

Electronic Supplementary Information (ESI)

Cyclen-based cationic lipids containing pH-sensitive moiety as gene delivery vectors

Zheng Huang, Yan-Hong Liu, Yi-Mei Zhang, Ji Zhang*, Qiang Liu and Xiao-Qi Yu*

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

* Correspondence to: Fax: + 86 28 85415886; Tel: + 86 28 85415886
E-mail: xqyu@scu.edu.cn (X.-Q. Yu); jzhang@scu.edu.cn (J. Zhang)

Table S1 The buffer capacity of newly synthesized lipids and PEI

| Compounds | 3b | 3d | 3e | 3f | 3g | 3h | PEI |
|------------------------|-----------|-----------|-----------|-----------|-----------|-----------|------|
| Buffering capacity (%) | 15.9 | 18.7 | 18.8 | 17.3 | 19.2 | 9.8 | 17.2 |

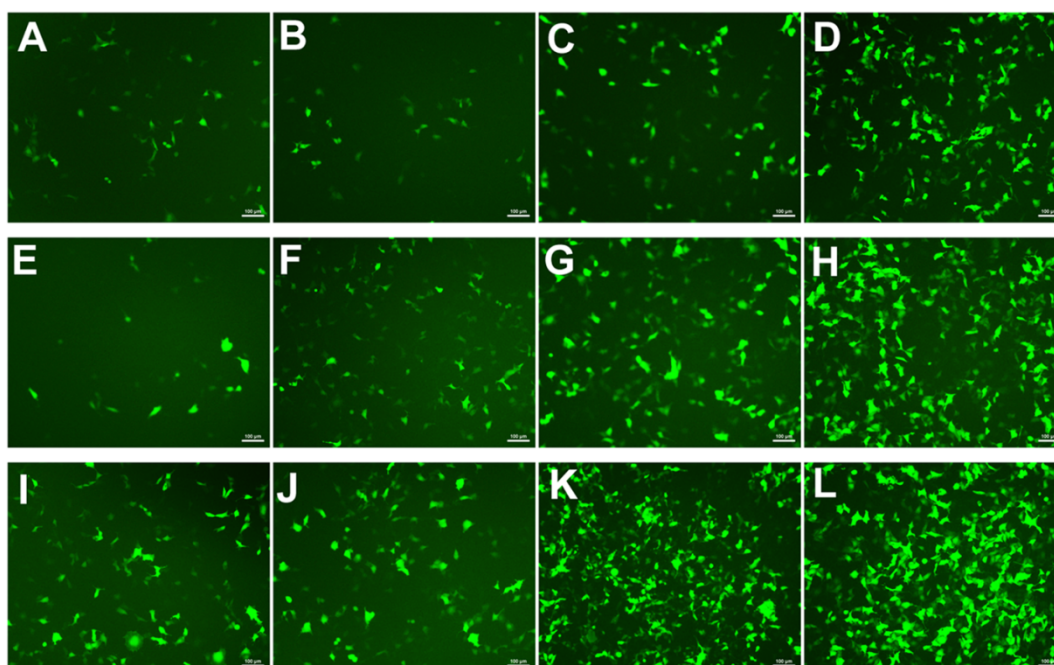


Fig. S1 Fluorescent microscope images of HEK293 cells transfected by **3f** with different Lipid/DOPE ratio. Lipid/DOPE ratio was 1:1 (A, B, C, D), 1:2 (E, F, G, H), 1:3 (I, J, K, L), and from the left to right, the N/P ratios were 2, 4, 6 and 8, respectively. The cells were observed by fluorescence microscopy after 24 h transfection.

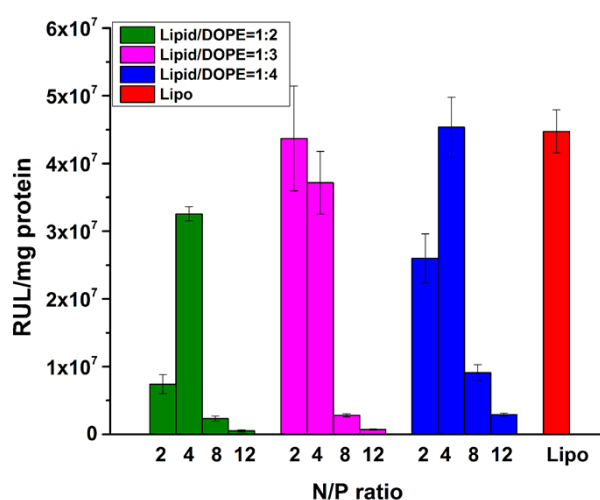


Fig. S2 Luciferase expression in HEK 293 cells transfected by **3f**/DOPE/DNA lipoplexes at various N/P ratios and different Lipid/DOPE ratios. The values were the means \pm SD for three independent transfection repeats.

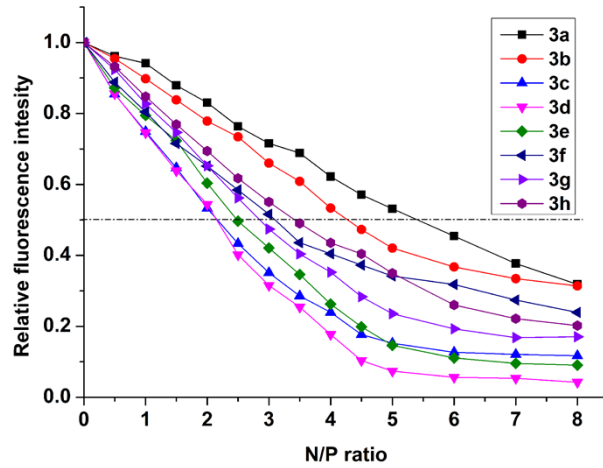


Fig. S3 Fluorescent quenching assay of EB/DNA by the addition of lipid/DOPE liposomes. The molar ratio of lipid/DOPE was 1:3.

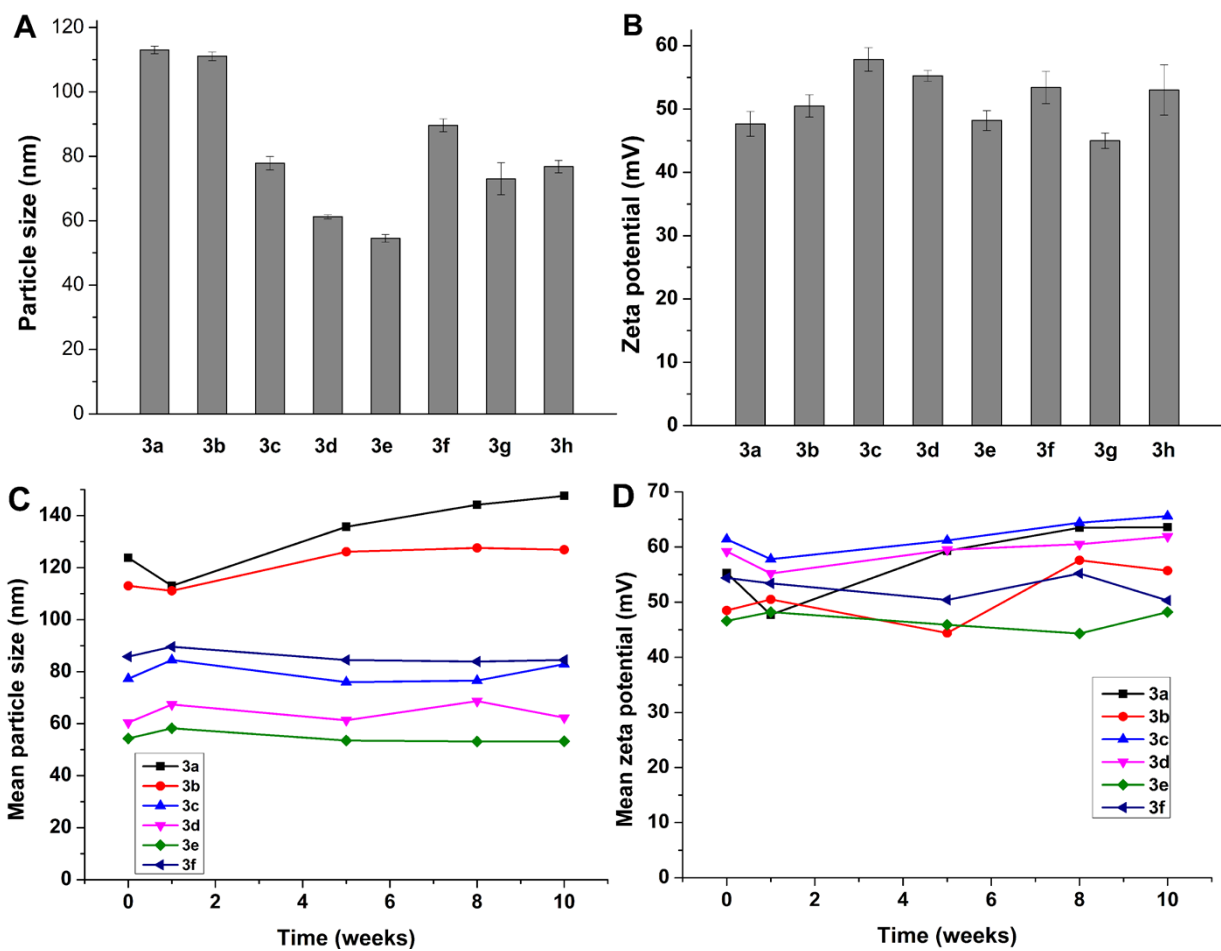


Fig. S4 Mean particle size (A) and zeta potential (B) of liposomes formed from **3a-3h**. Mean particle sizes (C) and zeta-potentials (D) of the liposomes after storage under 4°C for different durations.

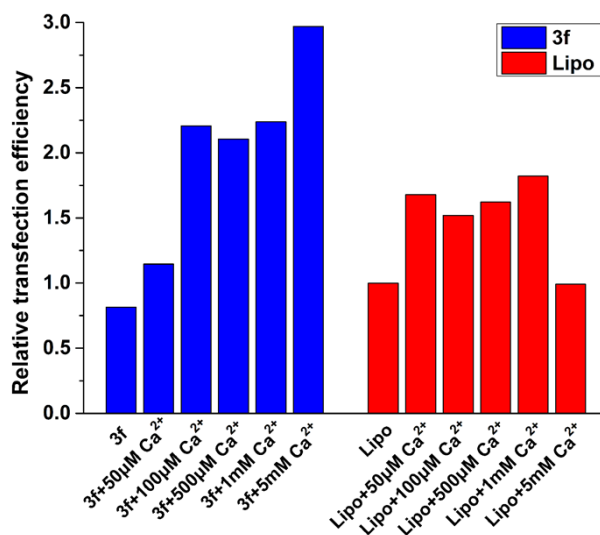


Fig. S5 Relative fluorescence intensity of eGFP after 24 h of transfection mediated by **3f** and lipofectamine 2000. Excitation wavelength was 485 nm and the emission wavelength was 538 nm.

Analysis data of Compounds 2a-2g

2a (306 mg, yield: 65.1%): ¹H NMR (CDCl₃, 400 MHz) δ = 7.52 (s, 1H, imidazole), 7.40 (s, 1H, CON-H), 6.78 (s, 1H, imidazole), 4.79 (s, 1H, NHC-H), 4.07 (t, 2H, *J*=6.8 Hz, COO-CH₂), 3.63-2.59 (m, 20H, cyclen-H, cyclen-CH₂, imidazole-CH₂), 1.63-1.55 (m, 2H, COOC-CH₂), 1.49-1.41 (m, 27H, Boc-H), 1.31-1.22 (m, 18H, (CH₂)₉), 0.87 (t, 3H, *J*=6.8 Hz, -CH₃). ¹³C NMR (CDCl₃, 100 MHz): δ = 171.24, 170.13, 156.21, 155.60, 135.06, 79.82, 79.47, 77.37, 77.26, 77.05, 76.74, 65.57, 56.29, 54.46, 53.40, 52.21, 49.66, 47.60, 31.87, 29.61, 29.60, 29.56, 29.49, 29.31, 29.20, 28.64, 28.50, 25.95, 25.80, 25.31, 25.07, 24.61, 22.64, 14.08. HR-MS (ESI): *m/z* calcd for [C₄₃H₇₈N₇O₉]⁺ ([M+H]⁺): 836.5861, found: 836.5857.

2b (260 mg, yield: 62.5%): ¹H NMR (CDCl₃, 400 MHz) δ = 7.54 (s, 1H, CON-H), 7.51 (s, 1H, imidazole), 7.13 (s, 1H, CON-H), 6.81 (s, 1H, imidazole), 4.63 (s, 1H, NHC-H), 3.60-2.58 (m, 22H, cyclen-H, cyclen-CH₂, imidazole-CH₂, CON-CH₂), 1.53-1.36 (m, 29H, Boc-H, CON-CH₂), 1.30-1.19 (m, 18H, (CH₂)₉), 0.88 (t, 3H, *J*=6.8 Hz, -CH₃). ¹³C NMR (CDCl₃, 100 MHz): δ = 171.13, 170.73, 156.22, 155.58, 134.79, 79.93, 79.63, 77.37, 77.25, 77.05, 76.73, 53.43, 53.06, 49.80, 47.46, 39.63, 31.90, 29.65, 29.63, 29.61, 29.57, 29.36, 29.34, 29.28, 28.63, 28.51, 26.88, 22.67, 14.11. HR-MS (ESI): *m/z* calcd for [C₄₃H₇₉N₈O₈]⁺ ([M+H]⁺): 835.6021, found: 835.6059.

2c (268 mg, yield: 60.2%): ^1H NMR (CDCl_3 , 400 MHz) δ = 7.58 (s, 1H, CON-H), 7.50 (s, 1H, imidazole), 7.18 (s, 1H, CON-H), 6.79 (s, 1H, imidazole), 4.63 (s, 1H, NHC-H), 3.75-2.53 (m, 22H, cyclen-H, cyclen- CH_2 , imidazole- CH_2 , CON- CH_2), 1.63-1.32 (m, 29H, Boc-H, CONC- CH_2), 1.31-1.15 (m, 26H, $(\text{CH}_2)_{13}$), 0.87 (t, 3H, $J=6.8$ Hz, $-\text{CH}_3$). ^{13}C NMR (CDCl_3 , 100 MHz): δ = 171.12, 170.67, 156.15, 155.58, 134.77, 79.91, 79.63, 77.38, 77.06, 76.74, 53.09, 49.74, 47.51, 39.62, 31.89, 29.68, 29.63, 29.57, 29.33, 29.28, 28.63, 28.50, 26.89, 22.66, 14.10. HR-MS (ESI): m/z calcd for $[\text{C}_{47}\text{H}_{87}\text{N}_8\text{O}_8]^+$ ($[\text{M}+\text{H}]^+$): 891.6647, found: 891.6686.

2d (268 mg, yield: 58.3%): ^1H NMR (CDCl_3 , 400 MHz) δ = 7.56 (s, 1H, CON-H), 7.50 (s, 1H, imidazole), 7.16 (s, 1H, CON-H), 6.78 (s, 1H, imidazole), 4.62 (s, 1H, NHC-H), 3.62-2.65 (m, 22H, cyclen-H, cyclen- CH_2 , imidazole- CH_2 , CON- CH_2), 1.50-1.18 (m, 59H, Boc-H, CONC- CH_2 , $(\text{CH}_2)_{15}$), 0.87 (t, 3H, $J=6.4$ Hz, $-\text{CH}_3$). ^{13}C NMR (CDCl_3 , 100 MHz): δ = 171.12, 170.67, 156.18, 155.57, 134.76, 79.90, 79.61, 77.37, 77.25, 77.05, 76.73, 54.93, 53.40, 53.09, 49.70, 47.47, 39.63, 31.89, 29.67, 29.62, 29.57, 29.36, 29.32, 29.27, 28.62, 28.50, 26.88, 22.65, 14.08. HR-MS (ESI): m/z calcd for $[\text{C}_{49}\text{H}_{91}\text{N}_8\text{O}_8]^+$ ($[\text{M}+\text{H}]^+$): 919.6960, found: 919.6995.

2e (236 mg, yield: 51.4%): ^1H NMR (CDCl_3 , 400 MHz) δ = 7.55 (s, 1H, CON-H), 7.50 (s, 1H, imidazole), 7.17 (s, 1H, CON-H), 6.80 (s, 1H, imidazole), 5.40-5.28 (m, 2H, $-\text{CH}=\text{CH}-$), 4.62 (s, 1H, NHC-H), 3.80-2.54 (m, 22H, cyclen-H, cyclen- CH_2 , imidazole- CH_2 , CON- CH_2), 2.07-1.91 (m, 4H, $-\text{CH}_2-\text{C}=\text{C}-\text{CH}_2-$), 1.53-1.17 (m, 51H, Boc-H, CONC- CH_2 , $(\text{CH}_2)_{11}$), 0.87 (t, 3H, $J=6.8$ Hz, $-\text{CH}_3$). ^{13}C NMR (CDCl_3 , 100 MHz): δ = 171.12, 170.68, 156.20, 155.58, 134.76, 130.40, 130.21, 129.94, 129.75, 79.92, 79.63, 77.36, 77.05, 76.73, 54.41, 53.09, 49.72, 47.49, 39.63, 32.58, 31.87, 29.74, 29.68, 29.63, 29.57, 29.49, 29.47, 29.36, 29.33, 29.28, 29.25, 29.16, 29.11, 28.63, 28.50, 27.19, 26.88, 22.64, 14.08. HR-MS (ESI): m/z calcd for $[\text{C}_{49}\text{H}_{89}\text{N}_8\text{O}_8]^+$ ($[\text{M}+\text{H}]^+$): 917.6803, found: 917.6812.

2f (284 mg, yield: 62.9%): ^1H NMR (CDCl_3 , 400 MHz) δ = 7.57 (s, 1H, CON-H), 7.53 (s, 1H, imidazole), 7.17 (s, 1H, CON-H), 6.81 (s, 1H, imidazole), 5.42-5.27 (m, 4H, $-\text{CH}=\text{CH}-$), 4.63 (s, 1H, NHC-H), 3.62-2.63 (m, 24H, cyclen-H, cyclen- CH_2 , imidazole- CH_2 , CON- CH_2 , $\text{C}=\text{C}-\text{CH}_2-\text{C}=\text{C}$), 2.04 (m, 4H, $\text{C}=\text{C}-\text{CH}_2-$), 1.49-1.40 (m, 29H, Boc-H, CONC- CH_2), 1.34-1.23 (m, 16H, $(\text{CH}_2)_8$), 0.88 (t, 3H, $J=6.8$ Hz, $-\text{CH}_3$). ^{13}C NMR (CDCl_3 , 100 MHz): δ = 171.09, 170.70, 156.25, 155.58, 134.72, 130.19, 130.05, 129.94, 129.75, 127.99, 127.89, 79.91, 79.61, 77.37, 77.05, 76.73, 55.23, 53.09, 49.69, 47.51, 39.63, 31.87, 31.49, 29.74, 29.64, 29.47,

29.36, 29.31, 29.25, 29.23, 28.63, 28.50, 27.21, 27.17, 26.88, 25.61, 22.64, 22.53, 14.04. HR-MS (ESI): m/z calcd for $[C_{49}H_{87}N_8O_8]^+$ ($[M+H]^+$): 915.6647, found: 915.6647.

2g (229 mg, yield: 50.0%), 1H NMR ($CDCl_3$, 400 MHz) δ = 7.52 (s, 1H, imidazole), 6.78 (s, 1H, imidazole), 5.41-5.27 (m, 4H, -CH=CH-), 4.78 (s, 1H, NHC-H), 4.07 (t, 2H, $J=6.8$ Hz, COO-CH₂), 3.69-2.56 (m, 22H, cyclen-H, cyclen-CH₂, imidazole-CH₂, C=C-CH₂-C=C), 2.03 (m, 4H, C=C-CH₂-), 1.63-1.55 (m, 2H, COOC-CH₂), 1.49-1.39 (m, 27H, Boc-H), 1.35-1.23 ((m, 16H, (CH₂)₈), 0.88 (t, 3H, $J=6.8$ Hz, -CH₃). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.22, 170.16, 156.17, 155.59, 135.02, 130.18, 130.03, 129.73, 128.00, 127.88, 79.80, 79.44, 77.39, 77.28, 77.07, 76.75, 65.56, 56.07, 53.91, 52.22, 50.38, 49.69, 47.60, 33.06, 31.86, 31.48, 29.72, 29.62, 29.49, 29.41, 29.31, 29.22, 29.18, 28.65, 28.50, 27.19, 27.17, 25.91, 25.79, 25.60, 25.29, 25.09, 24.39, 22.65, 22.54, 14.06. HR-MS (ESI): m/z calcd for $[C_{49}H_{86}N_7O_9]^+$ ($[M+H]^+$): 916.6487, found: 916.6485.

Analysis data of Compounds 3a-3g

3a (343 mg, yield: 98.2%), 1H NMR (CD_3OD , 400 MHz) δ = 8.86 (s, 1H, imidazole), 7.41 (s, 1H, imidazole), 4.86-4.83 (m, 1H, NHC-H), 4.24-4.09 (m, 2H, COO-CH₂), 3.40-2.73 (m, 20H, cyclen-CH₂, cyclen-H, imidazole-CH₂), 1.69-1.61 (m, 2H, COOC-CH₂), 1.37-1.28 ((m, 18H, (CH₂)₉), 0.92 (t, 3H, $J=6.8$ Hz, -CH₃). ^{13}C NMR (CD_3OD , 100 MHz): δ = 173.2, 170.2, 133.7, 129.4, 117.1, 65.9, 55.3, 51.9, 50.0, 48.2, 48.0, 47.8, 47.6, 47.4, 47.2, 47.0, 44.4, 42.7, 31.7, 29.4, 29.3, 29.1, 29.0, 28.2, 26.1, 25.5, 22.3, 13.0. HR-MS (ESI): m/z calcd for $[C_{28}H_{54}N_7O_3]^+$ ($[M+H]^+$): 536.4288, found: 536.4285.

3b (330 mg, yield: 95.7%), 1H NMR (CD_3OD , 400 MHz) δ = 8.83 (s, 1H, imidazole), 7.37 (s, 1H, imidazole), 4.77-4.72 (m, 1H, NHC-H), 3.35-2.81 (m, 22H, cyclen-CH₂, cyclen-H, imidazole-CH₂, CON-CH₂), 1.54-1.42(m, 2H, CONC-CH₂), 1.35-1.27 ((m, 18H, (CH₂)₉), 0.92 (t, 3H, $J=6.8$ Hz, -CH₃). ^{13}C NMR (CD_3OD , 100 MHz): δ = 172.9, 170.2, 133.6, 129.5, 117.1, 55.3, 52.5, 50.0, 48.2, 48.0, 47.8, 47.6, 47.4, 47.2, 47.0, 44.4, 42.7, 42.5, 39.3, 31.7, 29.4, 29.1, 29.0, 28.9, 26.9, 26.6, 22.3, 13.0. HR-MS (ESI): m/z calcd for $[C_{28}H_{55}N_8O_2]^+$ ($[M+H]^+$): 535.4448, found: 535.4451.

3c (324 mg, yield: 93.2%), 1H NMR ($DMSO-d_6$, 400 MHz) δ = 8.97 (s, 1H, imidazole), 8.13 (s, 1H, CON-H), 7.34 (s, 1H, imidazole), 4.59 (s, 1H, NHC-H), 4.10-3.60 (m, 4H, cyclen-CH₂,

CON-CH₂), 3.47-3.29 (t, 2H, imidazole-CH₂), 3.14-2.78 (m, 16H, cyclen-H), 1.39-1.01 ((m, 28H, (CH₂)₁₄), 0.84 (t, 3H, *J*=6.4 Hz, -CH₃). ¹³C NMR (DMSO-d₆, 100 MHz): δ = 172.1, 169.7, 134.1, 129.7, 117.3, 54.8, 52.3, 52.2, 49.7, 44.6, 43.1, 42.8, 40.5, 40.3, 40.1, 39.8, 39.6, 39.4, 39.2, 31.7, 29.5, 29.4, 29.3, 29.2, 27.7, 26.8, 22.5, 14.3. HR-MS (ESI): *m/z* calcd for [C₃₂H₆₃N₈O₂]⁺ ([M+H]⁺): 591.5074, found: 591.5069.

3d (319 mg, yield: 95.2%), ¹H NMR (DMSO-d₆, 400 MHz) δ = 8.97 (s, 1H, imidazole), 8.64 (s, 1H, CON-H), 8.12 (s, 1H, CON-H), 7.35 (s, 1H, imidazole), 4.63-4.52 (m, 1H, NHC-H), 3.57-2.56 (m, 22H, cyclen-H, cyclen-CH₂, imidazole-CH₂, CON-CH₂), 1.41-1.11 ((m, 30H, (CH₂)₁₅), 0.84 (t, 3H, *J*=6.0 Hz, -CH₃). ¹³C NMR (DMSO-d₆, 100 MHz): δ = 172.1, 169.6, 134.2, 129.8, 117.3, 54.8, 52.3, 49.7, 44.6, 43.1, 42.8, 40.5, 40.3, 40.1, 39.9, 39.7, 39.5, 39.3, 31.7, 29.5, 29.3, 29.2, 26.8, 22.5, 14.4. HR-MS (ESI): *m/z* calcd for [C₃₄H₆₇N₈O₂]⁺ ([M+H]⁺): 619.5387, found: 619.5383.

3e (294 mg, yield: 99.4%), ¹H NMR (DMSO-d₆, 400 MHz) δ = 8.97 (s, 1H, imidazole), 8.64 (s, 1H, CON-H), 8.12 (s, 1H, CON-H), 7.34 (s, 1H, imidazole), 5.42-5.01 (m, 2H, -CH=CH-), 4.62-4.55 (m, 1H, NHC-H), 3.47-2.59 (m, 22H, cyclen-H, cyclen-CH₂, imidazole-CH₂, CON-CH₂), 2.07-1.52 (m, 4H, C=C-CH₂), 1.34-1.12 ((m, 24H, (CH₂)₁₂), 0.85 (t, 3H, *J*=5.6 Hz, -CH₃). ¹³C NMR (DMSO-d₆, 100 MHz): δ = 172.1, 169.6, 134.2, 130.1, 129.7, 117.3, 54.8, 52.3, 49.7, 44.6, 43.2, 42.8, 40.3, 40.1, 39.9, 39.7, 39.5, 31.7, 29.6, 29.5, 29.3, 29.1, 29.0, 27.6, 27.1, 27.0, 26.8, 22.5, 14.4. HR-MS (ESI): *m/z* calcd for [C₃₄H₆₅N₈O₂]⁺ ([M+H]⁺): 617.5230, found: 617.5235.

3f (335 mg, yield: 95.8%) ¹H NMR (DMSO-d₆, 400 MHz) δ = 8.99 (s, 1H, imidazole), 8.65 (s, 1H, CON-H), 8.11 (s, 1H, CON-H), 7.35 (s, 1H, imidazole), 5.53-5.03 (m, 4H, -CH=CH-), 4.61-4.53 (m, 1H, NHC-H), 3.49-2.65 (m, 24H, cyclen-H, cyclen-CH₂, imidazole-CH₂, CON-CH₂, -C=C-CH₂-C=C-), 2.09-1.88 (m, 4H, -CH₂-C=C-), 1.37-1.12 ((m, 18H, (CH₂)₉), 0.86 (t, 3H, *J*=6.8 Hz, -CH₃). ¹³C NMR (DMSO-d₆, 100 MHz): δ = 172.1, 169.6, 134.8, 134.2, 130.1, 129.8, 128.2, 117.3, 54.8, 52.3, 49.7, 44.6, 43.2, 42.8, 40.3, 40.1, 39.9, 39.7, 39.5, 31.3, 29.5, 29.3, 29.2, 27.7, 27.1, 27.0, 26.8, 25.6, 22.4, 14.3. HR-MS (ESI): *m/z* calcd for [C₃₄H₆₃N₈O₂]⁺ ([M+H]⁺): 615.5074, found: 615.5071.

3g (279 mg, yield: 94.2%) ¹H NMR (DMSO-d₆, 400 MHz) δ = 9.00 (s, 1H, imidazole), 8.90 (d, 2H, *J*=7.6 Hz, CON-H), 7.43 (s, 1H, imidazole), 5.50-4.99 (m, 4H, -CH=CH-), 4.70-4.58

(m, 1H, NHC-H), 4.13-3.94 (m, 2H, COOCH₂), 3.50-2.55 (m, 22H, cyclen-H, cyclen-CH₂, imidazole-CH₂, -C=C-CH₂-C=C-), 2.34-1.70 (m, 4H, -CH₂-C=C-), 1.57-1.13 (m, 18H, (CH₂)₉), 0.85 (t, 3H, *J*=6.4 Hz, -CH₃). ¹³C NMR (DMSO-d₆, 100 MHz): δ = 172.0, 170.7, 134.5, 130.2, 129.4, 128.2, 117.6, 65.5, 54.6, 51.8, 49.5, 44.7, 43.1, 42.7, 40.6, 40.4, 40.1, 40.0, 39.7, 39.5, 39.3, 31.3, 29.5, 29.3, 29.2, 28.4, 27.1, 27.0, 26.6, 25.6, 22.4, 14.3. HR-MS (ESI): *m/z* calcd for [C₃₄H₆₂N₇O₃]⁺ ([M+H]⁺): 616.4914, found: 616.4917.

Analysis data of Compounds 5, 6 and 3h

5 (1.10 g, yield: 82.7%) ¹H NMR (CDCl₃, 400 MHz) δ = 5.41-5.31 (m, 4H, -CH=CH-), 3.83-3.70 (m, 2H, Boc-N-CH₂), 3.34-3.15 (m, 2H, CON-CH₂), 2.92-3.65 (m, 2H, -C=C-CH₂-C=C-), 2.13-1.97 (m, 4H, -CH₂-C=C-), 1.56-1.48 (m, 2H, CON-C-CH₂), 1.47-1.45 (s, 9H, Boc-H), 1.38-1.26 (m, 16H, (CH₂)₈), 0.90 (t, 3H, *J*=6.8 Hz, -CH₃). ¹³C NMR (CDCl₃, 100 MHz): δ = 169.57, 156.23, 130.01, 129.89, 127.91, 127.82, 79.65, 44.14, 39.41, 31.82, 31.43, 29.67, 29.57, 29.44, 29.40, 29.25, 29.17, 28.23, 27.12, 27.10, 26.85, 25.53, 22.59, 22.48, 13.98. HR-MS (ESI): *m/z* calcd for [C₂₅H₄₇N₂O₃]⁺ ([M+H]⁺): 423.3587, found: 423.3592.

6 (601 mg, yield: 71.9%) ¹H NMR (CDCl₃, 400 MHz) δ = 5.43-5.24 (m, 4H, -CH=CH-), 3.81 (s, 2H, CON-CH₂-CON), 3.72-2.30 (m, 22H, cyclen-H, cyclen-CH₂, CON-CH₂, -C=C-CH₂-C=C-), 2.09-1.95 (m, 4H, -C=C-CH₂), 1.51-1.23 (m, 45H, Boc-H, (CH₂)₉), 0.88 (t, 3H, *J*=6.8 Hz, -CH₃). ¹³C NMR (CDCl₃, 100 MHz): δ = 171.60, 168.90, 155.59, 130.18, 130.09, 129.90, 129.79, 127.95, 127.90, 80.04, 77.39, 77.27, 77.07, 76.75, 52.11, 50.21, 47.26, 43.57, 39.46, 31.87, 31.49, 29.74, 29.65, 29.45, 29.43, 29.32, 29.24, 28.53, 28.40, 27.21, 27.17, 26.85, 25.60, 22.65, 22.54, 14.10. HR-MS (ESI): *m/z* calcd for [C₄₅H₈₂N₆NaO₈]⁺ ([M+Na]⁺): 857.6092, found: 857.6091.

3h (662 mg, yield: 93.1%) ¹H NMR (CDCl₃, 400 MHz) δ = 8.61 (s, 1H, CON-H), 5.66-5.03 (m, 4H, -CH=CH-), 3.80 (s, 2H, CON-CH₂-CON), 3.44-3.32 (m, 2H, cyclen-CH₂), 3.28-2.67 (m, 20H, cyclen-H, CON-CH₂, -C=C-CH₂-C=C-), 2.16-1.60 (m, 4H, -C=C-CH₂), 1.47-1.11 (m, 18H, (CH₂)₉), 0.85 (t, 3H, *J*=6.4 Hz, -CH₃). ¹³C NMR (DMSO-d₆, 100 MHz): δ = 172.8, 168.6, 130.2, 128.2, 55.6, 50.0, 44.6, 43.3, 42.9, 42.4, 40.6, 40.4, 40.1, 39.9, 39.7, 39.5, 39.3, 31.3, 29.6, 29.4, 29.2, 27.1, 27.0, 26.9, 25.7, 22.5, 22.4, 14.3. HR-MS (ESI): *m/z* calcd for [C₃₀H₅₉N₆O₂]⁺ ([M+H]⁺): 535.4700, found: 535.4702.