Supplementary Materials

Effective array of amines on the transfection efficiency of cationic peptidomimetic lipid molecules into neural cells

Xiang Yu, Hai Xiao, Muqier Muqier, Shuqin Han*, Huricha Baigude*

Institute of Mongolian Medicinal Chemistry, School of Chemistry & Chemical Engineering, Inner Mongolia University, Hohhot, Inner Mongolia, 010020, P.R. China

**Correspondence to: Prof. Huricha Baigude (hbaigude@imu.edu.cn): Inner Mongolia Key Laboratory of Mongolian Medicinal Chemistry, School of Chemistry & Chemical Engineering, Inner Mongolia University, Hohhot, 010020 and P.R. China. Tel.: +86 471 4992511; fax: +86 471 4992511*

Synthesis of N3 lipid:

Step 1: Lysine methyl ester hydrochloride (700 mg, 2.7 mmol), 2-(Boc-amino) ethyl bromide (783 mg, 3.5 mmol) and K_2CO_3 (1.8 g, 13.4 mmol) were dissolved in DMF (2 mL), and the mixture was stirred at 60°C for 6 h, followed by washing with 5% citric acid, distilled water, brine, and dried on anhydrous Na_2SO_4, concentrated, and purified by silica gel chromatography using dichloromethane and methanol (10:1) as eluent. The compound 1 was obtained as oil in 74% yield. ^{1}H NMR (DMSO-d6, ppm) (Figure S1): 1.3-1.6 (m, 24H, -CH_2-, -NHCOOC$(CH_3)_3$); 2.4 (m, 2H, -NHCH_2-); 2.8-3.1 (m, 5H, -COCH(NH)-, -CH_2NHCOO-); 3.2 (m, 1H, -NH-); 3.6 (s, 3H, -CH_3). HRMS (ESI+) (Figure S2): 404.2751 $[M + H]^+$. (Calcd for $C_{19}H_{37}N_3O_6$, 403.2682).

Step 2: The compound 1 was dissolved in 1,4-dioxane (2 mL) in an ice bath, 1 M NaOH (5 mL) and di-tert-butyl dicarbonate (685 mL, 2.98 mmol) reagents were added, and reaction continued for 24 h at room temperature. Then, the mixture was washed with 5% citric acid, distilled water, brine, and dried on anhydrous Na_2SO_4. The organic layer was concentrated and purified by silica gel chromatography using dichloromethane and methanol (20:1) as eluent. The compound 2 was obtained as oil in 86% yield. ^{1}H NMR (DMSO-d6, ppm) (Figure S3): 1.3-1.6 (m, 33H, -CH_2-, -NHCOOC$(CH_3)_3$); 1.7-1.8 (t, 2H, -N(COO)CH_2-); 2.8-3.1 (m, 5H, -CH_2NH-, -COCH-); 3.6 (s, 3H, -CH_3); 6.7 (t, 2H, -NHCOOC$(CH_3)_3$). HRMS (ESI+) (Figure S4): 526.2771 $[M + Na]^+$. (Calcd for $C_{24}H_{45}N_3O_8$, 503.3207).

Step 3: The compound 2 was dissolved in a mixture of MeOH/NaOH. After vigorously stirring at room temperature for 3 h, the mixture was neutralized by 1 M HCl and the solvents were evaporated, then washed twice with distilled water, saturated brine, dried on anhydrous Na_2SO_4 and concentrated to afford compound 3 as white powder in 61% yield. ^{1}H NMR (DMSO-d6, ppm) (Figure S5): 1.3-1.5 (m, 33H, -CH_2-, -NHCOOC$(CH_3)_3$); 1.7-1.8 (t, 2H, -NHCH_2-); 2.8-3.1 (m, 5H, -NHCH_2-, -OCOCH(NH)CH_2-); 6.7 (t, 2H, -NHCOOC$(CH_3)_3$); 12.6 (s, 1H, -COOH).

Step 4: DoGo1[41] (972.7 mg, 1.50 mmol) and compound 3 (452.4 mg, 1.37 mmol) were dissolved in dichloromethane (2.6 mL). After triethylamine (247 μL, 1.78 mmol) and EDCI (341.4 mg, 1.78 mmol) reagents were added, the reaction was performed under nitrogen atmosphere at room temperature for 24 h. The reaction solution was washed with 5% citric acid, distilled water, brine, and dried on anhydrous Na_2SO_4. The organic layer was concentrated and purified by silica gel chromatography using dichloromethane and methanol (15:1) as eluent. The N3 was obtained as oil after the evaporation of the purified solution into dryness. The yield was 59%. N3 was dissolved in hydrogen chloride-1,4-dioxane and the solution was vigorously stirred in an ice bath for 30 min to give N3 lipid as oil. ^{1}H NMR (DMSO-d6, ppm) of protected N3 (Figure S6): 0.8-0.9 (t, 6H, -CH_3); 1.1-1.4 (m, 83H, -CH_2-, -NHCOOC(CH_3)$_3$); 1.5-1.8 (m, 4H, -NHCOCH_2CH_2CH(NH_2)-); 2.0 (m, 8H, -CH_2CH=CHCH_2-); 3.0-3.2 (m, 8H, -CH_2NHCO-, -CH_2NHCOO(CH_3)$_3$); 4.3 (m, 2H, -COCH(NH)CH_2-); 5.4 (m, 4H, -CH=CH-); 6.7 (m, 2H, -NHCOOC(CH_3)$_3$); 7.8 (t, 2H, -CONH-). ^{13}C NMR (DMSO-d6, ppm) of N3 (Figure S7): 14.0 (q, 2C, -CH_3); 27.7 (t, 5C, -CH_2CH=CHCH_2-, -CH_2CH(NH)CO-); 32.4 (t, 1C, -COCH_2-); 39.2 (t, 2C, -CH_2NHCO-); 42.0 (t, 2C, -CH_2NH_2); 52.0 (t, 1C, -NHCH_2-); 61.0 (d,1C, -CH(NH)CO-); 73.0 (d, 1C, -COCH(NH)-); 130.0 (d, 4C, -CH=CH-);172.0 (s, 2C, -CO-). HRMS (ESI+) of N3 (Figure S8): $[M + Na]^+$: 817.7557. (Calcd for $C_{64}H_{120}N_6O_9$, 816.7544).

Synthesis of N4 lipid:

Step 1: Lysine methyl ester hydrochloride (300 mg, 1.0 mmol) and Alloc-OSu (171.9 μL, 1.11 mmol) were dissolved in dichloromethane (2. mL), and then triethylamine (350 mL, 2.52 mmol) reagent was added, followed by stirring at room temperature under nitrogen atmosphere for 24 h. After the completion of the reaction, the mixture was washed with 5% citric acid, distilled water, saturated brine and dried on anhydrous Na_2SO_4. The organic layer was concentrated and purified by silica gel chromatography using dichloromethane

and methanol (15:1) as eluent. The compound 4 was obtained as oil with the yield of 65%. HRMS (ESI+): $[M + Na]^+$ (Figure S9): 368.1895. **(**Calcd for $C_{16}H_{28}N_2O_6$**,** 344.1947**).**

Step 2: The compound 4 was dissolved in a mixture of MeOH/NaOH. After vigorously stirring at room temperature for 3 h, the mixture was neutralized by 1 M HCl and the solvents were evaporated, then washed twice with distilled water, saturated brine, dried on anhydrous Na_2SO_4 and concentrated to afford compound 5 as oil in 70% yield. ^{1}H NMR (DMSO-d6, ppm) (Figure S10): 1.3-1.7 (m, 15H, -CH_2-, -NHCOOC$(CH_3)_3$); 2.9-3.0 (m, 2H, -CH_2NHCOO$(CH_3)_3$); 3.7-3.9 (m, 1H, -COCH(NH)-); 4.5 (d, 2H, -OCH_2-); 5.2-5.4 (d, 2H, -CH=CH_2); 5.7-6.0 (m,1H, -CH=CH_2); 6.7-6.9 (t, 3H, -NHCOO$(CH_3)_3$); 7.5 (d, 1H, -NHCO-); 12.5 (s, 1H, -COOH).

Step 3: DoGo1 (650 mg, 1.0 mmol) and compound 5 (332 mg, 1.0 mmol) were dissolved in dichloromethane (2.6 mL), and triethylamine (208 μL, 1.55 mmol) and EDCI (297.30 mg，1.55 mmol) reagents were added, followed by stirring at room temperature under nitrogen atmosphere for 24 h. After the completion of the reaction, the mixture was washed with 5% citric acid, distilled water, saturated brine and dried on anhydrous Na_2SO_4. The organic layer was concentrated and purified by silica gel chromatography using dichloromethane and methanol (15:1) as eluent. The compound 6 was obtained as oil with the yield of 50%. The compound 6 was dissolved in a mixture of dichloromethane and trifluoroacetic acid (v/v=1/1), and vigorously stirred in room temperature for 30 min, the solvents were removed by evaporation and then washed with dichloromethane twice. Compound 6 was obtained as oil. ^{1}H NMR (DMSO-d6, ppm) (Figure S11): 0.8-0.9 (t, 6H, -CH_3); 1.1-1.4 (m, 63H, -CH_2-, -NHCOOC$(CH_3)_3$); 1.5-1.8 (m, 4H, -NHCOCH_2CH_2CH(NH_2)-); 2.0 (m, 8H, -CH_2CH=CHCH_2-); 3.0-3.2 (m, 6H, -CH_2NHCO-, -CH_2NHCOO$(CH_3)_3$); 3.9-4.3 (m, 2H, -COCH(NH)-, -COCH(NH)-); 4.5 (d, 2H, -OCH_2-); 5.3-5.5 (m, 6H, -CH=CH-, -CH=CH_2); 5.9 (m, 1H, -CH=CH_2); 6.7 (m, 1H, -NHCOOC$(CH_3)_3$);

7.3-8.0 (t, 4H, -CONH-). HRMS (ESI+): $[M + Na]^+$ (Figure S12): 980.7707. (Calcd for $C_{56}H_{103}N_5O_7$, 957.7858).

Step 4: Compound 6 (496.8 mg, 0.58 mmol) and compound 3 (283.6 mg, 0.58 mmol), triethylamine (120.4 μL, 0.87 mmol) and EDCI (144.4 mg，0.75 mmol) were dissolved in dichloromethane (2 mL), and the solution was stirred at room temperature under nitrogen atmosphere for 24 h, followed by washed with 5% citric acid, distilled water, saturated brine and dried on anhydrous Na_2SO_4. The product was purified with silica gel chromatography using dichloromethane and methanol (10:1) as eluent. The compound 7 was obtained in 45% yield. ^{1}H NMR (DMSO-d6, ppm) (Figure S13): 0.8-0.9 (t, 6H, $-CH_3$); 1.1-1.4 (m, 87H, $-CH_2-$, $-NHCOOC(CH_3)_3$); 1.5-1.8 (m, 4H, $-NHCOCH_2CH_2CH(NH_2)-$); 2.0 (m, 8H, $-CH_2CH=CHCH_2-$); 3.0-3.2 (m, 10H, $-CH_2NHCO-$, $-CH_2NHCOO(CH_3)_3$); 3.9-4.3 (m, 3H, -COCH(NH)-, -COCH(NH)-); 4.5 (d, 2H, $-OCH_2-$); 5.3-5.5 (m, 6H, $-CH=CH-$, $-CH=CH_2$); 5.9 (m, 1H, $-CH=CH_2$); 6.7 (m, 2H, $-NHCOOC(CH_3)_3$); 7.3-8.0 (t, 5H, -CONH-). HRMS (ESI+): $[M + Na]^+$ (Figure S14): 1353.0155. (Calcd for $C_{74}H_{136}N_8O_{12}$, 1329.0278).

Step 5: Compound 7 (160 mg, 0.12 mmol) was dissolved in tetrahydrofuran (1 mL), and then $Pd[P(C_6H_5)_3]_4$ (12 mg, 0.04 mmol) and $NaBH_4$ (9.1 mg, 0.24 mmol) reagents were added, and the mixture was vigorously stirred at room temperature under nitrogen atmosphere for 1 h. After the completion of the reaction, the mixture was washed with saturated $NaHCO_3$, saturated brine and dried on anhydrous Na_2SO_4. The mixture was purified by silica gel chromatography using dichloromethane and methanol (10:1) as eluent. The N4 was obtained as oil after the evaporation of the purified solution into dryness. The yield was 60%. N4 was dissolved in hydrogen chloride-1,4-dioxane solution vigorously stirring in an ice bath for 30 min, the solvents were removed by evaporation and obtained the N4 as oil. ^{1}H NMR (DMSO-d6, ppm) of protected N4 (Figure S15): 0.8-0.9 (t, 6H, $-CH_3$); 1.1-1.4 (m, 87H, $-CH_2-$, $-NHCOOC(CH_3)_3$); 1.5-1.8 (m, 6H, $-CH_2CH_2NH-$, $-NHCOCH_2CH_2CH(NH_2)-$); 2.0 (m, 8H, $-CH_2CH=CHCH_2-$); 3.0-3.2 (m, 10H, $-CH_2NHCO-$, -

$CH_2NHCOO(CH_3)_3$); 4.1-4.3 (m, 3H, -COCH(NH)-); 5.4 (m, 4H, -CH=CH-); 6.7 (m, 2H, -NHCOOC$(CH_3)_3$); 7.6-8.0 (t, 5H, -CONH-). ^{13}C NMR (DMSO-d6, ppm) of N4: 14.0 (q, 2C, -CH_3); 27.7 (t, 5C, -CH_2CH=CHCH_2-, -CH_2CH(NH)CO-); 32.0 (t, 1C, -COCH_2-); 39.2 (t, 2C, -CH_2NHCO-); 43.0 (t, 2C, -CH_2NH_2); 52.0 (t, 1C, -NHCH_2-); 61.0 (d, 2C, -CH(NH)CO-); 73.0 (d, 1C, -COCH(NH)-); 130.0 (d, 4C, -CH=CH-); 172.0 (s, 4C, -CO-). HRMS (ESI+) of N4 (Figure S17): $[M + H]^+$ 945.8519. (Calcd for $C_{55}H_{108}N_8O_4$, 944.8494).

Synthesis of N5 lipid:

N4 (110 mg, 0.09 mmol), 2-(Boc-amino) ethyl Bromide (783 mg, 3.5 mmol) and K_2CO_3 (1.8 g, 13.4 mmol) were dissolved in N,N-dimethylformamide (1.5 mL). The reaction was performed at 60°C for 6 h. After the completion of the reaction, the mixture was washed with 5% citric acid, distilled water, saturated brine and dried on anhydrous Na_2SO_4. The product was purified with silica gel chromatography using dichloromethane and methanol (10:1) as eluent. The N5 was obtained as oil after the evaporation of the purified solution into dryness. The yield was 40%. N5 was dissolved in hydrogen chloride-1,4-dioxane solution vigorously stirring in an ice bath for 30 min, the solvents were removed by evaporation and obtained the N5 as oil. ^{1}H NMR (DMSO-d6, ppm) of protected N5 (Figure S18): 0.8-0.9 (t, 6H, -CH_3); 1.1-1.4 (m, 100H, -CH_2-, -NHCOOC$(CH_3)_3$); 1.5-1.8 (m, 4H, -NHCOCH_2CH_2CH(NH_2)-); 2.0 (m, 8H, -CH_2CH=CHCH_2-); 3.0-3.2 (m, 12H, -CH_2NHCO-, -CH_2NHCOO$(CH_3)_3$); 4.1-4.3 (m, 3H, -COCH(NH)-); 5.4 (m, 4H, -CH=CH-); 6.7 (m, 3H, -NHCOOC$(CH_3)_3$); 7.6-8.0 (t, 4H, -CONH-); 8.7-8.9(t, 1H, -CONH-). ^{13}C NMR (DMSO-d6, ppm) of N5 (Figure S19): 14.0 (q, 2C, -CH_3); 27.7 (t, 5C, -CH_2CH=CHCH_2-, -CH_2CH(NH)CO-); 32.0 (t, 1C, -COCH_2-); 39.2 (t, 2C, -CH_2NHCO-); 43.0 (t, 3C, -CH_2NH_2); 52.0 (t, 2C, -NHCH_2-); 61.0 (d, 2C, -CH(NH)CO-); 73.0 (d, 1C, -COCH(NH)-); 130.0 (d, 4C, -CH=CH-); 172.0 (s, 4C, -CO-). HRMS (ESI+) of N5 (Figure S20): $[M + H]^+$ 988.8965. (Calcd for $C_{57}H_{113}N_9O_4$, 987.8916).

Synthesis of N6 lipid:

DoGo2[41] (155.2 mg, 0.20 mmol) and compound 3 (94.6 mg, 0.41 mmol) were dissolved in dichloromethane (2 mL), and then triethylamine (65 μL, 0.47 mmol) and EDCI (90 mg，0.47 mmol) reagents were added, and the mixture was stirred at room temperature under nitrogen atmosphere for 24 h. The reaction mixture was then washed with 5% citric acid, distilled water, saturated brine and dried on anhydrous Na_2SO_4. The organic layer was concentrated and purified by silica gel chromatography using dichloromethane and methanol (10:1) as eluent. The N6 was obtained as oil after the evaporation of the purified solution into dryness. The yield was 65%. N6 lipid was dissolved in hydrogen chloride-1,4-dioxane solution and was vigorously stirred in an ice bath for 30 min, the solvents were removed by evaporation and the N6 was obtained as oil. ^{1}H NMR (DMSO-d6, ppm) of protected N6 (Figure S21): 0.8-0.9 (t, 6H, -CH_3); 1.1-1.4 (m, 118H, -CH_2-, -NHCOOC$(CH_3)_3$); 1.5-2.2 (m, 12H, -NHCOCH_2CH_2CH(NH_2)-, -CH_2CH=CHCH_2-); 2.8-3.5 (m, 14H, -CH_2NH-, -CH_2NHCOOC$(CH_3)_3$); 4.0-4.5 (m, 4H, -COCH(NH)CH_2-, -COCH(NH_2)-); 5.4 (m, 4H, -CH=CH-); 6.6-6.9 (t, 4H, -NHCOOC$(CH_3)_3$); 7.8-8.1 (t, 4H, -CONH-). ^{13}C NMR (DMSO-d6, ppm) of N6 (Figure S22): 14.0 (q, 2C, -CH_3); 27.7 (t, 5C, -CH_2CH=CHCH_2-, -CH_2CH(NH)CO-); 32.0 (t, 1C, -COCH_2-); 39.2 (t, 2C, -CH_2NHCO-); 43.0 (t, 4C, -CH_2NH_2); 52.0 (t, 2C, -NHCH_2-); 61.0 (d, 2C, -CH(NH)CO-); 73.0 (d, 1C, -COCH(NH)-); 130.0 (d, 4C, -CH=CH-); 172.0 (s, 6C, -CO-). HRMS (ESI+) of N6 (Figure S23): $[M + H]^+$: 1102.9760. (Calcd for $C_{62}H_{123}N_{11}O_5$, 1101.9709).

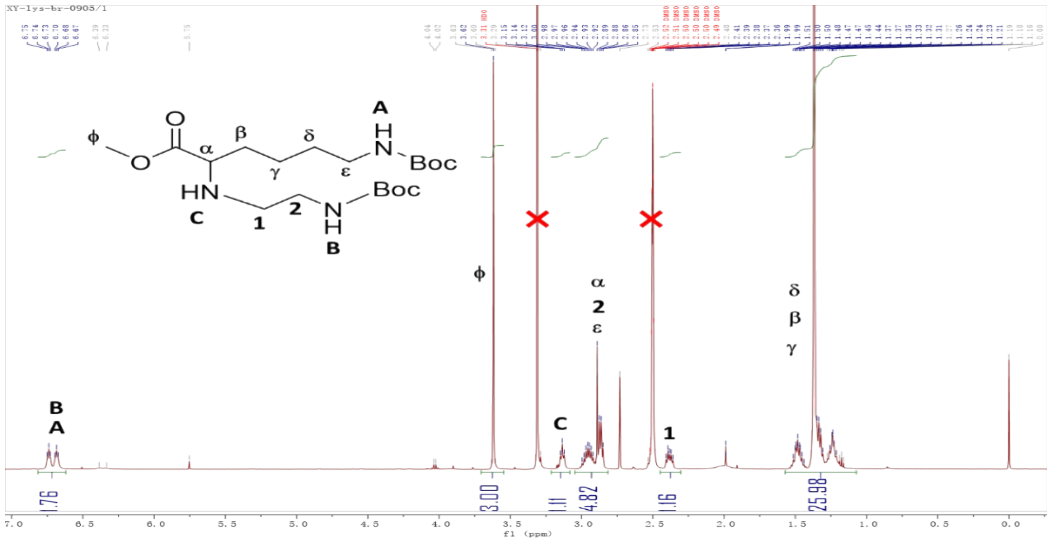

Figure S1 ^{1}H NMR spectrum of compound 1 (Solvent: DMSO-d6).

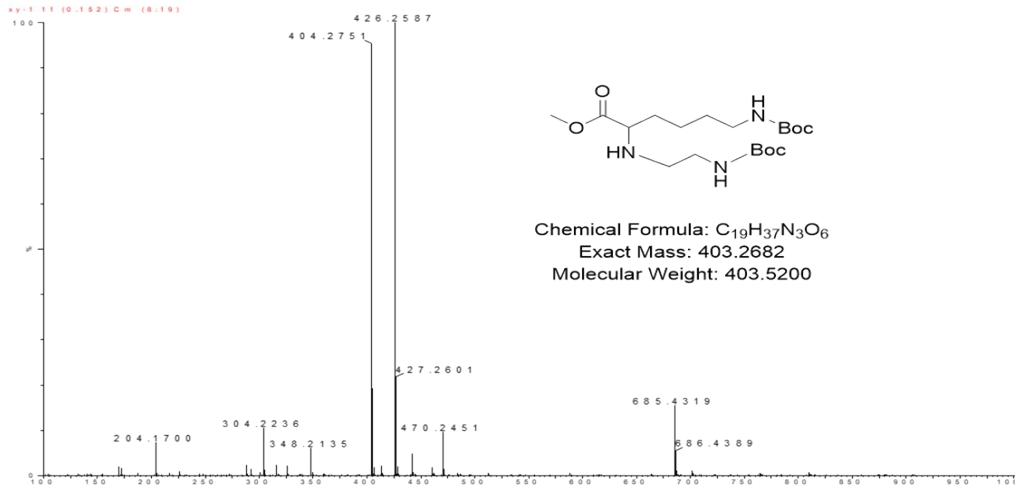

Figure S2 ~~19~~ Mass spectrum of compound 1.

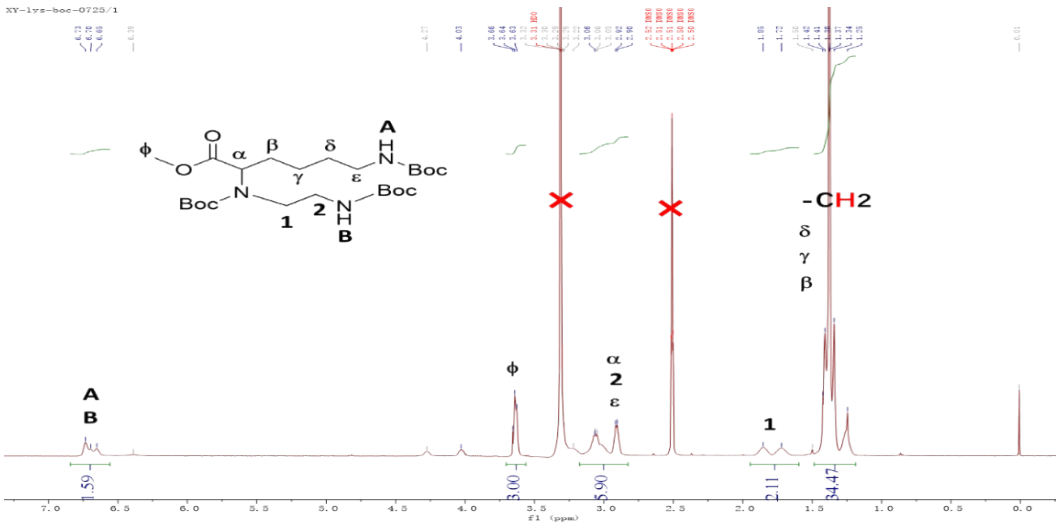

Figure S3 ^{1}H NMR spectrum of compound 2 (Solvent: DMSO-d6).

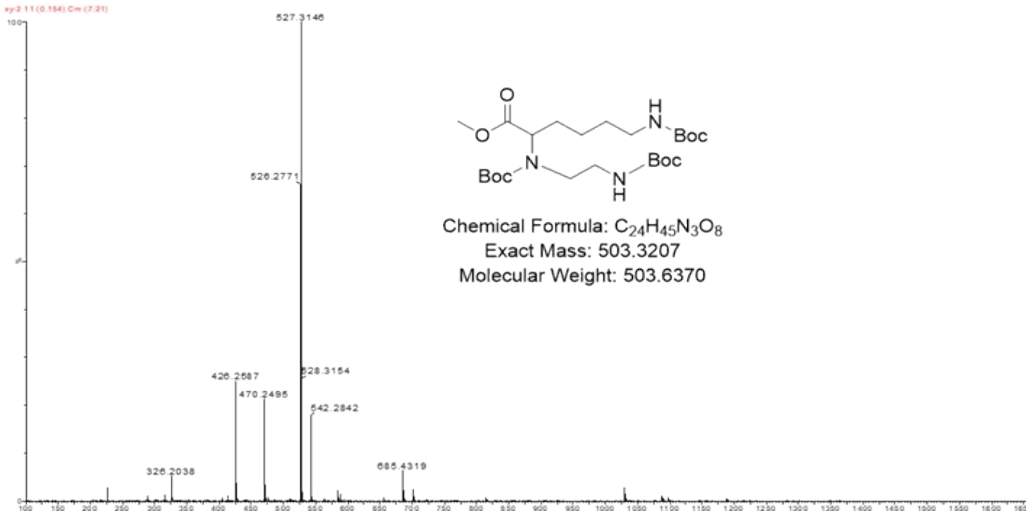

Figure S4 Mass spectrum of compound 2.

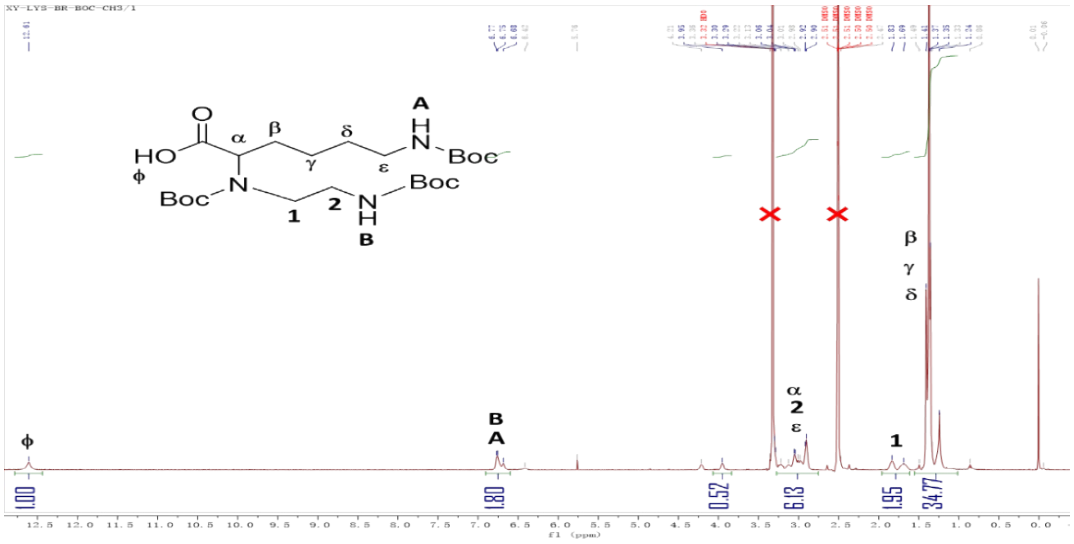

Figure S5 ^{1}H NMR spectrum of compound 3 (Solvent: DMSO-d6).

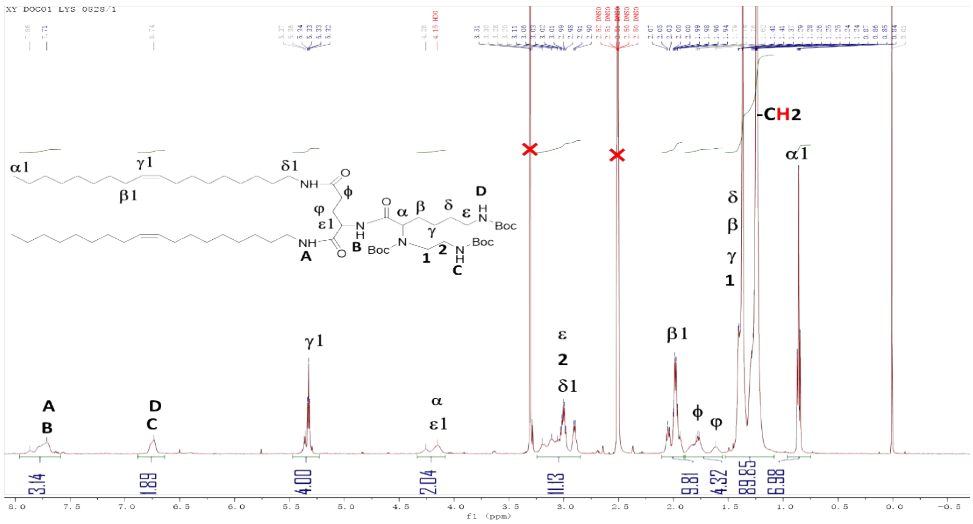

Figure S6 ^{1}H NMR spectrum of N3 (Solvent: DMSO-d6).

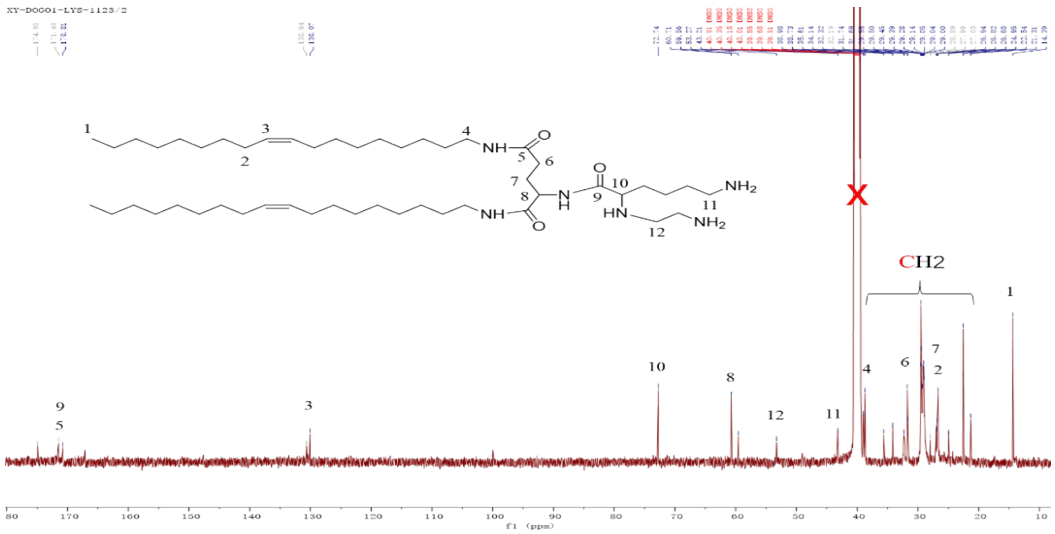

Figure S7 ^{13}C NMR spectrum of N3 (Solvent: DMSO-d6).

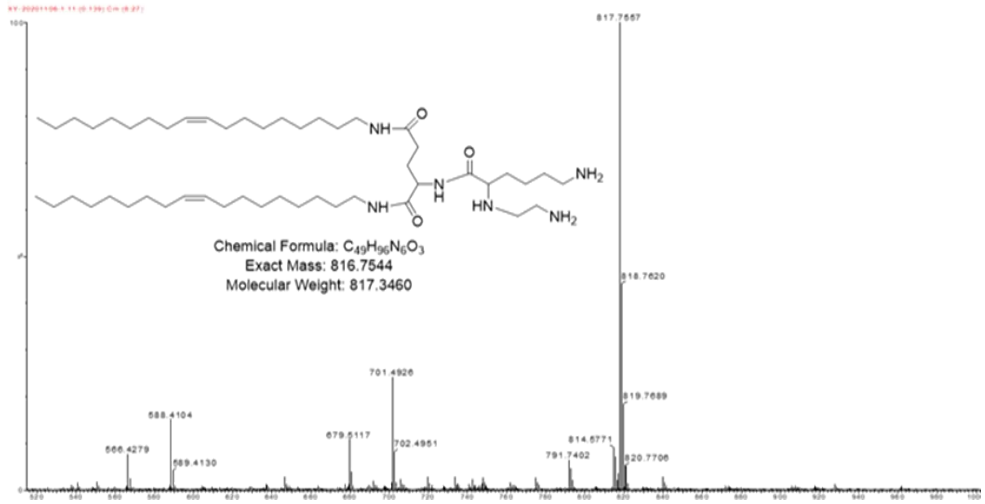

Figure S8 Mass spectrum of N3.

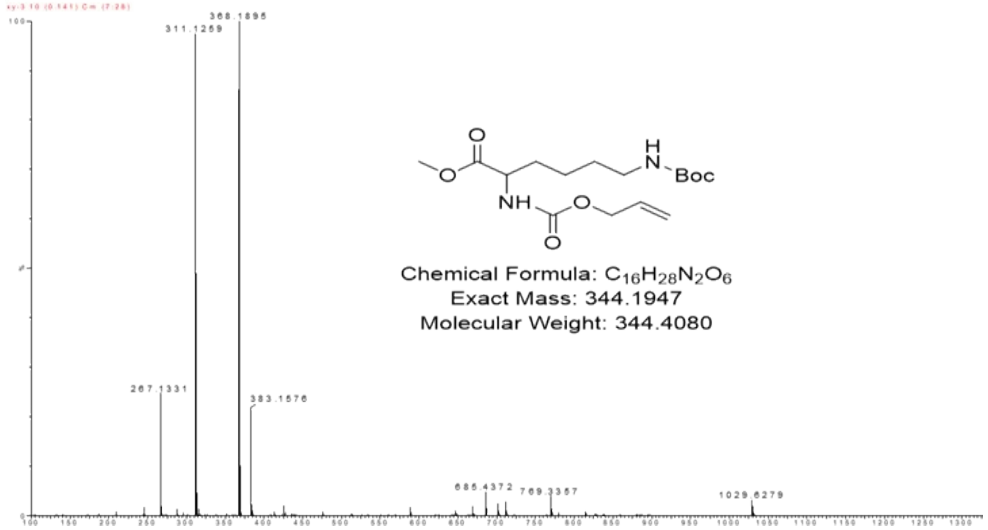

Figure S9 Mass spectrum of compound 4.

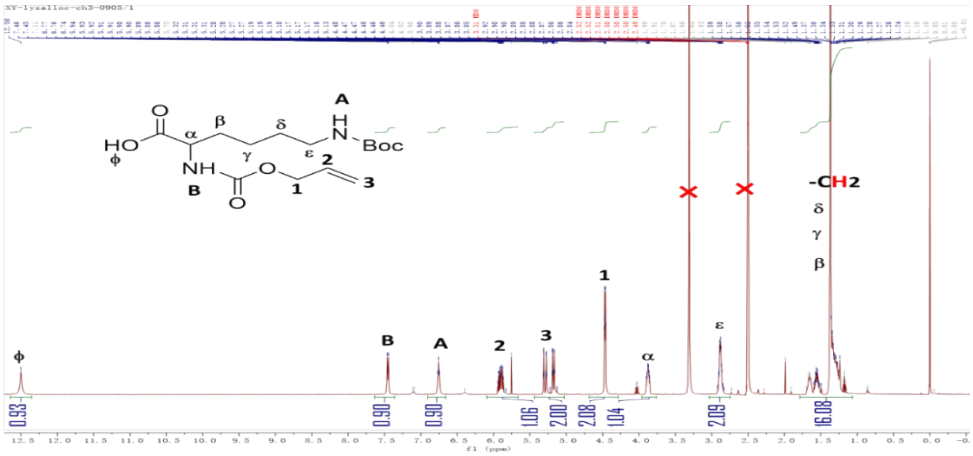

Figure S10 ^{1}H NMR spectrum of compound 5 (Solvent: DMSO-d6).

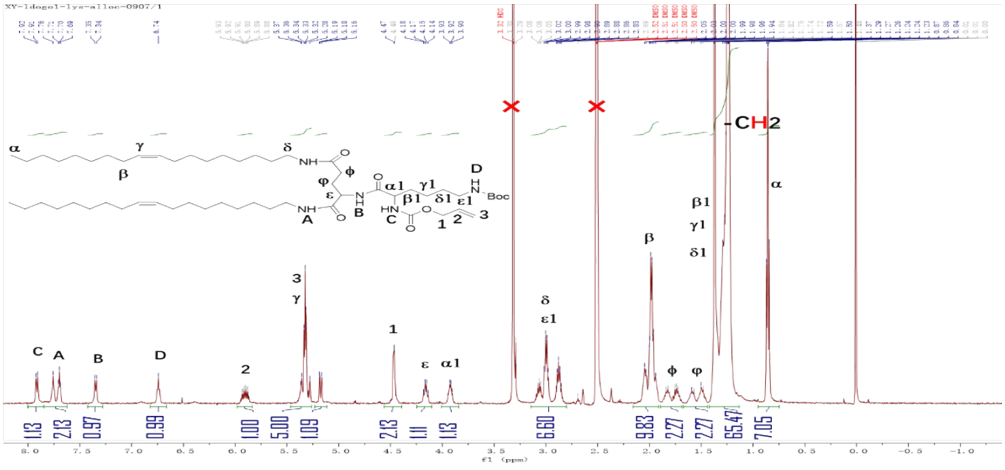

Figure S11 ^{1}H NMR spectrum of compound 6 (Solvent: DMSO-d6).

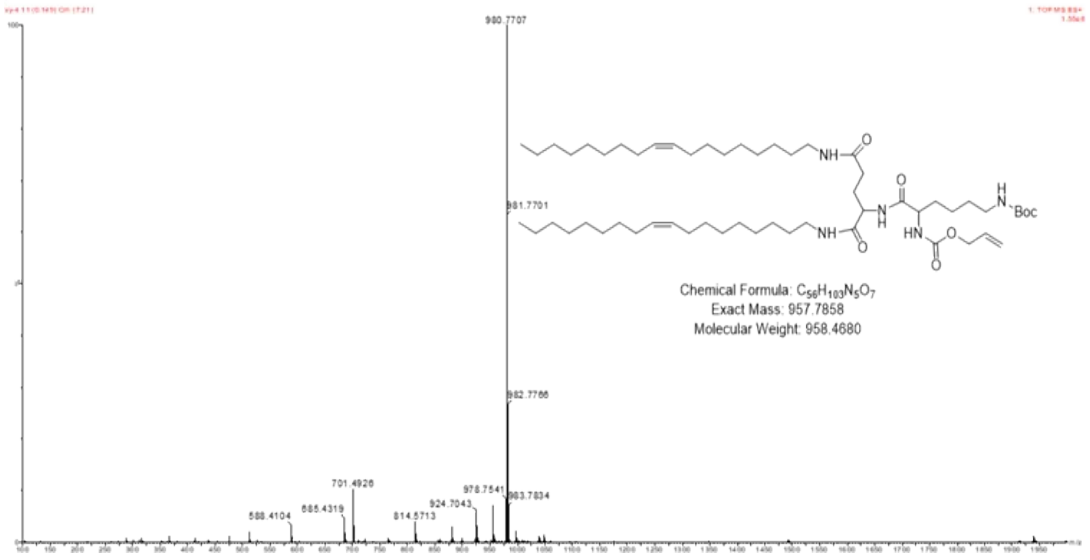

Figure S12 Mass spectrum of compound 6.

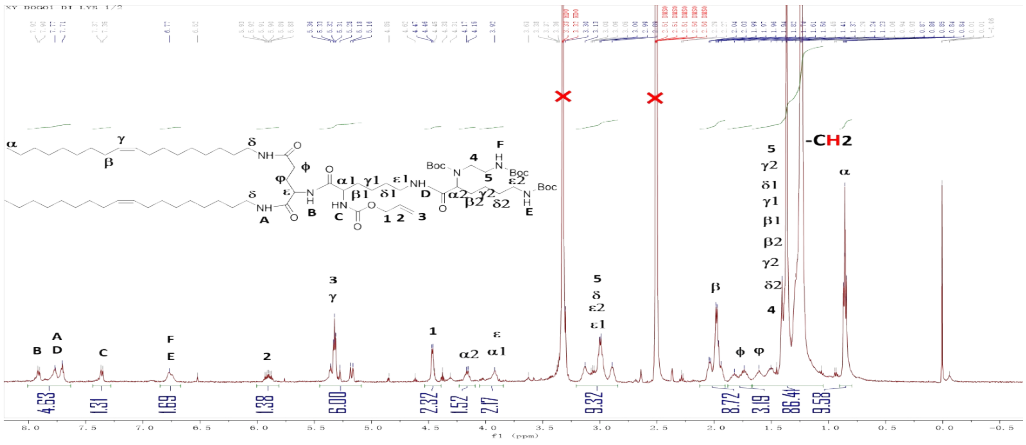

Figure S13 ^{1}H NMR spectrum of compound 7 (Solvent: DMSO-d6).

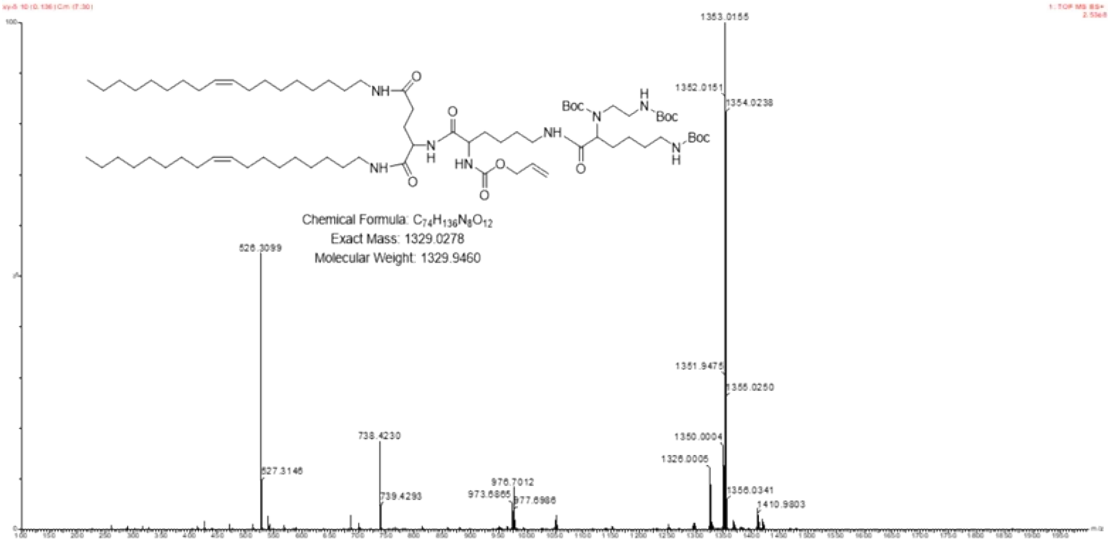

Figure S14 Mass spectrum of compound 7.

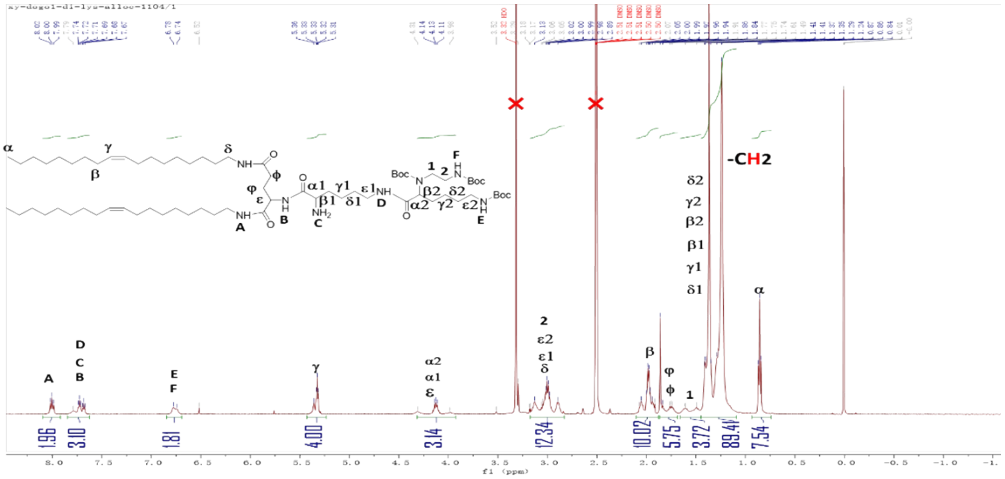

Figure S15 ^{1}H NMR spectrum of N4 (Solvent: DMSO-d6).

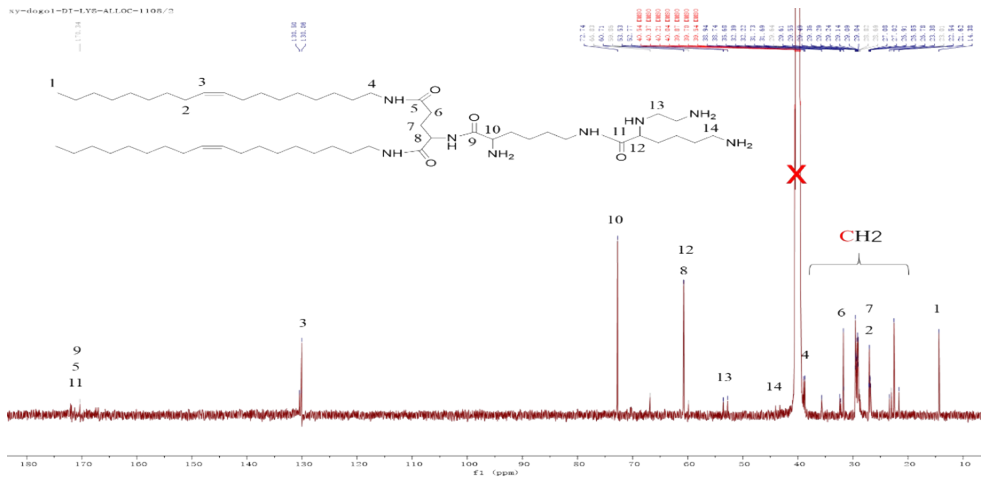

Figure S16 ^{13}C NMR spectrum of N4 (Solvent: DMSO-d6).

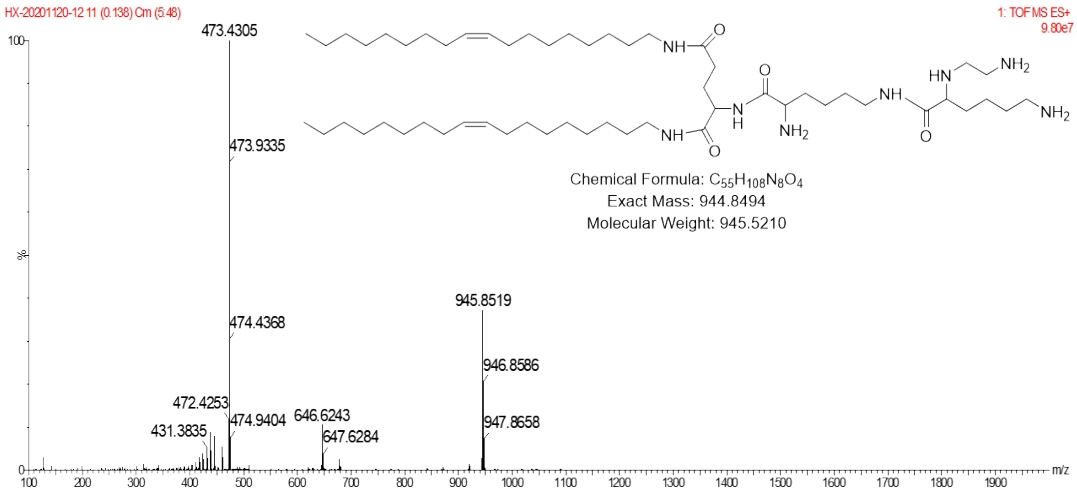

Figure S17 Mass spectrum of N4.

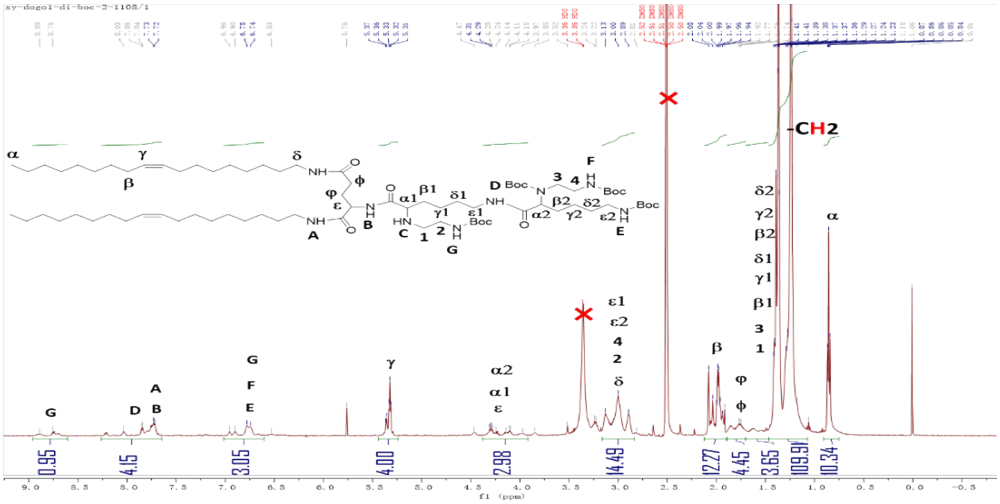

Figure S18 ^{1}H NMR spectrum of N5 (Solvent: DMSO-d6).

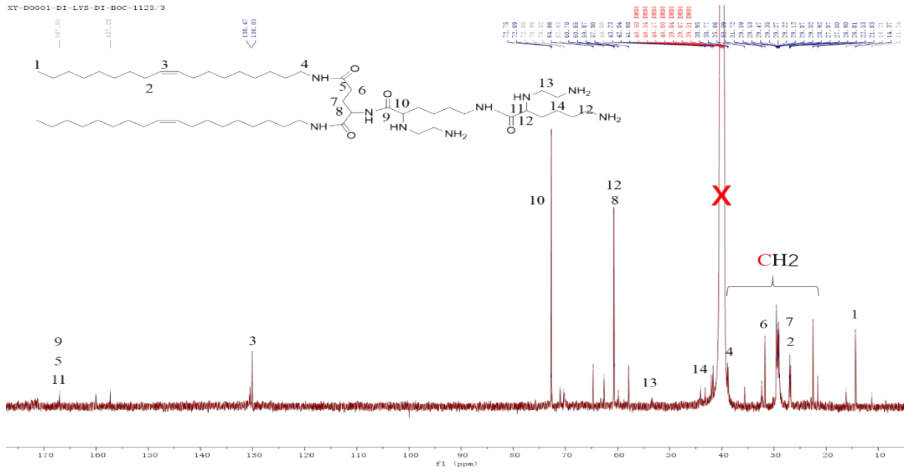

Figure S19 ^{13}C NMR spectrum of N5 (Solvent: DMSO-d6).

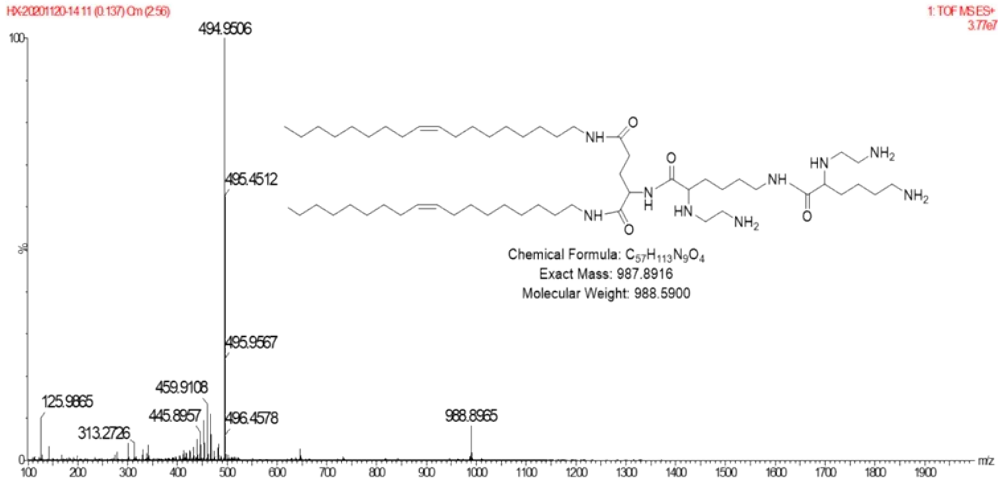

Figure S20 Mass spectrum of N5.

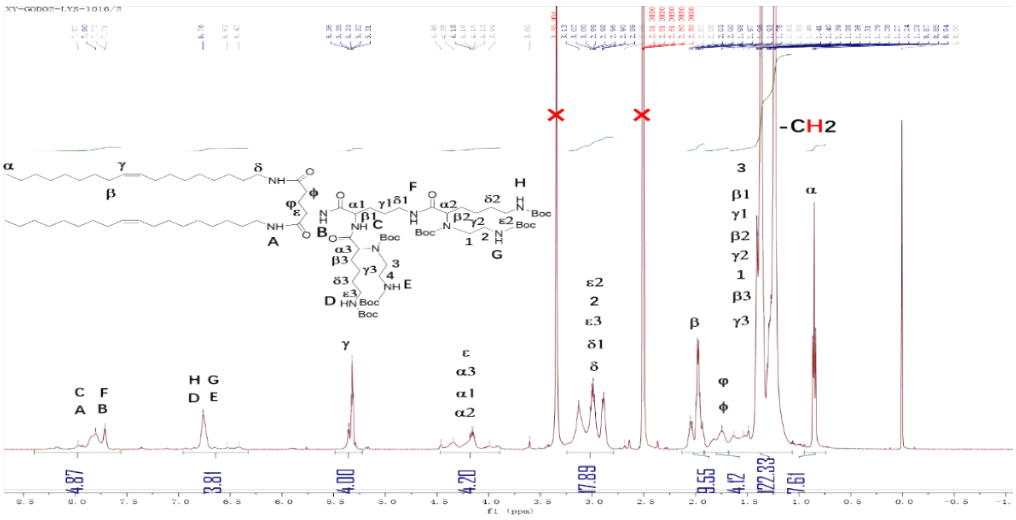

Figure S21 ^{1}H NMR spectrum of N6 (Solvent: DMSO-d6).

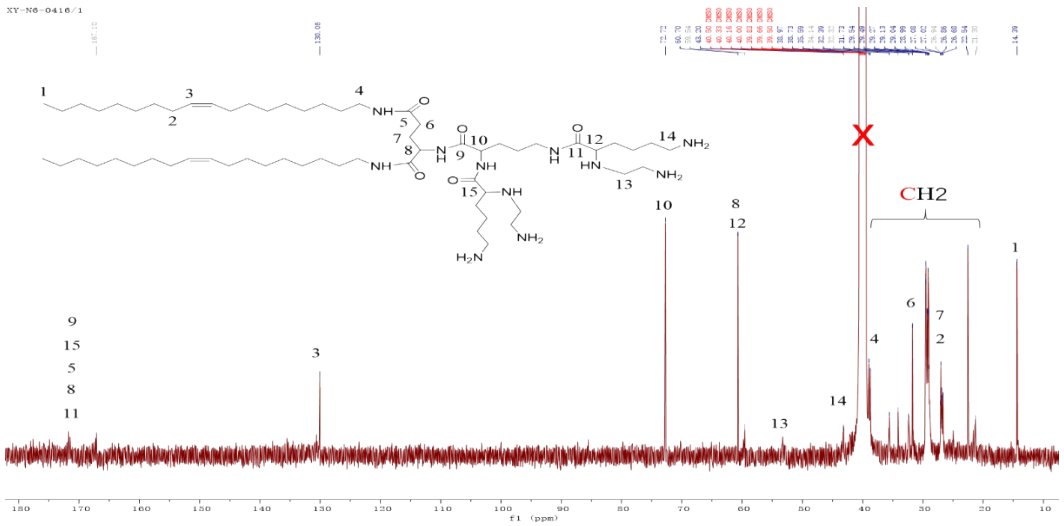

Figure S22 ^{13}C NMR spectrum of N6 (Solvent: DMSO-d6).

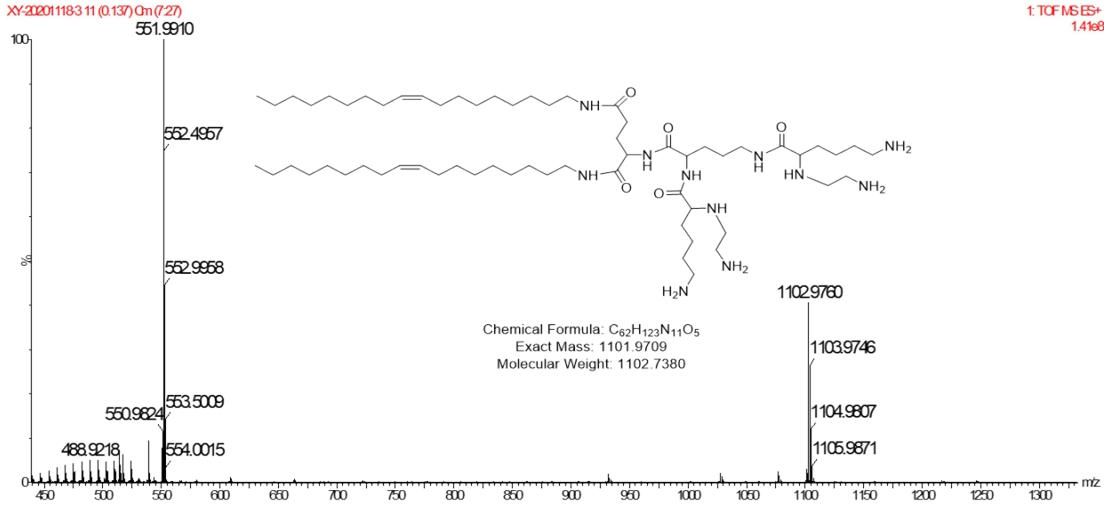

Figure S23 Mass spectrum of N6.