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The influence of aromatic residues in hydraphile spacer units: assay by ion selective electrode methods and in bacteria

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Abstract—A small library of hydraphiles has been prepared that incorporates either 1,4-phenylenedioxy or 2,6-naphthalenedioxy within the spacer chains. The side chains attached to the distal macrocycles in these tris(macrocyclic) compounds are either *n*-dodecyl or benzyl. The presence of the arenes subunits significantly affect sodium cation release from vesicles. The efficacy of ion transport is paralleled by the toxicity of these compounds to *Bacillus subtilis*.

1. Introduction

The hydraphiles[1] are a family of synthetic ion-conducting channels that function in phospholipid bilayers. Extensive studies have confirmed their placement and overall conformation in liposomal bilayers.[2] As a result of these studies, the roles of the spacers, the head groups, and of the side chains were confirmed, at least in general terms. It was particularly gratifying when the function of our central relay[3] was confirmed by the structure of the KcsA protein channel isolated from *Streptomyces lividans*,[4] a feat that earned MacKinnon the 2003 Nobel Prize in Chemistry.[5]

Before the KcsA potassium selective protein channel structure was reported, Kumpf and Dougherty put forth a hypothesis[6] that cation–pi interactions controlled Na^+/K^+ selection. This seemed plausible in light of the conserved GYG sequence that is present in the channel's selectivity filter. MacKinnon and co-workers showed by site-directed mutagenesis experiments that the tyrosine implicated by Dougherty was not serving this function.[7] Even so, examples of cation–pi interactions have mounted dramatically during the past decade[8] and its potential as a supramolecular force in biology is enormous.[9]

In the present study, we sought to examine the possibility of cation–pi interactions between alkali metal cations and arenes. Our extensive work in this area during the past few years[10] has confirmed that these ion–dipole interactions are formidable and predictable.[11] The plan was to incorporate arenes within the hydraphile spacer units. This would place the arenes within the ion path in the region of lowest polarity. This, in turn, should maximize the cation–pi interaction, if present. We report here the preparation of a small library of compounds to examine this hypothesis. The efficacy of these compounds has been assayed in vitro using phospholipid liposomes and in the bacterium *Bacillus subtilis*.

2. Results and discussion

2.1. Compounds used in this study

Six hydraphile channels were used in the present study. Compounds **1** and **2** have been previously reported and extensively studied. The remaining four compounds have not previously been reported. The general strategy[12] for the preparation of tris(macrocycles) has been to monoalkylate 4,13-diaza-18-crown-6 (H⟨N18N⟩H).[13] The synthesis of **2** begins with the reaction of H⟨N18N⟩H and $PhCH_2Cl$ to give $PhCH_2$⟨N18N⟩H. Reaction of this with $Br(CH_2)_{12}$⟨N18N⟩$(CH_2)_{12}Br$ give the tris(macrocycle) $PhCH_2$(⟨N18N⟩$(CH_2)_{12})_2$-⟨N18N⟩CH_2Ph, **2**. Compounds **3**–**6** require construction

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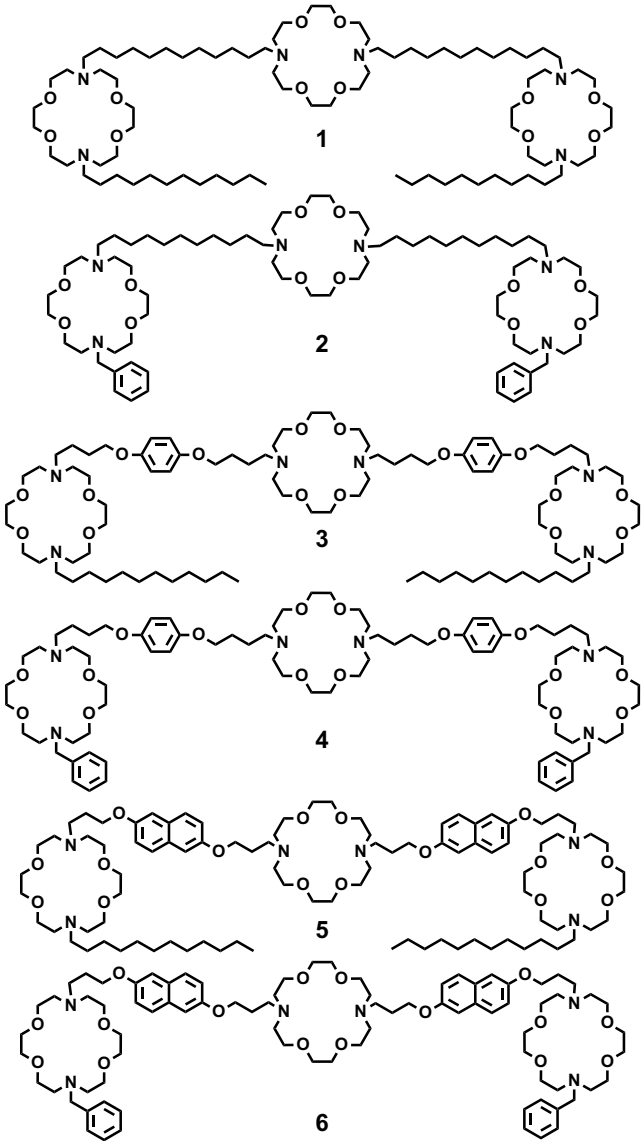
of the inner spacer chains that contain the aromatic residues.

The preparation of **5**, for example, was accomplished as follows. Commercially available 2,6-dihydroxynaphthalene was converted into 2,6-bis(3-bromopropoxy)naphthalene by reaction with excess 1,3-dibromopropane (MeCN, K_2CO_3, 64%). The *O*-alkylated naphthol was contaminated by 2-allyloxy-6-(3-bromopropoxy)naphthalene (~6%, NMR). The mixture was used in the next step. 4,13-Diaza-18-crown-6 was then treated with an excess of the dibromopropoxynaphthalene in the presence of Na_2CO_3 and catalytic KI. $Br(CH_2)_3O$-$C_{10}H_6O(CH_2)_3\langle N18N\rangle(CH_2)_3OC_{10}H_6O(CH_2)_3Br$ was obtained in 40% yield. This dibromide was treated with 2 equiv of *N*-dodecyl-4,13-diaza-18-crown-6 to give **5** (13%) as a nearly colorless solid, mp 103–104.5 °C. Details are recorded in Section 4 and the structures of **1**–**6** are shown.



2.2. Assay of hydraphile efficacy

The ion transport ability of hydraphiles **1**–**6** was assessed by measuring Na^+ release from phospholipid vesicles. In earlier studies, sodium cation exchange was monitored by using the ^{23}Na NMR method of Riddell and Hayer.[14] Recently, we have developed an ion selective electrode (ISE) methodology that was used in the present study.[15] The ion transport results were compared with those obtained from biological testing and the data obtained from the two sources was found to be remarkably consistent.

2.3. Ion selective electrode assay in phospholipid vesicles

Ion selective electrodes have been used for many years to assay the complexation behavior of crown ethers. The application of these methods to vesicular efflux is more recent. Breukink et al. used a potassium selective electrode to assay the effect of the antibiotic nisin on membranes.[16] Similar studies were reported by Silberstein et al.[17] The method we have reported and used in these studies relies on a sodium selective electrode to quantitate ion channel activity.

Briefly, the vesicles used in the studies reported here were prepared by sonication of an aqueous suspension of 1,2-dioleoyl-*sn*-phosphocholine followed by filtration through a 0.2 mm filter. The aqueous suspension contained 750 mM NaCl/15 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) at pH 7.0 so $[Na^+]_{internal} = 750$ mM. Exchange of the external buffer solution was accomplished by passing the liposomes over a Sephadex G25 column, equilibrated with sodium-free buffer (750 mM cholineCl/15 mM HEPES, pH 7.0). Lipid concentration was measured as reported[18] and vesicle size was confirmed by using a Coulter N4MD submicron particle analyzer.

Sodium release was measured by inserting a combination Na^+/pH microelectrode in a disposable beaker that contained the buffered liposome suspension (lipid concentration 0.4 mM, total volume 2 mL). After obtaining a baseline recording (5 min), the hydraphile was added as a 2-propanol solution. Voltage changes reflecting sodium efflux were recorded for 25 min. The vesicles were lysed by treatment with *n*-octylglucoside to achieve a final sodium release value, which was used to normalize the data. Additional details are recorded in Section 4.

2.4. Assessment of sodium transport rates

We have previously reported the activity profile for **2**, which shows maximal concentration-dependent release at [**2**] = 20 μM in about 900 s. The release profile for **1** (see Fig. 1) is similar to that previously observed for **2**. In the case of **1**, however, 72 μM channel (rather than 20 μM) is required to achieve full Na^+ release. Compounds **1** and **2** differ only in the identity of the side arm, but this small structural change significantly impacts the ion transport ability.

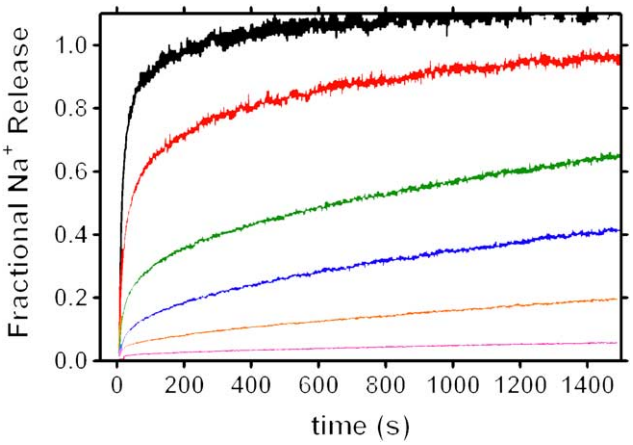


Figure 1. Fractional sodium ion release from phospholipid vesicles mediated by **1**.

The concentrations are, from bottom to top, 12, 24, 36, 48, 60, and 72 μM. In order to better understand the impact on hydraphile channel activity of arene subunits within the structure, we monitored the transport activity of **2**–**6**. The top panel of Figure 2 shows the Na^+ release of 12 μM of **1**–**6**, as monitored by the ISE. Clearly, benzyl channel **2** is by far the most active of this small library of compounds. At this concentration, sodium release is rapid and goes to approximately 70% completion in 1500 s. The other channels, **1** and **3**–**6**, show <10% release in the presence of 12 μM hydraphile. The bottom panel of Figure 2 shows Na^+ release for **1** and **3**–**6** at a fivefold higher concentration of 60 μM.

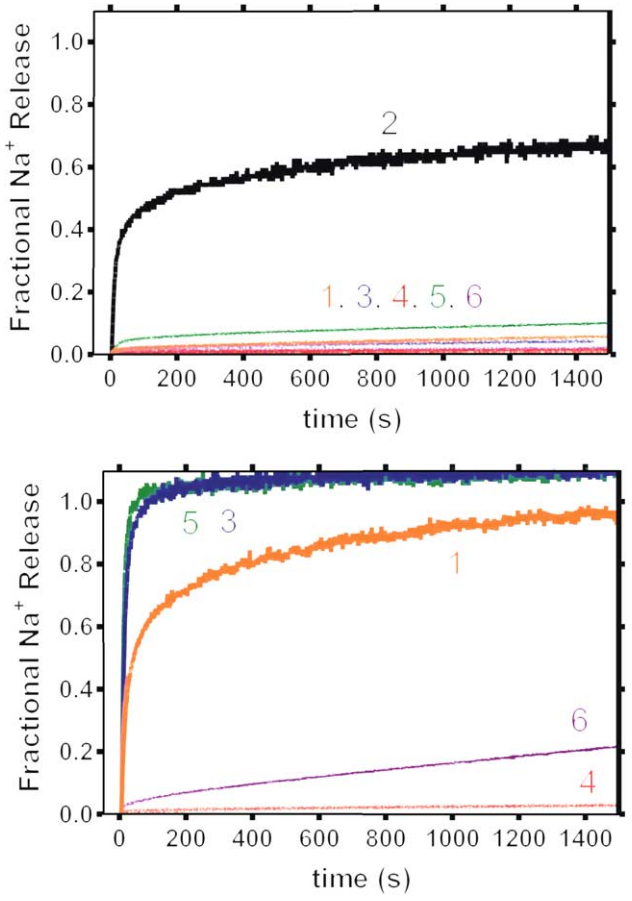


Figure 2. Comparison of Na^+-release activity from liposomes mediated by **1**–**6** at concentrations of 12 μM (upper panel) and 60 μM.

Compounds **4** and **6** have arenes in both the spacer units and the side arms. While **4** remains inactive at 60 μM, **6** shows a total release of ~20% at 1500 s. As shown in Figure 1, hydraphile **1** (no arene subunits) releases ~95% of Na^+ at [**1**] = 60 μM. Channels **3** and **5** (arene subunits in the spacer chains only) go to complete release immediately after addition of the channel solution to the liposome suspension. Compounds **3** and **5** are significantly more active than **1**. Release of Na^+ was measured for **1**, **3**, and **5** at a channel concentration of 36 μM (data not shown) and **3** and **5** demonstrated higher transport efficacy at this intermediate concentration as well.

Qualitatively, the Na^+-release efficacy of compounds **1**–**6** may be summarized as follows: **2** ≫ **1**, **3**–**6**. When only **1** and **3**–**6** are compared, the activity order is **3** ~ **5** > **1** > **4** ~ **6**. From data obtained at the higher concentration, it is clear that activity is affected more by side-arm identity than by the arene within the spacer chain. Thus, **3** and **5** have benzene and naphthalene subunits, respectively, in their spacer chains but they both have dodecyl side arms. Likewise, **4** and **6** have benzene and naphthalene subunits, respectively, in their spacer chains but they both have benzyl side arms. When there is no arene within the spacer chains, hydraphile **2**, which has benzyl side arms, is more active than **1**, which contains only aliphatic components. This contrasts with the higher activity of dodecyl-side-armed **3** and **5**, which are more active than the benzyl-side-armed counterparts, **4** and **6**.

Hydraphile **1** contains dodecyl units in both the side arm and backbone, and is expected to be the most flexible of these three systems. Incorporation of the arene backbone, in the form of a benzyl group (3) or a naphthalene group (5) will decrease the channel's conformational flexibility. It seems reasonable to expect the more flexible molecule to better adapt to the membrane environment. On the other hand, when an ion-conducting conformation is achieved within the bilayer, the more rigid arene spacers should help to maintain it. We infer from the experimentally determined transport rates that maintaining an active channel-forming conformation is more important for ion conduction than is the inherent flexibility of the compound.

Because benzyl-side-armed **2** is more active than dodecyl-side-armed **1**, we expected **4** and **6** to be more active than **3** and **5**. The evidence suggests that the pair of aromatic systems on each side of the distal macrocycle inhibits ion transport. Corey–Pauling–Koltun (CPK) molecular models show that there is sufficient conformational flexibility in either **4** or **6** to form either a pi–pi stack between the arenes or a pi-stacked 'sandwich' that includes a cation. The models suggest that **6** is less flexible than is **4**, which may explain the modest difference in

Table 1. Biological activity of hydraphiles to *B. subtilis*

Compd No.	MIC (μM)
3	1
4	8.9
5	0.5
6	4.3

activity. Specifically, **6** shows ~20% Na^+ release at 1500 s compared to ~2% for **4**. Naphthalene is larger and the spacer containing it is less flexible than the benzene-containing spacer. Thus, **6** should be less flexible and less able to form an obstructive pi-stacking interaction.

2.5. Biological assays

In previous studies, we noted that many of the hydraphiles showed toxicity to various organisms including the bacterium *B. subtilis*. We have noted an excellent correlation between transport efficacy as assessed in vitro and toxicity to bacteria cells in vivo.[19] We therefore surveyed the activity of **3**–**6** to see if a similar correlation was apparent. The minimum inhibitory concentration (MIC) data (in μM) are recorded in Table 1.

Remarkably, the same trends in activity as observed in the ISE transport data can be seen in the toxicity profiles of **3**–**6** to *B. subtilis*. Compounds **3** and **5**, which have arene spacer units and dodecyl side arms, were determined to have MIC values of 1 and 0.5 mM, respectively, which are comparable to **1**. However, when the hydraphile has an arene present in both the spacer unit and the side arm, the MIC values are at least eightfold higher.

3. Conclusions

We have incorporated arenes into the spacer chains of a small library of hydraphiles. Specifically, the arenes are 1,4-phenylenedioxy (**3**, **4**) or 2,6-naphthalenedioxy (**5**, **6**). The side arms are either *n*-dodecyl (**3**, **5**) or benzyl (**4**, **6**). The presence of the arenes gives mixed effects on ion transport but both increases and decreases in Na^+ release from liposomes is paralleled by changes in toxicity to *B. subtilis*.

Apparently, some rigidification of the ion path is beneficial to transport but when arenes are present in both the spacer and side chain, interactions apparently between them significantly diminish transport and toxicity.

4. Experimental

4.1. General

^{1}H NMR were recorded at 300 MHz in $CDCl_3$ solvents and are reported in ppm downfield from internal $(CH_3)_3Si$ unless otherwise noted. ^{13}C NMR were recorded at 75 MHz in $CDCl_3$ unless otherwise stated. Infrared spectra were recorded on a Perkin–Elmer 1710 Fourier Transform Infrared Spectrophotometer and were calibrated against the 1601 cm^{-1} band of polystyrene. Melting points were determined on a Thomas Hoover apparatus in open capillaries and are uncorrected. Thin layer chromatographic (TLC) analyses were performed on aluminum oxide 60 F-254 neutral (type E) with a 0.2 mm layer thickness or on silica gel 60 F-254 with a 0.2 mm layer thickness. Preparative chromatography columns were packed with activated aluminum oxide (MCB 80–325 mesh, chromatographic grade, AX 611) or with Kieselgel 60 (70–230 mesh). Chromatotron chromatography was performed on a Harrison Research Model 7924 Chromatotron with 2 mm thick circular plates prepared from Kieselgel 60 PF-254.

All reactions were conducted under dry N_2 unless otherwise stated. All reagents were the best (non-LC) grade commercially available and were distilled, recrystallized, or used without further purification, as appropriate. Molecular distillation temperatures refer to the oven temperature of a Kugelrohr apparatus. Combustion analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are reported as percents. Where water is factored into the analytical data, spectral evidence is presented for its presence.

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids as chloroform solutions. Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and the inorganic salts NaCl and cholineCl were all purchased from Sigma–Aldrich. The water that was used for all buffer preparation was of Milli-Q Plus quality, which is essential to avoid salt contamination in the buffer systems. *N*-Octylglucoside was purchased from CalBioChem.

4.2. *N*,*N*′-Bis{12-[*N*-(*N*′-dodecyl)-diaza-18-crown-6]-dodecyl}-diaza-18-crown-6, $C_{12}H_{25}\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle C_{12}H_{25}$ (1)

Compound **1** was prepared as previously reported.[2a]

4.3. *N*,*N*′-Bis{*N*-[12-(*N*′-benzyldiaza-18-crown-6)dodecyl]}-diaza-18-crown-6, $PhCH_2\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle(CH_2)_{12}\langle N18N\rangle CH_2Ph$ (2)

Compound **2** was prepared as previously reported.[2a]

4.4. $C_{12}H_{25}\langle N18N\rangle(CH_2)_4OC_6H_4O(CH_2)_4\langle N18N\rangle(CH_2)_4OC_6H_4O(CH_2)_4\langle N18N\rangle C_{12}H_{25}$ (3)

4.4.1. 1,4-Bis-(4-chlorobutoxy)benzene. A solution of 1,4-dichlorobutane (63.50 g, 0.5 mol), hydroquinone (2.75 g, 25 mmol), K_2CO_3 (8.30 g, 0.6 mol), in *n*-PrCN (100 mL) was heated at reflux for 48 h and then cooled and filtered. The solids were washed with $CHCl_3$ (2×20 mL) and the combined organic material was evaporated in vacuo. Column chromatography (SiO_2, 95% hexanes/ethyl acetate) gave 1,4-bis(4-chlorobutoxy)benzene (6.84 g, 94%) as a colorless solid, mp 78–79 °C.

^{1}H NMR: 1.85–2.00 (8H, m, $OCH_2CH_2CH_2CH_2Cl$), 3.62 (4H, t, $J = 6.0$ Hz, $OCH_2CH_2CH_2CH_2Cl$), 3.94 (4H, t, $J = 6.0$ Hz, $OCH_2CH_2CH_2CH_2Cl$), 6.82 (4H, s, H_{Ar}). ^{13}C NMR: 26.7, 29.3, 44.7, 67.5, 115.4, 153.0. IR (KBr): 2957, 2922, 2878, 1510, 1470, 1455, 1440, 1420, 1400, 1352, 1302, 1283, 1235, 1116, 1049, 1015, 823, 774, 738, 722 cm^{-1}.

4.4.2. $Cl(CH_2)_4OC_6H_4O(CH_2)_4\langle N18N\rangle(CH_2)_4OC_6H_4O(CH_2)_4Cl$. A solution of 1,4-bis(4-chlorobutoxy)benzene (4.40 g, 15.1 mmol), 4,13-diaza-18-crown-6 (1.00 g, 3.8 mmol), Na_2CO_3 (2.12 g, 20 mmol), and KI (30 mg, 0.2 mmol) in *n*-PrCN (30 mL) was heated to reflux for 72 h, cooled, and filtered. The solids were washed with $CHCl_3$ (2×20 mL) and the combined organic material was washed with brine (50 mL) and concentrated in vacuo. Column chromatography (SiO_2, 0–1% Et_3N in 50% hexanes/acetone), gave $Cl(CH_2)_4O$-$C_6H_4O(CH_2)_4\langle N18N\rangle(CH_2)_4OC_6H_4O(CH_2)_4Cl$ (1.20 g, 41%) as a slightly yellow oil. ^{1}H NMR $CDCl_3$: 1.52–1.64 (4H, m, NCH_2CH_2), 1.66–1.74 (4H, m, CH_2CH_2Cl), 1.82–1.98 (8H, m, CH_2CH_2OPh), 2.55 (4H, t, $J = 6$ Hz, NCH_2CH_2), 2.77 (8H, t, $J = 6$ Hz, NCH_2CH_2O), 3.50–3.65 (20H, overlapping signals due to CH_2CH_2Cl and $NCH_2CH_2OCH_2$), 3.85–3.95 (8H, m, CH_2CH_2OPh), 6.80 (8H, s, H_{Ar}). ^{13}C NMR: 23.9, 26.7, 27.2, 29.3, 44.7, 54.0, 55.6, 67.6, 68.4, 70.0, 70.7, 115.4, 152.9, 153.3. IR (neat): 2943, 2868, 1509, 1473, 1391, 1353, 1284, 1230, 1122, 1059, 826, 728.

4.4.3. Preparation of 3. A mixture of $H\langle N18N\rangle(CH_2)_{11}CH_3$ (0.56 g, 1.30 mmol), $[Cl(CH_2)_4(OC_6H_4O)(CH_2)_4]\langle N18N\rangle[(CH_2)_4(OC_6H_4O)(CH_2)_4Cl]$ (0.40 g, 0.518 mmol), $NaCO_3$ (0.55 g, 5.18 mmol), KI (cat.), and 20 mL of butyronitrile were heated to reflux for 4 days, cooled, filtered (Celite), and evaporated. The residue was dissolved in $CHCl_3$ (50 mL), washed with 10% aq Na_2CO_3 (3×75 mL), brine (75 mL), and evaporated (high vacuum). Column chromatography (SiO_2, 2% Et_3N in acetone) gave **3** (120 mg, 15%) as a waxy, tan solid, mp 40.5–41.5 °C. ^{1}H NMR: 0.87 (6H, t, $J = 6.6$ Hz, CH_3), 1.25 (38H, m, $CH_2(CH_2)_9CH_3$), 1.61 (8H, m, $NCH_2CH_2CH_2$), 1.74 (8H, m, $OCH_2CH_2CH_2$), 2.56 (12H, m, $NCH_2CH_2CH_2$), 2.78 (24H, t, $J = 6.0$ Hz, NCH_2CH_2O), 3.6 (48H, t, $J = 6.0$ Hz, NCH_2CH_2O, OCH_2CH_2O), 3.89 (8H, t, $J = 6.0$ Hz, OCH_2CH_2), 6.79 (8H, s, *OPhO*). ^{13}C NMR: 14.3, 22.9, 24.0, 27.2, 27.4, 27.7, 29.5, 29.8, 32.1, 54.1, 55.7, 56.2, 68.5, 70.1, 70.9, 115.5, 153.3. IR ($CHCl_3$): 730, 770, 825, 925, 1072, 1127, 1230, 1289, 1352, 1469, 1508, 1589, 1675, 2855, 2924, 3045, 3369. Elem. Anal. Calcd for $C_{88}H_{164}N_6O_{17}\cdot H_2O$: C, 66.97; H, 10.47; N, 5.32. Found: C, 67.05; H, 10.34; N, 5.36.

4.5. $C_6H_5CH_2\langle N18N\rangle(CH_2)_4OC_6H_4O(CH_2)_4\langle N18N\rangle(CH_2)_4OC_6H_4O(CH_2)_4\langle N18N\rangle CH_2C_6H_5$ (4)

A solution of $Cl(CH_2)_4OC_6H_4O(CH_2)_4\langle N18N\rangle(CH_2)_4OC_6H_4O(CH_2)_4Cl$ (0.60 g, 0.78 mmol), *N*-benzyl-4,13-diaza-18-crown-6 (0.74 g, 2.10 mmol), Na_2CO_3 (0.8 g, 7.5 mmol), and KI (30 mg, 0.2 mmol) in *n*-PrCN (20 mL) was heated at reflux for 72 h, cooled, and filtered. The salts were washed with $CHCl_3$ (2×20 mL) and the combined organics were washed with brine (50 mL) and concentrated in vacuo. Column chromatography (SiO_2, 0–2% Et_3N/acetone) gave $PhCH_2\langle N18N\rangle(CH_2)_4OC_6H_4O(CH_2)_4\langle N18N\rangle(CH_2)_4OC_6H_4O(CH_2)_4\langle N18N\rangle CH_2Ph$ (1.14 g, 70%) as a slightly yellow oil. ^{1}H NMR $CDCl_3$: 1.52–1.64 (8H, m, NCH_2CH_2), 1.66–1.80 (8H, m, CH_2CH_2OPh), 2.55 (8H, m, NCH_2CH_2), 2.72–2.84 (24H, m, NCH_2CH_2O), 3.56–3.62 (48H, m, $NCH_2CH_2OCH_2$), 3.65 (4H, s, CH_2Ph), 3.88 (8H, t, $J = 6.0$ Hz, CH_2CH_2OPh), 6.80 (8H, s, H_{Ar}), 7.20–7.40 (10H, m, CH_2PhHAr). ^{13}C NMR: 23.9, 27.3, 29.3, 53.8, 54.0, 55.6, 60.0, 70.0, 70.7, 115.4, 126.8, 128.1, 128.8, 139.7, 153.2. IR (neat): 2942, 2866, 1704, 1509, 1472, 1455, 1353, 1353, 1294, 1230, 1126, 1061, 853, 736, 700. Anal. Calcd for $C_{78}H_{126}N_6O_{16}$: C, 66.73; H, 9.05; N, 5.99. Found: C, 66.52; H, 9.05; N, 6.12.

4.6. $C_{12}H_{25}\langle N18N\rangle(CH_2)_3OC_{10}H_6O(CH_2)_3\langle N18N\rangle(CH_2)_3OC_{10}H_6O(CH_2)_3\langle N18N\rangle C_{12}H_{25}$ (5)

4.6.1. 2,6-Bis(3-bromopropoxy)naphthalene (5A). A mixture of 2,6-dihydroxynaphthalene (3.20 g, 20 mmol) and K_2CO_3 (13.8 g, 0.1 mol) in MeCN (300 mL) was heated at reflux for 1 h and 1,3-dibromopropane (40.4 g, 0.2 mol) was added at once. After 8 h heating, the hot mixture was filtered and reduced to about 30% of the previous volume. After a second filtration, CH_2Cl_2 (100 mL) was added, the mixture was filtered again, and then evaporated to dryness. Crystallization from EtOH gave **5A** (5.12 g, 64%) as a light orange powder. ^{1}H NMR indicated that this isolated materials contained 6% of allyloxy residue, and used to the next step without further purification. ^{1}H NMR: 2.378 (4H, quintet, $J = 6.0$ Hz, $BrCH_2CH_2CH_2O$), 3.653 (4H, t, $J = 6.6$ Hz, $BrCH_2CH_2CH_2O$), 4.202 (4H, t, $J = 6.0$ Hz, $BrCH_2CH_2CH_2O$), 7.10–7.14 (4H, m, aromatics), 7.638 (2H, d, $J = 9.6$ Hz, aromatics). IR (KBr disk): 3059, 2951, 2932, 2908, 1706, 1605, 1508, 1469, 1433, 1417, 1397, 1337, 1286, 1272, 1256, 1234, 1201, 1166, 1149, 1115, 1026, 964, 914, 805, 785, 852, 785, 693, 663, 625, 559, 473 cm^{-1}.

4.6.2. *N,N′*-Bis[6-(3-bromopropoxy(2-naphthoxy)propyl)]-4,13-diaza-18-crown-6 (5B). To a mixture of 4,13-diaza-18-crown-6 (525 mg, 2.0 mmol), Na_2CO_3 (7.32 g, 69.1 mmol), and KI (1.65 mg, 0.01 mmol), a warmed solution of **5A** (4.42 g, 11.0 mmol) in *n*-PrCN (25 mL) was added and the resultant suspension was heated at reflux for 3.5 h, cooled, filtered, and concentrated. Toluene (5 mL) was added and evaporated to assure the complete removal of *n*-PrCN. Residual solid was dissolved in minimal CH_2Cl_2. Column chromatography (Al_2O_3, eluant CH_2Cl_2, then 2% *i*-PrOH–CH_2Cl_2) gave **5B** (0.73 g, 40%, NMR purity ~80%) as a light yellow solid. This material was used directly in the next step without further purification. ^{1}H NMR: 1.949 (4H, quintet, $J = 6.7$ Hz, $NCH_2CH_2CH_2O$), 2.365 (4H, quintet, $J = 6.1$ Hz, $BrCH_2CH_2CH_2O$), 2.707 (4H, t, $J = 7.0$ Hz, $NCH_2CH_2CH_2O$), 2.777 (8H, t, $J = 5.8$ Hz, NCH_2CH_2O), 3.50–3.70 (20H, m, $BrCH_2CH_2$, CH_2OCH_2), 4.084 (4H, t, $J = 6.2$ Hz, $NCH_2CH_2CH_2O$),

4.185 (4H, t, $J = 5.6$ Hz, $BrCH_2CH_2CH_2O$), 7.06–7.14 (8H, m, aromatics), 7.621 (4H, d, $J = 10.0$ Hz, aromatics). IR (KBr disk): 3067, 3030, 2927, 2849, 2821, 1605, 1510, 1466, 1396, 1351, 1291, 1270, 1234, 1161, 1143, 1119, 1074, 1030, 967, 936, 853, 816, 692, 625, 573, 474 cm^{-1}.

4.6.3. 4,13-Bis{4-[6-(13-dodecyl-4,13-diaza-18-crown-6)-4-yl-1-oxabutyl]naphth-2-yl-4-oxabutyl}-4,13-diaza-18-crown-6 (5). A mixture of **5B** (0.62 g, 0.69 mmol), *N*-dodecyl-4,13-diaza-18-crown-6 (0.61 g, 1.41 mmol), Na_2CO_3 (2.99 g, 28.1 mmol), and KI (20 mg, 0.12 mmol) in *n*-PrCN (20 mL) was heated at reflux for 28 h. After cooling and filtering, the solids were extracted (CH_2Cl_2, 30 mL), and the combined organic solutions were concentrated. Toluene was added and evaporated to assure complete removal of *n*-PrCN. This procedure was repeated twice. The solids thus obtained were taken up in CH_2Cl_2 washed with H_2O (3×15 mL), dried ($MgSO_4$), and the solvent was evaporated. Column chromatography (Al_2O_3, CH_2Cl_2–*i*-PrOH–MeOH, 96:2.4:1.6). Fractions containing **5** were collected and solvent was evaporated. Alternate crystallization from 95% EtOH or AcOEt afforded pure **5** (143 mg, 13%) Mp 103–104.5 °C; ^{1}H NMR: 0.878 (6H, t, $J = 6.6$ Hz, methyl), 1.18–1.34 (36H, m, alkyl), 1.36–1.48 (4H, br, $NCH_2CH_2CH_2CH_2$), 1.90–2.00 (8H, br, $NCH_2CH_2CH_2O$), 2.461 (4H, t, $J = 6.7$ Hz, $NCH_2CH_2CH_2CH_2$), 2.65–2.85 (32H, m, NCH_2CH_2), 3.55–3.75 (48H, m, CH_2OCH_2), 4.05–4.10 (8H, m, $NCH_2CH_2CH_2O$), 7.05–7.15 (8H, m, aromatics), 7.603 (4H, pseudo-d, $J = 8.7$ Hz, aromatics). IR (KBr disk): 2918, 2850, 1604, 1510, 1466, 1450, 1396, 1381, 1351, 1292, 1267, 1234, 1160, 1143, 1119, 1073, 1023, 989, 970, 935, 854, 722, 691, 624, 577, 476. Anal. Calcd for $C_{92}H_{158}N_6O_{16}$: C, 68.88; H, 9.93; N, 5.24. Found: C, 68.97; H, 9.88; N, 5.27.

4.7. $C_6H_5CH_2\langle N18N\rangle(CH_2)_3OC_{10}H_6O(CH_2)_3\langle N18N\rangle(CH_2)_3OC_{10}H_6O(CH_2)_3\langle N18N\rangle CH_2C_6H_5$ (6)

A mixture of $H\langle N18N\rangle CH_2Ph$ (0.43 g, 0.475 mmol), $[Br(CH_2)_3OC_{10}H_6O(CH_2)_3]\langle N18N\rangle[(CH_2)_3OC_{10}H_6O(CH_2)_3Br]$ (0.59 g, 1.66 mmol), $NaCO_3$ (1.01 g, 9.5 mmol), KI (cat.), and butyronitrile (20 mL) was refluxed for 2 days. The reaction was then cooled, filtered (Celite), and evaporated. Column chromatography (SiO_2, 2% Et_3N in acetone) followed by crystallization from acetone gave **6** (98 mg, 14%) as a pink granular solid, mp 90–91 °C. ^{1}H NMR: 1.89 (8H, d, $J = 6.0$ Hz, $NCH_2CH_2CH_2O$), 2.72 (32H, m, NCH_2), 3.51 (52H, m, $CH_2OCH_2CH_2OCH_2$, NCH_2Bz), 4.01 (8H, d, 6.0 Hz, $NCH_2CH_2CH_2O$), 7.03 (8H, d, $J = 9$ Hz, OCCHCHC), 7.23 (10H, m, CH_2Ph), 7.51 (4H, d, $J = 9$ Hz, OCCHC). ^{13}C NMR: 27.5, 52.4, 53.9, 53.9, 54.4, 60.1, 66.2, 70.2, 70.9, 107.2, 119.3, 127.0, 128.3, 129.0, 129.9, 155.6. IR ($CHCl_3$): 623, 661, 699, 734, 801, 850, 912, 973, 1115, 1161, 1234, 1296, 1353, 1394, 1453, 1509, 1604, 2242, 2865, 3027, 3059, 3371 cm^{-1}. Anal. Calcd for $C_{82}H_{122}N_6O_{17}\cdot H_2O$: C, 67.19; H, 8.53; N, 5.73. Found: C, 67.22; H, 8.38; N, 5.51.

4.8. Vesicle preparation

Vesicles were prepared from 1,2-dioleoyl-*sn*-glycero-3-phosphocholine by using the reverse evaporation method of Szoka and Papahadjopoulos.[20] The phospholipid were obtained as $CHCl_3$ solutions, which were dried to lipid films and stored under vacuum at ambient temperature. The vesicles were prepared by dissolving a dry lipid film in 0.3 mL diethyl ether and 0.3 mL buffer (750 mM NaCl/15 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.0). The mixture was sonicated for ~20 s to give an opaque solution. The organic solvent was removed under reduced pressure and the solution was passed through a mini-extruder containing a 0.2 mm polycarbonate membrane filter. The residual, external buffer solution was exchanged for a sodium-free buffer (750 mM cholineCl/15 mM HEPES, pH 7.0) via passage over a Sephadex G25 column. Vesicle concentration was measured as reported[18] and vesicle size was measured by a Coulter N4MD submicron particle analyzer. The vesicles used in the transport studies had diameters of ~200–250 nm depending on lipid tail length.

4.9. Sodium efflux measurements

Sodium cation efflux from liposomes was measured using a pH/Na^+ combination microelectrode (Thermo-Orion). The electrode was equilibrated in sodium-free buffer (external buffer) in a small, disposable beaker while stirring. Vesicles suspended in external buffer were added to achieve a total lipid concentration of 0.4 mM and a total solution volume of 2.0 mL. A baseline measurement was recorded for 5 min. The channel solution was introduced as a 2-propanol solution and Na^+ efflux was monitored for 25 min. The vesicles were lysed with a 10% solution of *n*-octylglucoside to achieve total Na^+ release. This final release value was used to normalize all data (converted from mV to units of concentration).

4.10. Determination of minimum inhibitory concentration

We determined the minimum inhibitory concentration (in M) as the lowest twofold dilution of hydraphile that prevented bacterial growth, as outlined by the NCCLS.[21] In short, 5×10^5 colony forming units of *B. subtilis* (JH642 WT) cells were grown at 30 °C in 2 mL of Luria Bertani (LB) Miller media (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, 100 g/mL ampicillin) that were twofold serially diluted with hydraphile test compound. The MIC was taken as the lowest hydraphile concentration that inhibited growth after 24 h as judged by visual turbidity.

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References and notes

1. Gokel, G. W. *Chem. Commun.* **2000**, 1–9.
2. (a) Murillo, O.; Abel, E.; Maguire, G. E. M.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1996**, 2147–2148; (b) Murillo, O.; Suzuki, I.; Abel, E.; Gokel, G. W. *J. Am. Chem. Soc.* **1996**, *118*, 7628–7629; (c) Abel, E.; Maguire, G. E. M.; Meadows, E. S.; Murillo, O.; Jin, T.; Gokel, G. W. *J. Am. Chem. Soc.* **1997**, *119*, 9061–9062.
3. Murray, C. L.; Shabany, H.; Gokel, G. W. *Chem. Commun.* **2000**, 2371–2372.
4. Doyle, D. A.; Cabral, J. M.; Pfuetzner, R. A.; Kuo, A.; Gulbis, J. M.; Cohen, S. L.; Chait, B. T.; MacKinnon, R. *Science* **1998**, *280*, 69–77.
5. MacKinnon, R. *Angew. Chem., Int. Ed. Engl.* **2004**, *43*, 4265–4277.
6. Kumpf, R. A.; Dougherty, D. A. *Science* **1993**, *261*, 1708–1710.
7. Heginbotham, L.; Lu, Z.; Abramson, T.; MacKinnon, R. *Biophys. J.* **1994**, *66*, 1061–1067.
8. (a) Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303–1324; (b) Gallivan, J. P.; Dougherty, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9459–9464.
9. (a) Gokel, G. W.; Wall, S. L. D.; Meadows, E. S. *Eur. J. Org. Chem.* **2000**, 2967–2978; (b) Gokel, G. W.; Barbour, L. J.; De Wall, S. L.; Meadows, S. L. *Coord. Chem. Rev.* **2001**, *222*, 127–154; (c) Gokel, G. W.; Barbour, L. J.; Ferdani, R.; Hu, J. *Acc. Chem. Res.* **2002**, *35*, 878–886.
10. Gokel, G. W. *Chem. Commun.* **2003**, 2847–2852.
11. (a) De Wall, S. L.; Meadows, E. S.; Barbour, L. J.; Gokel, G. W. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6271–6276; (b) Meadows, E. S.; De Wall, S. L.; Barbour, L. J.; Gokel, G. W. *J. Am. Chem. Soc.* **2001**, *123*, 3092–3107; (c) Hu, J.; Barbour, L. J.; Gokel, G. W. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5121–5126.
12. Murillo, O.; Watanabe, S.; Nakano, A.; Gokel, G. W. *J. Am. Chem. Soc.* **1995**, *117*, 7665–7679.
13. Hernandez, J. C.; Trafton, J. E.; Gokel, G. W. *Tetrahedron Lett.* **1991**, 6269–6272.
14. Riddell, F. G.; Hayer, M. K. *Biochem. Biophys. Acta* **1985**, *817*, 313–317.
15. Weber, M. E.; Schlesinger, P. H.; Gokel, G. W. *J. Am. Chem. Soc.* **2004**, *126*, 636–642.
16. Breukink, E.; Kraaij, C. v.; Dernel, R. A.; Siezen, R. J.; Kuipers, O. P.; Kruijff, B. D. *Biochemistry* **1997**, *36*, 6968–6976.
17. Silberstein, A.; Mirzabekov, T.; Anderson, W. F.; Rozenberg, Y. *Biochim. Biophys. Acta* **1999**, *1461*, 103–112.
18. Stewart, J. C. *Anal. Biochem.* **1980**, *104*, 10–14.
19. Leevy, W. M.; Weber, M. E.; Schlesinger, P. H.; Gokel, G. W. *Chem. Commun.* **2004**, 89–91.
20. Szoka, F.; Papahadjopoulos, D. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4194–4198.
21. National Committee for Clinical Laboratory Standards. In *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow Aerobically*, 5th ed.; M7-A5 (NCCLS, Wayne, Pennsylvania, 2000).