which possess polar carbonyl groups differed significantly from **2**, which incorporates only ether residues. The general conclusion must be that, when polar

(23) Lynn, B. C.; Tsesarskaja, M.; Schall, O. F.; Hernandez, J. C.; Watanabe, S.; Kaifer, A.; Atwood, J. L.; Gokel, G. W. *Supramol. Chem.* **1993**, *1*, 253−260.

(24) Gatto, V. J.; Arnold, K. A.; Viscariello, A. M.; Miller, S. R.; Morgan, C. R.; Gokel, G. W. *J. Org. Chem.* **1986**, *51*, 5373−5384.

(25) Gatto, V. J.; Gokel, G. W. *J. Am. Chem. Soc.* **1984**, *106*, 8240−8244.

(26) Kano, K.; Fendler, J. H. *Biochim. Biophys. Acta* **1978**, *509*, 289.

(27) Menger, F. M.; Lee, J. J.; Aikens, P.; Davis, S. *J. Colloid Interface Sci.* **1989**, *129*, 185.

(28) (a) Xie, Q.; Gokel, G. W.; Hernandez, J. C.; Echegoyen, L. *J. Am. Chem. Soc.* **1994**, *116*, 690−696. (b) Li, Y.; Gokel, G. W.; Hernandez, J.; Echegoyen, L. *J. Am. Chem. Soc.* **1994**, *116*, 3087−3096.

residues are present within the channel former's interior, proton flux is diminished. This is despite the fact that **1**−**4** all possess six nitrogen atoms that can be protonated to some degree at physiologic pH (see below).

^{23}Na-NMR Rate Studies. Although proton flux determination is an informative and standard method for assessing cation flux in bilayers, a method was sought that could readily be quantitated. It was hoped that by so doing data obtained for new structures could be compared directly to a control compound such as gramicidin. It was anticipated that this approach would also permit comparison with data obtained in other laboratories in which gramicidin was one of several structures studied.

Sodium cation flux was measured for most of the systems described here by using the ^{23}Na-NMR-based method of Riddell.[29] Although this is not the technique used by electrophysiologists, it permits quantitative detection of Na^+ and comparisons of rates among compounds and with a standard. Vesicles for the ^{23}Na-NMR studies were prepared from phosphatidylcholine and phosphatidylglycerol (4:1 w/w). The total Na^+ concentration was adjusted to 100 mM by addition of NaCl. The solutions were buffered using a phosphate buffer held at pH = 7.3. The vesicles were prepared by the procedure described by Papahadjopoulos[30] with certain modifications described in the Experimental Section. Vesicle size was determined by laser light scattering. The preparation used here afforded vesicles having an average diameter of 1750−2000 Å. The total aqueous encapsulation volume in this preparation was 3% as judged by ^{23}Na-NMR.

NMR Measurements. The shift reagent was prepared according to the procedure of Gupta and Gupta[31] from sodium tris(polyphosphate) and Dy^{3+}. The ^{23}Na-NMR chemical shifts were measured as differences between the resonance position in the presence and absence of the Dy^{3+} shift reagent. Gramicidin or the compound under study was incorporated into the vesicles as a CF_3CH_2OH solution [*e.g.*, gramicidin (0.2−0.4 mM), other compounds (0.5−0.9 mM)]. After addition of the subject compound, the samples were agitated and warmed (50−60 °C) for 1 h, cooled to room temperature, and then diluted with D_2O (lock signal) and shift reagent solution (see the Experimental Section for additional details). Each solution was allowed to equilibrate for ≥1 h before data acquisition. Typically, 240 FID transients were accumulated per data set at 25 °C.

It is worth noting that there are two obvious means by which the putative channel formers can be inserted into a membrane. The "incubation" technique described above requires that the ionophore insert during a period of warming. Alternatively, the vesicles may be prepared in the presence of predetermined concentrations of ionophore. Both approaches were used and the identity (within experimental error) of the NMR results obtained by either method was confirmed. This experiment shows that differences in flux rates correlate directly to differences in the amount of compound inserted into the bilayer.

Rate Constants. Incorporation of an active ionophore into the vesicles, induced line broadening of both signals in the ^{23}Na-NMR spectrum. In the slow exchange region, the rate constant, $k = 1/\tau$, is directly proportional to the line broadening observed, $(\Delta\nu - \Delta\nu_o)$, where $\Delta\nu$ is the line width at half-height of the observed resonance line in the presence of the ionophore and $\Delta\nu_o$ is the corresponding value in its absence. If the line width is measured for the Na^+_{inside} resonance in the series of dynamic NMR spectra, then the rate for efflux of Na^+ from the vesicles is determined by the equation $k = 1/\tau = \pi(\Delta\nu - \Delta\nu_0)$. The line broadening reflects dynamic exchange between Na^+_{inside} and $Na^+_{outside}$ through the membrane channels. Typical stacked spectral plots are shown in Figure 4. The exchange rate constant in each case was determined from the slope of a plot of $1/\tau$ *vs* channel concentration.[32] Typical data for compounds **1**, **6**, **7**, and **9** are shown in Figure 5. In the graph are plotted $1/\tau$ (in Hz) as a function of ionophore concentration. Note the baseline data for <18N>C_{12}<N18N>C_{12}<N18> (**9**), which is interpreted as exhibiting non-ionophoretic activity.

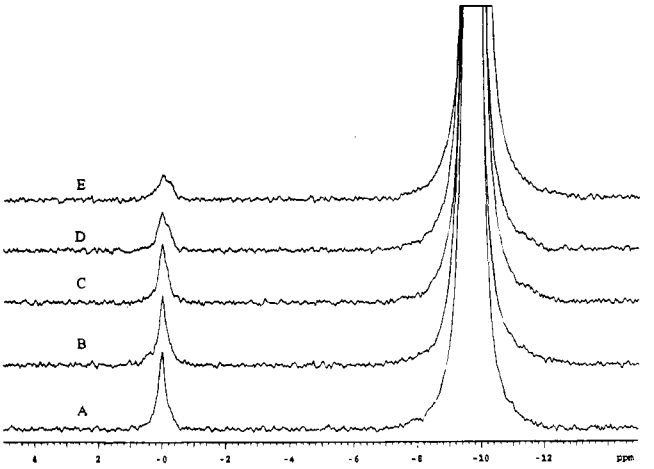


Figure 4. Observed changes in ^{23}Na-NMR spectra of vesicles upon addition of increasing amounts of 0.468 mM solution of **7** in CF_3CH_2OH. (A) 0 μL, (B) 5 μL, (C) 10 μL, (D) 15 μL, and (E) 20 μL.

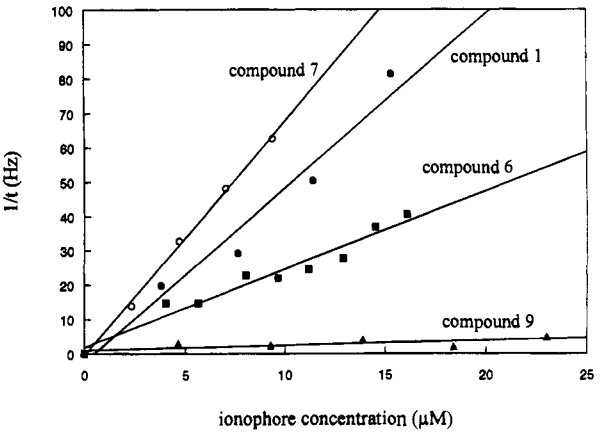


Figure 5. Mean lifetime $(1/\tau_{in})$ of the $^{23}Na^+$ "inside" spin site as a function of ionophore concentration for compounds **1**, **6**, **7**, and **9**.

Each set of experiments requires the preparation of a new phosphatidylcholine/phosphatidylglycerol vesicle system. In order to standardize the system, we obtained line width measurements on a gramicidin sample each time a new structure was studied. By settings the rate observed for gramicidin[33] to an arbitrary value of 100, we found that compound **1** transports Na^+ with a relative rate of 28. The data obtained by this technique for several of the compounds studied are recorded in Table 2.

(29) (a) Riddell, F. G.; Hayer, M. K. *Biochim. Biophys. Acta* **1985**, *817*, 313−317. (b) Buster, D. C.; Hinton, J. F.; Millett, F. S.; Shungu, D. C. *Biophys. J.* **1988**, *53*, 145−152. (c) Riddell, F. G.; Arumugam, S.; Brophy, P. J.; Cox, B. G.; Payne, M. C. H.; Southon, T. E. *J. Am. Chem. Soc.* **1988**, *110*, 734−738. (d) Riddell, F. G.; Arumugam, S. *Biochim. Biophys. Acta* **1989**, *984*, 6−10. (e) Riddell, F. G.; Tompsett, S. J. *Biochim. Biophys. Acta* **1990**, *1024*, 193−197.

(30) Papahadjopoulos, D.; Szoka, F. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4194.

(31) Gupta, R.; Gupta, P. *J. Magn. Reson.* **1982**, *47*, 344.

(32) Sandstrom, J. *Dynamic NMR Spectroscopy*; Academic Press: London, 1982; Chapter 6.

(33) All values determined at 10 μM.

Table 2. Cation Flux Rates in Egg Lecithin Vesicles Determined by ^{23}Na-NMR Spectroscopy[a]

no.	compd	rate
	gramicidin	100
1	C_{12}<N18N>C_{12}<N18N>C_{12}<N18N>C_{12}	28
2	C_{12}<N18N>EOEOEOE<N18N>EOEOEOE<N18N>C_{12}	3
3	C_{12}<N18N>pip<N18N>pip<N18N>C_{12}	ND[b]
4	C_{12}<N18N>AQ<N18N>AQ<N18N>C_{12}	ND
5	C_{12}<N18N>C_{12}<N15N>C_{12}<N18N>C_{12}	25
6	C_{12}<N18N>C_{12}OEOEOEOC$_{12}$<N18N>C_{12}	14
7	$PhCH_2$<N18N>C_{12}<N18N>C_{12}<N18N>CH_2Ph	39
8	H<N18N>C_{12}<N18N>C_{12}<N18N>H	28
9	<18N>C_{12}<N18N>C_{12}<N18>	<2
10	St-E<N18N>C_{12}<N18N>C_{12}<N18N>E-St[c]	<2
11	St-OCOM<N18N>C_{12}<N18N>C_{12}<N18N>MCOO-St	5
12	C_{12}<N18N>C_{12}	<2
13	$C_6H_5CH_2$<N18N>$CH_2C_6H_5$	<2
14	C_{12}<N18N>C_{12}<N18N>C_{12}	<2

[a] See compound structures, above. [b] ND means not determined by this method. [c] St represents the steroid 3-cholestanyl.

General Observations. The most important finding of this work is that several of these simple, property-designed structures do conduct cations with a rate of the same order of magnitude as that for gramicidin. It is also the case that structural variations lead to differences in flux rate that are well outside experimental error. From a comparison of **1** with **12**, **13**, or **14**, which are essential fragments of the structure, it is clear that all of the structural features of **1** are required for cation conduction.

The "Tunnel" Model. The solid state structures known for gramicidin and for bacteriorhodopsin (seven transmembrane protein segments organized into a porelike array) has popularized the concept of a channel as a tunnel or tube. Indeed, there is no evidence either to confirm or to exclude this "tunnel" possibility in the many known functional channels for which detailed structures have not yet been determined. The well-studied gramicidin system is a particularly appealing example of "tunnel" formation as is the "barrel-stave" model proposed for amphotericin channels.[34] In the former case, cations flow through the dimerized pentadecapeptide using inward-turned carbonyl groups as cation relays.[9]

Compound **1** was designed to serve as a tunnel-like, unimolecular ion channel. At the present time, there is no structural evidence to confirm this design hypothesis any more than highly useful hydropathy plots[35] generated for transmembrane proteins unequivocally confirm the specific identity of membrane-inserted, α-helical amino acid chains.[5] In both cases, the best current guesses may be true but the difficulty of evaluating these systems precludes certainty at the present time. We have now obtained kinetic evidence that permits a clear inference to be drawn concerning the "tunnel-like" structure originally proposed for **1**.

Compounds **1**, **5**, and **6** differ only in the identity of the central "relay" unit. It is diaza-18-crown-6, diaza-15-crown-5, and tris-(ethylene glycol) diether in **1**, **5**, and **6**, respectively. If sodium passes through and not over the central element, cation flux should diminish in the order **1** > **5** > **6**. This is anticipated for three reasons. First, the total number of donor groups available in the central unit diminishes in the order **1**, **5**, **6**. Second, the donicities of **1** or **5** and **6** toward Na^+ are known to diminish in the same order with the open-chained systems normally showing cation complexation constants several orders of magnitude below those of crown ethers.[36] Third, passage of a hydrated sodium cation *through* a 15-membered ring must surely be much more sterically demanding than through an 18-membered macrocycle.[37]

It is possible that an accidental cancellation of factors could affect the flux rates of **1** and **5**. This would be so if the larger ring of **1** bound Na^+ more strongly (cation release would be slowed) than did the smaller ring of **5**. On the other hand, the larger ring of **1** could pass Na^+ more readily than could the smaller ring of **5** and the overall rates might be similar. It is therefore interesting to note that Na^+ complexation constants (K_S) for dibenzyldiaza-15- and 18-membered crowns are known to be $10^{3.59}$ and $10^{2.68}$, respectively (in anhydrous methanol at 25 °C).[38]

The observed sodium transport rates for **1** and **5** are, within experimental error, the same (28 *vs* 25, relative to gramicidin). The flux rate drops to approximately one-half (14) when the central ring is opened. Obviously, there are other variables inherent in the latter comparison. Most prominent is the absence of nitrogen atoms which are replaced by oxygen although fewer donor groups are present in this central array than is the case for either **1** or **5**. It seems clear from the rate data that the central relay does not *necessarily* serve as a tunnel. An alternate view is that the ionophore may adopt an elongated conformation in which the central macrocycle is disposed approximately parallel to the membrane's hydrocarbon chains. This conformation would diminish the local polarity at the bilayer's midplane and also reduce the "jump" distance over which a cation is required to pass unaided by a channel donor group.

Based largely on the recently confirmed gramicidin structural postulate, most synthetic, small-molecule channel models have used the tunnel notion as a starting point. The data obtained thus far and presented above suggest that a unimolecular "tunnel" model is inappropriate for the present compounds. It is possible, however, that individual monomers could associate to form a transmembrane, tunnel-like domain in a fashion reminiscent of that supposed generally for transmembrane proteins. It seems likely that in the "monomer" channels reported by Menger[15h] and by Kobuke[15i] that aggregation is likely. The latter cases involve amphiphilic monomers. It is known that membrane lipids, and presumably alien amphiphiles or ionophores,[39] readily undergo lateral diffusion many powers of 10 faster than they undergo transverse diffusion (flip-flop).[40] Indeed, a phospholipid monomer in a PC vesicle is known to undergo flip-flop only once in several hours. Unlike the Menger or Kobuke monomers, the present systems are bola-amphiphilic, *i.e.* they span both sheets of the bilayer. It is not unreasonable to think that ionophores such as **1** could be highly mobile within the lipid bilayer since diffusion coefficients of 10^{-8} cm^2 s^{-1} are typically observed for lipid monomers and embedded proteins.[41] Thus, it seems possible that two or more molecules of **1** may be involved in each cation conduction event although we note that the plots of $1/\tau$ *vs* [ionophore] are first order.

(34) (a) Kleinberg, M. E.; Finkelstein, A. *J. Membr. Biol.* **1984**, *80*, 257. (b) Medoff, G.; Kobayashi, G. A. In *The Polyenes. Antifungal Chemotherapy*; Speller, D. C. E., Ed.; John Wiley: New York, 1980; p 3.

(35) (a) Kyte, J.; Doolittle, R. F. *J. Mol. Biol.* **1982**, *157*, 105. (b) Engelman, D. M.; Steitz, T. A.; Goldman, A. *Ann. Rev. Biophys. Biophys. Chem.* **1986**, *15*, 321.

(36) Gokel, G. W.; Goli, D. M.; Schultz, R. A. *J. Org. Chem.* **1983**, *48*, 2837.

(37) The internal cavities ("holes") of 15-crown-5 and 18-crown-6 are approximately 2.0 and 2.7–3.0 Å, respectively. The "fits" between CPK molecular models of these respective crowns with Na^+ (1.95 Å) and K^+ (2.66 Å) are excellent.

(38) Gatto, V. J.; Arnold, K. A.; Viscariello, A. M.; Miller, S. R.; Morgan, C. R.; Gokel, G. W. *J. Org. Chem.* **1986**, *51*, 5373–5384.

(39) Cherry, R. J. *Biochim. Biophys. Acta*, **1979** *559*, 289.

(40) Edidin, M. In *Molecular Motions and Membrane Organization and Function. Membrane Structure*; Finean, J. B., Mitchell, R. H., Eds.; Elsevier: New York, 1981; p 37.

(41) Gennis, R. B. *Biomembranes Molecular Structure and Function*; Springer Verlag: New York, 1989; p 183.

π-Donor Groups. A fundamental question related to the function of natural channels is how cation selectivity is achieved. MacKinnon and co-workers[42] postulated that aromatic amino acid side chains could serve as "relays" that might also foster cation selectivity. This is an appealing concept. Kumpf and Dougherty,[43] referring particularly to the K^+ selectivity of the shaker channel of *Drosophila melanogaster*,[44] showed by theoretical calculations that significant differences in stabilization energies for $(C_6H_6)_2M^+$ (M^+ = alkali metals) complexes, *i.e.* the inherent selectivity of π-complexation, could account for channel selectivity. Complexation between arenes and alkali metals, while certainly documented, is weaker than for oxygen donors. Conduction of a cation through a channel must involve stabilization by donor group relays at certain points between the two membrane surfaces. This stabilization must be relatively weak or the flow of cations would be stanched by complex stability. The π-donor group array offers a plausible, transient stabilization mechanism. In addition, molecular mechanics calculations showed that the selectivity order for stabilization between benzene and M^+ is expected to be in the order $Li^+ < Na^+ < K^+$ as observed for the shaker channel.

We prepared a three-ring channel compound (**7**) having benzyl group side arms. Molecular models suggested that, if the system exhibited any flexibility at all, the benzyl groups could reach into the membrane to a significant degree. Cation flux, assessed as described above, was found to be about 40% greater than for dodecyl channel **1**. This is a significant increase in cation flux and the efficacy of this very simple compound is approximately 40% of that of the gramicidin dimer.

Subsequent to the effort involving **7**, a study involving site-directed mutagensis of the shaker channel "signature sequence" (specifically a Y7 → F mutation therein) showed that the channel is more sensitive to removal of a hydroxyl than to deletion of an arene.[45] This suggests that **7** is not using the arene residues as relays but possibly as membrane anchors. If the arenes remain outside the membrane, they may interact with the glycolipid's polar head-group residues. Such interactions are known[46] and could stabilize the extended conformation of **7**. In such a case, cation flux would be enhanced by organization within the bilayer, *i.e.*, by forging a defined conduit. Indeed, this appears to be what is observed for bacteriorhodopsin,[47] which is rigidified by an α-helical structure and ionophore **7** may be anchored at the membrane surface as well.[48]

An alternative mechanism for anchoring may involve protonation. It is expected that these azacrown compounds can all be protonated and can be protonated at all nitrogen atoms. Considering the amphiphilic nature of the bilayer, it seems most likely that protonation of an inserted structure such as **1** would occur at the distal rings rather than the proximal ring which must reside at all times within the bilayer. Two questions can be posed regarding this possibility. First, are the nitrogens accessible for protonation at physiologic pH? Second, can a simple structural manipulation be used to demonstrate a significant difference in conductivity in closely related compounds?

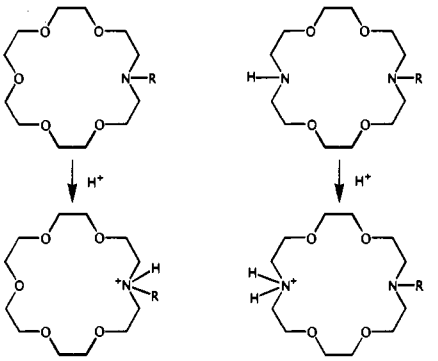


Figure 6. Protonation of azacrown derivatives having one or two nitrogen atoms in the macroring.

The pK_a values for a number of azacrowns have been reported, and we undertook the measurement of several others. The following have been reported in the literature: aza-18-crown-6, $pK_a = 9.40$[49] and diaza-18-crown-6, $pK_1 = 8.94$, $pK_2 = 7.81$.[50] These data are particularly relevant to compounds **8** and **9**, which differ only in a single heteroatom in the two distal rings. Thus compound **8** is terminated at each end by a diaza-18-crown-6 structure whereas **9** is terminated by aza-18-crown-6. Because of the potential implications of protonation, we determined the pK_a values for both *N,N'*-bis(*n*-butyl)diaza-18-crown-6, a simple analog of **12**, and *N,N'*-bis(*n*-benzyl)diaza-18-crown-6 (**13**). The protonation constants were as follows: dibutyl, $pK_1 = 9.40$, pK_2 7.97; **13**, $pK_1 = 7.5$, $pK_2 = 6.83$.

Protonation of aza-18-crown-6 is possible at only one nitrogen atom. In the channel formers, this is the position to which the alkyl chain is attached (see figure). If the azacrown functions as the head group for this amphiphilic molecule, polarity is increased at a portion of the molecule that is expected to penetrate the bilayer's hydrophobic domain. Indeed, we have previously speculated that the high stability we observed for membranes formed from bola-amphiphilic bis(crown) compounds of the type <18N>C_{12}<N18> was due to protonation of the head groups with concurrent formation of a hydrogen-bonded network.[13]

Protonation of a diazacrown can occur at either nitrogen atom. The protonated diazacrown shown in Figure 6 represents a structure that has a more appropriate amphiphilic arrangement than does that shown for the azacrown. The preparation of a drawing or molecular models certainly does not prove that the diazacrown protonates in the "more favorable" position, but it is inescapable that protonation of the azacrown must occur at a position favorable to channel function or at least in a way that does not hinder function. Cation flux rates were determined for compounds **8** and **9** and found to differ dramatically. Removal of the two dodecyl chains of **1** to give **8** did not, within experimental error, affect the ability of this structure to conduct cations. In marked contrast, the NH → O mutation (**8** → **9**) obliterated cation channel function in the latter. This finding is comprehensible if protonation of **8** at the two distal nitrogens stabilizes the active, transmembrane conformation and protonation of **9** at the only available nitrogens disrupts this structural arrangement.

Steroidal Sidearms. It is well-known that steroids are hydrophobic, membrane insertable substances. Indeed, the role

(42) Heginbotham, L.; MacKinnon, R. *Neuron* **1992**, *8*, 483.
(43) Kumpf, R. A.; Dougherty, D. A. *Science* **1993**, *261*, 1708.
(44) (a) Yellen, G.; Yurman, M. E.; Abramson, T.; MacKinnon, R. *Science* **1990**, *251*, 939. (b) MacKinnon, R.; Yellen, G. *Science* **1990**, *250*, 276, 279. (c) Yool, A. J.; Schwartz, T. L. *Nature* **1991**, *349*, 700. (d) MacKinnon, R. *Nature* **1991**, *350*, 232.
(45) Heginbotham, L.; Lu, Z.; Abramson, T.; MacKinnon, R. *Biophys. J.* **1994**, *66*, 1061.
(46) (a) Petti, M. A.; Shepodd, T. J.; Barrans, R. E., Jr.; Dougherty, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 6825. (b) Kearney, P. C.; Mizoue, L. S.; Kumpf, R. A.; Forman, J. E.; McCurdy, A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 9907.
(47) Ovchinnikov, Yu.; Abdulaev, N.; Feizina, M.; Lobanov, N. *FEBS Lett.* **1979**, *100*, 219.
(48) Abel, E.; Fedders, M. F.; Gokel, G. W. *J. Am. Chem. Soc.* 1995, *117*, 1265–1270.
(49) Kataky, R.; Matthes, K.; Nicholson, P.; Parker, D.; Buschmann, H. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1425.
(50) Suh, M.; Eom, T.; Kim, S. *Bull. Korean Chem. Soc.* **1983**, *4*, 231.

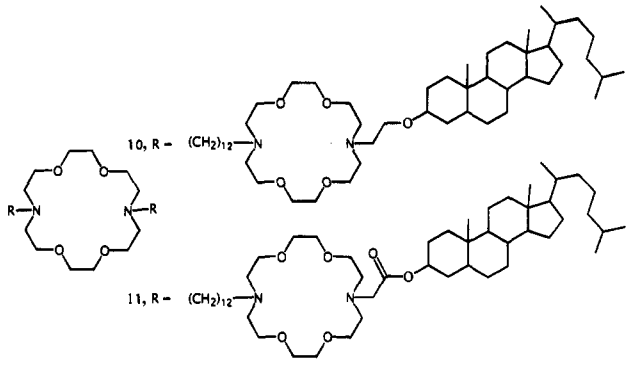


Figure 7. Structures of compounds **10** and **11**.

of steroids in stabilizing membranes has been extensively studied.[51] We incorporated cholestanol as sidearms in two ionophores. The steroids were attached in one case (**10**) by an ethylene chain terminated at the steroid's 3-oxygen. In the other case (**11**), attachment was by a glycine unit. The compounds are illustrated in Figure 7.

The ability of **10** and **11** to conduct Na^+ was dramatically lower than observed for **1**, which possesses dodecyl side chains. Indeed, the flux rate for **10** is below our detection limit. Perhaps more important, however, is that the cation flux rates for the two compounds differ from each other despite the fact that the only structural difference is attachment of the steroid in one case by an ester group and in the other by an ether linkage. The ester link is known to be more rigid than the ether, but the effect on overall channel structure is impossible to determine in the absence of structural information. We have thus constructed CPK molecular models of the two compounds in order to assess whether there are obvious differences.

For both compounds **10** and **11**, the models can be placed in an overall extended conformation in which the steroids are aligned with the dodecyl chains. Indeed, both the spacers and the steroidal sidearms are almost identical in overall length. The critical difference appears to be in the flexibility of the link by which the steroid is attached to the crown. We presume that the crown is "open" to an equivalent extent in each case. In both cases, the α-surface of the steroid can lie flush with the dodecyl spacer. This is expected to be a favorable, hydrophobic–hydrophobic interaction. It is also expected to exclude water since the contact appears intimate. Because of the more rigid ester function in **11**, the contact does not appear (again by models) to be as extensive as in the case of **10**.

In studies designed to assess hydrophobic interactions between hydrocarbon chains of ammonium salts ($R\text{-}NH_3^+$) and steroidal crown ethers, we have obtained evidence that steroid chains can "stack" and exclude the ammonium salt.[52] Similarly, stacking of the alkyl spacer of either **10** or **11** with the steroid would create an obstruction to the passage of cations.

The plausibility of the interactions suggested above is enhanced by the known stabilization of phospholipid membranes by cholesterol, presumably by contacts of the sort described. It should also be noted that the steroid side chains of **10** and **11** can interact with the alkyl chains of membrane lipids and a disruption of a functional channel structure analogous to that of **1** may thus be precluded. At the present time, we cannot distinguish these related possibilities.

Control Experiments. The NMR studies described here purport to assess the equilibrium between sodium either inside or outside of the vesicle. A possible weakness of the method is that the equilibrium could detect ^{23}Na in the bilayer but that is not completely transferred to the vesicle's interior. Using our experimental setup (see the Experimental Section), we searched for magnetization transfer.[53] This was done first using gramicidin as the channel. The NMR cross peaks, indicating that sodium within the vesicle and sodium outside it are in chemical equilibrium, are shown in Figure 8. We note that magnetization transfer has been previously observed for gramicidin by Hinton and co-workers.[54]

An identical experiment was attempted using compound **8** as the channel former. Despite an extensive effort, neither magnetization transfer nor cross peaks could be observed. This is probably due to the lower activity of **8** relative to gramicidin which manifests iteself as more rapid signal decay. Although this is a complex experiment and a failure can be rationalized, it is still troubling. We thus compared the data obtained from the NMR experiments described above with those obtained from fluorescence studies. The fluorescence spectrum of pyranine is dependent upon its environment. The observed spectra show, in each case, a gradual change in environment as protons diffuse across the bilayer. Vesicular lysis is an abrupt process. A comparison of the data obtained by both methods shows that correlations are good. In both cases, **1** is superior to **2** and to carrier **13**. If the equilibrium involved only a rapid partitioning of the compound between the aqueous phase and the membrane without penetrating into the latter, the apparent efficacy of **13** should exceed that of **1** and the efficacy order for these compounds would probably not be the same. In fact, the order is the same and the carrier is poorer in both cases. This is not definitive evidence but it is strongly suggestive.

Molecular Controls. Several molecules were prepared for the express purpose of determining if all of the elements incorporated into the channel formers were required for efficacy. It has been noted above that two simple carrier molecules that represent subunits of the ionophore (**12** and **13**) are ineffective in the present experimental system. The dramatic difference in efficacy between **8** and **9**, which differ only by an NH → O mutation in the distal ring, was noted above. A further control experiment was conducted by comparing the activity of **1** with **14**. The latter comprises essentially two-thirds of the active compound **1**. The two molecules are illustrated in Figure 9. Compound **14** is missing only one distal ring and the dodecyl side chain, and yet its ability to conduct cations is below our ability to detect it.

Detergent Effects. Although the data presented above show conclusively that the compounds prepared function as cation-conducting channels, one might be tempted to suggest that these structures are simply detergents that render the lipid bilayer "leaky." Indeed, in a sense, this is true. So far as is known, this is what occurs when a protein inserts in a bilayer. The fact that a protein is inherently more complicated than the compounds studied here does not alter the fact that both compounds appear to be active at low concentrations and to exhibit a reproducible effect.

In order to exclude a simple detergent effect, however, two ^{23}Na-NMR studies were undertaken. In each study, the egg lecithin vesicles described above were prepared and incremental amounts (0, 5, 10, 15, and 20 μM) of the known detergents sodium dodecyl sulfate and Triton X-100 were added. No line broadening was observed for any of these individually studied cases. In the most concentrated system, microliter additions

(51) (a) Yeagle, P. L. *The Membranes of Cells*, 2nd rd; Academic Press: San Diego, CA, 1993; p 241. (b) Finegold, L., Ed. *Cholesterol in Membrane Models*; CRC Press, Inc.: Boca Raton, FL, 1993.

(52) Wang, K.; Han, X.; Gross, R. W.; Gordon, J. I.; Gokel, G. W. Unpublished work.

(53) (a) Shungu, D.; Briggs, R. *J. Magn. Reson.* **1989**, *77*, 491. (b) Shungu, D.; Buster, D.; Briggs, R. *J. Magn. Reson.* **1990**, *77*, 491.

(54) Buster, D.; Hinton, J.; Millett, F.; Shungu, D. *Biophys. J.* **1988**, *53*, 145.

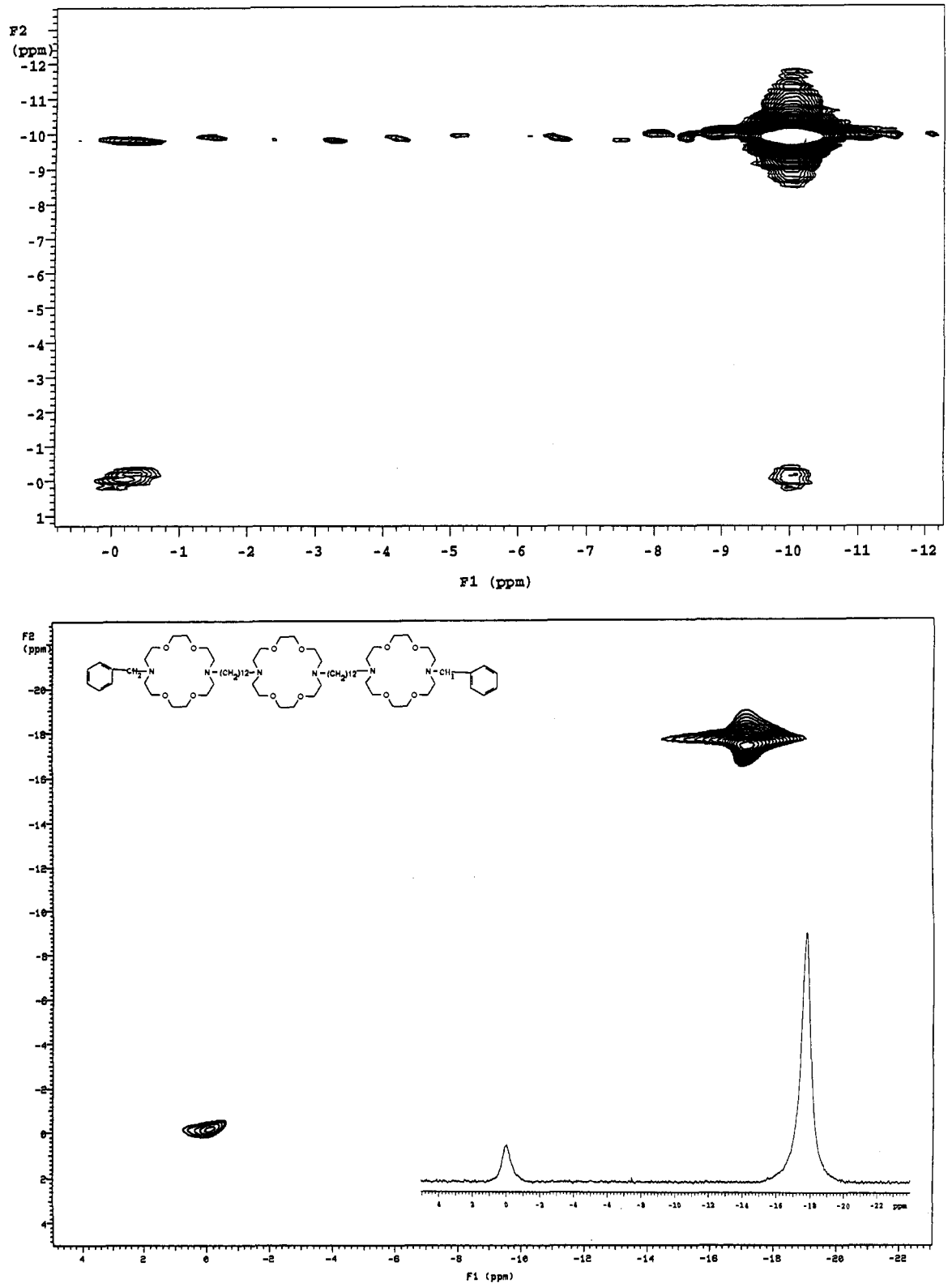


Figure 8. ^{23}Na 2D NOESY spectrum of a vesicle preparation in the presence of (a) 2.22 μM gramicidin and (b) 14.0 μM compound **7**.

of detergent solution were made until a final concentration of 190 μM was reached. This most concentrated sample did not show vesicular lysis nor did it show any line broadening. Membrane lysis was observed in the above vesicle system when either sodium dodecyl sulfate or Triton X-100 was present at a concentration of 2 mM. Admittedly, these experiments do not exclude an undefined "detergent effect" but they confirm that the compounds of the present study in the present system do not behave in the same way as do "standard" detergents.

Mass Spectral Analyses. There are two questions which can be posed concerning the macrorings. The first, whether the macrorings can actually serve as head groups is resolved. The second is that, although it seems obvious that the rings must interact with cations within the membrane, it is unknown whether one, two, or all three interact during transport. Indeed, it is currently unclear how the question of whether all sites are simultaneously occupied during *in situ* channel function can be resolved. We have, however, been able to address this question

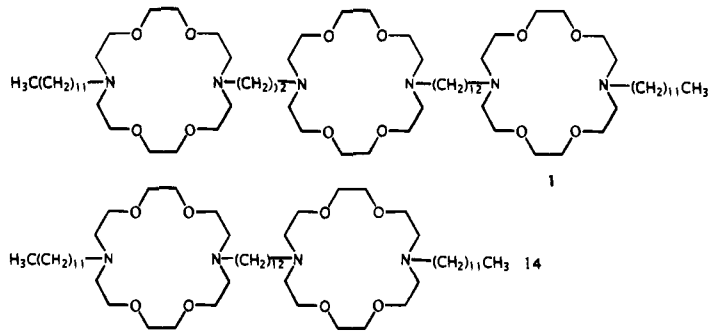


Figure 9. Structures of compounds **1** and **14** drawn to illustrate the similarity.

by examining the complexation between **1** and Na^+ using the electrospray ionization mass spectrometry technique. The results have recently been reported elsewhere[55] and show clear evidence for a complex corresponding to $[\mathbf{1}\cdot Na_3]^{3+}$.

Conclusions. More than a dozen novel ionophores have been designed and synthesized and their cation transport ability assessed either by fluorescence or by a $^{23}Na^+$ dynamic NMR technique. Where the same compounds have been studied by both methods, there is reasonable correspondence in activity even though the cations (H^+ and Na^+) differ. The data suggest that the effective ionophores span the membrane in which they are anchored by a variety of mechanisms. When the potential for anchoring is absent, efficacy is lost. The central macroring is found to enhance flux compared to an open-chained analog, but size variations within the ring suggest that the ionophore does not require a tunnel-like conformation in order to function. Evidence has also been obtained for strong hydrophobic contacts that act to "close" the channel or at least dramatically impede cation transport. Relatively small changes in the channel structure are manifested by significant changes in flux rate and known carrier molecules, closely related to the channel formers failure to transport cations under the present conditions. Studies to assess the importance of "anchoring" using compounds more closely related to transmembrane proteins are underway.

Experimental Section

^{1}H-NMR spectra were recorded on a Varian VXR-500 high-resolution spectrometer or on a Gemini 300 MHz spectrometer. $CDCl_3$ was used as solvent unless otherwise specified. Chemical shifts (δ) are given in ppm downfield from internal TMS. NMR data are given in the following order: chemical shift, peak multiplicity (b = broad, s = singlet, d = doublet, t = triplet, m = multiplet, bs = broad singlet, etc.), integration, and assignment. Infrared spectra were recorded on a Perkin-Elmer 1310 infrared spectrophotometer (KBr unless otherwise noted) and were calibrated against the 1601 cm^{-1} band of polystyrene. FAB mass spectra were recorded on a VG Trio 2 instrument using *m*-nitrobenzyl alcohol as the matrix. ESI mass spectra were acquired on a triple-quadrupole tandem mass spectrometer (Finnigan MAT TSQ 700, San Jose, CA) equipped with an electrospray interface (Analytica of Branford, Branford, CT). Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter in a glass microcell (100 mm path length, 1 mL volume) with a Na gas discharge lamp as the light source. Fluorescence intensity was measured on Hitachi 650-10M Fluorescence spectra (excitation, 450 nm; emission, 510 nm) were obtained using 2.5 mm slits on both the excitation and emission sides. The pH of the buffer solutions was adjusted and monitored by means of a HORIBA M-8 pH Meter. Melting points were determined on a Thomas Hoover apparatus in open capillaries and are uncorrected. Thin layer chromatographic (TLC) analyses were performed on aluminum oxide 60 F-254 neutral (type E, 0.2 mm thickness, Merck). Preparative chromatography columns were packed with alumina [neutral, Brockman 1, standard grade (150 mesh, Aldrich)], Sephadex LH-20, or silica gel (Merck, 230–400 mesh, 60 Å).

All reactions were conducted under dry N_2 unless otherwise noted. All reagents were the best grade commercially available and were distilled, recrystallized, or used without further purification, as appropriate. Molecular distillation temperatures refer to the oven temperature of a Kugelrohr apparatus. Combustion analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are reported as percents.

Aqueous Potentiometric Acidity Measurments. Successive acid dissociation constants for aza-18-crown-6, 4,13-diaza-18-crown-6, *N,N'*-dibenzyl-18-crown-6, and *N,N'*-bis(*n*-butyl)diaza-18-crown-6 were determined by pH-metric titration using an autotitrator (Man-Tech Associates, Inc.) according to the procedure reported by Parker and co-workers.[48,56]

Proton Transport Studies. Vesicle Preparation. Stock solutions of distearoyl-D,L-α-phosphatidylcholine (DPSC, 247.8 mg/25 mL, 1.23 × 10^{-2} mol dm^3) and sodium dicetyl phosphate (SDP, 16.4 mg/25 mL, 1.19 × 10^{-3} mol dm^3) in $CHCl_3$ were freshly prepared every 14 d and stored under refrigeration. In a typical experiment, 2.0 mL of SPC solution and 2.0 mL of DSPC solution were mixed in a 100 mL round-bottomed flask and the $CHCl_3$ was evaporated *in vacuo*. The remaining thin film was dried *in vacuo* for 2 h. The film was dispersed into the buffer solution (2.0 mL, 0.1 mol dm^3 KH_2PO_4–0.05 mol dm^3 $Na_2B_4O_7$ (pH = 7.70); KCl, 0.1 mol dm^3) that includes pyranine (1.0 × 10^{-4} mol dm^3), and the dispersion was sonicated for 5 min (Bransonic 12 bath sonicator). The turbid solution, which includes multicompartment liposomes, was sonicated for 15 min under N_2 at 50–55 °C using a tip sonicator (Nissea Ultrasonic Generator US-150). After cooling to room temperature, the resulting clear solution was passed through a Sephadex G-50 column (2.5 × 17 cm). The DSPC/DSP vesicles that included fluorescence probes were recovered completely, and the solution was adjusted to an appropriate volume (10–25 mL).

Rate Studies. The proton-transporting abilities of these channels were estimated by the fluorescence techniques developed by Kano and Fendler[26] and modified by Menger.[27] Unilamellar vesicles (DSPG/DSP) were prepared [phosphate buffer, pH = 7.7, μ = 0.1 (KCl)] to include the fluorescence-sensitive dye pyranine (8-hydroxy-1,3,6-pyrenetrisulfonic acid). The dye located in the outer aqueous phase was removed by gel filtration through a Sephadex G-50 column. The vesicle solution (2 mL) was placed in a thermostated cell held at 25.0 ± 0.1 °C. A CH_3OH solution (20 μL) of the channel compound was injected into the vesicle solution and incubated for 10 min. An acid pulse (2 N HCl, 20 μL) was added to the solution, and the change of fluorescence intensity was monitored during 10–15 min. After the measurements were complete, vesicular lysis was induced by addition of Triton X-100 (1.2% aqueous solution, 50 μL). The final pH in the solution was 6.60.

Measurement of Cation Transport Rates by ^{23}Na-NMR. Vesicle Preparation. Phosphatidylcholine (chloroform–methanol solution), phosphatidylglycerol ($CHCl_3$–MeOH solution), Na_2HPO_4, NaH_2PO_4, KH_2PO_4, NaCl (molecular grade reagents), gramicidin D (85% gramicidin A, 15% gramicidin B and C), and sodium tris(polyphosphate) (90–95%) were purchased from Sigma, St. Louis, MO, and used without further purification. Diethyl ether (anhydrous, Mallinckrodt) was distilled over Na°/benzophenone prior to use. Distilled, deionized

(55) Wang, K.; Han, X.; Gross, R. W.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1995**, in press.

(56) Kataky, R.; Parker, D.; Teasdale, A.; Hutchinson, J.; Buschmann, H. *J. Chem. Soc., Perkin Trans 2* **1992**, 1347.

water was used to prepare the buffer solution. 2,2,2-Trifluoroethanol (TFE), NMR grade, $DyCl_3 \cdot 6H_2O$ (99.99%), D_2O, KCl (ACS grade), and $CHCl_3$ (HPLC grade) were purchased from Aldrich and used without further purification. The sonication was done using either a Branson model 1200 or Bransonic 12 water bath. The mean diameters of the vesicles were determined by using a Coulter N4MD submicron particle analyzer.

To prepare the vesicles, the procedure described by Papahadjopoulos[30] was followed with certain modifications. The concentration of Na^+ in the phosphate-buffered saline solution (1:10 dilution) was increased by addition of NaCl until $[Na^+]_{total}$ = 100 mM. Phophatidylglycerol (PtdGro) and phosphatidylcholine (PtdCho) solutions were allowed to reach room temperature before utilization. A solution of phosphatidylglycerol (1.30 mL) and phosphatidylcholine (0.52 mL) was diluted in a volumetric flask to 25.00 mL with $CHCl_3$. This solution was poured into a 250 mL round-bottomed flask, and the solvent was evaporated *in vacuo*. The system was maintained under vacuum (<0.02 mmHg) overnight, and then it was purged with nitrogen. The film of lipids was dissolved in 25.0 mL of ether, and 10.00 mL of buffered saline solution (1:10 dilution) was added.

The resulting two-phase suspension was sonicated at 2−5 °C in a water bath sonicator for 40 min. All of the organic phase was removed by rotary evaporation as described in the sequence following. The rotary evaporator was gradually evacuated by reducing the pressure sequentially as follows: ≈500 mm for 10 min (no heating), ≈200 mm (10 min, no heat), then warm in a 40 °C water bath (≈30−60 s), then remove water bath and evacuate (high vacuum, ≈5 min, solution becomes translucent). The resulting vesicle solution was either filtered through a polycarbonate membrane (Poretics, 0.4 μm) or centrifuged for 15 min. Vesicle size was determined by laser light scattering. The sample was diluted in the cuvette to a concentration appropriate for the instrument using 1:10 buffer (see below). This preparation produces vesicles with an average diameter of 175−200 nm (≈2000 Å). Integration the ^{23}Na signals inside and outide the vesicle showed ≈3% aqueous volume entrapment.

Buffer Composition. The composition of the buffered saline solution employed to prepare the 1:10 buffered saline solution was as follows (at pH 7.3 (25 °C)): NaCl, 0.137 M; KCl, 0.0026 M (2.6 mM); Na_2HPO_4, 0.0064 M (6.4 mM); KH_2PO_4, 0.00143 M (1.43 mM). A 10.0 mL aliquot was taken and diluted to 100.0 mL with distilled water. The total Na^+ concentration was adjusted to 100 mM by addition of NaCl.

Insertion of Ionophores. Ionophores were inerted into the membranes either during vesicle formation or afterward. When the vesicles were preformed as described above, a trifluoroethanol solution of the ionophore (≈0.5−1 mM) was added in an amount appropriate to afford the required 5−40 μM concentration. A mixture of the vesicle and trifluoroethanol solutions in an Eppendorf vial was warmed at 60 °C (water bath) for 1 h. This solution was then transferred to an NMR tube for study. Preparation of samples directly in 5 mm NMR tubes was normally successful, but vortexing the Eppendorf vials generally produced more homogeneous samples.

Alternately, the vesicle preparation described above could be conducted in the presence of the trifluoroethanol solution of ionopohore. In this way, different concentrations of ionophore could be incorporated directly during vesicle formation. Thus, prior to formation of the vesicles, an amount of ionophore suitable to give the desired final concentration (in CF_3CH_2OH) was added to the vesicle preparation solution. Vesicles were prepared in the normal way and ionophore concentrations were calculated on the basis of the assumption that no material was lost during vesicle preparation. Vesicles containing **7** were prepared by both methods, and data obtained were within experimental error.

Shift Reagent. The shift reagent was prepared by mixing an aliquot of 2.00 mL of a tris(polyphosphate) solution (0.200 M) and 1.00 mL of a Dy^{3+} solution (0.100 M). No correction for purity was made in the case of the tris(polyphosphate).

NMR Measurements. ^{23}Na-NMR spectra were obtained on a Varian VXR 500 high-resolution NMR spectrometer operating at 132.218 MHz. Chemical shifts (isotropic hyperfine shifts) were measured as differences between the frequencies for Na^+ inside and outside of the vesicle. Upfield hyperfine shifts are defined as negative. Samples for NMR measurements were prepared in Eppendorf vials (1.7 mL) and then transferred to 5 mm NMR sample tubes. After the incubation period (see above), the samples were cooled to room temperature and then diluted with 0.100 mL of D_2O and 0.100 mL of shift reagent. The 10% D_2O was necessary for optimizing the static field homogeneity and for field/frequency locking of the spectrometer. The solution was then allowed to equilibrate for at least 1 h before data acquisition. Typically, 240 FID transients were accumulated per data set at a probe temperature of 25 °C.

Rate Constants. Once the potential ionophore was incorporated into the vesicles, any line broadening of the ^{23}Na signal was determined. In the slow exchange region, the rate constant, $k = 1/\tau$, is directly proportional to the observed line broadening, $(\Delta\nu - \Delta\nu_o)$: $k = 1/\tau = \pi(\Delta\nu - \Delta\nu_0)$. The rate constant is determined from the slope of a plot of $1/\tau$ *vs* ionophore concentration.

Ionophore Syntheses. Butyronitrile (98%), 1-bromododecane (97%), 1,12-dibromododecane (97%), sodium dodecyl sulfate, Triton X-100, and pyranine were purchased from Aldrich and distilled or recrystallized prior to use. The synthesis *N,N'*-didodecyl-4,13 diaza-18-crown-6 is reported elsewhere.[24] The following four compounds are fragments required for various syntheses.

***N*-Dodecyl-4,13-diaza-18-crown-6 ($C_{12}H_{25}$<N18N>H).** A solution of 4,13-diaza-18-crown-6 (1.0 g, 3.84 mmol), 1-bromododecane (765 mg, 3.07 mmol), Na_2CO_3 (5.8 g, 54.8 mmol), and KI (20 mg, 0.12 mmol) in PrCN (30 mL) was heated under reflux for 4 d. The mixture was cooled, filtered, and concentrated *in vacuo*. Column chromatography (5% deactivated Al_2O_3, 0.5−5% 2-PrOH/CH_2Cl_2) afforded C_{12}<N18N> (449 mg, 27%) as a waxy solid (lit. mp[57] 33.5 °C) and *N,N'*-bis(dodecyl)-4,13-diaza-18-crown-6 (C_{12}<N18N>C_{12}, 320 mg, 14%) also as a waxy solid, mp 46−48 °C (lit.[24] mp 46−48 °C). ^1H-NMR: 0.84 (t, 3H), 1.21 (s, 18H), 1.50 (bt, 2H), 2.48 (t, 2H), 2.70−2.84 (m, 8H), 3.52−3.65 (m, 16H). Note that the amine proton was not observed. IR (neat): 3560 (NH), 2960 (CH), 2880 (CH) cm^{-1}. Anal. Calcd for $C_{24}H_{50}N_2O_4$: C, 66.93; H, 11.70; N, 6.50%. Found: C, 67.00; H, 11.65; N, 6.55%.

***N*-(12-Bromododecyl)aza-18-crown-6 [Br(CH_2)$_{12}$<N18>].** A solution of aza-18-crown-6 (4.0 g, 15 mmol), 1,12-dibromododecane (5 g, 1 equiv), Na_2CO_3 (31.8 g, 20 equiv) and KI (0.31 g) in PrCN (150 mL) was heated under reflux for 4 h. The mixture was cooled, filtered, and concentrated *in vacuo*. The oil obtained (7.25 g) was dissolved in 100 mL of CH_2Cl_2 and washed with water (5 × 30 mL). The organic layer was dried over $MgSO_4$, the solvent was removed *in vacuo*, and the residue was chromatographed (alumina column, 5% 2-PrOH in hexane) to afford the title compound (BrC_{12}<N18N>, 1.75 g, 22%) as a pale yellow oil. ^1H-NMR: 1.1−1.4 (s, 18H), 1.7−1.8 (q, 2H), 2.4 (t, 2H), 2.7 (t, 4H), 3.35 (t, 2H), 3.6 (m, 20H).

***N*-Dodecyl-*N'*-(12-bromododecyl)-4,13-diaza-18-crown-6 [$C_{12}H_{25}$<N18N>(CH_2)$_{12}$Br].** A solution of *N*-dodecyl-4,13-diaza-18-crown-6 (421 mg, 0.98 mmol), 1,12-dibromodecane (961 mg, 2.93 mmol), Na_2CO_3 (3.07 g, 29 mmol), and KI (20 mg, 0.12 mmol) in PrCN (20 mL) was stirred at reflux temperature for 90 min. The mixture was cooled, filtered, and concentrated *in vacuo*. Column chromatography (5% deactivated Al_2O_3, 0−5% 2-PrOH/CH_2Cl_2) afforded C_{12}<N18N>C_{12}-Br (440 mg, 66%) as a pale yellow oil. ^1H-NMR: 0.86 (t, 3H), 1.25 (s, 34H), 1.42 (br t, 4H), 1.81−1.90 (m, 2H), 2.49 (t, 4H), 2.73 (t, 8H) 3.39 (t, 2H), 3.52−3.78 (m, 16H). IR (neat): 2960 and 2880 cm^{-1} (ν_{CH}). Anal. Calcd for $C_{36}H_{73}N_2O_4Br$: C, 63.78; H, 10.85; N, 4.13%. Found: C, 63.85; H, 10.82; N, 4.07%.

***N,N'*-Bis(12-bromododecyl)diaza-18-crown-6 [Br(CH_2)$_{12}$<N18N>(CH_2)$_{12}$Br].** A solution of 12-bromododecanoic acid (8.4 g, 30 mmol) and $SOCl_2$ (50 mL) was heated at 40 °C under N_2 for 1 h. The excess of $SOCl_2$ was removed by azeotropic distillation of benzene (3 × 50 mL). The product was used in the subsequent reaction without further purification.

To a solution of 12-bromododecanoyl chloride (8.5 g, 29 mmol) prepared as described above and a catalytic amount 4-(*N,N*-dimethylamino)pyridine in benzene (30 mL) was added a solution of diaza-18-crown-6 (3.6 g, 14 mmol) and Et_3N (2.5 mL) in benzene (100 mL) at 0 °C under N_2. The temperature of the mixture was allowed to reach ambient temperature and was then stirred for 72 h. The mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was

(57) LeMoigne, J.; Simon, J. *J. Phys. Chem.* **1980**, *84*, 170.

dissolved in CH_2Cl_2 (100 mL), washed with saturated $NaHCO_3$ (3 × 50 mL) and brine (2 × 100 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography (alumina, 10% 2-PrOH/*n*-hexane) followed by crystallization (EtOAc) gave $Br(CH_2)_{11}CO<N18N>CO(CH_2)_{11}Br$ (8.0 g, 75%) as colorless crystals, mp 70–72 °C. ^{1}H-NMR: 1.28 (pseudo-s, 24H, CH_2), 1.35–1.45 (m, 4H, $BrCH_2$), 1.59–1.68 (m, 4H, $COCH_2CH_2CH_2$), 2.31 (dt, J = 3 and 8 Hz, 4H, $COCH_2$), 3.41 (t, J = 7 Hz, 4H, Br CH_2), 3.55–3.69 (m, 24H, NCH_2 and OCH_2). IR (KBr): 2920, 2855, 1628, 1440, 1350, 1325, 1290, 1220, 1100, 1030 cm^{-1}. Mass spectrum (FAB): *m/z* 785 ($M^+ + 1$). Anal. Calcd for $C_{36}H_{68}Br_2N_2O_6$: C, 55.10; H, 8.73; N, 3.57%. Found: C, 54.89; H, 8.79; N, 4.48%.

To a solution of BH_3·THF (1.0 M, 50 mL) was added $Br(CH_2)_{11}CO<N18N>CO(CH_2)_{11}Br$ (3.6 g, 4.6 mmol) as a solid at 0 °C. The temperature of the mixture was brought to room temperature and stirred for 72 h. Water was added dropwise until the liberation of hydrogen ceased. The mixture was concentrated *in vacuo* and 6 N HBr (50 mL) was added. The aqueous solution was heated at reflux for 4 h, cooled, and adjusted to pH = 9 with 3 M NaOH. The aqueous phase was diluted with water until all of the salts dissolved and then extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layer was washed with brine (2 × 50 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography (alumina, 2% iPrOH/CH_2Cl_2) gave $Br(CH_2)_{12}<N18N>(CH_2)_{12}Br$ (2.4 g, 70%) as a colorless oil which solidified on standing. ^{1}H-NMR: 1.23 (pseudo-s, 28H), 1.35–1.48 (m, 8H), 1.78–1.85 (m, 4H), 2.45 (t, 4H), 2.74 (t, 8H), 3.38 (t, 4H), 3.55–3.65 (m, 16H). FAB-MS: *m/z* 757 $(M + 2)^+$. Anal. Calcd for $C_{36}H_{72}O_4N_2Br$: C, 57.14; H, 9.59; N, 3.70%. Found: C, 57.38; H, 9.68; N, 3.63%.

***N,N'*-Bis{12-(*N'*-dodecyldiaza-18-crown-6)-dodecyl]diaza-18-crown-6 [$C_{12}H_{25}<N18N>(CH_2)_{12}<N18N>(CH_2)_{12}<N18N>C_{12}H_{25}$, 1].** A solution of $C_{12}<N18N>C_{12}Br$ (*see above*, 460 mg, 0.68 mmol), 4,13-diaza-18-crown-6 (87 mg, 0.33 mmol), Na_2CO_3 (690 mg, 6.5 mmol), and KI (20 mg, 0.12 mmol) in a mixture of CH_3CN and PrCN (2:3 v/v, 10 mL total) was stirred at reflux for 4 d. The mixture was cooled, filtered, and concentrated *in vacuo*, and the crude mixture was chromatographed over Sephadex LH-20 (CH_3OH solvent) followed by chromatography over 5% deactivated Al_2O_3 (0–5% 2-PrOH/CH_2Cl_2). The product, **1**, was obtained (111 mg, 23%) as a white solid, mp 61–63 °C. ^{1}H-NMR: 0.85 (t, 6H), 1.23 (s, 68H), 1.44 (bt, 12H), 2.48 (t, 12H), 2.77 (t, 24H), 3.52–3.71 (m, 48H). IR: 3460 cm^{-1} (ν_{OH} of H_2O, *see analysis*), 2950 and 2890 cm^{-1} (ν_{CH}). Anal. Calcd for $C_{84}H_{170}N_6O_{12}$·H_2O: C, 68.43; H, 11.76; N, 5.70%. Found: C, 68.39; H, 11.69; N, 5.73%.

***N,N'*-Bis[11-(4'-dodecyl-4',13'-diaza-18-crown-6)-3,6,9-trioxaundec-1-yl]-4,13-diaza-18-crown-6 [$C_{12}<N18N>EOEOEOE<N18N>EOEOEOE<N18N>C_{12}$, 2].** ***N*-Dodecyl-*N'*-(3,6,9-trioxa-12-iodoundec-1-yl)-4,13-diaza-18-crown-6.** *N*-Dodecyl-4,13-diaza-18-crown-6 (900 mg, 2.08 mmol), 1,12-diiodo-3,6,9-trioxaundecane (2.59 g, 6.27 mmol), and Na_2CO_3 (13.2 g, 124 mmol) were suspended in $CH_3CH_2CH_2CN$ (80 mL) and then heated at reflux for 2 h. The mixture was cooled, filtered, concentrated *in vacuo*, and then chromatographed (Al_2O_3, CH_2Cl_2) to afford the product (612 mg, 41%) as a pale brown oil which gradually solidifed on standing. ^{1}H-NMR: 0.87 (br t, 3H), 1.26 (s, 20H), 2.79 (dd, 10H), 3.13–3.42 (m, 2H), 3.42–3.93 (m, 28H). IR (neat): 2910, 2850 cm^{-1}.

***N,N'*-Bis[12-(4'-dodecyl-4',13'-diaza-18-crown-6)-3,6,9-trioxaundec-1-yl]-4,13-diaza-18-crown-6 (2).** The above compound (1.18 g, 1.64 mmol), 4,13-diaza-18-crown-6 (215 mg, 0.82 mmol), and Na_2CO_3 (10.4 g, 98 mmol) were suspended in $CH_3CH_2CH_2CN$ (40 mL) and then stirred and heated at reflux for 5 d. The mixture was cooled, filtered, concentrated *in vacuo*, and then chromatographed (5% deactivated Al_2O_3, 0–3% CH_3OH/CH_2Cl_2, then Sephadex LH-20, CH_3OH). Compound **2** was obtained (320 mg, 13%) as a slightly brown oil. ^{1}H-NMR: 0.87 (br t, 6H), 1.26 (s, 40H), 2.45 (dd, 4H), 2.80 (br dd, 32H), 3.61 (s, 72H). IR (neat): 3450 (ν_{OH} H_2O), 2940, 2860 cm^{-1} (ν_{CH}). Anal. Calcd for $C_{76}H_{154}N_6O_{18}$·$4H_2O$: C, 60.36; H, 10.80; N, 5.55%. Found: C, 60.24; H, 10.74; N, 6.01%.

***N,N'*-Bis[[[[[5-(4'-dodecyl-4',13'-diaza-18-crown-6)-pentyl]carbonyl]piperazinyl]carbonyl]pentyl]-4,13-diaza-18-crown-6[$C_{12}<N18N>C_5CONE_2NCOC_5<N18N>C_5CONE_2NCOC_5<N18N>C_{12}$, 3].** ***N,N'*-Bis(6-bromohexanoyl)piperazine.** A solution of piperazine (2.0 g, 23.1 mmol) and Et_3N (11.7 g, 116 mmol) in dry C_6H_6 (40 mL) was added dropwise to a 5 °C solution of 6-bromohexanoyl chloride (11.2 g, 52 mmol) in dry C_6H_6 (30 mL). The solution was stirred for 2 h at 5 °C and then at ambient temperature for 2 h. Workup and crystallization from EtOAc afforded the title compound (6.08 g, 60% as a white powder, mp 61.5–63.5 °C. ^{1}H-NMR: 1.30–2.10 (m, 12H), 2.36 (t, 4H), 3.42 (t, 4H), 3.76 (m, 8H). IR (neat): 2940 and 2850 cm^{-1} (ν_{CH}), 1640 cm^{-1} ($\nu_{C=O}$, amide).

***N*-Dodecyl-*N'*-(16-bromo-6,11-dioxo-7,10-diaza-8,9-ethylenehexadecyl)-4,13-diaza-18-crown-6 ($C_{12}<N18N>C_5CONE_2NCOC_5Br$).** *N*-Dodecyl-4,13-diaza-18-crown-6 (0.4 g, 0.93 mmol) was dissolved in hot MeCN (20 mL) and Na_2CO_3 (5.0 g, 47 mmol) and KI (20 mg) and *N,N'*-bis(6-bromohexanoyl)piperazine (2.03 g, 4.65 mmol) were added. The mixture was stirred and heated at reflux for 2 d and then cooled, filtered, and concentrated *in vacuo*. The crude product was chromatographed (Al_2O_3, 0–5% CH_3OH/CH_2Cl_2, then Sephadex LH-20, CH_3OH, repeated a second time). The product was obtained as a pale yellow oil (374 mg, 51%). ^{1}H-NMR: 0.89 (br t, 3H), 1.88 (m, 32H), 2.41 (br t, 8H), 2.81 (bt, 8H), 3.06–3.88 (m, 24H). IR (neat): 3400 (ν_{OH}, H_2O), 2900, 2825 (ν_{CH}) and 1630 cm^{-1} ($\nu_{C=O}$, amide).

$C_{12}<N18N>C_5CONE_2NCOC_5<N18N>C_5CONE_2NCOC_5<N18N>C_{12}$ 3. The compound described above (374 mg, 0.47 mmol) 4,13-diaza-18-crown-6 (63 mg, 0.24 mmol), Na_2CO_3 (2.53 g, 23 mmol), and KI (20 mg, 0.12 mmol) were suspended in CH_3CN (20 mL) and stirred at reflux (under N_2) for 8 d. The mixture was cooled, filtered, reduced in volume, and chromatographed (2×) over Sephadex LH-20 (CH_3OH eluant) to afford **3** as a yellow wax (64 mg, 16%). ^{1}H-NMR: 0.86 (br t, 6H, CH_3), 1.25 (br s, 64H, $CH_2(CH_2)_{10}CH_3$ and $COCH_2(CH_2)_3CH_2$–), 2.02–2.06 (m, 20H, $COCH_2$ and CH_2N=), 2.75 (t, 24H, CH_2N of crown), 3.61 (br s, 64H, piperazine, crown CH_2's adjacent to oxygen). IR: 3420 (ν_{OH} H_2O), 2910, 2850 (ν_{CH}), 1630 cm^{-1} ($\nu_{C=O}$, amide). Anal. Calcd for $C_{92}H_{178}O_{16}N_{10}$·$4H_2O$ (see IR for evidence of water): C, 63.05; H, 10.70; N, 7.99%. Found: C, 62.95; H, 10.12; N, 7.83%.

***N,N'*-Bis{6-[[8'-[[6''-(4'''-dodecyl-4''',13'''-diaza-18-crown-6)-hexyl]oxy]anthraquinone-1'-yl]oxy]hexyl}-3,14-diaza-18-crown-6 [$C_{12}<N18N>C_6OAQOC_6<N18N>C_6OAQOC_6<N18N>C_{12}$, 4].** **1,8-Bis[*O*-(6-iodohexyl)oxy]anthraquinone (IC_6OAQOC_6I).** 1,8-Dihydroxyanthraquinone (1.06 g, 4.4 mmol) and Cs_2CO_3 (5.4 g, 16 mmol) were suspended in DMF (40 mL) and heated to 80 °C while stirring during 5 min. 1,6-Diiodohexane (7.75 g, 23 mmol) was added, and the mixture was stirred for an additional 1.5 h (80 °C). The solids were removed by filtration, the solution was concentrated *in vacuo*, and CH_2Cl_2 (200 mL) was added to the brown, oily residue. The solution was washed (H_2O, 5×), dried over Na_2SO_4, and concentrated *in vacuo*. The residual oil was chromatographed over SiO_2 (CH_2Cl_2). The first eluant fraction contained monoalkylated compound IC_6OAQOH. Evaporation of the solvent gave a yellow solid that was crystallized from CH_2Cl_2/EtOH (3:10 v/v) to afford IC_6OAQOC_6I (1.5 g, 39% yield) as yellow needles, mp 57.0–58 °C. ^{1}H-NMR: 1.20–2.10 (m, 16H), 3.23 (t, 4H), 4.13 (t, 4H), 7.26 (dd, 2H), 7.60 (t, 2H), 7.83 (dd, 2H). IR: 2940, 2860 (ν_{CH}), 1670 ($\nu_{C=O}$), 1590 cm^{-1} ($\nu_{C=C}$). The monoalkylated compound was also obtained (110 mg, 19%) as orange crystals, mp 105–107 °C.

***N,N'*-Bis{6-[[8'-[(6''-iodohexyl)oxy]anthraquinone-1'-yl]oxy]hexyl}-4,13-diaza-18-crown-6 ($IC_6OAQOC_6<N18N>C_6OAQOC_6I$].** $CsCO_3$ (12.6 g, 65 mmol) was added to a solution of 4,13-diaza-18-crown-6 (0.35 g, 1.3 mmol) in hot $CH_3CH_2CH_2CN$ (30 mL). 1,8-Bis[*O*(6-iodohexyl)oxy]anthraquinone (4.5 g, 7.2 mmol) was dissolved in minimum PrCN and added to the above, and the mixture was heated and stirred at 80 °C for 4 h. A yellow oil was obtained after workup which was purified by column chromatography (Al_2O_3, CH_2Cl_2 eluant). The product was obtained (0.61 g, 35%) as a yellow oil. ^{1}H-NMR: 1.10–2.13 (bm, 32H), 2.55 (bt, 4H), 2.80 (bt, 8H), 3.23 (t, 4H), 3.60 (bs, 16H), 4.14 (t, 8H), 7.26 (dd, 4H), 7.60 (t, 4H), 7.83 (dd, 4H). IR (neat): 3420 (ν_{OH} H_2O), 2950, 2870 (ν_{CH}), 1665 ($\nu_{C=O}$), and 1585 cm^{-1} (ν_{C-C} aromatic ring).

$C_{12}<N18N>C_6OAQOC_6<N18N>C_6OAQOC_6<N18N>C_{12}$ (4). The diiodide prepared above (0.71 g, 0.54 mmol) and *N*-dodecyl-4,-13-diaza-18-crown-6 (0.81 g, 1.88 mmol) were dissolved in hot PrCN (30 mL). Cs_2CO_3 (5 g, 47 mmol) was added, and the mixture was stirred for 21 h at 80 °C. The mixture was cooled, filtered, and concentrated *in vacuo*, and the crude product was chromatographed (2×, Al_2O_3, eluant CH_2Cl_2 → 2% CH_3OH/CH_2Cl_2). The product was isolated (70

mg, 10%) as a dark yellow oil. ^{1}H-NMR: 0.88 (bt, 6H), 1.26 (s, 40H), 2.10 (bm, 32H), 2.50 (bt, 12H), 2.78 (t, 24H), 3.62 (bs, 48H), 4.15 (t, 8H), 7.26 (dd, 4H), 7.60 (t, 4H), 7.83 (dd, 4H). IR (neat): 3420 (ν_{OH} H_2O), 2950, 2870 (ν_{CH}), 1665 ($\nu_{C=O}$), 1585 cm^{-1} ($\nu_{C=C}$). Anal. Calcd for $C_{112}H_{182}O_{20}N_6 \cdot 5H_2O$: C, 66.51; H, 9.56; N, 4.16%. Found: C, 66.30; H, 9.70; N, 3.88%.

N,N'-Bis[12-(N'-dodecyldiaza-18-crown-6)-dodecyl]diaza-15-crown-5 [$C_{12}H_{25}$<N18N>$(CH_2)_{12}$<N15N>$(CH_2)_{12}$<N18N>$C_{12}H_{25}$, 5]. A solution of *N*-dodecyl-*N'*-(12-bromododecyl)diaza-18-crown-6 (BrC_{12}<N18N>C_{12}, see above, 341 mg, 0.50 mmol), 4,10-diaza-15-crown-5 (53 mg, 0.24 mmol), Na_2CO_3 (610 mg, 5.8 mmol), and KI (2 mg, 0.01 mmol) in PrCN (5 mL) was heated at reflux for 24 h. The mixture was cooled and filtered, and the filtrate was concentrated *in vacuo*. Column chromatography (alumina, 0–2% 2-PrOH/CH_2Cl_2, then Sephadex LH-20, MeOH) gave **5** (74 mg, 21%) as a slightly yellow oil which solidified on standing. ^{1}H-NMR: 0.88 (t, J = 7 Hz, 6H), 1.25 (pseudo-s, 68H), 1.40–1.52 (br, 12H), 2.49–2.59 (m, 12H), 2.75–2.88 (m, 24H), 3.55–3.72 (m, 44H). IR (neat): 3400, 2920, 2850, 1460, 1350, 1295, 1120 cm^{-1}. Mass spectrum (FAB): *m/z* 1412 (M^+). Electrospray MS: $[M + Na]^+$ = 1434.6, $[M + 2Na]^{2+}$ = 720.7, $[M + H + Na]^{2+}$ = 717.8, $[M + 2H]^{2+}$ = 707.0. Anal. Calcd for $C_{82}H_{172}N_6O_{14} \cdot 3H_2O$: C, 67.17; H, 11.82; N, 5.73%. Found: C, 66.82; H, 11.38; N, 5.46%.

O,O'-Bis[12-(N'-dodecyldiaza-18-crown-6)dodecyl]tris(ethylene glycol) [$C_{12}H_{25}$<N18N>$(CH_2)_{12}$OEOEOEO$(CH_2)_{12}$<N18N>$C_{12}H_{25}$, 6].

12-(Bromododecyl)-3,4-dihydro-2*H*-pyran $BrC_{12}OTHP$. A solution of 3,4-dihydro-2*H*-pyran (1.126 g, 13.3 mmol) in CH_2Cl_2 (7.5 mL) was added dropwise to a solution of 12-bromododecanol (3.0 g, 11.3 mmol) and *p*-toluensulfonic acid monohydrate (0.221 g, 1.16 mmol) in CH_2Cl_2 (45 mL) at 0 °C. After addition, the mixture was stirred at 0 °C for 3.5 h, poured into a saturated aqueous solution of $NaHCO_3$, and extracted with CH_2Cl_2 (50 mL). The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (4 × 25 mL) and brine, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography (silica gel, CH_2Cl_2) gave the THP derivative as a colorless oil (3.31 g, 84%). NMR: 1.27 (pseudo-s, 16H), 1.38–1.54 (m, 2H), 1.54–1.61 (m, 4H), 1.69–1.74 (m, 1H), 1.82–1.88 (m, 3H), 3.35–3.42 (m, 3H), 3.48–3.51 (m, 1H), 3.70–3.74 (m, 1H), 3.73–3.89 (m, 1H), 4.58 (pseudo-s, 1H). Mass spectrum DCI (CH_4): 349. Anal. Calcd for $C_{17}H_{33}BrO_2$: C, 58.45; H, 9.52%. Found: C 58.43; H 9.47%.

$THPOC_{12}OEOEOEOC_{12}OTHP$. To a solution of $BrC_{12}OTHP$ ether (8.8 g, 28 mmol), tris(ethylene glycol) (2.1 g, 14 mmol), and tetrabutylammonium sulfate (0.1 g 0.3 mmol) was added dropwise 50% NaOH (0.3 mol) at 70 °C. The two-phase mixture was stirred vigorously for 24 h. The reaction was cooled, and CH_2Cl_2 was added. The organic layer was washed with water, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography (1:1 EtOAc/*n*-hexane, silica gel) gave the disubstituted compound (1.565 g, 17%) as a tranparent oil. NMR: 1.26 (pseudo-s, 36H), 1.47–1.63 (m, 12H), 1.65–1.76 (m, 2H), 1.78–1.90 (m, 2H), 3.35–3.55 (m, 4H), 3.44 (t, 4H), 3.55–3.78 (m, 14H), 3.81–3.91 (m, 2H), 4.58 (s, 2H). Mass spectrum: 687 (M^+). Anal. Calcd for $C_{40}H_{78}O_8$: C, 69.93; H, 11.44%. Found, C, 70.06; H, 11.39%.

Preparation of the Alcohol, $HOC_{12}OEOEOEOC_{12}OH$. A solution of the bis(THP) derivative (1.1 g, 1.6 mmol) and *p*-toluensulfonic acid monohydrate (0.4 g, 2.1 mmol) in 30% H_2O/MeOH (30 mL) was heated at 60 °C for 8 h. The reaction was cooled and poured into ice water (50 mL). The precipitate was filtered. Chromatography (silica gel, 5% MeOH/CH_2Cl_2) gave two products. The first fraction was crystallized from EtOAc to give the mono(THP) ether (0.156 g, 16%) as colorless crystals. The second fraction was crystallized from EtOAc to give the dihydroxy compound (0.320 g, 37%) as colorless crystals. NMR: 1.28 (s, 32H), 1.52–1.66 (m, 8H), 3.46 (t, 4H), 3.56–3.74 (m, 16H); no hydroxyl proton was observed in this case. MS (DCI, CH_4): 520 $(M + 2)^+$. Anal. Calcd for $C_{30}H_{62}O_6$: C, 69.45; H, 12.04%. Found: C, 69.44; H, 11.99%.

Preparation of the Ditosylate, $TsOC_{12}OEOEOEOC_{12}OTs$. The diol described above (1.8 g, 3.2 mmol) was dissolved in $CHCl_3$ (15 mL) and cooled in an ice bath (0 °C). Pyridine (1.5 g, 18.0 mL) was then added, followed by the addition of *p*-toluenesulfonyl chloride (6.2 g, 32.5 mmol) in small portions, with stirring. The reaction was brought to ambient temperature and stirred for 7 d. Chloroform (50 mL) and water (10 mL) were added, and the organic layer was washed with 2 N HCl (50 mL), 5% $NaHCO_3$ (3 × 25 mL), and brine (2 × 50 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography (silica gel, 2% MeOH/CH_2Cl_2 and 20%EtOAc/*n*-hexane) gave three major products. The first fraction gave the chloro/tosylate ($TsOC_{12}$-$OEOEOEOC_{12}OCl$, 0.581 g, 26%) as a transparent oil which solidified on standing. Anal. Calcd for $C_{37}H_{67}O_7ClS$: C, 64.27; H, 9.77%. Found: C 64.22; H, 9.82%.

The second fraction was the desired ditosylate ($TsOC_{12}OEOEOEOC_{12}$-OTs, 1.483 g, 16%) as a transparent oil which solidified on standing, mp 61–63 °C. NMR: 1.21–1.28 (s, 32H), 1.57 (t, 4H), 1.63 (t, 4H), 2.45 (s, 3H), 3.43–3.46 (t, 4H), 3.56–3.66 (m, 12H),7.33–7.35 (d, 2H), 7.78–7.80 (d, 2H). Anal. Calcd for $C_{44}H_{74}O_{10}S_2$: C, 63.89; H, 9.02%. Found: C, 63.97; H, 9.07%.

The third fraction was the monotosylate ($HOC_{12}OEOEOEOC_{12}OTs$, 0.028 g, 1.3%) as a transparent oil, which solidified on standing. Anal. Calcd for $C_{37}H_{68}O_8S$: C, 66.03; H, 10.18%. Found: C, 65.84; H, 10.20%.

O,O'-Bis[12-(N'-dodecyldiaza-18-crown-6)dodecyl]tris(ethylene glycol) [$C_{12}H_{25}$<N18N>$(CH_2)_{12}$OEOEOEO$(CH_2)_{12}$<N18N>$C_{12}H_{25}$, 6]. A solution of C_{12}<N18N>H (1.6 g, 3.7 mmol), the tosyl ether (0.8 g, 1 mmol), and Na_2CO_3 (2.7 g, 25.5 mmol) in CH_3CN (15 mL) was heated under reflux for 72 h. The solution was cooled and filtered. The filtrate was concentrated *in vacuo*. Column chromatography (alumina, 2% *i*-PrOH in CH_2Cl_2) gave two major products. The first fraction gave the monosubstituted compound (0.24 g, 7%) as a slightly yellow oil that solidified on standing. The second fraction gave, after crystallization from EtOAc, **6** (451 mg, 35%) as a colorless solid, mp 51–53 °C. NMR: 0.881 (t, 6H), 1.25–1.43 (s, 34H), 1.44 (broad-s, 8H), 1.58 (m, 4H), 2.48 (broad-s, 8H), 2.79 (s, 16H), 3.44 (t, 4H), 3.56–3.60 (m, 44H). Anal. Calcd for $C_{78}H_{158}N_4O_{12}$: C, 69.70; H, 11.85; N, 4.17%. Found: C, 69.61; H, 11.82; N, 4.14%.

N,N'-Bis[12-(N'-benzyldiaza-18-crown-6)dodecyl]diaza-18-crown-6 [$PhCH_2$<N18N>$(CH_2)_{12}$<N18N>$(CH_2)_{12}$<N18N>CH_2Ph, 7].

$PhCH_2$<N18N>H. A solution of diaza-18-crown-6 (3.6 g, 13.7 mmol), benzyl bromide (2.1 g, 12.3 mmol), Na_2CO_3 (14 g, 132 mmol), and KI (50 mg, 0.3 mmol) in butyronitrile (300 mL) was heated under reflux for 24 h. The reaction was cooled and filtered, and the filtrate was concentrated *in vacuo*. Column chromatography (alumina, 2% *i*-PrOH in CH_2Cl_2) gave two major products. The first fraction gave, after crystallization from hexane, $PhCH_2$<N18N>CH_2Ph (1.07 g, 18%) as colorless needles.[25] The second fraction consisted of 1.58 g (33%) of $PhCH_2$<N18N>H as a slightly yellow oil which solidified on standing.

A solution of BrC_{12}<N18N>$C_{12}Br$ (*see above*, 1.7 g, 2.0. mmol), *N*-benzyl-4,13-diaza-18-crown-6 (1.6 g, 4.5 mmol), Na_2CO_3 (4.8 g, 45 mmol), and KI (2 mg, 0.01 mmol) was heated at reflux for 24 h. The mixture was cooled and filtered, and the filtrate was concentrated *in vacuo*. Column chromatography (alumina, 2–5% *i*-PrOH/CH_2Cl_2) gave **7** (1.4 g, 48%) as a slightly yellow oil which solidified on standing, mp 53–55 °C. NMR: 1.25 (pseudo-s, 32H), 1.38–1.48 (bs), 2.48 (dt, J = 3 and 7 Hz, 8H), 2.74–2.84 (m, 24H), 3.59 (pseudo-s, 48H), 3.62 (s, 4H), 7.15–7.30 (m, 10H). IR (neat): 2900, 2830, 1445, 1340, 1285, 1250, 1120, 1070 cm^{-1}. Anal. Calcd for $C_{74}H_{134}N_6O_{12}$: C, 68.38; H, 10.39; N, 6.47%. Found: C, 68.35; H, 10.36; N, 6.41%.

N,N'-Bis[12-(diaza-18-crown-6)dodecyl]diaza-18-crown-6 [<N18N>$(CH_2)_{12}$<N18N>$(CH_2)_{12}$<N18N>, 8]. A solution of **7** ($PhCH_2$<N18N>C_{12}<N18N>C_{12}<N18N>CH_2Ph, 1.0 g, 0.8 mmol) and Pd/C (10%, Degussa type, 0.11 g) in EtOH (20 mL) was shaken in a Parr series 3900 hydrogenation apparatus at 70 psi H_2 pressure and 25 °C for 72 h. The mixture was filtered through a pad of Celite and concentrated *in vacuo*. Crystallization from EtOAc gave **8** (0.67 g, 92%) as a colorless solid: mp 63–65 °C. ^{1}H-NMR: 1.25 (pseudo-s, 32H), 1.38–1.48 (br, 8H), 2.45–2.52 (m, 8H), 2.75–2.83 (m, 24H), 4.57–3.63 (m, 48H). Anal. Calcd for $C_{60}H_{122}N_6O_{12}$: C, 64.36; H, 10.88; N, 7.51%. Found: C, 64.13; H, 10.96; N, 7.48%. FAB-MS: *m/z* 1119 $(M + 1)^+$.

N,N'-Bis[12-(aza-18-crown-6)dodecyl]diaza-18-crown-6 [<18N>$(CH_2)_{12}$<N18N>$(CH_2)_{12}$<N18>, 9]. A solution of *N*-(12-bromododecyl)aza-18-crown-6 (see above, 0.142 g, 2.8 mmol), 4,13-diaza-18-crown-6 (0.37 g, 1.4 mmol), Na_2CO_3 (2.96 g, 28 mmol equiv), and

KI (30 mg) in PrCN (35 mL) was heated at reflux for 5 h. The solution was cooled, filtered, and concentrated *in vacuo*. The oily residue (3.25 g) was dissolved in 100 mL of CH_2Cl_2 and washed with water (100 mL, 4 × 25), the organic layer was dried over $MgSO_4$, and the solvent was evaporated *in vacuo*. The pale yellow oil thus obtained (2.25 g) was chromatographed over alumina (10% 2-PrOH in hexanes) to afford a waxy solid. This solid was chromatographed again over alumina (2% 2-PrOH/CH_2Cl_2, then MeOH/CH_3CN) and chromatographed a third time (alumina, 5% MeOH/CH_2Cl_2) to afford **9** (0.23 g, 15%) as a waxy, somewhat unstable, slightly yellow solid. ^{1}H-NMR: 1.25 (s, 32H), 1.45 (bs, 8H), 2.50 (t, 8H), 2.80 (t, 16H), 3.65 (m, 56H). ^{13}C-NMR: 26.56, 27.32, 29.43, 53.60, 55.60, 68.57, 69.20, 70.17, 70.60 ppm. Electrospray mass spectrum: $(M + Na^+) = 1144$, $(MW + 2H)^{2+} = 561.5$, $(MW + H^+ + Na)^{2+} = 572.5$, $(M + 2Na)^{2+} = 583.4$. Anal. Calcd for $C_{60}H_{120}O_{14}N_4$: C, 64.25; H, 10.78; N, 4.99%. Found: C, 63.61; H, 10.55; N, 4.93%.

CholOE<N18N>C_{12}<N18N>C_{12}<N18N>EOChol (10). A solution of 5-α-cholesten-3-one (1.0 g, 2.6 mmol), ethylene glycol (4 mL, 72 mmol), and *p*-toluenesulfonic acid (0.1 g, 0.6 mmol) in C_6H_6 (60 mL) was heated at reflux for 24 h. The water formed during the reaction was removed by a Dean–Stark trap. The mixture was cooled, washed with saturatedNaHCO$_3$ (2 × 50 mL) and water (2 × 50 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Recrystallization from *n*-hexane gave the ketal "**A**" (0.961 g, 85%) as colorless crystals (mp 115–117 °C). ^{1}H-NMR: 0.62 (s, 3H), 0.78 (s, 3H), 0.83 (dd, 3H), 0.86 (s, 3H), 0.89 (s, 3H), 0.60–2.00 (m, 31H), 3.91 (s, 4H). IR: 2930, 2865, 1440, 1365, 1335 (s), 1300, 1280, 1255, 1230, 1255, 1230, 1190, 1175, 1135, 1100, 1070, 1040, 1020 cm^{-1}. Mass spectrum: *m/z* 430 (M^+). Anal. Calcd for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70%. Found, C, 80.94: H, 11.70%.

To a stirred solution of BH_3·THF (1.0 M, 2.4 mL) was added **A** (0.694 g, 1.6 mmol) as a solid at 0 °C. The reaction was stirred at ambient temperature for 7 d. Water was then added dropwise until H_2 evolution ceased, and then enough water was added to complete hydrolysis and dissolve the salts. The aqueous solution was extracted (Et_2O), and the combined extracts were washed with water, dried ($MgSO_4$), and evaporated. MeOH was added, and the mixture was evaporated again. The crude residue was chromatographed (silica gel, 2% MeOH/CH_2Cl_2) to give two major products. The first fraction (**B**, 0.122 g, 18%) was a transparent oil which solidified on standing (mp 73–73 °C). The second fraction gave a solid (**C**) which was crystallized from EtOAc to give C ($HOCH_2CH_2O$-3-cholestanyl, 0.358 g, 51%) as colorless crystals (mp 148–150 °C). NMR: 0.60–2.0 (m, 46H), 3.25 (m, 1H), 3.57 (t, 2H), 3.71 (t, 2H). Anal. Calcd for $C_{29}H_{52}O_2$: C, 80.49; H, 12.11%. Found: C, 80.24; H, 12.06%.

To a stirred solution of $HOCH_2CH_2O$-3-cholestanyl (**C**, 5.7 g, 13 mmol) in pyridine (20 mL) was added solid *p*-toluensulfonyl chloride (2.6 g, 14 mmol) at 0 °C. The reaction was brought to room temperature and stirred for 4 h. The mixture was poured into water (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with 2 N HCl (2 × 25 mL), 5% $NaHCO_3$ (2 × 25 mL), and brine (2 × 50 mL) and then concentrated *in vacuo*. The residue was extracted with EtOAc. Filtration produced a solid material that was washed with EtOAc to give pyridinium salt **D** (0.042 g, 0.5%) as colorless crystals (mp 210–212 °C). The filtrate was concentrated *in vacuo*. Column chromatography gave two products. The first fraction was cholOCH$_2$CH$_2$Cl (0.103 g, 2%). The second fraction, obtained by recrystallization from EtOAc, was the desired tosylate (cholOCH$_2$CH$_2$OTs, **F**, 5.787 g, 75%) as colorless crystals (mp 91–93 °C). NMR: 0.6–2.0 (m, 46H), 2.45 (s, 3H), 3.16 (t, 2H), 4.14 (t, 2H), 7.32–7.34 (dd, 2H), 7.79–7.81 (dd, 2H). Anal. Calcd for $C_{36}H_{58}O_4S$: C, 73.67; H, 9.96%. Found: C, 73.74; H, 9.96%.

A solution of compound **8** (see above, 0.476 g, 0.4 mmol), **F** (0.751 g, 1.3 mmol), and Na_2CO_3 (0.910 g, 8.9 mmol) in THF (10 mL) was heated at reflux for 96 h. The reaction mixture was cooled, filtered, and concentrated *in vacuo*. Column chromatography (alumina, 0–2% *i*-PrOH:CH_2Cl_2), followed by recrystallization from EtOAc, gave **10** (0.188 g, 24%) as a colorless solid (71–72 °C). NMR: 0.6–2.0 (m, 92H), 1.25 (s, 32H), 1.32 (m, 4H), 1.43 (m, 4H), 2.47 (t, 8H), 2.75 (m, 20H), 2.84 (t, 8H), 3.2 (m, 2H), 3.54 (t, 4H), 3.58–3.61 (m, 48H). Anal. Calcd for $C_{118}H_{222}N_6O_{14}$: C, 72.72; H, 11.48; N, 4.31%. Found: C, 72.58; H, 11.44; N, 4.25%.

CholOCOCH$_2$<N18N>C_{12}<N18N>C_{12}<N18N>CH$_2$COOChol (11). To a solution of chloroacetyl chloride (8.7 g, 77 mmol) and a catalytic amount of 4-(dimethylamino)pyridine in C_6H_6 (80 mL) at 5 °C was added a solution of dihydrocholesterol (25 g, 64 mmol) and Et_3N (6.8 g, 68 mmol) in C_6H_6 (400 mL). The reaction temperature was maintained at 0–5 °C during the addition and then stirred at ambient temperature for 24 h. The mixture was filtered, the filtrate was concentrated *in vacuo*, and the crude material was crystallized from EtOH to give **G**, ClCH$_2$COO-3-cholesteryl, (23.3 g, 78%) as white crystals (mp 184–185 °C).

Preparation of CholOOCCH$_2$<N18N> (H). A solution of 4,13-diaza-18-crown-6 (2.0 g, 7.6 mmol), **G** (3.4 g, 7.3 mmol), Na_2CO_3 (8.7 g, 82 mmol), and KI (0.05 g, 0.3 mmol) in PrCN (200 mL) was heated at reflux for 15 h. The reaction was cooled and filtered, and the filtrate was concentrated *in vacuo*. Column chromatography (3% *i*-PrOH/CH_2Cl_2) over a short column of alumina afforded two products. The first fraction was recrystallized from EtOAc to give the disubstituted compound cholOOCCH$_2$<N18N>CH$_2$COOchol (1.7 g, 20%) as colorless crystals (mp 120–121 °C). Mass spectrum: *m/z* 1119 [$(M + 1)^+$]. Anal. Calcd for $C_{70}H_{122}N_2O_8$: C, 75.09; H, 10.98; N, 2.50%. Found: C, 74.96; H, 10.92; N, 2.48%. The second fraction was crystallized from EtOAc to give **H**, cholOOCCH$_2$<N18N> (1.4 g, 27%) as a colorless solid (mp 97–98 °C). ^{1}H-NMR: 0.64 (s, 3H), 0.81 (s, 3H), 0.86 (dd, 3H), 0.89 (s, 3H), 0.90 (s, 3H), 1.91–2.00 (m, 31H), 2.85 (s, 1H), 2.96 (t, 8H), 3.49 (s, 2H), 3.56–3.72 (m, 16H), 4.65–4.79 (m, 1H). DCI-MS (CH_4): *m/z* 692 ($M^+ + 1$). Anal. Calcd for $C_{41}H_{74}N_2O_6$: C, 71.26; H, 10.79; N, 4.05%. Found: C, 71.51; H, 10.74; N, 3.83%

Preparation of CholOOCCH$_2$<N18N>C_{12}Br (I). A solution of **H** (1.5 g, 2.4 mmol), 1,12-dibromododecane (2.4 g, 7.3 mmol), Na_2CO_3 (7.6 g, 72 mmol), and KI (0.03 g, 0.2 mmol) in PrCN (10 mL) was heated at reflux for 1.5 h. The mixture was cooled and filtered, and the filtrate was concentrated *in vacuo*. Column chromatography (alumina, 10% *i*-PrOH-CH_2Cl_2) gave **I** (0.45 g, 20%) as a transparent oil which solidified on standing (mp 54–55 °C). ^{1}H-NMR: 0.64 (s, 3H), 0.81 (s, 3H), 0.86 (dd, 3H), 0.89 (s, 3H), 0.90 (s, 3H), 1.91–2.00 (m, 31H), 2.48 (t, 2H), 3.46 (s, 2H), 3.53 (t, 2H), 3.57–3.63 (m, 16H). MS (FAB): *m/z* 939 ($M^+ + 1$). Anal. Calcd for $C_{53}H_{97}BrN_2O_6$: C, 67.85; H, 10.42; N, 2.99%. Found: C, 67.91; H, 10.34; N, 2.95%.

Preparation of CholOCOCH$_2$<N18N>C_{12}<N18N>C_{12}<N18N>CH$_2$COOChol (11). A solution of I (0.69 g, 0.7 mmol), diaza-18-crown-6 (0.094 g, 0.4 mmol), Na_2CO_3 (0.787 g, 7 mmol), and KI (2 mg, 0.01 mmol) in PrCN (8mL) was heated at reflux for 12 h. The mixture was cooled, filtered, and concentrated *in vacuo*. The crude mixture was purified by column chromatography (alumina, 2–5% *i*-PrOH/CH_2Cl_2) followed by recrystallization from EtOAc to give compound **12** (80 mg, 11%) as a colorless solid (mp 71–73 °C). NMR: 0.6–2.0 (m, 92H), 1.25 (s, 32H), 1.34 (m, 4H), 1.45 (m, 4H), 2.49 (broad-s, 8H), 2.78 (broad-s, 16H), 2.95 (t, 8H), 3.46 (s, 4H), 3.61 (s, 48H), 4.72 (m, 2H). Anal. Calcd for $C_{118}H_{218}N_6O_{16}$: C, 71.69; H, 11.11; N 4.25%. Found: C, 71.47; H, 11.16; N, 4.21%.

***N,N'*-Didodecyldiaza-18-crown-6 (12)** was prepared as described previously.[24]

***N,N'*-Dibenzyldiaza-18-crown-6 (13)** was prepared as described previously.[25]

Synthesis of C_{12}<N18N>C_{12}<N18N>C_{12} (14). Compound **14** was obtained from the reaction of C12<N18N>H and 1,12-dibromododecane. A solution of C12<N18N>H (0.885 g, 2.06 mmol), 1,12-dibromododecane (0.676 g, 2.06 mmol), Na_2CO_3 (4.37 g, 41.1 mmol), KI (0.046 g, 0.28 mmol), and PrCN (50 mL) was set to reflux for 2.5 h. After column chromatography (alumina, 10% *i*-PrOH/hexane, then alumina, 5%MeOH/CH_2Cl_2) **14** was isolated as a waxy solid (0.025 g). ^{1}H-NMR 0.9 (t, 6H), 1.35–1.45 (s, b, 59H), 2.5 (t, 8H), 3.6 (t, 32H). ^{13}C-NMR: 14.11, 22.68, 27.47, 29.34, 29.63, 31.919, 53.818, 55.901, 69.736, 70.711. Electrospray MS: 536.6 $(MW + 2Na^+)^{2+}$, 1050 $(MW + Na^+)^+$.

Acknowledgment. We thank the NIH for a grant (GM-36262) that supported this work. We are also grateful to Drs. D. André d'Avignon and Jeff Kao of the Washington University NMR facility for advice and assistance.

JA944004D