Supplementary Information

Cationic lipids with cyclen headgroup: Synthesis and structure-activity relationship studies as non-viral gene vectors

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Figure S1. Ethidium bromide displacement assay of plasmid DNA binding abilities for the liposomes S1-S4 (A), D1-D6 (B) under various N/P ratios in Hepes buffer solution (pH = 7.4, 10 mM). The molar ratio of lipid/DOPE was 1 : 1.

Figure S2. Protection and SDS-induced release of DNA from liposomes D1-D6, S1 and S4 at N/P ratio of 8 visualized by agarose gel electrophoresis. The first 4 lanes are DNA control.
Figure S3. Mean particle sizes (columns) and zeta-potentials (dots) of the liposomes formed from S1-S4 (A) and D1-D6 (B).

Figure S4. Fluorescent microscope images of HeLa cells transfected by double-tailed lipid/DOPE/DNA lipoplexes. Lipid/DOPE ratio was 1 : 1. The cells were observed by inversion fluorescence microscope after 24 h transfection. (A, C, E, G: N/P = 6; B, D, F, H, N/P = 8; I: lipofectamine 2000).

Figure S5. Electrophoretic gel retardation assays of lipids/DOPE/pDNA complexes at different N/P ratios. The molar ratio of lipid/DOPE was 1 : 1.
Figure S6. Luciferase expression in HeLa cells transfected by lipid/DOPE/DNA lipoplexes at various N/P ratios under lipid/DOPE ratio of 1 : 1. Data represent mean ± SD (n = 3).

Figure S7. Cytotoxicity of the lipoplexes D2-10–D2-18 prepared at various N/P ratios (2, 4, 6, 8 and 12) and D2 prepared at N/P ratios (4, 6, 8, 10 and 12) in HeLa cells. Data represent mean ± SD (n = 3).
Synthesis and characterization

**Scheme S1.** Detailed synthetic routes of single-tailed lipids S1-S4.

**Preparation of compounds 1a, 2a, 3a, 4a**

Boc protected amino acids were synthesized according to general procedures. Briefly, to a solution of L-amino acids (1, 2, 3, 4, 0.050 mol) in 70 mL tetrahydrofuran (THF) and 70 mL 1 N aqueous NaOH, (Boc)$_2$O (10.90 g, 0.050 mmol) was added respectively. The resulting solution was left stirring at room temperature for 24 h. The THF was then evaporated and the residue was adjusted to pH 2 with 2 N aqueous HCl and then extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried over anhydrous sodium sulfate (NaSO$_4$), filtered, and concentrated in vacuo.

**Preparation of compounds 1b-4b**

To a mixing solution of compound 1a, 2a, 3a and 4a (0.01 mol) and N-methylmorpholine (2.20 mL, 0.02 mmol) in CHCl$_3$ at 0 °C, isobutylchloroformate (1.78 mL, 0.01 mol) in chloroform (CHCl$_3$) was added dropwise for activation of carboxyl respectively. After 0.5 h,
oleylamine (2.67 g, 0.01 mol) was added, and the reaction was slowly warmed to room temperature. After 40 h of reaction, the mixture was washed with saturated aqueous NaHCO$_3$ (2 × 50 mL) and brine (2 × 50 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$ and then filtered. The solvent was evaporated under reduced pressure to give the crude products and then the residue was purified by silica gel column chromatography to obtain compound 1b, precursor 2b, 3b and 4b. Yield: 49.0%-50.3%.

As for 2b, oleic acid (1.41g, 0.005 mol) was mixed with Dicyclohexylcarbodiimide (DCC) (1.03 g, 0.005 mol) and 4-Dimethylaminopyridine (DMAP) (0.06 g, 0.0005 mol) in dichloromethane (DCM) at 0 ºC for 1 h, then precursor 2b was added and the reaction was slowly warmed to room temperature. After 40 h of reaction, the solution was cooled to 0 ºC, the precipitate formed was filtered off, evaporated the solvent. The residue was purified by silica gel column chromatography (PE/EA=4/1, v/v) to obtain 4b. Yield: 55%

Preparation of compounds 1c-4c

The protection groups of compounds 1b-4b were removed by trifluoroacetic acid in anhydrous DCM to obtain deprotected 1b-4b. To a mixing solution of compound 6 (0.005 mol), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.005 mol), 1-Hydroxybenzotriazole (HOBt) (0.005 mol) and N, N- diisopropylethylamine (DIEA) (0.05 mol) in DCM, cooled down to 0 ºC for 0.5 h, deprotected 1b-4b (0.005 mol) were added, and the reaction was slowly warmed to room temperature. After 24 h of reaction, the solvent was removed under reduced pressure. The mixture was washed with saturated aqueous NaHCO$_3$ (2 × 50 mL) and brine (2 × 50 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$ and then filtered. The solution was evaporated and the residue was purified by silica gel column chromatography to obtain compound 1c, precursor 2c, 3c, 4c, Yield: 45~55%

As for 2c, precursor 2c was dissolved in Methanol (CH$_3$OH) (25 mL) and H$_2$O (25 mL), then 2N NaOH was added to it. The reaction kept stirring at room temperature for 2 h. After reaction, the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography to obtain compound 2c (PE/E= 4/1, v/v), Yield: 80%

Preparation of compounds S1-S4

Compound 1c-4c (250 mg) was dissolved in anhydrous DCM (2.5 mL), then trifluoroacetic acid (CF$_3$COOH) (2.5ml) in anhydrous DCM (2.5 mL) was added at 0 ºC. After stirring for 6 h,
the solvent was removed under reduced pressure. The residue was washed with anhydrous ether twice to get pure compound S1-S4. Yield: 82%-90%

Analytical data for novel compounds.

Compound 1c: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.46 – 5.34 (m, 2H, -CH=CH-), 3.84 (d, $J = 5.2$ Hz, 2H, -CH$_2$- glycine), 3.74 (dd, $J = 13.9, 6.9$ Hz, 1H, cyclen-H), 3.45 (d, $J = 46.4$ Hz, 11H, cyclen-H), 3.24 (dd, $J = 13.3, 6.5$ Hz, 4H, cyclen-H), 2.56 (s, 4H, cyclen-CH$_2$), 2.08 – 1.93 (m, 2H, -CH$_2$-CONH-), 1.77 – 1.58 (m, 3H, -CH$_2$-CH=CH-), 1.47 (d, $J = 19.5$ Hz, 27H, BOC), 1.28 (s, 22H, -CH$_2$-), 0.90 (t, $J = 6.8$ Hz, 3H, -CH$_2$CH$_3$).

Compound S1: $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 7.99 (s, 1H, -CONH-), 5.36 (ddd, $J = 13.9, 9.0, 2.3$ Hz, 2H, -CH=CH-), 3.95 (s, 2H, -CH$_2$- glycine), 3.47 (s, 2H, cyclen-H), 3.03 (dd, $J = 139.4, 63.2$ Hz, 17H, cyclen-H), 2.00 (dd, $J = 14.0, 7.4$ Hz, 2H, -CH$_2$-CONH-), 1.53 – 1.40 (m, 3H, -CH$_2$-CH=CH-), 1.27 (s, 22H, -CH$_2$-), 0.89 (t, $J = 6.6$ Hz, 3H, -CH$_2$CH$_3$); $^{13}$C - NMR (101 MHz, CDCl$_3$): $\delta$ / ppm: 173.11, 160.63, 130.20, 129.95, 129.73, 119.76, 116.88, 114.02, 111.16, 64.35, 56.53, 50.50, 44.77, 43.35, 42.48, 40.23, 33.63, 32.61, 31.89, 29.85, 28.73, 28.73, 27.95, 27.18, 26.79, 24.96, 22.66, 14.05, 13.76; HR-MS (ESI): C$_{30}$H$_{60}$N$_6$O$_2$,[M+H]$^+$, 537.4856, found: 537.4851.

Compound 2c: $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 7.54 (s, 1H, -CONH-), 5.45 – 5.31 (m, 2H, -CH=CH-), 4.48 (s, 1H, serine-CH-), 4.12 (d, $J = 10.4$ Hz, 2H, serine-CH$_2$-), 3.77 – 2.67 (m, 21H, cyclen-H), 2.30 (s, 2H, -CH$_2$-CONH-), 2.06 – 1.94 (m, 4H, -CH$_2$-CH=CH-), 1.48 (d, $J = 16.8$ Hz, 27H, BOC), 1.38 – 1.21 (m, 22H, -CH$_2$-), 0.90 (t, $J = 6.8$ Hz, 3H, -CH$_2$CH$_3$); HR-MS (ESI): C$_{46}$H$_{86}$N$_6$O$_9$, [M+Na]$^+$, 889.6354, found: 889.6349.

Compound S2: $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 7.39 (d, $J = 48.6$ Hz, 1H, -CONH-), 5.42 – 5.34 (m, 2H, -CH=CH-), 4.52 (s, 1H, serine-CH-), 4.12 (d, $J = 10.4$ Hz, 2H, serine-CH$_2$-), 3.93 – 3.78 (m, 1H, serine-CH$_2$-), 3.62 – 2.84 (m, 19H, cyclen-H), 2.01 (dd, $J = 13.9, 7.4$ Hz, 3H, -CH$_2$-CONH-), 1.45 (d, $J = 22.6$ Hz, 2H, -CH$_2$-CH=CH-), 1.31 (d, $J = 29.4$ Hz, 23H, -CH$_2$-), 0.89 (t, $J = 6.8$ Hz, 3H, -CH$_2$CH$_3$); $^{13}$C - NMR (101 MHz, CDCl$_3$): $\delta$ / ppm: 161.21, 137.64, 129.90, 31.86, 29.72, 29.38, 27.17, 22.63, 14.04. HR-MS (ESI): C$_{31}$H$_{60}$N$_6$O$_3$, [M+H]$^+$, 567.4962, found: 567.4966.

Compound 3c: $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 7.29 (s, 5H, Ph-H), 5.36 (t, $J = 14.5$ Hz, 2H, -CH=CH-), 4.48 (s, 1H, Phenylalanine-CH-), 3.35 (dd, $J = 165.0, 61.0$ Hz, 14H, Phenylalanine-
CH₂_, cyclen-H), 2.78 (s, 4H, cyclen-H), 2.02 (d, J = 7.0 Hz, 4H, cyclen-H), 1.63 (s, 8H, -
CH₂-CONH-, -CH₂-CH=CH-), 1.48 (d, J = 16.9 Hz, 22H, BOC), 1.29 (dd, J = 10.2, 4.0 Hz,
27H, -CH₂-), 0.90 (t, J = 6.6 Hz, 3H, -CH₂CH₃); HR-MS (ESI): C₅₂H₉₀N₆O₈, [M+Na]⁺,
949.6718, found: 949.6712.

Compound S3: ¹H - NMR (400 MHz, CDCl₃) δ 7.21 – 7.08 (m, 3H, Ph-H), 5.32 (t, J = 15.6 Hz,
2H, -CH=CH-), 4.71 – 4.43 (m, 2H, Phenylnaline-CH₂-), 3.70 – 2.64 (m, 23H, Phenylnaline-
CH-, cyclen-H), 1.99 (d, J = 4.8 Hz, 3H, cyclen-H), 1.47 – 1.33 (m, 3H, -CH₂-CH=CH-), 1.15
(d, J = 65.1 Hz, 24H, -CH₂-), 0.85 (t, J = 6.0 Hz, 3H, -CH₂CH₃).

¹³C - NMR (101 MHz, CDCl₃): δ / ppm: 161.23, 129.93, 129.88, 129.79, 129.46, 128.58, 117.40,
114.51, 32.58, 31.86, 29.89, 29.43, 29.28, 27.18, 26.72, 22.64 14.07; HR-MS (ESI): C₃₇H₆₆N₆O₂,
[M+H]⁺, 627.5326, found: 627.5325.

Compound 4c: ¹H - NMR (400 MHz, CDCl₃) δ 7.26 (d, J = 7.5 Hz, 2H, Ph-H), 7.06 (dd, J =
38.3, 8.5 Hz, 2H, Ph-H), 6.77 (d, J = 7.4 Hz, 1H, -CONH-), 5.36 (t, J = 14.9 Hz, 2H, -CH=CH-
), 4.57 (s, 1H, tyrosine-CH-), 3.27 (ddd, J = 75.1, 66.7, 29.7 Hz, 20H, cyclen-H, tyrosine-CH₂-),
2.91 (s, 1H, cyclen-H), 2.70 (d, J = 66.0 Hz, 4H, cyclen-H), 2.01 (dd, J = 14.5, 8.4 Hz, 4H, -
CH₂-CH=CH-), 1.48 (d, J = 5.4 Hz, 27H, BOC), 1.27 (s, 22H, -CH₂-), 0.90 (t, J = 6.7 Hz, 3H,
-CH₂CH₃); HR-MS (ESI): C₅₂H₉₀N₆O₉, [M+Na]⁺, 965.6667, found: 965.6670.

Compound S4: ¹H - NMR (400 MHz, MeOD) δ 7.32 (d, J = 8.4 Hz, 1H, Ph-H), 7.11 (dd, J =
23.6, 8.4 Hz, 2H, Ph-H), 6.73 (d, J = 8.4 Hz, 1H, Ph-H), 5.44 – 5.31 (m, 2H, -CH=CH-), 4.58
(dd, J = 8.5, 6.5 Hz, 1H, tyrosine-CH-), 4.50 (dd, J = 8.6, 6.6 Hz, 1H, tyrosine-CH₂-), 3.87 (s,
1H, cyclen-H), 3.50 (d, J = 4.7 Hz, 1H, cyclen-H), 3.46 (d, J = 4.7 Hz, 1H, cyclen-H), 3.29 –
2.78 (m, 24H, cyclen-H), 2.09 – 1.94 (m, 3H, -CH₂-CH=CH-), 1.47 – 1.20 (m, 25H, -CH₂-),
0.92 (t, J = 6.8 Hz, 3H, -CH₂CH₃); ¹³C - NMR (101 MHz, MeOD): δ / ppm: 172.54, 129.85,
114.84, 49.98, 44.40, 42.71, 31.62, 29.35, 28.89, 26.58, 22.29, 13.01; HR-MS (ESI):
C₃₇H₆₆N₆O₃, [M+H]⁺, 643.5275, found: 643.5273.
Scheme S2. Detailed synthetic routes of double-tailed lipids D1-D6.

Preparation of compound 4a’
To a mixing solution of compound 4a (0.005 mol) and imidazole (0.005 mol) in DCM at 0 °C, tertiarybutyldimethylchlorosilane (TBSCl, 0.005 mol) was added dropwise, and the reaction was slowly warmed to room temperature. After 24 h of reaction, the precipitate formed was filtered off, evaporated the solvent. The residue was purified by silica gel column chromatography (DCM/CH$_3$OH=10/1, v/v) to obtain yellow oil 4a'. Yield: 55%

Preparation of compound 5a

Step a: Ethyl trifluoroacetate (11.57 g; 0.081 mol) was dropped into a solution of diethylenetriamine (4 g, 0.039 mol) in DCM (180 mL) at 0°C for 1 h. Then triethylamine (10.96 mL, 0.078mmol) and (Boc)$_2$O (10.21 g, 0.047 mmol) were added. The resulting solution was stirring at room temperature for overnight. The mixture was evaporated and recrystallized by PE/DCM.

Step b: Four grams of the above product was then refluxed in 150 mL of methanol/water (volume ratio, 20:1, containing 3.90 g K$_2$CO$_3$) for 4 h to remove the trifluoroacetyl groups and liberate the primary amines. The methanol was removed under reduce pressure, and the residue was extracted with DCM (3 × 50 mL). The organic layers were combined, dried over Na$_2$SO$_4$ and then filtered. The solution was evaporated to yield the title compound 5a as a waxy solid. Yield: 92%.

Preparation of compound 5b

To a mixing solution of oleic acid (11.18g, 0.04 mol), EDCI (7.67g, 0.04 mol), HOBt (6.12 g, 0.04 mol) and DIEA (5.18 g, 0.04 mol) in DCM, cooled down to 0 °C for 0.5 h, 5a (0.018 mol) in CH$_3$OH was added, and the reaction was slowly warmed to room temperature. After 24 h of reaction, the solvent was removed under reduced pressure. The mixture was washed saturated aqueous NaHCO$_3$ (2 × 50 mL), and brine (2 × 50 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$ and then filtered. The solvent was evaporated and the residue was purified by silica gel column chromatography (PE/EA = 2:1 v/v) to obtain compound 5b, Yield: 15%.

Preparation of compound 8

The protection group of compound 5b was removed by trifluoroacetic acid in anhydrous DCM to obtain deprotected 5b. To a mixing solution of compound 6 (0.005 mol), EDCI (0.005 mol), HOBt (0.005 mol) and DIEA (0.05 mol) in DCM, cooled down to 0 °C for 0.5 h,
deprotected 5b (0.005 mol) was added, and the reaction was slowly warmed to room temperature. After 24 h of reaction, the solvent was removed under reduced pressure. The mixture was washed with saturated aqueous NaHCO₃ (2 × 50 mL), and brine (2 × 50 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and evaporated the solvent to yellow oil. The residue was purified by silica gel column chromatography to obtain compound 8 (DCM/CH₃OH = 10:1 v/v), Yield: 45%.

Preparation of compound 9

Deprotected 5b was suspended in 100 mL ethyl acetate. Added 1 g K₂CO₃ (3 equiv.) and compound 7 (1 equiv.). The mixture was refluxed overnight. The solution was filtered and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (PE/EA = 1:2 v/v) to obtain compound 9, white solid, Yield: 55%

Preparation of compounds 10, 11, 12 and 13

To a mixing solution of compound 3a, 4a’ (0.005 mol), EDCI (0.005 mol), HOBt (0.005 mol) and DIEA (0.05 mol) in DCM, cooled down to 0 ºC for 0.5 h, deprotected 2b and 5b (0.005 mol) was added respectively, and the reaction was slowly warmed to room temperature. After 24 h of reaction, the solvent was removed under reduced pressure. The mixture was washed with saturated aqueous NaHCO₃ (2 × 50 mL), and brine (2 × 50 mL). The organic layer was dried over anhydrous Na₂SO₄ and evaporated the solvent to yellow oil. The residue was purified by silica gel column chromatography to obtain compound 10, 11, 12 and 13, Yield: 45~50%

Preparation of compounds D1-D6

Step a: The connection between compound 6 and 10, 11, 12, 13 were in similar method. As for D4 and D6, after the connection with compound 6, the TBS protection groups of 11 and 13 were removed by tetrabutylammonium fluoride (TBAF) in anhydrous THF.

Step b: all the compound (250 mg) was dissolved in anhydrous DCM (2.5 mL), then CF₃COOH (2.5ml) in anhydrous DCM (2.5 mL) was added at 0 ºC. After stirring for 6 h, the solvent was removed under reduced pressure. The residue was washed with anhydrous ether twice to get pure compound D1-D6. Yield: 82%-90%

Analytical data for novel compounds.
Compound 5b: $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 6.59 (s, 1H, -CONH-), 6.30 (s, 1H, -CONH-), 5.37 – 5.25 (m, 4H, -CH=CH-), 3.32 (s, 8H, diethylenetriamine-H), 2.14 (dd, J = 12.6, 7.3 Hz, 4H, -CH$_2$-CONH-), 2.03 – 1.89 (m, 7H, -CH$_2$-CH=CH-), 1.58 (s, 4H, -CH$_2$-), 1.42 (s, 9H, Boc), 1.32 – 1.18 (m, 40H, -CH$_2$-), 0.85 (dd, J = 8.8, 4.8 Hz, 6H, -CH$_2$CH$_3$). $^{13}$C - NMR (101 MHz, CDCl$_3$): $\delta$ 174.09, 173.58, 156.80, 130.16, 129.92, 129.68, 127.91, 80.36, 48.86, 47.35, 39.52, 38.64, 36.64, 32.56, 31.87, 31.47, 29.82 – 29.04 (m), 28.34, 27.17, 25.64, 22.58, 14.07.

Compound 2b: $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 6.43 (s, 2H, -CONH-), 5.38 – 5.24 (m, 4H, -CH=CH-), 4.34 (d, J = 9.3 Hz, 2H, serine-CH$_2$-), 4.22 (d, J = 6.5 Hz, 1H, serine-CH-), 3.20 (s, 2H, -CH$_2$-CONH-), 2.25 (dd, J = 14.7, 7.1 Hz, 2H, -CH$_2$-COOCH$_2$-), 2.04 – 1.89 (m, 7H, -CH$_2$-CH=CH-), 1.56 (s, 3H, -CH$_2$-), 1.40 (s, 9H, BOC), 1.21 (s, 42H, -CH$_2$-), 0.83 (t, J = 6.1 Hz, 6H, -CH$_2$CH$_3$). $^{13}$C - NMR (101 MHz, CDCl$_3$): $\delta$ 173.53, 168.85, 155.48, 130.23, 129.89, 129.67, 80.31, 64.05, 53.56, 39.59, 34.01, 32.56, 31.86, 31.47, 29.71, 29.48, 29.21, 28.22, 27.16, 26.81, 24.76, 22.63, 14.06.

Compound 7: $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 7.30 (d, J = 7.6 Hz, 2H, Ph-H), 7.20 (d, J = 7.6 Hz, 2H, Ph-H), 4.45 (s, 2H, Ph-CH$_2$), 3.70 (s, 2H, Ph-CH$_2$), 3.55 (s, 4H, cyclen-CH$_2$), 3.30 (d, J = 58.6 Hz, 8H, cyclen-CH$_2$), 2.63 (s, 4H, cyclen-CH$_2$), 1.49 – 1.21 (m, 27H, BOC). $^{13}$C - NMR (101 MHz, CDCl$_3$): $\delta$ 155.70, 137.22, 136.78, 130.66, 128.88, 79.62, 56.81, 49.90, 48.20, 33.23, 28.44. HR-MS (ESI): C$_{31}$H$_{51}$BrN$_4$O$_6$, [M+Na]$^+$, 677.2980, found: 677.2885.

Compound 8: $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 7.04 (s, 1H, -CONH-), 6.42 (s, 1H, -CONH-), 5.38 – 5.24 (m, 4H, -CH=CH-), 3.41 (ddd, J = 135.6, 75.5, 63.5 Hz, 19H, diethylenetriamine-H, Cyclen-CH$_2$), 3.03 (s, 4H, Cyclen-H), 2.17 – 2.05 (m, 4H, -CH$_2$-CONH-), 1.96 (dd, J = 16.8, 10.6 Hz, 8H, cyclen-CH$_2$), 2.63 (s, 4H, cyclen-CH$_2$), 1.60 – 1.51 (m, 4H, -CH$_2$-), 1.42 (d, J = 9.0 Hz, 27H, BOC), 1.22 (s, 42H, -CH$_2$-), 0.84 (t, J = 6.3 Hz, 6H, -CH$_2$CH$_3$). $^{13}$C - NMR (101 MHz, CDCl$_3$): $\delta$ 174.06, 173.81, 171.44, 155.95, 155.13, 129.93, 129.67, 79.32, 53.06, 49.89, 47.77, 44.59, 38.67, 36.65, 36.41, 32.56, 31.86, 29.82, 28.82, 28.57, 28.24, 28.17, 27.16, 25.89, 25.43, 22.58, 14.08.

Compound 9: $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 7.14 (s, 4H, benzene-H), 6.33 (s, 1H, -CONH-), 5.32 (t, J = 14.9 Hz, 3H, -CH=CH-), 3.71 – 3.16 (m, 20H, Cyclen-CH$_2$), 2.76 – 2.45 (m, 8H, diethylenetriamine-H), 2.17 (t, J = 7.0 Hz, 3H, -CH$_2$-CONH-), 1.97 (dd, J = 13.5, 6.7 Hz, 4H, -CH$_2$-), 1.62 (d, J = 7.1 Hz, 9H, -CH$_2$-CH=CH-), 1.48 – 1.33 (m, 26H, BOC), 1.33 – 1.17 (m,
Compound 10: 

$^{1}H$ NMR (400 MHz, CDCl$_3$) $\delta$ 7.30 – 7.25 (m, 2H, Ph-H), 7.16 (t, $J$ = 7.6 Hz, 3H, Ph-H), 6.82 (s, 1H, -CONH-), 5.32 (dd, $J$ = 12.9, 6.3 Hz, 3H, -CH=CH-), 5.23 (d, $J$ = 7.9 Hz, 1H, Phenylalanine-CH-), 4.67 (dd, $J$ = 15.3, 7.7 Hz, 1H, Phenylalanine-CH$_2$-), 3.51 – 3.15 (m, 8H, diethylenetriamine-H), 2.93 – 2.82 (m, 2H, -CH$_2$-), 2.11 (t, $J$ = 7.5 Hz, 4H, -CH$_2$-CONH-), 2.00 (dd, $J$ = 16.2, 4.5 Hz, 6H, -CH$_2$-CH=CH-), 1.55 (d, $J$ = 5.9 Hz, 4H, -CH$_2$-). HR-MS (ESI): C$_{71}$H$_{127}$N$_7$O$_8$, [M+H]$^+$, 1228.9644, found:1228.9551.

Compound 11: 

$^{1}H$ NMR (400 MHz, CDCl$_3$): $\delta$ 7.01 (d, $J$ = 8.2 Hz, 2H, Ph-H), 6.74 (d, $J$ = 8.1 Hz, 2H, Ph-H), 6.35 (s, 1H, -CONH-), 5.40 – 5.26 (m, 4H, -CH=CH-), 4.65 – 4.58 (m, 1H, tyrosine-CH-), 3.39 – 3.16 (m, 8H, diethylenetriamine-H), 2.87 – 2.78 (m, 2H, tyrosine-CH$_2$-), 2.11 (t, $J$ = 7.6 Hz, 4H, -CH$_2$-CONH-), 1.98 (dd, $J$ = 20.6, 15.0 Hz, 7H, -CH$_2$-CH=CH-), 1.59 (d, $J$ = 15.4 Hz, 10H, -CH$_2$-), 1.39 (s, 9H, BOC), 1.23 (s, 41H, -CH$_2$-), 0.95 (s, 9H, TBS-CH$_3$), 0.86 (t, $J$ = 6.6 Hz, 6H, -CH$_2$CH$_3$), 0.15 (d, $J$ = 4.6 Hz, 6H, TBS-CH$_3$). HR-MS (ESI): C$_{74}$H$_{130}$N$_8$O$_{10}$, [M+Na]$^+$, 1313.9808, found:1313.9770.

Compound 12: 

$^{1}H$ NMR (400 MHz, CDCl$_3$): $\delta$ 7.26 (dd, $J$ = 17.5, 6.2 Hz, 4H, Ph-H), 6.98 (d, $J$ = 6.2 Hz, 1H, -CONH-), 6.63 (s, 1H, -CONH-), 5.40 – 5.28 (m, 4H, -CH=CH-), 4.99 (s, 2H, Phenylalanine-CH$_2$-), 4.64 (d, $J$ = 2.5 Hz, 1H, Phenylalanine-CH-), 4.43 (s, 1H, serine-CH-), 4.30 (s, 1H, serine-CH$_2$-), 4.18 (d, $J$ = 11.4 Hz, 1H, serine-CH$_2$-), 3.15 (dd, $J$ = 24.1, 9.8 Hz, 4H, -CH$_2$-), 2.98 – 2.90 (m, 1H, -CH$_2$-CONH-), 2.72 (d, $J$ = 20.4 Hz, 1H, -CH$_2$-CONH-), 2.22 (t, $J$ = 5.5 Hz, 2H, -CH$_2$-COOCH$_2$-), 1.97 (t, $J$ = 14.7 Hz, 7H, -CH$_2$-CH=CH-), 1.54 (s, 2H, -CH$_2$-), 1.44 (s, 2H, -CH$_2$-), 1.38 (s, 9H, BOC), 1.23 (s, 43H, -CH$_2$-), 0.85 (d, $J$ = 3.6 Hz, 6H, -CH$_2$CH$_3$). 

$^{13}C$ NMR (101 MHz, CDCl$_3$): $\delta$ 173.92, 171.55, 168.09, 155.91, 135.95, 129.81, 129.43, 129.06, 128.83, 127.23, 80.82, 63.84, 56.37, 53.39, 52.80, 39.82, 37.74, 33.98, 32.58, 31.87, 29.74, 29.49, 29.41, 28.88, 28.17, 26.99, 26.80, 26.70, 24.76, 22.65, 14.08. HR-MS (ESI): C$_{53}$H$_{91}$N$_3$O$_6$, [M+Na]$^+$, 888.6808, found:888.6810.

Compound 13: 

$^{1}H$ NMR (400 MHz, CDCl$_3$): $\delta$ 7.07 (t, $J$ = 8.1 Hz, 2H, Ph-H), 6.91 – 6.77 (m, 2H, Ph-H), 6.59 (d, $J$ = 8.0 Hz, 1H, -CONH-), 5.37 (dd, $J$ = 11.4, 5.8 Hz, 3H, -CH=CH-), 4.69 – 4.61 (m, 1H, tyrosine-CH-), 4.59 – 4.50 (m, 1H, tyrosine-CH$_2$-), 4.24 (dt, $J$ = 6.0, 4.1 Hz, 1H,
tyrosine-CH₂), 4.12 – 4.02 (m, 1H, serine-CH-), 3.31 – 3.08 (m, 3H, serine-CH₂), 3.07 – 2.86 (m, 2H, -CH₂-CONE-), 2.31 – 2.24 (m, 2H, -CH₂-COOCH₂-), 2.08 – 1.94 (m, 6H, -CH₂-CH=CH-), 1.72 (s, 1H, -CH₂-), 1.59 (s, 2H, -CH₂-), 1.50 (d, J = 6.3 Hz, 2H, -CH₂-), 1.43 (d, J = 2.3 Hz, 9H, BOC), 1.26 (t, J = 6.8 Hz, 46H, -CH₂), 0.99 (d, J = 1.7 Hz, 9H, TBS-CH₃), 0.90 (t, J = 6.8 Hz, 6H, -CH₂CH₃), 0.20 (d, J = 3.4 Hz, 6H, TBS-CH₃). HR-MS (ESI): C₅₉H₁₀₅N₃O₇Si, [M+Na]⁺, 1018.7619, 1018.7519.

Compound triBoc-cyclen-10: ¹H - NMR (400 MHz, CDCl₃) δ 7.29 – 7.25 (m, 2H, Ph-H), 7.17 (d, J = 7.0 Hz, 2H, Ph-H), 7.17 – 7.01 (m, 1H, Ph-H), 6.80 (s, 1H, -CONH-), 6.28 (s, 1H, -CONH-), 5.37 – 5.30 (m, 3H, -CH=CH-), 4.91 (d, J = 7.2 Hz, 1H, Phenylalanine-CH-), 3.52 (d, J = 20.0 Hz, 9H, Phenylalanine-CH₂, cyclen-CH₂), 3.36 – 3.24 (m, 7H, diethylenetriamine-H), 3.20 (d, J = 8.3 Hz, 4H, cyclen-CH₂), 2.94 (ddd, J = 34.7, 25.2, 17.3 Hz, 4H, cyclen-CH₂), 2.73 (d, J = 6.3 Hz, 4H, cyclen-CH₂) 2.13 – 2.06 (m, 4H, -CH₂-CONE-), 2.02 – 1.90 (m, 4H, -CH₂-), 1.69 (s, 6H, -CH=CH-CH₂-), 1.62 – 1.49 (m, 4H, -CH₂-), 1.43 (t, J = 12.0 Hz, 25H, BOC), 1.22 (s, 43H, -CH₂), 0.85 (t, J = 6.7 Hz, 6H, -CH₂CH₃). HR-MS (ESI): C₇₄H₁₄₅N₈O₁₂, [M+Na]⁺, 1313.9808, 1313.9770.

Compound triBoc-cyclen-11: ¹H - NMR (400 MHz, CDCl₃) δ 6.97 (d, J = 7.9 Hz, 2H, Ph-H), 6.73 (d, J = 8.0 Hz, 2H, Ph-H), 6.56 (s, 1H, -CONH-), 5.38 – 5.25 (m, 4H, -CH=CH-), 4.86 (d, J = 7.2 Hz, 1H, tyrosine-CH-), 3.63 – 3.54 (m, 2H, tyrosine-CH₂), 3.46 (d, J = 20.1 Hz, 8H, diethylenetriamine-H), 3.30 (d, J = 15.5 Hz, 8H, cyclen-CH₂), 3.18 (s, 4H, cyclen-CH₂), 2.75 – 2.63 (m, 4H, cyclen-CH₂), 2.12 (dd, J = 16.7, 8.6 Hz, 4H, cyclen-CH₂), 1.98 (dd, J = 22.5, 16.9 Hz, 5H, -CH₂-), 1.85 (d, J = 16.0 Hz, 5H, -CH=CH-CH₂-), 1.62 – 1.50 (m, 5H, -CH=CH-CH₂-), 1.45 (d, J = 4.2 Hz, 24H, BOC), 1.23 (s, 41H, -CH₂), 0.85 (t, J = 6.5 Hz, 6H, -CH₂CH₃). HR-MS (ESI): C₇₄H₁₃₀N₈O₁₁, [M+Na]⁺, 1329.9757, 1329.9720.

Compound triBoc-cyclen-12: ¹H - NMR (400 MHz, CDCl₃): δ 7.21 – 7.12 (m, 4H, Ph-H), 7.11 – 7.01 (m, 1H, Ph-H), 6.93 (d, J = 5.3 Hz, 1H, -CONH-), 6.23 (s, 1H, -CONH-), 5.39 – 5.23 (m, 4H, -CH=CH-), 4.60 – 4.48 (m, 2H, Phenylalanine-CH₂-), 4.37 (dd, J = 11.3, 5.3 Hz, 1H, Phenylalanine-CH-), 4.19 (dd, J = 11.2, 3.8 Hz, 2H, serine-CH₂-), 4.11 – 3.99 (m, 1H, serine-CH-), 3.57 – 2.95 (m, 22H, cyclen-H, -CH₂), 2.71 (d, J = 5.3 Hz, 3H, -CH₂-), 2.22 (t, J = 7.5 Hz, 2H, -CH₂-COOCH₂-), 2.03 – 1.88 (m, 8H, -CH=CH-CH₂-), 1.50 (d, J = 14.7 Hz, 3H,
-CH₂), 1.42 (d, J = 9.5 Hz, 27H, BOC), 1.22 (d, J = 3.7 Hz, 43H, -CH₂), 0.84 (t, J = 6.6 Hz, 6H, -CH₂CH₃). ¹³C - NMR (101 MHz, CDCl₃): δ 173.65, 171.50, 170.83, 167.92, 136.37, 129.80, 129.04, 128.63, 127.02, 125.71, 79.72, 63.70, 55.02, 52.68, 39.66, 37.43, 33.96, 32.56, 31.85, 31.46, 29.61, 29.18, 28.52, 27.17, 26.83, 25.58, 24.73, 22.58, 14.08.


Compound triBoc-cyclen-13: ¹H - NMR (400 MHz, CDCl₃): δ 7.02 (t, J = 7.3 Hz, 2H, Ph-H), 6.78 – 6.73 (m, 2H, Ph-H), 6.30 (s, 1H, -CONH-), 5.41 – 5.29 (m, 3H, -CH=CH-), 4.58 (dt, J = 14.3, 7.1 Hz, 2H, tyrosine-CH₂-), 4.41 (dd, J = 11.3, 5.4 Hz, 1H, tyrosine-CH-), 4.24 (dd, J = 11.3, 4.2 Hz, 1H, serine-CH-), 3.50 (s, 6H, cyclen-H), 3.34 (s, 4H, cyclen-H), 3.26 – 3.05 (m, 6H, cyclen-H), 2.91 (d, J = 7.5 Hz, 1H, -CH₂-), 2.65 (s, 3H, -CH₂-), 2.27 (t, J = 7.6 Hz, 2H, -CH₂-COOCH₂-), 2.05 – 1.93 (m, 6H, -CH₂-), 1.70 (s, 8H, -CH=CH-CH₂-), 1.56 (d, J = 4.1 Hz, 3H, -CH₂), 1.47 (d, J = 7.7 Hz, 25H, BOC), 1.24 (t, J = 9.3 Hz, 43H, -CH₂), 0.88 (t, J = 6.9 Hz, 6H, -CH₂CH₃). HR-MS (ESI): C₇₃H₁₃₂N₇O₁₁, [M+Na]⁺, 1316.9440, found: 1316.9443.

Compound D1: ¹H - NMR (400 MHz, CDCl₃): δ 5.33 (d, J = 14.8 Hz, 3H, -CH=CH-), 3.83 – 2.57 (m, 24H, diethylenetriamine-H, cyclen-CH₂), 2.17 (s, 4H, -CH₂-CONH-), 1.95 (d, J = 16.1 Hz, 4H, -CH₂-), 1.46 (d, J = 49.6 Hz, 8H, -CH₂-CH=CH-), 1.23 (s, 40H, -CH₂-), 0.85 (s, 6H, -CH₂CH₃). ¹³C - NMR (101 MHz, CDCl₃): δ 176.59, 161.18, 129.97, 117.16, 114.27, 35.93, 33.63, 32.58, 31.89, 30.11, 27.36, 27.16, 25.76, 24.96, 22.64, 14.06. HR-MS (ESI⁺): C₅₀H₉₇N₇O₃, [M+H]⁺, 844.7731, found: 844.7584.

Compound D2: ¹H - NMR (400 MHz, CD₃OD): δ 7.53 (d, J = 7.8 Hz, 2H, Ph-H), 7.44 (d, J = 7.3 Hz, 2H, Ph-H), 5.31 (d, J = 15.0 Hz, 3H, -CH=CH-), 4.46 (s, 2H, Ph-CH₂-), 3.84 (s, 2H, CH₂-Ph), 3.55 (d, J = 50.5 Hz, 7H, diethylenetriamine-H), 3.30 (s, 4H, cyclen-CH₂), 3.15 (d, J = 27.2 Hz, 9H, cyclen-CH₂), 3.00 – 2.63 (m, 12H, cyclen-CH₂), 2.16 (s, 4H, -CH₂-CONH-), 1.96 (d, J = 21.5 Hz, 4H, -CH₂-), 1.58 (d, J = 29.2 Hz, 7H, -CH₂-CH=CH-), 1.24 (s, 41H, -CH₂-), 0.85 (t, J = 6.0 Hz, 6H, -CH₂CH₃). ¹³C - NMR (101 MHz, CD₃OD): δ 177.04, 131.31, 130.61, 129.50, 113.18, 56.15, 54.75, 44.43, 41.81, 35.28, 34.78, 32.24, 31.66, 29.57 – 28.15 (m), 26.74, 25.18, 22.32, 13.03. HR-MS (ESI⁺): C₇₁H₁₂₇N₇O₈, [M+H]⁺, 1228.9644, found: 1228.9551.

Compound D3: ¹H - NMR (400 MHz, CDCl₃): δ 7.27 (s, 3H, Ph-H), 7.18 (s, 2H), 5.34 (d, J = 13.2 Hz, 3H, -CH=CH-), 5.04 (s, 1H, Phenylalanine -CH-), 3.60 – 2.71 (m, 32H, cyclen-H,
diethylenetriamine-H, Phenylalanine-CH$_2$-, 2.17 (d, J = 7.4 Hz, 4H, -CH$_2$-CONH-), 2.06 – 1.90 (m, 4H, -CH$_2$-), 1.51 (s, 6H, -CH=CH-CH$_2$-), 1.23 (s, 41H, -CH$_2$-), 0.86 (t, J = 6.2 Hz, 6H, -CH$_2$CH$_3$). $^{13}$C - NMR (101 MHz, CDCl$_3$): δ 160.68, 160.27, 135.12, 130.03, 129.50, 128.75, 127.58, 116.70, 113.82, 50.73, 47.28, 44.82, 43.42, 42.87, 38.75, 37.71, 35.94, 32.56, 31.88, 29.63, 29.32, 29.03, 27.16, 25.57, 24.65, 22.62, 14.02. HR-MS (ESI): C$_{59}$H$_{106}$N$_8$O$_4$, [M+H]$^+$, 991.8415, found:991.8406.

Compound D4: $^1$H - NMR (400 MHz, CDCl$_3$): δ 7.53 (s, 2H, -CONH-), 6.94 (d, J = 38.9 Hz, 2H, Ph-H), 6.74 (d, J = 6.5 Hz, 2H, Ph-H), 5.40 – 5.24 (m, 3H, -CH=CH-), 5.04 (s, 1H, tyrosine-CH-), 3.63 (dd, J = 13.9, 6.9 Hz, 2H, tyrosine-CH$_2$-, 2.99 (ddd, J = 86.0, 67.8, 21.3 Hz, 26H, diethylenetriamine-H, cyclen-H), 2.22 (d, J = 6.1 Hz, 4H, -CH$_2$-CONH-), 2.07 – 1.88 (m, 4H, -CH$_2$-), 1.56 (t, J = 17.6 Hz, 6H, -CH=CH-CH$_2$-), 1.23 (s, 41H, -CH$_2$-), 0.85 (d, J = 6.7 Hz, 6H, -CH$_2$CH$_3$). $^{13}$C - NMR (101 MHz, CDCl$_3$): δ 160.53, 160.15, 155.29, 130.83, 130.02, 129.56, 116.46, 115.56, 113.60, 55.41, 50.89, 44.72, 43.40, 42.74, 38.16, 37.90, 35.76, 32.55, 31.87, 29.73 – 29.31 (m), 29.12, 27.14, 25.55, 22.61, 14.45, 14.02. HR-MS (ESI): C$_{59}$H$_{106}$N$_8$O$_5$, [M+H]$^+$, 1007.8364, found:1007.8367.

Compound D5: $^1$H - NMR (400 MHz, CDCl$_3$): δ 7.83 (s, 1H, -CONH-), 7.49 (s, 1H, -CONH-), 7.25 (s, 4H, Ph-H), 6.58 (s, 1H, Ph-H), 5.33 (s, 4H, -CH=CH-), 4.61 (s, 2H, Phenylalanine-CH$_2$-), 4.21 (s, 3H, Phenylalanine-CH-, serine-CH$_2$-), 3.47 – 2.68 (m, 27H, cyclen-H, -CH$_2$-CONH-), 2.24 (s, 2H, -CH$_2$-COOCH$_2$-), 1.97 (d, J = 19.2 Hz, 7H, -CH=CH-CH$_2$-), 1.53 (d, J = 14.7 Hz, 3H, -CH$_2$-), 1.42 (s, 3H, -CH$_2$-), 1.24 (s, 42H, -CH$_2$-), 0.85 (d, J = 6.4 Hz, 6H, -CH$_2$CH$_3$). $^{13}$C - NMR (101 MHz, CDCl$_3$): δ 134.82, 129.98, 129.63, 128.91, 128.72, 128.45, 127.51, 55.57, 44.74, 40.38, 33.81, 31.86, 29.44, 28.80, 27.15, 26.70, 24.59, 22.63, 14.03. HR-MS (ESI): C$_{58}$H$_{103}$N$_7$O$_5$, [M+H]$^+$, 978.8099, found:978.8096.

Compound D6: $^1$H - NMR (400 MHz, CDCl$_3$): δ 6.99 (d, J = 45.2 Hz, 4H, Ph-H), 6.67 (s, 2H, -CONH-), 5.42 – 5.25 (m, 4H, -CH=CH-), 4.54 (d, J = 45.6 Hz, 4H, tyrosine-CH$_2$-, serine-CH$_2$-), 4.23 (s, 2H, tyrosine-CH-, serine-CH-)), 4.00 (s, 1H, -OH), 2.90 (dd, J = 120.6, 29.2 Hz, 25H, cyclen-H), 2.25 (s, 2H, -CH$_2$-COOCH$_2$-), 1.96 (dd, J = 13.5, 7.2 Hz, 7H, -CH=CH-CH$_2$-), 1.53 (dd, J = 39.8, 29.7 Hz, 7H, -CH$_2$-), 1.23 (s, 44H, -CH$_2$-), 0.85 (t, J = 6.8 Hz, 6H, -CH$_2$CH$_3$). $^{13}$C - NMR (101 MHz, CDCl$_3$): δ 155.26, 130.36, 129.94, 129.61, 127.14, 116.82, 115.56, 113.90, 44.75, 43.50, 40.24, 33.85, 32.56, 31.87, 29.89, 28.56, 27.16, 26.79, 24.65, 22.62, 14.02. HR-MS (ESI): C$_{59}$H$_{106}$N$_8$O$_4$, [M+H]$^+$, 991.8415, found:991.8406.
MS (ESI): $C_{58}H_{103}N_7O_6$, [M+H]$^+$, 994.8048, found: 994.7985.

for target lipids D2-10 to D2-18 with different alkyl chain

Scheme S3. Detailed synthetic routes of double-tailed lipids D2-10 to D2-18 (synthetic method of these lipids is similar to that of D2).

Analytical data for novel compounds.

Compound 5a-10 (yield: 45%): $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 6.68 (s, 1H, -CONH-), 6.39 (s, 1H, -CONH-), 3.33 (s, 7H, diethylenetriamine-H), 2.30 (dd, $J = 13.5$, 6.0 Hz, 2H), 2.15 (dd, $J = 15.1$, 6.9 Hz, 3H, -CH$_2$-CONH-), 1.64 – 1.53 (m, 4H, -CH$_2$-), 1.43 (s, 7H, BOC), 1.23 (s, 26H, -CH$_3$), 0.85 (t, $J = 6.7$ Hz, 6H, -CH$_2$CH$_3$). $^{13}$C - NMR (101 MHz, CDCl$_3$): $\delta$ 177.65, 174.33, 156.85, 80.49, 66.01, 54.99, 48.91, 47.38, 39.51, 38.65, 36.69, 34.11, 31.83, 29.53 – 29.02 (m), 28.34, 25.68, 24.86, 22.63, 14.07. HR-MS (ESI): $C_{20}H_{57}N_7O_4$, [M+Na]$^+$, 534.4247, found: 534.4248.

Compound 5a-12 (yield: 47%): $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 6.53 (s, 1H, -CONH-), 6.24 (s, 1H, -CONH-), 3.33 (s, 8H, diethylenetriamine-H), 2.22 – 2.08 (m, 4H, -CH$_2$-CONH-), 1.61 (s, 8H, -CH$_2$-), 1.44 (s, 8H, BOC), 1.23 (s, 32H, -CH$_2$), 0.86 (t, $J = 6.7$ Hz, 6H, -CH$_2$CH$_3$). HR-MS (ESI): $C_{33}H_{69}N_7O_4$, [M+Na]$^+$, 590.4867, found: 590.4884.

Compound 5a-14 (yield: 43%): $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 6.53 (s, 1H, -CONH-), 6.24 (s, 1H, -CONH-), 3.33 (s, 8H, diethylenetriamine-H), 2.21 – 2.09 (m, 4H, -CH$_2$-CONH-), 1.59 (s, 4H, -CH$_2$-), 1.43 (s, 9H, BOC), 1.23 (s, 43H, -CH$_2$), 0.85 (t, $J = 6.6$ Hz, 6H, -CH$_2$CH$_3$). HR-MS (ESI): $C_{37}H_{73}N_7O_4$, [M+Na]$^+$, 646.5493, found: 646.5513.

Compound 5a-16 (yield: 41%): $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 6.57 (s, 1H, -CONH-), 6.25 (s,
1H, -CONH-), 3.33 (s, 8H, diethylenetriamine-H), 2.16 (dd, J = 15.0, 7.1 Hz, 4H, -CH₂-CONH-), 1.59 (s, 4H, -CH₂-), 1.43 (s, 9H, BOC), 1.23 (s, 45H, -CH₂-), 0.86 (t, J = 6.3 Hz, 6H, -CH₃CH₃). ¹³C - NMR (101 MHz, CDCl₃): δ 156.84, 80.38, 48.93, 47.34, 39.56, 38.63, 36.74, 31.91, 29.55, 28.36, 25.73, 22.68, 14.12. HR-MS (ESI): C₄₁H₈₁N₃O₄, [M+Na]⁺, 702.6119, found: 702.6125.

Compound 5a-18 (yield: 45%): ¹H - NMR (400 MHz, CDCl₃) δ 6.57 (s, 1H, -CONH-), 6.25 (s, 1H, -CONH-), 3.33 (s, 8H, diethylenetriamine-H), 2.16 (dd, J = 15.0, 7.1 Hz, 4H, -CH₂-CONH-), 1.59 (s, 4H, -CH₂-), 1.43 (s, 9H, BOC), 1.23 (s, 45H, -CH₂-), 0.86 (t, J = 6.3 Hz, 6H, -CH₃CH₃). ¹³C - NMR (101 MHz, CDCl₃): δ 173.61, 156.84, 80.38, 48.93, 47.36, 39.58, 38.65, 36.65, 31.91, 29.79 – 29.20 (m), 28.36, 25.74, 22.68, 14.12. HR-MS (ESI): C₄₅H₉₉N₃O₄, [M+Na]⁺, 758.6742, found: 758.6748.

Compound 7-5a-10 (yield: 75%): ¹H - NMR (400 MHz, CDCl₃) δ 7.17 – 7.05 (m, 4H, Ph-H), 3.68 – 3.18 (m, 21H, Ph-CH₂, cyclen-CH₂, diethylenetriamine-H), 2.53 (d, J = 31.0 Hz, 8H, cyclen-CH₂), 2.16 (t, J = 7.4 Hz, 4H, -CH₂-CONH-), 1.65 – 1.56 (m, 4H, -CH₂-), 1.41 (d, J = 20.2 Hz, 24H, BOC), 1.25 (d, J = 15.8 Hz, 26H, -CH₂-), 0.84 (t, J = 6.6 Hz, 6H, -CH₂CH₃). ¹³C - NMR (101 MHz, CDCl₃): δ 173.87, 155.69, 138.31, 130.03, 128.99, 234.29 – 28.61 (m), 113.46 – 29.61, 28.43, 25.84, 22.64, 14.08. HR-MS (ESI): C₅₅H₁₀₉N₇O₈, [M+Na]⁺, 1008.7453, found: 1008.7450.

Compound 7-5a-12 (yield: 70%): ¹H - NMR (400 MHz, CDCl₃) δ 7.10 (s, 4H, Ph-H), 6.43 (s, 2H, -CONH-), 3.67 – 3.13 (m, 24H, Ph-CH₂, cyclen-CH₂, diethylenetriamine-H), 2.52 (d, J = 29.5 Hz, 9H, cyclen-CH₂), 2.16 (s, 4H, -CH₂-CONH-), 1.59 (s, 4H, -CH₂-), 1.45 – 1.32 (m, 26H, BOC), 1.23 (d, J = 18.6 Hz, 42H, -CH₂-), 0.86 – 0.80 (m, 6H, -CH₂CH₃). ¹³C - NMR (101 MHz, CDCl₃): δ 173.87, 155.71, 138.30, 129.98, 128.95, 79.43, 57.91, 53.43, 49.84, 47.91, 37.07, 36.44, 31.88, 29.78 – 29.26 (m), 28.51, 25.84, 22.64, 14.08. HR-MS (ESI): C₅₉H₁₀₇N₇O₈, [M+Na]⁺, 1064.8079, found: 1064.8049.

Compound 7-5a-14 (yield: 60%): ¹H - NMR (400 MHz, CDCl₃) δ 7.17 – 7.02 (m, 4H, Ph-H), 6.43 (s, 2H, -CONH-), 3.67 – 3.13 (m, 24H, Ph-CH₂, cyclen-CH₂, diethylenetriamine-H), 2.52 (d, J = 29.5 Hz, 9H, cyclen-CH₂), 2.16 (s, 4H, -CH₂-CONH-), 1.59 (s, 4H, -CH₂-), 1.45 – 1.32 (m, 26H, BOC), 1.23 (d, J = 18.6 Hz, 42H, -CH₂-), 0.86 – 0.80 (m, 6H, -CH₂CH₃). ¹³C - NMR (101 MHz, CDCl₃): δ 173.87, 155.71, 138.30, 129.98, 128.93, 79.43, 57.92, 57.62, 53.43,
Compound 7-5a-16 (yield: 77%): 1H - NMR (400 MHz, CDCl₃) δ 7.13 (s, 4H, Ph-H), 6.38 (s, 2H, -CONH-), 3.48 (dd, J = 99.3, 49.0 Hz, 24H, Ph-CH₂, cyclen-CH₂, diethylenetriamine-H), 2.70 – 2.45 (m, 10H, cyclen-CH₂), 2.17 (t, J = 6.8 Hz, 4H, -CH₂-CONH-), 0.85 (t, J = 6.4 Hz, 6H, -CH₂CH₃). 13C - NMR (101 MHz, CDCl₃): δ 173.91, 138.32, 129.97, 129.04, 79.47, 57.88, 53.43, 37.07, 36.48, 31.91, 29.82 – 29.26 (m), 28.54, 28.40 – 28.00 (m), 25.86, 22.67, 14.12. HR-MS (ESI): C₆₇H₁₂₃N₇O₈, [M+H]+, 1154.9511, found: 1154.9512.

Compound 7-5a-18 (yield: 79%): 1H - NMR (400 MHz, CDCl₃) δ 7.13 (s, 4H, Ph-H), 6.39 (s, 2H, -CONH-), 3.48 (dd, J = 99.4, 48.7 Hz, 24H, Ph-CH₂, cyclen-CH₂, diethylenetriamine-H), 2.69 – 2.45 (m, 9H, cyclen-CH₂), 2.16 (d, J = 6.6 Hz, 4H, -CH₂-CONH-), 0.85 (t, J = 6.3 Hz, 6H, -CH₂CH₃). 13C - NMR (101 MHz, CDCl₃): δ 173.91, 155.67, 138.32, 130.02, 128.95, 79.53, 57.83, 53.43, 37.07, 36.48, 31.90, 29.82 – 29.26 (m), 28.43, 25.86, 22.67, 14.12. HR-MS (ESI): C₇₁H₁₃₁N₇O₈, [M+H]+, 1211.0134, found: 1211.0137.

Compound D2-10 (yield: 99%): 1H - NMR (400 MHz, CD₃OD) δ 7.55 (d, J = 7.5 Hz, 2H, Ph-H), 7.46 (d, J = 7.6 Hz, 2H, Ph-H), 4.47 (s, 2H, Ph-CH₂), 3.86 (s, 2H, Ph-CH₂), 3.51 (s, 5H, cyclen-CH₂), 3.32 (s, 4H, cyclen-CH₂), 3.17 (d, J = 26.6 Hz, 8H, diethylenetriamine-H), 2.96 (s, 4H, cyclen-CH₂), 2.83 (s, 4H, cyclen-CH₂), 2.17 (t, J = 7.4 Hz, 4H, -CH₂-CONH-), 1.56 (s, 4H, -CH₂-), 1.28 (s, 25H, -CH₂-), 0.87 (t, J = 6.1 Hz, 6H, -CH₂CH₃). 13C - NMR (101 MHz, CD₃OD): δ 180.97, 141.06, 135.24, 134.55, 133.16, 60.86, 60.09, 58.57, 48.37, 45.75, 39.22, 38.71, 35.58, 33.05, 29.12, 26.26, 16.96. HR-MS (ESI): C₄₈H₉₁N₇O₂, [M+Na]+, 686.6060, found: 686.6053.

Compound D2-12 (yield: 99%): 1H - NMR (400 MHz, CD₃OD) δ 7.55 (d, J = 7.6 Hz, 2H, Ph-H), 7.46 (d, J = 7.6 Hz, 2H, Ph-H), 4.48 (s, 2H, Ph-CH₂), 3.87 (s, 2H, Ph-CH₂), 3.52 (s, 4H, cyclen-CH₂), 3.33 (s, 4H, cyclen-CH₂), 3.21 (s, 3H, diethylenetriamine-H), 3.15 (s, 4H, diethylenetriamine-H), 2.98 (s, 4H, cyclen-CH₂), 2.85 (s, 4H, cyclen-CH₂), 2.18 (t, J = 7.4 Hz, 4H, -CH₂-CONH-), 1.57 (s, 4H, -CH₂-), 1.27 (s, 32H, -CH₂-), 0.87 (t, J = 6.3 Hz, 6H, -CH₂CH₃). 13C - NMR (101 MHz, CD₃OD): δ 180.94, 163.93, 163.54, 141.06, 135.27, 134.53.
Compound **D2-14**(yield: 99%): $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 7.37 (s, 4H, Ph-H), 4.35 (s, 2H, Ph-CH$_2$), 3.90 – 2.81 (m, 31H, Ph-CH$_2$, diethylenetriamine-H, cyclen-CH$_2$), 2.20 (s, 4H, -CH$_2$-CONH-), 1.53 (d, $J = 11.8$ Hz, 4H, -CH$_2$-), 1.22 (s, 38H, -CH$_2$-), 0.85 (t, $J = 6.7$ Hz, 6H, -CH$_2$CH$_3$). $^{13}$C - NMR (101 MHz, CDCl$_3$): $\delta$ 178.64, 160.87, 160.49, 130.95, 128.32, 116.73, 113.87, 111.00, 54.13, 48.60, 35.67, 31.86, 29.73 – 28.75 (m), 25.33, 22.61, 13.98. HR-MS (ESI): C$_{44}$H$_{83}$N$_7$O$_2$, [M+H]$^+$, 742.6686, found: 742.6696.

Compound **D2-16**(yield: 99%): $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 7.38 (s, 4H, Ph-H), 4.37 (s, 2H, Ph-CH$_2$), 3.67 – 2.86 (m, 26H, diethylenetriamine-H, cyclen-CH$_2$), 2.21 (s, 4H, -CH$_2$-CONH-), 1.52 (s, 4H, -CH$_2$-), 1.24 (d, $J = 11.3$ Hz, 46H, -CH$_2$-), 0.85 (t, $J = 6.0$ Hz, 6H, -CH$_2$CH$_3$). $^{13}$C - NMR (101 MHz, CDCl$_3$): $\delta$ 160.47, 116.67, 113.82, 66.15, 35.60, 31.88, 29.73 – 28.91 (m), 25.28, 22.63, 14.44, 14.00. HR-MS (ESI): C$_{52}$H$_{99}$N$_7$O$_2$, [M+H]$^+$, 854.7939, found: 854.7974.

Compound **D2-18**(yield: 99%): $^1$H - NMR (400 MHz, CDCl$_3$) $\delta$ 7.46 (s, 2H, -CONH-), 7.36 (s, 4H, Ph-H), 4.33 (s, 2H, Ph-CH$_2$), 3.75 (s, 2H, Ph-CH$_2$), 3.64 – 2.73 (m, 29H, diethylenetriamine-H, cyclen-CH$_2$), 2.19 (s, 4H, -CH$_2$-CONH-), 1.52 (s, 4H, -CH$_2$-), 1.23 (s, 53H, -CH$_2$-), 0.85 (t, $J = 6.2$ Hz, 6H, -CH$_2$CH$_3$). $^{13}$C - NMR (101 MHz, CDCl$_3$): $\delta$ 178.14, 160.96, 114.03, 35.75, 31.89, 29.69, 29.30, 25.34, 22.65, 14.06. HR-MS (ESI$^+$): C$_{56}$H$_{107}$N$_7$O$_2$, [M+H]$^+$, 910.8565, found: 910.8568.