Synthesis of New Polymerizable Metal-Chelating Lipids

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The attractive properties of functionalized lipids (bioadaptability, two-dimensional fluidity, inherent potential of self-organization, and pattern formation) make them aptly suited for numerous biological applications, e.g., for functionalization of membranes,[1] for drug delivery systems,[2] for immobilizing receptors on lipid surfaces,[3] for liposome targeting with antibodies,[4] and for biosensor applications.[5]

Interest in fabricating stable lipid-based systems (monolayers, supported bilayers, and liposomes) has led to the synthesis of polymerizable lipids. A variety of polymerizable moieties, e.g., conjugated diene, conjugated diyne, styrene, and acrylate moieties, have been incorporated. The polymerizable fragment can be positioned near the headgroup, near the hydrophobic tail, or at the middle of the fatty acids.[6] The nature as well as position of the polymerizable moiety determines the supramolecular structure formed and polymerization properties of the lipids.

Recent interest in protein targeting to lipid monolayers[7] and liposomes[8] requires the synthesis of metal-chelating lipids. Though some saturated metal-chelating lipids are reported in the literature,[9] reports for the synthesis of polymerizable counterparts are relatively few.[10] Usually, these synthetic routes provide substantially low yields and the structural diversity of the metal-chelating lipids are often limited.

In this paper, we report the synthesis of several metal-chelating polymerizable lipids. The procedures are general enough to allow for the variation of the metal-chelating headgroup as well as the polymerizable fatty acid moiety. We routinely prepare these lipids in our laboratory in moderately good quantity (>250 mg) in high purity.

Results and Discussion

We have successfully prepared the polymerizable lipids **1**−**5** (Figure 1). These lipids have the conjugated diyne moiety as the polymerizable functionality. Conjugated dialkynes polymerize efficiently under ultraviolet radiation to generate crystalline cross-linked polymers.[11] Lipid **1** is chiral with nitrilotriacetate as the headgroup. The compound **2** is a chiral lipid with iminodiacetate as the metal-chelating headgroup. Lipids **3** and **4** have one fatty acid chain; lipids **4** and **5** have the capability to position two metal ions 8−12 Å apart in the headgroup. For this study, the lipid **1** has been prepared as a mixture of diastereomers and lipids **2** and **5** have been synthesized in racemic forms using the commercially available racemic 2,3-diaminopropanoic acid.

Syntheses of the lipids **1** and **2** are depicted in Scheme 1. The nonpolymerizable counterpart of the lipid **1** has been reported in the literature.[12] Recently, the synthesis of a polymerizable lipid similar to **1** has been reported.[10] A modified procedure was followed to accomplish compatibility amongst the protecting groups. The Cbz protected L-lysine (**6**) was alkylated with bromoacetic acid to produce the nitrilotriacetate group. The three carboxylic acid groups were protected as methyl esters to improve solubility of the product in organic solvents. The hydrogenation reaction was carried out in the presence of a dilute formic acid solution (4% in methanol) to prevent oligomerization of the amino ester **7**.

The amino ester **7** was reacted with Boc-protected racemic 2,3-diaminopropanoic acid (**9**)[13] using BOP (Castro's reagent)[14] as the coupling reagent. After deprotection, the diamine **8** was combined with the polymerizable acid (commercially available from GFS Chemicals, Inc., Gainesville, FL) to afford the ester protected lipid. Ester groups were hydrolyzed under mild conditions (LiOH, MeOH−THF)[15] to yield the polymerizable lipid **1** as a waxy white solid. The synthesis of the racemic lipid **2** started from the selectively functionalized diamine **10**.[16] A strategy similar to that of **1** afforded the lipid **2** as a white waxy solid.

Scheme 2 shows the synthesis of the polymerizable lipid **3**. This lipid has only one polymerizable fatty acid moiety. For **3**, the polymerizable acid was linked to the metal-chelating headgroup through the amide linkage.

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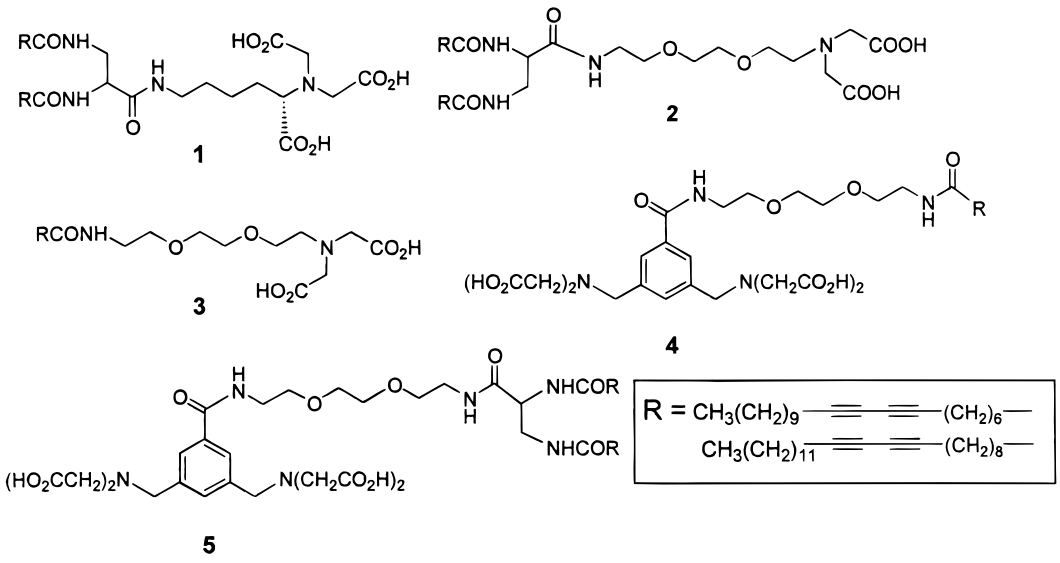

Figure 1. Structures of the metal-chelating polymerizable lipids synthesized.

The lipids discussed thus far have one metal-chelating moiety in the headgroup. We are interested in positioning more than one transition metal ion in a defined pattern using organic molecules.[17] Lipids **4** and **5** were designed (using the molecular modeling software Spartan 5.0.3, Wavefunction, Inc., Irvine, CA) to place two metal ions 8−12 Å apart. The synthesis started by reacting *tert*-butyl 3,5-bis(bromomethyl)benzoate (**14**)[18] with diethyl iminodiacetate (Scheme 3). It should be noted that the bromide can be displaced by a variety of other nucleophilic ligands. After removal of the *tert*-butyl groups, the resultant carboxylic acid **15** was combined with the monoprotected diamine **10**[16] using BOP reagent. Further elaboration of the intermediate **16** afforded the lipids **4** and **5** as waxy white solids.

Experimental Section

General Methods. Commercial reagents were purchased from either Aldrich or Acros Chemical Co. and were used without further purification unless specified otherwise. Experiments were conducted under an atmosphere of dry nitrogen. Melting points were determined on a micromelting point apparatus, and all melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra are recorded using 270, 300, or 400 MHz spectrometers in one of the following solvents: D_2O, $CDCl_3$, CD_3OD, and DMSO-d_6 with TMS internal standard. Many of the reported lipids here contained exchangeable protons (from amine and amide groups). To remove these signals from the ^{1}H NMR spectra, one drop of D_2O was added to the solvents $CDCl_3$ and DMSO-d_6. MS analyses were obtained from the Mass Spectroscopy facilities at University of California, Riverside or at the University of Minnesota, Minneapolis. Elemental analyses were carried out by in-house materials characterization laboratory. TLC was performed with Adsorbosil Plus 1P, 20 × 20 cm plate, 0.25 mm (Alltech Associates, Inc.). Chromatography plates were visualized by either UV light or iodine chamber. For workup, the organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The syntheses of polymerizable lipids were carried out at low temperature in the dark. The compounds were stored at −20 °C in solution under nitrogen to avoid the initiation of polymerization.

Methyl 6-Amino-2-(bis((methoxycarbonyl)methyl)amino)hexanoate (7). The reaction of ϵ-*N*-Cbz-lysine (**6**) with bromoacetic acid was carried out according to literature procedure.[9] The resultant triacid (3.0 g, 7.57 mmol) was dissolved in 80 mL of dry methanol, *p*-toluenesulfonic acid (8.69 g, 45.65 mmol) was added, and the solution was heated under reflux for 36 h. Methanol was removed in vacuo. The viscous colorless liquid was extracted with chloroform, and the combined organic phase was successively washed with water, 5% aqueous $NaHCO_3$, and water and then dried (Na_2SO_4). The crude product was purified by silica gel column chromatography with 2% methanol/chloroform as the solvent (R_f = 0.3) to afford the pure product as a viscous liquid. Yield (Cbz-**7**): 2.80 g (84%). ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 1.21−1.37 (m, 4H), 1.51−1.54 (m, 2H), 2.97−3.02 (m, 2H), 3.24 (t, 1H, J = 7.1 Hz), 3.35−3.38 (m, 4H), 3.47 (s, 3H), 3.51 (s, 6H), 4.96 (s, 2H), 7.15−7.20 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.1, 29.5, 30.1, 41.0, 51.6, 51.9, 52.7, 64.8, 66.7, 128.2, 128.7, 136.9, 156.6, 171.9, 173.1.

The triester from the above reaction (Cbz-**7**, 2.25 g, 5.15 mmol) was dissolved in 4.4% formic acid solution in MeOH. A small portion of Pd-black was added, and hydrogen was bubbled through the solution. Reaction was carried out at room temperature for 6 h. Pd-black was filtered off, and solvent was removed in vacuo to give the product **7** as a viscous oil. This was taken directly to the next step. Yield (**7**): 1.75 g (88%). ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 1.35−1.60 (m, 4H), 1.65−1.72 (m, 2H), 2.95−3.00 (m, 2H), 3.42 (t, 1H, J = 6.7 Hz), 3.58 (s, 4H), 3.67 (s, 6H), 3.70 (s, 3H).

Methyl 6-(2,3-Diaminopropanoylamino)-2-bis((methoxycarbonyl)methyl) aminohexanoate (8). To a solution of acid **9**[13] (1.48 g, 4.89 mmol) and **7**·$(HCOOH)_2$ (1.7 g, 4.89 mmol) was added BOP reagent (2.16 g, 4.89 mmol) followed by the addition of Et_3N (2.27 mL, 19.55 mmol). The reaction was continued for 12 h at room temperature and then quenched with a saturated solution of NaCl. The product was extracted with ethyl acetate. The combined organic layer was washed successively with 5% citric acid, water, 5% $NaHCO_3$, and water. The organic layer was dried (Na_2SO_4) and evaporated to provide the crude product. Purification was achieved by silica gel column chromatography with 2% MeOH/$CHCl_3$ as the eluant (R_f = 0.4) to give the pure product as a viscous liquid. Yield (Boc-**8**): 2.22 g (77%). ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 1.41−1.57 (m, 22H), 1.67−1.72 (m, 2H), 3.22−3.30 (m, 3H), 3.42−3.48 (m, 2H), 3.67 (m, 4H), 3.66−3.82 (m, 9H), 4.17−4.20 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.87, 28.37, 28.39, 29.71, 39.19, 39.24, 51.59, 51.86, 52.49, 64.74, 170.57, 171.65, 171.93, 173.12, 173.15.

The di-Boc protected compound (Boc-**8**, 2 g, 3.40 mmol) was dissolved in cold trifluoroacetic acid (0−5 °C, 10 mL) and stirred at room temperature for 2.5 h. TFA was evaporated to dryness in high vacuo. To remove the residual TFA, the viscous oil was repeatedly treated with dry CH_2Cl_2 (10 × 2 mL per batch) and the dichloromethane was removed under high vacuo. The resultant oily liquid was quite pure and was taken to the next

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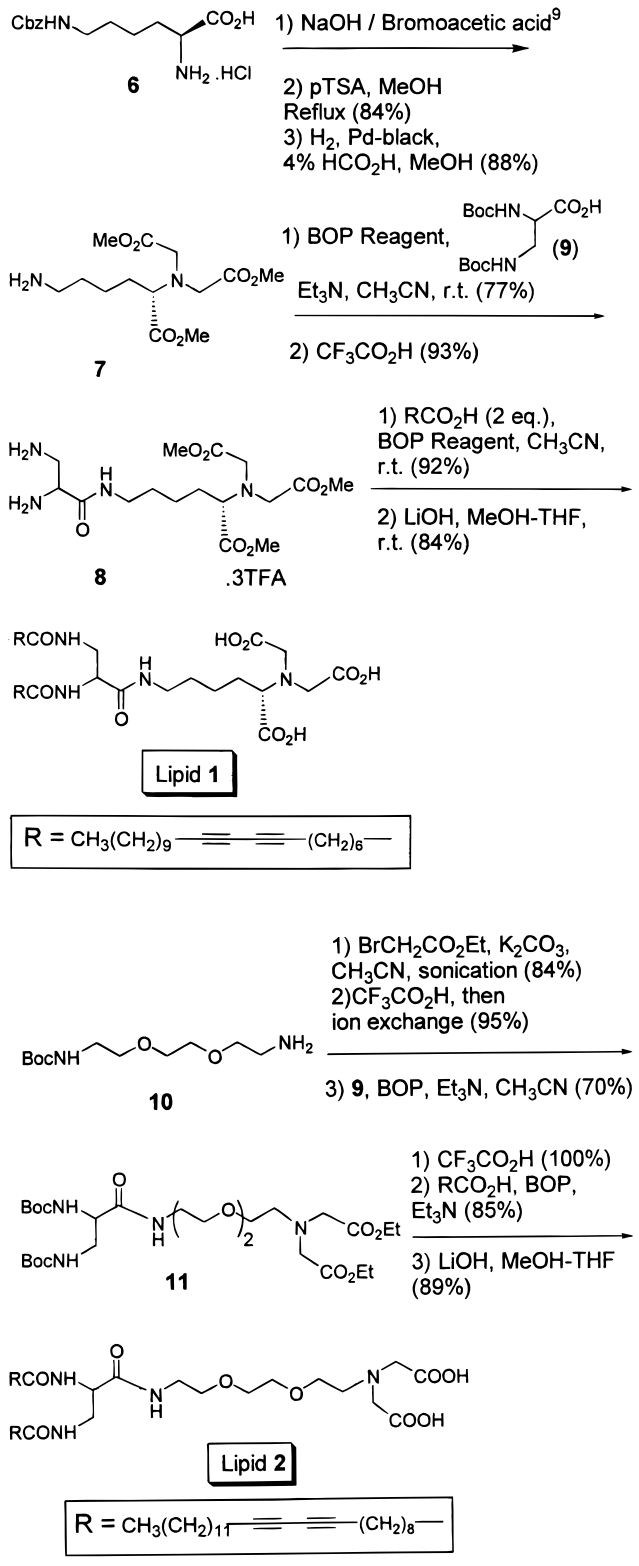
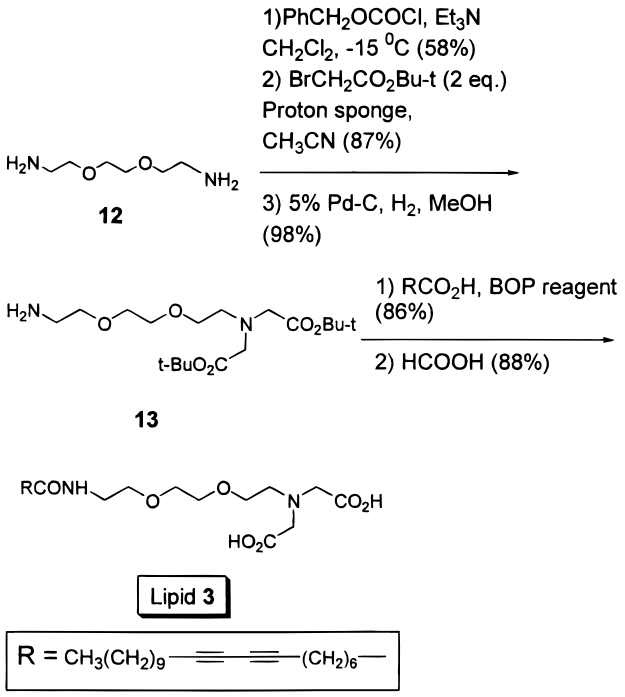
Scheme 1. Synthesis of the Polymerizable Metal-Chelating Lipids 1 and 2



step. Yield (**8**): 2.30 g (93%). ^{1}H NMR (400 MHz, DMSO-d_6/D_2O) δ1.23−1.47 (m, 4H), 1.58−1.62 (m, 2H), 3.00−3.09 (m, 1H), 3.13−3.32 (m, 3H), 3.39 (t, 1H, J = 7.2 Hz), 3.55−3.61 (m, 13H), 4.05−4.08 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 22.72, 23.00, 29.41, 39.55, 50.63, 50.76, 50.91, 52.18, 52.23, 172.03, 173.29, 173.31.

2-Bis((carboxymethyl)amino)-6-(2,3-bis(8′10′-heneicosadiynoylamino)propanoylamino)hexanoic Acid (Lipid 1). Under nitrogen, the above-mentioned amine salt (**8**, 0.62 g, 0.86 mmol) and polymerizable acid (8,10-heneicosadiynoic acid,

Scheme 2. Synthesis of the Lipid 3

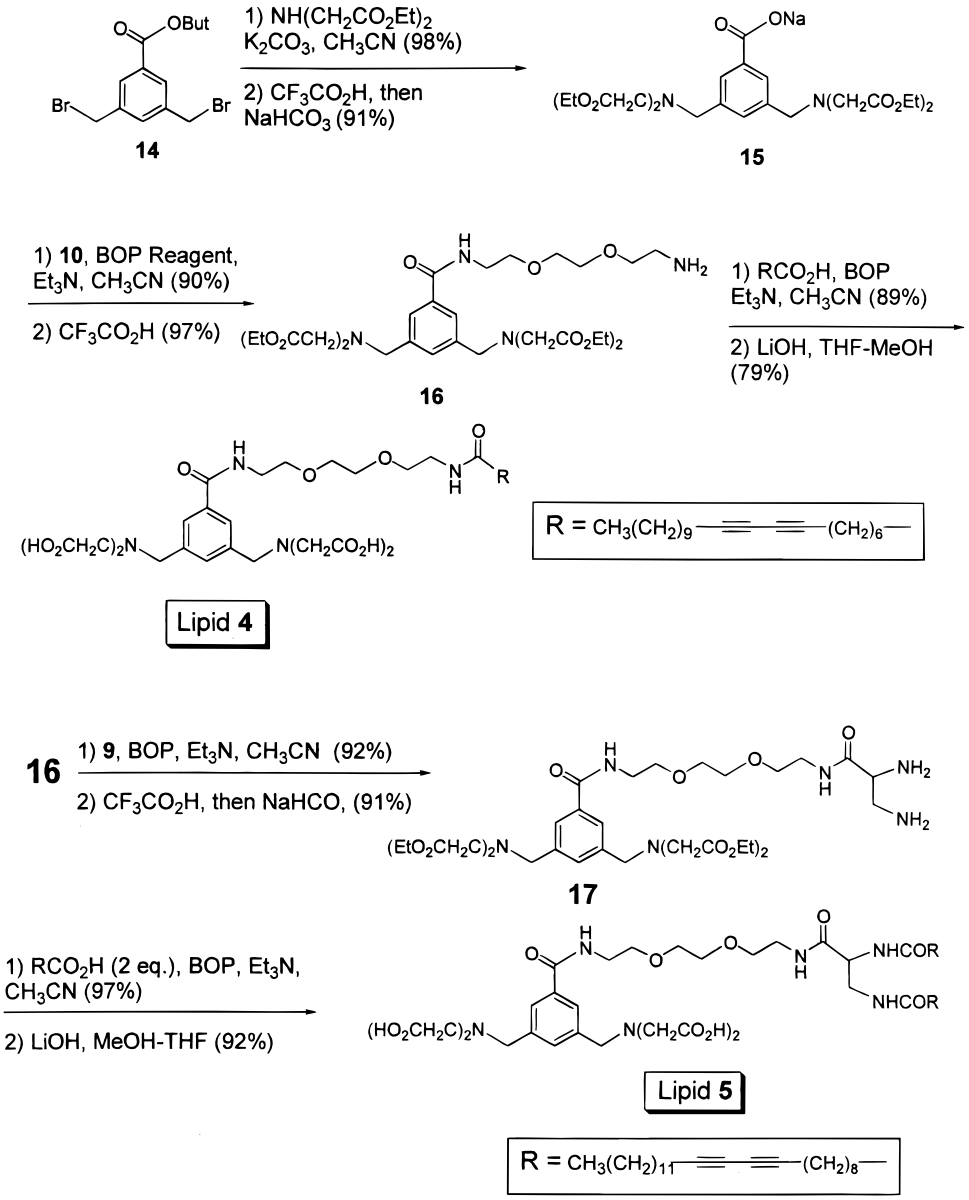


0.55 g, 1.73 mmol) were dissolved in MeCN (30 mL) in the presence of Et_3N (1.2 mL). BOP reagent (0.76 g, 1.73 mmol) was added, and the reaction was stirred for 24 h at room temperature. The workup procedure was same as described for **8**. The crude product was purified by column chromatography (silica gel, 3% MeOH/$CHCl_3$, R_f = 0.4). Yield (ester-**1**): 0.70 g (92%). ^{1}H NMR (400 MHz, $CDCl_3$/D_2O): δ 0.85 (t, 6H, J = 6.8 Hz), 1.22−1.39 (m, 36H), 1.48−1.52 (m, 10H), 1.53−1.65 (m, 6H), 2.15−2.25 (m, 12H), 3.19−3.26 (m, 2H), 3.37−3.41 (m, 2H), 3.59−3.63 (m, 5H), 3.66−3.69 (m, 10H), 3.71−3.74 (m, 1H), 4.38−4.42 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.20, 19.22, 19.28, 22.75, 24.73, 25.40, 25.66, 28.12, 28.20, 28.27, 28.42, 28.54, 28.56, 28.65, 28.91, 28.94, 29.17, 29.38, 29.56, 29.66, 31.91, 33.48, 36.48, 51.61, 51.87, 52.53, 64.24, 65.22, 65.48, 77.24, 77.76, 77.79, 170.16, 172.00, 174.59, 176.94.

A solution of lithium hydroxide (0.32 g, 7.7 mmol) in 1:1 MeOH/THF (30 mL) was added to a solution of the trimethyl ester (ester-**1**, 0.85 g, 0.86 mmol) at 5 °C under nitrogen. The reaction was stirred at room temperature for 24 h. The solvents were evaporated in vacuo, and the white solid was dissolved in water and acidified to pH = 3.0 with 1 N HCl. The white precipitate was filtered and washed with water (250 mL) to afford the product **1** as a waxy white solid. Yield (lipid-**1**): 710 mg (84%). ^{1}H NMR (400 MHz, DMSO-d_6/D_2O): δ 0.85 (t, 6H, J = 6.6 Hz), 1.24−1.31 (m, 42H), 1.42−1.46 (m, 14H), 2.03 (t, 2H, J = 7.1 Hz), 2.10 (t, 2H, J = 7.1 Hz), 2.25−2.29 (m, 8H), 2.95−3.05 (m, 1H), 3.25−3.33 (m, 2H), 3.40−3.59 (m, 4H), 4.25−4.31 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.51, 18.86, 22.67, 23.65, 24.92, 25.51, 25.68, 28.24, 28.27, 28.30, 28.60, 28.64, 28.66, 28.76, 28.97, 29.00, 29.25, 29.47, 29.51, 29.93, 31.87, 35.88, 54.53, 55.12, 65.91, 65.93, 78.41, 78.46, 170.17, 172.68, 173.27, 173.92, 174.45. FAB m/z (M + H) calcd for $C_{55}H_{88}N_4O_9$ 949.5, found 949.5. Anal. Calcd for $C_{55}H_{88}N_4O_9$·HCl: C, 67.01; H, 9.10; N, 5.68. Found: C, 66.79; H, 8.75; N, 5.46.

Ethyl 2-(2-(2-(2-(2,3-bis((*tert*-butoxy)carbonylamino)-propanoylamino)ethoxy)ethoxy)ethyl)((ethoxycarbonyl)-methyl)amino)acetic Acid (11). The mono-Boc-protected amine **10**[16] (2.38 g, 10.44 mmol), ethylbromoacetate (3.48 g, 20.88 mmol), and anhydrous K_2CO_3 were mixed in MeCN, and the resulting mixture was sonicated (125 W bath sonicator) at room temperature for 12 h. Suspended solids were filtered off, and solvent was removed under vacuo. Crude viscous oil was purified by silica gel column chromatography (2% MeOH/$CHCl_3$, R_f = 0.4) to afford the pure product. Yield (Boc-**11**): 3.60 g (82%). ^{1}H NMR (400 MHz, $CDCl_3$/D_2O): δ 1.18 (t, 6H, J = 7.0 Hz), 1.35 (s, 9H), 2.89 (t, 2H, J = 5.5 Hz), 3.20−3.24 (m, 2H), 3.42−3.46

Scheme 3. Synthesis of the Lipids 4 and 5



(m, 2H), 3.50 (s, 4H), 3.52−3.58 (m, 6H), 4.15 (q, 4H, J = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.26, 28.42, 40.36, 53.55, 55.84, 60.47, 70.17, 70.21, 70.24, 70.33, 79.05, 156.07, 171.35.

The Boc group of the amine ester (Boc-**11**, 2.22 g, 5.28 mmol) was cleaved with TFA (10 mL). The workup procedure was the same as described for **8**. The free amine was generated from the TFA salt by passing through a weakly basic anion-exchange column (Dowex 66) and eluting with methanol. Eluant was collected until pH = 7.0, and methanol was evaporated in vacuo to afford the free amine. Yield (*t*-Bu-**11**): 2.88 g (100%). ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 1.27 (t, 6H, J = 7.2 Hz), 3.21−3.26 (m, 2H), 3.61−3.68 (m, 6H), 3.75 (t, 2H, J = 4.8 Hz), 3.81−3.86 (m, 2H), 4.14 (q, 4H, J = 7.2 Hz), 4.30 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.7, 40.2, 54.0, 55.82, 63.1, 65.3, 66.5, 70.3, 70.4, 166.0.

The coupling of amine (*t*-Bu-**11**, 2.25 g, 4.12 mmol) and acid **9** (1.25 g, 4.12 mmol) was carried out with BOP reagent (1.82 g, 4.12 mmol) in the presence of excess Et_3N (5.81 g, 57.5 mmol) in MeCN. The workup procedure was the same as **8**. The crude oily liquid was purified by silica gel column chromatography (5% MeOH/$CHCl_3$, R_f = 0.4) to give compound **11** as viscous oily liquid. Yield (**11**): 2.46 g, (98%). ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 1.21 (t, 6H, J = 7.2 Hz), 1.37 (s, 18H), 3.00 (t, 2H, J = 5.2 Hz), 3.35−3.41 (m, 3H), 3.42−3.49 (m, 3H), 3.51−3.54 (m, 4H), 3.59 (t, 2H, J = 5.2 Hz), 3.64 (s, 4H), 4.09−4.19 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.21, 23.03, 23.75, 28.34, 28.39, 28.91, 30.45, 38.72, 39.18, 53.75, 55.37, 60.92, 69.67, 69.75, 70.34, 170.57, 170.61, 170.93.

2-((2-(2-(2-(2,3-Bis(10′,12′-pentacosadiynoylamino)propanoylamino)ethoxy)ethoxy)ethyl)(carboxymethyl)amino)-acetic Acid (Lipid 2). The di-Boc-protected compound **11** (2.00 g, 3.30 mmol) was cleaved with TFA (8 mL). The workup procedure was same as described for **8**. The crude product was used in the next step without any further purification. Yield (TFA-**3**): 2.40 g (97%). ^{1}H NMR (400 MHz, DMSO-d_6/D_2O): δ 1.23 (t, 6H, J = 7.0 Hz), 3.19 (t, 2H, J = 5.0 Hz), 3.24−3.30 (m, 3H), 3.32−3.39 (m, 1H), 3.46−3.50 (m, 6H), 3.63 (t, 2H, J = 5 Hz), 3.94 (s, 4H), 4.10−4.20 (m, 5H).

The coupling of amine (TFA-**3**) salt (0.7 g, 0.94 mmol) and 10,12-pentacosadiynoic acid (0.7 g, 1.88 mmol) was achieved with BOP reagent (0.83 g, 1.88 mmol) in the presence of excess Et_3N (1.5 mL, 9.42 mmol) in MeCN/$CHCl_3$ (1:1). The workup procedure is the same as described for **8**. The crude product was purified by silica gel column chromatography with 4% MeOH in $CHCl_3$ (R_f = 0.4) to give a colorless waxy semisolid. Yield (ester-**2**): 0.95 g (90%). ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 0.82 (t, 6H, J = 7.0 Hz), 1.05−1.25 (m, 50H), 1.27−1.35 (m, 8H), 1.40−1.48 (m, 8H), 1.51−1.56 (m, 4H), 2.10−2.19 (m, 12H), 2.93 (t, 2H, J = 5.4 Hz), 3.32−3.39 (m, 2H), 3.42−3.48 (m, 4H), 3.52 (s, 4H), 3.54−3.58 (m, 6H), 4.08−4.13 (m, 4H), 4.39−4.43 (m,

1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.17, 14.30, 19.22, 19.23, 22.73, 25.55, 25.69, 28.36, 28.38, 28.40, 28.84, 28.90, 29.00, 29.14, 29.24, 29.26, 29.30, 29.38, 29.52, 29.65, 29.66, 29.69, 31.95, 36.49, 36.52, 39.31, 42.25, 53.67, 54.83, 55.84, 60.62, 65.28, 65.30, 65.36, 65.38, 69.59, 70.20, 70.25, 70.29, 77.37, 77.40, 77.57, 77.59, 170.22, 171.40, 174.31, 175.23.

Selective hydrolysis of the ester (0.4 g, 0.38 mmol) was carried out with lithium hydroxide (0.08 g, 1.90 mmol) in THF−MeOH mixture. After standard workup (same as lipid **1**), a white waxy solid of lipid **2** was obtained. Yield 0.34 g (89%). ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 0.85 (t, 6H, J = 7.0 Hz), 1.18−1.29 (m, 44H), 1.32−1.38 (m, 8H), 1.44−1.52 (m, 8H), 1.54−1.59 (m, 4H), 2.14−2.23 (m, 12H), 3.33−3.38 (m, 2H), 3.42−3.45 (m, 2H), 3.50−3.55 (m, 4H), 3.57−3.61 (m, 5H), 3.80−3.85 (m, 2H), 4.15 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.23, 19.29, 22.78, 25.62, 25.74, 28.44, 28.45, 28.94, 28.95, 29.08, 29.20, 29.30, 29.35, 29.44, 29.58, 29.71, 29.73, 29.74, 36.47, 36.53, 54.53, 65.29, 65.37, 69.65, 70.07, 77.32, 77.51, 77.68, 77.70, 170.29, 170.76, 174.92, 175.56. FAB m/z (M + H): calcd for $C_{63}H_{106}N_4O_9$ 1063.7, found 1063.7. Anal. Calcd for $C_{63}H_{106}N_4O_9$: C, 71.15; H, 10.05; N, 5.27. Found: C, 70.79; H, 9.99; N, 5.12.

***tert*-Butyl 2-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)((*tert*-butyloxycarbonyl)methylamino) acetate (13).** Excess diamine **12** (40 g, 270 mmol) and Et_3N (11 mL, 77 mmol) were dissolved in chloroform (100 mL) and cooled to −45 °C. Cbz chloride (13.13 g, 77 mmol in 70 mL of $CHCl_3$) was added by syringe pump over 3 h. The resulting solution was stirred at room temperature for 12 h, and the organic layer was washed with water, dried (Na_2SO_4), and then evaporated. The crude product was purified by silica gel column chromatography (20% MeOH/$CHCl_3$, R_f = 0.2) to afford the pure compound as a colorless viscous oil. Yield (Cbz-**13**): 12.61 g (58%). ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 2.82 (t, 2H, J = 5.1 Hz), 3.35−3.39 (m, 2H,), 3.47 (t, 2H, J = 5.1 Hz), 3.53−3.66 (m, 6H), 5.07 (s, 2H), 7.31−7.34 (m, 5H).

To a solution of mono-Cbz-diamine (4.00 g, 15.02 mmol) and Proton Sponge (6.42 g, 30.00 mmol) in $CHCl_3$ (30 mL) at 5 °C was added dropwise a solution of *tert*-butyl bromoacetate in $CHCl_3$. After 12 h, the precipitate was filtered, and the solvent was removed in vacuo. The solid was suspended in AcOEt, cooled, and filtered. The AcOEt layer was washed with water, and the crude product was purified by silica gel column chromatography (with 17% MeOH/chloroform, R_f = 0.3) to afford the pure compound as a viscous oil. Yield (*t*-Bu-ester-**13**): 6.41 g (87%). ^{1}H NMR (270 MHz, $CDCl_3/D_2O$): δ 1.42 (s, 18H), 2.92 (t, 2H, J = 5 Hz), 3.34−3.40 (m, 2H), 3.46 (s, 4H), 3.51−3.62 (m, 8H), 5.08 (s, 2H), 7.34 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 41.1, 53.4, 56.8, 66.7, 70.2, 70.3, 70.5, 70.6, 81.0, 128.2, 128.6, 128.6, 136.8, 156.8, 171.0.

This compound from the previous reaction (*t*-Bu-ester-**13**) was dissolved in 60 mL of MeOH/H_2O (20:1), a spatula tip of 5% wet Pd/C was added, and H_2 was bubbled. Total removal of the Cbz group was achieved after 6 h as monitored by TLC (same solvent as the reaction). The catalyst was filtered, and the solvent was removed in a rotary evaporator. The solid was recrystallized from hexane−chloroform mixture to provide compound **13** as a white solid. Yield (**13**): 2.78 g (98%); mp: 60−61 °C. ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 1.42 (s, 18H), 2.89 (t, 2H, J = 4.6 Hz), 3.15 (t, 2H, J = 4.8 Hz), 3.51−3.55 (m, 6H), 3.65 (t, 2H, J = 4.8 Hz), 3.74 (t, 2H, J = 4.8 Hz), 3.94 (t, 2H, J = 4.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.2, 40.1, 53.4, 56.0, 67.6, 68.0, 70.4, 70.6, 81.8, 171.0.

2-((Carboxymethyl)(2-(2-(2-(8′,10′-heneicosadiynoylamino)ethoxy)ethoxy)ethyl)amino)acetic Acid (Lipid 3). The coupling of amine **13** (0.93 g, 2.51 mmol) and polymerizable acid (8,10-heneicosadiynoic acid, 0.80 g, 2.51 mmol) was achieved with BOP reagent (1.11 g, 2.51 mmol) as described for **8**. The crude product was purified by silica gel column chromatography (with 2% MeOH/chloroform, R_f = 0.4) to give a waxy solid. Yield (ester-**3**): 1.40 g (86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, 3H, J = 6.8 Hz), 1.21−1.34 (broad s, 18H), 1.44−1.51 (m, 22H), 1.53−1.62 (m, 2H), 2.14−2.24 (m, 6H), 2.94 (t, 2H, J = 5.9 Hz), 3.43 (t, 2H, J = 5.0 Hz), 3.46 (s, 4H), 3.51 (t, 2H, J = 4.8 Hz), 3.58−3.60 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.21, 19.18, 19.27, 22.76, 24.10, 25.65, 28.10, 28.22, 28.39, 28.40, 28.41, 28.64, 28.82, 28.93, 29.17, 29.39, 29.56, 29.64, 31.97, 35.23, 36.58, 39.25, 65.20, 65.28, 65.53, 70.17, 70.21, 70.39, 77.17, 77.79, 159.50, 173.80

The ester (ester-**3**, 0.25 g, 0.37 mmol) was dissolved in 88% cold formic acid (5 mL) and stirred for 48 h under nitrogen at room temperature. Formic was evaporated in high vacuo, the resultant waxy solid was repeatedly treated with dry CH_2Cl_2 (10 × 2 mL each time), and the dichloromethane was removed under vacuo. The product was obtained as a yellowish semisolid. Yield (lipid-**3**·formate): 0.18 g (88%). ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 0.86 (t, 3H, 7.0 Hz), 1.17−1.42 (m, 20H), 1.47−1.65 (m, 6H), 2.15−2.33 (m, 10H), 3.42−3.46 (m, 2H), 3.53−3.64 (m, 8H), 8.05 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.22, 19.20, 19.26, 22.76, 24.68, 25.75, 28.19, 28.26, 28.42, 28.45, 28.53, 28.64, 28.71, 28.93, 28.97, 29.19, 29.39, 29.58, 29.65, 31.95, 34.05, 65.23, 65.29, 65.48, 69.82, 69.94, 70.02, 77.37, 77.77, 168.64, 174.93, 178.39. FAB m/z (M + H) calcd for $C_{31}H_{52}N_2O_7$ 581.4, found 581.4. Anal. Calcd for $C_{31}H_{52}N_2O_7$: C, 65.93; H, 9.28; N, 4.96. Found: C, 65.69; H, 9.18; N, 4.70.

Sodium 3,5-Bis(bis((ethoxycarbonyl)methyl)aminomethyl)benzoate (15). *tert*-Butyl 3,5-bis(bromomethyl)benzoate[18] (1.21 g, 3.28 mmol) and diethyl iminodiacetate (1.25 g, 6.61 mmol) were dissolved in MeCN, and excess K_2CO_3 (4.6 g, 32.87 mmol) was added. The resulting reaction mixture was sonicated (125 W bath sonicator) overnight at room temperature. Solids were filtered, and solvent was removed in vacuo. The crude oily product was purified via silica gel column chromatography (2% MeOH/$CHCl_3$, R_f = 0.3). Yield (*t*-Bu-ester-**15**): 1.89 g (98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, 12H, J = 10.4 Hz) 1.57 (s, 9H), 3.54 (s, 8H), 3.95 (s, 4H), 4.15 (q, 8H, J = 10.4 Hz), 7.62 (s, 1H), 7.86 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3, 28.2, 54.3, 57.5, 60.5, 76.9, 77.2, 81.0, 129.2, 132.4, 133.8, 138.8, 165.8, 171.0.

The ester (*t*-Bu-ester-**15**, 1.26 g, 2.17 mmol) was dissolved in 10 mL of TFA and stirred at room temperature for 2.5 h. TFA was evaporated in vacuo. It was then dissolved in $CHCl_3$ (30 mL) and stirred with an aqueous 5% solution of $NaHCO_3$ for 0.5 h. The organic layer was washed with water, dried (Na_2SO_4), and evaporated to give compound **15**, which was used in the next step without any further purification. Yield (**15**): 1.08 g (91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (t, 12H, J = 7.1 Hz), 3.56 (s, 8H), 3.98 (s, 4H), 4.07 (q, 8H, J = 7.1 Hz) 7.65 (s, 1H), 7.80 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2, 54.2, 57.6, 60.5, 129.7, 130.9, 134.3, 138.8, 169.7, 171.0.

Ethyl 2-(((5-(*N*-(2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamoyl)-3-((bis(ethoxycarbonyl) methylamino)methyl)-phenyl)methyl)(ethoxycarbonyl)methylamino)acetate (16). The coupling reaction between **15** (1.35 g, 1.82 mmol) and amine **10** (0.45 g, 1.82 mmol) was achieved with BOP reagent (1.09 g, 2.47 mmol) using Et_3N (0.7 mL) as a base and MeCN (30 mL) as the solvent. The workup procedure was the same as **8**. The crude product was purified with column chromatography (silica gel, 5% MeOH/$CHCl_3$, R_f = 0.5) to afford the pure compound as a viscous oil. Yield (Boc-**16**): 1.74 g (90%). ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 1.24 (t, 12H, J = 7.3 Hz), 1.41 (s, 9H), 3.28−3.30 (m, 2H), 3.50−3.54 (m, 10H), 3.63 (s, 4H), 3.65 (s, 4H), 3.90 (s, 4H), 4.13 (q, 8H, J = 7.3 Hz), 7.47 (s, 1H), 7.82 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3, 28.4, 28.5, 40.3, 54.3, 57.7, 60.6, 70.2, 70.3, 127.2, 132.6, 135.1, 138.8, 156.0, 171.1.

The Boc group of Boc-protected amine-ester (Boc-**16**, 1.60 g, 2.12 mmol) was cleaved with TFA (5 mL) using the same procedure as **15**. The resultant TFA salt was treated with 4% aqueous $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was dried and evaporated to afford the pure **16** as a viscous pale yellow liquid. Yield (**16**): 1.35 g (97%). ^{1}H NMR (400 MHz, $CDCl_3/D_2O$): δ 1.26 (t, 12H, J = 7.1 Hz), 3.17 (t, 2H, J = 4.7 Hz), 3.63−3.71 (m, 10H), 3.88 (s, 8H), 4.19 (q, 8H, J = 7.1 Hz), 4.31 (s, 4H), 7.67 (s, 1H), 8.01 (s, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 13.01, 53.69, 58.27, 58.30, 61.82, 66.53, 69.98, 129.20, 132.05, 136.02, 138.11, 159.77, 168.26.

2-((3-((Bis(carboxymethyl)amino)methyl)-5-(*N*-(2-(2-(2-(8′,10′-heneicosadiynoylamino)ethoxyethoxy)ethyl)carbamoyl)phenyl)methyl)(carboxymethyl)amino)acetic Acid (Lipid 4). Polymerizable acid (8,10-heneicosadiynoic acid, 0.226 g, 0.71 mmol) and free amine **16** (0.46 g, 0.71 mmol) were coupled with BOP reagent (0.315 g, 0.712 mmol) in the presence of Et_3N (7.12 mmol). After standard workup (same as lipid **3**), the crude

product was purified by column chromatography (silica gel, 2% MeOH/$CHCl_3$, $R_f = 0.3$) to give the pure amide as a waxy white solid. Yield (ester-**4**): 0.55 g (89%). ^{1}H NMR (400 MHz, $CDCl_3$/D_2O): δ 0.86 (t, 3H, $J = 6.8$ Hz), 1.22−1.36 (m, 34H), 2.02−2.29 (m, 8H), 3.41−3.43 (m, 2H), 3.53−3.57 (m, 8H), 3.64−3.67 (m, 10H), 4.00 (s, 4H), 4.15 (q, 8H, $J = 6.8$ Hz), 7.54 (s, 1H), 7.85 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.21, 19.25, 19.39, 22.76, 24.68, 25.05, 28.21, 28.41, 28.43, 28.50, 28.56, 28.61, 28.63, 28.65, 28.94, 28.96, 29.18, 29.39, 29.56, 29.66, 30.64, 31.22, 31.97, 33.32, 65.22, 65.25, 65.42, 77.31, 129.64, 133.48, 134.73, 141.78, 152.66, 158.09, 173.43.

Selective hydrolysis of the ester groups (ester-**4**, 0.52 g, 0.58 mmol) was carried out with LiOH (0.29 g, 7.0 mmol) in THF/H_2O as described for lipid **1**. Lipid **4** was obtained as a waxy white solid. Yield (**4**): 0.35 g (79%). ^{1}H NMR (400 MHz, DMSO-d_6/D_2O): δ 0.84 (t, 3H, $J = 4.0$ Hz), 1.20−1.29 (m, 16H), 1.39−1.45 (m, 6H), 2.02 (t, 2H, $J = 7.2$ Hz), 2.17−2.25 (m, 6H), 3.16 (t, 2H, $J = 5.6$ Hz), 3.34−3.40 (m, 12H), 3.47−3.51 (m, 6H), 3.87 (s, 4H), 7.46 (s, 1H), 7.67 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.52, 18.80, 18.83, 22.66, 25.64**,** 28.19, 28.25, 28.53, 28.60, 28.74, 28.96, 29.22, 29.44, 29.48, 31.86, 35.78, 38.99, 54.02, 57.50, 65.89, 69.48, 69.72, 70.10, 78.49, 78.57, 127.08, 132.31, 135.11, 139.42, 167.01, 172.75, 172.78. FAB *m*/*z* (M + H) calcd for $C_{44}H_{66}N_4O_{12}$ 843.4, found 843.4. Anal. Calcd for $C_{44}H_{66}N_4O_{12}$: C, 62.69; H, 7.89; N, 6.65. Found: C, 62.40; H, 7.87; N, 6.50.

Ethyl 2-(5-(*N*-(2-(2-(2-(2,3-Diaminopropanoylamino)-ethoxy)ethyl)carbamoyl)-3-bis (ethoxycarbonyl)methyl)-amino)acetate (17). Coupling of amine **16** (1.30 g, 1.98 mmol) and acid **9** (0.61 g, 1.98 mmol) was carried out with BOP reagent (0.88 g, 1.98 mmol). The crude product was purified by silica gel column chromatography (2% MeOH/$CHCl_3$, $R_f = 0.4$) to afford the amide as a colorless viscous liquid. Yield (Boc-**17**): 1.72 g (92%). ^{1}H NMR (400 MHz, $CDCl_3$/D_2O): δ 1.22 (t, 12H, $J = 7.1$ Hz), 1.37 (s, 18H), 3.31−3.39 (m, 3H), 3.41−3.51 (m, 11H), 3.56−3.63 (m, 8H), 3.87 (s, 4H), 4.08−4.16 (m, 9H), 7.45 (s, 1H), 7.78 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.28, 28.34, 28.36, 39.28, 39.89, 42.67, 54.31, 57.70, 60.60, 69.64, 70.02, 70.30, 70.34, 76.32, 126.91, 132.51, 134.99, 138.93, 167.68, 170.71, 171.11.

The Boc groups (Boc-**17**, 1.65 g, 1.76 mmol) were cleaved with TFA (8 mL). After evaporation of TFA, the resultant viscous oil was dissolved in excess $CHCl_3$, washed with 4% $NaHCO_3$ solution, dried, and evaporated to afford compound **17** as a viscous yellow liquid. Yield (**17**): 1.2 g (92%). ^{1}H NMR (400 MHz, $CDCl_3$/D_2O): δ 1.24 (t, 12H, $J = 7.2$ Hz), 3.42−3.46 (m, 3H), 3.49 (s, 9H), 3.56 (t, 2H, $J = 5.1$ Hz), 3.61−3.67 (m, 8H), 3.89 (s, 4H), 4.00−4.16 (m, 9H), 7.46 (s, 1H), 7.81 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.25, 38.64, 38.76, 39.82, 39.94, 54.35, 57.74, 60.64, 69.68, 70.15, 70.17, 70.24, 77.38, 127.07, 132.43, 134.99, 138.86, 167.67, 167.75, 171.24.

2-(((3-(Bis(carboxymethylamino)methyl)-5-(*N*-(2-(2-(2-(2,3-bis-(10′,12′-pentacosadiynoylamino)propanoylamino)ethoxy)-ethoxy)ethyl)carbamoyl)phenyl)methyl)(carboxymethyl)-amino)acetic Acid (Lipid 5). The polymerizable acid (10,12-pentacosadiynoic acid, 0.35 g, 0.47 mmol) and the amine **17** (0.35 g, 0.47 mmol) were coupled with BOP reagent (0.47 g, 47 mmol) in the presence of Et_3N (0.3 mL, 14.10 mmol), as described for lipid **3**. The crude product was purified by column chromatography (silica gel, 5% MeOH/$CHCl_3$, $R_f = 0.6$), and the pure product was obtained as a waxy white solid. Yield (ester-**5**): 0.67 g (97%). ^{1}H NMR (400 MHz, $CDCl_3$/D_2O): δ 0.84 (t, 6H, $J = 7.0$ Hz), 1.15−1.24 (m, 56H), 1.30−1.35 (m, 8H), 1.40−1.48 (m, 8H), 1.53−1.56 (m, 4H), 2.11−2.21 (m, 12H), 3.34−3.39 (m, 2H), 3.44−3.51 (m, 11H), 3.55−3.65 (m, 9H), 3.92 (s, 4H), 4.11 (q, 8H, $J = 7.0$ Hz), 4.34−4.38 (m, 1H),7.49 (s, 1H), 7.75 (s,2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.18, 14.28, 19.23, 19.25, 22.74, 25.55, 25.69, 28.37, 28.41, 28.84, 28.91, 29.00, 29.15, 29.23, 29.26, 29.29, 29.39, 29.53, 29.66, 29.67, 29.69, 31.97, 36.50, 36.56, 39.22, 39.92, 42.15, 43.35, 54.30, 55.18, 57.70, 60.62, 65.28, 65.36, 65.38, 69.56, 70.04, 70.32, 70.35, 77.35, 77.63, 126.95, 132.55, 134.94, 138.84, 167.67, 170.22, 171.08, 174.48, 175.52.

Selective hydrolysis of the ester groups (ester-**5**, 0.16 g, 0.117 mmol) was carried out with LiOH (0.04 g, 0.95 mmol) in 4 mL of THF−MeOH at room temperature. The workup procedure was same as that described for lipid **1**. The lipid **5** was a waxy white solid. Yield (**5**): 0.128 g (92%). ^{1}H NMR (400 MHz, DMSO-d_6/D_2O): δ 0.85 (t, 6H, $J = 6.8$ Hz), 1.20−126 (m, 44H), 1.28−1.31 (m, 8H) 1.38−1.46 (m, 12H), 2.02 (t, 2H, $J = 7.4$ Hz), 2.09 (t, 2H, $J = 7.4$ Hz), 2.25 (t, 8H, $J = 6.9$ Hz), 3.16−3.21 (m, 2H), 3.22−3.29 (m, 3H), 3.34−3.44 (m, 9H), 3.48−3.53 (m, 8H), 3.87 (s, 4H), 4.27−4.31 (m, 1H), 7.48 (s, 1H), 7.69 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.54, 18.79, 22.70, 25.65, 25.83, 28.28, 28.36, 28.75, 28.81, 28.84, 28.95, 28.97, 28.99, 29.22, 29.24, 29.31, 29.32, 29.33, 29.47, 29.56, 29.61, 31.89, 54.05, 57.54, 65.91, 69.42, 69.45, 69.51, 70.10, 78.49, 127.13, 127.66, 135.15, 139.44, 172.75, 172.79, 172.81. FAB *m*/*z* (M + H) calcd for $C_{76}H_{120}N_6O_{14}$ 1341.9, found 1341.9. Anal. Calcd for $C_{76}H_{120}N_6O_{14}$·HCl: C, 66.23; H, 8.84; N, 6.09. Found: C, 66.56; H, 8.64; N, 6.20.

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