ChemComm

Enhancement of cation transport in synthetic hydraphile channels having covalently-linked headgroups

Hossein Shabany and George W. Gokel*

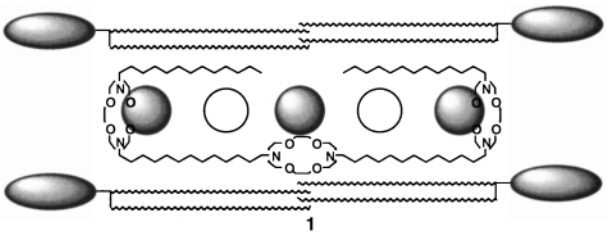
Bioorganic Chemistry Program and Dept. of Molecular Biology & Pharmacology, Washington University School of Medicine, 660 South Euclid Ave., Campus Box 8103, St. Louis, MO 63110, USA. E-mail: ggokel@molecool.wustl.edu

Received (in Columbia, MO, USA) 28th June 2000, Accepted 16th October 2000
First published as an Advance Article on the web

A novel, pentamacrocyclic host molecule has been prepared in which four symmetry-equivalent diazacrown ethers lead to a dramatic enhancement in Na^+ transport, across a phospholipid bilayer, relative to open-chained analogs lacking the fourth crown.

Our strategy[1] to design functional, non-peptide cation-conducting channels[2] has two key elements. First, the system was designed to be flexible so that the structural features could adapt if their exact physical organization had not been correctly evaluated. Second, the planned design was modular so that a compound exhibiting ionophoretic activity in a phospholipid bilayer membrane could be selectively and readily altered. The incorporation of flexibility into the framework could permit minor structural adjustments to compensate for design flaws but could also lead to a non-optimal structural arrangement. This, in turn, could result in poorer function than that of which the system was actually capable.

The first hydraphile structure **1**,[3] a tris(macrocycle), was

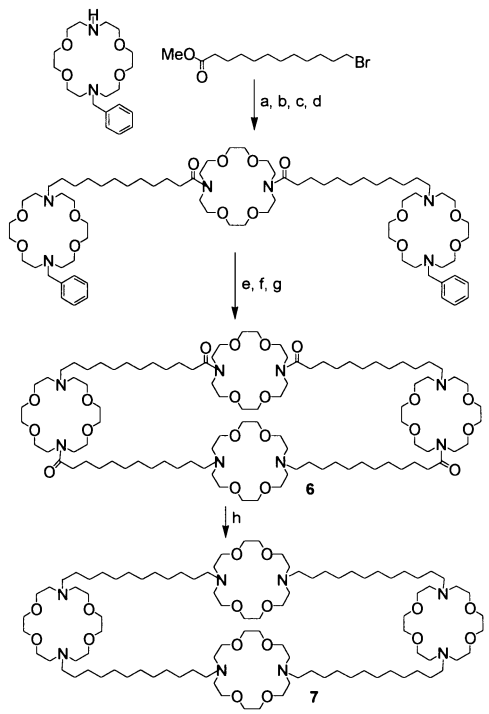


1

covalently-linked to define the overall transmembrane distance but the corresponding opposite side pendant chains were attached only at the distal macrocycles. The conformation shown for structure **1** has been inferred by changing the macroring sizes,[4] by incorporating fluorescent residues,[5] and by other methods.[6] The filled and open circles in the figure are meant to represent Na^+ and H_2O, respectively, but details of the structure within the membrane are not in hand nor are they implied. Note that we use shorthand developed for the purpose[7] to represent **1** as follows: C_{12} < N18N > C_{12} < N18N > C_{12} < N18N > C_{12}.

Studies to determine optimal overall length,[8] coupled with the efforts described above, have led to the conviction that we could connect the two flexible sidechains to form a more rigid and more efficacious structure. Compound **7** (Scheme 1) was a target of this approach. The synthetic plan required a strategy different from that that we had used previously. Compound **1**, which is not cyclic, can be constructed without the requirement for a cyclization reaction.[9] Tetramacrocycle **7**, the key compound in this report, was prepared by a sequence that is detailed in Scheme 1. Monobenzyl-4,13-diaza-18-crown-6[10] ($PhCH_2$ < N18N > H) was alkylated with methyl 12-bromododecanoate. The ester was hydrolyzed, treated with oxalyl chloride to give $PhCH_2$ < N18N > $(CH_2)_{11}COCl$, and then treated with diaza-18-crown-6 to give $PhCH_2$ < N18N > $(CH_2)_{11}CO$ < N18N > - $CO(CH_2)_{11}$ < N18N > CH_2Ph. Debenzylation was accomplished by hydrogenolysis to afford the tris(macrocycle). The diacid, $HO_2C(CH_2)_{11}$ < N18N > $(CH_2)_{11}CO_2H$, a compound that was in hand from previous studies[11] was converted into the corresponding dichloride. The cyclization reaction between H < N18N > $(CH_2)_{11}CO$ < N18N > $CO(CH_2)_{11}$ < N18N > H and $ClCO(CH_2)_{11}$ < N18N > $(CH_2)_{11}COCl$ formed tetramide **6**. Reduction of **6** (BH_3/THF) gave tetraamine **7** as a yellow oil (76% overall from tris(macrocycle). Treatment of Ph_2CH_2 < N18N > H with $ClCOC_{11}O–C_6H_4–C_6H_4–O–C_{11}COCl$ gave $PhCH_2$ < N18N > $CO(CH_2)_{11}O–C_6H_4–C_6H_4–O–(CH_2)_{11}CO$ < N18N > CH_2Ph (65%, yellow oil). Hydrogenolytic debenzylation afforded the biphenyl-bridged bis(macrocycle). The latter underwent cyclization with $ClCO-C_{11}OC_6H_4C_6H_4OC_{11}COCl$ to give tetraamide **2**. Reduction of **2** (BH_3/THF) afforded tetraamine **3** (72%, yellow oil). Compounds **4** (40%), **5** (60%) and **8** (62%) were prepared in a fashion similar to that shown in Scheme 1 and were characterized by standard chemical methods (^1H, ^{13}C NMR and FAB-MS). Dansyl channel **9** has been previously described.[5]

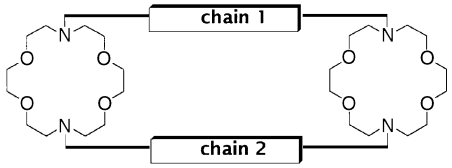
The Na^+ transport efficacies of the compounds prepared as part of this study were assessed in phospholipid bilayers by using the dynamic ^{23}Na NMR method of Riddell *et al.*[12] This method involves the formation of liposomes from phosphatidyl glycerol and phosphatidyl choline in the presence of 100 mM NaCl. The salt is present within the liposomes and in the



Scheme 1 *Reagents and conditions*: (a) Na_2CO_3, cat. KI, reflux 24 h, 83%; (b) 2 M NaOH, reflux, 18 h, 98%; (c) $(COCl)_2$, cat. DMF, 2 h; (d) diazacrown, Et_3N, cat. DMAP, 48 h, 71%; (e) Pd/C, 24 h, 95%; (f) step (c), $HO_2C(CH_2)_{11}$ < N18N > $(CH_2)_{11}CO_2H$; (g) Et_3N, cat. DMAP, 48 h, 70%; (h) BH_3/THF, −5 °C to rt, 48 h, 65%.

DOI: 10.1039/b005436o

Table 1 Sodium transport by hydraphiles in phospholipid liposomes



	Chain 1	Chain 2	k_{rel}[a] (%)
1	C_{12}<N18N>C_{12}	$(CH_2)_{11}Me$	105[b]
2	$COC_{11}O$–C_6H_4–C_6H_4–O–$C_{11}CO$	$COC_{11}O$–C_6H_4–C_6H_4–O–$C_{11}CO$	<2
3	$C_{12}O$–C_6H_4–C_6H_4–O–C_{12}	$C_{12}O$–C_6H_4–C_6H_4–O–C_{12}	<2
4	$C_{11}CO$<N18N>COC_{11}	$COC_{11}O$–C_6H_4–C_6H_4–O–$C_{11}CO$	30
5	C_{12}<N18N>C_{12}	$C_{12}O$–C_6H_4–C_6H_4–O–C_{12}	170
6	$C_{11}CO$<N18N>COC_{11}	COC_{11}<N18N>$C_{11}CO$	250
7	C_{12}<N18N>C_{12}	C_{12}<N18N>C_{12}	340
8	C_{12}<N18N>C_{12}	$(CH_2)_{12}S(CH_2)_8S(CH_2)_{12}$	190
9	C_{12}<N18N>C_{12}	Dansyl	100[c]

[a] We estimate the experimental error to be ±10%. [b] The rate relative to gramicidin is 27%. [c] The rate relative to gramicidin is 24%.

surrounding bulk aqueous phase. The ^{23}Na NMR spectrum observed under these conditions is a singlet. Addition of a Dy^{3+} shift reagent to the aqueous phase renders the two Na^+ ions magnetically non-equivalent and two signals are observed. Addition of an ionophore to the bilayer permits exchange of Na^+ ions with a concomitant change in linewidth. The exchange rate constant (k) can be evaluated from the linewidth change according to the relation $k = 1/\tau = \pi[(\Delta\nu - \Delta\nu_0)]$. In this relationship, τ is the half-life and ν represents the linewidth. The experimentally determined values of K are then compared with the value determined for the dansyl channel (Dn<N18N>C_{12}<N18N>C_{12}<N18N>Dn **9**).[13] The compounds that were studied for the present report are recorded in Table 1. Sodium cation transport by ionophores **1**–**9** was investigated at very low concentration (0–20 μM). The rate is reported as the relative rate $k_{rel} = 100\ k_{obs}/k_9$. A value of <2 means that the compound does not transport Na^+ at a rate sufficient to be observed under the experimental conditions. Dansyl channel **9** is used as the standard rather than gramicidin because the latter is 'too robust'. When experiments are not properly executed, gramicidin will still show transport behavior but **9** requires a proper experimental environment and is therefore a better control and standard.

Channel **1** transports Na^+ with a rate 27% that of gramicidin while its rate is 105% relative to **9**, the standard used throughout this study. The most important finding of the study is that when an additional crown ether is present as a central unit to organize water (see preceding communication), joining the flexible sidechains with a fourth crown produces an ionophore significantly (3.5-fold) more active than **1**. The very high efficacy of this structure supports previous conclusions concerning distance and polarity requirements. The family of structures also permits us to probe the effects of certain of the modular subunits.

First, we note that both compounds **2** and **3** are inactive in the ^{23}Na NMR experiment. We attribute this to the lack of a water-organizing central unit (see preceding communication).[14] When the center of the channel possesses one biaryl unit and an amide-substituted diazacrown **4**, activity is restored but it is modest (30% of **9**). Reduction of the amide residues in **4** affords **5** which has substantially increased Na^+ transport activity (150% of **9**). An increase in transport is expected because the amide residues conformationally restrict the macrocycle to which they are attached. The tetraamide precursor to **7**, *i.e.* **6**, is considerably less flexible than **7** but is also 2.5-fold more active than **1**. There may also be a deleterious effect of an arene on cation transport. In the low polarity environment of the phospholipid bilayer, a cation–π interaction between Na^+ and benzene[15] could substantially diminish the transport rate. Thus, the rate for **8**, which lacks an arene, exceeds that of **5** by a small but significant amount.

An important question is whether the increase in Na^+ transport may be attributed to the presence of the crown, to the higher level of organization present where the two flexible sidechains were, or to both. We know from the results reported in the preceding communication that 4,4′-dihydroxybiphenyl is inactive as a 'central ion capsule.' Thus, the fact that ionophoretic activity of **7** is twice that of **5** confirms that both variables are important. When only arenes are present as the central units, no Na^+ transport is measured in the NMR experiment.

The present effort demonstrates that a tetramacrocyclic hydraphile is more efficacious than its flexible counterpart. This confirms that distance relationships, headgroup functions, and ion–capsule interactions occur as previously surmised.

We thank the NIH (GM-36262) and the NSF (CHE-9805840) for grants that supported this work.

Notes and references

1 G. W. Gokel, *Chem. Commun.*, 2000, 1.
2 D. J. Aidley and P. R. Stanfield, *Ion Channels: Molecules in Action*, Cambridge University Press, Cambridge, 1996; G. W. Gokel and O. Murillo, *Acc. Chem. Res.*, 1996, **29**, 425; B. Hille, *Ionic Channels of Excitable Membranes*, Sinauer Press, Sunderland, MA, 2nd edn., 1992.
3 A. Nakano, Q. Xie, J. V. Mallén, L. Echegoyen and G. W. Gokel, *J. Am. Chem. Soc.*, 1990, **112**, 1287.
4 C. L. Murray, E. S. Meadows, O. Murillo and G. W. Gokel, *J. Am. Chem. Soc.*, 1997, **119**, 7887.
5 E. Abel, G. E. M. Maguire, E. S. Meadows, O. Murillo, T. Jin and G. W. Gokel, *J. Am. Chem. Soc.*, 1997, **119**, 9061; E. Abel, G. E. M. Maguire, O. Murillo, I. Suzuki and G. W. Gokel, *J. Am. Chem. Soc.*, 1999, **121**, 9043.
6 O. Murillo, I. Suzuki, E. Abel and G. W. Gokel, *J. Am. Chem. Soc.*, 1996, **118**, 7628.
7 J. C. Hernandez, J. E. Trafton and G. W. Gokel, *Tetrahedron Lett.*, 1991, 6269.
8 C. L. Murray and G. W. Gokel, *Chem. Commun.*, 1998, 2477.
9 O. Murillo, S. Watanabe, A. Nakano and G. W. Gokel, *J. Am. Chem. Soc.*, 1995, **117**, 7665.
10 J. de Mendoza, F. Cuevas, P. Prados, E. S. Meadows and G. W. Gokel, *Angew. Chem., Int. Ed.*, 1998, **37**, 1534.
11 Synthesis of $HO_2C(CH_2)_{11}$<N18N>$(CH_2)_{11}CO_2H$ was achieved from the reaction of H<N18N>H with methyl 12-bromododecanoate, followed by base hydrolysis.
12 F. G. Riddell and M. K. Hayer, *Biochem. Biophys. Acta*, 1985, **817**, 313; F. G. Riddell, S. Arumugam and B. G. Cox, *J. Chem. Soc., Chem. Commun.*, 1987, 1890.
13 E. Abel, G. E. M. Maguire, O. Murillo, I. Suzuki and G. W. Gokel, *J. Am. Chem. Soc.*, 1999, **121**, 9043.
14 C. L. Murray, H. Shabany and G. W. Gokel, *Chem. Commun.*, 2000, 2371.
15 S. L. De Wall, E. S. Meadows, L. J. Barbour and G. W. Gokel, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 6271.