Supplementary Information

Cyclen-based cationic lipids with double hydrophobic tails for efficient gene delivery

Hai-Jiao Wang, Yan-Hong Liu, Ji Zhang*, Yang Zhang, Yan Xia and Xiao-Qi Yu*

Key Laboratory of Green Chemistry and Technology (Ministry of Education), College of Chemistry, Sichuan University, Chengdu 610064, PR China

*Corresponding authors: xqyu@scu.edu.cn (X.-Q. Yu); jzhang@scu.edu.cn (J. Zhang)

Table S1 Particle size and zeta-potential of **5a** and **5b** against two weeks. The molar ratio of lipid/DOPE was 1 : 2.

Liposome	Particle size (nm)		Zeta-potential (mV)	
	1 day	15 days	1 day	15 days
5a	127.3 ± 1.6	126.1 ± 0.9	61.1 ± 0.3	61.8 ± 1.3
5b	133.9 ± 1.0	137.8 ± 1.5	54.6 ± 3.3	61.3 ± 6.8

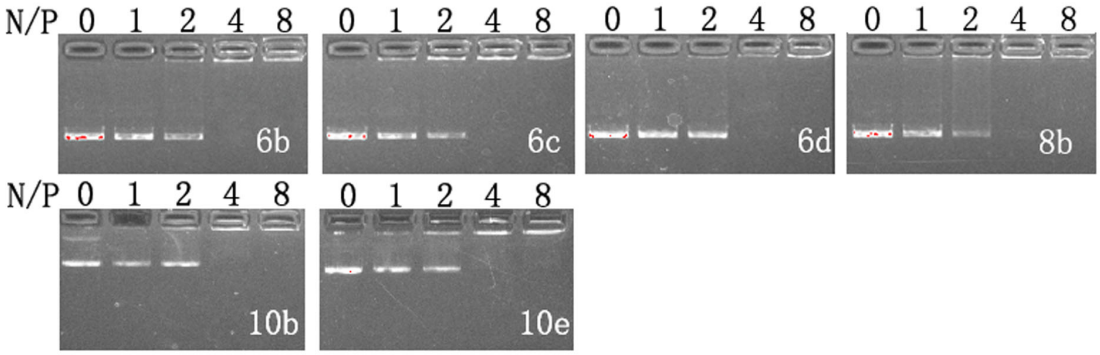

Fig. S1 Electrophoretic gel retardation assays of lipid**s**/DOPE/pDNA complexes at different N/P ratios. The molar ratio of lipid/DOPE was 1 : 1.

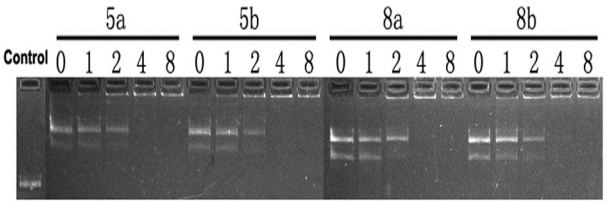

Fig. S2 Electrophoretic gel retardation assays of lipoplexes at different N/P ratios in the presence of 10% serum. The molar ratio of lipid/DOPE was 1 : 1.

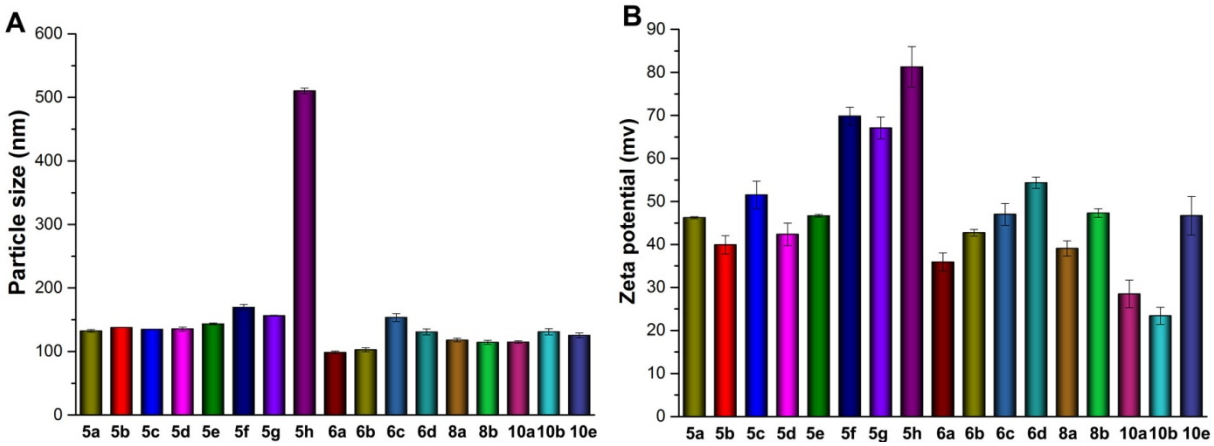

Fig. S3 Mean particle size (A) and Zeta-potential (B) of the liposomes without DNA (DLS at room temperature). The molar ratio of lipid/DOPE was 1 : 1.

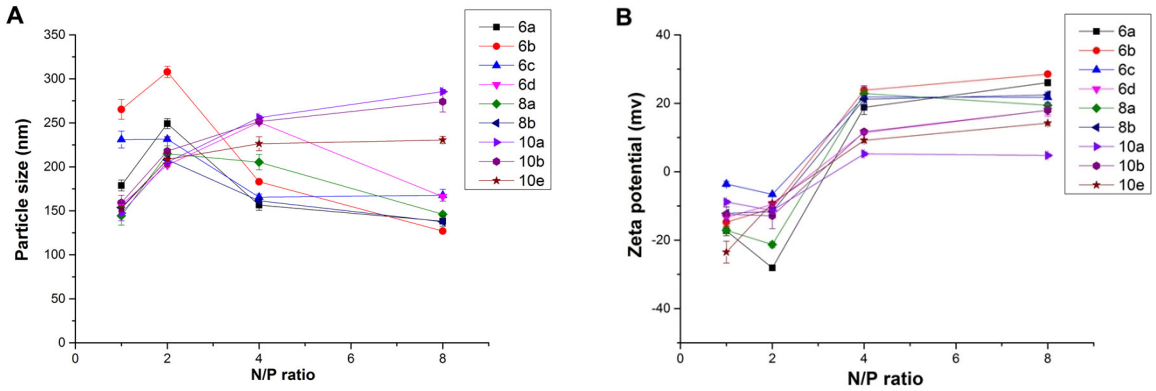

Fig. S4 Mean particle size (A) and Zeta-potential (B) of the lipoplexes formed from lipids under various N/P ratios (DLS at room temperature). The molar ratio of lipid/DOPE was 1 : 1.

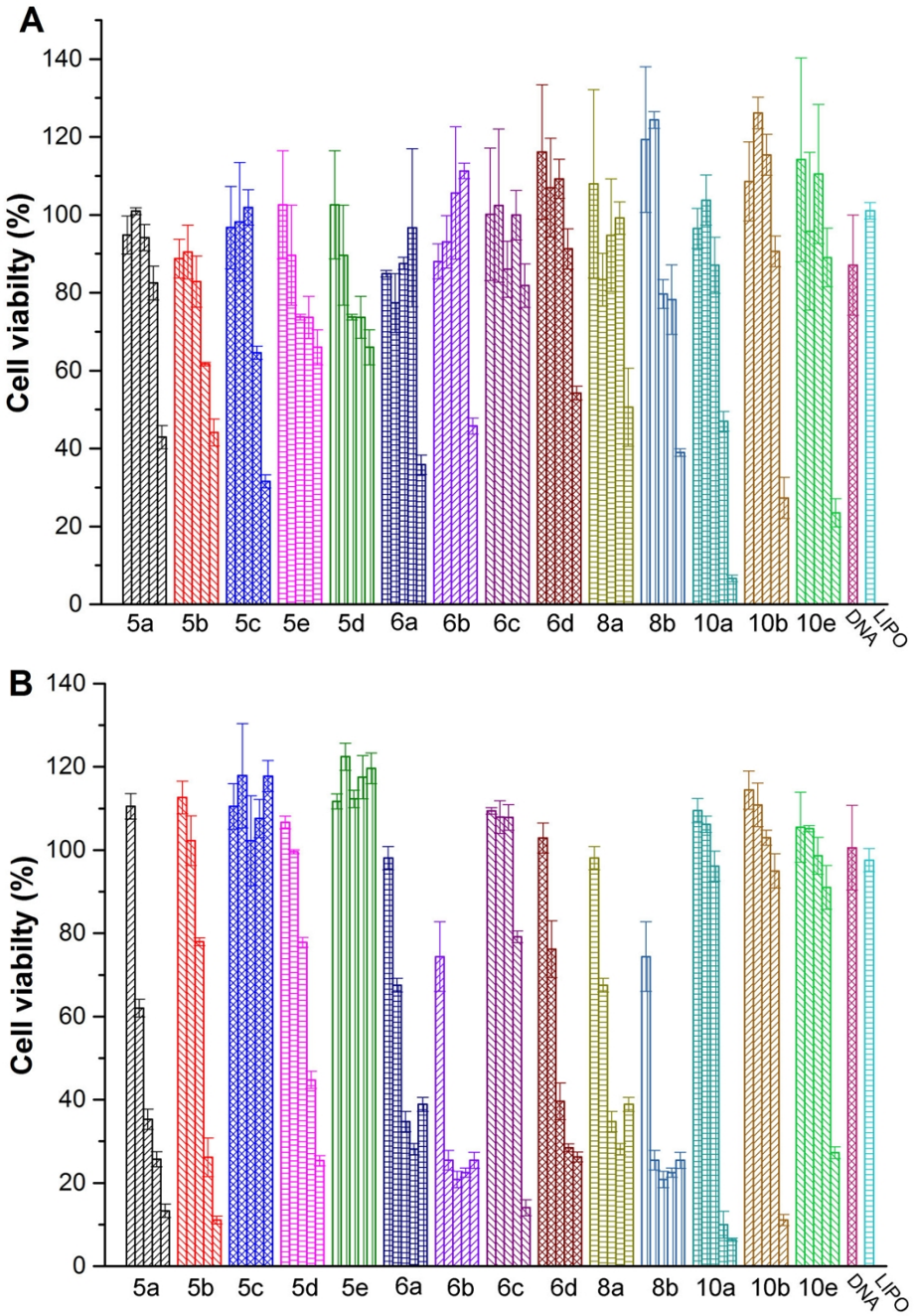

Fig. S5 Cytotoxicity of the lipoplexes at various N/P (1, 2, 4, 8 and 16) ratios in A549 (A) and 7402 (B) cells. Data represent mean ± SD (n = 3). The molar ratio of lipid/DOPE was 1 : 1.

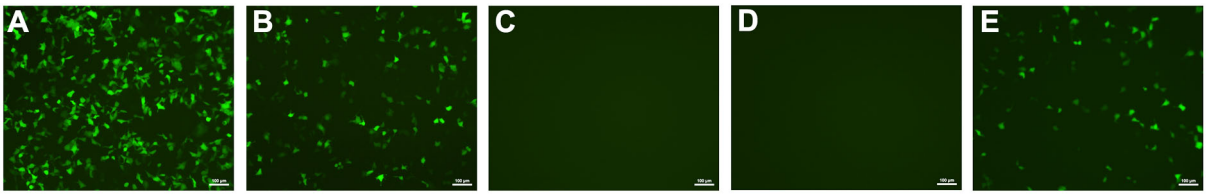

Fig. S6 Fluorescent microscope images of HEK293 (N/P = 4) cells transfected by **5a** (A), **8a** (B), **10a** (C), null (naked DNA) and lipofectamine 2000 (E) in the presence of serum. The molar ratio of lipid/DOPE was 1 : 1. The cells were observed by fluorescence microscopy after 24 h transfection.

Analytical data for novel compounds:

Compound **3** (yield 80%): ^{1}H NMR($CDCl_3$, 400 MHz): δ = 1.26 (t, *J* = 7.1 Hz, 6H, -*CH3*), 1.44 (s, 18H, Boc-*H*), 2.87 (s, 8H, Cyclen-H), 3.38 (s, 8H, Cyclen-*H*), 3.43 (s, 4H, CO-*CH_2*-N), 4.14 (t, 4H, CO-*CH_2*-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.3, 155.9, 79.4, 60.3, 55.2, 54.5, 46.6, 28.4, 14.2.

Compound **4a** (yield 60%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, *J* = 6.4 Hz, 6H, -*CH_3*), 1.28 (m, 36H, -*CH_2*-), 1.44 (s, 18H, Boc-*H*), 1.62 (s, 4H, -*CH_2*-), 2.88 (s, 8H, Cyclen-*H*), 3.37 (s, 8H, Cyclen-*H*), 3.45 (s, 4H, CO-*CH_2*-N), 4.07 (t, *J* = 6.5 Hz, 4H, CO-*CH_2*-CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.43, 155.92, 79.43, 64.51, 55.08, 54.45, 46.68, 31.92, 29.64, 29.59, 29.53, 29.36, 29.25, 28.65, 28.49, 25.95, 22.69, 14.13. HR-MS (ESI): $C_{46}H_{88}N_4NaO_8$ $[M+Na]^+$, 847.6500, found 847.6500.

Compound **4b** (yield 64%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, *J* = 6.8 Hz, 6H, -*CH_3*), 1.28 (m, 44H, -*CH_2*-), 1.44 (s, 18H, Boc-*H*), 1.62 (s, 4H, -*CH_2*-), 2.88 (s, 8H, Cyclen-*H*), 3.37 (s, 8H, Cyclen-*H*), 3.45 (s, 4H, CO-*CH_2*-N), 4.07 (t, *J* = 6.7 Hz, 4H, CO-*CH_2*-CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.42, 155.92, 79.42, 64.50, 54.99, 54.49, 46.68, 31.92, 29.67, 29.59, 29.53, 29.36, 29.25, 28.65, 28.49, 25.95, 22.69, 14.12. HR-MS (ESI): $C_{50}H_{97}N_4O_8$ $[M+H]^+$, 881.7306, found 881.7296.

Compound **4c** (yield 62%): ^{1}H NMR($CDCl_3$, 400 MHz): δ = 0.88 (t, *J* = 6.8 Hz, 6H, -*CH_3*), 1.26 (m, 52H, -*CH_2*-), 1.44 (s, 18H, Boc-*H*), 1.62 (m, 4H, -*CH_2*-), 2.88 (s, 8H, Cyclen-*H*), 3.37 (s, 8H, Cyclen-*H*), 3.43 (s, 4H, CO-*CH_2*-N), 4.07 (t, *J* = 6.7 Hz, 4H, CO-*CH_2*-CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.42, 155.91, 79.42, 64.50, 54.98, 54.72, 46.70, 31.93, 29.68, 29.60, 29.53, 29.36, 29.25 28.65, 28.49, 25.95, 22.69, 14.12. HR-MS (ESI): $C_{54}H_{105}N_4O_8$ $[M+H]^+$, 937.7932, found 937.7996.

Compound **4d** (yield 58%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, *J* = 6.7 Hz, 6H, -*CH_3*), 1.26(m, 44H, -*CH_2*-), 1.44 (s, 18H, Boc-*H*), 1.60 (m, 4H, -*CH_2*-), 2.01 (m, 8H, CH=CH-*CH_2*), 2.88 (s, 8H, Cyclen-*H*), 3.37 (s, 8H, Cyclen-*H*), 3.45 (s, 4H, CO-*CH_2*-N), 4.07 (t, *J* = 6.7 Hz, 4H, CO-*CH_2*-CH_2), 5.36(m, 4H, -*CH=CH*-). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.39, 155.91, 129.87, 79.44, 64.50, 54.98, 54.45, 46.67, 31.91, 29.77, 29.74, 29.70, 29.66, 29.53, 29.43, 29.32, 29.23, 28.65, 28.49, 27.21, 25.94, 22.68, 14.12. HR-MS (ESI): $C_{58}H_{109}N_4O_8$ $[M+H]^+$, 989.8245, found 989.8444.

Compound **4e** (yield 55%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.89 (t, *J* = 6.8 Hz, 6H, -*CH*$_3$), 1.29 (m, 60H, -*CH*$_2$-), 1.45 (s, 18H, Boc-*H*), 1.62 (m, 4H, -*CH*$_2$-), 2.89 (s, 8H, Cyclen-*H*), 3.38 (s, 8H, Cyclen-*H*), 3.46 (s, 4H, CO-*CH*$_2$-N), 4.08 (t, *J* = 6.8 Hz, 4H, CO-*CH*$_2$-CH$_2$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.40, 155.90, 79.42, 64.51, 54.95, 54.48, 46.70, 31.93, 29.68, 29.60, 29.54, 29.36, 29.25, 28.65, 28.48, 25.95, 22.69, 14.13. HR-MS (ESI): $C_{58}H_{113}N_4O_8$ $[M+H]^+$, 993.8558, found 993.8561.

Compound **4f** (yield 58%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ= 0.68 (s, 6H, cholesterol-*H*), 0.86-2.05 (m, 94H, cholesterol-*H* , Boc-*H*), 2.31 (d, *J* = 7.8 Hz, 4H, cholesterol-*H*), 2.87 (s, 8H, Cyclen-*H*), 3.38-3.41 (m, 12H, Cyclen-*H*, CO-*CH*$_2$-N), 4.63 (m, 2H, cholesterol-H), 5.37 (d, *J* = 3.9 Hz, 2H, cholesterol-*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.69, 155.93, 139.52, 122.74, 79.42, 74.15, 56.68, 56.13, 55.55, 54.60, 50.01, 46.75, 42.31, 39.73, 39.52, 38.24, 36.98, 36.58, 36.18, 35.80, 31.90, 31.85, 28.52, 28.24, 28.02, 27.91, 24.29, 23.83, 22.83, 22.57, 21.03, 19.33, 18.72, 11.86. HR-MS (ESI): $C_{76}H_{129}N_4O_8$ $[M+H]^+$, 1225.9810, found 1225.9813.

Compound **4g** (yield 60%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.79 (m, 12H, diosgenin-*H*), 0.98-2.01 (m, 76H, diosgenin-*H* , Boc-*H*), 2.32 (d, *J* = 7.0 Hz, 4H, diosgenin-*H*), 2.87 (s, 8H, Cyclen-*H*), 3.44 (m, 16H, Cyclen-*H*, CO-*CH*$_2$-N, diosgenin-*H*), 4.63 (m, 2H, diosgenin-*H*), 5.37 (d, *J* = 4.1 Hz, 2H, diosgenin-*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.63, 155.92, 139.56, 122.46, 109.28, 80.80, 79.42, 74.06, 71.68, 66.85, 62.07, 56.42, 55.56, 54.58, 49.92, 46.68, 41.61, 40.26, 39.72, 38.22, 36.95, 36.72, 32.04, 31.84, 31.39, 30.30, 28.81, 28.52, 27.88, 20.82, 19.35, 17.15, 16.29, 14.54. HR-MS (ESI): $C_{76}H_{121}N_4O_{12}$ $[M+H]^+$, 1281.8981, found 1281.9133.

Compound **4h** (yield 61%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.85 (m, 24H, -*CH*$_3$), 1.06-1.58 (m, 66H, tocopherol-*H*), 1.77 (m, 4H, C–*CH*$_2$), 1.96 (s, 6H, Ph-*CH*$_3$), 2.00 (s, 6H, Ph-*CH*$_3$), 2.08 (s, 6H, Ph- *CH*$_3$), 2.58 (t, *J* = 6.4 Hz, 4H, tocopherol-*H*), 3.01 (s, 8H, Cyclen-*H*), 3.47 (s, 8H, Cyclen-*H*), 3.80 (s, 4H, CO-*CH*$_2$-N), ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.22, 156.01, 149.44, 140.29, 126.51, 124.76, 123.10, 117.42, 79.54, 75.07, 54.73, 46.44, 39.39, 37.48, 37.38, 32.81, 32.73, 31.08, 28.54, 27.99, 24.82, 24.46, 22.75, 22.65, 21.04, 20.63, 19.77, 19.67, 13.22, 12.38, 11.85. HR-MS (ESI): $C_{80}H_{137}N_4O_{10}$ $[M+H]^+$, 1314.0335, found 1314.0331.

Lipid **5a** (yield 99%): ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.85 (t, *J* = 6.6 Hz, 6H, -*CH*$_3$), 1.26 (m, 36H, -*CH*$_2$-), 1.57 (m, 4H, -*CH*$_2$-), 2.62-3.29 (m, 16H, Cyclen-*H*,), 3.51 (s, 4H, CO-

CH$_2$-N), 4.05 (t, *J* = 6.7 Hz, 4H, CO-*CH*$_2$-CH$_2$). ^{13}C NMR (DMSO-d$_6$, 100 MHz): δ = 171.68, 64.79, 53.29, 48.76, 42.68, 31.76, 29.47, 29.19, 29.15, 28.52, 22.56, 14.34. HR-MS (ESI): $C_{36}H_{73}N_4O_4$ [M+H]$^+$, 625.5632, found 625.5636.

Lipid **5b** (yield 98%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, *J* = 6.7 Hz, 6H, -*CH*$_3$), 1.26 (m, 44H, -*CH*$_2$-), 1.62 (s, 4H, -*CH*$_2$-), 2.98-3.51 (m, 20H, Cyclen-*H*, CO-*CH*$_2$-N), 4.06 (t, *J* = 6.9 Hz, 4H, CO-*CH*$_2$-CH$_2$). ^{13}C NMR (DMSO-d$_6$, 100 MHz): δ = 171.70, 64.80, 53.31, 48.80, 42.68, 31.77, 29.49, 29.19, 29.17, 28.53, 22.56, 14.32. HR-MS (ESI): $C_{40}H_{81}N_4O_4$ [M+H]$^+$, 681.6258, found 681.6262.

Lipid **5c** (yield 99%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, *J* = 6.7 Hz, 6H, -*CH*$_3$), 1.27 (m, 52H, -*CH*$_2$-), 1.60 (s, 4H, -*CH*$_2$-), 3.04-3.52 (m, 20H, Cyclen-*H*, CO-*CH*$_2$-N), 4.07 (t, *J* = 6.9 Hz, 4H, CO-*CH*$_2$-CH$_2$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.79, 66.32, 53.77, 49.88, 43.03, 31.92, 29.70, 29.66, 29.59, 29.49,29.36, 29.23, 28.25, 25.72, 22.68, 14.09. HR-MS (ESI): $C_{44}H_{89}N_4O_4$ [M+H]$^+$, 737.6884, found 737.6891.

Lipid **5d** (yield 98%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, *J* = 6.6 Hz, 6H, -*CH*$_3$), 1.27 (m, 44H, -*CH*$_2$-), 1.60 (m, 4H, -*CH*$_2$-), 2.00 (m, 8H, CH=CH-*CH*$_2$), 3.02-3.51 (m, 20H, Cyclen-*H*, CO-*CH*$_2$-N), 4.06 (t, *J* = 6.9 Hz, 4H, CO-*CH*$_2$-CH$_2$), 5.35 (m, 4H, -*CH*=*CH*-). ^{13}C NMR (DMSO-d$_6$, 100 MHz): δ =: 171.69, 130.18, 64.79, 53.34, 48.81, 42.70, 31.75, 29.47, 29.31, 29,16, 29.09, 28.52, 27.06, 27.02, 25.83, 22.55, 14.35. HR-MS (ESI): $C_{48}H_{93}N_4O_4$ [M+H]$^+$, 789.7197, found 789.7196.

Lipid **5e** (yield 98%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, *J* = 6.8 Hz, 6H, -*CH*$_3$), 1.27 (d, 60H, -*CH*$_2$-), 1.64 (m, 4H, -*CH*$_2$-), 3.02-3.54 (m, 20H, Cyclen-*H*, CO-*CH*$_2$-N), 4.09 (t, *J* = 6.8 Hz, 4H, CO-*CH*$_2$-CH$_2$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ =: 171.82, 66.30, 53.97, 50.06, 43.09, 31.92, 29.74, 29.66, 29.60, 29.51,29.36, 29.24, 28.29, 25.75, 22.68, 14.10. HR-MS (ESI): $C_{44}H_{97}N_4O_4$ [M+H]$^+$, 793.7510, found 793.7518.

Lipid **5f** (yield 99%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.68 (s, 6H, cholesterol-*H*), 0.87-1.99 (m, 76H, cholesterol-*H*), 2.31 (d, *J* = 7.7 Hz, 4H, cholesterol-*H*), 3.05-3.47 (m, 20H, Cyclen-*H*, CO-*CH*$_2$-N), 4.56 (d, 2H, cholesterol-*H*), 5.37 (s, 2H, cholesterol-*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.00, 138.99, 123.05, 78.27, 56.71, 56.18, 50.03, 42.33, 39.74, 39.52, 37.68, 36.85, 36.49, 36.20, 35.81, 31.93, 31.82, 28.42, 28.24, 28.02, 27.43, 24.29, 23.85, 22.81, 22.55, 20.97, 19.20, 18.75, 11.84. HR-MS (ESI): $C_{66}H_{113}N_4O_4$ [M+H]$^+$, 1025.8762, found

1025.8770.

Lipid **5g** (yield 99%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.79 (m, 12H, diosgenin-*H*), 0.96-1.98 (m, 58H, diosgenin-*H*), 2.32 (d, *J* = 7.3 Hz, 4H, diosgenin-*H*), 2.87-3.44 (m, 24H, Cyclen-*H*, CO-*CH*$_2$-N, diosgenin-*H*), 4.59 (s, 2H, diosgenin-*H*), 5.38 (s, 2H, diosgenin-*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.04, 139.05, 122.77, 109.30, 80.78, 76.14, 71.83, 66.84, 62.14, 56.46, 54.21, 49.96, 41.60, 40.26, 39.71, 37.67, 36.80, 36.63, 32.05, 31.83, 31.36, 30.28, 28.79, 27.41, 20.83, 19.18, 17.12, 16.26, 14.51. HR-MS (ESI): $C_{66}H_{105}N_4O_8$ $[M+H]^+$, 1081.7932, found 1081.7931.

Lipid **5h** (yield 99%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.85 (t, 24H, -*CH*$_3$), 1.06-1.51 (m,48H, Tocopheryl-*H*), 1.77 (m, 4H, C–*CH*$_2$), 1.89 (s, 6H, Ph-*CH*$_3$), 1.92 (s, 6H, Ph-*CH*$_3$), 2.07 (s, 6H, Ph-*CH*$_3$), 2.56 (t, *J* = 6.3 Hz, 4H, Tocopheryl -*H*), 3.04-3.47 (m, 16H, Cyclen-*H*), 3.86 (s, 4H, CO-*CH*$_2$-N). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.44, 149.78, 140.02, 126.20, 124.57, 123.24, 117.62, 75.26, 53.73, 39.38, 37.45, 37.28, 32.79, 32.73, 31.01, 28.41, 27.98, 24.80, 24.45, 22.71, 22.62, 21.04, 20.50, 19.74, 19.62, 12.81, 11.99, 11.77. HR-MS (ESI): $C_{70}H_{121}N_4O_6$ $[M+H]^+$, 1113.9286, found 1113.9281.

Lipid **6a** (yield 99%): ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.85 (t, *J* = 6.7 Hz, 6H, -*CH*$_3$), 1.24 (m, 36H, -*CH*$_2$-), 1.41 (s, 4H, -*CH*$_2$-), 2.68-3.12 (m, 20H, Cyclen-*H*, CONH-*CH*$_2$-), 3.28 (s, 4H, CO-*CH*$_2$-N). ^{13}C NMR (CD_3OD_3, 100 MHz):δ = 171.24, 55.24, 49.77, 42.81, 39.38, 31.68, 29.41, 29.37, 29.15, 29.09, 29.03, 26.84, 22.33, 13.04. HR-MS (ESI): $C_{36}H_{75}N_6O_2$ $[M+H]^+$, 623.5952, found 623.5950.

Lipid **6b** (yield 98%): ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.85 (t, *J* = 6.7 Hz, 6H, -*CH*$_3$), 1.23 (m, 44H, -*CH*$_2$-), 1.40 (s, 4H, -*CH*$_2$-), 2.66-3.12 (m, 20H, Cyclen-*H*, CONH-*CH*$_2$-), 3.28 (s, 4H, CO-*CH*$_2$-N). ^{13}C NMR (CD_3OD_3, 100 MHz): δ = 171.22, 55.22, 49.76, 42.79, 39.38, 31.69, 29.42, 29.39 29.17, 29.09, 29.04, 26.86, 22.34, 13.05. HR-MS (ESI): $C_{40}H_{83}N_6O_2$ $[M+H]^+$, 679.6578, found 679.6571.

Lipid **6c** (yield 98%): ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.85 (t, *J* = 6.7 Hz, 6H, -*CH*$_3$) 1.23 (m, 52H, -*CH*$_2$-), 1.40 (s, 4H, -*CH*$_2$-), 2.68-3.12 (m, 20H, Cyclen-*H*, CONH-*CH*$_2$-), 3.28 (s, 4H, CO-*CH*$_2$-N). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 171.03, 65.48, 56.16, 49.96, 42.96, 31.76, 29.50, 29.35 27.10, 26.55, 22.34, 14.37. HR-MS (ESI): $C_{44}H_{91}N_6O_2$ $[M+H]^+$, 735.7204, found 735.7203.

Lipid **6d** (yield 97%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, *J* = 6.7 Hz, 6H, -*CH*$_3$), 1.23 (m, 44H, -*CH*$_2$-), 1.40 (s, 4H, -*CH*$_2$-), 2.00 (m, 8H, CH=CH-*CH*$_2$), 3.02-3.45 (m, 24H, Cyclen-*H*, CONH-*CH*$_2$-), 5.35 (m, 4H, -*CH*=*CH*-). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 171.02, 129.99, 56.18, 49.93, 42.94, 31.75, 29.36, 27.09, 27.02, 22.55, 14.33. HR-MS (ESI): $C_{48}H_{95}N_6O_2$ [M+H]$^+$, 787.7517, found 787.7514.

Lipid **8a** (yield 99%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, *J* = 6.8 Hz, 6H, -*CH*$_3$), 1.28 (m, 32H, -*CH*$_2$-), 1.60 (m, 4H, -*CH*$_2$-), 2.36 (t, *J* = 7.7 Hz, 4H, CO-*CH*$_2$-CH_2), 2.71-3.44 (m, 20H, Cyclen-*H*,), 4.28 (t, *J* = 4.4 Hz, 4H, COO-*CH*$_2$). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 173.33, 60.71, 50.57, 47.70, 42.59, 33.89, 31.77, 29.48, 29.38, 19.19, 28.91, 24.82, 22.57, 14.38. HR-MS (ESI): $C_{36}H_{73}N_4O_4$ [M+H]$^+$, 625.5632, found 625.5629.

Lipid **8b** (yield 99%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, *J* = 6.8 Hz, 6H, -*CH*$_3$), 1.28 (m, 40H, -*CH*$_2$-), 1.60 (m, 4H, -*CH*$_2$-), 2.36 (t, *J* = 7.7 Hz, 4H, CO-*CH*$_2$-CH_2), 2.71-3.44 (m, 20H, Cyclen-*H*,), 4.28 (t, *J* = 4.4 Hz, 4H, COO-*CH*$_2$). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 173.33, 60.74, 50.61, 47.73, 42.58, 33.89, 31.78, 29.51, 29.39, 19.20, 28.92, 24.82, 22.57, 14.38. HR-MS (ESI): $C_{40}H_{81}N_4O_4$ [M+H]$^+$, 681.6258, found 681.6263.

Lipid **10a** (yield 99%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, *J* = 6.8 Hz, 3H, -*CH*$_3$), 1.29 (m, 18H, -*CH*$_2$-), 1.64 (m, 2H, -*CH*$_2$-), 3.14 (m, 16H, Cyclen-*H*), 3.51 (s, 2H, CO- *CH*$_2$-N), 4.11 (t, *J* = 6.9, 2H, COO- *CH*$_2$). ^{13}C NMR (DMSO-d_6, 100 MHz):δ = 172.13, 64.87, 53.35, 48.99, 44.57, 42.51, 31.76, 29.50, 29.47, 29.45, 29.40, 29.18, 29.12, 28.49, 25.83, 22.56, 14.38. HR-MS (ESI): $C_{22}H_{47}N_4O_2$ [M+H]$^+$, 399.3699, found 399.3694.

Lipid **10b** (yield 99%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, *J* = 6.8 Hz, 3H, -*CH*$_3$), 1.29 (m, 22H, -*CH*$_2$-), 1.64 (m, 2H, -*CH*$_2$-), 3.12 (m, 16H, Cyclen-*H*), 3.50 (s, 2H, CO- *CH*$_2$-N), 4.12 (t, *J* = 6.9, 2H, COO-*CH*$_2$). ^{13}C NMR (DMSO-d_6, 100 MHz):δ = 172.16, 64.89, 53.35, 48.99, 44.59, 42.52, 31.76, 29.52, 29.50, 29.48, 29.45, 29.41, 29.18, 29.13, 28.50, 25.83, 22.56, 14.40. HR-MS (ESI): $C_{24}H_{51}N_4O_2$ [M+H]$^+$, 427.4012, found 427.4004.

Lipid **10e** (yield 98%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, *J* = 6.8 Hz, 3H, -*CH*$_3$), 1.30 (m, 30H, -*CH*$_2$-), 1.64 (m, 2H, -*CH*$_2$-), 3.12 (m, 16H, Cyclen-*H*), 3.50 (s, 2H, CO- *CH*$_2$-N), 4.13 (t, *J* = 6.9, 2H, COO-*CH*$_2$). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 172.13, 64.88, 53.34, 48.99, 44.58, 42.52, 31.77, 29.51, 29.48, 29.42, 29.19, 29.15, 28.50, 25.84, 22.57, 14.38 HR-MS (ESI): $C_{28}H_{59}N_4O_2$ [M+H]$^+$, 483.4638, found 483.4638.