The effect of midpolar regime mimics on anion transport mediated by amphiphilic heptapeptides

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Received (in Durham, UK) 4th April 2007, Accepted 22nd June 2007
First published as an Advance Article on the web 1st August 2007
DOI: 10.1039/b705179b

Nine amphiphilic heptapeptides, synthetic anion transporters (SATs) of the form $(C_{18}H_{37})_2N$–Y–$(Gly)_3$-Pro-$(Gly)_3$–OCH_2Ph were prepared. The unit $(OH_2)Y$ represents the diacids succinic, glutaric, diglycolic, 3-thiaglutaric, *N*-methyliminodiglycine, isophthalic, and terephthalic acids. Additionally, Y was absent or present as acetic acid affording the structure $(C_{18}H_{37})_2N$–$(Gly)_3$-Pro-$(Gly)_3$–OCH_2Ph or $(C_{18}H_{37})_2N$–$(Gly)_4$-Pro-$(Gly)_3$–OCH_2Ph. The diglycolic acid derivative was reported previously but the remaining compounds are new. These nine peptides mediated release of Cl^- from DOPC/DOPA vesicles with varying efficacy. Chloride release diminished for the Y-containing amphiphilic heptapeptides in the order glutaric, succinic > 3-thiaglutaric > terephthalic > acetic, *N*-methyliminodiacetic, > no Y, isophthalic. The release of Cl^- was generally exponential over time but the curve shapes were distinctly sigmoidal for the more flexible diacids. Computational studies were undertaken to assess differences in conformation. Overall, it appeared that Cl^- release for those SATs that could adopt a linear conformation on the *N*-terminal side of proline correlated best with the polarity of the diacid.

Introduction

The efficiency and complexity of modern ion channel proteins has led chemists to emulate the transport function with various model systems.[1–6] Synthetic models of cation-conducting channels have been developed and studied in a number of laboratories. Notable examples have been reported by Tabushi,[7] Fyles,[8] Kobuke,[9] Ghadiri,[10] Voyer,[11] Koert,[12] Matile,[13] and their co-workers. We have also reported extensive studies in the cation channel area.[14] The important efforts of others are included in the reviews cited as refs. 1–6.

Just as structural studies of protein chloride transporters[15] lagged behind those of cation channels,[16] model systems for anion conductors have only recently been developed. Several models have been reported including modified natural peptides[17] and the amphiphilic oligopeptide systems reported here.[18] Additional anion transporters have been reported by Davis,[19] Smith,[20] and Gin[21] and their co-workers. There is ambiguity in the mechanism(s) by which these model anion transporters function as there is for the natural ClC family of "channel" proteins.[22–26]

Our anion channel design began from the notion that the earliest channels must have resembled the monomers of membranes in which they functioned. It is, of course, unknown whether or not the earliest encapsulating membranes were constructed of phospholipids or, indeed, if they were even bilayers. Nevertheless, our design began from the general character of a modern phospholipid. We recognized that the reaction of $(C_{18}H_{37})_2NH$ with the anhydride of diglycolic acid $[O(CH_2COOH)_2]$ should directly give $(C_{18}H_{37})_2NCOCH_2OCH_2COOH$. This single step reaction afforded twin alkyl chains akin to those present in many phospholipids. Further, the pattern of polar oxygen atoms arising from diglycolic acid corresponded well to that present in the midpolar regime. This single step reaction would, in principle, afford two of the modules critical to emulate a phospholipid structure.

We devised a heptapeptide headgroup based on ClC channel protein sequences and the known GxxP hinge-bend sequence.[27,28] Taken together, the simplest peptide sequence that seemed to hold promise was $\sim(Gly)_3$-Pro-$(Gly)_3\sim$. The heptapeptide was capped at its *C*-terminus with a benzyl ester. The resulting structure was $(C_{18}H_{37})_2NCOCH_2OCH_2$-CO–$(Gly)_3$-Pro-$(Gly)_3$–$OCH_2Ph$.[18] We found that this compound mediated Cl^- transport, it was selective for Cl^- over K^+, and transport showed the open-close behavior typical of ion channels.

In subsequent studies, we explored the effect on ion transport of changing the *C*-terminal ester,[29] varying the alkyl chain lengths,[30] changing proline to pipecolic acid,[31] and varying the number of amino acids in the peptide.[32] It was also found that these compounds exhibited biological activity.[33] An aspect of the system that remained unstudied was the subunit that connects the dialkylamine residue to the heptapeptide. In a phospholipid monomer, we have referred to the fragment linking the fatty acids to the phosphate (*i.e.*, the glyceryl unit) as the "midpolar regime." The goal of this study was to

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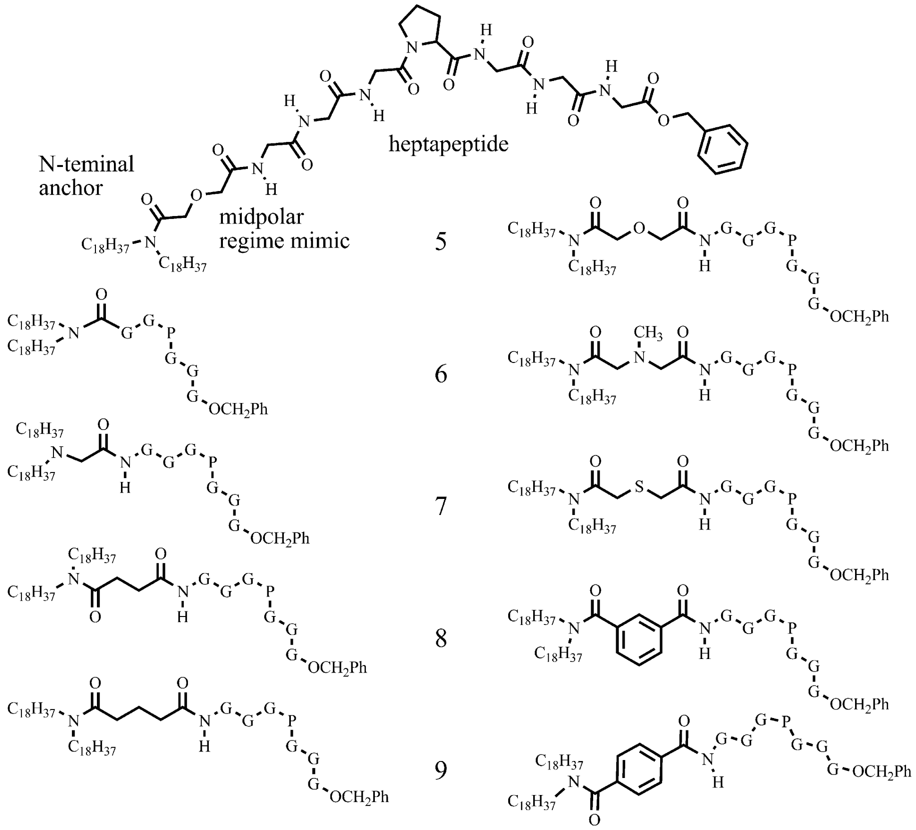
evaluate changes in the structure of the midpolar regime mimic and the effect of such alterations on ion transport efficacy.

Results and discussion

The compounds studied are variants of the general structure $(C_{18}H_{37})_2N$–acid–CO–$(Gly)_3$-Pro-$(Gly)_3$–OCH_2Ph. A structure of the synthetic anion transporter (SAT) is shown. Its essential elements are identified on the top structure. In order to gain insight into the effect on transport of variations in the midpolar regime mimic, compounds were prepared that varied in flexibility, in chain length, in the presence or absence of donor groups, and the presence of arenes. The structures used in this study are shown as **1–9**. Compound **1** is a monoacid that reacts to incorporate an acetic acid amide (glycine) unit into the SAT. Compounds **2–8** are diacids, ultimately incorporating two amide linkages.



Syntheses

Conceptually, the preparation of the various $(C_{18}H_{37})_2$-N–Y–CO–$(Gly)_3$-Pro-$(Gly)_3$–OCH_2Ph molecules requires assembly of three modules. The midpolar element needs to be connected to the hydrocarbon chains comprising the *N*-terminus and originating from dioctadecylamine. The heptapeptide must be assembled and esterified at its *C*-terminal end. This peptide module must then be connected to the R_2N–Y–COOH fragment. In practice, the synthesis requires a peptide fragment to be connected to R_2N–Y–COOH and the remaining ester-terminated peptide added on. Such a procedure was used to construct the first example of this family and the approach has been described in detail.[31a]

In the present work, three coupling approaches have been taken. The first parallels the initial synthetic approach, reported in detail for **5**.[31a] In this case, diglycolic acid was converted to diglycolic anhydride by heating with acetic anhydride. Diglycolic anhydride was then heated with dioctadecylamine to form the half-amide acid $(C_{18}H_{37})_2NCO$-CH_2OCH_2COOH, precursor to **5**. An essentially identical approach was used to form $(C_{18}H_{37})_2NCOCH_2CH_2COOH$, $(C_{18}H_{37})_2NCOCH_2CH_2CH_2COOH$, $(C_{18}H_{37})_2NCOCH_2$-$N(CH_3)CH_2COOH$ and $(C_{18}H_{37})_2NCOCH_2SCH_2COOH$, the precursors to **3**, **4**, **6** and **7**, respectively.

The dialkylamido acid module required for **2** was prepared by first converting bromoacetic acid into its ethyl ester. Heating $BrCH_2COOEt$ with $(C_{18}H_{37})_2NH$ and Na_2CO_3 in $CH_3CH_3CH_2CN$ gave $(C_{18}H_{37})_2NCH_2COOEt$ in 95% yield. The precursors to **8** and **9**, which have aromatic midpolar mimics, were prepared by treating dioctadecylamine with an excess of either isophthaloyl chloride (→**8**) or terephthaloyl chloride (→**9**). The approach taken in the latter two cases afforded $(C_{18}H_{37})_2NCO$–Ar–COOH in 41 and 37% yields, respectively. The lower yields obtained in these condensations were compensated by the ready availability of the starting materials and the ease of purification.

We note that our efforts to prepare the corresponding derivatives of maleic and butynedioic acids failed. In the former case, a product was obtained but no double bond could be detected by NMR suggesting a possible Michael addition. Only a tarry residue was obtained from reactions using butynedioic acid as the starting material.

Once the dialkylamido acids were in hand, construction of the heptapeptide proceeded as previously described.[30b] In short, the acid was coupled to triglycine, which was then linked to proline. The three additional glycines were then coupled to the existing unit to afford the entire structure: $(C_{18}H_{37})_2NCO–Y–CO–(Gly)_3$-Pro-$(Gly)_3–OCH_2Ph$. Details of the syntheses are reported in the Experimental section. The SATs were typically waxy, white solids that were poorly soluble in water. Attempts to crystallize them have thus far failed to produce samples suitable for X-ray analysis.

The process of ion release

The bilayer membranes of liposomes prepared from phospholipid monomers do not pass ions such as Na^+ or Cl^-. Compounds **1–9** are amphiphilic peptides that must insert into the liposomal bilayer and form a stable pathway through the membrane. We surmise that the peptides in aqueous suspension associate with the outer leaflet of the liposomal membrane and then the twin hydrocarbon tails insert into it. The various amphiphilic peptide monomers present in the outer leaflet diffuse laterally, presumably in a random pattern. When two (or more) of the monomers come into proximity, they form an opening through which water and ions may pass. We surmise that monomer headgroups in the lower leaflet of the bilayer reorganize to form the lower part of the conduction pathway. There is precedence for this and transverse relaxation (''flip-flop'') of the amphiphilic peptide is expected to be slow.

Once the conducting pore is formed, the vesicle is expected to empty its contents quite rapidly. The open-close behaviors of certain amphiphilic peptides of this type have been recorded and reported previously.[18] The bilayer voltage clamp experiment is an excellent analytical technique but it requires both special equipment and expertise. In the present case, we have chosen to use ion selective electrodes (ISEs) to record the release of ions from a collection of vesicles. The method comprises a reproducible assay of overall efficacy so that comparisons among different compounds can be made on a quantitative basis.

Analysis of ion release

The compounds studied for the present report were designed to be Cl^- transporters. In order to test their efficacy in that role, we developed a technique involving a chloride selective electrode.[18] Thus, vesicles were prepared from a 7 : 3 w/w (2 : 1 mol/mol) mixture of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphate (DOPA). The vesicles were prepared in a solution containing 600 mM KCl and HEPES buffer (pH 7). After brief sonication, the suspension was passed through a 200 nm filter membrane and a Sephadex G25 column. The resulting vesicles were found, by using laser light scattering, to be ~200 nm.

An Accumet chloride combination electrode was introduced into a vesicle suspension (2 mL) and allowed to equilibrate for ~5 min. The ionophore was added (~9 mM in 2-propanol) and data recording commenced. The vesicles were lysed after 1 h by addition of Triton X-100. The values for ion release were then normalized to the value determined after lysis. The graphs shown below plot the fraction of Cl^- released over time, typically 1500 s. The reproducibility of these determinations is confirmed by the fact that each release curve shown is the average of at least three independent experiments.

Chloride ion release mediated by diglycolic acid derivative 5

In previously reported studies, we found that release of Cl^- from liposomes was mediated in a concentration dependent fashion by $(C_{18}H_{37})_2NCOCH_2OCH_2CO–(Gly)_3$-Pro-$(Gly)_3–OCH_2Ph$. Replacing proline with leucine changed the fourth amino acid from cyclic to acyclic while changing the carbon atom number by only one. When the structure was thus changed to $(C_{18}H_{37})_2N–COCH_2OCH_2CO–(Gly)_3$-Leu-$(Gly)_3–OCH_2Ph$, chloride ion transport was dramatically reduced. Significant differences in Cl^- transport were also apparent when proline was changed to pipecolic acid, its six-membered ring homolog, although every other feature remained identical.[31a,34] In the studies below, structures **3–9** may all be described by the semi-schematic formula $(C_{18}H_{37})_2N$–diacid–$(Gly)_3$-Pro-$(Gly)_3–OCH_2Ph$. Compound **2** is $(C_{18}H_{37})_2N–(Gly)_4$-Pro-$(Gly)_3–OCH_2Ph$ and **1** is $(C_{18}H_{37})_2N–(Gly)_3$-Pro-$(Gly)_3–OCH_2Ph$. It was intended that **1** and **2** would serve as controls or benchmarks for the remaining compounds, whose structures were systematically varied.

Overview of transport efficacy

Data for the release of chloride ion from vesicles by closely related structures are illustrated in several graphs in the sections below. Because different compounds give different Cl^- release profiles, we have compared **1–9** in the table below. The release values presented are for 1500 s, an arbitrarily selected time point. Such data are useful to gage the overall effect of midpolar regime changes on transport. The values shown were obtained by averaging the release values observed over a 20 s interval (1490–1510 s). Data points were collected each second during the experiment.

Replacement of ether oxygen in 5 by NMe (6) or S (7)

Fig. 1 shows the fractional release of Cl^- mediated by $(C_{18}H_{37})_2N–Y–(Gly)_3Pro(Gly)_3–OCH_2Ph$ structures in which Y comprises $\sim CO(CH_2)_3CO\sim$ (**4**), $O(CH_2CO\sim)_2$ (**5**), $CH_3N(CH_2CO\sim)_2$ (**6**), or $S(CH_2CO\sim)_2$ (**7**). The release traces for the heteroatom containing diacids (**5–7**) generally show similar exponential shapes while the ion release curve for glutaric acid derivative **4** is distinctly sigmoidal. When Cl^- transport is compared at 1500 s, the fraction of entrapped ion released in each case is as follows: **4**, 0.53; **5**, 0.30; **6**, 0.33; and **7**, 0.49.

The initial Cl^- release rate is presumably related to the kinetics of insertion and pore formation. If the nitrogen atom is protonated at physiological pH, it would be attracted to the vesicle surface, which incorporates a significant concentration of phosphoric acid headgroups. We were unable to find a literature pK_A value for the nitrogen of *N*-methyldiglycine. We

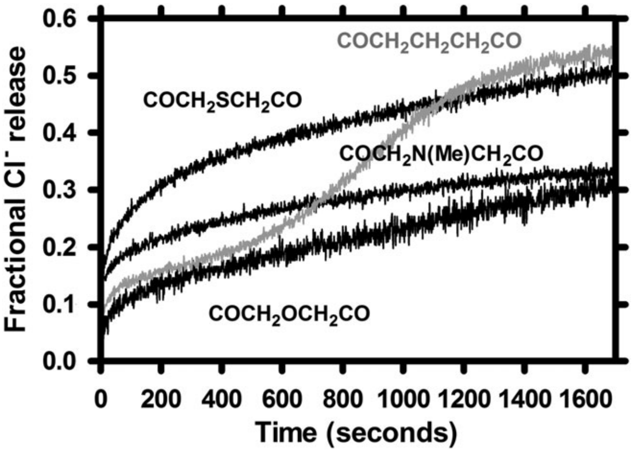


Fig. 1 Chloride anion release from 7 : 3 w/w DOPC/DOPA vesicles mediated by **4**, **5**, **6** and **7**. Ion release was detected by ion selective electrode methods (see text for details).

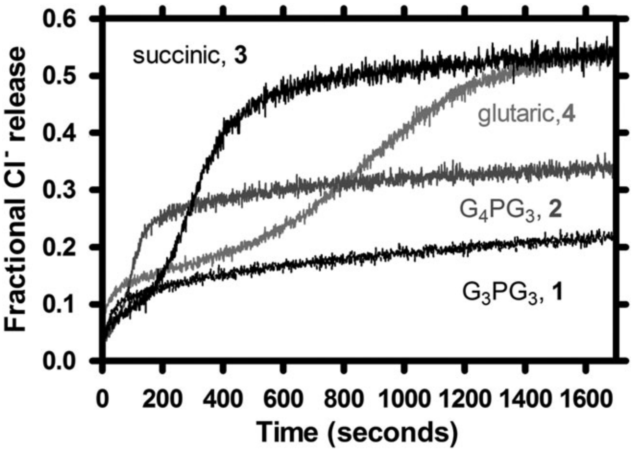


Fig. 2 Chloride anion release from 7 : 3 w/w DOPC/DOPA vesicles mediated by **1**, **2**, **3** and **4**. Ion release was detected by ion selective electrode methods (see text for details).

therefore estimated the nitrogen basicity from the pK_A of trimethylamine: 9.80. A nitrogen having this pK_A will be fully protonated at pH 7.4 or lower. At a minimum, the nitrogen of *N*-methyldiglycine in the SAT must be substantially protonated.

Diminished Cl^- transport was observed with **5** and **6**, which are structurally similar to, but more polar than, **4** and **7**. The similarity in curve shape suggests that these three compounds can be reasonably compared. Divalent sulfur is somewhat, but not dramatically, larger than either O or N. The difference in electronegativity is in the order $O > N > S \approx C$. The Pauling electronegativity values are 3.5, 3.0 and 2.5, respectively. These observations suggest that a lower polarity midpolar regime may be more efficacious in this structural framework. A lower polarity conduction path is expected to be more favorable than one in which interactions can take place between it and the fluxional ion. Repulsion would obviously be unfavorable but so would a strongly stabilizing contact that favored complexation over transport.

The curve shape is significantly different for glutaric acid derivative **4** than for the other ionophores in this series. Initial insertion leads to ion transport by **4** that is as rapid as that observed for diglycolic acid derivative **5**. It may be that many of the **4** monomers require a conformational adjustment before they can insert and function effectively. The three adjacent methylene units of glutaric acid present a more complex conformational situation than the two oxygen-separated methylenes of diglycolic acid in **5**.

Transport by succinic and glutaric acid derivatives

The graph of Fig. 2 compares compounds **1–4**. In **1**, there is no midpolar regime mimic. The twin octadecyl chains are attached directly to the *N*-terminal glycine. In **2**, the peptide sequence is $(Gly)_4$-Pro-$(Gly)_3$. The *N*-terminal glycine may be considered an acetic acid midpolar regime mimic in this case, making the sequence $R_2N–CH_2CO–(Gly)_3$-Pro-$(Gly)_3–OCH_2Ph$. In **3** and **4**, the midpolar regime mimics are succinic and glutaric acids, respectively.

Two points seem apparent

Compound **1**, which lacks a midpolar regime mimic, shows poorer transport than do **2–7**, which incorporate such a module. This observation supports the initial design criteria. Second, even a simple acetic acid residue (**2**) is superior to no midpolar mimic, although it is inferior to the original design (**5**) and most of the variants explored here. An additional conclusion that can be drawn is that although there is little difference in ultimate transport by the succinic (**3**) and glutaric (**4**) acid derivatives, it seems that an efficacious conductance pathway is achieved more rapidly in less flexible **3** than in **4**.

Aromatic diacids

Isophthalic (**8**) and terephthalic (**9**) acids differ only in the relative positions of their carboxylate groups. Fractional Cl^- release mediated by **1**, **8** and **9** is compared in Fig. 3. Compound **8**, in which isophthalic acid comprises the midpolar element, is a very poor Cl^- transporter. Its isomer, **9**, which incorporates terephthalic acid, is similar in transport efficacy to the derivatives containing succinic (**3**), glutaric (**4**) and 3-thiaglutaric acids (**7**).

Terephthalic acid is not a flexible residue but **9** shows a slightly sigmoidal Cl^- release curve. This suggests that it experiences some of the same organizational issues apparent in the release curves for the succinic (**3**) and glutaric (**4**) acid derivatives. Such a profile was not apparent in the ionophores incorporating heteroatom-containing diacids, *i.e.*, **5–7**. Since the origin of the sigmoidal shape is not known, any comparison is speculative. Still, it appears that greater conformational variability correlates to a delayed onset of transport activity. For this reason, conformational minima were calculated for each of the diacids as discussed below.

Acid conformations

The Gaussian 03 program suite was used to calculate minimum energy conformations for the acid components of **2–9**. Dimethylamino, rather than dioctadecylamino, comprised the *N*-terminal amide. The *C*-terminal carboxyl was considered as an *N*-methylamide, to represent the peptide chain's

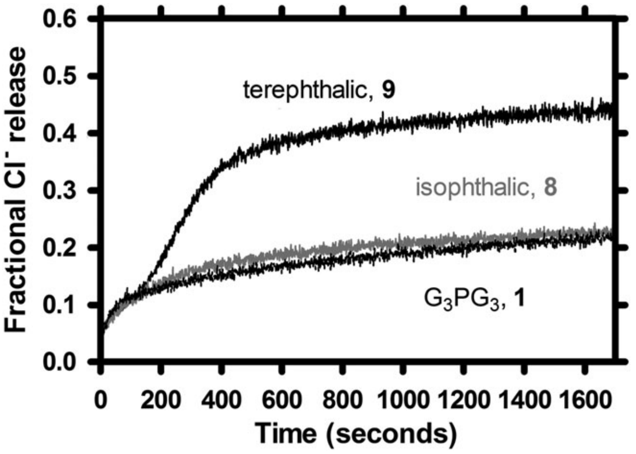


Fig. 3 Chloride anion release from 7 : 3 w/w DOPC/DOPA vesicles mediated by **1**, **8** and **9**. Ion release was detected by ion selective electrode methods (see text for details).

N-terminus. Calculations that included the 36 additional carbons of the two octadecyl chains would have required an inordinate amount of computation. Similar reasoning led to replacement of the heptapeptide by a simple methylamide residue. Thus, each $(C_{18}H_{37})_2NCO–Y–CO–(Gly)_3$-Pro-$(Gly)_3–OCH_2Ph$ was reduced to $(CH_3)_2NCO–Y–CO–NHCH_3$ for the purpose of comparison. The calculated $(CH_3)_2NCO–Y–CO–NHCH_3$ structures are shown on the first three lines of Fig. 4. The ultimate product in each case is indicated by an arrow and the number of the compound that incorporates the particular diacid.

The fourth line of Fig. 4 shows the calculated overlays of compounds **3** and **4** (lower left panel), **5–7** (lower center panel) and **8** and **9** (lower right panel). Differences in the orientations of the carboxylic acid residues are clearly discernible in the comparisons of succinic (**3**) *vs.* glutaric (**4**) acids and in the *meta*- (**8**) and *para*-substitution (**9**) patterns of dicarboxybenzene. When isophthalic acid is the midpolar element, the SAT shows about half the Cl^- release activity of the compound incorporating terephthalic acid.

The results obtained for $(CH_3)_2NCO–CH_2$–Het–CH_2–CO–$NHCH_3$, where Het is O (**5**), S (**7**) or NCH_3 (**6**) vary more than expected. Of course, the more acute angles of the C–S–C bond were anticipated, but the large conformational differences shown in Fig. 4 were not. Admittedly, these systems may be too truncated to adequately reflect the amphiphile's conformation. An additional attempt was made to gain insight into this question. Thus, the calculations were repeated for the $(CH_3)_2NCO–Y–CO–(Gly)_3–OCH_3$ analogs of **3**, **4**, **5**, **8** and **9** (data not shown). In all cases, the diacid conformations were similar or identical to those shown in Fig. 4 and the tripeptide was linear, extended, and essentially invariant in each calculation.

Overall comparisons

Table 1 (above) shows that compounds **1–9** mediate the release of Cl^- to extents ranging from 21 to 53% of the total enclosed

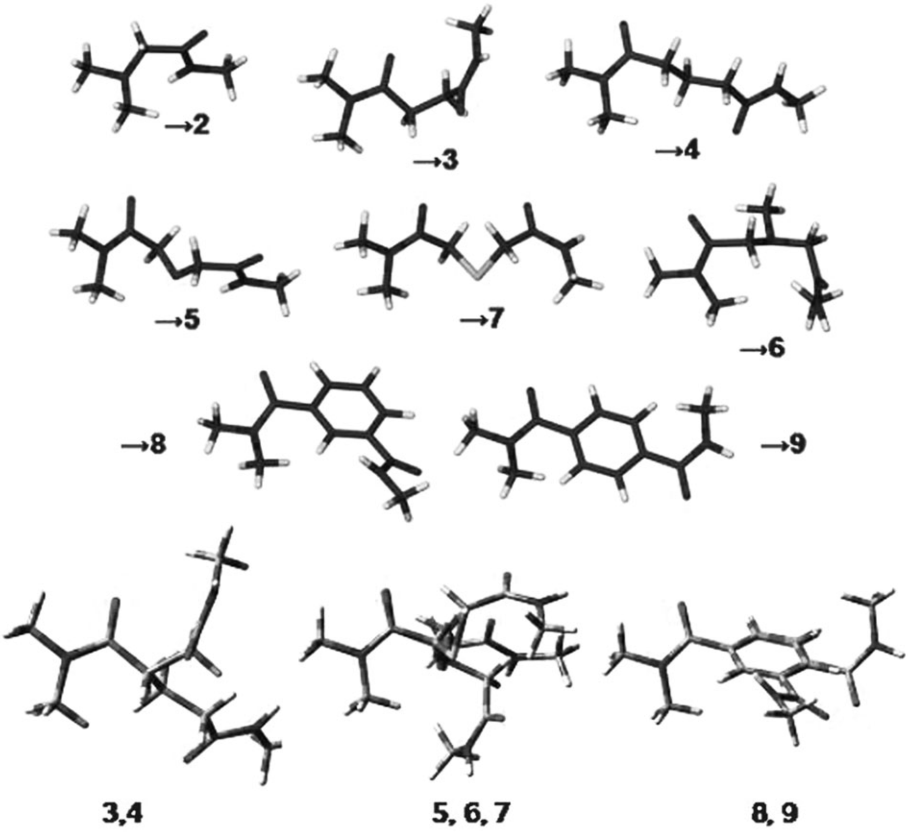


Fig. 4 Calculated (Gaussian 03) gas phase conformations for acids or diacids incorporated into **2–9** (first three lines). Bottom line: superimposition of (left) calculated structures of precursors to **3** and **4**; (middle) **5**, **6** and **7**; and (bottom) **8** and **9** (see text for details).

within phospholipid vesicles. The two poorest midpolar elements are none (**1**) and isophthalic acid (**8**). It seems likely that the *N*-terminal glycine in **1** plays the role of a midpolar element but this reduces the peptide component from G_3PG_3 to G_2PG_3. We have previously found that smaller peptides are less efficacious Cl^- transporters.[32] Compound **9** possesses a midpolar element but its poor transport efficacy is likely due to the orientation of its carboxyl groups. Not only are they held rigidly at a 120° angle, but if the two amide carbonyls exist in the *anti* conformation (see Fig. 4), the alkyl chains and peptide are oriented nearly at a right angle to each other.

There is little structural difference expected in the closely related SATs **5–7**. Despite the more acute C–S–C bond angle in **7**, Fig. 4 shows that the heteroatoms of **5**, **6** and **7** are nearly overlapped. Since these molecules are flexible, it appears that the greater polarity of oxygen or methylamino is the key factor in their diminished transport efficacy. Glutaric acid derivative **4** replaces the heteroatoms of **5–7** by a methylene. This increases the number of possible conformations for the molecule and likely contributes to the oddly shaped ion release curve (see Fig. 1 and 2). Still, the polarity is low and transport by both **4** and **7** is identical within experimental error at 1500 s. The transport efficacy of **4** (and **3**) is consistent with the low polarity hypothesis.

Experimental

General

^1H NMR spectra were recorded at 300 MHz in $CDCl_3$ solvent and are reported in ppm (δ) downfield from internal $(CH_3)_4Si$. ^{13}C NMR spectra 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. Optical rotations were measured on a Perkin-Elmer Model 241 Polarimeter in a glass microcell (100 mm path length, 1 mL volume) with a Na gas discharge lamp as the light source. 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 aluminium 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 percentages. Where water is factored into the analytical data, spectral evidence is presented for its presence. Samples suitable for single crystal X-ray analysis were grown by slow cooling of a warm saturated solution, by evaporation of a saturated solution, or by vapor diffusion.

Table 1 Comparison of chloride release from phospholipid vesicles mediated by **1–9**

No.	Midpolar mimic[a]	Release[b]
1	None (G_3PG_3)	0.21
2	CH_2CO (G_4PG_3)	0.33
3	Succinic	0.53
4	Glutaric	0.53
5	Diglycolic	0.30
6	*N*-Methyliminodiacetic	0.33
7	3-Thiaglutaric	0.49
8	Isophthalic	0.22
9	Terephthalic	0.44

[a] Acid connecting dialkylamino and peptide chains. [b] Fractional Cl^- release at 1500 s.

Preparation of BocPGGG–OCH_2Ph

Boc-L-Proline (1.43 g, 6.7 mmol), TsOH–GGG–O–CH_2Ph (3.0 g, 6.7 mmol), and Et_3N (2.80 mL) were dissolved in CH_2Cl_2 (40 mL) and cooled to 5 °C. EDCI (1.34 g, 7 mmol) was added and the reaction was stirred at rt for 3 days. Solvent was evaporated and the residue was dissolved in EtOAc (50 mL) and washed with aq. NH_4Cl (25 mL) and brine (25 mL), dried over $MgSO_4$ and evaporated. The crude, oily product was chromatographed (SiO_2, 5% MeOH–CH_2Cl_2) and afforded colorless crystals (2.25 g, 71%, mp 54–55 °C). ^1H NMR: 1.42 (9H, s, $C(CH_3)_3$), 1.80–2.20 (4H, m, Pro $NCH_2CH_2CH_2$), 3.35–3.55 (2H, m, Pro $NCH_2CH_2CH_2$), 3.85–4.20 (7H, m, Gly NCH_2, Pro NCH), 5.15 (2H, s, $PhCH_2O$), 7.05 (2H, br s, Gly CONH), 7.30–7.35 (5H, m, H_{Ar}), 7.80 (1H, br s, Gly CONH). ^{13}C NMR: 24.6, 28.3, 29.4, 41.1, 43.0, 43.3, 47.2, 60.7, 66.9, 80.9, 128.4, 128.5, 128.7, 135.4, 155.8, 169.6, 170.0, 173.9. IR (KBr): 3310, 3066, 2976, 2933, 1753, 1667, 1540, 1455, 1408, 1366, 1245, 1174, 1129, 1031, 974, 912, 773, 739, 698 cm^{-1}. Anal. Calc. for $C_{23}H_{32}N_4O_7$: C, 57.97; H, 6.77; N, 11.76%. Found: C, 57.87; H, 6.76; N, 11.39%.

Preparation of PGGG–$OCH_2Ph \cdot HCl$

Boc–PGGG–OCH_2Ph (0.2 g, 0.42 mmol) was dissolved in 4 M HCl in dioxane (10 mL) at 5 °C and the reaction mixture was stirred for 1 h. The solvent was evaporated *in vacuo* and the residue was crystallized from MeOH–Et_2O (0.18 g, 100%) to give a colorless solid (mp 145–146 °C). ^1H NMR (CD_3OD): 2.00–2.25 (4H, m, Pro $NCH_2CH_2CH_2$), 3.35–3.45 (2H, m, Pro $NCH_2CH_2CH_2$), 3.90–4.05 (6H, m, Gly NCH_2), 4.30–4.40 (1H, m, Pro NCH), 5.18 (2H, s, $PhCH_2O$), 7.30–7.40 (5H, m, H_{Ar}). ^{13}C NMR: 25.2, 30.9, 42.1, 43.3, 43.7, 47.6, 61.4, 68.1, 129.5, 129.6, 129.9, 137.5, 170.9, 171.4, 171.8, 172.4.

Compound 1: (2-{2-[(1-{2-[2-(2-dioctadecylamino-acetylamino)-acetylamino]acetyl}pyrrolidine-2-carbonyl)amino]acetylamino}-acetylamino)acetic acid benzyl ester

18_2NCH_2CO–GG–OCH_2Ph. To 18_2NCH_2COOH (0.39 g, 0.63 mmol) dissolved in CH_2Cl_2 (20 mL), PyBroP (0.34 g, 0.73 mmol) and HOBt (0.1 g, 0.74 mmol) were added and the mixture was stirred at rt. After 0.5 h, TsOH–GGG–OCH_2Ph

(0.27 g, 0.68 mmol) and DIEA (0.27 mL) were added and the reaction mixture was stirred at rt for 2 days. The solvent was evaporated and the residue chromatographed (SiO_2, 2% MeOH–$CHCl_3$) to give a colorless oil (0.44 g, 89%). ^{1}H NMR: 0.88 (6H, t, J = 6.9 Hz, $-CH_2C\mathit{H}_3$), 1.25 (60H, *pseudo*-s, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.43 (2H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 1.64 (2H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 2.48 (4H, br s, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.10 (2H, br s, NCH_2CO), 3.98 (2H, d, J = 5.7 Hz, Gly NCH_2), 4.07 (2H, d, J = 5.7 Hz, Gly NCH_2), 5.18 (2H, s, $PhC\mathit{H}_2O$), 6.66 (1H, br s, Gly CONH), 7.30–7.40 (5H, m, H_{Ar}), 7.96 (1H, br s, Gly CONH). ^{13}C NMR: 14.1, 22.7, 27.4, 29.3, 29.5, 29.7, 31.9, 41.3, 43.1, 55.5, 67.2, 128.4, 128.6, 128.7, 135.4, 169.2, 169.4, 173.5.

18_2NCH_2CO–GGPGGG–OCH_2Ph, 1. 18_2NCH_2CO–GG–OCH_2Ph (0.40 g, 0.51 mmol) was dissolved in EtOH (30 mL), 10% Pd/C (0.14 g) was added, and this mixture was shaken (60 psi H_2) for 3 h. The mixture was heated to reflux, filtered (Celite pad), and the solvent was evaporated to give a white solid (0.35 g, 100%). This product was used in the next step without further purification. 18_2NCH_2CO–GG–OH (0.35 g, 0.5 mmol) was suspended in CH_2Cl_2 (30 mL) at 0 °C. EDCI (0.11 g, 0.57 mmol), PGGG–$OCH_2Ph \cdot HCl$ (0.22 g, 0.52 mmol), HOBt (0.08 g, 0.57 mmol) and DIEA (0.1 mL) were added and the reaction was stirred for 48 h at rt. The solvent was evaporated, the residue was chromatographed (SiO_2, 2% MeOH–$CHCl_3$), and crystallized (MeOH) to give a white solid (0.29 g, 51%), mp 116–117 °C. ^{1}H NMR: 0.87 (6H, t, J = 6.3 Hz, $C\mathit{H}_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, m, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.46 (2H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 1.65 (2H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 1.80–2.20 (4H, m, Pro $NCH_2C\mathit{H}_2C\mathit{H}_2$), 2.47 (4H, br s, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.07 (2H, br s, NCH_2CO), 3.30–3.55 (2H, m, Pro $NC\mathit{H}_2CH_2CH_2$), 3.60–4.15 (10H, overlapping signals due to Gly CH_2), 4.35 (1H, t, J = 6.3 Hz, Pro CH), 5.15 (2H, m, $OC\mathit{H}_2Ph$), 7.30–7.35 (5H, m, H_{Ar}), 7.60 (1H, br s, NH), 7.81 (2H, br s, NH), 8.00 (1H, br s, NH). ^{13}C NMR: 14.0, 22.7, 25.1, 26.9, 27.4, 28.9, 29.3, 29.5, 29.7, 31.9, 41.3, 42.1, 42.6, 42.8, 43.6, 46.9, 55.2, 58.3, 61.1, 67.1, 128.2, 128.4, 128.6, 135.3, 168.7, 169.4, 169.8, 170.0, 172.9, 173.7. Anal. Calc. for $C_{60}H_{105}N_7O_8$: C 68.47, H 10.06, N 9.32%. Found: C 67.10, H 9.53, N 9.59%.

Compound 2: [2-(2-{[1-(2-{2-[2-(2-dioctadecylaminoacetylamino)-acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}acetylamino)acetylamino]acetic acid benzyl ester

Dioctadecylaminoacetic acid ethyl ester $(C_{18}H_{37})_2NCH_2COOCH_2CH_3$. A solution of dioctadecylamine (5.20 g, 10.0 mmol), ethyl bromoacetate (1.43 g, 8.5 mmol), Na_2CO_3 (1.6 g, 10.0 mmol), and KI (30 mg, 0.2 mmol) in *n*-PrCN (75 mL) was heated at reflux for 2 h. The mixture was cooled and filtered to remove salts. This insoluble material was washed with $CHCl_3$ (2 × 20 mL) and the combined organic layer was washed with brine (50 mL) and concentrated *in vacuo*. Chromatography (SiO_2, 10% hexanes in $CHCl_3$ then 100% $CHCl_3$) gave 4.94 g (95%) of a slightly yellow oil. ^{1}H NMR: 0.87 (6H, t, J = 6.6 Hz, $C\mathit{H}_3(CH_2)_{15}CH_2CH_2N$), 1.26 (63H, overlapping signals due to $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$ and $OCH_2C\mathit{H}_3$), 1.43 (4H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 2.54 (4H, t, J = 6.6 Hz, $CH_3(CH_2)_{15}CH_2C\mathit{H}_2N$), 3.30 (2H, s, NCH_2CO), 4.16 (2H, q, J = 7.2 Hz, $OC\mathit{H}_2CH_3$). ^{13}C NMR: 14.1, 14.3, 22.7, 27.4, 27.5, 29.3, 29.5, 29.6, 29.7, 31.9, 54.5, 55.3, 60.2, 171.7.

Dioctadecylaminoacetic acid $(C_{18}H_{37})_2NCH_2COOH$. A solution of $(C_{18}H_{37})_2NCH_2COOCH_2CH_3$ (2.0 g, 3.3 mmol) in EtOH (30 mL) containing 2 N NaOH (20 mL) was refluxed overnight. After cooling, the pH was adjusted to 6 and solids were removed by filtration. The solid material was dissolved in $CHCl_3$ (50 mL), filtered, and the solvent evaporated. The product was dried (high vacuum) to give a white solid (1.9 g, 100%), mp 102–103 °C. ^{1}H NMR: 0.88 (6H, t, J = 6.6 Hz, $C\mathit{H}_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, pseudo-s, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.70 (4H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 3.13 (4H, t, J = 7.8 Hz, $CH_3(CH_2)_{15}CH_2C\mathit{H}_2N$), 3.58 (2H, s, NCH_2CO), 9.95 (1H, br s, COOH). ^{13}C NMR: 14.1, 22.7, 24.0, 26.8, 29.2, 29.3, 29.4, 29.5, 29.7, 31.9, 54.2, 55.2, 167.6.

18_2NCH_2CO–GGG–OCH_2Ph. 18_2NCH_2COOH (0.4 g, 0.65 mmol), EDCI (0.14 g, 0.72 mmol), and HOBt (0.1 g, 0.74 mmol) were dissolved in CH_2Cl_2 (20 mL) and stirred at rt. After 0.5 h, TsOH–GGG–OCH_2Ph (0.3 g, 0.65 mmol) and DIEA (0.26 mL) were added and the reaction mixture was stirred at rt for 2 days. The solvent was evaporated and the residue chromatographed (SiO_2, 2% MeOH–$CHCl_3$) to give a colorless oil (0.42 g, 76%). ^{1}H NMR: 0.88 (6H, t, J = 6.9 Hz, $CH_2C\mathit{H}_3$), 1.25 (60H, pseudo-s, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.45 (4H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 2.53 (4H, br s, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.16 (2H, br s, NCH_2CO), 3.95 (2H, d, J = 5.7 Hz, Gly NCH_2), 3.99 (2H, d, J = 5.7 Hz, Gly NCH_2), 4.06 (2H, d, J = 5.7 Hz, Gly NCH_2), 5.16 (2H, s, $PhC\mathit{H}_2O$), 6.96 (1H, br s, Gly CONH), 7.05 (1H, br s, Gly CONH), 7.30–7.35 (5H, m, H_{Ar}), 8.05 (1H, br s, Gly CONH). ^{13}C NMR: 14.1, 22.7, 27.3, 29.4, 29.5, 29.6, 31.9, 41.2, 41.8, 43.1, 55.5, 67.2, 128.4, 128.6, 128.7, 135.4, 169.2, 169.4, 173.5.

18_2NCH_2CO–GGGPGGG–OCH_2Ph. 18_2NCH_2CO–GGG–OCH_2Ph (0.4 g, 0.47 mmol) and 10% Pd/C (0.13 g) were dissolved in EtOH (30 mL) shaken (60 psi H_2) for 3 h. The mixture was heated to reflux, filtered (Celite pad), and the solvent evaporated to give a white solid (0.25 g, 72%). This product was used in the next step without purification. 18_2NCH_2CO–GGG–OH (0.25 g, 0.33 mmol) was suspended in CH_2Cl_2 (30 mL) at 0 °C. EDCI (0.07 g, 0.36 mmol), PGGG–$OCH_2Ph \cdot HCl$ (0.14 g, 0.33 mmol), HOBt (0.05 g, 0.36 mmol) and DIEA (0.06 mL) were added and the mixture was stirred for 48 h at rt. The solvent was evaporated, the residue was chromatographed (SiO_2, 5% MeOH–$CHCl_3$), and then crystallized from MeOH to give a white solid (0.29 g, 78%), mp 137–138 °C. ^{1}H NMR: 0.87 (6H, t, J = 6.3 Hz, $C\mathit{H}_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, m, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.50 (2H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 1.80–2.20 (4H, m, Pro $NCH_2C\mathit{H}_2C\mathit{H}_2$), 2.47 (4H, br s, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.07 (2H, br s, NCH_2CO), 3.30–3.55 (2H, m, Pro $NC\mathit{H}_2CH_2CH_2$), 3.60–4.15 (12H, overlapping signals due to Gly CH_2), 4.32 (1H, t, J = 6.3 Hz, Pro CH), 5.14 (2H, m, $OC\mathit{H}_2Ph$), 7.30–7.35 (5H, m, H_{Ar}), 7.42 (1H, br s, NH), 7.51 (2H, br s, NH), 7.80 (1H, br s, H_{Ar}), 7.94 (1H, br s, NH), 8.00 (1H, br s, NH). ^{13}C NMR: 14.1, 22.7, 25.1, 26.9, 27.4, 29.0, 29.3, 29.5, 29.7, 31.9, 41.3, 41.8, 42.7, 43.5, 46.9, 55.1, 58.4, 61.3, 67.1, 128.2, 128.4, 128.6, 135.3, 168.7, 169.6, 170.0, 170.1, 170.3, 170.1, 173.3. Anal. Calc. for $C_{62}H_{108}N_8O_{10}$: C 67.11, H 9.81, N 10.10%. Found: C 67.13, H 9.79, N 10.30%.

Compound 3: [2-(2-{[1-(2-{2-[2-(3-dioctadecylcarbamoylpropionylamino)acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]-amino}acetylamino)acetylamino]acetic acid benzyl ester, 3

$18_2COCH_2CH_2COOH$. A solution of dioctadecylamine (4.0 g, 7.6 mmol) and succinic anhydride (0.76 g, 7.6 mmol) was refluxed in toluene (100 mL) for 48 h. The solvent was evaporated and the residue was crystallized from Et_2O to give the product as a white solid (3.88 g, 82%), mp 63–64 °C. ^{1}H NMR: 0.88 (6H, t, J = 6.6 Hz, $C\mathit{H}_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, pseudo-s, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.54 (4H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 2.68 (4H, s, $COCH_2$), 3.22 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.31 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}C\mathit{H}_2N$). ^{13}C NMR: 14.1, 22.7, 26.9, 27.0, 27.6, 28.0, 28.8, 29.3, 29.7, 30.5, 31.9, 46.7, 48.3, 172.3, 174.8.

TsOH–GGG–OCH_2Ph. GGG (3.0 g, 15.9 mmol) and *p*-toluenesulfonic acid monohydrate (3.6 g, 18.9 mmol) were added to a mixture of benzyl alcohol (20 mL) and toluene (30 mL). The mixture was dehydrated by heating under reflux with a Dean–Stark trap (~8 h). The mixture was cooled to rt, diluted with Et_2O (50 mL), and cooled in an ice water bath for 2 h. The solid *p*-toluenesulfonate of GGG–OCH_2Ph was collected, washed with Et_2O (50 mL) and dried. After crystallization from MeOH–Et_2O the salt (5.5 g, 77%) melted at 176–177 °C. ^{1}H NMR (DMSO-d_6): 2.34 (3H, s, $C\mathit{H}_3Ph$), 3.74 (2H, s, Gly NCH_2), 3.97 (4H, s, Gly NCH_2), 5.14 (2H, s, $PhC\mathit{H}_2O$), 7.21 (2H, d, J = 8.4 Hz, Tosyl H_{Ar}), 7.30–7.35 (5H, m, Ph H_{Ar}), 7.69 (2H, d, J = 8.4 Hz, Tosyl H_{Ar}). ^{13}C NMR: 21.4, 41.7, 42.1, 43.2, 68.1, 127.2, 129.5, 129.6, 129.9, 130.2, 137.5, 142.1, 143.7, 168.4, 171.4, 172.2.

$18_2COCH_2CH_2CO$–GGG–OCH_2Ph. $18_2COCH_2CH_2$-COOH (1.87 g, 3.0 mmol) and EDCI (0.62 g, 3.2 mmol) were stirred in CH_2Cl_2 (60 mL) at rt. After 0.5 h, TsOH–GGG–OCH_2Ph (1.32 g, 3.0 mmol) and Et_3N (1.2 mL) were added and the mixture was stirred at rt overnight. Evaporation of the solvent followed by chromatography (SiO_2, 5% MeOH–$CHCl_3$) gave a white solid (2.26 g, 85%), mp 72–73 °C. ^{1}H NMR: 0.88 (6H, t, J = 6.9 Hz, $C\mathit{H}_3(CH_2)_{15}$-CH_2CH_2N), 1.24 (60H, pseudo-s, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.40–1.55 (4H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 2.41 (2H, t, J = 5.6 Hz, $COC\mathit{H}_2$), 2.78 (2H, t, J = 5.6 Hz, $COC\mathit{H}_2$), 3.10–3.25 (4H, overlapping signals due to $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.93 (4H, d, J = 5.7 Hz, Gly NCH_2), 4.03 (2H, d, J = 5.7 Hz, Gly NCH_2), 5.14 (2H, s, $PhC\mathit{H}_2O$), 6.58 (1H, br s, Gly CONH), 7.16 (1H, t, J = 5.7 Hz, Gly CONH), 7.30–7.35 (5H, m, H_{Ar}), 8.17 (1H, t, J = 5.7 Hz, Gly CONH). ^{13}C NMR: 14.1, 22.7, 27.0, 27.1, 27.7, 28.7, 29.0, 29.3, 29.6, 29.7, 31.7, 31.9, 41.0, 43.1, 43.9, 46.5, 48.2, 66.8, 128.1, 128.3, 128.6, 135.4, 169.4, 169.6, 169.9, 171.9, 174.6.

$18_2COCH_2CH_2CO$–GGG–OH. $18_2COCH_2CH_2CO$–GGG–OCH_2Ph (2.2 g, 2.5 mmol) and 10% Pd/C (0.2 g) in EtOH (100 mL) were shaken (60 psi H_2) for 3 h, heated to reflux, and then filtered (Celite pad). Evaporation of the solvent gave a white solid (1.86 g, 94%), mp 151–152 °C. ^{1}H NMR (CD_3OD): 0.90 (6H, t, J = 6.9 Hz, $C\mathit{H}_3(CH_2)_{15}$-CH_2CH_2N), 1.29 (60H, pseudo-s, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.40–1.60 (4H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 2.52 (2H, t, J = 6.3 Hz, $COCH_2$), 2.74 (2H, t, J = 6.3 Hz, $COCH_2$), 3.80–3.90 (6H, overlapping signals due to Gly NCH_2).

$18_2COCH_2CH_2CO$–GGGPGGG–OCH_2Ph. $18_2COCH_2CH_2CO$–GGG–OH (0.66 g, 0.83 mmol) was suspended in CH_2Cl_2 (30 mL) at 0 °C. EDCI (0.17 g, 0.91 mmol), PGGG–$OCH_2Ph\cdot HCl$ (0.34 g, 0.83 mmol), HOBt (0.12 g, 0.91 mmol) and Et_3N (0.16 mL) were added and the mixture was stirred for 48 h at rt. The solvent was evaporated, the residue was chromatographed (SiO_2, 10–20% MeOH–$CHCl_3$), and then crystallized from MeOH to give a white solid (0.57 g, 82%), mp 150–152 °C. ^{1}H NMR: 0.87 (6H, t, J = 6.3 Hz, $C\mathit{H}_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, m, $CH_3(C\mathit{H}_2)_{15}$-CH_2CH_2N), 1.42 (2H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 1.55 (2H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 1.80–2.40 (4H, m, Pro $NCH_2C\mathit{H}_2C\mathit{H}_2$), 2.40–2.80 (4H, m, $COC\mathit{H}_2C\mathit{H}_2CO$), 3.20 (4H, br s, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.25–4.40 (15H, overlapping signals due to Pro CH and $NC\mathit{H}_2CH_2CH_2$, and Gly CH_2), 5.15 (2H, m, $OC\mathit{H}_2Ph$), 7.30–7.40 (5H, m, H_{Ar}), 7.46 (2H, br s, NH), 7.73 (1H, br s, NH), 7.90 (1H, br s, H_{Ar}), 8.14 (2H, br s, NH). ^{13}C NMR: 14.1, 22.7, 25.3, 27.0, 27.1, 27.8, 28.7, 28.9, 29.3, 29.7, 30.9, 31.9, 41.4, 42.6, 43.3, 43.9, 46.2, 46.7, 48.1, 61.4, 67.4, 128.3, 128.4, 128.6, 135.3, 168.7, 169.7, 170.2, 170.4, 171.4, 171.7, 171.9, 173.8, 174.5. Anal. Calc. for $C_{64}H_{110}N_8O_{10}$: C 66.75, H 9.63, N 9.73%. Found: C 67.00, H 9.78, N 9.68%.

Compound 4: [2-(2-{[1-(2-{2-[2-(4-dioctadecylcarbamoylbutyrylamino)acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]-amino}acetylamino)acetylamino]acetic acid benzyl ester

$18_2COCH_2CH_2CH_2COOH$. Dioctadecylamine (3.0 g, 5.75 mmol) and glutaric anhydride (0.85 g, 7.5 mmol) in THF (40 mL) was heated at reflux for 48 h. The solvent was evaporated and the product was crystallized from Et_2O to give a white solid (3.38 g, 92%), mp 62–63 °C. ^{1}H NMR: 0.88 (6H, t, J = 6.6 Hz, CH_3), 1.25 (60H, pseudo-s, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.53 (4H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 1.97 (2H, m, $COCH_2C\mathit{H}_2CH_2CO$), 2.44 (4H, m, $COC\mathit{H}_2CH_2C\mathit{H}_2CO$), 3.20 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.29 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}C\mathit{H}_2N$). ^{13}C NMR: 14.1, 20.5, 22.7, 26.9, 27.1, 27.7, 28.0, 29.1, 29.3, 29.4, 29.6, 29.7, 31.7, 31.9, 33.5, 46.2, 48.1, 172.2.

$18_2COCH_2CH_2CH_2CO$–GGG–OCH_2Ph. $18_2COCH_2CH_2CH_2COOH$ (1.5 g, 2.36 mmol), EDCI (0.5 g, 2.6 mmol), and HOBt (0.35 g, 2.6 mmol) were dissolved in CH_2Cl_2 (60 mL) and stirred at rt. After 0.5 h, TsOH·GGG–OCH_2Ph (1.06 g, 2.36 mmol) and Et_3N (1.0 mL) were added and the mixture was stirred at rt for 2 days. The solvent was evaporated and the residue was chromatographed (SiO_2, 2% MeOH–$CHCl_3$) to give a white solid (1.82 g, 87%), mp 81–82 °C. ^{1}H NMR: 0.88 (6H, t, J = 6.9 Hz, –$CH_2C\mathit{H}_3$), 1.24 (60H, pseudo-s, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.40–1.55 (4H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 1.95 (2H, m, $COCH_2C\mathit{H}_2CH_2CO$), 2.25–2.40 (4H, m, $COC\mathit{H}_2CH_2C\mathit{H}_2CO$), 3.10–3.25 (4H, overlapping signals due to $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.92 (2H, d,

J = 5.7 Hz, Gly NCH_2), 3.96 (2H, d, J = 5.7 Hz, Gly NCH_2), 4.02 (2H, d, J = 5.7 Hz, Gly NCH_2), 5.13 (2H, s, $PhCH_2O$), 7.17 (1H, t, J = 5.7 Hz, Gly CONH), 7.30–7.35 (5H, m, H_{Ar}), 7.39 (1H, t, J = 5.7 Hz, Gly CONH), 7.74 (1H, t, J = 5.7 Hz, Gly CONH). ^{13}C NMR: 14.0, 21.2, 22.6, 26.9, 27.1, 27.8, 29.1, 29.3, 29.4, 29.5, 29.6, 31.9, 35.2, 41.2, 43.0, 43.4, 46.0, 48.2, 67.0, 128.2, 128.3, 128.5, 135.3, 169.6, 169.7, 170.1, 172.1, 173.9.

$18_2COCH_2CH_2CH_2CO$–GGGPGGG–OCH_2Ph. $18_2COCH_2CH_2CH_2CO$–GGG–OCH_2Ph (1.17 g, 1.31 mmol) and 10% Pd/C (0.2 g) in EtOH (40 mL) were shaken (60 psi H_2) for 3 h. The mixture was heated to reflux, filtered (Celite pad), and the solvent was evaporated to afford a white solid (0.99 g, 93%). This product was used in the next step without further purification. $18_2COCH_2CH_2CH_2CO$–GGG–OH (0.52 g, 0.65 mmol) was suspended in CH_2Cl_2 (30 mL) at 0 °C. EDCI (0.14 g, 0.72 mmol), PGGG–$OCH_2Ph \cdot HCl$ (0.27 g, 0.65 mmol), HOBt (0.1 g, 0.72 mmol) and Et_3N (0.3 mL) were added and the mixture was stirred for 48 h at rt. The solvent was evaporated, the residue was chromatographed (SiO_2, 10% MeOH–$CHCl_3$) and then crystallized from MeOH to give a white solid (0.4 g, 53%), mp 160–161 °C. ^1H NMR: 0.87 (6H, t, J = 6.3 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.50 (4H, br s, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.80–2.40 (10H, overlapping signals due to $COCH_2CH_2CH_2CO$ and Pro $NCH_2CH_2CH_2$), 3.10–3.20 (4H, m, $CH_3(CH_2)_{16}CH_2N$), 3.25–4.30 (15H, overlapping signals due to Pro CH and $NCH_2CH_2CH_2$, and Gly CH_2), 5.14 (2H, m, OCH_2Ph), 7.30–7.40 (5H, m, H_{Ar}), 7.47 (1H, br s, NH), 7.57 (1H, br s, NH), 7.77 (1H, br s, H_{Ar}), 8.12 (1H, br s, NH), 8.28 (1H, br s, NH). ^{13}C NMR: 14.0, 21.2, 22.7, 25.2, 27.0, 27.1, 27.8, 29.0, 29.1, 29.3, 29.4, 29.6, 29.7, 31.9, 35.2, 41.4, 41.7, 43.0, 46.2, 46.8, 48.3, 61.3, 67.2, 128.2, 128.4, 128.6, 128.8, 135.4, 168.6, 170.1, 170.2, 170.6, 171.3, 172.4, 173.9, 174.0. Anal. Calc. for $C_{65}H_{112}N_8O_{10}$: C 66.98, H 9.68, N 9.61%. Found: C 66.70, H 9.70, N 9.74%.

Compound 5: [2-(2-{[1-(2-{2-[2-(2-dioctadecylcarbamoylmethoxyacetylamino)acetylamino]acetylamino}acetyl)pyrrolidine2-carbonyl]-amino}acetylamino)acetylamino]acetic acid benzyl ester, 5

A detailed preparation of this compound has previously appeared.[31a]

Compound 6: {2-[2-({1-[2-(2-{2-[2-(dioctadecylcarbamoylmethylmethylamino)acetylamino]acetylamino}acetylamino)acetyl]-pyrrolidine-2-carbonyl}amino)acetylamino]acetylamino}acetic acid benzyl ester

$18_2COCH_2(CH_3)NCH_2COOCH_2Ph$. Methyliminodiacetic acid 1.15 g, 7.82 mmol) was suspended in acetic anhydride (5.5 g) and heated to reflux for 0.5 h. The mixture was cooled and the solvent removed *in vacuo*. The residue was dissolved in THF (40 mL), dioctadecylamine (4.0 g) was added, and the reaction was heated to reflux overnight. The hot solution was filtered (Celite pad). The solvent was evaporated and the residue dissolved in toluene (50 mL). Benzyl alcohol (1.92 g, 17.7 mmol) and *p*-toluenesulfonic acid monohydrate (3.38 g, 17.8 mmol) were added. The mixture was heated to reflux and water was removed by using a Dean–Stark trap (overnight). The mixture was cooled to rt, solvent evaporated, the residue was dissolved in $CHCl_3$ (40 mL), washed with aq. Na_2CO_3 (2 × 25 mL), brine (25 mL), dried over $MgSO_4$, and evaporated. The oily product was chromatographed (SiO_2, 5–15% ethyl acetate–hexanes) to give a slightly yellow oil (2.98 g, 52%). ^1H NMR: 0.88 (6H, t, J = 6.9 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, pseudo-s, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.50 (4H, br s, $CH_3(CH_2)_{15}CH_2CH_2N$), 2.50 (3H, s, CH_3N), 3.20–3.30 (4H, overlapping signals due to $CH_3(CH_2)_{16}CH_2N$), 3.45 (2H, s, $COCH_2$), 3.53 (2H, s, $COCH_2$), 5.15 (2H, s, $PhCH_2O$), 7.30-7.35 (5H, m, H_{Ar}). ^{13}C NMR: 14.1, 22.7, 26.9, 27.1, 27.7, 29.0, 29.3, 29.4, 29.6, 29.7, 31.9, 42.5, 45.8, 47.4, 57.6, 57.9, 66.1, 128.1, 128.2, 128.5, 135.9, 169.2, 170.9.

$18_2COCH_2(CH_3)NCH_2CO$–GGG–OCH_2Ph. $18_2COCH_2(CH_3)NCH_2COOCH_2Ph$ (2.98 g, 4.02 mmol) and 10% Pd/C (0.2 g) in EtOH (30 mL) were shaken (60 psi H_2) for 3 h. The mixture was heated to reflux, filtered (Celite pad), and the solvent was evaporated to give a white solid (2.45 g, 93%). This product was used without further purification.

$18_2COCH_2(CH_3)NCH_2COOH$. (1.0 g, 1.53 mmol) was suspended in CH_2Cl_2 (30 mL) at 0 °C. EDCI (0.31 g, 1.62 mmol), TsOH · GGG–OCH_2Ph (0.69 g, 1.53 mmol), HOBt (0.22 g, 1.63 mmol) and DIEA (0.9 mL) were added and the reaction was stirred for 48 h at rt. The solvent was evaporated and the residue was chromatographed (SiO_2, 2–5% MeOH–$CHCl_3$) to give a white solid (1.21 g, 87%), mp 63–64 °C. ^1H NMR: 0.88 (6H, t, J = 6.3 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.40–1.60 (4H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 2.50 (3H, s, CH_3N), 3.10–3.15 (4H, overlapping signals due to $CH_3(CH_2)_{16}CH_2N$ and $COCH_2$), 3.22 (2H, t, J = 6.3 Hz, $COCH_2$), 3.41 (2H, s, $COCH_2$), 3.90–4.10 (6H, overlapping signals due to Gly $4CH_2$), 5.14 (2H, s, OCH_2Ph), 6.99 (1H, t, J = 5.7 Hz, NH), 7.30–7.35 (5H, m, H_{Ar}), 8.18 (1H, t, J = 5.7 Hz, NH), 8.59 (1H, t, J = 5.7 Hz, NH). ^{13}C NMR: 14.1, 22.7, 26.9, 27.1, 27.6, 28.9, 29.3, 29.4, 29.7, 31.9, 41.2, 43.3, 44.9, 46.6, 47.3, 58.9, 61.0, 66.9, 128.2, 128.4, 128.6, 135.4, 169.2, 169.5, 170.3, 172.9.

$18_2COCH_2(CH_3)NCH_2CO$–GGG–OH. $18_2COCH_2(CH_3)NCH_2CO$–GGG–OCH_2Ph (1.2 g, 1.31 mmol) and 10% Pd/C (0.12 g) in EtOH (30 mL) was shaken (60 psi H_2) for 3 h. The reaction mixture was heated to reflux, filtered (Celite pad), and the solvent was evaporated to afford a white solid (1.06 g, 98%). This product was used without further purification. ^1H NMR: 0.87 (6H, t, J = 6.3 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.51 (4H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 2.66 (3H, s, CH_3N), 3.15 (2H, t, J = 6.3 Hz, $CH_3(CH_2)_{16}CH_2N$), 3.26 (2H, t, J = 6.3 Hz, $CH_3(CH_2)_{16}CH_2N$), 3.59 (2H, s, $COCH_2$), 3.72 (2H, s, $COCH_2$), 3.80–4.10 (6H, overlapping signals due to Gly CH_2), 7.67 (1H, br s, NH), 8.14 (1H, br s, NH), 8.60 (1H, br s, NH). ^{13}C NMR: 14.1, 22.7, 26.9, 27.1, 27.7, 28.9, 29.3, 29.6, 29.7, 31.9, 42.2, 43.0, 43.7, 46.6, 47.5, 57.8, 59.5, 167.4, 170.2, 170.3, 173.3.

$18_2COCH_2(CH_3)NCH_2CO$–GGGPGGG–OCH_2Ph. $18_2COCH_2(CH_3)NCH_2CO$–GGG–OH (1.03 g, 1.25 mmol) was suspended in CH_2Cl_2 (30 mL) at 0 °C. EDCI (0.25 g, 1.30 mmol), PGGG–$OCH_2Ph\cdot HCl$ (0.52 g, 1.25 mmol), HOBt (0.18 g, 1.33 mmol) and DIEA (0.5 mL) were added and the reaction was stirred for 48 h at rt. The solvent was evaporated and the residue chromatographed (SiO_2, 5–10% MeOH–$CHCl_3$) and recrystallized from MeOH to give a white solid (1.23 g, 83%), mp 106–107 °C. ^1H NMR: 0.87 (6H, t, J = 6.3 Hz, $C\mathit{H}_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, m, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.51 (4H, m, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 1.85–2.20 (4H, m, Pro $NCH_2C\mathit{H}_2C\mathit{H}_2$), 2.77 (3H, s, CH_3N), 3.13 (2H, t, J = 6.3 Hz, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.26 (2H, t, J = 6.3 Hz, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.50–4.20 (18H, overlapping signals due to Pro $NC\mathit{H}_2CH_2CH_2$, Gly CH_2, $COCH_2$), 4.36 (1H, m, Pro CH), 5.14 (2H, m, $OC\mathit{H}_2Ph$), 7.30–7.35 (5H, m, H_{Ar}), 7.64 (1H, br s, NH), 7.80 (1H, br s, NH), 7.93 (1H, br s, NH), 8.09 (1H, br s, NH), 8.31 (1H, br s, NH), 8.78 (1H, br s, NH). ^{13}C NMR: 14.1, 22.7, 25.1, 26.7, 27.1, 27.7, 28.9, 29.1, 29.3, 29.4, 29.6, 29.7, 31.9, 41.3, 41.9, 42.9, 43.1, 43.4, 43.9, 46.5, 47.0, 47.4, 57.9, 61.4, 67.2, 128.2, 128.4, 128.6, 135.3, 169.2, 170.2, 170.3, 170.4, 171.0, 173.4. Anal. Calc. for $C_{65}H_{113}N_9O_{10}$: C 66.12, H 9.65, N 10.68%. Found: C 65.93, H 9.61, N 10.48%.

Compound 7: [2-(2-{[1-(2-{2-[2-(2-dioctadecylcarbamoylmethylsulfanylacetylamino)acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}acetylamino)acetylamino]acetic acid benzyl ester, 7

$18_2COCH_2SCH_2COOH$. Dioctadecylamine (7.9 g, 15.1 mmol) and thiodiacetic anhydride (2.0 g, 15.1 mmol) in THF (100 mL) was heated at reflux for 48 h. The solvent was evaporated and the product was crystallized (Et_2O) to give the amido acid as a white solid (9.9 g, 70%), mp 59–61 °C. ^1H NMR: 0.88 (6H, t, J = 6.6 Hz, $C\mathit{H}_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, pseudo-s, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.54 (4H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 3.26 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.30–3.40 (4H, overlapping signals due to $CH_3(CH_2)_{16}C\mathit{H}_2N$ and $COCH_2S$), 3.48 (2H, s, $COCH_2S$). ^{13}C NMR: 14.1, 22.7, 26.9, 27.6, 29.1, 29.2, 29.3, 29.5, 29.7, 31.9, 32.45, 34.6, 47.1, 49.0, 169.8, 170.2.

$18_2COCH_2SCH_2CO$–GGG–OCH_2Ph. To $18_2COCH_2SCH_2COOH$ (2.0 g, 3.0 mmol) dissolved in CH_2Cl_2 (30 mL), EDCI (0.62 g, 3.2 mmol) and HOBt (0.43 g, 3.2 mmol) were added and the mixture was stirred at rt. After 0.5 h, $TsOH\cdot GGG$–OCH_2Ph (1.32 g, 3.0 mmol) and DIEA (1.7 mL) were added and the reaction mixture was stirred at rt overnight. The solvent was evaporated and the residue chromatographed (SiO_2, 2% MeOH–$CHCl_3$) to give a colorless oil (2.20 g, 79%). ^1H NMR: 0.87 (6H, t, J = 6.9 Hz, $C\mathit{H}_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, pseudo-s, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.40–1.60 (4H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 3.20–3.35 (6H, overlapping signals due to $CH_3(CH_2)_{16}C\mathit{H}_2N$ and $COC\mathit{H}_2S$), 3.44 (2H, s, $COC\mathit{H}_2S$), 3.90–4.10 (6H, overlapping signals due to Gly NCH_2), 5.15 (2H, s, $PhC\mathit{H}_2O$), 7.11 (1H, t, J = 5.7 Hz, Gly CONH), 7.30–7.35 (5H, m, H_{Ar}), 7.74 (1H, t, J = 5.7 Hz, Gly CONH), 8.07 (1H, t, J = 5.7 Hz, Gly CONH). ^{13}C NMR: 14.0, 22.6, 26.9, 27.0, 27.7, 29.2, 29.3, 29.5, 29.6, 29.7, 31.9, 33.1, 35.7, 41.2, 43.1, 44.0, 46.6, 48.8, 67.0, 128.2, 128.4, 128.6, 135.4, 168.7, 169.4, 169.6, 169.7, 170.1.

$18_2COCH_2SCH_2CO$–GGGPGGG–OCH_2Ph. $18_2COCH_2SCH_2CO$–GGG–OCH_2Ph (2.0 g, 2.2 mmol) was dissolved in MeOH (10 mL) and 15% NaOH (30 mL) was added. The mixture was heated to reflux overnight, then cooled and the pH was adjusted to 2 (conc. HCl). The mixture was filtered and the precipitate was dried *in vacuo* to give a white solid (1.7 g, 94%). This product was used without further purification.

$18_2COCH_2SCH_2CO$–GGG–OH. (1.33 g, 1.61 mmol) was suspended in CH_2Cl_2 (30 mL) at 0 °C. EDCI (0.33 g, 1.72 mmol), PGGG–$OCH_2Ph\cdot HCl$ (0.67 g, 1.61 mmol), HOBt (0.23 g, 1.70 mmol) and DIEA (0.3 mL) were added and the reaction was stirred for 48 h at rt. The solvent was evaporated and the residue chromatographed (SiO_2, 5–10% MeOH–$CHCl_3$) to give a white solid (1.58 g, 83%), mp 59–60 °C. ^1H NMR: 0.87 (6H, t, J = 6.3 Hz, $C\mathit{H}_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, m, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.40–1.60 (4H, m, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 1.80–2.10 (4H, m, Pro $NCH_2C\mathit{H}_2C\mathit{H}_2$), 3.00–4.10 (22H, overlapping signals due to $CH_3(CH_2)_{15}CH_2C\mathit{H}_2N$, Pro $NC\mathit{H}_2CH_2CH_2$, Gly CH_2, $COCH_2S$), 4.38 (1H, m, Pro CH), 5.14 (2H, s, $OC\mathit{H}_2Ph$), 7.30–7.35 (5H, m, H_{Ar}), 7.70 (2H, br s, NH), 8.48 (1H, t, J = 5.7 Hz, NH). ^{13}C NMR: 14.1, 22.7, 25.0, 26.9, 27.0, 27.6, 28.9, 29.1, 29.4, 29.7, 31.7, 31.9, 34.1, 41.1, 42.6, 42.9, 43.6, 46.4, 47.1, 48.8, 61.3, 66.9, 128.2, 128.3, 128.5, 135.4, 168.7, 169.0, 169.8, 169.9, 170.0, 170.2, 173.0. Anal. Calc. for $C_{64}H_{110}N_8O_{10}S\cdot H_2O$: C 63.97, H 9.39, N 9.32%. Found: C 63.87, H 9.37, N 9.12%.

Compound 8: [2-(2-{[1-(2-{2-[2-(3-dioctadecylcarbamoylbenzoylamino)acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}acetylamino)acetylamino]acetic acid benzyl ester

***N*,*N*-Dioctadecylisophthalamic acid.** Isophthaloyl dichloride (4.25 g, 20.9 mmol) was dissolved in a mixture of dry DMF (150 mL) and CH_2Cl_2 (300 mL). Dioctadecylamine (2.0 g, 3.9 mmol) and Et_3N (6.6 mL) were dissolved in a mixture of dry DMF (150 mL) and CH_2Cl_2 (300 mL) and added dropwise to the solution of isophthaloyl dichloride during 15 min. The mixture was stirred at rt for 40 min and evaporated *in vacuo*. The product was dissolved in CH_2Cl_2 (150 mL) and washed with dilute HCl (3 × 50 mL), brine (25 mL), dried over $MgSO_4$ and evaporated. The oily product was chromatographed (SiO_2, 25% EtOAc–hexanes) to give a white solid (1.07 g, 41%, mp 71–72 °C). ^1H NMR: 0.87 (6H, t, J = 6.6 Hz, CH_3), 1.25 (60H, pseudo-s, $CH_3(C\mathit{H}_2)_{15}CH_2CH_2N$), 1.50 (2H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 1.66 (2H, br s, $CH_3(CH_2)_{15}C\mathit{H}_2CH_2N$), 3.17 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 3.49 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}C\mathit{H}_2N$), 7.40–7.45 (1H, m, H_{Ar}), 7.50–7.57 (1H, m, H_{Ar}), 8.00–8.10 (2H, m, H_{Ar}). ^{13}C NMR: 14.1, 22.7, 26.5, 27.1, 27.5, 29.3, 29.7, 31.9, 45.1, 49.2, 128.2, 128.8, 129.4, 130.7, 131.8, 137.7, 170.1, 170.4.

18_2CO–*m*-C_6H_4–CO–GGG–OCH_2Ph. To *N*,*N*-dioctadecylisophthalamic acid (0.48 g, 0.7 mmol) was dissolved in CH_2Cl_2 (25 mL), EDCI (0.15 g, 0.77 mmol) and HOBt (0.11 g, 0.77 mmol) were added and the mixture was stirred at rt. After 0.5 h, TsOH · GGG–OCH_2Ph (0.32 g, 0.7 mmol) and Et_3N (0.3 mL) were added and the reaction mixture was stirred at rt for 48 h. The solvent was evaporated and the residue chromatographed (SiO_2, 2% MeOH–$CHCl_3$) to give a white solid (0.43 g, 66%, mp 48–49 °C). ^1H NMR: 0.87 (6H, t, J = 6.9 Hz, –CH_2CH_3), 1.25 (60H, pseudo-s, $CH_3(CH_2)_{15}$-CH_2CH_2N), 1.54 (2H, br s, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.63 (2H, br s, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.13 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}CH_2N$), 3.44 (2H, t, J = 7.8 Hz, CH_3-$(CH_2)_{16}CH_2N$), 3.83 (2H, d, J = 5.7 Hz, Gly NCH_2), 3.80–4.00 (4H, overlapping signals due to Gly NCH_2), 5.13 (2H, s, $PhCH_2O$), 7.30–7.50 (10H, m, H_{Ar}), 7.87 (2H, s, H_{Ar}), 7.96 (1H, br s, Gly CONH). ^{13}C NMR: 14.1, 22.7, 26.6, 27.1, 27.5, 28.9, 29.1, 29.3, 29.4, 29.6, 29.7, 31.9, 41.2, 42.7, 43.8, 45.2, 49.3, 67.2, 125.4, 128.2, 128.4, 128.6, 128.7, 129.3, 134.0, 135.2, 137.2, 167.5, 169.6, 169.9, 170.0, 170.9.

18_2CO–*m*-C_6H_4–CO–GGGPGGG–OCH_2Ph. 18_2CO–*m*-C_6H_4-CO–GGG–OCH_2Ph (0.85 g, 0.91 mmol) and 10% Pd/C (0.14 g) in EtOH (30 mL) were shaken (60 psi H_2) for 3 h. The reaction mixture was heated to reflux, filtered, and evaporated to afford a white solid (0.68 g, 90%). This product was used without further purification.

18_2CO–*m*-C_6H_4–CO–GGG–OH. (0.5 g, 0.59 mmol) was suspended in CH_2Cl_2 (30 mL) at 0 °C. EDCI (0.12 g, 0.65 mmol), PGGG–OCH_2Ph · HCl (0.24 g, 0.59 mmol), HOBt (0.09 g, 0.65 mmol) and Et_3N (0.25 mL) were added and the reaction was stirred for 48 h at rt. The solvent was evaporated and the residue chromatographed (SiO_2, 10% MeOH–$CHCl_3$) to give **8** as a white solid (0.34 g, 48%), mp 138–139 °C. ^1H NMR: 0.87 (6H, t, J = 6.3 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.54 (2H, br s, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.63 (2H, br s, $CH_3(CH_2)_{15}$-CH_2CH_2N), 1.80–2.10 (4H, m, Pro $NCH_2CH_2CH_2$), 3.10 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}CH_2N$), 3.10–4.20 (17H, overlapping signals due to $CH_3(CH_2)_{15}CH_2CH_2N$, Pro CH and $NCH_2CH_2CH_2$, and Gly CH_2), 5.09 (2H, m, OCH_2Ph), 7.30–7.40 (10H, m, H_{Ar}), 7.55 (1H, br s, NH), 7.68 (1H, br s, NH), 7.85–7.95 (2H, m, H_{Ar}), 8.30 (1H, br s, NH), 8.38 (1H, br s, NH). ^{13}C NMR: 14.1, 22.6, 25.2, 26.6, 27.1, 27.5, 28.6, 28.9, 29.1, 29.4, 29.6, 29.7, 31.9, 41.2, 41.7, 42.6, 42.7, 43.4, 44.1, 45.2, 46.8, 49.3, 61.0, 67.2, 125.8, 128.2, 128.4, 128.6, 128.8, 129.1, 133.8, 135.3, 137.2, 167.2, 168.3, 170.0, 170.1, 170.3, 170.5, 171.1, 171.3, 173.9. Anal. Calc. for $C_{68}H_{110}N_8O_{10}$: C 68.08, H 9.24, N 9.34%. Found: C 67.87, H 9.17, N 9.48%.

Compound 9: [2-(2-{[1-(2-{2-[2-(4-dioctadecylcarbamoylbenzoyl-amino)acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]-amino}acetylaminoacetylamino]acetic acid benzyl ester

***N*,*N*-Dioctadecylterephthalamic acid.** Terephthalic acid (3.8 g, 22.8 mmol) was dissolved in toluene (20 mL) and $SOCl_2$ (15 mL) was added in one portion. The reaction mixture was heated at 40 °C overnight and then evaporated *in vacuo*. The resulting yellow oil was dissolved in dry DMF (150 mL) and CH_2Cl_2 (300 mL). Dioctadecylamine (2.2 g, 4.2 mmol) and Et_3N (7.2 mL) were dissolved in dry DMF (150 mL) and CH_2Cl_2 (300 mL) and added dropwise to the solution of terephthaloyl chloride during 15 min. The reaction mixture was stirred at rt for 40 min and the solvent was removed *in vacuo*. The product was dissolved in CH_2Cl_2 (150 mL) and washed with 10% HCl (3 × 50 mL), brine (25 mL), dried over $MgSO_4$, and evaporated. The oily product was chromatographed (SiO_2, 25% EtOAc–hexanes) to afford a white solid (1.07 g, 37%, mp 51–52 °C). ^1H NMR: 0.87 (6H, t, J = 6.6 Hz, CH_3), 1.25 (60H, pseudo-s, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.45–1.70 (4H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.13 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}CH_2N$), 3.49 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}CH_2N$) 7.44 (2H, d, J = 8.1 Hz, H_{Ar}), 8.13 (2H, d, J = 8.1 Hz, H_{Ar}). ^{13}C NMR: 14.1, 22.7, 26.5, 27.1, 27.5, 27.7, 28.7, 29.1, 29.3, 29.7, 31.9, 44.9, 49.0, 126.6, 130.0, 130.3, 142.3, 170.5, 170.6.

18_2CO–*p*-C_6H_4–CO–GGG–OCH_2Ph. To *N*,*N*-dioctadecyl-terephthalamic acid (0.38 g, 0.55 mmol) dissolved in CH_2Cl_2 (25 mL), EDCI (0.12 g, 0.6 mmol) and HOBt (0.08 g, 0.6 mmol) were added and the mixture was stirred at rt. After 0.5 h, TsOH · GGG–OCH_2Ph (0.25 g, 0.55 mmol) and Et_3N (0.23 mL) were added and the reaction mixture was stirred at rt for 48 h. The solvent was evaporated and the residue chromatographed (SiO_2, 2% MeOH–$CHCl_3$) to give a white solid (0.42 g, 83%, mp 83–84 °C). ^1H NMR: 0.87 (6H, t, J = 6.9 Hz, -CH_2CH_3), 1.25 (60H, pseudo-s, $CH_3(CH_2)_{15}$-CH_2CH_2N), 1.40–1.65 (4H, br s, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.12 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}CH_2N$), 3.46 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}CH_2N$), 3.87 (2H, d, J = 5.7 Hz, Gly NCH_2), 4.00–4.10 (4H, overlapping signals due to Gly NCH_2), 5.15 (2H, s, $PhCH_2O$), 7.16 (2H, m, Gly CONH), 7.34 (5H, pseudo-s, H_{Ar}), 7.37 (2H, d, J = 8.1 Hz, H_{Ar}), 7.65 (1H, m, Gly CONH) 7.87 (2H, d, J = 8.1 Hz, H_{Ar}). ^{13}C NMR: 14.0, 22.6, 27.1, 27.5, 28.8, 29.3, 29.6, 29.7, 31.9, 41.3, 42.8, 43.7, 45.0, 49.1, 67.2, 126.5, 127.7, 128.2, 128.5, 128.6, 134.2, 135.2, 140.5, 167.6, 169.4, 169.7, 170.7.

18_2CO–*p*-C_6H_4-CO–GGGPGGG–OCH_2Ph. 18_2CO–pC_6H_4–CO–GGG–OCH_2Ph (0.7 g, 0.75 mmol) and 10% Pd/C (0.12 g) in EtOH (30 mL) was shaken (60 psi H_2) for 3 h. The mixture was heated to reflux, filtered (Celite pad) and the solvent was evaporated to afford a white solid (0.61 g, 97%). This product was used in without further purification. 18_2CO–pC_6H_4-CO–GGG–OH (0.4 g, 0.48 mmol) was suspended in CH_2Cl_2 (30 mL) at 0 °C. EDCI (0.1 g, 0.53 mmol), PGGG–OCH_2Ph · HCl (0.2 g, 0.48 mmol), HOBt (0.07 g, 0.53 mmol) and Et_3N (0.2 mL) were added and the reaction was stirred for 48 h at rt. The solvent was evaporated and the residue was chromatographed (SiO_2, 5% MeOH–$CHCl_3$) to give **9** as a white solid (0.34 g, 59%), mp 150–151 °C. ^1H NMR: 0.87 (6H, t, J = 6.3 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.25 (60H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.40–1.60 (4H, m, CH_3-$(CH_2)_{15}CH_2CH_2N$), 1.80–2.10 (4H, m, Pro NCH_2-CH_2CH_2), 3.07 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{16}CH_2N$), 3.10–4.25 (17H, overlapping signals due to $CH_3(CH_2)_{15}$-CH_2CH_2N, Pro CH and $NCH_2CH_2CH_2$, and Gly CH_2), 5.14 (2H, m, OCH_2Ph), 7.30–7.35 (5H, m, H_{Ar}), 7.38 (2H, d, J = 8.1 Hz, H_{Ar}), 7.54 (2H, br s, NH), 7.95 (2H, d, J = 8.1 Hz, H_{Ar}), 8.04 (1H, br s, NH), 8.52 (2H, br s, NH). ^{13}C NMR: 14.0, 22.6, 25.1, 26.6, 27.1, 27.5, 28.7, 28.9, 29.2, 29.3, 29.4, 29.7, 31.9, 41.3, 41.5, 42.6, 42.8, 43.4, 43.6, 45.0, 46.7, 49.2, 61.0, 67.3, 126.4, 127.9, 128.2, 128.4, 128.6, 134.4, 135.3, 140.1, 167.7, 168.0, 170.0, 170.3, 170.4, 170.5, 171.0, 171.6, 174.2. Anal. Calc. for $C_{68}H_{110}N_8O_{10}$: C 68.08, H 9.24, N 9.34%. Found: C 67.86, H 9.27, N 9.59%.

Formation of vesicles. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphate (DOPA) were obtained from Avanti Polar Lipids as $CHCl_3$ solutions (vials with 25 mg in 2.5 mL). Dry lipid films of DOPC-DOPA (20 mg, 7 : 3 w/w, *i.e.*, 2 : 1 mol/mol) were dissolved in Et_2O (0.5 mL) and then 0.5 mL of internal buffer (600 mM KCl : 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH adjusted to 7.0) were added. The mixture was sonicated for approximately 10 s to give an opalescent dispersion. The organic solvent was then removed by evaporation under mild vacuum at 30 °C. The suspension was filtered through a 200 nm filter membrane (five times) using a mini extruder and passed through a Sephadex G25 column, which had been equilibrated with external buffer (400 mM K_2SO_4 : 10 mM HEPES, pH adjusted to 7.0). The vesicles collected were subsequently characterized as ~200 nm using laser light scattering. The final lipids concentration was assessed through colorimetric determination of the phospholipid-ammonium ferrothiocyanate complex.

Studies of chloride release from liposomes. Chloride release was assayed directly on ~200 nm, 0.23 mM phospholipid vesicles by using a chloride selective electrode (Accumet Chloride Combination Electrode). The electrode was introduced in a 2 mL vesicle solution and allowed to equilibrate. The voltage output was recorded, and after 5 min, aliquots of the compound solution (~9 mM in 2-propanol) were added. The amount of 2-propanol was limited to 20 μL to minimize its effect on the liposomes in any experiment. Complete lysis of the vesicles was induced by addition of a 2% aqueous solution of Triton X-100 (100 μL) and the data collected (DigiData 1322A series interface and Axoscope 9.0 software) were normalized to this value.

Computational details. All calculations were performed using the Gaussian 03 suite of programs. The B3LYP as one of Density Functional Theory methods was used for all geometry optimization. The method combines Beck's three-parameter functional,[35] with the nonlocal correlation provided by the correlation functional of Lee, Yang, and Parr,[36] which has been shown to be suitable for vibrational frequency calculations and geometry optimization. We performed the restricted B3LYP with 6-31+G* basis set. For each molecule, several reasonable conformations were calculated to compare the final energies of stable conformations and the one having the lowest energy was chosen as the final structure. For the chosen structure, all geometries were completely optimized in gas phase.

Conclusions

The original SAT design[18] incorporated diglycolic acid for two reasons. First, its overall size and polarity corresponded well to the glyceryl unit present in phospholipid monomers. Second, it was a convenient synthetic module that was easy to incorporate and to confirm analytically in the product. We conclude from the studies described here that 3-thiaglutaric acid is equally convenient to incorporate and gives a SAT that typically has higher Cl^- transport efficacy. Terephthalic acid is also a candidate for a midpolar regime mimic but it leads to a somewhat less efficacious SAT (**9**) than **7**. The location of the carboxyl groups in terephthalic acid prevents formation of a cyclic anhydride, making the diacid less convenient to incorporate. Future designs will incorporate 3-thiaglutaric acid. Polar elements and very high flexibility will be avoided in designing new synthetic anion transporters of this class.

Acknowledgements

We thank the NIH for grants (GM 36262 and GM 63190) that supported this work.

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