A tris(macrocycle) that exhibits H-bond-induced blockage of the cation channel function in a phospholipid bilayer

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An indole-terminated tris(macrocyclic) compound, designed to be a channel-former, is an effective carrier in bulk membranes but fails to function in a lipid bilayer owing to hydrogen bond formation which blocks the channel.

Because of the overwhelming importance of cation channels to biology, the study of cation-conducting, transmembrane proteins is receiving enormous attention from the biochemical, biophysical and molecular biological communities.[1] In addition, a number of chemical research groups have designed, synthesized and studied model channel systems. Our cation channel model system involves crown ethers that function as head groups and cation entry points. The headgroups are connected *via* a spacer to a 'relay' unit that is midway between the two.[2]

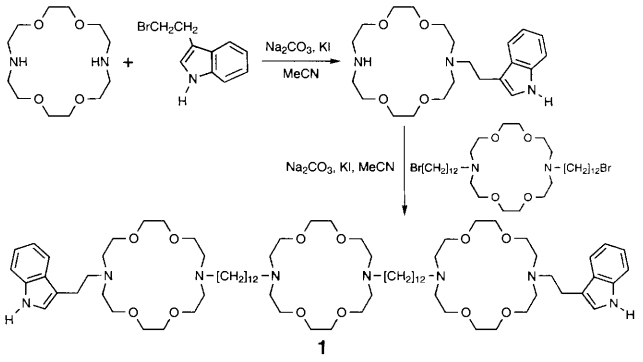
Expanding evidence indicates that the distal crowns in our tris(macrocyclic) channel formers serve both as headgroups and as cation entry points. Crown ethers are now known to function as headgroups for the formation of micelles[3] and stable vesicles[4] and are assumed in our cases of functional channel-formers to be near the surface of the bilayer. In previous studies, we have demonstrated transport functionality for both protons and Na^+.[2] We speculated that certain amino acids may function in transmembrane proteins to stabilize the position of the multihelix pore with respect to the bilayer. A candidate for this stabilization was indole, the sidechain of tryptophan. Cross and co-workers showed by oriented NMR experiments that the multiple tryptophan residues of gramicidin are directed to the aqueous–bilayer interface.[5] We demonstrated that *N*-alkylindoles could form stable vesicles[6] even though (i) the headgroups are not charged, (ii) the alkyl chain is attached at the H-bonding nitrogen atom, and (iii) only a single alkyl chain is present in the amphiphile. In an effort to explore this potentially stabilizing headgroup effect, we prepared a tris(macrocycle) terminated at each end by indol-3-yl groups, $InCH_2CH_2<N18N>C_{12}<N18N>C_{12}<N18N>CH_2CH_2In$,[8] **1**. The behaviour of this compound is compared and contrasted with that of $PhCH_2<N18N>C_{12}<N18N>C_{12}<N18N>CH_2Ph$, **2**.

Compound **1** was prepared by the approach illustrated in Scheme 1. 4-*N*-[2-(Indol-3-yl)ethyl]-4,13-diaza-18-crown-6 was obtained (38%) by monoalkylation of diaza-18-crown-6 (Na_2CO_3, KI, MeCN). Alkylation of the monosubstituted crown by using previously reported[2] *N,N'*-bis(12-bromododecyl)-4,13-diaza-18-crown-6 (Na_2CO_3, KI, MeCN) afforded the channel, (**1**, 19%) as an oil that was purified by repeated chromatography over silica.‡ The analogous benzyl channel, **2**, has been previously described.[2]

The channel function of compounds **1** and **2** was assessed in a phosphatidylcholine–phosphatidylglycerol vesicle system using a dynamic NMR method developed by Riddell and Hayer.[8] The results of our NMR experiments are always correlated directly to those obtained simultaneously for gramicidin and normalized to a relative rate of 1.00.[2] A number of channel formers having the general structure $R<N18N>C_{12}<N18N>C_{12}<N18N>R$ exhibited transport rates in the range 0.15–0.45 on this scale.[2] The limit of detection in these experiments is approximately 2% of the transport rate of gramicidin. Benzyl channel **2** was found to have a Na^+ transport rate relative to gramicidin of 0.39. Remarkably, indolyl channel **1** showed no detectable Na^+ flux under comparable experimental conditions.

An explanation for this difference was sought in the unique properties of indole. The heterocycle indole is known to organize solvent about the pyrrole ring, often using N–H as a H-bond donor.[9] It was originally anticipated that this property would stabilize the tris(macrocycle) within the membrane. Alternately, the indole N–H could hydrogen-bond internally (transannularly to a macroring oxygen or nitrogen atom) and block the orifice created by the diazacrown. Molecular models suggested clearly that a snug, intramolecular H-bond could readily form between the indole NH and the second macroring oxygen atom. Hydrogen bond formation appears from CPK models to hold the indole in a rigid, perpendicular relationship to the plane of the macroring. This H-bonding possibility was further explored by subjecting the headgroup fragment of **1**, *i.e.* $InCH_2CH_2<N18N>[CH_2]_5Me$, to a Monte Carlo (conformational) search routine using the molecular modeling program MacroModel.[10] Calculations were also done for the corresponding headgroup fragment $PhCH_2<N18N>[CH_2]_5Me$ of the benzyl analogue of **1**, $PhCH_2<N18N>C_{12}<N18N>C_{12}<N18N>CH_2Ph$, **2**. The Monte Carlo calculations afforded numerous low energy structures. The 10 lowest energy structures (all within 1 kcal of the global minimum§) for **1** and **2** are overlaid in Fig. 1(*a,b*).

It is obvious from the calculated structures, that all low energy conformers of **1** are transannularly H-bonded, as anticipated from the CPK models, effectively closing the molecular orifice. In contrast, the *N*-benzyl group of **2** is turned away from the opening in all equivalent conformers. We have previously demonstrated[11] that when the benzyl group of **2** is substituted in the 4-position by either a nitro or a methoxy group, Na^+ transport in the bilayer is, respectively, slower and faster. Indeed, the transport rates correlate ($r > 0.96$) with the Hammett σ^0 constant for the substituent. Thus, the lack of activity of electron-rich, indole-substituted **1** would be



1

Scheme 1

extremely unusual were it not for the possibility of physical closure of the cation's entry point.

The presence of an intramolecular hydrogen bond was confirmed experimentally by examining the IR spectrum of **1** in CCl_4 at concentrations ranging from 10 to 0.1 mmol dm^{-3}. The only vibration apparent in the 3000–2000 cm^{-1} range was a sharp doublet observed at 2927 ± 1 and 2855 ± 1 cm^{-1} which was not altered either in position or shape by the 100-fold concentration change. In contrast, the IR spectrum of 3-(2-*N*,*N*-dimethylamino)indole exhibits a single, broad band in the range *ca.* 3000–2850 cm^{-1}.[12] Note that for the channel studies, **1** is dissolved in trifluoroethanol and then added to an aqueous vesicle suspension. These conditions suggest that the H-bonded form of **1** is quite stable.

The indolyl residue is known to be fluorescent. Indeed, the Stokes shift of the indolyl sidechain of tryptophan has often been used to assess the position of this residue in proteins embedded in a bilayer membrane.[13] The fluorescence emission spectrum of **1** was thus determined in various solvents and in a phospholipid bilayer. The fluorescence maximum ranged from 330 nm in hexane to *ca.* 345 in ethanol. A plot of the solvent dependence of the emission maximum *vs.* E_T gave a straight line relationship (r = 0.96).[14] The fluorescence maximum observed for **1** in phospholipid vesicles was *ca.* 334 nm. Thus, the environment of the indolyl residues is nonpolar rather than similar to ethanol as would be expected if this residue served as the headgroup. This suggests that the 'channel' is not in a fully extended conformation but in what might be called a 'globular' arrangement. It is interesting to note that 3-methylindole undergoes solvent-induced Stokes shifts of *ca.* 40 nm from cyclohexane to butanol.[9b] The corresponding change for **1** is about half of that (*ca.* 20 nm), which suggests that the indolyl residue is confined to an environment that is less readily solvent accessible [see Fig. 1(*a*)].

The combined evidence, (i) carrier rather than channel function, (ii) calculations showing a 'closed' conformation, (iii) IR evidence confirming the presence of an intramolecular hydrogen bond and (iv) fluorescence data confirming a nonpolar environment for the 'headgroup' demonstrates that the function of channel model **1** can be understood in structural terms. The ability of **1** to serve as a carrier in a bulk chloroform membrane clearly differentiates the carrier and channel mechanisms for this family of tris(macrocyclic) structures. Taken together, the observations described above show that **1** is embedded in the bilayer. The indolyl residues are in a nonpolar environment suggesting that they are deeply embedded rather than serving as headgroups. The efficacy of carrier, and lack of channel, function correlate with this observation. Molecular models and calculations support the notion that entry of the cation is blocked by formation of a transannular hydrogen bond between the indole NH and a macrocycle heteroatom. These findings buttress the presumption that the distal macrorings of **1** must serve as headgroups through which the cations enter and exit.

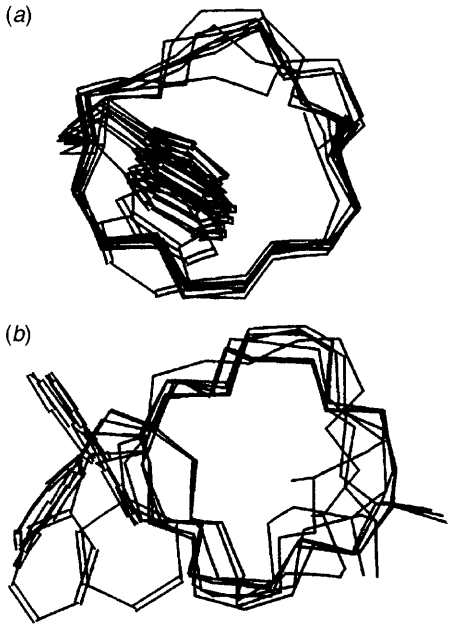


Fig. 1 Monte Carlo search of low energy conformations of (*a*) **1** and (*b*) **2**

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Footnotes

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‡ *Selected data* for **1** ^1H NMR ($CDCl_3$): 1.245, 1.45–1.47 (s, 42 H), 2.51–2.52 (m, 8 H), 2.80–2.81, 2.81–2.90 (s, m, 32 H), 3.60–3.61 (m, 48 H), 7.06–7.2 (m, 6 H), 7.3 (d, 2 H), 7.56–7.60 (d, 2 H) and 8.68 (br s, 2 H); ^{13}C NMR: 27.086, 23.023, 26.855, 27.477, 29.587, 53.837, 54.032, 54.200, 55.935, 56.142, 56.297, 69.853, 70.682, 111.103, 114.0, 118.548, 118.846, 121.474, 122.510, 126.7 and 136.2. Electrospray mass spectrum: $(M + 1)^+$ 1407.00, $(M + 2)^{2+}$ 704.38, $(M + 3)^{3+}$ 470.13. High resolution FAB mass spectrum: calculated for $C_{80}H_{140}O_{12}N_8$: 1406.0669. Found: 1406.0669, 1406.0610.

§ The conformational search program produced the structure shown here as the minimum after five separate search cycles.

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