Supplementary Information

SUPPLEMENTARY METHODS

Acrylamide synthesis. Acrylamides were synthesized by the drop-wise addition of acryloyl chloride to the appropriate 1-aminoalkane in dry tetrahydrofuran at -15ºC with stirring for several hours. Excess triethylamine was added to neutralize the hydrochloric acid that was generated. The product was extracted in ethyl acetate, the organic phase was dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The product was resuspended in petroleum ether, and the solution was filtered over charcoal to remove impurities. The crude material was purified using silica gel column chromatography, running a solvent gradient (petrol ether:ethyl acetate = 1:0; 3:1; 1:1; 0:1).

Library purification and characterization. Representative library members were purified and characterized. Chromatographic purification was performed by flash chromatography using Merck Silica Gel 60. Mixtures of dichloromethane (75%), methanol (22%), and ammonium hydroxide (3%) with varying amounts of additional dichloromethane (depending on the lipid) yielded pure products. Solvent removal was performed by evaporation on a Büchi rotavapor, with heating to 40°C. TLC was performed on Merck silica gel 60 F254 TLC glass plates and visualized with ninhydrin stain or UV 254. IR spectra were recorded on a Nicolet Magna-IR 550 spectrometer using polyethylene sheets or ATR technology. NMR spectra were recorded on Varian Mercury-300 or Varian Inova-500 spectrometers with the internal signal of the deuterated solvent as standard. Fast Atom Bombardment analysis and Positive-ion Electrospray analysis was carried out by M-Scan (West Chester, PA), on a VG Analytical ZAB 2-SE high field mass spectrometer and a Micromass Q-Tof API US hybrid quadrupole/time of flight mass spectrometer.

Synthesis of PEG-DMG lipid. PEG-DMG was synthesized from (*S*)-1,2-di-*O*-tetradecyl-*sn*-glycerol and ω-methoxy-PEG2000-amine (mPEG2000-NH_2). Treatment of (*S*)-1,2-di-*O*-tetradecyl-*sn*-glycerol with *N*,*N'*-disuccinimidyl carbonate in the presence of triethylamine in dichloromethane under argon atmosphere and subsequent reaction of the intermediate formed with mPEG2000-NH_2 in the presence of pyridine in dichloromethane afforded the desired compound (*R*)-3-[(ω-methoxy-PEG2000-carbamoyl)]-1,2-di-*O*-tetradecyl-*sn*-glyceride (PEG-DMG) in 90 % yield.

Formulation of lyophilized materials for use *in vivo*. For acrylate-based lipidoids, 15 mg of lipidoid, 0.8 mg of cholesterol (Avanti Polar Lipids, Alabaster, AL), and 7 mg of mPEG2000-ceramideC16 (Avanti Polar Lipids) were dissolved in 2 mL of 25 mM sodium acetate in a 15 mL conical tube with vortexing for 5-10 minutes. For acrylamide based lipidoids, 15 mg of lipidoid and 0.8 mg of cholesterol were first dissolved in 0.8 mL of ethanol. 7 mg of mPEG2000-ceramideC16 and 1.2 mL of 25 mM sodium acetate were added subsequently with continued vortexing. 20 mg of sucrose was added with vortexing. 0.1 mL of 10 mg/mL siRNA solution was added to 1.9 mL of 25 mM sodium acetate. This solution was added to the solution containing the complexes and vortexed for an additional 20 min. The solution was extruded 11 times through 400 nm polycarbonate membranes and 11 times through 200 nm polycarbonate membranes. An additional 10 mg/mL of sucrose was added to the extruded samples. The samples were then frozen at -80°C for 2 h and lyophilized overnight.

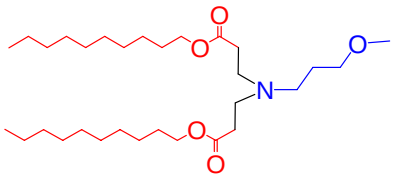
Determination of entrapment efficiency. A modified Ribogreen (Invitrogen) assay was used to quantify entrapment of siRNA. Samples were diluted 1:200 in TE buffer, and mixed with Ribogreen reagent (1:200 in TE buffer) at 37 °C in the presence or absence of 0.5 %

final w/v Triton X-100. Entrapment efficiency of siRNA in lipidoid complexes was determined by comparing the fluorescent signal of the lipidoid-siRNA sample in the presence (total siRNA) and absence (free siRNA) of Triton X-100 detergent. Signal in the absence of detergent yields the "free" or accessible siRNA fraction, while signal in the presence of detergent yields the total siRNA content.

siRNA quantification by UV absorption. Formulated siRNA samples were diluted 1:10 in PBS (200 µL). 800 µL of a 2.5:1 mixture of methanol:chloroform was added to the diluted siRNA sample and mixed, resulting in a single clear phase. The absorbance at 260 nm was measured on a UV spectrophotometer. siRNA was quantified based on an experimentally determined extinction coefficient for the duplex siRNA.

SELECTED CHARACTERIZED LIPIDOIDS

1L$_{10}$



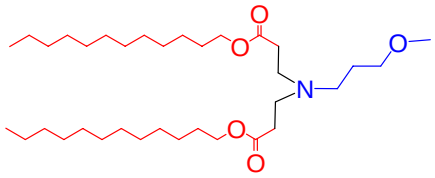
IR (ATR): 2956, 2927, 2856, 1725, 1465, 1388, 1266, 1182, 1115, 1045.

^{1}H (500 MHz, $CDCl_3$, δ): 3.98–3.96 (m, 4H, OCH_2), 3.30-3.28 (m, 2H, CH_3OCH_2), 3.24-3.23 (m, 3H, OCH_3), 2.67 (t, J = 7.21 Hz, 4H, NCH_2), 2.41 (t, J = 7.05 Hz, 2H, NCH_2), 2.36-2.33 (m, 4H, $C(O)CH_2$), 1.61-1.51 (m, 8H, $CH_2CH_2CH_2$), 1.23-1.19 (m, 28H, $CH_2CH_2CH_2$), 0.81-0.79 (m, 6H, CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 172.8 (C=O), 70.7 (CH_2), 64.6 (CH_2), 58.6 (CH_3), 50.6 (CH_2), 49.6 (CH_2), 32.9 (CH_2), 32.1 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 28.8 (CH_2), 27.7 (CH_2), 26.1 (CH_2), 22.8 (CH_2), 14.3 (CH_3).

ESI (m/z): $[M + H]^+$ calcd for $C_{30}H_{60}NO_5$, 514.4471; found, 514.4464.

1L$_{12}$



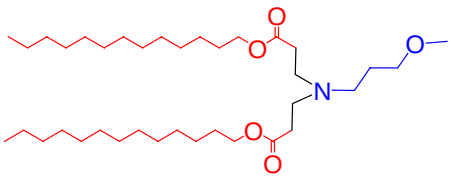
IR (ATR): 3022, 2935, 2858, 2401, 1730, 1468, 1222, 1182, 1116.

^{1}H (500 MHz, $CDCl_3$, δ): 4.04–3.98 (m, 4H, OCH_2), 3.40-3.38 (m, 0.5H, CH_3OCH_2), 3.23-3.31 (m, 2H, CH_3OCH_2) 3.27-3.26 (m, 3H, OCH_3), 2.83 (t, J = 6.41 Hz, 0.5H, NCH_2), 2.71-2.67 (m, 4H, NCH_2), 2.48-2.42 (m, 2H, NCH_2), 2.37 (t, J = 7.05 Hz, 4H, $C(O)CH_2$), 1.72-1.53 (m, 8H, $CH_2CH_2CH_2$), 1.26-1.21 (m, 36H, $CH_2CH_2CH_2$), 0.84-0.81 (m, 6H, CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 172.9 (C=O), 70.8 (CH_2), 64.7 (CH_2), 58.7 (CH_3), 50.6 (CH_2), 49.6 (CH_2), 32.9 (CH_2), 32.1 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 28.8 (CH_2), 27.7 (CH_2), 26.1 (CH_2), 22.9 (CH_2).

ESI (m/z): $[M + H]^+$ calcd for $C_{34}H_{68}NO_5$, 570.5097; found, 570.5096.

1L$_{14}$



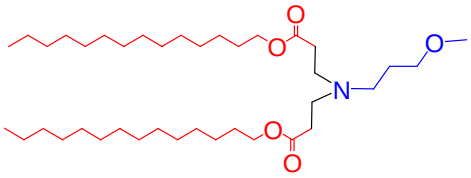
IR (ATR): 2959, 2929, 2873, 1726, 1464, 1381, 1255, 1181, 1115, 1049.

^{1}H (500 MHz, $CDCl_3$, δ): 4.05–3.98 (m, 4H, OCH_2), 3.32-3.30 (m, 2H, CH_3OCH_2), 3.26-3.24 (m, 3H, OCH_3), 2.71-2.68 (m, 4H, NCH_2), 2.45-2.42 (m, 2H, NCH_2), 2.3-2.36 (m, 4H, $C(O)CH_2$), 1.65-0.70 (m, 54H, $CH_2CH_2CH_2$, $CH_2CH_2CH_2$, CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 172.8 (C=O), 70.7 (CH_2), 65.8 (CH_2), 58.7 (CH_3), 50.6 (CH_2), 49.5 (CH_2), 32.9 (CH_2), 31.7 (CH_2), 31.7 (CH_2), 29.6 (CH_2), 27.7 (CH_2), 26.9 (CH_2), 24.8 (CH_2), 22.9 (CH_2), 18.1 (CH_2), 14.3 (CH_2).

ESI (m/z): $[M + H]^+$ calcd for $C_{36}H_{72}NO_5$, 598.5410; found, 598.5410.

1L$_{15}$



IR (ATR): 2926, 2855, 1726, 1466, 1393, 1266, 1182, 1114, 1047..

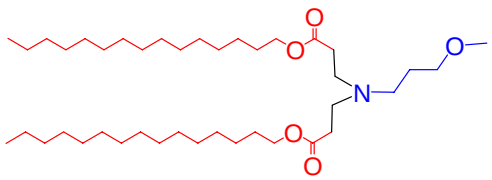
^{1}H (500 MHz, $CDCl_3$, δ): 4.03–4.00 (m, 4H, OCH_2), 3.33 (t, J = 6.51Hz, 2H, CH_3OCH_2), 3.28 (s, 3H, OCH_3), 2.73-2.71 (m, 4H, NCH_2), 2.47-2.44 (m, 2H, NCH_2), 2.41-2.38 (m, 4H,

C(O)CH_2), 1.66-1.56 (m, 6H, $CH_2CH_2CH_2$), 1.28-1.22 (m, 42H, $CH_2CH_2CH_2$), 0.86-0.83 (m, 6H, CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 172.9 (C=O), 70.8 (CH_2), 64.7 (CH_2), 58.7 (CH_3), 50.6 (CH_2), 49.6 (CH_2), 32.9 (CH_2), 32.2 (CH_2), 31.8 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 28.9 (CH_2), 27.7 (CH_2), 26.2 (CH_2), 22.9 (CH_2), 14.3 (CH_2).

ESI (m/z): $[M + H]^+$ calcd for $C_{38}H_{76}NO_5$, 626.5723; found, 626.5714.

1L$_{16}$



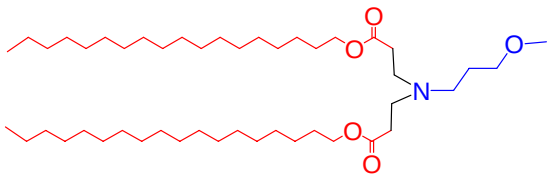
IR (ATR): 2926, 2855, 1726, 1466, 1394, 1266, 1182, 1114, 1038.

^{1}H (500 MHz, $CDCl_3$, δ): 4.03–3.92 (m, 4H, OCH_2), 3.35-3.32 (m, 2H, CH_3OCH_2), 3.29-3.26 (m, 3H, OCH_3), 2.74-2.70 (m, 4H, NCH_2), 2.50-2.41 (m, 2H, NCH_2), 2.39 (t, J = 7.05Hz, 4H, C(O)CH_2), 1.66-1.55 (m, 6H, $CH_2CH_2CH_2$), 1.27-1.22 (m, 46H, $CH_2CH_2CH_2$), 0.89-0.83 (m, 6H, CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 172.9 (C=O), 70.8 (CH_2), 64.7 (CH_2), 58.7 (CH_3), 50.6 (CH_2), 49.6 (CH_2), 32.9 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 28.9 (CH_2), 27.7 (CH_2), 26.2 (CH_2), 22.9 (CH_2), 14.3 (CH_2).

ESI (m/z): $[M + H]^+$ calcd for $C_{40}H_{80}NO_5$, 654.6036; found, 654.6027.

1L$_{18}$



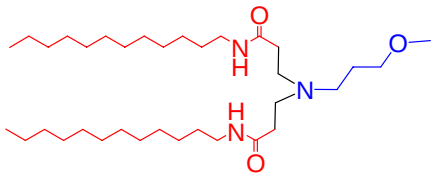
IR (ATR): 2927, 2855, 1726, 1467, 1392, 1267, 1181, 1113..

^{1}H (500 MHz, $CDCl_3$, δ): 4.06–4.03 (m, 4H, OCH_2), 3.38-3.35 (m, 2H, CH_3OCH_2), 3.33-3.31 (m, 3H, OCH_3), 2.75 (t, J = 7.2Hz, 4H, NCH_2), 2.50-2.47 (m, 2H, NCH_2), 2.42 (t, J = 7.21Hz, 4H, $C(O)CH_2$), 1.69-1.58 (m, 6H, $CH_2CH_2CH_2$), 1.30-1.25 (m, 54H, $CH_2CH_2CH_2$), 0.89-0.86 (m, 6H, CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 173.0 (C=O), 70.9 (CH_2), 64.8 (CH_2), 58.8 (CH_3), 50.6 (CH_2), 49.6 (CH_2), 33.0 (CH_2), 32.2 (CH_2), 31.7 (CH_2), 30.0 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 28.9 (CH_2), 27.7 (CH_2), 26.2 (CH_2), 23.0 (CH_2), 14.4 (CH_2).

ESI (m/z): $[M + H]^+$ calcd for $C_{46}H_{92}NO_5$, 738.6975; found, 738.6968.

1N$_{12}$



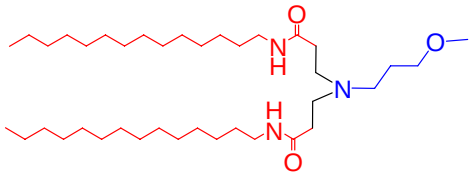
IR (ATR): 2928, 2855, 1710, 1657, 1649, 1519, 1466, 1379, 1224, 1112.

^{1}H (500 MHz, $CDCl_3$, δ): 6.7 (s, 2H, N*H*), 6.28-6.24 (m, 0.6H, CH_2CH(C)), 6.12-6.10 (m, 0.6H, C*H*HCH(C)), 5.62-5.61 (m, 0.6H, N*H*), 5.60 (d, *J* = 1.44Hz, 0.6H, C*H*HCH(C)), 3.46-3.36 (m, 2H, CH_3OCH_2), 3.33-3.29 (m, 3H, OCH_3), 3.19-3.15 (m, 2H, NCH_2), 2.69-2.67 (m, 4H, NCH_2), 2.48-2.45 (m, 2H, NCH_2), 2.30-2.28 (m, 4H, $C(O)CH_2$), 1.68-1.47 (m, 6H, $CH_2CH_2CH_2$), 1.27-1.24 (m, 36H, $CH_2CH_2CH_2$), 0.88-0.85 (m, 6H, CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 172.5 (C=O), 70.4 (CH_2), 58.5 (CH_3), 50.2 (CH_2), 50.0 (CH_2), 39.7 (CH_2), 34.2 (CH_2), 32.2 (CH_2), 29.9 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 27.3 (CH_2), 27.2 (CH_2), 26.9 (CH_2), 24.9 (CH_2), 22.9 (CH_2), 14.4 (CH_2).

ESI (m/z): $[M + H]^+$ calcd for $C_{34}H_{70}N_3O_3$, 568.5417; found, 568.5414.

1N$_{14}$



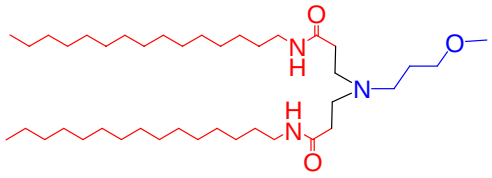
IR (ATR): 2928, 2855, 1658, 1650, 1643, 1518, 1467, 1098.

^{1}H (500 MHz, $CDCl_3$, δ): 6.67–6.66 (m, 2H, N*H*), 6.29 (d, *J* = 1.4Hz, 0.8H, CH_2CH(C)), 6.25-6.06 (m, 0.8H, C*H*HCH(C)), 5.75 (*br*s, 0.8H, N*H*), 5.63-5.60 (m, 0.8H, C*H*HCH(C)), 3.39-3.37 (m, 2H, CH_3OCH_2), 3.33-3.30 (m, 3H, OCH_3), 3.21-3.16 (m, 2H, NCH_2), 2.70-2.67 (m, 4H, NCH_2), 2.49-2.46 (m, 2H, NCH_2), 2.31-2.28 (m, 4H, $C(O)CH_2$), 1.53-1.47 (m, 6H, $CH_2CH_2CH_2$), 1.28-1.25 (m, 44H, $CH_2CH_2CH_2$), 0.89-0.86 (m, 6H, CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 172.5 (C=O), 71.6 (CH_2), 64.3 (CH_3), 50.2 (CH_2), 39.7 (CH_2), 34.2 (CH_2), 32.2 (CH_2), 31.8 (CH_2), 29.9 (CH_2), 29.6 (CH_2), 27.4 (CH_2), 24.9 (CH_2), 22.9 (CH_2), 14.4 (CH_2).

ESI (m/z): $[M + H]^+$ calcd for $C_{38}H_{78}N_3O_3$, 624.6043; found, 624.6038.

1N$_{15}$



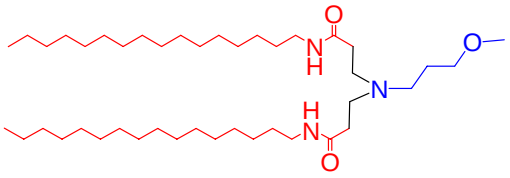
IR (ATR): 2927, 2855, 1858, 1650, 1643, 1519, 1467.

^{1}H (500 MHz, $CDCl_3$, δ): 6.64 (s, 2H, N*H*), 6.26 (d, *J* = 1.4Hz, 1H, CH_2CH(C)), 6.11-6.01 (m, 1H, C*H*HCH(C)), 5.63 (*br*s, 1H, N*H*), 5.61-5.60 (m,1H, C*H*HCH(C)), 3.40-3.37 (m, 2H, CH_3OCH_2), 3.35-3.30 (m, 3H, OCH_3), 3.20-3.16 (m, 2H, NCH_2), 2.74-2.68 (m, 4H, NCH_2), 2.49-2.46 (m, 2H, NCH_2), 2.30 (t, *J* = 6.3Hz, 4H, $C(O)CH_2$), 1.80-1.68 (m, 2H), 1.56-1.45 (m, 6H, $CH_2CH_2CH_2$), 1.37-1.13 (m, 64H, $CH_2CH_2CH_2$), 0.89-0.86 (m, 6H, CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 172.5 (C=O), 71.6 (CH_2), 64.3 (CH_3), 50.3 (CH_2), 39.7 (CH_2), 34.2 (CH_2), 32.2 (CH_2), 31.8 (CH_2), 30.0 (CH_2), 29.6 (CH_2), 27.3 (CH_2), 24.9 (CH_2), 22.9 (CH_2), 18.1 (CH_2), 14.4 (CH_2).

ESI (m/z): $[M + H]^+$ calcd for $C_{40}H_{82}N_3O_3$, 652.6356; found, 652.6345.

1N$_{16}$



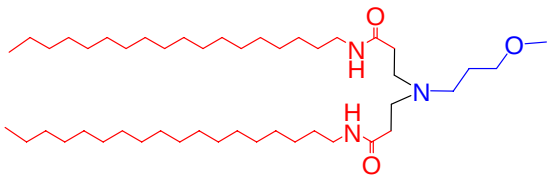
IR (ATR): 2927, 2855, 1794, 1659, 1650, 1643, 1518, 1467, 1388, 1099.

^{1}H (500 MHz, $CDCl_3$, δ): 6.73–6.71 (m, 1H, N*H*), 6.26 (d, *J* = 17Hz, 1H, CH_2CH(C)), 6.11-6.06 (m, 1H, C*H*HCH(C)), 5.88 (*br*s, 1H, N*H*), 5.60 (d, *J* = 10.3Hz, 1H, C*H*HCH(C)), 3.48-3.36 (m, 2H, CH_3OCH_2), 3.34-3.28 (m, 3H, OCH_3), 3.25-3.15 (m, 2H, NCH_2), 2.87-2.85 (m, 1H), 2.82-2.60 (m, 4H, NCH_2), 2.48 (t, *J* = 6.9Hz, 2H, NCH_2), 2.35-2.33 (m, 1H), 2.29 (t, *J* = 6.1Hz, 2H, $C(O)CH_2$), 1.79-1.73 (m, 1H), 1.69-1.64 (m, 1H), 1.54-1.47 (m, 6H, $CH_2CH_2CH_2$), 1.36-1.78 (m, 54H, $CH_2CH_2CH_2$), 0.88-0.85 (m, 6H, CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 172.6 (C=O), 165.8 (C=O), 131.3 ($C=CH_2$), 126.3 ($C=CH_2$), 70.4 (CH_2), 64.3 (CH_3), 59.0 (CH_2), 58.5 (CH_2), 50.3 (CH_2), 39.9 (CH_2), 39.7 (CH_2), 38.6 (CH_2), 34.2 (CH_2), 32.2 (CH_2), 31.8 (CH_2), 30.0 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 27.3 (CH_2), 27.2 (CH_2), 25.0 (CH_2), 22.9 (CH_2), 14.4 (CH_2).

ESI (m/z): $[M + H]^+$ calcd for $C_{42}H_{86}N_3O_3$, 680.6669; found, 680.6671.

1N$_{18}$



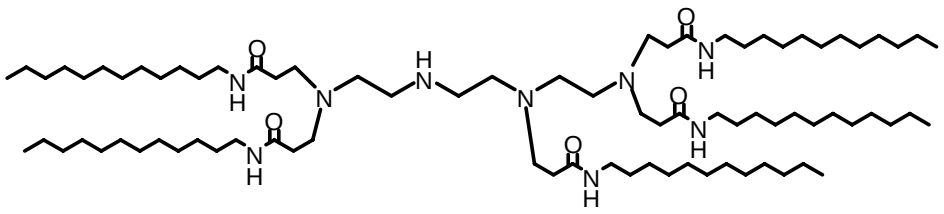
IR (ATR): 2927, 2855, 1659, 1650, 1643, 1518, 1467. 1388, 1297, 1097.

^{1}H (500 MHz, $CDCl_3$, δ): 6.71−6.70 (m, 2H, N*H*), 3.49-3.29 (m, 5H, CH_3OCH_2, OCH_3), 3.20-3.15 (m, 4H, (m, 2H, NCH_2), 2.69-2.67 (m, 4H, NCH_2), 2.49-2.42 (m, 2H, NCH_2), 2.35-2.28 (m, 4H, $C(O)CH_2$), 1.69-1.66 (m, 2H), 1.70-1.66 (m, 6H, $CH_2CH_2CH_2$), 1.25-1.24 (m, 54H, $CH_2CH_2CH_2$), 0.89-0.85 (m, 6H, CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 172.5 (C=O),131.2 (CH_2), 126.4 (CH_2), 70.4 (CH_2), 58.5 (CH_3), 50.3 (CH_2), 50.0 (CH_2), 39.7 (CH_2), 38.6 (CH_2), 34.2 (CH_2), 32.2 (CH_2), 31.7 (CH_2), 30.0 (CH_2), 29.9 (CH_2) 29.6, (CH_2), 27.3 (CH_2), 27.2 (CH_2), 26.9 (CH_2), 24.9 (CH_2), 23.0 (CH_2), 18.1 (CH_2), 14.4 (CH_2).

ESI (m/z): $[M + H]^+$ calcd for $C_{46}H_{94}N_3O_3$, 736.7295; found, 736.7283.

98N$_{12}$-5(1)



IR (ATR): 2928, 2855, 1794, 1658, 1642, 1552, 1467, 1387, 1284, 1096.

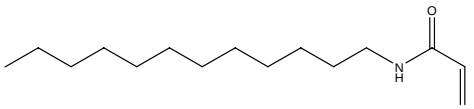
^{1}H (500 MHz, $CDCl_3$, δ): 7.62 (s, 1H, N*H*), 7.26−7.13 (m, 1H, N*H*), 7.00 (s, 1H, N*H*), 6.77 (s, 1H, N*H*), 6.57 (s, 1H, N*H*), 3.24-3.13 (m, 10H, NCH_2), 2.89-2.86 (m, 2H, NCH_2), 2.74-2.24 (m, 30H, NCH_2, $C(O)CH_2$), 1.48-1.36 (m, 10H, CH_2), 1.28-1.25 (m, 90H, CH_2), 0.89-0.86 (m, 15H CH_3).

^{13}C (500 MHz, $CDCl_3$, δ): 172.8 (C=O), 39.8 (CH_2), 32.2 (CH_2), 31.8 (CH_2), 30.0 (CH_2), 29.9 (CH_2), 29.7 (CH_2), 27.4 (CH_2), 22.9 (CH_2), 14.4 (CH_3).

ESI (m/z): $[M + H]^+$ calcd for $C_{81}H_{164}N_9O_5$, 1343.2855; found, 1343.2833.

SYNTHESIZED STARTING MATERIALS

N-dodecylacrylamide (N_{12})



To a solution of *N*-dodecylamine (100 mL, 430 mmol) and triethylamine (80 mL, 570 mmol in tetrahydrofuran (200 mL) at 0°C was slowly added acryloyl chloride (80 mL, 1.1 mol, 3 eq.). The resulting suspension was stirred at r.t. for 14h. Then ethylacetate (500 mL) and 1 M HCl (250 mL) were added. The organic phase was separated and washed with 1 M HCl (250 mL) and brine. The organic phase was dried under Na_2SO_4 and charcoal and filtered. The solvent was evaporated under reduced pressure. Then petrol ether was added and the insoluble product passed through a glass sintered filter. The product was resuspended in petrol ether and filtered. The crude material was purified using silica gel chromatography (Petrolether/EtOAc gradient, 1:0; 3:1; 1:1; 0:1). After evaporation the product was isolated as a white powder (13.2 g, 55 mmol, 13%).

R_f: 0.53 (in petrolether/ethyl acetate 1:1).

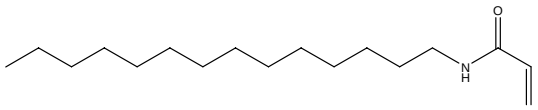
IR (Polyethylene): 3268, 3071, 2955, 2931, 2922, 2913, 2872, 2847, 1668, 1652, 1621, 1450, 1475, 1470.

^{1}H (300 MHz, $CDCl_3$, δ): 6.5 (s, 1H, amideN*H*), 6.27-6.09 (m, 2H, CH*H*C*H*R), 5.56 (dd, *J* = 6.98 Hz, 2.5 Hz, 1H, C*H*HCHR), 3.30-3.23 (m, 2H, CH_2NHR), 1.51-1.45 (m, 2H RC$H_2$$CH_2$NHR), 1.25-1.22 (m, 18H CH_3CH_2R), 0.86-0.82 (m, 3H, CH_3).

^{13}C (300 MHz, $CDCl_3$, δ): 166.7 (C=O), 131.2 (CH), 126.4 (CH_2), 39.9 ($NHCH_2$), 32.1 (CH_2), 29.9-29.5 (m, CH_2), 27.2 (CH_2), 22.9 (CH_2CH_3), 14.4 (CH_3).

FAB (m/z): $[M + H]^+$ calcd for $C_{15}H_{30}NO$, 240.2327; found, 240.2335.

***N*-tetradecylacrylamide (N_{14})**



To a solution of *N*-tetradecylamine (25 g, 120 mmol) and triethylamine (50 mL, 360 mmol in THF (250 mL) at 4°C was slowly added acryloyl chloride (20 mL, 250 mmol). The resulting suspension was stirred at r.t. for 14h. Then ethylacetate (500 mL) and H_2O (500 mL) were added. The organic phase was separated. The aqueous phase was acidified with HCl and extracted twice with ethyl acetate. The combined organic solvents were evaporated under reduced pressure. Then petrol ether was added and the insoluble product passed through a glass sintered filter. The title compound was purified using silica gel chromatography, running a solvent gradient (petrolether/ethyl acetate: 1:0; 3:1; 1:1; 0:1, 1 L each). *N*-tetradecylacrylamide was isolated as a yellowish powder (11 g, 42 mmol, 34%).

R_f: 0.59 (in petrolether/ethyl acetate 1:1).

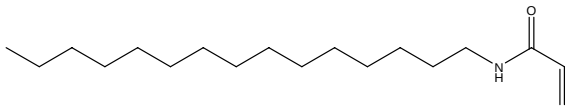
IR (Polyethylene): 3298, 2953, 2917, 2841, 1655, 1624, 1543, 1473, 1403, 1248. ^{1}H (300 MHz, $CDCl_3$, δ):.

^{1}H (300 MHz, $CDCl_3$, δ): 6.29–6.22 (m, 1H, CHHC*H*R), 6.16-6.03 (m, 2H, CH*H*CHR, amideN*H*), 5,62-5.58 (m, 1H, C*H*HCHR), 3.33-3.27 (m, 2H, C*H*$_2$NHR), 1.54-1.47 (m, 2H RC*H*$_2$CH$_2$NHR), 1.27-1.24 (m, 24H CH_3C*H*$_2$R), 0.89-0.84 (m, 3H, C*H*$_3$).

^{13}C (300 MHz, $CDCl_3$, δ): 165.8 (C=O), 131.2 (CH), 126.3 (CH_2), 39.9 ($NHCH_2$), 32.1 (CH_2), 29.9-29.5 (m, CH_2), 27.2 (CH_2), 22.9 (CH_2CH_3), 14.3 (CH_3).

FAB (m/z): $[M + H]^+$ calcd for $C_{17}H_{34}NO$, 268.2640; found, 268.2656..

N-pentadecylacrylamide (N$_{15}$)



To a solution of *N*-pentadecylamine (50 g, 220 mmol), triethylamine (100 mL, 720 mmol), and DMAP (5 mg) in THF (250 mL) at 4°C was slowly added acryloyl chloride (40 mL, 500 mmol). The resulting suspension was stirred at r.t. for 16h. Then ethylacetate (400 mL) and H_2O (400 mL) were added. The organic phase was separated. The aqueous phase was acidified with HCl and extracted twice with ethyl acetate. The combined organic solvents were evaporated under reduced pressure. Then petrol ether was added and the insoluble product passed through a glass sintered filter. The filtercake was dissolved in ethylacetate and silica gel (100 g) was added. After vigorous shaking the silica gel was removed on a glass sintered filter. The ethyl acetate was removed under reduced pressure to give the title compound as a yellowish powder (27 g, 97 mmol, 44%).

R_f: 0.54 (in petrolether/ethyl acetate 1:1).

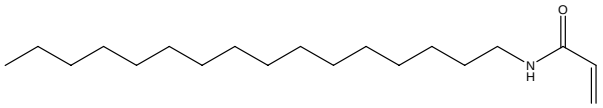
IR (Polyethylene): 3300, 2920, 2915, 2849, 1654, 1623, 1541, 1470, 1408, 1244, 1233, 996, 954.

^{1}H (300 MHz, $CDCl_3$, δ): 6.27–6.06 (m, 3H, amideN*H*, CH*H*C*H*R), 5.58 (dd, *J* = 8.0 Hz, 1.9 Hz, 1H, C*H*HCHR), 3.32-3.30 (m, 2H, CH_2NHR), 1.53-1.46 (m, 2H RC$H_2$$CH_2$NHR), 1.26-1.23 (m, 22H CH_3CH_2R), 0.87-0.83 (m, 3H, CH_3).

^{13}C (300 MHz, $CDCl_3$, δ): 165.8 (C=O), 131.3 (CH), 126.2 (CH_2), 39.9 ($NHCH_2$), 32.1 (CH_2), 29.9-29.6 (m, CH_2), 27.2 (CH_2), 22.9 (CH_2CH_3), 14.3 (CH_3).

FAB (m/z) $[M + H]^+$ calcd for $C_{18}H_{36}NO$, 282.2797; found, 282.2792.

N-hexadecylacrylamide (N_{16})



To a solution of *N*-hexadecylamine (25 g, 100 mmol) and triethylamine (50 mL, 360 mmol in THF (300 mL) at 4°C was slowly added acryloyl chloride (17 mL, 210 mmol). The suspension was heated to reflux for 0.5h the allowed to cool to r.t. Then methanol (200 mL) was added and the resulting solution was stirred at r.t. for 14h. Then the solvents were evaporated under reduced pressure. The title compound was purified using silica gel chromatography, running a solvent gradient (petrolether/ethyl acetate: 1:0; 3:1; 1:1; 0:1, 1 L each). *N*-hexadecylacrylamide was isolated as a yellowish powder (15 g, 50 mmol, 49%).

R_f: 0.52 (in petrolether/ethyl acetate 1:1).

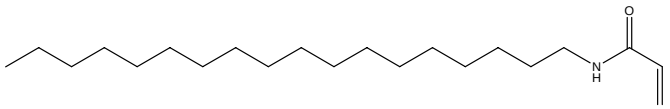
IR (Polyethylene): 3298, 2916, 2849, 1656, 1622, 1553, 1473, 1463, 1413, 1244.

^{1}H (300 MHz, $CDCl_3$, δ): 6.27 (dd, *J* = 15.4 Hz, 1.7 Hz, 1H, CHHC*H*R), 6.14-6.05 (m, 1H, CH*H*CHR), 5.73 (s, 1H, amideN*H*), 5,73-5.60 (m, 1H, C*H*HCHR), 3.38-3.29 (m, 2H, CH_2NHR), 1.56-1.49 (m, 2H RCH_2CH$_2$NHR), 1.29-1.17 (m, 23H CH_3CH_2R), 0.90-0.86 (m, 3H, CH_3).

^{13}C (300 MHz, $CDCl_3$, δ): 165.7 (C=O), 131.2 (CH), 126.1 (CH_2), 39.9 ($NHCH_2$), 32.2 (CH_2), 29.9-29.5 (m, CH_2), 27.2 (CH_2), 22.9 (CH_2CH_3), 14.3 (CH_3).

FAB (m/z) $[M + H]^+$ calcd for $C_{19}H_{38}NO$, 296.2953; found, 296.2960.

N-octadecylacrylamide (N_{18})



To a solution of *N*-octadecylamine (25 g, 93.0 mmol) and triethylamine (50 mL, 360 mmol in THF (250 mL) at 4°C was slowly added acryloyl chloride (20 mL, 250 mmol) and DMAP (ca. 5 mg). The resulting suspension was stirred at r.t. for 14h. Then ethylacetate (500 mL) and H_2O (500 mL) were added. The organic phase was separated and the solvent was evaporated under reduced pressure. The title compound was purified using silica gel chromatography, running a solvent gradient (petrolether/ethyl acetate: 1:0; 3:1; 1:1; 0:1, 1 L each). *N*-octadecylacrylamide was isolated as a yellowish powder (8.5 g, 26 mmol, 28%).

R_f: 0.59 (in petrolether/ethyl acetate 1:1).

IR (Polyethylene): 3299, 2952, 2916, 2847, 1657, 1623, 1556, 1473, 1463, 1412, 1241, 1160, 1069.

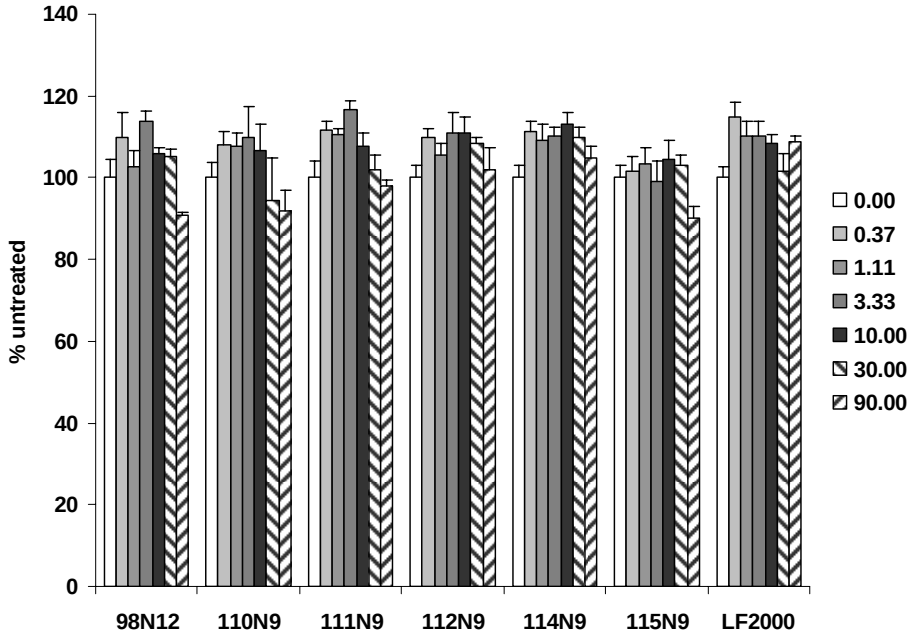
^{1}H (300 MHz, $CDCl_3$, δ): 6.26 (dd, *J* = 15.4 Hz, 1.7 Hz, 1H, CHHC*H*R), 6.15-6.06 (m, 1H, CH*H*CHR), 5.90 (s, 1H, amideN*H*), 5,62-5.58 (m, 1H, C*H*HCHR), 3.34-3.27 (m, 2H, CH_2NHR), 1.54-1.48 (m, 2H RC$H_2$$CH_2$NHR), 1.28-1.24 (m, 28H CH_3CH_2R), 0.89-0.85 (m, 3H, CH_3).

^{13}C (300 MHz, CDCl3, δ): 165.7 (C=O), 131.2 (CH), 129.1 (CH_2), 126.4 (CH_2), 59.5, 47.4, 39.9 ($NHCH_2$), 32.2 (CH_2), 29.9-29.5 (m, CH_2), 27.2 (CH_2), 22.9 (CH_2CH_3), 14.4 (CH_3).

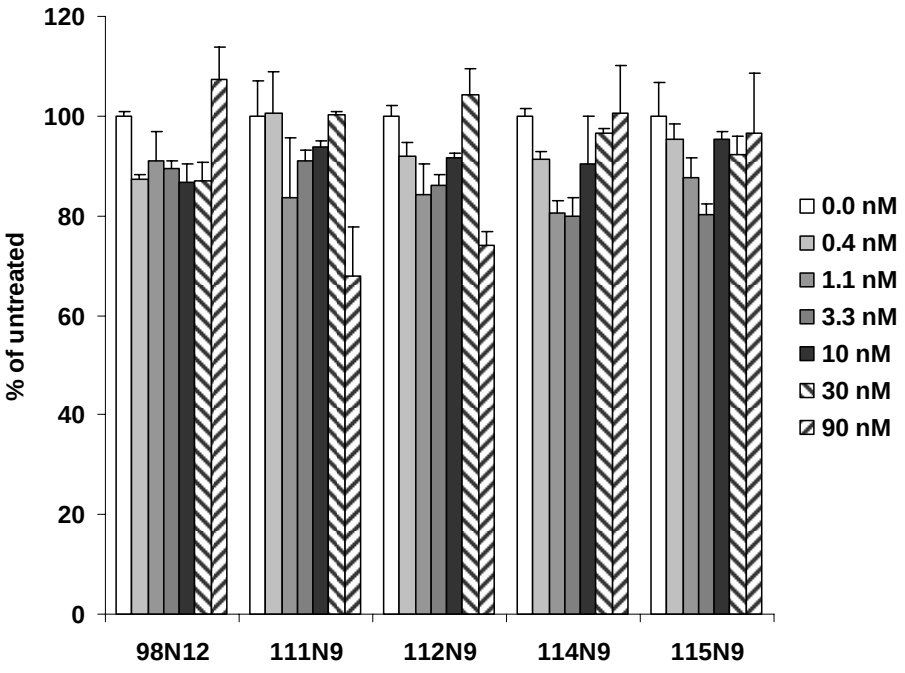
FAB (m/z) $[M + H]^+$ calcd for $C_{21}H_{42}NO$, 324.3266; found, 324.3271.

SUPPLEMENTARY FIGURES

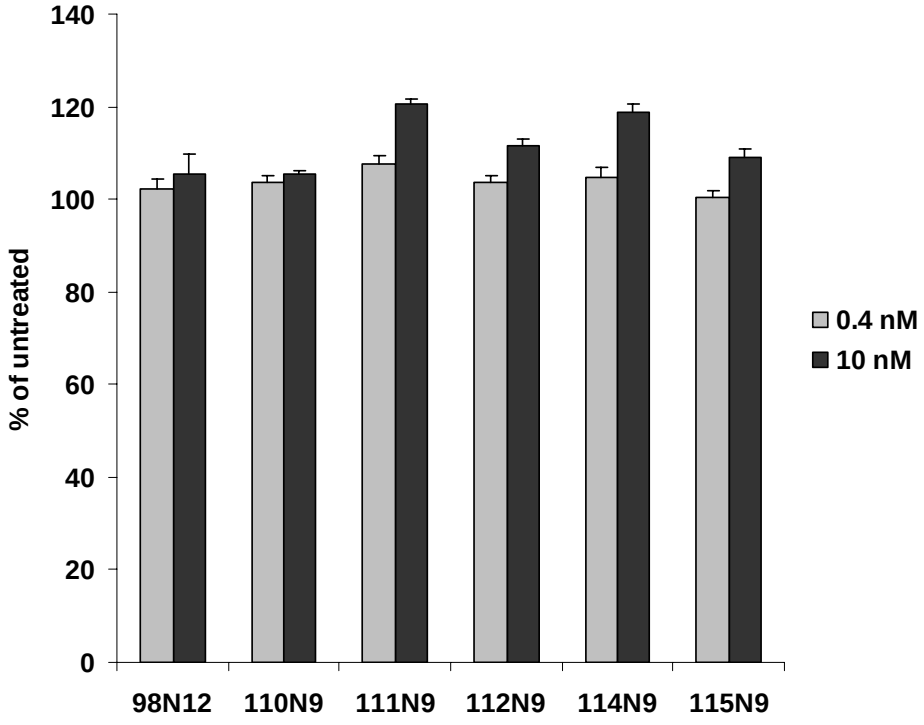
a)



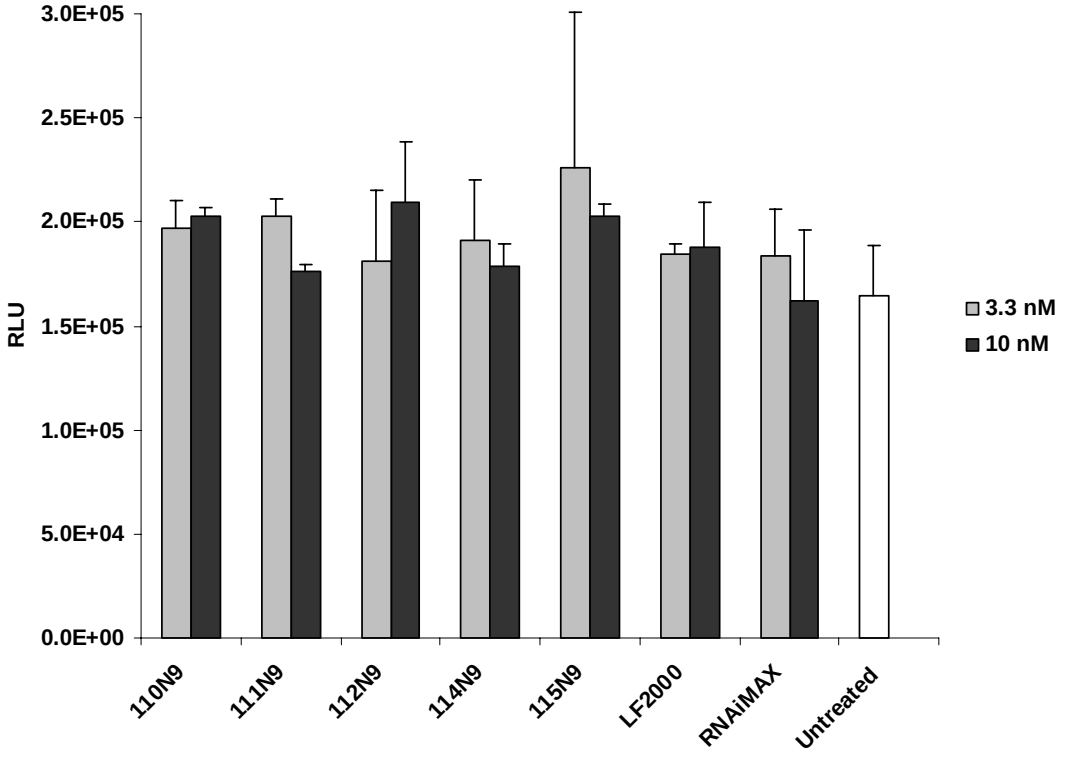
b)


120
100
80
60
40
20
0
% of untreated
98N12
111N9
112N9
114N9
115N9
0.0 nM
0.4 nM
1.1 nM
3.3 nM
10 nM
30 nM
90 nM

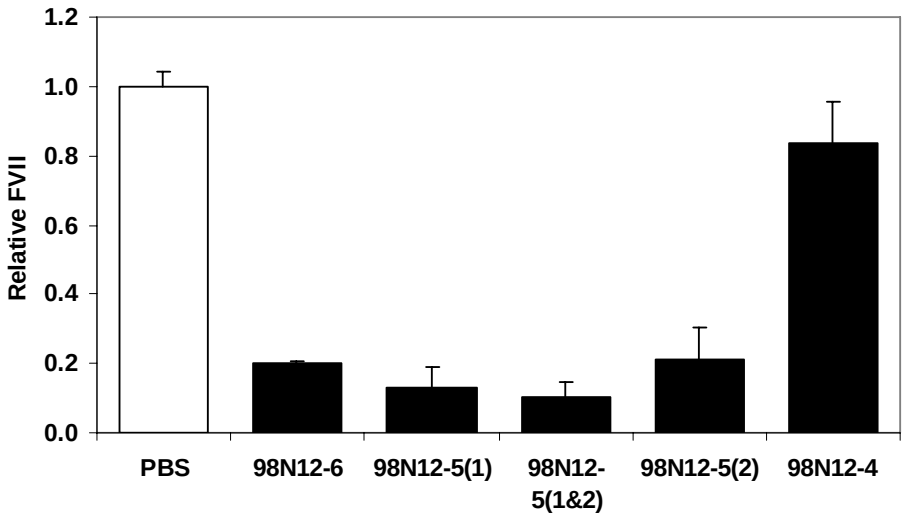
c)


140
120
100
80
60
40
20
0
% of untreated
98N12
110N9
111N9
112N9
114N9
115N9
0.4 nM
10 nM

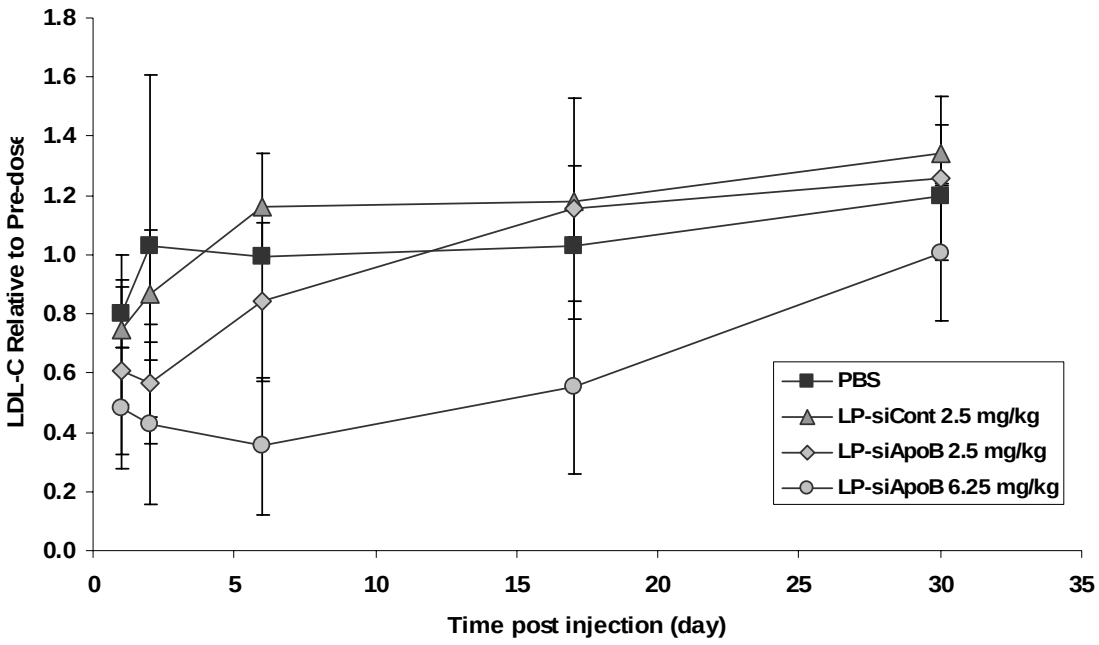
d)



Supplementary Figure 1. Lack of *in vitro* toxicity of lipidoids used for siRNA delivery. Dose response of control siRNAs formulated with selected lipidoids demonstrating lack of significant toxicity under conditions used for gene silencing. **a**) Firefly luciferase levels of HeLa cells treated with lipidoid-formulated control siRNA. Levels expressed of % of untreated cultures. Date points represent mean ± s.d. **b**) *Apob* levels of HepG2 cells treated with lipidoid-formulated control siRNA. Levels expressed of % of untreated cultures. Date points represent mean ± s.d. **c**) GFP levels in primary macrophage culture derived from GFP transgenic mice treated with lipidoid-formulated control siRNA. Levels expressed of % of untreated cultures. Date points represent mean ± s.d. **d**) Metabolic activity of primary macrophage cultures treated with lipidoid-formulated siRNA, as measured using the CellTiter Glo assay (Promega). Date points represent mean ± s.d.



Supplementary Figure 2. ***In vivo*** **efficacy of lipidoid-formulated siRNA employing 98N12 with different tail numbers.** Lipidoid reaction products of 98N12 were fractionated to isolate 98N12 compounds with different tail numbers (6-, 5-, and 4-tail versions). The following compounds were isolated: the single possible 6-tail compound (98N12-6), both possible isomers of the 5-tail compound (denoted 98N12-5(1) and -5(2)), as well as mixture of the two 5-tail isomers (98N12-5(1&2)), and a mixture of the 4-tail isomers (98N12-4). Factor VII-targeting siRNA was formulated using these compounds and administered to C57BL6 mice at 2.5 mg/kg via single i.v. bolus injection. Twenty-four hours after administration, serum Factor VII protein levels were quantified (n = 3, data points represent mean ± s.d.).



Supplementary Figure 3. Extent and persistence of LDL-C reduction in cynomolgus monkeys. Animals received either PBS, formulated siCont at 2.5 mg/kg or formulated siApoB at 2.5 or 6.25 mg/kg as bolus i.v. injections. For all groups except saline control, n = 6 for data points up to and including 2 d and n = 3 for data points beyond 2 d. For saline control, n = 4 for data points up to and including 2 d and n = 2 for data points beyond 2 d. Data points represent group mean ± s.d. No error bars shown for saline group where n = 2.

Supplementary Table 1. Molar ratios of acrylate or acrylamide to amine for lipidoids shown in Figure 2b.

Lipidoid	Ratio
$109O_{15}$	3:1
$99O_{12+}$	4:1
$115N_{11}$	4:1
$110N_{13}$	6:1
$115N_{13}$	4:1
$113N_{13}$	4:1
$113O_{14}$	4:1
$98O_{10}$	5:1
$100N_{11}$	4:1
$98N_{11}$	6:1
$103N_{12}$	2:1
$112O_{12}$	5:1
$28N_{12}$	2:1
$100N_{14}$	3:1
$95N_{13}$	3:1
$117N_{12}$	4:1
$109O_{12}$	3:1
$95O_{14}$	3:1
$99N_{11}$	4:1
$99O_{14+}$	4:1
$113N_{12}$	4:1
$100O_{15}$	4:1
$95N_{12}$	3:1
$100N_{12}$	4:1
$31O_{14}$	2:1

$32O_{15}$	2:1
$99N_{12}$	4:1
$110N_{11}$	6:1
$113O_{10}$	4:1
$109N_{14}$	3:1
$113O_{11}$	4:1
$113N_{8}$	4:1
$64N_{14}$	2:1
$87O_{13}$	2:1
$77O_{15}$	2:1
$96O_{15}$	3:1
$96N_{13}$	3:1
$109N_{11}$	3:1
$96N_{12}$	3:1
$31O_{12}$	2:1
$115N_{8}$	4:1
$98N_{12}$	6:1
$96N_{11}$	3:1
$114N_{8}$	5:1
$98N_{8}$	6:1
$64N_{15}$	2:1
$111N_{8}$	7:1
$98O_{12}$	5:1
$96N_{14}$	3:1
$111N_{11}$	6:1
$110O_{11}$	6:1
$110O_{12}$	6:1
$109N_{12}$	3:1
$80O_{15}$	2:1
$110N_{8}$	6:1

$112N_8$	5:1

Supplementary Table 2. Clinical chemistry and hematology parameters for lipidoid-siRNA treated rats at 15 min post administration. Sprague-Dawley rats (n = 4) were given a bolus i.v. injections of formulated siCont at 5 mg/kg. Blood samples were taken at 15 min post administration.

Group	ALT (U/L)	AST (U/L)	RBC (x 10^6/μL)	Hemo-globin (g/dL)	WBC (x 10^3/μL)	PLT (x 10^3/μL)
Saline	38 ± 15	81 ± 21	7.2 ± 0.2	13.8 ± 0.5	8.6 ± 0.7	1189 ± 114
siCont	39 ± 8	103 ± 42	6.8 ± 0.9	13.9 ± 0.6	9.7 ± 2.1	1218 ± 235

Supplementary Table 3.

a. ALT, AST, total bilirubin, and blood urea nitrogen (BUN) levels for lipidoid-siRNA treated cynomolgus monkeys

	ALT (U/L)	AST (U/L)	Bilirubin (μmol/L)	BUN (mg/dL)
siCont 2.5 mg/kg				
Pre-dose	50 ± 10	53 ± 14	3.1 ± 1.1	22 ± 4.6
24 h	76 ± 5	100 ± 5	2.5 ± 1.0	19 ± 2.5
48 h	64 ± 27	61 ± 19	2.7 ± 1.9	19 ± 2.0
siApoB 2.5 mg/kg				
Pre-dose	40 ± 21	45 ± 42	2.9 ± 0.5	18 ± 4
24 h	61 ± 10	65 ± 13	2.6 ± 0.8	17 ± 0
48 h	53 ± 38	48 ± 45	3.1 ± 1.1	18 ± 2

siApoB 6.25 mg/kg				
Pre-dose	44 ± 19	53 ± 27	3.9 ± 0.2	21 ± 3
24 h	70 ± 6	127 ± 10	3.4 ± 1.0	18 ± 2
48 h	67 ± 32	81 ± 38	3.7 ± 1.0	19 ± 1

b. PT, APTT, and fibrinogen for lipidoid-siRNA treated cynomolgus monkeys

	PT (s)	**APTT (s)**	**Fibrinogen (g/L)**
siCont 2.5 mg/kg			
Pre-dose	9.4 ± 0.2	19.1 ± 1.2	3.6 ± 0.6
48 h	9.2 ± 0.3	19.6 ± 0.6	3.2 ± 0.7
siApoB 2.5 mg/kg			
Pre-dose	10.2 + 0.3	19.1 ± 1.2	4.2 ± 0.4
48 h	9.7 ± 0.2	20.4 ± 0.9	3.6 ± 0.4
siApoB 6.25 mg/kg			
Pre-dose	10.7 ± 1.0	21.8 ± 3.4	3.2 ± 1.0
48 h	10.0 ± 0.2	19.4 ± 0.3	2.7 ± 0.4

c. Red blood cells (RBC), hemoglobin, white blood cells (WBC), and platelets (PLT) for lipidoid-siRNA treated cynomolgus monkeys

	RBC (x 10^9/L)	Hemoglobin (g/L)	WBC (x 10^9/L)	PLT (x 10^9/L)
siCont 2.5 mg/kg				
Pre-dose	5.3 ± 0.5	128 ± 8	14.8 ± 1.3	388 ± 114
48 h	4.8 ± 0.7	117 ± 12	15.5 ± 0.9	414 ± 108
siApoB 2.5 mg/kg				
Pre-dose	5.4 ± 0.4	132 ± 6	14.7 ± 8.7	427 ± 212
48 h	4.9 ± 0.4	119 ± 8	13.2 ± 5.6	424 ± 203
siApoB 6.25 mg/kg				
Pre-dose	5.5 ± 0.6	132 ± 12	16.9 ± 1.7	480 ± 189
48 h	4.7 ± 0.3	114 ± 5	17.4 ± 4.5	274 ± 105