Biomimetic Macromolecular Chemistry: Design and Synthesis of an Artificial Ion Channel Based on a Polymer Containing Cofacially Stacked Crown Ether Rings. Incorporation in Dihexadecyl Phosphate Vesicles and Study of Cobalt Ion Transport

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ABSTRACT: We describe a synthetic model of a natural channel-forming ionophore. It is based on a polymer with a rigid backbone, viz. a polymer of an isocyanide, $[R—N{=}C<]_n$. Each of the polymer side chains R contains a crown ether ring. Due to the rigid 4/1 helical structure of the polymer the crown ether rings are cofacially stacked and form four channels which run parallel to the polymer helix axis. The channels are better ion-complexing agents than low molecular weight crown ethers. Evidence is presented that the channel structures can be incorporated in the bilayers of dihexadecyl phosphate vesicles. Electron microscopic data, fluorescence experiments, as well as entrapment studies indicate that the vesicle bilayers are not disrupted by the channels. Ion transport studies are presented which reveal that the channel structures enhance the permeability of the dihexadecyl phosphate membranes by forming transmembranous pores.

Introduction

Many vital functions of the living cell are controlled by processes that take place at the cell membrane–water interface.[2] These processes are commonly catalyzed by molecules located in the membrane matrix. An example is the transport of ions across the cell membrane. The activation of nerve cells, heart muscle cells, etc. is directly linked to processes regulating the diffusion of ions through the cell membranes.[3] The uncatalyzed transport of ions is very slow: reported permeation coefficients for K^+ and Na^+ are 7×10^{-13} [4a] and 2×10^{-13} cm/s,[4b] respectively.

Generally, there are two ways by which nature facilitates the transport of ions across a bilayer membrane.[3] One way is by means of carrier molecules, e.g. the depsipeptide antibiotics valinomycin and nonactin. These ionophores form a lipophilic complex with a cation at one interface of the membrane. Via diffusion, this complex migrates to the other interface where the cation is released. A second, more frequently encountered mode of transport is by proteins that form a transmembrane ion channel.[5] A well-studied example is the gramicidin A ion channel.[5d,e] Two gramicidin molecules dimerize head-to-head in the membrane to form a solvent-filled, transient pore.

Ion transport by means of synthetic carrier-type ionophores has been studied in great detail.[6] On the contrary, studies of ion transport via synthetic channel-type ionophores are scarce.[7] The reason for this probably is that designing an ion channel poses more problems than designing an ion carrier. Fuhrhop and associates have succeeded in modifying the natural carrier monensin by substituting one of the OH end groups of this molecule by carboxylic acid functions.[7b] This modification enables the molecule to span a bilayer membrane and to function as a pore for lithium ions. In recent papers it was shown that such pores can be sealed by stoppers.[7e,f] Fyles and coworkers have reported the synthesis of a biomimetic ion channel structure based on a crown ether containing six pendant amphiphilic chains.[7g]

We are interested in designing models of biomolecules and biological systems using polymers as building blocks.[8] Here we describe an ion channel based on a rigid polymer containing stacked crown ether rings. We present evidence that this channel compound can be incorporated into the bilayer membrane of dihexadecyl phosphate vesicles and is able to facilitate the transport of cobalt ions.[9]

Results and Discussion

Strategy. A synthetic ion channel modeled upon naturally occurring channel-type ionophores should have a porelike structure with a polar interior, an apolar exterior, and a hydrophilic top and bottom. From a topological point of view there are several ways to construct such a channel. If a linear molecule is spiralized, a gramicidin-like channel is obtained. Work according to this principle was reported by Spach et al.[7c] Self-assembly of appropriately designed molecules is another route to the formation of a channel. In this way an interstitial channel reminiscent of alamethicin is created. Fuhrhop has reported on the formation of synthetic channels of this type.[7a,b,e,f] A channel can also be constructed by stacking a number of ringlike species. As far as we know, this method is not used by nature. Building blocks in the form of crown ether rings are readily available. The main problem is how to interconnect the macrocyclic rings. Piling them stepwise via lateral appendages is difficult to realize and will probably go no further than two rings.[10] We have solved this problem by anchoring the crown ether rings onto a rigid polymer support. Two procedures have been followed: A dihydrosilicon phthalocyanine containing four crown ether rings was synthesized and polymerized to give a crown ether substituted poly(silicon phthalocyanine).[11] In this polymer the crowns are stacked at a distance of 4 Å to form four ion-conducting channels. This way of forming a channel-type ionophore is described in a separate paper.[12] In the second procedure, which is discussed in this paper, a polymer of an isocyanide is used as the polymer support. A third procedure using a polypeptide backbone was recently described by Voyer.[7i]

Polymers of isocyanides are easily prepared from isocyanides by the catalytic action of, e.g., nickel(II) salts.[13]

$$n\mathrm{R—N^+{\equiv}C^-} \rightarrow [\mathrm{R—N{=}C<}]_n$$

The polymers are unusual in the sense that each main-

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chain carbon atom carries a side chain. The backbone of a poly(isocyanide) has a helical structure (Figure 1A). This was proven by the complete resolution of poly(*tert*-butyl isocyanide) into left-handed and right-handed helices.[13a,c] This helical structure is the result of steric repulsion by the side groups R.[13d] Electronic effects also seem to play a role.[13e] Molecular orbital calculations have shown that the number of repeating units per helical turn for poly(*tert*-butyl isocyanide) is close to 4.[13e] A similar number was obtained from molecular mechanics calculations (viz. 3.8[13d,f]) and from a comparison of calculated and experimental circular dichroism spectra (between 3.6 and 4.6[13g], and 3.8[13d]). The bulkiness of the R group influences the helical structure of the polymer. For R = tertiary or secondary alkyl the helix is very rigid. When the steric bulk decreases (R = primary alkyl) more structures with different degrees of helicity are possible.[13d,e] In principle, the side groups R in a poly(isocyanide) can adopt syn- or anti-configurations. The most stable polymer structure is suggested to be the one in which the groups are all syn or, what is equivalent, all anti.[13d,f,14]

The support which we have used for the anchoring of crown ether rings is poly[(*R*,*S*)-α-phenylethyl isocyanide]. This polymer has a very stiff backbone and contains 3.8 monomeric units per helical turn.[13d] Its side chains form four stacks which run parallel to the helix axis.[13b,d] In the stacks the phenyl groups are situated on top of each other at a distance of 4 Å. They are locked in this position by the tightly packed polymer chain and by the methyl substituents on the α-carbon atoms of the side chains. If the phenyl groups in poly(α-phenylethyl isocyanide) are incorporated into a crown ether ring system, these crown ethers will be positioned on top of each other and form four channels, as is depicted in Figure 1B,C.

The polymerization of isocyanides by nickel(II) is believed to proceed with a high degree of stereoselection,[13ij] although not always with absolute stereoselection.[13l] Chiral isocyanides give polymers with an excess of one screw sense, either left-handed or right-handed depending on the chirality of the side group R.[13h,i] Polymerization of achiral isocyanides with optically active nickel(II) catalysts yields optically active polymers with an enantiomeric excess of helical chains or helical segments up to 83%.[13j]

Theoretical calculations[13d] have predicted that on polymerization each of the enantiomers of α-phenylethyl isocyanide forms one particular helix. Spectroscopic studies on optically active poly(α-phenylethyl isocyanide) samples have confirmed this: the (*R*)-monomer forms a left-handed helix and the (*S*)-monomer a right-handed helix.[13h,k] The (*R*,*S*)-monomer mixture was recently shown to polymerize with a certain degree of stereoselection, but the reaction is not absolute stereoselective: (*R*)-monomers are incorporated into the (*S*)-chains and vice versa.[13l] We believe that the much more bulky crown ether substituted derivatives of α-phenylethyl isocyanide will polymerize more stereoselectively than α-phenylethyl isocyanide itself, although this remains to be proven experimentally.[13i] The results which will be presented in the following are in line with a higher stereoselection: both the ion-binding properties of the crown ether polymers and the ion transport experiments suggest that the number of defects in the chains are relatively small.

Synthesis and Complexation Studies. Channel compounds **5a–d** (pb15c5, pb18c6, pb21c7, and pb24c8, respectively) were prepared as shown in Scheme I. Benzocrown ethers **1a–d** (b15c5, b18c6, b21c7, b24c8, respectively) were obtained by ring closure of catechol with the appropriate oligo(ethylene glycol) dichlorides.[15] In the next step compounds **1** were treated with acetic anhydride in poly(phosphoric acid) to give the acetyl derivatives **2a–d**.[16] The latter compounds were converted into the formamides **3a–d** by means of a Leuckart reaction.[17] These formamides were dehydrated with phosphorus oxychloride and base to give the isocyanides **4a–d**.[18] Polymerization of **4a–d** was performed by heating the monomer neat at 60 °C with 1 mol % $NiCl_2 \cdot 6H_2O$ for 3 days. For comparison a copolymer (**6**) of **4b** and α-phenylethyl isocyanide was prepared by treating a 1:3 mixture of these compounds with $NiCl_2 \cdot 6H_2O$ at 25 °C. From elemental analysis it appeared that in this copolymer **4b** and α-phenylethyl isocyanide are present in a molar ratio of 1:4.

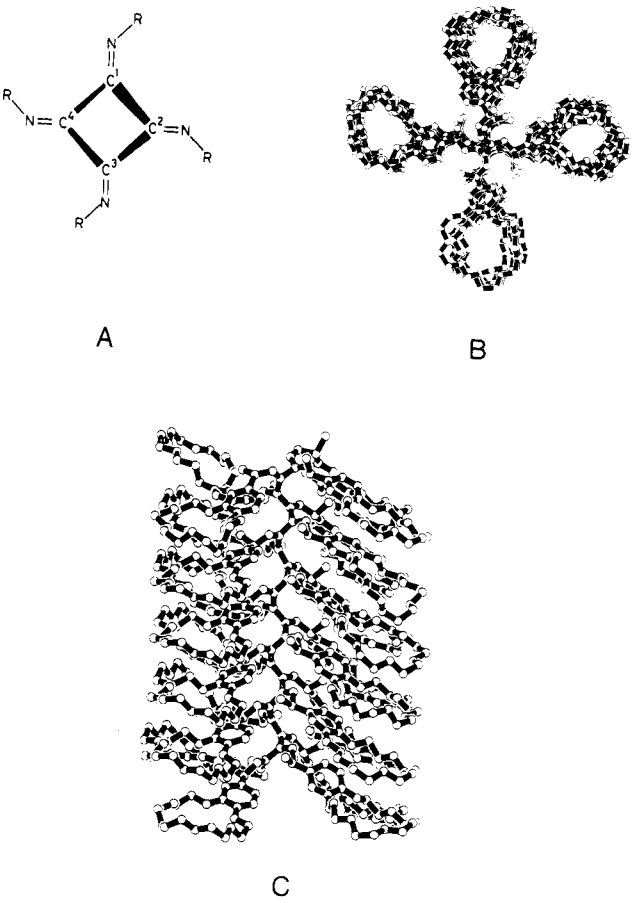
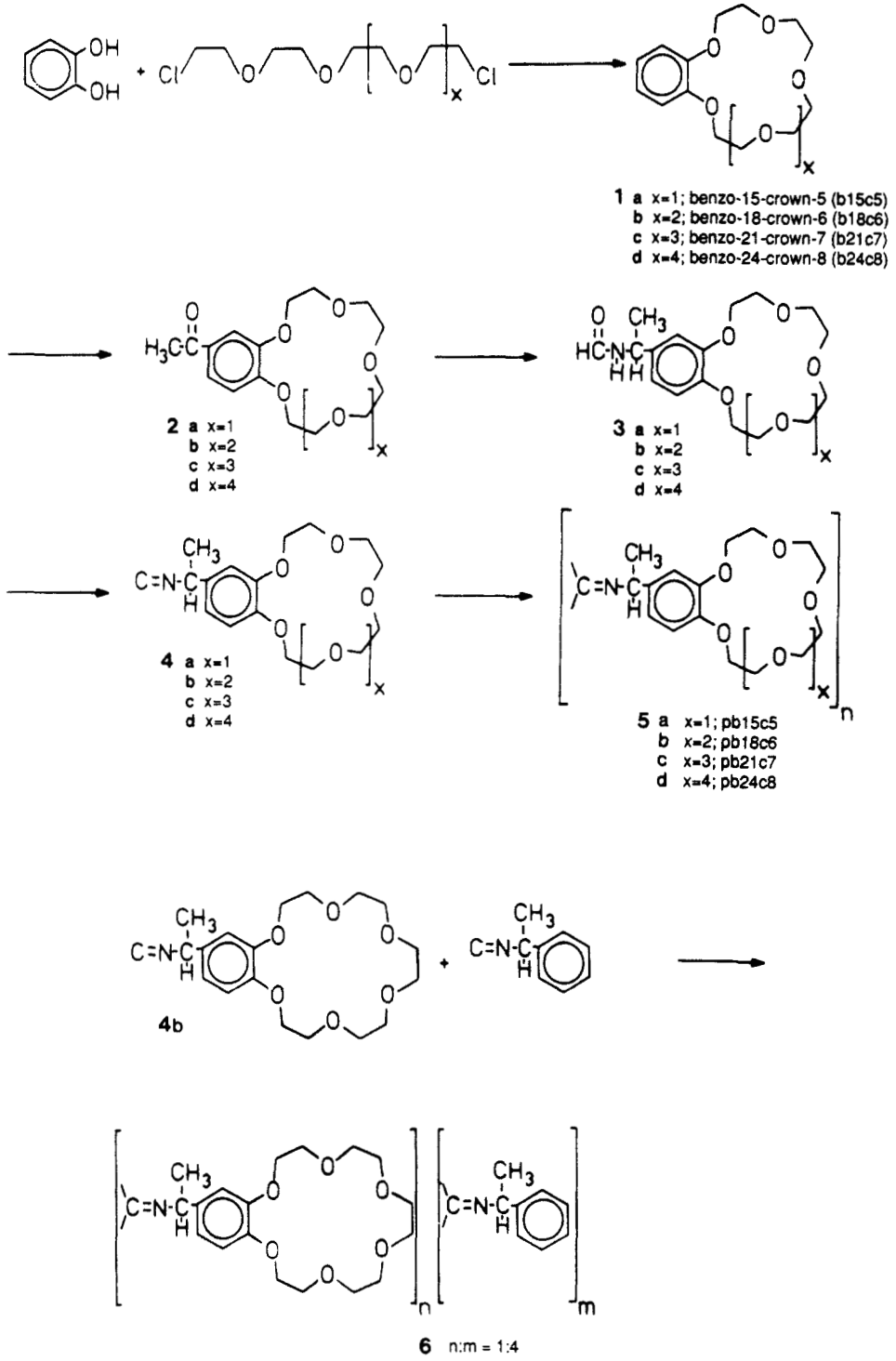


Figure 1. (A) Helical structure of a poly(isocyanide), projection along the helix axis. C^1 is above C^5, C^2 is above C^6, etc. (B) Calculated structure of a poly(α-phenylethyl isocyanide) with 18-crown-6 rings, top view. (C) Same as (B) except side view. The structure was calculated using the SYBYL 5.4 package (TRIPOS Associates, Inc., 1991).

Channel compounds **5b–d** are soluble in chloroform, toluene, and THF. They are slightly soluble in water and insoluble in hexane. Compounds **5a** and **6** have similar solubility properties, with the exception that **5a** is poorly soluble in toluene and **6** is insoluble in water.

The intrinsic viscosities of the polymers are in the range 0.03–0.05 dL/g (see Experimental Section). The number-averaged molecular weight of **5b** was determined by vapor pressure osmometry in toluene and in water; it appeared to be $M_n \simeq 4000$. To get an indication of the molecular weight distribution, membrane filtration experiments were performed. It appeared that the channel compounds could pass filters with a molecular weight cutoff of 20 000, whereas only very small amounts could pass filters with a molecular weight cutoff of 5000. Molecular weight determinations by light-scattering measurements could not be carried out because the polymers are colored and

Scheme I



because they have a relatively low molecular weight. On the basis of an assumed molecular weight range of 4000–20 000 and taking into account the 4-Å pitch of the polymer helices, the lengths of the channels can be calculated to vary from 10 to 50 Å.

The picrate extraction technique[19] was applied to determine the cation-binding properties of the channel compounds **5a–d** and their monomeric analogues **1a–d**. The apparent association constants K_a and the corresponding free energies of complexation ($-\Delta G°$) are listed in the Experimental Section. In Figure 2 the $-\Delta G°$ values of the various complexes are presented graphically.

The ratio of the K_a values of polymer-bound crown ether–cation complexes to that of the corresponding benzo-crown ether–cation complexes, $Q = (K_{a,polymer}/K_{a,monomer})_{M+}$, can serve as a measure of the polymer effect on cation binding. This ratio was calculated for the various complexes (Table I). It appears that in all cases the binding efficiency is higher for the polymers than for the corresponding benzo-crown ethers. An explanation may be that the conformation of the crown ethers in the channels is different from that in the free crown ethers. An isolated crown ether is not planar but has a folded conformation, which opens when it binds a cation. Because of the tightly packed structure of poly(isocyanides) it can be imagined that the crown ether rings in the polymer are forced into a more planar conformation. Such a preorganized conformation will facilitate cation complexation for entropy reasons.[20] Additional binding by neighboring crowns could

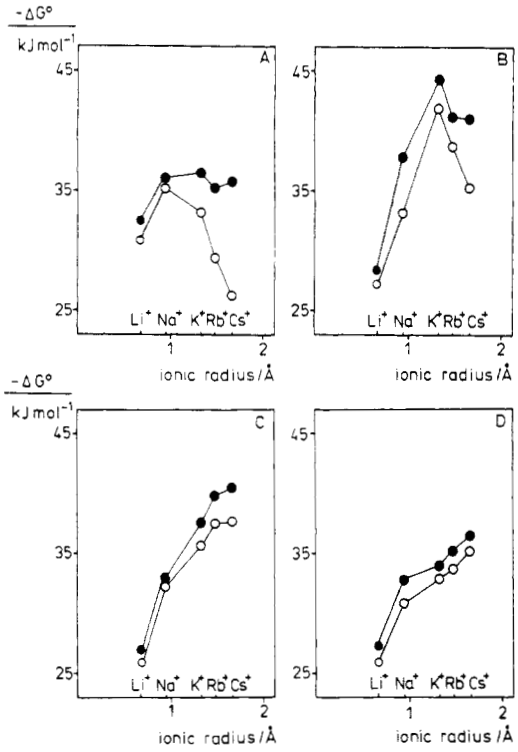


Figure 2. Plots of free energies of association versus the radius of the complexed cation: benzo-15-crown-5 (A); benzo-18-crown-6 (B); benzo-21-crown-7 (C); benzo-24-crown-8 (D); (open circles) low molecular weight crown ethers; (filled circles) channel compounds.

Table I
Polymer Effects (Q) on Cation Complexation

	5a/1a	5b/1b	6/1b	5c/1c	5d/1d
Li^+	1.8	1.6		1.5	1.7
Na^+	1.4	6.6	1.9	1.4	2.2
K^+	3.8	2.6	1.7	2.1	1.5
Rb^+	10.9	2.8	1.5	2.5	1.8
Cs^+	46.0	10.5	2.2	3.0	1.7
NH_4^+	17.0	5.1		1.2	1.8

be another reason for the increased ion-binding efficiencies of the channels.

The differences in association constants between polymers and monomers are not equal in all cases (Figure 2). The binding of the larger cations increases considerably on going from b15c5 to pb15c5 and from b18c6 to pb18c6. The differences in binding properties between b21c7 and pb21c7 and between b24c8 and pb24c8 are less pronounced. Here the polymer effect on cation binding is rather small. The large polymer effect on the binding of the larger cations by the smaller crowns can be explained as follows. It is known from literature that crown ethers form 2:1 sandwich complexes, when the size of the cation exceeds that of the hole in the crown.[21] In these complexes two crowns are coordinated to one cation. It is likely that in the channel compounds binding of large cations also occurs by 2:1 complexation. The stacking of the crown ethers in the polymers would facilitate such a complexation. It would explain why the polymer effect increases for the 15-crown-5 and—with some exceptions—for the 18-crown-6 derivative when going from small to large cations. Further support for the occurrence of sandwichlike complexes in pb15c5 and pb18c6 is obtained from the binding properties of copolymer **6**. In this polymer phenyl and benzo-18-crown-6 side groups are present in a ratio of 4:1. As the crown ether units in **6** are probably randomly distributed, the average distance between the crowns will be larger in this copolymer than in the homopolymer pb18c6. Therefore 2:1 complexation will less easily occur in **6** than in pb18c6. Indeed, the K_a and $-\Delta G°$ values of **6** are intermediate between those of b18c6 and pb18c6 (see Experimental Section, Table VI). The cavities of b21c7 and b24c8 are sufficiently large to accommodate the larger cations. Therefore 2:1 complex formation is not likely to occur with these crown ethers. Thus the K_a and $-\Delta G°$ values of the polymer-bound crowns do not differ very much from those of the free crowns.

Additional information on the complex stoichiometry was obtained by measuring the saturation level of the channels. Two procedures were followed. In the first procedure picrate extraction experiments were performed using a fixed concentration of the cation in the aqueous phase and a varying concentration of the crown ether in the organic phase.[22] The results are presented in Figure 3. As appears from this figure, the data, especially those of pb15c5, show considerable scattering. This scattering is due to precipitation of the complexes at higher overall cation to crown ether ratios. In the second procedure chloroform solutions of **5a–d** were shaken with solid picrates for 1.5 h. The results of these experiments are also represented in Figure 3. For pb15c5 the complexed cation to crown ether ratio does not exceed 0.5 when this polymer is saturated with K^+, Rb^+, and Cs^+. This is indicative of 2:1 crown ether–cation stoichiometry. With Na^+ a saturation level of 0.89 is reached, which suggests that 1:1 complexes are formed. The maximum complexed cation to crown ether ratio of 1:1 is not reached, probably because of electrostatic repulsion between the cations. The saturation level of pb18c6 with K^+ amounts to 0.84, again indicative of 1:1 complexation. With Rb^+, ultimately, a similar saturation level is reached but at a much higher overall cation to crown ratio, because this cation is less effectively bound by the 18-crown-6 rings than K^+. The saturation level of pb18c6 for Cs^+ amounts to approximately 0.4, again indicative of 2:1 complexation. For complexes of pb21c7 and pb24c8 with K^+, Rb^+, and Cs^+ the complexed cation to crown ether ratio does not exceed 0.3 and 0.5, respectively, although saturation levels of approximately 0.85 are to be expected. The reason for this difference is the relatively low binding efficiency of the 21-crown-7 and 24-crown-8 rings. To illustrate this, the saturation curves for b21c7 and b24c8 are also presented in Figure 3. For these compounds the complexed cation to crown ether ratios do not exceed those of the corresponding polymers.

Incorporation in Vesicle Bilayers. Vesicles of dihexadecyl phosphate (DHP) were prepared by ultrasonic dispersal of the amphiphile in water at 80 °C either in the presence or in the absence of channel compounds **5**. The dispersion process was followed by taking samples after varying the sonication times. Dynamic light-scattering measurements were performed to estimate the hydrodynamic radii of the vesicles. Table II reveals that these hydrodynamic radii decrease with increasing sonication time down to a plateau value. This plateau value is reached after 20 min and is the same for vesicles with and without channels.

For ion transport experiments it is important to know whether the vesicles are unilamellar or not. In multilamellar systems the apparent transport rate will be lower than in unilamellar systems. The lamellarity was determined by applying a method described by Barrow and Lentz.[23] In this method the absorbance of vesicle dispersions is measured at several wavelengths between 300 and 650 nm. These absorbances, which are a measure of the turbidity, are plotted versus the reciprocal fourth power

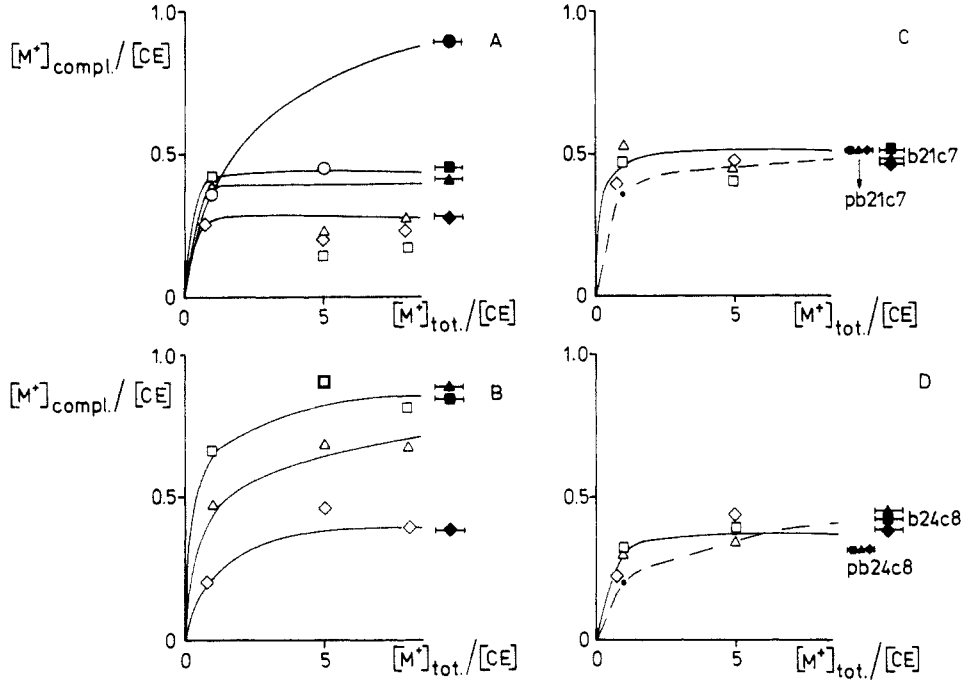


Figure 3. Saturation behaviour of pb15C5 (A), pb18C6 (B), pb21C7 (C), and pb24C8. The broken curves in (C) and (D) reflect the behavior of the low molecular weight analogues. Key: Na^+ (○), K^+ (□), Rb^+ (△), Cs^+ (◇). The open and filled symbols refer to the liquid–liquid and to the solid–liquid extraction experiments, respectively.

Table II
Hydrodynamic Radii of DHP Vesicles and Percentage of Incorporation of Channel Compounds[a]

sonication time (min)	R_H (nm)[b] with **5b**	R_H (nm)[b] without **5b**	sonication time (min)	R_H (nm)[b] with **5b**	R_H (nm)[b] without **5b**
9		118	20	51	
11	100		25	46	46
15		82	40	45	45

channel compd	% incorporation	channel compd	% incorporation
5a	<10	**5c**	85 ± 9
5b	85 ± 10	**5d**	50 ± 10

[a] pH 3–6. [b] Hydrodynamic radius; standard deviation ≥50%.

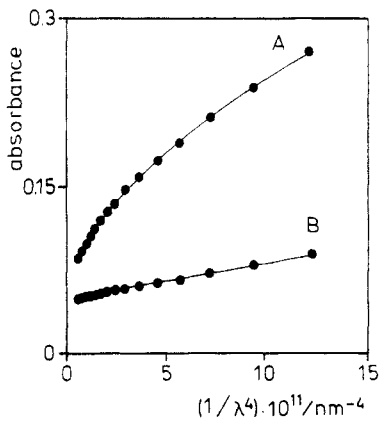


Figure 4. Plots of absorbance versus $1/\lambda^4$ for DHP dispersions sonicated during 5 (A) and 45 min (B) (pH = 3).

of the corresponding wavelength. In fact this method measures to what extent the vesicles behave as Rayleigh scatterers. Typical plots are shown in Figure 4. The correlation coefficient (r_c) of the absorption versus $1/\lambda^4$ plot is a measure of the unilamellarity of the vesicles. The maximum value that can be reached for r_c is 1.000. For DHP vesicles with and without channel compound **5b**, sonicated for 45 min, the correlation coefficients had increased to 0.995, indicating that the aggregates have a high degree of unilamellarity.

Whether channel compounds **5** were really incorporated into the vesicle bilayers was tested by ultrafiltration experiments. Dispersions containing vesicles and channel compounds were filtered through an ultrafiltration membrane. As the channel compounds display an absorption band at 280 nm, the presence of channels could be checked by recording UV–vis spectra of the residues and the filtrates. In separate experiments it was confirmed that the channel compounds could and the vesicles could not pass the ultrafiltration membrane. The results of the experiments are summarized in Table II. This table shows that channel compound **5a** cannot be incorporated into the vesicle bilayers. Table II further shows that compound **5d** is less effectively incorporated than **5b** and **5c**. The reason could be the higher solubility of **5d** in water.

Electron micrographs taken by the freeze-fracture technique revealed that the vesicles with channel compounds **5** have a flattened spherelike morphology. The diameters of the aggregates ranged from 100 to 200 nm, which is in good agreement with the results from the light-scattering experiments. The same features were observed for the vesicles without channel compounds. To check whether the vesicles are closed aggregates entrapment studies were performed using the water-soluble fluorescent dye pyranine.[24] It was found that vesicles with and without channels entrapped approximately the same amount of pyranine, suggesting that the aggregates indeed are closed aggregates and that they remain intact on incorporation of the channels.

The effect of incorporation of channel compound **5b** on the structure of the DHP bilayers was investigated with fluorescence techniques using the polarity probe *N*-phenyl-1-naphthylamine (NPN) and the viscosity probe 1,6-diphenyl-1,3,5-hexatriene (DPH).[25] The results of these experiments are given in Table III. The fluorescence maximum of NPN shifts from 433 to 422 nm when **5b** is incorporated. At the same time the solvent isotope effect[25,26] changes from 1.4 to 1.2. These two observations suggest that the polarity and the water content of the

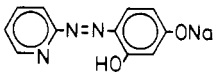
Table III
Fluorescence Data of Vesicles with and without Channel Compound 5b[a]

vesicle composition	fluorescent probe: DPH r^b	NPN λ_F (nm)[c]	NPN sie[d]
DHP	0.31	433	1.4
DHP + **5b**[e]	0.32	422	1.2

[a] pH = 5.5–5.7. [b] Fluorescence anisotropy value. [c] Wavelength of maximum fluorescence. [d] Solvent isotope effect. [e] Molar ratio **5b**/DHP = 2.8×10^{-3} based on a channel molecular weight of 15 000.

DHP bilayer are lowered on incorporation of **5b**. It may be that NPN penetrates deeper in bilayers containing **5b** than in pure DHP bilayers. This can occur if the former bilayers are more fluid than the latter ones. If this were the case a decrease of the fluorescence anisotropy would have been expected on incorporation of **5b**. This, however, was not observed (Table III). Our fluorescence experiments indicate that the channel compounds do not disrupt the structure of the DHP bilayer. The channels rather have a structure-making effect, as less water molecules are allowed to penetrate in the bilayer. This result is important for the ion transport experiments (vide infra).

Ion Transport. Ion transport across vesicle bilayers can be measured in two ways: (i) ions are entrapped in the inner aqueous compartments of the vesicles and the ion efflux is measured. (The process can be followed e.g. by using an ion-selective electrode); (ii) ions are added externally and ion influx is measured, e.g., by monitoring spectral changes of an entrapped indicator. Initial experiments using the first procedure were unsuccessful as the DHP vesicles could not withstand the relatively high salt concentrations that are required to entrap a measurable amount of cations in the inner aqueous compartments. Therefore, the second procedure was chosen. The dye 4-(2-pyridylazo)resorcinol monosodium salt (PAR) was



selected as the indicator and Co^{2+} as the ion to be transported. PAR forms a stable 2:1 complex with Co^{2+}. This complex strongly absorbs at 510 nm, whereas free PAR has an absorption band at 390 nm.

Vesicles with entrapped PAR were obtained by sonicating DHP in an aqueous solution of this dye. The pH of the vesicle dispersion was set to 5.5–5.7 by neutralizing 50% of the DHP with NaOH prior to sonication. DHP vesicles prepared in this way have been found to have relatively high trapping efficiencies.[27] The exovesicular PAR was removed by repeated ultrafiltration. According to UV–vis absorbance measurements the trapping percentage amounted to ~4%.

Ion transport was measured by adding an aqueous solution of $Co(NO_3)_2$ to the vesicle dispersions and recording the increase in absorption of the cobalt–PAR complex at 510 nm. We checked separately that cobalt ions do not disrupt the vesicle membrane. By repeatedly dialysing after addition of cobalt ions it appeared that PAR remained entrapped both in vesicles with and in vesicles without channel compounds. If after addition of cobalt ions the vesicles without channels were destroyed with Triton X-100, a drastic increase in absorbance at 510 nm and a decrease in absorbance at 390 nm was observed. This also indicates that under our experimental conditions the vesicle structure is not affected by Co^{2+}.

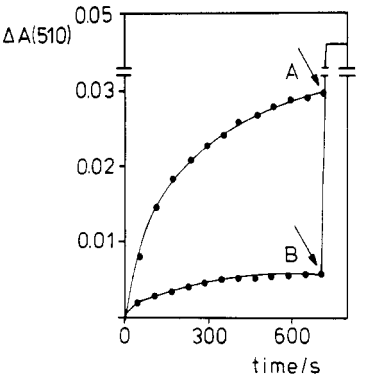


Figure 5. Plots of the change in absorbance at 510 nm versus time for vesicles with (A) and without (B) channel compound pb18C6 (**5b**). The arrows indicate the addition of Triton X-100; $[Co^{2+}] = 2.03 \times 10^{-5}$ M.

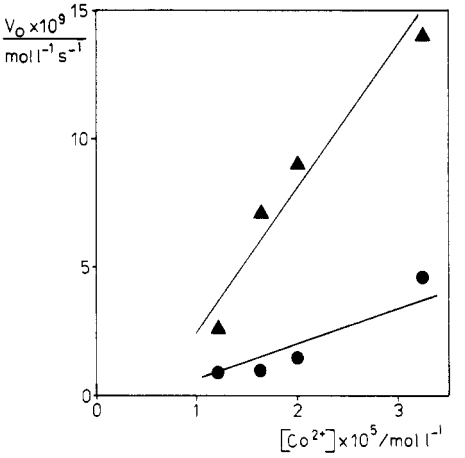
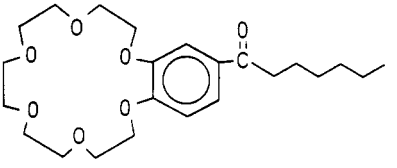


Figure 6. Co^{2+} influx as a function of the cobalt concentration: molar ratio **5b**/DHP 1.5×10^{-3} (●), 2.8×10^{-3} (▲); T = 25 °C.

Plots of the change in absorbance at 510 nm versus time are shown in Figure 5. It appears that the Co^{2+} influx rate is considerably higher if channels are present in the bilayers. At several concentrations of vesicles the rate increases with increasing number of channels per vesicle and with increasing concentration of Co^{2+} ions in the outer aqueous phase (Figure 6; see also ref. 9). In Table IV the estimated permeabilities of the DHP vesicles containing channel compounds **5** toward Co^{2+} ions are given. For comparison, the permeation constant (P) was also measured for DHP vesicles in which the low molecular weight carrier-type ionophore **7** had been incorporated. Table



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IV shows that in the presence of **7** a much lower permeability is observed. This suggests that the channel compounds do not act as carriers for Co^{2+} and that the observed effects are not due to low molecular weight fractions present in the polymer samples.

In order to maintain electroneutrality, transmembrane cation transport should be accompanied by anion transport in the same direction (anion symport) or cation transport in the opposite direction (cation antiport). Anion symport easily occurs with anions in which the charge is delocalized, e.g. SCN^-.[28] Highly charged ions such as NO_3^- cannot

Table IV
Estimated Permeabilities of DHP Vesicles toward Co^{2+} Ions in the Presence of Channel Compounds 5 and Carrier Compound 7[a]

entry	compd	$10^{11}P$ (cm/s)
1	**5b** + $Co(NO_3)_2$	56.9 ± 14
2	**5b** + $Co(SCN)_2$	69.0 ± 24
3	**5b** + $Co(NO_3)_2$ + FCCP[b]	73.6 ± 26
4	**5b** + $Co(NO_3)_2$ + MOH[c]	55–74[d]
5	**5c** + $Co(NO_3)_2$	69.4 ± 11
6	**5d** + $Co(NO_3)_2$	24.0 ± 2
7	**7** + $Co(NO_3)_2$	0.77 ± 0.2

[a] Molar ratio 5 (or 7)/DHP = 2.8×10^{-3}. [b] Proton decoupler carbonyl cyanide [4-(trifluoromethoxy)phenyl]hydrazone (1.3 mmol) was added before addition of $Co(NO_3)_2$. [c] pH adjustment was effected by addition of KOH, RbOH, or CsOH (see text). [d] Estimated error ±35%.

pass the bilayer membrane. Table IV shows that within experimental error equal permeabilities are found for $Co(NO_3)_2$ and $Co(SCN)_2$. Apparently, the anion is not involved in the ion transport process. In our system electroneutrality is most likely maintained by antiport of H^+ or Na^+. Proton antiport may sometimes be facilitated by proton uncouplers. We measured the influence of the proton uncoupler carbonyl cyanide [4-(trifluoromethoxy)-phenyl]hydrazone (FCCP)[29] and found that it had no marked effect on the transport of the Co^{2+} ions (Table IV). This result is not surprising since in our system protons and sodium ions can be expected to diffuse freely through the channels.

In a separate series of experiments we investigated the influence of the channel diameter on the cobalt ion transport rate. Table IV reveals that the permeability of the vesicles toward cobalt ions only slightly increases when the channel diameter is changed from 2.9 Å (**5b**, entry 1) to 3.8 Å (**5c** entry 5). Compound **5d**, which has a channel diameter of approximately 4.5 Å[30] displays a significantly lower transport rate (Table IV, entry 6). We attribute this to the fact that channel compound **5d** is less efficiently incorporated in DHP vesicles than **5b** and **5c** (Table II). Unfortunately, we could not test the effect of a channel diameter smaller than 2.9 Å. Compound **5a**, which has such a small diameter, cannot be incorporated into the vesicle bilayers (vide supra).

A number of experiments were performed to see whether ion transport by **5b** could be blocked by large cations, e.g. Rb^+ or Cs^+. For these experiments vesicles were prepared as described above. However, the NaOH added for pH adjustment, was replaced by RbOH or CsOH. Table IV (entry 4) shows that the effect of Rb^+ or Cs^+ is small. The reason probably is that at the applied concentrations (2.5×10^{-3} M) the ion stoppers are not sufficiently bound to the channels. Higher concentrations of these ions could not be used, because DHP vesicles cannot withstand these conditions.

The activation energy for ion transport (E_a) by **5b** was calculated from transport experiments carried out in the temperature range 25–60 °C. The result is presented in Table V. This table also includes E_a values for the natural channel compound gramicidin A and a number of natural ion carriers. These data support the idea that **5b** acts as a channel type ionophore and not as a carrier type ionophore.

Concluding Remarks

In this paper we have shown that channel type compounds can be synthesized by stacking crown ether rings in a face-to-face fashion. When incorporated into vesicle bilayers these channels display ion transport properties reminiscent of natural channel type ionophores. The channels are polydisperse polymer molecules having an average length more or less similar to the thickness of the DHP bilayer. Various arrangements of the molecules in the bilayer may occur. One molecule may completely span the membrane, or two molecules may form dimers like gramicidin does. Another possibility is that several channel molecules of short length are arranged in a partly overlapping and parallel orientation. Our data do not provide information about the exact translocation mechanism of the ions. We believe that transport via defective pores can be excluded in view of the results presented above. Cobalt ions do not easily lose their hydration shell, and as a result their complexation to a crown ether ring is relatively weak. We propose that in our system ion translocation occurs via water molecules present in the interior of the channels. A migration process in which the ion hops from a water molecule bound at a crown ether ring to a water molecule bound at an another ring along the exterior of the channels, is less probable. Such a process would require a high energy of activation (probably E_a = 60–120 kJ/mol)[31] owing to a less effective screening of the ion charge in the lipid environment. With regard to the translocation mechanism it is of interest to mention recent NMR studies by Dickert et al. on the process of ligand exchange in sandwich complexes of Co^{2+} and 12-crown-4.[32] According to these studies ligand exchange proceeds via a mechanism in which a free crown ether lands on one side of the sandwich complex forming a cofacial stack of three rings. In this stack the cobalt ion hops through the middle ring to the side where the new ring had been bound. The crown ether ring at the other side is released and a new sandwich complex is formed.

Table V
Arrhenius Activation Energy for Ion Transport by Various Ionophores

ionophore	E_a (kJ/mol)	ref
5b	24[a]	this work
gramicidin A	20.5–22.5[b]	37
valinomycin	50–90[b]	38
etheromycin	120[c]	39
passive (uncatalyzed) transport	50–125[d]	40

[a] Calculated from ion transport experiments carried out at 25–60 °C. Estimated error 4 kJ/mol. [b] Black lipid membrane of glyceryl monooleate. [c] Phosphatidylcholine vesicles. [d] Phosphatidylserine vesicles, human erythrocyte membrane.

Experimental Section

Intrinsic viscosities were obtained with a Cannon Ubbelohde viscometer in a constant-temperature bath at 30.00 °C. TLC was performed on silica (Merck, DC-Platikrolle Kieselgel 60 F 254) or alumina (Merck, DC Alufolien Alumiumoxid 60 F 254 neutral (Type E)), and detection was effected by UV and/or iodine. Column chromatography was performed on silica (Merck, Kieselgel 60, 230-400 mesh) or alumina (Janssen, aluminum oxide, neutral, 70–290 mesh).

Electron microscopy was carried out according to standard procedures[33] in the group of Prof. A. J. Verkley, University of Utrecht.

Fluorescence depolarization experiments were carried out on a home-built apparatus in the group of Prof. Y. K. Levine, University of Utrecht.[34]

Ultrafiltrations were performed with Sartorius SM 145 29 (M_w cutoff 5000) and SM 145 49 (M_w cutoff 20 000) filters.

Dynamic light-scattering measurements were performed on a home-built apparatus by Dr. C. G. de Kruif, University of Utrecht.

Tetraethylene Glycol Dichloride, Pentaethylene Glycol Dichloride, and Heptaethylene Glycol Dichloride. These compounds were prepared by treating the corresponding diols

with thionyl chloride in pyridine and toluene according to a literature procedure.[35] **Tetraethylene glycol dichloride**: yield 65%; bp 110 °C (0.05 mmHg); $n_D^{20.5}$ 1.4671; ^{1}H NMR ($CDCl_3$) δ 3.65 (s, 16 H, CH_2O). **Pentaethylene glycol dichloride**: yield 80%; bp 99 °C (0.05 mmHg); n_D^{24} 1.4618; ^{1}H NMR ($CDCl_3$) δ 3.65 (s, 20 H, CH_2O). **Hexaethylene glycol dichloride**: yield 74%; bp 150–155 °C (0.1 mmHg); $n_D^{20.5}$ 1.476; ^{1}H NMR ($CDCl_3$) δ 3.65 (s, 24 H, CH_2O). **Heptaethylene glycol dichloride**: yield 58%; bp 180 °C (0.1 mmHg); n_D^{20} 1.4665; ^{1}H NMR ($CDCl_3$) δ 3.65 (s, 28 H, CH_2O).

2,3,5,6,8,9,11,12-Octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin (1a). A mixture of 15.4 g (0.14 mol) of catechol, 200 mL of *n*-butanol, and 12 g (0.3 mol) of sodium hydroxide, dissolved in 15 mL of water, was stirred for 5 min under nitrogen and treated with 32 g (0.14 mol) of tetraethylene glycol dichloride. The mixture was refluxed with stirring for 40 h. The reaction mixture was cooled to room temperature, acidified with 4 mL of concentrated hydrochloric acid and filtered. The residue was washed with methanol. The filtrate and the washings were combined and evaporated to dryness. The crude product was extracted repeatedly with petroleum ether 80–110. After evaporation a white solid was obtained which subsequently was recrystallized from *n*-hexane: yield 19.5 g (52%); IR (KBr) 1250 and 1130 (ether) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.6–4.3 (m, 16 H, CH_2O), 6.90 (s, 3 H, ArH).

2,3,5,6,8,9,11,12-Octahydro-1,4,7,10,13-(4′-acetylbenzopentaoxacyclopentadecin (2a). To a mixture of 60 g of polyphosphoric acid and 9.0 g (0.09 mol) of acetic anhydride, 15.3 g (0.056 mol) of **1a** was added. After stirring for a short time at 60 °C the color changed from colorless to red. Stirring and heating were continued for 5 h. The reaction mixture was diluted with water and extracted three times with chloroform. The combined chloroform layers were washed with a dilute aqueous solution of Na_2CO_3 and with water, dried (Na_2SO_4), filtered, and evaporated. The residual red oil was extracted repeatedly with petroleum ether 80–110. After evaporation a white solid was obtained which subsequently was recrystallized from *n*-hexane: yield 14.2 g (0.05 mol; 80%); IR (neat) 1670 (CO), 1250 and 1130 (ether) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.5 (s, 3 H, CH_3), 3.6–4.3 (m, 16 H, CH_2O), 6.90 (d, 1 H, ArH), 7.55 (m, 2 H, ArH).

2,3,5,6,8,9,11,12-Octahydro-1,4,7,10,13-[4′-(1-(*N*-formylamino)ethyl)benzo]pentaoxacyclopentadecin (3a). Compound **2a** (14.2 g, 0.05 mol) and ammonium formate (≈42 g, 0.66 mol) were heated in a nitrogen atmosphere for 7 h. During this period the temperature was slowly raised to 190 °C while water and ammonium carbonate were distilled off. After cooling to room temperature the mixture was diluted with water and chloroform. The organic layer was separated, and the aqueous layer was extracted twice with chloroform. The combined chloroform layers were washed with water, dried (Na_2SO_4, $MgSO_4$), filtered, and concentrated. The resulting orange-red oil was purified by column chromatography using a short column of neutral alumina (activity grade II; eluent ethyl acetate–diethyl ether, 9:1, v/v) and recrystallization from ethyl acetate–diethyl ether: yield 14.1 g (0.04 mol; 88%); IR (KBr) 1675 (CO), 1250 and 1130 (ether) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (d, 3 H, CH_3), 3.7–4.1 (m, 16 H, CH_2O), 5.10 (m, 1 H, CH), 6.80 (m, 3 H, ArH), 7.0 (b, 1 H, NH), 8.13 (s, 1 H, CHO).

2,3,5,6,8,9,11,12-Octahydro-1,4,7,10,13-[4′-(1-isocyanoethyl)benzo]pentaoxacyclopentadecin (4a). Phosphorus oxychloride (7.8 g, 0.05 mol) dissolved in 15 mL of dichloromethane was added dropwise over a 1-h period to a solution of **3a** (14.1 g, 0.04 mol) and triethylamine (12.6 g, 0.13 mol) in dichloromethane, which was kept at 0 °C. Subsequently, the temperature was slowly raised (0.5 h) to room temperature. An aqueous solution of Na_2CO_3 was added and the organic layer was separated, dried (Na_2SO_4, $MgSO_4$), and concentrated under vacuum. The product was purified by column chromatography over neutral alumina (activity grade II; eluent ethyl acetate–diethyl ether, 1:1, v/v)): yield 8.6 g (0.03 mol; 66%) of an almost colorless oil; IR (neat) 2138 (NC), 1260 and 1130 (ether) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.62 (m, 3 H, CH_3), 3.65–4.15 (m, 16 H, CH_2O), 4.74 (q, 1 H, CH), 6.90 (s, 3 H, ArH); MS *m/e* 321 (M^+).

Poly[2,3,5,6,8,9,11,12-Octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-4′-ylethyliminomethylene] (5a). Compound **4a** (0.15 g, 0.47 mmol) and $NiCl_2 \cdot 6H_2O$ (1.5 mg, 0.005 mmol) were dissolved in dichloromethane. The solvent was evaporated and the residual mixture heated neat at 60 °C for 3 days. The reddish glassy polymerization product was dissolved in chloroform. The latter solution was washed with water, dried (Na_2SO_4), concentrated to a smaller volume, and added dropwise to a well-stirred mixture of diethyl ether–hexane (1:1, v/v). The flocky precipitate was filtered out, washed with ether–hexane (1:1, v/v), and dried to give pale yellow **5a**: yield 0.1 g (65%); [η] (0.04 dL/g) (chloroform, 30.00 °C); IR 1625 (NC), 1130 and 1260 (ether) cm^{-1}. Anal. Calcd for $C_{17}H_{23}NO_5 \cdot {}^1/_3H_2O$: C, 62.37; H, 7.29; N, 4.28; O, 26.06. Found: C, 62.28; H, 7.28; N, 4.39; O, 26.23.

2,3,5,6,8,9,11,12,14,15-Decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin (1b). This compound was prepared as described for **1a**: yield 43% of a white solid; IR (KBr) 1250 and 1130 (ether) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.6–4.3 (m, 20 H, CH_2O), 6.90 (s, 3 H, ArH).

2,3,5,6,8,9,11,12,14,15-Decahydro-1,4,7,10,13,16-(4′-acetylbenzo)hexaoxacyclooctadecin (2b). This compound was prepared from **1b** as described for **2a**: yield 81% of a white solid; mp 68–73 °C; IR (neat) 1670 (CO), 1250 and 1130 (ether) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.5 (s, 3 H, CH_3), 3.6–4.3 (m, 20 H, CH_2O), 6.90 (d, 1 H, ArH), 7.55 (m, 2 H, ArH).

2,3,5,6,8,9,11,12,14,15-Decahydro-1,4,7,10,13,16-[4′-(1-(*N*-formylamino)ethyl)benzo]hexaoxacyclooctadecin (3b). This compound was prepared from **2b** as described for **3a**: yield 88% of a white solid; IR (KBr) 1675 (CO), 1250 and 1130 (ether) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (d, 3 H, CH_3), 3.67–4.10 (m, 20 H, CH_2O), 5.10 (m, 1 H, CH), 6.80 (m, 3 H, ArH), 7.0 (b, 1 H, NH), 8.13 (s, 1 H, CHO).

2,3,5,6,8,9,11,12,14,15-Decahydro-1,4,7,10,13,16-[4′-(1-isocyanoethyl)benzo]hexaoxacyclooctadecin (4b). This compound was prepared from **3b** as described for **4a**: yield 80% of an almost colorless oil; IR (neat) 2138 (NC), 1260 and 1130 (ether) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.62 (m, 3 H, CH_3), 3.65–4.15 (m, 20 H, CH_2O), 4.74 (q, 1 H, CH), 6.90 (s, 3 H, ArH); MS *m/e* 365 (M^+).

Poly[2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin-4′-ylethyliminomethylene] (5b). This compound was prepared from **4b** as described for **5a**: yield 0.39 g (78%) of a pale yellow solid; [η] 0.03 (toluene, 30.00 °C); M_n 3670 (VPO, water, 50.6 °C), 4340 (VPO, toluene, 54.6 °C); IR (KBr) 1625 (NC), 1260 and 1130 (ether) cm^{-1}. Anal. Calcd for $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83; O, 26.27. Found: C, 62.13; H, 7.19; N, 3.96; O, 26.72.

2,3,5,6,8,9,11,12,14,15,17,18-Dodecahydro-1,4,7,10,13,16,19-benzoheptaoxacycloheneicosin (1c). This compound was prepared as described for **1a**: yield 44% of a colorless oil; IR (neat) 1250 and 1130 (ether) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.6–4.3 (m, 24 H, CH_2O), 6.90 (s, 3 H, ArH).

2,3,5,6,8,9,11,12,14,15,17,18-Dodecahydro-1,4,7,10,13,16,19-(4′-acetylbenzo)heptaoxacycloheneicosin (2c). This compound was prepared from **1c** as described for **2a**: yield 79% of a white solid; mp 53 °C; ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3 H, CH_3), 3.55–4.35 (m, 24 H, CH_2O), 6.87 (d, 1 H, ArH), 7.58 (m, 2 H, ArH); MS *m/e* 398 (M^+).

2,3,5,6,8,9,11,12,14,15,17,18-Dodecahydro-1,4,7,10,13,16,19-[4′-(1-(*N*-formylamino)ethyl)benzo]heptaoxacycloheneicosin (3c). This compound was prepared form **2c** as described for **3a** with the exception that the chromatographic purification was performed with ethyl acetate as the eluent: yield 74% of a colorless oil; IR (neat) 1680 (CO), 1260 and 1130 (ether) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.53 (d, 3 H, CH_3), 1.60–4.25 (m, 24 H, CH_2), 5.10 (m, 1 H, CH), 6.7 (b, 1 H, NH), 6.85 (m, 3 H, ArH), 8.15 (s, 1 H, CHO); MS *m/e* 427 (M^+).

2,3,5,6,8,9,11,12,14,15,17,18-Dodecahydro-1,4,7,10,13,16,19-[4′-(1-isocyanoethyl)benzo]heptaoxacycloheneicosin (4c). This compound was prepared from **3c** as described for **4a** with the exception that the chromatographic purification was performed with ethyl acetate as the eluent: yield 83% of a colorless oil; IR (neat) 2140 (NC), 1260 and 1130 (ether) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.65 (d, 3 H, CH_3), 3.68–4.35 (m, 24 H, CH_2O), 4.80 (q, 1 H, CH), 6.92 (s, 3 H, ArH); MS *m/e* 409 (M^+).

Poly[2,3,5,6,8,9,11,12,14,15,17,18-dodecahydro-1,4,7,10,13,16,19-benzoheptaoxacycloheneicosin-4′-ylethyliminomethylene] (5c). This compound was prepared form **4c** as described for **5a**: yield 60% of an orange-red flocky solid; [η]

Table VI
Association Constants (M^{-1}) and Free Energies of Complexation (kJ/mol) of Crown Ether–Cation Complexes

	b15c5		pb15c5		b18c6		pb18c6		copolymer 6		b21c7		pb21c7		b24c8		pb24c8	
	$10^{-5}K_a$	$-\Delta G°$	$10^{-5}K_a$	$-\Delta G°$	$10^{-5}K_a$	$-\Delta G°$	$10^{-5}K_a$	$-\Delta G°$	$10^{-5}K_a$	$-\Delta G°$	$10^{-5}K_a$	$-\Delta G°$	$10^{-5}K_a$	$-\Delta G°$	$10^{-5}K_a$	$-\Delta G°$	$10^{-5}K_a$	$-\Delta G°$
Li^+	2.2	30.8	4.0	32.4	0.5	27.2	0.8	28.3			0.3	25.9	0.45	26.9	0.3	25.9	0.5	27.2
Na^+	14.5	35.2	21.4	36.1	6.5	33.2	43.7	37.9	12.5	34.8	4.5	32.2	6.1	33.0	2.6	30.9	5.7	32.8
K^+	6.6	33.2	24.9	36.5	231	42.0	608	44.4	392	43.4	18.0	35.7	38.0	37.6	5.8	32.9	8.9	34.0
Rb^+	1.4	29.4	15.2	35.3	62.6	38.8	172	41.3	90.8	39.7	37.6	37.5	95.1	39.8	8.0	33.7	14.6	35.2
Cs^+	0.4	26.3	18.4	35.8	15.5	35.3	162	41.1	33.4	37.2	41.1	37.7	124	40.5	14.5	35.2	25.0	36.5
NH_4^+	0.3	25.7	5.1	32.6	40.3	37.7	202	41.7			16.6	35.5	19.3	35.9	3.5	31.7	6.3	33.1

0.05 (toluene, 30.00 °C); IR (neat) 1630 (NC), 1260 and 1130 (CO) cm^{-1}. Anal. Calcd for $C_{21}H_{31}NO_7 \cdot {}^1/_2H_2O$: C, 60.27; H, 7.70; N, 3.35; O, 28.68. Found: C, 60.11; H, 7.77; N, 3.38; O, 28.50.

2,3,5,6,8,9,11,12,14,15,17,18,20,21-Tetradecahydro-1,4,7,10,13,16,19,21-benzooctaoxacyclotetraeicosin (1d). This compound was prepared as described for **1a**: yield 40% of a colorless oil; IR 1250 and 1130 (ether) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.6–4.3 (m, 28 H, CH_2O), 6.90 (s, 3 H, ArH).

2,3,5,6,8,9,11,12,14,15,17,18,20,21-Tetradecahydro-1,4,7,10,13,16,19,21-(4′-acetylbenzo)octaoxacyclotetraeicosin (2d). This compounds was prepared from **1d** as described for **2a**: yield 67% of a colorless oil; ^1H NMR ($CDCl_3$) δ 2.60 (s, 3 H, CH_3), 3.60–4.45 (m, 28 H, CH_2O), 6.95 (d, 1 H, ArH), 7.63 (m, 2 H, ArH); MS *m/e* 442 (M^+).

2,3,5,6,8,9,11,12,14,15,17,18,20,21-Tetradecahydro-1,4,7,10,13,16,19,21-[4′-(1-(*N*-formylamino)ethyl)benzo]octaoxacyclotetraeicosin (3d). This compound was prepared from **2d** as described for **3a** with the exception that the chromatographic purification was performed with ethyl acetate as the eluent: yield ≈80% of an oil; ^1H NMR ($CDCl_3$) δ 1.35 (d, 3 H, CH_3), 3.45–4.25 (m, 28 H, CH_2O), 5.13 (q, 1 H, CH), 6.70 (b, 1 H, NH), 6.83 (s, 3 H, ArH), 8.10 (s, 1 H, CHO); MS *m/e* 472 (M^+).

2,3,5,6,8,9,11,12,14,15,17,18,20,21-Tetradecahydro-1,4,7,10,13,16,19,21-[4′-(1-isocyanoethyl)benzo]octaoxacyclotetraeicosin (4d). This compound was prepared from **3d** as described for **4a** with the exception that the chromatographic purification was performed with ethyl acetate as the eluent: yield 60% of a colorless oil; IR (neat) 2140 (NC), 1260 and 1130 (ether) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.61 (d, 3 H, CH_3), 3.60–4.27 (m, 28 H, CH_2O), 4.73 (q, 1 H, CH), 6.90 (s, 3 H, ArH); MS *m/e* 453 (M^+).

Poly[2,3,5,6,8,9,11,12,14,15,17,18,20,21-tetradecahydro-1,4,7,10,13,16,19,21-benzooctaoxacyclotetraeicosin-4′-ylethyliminomethylene] (5d). This compound was prepared from **4d** as described for **5a**: yield 60% of a red-brown oil; [η] 0.04 (toluene, 30.00 °C); IR (neat) 1630 (NC), 1260 and 1120 (ether) cm^{-1}. Anal. Calcd for $C_{23}H_{35}NO_8 \cdot {}^3/_4H_2O$: C, 59.15; H, 7.88; N, 3.00; O, 29.98. Found: C, 59.29; H, 7.90; N, 2.82; O, 29.98.

Copolymer of 4b and α-Phenylethyl Isocyanide (6). To a mixture of compound **4b** (0.4 g, 1 mmol) and α-phenylethyl isocyanide (0.4 g, 3 mmol) a small amount (±1 mol %) of $NiCl_2 \cdot 6H_2O$ was added. After 5 days at room temperature the reaction mixture was worked up as described for **5a**. After the precipitation step the product was washed with methanol to remove any homopolymer **5b** formed: yield ≈0.6 g (75%) of a pale yellow solid: IR 1625 (NC), 1260 and 1120 (ether) cm^{-1}. Anal. Calcd for $C_{55.9}H_{64.0}N_{5.1}O_{6.0}$: C, 74.37; H, 7.09; N, 7.91; O, 10.64. Found: C, 74.36; H, 7.15; N, 7.86; O, 10.63.

2,3,5,6,8,9,11,12,14,15-Decahydro(4′-heptanoylbenzo)-1,4,7,10,13,16-hexaoxacyclooctadecin (7). This compound was prepared from **1b** and heptanoic anhydride as described for **2a**: yield 76% of a semisolid; IR (neat) 1680 (CO), 1250 and 1130 (ether) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (t, 3 H, CH_3), 1.4 (m, 8 H, CH_2), 2.8 (t, 2 H, CH_2CO), 3.5–4.3 (m, 20 H, CH_2O), 6.9 (d, 1 H, ArH), 7.5 (m, 2 H, ArH).

Determination of K_a and $\Delta G°$ Values. The K_a values were determined by the picrate salt extraction technique from H_2O into $CHCl_3$ described by Cram et al.[19] In a typical experiment an aqueous 0.015 M metal picrate solution was extracted with a 0.015 M solution of a crown ether in chloroform (concentration based on monomer molecular weight). The picrate concentrations of the organic and of the aqueous layer were measured spectrophotometrically. The equations for calculating the K_a and $\Delta G°$ values have been published.[19] The results are presented in Table VI.

Saturation Experiments. Method 1. Aqueous picrate solutions (0.015 M) were extracted with equal volumes of 0.0015–0.015 M solutions of **5a–d** in chloroform (concentrations based on monomer molecular weight).

Method 2. Solutions of **5a–d** in chloroform (0.005 M) were shaken with solid picrates for 1.5 h using a Griffin flask shaker. The picrate concentrations were measured spectrophotometrically. The solubilities of the uncomplexed picrates in chloform were negligible, as was checked separately.

Experiments with Dihexadecyl Phosphate Vesicles. Preparation of Vesicles with and without Channels. Vesicles of dihexadecyl phosphate (Sigma) were prepared by sonication at 80 °C using a microtip of a Branson Sonifier B-12 set at 75 W. In a typical experiment 27.0 mg (0.05 mmol) of DHP was added to 10 mL of preheated distilled water containing 0.0–2.1 mg of **5a–d**. In all experiments, except for the light-scattering experiments, 0.25 mL of 0.100 M NaOH was added just before sonication. Sonication was continued for 45 min. After cooling to room temperature the dispersion was centrifuged at 8000 rpm for 15 min to remove titanium particles originating from the tip.

Incorporation of Channels: Ultrafiltration Experiments. Vesicles prepared as described above were dialysed by ultrafiltration (Amicon Diaflo YM 100 (M_w 1 000 000 cutoff). UV–vis spectra of the residue and the filtrate were recorded. In control experiments aqueous solutions of **5a–d** were dialysed in the same way.

Light-Scattering Measurements. At several sonication times, samples were taken from aqueous DHP dispersions (0.73 mM) containing 0.0–2.1 mg of compounds **5b–d**. The samples were filtered through 0.6-μm Millipore filters before light-scattering measurements were performed. The same samples were also used in the turbidity measurements. To this end the absorption of the samples was measured at various wavelengths between 300 and 650 nm. A similar procedure was followed for the vesicle systems without channels.

Entrapment of Pyranine. DHP vesicles were prepared by dispersing DHP in 5.0 mL of water to which 0.0–2.1 mg of **5b–d** and 0.25 mL of 0.100 M NaOH had been added. After 5 min sonication 5.0 mL of a 10^{-3} M aqueous solution of pyranine was added and sonication was continued for another 40 min. The procedure and further conditions were the same as described above. The external pyranine was removed by gel filtration (Sephadex G 25 fine; column dimensions 1.5 × 20 cm; eluent water). The residual fluorescence in the vesicles was measured (λ_{ex} = 400 nm, λ_{em} = 510 nm). The same procedure was followed for the systems without channels.

Co^{2+} Influx Measurements. DHP vesicles containing channels were obtained by dispersing DHP in 5.0 mL of water to which 0.0–2.1 mg of **5b–d** and 0.25 mL of 0.100 M NaOH had been added. After 5 min of sonication 5.0 mL of a 5.5 × 10^{-3} M aqueous solution of PAR was added and sonication was continued for another 40 min. The procedure and further conditions were the same as described above. The exovesicular PAR was removed by ultrafiltration (Amicon Diaflo YM 100; 5 × 40 mL of water). To 0.7-mL aliquots of vesicles thus obtained was added (7.5–15.0) × 10^{-3} mL of an aqueous 1.17 × 10^{-3} M $Co(NO_3)_2$ solution. The increase in absorbance at 510 nm was recorded. To determine the final value of $A(510)$ the vesicles were destroyed after 15–30 min by addition of 0.010 mL of a 10% aqueous solution of Triton X-100. A similar procedure was followed for the systems without channels. The observed rates followed pseudo-first-order ki-

netics. Permeation coefficients were calculated by using a mean vesicle diameter of 45 nm.[36]

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