Synthetic Transmembrane Channels: Functional Characterization Using Solubility Calculations, Transport Studies, and Substituent Effects

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Abstract: Dibenzyldiaza-18-crown-6 ($PhCH_2\langle N18N\rangle CH_2Ph$, **1**), di(dodecyldiaza-18-crown-6 ($C_{12}H_{25}\langle N18N\rangle C_{12}H_{25}$, **2**), $HOOC(CH_2)_{11}\langle N18N\rangle(CH_2)_{11}COOH$ (**3**), $\langle 18N\rangle(CH_2)_{12}\langle N18N\rangle(CH_2)_{12}\langle N18\rangle$ (**4**), $\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle$ (**5**), $C_{12}H_{25}\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle C_{12}H_{25}$ (**6**), $PhCH_2\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle CH_2Ph$ (**7**), 4-(p-$MeOC_6H_4CH_2\langle N18N\rangle C_{12})_2\langle N18N\rangle$ (**8**), (p-$NO_2C_6H_4CH_2\langle N18N\rangle C_{12})_2\langle N18N\rangle$ (**9**), and [chol-O-$(CH_2)_2\langle N18N\rangle$-$C_{12}]_2\langle N18N\rangle$ (**10**) were studied. Octanol−water partition coefficients were determined for **1**, **6**, **7**, **8**, **10**, and 3-cholestanyl-$OCOCH_2\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle COCH_2O$-3-cholestanyl (**11**). All were found to favor octanol, and by implication the phospholipid bilayer membrane, by at least 10^4-fold. Transport of Na^+ was assessed in both a phospholipid bilayer and in a bulk $CHCl_3$ membrane phase. Addition of ionophores to the latter was found in some cases to strongly enhance $CHCl_3$ phase hydration. An attempt to correlate transport rates determined in the two systems failed, suggesting that the carrier mechanism, required in the $CHCl_3$ phase, does not apply to the tris(macrocyclic) compounds in the bilayer. Sodium transport rates were also assessed for these compounds by using the bilayer clamp technique. Although Na^+ flux rates thus determined for **7**−**9** in the phospholipid bilayer did not correlate with results obtained by the ^{23}Na-NMR technique, the traces are similar to those obtained with protein channels, further supporting the function of tris(macrocycle)s as channel formers.

Introduction

One of the most intensely studied areas of modern biology concerns the function and mechanism of transmembrane ion channels.[1] Numerous proteins span bilayer membranes and effect the transport of cations, anions, and molecules across the boundaries of cells and organelles. During the past decade, an enormous amount of information has accumulated on which proteins are responsible for the transport of ionic or molecular species[2] but physical chemical details of transport and selectivity remain largely obscure.[3]

This ardent biological interest has fostered attempts to develop chemical models designed to help to understand the structural and kinetic requirements of cation transport. The various model systems reflect unique approaches[4] to the design and synthetic problems. Synthetic peptides have been prepared that span membranes and associate into "bundles" capable of ionophoretic activity.[5] A number of "half-channel elements," perhaps inspired by the natural ionophores gramicidin[6] or amphotericin,[7] have been prepared. In these cases, the amphiphilic monomers are presumably in flux within their respective bilayer leaflets (lateral relaxation) and conduction occurs when two elements occupy positions opposite to each other. The monomer systems of Tabushi,[8] Menger,[9] Kobuke,[10] and Regen[11] are representative of this approach.

A number of these compounds have been designed as "tunnel-like" structures intended to emulate the structure of bacteriorhodopsin,[12] which forms a pore from seven transmembrane helical protein segments. Notable examples of this include the pioneering designs of Fyles[13] and Lehn.[14] Recently Ghadiri and co-workers[15] have constructed cyclic, peptidic "nanotubes" that conduct cations. Voyer and Robitaille[16] have used an α-helical amino acid backbone to which are appended appropriately

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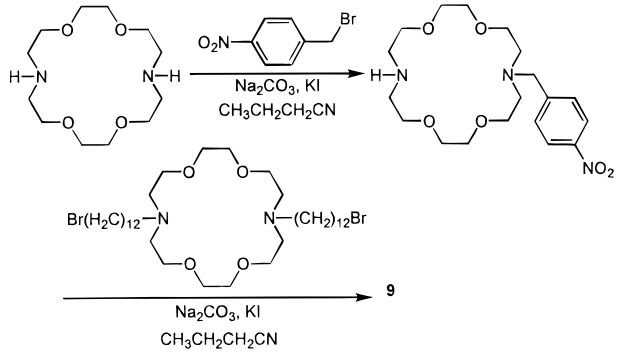
positioned crown ethers so that a "tunnel" of macrocycles is available to the transmigrating cation.

Our own approach involved the use of crown ether compounds to function both as "head groups" and as a cation relay positioned within the phospholipid bilayer.[17] The most important feature of our design is the use of a "flexible framework" which possesses what we believe are the essential elements for function. The system still has enough flexibility to adapt to unanticipated requirements. This approach is based upon a recognition that the most rigid and stable structures often lack the dynamics required to achieve the desired function. We have recently reported a number of structural variations on our original design, and we have correlated these changes with differences in sodium cation flux.[18] The emergence of "single-strand" channels such as the Isk protein[19] suggests that nontubular, membrane-spanning monomers are an important natural alternative to bacteriorhodopsin or the acetylcholine receptor. The "7-TM" proteins clearly form a tunnel-like physical arrangement in the bilayer that is impossible with shorter peptides. Even so, certain single-strand compounds exhibit ion channel function. Although many channel compounds have been characterized in remarkable detail, the mechanism of transport for either multipass proteins or single-strand peptides remains unknown. It is therefore unclear whether the mechanism is the same or different.

In the present work, we have undertaken a three-part study in an effort to further characterize our synthetic channel model system. First, we have measured and/or calculated octanol−water partition coefficients for several simple crown ethers and channel model compounds. It is possible that the tris(macrocyclic) amphiphiles transport cations to the same extent and the differences observed in activity reflect only differential solubility. Second, we have examined transport rates for channel formers and known carriers in both lipid bilayers and in a bulk organic membrane system. Transport in the latter system cannot occur by a channel mechanism because the membrane thickness is, by molecular standards, enormous. Assessment of transport rates using the two membrane systems and comparisons among compounds shed light on the function of these synthetic ionophores. Third, we have used the bilayer clamp technique to assess the Na^+ flux rates for three benzyl-substituted tris(macrocycle) derivatives having different substituents in the benzyl group's 4-position. The single-channel conductivity data were acquired by using an asolectin phospholipid bilayer membrane and a patch clamp amplifier equipped with a bilayer head stage. The conductivity traces are identical in most respects to those observed for protein channels.

Scheme 1



Results and Discussion

Compounds Studied. Several of the compounds used in the present study possess somewhat complicated structures. We use here a shorthand system that we have employed previously[20] to describe these and related systems. A crown is represented using angle brackets and a number to indicate ring size. Thus, ⟨18⟩ is shorthand for 18-crown-6. Aza-15-crown-5 can be represented as ⟨N15⟩ or ⟨15N⟩ and hydrogen (normally understood) or an additional abbreviation may be added to indicate the presence of a substituent, *e.g.* ⟨15N⟩H or ⟨15N⟩R. 4,13-Diaza-18-crown-6, the most common macrocycle used in this study, is represented as ⟨N18N⟩.

Gramicidin, a naturally occurring channel former, and valinomycin, a mitochondrial potassium carrier,[21] were obtained commercially. Dibenzyldiaza-18-crown-6 ($PhCH_2$⟨N18N⟩CH_2Ph, **1**), and didodecyldiaza-18-crown-6 ($C_{12}H_{25}$⟨N18N⟩$C_{12}H_{25}$, **2**), ⟨18N⟩$(CH_2)_{12}$⟨N18N⟩$(CH_2)_{12}$⟨N18⟩ (**4**), ⟨N18N⟩$(CH_2)_{12}$⟨N18N⟩$(CH_2)_{12}$⟨N18N⟩ (**5**), $C_{12}H_{25}$⟨N18N⟩$(CH_2)_{12}$⟨N18N⟩$(CH_2)_{12}$⟨N18N⟩$C_{12}H_{25}$ (**6**), [$PhCH_2$⟨N18N⟩$(CH_2)_{12}$]$_2$⟨N18N⟩ (**7**), [chol-O-$(CH_2)_2$⟨N18N⟩C_{12}]$_2$⟨N18N⟩ (**10**), and [chol-O-CH_2CO⟨N18N⟩C_{12}]$_2$⟨N18N⟩ (**11**) have been previously reported.[17] The carboxy-terminated derivative of **2**, $HOOC(CH_2)_{11}$⟨N18N⟩$(CH_2)_{11}COOH$ (**3**), and the *p*-methoxy and *p*-nitro derivatives of **7**, *i.e.*, **8** and **9**, respectively, are described in the Experimental Section.

Synthetic Access. The tris(macrocycle)s described herein were prepared by a sequence similar to that previously reported.[17] Thus, 4,13-diaza-18-crown-6 was treated with 1,2-dibromododecane to afford $Br(CH_2)_{12}$⟨N18N⟩$(CH_2)_{12}Br$. 4,13-Diaza-18-crown-6 was monoalkylated by treatment with 4-nitrobenzyl bromide, sodium carbonate, and potassium iodide in butyronitrile. Along with the expected dialkylated derivative, the desired product was obtained in 44% yield. Reaction of 2 equiv of $ArCH_2$⟨N18N⟩H with $Br(CH_2)_{12}$⟨N18N⟩$(CH_2)_{12}Br$, Na_2CO_3 and KI in $CH_3CH_2CH_2CN$ afforded tris(macrocycle) **9** in 39% yield as a yellow solid, mp 61.5−62 °C. The approach is shown in Scheme 1.

Compound **3** ($HOOC(CH_2)_{11}$⟨N18N⟩$(CH_2)_{11}COOH$), was prepared from 12-bromododecanoic acid, which was protected as its benzyl ester. Bis(alkylation) gave the diester $PhCH_2OOC(CH_2)_{11}$⟨N18N⟩$(CH_2)_{11}COOCH_2Ph$. Hydrogenolysis afforded the diacid (**3**), which could be further functionalized but was used as obtained in the present studies.

Compound Solubility Properties. It might be argued that the compounds of the present study are all amphiphiles that behave as detergents. The mode of action would presumably be to create a defect in the bilayer. Differences in transport might then be due to differential membrane solubility rather

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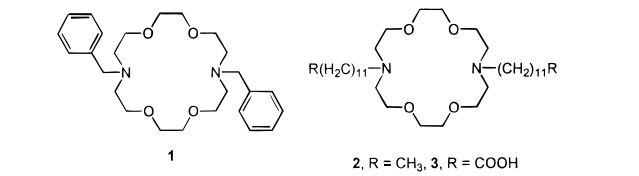

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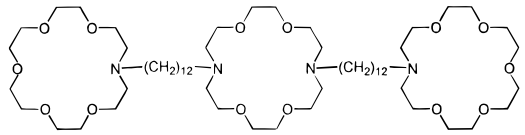
2, R = CH_3, **3**, R = COOH



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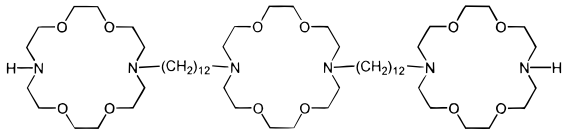

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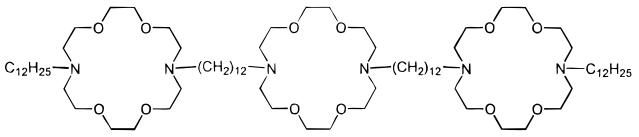

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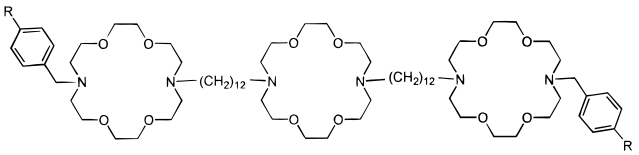
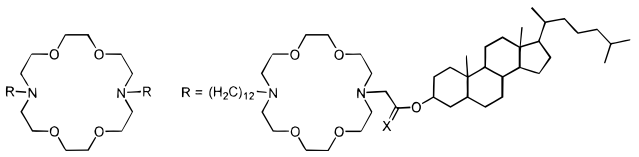


7, R = H; **8**, R = CH_3O; **9**, R = NO_2



10, X = H_2; **11**, X = O

than any intrinsic transport efficacy. Whatever variations in solubility may be observed for these compounds, it is clear that the ionophore must partition into the membrane to effect transport. Relatively few standard solubility data are available in the literature for crown systems, and none is recorded for the compounds of interest here. The determination of solubility data for all-aliphatic channel **6** is problematic because it lacks UV- or fluorescence-active residues that would permit the detection of very low concentrations of material in water.

Solubility was determined by a comparison of experimental determined octanol−water partition coefficients ("*P* values") with values calculated by using the HINT (solubility) module of the SYBYL molecular modeling package. The calculations take into account all atoms (including H). The "solvent condition" parameter in this program was set to neutral, and the "polar proximity" was calculated using the through-space function $me^{(-nr)}$, where e = exponential, $m = 5$, $n = 1$, and r = radius.

Five octanol−water partition coefficients for crown ethers are reported in the literature. These are for 3*n*-crown-*n* (n = 4−6),[22] *p-tert*-butyl-benzo-27-crown-9, and *p-tert*-butyl-benzo-30-crown-10.[23] We experimentally determined the partition coefficient for dibenzyldiaza-18-crown-6. These solubilities were then compared with the experimentally determined values for calibration. Keeping in mind that *P* values are reported on a logarithmic scale, the agreement apparent in the data shown in Table 1 is excellent.

Table 1. Octanol−Water Partitioning Coefficients for Crown Ether Derivatives

no.	compd	P_{oct}(exptl)	P_{oct}(calcd)[d]
	12-crown-4	0.92[a]	0.57
	15-crown-5	0.33[a]	0.34
	18-crown-6	0.21[a]	0.17
	benzo-15-crown-5	1.60[c]	0.91
	benzo-33-crown-11	0.24[c]	0.23
	p-tert-butylbenzo-27-crown-9	1.89[b]	2.15
	p-tert-butylbenzo-30-crown-10	1.69[b]	1.65
	dibenzo-18-crown-6	2.20[c]	3.70
	dibenzo-24-crown-8	3.54[c]	2.11
	dibenzo-27-crown-9	3.30[c]	1.63
	5,5-dibenzo-30-crown-10	1.94[c]	1.80
	2,8-dibenzo-30-crown-10	3.32[c]	1.82
	4,7-dibenzo-33-crown-11	3.39[c]	1.45
	8,8-dibenzo-48-crown-16	1.43[c]	0.52
1	*N,N*-dibenzyl-4,13-diaza-18-crown-6	4.21	4.10
6	$C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}$	−	18.48
7	$[PhCH_2\langle N18N\rangle C_{12}]_2\langle N18N\rangle$	−	11.23
8	$(p\text{-}MeOC_6H_4CH_2\langle N18N\rangle C_{12})_2\langle N18N\rangle$	−	11.26
9	$(p\text{-}O_2NC_6H_4CH_2\langle N18N\rangle C_{12})_2\langle N18N\rangle$	−	11.76
10	$[chol\text{-}O\text{-}(CH_2)_2\langle N18N\rangle C_{12}]_2\langle N18N\rangle$	−	30.08
11	$(chol\text{-}O\text{-}CO\text{-}CH_2\langle N18N\rangle C_{12})_2\langle N18N\rangle$	−	30.83

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The agreement between calculated and experimentally determined values for simple crown ethers is generally good. In particular, the agreement for dibenzyl-4,13-diaza-18-crown-6 (**1**, 4.2 *vs* 4.1) is excellent. A value of 4 means that the compound favors octanol over water by a factor of 10^4. Thus, a compound having a *P* value of 2 would partition 99% into *n*-octanol and one with a value of 4 would partition 99.99% into octanol. In terms of ionophore function, any value greater than 2 would leave almost no ionophore in the aqueous phase.

We have been unable to experimentally determine log *P* values for channel compounds (**6**−**11**) because there is no detectable concentration of them in water. The calculated values show clearly that this is expected. Compounds **6**−**11** all favor the hydrophobic solvent by $>10^{10}$. The suggestion that differences in transport efficacy are due to differential solubility of the ionophores in the bilayer is therefore not supported.

Picrate Extraction Constants. The ability of the compounds studied here to complex Na^+ can be assessed by the picrate extraction method.[24] In this experiment, sodium picrate is contacted by a mixture of H_2O and $CHCl_3$. The yellow picrate salt remains in water. Addition of a host that can complex cations and partition into $CHCl_3$ causes a yellow color to develop in the lipophilic phase. The amount of picrate anion in $CHCl_3$ can be assessed quantitatively by UV−vis spectroscopy. The

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Table 2. Sodium Cation Binding and Transport by Ionophores[a]

no.	ionophore	rate × 10^8 mol·h^{-1}	rel rate ($CHCl_3$)	picrate extn %	rel rate (bilayer)
	valinomycin	4.17	1.0	16.6	0.14
	gramicidin	0.08	0.02	na	1.00
1	$PhCH_2\langle N18N\rangle CH_2Ph$	2.22	0.53	13.7	0.01
2	$C_{12}\langle N18N\rangle C_{12}$	2.01	0.48	17	0.01
3	$HOOC(CH_2)_{11}\langle N18N\rangle (CH_2)_{11}COOH$	ND[b]	0	1.5	0.01
4	$\langle 18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18\rangle$	2.4	0.58	37.3	0.02
5	$H\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle H$	1.13	0.27	27.9	0.28
6	$C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}$	1.1	0.26	37	0.28
7	$(PhCH_2\langle N18N\rangle C_{12})_2\langle N18N\rangle$	1.9	0.46	26.2	0.38
8	$(p\text{-}MeOC_6H_4CH_2\langle N18N\rangle C_{12})_2\langle N18N\rangle$	1.8	0.43	26	0.43
9	$(p\text{-}O_2NC_6H_4CH_2\langle N18N\rangle C_{12})_2\langle N18N\rangle$	1.85	0.44	16.4	0.30
10	[chol-O-$(CH_2)_2\langle N18N\rangle C_{12}]_2\langle N18N\rangle$[c]	2.6	0.62	29.1	0.02

[a] See text for details. [b] ND is "not determined." [c] "Chol" is 3-dihydrocholestanyl.

amount of Na^+ is assumed to be equimolar, so its concentration is known as well. Extraction constants are normally expressed as a percentage of available cation. Note that the extraction constant tells only how much of the available Na^+ is extracted (complexed) and not how many of the macrorings are involved in the process.

Extraction constants were determined for valinomycin and compounds **1**−**10**. The method used 3.0 mL each of H_2O and $CHCl_3$; the compound under study was 1.0 mM in $CHCl_3$. The aqueous sodium picrate solution was 1.0 mM in $Na^+(picrate)^-$ and 0.0975 M in NaOH. The UV spectrum ($\lambda = 354$ nm) was evaluated quantitatively to obtain the extraction constants which are recorded in Table 2. Further details of the procedure may be found in the Experimental Section.

We may use diaza-18-crown-6 derivatives **1** and **2** as "baseline" compounds for our consideration of cation binding. These two structures possess the diaza-18-crown-6 framework that is the basic structural unit of the other systems. Whether the sidearms are benzyl (**1**) or *n*-dodecyl (**2**), the extraction constant is 15 ± 2%. Adding two aza-18-crown-6 units at the end of each dodecyl chain increases the extraction constant to ∼37%, or about 2.5-fold. A similar increase is observed when $\langle N18N\rangle C_{12}$ is added to the end of each dodecyl chain (→**6**). Surprisingly, the corresponding substitution by $\langle N18N\rangle CH_2Ph$ (→**7**) leads to an extraction constant of only 26%. A similar value is found if benzyl is altered to 4-methoxybenzyl (**8**, 26%) or if the chain is eliminated entirely and the crown left as $\langle N18N\rangle H$ (**5**, 28%). The cholestanyl sidearm of **10** gave slightly increased (29%) extraction, but the only major difference resulted from attachment of a 4-NO_2 group to **7** to give **9** (16%).

Transport Experiments. We have previously reported that sodium transport in a phospholipid bilayer is effected by tris(macrocycle)s such as **5** and **6**.[20] The inability of **4** to function as a channel may be due to the lack of an additional residue or functional group that would stabilize it within the bilayer. In **6**, this residue is the *N*-dodecyl chain that is held in the membrane. In **7**, it is a benzyl group that may interact with phospholipid head groups. Compound **5** may be stabilized at the membrane head group by protonation of a nitrogen atom, only one of which is available in **4**.

We expected **6** to transport cations by a channel, rather than by a carrier, mechanism for three reasons. First, cation transport mediated by protein channels is usually 10^3−10^4 greater than observed for even the best carriers.[25] The difference in efficacy is apparent from a comparison of bilayer transport rates for two naturally-occurring peptides: gramicidin (channel) and valinomycin (carrier). We have measured the valinomycin-mediated transport rate for Na^+ in a phospholipid bilayer. To our knowledge, this has never before been reported. The observed rate in the present experimental system is ∼25 s^{-1}. This corresponds to about 14% of the rate exhibited by gramicidin under identical conditions.

Second, compounds such as **6** are amphiphilic and are known to form stable membranes[26] using the diazacrown residues as head groups. The mobility of an amphiphile in a membrane is normally high within a bilayer leaflet (lateral relaxation). If the present compounds are functioning as carriers, they would be required to pass through to the opposite leaflet, an obviously unfavorable process akin to transverse relaxation. Such chemical processes are known to occur in membranes, but the difference in rates for the two is usually many powers of 10 (lateral > transverse).

Third, if **6** [$C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}$] functions as a carrier, then analogues of it such as $C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}$ should be almost as active. We have previously shown that transport of Na^+ by this bolaamphiphile (NMR method) does not occur at a detectable rate in the phospholipid bilayer system.[18] The same is true for $C_{12}\langle N18N\rangle C_{12}$ (**2**).

Notwithstanding these arguments, we felt it would be worthwhile to assess the transport ability of this family of compounds in a bulk organic membrane since many potential ionophores have been studied using this technique (see Table 2).

Techniques Used in Transport Studies. The ability of valinomycin, gramicidin, and compounds **1**−**10** to transport Na^+ was assessed by using two different techniques. Sodium cation transport through a phosphatidylcholine/phosphatidyl glycerol bilayer can be assessed directly by using the ^{23}Na-NMR technique developed originally by Riddell and co-workers.[27] The vesicle system is prepared in the presence of NaCl and then Dy^{3+} is added to the external medium. Two signals are observed for ^{23}Na, their exchange rate can be measured, and

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an exchange rate constant can be obtained in the presence of a suitably active ionophore. In all cases, gramicidin was run concurrently with any new sample and rate constants obtained were normalized to a value of 1.00 corresponding to its observed exchange rate ($\sim$175 s^{-1}).

Bulk liquid membrane transport rates were measured using the concentric tube apparatus developed by Izatt and coworkers.[28] The device consists of a hollow glass tube (10 mm o.d.) inserted into a tall-form beaker (25 mL). The bulk organic membrane used was either $CHCl_3$ or $CDCl_3$ (6 mL). The source phase was aqueous sodium picrate (1 mM) in the presence of excess NaOH (100 mM). The receiving phase was initially distilled, deionized water. The membrane was stirred magnetically. Aliquots were withdrawn at the specified intervals and analyzed by UV−visible spectroscopy.

The ability of such compounds as dibenzyldiaza-18-crown-6 (**2**) and didodecyldiaza-18-crown-6 (**3**) to transport cations through a bulk liquid membrane is well-established by numerous experimental studies.[29] As expected, both **2** and **3** transported sodium picrate (as judged by measurement of changes in picrate concentration) with rates of 2.22×10^{-8} and 2.01×10^{-8} mol·h^{-1}, respectively. These values compare with an experimentally determined rate of 4.17×10^{-8} mol·h^{-1} for valinomycin. Under identical conditions, gramicidin exhibited a negligible transport rate of 0.08×10^{-8} mol·h^{-1}, which was barely above simple diffusion (blank value: $0.013 \times \%10^{-8}$ mol·h^{-1}). Channel former **6** was also found to transport Na^+, but its rate (1.1×10^{-8} mol·h^{-1}) was less than one-half that for either **2** or **3**. Compounds **4** and **5**, which constitute major fragments of the channel structure, were found to transport sodium picrate with rates of 2.4×10^{-8} and 1.13×10^{-8} mol·h^{-1}, respectively. It is noteworthy that although **5** and **6** function similarly as carriers and are both inferior to **4**, exactly the opposite relationship is observed in a phospholipid bilayer (see column 4 of Table 2).

Bulk Membrane Hydration. During the course of transport studies with **6**, a distinct cloudiness developed in the bulk $CHCl_3$ membrane. It seemed reasonable to assume that water was partitioning into the $CHCl_3$ phase. This membrane hydration was not apparent in most other cases, so the extent and rate of hydration were determined experimentally by using NMR methods.

The extent of hydration was quantified as follows. During the transport experiment involving 1 mM ionophore, 100-μL aliquots were withdrawn from the $CHCl_3$ ($CDCl_3$ in this case) phase. The samples were diluted with 500 μL of additional $CDCl_3$, and a stock solution (25 μL) of 1,4-dinitrobenzene (75 mM) in $CDCl_3$ was added. Comparison of the integrals of water and the single, downfield (8.4 ppm) resonance of dinitrobenzene permitted quantitative measurement of the former. The data are plotted in Figure 1 for compounds **5**−**7** along with data for the blank.

Chloroform is reported[30] to absorb temperature-dependent percentages of water. In the range 20−25 °C, the equilibrium amount of water reported to be present in $CHCl_3$ (presumably ethanol-free) is 60−80 mM. In our own experimental system

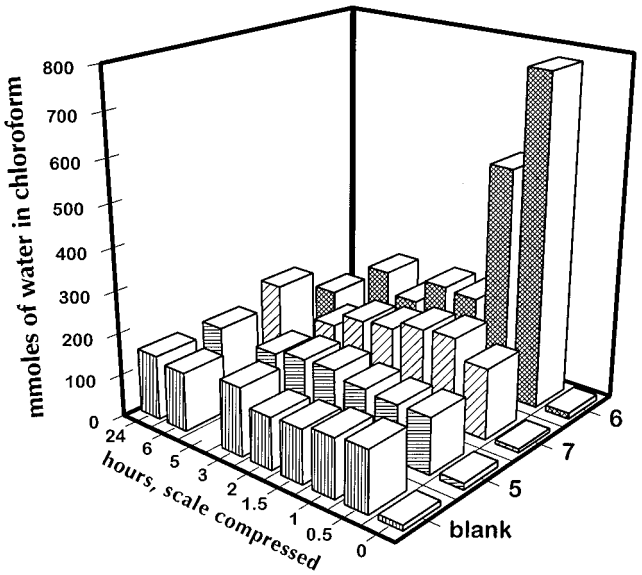

Figure 1. Plot of H_2O content in $CHCl_3$ as a function of time in the absence and in the presence of compounds **5**−**7**.

($\sim$22 °C), "dry" $CHCl_3$ was found to quickly hydrate to an equilibrium water concentration of $\sim$140 mM. Our system is in contact with aqueous sodium picrate, and this could have a 2-fold effect on hydration, although contact with a salt might be expected to reduce the chloroform aquation. A slight solubility of the picrate in the absence of carrier might account for a small, additional amount of water brought into the chloroform phase along with the sodium cation.

In the presence of **5**, a similar extent of hydration was observed. In the presence of compound **7** ($PhCH_2\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle CH_2Ph$), the membrane hydrated to the extent of about 250 mM but had reached the equilibrium level of 140 mM by 24 h. The most dramatic result was observed for **6** ($C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}$), which caused the membrane to reach a water concentration of $\sim$750 mM within 30 min. The water was present as $\sim$3000 Å droplets as judged by laser light scattering. After several hours, the concentration of water diminished and, by 24 h, the H_2O and $CHCl_3$ were essentially in equilibrium.

There are two obvious questions related to these observations. First, why does the membrane hydrate, and second, why does the amount of water diminish in an apparent return to equilibrium? The answer to the second question is probably that the water is gradually "salted out" of the membrane by an increasing concentration of sodium picrate in the receiving side phase. Why the membrane hydrates so extensively in the presence of **6** is a more difficult and intriguing question, the answer to which is currently unclear but may be due to the formation of a hydrated pseudo-micelle.

Comparison of Transport Rates in a Phospholipid Bilayer and in Chloroform. An important purpose of the studies described here was to determine if the use of a bulk liquid ($CHCl_3$) membrane transport system could provide any information about the chemical behavior of single-stranded, synthetic channel compounds. In previous work in our own group, we studied a series of 12-, 15-, and 18-membered ring azamacrocycles having alkyl-substituted ester or amide sidearms appended at nitrogen [*e.g.*, $\langle 15N\rangle CH_2COO(CH_2)_9CH_3$].[20] The sidearms ranged from 5 to 18 carbon atoms in length. In that study, picrate extraction constants and homogeneous equilibrium binding constants (log K_S) were both found to correlate reasonably well with transport rates in a bulk chloroform membrane. The correlation (r factor) between transport and

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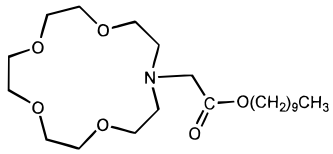
either extraction constants or binding constants was, respectively, 0.89 (10 points) and 0.93 (17 log K_S values).



Among compounds **1**−**10**, valinomycin is expected to be the best carrier in $CHCl_3$ and gramicidin is expected to be the most effective channel former in a bilayer. All rate data are therefore normalized to values of 1.0 for these respective standards. We anticipated that compounds such as *N*,*N*′-dibenzyl-4,13-diaza-18-crown-6 (**1**) and *N*,*N*′-didodecyl-4,13-diaza-18-crown-6 (**2**) would be excellent carriers when studied in this system. Gramicidin, in order to transport cations, must dimerize to form a membrane-spanning, channel-like structure within a bilayer. Since this is impossible in the bulk $CHCl_3$ membrane, gramicidin was expected to function poorly in this experimental system.

Dialkyl crowns such as **1** and **2** were not expected to be as effective channels in a bilayer, but compounds such as **6** ($C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}$) might exhibit efficacy in both types of membranes. From the data in Table 1, it is clear that certain of the above expectations are met. It is especially interesting to note that valinomycin exhibits transport activity in a lipid bilayer that is about 14% that of gramicidin. To our knowledge, valinomycin transport has not been previously studied in a bilayer using NMR methods. Also as anticipated, the efficacy of gramicidin in $CHCl_3$ is poor (8% that of valinomycin).

A comparison of compounds **4** and **5** is particularly interesting. The Na^+ transport rate by **5** ($\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle$) in a bilayer is identical to that of **6**. On the other hand, **4** ($\langle 18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18\rangle$) does not transport cations in the bilayer system. The order of efficacy in the bilayer is **5**;**6** > **4**. In contrast, in the $CHCl_3$ membrane, the order is **4** > **5**;**6**. Compound **7** ($PhCH_2\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle CH_2Ph$) shows transport activity in the bilayer about 40% of gramicidin and about 35% better than for **6**. In the bulk membrane system, its rate is about 46% that of valinomycin, nearly 6-fold that of gramicidin, and ∼75% better than for **6**. These differences in relative efficacy in the various membrane systems strongly imply that different mechanisms operate.

One of the most dramatic examples of this efficacy difference is observed for **11**. In the lipid bilayer, its activity is below our ability to detect even though structurally it is quite similar to **6**. In the bulk $CHCl_3$ membrane, its activity is second only to valinomycin itself (66%). A carrier mechanism operating in both membrane systems is expected to give similar, if not identical, efficacy orders.

A plot of relative transport rate *vs* picrate extraction constant is shown in Figure 2. The correlation coefficient for a calculated linear fit of the 10 points shown is 17%. Unlike data obtained from a variety of previous comparisons, this is essentially a scatter plot. When data for nonchannel compounds **1**−**3**, gramicidin, and valinomycin are removed, the best linear fit of the data is a line through them having a slope of 0.

It might be argued that the difference in media ($CHCl_3$ *vs* phospholipid bilayers) is too great for there to be any meaningful relationship between the function of a set of compounds in either. Using the family of compounds noted above [*e.g.*, $\langle 15N\rangle CH_2COO(CH_2)_9CH_3$], we studied transport in the bilayer[31] and demonstrated that this did, indeed, correlate to carrier transport determined by the bulk membrane approach.[32]

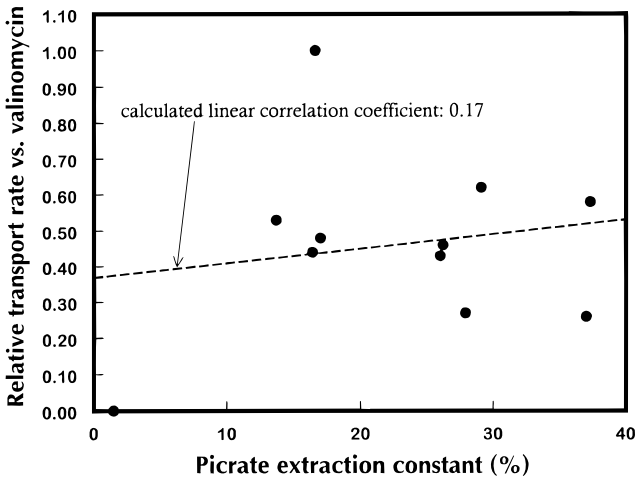


Figure 2. Plot of transport rate *vs* extraction constant for compounds **1**−**10**.

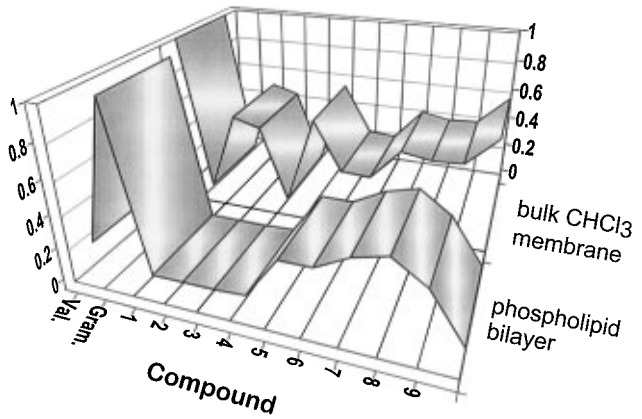


Figure 3. Comparison of Na^+ transport effected by **1**−**10** in a $CHCl_3$ membrane or a phospholipid bilayer.

The data obtained for transport of Na^+ by this group of compounds either in a bulk chloroform membrane or in a phospholipid bilayer show that there is no correlation between the two. If the tris(macrocycle) conducts cations by functioning as a carrier in $CHCl_3$ and a "supercarrier" in the bilayer, there ought to be a clear rate correlation. It is simply unreasonable to think that carrier transport is fundamentally different in a bilayer than it is in a bulk membrane. The lack of correlation is shown clearly in Figure 3 in which rates determined for a range of compounds in both the bilayer and chloroform membranes are compared. In this figure, values are normalized to 1 *vs* gramicidin in the bilayer and *vs* valinomycin in $CHCl_3$.

The construction of the concentric tube transport device is such that the shortest distance between the two aqueous phases through $CHCl_3$ is $\sim 10^8$C. It is clearly impossible for any of these compounds to bridge this distance as gramicidin spans a ∼30 Å biological bilayer membrane by dimerizing. The only known transport mechanism available to the compounds under study in this system is the carrier mechanism. The complete absence of any correlation between Na^+ transport efficiencies assessed in the $CHCl_3$ and phospholipid membranes suggests that the transport mechanisms differ.

The channel mechanism is clearly impossible in the $CHCl_3$ membrane. The carrier mechanism may be possible for the tris(macrocycle)s in the bilayer, but the behavior of valinomycin

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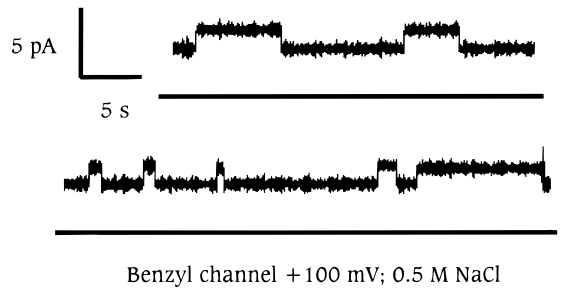

Figure 4. Bilayer clamp data trace for **7** in a phospholipid membrane.

argues against it. Valinomycin is the most efficient of all the carrier compounds studied. Compounds **1**, **4**, and **10** exhibit about one-half of its activity in $CHCl_3$. Valinomycin's natural environment is the mitochondrial membrane, a phospholipid bilayer. In the model phospholipid bilayer, valinomycin shows a transport efficacy only 14% that of gramicidin. Compounds **1**, **4**, and **10** show no detectable activity. Our experimental system is such that we could easily detect one-half the activity of valinomycin, *i.e*. 7% of the activity of gramicidin. That the best synthetic carriers are the poorest channels does not comport with the postulate that these compounds may function by a carrier mechanism. Obviously, this does not prove that the remaining compounds are channel formers but the implication is clear.

Attempted Correlation of Transport Rates to Substituent Constants. We have previously shown that tris(macrocycle)s **7**−**9**, which differ only by the *para*-substituent present on the distal benzyl groups, exhibit Na^+ transport rates that correlate with the appropriate Hammett parameters.[33] The previously reported rates were obtained by using the dynamic ^{23}Na-NMR method described above. Our plan was to determine whether data obtained by using the bilayer clamp method would give corresponding results.

The bilayer clamp method uses a patch clamp amplifier and a bilayer head stage[34] to assess single ion currents in a phospholipid bilayer. Results obtained in this way therefore differ from those obtained by using the NMR method because the latter affords an "average" or "macroscopic" result. A typical data trace obtained for $[PhCH_2\langle N18N\rangle C_{12}]_2\langle N18N\rangle$ (**7**) is shown in Figure 4. The data were obtained at different applied potentials in the presence of 500 mM NaCl. The discrete, integral opening and closing behavior observed for this system is also typical of naturally occurring protein channels. The conductance traces are strongly suggestive of the channel mechanism. This is important evidence that bears directly on the channel mechanism, notwithstanding the original intent only to demonstrate a substituent effect.

The cation flux for the channel molecules in the bilayer can be determined by dividing the observed peak height (amperage) by the applied voltage. In this case, a value of 12.8 picosiemens (pS) was obtained for **7**. It was anticipated, based upon the results obtained by the ^{23}Na-NMR method, that different, substituent-dependent fluxes would be observed for **7**, **8**, and **9**. It was also expected that these flux rates would correlate to the Taft σ^0 constant. In fact, very little difference was observed in the cation fluxes. Instead, preliminary studies suggest that the open times observed for the channels are different. Thus, open times appear to be in the order *p*-CH_3O > *p*-H > *p*-NO_2 (**8** > **7** > **9**). In principle, the longer a channel is open, the greater the flux of sodium is expected to be. In this sense, the results obtained by this method correlate with those previously obtained. Still, this correlation is neither quantitative nor the expected one and the meaning of open time as a parameter is currently unclear.

Another difficulty we encountered in comparing data obtained by the bilayer clamp and NMR methods involves the use of gramicidin as an internal standard. In our previous efforts, we have determined Na^+ flux mediated by gramicidin in parallel with the corresponding determination for a tris(macrocycle). This was done to minimize experimental variations in these relatively complex analyses. It was found, however, that the flux rates determined for gramicidin by using the bilayer clamp method varied depending upon the concentration of NaCl present in the medium. Thus, when [NaCl] varied from 100−500 mM, transport of Na^+ by gramicidin varied sufficiently that it was either faster or slower than the nearly constant rate for **7**.

The use of gramicidin also presents a problem in the ^{23}Na-NMR method of which we were not previously aware. In the dynamic ^{23}Na-NMR experiment developed by Riddell, line broadening of the $Na^+_{(in)}$ is observed relative to [ionophore]. In our system, the Na^+ line width is typically determined at 4−8 ionophore concentrations (0∼20 μM). Changes in the half-height line width (multiplied by π) are plotted against ionophore concentration. The rate is determined from the equation for the line and expressed as its "standard" value at 10 μM.

It is of interest to consider the slope of the log log plot of the data obtained as described above. According to Hinton,[35] this should give the kinetic order of the reaction. For most of the examples we have studied, the slope of the plot of $\log_{10} k$ [or $\log_{10}(1/\tau)$] *vs* $\log_{10}$ [ionophore] lies in the range 1−1.2. The value of approximately 1 was not surprising to us since our tris(macrocycle)s were designed to function as unimolecular ion transporters. The fact that the slopes we observed were in a range rather than exactly unity was troubling, but we were comforted by the fact that the slope reported for the gramicidin dimer is 1.8 rather than 2.0.[35] Unfortunately, in our work, gramicidin gave a slope of approximately 1 rather than nearly 2 even though the experiment was repeated more than 50 times.

In an effort to understand this discrepancy, we examined the published data for the gramicidin experiment. The graph presented in the report shows seven points plus zero while the data table shows only five points plus zero. We plotted the five data points and obtained, as published, an excellent line. The corresponding log log plot gave a slope of ∼1.8, as previously published. Surprisingly, R^2 for the original data set plotted as $1/\tau$ *vs* [ionophore] was 1.00. Unfortunately, none of our data were of this remarkably high quality.

The similarity of the rates observed by using the bilayer clamp method is demonstrated for compounds **7**−**9** in the current−voltage plot shown as Figure 5. The applied voltages ranges from ±60 to ±140 mV, and the calculated conductance was ∼13 pS.

The above results show that ^{23}Na-NMR method has proved to be experimentally cumbersome and that the data that are obtained do not correlate exactly with those obtained by using the bilayer clamp method. Our inability to corroborate the NMR method Hammett study with bilayer clamp data makes suspect the previous results. The fact that the bilayer clamp data appear typical in most ways of data obtained from Na^+-conducting protein channels strongly suggests that the tris(macrocycle)s function as channels rather than as carriers or by some other, as yet undefined, mechanism.

Conformation of 6 in the Phospholipid Bilayer. Previous studies have confirmed that the central macrocycle does not

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(34) Warner Instrument PC-505 amplifier equipped with a HB-202 50 gΩ head stage.

(35) Buster, D. C.; Hinton, J. F.; Millett, F. S.; Shungu, D. C. *Biophys. J.* **1988**, *53*, 145−152.

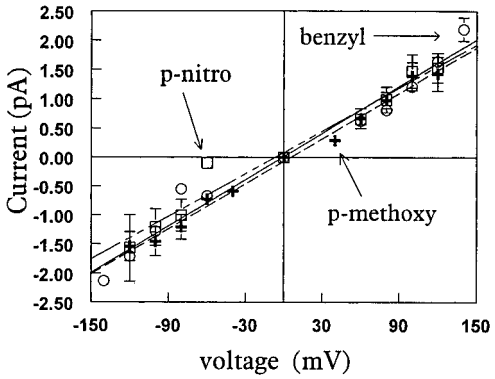

Figure 5. Plot of voltage *vs* amperage for compounds **7**−**9**.

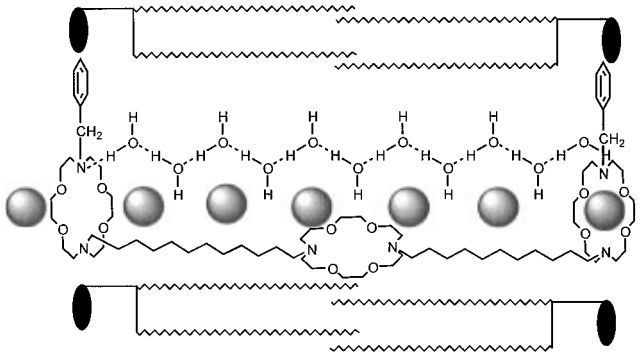

Figure 6. Postulated channel conformation for **7** in a phospholipid bilayer.

require to be parallel to the distal rings. The distal macrocycles are expected to be head groups in these structures. This is anticipated from studies of crown ethers as head groups for stable vesicles.[36] Upon the basis of the evidence reported here and accumulated thus far, we suggest the conformation shown in Figure 6 as the most likely arrangement of **7** in a lipid bilayer. Presumably, water infiltrates the channel and assists in transport of the cation through the transmembrane "groove."

Conclusions

The present study addressed three basic issues concerning the tris(macrocyclic) Na^+-transporting system. The possibility that differences in Na^+ transport rate may correlate with differential membrane solubility of the ionophores was addressed. Calculations, verified where possible, of the octanol−water partition coefficients show that all of the channel formers favor a hydrophobic environment by $>10^{10}$. Second, Na^+ transport rates were determined in a bulk $CHCl_3$ membrane. The channel mechanism is not possible in this case due to the large distance separating the source and receiving aqueous phases. A plot of transport rates determined in $CHCl_3$ and in a bilayer showed no correlation. It was interesting to note that several of the tris(macrocyclic) ionophores caused rapid hydration of the $CHCl_3$ phase although water was lost during equilibration over several hours. Finally, a postulated channel-like conformation for the tris(macrocycle)s should show "channel-like" conductivity when studied by using a bilayer clamp method. Such data traces were, in fact, obtained and show remarkable similarity to those obtained for natural, Na^+-conducting protein channels. We thus conclude that the tris(macrocyclic) systems span the bilayer and function as model cation channels.

Experimental Section

^{1}H-NMR spectra were recorded at 300, 500, or 600 MHz in $CDCl_3$ solvent and are reported in ppm (δ) downfield from internal $(CH_3)_4Si$ unless otherwise specified. ^{13}C-NMR were recorded at proportional frequencies as noted above. Infrared spectra were recorded on a Perkin-Elmer 1710 Fourier transform infrared spectrophotometer and were calibrated against the 1601 cm^{-1} band of polystyrene. 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) with a 0.2-mm layer thickness or on silica gel 60 F-254 with a 0.2-mm layer thickness. Preparative chromatography columns were packed with activated aluminum oxide (MCB 80−325 mesh, chromatographic grade, AX 611) or with Kieselgel 60 (70−230 mesh). Rotating disk chromatography was performed on a Harrison Research Model 7924 Chromatotron with 2-mm thick circular plates prepared from Kieselgel 60 PF-254.

All reactions were conducted under dry N_2 unless otherwise stated. All reagents were the best (non-LC) 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.

Reagents. Phophatidylcholine ($CHCl_3$ solution), phosphatidylglycerol ($CHCl_3$:CH_3OH solution), Na_2HPO_4, KH_2PO_4, NaCl, gramicidin D (85% gramicidin A, 15% gramicidin B and C), and sodium tripolyphosphate (98%) were purchased from Sigma, St. Louis, MO, and used without further purification. Diethyl ether (anhydrous) was purchased from Mallinckrodt and distilled over sodium/benzophenone prior to use. The water used in the buffer solution was distilled and deionized. 2,2,2-Trifluoroethanol (TFE, NMR grade), $DyCl_3 \cdot H_2O$ (99.99%), D_2O, KCl, $CHCl_3$ (HPLC grade), picric acid, NaOH volumetric solution (0.0975 M), and 1,4-dinitrobenzene (98%) were purchased from Aldrich and used without further purification. Chloroform-*d* (99.8%) was purchased from Isotec, Ohio. Polycarbonate membranes (1.0 μm, 2.5 cm diameter) and membrane holders were obtained from Poretics Corp., Livermore, CA. The sonication was done in a water sonication bath, Branson, model 1200, or in a Branson, Bransonic 12. The mean diameters of the vesicles were determined in a Coulter, submicron particle analyzer, model N4 ND.

Compounds Studied. Gramicidin and valinomycin were purchased from Sigma and Aldrich and used as received.

***N,N′*-Dibenzyl-4,13-diaza-18-crown-6 (1)** was prepared as previously reported.[37]

***N,N′*-Dididecyl-4,13-diaza-18-crown-6 (2)** was prepared as previously reported.[38]

***N,N′*-Bis(11-carboxyundecyl)-4,13-diaza-18-crown-6 (3). Benzyl 12-Bromodedecanoate (3A).** To a mixture of 12-bromododecanoic acid (4.75 g, 17.0 mmol), DCC (3.86 g, 18.7 mmol), and DMAP (0.23 g, 1.87 mmol) in ether (50 mL) was added benzyl alcohol (2.02 g, 18.7 mmol) at room temperature. After 2 h, the precipitate that formed was removed by filtration and the filtrate was washed successively with 5% $NaHCO_3$ (2 × 15 mL), water (2 × 15 mL), 10% citric acid (2 × 15 mL), water (15 mL), and brine (15 mL). The ether layer was dried ($MgSO_4$), concentrated, and chromatographed (SiO_2 column) to give **3A** (4.03 g, 64%) as a colorless oil, bp 197−200 °C (0.5 Torr). ^{1}H-NMR: 1.20−1.35 (12H, m, alkyl), 1.35−1.50 (2H, m, $BrCH_2CH_2CH_2CH_2CH_2$), 1.57−1.70 (2H, m, $COCH_2CH_2CH_2CH_2CH_2$), 1.851 (2H, quintet, J = 7.3 Hz, $BrCH_2CH_2CH_2CH_2CH_2$), 2.354 (2H, t, J = 7.5 Hz, $COCH_2CH_2CH_2CH_2$), 3.408 (2H, t, J = 6.9 Hz, $BrCH_2CH_2CH_2$), 5.116 (2H, s, $ArCH_2O$), 7.32−7.38 (5H, m, aromatics). IR (neat film): 3066, 3034, 2927, 2854, 1737, 1498, 1456, 1383, 1353, 1256, 1214, 1164, 1120, 1003, 908, 736, 698 cm^{-1}. Anal. Calcd for $C_{19}H_{29}BrO_2$: C, 61.79; H, 7.91; Br, 21.63%. Found: C, 61.93; H, 7.91; Br, 21.48%.

***N,N′*-Bis(11-benzyloxycarbonylundecyl)-4,13-diaza-18-crown-6 (3B).** A mixture of 4,13-diaza-18-crown-6 (1.20 g, 4.57 mmol), **3A** (3.38 g,

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9.15 mmol), Na_2CO_3 (9.69 g, 91.4 mmol), and KI (30 mg, 0.18 mmol) in *n*-PrCN (50 mL) was heated at reflux for 24 h. After the mixture was cooled, the insoluble materials were removed by filtration and the resulting solution was concentrated to an oil which was dissolved in CH_2Cl_2 (40 mL), washed with water (4 × 15 mL), dried ($MgSO_4$), and concentrated. Toluene was added and evaporated to assure complete removal of *n*-PrCN. The crude, oily product was purified by chromatography (alumina, 3% *i*-PrOH−CH_2Cl_2) and afforded **3B** (3.04 g, 79%) as colorless oil, mp <25 °C, $bp_{0.5}$ > 300 °C. ^{1}H-NMR: 1.20−1.36 (28H, m, alkyl), 1.36−1.48 (4H, br, $NCH_2CH_2CH_2$), 1.638 (4H, quintet, J = 7.4 Hz, $COCH_2CH_2CH_2$), 2.352 (4H, t, J = 7.6 Hz, $COCH_2CH_2$), 2.475 (4H, t, J = 7.8 Hz, $NCH_2CH_2CH_2$), 2.771 (8H, t, J = 6.0 Hz, NCH_2CH_2O), 3.56−3.64 (16H, m, CH_2OCH_2), 5.114 (4H, s, $ArCH_2O$), 7.31−7.37 (10H, m, aromatics). IR (neat film): 3065, 3034, 2927, 2854, 1737, 1456, 1352, 1255, 1163, 1127, 994, 751, 698 cm^{-1}. Anal. Calcd for $C_{50}H_{82}N_2O_8$: C, 71.56; H, 9.85; N, 3.34%. Found: C, 71.60; H, 9.88; N, 3.25%.

***N,N′*-Bis(11-carboxyundecyl)-4,13-diaza-18-crown-6 (3).** A pressure bottle was charged with **3B** (2.00 g, 2.38 mmol) in *i*-PrOH (70 mL). To this solution was carefully added water (20 mL) to keep the solution clear. The vessel was purged with N_2, 10% Pd on carbon was added, and the resulting suspension was shaken on a Parr hydrogenator under H_2 (2 atm) at room temperature. The catalyst was removed (pad of Celite), and the mixture was evaporated to dryness, leaving **3** (1.54 g, 97%) as a colorless solid, mp 77−79 °C. ^{1}H-NMR: 1.20−1.38 (28H, m, alkyl), 1.48−1.66 (8H, br, $NCH_2CH_2CH_2$, $COCH_2CH_2CH_2$), 2.269 (4H, t, J = 6.8 Hz, $COCH_2CH_2$), 2.746 (4H, t, J = 8.0 Hz, $NCH_2CH_2CH_2$), 3.066 (8H, t, J = 5.3 Hz, NCH_2CH_2O), 3.592 (8H, s, NCH_2CH_2O), 3.726 (8H, t, J = 5.4 Hz, OCH_2CH_2O). IR (KBr disk): 3837, 2914, 2850, 1698, 1472, 1358, 1343, 1266, 1127, 1062, 1016, 974, 866, 832, 805, 720, 600, 562, 513 cm^{-1}. Anal. Calcd for $C_{36}H_{70}N_2O_8$: C, 65.62; H, 10.71; N, 4.25%. Found: C, 65.36; H, 10.66; N, 4.25%.

***N,N′*-Bis[(*N*-4,13-diaza-18-crown-6)dodecyl]-4,13-diaza-18-crown-6 (4)** was prepared as previously reported.

Compounds 5−7 were prepared as previously described.[17]

4,13-Bis[12-{13-(4-methoxybenzyl)-4,13-diaza-18-crown-6-4-yl}-dodecyl]-4,13-diaza-18-crown-6 (8). *N*-(4-Methoxybenzyl)-4,13-diaza-18-crown-6 (8A). A mixture of 4,13-diaza-18-crown-6 (3.54 g, 13.5 mmol), 4-methoxybenzyl chloride (1.90 g 12.2 mmol), Na_2CO_3 (14.3 g, 135 mmol), and KI (48 mg, 0.3 mmol) in *n*-PrCN (300 mL) was heated at reflux for 2 h. After cooling, the mixture was filtered and the filtrate was concentrated to leave a slightly yellow oil. Toluene was added and then evaporated (3×) to remove any residual *n*-PrCN. The resulting oil was chromatographed over alumina and eluted with 2% MeOH−CH_2Cl_2. After removal of the solvent, **8A** was obtained (2.38 g, 46% based on 4,13-diaza-18-crown-6) as a slightly yellow oil which gradually gelled. ^{1}H-NMR: 2.699 (4H, t, J = 4.8 Hz, NCH_2CH_2O), 3.123 (4H, t, J = 4.5 Hz, $NHCH_2CH_2O$), 3.50−3.96 (21H, m, N*H*, $CH_2OCH_2CH_2OCH_2$, $PhCH_2N$, CH_3OPh), 6.910 (2H, d, J = 8.4 Hz), 7.236 (2H, d, J = 8.4 Hz). IR (KBr): 3467, 3241, 3056, 3025, 2876, 1612, 1582, 1513, 1474, 1458, 1367, 1352, 1301, 1268, 1242, 1173, 1113, 1033, 954, 836, 793, 756, 574, 524 cm^{-1}. Compound **8A** was used in the next step without additional purification.

4,13-Bis[12-{13-(4-methoxybenzyl)-4,13-diaza-18-crown-6-4-yl}-dodecyl]-4,13-diaza-18-crown-6 (8). A mixture of **8A** (1.04 g, 2.71 mmol), 4,13-bis(12-bromododecyl)-4,13-diaza-18-crown-6 (1.00 g, 1.32 mmol), Na_2CO_3 (2.875 g, 27.1 mmol), and KI (20 mg, 0.12 mmol) in *n*-PrCN (20 mL) was heated under reflux for 7.5 h. After being cooled, the mixture was filtered and the filtrate was concentrated to a yellow oil. Toluene was added and then evaporated (2×) to assure removal of *n*-PrCN. The resulting oil was chromatographed over alumina (*i*-PrOH−hexanes-CH_2Cl_2 (1:20:5)). Evaporation of the solvent gave the crude product which solidified on standing. Repeated crystallization (95% EtOH) gave **8** (0.158 g, 9% based on 4,13-bis(12-bromododecyl)-4,13-diaza-18-crown-6) as a slightly yellow powder, mp 68.5−69 °C. ^{1}H-NMR: 1.22−1.28 (32H, *pseudo*-s, alkyl), 1.42−1.54 (8H, br, $NCH_2CH_2CH_2$), 2.482 (8H, t, J = 6.0 Hz, $NCH_2CH_2CH_2$), 2.74−2.82 (24H, m, NCH_2CH_2O) 3.58−3.64 (52H, m, $CH_2OCH_2CH_2OCH_2$, $PhCH_2O$), 3.795 (6H, s, CH_3OPh), 6.834 (4H, d, J = 8.7 Hz), 7.234 (4H, d, J = 8.7 Hz). IR (KBr): 2918, 2871, 1619, 1518, 1536, 1333, 1297, 1265, 1240, 1172, 1125, 1072, 1039, 966, 942, 881, 844, 831, 817, 803, 718, 605, 573 cm^{-1}. Anal. Calcd for $C_{76}H_{138}N_6O_{14}$: C, 67.12; H, 10.23; N, 6.18. Found: C, 67.02; H, 10.21; N, 6.12%.

4,13-Bis[12-{13-(4-Nitrobenzyl)-4,13-diaza-18-crown-6-4-yl}dodecyl]-4,13-diaza-18-crown-6 (9). *N*-(4-Nitrobenzyl)-4,13-diaza-18-crown-6 (9A). A mixture of 4,13-diaza-18-crown-6 (3.54 g, 13.5 mmol), 4-nitrobenzyl bromide (2.62 g 12.2 mmol), Na_2CO_3 (14.3 g, 135 mmol), and KI (48 mg, 0.3 mmol) in *n*-PrCN (300 mL) was heated at reflux for 4.5 h. After being cooled, the mixture was filtered and the filtrate was concentrated to a yellow oil. Toluene was added and then evaporated (2×) to assure removal of *n*-PrCN. The resulting oil was chromatographed over alumina (3% MeOH-CH_2Cl_2). Compound **9A** eluted second; solvent was removed, and the yellow oil thus obtained (2.36 g, 44% based on 4,13-diaza-18-crown-6) solidified on standing. ^{1}H-NMR: 2.802 (4H, t, J = 4.7 Hz, NCH_2CH_2O), 3.168 (4H, t, J = 3.9 Hz, $NHCH_2CH_2O$), 3.52−3.66 (13H, m, N*H*, $CH_2OCH_2CH_2O$), 3.756 (2H, s, $PhCH_2N$), 3.875 (4H, t, J = 4.2 Hz, $HNCH_2CH_2O$), 7.584 (2H, d, J = 8.1 Hz), 8.185 (2H, d, J = 7.8 Hz). IR (KBr disk) 3467, 2884, 2499, 1625, 1605, 1515, 1459, 1346, 1278, 1244, 1106, 1084, 974, 937, 855, 829, 802, 744, 737, 708, 648, 523, 442 cm^{-1}. Compound **9A** was used in the next step without further purification.

4,13-Bis[12-{13-(4-nitrobenzyl)-4,13-diaza-18-crown-6-4-yl}dodecyl]-4,13-diaza-18-crown-6 (9). The mixture of **9A** (1.077 g, 2.71 mmol), 4,13-bis(12-bromododecyl)-4,13-diaza-18-crown-6 (1.00 g, 1.32 mmol), Na_2CO_3 (2.875 g, 27.1 mmol), and KI (20 mg, 0.12 mmol) in *n*-PrCN (20 mL) was heated under reflux for 7.5 h. After cooling, the mixture was filtered and the filtrate was concentrated to a yellow oil. Toluene was added and evaporated (2×) to assure removal of *n*-PrCN. The resulting yellow oil was chromatographed (alumina, 10% *i*-PrOH: hexanes, then *i*-PrOH−hexanes−CH_2Cl_2 (2:40:10)). Evaporation of the solvent gave a yellow oil which solidified on standing. Crystallization (95% EtOH) gave **9** (0.72 g, 39% based on 4,13-bis(12-bromododecyl)-4,13-diaza-18-crown-6) as a yellow solid, mp 61.5−62 °C. ^{1}H-NMR: 1.22−1.28 (32H, *pseudo*-s, alkyl), 1.42−1.56 (8H, br, $NCH_2CH_2CH_2$), 2.50−2.60 (8H, br, $NCH_2CH_2CH_2$), 3.58−3.70 (48H, m, $CH_2OCH_2CH_2OCH_2$), 3.794 (4H, s, $PhCH_2O$), 7.553 (4H, d, J = 8.7 Hz), 8.162 (4H, d, J = 8.7 Hz). IR (KBr disk) 2918, 2870, 1607, 1518, 1473, 1356, 1296, 1239, 1125, 1072, 1045, 965, 843, 740, 718, 694, 605, 422 cm^{-1}. Anal. Calcd for $C_{74}H_{132}N_8O_{16}$: C, 63.95; H, 9.57; N, 8.06%. Found: C, 63.85; H, 9.56; N, 8.07%.

NMR Studies of Transport in Bilayers. The procedure is as reported in ref 17.

log P_{oct} Determination for *N,N′*-Dibenzyldiaza-18-crown-6. 1-Octanol (200 mL, ACS spectroscopic grade) was saturated with deionized water. To saturate the octanol, 200 mL of alcohol were placed in a 1.0-L bottle with 400 mL of deionized water. The two-phase system was gently shaken for 3 min and then placed in a thermostatic water bath at 21.0 °C overnight. Doubly deionized water was similarly saturated with 1-octanol.

A solution of *N,N′*-dibenzyldiaza-18-crown-6 (10^{-2} M, 3.00 mL) in water-saturated 1-octanol was placed in a separatory funnel, and 100 mL of the water-saturated octanol were added. The funnel was gently shaken for 3 min. The aqueous layer was drained, and the octanol layer was removed with the aid of a Pasteur pipette. A 0.2-mL sample of this octanol layer was placed in a spectroscopic cuvette and diluted with 1-octanol (1.8 mL). The partition coefficient was determined by comparison of the absorbance of this solution with that of a freshly prepared 10^{-3} M solution of *N,N′*-dibenzyldiaza-18-crown-6 in 1-octanol.

Bulk Membrane Transport Experiments. UV−vis spectra were recorded on a Beckman DU-8 spectrophotometer. To obtain the transport rate of sodium picrate, the previously described procedure has been followed with the following modifications: 20-mL Beakers were used instead of the 18-mm vials. The internal diameter of the concentric tube was 12 mm. Sodium picrate transport was followed by measurement of the %T in the receiving phase at a wavelength of 354 nm. A layer of 6.0 mL of $CHCl_3$ in a 20-mL beaker was stirred (synchronous stirrer, power 1) using a 7-mm Teflon-coated magnetic bar. The source and the receiving phases were separated by a glass tube (10 mm i.d.) suspended 5 mm above the bottom of the beaker, but below the surface of the $CHCl_3$ layer. The source phase was placed inside the concentric tube. The receiving phase was in the external

compartment. 1.0 mL of the sodium picrate solution (1.0 mM in 0.100 M NaOH) (source phase) and 6.0 mL of distilled and deionized water (receiving phase) were placed on top of the chloroform layer. The concentration of all the compounds studied was 1.0 mM. The transport rate is reported as moles transported/24 h. Ion transport was determined by following the change in absorbance (%T) of the receiving phase at a wavelength of 354 nm. The concentration of sodium picrate was determined from the calibration curve.[29]

Procedure for Bilayer Clamp Studies. Phospholipid membranes were prepared by the painted bilayer method.[39] A lipid solution was prepared by dissolving 30 mg of asolectin[40] in 1.0 mL of decane. This solution was used to pretreat the pinhole in the Teflon cup of the bilayer setup. Normally, 1−5 μL was placed on top of the pinhole and allowed to dry. The cup and cup-holder setup was then assembled. A NaCl solution (0.5 M in H_2O) was added to both the cup and cupholder, taking care that the water level was the same on both sides. A glass rod was dipped into the lipid/decane solution, and the tip of the rod was rubbed across the pinhole in the Teflon cup. Bilayer formation was detected by a sudden drop in conductance. A 10-mV square wave train pulse was applied to the membrane to monitor the membrane capacitance. All of the membrane systems studied exhibited a capacitance of more than 100 pF and a conductance of less than 3.0 pA when a +100-mV holding voltage was applied.

The aqueous solution in the cup holder was carefully stirred, and 10−50 μL of a 10^{-6} M channel solution (in TFE) was added. The solution was continuously stirred for 1−10 min. A holding potential was applied and channel responses recorded. Single-channel signals were detected and recorded using a Digidata 1200A A/D converter and the data acquisition software Axoscope. The holding voltage was changed manually to determine the channel's response at different voltages.

Single-channel current amplitudes and standard deviation values were also determined using Axoscope software.

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