Anionic Amphiphilic Dendrimers as Antibacterial Agents

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Materials and Methods

Endothelial Cell (HUVEC) Culture

Human umbilical vein endothelial cells were purchased from Cambrex Corp. (East Rutherford, NJ) and used between passages 3 and 6 for all experiments. Cells were maintained in M199 medium (Mediatech, Inc.; Manasas, VA) with 20% (v/v) fetal bovine serum (BioWhittaker, Inc.; Walkersville, MD) and 1% (v/v) glutamine-penicillin-streptomycin (ATCC; Manassas, VA) and containing 4000 Units/mL heparin (Sigma-Alderich; St. Louis, MO) and 2.5 mg/mL endothelial mitogen (Biomedical Technologies, Inc.; Stoughton, MA). Cells were maintained at all times in a humidified incubator at 37 °C and 5% CO_2 when not under direct experimentation.

Bacterial (B. subtilis) Culture

Wild type *Bacillus subtilis* AG174 (JH642) cultures (donated by the Collins lab at Boston University) used in gram-positive bacterial viability tests were grown in Luria-Bertani broth (LB) (Fisher Scientific; Pittsburg, PA) and incubated at 37 °C. Prior to experimentation, cultures were allowed to saturate for 24 hours.

Endothelial (HUVEC) Cytotoxicity

5000 HUVEC were placed into each well of a 96-well tissue culture polystyrene plate (Corning Life Sciences; Lowell, MA) containing 100 µL of medium and left to equilibrate overnight. The next day the medium was removed and 100 µL of fresh medium containing varied concentrations of SDS, Triton X-100, dendrimers **1** and **2**, glycerol, succinic acid, and myristic acid was added to the wells and incubated for 24 hours. Following the exposure the liquid was removed and 100 µL fresh medium containing 20 µL of an MTS tetrazolium proliferation assay (Promega Corp.; Madison, WI). After an additional hour subsequent incubation the absorbance at 492 nm was read on an AD 340C platereader (Beckman Coulter, Inc.; Fullerton, CA). Viabilities were calculated (equation 1) by subtracting the background absorbance reading for blank media with MTS from all values and then dividing the experimental value by the untreated control reading. EC_{50} values were calculated by fitting a 4-parameter sigmoidal curve to the dataset and reporting the inflection point.

$$\%\,\text{Control Absorbance} = \left(\frac{Unknown - Background}{Neg.Control - Background} \right) * 100\% \qquad \textit{(equation 1)}$$

Bacterial (B. subtilis) Cytotoxicity

Solutions of LB media doped with varied concentrations of the compounds under investigation as well as an untreated negative control were prepared. Prior to inoculation, *B.subtilis* cultures in early log phase growth were diluted 1:1000 into fresh LB broth. These diluted cultures were inoculated into each of the test solutions, in triplicate, to a final volume of 80 µL in a 384 well clear polystyrene plate (NUNC; Rochester, NY). Following inoculation, samples were incubated at 37°C for 9 hours. The *B.subtilis* growth was then measured by taking an absorbance reading at 600 nm on a Spectrafluor Plus platereader with appropriate filters (Tecan; Durham NC). Viabilities were calculated (equation 1) by subtracting the background absorbance reading for blank medium from all values and then by dividing the experimental

value by the untreated control reading. EC_{50} values were calculated by fitting a 4-parameter sigmoidal curve to the dataset and reporting the inflection point.

CAC Measurements:

The critical aggregation concentration (cac) for compounds **1** and **2** was determined using a tensiometric technique at 24 ^{o}C. The data were obtained using a Kruss K12 Tensiometer in conjunction with a Metrohm Desimate 665. Specifically, the surface tension (σ, mN/m) was plotted as a logarithmic function of surfactant concentration in PBS buffer (8.3 mmol, pH = 7.2) and a break in the curve occurs at the threshold aggregation concentration. The critical aggregation concentrations for compound **2**, and **4** are 2.0 x 10^{-4} M and 1.1 x 10^{-5} M, respectively. For comparison, dendritic-linear hybrids and poly(propylene imine) modified dendrimers have been reported to have cacs of 10^{-6} - 10^{-7} M and poly(amido amine) modified dendrimers have been reported with cacs of 2 - 6 x 10^{-4} M.[1]

Chemical Reagents

All solvents were dried and freshly distilled prior to use (DCM and pyridine with CaH, and THF with Na). All chemicals were purchased from Aldrich or Acros at highest purity grade and used without further purification (DCC 99%; DMAP 99%) except Triton X-100 which was purchased from Lancaster Synthesis (Windham, NH). All reactions were performed under nitrogen atmosphere and at room temperature unless otherwise specified.

Abbreviations

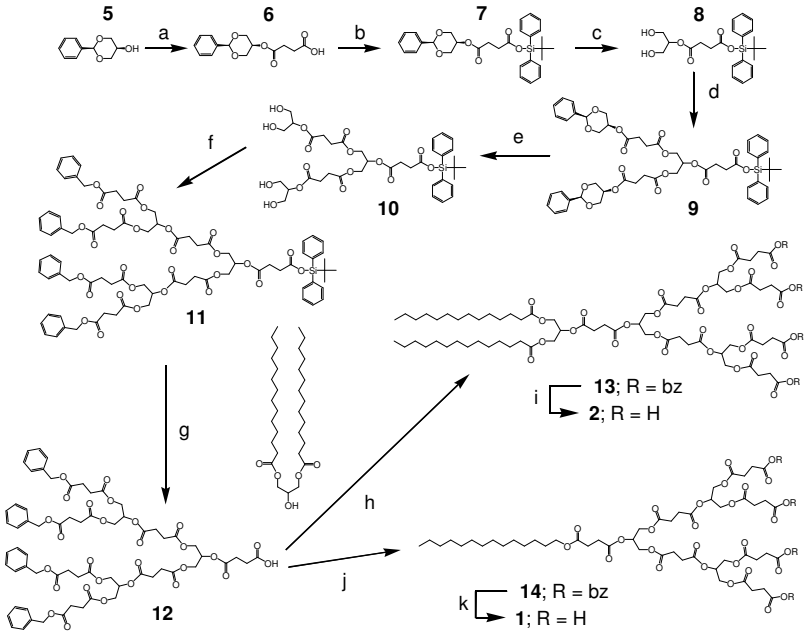
EDCI = 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, EtOAc = ethyl acetate, DCC = dicyclohexylcarbodiimide, DCM = dichloromethane, DCU = 1,3-dicyclohexylurea, DMAP = 4-

(dimethylamino)pyridine, DPTS = 4-(dimethylamino)-pyridinium *p*-toluenesulfonate, Pd/C = 10 % palladium on activated carbon, THF = tetrahydrofuran.

Instrumentation

NMR spectra were recorded on a Varian INOVA spectrometer (for ^{1}H and ^{13}C NMR, 400 MHz and 100.6 MHz respectively). Fast atom bombardment mass spectra (FAB-MS) were obtained on a JEOL JMS-SX102A spectrometer using a 3-nitrobenzyl alcohol matrix. MALDI-TOF mass spectra were obtained using a PerSpective Biosystems Voyager-DE Biospectrometry Workstation operating in the positive ion mode using 2-(4-hydroxyphenylazo)-benzoic acid (HABA). Elemental analysis was obtained from Atlantic Microlab, Inc. Size exclusion chromatography was performed using THF as the eluent on a Polymer Laboratories PLgel 3 µm MIXED-E column (3 µm bead size) and a Rainin HPLC system (temp = 25 °C; flow rate = 1.0 mL/min). Polystyrene standards (0.93K, 4.45K, and 12K; PDI = 1.03 - 1.05) were used for calibration.

*Synthesis of Dendritic Dendrimers **1** and **2***



*Compound **5**;*

Glycerol (500 g, 5.42 mol), benzaldehyde (500 g, 4.77 mol), and concentrated sulfuric acid (0.5 mL) were stirred for 4 hours. The solution was then heated to 55 °C under vacuum to remove water that was produced during the reaction. After approximately 4 hours no more water evolved. The reaction was cooled and stirred for 1 hour. Diethyl ether (0.75 L) was added and the solution was washed twice with saturated sodium carbonate solution and twice with brine. It was then dried over anhydrous sodium sulfate and filtered. An addition 0.5 L of diethyl ether was added and the solution was cooled to -20 °C. The solution was filtered and the precipitate was crystallized from ether two additional times to afford compound **5** in 8% yield. ^{1}H NMR ($CDCl_3$): δ=3.18 (d, 1H), 3.58 (d, 1H), 4.10 (m, 4H), 5.53 (s, 1H), 7.37 (m, 3H), 7.49 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$): δ=64.17, 72.47, 101.83, 126.12, 128.52, 129.29, 138.08 ppm. GC-MS: 179 *m/z* (MH^+) (theory: 180 *m/z* (M^+)).

*Compound **6**; Reaction 'a'*

cis-1,3-*O*-Benzylidene glycerol (**5**; 17.0 g, 0.094 mol) and succinic anhydride (14.42 g, 0.144 mol) were stirred in pyridine (150 mL) for 18 h. The pyridine was removed and the white powder was dissolved in H_2O. The pH of the water was adjusted to 7.0 with 1N NaOH. The water layer was washed with CH_2Cl_2 to remove impurities. The water layer was then adjusted to pH 4.0 with 1N HCl. The product was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered, and concentrated to yield 25.02 g of pure product as a white powder (95% yield). ^{1}H NMR ($CDCl_3$): δ=2.68-2.72 (m, 4H; -C*H*$_2$-C*H*$_2$-), 4.11-4.14 (m, 2H; -C*H*$_2$-CH-C*H*$_2$-), 4.24-4.27 (m, 2H; -C*H*$_2$-CH-C*H*$_2$-), 4.71-4.72 (m, 1H; -CH$_2$-C*H*-CH$_2$-), 5.53 (s, 1H; C*H*), 7.34-7.36 (m, 3H; arom. C*H*), 7.48-7.50 (m, 2H; arom. C*H*) ppm. ^{13}C NMR ($CDCl_3$): δ =29.05 (*C*H$_2$), 29.24 (*C*H$_2$), 66.57 (*C*H), 69.15 (*C*H$_2$), 101.43 (*C*H), 126.26 (*C*H), 128.51 (*C*H), 129.33 (*C*H), 137.95 (*C*H), 172.38 (*C*OOR), 178.07 (*C*OOH) ppm. GC-MS: 281 *m/z* (MH^+) (theory: 280 *m/z* (M^+)). Elemental analysis: C, 60.07%; H, 5.80% (theory: C, 59.99%; H, 5.75%).

*Compound **7**; Reaction 'b'*

Compound **6** (4.00 g, 0.014 mol) and imidazole (3.24 g, 0.048 mol) were stirred in DMF (150 mL). Next, diphenyl-*t*-butyl silyl chloride (6.4 mL, 0.024 mol) was added, and the reaction was stirred for 48 h. The DMF was removed, the product was dissolved in CH_2Cl_2, washed with sat. $NaHCO_3$ and water, dried over Na_2SO_4, filtered, concentrated, and dried on the vacuum line. The product was purified by column chromatography (4:1 hexanes:EtOAc) affording 6.38 g of product as a viscous opaque oil (86% yield). R_f=0.13 (4:1 hexanes:EtOAc). ^{1}H NMR ($CDCl_3$): δ=1.09 (s, 9H; *t*Bu), 2.78-2.84 (m, 4H; -C*H*$_2$-C*H*$_2$), 4.11-4.15 (m, 2H; -C*H*$_2$-CH-C*H*$_2$-), 4.23-4.26 (m, 2H; -C*H*$_2$-CH-C*H*$_2$-), 4.70-4.71 (m, 1H; -CH$_2$-C*H*-CH$_2$-), 5.54 (s, 1H; C*H*), 7.33-7.42, 7.48-7.50, 7.67- 7.68 (m, 15H; arom. bzld and phenyl C*H*) ppm. ^{13}C NMR ($CDCl_3$): δ=19.34 (-*C*-(CH$_3$)$_3$), 27.07 (-C-(*C*H$_3$)$_3$), 29.72, 30.96 (succ. -*C*H$_2$-), 66.46, 69.18 (glycerol, 2C, -*C*H$_2$-), 101.39 (O-*C*H-O), 126.23, 127.94, 128.50, 129.28, 130.29, 131.93, 135.51 (arom. *C*H), 137.99 (arom. bzld -*C*-), 171.53, 172.52 (succ. -*C*(=O)-) ppm. GC-MS: 519.2 *m/z* (MH$^+$) (theory: 518.2 *m/z* (M$^+$)). HR-FAB: 517.2028 *m/z* (M-H$^+$) (theory: 518.2125 *m/z* (M$^+$)). Elemental Analysis: C, 69.18%; H, 6.69% (theory: C, 69.47%; H, 6.61%).

*Compound **8**; Reaction 'c'*

Compound **7** (2.41 g, 4.65 mmol) was dissolved in THF (45 mL), and 20% $Pd(OH)_2$/C (1.0 g) was added. The solution was then placed in a Parr tube on a hydrogenator, evacuated, flushed with hydrogen, and shaken under 50 psi H_2 for 3 h. The solution was then filtered over wet celite and the solvent removed by rotoevaporation. The product was purified by column chromatography (1:1 hexanes:EtOAc increasing to 1:4 hexanes:EtOAc) to yield 1.9 g of a clear oil (95% yield). R_f=0.24 (1:4 hexanes:EtOAc). ^{1}H NMR ($CDCl_3$): δ=1.08 (s, 9H; *t*Bu), 2.02 (b s, 2H; -O*H*), 2.64-2.85 (m, 4H; -C*H*$_2$-C*H*$_2$), 3.70-3.72, 4.07-4.14 (m, 4H; -C*H*$_2$-CH-C*H*$_2$-), 4.83-4.86 (m, 1H; -CH$_2$-C*H*-CH$_2$-), 7.33-7.44, 7.62-7.65 (m, 10H; arom. phenyl C*H*) ppm. ^{13}C NMR ($CDCl_3$): δ=19.30 (-*C*-(CH$_3$)$_3$), 27.03 (-C-(*C*H$_3$)$_3$), 29.77, 31.37 (succ. -*C*H$_2$-), 62.45 (glycerol, -*C*H$_2$-), 75.86 (CH$_2$-*C*H-CH$_2$), 127.97, 130.36, 132.67, 135.49 (phenyl *C*H), 172.65, 178.24 (succ. -*C*(=O)-) ppm. FAB-MS: 431 *m/z* (M-H$^+$) (theory: 430.57 *m/z* (M$^+$)).

Compound **8** was a hydroscopic oil and repeated attempts to obtain satisfactory elemental analysis failed. Thus, we decided to prepare the acetyl analogue for elemental analysis. Compound **4** (0.44 g, 1.02 mmol) was stirred in CH_2Cl_2 (30 mL) with DPTS (0.30 g, 1.02 mmol), freshly distilled acetic acid (0.15 mL, 2.66 mmol), and DCC (0.63 g, 3.07 mmol). The solution was stirred for 18 h. The DCU precipitate was filtered and the solvent was evaporated. A solution of 1:1 hexanes:EtOAc was added and impurities precipitated. The solution was filtered, concentrated and further purified by column chromatography (3:1 hexanes:EtOAc), to afford 0.44 g of product (83% yield). R_f=0.19 (4:1 hexanes:EtOAc). ^{1}H NMR ($CDCl_3$): δ=1.08 (s, 9H; *t*Bu), 1.87-1.93 (m, 6H; -C*H*$_3$), 2.50-2.71 (m, 4H; -C*H*$_2$-C*H*$_2$), 3.96-4.19 (m, 4H; -C*H*$_2$-CH-C*H*$_2$-), 5.06-5.18 (m, 1H; -CH$_2$-C*H*-CH$_2$-), 7.22-7.33, 7.51-7.56 (m, 10H; phenyl C*H*) ppm. ^{13}C NMR ($CDCl_3$): δ=19.10 (-*C*-(CH$_3$)$_3$), 20.61 (OC-*C*H$_3$), 26.82 (-C-(*C*H$_3$)$_3$), 29.14, 30.62 (succ. -*C*H$_2$-), 62.12, 69.28 (glycerol, -*C*H$_2$-), 127.71, 130.09, 131.65, 135.27 (arom. *C*H), 170.52, 171.19, 171.58 (-*C*(=O)-) ppm. FAB-MS: 515.4 *m/z* (MH$^+$) (theory: 514.6 *m/z* (M$^+$)). Elemental analysis: C, 62.76%; H, 6.69% (theory: C, 63.01%; H, 6.66%). SEC: M_w=547, M_n=528, PDI=1.04.

*Compound **9**; Reaction 'd'*

Compound **8** (1.90 g, 4.41 mmol) was stirred in CH_2Cl_2 (100 mL) with DPTS (1.30 g, 4.41 mmol), compound **6** (2.72 g, 9.70 mmol), and DCC (2.00 g, 9.70 mmol). The solution was stirred for 18 h. The DCU precipitate was filtered and the solvent was evaporated. A solution of 1:1 hexanes:EtOAc was added and impurities precipitated. The solution was filtered, concentrated and further purified by column chromatography (1:1 hexanes:EtOAc) to afford 3.70 g of product (88% yield). R_f=0.22 (1:1 hexanes:EtOAc). ^{1}H NMR ($CDCl_3$): δ=1.08 (s, 9H; *t*Bu), 2.57-2.79 (m, 12H; -C*H*$_2$-C*H*$_2$), 4.08-4.14, 4.16-4.22 (m, 12H; -C*H*$_2$-CH-C*H*$_2$-), 4.70-4.71 (m, 2H; -CH$_2$-C*H*-CH$_2$-), 5.21 (m, 1H; C*H*), 5.49-5.54 (m, 1H; C*H*), 7.32-7.41, 7.47-7.49, 7.62-7.67 (m, 20H; arom. bzld and phenyl C*H*) ppm. ^{13}C NMR ($CDCl_3$): δ=19.31 (-*C*-(CH$_3$)$_3$), 27.04 (-C-(*C*H$_3$)$_3$), 28.98, 29.33, 30.81 (succ. -*C*H$_2$-), 62.48, 66.50, 69.16, 69.43 (glycerol, -*C*H$_2$-), 101.33 (O-*C*H-O), 126.22, 127.95, 128.49, 129.26, 130.32, 131.92, 135.49 (arom. *C*H), 138.02 (arom. bzld -*C*-), 171.93, 172.28 (succ. -*C*(=O)-) ppm. GC-MS: 955.3 *m/z* (MH$^+$) (theory: 954.4

m/z (M^+)). Elemental analysis: C, 64.35%; H, 6.29% (theory: C, 64.14%; H, 6.12%). SEC: M_w=940, M_n=930, PDI=1.01.

*Compound **10**; Reaction 'e'*

Compound **9** (1.55 g, 1.62 mmol) was dissolved in THF (40 mL) and 20% $Pd(OH)_2$/C (1.0 g) was added. The solution was then placed in a Parr tube on a hydrogenator and shaken under 50 psi H_2 for 4 h. The solution was then filtered over wet celite, concentrated, and purified by column chromatography (0-25% acetone in EtOAc) to yield 1.12 g of product (95% yield). R_f=0.25 (1:3 acetone:EtOAc). ^{1}H NMR ($CDCl_3$): δ=1.07 (s, 9H; *t*Bu), 2.25 (b s, 4H; -O*H*), 2.58-2.82 (m, 12H; -CH_2-CH_2), 3.71-3.74, 4.09-4.26 (m, 12H; -CH_2-CH-CH_2-), 4.87-4.99, 5.24-5.25 (m, 3H; -CH_2-C*H*-CH_2-), 7.34-7.43, 7.63-7.68 (m, 10H; phenyl C*H*) ppm. ^{13}C NMR ($CDCl_3$): δ=14.52 (-*C*-$(CH_3)_3$), 25.78 (-C-(*C*$H_3)_3$), 26.99, 29.30, 30.51, 30.81 (succ. -*C*H_2-), 62.08, 63.44, 68.17, 70.23 (glycerol, -*C*H_2-), 125.71, 127.96, 130.35, 135.45 (phenyl), 171.94, 172.40 (succ. -*C*(=O)-) ppm. GC-MS: 779.5 *m/z* (MH^+) (theory: 778.3 *m/z* (M^+)). SEC: M_w=800, M_n=792, PDI=1.01.

Compound **10** was a hydroscopic oil and repeated attempts to obtain satisfactory elemental analysis failed. Thus, we decided to prepare the acetyl analogue. Compound **10** (0.55 g, 0.70 mmol) was stirred in CH_2Cl_2 (40 mL) with DPTS (0.39 g, 1.34 mmol), freshly distilled acetic acid (0.19 mL, 3.36 mmol), and DCC (0.87 g, 4.20 mmol). The solution was stirred for 18 h. The DCU precipitate was filtered and the solvent was evaporated. The residue was resuspended in a minimum of CH_2Cl_2, cooled to 10 °C and filtered. The resulting solution was concentrated and further purified by column chromatography (0-5% acetone in CH_2Cl_2) to afford 0.49 g of product (66% yield). R_f=0.17 (5% acetone in CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ=1.07 (s, 9H; *t*Bu), 2.04 (s, 12H; -CH_3), 2.55-2.83 (m, 12H; -CH_2-CH_2), 4.09-4.32 (m, 12H; -CH_2-CH-CH_2-), 5.20-5.29 (m, 3H; -CH_2-C*H*-CH_2-), 7.32-7.44, 7.61-7.67 (m, 10H; phenyl C*H*) ppm. ^{13}C NMR ($CDCl_3$): δ=19.10 (-*C*-$(CH_3)_3$), 20.67 (OC-*C*H_3), 26.82 (-*C*-$(CH_3)_3$), 28.60, 28.80, 29.10, 30.59 (succ. -*C*H_2-), 62.11, 62.31, 69.39 (glycerol, -*C*H_2-), 127.72, 130.09, 131.67, 135.27 (arom. *C*H), 170.50,

171.33, 171.61 (-*C*(=O)-) ppm. FAB-MS: 947.9 *m/z* (MH^+) (theory: 947.0 *m/z* (M^+)). Elemental analysis C, 57.15%; H, 6.26% (theory:C, 57.07%; H, 6.17%). SEC: M_w=1075, M_n=1041, PDI=1.03.

*Compound **11**; Reaction 'f'*

Compound **10** (0.77 g, 0.99 mmol) was dissolved in 75 mL of CH_2Cl_2 with 0.99 g (4.76 mmol) of succinic acid monobenzyl ester, 0.58 g (1.98 mmol) of DPTS, and 1.23 g (5.91 mmol) of DCC. The reaction was stirred for 16 hours. The DCU precipitate was filtered and the solution was evaporated. The residue was resuspended in a minimum of CH_2Cl_2, cooled to 10 °C for 1 hour and filtered. The solution was concentrated under reduced pressure and purified by column chromatography (30-50% EtOAc in hexanes) to afford 1.21 g of product (79% yield). R_f = 0.18 (40% EtOAc in hexanes). ^{1}H NMR ($CDCl_3$): δ=1.08 (s, 9H, *t*bu), 2.55-2.81 (m, 28H, succinic -C*H*$_2$-C*H*$_2$), 4.06-4.37 (m, 12H, -C*H*$_2$-CH-C*H*$_2$-), 5.11 (s, 8H, benzyl -C*H*$_2$-), 5.18-5.29 (m, 3H, -CH$_2$-C*H*-CH$_2$-), 7.22-7.44, 7.61-7.67 (m, 30H, aromatic C*H*) ppm. ^{13}C NMR ($CDCl_3$): δ=19.13, 26.81, 28.42, 28.64, 28.70, 28.91, 29.07, 30.56, 62.68, 66.72, 69.07, 73.69, 127.68, 128.23, 128.54, 130.06, 131.73, 135.21, 135.77, 171.64, 171.73, 171.90 ppm. FAB-MS: 1539.6 *m/z* (MH^+) (theory: 1539.7 *m/z* (M^+)). Elemental analysis: C, 63.35%; H, 6.02% (theory: C, 63.19%; H, 5.89%). SEC: M_w = 1739, M_n = 1704, PDI = 1.02.

*Compound **12**; Reaction 'g'*

Compound **11** (1.12 g, 0.73 mmol) was dissolved in 100 mL of THF. Next, 0.89 g (2.76 mmol) of tetrabutylammonium fluoride trihydrate was added to the solution. The mixture was stirred for 1 hour. After one hour the reaction was complete as indicated by TLC. The solution was diluted with 25 mL of H_2O and acidified with 1N HCl to a pH of 3. The product was extracted into EtOAc, dried over Na_2SO_4, rotoevaporated and dried on the vacuum line. The product was purified by column chromatography (0-3% MeOH in CH_2Cl_2) to afford 0.71 g of product (75% yield). R_f = 0.18 (3% MeOH in CH2Cl2). ^{1}H NMR ($CDCl_3$): δ=2.54-2.69 (m, 28H, -C*H*$_2$-C*H*$_2$), 4.11-4.31 (m, 12H, -C*H*$_2$-CH-C*H*$_2$-), 5.09 (s, 8H, benzyl –C*H*$_2$-), 5.18-5.25 (m, 3H, -CH$_2$-C*H*-CH$_2$-), 7.25-7.36 (m, 20H, aromatic C*H*) ppm. ^{13}C NMR

($CDCl_3$): δ=28.57, 28.78, 28.94, 62.28, 62.43, 66.60, 69.16, 69.37, 128.24, 128.29, 128.61, 128.57, 171.33, 171.79, 171.95 ppm. FAB-MS: 1301.5 *m/z* ($M\text{-}H^+$) (theory: 1301.3 *m/z* (M^+)). Elemental analysis: C, 60.23%; H, 5.81% (theory: C, 60.00%; H, 5.58%). SEC: M_w = 1415, M_n = 1379, PDI = 1.03.

*Compound **13**; Reaction 'h'*

Compound **12** (0.97 g, 0.75 mmol) was dissolved in 75 mL of CH_2Cl_2 with 0.46 g (0.90 mmol) of 1,3-di-*O*-tetradecanoylglycerol, 0.07 g (0.56 mmol) of DMAP, and 0.22 g (1.12 mmol) of EDCI. The reaction was stirred for 48 hours. The solution was concentrated under reduced pressure and purified by column chromatography (10-50% EtOAc in hexanes) to afford 1.00 g of product (74% yield). R_f = 0.12 (40% EtOAc in hexanes). ^{1}H NMR ($CDCl_3$): δ=0.82-0.89 (t, 6H, -C*H*$_3$), 1.17-1.31 (m, 40H, -C*H*$_2$-), 1.54-1.62 (m, 4H, C(=O)-CH$_2$-C*H*$_2$-), 2.25-2.32 (t, 4H, C(=O)-C*H*$_2$-), 2.57-2.68 (m, 28H, succinic -C*H*$_2$-C*H*$_2$), 4.09-4.30 (m, 16H, -C*H*$_2$-CH-C*H*$_2$-), 5.11 (s, 8H, benzyl –C*H*$_2$-), 5.18-5.26 (m, 4H, -CH$_2$-C*H*-CH$_2$-), 7.28-7.37 (m, 20H, aromatic C*H*) ppm. ^{13}C NMR ($CDCl_3$): δ=14.02, 22.60, 24.75, 28.55, 28.71, 28.78, 28.92, 29.05, 29.20, 29.27, 29.41, 29.57, 31.85, 33.92, 61.78, 62.23, 66.55, 69.26, 69.54, 126.29, 128.35, 128.46, 128.63, 135.82, 171.41, 171.87, 172.04, 173.41 ppm. MALDI-MS: 1818.27 *m/z* ($M+Na^+$) (theory: 1796.04 *m/z* (M^+)). Elemental analysis: C, 64.29%; H, 7.35% (theory: C, 64.20%; H, 7.30%). SEC: M_w =2034, M_n = 1978, PDI = 1.03.

*Compound **2**; Reaction 'i'*

Compound **13** (0.10 g, 0.05 mmol) was dissolved in THF (20 mL) and 10% Pd/C (0.1 g) was added. The solution was then placed in a Parr tube on a hydrogenator and shaken under 50 psi. H_2 for 12 hours. The solution was then filtered over wet celite, concentrated, and placed on a high vacuum line at 50 °C for 48 hours to yield 0.08 g of product (97% yield). ^{1}H NMR ($CDCl_3$): δ=0.82-0.88 (t, 6H, -C*H*$_3$), 1.20-1.33 (m, 40H, myristic -C*H*$_2$-), 1.53-1.62 (m, 4H, C(=O)-CH$_2$-C*H*$_2$-CH$_2$-), 2.67-2.33 (t, 4H, C(=O)-C*H*$_2$-CH$_2$-), 2.56-2.72 (m, 21H, succinic -C*H*$_2$-C*H*$_2$), 4.08-4.38 (m, 16H, -C*H*$_2$-CH-C*H*$_2$-), 5.18-5.30 (m, 4H, -CH$_2$-C*H*-CH$_2$-) ppm. ^{13}C NMR ($CDCl_3$): δ=14.01, 22.59, 28.65, 28.79, 28.85, 29.03, 29.18, 29.27, 29.39,

29.57, 29.60, 31.83, 33.93, 61.79, 62.26, 69.34, 69.57, 171.59, 171.82, 172.20, 173.60, 177.16 ppm. MALDI-MS: 1458.34 *m/z* ($M+Na^+$) (theory: 1435.55 *m/z* (M^+)). Elemental analysis: C, 56.93%; H, 7.52% (theory: C, 56.89%; H, 7.44%). SEC: M_w = 1872, M_n = 1840, PDI = 1.02.

*Compound **14**; Reaction 'j'*

Compound **12** (0.97 g, 0.75 mmol) was dissolved in 75 mL of CH_2Cl_2 with 0.19 g (0.90 mmol) of tetradecanol, 0.07 g (0.56 mmol) of DMAP, and 0.22 g (1.12 mmol) of EDCI. The reaction was stirred for 48 hours. The solution was concentrated under reduced pressure and purified by column chromatography (10-50% EtOAc in hexanes) to afford 0.88 g of product (79% yield). R_f = 0.15 (40% EtOAc in hexanes). ^{1}H NMR ($CDCl_3$): δ=0.82-0.90 (t, 3H, -C*H*$_3$), 1.16-1.37 (m, 22H, alkyl -C*H*$_2$-), 1.53-1.63 (m, 2H, -C*H*$_2$-CH$_2$-O-), 2.55-2.75 (m, 28H, succinic -C*H*$_2$-C*H*$_2$), 4.00-4.07 (t, 2H, -C*H*$_2$-O-), 4.10-4.32 (m, 12H, -C*H*$_2$-CH-C*H*$_2$-), 5.11 (s, 8H, benzyl –C*H*$_2$-), 5.18-5.27 (m, 3H, -CH$_2$-C*H*-CH$_2$-), 7.26-7.40 (m, 20H, aromatic C*H*) ppm. ^{13}C NMR ($CDCl_3$): δ=14.01, 22.59, 25.80, 28.55, 28.71, 28.76, 28.83, 28.90, 29.19, 29.26, 29.44, 29.51, 31.83 , 62.23, 62.34, 64.94, 66.54, 69.13, 69.24, 128.28, 128.34, 128.50, 128.62, 135.81, 171.45 , 171.67, 171.76, 171.87, 172.04, 172.25 ppm. MALDI-MS: 1519.96 *m/z* ($M+Na^+$) (theory: 1497.62 *m/z* (M^+)). Elemental analysis: C, 63.49%; H, 6.88% (theory: C, 63.36%; H, 6.73%). SEC: M_w =1708, M_n = 1664, PDI = 1.03.

*Compound **1**; Reaction 'k'*

Compound **14** (0.17 g, 0.11 mmol) was dissolved in THF (20 mL) and 10% Pd/C (0.1 g) was added. The solution was then placed in a Parr tube on a hydrogenator and shaken under 50 psi. H_2 for 12 hours. The solution was then filtered over wet celite, concentrated, and placed on a high vacuum line at 50 °C for 48 hours to yield 0.12 g of product (98% yield). ^{1}H NMR ($CDCl_3$): δ=0.82-0.88 (t, 3H, -C*H*$_3$), 1.20-1.35 (m, 22H, alkyl -C*H*$_2$-), 1.55-1.64 (m, 2H, -C*H*$_2$-CH$_2$-O-), 2.58-2.71 (m, 28H, succinic -C*H*$_2$-C*H*$_2$), 4.02-4.08 (t, 2H, -C*H*$_2$-O-), 4.13-4.38 (m, 12H, -C*H*$_2$-CH-C*H*$_2$-), 5.21-5.31 (m, 3H, -CH$_2$-C*H*-CH$_2$-) ppm. ^{13}C NMR ($CDCl_3$): δ=14.01, 22.59, 25.79, 28.48, 28.78, 28.86, 29.19, 29.27, 29.44, 29.51, 29.57, 30.23,

31.83, 62.28, 65.13, 69.20, 69.34, 171.59, 171.84, 171.92, 172.19, 172.62, 177.24 ppm. MALDI-MS: 1158.72 *m/z* ($M+Na^+$) (theory: 1137.13 m/z (M^+)). Elemental analysis: C, 53.98%; H, 6.86% (theory: C, 53.87%; H, 6.74%). SEC: M_w = 1482, M_n = 1451, PDI = 1.02.