Biologically active, synthetic ion transporters

George W. Gokel*[ab] and I. Alexandru Carasel[b]

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The compelling chemical goal of modeling protein channel behavior has led to synthetic compounds that are true ion channels. Although they largely lack the selectivity and sophistication of highly evolved proteins, they successfully perform a variety of biological functions. This *tutorial review* describes these novel structures and their activity in living systems. Different channel structures show antibacterial to anticancer activity when tested against a variety of cell types.

1. Introduction

Cells are chemically complex capsules that contain the essential elements and features required for life. The vital cellular constituents must interact with each other within the cell, but they must also be in contact, and in communication, with the external environment. The evolutionary problem that confronted early cells or capsules was how to incorporate nutrients and excrete waste products while remaining mostly separate from the surrounding medium. In the earliest forms of life, this must have been accomplished largely by diffusion. The earliest membranes could not have possessed the complex structure and properties of modern membranes, although their composition is unknown. It would not be surprising if they were ''leaky'' and this poor insulation accrued to the advantage of the evolving cells.

As cellular systems became better defined and their membranes more secure, mechanisms had to evolve to permit transport of molecules and ions. The molecules that permitted transport had to exhibit selectivity at some level for ions or molecules. A transporter that exhibited selectivity for charged or non-charged species would ultimately require refinement to pass certain examples of the species. For instance, a transporter selective for cations should favor only Na^+, K^+, *etc.*, in order to comprise a regulatory mechanism.

We speculate that early in evolution, relatively simple molecules served as transporters or channels. These evolved over time into compounds that are not only selective for certain ions or molecules but selective for the membranes in which they function. Protein ion channels are highly complex and selective molecules that are exquisitely regulated. Much about them is known from more than 100 years of intense and productive study. A number of breakthroughs marked the end of the previous century.[1] Notable among these were solid state structures of the KcsA K^+ selective ion channel[2] isolated from *Streptomyces lividans* and the water-regulating channel[3] observed in *Xenopus* oocytes. Indeed, so important were the structural advances that MacKinnon and Agre shared the 2003 Nobel Prize in Chemistry for their efforts.[4]

Notwithstanding the remarkable structural advances made in the past decade, much remains to be resolved concerning the

[a]*Department of Chemistry, University of Missouri, One University Blvd, Saint Louis, MO 63121, USA. E-mail: ggokel@wustl.edu; Tel: 314-362-9297*
[b]*Departments of Chemistry and of Molecular Biology & Pharmacology, Washington University School of Medicine, St. Louis, MO 63110, USA*

George W. Gokel

George Gokel earned the BS at Tulane University and the PhD (chemistry) at the University of Southern California, and did post-doctoral work with Donald Cram at UCLA. He has held faculty positions in the chemistry departments at the Pennsylvania State University, the University of Maryland, the University of Miami, and the Washington University in St. Louis. He is currently Distinguished Professor in the Departments of Chemistry and Biology at the University of Missouri in St. Louis.

Ionut Alexandru Carasel

Ionut Alexandru Carasel was born in Craiova, Romania. He earned his undergraduate degree in pharmacy from the University of Medicine and Pharmacy Cluj-Napoca, Romania in September 2005. He is currently pursuing a doctoral degree in chemistry from Washington University in St. Louis under the supervision of G. W. Gokel. His research focuses on synthesis and characterization of peptide-based anion channels. He is studying the influence of size of the peptidic chain on the selectivity and activity of synthetic chloride channels.

mechanisms of channel function. An example is how the opening and closing of channels is regulated. Channels and carriers are fundamentally different even though both may be considered transporters. A carrier molecule is sometimes described as a ''molecular ferry boat.'' The host or receptor molecule complexes the ion or molecule to be transported and ''carries'' it like a passenger across the membrane. The guest is then deposited at the opposite side of the membrane and the host molecule diffuses back across the membrane. A channel inserts in the membrane and forms a tunnel or pore through which the species it selects can pass. When the channel is open (in the open state), many ions or molecules pass through. When the channel is in a closed state, the membrane is non-conducting. How the opening and closing of ion channels is regulated remains an area of vigorous investigation. Much work will also be required to completely understand the various mechanisms that control the selectivity of protein channels.

1.1 *De novo* designs

The challenge of understanding the chemical mechanisms of channel function has inspired several attempts to design *de novo* synthetic channel compounds. Extensive efforts have been made in the biochemical community where peptides have typically comprised the basic structures or scaffolds. Notable among these early efforts were the approaches of Mutter *et al.* and DeGrado *et al.* Many of the early efforts were reviewed previously in this Journal.[5] Imaginative and extensive as these studies were, these peptides are more closely related to natural pore-forming peptides than the abiotic structures developed by chemists. It is the latter that is the focus of this article.

Shortly after crown ethers were discovered,[6] they were applied as carriers of various cations through bulk organic membranes. Almost simultaneously in the 1980s, several investigators began to contemplate the possibility of building a synthetic alkali metal cation channel that would function in bilayers. This was a daunting challenge at the time because no protein channel structure was known at high resolution. Then, as now, the actual thickness of membranes could only be estimated based on the phospholipid monomers to be used. Further, chemical intuition had to be applied to fill the mechanistic gaps in the design. Most of the early designs were based in one way or another on macrocyclic compounds.

The two earliest synthetic ion channel structures were characterized by divalent cobalt transport and will not be discussed further here.[5] Jullien and Lehn reported a channel-like structure based on a crown ether but did not demonstrate ion transport.[5] The first synthetic ion channels that demonstrated Na^+ transport were reported months apart by Fyles *et al.*[7] and Gokel *et al.*[8] Shortly thereafter, Kobuke reported an oligoethylene glycol-based compound that showed single channel behavior.[5] The Fyles channel (**1**) is shown in Fig. 1. 18-Crown-6 serves as the central ion relay and as the key structural element. The stereochemical arrangement is defined by the tartaric acid residues from which the macrocycle was constructed. The ''walls'' of the channel, which are linked through these carboxyl groups, are bola-amphiphiles (or bolytes) linked at either end by succinic acid residues. The ''Head'' groups shown at each end of the molecule were varied and could be carboxyl or sugar groups. The headgroups were the polar elements expected to align with the bilayer headgroups. The elements labeled ''A'' and ''B'' were hydrocarbon or oligoethylene glycol chains that could be mixed or matched.

Although produced completely independently, the channel developed in the author's lab (**2**) and shown in Fig. 2 bears some similarity to this one. In our effort, crown ethers were intended to function both as headgroups in the amphiphilic sense and as entry portals for ions. It was expected that the crown would impose selectivity upon the ion transport process. Chemical intuition in our case (and clearly also in Fyles') suggested that a central macrocycle was required to lower the energy of transporting an ion 30 Å or more across a low polarity regime.

Some comment is in order concerning the structural features of the designs shown in Fig. 1 and 2. Although both were conceived approximately a decade before the appearance of the first solid state structure, they can be described in the same terms used by MacKinnon and coworkers[2] to characterize the KcsA channel. They stated as follows. ''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 ($\sim$10 Å across) near the middle of the membrane.'' The central macrocycle serves the purpose of lowering the energy required to traverse the long insulator regime of the bilayer. Mackinnon *et al.* refer to this structural element as a ''water and ion filled

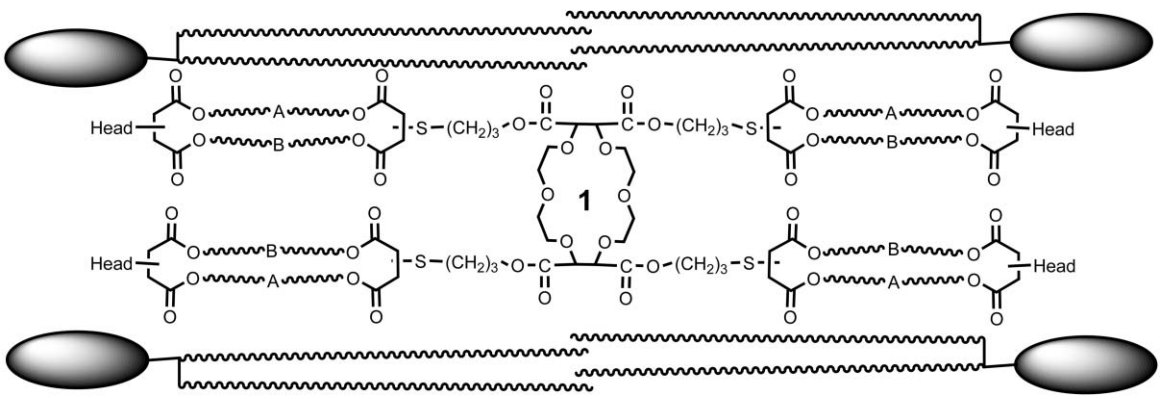

Fig. 1 Semi-schematic representation of a synthetic ion channel using a crown ether central element and bolyte walls.

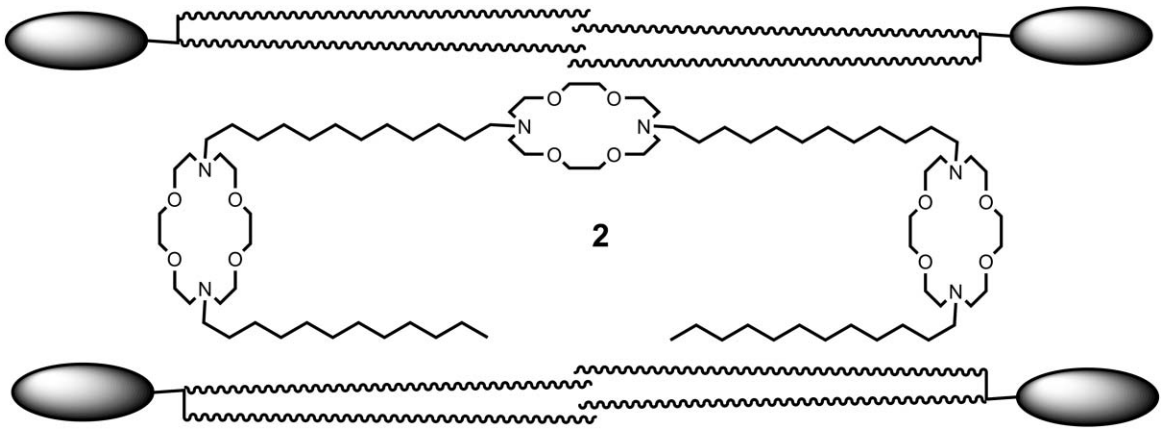

Fig. 2 Semi-schematic representation of a synthetic, tris(macrocycle) ion channel.

capsule." We call it the "central relay." In either case, it accomplishes the same purpose.

A finding made with both channels (Fig. 1 and 2) concerns the chains that extend from the central relay (or central macrocycle) to the membrane boundaries. In both efforts, channel variants were prepared in which oligoethylene glycol units replaced hydrocarbon chains. The reasoning was that shorter spacings between donor groups would be energetically favorable and lead to higher transport rates. In fact, such replacements in both channels had the opposite effect. Indeed, MacKinnon and coworkers note that in the KcsA channel, the "chemical composition of the wall lining the internal pore and cavity is predominantly hydrophobic."

We have studied the efficacy of transport when different residues are present at the midplane of the bilayer.[9] These include diaza-18-crown-6 as illustrated in Fig. 2. That macrocycle was replaced by 15- and 12-membered rings and by a variety of other polar residues. We concluded that in the hydraphile family of synthetic ion channels, residues that bound water were good central relays and compounds that were strong ion binders diminished transport efficacy. When oligoethylene glycol chains are added in the channel spans, they interact with the transient cation and diminish transport rate compared to situations in which the spans are hydrocarbon chains.

Extensive biophysical studies were undertaken to determine if the channel had the conformation illustrated in Fig. 2. These studies included fluorescence techniques such as depth quenching and fluorescence resonance energy transfer (FRET).[10] The confirmation was also validated by study of a synthetic modification. Thus the two sidechains in the channel shown were linked by a fourth diaza-18-crown-6 macrocycle. This gave an overall symmetrical structure ($[(CH_2)_{12}<N18N>]_4$) that required the two intramembrane macrocycles to be parallel, thus enforcing the conformation illustrated. This tunnel-like structure proved to be the most active of all of the hydraphiles prepared to date.[11]

1.2 Semi-synthetic peptides

An alternate approach to the preparation of synthetic ion channels is to prepare peptide-abiotic hybrids that can insert in a bilayer. Voyer and coworkers used the helical peptides devised by DeGrado[5] as a scaffold to support a chain of crown ether compounds.[5] By incorporating a crown-modified phenylalanine on every fourth residue, the macrocycles were approximately aligned. The peptide sequence was *N*-*t*-Boc-(Leu-FCr-Leu-Leu-Leu-FCr-Leu)$_3$ in which the phenylalanine-crown ether amino acid is designated FCr. This produced a sodium-conducting channel, **3**, that functioned in phospholipid bilayers.[5]

Koert and coworkers took a different, but somewhat related, approach. They prepared oligo(tetrahydrofuran) compounds (**4**). These proved not to be well behaved transporters and were subsequently modified.[5,12] The new channel (**5**) incorporated four tetrahydrofuran residues, but these were linked to elements derived from the bacterial peptide gramicidin. Thus the *C*- and *N*-terminal ends of the structure incorporated a Leu-Trp repeats characteristic of the gramicidin family of channels. When the latter "stabilizing groups" were added, the channels exhibited stable open–close behavior rather than spiking characteristics.

1.3 Cyclic peptides and nanotubes

Ghadiri and coworkers prepared various cyclic peptides that could stack to form nanotubes.[5] In particular, the sequence *cyclo*[Trp-D-Leu-Trp-D-Leu-Trp-D-Leu-Gln-D-Leu] was prepared. The stereochemistry of the amino acids alternated between the common L-configuration and the opposite

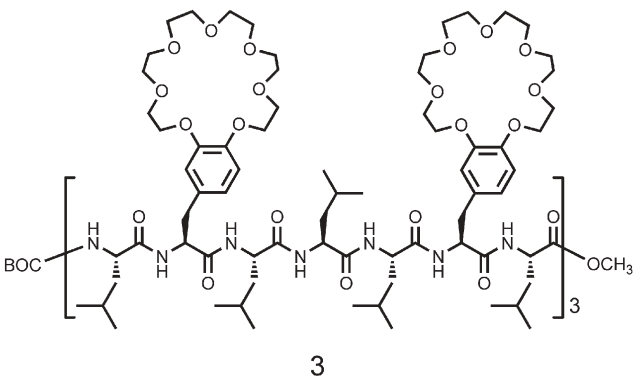

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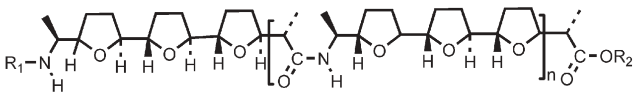
Fig. 3 Synthetic ion channel incorporating a helical peptide backbone and oriented crown ethers to form a unimolecular pore.



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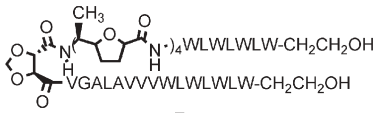

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Fig. 4 Oligo(tetrahydrofuran)-based synthetic ion channels.

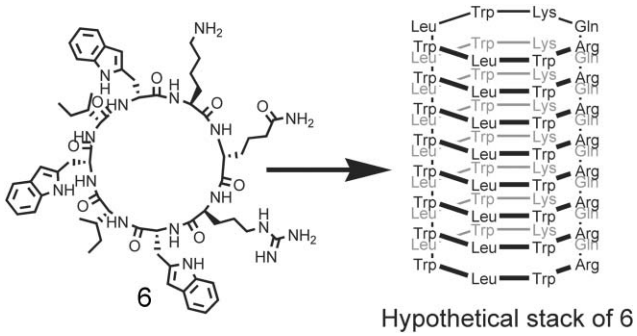

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Fig. 5 Membrane active nanotubes formed by stacks of cyclic peptides.

D-configuration. As a result, the amino acid sidearms extended from the cycle and the amide residues within the ring could form a hydrogen bonded stack. The result was a nanotube thought to possess a stack of 8 cyclic peptides. The formation of channels by these nanotubes was confirmed by single channel conductance measurements determined in planar lipid bilayers. Clear open–close behavior was observed in lipid bilayers formed in the tip of a patch pipette. The cyclic peptide and the presumed octapeptide stack is illustrated in Fig. 5.

1.4 Oligophenyl "rigid rod" channel assemblies

A unique "rigid rod" assembly[5,13] was designed and prepared by Matile and coworkers. In their approach, eight benzene rings are connected in their *para*-positions to form a rigid oligophenylene assembly. The benzene rings are substituted by oxygen atoms which, in turn, are connected to a tripeptide sequence through an oxyacetamide connector. The backbone minimizes energy by alternating the peptide chains. The overall structure organizes into an approximate cylinder, which exhibits channel properties. The channel properties were typically assessed by planar bilayer conductance experiments.[14]

The focus of this work has primarily been the development of channel models and their use in understanding transport mechanisms. In very recent work, however, studies related to pore formation by natural toxins have been reported.[15]

1.5 Synthetic peptides and other synthetic ion channels

A number of diverse channel systems have been designed and prepared. Many of these have been studied in bilayer membranes and shown to effect transport to a greater or lesser extent. Many of these may ultimately reveal biological activity but, to date, such activity has not been reported.

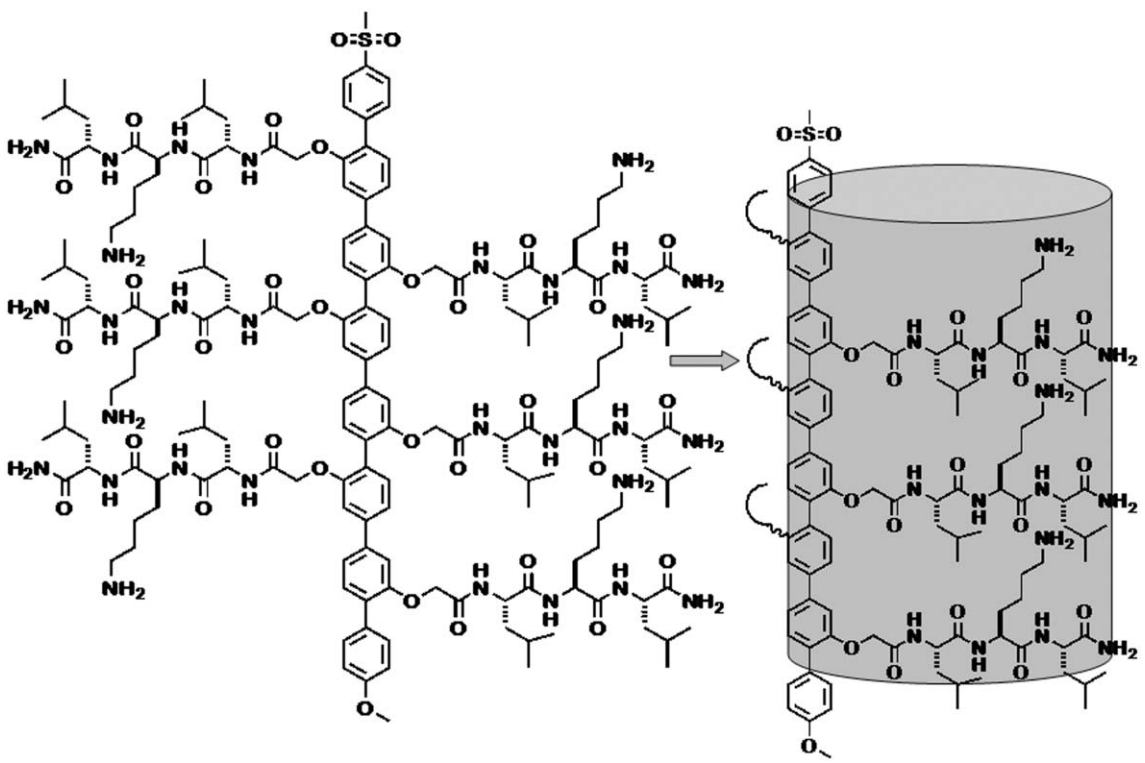


Fig. 6 Membrane active channels based on an oligophenyl backbone and peptide sidechains

Channel activity was demonstrated by Mutter in synthetic peptides that assembled into four-helix bundles.[5] DeGrado and coworkers developed a "minimalist" approach to protein design. This resulted in compounds exhibiting ion channel activity in bilayers.[5] Montal *et al.* developed a family of molecules they referred to as "Synporins."[5] Channel designs that have revealed the ability to transport protons, metallic cations, or anions have been reported by several laboratories, but the potential biological activity of these designs does not appear to have been explored. Synthetic ion transporters have been reported by Tabushi,[5] Fuhrhop,[5] Menger,[5] Lehn,[5] Nolte,[5] Kobuke,[5] Frye,[5] Regen,[5] de Mendoza,[5] Tomich,[16] Hall,[5] Davis,[17] Smith,[18] Gin,[19] and their coworkers. Notable as these accomplishments are, they are beyond the focus of the present article.

2. Characterization of transport

The design of compounds that are tubular or channel-like is a reasonable entry-level approach to synthetic transporters. The designs may be simple or complex but the critical issue is always function. A novel compound that looks like a channel but does not conduct ions or molecules is simply an interesting structure. Thus, a significant challenge for researchers in the synthetic channel area has been the question of characterization. A number of analytical methods have been developed or adopted to characterize ion transport. Some of these are described briefly below.

2.1 Proton transport assayed by fluorescence

Various proton sensitive dyes or other compounds have been included in phospholipid vesicles. In early work, Kano and Fendler used a sulfonated pyranine dye to detect pH gradients across liposomal membranes.[20] Compounds capable of transporting protons obviously affect the pH gradient and reflect transport. Variations on this methodology have been used by Menger[5] and in our lab.[21] Fyles and coworkers[5] exploited the method developed by Gary-Bobo, *et al.*[22] by competing protons and various alkali metal cations.

2.2 ^{23}Na-NMR methods

The use of NMR has proved to be valuable in the study of synthetic ion channels. The method is reproducible if somewhat cumbersome. Still, it is more familiar and accessible to the organic chemist than are planar bilayer conductance or whole cell patch methods. The method pioneered by Riddell and coworkers has proved particularly useful.[23] In this approach, phospholipid vesicles are prepared in the presence of NaCl. The ^{23}Na NMR spectrum shows a single resonance because the cations inside and outside the vesicles are equivalent. When a shift reagent such as Dy^{3+} is added to the aqueous suspension, the external Na^+ is affected but the Na^+ within the vesicles is insulated. Thus, two resonances are observed instead of one. When a channel molecule is added to the suspension and inserts into the bilayer, Na^+ inside and outside exchange. The exchange rate constant can be calculated from the changes in the sodium resonance linewidths. Higher exchange rates suggest more effective transport or channel function.

2.3 Ion-selective electrodes

The NMR methods used to assay transport are useful but both experimentally cumbersome and somewhat operator dependent. We therefore developed methodology based on ion selective electrodes and on previous efforts[24] that permitted assay of either Na^+ or Cl^- transport.[25] In this method, vesicles must be prepared in the presence of the ion of interest. The external solution must be replaced by one lacking in the ion to be studied. A microelectrode is inserted in the vesicular suspension and then the ionophore of interest is added. Ion release is typically measured over 500–3600 s, depending on the rate of release. At the end of each experiment, the vesicles are lysed by addition of detergent and a 100% release value is established. The data are then normalized to this point. Release rates are determined over at least a 10-fold concentration range, if possible. Often, but not always, the curve at each concentration is exponential. In some cases, however, complex undulations are observed. In the latter situation, an arbitrary time point is chosen for comparison of release values.

2.4 Carboxyfluorescein release

The analysis of anion release from phospholipid vesicles can be conducted by using carboxyfluorescein (CF) anion.[26] Such methods are widely used in biological studies.[27] In the synthetic ion transport studies that are the focus of this review, vesicles are prepared in the presence of CF and the external solution is then exchanged for a CF free medium. Within the vesicles, the highly fluorescent anion self-quenches. When an anion transporter is added to the suspension and inserts in the bilayer, release of CF can be quantitated by fluorescence. This constitutes a very convenient and quantitative means to assay anion release. As above, the data are normalized to a 100% value determined after detergent lysis of the vesicles.

The CF release method has the advantage of being rapid and sensitive. Carboxyfluorescein may seem an unlikely anion to pass through a channel designed for chloride, but the difference in size is not so large as one might think. When chloride is hexahydrated, it is calculated to be an approximate sphere of diameter 6.6 Å. Carboxyfluorescein is about 10 Å wide and about 6.5 Å thick. If a channel has some flexibility, CF may pass through the pore. Because the sensitivity of CF fluorescence detection is high, the range of ionophore concentrations that can be used is also large. When a large enough range of concentrations shows appropriate behavior, a Hill plot may be used to determine the molecularity of the pore.

2.5 Planar bilayer conductance

In this technique, a Teflon disk separates two cuvettes or chambers. The chambers are sometimes referred to as cis and trans. A very small hole in the Teflon disk is painted with a solution of phospholipid monomers. These monomers spontaneously form into a planar bilayer membrane within the hole and form a barrier between the two solutions. Addition of an

ionophore permits ions to pass through the bilayer and the current is directly detected with a sensitive amplifier and recorder.

2.6 Whole cell patch

When an almost microscopically fine pipette tip contacts a cell, a ''patch'' of the cell membrane becomes exposed. An electrical current can pass into the cell through the electrode embedded within the micropipet. Typically, no current will pass because the cellular membrane is insulating. If a channel inserts into the membrane, current can pass from the embedded electrode into the cell and out through the channel *via* the conducted ions. The cell normally remains vital during this study and current is conducted according to the open–close behavior of the channel. Hydraphiles have recently been shown to behave in a fashion similar to natural protein channels in conducting current in vital cells.[28]

3.0 Characterization of biological activity

A straightforward way to obtain information about biological activity is to use the disk diffusion method. In this approach, a Petri dish is filled with growth medium, typically agar. The medium is seeded with bacteria, which are allowed to grow to a confluent ''lawn.'' One or more cellulose disks is impregnated with the compound of interest and the disks are placed on the surface of the medium. Antibiotic activity (toxicity to the organism) is apparent by the appearance of a zone of clearing or ''halo'' around a disk. The halo is an area of little or no growth, suggesting toxicity of the compound under study to the organism.

A more quantitative method is to determine the minimum inhibitory concentration (MIC). A group of tubes or flasks is charged with a mixture of the bacterium and growth medium. The compound of interest is then added to the first flask. The second flask receives half the concentration of the first. The third flask receives half the concentration of the second, and so on. A typical range of concentrations would be 500 nM, 1 μM, 2 μM, 4 μM, 8 μM, 16 μM, 32 μM, 64 μM, 128 μM, and 256 μM. If the activity of the compound is high, dilutions would continue into the lower nanomolar range. Concentrations higher than 250 μM can also be tested, but at such levels the biological activity is generally considered not to be significant.

The MIC is determined in this experiment by visual inspection. Where the organism is growing successfully, the medium will be cloudy or opaque. When the compound under study effectively stops growth or kills the organism, the growth medium will appear beige to brown but it will be clear. The MIC is determined to be the concentration at which the cloudy to clear transition occurs.

4.0 Biological activity of simple crown ethers

Not long after Pedersen discovered crown ethers, they were tested for biological activity.[29] The toxicity of 12-crown-4 [$(CH_2CH_2O)_4$] was tested in rats[30] and 18-crown-6 was evaluated in dogs.[31] In the latter case, tremulous motion, salivation, and paralysis of the hind legs were all observed when 18-crown-6 was administered, but the symptoms proved to be reversible within 24 h. The oral toxicity to mice of 12-crown-4, 15-crown-5, and 18-crown-6 was reported as LD_{50} in grams per Kg body weight.[32] The values were, respectively, 3.15, 1.02, and 0.7 g Kg^{-1}. This means that a 180 pound (82 Kg) human would have to ingest nearly 60 g of 18-crown-6 to realize the LD_{50}, if the mouse results were extensible to humans. Detrimental neurological effects were observed in mice and rabbits upon either intravenous or intraperitoneal administration, but the effects were reversible when exposure was discontinued.[33] More extensive work from the same laboratory surveyed 13 ionophores, including nine crown ethers. These compounds were administered by multiple routes to mice, rats, and rabbits, and evaluated for various biological effects and their effect on the membrane permeability of physiologically relevant cations.[34]

5.0 Biological activity of synthetic ion channels

The biological activity of chemical substances is often revealed by general screening programs. Compounds prepared for one purpose may have value in a context that was not anticipated. Indeed, why a compound exhibits biological activity in a different context may not even be understood. As noted above, it was recognized almost immediately that crown ethers are biologically active. The surmise was that these crowns bound cations and that metal cation-dependent biological processes were therefore affected in some way.

The notion that synthetic ion channels may have utility as antibiotics was based on their ability to transport alkali metal cations. In many cellular systems, the concentration of Na^+ ions is >100 mM in the external medium (periplasm) and <10 mM in the cytosol. The reverse is true for K^+: the cytosolic concentration is typically >100 mM and <10 mM in the periplasm. These ionic balances are closely regulated in mammalian cells by the sodium–potassium pump. A synthetic ion channel that inserts into the bilayer and permits unregulated transport of cations through the membrane will disrupt the cell's osmotic balance. If the disruption is severe enough and uncompensated by the organism, cell death will result.

Of course, it may be that compounds thought to function in a certain way will be efficacious for reasons different from those formulated in the design criteria. This does not diminish their value but it makes structural refinement a more difficult matter. To date, the evidence suggests that the major mechanism by which synthetic ion channels manifest toxicity is disruption of cellular ion balance.

5.1 Biological activity of stacked peptide nanotube channels

The six- and eight-residue cyclic D,L-α-peptides compounds developed in the Ghadiri laboratory and described in Section 1.3 above were found to exhibit biological activity. Granja, Ghadiri, and their coworkers report that these peptides ''act preferentially on Gram-positive and/or Gram-negative bacterial membranes compared to mammalian cells, increase membrane permeability, collapse transmembrane ion potentials, and cause rapid cell death.''[35] The authors further proposed that ''appropriately designed cyclic D,L-α-peptides

may be able to selectively target and self assemble in bacterial membranes and exert antibacterial activity by increasing the membrane permeability.'' The compounds tested have amino acids having both the D- and L-configurations. When cyclic peptides of similar structure but different chirality were studied, they exhibited similar *in vitro* activities. This does not prove the mechanism, but it is inconsistent with a receptor/ligand-mediated mode of action. It was also noted that the rates of bacterial killing were consistent with disruption of ion balance. Further, the authors noted that in ''fluorescence-based cell depolarization studies, the activity of the cyclic peptides compared to that of analogous linear peptides, the rate of bacteria killing, and attenuated total reflectance (ATR) FT-IR studies in synthetic lipids are all consistent with a membrane permeation mode of action for this class of antibacterial peptides.''

Preliminary screening was done with the cyclic peptide having the sequence [D-Lys-Gln-D-Arg-Trp-D-Leu-Trp-D-Leu-Trp] (kQrWlWlW). This compound ''displayed potent *in vitro* activity'' against *Bacillus subtilis* (Gram-positive), *Staphylococcus aureus* (Gram-positive), and against *Streptococcus pneumoniae* (Gram-negative), and vancomycin-resistant *Enterococcus faecalis* (Gram-negative). In subsequent studies, they focused on two organisms: *Escherichia coli* and methicillin-resistant *S. aureus* (MRSA). This was because these microbes are responsible for a large number of hospital acquired infections in the United States.

The effect of single site changes was established by preparing some 25 structural variants. Mammalian cells are typically bounded by a plasma membrane. Bacteria have a plasma membrane but also have lipopolysaccharides and various surface proteins that present both a barrier and an external charge. Amino acid replacements, particularly of serines, were made with amino acids containing charged sidechains. These included arginine, lysine, and aspartic acid. Activity was generally enhanced by the presence of positive arginine or lysine, and diminished when negatively charged aspartic acid was present. These changes in activity were attributed to surface interactions. Certain variations in amino acids also produced compounds that were highly haemolytic.

Although numerous minimum inhibitory concentrations (MIC values) were reported, the general efficacy can be summarized for the peptide kQrWlWlW. Uppercase single letter amino acid codes refer to the L-form of the amino acid that occurs in proteins. The opposite configuration (*i.e.*, the D-amino acid) is represented by using a lower case single letter code. The MIC values are reported in $\mu g\ mL^{-1}$. For Gram-positive bacteria, they were: *Bacillus subtilis*, 3; *Bacillus cereus*, 2; *Staphylococcus aureus*, 3; and *Listeria monocytogenes*, 20 $\mu g\ mL^{-1}$. For Gram-negative bacteria, the values were: *Enterococcus faecalis*, 10; *Streptococcus pneumoniae*, 10; *Salmonella typhimurium*, 70 $\mu g\ mL^{-1}$.

5.2 Biological activity of crown-peptide hybrids

Voyer and coworkers recognized that pore-forming compounds[36] can upset an organism's osmotic balance and may exhibit biological effects such as antimicrobial activity and cytotoxicity.[37]

The biological activity of compound **3**, shown above, was assessed against both bacteria and mammalian cells. No activity was found against either Gram-positive or Gram-negative bacteria. However, cytotoxic behavior against breast cancer cells (MDA) and mouse leukemia cells (P388) was observed for several different crown peptides. These compounds differed from **3** in the size of the macrocycle pendant from the Ser-Leu backbone (see Fig. 3). The authors noted that the macrocyclic amino acid that is a critical component of the channel design was inactive when studied as a control. Further, analogs of **3** were inactive when only a single repeat of the structure shown (*i.e.*, a 7-mer rather than a 21-mer) was present. This was attributed to the compound's inability to span the phospholipid bilayer.

The cytotoxicity data in this report are expressed as LD_{50} values for killing MDA and P388 cells. Compound **3** was active (LD_{50}) against MDA and P388 at 15 μM and 8.5 μM concentrations, respectively. When the compound was identical except that the six macrocycles were 15-membered rings rather than 21-membered, the activity was 10 μM and 2.5 μM.

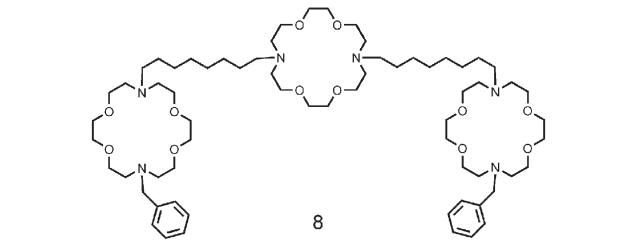
5.3 Biological activity of hydraphiles

Extensive biophysical studies characterized the hydraphiles as ion channels.[5] Fluorescent probes incorporated into the hydraphiles were used to determine the positions of various residues within the bilayer. Fluorescence resonance energy transfer was used to establish that the channels functioned as monomers. A series of compounds that were identical except for overall length established that a minimum channel length was required for transport. Further, when the channels were ''too long,'' transport was reduced, perhaps owing to difficulty in adopting an appropriate conductance state. Channel function was demonstrated by planar bilayer conductance methods and, as noted above, by whole cell patch.[28]

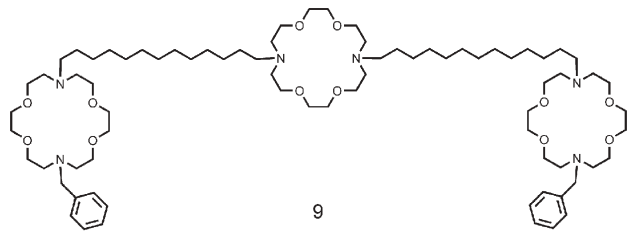
The symmetrical hydraphiles are non-rectifying. Thus, they potentially have the ability to transport ions in both directions. The concentration of K^+ is typically high within the cell and lower outside. The reverse is true for Na^+. If hydraphiles insert into a microbe's bilayer, sodium (and perhaps other cations) should be readily equilibrated, disrupting the organism's osmotic balance. Absent rapid recovery, the organism should perish.

The expectation of biological activity was confirmed first by disk diffusion studies and then by MIC measurements as described above. In the first experiments, *E. coli* were exposed to disks impregnated with hydraphiles having either C_8 (**8**) or C_{12} (**9**) spacer units. Previous studies using liposomes showed that the C_8 compound was ineffective at transporting Na^+. The expectation was that the C_{12} channel (**9**) would be toxic and the C_8 compound (**8**) would not. This was, in fact, the result, although slight activity was observed for C_8 (**8**). When the results were quantitated by MIC experiments, it was found that C_{12} (**9**) was about 13-fold more active than C_8 (**8**) against *E. coli* and that MIC for the former was $\sim$10 μM.[38] A fluorescent variant of the C12 channel (**9**) was used in concert with fluorescence microscopy to confirm that the channel was localized in the *E. coli's* external membrane.

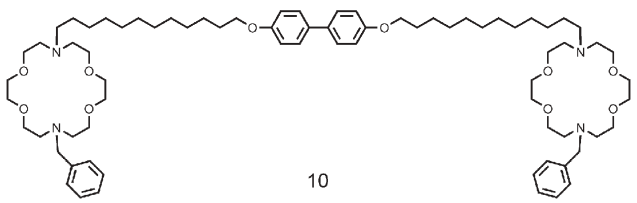
5.3.1 Control experiments. The hydraphiles shown as structures **9** and **10** are identical except for the "central relay" unit. This is a water-organizing element that has been referred to in voltage gated protein channels as the "water and ion-filled capsule."[2] In previous studies, we have shown that diaza-18-crown-6 functions effectively in this role but 4,4′-biphenol does not.[39] In this study, transport rates were determined by a variation of the ^{23}Na NMR method of Riddell and coworkers.[13] The relative transport rates for NaCl through a phospholipid bilayer membrane mediated by **9** and **10** were 200 and <2. So far as could be determined by the NMR method, the biphenol channel failed to transport Na^+ at a detectable rate.



8



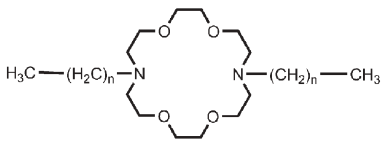
9



10

The biphenol compound is a good control compound precisely because it does not transport cations. The two compounds are similar (±10%) in overall length. The two compounds contain the same number of carbon atoms and their respective molecular weights are 1300 and 1224 Daltons. This is a difference in molecular weight of only about 6%. The distal macrocycles, the spacer chains, and the terminal residues (side chains) are identical in both cases. Overall, they are quite structurally similar. If the compound that functions as a channel is toxic to bacteria and the non-channel is non-toxic, the evidence would support a channel mechanism for toxicity. In the disk diffusion assay, no zone of clearing was observed surrounding the C_8 channel (**8**), the biphenol channel, or the disk impregnated only with DMSO solvent. As noted above, MIC values revealed that the C_8 channel (**8**) was 13-fold less toxic than the C_{12} channel (**9**).

5.3.2 Biological activity of lariat ethers. Although considerable evidence for crown ether toxicity was present in the literature (see Section 4), the compounds studied did not always represent a systematically chosen array. Further, data were lacking for simple dialkyl-sidearmed lariat ethers. These were of special significance to us because they comprise subunits within the hydraphile channels.



11, n=7 (octyl)
12, n=9 (decyl)
13, n=11 (dodecyl)
14, n=13 (tetradecyl)
15, n=15 (hexadecyl)
16, n=17 (octadecyl)

A family of *N*,*N*′-dialkyldiaza-18-crown-6 derivatives was studied. The compounds evaluated had the following side-chains: *n*-octyl (**11**), *n*-decyl (**12**), *n*-dodecyl (**13**), *n*-tetradecyl (**14**), *n*-hexadecyl (**15**), and *n*-octadecyl (**16**). The known K^+-selective transporter valinomycin was also studied. The values reported in Table 1 are MIC values and are given as the toxic concentration in μM.

An interesting observation is that valinomycin, one of the most effective transmembrane carrier molecules known, is less toxic to these three microbes than is didecyldiaza-18-crown-6. Valinomycin does exhibit toxicity, to be sure, but it is substantially less active than the hydraphiles described in Section 5.3.2, below. These experiments serve as controls for the hydraphile biological activity studies in another way. Compounds **13** and **14** have the structure $CH_3(CH_2)_n$<N18N>$(CH_2)_nCH_3$. in which n = 11 or 13. These comprise the central units of the corresponding hydraphiles. The hydraphiles exhibit considerably different toxicity profiles showing that it is not the simple chemical entity that is critical for activity.

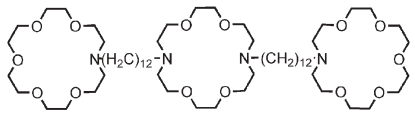
5.3.3 Side chain dependence of toxicity. A family of hydraphiles was prepared in which the spacer chains were dodecylene units and the macrocycles were all diaza-18-crown-6. The structures may be abbreviated as Y-<N18N>C_{12}<N18N>C_{12}<N18N>-Y. An additional compound was prepared in which the terminal macrocycles were aza-18-crown-6. This may be illustrated similarly: <18N>C_{12}<N18N>C_{12}<N18> (**17**). In the former structure, the letter Y represents the side chains that were varied. These include Y = H (**18**), Y = $CH_2C_6H_5$ (**9**), Y = $(CH_2)_{11}CH_3$ (**19**), and a unit that replaced both Y groups. The latter compound

Table 1 Toxicity of several lariat ethers to three organisms

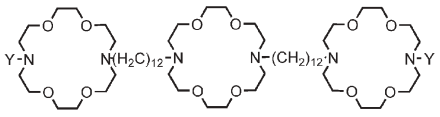
		Toxicity to organism/μM[a]		
Cpd. No.	Side chain[b]	*B. subtilis*	*E. coli*	*S. cerevisiae*
11	*n*-octyl	26	206	103
12	*n*-decyl	2.8	11	2.8
13	*n*-dodecyl	2.5	>300	2.5
14	*n*-tetradecyl	>300	>300	>300
15	*n*-hexadecyl	>300	>300	>300
16	valinomycin	50	50	no data

[a] MIC. [b] Alkyl chain attached to the nitrogen atoms of 4,13-diaza-18-crown-6.

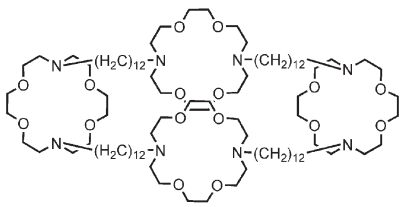
incorporated a connector unit having the structure C_{12}<N18N>C_{12}, which produced a symmetrical tetramacrocyclic structure (**20**). The compounds are illustrated below.



17



9 Y=CH_2Ph 18 Y=H 19 Y=$(CH_2)_{11}CH_3$



20

Compound **20** is unique among those prepared because the two sidearms (Y groups) are linked covalently. In addition, a fourth macrocycle is present, making this system overall symmetrical. In studies of sodium transport rates, **20** proved to be more active than **17–19**.

The three organisms surveyed in this study were the Gram-negative bacterium *Escherichia coli*, the Gram positive bacterium *Bacillus subtilis*, and the yeast *Saccharomyces cerevisiae*. The data are summarized in Table 2. When the distal macrocycles are aza-18-crown-6 (**17**), little or no activity is manifested against any of the three microbes. The situation changes dramatically when diaza-18-crown-6 replaced aza-18-crown-6. Compound **18** shows little activity against yeast, but has modest to good potency against both Gram-positive and Gram-negative bacteria.

The highest toxicities are observed for compounds **19** and **20** against *E. coli* and *B. subtilis*. These MIC values compare with the published value of 8 μM for penicillin. Among the five compounds, tetramacrocycle **20** shows the highest and excellent toxicity against bacteria and only toxicity to *S. cerevisiae*. This is encouraging because yeast, a primitive eukaryote, is often considered to be a model for the behavior of higher organisms. In this case, a 60-fold higher concentration of **20** must be administered to kill yeast compared to the two bacteria.

Table 2 Toxicity of hydraphile channels to microbes

		MIC/μM[a]		
Cpd. No.	Y	*E. coli*	*B. subtilis*	*S. cerevisiae*
17	None	>175	80	170
18	H	22	11	170
9	benzyl	4.7	1.2	38
19	dodecyl	2.1	0.5	8
20	cycle[b]	0.99	0.5	64

[a] Minimum inhibitory concentration in μM. [b] Cyclic tetramacrocycle (see structure above).

5.3.4 Chain length dependence of toxicity. A series of compounds was prepared that are analogs of **9**. These hydraphiles all have benzyl sidechains attached to the distal macrocycles. They differ only in the number of methylene groups present in the spacer chains. All may be abbreviated as $PhCH_2$<N18N>C_n<N18N>C_n<N18N>CH_2Ph. The compounds studied had spacer chains as follows: **11**, $(CH_2)_8$; **21**, $(CH_2)_{10}$; **9**, $(CH_2)_{12}$; **22**, $(CH_2)_{14}$; **23**, $(CH_2)_{16}$; **24**, $(CH_2)_{18}$; and **25**, $(CH_2)_{20}$. Data for the toxicity of these hydraphiles to *E. coli*, *B. subtilis*, and *S. cerevisiae* are recorded in Table 3. The structures of compounds **11** and **9** are illustrated above. They were the first compounds to be studied in this series.

Two trends emerge from the data shown in this table. First, this family of hydraphiles does not exhibit any useful selectivity. Compounds **22** and **23** show the greatest toxicity to *E. coli* and to *B. subtilis*, but they are almost as toxic to yeast. Yeast is far from a perfect indicator of mammalian response but there is too little difference in toxic effect for these compounds to be considered promising. The data of Table 3 are graphed in Fig. 7. It is apparent from the graph that both the C14 and C16 channels are highly toxic. They are not, however, selective.

Second, the family of hydraphiles shows a remarkable parallel between toxicity to *E. coli* and transport efficacy. Sodium transport through a liposomal membrane is plotted as open circles on the graph (Fig. 8). The data shown were obtained by measuring release from phospholipid vesicles mediated by hydraphiles and detected by ion selective electrode methods. Note that when the hydraphile spacer chains are 12, 14, or 16 carbon atoms, similar Na^+ release is apparent. Release is not identical, but the difference (see

Table 3 Length dependence of hydraphile toxicity to *E. coli*, *B. subtilis, and S. cerevisiae*

Compound	**11** (C_8)	**21** (C_{10})	**9** (C_{12})	**22** (C_{14})	**23** (C_{16})	**24** (C_{18})	**25** (C_{20})
Organism	MBC value (μM)[a]						
E. coli	170	80	9.4	2.3	4.6	75	160
B. subtilis	42	10	1.2	0.56	0.6	1	2
S. cerevisiae	170	160	38	4.6	2.3	—[b]	—[b]

[a] Minimum bactericidal concentration. [b] Not tested.

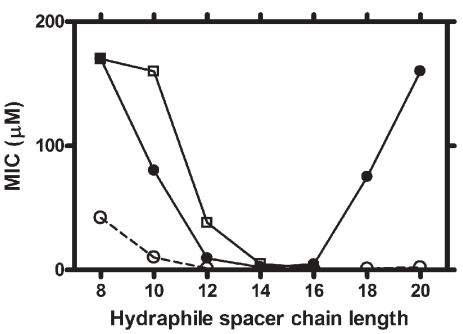


Fig. 7 Graph showing toxicity of hydraphiles to *E. coli* (filled circles), *B. subtilis* (open circles), and *S. cerevisiae* (open squares).

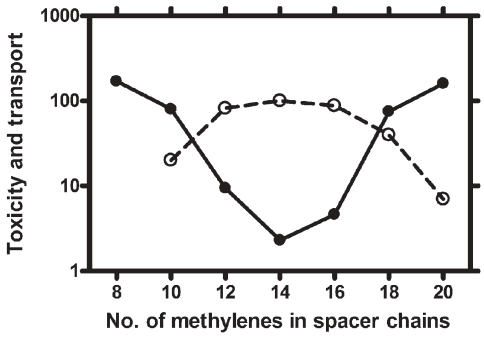


Fig. 8 Comparison of Na^+ transport rate in phospholipid vesicles (maximum 100%) with toxicity to *E. coli* (MIC in μM).

Table 3) is masked by the logarithmic ordinate. The toxicity data for *E. coli* was taken from Table 3. More effective Na^+ release should translate to more rapid occurrence of osmotic imbalance. If the organism cannot recover or cannot recover quickly enough, it will die. Thus, the inverse relationship between toxicity to *E. coli* and Na^+ transport is expected.

It is remarkable that the Na^+ transport rate determined in synthetic liposomes correlates so well with the toxicity to a bacterium. Bacterial membranes are profoundly different from those of simple phospholipid vesicles. There are three mechanisms by which antimicrobials are thought to act. One of these is to alter membrane permeability. Compounds such as the channel-forming peptide gramicidin and the cyclic depsipeptide carrier valinomycin are thought to function by this mechanism. Compounds such as nystatin and amphotericin have been described as forming ''barrel stave'' pores. These may alter membrane permeability but may also function by a second mechanism: disruption of membrane structure and organization. A third possibility is that the hydraphiles interact with membrane enzymes in a deleterious way.

In recent studies, bioluminescent bacteria[40] were used to assess the speed with which the microbe was killed by hydraphiles.[41] The bioluminescent *E. coli* emit light during log phase growth. Cell death can be assayed directly by loss of light emission. Compounds **9** (C_{12} hydraphile) and **22** (C_{14} hydraphile) were administered at their minimum bactericidal concentrations (MBCs). Half the *E. coli* population was killed by **9** in 8.5 minutes and by 22 in 9.1 minutes. The known antibiotic kanamycin (MBC = 1.3 μM) has a halftime for killing *E. coli* of 44.8 minutes. Such rapid killing is consistent with disruption of osmotic balance, but membrane disruption and interaction with membrane enzymes cannot be ruled out.[42]

5.4 Membrane depolarization studies

Disruption of osmotic balance leads to membrane depolarization. Fernandez-Lopez *et al.*[35] and we have used a combination of bilayer conductance and membrane depolarization to help understand the mechanism of action. Such studies typically use the membrane dye ''diSC3(5)'' (3,3′-dipropylthiadicarbocyanine iodide).[43] Organisms whose interior membranes exhibit a negative internal potential can absorb this dye. When this occurs, self-quenching of the dye results in little detectable fluorescence. When ion transport occurs, there is a membrane polarization change that is detected as increased fluorescence intensity. The results of such experiments comport with an ion transport mechanism for the cyclic peptide nanotubes[35] and the hydraphiles.[42]

5.5 Amphiphilic peptides

A number of efforts have been made to develop synthetic peptides as antibiotics. Some of these efforts are extensions of work with antibiotic peptides isolated from natural sources.[33] In others, semi-synthetic derivatives have been prepared.[44] A family of synthetic peptides having antibiotic activity has been reported by Tirrell and coworkers.[45] Inspiration has come from several quarters, but an important goal has been to address the re-emergence of bacterial infection resulting from antibiotic resistance.[46]

5.5.1 Synthesis of synthetic anion transporters. The hydraphile compounds described above were designed to be cation transporters and found to have significant biological activity. A family of synthetic amphiphilic peptides was designed to be a chloride selective transporter. The design was modular and had several components. Twin hydrocarbon tails comprised the *N*-terminus. Twin octadecyl chains are highly lipophilic and easily appended as the dialkylamine. A heptapeptide sequence was designed based on the amino acid sequence of the putative ion pathway in the ClC family proteins. Finally, the heptapeptide and hydrocarbon tails were linked through a diglycolic acid unit that approximates the length and polarity of the glyceryl portion of a phospholipid.

The initial compound resulting from this design was $(H_{37}C_{18})_2NHCOCH_2OCH_2CONH(Gly)_3Pro(Gly)_3OCH_2Ph$, **26**. It proved to be effective at transporting both Cl^- ion and carboxyfluorescein (CF^-).[25a] Extensive studies were undertaken to characterize the roles of the *C*- (ester) and *N*-terminal (dialkylamine) residues.[47] The central amino acid was varied[48,49] as well as the peptide chain length.[50] In several studies, the interaction of the peptide with Cl^- was clarified.[51,52] The preparation and activity of a synthetic dimer supported the conclusion from other studies that ion transport involved at least the formation of a dimeric pore.[53]

The lack of effective chloride transport is an issue in patients suffering from cystic fibrosis. The synthetic ion channels that are the subject of this review are generally of too high molecular weight to be serious drug candidates. Moreover, many are peptides, which are readily hydrolyzed by endogenous peptidases. Our hope that **26** might prove useful lay in the fact that the lungs are accessible by airway passages. Direct administration of **26** by inhaler seemed possible, if the compound itself was biologically active. We therefore assayed the efficacy of **26** in mouse airway epithelial cells by using an Ussing chamber.[54]

The Ussing chamber study confirmed that the synthetic, amphiphilic heptapeptide **26** stimulated C_{12} transport in mouse trachea epithelial cells. The membrane was not disrupted by the voltage change. Compound **26** appeared to establish a new chloride transport pathway in the apical membrane for chloride transport. This pathway appears to function independent of any chloride channels endogenous to the mouse tracheal cells.

We note that Tomich and coworkers have developed a family of semi-synthetic peptides that have also been studied in epithelial cells.[55] These compounds may be considered modified peptides rather than the synthetic receptors described above but the very different approaches have clearly borne similar fruit. The potential diversity of design and compounds is apparent.

6.0 Conclusions

The early impetus to develop synthetic ion transporters came largely from the challenge of preparing biofunctional molecules. During the past decade or more, property-directed synthesis has begun to compete seriously as an intellectual challenge with the more traditional target-directed synthesis. The latter will always be a major focus of chemistry and it is an essential part of biological model design. The novel compounds described in this review were designed to have a particular function: ion transport. The synthetic ion transporters are not only models for their biological counterparts, they are themselves functional ion transporters. As such, they exhibit biological properties tangential to those for which they were designed. The success of multiple laboratories in this endeavor foreshadows both great interest and future effort in designing molecules that exhibit substantial and selective biological activity while being relatively simple to prepare and of modest molecular weight. The potential applications of such compounds can only be imagined, but their future existence can be predicted with confidence.

Acknowledgements

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References

1 B. Hille, *Ionic Channels of Excitable Membranes (Third Edition)*, Sinauer Associates, Sunderland, MA, USA, 2001, 814 pp.
2 D. A Doyle, J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait and R. MacKinnon, *Science*, 1998, **280**, 69–77.
3 P. Agre, L. S. King, M. Yasui, W. B. Guggino, O. P. Ottersen, Y. Fujiyoshi, A. Engel and S. Nielsen, *J. Physiol.*, 2002, **542**, 3–16.
4 R. MacKinnon, *Angew. Chem., Int. Ed.*, 2004, **43**(33), 4265–77.
5 G. W. Gokel and A. Mukhopadhyay, *Chem. Soc. Rev.*, 2001, **30**, 274–286.
6 C. J. Pedersen, *J. Am. Chem. Soc.*, 1967, **89**, 7017–36.
7 V. E. Carmichael, P. J. Dutton, T. M. Fyles, T. D. James, J. A. Swan and M. Zojaji, *J. Am. Chem. Soc.*, 1989, **111**, 767–769.
8 A. Nakano, Q. Xie, J. V. Mallen, L. Echegoyen and G. W. Gokel, *J. Am. Chem. Soc.*, 1990, **112**, 1287–9.
9 C. L. Murray, H. Shabany and G. W. Gokel, *Chem. Commun.*, 2000, 2371–2372.
10 E. Abel, G. E. M. Maguire, O. Murillo, I. Suzuki and G. W. Gokel, *J. Am. Chem. Soc.*, 1999, **121**, 9043–9052.
11 H. Shabany and G. W. Gokel, *Chem. Commun.*, 2000, 2373–2374.
12 H. D. Arndt, D. Bockelmann, A. Knoll, S. Lamberth, C. Griesinger and U. Koert, *Angew. Chem., Int. Ed.*, 2002, **41**, 4062–5.
13 N. Sakai, J. Mareda and S. Matile, *Acc. Chem. Res.*, 2005, **38**, 79–87.
14 Y. Baudry, D. Pasini, M. Nishihara, N. Sakai and S. Matile, *Chem. Commun.*, 2005, 4798–800.
15 I. Iacovache, P. Pauard, H. Scheib, C. Lesieur, N. Sakai, S. Matile, M. W. Parker and F. G. van der Goot, *EMBO J.*, 2006, **25**, 457–66.
16 J. M. Tomich, D. Wallace, K. Henderson, K. E. Mitchell, G. Radke, R. Brandt, C. A. Ambler, A. J. Scott, J. Grantham, L. Sullivan and T. Iwamoto, *Biophys. J.*, 1998, **74**, 256–267.
17 V. Sidorov, F. W. Kotch, J. L. Kuebler, Y.-F. Lam and J. T. Davis, *J. Am. Chem. Soc.*, 2003, **125**, 2840–2841.
18 A. V. Koulov, T. N. Lambert, R. Shukla, M. Jain, J. M. Boon, B. D. Smith, H. Li, D. N. Sheppard, J. B. Joos, J. P. Clare and A. P. Davis, *Angew. Chem., Int. Ed.*, 2003, **42**, 4931–4933.
19 N. Madhavan, E. C. Robert and M. S. Gin, *Angew. Chem., Int. Ed.*, 2005, **44**, 7584–7587.
20 K. Kano and J. H. Fendler, *Biochim. Biophys. Acta*, 1978, **509**, 289–299.
21 O. Murillo, S. Watanabe, A. Nakano and G. W. Gokel, *J. Am. Chem. Soc.*, 1995, **117**, 7665–7679.
22 M. Hervé, B. Cybulska and C. M. Gary-Bobo, *Eur. Biophys. J.*, 1985, **12**, 121–128.
23 F. G. Riddell and M. K. Hayer, *Biochem. Biophys. Acta*, 1985, **817**, 313–317.
24 (*a*) S. L. Schendel and J. C. Reed, *Methods Enzymol.*, 2000, **322**, 274–82; (*b*) A. Lynch, D. Diamond and M. Leader, *Analyst*, 2000, **125**, 2264–7.
25 (*a*) P. H. Schlesinger, R. Ferdani, J. Liu, J. Pajewska, R. Pajewski, M. Saito, H. Shabany and G. W. Gokel, *J. Am. Chem. Soc.*, 2002, **124**, 1848–9; (*b*) M. E. Weber, P. H. Schlesinger and G. W. Gokel, *J. Am. Chem. Soc.*, 2005, **126**, 636–642.
26 J. N. Weinstein, S. Yoshikami, P. Henkart, R. Blumenthal and W. A. Hagins, *Science*, 1977, **195**, 489–92.
27 M. L. Graber, D. C. DiLillo, B. L. Friedman and E. Pastoriza-Munoz, *Anal. Biochem.*, 1986, **156**, 202–12.
28 W. M. Leevy, J. E. Huettner, R. Pajewski, P. H. Schlesinger and G. W. Gokel, *J. Am. Chem. Soc.*, 2004, **126**, 15747–15753.
29 C. J. Pedersen, *Org. Synth.*, 1972, **52**, 66–74.
30 B. K. J. Leong, T. O. T. Ts'o and M. B. Chenoweth, *Toxicol. Appl. Pharmacol.*, 1974, **27**, 342–54.
31 K. Takayama, S. Hasegawa, S. Sasagawa, N. Nambu and T. Nagai, *Chem. Pharm. Bull.*, 1977, **25**, 3125–3130.
32 R. R. Hendrixson, M. P. Mack, R. A. Palmer, A. Ottolenghi and R. G. Ghirardelli, *Toxicol. Appl. Pharmacol.*, 1978, **44**, 263.
33 S. C. Gad, W. J. Conroy, J. A. McKelvey and K. A. Turney, *Drug Chem. Toxicol.*, 1978, **1**, 339–53.
34 S. C. Gad, C. Reilly, K. Siino, F. A. Gavigan and G. Witz, *Drug Chem. Toxicol.*, 1985, **8**, 451–68.
35 S. Fernandez-Lopez, H.-S. Kim, E. C. Choi, M. Delgado, J. R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D. A. Weinberger, K. M. Wilcoxen and M. R. Ghadiri, *Nature*, 2001, **412**, 452–455.
36 H. W. Huang, in Gramicidin and related Ion Channel-forming Peptides, Novartis Foundation Symposyum 225, ed. D. J. Chadwick and G. Cardew, John Wiley & Sons, Chichester, UK, 1999, 188–206.
37 E. Biron, F. Otis, J. C. Meillon, M. Robitaille, J. Lamothe, P. Van Hove, M. E. Cormier and N. Voyer, *Bioorg. Med. Chem.*, 2004, **12**, 1279–90.
38 W. M. Leevy, G. M. Donato, R. Ferdani, W. E. Goldman, P. H. Schlesinger and G. W. Gokel, *J. Am. Chem. Soc.*, 2002, **124**, 9022–3.
39 C. L. Murray, H. Shabany and G. W. Gokel, *Chem. Commun.*, 2000, 2371–2372.
40 S. T. Gammon, W. M. Leevy, S. Gross, G. W. Gokel and D. Piwnica-Worms, *Anal. Chem.*, 2006, **78**, 1520–7.
41 W. M. Leevy, S. T. Gammon, T. Levchenko, D. D. Daranciang, O. Murillo, V. Torchilin, D. Piwnica-Worms, J. E. Huettner and G. W. Gokel, *Org. Biomol. Chem.*, 2005, **3**, 3544–3550.
42 W. M. Leevy, M. R. Gokel, G. B. Hughes-Strange, P. H. Schlesinger and G. W. Gokel, *New J. Chem.*, 2005, **29**, 205–209.
43 W. M. Leevy, M. E. Weber, P. H. Schlesinger and G. W. Gokel, *Chem. Commun.*, 2005, 89–91.
44 B. al-Nawas and P. M. Shah, *Infection*, 1998, **26**, 165–7.
45 A. F. Chu-Kung, K. N. Bozzelli, N. A. Lockwood, J. R. Haseman, K. H. Mayo and M. V. Tirrell, *Bioconjugate Chem.*, 2004, **15**, 530–5.

46 P. M. Shah, *Clin. Microb. Infect.*, 2005, **11**, 36–42.
47 N. Djedovic, R. Ferdani, E. Harder, J. Pajewska, R. Pajewski, M. E. Weber, P. H. Schlesinger and G. W. Gokel, *New J. Chem.*, 2005, **29**, 291–305.
48 P. H. Schlesinger, R. Ferdani, J. Pajewska, R. Pajewski and G. W. Gokel, *New J. Chem.*, 2003, **27**, 60–67.
49 R. Ferdani, R. Pajewski, N. Djedovic, J. Pajewska, P. H. Schlesinger and G. W. Gokel, *New J. Chem.*, 2005, **29**, 673–680.
50 R. Ferdani, R. Pajewski, J. Pajewska, P. H. Schlesinger and G. W. Gokel, *Chem. Commun.*, 2006, 439–441.
51 R. Pajewski, R. Ferdani, J. Pajewska, R. Li and G. W. Gokel, *J. Am. Chem. Soc.*, 2005, **126**, 18281–18295.
52 G. A. Cook, R. Pajewski, M. Aburi, P. E. Smith, O. Prakash, J. M. Tomich and G. W. Gokel, *J. Am. Chem. Soc.*, 2006, **128**, 1633–1638.
53 R. Pajewski, R. Ferdani, J. Pajewska, N. Djedovic, P. H. Schlesinger and G. W. Gokel, *Org. Biomol. Chem.*, 2005, **3**, 619–625.
54 R. Pajewski, R. Garcia-Medina, S. L. Brody, P. H. Schlesinger and G. W. Gokel, *Chem. Commun.*, 2006, 329–331.
55 K. E. Mitchell, T. Iwamoto, J. Tomich and L. C. Freeman, *Biochim. Biophys. Acta*, 2000, **1466**, 47–60.