

Electronic Supplementary Information (ESI)

Structure-activity relationship studies of symmetrical cationic bolasomes as non-viral gene vectors

Zheng Huang, Yi-Mei Zhang, Qian Cheng, Ji Zhang*, Yan-Hong Liu, Bing Wang and Xiao-Qi
Yu*

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

*Corresponding authors: xqyu@scu.edu.cn (X.-Q. Yu); jzhang@scu.edu.cn (J. Zhang); Fax: + 86 28
85415886

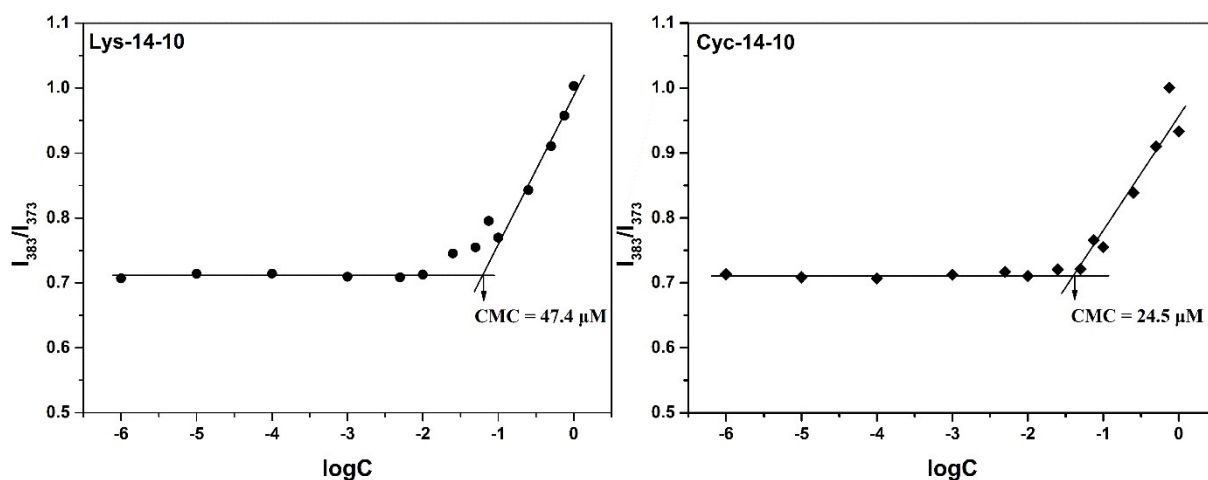


Fig. S1 Plots of the intensity ratio I_{383}/I_{373} from the pyrene emission spectra *versus* the logarithm of the concentration for self-assembling micelles in aqueous media from **Lys-14-10** and **Cyc-14-10**.

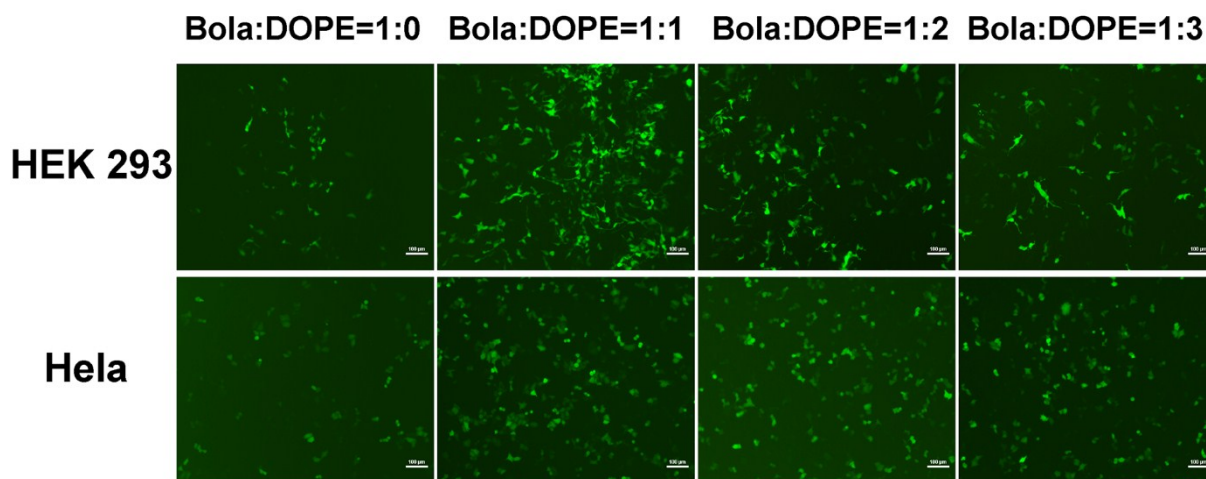


Fig. S2 Fluorescent microscope images of HEK 293 cells and Hela cells transfected by **Lys-14-10** at various bolalipid/DOPE ratios, N/P ratio was 4. The cells were observed by fluorescence microscopy after 24 h transfection.

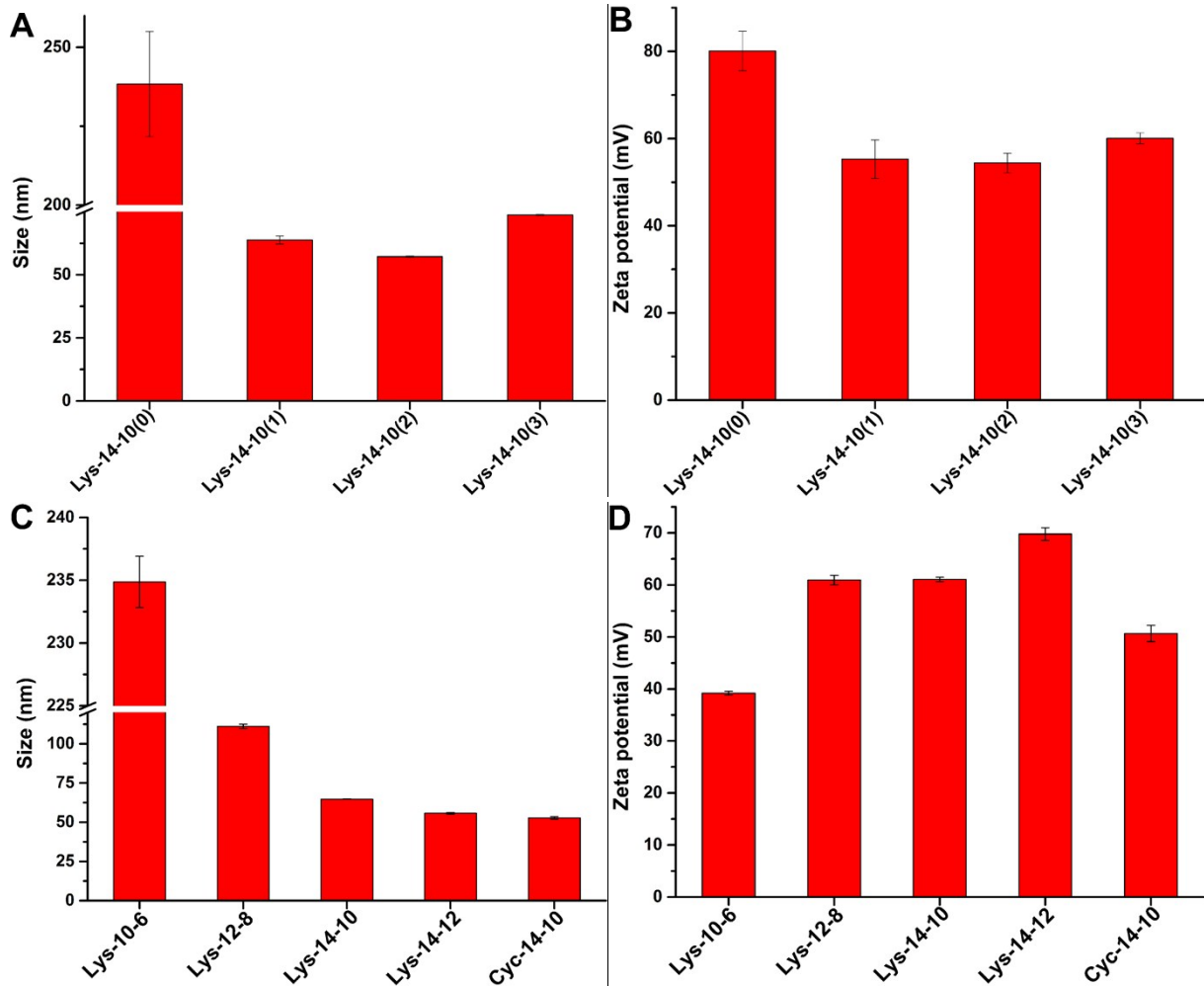


Fig. S3 Mean particle sizes (A) and zeta-potentials (B) of the bolosomes formed from **Lys-14-10** with various DOPE/bolalipid ratios (0, 1, 2, 3, respectively). Mean particle sizes (C) and zeta potentials (D) of the five bolosomes with the DOPE/bolalipid ratio of 1 : 1. DLS at room temperature. Data represent mean \pm SD (n = 3).

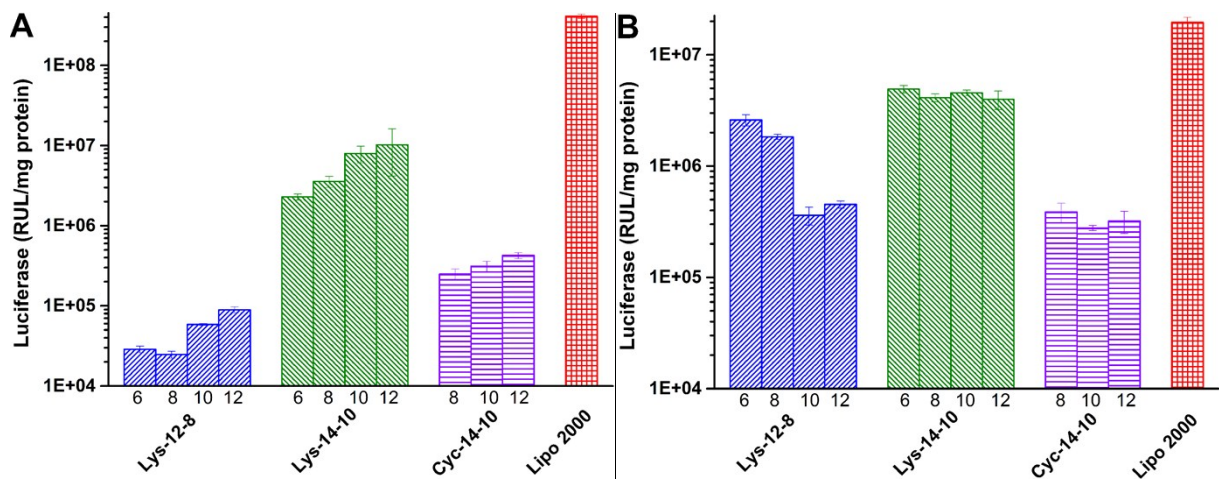


Fig. S4 Luciferase expression in HEK 293 (A) and HeLa (B) cells transfected by **Lys-12-8**, **Lys-14-10** and **Cyc-14-10** at various N/P ratios in the presence of 10% serum. Data represent mean \pm SD (n = 3).

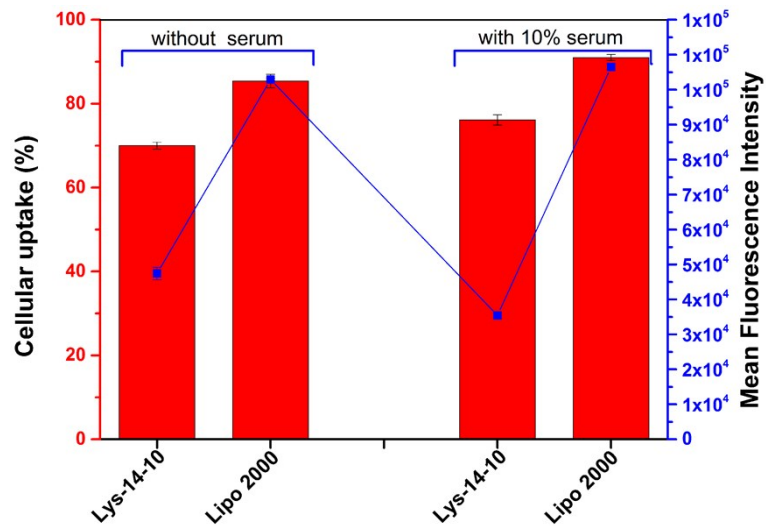


Fig. S5 Cellular uptake (columns, percentage of Cy5-positive cells) and mean fluorescence intensity (dots and lines) of **Lys-14-10**/DNA complex at optimal N/P ratio in the absence of or presence of 10% serum in HeLa cells quantified by flow cytometry. Lipofectamine 2000 was used as control. Data represent mean \pm SD (n = 3).

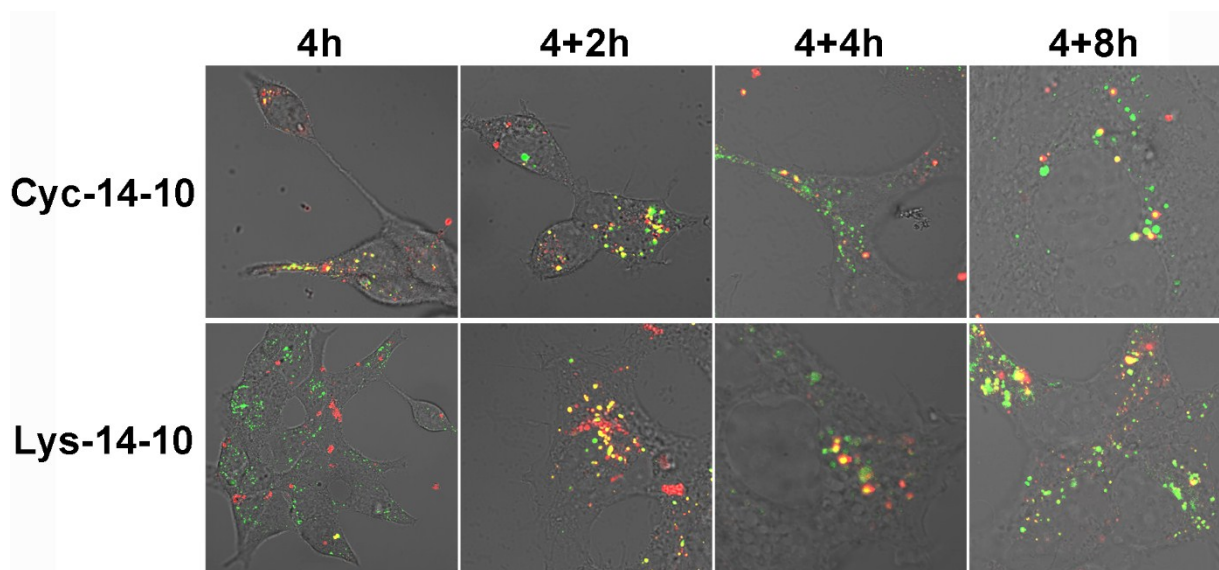


Fig. S6 Intracellular trafficking of Cy5-labelled DNA transfected by **Lys-14-10** and **Cyc-14-10** in HEK 293 cells at various time observed by CLSM. Endosomes were stained by LysoTracker Green (green); pDNA was labelled by Cy-5 (red).

Analysis data of Compounds 2

6-((tert-butyldimethylsilyl)oxy)hexan-1-ol (C6): Colourless liquid, 3.26 g, yield:70.1%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) =3.58-3.54 (m, 4H, HO-CH₂-, Si-O-CH₂-), 1.56-1.30 (m, 8H, (CH₂)₄), 0.85 (s, 9H, -C(CH₃)₃), 0.05 (s, 6H, Si-CH₃). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 63.13, 62.74, 32.73, 32.68, 26.85, 25.91, 25.57, 25.50, -5.33.

8-((tert-butyldimethylsilyl)oxy)octan-1-ol (C8): Colourless liquid, 3.40 g, yield:65.2%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 3.62-3.55 (m, 4H, HO-CH₂-, Si-O-CH₂-), 1.55-1.29 (m, 12H, (CH₂)₆), 0.87 (s, 9H, -C(CH₃)₃), 0.02 (s, 6H, Si-CH₃). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 63.26, 62.99, 32.80, 32.74, 29.36, 29.35, 25.94, 25.69, 25.65, 18.34, -5.30.

10-((tert-butyldimethylsilyl)oxy)decan-1-ol (C10): Colourless liquid, 3.72 g, yield:64.5%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 3.60-3.54 (m, 4H, HO-CH₂-, Si-O-CH₂-), 1.54-1.25 (m, 16H, (CH₂)₈), 0.86 (s, 9H, -C(CH₃)₃), 0.03 (s, 6H, Si-CH₃). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 63.28, 62.97, 32.82, 32.74, 29.50, 29.37, 26.87, 25.95, 25.93, 25.74, 25.69, 18.32, -5.31.

12-((tert-butyldimethylsilyl)oxy)dodecan-1-ol (C12): Colourless liquid, 3.24 g, yield:51.2%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 3.66-3.59 (m, 4H, HO-CH₂-, Si-O-CH₂-), 1.59-1.28 (m, 20H, (CH₂)₁₀), 0.90 (s, 9H, -C(CH₃)₃), 0.06 (s, 6H, Si-CH₃). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 63.30, 62.94, 32.83, 32.75, 29.58, 29.56, 29.53, 29.39, 25.94, 26.73, 25.75, 25.71, 25.70, 18.33, -5.31.

Analysis data of Compounds 4 (Diol-(m+2)-n)

Bis(6-hydroxyhexyl) adipate (Diol-6-6): White powder, 830.9 mg, yield: 53.8%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.09-3.95 (m, 4H, COO-CH₂), 3.66-3.49 (m, 4H, HO-CH₂), 2.29 (s, 4H, CH₂-COO), 1.69-1.27 (m, 20H, (CH₂)₁₀). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.51, 64.35, 62.65, 33.92, 32.46, 28.53, 25.67, 25.31, 24.38.

Bis(6-hydroxyhexyl) octanedioate (Diol-8-6): White powder, 236.3 mg, yield: 31.6%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.01 (t, 4H, *J*=8.0 Hz, COO-CH₂), 3.58 (t, 4H, *J*=8.0 Hz, HO-CH₂), 2.24 (t, 4H, *J*=8.0 Hz, CH₂-COO), 1.65-1.21 (m, 24H, (CH₂)₁₂). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.91, 64.25, 62.61, 34.23, 32.52, 28.70, 28.55, 25.69, 25.33, 24.75.

Bis(6-hydroxyhexyl) decanedioate (Diol-10-6): White powder, 430.3 mg, yield: 41.7%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.08 (t, 4H, *J*=6.6 Hz, COO-CH₂), 3.65 (t, 4H, *J*=6.6 Hz,

HO-CH₂), 2.30 (t, 4H, *J*=7.5 Hz, CH₂-COO), 1.68-1.31 (m, 28H, (CH₂)₁₄). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 174.02, 64.24, 62.77, 34.35, 32.60, 29.05, 28.62, 25.75, 25.38, 24.95.

Bis(6-hydroxyhexyl) dodecanedioate (Diol-12-6): White powder, 500.4 mg, yield: 40.3%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.05 (t, 4H, *J*=6.6 Hz, COO-CH₂), 3.62 (t, 4H, *J*=6.6 Hz, HO-CH₂), 2.28 (t, 4H, *J*=7.5 Hz, CH₂-COO), 1.64-1.27 (m, 32H, (CH₂)₁₆). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 174.05, 64.23, 62.66, 34.35, 32.56, 29.34, 29.19, 29.09, 28.60, 25.73, 25.38, 24.97.

Bis(8-hydroxyoctyl) dodecanedioate (Diol-12-8): White powder, 750.6 mg, yield: 51.3%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.02 (t, 4H, *J*=8.0 Hz, COO-CH₂), 3.60 (t, 4H, *J*=8.0 Hz, HO-CH₂), 2.25 (t, 4H, *J*=8 Hz, CH₂-COO), 1.58-1.24 (m, 40H, (CH₂)₂₀). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 174.00, 64.31, 62.91, 34.35, 32.69, 29.34, 29.25, 29.19, 29.15, 29.09, 28.58, 25.82, 25.62, 24.96.

Bis(8-hydroxyoctyl) tetradecanedioate (Diol-14-8): White powder, 484.7 mg, yield: 55.4%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.03 (t, 4H, *J*=8.0 Hz, COO-CH₂), 3.62 (t, 4H, *J*=6.0 Hz, HO-CH₂), 2.26 (t, 4H, *J*=6.0 Hz, CH₂-COO), 1.61-1.27 (m, 44H, (CH₂)₂₂). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 174.01, 64.35, 63.02, 34.38, 32.75, 29.53, 29.46, 29.39, 29.34, 29.24, 29.17, 28.60, 25.87, 25.68, 24.99.

Bis(10-hydroxydecyl) tetradecanedioate (Diol-14-10): White powder, 420.7 mg, yield: 58.7%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.01 (t, 4H, *J*=8.0 Hz, COO-CH₂), 3.58 (t, 4H, *J*=8.0 Hz, HO-CH₂), 2.24 (t, 4H, *J*=8.0 Hz, CH₂-COO), 1.58-1.21 (m, 52H, (CH₂)₂₆). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 174.02, 64.34, 62.91, 34.36, 32.72, 29.51, 29.44, 29.40, 29.37, 29.34, 29.22, 29.16, 29.10, 28.58, 25.86, 25.68, 24.97.

Bis(12-hydroxydodecyl) tetradecanedioate (Diol-14-12): White powder, 500.7 mg, yield: 48.2%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.02 (t, 4H, *J*=6.0 Hz, COO-CH₂), 3.60 (t, 4H, *J*=6.0 Hz, HO-CH₂), 2.26 (t, 4H, *J*=6.0 Hz, CH₂-COO), 1.60-1.24 (m, 60H, (CH₂)₃₀). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 174.01, 64.36, 63.00, 34.38, 32.76, 29.54, 29.53, 29.49, 29.46, 29.42, 29.39, 29.24, 29.19, 29.12, 28.60, 25.88, 25.70, 24.99.

Analysis data of Compounds 6

3Boc-Cyc-6-6: White powder, yield: 60.4%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.11-4.05

(m, 8H, COO-CH₂), 3.56-2.92 (m, 36H, cyclen-H, cyclen-CH₂), 2.30 (t, 4H, *J*=8.0 Hz, CH₂-COO), 1.69-1.59, 1.40-1.27 (m, 20H, (CH₂)₁₀), 1.46 (s, 54H, Boc-H). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.77, 170.73, 155.78, 79.53, 79.27, 64.31, 64.14, 54.94, 53.52, 49.91, 47.72, 47.40, 47.07, 34.23, 28.79, 28.72, 28.52, 28.48, 25.62, 25.57, 24.78. HR-MS (ESI): C₆₈H₁₂₂N₈O₂₀Na [M+Na]⁺, 1393.8673, found: 1393.8598.

3Boc-Cyc-8-6: White powder, yield: 54.7%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.10-4.06 (m, 8H, COO-CH₂), 3.56-2.93 (m, 36H, cyclen-H, cyclen-CH₂), 2.34 (t, 4H, *J*=8.0 Hz, CH₂-COO), 1.68-1.65, 1.41-1.37 (m, 24H, (CH₂)₁₂), 1.46 (s, 54H, Boc-H). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.42, 170.77, 156.13, 155.78, 155.28, 79.32, 79.54, 79.28, 64.32, 64.27, 60.41, 54.92, 53.45, 50.94, 49.99, 47.73, 47.39, 33.92, 28.74, 28.51, 28.49, 25.64, 25.58, 24.41. HR-MS (ESI): C₇₀H₁₂₆N₈O₂₀Na [M+Na]⁺, 1421.8986, found: 1421.8922.

3Boc-Cyc-10-6: White powder, yield: 58.2%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.11-4.05 (m, 8H, COO-CH₂), 3.56-2.93 (m, 36H, cyclen-H, cyclen-CH₂), 2.30 (t, 4H, *J*=8.0 Hz, CH₂-COO), 1.69-1.58, 1.40-1.28 (m, 28H, (CH₂)₁₄), 1.46 (s, 54H, Boc-H). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.92, 170.73, 155.78, 79.71, 79.53, 79.27, 64.32, 64.12, 54.93, 53.50, 51.11, 50.00, 47.72, 47.39, 47.07, 34.32, 29.10, 28.73, 28.52, 28.48, 25.63, 25.58, 24.94. HR-MS (ESI): C₇₂H₁₃₀N₈O₂₀Na [M+Na]⁺, 1449.9299, found: 1449.9218.

3Boc-Cyc-12-6: White powder, yield: 46.7%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.11-4.02 (m, 8H, COO-CH₂), 3.53-2.85 (m, 36H, cyclen-H, cyclen-CH₂), 2.29 (t, 4H, *J*=8.0 Hz, CH₂-COO), 1.69-1.58, 1.40-1.25 (m, 32H, (CH₂)₁₆), 1.45 (s, 54H, Boc-H). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.94, 170.74, 156.10, 155.77, 155.29, 79.71, 79.52, 79.25, 64.31, 64.09, 54.93, 53.51, 53.42, 51.00, 50.18, 49.99, 47.70, 47.38, 47.06, 34.33, 29.39, 29.24, 29.15, 28.72, 28.51, 28.47, 25.62, 25.57, 24.97. HR-MS (ESI): C₇₄H₁₃₄N₈O₂₀Na [M+Na]⁺, 1477.9612, found: 1477.9537.

3Boc-Cyc-12-8: White powder, yield: 49.3%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.13-4.00 (m, 8H, COO-CH₂), 3.61-2.80 (m, 36H, cyclen-H, cyclen-CH₂), 2.28 (t, 4H, *J*=8.0 Hz, CH₂-COO), 1.67-1.56, 1.36-1.25 (m, 40H, (CH₂)₂₀), 1.44 (s, 54H, Boc-H). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 174.00, 170.78, 155.32, 79.72, 79.55, 79.28, 64.50, 64.31, 54.96, 53.54, 49.96, 47.72, 47.41, 47.04, 34.38, 29.41, 29.26, 29.17, 29.12, 28.73, 28.61, 28.60, 28.49, 25.89, 25.86, 25.00.

3Boc-Cyc-14-6: White powder, yield: 56.8%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.13-4.01 (m, 8H, COO-CH₂), 3.70-2.76 (m, 36H, cyclen-H, cyclen-CH₂), 2.29 (t, 4H, *J*=8.0 Hz, CH₂-COO), 1.68-1.58, 1.40-1.23 (m, 36H, (CH₂)₁₈), 1.45 (s, 54H, Boc-H). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.99, 170.76, 156.11, 155.80, 155.32, 79.73, 79.55, 79.28, 64.33, 64.10, 54.93, 53.52, 53.43, 51.05, 49.99, 47.73, 47.40, 47.06, 34.36, 29.59, 29.48, 29.29, 29.19, 28.73, 28.53, 28.49, 25.64, 25.59, 25.00.

3Boc-Cyc-14-8: White powder, yield: 60.3%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.09-3.97 (m, 8H, COO-CH₂), 3.58-2.80 (m, 36H, cyclen-H, cyclen-CH₂), 2.25 (t, 4H, *J*=8.0 Hz, CH₂-COO), 1.63-1.55, 1.35-1.15 (m, 44H, (CH₂)₂₂), 1.42 (s, 54H, Boc-H). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.95, 170.71, 156.08, 155.80, 155.24, 79.23, 78.75, 64.45, 64.25, 53.50, 53.42, 49.98, 47.72, 47.36, 47.08, 34.35, 29.54, 29.44, 29.25, 29.14, 29.07, 28.69, 28.56, 28.44, 25.82, 24.98. HR-MS (ESI): C₈₀H₁₄₆N₈O₂₀Na [M+Na]⁺, 1562.0551, found: 1562.0518.

3Boc-Cyc-14-10: White powder, yield: 45.7%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.10-3.98 (m, 8H, COO-CH₂), 3.56-2.80 (m, 36H, cyclen-H, cyclen-CH₂), 2.26 (t, 4H, *J*=8.0 Hz, CH₂-COO), 1.70-1.54, 1.32-1.19 (m, 52H, (CH₂)₂₆), 1.42 (s, 54H, Boc-H). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.96, 170.60, 15.15, 155.47, 155.03, 79.47, 79.22, 64.52, 64.33, 54.93, 53.52, 53.09, 49.88, 47.68, 47.36, 34.36, 29.54, 29.40, 29.25, 29.17, 28.62, 28.45, 25.88, 24.98. HR-MS (ESI): C₈₄H₁₅₄N₈O₂₀Na [M+Na]⁺, 1618.1177, found: 1618.1146.

2Boc-Lys-10-6: White powder, yield: 62.3%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.63 (s, 2H, CONH-CH), 4.06 (t, 4H, *J*=8.0 Hz, COO-CH₂), 4.00 (t, 4H, *J*=8.0 Hz, Lys-COO-CH₂), 3.05 (d, 4H, CONH-CH₂), 2.23 (t, 4H, *J*=8.0 Hz, OOC-CH₂), 1.66-1.46 (m, 16H, CONH-CH₂-CH₂, COO-CH₂-CH₂, OOC-CH₂-CH₂), 1.38 (s, 36H, Boc-H), 1.35-1.27 (m, 12H, (CH₂)₆). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.84, 172.81, 156.01, 155.43, 79.71, 78.99, 65.12, 64.05, 60.32, 53.38, 53.26, 40.03, 34.24, 32.30, 29.51, 29.01, 28.45, 28.36, 28.27, 25.49, 25.43, 24.87, 22.43. HR-MS (ESI): C₅₄H₉₈N₄O₁₆Na [M+Na]⁺, 1081.6876, found: 1081.6864.

2Boc-Lys-12-8: White powder, yield: 59.6%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.58 (s, 2H, CONH-CH), 4.08 (t, 4H, *J*=8.0 Hz, COO-CH₂), 4.02 (t, 4H, *J*=8.0 Hz, Lys-COO-CH₂), 3.08 (d, 4H, CONH-CH₂), 2.25 (t, 4H, *J*=8.0 Hz, OOC-CH₂), 1.85-1.71 (m, 4H, CONH-CH₂-CH₂-CH₂-CH₂), 1.64-1.47 (m, 16H, CONH-CH₂-CH₂, COO-CH₂-CH₂, OOC-CH₂-CH₂), 1.41 (s, 36H, Boc-H), 1.35-1.21 (m, 32H, (CH₂)₁₆). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) =

173.93, 172.82, 155.99, 79.72, 79.02, 65.32, 64.26, 53.38, 53.26, 40.09, 34.33, 32.41, 29.53, 29.36, 29.21, 29.11, 29.07, 29.04, 28.57, 28.47, 28.39, 28.29, 25.81, 25.71, 24.95, 22.44. HR-MS (ESI): C₆₀H₁₁₀N₄O₁₆Na [M+Na]⁺, 1165.7815, found: 1165.7816.

2Boc-Lys-14-10: White powder, yield: 65.7%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.59 (s, 2H, CONH-CH), 4.07 (t, 4H, *J*=8.0 Hz, COO-CH₂), 4.01 (t, 4H, *J*=8.0 Hz, Lys-COO-CH₂), 3.07 (d, 4H, CONH-CH₂), 2.24 (t, 4H, *J*=8.0 Hz, OOC-CH₂), 1.80-1.50 (m, 20H, CONH-CH₂CH₂CH₂-CH₂, CONH-CH₂-CH₂, COO-CH₂-CH₂, OOC-CH₂-CH₂), 1.40 (s, 36H, Boc-*H*), 1.35-1.15 (m, 44H, (CH₂)₂₂). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.96, 172.82, 155.99, 155.43, 79.72, 79.03, 78.43, 65.37, 64.31, 53.38, 53.25, 40.08, 34.34, 33.91, 32.39, 29.52, 29.41, 29.38, 29.36, 29.23, 29.17, 29.12, 28.59, 28.49, 28.38, 28.28, 28.22, 25.86, 25.76, 24.96, 22.43. HR-MS (ESI): C₆₆H₁₂₂N₄O₁₆Na [M+Na]⁺, 1249.8754, found: 1249.8744.

2Boc-Lys-14-12: White powder, yield: 47.8%. ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 4.57 (s, 2H, CONH-CH), 4.07 (t, 4H, *J*=8.0 Hz, COO-CH₂), 4.01 (t, 4H, *J*=8.0 Hz, Lys-COO-CH₂), 3.07 (d, 4H, CONH-CH₂), 2.25 (t, 4H, *J*=8.0 Hz, OOC-CH₂), 1.83-1.52 (m, 20H, CONH-CH₂CH₂CH₂-CH₂, CONH-CH₂-CH₂, COO-CH₂-CH₂, OOC-CH₂-CH₂), 1.40 (s, 36H, Boc-*H*), 1.34-1.15 (m, 52H, (CH₂)₂₆). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) = 173.95, 172.81, 155.98, 155.41, 79.72, 79.04, 65.04, 64.34, 53.38, 53.25, 40.09, 34.35, 32.41, 29.52, 29.49, 29.47, 29.44, 29.41, 29.23, 29.21, 29.16, 29.12, 28.60, 28.50, 28.38, 28.29, 25.88, 25.78, 24.97, 22.43. HR-MS (ESI): C₇₀H₁₃₀N₄O₁₆Na [M+Na]⁺, 1305.9380, found: 1305.9570.

Analysis data of Compounds 7

Cyc-6-6: Light yellow viscous liquid, yield: 93.6%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 4.06 (t, 4H, *J*=6.0 Hz, COO-CH₂), 3.96 (t, 4H, *J*=6.0 Hz, cyclen-CH₂-COO-CH₂), 3.44 (s, 4H, Cyclen-CH₂), 3.11-2.80 (m, 32H, cyclen-*H*), 2.24-2.18 (m, 4H, -OOC-CH₂), 1.61-1.25 (m, 20H, (CH₂)₁₀). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.74, 172.93, 65.25, 63.93, 53.64, 49.57, 49.33, 44.09, 42.32, 42.18, 42.12, 41.96, 33.23, 28.02, 27.62, 25.13, 24.86, 24.04. HR-MS (ESI): C₃₈H₇₅N₈O₈ [M+H]⁺, 771.5708, found: 771.5701.

Cyc-8-6: Light yellow viscous liquid, yield: 95.2%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 4.16 (t, 4H, *J*=6.0 Hz, COO-CH₂), 4.05 (t, 4H, *J*=8.0 Hz, cyclen-CH₂-COO-CH₂), 3.54 (s, 4H, Cyclen-CH₂), 3.20-2.88 (m, 32H, cyclen-*H*), 2.29 (t, 4H, *J*=6.0 Hz, -OOC-CH₂), 1.69-1.30 (m,

24H, (CH₂)₁₂). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.11, 172.95, 65.27, 63.87, 53.63, 49.57, 49.33, 44.09, 42.31, 42.17, 42.12, 41.97, 33.54, 28.39, 28.16, 28.04, 27.63, 25.21, 25.15, 24.87, 24.45. HR-MS (ESI): C₄₀H₇₉N₈O₈ [M+H]⁺, 799.6021, found: 799.6015

Cyc-10-6: Light yellowviscous liquid, yield: 92.1%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 4.17 (t, 4H, *J*=6.0 Hz, COO-CH₂), 4.04 (t, 4H, *J*=6.0 Hz, cyclen-CH₂-COO-CH₂), 3.54 (s, 4H, Cyelen-CH₂), 3.25-2.69 (m, 32H, cyclen-*H*), 2.29 (t, 4H, *J*=6.0 Hz, -OOC-CH₂), 1.70-1.27 (m, 28H, (CH₂)₁₄). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.20, 172.93, 65.27, 63.86, 53.63, 49.33, 44.08, 42.18, 41.96, 33.63, 28.78, 28.67, 28.16, 28.04, 25.22, 25.15, 24.87, 24.62. HR-MS (ESI): C₄₂H₈₃N₈O₈ [M+H]⁺, 827.6334, found: 827.6332.

Cyc-12-6: Light yellowviscous liquid, yield: 90.9%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 4.15 (t, 4H, *J*=8.0 Hz, COO-CH₂), 4.04 (t, 4H, *J*=6.0 Hz, cyclen-CH₂-COO-CH₂), 3.54 (s, 4H, Cyelen-CH₂), 3.21-2.87 (m, 32H, cyclen-*H*), 2.28 (t, 4H, *J*=8.0 Hz, -OOC-CH₂), 1.69-1.25 (m, 32H, (CH₂)₁₆). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.23, 172.91, 65.26, 63.86, 53.64, 49.99, 49.33, 44.30, 44.08, 42.65, 42.45, 42.19, 41.96, 33.66, 29.08, 28.92, 28.74, 28.16, 28.04, 25.22, 25.15, 24.65. HR-MS (ESI): C₄₄H₈₇N₈O₈ [M+H]⁺, 855.6647, found: 855.6645.

Cyc-12-8: Light yellowviscous liquid, yield: 93.4%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 4.14 (t, 4H, *J*=8.0 Hz, COO-CH₂), 4.04 (t, 4H, *J*=6.0 Hz, cyclen-CH₂-COO-CH₂), 3.53 (s, 4H, Cyelen-CH₂), 3.25-2.60 (m, 32H, cyclen-*H*), 2.28 (t, 4H, *J*=8.0 Hz, -OOC-CH₂), 1.69-1.20 (m, 40H, (CH₂)₂₀). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.23, 172.92, 65.38, 64.02, 53.64, 49.33, 44.08, 42.18, 41.96, 33.68, 29.10, 28.93, 28.84, 28.79, 28.74, 28.28, 28.11, 25.55, 25.47, 24.68. HR-MS (ESI): C₄₈H₉₅N₈O₈ [M+H]⁺, 911.7273, found: 911.7272.

Cyc-14-6: Light yellowviscous liquid, yield: 92.3%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 4.15 (t, 4H, *J*=6.0 Hz, COO-CH₂), 4.04 (t, 4H, *J*=6.0 Hz, cyclen-CH₂-COO-CH₂), 3.53 (s, 4H, Cyelen-CH₂), 3.26-2.59 (m, 32H, cyclen-*H*), 2.28 (t, 4H, *J*=8.0 Hz, -OOC-CH₂), 1.70-1.20 (m, 36H, (CH₂)₁₈). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.23, 172.90, 65.25, 63.86, 53.63, 49.32, 44.08, 42.18, 41.95, 33.67, 29.24, 29.16, 28.96, 28.76, 28.17, 28.04, 27.46, 25.22, 25.16, 24.66. HR-MS (ESI): C₄₆H₉₁N₈O₈ [M+H]⁺, 883.6960, found: 883.6951.

Cyc-14-8: Light yellowviscous liquid, yield: 91.3%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 4.15 (t, 4H, *J*=6.0 Hz, COO-CH₂), 4.04 (t, 4H, *J*=6.0 Hz, cyclen-CH₂-COO-CH₂), 3.53 (s, 4H, Cyelen-CH₂), 3.21-2.85 (m, 32H, cyclen-*H*), 2.28 (t, 4H, *J*=8.0 Hz, -OOC-CH₂), 1.70-1.25 (m,

44H, (CH₂)₂₂). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.24, 173.04, 65.47, 64.00, 53.67, 49.37, 44.10, 42.18, 42.00, 33.67, 29.24, 29.16, 28.95, 28.83, 28.78, 28.75, 28.28, 28.12, 25.52, 25.48, 24.66. HR-MS (ESI): C₅₀H₉₉N₈O₈ [M+H]⁺, 939.7586, found: 939.7492.

Cyc-14-10: Light yellowviscous liquid, yield: 92.5%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 4.15 (t, 4H, *J*=6.0 Hz, COO-CH₂), 4.04 (t, 4H, *J*=6.0 Hz, cyclen-CH₂-COO-CH₂), 3.53 (s, 4H, Cyelen-CH₂), 3.24-2.80 (m, 32H, cyclen-*H*), 2.28 (t, 4H, *J*=8.0 Hz, -OOC-CH₂), 1.69-1.23 (m, 52H, (CH₂)₂₆). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.24, 173.00, 65.47, 64.04, 53.67, 49.36, 44.10, 42.19, 41.98, 33.69, 29.23, 29.15, 29.13, 28.93, 28.89, 28.73, 28.31, 28.16, 25.60, 25.54, 24.68. HR-MS (ESI): C₅₄H₁₀₇N₈O₈ [M+H]⁺, 995.8212, found: 995.8119.

Lys-10-6: Light yellowviscous liquid, yield: 95.8%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 4.28-4.19 (m, 4H, COO-CH₂), 4.05 (t, 4H, *J*=8.0 Hz, Lys-COO-CH₂), 3.50-3.42 (m, 2H, NH₂-CH), 2.92 (t, 4H, *J*=6.0 Hz, NH₂-CH₂), 2.28 (t, 4H, *J*=6.0 Hz, OOC-CH₂), 2.01-1.84 (m, 4H, NH₂-CH-CH₂), 1.75-1.25 (m, 36H, (CH₂)₁₈). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.24, 168.97, 66.09, 63.85, 52.27, 38.77, 33.63, 29.61, 28.77, 28.68, 28.14, 27.99, 26.58, 25.13, 25.02, 24.60, 21.68. HR-MS (ESI): C₃₄H₆₇N₄O₈ [M+H]⁺, 659.4959, found: 659.4949.

Lys-12-8: Light yellowviscous liquid, yield: 94.7%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 4.26-4.19 (m, 4H, COO-CH₂), 4.04 (t, 4H, *J*=8.0 Hz, Lys-COO-CH₂), 3.50-3.42 (m, 2H, NH₂-CH), 2.92 (t, 4H, *J*=8.0 Hz, NH₂-CH₂), 2.28 (t, 4H, *J*=8.0 Hz, OOC-CH₂), 2.01-1.83 (m, 4H, NH₂-CH-CH₂), 1.75-1.23 (m, 48H, (CH₂)₂₄). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.27, 168.98, 66.20, 64.01, 52.26, 38.77, 33.67, 29.61, 29.07, 28.91, 28.76, 28.73, 28.27, 28.07, 26.57, 25.51, 25.35, 24.65, 21.67. HR-MS (ESI): C₄₀H₇₉N₄O₈ [M+H]⁺, 743.5898, found: 743.5902.

Lys-14-10: Light yellowviscous liquid, yield: 93.2%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 4.28-4.18 (m, 4H, COO-CH₂), 4.04 (t, 4H, *J*=6.0 Hz, Lys-COO-CH₂), 3.50-3.43 (m, 2H, NH₂-CH), 2.92 (t, 4H, *J*=8.0 Hz, NH₂-CH₂), 2.28 (t, 4H, *J*=8.0 Hz, OOC-CH₂), 2.00-1.86 (m, 4H, NH₂-CH-CH₂), 1.78-1.21 (m, 60H, (CH₂)₃₀). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.26, 168.98, 66.24, 64.04, 52.26, 38.77, 33.69, 29.62, 29.22, 29.14, 29.12, 28.93, 28.88, 28.86, 28.73, 28.31, 28.12, 26.58, 25.59, 25.42, 24.67. HR-MS (ESI): C₄₆H₉₁N₄O₈ [M+H]⁺, 827.6837, found: 827.6833.

Lys-14-12: Light yellowviscous liquid, yield: 94.2%. ¹H NMR (CD₃OD, 400 MHz): δ (ppm) =

4.28-4.16 (m, 4H, COO-CH₂), 4.04 (t, 4H, *J*=6.0 Hz, Lys-COO-CH₂), 3.49-3.43 (m, 2H, NH₂-CH), 2.92 (t, 4H, *J*=8.0 Hz, NH₂-CH₂), 2.28 (t, 4H, *J*=8.0 Hz, OOC-CH₂), 2.01-1.86 (m, 4H, NH₂-CH-CH₂), 1.84-1.21 (m, 68H, (CH₂)₃₄). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) = 174.24, 168.99, 66.23, 64.06, 52.26, 38.76, 33.72, 29.59, 29.27, 29.24, 29.21, 29.15, 28.94, 28.92, 28.73, 28.32, 28.12, 26.54, 25.63, 25.44, 24.69, 21.65. HR-MS (ESI): C₅₀H₉₉N₄O₈ [M+H]⁺, 883.7463, found: 883.7471.