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Cation flux dependence on carbon chain length in hydraphile channels as assessed by dynamic ^{23}Na NMR methods in phospholipid bilayers

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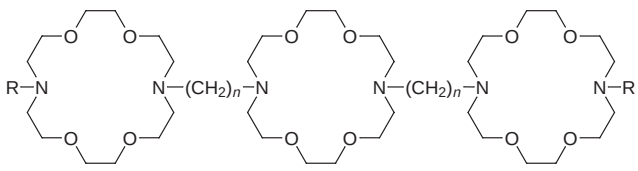
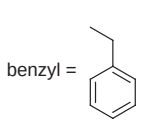
The length dependence for the family of tris(macrocycle) channels has been defined by a combination of synthetic and kinetic studies conducted in phospholipid bilayers.

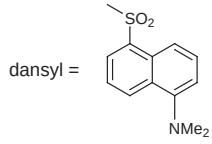
Two modes of cation transport are known to occur *in vivo*: they are designated carrier and channel mechanisms. Both kinds of transport have been studied extensively. The dynamics of carrier transport are generally understood for the limited natural carriers and numerous synthetic ionophores.[1] In the channel mechanism, extensive biophysical and molecular biological studies have defined structural, functional, and selectivity aspects of naturally occurring protein channels.[2] The recent crystal structure of the potassium-selective transmembrane shaker channel has given a view of how the transmembrane helices are positioned within the phospholipid bilayer but mechanistic details in this and other transmembrane proteins remain elusive.[3] The complexity of natural channel compounds has fostered the invention and study of a variety of synthetic model systems that have proved to be more or less successful as ionophores.[4]

We have developed a family of synthetic, transmembrane ionophores based upon crown ethers which have been proved to transport sodium.[5] These compounds consist of three macrocycles connected by hydrocarbon spacers and are terminated by flexible sidechains. We call these compounds 'hydraphiles' in reference to the monster slain by Hercules that had two heads on each neck.[6] The general structure is shown below.



1 *n* = 8, R = benzyl
2 *n* = 10, R = benzyl
3 *n* = 12, R = benzyl
4 *n* = 14, R = benzyl
5 *n* = 16, R = benzyl
6 *n* = 12, R = dansyl





Several of these macrocycle-based channel compounds have been shown by fluorescence methods to transport protons and by dynamic NMR methods to transport alkali metal cations.[5] Considerable evidence has accumulated on the function of these compounds. (1) The central macrocycle is beneficial but not essential for cation transport in the cases where that issue was studied and is probably oriented along the lipid axis in the bilayer. (2) The ionophoretic activity of hydraphiles cannot be explained either by a simple carrier mechanism or by unadorned detergent action.[7] Specifically, neither bis(benzyl)diaza-18-crown-6 nor bis(dodecyl)diaza-18-crown-6 shows any measurable transport in the ^{23}Na NMR experiment. (3) Transport rates generally show a comprehensible structure–activity relationship[5] and follow the Hammett principle.[8] (4) The channel can be blocked by the presence of a hydrogen bond donor attached to the distal macrocycles.[9] In the present work, we wished to gain insight into the structural requirement for spanning the phospholipid membrane's 'hydrocarbon slab.' This insulating portion of the larger bilayer is 30–34 Å across as judged from work reported by Wiener and White[10] and the shaker potassium channel crystal structure.[11] We thus prepared five tris(macrocyclic) hydraphiles that are identical except for the lengths of the spacer chains connecting the central macrocycle to its distal counterpart. It was anticipated that the synthetic channel compounds would show a higher level of cation flux when the length was optimal than when the spacer chains were either too long or too short. In the latter case, a complete shutdown of cation transport was anticipated if the ionophore could not span the insulating regime of the membrane. If the synthetic channel functioned by a carrier mechanism, such a cut-off would not be expected.

Macrocycles **1**–**5** were prepared by a three-step sequence.[12] First, monobenzyldiaza-18-crown-6 ($PhCH_2 < N18N > H$) was prepared either by benzylation of diaza-18-crown-6 or by partial hydrogenolysis of dibenzyldiaza-18-crown-6.[13] Alkylation of $PhCH_2 < N18N > H$ with excess $Br(CH_2)_nBr$ afforded $PhCH_2 < N18N > (CH_2)_nBr$ which was then allowed to react with $H < N18N > H$.[14] Compound **6** was prepared in an analogous fashion except that $H < N18N > H$ was monoalkylated with dansyl chloride rather than benzyl bromide.

Sodium cation flux was measured by using the ^{23}Na NMR-based method of Riddell and co-workers.[15] This technique permits quantitative evaluation of Na^+ transport. The observed rates may be compared with each other and with a standard. Vesicles for the ^{23}Na NMR studies were prepared from phosphatidylcholine (0.14 mmol) and phosphatidylglycerol (0.037 mmol, 4 : 1 w/w). The total Na^+ concentration was adjusted to 100 mM by addition of NaCl. The solutions were buffered using a phosphate buffer held at pH 7.3. The vesicles were prepared by a procedure similar to that described by Papahadjopoulos and Szoka.[16] The preparation used here afforded vesicles having an average diameter of 1750–2000 Å. The total aqueous encapsulation volume in this preparation was 3% as judged by ^{23}Na NMR spectroscopy.

The shift reagent was prepared according to the procedure of Gupta and Gupta[17] from sodium tripolyphosphate and Dy^{3+}. The ^{23}Na NMR chemical shifts were measured as differences between the resonance position in the presence and absence of the Dy^{3+} shift reagent. Compound **6** (standard) or **1**–**5** was incorporated into the vesicles as a CF_3CH_2OH solution ([**1**–**6**] = 5–20 μM). After addition of the subject compound, the samples were agitated and warmed (50–60 °C) for 1 h, cooled to room temperature, and then diluted with D_2O (lock signal) and shift reagent solution. Each solution was allowed to equilibrate for ~1 h before data acquisition. Typically, 240 FID transients were accumulated per data set at 25 °C. The rate results, relative to those determined for **6** (simultaneous with each sample) are recorded in Table 1 and shown graphically in Fig. 1.

The NMR experiment is somewhat complex and we have therefore chosen to normalize the data relative to a simultaneously determined standard (**6**). Transport rates for **6** have

Table 1 Sodium cation transport by tris(macrocycle) ionophores[a]

Cpd.	Structure	Rel. rate[a]
1	$PhCH_2 < N18N > C_8 < N18N > C_8 < N18N > CH_2Ph$	< 2
2	$PhCH_2 < N18N > C_{10} < N18N > C_{10} < N18N > CH_2Ph$	96
3	$PhCH_2 < N18N > C_{12} < N18N > C_{12} < N18N > CH_2Ph$	211[b]
4	$PhCH_2 < N18N > C_{14} < N18N > C_{14} < N18N > CH_2Ph$	201
5	$PhCH_2 < N18N > C_{16} < N18N > C_{16} < N18N > CH_2Ph$	109
6	$Dn < N18N > C_{12} < N18N > C_{12} < N18N > Dn$	100[c]

[a] Rate relative to compound **6**, arbitrarily set at 100. Comparative rates are recorded for 10 μM ionophore concentration. [b] The Na^+ transport rate determined relative to gramicidin (= 100) was 39.[18] [c] The Na^+ transport rate determined relative to gramicidin (= 100) was 23.[19]

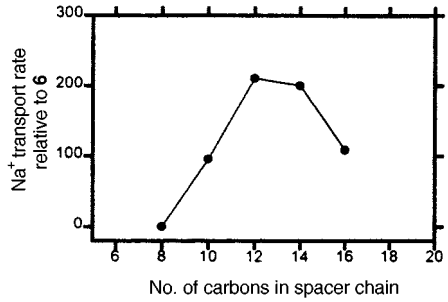


Fig. 1 Na^+ transport *vs.* spacer chain length.

been determined independently more than 10 times and each of the values shown in Table 1 for **1**–**5** represents at least three independent experiments.

Hydraphiles **3** and **6** are both of the form $R < N18N > C_{12} < N18N > C_{12} < N18N > R$ in which R is either benzyl (**3**) or dansyl (**6**). In previous work we noted that the rates for these compounds, relative to gramicidin (= 100), were 39[18] and 23, respectively. The most important observation is that cation flux exhibits clear spacer chain length dependence. Spacer chains having 12 or 14 carbons appear optimal in this system showing relative Na^+ transport rates of 201 and 211, respectively. When the spacer chain length is either increased or decreased by two carbons from the 12–14 range to give **2** or **5**, cation flux is reduced to about half of the previous value. The most striking result, however, is that when the chain length is reduced a further two carbons, $PhCH_2 < N18N > C_8 < N18N > C_8 < N18N > CH_2Ph$ (**1**) proves completely ineffective as an ionophore. Since the hydraphiles are flexible compounds, it seems reasonable that conformational adaptability would still allow function as the chain length increased. If the ionophore must be extended in order to function, the compound must pass a certain size beyond which it simply cannot span enough of the hydrocarbon slab to be functional. This limit appears to have been reached for the octyl spacer chain.

The experimental results presented here resolve two important issues about the hydraphile channel compounds. First, in accord with previous evidence, the carrier mechanism as a possible mode of transport is ruled out. Second, the two-fold changes in transport rates for **2** and **5** compared to **3** or **4** show the sensitivity of this system to dimensional changes of only 4 Å in either direction. This strongly suggests an extended conformation and that the critical membrane span is the insulating hydrocarbon slab rather than the entire phospholipid bilayer.

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Notes and references

1 B. A. Moyer, *Complexation and Transport*, in *Comprehensive Supramolecular Chemistry*, vol. 1, ed. G. W. Gokel, Elsevier Science, Oxford, 1996, p. 377.
2 B. Hille, *Ionic Channels of Excitable Membranes*, 2nd edn., Sinauer Press, Sunderland, MA, 1992; D. G. Nicholls, *Proteins, Transmitters, and Synapses*, vol. 1994, Oxford, Blackwell, 1994; D. J. Aidley and P. R. Stanfield, *Ion Channels: Molecules in Action*, Cambridge University Press, Cambridge 1996.
3 D. A. Doyle, J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait and R. MacKinnon, *Science*, 1998, **280**, 69.
4 G. W. Gokel and O. Murillo, *Acc. Chem. Res.*, 1996, **29**, 425.
5 O. Murillo, S. Watanabe, A. Nakano and G. W. Gokel, *J. Am. Chem. Soc.*, 1995, **117**, 7665.
6 Hydra is defined in *The American Heritage Dictionary* as 'any of several small freshwater polyps of the genus *Hydra* and related genera, having a naked cylindrical body and an oral opening surrounded by tentacles.' This is structurally appropriate to the present systems. The term hydraphile also connotes to us the concept of hydrophilicity. We favor 'hydraphile' over the more cumbersome 'bolaamphiphile' which evokes a vision of random spheres at the ends of swirling and disorganized tethers.
7 O. Murillo, I. Suzuki, E. Abel, C. L. Murray, E. S. Meadows, T. Jin and G. W. Gokel, *J. Am. Chem. Soc.*, 1997, **119**, 5540.
8 O. Murillo, I. Suzuki, E. Abel and G. W. Gokel, *J. Am. Chem. Soc.*, 1996, **118**, 7628.
9 O. Murillo, E. Abel, G. E. M. Maguire and G. W. Gokel, *Chem. Commun.*, 1996, 2147.
10 M. C. Wiener and S. H. White, *Biophys. J.*, 1992, **61**, 434.
11 D. A. Doyle, J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait and R. MacKinnon, *Science*, 1998, **280**, 69.
12 **1**: Anal. calc. for $C_{66}H_{118}O_{12}N_6$: C, 66.74; H, 10.01; N, 7.08%. Found: C, 66.52; H, 9.92; N, 6.98%. **2**: [ESI, $(M + H)^+$] Calc. for $C_{70}H_{126}N_6O_{12}$: 1243.94; Found: 1243.90. **4**: [ESI, $(M + H)^+$] Calc. for $C_{78}H_{143}N_6O_{12}$: 1356.07; Found: 1356.10. **5**: [ESI, $(M + H)^+$] Calc. for $C_{82}H_{151}N_6O_{12}$: 1412.10; Found: 1412.10.
13 F. Cuevas and J. de Mendoza, personal communication, 1998.
14 Compounds **3** (ref. 5) and **6** (ref. 7) were previously reported. The remaining compounds had ^1H NMR, ^{13}C NMR, and high resolution mass spectral data in accord with their structures.
15 F. G. Riddell and M. K. Hayer, *Biochim. Biophys. Acta*, 1985, **817**, 313; D. C. Buster, J. F. Hinton, F. S. Millett and D. C. Shungu, *Biophys. J.*, 1988, **53**, 145; F. G. Riddell, S. Arumugam, P. J. Brophy, B. G. Cox, M. C. H. Payne and T. E. Southon, *J. Am. Chem. Soc.*, 1988, **110**, 734; F. G. Riddell and S. Arumugam, *Biochim. Biophys. Acta*, 1989, **984**, 6; F. G. Riddell and S. J. Tompsett, *Biochim. Biophys. Acta*, 1990, **1024**, 193.
16 D. Papahadjopoulos and F. Szoka, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 4194.
17 R. Gupta and P. Gupta, *J. Magn. Reson.*, 1982, **47**, 344.
18 In previous work we have used gramicidin as standard. This is described in detail in ref. 5. With increasing experience and confidence, we have adopted a hydraphile as standard because of its greater structural similarity to the compounds under study.
19 E. Abel, G. E. M. Maguire, E. S. Meadows O. Murillo, T. Jin and G. W. Gokel, *J. Am. Chem. Soc.*, 1997, **119**, 9061.

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