Published in final edited form as:

New J Chem. 2006 ; 30(2): 177–184. doi:10.1039/b510863m.

The Influence of Varied Amide Bond Positions on Hydrphile Ion Channel Activity

Michelle E. Weber[†], Wei Wang[§], Sarah E. Steinhardt[§], Michael R. Gokel[†], W. Matthew Leevy[†], and George W. Gokel^{†,§}

[§]Department of Chemistry, Washington University, 1 Brookings Drive, St. Louis, MO 63130

[†]Department of Molecular Biology & Pharmacology, Washington University School of Medicine, Campus Box 8103, 660 S. Euclid Ave., St. Louis, MO 63110 USA; E-mail: ggokel@molecool.wustl.edu

Abstract

Hydrphile compounds have been prepared in which certain of the amine nitrogens have been replaced by amide residues. The amide bonds are present either in the sidearm, the side chain, or the central relay. Sodium cation transport through phospholipid vesicles mediated by each hydrphile was assessed. All of the amide-containing hydrphiles showed increased levels of Na⁺ transport compared to the parent compound, but the most dramatic rate increase was observed for sidearm amine to amide replacement. We attribute this enhancement to stabilization of the sidearm in the bilayer to achieve a better conformation for ion conduction. Biological studies of the amide hydrphiles with *E. coli* and *B. subtilis* showed significant toxicity only with the latter. Further, the consistency between the efficacies of ion transport and toxicity previously observed for non-amidic hydrphiles was not in evidence.

Introduction

Crown ethers are known to complex metal and organic cations.¹ They can also complex or include in their crystalline lattices neutral compounds such as nitromethane, acetonitrile, and urea.² The donor elements that best complement alkali metal or alkaline earth cations are oxygen and nitrogen. Complexation to transition metal cations is favored by the presence of sulfur, but nitrogen is often an effective donor element for these cations as well. Oxygen and sulfur are typically neutral and divalent in their complexing role, but nitrogen within a macrocycle may be secondary or tertiary and neutral or protonated. Further, nitrogen may be present either as an amine or as an amide residue.

Amine nitrogen is electron rich and, unless it is sterically hindered, it is readily accessible to a Lewis acid. The donicity of nitrogen is diminished by proximate electron withdrawing substituents and rendered partially positive by resonance when it is in the amide (R₂N-CO-R') form. An amide is thus a poorer donor at nitrogen but a strong donor at oxygen by virtue of its formal negative charge in the enolate canonical form.

Numerous macrocyclic amides (lactams) are known.^{3,4,5,6} In principle, incorporation of an amide into a typical 15- or 18-membered ring macrocycle will rigidify the ring. The utility of an amide as a donor in a macrocycle depends upon whether or not the carbonyl residue is available for complexation. Figure 1 shows 3-oxo-4-aza-15-crown-5 in its two resonance forms

(A and B). The amide oxygen is a more powerful donor in form B, but cation complexation by the macroring is expected to be lowered by the presence of the formal positive charge. In either event, however, the oxygen donors are directed away from the internal, complexing cavity. This is illustrated in the CPK molecular model shown with the resonance forms.

The cation complexing ability of relatively few macrocyclic lactams has been studied. Even when such studies have been undertaken, direct comparisons to complexation strengths and selectivities of the corresponding crowns or azacrowns have not been reported.⁴⁻⁶ When tertiary amide donors were present in flexible lariat ether sidearms, and therefore accessible to ring-bound cations, complexation for Na⁺ in methanol increased by an order of magnitude compared to the macrocycle lacking a sidearm.^{7,8} In other studies, it was found that when two amide residues are present in lariat ether sidearms, calcium over sodium cation selectivity is enhanced.⁹

When crown ethers are incorporated into a synthetic channel system¹⁰ such as the hydraphiles,¹¹ transport dynamics and complexation strengths are at odds with each other. Channels must obviously recognize a cation, but selectivity in the channel context means that a particular cation is passed most readily, not held most strongly. The hydraphiles are less structurally complex than are protein channels but many of their functions are similar. For example, they transport ions in planar bilayers,¹² in liposomes,¹³ and in the membranes of live organisms.¹⁴

The hydraphiles are constructed in four modules. Two distal diaza-18-crown-6 macrocycles serve as head groups for ion entry and egress. Covalent spacer chains link the two distal macrocycles to the central relay¹⁵ and define the channel's overall length. In the compounds studied here, the spacers are 1,12-dodecyl. Finally, sidearms are appended to the distal macrocycles. These sidearms were designed to serve as intra-membrane anchors to stabilize the channel in the bilayer.¹⁶ The compounds reported here incorporate the features described above but integrate amide residues at various positions within the channel. The influence of these strategically placed amide residues on sodium cation transport has been assayed analytically and *in vivo* and the results are reported below.

Results and Discussion

Compounds studied

Three new hydraphile structures were prepared for this study. They are illustrated as **2–4** along with the parent structure (**1**),¹⁷ which contains all of the same structural features but lacks any amide function. In compound **2** (C₁₂<N18N>C₁₁CO<N18N>COC₁₁<N18N>C₁₂), the amide residues are adjacent to the central relay. In **3** (C₁₂<N18N>COC₁₁<N18N>C₁₁CO<N18N>C₁₂), the amides are part of the proximal nitrogen atoms in the two distal macrocycles. The amides in **4** (C₁₁CO<N18N>C₁₂<N18N>C₁₂<N18N>COC₁₁) are part of the distal nitrogens in the same macrocycles. Thus, the three structurally distinct positions adjacent to macrocycles have been cataloged by this small library.

Synthetic access to 1–4

Different synthetic strategies were required for the preparation of **2**, **3**, and **4**. Channel **1**, although previously described,¹⁷ was synthesized for this study by the reduction of **4**. Channel **4** was made from the reaction of H<N18N>C₁₂<N18N>C₁₂<N18N>H with commercially available dodecanoic acid to afford a yellow solid in 79% yield. This compound was then reduced with BH₃ to afford **1** in 67% yield. Channel **2** was prepared by acylation of diazacrown¹⁸ with 12-bromododecanoyl chloride. The resulting dibromide was then alkylated with CH₃(CH₂)₁₁<N18N>H to afford **2** as a white powder. The route to hydraphile **3** reversed

the first steps used for compound **2**: diazacrown was dialkylated with 12-bromododecanoic acid to afford the diacid. This product was then converted to the acid chloride and reacted with C₁₂<N18N>H to afford **3** as an orange oil. All compounds were characterized by ¹H and ¹³C NMR and had satisfactory elemental analyses. Synthetic details are recorded in the Experimental Section.

Ion transport studies for 1–4

We have recently developed a method that uses ion selective electrodes (ISEs) to monitor sodium cation release from phospholipid vesicles (liposomes).¹³ Using this technique, a concentration-dependent set of release curves was obtained for each hydrophile (Na⁺ selective electrode, *see* Experimental Section for details). We note that the Na⁺ release profile for **1** has previously been reported in another context.¹⁹

The release profile for **4** is shown in Figure 2. Each line is the average of at least three separate determinations. The graph clearly shows the release of sodium cation from these phospholipid liposomes mediated by **4** in a concentration dependent fashion. When the concentration of **4** is ([**4**] =) 12 μM, ~60% of the total Na⁺ is released under these conditions. Total release is achieved in ~500 s when [**4**] = 36 μM. The concentration range in this experiment is from 2 μM to 36 μM or nearly 20-fold. The quality of the data and the broad concentration range give us considerable confidence in this method for comparing compound activity. It is interesting to note that channel **4** showed 10-fold higher Na⁺ transport at 12 μM than does the same concentration of the parent channel (**119**), which lacks amide bonds.

Since amide-containing channel **4** proved to be more active than **1**,¹⁹ comparisons with channels **2** and **3** were undertaken. These are structural variations in which the amide residues are adjacent to the central macrocycle (**2**) or on the proximal sides of the distal macrocycles (**3**). Sodium release from phospholipid vesicles mediated by **1–4** is shown in the graph of Figure 3.

At 12 μM , the percent release values determined for **1**, **2**, **3**, and **4** were 6%, 8%, 11%, and 60%, respectively. Based on these values, we can assign the following activity trend: **4** >> **3** > **2** > **1**. The concentration of 12 μM was chosen arbitrarily in order to simplify comparisons. As noted above, total release of Na^+ is observed in ~ 500 s when [**4**] = 36 μM . In contrast, **1**¹⁹ and **3** give full release in 500 s when their concentrations are 72 μM and 48 μM , respectively. While **4** is by far the most active compound in this series, the efficacies of compounds **1–3** can be differentiated at higher concentrations. Thus, while **2** and **3** are both more active than parent compound **1**, **3** exhibits higher Na^+ transport than does **2**.

Comparison with previously studied hydrapiles

We have previously obtained transport data for a few amide-containing hydrapile compounds.²⁰ In our two earlier studies, the goals were to assess sub-structural units other than the amide residue(s). As a consequence, comparisons of only two related structures are available. Moreover, the methods used to assay transport differed from the ISE technique used here. A final and problematic variable is that comparisons made by different methods were made against different standards. Notwithstanding these difficulties, the data are valuable because the syntheses of these compounds are complicated enough that a fully consistent series will be difficult to obtain. The compounds shown as **5–10** have previously been reported and may be compared pairwise.

Sodium transport was determined for **5–10** by using the method of Riddell and Hayer.²¹ In this approach, phospholipid vesicles are created in a sodium-containing buffer. Sodium-23 is an NMR active nucleus that may be observed directly. The vesicle suspension shows a single ²³Na line spectrum even though some cations are within the liposomes and most are in the bulk aqueous phase. When a dysprosium shift reagent is added to the suspension, external and internal Na⁺ are apparent as different NMR resonances. When the hydraphile is added, it inserts in the liposomal bilayer, and allows passive, equilibrium transport of both internal and external Na⁺ through the membrane. From changes in the two linewidths, an exchange constant may be calculated. This constant is compared with the exchange rate observed for the same system with a standard. In most of our previous work, the standard was the bacterial ion channel gramicidin. In other studies, it was the hydraphile we call “dansyl channel.” The latter is identical to **5** except that the terminal benzyl groups are replaced by dansyl (dimethylaminonaphthylsulfonyl) residues.

Data were obtained in two previous studies (shown in Table 1).²⁰ In the three cases where direct comparisons can be made, the presence of amide functions reduced sodium transport activity. Amide residues are adjacent to the medial macrocycle in all three of these cases (**6**, **8**, **10**). Compound **10** has carbonyl groups in positions that correspond to those in **4**. If the medial amides deactivate and the distal amides activate, then **9** and **10** should show similar activities. Indeed, **10** retains 74% of the activity of **9**. The amide position is not immediately adjacent to the distal macroring in **8**, which is only 65% as active as **7**. The most striking contrast in activity is between **5** and **6**. The latter loses two thirds of its activity when the medial macrocycle's nitrogens are amidated.

Cation binding data for macrocyclic lactams and lactones

Numerous macrocyclic amides have been reported and a lesser number of macrocyclic lactones have been prepared. Cation binding data have been reported for several of the latter, but the cation complexing ability of relatively few macrocyclic lactams is represented in the literature. One reason for this is that many lactams have been prepared as intermediates in the syntheses either of azacrowns or cryptands (D → C).^{3,22} As such, they were not studied directly for cation complexation. Moreover, an unsubstituted lactam reduces to a secondary azacrown and these typically show low binding affinities for cations in polar solvents.

The presence of an ester residue within the macrocycle does not significantly alter cation complexation strength or selectivity (*cf.* A, B).²³ This is expected because the carbonyl group is turned outward and the ester's ether oxygen is not strongly affected by the adjacent carbonyl. In contrast, an embedded amide confers upon the linkage both a partial positive charge and partial double bond character (*cf.* E, F). Thus, the principal donor is altered from nitrogen within the ring to carbonyl, which is turned outward. The formal double bond in a macrocyclic lactam also affects the macrocycle's rigidity. The lack of flexibility and structural adaptation is often detrimental to cation complexation. Of course, when the amide is present in a sidearm that can serve as a lariat ether donor, the effect is favorable.²⁴

Amide and amine geometry

In order to better understand the influence of amide and amine residues, we energy minimized *N*-butylmorpholine and *N*-morpholinobutyramide. Their structures are shown in Figure 4. The results are unsurprising but the key points deserve comment. First, in the case of *N*-butylmorpholine, the morpholine ring is in the chair conformation and the butyl chain is equatorial and fully staggered. The nitrogen is approximately tetrahedral and its electron pair is unhindered. The structure of *N*-morpholinobutyramide is quite different. The 6-membered ring is nearly planar, as is the amide residue. The amide oxygen is roughly in a plane with the morpholine ring but it is pointed away from it. Inversion of the morpholine ring would give a bidentate O and N donor array focused in the same direction. No conformational variation of the amide will permit the two oxygen donors to comprise a bidentate coordination array.

Amide residues and the hydrophile central relay

We have prepared several hydrophile derivatives in which the central macrocycle ("central relay") is replaced by other residues.¹⁵ From these studies, we concluded that the central relay served a water-binding function rather than directly coordinating a transient cation. This analysis comports with the studies conducted by MacKinnon and coworkers on the KcsA K⁺ channel of *Streptomyces lividans*.²⁵ Crown ether derivatives are known to bind water²⁶ as well as various cations.¹ Binding occurs by hydrogen bond formation between water and alternate oxygen or nitrogen donors within the macrocycle.

An 18-membered ring macrocycle is shown in which the donors are numbered 1–6. Water may bridge positions 1–3, 1–5, 2–4, 2–6, 3–5, and 4–6. If donors 1 and 4 are removed as by amidation of nitrogens at those positions, the only alternate H-bond sites remaining for water would be 2–6 and 3–5. Thus, binding of water would be less effective when the amides are part of the central macrocycle. In turn, the absence of an effective central relay for cations should diminish transport, as observed in all of these cases.

Sidearm amide residues

Conversion of a sidearm amine to its corresponding amide will have two obvious consequences. We know from previous studies that the macroring nitrogens of dibutyldiaza-18-crown-6 have pK₁ and pK₂ values in the range 7–9.¹⁷ Protonation of macroring nitrogen is expected to diminish cation transport. Transport reduction most likely would result from charge-charge

repulsion between the protonated nitrogen and the transient cation. Protonation will also quaternize the nitrogen to which it is attached, diminishing flexibility. This could have either positive or negative consequences depending upon the conformational requirements for transport. In these dynamic systems, as for protein channels, this issue remains largely unresolved.

The dramatic difference in transport efficacy between **3** and **4** suggests that stabilization and rigidification of the sidearm is more important than a corresponding alteration to the inner distal nitrogen atoms. The presence of an amide in either position should make the amide nitrogen atom both a poorer donor and a more rigid functional group. Indeed, both effects may be operating but the influence is clearly greater in the outer distal nitrogen position. The hydrapiles were conceived as flexible molecules that could adapt to the existing bilayer structure as necessary. This study suggests that the flexibility of the dodecyl sidechain is not necessarily a positive attribute in these systems. Better Na⁺ transport is observed when the sidearm is conformationally restricted, even marginally.

Biological studies

Studies of sodium cation transport **1–10** in synthetic liposomes show that amide bonds can dramatically affect hydrapile activity. In previous studies of non-amide hydrapiles, bacterial toxicity data showed a remarkable correspondence to hydrapile activity.²⁷ We thus undertook similar biological studies using compounds **1–4**. The minimum inhibitory concentrations for **1–4** against *E. coli* and *B. subtilis* are recorded in Table 2.

The results shown in Table 2 do not correspond well with Na⁺ transport rate data, as was the case for their saturated analogs.²⁸ While compound **1** is active against *E. coli* (2 μM), its amide-containing counterparts (**2–4**) are at least 16-fold less cytotoxic (34–67 μM) to this bacterium. We note that in a previous comparative study²⁸ involved hydrapiles in which the sidechains were benzyl groups and only the overall lengths were varied. The compounds examined here have alkyl chain side arms and also possess integral amides. The results obtained in the present study confirm the expectation that small structural changes in hydrapile structure can mediate large changes in biological activity. Even so, it is surprising that compounds **2–4** were active in the 0.5 to 2 μM range to *B. subtilis* but inactive to *E. coli*. The differences in activity are shown in the graph of Figure 5. Note that a higher value indicates better transport but lower toxicity.

The differences in toxicity to these two microorganisms may reflect the disparity in membrane structure. Gram-positive *B. subtilis* have a cytoplasmic membrane surrounded by a thick peptidoglycan cell wall. Gram-negative *E. coli* have a cytoplasmic membrane, thin peptidoglycan layer, and an additional lipopolysaccharide membrane.²⁸ These membranes are very different from each other and from the synthetic liposomes tested previously. Notwithstanding these differences in organisms, the correspondence between ion transport activity and biological activity for non-amide containing crowns stands in contrast to the results obtained previously for non-amidic, benzyl side armed hydrapiles.

Conclusion

The studies reported here present evidence for enhanced Na⁺ ion transport activity of amide-containing hydrapiles compared to analogous structures lacking the amide residues. The data obtained presented two surprises. First, we anticipated that when amide donors replaced amines, the diminished molecular flexibility would impair ion transport. Instead, more flexible **1** showed significantly lower transport than that observed for **4**. Second, the previously observed correspondence between cation transport rates and biological activity is not apparent in this series of structures. The compounds previously studied possessed only saturated oxygen

and/or nitrogen and the present channels contain amide functional groups. This accounts for, but does not explain, the differences in activity, which are under further investigation.

Experimental Section

General

^1H -NMR were recorded at 300 MHz in CDCl_3 solvents and are reported in ppm (δ) downfield from internal TMS 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).

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 MWH Laboratories, Phoenix, AZ, and are reported as percents. Where sodium is factored into the analytical data, high resolution mass spectroscopy (Washington University) confirmed its presence.

Vesicle experiments

Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and the inorganic salts NaCl and cholineCl were all purchased from Sigma-Aldrich. The water used was Milli-Q Plus quality, which is essential to avoid salt contamination in the buffer systems. *n*-Octylglucoside was purchased from CalBioChem.

The vesicles used were prepared using the reverse evaporation method of Szoka and Papahadjopoulos.²⁹ 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids as a chloroform solution, which was dried to a lipid film and stored at ambient temperature under high vacuum. 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. Solvent was removed (*in vacuo*) and the solution was passed through a mini extruder containing a 0.2 μm 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 reported³⁰ and vesicle size was measured by a Coulter N4MD submicron particle analyzer. The vesicles used in the transport studies had diameters of ~200 nm.

Na^+ transport measurements

All data were collected by Axoscope 7.0 using a Digidata 1322A series interface. Sodium transport was measured using a Micro-Combination pH/sodium electrode (Thermo-Orion) in aqueous sodium-free buffer (750 mM cholineCl/15 mM HEPES, pH 7.0). After equilibration in external buffer, the electrode was placed in a 5-mL disposable beaker containing external buffer and vesicle solution to achieve a lipid concentration of 0.4 mM and a total volume of 2 mL. In order to set the baseline, the voltage output was recorded for five minutes prior to addition of the channel. The channel solution in 2-propanol was added and channel conductivity

was measured over an appropriate time range, typically 25 minutes. Addition of 200 μL 10% aqueous *n*-octylglucoside induced complete lysis of the vesicles to achieve total sodium release. All experiments were performed at room temperature.

Data analysis

The data were collected in units of mV and converted to units of concentration, using the appropriate electrode calibration curve. The data were normalized (OriginPro 7) to the total sodium concentration, as determined by lysing the vesicles with *n*-octylglucoside.

Biological studies

A series of test tubes was prepared, in which each contained 2 mL of Luria Bertani (LB) Miller media (10 $\text{g}\cdot\text{L}^{-1}$ peptone, 5 $\text{g}\cdot\text{L}^{-1}$ yeast extract, 10 $\text{g}\cdot\text{L}^{-1}$ NaCl). Each series contained a set of serial 2-fold dilutions of an individual hydrophile compound. 1×10^6 colony forming units (C.F.U.) of bacteria were added to the 2 mL aliquots to give an inoculum of 5×10^5 C.F.U./mL, as outlined by the NCCLS.³¹ The MIC was taken as the lowest hydrophile concentration that inhibited growth after 24 h, as judged by visual turbidity. Each MIC value was confirmed by three independent trials for each concentration and bacterium tested.

N,N-bis{12-[*N*-(*N*-dodecyl)-diazia-18-crown-6]-dodecyl}-diazia-18-crown-6

$\text{C}_{12}\text{H}_{25}\langle\text{N18N}\rangle(\text{CH}_2)_{12}\langle\text{N18N}\rangle(\text{CH}_2)_{12}\langle\text{N18N}\rangle\text{C}_{12}\text{H}_{25}$, 1—was prepared from the reduction of **4** (0.35 g, 0.23 mmol, see below) in $\text{BH}_3\cdot\text{THF}$ (6 mL). The reaction was stirred (rt, N_2) for 3 d. Dropwise addition of H_2O quenched the remaining hydride and the reaction was concentrated under reduced pressure. The resulting white solid was dissolved in 24% HBr (10 mL) and heated to reflux for 3 h. The pH was adjusted to ~ 9 by addition of NaOH pellets at 0 $^\circ\text{C}$. The aqueous solution was extracted 3x with CH_2Cl_2 , washed with brine (2×50 mL) and NaHCO_3 (50 mL), dried over Mg_2SO_4 , and the solvent removed *in vacuo* to afford **1** (240 mg, 67%) as white crystals, mp 60–61 $^\circ\text{C}$ (lit: 61–63 $^\circ\text{C}$ ¹⁷). ^1H -NMR: 0.86 (6H, t, $J = 6.9$), 1.24 (68H, s), 1.42 (12H, m), 2.46–2.51 (12H, m), 2.76–2.80 (24H, m), 3.58–3.62 (48H, m). ^{13}C -NMR: 14.01, 22.60, 27.03, 27.44, 29.26, 29.57, 31.84, 53.93, 55.90, 69.81, 70.67.

$\text{H}_{25}\text{C}_{12}\langle\text{N18N}\rangle(\text{CH}_2)_{11}\text{CO}\langle\text{N18N}\rangle\text{CO}(\text{CH}_2)_{11}\langle\text{N18N}\rangle\text{C}_{12}\text{H}_{25}$, 2

***N*-Dodecyl-4,13-diazia-18-crown-6, $\text{C}_{12}\langle\text{N18N}\rangle\text{H}$** —A solution of 4,13-diazia-18-crown-6 (1.0 g, 3.81 mmol), 1-bromododecanoic acid (0.85 mL, 3.43 mmol), Na_2CO_3 (4.08 g, 0.69 mol), and KI (cat.) in *n*-PrCN was heated to reflux for 1.5 h. The reaction was cooled, filtered and concentrated. The product was purified (SiO_2 , 2% Et_3N /acetone $\rightarrow$ 10% Et_3N /acetone) to afford 500 mg (32%) of a yellow solid (mp 34 $^\circ\text{C}$, lit¹⁷ 33.5 $^\circ\text{C}$) ^1H NMR: 0.84 (3H, t), 1.22 (18H, s), 1.50 (2H, bt), 2.49 (2H, t), 2.72–2.81 (16H, m). ^{13}C -NMR: 14.10, 22.78, 27.41, 28.43, 29.70, 31.92, 50.01, 55.87, 69.07, 70.28, 71.25.

$\text{Br}(\text{CH}_2)_{11}\text{CO}\langle\text{N18N}\rangle\text{CO}(\text{CH}_2)_{11}\text{Br}$ —Oxaloyl chloride (2.72 g, 21.5 mmol) was added (0 $^\circ\text{C}$, N_2) to a solution of 12-bromododecanoic acid (1.00 g, 3.58 mmol) in anhydrous CH_2Cl_2 (20 mL). The reaction was stirred at ambient temperature for 2 h. The reaction was concentrated and dried under high vacuum for 2 h. The residue was dissolved in CH_2Cl_2 and added (0 $^\circ\text{C}$, N_2) to a solution of 4,13-diazia-18-crown-6¹⁸ (0.57 g, 1.63 mmol), Et_3N (0.45 g, 6.52 mmol), and 4-dimethylaminopyridine (cat.) in anhydrous CH_2Cl_2 (30 mL). The reaction was stirred (rt) for 20 h. The reaction mixture was concentrated, dissolved in CHCl_3 , washed with aq. citric acid (2×25 mL), satd Na_2CO_3 (2×25 mL), and brine solution (2×25 mL). The filtrate was dried over Mg_2SO_4 and concentrated to afford a slightly yellow solid (1.10 g, 77%, mp 77–78 $^\circ\text{C}$) that was used without further purification. ^1H NMR: 1.27–1.43 (32H, *pseudo*-s), 1.61 (4H, quartet), 1.84 (4H, quartet), 2.31 (4H, d), 3.40 (4H, t), 3.57–3.70 (16H, m).

$\text{H}_{25}\text{C}_{12}<\text{N18N}>(\text{CH}_2)_{11}\text{CO}<\text{N18N}>\text{CO}(\text{CH}_2)_{11}<\text{N18N}>\text{C}_{12}\text{H}_{25}$, **2**

A solution of $\text{Br}(\text{CH}_2)_{11}\text{CO}<\text{N18N}>\text{CO}(\text{CH}_2)_{11}\text{Br}$ (0.76 g, 0.87 mmol), $\text{C}_{12}\text{H}_{25}<\text{N18N}>\text{H}$ (0.75 g, 1.74 mmol), Na_2CO_3 (3.69 g, 34.8 mmol), and KI (20 mg) in *n*-PrCN (40 mL) was heated at reflux (N_2) for 4 days. The reaction mixture was cooled, filtered, and concentrated. The resulting residue was dissolved in CHCl_3 (50 mL), washed with aqueous 5% NaHCO_3 (2×25 mL), brine (2×25 mL), and dried over Mg_2SO_4 . The filtrate was concentrated to obtain a brown oil. The product was purified by column chromatography (SiO_2 , acetone $\rightarrow$ 2% Et_3N /acetone) and crystallized from acetone at 4°C to afford 100 mg of white crystals (mp $51-52^\circ\text{C}$). ^1H NMR: 0.88 (6H, t), 1.25 (68H, s), 1.46 (8H, t), 1.66 (4H, quartet), 2.31 (4H, t), 2.50 (8H, t), 2.80 (16H, m), 3.58–3.64 (56H, m). ^{13}C -NMR: 14.10, 22.67, 22.95, 23.71, 25.33, 27.27, 28.88, 29.48, 29.52, 29.60, 30.33, 31.89, 33.15, 38.69, 46.81, 48.71, 53.44, 68.12, 69.73, 70.15, 70.50, 70.82, 173.31. *Anal.* calcd for $\text{C}_{84}\text{H}_{166}\text{N}_6\text{O}_{14}$: C, 67.97; H, 11.27; N, 5.66%. Found: C, 67.77; H, 11.36; N, 5.70%.

$\text{C}_{12}\text{H}_{25}<\text{N18N}>\text{CO}(\text{CH}_2)_{11}<\text{N18}>(\text{CH}_2)_{11}\text{CO}<\text{N18N}>\text{C}_{12}\text{H}_{25}$, **3**

$\text{CH}_3\text{OCO}(\text{CH}_2)_{11}<\text{N18N}>(\text{CH}_2)_{11}\text{COOCH}_3$ —A solution of 4,13-diaza-18-crown-6¹⁸ (0.406 g, 1.55 mmol), $\text{Br}(\text{CH}_2)_{11}\text{COOCH}_3$ (1.00 g, 3.41 mmol), Na_2CO_3 (3.62 g, 34.1 mmol), and KI (20 mg) in 40 mL *n*-PrCN was heated to reflux for 2 days. The mixture was filtered and concentrated. The residue was dissolved in Et_2O and the resulting solid removed by filtration. The product was purified by column chromatography (SiO_2 , acetone $\rightarrow$ 2% Et_3N /acetone) to afford a white solid (0.63 g, 59%, mp $45-46^\circ\text{C}$). ^1H NMR: 1.22 (28H, s), 1.42 (4H, t), 1.58 (4H, t), 2.27 (4H, t), 2.49 (4H, t), 2.78–2.80 (8H, m), 3.58–3.63 (16H, m). ^{13}C NMR: 24.81, 27.05, 27.30, 29.08, 29.11, 29.32, 29.40, 33.91, 51.34, 53.89, 55.90, 69.99, 70.60, 174.41.

$\text{HOOC}(\text{CH}_2)_{11}<\text{N18N}>(\text{CH}_2)_{11}\text{COOH}$ —The diazacrown diester (0.63 g, 0.91 mmol) was dissolved in 2N NaOH (20 mL) and heated to reflux for 24 h. The pH was adjusted to ~ 4 with 2N HCl . The organic layer was extracted with MeCN (3×50 mL), dried over Mg_2SO_4 and concentrated to afford 0.56 g (93%) of a white solid, mp $242-244^\circ\text{C}$. ^1H NMR (D_2O): 1.05–1.10 (28H, bs), 1.34 (4H, quartet), 1.49 (4H, m), 2.12 (4H, t), 3.00 (4H, t), 3.24 (8H, t), 3.50 (8H, s), 3.62 (8H, s). ^{13}C NMR (CD_3OD): 24.4, 26.1, 27.6, 30.2, 30.4, 35.1, 54.2, 55.4, 65.8, 71.4, 174.4.

$\text{C}_{12}\text{H}_{25}<\text{N18N}>\text{CO}(\text{CH}_2)_{11}<\text{N18}>(\text{CH}_2)_{11}\text{CO}<\text{N18N}>\text{C}_{12}\text{H}_{25}$, **3**

DMF (2 drops) and oxaloyl chloride (213 mg, 1.68 mmol) were added (N_2 , 0°C , dropwise) into a solution of the diazacrown diacid (180 mg, 0.27 mmol) in CH_2Cl_2 (20 mL). The reaction was stirred at rt for 2 h. The yellow solution was concentrated under vacuum and dried under high vacuum for 1 h. The residue was added (N_2 , 0°C , dropwise) to a solution of *N*-dodecyl-4,13-diaza-18-crown-6 (260 mg, 0.6 mmol), Et_3N (165.8 mg, 2.4 mmol), and 4-dimethylaminopyridine (20 mg, cat.) in anhydrous CH_2Cl_2 (20 mL). The reaction was stirred at rt for 2 d. The solvent was removed and the residue washed with 5% Na_2CO_3 (2×25 mL), followed by brine solution (2×25 mL). The organic phase was dried over Mg_2SO_4 , filtered and concentrated. The product was purified over SiO_2 (2% Et_3N /acetone) to afford the product as a yellow oil (40 mg). ^1H -NMR: 0.87 (6H, t), 1.25 (64H, bs), 1.43 (8H, quartet), 1.61 (4H, quartet), 2.31 (4H, t), 2.48 (8H, t), 2.78 (16H, m), 3.59–3.64 (56H, m). ^{13}C -NMR: 14.10, 22.66, 25.32, 27.20, 27.49, 29.32, 29.51, 29.61, 31.89, 33.18, 46.84, 46.98, 48.81, 53.87, 56.02, 69.56, 69.99, 70.43, 70.53, 70.72, 173.34 ppm. *Anal.* calcd for $\text{C}_{84}\text{H}_{166}\text{N}_6\text{O}_{14} \cdot 2\text{Na}$: C, 65.93; H, 10.93; N, 5.49%. Found: C, 66.00; H, 11.04; N, 5.49%. Complexation of 2 Na^+ ions was confirmed by high-resolution mass spectroscopy.

$C_{11}H_{23}CO<N18N>(CH_2)_{12}<N18N>(CH_2)_{12}<N18N>COC_{11}H_{23}$, **4**

Dodecanoic acid (0.2 g, 1.03 mmol) was dissolved by the slow addition of $SOCl_2$ (4 mL, 6.5 mmol) at 0 °C. The reaction was heated to reflux (N_2) for 1 h. The $SOCl_2$ was removed *in vacuo* and the residue washed with toluene (2×5 mL). The resulting acid chloride was dissolved in toluene (5 mL) and added dropwise (0 °C, N_2) to a toluene solution of $H<N18N>(CH_2)_{12}<N18N>(CH_2)_{12}<N18N>H^{17}$ (0.5 g, 0.45 mmol), dry Et_3N (100 μ L) and 4-dimethylaminopyridine (cat.). The reaction was stirred at rt for 2 d. It was filtered, concentrated *in vacuo*, and washed with satd. $NaHCO_3$ (2×25 mL) and brine (2×25 mL). The product was purified by column chromatography (SiO_2 , 2% Et_3N in acetone) to afford **4** (520 mg, 79%) as a yellow solid, mp 48–49 °C. 1H -NMR: 0.87 (6H, t, $J = 6.9$), 1.24 (64H, *pseudo-s.*), 1.42 (8H, m), 1.61 (4H, m), 2.28–2.33 (4H, m), 2.44–2.49 (8H, m), 2.75–2.80 (16H, m), 3.58–3.66 (56H, m). ^{13}C -NMR: 14.07, 22.63, 25.33, 27.18, 27.47, 29.29, 29.46, 29.58, 31.86, 33.12, 46.89, 48.77, 53.87, 56.01, 69.62, 69.96, 70.08, 70.25, 70.32, 70.47, 70.58, 70.70, 70.96, 173.34. *Anal.* calculated for $C_{84}H_{166}N_6O_{14}$: C, 67.97; H, 11.27; N, 5.66%. Found: C, 68.02; H, 11.36; N, 5.60%.

Acknowledgement

We thank the NIH for a grant (GM-36262) that supported this work and for Training Grant Fellowships under grants T32-GM08785 and T32-GM04892 to MEW and WML, respectively.

References

- Inoue, Y.; Gokel, GW., editors. *Cation Binding by Macrocycles*. Marcel Dekker; New York: 1990. p. 761
- Gokel, GW., editor. *Crown Ethers and Cryptands*. 3. The Royal Society of Chemistry; London, England: 1991. p. 190
- Gokel, GW.; Korzeniowski, SH. *Macrocyclic Polyether Syntheses*. Springer-Verlag; Berlin: 1982. p. 410
- Choi H-J, Kwak M-O, Kim J-M. *Tetrahedron Lett* 1997;38:6217–6220.
- Kumar S, Hundal MS, Kaur N, Singh R, Singh H. *J. Org. Chem* 1996;61:7819–7825. [PubMed: 11667739]
- Kumar S, Singh R, Singh H. *J. Chem. Soc. Perkin Trans. 1* 1992:3049–3053.
- Kataky R, Parker D, Teasdale A, Hutchinson JP, Buschmann H-J. *J. Chem. Soc. Perkin Trans. 1* 1992:1347–1351.
- Hernandez JC, Trafton JE, Gokel GW. *Tetrahedron Lett* 1991:6269–6272.
- a White BD, Arnold KA, Gokel GW. *Tetrahedron Lett* 1987:1749–1752. b White BD, Fronczek FR, Gandour RD, Gokel GW. *Tetrahedron Lett* 1987:1753–1756.
- a Gokel GW, Murillo O. *Acc. Chem. Res* 1996;29:425–432. b Gokel GW, Mukhopadhyay A. *Chem. Soc. Reviews* 2001;30:274–286. c Gokel GW, Schlesinger PH, Djedovic NK, Ferdani R, Harder EC, Hu J, Leevy WM, Pajewska J, Pajewski R, Weber ME. *Bioorg. Med. Chem* 2004;12(6):1291–304. [PubMed: 15018901]
- a Gokel GW. *Chem. Comm* 2000:1–9. b Gokel GW. *Cell Biochem Biophys* 2001;35(3):211–31. [PubMed: 11894842]
- Murillo O, Suzuki I, Abel E, Murray CL, Meadows ES, Jin T, Gokel GW. *J. Am. Chem. Soc* 1997;119:5540–5549.
- Weber ME, Schlesinger PH, Gokel GW. *J. Am. Chem. Soc* 2005;127:636–642. [PubMed: 15643888]
- Leevy WM, Huettner JE, Pajewski R, Schlesinger PH, Gokel GW. *J. Am. Chem. Soc* 2004;126:15747–15753. [PubMed: 15571397]
- Murray CL, Shabany H, Gokel GW. *Chem. Comm* 2000:2371–2372.
- Nakano A, Xie Q, Mallen JV, Echegoyen L, Gokel GW. *J. Am. Chem. Soc* 1990;112:1287–9.
- Murillo O, Watanabe S, Nakano A, Gokel GW. *J. Am. Chem. Soc* 1995;117:7665–7679.

18. Gatto VJ, Miller SR, Gokel GW. *Organic Syntheses* 1989;68:227–233.
19. Meyer AE, Leevy WM, Pajewski R, Suzuki I, Weber ME, Gokel GW. *Bioorg. Med. Chem* 2005;13:3321–3327. [PubMed: 15809167]
20. a Shabany H, Gokel GW. *Chem. Comm* 2000:2373–2374. b Shabany H, Pajewski R, Abel E, Mukhopadhyay A, Gokel GW. *J. Heterocyclic Chem* 2001;38:1393–1400.
21. Riddell FG, Hayer MK. *Biochem. Biophys. Acta* 1985;817:313–317. [PubMed: 4016108]
22. Bradshaw, JS.; Krakowiak, KE.; Izatt, RM. *Aza-Crown Compounds*. 51. Wiley; New York: 1993. p. 885
23. Izatt RM, Pawlak K, Bradshaw JS. *Chem. Rev* 1995;95:2529–2586.
24. Arnold KA, Hernandez JC, Li C, Mallen JV, Nakano A, Schall OF, Trafton JE, Tsesarskaja M, White BD, Gokel GW. *Supramol. Chem* 1995;5:45–60.
25. Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R. *Science* 1998;280:69–77. [PubMed: 9525859]
26. Gokel GW, Garcia BJ. *Tetrahedron Lett* 1977:317–20.
27. Leevy WM, Weber ME, Schlesinger PH, Gokel GW. *Chem. Commun. (Camb)* 2005:89–91. [PubMed: 15614382]
28. Reaveley DA, Burge RE. *Adv. Microb. Phys* 1972:1–81.
29. Szoka FJ, Papahadjopoulos D. *Proc. Natl. Acad. Sci. USA* 1978;75:4194–4198. [PubMed: 279908]
30. Stewart JCM. *Anal. Biochem* 1980;104:10–14. [PubMed: 6892980]
31. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow Aerobically*. 5th edn.. NCCLS, Wayne; Pennsylvania: 2000. p. M7-A5.

Figure 1.

Caption: Resonance forms (A and B) and CPK molecular model of 3-oxo-4-aza-15-crown-5.

Figure 2.

Caption: Fractional Na⁺ release from phospholipid vesicles mediated by **4**. Transporter concentrations are, from bottom to top, 2, 4, 8, 12, 20, and 36 μM. The concentration of DOPC vesicles was 0.4 mM.

Figure 3.

Caption: Fractional Na⁺ release, determined by the ISE method, for **1–4** (12 μ M each). The concentration of DOPC vesicles was 0.4 mM. Total Na⁺ release of $\leq 11\%$ was observed for **1–3**.

Figure 4.

Caption: Framework molecular models of *N*-butylmorpholine (top) and *N*-morpholinobutyramide (bottom)

Figure 5.

Caption: Graphical illustration of the relationship between Na^+ transport (open squares) and toxicity of compounds **1–4** (X axis) to *E. coli* (filled circles) and *B. subtilis* (open circles). The units for biological activity are micromolar and for ion transport that are percent release from liposomes at 1500 s.

Table 1

Sodium transport activity loss between hydrophile pairs in which amides replace amine residues

<i>Cpd. No.</i>	<i>Function</i>	<i>Amide placement</i>	<i>Relative Activity</i>
5	X=H ₂	None	100% ^a
6	X=O	Medial	33% ^a
7	X=H ₂	None	100% ^a
8	X=O	Medial, lariat	65% ^a
9	X=H ₂	None	100% ^b
10	X=O	Medial, distal	74% ^b

^a See ref. 20a.^b See ref. 20b.

Table 2MIC^a (μM) of 1–4 to *E. coli* and *B. subtilis*

Compound Number	<i>E. coli</i>	<i>B. subtilis</i>
1	2.1	0.5
2	>67	0.5
3	34	2
4	>67	1

^aMinimum Inhibitory Concentration