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Supporting Information for

Development of tertiary amine cationic lipids achieves safe and efficient siRNA delivering

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Abbreviations used:

EDCI: 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride

HOBt: N-Hydroxybenzotriazole

TEA: Triethylamine

DCM: Dichloromethane.

THF: Tetrahydrofuran

MTT: Methylthiazolyldiphenyl-tetrazolium bromide

FDA: US Food and Drug Administration

PMSF: Phenylmethanesulfonyl fluoride

DOPE: 2-dioleoyl-sn-glycero-3-phosphoethanolamine

LDL-c: Low Density Lipoprotein cholesterol

TCHO: Total Cholesterol

HDL-c: High Density Lipoprotein cholesterol

TG: Total triglyceride

AST: Aspartate aminotransferase

ALT: Alanine aminotransferase

CREA: Creatinine

BUN: Urea Nitrogen

Method Section

Synthesis of tertiary amine-based cationic lipids

1.1. ditetradecyl glutamate (**a**)

A stirred solution of L-glutamic acid (20.0 g, 0.14 mol) in toluene (500 mL) was added with *p*-toluenesulfonic acid (28.5 g, 0.15 mol), followed by refluxing for 3 h in an ice bath. Then, the stirred solution was added with myristyl alcohol (61.2 g, 0.29 mol) and refluxed overnight. The solution was evaporated and the residue was dissolved in $CHCl_3$ (400 mL). The organic phase was sequentially washed with saturated $NaHCO_3$ solution, brine and dried over anhydrous Na_2SO_4, followed by filtration and concentration. The residue was recrystallized in methanol (300 mL) for twice and dried to obtain the compound **a** as white solid (40.8 g, 56% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 4.11 (t, *J* = 6.8 Hz, 2H, COOCH_2), 4.07 (t, *J* = 6.8 Hz, 2H, COOCH_2), 3.52 – 3.43 (m, 1H, NH_2C*H*), 2.46 (t, *J* = 7.6 Hz, 2H, CH_2CO), 2.15 – 2.01 (m, 1H, NH_2CHCH_2), 1.92 – 1.80 (m, 1H, NH_2CHCH_2), 1.68 – 1.58 (m, 4H, $COOCH_2CH_2$), 1.33 – 1.23 (m, 44H, C$H_{2\ (myristoyl)}$), 0.88 (t, *J* = 6.9 Hz, 6H, CH_2CH_3). HRMS, ESI^+, m/z: Calcd for $C_{33}H_{66}NO_4$ $[M+H]^+$, 540.4914; found, 540.5006.

1.2. 4-((1,5-dioxo-1,5-bis(tetradecyloxy)pentan-2-yl)amino)-4-oxobutanoic acid (**b**)

A stirred solution of compound **a** (40.8 g, 75.6 mmol) in THF/DCM (200 mL/200 mL) was added with succinic anhydride (11.3 g, 113.3 mmol) and reacted at room temperature overnight. The solution was evaporated and the residue was recrystallized in methanol (150 mL) for twice and dried to obtain the compound **b** as white solid (40.2 g, 83% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 6.62 (d, *J* = 7.8 Hz, 1H, N*H*), 4.66 – 4.56 (m, 1H, NHC*H*), 4.13 (t, *J* = 6.8 Hz, 2H, COOCH_2), 4.06 (t, *J* = 6.8 Hz, 2H, COOCH_2), 2.75 – 2.65 (m, 2H, COOHCH_2), 2.56 (t, *J* = 6.5 Hz, 2H, CH_2COO), 2.46 – 2.33 (m, 2H, CH_2CONH), 2.25 – 2.13 (m, 1H, NHCHCH_2), 2.07 – 1.93 (m, 1H, NHCHCH_2), 1.68 – 1.56 (m, 4H, $COOCH_2CH_2$), 1.33 – 1.23 (m, 44H, C$H_{2\ (myristoyl)}$), 0.88 (t, *J* = 6.7 Hz, 6H, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 176.27 (1C, *C*OOH), 173.08 (1C, *C*ONHCH), 171.92 (1C, CH_2COOCH_2), 171.79

(1C, NHCH*C*O), 65.94 (1C, COO*C*H_2), 65.07 (1C, COO*C*H_2), 51.91 (1C, NH*C*H), 31.90 (2C, *C*$H_2$$CH_2$$CH_3$), 30.56 (1C, *C*$H_2COOCH_2$), 30.28 (1C, COOH*C*$H_2$), 29.63 (8C, *C*$H_{2(myristoyl)}$), 29.57 (2C, *C*$H_{2(myristoyl)}$), 29.51 (1C, *C*$H_{2(myristoyl)}$), 29.48 (1C, *C*$H_{2(myristoyl)}$), 29.33 (2C, *C*$H_{2(myristoyl)}$, 1C, NHCH*C*$H_2$), 29.25 (1C, *C*$H_{2(myristoyl)}$), 29.19 (1C, *C*$H_{2(myristoyl)}$), 28.56 (1C, OCH$_2$*C*$H_2$), 28.47 (1C, OCH$_2$*C*$H_2$), 27.37 (1C, COOHCH$_2$*C*$H_2$), 25.87 (1C, OCH$_2CH_2$*C*$H_2$), 25.79 (1C, OCH$_2CH_2$*C*$H_2$), 22.65 (2C, *C*$H_2$$CH_3$), 14.06 (2C, CH_2*C*H_3). HRMS, ESI$^+$, m/z: Calcd for $C_{37}H_{70}NO_7$ $[M+H]^+$, 640.5074; found, 640.5127.

1.3. General procedure - synthesis of compounds **TA1-TA21**

A stirred solution of compound **b** (500 mg, 0.78 mmol) in $CHCl_3$ (50 mL) was added with EDCI (240 mg, 1.25 mmol) and HOBt (170 mg, 1.25 mmol) in an ice bath. After stirring for 3 h at room temperature, the solution was added with appropriate primary amine (0.8 mmol) and TEA (330 μL, 2.35 mmol). The reaction mixture was stirred at room temperature for 12 h, and washed with water, 10% citric acid solution and brine successively and dried over anhydrous Na_2SO_4, followed by filtration and concentration. The residue was purified by silica gel column chromatography eluting with appropriate mixture as indicated in each case.

*1.3.1. ditetradecyl (4-((2-(dimethylamino)ethyl)amino)-4-oxobutanoyl)glutamate (**TA1**)*

DCM/methanol (20/1). Yellow granular solid. 39% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.99 (brs, 1H, N*H*COCH$_2$), 7.38 (d, *J* = 7.5 Hz, 1H, CON*H*CH), 4.59 – 4.47 (m, 1H, NHC*H*), 4.10 (t, *J* = 6.6 Hz, 2H, COOC*H*$_2$), 4.05 (t, *J* = 6.7 Hz, 2H, COOC*H*$_2$), 3.68 (brs, 2H, CH$_2$C*H*$_2$NH), 3.31 (brs, 2H, C*H*$_2$CH$_2$NH), 2.92 (s, 6H, (C*H*$_3$)$_2$N), 2.80 – 2.66 (m, 2H, NHCOC*H*$_2$), 2.65 – 2.57 (m, 2H, C*H*$_2$COO), 2.48 – 2.38 (m, 2H, C*H*$_2$CONH), 2.22 – 2.11 (m, 1H, NHCHC*H*$_2$), 2.08 – 1.97 (m, 1H, NHCHC*H*$_2$), 1.67 – 1.57 (m, 4H, COOCH$_2$C*H*$_2$), 1.33 – 1.24 (m, 44H, C*H*$_{2\ (myristoyl)}$), 0.88 (t, *J* = 6.8 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 174.13 (1C, NH*C*OCH$_2$), 173.17 (1C, *C*ONHCH), 172.96 (1C, CH$_2$*C*OOCH$_2$), 172.28 (1C, NHCH*C*O), 65.79 (1C, COO*C*H_2), 64.97 (1C, COO*C*H_2), 57.35 (1C, $(CH_3)_2$N*C*H_2),

51.95 (1C, NH*C*H), 43.65 (2C, (CH$_3$)$_2$N), 31.92 (2C, CH$_2$CH$_2$CH$_3$), 31.35 (1C, (CH$_3$)$_2$NCH$_2$$CH_2$), 31.00 (1C, CH$_2$COOCH$_2$), 30.38 (1C, NHCOCH$_2CH_2$), 29.71 (8C, CH$_{2(myristoyl)}$), 29.66 (2C, CH$_{2(myristoyl)}$), 29.58 (2C, CH$_{2(myristoyl)}$), 29.36 (4C, CH$_{2(myristoyl)}$, 1C, NHCHCH$_2$), 28.58 (1C, OCH$_2$$CH_2$), 28.49 (1C, OCH$_2$$CH_2$), 27.00 (1C, COCH$_2$$CH_2$CO), 25.91 (1C, OCH$_2CH_2$$CH_2$), 25.88 (1C, OCH$_2CH_2$$CH_2$), 22.68 (2C, CH$_2CH_3$), 14.10 (2C, CH$_2$$CH_3$). HRMS, ESI$^+$, m/z: Calcd for $C_{41}H_{80}N_3O_6$ [M+H]$^+$, 710.5969; found, 710.5986.

*1.3.2. ditetradecyl (4-((3-(dimethylamino)propyl)amino)-4-oxobutanoyl)glutamate (**TA2**)*

DCM/methanol (30/1). Yellow-white granular solid. 51% yield. ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm) 7.41 (brs, 1H, N*H*COCH$_2$), 6.96 (d, *J* = 7.2 Hz, 1H, CON*H*CH), 4.55 – 4.51 (m, 1H, NHC*H*), 4.11 (t, *J* = 6.7 Hz, 2H, COOC*H*$_2$), 4.05 (t, *J* = 6.7 Hz, 2H, COOC*H*$_2$), 3.41 – 3.32 (m, 2H, (CH$_3$)$_2$NCH$_2$CH$_2$C*H*$_2$), 2.88 (t, *J* = 6.7 Hz, 2H, NHCOC*H*$_2$), 2.62 (s, 6H, (C*H*$_3$)$_2$N), 2.59 – 2.49 (m, 2H, C*H*$_2$COO， 2H, (CH$_3$)$_2$NC*H*$_2$), 2.44 – 2.33 (m, 2H, C*H*$_2$CONH), 2.24 – 2.11 (m, 1H, NHCHC*H*$_2$), 2.07 – 1.96 (m, 1H, NHCHC*H*$_2$), 1.96 – 1.89 (m, 2H, (CH$_3$)$_2$NCH$_2$C*H*$_2$), 1.67 – 1.56 (m, 4H, COOCH$_2$C*H*$_2$), 1.34 – 1.22 (m, 44H, C*H*$_{2(myristoyl)}$), 0.88 (t, *J* = 6.8 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (75 MHz, CDCl$_3$): δ (ppm) 172.82 (1C, NH*C*OCH$_2$), 172.58 (1C, *C*ONHCH), 172.24 (1C, CH$_2$*C*OOCH$_2$), 171.93 (1C, NHCH*C*O), 65.69 (1C, COO*C*H$_2$), 64.89 (1C, COO*C*H$_2$), 56.43 (1C, (CH$_3$)$_2$N*C*H$_2$CH$_2$CH$_2$), 51.85 (1C, NH*C*H), 43.78 (2C, (*C*H$_3$)$_2$N), 37.08 (1C, (CH$_3$)$_2$NCH$_2$CH$_2$*C*H$_2$), 31.86 (2C, *C*H$_2$CH$_2$CH$_3$), 31.37 (1C, *C*H$_2$COOCH$_2$), 30.31 (1C, NHCO*C*H$_2$CH$_2$), 29.60 (8C, *C*H$_{2(myristoyl)}$), 29.55 (2C, *C*H$_{2(myristoyl)}$), 29.49 (1C, *C*H$_{2(myristoyl)}$), 29.46 (1C, *C*H$_{2(myristoyl)}$), 29.29 (2C, *C*H$_{2(myristoyl)}$, 1C, NHCH*C*H$_2$), 29.23 (1C, *C*H$_{2(myristoyl)}$), 29.18 (1C, *C*H$_{2(myristoyl)}$), 28.56 (1C, OCH$_2$*C*H$_2$), 28.47 (1C, OCH$_2$*C*H$_2$), 27.26 (1C, COCH$_2$*C*H$_2$CO), 25.86 (1C, OCH$_2$CH$_2$*C*H$_2$), 25.78 (1C, OCH$_2$CH$_2$*C*H$_2$), 25.03 (1C, (CH$_3$)$_2$NCH$_2$*C*H$_2$), 22.62 (2C, *C*H$_2$CH$_3$), 14.04 (2C, CH$_2$*C*H$_3$). HRMS, ESI$^+$, m/z: Calcd for $C_{42}H_{82}N_3O_6$ [M+H]$^+$, 724.6125; found, 724.6164.

*1.3.3. ditetradecyl (4-((2-(diethylamino)ethyl)amino)-4-oxobutanoyl)glutamate (**TA3**)*

DCM/methanol (30/1). Yellow-white solid. 41% yield. ^{1}H NMR (300 MHz,

$CDCl_3$): δ (ppm) 6.79 (d, *J* = 7.2 Hz, 1H, CON*H*CH), 6.52 (brs, 1H, N*H*$COCH_2$), 4.63 – 4.53 (m, 1H, NHC*H*), 4.12 (t, *J* = 6.8 Hz, 2H, $COOCH_2$), 4.05 (t, *J* = 6.8 Hz, 2H, $COOCH_2$), 3.38 – 3.28 (m, 2H, $(CH_3CH_2)_2NCH_2CH_2$), 2.63 – 2.54 (m, 6H, $(CH_3CH_2)_2NCH_2$, 2H, $NHCOCH_2CH_2$), 2.49 – 2.30 (m, 2H, CH_2CONH, 2H, CH_2COO), 2.26 – 2.12 (m, 1H, $NHCHCH_2$), 2.05 – 1.91 (m, 1H, $NHCHCH_2$), 1.70 – 1.55 (m, 4H, $COOCH_2CH_2$), 1.40 – 1.20 (m, 44H, $CH_{2(myristoyl)}$), 1.04 (t, *J* = 7.2 Hz, 6H, CH_2CH_3), 0.88 (t, *J* = 6.8 Hz, 6H, $CH_2CH_{3\ (myristoyl)}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 172.83 (1C, $NHCOCH_2$), 172.11 (1C, *C*ONHCH), 171.89 (1C, CH_2COOCH_2, 1C, NHCH*C*O), 65.72 (1C, $COOCH_2$), 64.91 (1C, $COOCH_2$), 51.77 (1C, NH*C*H), 51.47 (1C, $(CH_3CH_2)_2NCH_2$), 46.70 (1C, $(CH_3CH_2)_2NCH_2CH_2$), 36.78 (2C, CH_2CH_3), 31.91 (2C, $CH_2CH_2CH_3$), 31.52 (1C, CH_2COOCH_2), 30.30 (1C, $COCH_2CH_2CO$), 29.64 (8C, $CH_{2(myristoyl)}$), 29.58 (2C, $CH_{2(myristoyl)}$), 29.52 (1C, $CH_{2(myristoyl)}$), 29.49 (1C, $CH_{2(myristoyl)}$), 29.34 (2C, $CH_{2(myristoyl)}$, 1C, $NHCHCH_2$), 29.27 (1C, $CH_{2(myristoyl)}$), 29.21 (1C, $CH_{2(myristoyl)}$), 28.59 (1C, OCH_2CH_2), 28.49 (1C, OCH_2CH_2), 27.44 (1C, $COCH_2CH_2CO$), 25.89 (1C, $OCH_2CH_2CH_2$), 25.80 (1C, $OCH_2CH_2CH_2$), 22.67 (2C, $CH_{2(myristoyl)}CH_3$), 14.09 (2C, $CH_2CH_{3(myristoyl)}$), 11.33 (2C, CH_2CH_3). HRMS, ESI^+, m/z: Calcd for $C_{43}H_{83}N_3O_6Na$ $[M+Na]^+$, 760.6180; found, 760.6196.

1.3.4. ditetradecyl (4-((3-(diethylamino)propyl)amino)-4-oxobutanoyl)glutamate ***(TA4)***

DCM/methanol (40/1). White solid. 48% yield. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.58 (t, *J* = 5.8 Hz, 1H, N*H*$COCH_2$), 7.03 (d, *J* = 7.5 Hz, 1H, CON*H*CH), 4.58 – 4.47 (m, 1H, NHC*H*), 4.11 (t, *J* = 6.8 Hz, 2H, $COOCH_2$), 4.05 (t, *J* = 6.8 Hz, 2H, $COOCH_2$), 3.46 – 3.33 (m, 2H, $(CH_3CH_2)_2NCH_2CH_2CH_2$), 3.20 – 3.03 (m, 4H, $(CH_3CH_2)_2NCH_2$, 2H, $(CH_3CH_2)_2NCH_2$), 2.69 – 2.48 (m, 2H, CH_2COO, 2H, $NHCOCH_2CH_2$), 2.47 – 2.32 (m, 2H, CH_2CONH), 2.24 – 2.12 (m, 1H, $NHCHCH_2$), 2.12 – 1.92 (m, 1H, $NHCHCH_2$, 2H, $(CH_3CH_2)_2NCH_2CH_2$), 1.70 – 1.54 (m, 4H, $COOCH_2CH_2$), 1.37 (t, *J* = 7.2 Hz, 6H, CH_2CH_3), 1.33 – 1.23 (m, 44H, $CH_{2(myristoyl)}$), 0.88 (t, *J* = 6.9 Hz, 6H, $CH_2CH_{3(myristoyl)}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 173.03 (1C, $NHCOCH_2$), 172.83 (1C, *C*ONHCH), 172.23 (1C, CH_2COOCH_2), 172.01 (1C, NHCH*C*O), 65.68 (1C, $COOCH_2$), 64.88 (1C, $COOCH_2$), 51.89 (1C, NH*C*H),

49.50 (1C, $(CH_3CH_2)_2NCH_2$), 46.70 (1C, $(CH_3CH_2)_2NCH_2CH_2CH_2$), 36.29 (2C, CH_2CH_3), 31.87 (2C, $CH_2CH_2CH_3$), 31.34 (1C, CH_2COOCH_2), 30.35 (1C, $COCH_2CH_2$), 29.61 (8C, $CH_{2(myristoyl)}$), 29.56 (2C, $CH_{2(myristoyl)}$), 29.49 (1C, $CH_{2(myristoyl)}$), 29.48 (1C, $CH_{2(myristoyl)}$), 29.30 (2C, $CH_{2(myristoyl)}$, 1C, $NHCHCH_2$), 29.24 (1C, $CH_{2(myristoyl)}$), 29.19 (1C, $CH_{2(myristoyl)}$), 28.57 (1C, OCH_2CH_2), 28.48 (1C, OCH_2CH_2), 27.22 (1C, $COCH_2CH_2CO$), 25.87 (1C, $OCH_2CH_2CH_2$), 25.79 (1C, $OCH_2CH_2CH_2$), 23.97 (1C, $(CH_3CH_2)_2NCH_2CH_2$), 22.63 (2C, $CH_{2(myristoyl)}CH_3$), 14.05 (2C, $CH_2CH_{3(myristoyl)}$), 8.41 (2C, CH_2CH_3). HRMS, ESI^+, m/z: Calcd for $C_{44}H_{86}N_3O_6$ $[M+H]^+$, 752.6517; found, 752.6537.

*1.3.5. ditetradecyl (4-oxo-4-((2-(pyrrolidin-1-yl)ethyl)amino)butanoyl)glutamate (**TA5**)*

DCM/methanol (40/1). White solid. 46% yield. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.75 (brs, 1H, $NHCOCH_2$), 7.18 (d, J = 7.6 Hz, 1H, $CONHCH$), 4.57 – 4.51 (m, 1H, $NHCH$), 4.10 (t, J = 6.8 Hz, 2H, $COOCH_2$), 4.05 (t, J = 6.7 Hz, 2H, $COOCH_2$), 3.68 – 3.56 (m, 2H, $(CH_2CH_2)_2NCH_2CH_2$), 3.24 (brs, 4H, $(CH_2CH_2)_2N$), 3.20 – 3.13 (m, 2H, $(CH_2CH_2)_2NCH_2$), 2.73 – 2.62 (m, 2H, $NHCOCH_2$), 2.62 – 2.54 (m, 2H, CH_2COO), 2.48 – 2.34 (m, 2H, CH_2CONH), 2.22 – 2.14 (m, 1H, $NHCHCH_2$), 2.11 – 2.05 (m, 4H, $CH_2CH_2NCH_2CH_2$), 2.05 – 1.96 (m, 1H, $NHCHCH_2$), 1.67 – 1.57 (m, 4H, $COOCH_2CH_2$), 1.32 – 1.25 (m, 44H, $CH_{2(myristoyl)}$), 0.88 (t, J = 7.1 Hz, 6H, CH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 172.86 (1C, $NHCOCH_2$), 172.78 (1C, $CONHCH$), 172.27 (1C, CH_2COOCH_2), 172.16 (1C, $NHCHCO$), 65.65 (1C, $COOCH_2$), 64.85 (1C, $COOCH_2$), 55.20 (1C, $(CH_2CH_2)_2NCH_2$), 54.28 (2C, $CH_2CH_2NCH_2CH_2$), 51.87 (1C, $NHCH$), 36.13 (1C, $(CH_2CH_2)_2NCH_2CH_2$), 31.86 (2C, $CH_2CH_2CH_3$), 31.24 (1C, CH_2COOCH_2), 30.39 (1C, $COCH_2CH_2CO$), 29.64, 29.62, 29.60 (8C, $CH_{2(myristoyl)}$), 29.55 (2C, $CH_{2(myristoyl)}$), 29.49 (1C, $CH_{2(myristoyl)}$), 29.46 (1C, $CH_{2(myristoyl)}$), 29.30 (2C, $CH_{2(myristoyl)}$, 1C, $NHCHCH_2$), 29.24 (1C, $CH_{2(myristoyl)}$), 29.19 (1C, $CH_{2(myristoyl)}$), 28.57 (1C, OCH_2CH_2), 28.48 (1C, OCH_2CH_2), 27.23 (1C, $COCH_2CH_2CO$), 25.87 (1C, $OCH_2CH_2CH_2$), 25.80 (1C, $OCH_2CH_2CH_2$), 23.27 (2C, $(CH_2CH_2)_2N$), 22.62 (2C, CH_2CH_3), 14.03 (2C, CH_2CH_3). HRMS, ESI^+, m/z: Calcd for $C_{43}H_{82}N_3O_6$ $[M+H]^+$, 736.6204; found, 736.6209.

*1.3.6. ditetradecyl (4-oxo-4-((3-(pyrrolidin-1-yl)propyl)amino)butanoyl)glutamate (**TA6**)*

DCM/methanol (20/1). Yellow-white solid. 35% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.61 (brs, 1H, N*H*COCH$_2$), 7.16 (d, *J* = 7.3 Hz, 1H, CON*H*CH), 4.55 – 4.48 (m, 1H, NHC*H*), 4.15 – 4.07 (m, 2H, COOC*H$_2$*), 4.05 (t, *J* = 6.7 Hz, 2H, COOC*H$_2$*), 3.76 (brs, 2H, (CH$_2$CH$_2$)$_2$NCH$_2$CH$_2$C*H$_2$*), 3.38 (d, *J* = 4.9 Hz, 2H, CH$_2$C*H$_2$*NCH$_2$CH$_2$), 3.20 (d, *J* = 5.6 Hz, 2H, CH$_2$CH$_2$NC*H$_2$*CH$_2$), 2.96 – 2.86 (m, 2H, (CH$_2$CH$_2$)$_2$NC*H$_2$*), 2.64 – 2.54 (m, 2H, NHCOC*H$_2$*, 2H, C*H$_2$*COO), 2.47 – 2.30 (m, 2H, C*H$_2$*CONH), 2.23 – 2.12 (m, 1H, NHCHC*H$_2$*, 2H, (CH$_2$CH$_2$)$_2$NCH$_2$C*H$_2$*), 2.12 – 1.96 (m, 1H, NHCHC*H$_2$*, 4H, C*H$_2$*CH$_2$NCH$_2$C*H$_2$*), 1.67 – 1.57 (m, 4H, COOCH$_2$C*H$_2$*), 1.32 – 1.25 (m, 44H, C*H$_{2(myristoyl)}$*), 0.88 (t, *J* = 7.0 Hz, 6H, CH$_2$C*H$_3$*). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 173.30 (1C, NH*C*OCH$_2$), 172.85 (1C, *C*ONHCH), 172.64 (1C, CH$_2$*C*OOCH$_2$), 171.95 (1C, NHCH*C*O), 65.73 (1C, COO*C*H$_2$), 64.92 (1C, COO*C*H$_2$), 53.77 (2C, (CH$_2$*C*H$_2$)$_2$N), 52.88 (1C, (CH$_2$CH$_2$)$_2$N*C*H$_2$), 51.94 (1C, NH*C*H), 36.26 (1C, (CH$_2$CH$_2$)$_2$NCH$_2$CH$_2$*C*H$_2$), 31.87 (2C, *C*H$_2$CH$_2$CH$_3$), 31.33 (1C, *C*H$_2$COOCH$_2$), 30.34 (1C, CO*C*H$_2$CH$_2$CO), 29.63, 29.61 (8C, *C*H$_{2(myristoyl)}$), 29.56 (2C, *C*H$_{2(myristoyl)}$), 29.50 (1C, *C*H$_{2(myristoyl)}$), 29.48 (1C, *C*H$_{2(myristoyl)}$), 29.30 (2C, *C*H$_{2(myristoyl)}$, 1C, NHCH*C*H$_2$), 29.25 (1C, *C*H$_{2(myristoyl)}$), 29.20 (1C, *C*H$_{2(myristoyl)}$), 28.57 (1C, OCH$_2$*C*H$_2$), 28.48 (1C, OCH$_2$*C*H$_2$), 27.18 (1C, COCH$_2$*C*H$_2$CO), 25.87 (1C, OCH$_2$CH$_2$*C*H$_2$), 25.80 (1C, OCH$_2$CH$_2$*C*H$_2$), 25.65 (1C, (CH$_2$CH$_2$)$_2$NCH$_2$*C*H$_2$), 23.25 (2C, (*C*H$_2$CH$_2$)$_2$N), 22.62 (2C, *C*H$_2$CH$_3$), 14.03 (2C, CH$_2$*C*H$_3$). HRMS, ESI$^+$, m/z: Calcd for $C_{44}H_{84}N_3O_6$ $[M+H]^+$, 750.6360; found, 750.6360.

*1.3.7. ditetradecyl (4-((2-(1-methylpyrrolidin-2-yl)ethyl)amino)-4-oxobutanoyl) glutamate (**TA7**)*

DCM/methanol (25/1). Milky-white solid. 37% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.62 (brs, 1H, N*H*COCH$_2$), 7.35 (t, *J* = 8.7 Hz, 1H, CON*H*CH), 5.46 (brs, 6H, C*H$_2$*N(C*H$_3$*)C*H*), 4.54 – 4.47 (m, 1H, NHC*H*), 4.17 – 3.97 (m, 4H, COOC*H$_2$*), 3.42 – 3.26 (m, 2H, N(CH$_3$)CHCH$_2$C*H$_2$*NH), 3.08 – 2.92 (m, 2H, N(C*H$_3$*)CHC*H$_2$*CH$_2$NH), 2.91 – 2.85 (m, 2H, NHCOC*H$_2$*), 2.64 – 2.52 (m, 2H, C*H$_2$*COO, 1H,

N(CH_3)CHCH_2CH_2), 2.47 – 2.29 (m, 2H, CH_2CONH, 1H, N(CH_3)CHCH_2), 2.17 – 1.97 (m, 2H, NHCHCH_2, 1H, N(CH_3)CHCH_2, 1H, N(CH_3)CHCH_2CH_2), 1.69 – 1.53 (m, 4H, COOCH_2CH_2), 1.37 – 1.19 (m, 44H, C$H_{2(myristoyl)}$), 0.88 (t, J = 7.0 Hz, 6H, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 173.54 (1C, NH*C*OCH_2), 173.00 (1C, *C*ONHCH), 172.42 (1C, CH_2*C*OOCH_2), 172.07 (1C, NHCH*C*O), 71.26 (1C, N(CH_3)*C*H), 67.42 (1C, N(CH_3)*C*H_2), 65.84 (1C, COO*C*H_2), 65.04 (1C, COO*C*H_2), 56.16 (1C, N(*C*H_3)), 51.96 (1C, NH*C*H), 39.74 (1C, N(CH_3)CHCH_2*C*H_2NH), 36.43 (1C, N(CH_3)CH*C*H_2CH_2NH), 31.92 (2C, *C*H_2CH_2CH_3), 31.28 (1C, *C*H_2COOCH_2), 31.25 (1C, N(CH_3)CH*C*H_2CH_2CH_2), 30.34 (1C, CO*C*H_2CH_2), 29.69 (8C, *C*$H_{2(myristoyl)}$), 29.66 (2C, *C*$H_{2(myristoyl)}$), 29.57 (1C, *C*$H_{2(myristoyl)}$), 29.55 (1C, *C*$H_{2(myristoyl)}$), 29.36 (2C, *C*$H_{2(myristoyl)}$, 1C, NHCH*C*H_2), 29.32 (1C, *C*$H_{2(myristoyl)}$), 29.29 (1C, *C*$H_{2(myristoyl)}$), 28.58 (1C, OCH_2*C*H_2), 28.49 (1C, OCH_2*C*H_2), 27.09 (1C, COCH_2*C*H_2CO), 25.91 (1C, OCH_2CH_2*C*H_2), 25.86 (1C, OCH_2CH_2*C*H_2), 22.68 (2C, *C*H_2CH_3), 21.60 (1C, N(CH_3)CHCH_2*C*H_2), 14.10 (2C, CH_2*C*H_3). HRMS, ESI^+, m/z: Calcd for $C_{44}H_{84}N_3O_6$ $[M+H]^+$, 750.6360; found, 750.6367.

*1.3.8. ditetradecyl (4-((2-(4-methylpiperazin-1-yl)ethyl)amino)-4-oxobutanoyl) glutamate (**TA8**)*

DCM/methanol (15/1). White granular solid. 76% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.70 (brs, 1H, N*H*COCH_2), 7.43 (brs, 1H, CON*H*CH), 4.53 – 4.46 (m, 1H, NHC*H*), 4.15 – 4.06 (m, 2H, COOCH_2), 4.04 (t, J = 6.8 Hz, 2H, COOCH_2), 3.53 (m, 8H, N(CH_2CH_2)$_2$NCH_3, 2H, NCH_2CH_2NH), 3.22 (brs, 2H, NCH_2CH_2NH), 2.90 (brs, 3H, NCH_3), 2.71 – 2.50 (m, 2H, CH_2COO, 2H, COCH_2CH_2CO), 2.48 – 2.34 (m, 2H, CH_2CONH), 2.19 – 2.09 (m, 1H, NHCHCH_2), 2.06 – 1.96 (m, 1H, NHCHCH_2), 1.67 – 1.57 (m, 4H, COOCH_2CH_2), 1.31 – 1.25 (m, 44H, C$H_{2(myristoyl)}$), 0.88 (t, J = 7.1 Hz, 6H, CH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 173.51 (1C, NH*C*OCH_2), 172.82 (1C, *C*ONHCH), 172.78 (1C, CH_2*C*OOCH_2), 172.07 (1C, NHCH*C*O), 65.71 (1C, COO*C*H_2), 64.91 (1C, COO*C*H_2), 56.27 (1C, N*C*H_2CH_2NH), 51.96 (1C, NH*C*H), 51.28 (2C, N(CH_2*C*H_2)$_2$NCH_3), 49.56 (2C, N(*C*H_2CH_2)$_2$NCH_3), 43.45 (1C, N*C*H_3), 34.73 (1C, NCH_2*C*H_2NH), 31.87 (2C, *C*H_2CH_2CH_3), 31.13 (1C, *C*H_2COOCH_2), 30.35 (1C, CO*C*H_2CH_2CO), 29.67 (2C, *C*$H_{2(myristoyl)}$), 29.65 (8C,

C$H_{2(myristoyl)}$), 29.61 (2C, *C*$H_{2(myristoyl)}$), 29.54 (2C, *C*$H_{2(myristoyl)}$, 1C, NHCH*C*H_2), 29.30 (1C, *C*$H_{2(myristoyl)}$), 29.28 (1C, *C*$H_{2(myristoyl)}$), 28.58 (1C, OCH_2*C*H_2), 28.50 (1C, OCH_2*C*H_2), 27.06 (1C, COCH_2*C*H_2CO), 25.89 (1C, OCH_2CH_2*C*H_2), 25.86 (1C, OCH_2CH_2*C*H_2), 22.62 (2C, *C*H_2CH_3), 14.02 (2C, CH_2*C*H_3). HRMS, ESI^+, m/z: Calcd for $C_{44}H_{85}N_4O_6$ $[M+H]^+$, 765.6469; found, 765.6464.

*1.3.9. ditetradecyl (4-((3-(4-methylpiperazin-1-yl)propyl)amino)-4-oxobutanoyl) glutamate (**TA9**)*

DCM/methanol (15/1). Milky white gel-like solid. 53% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.63 (brs, 1H, N*H*COCH_2), 7.39 (brs, 1H,CON*H*CH), 4.51 – 4.45 (m, 1H, NHC*H*), 4.15 – 4.06 (m, 2H, COOC*H*$_2$), 4.04 (t, *J* = 7.0 Hz, 2H, COOC*H*$_2$), 3.55 (brs, 8H, N(C*H*$_2$C*H*$_2$)$_2$NCH_3), 3.33 (brs, 2H, NC*H*$_2$CH_2CH_2NH), 3.18 (brs, 2H, NCH_2CH_2C*H*$_2$NH), 2.89 (s, 3H, NC*H*$_3$), 2.66 – 2.49 (m, 2H, C*H*$_2$COO, 2H, COC*H*$_2$CH_2CO), 2.47 – 2.34 (m, 2H, C*H*$_2$CONH), 2.19 – 2.09 (m, 1H, NHCHC*H*$_2$), 2.06 – 1.95 (m, 1H, NHCHC*H*$_2$, 2H, NCH_2C*H*$_2$CH_2NH), 1.65 – 1.57 (m, 4H, COOCH_2C*H*$_2$), 1.30 – 1.25 (m, 44H, C*H*$_{2(myristoyl)}$), 0.88 (t, *J* = 6.7 Hz, 6H, CH_2C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 173.29 (1C, NH*C*OCH_2), 172.83 (1C, *C*ONHCH), 172.71 (1C, CH_2*C*OOCH_2), 172.08 (1C, NHCH*C*O), 65.72 (1C, COO*C*H_2), 64.93 (1C, COO*C*H_2), 54.66 (1C, N*C*H_2CH_2*C*H_2NH), 51.95 (1C, NH*C*H), 50.94 (2C, N(CH_2*C*H_2)$_2$NCH_3), 49.38 (2C, N(*C*H_2CH_2)$_2$NCH_3), 43.52 (1C, N*C*H_3), 36.66 (1C, NCH_2CH_2*C*H_2NH), 31.88 (2C, *C*H_2CH_2CH_3), 31.22 (1C, *C*H_2COOCH_2), 30.38 (1C, CO*C*H_2CH_2CO), 29.68 (2C, *C*$H_{2(myristoyl)}$), 29.67 (8C, *C*$H_{2(myristoyl)}$), 29.30 (2C, *C*$H_{2(myristoyl)}$), 29.25 (2C, *C*$H_{2(myristoyl)}$, 1C, NHCH*C*H_2), 29.21 (2C, *C*$H_{2(myristoyl)}$), 28.58 (1C, OCH_2*C*H_2), 28.48 (1C, OCH_2*C*H_2), 27.25 (1C, COCH_2*C*H_2CO), 25.91 (1C, OCH_2CH_2*C*H_2), 25.87 (1C, OCH_2CH_2*C*H_2), 24.20 (1C, NCH_2*C*H_2CH_2NH), 22.63 (2C, *C*H_2CH_3), 14.03 (2C, CH_2*C*H_3). HRMS, ESI^+, m/z: Calcd for $C_{45}H_{87}N_4O_6$ $[M+H]^+$, 779.6626; found, 779.6631.

*1.3.10. ditetradecyl (4-((3-(bis(2-hydroxyethyl)amino)propyl)amino)-4-oxobutanoyl) glutamate (**TA10**)*

DCM/methanol (10/1). White granular solid. 51% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.85 (brs, 1H, N*H*COCH_2), 7.60 (d, *J* = 7.1 Hz, 1H, CON*H*CH),

4.78 (brs, 6H, (HOCH_2CH$_2$)$_2$NCH$_2$CH$_2$CH_2), 4.51 – 4.44 (m, 1H, NHCH), 4.09 – 3.97 (m, 4H, COOCH_2, 2H, (HOCH$_2$CH$_2$)$_2$N), 3.52 – 3.35 (m, 6H, (HOCH$_2$CH_2)$_2$NCH_2CH$_2$CH$_2$), 2.68 – 2.50 (m, 2H, NHCOCH_2, 2H, CH_2COO), 2.46 – 2.35 (m, 2H, CH_2CONH), 2.19 – 1.95 (m, 2H, NHCHCH_2, 2H, (HOCH$_2$CH$_2$)$_2$NCH$_2$CH_2CH$_2$), 1.67 – 1.56 (m, 4H, COOCH$_2$CH_2), 1.34 – 1.22 (m, 44H, C$H_{2(\text{myristoyl})}$), 0.88 (t, J = 6.9 Hz, 6H, CH$_2$CH_3). ^{13}C NMR (75 MHz, CDCl$_3$): δ (ppm) 173.87 (1C, NHCOCH$_2$), 173.05 (1C, CONHCH), 172.90 (1C, CH$_2$$C$OOCH$_2$), 172.14 (1C, NHCH$C$O), 65.73 (1C, COO$CH_2$), 64.95 (1C, COO$CH_2$), 55.91 (2C, (HOCH$_2$$CH_2$)$_2$N), 55.65 (2C, (HO$CH_2CH_2$)$_2$N), 52.14 (1C, (HOCH$_2CH_2$)$_2NCH_2$), 51.96 (1C, NH$C$H), 36.33 (1C, (HOCH$_2CH_2$)$_2NCH_2CH_2$$CH_2$), 31.89 (2C, CH$_2CH_2CH_3$), 31.20 (1C, CH$_2$COOCH$_2$), 30.39 (1C, COCH$_2CH_2$CO), 29.68 (8C, CH$_{2(\text{myristoyl})}$), 29.64 (2C, CH$_{2(\text{myristoyl})}$), 29.57 (2C, CH$_{2(\text{myristoyl})}$), 29.33 (4C, CH$_{2(\text{myristoyl})}$, 1C, NHCHCH$_2$), 28.59 (1C, OCH$_2$$CH_2$), 28.51 (1C, OCH$_2$$CH_2$), 27.00 (1C, COCH$_2$$CH_2$CO), 25.90 (1C, OCH$_2CH_2$$CH_2$), 25.89 (1C, OCH$_2CH_2$$CH_2$), 23.87 (1C, (HOCH$_2CH_2$)$_2NCH_2$$CH_2$), 22.64 (2C, CH$_2CH_3$), 14.04 (2C, CH$_2$$CH_3$). HRMS, ESI$^+$, m/z: Calcd for $C_{44}H_{86}N_3O_8$ [M+H]$^+$, 784.6415; found, 784.6407.

1.3.11. ditetradecyl (4-((3-(1H-imidazol-1-yl)propyl)amino)-4-oxobutanoyl)glutamate ***(TA11)***

DCM/methanol (10/1). White gel-like solid. 59% yield. ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm) 8.02 (brs, 1H, Imidazole), 7.27 (brs, 2H, Imidazole), 4.66 – 4.47 (m, 1H, NHCH), 4.32 – 3.94 (m, 4H, COOCH_2, 2H, NCH_2)， 3.23 (brs, 2H, NCH$_2$CH$_2$CH_2), 2.64 (brs, 2H, NCH$_2$CH_2, 2H, COCH_2CH$_2$CO), 2.44 (brs, 1H, NHCHCH_2, 2H, CH_2COO), 2.17 – 1.98 (m, 1H, NHCHCH_2, 2H, CH_2CONH), 1.65 – 1.55 (m, 4H, COOCH$_2$CH_2), 1.32 – 1.24 (m, 44H, C$H_{2\ (\text{myristoyl})}$), 0.88 (t, J = 6.9 Hz, 6H, CH$_2$CH_3). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) 173.72 (1C, NHCOCH$_2$), 173.67 (1C, CONHCH), 173.04 (1C, CH$_2$$C$OOCH$_2$), 172.45 (1C, NHCH$C$O), 139.22 (1C, Imidazole), 138.81 (1C, Imidazole), 114.01 (1C, Imidazole), 66.06 (1C, COOCH$_2$), 65.03 (1C, COOCH$_2$), 52.10 (1C, NHCH), 48.69 (1C, NCH$_2$CH$_2$CH$_2$NH), 33.77 (1C, NCH$_2$CH$_2$$CH_2$NH), 31.90 (2C, CH$_2CH_2CH_3$), 31.60 (1C, CH$_2$COOCH$_2$), 30.46 (1C, COCH$_2CH_2$CO), 29.72 (6C, CH$_{2(\text{myristoyl})}$), 29.70 (2C, CH$_{2(\text{myristoyl})}$), 29.66

(2C, *C*$H_{2(myristoyl)}$), 29.65 (2C, *C*$H_{2(myristoyl)}$), 29.59 (2C, *C*$H_{2(myristoyl)}$, 1C, NHCH*C*H_2), 29.35 (1C, *C*$H_{2(myristoyl)}$), 29.34 (1C, *C*$H_{2(myristoyl)}$), 28.92 (1C, COCH_2*C*H_2CO), 28.60 (1C, OCH_2*C*H_2), 28.50 (1C, OCH_2*C*H_2), 26.84 (1C, NCH_2*C*H_2CH_2NH), 25.93 (1C, OCH_2CH_2*C*H_2), 25.91 (1C, OCH_2CH_2*C*H_2), 22.65 (2C, *C*H_2CH_3), 14.04 (2C, CH_2*C*H_3). HRMS, ESI$^+$, m/z: Calcd for $C_{43}H_{79}N_4O_6$ $[M+H]^+$, 747.6000; found, 747.6002.

1.3.12. ditetradecyl (4-((1-methylpiperidin-4-yl)amino)-4-oxobutanoyl)glutamate ***(TA12)***

DCM/methanol (20/1). White gel-like solid. 38 % yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.01 (brs, 1H, CON*H*CH), 4.57 – 4.51 (m, 1H, NHC*H*), 4.15 – 4.08 (m, 2H, COOC*H*$_2$), 4.05 (t, *J* = 6.8 Hz, 2H, COOC*H*$_2$), 3.93 (brs, 1H, $CH_3N(CH_2CH_2)_2CH$), 3.28 – 3.19 (m, 2H, $CH_3N(CH_2CH_2)_2CH$), 2.73 – 2.64 (m, 2H, $CH_3N(CH_2CH_2)_2CH$), 2.61 (s, 3H, NC*H*$_3$), 2.59 – 2.52 (m, 2H, COC*H*$_2$CH$_2$CO, 2H, C*H*$_2$COO), 2.45 – 2.32 (m, 2H, C*H*$_2$CONH), 2.21 – 2.12 (m, 1H, NHCHC*H*$_2$), 2.11 – 2.04 (m, 2H, $CH_3N(CH_2CH_2)_2CH$), 2.03 – 1.95 (m, 1H, NHCHC*H*$_2$), 1.94 – 1.85 (m, 2H, $CH_3N(CH_2CH_2)_2CH$), 1.67 – 1.57 (m, 4H, COOCH$_2$C*H*$_2$), 1.32 – 1.25 (m, 44H, C*H*$_{2(myristoyl)}$), 0.88 (t, *J* = 6.8 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 172.81 (1C, NH*C*OCH$_2$), 172.28 (1C, *C*ONHCH), 171.91 (1C, NHCH*C*O, 1C, CH$_2$*C*OOCH$_2$), 65.74 (1C, COO*C*H$_2$), 64.92 (1C, COO*C*H$_2$), 53.60 (2C, $CH_3N(CH_2CH_2)_2CH$), 51.84 (1C, NH*C*H), 44.43 (1C, N*C*H$_3$, 1C, $CH_3N(CH_2CH_2)_2CH$), 31.87 (2C, *C*H$_2$CH$_2$CH$_3$), 31.50 (1C, *C*H$_2$COOCH$_2$), 30.34 (1C, CO*C*H$_2$CH$_2$CO), 29.81 (2C, $CH_3N(CH_2CH_2)_2CH$), 29.64, 29.62 (8C, *C*$H_{2(myristoyl)}$), 29.57 (2C, *C*$H_{2(myristoyl)}$), 29.51 (1C, *C*$H_{2(myristoyl)}$), 29.48 (1C, *C*$H_{2(myristoyl)}$), 29.30 (2C, *C*$H_{2(myristoyl)}$, 1C, NHCH*C*H_2), 29.25 (1C, *C*$H_{2(myristoyl)}$), 29.21 (1C, *C*$H_{2(myristoyl)}$), 28.56 (1C, OCH_2*C*H_2), 28.47 (1C, OCH_2*C*H_2), 27.37 (1C, COCH_2*C*H_2CO), 25.87 (1C, OCH_2CH_2*C*H_2), 25.80 (1C, OCH_2CH_2*C*H_2), 22.62 (2C, *C*H_2CH_3), 14.03 (2C, CH_2*C*H_3). HRMS, ESI$^+$, m/z: Calcd for $C_{43}H_{82}N_3O_6$ $[M+H]^+$, 736.6204; found, 736.6199.

1.3.13. ditetradecyl (4-((1-methylpyrrolidin-3-yl)amino)-4-oxobutanoyl)glutamate ***(TA13)***

DCM/methanol (25/1). White gel-like solid. 34% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.08 (t, *J* = 7.3 Hz, 1H, N*H*COCH$_2$), 7.35 (d, *J* = 8.3 Hz, 1H, CON*H*CH), 4.82 (brs, 1H, CH$_3$N(CH$_2$CH$_2$)(CH$_2$)C*H*), 4.59 – 4.50 (m, 1H, NHC*H*), 4.11 – 4.02 (m, 4H, COOC*H*$_2$), 3.89 – 3.61 (m, 2H, CH$_3$N(CH$_2$CH$_2$)(C*H*$_2$)CH), 3.30 – 3.04 (m, 2H, CH$_3$N(C*H*$_2$CH$_2$)(CH$_2$)CH), 2.94 (s, 3H, NC*H*$_3$), 2.66 – 2.53 (m, 2H, COC*H*$_2$CH$_2$CO, 2H, C*H*$_2$COO), 2.47 – 2.36 (m, 2H, C*H*$_2$CONH), 2.36 – 2.09 (m, 1H, NHCHC*H*$_2$, 2H, CH$_3$N(CH$_2$C*H*$_2$)(CH$_2$)CH), 2.07 – 1.95 (m, 1H, NHCHC*H*$_2$), 1.66 – 1.57 (m, 4H, COOCH$_2$C*H*$_2$), 1.33 – 1.24 (m, 44H, C*H*$_{2(\text{myristoyl})}$), 0.88 (t, *J* = 6.9 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 172.80 (1C, NH*C*OCH$_2$), 172.75 (1C, *C*ONHCH), 172.27 (1C, CH$_2$*C*OOCH$_2$), 172.17 (1C, NHCH*C*O), 65.95 (1C, CH$_3$N(CH$_2$CH$_2$)(*C*H$_2$)CH), 65.86 (1C, COO*C*H$_2$), 64.99 (1C, CH$_3$N(*C*H$_2$CH$_2$)(CH$_2$)CH), 64.97 (1C, COO*C*H$_2$), 56.32 (1C, *C*H$_3$N), 51.94 (1C, CH$_3$N(CH$_2$CH$_2$)(CH$_2$)*C*H), 51.80 (1C, NH*C*H), 48.89 (1C, CH$_3$N(CH$_2$*C*H$_2$)(CH$_2$)CH), 31.88 (2C, *C*H$_2$CH$_2$CH$_3$), 31.38 (1C, *C*H$_2$COOCH$_2$), 30.62 (1C, CO*C*H$_2$CH$_2$CO), 29.65 (8C, *C*H$_{2(\text{myristoyl})}$), 29.61 (2C, *C*H$_{2(\text{myristoyl})}$), 29.58 (1C, *C*H$_{2(\text{myristoyl})}$), 29.52 (1C, *C*H$_{2(\text{myristoyl})}$), 29.31 (2C, *C*H$_{2(\text{myristoyl})}$, 1C, NHCH*C*H$_2$), 29.28 (1C, *C*H$_{2(\text{myristoyl})}$), 29.26 (1C, *C*H$_{2(\text{myristoyl})}$), 28.63 (1C, OCH$_2$*C*H$_2$), 28.56 (1C, OCH$_2$*C*H$_2$), 27.50 (1C, COCH$_2$*C*H$_2$CO), 25.91 (1C, OCH$_2$CH$_2$*C*H$_2$), 25.87 (1C, OCH$_2$CH$_2$*C*H$_2$), 22.64 (2C, *C*H$_2$CH$_3$), 14.05 (2C, CH$_2$*C*H$_3$). HRMS, ESI$^+$, m/z: Calcd for $C_{42}H_{80}N_3O_6$ $[M+H]^+$, 722.6047; found, 722.6062.

*1.3.14. ditetradecyl (4-((2-morpholinoethyl)amino)-4-oxobutanoyl)glutamate (**TA14**)*

DCM/methanol (25/1). White gel-like solid. 51% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.12 (brs, 1H, N*H*COCH$_2$), 7.32 (d, *J* = 3.7 Hz, 1H, CON*H*CH), 4.54 – 4.47 (m, 1H, NHC*H*), 4.17 – 3.97 (m, 4H, COOC*H*$_2$, 4H, O(C*H*$_2$CH$_2$)$_2$N), 3.70 (brs, 4H, O(CH$_2$C*H*$_2$)$_2$N), 3.34 (brs, 2H, NCH$_2$C*H*$_2$NH), 3.04 (brs, 2H, NC*H*$_2$CH$_2$NH), 2.72 – 2.56 (m, 2H, COC*H*$_2$CH$_2$CO, 2H, C*H*$_2$COO), 2.48 – 2.35 (m, 2H, C*H*$_2$CONH), 2.19 – 2.11 (m, 1H, NHCHC*H*$_2$), 2.06 – 1.96 (m, 1H, NHCHC*H*$_2$), 1.66 – 1.59 (m, 4H, COOCH$_2$C*H*$_2$), 1.31 – 1.25 (m, 44H, C*H*$_{2(\text{myristoyl})}$), 0.88 (t, *J* = 7.0 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 176.27 (1C, NH*C*OCH$_2$), 173.08 (1C, *C*ONHCH), 171.92 (1C, CH$_2$*C*OOCH$_2$), 172.09 (1C, NHCH*C*O), 65.80 (1C,

COO*C*H_2), 64.98 (1C, COO*C*H_2), 63.69 (2C, O(*C*H_2CH_2)$_2$N), 57.49 (1C, N*C*H_2CH_2NH), 52.67 (1C, O(CH_2*C*H_2)$_2$N), 52.59 (1C, O(CH_2*C*H_2)$_2$N), 52.01 (1C, NH*C*H), 33.73 (1C, NCH_2*C*H_2NH), 31.88 (2C, *C*H_2CH_2CH_3), 31.10 (1C, *C*H_2COOCH_2), 30.34 (1C, CO*C*H_2CH_2CO), 29.65, 29.64, 29.62(8C, *C*$H_{2(myristoyl)}$), 29.59 (2C, *C*$H_{2(myristoyl)}$), 29.52 (1C, *C*$H_{2(myristoyl)}$), 29.50 (1C, *C*$H_{2(myristoyl)}$), 29.31 (2C, *C*$H_{2(myristoyl)}$, 1C, NHCH*C*H_2), 29.27 (1C, *C*$H_{2(myristoyl)}$), 29.24 (1C, *C*$H_{2(myristoyl)}$), 28.57 (1C, OCH_2*C*H_2), 28.48 (1C, OCH_2*C*H_2), 27.07 (1C, COCH_2*C*H_2CO), 25.88 (1C, OCH_2CH_2*C*H_2), 25.82 (1C, OCH_2CH_2*C*H_2), 22.63 (2C, *C*H_2CH_3), 14.04 (2C, CH_2*C*H_3). HRMS, ESI$^+$, m/z: Calcd for $C_{43}H_{82}N_3O_7$ $[M+H]^+$, 752.6153; found, 752.6149.

*1.3.15. ditetradecyl (4-((3-morpholinopropyl)amino)-4-oxobutanoyl)glutamate (**TA15**)*

DCM/methanol (25/1). White gel-like solid. 46% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.70 (brs, 1H, N*H*COCH_2), 7.19 (brs, 1H, CON*H*CH), 4.52 – 4.47 (m, 1H, NHC*H*), 4.20 – 3.98 (m, 4H, COOC*H*$_2$, 4H, O(C*H*$_2$CH$_2$)$_2$N), 3.53 (brs, 2H, O(CH$_2$C*H*$_2$)$_2$N), 3.37 (brs, 2H, NCH$_2$CH$_2$C*H*$_2$NH), 3.18 (brs, 2H, NC*H*$_2$CH$_2$CH$_2$NH), 2.96 (brs, 2H, O(CH$_2$C*H*$_2$)$_2$N), 2.63 – 2.58 (m, 2H, COC*H*$_2$CH$_2$CO), 2.57 (brs, 2H, C*H*$_2$COO), 2.47 – 2.33 (m, 2H, C*H*$_2$CONH), 2.19 – 2.12 (m, 1H, NHCHC*H*$_2$), 2.09 (brs, 2H, NCH$_2$C*H*$_2$CH$_2$NH), 2.04 – 1.96 (m, 1H, NHCHC*H*$_2$), 1.66 – 1.59 (m, 4H, COOCH$_2$C*H*$_2$), 1.31 – 1.25 (m, 44H, C*H*$_{2(myristoyl)}$), 0.88 (t, *J* = 7.0 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 173.41 (1C, NH*C*OCH$_2$), 172.85 (1C, *C*ONHCH), 172.63 (1C, CH$_2$*C*OOCH$_2$), 172.01 (1C, NHCH*C*O), 65.74 (1C, COO*C*H_2), 64.94 (1C, COO*C*H_2), 63.74 (2C, O(*C*H_2CH$_2$)$_2$N), 55.17 (1C, N*C*H_2CH$_2$CH$_2$NH), 52.03 (2C, O(CH$_2$*C*H_2)$_2$N), 51.94 (1C, NH*C*H), 36.24 (1C, NCH$_2$CH$_2$*C*H_2NH), 31.87 (2C, *C*H_2CH$_2$CH$_3$), 31.16 (1C, *C*H_2COOCH$_2$), 30.33 (1C, CO*C*H_2CH$_2$CO), 29.64, 29.62, 29.60(8C, *C*$H_{2(myristoyl)}$), 29.57 (2C, *C*$H_{2(myristoyl)}$), 29.51 (1C, *C*$H_{2(myristoyl)}$), 29.49 (1C, *C*$H_{2(myristoyl)}$), 29.30 (2C, *C*$H_{2(myristoyl)}$, 1C, NHCH*C*H_2), 29.26 (1C, *C*$H_{2(myristoyl)}$), 29.22 (1C, *C*$H_{2(myristoyl)}$), 28.57 (1C, OCH_2*C*H_2), 28.48 (1C, OCH_2*C*H_2), 27.14 (1C, COCH_2*C*H_2CO), 25.87 (1C, OCH_2CH$_2$*C*H_2), 25.81 (1C, OCH_2CH$_2$*C*H_2), 23.46 (1C, NCH$_2$*C*H_2CH$_2$NH), 22.62 (2C, *C*H_2CH$_3$), 14.03 (2C, CH$_2$*C*H_3). HRMS, ESI$^+$, m/z: Calcd for $C_{44}H_{84}N_3O_7$ $[M+H]^+$, 766.6309; found,

766.6308.

*1.3.16. ditetradecyl (4-oxo-4-((2-(piperidin-1-yl)ethyl)amino)butanoyl)glutamate (**TA16**)*

DCM/methanol (10/1). White gel-like solid. 52% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 6.78 (d, *J* = 7.7 Hz, 1H, CON*H*CH), 6.59 (brs, 1H, N*H*$COCH_2$), 4.60 – 4.54 (m, 1H, NHC*H*), 4.11 (t, *J* = 6.7 Hz, 2H, $COOCH_2$), 4.05 (t, *J* = 6.7 Hz, 2H, $COOCH_2$), 3.42 – 3.33 (m, 2H, NCH_2CH_2NH), 2.64 – 2.45 (m, 2H, $COCH_2CH_2CO$, 2H, CH_2COO, 6H, $CH_2(CH_2CH_2)_2NCH_2$), 2.43 – 2.32 (m, 2H, CH_2CONH), 2.23 – 2.15 (m, 1H, NHCHCH_2), 2.03 – 1.94 (m, 1H, NHCHCH_2), 1.66 – 1.57 (m, 4H, $COOCH_2CH_2$, 4H, $CH_2(CH_2CH_2)_2N$), 1.50 – 1.43 (m, 2H, $CH_2(CH_2CH_2)_2N$), 1.31 – 1.24 (m, 44H, $CH_{2(myristoyl)}$), 0.88 (t, *J* = 7.1 Hz, 6H, CH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 172.78 (1C, NH*C*OCH_2), 172.11 (1C, *C*ONHCH), 171.91 (1C, CH_2COOCH_2), 171.86 (1C, NHCH*C*O), 65.68 (1C, $COOCH_2$), 64.87 (1C, $COOCH_2$), 57.19 (1C, NCH_2CH_2NH), 54.25 (2C, $CH_2(CH_2CH_2)_2N$), 51.78 (1C, NH*C*H), 35.86 (1C, NCH_2CH_2NH), 31.88 (2C, $CH_2CH_2CH_3$), 31.52 (1C, CH_2COOCH_2), 30.30 (1C, $COCH_2CH_2CO$), 29.65, 29.63, 29.61 (8C, $CH_{2(myristoyl)}$), 29.56 (2C, $CH_{2(myristoyl)}$), 29.50 (1C, $CH_{2(myristoyl)}$), 29.46 (1C, $CH_{2(myristoyl)}$), 29.31 (2C, $CH_{2(myristoyl)}$, 1C, NHCH*C*H_2), 29.24 (1C, $CH_{2(myristoyl)}$), 29.18 (1C, $CH_{2(myristoyl)}$), 28.58 (1C, OCH_2CH_2), 28.49 (1C, OCH_2CH_2), 27.43 (1C, $COCH_2CH_2CO$), 25.87 (1C, $OCH_2CH_2CH_2$), 25.78 (1C, $OCH_2CH_2CH_2$), 25.41 (2C, $CH_2(CH_2CH_2)_2N$), 24.01 (1C, $CH_2(CH_2CH_2)_2N$), 22.64 (2C, CH_2CH_3), 14.04 (2C, CH_2CH_3). HRMS, ESI^+, m/z: Calcd for $C_{44}H_{84}N_3O_6$ $[M+H]^+$, 750.6360; found, 750.6367.

*1.3.17. ditetradecyl (4-oxo-4-((2-(pyridin-4-yl)ethyl)amino)butanoyl)glutamate (**TA17**)*

DCM/methanol (40/1). White gel-like solid. 28% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.55 (brs, 2H, $N(CHCH)_2C$), 7.34 (brs, 2H, $N(CHCH)_2C$), 6.83 (brs, 1H, N*H*$COCH_2$), 6.64 (brs, 1H, CON*H*CH), 4.58 – 4.52 (m, 1H, NHC*H*), 4.12 (t, *J* = 6.3 Hz, 2H, $COOCH_2$), 4.05 (t, *J* = 6.8 Hz, 2H, $COOCH_2$), 3.54 (brs, 2H, CCH_2CH_2NH), 2.91 (brs, 2H, CCH_2CH_2NH), 2.55 (brs, 2H, CH_2COO), 2.48 (brs, 2H, CH_2CONH), 2.44 – 2.32 (m, 2H, CH_2CONH), 2.23 – 2.14 (m, 1H, NHCHCH_2), 2.04

– 1.95 (m, 1H, NHCHCH_2), 1.66 – 1.58 (m, 4H, COOCH$_2$CH_2), 1.31 – 1.25 (m, 44H, C$H_{2(\text{myristoyl})}$), 0.88 (t, J = 7.0 Hz, 6H, CH$_2$CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 172.83 (1C, NH*C*OCH$_2$), 172.31 (1C, *C*ONHCH), 172.21 (1C, CH$_2$*C*OOCH$_2$), 171.81 (1C, NHCH*C*O), 151.64 (2C, N(*C*HCH)$_2$C), 147.23 (1C, N(CHCH)$_2$*C*), 125.23 (2C, N(CH*C*H)$_2$C), 65.80 (1C, COO*C*H$_2$), 64.98 (1C, COO*C*H$_2$), 51.94 (1C, NH*C*H), 39.53 (1C, CCH$_2$*C*H$_2$NH), 35.30 (1C, C*C*H$_2$CH$_2$NH), 31.87 (2C, *C*H$_2$CH$_2$CH$_3$), 31.39 (1C, *C*H$_2$COOCH$_2$), 30.32 (1C, CO*C*H$_2$CH$_2$CO), 29.65, 29.63, 29.61 (8C, *C*H$_{2(\text{myristoyl})}$), 29.55 (2C, *C*H$_{2(\text{myristoyl})}$), 29.49 (1C, *C*H$_{2(\text{myristoyl})}$), 29.47 (1C, *C*H$_{2(\text{myristoyl})}$), 29.30 (2C, *C*H$_{2(\text{myristoyl})}$, 1C, NHCH*C*H$_2$), 29.24 (1C, *C*H$_{2(\text{myristoyl})}$), 29.18 (1C, *C*H$_{2(\text{myristoyl})}$), 28.57 (1C, OCH$_2$*C*H$_2$), 28.49 (1C, OCH$_2$*C*H$_2$), 27.24 (1C, COCH$_2$*C*H$_2$CO), 25.87 (1C, OCH$_2$CH$_2$*C*H$_2$), 25.79 (1C, OCH$_2$CH$_2$*C*H$_2$), 22.63 (2C, *C*H$_2$CH$_3$), 14.04 (2C, CH$_2$*C*H$_3$). HRMS, ESI$^+$, m/z: Calcd for $C_{44}H_{78}N_3O_6$ $[M+H]^+$, 744.5891; found, 744.5899.

*1.3.18. ditetradecyl (4-oxo-4-((3-(piperidin-1-yl)propyl)amino)butanoyl)glutamate (**TA18**)*

DCM/methanol (30/1). White gel-like solid. 32% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.81 (brs, 1H, N*H*COCH$_2$), 7.22 (brs, 1H, CON*H*CH), 4.55 – 4.48 (m, 1H, NHC*H*), 4.10 (t, J = 6.7 Hz, 2H, COOCH_2), 4.05 (t, J = 6.7 Hz, 2H, COOCH_2), 3.58 (brs, 2H, NCH$_2$CH$_2$CH_2NH), 3.38 (brs, 2H, CH$_2$(CH$_2$CH_2)$_2$N), 3.10 (brs, 2H, CH$_2$(CH$_2$CH_2)$_2$N), 2.75 – 2.65 (m, 2H, NCH_2CH$_2$CH$_2$NH), 2.61 (brs, 2H, COCH_2CH$_2$CO, 2H, CH_2COO), 2.48 – 2.34 (m, 2H, CH_2CONH), 2.26 – 2.15 (m, 1H, NHCHCH_2, 2H, CH$_2$(CH_2CH$_2$)$_2$N), 2.15 – 2.07 (m, 2H, CH$_2$(CH_2CH$_2$)$_2$N), 2.06 – 1.97 (m, 1H, NHCHCH_2), 1.93 – 1.83 (m, 1H, CH_2(CH$_2$CH$_2$)$_2$N, 2H, NCH$_2$CH_2CH$_2$NH), 1.67 – 1.58 (m, 4H, COOCH$_2$CH_2), 1.49 – 1.38 (m, 1H, CH_2(CH$_2$CH$_2$)$_2$N), 1.32 – 1.25 (m, 44H, C$H_{2(\text{myristoyl})}$), 0.88 (t, J = 6.8 Hz, 6H, CH$_2$CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 173.24 (1C, NH*C*OCH$_2$), 172.79 (1C, *C*ONHCH), 172.41 (1C, CH$_2$*C*OOCH$_2$), 171.98 (1C, NHCH*C*O), 65.67 (1C, COO*C*H$_2$), 64.86 (1C, COO*C*H$_2$), 54.55 (2C, CH$_2$(CH$_2$*C*H$_2$)$_2$N), 53.37 (1C, NCH$_2$CH$_2$*C*H$_2$NH), 53.28 (1C, N*C*H$_2$CH$_2$CH$_2$NH), 51.95 (1C, NH*C*H), 36.20 (1C, NCH$_2$*C*H$_2$CH$_2$NH), 31.86 (2C, *C*H$_2$CH$_2$CH$_3$), 31.32 (1C, *C*H$_2$COOCH$_2$), 30.39 (1C,

CO*C*H_2CH$_2$CO), 29.62, 29.60 (8C, *C*$H_{2(myristoyl)}$), 29.55 (2C, *C*$H_{2(myristoyl)}$), 29.49 (1C, *C*$H_{2(myristoyl)}$), 29.47 (1C, *C*$H_{2(myristoyl)}$), 29.29 (2C, *C*$H_{2(myristoyl)}$, 1C, NHCH*C*H_2), 29.24 (1C, *C*$H_{2(myristoyl)}$), 29.20 (1C, *C*$H_{2(myristoyl)}$), 28.57 (1C, OCH$_2$*C*H_2), 28.49 (1C, OCH$_2$*C*H_2), 27.16 (1C, COCH$_2$*C*H$_2$CO), 25.87 (1C, OCH$_2$CH$_2$*C*H_2), 25.80 (1C, OCH$_2$CH$_2$*C*H_2), 23.77 (2C, CH$_2$(*C*H$_2$CH$_2$)$_2$N), 22.62 (2C, *C*H_2CH$_3$), 22.11 (1C, *C*H_2(CH$_2$CH$_2$)$_2$N), 14.03 (2C, CH$_2$*C*H_3). HRMS, ESI$^+$, m/z: Calcd for $C_{45}H_{86}N_3O_6$ [M+H]$^+$, 764.6517; found, 764.6527.

*1.3.19. ditetradecyl (4-(((1-methylpiperidin-4-yl)methyl)amino)-4-oxobutanoyl) glutamate (**TA19**)*

DCM/methanol (25/1). White gel-like solid. 44% yield. ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) 7.36 (brs, 1H, N*H*COCH$_2$), 7.30 (brs, 1H, CON*H*CH), 4.53 – 4.47 (m, 1H, NHC*H*), 4.15 – 4.07 (m, 2H, COOC*H*$_2$), 4.04 (t, *J* = 7.1 Hz, 2H, COOC*H*$_2$), 3.43 (d, *J* = 11.4 Hz, 2H, CH$_3$N(CH$_2$CH$_2$)$_2$CHC*H*$_2$), 3.24 – 3.15 (m, 2H, CH$_3$N(C*H*$_2$CH$_2$)$_2$CH), 2.82 – 2.75 (m, 2H, CH$_3$N(C*H*$_2$CH$_2$)$_2$CH), 2.74 (s, 3H, NC*H*$_3$), 2.64 – 2.59 (m, 2H, COC*H*$_2$CH$_2$CO), 2.59 – 2.55 (m, 2H, C*H*$_2$COO), 2.47 – 2.35 (m, 2H, C*H*$_2$CONH), 2.20 – 2.11 (m, 1H, NHCHC*H*$_2$), 2.05 – 1.98 (m, 1H, NHCHC*H*$_2$), 1.96 – 1.90 (m, 2H, CH$_3$N(CH$_2$C*H*$_2$)$_2$CH), 1.87 – 1.75 (m, 1H, CH$_3$N(CH$_2$CH$_2$)$_2$C*H*, 2H, CH$_3$N(CH$_2$C*H*$_2$)$_2$CH), 1.67 – 1.57 (m, 4H, COOCH$_2$C*H*$_2$), 1.32 – 1.25 (m, 44H, C*H*$_{2(myristoyl)}$), 0.88 (t, *J* = 7.0 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) 172.85 (1C, NH*C*OCH$_2$), 172.80 (1C, *C*ONHCH), 172.47 (1C, CH$_2$*C*OOCH$_2$), 171.94 (1C, NHCH*C*O), 65.63 (1C, COO*C*H$_2$), 64.87 (1C, COO*C*H$_2$), 54.31 (2C, CH$_3$N(CH$_2$*C*H$_2$)$_2$CH), 51.93 (1C, NH*C*H), 43.69 (1C, *C*H$_3$N), 43.56 (1C, CH$_3$N(CH$_2$CH$_2$)$_2$CH*C*H$_2$), 33.37 (1C, CH$_3$N(CH$_2$CH$_2$)$_2$*C*H), 31.85 (2C, *C*H$_2$CH$_2$CH$_3$), 31.57 (1C, *C*H$_2$COOCH$_2$), 30.39 (1C, CO*C*H$_2$CH$_2$CO), 29.62, 29.59 (8C, *C*H$_{2(myristoyl)}$), 29.55 (2C, *C*H$_{2(myristoyl)}$), 29.48 (1C, *C*H$_{2(myristoyl)}$), 29.46 (1C, *C*H$_{2(myristoyl)}$), 29.28 (2C, *C*H$_{2(myristoyl)}$, 1C, NHCH*C*H$_2$), 29.23 (1C, *C*H$_{2(myristoyl)}$), 29.19 (1C, *C*H$_{2(myristoyl)}$), 28.56 (1C, OCH$_2$*C*H$_2$), 28.47 (1C, OCH$_2$*C*H$_2$), 27.11 (1C, COCH$_2$*C*H$_2$CO), 26.99 (2C, CH$_3$N(CH$_2$*C*H$_2$)$_2$CH), 25.86 (1C, OCH$_2$CH$_2$*C*H$_2$), 25.80 (1C, OCH$_2$CH$_2$*C*H$_2$), 22.60 (2C, *C*H$_2$CH$_3$), 14.02 (2C, CH$_2$*C*H$_3$). HRMS, ESI$^+$, m/z: Calcd for $C_{44}H_{84}N_3O_6$ [M+H]$^+$, 750.6360; found, 750.6379.

*1.3.20. ditetradecyl (4-((1-benzylpyrrolidin-3-yl)amino)-4-oxobutanoyl)glutamate **(TA20)***

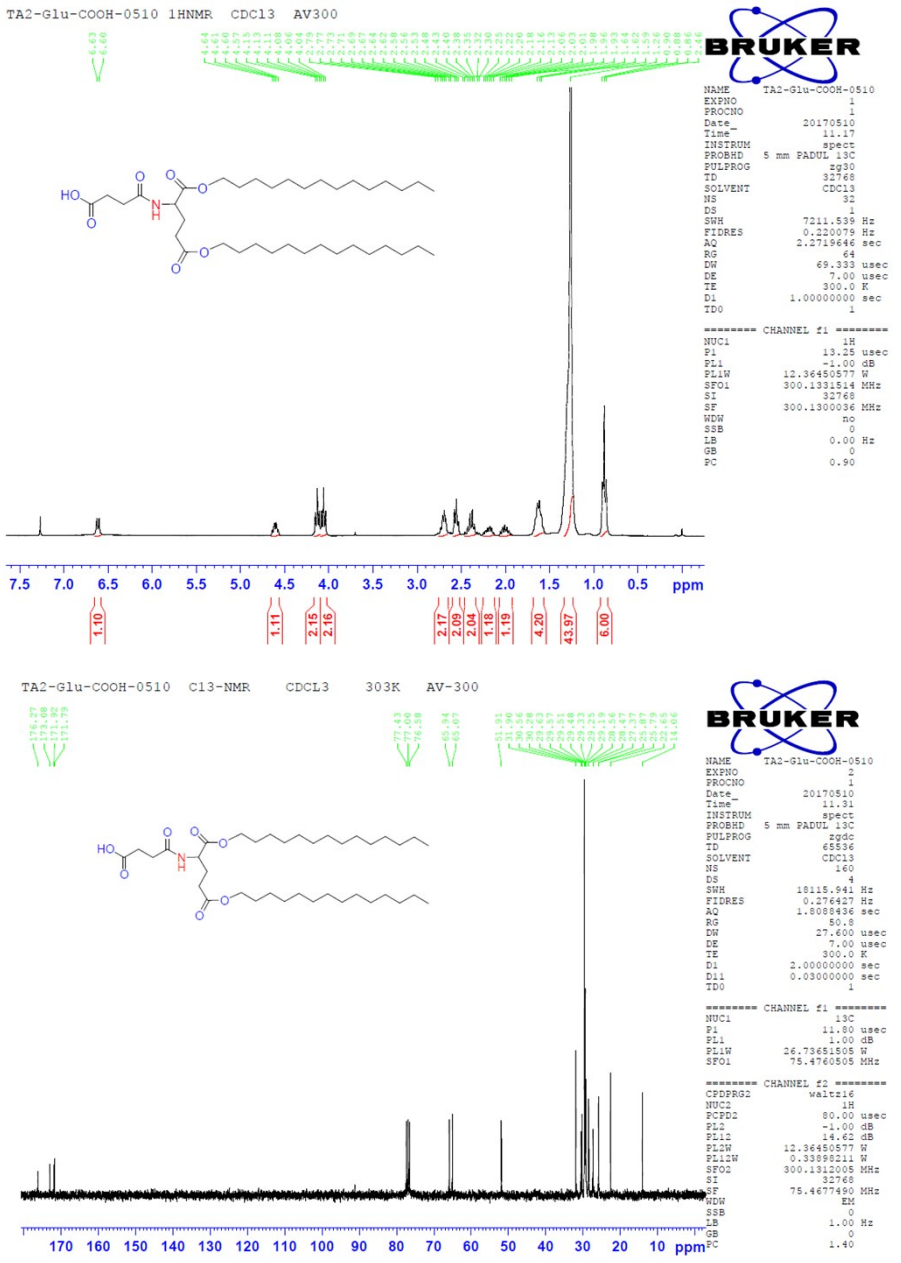
DCM/methanol (40/1). White solid. 40% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.32 (s, 2H, $C_6H_5CH_2$), 7.31 (s, 2H, $C_6H_5CH_2$), 7.26 (s, 1H, $C_6H_5CH_2$), 6.64 (t, *J* = 8.2 Hz, 1H, N*H*COCH$_2$), 6.27 (brs, 1H, CON*H*CH), 4.59 – 4.54 (m, 1H, NHC*H*), 4.48 – 4.40 (m, 1H, N(CH$_2$CH$_2$)(CH$_2$)C*H*), 4.13 – 4.08 (m, 2H, COOC*H$_2$*), 4.05 (t, *J* = 6.7 Hz, 2H, COOC*H$_2$*), 3.68 – 3.62 (m, 2H, C$_6$H$_5$C*H$_2$*), 2.93 – 2.85 (m, 1H, N(CH$_2$CH$_2$)(C*H$_2$*)CH), 2.64 – 2.59 (m, 2H, N(CH$_2$C*H$_2$*)(C*H$_2$*)CH), 2.57 – 2.52 (m, 2H, COC*H$_2$*CH$_2$CO), 2.49 – 2.45 (m, 2H, C*H$_2$*COO), 2.41 – 2.30 (m, 2H, C*H$_2$*CONH, 1H, N(C*H$_2$*CH$_2$)(CH$_2$)CH), 2.29 – 2.22 (m, 1H, N(CH$_2$C*H$_2$*)(CH$_2$)CH), 2.21 – 2.13 (m, 1H, NHCHC*H$_2$*), 2.01 – 1.93 (m, 1H, NHCHC*H$_2$*), 1.65 – 1.58 (m, 4H, COOCH$_2$C*H$_2$*, 1H, N(CH$_2$C*H$_2$*)(CH$_2$)CH), 1.29 – 1.25 (m, 44H, C*H$_{2(myristoyl)}$*), 0.88 (t, *J* = 7.1 Hz, 6H, CH$_2$C*H$_3$*).^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 172.80 (1C, NH*C*OCH$_2$), 172.00 (1C, *C*ONHCH), 171.82 (1C, CH$_2$*C*OOCH$_2$), 171.17 (1C, NHCH*C*O), 128.91 (1C, *C*$_6$H$_5$CH$_2$), 128.37 (4C, *C*$_6$H$_5$CH$_2$), 127.30 (1C, *C*$_6$H$_5$CH$_2$), 65.74 (1C, COO*C*H$_2$), 64.91 (1C, COO*C*H$_2$), 60.46 (1C, N(CH$_2$CH$_2$)(*C*H$_2$)CH), 59.91 (1C, C$_6$H$_5$*C*H$_2$), 52.49 (1C, N(*C*H$_2$CH$_2$)(CH$_2$)CH), 51.82 (1C, NH*C*H), 48.69 (1C, N(CH$_2$CH$_2$)(CH$_2$)*C*H), 32.36 (1C, N(CH$_2$*C*H$_2$)(CH$_2$)CH), 31.91 (2C, *C*H$_2$CH$_2$CH$_3$), 31.51 (1C, *C*H$_2$COOCH$_2$), 30.30 (1C, CO*C*H$_2$CH$_2$CO), 29.68, 29.64 (8C, *C*H$_{2(myristoyl)}$), 29.59 (2C, *C*H$_{2(myristoyl)}$), 29.52 (1C, *C*H$_{2(myristoyl)}$), 29.49 (1C, *C*H$_{2(myristoyl)}$), 29.34 (2C, *C*H$_{2(myristoyl)}$, 1C, NHCH*C*H$_2$), 29.27 (1C, *C*H$_{2(myristoyl)}$), 29.20 (1C, *C*H$_{2(myristoyl)}$), 28.61 (1C, OCH$_2$*C*H$_2$), 28.51 (1C, OCH$_2$*C*H$_2$), 27.45 (1C, COCH$_2$*C*H$_2$CO), 25.90 (1C, OCH$_2$CH$_2$*C*H$_2$), 25.81 (1C, OCH$_2$CH$_2$*C*H$_2$), 22.66 (2C, *C*H$_2$CH$_3$), 14.07 (2C, CH$_2$*C*H$_3$). HRMS, ESI$^+$, m/z: Calcd for $C_{48}H_{84}N_3O_6$ $[M+H]^+$, 798.6360; found, 798.6368.

*1.3.21. ditetradecyl (4-((1-benzylpiperidin-4-yl)amino)-4-oxobutanoyl)glutamate **(TA21)***

DCM/methanol (35/1). Yellow-white solid. 29% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.30 (s, 2H, $C_6H_5CH_2$), 7.29 (s, 2H, $C_6H_5CH_2$), 7.26 – 7.22 (m, 1H, $C_6H_5CH_2$), 6.66 (t, *J* = 7.0 Hz, 1H, N*H*COCH$_2$), 5.89 (brs, 1H, CON*H*CH), 4.59 –

4.54 (m, 1H, NHC*H*), 4.11 (t, *J* = 6.7 Hz, 2H, COOCH_2), 4.04 (t, *J* = 6.9 Hz, 2H, COOCH_2), 3.82 – 3.73 (m, 1H, $CH_3N(CH_2CH_2)_2CH$), 3.49 (s, 2H, $C_6H_5CH_2$), 2.83 – 2.75 (m, 2H, $N(CH_2CH_2)_2CH$), 2.58 – 2.53 (m, 2H, $COCH_2CH_2CO$), 2.53 – 2.44 (m, 2H, CH_2COO), 2.43 – 2.29 (m, 2H, CH_2CONH), 2.22 – 2.16 (m, 1H, NHCHCH_2), 2.16 – 2.09 (m, 2H, $N(CH_2CH_2)_2CH$), 2.02 – 1.93 (m, 1H, NHCHCH_2), 1.91 – 1.84 (m, 2H, $N(CH_2CH_2)_2CH$), 1.67 – 1.56 (m, 4H, $COOCH_2CH_2$), 1.51 – 1.42 (m, 2H, $N(CH_2CH_2)_2CH$), 1.31 – 1.23 (m, 44H, $CH_{2(myristoyl)}$), 0.88 (t, *J* = 6.7 Hz, 6H, CH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 172.79 (1C, $NHCOCH_2$), 172.10 (1C, *C*ONHCH), 171.77 (1C, CH_2COOCH_2), 171.18 (1C, NHCH*C*O), 129.06 (1C, $C_6H_5CH_2$), 128.19 (4C, $C_6H_5CH_2$), 127.04 (1C, $C_6H_5CH_2$), 65.74 (1C, $COOCH_2$), 64.91 (1C, $COOCH_2$), 62.98 (1C, $C_6H_5CH_2$), 52.14 (2C, $N(CH_2CH_2)_2CH$), 51.82 (1C, NHCH), 46.56 (1C, $N(CH_2CH_2)_2CH$), 32.02 (2C, $N(CH_2CH_2)_2CH$), 31.89 (2C, $CH_2CH_2CH_3$), 31.62 (1C, CH_2COOCH_2), 30.29 (1C, $COCH_2CH_2CO$), 29.66, 29.64, 29.62 (8C, $CH_{2(myristoyl)}$), 29.57, 29.56 (2C, $CH_{2(myristoyl)}$), 29.51 (1C, $CH_{2(myristoyl)}$), 29.47 (1C, $CH_{2(myristoyl)}$), 29.31 (2C, $CH_{2(myristoyl)}$, 1C, NHCHCH_2), 29.25 (1C, $CH_{2(myristoyl)}$), 29.18 (1C, $CH_{2(myristoyl)}$), 28.58 (1C, OCH_2CH_2), 28.49 (1C, OCH_2CH_2), 27.39 (1C, $COCH_2CH_2CO$), 25.87 (1C, $OCH_2CH_2CH_2$), 25.78 (1C, $OCH_2CH_2CH_2$), 22.64 (2C, CH_2CH_3), 14.05 (2C, CH_2CH_3). HRMS, ESI^+, m/z: Calcd for $C_{49}H_{86}N_3O_6$ $[M+H]^+$, 812.6517; found, 812.6504.

Supplemental Figures



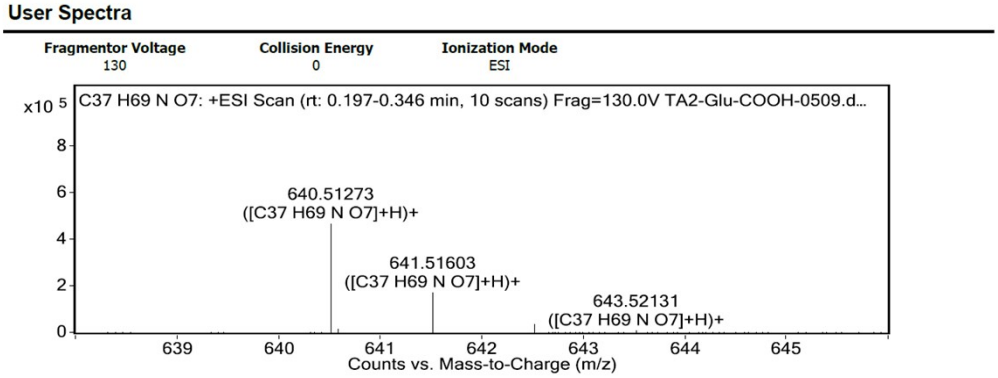
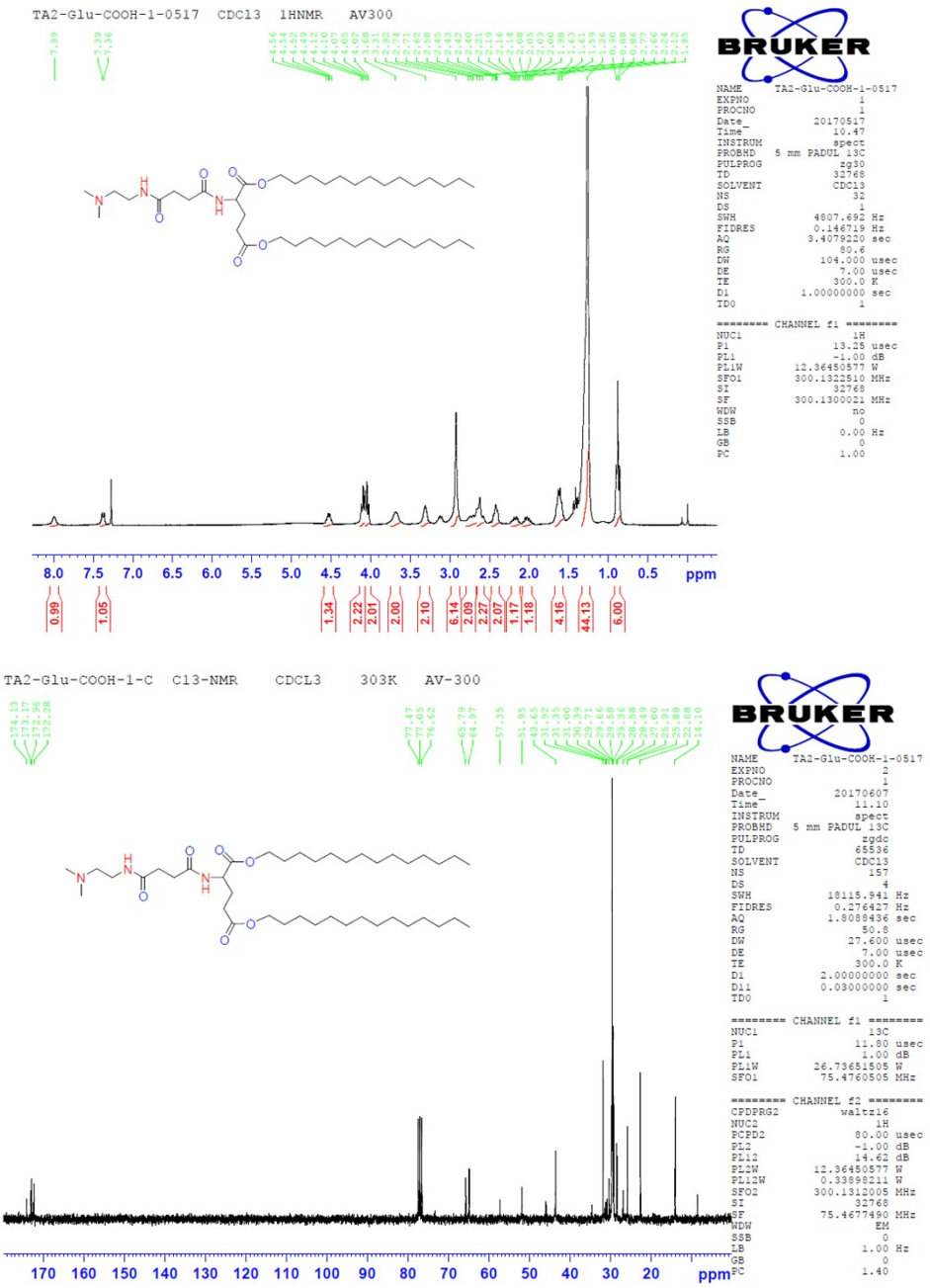


Figure S1. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **b**



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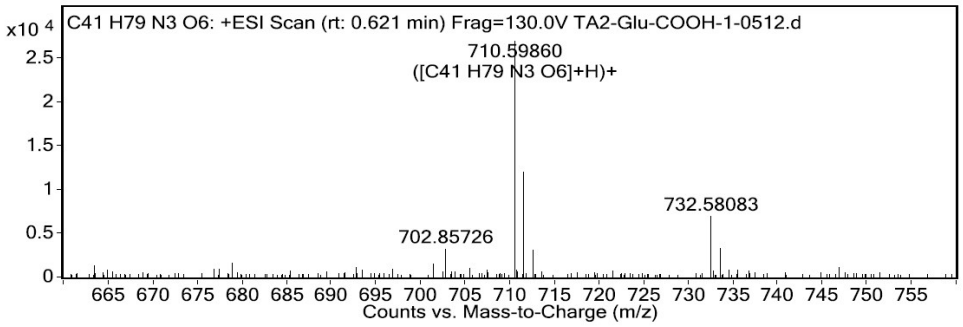
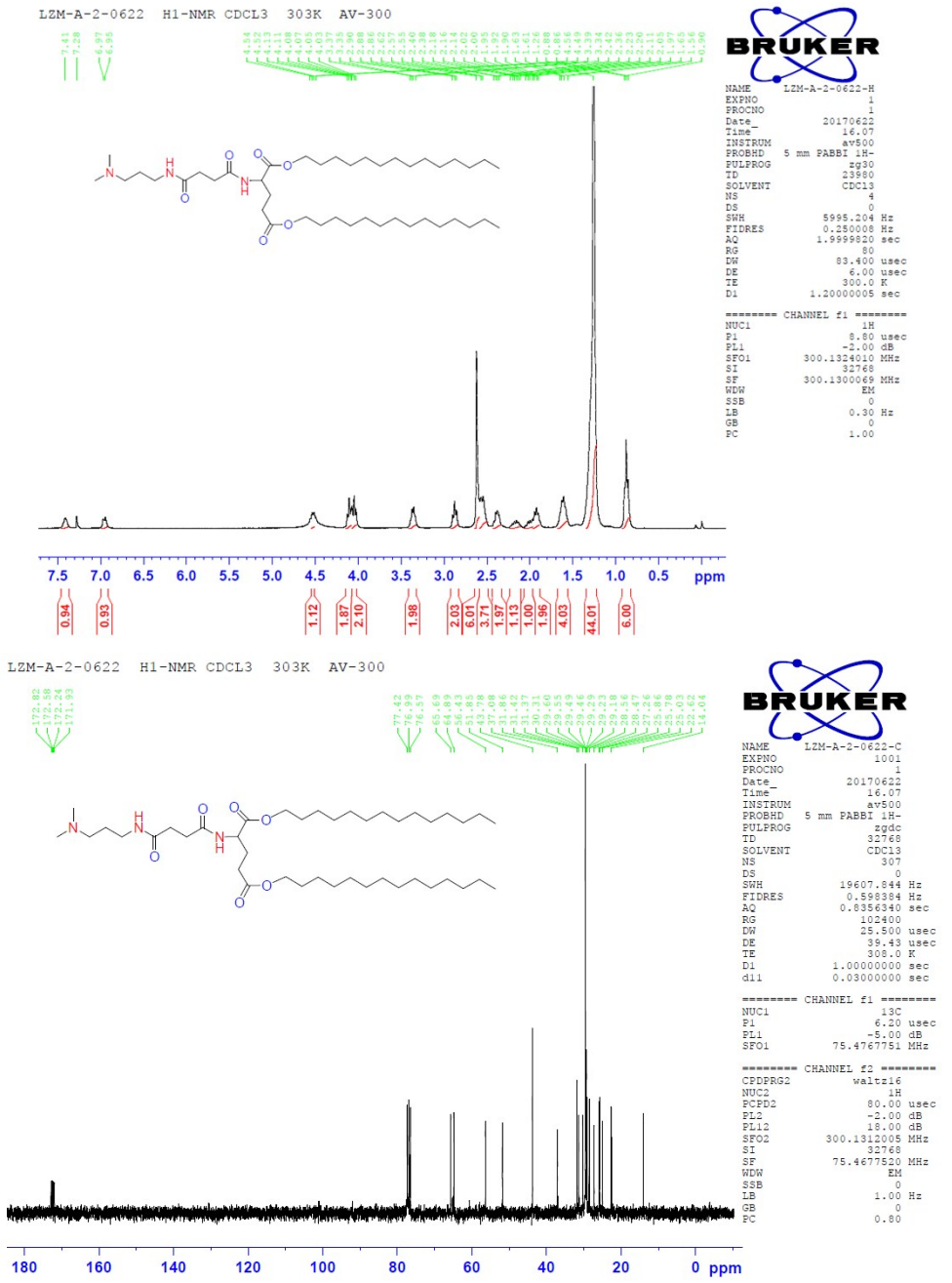


Figure S2. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA1**



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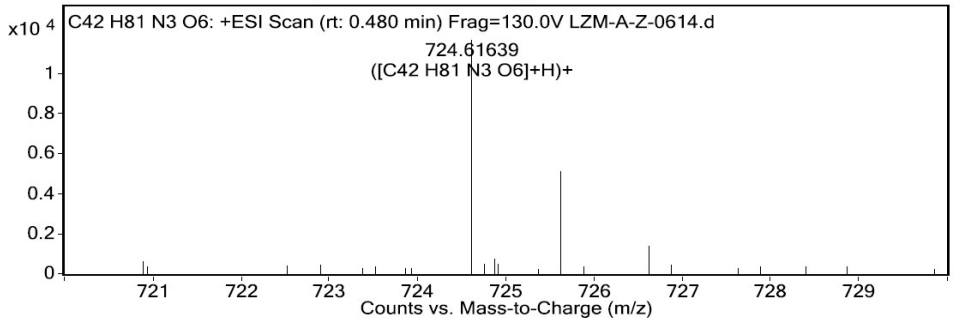
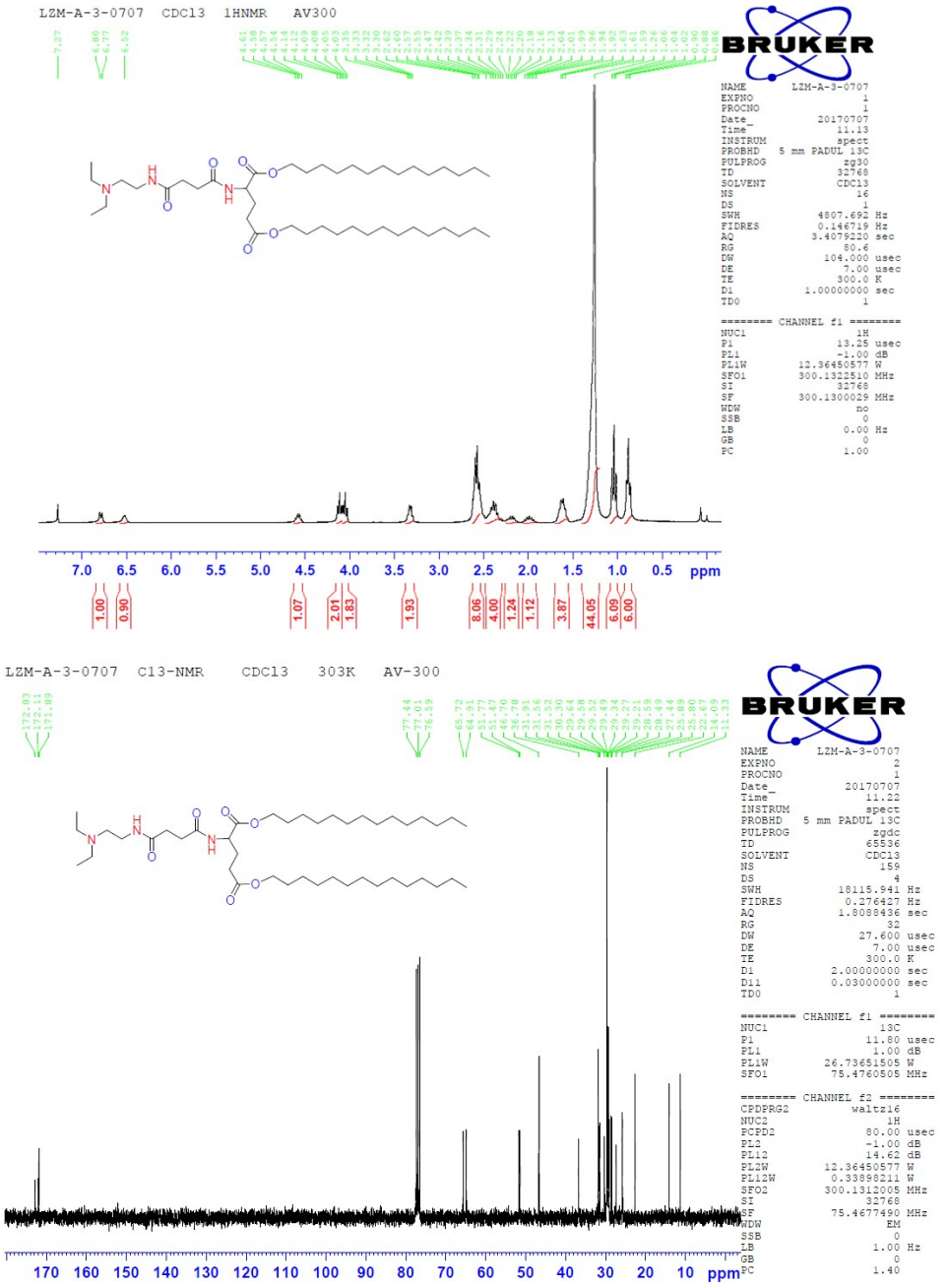


Figure S3. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA2**



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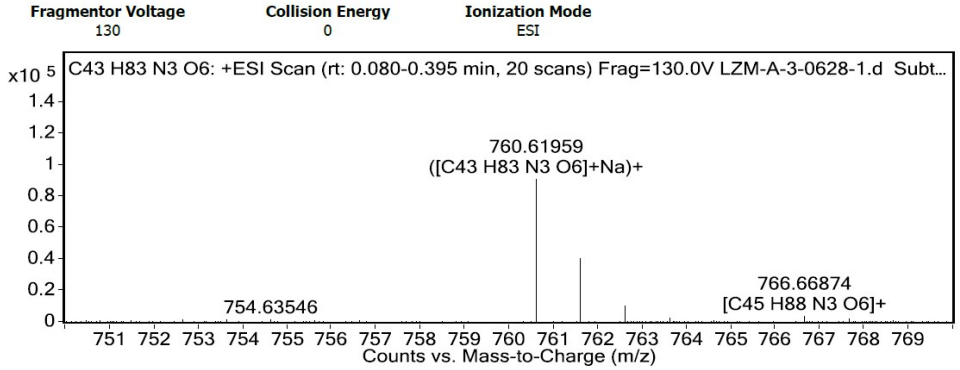
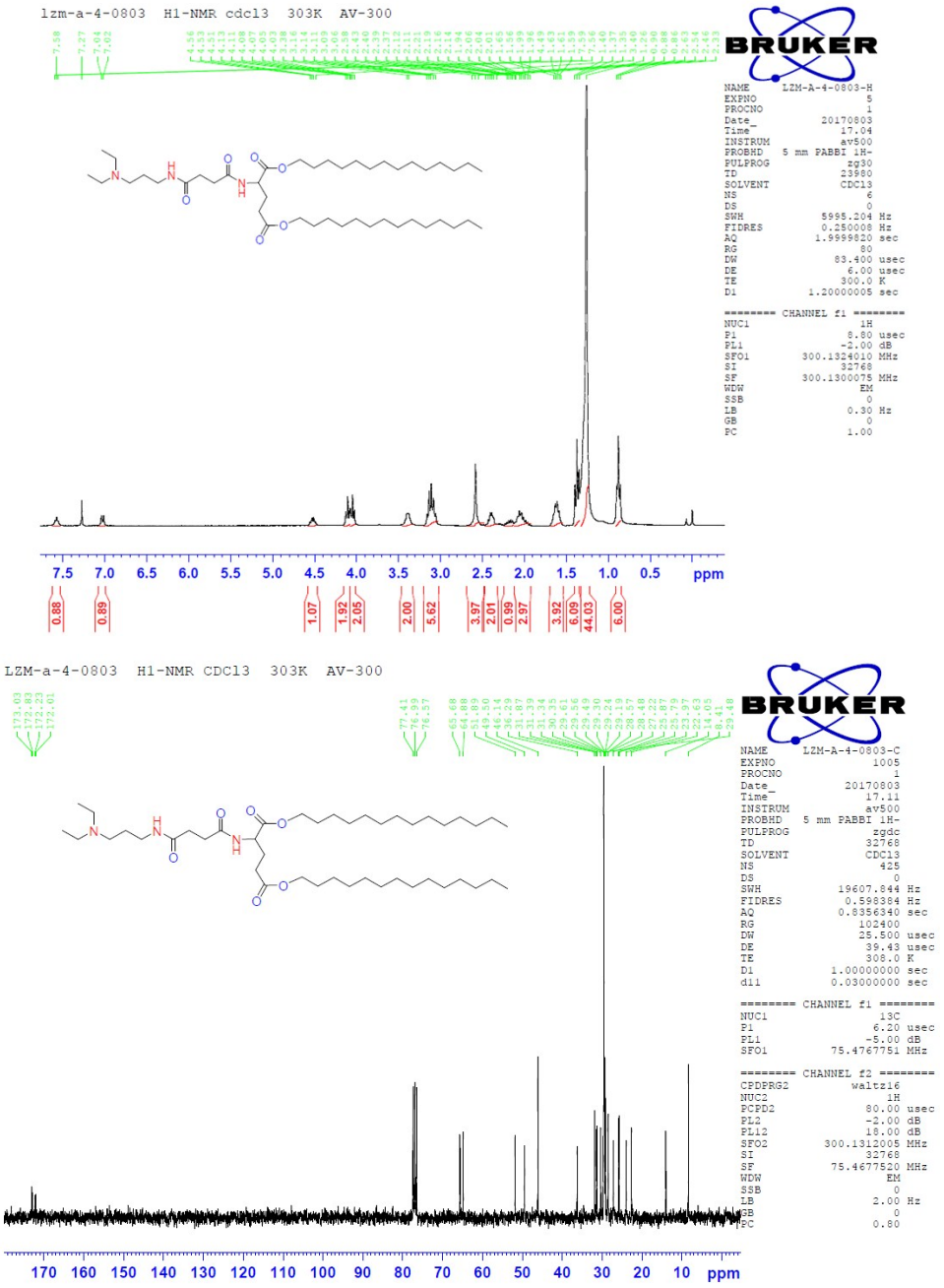


Figure S4. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA3**



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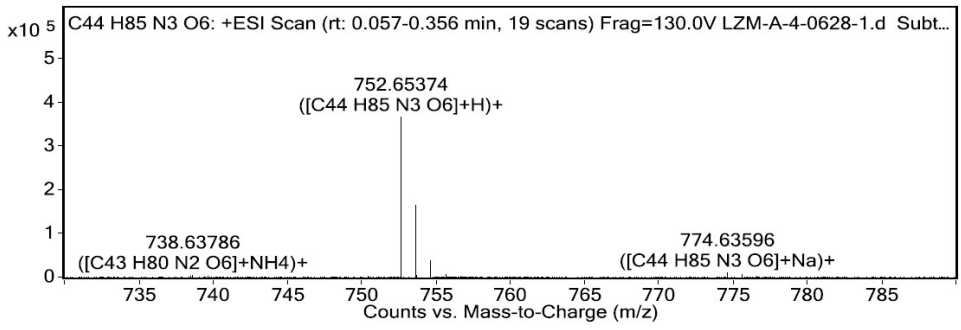
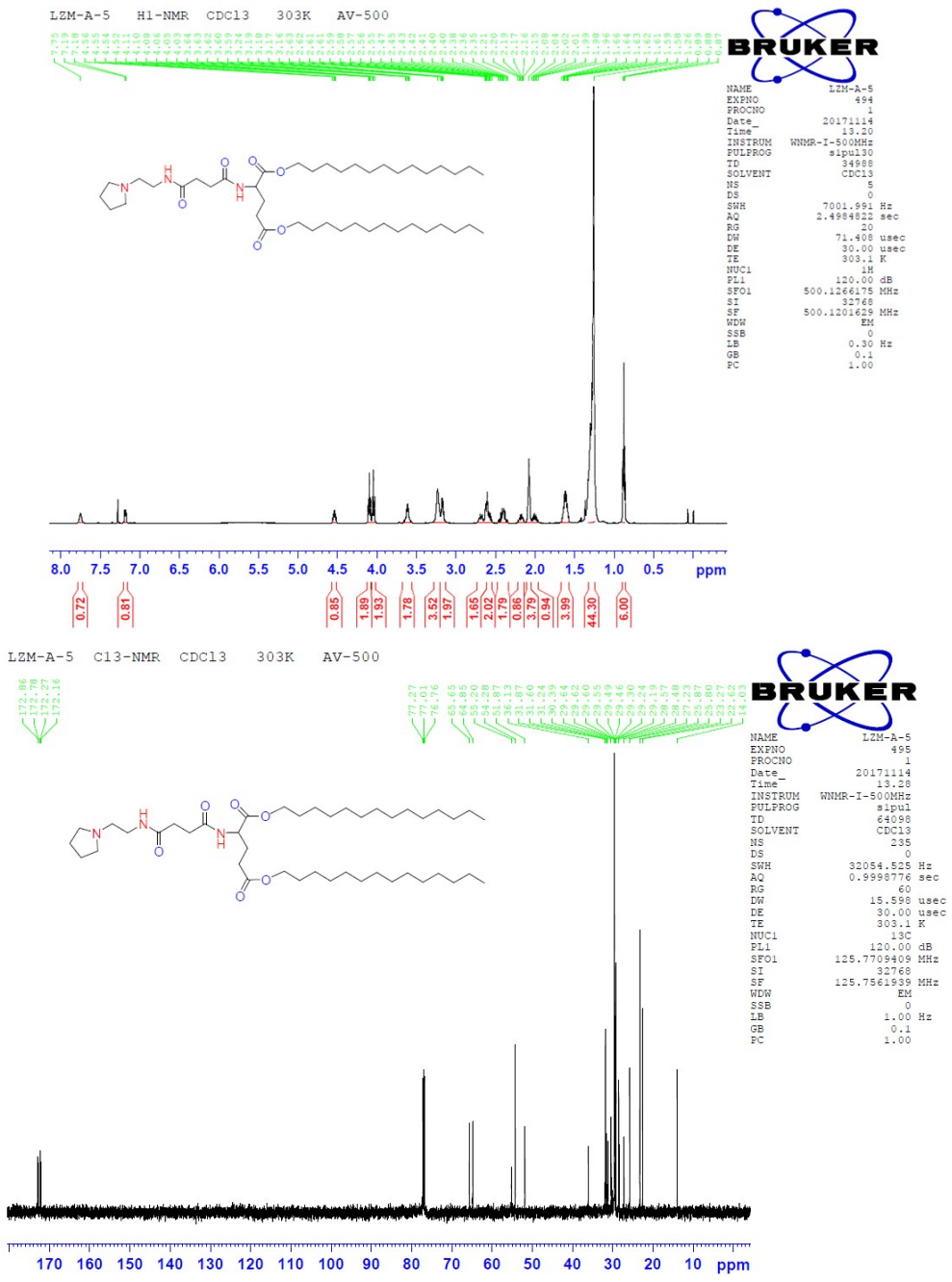


Figure S5. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA4**



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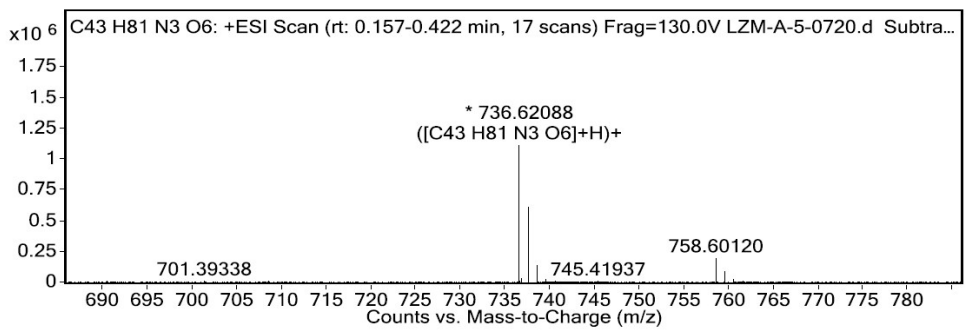
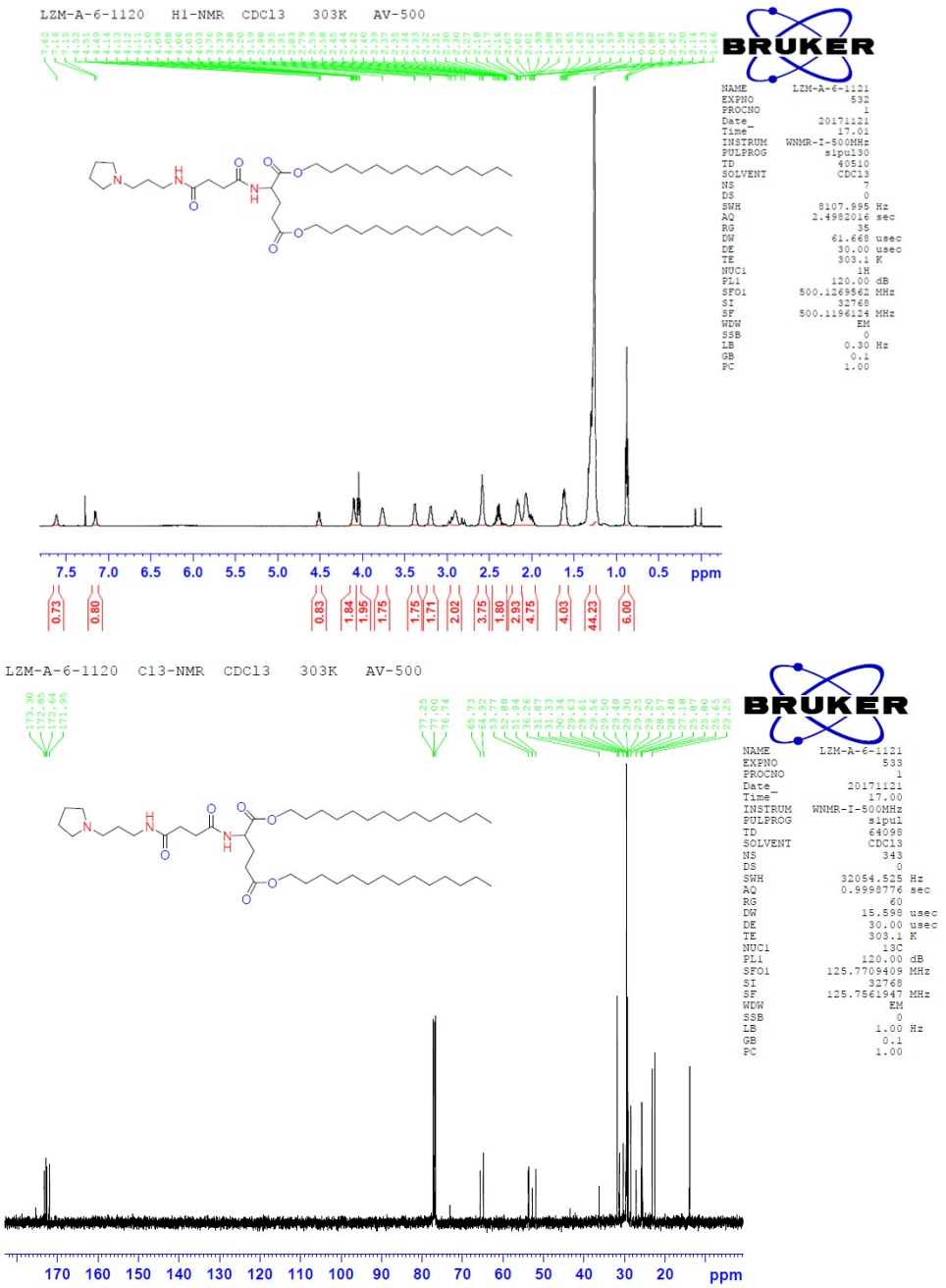


Figure S6. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA5**



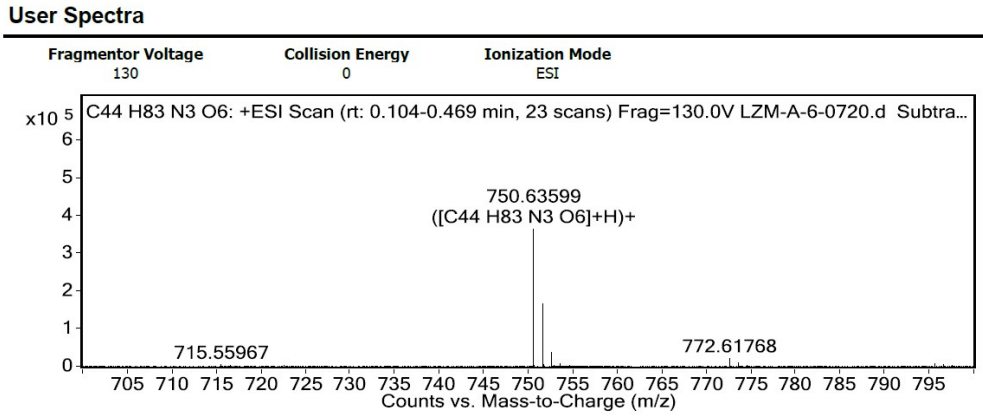
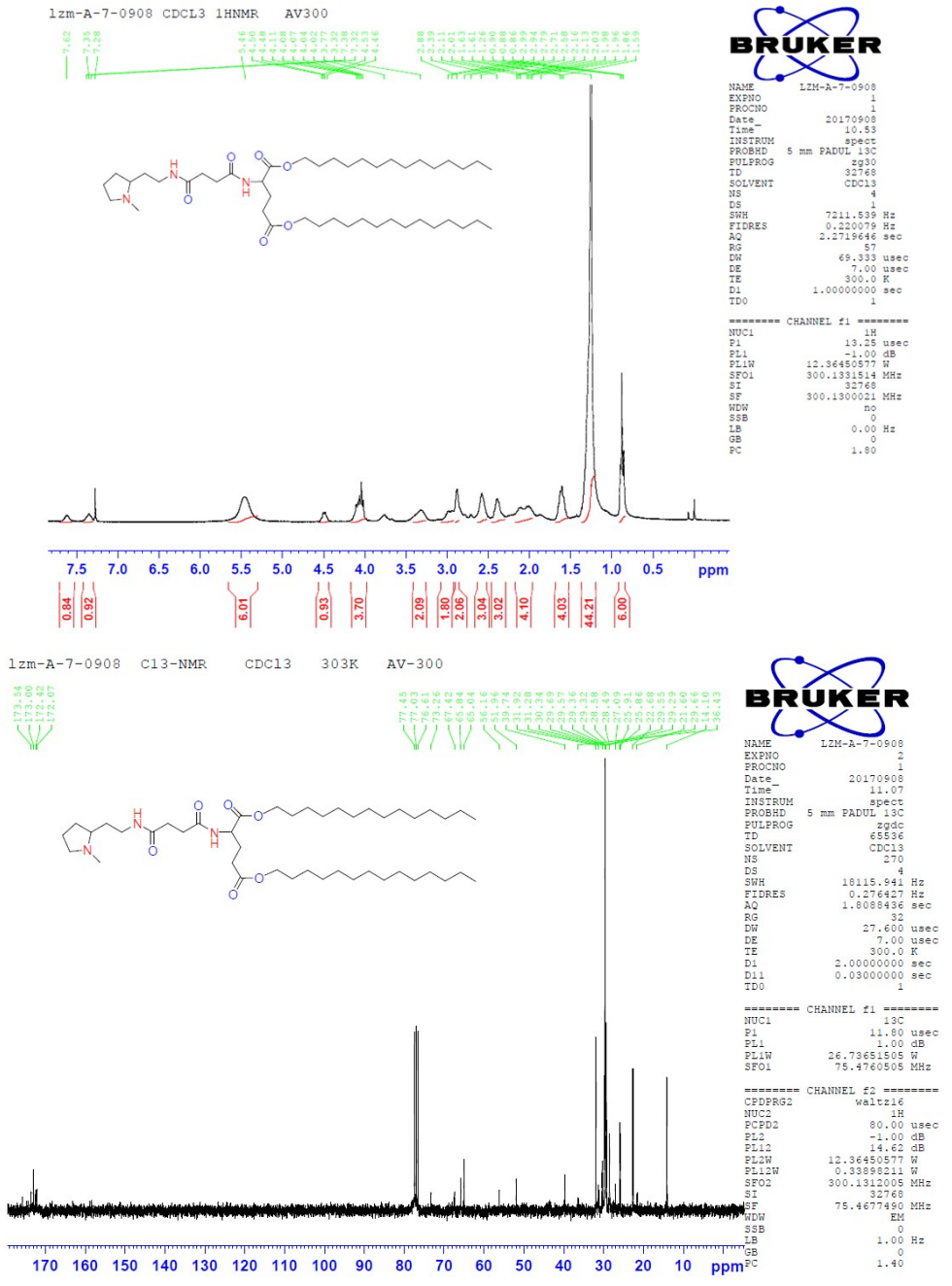


Figure S7. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA6**



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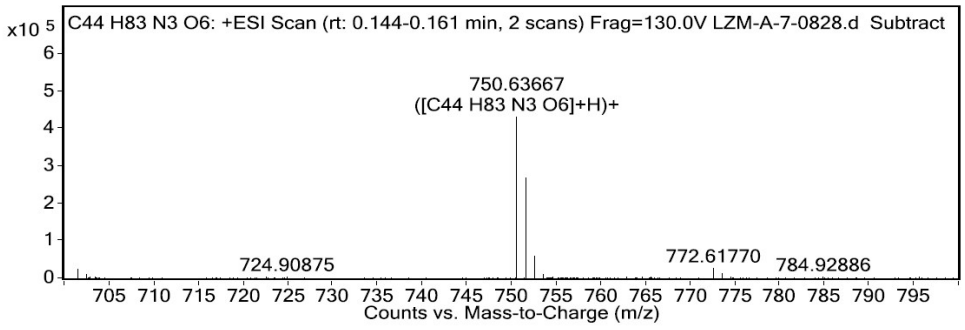
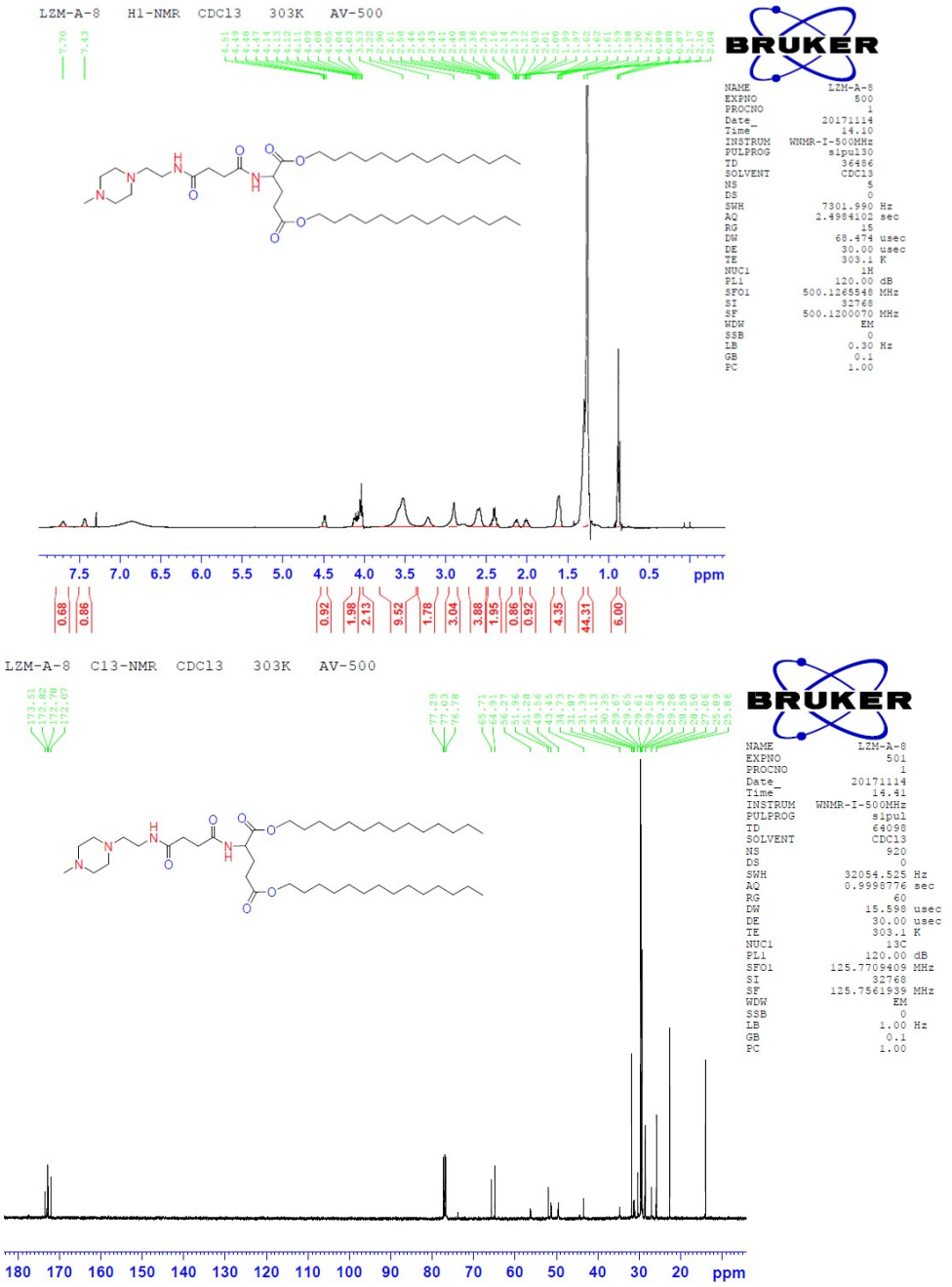


Figure S8. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA7**



User Spectra

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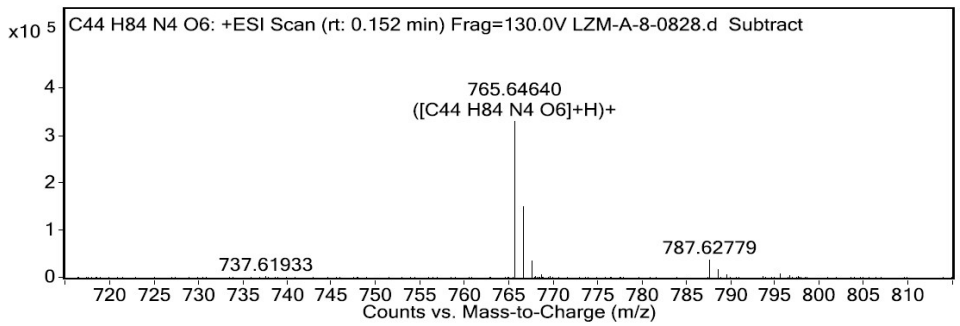
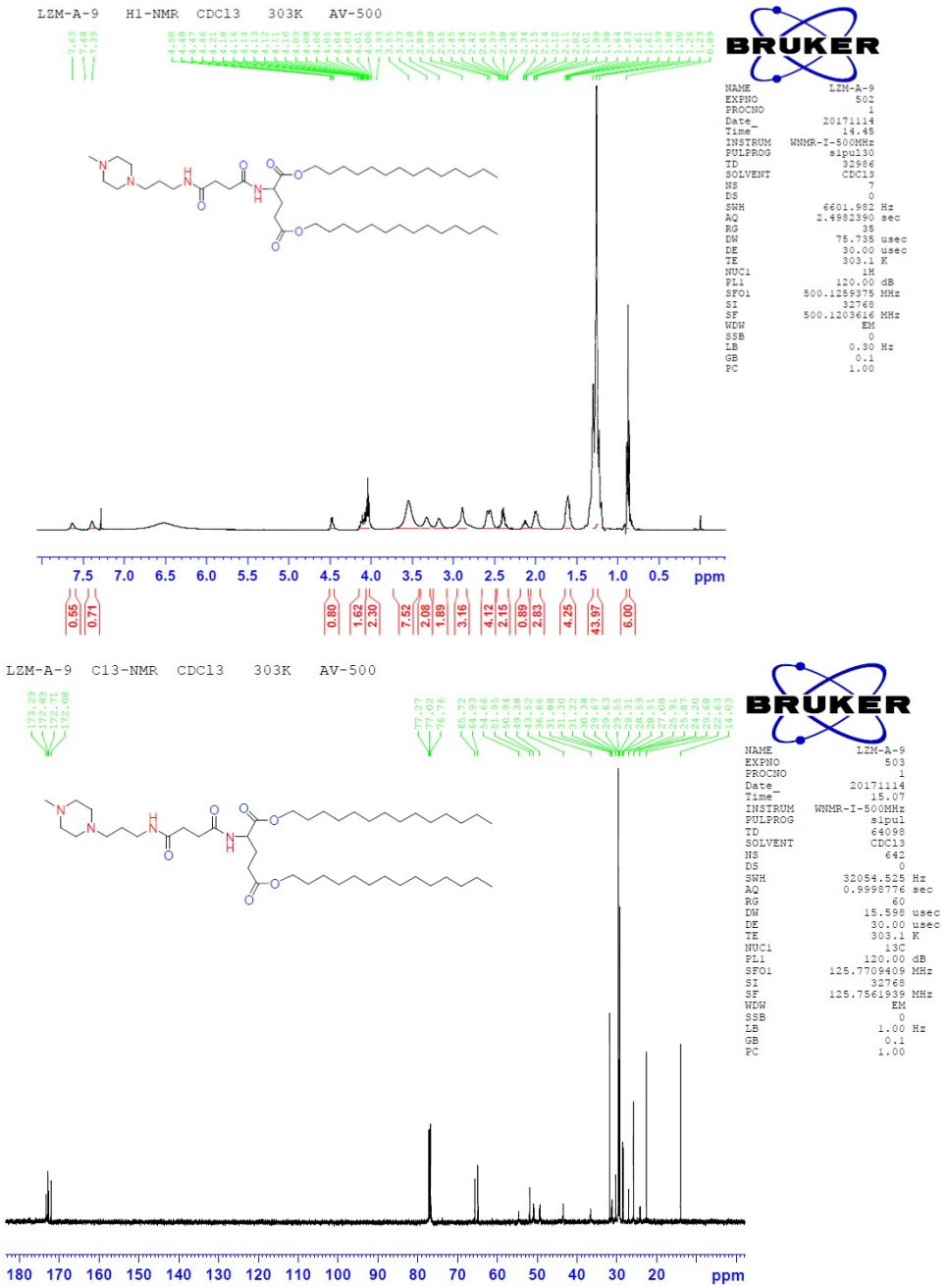


Figure S9. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA8**



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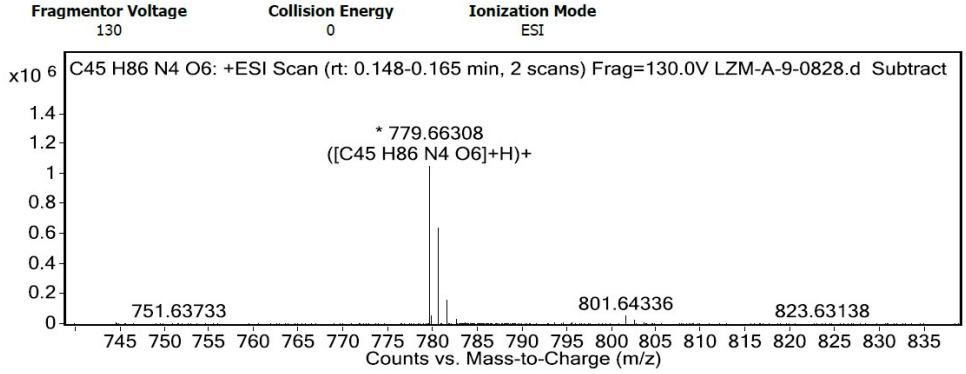


Figure S10. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA9**

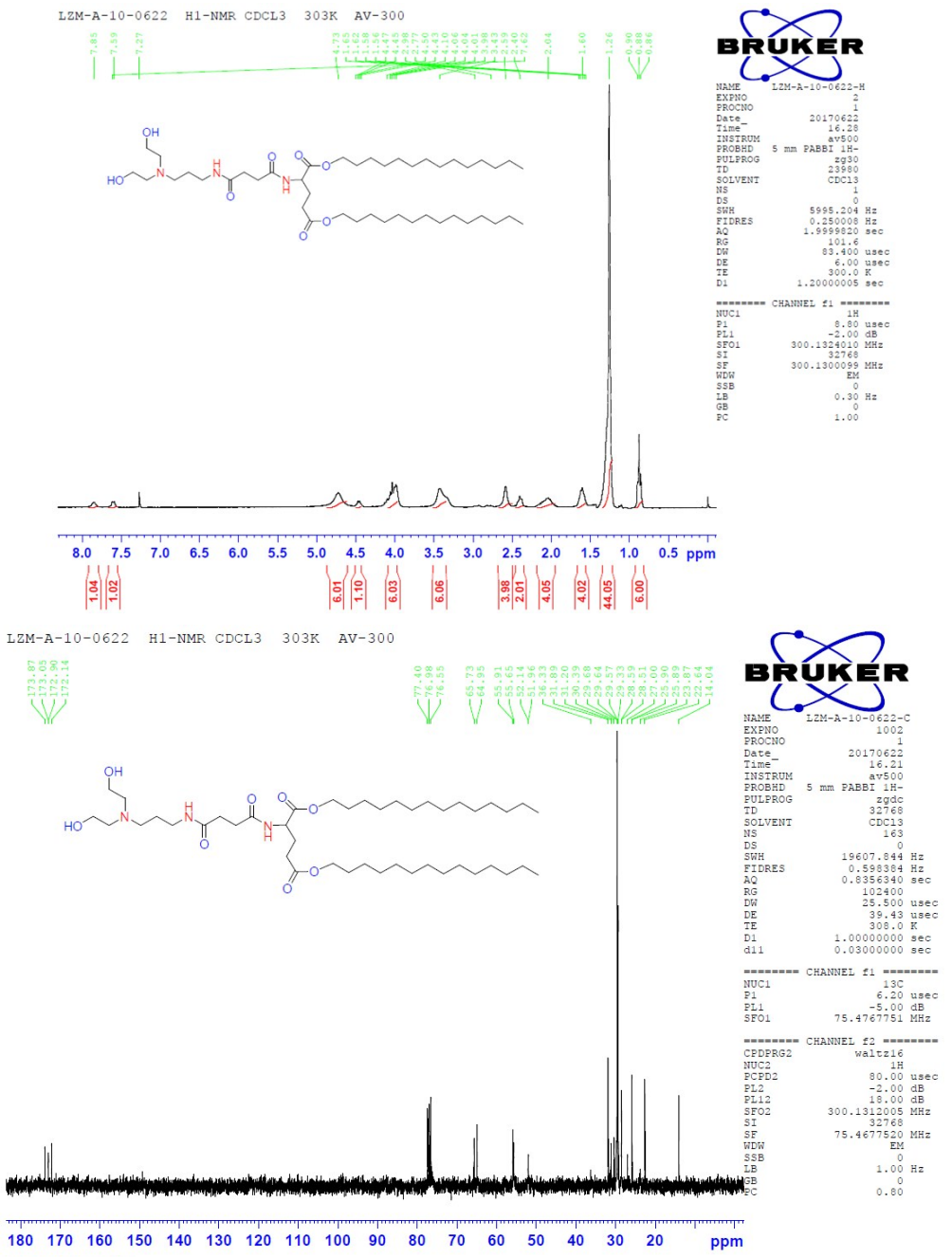
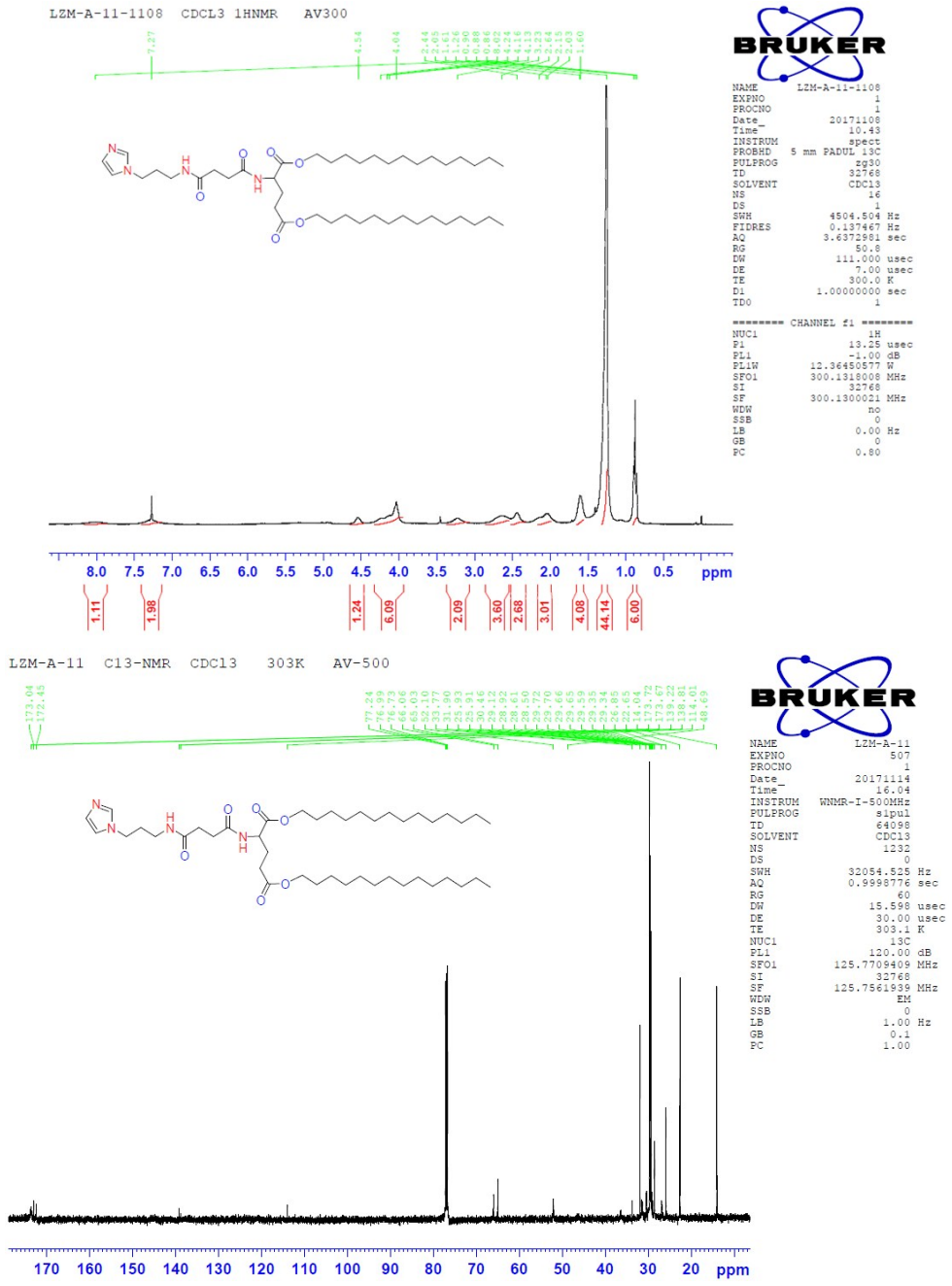


Figure S11. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA10**



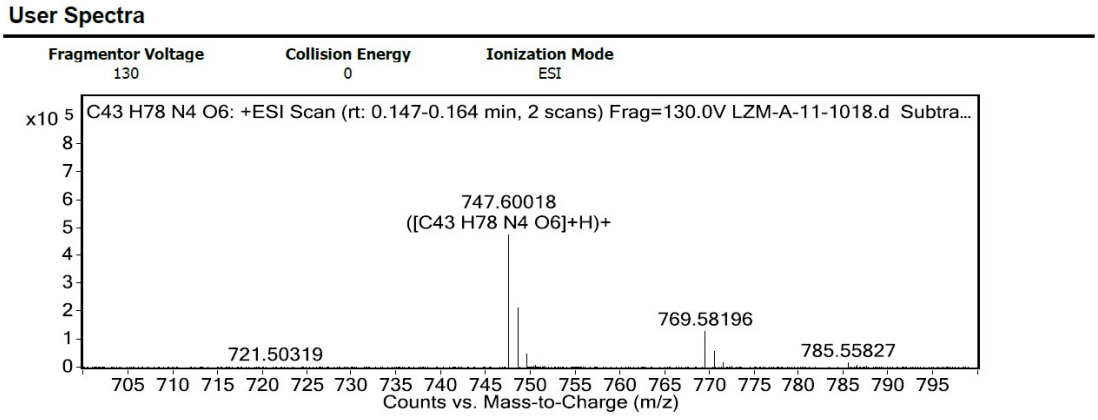
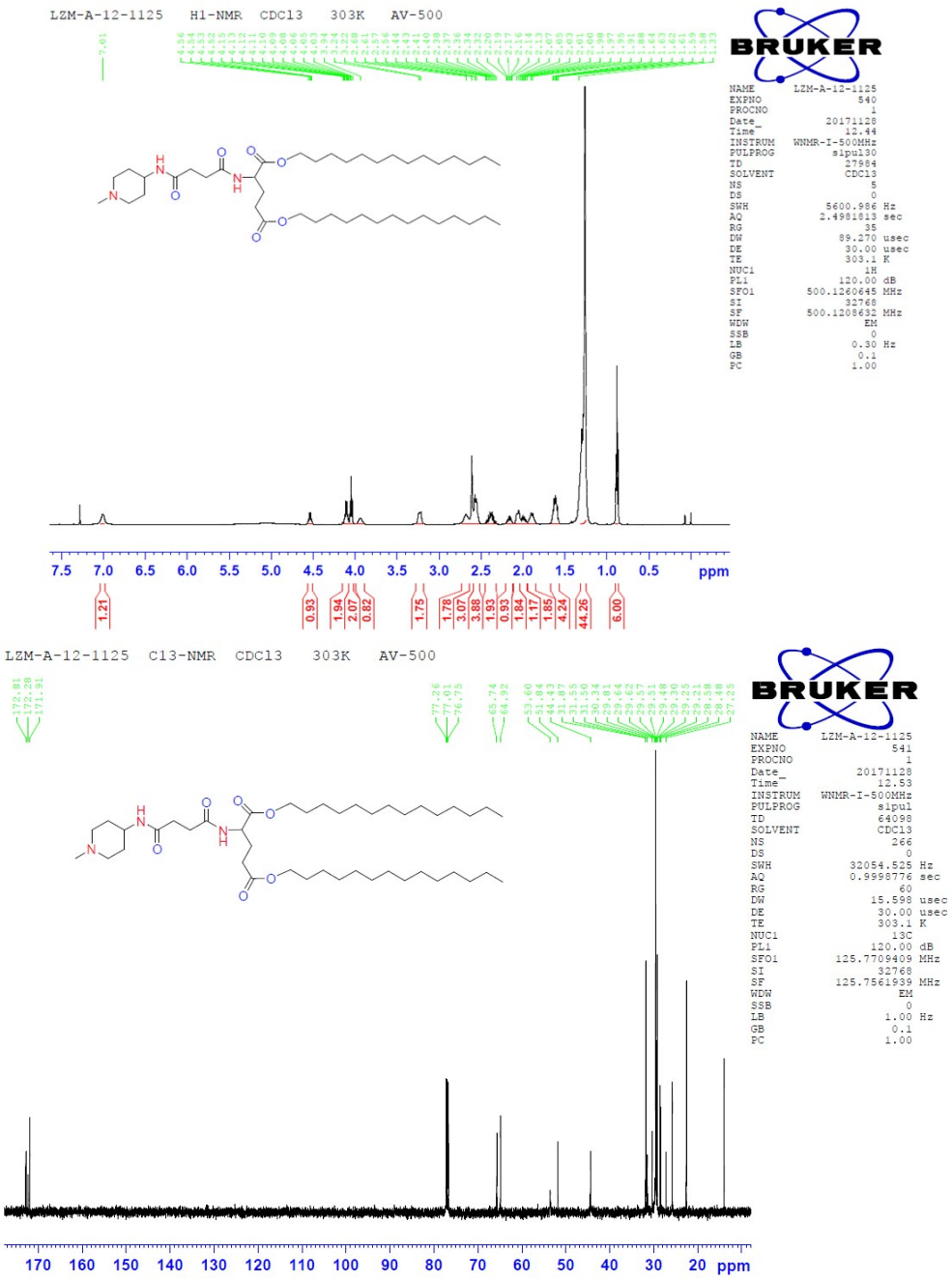


Figure S12. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA11**



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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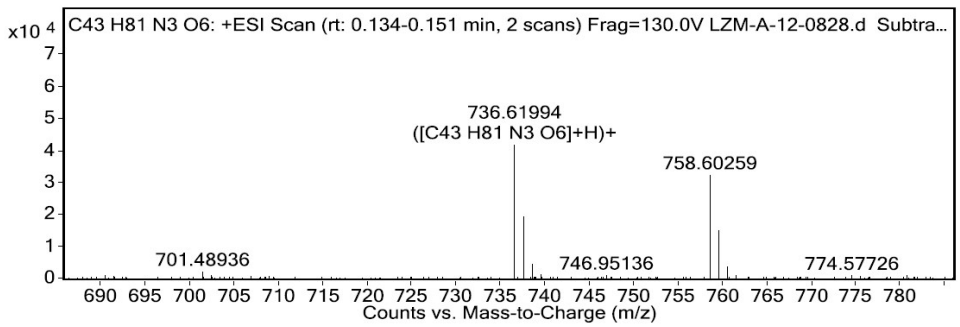
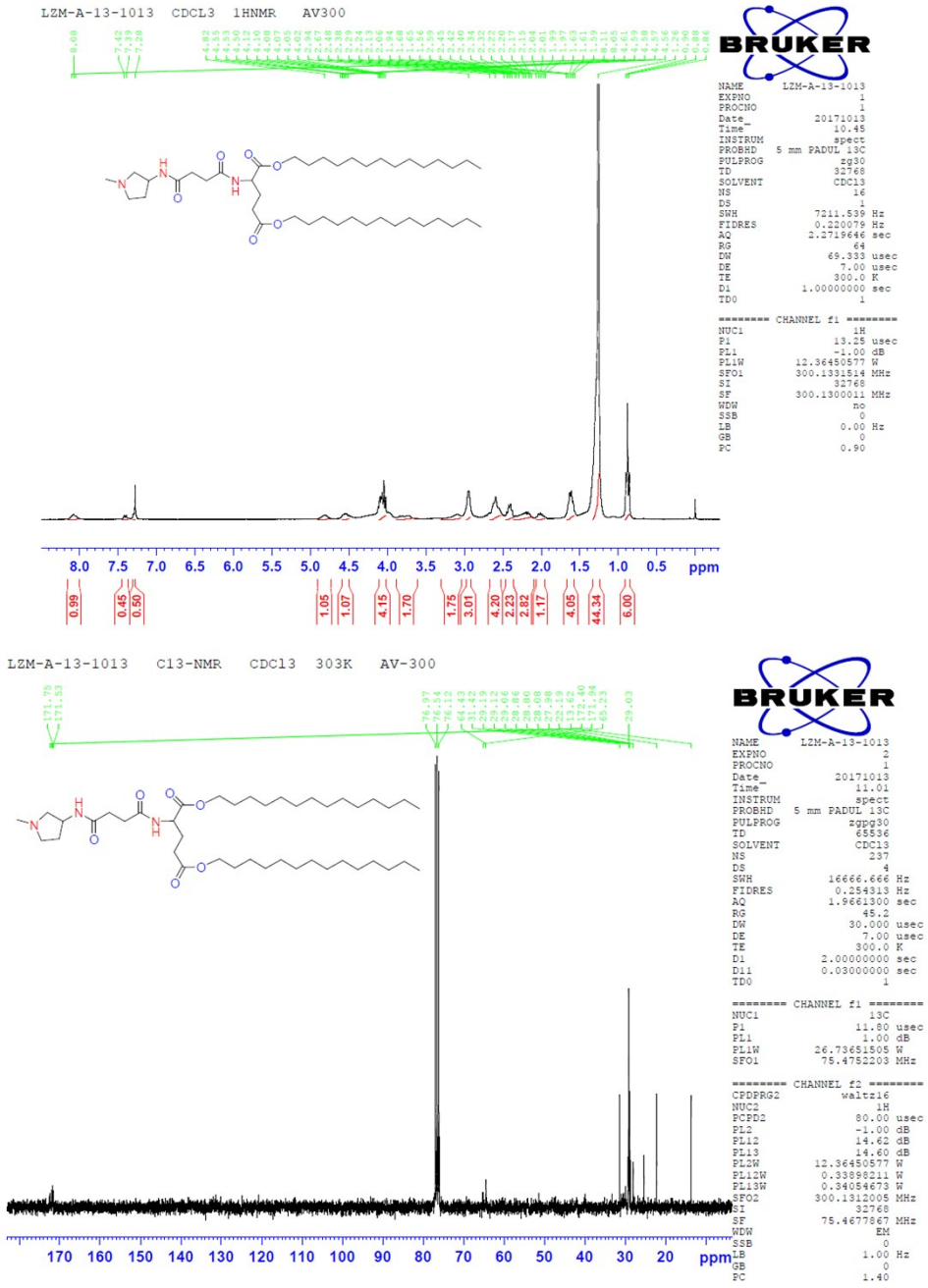


Figure S13. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA12**



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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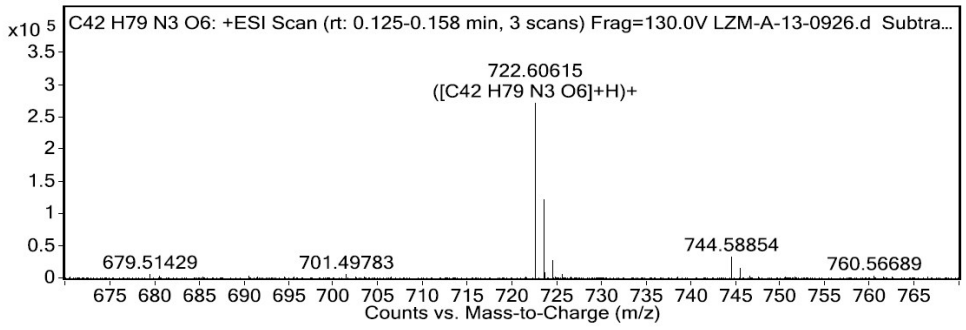
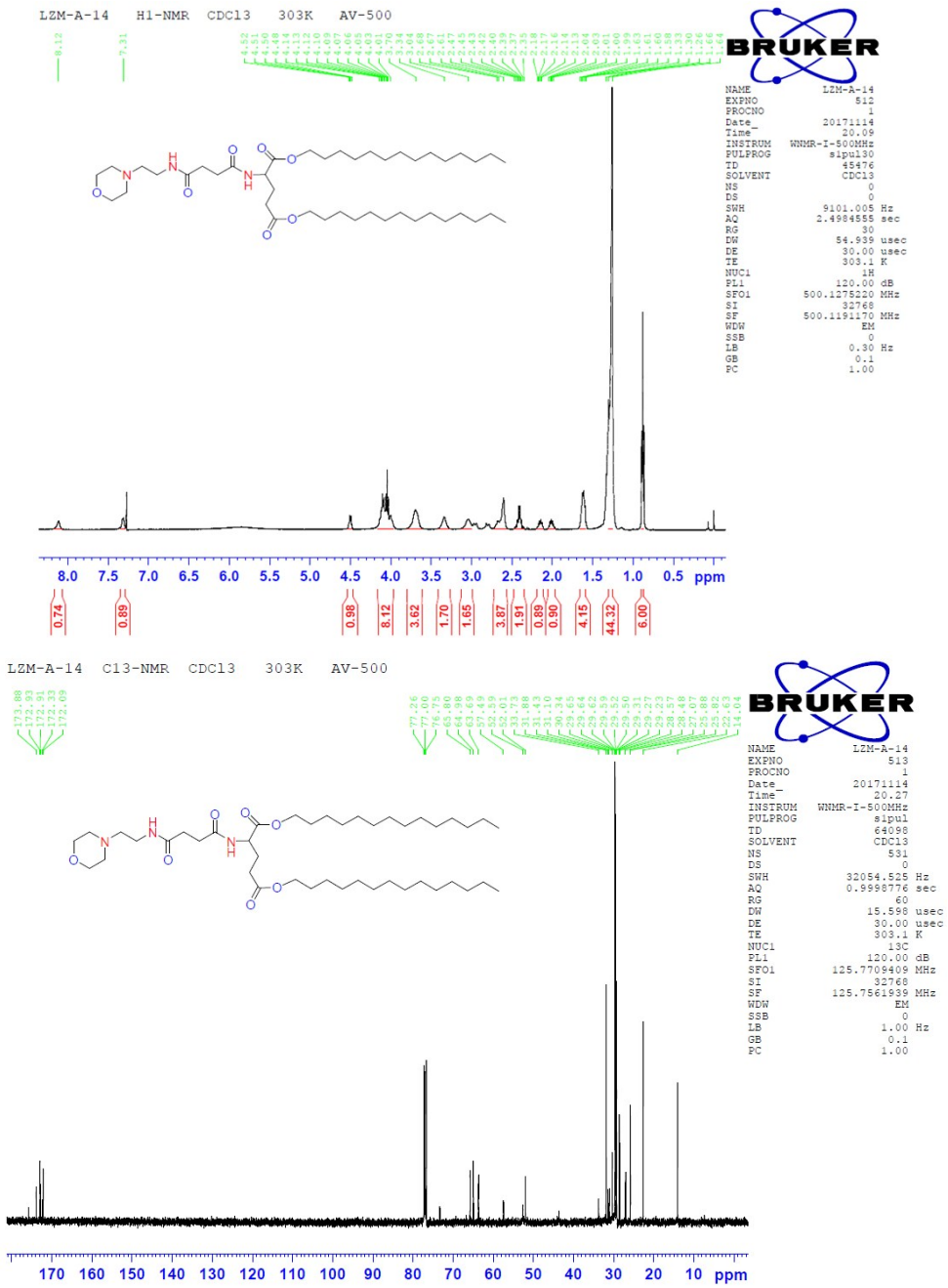


Figure S14. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA13**



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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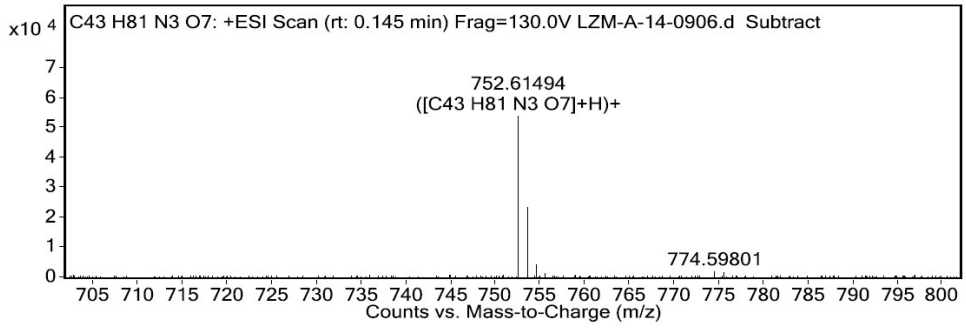
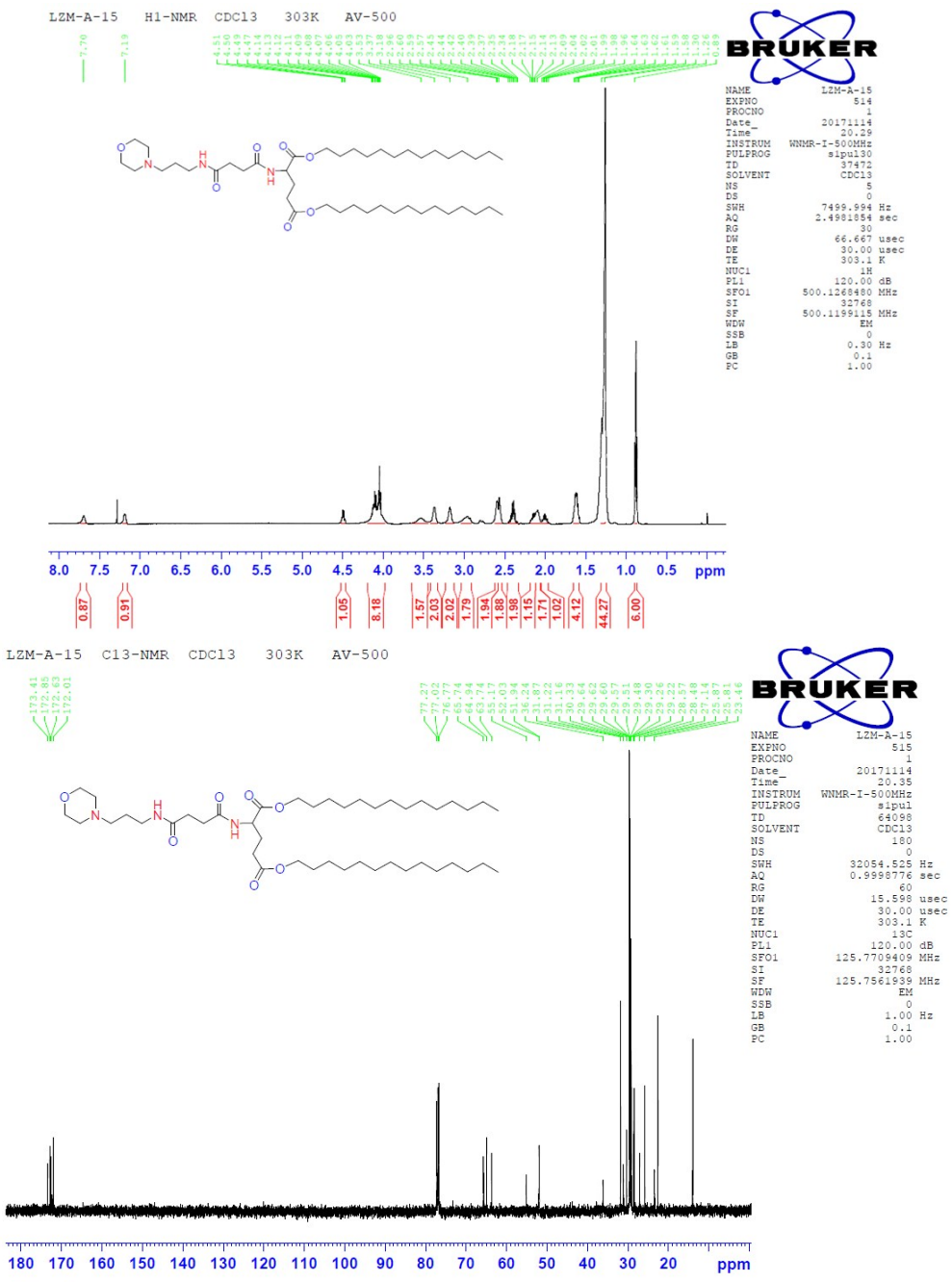


Figure S15. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA14**



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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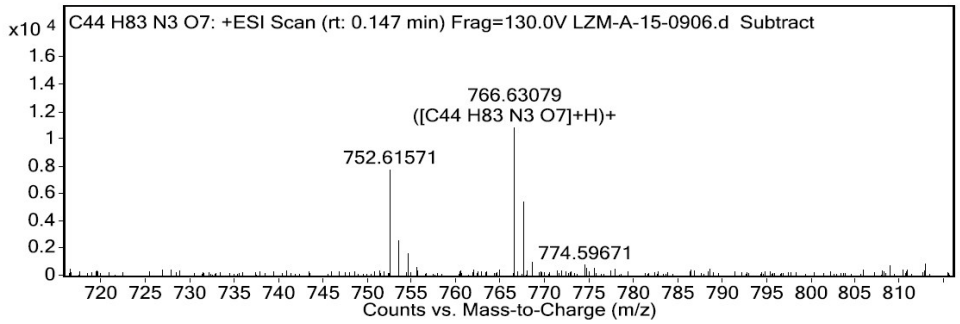
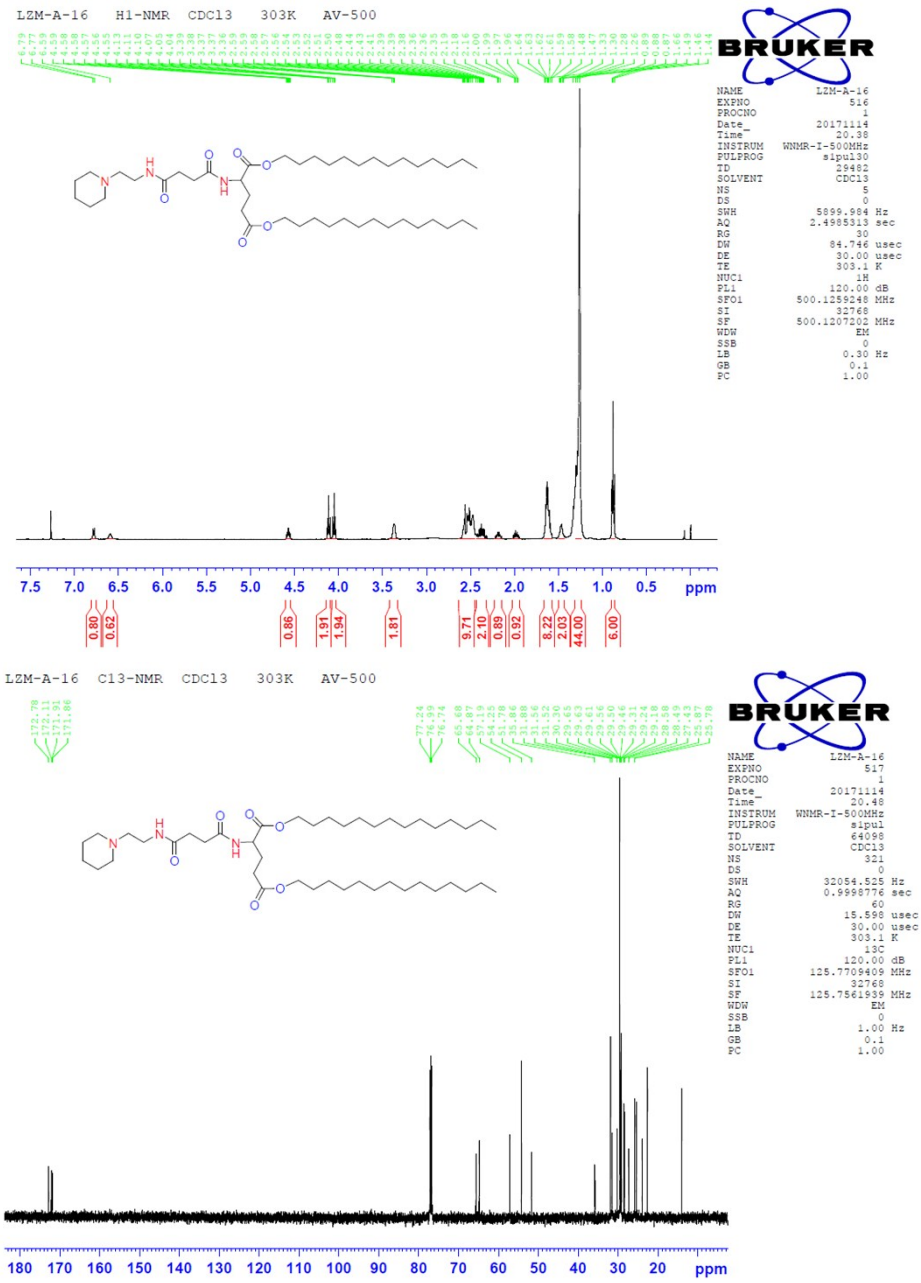


Figure S16. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA15**



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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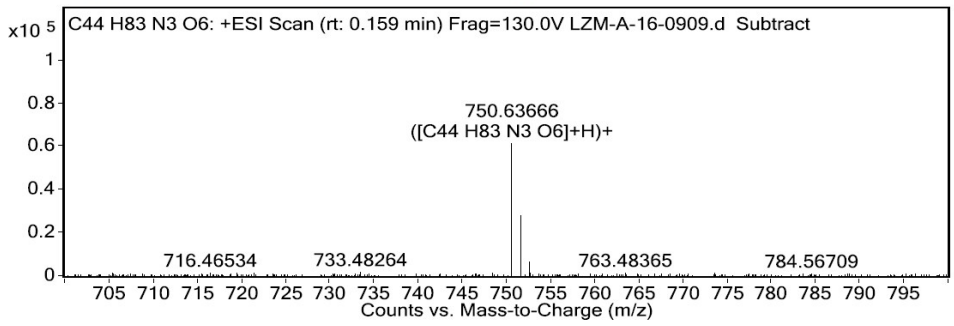
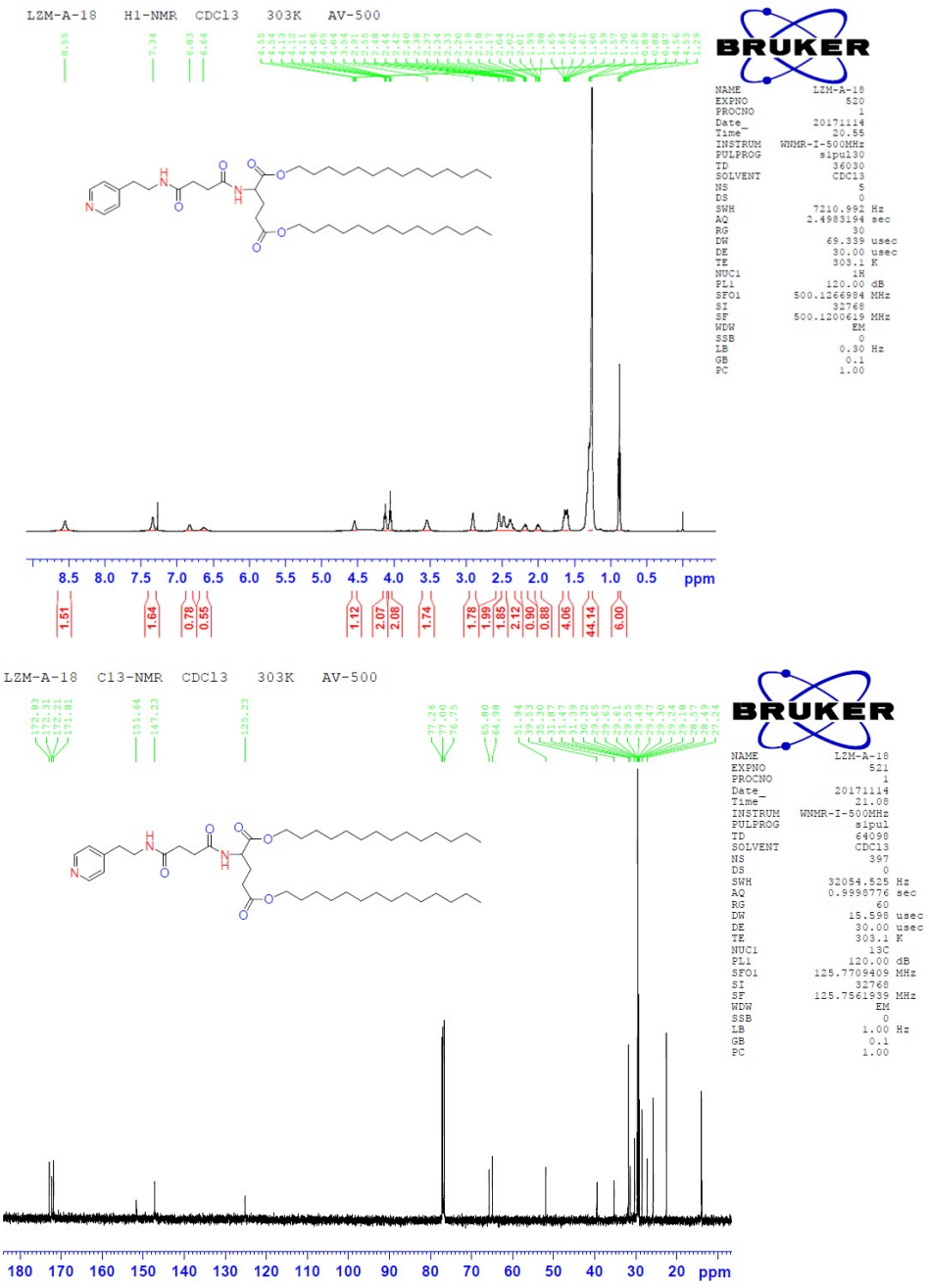


Figure S17. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA16**



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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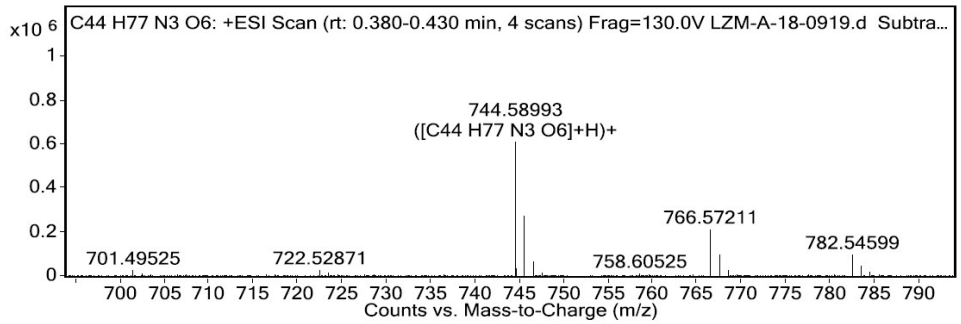
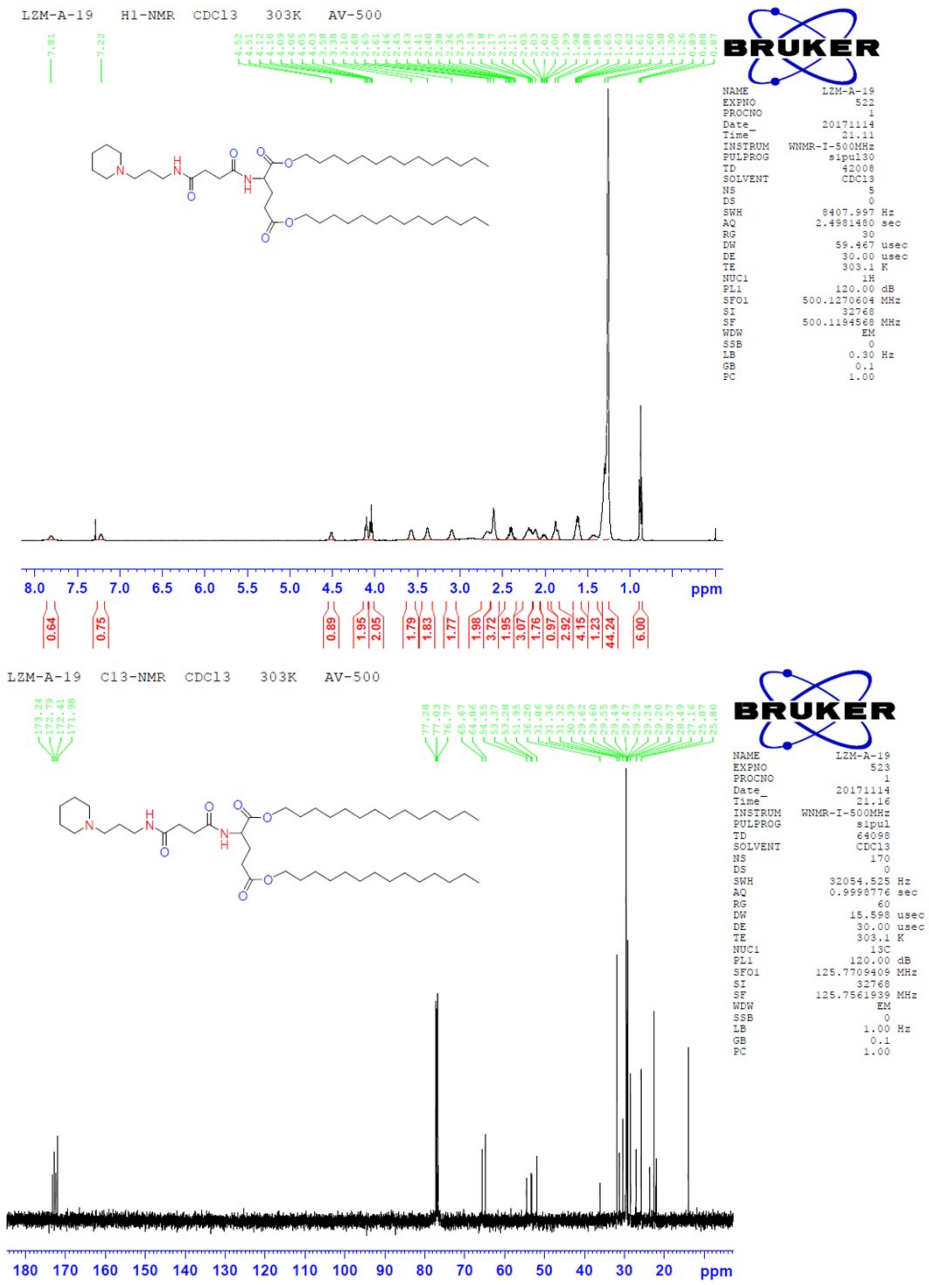


Figure S18. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA17**



User Spectra

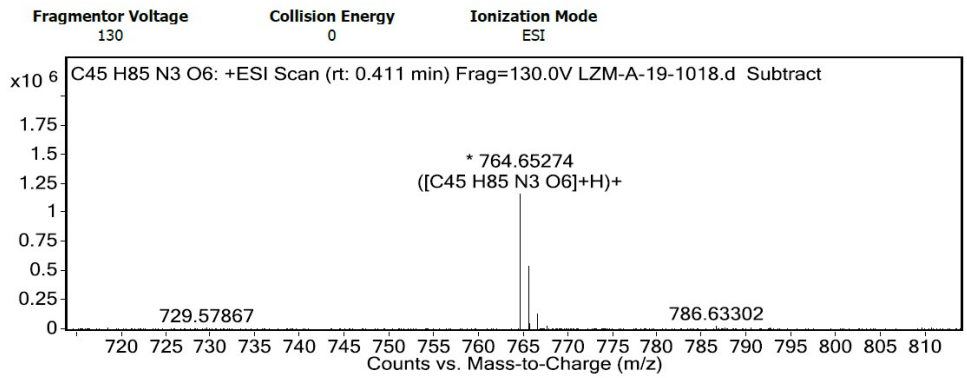
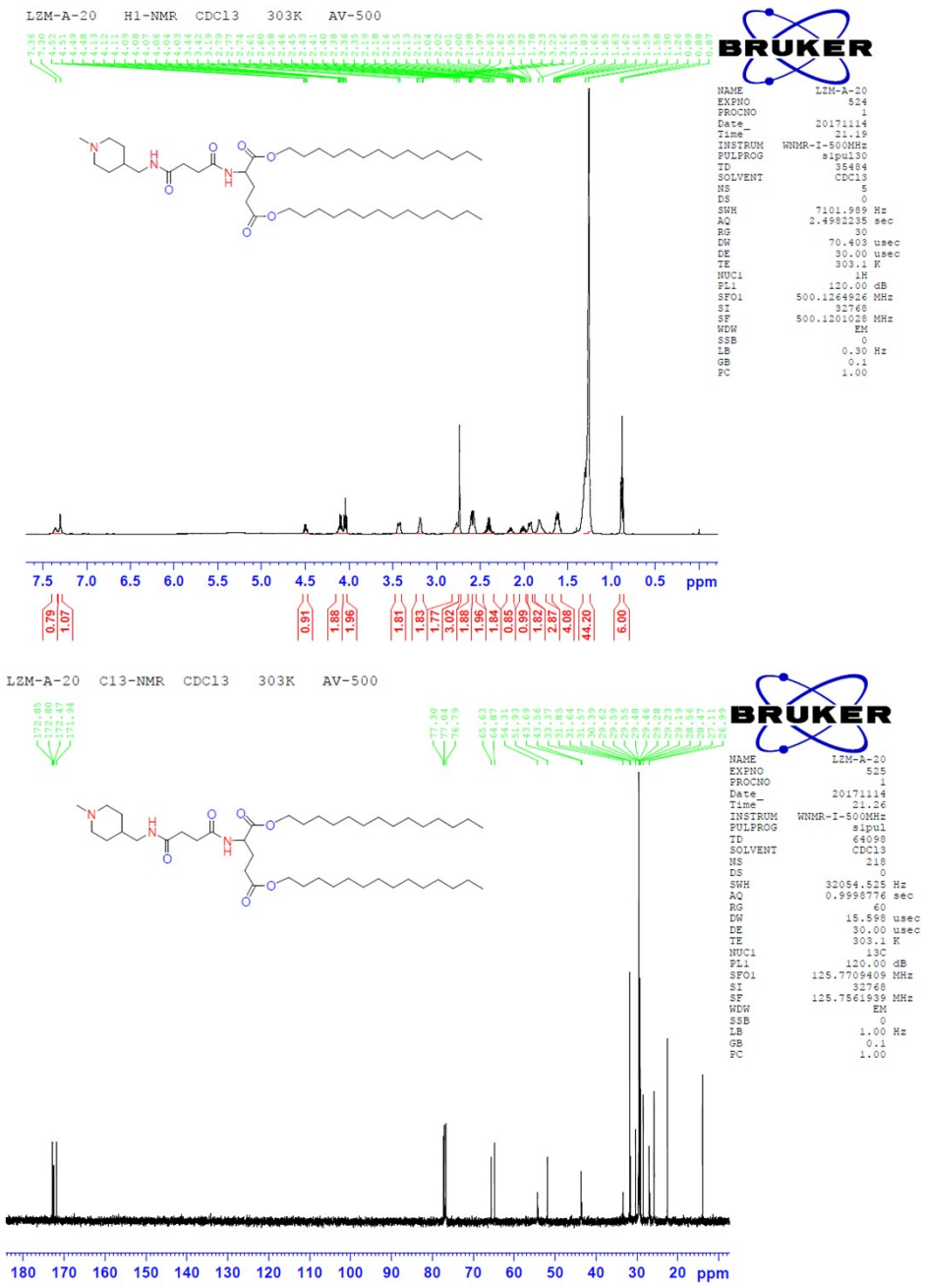


Figure S19. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA18**



User Spectra

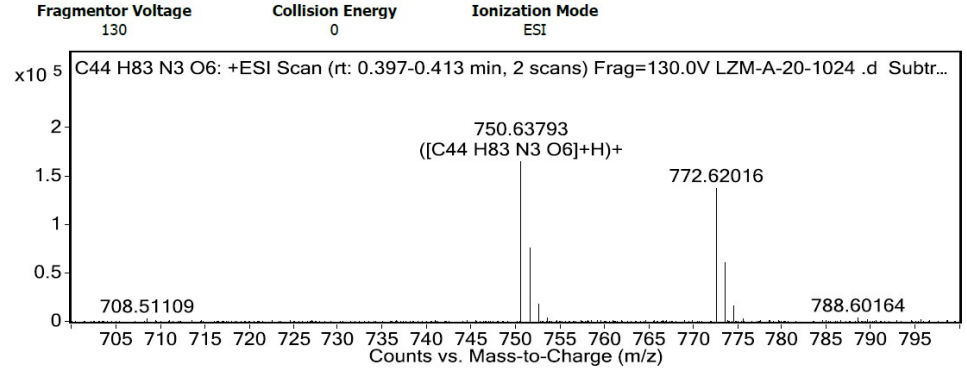
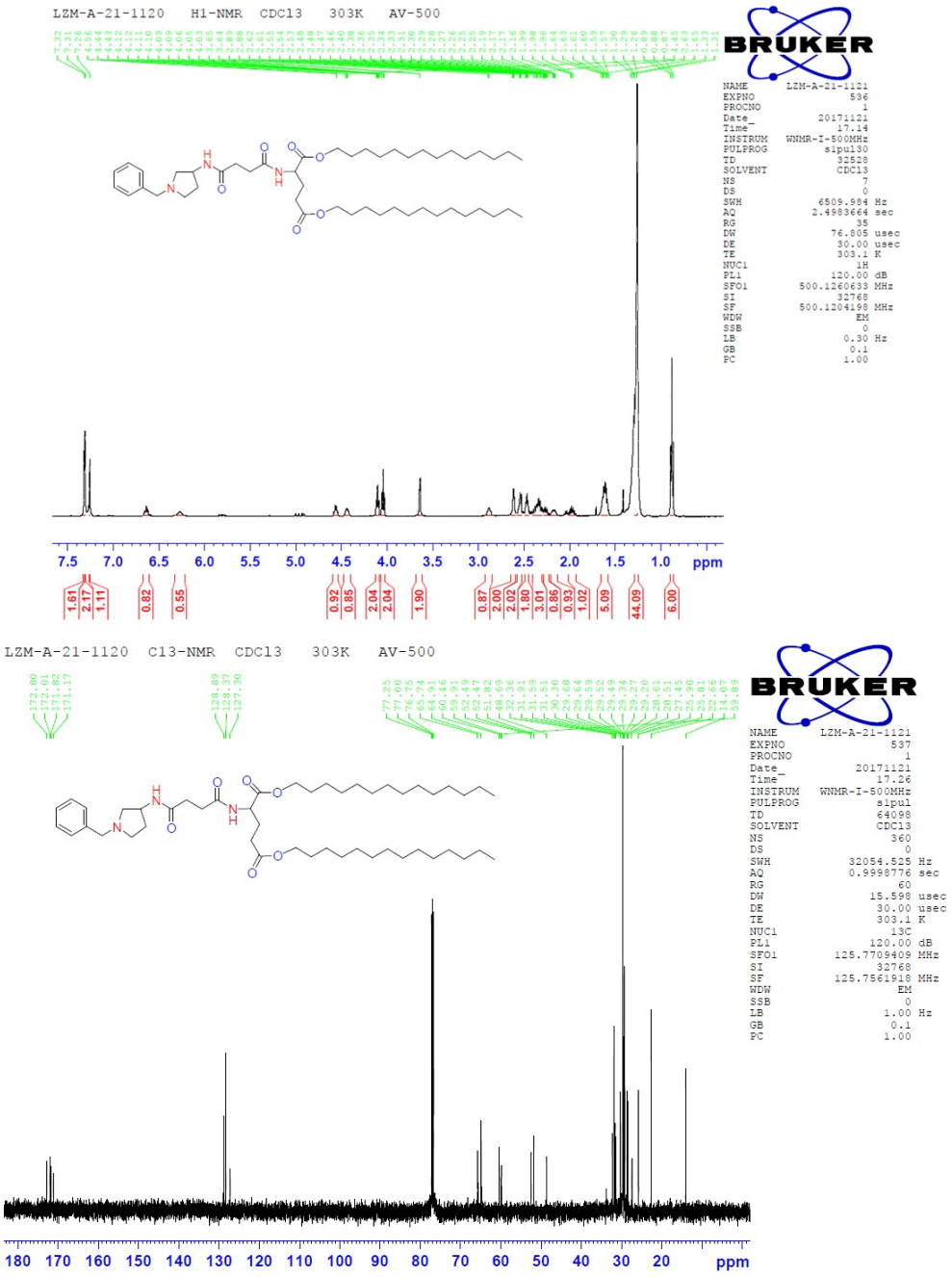


Figure S20. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA19**



User Spectra

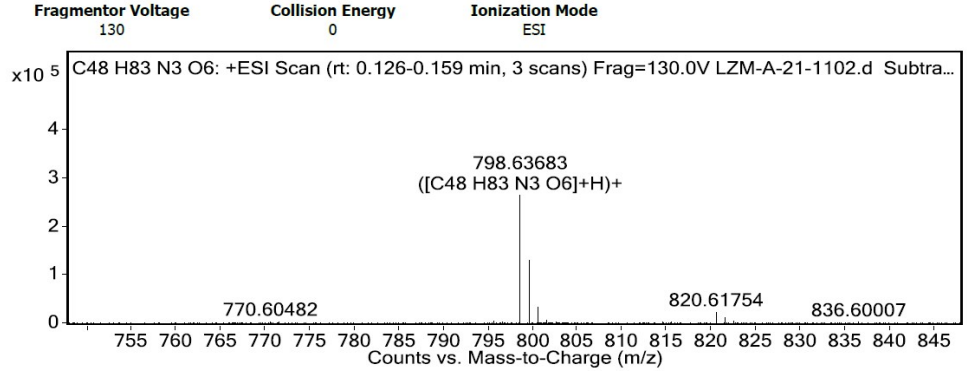
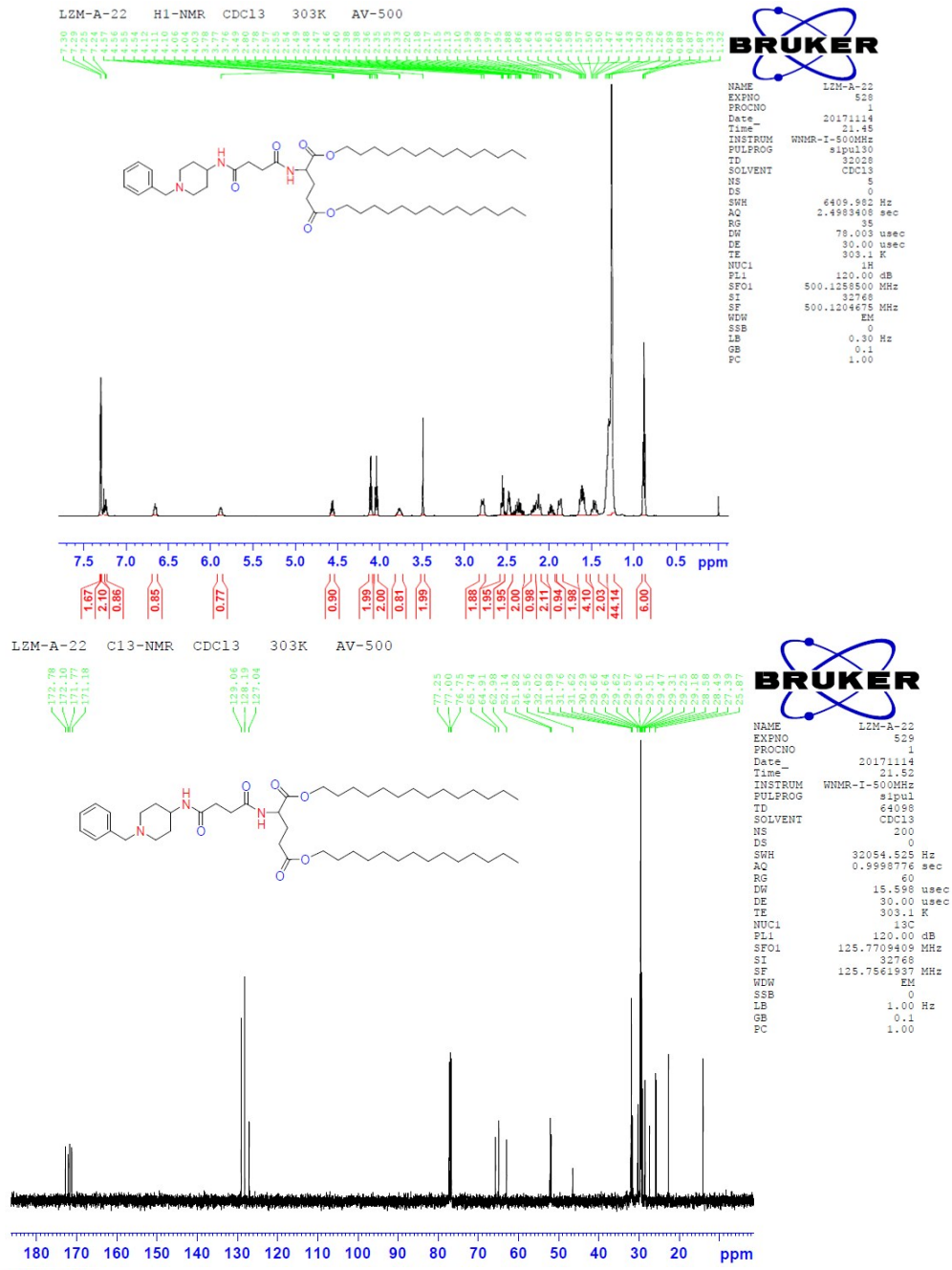


Figure S21. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA20**



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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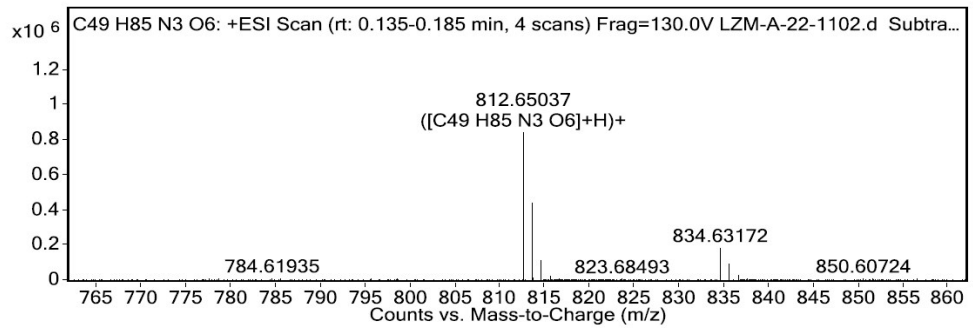

Figure S22. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **TA21**

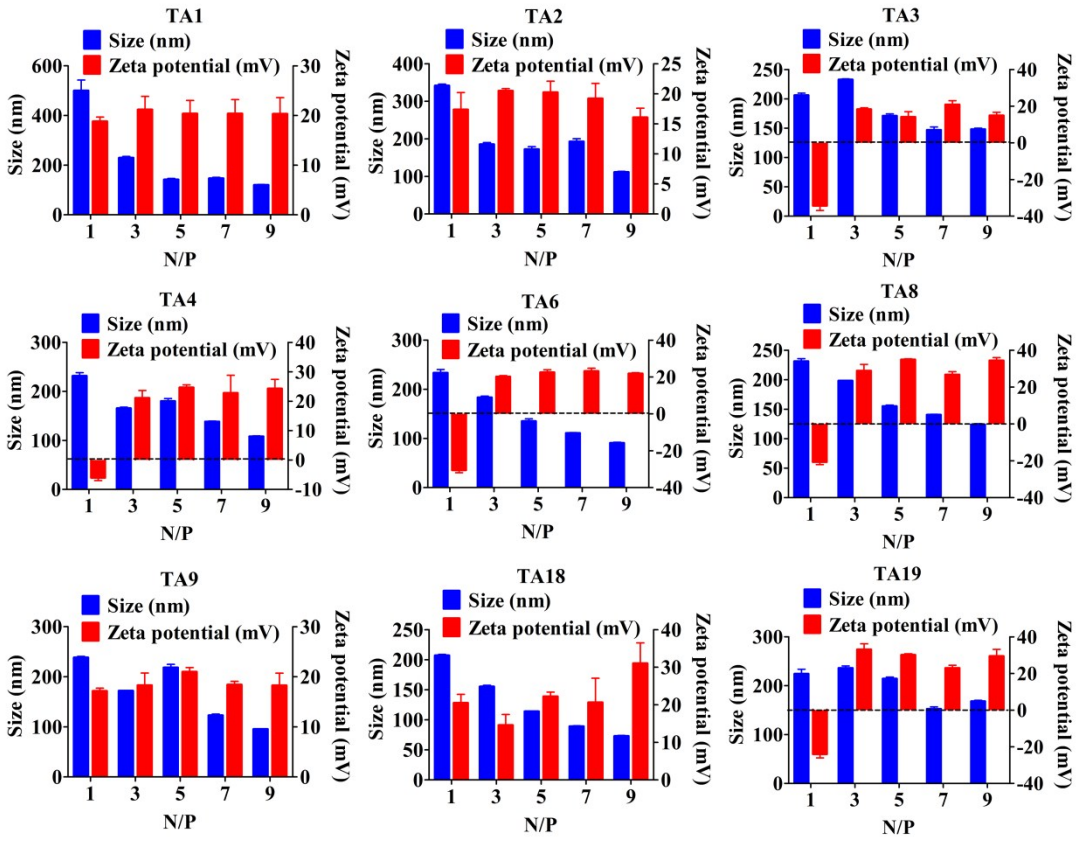

Figure S23. Mean particle sizes and zeta potentials of lipoplexes at N/P ratios of 1, 3, 5, 7 and 9.

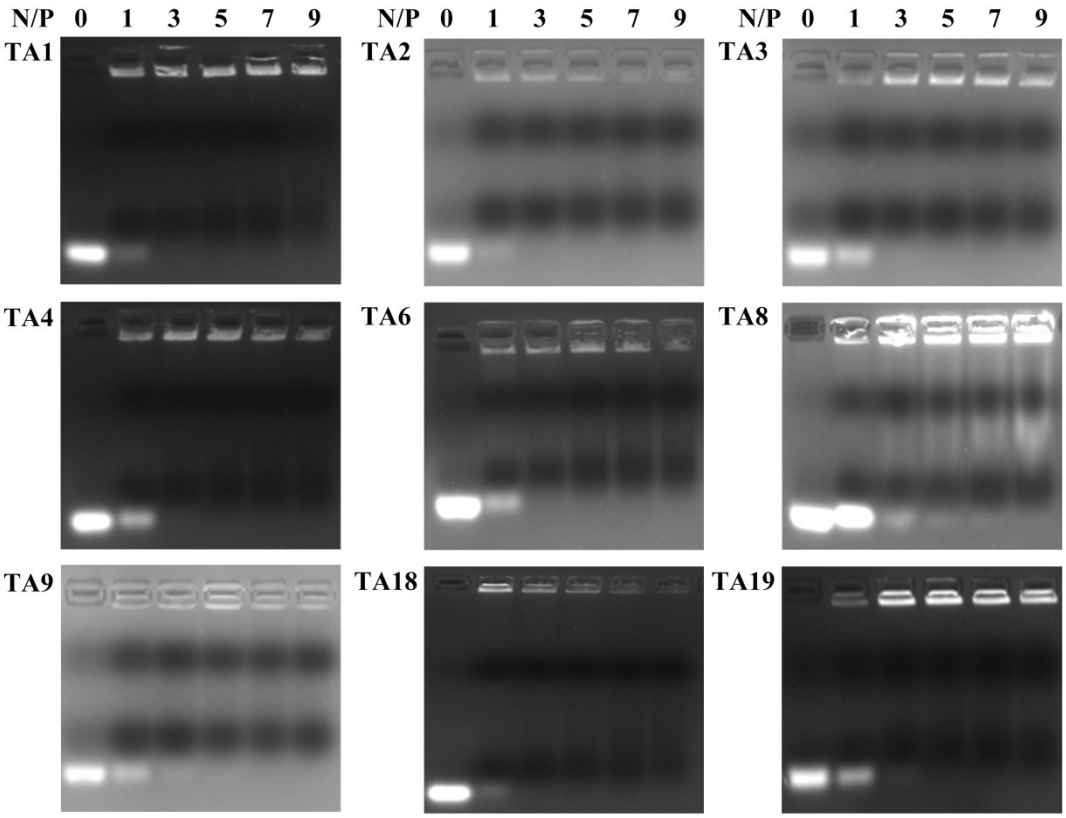

Figure S24. Agarose gel electrophoresis of lipoplexes at N/P ratios of 1, 3, 5, 7 and 9, indicating the successful siRNA encapsulation at the N/P more than 5. (N/P = 0 stands for naked siRNA)

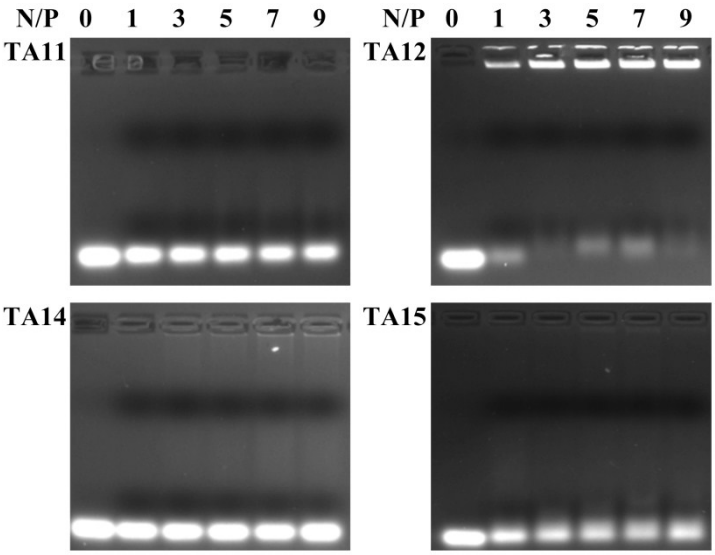

Figure S25. Agarose gel electrophoresis of lipoplexes at N/P ratios of 1, 3, 5, 7 and 9. However, the lipoplexes of TA11, TA12, TA14 and TA15 cannot successfully encapsulate siRNA at all N/P ratios. (N/P = 0 stands for naked siRNA)

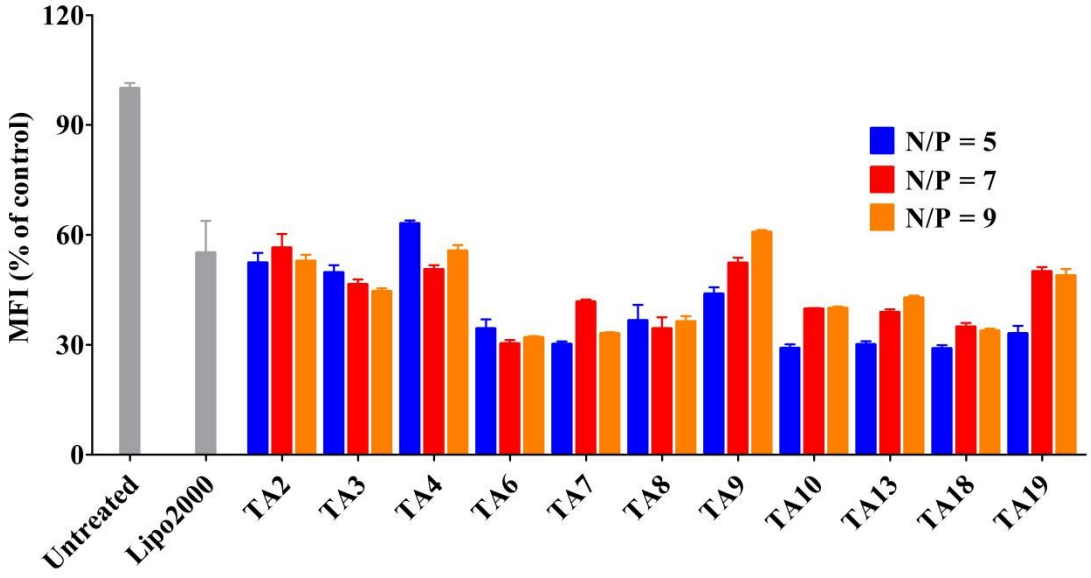

Figure S26. *In vitro* gene silencing efficiency of lipoplexes in Hela-eGFP cells using flow cytometry. Cells were treated with different lipoplexes at recommended siRNA transfection concentration (200 nM) of Lipofectamine 2000 at N/P ratios of 5, 7 and 9. Lipofectamine 2000 served as a positive control. Untreated cells served as a negative control.

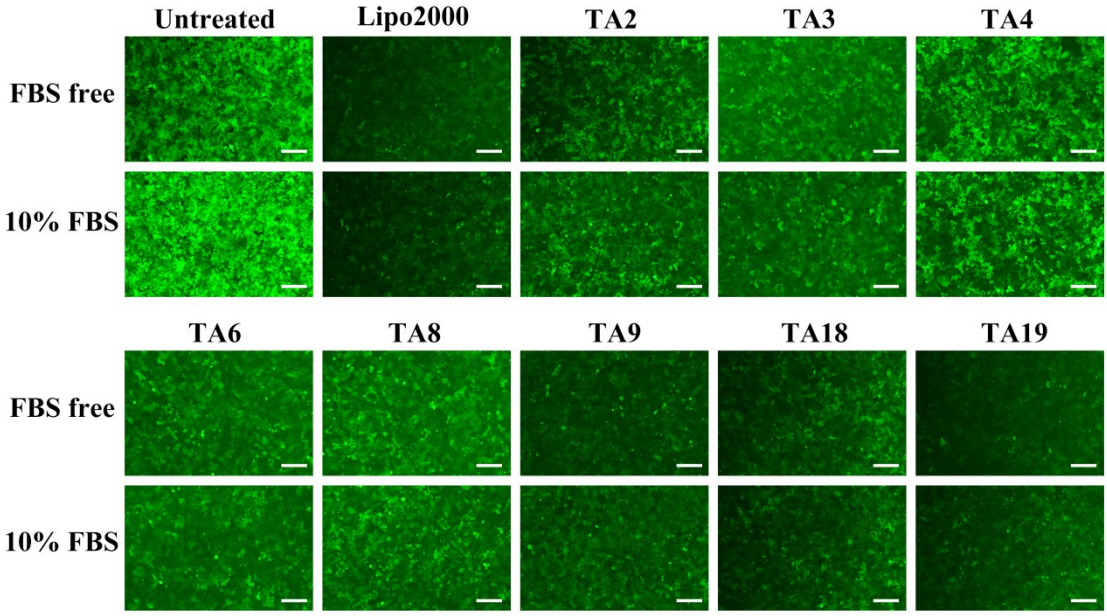

Figure S27. *In vitro* gene silencing efficiency of the lipoplexes in Hela-eGFP cells by inverted fluorescence microscope. Cells were treated with different lipoplexes at N/P ratios of 5 at recommended siRNA transfection concentration (200 nM) of Lipofectamine 2000 in the absence and presence of 10% (v/v) serum. Lipofectamine 2000 served as a positive control. Untreated cells served as a negative control. Scale bar: 100 μm.

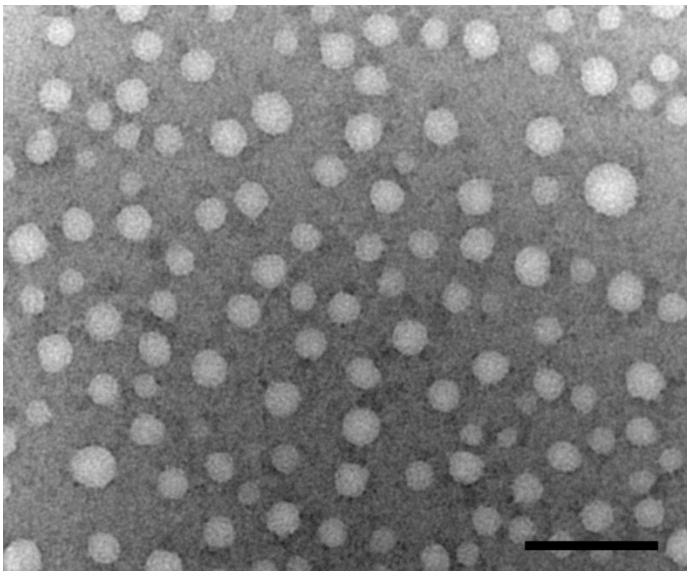

Figure S28. TEM image of TA13 cationic liposomes, indicating uniform and sphere nanoparticle. Scale bar: 100 nm.

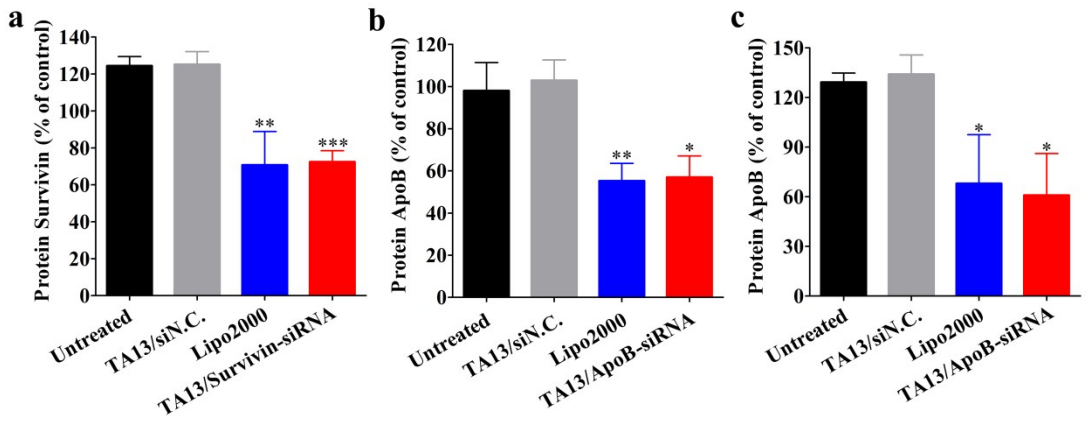

Figure S29. Semiquantitative determination of western blotting images. **(a)** MCF-7 cells were treated with TA13/Survivin-siRNA and the expression of Survivin protein was determined by western blotting. **(b)** HepG2 cells were treated with TA13/ApoB-siRNA and the expression of Survivin protein was determined by western blotting. **(c)** L02 cells were treated with TA13/ApoB-siRNA and the expression of Survivin protein was determined by western blotting. Lipo2000 was used as a positive control. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, compared with the untreated groups.

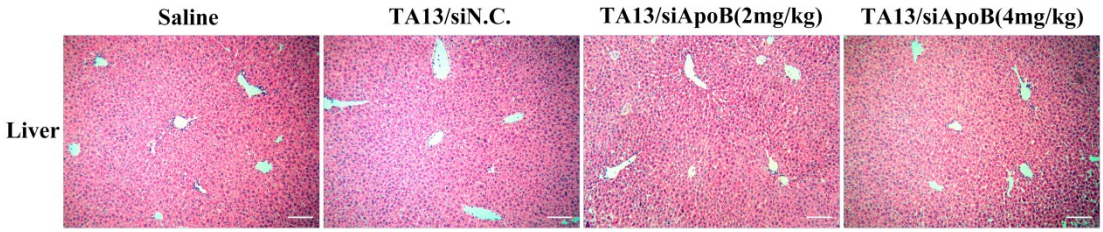

Figure S30. Histopathological analysis of the livers that collected from the C57BL/6 mice treated with saline, TA13/siN.C. at 2 mg siRNA/kg and TA13/ApoB-siRNA at 2 mg siRNA/kg or 4 mg siRNA/kg. H&E-stained liver sections revealed that no significant change was observed for all four groups of mice. Scale bar: 100 μm.

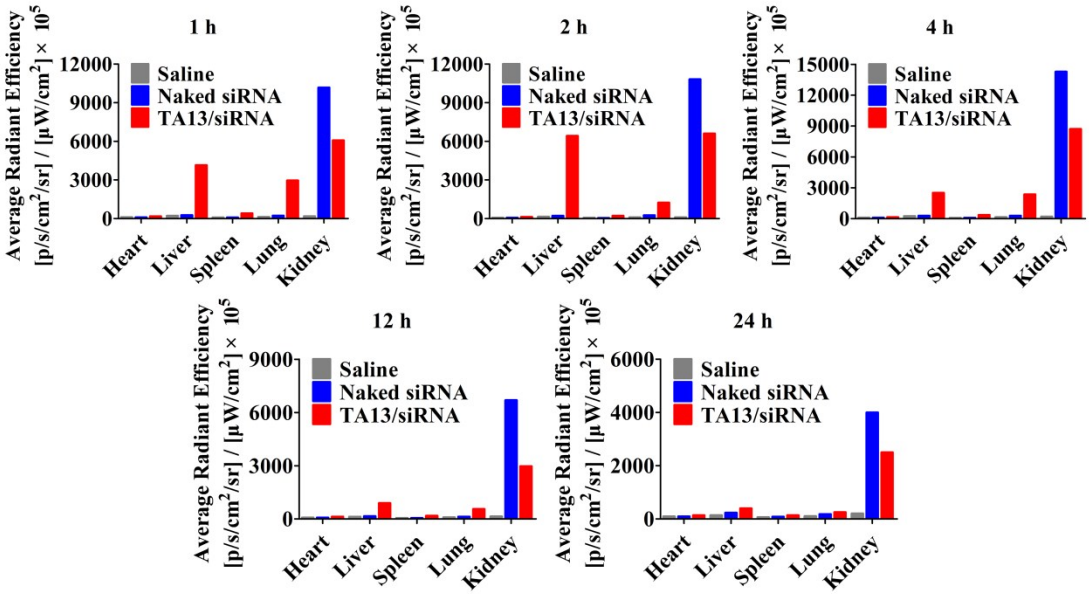

Figure S31. Semiquantitative analyses of fluorescence intensity of Cy5 within isolated major organs from C57BL/6 mice treated with TA13/Cy5-siRNA (1 mg/kg) at 1 h, 2 h, 4 h, 12 h, and 24 h post administration using a software package included with the *in vivo* imaging system.

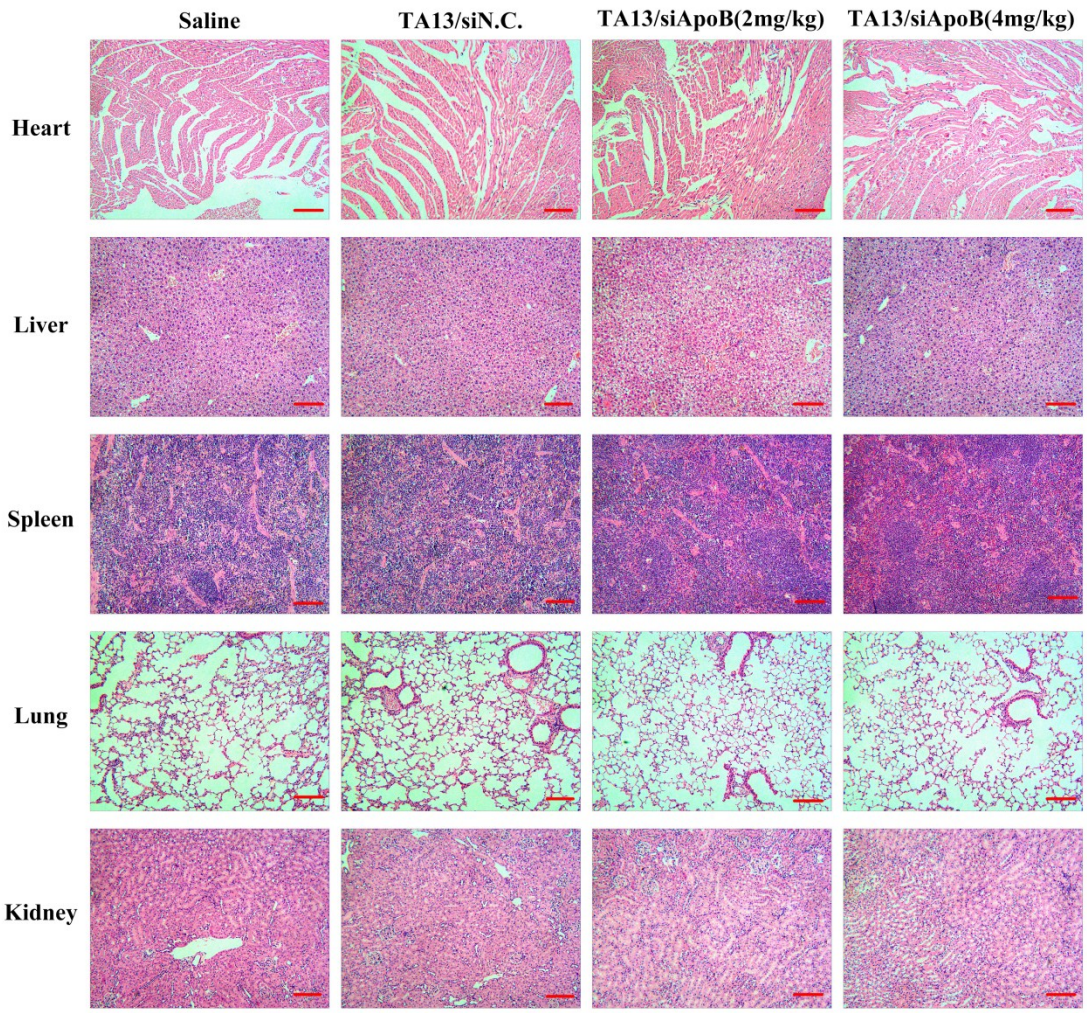

Figure S32. Histopathological analysis of the major organs that collected from the hypercholesterolemia mice treated with saline, TA13/siN.C. at 2 mg siRNA/kg and TA13/ApoB-siRNA at 2 mg siRNA/kg or 4 mg siRNA/kg. H&E-stained organs sections revealed that liver sections of four groups showed a vacuole-like structure, due to fatty liver in hypercholesterolemia mice, and no significant change was observed in other organs sections for all four groups of mice. Scale bar: 100 μm.