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Artificial ion channels

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Abstract. The approaches to the design of artificial ion channels are systematised and the data on their structure, stability and electrical conduction are summarised. The bibliography includes 104 references.

I. Introduction

The transport of ions through phospholipid membranes is among the most important requisites of functioning of living organisms.[1–4] The key function of the phospholipid bilayer is to create a barrier preventing the hydrophilic molecules and ions present in the aqueous phases near the cell from penetrating inside the cell. This function is performed by the hydrophobic internal layer of the phospholipid membrane. Nevertheless, some polar compounds and ions are able to penetrate the membrane at a high rate. This selective permeability of natural membranes for certain particles is needed for many biological processes to occur.

The transport of ions through a membrane can be accomplished by carrier molecules (transporters) or through transmembrane channels. In biological systems, the ion transfer (biological transport) takes place mainly through ion channels formed most often by appropriate proteins (channel proteins), which produce channel structures in phospholipid membranes; the sites of binding of the transported substances in such proteins are accessible simultaneously from both sides of the membrane. In this case, the ion transport occurs very rapidly ($10^6 – 10^8$ ion s^{-1} per channel protein).[4–7]

The rate of ion transport through a membrane by carrier molecules is lower by several orders of magnitude. This difference between the rates is due to several reasons. An important reason is the fact that channel proteins do not undergo serious conformational changes upon the transport of ions from one side of the membrane to the other, whereas carrier molecules do undergo conformational changes when they bind the ions to be transported on one side of the membrane and liberate them on the other side. In addition, the binding sites for molecules being transported in the carrier molecules are accessible only on one side of the membrane.

The carrier molecules are used more often for artificial transport of ions than transmembrane channels. This is due to the fact that artificial transmembrane channels are compounds with complex organisation. The synthesis and elucidation of the three-dimensional structure of such compounds are faced with substantial difficulties. Nevertheless, artificial transport systems are very important because study of these systems is favourable for deeper understanding of the properties of channel proteins at the molecular level and forms the basis for the development of new pharmaceuticals and drug delivery systems.[1,8] Transmembrane channels perform the ion transport through membranes by a flow or jumping mechanism.[1–4]

The last decade has seen intensive research into biomimetic tubular structures mimicking natural ion channels[9–17] which can be inserted into 3 – 5-nm thick bilayer membranes. Depending on the type of molecule that forms the channel and general features of its structure and also on the nature and mutual arrangement of the ion binding sites, synthetic ion channels are classified into different types. Some of them are sketched in Fig. 1.

The natural channel proteins consist of two key functional units, namely, the 'gate' and the 'pore'.[8] The 'pore' implies the way of ion penetration through the membrane, while the 'gate' means the mechanism of switching on and off the ion flow. The ions that pass through the channel are considered as an ion flux. The ion flux through the membrane depends on the difference between the electrochemical potentials on the two sides of the membrane. The conductance, *i.e.*, the reciprocal of the resistance, serves as the measure of the efficiency of the ion flux.

Let us consider three main characteristics of ion channels:[2,8] (i) the time it takes for the channel to attain a constant conductance (*i.e.*, the time of maintenance of an invariable geometric structure of the channel, for example, the critical length and pore diameter); (ii) ion selectivity caused by the energetic and geometric complementarity of the ions and the channel pores; and

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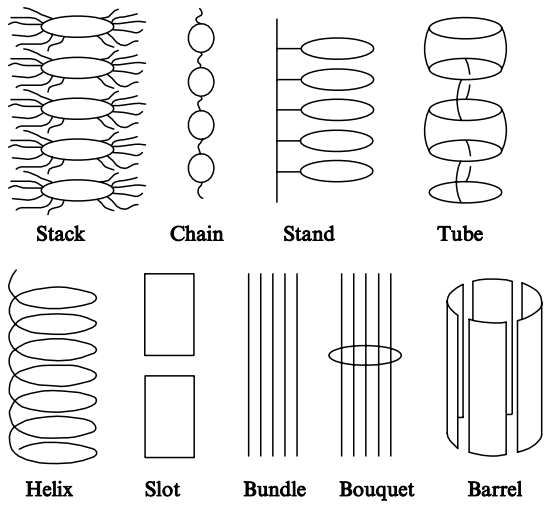


Figure 1. Types of structures of ion channels.

(iii) the type of gate mechanism (gate opening–closing) acting in the channel.

The conductance of an ion channel depends on the pore size and length and on the dielectric constant. The ion selectivity also depends on many factors such as the size of the pores, the energy of ion dehydration, the electrostatic interaction between the ions and the amino acid residues that form the pore. (The channel activity of synthetic peptides is often measured using the method of planar bilayers.[8])

Depending on the type of gate mechanism, ion channels are classified into sensing, ligand and mechanical ones. In the sensing (voltage-controlled or electrically excitable) channels, the channel opening is induced by an electric signal. Examples of such channels are fast Na^+-channels[2] or voltage-gated K^+-channels, which respond to the change in the potential of nerve cells (Fig. 2 *a*).[8] In the ligand (ligand-gated or chemically excitable) channels, opening or closing of the channel is induced by the chemical changes that take place under the action of an appropriate ligand. These channels can be exemplified by nicotinic cholinergic receptor (Fig. 2 *b*). In mechanical (mechanosensitive) channels, opening of the channel is due to expansion of the cell membrane. Channels of this type participate in muscle contraction and in the control of the osmotic pressure and cell volume.

A number of approaches to the design of ion channels and ion-sensitive membrane systems distinguished by high sensitivity and selectivity have now been proposed.[18] The increase in interest in detecting biologically active substances *in vitro* and *in vivo* (for example, in individual cells and in tissue sections and organs) and in the development of nano-sized drugs and ion–molecular devices is stimulating studies aimed at the design of synthetic ion channels.

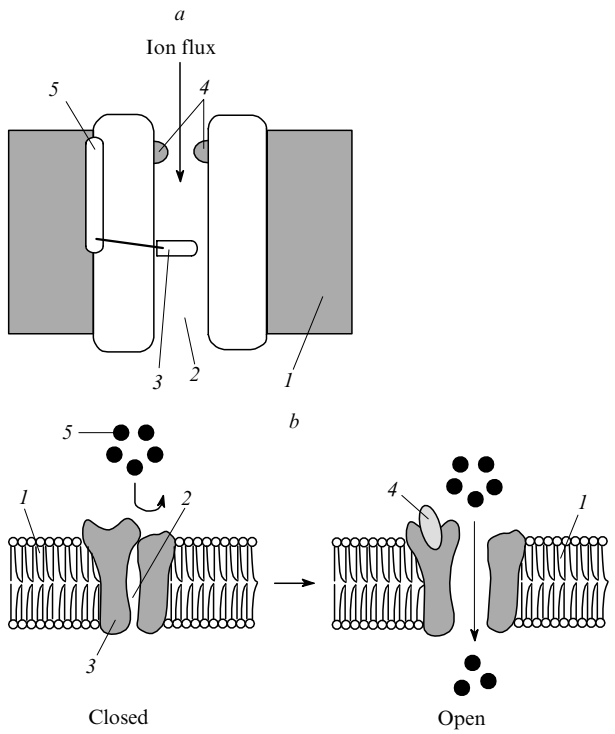


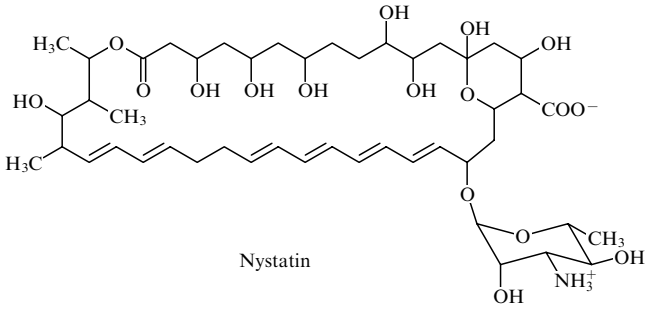
Figure 2. Schematic view of ion voltage-gated (*a*) and ligand-gated (*b*) channels.
Designations in (*a*): (*1*) membrane, (*2*) pore, (*3*) gate, (*4*) ionic filter, (*5*) potential-sensitive fragment.
Designations in (*b*): (*1*) membrane, (*2*) pore, (*3*) ligand-gated fragment; (*4*) ligand; (*5*) ions.

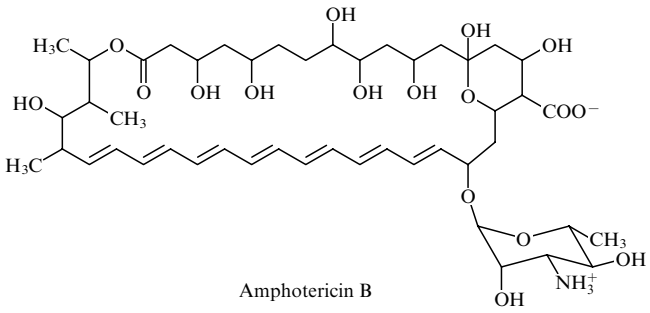
II. Compounds used as models of natural channels

A lot of data on the structure and properties of channel proteins which perform biological transport has been collected.[1–4] The amino acid sequences in many channel proteins have been decoded. However, in investigations of the structure and the mechanism of channel operation, it was necessary to resort to model systems, due to the complexity of X-ray diffraction studies of transport proteins. Considerable capabilities for this modelling are provided by the use of oligo- and polypeptides [for example, some toxins[19,20] and polyene (nystatin, amphotericin B) and peptide (gramicidin A, alamethicin and mellitin)[21–23] antibiotics] capable of forming channel-like associates in bilayer phospholipid membranes.

1. Nystatin and amphotericin B

The polyene antibiotics — amphotericin B,[24–26] filipin[25] and nystatin[27] — are membrane-active antimycotic agents.





Apparently, the lytic activity† and the ability of this type of antibiotic to induce the removal of low-molecular-weight compounds from cells with low selectivity are due to the association of

† Lytic activity is the ability of a compound to cause rupture of the cell wall.

these agents with steroid membranes to form pores. The formation of these complexes has been detected by NMR.[21,26]

The molecules of polyene antibiotics 8 to 10 in number form a tubular structure (pore) with the hydroxy groups directed inwards in the membrane bilayer.[22,28] The diameter of this pore is about 8 Å and water molecules are loosely accommodated inside. For the formation of a channel in the phospholipid bilayer, two cylindrical structures arranged according to the tail-to-tail pattern are required. This channel would be nonpolar from the outside but hydrophilic from the inside and would end with polar fragments of amino sugars. The association and dissociation of the tubular ensemble of polyene molecules result in opening and closing of the channel, respectively.[22–28]

2. Gramicidin A and alamethicin

The best known ion channel is that formed by gramicidin A,[2,29,30] which is a hydrophobic peptide composed of alternating L- and D-amino acids[31] (HCO–L-Val–Gly–L-Ala–D-Leu–L-Ala–D-Val–L-Val–D-Val–L-Trp–D-Leu–L-Trp–D-Leu–L-Trp–D-Leu–L-Trp–$NHCH_2CH_2OH$). This ion channel is formed by two gramicidin A molecules arranged in the head-to-head mode by their N-end fragments to form a β(LD)-helical configuration[32] (Fig. 3 *a*). In this helical structure, all the side chains are located outside, while the carbonyl groups are inside the channel. The total length of the gramicidin channel is equal to ~30 Å; the external and internal diameters are 15 and 5 Å, respectively. Owing to the small internal diameter of the channel, the transport through the channel can be performed only by a single molecule. The gramicidin channel is cation selective,[33] although gramicidin A itself is extremely hydrophobic and contains no polar amino acid. The conductance of a single channel formed by gramicidin is rather high. When the concentration of RbCl in the solution surrounding the membrane is 100 mmol litre^{-1}, the cationic conductance of the channel (for Rb) amounts to 30×10^{-12} Ω^{-1} per channel,[2] whereas the permeability for chloride anions is zero.[30] This selectivity is related to the electrostatic repulsion of the anions by the carbonyl group dipoles at the gate of the channel. The permeability of the gramicidin channel for single-charged cations changes in the following sequence: $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$. The selectivity exhibited by the gramicidin channel with respect to these cations is not very high: it corresponds to a fivefold change in the permeability coefficients. The gramicidin channel is opened due to association of monomers to form a dimer and closed upon dissociation of the dimer (Fig. 3 *a*). The lifetime of the dimer is ~10^{-3} to 10 s, depending on the lipid composition of the membrane and other factors.[2,29,30] It is noteworthy that the gramicidin channel is not voltage-dependent.

Alamethicin (Alm) is a peptide antibiotic consisting of 20 amino acid residues, which forms transmembrane channels with a variable number of associated subunits in artificial[34–37] and biological[38] membranes. The mechanism of ion transport by alamethicin has been studied rather comprehensively. The peptide forms α-helices in the bilayer, which are associated with each other forming a channel which consists of several interconnected α-helices[39–43] (Fig. 3 *b*). The conductance of this channel, unlike that of the gramicidin channel, depends on the potential applied to the surface of the bilayer membrane. Alamethicin channels start conducting ions (Na^+, K^+) only at a positive transmembrane potential (> 60 mV). Thus, they represent nano-sized ionic diodes which can conduct current only in one direction, like macroscopic diodes. The voltammetric characteristics of the alamethicin channel formed in a planar bilayer on the basis of a mixed lipid (egg phosphatidylcholine–egg phosphatidic acid–cholesterol in a molar ratio of 8 : 2 : 1) are shown in Fig. 4.

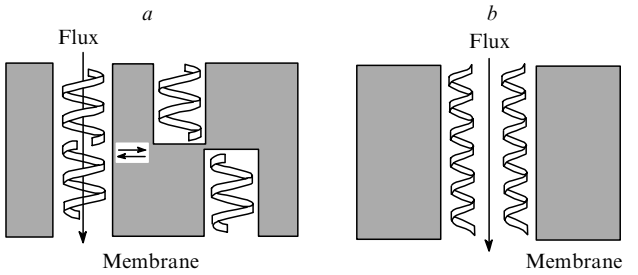


Figure 3. Schematic view of the ion channels formed in phospholipid membranes by peptide antibiotics, gramicidin A (*a*) and alamethicin (*b*) under the action of osmotic pressure.

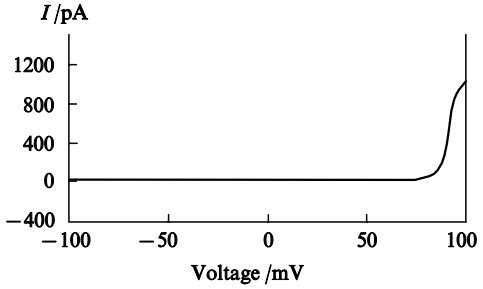


Figure 4. Average macroscopic current–voltage relationships for alamethicin channel (130 pmol of alamethicin, 1 M KCl, egg phosphatidylcholine–egg phosphatidic acid–cholesterol, 10 cycles from −100 to 100 mV at 5.7 mV s^{-1}).

3. Ion channels based on modified peptides and antibiotics

Futaki *et al.*[34] have prepared the channel peptide Alm–LeuZ by covalent linking of a leucine-enriched oligopeptide (LeuZ) to an ionophore, namely, alamethicin (Alm). As a result of this linking, it is possible to perform the extramembrane control of the association of the channel peptides Alm–LeuZ (Fig. 5) and thus to change the channel conductance. Here Alm–LeuZ = Ac–Alm–Gly–Gly–Gly–Gly–LeuZ–$CONH_2$, Alm = UP.UAUAQUVUGLUPVUUEQF, LeuZ = RXKQLEDKVEEL.

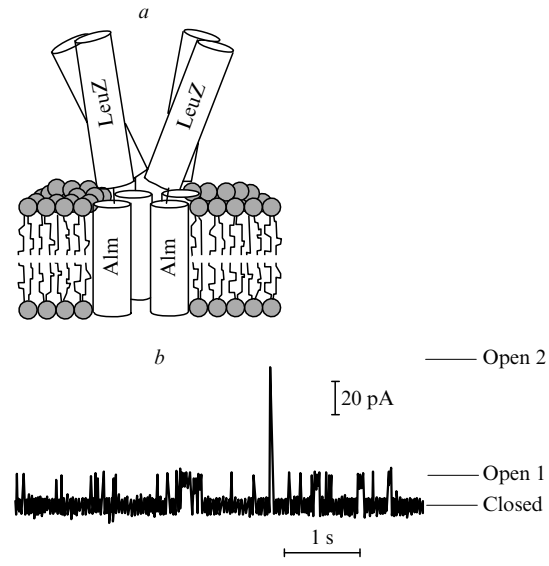
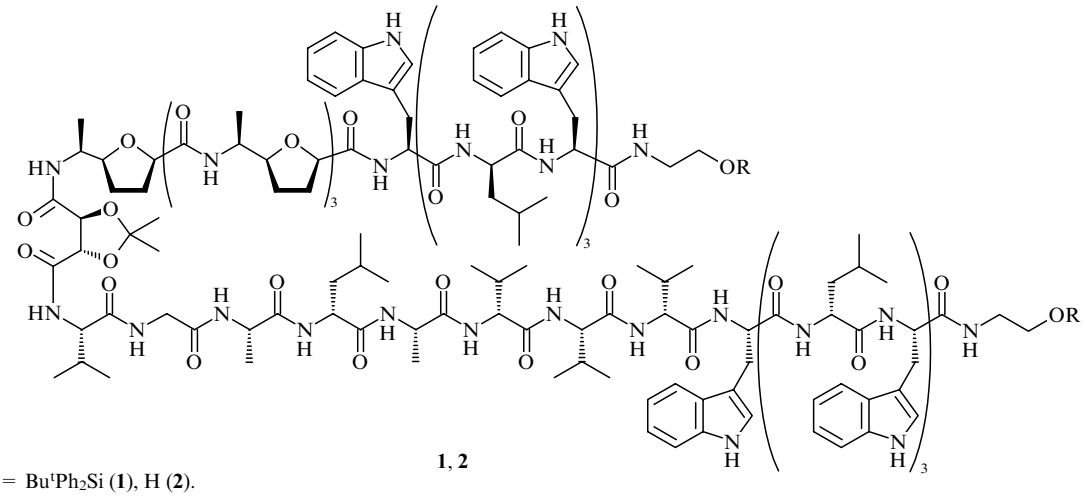


Figure 5. Schematic view of a single channel formed in a lipid bilayer by an association of modified alamethicin subunits Alm–LeuZ (50 nmol Alm–LeuZ) (*a*) and current through this single channel at 100 mV (*b*).[34] Open 1 and Open 2 are the current levels observed for two different conformations of the channel.



1, **2**

R = Bu^tPh_2Si (**1**), H (**2**).

.LSKNYHLENEVARLKKLVGE, where U is (*R*)-aminoisobutyric acid (Aib), X is norleucine (Nle), A is Ala, D is Asp, E is Glu, F is Phe, G is Gly, H is His, K is Lys, L is Leu, N is Asn, P is Pro, Q is Gln, R is Arg, S is Ser, V is Val, and Y is Tyr.

The conductance of this single ion channel in 1 M KCl is $120 \times 10^{-12}\ \Omega^{-1}$. The channel behaviour is voltage dependent. The conformational transitions from the helical to the disordered structure which take place in extramembrane segments regulate the ionic conduction of the channel. Thus, it is possible to control the functioning of the channel by varying the conformation of the attached synthetic extramembrane fragment.

In 1977, Bamberg and Janko [44] connected two gramicidin A molecules by a malonic acid bridge and thus obtained malonylbis(deformyl)gramicidin. It was shown that this covalent dimer forms a channel in the bilayer lipid membrane, the conductance and the ion selectivity of this channel being the same as those of the gramicidin channel, while the time the channel remains open significantly increases. Whereas the open lifetime is 1–2 s for the single gramicidin channel, in the case of a single malonylbis(deformyl)gramicidin channel, it is several minutes.

Arndt *et al.*[45] also used gramicidin A molecules as building blocks for the design of artificial ion channels with a long-lived active conformation (unlike the natural short-lived gramicidin dimers). For this purpose, two gramicidin molecules were cross-linked by C_4-linkers based on succinic acid. To study the phenomenon of the active conformation of the channel protein, a series of gramicidin-based synthetic channels with a long open duration in solution has been developed. The compounds synthesised for this purpose contained gramicidin molecules connected by various C_4-linkers. The influence of the linkers on the channel behaviour was studied.[45]

In the last decade, ion channels based on gramicidin and other antibiotics and on modified peptides have been used as sensing elements. These channels incorporated in a bilayer lipid membrane are highly sensitive and selective.[18] Sensors making use of chemically modified gramicidin and alamethicin have been reported.[46,47] Gramicidin ion channels bound to antibodies by covalent bonds were used to design biosensors for determination of proteins, viruses, antibodies, DNA, electrolytes, drugs, and pesticides. The ionic conductance of these channels is regulated by the interaction of substrates with the antibodies connected to gramicidin molecules through biotin bridges.

It is well known that tetrahydrofuran can efficiently solvate metal cations. Therefore, in the development of cation-conductive synthetic peptide channels, it has been proposed to use oligotetrahydrofurans and THF-substituted amino acids. On the basis of THF-containing amino acids, cation channels with biomimetic entrance and exit have been synthesised.[48]

The biomimetic fragment present in the obtained structures **1** and **2** is gramicidin A. The entrance and the exit of the channels **1** and **2** contain four tryptophan residues. Apparently, the indole side groups of tryptophan contribute to stabilisation of the structures of channels **1** and **2** through hydrogen bonding to the polar groups of the lipid membrane and electrostatic dipole–dipole interaction with the surface charge of the membrane. The compounds **1** and **2** contain a heptapeptide (L-Trp–D-Leu)$_3$–L-Trp, a tetra-THF-peptide fragment, a linker based on tartaric acid ‡ and a pentadecapeptide, namely, gramicidin A.

In the compound **1**, the terminal fragments of ethanolamine are protected by lipophilic *tert*-butyldiphenylsilyl groups, while in the compound **2**, they are free. Upon incorporation in the lipid bilayer, the compound **1** forms a channel with different levels of conductance, indicating the existence of several active conformations of the channel. The first conformation with a conductance of $15.4 \times 10^{-12}\ \Omega^{-1}$ and an average open-state duration of 500 ms is responsible for passage of ~30% of the cations through the channel. The second conformation ($19.7 \times 10^{-12}\ \Omega^{-1}$) is more rarely encountered; however, its contribution to the total cation flux is greater, as its lifetime in the open state is ~15 s. Channel **1** demonstrated selectivity with respect to single-charged cations ($NH_4^+ > Cs^+ > K^+ > Na^+$). Since the cations lose their hydration shells, at least partially, when passing through the channel, this selectivity is due to the difference in the dehydration energies of the cations.

The electrochemical experiments with gramicidin D carried out by Wang's group [50] have dealt with the effect of the gramicidin concentration in the bilayer lipid membrane on the properties of the channels formed by gramicidin. When the concentration of gramicidin D is high, its structure in the bilayer lipid membrane is represented only by dimers.

Futaki [8] reported a new approach to the design of sodium ion channels on the basis of the peptide template H–Cys(Acm)–Lys–Cys(SH)–Pro–Gly–Cys(MBzl)–Glu–Cys(Ad)–Gly–OH (Fig. 6). He synthesised a compound containing four different α-peptides ([NaI]–[NaIV]) attached to the given peptide template.

The assembly of such a peptide ion channel is shown in Fig. 7. Chloroacetyl-γ-aminobutyric acid was attached as a spacer to the N-end fragments of the amino acid sequences of the [NaI]–[NaIV] peptides. All the [NaI]–[NaIV] α-peptides were linked to the template through the interaction of chloroacetyl fragments with the free SH groups of the template after selective removal of the S-protective groups. The protein thus produced showed an ion-channel activity in lipid bilayers, dependent on the conformational changes in the channel induced by the change in the voltage applied to the membrane.

‡ (*S*,*S*)-Tartaric acid has already been successfully used as the bridge for the head-to-head covalent attachment for the gramicidin dimer.[49]

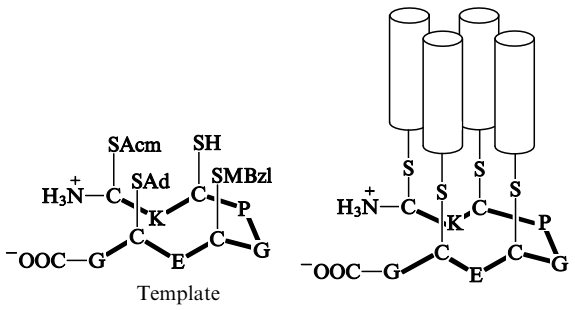


Figure 6. Nonapeptide template and artificial ion channel assembled from it.
Designations: C is Cys, K is Lys, P is Pro, G is Gly, E is Glu, Acm is acetamidomethyl, MBzl is *p*-methoxybenzyl, Ad is 1-adamantyl.

4. Toxins

Toxins capable of forming channels in a bilayer membrane are often used to model ion channels. The problem of spontaneous incorporation of these water-soluble hydrophilic proteins in the membrane bilayer appears to be most interesting. This problem has been studied using thionins and ε-toxin.[4,19] Thionins are small cysteine-containing amphipathic plant proteins. Many of them are toxic for microorganisms and mammal cells. Hughes *et al.*[19] have shown that β-purothionin isolated from wheat flour can form cation-selective ion channels in artificial and biological bilayer lipid membranes (plasma membrane of neurons of the rat hippocamp); this apparently is responsible for the toxicity of β-purothionin and other thionins with respect to cells.

Petit *et al.*[20] demonstrated that the ε-toxin produced by the *Clostridium perfringens* microbe forms in lipid membranes wide channels capable of carrying substances with a molecular mass of up to 1 kDa, for example, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The ionic conductance of this toxin in a synthetic bilayer membrane amounts to 50×10^{-12} Ω^{-1} at a KCl concentration of 100 μmol litre^{-1}.

The method of template assembling of synthetic peptides has also been used to prepare a branched peptide containing a δ-toxin fragment.[51] This peptide was used as a model to study the structure and the transport properties of the δ-toxin.

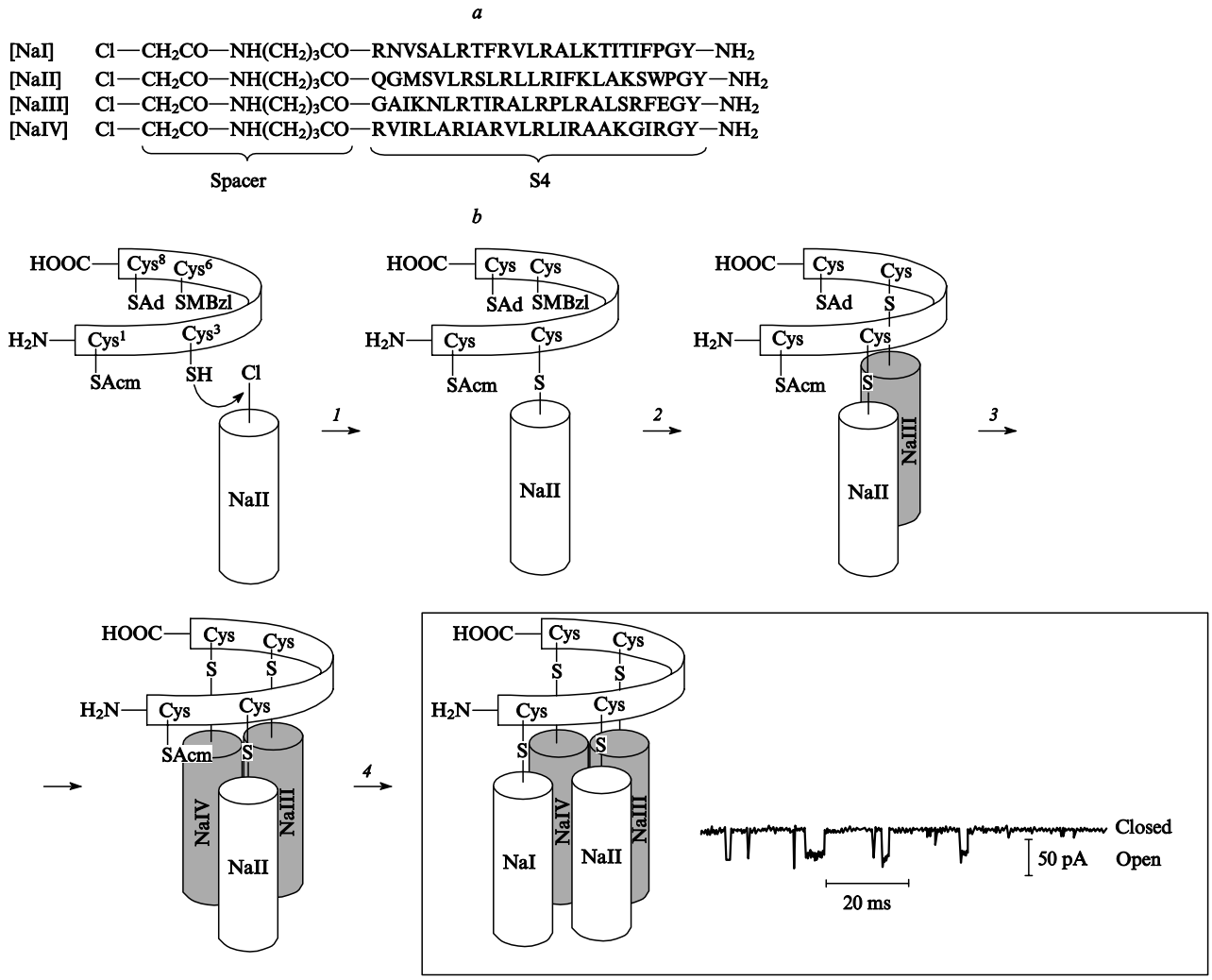


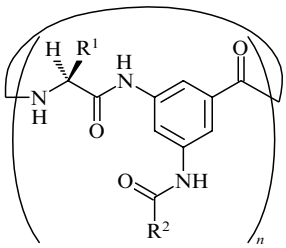
Figure 7. Synthesis of four-helical bundle-type peptide sodium channel [NaI]–[NaIV] containing peptide potential-sensitive segments (S4). For designations of amino acid residues, see the text, I is isoleucine, T is threonine.
(*a*) Design of peptide segments, (*b*) assembling and profile of a single peptide channel.
(*1*) Introduction of [NaII] in a 6 M solution of guanidine hydrochloride–0.1 M Tris-buffer (pH 8) system, (*2*) removal of the MBzl-protective group by treatment with 1 M Me_3SiBr–thioanisole in THF and introduction of [NaIII] (under conditions *1*), (*3*) removal of the Ad-protection on treatment with 1 M CF_3SO_3H–thioanisole in THF and introduction of [NaIV] (see *1*), (*4*) removal of the Acm protection on treatment with $AgOSOCF_3$ in THF and introduction of [NaI] (see *1*).

III. Artificial transmembrane ion channels based on cyclic peptides

1. Cyclic peptides consisting of alternating natural and abiotic amino acids

Usually, cyclic peptides form hydrophilic ion channels shaped like stacks in bilayer membranes. These channels are formed as a result of specific intermolecular interactions either between the peptide fragments of the macrocycle or between the amino acid residues of the side chains. The possibility of formation of this type of associate by cyclic peptides (see Fig. 1) was predicted back in 1972 by Hassal *et al.*[52] Later, it was shown by X-ray diffraction[11] that cyclic tetrapeptides composed of alternating α- and β-amino acid residues are packed into stacks through hydrogen bonding of the cyclic fragments thus forming hollow cylindrical structures.

Japanese researchers [53] have synthesised cyclic peptides **A**–**C** containing 3 to 5 dipeptide units on the basis of one abiotic [5-(n-alkanoylamino)-3-aminobenzoic acid] and one natural (L-alanine, L-aspartic acid, L-lysine and L-serine) amino acids (Fig. 8). The unnatural amino acid makes the cyclic peptides structurally rigid.



A–**C**

R^1 = Me (L-Ala), CH_2COOH (L-Asp), $(CH_2)_3NH_2$ (L-Lys), CH_2OH (L-Ser); $R^2 = C_9H_{19}-C_{17}H_{35}$; $n = 3-5$.

Analysis of the structure of the possible associates formed by such peptides and the data on the voltage-dependent blockade of the channels based on them by Ca^{2+} ions allowed the researchers [53] to conclude that ion channels consist of two peptide rings packed according to the tail-to-tail pattern. The peptide parts of the rings form the entrance and the exit of the channel, while the long alkanoyl chains penetrate the membrane. It was also shown that variation of the natural amino acid incorporated in the cyclopeptide, *e.g.*, replacement of the neutral L-alanine by either positively charged L-lysine or negatively charged L-aspartic acid, does not influence the channel selectivity.

The ion channels formed by cyclic peptides **A**–**C** with systematically varied pore size, shape and lipophilicity had a conductance of $\sim 9 \times 10^{-12}\ \Omega^{-1}$ in a 500 μM aqueous solution of KCl. The selectivity of transport of the potassium cation with respect to the chloride anion (P_{K^+}/P_{Cl^-}) was 5.9, while that with respect to the sodium cation (P_{K^+}/P_{Na^+}) was ~12.5.

Under conditions that mimic the biological conditions (addition of equal amounts of solutions of sodium and potassium gluconates), the sodium/potassium selectivity (P_{Na^+}/P_{K^+}) for the tetradipeptide $n = 4$ (Fig. 8, structure **B**) is equal to 0.40. The Li^+ cation cannot pass through this channel, despite the substantially smaller size (1.56 Å) compared to the K^+ cation (2.66 Å), because of the great energy expenditure required for dehydration (it has been noted above that dehydration is a necessary stage involved in the transport of cations through the channel [2]).

2. β³-Peptides

β³-Peptides represent a class of biopolymers of abiotic origin, which tend to form unusual types of secondary structures.[11, 54–57] Gellman and co-workers [54, 56] have shown that homooligomers of *trans*-2-aminocyclohexane- and -cyclopentanecarboxylic acids form helical structures. The cyclic oligomers formed by α-unsubstituted β-amino acids with the β-chiral carbon atom can adopt planar ring conformations; in the solid state, these can be combined through hydrogen bonding to form hollow tubular ensembles (cyclic β³-peptide nanotubes).[57] These ensembles are structurally similar to self-assembling associates of cyclic peptides consisting of alternating D- and L-α-amino acids (cyclic DL-α-peptide nanotubes).[58–62] These two classes of nanotubes supplement each other in the structure and functions.[11]

Ghadiri and co-workers [11] have shown that β³-peptides **3**–**5** with appropriately selected hydrophobic side chains can be self-assembled in the lipid bilayer membranes to form highly efficient transmembrane ion channels (Fig. 9).

Owing to their unusual structure, cyclic β³-peptide nanotubes of abiotic origin exhibit unique properties (selectivity, solubility). In particular, due to the unidirectional arrangement of the polar amide groups in space, the nanotubes based on cyclic β³-tetrapeptide subunits possess a macrodipole moment (Fig. 10 *a*), whereas the nanotubes based on cyclic DL-α-octapeptides (Fig. 10 *b*) are not dipoles. The dipole moment of the amide groups (macrodipole) enhanced by the parallel arrangement of the macrocycles

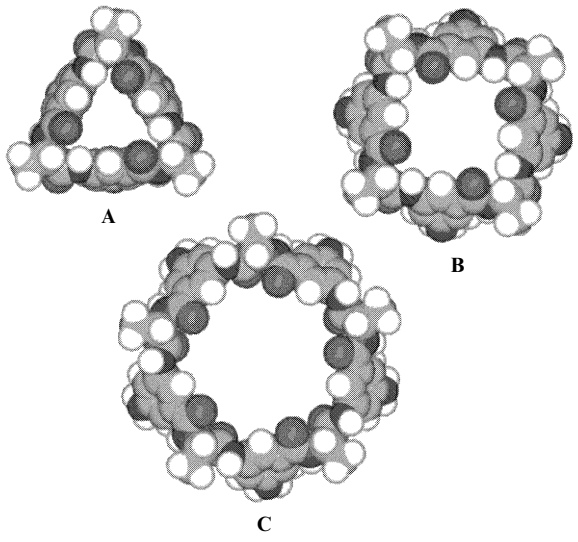


Figure 8. Models of peptides containing 3 (**A**), 4 (**B**) and 5 (**C**) dipeptide units (vertical view).[53]
R^1 = Me; for clarity, the alkanoyl chains $R^2C(O)$ are not shown.

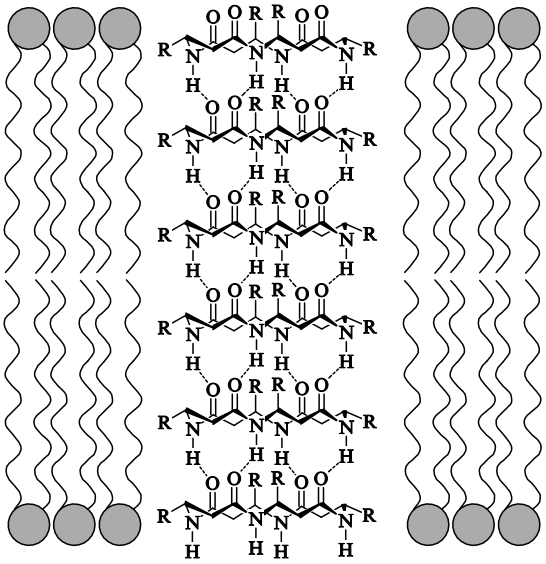


Figure 9. Presumptive structure of self-assembling transmembrane channels formed by β³-peptides **3**–**5**.[11]

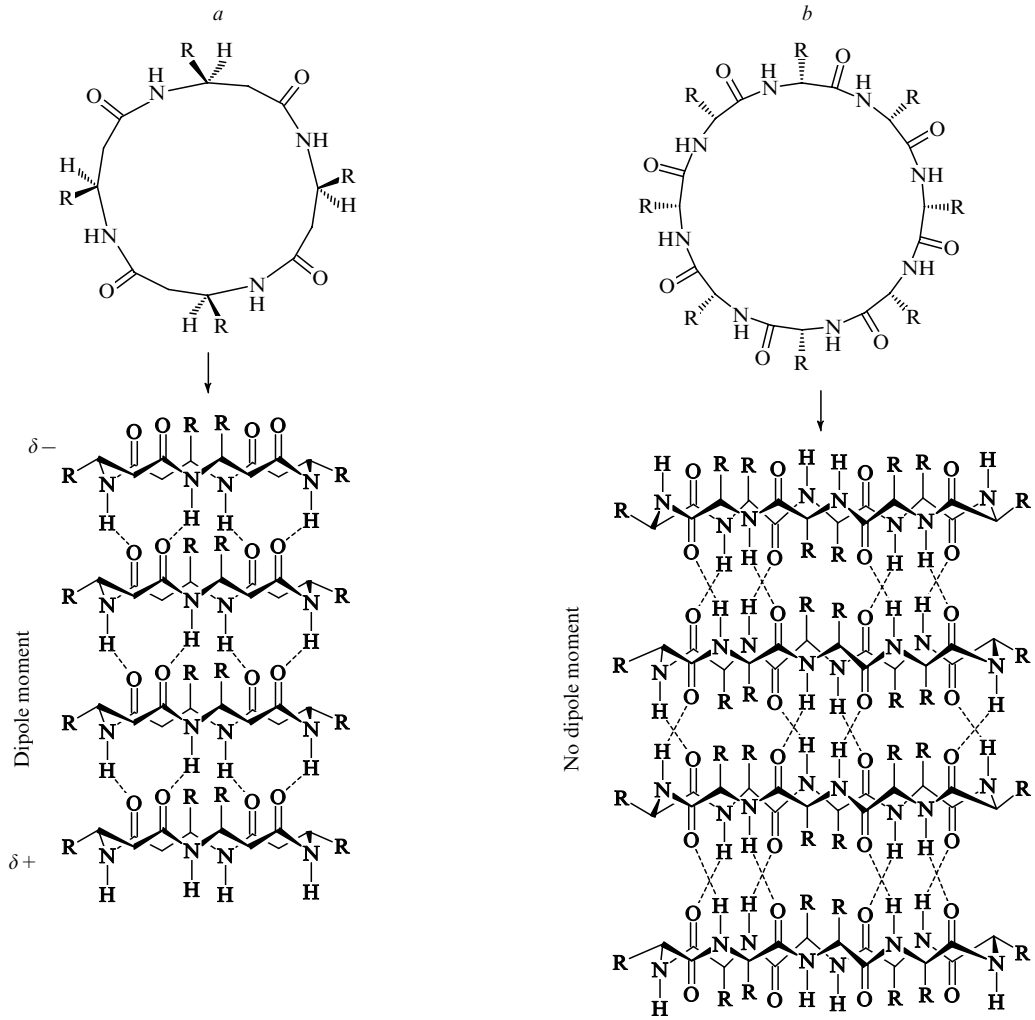
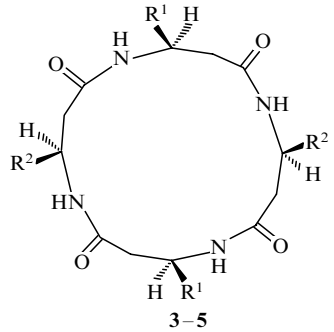
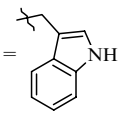


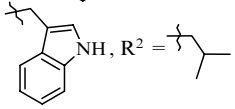
Figure 10. Cyclic peptide nanotubes consisting of cyclic abiotic β^3-tetrapeptides (*a*) and cyclic DL-α-octapeptides (*b*).[11]

increases the selectivity of the ion channel formed by β^3-tetrapeptides. The β^3-tetrapeptide **3**, containing indole groups in side fragments, is readily soluble in polar solvents such as DMSO and DMF.



3–**5**

$R^1 = R^2 =$  (**3**, β^3-HTrp);

$R^1 =$ , $R^2 =$ (**4**, β^3-HTrp-β^3-HLeu);

$R^1 = R^2 =$  (**5**, β^3-HLeu).

Cyclic β^3-tetrapeptides possess a higher conformational rigidity and a higher degree of pre-organisation for self-assembly than larger β^3-penta- and β^3-hexapeptides. Powder X-ray diffraction and molecular modelling have shown that the β^3-tetrapeptide molecule can exist as both C_2- and C_4-symmetrical conformations (Fig. 11).

The results of calculations of the diameters of macrocycles suggest that in the lipid membrane, β^3-tetrapeptides occur in the C_4-symmetrical conformation, because in this case, the ring diameter equals 2.6–2.7 Å, which allows water molecules and

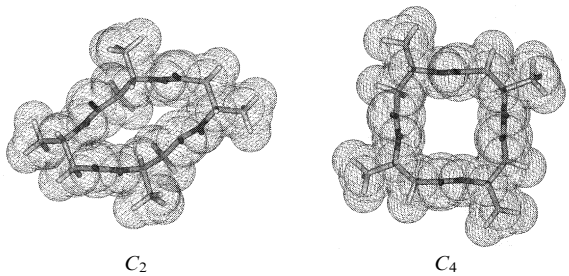


Figure 11. Cyclic β^3-tetrapeptides in C_2- and C_4-symmetrical conformations.[11] For clarity, the side groups are shown as methyl groups.

relatively small ions to pass through the central pore of the transmembrane channel.

A synthetic transmembrane channel based on the cyclic β^3-tetrapeptide **3** has demonstrated a K^+-conductance of $56 \times 10^{-12}\ \Omega^{-1}$ (Fig. 12), which is higher than the K^+-conductance of the channel based on gramicidin A under the same conditions. The peptides **3** and **5** ensure a proton conductance through a liposome membrane.



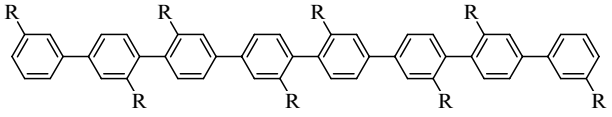
Figure 12. Conductance of the K^+ ions at 60 mV (peptide **3**, the probability of channel opening is 0.14).[11]

Experiments confirmed the possibility of using cyclic peptides as channels for water molecules and small ions. The jumps of the measured current (see Fig. 12) point to opening or closure of the channel caused by fast conformational transitions (see Fig. 11) or changes in the channel structure.

IV. Supramolecular ion channels based on extended linear molecules

1. β-Barrels based on functionalised oligo(*p*-phenylene)

Matile and co-workers [14–17] have reported the development of giant transmembrane channels (β-barrels) (see Fig. 1), which consist of rigid bars of octa-*p*-phenylenes **6** and **7** connected by short antiparallel β-sheet structures (Fig. 13). The rigidity of the channel structure is based on the electronic repulsion of the phenylene groups which form the internal surface of the β-barrel. Recording of the current in a single channel at −50 mV revealed a fluctuation-induced appearance of fluxes amounting to 178 pA.



6, **7**

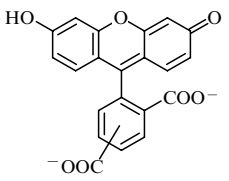
R = Leu–Lys–Leu–NH_2 (**6**), Leu–NH_2 (**7**).

The channel **6** incorporated into monolamellar vesicles can convey an anionic fluorophore, 5(6)-carboxyfluorescein, by means of the cationic NH_3^+ groups of lysine, which line the internal surface of the hexamer of the compound **6** (see Fig. 13). For estimating the internal diameter of the β-barrel self-assembled in the membrane, the transport of carboxyfluorescein was studied (Fig. 14). Since a carboxyfluorescein molecule has a diameter of more than 9 Å, it can be carried through channels with a diameter of at least 20 Å. This nanopore is comparable in size with the natural β-barrel formed by the polypeptide α-hemolysin containing 293 amino acids.

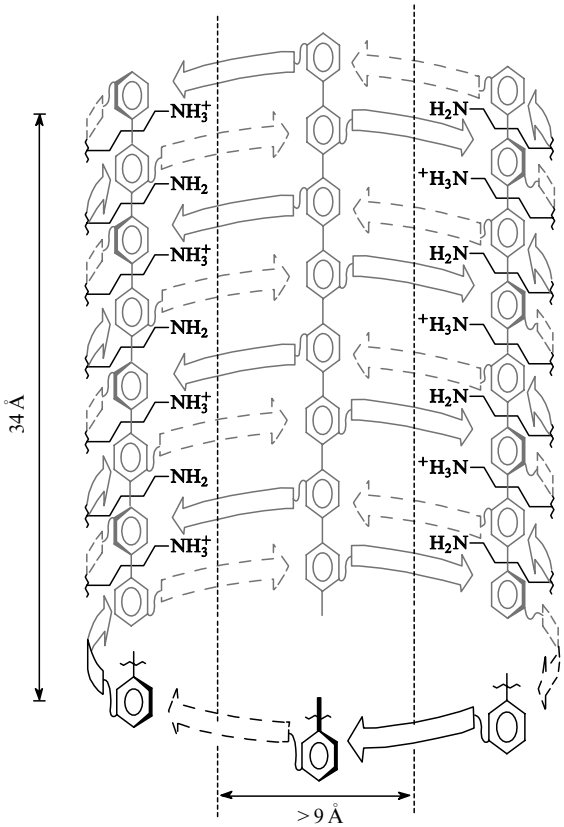
2. Biomimetic oligo(oxyethylene) esters of carboxylic acids

Not only peptides but also various non-peptide structures can be used to construct artificial ion channels. In 1990, Menger *et al.*[63] synthesised a number of compounds with the general formula $R^1O(CH_2CH_2O)_nR^2$ (**8**), which facilitated the transport of ions through bilayer membranes.

The highest activity was found for compound **8a** [R^1 = n-$C_{11}H_{23}C(O)$; R^2 = $PhCH_2$, n = 5], whose molecules are



5(6)-Carboxyfluorescein



6[6]: ⇄ = NH_2–Leu–Lys–Leu–$COCH_2O$–OCH_2CO–Leu–Lys–Leu–NH_2.

Figure 13. Structures of self-assembling hexamer **6**.[15]

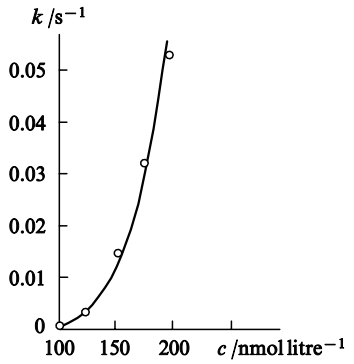
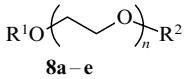


Figure 14. Observed fluxes of carboxyfluorescein as a function of the concentration of octa-*p*-phenylene **6**.



8a–**e**

8a: R^1 = n-$C_{11}H_{23}C(O)$; R^2 = $PhCH_2$, n = 5;
8b: R^1 = n-$C_{13}H_{27}C(O)$; R^2 = $PhCH_2$, n = 4;
8c: R^1 = n-$C_{11}H_{23}C(O)$; R^2 = $PhCH_2$, n = 4;
8d: R^1 = n-$C_9H_{19}C(O)$; R^2 = $PhCH_2$, n = 5;
8e: R^1 = n-$C_7H_{15}C(O)$; R^2 = $PhCH_2$, n = 5.

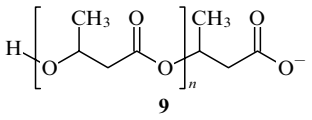
stretched throughout the whole thickness of the lipid membrane thus forming channels. The spontaneous inclusion of compound **8a** in the lipid membrane is due to both the hydrophobic section of the fatty acid and the interaction of the π-electron system of the benzyl substituent with the quaternary ammonium group of the membrane lipids. The ion flux through the vesicle membrane was determined by time-resolved fluorescence spectroscopy using a fluorescent dye (pyranine) as the pH indicator. It was found that for a concentration of **8a** equal to 75 molecules per vesicle 30 ± 5 nm in diameter, the equilibrium between the exterior and interior ions is established almost instantaneously. Comparison of the efficiency of the transport of protons by compound **8a**, gramicidin D and 18-crown-6 ether has shown that compound **8a** surpasses gramicidin, while 18-crown-6 does not influence at all the ion flux through the bilayer lipid membrane. The last-mentioned fact deserves particular attention, because experiments with bulky liquid chloroform membranes (in this case, the ion transport is accomplished by ion carriers) give the opposite result, namely, 18-crown-6 transports the K^+ cations through a chloroform liquid membrane, whereas compound **8a** is completely inert. This is due to the fact that crown ethers are potent ligands, able to carry cations through a chloroform membrane,[64] while compound **8a** is a weak ligand for K^+. This finding supports the assumption that compound **8a** carries the cations due to the formation of channels in the bilayer membrane. Some other facts also attest to the formation of an ion channel in the membrane. Indeed, ether **8a** is active even when present in nanomolar concentrations; in addition, the efficiency of cation transport is structurally specific, in particular, analogues of compound **8a** having either higher ($n > 5$) or lower ($n < 5$) hydrophilicity do not change the membrane permeability.

In order to elucidate the influence of the length of the fatty acid residue and the length of the oligo(oxyethylene) fragment on the efficiency of cation transport by compounds **8**, Schafer and co-workers[65] have synthesised and studied derivatives **8b**–**e**. Study of the transport of the Li^+, K^+ and Cs^+ cations through the phospholipid membrane showed that only compounds **8b**–**d** form single channels (determined on the basis of the current). The highest efficiency was found for compound **8d**, which is probably due to the most favourable ratio of the total chain length and the number of ethylene glycol fragments. The increase in the conductance of this channel in the series $Li^+ < K^+ < Cs^+$ is correlated with the increase in the ion radius of the cation (Li^+, 78; K^+, 133; and Cs^+, 169 pm) and also with the decrease in the radius of the hydrated cation (Li^+, 382; K^+, 331; Cs^+, 329 pm). Apparently, in this case, the pore diameter varies from 340 to 660 pm.

When comparing the compound **8d** and gramicidin D regarding their ability to carry cations, it should be noted that in the case of gramicidin D, the highest ionic conductance of vesicles is observed when its concentration is as low as 1 μmol litre^{-1} (further increase in the gramicidin D concentration to 5 μmol litre^{-1} does not increase the ionic conductance). Conversely, an increase in the concentration of compound **8d** up to 20 μmol litre^{-1} still induces an increase in the conductance of the vesicle. This implies that the formation of the ion channel involves more than two molecules of **8d**, unlike gramicidin D. It was assumed[65] that the molecules of **8** are associated according to the 'head-to-tail' pattern, because only in this case can the hydrophilic part of the channel-forming compound extend throughout the whole membrane.

3. Biomimetic oligo-(*R*)-3-hydroxybutyrate

The low-molecular-mass polyhydroxybutyrates composed of 50–150 residues of (*R*)-3-hydroxybutyrate and oligohydroxybutyrates composed of less than 50 residues are common in prokaryotic and eukaryotic cells, where they form numerous coordination bonds with cations. They are able to transport ions through a phospholipid membrane in model planar bilayer systems and in liposomes. However, these channels have low selectivity and low sensitivity to the change in the transmembrane potential.[66] In addition, for ion transport, it is necessary to build-in large amounts of these substances (>0.1%) in the membrane.[4] Das *et al.*[66] have gained a higher efficiency from this transport system. They prepared complexes of oligo-(*R*)-3-hydroxybutyrates **9** ($n = 18-22$) with inorganic polyphosphates, which demonstrate a higher cationic conductance (275×10^{-12} Ω^{-1}) compared to that of the complexes formed by poly-(*R*)-3-hydroxybutyrates ($n = 120-140$) with polyphosphates (100×10^{-12} Ω^{-1}).



9

$n = 18-22$.

However, the selectivity of the transport of Ca^{2+} cations with respect to Na^+ cations by means of these oligomers is much lower than the selectivity of transport by polymers: 4:1 and >90:1, respectively. These results confirm the hypothesis that polyphosphate fragments play the crucial role in the cation selectivity, while oligo- and poly-(*R*)-3-hydroxybutyrates are solvating agents (Fig. 15). Thus, the monodisperse biomimetic oligo-(*R*)-3-hydroxybutyrate/polyphosphate system can be efficiently used for the development of voltage-activated synthetic ion channels with a limited cationic selectivity (Fig. 16).

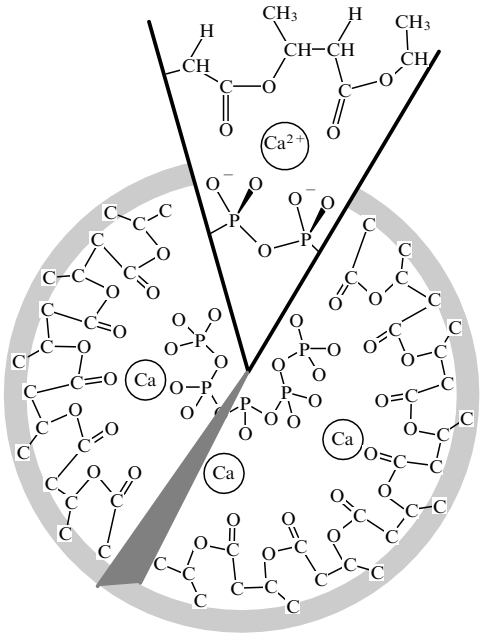


Figure 15. Structure of the channel formed by oligo-(*R*)-3-hydroxybutyrates with inorganic polyphosphates (vertical view).[68]

The formation of ion-selective voltage-activated channels by poly-(*R*)-3-hydroxybutyrate/polyphosphate complexes has been considered in detail.[67] Complexes of these two polymers isolated from the plasmatic membranes of bacteria or obtained from synthetic polymers form voltage-dependent Ca^{2+}-selective channels in a planar bilayer membrane. The key factor in the solvation of calcium polyphosphates by polyhydroxybutyrates is the flexible core of the polymer and the optimal distance between the carbonyl groups of the ester fragments. In the models of channel complexes proposed by Reusch[68] and Seebach,[69] the highly polar polyanionic polyphosphates are shielded from the hydrophobic region of the bilayer by amphiphilic polyhydroxybutyrates. As a result, numerous sites of cation binding are formed between the two polymers, *i.e.*, irregular binding cavities formed by the phosphoryl oxygen atoms belonging to the polyphosphate and the oxygen

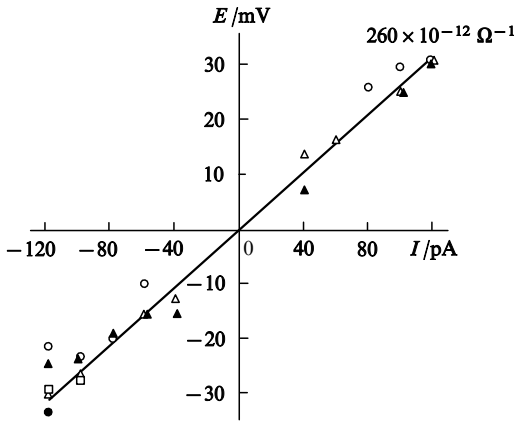


Figure 16. Current–applied voltage dependence for the channel complexes of oligo-(*R*)-3-hydroxybutyrates with polyphosphates.
Different designations of experimental points correspond to values obtained in parallel experiments.

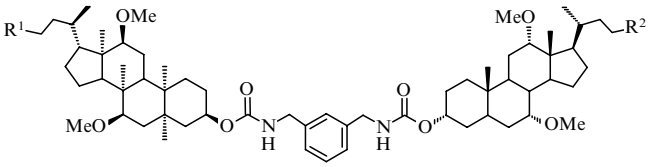
atoms of the ester groups of poly-(*R*)-3-hydroxybutyrate, which bind more readily the Ca^{2+} cation than the Mg^{2+} cation.

4. Voltage-dependent channels based on cholic acid derivatives

The natural transport proteins are characterised by a high degree of controllability. Transition from the closed to the open (or conductive) state is usually induced by a particular process: phosphorylation, binding of small molecules (acetylcholine, Ca^{2+} and so on) or a change in the transmembrane potential. Conversely, most synthetic ion channels cannot be controlled: they switch from the closed to the open state as a result of random aggregation processes in the lipid bilayer.

The regulation of the permeability of bilayer membranes by applying a voltage is one of the fundamental approaches to the control of the overall conductance of ion channels and to the design of new supramolecular ionic devices (selective filters, conductors and so on) and pharmaceuticals. Voltage-dependent channels are opened or closed upon application of a voltage higher or lower than a certain critical value. Only a few studies dealing with the preparation of synthetic voltage-dependent ion channels have been published to date.

The design of voltage-dependent ion channels **10**–**13** on the basis of cholic acid and its derivatives has been reported.[70,71] Compounds **10**–**13** are formed upon cross-linking of two fragments of cholic acid or its derivatives by a *m*-xylylenedicarbamate bridge.



10–**13**

$R^1 = R^2 = CO_2H$ (**10**), $CH_2OC(O)CH_2N^+(CH_3)_3Cl^-$ (**11**);
$R^1 = OP(O)(OH)_2$, $R^2 = CO_2H$ (**12**); $R^1 = CO_2H$, $R^2 = OH$ (**13**).

The length of channels formed by these compounds approximately corresponds to the thickness of the bilayer membrane (~40 Å). The rigid amphiphilic steroidal frameworks of compounds **10**–**13** have a favourable effect on the stability of the resulting channels. In order to elucidate the effect of the electric asymmetry of the ion channel on the possibility of controlling the channel by means of an applied transmembrane potential, two types of compounds have been prepared, those with identical (compounds **10**, **11**) and different (compounds **12**, **13**) terminal hydrophilic groups. The electric asymmetry of the structures of compounds **12** and **13** was attained by introducing different terminal hydrophilic groups, namely, a carboxy group and a phosphoric acid fragment and a hydroxy and carboxy groups, respectively.

In a bilayer membrane, compounds **10**–**13** formed stable single ion channels having long open durations (200 ms to 4.7 s); in addition, with a positive potential applied, higher ion currents were observed than on application of a negative potential, *i.e.*, an essential non-linearity of voltammetric characteristics was observed. Taking into account the cation selectivity of the channels formed by compounds **12** and **13** (for example, for channel **12**, the P_{K^+}/P_{Cl^-} ratio is equal to 11), the researchers suggested that in a channel formed by compound **12**, the potassium cations move from the phosphoryl end of the molecule to the carboxylate one. The functioning of the channel based on compound **12** is schematically illustrated in Fig. 17.

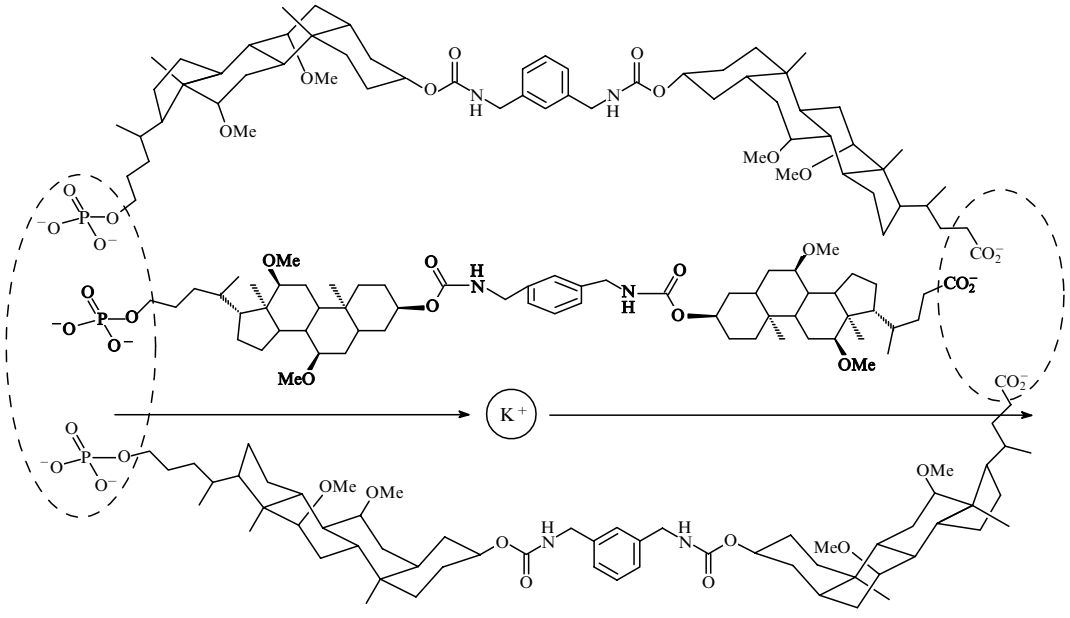
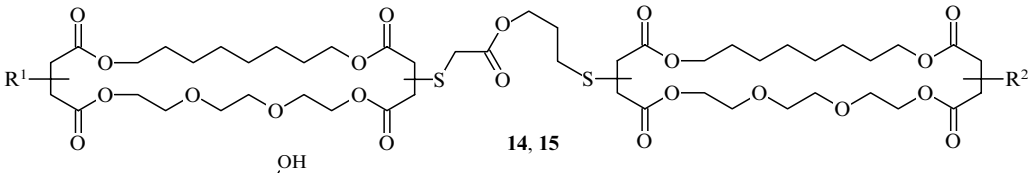
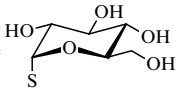


Figure 17. Movement of the potassium cation in channel **12**.[71]



14, **15**

$R^1 = SCH_2CO_2H$: R^2 =  (**14**), $SCH(CO_2H)CH_2CO_2H$ (**15**).

5. Voltage-dependent ion channels based on bismacrocyclic amphiphiles

Fyles *et al.*[12] described the synthesis of bismacrocyclic amphiphilic compounds **14** and **15**, where groups able to carry different charges are located on the opposite ends of the molecule, in particular, a mercaptoacetate (1−) and a thioglucose (neutral) group in compound **14** and a mercaptoacetate (1−) and a mercaptosuccinate (2−) group in compound **15**. The presence of such groups imparts a substantial dipole moment to compounds **14** and **15** and favours fixing of these molecules on the surface of a bilayer membrane.

It was found that the presence of a negatively charged (1−) and neutral end groups in compound **14** gives rise to an ion channel, characterised by regular changes in the ionic conductance. However, the voltammetric characteristics of such a channel proved to be almost linear, *i.e.*, opening of the channel did not depend on the voltage applied. This was attributed to a random orientation of the channel-forming molecules **14** in the membrane. Meanwhile, compound **15** forms a channel due to asymmetric orientation in the diphytanoyl phosphatidylcholine bilayer membrane such that the succinate group is located predominantly on the *cis*-face of the bilayer. As a result, a non-linear voltammetric characteristic of the channel is observed (similar to that typical of the alamethicin channel) with an opening threshold of about −30 mV (Fig. 18, curve *1*). The drawbacks of this channel include the unstable behaviour of its opening and functioning. The non-linear voltammetric characteristic is observed only for the first cycles and disappears after 10–15 min of channel operation (Fig. 18, curve *2*). The time the channel remains in the open state is exceptionally short, being equal to 0.5–10 ms. The current amplitude is variable and can change by an order of magnitude (1–10 pA). Thus, the qualitative characteristics of these channels, namely, the 'explosive' opening, variable flux values per opening, and low cationic selectivity, are indicative of a relatively low degree of structuring of the resulting channels.

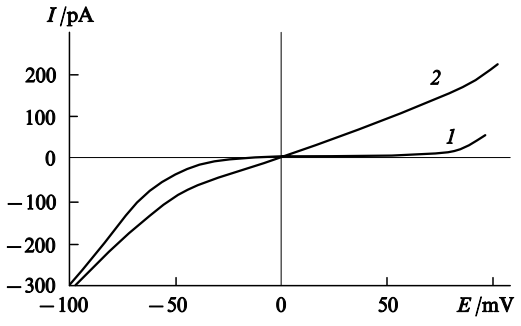


Figure 18. Voltammetric characteristics of the ion channel based on compound **15** (118 nmol of **15**, 1 M LiCl, diphytanoyl phosphatidylcholine) with 1–7 (*1*) and 8–20 (*2*) cycles.

The presumptive mechanism of the functioning of voltage-dependent channel **15** is shown in Fig. 19.

Apart from the non-specific pre-association of compound **15** with the bilayer (structure **A**), the researchers also considered

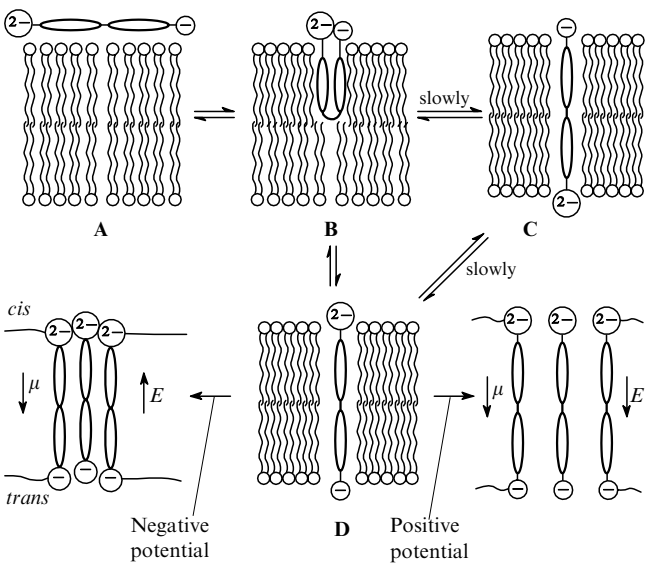


Figure 19. Presumptive mechanism of operation of the voltage-dependent channel formed by **15**.

other types of association of this compound with the bilayer giving intermediate U-shaped (**B**) and transmembrane (**C** and **D**) structures. Since the processes involving penetration of the succinate dianion terminal group through the lipophilic membrane (transition from structure **B** to structure **C** and from structure **D** to structure **C**) occur more slowly than the processes of penetration of the acetate monoanion group (transition from **B** to **D**), it can be suggested that structure **D** with the succinate terminal group located on the *cis*-face of the bilayer is the first to be formed (see Fig. 18). In this case, opening or closure of the channel is due to parallel or antiparallel arrangement of the molecular dipole moment and external field vectors. Since the electrostatic repulsion of the succinate groups should be substantial, the degree of aggregation would depend appreciably on the stabilisation of the resulting supramolecular system due to the external electric field. In the case of antiparallel orientation of the dipole moment and external field vectors, ion-conductive aggregates are formed at negative potentials on the bilayer surface and the system proves to be more stable. The parallel orientation results in destabilisation, predominance of the electrostatic repulsion of likely charged terminal groups and destruction of ion-conductive aggregates.

6. The formation of supramolecular ion channels by amphiphilic ion pairs

Kobuke *et al.*[72–74] have designed amphiphilic compounds able to be incorporated into bilayer membranes by combining a hydrophilic oligoether and a hydrophobic alkyl chain in an ion pair formed by an ammonium cation and carboxylate or phosphate anions. The researchers synthesised two types of amphiphilic ion pairs with the hydrophilic component represented by either an anion (compounds **16** and **17**) or a cation (compounds **18**–**21**).

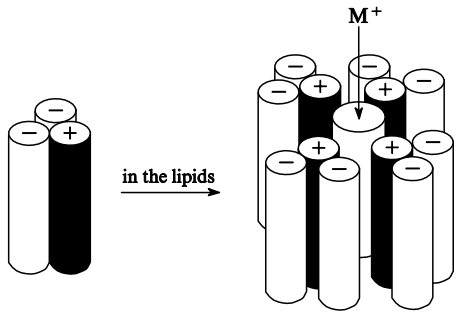


Figure 20. Schematic view of the structure of supramolecular ion half channel formed by an amphiphilic ion pair. The black and white columns designate the hydrophilic and hydrophobic parts of the ion pair, respectively.

The presence of the hydrophilic oligoether moiety in the ion pair results in supramolecular aggregation of ion pairs. The hydrophilic parts of ion pairs in the aggregates are 'expelled' from the hydrophobic lipid membrane to give a half ion channel (Fig. 20). Further tail-to-tail dimerisation of these half channels forms a full single channel. It is evident that the stability of these supramolecular ensembles would depend on the hydrophilic–lipophilic balance within the amphiphilic ion pairs. The most stable channels were found to appear when the hydrophobic parts of the ion pairs contain two alkyl chains (compounds **17**, **19**, and **20**). The ion pairs containing one alkyl chain (compounds **16** and **18**) form channels with low stability. The ion pair **21** does not form channels at all. Probably, in this case, the four hydrophobic chains of cardiolipin mask the oligoether chain of the cation, thus hampering the formation of a supramolecular ensemble in the lipid membrane.

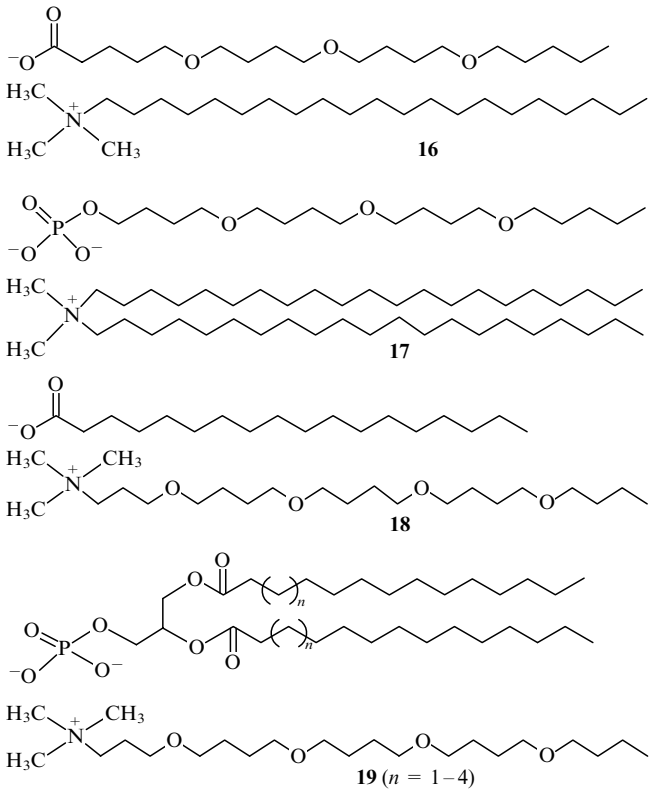
All the ion channels studied showed a cation selectivity (cations are transferred more efficiently than anions). For example, for channels **19** and **20**, the P_{K^+}/P_{Cl^-} values are equal to 4.9 and 16.8, respectively. Moreover, this is observed despite the different characters of the anionic terminal groups in the hydrophobic component of the ion pair. Thus, the pattern of selectivity is determined by the nature of the intramembrane walls of the channel rather than by the type of terminal groups which form the entrance of the channel.

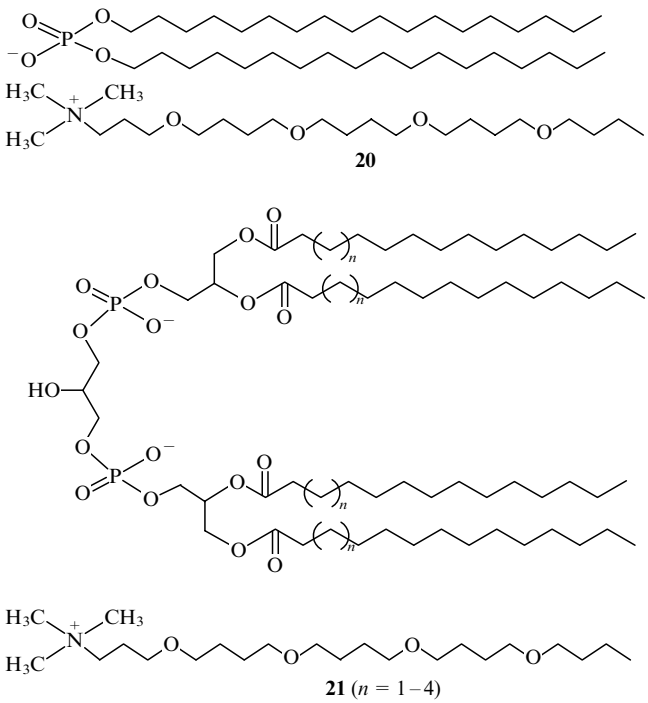
The discovery of voltage-dependent ion channels represented one more important result of this study. The probability of opening and closure of the channels formed from ion pairs of the monoammonium cation and the alkyl phosphate dianion (compounds **17** and **19**) depends on the voltage applied. Meanwhile, the ion pairs consisting of the monoammonium cation and the carboxylate (compounds **16**, **18**) or dialkyl phosphate (compound **20**) monoanions do not form voltage-dependent ion channels.

As noted previously, the presence of different charges (dipole moment) on the opposite ends of the channel is a necessary condition for the functioning of voltage-dependent ion channels. It is clear that the degree of aggregation of amphiphilic ion pairs in the half channel depends on many factors, and aggregates of diverse compositions are formed in the membrane. As a result, a single channel assembled from two such half channels has different negative charges on the opposite ends; in other words, it has a dipole moment. However, the dipole moments for different single channels assembled from two half channels in a random way are dissimilar and cannot be controlled. Therefore, the voltammetric characteristics of the resulting channels vary from one experiment to another.

7. Photochemical control of the ion transport using compounds containing an azobenzene fragment

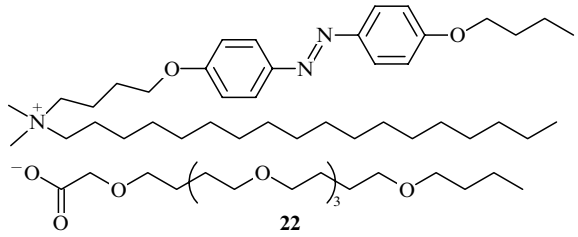
The photochemical method for controlling ion channels based on modified gramicidin peptides has been reported in several publications.[75–77] Schreiber and coworkers [75] have found that the gramicidin dimer containing an azobenzene bridging fragment in the *cis*-form acts as an ion channel. Higuchi *et al.*[76] have shown that in the case of azobenzenes linked to the α-helix of bispolyglutamate, the overall ion flux is controlled by photoisomerisation. Woolley and co-workers [77] have observed the variation of a single ion flux induced by isomerisation of the azobenzene fragments attached to the channel gate of gramicidin. On exposure to





laser radiation with a wavelength of 337 nm, the channels pass from the *trans*-state characterised by the highest flux levels of 14.9 ± 0.4 pA and lower current levels of 0.6 ± 0.1 pA to the *cis*-state with a flux level of 12.8 ± 0.4 pA and three current levels, 1.1 ± 0.1 pA (first level), 2.6 ± 0.1 pA (second level) and 3.5 ± 0.2 pA (third level). The subsequent illumination of the *cis*-channels by a 100-W table lamp for 10 min resulted in the transition into the *trans*-form.

Kobuke and Ohgoshi [78] have synthesised and studied a supramolecular synthetic ion channel based on *N*,*N*-dimethyl-*N*-octadecyl-*N*-[4-(4-butoxy)-*trans*-azophenoxy]butylammonium 3,8,13,18,23-pentaoxaheptacosanoate (**22**). The channel is an amphiphilic ion pair which isomerises on exposure to photoradiation.



22

After this ion pair (single channel) had been incorporated into a planar phospholipid membrane, an ion flux was passed through it (Fig. 21). An important characteristic of this channel is that the period when the channel is open is longer than the period when the channel is closed. The channel based on compound **22** has shown different values of electrical conductivity, most of all, in the range of $(2-13) \times 10^{-12}\ \Omega^{-1}$. The channel was found to be selective for the potassium cation with respect to both the chloride anion and the sodium cation: the permeability ratios are as follows: $P_{K^+}/P_{Cl^-} = P_{K^+}/P_{Na^+} = 6.1$.

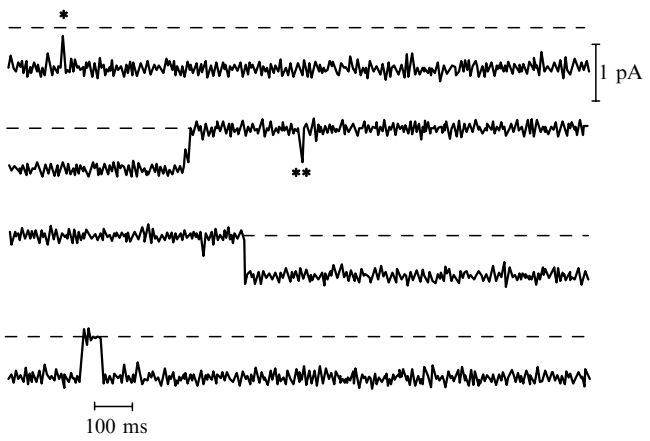


Figure 21. Current created by *trans*-azobenzene ion pair **22** at a voltage of 96.9 mV in a 500 mM solution of KCl and 5 mM Tris–HEPES buffer (pH 7.2) [Tris is tris(hydroxymethyl)aminomethane, HEPES is *N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonic acid]. The dashed line corresponds to a closed channel. The short-lived opening and closure of the channel were observed at the points marked by one and two asterisks, respectively.

Upon photoirradiation at 367 nm, the *trans*-azobenzene fragment passes to the *cis*-form (Fig. 22). This isomerisation results in channel closure and the current does not pass through it. The subsequent transition of the *cis*-azobenzene fragment to the *trans*-form resumes the ion flux through single channels. Thus, the photocontrol of the channel throughput capacity was attained.[78]

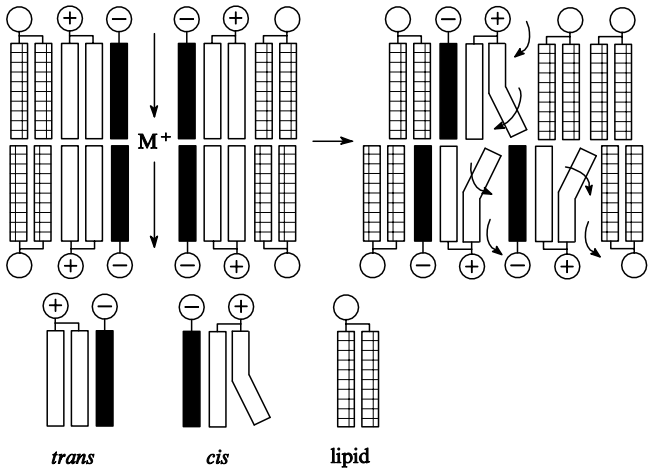


Figure 22. Possible mechanism of the photochemical control of the ion flux with the use of ion pair **22**.

V. Ion channels based on crown ethers

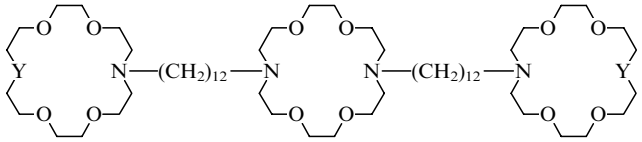
1. Tris- and tetrakis(diazacrown ether) channel-forming structures

Crown ether derivatives are convenient models for investigating the mechanism of functioning of ion channels in bilayer phospholipid membranes. In artificial channel-forming systems, crown ethers are used as either head groups or binding sites for the corresponding ion.

It has been shown [79, 80] that *N*,*N*′-bisalkylated and *N*,*N*′-bisbenzylated diaza-18-crown-6 ethers are self-aggregated in the solid phase to form channel-like ensembles. The length of the supramolecular structures thus formed determined by laser light scattering was 2000–3000 Å; the aggregates of alkylated diaza-18-crown-6 derivatives proved to be more stable than the aggregates of benzylated derivatives.

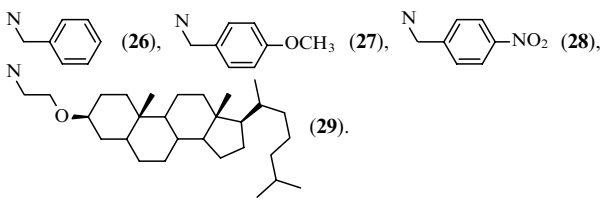
Meadows *et al.*[81] reported that complexes of propargyl-substituted diaza-18-crown-6 with KBF_4 form a channel-like extended structure whose supramolecular organisation indicates that the diaza-18-crown-6 molecule within synthetic channels can play the same role as water that forms the hydration shell in natural proteins. This structure formed by cations and donor groups can mimic the natural cationic channels located in the lipid bilayer.

The amphiphilic nature of crown ethers allows one to use them as the channel gate. A substantial contribution to the understanding of the principles of design and functioning of artificial ion channels based on crown ethers was made by Gokel and coworkers.[10, 13, 82, 83] In particular, they synthesised a series of tris(diaza-18-crown-6) compounds **23**–**29**,[82] which demonstrated the ability to be incorporated into lipid membranes to form ion channels.



23–**29**

Y = O (**23**), NH (**24**), $N(CH_2)_{11}CH_3$ (**25**),



(**26**), (**27**), (**28**), (**29**).

For estimating the efficiency of the ion transport, two methods have been used most often, namely, determination of the exchange rate of the sodium cation in the vesicles by ^{23}Na NMR dynamic spectroscopy using shift reagents and determination of the conductance of planar bilayer membranes.

To establish the mechanism of action of synthetic channels based on tris(macrocyclic) systems, one should answer three main questions. The first one concerns the possibility of explaining the differences in the fluxes of the Na^+ cation through a bilayer membrane by different solubilities of ionophores in the membrane. For this purpose, the distribution coefficients of tris(crown ethers) **23**–**29** in the octanol–water system were calculated. The calculation showed that all the compounds studied occur preferably in the lipid bilayer: the ratio of the distribution coefficients is $10^{10}:1$ (*i.e.*, the crown ethers occur entirely in the membrane phase).[60]

The second question concerns the ion transport, because the ability of crown ethers to function as cation carriers is well known.[84] In order to answer this question, it was necessary to determine the mode of functioning of the systems of interest, whether they are ion carriers or ion channels. Therefore, experiments were carried out on Na^+ transport through lipid and bulky chloroform membranes by means of crown ethers **23**–**29**. It is evident that the transport through a chloroform membrane cannot occur by the channel mechanism, because in this case, the distance between the source and receiving aqueous phases is too great, unlike that in a bilayer lipid membrane. Figure 23 presents the measured rate constants for the transport of the Na^+ cations by crown ethers **23**–**29** in the lipid bilayer and chloroform membranes. For comparison, similar experiments were carried out with gramicidin (the natural channel-forming peptide) and valinomycin (a natural receptor of Na^+ ions).

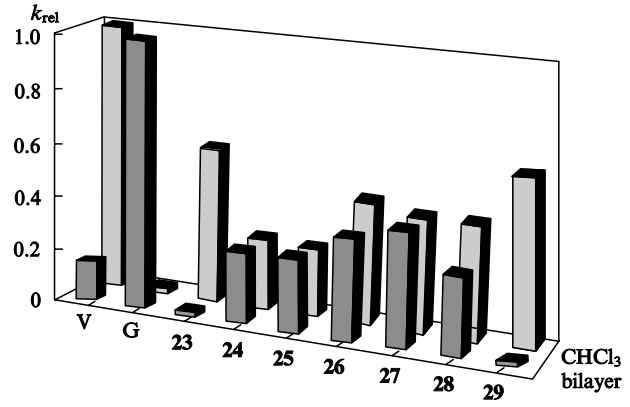


Figure 23. Comparison of the efficiency of Na^+ transport by compounds **23**–**29**, gramicidin (G) and valinomycin (V) through chloroform and lipid bilayer membranes.

It can be seen from Fig. 23 that most of the compounds **23**–**29** studied are less active ion carriers than natural compounds. As expected, valinomycin proved to be the most efficient carrier, while gramicidin was the most effective channel-forming compound. The lack of correlation between the rates of transport of Na^+ ions through phospholipid and chloroform membranes by tris(crown ethers) **23**–**29** confirmed that they function in a bilayer membrane as channel-forming agents rather than mere ion carriers.

Finally, the last question concerns the role of the terminal residues of the crown ethers, whether they are the head groups through which the cations pass when they enter or leave the membrane? If the terminal crown-ether fragments function as the input elements of the channel, the transport efficiency should depend on the electron density on the macrocycle and on the nature of the substituents at nitrogen. Experiments with compounds **26**–**28**, which differ only in substituents (H, OCH_3 and NO_2) in the benzene ring, showed a positive effect of electron-donating substituents on the ion flux. This influence is quite understandable, as an increase in the electron density at the entrance of the channel is expected to accelerate the transport of cations through the channel. On that ground, Murillo *et al.*[82] suggested that the terminal residues of crown ethers are the head groups of the ion channel through which the cations pass.

Further investigation of tris(diaza-18-crown-6 ethers) as channel-forming structures in bilayer phospholipid membranes allowed researchers to propose a structure for the ion channel formed (Fig. 24).[83] They suggested that water molecules fill the channel and assist in the cation transport. This suggestion was based on the known fact that a chloroform membrane containing some tris(macrocyclic) compounds becomes rapidly saturated with water.

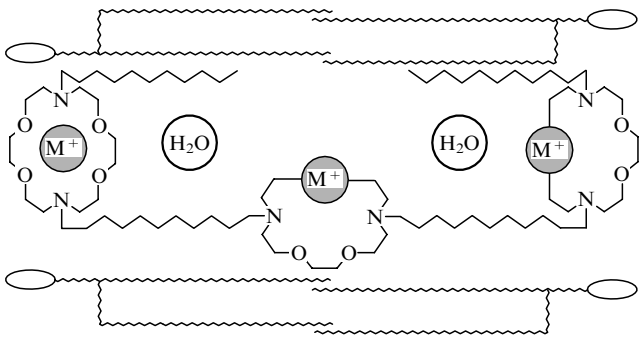
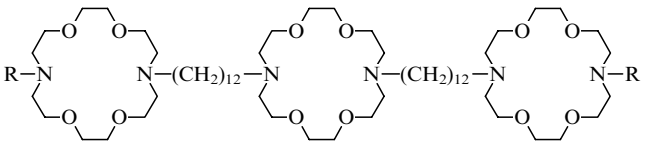
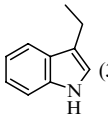
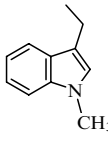
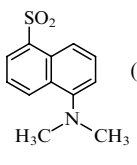


Figure 24. Structure of the ion channel formed by tris(diaza-18-crown-6 ether) **25**.[13]

Subsequently, Gokel and co-workers[13] proposed using heterocyclic compounds as the terminal fragments of tris(macrocyclic) structures based on diazacrown ethers. It is known that side groups of some amino acid residues in transmembrane proteins serve to fix the protein helices relative to each other and relative to the bilayer membrane.[4] This role is often performed by the indole fragment of tryptophan. It was shown by NMR spectroscopy that numerous tryptophan residues present in the gramicidin channel are directed toward the water–bilayer membrane interface. Therefore, Gokel chose indole as the terminal heterocyclic fragment. He prepared a series of compounds with the general formulae **30**–**32**, which were used to create ion channels in phospholipid vesicles. It was demonstrated experimentally that *N*-alkylindoles are able to form stable vesicles with neutral head groups represented by crown-ether fragments which control the cation transport (channel gate). The C_{12} hydrophobic bridge in tris(diazacrown ethers) **30**–**32** can be oriented in the membrane parallel to the lipid chains. Generally, the length of these tris(macrocyclic) channels proved to be sufficient to bridge the hydrocarbon region of the membrane and to form a channel.



30–**32**

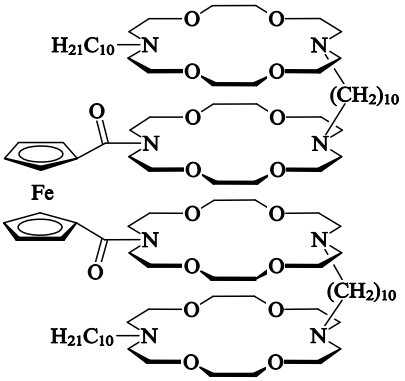
R =  (**30**),  (**31**),  (**32**).

Compounds **31** and **32** are able to transfer the sodium cation in the bilayer membrane, unlike compound **30**. This can be attributed to the fact that compound **30** forms in the membrane a hydrophilic globular conformation due to strong intramolecular hydrogen bonding between the NH groups of the indole substituent and heteroatoms of the macrocycle.

The synthesis of tetrakis(diazacrown ether) **33** has been described.[85] The researchers linked two macrocyclic fragments connected by long aliphatic chains to the central ferrocene fragment capable of functioning as a cationic 'filter'.

The length of the resulting channel was ~30 Å, which corresponded to the thickness of a typical biological membrane. It was assumed that variation of the head groups would create the basis for controlling the cationic selectivity.

Cyclic voltammetry studies of compound **33** showed that the strength of the coordination interaction of the Na^+ ion with the amide group of the ferrocene fragment decreases as the channel becomes filled with cations, which can be explained by charge repulsion. This does not occur for Ca^{2+} ions, apparently, due to their stronger coordination to the amide groups.

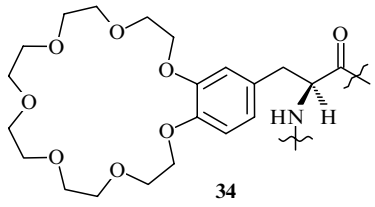


33

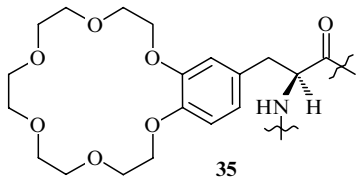
The transport of ions through such channels has been studied using biological membranes (fragments of hamster brain cells). The change in the membrane potential from −60 to +60 mV decreased the transmembrane cation flux following the accumulation of positive charge on the inner side of the cell. At −80 mV, the amplitude of the mean K^+ flux through a single channel was −4.0 pA, which corresponded to a conductance of $50 \times 10^{-12}\ \Omega^{-1}$. For a potential of 80 mV, this value was 2.4 pA ($30 \times 10^{-12}\ \Omega^{-1}$). These results indicate that the activity of these channels can be controlled to some extent by varying the applied voltage.

2. Artificial ion channels based on peptides containing crown-ether fragments

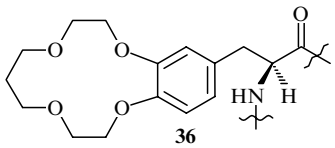
Voyer and co-workers[86,87] proposed that crown ether fragments **34**–**36** be used for the creation of ion channels with a new architecture on the basis of synthetic peptides.



34



35



36

They synthesised a series of peptides **37**–**41** consisting of 21 amino acid residues carrying six crown ether fragments:

N-BOC–(Leu–**34**–Leu$_3$–**34**–Leu)$_3$–OMe (**37**),
N-BOC–(Leu–**35**–Leu$_3$–**35**–Leu)$_3$–OMe (**38**);
N-BOC–(Leu–**36**–Leu$_3$–**36**–Leu)$_3$–OMe (**39**);
N-BOC–(Leu–**36**–Leu$_3$–**36**–Leu)$_3$–OH (**40**);
H_2N–(Leu–**36**–Leu$_3$–**36**–Leu)$_3$–OH (**41**).

Since peptide **37** contains 15 L-leucine residues of the total 21 amino acid residues, it is sufficiently hydrophobic to be incorporated in a lipid membrane. Therefore, the channel is formed most likely due to stacking of crown ether rings (Fig. 25).

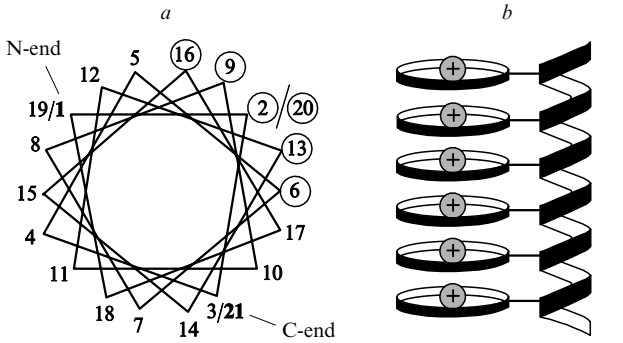


Figure 25. Axial projection of the α-helix of the hexacrown peptides **37**–**41** (*a*, the circles mark the sites of attachment of the crown ethers) and scheme of the channel formed by these peptides in the bilayer membrane (*b*).

Studies of the conformations of hexacrown peptide **37** and its analogues **38**–**41** by polarimetry and FT IR spectroscopy have shown that they exist as two forms, **A** and **B** (Fig. 26), which occur in equilibrium. Peptides existing in the inactive form **A** are adsorbed on the bilayer surface, while peptides in the active form **B** are arranged parallel to the aliphatic tails of lipids.

Aggregated crown peptide is adsorbed from a solution on the surface of a bilayer membrane. The adsorbed inactive form **A** of the peptide, partially immersed in the membrane, is reorganised to the active form **B** (see Fig. 26). It is noteworthy that in both the membrane and the solution, peptides occur in the stable α-helical conformation.

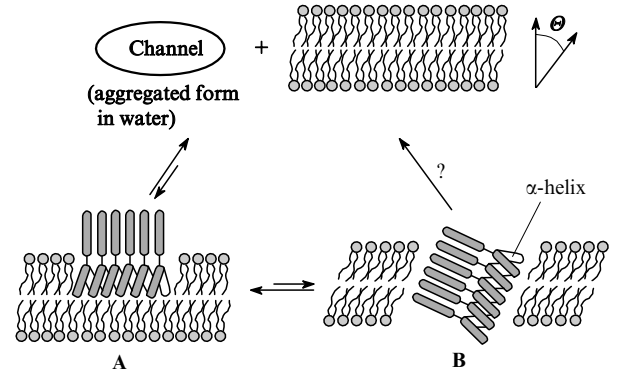


Figure 26. Model of the formation of the channel from peptides **37**–**41**. **A** is the inactive form, $\Theta = 90°$; **B** is the active form, $\Theta = 30°$.

VI. The use of 'bouquet' structures for the design of synthetic ion channels

1. 'Bouquets' based on 18-crown-6 and β-cyclodextrin

Lehn and co-workers[88–91] used polyfunctional macrocycles, β-cyclodextrin and 18-crown-6 as the 'platform' for the design of artificial ion channels. It is common knowledge that crown ethers form labile complexes with alkali metal cations and that cyclodextrins have rigid cavities which are sufficiently large for hydrated alkali metal cations to pass through them. To make these channels amphiphilic, polyoxyethylene or polymethylene chains with polar terminal groups were linked to the macrocyclic 18-crown-6 and β-cyclodextrins.[88,89] Four compounds differing in the type of central structure and the type of podand chains have been prepared in this way (the structures of two such compounds **42** and **43** are presented below). These compounds have been called 'bouquets'.

The ability of compounds **42** and **43** to be incorporated into phosphatidylcholine bilayers of the vesicles was studied by a number of methods (circular dichroism, UV/Vis spectroscopy, differential scanning calorimetry and so on).[88,89]

It was found that the 'bouquets' are incorporated in the bilayers in two modes: in the U-mode (both hydrophilic ends are arranged on one side of the membrane) and in the transmembrane mode.

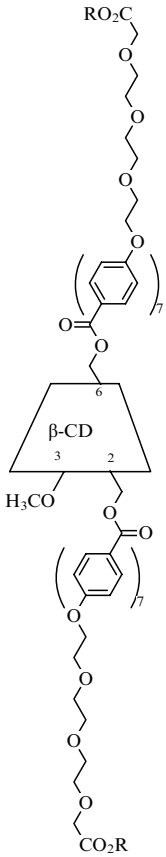
The transport of metal cations (Li^+ and Na^+) induced by the 'bouquet' compounds incorporated in liposomes[90] has been studied by NMR on alkali metal nuclei with a shift reagent (a 3 : 1 mixture of tetramethylammonium tripolyphosphate and dysprosium chloride), which allows one to distinguish between the signals from intraliposome metal cations (no shift) and those from extraliposome metal cations (a shift is observed). Upon creation of opposite gradients for the concentrations of Li^+ and Na^+ cations, the 'bouquets' induced the cation transport according to the antiport mechanism. The rate constants for ion transport calculated for the 'bouquets' based on 18-crown-6 with polyoxyethylene and polymethylene chains were, on average, 6×10^{-3} and 10×10^{-3} min^{-1}, respectively. While studying the cation transport along channels based on 18-crown-6, the researchers found out that an increase in the concentration of a compound in a membrane enhanced the transport only for the channel with polymethylene chains.

In order to distinguish between two transport mechanisms (an ion channel or an ion carrier), the transport rate through the membrane under usual conditions was compared with the transport rate through a frozen membrane. Switching to the gel state is expected to cause a substantial decrease in the rate of the ion flux for a carrier, which executes shuttle movements in the membrane, but to have almost no influence on the rate of the transport induced by a static channel structure. It was shown that the liquid crystal – gel phase transition did not result in a decrease in the transport rate only for two of the four 'bouquets', those based on cyclodextrin with polyoxyethylene chains and 18-crown-6 with hydrocarbon chains. The behaviour demonstrated by the compounds derived from 18-crown-6 with polyoxyethylene chains and from β-cyclodextrin with hydrocarbon chains does not allow one to classify them as channel structures; however, the results obtained do not indicate that they are ion carriers either.[91]

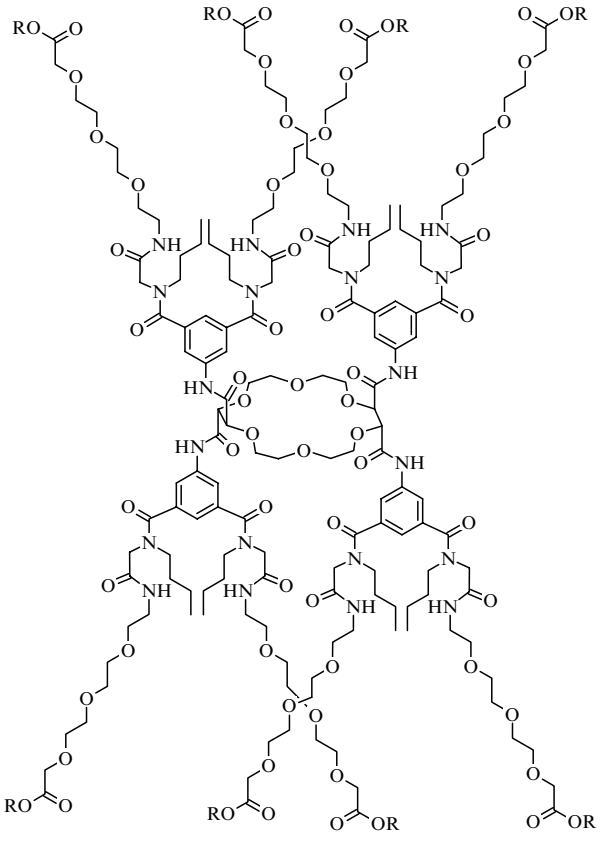
2. 'Bouquet' structures based on calixarenes

A few examples of using calixarenes for constructing artificial ion channels have been reported. A cyclic resorcinol tetramer with long alkyl chains has been synthesised and studied as an ion channel.[92] It was found that this compound functions as a half channel with K^+-selectivity. This channel is of interest as a model of natural ion-channel proteins having hydrophobic fragments. The uncharged and highly hydrophobic M2-segment of the nicotine acetylcholine receptor ...MTLSISVLLSLTVFLLVIV... can serve as an example.[93]

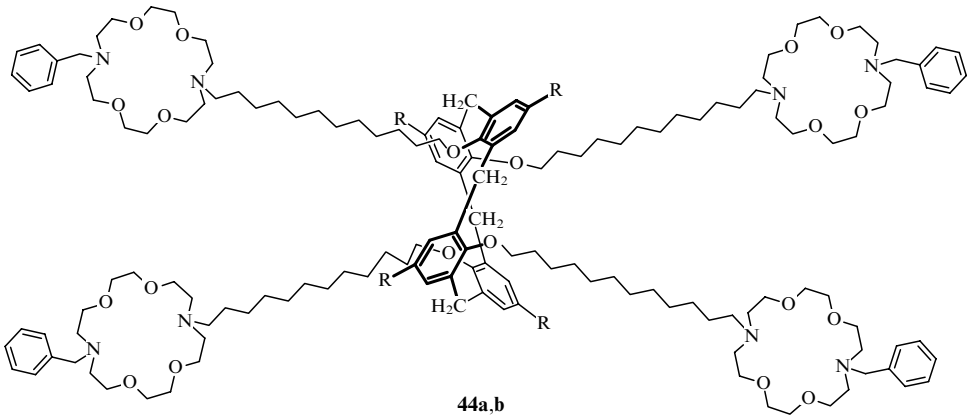
The synthesis of two compounds based on calix[4]arene in the 1,3-*alternate* conformation (**44a**,**b**) has been described. In compounds **44a**,**b**, each phenol group of calixarene is connected



42 (R = H)



43 (R = Na)



44a,**b**

R = H (**a**), Bu^t (**b**).

through an oxygen atom to one dodecyl chain which ends with a 10-benzyl-1,10-diaza-18-crown-6 fragment.

The side chains in compounds **44a**,**b** are arranged on both sides of the central fragment, two chains on each side. The diazacrown fragments acting as head groups stabilise the distribution of side chains within the membrane. The total length of the channel is 34–60 Å, which is sufficient for bridging the lipid part of a typical bilayer membrane, whose average thickness is ~30 Å.[9]

The incorporation of compounds **44a**,**b** (1 mmol) in a bilayer membrane increases the membrane permeability for alkali metal cations [with smaller amounts of these compounds (1 nmol), the membranes show unstable permeability] (Fig. 27). The repeated introduction of solutions of compounds **44a**,**b** in the membrane increases the intensity and the frequency of the observed transitions (up to 300×10^{-12} Ω^{-1}).

The probability for Na^+ and K^+ cations to flow through a calixarene platform has been estimated. In the solid state, the average distance between the centres of the aryl fragments in the 1,3-*alternate* calixarene conformation is 5.9 Å. This distance is sufficient for penetration of Na^+ and K^+ cations, because according to theoretical calculations, the benzene–M^+–benzene distance in an ideal sandwich structure is 4.96 Å (for M = Na) or 5.84 Å (for M = K). Thus, it was shown that Na^+ and K^+ cations can flow through calix[4]arenes in the 1,3-*alternate* conformation.[94, 95]

However, in the case of tetra-*tert*-butylcalix[4]arene **44b**, the calixarene platform is completely blocked from both sides by bulky *tert*-butyl groups. To enable the flowing of cations through calixarene **44b**, the conformation must undergo a definite sequence of modifications: two aryl fragments should pass into the open state, to let the cation through, while the other two fragments are closed. Similar changes should take place at the other end of the molecule to let the cations out. However, at room temperature, calix[4]arene is unable to change its conformation. The results obtained by measuring the channel conductance for compounds **44a**,**b** did not allow the researchers to decide how the ions flow in such a channel, either through the macrocyclic ring of calixarene or outside the ring.

Compounds **44a**,**b** represent a new class of cation-conductive channels with a high level of conductance for the sodium and potassium cations. In these compounds, calixarenes in the 1,3-*alternate* conformation were used as the basic compounds for the design of ion channels.

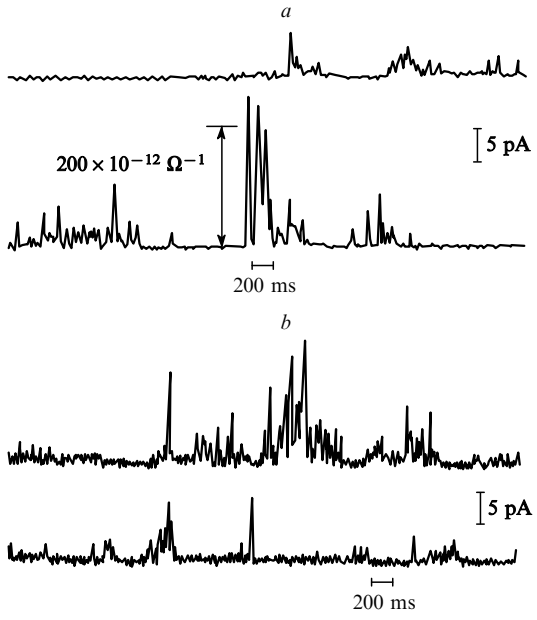
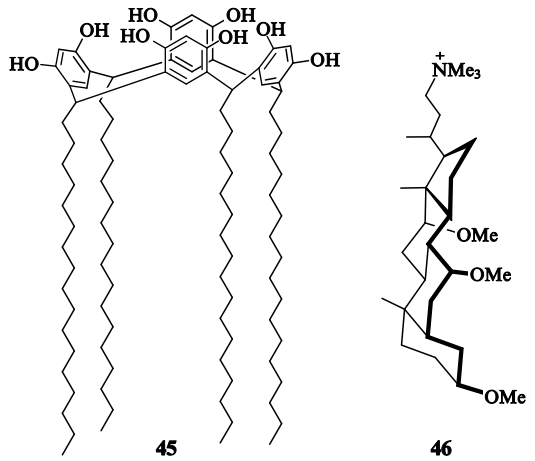


Figure 27. Conductance of compounds **44a** (*a*) and **44b** (*b*) (1 pmol each in THF, a 0.5 M solution of NaCl) in a planar bilayer membrane at 100 mV. For each compound, two different time sections are shown. The different patterns of these sections point to an unstable permeability regime.

VII. The use of the calixarene platform for the design of ion channels

1. The artificial ion channel based on a macrocyclic calix[4]resorcinarene with amphiphilic ester groups of cholic acid

As a result of further development of approaches to the preparation of nonpeptide ion channels — supramolecular and molecular half channels — Kobuke and co-workers [96] synthesised calix[4]resorcinarene **45** with four alkyl substituents. This compound forms a single ion channel with one conductance level. They also obtained a supramolecular ion channel on the basis of an amphiphilic derivative of rigid methyl cholate **46**. The channel



45 **46**

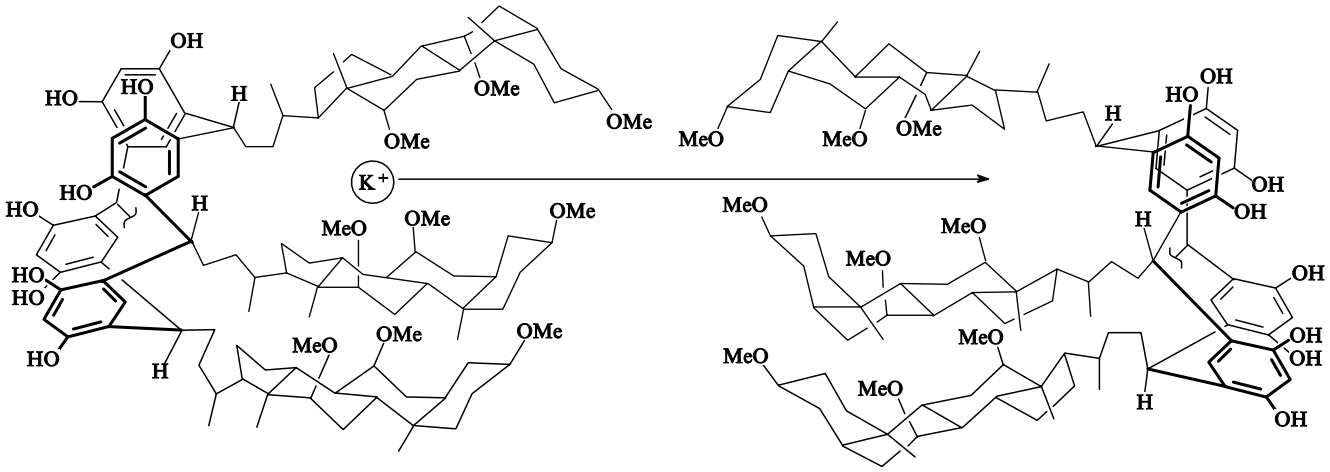
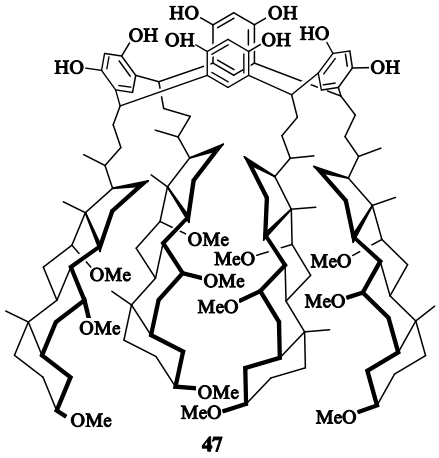


Figure 28. Ion channel formed from two calix[4]resorcinarene **47** molecules connected in the tail-to-tail mode (one methyl cholate residue is omitted for clarity).



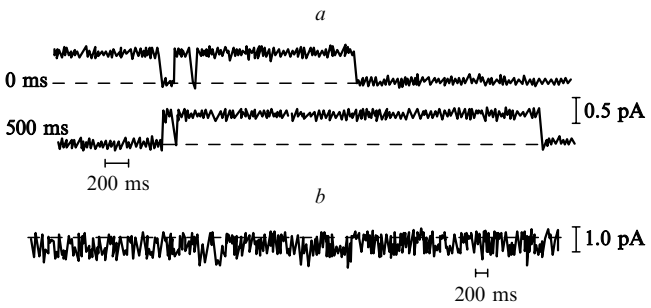


Figure 29. Time profiles of single channels **47** (*a*) and **45** (*b*).
(*a*) 0.5 mol of KCl, pH 7.2, 70 mV; (*b*) 0.5 mol of KCl, pH 7.2, −74.4 mV.

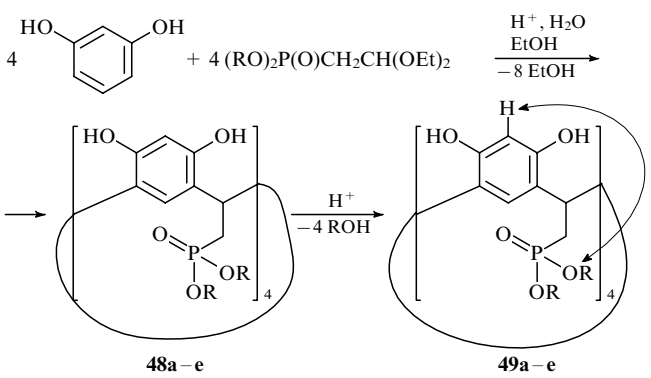
formed by compound **46** in the bilayer membrane has a long open duration.

To prepare a molecular ion channel having one conductance level and a long open-state duration, calix[4]resorcinarene **47**, combining calixarene and methylcholine fragments, was synthesised. A study by ^{13}C NMR spectroscopy showed that compound **47** exists in a C_4-symmetric conformation. The presence of hydrophilic head groups and amphiphilic steroidal fragments in calix[4]resorcinarene **47** allows the molecules in a bilayer membrane to form a tail-to-tail pair, which gives rise to a transmembrane channel (Fig. 28). The hydrophilic parts of the steroid groups form a hydrophilic pore around the central axis, whereas the hydrophobic parts are directed toward the lipid alkyl groups surrounding the channel. The ion channel formed by the molecules of compound **47** exists in the open state for a long time (Fig. 29).

On passing from compound **45** to compound **47**, the electrical conductance increases by 60%, but the cation/anion selectivity decreases simultaneously by 60%: the P_{K^+}/P_{Cl^-} ratio decreases from 20 to 8. These changes attest to the formation of a more hydrophilic medium at the centre of the pore when steroidal fragments are used as side groups. The Na^+ and K^+ selectivities found for the channels based on calix[4]resorcinarenes **45** and **47** do not differ much, $P_{K^+}/P_{Na^+} = 3$ (for channel **45**) and 2.8 (for channel **47**).

The ion channels constructed from calix[4]resorcinarenes proved to be appropriate models for investigating the structure–function relationship in ion channels, as they exhibit one conductance level and the tail section can be varied. It should be noted that compound **47** has certain advantages over compound **45**, because the steroidal tail stabilises the open state of the channel and promotes modification of the hydrophilic pore of the channel without a change in the general structure, which is confirmed by the values of conductance and selectivity of this channel with respect to cations.

A new approach to the design of ion channels on the basis of nanotubular structures has been developed by chemists from Kazan'.[97–100] It is based on self-stacking of functionalised calix[4]arenes through specific interactions (mainly, hydrogen bonding) between groups appropriately oriented in space. A series of amphiphilic organophosphorus calix[4]resorcinarenes self-assembled due to the presence of groups, linked to each other by strong hydrogen bonds, on the lower and upper rims of the macrocycle have been synthesised. An original method for the synthesis of calix[4]resorcinarenes **48a–e**, containing dialkoxy-



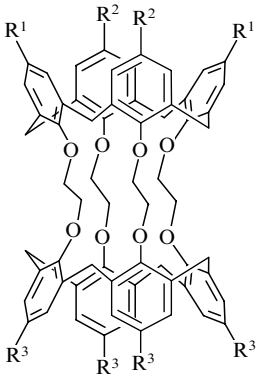
R = Et (**a**), Pr^n (**b**), Pr^i (**c**), Bu^n (**d**), Bu^i (**e**).

phosphoryl groups on the lower rim has been proposed.[99] Partial hydrolysis of these compounds yielded phosphonic monoesters **49a**–**e**.

Studies of self-aggregation of compounds **49a**–**e** in deuteriochloroform performed by 2D ^{1}H NMR techniques (NOESY and ROESY) confirmed the formation of tubular aggregates due to head-to-tail association of molecules in solution. This is also indicated by the presence of cross-peaks between the hydrogen atoms of the alkyl groups of the phosphorus fragments located on the lower rim of the macrocycle and the aromatic hydrogen atom located between two hydroxy groups on the upper rim (marked by arrows).

2. Calix[4]arene tubes as a new class of selective potassium ionophores

Determination of the structure of the potassium channel from *Streptomyces lividans* encouraged researchers to study the structural features of ion channels by using synthetic mimetics. Although the known synthetic receptors for K^+ cations are rather good, they still do not demonstrate sufficient selectivity. High selectivity was attained by Beer and co-workers.[101] They synthesised a new class of cryptand-like ionophores **50**–**58** derived from the biscalix[4]arene structure. Compounds **50**–**58** are composed of two calixarene fragments connected by four ethylene groups located on the lower rim. The ion channels based on these compounds show high selectivity for K^+ cations over other Group I metal cations and Ba^{2+} cations.



50–**58**

50: $R^1 = R^2 = R^3 = Bu^t$; **51**: $R^1 = R^2 = R^3 = Oct^t$;
52: $R^1 = R^2 = R^3 = H$; **53**: $R^1 = R^2 = H$, $R^3 = Bu^t$;
54: $R^1 = R^2 = Oct^t$, $R^3 = Bu^t$; **55**: $R^1 = R^2 = Pr^i$, $R^3 = Bu^t$;
56: $R^1 = R^2 = Ph$, $R^3 = Bu^t$; **57**: $R^1 = R^2 = Oct^t$, $R^3 = H$;
58: $R^1 = Bu^t$, $R^2 = R^3 = H$.

Two calixarene fragments connected by four ethylene spacers provide a rigid ensemble of eight electron-donating oxygen atoms for complexation with the metal. The calixarene fragments endow the structure with a filter restricting the size of the incoming cations, similar to the tyrosine filter which exists in the cell potassium channels.

Variation of substituents on the upper rim ensures fine adjustment of the rate of K^+ capture by the calixarene fragment; this was evaluated using NMR data on the conformational changes during binding. The selectivity of complex formation by compounds **50**–**58** with K^+ was demonstrated by NMR and electrospray mass spectrometry. According to X-ray diffraction data (Fig. 30), the potassium ion in compound **50** is localised at the centre of a partially flattened cube formed by oxygen atoms, the K–O distances varying from 2.759(6) to 2.809(6) Å. The cone cavities contain two methanol molecules.

For elucidating the influence of the nature of the counter-ion on binding of the K^+ cation, complexation of compound **50** with a number of potassium salts has been studied. Substantial differences in binding of the K^+ cation were found. In particular, the complexation was most efficient with the iodide, hexafluorophosphate, benzoate and triflate anions present as counter-ions. The least pronounced or even zero binding was found for the nitrate, perchlorate and picrate anions. A competitive experiment for binding of the potassium cations by compound **50** and by [2.2.2]cryptand showed that the complex with the calix[4]arene tube **50** is exceptionally stable, whereas in the case of cryptand, 28% of potassium ions remain free after 15 h.

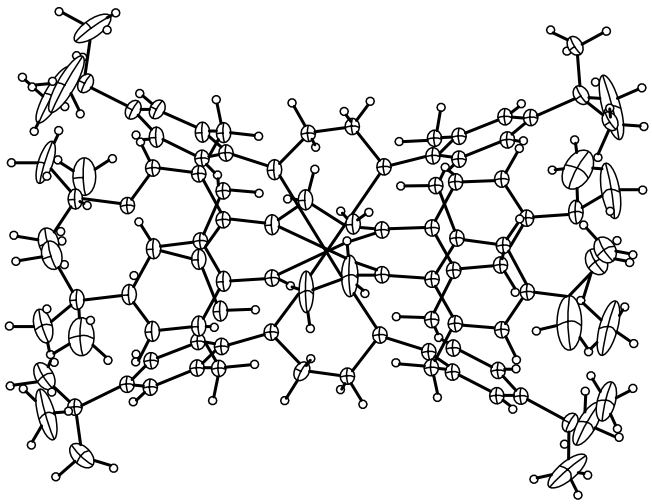


Figure 30. Structure of the crystalline complex of calix[4]arene **50** with K^+.

The selectivity of complexation of calix[4]arene tubes **50**–**58** with the potassium cation over other alkali metal cations and Ba^{2+} in a chloroform–methanol mixture (4 : 1) was determined at a 20-fold excess of Na^+, Rb^+, K^+, Cs^+, and Ba^{2+} iodides. Only K^+ cations are bound by any of compounds **52**–**58**. Nanotube **50** binds less than 5% of Rb^+, and tube **51** showed a 10% binding of Na^+ cations after 48 h; over the same period of time, these compounds bind 100% of potassium. Thus, calix[4]arene tubes are highly selective for potassium cation and surpass in selectivity ionophores based on calix[4]crowns, which, despite the Na^+/K^+ selectivity, still capture substantial amounts of Rb^+ cations.

Competitive complexation has also been studied by electrospray mass spectrometry. All spectra of equimolar mixtures of compounds exhibited peaks only for the potassium complexes $[M + K^+]$ and $[M + K^+ + 2H_2O]$. Even in the case where the concentration of K^+ decreased to 2.5 μmol litre^{-1} and the concentrations of competing ions were below 10 μmol litre^{-1}, the mass spectrum displayed the presence of only potassium-containing complexes, which proves substantial K^+ selectivity of all of the calix[4]arene tubes.

In order to clarify how the K^+ cation enters the calix[4]arene tube, molecular modelling of this process was performed. Two routes are possible for the potassium cations to flow through the tube, namely (i) the axial route where the cation passes the calixarene filter toward the polyether binding sites and (ii) the equatorial route where the cation is coordinated directly to groups of the cryptand-like binding sites. First, the influence of substituents occurring on the upper rim of the macrocycle (H, Bu^t, Oct^t, OH) was studied. It was found that the *tert*-butyl groups play an important role in the complexation through a change in the conformation of calix[4]arene tubes, which does not take place in the presence of sterically crowded *tert*-octyl groups, which block the *gggg*-conformation of the cone. The lack of alkyl groups on the upper rim (**52**, $R^1 = R^2 = H$) also deprives the cavity of any incentive to change the conformation.

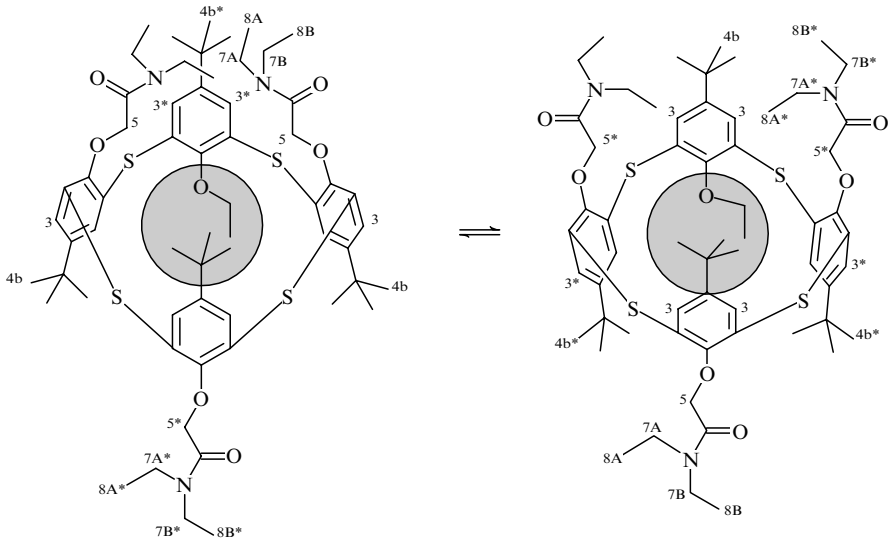


Figure 31. Passage of a potassium cation through the macrocyclic ring of compound **59** in the 1,3-*alternate* conformation.

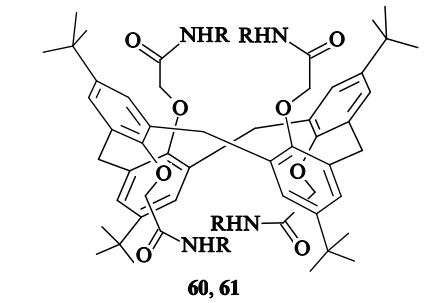
Data about the route travelled by the cation were obtained by molecular mechanics calculation of the energy of the cationic complex depending on the position of the potassium ion relative to the axis (along and across) of the calix[4]arene tube with a step of 0.5 Å. The flow of the cation through the *tert*-butyl groups of calix[4]arene with further movement along the axis toward the centre of the nanotube was found to be most favourable from the energy standpoint. Thus, the molecular modelling on the basis of crystallographic data confirmed that the potassium cation approaches the oxygen atoms along the axial plane of the calixarene dimer, passing through one of its lipophilic molecular cavities.

The possibility of passage of K^+ through the macrocyclic ring of thiacalix[4]arene has been confirmed experimentally by Solovieva *et al.*,[102] who studied the 1 : 1 potassium complex (with chloride as the counter-ion) of the tetraamide derivative **59** in the 1,3-*alternate* conformation by 1D and 2D ^{1}H NMR spectroscopy in $CDCl_3$. They demonstrated the presence of chemical exchange between the amide fragments located on different sides of the macrocyclic plane, which are magnetically inequivalent in pairs (due to complexation with K^+). It is evident that such exchange is possible only in two cases: in the case of intramolecular migration of K^+ between the amide fragments located on different sides of the macrocyclic plane of 1,3-*alternate* **59** (Fig. 31) and upon dissociation of the complex formed on one side of the macrocycle and subsequent complexation on the other side of the macrocycle. The second route appears unlikely in nonpolar solvents ($CDCl_3$) due to the very low concentration of free ions in these solutions. Moreover, on heating in a nonpolar medium, this complex is destroyed and KCl precipitates.

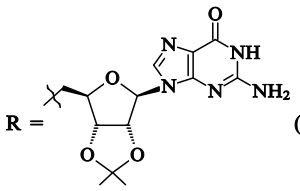
3. Self-assembling nanotubes from combined calix[4]arene and guanosine fragments

It has been shown[103] that in the presence of sodium cations, calix[4]arene **60** containing guanosine fragments in the 1,3-*alternate* conformation forms self-assembling nanotubes.

It has been shown previously that telomeric DNA forms G-complexes with channels filled by the cations. The G-wires that reach 1000 nm in length can be visualised using atomic force



60, 61



R = **(60), $(CH_2)_3CH_3$ (61).**

microscopy. This approach is based on the template self-assembly of guanosine on metal cations, in particular, in the presence of metal cations, guanosine forms a quartet with hydrogen bonds (Fig. 32 *a*). The cyclic G-quartet stabilised by the Na^+ or K^+ cation is capable of further self-assembly through stacking.

The Na^+ cations initiate one-dimensional association of G_4-calix[4]arene **60** (Fig. 32 *b*). Calix[4]arene **60** in the 1,3-*alternate* conformation orients two orthogonal pairs of self-complementary G-nucleosides in such a way that they become capable of efficient intermolecular hydrogen bonding with neighbouring macrocycles. The process of assembling is fully reversible. The cation-template aggregation of calix[4]arene **60** can be controlled by varying the pH value and the temperature.

In the micrographs of compound **60** obtained by electron transmission microscopy, one can see extended structures about 1 μm long, which are arranged either as separate tubes or as 3 to 50 nm thick bundles of tubes. These structures are relatively straight and have no sharp turns. The thickness of separate tubes is close to the resolving power of the microscope, *i.e.*, it is about 3 nm.

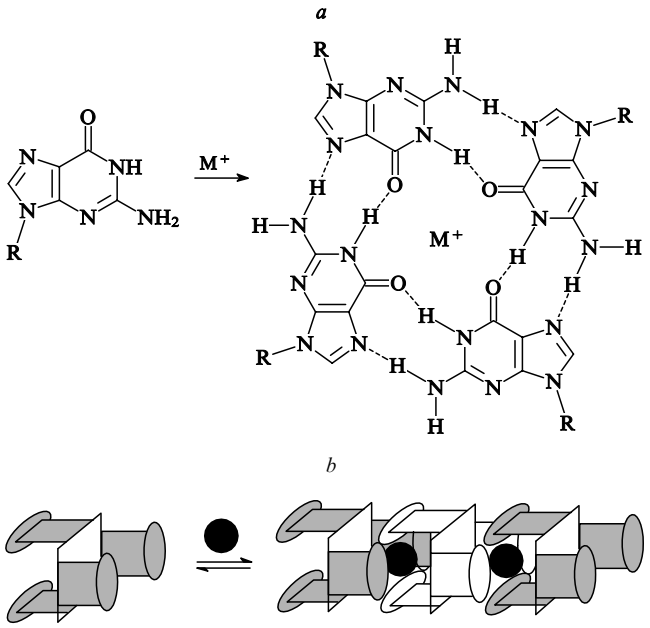


Figure 32. Template self-organisation of guanosine on metal cations (*a*) and cation-template self-assembling of calix[4]arene **60** to form a nanotube (*b*).
The squares stand for the calix[4]arene in the 1,3-*alternate* conformation, ellipses are the guanosine fragments and circles show the Na^+ cation.

4. The anionic channel based on aminocalix[4]arene

Sidorov *et al.*[104] have studied the transport activity of the tetrabutylamide derivative of calix[4]arene in the 1,3-*alternate* conformation (compound **61**) with respect to Cl^- and HSO_4^- anions in liposomes, planar bilayer lipid membranes and cells of the HEK-293 line.

The results of ionic conductance measurements, together with ^1H NMR and X-ray diffraction data indicate that compound **61**, first, forms ion channels in bilayer membranes; second, performs ion transport through a cell membrane with a positive holding potential; third, inverts the pH inside liposomes at a concentration gradient of chloride anions; fourth, demonstrates a substantial Cl^-/SO_4^{2-} selectivity. Calix[4]arene **61**, which binds HCl and forms an ion channel in a bilayer lipid membrane, is a rare example of a synthetic nonpeptide ion channel demonstrating anion selectivity. The ability to bind HCl and the transporting activity of this compound can be explained by the three-dimensional structure and hydrophobicity of calixarene and the presence of amide side chains in the molecule. The change of any of these parameters either shifts the selectivity from the anion to the cation or deprives the molecule of the transport activity. The transport of anions by compound **61** takes place through the H^+/Cl^- symport or Cl^-/OH^- antiport. Some crystal data indicate that calix[4]arene **61** forms self-assembling channels able to transmit Cl^- anions and water molecules. In addition, compound **61** performs the voltage-dependent selective transport of Cl^- anions through the membranes of the HEK-293 cells. This opens up broad prospects for investigating compound **61** as an antibiotic capable of selective interaction with the bacterial cell.

VIII. Conclusion

It is known that cell membranes have a common structural organisation. They are constructed as phospholipid bilayers with associated membrane proteins responsible for specific functions. Some proteins function as receptors responding to an external signal by activation of a number of intracell signal cascades, some others are involved in the transport of ions and molecules through the cell membrane. The design of models that mimic the ion transport in biological systems is needed for the understanding of very complex membrane processes that regulate the relationship of living organisms with the environment. The research into transmembrane ion channels is among the most vigorously progressing routes of modern biochemistry, nanotechnology and supramolecular chemistry. The design of ion–molecular devices that mimic the principles of transmembrane signal transfer opens up absolutely new opportunities for the development of natural science and technology. The use of simple artificial channel-like systems representing an alternative to natural peptides and proteins would result in the development of new nano-sized structures (nanotubes, ionic wires, *etc.*) with the properties of abiotic transmembrane channels ensuring the ionic permeability in natural systems (cells). The use of such systems in biosensors is a new engineering approach to the design of sensors for detection of various substrates both *in vitro* and *in vivo*.

During the last decade, significant progress has been attained in the study of properties of artificial ion channels and the key requirements of the design have been developed rather thoroughly. Any transmembrane ion channel has contacts with both the hydrophobic core of the lipid bilayer and aqueous solutions. In this connection, the channel-forming structures should be amphiphilic. The sites of a compound or an associate that are in contact with the aqueous medium surrounding the membrane should be enriched with polar and readily ionisable functional groups and the residues in contact with the lipid chains should contain long non-polar segments whose length must be sufficient to bridge the bilayer. Thus, when designing channel-forming structures, one should bear in mind that molecules incorporated in the lipid membrane should consist of a hydrophilic head in contact with the polar environment and the non-polar tail, which is incorporated in the membrane due to hydrophobic interactions. This follows from energy principles. In addition, for binding and transport of ions, charged or polar groups must be present inside the bilayer, *i.e.*, ion channels should be polar from the inside and hydrophobic from the outside.

Two key approaches to the design of channel-forming structures can be distinguished. One approach is the creation of molecules or various ion pairs that are able to form channel-like associates upon incorporation into a phospholipid membrane. The degree of aggregation of chemical species to form a half channel or a channel depends on a large number of factors; therefore aggregates of a variety of compositions can appear in the membrane.

Most artificial ion channels switch from the closed state to the open state as a result of random aggregation processes in the lipid bilayer. Self-assembling oligomeric associates immobilised on an electrode surface and mimicking the properties of biomolecules represent the first step in the creation of new ion–molecular devices used as sensors for various types of chemical species. This equipment is needed first of all for the diagnostics and analysis of the state of the environment.

The second approach is the design of a single pore-forming molecular structure, most often, containing a macrocyclic system which can be spontaneously incorporated into the membrane. However, in the case of these structures, two mechanisms of ion transport may compete (an ion channel or an ion carrier). The molecular structures whose length is sufficient to bridge the phospholipid membranes are nano-sized. This opens up broad prospects for using such structures for miniaturisation of electronic devices.

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