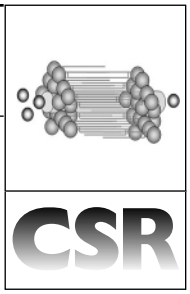
Synthetic models of cation-conducting channels



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Modern channel proteins are complex in structure and marvelous in function. The earliest channels cannot possibly have been so complex but they must have functioned at some modest level. Synthetic chemists have designed a variety of novel structures with the goal of creating a mimic of channel function that is complex enough to function in a bilayer but simple enough to be understood, dissected, and modified. These synthetic model systems are the subject of this article.

1 Introduction

Membranes are ubiquitous barriers that define, bound, and protect cells. They prevent the contents of the cell from being lost and they also prevent intrusion of inappropriate chemicals. The critical importance of this is apparent from the typical asymmetry of a cell: the concentration of sodium cation is 150 mM outside and 10 mM inside. In contrast, $[K^+]_{out}$ = 5 mM and $[K^+]_{in}$ = 150 mM. The bilayer must serve as a barrier to equilibration of these closely related cations while permitting the appropriate concentrations to be maintained. In most cells, it is complex channel-forming proteins that mediate the passage of such cations as Na^+, K^+, and Ca^{2+} as well as organic species such as sugars. During more than a century, the function of channels has been studied by a variety of techniques and much is now known about their biochemistry and biophysics.[1]

A breakthrough in protein channel biochemistry occurred in 1998 when the X-ray crystal structure of the KcsA K^+-selective channel of *Streptomyces lividans* was reported.[2] For the first time, the inferences drawn from biophysical studies could be placed in a structural context. It was gratifying to note that many of the inferred elements of protein channels were apparent in the structure. The channel inserts asymmetrically into the bilayer to form a pore defined by four α-helical segments of the protein. The pore is open at both ends as required for cation transport but it has an overall 'teepee' shape. The crystal structure shows a restriction within the pore thought to be the 'selectivity filter' and both cations and water molecules, arranged single-file, are apparent.

Many issues concerning channel structure and function remain to be resolved, however. This is so not only for the channel biologist but also for the bioorganic chemist. The intimate chemical details of cation transport, gating, and selectivity remain elusive. How the channel passes cations in a single direction ('rectification') also remains unclear. The bioorganic chemist, who is equipped to prepare model systems to selectively assess function, can address such questions. In this article, we describe chemical attempts to model and to understand cation channel function.

1.1 Carrier *vs.* channel transport

A cation carrier is a host molecule that complexes a cation and 'ferries' it across the membrane. This is illustrated schematically in Fig. 1. The box marked 'H' is a membrane-resident

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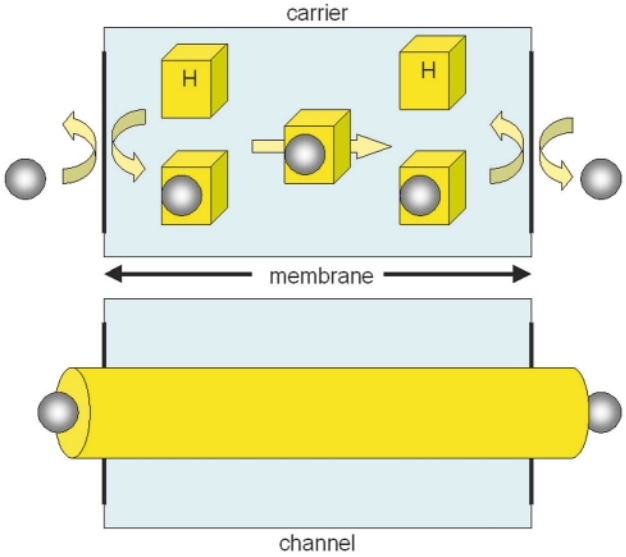


Fig. 1 Schematic of the carrier and channel mechanisms of ion transport.

host. At the aqueous phase–membrane interface, the cation is captured. The complex diffuses across the membrane where the cation is released at the opposite aqueous interface. The cyclic depsipeptide valinomycin is a natural carrier molecule that selectively binds K^+ over Na^+ and the complex moves through the membrane to shuttle the ion from one side to the other. Such 'ionophores' are inherently limited by diffusion rates in the speed at which the host–guest complex of the ion can be transported.

A channel-forming compound functions differently. Like a carrier, the channel is resident in the bilayer membrane but it forms a pore within which cations and water (probably single-file) align throughout. This is shown schematically in the bottom panel of Fig. 1. Like the illustration, the details of the transport process remain obscure. When the channel opens (an ill-defined process called 'gating' that may vary according to channel type), cations flow through the pore and thus through the membrane. Since the ions are presumably in a chain, the cation that exits the membrane is not the one that enters at the opposite side. This 'billiard ball' process makes channel transport inherently faster than the carrier process. Indeed, the only 'definition' available for a channel is that it transports cations 10^3–10^4 faster than does a carrier. Typical protein channels can conduct an astonishing 10^7–10^8 ions per second across a phospholipid bilayer. An important motivation to develop chemical models of channel function is to understand the chemical details of how the ions are conducted, how the direction of transport is selected, and how the 'gate' opens and closes.

1.2 Structure of lipid bilayers

The channel models most relevant to biology will clearly be those that function in a phospholipid bilayer rather than in a bulk solvent or polymer membrane. A brief discussion of phospholipid bilayers may therefore be useful.

A natural bilayer is comprised of numerous phospholipid monomers that organize into a pair of 'leaflets'. The membrane-forming monomers typically consist of two fatty acid groups esterified to the 1° and 2° hydroxy groups of glycerol. The remaining 1° hydroxy is esterified to phosphoric acid, which, in turn, is esterified by another group such as ethanolamine, choline, or serine. The structure of two pairs of membrane monomers is shown in Fig. 2. Each phosphatidylethanolamine has two myristic (tetradecanoic) acid chains. The phospholipids align along the hydrocarbon axes and the two leaflets form a membrane by interdigitation of the fatty acid chains. These chains constitute the 'hydrocarbon slab' or insulator regime, a low polarity barrier that is 30–35 Å thick. The combination of glyceryl esters, phosphoryl groups, and terminal groups adds another 20 Å or so making typical bilayer membranes 50–55 Å thick overall.

The most polar part of the membrane is the headgroup, which interacts with the external aqueous phase and the internal aqueous compartment of a cell. There is, however, a region of intermediate polarity that we have designated the 'midpolar regime' that may be important for channels. This region comprises the carboxylic acid esters of glycerol. The carbonyl groups are potential donors to cations and hydrogen bond formers. Structural evidence suggests that natural channels are anchored in this region of the bilayer. Thus, any model channel must be commensurate with these distances and structural features.

1.3 Chemical models of channel function

With the advent of crown ether chemistry and the numerous derivatives and relatives that it spawned, it became possible to examine the transport of alkali metal cations in membranes. In virtually all of these studies, the carrier mechanism was involved. Thus, the crown ether ('H' for 'host' in Fig. 1) complexes the cation at one membrane–water interface, conducts it through the membrane as the host–guest complex, and releases it at the opposite membrane–water interface. Crown ethers, lariat ethers, cryptands, calixarenes, and other host molecules have all been studied in this context. Informative as such studies were, the relevance to biology was limited because most transport processes *in vivo* involve channels. The challenge of preparing an artificial chemical model of a protein channel was a daunting prospect for two reasons. First, as

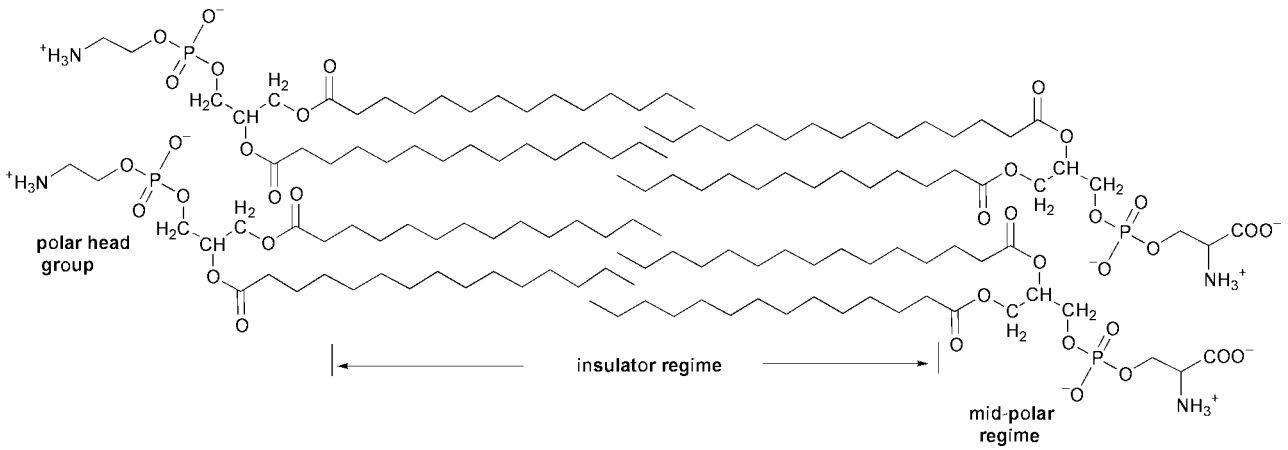


Fig. 2 Partial structure of an asymmetric bilayer membrane.

recently as five years ago, no protein structure was available to serve as an intellectual starting point. Second, the model would have to mimic a natural system in which the chemical details are barely adumbrated. Notwithstanding, that gauntlet was taken up in several laboratories. The approaches and successes are described below.

2 Chemistry, biology, and a primordial channel

An unusual channel system that deserves special note is the Ca^{2+}-conducting channel found in bacteria. It is neither a synthetic model nor a protein. It is important as a starting point for our discussion because it may be a primordial channel that is remote in an evolutionary sense. The channel itself is comprised of poly(3-hydroxybutyrate) (PHB) and inorganic polyphosphate. High molecular weight (10^5–10^6 Da) PHB's were discovered 75 years ago in *Bacillus megaterium* and are now known to occur in a range of organisms from primitive bacteria to highly evolved plants and animals. The biophysics of this channel was worked out in studies conducted by Reusch and co-workers.[3] Seebach and co-workers, in the classical tradition of organic chemistry, realized confirmation of structure and function by total synthesis (Fig. 3). These channels are

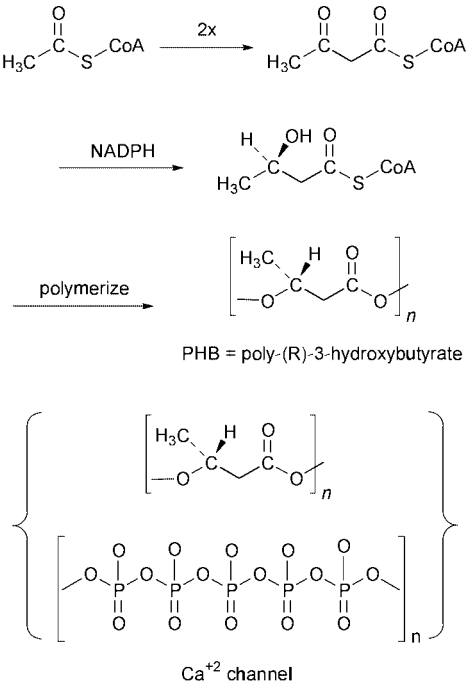


Fig. 3 Preparation of the poly(hydroxybutyrate)–polyphosphate calcium channel.

structurally simple, readily formed biosynthetically, and they occur widely. It has been speculated that polyphosphate and PHB were components of very early cells, possibly preceding the evolution of RNA.

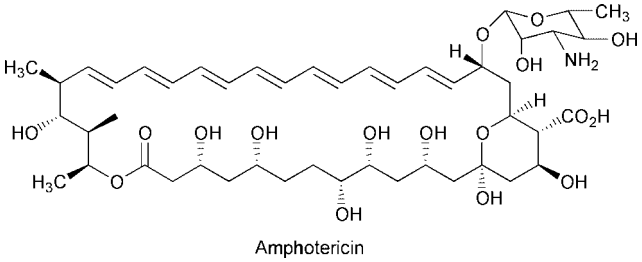
The existence of such channels is an important reminder that elaborate proteins having complex functions must have evolved from much simpler entities. Like primordial channels, artificial model systems may exhibit simplicity in structure but their function will define their value.

3 Low molecular weight, natural channel-formers

Naturally occurring compounds that form channels in bilayers are advantageous for biological study. First, channel formation may be their primary biological function, making their study self-validating. Second, as natural compounds, they can be obtained in a known form suitable for direct study. Peptides are particularly attractive as channel models because they are compositionally identical to proteins, except for the appearance of the occasional D- or uncommon amino acid. Other substances have been studied, however. In the sections that follow, we focus on some of the best-studied natural models, from which an abundance of data has been obtained.

3.1 Polyene-polyols: nystatin and amphotericin B

The channel-forming antibiotics nystatin and amphotericin are closely related in structure. Amphotericin is an important systemic anti-fungal agent that is thought to form channels or pores in fungal membranes. As shown in the illustration, the right 'end' of the molecule has hydrophilic residues that could be considered headgroups. The left end of the molecule is less polar and the interior contains a polyene that rigidifies the structure. The multiple hydroxy groups could serve as coordination sites for a transient cation. A molecule of amphotericin is similar in width to a single leaflet of a phospholipid bilayer. The flat, rectangular molecules may form a pore in much the same way that the staves of a barrel form a cylinder—hence the 'barrel stave model'. Although much studied, it must be admitted that the barrel stave structure remains to be confirmed and the mechanism of transport remains unknown.



3.2 Gramicidin and alamethicin

The name gramicidin is applied to a peptide mixture isolated from *Bacillus brevis*. Gramicidin D (Dubos) contains three individual peptides (gramicidins A, B, and C) in an approximate ratio of 80 : 5 : 15,[4] respectively. The structures may be summarized as OHCNH-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-xxx-D-Leu-L-Trp-D-Leu-L-Trp-CONH-CH_2CH_2OH, in which the xxx residue is Trp in gramicidin A (gA), Phe in gB, and Tyr in gC. The peptide dimerizes in a tail-to-tail (end-to-end, sometimes called head-to-head, N-terminus to N-terminus) fashion to form a helix of approximately 4 Å diameter through which cations pass. This structure, known as a $\beta^{6.3}$-helix or π-helix, forms a single internal pore. This is unlike most protein channels, which arrange multiple α-helices into a structure that forms a pore surrounded by them. Individual α-helices have essentially no interior cavity that could accommodate a cation within it and pores involving α-helices typically consist of 4–7 transmembrane segments. The tail-to-tail arrangement was confirmed by a bioorganic model approach as described in section 4.1.

The features that commend gramicidin for study as a channel model are that it is a peptide of appropriate span, it conducts monovalent cations, and it is blocked by divalent cations. Transport rates observed for Na^+ and K^+ are $\sim 10^7$ ions s^{-1}, which are similar to protein channel values. The presence of alternate D-amino acids, the end-to-end dimerization, and the fact that the pore is inside the helix all detract from the realism of this model.

Alamethicin is in the family of aminoisobutyric acid (Aib) containing peptides. The presence of this amino acid fosters

helix formation. The peptides in this family form voltage-gated channels across membranes (perhaps using the barrel stave mode) and are therefore models for potential-mediated channel opening and closing. Alamethicin has the sequence Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol (Phol or Phl is L-phenylalaninol). Although these peptides have proved to be important model systems for the study of channels, they are generally beyond the scope of this review. They have, however, been used in a few cases as components in semi-synthetic channels. This is described in the next section.

4 Structurally modified peptides

Although gramicidin, the prototype channel-forming peptide, has been known for decades, its study continues unabated. At this writing, more than 7000 references to gramicidin may be found on the American Chemical Society's SciFinder database. Structural alterations of this molecule have been used to help understand its chemical mechanism of action and to alter its properties. The well-studied alamethicin has also been modified and is discussed in section 4.3 below.

4.1 A covalently-linked gramicidin dimer

As noted above, gramicidin is thought to function as an end-to-end dimer in which each peptide effectively spans one leaflet of the bilayer. Efforts by Heinemann, Schreiber, and their co-workers resulted in the preparation of a covalently-linked dimer. Thus, the amino termini of two gramicidin A molecules were linked to each of two carboxy groups in a hydroxy-protected tartaric acid derivative. The (*S*,*S*)-tartaric acid dioxolane is shown in Fig. 4 with attached peptide chains. Cation

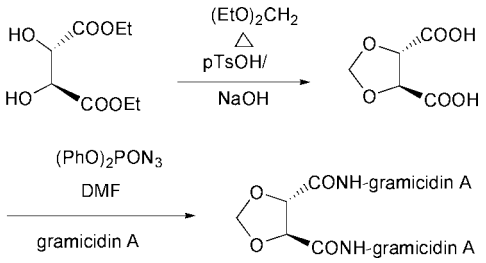


Fig. 4 A covalently-linked gramicidin channel.

conduction in bilayers was confirmed by patch clamp measurements.[5] This provided excellent confirmation of the presumed dimer structure. In later work, with an eye toward developing a molecular switch, stereochemical and steric variation was used to alter gating behavior.

4.2 Molecular modifications in gramicidin channels

As noted above, the structure and behavior of gramicidin are well known and the channels it forms are active and robust. Gramicidin may therefore provide an especially favorable scaffold for assessing the influence of structural modifications. Moreover, the remarkably sensitive patch clamp technique permits evaluation of the function of a single molecule within the bilayer. Woolley and co-workers have used this idea to advantage in two different molecular switches.[6] In the first, the C-terminus of gramicidin was converted from –OH to the peptide carbamate gramicidin-O-CO-NHCH$_2$CH$_2$NH$_2$. The barrier to rotation about the carbamate's CN bond is reported to be in the range of ~13–17 kcal mol^{-1}. Such isomerization alters the position of the terminal aminoethyl group and thus the channel's entry portal. The terminal amino group was protonated in the studies reported so pH was not a variable in the studies. Isomerization was detected at the molecular level by monitoring Cs^+ transport in single channels.

An attempt to achieve greater control of transport involved functionalizing gramicidin's C-terminus with an azobenzene derivative (top panel of Fig. 5). Ground state *trans*-azobenzene can be photoisomerized to the *cis* form. This isomerization changes the molecule's overall conformation and length. Again, single channel recordings of Cs^+ flux were used to monitor the behavior of the two major channels, *cis–cis* and *trans–trans*. Irradiation with a nitrogen laser (λ = 337 nm) afforded a photostationary state (>85% *cis*). This work presents a clear example of a photo-switched, gated channel molecule.

4.3 A redox-sensitive alamethicin derivative

Voltage sensitivity was added to the channel-forming ability of alamethicin by modifying it with a terminal ferrocene (Fc-ALM, bottom panel of Fig. 5).[7] Ferrocene undergoes facile oxidation from the neutral Fe(II) state (yellow–orange) to the positive Fe(III) state (blue). The presence of an integral positive charge was expected to alter the channel activity. When the compound shown in the bottom panel of Fig. 5 was studied in a planar phospholipid bilayer (0.5 M KCl), channels showing ion transport behavior similar to that of unmodified alamethicin were observed although a somewhat higher potential was required for activation. When the structurally-modified channels were oxidized using ceric ammonium nitrate, a 'time dependent diminution of Fc-ALM channels at constant bilayer potential' was observed. Increasing the membrane potential rescued channel function. Such model systems may prove to be useful in understanding the chemical details of voltage gating.

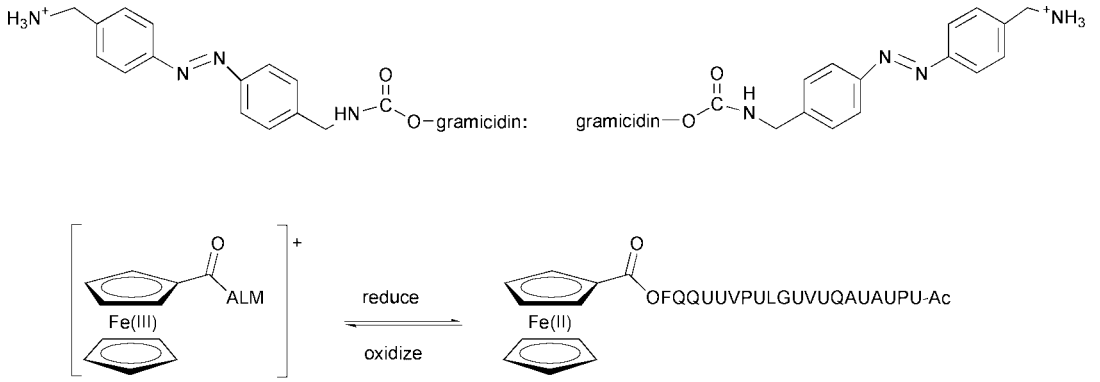


Fig. 5 Schematic of the photo-switchable *trans*-azobenzene–gramicidin dimer channel (top) and a redox-switchable alamethicin derivative.

5 Peptidic ion-channel models

The compounds and concepts discussed in this section should be distinguished from peptides that form ion channels. The structures described here fall into two groups: peptides that organize or are organized into channels and channel-forming structures that involve integral peptides.

5.1 Template-assembled synthetic proteins (TASP's) of Mutter and Montal

The strategy in this work was to determine sequences within proteins that were likely to be part of the channel pore. These sequences would then be prepared as peptides and assembled on an appropriate scaffold into active channel compounds.[8] The concept is illustrated by a synthetic structure assembled from a cyclic decapeptide having the sequence [–CKAKPGKAKC–] in which the two cysteines are linked to each other by a disulfide bridge.[9] Attached to the amino terminus of each of the four lysines is the melittin sequence GIGAVLKVLTTGLPALIS-WIKRKRQQ. Melittin, the bee-sting peptide, is thought to form a channel by aggregating into a tetramer (Fig. 6). The synthetic

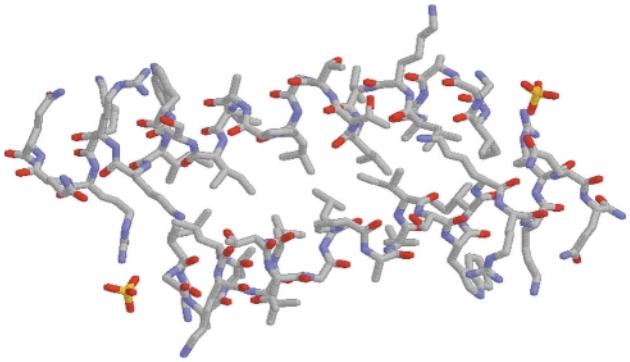


Fig. 6 Solid-state structure of two mellitin molecules.

peptide formed by this approach exhibited channel activity and was studied in detail by a variety of biochemical techniques.

Asami and co-workers used an alternating lysine-3-amino-benzoic acid cyclic template to link four alamethicin peptides in parallel. The structure was intended to mimic the putative 'barrel stave' organization of alamethicin and did, indeed, exhibit channel-like properties.[10] A cyclic alanine—3-amino-benzoic acid (Ala-Aba)$_n$ scaffold (n = 3–5)—was used by Ishida and co-workers.[11] In this case, the benzoic acid's 5-position was substituted by a fatty acid amide of 10 or 16 carbons. Single channel K^+ (0.5 M KCl) currents were observed for these compounds. Conductances were all in the 9 pS range for the four compounds studied.

5.2 A synthetic, channel-forming peptide channel

Extensive work by DeGrado and co-workers[12] has involved synthetic peptides constructed from leucine and serine residues. A typical 21-residue peptide prepared as part of these studies is H_2N-(Leu-Ser-Ser-Leu-Leu-Ser-Leu)$_3$-$CONH_2$. This compound was shown to form ion channels, the ion permeability and open lifetime properties of which resembled those known for the acetylcholine receptor. These efforts were inspirational for the work of Voyer (section 5.4) but are otherwise beyond the scope of this review.

5.3 Self-assembling peptide nano-tubes as ion-channels

Ghadiri and co-workers have prepared cyclic peptides that stack upon each other and organize into 'nano-tubes'. The cyclopeptides were constructed of alternating D- and L-amino acids, by virtue of which the sidechains turn 'outward' and the amides may align to form stacked hydrogen bonds. One of the sequences that was studied was cyclo[-(D-Ala-Glu-D-Ala-Gln)$_2$-]. The cyclic octapeptide chosen for channel formation, cyclo[-(Trp-D-Leu)$_3$-Gln-D-Leu-], is shown in Fig. 7 and was

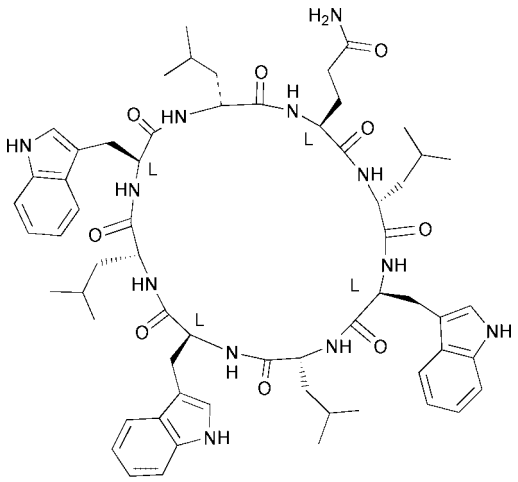


Fig. 7 Structure of the octapeptide cyclo[-(Trp-D-Leu)$_3$-Gln-D-Leu-] that forms nanotube channels.

estimated to have an internal diameter of 7–8 Å. The nano-tube showed clear evidence of channel activity when incorporated (presumably stacked) within a phospholipid bilayer. The relatively large pore size (compared to the diameter of Na^+ or K^+) afforded significant ion transport (the conductance of 0.5 M NaCl was 55 pS, and 65 pS for 0.5 M KCl). The open–close behavior of these nano-tubes was very reminiscent of natural protein channels. Of course, the similarity in conductances for Na^+ and K^+ bespeaks relatively low cation selectivity.[13]

5.4 Crown ether-containing peptides

The helix-forming proclivity of aminoisobutyric acid ('Aib', methylalanine or dimethylglycine) is well documented. It has been incorporated into (Ala-Aib)$_{4,8}$ sequences C-terminated with an additional alanine attached to benzo-18-crown-6.[14] The latter was appended to serve as an 'ion-binding site'. Different ion currents ([KCl] = 1 M) were observed for the two peptides but the recording conditions were such that the data were not comparable. Significant ion transport activity was, however, apparent in both cases.

The combination of helical peptide and crown ether was used in a different fashion by Voyer and co-workers in the formation of a poly(crown) channel.[15] Every fourth residue of a DeGrado-like peptide (see above) was substituted by benzo-21-crown-7 (Fig. 8). Modeling studies suggested that the crowns were aligned in a columnar fashion because the helical peptide backbone oriented them to the same side of the chain. In the earliest stage of the work, proton transport was demonstrated. In later studies, the patch clamp technique was used to demonstrate Na^+ transport through the bilayer. Significant ion transport was observed from +60 to −60 mV using 100 mM NaCl. Behavior most typical of natural channels was obtained for H_2N-A-CE-AAA-CE-AA-CE-AAA-CE-AA-CE-AAA-CE-A-OH in which A is alanine and CE is the benzo-21-crown-7 derivative of phenylalanine.

5.5 An oligo(THF) peptide model

A relatively recent contribution by Koert and co-workers used oligomers of tetrahydrofuran (THF) linked at the 2- and 5-positions. A number of derivatives were reported having as many as 30 linked THF units. In some cases, groups of three

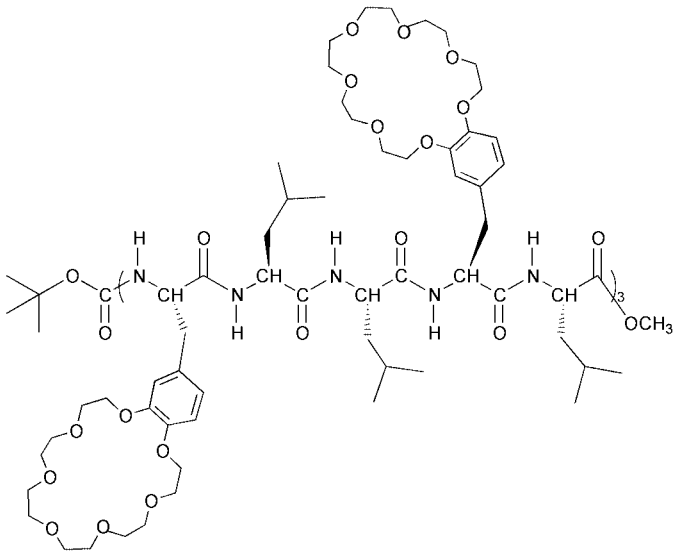


Fig. 8 A poly(crown) channel supported on a helical peptide backbone.

THF units were connected by an amide link. Evidence from NMR (NOESY) analysis suggested that the amides afforded turns in the chains and the overall conformation was suggested to be a helix.

Planar bilayer conductance methods were used to assess the transport efficacy of these systems. In this method, a planar phospholipid bilayer is created in a pinhole in Teflon separating two reservoirs. The reservoir chambers are charged with different solutions so that an ionic gradient is created. A sensitive electrode and amplifier assembly then measures conductance when a transporter is present in the otherwise insulating bilayer membrane. The oligo(THF) derivatives showed evidence for rapid opening and closing in the presence of 1 M aqueous KCl. Peak conductances were approximately 150 pS. The type of membrane activity observed is often called 'spiking' or 'bursting' behavior because the well-behaved flat-topped, integral open states observed for gramicidin and most protein channels is not apparent.

A further elaboration of this system incorporated the tartaric acid link of Schreiber (see below) to connect a tetrakis(THF) unit and peptides.[16] Each of the chains was terminated by an oligomer of tryptophan (Trp, W) and leucine (Leu, L; *i.e.*, WLWLWLW). As noted above, gramicidin A has a terminal WLWLWLW sequence. The tryptophans are thought to serve, at least in part, as membrane anchors to stabilize the channel's position in the bilayer. The final structure of the active compound may be illustrated schematically as shown in Fig. 9.

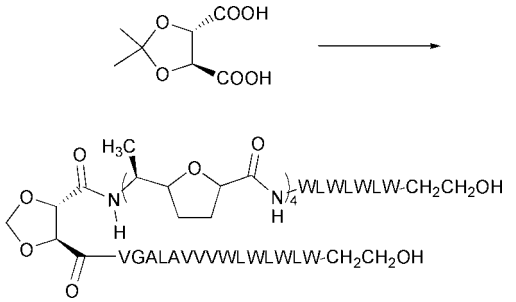


Fig. 9 An oligo(THF) peptide based channel modeled in part on the structure of gramicidin.

6 Non-peptidic organic synthetic channel models

Such models of protein channel function as gramicidin and alamethicin are of profound importance and the greatest interest. Synthetic peptides or peptide derivatives that show channel activity are also fascinating model systems. The main focus of this review, however, is synthetic organic model systems that have been deliberately designed to function as channels in bilayers. More particularly, compounds that have been designed to conduct the biologically relevant ions Na^+, K^+, and Ca^{2+} hold the greatest interest. In the sections that follow, we describe these efforts and their relative levels of success.

6.1 A solid-state channel model

In 1982, Behr, Lehn, Dock, and Moras reported the solid-state structure of 2,3,11,12-tetracarboxamido-18-crown-6.[17] The compound was prepared from tartaric acid and adjacent $CONH_2$ sidearms were disposed *trans* diaxial with respect to the mean plane of the macroring. The crystal structure showed stacked macrocycles that formed a channel having K^+ and water within it. The authors concluded that 'the present structure possesses three main features, which are expected to have a role in ion flow through membrane protein channels: a chain of cation binding units; a polar inside/apolar outside orientation of residues; and mixed site cation binding. It may therefore be considered a solid-state model of a molecular channel with cation propagation through the channel from one binding site to the next'. This assessment has proved to be only partly correct. Even so, converting this intriguing model to a functioning channel has proved to be a daunting task.

6.2 A β-cyclodextrin-based, half channel compound

A discussion of synthetic organic channel model systems must necessarily begin with a description of Tabushi's pioneering channel compound.[18] Like several of the structures discussed above, the tetra-chained cyclodextrin was designed to reside in only one of the bilayer's leaflets. Gating (opening and closing) was expected to result from the tubular structures passing by each other and momentarily creating a transmembrane tunnel or pore. With that in mind, the structure was designed to have a polar headgroup that also constituted an entry portal. The β-cyclodextrin unit has 14 hydroxy groups on its secondary rim (Fig. 10). These hydroxy groups will surely face the aqueous

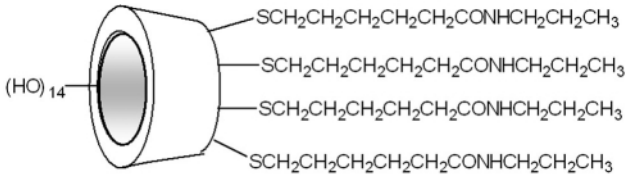


Fig. 10 Tabushi's cyclodextrin-headgrouped cation transporter.

medium. Cations may enter the hole within the cyclodextrin, the diameter of which is ~8 Å. Four of the 7 primary hydroxy groups are substituted by $S(CH_2)_6CONH(CH_2)_2CH_3$ chains. These chains span about 13 Å. The depth of a cyclodextrin molecule is nearly 8 Å. The hydroxy groups add another ~2–3 Å so the overall monomer measures >20 Å in 'head-to-tail' length. Tabushi and co-workers estimated the overall dimer dimension to be 48 Å. In either case, two such structures would readily span the 30–35 Å of a typical bilayer's insulator regime.

Assessment of cation transport posed an obvious problem. Ideally, one would like to know the rate at which Na^+ or K^+ is transported through a phospholipid bilayer. Organic chemists typically do not possess patch clamp amplifiers that can monitor single channel events in phospholipid membranes. In this case, Tabushi and co-workers assessed transport of Cu^{2+} and Co^{2+} in egg lecithin membranes. The channel-former was present in the

membrane at concentrations in the range 0–55 μM. It was found that Co^{2+} transport followed second order kinetics with respect to channel concentration but Cu^{2+} transport was first order. The channel transport rate (at 55 μM) was reported to be 4.5×10^4 s^{-1}. It was noted that this rate was 'much faster than the specific carriers; *e.g.*, 5.4×10^5 s^{-1} under the corresponding conditions for 18-azacrown-6'. Both the channel and carrier rates reported are relatively slow but comparison of either with transport of alkali metal cations has not, to our knowledge, been done.

6.3 A synthetic, H^+-flux promoting compound

An early and remarkable success in developing a proton-transporter was reported by Menger and co-workers.[19] They were attempting to prepare model phospholipids that would have two different tails: stearic acid and $HOOCCH_2(OCH_2CH_2)_nOCH_3$. During their studies, they obtained the compound $CH_3(CH_2)_{10}COO(CH_2CH_2O)_5CH_2Ph$. Its ability to transport protons was assessed in phospholipid bilayers by using a previously published pyranine dye-based technique. This is a spectroscopic method that involves trapping fluorescent pyranine dye inside phospholipid liposomes (vesicles). When the transporter inserts in the bilayer, the pH change that accompanies proton loss is followed by a change in fluorescence.

The mechanism by which this proton transporter functions is unknown. Its ability to transport protons exceeds that of gramicidin (see above). The transport of alkali metal cations was not reported so the comparison with gramicidin is limited. Such issues as orientation and aggregation of the monomer have not been studied and this pioneering work remains a curiosity.

6.4 Synthetic systems that transport metal cations

It was noted above that this article would emphasize the transport of biologically relevant cations. In early channel model systems such as that designed by Tabushi, cobalt transport was successfully monitored. Two other synthetic efforts deserve mention but will not be discussed extensively because the relevance of cobalt to sodium or potassium cation transport is unclear.

Nolte and co-workers[20] polymerized an isocyanide derivative of benzo-18-crown-6. An internal helical backbone organized the macrocycles into four 'stacks' (Fig. 11). This pseudo-

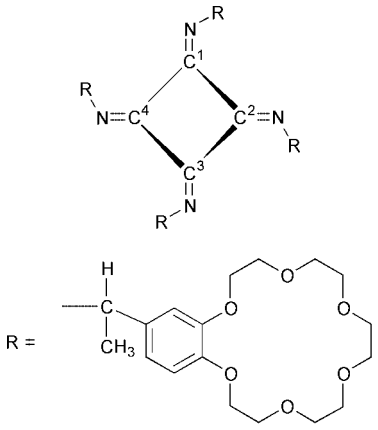


Fig. 11 A poly(isonitrile), stacked ion transporter.

tetramer was inserted into liposomes formed from a synthetic surfactant and Co(II) transport efficacy was assessed by using a fluorescent dye. Transport was monitored over 1 h and a steady increase in cobalt was detected. Triton X-100 was added after 1 h. The neutral detergent ruptured the vesicles permitting the remaining cobalt to be rapidly lost. This observation confirms that transport was controlled and that the bilayer remained intact throughout the experiment.

A 38-membered, tetraether macrocycle was prepared by heating $S(CH_2CH_2OH)_2$ and $HO(CH_2)_{12}OH$ in the presence of toluene-*p*-sulfonic acid. This macrocycle was converted into a two-headed amphiphile (a 'bola-amphiphile') by oxidation or alkylation of the sulfur atoms. Vesicles were formed from these amphiphiles by sonication. Transport of Li^+ or Fe^{2+} was assessed by fluorescent dye methods after addition of a dicarboxylated hexamine (shown in Fig. 12). It was possible to block the pores by using various organic ions.[21]

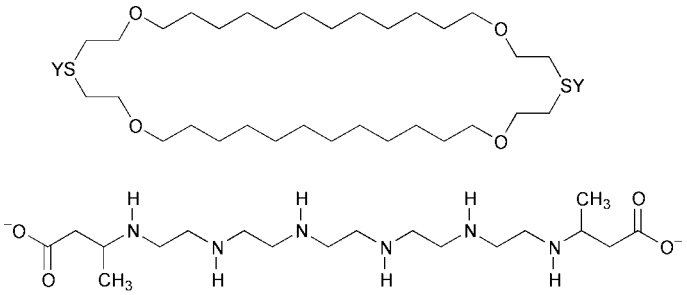


Fig. 12 Bola-amphiphilic elements of a pore-forming system that transports Li^+ and Fe^{2+}.

In another study involving a 'bola-amphiphilic' structure, a substituted octaamine was prepared. This structure involves a 40-atom chain in which $(CH_2)_3$ or $(CH_2)_4$ alternates with NAda (Ada = adamantanylmethyl). The structure may be represented schematically as $^-O_3S(CH_2)_3[NAda(CH_2)_{3,4}]_8(CH_2)_3SO_3^-$. The sulfonic acid residues presumably served as headgroups and the adamantane groups may have disrupted the packing of the fatty acid chains. In any event, insertion of the amphiphile into phosphatidylcholine liposomes led to measurable proton efflux during a period of approximately 2 h.[22]

Kobuke and co-workers[23] used a combination of monoalkyloligo(1,4-butylene glycol) glycolate ethers and dioctadecyldimethylammonium cation to transport Na^+ and K^+. It was suggested that the anionic carboxylate of $BuO[(CH_2)_4O]_n(CH_2)_4COO^+$ associated with the cationic nitrogen of $Me_2N^+(C_{16}H_{33})_2$. The 'tail' chains of each structure were presumably aligned as shown in Fig. 13. Cation transport was

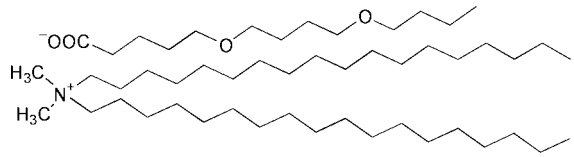


Fig. 13 Carboxylate and quaternary ammonium ions that engender ion transport in bilayers.

demonstrated by using the patch clamping methodology common in electrophysiology laboratories but not, to our knowledge, applied previously to synthetic chemical channel model systems. Using these methods, these relatively simple adducts were found to exhibit (similar) single channel conductances for Na^+ and K^+. The remarkable transport efficacy of these simple structures must be tempered by recognition of relatively low ion selectivity.

6.5 Central scaffold molecules

Some efforts to develop channels have used what might be referred to as a central scaffold strategy. The basic notion is that a cyclic structural unit such as a crown ether or a cyclodextrin comprises a building block from which the channel walls or

tendrils radiate. A particularly popular unit has been an 18-crown-6 incorporating 1–3 tartaric acid units. Tartaric acid has the structure HOOC–CHOH–CHOH–COOH. The two central carbons comprise a substituted ethyleneoxy unit that can be interchanged with ethylene glycol in the crown structure. This leaves two carboxy groups having specific stereochemistry on which to anchor other strands or residues.

Jullien and Lehn reported one of the earliest 'full-span pore channel model'. The centerpiece of this structure was the tartaric acid-based crown ether of which the channel-like structure had been obtained some years before. The design was a 'molecular sheaf…formed by bundles of oligo(oxyethylene) chains grafted onto' the macrocycle. The macrocycle was expected to reside at the midplane of the bilayer and the oxyethylene fibers terminated by polar groups would radiate from it in both directions to form a membrane-spanning pore. The compound was named 'chundle,' a contraction of *ch*annel and b*undle*. No transport data were included in the first report of the structure. In later work, the central scaffold evolved to a cyclodextrin residue and the cognomen was altered to 'bouquet molecule', but the overall strategy remained similar. For all their structural complexity, alkali metal ion transport for these systems was relatively poor.[24] These intricate and structurally complex molecules are illustrated in Fig. 14.

The 'tartaric acid–crown' strategy was used by Frye and co-workers who appended six cholesterol units to a central macrocycle *via* the steroid's tail chain.[25] In this design, three of the steroids faced in one direction from the planar macrocycle and three faced in the opposite direction (see Fig. 15). In principle, this formed a cylinder, the axis of which comprised cholesteryl walls that could interact with the lipid chains of the phospholipid bilayer. It was anticipated that the 3-hydroxy groups in the cholesterol A-ring would serve as headgroup anchors.

Transport of both Li^+ and Na^+ was assessed by using the dynamic NMR method developed originally by Riddell and Hayer.[26] The studies were conducted in phospholipid vesicles and several control experiments were also undertaken. The authors noted that the 'relatively slow rate observed for sodium ion translocation by [the] potential ion channel…may be a result of known interactions between crown ethers and alkali metal cations'. In any event, the cation transport efficacy was low and additional studies of this potentially interesting structure have not appeared.

The tartaric acid–18-crown-6 scaffold was also used in the development of a family of channel-forming structures reported by Fyles and co-workers.[27] The design was of the tunnel or pore type. An effort was made to create a pathway in the membrane using the structural properties of the macrocycle. The inherent selectivity of the macrocycle was expected to affect which cations were transported most effectively.

Fig. 16 shows the chemical structure of one of the molecules prepared for these studies. Below it is a schematic of the overall pore structure as it was envisioned. Although conceived and executed separately from and either prior to or simultaneously with other tartaric acid-based structures, the basic design is similar in some respects to the efforts of Lehn and Frye[24] although it preceded the latter.[25]

The Fyles design uses twin-stranded bola-amphiphiles as the 'walls' of the channel. These are compounds that have two headgroups and two chains connecting them. In this case, they are actually macrocycles similar in some respects to those discussed in section 6.4. These bola-amphiphiles are secured to the crown by ester linkages. The carboxy groups at each terminus constitute headgroups that face the polar surfaces of the bilayer membrane.[27]

The family of compounds that was prepared used two, four, or six 'bola-amphiphile walls' attached to 2, 4, or 6 carboxy groups (1, 2, or 3 tartaric acid units in the crown ether). A number of different wall units were incorporated that varied primarily in polarity. The transport efficacy of the channel models was assessed by using a competition between metal cations and protons. Although the experimental system is somewhat complicated, it permitted a study of Li^+, Na^+, K^+, Rb^+, and Cs^+ and was not limited to Li^+ or Na^+, ions which could readily be studied by dynamic NMR methods. By varying

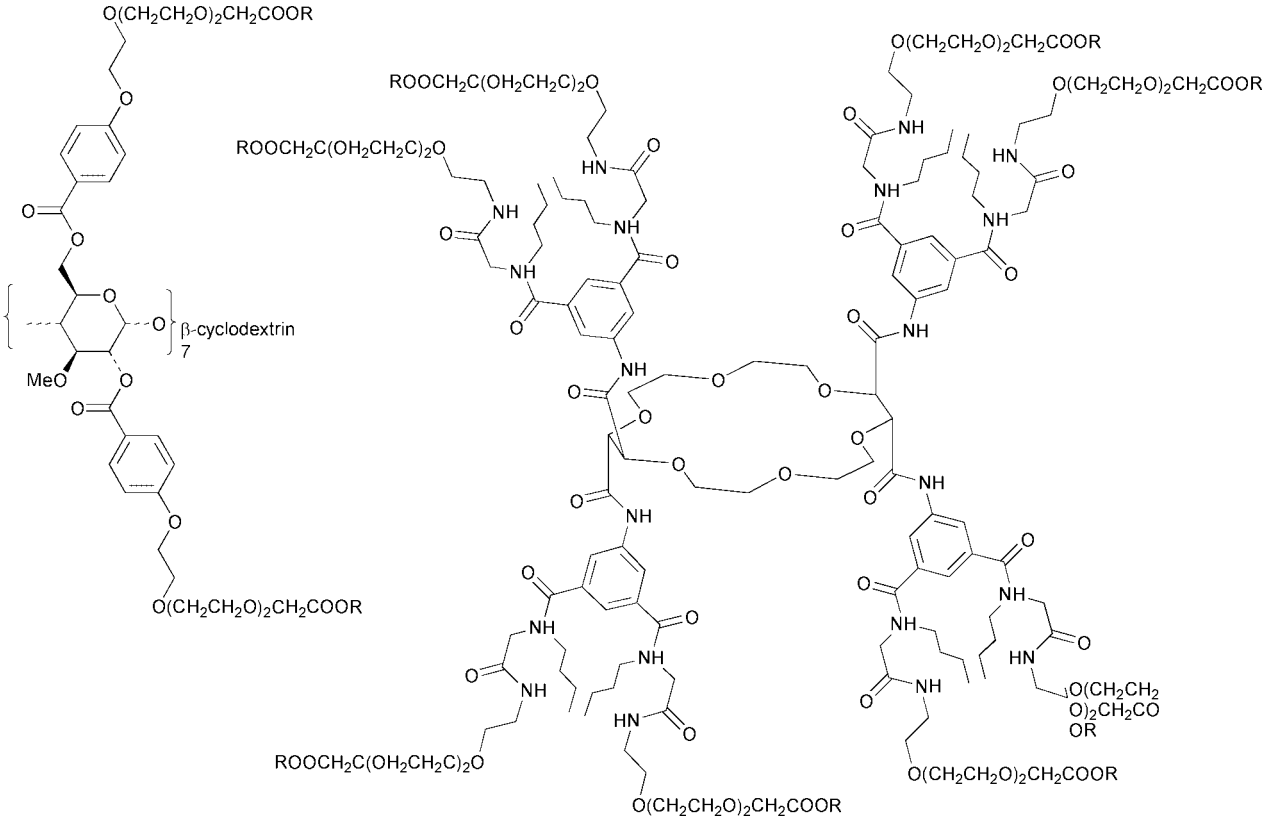


Fig. 14 'Chundle' and 'bouquet' ion transporters using, respectively, crown ether and cyclodextrin central scaffolds.

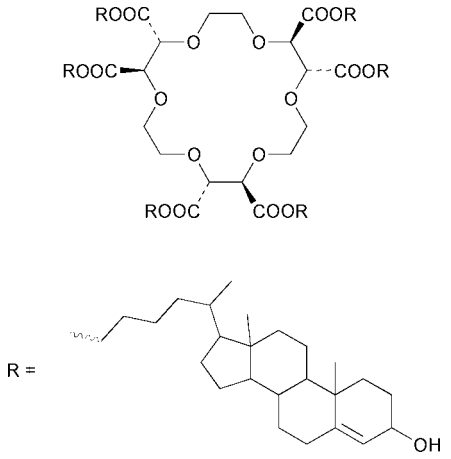


Fig. 15 An ion transporter based on a crown ether central scaffold having cholesteryl 'walls'.

the number and identity of walls and the headgroups with which they were terminated, a structure–activity relationship was developed.

An interesting result that can now be appreciated in terms of a potassium channel structure[2] is that transport efficacy was greater when the 'walls' were non-polar than when they were constructed from ethyleneoxy units. The KcsA channel of *Streptomyces lividans*, as described by Doyle *et al*.,[2] possesses two hydrophobic regions: one either side of a water and ion-filled capsule. These 'tubes' or 'tunnels' correspond to the bola-amphiphile walls of Fyles' channels. Doyle and co-workers have asked a pointed question with respect to the natural structure. 'What is the significance of the hydrophobic lining? We suggest that it would be counterproductive to achieving a high throughput of K^+ ions were the lining of the channel to interact strongly with ions outside of the selectivity filter. The hydrophobic lining presents a relatively inert surface to a diffusing ion over most of the length of the pore.'[2] An increase in polarity is expected therefore to attenuate transport.

Because a number of structural variants were available, a combination of kinetic measurements and inhibition studies were undertaken in an effort to classify these transporters as either carriers or channels. It was concluded that approximately half of the compounds were carriers rather than channels. For at least two of the channel structures, the transport selectivity order of $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$ was observed. A similar order was reported for the structures thought to be functioning as carriers.

6.6 Central relay molecules

The family of molecules we have called 'hydraphiles' was based on a three-macrocycle concept.[28] The design schematic is shown in Fig. 17. As was the case in other laboratories, our design used a target span of 30–35 Å, the width of the insulator regime in a typical phospholipid bilayer. Crown ethers were selected as combination headgroups and cation entry portals. It was thought that these residues would stabilize the position of the channel structure within the bilayer. Entry of a cation through the macrocycle was also expected to confer a modicum of selectivity upon the system. The fact that the sidearms did not make a covalent connection was deliberate. It was hoped that by deliberately incorporating flexibility, the channel would be active even if not all of the design features were optimal.

The channel was designed to be largely hydrophobic but with what we dubbed a 'central relay'. Chemical intuition suggested that a cation, whether or not it was hydrated, could not 'jump' a distance of >30 Å without some polar stabilization. In our original design, we chose a macrocycle identical to those used as the headgroups to serve this purpose. We originally envisioned that the transient cation would pass through the macrocycle but accumulated evidence showed that the macrocycle is actually parallel to the axis of the fatty acid chains rather than parallel to the membrane's surface. This conformation is shown for the first of the series of hydraphile channels in semi-schematic form in Fig. 18. The spacer chains that define the channel's overall length are $(CH_2)_{12}$. The sidearms are also dodecyl units.

It seems obvious that a chain of cations must be present within the pore structure (as shown in Fig. 18) in order to be transported across the membrane. Further, some level of hydration for each cation seems mandatory. Although the cations are represented in the illustration as an orderly chain, the details of hydration and cation organization within the pore

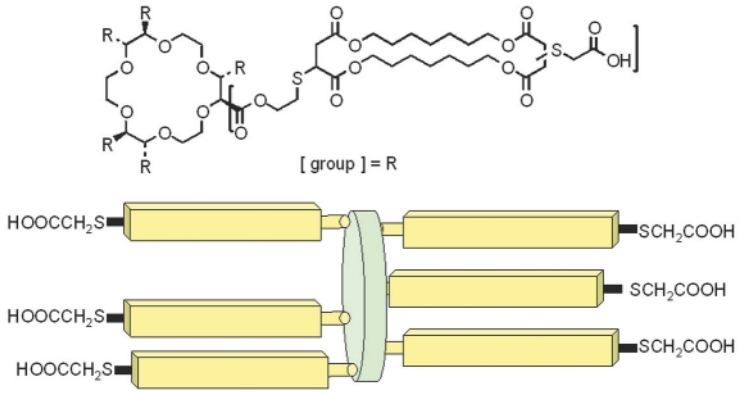


Fig. 16 Schematic of the Fyles family of ion transporters.

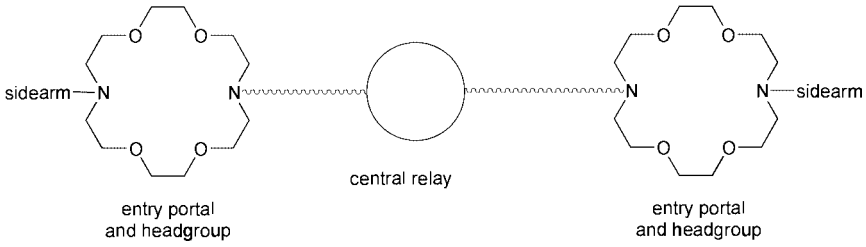


Fig. 17 The 'hydraphile' channel family design schematic.

remain unclear. Indeed, these details of the transport phenomenon remain obscure in all of the model systems and in the numerous protein channels that have been studied.

It is important to note, however, that the conformation shown in Fig. 18 is not merely speculation. Instead, it is the result of extensive structural and fluorescence studies.[29] The accumulated data place the distal macrocycles (headgroups) at a distance of 14 Å from the midplane of the bilayer. This value suggests that the headgroups are separated from each other by the expected 30 Å distance. The evidence also shows that the fluorescent headgroups experience a polar environment (relative permittivity) greater than that of ethanol (ε = 24). Clearly, the hydrocarbon regime of the membrane must have a relative permittivity far less than that. Another important conclusion that can be drawn from this model system is that the 'headgroups' are not interacting with the aqueous phase surroundings or within the phospholipid liposomes as the relative permittivity of water is ~80.

A variation on the hydraphile theme used a calixarene as the central relay.[30] Two potential channel compounds were prepared. In both cases, the spacer chains and headgroups were those that had proved efficacious in the hydraphile family. Calix[4]arene, however, is conformationally rigid and the chains could radiate from this 'central' unit either to the same (left panel of Fig. 19) or to opposite sides. The latter was expected to be an active cation transporter and the former was not expected to be effective. In addition to the conformational question, a second issue could be addressed: does the cation pass through or around the calixarene?

Planar bilayer conductance data showed that the *syn* (left structure in Fig. 19) was inactive. The *anti* structure showed bursting behavior and was clearly providing a cation transport pathway through the bilayer. When the calixarene was substituted by *tert*-butyl groups, so that the cation could not pass through the central relay, the modified structure remained active.

The hydraphile channel design was adapted by Hall *et al*. to incorporate an electrochemical switch.[31] In this channel model, two diaza-18-crown-6 residues were connected by either ferrocene or cobaltocene (M = Fe, Co in Fig. 20). The spacer chains were decamethylene, a length appropriate to accommodate the additional macrocycle. The metallocene can undergo oxidative loss of an electron, affording a ferricinium [Fe(III)] species (see section 4.3). Cation transport was assessed by using the patch clamp method common in electrophysiology. A so-called 'inside-out' patch was excised from hamster brain cells exposed to the channel model in solution. Potassium transport was assessed at transmembrane potentials ranging from +60 to 260 mV. The data showed ion transport apparently promoted by the channel. Evidence was obtained at both negative and positive potentials and some rectification was also reported.

7 The polyphenylene scaffold

A number of synthetic systems have been reported recently by Matile and co-workers[32] in which a key structural element is the 'rigid-rod' polyphenylene residue shown in Fig. 21. By attaching a tripeptide unit to a *meta* position in each benzene of the octaphenylene chain, a structure is formed that resembles a ladder from which one rail has been removed. These 'hemiladders' organize into a rigid-rod β-barrel that constitutes a membrane resident nanotube. Fluorescence methods, especially transport of the dye carboxyfluorescein, were used to characterize the pore system. These remarkable pores may be too large to selectively transport alkali metal cations but other important possibilities can readily be imagined for them.

8 The value of synthetic channel models

There are two obvious reasons to prepare chemical models of biological systems. The first is to mimic function and perhaps substitute the (presumably simpler) synthetic compound for the natural product in a therapeutic application. The model systems described here are more complex than typical pharmaceuticals but the principle is similar. The second utility of models is that they can often be modified more readily than the natural product and specific aspects of the function can be assessed.

An interesting series of studies was reported by Regen and co-workers who were inspired to develop amphotericin B mimics.[33] They developed a steroid derivative that was pegylated at the 3- and 17-positions. These showed clear but low ionophoretic activity in bilayers. Regen surmised that the ionophore might function differently if acting as a monomer (perhaps as a carrier at low concentration) compared to an aggregate (pore at high concentration) such as noted for amphotericin above in section 3.1. These workers reported that the ion transport obeyed different kinetics depending on the concentration of the ionophore. Thus, carrier-type transport appeared to prevail at lower concentration. Such studies are of obvious importance in characterizing models systems. Not only is concentration an issue, studies of the conformation and ion selectivity are important as well. In our own work, we have made extensive use of fluorescence techniques borrowed from the biological community to characterize headgroup position, headgroup separation, and the aggregations state of the monomer molecules.

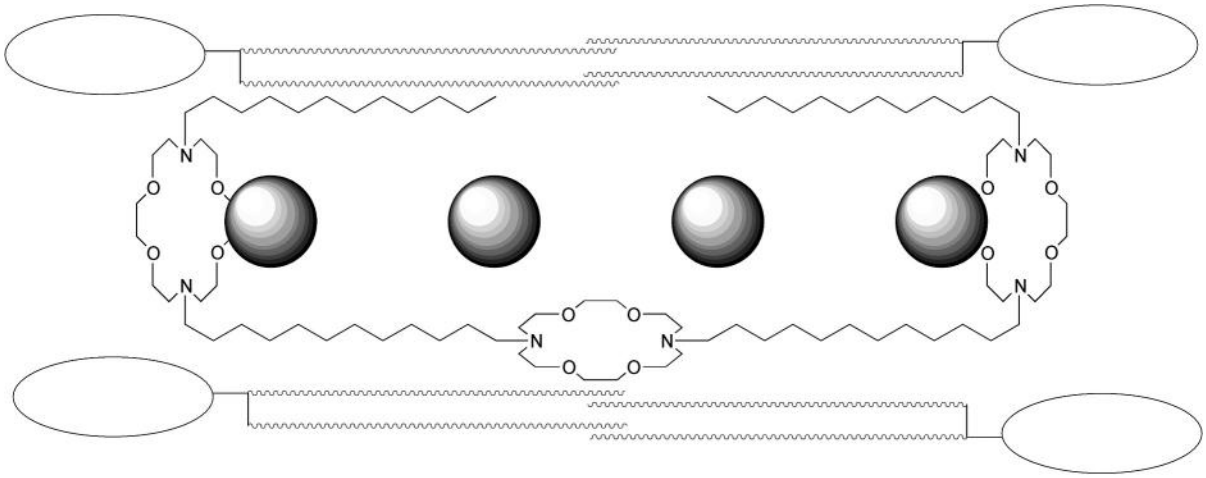


Fig. 18 The conformation adopted by hydraphile channels having three macrocycles, as deduced from experimental evidence.

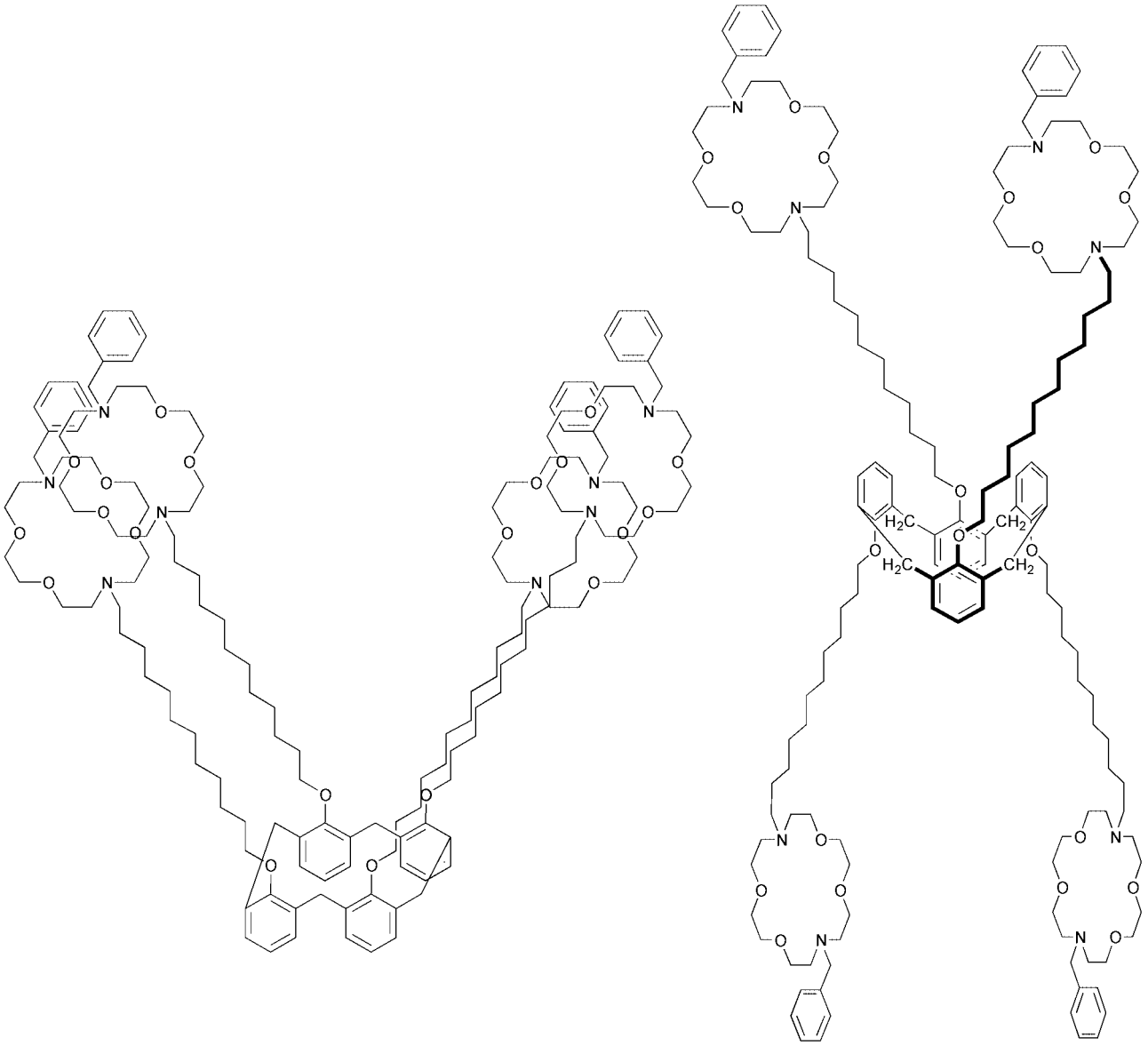


Fig. 19 Hydraphile channels using calix[4]arene as a central scaffold/relay.

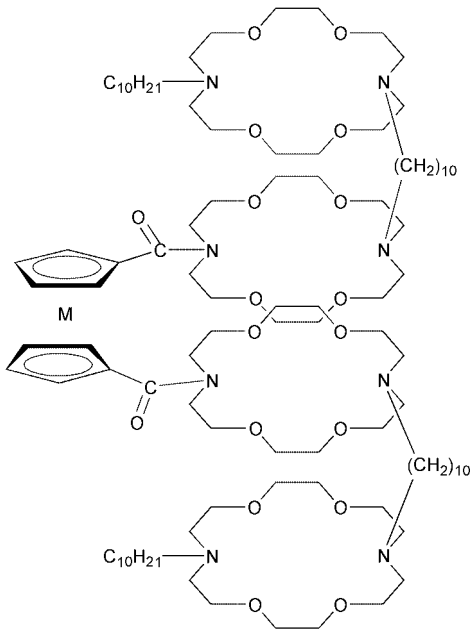


Fig. 20 An electrochemically-switchable channel based on the hydraphile design.

We noted in section 6.5 that Fyles and co-workers[27] found that the efficacy of channel formation was greater when the 'walls' were hydrophobic, rather than more polar. Our own work showed similar results: hydraphiles having hydrocarbon spacer chains were more effective ion transporters than were otherwise identical molecules in which more donor groups were present.[29] Our expectations were contrary to this at the outset but these findings, if we had greater confidence in them at the time, would have predicted something important about natural channel structures.

Doyle *et al*. made the following obsevation when they reported the solid-state structure of the KcsA channel. 'The overall length of the pore is 45 Å, and its diameter varies along its distance… From inside the cell (bottom) the pore begins as a tunnel 18 Å in length (the internal pore) and then opens into a wide cavity (~10 Å across) near the middle of the membrane… The chemical composition of the wall lining the internal pore and cavity is predominantly hydrophobic…' We note that in advance of this structural knowledge, a number of synthetic channel model systems (Fyles, Gokel) used hydrophobic 'pore linings' of a similar length.

The 'cavity' was not anticipated and has required some study and explanation. We have been particularly interested in this aspect of the KcsA channel structure because this element corresponds to the 'central relay' we designed into our first

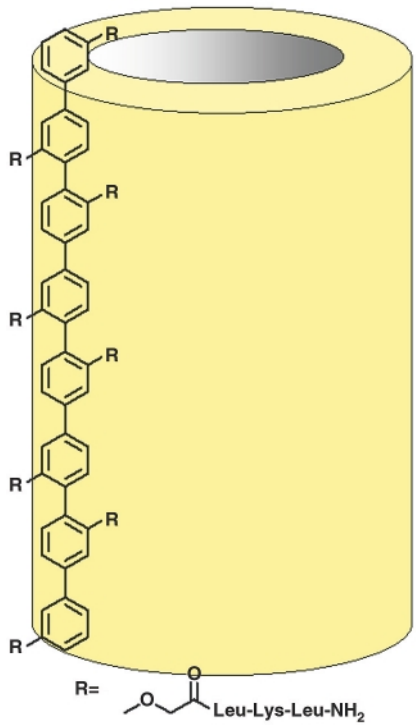


Fig. 21 A large-pore, β-barrel formed from multiple, tripeptide-substituted, octaphenylene rigid rods.

tris(macrocycle) hydraphile. Indeed, a comparison of the KcsA channel's overall structure, in which the pore is emphasized, with a hydraphile channel shows that the structures correspond remarkably well (Fig. 22).

A successful model design may well involve some luck. In the present case, the existence of a central cavity was not known when several of the model systems described here were designed. Both structural needs and chemical intuition dictated incorporation of the 'central scaffold' or 'central relay'. The chemical consideration was that the midplane of the bilayer is extremely non-polar and some charge accommodation mechanism must be required for transport.

One may use a functioning model to ask chemical and biological questions. In the hydraphile case, we have asked whether the central cavity or relay unit binds cations or water. We addressed this question experimentally by varying the central relay unit so that its accommodation of water or cations was altered. We concluded from the experimentally observed effect of Na^+ transport in phospholipid bilayers that organizing water to interact with cations was the function of the central unit.[34]

8 Conclusions and challenges

The quest for cation channel mimics has resulted in the design and synthesis of several functional ion transporters. Questions of hydrophilicity *vs*. hydrophobicity of the pore walls, the function of the central scaffold or relay, and aggregation state have all been addressed using these synthetic models. A challenge for chemists is characterization. Of course, the chemical purity and the structure of any compound must be established. In addition, however, the structure of an aggregate or supramolecular assembly that forms from the designed monomers must also be characterized. Often, this is difficult because the system may aggregate in a phospholipid bilayer in a way different from that revealed by a crystal structure. To be sure, the solid-state structure gives critical information about supramolecular interactions but such techniques as fluorescence must be used to connect the solid state to a membrane-inserted phase.

A further challenge for chemists is to understand the biologist's perspective. The current biological view of channel function is based on complex proteins that conduct cations with remarkable speed and selectivity. The channel models being

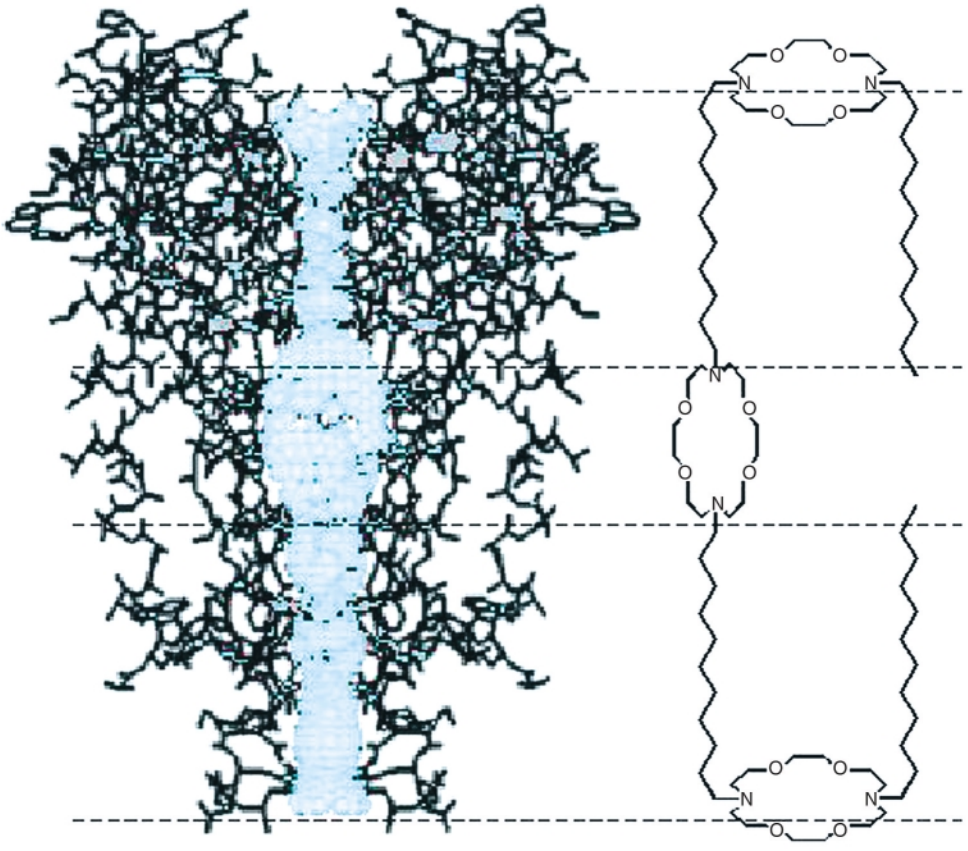


Fig. 22 Structural comparison of a hydraphile channel with the solid-state structure of the KcsA channel of *Streptomyces lividans*.

designed by chemists are primitive indeed compared to these. Still, the first natural channels must have been primitive as well. As chemical efforts evolve to greater structural and functional complexity, the relevance to modern proteins will surely increase. For now, it is important to understand the limitations of chemical models but to use their strengths to address questions of fundamental importance to both the chemical and biological communities.

9 Acknowledgments

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