

Electronic Supplementary Information

Cationic gemini lipids with cyclen headgroups: Interaction with DNA and gene delivery abilities

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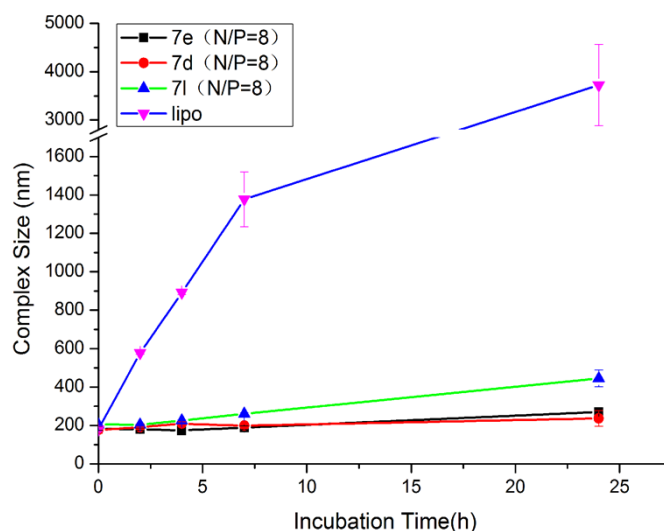


Fig. S1 Change of particle size along with the time in Tris buffer.

Analytical data for novel compounds:

4a: (0.66 g, 0.512 mmol, yield 33%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.88 (t, J=6.6 Hz, 6H, CH₂-CH₃), 1.25-1.34 (m, 36H, (CH₂)₉-CH₃), 1.46 (s, 18H, boc), 1.49-1.59 (d, J=6.7 Hz, 4H, -NHCH₂CH₂-), 2.80-3.0 (m, 4H, NHCH₂), 3.12-3.35 (m, 4H, -CH₂- cystine), 4.79 (d, J=3.9 Hz, 2H, -CH- of cystine);

4b: (0.65 g, 0.789 mmol, yield 39.3%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.88 (t, J=6.6 Hz, 6H, CH₂-CH₃), 1.25-1.34 (m, 44H, -CH₂(CH₂)₁₁CH₃), 1.46 (s, 18H, boc), 1.49-1.59 (m, 4H, NHCH₂CH₂), 2.80-3.0 (m, 4H, -NHCH₂-), 3.12-3.35 (m, 4H, -CH₂- cystine), 4.79 (d, J=3.9 Hz, 2H, -CH-, cystine);

4c: (0.588 g, 0.664 mmol, yield 33.2%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.88 (t, J=6.6 Hz, 6H, CH₂-CH₃), 1.25-1.34 (m, 52H, -(CH₂)₁₃-CH₃), 1.46 (s, 18H, boc), 1.49-1.59 (m, 4H, -NHCH₂CH₂-), 2.80-3.0 (m, 4H, -NHCH₂), 3.12-3.35 (m, 4H, -CH₂-cystine), 4.79 (d, J=3.9 Hz, 2H, -CH- cystine);

4d: (0.835g, 0.886 mmol, yield 44.3%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.88 (t, J=6.4 Hz, 6H, -CH₂-CH₃), 1.25-1.34 (m, 60H, -(CH₂)₁₅-CH₃), 1.46 (s, 18H, boc), 1.49-1.59 (m, 4H, -NHCH₂CH₂), 2.80-3.0 (m, 4H, -NHCH₂-), 3.12-3.35 (m, 4H, CH₂-cystine), 4.79 (d, J=4.1 Hz, 2H, CH-cystine);

4e: (0.944 g, 1.006 mmol, yield 50.3%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.88 (t, J=6.4 Hz, 6H, -CH₂CH₃), 1.25-1.34 (m, 44H, -CH=CHCH₂(CH₂)₆CH₃ and -CH=CHCH₂(CH₂)₅CH₂NH- of double chains), 1.46 (s, 18H, Boc), 1.49-1.59 (m, 4H, -NHCH₂CH₂-), 2.0 (s, 8H, -CH₂CH=CHCH₂-), 2.80-3.0 (m, 4H, -CH₂- cystine), 3.12-3.35 (m,

4H, -NHCH₂-), 4.79 (d, J=4.0 Hz, 2H, CH-cystine), 5.27-5.41 (s, 4H, -CH=CH-), 7.69 (s, 2H, -CONH-);

4f: (0.846 g, 0.904 mmol, yield 45.2%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.90 (t, J=6.9 Hz, 6H, -CH₂CH₃), 1.25-1.41 (m, 32H, CH₃(CH₂)₃CH₂- and -NHCH₂CH₂(CH₂)₅- of double chains), 1.48 (s, 18H, Boc), 1.49-1.59 (m, 4H, -NHCH₂CH₂-), 2.0-2.1 (m, 8H, -CH₂CH=CH- and -CH=CHCH₂- of double chains), 2.75-2.82 (t, J=6.5 Hz, 4H, -CH=CHCH₂CH=CH-), 2.86-3.01 (m, 4H, CH₂-cystine), 3.1-3.3 (m, 4H, -NHCH₂-), 4.75-4.85 (d, J=4.0 Hz, 2H, CH-cystine), 5.30-5.44 (m, 8H, -CH=CH-), 7.65-7.75 (t, 2H, -CONHCH₂-)

4g: (0.499 g, 0.642 mmol, yield 32.1%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.88 (t, J=6.6 Hz, 6H, -CH₂CH₃), 1.23-1.34 (m, 40H, -CH₃(CH₂)₁₀CH₂- of double chains), 1.45 (s, 18H, Boc), 1.60-1.68 (m, 4H, -OCH₂CH₂-), 3.08-3.23 (m, 4H, CH₂-cystine), 4.07-4.20 (m, 4H, -COOCH₂-), 4.5-4.68 (m, 2H, CH-cystine);

4h: (0.719 g, 0.764 mmol, yield 38.2%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.90 (t, J=6.6 Hz, 6H, CH₂-CH₃), 1.27-1.40 (m, 44H, -CH=CHCH₂(CH₂)₆CH₃ and -CH=CHCH₂(CH₂)₅CH₂NH- of double chains), 1.46 (s, 18H, Boc), 1.59-1.71 (m, 8H, -OCH₂CH₂CH₂-), 2.07 (q, J=6.8 Hz, 8H, -CH₂CH=CHCH₂-), 2.76-2.82 (t, J=6.5 Hz, 4H, -CH=CHCH₂CH=CH-), 3.12-3.35 (m, 4H, CH₂-cystine), 4.12-4.22 (m, 4H, -COOCH₂-), 4.60 (dd, J=12.8, 5.2 Hz, 2H, CH-cystine), 5.27-5.41 (m, 4H, -CH=CH-);

4i: (0.664 g, 0.708 mmol, yield 35.4%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.9 (t, J=6.8 Hz, 6H, -CH₂CH₃), 1.25-1.41 (m, 28H, CH₃(CH₂)₃CH₂- and -OCH₂CH₂CH₂(CH₂)₄- of double chains), 1.48 (s, 18H, Boc), 1.54-1.71 (m, 8H, -OCH₂CH₂CH₂-), 1.99-2.05 (dd, J=12.1, 6.3 Hz, 8H, -CH₂CH=CH- and -CH=CHCH₂- of double chains), 3.10-3.21 (d, J=4.9 Hz, 4H, CH₂-cystine), 4.08 (m, 4H, -COOCH₂-), 4.54-4.64 (m, 2H, CH-cystine), 5.30-5.46 (m, 8H, -CH=CH-);

4j: (0.598 g, 0.508 mmol, yield 25.4%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.55-2.40 (m, 86H, cholesterol skeleton), 1.48 (s, 18H, Boc), 3.1-3.2 (m, 2H, chol), 3.07-3.5 (m, 4H, -CH₂-cystine), 4.7-4.9 (m, 2H, -CH-, cystine), 5.3-5.4 (m, 2H, chol);

4k: (0.679 g, 0.536 mmol, yield 26.8%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.80-0.88 (m, 24H, -CHCH₃, tocopheryl), 1.1-1.4 (m, 36H, -(CH₂)₉- tocopheryl), 1.45 (s, 6H, -CH₃ tocopheryl, 18H, Boc), 1.67-1.86 (m, 4H, CH₂- tocopheryl), 1.93 (s, 6H, -CH₃ tocopheryl), 2.00 (s, 6H, -CH₃, tocopheryl), 2.12 (s, 6H, CH₃- tocopheryl), 2.58-2.62 (t, J=6.5 Hz, 4H, -CH₂- tocopheryl), 3.2-3.55 (dd, J=13.7, 6.8 Hz, 4H, -CH₂, cystine), 4.9-5.0 (d, J=5.6 Hz, 2H, CH-cystine);

6a: (0.723 g, 0.452 mmol, yield 45.2%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.88 (t, J=6.8 Hz, 6H, -CH₂-CH₃), 1.22-1.35 (m, 36H, -(CH₂)₉CH₃), 1.40-1.48 (d, J=9.5 Hz, 54H, Boc), 2.7-3.0 (m, 4H, -NHCH₂-), 8H, -CH₂- cyclen), 3.08-3.57 (m, 24H, -CH₂- cyclen, 4H, -CH₂- cystine, 4H, -NHCH₂CO-), 5.2 (d, J=4.5 Hz, 2H, -CH-, cystine); ¹³C NMR (100 MHz, CDCl₃): δ (ppm): 14.2, 22.5, 27.3, 28.4, 29.5, 32.1, 39.6, 53.2, 79.8, 155.9, 169.3, 169.5, 170.9; HR-MS (ESI): C₈₀H₁₅₀N₁₂O₁₆S₂, [M+H]⁺: 1600.0812, found: 1600.0809;

6b: (0.728 g, 0.44 mmol, yield 44.0%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.88 (t, J=6.7 Hz, 6H, -CH₂CH₃), 1.25-1.34 (m, 44H, -(CH₂)₁₁CH₃), 1.46 (d, J=9.4 Hz, 54H, Boc), 1.49-1.59 (m, 4H, -NHCH₂CH₂-), 2.80-3.0 (m, 4H, -NHCH₂-), 8H, -CH₂- cyclen), 3.12-3.35 (m, 4H, -CH₂-, cystine, 24H, -CH₂-, cyclen, 4H, -NCH₂CO-), 4.79 (dd, J=14.0, 9.4 Hz, 2H, -CH-, cystine); ¹³C NMR (100 MHz, CDCl₃): δ (ppm): 13.9, 22.0, 26.3, 28.0, 28.3, 28.7, 28.78, 28.9, 29.0, 29.9, 31.3, 51.4, 65.1, 71.2, 78.5, 155.4, 170.9; HR-MS (ESI): C₈₄H₁₅₈N₁₂O₁₆S₂ [M+H]⁺: 1656.1438, found: 1656.1433;

6c: (0.684 g, 0.4 mmol, yield 40.0%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.88 (t, J=6.7 Hz, 6H, -CH₂CH₃), 1.19-1.37 (m, 52H, -(CH₂)₁₃CH₃), 1.40-1.48 (d, J=13.5 Hz, 54H, Boc), 1.49-1.52 (m, 4H, -NHCH₂CH₂-), 2.7-3.0 (m, 4H, -NHCH₂-), 8H, -CH₂-, cyclen), 3.08-3.57 (m, 24H, -CH₂-, cyclen, 4H, -CH₂-, cystine, 4H, -NHCH₂CO-), 5.0-5.28 (dd, J=14.0, 9.5 Hz, 2H, -CH-, cystine); ¹³C NMR (100 MHz, CDCl₃): δ (ppm): 13.9, 22.0, 28.0, 28.7, 29.0, 31.3, 51.7, 78.2, 155.1, 169.5; HR-MS (ESI): C₈₈H₁₆₆N₁₂O₁₆S₂, [M+H]⁺: 1712.2064, found: 1712.2064;

6d: (0.864 g, 0.489 mmol, yield 48.9%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.88 (t, J=6.7 Hz, 6H, -CH₂CH₃), 1.22-1.35 (m, 60H, -(CH₂)₁₅CH₃), 1.40-1.48 (d, J=10.1 Hz, 54H, Boc), 1.48-1.52 (m, 4H, -NHCH₂CH₂-), 2.7-3.0 (m, 4H, -NHCH₂-), 8H, -CH₂-, cyclen), 3.08-3.57 (m, 24H, -CH₂-, cyclen, 4H, -CH₂-cystine, 4H, -NHCH₂CO-), 5.2 (dd, J=14.2, 9.4 Hz, 2H, -CH-, cystine); ¹³C NMR (100 MHz, CDCl₃): δ (ppm): 13.9, 22.2, 28.9, 31.1, 54.4, 79.0, 155.2, 170.1; HR-MS (ESI): C₉₂H₁₇₄N₁₂O₁₆S₂, [M+H]⁺: 1768.2690, found: 1768.2673;

6e: (0.917 g, 0.52 mmol, yield 52.0%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.88 (t, J=6.5 Hz, 6H, -CH₂CH₃), 1.22-1.35 (m, 44H, -(CH₂)₁₁CH₃), 1.40-1.48 (d, J=7.4 Hz, 54H, Boc), 1.48-1.55 (m, 4H, -NHCH₂CH₂-), 1.92-2.08 (m, 8H, -CHCH₂), 2.8-3.0 (m, 4H, -NHCH₂-), 8H, -CH₂-cyclen), 3.08-3.73 (m, 24H, -CH₂- cyclen, 4H, -CH₂- cystine, 4H, -NHCH₂CO-), 5.2 (dd, J=13.9, 9.2 Hz, 2H, -CH- cystine), 5.2-5.4 (m, 4H, -CH=CH-); ¹³C NMR (100 MHz, CDCl₃): δ (ppm): 13.9, 22.0, 28.0, 28.3, 28.7, 29.0, 30.0, 31.3, 51.7, 78.2, 128.6, 131.8, 155.1, 169.8, 170.9; HR-MS (ESI): C₉₂H₁₇₀N₁₂O₁₆S₂, [M+H]⁺: 1764.2377, found: 1764.2291;

6f: (0.739 g, 0.42 mmol, yield 42.0%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.9 (t, J=6.7 Hz, 6H, -CH₂CH₃), 1.22-1.39 (m, 32H, CH₃(CH₂)₃CH₂- and -NHCH₂CH₂(CH₂)₅- of double

chains), 1.41-1.55 (d, $J=9.7$ Hz, 54H, Boc), 2.0-2.1 (m, 8H, $-\text{CH}_2\text{CH}=\text{CH}-$ and $-\text{CH}=\text{CHCH}_2-$ of double chains), 2.75-2.82 (t, $J=6.4$ Hz, 4H, $-\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}-$), 2.82-3.04 (m, 4H, $-\text{NHCH}_2-$, 8H, $-\text{CH}_2-$ cyclen), 3.08-3.73 (m, 24H, $-\text{CH}_2-$ cyclen, 4H, $-\text{CH}_2-$ cystine, 4H, $-\text{NHCH}_2\text{CO}-$), 4.75-4.85 (m, 2H, $-\text{CH}-$ cystine), 5.20-5.44 (m, 8H, $-\text{CH}=\text{CH}-$), 7.8 (s, 2H, $-\text{CONHCH}_2-$); ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 14.1, 22.5, 25.6, 27.1, 28.5, 29.3, 31.5, 39.7, 53.2, 79.8, 127.9, 130.0, 130.2, 156.2, 169.5, 170.8; HR-MS (ESI): $\text{C}_{92}\text{H}_{166}\text{N}_{12}\text{O}_{16}\text{S}_2$, $[\text{M}+\text{H}]^+$: 1760.2064, found: 1760.2063;

6g: (0.711 g, 0.44 mmol, yield 44.4%). ^1H NMR (400 MHz, CDCl_3): δ (ppm) 0.88 (t, $J=6.8$ Hz, 6H, $-\text{CH}_2\text{CH}_3$), 1.25-1.36 (m, 40H, $-\text{CH}_3(\text{CH}_2)_{10}\text{CH}_2-$ of double chains), 1.39-1.51 (d, $J=11.1$ Hz, 54H, Boc), 1.60-1.70 (m, 4H, $-\text{OCH}_2\text{CH}_2-$), 2.82-3.04 (m, 8H, $-\text{CH}_2-$, cyclen), 3.08-3.73 (m, 24H, $-\text{CH}_2-$ cyclen, 4H, $-\text{CH}_2-$ cystine, 4H, $-\text{NHCH}_2\text{CO}-$), 4.07-4.20 (t, $J=6.8$ Hz, 4H, $-\text{COOCH}_2-$), 4.5-4.68 (m, 2H, $\text{CH}-$ cystine); ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 14.1, 22.7, 25.8, 28.5, 29.6, 31.9, 40.3, 51.6, 66.0, 79.6, 156.1, 170.1, 170.7; HR-MS (ESI): $\text{C}_{80}\text{H}_{148}\text{N}_{10}\text{O}_{18}\text{S}_2$, $[\text{M}+\text{H}]^+$: 1602.0493, found: 1602.0491;

6h: (0.797 g, 0.45 mmol, yield 45.1%). ^1H NMR (400 MHz, CDCl_3): δ (ppm) 0.89 (t, $J=6.7$ Hz, 6H, $-\text{CH}_2\text{CH}_3$), 1.23-1.36 (m, 44H, $-\text{CH}=\text{CHCH}_2(\text{CH}_2)_6\text{CH}_3$ and $-\text{CH}=\text{CHCH}_2(\text{CH}_2)_5\text{CH}_2\text{NH}-$ of double chains), 1.40-1.56 (d, $J=11.5$ Hz, 54H, Boc), 1.59-1.69 (m, 4H, $-\text{OCH}_2\text{CH}_2-$), 2.0 (m, 8H, $-\text{CH}_2\text{CH}=\text{CHCH}_2-$), 2.69-2.95 (m, 8H, $-\text{CH}_2-$, cyclen), 3.06-3.24 (m, 4H, $-\text{CH}_2-$, cystine), 3.24-3.68 (m, 24H, $-\text{CH}_2-$, cyclen, 4H, $-\text{NHCH}_2\text{CO}-$), 4.12-4.22 (q, $J=6.5$ Hz, 4H, $-\text{COOCH}_2-$), 4.80 (dd, $J=12.8, 6.5$ Hz, 2H, $-\text{CH}-$ cystine), 5.29-5.41 (m, 4H, $-\text{CH}=\text{CH}-$); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm): 14.1, 22.7, 25.8, 27.2, 28.5, 29.3, 29.7, 32.9, 40.3, 51.6, 58.4, 66.0, 79.7, 129.7, 129.9, 170.1, 170.7; HR-MS (ESI): $\text{C}_{92}\text{H}_{168}\text{N}_{10}\text{O}_{18}\text{S}_2$, $[\text{M}+\text{H}]^+$: 1766.2058, found: 1766.2050;

6i: (0.670 g, 0.38 mmol, yield 38.0%). ^1H NMR (400 MHz, CDCl_3): δ (ppm) 0.9 (t, $J=6.3$ Hz, 6H, CH_2-CH_3), 1.22-1.40 (m, 28H, $\text{CH}_3(\text{CH}_2)_3\text{CH}_2-$ and $-\text{OCH}_2\text{CH}_2\text{CH}_2(\text{CH}_2)_4-$ of double chains), 1.48 (d, $J=11.3$ Hz, 54H, Boc), 1.60-1.70 (dd, $J=13.3, 7.0$ Hz, 4H, $-\text{OCH}_2\text{CH}_2-$), 1.99-2.12 (m, 8H, $-\text{CH}_2\text{CH}=\text{CH}-$ and $-\text{CH}=\text{CHCH}_2-$ of double chains), 2.68-3.00 (m, 4H, $-\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}-$, 8H, $-\text{CH}_2-$, cyclen), 3.10-3.21 (m, 4H, CH_2- cystine), 3.25-3.66 (m, 24H, $-\text{CH}_2-$, cyclen, 4H, $-\text{NHCH}_2\text{CO}-$), 4.13 (t, $J=6.4$ Hz, 4H, $-\text{COOCH}_2-$), 4.80 (m, 2H, $\text{CH}-$ cystine), 5.27-5.46 (m, 8H, $-\text{CH}=\text{CH}-$); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm): 14.2, 22.5, 25.8, 27.2, 29.2, 29.6, 31.5, 40.3, 51.6, 66.0, 79.7, 127.9, 130.0, 130.2, 156.4, 170.2, 170.7; HR-MS (ESI): $\text{C}_{92}\text{H}_{164}\text{N}_{10}\text{O}_{18}\text{S}_2$, $[\text{M}+\text{H}]^+$: 1762.1745, found: 1762.1740;

6j: (0.741 g, 0.37 mmol, yield 37.0%). ^1H NMR (400 MHz, CDCl_3): δ (ppm) 0.55-2.40 (m, 86H, cholesterol skeleton), 1.48 (d, $J=10.6$ Hz, 54H, Boc), 2.69-2.95 (m, 8H, $-\text{CH}_2-$ cyclen),

3.06-3.24 (m, 4H, -CH₂-,cystine), 3.24-3.68 (m, 24H, -CH₂-, cyclen), 4.79 (dd, J=13.0, 6.1 Hz, 2H, -CH-, cystine), 5.3-5.4 (m, 2H, chol); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 11.8, 18.7, 19.3, 21.0, 22.5, 22.8, 23.8, 24.3, 28.0, 28.5, 31.8, 35.6, 36.2, 36.6, 36.9, 37.9, 39.5, 42.3, 50.0, 51.8, 56.2, 56.7, 75.8, 79.9, 123.0, 139.2, 156.2, 169.5, 170.6; HR-MS (ESI): C₁₁₀H₁₈₈N₁₀O₁₈S₂, [M+H]⁺ : 2003.3656, found: 2003.3658;

6k: (0.926 g, 0.443 mmol, yield 44.3%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.68-0.95 (m, 24H, -CHCH₃, tocopheryl), 0.97-1.33 (m, 36H, -(CH₂)₉- tocopheryl), 1.34-1.60 (s, 6H, -CH₃ tocopheryl, 54H, Boc), 1.70-1.86 (m, 4H -CH₂- tocopheryl), 1.96 (s, 6H, -CH₃ tocopheryl), 1.99 (s, 6H, -CH₃, tocopheryl), 2.06 (s, 6H, -CH₃ tocopheryl), 2.58-2.62 (d, J=3.2 Hz, 4H, -CH₂-, tocopheryl), 2.66–2.95 (m, 8H, -CH₂-, cyclen), 3.11-3.66 (m, 4H, -CH₂- cystine, 24H, -CH₂-, cyclen.), 5.14 (dd, J=12.1, 6.9 Hz, 2H, -CH-,cystine); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 11.8, 12.3, 13.1, 18.4, 19.6, 20.6, 22.6, 24.4, 24.8, 28.0, 31.0, 32.8, 37.4, 39.4, 40.0, 51.8, 58.3, 79.7, 117.4, 123.1, 140.3, 149.6, 169.1, 171.1; HR-MS (ESI): C₁₁₄H₁₉₆N₁₀O₂₀S₂, [M+H]⁺: 2091.4181, found: 2091.4175;

7a: (245 mg, 0.128 mmol, yield 82.0%). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 0.85 (t, J=6.6 Hz, 6H, -CH₂CH₃), 1.17-1.30 (m, 36H, (CH₂)₉-CH₃), 1.33-1.42 (m, 4H, -NHCH₂CH₂-), 2.7-3.2 (m, 4H, -NHCH₂-, 32H, -CH₂-cyclen, 4H, CH₂-cystine), 3.27-3.43 (m, 4H, -NCH₂CO-), 5.2 (s, 2H, -CH-cystine); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 13.9, 22.0, 26.2, 28.9, 31.3, 40.75, 42.37, 42.66, 44.25, 49.13, 52.21, 54.35, 169.2, 171.4; HR-MS (ESI): C₅₀H₁₀₂N₁₂O₄S₂, [M+H]⁺: 998.7588, found: 999.7664.

7b: (245 mg, 0.124 mmol, yield 82.4%). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 0.85 (t, J=6.6 Hz, 6H, -CH₂CH₃), 1.2-1.27 (m, 44H, -(CH₂)₁₁CH₃), 1.31-1.45 (m, 4H, -NHCH₂CH₂), 2.66-3.21 (m, 4H, -NHCH₂, 32H, -CH₂- cyclen, 4H, -CH₂- cystine), 3.27-3.43(m, 4H, -NCH₂CO-), 4.44-4.54 (s, 2H, -CH- cystine); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 13.9, 22.1, 26.4, 28.7, 28.76, 28.9, 29.0, 31.34, 40.8, 42.35, 42.7, 44.5, 49.0, 52.2, 54.4, 169.0, 171.7; HR-MS (ESI): C₅₄H₁₁₀N₁₂O₄S₂, [M+H]⁺ : 1055.8293, found: 1055.8289.

7c: (254 mg, 0.126 mmol, yield 86.0%). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 0.85 (t, J=6.6 Hz, 6H, -CH₂CH₃), 1.2-1.27 (m, 52H, -(CH₂)₁₃CH₃), 1.31-1.45 (m, 4H, -NHCH₂CH₂), 2.66-3.21 (m, 4H, -NHCH₂, 32H, -CH₂- cyclen, 4H, -CH₂- cystine), 3.27-3.43 (m, 4H, -NCH₂CO-), 4.44-4.54 (dd, J=13.8, 7.9 Hz, 2H, -CH-cystine); ¹³C NMR(100 MHz, DMSO-d₆) δ (ppm): 13.9, 22.0, 26.5, 28.9, 31.3, 40.7, 42.37, 42.71, 44.3, 49.1, 52.2, 54.4, 169.0, 171.7; HR-MS (ESI): C₅₈H₁₁₈N₁₂O₄S₂, [M+H]⁺ : 1111.8919 , found: 1111.8909;

7d: (262 mg, 0.126 mmol, yield 89.0%). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 0.85 (t, J=6.7 Hz, 6H, -CH₂CH₃), 1.2-1.27 (m, 60H, -(CH₂)₁₅CH₃), 1.31-1.45(m, 4H, -NHCH₂CH₂-),

2.69-3.22 (m, 4H, -NHCH₂-, 32H, -CH₂- cyclen, 4H, -CH₂- cystine), 3.27-3.43 (m, 4H, -NCH₂CO-), 4.44-4.54 (dd, J=13.9, 8.1 Hz, 2H, -CH- cystine); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 13.7, 22.2, 26.5, 28.9, 31.3, 40.8, 42.4, 42.8, 44.3, 49.2, 52.2, 54.5, 169.3, 171.4, HR-MS (ESI): C₆₂H₁₂₆N₁₂O₄S₂, [M+H]⁺ : 1167.9545, found: 1167.9547;

7e: (252 mg, 0.121 mmol, yield 85.6%). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 0.85 (t, J=6.4 Hz, 6H, -CH₂CH₃), 1.2-1.27 (m, 44H, -(CH₂)₁₁CH₃), 1.33-1.42 (m, 4H, -NHCH₂CH₂), 1.81-2.02 (m, 8H, -CH₂CH=CHCH₂), 2.64-3.22 (m, 4H, -NHCH₂-, 32H, -CH₂- cyclen, 4H, -CH₂- cystine), 3.22-3.47 (m, 4H, -NCH₂CO), 4.44-4.54 (d, J=5.8 Hz, 2H, -CH- cystine), 5.07-5.58 (m, 4H, -CH=CH-); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 13.9, 22.2, 26.5, 28.9, 31.3, 40.7, 42.3, 42.7, 44.3, 49.2, 52.2, 54.4, 128.6, 132.0, 169.7, 171.0; HR-MS (ESI): C₆₂H₁₂₂N₁₂O₄S₂, [M+H]⁺: 1163.9232, found: 1163.9233;

7f: (258 mg, 0.125 mmol, yield 87.7%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.9 (t, J=6.7 Hz, 6H, -CH₂CH₃), 1.17-1.41 (m, 32H, CH₃(CH₂)₃CH₂- and -NHCH₂CH₂(CH₂)₅- of double chains), 1.41-1.54 (m, 4H, -NHCH₂CH₂-), 2.0-2.1 (m, 8H, -CH₂CH=CH- and -CH=CHCH₂- of double chains), 2.73-2.82 (t, J=6.5 Hz, 4H, -CH=CHCH₂CH=CH-), 2.82-2.94 (m, 4H, -CH₂-, cystine), 2.94-3.73 (m, 32H, -CH₂-cyclen, 4H, -NHCH₂-, 4H, -NHCH₂CO-), 4.76 (m, 2H, -CH-, cystine), 5.20-5.44 (m, 8H, -CH=CH-), 7.91 (s, 2H, -CONH-), 8.5 (s, 2H, -CONH-, cystine); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 14.4, 22.4, 25.6, 26.9, 27.0, 29.4, 31.4, 42.9, 44.8, 49.6, 115.8, 118.8, 128.2, 130.2, 158.8, 159.1, 169.5, 171.9; HR-MS (ESI): C₅₀H₁₀₀N₁₀O₆S₂, [M+H]⁺ : 1001.7341, found: 1001.7344;

7g: (257 mg, 0.135 mmol, yield 86.2%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.90 (t, J=6.8 Hz, 6H, -CH₂CH₃), 1.22- 1.36 (m, 40H, -CH₃(CH₂)₁₀CH₂- of double chains), 1.60-1.68 (m, 4H, -OCH₂CH₂-), 2.82-2.97 (m, 4H, -CH₂-,cystine), 2.97-3.73(m, 32H, -CH₂- cyclen, 4H, -NHCH₂CO-), 4.13 (t, J=6.5 Hz, 4H, -COOCH₂-), 4.77 (d, J=4.9 Hz, 2H, CH-cystine), 8.15 (s, 2H, -CONH-, cystine); ¹³C NMR (100MHz, DMSO-d₆) δ (ppm): 14.4, 22.5, 25.7, 28.4, 29.5, 32.8, 42.8, 44.8, 49.5, 65.6, 158.7, 170.5, 171.9; HR-MS (ESI): C₅₀H₁₀₀N₁₀O₆S₂, [M+H]⁺ : 1001.7341, found: 1001.7344;

7h: (265 mg, 0.127 mmol, yield 90.0%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.89 (t, J=6.6 Hz, 6H, -CH₂CH₃), 1.17-1.39 (m, 44H, -CH=CHCH₂(CH₂)₆CH₃ and -CH=CHCH₂(CH₂)₅CH₂NH- of double chains), 1.55-1.72 (m, 4H, -OCH₂CH₂-), 2.0 (m, 8H, -CH₂CH=CHCH₂-), 2.67-3.38 (m, 32H, -CH₂-, cyclen, 4H, -CH₂-,cystine), 3.38-3.64 (m, 4H, -NHCH₂CO-), 4.10 (s, 4H, -COOCH₂-), 4.76 (m, 2H, -CH-cystine), 5.30-5.43 (m, 4H, -CH=CH-), 7.80-8.00 (s, 2H, -CONH-); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 14.3, 22.5,

25.7, 27.0, 28.4, 29.0, 29.6, 31.7, 42.8, 43.1, 44.7, 49.5, 51.8, 54.6, 65.6, 115.2, 118.2, 121.1, 130.1, 158.8, 170.5, 171.9; HR-MS (ESI): $C_{62}H_{120}N_{10}O_6S_2$, $[M+H]^+$: 1165.8906, found:1165.8916;

7i: (240 mg, 0.116 mmol, yield 81.5%). 1H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.9 (t, $J=6.8$ Hz, 6H, $-CH_2CH_3$), 1.26-1.35 (m, 28H, $CH_3(CH_2)_3CH_2-$ and $-OCH_2CH_2CH_2(CH_2)_4-$ of double chains), 1.60-1.70 (m, 4H, $-OCH_2CH_2-$), 2.0-2.12 (m, 8H, $-CH_2CH=CH-$ and $-CH=CHCH_2-$ of double chains), 2.73-3.28 (m, 4H, $-CH=CHCH_2CH=CH-$), 2.8-3.39 (m, 32H, $-CH_2-$, cyclen), 4.11 (s, 4H, $-COOCH_2-$), 4.77 (m, 2H, CH-cystine), 5.27-5.45 (m, 8H, $-CH=CH-$), 7.87 (s, 2H, $-CONH-$); ^{13}C NMR (100 MHz, $DMSO-d_6$) δ (ppm): 14.3, 19.0, 22.4, 25.7, 27.0, 28.4, 29.0, 29.5, 31.4, 42.8, 43.1, 44.8, 49.5, 56.5, 65.6, 115.1, 118.1, 158.9, 159.2, 170.5, 171.8; HR-MS (ESI): $C_{62}H_{116}N_{10}O_6S_2$ $[M+H]^+$: 1161.8593; found: 1161.8604;

7j: (260 mg, 0.112 mmol, yield 90.0%). 1H NMR (400 MHz, $DMSO-d_6$): δ (ppm) 0.55-2.40 (m, 86H, cholesterol skeleton), 2.69-3.2 (m, 32H, $-CH_2-$, cyclen, 4H, $-CH_2-$, cystine), 3.34 (m, 4H, $-CH_2CONH-$), 4.54 (m, 2H, $-CH-$, cystine), 5.33 (m, 2H, chol), 8.90 (s, 2H, $-CONH-$); ^{13}C NMR (100 MHz, $DMSO-d_6$) δ (ppm): 12.1, 19.0, 19.4, 22.8, 23.1, 23.7, 27.8, 31.8, 35.7, 36.5, 42.3, 42.8, 44.7, 49.5, 49.9, 56.6, 139.6, 158.7, 159.0, 169.9, 171.9; HR-MS (ESI): $C_{80}H_{140}N_{10}O_6S_2$, $[M+H]^+$:1402.0477, found: 1402.0465;

7k: (238 mg, 0.099 mmol, yield 83.0%). 1H NMR (400 MHz, $DMSO-d_6$): δ (ppm) 0.68-0.95 (m, 24H, $-CHCH_3$, tocopheryl), 0.85-1.22 (m, 36H, $-(CH_2)_9-$ tocopheryl), 1.36-1.48 (s, 6H, $-CH_3$ tocopheryl), 1.59-1.71 (m, 4H, CH_2- , tocopheryl), 1.71-1.96 (s, 18H, $-CH_3$, tocopheryl), 2.58-2.62 (t, 4H, $-CH_2-$, tocopheryl), 2.66-3.46 (m, 32H, $-CH_2-$ cyclen, 4H, $-CH_2-$, cystine, 4H, $-CH_2CONH-$), 4.8 (m, 2H, $-CH-$, cystine), 9.0 (s, 2H, $-CONH-$); ^{13}C NMR (100 MHz, $DMSO-d_6$) δ (ppm): 11.8, 12.3, 13.1, 19.9, 20.1, 22.9, 24.6, 27.8, 32.5, 37.1, 42.8, 44.8, 49.2, 75.3, 115.4, 118.4, 140.4, 158.7, 159.1, 169.2, 171.1; HR-MS (ESI): $C_{84}H_{148}N_{10}O_8S_2$, $[M+H]^+$: 1490.1001, found: 1490.1001;

9: (621 mg, 1 mmol, yield 51.6%). 1H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.25 (t, $J = 7.1$ Hz, 3H, $-CH_3$), 1.42-1.46 (d, $J=14.4$ Hz, 27H, $-Boc$), 2.52-2.91 (s, 4H, cyclen), 3.24 (s, 2H, $-NCH_2CO-$), 3.31-3.62 (m, 12H, cyclen), 3.97 (d, $J=5.7$ Hz, 2H, $-NHCH_2CO-$), 4.17 (q, $J=7.1$ Hz, $-COOCH_2CH_3$), HR-MS (ESI): $C_{29}H_{53}N_5O_9$, $[M+H]^+$: 616.3922, found: 616.3926;

10: (589 mg, 0.662 mmol, yield 84.9%). 1H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.89 (t, $J=6.8$ Hz, 3H, $-CH_3$), 1.22-1.33 (m, 18H, $-CH_2-(CH_2)_9-CH_3$), 1.40-1.54 (d, $J = 18.9$ Hz, 27H, $-Boc$), 1.64-1.73 (m, 2H, $-NHCH_2CH_2-$), 2.45-2.79 (m, 4H, cyclen), 3.14-3.29 (m, 4H, $-NCH_2CO-$ and $-CONHCH_2-$), 3.29-3.78 (m, 12H, cyclen), 3.97 (d, $J=21.7$ Hz, 2H, $-NHCH_2CO-$); ^{13}C

NMR (100 MHz, CDCl₃) δ (ppm) 14.1, 18.4, 22.7, 26.8, 29.2, 29.6, 31.9, 39.5, 43.6, 58.3, 168.9, 171.6; HR-MS (ESI): C₃₉H₇₄N₆O₈, [M+H]⁺: 755.5645, found: 755.5641;

7I: (145 mg, 0.159 mmol, yield 80.0%). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 0.88 (t, J=6.7 Hz, 3H, -CH₃), 1.22-1.33 (m, 18H, -CH₂-(CH₂)₉-CH₃) 1.45 (m, 2H, -NHCH₂CH₂-), 2.54-3.45 (m, 4H, -NCH₂CO-, 2H, -CONHCH₂-, 16H, cyclen), 3.87 (d, 2H, -NHCH₂CO-); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 14.4, 22.5, 26.9, 29.2, 29.5, 31.7, 42.4, 42.9, 43.3, 44.7, 50.0, 55.6, 168.6, 172.8; HR-MS (ESI): C₂₄H₅₀N₆O₂, [M+H]⁺: 455.4073, found: 455.4071;