The Effect of Twin-tailed Sidearms on Sodium Cation Transport in Synthetic Hydraphile Cation Channels

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The preparation of novel tris(macrocycle)s that transport Na^+ through phospholipid bilayers is reported. All of the reported structures have the following structural elements: sidechain-macrocycle-spacer-macrocycle-spacer-macrocycle-sidechain. The overall extension of the channel system is defined by the lengths of the spacer chains. The flexible sidearms are thought to be aligned with the lipid axis and opposite to the spacer chains. To the extent the spacer chains contribute to organization of the structure and isolation of the cation-containing pore from the surrounding lipid chains, more hydrophobic chains are expected to enhance cation transport. A comparison of $C_{12}H_{25}$<N18N>$(CH_2)_{12}$<N18N>$(CH_2)_{12}$<N18N> $C_{12}H_{25}$, **1**, with $(C_{12}H_{25})_2N(CH_2)_2$<N18N>$(CH_2)_{12}$<N18N>$(CH_2)_{12}$<N18N>$(CH_2)_2N(C_{12}H_{25})_2$, **11**, shows that the Na^+ transport is, indeed, enhanced.

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Introduction.

During the past decade, a number of synthetic channel compounds have been developed. These include synthetic peptides, non-peptide channels and hybrid peptide/-non-peptide structures. The types of compounds that have been prepared and studied can generally be grouped into relatively few categories. Synthetic peptides have been studied most extensively in the laboratories of Mutter [1], Montal [2], and DeGrado [3]. Tomich and coworkers have recently reported related efforts [4]. These important efforts are less relevant to the subject of the present work and will not be further discussed despite their obvious importance.

The first non-peptidic, synthetic ion channel was designed and prepared by Tabushi and coworkers [5]. This pioneering effort used β-cyclodextrin as a "head-group" element, attached to hydrocarbon tails that served to insert in and presumably disrupt the fatty acid chains of membrane lipids. Each subunit comprised a "pore" or "tunnel" within a single leaflet of the bilayer. It was speculated that two of these synthetic, cyclodextrin-based channel elements aligned to afford a transmembrane pore. Tabushi and coworkers demonstrated transmembrane Co(II) flux in this system. Although Co(II) transport is of marginal biological relevance, the effort remains significant and visionary.

An interesting group of ionophores has been reported that mediate proton and alkali metal cation transport across phospholipid bilayers. These are remarkably effective considering their structural simplicity. Despite their efficacy, to our knowledge, the mechanism(s) by which they function remains unknown. In an early example, Menger and coworkers reported that $CH_3(CH_2)_{10}COO(CH_2CH_2O)_5CH_2Ph$ [6] promoted greater proton flux than did gramicidin under comparable conditions when using the method of Fendler and Kano to assess it [7].

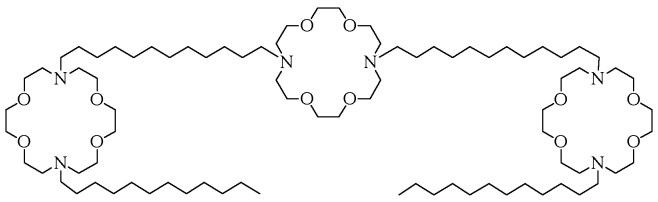
Kobuke and coworkers reported a further example of a strikingly simple ion transporter. It involved a complex of glycolate ethers of monoalkyloligo(1,4-butylene glycol) and dioctadecyldimethylammonium cation to foster Na^+ and K^+ transport in a phospholipid bilayer [8]. The latter studies have been extended to channels that have an oligoether chain attached to the ammonium head group and the hydrophobic counteranion is provided either by stearic acid or by phospholipid headgroups [9]. Regen and coworkers reported a steroid-based ionophore in which 5-androstene was esterified in the 3β and 17β positions by $OCO(CH_2CH_2O)_nH$ groups. The pegylated steroid exhibited only modest Na^+ transport [10].

The structures described above are essentially "thread-like" molecules speculated to span the bilayer and to provide a diffusion pathway for cations. In this connection, the poly-THF compound prepared by Koert and coworkers should be mentioned [11]. This compound was characterized by using the bilayer clamp technique and exhibited "spiking" behavior instead of the well-behaved properties of gramicidin. The structural design was refined by incorporating gramicidin-like Leu-Trp repeats [12] and a covalent tartaric acid scaffold [13].

A number of more complex channel-forming structures have also been devised and reported. Many of these incorporate crown ethers [14] as basic elements. Nolte and coworkers prepared a tunnel-like oligomer of a crown ether-isonitrile that was one of the earliest examples of this ilk. As Tabushi and coworkers had done, they demonstrated Co(II) transport for this system [15].

Voyer and Robitaille [16] devised a molecular tunnel in which 21-crown-7 residues were incorporated as peptide sidechains in a Leu-(CrF-Leu-Leu-Leu-CrF- Leu)$_3$ peptide. The peptide was known to prefer an α-helical conformation, which meant that the crown amino acids (CrF) were aligned on the same side of the chain. Cation

transport was assessed in phospholipid bilayers but relatively little selectivity was observed among the cations Li^+, Na^+, K^+, and Rb^+. This is presumably due to the large size of 21-crown-7 relative to the cations.



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Lehn and Fyles and their respective coworkers reported macrocycle-based channel systems almost simultaneously. In the Lehn and Jullien channel, a tartaric acid derived macrocycle was used as a central scaffold to which were appended multiple, membrane-disrupting chains [17]. The Fyles channel also used a tartaric-acid-derived macrocycle but the overall structure was inspired by the putative channel formed by the antifungal agent amphotericin. Fyles and coworkers developed a family of ionophore structures and reported their proton transport rates in a phosphoplipid bilayer [18].

Quite a different approach was adopted by Ghadiri and coworkers who used alternating D- and L-amino acids to construct cyclic peptides. These organized into "nanotubes" that exhibited cation channel properties. An example is cyclo[(Trp-D-Leu)$_3$Gln-D-Leu-] which, if planar, would have an internal diameter of about 7.5 Å. It was surmised that these "disks" stacked into a transmembrane tube as a result of H-bond interactions [19].

Another family of structures has been reported by Matile and coworkers, who have demonstrated ion transport in some cases [20]. These innovative molecules have been called "rigid-rods" and "π-slides." Cation transport has been studied in phospholipid membranes.

Our own cation channel design resulted in a family of molecules that we have called "hydraphiles" [21]. The first examples of this family used three 4,13-diaza-18-crown-6 macrocycles as key elements connected by covalent spacers and terminated by flexible sidechains. The first compound we prepared had the structure shown as **1**, which may also be represented in shorthand as C_{12}<N18N>C_{12}<N18N>C_{12}<N18N>C_{12}. Both the connector and spacer chains are *n*-dodecyl groups and span a distance, when fully extended, of about 14 Å. This means that the entire structure is about 40 Å long and spans the insulator regime of the phospholipid bilayer [22]. We have recently demonstrated that when the two sidearms are covalently linked, a substantial enhancement of ionophoretic activity is observed [23]. Such compounds, although more effective ionophores, are significantly more difficult to prepare because they are such large macroheterocycles. In the present report, we explore the effect of sidearm alterations as a means of enhancing ionophoretic activity.

Results and Discussion.

Compound **1**, shown above, was the first representative of our hydraphile family of channels. Extensive studies have characterized the headgroup position [24] and function [25], the orientation [26] of the central macrocycle, and the channel's aggregation state within the phospholipid bilayer [27]. As noted above, linking the sidechains improved ionophoretic activity. This was accomplished by spanning the unlinked chains of **1** with a fourth diaza-18-crown-6 unit. Thus, inferences concerning restrictions of the sidechains involve two variables.

In order to address this question directly, we proposed to prepare the analog of **1** in which twin sidechains replaced the single dodecyl unit. The target molecule was **11**, which we anticipated would be accessible from **8** by alkylation and reduction.

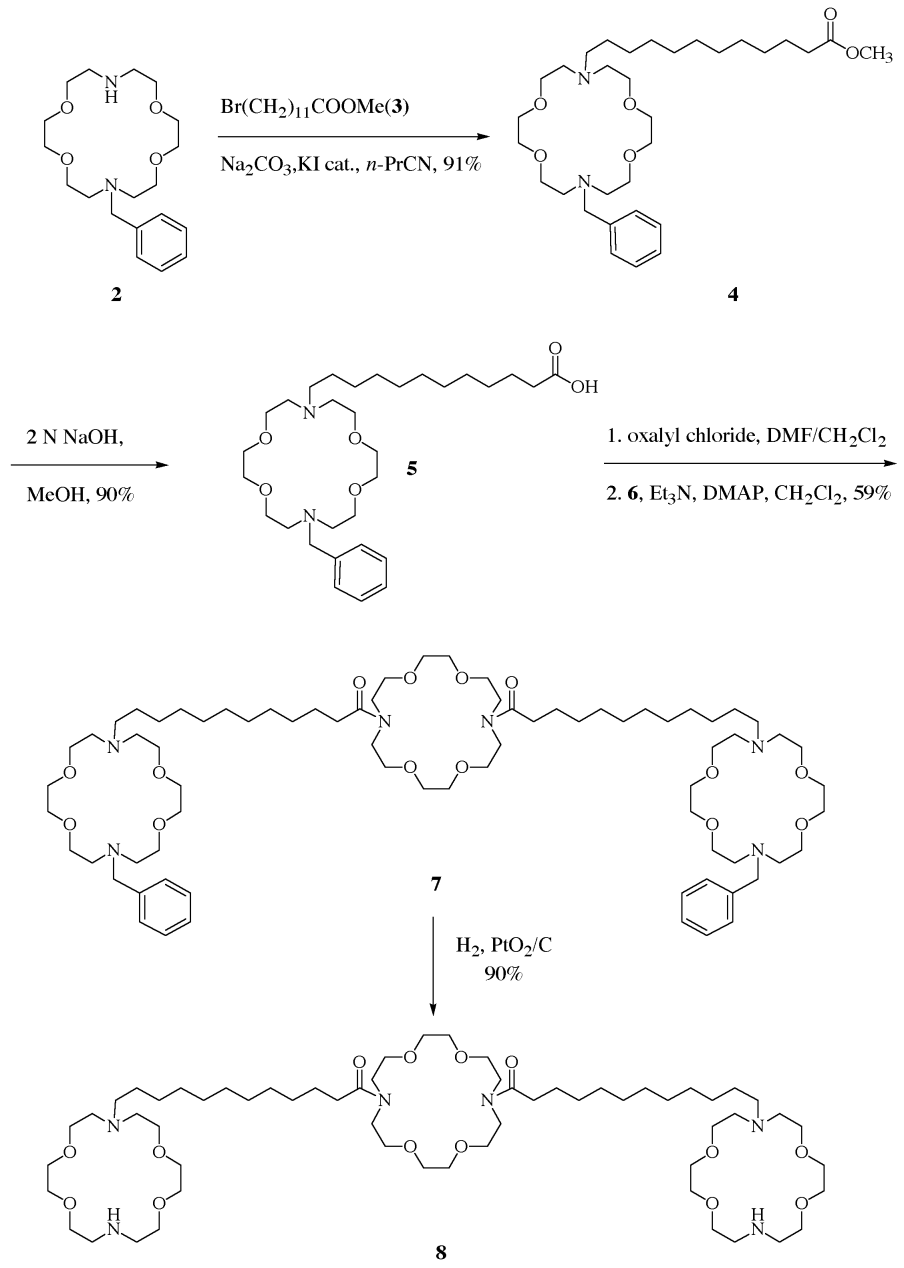
Synthetic Access.

The preparation of **11** (Schemes 1 and 2) was accomplished as follows. Monobenzyl-4,13-diaza-18-crown-6 [28] (**2**, PhCH$_2$<N18N>H) was alkylated with methyl 12-bromododecanoate [29] **3** in the presence of Na_2CO_3 and a catalytic amount of KI. Ester **4** was hydrolyzed (aqueous NaOH) to afford free acid **5**. The tris(macrocyclic) diamide, **7**, was synthesized by acylation of 4,13-diaza-18-crown-6 (H<N18N>H, **6**) by reaction with the acyl chloride derivative of **5**, which was obtained by treatment of **5** with oxalyl chloride (catalytic DMF). Hydrogenolysis deprotected the macrocycle **7** to afford **8**.

Alternate Synthesis of Channel **7**.

The central unit, Br(CH$_2$)$_{11}$CO<N18N>CO(CH$_2$)$_{11}$Br was prepared by acylation of 4,13-diaza-18-crown-6 with 2 equivalents of 12-bromododecanoic acid in the presence of thionyl chloride. The resulting dibromide was then treated with **2** in butyronitrile in the presence of Na_2CO_3 and KI. After purification by chromatography, channel **7** was isolated as a yellow oil in 59% yield.

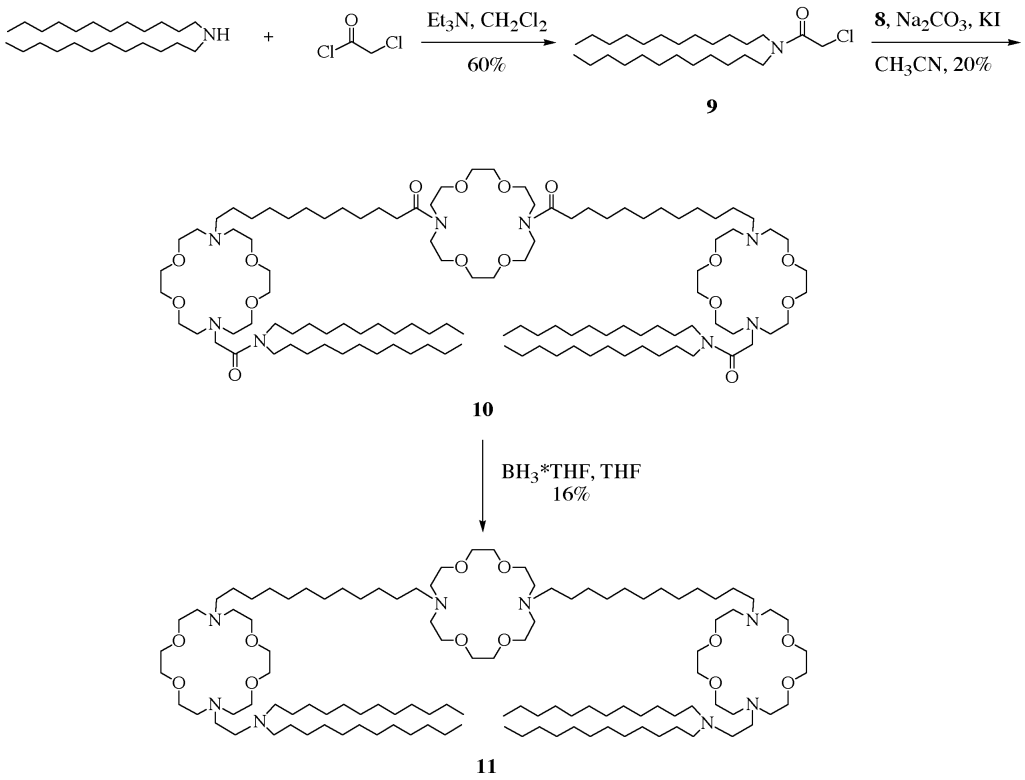
Scheme 1



The infrared (IR) spectrum of this compound showed a peak at 1650 cm^{-1} corresponding to the amide carbonyl stretching. In addition to the usual signals, a multiplet was observed in the ^{1}H-NMR spectrum at 3.56 ppm. These signals correspond to the hydrogens adjacent to the crown oxygens, the crown hydrogen α to the amidic nitrogens and the benzylic methylene protons. Another unresolved multiplet is observed at 7.24. These are assigned to the aromatic protons of the benzyl group.

The sidearm precursor, **9**, was prepared by treatment of bis(dodecyl)amine with chloroacetyl chloride in the presence of a catalytic amount of DMAP. Alkylation of tris(macrocycle) **8** with **9** in the presence of Na_2CO_3 and catalytic KI gave tris(macrocycle) tetraamide **10**. The saturated tris(macrocycle) **11** was obtained (90%) by reduction using BH_3•THF. Several presumed amide rotamers (^{13}C-NMR peaks at 46.5-48.6 ppm and 69.3-70.5) were detected for **10**. There was no evidence of multiple conformations in **11** after reduction of **10**.

Scheme 2



Assessment of Sodium Transport by NMR.

Cation transport efficacy was assessed by using the dynamic NMR method developed by Riddell and Hayer [28]. The experiment is conducted in a phosphatidylcholine/phosphatidylglycerol vesicle system, created in the presence of NaCl. In the absence of any NMR shift reagent, a single signal is observed for $^{23}Na^+$. However, Na^+ within the vesicle is readily distinguished from Na^+ present in the bulk phase *(i.e.* two peaks are observed) when the NMR shift reagent Dy^{3+} is present in the extra-vesicular aqueous phase. The presence of a Na^+-transporting ionophore causes the linewidths to alter if cation transport through the bilayer is sufficiently rapid. The variation can be quantitated and a transport rate can be calculated. This methodology has been successfully applied to a number of ionophores including gramicidin [29].

We have used gramicidin as a standard for these experiments in previous studies but now prefer a synthetic ionophore, our dansyl channel (Dan<N18N>C_{12}-<N18N>C_{12}<N18N>Dan), as the standard. The dansyl channel has the typical tris(macrocycle) structure but has dansyl sidearms [27]. Gramicidin is a robust channel-former. It will function even when the vesicle system is not well formed. The dansyl channel forms less robust but reproducible channels in the phospholipid vesicle system. Thus, failure of a novel ionophore to transport sodium cation when the dansyl standard is successful is a more credible negative observation than when gramicidin is used as standard.

The vesicles are prepared in the presence of aqueous NaCl from natural phospholipids and characterized by laser light scattering to be sure that uniform liposomes of the expected size have been prepared. As noted above, ^{23}Na-NMR is used to assess the concentrations of Na^+ (Na^+_{inside} and $Na^+_{outside}$) inside and outside of the vesicles after the addition of Dy^{3+}. The ionophore is added to individual samples in concentrations typically ranging from 1-2 μ*M* to 15 μ*M*. The vesicle and reagent suspension is allowed to stand for ≥0.5 h at ambient temperature to be certain that the ionophore has penetrated the membrane. Clearly, it is unknown in advance of each experiment whether the ionophore under study will be efficacious. If no Na^+ transport is observed, it could be due to poor transport qualities of the ionophore or it could be due to unexpected difficulty in the complex experimental system. Thus, an identical set of experiments is run simultaneously in which a known ionophore is added.

In each of the NMR experiments reported here, the results are correlated to those obtained simultaneously for one of the two standard compounds and normalized to a

relative rate of 100. As conducted in our laboratory, the limit of detection in these experiments is approximately 2% of the transport rate of gramicidin. Previously reported [25] exchange rate constants were: dodecyl channel, **1**, 27 and benzyl channel, **12**, 39 relative to gramicidin (100). The results of ^{23}Na-NMR transport experiments conducted on **10** and **11** are shown in Table 1 along with data for several previously studied and related compounds.

Table 1

Sodium Cation Transport Rates in Phospholipid Vesicles

No.	Spacer	Sidearm	Rel. rate	Rel. to
1	$(CH_2)_{12}$	$(CH_2)_{11}CH_3$	27	gramicidin
1	$(CH_2)_{12}$	$(CH_2)_{11}CH_3$	40	**13**
7	$(CH_2)_{11}CO$	$CH_2C_6H_5$	13	gramicidin
10	$(CH_2)_{11}CO$	$COCH_2N[(CH_2)_{11}CH_3]_2$	50	**13**
11	$(CH_2)_{12}$	$CH_2CH_2N[(CH_2)_{11}CH_3]_2$	77	**13**
12	$(CH_2)_{12}$	$CH_2C_6H_5$	39	gramicidin
12	$(CH_2)_{12}$	$CH_2C_6H_5$	211	**13**
13	$(CH_2)_{12}$	dansyl	23	gramicidin
13	$(CH_2)_{12}$	dansyl	100	standard

The data reported in Table 1 may be compared in different ways to address different questions. First, dodecyl channel **1** shows a higher transport rate relative to dansyl channel **13** than compared to gramicidin. This is expected because gramicidin is a potent, channel-forming ionophore.

The effect of replacing methylene groups by carbonyls within the channel can be assessed by comparing compounds **7** and **12**. The two structures are identical except that the point of attachment of the central macrocycle is a bis(amide) in **7** (~CO<N18NCO~) and this linkage is saturated in **12** (~CH_2<N18N>CH_2~). The Na^+ transport rate for bis(amide) **7** is only about a third that of the saturated structure. Thus, we anticipate that incorporation of amide residues within the tris(macrocycle) channels will have a deleterious effect.

The point of the present study was to determine if a twin-chained sidearm would afford greater organization within the bilayer and thus enhanced transport efficacy. Current experimental data suggest that the two distal macrocycles are in the phospholipid's midpolar regime. Additional evidence indicates that the central macrocycle and the hydrocarbon chains are all parallel to lipid axis in the bilayer. If better organization of the channel structure within the bilayer is important for transport efficacy, then twin "flexible sidechains" are expected to afford faster ion transport. Rate data for several hydraphiles are recorded in Table 1. Compound **1** (see above) transports Na^+ at 40% the rate of dansyl channel **13** in an otherwise identical experimental system. Compounds **10** and **11** can be compared directly, as they are identical in all respects except for the presence of four amide residues in **10** that are saturated in **11**. We expect the saturated system to be more flexible than the tetraamide. This should be especially important for the central macrocycle where the weakly donating nitrogen atoms are converted into potent amide donors. We expect the more polar donors to interact more strongly with the transient cation, slowing its progress. Indeed, this appears to be the case for **10** (k_{rel} = 50) compared to **11** (k_{rel} = 77). The latter value is certainly well beyond experimental error of the value obtained for **1** (40).

These rate differences between **10** and **11** are modest but they clearly show that cation transport rates are enhanced by removal (reduction) of amide residues. More importantly, they provide the first evidence that the presence of additional lipophilic chains enhances the organization of the ionophore system (*i.e.* **11** *vs.* **1**). This, in turn, leads to higher transport efficacy.

Conclusion.

Two novel but complicated channel-forming molecules have been prepared in order to gain additional insight into sidechain interactions. Compounds **10** and **11** may be compared directly. The latter is a better ion transporter, demonstrating the deleterious effect of the amide functions. Remarkably, additional sidechain hydrocarbons appear to have little effect on sodium cation transport efficacy. Replacing the two dodecyl sidechains with a twin-stranded ~$CH_2CH_2N[(CH_2)_{11}CH_3]_2$ sidearm may change the rate at which **11** penetrates the liposome compared to **1** or a different percentage of the ionophore may insert in the membrane. These issues are clearly important and will be reported as additional data are obtained.

EXPERIMENTAL

General Methods.

All reaction solvents were freshly distilled and reactions conducted under N_2 unless otherwise stated. Et_2O and THF were distilled from a sodium/benzophenone. Et_3N was distilled from KOH and stored over KOH. CH_2Cl_2 distilled from CaH_2. Column chromatograph was performed with silica gel 60 (230-400 mesh). Thin layer chromatography was performed with silica gel 60 F_{254} plates with visualization by UV light (254 nm) and/or by phosphomolybdic acid (PMA) spray. Starting materials were purchased from Aldrich Chemical Co, and used as is. ^{1}H-NMR spectra were recorded at 300 MHz and are reported in the following manner: Chemical shift in ppm down field from internal Me_4Si (multiplicity, integrated intensity, coupling constants in Hertz, assignment). ^{13}C-NMR spectra were obtained at 75 MHz and referenced to $CDCl_3$ (77.0). NMR data are recorded as follows: chemical shift, peak multiplicity (b = broad; s = singlet; d = doublet; t = triplet; m = multiplet, bs = broad singlet, etc.), integration.

N,N'-Bis{12-[*N*-(*N'*-dodecyl)-diaza-18-crown-6]-dodecyl}-diaza-18-crown-6, ($C_{12}H_{25}$<N18N>$(CH_2)_{12}$<N18N>$(CH_2)_{12}$-<N18N>$C_{12}H_{25}$, **1**).

Compound **1** was prepared as previously reported [26].

12-Bromododecanoic Acid.

This compound was obtained from the Aldrich Chemical Company and used without further purification.

4,13-Diaza-18-crown-6, and *N*-benzyl-4,13-diaza-18-crown-6 (**2**).

These compounds were prepared as previously described [28].

Methyl 12-Bromododecanoate (**3**).

12-Bromododecanoic acid (8.0 g, 14.30 mmol) and concentrated H_2SO_4 (0.5 mL) in anhydrous MeOH (40 mL) were heated to reflux for 24 hours while monitoring by TLC (10% EtOAc/Hexane, SiO_2). The solvent was removed *in vacuo* and the residue dissolved in hexane (50 mL), washed with aqueous $NaHCO_3$ (10%, 25 mL), brine (25 mL), and dried over $MgSO_4$. The mixture was filtered and concentrated *in vacuo* to afford the target molecule as a colorless oil (7.54 g, 93%). ^{1}H-NMR: δ 1.24 (bs, 12H), 1.38 (m, 2H), 1.57 (m, 2H), 1.84 (m, 2H), 2.26 (t, *J* = 7.8 Hz, 2H), 3.37 (t, *J* = 6.9 Hz, 2H), 3.62 (s, 3H); ^{13}C-NMR: δ 24.7, 28.0, 28.5, 28.9, 29.0, 29.2, 29.24, 32.6, 33.8, 33.9, 51.2, 174.3.

12-(16-Benzyl-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl)-dodecanoic Acid Methyl Ester($CH_3OOC(CH_2)_{11}$<N18N>-CH_2Ph, **4**).

A solution of mono-benzyldiazacrown **2** (1.23 g, 3.5 mmol), methyl 12-bromododecanoate **3** (1.10 g, 3.75 mmol), Na_2CO_3 (1.96 g, 18.5 mmol), and KI (20 mg, 0.12 mmol) in $CH_3(CH_2)_2CN$ (50 mL) was heated to reflux for 24 hours. The mixture was cooled, filtered and concentrated *in vacuo*. Column chromatography (SiO_2, 10% Et_3N-EtOAc) afforded target molecule (1.80 g, 91%). ^{1}H-NMR: δ 1.26 (bs, 14H), 1.35-1.50 (m, 2H), 1.55-1.65 (m, 2H), 2.28 (t, *J* = 7.5 Hz, 2H), 2.47 (t, *J* = 7.5 Hz, 2H), 2.81 (m, 8H), 3.50-3.70 (m, 20H), 7.20-7.30 (m, 5H); ^{13}C-NMR: δ ~24.8, 27.0, 27.0, 27.3, 29.0, 29.1, 29.3, 29.4, 33.9, 51.3, 53.6, 53.8, 55.9, 59.8, 69.9, 70.6, 126.8, 128.1, 128.8, 139.7, 174.4.

12-(16-Benzyl-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl)-dodecanoic Acid ($HOOC(CH_2)_{11}$<N18N>CH_2Ph, **5**).

The azacrown ester **4** (1.44 g, 2.55 mmol) was suspended in 2 *N* NaOH (20 mL), and heated to reflux for 24 hours. The mixture was acidified to pH 4-5 with 2 *N* HCl. The product was extracted with CH_3CN (3×50 mL), dried over $MgSO_4$ and the solvent was evaporated under reduced pressure leaving a colorless solid (1.26 g, 90%), mp 140-142 °C. ^{1}H-NMR: δ 1.24 (bs, 14H), 1.30-1.45 (m, 2H), 1.50-1.65 (m, 2H), 2.28 (t, *J* = 7.5 Hz, 2H), 2.47 (t, *J* = 7.5 Hz, 2H), 2.70-2.81 (m, 8H), 3.55-3.70 (m, 20H), 7.20-7.35 (m, 5H); ^{13}C-NMR: δ 24.8, 27.0, 27.3, 28.9, 29.1, 29.3, 29.4, 29.5, 33.9, 51.3, 53.6, 53.7, 55.9, 59.8, 69.9, 70.5, 126.8, 128.1, 128.8, 139.7, 174.4.

Diaza-18-crown-6 (**6**).

Compound **6** was obtained commercially or as described in reference 30.

12-(16-Benzyl-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl)-1-{16-[12-(16-benzyl-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl)-dodecanoyl]-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl}-dodecan-1-one ($PhCH_2$<N18N>-$(CH_2)_{11}CO$<N18N>$CO(CH_2)_{11}$<N18N>CH_2Ph, **7**).

The acid **5** (0.180 g, 0.33 mmol) was suspended in CH_2Cl_2 (20 mL), at 0 °C. To this heterogeneous solution DMF (2 drops) was added followed by oxalyl chloride (0.175 mL, 1.98 mmol). After 20 minutes the reaction was allowed to reach room temperature, and was stirred for an additional 2 hours. The yellow homogeneous solution was concentrated *in vacuo*. The product was used in the subsequent reaction without further purification. To the solution of acyl chloride in CH_2Cl_2 (20 mL) was added a solution of diazacrown (0.050 g, 0.165 mmol), 4-*N,N*-dimethylaminopyridine (DMAP, 20 mg), and Et_3N (0.4 mL) in CH_2Cl_2 (20 mL) at 0 °C. The temperature of the mixture was allowed to reach ambient temperature and was then stirred for 48 hours. The mixture was filtered, and the solvent was evaporated. Column chromatography (Al_2O_3, 10% MeOH-$CHCl_3$) afforded the target product as yellow oil (0.130 g, 30%). ^{1}H-NMR: δ 1.23 (bs, 28H), 1.40 (m, 4H), 1.62 (m, 4H), 2.31(t, *J* = 6.3 Hz, 4H), 2.49 (t, *J* = 7.5 Hz, 4H), 2.75-2.85 (m, 16H), 3.55-3.70 (m, 60H), 7.27-7.35 (m, 10H); ^{13}C-NMR: δ 24.9, 25.0, 26.8, 27.2, 29.3, 29.4, 32.9, 32.8, 33.1, 46.6, 46.7, 48.5, 53.5, 53.6, 55.8, 59.6, 68.9, 70.2, 70.3, 70.5, 126.7, 128.1, 128.8, 139.5, 173.4.

Alternate Synthesis of (**7**).

Monobenzyl diaza-18-crown-6 (0.353 g, 1.0 mmol), ($BrC_{11}CO$<N18N>$COC_{11}Br$) (0.392 g, 0.5 mmol), Na_2CO_3 (0.53 g, 5.0 mmol), and KI (0.017 g, 0.1 mmol) were suspended in $CH_3(CH_2)_2CN$ (3.0 mL). After 48 hours heating, the reaction mixture was allowed to cool, filtered, and concentrated *in vacuo*. Toluene (50 mL) was added to the resulting syrup and the solution again reduced *in vacuo*. The residue was dissolved in ethyl acetate (50 mL) and washed with H_2O (3×20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting yellow solid was purified by flash column chromatography (silica, 50-100% CH_2Cl_2/acetone and 0-2% Et_3N/acetone) to give compound **7** as a yellow oil (0.39 g, 59 % yield). ^{1}H-NMR ($CDCl_3$): δ 1.21, 1.39, 1.57, 2.0 (s, acetone) 2.26, 2.44, 2.74, 3.56, 3.62, 7.24. IR (KCl): 2926, 1650 1455, 1353, 1057 cm^{-1}. High resolution FAB mass spectra for $C_{74}H_{130}N_6O_{14}$: calculated 1327.9723 g/mol, found: 1327.9700 g/mol.

12-(1,4,10,13-Tetraoxa-7,16-diaza-cyclooctadec-7-yl)-1-[16-(12-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl-dodecanoyl)-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl]-dodecan-1-one (H<N18N>$(CH_2)_{11}CO$<N18N>$CO(CH_2)_{11}$<N18N>H, **8**).

Dibenzyl tris(macrocyclic) diamide **7** (0.130 g, 0.097 mmol) was dissolved in absolute EtOH (10 mL). To the solution was added 10% PtO_2/C, (26 mg) and this mixture was shaken under 60 psi for 48 hours. The reaction mixture was filtrated through celite and concentrated under reduced pressure to dryness (yellow oil, 0.100 g, 90%). ^{1}H-NMR: δ (bs, 28H), 1.30-1.50 (m, 8H), 2.21 (t, *J* = 7.0 Hz, 4H), 2.68 (t, *J* = 7.5 Hz, 4H), 2.86 (bs, 8H), 3.03 (m, 16H), 3.60 (bs, 48H); ^{13}C-NMR: δ 23.6, 25.0, 26.8, 29.0, 29.1, 29.2, 32.7, 32.6, 46.5, 46.6, 48.0, 48.3, 48.5, 52.3, 53.3, 57.6, 66.5, 67.2, 69.3, 69.7, 69.8, 69.9, 70.0, 70.2, 704, 70.6, 70.2, 70.3, 70.5, 173.3, 173.4.

N,N-Didodecyl-2-chloroacetamide (**9**).

To a solution of *N,N*-didodecyl amine (0.707 g, 2.0 mmol), Et_3N (0.606 g, 6.0 mmol) and DMAP (20 mg) in CH_2Cl_2 (20 mL) pre-cooled to 5 °C was added chloroacetyl chloride (0.249 mg, 2.2 mmol). After 20 minutes, the reaction mixture was allowed to come to room temperature, and was stirred for 18 hours. The brown solution was washed with aqueous $NaHCO_3$ (10%, 10 mL), the organic layer dried over $MgSO_4$ and evaporated to dryness (0.52 g, 60% yield). ^{1}H-NMR: δ 0.88 (t, *J* = 6.9 Hz, 6H), 1.26 (bs, 42H), 1.56 (m, 4H), 3.20-3.35 (m, 4H), 4.01 (s, 2H); ^{13}C-NMR: δ 14.0, 22.5, 26.7, 26.8, 27.2, 29.0, 29.2, 29.3, 29.4, 29.5, 31.8, 41.2, 46.1, 48.2, 166.1.

2-[16-(12-{16-[12-(16-Didodecylcarbamoylmethyl-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl)-dodecanoyl]-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl}-12-oxo-dodecyl)-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl]-*N,N*-didodecyl-acetamide ($(C_{12}H_{25})_2NCOCH_2$<N18N>$(CH_2)_{11}CO$<N18N>$CO(CH_2)_{11}$<N18N>$CH_2CON(C_{12}H_{25})_2$, **10**).

To a solution of chloroacetamide **9** (0.083 g , 0.192 mmol) in acetonitrile (20 mL) was added tris(macrocyclic) diamide **8** (0.100, 0.087 mmol), KI (20 mg), and Na_2CO_3 (0.055 g, 0.55 mmol). The reaction mixture was heated to reflux for 48 hours. The solvent was removed under reduced pressure and the residue purified by column chromatography (SiO_2, 20% MeOH-$CHCl_3$). Yellow oil (30 mg, 20%). ^{1}H-NMR: δ 0.88 (t, *J* = 6.6 Hz, 6H), 1.26 (bs, 104H), 1.42- 1.59 (m, 16H), 2.32 (t, *J* = 7.5 Hz, 4H), 2.68-2.75 (m, 16H), 2.93 (t, *J* = 5.5 Hz, 4H), 3.27 (t, *J* = 7.5 Hz, 8H), 3.46 (bs, 4H), 3.63 (bs, 64H); ^{13}C-NMR: δ 14.0, 22.5, 23.5, 25.2, 26.7, 27.2, 27.7, 29.2, 29.3, 29.4, 29.5, 29.5, 31.8, 33.02, 46.7, 47.3, 48.7, 51.8, 53.8, 54.8, 56.6, 66.9, 67.4, 68.4, 69.0, 69.9, 70.3, 70.4, 70.7, 70.8, 170.8, 173.4. FAS-MAS *m/z*: $(M+Na)^+$ 1957.0, $(M+2Na)^{2+}$ 990.3, $(M+3Na)^{3+}$ 667.8, calcd for $C_{112}H_{220}N_8O_{16}$, 1933.66.

2-[16-(12-{16-[12-(16-Didodecylcarbamoylmethyl-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl)-dodecyl]-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl}-dodecyl)-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-yl]-*N,N*-didodecylacetamide **(**$(C_{12}H_{25})_2N(CH_2)_2$<N18N>$(CH_2)_{12}$<N18N>$(CH_2)_{12}$<N18N>$(CH_2)_2N(C_{12}H_{25})_2$, **11**).

To a solution of tetraamide **10** (0.200 g, 0.103 mmol) in THF (5 mL), BH_3•THF (3 mL, 2 *M* in THF) was added and the resulting solution was stirred at room temperature for 72 hours. The reaction mixture was cooled to 5 °C, quenched with ice-water, and the solvent removed *in vacuo*. The solid residue was heated to reflux with 2 *N* HCl (4 mL) for 4 hours. After cooling, the solution was brought to pH = 10 (2 *N* NaOH), extracted with $CHCl_3$ (20 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified by column chromatography (SiO_2, 10% MeOH-$CHCl_3$) to afford **11** as a yellow oil (0.030 g, 16%). ^{1}H-NMR: δ 0.88 (t, *J* = 6.6 Hz, 6H), 1.26 (bs, 104H), 1.42-1.59 (m, 22H), 2.49 (t, *J* = 7.0 Hz, 4H), 2.82 (t, *J* = 5.5 Hz, 16H), 2.91 (t, *J* = 5.4 Hz, 28H), 3.62 (bs, 24H), 3.74 (bs, 24H): ^{13}C-NMR: 14.0, 22.6, 27.0, 27.2, 29.2, 29.4, 29.5, 31.8, 53.6, 70.5: FAS-MAS *m/z* $(M+Na)^+$ 1901.8, $(M+2Na)^{2+}$ 962.4, calcd for $C_{112}H_{228}N_8O_{12}$, 1877.75.

$PhCH_2$<N18N>$(CH_2)_{12}$<N18N>$(CH_2)_{12}$<N18N>CH_2Ph (**12**) was prepared as reported in reference 26.

Dansyl<N18N>$(CH_2)_{12}$<N18N>$(CH_2)_{12}$<N18N>Dansyl (**13**) was prepared as reported in reference 24.

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