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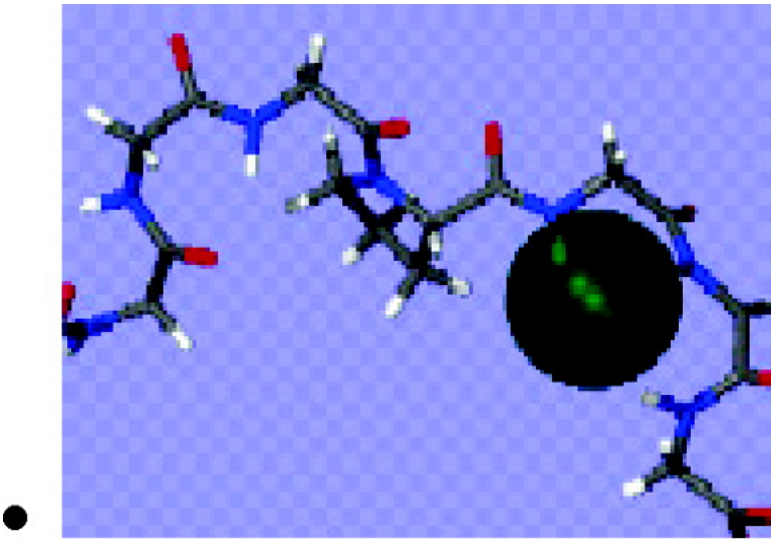

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Cation Dependence of Chloride Ion Complexation by Open-Chained Receptor Molecules in Chloroform Solution

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Abstract: Seventeen peptides, most having the sequence GGGPGGG, but differing in the C- and N-terminal ends, have been studied as anion-complexing agents. These relatively simple, open-chained peptide systems interact with both chloride and the associated cation. Changes in the N- and C-terminal side chains appear to make little difference in the efficacy of binding. NMR studies suggest that the primary interactions involve amide NH contacts with the chloride anion, and CD spectral analyses suggest a concomitant conformational change upon binding. Changes in binding constants, which are expected in different solvents, also suggest selective solvent interactions with the unbound host that helps to preorganize the open-chained peptide system. Significant differences are apparent in complexation strengths when the heptapeptide chain is shortened or lengthened or when the relative position of proline within the heptapeptide is varied.

Introduction

In a recent and excellent review by Kubik and co-workers titled "Recognition of Anions by Synthetic Receptors in Aqueous Solution,"[1] the authors state that "[t]he chemistry of life mainly takes place in water..." While this is true in the broadest sense, relatively little biological chemistry takes place in bulk water per se. Instead, biological reactions and interactions occur in or between proteins or membranes or both. Assuredly, cytosolic proteins interact with numerous species, but most recognition, catalysis, signaling, and other processes occur in enzyme pockets or in or on membranes. This contradiction in medium requirements presents the organic chemist with the challenge of developing a model system that is truly suitable for the study of biological phenomena.

Bilayer lipid membranes are impermeable to cations and most anions. The complex proteins that transport ions and regulate ionic concentrations in vivo insert into bilayers and create an ion conduction pathway.[2] Ion channel proteins have been investigated for decades, but it is only recently that molecular structures have been obtained.[3] In a short time following the first channel X-ray structure, the Nobel Prize was awarded for these important contributions.[4] The protein channels that transport chloride ions have likewise been extensively studied,[5] but the first structural details emerged only in 2002.[6] The complexity of the ClC protein channel, which is evident from the crystal structure, is remarkable. Despite the structural information available, details of the transport mechanism remain speculative.[7] It is clear, however, that chloride ions enter the protein channel, which is embedded within the bilayer membrane, from an aqueous phase.

Because transmembrane ion transport is so intricate, synthetic model systems have been developed in several laboratories throughout the world.[8] We have designed and prepared both cation-[9] and anion-selective channels. The present report deals with the latter: a membrane-anchored peptide that exhibits both selective transport and complex gating behavior.[10]

An intriguing paradox in channel behavior is that ion selectivity requires both recognition and transport. Molecular recognition implies at least contact, but channels still pass $\geq 10^7$ ions per second through a bilayer. Recognition suggests at least a transient supramolecular interaction, which, in turn, implies host−guest complex formation. Numerous complexes have been reported that involve various anions and a wide range of receptor

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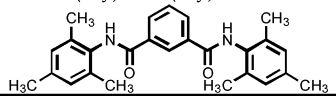
Table 1. Anion Binders Prepared for the Present Study

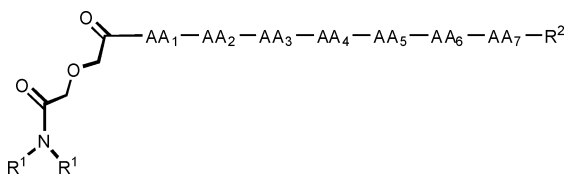
Cpd. No.	*R¹*	*AA1*	*AA2*	*AA3*	*AA4*	*AA5*	*AA6*	*AA7*	*R²*
1	*n*-C_3H_7	$GlyH_2$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	$GlyH_2$	OCH_2Ph
2	*n*-C_3H_7	$GlyD_2$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	$GlyH_2$	OCH_2Ph
3	*n*-C_3H_7	$GlyH_2$	$GlyD_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	$GlyH_2$	OCH_2Ph
4	*n*-C_3H_7	$GlyH_2$	$GlyH_2$	$GlyD_2$	Pro	$GlyH_2$	$GlyH_2$	$GlyH_2$	OCH_2Ph
5	*n*-C_3H_7	$GlyH_2$	$GlyH_2$	$GlyH_2$	Pro	$GlyD_2$	$GlyH_2$	$GlyH_2$	OCH_2Ph
6	*n*-C_3H_7	$GlyH_2$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyD_2$	$GlyH_2$	OCH_2Ph
7	*n*-C_3H_7	$GlyH_2$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	$GlyD_2$	OCH_2Ph
8	*n*-$C_{10}H_{21}$	$GlyH_2$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	$GlyH_2$	OCH_2Ph
9	*n*-$C_{18}H_{37}$	$GlyH_2$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	$GlyH_2$	OCH_2Ph
10	*n*-$C_{18}H_{37}$	$GlyH_2$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	$GlyH_2$	OCH_2CH_3
11	*n*-$C_{18}H_{37}$	$GlyH_2$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	$GlyH_2$	$O(CH_2)_6CH_3$
12	*n*-C_3H_7	$GlyH_2$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	$GlyH_2$	$O(CH_2)_{17}CH_3$
13	*n*-$C_{18}H_{37}$	$GlyH_2$	$GlyH_2$	$GlyH_2$	Pip	$GlyH_2$	$GlyH_2$	$GlyH_2$	OCH_2Ph
14	*n*-$C_{18}H_{37}$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	$GlyH_2$	$GlyH_2$	OCH_2Ph
15	*n*-$C_{18}H_{37}$	$GlyH_2$	$GlyH_2$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	OCH_2Ph
16	*n*-$C_{18}H_{37}$	$GlyH_2$	$GlyH_2$	Pro	$GlyH_2$	$GlyH_2$	–	–	OCH_2Ph
17	*n*-$C_{18}H_{37}$	(Gly)$_4$-Pro-(Gly)$_4$							OCH_2Ph
18									

molecules.[11] Most of these are fairly rigid, macrocyclic receptors. There are also a few examples of cyclic peptide hosts for anions,[12] but these often incorporate unnatural amino acids, nonamidic linkages, or alternating D,L-stereochemistry.

Recent work has shown that appropriate ditopic receptor molecules can selectively bind ion pairs.[13] A host system that combined a Crabtree-type anion binding site[14] with a diaza-18-crown-6 cation binding site mediated chloride release from vesicles[15] and exhibited methylammonium chloride recognition.[16] The fact that anions are bound with different selectivities by individual receptors is well established. Equilibrium complexation constants for anions also depend, sometimes dramatically, on the associated cation, as we have previously communicated.[17] We recount below the chloride ion complexation behavior of a family of heptapeptides, analogous to that originally reported as a chloride-selective ion channel: $(C_{18}H_{37})_2$-$NCOCH_2OCH_2CO$-$(Gly)_3$-Pro-$(Gly)_3$-OCH_2Ph.[10] We have established complexation sites within the peptide and we report them here along with a survey of anion complexation and the effect of counterion thereupon.

Results and Discussion

Compounds Prepared for the Present Study. Eighteen compounds were prepared for the studies that are presented here. The preparation of compounds **1**−**17** is detailed in the Experimental Section; **18** was previously reported by others (Table 1).[14]



The peptide derivatives shown were prepared sequentially. The appropriate amine $[(R^1)_2NH]$ was heated with diglycolic anhydride in THF or toluene (see Experimental Section) to afford $(R^1)_2NCOCH_2OCH_2COOH$ (abbreviated R_2[DGA]-OH), which was obtained by evaporation of the solvent followed by crystallization of the product. Typically, the N-terminal tripeptide was coupled to R_2[DGA]-OH to give a fragment of the type $(R^1)_2$[DGA]-$(Aaa)_m$-PrGp, where PrGp is a protecting group. Removal of the protecting group, usually an ester, reveals an acid that is coupled to the C-terminal fragment, of the form H-$(Aaa)_n$-PrGp, giving $(R^1)_2$[DGA]-$(Aaa)_m$-$(Aaa)_n$-PrGp. In **1**, the N-terminal residues were −Gly-Gly-Gly− and the C-terminal unit was −Pro-Gly-Gly-Gly−.

Deuterated Heptapeptides, 2−7. Solution complexation studies that were undertaken by using ^{1}H NMR methods are described below. To unequivocally assign the various proton resonances, a series of six analogues of **1**, i.e., **2**−**7**, was prepared. Each of these compounds is identical to **1** except that one of the six glycines has been replaced by a dideuterated glycine analogue (i.e., $-NHCD_2CO-$). The requirement of individual deuterated amino acids in the peptide chain precluded the use of commercial triglycine in constructing the heptapeptides in some cases. Details of the sequential syntheses by standard coupling methods and in analogy to Scheme 1 are recorded in the Experimental Section.

NMR Spectrum of 1 in $CDCl_3$. We report here studies of $(C_{18}H_{37})_2NCOCH_2OCH_2CO$-$(Gly)_3$-Pro-$(Gly)_3$-$OCH_2Ph$ (**9**), to which we have previously referred as SCMTR.[10] The long, N-terminal dialkyl chains complicate the ^{1}H NMR spectrum, so the derivatives studied here used dipropyl, rather than

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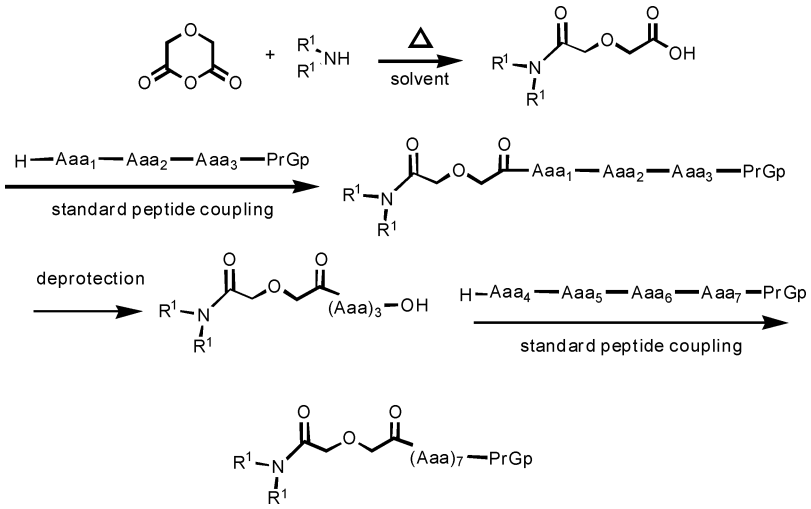
Scheme 1. Synthesis of Ion Binders **1**−**17**



dioctadecyl, chains. The ^{1}H NMR spectra of **1** in the absence and presence of Bu_4NCl are shown in the lower and middle panels of Figure 1. The top trace in Figure 1 shows the spectrum of **7**. Compound **7** is identical to **1** except that Gly-7 (H_2NCH_2-COOH) has been replaced by H_2NCD_2COOH, decoupling the NH proton.

When tetrabutylammonium chloride (Bu_4NCl, 10 equiv) was added to **1**, the peak positions of all six amide hydrogens were altered. The two NH signals most affected were those on Gly-5 and Gly-7 ($^5G_{NH}$, $^7G_{NH}$). When Bu_4NCl was added to **1**, the $^5G_{NH}$ shifted from 7.63 to 9.30 ppm ($\Delta\delta$ 1.67 ppm) and $^7G_{NH}$ changed from 7.35 to δ 8.63 ppm ($\Delta\delta$ 1.28 ppm). The chemical shift changes were interpreted as reflecting the interaction between **1** and Bu_4NCl. These large shifts suggested that the amide hydrogens were forming direct H-bond interactions with chloride anions. Main chain amide NH to anion interactions have been recognized as a structural motif in protein chemistry and given the name "nest" by Watson and Wilner-White.[18] The studies presented here provide insufficient structural information to confirm a nest structural motif in the synthetic peptides.

Chloride Ion Complexation of 1 Assayed by ^{1}H NMR. The multiple amide NH bonds appeared from the ^{1}H NMR spectrum in $CDCl_3$ to serve as donors to complex Cl^-. Heptapeptide **1** ([**1**] ∼ 4 mM in $CDCl_3$) was therefore titrated with Bu_4NCl (∼80 mM in $CDCl_3$) and the chemical shift changes were monitored. Figure 1, above, shows the maximal changes observed for **1** in the presence of 10 equiv of Bu_4NCl. Changes in the $^5G_{NH}$ and $^7G_{NH}$ resonances were monitored. Titration curves, in which $^7G_{NH}$ of **1**, **8**, and **9** was monitored upon addition of Bu_4NCl, are shown in Figure 2.

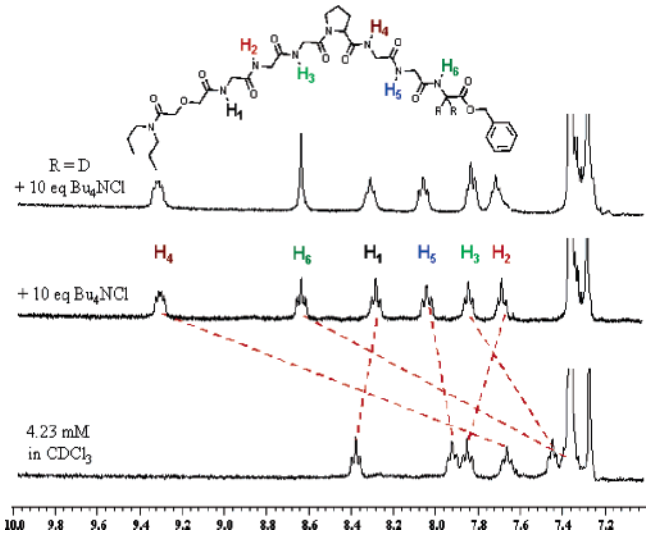


Figure 1. ^{1}H NMR spectra in $CDCl_3$ of **7** (top) and **1** (middle) in the presence of 10 equiv of Bu_4NCl. ^{1}H NMR spectrum in $CDCl_3$ of **1** (4.28 mM) in the absence of salt.

These three compounds have the general structure $(R_1)_2$-$NCOCH_2OCH_2CO$-$(Gly)_3$-Pro-$(Gly)_3$-OCH_2Ph (abbreviated 3_2[DGA]-GGGPGGG-OCH_2Ph when the N-terminal groups are propyl). They differ only in the N-terminal alkyl groups, which are *n*-propyl, *n*-decyl, and *n*-octadecyl for **1**, **8**, and **9**, respectively. We note that the three curves are nearly superimposed upon each other and the binding constants derived from them must, therefore, be nearly identical. The magnitudes of the changes indicated little effect of the side chain residues in these experiments.

In principle, the side chains could interact with the cation, the anion, or with other molecules of the host. Intermolecular interaction of hosts constitutes aggregation, which should be concentration dependent. Binding constants for **9**, which has the longest side chains studied, were determined at concentrations ranging from about 1 to 5 mM. The results recorded in Table 2 confirm the absence of any detectable aggregation or other side chain effect and provide a sample of the excellent data obtained in these experiments. The average of all runs is 1757 ± 23. This corresponds to a $\log_{10} K_S$ of 3.24.

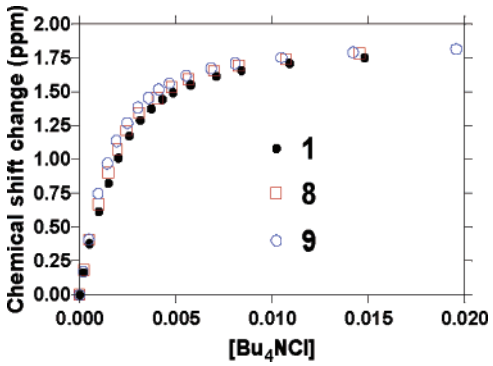


Figure 2. Titration curves in which the $^7G_{NH}$ ^{1}H NMR chemical shift of **1**, **8**, and **9** was monitored upon addition of Bu_4NCl (concentration in molar).

Table 2. Concentration Dependence of Bu_4NCl Binding by 9 in $CDCl_3$[a]

	$^7G_{NH}$[b]		$^5G_{NH}$[b]	
concn (mM)	$\Delta\delta_{max}$ (ppm)	K_S[c]	$\Delta\delta_{max}$ (ppm)	K_S[c]
0.88	1.95	1780 ± 65	1.33	1775 ± 52
1.73	1.86	1750 ± 61	1.32	1738 ± 58
4.28	1.67	1735 ± 52	1.28	1763 ± 20

[a] Determined by ^1H NMR at 25 °C. [b] The amide proton of the indicated glycine was monitored. [c] Standard deviation of three independent measurements.

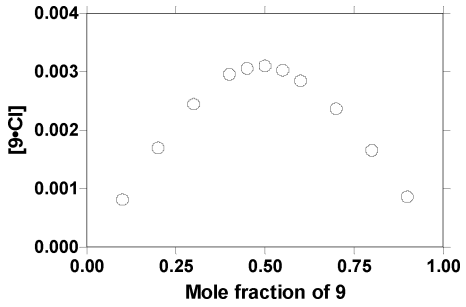


Figure 3. Job's plot for **9** (using a 8.56×10^{-3} M stock solution) and Bu_4NCl, for NH at δ 7.63 ppm.

Binding of ions by host molecules is typically weaker in media of higher polarity compared to nonpolar solvents. The insulating regime ("hydrocarbon slab") of a bilayer is nonpolar but probably rich in water. Experiments identical to those described above were thus undertaken in wet solvent. When **9** was studied in $CDCl_3$ saturated with H_2O (1.8 mM solution of 18_2DGA-GGGPGGG-OBz, ~100 mM Bu_4NCl), the complexation constant determined by NMR fell from 1750 to 720.

Complexation Stoichiometry. Confirmation of 1:1 stoichiometry was obtained from an analysis using the method of continuous variations, or Job's plot.[19] Solutions of host (H) and guest (G) were mixed in varying ratios such that the total concentration, [H] + [G], remained constant. For 1:1 stoichiometry, the concentration of complex, [H·G], is maximal when [H] = [G]. Other stoichiometries produce plots with different maxima. The amide residue of **9** at δ 7.63 was monitored by ^1H NMR as Bu_4NCl was added. The plot, clearly indicating 1:1 complex stoichiometry, is shown as Figure 3.

It should also be noted that eq 1 (see Experimental Section), as used above, assumed 1:1 complexation stoichiometry. Complexation of Bu_4NCl by **17** was studied as above. Equation 1 was applied by assuming both 1:1 and 1:2 stoichiometries. Including the latter did not improve the error. The second binding constant was <100.

Confirmation of NMR Titration Results by Using Circular Dichroism (CD). Titration experiments were also conducted by evaluating the circular dichroism spectra of **9** in $CHCl_3$. Heptapeptide **9** ([**9**] ~1.7 mM in $CDCl_3$) was titrated with Bu_4NCl (~100 mM in $CDCl_3$) and changes in the CD spectrum were observed. Figure 4 shows CD spectra of a 1.7 mM solution of 18_2[DGA]-GGGPGGG-OCH_2Ph (**9**) in $CHCl_3$ (black) and after addition of 1 equiv (green) and 10 equiv (red) of Bu_4NCl. The negative band at approximately 230 nm, which is characteristic of nonordered peptides,[20] is affected by addition of Bu_4NCl and nearly disappears when 10 equiv of salt are present. It is the latter change that was monitored when using this method to determine complexation constants, as described below.

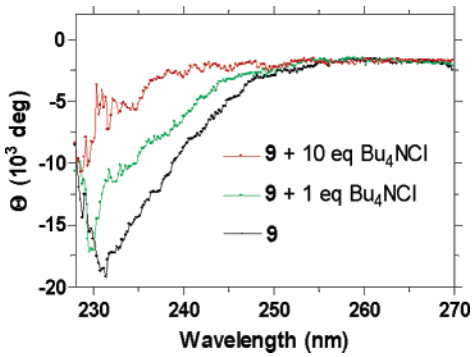


Figure 4. Circular dichroism (CD) spectrum of **9** in the absence and presence of Bu_4NCl.

Fitting of the CD data in $CHCl_3$, as done for the NMR data obtained in $CDCl_3$, to eq 1 (see Experimental Section), gave a binding constant of 1848 ± 439. The fitting error (~25%) is higher than that observed in the NMR experiments (<10%), but the two values are within the error of the CD experiments. This experiment provides independent confirmation of the results obtained by NMR.

Effect of "Secondary Anchors" on Bu_4NCl Complexation. In other work, we have closely examined the effect of differences in C- and N-terminal chain identity and length on anion transport through phospholipid bilayer membranes.[21−23] Release of both chloride and carboxyfluorescein (CF) anions from vesicles was monitored by ion selective electrode or fluorescent methods, respectively. In that work,[23] C- and N-terminal variants of −GGGPGGG− were prepared and assayed. When the C-terminal ester was $-OCH_2Ph$, chloride anion release from liposomes observed the following general pattern for variations in the N-terminal residues [i.e., $(R^1)_2$]: bis(octyl) > bis(decyl) > bis(hexyl) > bis(dodecyl) > bis(propyl) ≈ bis(tetradecyl) ≈ bis(octadecyl) > bis(hexadecyl). The results obtained for CF were similar, although not identical.[23]

A smaller compound sample was surveyed in the present work than in the previous study using CF or Cl^- ion. We assumed that the major interactions with chloride anion would involve the "main chain" amide groups. If so, changes at either the C- or N-terminal end of the molecule should not be important unless they influenced solubility. This expectation is largely confirmed by the data presented in Table 3. The variation in K_S for the formation of a host·Bu_4NCl complex is about 25% over the range of compounds chosen.

Cation Dependence of Complexation by 9. Relatively few examples of open-chained anion-complexing agents have been reported. The tris(aromatic) diamide of Crabtree and co-workers (**18**) is exceptional, as it is an anion-complexing agent in its

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Table 3. Complexation by $(R^1)_2NCOCH_2OCH_2CO$-GGGPGGG-R^2 of Bu_4NCl in $CDCl_3$[a]

no.	dialkyl groups (R^1)	ester (R^2)	NH peak 1	NH peak 2	$\log_{10} K$[b]
1	$CH_2CH_2CH_3$	$OCH_2C_6H_5$	1340 ± 44	1379 ± 54	3.13
8	$(CH_2)_9CH_3$	$OCH_2C_6H_5$	1530 ± 63	1527 ± 50	3.18
9	$(CH_2)_{17}CH_3$	$OCH_2C_6H_5$	1735 ± 52	1763 ± 20	3.25
10	$(CH_2)_{17}CH_3$	OCH_2CH_3	1756 ± 99	1761 ± 93	3.25
11	$(CH_2)_{17}CH_3$	$O(CH_2)_6CH_3$	1811 ± 150	1768 ± 115	3.25
12	$CH_2CH_2CH_3$	$O(CH_2)_{17}CH_3$	1479 ± 38	1455 ± 33	3.16

[a] All titration experiments were conducted at 25 °C in $CDCl_3$ with Bu_4NCl using 1.8 mM initial concentrations of **1**−**7**. [b] $\log_{10}$ of the average of the two (1 and 2) peak values.

Table 4. Ion Pair Binding by 9 and 18 in $CDCl_3$[a]

salt	log K_S[b] **9**	**18**
Bu_4NCl	3.24	3.39
Me_3NCH_2PhCl	3.35	2.93
Et_3NCH_2PhCl	3.39	—[c]
Bu_3NCH_2PhCl	3.23	—[c]
Bu_4PCl	2.71	—[c]
Ph_4PCl	4.20[d]	3.70

[a] At 25 °C, determined by ^1H NMR titration. [b] Standard deviation of three independent measurements. [c] Not determined in this work. [d] 0.60 mM solution.

own right and it has been used as the basis for other, more elaborate anion receptors.[14,24] We, therefore, reproduced the complexation studies with **18** to calibrate our own efforts. The NMR titration method paralleled that previously reported. We successfully reproduced the data for **18** and Ph_4PCl. In our hands, the constant for formation of **18**·Ph_4PCl was ∼5000 (log K_S 3.70). This is similar to that previously reported (∼5300, log K_S 3.72).[14] The calculations used the reported[14] equation (eq 1), which is reproduced in the Experimental Section.

Table 4 records ion pair complexation data for compounds **9** and **18**. Six different chloride salts having organic countercations were studied. The cations were tetrabutylammonium (Bu_4N^+), benzyltrimethylammonium ($Me_3NCH_2Ph^+$), benzyltriethylammonium ($Et_3NCH_2Ph^+$), benzyltributylammonium ($Bu_3NCH_2Ph^+$), tetrabutylphosphonium (Bu_4P^+), and tetraphenylphosphonium (Ph_4P^+). The variations in log K_S for complexation of Me_3NCH_2PhCl, Et_3NCH_2PhCl, Bu_3NCH_2PhCl, and Bu_4NCl were well outside experimental error but certainly in a similar range (log K_S = 3.31 ± 0.08). Complexation of the phosphonium salts, however, was significantly different. Tetrabutylphosphonium chloride and tetraphenylphosphonium chloride had markedly different complexation constants with both **9** and **18**. The binding constant (log K_S) for **18** was reproducibly ∼3.7 with Ph_4PCl, but fell to 3.4 with Bu_4NCl and to 2.9 with Me_3NCH_2PhCl. Since the anion is identical in all three cases, these values suggest an interaction with the cation as well. Indeed, such differences have been documented for a ditopic receptor based upon **18**.[16]

The apparent constants, K_S, for **1** were ∼1700 for Bu_3NCH_2PhCl and ∼2400 for Et_3NCH_2PhCl. For Me_3NCH_2PhCl, the K_S values calculated from $\Delta\delta$ for the δ 7.35 and δ 7.63 ppm protons were ∼1000 and ∼2500, respectively. The data obtained for Ph_4PCl gave a smooth titration curve but it could not be fitted to the published equation.[14] When forced to fit, K = ∼34 000 ± 51 000. This large error resulted from too high a concentration of **1** because the equilibrium binding constant was so much larger than expected. Titration of 0.6 mM solutions of **1** with Ph_4PCl gave K_S values of 16 000 ± 1380 and 16 300 ± 1420 when fitted.

The influence of cation structure on anion activity is well-known. Indeed, it is the basis of both ion pair extraction[25] and of phase transfer catalysis[26,27] and has been explored extensively.[28] For example, 22 quaternary halides were used as catalysts for the nucleophilic substitution reaction of PhS with BrC_8H_{17} to give $PhSC_8H_{17}$. The reactions were all conducted in a two-phase, water−benzene mixture, and all used quaternary halides.[29] Reaction rates for the 22 different quaternary halides varied by nearly 5 orders of magnitude. The relative reaction rates for Bu_4NCl, Bu_4PCl, and Ph_4PCl were, respectively, 1.0, 7.1, and 0.5.

To our knowledge, the question of cation effect on anion receptor binding has been addressed only to a very limited extent. Tuntulani, Vicens, and their co-workers studied the influence of cations on the binding of various anions by tripodal azacrown-calix[4]arenes.[30] In this study, cations (primarily metallic) were varied to determine the effect on overall binding. It was concluded that these calixarene derivatives could potentially be used either as transition metal ion or anion receptors and that control could be effected by pH changes. Kubik and co-workers[12c] examined Na^+, K^+, and Me_4N^+ cations and Cl^-, Br^-, I^-, NO_3^-, and SO_4^{2-} in conjunction with their cyclic hexapeptide receptor system in water. To our knowledge, no acyclic system has been surveyed as done here, in any solvent.

Solvent Dependence of Bu_4NCl Complexation by 1. Compound **9**, $(C_{18}H_{37})_2NCOCH_2OCH_2CO$-$(Gly)_3$-Pro-$(Gly)_3$-$OCH_2Ph$, is quite soluble in $CHCl_3$ but less so in polar solvents. Indeed, solutions as concentrated as those used for the ^1H NMR studies performed in $CDCl_3$ could not be obtained in either CH_3COCH_3 (dielectric constant, ϵ = 20.7) or CH_3CN (ϵ = 36.6). Compound **1** has shorter alkyl chains than **9**, is less hydrophobic, and is correspondingly more soluble in a range of solvents. Compound **1** is sufficiently soluble in both CD_3COCD_3 and CD_3CN to perform complexation studies with Bu_4NCl, assayed by ^1H NMR titration. Thus, 1.8 mM solutions of $(C_3H_7)_2NCOCH_2OCH_2CO$-$(Gly)_3$-Pro-$(Gly)_3$-OCH_2Ph (**1**) were prepared and titrated with Bu_4NCl. The results are collected in Table 5.

The association constant for **1** in $CDCl_3$ is 1360, lower than K_S for **9**. A significant increase in chloride binding was observed in CD_3COCD_3, and a value of 2170 was observed. The same experiment performed in CD_3CN gave K_S = 950. The significant counterion effect observed in $CHCl_3$ disappears when more strongly competing solvents are used, and larger ion separation is expected. Titration in CD_3CN with tetraphenylphosphonium

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Table 5. Association Constants between 1 and Bu_4NCl at 25 °C in Solvents of Different Polarity[a]

solvent	$\Delta\delta_{max}$ (ppm)	K_S ($^7G_{NH}$)	$\Delta\delta_{max}$ (ppm)	K_S ($^5G_{NH}$)	av K_S	log K_S
$CDCl_3$	1.83	1340 ± 44	1.34	1379 ± 54	1360	3.13
CD_3COCD_3	1.30	2134 ± 132	1.30	2217 ± 133	2175	3.33
CD_3CN	1.68	949 ± 81	1.42	956 ± 87	953	2.98
CD_3CN[b]	1.68	977 ± 67	1.40	909 ± 54	943	2.97

[a] Assay by ^1H NMR; $^7G_{NH}$ indicates the NH proton of glycine-7. [b] Titration with Ph_4PCl.

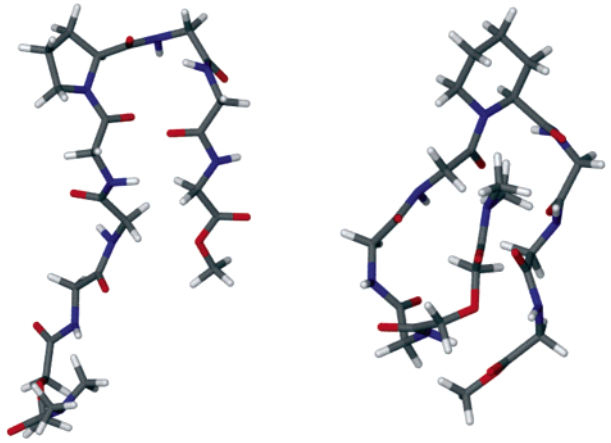

Figure 5. Calculated structures of Me_2[DGA]-GGGPGGG-OCH_3 (**A**, left) and Me_2[DGA]-GGGPipGGG-OCH_3 (**B**, right) in the gas phase in the absence of salt. The images shown were obtained from Gaussian 03W using the semiempirical method.

chloride instead of tetrabutylammonium chloride gave the same association value within experimental error.

The binding constant for **1**·Bu_4NCl in acetone is higher than expected on the basis of the differences in dielectric constants among these solvents. It is probably inappropriate to compare interactions involving Bu_4NCl in chloroform with those in either acetone or acetonitrile. In chloroform, the salt probably exists primarily as an ion pair, whereas the higher polarity solvents will give solvent separated ion pairs. Purely on the basis of the dielectric constants, we would expect binding to be highest in chloroform and lowest in acetonitrile. A possible, but speculative, explanation is that the carbonyl group of acetone could fit into the "v" formed at the apex of **1** (see the calculated structure in Figure 5 below) and help to preorganize the host's amide NH bonds for interaction with chloride.

Voltage clamp experiments performed on **9** in planar bilayers[31] revealed that selective chloride transport is observed in the presence of a high concentration of K^+. However, when Na^+ was the only cation present, both Na^+ and Cl^- ions were transported simultaneously. Simultaneous cation and anion transport is not unreasonable, as both carbonyl and amide H-bond donors are present in the structure. Titration experiments were conducted (^1H NMR, CD_3CN) with 3_2[DGA]-GGGPGGG-OCH_2Ph (**1**) and $NaBPh_4$ or $KBPh_4$. Tetraphenylborate was chosen because it is a less interactive anion than chloride, and $NaBPh_4$ and $KBPh_4$ are soluble in CD_3CN. The binding constants for **1**·$NaBPh_4$ and **1**·$KBPh_4$ were ~310 and ~57, respectively. This compares to $K_S = 950 \pm 80$ for **1**·Bu_4NCl.

Table 6. Peptide Sequence Dependence of Bu_4NCl Binding in $CDCl_3$[a]

no.	peptide sequence	K_S	log K_S
9	$(Gly)_3Pro(Gly)_3$	1755 ± 55	3.25
13	$(Gly)_3Pip(Gly)_3$	400 ± 40	2.60
16	$(Gly)_2Pro(Gly)_2$	56 ± 3	1.75
14	$(Gly)_2Pro(Gly)_4$	1330 ± 81	3.12
15	$(Gly)_4Pro(Gly)_2$	406 ± 43	2.61
17	$(Gly)_4Pro(Gly)_4$	9630 ± 2400	3.98

[a] Determined by the previously described NMR method at 25 °C.

It seems reasonable that any interaction between the carbonyl groups of **1** and Na^+ would be stronger than with K^+, owing to the former's greater charge density. We note that the even larger binding constant observed for **1**·Bu_4NCl involves both an anion and cation that interact with the peptide chain.

The variations observed in binding constants by the heptapeptides were clearly of interest per se, but a possible cation−π effect[32] in the system made it additionally intriguing. If a single arene significantly altered the binding behavior, four arenes might have an even more dramatic effect. Thus, **10** (18_2[DGA]-GGGPGGG-OCH_2CH_3) was titrated with Ph_4PCl in $CDCl_3$. As before, the proton signals for $^7G_{NH}$ and $^5G_{NH}$ were evaluated to obtain association constants. The values obtained for K_S were 15 400 ± 1000 and 16 000 ± 1200. These are identical within experimental error but an order of magnitude higher than observed for Bu_4NCl. Stronger binding clearly indicates a more favorable overall interaction. We were, however, unable to identify any dramatic upfield shifts in other parts of the host molecule that would suggest an intimate π-contact between an arene in the salt and the host.

Effect of Length and Sequence Changes. Table 6 records complexation constants for compounds **9** and **13**−**17** with Bu_4NCl in $CDCl_3$, determined as described above. The structures shown represent two variations. First, the fourth or "central" amino acid in **9** is altered from proline to pipecolic acid in **13**. From the structural perspective, this is a minor change as the ring size is increased by a methylene, but the stereochemistry and functionality remain the same. The binding constant (K_S) for **9**·Bu_4NCl in $CDCl_3$ is ~1750. When proline in **9** is replaced by pipecolic acid to give **13**, K_S falls to ~400. Previous studies[31] compared the ability of these pore formers to release the anion carboxyfluorescein from phospholipid liposomes. In that case, the change in release rate was about 20-fold with **9** being more effective than **13**. The anions and conditions are different in the former and present studies, but the trend is similar.

Gaussian 03W and Spartan calculations (gas phase, alkyl chains (R^1) and C-terminal ester (R^2) = CH_3, Spartan data not shown) both suggest a significant difference in the preferred conformations of **A** and **B**. Computational models of **A** and **B** are shown in Figure 5 (left and right panels, respectively) with the cyclic amino acid at the apex. The conformation of **B** (pipecolic) is calculated to be much more compact than **A** (proline). The positions of the amide NH bonds are significantly different in the two conformations. Although these calculations may not accurately reflect the situation when either **A** or **B** is in contact with a bilayer membrane or in solution, they do comport with the significant differences in ion transport activity observed for these two close relatives.

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In addition to the proline−pipecolic acid variation, Table 6 shows the effect of changes in the number and arrangement of amino acids in the peptide sequence. Compound **16** is a pentapeptide, **14** and **15** are heptapeptides, and **17** is a nonapeptide. Shortening the heptapeptide sequence that is the central theme of this study dramatically lowers K_S for its interaction with Bu_4NCl in $CDCl_3$. A significant, but not as large, decrease in K_S is observed for **15**. The latter is a heptapeptide rather than a pentapeptide but has the same −Pro-Gly-Gly− C-terminal sequence as **13**.

The most dramatic result is observed for 18_2[DGA]-GGGG-PGGGG-OCH_2Ph, **17**. The binding constant (K_S) for **17**·Bu_4-NCl in $CDCl_3$ is 9630 ± 2400. Three separate complexation studies gave values for K_S of 7134, 9827, and 11 924, resulting in the ∼25% error limits. As noted above, incorporation of 1:2 stoichiometry in the calculation gave no significant difference in the values quoted. The high binding constant would require low concentrations of **17** for optimal NMR evaluation. Appropriately low concentrations, however, would require excessive acquisition times. Thus, we compromised on the acquisition times and feel that the error range is acceptable. The critical finding for this study is that even the lowest of the three K_S values obtained in individual experiments is more than 4-fold higher than the value of 1750 observed for **9**. In the absence of a structure determination, the enhanced binding interactions between host and salt cannot be analyzed. Clearly, however, the longer peptide sequence is of significant value in the binding context.

Cyclic and Open-Chained Cation Binders. The concept of preorganization is well-established[33] and extensively documented for cation complexing systems. Thus, pentaethylene glycol dimethyl ether binds Na^+ in CH_3OH with log K_S of 1.52, and for 18-crown-6, the value is 4.35, a difference of more than 600-fold.[34] Similar principles apply in the complexation of anions. Numerous examples now exist of a similar survey of anion properties. The extremes of such studies are rigid macrocycles such as porphyrins,[35] calixarenes,[36] or hybrids thereof,[37] and the charge-dense anion fluoride.[38] Examples are also abundant of H-bond donors that are appended to (or pendant from) a rigid scaffold.[39] Examples of cyclic and open-chained peptides are less common. Indeed, the principal examples of cyclic peptides incorporate unnatural amino acids (e.g. aminoxy amino acids) or alternating D,L-stereochemistry.[40] For example, in the work reported by Kubik and co-workers noted above, the cyclic system was constructed from L-proline and 6-aminopicolinic acid.[12a]

Several studies of macrocyclic and open-chained compounds in which chloride complexation is addressed have appeared. Indeed, the area has recently been reviewed several times.[11,41] Bowman-James and co-workers studied chloride complexation by a macrocycle incorporating two isophthalic acid units, six nitrogens, and four amides. In $CDCl_3$, the complexation constant was about 500.[42] Hamilton and co-workers compared a macrocycle containing three amide residues with its open-chained counterpart. Binding was lower for open-chained analogues complexing various ions, but no chloride association constant was reported. The macrocycle bound Bu_4NCl in 2% DMSO-d_6/$CDCl_3$ with K_S = 8800.[43] A tetraamide-containing ditopic calixarene reported by Ungaro et al. bound chloride in CD_3-$COCD_3$ with K_S = 2800.[44] A bis(calixarene), linked by two amide residues, bound chloride in CD_2Cl_2 with K_S = 172.[45]

A number of open-chained anion binders that use amides as donors have also been reported. A pyrrole-2,5-dicarboxamide was reported to bind Cl^- in CD_3CN with K_S = 138.[46] A family of open-chained amides was assayed for chloride binding in $CDCl_3$, but the highest K_S value among the eight compounds studied was 395.[47] In addition, numerous anion binders that use organometallic scaffolds such as ferrocene or cobalticene have been reported. In most cases, the complexation was done in CD_3CN for solubility reasons and cations were not varied.[48]

From the biological perspective, rigidity and charge density represent extremes in interactions. Most forces in nature are weak and transient because very strong interactions tend to be irreversible. To our knowledge, no assessment of chloride binding has previously been done with an open-chained peptide assembled only from natural amino acids. The closest study to our own effort involves the cyclic aminoxy receptors noted above.[40] The authors report that the association constants K_S for the "cyclohexapeptide" complexes with Cl^- and F^- were 11 880 and 30 M^{-1}, respectively (298 K). It is unclear what cation was used in the NMR studies, but the salt studied by mass spectrometry was Ph_4PCl. We obtained a value of K_S for complexation of open-chained **1** with Ph_4PCl (see above) of about 16 000.

Conclusions

Three major findings emerge from the data acquired in this study. First, open-chained peptides exhibit strong chloride binding in solvents of low and moderate polarity. Compared to values reported previously in the literature, the open-chained compounds reported here are as good as or better than many amide-containing macrocycles. The acyclic peptides are flexible and therefore adaptable. In principle, higher binding and selectivity can be achieved by a rigid host of the correct size. In general, however, the synthetic approaches prevent most macrocycles from being adjustable in small increments of ring size. Second, the associated cation may dramatically influence

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how strongly the anion is bound. Of course, the differences may simply reflect how effectively the ion pair is bound. In most published cases to date, however, the cation effect is either unspecified or unrecognized. Finally, the binding studies reported here correlate with the channel activity. Thus, binding strength increases in the series $-(Gly)_2Pro(Gly)_2-$ (**16**) < $-(Gly)_3Pro(Gly)_3-$ (**9**) < $-(Gly)_4Pro(Gly)_4-$ (**17**), which is consistent with the increase in chloride transport activity in liposomes among these three compounds. In addition, pipecolic acid derivative **13** ($-(Gly)_3Pip(Gly)_3-$) shows poorer chloride binding in the present study and was found to be a less effective ion channel than **9** in previous studies.

Experimental Section

General Methods. All reaction solvents were freshly distilled and the reactions were conducted under N_2 unless otherwise stated. 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 F_{254} plates with visualization by UV light (254 nm) and/or by phosphomolybdic acid (PMA) spray. Starting materials were purchased from Aldrich Chemical Co. and used as received. ^{1}H 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. Combustion analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are reported as percents. EDCI and HOBt are abbreviations for 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride and 1-hydroxybenzotriazole hydrate, respectively. Q^+X^- salts used for the NMR titrations were dried overnight (80 °C) under vacuum prior to the experiments.

Continuous Variation Method (Job's plot). Stock solutions of the host (8.56 mM) and Bu_4NCl (8.56 mM) in $CDCl_3$ were prepared. Ten NMR tubes were filled with 1 mL of solution containing the host and guest in the following volume ratios (in mL): 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4, 0.55:0.45, 0.5:0.5, 0.45:0.55, 0.4:0.6, 0.3:0.7, 0.2:0.8, 0.1:0.9. ^{1}H NMR spectra were recorded, and the concentration of the complex was calculated as $[\mathbf{9}] = [H]_t \times (\delta_{obs} - \delta_0)/(\delta_c - \delta_0)$, where $[H]_t$ is the total concentration of host in the solution, δ_{obs} is the observed chemical shift for the NH signal, and δ_c is the chemical shift of the NH signal in the complex.

^{1}H NMR Titrations. Solutions of host in $CDCl_3$ (CD_2Cl_2 for **2**) were prepared in the concentration range 0.88−4.30 mM (0.30 mM for **2**). Deuterated solvents were dried over 4 Å molecular sieves. One mL of this solution was titrated in NMR tubes with 35−170 mM (12 mM for **2**) solutions of Q^+Cl^-, which also contained host in the same concentration as the titrated solution. The signals assigned to the NH protons were monitored as a function of the anion concentration to the point at which the chemical shift change reached saturation. The association constant K_a was calculated from the obtained isotherms ($\Delta\delta$NH vs [Cl^-]) by nonlinear regression analysis carried out with Origin 7 and using the curve fit for 1:1 binding (eq 1). All the runs were carried out for at least three independent samples.

Complexation between **1**−**18** and various salts was conducted as described by Kavallieratos et al.[14] using the equation reproduced below, where H and G represent host and guest.

$$\Delta\delta = \frac{\left([H]_0 + [G]_0 + \frac{1}{K_a} - \left(\sqrt{\left(\left([H]_0 + [G]_0 + \frac{1}{K_a}\right)^2 - 4[G]_0[H]_0\right)}\right)\right)\Delta\delta_{max}}{2[H]_0} \quad (1)$$

[2-(2-{[1-(2-{2-[2-(2-Dipropylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}-acetylamino)acetylamino]acetic acid benzyl ester, 1, was prepared as previously described.[31]

[2-(2-{[1-(2-{2-[2-(2-Dipropylcarbamoylmethoxyacetylamino)-dideutoeroacetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]-amino}acetylamino)acetylamino]acetic Acid Benzyl Ester, 2, 3_2-[DGA]-Gd_2-GGPGGG-OCH_2Ph. Dipropylcarbamoylmethoxyacetic acid (3_2[DGA]-OH). A solution of dipropylamine (2.0 g, 19.8 mmol) and diglycolic anhydride (2.5 g, 21.7 mmol) was refluxed in THF (30 mL) for 48 h. The solvent was evaporated and the crude product dissolved in $CHCl_3$ and washed with dilute aq HCl. The solvent was removed and the residue recrystallized from Et_2O to give the final product as a white solid (3.2 g, 75%), mp 55−56 °C. ^{1}H NMR: 0.91 (6H, m, CH_3), 1.59 (4H, m, $CH_3CH_2CH_2N$), 3.07 (2H, t, J = 7.8 Hz, $CH_3CH_2CH_2N$), 3.23 (2H, t, J = 7.8 Hz, $CH_3CH_2CH_2N$), 4.20 (2H, s, $C(O)CH_2O$), 4.40 (2H, s, $C(O)CH_2O$). ^{13}C NMR: δ 11.1, 11.2, 20.6, 21.7, 48.4, 71.2, 73.0, 170.8, 171.8.

***N-tert*-Butoxycarbonylglycine-2,2-d_2.** Glycine-2,2-d_2 was suspended in mixture of H_2O (10 mL), dioxane (10 mL), and Et_3N (2.0 g 19.8 mmol) followed by Boc-OH (3.20 g, 13.0 mmol). The reaction was stirred at room temperature for 2 h. H_2O (25 mL) was added and the aqueous solution was extracted with EtOAc (30 mL). The residue was acidified with 5% citric acid and extracted with EtOAc (3 × 25 mL). The organic layer was dried over $MgSO_4$ and the solvent evaporated. The crude oily product was recrystallized from hexanes−EtOAc to give a white solid (1.83 g, 80%), mp 89−90 °C.^{1}H NMR: 1.44 (9H, s, $C(CH_3)_3$), 5.13 (bs, Gly CO*NH*), 6.75 (bs, Gly CO*NH*), 10.41 (1H, CO*OH*). ^{13}C NMR: 28.2, 41.0, 80.4, 81.7, 156.0, 157.3, 174.0, 174.8. IR ($CHCl_3$): 3354, 1700, 1516, 1453, 1395, 1369, 1287, 1255, 1166, 1077, 1055, 885, 847, 782 cm^{-1}.

Boc-Gd_2-OCH_2Ph. Boc-glycine-2,2-d_2 (0.4 g, 2.26 mmol), benzyl alcohol (0.24 g, 2.26 mmol), and DMAP (0.03 g, 0.23 mmol) were dissolved in CH_2Cl_2 (30 mL) and cooled to 5 °C. *N*,*N*′-Diisopropylcarbodiimide (0.37 mL, 2.39 mmol) was added and the reaction was stirred at room temperature overnight. The solvent was evaporated and the residue was chromatographed (SiO_2, 1% MeOH−$CHCl_3$) to give a white solid (0.50 g, 83%), mp 72−73 °C. ^{1}H NMR: 1.44 (9H, s, $C(CH_3)_3$), 4.99 (1H, bs, Gly CO*NH*), 5.18 (2H, s, PhCH_2O), 7.30−7.38 (5H, m, H_{Ar}). ^{13}C NMR: 28.3, 67.0, 80.0, 128.4, 128.5, 128.6, 135.2, 155.7, 170.2. IR ($CHCl_3$): 3370, 2977, 1751, 1714, 1499, 1455, 1392, 1367, 1274, 1254, 1166, 1076, 1052, 1030, 884, 845, 752, 698 cm^{-1}.

HCl·Gd_2-OCH_2Ph. Boc-Gd_2-OCH_2Ph (0.21 g, 0.79 mmol) was dissolved in 4 N HCl in dioxane (5 mL) at 5 °C and the reaction mixture was stirred for 0.5 h. The solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

3_2[DGA]-Gd_2-OCH_2Ph. To dipropylcarbamoylmethoxyacetic acid (0.17 g, 0.79 mmol) dissolved in CH_2Cl_2 (30 mL) were added EDCI (0.17 g, 0.89 mmol) and HOBt (0.12 g, 0.89 mmol) (at 5 °C, ice bath), and the mixture was stirred at room temperature. After 0.5 h, HCl·Gd_2-OCH_2Ph (0.16 g, 0.79 mmol) and NMM (0.1 mL) were added, and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was purified on column (SiO_2, 2% MeOH−$CHCl_3$) to afford a colorless oil (0.24 g, 83%).^{1}H NMR: 0.80−0.95 (6H, m, $-CH_2CH_3$), 1.50−1.65 (4H, m, CH_3CH_2-CH_2N), 3.06 (2H, t, J = 7.8 Hz, $CH_3CH_2CH_2N$), 3.28 (2H, t, J = 7.8 Hz, $CH_3CH_2CH_2N$), 4.14 (2H, s, $COCH_2O$), 4.27 (2H, s, $COCH_2O$), 5.16 (2H, s, $PHCH_2O$), 7.30−7.40 (5H, m, H_{Ar}), 8.17 (1H, pseudo-s,

Gly CO*NH*). ^{13}C NMR: 11.1, 11.3, 20.7, 22.0, 47.7, 48.4, 66.9, 69.5, 71.6, 128.3, 128.4, 128.5, 135.3, 168.3, 169.4, 170.2. IR ($CHCl_3$): 3307, 2964, 2934, 2875, 1751, 1646, 1526, 1432, 1380, 1269, 1152, 1128, 1048, 814, 739, 698 cm^{-1}.

3_2[DGA]-Gd_2-OH. 3_2[DGA]-Gd_2-OCH_2Ph (0.16 g, 0.44 mmol) was dissolved in abs EtOH (20 mL) and 10% Pd/C (0.10 g) was added, and this mixture was shaken under 60 psi pressure of H_2 for 3 h. The reaction mixture was filtered (Celite pad). The solvent was evaporated to afford a white solid (0.12 g, 100%), mp 87−89 °C. ^{1}H NMR: 0.75−0.95 (6H, m, $-CH_2CH_3$), 1.45−1.65 (4H, m, $CH_3CH_2CH_2N$), 3.05 (2H, t, J = 7.8 Hz, $CH_3CH_2CH_2N$), 3.24 (2H, t, J = 7.8 Hz, $CH_3CH_2CH_2N$), 4.08 (2H, s, $COCH_2O$), 4.25 (2H, s, $COCH_2O$), 7.87 (1H, pseudo-s, Gly *NH*), 10.26 (1H, bs, CO*OH*). ^{13}C NMR: 11.0, 11.1, 20.5, 21.7, 40.1, 47.8, 48.4, 68.9, 70.7, 168.6, 170.1, 171.3. IR ($CHCl_3$): 3361, 2966, 2936, 2877, 1744, 1642, 1535, 1467, 1435, 1382, 1344, 1244, 1158, 1131, 1047, 901, 826, 732, 644 cm^{-1}.

BocGPGGG-OCH_2Ph. Boc-glycine (0.16 g, 0.94 mmol), PGGG-OCH_2Ph·HCl (0.39 g, 0.94 mmol), and NMM (0.12 mL) were dissolved in CH_2Cl_2 (30 mL) and cooled to 5 °C. EDCI (0.20 g, 1.04 mmol) and HOBt (0.14 g, 1.04 mmol) were added and the reaction was stirred at room temperature overnight. The solvent was evaporated, and the residue was dissolved in $CHCl_3$ (50 mL); washed with 5% citric acid (2 × 25 mL), 5% $NaHCO_3$ (2 × 25 mL), and brine (25 mL); dried over $MgSO_4$; and evaporated to afford a white solid (0.47 g, 90%), mp 80−81 °C. ^{1}H NMR: 1.41 (9H, s, $C(CH_3)_3$), 1.80−2.15 (4H, m, Pro $NCH_2CH_2CH_2$), 3.40−3.55 (2H, m, Pro $NCH_2CH_2CH_2$), 3.75−4.20 (8H, overlapping sigmals due to Gly NCH_2), 4.35 (1H, t, J = 6.6 Hz, Pro N*CH*), 5.10−5.14 (2H, m, $PhCH_2O$), 5.67 (1H, t, J = 5.7 Hz, Gly CO*NH*), 7.23 (1H, bs, Gly CO*NH*), 7.30−7.36 (5H, m, H_{Ar}), 7.65 (1H, t, J = 5.7 Hz, Gly CO*NH*), 7.83 (1H, t, J = 5.7 Hz, Gly CO*NH*). ^{13}C NMR: 25.1, 28.3, 28.6, 41.1, 42.8, 43.0, 43.5, 46.7, 60.9, 67.0, 79.8, 128.2, 128.4, 128.5, 135.3, 156.5, 169.6, 169.7, 169.8, 169.9, 173.2. IR ($CHCl_3$): 3311, 2978, 1750, 1533, 1454, 1410, 1392, 1366, 1250, 1175, 1030, 919, 866, 732, 698 cm^{-1}.

HCl·GPGGG-OCH_2Ph. Boc-GPGGG-OCH_2Ph (0.35 g, 0.65 mmol) was dissolved in 4 N HCl in dioxane (5 mL) at 5 °C and the reaction mixture was stirred for 0.5 h. The solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

BocGGPGGG-OCH_2Ph. Boc-glycine (0.14 g, 0.81 mmol), GPGGG-OCH_2Ph·HCl (0.38 g, 0.81 mmol), and NMM (0.10 mL) were dissolved in CH_2Cl_2 (30 mL) and cooled to 5 °C. EDCI (0.17 g, 0.89 mmol) and HOBt (0.12 g, 0.89 mmol) were added, and the mixture was stirred at room temperature overnight. Solvent was evaporated, and the residue was dissolved in $CHCl_3$ (30 mL); washed with 5% citric acid (2 × 25 mL), 5% $NaHCO_3$ (2 × 25 mL), and brine (25 mL); dried over $MgSO_4$; and then evaporated to afford a white solid (0.32 g, 68%), mp 68−69 °C. ^{1}H NMR: 1.42 (9H, s, $C(CH_3)_3$), 1.90−2.30 (4H, m, Pro $NCH_2CH_2CH_2$), 3.40−4.25 (12H, overlapping signals due to Gly NCH_2 and Pro $NCH_2CH_2CH_2$), 4.32 (1H, t, J = 6.6 Hz, Pro N*CH*), 5.05−5.15 (2H, m, $PhCH_2O$), 5.90 (1H, bs, Gly CO*NH*), 7.30−7.40 (6H, overlapping signals due to H_{Ar} and Gly CO*NH*), 7.60 (1H, bs, Gly CO*NH*), 7.72 (1H, bs, Gly CO*NH*), 8.00 (1H, bs, Gly CO*NH*). ^{13}C NMR: 25.1, 28.3, 28.9, 41.2, 41.9, 42.8, 43.5, 43.9, 46.9, 61.3, 67.2, 128.2, 128.4, 128.6, 135.2, 156.1, 170.0, 170.5, 170.8, 173.2. IR ($CHCl_3$): 3309, 2978, 2933, 1750, 1659, 1531, 1455, 1410, 1392, 1367, 1332, 1249, 1175, 1029, 912, 732, 698 cm^{-1}.

HCl·GGPGGG-OCH_2Ph. Boc-GGPGGG-OCH_2Ph (0.25 g, 0.43 mmol) was dissolved in 4 N HCl in dioxane (5 mL) at 5 °C and the reaction mixture was stirred for 0.5h. The solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

3_2[DGA]-Gd_2GGPGGG-OCH_2Ph. To 3_2[DGA]-Gd_2-OH (0.12 g, 0.43 mmol) suspended in CH_2Cl_2 (20 mL) were added PyBroP (0.13 g, 0.47 mmol) and HOBt (0.06 g, 0.47 mmol), and the reaction was stirred for 0.5 h. HCl·Gd_2PGGG-OCH_2Ph (0.22 g, 0.43 mmol) in CH_2Cl_2 (10 mL) containing DIEA (0.08 mL) was added and the mixture was stirred for 48 h at room temperature. The solvent was evaporated and the residue was chromatographed (SiO_2, 10−30% MeOH−$CHCl_3$) to give 0.23 g (72%) of a white solid, mp 111−112 °C. ^{1}H NMR: 0.80−0.96 (6H, m, $-CH_2CH_3$), 1.40−1.60 (4H, m, $CH_3CH_2CH_2N$), 1.90−2.25 (4H, m, Pro $NCH_2CH_2CH_2$), 3.04 (2H, t, J = 7.5 Hz, $CH_3CH_2CH_2N$), 3.24 (2H, t, J = 7.5 Hz, $CH_3CH_2CH_2N$), 3.45−3.65 (2H, m, Pro $NCH_2CH_2CH_2$), 3.75−4.20 (12H, overlapping signals due to Gly NCH_2, and $COCH_2O$) 4.30 (2H, s, $COCH_2O$), 4.33 (1H, t, J = 6.6 Hz, and Pro N*CH*), 5.14−5.16 (2H, m, $PhCH_2O$), 7.30−7.38 (5H, m, H_{Ar}), 7.49 (1H, bt, Gly CO*NH*), 7.55 (1H, bs, Gly CO*NH*), 7.84−7.98 (3H, overlapping signals due to Gly CO*NH*), 8.35 (1H, pseudo-s, $Glyd_2$ *NH*). ^{13}C NMR: 11.2, 11.4, 20.7, 21.9, 22.7, 25.1, 29.0, 29.7, 31.9, 41.2, 41.9, 42.7, 43.0, 43.4, 46.9, 47.8, 48.4, 61.3, 67.2, 69.4, 71.3, 128.3, 128.4, 128.6, 135.3, 168.7, 168.9, 170.2, 170.4, 170.8, 171.2, 173.5. IR ($CHCl_3$): 3301, 2924, 2852, 1747, 1648, 1534, 1455, 1242, 1192, 1128, 1031, 736 cm^{-1}.

[2-(2-{[1-(2-{2-[2-(2-Dipropylcarbamoylmethoxyacetylamino)-acetylamino]dideuterioacetylamino}acetyl)pyrrolidine-2-carbonyl]-amino}acetylamino)acetylamino]acetic Acid Benzyl Ester, 3, 3_2-[DGA]-G-Gd_2-GPGGG-OCH_2Ph. 3_2[DGA]-G-OCH_2Ph. To dipropylcarbamoylmethoxyacetic acid (0.5 g, 2.3 mmol) dissolved in CH_2Cl_2 (30 mL) were added EDCI (0.48 g, 2.5 mmol) and HOBt (0.34 g, 2.5 mmol, at 5 °C, ice bath). The mixture was stirred at room temperature. After 0.5 h, TsOH·G-OCH_2Ph (0.78 g, 2.3 mmol) and Et_3N (1 mL) were added, and the mixture was stirred at room temperature for 48 h. The solvent was evaporated and the residue was dissolved in EtOAc (40 mL). The mixture was successively washed with 5% citric acid (2 × 20 mL), 5% $NaHCO_3$ (2 × 20 mL), and brine (20 mL); dried ($MgSO_4$); and evaporated to afford a colorless oil (0.67 g, 80%). ^{1}H NMR: 0.80−0.95 (6H, m, $-CH_2CH_3$), 1.50−1.65 (4H, m, $CH_3CH_2CH_2N$), 3.06 (2H, t, J = 7.8 Hz, $CH_3CH_2CH_2N$), 3.27 (2H, t, J = 7.8 Hz, $CH_3CH_2CH_2N$), 4.08−4.55 (4H, overlapping signals due to Gly NCH_2 and $COCH_2O$), 4.27 (2H, s, $COCH_2O$), 5.16 (2H, s, $PhCH_2O$), 7.30−7.36 (5H, m, H_{Ar}), 8.18 (1H, bt, Gly CO*NH*). ^{13}C NMR: 11.1, 11.3, 20.7, 22.0, 40.7, 47.7, 48.4, 66.9, 69.5, 71.6, 128.3, 128.4, 128.5, 135.3, 168.3, 169.4, 170.2. IR ($CHCl_3$): 3307, 2964, 2934, 2875, 1752, 1646, 1531, 1456, 1432, 1383, 1357, 1237, 1189, 1128, 740, 698 cm^{-1}.

3_2[DGA]-G-OH. 3_2[DGA]-G-OCH_2Ph (0.66 g, 1.81 mmol) was dissolved in abs EtOH (30 mL), 10% Pd/C (0.10 g) was added, and the mixture was shaken under 60 psi pressure of H_2 for 3 h. The mixture was filtered (Celite pad). The solvent was evaporated to afford a white solid (0.50 g, 100%), mp 87−89 °C. ^{1}H NMR: 0.80−1.00 (6H, m, $-CH_2CH_3$), 1.45−1.65 (4H, m, $CH_3CH_2CH_2N$), 3.07 (2H, t, J = 7.8 Hz, $CH_3CH_2CH_2N$), 3.27 (2H, t, J = 7.8 Hz, $CH_3CH_2CH_2N$), 4.07 (2H, d, J = 5.7 Hz, Gly NCH_2), 4.11 (2H, s, $COCH_2O$), 4.27 (2H, s, $COCH_2O$), 7.88 (1H, t, J = 5.7 Hz, Gly CO*NH*), 11.40 (1H, bs, CO*OH*). ^{13}C NMR: 11.1, 11.2, 20.6, 21.8, 40.6, 47.9, 48.5, 68.9, 70.9, 168.7, 170.0, 171.6. IR ($CHCl_3$): 3352, 2966, 2936, 2877, 1741, 1644, 1540, 1466, 1433, 1383, 1343, 1238, 1207, 1130, 1041, 893, 816, 748 cm^{-1}.

BocGd$_2$GPGGG-OCH_2Ph. Boc-glycine-2,2-d_2 (0.12 g, 0.67 mmol), GPGGG-OCH_2Ph·HCl (0.31 g, 0.67 mmol), and NMM (0.10 mL) were dissolved in CH_2Cl_2 (30 mL) and cooled to 5 °C. EDCI (0.13 g, 0.68 mmol) and HOBt (0.01 g, 0.70 mmol) were added, and the reaction was stirred at room temperature overnight. Solvent was evaporated, and the residue was dissolved in $CHCl_3$ (50 mL); washed with 5% citric acid (2 × 25 mL), 5% $NaHCO_3$ (2 × 25 mL), and brine (25 mL); dried over $MgSO_4$; and evaporated to afford a white solid (0.32 g, 82%), mp 67−69 °C. ^{1}H NMR: 1.421 (9H, s, $C(CH_3)_3$), 1.90−2.20 (4H, m, Pro $NCH_2CH_2CH_2$), 3.45−3.60 (2H, m, Pro $NCH_2CH_2CH_2$), 3.75−4.25 (8H, overlapping signals due to Gly NCH_2), 4.32 (1H, t, J = 6.6 Hz, Pro N*CH*), 5.10−5.14 (2H, m, $PhCH_2O$), 5.89 (1H, pseudo-s, $Glyd_2$ CO*NH*), 7.30−7.38 (6H, overlapping signals due to H_{Ar} and Gly CO*NH*), 7.60 (1H, bs, Gly CO*NH*), 7.80 (1H, t, J = 5.7 Hz, Gly

CO*NH*), 8.01 (1H, t, J = 5.7 Hz, Gly CO*NH*). ^{13}C NMR: 25.1, 28.3, 28.9, 41.1, 41.8, 42.7, 43.5, 46.9, 61.2, 67.1, 79.9, 128.2, 128.4, 128.6, 135.2, 156.3, 168.6, 169.9, 170.4, 170.7, 173.3. IR ($CHCl_3$): 3310, 3070, 2978, 2934, 1751, 1660, 1534, 1455, 1410, 1392, 1367, 1334, 1252, 1190, 1120, 1074, 1031, 912, 732, 698 cm^{-1}.

HCl·Gd$_2$GPGGG-OCH$_2$Ph. Boc-Gd$_2$GPGGG-OCH$_2$Ph (0.35 g, 0.65 mmol) was dissolved in 4N HCl/dioxane (5 mL) at 5 °C and the reaction mixture was stirred for 0.5 h. The solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

3$_2$[DGA]-GGd$_2$GPGGG-OCH$_2$Ph. To 3$_2$[DGA]-G-OH (0.13 g, 0.49 mmol) suspended in CH_2Cl_2 (20 mL) were added PyBroP (0.25 g, 0.54 mmol) and HOBt (0.07 g, 0.52 mmol), and the mixture was stirred for 0.5 h. Then HCl·Gd$_2$PGGG-OCH$_2$Ph (0.26 g, 0.49 mmol) in CH_2Cl_2 (10 mL) containing DIEA (0.1 mL) was added and the mixture was stirred for 48 h at room temperature. The solvent was evaporated and the residue was chromatographed (SiO_2, 10−30% MeOH−$CHCl_3$) to give 0.32 g (87%) of pure product as a white solid, mp 111−112 °C. ^{1}H NMR: 0.80−0.96 (6H, m, −CH$_2$*CH$_3$*), 1.40−1.60 (4H, m, CH$_3$*CH$_2$*CH$_2$N), 1.90−2.25 (4H, m, Pro NCH$_2$*CH$_2$CH$_2$*), 3.05 (2H, t, J = 7.5 Hz, CH$_3$CH$_2$*CH$_2$*N), 3.24 (2H, t, J = 7.5 Hz, CH$_3$-CH$_2$*CH$_2$*N), 3.45−4.20 (13H, overlapping signals due to Gly N*CH$_2$*, Pro N*CH$_2$*CH$_2$CH$_2$, and Pro N*CH*), 4.11 (2H, s COC*H$_2$*O), 4.31 (2H, s, CO*CH$_2$*O), 5.14−5.16 (2H, m, PhCH$_2$O), 7.30−7.38 (5H, m, *H$_{Ar}$*), 7.40 (1H, bt, Gly CO*NH*), 7.42 (1H, bt, Gly CO*NH*), 7.66 (1H, bt, Gly CO*NH*), 7.86−7.94 (2H, overlapping signals due to Gly CO*NH*), 8.33 (1H, bt, Gly CO*NH*). ^{13}C NMR: 11.2, 11.4, 20.7, 21.9, 25.1, 29.0, 41.2, 42.7, 42.8, 42.9, 43.5, 46.9, 47.8, 48.4, 61.2, 67.2, 69.5, 71.4, 128.31, 128.4, 128.6, 135.3, 168.6, 168.8, 170.0, 170.1, 170.3, 170.4, 171.1, 173.6. IR ($CHCl_3$): 3301, 2964, 1750, 1648, 1535, 1437, 1241, 1192, 1128, 1030, 733 cm^{-1}. Anal. Calcd for $C_{34}H_{48}N_8O_{11}$·H_2O: C, 53.25; H, 7.10; N, 14.61. Found: C, 53.31; H, 7.08; N, 14.63.

[2-(2-{[1-(2-{2-[2-(2-Dipropylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}dideuterioacetyl)pyrrolidine-2-carbonyl]-amino}acetylamino)acetylamino]acetic Acid Benzyl Ester, 4, 3$_2$-[DGA]-GG-Gd$_2$-PGGG-OCH$_2$Ph. TsOH·GG-OCH$_2$Ph. H-(Gly)$_2$OH (5.0 g, 37.9 mmol) and *p*-toluenesulfonic acid monohydrate (7.9 g, 41.6 mmol) were added to a mixture of benzyl alcohol (45 mL) and toluene (70 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 (after 8 h), 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. The crystalline *p*-toluenesulfonate of GG-OCH$_2$Ph was collected on a filter, washed with ether (50 mL), dried, and recrystallized from MeOH−ether (11.72 g, 78%), mp 157−159 °C. ^{1}H NMR (CD_3-OD): 2.34 (3H, s, *CH$_3$*Ph), 3.72 (2H, s, Gly N*CH$_2$*), 4.05 (2H, s, Gly N*CH$_2$*), 5.16 (2H, s, PH*CH$_2$*O), 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, 41.6, 42.1, 68.2, 127.3, 129.6, 129.7, 129.9, 130.1, 130.2, 137.4, 168.3, 171.3. IR (KBr): 3332, 3081, 1747, 1671, 1544, 1455, 1407, 1363, 1202, 1126, 1034, 1011, 912, 736, 685 cm^{-1}.

3$_2$[DGA]-GG-OCH$_2$Ph. To dipropylcarbamoylmethoxyacetic acid (1 g, 4.60 mmol) dissolved in CH_2Cl_2 (30 mL) were added EDCI (0.90 g, 4.69 mmol) and HOBt (0.63 g, 4.66 mmol) (at 5 °C, ice bath), and the mixture was stirred at room temperature. After 0.5 h, TsOH·GG-OCH$_2$Ph (1.81 g, 4.6 mmol) and NMM (0.8 mL) were added, and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was dissolved in EtOAc (50 mL). The mixture was successively washed with 5% citric acid (2 × 20 mL), 5% $NaHCO_3$ (2 × 20 mL), and brine (20 mL); dried ($MgSO_4$); and evaporated to afford a colorless oil (1.60 g, 82%). ^{1}H NMR: 0.80−0.95 (6H, m, −CH$_2$*CH$_3$*), 1.45−1.60 (4H, m, CH$_3$*CH$_2$*CH$_2$N), 3.04 (2H, t, J = 7.8 Hz, CH$_3$CH$_2$*CH$_2$*N), 3.22 (2H, t, J = 7.8 Hz, CH$_3$CH$_2$*CH$_2$*N), 4.00−4.05 (4H, overlapping signals due to Gly N*CH$_2$*), 4.10 (2H, s, COCH$_2$O), 4.30 (2H, s, COCH$_2$O), 5.15 (2H, s, PH*CH$_2$*O), 7.30−7.40 (5H, m, *H$_{Ar}$*), 7.52 (1H, t, J = 5.7 Hz, Gly CO*NH*), 8.22 (1H, t, J = 5.7 Hz, Gly CO*NH*). ^{13}C NMR: 11.1, 11.3, 20.7, 22.0, 41.1, 42.6, 47.8, 48.3, 67.0, 69.6, 71.9, 128.2, 128.4, 128.6, 135.2, 168.5, 169.5, 170.5. IR ($CHCl_3$): 3315, 2964, 2934, 1750, 1651, 1532, 1457, 1432, 1384, 1358, 1271, 1238, 1189, 1128, 1032, 958, 815, 741, 698 cm^{-1}.

3$_2$[DGA]-GG-OH. 3$_2$[DGA]-GG-OCH$_2$Ph (1.57 g, 3.72 mmol) was dissolved in abs EtOH (30 mL), 10% Pd/C (0.20 g) was added, and this mixture was shaken under 60 psi pressure of H_2 for 3 h. The reaction mixture was filtered (Celite pad). The solvent was evaporated. The crude product was crystallized from a mixture of MeOH−ethyl ether (1:1 v/v) to afford a white solid (1.18 g, 96%), mp 129−131 °C. ^{1}H NMR 5% CD_3OD-$CDCl_3$: 0.75−0.90 (6H, m, −CH$_2$*CH$_3$*), 1.40−1.60 (4H, m, CH$_3$*CH$_2$*CH$_2$N), 3.03 (2H, t, J = 7.8 Hz, CH$_3$CH$_2$*CH$_2$*N), 3.20 (2H, t, J = 7.8 Hz, CH$_3$CH$_2$*CH$_2$*N), 3.92 (2H, d, J = 3.9 Hz, Gly N*CH$_2$*), 3.98 (2H, d, J = 3.9 Hz, Gly N*CH$_2$*), 4.04 (2H, s, COCH$_2$O), 4.25 (2H, s, COCH$_2$O), 6.42 (1H, bs, CO*OH*), 7.66 (1H, t, J = 3.9 Hz, Gly CO*NH*), 8.20 (1H, t, J = 3.9 Hz, Gly CO*NH*). ^{13}C NMR: 10.9, 11.1, 17.9, 20.5, 21.7, 41.0, 42.1, 47.7, 48.3, 57.8, 70.0, 70.8, 168.6, 169.7, 170.7, 171.4. IR ($CHCl_3$): 3319, 2962, 1722, 1667, 1645, 1600, 1554, 1466, 1415, 1342, 1295, 1256, 1219, 1157, 1129, 1045, 982, 935, 879, 811, 759, 738, 659 cm^{-1}.

BocGd$_2$PGGG-OCH$_2$Ph. Boc-glycine-2,2-d_2 (0.11 g, 0.63 mmol), PGGG-OCH$_2$Ph·HCl (0.26 g, 0.63 mmol), and NMM (0.10 mL) were dissolved in CH_2Cl_2 (30 mL) and cooled to 5 °C. EDCI (0.13 g, 0.68 mmol) and HOBt (0.09 g, 0.67 mmol) were added, and the reaction was stirred at room temperature overnight. Solvent was evaporated and the residue was dissolved in $CHCl_3$ (50 mL); washed with 5% citric acid (25 mL), 5% $NaHCO_3$ (25 mL), and brine (25 mL); dried over $MgSO_4$; and evaporated to afford a white solid (0.30 g, 89%), mp 80−81 °C. ^{1}H NMR: 1.42 (9H, s, C(*CH$_3$*)$_3$), 1.80−2.10 [4H, m, (Pro)-NCH$_2$*CH$_2$CH$_2$*], 3.45−3.60 (2H, m, Pro N*CH$_2$*CH$_2$CH$_2$), 3.80−4.20 (6H, overlapping signals due to Gly N*CH$_2$*), 4.35 [1H, t, J = 6.6 Hz, (Pro)N*CH*], 5.15−5.20 (2H, m, Ph*CH$_2$*O), 5.57 (1H, pseudo-s, Glyd$_2$ CO*NH*), 7.11 (1H, bs, Gly CO*NH*), 7.30−7.35 (5H, m, *H$_{Ar}$*), 7.50 (1H, bs, Gly CO*NH*), 7.80 (1H, bs, Gly CO*NH*). ^{13}C NMR: 25.4, 28.5, 28.8, 41.3, 43.0, 43.8, 47.0, 61.3, 67.3, 80.2, 128.5, 128.6, 128.8, 135.5, 156.8, 169.9, 170.2, 173.3. IR ($CHCl_3$): 3310, 2978, 29.35, 1750, 1651, 15278, 1447, 1392, 1366, 1252, 1176, 1082, 1067, 1030, 919, 733 cm^{-1}.

HCl·Gd$_2$PGGG-OCH$_2$Ph. Boc-Gd$_2$PGGG-OCH$_2$Ph (0.24 g, 0.45 mmol) was dissolved in 4 N HCl in dioxane (5 mL) at 5 °C and the reaction mixture was stirred for 0.5 h. The solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

3$_2$[DGA]-GGGd$_2$PGGG-OCH$_2$Ph. To 3$_2$[DGA]-GG-OH (0.15 g, 0.45 mmol) suspended in CH_2Cl_2 (20 mL) were added PyBroP (0.23 g, 0.49 mmol) and HOBt (0.07 g, 0.52 mmol), and the reaction was stirred for 0.5 h. Then HCl·Gd$_2$PGGG-OCH$_2$Ph (0.21 g, 0.45 mmol) in CH_2Cl_2 (10 mL) containing DIEA (0.09 mL) was added and the reaction mixture was stirred for 48 h at room temperature. The solvent was evaporated and the residue was chromatographed (SiO_2, 10−30% MeOH−$CHCl_3$) to give 0.26 g (79%) of pure product as a white solid, mp 111−112 °C. ^{1}H NMR: 0.75−0.90 (6H, m, −CH$_2$*CH$_3$*), 1.40−1.60 (4H, m, CH$_3$*CH$_2$*CH$_2$N), 1.85−2.20 (4H, m, Pro NCH$_2$*CH$_2$CH$_2$*), 3.02 (2H, t, J = 7.5 Hz, CH$_3$CH$_2$*CH$_2$*N), 3.24 (2H, t, J = 7.5 Hz, CH$_3$-CH$_2$*CH$_2$*N), 3.40−3.45 (1H, m, Pro N*CH$_2$*CH$_2$CH$_2$), 3.55−4.10 (13H, overlapping signals due to Gly N*CH$_2$*, Pro N*CH$_2$*CH$_2$CH$_2$, and Pro N*CH*), 4.11 (2H, s COC*H$_2$*O), 4.31 (2H, s, CO*CH$_2$*O), 5.15 (2H, d, J = 5.1 Hz, Ph*CH$_2$*O), 7.30−7.38 (5H, m, *H$_{Ar}$*), 7.39 (1H, pseudo-s, Glyd$_2$ CO*NH*), 7.48 (1H, bt, Gly CO*NH*), 7.72 (1H, bt, Gly CO*NH*), 7.84 (1H, bt, Gly CO*NH*), 7.91 (1H, bt, Gly CO*NH*), 8.31 (1H, bt, Gly CO*NH*). ^{13}C NMR: 11.1, 11.3, 20.7, 21.8, 25.0, 29.1, 41.2, 42.2, 42.6, 42.7, 42.8, 43.2, 46.8, 47.7, 48.3, 61.2, 67.1, 69.2, 70.1, 128.2, 128.3, 128.6, 135.3, 168.7, 168.8, 170.2, 170.3, 170.4, 170.9, 171.2, 173.6. IR ($CHCl_3$): 3303, 2966, 2936, 1750, 1649, 1534, 1437, 1241, 1191, 1129, 1030, 843, 731, 699 cm^{-1}. Anal. Calcd for $C_{34}H_{48}N_8O_{11}$·H_2O: C, 53.25; H, 7.10; N, 14.61. Found: C, 53.33; H, 7.07; N, 14.51.

[2-(2-{[1-(2-{2-[2-(2-Dipropylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}-dideuterioacetylamino)acetylamino]acetic Acid Benzyl Ester, 5, 3_2[DGA]-GGGP-Gd_2-GG-OCH_2Ph. 3_2[DGA]-GGG-OCH_2Ph. To a solution of 3_2[DGA] (0.5 g, 2.3 mmol) in CH_2Cl_2 (30 mL) cooled to 5 °C were added EDCI (0.48 g, 2.5 mmol), HOBt (0.34 g, 2.5 mmol), TsOH·GGG-OCH_2Ph (1.0 g, 2.3 mmol), and Et_3N (1.0 mL), and the reaction was stirred at room temperature for 2 days. The reaction was quenched and washed with a saturated solution of citric acid (20 mL), a saturated solution of $NaHCO_3$ (20 mL), and water (20 mL). The organic phase was then dried over $MgSO_4$ and evaporated, and the residue was purified by column chromatography (silica, 0−3% MeOH in CHCl3) to give the pure final product (0.87 g, 79%) as a deliquescent solid. ^{1}H NMR: 0.89 (6H, m, $-CH_2CH_3$), 1.40−1.60 (4H, m, CH_3CH_2-CH_2N), 3.04 (2H, t, J = 7.5 Hz, $CH_3CH_2CH_2$N), 3.23 (2H, t, J = 7.5 Hz, $CH_3CH_2CH_2$N), 3.98−4.06 (6H, overlapping signals due to Gly NCH_2), 4.09 (2H, s, COCH_2O), 4.33 (2H, s, COCH_2O), 5.14 (2H, s, PhCH_2O), 7.04 (1H, t, J = 5.7 Hz, Gly CONH), 7.32 (5H, m, H_{Ar}), 7.95 (1H, t, J = 5.7 Hz, Gly CONH), 8.22 (1H, t, J = 5.7 Hz, Gly CONH). ^{13}C NMR: 11.2, 11.3, 20.7, 21.9, 41.1, 43.1, 48.0, 48.4, 67.0, 69.8, 72.0, 128.2, 128.4, 128.6, 135.3, 168.7, 169.46, 169.55, 169.9, 171.5. IR ($CHCl_3$): 3307, 2966, 2935, 1748, 1652, 1540, 1457, 1360, 1241, 1194, 1129, 1032, 746, 699 cm^{-1}.

3_2[DGA]-GGG-OH. 3_2[DGA]-GGG-OCH2Ph (0.82 g, 1.7 mmol) was dissolved in abs EtOH (30 mL), 10% Pd/C (0.1 g) was added, and this mixture was shaken under 70 psi hydrogen pressure for 3 h in a Parr apparatus. The reaction mixture was heated to reflux and filtrated through a Celite pad. The solvent was evaporated to leave a white solid in a quantitative yield (0.66 g), mp 177−178 °C. This product was used in the subsequent reaction with no further purification.

BocGd_2GG-OCH_2Ph. Boc-glycine-2,2-d_2 (0.20 g, 1.13 mmol), TsOH·GG-O-CH_2Ph (0.45 g, 6.7 mmol), and NMM (0.14 mL) were dissolved in CH_2Cl_2 (20 mL) and cooled to 5 °C. EDCI (0.24 g, 1.24 mmol) and HOBt (0.17 g, 1.24 mmol) were added, and the reaction was stirred at room temperature for 48 h. Solvent was evaporated, and the residue was dissolved in $CHCl_3$ (50 mL); washed with 5% citric acid (25 mL), 5% $NaHCO_3$ (25 mL), and brine (25 mL); dried over $MgSO_4$; and evaporated to afford a white solid (0.37 g, 86%), mp 41−42 °C. ^{1}H NMR: 1.42 (9H, s, C(CH_3)$_3$), 4.00−4.20 (4H, overlapping signals due to Gly NCH_2), 5.15 (2H, s, PhCH_2O), 5.45 (1H, bs, Gly CONH), 7.19 (2H, overlapping signals due to Gly_2 CONH, and Gly CONH), 7.30−7.40 (5H, m, H_{Ar}). ^{13}C NMR: 20.2, 41.2, 42.8, 67.2, 80.4, 128.3, 128.5, 128.6, 135.1, 156.4, 169.3, 169.6, 170.4. IR ($CHCl_3$): 3317, 2978, 2934, 1749, 1662, 1530, 1456, 1392, 1367, 1278, 1253, 1175, 1074, 1031, 993, 735, 697 cm^{-1}.

HCl·Gd_2GG-OCH_2Ph. Boc-Gd_2GG-OCH_2Ph (0.36 g, 0.94 mmol) was dissolved in 4 N HCl in dioxane (5 mL) at 5 °C and the reaction mixture was stirred for 0.5 h. The solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

BocPGd_2GG-OCH_2Ph. Boc-L-proline (0.20 g, 0.94 mmol), HCl·GGd_2G-OCH_2Ph (0.30 g, 0.94 mmol), and NMM (0.18 mL) were dissolved in CH_2Cl_2 (20 mL) and cooled to 5 °C. EDCI (0.20 g, 1.03 mmol) and HOBt (0.14 g, 1.03 mmol) were added, and the reaction was stirred at room temperature overnight. Solvent was evaporated, and the residue was dissolved in ethyl acetate (50 mL); washed with 5% citric acid (25 mL), 5% $NaHCO_3$ (25 mL), and brine (25 mL); dried over $MgSO_4$; and evaporated to afford white solid (0.19 g, 42%), mp 54−55 °C. ^{1}H NMR: 1.38 (9H, s, C(CH_3)$_3$), 1.80−2.20 (4H, m, Pro NCH$_2$$CH_2CH_2$), 3.35−3.55 (2H, m, Pro NCH_2CH$_2$CH$_2$), 3.80−4.20 (5H, overlapping signals due to Gly NCH_2, and Pro NCH), 5.11 (2H, s, PhCH_2O), 7.20−7.40 (6H, overlapping signals due to Gly CONH, and H_{Ar}), 7.50 (1H, pseudo-s, Gly_2 CONH), 7.92 (1H, bt, Gly CONH). ^{13}C NMR: 24.5, 28.2, 29.5, 41.0, 42.8, 47.1, 60.5, 66.8, 80.6, 128.1, 128.3, 128.5, 135.2, 155.4, 169.3, 169.6, 173.9. IR ($CHCl_3$): 3313, 2977, 2934, 1751, 1669, 1534, 1455, 1404, 1367, 1248, 1172, 1128, 1029, 742, 699 cm^{-1}.

HCl·PGd_2GG-OCH_2Ph. Boc-PGd_2GG-OCH_2Ph (0.19 g, 0.40 mmol) was dissolved in 4 N HCl in dioxane (5 mL) at 5 °C, and the reaction mixture was stirred for 0.5 h. The solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

3_2[DGA]-GGGPGd_2GG-OCH_2Ph. To 3_2[DGA]-GGG-OH (0.16 g, 0.41 mmol) suspended in CH_2Cl_2 (20 mL) were added PyBroP (0.21 g, 0.44 mmol) and HOBt (0.06 g, 0.44 mmol), and the reaction was stirred for 0.5 h. Then HCl·PGd_2GG-OCH_2Ph (0.16 g, 0.40 mmol) in CH_2Cl_2 (10 mL) containing DIEA (0.08 mL) was added, and the reaction mixture was stirred for 48 h at room temperature. The solvent was evaporated and the residue was chromatographed (SiO_2, 10−30% MeOH−$CHCl_3$) to give 0.24 g (80%) of pure product as a white solid, mp 111−112 °C. ^{1}H NMR: 0.80−0.95 (6H, m, $-CH_2CH_3$), 1.45−1.65 (4H, m, $CH_3CH_2CH_2$N), 1.85−2.20 (4H, m, Pro NCH$_2$$CH_2CH_2$), 3.05 (2H, t, J = 7.5 Hz, $CH_3CH_2CH_2$N), 3.24 (2H, t, J = 7.5 Hz, CH_3-CH_2CH_2N), 3.45−3.65 (2H, m, Pro NCH_2CH$_2$CH$_2$), 3.75−4.20 (12H, overlapping signals due to Gly NCH_2, Pro and COCH_2O), 4.29 (2H, s, COCH_2O), 4.35 (1H, t, J = 6.5 Hz, Pro NCH), 5.15 (2H, m, PhCH_2O), 7.30−7.38 (5H, m, H_{Ar}), 7.50 (2H, bs, Gly CONH), 7.91 (1H, bt, Gly CONH), 7.95 (1H, bt, Gly CONH), 7.99 (1H, pseudo-s, Gly_2 CONH), 8.37 (1H, bt, Gly CONH). ^{13}C NMR: 11.1, 11.3, 20.7, 22.0, 25.1, 29.1, 29.7, 41.3, 41.9, 42.8, 42.9, 46.9, 47.8, 48.4, 61.2, 67.1, 69.4, 71.3, 128.2, 128.4, 128.6, 135.4, 168.7, 168.9, 170.1, 170.2, 170.3, 170.4, 170.8, 171.1. 173.5. IR ($CHCl_3$): 3300, 2964, 2934, 1747, 1645, 1630, 1533, 1435, 1241, 1193, 1128, 1029, 698 cm^{-1}. Anal. Calcd for $C_{34}H_{48}N_8O_{11}$·H_2O: C, 53.25; H, 7.10; N, 14.61. Found: C, 53.27; H, 7.11; N, 14.53.

[2-(2-{[1-(2-{2-[2-(2-Dipropylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}-acetylamino)dideuterioacetylamino]acetic Acid Benzyl Ester, 6, 3_2[DGA]-GGGPG-Gd_2-G-OCH_2Ph. BocPG-OCH_2Ph. Boc-L-proline (0.70 g, 3.25 mmol), TsOH·G-O-CH_2Ph (1.10 g, 3.25 mmol), and Et_3N (1.40 mL) were dissolved in CH_2Cl_2 (40 mL) and cooled to 5 °C. EDCI (0.69 g, 3.60 mmol) and HOBt (0.49 g, 3.60 mmol) were added, and the reaction was stirred at room temperature for 2 days. Solvent was evaporated, and the residue was dissolved in EtOAc (50 mL); washed with 5% citric acid (25 mL), 5% $NaHCO_3$, and brine (25 mL); dried over $MgSO_4$; and evaporated to give a colorless oil (0.98 g, 83%). ^{1}H NMR: 1.45 (9H, s, C(CH_3)$_3$), 1.80−2.20 (4H, m, Pro NCH$_2$$CH_2CH_2$), 3.35−3.55 (2H, m, Pro NCH_2CH$_2$CH$_2$), 3.90−4.20 (2H, m, Gly NCH_2), 4.32 (1H, bs, Pro NCH), 5.16 (2H, s, PhCH_2O), 6.55 (1H, bs, Gly CONH), 7.30−7.35 (5H, m, H_{Ar}). ^{13}C NMR: 23.9, 24.6, 28.5, 31.0, 41.4, 47.2, 60.1, 61.1, 67.2, 80.5, 128.3, 128.4, 128.6, 135.1, 154.6, 169.5, 172.3. IR ($CHCl_3$): 3317, 2976, 2881, 1752, 1698, 1531, 1479, 1455, 1392, 1366, 1256, 1172, 1125, 975, 739, 698 cm^{-1}.

HCl·PG-OCH_2Ph. Boc-PG-OCH_2Ph (0.41 g, 1.13 mmol) was dissolved in 4 N HCl in dioxane (5 mL) at 5 °C and the reaction mixture was stirred for 0.5 h. The solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

3_2[DGA]-GGGPG-OCH_2Ph. 3_2[DGA]-GGG-OH (0.44 g, 1.13 mmol), HCl·PG-O-CH_2Ph (0.34 g, 1.13 mmol), and NMM (0.14 mL) were dissolved in CH_2Cl_2 (20 mL) and cooled to 5 °C. EDCI (0.24 g, 1.24 mmol) and HOBt (0.17 g, 1.24 mmol) were added, and the reaction was stirred at room temperature for 2 days. Solvent was evaporated, and the residue was dissolved in $CHCl_3$ (50 mL); washed with 5% citric acid (25 mL), 5% $NaHCO_3$, and brine (25 mL); dried over $MgSO_4$; and evaporated to give colorless oil (0.70 g, 98%). ^{1}H NMR: 0.80−0.95 (6H, m, $-CH_2CH_3$), 1.40−1.60 (4H, m, $CH_3CH_2CH_2$N), 1.85−2.10 (4H, m, Pro NCH$_2$$CH_2CH_2$), 3.03 (2H, t, J = 7.5 Hz, CH_3-CH_2CH_2N), 3.12−3.34 (2H, m, $CH_3CH_2CH_2$N), 3.40−3.45 (1H, m, Pro NCH_2CH$_2$CH$_2$), 3.55−4.30 (13H, overlapping signals due to Gly NCH_2, Pro NCH_2CH$_2$CH$_2$, and COCH_2O), 4.45 (1H, t, J = 6 Hz), 5.00−5.15 (2H, m, PhCH_2O), 7.30−7.35 (5H, m, H_{Ar}), 7.47 (1H, t, J

= 5.7 Hz, Gly CO*NH*), 7.82 (1H, t, *J* = 5.7 Hz, Gly CO*NH*), 7.93 (1H, t, *J* = 5.7 Hz, Gly CO*NH*), 8.05 (1H, t, *J* = 5.7 Hz, Gly CO*NH*). ^{13}C NMR: 11.1, 11.3, 20.7, 21.9, 24.8, 29.1, 41.0, 41.1, 42.6, 42.8, 46.5, 47.6, 48.3, 60.2, 66.8, 69.4, 71.6, 128.2, 128.3, 128.5, 135.2, 168.2, 168.6, 170.1, 170.2, 170.4, 172.4. IR ($CHCl_3$): 3308, 2965, 2935, 2876, 1748, 1648, 1534, 1454, 1339, 1241, 1187, 1028, 733, 698 cm^{-1}.

3_2[DGA]-GGGPG-OH. 3_2[DGA]-GGGPG-OCH_2Ph (0.72 g, 1.14 mmol) was dissolved in abs EtOH (30 mL), 10% Pd/C (0.20 g) was added, and this mixture was shaken under 60 psi hydrogen pressure for 3 h in a Parr apparatus. The reaction mixture was heated to reflux and filtrated through a Celite pad. The solvent was evaporated to leave a white solid in a quantitative yield (0.62 g), mp 78−79 °C. This product was used in the subsequent reaction with no further purification.

BocGd_2-G-OCH_2Ph. Boc-glycine-2,2-d_2 (0.20 g, 1.13 mmol), TsOH·G-OCH_2Ph (0.38 g, 1.13 mmol), and NMM (0.14 mL) were dissolved in CH_2Cl_2 (30 mL) and cooled to 5 °C. EDCI (0.24 g, 1.24 mmol) and HOBt (0.17 g, 1.24 mmol) were added, and the reaction was stirred at room temperature overnight. Solvent was evaporated, and the residue was dissolved in EtOAc (50 mL); washed with 5% citric acid (25 mL), 5% $NaHCO_3$, and brine (25 mL); dried over $MgSO_4$; and evaporated to give a colorless oil (0.33 g, 87%). ^{1}H NMR: 1.45 (9H, s, C(*CH*$_3$)$_3$), 4.10 (2H, d, *J* = 5.4 Hz, Gly N*CH*$_2$), 5.10 (1H, pseudo-s, Glyd$_2$ CO*NH*), 5.17 (2H, s, Ph*CH*$_2$O), 6.62 (1H, bt, Gly CO*NH*), 7.30−7.40 (5H, m, *H*$_{Ar}$). ^{13}C NMR: 28.3, 41.2, 67.3, 128.4, 128.6, 128.7, 135.0, 169.5, 169.7. IR ($CHCl_3$): 3327, 2978, 1750, 1714, 1678, 1521, 1499, 1456, 1391, 1366, 1252, 1173, 1074, 737, 698 cm^{-1}.

HCl·Gd_2-G-OCH_2Ph. Boc-Gd_2-G-OCH_2Ph (0.19 g, 0.55 mmol) was dissolved in 4 N HCl in dioxane (5 mL) at 5 °C and the reaction mixture was stirred for 0.5 h. The solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

3_2[DGA]-GGGPG-Gd_2-G-OCH_2Ph. To 3_2[DGA]-GGGPG-OH (0.30 g, 0.55 mmol) suspended in CH_2Cl_2 (20 mL) were added PyBroP (0.28 g, 0.61 mmol) and HOBt (0.08 g, 0.61 mmol), and the reaction was stirred for 0.5 h. Then HCl·Gd_2-G-OCH_2Ph (0.14 g, 0.55 mmol) in CH_2Cl_2 (10 mL) containing DIEA (0.11 mL) was added and the reaction mixture was stirred for 48 h at room temperature. The solvent was evaporated, and the residue was chromatographed (SiO_2, 10−30% MeOH−$CHCl_3$) to give 0.34 g (83%) of pure product as a white solid, mp 111−112 °C. ^{1}H NMR: 0.80−0.95 (6H, m, −CH_2*CH*$_3$), 1.40−1.60 (4H, m, CH_3*CH*$_2$$CH_2$N), 1.85−2.20 (4H, m, Pro NCH_2*CH*$_2$*CH*$_2$), 3.04 (2H, t, *J* = 7.5 Hz, CH_3CH_2*CH*$_2$N), 3.23 (2H, t, *J* = 7.5 Hz, CH_3-CH_2*CH*$_2$N), 3.40−3.45 (1H, m, Pro N*CH*$_2$$CH_2$$CH_2$), 3.55−4.10 (11H, overlapping signals due to Gly N*CH*$_2$, Pro N*CH*$_2$$CH_2$$CH_2$), 4.12 (2H, s COC*H*$_2$O), 4.30 (2H, s, CO*CH*$_2$O), 4.35 (1H, bs, Pro N*CH*), 5.12−5.15 (2H, m, PhCH_2O), 7.30−7.38 (5H, m, *H*$_{Ar}$), 7.49 (1H, bs, Gly CO*NH*), 7.54 (1H, bs, Gly CO*NH*), 7.86 (1H, pseudo-s, Glyd$_2$ CO*NH*), 7.88 (1H, bs, Gly CO*NH*), 7.91 (1H, bs, Gly CO*NH*), 8.34 (1H, bs, Gly CO*NH*). ^{13}C NMR: 11.1, 11.3, 20.7, 21.8, 25.0, 29.1, 41.2, 41.9, 42.6, 42.8, 43.2, 46.8, 47.7, 48.3, 61.3, 67.1, 69.2, 70.9, 128.2, 128.3, 128.6, 135.3, 168.7, 168.9, 169.7, 170.3, 170.4, 170.5, 170.9, 171.2, 173.7. IR ($CHCl_3$): 3301, 2966, 2935, 1749, 1653, 1535, 1455, 1408, 1241, 1193, 1129, 1029, 914, 844, 732 cm^{-1}. Anal. Calcd for $C_{34}H_{48}N_8O_{11}$·H_2O: C, 53.25; H, 7.10; N, 14.61. Found: C, 53.31; H, 7.08; N, 14.63.

[2-(2-{[1-(2-{2-[2-(2-Dipropylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}-acetylamino)acetylamino]dideuterioacetic Acid Benzyl Ester, 7, 3_2[DGA]-GGGPGG-Gd_2-OCH_2Ph. 3_2[DGA]-GGGPGG-OCH_2Ph. 3_2[DGA]-GGGPG-OH (0.22 g, 0.41 mmol), TsOH·G-O-CH_2Ph (0.14 g, 0.41 mmol), and NMM (0.05 mL) were dissolved in CH_2Cl_2 (20 mL) and cooled to 5 °C. EDCI (0.09 g, 0.45 mmol) and HOBt (0.06 g, 0.45 mmol) were added, and the reaction was stirred at room temperature overnight. Solvent was evaporated, and the residue was dissolved in $CHCl_3$ (50 mL); washed with 5% citric acid (25 mL), 5% $NaHCO_3$, and brine (25 mL); dried over $MgSO_4$; and evaporated to give colorless crystals (0.19 g, 67%), mp 60−61 °C. ^{1}H NMR: 0.80−0.95 (6H, m, −CH_2*CH*$_3$), 1.40−1.60 (4H, m, CH_3*CH*$_2$$CH_2$N), 1.85−2.10 (4H, m, Pro NCH_2*CH*$_2$*CH*$_2$), 3.04 (2H, t, *J* = 7.5 Hz, CH_3-CH_2*CH*$_2$N), 3.25 (2H, t, *J* = 7.5 Hz, CH_3CH_2*CH*$_2$N), 3.40−3.45 (1H, m, Pro N*CH*$_2$$CH_2$$CH_2$), 3.55−4.30 (15H, overlapping signals due to Gly N*CH*$_2$, Pro N*CH*$_2$$CH_2$$CH_2$, and COC*H*$_2$O), 4.44 (1H, t, *J* = 6 Hz, Pro N*CH*), 5.15 (2H, s, Ph*CH*$_2$O), 7.30−7.35 (5H, m, *H*$_{Ar}$), 7.38 (1H, t, *J* = 5.7 Hz, Gly CO*NH*), 7.47 (1H, t, *J* = 5.7 Hz, Gly CO*NH*), 7.80 (2H, t, *J* = 5.7 Hz, overlapping signals due to Gly CO*NH*), 8.35 (1H, t, *J* = 5.7 Hz, Gly CO*NH*). ^{13}C NMR: 11.2, 11.4, 20.7, 21.9, 24.9, 29.0, 41.2, 42.0, 42.9, 43.1, 47.1, 47.9, 48.4, 61.1, 67.1, 69.6, 71.7, 128.1, 128.4, 128.6, 135.2, 168.7, 169.1, 169.9, 170.0, 170.2, 170.6, 171.5, 172.0. IR ($CHCl_3$): 3308, 2965, 2935, 2876, 1749, 1650, 1534, 1454, 1340, 1240, 1028, 733, 698 cm^{-1}.

3_2[DGA]-GGGPGG-OH. 3_2[DGA]-GGGPGG-OCH_2Ph (0.18 g, 0.26 mmol) was dissolved in abs EtOH (20 mL), 10% Pd/C (0.02 g) was added, and this mixture was shaken under 60 psi hydrogen pressure for 3 h in a Parr apparatus. The reaction mixture was heated to reflux and filtrated through a Celite pad. The solvent was evaporated to leave a white solid in a quantitative yield (0.16 g), mp 84−85 °C. ^{1}H NMR: 0.80−0.95 (6H, m, −CH_2*CH*$_3$), 1.40−1.60 (4H, m, CH_3*CH*$_2$$CH_2$N), 1.85−2.20 (4H, m, Pro NCH_2*CH*$_2$*CH*$_2$), 3.07 (2H, t, *J* = 7.5 Hz, CH_3-CH_2*CH*$_2$N), 3.24 (2H, t, *J* = 7.5 Hz, CH_3CH_2*CH*$_2$N), 3.40−3.50 (1H, m, Pro N*CH*$_2$$CH_2$$CH_2$), 3.55−4.20 (13H, overlapping signals due to Gly N*CH*$_2$, Pro N*CH*$_2$$CH_2$$CH_2$, and COC*H*$_2$O), 4.31 (2H, s, COC*H*$_2$O), 4.41 (1H, bs, Pro N*CH*), 7.70 (1H, bs, Gly CO*NH*), 7.76 (1H, bs, Gly CO*NH*), 7.99 (1H, bs, Gly CO*NH*), 8.02 (1H, bs, Gly CO*NH*), 8.26 (1H, bs, Gly CO*NH*). ^{13}C NMR: 11.1, 11.3, 20.7, 21.8, 24.9, 29.2, 41.1, 42.1, 42.7, 47.0, 47.8, 48.4, 61.2, 69.1, 70.8, 168.8, 169.0, 170.5, 170.6, 171.2, 172.3, 172.8. IR ($CHCl_3$): 3305, 2966, 2935, 2877, 1735, 1646, 1540, 1452, 1410, 1338, 1240, 1209, 1129, 1030, 909, 729 cm^{-1}.

3_2[DGA]-GGGPGG-Gd_2-OCH_2Ph. To 3_2[DGA]-GGGPGG-OH (0.16 g, 0.26 mmol) suspended in CH_2Cl_2 (20 mL) were added PyBroP (0.13 g, 0.29 mmol) and HOBt (0.04 g, 0.29 mmol), and the reaction was stirred for 0.5 h. Then HCl·Gd_2-OCH_2Ph (0.5 g, 0.26 mmol) in CH_2Cl_2 (10 mL) containing DIEA (0.05 mL) was added and the reaction mixture was stirred for 48 h at room temperature. The solvent was evaporated and the residue was chromatographed (SiO_2, 10−30% MeOH−$CHCl_3$) to give 0.16 g (84%) of pure product as a white solid, mp 111−112 °C. ^{1}H NMR: 0.80−0.95 (6H, m, −CH_2*CH*$_3$), 1.40−1.60 (4H, m, CH_3*CH*$_2$$CH_2$N), 1.85−2.20 (4H, m, Pro NCH_2*CH*$_2$*CH*$_2$), 3.05 (2H, t, *J* = 7.5 Hz, CH_3CH_2*CH*$_2$N), 3.24 (2H, t, *J* = 7.5 Hz, CH_3-CH_2*CH*$_2$N), 3.40−3.45 (1H, m, Pro N*CH*$_2$$CH_2$$CH_2$), 3.55−4.20 (13H, overlapping signals due to Gly N*CH*$_2$, Pro N*CH*$_2$$CH_2$$CH_2$, and COC*H*$_2$O), 4.31 (2H, s, CO*CH*$_2$O), 4.35 (1H, bs, Pro N*CH*), 5.12−5.15 (2H, m, PhCH_2O), 7.30−7.38 (5H, m, *H*$_{Ar}$), 7.45 (1H, bs, Gly CO*NH*), 7.72 (1H, pseudo-s, Glyd$_2$ CO*NH*), 7.90 (2H, overlapping signals due to Gly CO*NH*), 8.36 (1H, bt, Gly CO*NH*). ^{13}C NMR: 11.1, 11.3, 20.6, 21.8, 25.0, 29.1, 41.2, 41.8, 42.6, 42.8, 43.2, 46.8, 47.7, 48.3, 61.2, 67.1, 69.1, 70.9, 128.2, 128.3, 128.5, 135.3, 168.7, 168.9, 169.7, 170.3, 170.5, 170.6, 171.0, 171.1, 173.7. IR ($CHCl_3$): 3300, 2965, 1748, 1654, 1533, 1455, 1408, 1240, 1191, 1130, 1029, 915, 843, 732. Anal. Calcd for $C_{34}H_{48}N_8O_{11}$·H_2O: C, 53.25; H, 7.10; N, 14.61. Found: C, 53.16; H, 7.11; N, 14.53.

[2-(2-{[1-(2-{2-[2-(2-Didecylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}-acetylamino)acetylamino]acetic acid benzyl ester, 8, was prepared as previously reported.[31]

[2-(2-{[1-(2-{2-[2-(2-Dioctadecylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}-acetylamino)acetylamino]acetic acid benzyl ester, 9, was prepared as previously reported.[31]

[2-(2-{[1-(2-{2-[2-(2-Dioctadecylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}-acetylamino)acetylamino]acetic acid ethyl ester, 10, was prepared as previously reported.[49]

[2-(2-{[1-(2-{2-[2-(2-Dioctadecylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}-acetylamino)acetylamino]acetic acid heptyl ester, 11, was prepared as previously reported.[33]

[2-(2-{[1-(2-{2-[2-(2-Dipropylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}acetyl)pyrrolidine-2-carbonyl]amino}-acetylamino)acetylamino]acetic Acid Octadecyl Ester, 12, 3_2[DGA]-GGGPGGG-$OC_{18}H_{37}$. 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 heated to reflux and water was removed by using a Dean−Stark trap. When no more water appeared in the distillate (after 8 h), 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. The crystalline *p*-toluenesulfonate of GGG-OCH_2Ph was collected on a filter, washed with ether (50 mL), dried, and recrystallized from MeOH−ether (5.5 g, 77%), mp 176−177 °C. ^{1}H NMR: 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 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. IR (KBr): 3331, 3083, 1747, 1670, 1545, 1456, 1406, 1362, 1202, 1125, 1035, 1011, 913, 817, 736, 685 cm^{-1}.

Boc-PGGG-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 room temperature for 3 days. Solvent was evaporated, and the residue was dissolved in EtOAc (50 mL), 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. ^{1}H 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$_2$CH$_2$$CH_2$), 3.85−4.20 (7H, m, Gly NCH_2, Pro NCH), 5.15 (2H, s, PHCH_2O), 7.05 (2H, bs, Gly CONH), 7.30−7.35 (5H, m, H_{Ar}), 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 cm^{-1}. Anal. Calcd 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.

PGGG-OCH_2Ph·HCl. Boc-PGGG-OCH_2Ph (0.2 g, 0.42 mmol) was dissolved in 4 N 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). ^{1}H NMR (CD_3OD): 2.00−2.25 (4H, m, Pro NCH_2CH_2CH$_2$), 3.35−3.45 (2H, m, Pro NCH$_2$CH$_2$$CH_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.

Dioctadecylcarbamoylmethoxyacetic Acid (18_2[DGA]-OH). 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. ^{1}H NMR: 0.87 (6H, t, J = 6.9 Hz, −CH$_2$$CH_3$), 1.25 (60H, pseudo-s, CH$_3$(CH_2)$_{15}$CH$_2$CH$_2$N), 1.55 (4H, bs, CH$_3$(CH$_2$)$_{15}$$CH_2CH_2$N), 3.07 (2H, t, J = 7.8 Hz, CH$_3$-(CH$_2$)$_{16}$$CH_2$N), 3.34 (2H, t, J = 7.8 Hz, CH$_3$(CH$_2$)$_{16}$$CH_2$N), 4.21 (2H, s, COCH$_2$O), 4.38 (2H, s, COCH$_2$O). ^{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}.

18_2[DGA]-GGG-OCH_2Ph. To dioctadecylcarbamoylmethoxyacetic acid (1 g, 1.5 mmol) dissolved in CH_2Cl_2 (30 mL) was added EDCI (0.31 g, 1.6 mmol) and the mixture was stirred at room temperature. After 0.5 h, TsOH·GGG-OCH_2Ph (0.66 g, 1.5 mmol) and Et_3N (0.6 mL) were added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was successively washed with water (20 mL), 0.5 M HCl (20 mL), water (20 mL), 10% Na_2CO_3 (20 mL), and brine (20 mL); dried ($MgSO_4$); and evaporated, and the residue was crystallized from MeOH to afford a white solid (1.26 g, 89%), mp 41−42 °C. ^{1}H NMR: 0.86 (6H, t, J = 6.9 Hz, −CH$_2$$CH_3$), 1.24 (60H, pseudo-s, CH$_3$(CH_2)$_{15}$CH$_2$CH$_2$N), 1.49 (4H, bs, CH$_3$-(CH$_2$)$_{15}$$CH_2CH_2$N), 1.61 (1H, H$_2$O), 3.04 (2H, t, J = 7.5 Hz, CH$_3$-(CH$_2$)$_{16}$$CH_2$N), 3.24 (2H, t, J = 7.5 Hz, CH$_3$(CH$_2$)$_{16}$$CH_2$N), 3.95−4.05 (6H, m, Gly NCH_2), 4.09 (2H, s, COCH$_2$O), 4.29 (2H, s, COCH$_2$O), 5.12 (2H, s, PHCH_2O), 7.23 (1H, t, J = 6.0 Hz, Gly CONH), 7.30−7.35 (5H, m, H_{Ar}), 7.93 (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, 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 $C_{53}H_{94}N_4O_7$·0.5H_2O: C, 70.11; H, 10.54; N, 6.17. Found: C, 70.18; H, 10.55; N, 6.18.

18_2[DGA]-GGG-OH. 18_2[DGA]-GGG-OCH_2Ph (1.0 g, 1.1 mmol) was dissolved in abs EtOH (100 mL) and 10% Pd/C (0.2 g) was added, and this mixture was shaken under 60 psi pressure of H_2 for 3 h. The reaction mixture was heated to reflux and filtered (Celite pad). The solvent was evaporated to afford a white solid (0.86 g, 96%), mp 163−164 °C. ^{1}H NMR (CD_3OD): 0.90 (6H, t, J = 6.9 Hz, −CH$_2$$CH_3$), 1.29 (60H, pseudo-s, CH$_3$(CH_2)$_{15}$CH$_2$CH$_2$N), 1.57 (4H, bs, CH$_3$-(CH$_2$)$_{15}$$CH_2CH_2$N), 3.21 (2H, t, J = 7.8 Hz, CH$_3$(CH$_2$)$_{16}$$CH_2$N), 3.35 (2H, t, J = 7.8 Hz, CH$_3$(CH$_2$)$_{16}$$CH_2$N), 3.93 (2H, s, Gly N$CH_2$), 3.94 (2H, s, Gly N$CH_2$), 3.97 (2H, s, Gly N$CH_2$), 4.12 (2H, s, COCH$_2$O), 4.40 (2H, s, COCH$_2$O). IR (KBr): 3285, 3084, 2925, 2852, 1740, 1650, 1551, 1467, 1420, 1378, 1219, 1128, 1033, 1011, 721, 681 cm^{-1}. Anal. Calcd for $C_{46}H_{88}N_4O_7$: C, 68.28; H, 10.96; N, 6.92. Found: C, 67.97; H, 10.92; N, 6.81.

3_2[DGA]-GGGPGGG-$OC_{18}H_{37}$. To 18_2[DGA]-GGGPGGG-OH (0.33 g, 0.5 mmol) suspended in CH_2Cl_2 (20 mL) were added 1,3-diisopropylcarbodiimide (0.13 mL, 0.84 mmol) and DMAP (0.03 g, 0.24 mmol), and the mixture was stirred at room temperature. After 0.5 h, 1-octanol (0.16 g, 0.59 mmol) was added and the reaction was stirred at room temperature for 48 h. The reaction mixture was evaporated in vacuo, and the residue was chromatographed (SiO_2, 5%−10% MeOH−$CHCl_3$) and afforded a white solid (0.08 g, 17%), mp 135−137 °C. ^{1}H NMR: 0.85−1.00 (9H, overlapping signals due to −CH$_2$$CH_3$), 1.25 (30H, pseudo-s, CH$_3$(CH_2)$_{15}$CH$_2$CH$_2$O), 1.50−1.70 (6H, overlapping signals due to CH$_3$$CH_2CH_2$N, and CH$_3$(CH$_2$)$_{15}$$CH_2$-CH$_2$O), 1.90−2.25 (4H, m, Pro NCH_2CH_2CH$_2$), 3.07 (2H, t, J = 7.5 Hz, CH$_3$$CH_2$N), 3.26 (2H, t, J = 7.5 Hz, CH$_3$$CH_2$N), 3.50−3.60 (1H, m, Pro NCH$_2$CH$_2$$CH_2$), 3.65−3.70 (1H, m, Pro NCH$_2$CH$_2$$CH_2$), 3.70−4.10 (16H, overlapping signals due to Gly NCH_2, COCH$_2$O, and CH$_3$(CH$_2$)$_{15}$CH$_2$$CH_2$O), 4.33 (2H, s, COCH$_2$O), 4.35 (1H, bs, Pro N$CH$), 7.31 (1H, t, J = 6.0 Hz, Gly CONH), 7.46 (1H, t, J = 6.0 Hz, Gly CONH), 7.56 (1H, t, J = 6.0 Hz, Gly CONH), 7.87 (1H, t, J = 6.0 Hz, Gly CONH), 7.91 (1H, t, J = 6.0 Hz, Gly CONH), 8.42 (1H, t, J = 6.0 Hz, Gly CONH).^{13}C NMR: 11.2, 11.3, 14.1, 20.7, 21.9, 22.6, 25.1, 25.8, 28.5, 29.0, 29.2, 29.3, 29.5, 29.7, 31.9, 41.2, 41.9, 42.7, 42.8, 42.9, 43.4, 46.9, 47.8, 48.4, 61.2, 65.7, 69.4, 71.3, 168.6, 168.8, 170.1, 170.2, 170.3, 170.4, 170.7, 171.1, 173.5. IR ($CHCl_3$): 3306, 3080, 2957, 2919, 2851, 1748, 1659, 1647, 1548, 1534, 1467, 1410, 1378, 1340,

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1240, 1204, 1129, 1030, 722 cm^{-1}. Anal. Calcd for $C_{64}H_{110}N_8O_{11}\cdot H_2O$: C, 58.29; H, 8.91; N, 12.09. Found: C, 58.32; H, 8.87; N, 12.07.

[2-(2-{[1-(2-{2-[2-(2-Dioctadecylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}acetyl)piperidine-2-carbonyl]amino}-acetylamino)acetylamino]acetic acid benzyl ester, 13, was prepared as previously reported.[33]

[2-(2-{2-[(1-{2-[2-(2-Dioctadecylcarbamoylmethoxyacetylamino)-acetylamino]acetyl}pyrrolidine-2-carbonyl)amino]acetylamino}-acetylamino)acetylamino]acetic acid benzyl ester, 14, 18_2[DGA]-GGPGGGG-OCH$_2$Ph. 18_2[DGA]-GG-OH (0.45 g, 0.60 mmol) was suspended in CH_2Cl_2 (60 mL) at 0 °C. EDCI (0.15 g, 0.78 mmol), PGGGG-OCH$_2$Ph·HCl (0.28 g, 0.59 mmol), HOBt (0.10 g, 0.74 mmol), and Et_3N (0.5 mL) were added, and the reaction was stirred for 48 h at room temperature. The solvent was then evaporated and the residue purified by column chromatography (SiO_2, 10% MeOH−$CHCl_3$) to give a white solid (0.57 g, 82%), mp 169−171 °C. ^{1}H NMR: 0.79 (6H, t, J = 6.3 Hz, *CH*$_3$(CH$_2$)$_{15}$CH$_2$CH$_2$N), 1.17 (60H, m, CH$_3$(*CH*$_2$)$_{15}$-CH$_2$CH$_2$N), 1.42 (4H, m, CH$_3$(CH2)15*CH2*CH2N), 1.82−2.18 (4H, m, Pro NCH$_2$*CH*$_2$*CH*$_2$), 2.98 (2H, t, J = 7.2 Hz, CH$_3$(CH$_2$)$_{15}$CH$_2$*CH*$_2$N), 3.17 (2H, t, J = 7.2 Hz, CH$_3$(CH$_2$)$_{15}$CH$_2$*CH*$_2$N), 3.40 and 3.60 (2H, m, Pro N*CH*$_2$CH$_2$CH$_2$), 3.75−4.05 (16H, m, Gly CH$_2$, CO*CH*$_2$O), 4.18 (2H, s, COCH$_2$O), 4.30 (1H, m, Pro *CH*), 5.04 (2H, s, O*CH*$_2$Ph), 7.25 (5H, m, HAr), 7.69 (2H, m, *NH*), 7.78 (1H, bt, *NH*), 7.86 (1H, bt, *NH*), 8.08 (1H, bt, *NH*), 8.24 (1H, t, J = 5.4 Hz, *NH*). ^{13}C NMR: 14.3, 22.9, 25.3, 27.1, 27.3, 27.8, 29.0, 29.3, 29.5, 29.6, 29.8, 29.9, 32.1, 41.3, 42.3, 42.5, 43.0, 43.3, 43.5, 46.5, 47.1, 61.6, 67.2, 69.4, 71.3, 128.3, 128.6, 128.8, 135.6, 168.6, 169.3, 170.50, 170.55, 170.9, 171.0, 171.1, 173.3. Anal. Calcd for $C_{64}H_{110}N_8O_{11}$: C, 65.83, H, 9.50; N, 9.60. Found: C, 65.88; H, 9.88; N, 9.39.

[2-({1-[2-(2-{2-[2-(2-Dioctadecylcarbamoylmethoxyacetylamino)-acetylamino]acetylamino}acetylamino)acetyl]pyrrolidine-2-carbonyl}-amino)acetylamino]acetic Acid Benzyl Ester, 15, 18_2[DGA]-GGPGGGG-OCH$_2$Ph. 18_2[DGA]-GGGGPGG-OCH$_2$Ph. 18_2-DGA-GGGG-OH (prepared as detailed in the following procedure, 0.65 g, 0.75 mmol) was suspended in CH_2Cl_2 (80 mL) at 0 °C; EDCI (0.20 g, 1.04 mmol), PGG-OCH$_2$Ph·HCl (0.27 g, 0.75 mmol), 1-hydroxybenzotriazole (0.15 g, 1.11 mmol), and Et_3N (0.6 mL) were added; and the reaction was stirred for 48 h at room temperature. The solvent was evaporated and the residue purified by column chromatography (SiO_2, 10% MeOH−$CHCl_3$) to give a white solid (0.70 g, 80%), mp 175−177 °C. ^{1}H NMR: 0.79 (6H, t, J = 6.3 Hz, *CH*$_3$(CH$_2$)$_{15}$CH$_2$CH$_2$N), 1.18 (60H, m, CH$_3$(*CH*$_2$)$_{15}$CH$_2$CH$_2$N), 1.40 (4H, m, CH$_3$(CH$_2$)$_{15}$*CH*$_2$-CH$_2$N), 1.82−2.10 (4H, m, ProNCH$_2$*CH*$_2$*CH*$_2$), 3.00 (2H, t, J = 7.5 Hz, CH$_3$(CH$_2$)$_{15}$CH$_2$*CH*$_2$N), 3.17 (2H, t, J = 7.5 Hz, CH$_3$(CH$_2$)$_{15}$-CH$_2$*CH*$_2$N), 3.41 and 3.62 (2H, m, Pro N*CH*$_2$CH$_2$CH$_2$), 3.72−4.05 (16H, m, Gly CH$_2$, COCH$_2$O), 4.19 (2H, s, COCH$_2$O), 4.36 (1H, m, Pro *CH*), 5.05 (2H, s, O*CH*$_2$Ph), 7.25 (5H, m, *H*$_{Ar}$), 7.52 (2H, m, *NH*), 7.66 (1H, t, J = 5.7 Hz, *NH*), 7.88 (1H, t, J = 5.4 Hz, *NH*), 7.96 (1H, t, J = 5.7 Hz, *NH*), 8.16 (1H, t, J = 5.7 Hz, *NH*). ^{13}C NMR: 14.3, 22.7, 25.1, 27.1, 27.3, 27.8, 29.0, 29.5, 29.6, 29.9, 32.1, 41.5, 42.2, 43.0, 43.5, 46.0, 46.5, 47.1, 61.2, 67.2, 69.6, 71.6, 128.4, 128.6, 128.8, 135.5, 168.6, 168.9, 170.4, 170.6, 170.8, 171.2, 172.5. Anal. Calcd for $C_{64}H_{110}N_8O_{11}\cdot H_2O$: C, 64.83; H, 9.52; N, 9.45. Found: C, 65.05; H, 9.40; N, 9.39.

18_2[DGA]-GGPGGGG-OCH$_2$Ph. 18_2[DGA]-GG-OH (0.45 g, 0.60 mmol) was suspended in CH_2Cl_2 (60 mL) at 0 °C. EDCI (0.15 g, 0.78 mmol), PGGGG-OCH$_2$Ph·HCl (0.28 g, 0.59 mmol), HOBt (0.10 g, 0.74 mmol), and Et_3N (0.5 mL) were added, and the reaction was stirred for 48 h at room temperature. The solvent was then evaporated and the residue purified by column chromatography (SiO_2, 10% MeOH−$CHCl_3$) to give a white solid (0.57 g, 82%), mp 169−171 °C. ^{1}H NMR: 0.79 (6H, t, J = 6.3 Hz, *CH*$_3$(CH$_2$)$_{15}$CH$_2$CH$_2$N), 1.17 (60H, m, CH$_3$(*CH*$_2$)$_{15}$CH$_2$CH$_2$N), 1.42 (4H, m, CH$_3$(CH$_2$)$_{15}$*CH*$_2$CH$_2$N), 1.82−2.18 (4H, m, Pro NCH$_2$*CH*$_2$*CH*$_2$), 2.98 (2H, t, J = 7.2 Hz, CH$_3$(CH$_2$)$_{15}$-CH$_2$*CH*$_2$N), 3.17 (2H, t, J = 7.2 Hz, CH$_3$(CH$_2$)$_{15}$CH$_2$*CH*$_2$N), 3.40 and 3.60 (2H, m, Pro N*CH*$_2$CH$_2$CH$_2$), 3.75−4.05 (16H, m, Gly CH$_2$, CO*CH*$_2$O), 4.18 (2H, s, COCH$_2$O), 4.30 (1H, m, Pro *CH*), 5.04 (2H, s, O*CH*$_2$Ph), 7.25 (5H, m, H$_{Ar}$), 7.69 (2H, m, *NH*), 7.78 (1H, bt, *NH*), 7.86 (1H, bt, *NH*), 8.08 (1H, bt, *NH*), 8.24 (1H, t, J = 5.4 Hz, *NH*). ^{13}C NMR: 14.3, 22.9, 25.3, 27.1, 27.3, 27.8, 29.0, 29.3, 29.5, 29.6, 29.8, 29.9, 32.1, 41.3, 42.3, 42.5, 43.0, 43.3, 43.5, 46.5, 47.1, 61.6, 67.2, 69.4, 71.3, 128.3, 128.6, 128.8, 135.6, 168.6, 169.3, 170.50, 170.55, 170.9, 171.0, 171.1, 173.3. Anal. Calcd for $C_{64}H_{110}N_8O_{11}$: C, 65.83; H, 9.50; N, 9.60. Found: C, 65.88; H, 9.88; N, 9.39.

{2-[(1-{2-[2-(2-Dioctadecylcarbamoylmethoxyacetylamino)-acetylamino]acetyl}pyrrolidine-2-carbonyl)amino]acetylamino}-acetic Acid Benzyl Ester, 16, Boc-GGGG-OCH$_2$Ph. 18_2[DGA]-GG-OCH$_2$Ph. To a solution of 18_2[DGA]-OH (1.0 g, 1.5 mmol) in CH_2Cl_2 (30 mL) was added EDCI (0.31 g, 1.6 mmol), and the reaction was stirred at room temperature. After 0.5 h, GG-OCH$_2$Ph·TsOH (0.62 g, 1.5 mmol) and Et_3N (0.6 mL) were added, and the mixture was stirred overnight. The reaction was quenched; washed with water (20 mL), 0.5 M aq HCl (20 mL), water (20 mL), 10% Na_2CO_3(aq) (20 mL), and brine (20 mL); dried over $MgSO_4$; and evaporated. The residue was recrystallized from MeOH, yielding the desired product as a colorless oil (1.32 g, 94%). ^{1}H NMR: 0.86 (6H, t, J = 6.9 Hz, *CH*$_3$(CH$_2$)$_{15}$CH$_2$CH$_2$N), 1.24 (60H, m, CH$_3$(*CH*$_2$)$_{15}$CH$_2$CH$_2$N), 1.48 (4H, bs, CH$_3$(CH$_2$)$_{15}$*CH*$_2$CH$_2$N), 3.04 (2H, t, J = 7.5 Hz, CH$_3$(CH$_2$)$_{15}$-CH$_2$*CH*$_2$N), 3.21 (2H, t, J = 7.5 Hz, CH$_3$(CH$_2$)$_{15}$CH$_2$*CH*$_2$N), 4.02−4.07 (6H, m, Gly *CH*$_2$), 4.08 (2H, s, CO*CH*$_2$O), 4.28 (2H, s, CO*CH*$_2$O), 5.13 (2H, s, Ph*CH*$_2$O), 7.32 (5H, m, *H*$_{Ar}$), 7.58 (1H, t, J = 5.7 Hz, *NH*), 8.21 (1H, t, J = 5.7 Hz, *NH*). ^{13}C NMR: 14.0, 22.6, 26.7, 26.9, 27.4, 28.7, 29.2, 29.3, 29.4, 29.5, 29.6, 31.8, 41.1, 42.7, 46.7, 66.9, 69.5, 71.7, 128.1, 128.3, 128.5, 135.2, 168.3, 169.5, 170.4.

18_2[DGA]-GG-OH. 18_2[DGA]-GG-OCH$_2$Ph (2.3 g, 2.73 mmol) was dissolved in abs EtOH (30 mL), 10% Pd/C (0.15 g) was added, and this mixture was shaken under 60 psi pressure of H_2 for 3 h. The reaction mixture was heated to reflux and filtered (Celite pad). The solvent was evaporated to afford a white solid (2.0 g, 100%), mp 114−115 °C. ^{1}H NMR ($CDCl_3$/CD_3OD 95:5): 0.80 (6H, t, J = 6.9 Hz, *CH*$_3$(CH$_2$)$_{15}$CH$_2$CH$_2$N), 1.18 (60H, m, CH$_3$(*CH*$_2$)$_{15}$CH$_2$CH$_2$N), 1.45 (4H, m, CH$_3$(CH$_2$)$_{15}$*CH*$_2$CH$_2$N), 3.02 (2H, t, J = 7.8 Hz, CH$_3$(CH$_2$)$_{15}$-CH$_2$*CH*$_2$N), 3.22 (2H, t, J = 7.8 Hz, CH$_3$(CH$_2$)$_{15}$CH$_2$*CH*$_2$N), 3.91 (2H, s, Gly *CH*$_2$), 3.95 (2H, s, Gly *CH*$_2$), 4.02 (2H, s, CO*CH*$_2$O), 4.20 (2H, s, CO*CH*$_2$O). ^{13}C NMR ($CDCl_3$/CD_3OD 95:5): 13.9, 22.5, 26.7, 26.8, 27.3, 28.6, 29.2, 29.4, 29.5, 31.7, 40.8, 42.1, 46.1, 46.7, 68.8. 70.8, 168.2, 169.7, 170.5, 171.3.

Boc-PGG-OCH$_2$Ph. Boc-P-OH (0.55 g, 2.54 mmol) was dissolved in dry CH_2Cl_2 (50 mL) at 0 °C; GG-OCH$_2$Ph·TsOH (1.00 g, 2.54 mmol), EDCI (0.54 g, 2.8 mmol), HOBt (0.38 g, 2.8 mmol), and Et_3N (1.0 mL) were added; and the solution was stirred for 1 h at 0 °C and for 48 h more at room temperature. The solvent was removed in vacuo and the crude product purified by column chromatography (SiO_2, 5−10% MeOH−$CHCl_3$) to give 1.0 g of a deliquescent solid (93%). ^{1}H NMR: 1.41 (9H, s, C(*CH*$_3$)$_3$), 1.8−2.2 (4H, m, Pro NCH$_2$*CH*$_2$*CH*$_2$), 3.46 (2H, m, Pro N*CH*$_2$CH$_2$CH$_2$), 4.04 (4H, m, Gly *CH*$_2$), 4.20 (1H, m, Pro *CH*), 5.15 (2H, s, O*CH*$_2$Ph), 7.02 (1H, bt, *NH*), 7.34 (5H, m, H$_{Ar}$), 7.46 (1H, bt, *NH*). ^{13}C NMR: 24.6, 28.3, 29.3, 41.2, 42.9, 47.3, 60.7, 67.0, 80.7, 128.2, 128.4, 128.5, 135.2, 155.7, 169.6, 169.7, 172.9.

HCl·PGG-OCH$_2$Ph. Boc-PGG-OCH$_2$Ph (0.4 g, 0.95 mmol) was dissolved in 4 N HCl in dioxane (10 mL) at 5 °C and the reaction mixture was stirred for 1 h. The solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

18_2[DGA]-GGPGG-OCH$_2$Ph. 18_2[DGA]-GG-OH (0.71 g, 0.95 mmol), PGG-OCH$_2$Ph·HCl (0.25 g, 0.95 mmol), and DIEA (0.2 mL) were dissolved in CH_2Cl_2 (30 mL) and cooled to 5 °C. EDCI (0.2 g, 1.05 mmol) and HOBt (0.14 g, 1.05 mmol) were added, and the reaction was stirred at room temperature for 48 h. The solvent was evaporated and the residue purified by column chromatography (SiO_2, 5−10% MeOH−$CHCl_3$) to give an off-white solid (0.76 g, 76%), mp 55−56 °C. ^{1}H NMR: 0.86 (6H, t, J = 6.9 Hz, *CH*$_3$(CH$_2$)$_{15}$CH$_2$CH$_2$N), 1.25 (60H, m, CH$_3$(*CH*$_2$)$_{15}$CH$_2$CH$_2$N), 1.40−1.50 (4H, m, CH$_3$(CH$_2$)$_{15}$*CH*$_2$-

CH_2N), 1.80−2.20 (4H, m, Pro $NCH_2CH_2CH_2$), 3.08 (2H, t, J = 7.5 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.15−3.35 (2H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.50−4.40 (14H, overlapping signals due to Pro $NCH_2CH_2CH_2$, Gly CH_2, and $COCH_2O$), 4.43 (1H, m, Pro *CH*), 5.14 (2H, s, OCH_2Ph), 7.33 (5H, m, H_{Ar}), 7.42 (2H, bt, *NH*), 7.56 (1H, bt, *NH*), 7.88 (1H, bt, *NH*), 8.22 (1H, bt, *NH*). ^{13}C NMR: 14.1, 22.7, 24.9, 26.9, 27.1, 27.6, 28.9, 29.3, 29.4, 29.5, 29.7, 31.9, 41.0, 42.3, 42.6, 42.9, 46.3,46.8, 47.4, 61.2, 67.0, 69.4, 71.7, 128.2, 128.4, 128.6, 135.3, 168.2, 169.1, 169.8, 170.4, 170.6, 171.7. IR ($CHCl_3$): 3307, 2919, 2851, 1748, 1658, 1651, 1536, 1467, 1455, 1243, 1191, 1129, 1030, 754 cm^{-1}. Anal. Calcd for $C_{60}H_{104}N_6O_9$: C, 68.40; H, 9.95; N, 7.98. Found: C, 68.17; H, 9.95; N, 8.11.

(2-{2-[2-({1-[2-(2-{2-[2-(2-Dioctadecylcarbamoylmethoxyacetylamino)acetylamino]acetylamino}acetylamino)acetyl]pyrrolidine-2-carbonyl}amino)acetylamino]acetylamino}acetylamino)-acetic Acid Benzyl Ester, 17, 18_2[DGA]-GGGGPGGGG-OCH_2Ph. HCl·GGGG-OCH_2Ph. Boc-GGGG-OCH_2Ph. Boc-G-OH (0.50 g, 2.85 mmol) was suspended in CH_2Cl_2 (100 mL) at 0° C, and EDCI (0.70 g, 3.65 mmol), GGG-OCH_2Ph·TsOH (1.28 g, 2.84 mmol) and Et_3N (1.0 mL) were added. The reaction was stirred for 48 h at room temperature. The solvent was then evaporated and the residue purified by column chromatography (SiO_2, 5−10% MeOH−$CHCl_3$) to give an off-white solid (0.23 g, 99%), mp 189−90 °C. ^1H NMR 1.36 (9H, s, $(CH_3)_3$), 3.71 (2H, d, J = 5.7 Hz, Gly CH_2), 3.83 (2H, d, J = 5.7 Hz, Gly CH_2), 3.86 (2H, d, J = 5.7 Hz, Gly CH_2), 3.96 (2H, d, J = 5.7 Hz, Gly CH_2), 5.09 (2H, s, OCH_2Ph), 5.96 (1H, bs, *NH*), 7.27 (5H, m, H_{Ar}), 7.63 (1H, bt, *NH*), 7.80 (1H, bt, *NH*), 7.82 (1H, bt, NH). ^{13}C NMR: 28.0, 40.9, 41.0, 42.4, 42.8, 43.9, 67.1, 80.3, 128.1, 128.3, 128.5, 134.9, 169.8, 170.0, 170.1, 171.4.

Boc-GGGG-OCH_2Ph (0.73 g, 1.82 mmol) was dissolved in 4 N HCl in dioxane (10 mL) at 5 °C and the reaction mixture was stirred for 1 h. The solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

18_2[DGA]-GGGG-OCH_2Ph. 18_2[DGA]-OH (1.16 g, 1.82 mmol) and GGGG-OCH_2Ph·HCl (0.68 g, 1.82 mmol) were suspended in CH_2Cl_2 (100 mL) at 0 °C, diisopropylcarbodiimide (0.45 g, 2.35 mmol) and Et_3N (1.0 mL) were added, and then the reaction was stirred for 72 h at room temperature. The solvent was then evaporated and the residue purified by column chromatography (SiO_2, 5% MeOH−$CHCl_3$) to give the final product as an oil (1.51 g, 87%). ^1H NMR: 0.78 (6H, t, J = 6.6 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.16 (60H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.40 (4H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 2.95 (2H, t, J = 6.9 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.15 (2H, t, J = 6.9 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.80−3.93 (8 H, m, Gly CH_2), 3.99 (2H, s, $COCH_2O$), 4.18 (2H, s, $COCH_2O$), 5.02 (2H, s, OCH_2Ph), 7.23 (5H, m, H_{Ar}), 7.51 (1H, t, J = 5.7 Hz, *NH*), 7.60 (1H, t, J = 5.1 Hz, *NH*), 7.95 (1H, t, J = 5.1 Hz, *NH*), 8.17 (1H, t, J = 5.4 Hz, *NH*). ^{13}C NMR: 14.2, 22.8, 27.0, 27.2, 27.7, 28.9, 29.46, 29.52, 29.8, 32.0, 41.3, 42.9, 43.3, 46.4, 47.0, 67.1, 69.5, 71.5, 128.3, 128.4, 128.7, 135.5, 168.6, 170.1, 170.2, 170.7, 171.3.

18_2[DGA]-GGGG-OH. 18_2[DGA]-GGGG-OCH_2Ph (0.76 g, 0.79 mmol) was dissolved in abs EtOH (100 mL), 10% Pd/C (0.3 g) was added, and this mixture was shaken under 60 psi hydrogen pressure for 3 h in a Parr apparatus. The reaction mixture was heated to reflux and filtrated through a Celite layer. The solvent was evaporated to leave a white solid (0.66 g, 95%) that was used without further purification, mp 158−160 °C. ^1H NMR ($CDCl_3/CD_3OD$ 95:5): 0.77 (6H, t, J = 6.9 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.15 (60H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.44 (4H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.00 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.19 (2H, t, J = 7.8 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.80 (2H, s, Gly CH_2), 3.84 (2H, s, Gly CH_2), 3.86 (2H, s, Gly CH_2), 3.89 (2H, s, Gly CH_2), 3.99 (2H, s, $COCH_2O$), 4.18 (2H, s, $COCH_2O$). ^{13}C NMR ($CDCl_3/CD_3OD$ 95:5): 13.8, 22.4, 26.6, 26.8, 27.3, 28.5, 29.1, 29.3, 29.5, 31.7, 40.7, 42.3, 42.9, 46.2, 46.7, 68.6, 70.6, 168.4, 170.0, 170.2, 170.8, 171.0, 171.6.

Boc-PGGGG-OCH_2Ph. Boc-P-OH (0.29 g, 1.37 mmol), GGGG-O-CH_2Ph·HCl (0.51 g, 1.37 mmol), and DIEA (0.26 mL) were dissolved in CH_2Cl_2 (40 mL) and cooled to 5 °C. EDCI (1.34 g, 1.51 mmol) and HOBt (0.2 g, 1.51 mmol) were added, and the mixture was stirred at room temperature for 48 h and evaporated, and the crude, oily product was chromatographed (SiO_2, 5−10% MeOH−$CHCl_3$) to afford white crystals (0.72 g, 98%), mp 122−123 °C. ^1H NMR: 1.40 (9H, s, $(CH_3)_3$), 1.80−2.20 (4H, m, Pro $NCH_2CH_2CH_2$), 3.35−3.55 (2H, m, Pro $NCH_2CH_2CH_2$), 3.70−4.20 (9H, overlapping signals due to Gly NCH_2 and Pro *NCH*), 5.14 (2H, s, OCH_2Ph), 7.05 (2H, bs, *NH*), 7.30−7.35 (7H, overlapping signals due to H_{Ar} and *NH*), 7.45 (1H, bs, *NH*), 8.00 (1H, bs, *NH*). ^{13}C NMR: 24.9, 28.5, 30.0, 41.2, 42.9, 43.7, 47.4, 61.0, 67.2, 81.1, 128.3, 128.6, 128.7, 135.3, 155.8, 169.9, 170.9, 174.5. IR ($CHCl_3$): 3307, 3069, 2979, 2935, 1747, 1668, 1661, 1532, 1408, 1243, 1191, 1164, 1132, 1030, 975, 913, 751, 672 cm^{-1}.

HCl·PGGGG-OCH_2Ph. Boc-PGGG-OCH_2Ph (0.3 g, 0.56 mmol) was dissolved in 4 N HCl/dioxane (10 mL) at 5 °C, the mixture was stirred for 1 h, and the solvent was evaporated in vacuo. The product was used in the subsequent reaction without further purification.

18_2[DGA]-GGGGPGGGG-OCH_2Ph. 18_2[DGA]-GGGG-OH (0.48 g, 0.56 mmol), PGGGG-OCH_2Ph·HCl (0.21 g, 0.56 mmol), and DIEA (0.1 mL) were dissolved in CH_2Cl_2 (30 mL), and the mixture was cooled to 5 °C. EDCI (0.12 g, 0.62 mmol) and HOBt (0.08 g, 0.62 mmol) were added, and the mixture was stirred at room temperature for 48 h. The solvent was evaporated and the residue was crystallized from MeOH and then chromatographed (SiO_2, 5−10% MeOH−$CHCl_3$) to give an off-white solid (0.15 g, 21%), mp 206−208 °C. ^1H NMR: 0.87 (6H, t, J = 6.9 Hz, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.24 (60H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.40−1.55 (4H, m, $CH_3(CH_2)_{15}CH_2CH_2N$), 1.80−2.20 (4H, m, Pro $NCH_2CH_2CH_2$), 3.05 (2H, bs, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.24 (2H, bs, $CH_3(CH_2)_{15}CH_2CH_2N$), 3.45−4.00 (18H, overlapping signals due to Pro $NCH_2CH_2CH_2$, and Gly CH_2), 4.09 (2H, s $COCH_2O$), 4.27 (2H, s $COCH_2O$), 4.34 (1H, m, Pro *CH*), 5.11 (2H, s, OCH_2Ph), 7.32 (5H, m, H_{Ar}), 7.73 (2H, bs, *NH*), 7.78 (1H, bs, *NH*), 7.89 (1H, bs, *NH*), 7.95 (1H, bs, *NH*), 8.03 (1H, bs, *NH*), 8.26 (2H, bs, *NH*). ^{13}C NMR: 14.0, 22.6, 25.1, 26.9, 27.1, 27.6, 28.9, 29.1, 29.3, 29.4, 29.6, 29.7, 31.9, 41.2, 42.0, 42.8, 43.0, 46.3, 46.9, 61.3, 66.9, 69.4, 71.3, 128.1, 128.3, 128.6, 135.4, 168.4, 168.9, 170.1, 170.2, 170.4, 170.5, 170.6, 171.0, 171.1, 173.6. IR ($CHCl_3$): 3299, 3087, 2921, 2852, 1744, 1646, 1552, 1467, 1455, 1418, 1378, 1339, 1284, 1246, 1192, 1130, 1028, 696 cm^{-1}. Anal. Calcd for $C_{68}H_{14}N_{10}O_{13}·H_2O$: C, 62.84; H, 9.15; N, 10.78. Found: C, 62.64; H, 9.08; N, 10.78.

***N,N′*-Bis-(2,4,6-trimethylphenyl)isophthalamide, 18**, was prepared as previously reported by Kavallieratos et al.[14]

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