

Replacing proline at the apex of heptapeptide-based chloride ion transporters alters their properties and their ionophoretic efficacy

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A membrane anchored heptapeptide, (C₁₈H₃₇)₂NHCOCH₂OCH₂CO–NH–Gly–Gly–Gly–Pro–Gly–Gly–Gly–OCH₂Ph, has proved to be a selective chloride anion transporter that functions in phospholipid bilayer membranes. When Pro was replaced by the natural amino acid Leu, the activity decreased dramatically. In the present study, Pro is replaced by pipecolic acid (homoproline, Pip); the resulting anchored heptapeptide is a membrane active, chloride selective transporter.

Chloride membrane transport is a ubiquitous process in living systems. An array of chloride channels has been studied for some time and understanding their function is clearly an important goal.^{1,2} The only solid state structures of chloride transporters were reported very recently.³ The X-ray structures of the ClC Cl[−] channels from the prokaryotes *Salmonella enterica* serovar typhimurium and *Escherichia coli* were reported at 3.0 Å and 3.5 Å resolutions, respectively. These structures reveal that the chloride channels are quite complicated and distinct from those of voltage gated cation channels. Both ClC channels incorporate two subunits of 18 α-helices that function as independent channel pathways. Their association is coordinated through a 2300 Å² contact surface. To date, four major families of chloride channels have been described.^{4,5}

The complexity of the chloride channel structures results from many regulatory influences and a long evolutionary history as a fundamental cellular function. In early organisms, chloride transport essential to volume and pH control must have been achieved by significantly simpler structures. Certainly, chloride permeability in contemporary cells is essential for volume, pH, and membrane potential regulation and these same features must have been required, at least to some extent, in early organisms.^{6,7} The work reported here results from an effort to develop a simple, abiotic compound that would function as a chloride transporter in phospholipid bilayers.

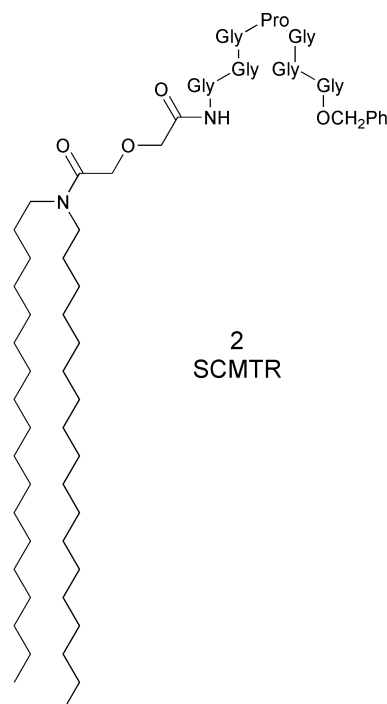
An effort by Tomich and coworkers^{8–10} has been reported to develop a chloride channel by using modified proteins. Specifically, C-K4-M2GlyR is a peptide prepared by modifying either the N- or C-terminus of the M2 transmembrane sequence of the brain glycine receptor by the addition of lysine residues. The resulting peptide, C-K4-M2GlyR, was found to act as a chloride channel when it was inserted into the apical membrane of cultured renal epithelial monolayers. Our effort to develop a chloride transporter differed. We used what is currently known about chloride channel proteins as a guide and inspiration for the design of a synthetic, chloride selective transporter. As a result, we recently reported the preparation and limited characterization of the first example of this new

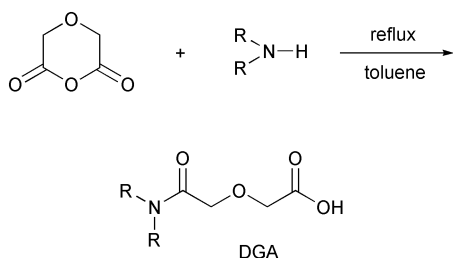
family of abiotic chloride channels.^{11,12} We now report related compounds that have quite different activities depending on what appear to be very subtle differences.

Results and discussion

Design and synthesis of SCMTR

Our design schematic for a chloride ion transporter (s_{ynthetic} c_{hloride} m_{embrane} t_ransporter, SCMTR or “scimitar”)





involved the development of three molecular sectors. These included a hydrocarbon anchor, a peptide fragment expected to serve as the channel portal and filter, and the connector. Diglycolic acid, $\text{HOCOCH}_2\text{OCH}_2\text{COOH}$, was selected to be the link between the peptide and the hydrocarbon anchors. The latter were constructed from a dialkylamine, giving an overall anchor of $\text{R}_2\text{NCOCH}_2\text{OCH}_2\text{COOH}$. The peptide was joined to the anchor's C-terminus to give, schematically, $\text{R}_2\text{NCOCH}_2\text{OCH}_2\text{CO-peptide}$.

The anchor unit was designed to correspond approximately to the size and shape of a phospholipid membrane monomer. The twin diamine chains mimicked the two fatty acid chains of a natural phospholipid. Phospholipids contain a glycerol unit, to which the fatty acids are attached by acyl links, resulting in a region of intermediate polarity. The midpolar regime was emulated by diglycolic acid, which contains carbonyl groups and an ether oxygen. Diglycolic acid is especially attractive for this application because its readily formed anhydride reacts cleanly with diamines as shown in Scheme 1.

The peptide's design was guided by structural patterns found in natural chloride transporters. It was hoped that these would lead to chloride selectivity in our abiotic compound. Based upon the following lines of evidence, we have identified a critical role for the peptide in the ion selectivity of naturally occurring chloride transporters.⁶ (1) Proline is present in the conserved motif GKxGPxxH in the anion pathway of all Cl^- chloride protein channels.^{13,14} (2) The cation selectivity of nicotinic acetylcholine receptor is altered to anion selectivity when proline is inserted into the native channel's selectivity filter.^{15,16} (3) The presence of a so-called "hinge-bend" regime in channel-forming peptides is attributed to the proline in the sequence GxxP .¹⁷ (4) Proline is reported to induce a surface "kink" in various proteins.¹⁸ (5) Studies with proinsulin's C-peptide show that the presence of a helix-loop-helix motif at the apex of the peptide's "loop" conformation is required for ion channel activity.¹⁹ The combination of structural observations and functional implications suggested to us the importance of incorporating this particular element into our ion portal design.

We settled on a heptapeptide sequence having proline as its central point for the reasons described above. The simplest heptapeptide containing a "central" proline is $\text{Gly-Gly-Gly-Pro-Gly-Gly-Gly}$ (GGGPGGG). Analyses using molecular models and simple computational methods suggested that GGGPGGG would have a v-shaped conformation. Further, distance measurements across a computer-generated model of the "v" show that the C- and N-termini have a maximum center-to-center separation of $\sim 8 \text{ \AA}$ and the apex of the "v" makes an angle of about 75° . Space-filling (CPK) molecular models show that the available space within the "v" is 6–8 Å, depending on how compressed are the two GGG chains. A computational model of $\text{CH}_3\text{CH}_2\text{CONH-GGGPGGG-OCH}_2\text{Ph}$ is shown in Fig. 1. Measurement of the model shows that the two remotest carbonyl groups are 8.8 Å apart in the illustrated conformation.

In **2**, $[\text{CH}_3(\text{CH}_2)_{17}]_2\text{NCOCH}_2\text{OCH}_2\text{CO-GGGPGGG-OCH}_2\text{Ph}$, the peptide sequence places proline at the pinnacle

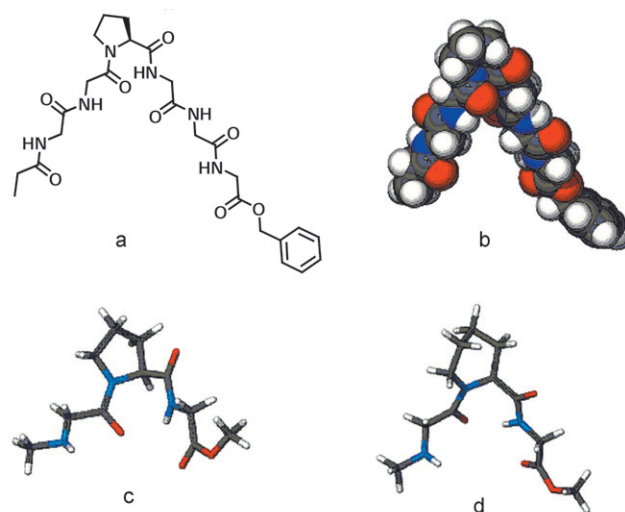


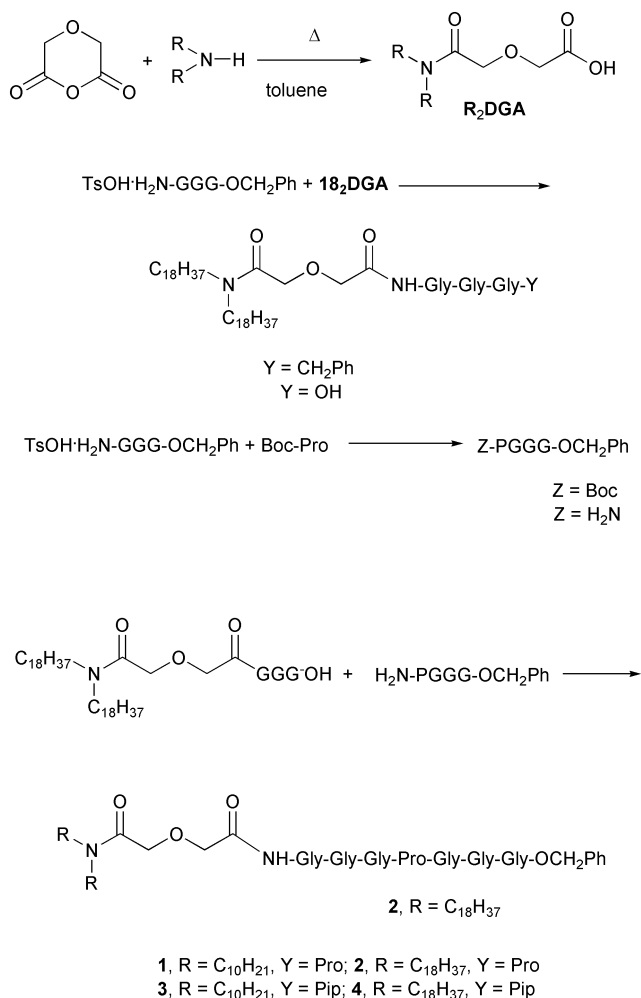
Fig. 1 Ac-GGGPGGG-Bzl shown in a structural figure (a) and rendered in the CPK representation (b). Calculated structures of $\text{CH}_3\text{NH-Gly-Pro-Gly-OCH}_3$ (c) and $\text{CH}_3\text{NH-Gly-Pip-Gly-OCH}_3$ (d).

of an "arch" flanked with glycine residues. We propose that having the heptapeptide reside at the top of the mid-polar regime of the membrane bilayer is critical. In this position it can form an uncharged, chloride-selective portal. The resulting formation of an aqueous pathway for ions to the interior of the bilayer was anticipated. If this pathway was maintained long enough for ion diffusion through the bilayer to occur, channel activity should result. We anticipated that by adding a hydrophobic membrane anchor of appropriate length, the peptide would be positioned to generate a functional "hinge-bend" portal at the membrane surface.¹⁹

The membrane anchor

As noted above, the membrane anchor was designed to mimic a phospholipid monomer in size, polarity, and functional group position. The anchor is a dialkylamino derivative of diglycolic acid: $\text{R}_2\text{NCOCH}_2\text{OCH}_2\text{COOH}$.²⁰ A related anchor system was reported several years ago^{21,22} but no experimental details were included and no yield or characterization data appear in the Beilstein database.²³ We were able to construct $\text{R}_2\text{NCOCH}_2\text{OCH}_2\text{COOH}$ conveniently by the reaction of one equivalent each of diglycolic anhydride and dialkylamine (*e.g.*, dioctadecylamine) in refluxing toluene. After heating for 48 hours, the solvent was removed and the nearly pure $\text{R}_2\text{NCOCH}_2\text{OCH}_2\text{COOH}$ was crystallized from CHCl_3 to afford the analytically pure product in nearly 90% yield. It is interesting to note that the reaction of bis(2-methoxyethyl)-amine with diglycolic anhydride is reported to be much faster than the rates we observed for the anhydride with simple dialkylamines.²¹ We confirmed this rate difference, although the experimental procedure in the literature does not work as reported for the methoxyethylamine.

We surmised that an anchored peptide would insert deeply into the outer leaflet of a phospholipid bilayer. This, we felt, would lead to a controlled membrane disruption of the second leaflet resulting in a transmembrane pore. The advantage of this approach was thought to be that the kinetics of insertion into the outer leaflet of the bilayer would be more favorable than any system that required transverse relaxation (flip-flop). Further, by limiting the length of our anchor to the thickness of a single bilayer, we anticipated that the detergent activity of this compound would be minimized, limiting the formation of membrane disks at high concentrations.²⁴



Scheme 2

Synthesis of the compounds studied

Four target compounds were prepared for the studies reported here. Each procedure began with the reaction of either didecylamine or dioctadecylamine with diglycolic anhydride. The product was $(\text{C}_{10}\text{H}_{21})_2\text{NCOCH}_2\text{OCH}_2\text{COOH}$ or $(\text{C}_{18}\text{H}_{37})_2\text{NCOCH}_2\text{OCH}_2\text{COOH}$, respectively. We use shorthand to designate these diglycolic amide (DGA) derivatives: 10_2DGA-OH and 18_2DGA-OH . The C-terminal tripeptide was prepared as a benzyl ester. It was coupled (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) with $\text{TsOH} \cdot \text{H}_2\text{N-GGG-OCH}_2\text{Ph}$ to give $\text{R}_2\text{DGA-Gly-Gly-Gly-OCH}_2\text{Ph}$. The C-terminal benzyl group was removed by hydrogenolysis in EtOH.

The tetrapeptide required to complete the heptapeptide sequence was prepared by coupling $\text{TsOH} \cdot \text{H}_2\text{N-GGG-OCH}_2\text{Ph}$ with N-Boc-Pro-OH to give $\text{Boc-Pro-Gly-Gly-Gly-OCH}_2\text{Ph}$ (Z-PGGG-Bzl). The Boc group was removed (HCl, dioxane) and the final coupling between $\text{R}_2\text{DGA-Gly-Gly-Gly-OH}$ and $\text{H}_2\text{N-PGGG-OCH}_2\text{Ph}$ gave either $10_2\text{DGA-GGGPGGG-Bzl}$ (**1**) or $18_2\text{DGA-GGGPGGG-Bzl}$ (**2**). Compounds **3** and **4** were prepared analogously except that pipecolic acid (homoproline) replaced proline in the heptapeptide. The sequence is shown below in Scheme 2 for the preparation of either **1** or **2** and details for the synthesis of compounds **1-4** are recorded in the Experimental Section.

Carboxyfluorescein dequenching and pore characterization

Carboxyfluorescein (CF) is a fluorescent dye that may readily be incorporated into liposomes. When concentrated within the

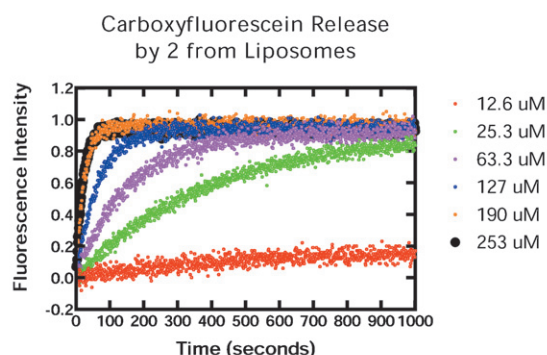


Fig. 2 Carboxyfluorescein release by **2** from liposomes. Concentrations of **2**: (bottom to top): 12.6 μM , 25.3 μM , 63.3 μM , 127 μM , 190 μM , and 253 μM . The two highest concentrations are nearly superimposed in this graph.

vesicle, CF self-quenches. Under appropriate conditions, its exit from vesicles may readily be detected. We prepared phospholipid liposomes in the presence of 20 mM carboxyfluorescein (CF). Dequenching of intravesicular CF was followed spectrofluorometrically in a thermostated cuvette (see Experimental Section). Data obtained for **2** over a range of concentrations are shown in Fig. 2. On the timescale shown, the data for **2** at 190 μM and 253 μM are nearly superimposable.

The CF transport data show that **2** forms well-behaved pores that release the dye in a controlled fashion. The critical test, however, of a putative chloride transporter is whether it fosters chloride release from liposomes. We used unilamellar liposomes (150 ± 16 nm) prepared from 30% (w/w) 1,2-dioleoyl-*sn*-glycero-3-phosphate and 70% 1,2-dioleoyl-*sn*-glycero-3-phosphocholine.

Compound **2** mediated chloride release that was rapid, complete, and concentration dependent.¹¹ Both **1** and **2** release carboxyfluorescein, a process related to chloride transport. We have recently noted that CF release by **1** (10Pro), which has shorter anchor chains, is about 10-fold greater²⁵ than for **2** (18Pro).¹¹ Thus CF release was complete in less than 100 s when either **1** or **2** was present at a concentration of ~ 15 μM or ~ 250 μM respectively. We infer from this that the as yet untested chloride transport rates will show a comparable relationship.

Replacement of proline by leucine in **2** ($18_2\text{DGA-GGGPGGG-Bzl} \rightarrow 18_2\text{DGA-GGGLGGG-Bzl}$, **5**) led to reduced CF release. Thus, equal CF release rates could be achieved when **[5]** = 154 μM and **[2]** = 24.1 μM .¹¹ This is at least a 6-fold reduction in activity, apparently resulting from the loss of the rigid “hinge-bend” enforced by proline’s cyclic structure.

Pore conductance and rectification in planar bilayers

Planar lipid bilayers were prepared from asolectin and studied under voltage clamp conditions (see Experimental Section and ref. 26). The addition and mixing of 89.7 μM $18_2\text{DGA-GGGPGGG-Bzl}$ (**2**, 18Pro, SCMT) on the *trans* side of the planar bilayer resulted in a current that was mildly rectifying at negative potentials. The I - V relationship (-60 mV to $+60$ mV) had a limiting slope of 349 pS. The $10_2\text{DGA-GGGPGGG-Bzl}$ (**1**, 10Pro, SCMT) at 5.2 μM was also mildly rectifying and had a limiting slope of 573 pS. We have previously noted that the 18Pro is selective for chloride over potassium by $> 10:1$. The larger conductance of 10Pro with an identical pore size as determined in Fig. 1 indicates that this pore is relatively non-selective for chloride *versus* potassium.

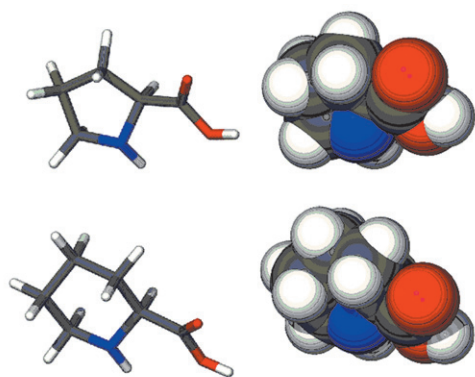


Fig. 3 Models of proline (top) and pipecolic acid in tube and space filling representations.

Substitution of proline in the heptapeptide sequence by leucine or by pipecolic acid

The known peptide sequence of Cl^- -conducting C-peptide²⁷ contains proline at the loop apex in humans and in rats.²⁸ In contrast, the pig sequence has leucine in this position and this peptide is not a chloride ion transporter.²⁷ As noted above, we found in previous studies that a Pro \rightarrow Leu replacement led to diminished (~ 6 -fold) activity. A more subtle variation is the change from proline to pipecolic acid.²⁹ This alteration formally involves a 1-carbon ring expansion of the essential amino acid. The structures of the bis(octadecyl) derivatives are shown below as **2** and **4**.

These two amino acids differ in several respects (Fig. 3).²⁹ The difference in ring size changes the N–C–CO angles from $\sim 112.5^\circ$ to $\sim 110.5^\circ$. The presence of the additional carbon atom in the ring also increases hydrophobicity but, as with the bond angles, the change is expected to be small. The molecular weights of these two compounds are 1168 Da and 1182 Da, respectively. This is a difference of only $\sim 1\%$. We also consider the differences in bond angles and hydrophobicity to be modest.

We have assessed the efficacy of the proline (**1**, **2**) and pipecolic acid (**3**, **4**) derivatives by CF dequenching (see above). Selected data are presented in Fig. 4. The graph shows four traces corresponding to various concentrations of **1–4**. The two nearly superimposed traces seen in the middle of the graph are for $10_2\text{DGA-GGGPGGGOBzl}$ (**1**) and $18_2\text{DGA-GGGPGGG-OBzl}$ (**2**). The CF release is essentially identical but the concentration difference is dramatic: their activities differ by nearly 400-fold ($[\mathbf{1}] = 0.167 \mu\text{M}$, $[\mathbf{2}] = 63.3 \mu\text{M}$).

We may compare the activity of $10_2\text{DGA-GGGPGGG-OBzl}$ (10Pro, **1**) with $10_2\text{DGA-GGGPipGGG-OBzl}$ (10Pip, **3**). If the traces were superimposed, the ratio of activity would

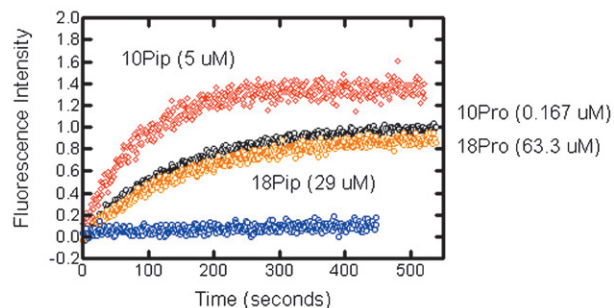
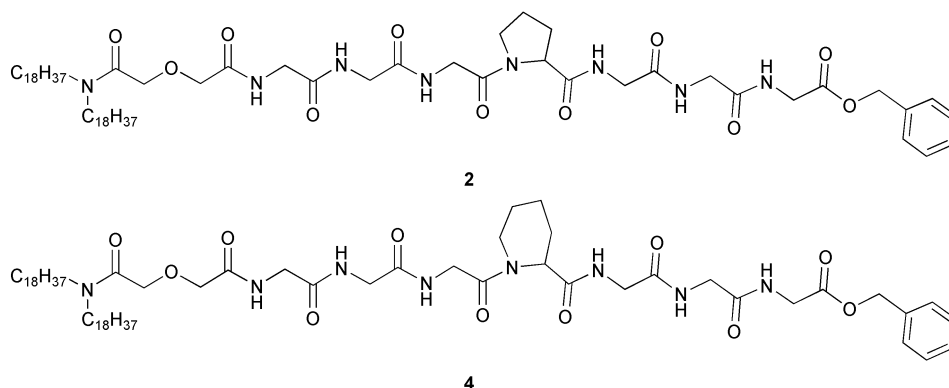


Fig. 4 Carboxyfluorescein release from phospholipid liposomes mediated by compounds **1–4**. The top (red, 10Pip, **3**, 5 μM) and bottom (blue, 18Pip, **4**, 29 μM) traces are for **3** (10Pip) pipecolic acid derivatives. The overlapping central traces (black above, **1**, 10Pro, 0.167 μM ; orange, **2**, 18Pro, 63.3 μM) are for the proline-containing heptapeptide.

be 5 $\mu\text{M}/0.167 \mu\text{M}$ or 29.9. The didecyl-pipecolic (10Pip) acid derivative (**3**) is somewhat more active at 5 μM than is the proline analog at 0.167 μM , so we estimate that 10Pip (**3**) is about 20-fold less active than the proline counterpart (10Pro, **1**). This is a significant reactivity difference that may result from variations in bond angles, hydrophobicity, and/or other factors. The more striking observation is that at a concentration of 29 μM , 18Pip (**4**) showed essentially no transport activity. A direct comparison of **2** with **4** at identical concentrations would have been more satisfactory. Unfortunately, when $[\mathbf{18Pip}] > 29 \mu\text{M}$, precipitation of the transporter occurred and the experiment was compromised. We therefore undertook the fluorescence dequenching experiment with 18Pro (**2**) at a concentration of 25.3 μM . Within the error of these experiments, this is an identical concentration. The results are shown in Fig. 4. At identical concentrations, the activity of **4** is unmeasurable compared to that of **2**.

A comparison of the data presented in the graphs of Figs. 2 and 4 raises several interesting questions. First, do we expect any activity from a compound in which pipecolic acid replaces proline? As noted above, previous work showed that the anchored heptapeptide lost nearly all of its activity when Leu replaced Pro.¹¹ This result correlated to known C-peptide activity (see above).²⁷ Pipecolic acid is far closer to proline²⁹ than is leucine, although Pro and Leu have the same number of carbon atoms. Proline and pipecolic acids are similar in size and shape and the latter should function to form a conformational kink in the heptapeptide chain as does proline. Indeed, in the C10-anchored system, pore formation for 10Pro (**1**) was ~ 20 -fold greater than for 10Pip (**3**). Significant activity for 10Pip was observed only at or above 5 μM concentrations, compared to 167 nM for 10Pro.



A second question is why no pore formation is observed for **4** when **2** shows reasonable kinetics at an identical concentration. Note that 18Pro (**2**) is significantly less active than is 10Pro (**1**). A more compelling question, given the design and conceptualization of these compounds, is why more effective transport is observed generally with shorter anchors (**1**, **3** are more active than **2** or **4**). We have experimentally confirmed this relationship in separate work but the explanation remains obscure.

Pore size for 18Pro, **2**, vs. 10Pip, **3**

Planar bilayer conductance studies permit us to assess the effective size of the pore that is formed. This is typically given in terms of conductance rather than diameter because ion selectivity as well as pore size can determine the current. As shown in the graph of Fig. 5, "C18" represents 18₂DGA-GGG-PGGG-OBzl, **2**, and "C10" corresponds to 10₂DGA-GGG-PipGGG-OBzl, **3**. The size of the resulting pores was determined to be the same when using CF (Fig. 2) so that the increase in conductance by 10Pro probably results from lowered ion selectivity. This pore passes both chloride and potassium; since they are traveling in opposite directions in response to the applied voltage, the resulting current is summed. The graph shows identical chloride selectivity (x -intercept), which indicates that the striking difference in slope results from a change in pore size. Admittedly, these compounds display large stability and activity differences in pore activation. However, these are single pore experiments in which thermodynamic and kinetic characteristics are only indirectly reflected. We have previously observed that pore sizes for **1** and **2** (C10Pro vs. C18Pro chains, proline derivative) do not differ significantly.²⁵ Thus, we did not expect ring size *per se* to lead to a difference in pore size. The data shown in Fig. 5 show that the pore sizes for **2** and **3** differ by nearly 4-fold. This is a remarkable difference considering that the peptide chains of **2** and **3** differ in such a subtle way.

Conclusion

Our study of these anchored heptapeptides was based upon the premise that a synthetic, chemically simple, chloride transporter would be instructive for the study of anion transport across bilayer lipid membranes. We hoped that defined changes in transporter structure would alter activity and provide insight

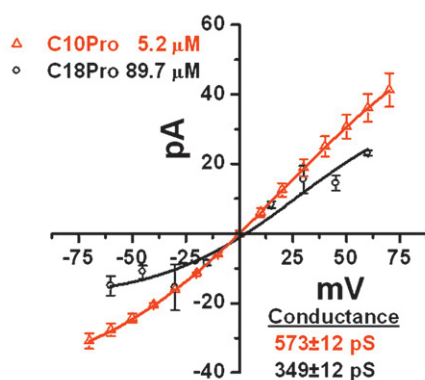


Fig. 5 Planar lipid bilayers were generated on a 0.25 mm orifice in a plastic cuvette. When a bilayer was present, C18Pro or C10Pip was added to the *cis* chamber at 40 or 9 μ M respectively in a 450:150 mM KCl gradient (*cis* to *trans*). Currents were then determined as the voltage across the bilayer was varied. The slope of the current-voltage plot is the conductance as determined from the inverse of Ohm's law ($1/R = I/E$). The x intercept is the reversal potential that is calculated by the Nernst equation to be ~ 29 mV for a 450:150 KCl gradient with a highly selective chloride channel. The slopes for the lines are shown in picoSiemens (pS) and millivolts (mV).

into important factors in anion transport. The results reported here support this hypothesis. The "SCMTR" family of molecules has solubility behavior with dramatic structural sensitivity and aggregation properties that are complex and structurally dependent. The pore activation step clearly depends upon the anchor chain length; pore size is altered dramatically by the subtle change from proline to pipecolate. We now anticipate that this novel family of compounds will be useful to explore in detail the chemistry and biophysics of pore formation and anion transport in lipid bilayers.

Experimental

General

All reaction solvents were freshly distilled and reactions were conducted under N_2 unless otherwise specified. Et_3N was distilled from KOH and stored over KOH. CH_2Cl_2 was distilled from CaH_2 . Column chromatography was performed on silica gel 60 (230–400 mesh). Thin layer chromatography was performed with silica gel 60 F254 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 received unless otherwise indicated. 1H -NMR spectra were recorded at 300 MHz and are reported in the following manner: chemical shifts are reported in ppm downfield from internal tetramethylsilane (integrated intensity, multiplicity (b = broad; s = singlet; d = doublet; t = triplet; m = multiplet, bs = broad singlet, etc.), coupling constants in Hertz, assignment). ^{13}C -NMR spectra were obtained at 75 MHz and referenced to $CDCl_3$ (δ 77.0). Infrared spectra were recorded in KBr unless otherwise noted and were calibrated against the 1601 cm^{-1} band of polystyrene. Melting points were determined on a Thomas Hoover apparatus in open capillaries and were uncorrected. Optical rotations were recorded on a Perkin-Elmer Model 214 polarimeter.

N,N-Didecyl-3-oxaglutaramide (10₂DGA)

A solution of didecylamine (5.0 g, 16.8 mmol) and diglycolic anhydride (2.2 g, 18.5 mmol) in THF (40 mL) was refluxed for 48 h. The solvent was evaporated and the crude product was dissolved in Et_2O (150 mL) and washed with 10% aqueous HCl (2×20 mL), dried ($MgSO_4$) and crystallized from Et_2O to give a white solid (5.61 g, 88%), mp 51–52 °C. 1H -NMR $CDCl_3$: 0.87 (6H, t, $J = 6.9$ Hz, $-CH_2CH_3$), 1.25 (28H, pseudo-s, $CH_3(CH_2)_7CH_2CH_2N$), 1.54 (4H, bs, $CH_3(CH_2)_7CH_2CH_2N$), 3.07 (2H, t, $J = 7.8$ Hz, $CH_3(CH_2)_7CH_2CH_2N$), 3.34 (2H, t, $J = 7.8$ Hz, $CH_3(CH_2)_7CH_2CH_2N$), 4.21 (2H, s, $COCH_2O$), 4.38 (2H, s, $COCH_2O$). ^{13}C -NMR: 14.1, 22.7, 26.8, 26.9, 27.4, 28.6, 29.2, 29.3, 29.4, 29.5, 31.8, 46.8, 71.3, 73.2, 170.6, 171.7. IR ($CHCl_3$): 3401, 3269, 2957, 2923, 2853, 1713, 1627, 1467, 1431, 1377, 1255, 1150, 1137, 1062, 923 cm^{-1} .

TsOH \cdot GGG-OCH₂Ph

H_2N -GGG-OH (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 heated to reflux and water was removed by using a Dean-Stark trap. When no more water appeared in the distillate, heating was stopped. The mixture was cooled to room temperature, diluted with ether (50 mL) and cooled in an ice water bath for 2 h. Crystalline TsOH \cdot H_2N -GGG-Bzl was collected on a filter, washed with ether (50 mL) and dried. After recrystallization from methanol-ether (1:1), the salt (5.5 g, 77%) melted at 176–177 °C. 1H -NMR $CDCl_3$: 2.34 (3H, s, CH_3Ph), 3.74 (2H, s, Gly NCH_2), 3.97 (4H, s, Gly NCH_2), 5.14 (2H, s, $PHCH_2O$), 7.21 (2H, d, $J = 8.4$ Hz, tosyl HAr), 7.30–7.35 (5H, m, Ph HAr), 7.69 (2H, d, $J = 8.4$ Hz, tosyl

HAr). ^{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. IR (KBr): 3331, 3083, 1747, 1670, 1545, 1456, 1406, 1362, 1202, 1125, 1035, 1011, 913, 817, 736, 685 cm^{-1} .

10₂DGA–GGG–OCH₂Ph

To 10₂DGA (2 g, 4.8 mmol) dissolved in CH_2Cl_2 (30 mL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.0 g, 5.3 mmol) was added and the mixture was stirred at room temperature. After 0.5 h, TsOH·GGG–OCH₂Ph (2.2 g, 4.8 mmol) and Et₃N (2.0 mL) were added and reaction was stirred at ambient temperature for two days. The reaction mixture was evaporated *in vacuo*, the residue successively washed with 5% citric acid (20 mL), water (20 mL), 5% NaHCO₃ (20 mL), and brine (20 mL), dried (MgSO₄), and evaporated. The crude, oily product was purified by chromatography (SiO₂, 2% MeOH–CH₂Cl₂) and afforded a colorless oil (2.42 g, 76%). ^1H -NMR CDCl_3 : 0.87 (6H, t, $J = 6.9$ Hz, $-\text{CH}_2\text{CH}_3$), 1.25 (28H, pseudo-s, $\text{CH}_3(\text{CH}_2)_7\text{CH}_2\text{CH}_2\text{N}$), 1.50 (4H, bs, $\text{CH}_3(\text{CH}_2)_8\text{CH}_2\text{CH}_2\text{N}$), 3.05 (2H, t, $J = 7.5$ Hz, $\text{CH}_3(\text{CH}_2)_7\text{CH}_2\text{CH}_2\text{N}$), 3.25 (2H, t, $J = 7.5$ Hz, $\text{CH}_3(\text{CH}_2)_7\text{CH}_2\text{CH}_2\text{N}$), 3.95–4.05 (6H, m, Gly NCH₂), 4.10 (2H, s, COCH₂O), 4.30 (2H, s, COCH₂O), 5.13 (2H, s, PHCH₂O), 7.30–7.35 (5H, m, HAr), 7.96 (1H, t, $J = 5.7$ Hz, Gly CONH), 8.27 (1H, t, $J = 5.7$ Hz, Gly CONH). ^{13}C -NMR: 13.9, 22.5, 26.7, 26.9, 27.4, 28.6, 29.2, 29.3, 31.8, 41.0, 42.9, 46.3, 46.7, 66.9, 69.6, 71.7, 128.2, 128.4, 128.6, 135.3, 168.6, 169.7, 169.8, 170.0, 171.5. IR (film): 3292, 2915, 2848, 1750, 1650, 1538, 1465, 1195, 1130, 1031, 721, 697 cm^{-1} .

10₂DGA–GGG–OH

10₂DGA–GGG–OCH₂Ph (2.3 g, 3.4 mmol) was dissolved in absolute ethanol (40 mL) and 10% Pd/C (0.15 g) was added and this mixture was shaken under 60 psi H₂ for 3 h. The reaction mixture was heated to reflux and filtered through a celite pad. The solvent was evaporated under reduced pressure to afford a white solid (1.92 g, 96%), mp 120–122 °C. ^1H -NMR CD_3OD : 0.90 (6H, t, $J = 6.9$ Hz, $-\text{CH}_2\text{CH}_3$), 1.29 (32H, pseudo-s, $\text{CH}_3(\text{CH}_2)_8\text{CH}_2\text{N}$), 1.57 (4H, bs, $\text{CH}_3(\text{CH}_2)_7\text{CH}_2\text{CH}_2\text{N}$), 3.21 (2H, t, $J = 7.8$ Hz, $\text{CH}_3(\text{CH}_2)_8\text{CH}_2\text{N}$), 3.35 (2H, t, $J = 7.8$ Hz, $\text{CH}_3(\text{CH}_2)_7\text{CH}_2\text{N}$), 3.93 (2H, s, Gly NCH₂), 3.94 (2H, s, Gly NCH₂), 3.97 (2H, s, Gly NCH₂), 4.12 (2H, s, COCH₂O), 4.40 (2H, s, COCH₂O). ^{13}C -NMR: IR (KBr): 3319, 3269, 3084, 2922, 2852, 1727, 1651, 1604, 1557, 1467, 1428, 1413, 1377, 1283, 1248, 1210, 1129, 1031, 955, 905, 722, 684 cm^{-1} .

Boc–PGGG–OCH₂Ph

Boc-L-Proline (1.43 g, 6.7 mmol), TsOH·GGG–O–CH₂Ph (3.0 g, 6.7 mmol), and Et₃N (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 reaction was stirred at ambient temperature for 3 days. Solvent was evaporated and residue was dissolved in EtOAc (50 mL) and washed with saturated aqueous NH₄Cl (25 mL) and brine (25 mL), dried over MgSO₄ and evaporated. The crude, oily product was purified by chromatography (SiO₂, 5% MeOH–CH₂Cl₂) and afforded colorless crystals (2.25 g, 71%, mp 54–55 °C). ^1H -NMR CDCl_3 : 1.42 (9H, s, C(CH₃)₃), 1.80–2.20 (4H, m, Pro NCH₂CH₂CH₂), 3.35–3.55 (2H, m, Pro NCH₂CH₂CH₂), 3.85–4.20 (7H, m, Gly NCH₂, Pro NCH), 5.15 (2H, s, PHCH₂O), 7.05 (2H, bs, Gly CONH), 7.30–7.35 (5H, m, HAr), 7.80 (1H, bs, 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. Anal.

Calcd for C₂₃H₃₂N₄O₇: C, 57.97; H, 6.77; N, 11.76%. Found: C, 57.87; H, 6.76; N, 11.39%.

HCl·H₂N–PGGG–OCH₂Ph

Boc–PGGG–OCH₂Ph (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 1:1 MeOH/Et₂O (0.18 g, 100%) to give the title compound as a colorless solid (mp 145–146 °C). ^1H -NMR (CD_3OD): 2.00–2.25 (4H, m, Pro NCH₂CH₂CH₂), 3.35–3.45 (2H, m, Pro NCH₂CH₂CH₂), 3.90–4.05 (6H, m, Gly NCH₂), 4.30–4.40 (1H, m, Pro NCH), 5.18 (2H, s, PHCH₂O), 7.30–7.40 (5H, m, HAr). ^{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.

10₂DGA–GGGPGGG–OCH₂Ph, 1

To 10₂DGA–GGG–OH (0.18 g, 0.31 mmol) suspended in CH_2Cl_2 (20 mL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.07 g, 0.34 mmol) was added and the reaction was stirred for 0.5 h. Then HCl·PGGG–OCH₂Ph (0.13 g, 0.31 mmol) in CH_2Cl_2 (10 mL) containing Et₃N (0.10 mL) was added and reaction mixture was stirred for 48 h at room temperature. Then solvent was evaporated and the crude, oily product was purified by chromatography (SiO₂, 10% MeOH–CH₂Cl₂) and afforded **1** as colorless crystals (0.17 g, 60%), mp 127–128 °C. $[\alpha]_D^{20} -11.4^\circ$ (c 1.265, CHCl_3). ^1H -NMR CDCl_3 : 0.87 (6H, t, $J = 6.9$ Hz, $-\text{CH}_2\text{CH}_3$), 1.25 (28H, pseudo-s, $\text{CH}_3(\text{CH}_2)_7\text{CH}_2\text{CH}_2\text{N}$), 1.47 (4H, bs, $\text{CH}_3(\text{CH}_2)_7\text{CH}_2\text{CH}_2\text{N}$), 1.80–2.20 (4H, m, Pro NCH₂CH₂CH₂), 3.03 (2H, t, $J = 7.5$ Hz, $\text{CH}_3(\text{CH}_2)_{16}\text{CH}_2\text{N}$), 3.23 (2H, t, $J = 7.5$ Hz, $\text{CH}_3(\text{CH}_2)_8\text{CH}_2\text{N}$), 3.40–3.45 (1H, m, Pro NCH₂CH₂CH₂), 3.50–3.55 (1H, m, Pro NCH₂CH₂CH₂), 3.90–4.05 (12H, m, Gly NCH₂), 4.12 (2H, s, COCH₂O), 4.26 (2H, s, COCH₂O), 4.35 (1H, bs, Pro NCH), 5.12 (2H, s, PHCH₂O), 7.33 (5H, s, HAr), 7.66 (1H, bs, Gly CONH), 7.76 (1H, br, Gly CONH), 7.85 (1H, br, Gly CONH), 8.06 (1H, bs, Gly CONH), 8.31 (1H, bs, Gly CONH), 8.47 (1H, bs, Gly CONH). ^{13}C -NMR: 13.9, 22.5, 24.9, 26.8, 26.9, 27.5, 28.6, 29.2, 29.5, 29.6, 31.8, 41.1, 41.8, 42.5, 42.6, 42.8, 43.1, 46.1, 46.7, 61.1, 67.0, 69.1, 70.9, 128.2, 128.4, 128.6, 135.4, 168.5, 169.0, 170.3, 170.5, 170.6, 170.9, 171.2, 173.7. IR (KBr): 3300, 2921, 2852, 1741, 1652, 1541, 1457, 1241, 1131, 1028, 720 cm^{-1} . Anal. Calcd for C₄₈H₇₈N₈O₁₁: C, 61.12; H, 8.34; N, 11.88%. Found: C, 60.87; H, 8.26; N, 11.65%.

N,N-Dioctadecyl 3-oxaglutaramide (18₂DGA)

A solution of dioctadecylamine (2.0 g, 3.8 mmol) and diglycolic anhydride (0.44 g, 3.8 mmol) in toluene (50 mL) was refluxed for 48 h. The solvent was evaporated and the crude product crystallized from CHCl_3 to give a white solid (2.12 g, 87%), mp 80–81 °C. ^1H -NMR CDCl_3 : 0.87 (6H, t, $J = 6.9$ Hz, $-\text{CH}_2\text{CH}_3$), 1.25 (60H, pseudo-s, $\text{CH}_3(\text{CH}_2)_{15}\text{CH}_2\text{CH}_2\text{N}$), 1.55 (4H, bs, $\text{CH}_3(\text{CH}_2)_{15}\text{CH}_2\text{CH}_2\text{N}$), 3.07 (2H, t, $J = 7.8$ Hz, $\text{CH}_3(\text{CH}_2)_{16}\text{CH}_2\text{N}$), 3.34 (2H, t, $J = 7.8$ Hz, $\text{CH}_3(\text{CH}_2)_{16}\text{CH}_2\text{N}$), 4.21 (2H, s, COCH₂O), 4.38 (2H, s, COCH₂O). ^{13}C -NMR: 14.2, 22.8, 26.9, 27.0, 27.5, 28.7, 29.4, 29.5, 29.6, 29.7, 29.8, 32.0, 47.0, 71.4, 73.2, 171.0, 172.2. IR (KBr): 2918, 2850, 1748, 1602, 1488, 1472, 1463, 1431, 1356, 1224, 1159, 1135, 1045, 1013, 990, 920, 885, 729, 720, 689, 643 cm^{-1} .

18₂DGA–GGG–OCH₂Ph

To 18₂DGA–OH (1g, 1.5 mmol) dissolved in CH_2Cl_2 (30 mL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.31 g, 1.6 mmol) was added and the mixture was stirred at room temperature. After 0.5 h, TsOH·H₂N–GGG–OCH₂Ph

(0.66 g, 1.5 mmol) and Et₃N (0.6 mL) were added and the mixture was stirred at ambient temperature overnight. The reaction mixture was successively washed with water (20 mL), 0.5 M HCl (20 mL), water (20 mL), 10% Na₂CO₃ (20 mL), and brine (20 mL), dried (MgSO₄), evaporated and the residue crystallized from MeOH to afford a white solid (1.26 g, 89%), mp 41–42 °C. ¹H-NMR CDCl₃: 0.86 (6H, t, *J* = 6.9 Hz, –CH₂CH₃), 1.24 (60H, pseudo-s, CH₃(CH₂)₁₅CH₂CH₂N), 1.49 (4H, bs, CH₃(CH₂)₁₅CH₂CH₂N), 1.61 (1H, H₂O), 3.04 (2H, t, *J* = 7.5 Hz, CH₃(CH₂)₁₆CH₂N), 3.24 (2H, t, *J* = 7.5 Hz, CH₃(CH₂)₁₆CH₂N), 3.95–4.05 (6H, m, Gly NCH₂), 4.09 (2H, s, COCH₂O), 4.29 (2H, s, COCH₂O), 5.12 (2H, s, PHCH₂O), 7.23 (1H, t, *J* = 6.0 Hz, Gly CONH), 7.30–7.35 (5H, m, HAr), 7.93 (1H, t, *J* = 5.7 Hz, Gly CONH), 8.27 (1H, t, *J* = 5.7 Hz, Gly CONH). ¹³C-NMR: 13.9, 22.5, 26.7, 26.9, 27.4, 28.6, 29.2, 29.3, 29.6, 31.8, 41.0, 42.9, 46.3, 46.7, 66.9, 69.6, 71.7, 128.2, 128.4, 128.6, 135.3, 168.6, 169.7, 169.8, 170.0, 171.5. IR (KBr): 3293, 2916, 2849, 1749, 1651, 1544, 1467, 1196, 1128, 1031, 721, 697 cm^{–1}. Anal. Calcd for C₅₃H₉₄N₄O₇ + 0.5 H₂O: C, 70.11; H, 10.54; N, 6.17%. Found: C, 70.18; H, 10.55; N, 6.18%.

18₂DGA–GGG–OH

18₂DGA–GGG–OCH₂Ph (1.0 g, 1.1 mmol) was dissolved in absolute ethanol (100 mL) and 10% Pd/C (0.2 g) was added and this mixture was shaken under 60 psi H₂ for 3 h. The reaction mixture was heated to reflux and filtered hot through a celite pad. The solvent was evaporated under reduced pressure to afford a white solid (0.86 g, 96%), mp 163–164 °C. ¹H-NMR CD₃OD: 0.90 (6H, t, *J* = 6.9 Hz, –CH₂CH₃), 1.29 (60H, pseudo-s, CH₃(CH₂)₁₅CH₂CH₂N), 1.57 (4H, bs, CH₃(CH₂)₁₅CH₂CH₂N), 3.21 (2H, t, *J* = 7.8 Hz, CH₃(CH₂)₁₆CH₂N), 3.35 (2H, t, *J* = 7.8 Hz, CH₃(CH₂)₁₆CH₂N), 3.93 (2H, s, Gly NCH₂), 3.94 (2H, s, Gly NCH₂), 3.97 (2H, s, Gly NCH₂), 4.12 (2H, s, COCH₂O), 4.40 (2H, s, COCH₂O). ¹³C-NMR: IR (KBr): 3285, 3084, 2925, 2852, 1740, 1650, 1551, 1467, 1420, 1378, 1219, 1128, 1033, 1011, 721, 681 cm^{–1}. Anal. Calcd for C₄₆H₈₈N₄O₇: C, 68.28; H, 10.96; N, 6.92%. Found: C, 67.97; H, 10.92; N, 6.81%.

18₂DGA–GGGPipGGG–OCH₂Ph, 2

18₂DGA–GGG–OH (0.31 g, 0.39 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.08 g, 0.42 mmol) were stirred for 0.5 h in CH₂Cl₂ (40 mL) and HCl·H₂N–PGGG–OCH₂Ph (0.16 g, 0.39 mmol) in CH₂Cl₂ (10 mL) containing Et₃N (0.15 mL) was added. The mixture was stirred for 48 h at room temperature, solvent was evaporated, and the residue was crystallized from MeOH to give **2** as a white solid (0.37 g, 82%), mp 116–118 °C. [α]_D²⁰ –8.9 (*c* 1.055, CHCl₃). ¹H-NMR CDCl₃: 0.87 (6H, t, *J* = 6.9 Hz, –CH₂CH₃), 1.25 (60H, pseudo-s, CH₃(CH₂)₁₅CH₂CH₂N), 1.47 (4H, bs, CH₃(CH₂)₁₅CH₂CH₂N), 1.80–2.20 (4H, m, Pro NCH₂CH₂CH₂), 3.03 (2H, t, *J* = 7.5 Hz, CH₃(CH₂)₁₆CH₂N), 3.23 (2H, t, *J* = 7.5 Hz, CH₃(CH₂)₁₆CH₂N), 3.40–3.45 (1H, m, Pro NCH₂CH₂CH₂), 3.50–3.55 (1H, m, Pro NCH₂CH₂CH₂), 3.90–4.05 (12H, m, Gly NCH₂), 4.12 (2H, s, COCH₂O), 4.26 (2H, s, COCH₂O), 4.35 (1H, bs, Pro NCH), 5.12 (2H, s, PHCH₂O), 7.33 (5H, s, HAr), 7.66 (1H, bs, Gly CONH), 7.76 (1H, br, Gly CONH), 7.85 (1H, br, Gly CONH), 8.06 (1H, bs, Gly CONH), 8.31 (1H, bs, Gly CONH), 8.47 (1H, bs, Gly CONH). ¹³C-NMR: 13.9, 22.5, 24.9, 26.8, 26.9, 27.5, 28.6, 29.2, 29.5, 29.6, 31.8, 41.1, 41.8, 42.5, 42.6, 42.8, 43.1, 46.1, 46.7, 61.1, 67.0, 69.1, 70.9, 128.2, 128.4, 128.6, 135.4, 168.5, 169.0, 170.3, 170.5, 170.6, 170.9, 171.2, 173.7. IR (KBr): 3301, 2922, 2853, 1740, 1653, 1540, 1457, 1242, 1131, 1029, 720. MS (ESI): *m/z* [M + Na]⁺ calculated for C₆₄H₁₁₀N₈O₁₁Na 1189, observed 1190. Anal. Calcd for

C₆₄H₁₁₀N₈O₁₁: C, 65.83; H, 9.50; N, 9.60%. Found: C, 65.38; H, 9.68; N, 9.16%.

Boc–PipGGG–Bzl

Boc-L-Pipecolic acid (0.50 g, 2.2 mmol), TsOH·GGG–O–CH₂Ph (0.98 g, 2.2 mmol), and Et₃N (1.50 mL) were dissolved in CH₂Cl₂ (20 mL) and cooled to 5 °C. EDCI (0.46 g, 2.4 mmol) was added and the reaction was stirred at ambient temperature for 3 days. Solvent was evaporated and the residue was dissolved in EtOAc (50 mL), washed with citric acid (5%, 25 mL), Na₂CO₃ (25 mL), brine (25 mL), dried over MgSO₄, and evaporated. The crude, oily product was purified by chromatography (SiO₂, 5% MeOH–CH₂Cl₂) to afford colorless crystals (0.97 g, 91%), mp 56–58 °C. [α]_D²⁰ –36.1° (*c* 1.045, CHCl₃). ¹H-NMR CDCl₃: 1.45 (9H, s, C(CH₃)₃), 1.59 (4H, bs, Pip CH₂), 2.15 (2H, bs, Pip CH₂), 3.00 (1H, bs, Pip CH₂), 3.85–4.15 (7H, overlapping signals due to Gly CH₂ and Pip CH₂), 4.69 (1H, s, Pip CH), 5.15 (2H, s, CH₂Ph), 6.96 (1H, bs, NH), 7.10 (1H, bs, NH), 7.30–7.50 (5H, m, HAr). ¹³C-NMR: 20.4, 24.7, 25.8, 28.3, 41.3, 42.6, 42.9, 43.2, 55.1, 67.2, 80.8, 128.3, 128.5, 128.6, 135.2, 156.1, 169.1, 169.6, 172.6. IR (KBr): 3325, 2938, 1752, 1664, 1530, 1457, 1409, 1366, 1253, 1189, 1164, 1032, 989, 870, 751, 699 cm^{–1}.

H₂N–PipGGG–OCH₂Ph·HCl

Boc–PipGGG–OCH₂Ph (0.2 g, 0.25 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 used in the next reaction without further purification.

10₂DGA–GGGPipGGG–OCH₂Ph, 3

To 10₂DGA–GGG–OH (0.36 g, 0.61 mmol) suspended in CH₂Cl₂ (20 mL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.12 g, 0.64 mmol) and HOBt (0.09 g, 0.64 mmol) were added and the reaction mixture was stirred for 0.5 h. Then HCl·PipGGG–OCH₂Ph (0.29 g, 0.61 mmol) in CH₂Cl₂ (20 mL) containing Et₃N (0.09 mL, 0.64 mmol) was added and the reaction mixture was stirred for 48 h at room temperature. The reaction mixture was evaporated *in vacuo*, the residue successively washed with 5% citric acid (20 mL), water (20 mL), 5% NaHCO₃ (20 mL), and brine (20 mL), dried (MgSO₄), and evaporated. The crude, oily product was purified by chromatography (SiO₂, 5% MeOH–CH₂Cl₂) and afforded **3** as a white solid (0.49 g, 84%), mp 78–80 °C. [α]_D²⁰ –22.5 (*c* 1.065, CHCl₃). ¹H-NMR CDCl₃: 0.87 (6H, t, *J* = 6.9 Hz, –CH₂CH₃), 1.25 (28H, pseudo-s, CH₃(CH₂)₇CH₂CH₂N), 1.50 (8H, bs, overlapping signals due to CH₃(CH₂)₇CH₂CH₂N and Pip CH₂), 2.13 (2H, bs, Pip CH₂), 2.85 (1H, bs, Pip CH₂), 3.05 (2H, t, *J* = 7.5 Hz, CH₃–(CH₂)₇CH₂CH₂N), 3.25 (2H, t, *J* = 7.5 Hz, CH₃(CH₂)₇CH₂CH₂N), 3.55–3.65 (1H, m, Pip CH₂), 3.80–4.15 (12H, m, Gly NCH₂), 4.10 (2H, s, COCH₂O), 4.27 (2H, s, COCH₂O), 5.06 (1H, bs, Pip CH), 5.14 (2H, s, PHCH₂O), 7.34 (5H, pseudo-s, HAr), 7.49 (1H, bs, Gly CONH), 7.55 (2H, bs, Gly CONH), 7.85 (1H, bs, Gly CONH), 7.98 (1H, bs, Gly CONH), 8.29 (1H, bs, Gly CONH). ¹³C-NMR: 14.0, 20.2, 22.6, 24.9, 25.7, 26.8, 27.0, 27.6, 28.8, 29.2, 29.3, 29.4, 29.5, 31.8, 41.2, 41.4, 42.7, 43.0, 43.3, 46.2, 46.8, 53.4, 67.0, 69.2, 71.2, 128.1, 128.3, 128.5, 135.3, 168.3, 169.1, 170.0, 170.1, 170.2, 170.4, 170.8, 171.9. IR (CHCl₃): 3309, 3069, 2926, 2854, 1748, 1651, 1540, 1457, 1259, 1193, 1129, 1029 cm^{–1}. Anal. Calcd for C₄₉H₈₀N₈O₁₁: C, 61.48; H, 8.42; N, 11.71%. Found: C, 61.12; H, 8.35; N, 11.43%.

18₂DGA–GGGPipGGG–OCH₂Ph, 4

18₂DGA–18–GGG–OH (0.20 g, 0.25 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.05 g,

0.28 mmol) and HOBt (0.04 g, 0.28 mmol) were suspended in CH_2Cl_2 (20 mL) and stirred for 0.5 h. $\text{HCl}\cdot\text{H}_2\text{N}\text{--PipGGG}\text{--OCH}_2\text{Ph}$ (0.12 g, 0.25 mmol) in CH_2Cl_2 (5 mL) containing *N*-methylmorpholine (0.05 mL, 0.46 mmol) was added and the mixture was stirred for 48 h at room temperature. The solvent was evaporated off and the residue was crystallized from MeOH to give **4** as a white solid (0.24 g, 82%), mp 164–165 °C. $[\alpha]_{\text{D}}^{20} -15.1^\circ$ (*c* 1.10, CHCl_3). $^1\text{H-NMR}$ CDCl_3 : 0.87 (6H, t, $J = 6.9$ Hz, $-\text{CH}_2\text{CH}_3$), 1.25 (60H, pseudo-s, $\text{CH}_3(\text{CH}_2)_{15}\text{CH}_2\text{CH}_2\text{N}$), 1.50 (8H, bs, overlapping signals due to $\text{CH}_3(\text{CH}_2)_{15}\text{CH}_2\text{CH}_2\text{N}$ and Pip CH_2), 2.13 (2H, bs, Pip CH_2), 2.85 (1H, bs, Pip CH_2) 3.05 (2H, t, $J = 7.5$ Hz, $\text{CH}_3(\text{CH}_2)_{16}\text{CH}_2\text{N}$), 3.25 (2H, t, $J = 7.5$ Hz, $\text{CH}_3(\text{CH}_2)_{16}\text{CH}_2\text{N}$), 3.55–3.65 (1H, m, Pip CH_2), 3.80–4.15 (12H, m, Gly NCH_2), 4.10 (2H, s, COCH_2O), 4.27 (2H, s, COCH_2O), 5.04 (1H, bs, Pip CH), 5.14 (2H, s, PHCH_2O), 7.34 (5H, pseudo s, HAr), 7.49 (1H, bs, Gly CONH), 7.55 (2H, bs, Gly CONH), 7.85 (1H, bs, Gly CONH), 7.98 (1H, bs, Gly CONH), 8.29 (1H, bs, Gly CONH). $^{13}\text{C-NMR}$: 14.1, 20.1, 22.7, 24.8, 25.8, 26.9, 27.1, 27.6, 29.3, 29.4, 29.6, 29.7, 31.9, 41.2, 41.4, 42.8, 43.0, 43.3, 46.3, 46.9, 50.7, 53.6, 67.0, 69.4, 71.3, 128.2, 128.4, 128.6, 135.4, 168.4, 169.4, 169.9, 170.0, 170.1, 170.3, 170.4, 170.9, 172.0. IR (KBr): 3315, 2921, 2851, 2359, 1749, 1656, 1541, 1468, 1262, 1130, 1029, 722, 698 cm^{-1} . Anal. Calcd for $\text{C}_{65}\text{H}_{112}\text{N}_8\text{O}_{11}$: C, 66.07; H, 9.55; N, 9.48%. Found: C, 66.34; H, 9.63; N, 9.32%.

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