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Hydraphile Synthetic Channel Compounds: Models for Transmembrane, Cation-conducting Transporters

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A family of novel cation-conducting, transmembrane channels has been designed and characterized. A number of functional channels are described along with an account of channel conformation and how the compounds are positioned in the phospholipid bilayer.

Keywords: Cation channel; Phospholipid bilayer; Membrane; Ion channel

1. INTRODUCTION

The cells of all higher organisms are bounded by structures such as membranes or cell walls that play at least two basic roles. They constitute the cellular boundary and they separate the cell's contents from the external environment. This barrier prevents the cell's contents from being lost but it also presents a formidable obstacle to the intake of nutrients and the expulsion of waste products. The passage of cations such as Na^+ and K^+ through the membrane must not only be permitted but also regulated as the intra- and extracellular concentrations of these critical ions differs substantially. The proteins that mediate cation transport are currently receiving enormous attention from the biochemical, biophysical, and molecular biological communities [1]. The challenge to understand the function of molecular channels is enormous and of the profoundest importance. In addition to the study of naturally occurring protein channels by biochemists and biophysicists, several chemical research groups have undertaken the development of synthetic model systems in the hope that such simplified structures will lead to an understanding of chemical function. A few groups have reported the design, synthesis, and study of model channel systems. These are considered in the following sections.

2. STRUCTURES OF CHANNEL PROTEINS

An enormous advance in the understanding of channel compounds has resulted from the publication recently of the first crystal structures of channel-forming proteins. Membrane-resident

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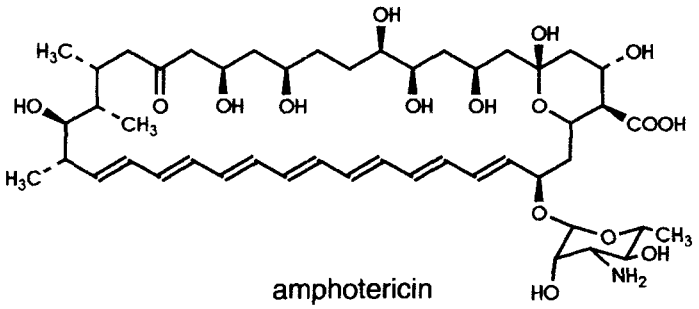
proteins are notoriously difficult to crystallize and the level of experimental achievement in these cases is of the highest order. The first structure to appear was reported by Doyle *et al.*, working in the MacKinnon laboratory [2]. This important work was followed by the structure of a mechanosensitive channel isolated from *mycobacterium tuberculosis* [3]. The keen insights offered by these structural studies are important and will be noted where appropriate in the following text. A detailed discussion of these important structural results is beyond the scope of the current work.



amphotericin

3. NATURALLY OCCURRING MODELS FOR CHANNEL FUNCTION

Several naturally occurring compounds have influenced the thinking of organic chemists interested in modeling channel function. They are the polyene antibiotics [4] such as nystatin and amphotericin B. Two peptides are alamethicin, which forms voltage-gated channels [5], and gramicidin [6], a pentadecapeptide that dimerizes to form the most studied of all cation-selective channel compounds. Unlike proteins, alternate amino acids have D-stereochemistry. Gramicidin D (a mixture of 3 closely related peptides designated A, B, and C) is OHCNH-L-val-gly-L-ala-D-leu-L-ala-D-val-L-val-D-val-L-trp-D-leu-L-trp-D-leu-L-trp-D-leu-L-trp-$CONHCH_2CH_2OH$. The functional form of gramicidin was thought to be the tail-to-tail dimer [7], but extensively studied gramicidin [8] continues to be the focus of controversy [9]. These low molecular weight (MW $\sim$ 1–2 kDa) structures exhibit behavior such as ion selectivity, voltage dependence, subconductance states, blocking, and modulation properties that are characteristic of proteins channels.

4. SYNTHETIC PEPTIDES AS MODELS FOR CHANNEL FUNCTION

Mutter and Montal [10] have used template-assembled synthetic proteins (TASPs) that were expected to form a four-helix bundle in a globular conformation that forms ion channels in lipid bilayers [11]. Tomich, a former collaborator of Montal, has extended this work recently [12].

Important studies conducted at about the same time were reported by DeGrado and coworkers. They studied the mechanism by which α-helices aggregate and they demonstrated that such aggregates could conduct ions. In particular, DeGrado and coworkers [13] prepared model peptides containing only leucine and serine residues. A 21-residue peptide H_2N-(Leu-Ser-Ser-Leu-Leu-Ser-Leu)$_3$-$CONH_2$ formed ion channels, with ion permeability and lifetime (opening and closing) characteristics resembling the acetylcholine receptor. This work has been reviewed recently [14].

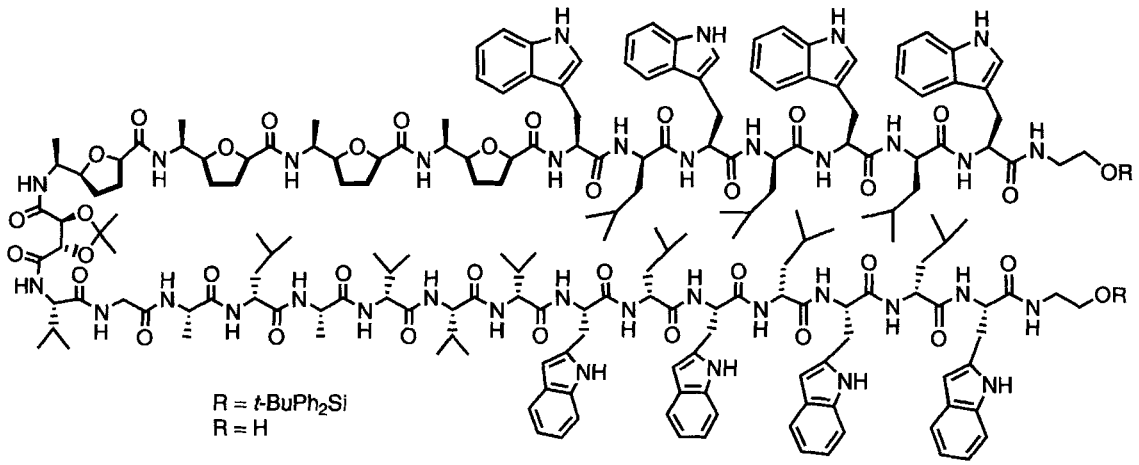
5. SYNTHETIC ORGANIC COMPOUNDS AS MODEL ION CHANNELS

The earliest non-peptidic, synthetic organic channel model was reported by Tabushi and coworkers [15]. In this effort, β-cyclodextrin was used as a "headgroup" in concert with hydrocarbon tails that comprised the channel's "tunnel" or pore element. This structure was designed to reside in each leaflet of the bilayer and provide transport when the two halves were appropriately aligned. Indeed, the transport of both copper and cobalt: $k_{Co(II)} = 4.5 \times 10^{-4}\,s^{-1}$ [channel monomer] = 55 μM were demonstrated.

5.1. Proton-conducting Channels

Several very simple organic structures have been shown to serve as cation transporters although the mechanisms by which these interesting compounds function remain largely obscure. Menger and coworkers prepared $CH_3(CH_2)_{10}$–$COO(CH_2CH_2O)_5CH_2Ph$ [16] and demonstrated dioctadecyldimethylammonium cation reported Na^+ and K^+ transport in a phospholipid bilayer. This work has recently been extended to ion pair channels in which an oligoether chain is attached to the ammonium head group and the hydrophobic counteranion is provided either by stearic acid or by phospholipid headgroups [19]. Regen and coworkers [20] esterified a 5-androstene derivative in the 3β and 17β positions by $OCO(CH_2CH_2O)_nH$ groups and demonstrated modest Na^+ transport.

An additional structural type that merits mention is the poly-THF compounds prepared by Koert and coworkers [21]. Characterization of channel function of the polyether channel by planar bilayer conductance methods showed "spiking" behavior rather than well-behaved gating. In a refinement, a poly-THF chain was terminated by gramicidin-like Leu-Trp repeats and a covalent tartaric acid unit, such as used by Schreiber, was incorporated [22]. This hybrid structure (1) showed channel activity in soybean lecithin bilayers.



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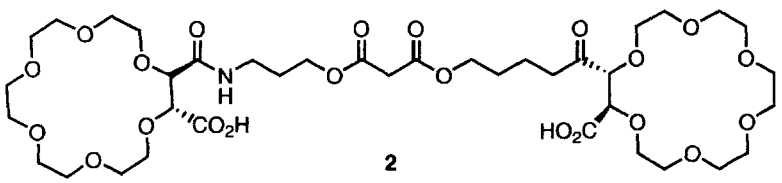
more effective proton transport than observed for gramicidin by using the method of Fendler and Kano [17]. Kobuke and coworkers [18], using a mixture of glycolate ethers of monoalkyloligo(1,4-butylene glycol) complexed with

5.2. Tubular Molecular Structures

Crown ethers have played a prominent role in the development of channel models. Nolte and coworkers [23] obtained an oligomer of a

crown-isonitrile that appeared to be tunnel-like. In vesicles prepared from cation impermeable dihexadecyl phosphate, the oligomer showed Co^{2+} transport with a rate of $k_{Co(II)} \approx 10^{-4} s^{-1}$. 21-Crown-7 ethers comprised the "tunnel" through which alkali metal cations pass in a membrane-active compounds reported by Voyer and Robitaille [24]. The macrocycles were attached to a DeGrado inspired peptide, ~Leu-(CrF-Leu-Leu-Leu-CrF-Leu)$_3$~, known to adopt an α-helical conformation [25]. The macrocycles are appended to every fourth residue aligning them to make the putative tunnel. Cation transport in phospholipid bilayers showed little selectivity among Li^+, Na^+, K^+, and Rb^+.

Macrocyclic polyethers derived from tartaric acid residues were the basis of two systems developed almost simultaneously by Lehn and Fyles and their coworkers. Lehn and Jullien [26] used a central, tartaric acid-derived crown to anchor dendrimer-like chains that radiated from it. Fyles [27] built an amphotericin-inspired tunnel using a similar crown central unit with bolaamphiphilic walls. Fyles produced a variety of ion-transporters and assessed their efficacy by using a proton transport system. Fyles also developed a bola-amphiphilic "membrane disruptor" that is shown below as compound **2**.



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Ghadiri and co-workers [28] reported channel formation from self-assembling peptide "nanotubes". Cyclic peptides incorporating alternating D- and L-amino acids such as *cyclo*[(Trp-D-Leu)$_3$Gln-D-Leu-] were prepared. If planar, the diameter of the internal orifice is 7.5 Å. The stacking of these cyclopeptides is envisioned as involving H-bond formation similar to those found in a β-pleated sheet. The conductance of sodium and potassium chlorides were found to be 60 ± 5 pS.

An interesting family of molecular structures that conduct cations have been reported by Matile and coworkers [29]. Molecules in this family have been referred to variously as "rigid-rods" and π-slides. Ionophoretic activity has been demonstrated in phospholipid membranes by using a valinomycin-coupled transport system.

6. DESIGN OF HYDRAPHILE CHANNEL COMPOUNDS

The challenge that confronts all those who attempt to design biomimetic compounds is to achieve function in a model system when the mode of action is imperfectly understood. An additional problem for the synthetic organic chemist is the desire to achieve elegance in the design and synthesis. In some cases, elegance is a luxury since achieving functionality is the overriding goal. A crude but functional system is clearly superior to an elegant but nonfunctional one.

6.1. Overall Strategy

The function to be mimicked in this case is straightforward: the channel model must be able to get the cation from one side of a bilayer membrane to the other. The advent of crown ethers permitted organic chemists to extensively

study the transport of cations across various membranes. Various crown ether carrier molecules function by complexing a cation on one side of a membrane, ferrying it to the opposite side, and then releasing it to the surrounding medium. Crown ethers have been especially well studied in bulk membrane systems such as chloroform or dichloromethane. Numerous macrocycles and combinations of salts and solvents have been studied [30].

Binding and transport lead one to an apparent paradox. The equilibrium constant, K_S for the reaction

$$\text{crown} + M^+ \rightleftharpoons [\text{crown} \cdot M]^+$$

is determined by the rates at which complexation and decomplexation occur, *i.e.*, $K_s = k_1/k_{-1} = k_c/k_d = k_{complex}/k_{decomplex}$. Crown ethers [31] such as 18-crown-6 bind and release cations with high rates as required for successful carrier transport. Since both binding and release rates are fast, overall binding strength and cation binding selectivity are both relatively poor. In contrast, cryptands are normally strong and selective binders but their binding and release kinetics are poor. The natural mitochondrial transport agent valinomycin achieves good binding strength and selectivity by having a flexible structure that can envelop a cation in a three-dimensional way. We developed the family of compounds we named "lariat ethers" [32] that could achieve both three-dimensional binding and good binding dynamics [33]. We adopted the name lariat ether in part because of the CPK models that resembled a looped rope. In addition, we envisioned that complexation of a cation would involve the ring and sidearm to "rope and tie" the guest. Structures of simple crown, cryptand, and lariat compounds are shown in Figure 1.

Our basic design philosophy has long been to combine structural features thought to be important with structural flexibility. A rigid compound, "perfectly designed" to fit a space or accommodate a certain guest is not adaptable should the design prove to be in error in any way. A structurally adaptable scaffold permits accommodation. Moreover, natural binding partners rarely exhibit perfect correspondence. This is because binding in natural systems is normally a means to an end rather than the end itself. If release was impossible, binding could occur but not, for example, catalysis.

6.2. The Phospholipid Bilayer

In the design of the hydraphile channels, we considered several issues. One was whether or not the channel would span the bilayer or only that part of it that constitutes the insulator. A phospholipid bilayer has dimensions that depend on the fatty acids that comprise it [34]. Generally, though, a phospholipid bilayer has three parts. The overall thickness from headgroup to headgroup is typically 50 Å. The insulator regime or "hydrocarbon slab", made up of the fatty acid tails, is about 30 Å thick [35]. Between the polar headgroups and the hydrocarbon tails lie the glyceryl esters that we have

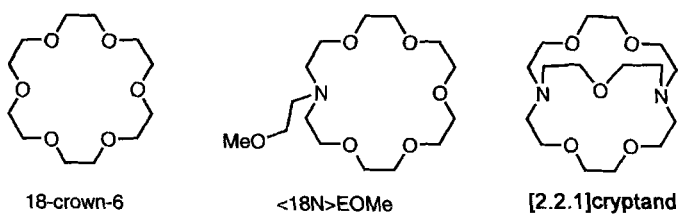


FIGURE 1 Structures of 18-crown-6, an aza-18-crown-6 lariat ether having a methoxyethyl tail, and the cryptand [2.2.1], which has the same number and arrangement of donor groups as has the lariat ether to its left.

called the "midpolar regime". These regions are illustrated in Figure 1.

6.3. Donor Groups and Headgroups

In order for the channel model to insert into and span the bilayer, it must be amphiphilic. If a single molecule spans the channel, it must be a twin-headed amphiphile. Such compounds are sometimes given the cumbersome cognomen "bolaamphiphile", often shortened to "bolyte". The headgroup of a channel must also serve as, or lead to, a cation entry portal. If the model channel is to function as do natural proteins, it must permit the entry and exit of cations but it must not otherwise disrupt the membrane's structure.

We decided early in our studies to position the channel's headgroup near the midpolar regime. Transmembrane segments of proteins for which structures are unavailable are typically identified by hydropathy analysis [36]. In this technique, a computer program examines the protein sequence and calculates how hydrophobic is each 20-amino acid sequence. The two assumptions are that (1) the transmembrane segment is hydrophobic and (2) it is about 20 amino acids if it is α-helical. The calculated hydrophobic sequence is expected to partition into the low polarity bilayer. We thus designed the system to have an overall span of about 30 Å. In the structure of the KcsA channel of *Streptomyces lividans* [2], the α-helices actually span about 34 Å.

Intuition suggests that some donor groups (O, N, or S) must be present or the channel model will have no means available to interact with the transient cation. On the other hand, an array of donors as potent and well organized as one finds in cryptands would doubtless prevent channel function by precluding cation release. Divalent oxygen is expected to be a good donor group for alkali metals. The choice of a crown ether as the organizing matrix for a donor group array was an easy one for us to make. It was based on our experience with these compounds and their excellent synthetic access ability [37].

One aspect of binding must be borne in mind. In aqueous solution, where crown ether binding constants are typically low, 18-crown-6 is selective for K^+ over Na^+ by 18-fold. The reported binding constants are: $K_s(Na^+) = 6.5$; $K_s(K^+) = 118$ [38]. The binding rates ($K_s = k_1/k_{-1}$) are known for these two cases and they differ by 2-fold: $k_1\ (Na^+) = 2.2 \times 10^8\ M^{-1}$ $k_1\ (K^+) = 4.4 \times 10^8\ M^{-1}$. The release rates differ by about 10-fold: $k_1\ (Na^+) = 3.4 \times 10^7\ s^{-1}$; $k_{-1}\ (K^+) = 3.7 \times 10^6\ s^{-1}$ comprising most of the observed selectivity.

A consideration of these rates is important because the channel must be dynamic to be successful. Cryptands are strong and selective binders but they are poor cation carriers. This is attributable to their low decomplexation rates that prevent rapid cation release. Using the rates discussed above, we conclude that Na^+ and K^+ are bound equally well but transport selectivity in the channel will be determined by the preferential release of Na^+. Thus, binding selectivity and transport selectivity are complementary. It is not cation capture that is important for a complexing agent but what we might call "permissivity" [39].

A ubiquitous source of divalent oxygen as a donor is water [40]. It is generally assumed that ions transported by channel proteins are only partly hydrated. Desolvation is an energetically expensive process. The cost of complete cation desolvation is also unnecessary if the cation will be dehydrated on the opposite side of the membrane. It therefore seems reasonable to assume that the cation will be partially hydrated and this, in turn, will afford a larger species.

Taken together, these ideas led to the design shown in Figure 3. The roles of amphiphile headgroup and entry portal were filled by diaza-18-crown-6. This macrocycle was known to exhibit modest K^+/Na^+ selectivity [41]. It had also been reported by Kuwamura [42] and by Okahara [43] that alkyl-substituted crown ethers

exhibited amphiphilic properties. We extended their efforts to twin-tailed diazacrowns and demonstrated that such amphiphiles could form stable liposomes. This observation supported our choice of diazamacrocycles as head groups [44].

6.4. Incorporation of a "Central Relay"

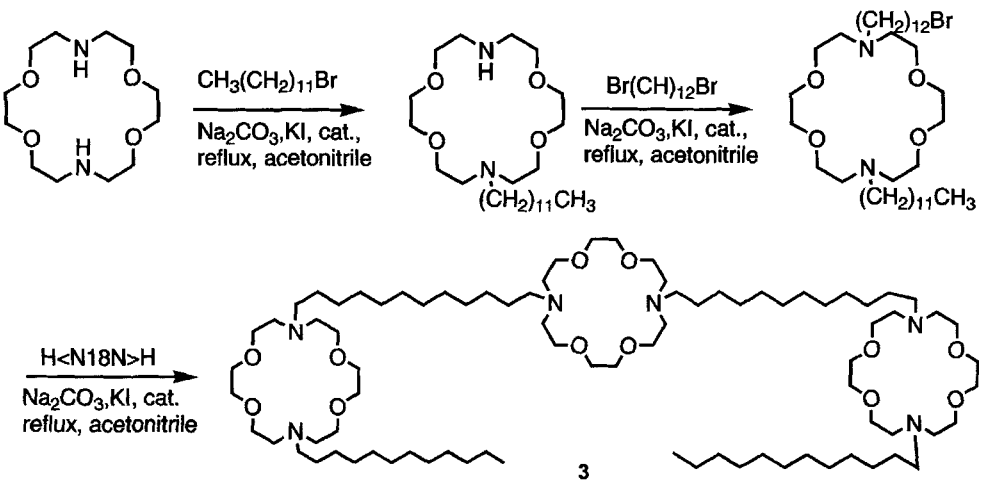
Chemical intuition suggested that a barren, nonpolar tunnel would not be conducive to cation transport through a bilayer. As noted above, the decision was made to incorporate a third diazamacrocycle at the center of the structure. The channel model would thus retain its symmetry but a modest level of polarity would be incorporated at the midplane of the bilayer. It was hoped that this would reduce the overall energy required for transport of the cation across the bilayer. At the time this design feature was incorporated, it was not known whether or how nature solved this perceived problem in protein channels [45].

6.5. Covalent Spacer Chains

Considering the estimated span distance of 30 Å, we chose dodecyl chains to function as the covalent spacers. Simple molecular modeling showed that the N↔N distance in *N,N,N′,N′*-tetramethyl-1,12-dodecanediamine was 16.2 Å. Two such spacer chains would give a span of about 32 Å not including the thickness of diaza-18-crown-6. We felt that covalent attachment among the three macrocycles was required on one side of the molecule only. By attaching the sidearms illustrated in Figure 2 at only one of their ends, we hoped to incorporate flexibility and thus adjustability. It should be noted that the span dimension is based on the notion that the channel model's headgroups will be proximate to the bilayer's midpolar regime. This decision was at worst arbitrary and at best a good guess.

6.6. Synthesis of Channel 1

The first channel we prepared may be represented in a shorthand we have used for some years [46] as $C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}-\langle N18N\rangle C_{12}$. The synthetic approach we use currently is as follows. First, diaza-18-crown-6 is treated with limited 1-bromododecane to afford $C_{12}\langle N18N\rangle$. The monoalkylated crown is then treated with 1,12-dibromododecane to give $C_{12}\langle N18N\rangle C_{12}Br$. Reaction of the latter with diaza-18-crown-6 yields channel 1 (**3**). An approach of this general type has now been applied to more than 30 representatives of this synthetic channel family.



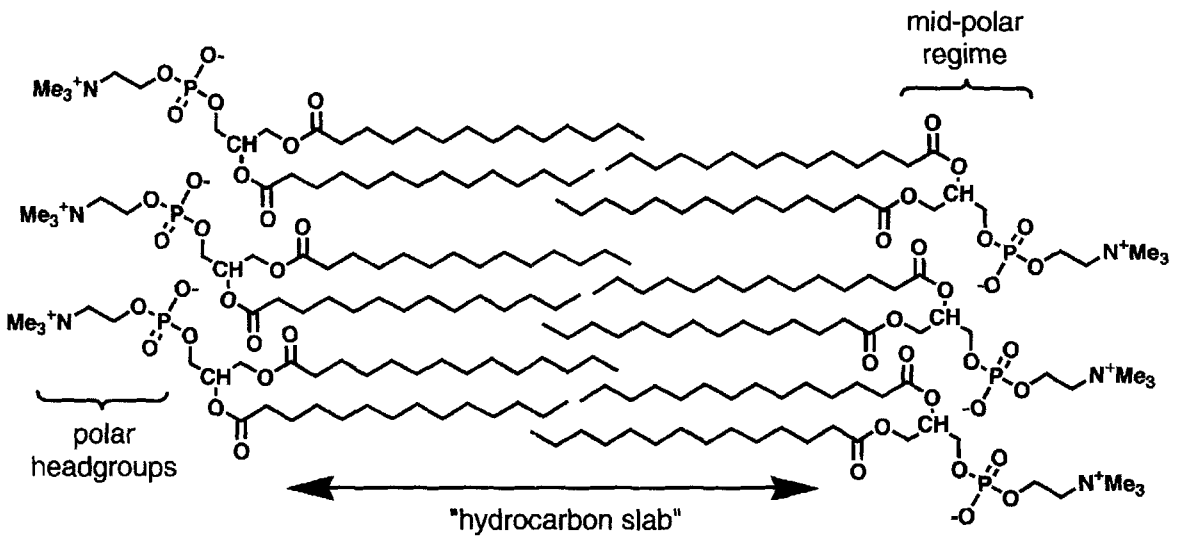


FIGURE 2 Schematic of a phospholipid bilayer membrane showing the insulator regime (hydrocarbon slab), the headgroups, and the midpolar regime.

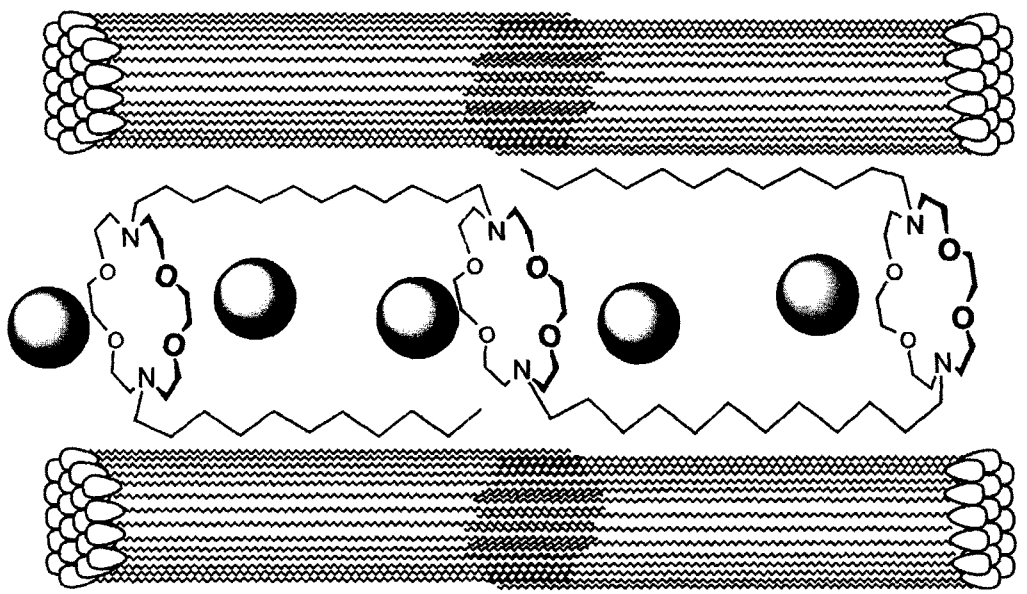


FIGURE 3 Design schematic for the tris(macrocycle) channel molecules.

6.7. Demonstrating Cation Conduction

During the course of the work described here, we have used three different experimental methods to assess ionophoretic activity. Proton transport was assessed by using fluorescence methods [16]. The standard electrophysiological method of "patch clamping" or more correctly in this case, planar bilayer conductance

measurements was applied as well. The meaning of conductance traces is beyond the scope of the current paper and will not be discussed further here [47].

Most of the cation transport measurements conducted thus far utilize a dynamic NMR method developed originally by Riddell [48]. In this technique, phospholipid vesicles are prepared in the presence of 0.5 M NaCl. ^{23}Na-NMR reveals a single sodium line because the ion is identical whether inside or outside of the vesicle. Addition to the external medium of Dy^{3+}, a shift reagent, alters the chemical shift of external Na^+ ion. When an ionophore is present in the bilayer, $Na^+_{(inside)}$ can exchange with $Na^+_{(outside)}$. The equilibrium exchange is detected as a concentration dependent change: $K = 1/\tau = \pi(\Delta\nu_1 - \Delta\nu_0)$. Typically, these experiments were conducted using the channel models at concentrations in the range 0–20 μM. Thus, the channel models active at reasonably low concentration. Compared with the naturally occurring, channel-forming peptide gramicidin [49] (K = 100%) channel 1 (**3**) exhibits a Na^+ exchange rate of 27%. The exchange rate determined for gramicidin in this system is $\sim 175\ s^{-1}$. Cation exchange for compound **3** is also occurring on the millisecond time scale. When the dodecyl sidearms were replaced by benzyl groups (to give $PhCH_2\langle N18N\rangle C_{12}\langle N18N\rangle - C_{12}\langle N18N\rangle CH_2Ph$, **4**), cation transport increased to 39% that of gramicidin. Replacement of the dodecyl sidechains with the fluorescent dansyl group gave a channel with about the same Na^+ exchange rate.

6.8. Control Experiments

The tris(macrocycle) channels functioning in phospholipid bilayers represents a complex experimental system. A number of controls were then undertaken to increase our confidence in the way the data were being interpreted.

Replacement of the dodecylamino sidearms of **3** by an oxygen atom affords a compound that may be represented as follows: $\langle O18N\rangle - C_{12}\langle N18N\rangle C_{12}\langle N18O\rangle$. This molecule possesses three macrocycles and spacer chains of identical length to compound **4**. The central relay unit is intact but the compound is inactive when assessed by the ^{23}Na-NMR method described above. A shorter but sidearmed relative, $C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}$, also failed to show cation transport at a rate detectable by the NMR method.

Another concern was whether or not the tris(macrocycle) channels functioned by detergent action. By this is meant that the "channel" is really a random membrane disruption. Replacement of the tris(macrocycle) ionophore by either Triton X-100, a neutral detergent, or sodium dodecyl sulfate, an anionic detergent gave no detectable Na^+ transport in the NMR assessment. This was true even though a concentration range of 0–200 μM was used for either detergent.

In addition to a simple detergent effect, it seemed plausible that the tris(macrocycle)s were functioning as carriers rather than pore-formers. Indeed, *N,N'*-dibenzyldiaza-18-crown-6 is a reasonable ionophore although its activity as a carrier is not sufficient to be detectable by the ^{23}Na-NMR assay used here.

Assessment of carrier function was undertaken using a concentric tube apparatus [43]. Bulk $CHCl_3$ was used as the membrane for a comparison of 10 compounds. The same group of compounds was assessed by the ^{23}Na-NMR method in phospholipid liposomes. Ionophoretic activity for each compound was assigned in the carrier system relative to valinomycin and relative to gramicidin in the bilayer. The relative values were normalized and compared to each other. A comparison of the activities observed in each series is shown in Figure 4. The standards, valinomycin and gramicidin, are shown at the right and left sides of the graph respectively. The identities of the other compounds are not important except to clearly demonstrate the lack of any significant correlation. While this fails to

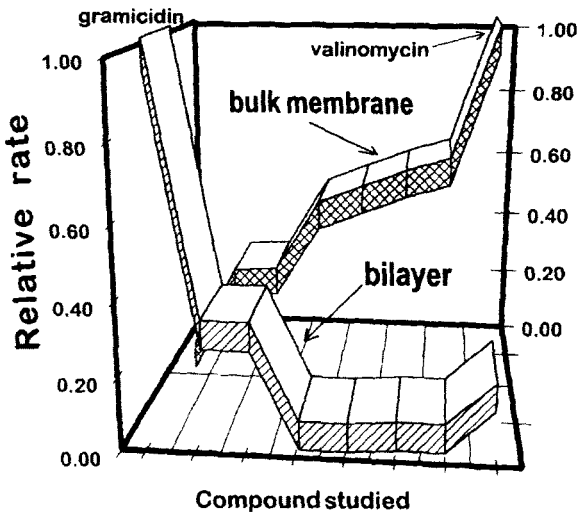


FIGURE 4 Graph comparing relative transport rates of 10 (unspecified) tris(macrocycle) channels. The standards are gramicidin (left) and valinomycin (right).

prove a channel mechanism, it clearly discredits a carrier mechanism within the bilayer [50].

An additional concern was whether the hydraphiles were hydrophobic enough to spontaneously insert in the membrane. The calculation of octanol-water partition coefficients [51] suggested that the tris(macrocycle)s favored octanol (*i.e.*, the membrane) by $>10^{10}$ and in some cases by $\sim 10^{30}$.

6.9. Hydraphile Conformation

When the central macrocycle of **3** was changed from an 18- to a 15-membered ring, the Na^+ transport rate was essentially unchanged. Opening the central macrocycle diminished, but did not prevent, transport. If Na^+ was passing through the macrocycle, it seemed reasonable to expect transport to be slowed by a smaller ring. The conformation shown in Figure 5 was thus inferred.

6.10. Assessment of the Conformation and Location of the Channel within the Bilayer

The sidechain of "channel 1" (**3**) can be altered as noted above. Replacement of the dodecyl sidechains of **3** with fluorescent dansyl groups $Dn\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle Dn$, **5**. The fluorescence spectrum of dansyl is expected to vary with solvent polarity and this expectation was confirmed for **5**. When **5** was assessed

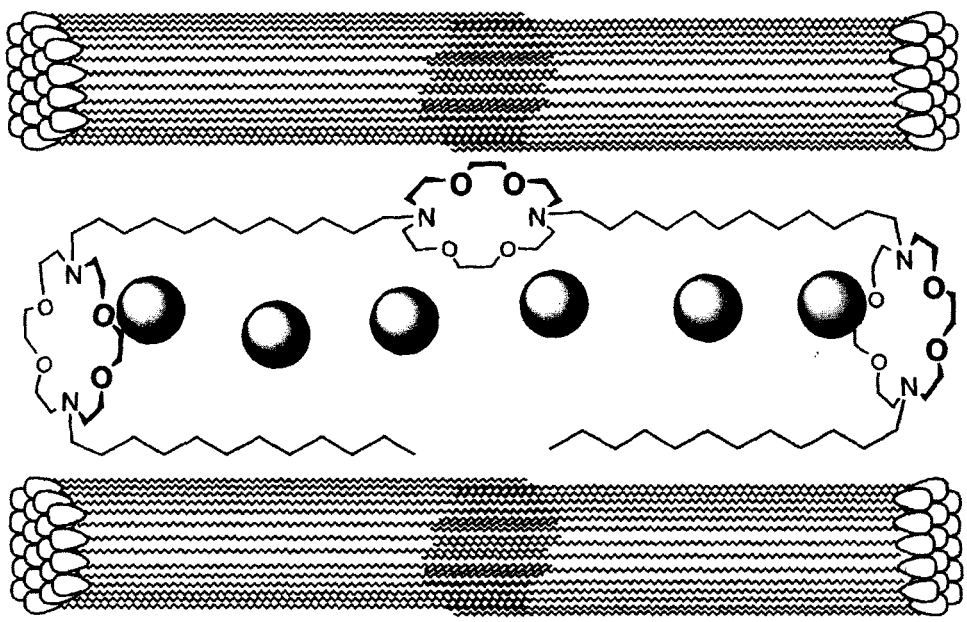
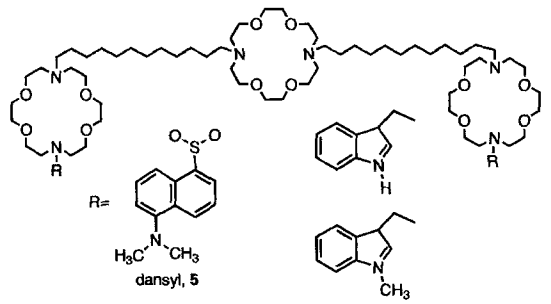


FIGURE 5 Presumed active conformation of "channel 1" (**3**).

in a phospholipid bilayer, the polarity of its environment was found to be between that of methanol and ethanol but closer to the latter. Clearly, the dansyl is not in a water-like environment nor is it buried within the hydrocarbon slab. The polarity generally comports with the notion expressed above that it is near the glyceryl residues [52].



An additional technique that can be used is fluorescence depth quenching. By placing the fluorescence quenching doxyl group at various positions on phospholipid fatty acid chains, it is possible to triangulate the location of the fluorescent residue. Using 7-doxyl- and 12-doxylpalmitoyl-substituted phosphatidylcholines along with **3**, we were (by application of the appropriate equations [53]) able to estimate the headgroup span at 28 Å [54].

Yet a further application of the fluorescent dansyl channel (**5**) can be undertaken in concert with an *N*-methylindolyl terminated analog, **7**. ($MeIndCH_2CH_2\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle - CH_2CH_2IndMe$). Methylindolyl channel **7** absorbs radiation at 283 nm and emits at 343 nm. Dansyl channel **5** is excited at this wavelength and fluorescence at about 515 nm in a phospholipid bilayer. By use of this known method, varying the concentrations of **5** and **7** such that [**5**] + [**7**] = 1 molal, we were able to determine the aggregation state. A plot of log of the fluorescence ratio *vs.* mole fraction gave a line with a slope of 1.12. This suggests that, at least for these two compounds, the channel functions as a monomer.

6.11. Channel Blockage Due to H-bond Formation

We [55] and others [56] have speculated that the indole residue of tryptophan (Trp, W) could function as a membrane anchor in integral membrane proteins. We had previously prepared $C_{12}OTrpNHC_{12}\langle N18N\rangle C_{12}NHTrpOC_{12}$, **8**, to see if tryptophan itself was sufficient to serve as a headgroup anchor and portal. This experiment failed. We then incorporated indole into $InCH_2CH_2\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle CH_2 - CH_2In$ (**6**). We were surprised to note that no Na^+ transport was observed in this case either. An examination of CPK models, confirmed by Monte Carlo simulations, suggested that a hydrogen bond could form between the indole NH and an oxygen atom in the macrocycle. This would block the "entry portal" and prevent channel function. The formation of such a hydrogen bond was confirmed by infrared analysis [57]. We were able to prevent H-bond formation by preparing **7** (discussed above). The *N*-methyl group eliminated the possibility of H-bond formation and the channel was fully functional.

6.12. Nomenclature for the Channel Compounds

We referred generically to the early structures described here as tris(macrocycle)s. Indeed, all of the channels studied in the first phase of this work were tris(macrocycle)s. As the work progressed, it was channel function rather than structure that intrigued us. We have adopted the name "hydraphile." Two references are relevant here. First, the term has an obvious association with the two-headed monster slain by Hercules. Second, the American Heritage Dictionary offers the following definition. A hydra is "any of several small freshwater polyps of the genus *Hydra* and related genera, having a naked cylindrical body and an oral opening surrounded by tentacles." Our compounds are cylindrical and possess sidearms that may be

likened to tentacles. A further definition states: "a persistent or multifaceted problem that cannot be eradicated by a single effort." The latter certainly describes this program, which is very much a work in progress.

6.13. Conclusions

We have demonstrated that a channel can be designed *de novo* and prepared without resorting to natural subunits such as peptides. We have undertaken numerous studies in an effort to characterize channel function and location within the phospholipid bilayer. Overall, the channel inserts into the phospholipid bilayer as do natural protein channels. We believe that the pore fills with water and ions; the host and guests cooperating to organize each other. When a cation enters the pore from one side, a cation in the chain at the opposite side is pushed out making transport of the ion a rapid process.

Now that the channel is reasonably well characterized, we hope to use the model to probe such significant biological questions as how channels transport ions, how they gate, and how they select ions or molecules.

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