Supporting information to accompany:

Branched-chain and dendritic lipids for lipid nanoparticles

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Synthetic procedures

Chemicals and solvents were used as received from known suppliers, except dry DCM which was obtained from a MBRAUN-SPS. NMR spectra were collected on a 300 MHz Bruker instrument. ESI-MS spectra were recorded on a LCQ-Classic instrument.

G1 Lipid Anchor Synthesis:

Scheme S1: Outline of the synthesis for the G1 lipid anchor

General Procedure: In a round bottom flask a stirred solution of thionyl chloride (10.0 equiv.) and **1a-d** (1.0 equiv.) was refluxed for 2 hours under a CaSO₄ drying tube. The reaction was monitored by ¹H-NMR analysis of aliquots. The reaction was allowed to cool and subsequently the thionyl chloride was removed under vacuum on a roto-evaporator to obtain the acid chloride. In a round bottom flask **2** (1.0 equiv.) was dissolved in pyridine (10.0 equiv.) and the acid chloride (2.5 equiv.) was added dropwise. The mixture was then heated to 70 °C and left to stir for 3 hours under a CaSO₄ drying tube. The reaction was monitored by ¹H-NMR analysis of aliquots. Once completed, the reaction was cooled and diluted with DCM. The solution was then washed 3 times with 1 M HCl and 3 times with 1 M NaOH. The organic layer was then dried over anhydrous sodium sulfate and then gravity filtered. The solvent was removed under vacuum to obtain the Boc-protected anchor. Excess TFA was added dropwise to the Boc-protected anchor in a round bottom flask. The reaction was left stirring for 1 hour at room temperature and was monitored by TLC (silica gel, EtOAc/ Hexanes as eluent, visualized by KMnO₄). Following completion of the reaction, TFA was removed on a roto-evaporator. NaOH (1 M) was then added and **3a-d** was extracted with DCM and washed 3 times with 1 M NaOH. The organic layer was dried with anhydrous sodium carbonate and subsequently gravity filtered. DCM was removed on a roto-evaporator yielding **3a-d**. No chromatography was necessary.

3a: By the general procedure: step 1, thionyl chloride (6.35 mL, 87.6 mmol) and **1a** (2.000 g, 8.757 mmol); step 2, **2** (0.596 g, 3.13 mmol) in pyridine (2.53 mL, 31.3 mmol) and the acid chloride (1.931 g, 7.835 mmol); step 3, TFA (5 mL) and the product of step 2; as a waxy yellow solid in a 66% yield (1.06 g). ¹H-NMR (300 MHz, CDCl₃) δ: 4.07 (m, 2H), 4.05 (m, 2H), 3.28 (m, 1H), 2.33 (t, 4H, 7.7 Hz), 1.62 (m, 4H), 1.48 (s(br), 2H), 1.26 (s, 40H), 0.88 (t, 6H, 7.0 Hz). ¹³C-NMR (75 MHz, CDCl₃): 173.6, 65.8, 49.3, 34.2, 31.9, 29.6, 29.6, 20.4, 29.3, 29.2, 29.1, 24.9, 22.7, 14.1 MS ((+) ESI): calc'd for C₃₁H₆₂NO₄⁺ = 512.47 amu, obtained = 512.33 amu.

3b: By the general procedure: step 1, thionyl chloride (5.61 mL, 78.0 mmol) and **1b** (2.000 g, 7.800 mmol); step 2, **2** (0.558 g, 2.92 mmol) in pyridine (2.31 mL, 29.2 mmol) and the acid chloride (2.000 g, 7.299 mmol); step 3 TFA (5 mL) and the product of step 2; as a waxy white solid in a 73% yield (1.21 g). ¹H-NMR (300 MHz, CDCl₃) δ: 4.06 (m, 2H), 4.05 (m, 2H), 3.28 (m, 1H), 2.32 (t, 4H, J=7.8 Hz), 1.61 (m, 4H), 1.24 (s, 48H), 0.87 (t, 6H). ¹³C-NMR (75

MHz, CDCl₃) δ : 173.8, 66.0, 49.5, 34.4, 32.1, 29.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 25.2, 22.9, 14.3. MS ((+) ESI): calc'd for C₃₅H₇₀NO₄⁺ = 568.53 amu, obtained = 568.33 amu.

3c: By the general procedure: step 1, thionyl chloride (5.66 mL, 78.2 mmol) and **1c** (2.30 mL, 7.82 mmol); step 2, **2** (0.488 g, 2.55 mmol) in pyridine (2.46 mL, 30.6 mmol) and the acid chloride (2.10 g, 7.64 mmol); step 3, TFA (5 mL) and the product of step 2; as a light yellow oil in a 75% yield (1.09 g). ¹H-NMR (300 MHz, CDCl₃) δ : 4.06 (m, 2H), 4.04 (m, 2H), 3.26 (m, 1H), 2.34 (m, 2H), 1.57, 1.44 (m, 8H), 1.24 (s, 40H), 0.86 (t, 12H, J=7.0 Hz). ¹³C-NMR (75 MHz, CDCl₃): 176.2, 65.5, 53.4, 49.4, 45.7, 32.4, 31.8, 31.6, 29.5, 29.4, 29.2, 29.2, 27.4, 27.4, 22.6, 22.6, 14.0, 14.0. MS ((+) ESI): calc'd for C₃₅H₇₀NO₄⁺ = 568.53 amu, obtained = 568.33 amu.

3d: By the general procedure: step 1, thionyl chloride (5.10 mL, 70.3 mmol) and **1d** (2.000 g, 7.030 mmol); step 2, **2** (0.510 g, 2.67 mmol) in pyridine (2.15 mL, 26.7 mmol) and the acid chloride (2.020 g, 6.668 mmol); step 3, TFA (5 mL) and the product of step 2; as a waxy white solid in a 67% yield (1.09 g). ¹H-NMR (300 MHz, CDCl₃) δ : 4.09 (m, 2H), 4.07 (m, 2H), 3.30 (m, 1H), 2.34 (t, 4H, J=7.5 Hz), 1.63 (m, 4H), 1.26 (s, 56H), 0.89 (t, 6H, J=6.8 Hz). MS ((+) ESI): calc'd for C₃₉H₇₈NO₄⁺ = 624.59 amu, obtained = 624.53 amu.

G2 Lipid Anchor Synthesis:

Scheme S2: Outline of the synthesis for the G2 lipid anchor

General Procedure: In a round bottom flask was dissolved **4** (1.0 equiv.) in dried DCM/DMF (1:1; 10mM). HBTU (2.4 equiv.), **3a-d** (2.2 equiv.) and triethylamine (4.0 equiv.) were subsequently added. The solution was stirred for 20 hours at room temperature and was monitored by ¹H-NMR. Once completed, the reaction solution was diluted with EtOAc and washed 3 times with 1M HCl and 3 times with H₂O. The organic layer was then dried over anhydrous sodium sulfate, followed by gravity filtration. EtOAc and DCM were roto-evaporated to yield a Boc-protected product. Excess TFA was added dropwise to the Boc-protected product in a round bottom flask. The reaction was left to stir for 1 hour and monitored by ¹H-NMR. Following completion of the reaction, TFA was removed on roto-evaporator and 1 M NaOH was added to the round bottom flask, the mixture was extracted with DCM and washed 3 times with 1 M NaOH. The organic layer was then dried over sodium carbonate, followed by gravity filtration. DCM was removed on a roto-evaporator and **5a-d** was isolated. The crude product was directly characterized; no purification was necessary. [Characterization- ¹H-NMR, ¹³C-NMR, ESI-MS] In a round bottom flask was dissolved Boc-glycine (1.1 equiv.) in dried DCM/DMF (1:1; 10mM). To this, HOBT (1.2 equiv.), HBTU (1.2 equiv.), **5a-d** (1.0 equiv.), and triethylamine (3.0 equiv.) were then added and the resulting solution was left stirring at room temperature for 20 hours. When the reaction was complete, visualized by ¹H-

NMR, the reaction solution was diluted with EtOAc, and washed once with 1 M HCl and 3 times with H₂O. The organic layer was then dried over anhydrous sodium sulfate and then gravity filtered. DCM and EtOAc were removed by roto-evaporation to yield a crude Boc-protected. Excess TFA was added in a round bottom flask and the reaction was left for 1 hour. Following completion, as monitored by ¹H-NMR, TFA was removed on a roto-evaporator. NaOH (1 M) was added to convert to the amine and **6a-d** was extracted with diethyl ether and subsequently washed with 3 times with 1 M NaOH. The organic layer was dried with anhydrous sodium carbonate and then gravity filtered. Diethyl ether was removed to obtain the crude product.

5a and 6a: By the general procedure: step 1, **4** (0.104 g, 0.444 mmol) in dried DCM/DMF (22.2 mL/22.2 mL; 10 mM). HBTU (0.404 g, 1.066 mmol), **3a** (0.500 g, 0.977 mmol) and triethylamine (0.25 mL, 1.8 mmol); step 2, TFA (5 mL) and the product of step 1; a waxy light yellow solid in 53% yield (0.264 g). Data for **5a**: ¹H-NMR (300 MHz, CDCl₃) δ: 7.02 (d, 2H, J=8.6 Hz), 4.46 (m, 2H), 4.24 (m, 4H), 4.12 (m, 4H), 3.25 (s, 4H), 2.31 (t, 8 H, J=7.8 Hz), 1.59 (m, 8H), 1.25 (s, 80H), 0.87 (t, 12H, J=6.9 Hz). ¹³C-NMR (75 MHz, CDCl₃) δ: 174.0, 170.8, 62.9, 52.8, 48.0, 34.3, 32.1, 29.9, 29.9, 29.7, 29.6, 29.5, 29.4, 25.1, 22.9, 14.3. MS ((+) ESI): calc'd for C₆₆H₁₂₆N₃O₁₀⁺ = 1120.94 amu, obtained = 1121.00 amu. Continuing the general procedure: step 3, Boc-glycine (0.033g, 0.19 mmol) in dried DCM/DMF (9.0 mL/9.0 mL; 10mM), HOBt (0.029 g, 0.21 mmol), HBTU (0.080 g, 0.21 mmol), **5a** (0.20 g, 0.179 mmol), and triethylamine (0.075 mL, 0.53 mmol); step 4, TFA (5 mL) and the Boc-protected product gave a light brown solid used without additional purification or characterization.

5b and 6b: By the general procedure: step 1, **4** (0.093 g, 0.40 mmol) in dried DCM/DMF (20.0 mL/20.0 mL; 10 mM). HBTU (0.364 g, 0.960 mmol), **3b** (0.500 g, 0.880 mmol) and triethylamine (0.22 mL, 1.6 mmol); step 2, TFA (5 mL) and the Boc-protected product of step 1; as a light brown waxy solid in 55% yield (0.272 g). Data for **5b**: ¹H-NMR (300 MHz, CDCl₃) δ: 7.03 (d, 2H, J=8.7 Hz), 4.47 (m, 2H), 4.26 (m, 4H), 4.14 (m, 4H), 3.27 (s, 4H), 2.33 (t, 8H, J=7.3 Hz), 1.60 (m, 8H), 1.27 (s, 96H), 0.89 (t, 12H, J=6.8). ¹³C-NMR (75 MHz, CDCl₃) δ: 174.0, 170.8, 62.8, 52.7, 48.0, 34.31, 32.1, 29.9, 29.7, 29.6, 29.5, 29.4, 25.1, 22.9, 14.3. MS ((+) ESI): calc'd for C₇₄H₁₄₂N₃O₁₀⁺ = 1233.07 amu, obtained = 1233.00. Continuing the general procedure: step 3, Boc-glycine (0.030 g, 0.17 mmol) in dried DCM/DMF (8.1 mL/8.1 mL; 10mM), HOBt (0.026 g, 0.20 mmol), HBTU (0.074 g, 0.20 mmol), **5b**: (0.201, 0.162 mmol), and triethylamine (0.068 mL, 0.49 mmol); step 4, TFA (5 mL) and the Boc-protected product of step 3 gave a light brown oil used without further purification. MS ((+) ESI): calc'd for C₇₆H₁₄₅N₄O₁₁⁺ = 1290.09 amu, obtained = 1290.00 amu.

5c and 6c: By the general procedure: step 1, **4** (0.093 g, 0.40 mmol) in dried DMF (20.0 mL), HBTU (0.363 g, 0.960 mmol), **3c** (0.500 g, 0.880 mmol) and triethylamine (0.22 mL, 1.6 mmol); step 2, TFA (5 mL) and the Boc-protected product of step 1; as a colorless oil in 60% yield (0.296 g). Data for **5c**: ¹H-NMR (300 MHz, CDCl₃) δ: 7.07 (d, 2H, J=8.6 Hz), 4.46 (m, 2H), 4.25 (m, 4H), 4.11 (m, 4H), 3.23 (s, 4H), 2.34 (m, 4H), 1.56, 1.45 (m, 16H), 1.24 (s, 80H), 0.86 (t, 24H, J=7.4 Hz). ¹³C-NMR (75 MHz, CDCl₃) δ: 176.4, 170.4, 62.3, 52.6, 48.0, 45.6, 34.6, 34.4, 32.9, 32.7, 32.5, 32.3, 32.2, 31.8, 31.6, 31.5, 29.6, 29.5, 29.4, 29.2, 29.2, 29.0, 27.4, 27.4, 25.2, 22.6, 22.6, 20.6, 14.0, 14.0, 11.3. MS ((+) ESI): calc'd for C₇₄H₁₄₂N₃O₁₀⁺ = 1233.07 amu, obtained = 1232.87 amu. Continuing the general procedure: Boc-glycine (0.030 g, 0.17 mmol) in dried DCM (1.62 mL), HOBt (0.026 g, 0.20 mmol), HBTU (0.074 g, 0.20 mmol), **5c** (0.200 g, 0.162 mmol), and triethylamine (0.068 mL, 0.49 mmol); step 4, TFA (5 mL) and the Boc-protected product of step 3 gave a light brown oil used without further purification. MS ((+) ESI): calc'd for C₇₆H₁₄₅N₄O₁₁⁺ = 1290.09 amu, obtained = 1289.93 amu.

5d and 6d: By the general procedure: step 1, **4** (0.088 g, 0.36 mmol) in dried DCM/DMF (18.2 mL/18.2 mL; 10 mM), HBTU (0.329 g, 0.867 mmol), **5d** (0.500 g, 0.801 mmol) and triethylamine (0.20 mL, 1.5 mmol); step 2, TFA (5 mL) and the Boc-protected product of step 1; as a white waxy solid in a 51% yield (0.248 g). Data for **5d**: ¹H-

NMR (300 MHz, CDCl₃) δ : 7.07 (d, 2H, J=8.6 Hz), 4.46 (m, 2H), 4.25 (m, 4H), 4.13 (m, 4H), 3.26 (s, 4H), 2.32 (t, 8H, J=7.8 Hz), 1.60 (m, 8H), 1.25 (s, 112H), 0.87 (t, 12H, J=6.8 Hz). ¹³C-NMR (75 MHz, CDCl₃) δ : 174.0, 170.8, 62.9, 52.8, 48.1, 34.3, 32.1, 29.9, 29.7, 29.6, 29.5, 29.4, 25.1, 22.9, 14.3. MS ((+) ESI): calc'd for C₈₂H₁₅₈N₃O₁₀⁺ = 1345.19 amu, obtained = 1345.07 amu. Continuing the general procedure: step 3, Boc glycine (0.013 g, 0.074 mmol) in dried DCM/DMF (3.7 mL/ 3.7 mL; 10 mM), HOBt (0.012 g, 0.085 mmol), HBTU (0.032 g, 0.085 mmol), **5d** (0.0950 g, 0.0706 mmol), and triethylamine (0.030 mL, 0.21 mmol); step 4, TFA (5 mL) and the Boc-protected product of step 3 gave a light brown oil used without further purification.

Asymmetric Disulfide Formation; preparation of **8**:

In a round bottom flask was dissolved 6-mercaptopurine monohydrate (0.500 g, 2.94 mmol) and 11-mercaptoundecanoic acid (0.641 g, 2.94 mmol) in DMSO (9.0 mL; 0.33M). While the reaction was stirred, solid DDQ (0.669 g, 2.94 mmol) was slowly added over 10 minutes and then left at room temperature for 1 hour. The reaction was monitored by TLC (silica gel, MeOH/DCM as eluent, visualized by iodine).

Following completion, the product was precipitated by adding 20 mL water to the reaction solution. The resulting mixture was left for 6 hours before being vacuum filtered and a red solid was isolated. The crude product was redissolved in hot methanol and cooled in an ice water bath then vacuum filtered. A white powder was afforded in a 60% yield (0.622 g). ¹H-NMR (300 MHz, DMSO-d₆) δ : 8.84 (s, 1H), 8.57 (s, 1H), 2.96 (t, 2H, J= 7.0 Hz), 2.21 (t, 2H, J=7.3 Hz), 1.67 (quintet, 2H, J=7.5 Hz), 1.50, 1.40 (m, 4H), 1.25 (s, 10H). ¹³C-NMR (75 MHz, DMSO-d₆) δ : 174.4, 151.7, (broad low intensity peaks), 39.5, 38.1, 33.6, 28.8, 28.7, 28.5, 28.1, 27.6, 24.5. (MS ((+) ESI): calc'd for C₁₆H₂₅N₄O₂S₂⁺ = 369.14 amu, obtained = 369.13 amu.

Similarly, in a round bottom flask was dissolved 6-thioguanine (0.500 g, 2.99 mmol) and 11-mercaptoundecanoic acid (0.652 g, 2.99 mmol) in DMSO (0.15M). While the reaction was stirred, solid DDQ (0.679 g, 2.99 mmol) was slowly added over 10 minutes and then left at room temperature for 1 hour. The reaction was monitored by TLC (silica gel, MeOH/DCM

as eluent, visualized by iodine). Following completion, the product was precipitated by adding water to the reaction solution. The resulting mixture was left for 6 hours before being vacuum filtered and a red solid was isolated. The crude product was redissolved in hot acetone and cooled in an ice water bath then vacuum filtered. A yellowish powder was afforded in 64% yield (0.707 g). ¹H-NMR (300 MHz, DMSO-d₆) δ : 7.98 (s, 1H), 6.45 (s, 2H), 2.90 (t, 2H, J=7.1 Hz), 2.17 (t, 2H, J=8.4 Hz), 1.62 (quintet, 2H, J=7.0), 1.46, 1.35 (m, 4H), 1.21 (s, 10H). ¹³C-NMR (75 MHz, CDCl₃) δ : 174.5, 159.9, 156.9, (2 broad low intensity peaks), 140.1, 39.5, 38.10, 33.6, 28.8, 28.7, 28.5, 28.01, 27.7, 24.5. MS ((+) ESI): calc'd for C₁₆H₂₆N₅O₂S₂⁺ = 384.15 amu, obtained = 384.27 amu.

LDC synthesis:

MP-C14:	R= C ₁₂ H ₂₅ ; R'= H
MP-C16:	R= C ₁₄ H ₂₉ ; R'= H
MP-dC16:	R= C ₈ H ₁₇ ; R'=C ₆ H ₁₃
MP-C18:	R= C ₁₆ H ₃₃ ; R'= H
TG-C14:	R= C ₁₂ H ₂₅ ; R'= H
TG-C16:	R= C ₁₄ H ₂₉ ; R'= H
TG-dC16:	R= C ₈ H ₁₇ ; R'=C ₆ H ₁₃

General Procedure: In a round bottom flask was dissolved **8** (1.1 equiv.) in dried DCM/DMF (1:1;10 mM). To this, HBTU (1.2 equiv.), **3(a-d)** (1.0 equiv.), and triethylamine (2.0 equiv.) were then added and the resulting solution was left stirring at room temperature for 20 hours. When the reaction was complete, visualized by ¹H-NMR, the reaction solution was diluted with EtOAc, and washed once with 1 M HCl and 3 times with H₂O. The organic layer was then dried over anhydrous sodium sulfate and then gravity filtered. DCM and EtOAc were removed by roto-evaporation. The crude product was purified by flash column chromatography on silica, using EtOAc/Hexanes as the eluent. Full characterization by ¹³C NMR was hampered by low intensity signals from quaternary carbons in the purine in many cases.

MP-C14: By the general procedure: **8-MP** (0.113 g, 0.308 mmol) in dried DCM/DMF (14.6 mL/ 14.6 mL;10 mM), HBTU (0.133 g, 0.352 mmol, 1.20 equiv.), **3a** (0.150 g, 0.293 mmol), and triethylamine (0.082 mL, 0.59 mmol); as a light yellow solid in a 28% yield (0.072 g) ¹H-NMR (300 MHz, CDCl₃) δ: 11.75 (s, 1H), 8.90 (s, 1H), 8.22 (s, 1H), 6.02 (d, 1H, J= 8.3 Hz) 4.52 (m, 1H), 4.30 (m, 2H), 4.11 (m, 2H), 2.91(t, 2H, J=7.0 Hz), 2.32 (t, 4H, J=7.9 Hz), 2.02 (t, 2H, J=7.9 Hz), 1.66 (m, 6H), 1.26, 1.12 (m, 54H), 0.88 (t, 6H, J=7.0). MS ((+) ESI): calc'd for C₄₇H₈₄N₅O₅S₂⁺ = 862.59 amu, obtained = 862.53 amu.

MP-C16: By the general procedure: **8-MP** (0.0648 g, 0.176 mmol) in dried DCM/DMF (9.0 mL/9.0 mL;10 mM), HBTU (0.112 g, 0.211 mmol), **3b** (0.0648 g, 0.176 mmol), and triethylamine (0.076 mL, 0.53 mmol); as a white solid in a 63% yield (0.102 g) ¹H-NMR (300 MHz, CDCl₃) δ: 12.88 (s, 1H), 8.91 (s, 1H), 8.29 (s, 1H), 6.05 (d, 1H, J=8.2 Hz), 4.50 (m, 1H), 4.26 (m, 2H), 4.09 (m, 2H), 2.91 (t, 2H, 7.1 Hz), 2.30 (t, 4H, J=7.6 Hz), 2.20 (t, 2H, J=7.1 Hz), 1.65 (m, 6H), 1.24, 1.17 (m, 62H), 0.87 (t, 6H, J=6.9 Hz). ¹³C-NMR (75 MHz, CDCl₃) δ: 174.1, 173.8, 160.5, 152.6, 149.9, 142.0, 131.4, 62.9, 48.0, 39.3, 36.9, 34.3, 32.1, 29.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.1, 28.7, 28.3, 25.9, 25.1, 22.9, 14.3. MS ((+) ESI): calc'd for C₅₁H₉₂N₅O₅S₂⁺ = 918.65 amu, obtained = 918.60 amu.

MP-bC16: By the general procedure: **8-MP** (0.136 g, 0.370 mmol) in dried DCM/DMF (17.5 mL/17.5 mL;10 mM), HBTU (0.160 g, 0.422 mmol), **3c** (0.200 g, 0.352 mmol), and triethylamine (0.126 mL, 0.704 mmol); As a light yellow oil in a 42% yield (0.137 g) ¹H-NMR (300 MHz, CDCl₃) δ: 12.8 (s, 1H), 8.90 (s, 1H), 8.29 (s, 1H), 5.97 (d, 1H, J=8.8 Hz), 4.48 (m, 1H), 4.26 (m, 2H), 4.09 (m, 2H), 2.90 (t, 2H, J=7.3 Hz), 2.32 (m, 2H), 2.2 (m, 2H), 1.68, 1.55, 1.42 (m, 14H), 1.22, 1.16 (m, 50H), 0.85 (t, 12H, J=6.9 Hz). ¹³C (75 MHz, CDCl₃) δ: 176.5, 173.3, 152.2, 142.0, 62.4, 48.0, 45.6, 39.1, 36.7, 32.3, 32.0, 31.8, 31.6, 29.7, 29.5, 29.4, 29.2, 29.2, 28.9, 28.5, 28.2, 27.4, 27.4, 25.6, 22.6, 22.6, 14.0, 14.0. MS ((+) ESI): calc'd for C₅₁H₉₂N₅O₅S₂⁺ = 918.65 amu, obtained = 918.53 amu.

MP-C18: By the general procedure: **8-MP** (0.062g, 0.168 mmol) in dried DCM/DMF (8.0 mL/8.0 mL;10 mM), HBTU (0.073 g, 0.19 mmol), **3d** (0.100 g, 0.160 mmol), and triethylamine (0.045 mL, 0.32 mmol)' as a white solid in a 32% yield (0.050 g). ¹H-NMR (300 MHz, CDCl₃) δ: 8.91 (s, 1H), 8.27 (s, 1H), 6.01 (d, 1H, J=8.3 Hz), 4.50 (m, 1H), 4.26 (m, 2H), 4.09 (m, 2H), 2.90 (t, 2H, J=7.2 Hz), 2.30 (t, 4H, J=7.8 Hz), 2.20 (t, 2H, J=7.8 Hz), 1.68, 1.59 (m, 6H), 1.24, 1.15 (m, 70H), 0.87 (t, 6H, J=6.9 Hz). ¹³C-NMR (75 MHz, CDCl₃) δ: 173.8, 173.5, 152.3, 62.7, 47.7, 39.1,

36.7, 34.1, 31.9, 29.7, 29.6, 29.5,, 29.3, 29.2, 29.1, 29.1, 29.0, 28.9, 28.8, 28.4, 28.0, 25.6, 24.9, 22.7, 14.1. MS ((+) ESI): calc'd for $C_{55}H_{100}N_5O_5S_2^+$ = 974.72 amu, obtained = 974.67 amu.

TG-C14: By the general procedure: **8-TG** (0.118 g, 0.308 mmol) in dried DCM/DMF (14.6 mL/ 14.6 mL;10 mM), HBTU (0.133 g, 0.352 mmol), **3a** (0.150 g, 0.293 mmol), and triethylamine (0.082 mL, 0.586 mmol); as a light yellow solid was afforded in 18% yield (0.047 g) 1H -NMR (300 MHz, $CDCl_3$) δ : 7.92 (s, 1H), 6.10 (d, 1H, $J=8.10$ Hz), 5.31 (s, 2H), 4.52 (m, 1H), 4.29 (m, 2H), 4.12 (m, 2H), 2.88 (t, 2H, $J=7.3$ Hz), 2.32 (t, 4H, $J=7.3$ Hz), 2.18 (t, 2H, $J=7.3$ Hz), 1.68, 1.59 (m, 6H), 1.25, 1.18 (m, 54H), 0.88 (t, 6H, $J=6.9$ Hz). ^{13}C -NMR (75 MHz, $CDCl_3$) δ : 174.1, 173.7, 159.7, 139.6, 63.0, 48.0, 39.4, 36.9, 34.3, 32.1, 29.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.4, 29.3, 29.1, 28.7, 28.3, 25.8, 14.3. MS ((+) ESI): calc'd for $C_{47}H_{85}N_6O_5S_2^+$ = 877.60 amu, obtained = 877.67 amu.

TG-C16: By the general procedure: **8-TG** (0.068 g, 0.18 mmol) in dried DCM/DMF (8.8 mL/ 8.8 mL;10 mM), HBTU (0.112 g, 0.211 mmol), **3b** (0.120 g, 0.18 mmol), and triethylamine (0.076 mL, 0.53 mmol); as a light yellow solid in a 49% yield (0.081 g). 1H -NMR (300 MHz, $CDCl_3$) δ : 11.23 (s, 1H), 7.87 (s, 1H), 6.08 (d, 1H, $J=8.4$ Hz), 5.24 (s, 2H), 4.51 (m, 1H), 4.29 (m, 2H), 4.11 (m, 2H), 2.87 (t, 2H, $J=7.0$ Hz), 2.31 (t, 2H, $J=7.5$ Hz), 2.17 (t, 2H, $J=7.6$ Hz), 1.68, 1.60 (m, 6H), 1.24, 1.17 (m, 62H), 0.87 (t, 6H, $J=7.0$ Hz). ^{13}C -NMR (75 MHz, $CDCl_3$) δ : 173.9, 173.5, 159.4, 62.8, 47.7, 39.2, 36.6, 34.1, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 29.0, 29.0, 28.8, 28.4, 28.1, 25.6, 24.9, 22.7, 14.1. MS ((+) ESI): calc'd for $C_{51}H_{93}N_6O_5S_2^+$ = 933.66 amu, obtained = 933.73 amu.

TG-bC16: By the general procedure: **8-TG** (0.071 g, 0.19 mmol) in dried DCM/DMF (8.8 mL/8.8 mL;10 mM), HBTU (0.080 g, 0.21), **3c** (0.100 g, 0.176 mmol), and triethylamine (0.049 mL, 0.35 mmol); as a light yellow liquid in a 37% yield (0.061 g) 1H -NMR (300 MHz, $CDCl_3$) δ : 7.88 (s, 1H), 6.05 (d, 1H, $J=8.2$ Hz), 5.20 (s, 2H), 4.50 (m, 1H), 4.30 (m, 2H), 4.12 (m, 2H), 2.87 (t, 2H, $J=7.1$ Hz), 2.35 (m, 2H), 2.15 (t, 2H, $J=7.4$ Hz), 1.68, 1.57, 1.45 (m, 14H), 1.24, 1.16 (m, 50H) 0.86 (t, 12H, $J=6.5$ Hz). ^{13}C -NMR (75 MHz, $CDCl_3$) δ : 176.7, 173.3, 159.4, 62.5, 48.0, 45.7, 39.1, 36.6, 32.3, 31.8, 31.6, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 29.0, 29.0, 28.8, 28.4, 28.0, 27.4, 27.4, 25.5, 22.6, 22.6, 14.1, 14.00. MS ((+) ESI): calc'd for $C_{51}H_{93}N_6O_5S_2^+$ = 933.66 amu, obtained = 933.60 amu.

Characterization of NHS-PEG:

PEG-lipids have a high sensitivity to ESI-MS since the ethylene chains possess a high affinity towards positively charged species such as protons, potassium or sodium ions. Information obtained from the ESI-MS spectra allowed for the determination of the degree of polymerization (n) in the starting sample (obtained from Sigma-Aldrich; n ~45).

Figure S1: Mass spectrum generated from ESI-MS of the starting material NHS-PEG. The sample was treated with 0.1% TFA and 0.1% NaCl.

ESI-MS of the NHS-PEG starting material shows five different series for multiply-charged species (Figure S1): 1, $[M + 3Na + K]^{3+}$ (orange squares); 2, $[M + 3Na]^{3+}$ (blue squares); 3, $[M + 2Na]^{2+}$ (red dots); 4, $[M + 2K]^{2+}$ (yellow dots); 5, $[M + Na + K]^{2+}$ (green dots). Series were identified by spacing (14.7 amu for triply charged; 22 amu for doubly charged). Figure S1 identifies 74 of the 87 ion clusters observed. Once the peaks were assigned, the intensities for a given n can then be summed, and the summed intensities plotted versus n with the data fit to a Gaussian with a high reliability (Figure S2). The experimental $n = 43$ by this method.

Figure S2: The intensities of a given n were summed and plotted versus n for the NHS-PEG. The plot was then fitted to a Gaussian distribution.

The ^1H -NMR of the NHS-PEG (Figure S3) suggested that the starting material contained impurities. The expected integration of the ethylene protons should be ~ 174 H; however, the observed integration was 297 H indicating that there may be polymeric impurities present. Although there were unassigned peaks in the ESI-MS (Figure S1), these peaks were not found to correspond to a polymeric series. The singlet for the terminal methoxy shows at 3.39 ppm with an integration of 5.2 H which is considerably greater than the expected 3 H. Based on the integrations it is most likely that both ends terminate with methoxy groups – suggesting an MeO-PEG-OMe like structure. Assuming this is a single impurity, purity calculations based on the ethylene proton signal and methoxy proton signal give mol % purities of 58 % and 63 %, respectively. Averaging of these two approximations gives 61 % purity for the NHS-PEG starting material.

Figure S3: The integration of the methylene protons in the ^1H NMR (300 MHz) of NHS-PEG, in CDCl_3 , was significantly different than the expected values.

Synthesis of PEG-Lipids:

Scheme S3: Amide formation between PEG-NHS and the free primary amine on the synthetic lipids

General Procedure: An equimolar mixture of **7** and the amino-lipid was made to a concentration of 0.12M in pyridine in a vial. The vial was then sealed, the solution heated to 55 °C and stirred for 48 hours. Following completion, as monitored by TLC (silica gel, MeOH/DCM as eluent, visualised by iodine), the pyridine was removed on a roto-evaporator and the resulting mixture was purified by flash column chromatography on silica gel, using MeOH/DCM as the eluent.

PEG-G1-C14: By the general procedure: **7** (0.111 g, 0.0488 mmol) and **3a** (0.025 g, 0.049 mmol); as a white solid in a 70% yield (0.087 g). $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 4.41 (m, 1H), 4.16 (m, 2H), 4.07 (m, 2H), 3.40-3.90 (m, 174H), 3.38 (s, 3H), 2.51 (s, 4H), 2.31 (t, 4H, 7.8 Hz), 1.59 (m, 4H), 1.24 (s, 40H), 0.87 (t, 6H, $J=7.0$ Hz). ESI-MS see Figure S4.

Figure S4: Mass spectrum generated from ESI-MS of **PEG-G₁-C14**. Sample was treated with 0.1% TFA and 0.1% NaCl. The compound shows $[\text{M}+3\text{Na}]^{3+}$, $[\text{M}+2\text{Na}+\text{H}]^{3+}$, $[\text{M}+2\text{Na}]^{2+}$, and $[\text{M}+\text{Na}+\text{H}]^{2+}$ ion series where 78 of the 85 observed ion clusters are assigned.

PEG-G1-C16: By the general procedure: **7** (0.100 g, 0.0440 mmol) and **3b** (0.025 g, 0.44 mmol); as a white solid in a 66% yield (0.076 g). $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 6.66 (d, 1H, $J=8.3$ Hz), 6.53 (m, 1H), 4.40 (m, 1H), 4.16 (m, 2H), 4.07 (m, 2H), 3.38-3.90 (m, 174H), 3.37 (s, 3H), 2.51 (s, 4H), 2.31 (t, 4H, $J=7.5$ Hz), 1.59 (m, 4H), 1.25 (s, 48H), 0.87 (t, 6H, $J=6.8$ Hz). ESI-MS see Figure S5

Figure S5: Mass spectrum generated from ESI-MS of **PEG-G₁-C16**. Sample was treated with 0.1% TFA and 0.1% NaCl. The compound shows $[\text{M}+3\text{Na}]^{3+}$, $[\text{M}+2\text{Na}]^{2+}$, and $[\text{M}+\text{Na}+\text{H}]^{2+}$ ion series where 60 of the 67 observed ion clusters are assigned.

PEG-G1-bC16: By the general procedure: **7** (0.100 g, 0.0440 mmol) and **3c** (0.025 g, 0.044 mmol). A gel-like colorless solid was isolated in a 68% yield (0.087 g). $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 4.40 (m, 1H), 4.19 (m, 2H), 4.04 (m, 2H), 3.39-3.90 (m, 174), 3.38 (s, 3H), 2.51 (s, 4H), 2.34 (m, 2H), 1.57, 1.45 (m, 8H), 1.25 (s, 40H), 0.87 (t, 12H, $J=7.2$ Hz). ESI-MS see Figure S6.

Figure S6: Mass spectrum generated from ESI-MS of **PEG-G1-bC16**. Sample was treated with 0.1% TFA and 0.1% NaCl. The compound shows $[\text{M}+3\text{Na}]^{3+}$, $[\text{M}+2\text{Na}]^{2+}$, and $[\text{M}+\text{Na}+\text{H}]^{2+}$ ion series where 56 of the 60 ion clusters are assigned.

PEG-G1-C18: By the general procedure: **7** (0.091 g 0.040 mmol) and **3d** (0.025 g, 0.040 mmol); as a white solid in a 67% yield (0.071 g). $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 6.69 (d, 1H, $J=8.9$ Hz), 6.61 (m, 1H), 4.41 (m, 1H), 4.16 (m, 2H), 4.07 (m, 2H), 3.38-3.90 (m, 174H), 3.37 (s, 3H), 2.51 (s, 4H), 2.31 (t, 4H, $J=7.8$ Hz), 1.61 (m, 4H), 1.24 (s, 56H), 0.87 (t, 6H, $J=7$ Hz). ESI-MS see Figure S7.

Figure S7: Mass spectrum generated from ESI-MS of **PEG-G1-C18**. Sample was treated with 0.1% TFA and 0.1% NaCl. The compound shows $[\text{M}+3\text{Na}]^{3+}$, $[\text{M}+2\text{Na}+\text{H}]^{3+}$, $[\text{M}+2\text{Na}]^{2+}$, and $[\text{M}+\text{Na}+\text{H}]^{2+}$ ion series where 79 of the 87 observed ion clusters are assigned.

PEG-G2-C14: By the general procedure: **7** (0.098 g, 0.043 mmol) and crude **6a** (0.051 g, 0.043 mmol); as a white solid in a 52% yield (0.072 g). $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 4.39 (m, 2H), 3.89-4.26 (m, 14H), 3.37-3.90 (m, 174H), 3.36 (s, 3H), 2.52 (m, 4H), 2.31 (t, 4H, $J=7.6$ Hz), 2.29 (t, 4H, $J=7.6$ Hz), 1.24 (s, 80H), 0.86 (t, 12H, 6.8 Hz). ESI-MS see Figure S8.

Figure S8: Mass spectrum generated from ESI-MS of **PEG-G2-C14**. Sample was treated with 0.1% TFA and 0.1% NaCl. The compound shows $[\text{M}+4\text{Na}]^{4+}$, $[\text{M}+3\text{Na}]^{3+}$, $[\text{M}+2\text{Na}+\text{H}]^{3+}$, and $[\text{M}+2\text{Na}]^{2+}$ ion series where 64 of the 71 observed ion clusters are assigned.

PEG-G2-C16: By the general procedure: **7** (0.123 g, 0.054 mmol) and crude **6b** (0.070 g, 0.054 mmol); as a white solid in 54% yield (0.096 g). $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 4.38 (m, 2H), 3.88-4.26 (m, 14H), 3.37-3.87 (m, 174H), 3.36 (s, 3H), 2.53 (m, 4H), 2.31 (t, 4H, $J=7.6$ Hz), 2.29 (t, 4H, $J=7.6$ Hz), 1.24 (s, 96H), 0.86 (t, 12H, $J=7.0$ Hz). ESI-MS see Figure S9.

Figure S9: Mass spectrum generated from ESI-MS of **PEG-G2-C16**. Sample was treated with 0.1% TFA and 0.1% NaCl. The compound shows $[M+4Na]^{4+}$, $[M+3Na]^{3+}$, and $[M+2Na+H]^{3+}$ ion series where 48 of the 56 observed ion clusters are assigned.

PEG-G2-bC16: By the general procedure: **7** (0.130 g, 0.0572 mmol) and crude **6c** (0.074 g, 0.057 mmol); as a white solid in 33% yield (0.061 g). $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 6.74 (s, 1H), 6.66 (d, 1H, $J = 7.5$ Hz), 6.47 (s, 1H), 4.40 (m, 2H), 3.87-4.22 (m, 14H), 3.36-3.87 (m, 174H), 3.35 (s, 3H), 2.51 (m, 4H), 2.31 (m, 4H), 1.42, 1.53 (m, 16H), 1.22 (s, 80H), 0.85 (t, 24H, $J = 7.0$ Hz). ESI-MS see Figure S10.

Figure S10: Mass spectrum generated from ESI-MS OF **PEG-G2-bC16**. Sample was treated with 0.1% TFA and 0.1% NaCl. The compound shows $[M+4Na]^{4+}$, $[M+3Na+H]^{4+}$, $[M+3Na]^{3+}$, $[M+2Na+H]^{3+}$, and $[M+Na+H]^{2+}$ ion series where 83 of the 92 observed ion clusters are assigned.

PEG-G2-C18: By the general procedure: **7** (0.065 g, 0.029 mmol) and crude **6d** (0.040 g, 0.029 mmol, 1.0 equiv.); as a gel-like solid in 47% yield (0.047 g). $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 4.39 (m, 2H), 3.89-4.27 (m, 14H), 3.38-3.89 (m, 174H), 3.37 (s, 3H), 2.53 (m, 4H), 2.32 (t, 4H, $J = 7.4$ Hz), 2.29 (t, 4H, $J = 7.4$ Hz), 1.24 (s, 112H), 0.87 (t, 12H, $J = 7.0$ Hz). ESI-MS see Figure S11.

Figure S11: Mass spectrum generated from ESI-MS of **PEG-G2-C18**. Sample was treated with 0.1% TFA and 0.1% NaCl. The compound shows $[M+4Na]^{4+}$, $[M+3Na]^{3+}$, $[M+2Na+H]^{3+}$, and $[M+2Na]^{2+}$ ion series where 73 of the 83 observed ion clusters were assigned.

Table S0 Summary of Gaussian fitting to the sum of intensities observed for a given N

	center	width	R ²
7	43.5 ± 0.12	14 ± 1.5	0.9796
PEG-G1-C14	43.0 ± 0.22	24 ± 15	0.912
PEG-G1-C16	43.6 ± 0.27	17 ± 4.5	0.9068
PEG-G1-brC16	43.1 ± 0.38	14 ± 3.9	0.829
PEG-G1-C18	43.7 ± 0.17	12 ± 1.1	0.9642
PEG-G2-C14	43.1 ± 0.14	11 ± 1.7	0.969
PEG-G2-C16	43.0 ± 0.17	9.0 ± 1.3	0.9517
PEG-G2-brC16	46.5 ± 0.8	17 ± 16	0.6933
PEG-G2-C18	43.0 ± 0.24	9.5 ± 2.1	0.9081

Preparation of LNPs

LNPs were prepared using a NanoAssemblr™ microfluidic mixer by the controlled mixing of ethanol solutions of lipid mixture with aqueous buffer.

For the preparation on LNPs from LDC, stock solutions of lipids were made up in ethanol with a lipid concentration of 10 mM. In an eppendorf tube, the lipid mixture of PEG-lipid (5.00 mol %), DMPC (5.00 mol %), and LDC (90.00 mol %) was made up to a total volume of 0.25 mL with additional ethanol. The mixture was then heated to 43 °C for 5 minutes in the oven. The volumetric compositions are given in Table S1

LDC	LDC [Stock] (mg/mL)	Volume of LDC Added (uL)	Volume of DSPE-PEG Added (uL)	Volume of DMPC Added (uL)	Volume of EtOH Added (uL)
MP-C14	10.0	204.0	36.9	8.9	0.0
MP-C16	10.4	199.0	35.0	8.5	7.9
MP-bC16	10.0	206.5	35.0	8.5	0.0
MP-C18	12.4	168.9	33.4	8.1	39.7
TG-C14	17.7	115.7	36.4	8.8	89.1
TG-C16	13.7	151.1	34.6	8.4	55.9
TG-bC16	16.6	124.8	34.6	8.4	82.3

Table S1: LDC composition with stock [DSPE-PEG] = 10.0 mg/mL and stock [DMPC] = 10.0 mg/mL where the composition was 90: 5: 5 (LDC monomer: DMPC: DSPE-PEG). The drug loading was 15 wt%.

For the evaluation of PEG-lipid in LNPs, the molar composition used in formulation was 1.5:10:38.5:50 (PEG-Lipid: Neutral Lipid: Cholesterol: Cationic/Ionizable Lipid) and with a lipid concentration of 12.5 mM. Stock solutions were made up in ethanol and the required amounts were transferred and then made to the required concentration by dilution with ethanol. The lipid mixture was made up in an Eppendorf tube from stock solutions of PEG-lipid (see Table A2.1), DSPC (20 mg/mL; volume added: 27.2 uL), DOTMA (40 mg/mL; volume added: 57.6 uL), and cholesterol (20 mg/mL; volume added: 51.2 uL). The final volume was made up to a total volume of 0.55 mL with additional ethanol (see Table S2). The mixture was then sealed and heated to 43 °C for 5 minutes in the oven. A stock solution of the dsDNA oligomer (5'-CGC GCG TAT ATA CGC GCG-3'; 50.00 mg/mL) was made up in PBS buffer (10.0 mM Na₃PO₄, 0.154 M NaCl, and made to pH 7.4 using concentrated HCl, in Millipore water). To a glass vial, 6.0 uL of the dsDNA stock solution was added and made up to a volume of 2.244 mL with sodium acetate buffer (25.00 mM; pH 4 using concentrated HCl to adjust the pH).

PEG-Lipid	PEG-Lipids [Stock] (mg/mL)	Volume of PEG-Lipid Added (uL)	Volume of EtOH Added (uL)
PEG-G1-C14	19.2	13.6	400.4
PEG-G1-C16	18.6	14.4	399.6
PEG-G1-bC16	17.4	15.4	398.6
PEG-G1-C18	17.9	15.3	398.8
PEG-G2-C14	30.6	10.8	403.3
PEG-G2-C16	29.6	11.6	402.5
PEG-G2-bC16	29.0	11.8	402.3
PEG-G2-C18	30.3	11.7	402.4
DSG-PEG	20.0	13.5	400.5
DSPE-PEG	10.0	28.9	385.1

Table S2: Concentrations of PEG-lipid solutions and required volumes for formulations containing 1.5 mol% of different PEG-lipids. The composition for these SLNPs was 50: 10: 38.5: 1.5 (DOTMA: DSPC: Cholesterol: PEG-lipid) with a DNA loading of 5.1 wt%.

Prior to using, the NanoAssemblr™ cartridge was washed with PBS buffer in a 3 mL syringe (left port) and ethanol in a 3 mL syringe (right port) with a 12 mL/min. flow rate and a 1:1 (aqueous:ethanol ratio) flow ratio. A total of 4 mL of wash was collected and discarded. The NanoAssemblr™ microfluidic mixer was then used to make the LNPs. In a 3 mL syringe (left port) was loaded 2 mL PBS buffer and in another 3 mL syringe (right port)

was loaded the 0.25 mL lipid mixture. The flow rate was set to 4 mL/min., the flow ratio to 3:1 (aqueous:EtOH), and the total volume collected 1 mL with the initial 300 uL at the beginning and 50 uL at the end being discarded.

For LNP containing LDCs, an aliquot (0.50 mL) of the collected formulation was then diluted to 0.40 mM (thiopurine) with PBS buffer, transferred to a 3 mL Slide-A-Lyzer® Dialysis Cassette G2 (10,000 molecular weight cutoff) and dialyzed against PBS buffer for 5 hours. The PBS was refreshed after 3 hours and the removal of ethanol from the formulation was monitored using potassium dichromate.

For PEG-lipid LNPs containing dsDNA, The collected formulation was immediately transferred to a 3 mL Slide-A-Lyzer® Dialysis Cassette G2 (10,000 molecular weight cutoff) and dialyzed against PBS buffer for 6 hours. The PBS was refreshed after 3 hours and the removal of ethanol from the formulation was monitored using potassium dichromate (stable orange colour without greenish reaction indicated EtOH removal).

Z_{avg} diameters of the LNPs were determined by dynamic light scattering (DLS) experiments (Brookhaven Instrument, ZetaPALS particle sizing software). The LNPs were then stored at 4 °C.

Particle stability assay methods

The stability of the formulated LNPs was assessed under 6 conditions: LNPs were stored at 3 different temperatures; 4 °C, RT, and 37 °C, and incubated in 2 different media; PBS buffer and PBS buffer with 10% serum (by volume). To a polystyrene cuvette, 200 uL of LNPs solution and 2.30 mL of PBS buffer were added and the diameters measured in a DLS experiment. Cuvettes were then sealed and incubated under the indicated conditions. Diameter measurements were taken regularly. To a polystyrene cuvette, 200 uL of LNPs solution, 2.05 mL of PBS buffer, and 0.25 mL of serum were added and the diameters measured in a DLS experiment. Cuvettes were sealed and incubated under the indicated conditions.

Serum proteins scatter light and therefore interfere with direct determination of LNP diameter and PDI. The DLS values for LNPs together with serum represent a weighted size average between the LNPs and the serum particles. The weighted average is sample specific; changes of a given sample with time have relevance to the particle stability, but comparisons between samples is not possible.

LDC particle stability

LDC	LDC Diameter (nm)	Polydispersity (PDI)	Volume of PBS buffer Added (mL)
MP-C14	227.5 ± 11.3	0.346 ± 0.009	2.59
MP-C16	216.7 ± 7.7	0.288 ± 0.004	2.44
MP-bC16	80.9 ± 0.9	0.277 ± 0.003	2.44
MP-C18	560.3 ± 10.1	0.358 ± 0.010	2.30
TG-C14	489.7 ± 14.1	0.343 ± 0.007	2.55
TG-C16	102.2 ± 2.2	0.237 ± 0.006	2.40
TG-bC16	64.8 ± 1.2	0.220 ± 0.007	2.40

Table S3: Physical Characterizations of LDC formulations immediately following dialysis where the composition was 90: 5: 5 (LDC monomer: DMPC: DSPE-PEG). The drug loading was 15 wt%.

	Day 1 Size(nm); PDI	Day 30 Size(nm); PDI	Day 60 Size(nm); PDI
MP-C14	227.5 ± 11.3; 0.346 ± 0.009	438.4 ± 5.4; 0.267 ± 0.004	--
MP-C16	216.7 ± 7.7; 0.288 ± 0.004	220.2 ± 2.3; 0.209 ± 0.013	205.4 ± 2.1; 0.231 ± 0.014
MP-bC16	80.9 ± 0.9; 0.277 ± 0.003	90.1 ± 0.5; 0.284 ± 0.002	100.5 ± 0.6; 0.296 ± 0.002
MP-C18	560.3 ± 10.1; 0.358 ± 0.010	807.4 ± 10.8; 0.371 ± 0.003	--
TG-C14	489.7 ± 14.1; 0.343 ± 0.007	1013.7 ± 85.4; 0.427 ± 0.036	--
TG-C16	102.2 ± 2.2; 0.237 ± 0.006	101.6 ± 0.3; 0.215 ± 0.008	113.9 ± 0.3; 0.279 ± 0.010
TG-bC16	64.8 ± 1.2; 0.220 ± 0.007	73.8 ± 1.3; 0.245 ± 0.009	93.8 ± 1.7; 0.314 ± 0.012

Table S4: LDC storage stability at 4 °C in PBS buffer where the composition was 90: 5: 5 (LDC monomer: DMPC: DSPE-PEG). The drug loading was 15 wt%.

LDC	Day 1 Size(nm); PDI	Day 30 Size(nm); PDI
MP-C16	160.7 ± 3.7; 0.399 ± 0.004	162.1 ± 4.8; 0.401 ± 0.007
MP-bC16	126.8 ± 2.3; 0.406 ± 0.002	128.7 ± 2.6; 0.396 ± 0.003
TG-C16	134.8 ± 3.1; 0.408 ± 0.002	138.4 ± 1.7; 0.405 ± 0.004
TG-bC16	128.6 ± 7.4; 0.413 ± 0.004	131.7 ± 2.5; 0.405 ± 0.003

Table S5: LDC storage stability at 4 °C in PBS buffer with 10% serum where the composition was 90: 5: 5 (LDC monomer: DMPC: DSPE-PEG). The drug loading was 15 wt%.

LDC	Day 1 Size(nm); PDI	Day 30 Size (nm); PDI	Day 60 Size (nm); PDI
MP-C16	216.7 ± 7.7; 0.288 ± 0.004	205.2 ± 7.3; 0.224 ± 0.003	220.5 ± 7.8; 0.212 ± 0.003
MP-bC16	80.9 ± 0.9; 0.277 ± 0.003	83.3 ± 0.9; 0.259 ± 0.003	81.3 ± 0.9; 0.261 ± 0.003
TG-C16	102.2 ± 2.2; 0.237 ± 0.006	121.6 ± 2.6; 0.147 ± 0.004	124.4 ± 2.7; 0.164 ± 0.004
TG-bC16	64.8 ± 1.2; 0.220 ± 0.007	73.8 ± 1.4; 0.275 ± 0.009	69.5 ± 1.3; 0.219 ± 0.007

Table S6: LDC storage stability at RT in PBS buffer where the composition was 90: 5: 5 (LDC monomer: DMPC: DSPE-PEG). The drug loading was 15 wt%.

LDC	Day 1 Size(nm); PDI	Day 30 Size (nm); PDI	Day 60 Size (nm); PDI
MP-C16	160.7 ± 3.7; 0.399 ± 0.004	230.8 ± 5.3; 0.413 ± 0.004	309.0 ± 7.1; 0.418 ± 0.004
MP-bC16	126.8 ± 2.3; 0.406 ± 0.002	163.5 ± 3.0; 0.397 ± 0.002	123.3 ± 2.3; 0.353 ± 0.002
TG-C16	134.8 ± 3.1; 0.408 ± 0.002	134.4 ± 3.1; 0.407 ± 0.002	139.6 ± 3.2; 0.413 ± 0.002
TG-bC16	128.6 ± 7.4; 0.413 ± 0.004	126.8 ± 7.3; 0.401 ± 0.004	199.8 ± 11.5; 0.433 ± 0.004

Table S7: LDC storage stability at RT in PBS buffer with 10% serum where the composition was 90: 5: 5 (LDC monomer: DMPC: DSPE-PEG). The drug loading was 15 wt%.

LDC	Day 1 Size(nm); PDI	Day 2 Size(nm); PDI	Day 3 Size (nm); PDI
MP-C16	216.7 ± 7.7; 0.288 ± 0.004	262.1 ± 5.0; 0.208 ± 0.007	260.5 ± 2.5 ; 0.221 ± 0.010
MP-bC16	80.9 ± 0.9; 0.277 ± 0.003	101.5 ± 4.2; 0.315 ± 0.007	95.0 ± 4.1; 0.309 ± 0.008
TG-C16	102.2 ± 2.2; 0.237 ± 0.006	189.3 ± 5.7; 0.208 ± 0.011	182.5 ± 0.7; 0.173 ± 0.005
TG-bC16	64.8 ± 1.2; 0.220 ± 0.007	72.9 ± 1.9; 0.251 ± 0.003	76.3 ± 1.8; 0.258 ± 0.013

Table S8: LDC storage stability at 37 °C in PBS buffer where the composition was 90: 5: 5 (LDC monomer: DMPC: DSPE-PEG). The drug loading was 15 wt%.

LDC	Day 1 Size(nm); PDI	Day 2 Size(nm); PDI	Day 3 Size (nm); PDI
MP-C16	160.7 ± 3.7; 0.399 ± 0.004	189.3 ± 2.3; 0.393 ± 0.003	186.0 ± 1.3; 0.402 ± 0.008
MP-bC16	126.8 ± 2.3; 0.406 ± 0.002	139.6 ± 16.8; 0.379 ± 0.001	134.4 ± 2.6; 0.388 ± 0.005
TG-C16	134.8 ± 3.1; 0.408 ± 0.002	143.3 ± 7.1; 0.378 ± 0.008	139.2 ± 3.0; 0.388 ± 0.004
TG-bC16	128.6 ± 7.4; 0.413 ± 0.004	154.3 ± 15.1; 0.387 ± 0.001	155.4 ± 2.8; 0.375 ± 0.004

Table S9: LDC storage stability at 37 °C in PBS buffer with 10% serum where the composition was 90: 5: 5 (LDC monomer: DMPC: DSPE-PEG). The drug loading was 15 wt%.

PEG-lipid particle stability

PEG-Lipids	SNP Diameter (nm)	Polydispersity
PEG-G1-C14	104.5 ± 1.5	0.307 ± 0.006
PEG-G1-C16	76.9 ± 0.7	0.259 ± 0.004
PEG-G1-bC16	156.1 ± 1.9	0.248 ± 0.008
PEG-G1-C18	146.1 ± 1.4	0.335 ± 0.003
PEG-G2-C14	181.4 ± 21.4	0.280 ± 0.137
PEG-G2-C16	211.1 ± 4.8	0.373 ± 0.016
PEG-G2-bC16	119.5 ± 7.1	0.351 ± 0.025
PEG-G2-C18	165.6 ± 11.3	0.394 ± 0.031
DSG-PEG	80.5 ± 0.9	0.268 ± 0.005
DSPE-PEG	89.0 ± 0.4	0.297 ± 0.002

Table S10: Physical characterization of the LNP formulations immediately following preparation for formulations containing 1.5 mol% of different PEG-lipids. The composition for these SLNPs was 50: 10: 38.5: 1.5 (DOTMA: DSPC: Cholesterol: PEG-lipid) with a DNA loading of 5.1 wt%.

Formulation	Day 1 Size (nm); PDI	Day 8 Size (nm); PDI
PEG-G1-C14	104.5 ± 1.5; 0.307 ± 0.006	166.7 ± 2.4; 0.222 ± 0.004
PEG-G1-C16	76.9 ± 0.7; 0.259 ± 0.004	144.2 ± 1.3; 0.212 ± 0.003
PEG-G1-bC16	156.1 ± 1.9; 0.248 ± 0.008	176.6 ± 2.1; 0.232 ± 0.007
PEG-G1-C18	146.1 ± 1.4; 0.335 ± 0.003	172.0 ± 1.6; 0.280 ± 0.002
DSG-PEG	80.5 ± 0.9; 0.268 ± 0.005	118.2 ± 1.3; 0.268 ± 0.005
DSPE-PEG	89.0 ± 0.4; 0.297 ± 0.002	126.2 ± 0.6; 0.293 ± 0.002

Table S11: LNP storage stability at RT in PBS buffer for formulations containing 1.5 mol% of different PEG-lipids. The composition for these SLNPs was 50: 10: 38.5: 1.5 (DOTMA: DSPC: Cholesterol: PEG-lipid) with a DNA loading of 5.1 wt%.

Formulation	Day 1 Size (nm); PDI	Day 8 Size (nm); PDI
PEG-G1-C14	134.1 ± 1.1 ; 0.350 ± 0.001	197.1 ± 1.6; 0.345 ± 0.001
PEG-G1-C16	129.6 ± 1.4; 0.340 ± 0.000	225.1 ± 2.4; 0.381 ± 0.005
PEG-G1-bC16	178.3 ± 5.5; 0.337 ± 0.005	249.1 ± 7.7; 0.338 ± 0.005
PEG-G1-C18	166.8 ± 4.4; 0.345 ± 0.003	290.7 ± 7.7; 0.376 ± 0.003
DSG-PEG	117.9 ± 2.2; 0.357 ± 0.004	223.7 ± 4.2; 0.383 ± 0.004
DSPE-PEG	129.0 ± 4.8; 0.349 ± 0.005	212.3 ± 7.9; 0.389 ± 0.006

Table S12: SLNP storage stability at RT in PBS buffer with 10% serum for formulations containing 1.5 mol% of different PEG-lipids. The composition for these SLNPs was 50: 10: 38.5: 1.5 (DOTMA: DSPC: Cholesterol: PEG-lipid) with a DNA loading of 5.1 wt%.

Formulation	Day 1 Size (nm); PDI	Day 2 Size (nm); PDI	Day 5 Size (nm); PDI
PEG-G1-C14	104.5 ± 1.5; 0.307 ± 0.006	175.2 ± 1.1; 0.194 ± 0.010	228.6 ± 1.4; 0.164 ± 0.008
PEG-G1-C16	76.9 ± 0.7; 0.259 ± 0.004	157.2 ± 1.4; 0.204 ± 0.002	194.3 ± 1.7; 0.131 ± 0.001
PEG-G1-bC16	156.1 ± 1.9; 0.248 ± 0.008	198.7 ± 7.5; 0.088 ± 0.042	180.5 ± 6.8; 0.142 ± 0.012
PEG-G1-C18	146.1 ± 1.4; 0.335 ± 0.003	173.0 ± 1.4; 0.245 ± 0.008	216.3 ± 1.8; 0.204 ± 0.007
DSG-PEG	80.5 ± 0.9; 0.268 ± 0.005	299.1 ± 16.0; 0.230 ± 0.004	192.1 ± 10.3; 0.187 ± 0.003
DSPE-PEG	89.0 ± 0.4; 0.297 ± 0.002	136.5 ± 1.7; 0.255 ± 0.005	175.4 ± 2.2; 0.146 ± 0.003

Table S13: LNP stability at 37°C in PBS buffer for formulations containing 1.5 mol% of different PEG-lipids. The composition for these SLNPs was 50: 10: 38.5: 1.5 (DOTMA: DSPC: Cholesterol: PEG-lipid) with a DNA loading of 5.1 wt%.

Formulation	Day 1 Size (nm); PDI	Day 2 Size (nm); PDI	Day 5 Size (nm); PDI
PEG-G1-C14	134.1 ± 1.1; 0.350 ± 0.001	190.1 ± 2.8; 0.291 ± 0.012	194.8 ± 2.9; 0.349 ± 0.014
PEG-G1-C16	129.6 ± 1.4; 0.340 ± 0.000	171.5 ± 1.9; 0.344 ± 0.005	165.3 ± 1.8; 0.351 ± 0.005
PEG-G1-bC16	178.3 ± 5.5; 0.337 ± 0.005	211.1 ± 16.8; 0.194 ± 0.069	220.0 ± 14.4; 0.363 ± 0.047
PEG-G1-C18	166.8 ± 4.4; 0.345 ± 0.003	237.9 ± 23.3; 0.320 ± 0.042	204.2 ± 19.9; 0.363 ± 0.048
DSG-PEG	117.9 ± 2.2; 0.357 ± 0.004	167.9 ± 9.4; 0.366 ± 0.002	120.5 ± 6.7; 0.356 ± 0.002
DSPE-PEG	129.0 ± 4.8; 0.349 ± 0.005	149.4 ± 6.0; 0.370 ± 0.005	197.8 ± 7.9; 0.365 ± 0.005

Table S14: LNP stability at 37°C in PBS buffer with 10% serum for formulations containing 1.5 mol% of different PEG-lipids. The composition for these SLNPs was 50: 10: 38.5: 1.5 (DOTMA: DSPC: Cholesterol: PEG-lipid) with a DNA loading of 5.1 wt%.

PEG-Lipid	Particle Diameter (nm)	PDI
DMG-PEG	65.2	0.249
PEG-G2-bC16	73.6	0.112
PEG-G2-C16	86.1	0.262

Table S15: The physical characterizations of formulations using the proprietary lipid mix are shown above. The DNA loading was 5.1% with a corresponding charge ratio of 5. The composition of the commercial mix was 50: 10: 38: 1.5 (Ionisable lipid: DSPC: Cholesterol: PEG-lipid) where the final 0.5 mol% was a fluorescent lipid-marker.

PEG-Lipid	Day 1 Size (nm); PDI	Day 8 Size (nm); PDI
DMG-PEG	65.1; 0.248	304.0; 0.263
PEG-G2-bC16	74.2; 0.122	124.3; 0.183
PEG-G2-C16	86.1; 0.262	259.1; 0.183

Table S16: LNP stability at 37°C in PBS buffer for formulations using the proprietary lipid mix. The DNA loading was 5.1% with a corresponding charge ratio of 5. The composition of the commercial mix was 50: 10: 38: 1.5 (Ionisable lipid: DSPC: Cholesterol: PEG-lipid) where the final 0.5 mol% was a fluorescent lipid-marker.

LNP imaging

Cryo-SEM images were prepared by applying the sample, doped with 5 nm gold particles for calibration, to a lacey carbon EM grid. Filter paper was then used to blot the sample and to dehydrate it. The grid was then frozen in liquid ethane. For the LNP containing LDCs, every sample preparation protocol resulted in dense particle aggregates (Figure S11). Individual particles are evident in these masses, with approximately the sizes expected from the DLS experiments; hydrophobic association apparently drives the aggregation during sample preparation.

Figure S11: SEM images of LDC formulated from **TG-C16**. An aliquot of the LDC mixture (440 μ M) in PBS (0.1 M NaCl; 10 mM phosphate pH 7.4) was dried on a lacey carbon grid prior to imaging. Panel A shows one of the large aggregates held on the grid; panel B shows an expanded section of this aggregate.

Preparation steps also damaged the samples from PEG-lipid compositions resulting in aggregation and coalescence. Some images (Figure S12 lower) show regions that are irregular on the particle surface suggesting that particle dehydration during the preparation has fractured the particle.

Figure S12: Cryo-SEM image of **PEG-G₁-C16** sample showing particles with a generally spherical morphology.

Bioassay methods

MCF7 cells were seeded on 24-well plates at 5.0×10^4 cells/well, 18 h later were treated with the indicated compounds in growth medium (Dulbecco's Modified Eagle's Medium [DMEM] supplemented with 10% Bovine Growth Serum [BGS]). DMSO was used at a concentration of 400 μ M in growth medium as a control for **6-TG** and **6-MP**, and Na_3PO_4 buffer (pH 7, $[\text{Na}_3\text{PO}_4] = 10$ mM $[\text{NaCl}] = 0.1$ M) was used at a concentration of 40 μ M in growth medium as a control for **6TG-C16**, **6TG-bC16**, **6MP-C16**, and **6MP-bC16**. After 72 h of treatment, cells were washed with phosphate-buffered saline (PBS), fixed for 10 mins with 4% paraformaldehyde in PBS, stained for 30 mins with 0.1% crystal violet in H_2O , washed twice with H_2O , and let dry. 10% acetic acid was added to wells and incubated on a shaker for 10 mins. Absorbance was measured at 590 nm using a PerkinElmer Victor³V 1420 multilabel plate counter. The absorbance from stained wells without cells was subtracted from experimental values to eliminate background absorbance from crystal violet adhering to the plate. % viability was calculated as (Absorbance compounds/Absorbance control buffer)*100.

A one-way ANOVA was performed for the experiments on MCF-7 cells: **TG**, $F(7, 16) = 243.8$ and $P < 0.001$; **MP**, $F(7, 16) = 18.77$ and $P < 0.001$; **TG-C16**, $F(5, 12) = 23.58$ and $P < 0.001$; **TG-bC16**, $F(5, 12) = 24.97$ and $P < 0.001$; **MP-C16**, $F(5, 12) = 18.21$ and $P < 0.001$; **MP-C16**, $F(5, 12) = 18.16$ and $P < 0.001$, followed by Tukey's multiple-comparison *post hoc* test to determine whether there were significant differences between groups. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Error bars represent standard deviation.

A one-way ANOVA was also performed for the experiments on HeLa cells: **TG**, $F(7, 16) = 24.69$, $P < 0.001$; **MP**, $F(7, 16) = 13.37$ and $P < 0.001$; **TG-C16**, $F(6, 14) = 26.51$ and $P < 0.001$; **TG-bC16**, $F(6, 14) = 2.06$ and $P > 0.05$; **MP-C16**, $F(6, 17) = 2.24$ and $P > 0.05$; **MP-bC16**, $F(6, 14) = 0.57$ and $P > 0.05$, followed by Tukey's multiple-comparison *post hoc* test to determine whether there were significant differences between groups. **, $P < 0.01$; ***, $P < 0.001$. Error bars represent standard deviation.

Cell viability data

Figure S13: Viability of MCF-7 cells with 6-mercaptopurine in the growth medium at the indicated concentrations. ANOVA statistics for are reported above; conditions marked *** are significantly different from untreated cells at $P < 0.001$.

Figure S14: Viability of MCF-7 cells with 6-thioguanine in the growth medium at the indicated concentrations. ANOVA statistics for this experiment are reported above; conditions marked *** are significantly different from untreated cells at $P < 0.001$.

Figure S15: Viability of MCF-7 cells with **MP-C16** in the growth medium at the indicated concentrations. Statistics for this experiment are reported above; conditions marked *** are significantly different from untreated cells at $P < 0.001$.

Figure S16: Viability of MCF-7 cells with **MP-bC16** in the growth medium at the indicated concentrations. ANOVA statistics for this experiment are reported above; conditions marked * and *** are significantly different from untreated cells at $P<0.05$ and $P<0.001$ respectively.

Figure S17: Viability of MCF-7 cells with **TG-C16** in the growth medium at the indicated concentrations. ANOVA statistics for this experiment are reported above; conditions marked ** and *** are significantly different from untreated cells at $P<0.01$ and $P<0.001$ respectively.

Figure S18: Viability of MCF-7 cells with **TG-bC16** in the growth medium at the indicated concentrations. ANOVA statistics for this experiment are reported above; conditions marked *, ** and *** are significantly different from untreated cells at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

Figure S19: Viability of HeLa cells with 6-mercaptopurine in the growth medium at the indicated concentrations. ANOVA statistics for this experiment are reported above; conditions marked ** and *** are significantly different from untreated cells at $P < 0.01$ and $P < 0.001$ respectively.

Figure S20: Viability of HeLa cells with 6-thioguanine in the growth medium at the indicated concentrations. ANOVA statistics for this experiment are reported above; conditions marked ** and *** are significantly different from untreated cells at $P < 0.01$ and $P < 0.001$ respectively.

Figure S21: Viability of HeLa cells with **MP-C16** in the growth medium at the indicated concentrations. ANOVA statistics for this experiment are reported above.

Figure S22: Viability of HeLa cells with **MP-bC16** in the growth medium at the indicated concentrations. ANOVA statistics for this experiment are reported above.

Figure S23: Viability of HeLa cells with **TG-C16** in the growth medium at the indicated concentrations. ANOVA statistics for this experiment are reported above; conditions marked *** are significantly different from untreated cells at $P < 0.001$.

Figure S24: Viability of HeLa cells with **TG-C16** in the growth medium at the indicated concentrations. ANOVA statistics for this experiment are reported above.

NMR spectra of compounds prepared

3a ^1H NMR (CDCl_3) 300 MHz

3a ^{13}C NMR (CDCl_3) 75 MHz

3b ^1H NMR (CDCl_3) 300 MHz

3b ^{13}C NMR (CDCl_3) 75 MHz

3c ^1H NMR (CDCl_3) 300 MHz

3c ^{13}C NMR (CDCl_3) 75 MHz

3d ^1H NMR (CDCl_3) 300 MHz

3d ^{13}C NMR (CDCl_3) 75 MHz

5a ^1H NMR (CDCl_3) 300 MHz

— 7.267
— 7.016

— 4.459
— 4.241
— 4.119

— 3.248

— 2.311

— 1.585
— 1.248
— 0.870

5a ^{13}C NMR (CDCl_3) 75 MHz

5b ¹H NMR (CDCl₃) 300 MHz

5b ^{13}C NMR (CDCl_3) 75 MHz

— 176.38
— 170.38

62.26
52.58
48.04
45.61
34.62
34.46
32.85
32.70
32.50
32.26
32.22
31.81
31.62
31.53
29.64
29.54
29.39
29.25
29.18
29.01
27.41
27.36
25.23

8MP ^1H NMR (DMSO-d_6) 300 MHz

8MP ^{13}C NMR(DMSO d_6) 75 MHz

—174.45

—151.67

39.52
38.13
33.64
28.78
28.66
28.49
28.11
27.63
24.46

8TG ^1H NMR (DMSO d_6) 300 MHz

8TG ^{13}C NMR (DMSO-d_6) 75 MHz

MP-C14 ^1H NMR (CDCl_3) 300 MHz

MP-C16 ^1H NMR (CDCl_3) 300 MHz

MP-C16 ^{13}C NMR (CDCl_3) 75 MHz

MP-C18 ^1H NMR (CDCl_3) 300 MHz

MP-C18 ^{13}C NMR (CDCl_3) 75 MHz

TG-C14 ^1H NMR (CDCl_3) 300 MHz

TG-C14 ^{13}C NMR (CDCl_3) 75 MHz

TG-C16 ¹H NMR (CDCl₃) 300 MHz

TG-bC16 ^1H NMR (CDCl_3) 300 MHz

TG-bC16 ^{13}C NMR (CDCl_3) 75 MHz

PEG-G₁-C14: R= C₁₂H₂₅; R'=H
¹H-NMR (CDCl₃) 300 MHz

PEG-G1-bC16 ^1H NMR (CDCl_3) 300 MHz

PEG-G₁-C18: R= C₁₆H₃₃; R'=H

¹H-NMR (CDCl₃) 300 MHz

PEG-G₂-C14: R= C₁₂H₂₅; R'=H

¹H-NMR (CDCl₃) 300 MHz

PEG-G₂-C18: R = C₁₆H₃₃; R' = H
¹H-NMR (CDCl₃) 300 MHz