Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

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

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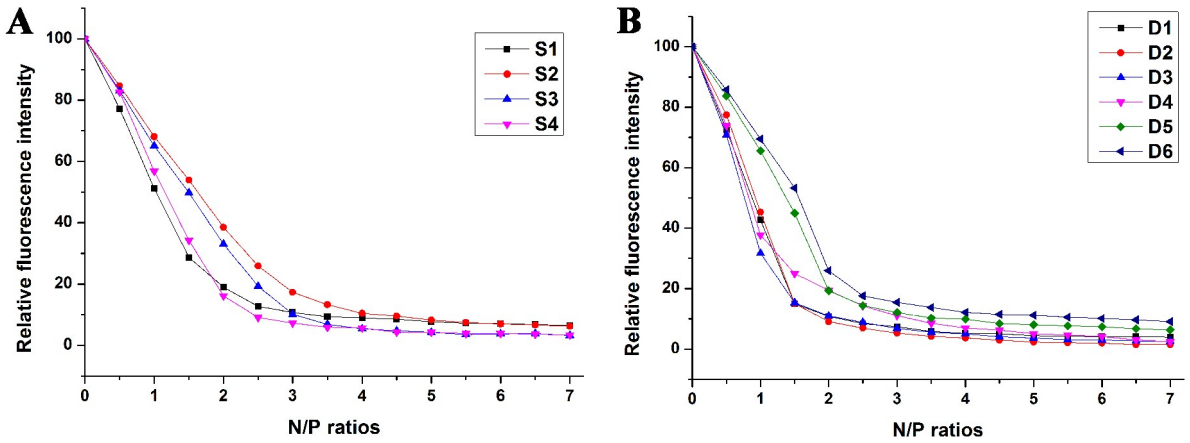


Figure S1. Ethidium bromide displacement assay of plasmid DNA binding abilities for the liposomes **S1**-**S4** (A), **D1**–**D6** (B) under various N/P ratios in Hepes buffer solution (pH = 7.4, 10 mM). The molar ratio of lipid/DOPE was 1 : 1.

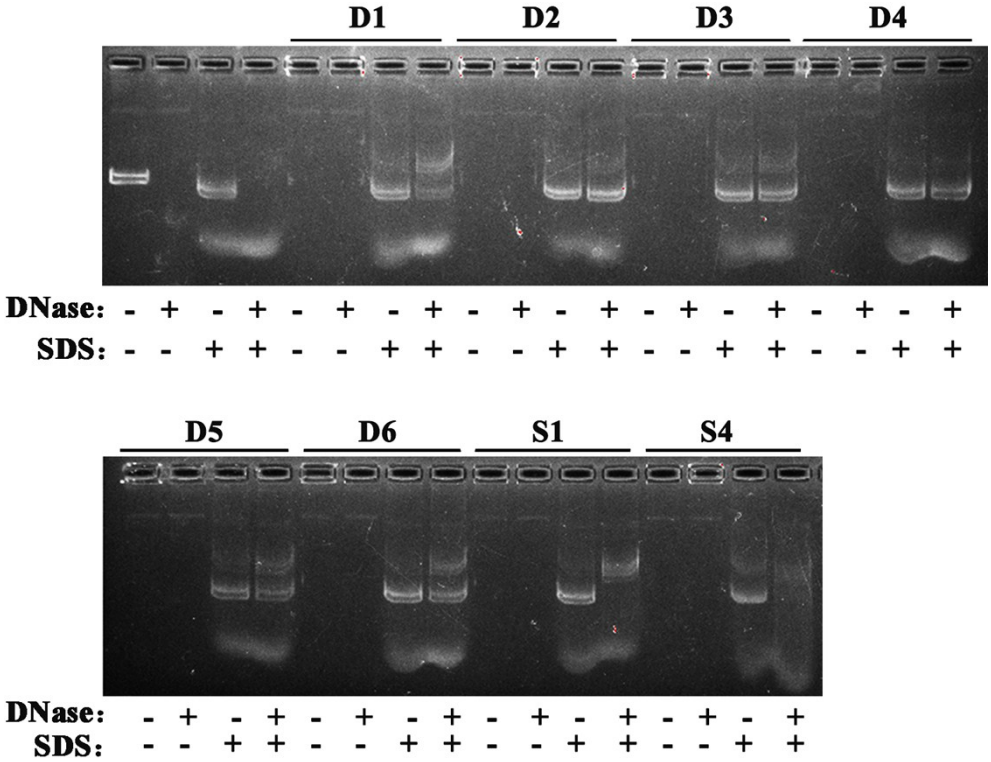


Figure S2. Protection and SDS-induced release of DNA from liposomes **D1**-**D6**, **S1** and **S4** at N/P ratio of 8 visualized by agarose gel electrophoresis. The first 4 lanes are DNA control.

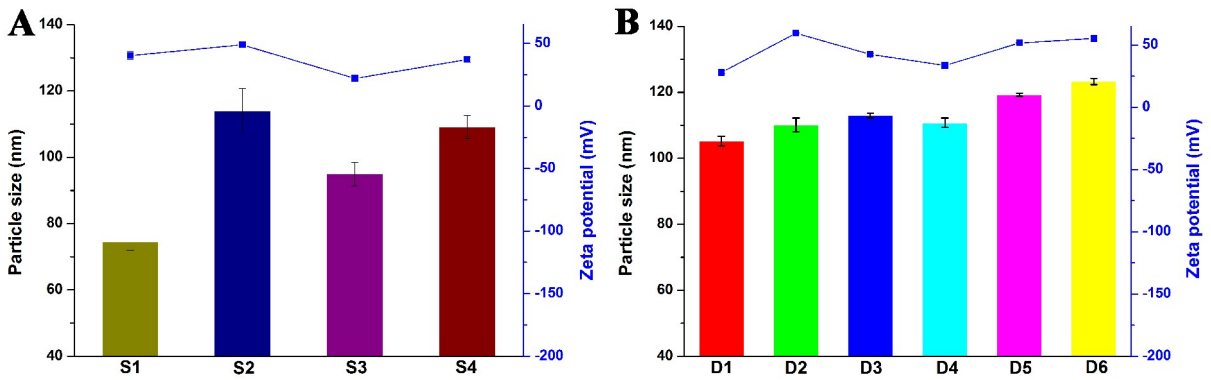


Figure S3. Mean particle sizes (columns) and zeta-potentials (dots) of the liposomes formed from **S1-S4** (A) and **D1-D6** (B).

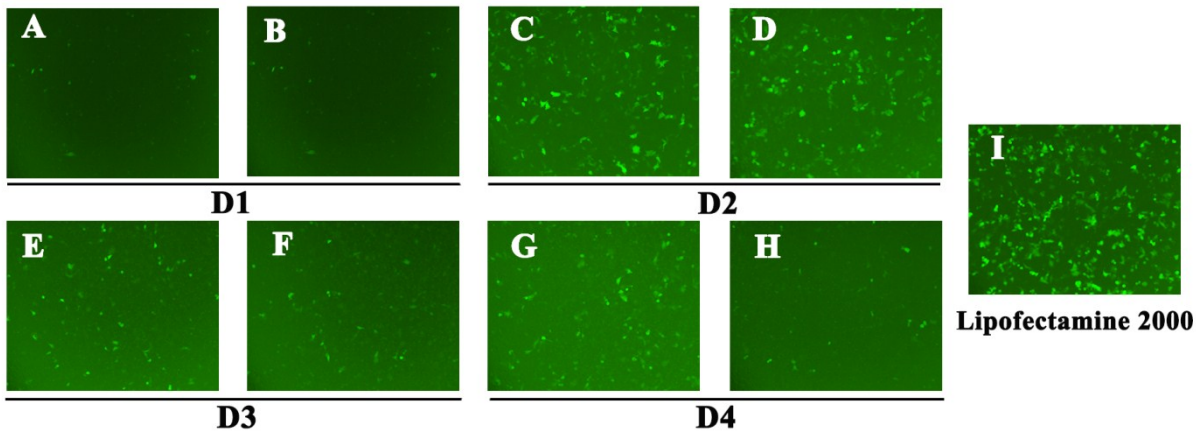


Figure S4. Fluorescent microscope images of HeLa cells transfected by double-tailed lipid/DOPE/DNA lipoplexes. Lipid/DOPE ratio was 1 : 1. The cells were observed by inversion fluorescence microscope after 24 h transfection. (A, C, E, G: N/P = 6; B, D, F, H, N/P = 8; I: lipofectamine 2000)**.**

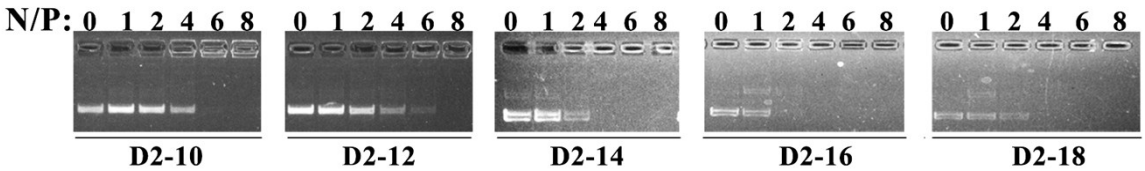


Figure S5. Electrophoretic gel retardation assays of lipids/DOPE/pDNA complexes at different N/P ratios. The molar ratio of lipid/DOPE was 1 : 1.

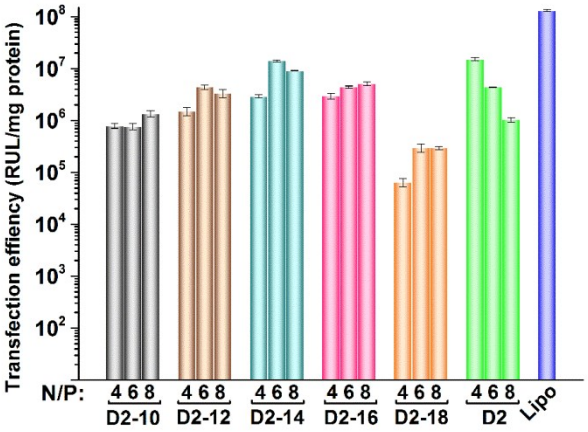


Figure S6. Luciferase expression in HeLa cells transfected by lipid/DOPE/DNA lipoplexes at various N/P ratios under lipid/DOPE ratio of 1 : 1. Data represent mean ± SD (n = 3).

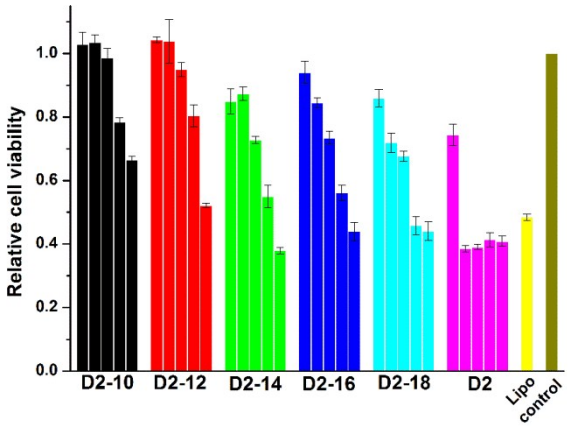
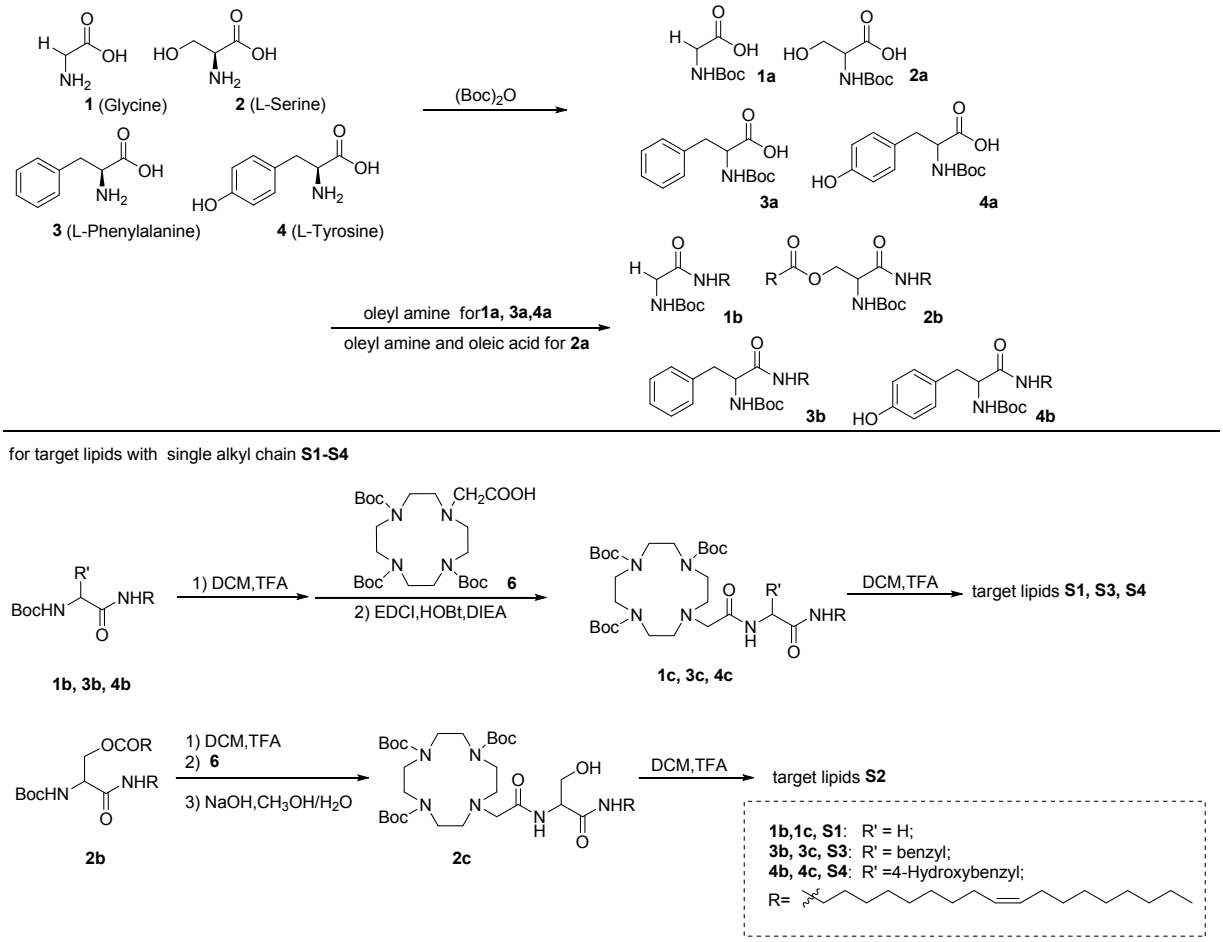


Figure S7. Cytotoxicity of the lipoplexes **D2-10**~**D2-18** prepared at various N/P ratios (2, 4, 6, 8 and 12) and **D2** prepared at N/P ratios (4, 6, 8, 10 and 12) in HeLa cells. Data represent mean ± SD (n = 3).

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, $(Boc)_2O$ (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 $NaSO_4$ 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 ℃ 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 ℃ 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_2SO_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_3OH) (25 mL) and H_2O (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/EA= 1:1, 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_3COOH) (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, $CDCl_3$) δ 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_2CH_3).

Compound **S1**: ^{1}H - NMR (400 MHz, $CDCl_3$) δ 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_2CH_3); ^{13}C - NMR (101 MHz, $CDCl_3$): δ / 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): $C_{30}H_{60}N_6O_2$,$[M+H]^+$, 537.4856, found: 537.4851.

Compound **2c**: ^{1}H - NMR (400 MHz, $CDCl_3$) δ 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_2CH_3); HR-MS (ESI): $C_{46}H_{86}N_6O_9$, $[M+Na]^+$, 889.6354, found: 889.6349.

Compound **S2**: ^{1}H - NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J = 48.6 Hz, 1H, -CONH-), 5.42 – 5.34 (m, 2H, -CH=CH-), 4.52 (s, 1H, serine-CH-), 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_2CH_3); ^{13}C - NMR (101 MHz, $CDCl_3$): δ / ppm: 161.21, 137.64, 129.90, 31.86, 29.72, 29.38, 27.17, 22.63, 14.04. HR-MS (ESI): $C_{31}H_{62}N_6O_3$, $[M+H]^+$, 567.4962, found: 567.4966.

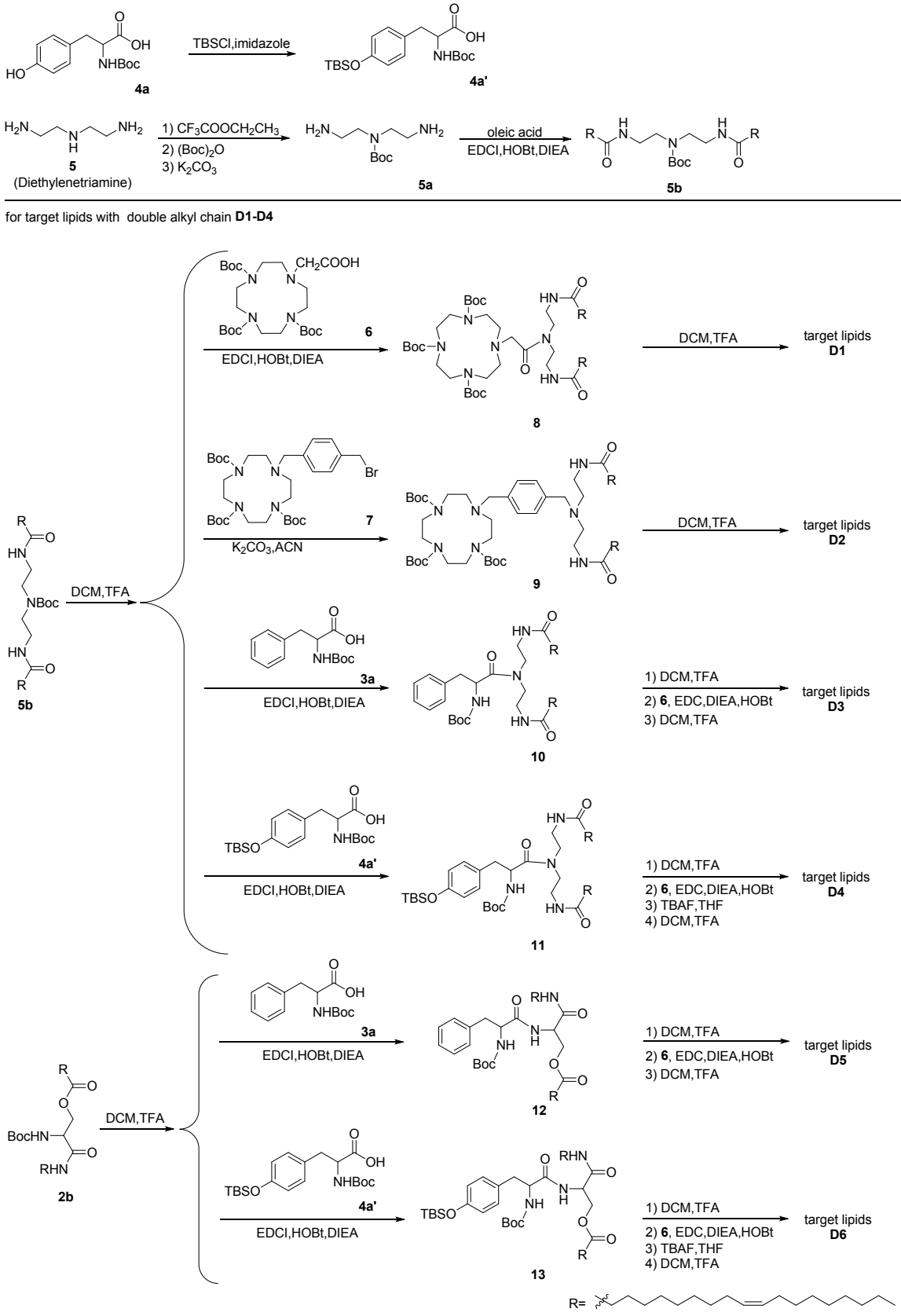
Compound **3c**: ^{1}H - NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 5H, Ph-H), 5.36 (t, J = 14.5 Hz, 2H, -CH=CH-), 4.48 (s, 1H, Phenylalanine-CH-), 3.35 (dd, J = 165.0, 61.0 Hz, 14H, Phenylalanine-

CH_2-, cyclen-H), 2.78 (s, 4H, cyclen-H), 2.02 (d, J = 7.0 Hz, 4H, cyclen-H)), 1.63 (s, 8H, -CH_2-CONH-, -CH_2-CH=CH-), 1.48 (d, J = 16.9 Hz, 22H, BOC), 1.29 (dd, J = 10.2, 4.0 Hz, 27H, -CH_2-), 0.90 (t, J = 6.6 Hz, 3H, -CH_2CH_3); HR-MS (ESI): $C_{52}H_{90}N_6O_8$, $[M+Na]^+$, 949.6718, found: 949.6712.

Compound **S3**:^{1}H - NMR (400 MHz, $CDCl_3$) 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, Phenylalanine-CH_2-), 3.70 – 2.64 (m, 23H, Phenylalanine-CH-, cyclen-H), 1.99 (d, J = 4.8 Hz, 3H, cyclen-H), 1.47 – 1.33 (m, 3H, -CH_2-CH=CH-)), 1.15 (d, J = 65.1 Hz, 24H, -CH_2-), 0.85 (t, J = 6.0 Hz, 3H, -CH_2CH_3)., ^{13}C - NMR (101 MHz, $CDCl_3$): 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): $C_{37}H_{66}N_6O_2$, $[M+H]^+$, 627.5326, found: 627.5325.

Compound **4c**: ^{1}H - NMR (400 MHz, $CDCl_3$) 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-CH_2-), 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, -CH_2-CH=CH-), 1.48 (d, J = 5.4 Hz, 27H, BOC), 1.27 (s, 22H, -CH_2-), 0.90 (t, J = 6.7 Hz, 3H, -CH_2CH_3); HR-MS (ESI): $C_{52}H_{90}N_6O_9$, $[M+Na]^+$, 965.6667, found: 965.6670.

Compound **S4**: ^{1}H - 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-CH_2-), 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, -CH_2-CH=CH-), 1.47 – 1.20 (m, 25H, -CH_2-), 0.92 (t, J = 6.8 Hz, 3H, -CH_2CH_3); ^{13}C - 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): $C_{37}H_{66}N_6O_3$, $[M+H]^+$, 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 imdazole (0.005 mol) in DCM at 0 ℃, 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_3OH=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 $(Boc)_2O$ (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_2CO_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_2SO_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 ℃ for 0.5 h, **5a** (0.018 mol) in CH_3OH 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_2SO_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 ℃ 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 $NaHCO_3$ (2 × 50 mL), and brine (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated the solvent to yellow oil. The residue was purified by silica gel column chromatography to obtain compound **8** (DCM/CH_3OH = 10:1 v/v), Yield: 45%.

*Preparation of compound **9***

Deprotected **5b** was suspended in 100 mL ethyl acetate. Added 1 g K_2CO_3 (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 ℃ 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 $NaHCO_3$ (2 × 50 mL), and brine (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4 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 CF_3COOH (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, $CDCl_3$) δ 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_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): δ 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, $CDCl_3$) δ 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_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): δ 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, $CDCl_3$) δ 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, $CDCl_3$): δ 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): $C_{31}H_{51}BrN_4O_6$, $[M+Na]^+$, 677.2980, found:677.2885.

Compound **8**: ^{1}H - NMR (400 MHz, $CDCl_3$) δ 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, -CH_2-CH=CH-), 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_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): δ 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, $CDCl_3$) δ 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,

48H, -CH_2-), 0.85 (t, J = 6.4 Hz, 6H, - CH_2CH_3). HR-MS (ESI): $C_{71}H_{127}N_7O_8$, $[M+H]^+$, 1228.9644, found:1228.9551.

Compound **10**: ^{1}H - NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.25 (m, 2H, Ph-H), 7.16 (t, J = 7.6 Hz, 3H, Ph-H), 6.82 (s, 1H, -CONH-), 6.40 (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-), 1.38 (s, 9H, BOC), 1.22 (d, J = 5.0 Hz, 41H, -CH_2-), 0.85 (t, J = 6.7 Hz, 6H, - CH_2CH_3).

Compound **11**: ^{1}H - NMR (400 MHz, $CDCl_3$): δ 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_2CH_3), 0.15 (d, J = 4.6 Hz, 6H, TBS-CH_3). HR-MS (ESI): $C_{74}H_{130}N_8O_{10}$, $[M+Na]^+$, 1313.9808, found:1313.9770.

Compound **12**: ^{1}H - NMR (400 MHz, $CDCl_3$): δ 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_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): δ 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): $C_{53}H_{91}N_3O_6$, $[M+Na]^+$, 888.6808, found:888.6810.

Compound **13**: ^{1}H - NMR (400 MHz, $CDCl_3$): δ 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-CH_2-), 4.12 – 4.02 (m, 1H, serine-CH-), 3.31 – 3.08 (m, 3H, serine-CH_2-), 3.07 – 2.86 (m, 2H, -CH_2-CONH-), 2.31 – 2.24 (m, 2H, -CH_2-$COOCH_2$-), 2.08 – 1.94 (m, 6H, -CH_2-CH=CH-), 1.72 (s, 1H, -CH_2-), 1.59 (s, 2H, -CH_2-), 1.50 (d, J = 6.3 Hz, 2H, -CH_2-), 1.43 (d, J = 2.3 Hz, 9H, BOC), 1.26 (t, J = 6.8 Hz, 46H, -CH_2-), 0.99 (d, J = 1.7 Hz, 9H, TBS-CH_3), 0.90 (t, J = 6.8 Hz, 6H, -CH_2CH_3), 0.20 (d, J = 3.4 Hz, 6H, TBS-CH_3). HR-MS (ESI): $C_{59}H_{105}N_3O_7Si$, $[M+Na]^+$, 1018.7619, found:1018.7519.

Compound **triBoc-cyclen-10**: ^1H - NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.25 (m, 2H, Ph-H), 7.17 (d, J = 7.0 Hz, 2H, 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-CH_2-, cyclen-CH_2), 3.36 – 3.24 (m, 7H, diethylenetriamine-H), 3.20 (d, J = 8.3 Hz, 4H, cyclen-CH_2), 2.94 (ddd, J = 34.7, 25.2, 17.3 Hz, 4H, cyclen-CH_2), 2.73 (d, J = 6.3 Hz, 4H, cyclen-CH_2) 2.13 – 2.06 (m, 4H, -CH_2-CONH-), 2.02 – 1.90 (m, 4H, -CH_2-), 1.69 (s, 6H, -CH=CH-CH_2-), 1.62 – 1.49 (m, 4H, -CH_2-), 1.43 (t, J = 12.0 Hz, 25H, BOC), 1.22 (s, 43H, CH_2-), 0.85 (t, J = 6.7 Hz, 6H, -CH_2CH_3). HR-MS (ESI): $C_{74}H_{130}N_8O_{10}$, $[M+Na]^+$, 1313.9808, found:1313.9770.

Compound **triBoc-cyclen-11**: ^1H - NMR (400 MHz, $CDCl_3$) 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-CH_2-), 3.46 (d, J = 20.1 Hz, 8H, diethylenetriamine-H), 3.30 (d, J = 15.5 Hz, 8H, cyclen-CH_2), 3.18 (s, 4H, cyclen-CH_2), 2.86 (dt, J = 19.3, 13.1 Hz, 4H, cyclen-CH_2), 2.75 – 2.63 (m, 4H, cyclen-CH_2), 2.12 (dd, J = 16.7, 8.6 Hz, 4H, -CH_2-CONH-), 1.98 (dd, J = 22.5, 16.9 Hz, 5H, -CH_2-), 1.85 (d, J = 16.0 Hz, 5H, -CH=CH-CH_2-), 1.62 – 1.50 (m, 5H, -CH=CH-CH_2-), 1.45 (d, J = 4.2 Hz, 24H, BOC), 1.23 (s, 41H, -CH_2-), 0.85 (t, J = 6.5 Hz, 6H, -CH_2CH_3). HR-MS (ESI): $C_{74}H_{130}N_8O_{11}$, $[M+Na]^+$, 1329.9757, found:1329.9720.

Compound **triBoc-cyclen-12**: ^1H - NMR (400 MHz, $CDCl_3$): 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-CH_2-), 4.37 (dd, J = 11.3, 5.3 Hz, 1H, Phenylalanine-CH-), 4.19 (dd, J = 11.2, 3.8 Hz, 2H. serine-CH_2-), 4.11 – 3.99 (m, 1H, serine-CH-), 3.57 – 2.95 (m, 22H, cyclen-H, -CH_2-), 2.71 (d, J = 5.3 Hz, 3H, -CH_2-), 2.22 (t, J = 7.5 Hz, 2H, -CH_2-$COOCH_2$-), 2.03 – 1.88 (m, 8H, -CH=CH-CH_2-), 1.50 (d, J = 14.7 Hz, 3H,

-CH_2-), 1.42 (d, J = 9.5 Hz, 27H, BOC), 1.22 (d, J = 3.7 Hz, 43H, -CH_2-), 0.84 (t, J = 6.6 Hz, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): 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. HR-MS (ESI): $C_{73}H_{127}N_7O_{11}$, $[M+K]^+$, 1316.9231, found:1316.9363.

Compound **triBoc-cyclen-13**: ^{1}H - NMR (400 MHz, $CDCl_3$): 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-CH_2-), 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, -CH_2-), 2.65 (s, 3H, -CH_2-), 2.27 (t, J = 7.6 Hz, 2H, -CH_2-$COOCH_2$-), 2.05 – 1.93 (m, 6H, -CH_2-), 1.70 (s, 8H, -CH=CH-CH_2-), 1.56 (d, J = 4.1 Hz, 3H, -CH_2-), 1.47 (d, J = 7.7 Hz, 25H, BOC), 1.24 (t, J = 9.3 Hz, 43H, -CH_2-), 0.88 (t, J = 6.9 Hz, 6H, -CH_2CH_3). HR-MS (ESI): $C_{73}H_{127}N_7O_{12}$, $[M+Na]^+$, 1316.9440, found:1316.9443.

Compound **D1**: ^{1}H - NMR (400 MHz, $CDCl_3$) δ 5.33 (d, J = 14.8 Hz, 3H, -CH=CH-), 3.83 – 2.57 (m, 24H, diethylenetriamine-H, cyclen-CH_2), 2.17 (s, 4H, -CH_2-CONH-), 1.95 (d, J = 16.1 Hz, 4H, -CH_2-), 1.46 (d, J = 49.6 Hz, 8H, -CH_2-CH=CH-), 1.23 (s, 40H, -CH_2-), 0.85 (s, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): 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 (ES^+): $C_{50}H_{97}N_7O_3$, $[M+H]^+$, 844.7731, found:844.7584.

Compound **D2**: ^{1}H - NMR (400 MHz, CD_3OD)) 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-CH_2-), 3.84 (s, 2H, CH_2-Ph), 3.55 (d, J = 50.5 Hz, 7H, diethylenetriamine-H), 3.30 (s, 4H, cyclen-CH_2), 3.15 (d, J = 27.2 Hz, 9H, cyclen-CH_2), 3.00 – 2.63 (m, 12H, cyclen-CH_2), 2.16 (s, 4H, -CH_2-CONH-), 1.96 (d, J = 21.5 Hz, 4H, -CH_2-), 1.58 (d, J = 29.2 Hz, 7H, -CH_2-CH=CH-), 1.24 (s, 41H, -CH_2-), 0.85 (t, J = 6.0 Hz, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, CD_3OD): 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): $C_{71}H_{127}N_7O_8$, $[M+H]^+$, 1228.9644, found:1228.9551.

Compound **D3**: ^{1}H - NMR (400 MHz, $CDCl_3$) 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,

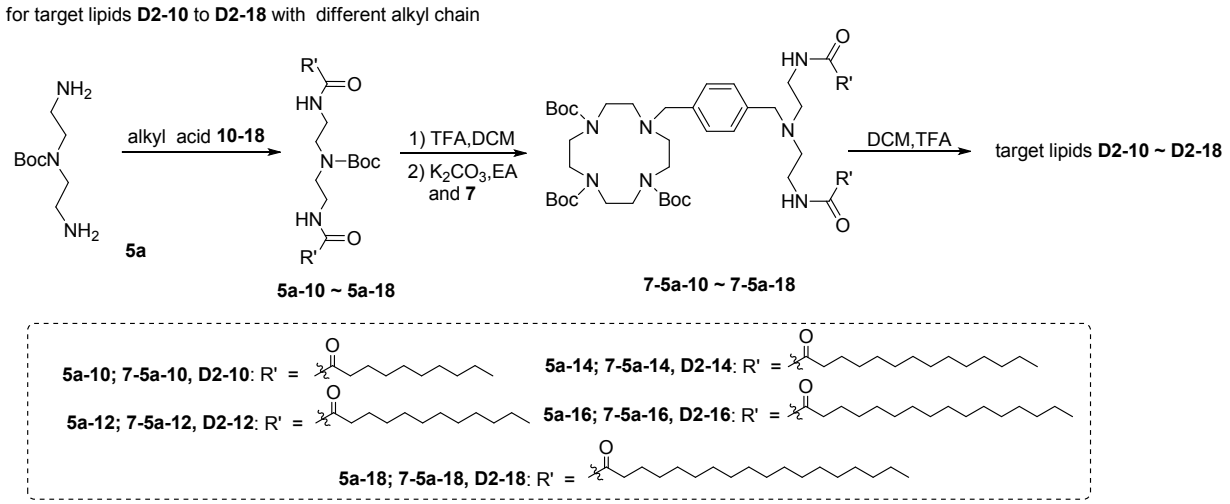
diethylenetriamine-H, Phenylalanine-CH_2-), 2.17 (d, J = 7.4 Hz, 4H, -CH_2-CONH-), 2.06 – 1.90 (m, 4H, -CH_2-), 1.51 (s, 6H, -CH=CH-CH_2-), 1.23 (s, 41H, -CH_2-), 0.86 (t, J = 6.2 Hz, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): δ 160.68, 160.27, 135.12, 130.03, 129.50, 128.75, 127.58, 116.70, 113.82, 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, 22.64, 14.77, 14.05. HR-MS (ESI): $C_{59}H_{106}N_8O_4$, $[M+H]^+$, 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_2CH_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): $C_{59}H_{106}N_8O_5$, $[M+H]^+$, 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_2CH_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): $C_{58}H_{103}N_7O_5$, $[M+H]^+$, 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_2CH_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): $C_{58}H_{103}N_7O_6$, $[M+H]^+$, 994.8048, found:994.7985.



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, $CDCl_3$) δ 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_2-), 0.85 (t, J = 6.7 Hz, 6H, -CH_2CH_3).^{13}C - NMR (101 MHz, $CDCl_3$):δ 177.65, 174.33, 156.85, 80.49, 66.01, 54.49, 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): $C_{29}H_{57}N_3O_4$, $[M+Na]^+$, 534.4247, found: 534.4248.

Compound **5a-12**(yield:47%): 1H - NMR (400 MHz, CDCl3) δ 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_2CH_3). HR-MS (ESI): $C_{33}H_{65}N_3O_4$, $[M+Na]^+$, 590.4867, found:590.4884.

Compound **5a-14**(yield:43%): ^{1}H - NMR (400 MHz, $CDCl_3$) δ 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_2CH_3). HR-MS (ESI): $C_{37}H_{73}N_3O_4$, $[M+Na]^+$, 646.5493, found:646.5513.

Compound **5a-16**(yield:41%): ^{1}H - NMR (400 MHz, $CDCl_3$)) 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, -CH_2-CONH-), 1.59 (s, 4H, -CH_2-), 1.43 (s, 9H, BOC), 1.23 (s, 45H, -CH_2-), 0.86 (t, J = 6.3 Hz, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): 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): $C_{41}H_{81}N_3O_4$, $[M+Na]^+$, 702.6119, found:702.6125.

Compound **5a-18**(yield:45%): ^{1}H - NMR (400 MHz, $CDCl_3$) 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, -CH_2-CONH-), 1.59 (s, 4H, -CH_2-), 1.43 (s, 9H, BOC), 1.23 (s, 45H, -CH_2-), 0.86 (t, J = 6.3 Hz, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): 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): $C_{45}H_{89}N_3O_4$, $[M+Na]^+$, 758.6742, found:758.6748.

Compound **7-5a-10**(yield:75%): ^{1}H - NMR (400 MHz, $CDCl_3$) δ 7.17 – 7.05 (m, 4H, Ph-H), 3.68 – 3.18 (m, 21H, Ph-CH_2, cyclen- CH_2, diethylenetriamine-H), 2.53 (d, J = 31.0 Hz, 8H, cyclen- CH_2), 2.16 (t, J = 7.4 Hz, 4H, -CH_2-CONH-), 1.65 – 1.56 (m, 4H, -CH_2-), 1.41 (d, J = 20.2 Hz, 24H, BOC), 1.25 (d, J = 15.8 Hz, 26H, -CH_2-), 0.84 (t, J = 6.6 Hz, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): 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): $C_{55}H_{99}N_7O_8$, $[M+Na]^+$, 1008.7453, found:1008.7450.

Compound **7-5a-12**(yield:70%): ^{1}H - NMR (400 MHz, $CDCl_3$) δ 7.10 (s, 4H, Ph-H), 3.66 – 3.15 (m, 24H, Ph-CH_2, cyclen- CH_2, diethylenetriamine-H), 2.51 (d, J = 31.2 Hz, 10H, cyclen-CH_2), 2.14 (t, J = 7.1 Hz, 4H, -CH_2-CONH-), 1.58 (s, 4H, -CH_2-), 1.39 (d, J = 19.8 Hz, 26H, BOC), 1.22 (d, J = 18.5 Hz, 36H, -CH_2-), 0.84 – 0.79 (m, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): δ 173.87, 155.65, 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): $C_{59}H_{107}N_7O_8$, $[M+Na]^+$, 1064.8079, found:1064.8049.

Compound **7-5a-14**(yield:60%): ^{1}H - NMR (400 MHz, $CDCl_3$) δ 7.17 – 7.02 (m, 4H, Ph-H), 6.43 (s, 2H, -CONH-), 3.67 – 3.13 (m, 24H, Ph-CH_2, cyclen-CH_2, diethylenetriamine-H), 2.52 (d, J = 29.5 Hz, 9H, cyclen- CH_2), 2.16 (s, 4H, -CH_2-CONH-), 1.59 (s, 4H, -CH_2-), 1.45 – 1.32 (m, 26H, BOC), 1.23 (d, J = 18.6 Hz, 42H, -CH_2-), 0.86 – 0.80 (m, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): δ 173.87, 155.71, 138.30, 129.98, 128.93, 79.43, 57.92, 57.62, 53.43,

49.84, 47.81, 37.07, 36.44, 31.88, 29.78 – 29.26 (m), 28.51, 25.84, 22.64, 14.08. HR-MS (ESI): $C_{63}H_{115}N_7O_8$, $[M+Na]^+$, 1120.8705, found:1120.8665.

Compound **7-5a-16**(yield:77%): ^1H - NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 4H, Ph-H), 6.38 (s, 2H, -CONH-), 3.48 (dd, J = 99.3, 49.0 Hz, 24H, Ph-CH_2, cyclen-CH_2, diethylenetriamine-H), 2.70 – 2.45 (m, 10H, cyclen-CH_2), 2.17 (t, J = 6.8 Hz, 4H, -CH_2-CONH-), 1.61 (s, 4H, -CH_2-), 1.42 (d, J = 19.7 Hz, 25H, BOC), 1.25 (d, J = 20.0 Hz, 50H, -CH_2-), 0.85 (t, J = 6.4 Hz, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): 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): $C_{67}H_{123}N_7O_8$, $[M+H]^+$, 1154.9511, found:1154.9512.

Compound **7-5a-18**(yield:79%): ^1H - NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 4H, Ph-H), 6.39 (s, 2H, -CONH-), 3.48 (dd, J = 99.4, 48.7 Hz, 24H, Ph-CH_2, cyclen-CH_2, diethylenetriamine-H), 2.69 – 2.45 (m, 9H, cyclen-CH_2), 2.16 (d, J = 6.6 Hz, 4H, -CH_2-CONH-), 1.61 (s, 4H, -CH_2-), 1.42 (d, J = 19.5 Hz, 25H, BOC), 1.25 (d, J = 20.0 Hz, 53H, -CH_2-), 0.85 (t, J = 6.3 Hz, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): δ 173.91, 155.67, 138.32, 130.02, 128.95, 79.53, 57.83, 53.43, 37.07, 36.47, 31.90, 29.82 – 29.25 (m), 28.43, 25.86, 22.67, 14.12. HR-MS (ESI): $C_{71}H_{131}N_7O_8$, $[M+H]^+$, 1211.0137, found:1211.0134.

Compound **D2-10**(yield:99%): ^1H - NMR (400 MHz, CD_3OD) 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-CH_2), 3.86 (s, 2H, Ph-CH_2), 3.51 (s, 5H, cyclen-CH_2), 3.32 (s, 4H, cyclen-CH_2), 3.17 (d, J = 26.6 Hz, 8H, diethylenetriamine-H), 2.96 (s, 4H, cyclen- CH_2), 2.83 (s, 4H, cyclen- CH_2), 2.17 (t, J = 7.4 Hz, 4H, -CH_2-CONH-), 1.56 (s, 4H, -CH_2-), 1.28 (s, 25H, -CH_2-), 0.87 (t, J = 6.1 Hz, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, CD_3OD): 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): $C_{48}H_{91}N_7O_2$, $[M+Na]^+$, 686.6060, found:686.6053.

Compound **D2-12**(yield:99%): ^1H - NMR (400 MHz, CD_3OD) 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-CH_2), 3.87 (s, 2H, Ph-CH_2), 3.52 (s, 4H, cyclen-CH_2), 3.33 (s, 4H, cyclen-CH_2), 3.21 (s, 3H, diethylenetriamine-H), 3.15 (s, 4H, diethylenetriamine-H), 2.98 (s, 4H, cyclen-CH_2), 2.85 (s, 4H, cyclen-CH_2), 2.18 (t, J = 7.4 Hz, 4H, -CH_2-CONH-), 1.57 (s, 4H, -CH_2-), 1.27 (s, 32H, -CH_2-), 0.87 (t, J = 6.3 Hz, 6H, -CH_2CH_3). ^{13}C - NMR (101 MHz, CD_3OD): δ 180.94, 163.93, 163.54, 141.06, 135.27, 134.53,

133.07, 60.85, 60.13, 58.45, 48.35, 45.89, 45.66, 39.24, 38.65, 35.60, 33.43 – 32.87 (m), 29.12, 26.26, 16.96. HR-MS (ESI): $C_{44}H_{83}N_7O_2$, $[M+H]^+$, 742.6686, found:742.6696.

Compound **D2-14**(yield:99%): ^1H - NMR (400 MHz, $CDCl_3$) δ 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_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): δ 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): $C_{48}H_{91}N_7O_2$, $[M+H]^+$, 798.7313, found:798.7307.

Compound **D2-16**(yield:99%): ^1H - NMR (400 MHz, $CDCl_3$) δ 7.38 (s, 4H, Ph-H), 4.37 (s, 2H, Ph-CH_2), 3.93 (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_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): δ 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): $C_{52}H_{99}N_7O_2$, $[M+H]^+$, 854.7939, found:854.7974.

Compound **D2-18**(yield:99%): ^1H - NMR (400 MHz, $CDCl_3$) δ 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_2CH_3). ^{13}C - NMR (101 MHz, $CDCl_3$): δ 178.14, 160.96, 114.03, 35.75, 31.89, 29.69, 29.30, 25.34, 22.65, 14.06. HR-MS (ES^+): $C_{56}H_{107}N_7O_2$, $[M+H]^+$, 910.8565, found:910.8568