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Choice of the optimal synthetic approach for the polypeptide ligands of prostatic specific membrane antigen preparation

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General information

All used solvents were purified according to described procedures. All starting compounds were commercially available reagents.

^{1}H and ^{13}C NMR spectra were registered on Bruker Avance 400 spectrometer (400 MHz for ^{1}H and 101 MHz for ^{13}C) in $CDCl_3$ or DMSO-d_6. Preparative column chromatography was performed on INTERCHIM puriFlash 430. For purification and analysis of samples we used Shimadzu Prominence LC-20 system with Phenomenex Luna 3μm C18 100A (150 x 4.6 mm) column in column oven at 40ºC and fraction collector coupled to single quadrupole mass-spectrometer Shimadzu LCMS-2020 with dual DUIS-ESI-APCI ionization source. Mobile phases: A - 0.1% formic acid in water, B - 10 mM ammonium formate in water, D - acetonitrile. LC parameters for analyses were: gradient flow of 1 ml/min (0-0.5 min - 5% D, 0.5 -10.5 min - 5% to 100% D, 10.5-12 min - 100% D, 12-14.5 min - 100% to 5% D) with optional UV detection for some compounds. MS parameters: drying gas 15.0 L/min, nebulizing gas 1.5 L min^{-1}, desolvation line temperature 250ºC, heat block temperature 400ºC, interface voltage -3.5 kV, corona needle voltage -3.5 kV. Positive (mass range 250-2000 Da, in some cases 155-2000 Da) and negative ions (mass range 100-2000 Da) were registered simultaneously. For purification we used identical LC parameters except gradient which was tailored for each compound (in some cases we used mobile phase B instead of A). Fractionation was based on UV detection only; fractions were collected based on UV signal level and slope. High resolution mass spectra were registered on Orbitrap Elite mass spectrometer (Thermo Scientific) with ESI ionization source. Compounds with concentration of 0.1-10 μg ml^{-1} (in 1% formic acid in acetonitrile) were directly infused into the ion source with syringe pump (5 μl min^{-1}). We did not use auxiliary and sheath gases, spray voltage was +-3.5 kV, capillary temperature was set to 275ºC. MS spectra were registered by Orbitrap analyzer with 480000 resolution (1 microscan, AGC target value of 1×106, max inject time 1000 ms, averaged on 10 spectra, MS range 100-2000 Da, in some cases 200-4000 Da). We used DMSO and di-*iso*-octyl phthalate as internal calibration signals (m/z 157.03515 and 413.26623) in positive mode and dodecyl sulfate (m/z 265.14790) in negative mode.

Synthesis

General techniques for solid-phase peptide synthesis

1) Activation of 2-CTC resin

2-CTC resin (1 eq., capacity 1.2-1.4 mmol g^{-1}) was stirred in DCM (10 ml per 1 g resin) for 10 min. Then, $SOCl_2$ (3 eq.) was added dropwise, followed by the addition of DMF (5% v/v, relative to $SOCl_2$). The resulting mixture was stirred at 40°C for 4 h. Then, the resin was filtered off, transferred to a polypropylene reactor and washed with DMF (3 times 1 min, 10 ml per 1 g resin) and methylene chloride (3 times 1 min, 10 ml per 1 g resin).

2) Immobilization of the first amino acid

To a mixture of activated 2-CTC resin (1 eq., capacity 1.0-1.5 mmol g^{-1}) in DMF (10 ml per 1 g of resin) was added Fmoc-protected amino acid (2 eq.) and diisopropylethylamine (DIPEA, 10 eq.). The resulting mixture was stirred for 2 h, after which the resin was filtered and successively washed with methanol (3 times 5 min, 10 ml per 1 g of resin), methylene chloride (3 times 1 min, 10 ml per 1 g of resin), DMF (3 times 1 min, 10 ml per 1 g of resin) and methylene chloride (3 times 1 min, 10 ml per1 g of resin).

3) Removal of the Fmoc protection group

The Fmoc-protected fragment immobilized on the 2-CTC resin was washed with DMF (2 times 1 min, 10 ml per 1 g resin), then a solution of 4-methylpiperidine in DMF (20% v/v, 10 ml per 1 g resin) was added and stirred for 15 min. The resin was filtered off, washed with DMF (3 times 1 min, 10 ml per 1 g of resin), added a solution of 4-methylpiperidine in DMF (20% v/v, 10 ml per 1 g of resin) and stirred for 15 minutes. Then the resin was filtered off, washed with DMF (3 times 1 min, 10 ml per 1 g of resin) and dichloromethane (3 times 1 min, 10 ml per 1 g of resin).

4) Acylation reaction using HBTU and HOBt

To the fragment immobilized on the resin (1 eq.) was added DMF (10 ml per 1 g of resin). Fmoc-protected amino acid (2 eq.), HOBt (0.5 eq.), HBTU (2 eq.) and DIPEA (3 eq.) were added to the resulting mixture, and the mixture was stirred for 2 h. Then the resin was filtered off and washed with DMF (3 times 1 min, 10 ml per 1 g resin) and dichloromethane (3 times 1 min, 10 ml per 1 g resin).

5) General methodology for acylation with a vector molecule **1a-d**

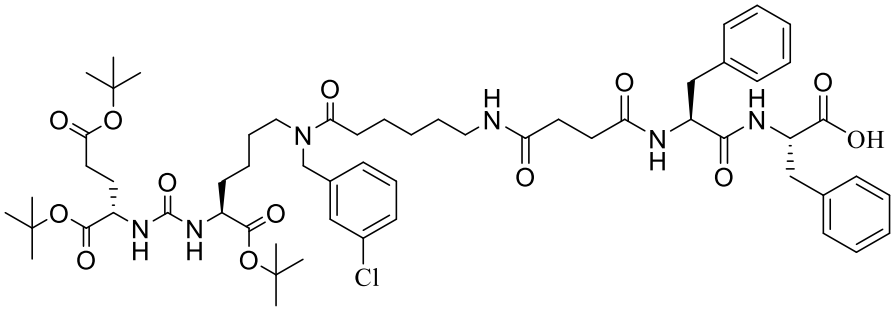
To the fragment immobilized on the resin (1 eq.) was added DMF (10 ml per 1 g of resin). vector molecule **1a-d** (1,2 eq.), HOBt (0.5 eq.), HBTU (2 eq.) and DIPEA (3 eq.) were added to the resulting mixture and the mixture was stirred for 16 h. Then the resin was filtered off and washed with DMF (4 times 2 min, 10 ml per 1 g resin) and dichloromethane (4 times 2 min, 10 ml per 1 g resin).

6) General methodology for the removal of immobilised PSMA ligands with a 1,3-diamine fragment from resins

The resin is stirred for 30 minutes in DCM (10 ml per 1 g of resin), after which the solvent is removed. Then 0.75% trifluoroacetic acid solution in DCM (15 ml per 1 g of resin) is added, and the resin is stirred for 15 minutes. The filtrate is collected in a flask, after which the resin is washed with DCM (3 times 5 minutes each, 10 ml per 1 g of resin), the wash fractions are also collected in a flask. The washing with TFA solution in DCM and DCM is continued until the immobilized fragment is completely removed from the resin. The filtrate is evaporated, and the target product is separated by preparative column chromatography in an elution system DCM/0.1% TFA in DCM/methanol (gradient: 100% DCM for 2 column volumes (cv), then in 3 cv methanol content is increased to 4% and then DCM is replaced by 0.1% trifluoroacetic acid solution in DCM. Then in 10 cv 4% to 10% methanol, 10% to 20% methanol in 0.5 cv, 20% to 100% methanol in 2 cv and 2 cv pure methanol)

Synthesis of ligands 4a-b using the first synthetic route

Synthesis of compound **2a**



From 1 g of unactivated 2-CTC resin, by successive steps: 1) 305 µl $SOCl_2$ (4.2 mmol), 16 µl DMF; 2) 1.085 g Fmoc-Phe(L)-OH (2.8 mmol), 2.44 ml DIPEA (14 mmol); 3) 15 ml 20% v/v solution of 4-methylpiperidine in DMF; 4) 1.085 g Fmoc-Phe(L)-OH (2.8 mmol), 95 mg HOBt (0. 7 mmol), 1.062 g HBTU (2.8 mmol) and 730 µl DIPEA (4.2 mmol); 3) 15 ml of 20% v/v solution of 4-methylpiperidine in DMF; 1.738 grams of resin with immobilized dipeptide was obtained. Then, from 751 mg of the resulting resin with dipeptide (0.54 mmol), step 5) was carried out: 535 mg of compound **1a** (0.648 mmol), 37 mg HOBt (0.27 mmol), 410 mg HBTU (1.08 mmol) and 282 µl DIPEA (1.62 mmol). Thus 1.095 g of resin with immobilized compound **2a** was obtained. After that, a mixture of DCM/TFA (99.25%/0.75%, 11 ml) was added to the resin and stirred for 15 minutes, then the solution was filtered off from the resin. The solvent was removed under reduced pressure and the residue was re-evaporated three times with DCM. The product was purified by column chromatography (Puriflash, column of PF-15C18AQ-F0025 (15µ40g), eluent: H_2O(80%)/MeCN(20%) => H_2O(0%)/MeCN (100%) for 15 minutes after MeCN (100%) for 5

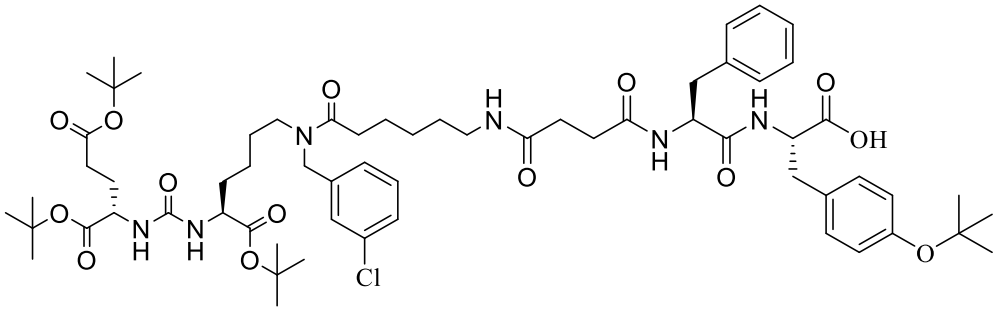
minutes. Compound **2a** was obtained (460 mg, yield 76% relative to resin and 63% relative to vector-molecule**1a**).

^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.71 (s, 1H, COOH), 8.27 (d, J = 8.2 Hz, 1H, NH), 8.03 – 8.09 (m, 1H, NH), 7.71 – 7.83 (m, 1H, NH), 7.08 – 7.47 (m, 14H, Ar), 6.24 – 6.33 (m, 2H, NH), 4.34 – 4.64 (m, 4H, CH+CH_2), 3.99-4.08 (m, 1H, CH), 3.90 – 3.99 (m, 1H, CH), 2.86 – 3.26 (m, 7H, CH_2), 2.58 – 2.75 (m, 1H, CH_2), 2.35 (t, J = 7.4 Hz, 1H, CH_2), 2.30 – 2.10 (m, 7H, CH_2), 1.80 – 1.92 (m, 1H, CH_2), 1.62 – 1.71 (m, 1H CH_2), 1.54 – 1.62 (m, 1H, CH_2), 1.40 – 1.54 (m, 5H, CH_2), 1.40 – 1.34 (m, 27H, CH_3), 1.10 – 1.34 (m, 6H, CH_2).

^{13}C NMR (400 MHz, DMSO-d_6, δ): 172.77, 172.25, 172.21, 172.14, 172.12, 171.93, 171.45, 171.42, 171.37, 171.16, 171.15, 157.15, 157.14, 141.19, 140.78, 138.13, 137.57, 133.44, 133.09, 130.61, 130.25, 129.18, 128.23, 127.99, 127.22, 127.16, 126.87, 126.45, 126.32, 126.18, 126.07, 124.96, 80.58, 80.41, 80.32, 79.76, 53.67, 53.63, 53.00, 52.87, 52.19, 49.61, 47.10, 46.80, 45.20, 38.53, 38.47, 37.34, 36.61, 32.33, 31.95, 31.83, 30.93, 30.82, 30.75, 29.12, 29.02, 27.75, 27.66, 27.63, 26.72, 26.29, 26.19, 24.76, 24.62, 22.44, 22.27.

HRMS (m/z, ESI): calculated for $C_{59}H_{83}ClN_6O_{13}$ – $[M+H]^+$ 1119.5779, found: 1119.5746

Synthesis of compound **2b**



From 1 g of unactivated 2-CTC resin, by successive steps: 1) 305 µl $SOCl_2$ (4.2 mmol), 16 µl DMF; 2) 1.287 g Fmoc-Tyr(L)-OH (2.8 mmol), 2.44 ml DIPEA (14 mmol); 3) 15 ml 20% v/v solution of 4-methylpiperidine in DMF; 4) 1.085 g Fmoc-Phe(L)-OH (2.8 mmol), 95 mg HOBt (0. 7 mmol), 1.062 g HBTU (2.8 mmol) and 730 µl DIPEA (4.2 mmol); 3) 15 ml of 20% v/v solution of 4-methylpiperidine in DMF; 1.614 grams of resin with immobilized dipeptide was obtained. Then, from 300 mg of the resulting resin with dipeptide (0.255 mmol), step 5) was carried out: 252 mg of compound **1a** (0.306 mmol), 17 mg HOBt (0.128 mmol), 193 mg HBTU (0,51 mmol) and 133 µl DIPEA (0,765 mmol). Thus 607 mg of resin with immobilized compound **2b** was obtained. After that, a mixture of DCM/TFA (99.25%/0.75%, 4 ml) was added to the resin and stirred for 15 minutes, then the solution was filtered off from the resin. The solvent was removed under reduced pressure and the residue was re-evaporated three times with DCM. The product was purified by column chromatography (Puriflash, column of PF-15C18AQ-F0025 (15µ40g), eluent: H_2O(80%)/MeCN(20%) => H_2O(0%)/MeCN (100%) for 15 minutes after MeCN (100%) for 5

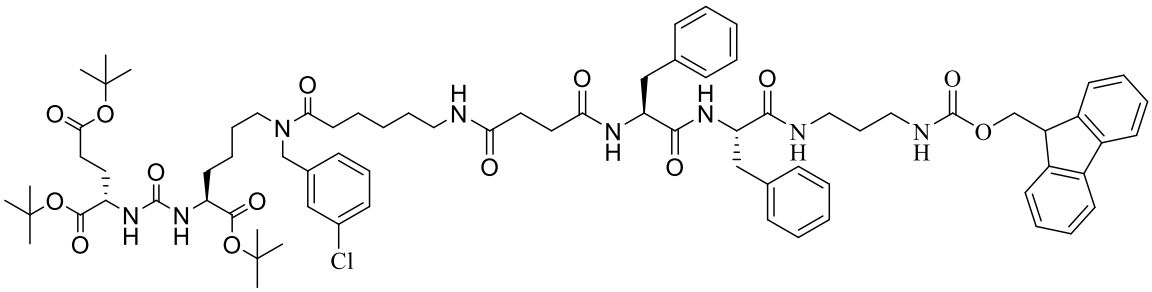
minutes. Compound **2b** was obtained (260 mg, yield 86% relative to resin and 72% relative to vector-molecule **1a**).

^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.68 (s, 1H, COOH), 8.26 (d, J = 7.8 Hz, 1H, NH), 8.05 (d, J = 8.3 Hz, 1H, NH), 7.69 – 7.83 (m, 1H, NH), 7.27 – 7.43 (m, 2H, Ar), 7.18 – 7.26 (M, 5H, Ar), 7.08 – 7.18 (m, 4H, Ar), 6.89 – 6.80 (m, 2H, Ar), 6.20 – 6.35 (m, 2H, NH), 4.42 – 4.59 (m, 3H, CH+CH_2), 4.33 – 4.42 (m, 1H, CH), 3.89 – 4.07 (m, 2H, CH), 3.12 – 3.25 (m, 2H, CH_2), 2.85 – 3.07 (m, 5H, CH_2), 2.69 – 2.57 (m, 1H, CH_2), 2.34 (t, J = 7.4 Hz, 1H, CH_2), 2.29 – 2.11 (m, 7H, CH_2), 1.91 – 1.80 (m, 1H, CH_2), 1.61 – 1.72 (m, 1H, CH_2), 1.61 – 1.54 (m, 1H, CH_2), 1.40 – 1.54 (m, 5H, CH_2), 1.34 – 1.40 (m, 27H, CH_3), 1.32 – 1.26 (m, 2H, CH_2), 1.22 – 1.26 (m, 9H, CH_3).

^{13}C NMR (400 MHz, DMSO-d_6, δ): 172.74, 172.20, 172.16, 172.14, 171.88, 171.42, 171.37, 171.32, 171.15, 171.14, 157.13, 157.12, 153.58, 141.15, 140.74, 138.09, 133.43, 133.08, 132.10, 130.59, 130.20, 129.67, 129.13, 127.96, 127.19, 127.13, 126.84, 126.29, 126.15, 126.05, 124.94, 123.43, 80.57, 80.40, 80.31, 79.74, 77.63, 53.72, 53.59, 53.00, 52.86, 52.21, 49.64, 47.12, 46.81, 45.19, 38.52, 38.45, 37.36, 35.97, 32.31, 31.94, 31.82, 30.91, 30.85, 30.76, 29.08, 28.97, 28.55, 27.73, 27.64, 27.63, 26.69, 26.26, 26.16, 24.74, 24.59, 22.42, 22.23.

HRMS (m/z, ESI): calculated for $C_{63}H_{91}ClN_6O_{14}$ – $[M+H]^+$ 1191.6355, found: 1191.6363

Synthesis of compound **3a**



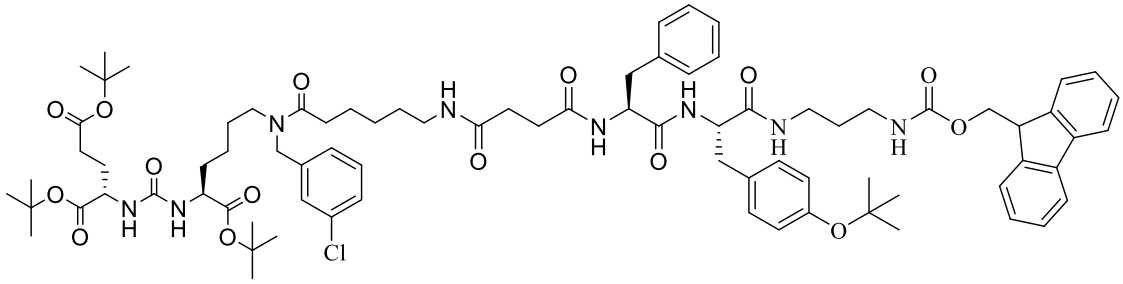
To a solution of compound **2a** (1 eq; 300 mg; 0.268 mmol) in 10 ml DMF was added TFA*$NH_2(CH_2)_3$NHFmoc (1.5 eq; 165 mg; 0. 402 mmol), DIPEA (2.5 eq; 117 μl; 0.67 mmol) in 5 ml DMF, followed by, HOBt (1,5 eq; 54 mg; 0.402 mmol), HBTU (1.5 eq; 152 mg; 0.402 mmol). The mixture was stirred for 12 hours in an inert atmosphere. The solvent was then removed under reduced pressure and reevaporated with DCM twice, the residue was dissolved in 30 ml DCM and extracted with H_2O (2*30 ml) and with saturated NaCl solution (2*30 ml). Then the organic fraction was dried over Na_2SO_4. Afterwards the solvent was removed. The residue was purified by column chromatography (Puriflash on a column (15μ 40g)); eluent: DCM(100%)/MeOH(0%) => DCM(90%)/MeOH(10%) for 30 min, after MeOH (100%) for 5 min. Compound **3a** was obtained as a pale yellow amorphous solid (330 mg, 88% yield).

^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.30 (d, J = 7.4 Hz, 1H, NH), 8.13 – 8.23 (m, 1H, NH), 7.74 – 7.97 (m, 3H, NH+Ar), 7.67 (d, J = 7.5 Hz, 2H, Ar), 7.52 – 7.60 (m, 1H, NH), 7.44 – 7.35 (m, 2H, Ar), 7.34 – 7.08 (m, 16H, Ar), 6.21 – 6.36 (m, 2H, NH), 4.44 – 4.58 (m, 2H, CH_2), 4.44 – 4.36 (m, 1H, CH), 4.24 – 4.36 (m, 3H, CH+CH_2), 4.20 (t, J = 6.9 Hz, 1H, CH), 3.99 – 4.08 (m, 1H, CH), 3.99 – 3.90 (m, 1H, CH), 3.12 – 3.25 (m, 2H, CH_2), 3.11 – 2.84 (m, 9H, CH_2), 2.60 – 2.70 (m, 1H, CH_2), 2.40 – 2.12 (m, 8H, CH_2), 1.80 – 1.92 (m, 1H, CH_2), 1.62 – 1.72 (m, 1H, CH_2), 1.55 – 1.62 (m, 1H, CH_2), 1.40 – 1.55 (m, 7H, CH_2), 1.40 – 1.35 (m, 27H, CH_3), 1.34 – 1.11 (m, 2H, CH_2).

^{13}C NMR (400 MHz, DMSO-d_6, δ): 172.79,, 172.76, 172.24, 172.20, 172.15, 172.13, 171.92, 171.57, 171.45, 171.11, 170.67, 157.15, 156.12, 143.94, 141.16, 140.77, 138.12, 138.02, 133.44, 133.09, 130.59, 130.23, 129.04, 128.16, 128.06, 127.63, 127.21, 127.15, 127.09, 126.86, 126.29, 126.24, 126.05, 125.17, 124.95, 120.13, 80.59, 80.42, 80.33, 79.77, 65.32, 54.94, 54.40, 53.01, 52.87, 52.20, 49.62, 47.11, 46.80, 45.19, 38.65, 38.60, 37.90, 37.11, 36.82, 36.39, 32.32, 31.95, 31.82, 30.93, 30.69, 30.58, 29.20, 29.05, 28.96, 27.74, 27.65, 27.62, 26.69, 26.31, 26.22, 24.73, 24.60, 22.43, 22.25.

HRMS (m/z, ESI): calculated for $C_{77}H_{101}ClN_8O_{14}$ – $[M+H]^+$ 1397.7199, found: 1397.7210

Synthesis of compound **3b**



To a solution of compound **2b** (1 eq; 258 mg; 0.217 mmol) in 10 ml DMF was added TFA*$NH_2(CH_2)_3$NHFmoc (1.05 eq; 93 mg; 0. 228 mmol), DIPEA (2.3 eq; 87 µl; 0.498 mmol) in 5 ml DMF, followed by, HOBt (1 eq; 29 mg; 0.217 mmol), HBTU (1.5 eq; 123 mg; 0.325 mmol). The mixture was stirred for 12 hours in an inert atmosphere. The solvent was then removed under reduced pressure and reevaporated with DCM twice, the residue was dissolved in 30 ml DCM and extracted with H_2O (2*30 ml) and with saturated NaCl solution (2*30 ml). Then the organic fraction was dried over Na_2SO_4. Afterwards the solvent was removed. The residue was purified by column chromatography (Puriflash on a column (15µ 40g)); eluent: DCM(100%)/MeOH(0%) => DCM(90%)/MeOH(10%) for 30 min, after MeOH (100%) for 5 min. Compound **3b** was obtained as a pale yellow amorphous solid (300 mg, 94% yield).

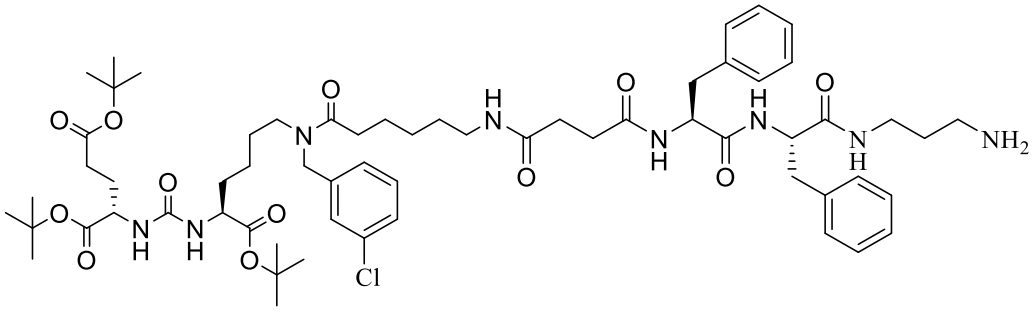
^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.34-8.26 (br.d., 1H, NH), 8.20-8.11 (br.d., 1H, NH), 7.84 – 7.97 (m, 3H, NH+Ar), 7.67 (d, J = 7.5 Hz, 2H, Ar), 7.58-7.50 (m, 1H, NH), 7.44-7.35 (m,

2H, Ar), 7.36-7.26 (m, 4H, Ar), 7.26-7.18 (m, 3H, Ar), 7.18-7.08 (m, 6H, Ar), 6.86 (d, J=7.9Hz, 2H, Ar), 6.35-6.20 (m, 2H, NH), 4.44 – 4.57 (m, 2H, CH_2), 4.40-4.24 (m, 3H, CH+CH_2), 4.20 (t, J = 6.9 Hz, 1H, CH), 4.07-3.99 (m, 1H, CH), 3.99-3.90 (m, 1H, CH), 3.21 (t, J=7.3 Hz) & 3.16 (t, J=7.3 Hz) (2H, K2Hε), 3.07-2.77 (m, 9H, CH_2), 2.68-2.57 (m, 1H, CH_2), 2.40-2.10 (m, 8H, CH_2), 1.91-1.79 (m, 1H, CH_2), 1.72-1.62 (m, 1H, CH_2), 1.62-1.54 (m, 1H, CH_2), 1.54-1.10 (m, 13H, CH_2), 1.40-1.34 (m, 27H, CH_3), 1.23 (s, 9H, CH_3).

^{13}C NMR (400 MHz, DMSO-d_6, δ): 172.72, 172.18, 172.08, 171.90, 171.51, 171.43, 171.03, 170.67, 157.12, 156.08, 153.40, 143.93, 141.15, 140.75, 138.00, 133.41, 133.06, 132.74, 130.56, 130.21, 129.53, 129.00, 128.04, 127.61, 127.19, 127.12, 127.06, 126.84, 126.29, 126.22, 126.04, 125.15, 124.93, 123.50, 120.12, 80.55, 80.38, 80.29, 79.74, 77.59, 65.29, 54.98, 54.40, 52.97, 52.84, 52.17, 49.58, 47.07, 46.77, 45.17, 38.62, 38.58, 37.88, 36.90, 36.49, 36.35, 32.30, 31.92, 31.81, 30.90, 30.67, 30.57, 29.21, 29.04, 28.94, 28.52, 27.72, 27.62, 26.68, 26.30, 26.21, 24.71, 24.57, 22.41, 22.24.

HRMS (m/z, ESI): calculated for $C_{81}H_{109}ClN_8O_{15}$ – $[M+H]^+$ 1469.7774, found: 1469.7751

Synthesis of compound **4a**



Compound **3a** (1 eq.; 562 mg; 0.402 mmol) was dissolved in mixture of Et_2NH/DMF (25 eq. of Et_2NH, 20 ml of DMF). The mixture was stirred for 20 minutes, then the solvent was removed under reduced pressure and reevaporated with DCM (0.1% TFA), then reevaporated with DCM twice. The product was precipitated with DEK and washed twice with ether (2 mL). The residue was purified by column chromatography (Puriflash 15μ 40g, eluent: DCM(100%)/MeOH(0%) => DCM(96%)/MeOH(4%) for 25 minutes, after DCM(0.1% TFA) (96%)/MeOH(4%) => DCM(0.1% TFA) (90%)/MeOH(10%) for 20 minutes, after MeOH (100%) for 5 minutes. Compound **4a** was obtained (493 mg, yield 87%).

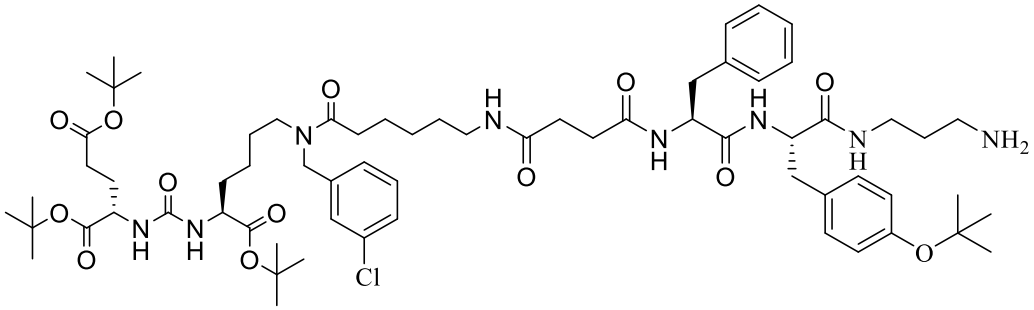
^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.37 (d, J = 7.3 Hz, 1H, NH), 8.20 (d, J = 8.2 Hz, 1H, NH), 8.04 – 7.92 (m, 1H, NH), 7.82 (br.s, 3H, NH_3^+), 7.69 – 7.77 (m, 1H, NH), 7.02 – 7.42 (m, 14H, Ar), 6.39 – 6.23 (m, 2H, NH), 4.43 – 4.59 (m, 2H, CH_2), 4.34 – 4.41 (m, 1H, CH), 4.22 – 4.33 (m, 1H, CH), 3.89 – 4.07 (m, 2H, CH), 3.11 – 3.27 (m, 3H, CH_2), 3.11 – 2.86 (m, 6H, CH_2), 2.78 – 2.60 (m, 3H, CH_2), 2.41 – 2.10 (m, 8H, CH_2), 1.79 – 1.92 (m, 1H, CH_2), 1.62 – 1.73 (m,

3H, CH_2), 1.61 – 1.55 (m, 1H, CH_2), 1.41 – 1.55 (m, 6H, CH_2), 1.34 – 1.40 (m, 27H, CH_3), 1.33 – 1.11 (m, 6H, CH_2).

^{13}C NMR (400 MHz, DMSO-d_6, δ): 172.91, 172.88, 172.24, 172.20, 172.15, 172.13, 171.92, 171.61, 171.46, 171.23, 171.09, 157.18, 157.16, 141.18, 140.78, 138.08, 138.02, 133.41, 133.07, 130.61, 130.25, 129.05, 128.20, 128.07, 127.20, 127.15, 126.86, 126.34, 126.31, 126.25, 126.06, 124.97, 80.55, 80.38, 80.30, 79.77, 55.10, 54.53, 53.01, 52.88, 52.19, 49.62, 47.10, 46.81, 45.22, 38.64, 38.58, 36.92, 36.79, 36.58, 35.81, 32.33, 31.95, 31.79, 30.92, 30.66, 30.57, 29.05, 28.97, 27.75, 27.66, 27.63, 27.57, 27.09, 26.70, 26.32, 26.23, 24.74, 24.60, 22.45, 22.26.

HRMS (m/z, ESI): calculated for $C_{62}H_{91}ClN_8O_{12}$ – $[M+H]^+$ 1175.6518, found: 1175.6520

Synthesis of compound **4b**



Compound **3b** (1 eq.; 200 mg; 0.143 mmol) was dissolved in a mixture of Et_2NH/DMF (20 eq. of Et_2NH, 10 ml of DMF). The mixture was stirred for 20 minutes, then the solvent was removed under reduced pressure and reevaporated with DCM (0.1% TFA), then reevaporated with DCM twice. The product was precipitated with DEK and washed twice with ether (2 mL). The residue was purified by column chromatography (Puriflash 15µ 40g, eluent: DCM(100%)/MeOH(0%) => DCM(96%)/MeOH(4%) for 25 minutes, after DCM(0.1% TFA) (96%)/MeOH(4%) => DCM(0.1% TFA) (90%)/MeOH(10%) for 20 minutes, after MeOH (100%) for 5 minutes. Compound **4b** was obtained (249 mg, yield 90%).

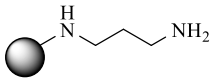
^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.35 (d, *J*=7.13 Hz, 1H, NH), 8.15 (d, *J*=8.02 Hz, 1H, NH), 7.98-7.89 (m, 1H, NH), 7.76-7.62 (m, 4H, NH+NH_3^+), 7.40-7.29 (m, 2H, Ar), 7.28-7.18 (m, 4H, Ar), 7.18-7.09 (m, 6H, Ar), 6.86 (d, *J*=8.41 Hz, 2H, Ar), 6.21-6.34 (m, 2H, NH), 4.56-4.44 (m, 2H, CH_2), 4.37-4.29 (m, 1H, CH), 4.29-4.23 (m, 1H, CH), 3.24-3.07 (m, 4H, CH_2), 3.08-2.90 (m, 5H, CH_2), 2.89-2.81 (m, 2H, CH_2), 2.78-2.70 (m, 2H, CH_2), 2.68-2.57 (m, 2H, CH_2), 2.37-2.30 (m, 3H, CH_2), 2.29-2.12 (m, 5H, CH_2), 1.91-1.79 (m, 1H, CH_2), 1.71-1.57 (m, 4H, CH_2), 1.56-1.40 (m, 7H, CH_2), 1.40-1.32 (m, 29H, CH_2+CH_3), 1.32-1.11 (m, 20H, CH_2+CH_3).

^{13}C NMR (400 MHz, DMSO-d_6, δ): 173.10, 172.19, 171.93, 171.69, 171.48, 171.25, 158.70, 158.37, 157.21, 153.48, 141.17, 138.00, 132.78, 128.36, 127.50, 124.33, 122.82, 80.59, 80.38, 79.79, 77.66, 56.02, 53.90, 51.59, 29.55, 29.16, 28.30, 27.95, 27.07, 26.73, 25.94.

HRMS (m/z, ESI): calculated for $C_{66}H_{99}ClN_8O_{13}$ – $[M+H]^+$ 1247.7020, found: 1247.7130

Synthesis of compounds **4a-i** and **8** using the second synthetic route

Preparation of 2-CTC resin with immobilized 1,3-diaminopropane fragment



From 1 g of inactivated 2-CTC resin, step 1) was performed. Then, the activated resin was washed with DMF 3 times 10 ml for 1 minute and DCM 3 times 10 ml for 1 minute. The resin was then stirred for 30 min in 10 ml DCM and then a solution of 1,3-diaminopropane (4 eq.) in DCM (6.24 ml DCM and 520 µL 1,3-diaminopropane) was added to the reactor and left to stir overnight. Afterwards the solvent was removed from the reactor. The resin was successively washed with DMF (4 times 1 min, 6 ml), DCM (4 times 1 min, 6 ml), a mixture of DCM, DIPEA and methanol (4 times 2 min, 6 ml, ratio: DCM:DIPEA:methanol=17:2.5: 1), methanol (3 times 1 min, 6 ml), triethylamine solution in DMF (Et3N:DMF=1:4 v/v ratio, 3 times 1 min, 6 ml), methanol (3 times 1 min, 6 ml) and DCM (3 times 1 min, 6 ml).

Optimization of synthesis of PSMA ligands on solid-phase carrier with immobilized 1,3-diaminopropane fragment

Using compound **5b** as an example, the possibility of efficiently removing the dipeptide fragment from the resin was tested. The immobilized dipeptide was obtained from 527 mg of modified 2-CTC resin by successive steps 4) 779 mg Fmoc-(L)-Tyr(OtBu)-OH, 443 µL DIPEA, 643 mg HBTU, 57 mg HOBt, 3) 6 mL 20% 4-methylpiperidine in DMF, 4) 657 mg Fmoc-(L)-Phe-OH, 443 µl DIPEA, 643 mg HBTU, 57 mg HOBt, 3) 7 mL 20% 4-methylpiperidine in DMF. Thus 948 mg of resin with immobilized dipeptide **5b** was obtained. Subsequently, based on 100 mg of resin with dipeptide, a test removal of the fragment from the resin was carried out. In contrast to standard procedures used to immobilize fragments on resin by the interaction of the carboxyl group with the chlorotrityl fragment, two successive washes for 15 minutes with 0.75% TFA solution in DCM were not sufficient to completely remove the compound from the resin. Increasing the acid concentration to 1% resulted in a more effective removal of the substance from the resin. However, as has been shown later, this step leads to an increase in the side processes of removing the side protecting groups. Two washes with 0.75% TFA solution and one wash with 1% TFA solution with 100 mg of modified resin yielded 35 mg of compound **6**.

Subsequently, using vector fragment **1b** and immobilized modified peptide **5b** as examples, a model reaction was carried out to produce protected ligand **4e**. To obtain this compound, the resin was stirred for 16 hours with the vector fragment in the presence of HBTU, HOBt and DIPEA. The substance was then removed from the solid-phase carrier. To do this, the resin was stirred with a 1% TFA solution in DCM for 15 minutes. Then, washed three times with DCM for one minute each. After 3 of these washes, a rather limited amount of substance was obtained. It

was decided to carry out 7 more rinses with TFA solution, with the intermediate rinses with methylene being increased to 5 minutes. Subsequently, the target compound **4e** was isolated by column chromatography in an elution system of 0.1% TFA in DCM/methanol. This reaction resulted in the formation of a side compound (about 17 w/w% of the total weight of the mixture obtained after removal from the resin) without a *tert*-butyl group in the structure of the tyrosine fragment, as shown by ^{1}H NMR spectroscopy.

In the next step, reactions were carried out to obtain protected ligands **4a** and **4b** with vector fragment **1a**. The acylation reaction conditions were left unchanged.

The removal of the immobilized fragments from the resin was carried out using the previously presented method. After each wash with TFA solution and three times with methylene chloride, the filtrate was evaporated and weighed. The data obtained after each such cycle are shown in Table S1 when a minimum of 6-7 washes with a 0.75% TFA solution are necessary to achieve a sufficiently high degree of removal from the resin.

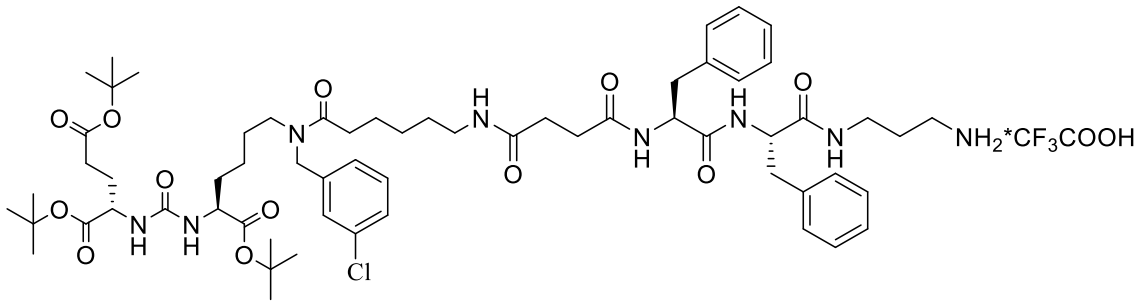
Table S1 Weight of the mixture upon removal of the substance from the solid phase carrier

No of washing	Weight of substance removed from the resin, mg		
	4a	**4b**	**8**
1	1	1	1
2	20	23	19
3	71	103	87
4	135	188	171
5	181	237	244
6	213	263	276
7	239	271	300
8	252	285	312
9	268	285	313
10	271	285	313

Subsequently, a chromatographic isolation of the target compounds was carried out. Despite a decrease in the concentration of trifluoroacetic acid, in the case of compound **4b** fractions containing the product without the *tert*-butyl protecting group at the tyrosine oxygen atom were isolated.

Protected ligand **8** was obtained using the methods described above. Also, as in the case of **4a-i** ligands the removal of 0.75% TFA from the resin was rather slow (see Table S1). Subsequent isolation of the target compound resulted in 111 mg of pure, according to thin layer chromatography (TLC). However, subsequent analysis of the HPLC-MS data of this fraction showed that a mixture of four components was obtained and the content of the target product is about 21% in positive ions. In this fraction as well as in some other fractions obtained by chromatographic separation the product without Boc-protective group was observed.

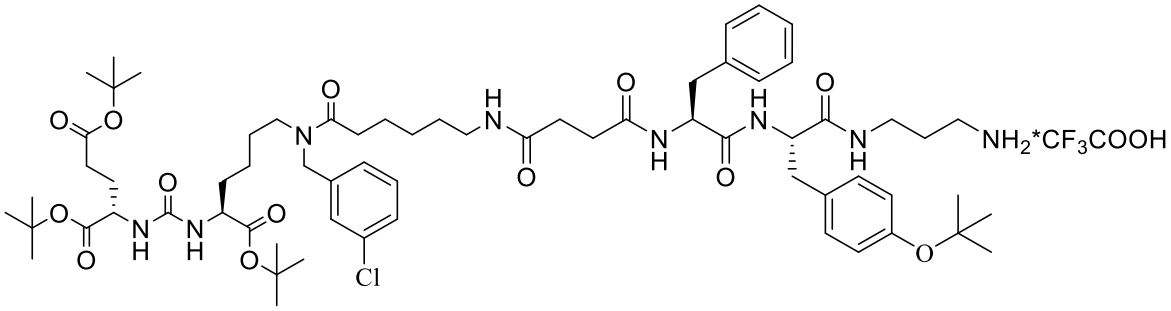
Synthesis of compound **4a**



From 195 mg 2-CTC resin (0.255 mmol) with immobilized 1,3-diaminopropane fragment by successive steps 4) 198 mg Fmoc-(L)-Phe-OH, 134 μL DIPEA, 193 mg HBTU, 17 mg HOBt, 3) 4 mL 20% 4-methylpiperidine in DMF, 4) 198 mg Fmoc-(L)-Phe-OH, 134 μl DIPEA, 193 mg HBTU, 17 mg HOBt, 3) 6 mL 20% 4-methylpiperidine in DMF, 5) 253 mg of compound **1a**, 134 μl DIPEA, 193 mg HBTU, 17 mg HOBt and 6) 178 mg (54% yield) compound **4a** as trifluoroacetate was obtained.

^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.37 (d, J = 7.3 Hz, 1H, NH), 8.20 (d, J = 8.2 Hz, 1H, NH), 8.04 – 7.92 (m, 1H, NH), 7.82 (br.s, 3H, NH_3^+), 7.69 – 7.77 (m, 1H, NH), 7.02 – 7.42 (m, 14H, Ar), 6.39 – 6.23 (m, 2H, NH), 4.43 – 4.59 (m, 2H, CH_2), 4.34 – 4.41 (m, 1H, CH), 4.22 – 4.33 (m, 1H, CH), 3.89 – 4.07 (m, 2H, CH), 3.11 – 3.27 (m, 3H, CH_2), 3.11 – 2.86 (m, 6H, CH_2), 2.78 – 2.60 (m, 3H, CH_2), 2.41 – 2.10 (m, 8H, CH_2), 1.79 – 1.92 (m, 1H, CH_2), 1.62 – 1.73 (m, 3H, CH_2), 1.61 – 1.55 (m, 1H, CH_2), 1.41 – 1.55 (m, 6H, CH_2), 1.34 – 1.40 (m, 27H, CH_3), 1.33 – 1.11 (m, 6H, CH_2).

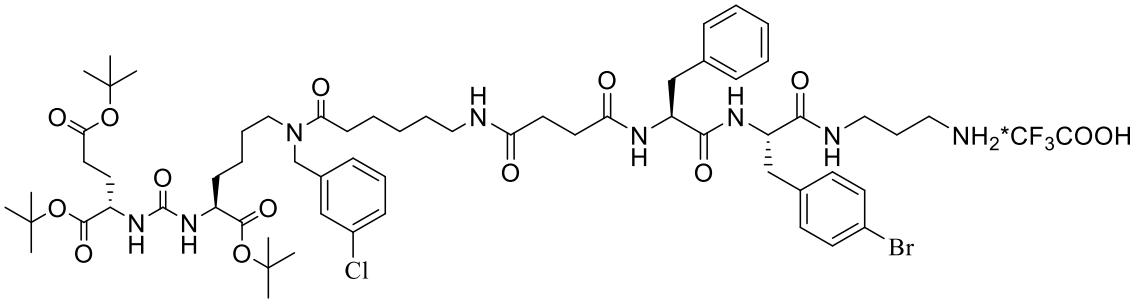
Synthesis of compound **4b**



From 205 mg 2-CTC resin (0.268 mmol) with immobilized 1,3-diaminopropane fragment by successive steps 4) 246 mg Fmoc-(L)-Tyr(OtBu)-OH, 140 μL DIPEA, 203 mg HBTU, 18 mg HOBt, 3) 4 mL 20% 4-methylpiperidine in DMF, 4) 208 mg Fmoc-(L)-Phe-OH, 140 μl DIPEA, 203 mg HBTU, 18 mg HOBt, 3) 6 mL 20% 4-methylpiperidine in DMF, 5) 266 mg of compound **1a**, 140 μl DIPEA, 203 mg HBTU, 18 mg HOBt and 6) 117 mg (32% yield) compound **4b** as trifluoroacetate was obtained.

^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.35 (d, *J*=7.13 Hz, 1H, NH), 8.15 (d, *J*=8.02 Hz, 1H, NH), 7.98-7.89 (m, 1H, NH), 7.76-7.62 (m, 4H, NH+NH_3^+), 7.40-7.29 (m, 2H, Ar), 7.28-7.18 (m, 4H, Ar), 7.18-7.09 (m, 6H, Ar), 6.86 (d, *J*=8.41 Hz, 2H, Ar), 6.21-6.34 (m, 2H, NH), 4.56-4.44 (m, 2H, CH_2), 4.37-4.29 (m, 1H, CH), 4.29-4.23 (m, 1H, CH), 3.24-3.07 (m, 4H, CH_2), 3.08-2.90 (m, 5H, CH_2), 2.89-2.81 (m, 2H, CH_2), 2.78-2.70 (m, 2H, CH_2), 2.68-2.57 (m, 2H, CH_2), 2.37-2.30 (m, 3H, CH_2), 2.29-2.12 (m, 5H, CH_2), 1.91-1.79 (m, 1H, CH_2), 1.71-1.57 (m, 4H, CH_2), 1.56-1.40 (m, 7H, CH_2), 1.40-1.32 (m, 29H, CH_2+CH_3), 1.32-1.11 (m, 20H, CH_2+CH_3).

Synthesis of compound **4c**



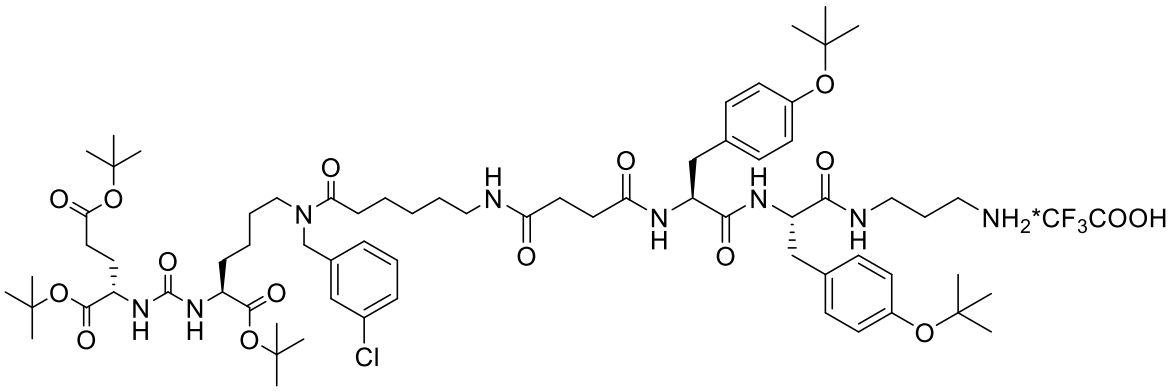
From 222 mg 2-CTC resin (0.25 mmol) with immobilized 1,3-diaminopropane fragment by successive steps 4) 140 mg Fmoc-(L)-Phe(4-Br)-OH, 128 μL DIPEA, 190 mg HBTU, 17 mg HOBt, 3) 4 mL 20% 4-methylpiperidine in DMF, 4) 193 mg Fmoc-(L)-Phe-OH, 128 μl DIPEA, 190 mg HBTU, 17 mg HOBt, 3) 6 mL 20% 4-methylpiperidine in DMF, 5) 247 mg of compound **1a**, 128 μl DIPEA, 190 mg HBTU, 17 mg HOBt and 6) 133 mg (39% yield) compound **4c** as trifluoroacetate was obtained.

^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.33 (d, *J*=7.29 Hz, 1H, NH), 8.14 - 8.23 (m, 1 H), 7.87 - 7.98 (m, 1H, NH), 7.68 (br.s., 4H, NH+NH^{3+}), 7.46 (d, *J*=8.28 Hz, 2H, NH), 7.26 - 7.35 (m, 2H,

Ar), 7.10 - 7.25 (m, 10H, Ar), 6.23 - 6.34 (m, 2H, NH), 4.54 (s, 1H, CH_2), 4.46 (s., 1 H), 4.36 – 4.31 (m., 1H, CH_2), 4.29 – 4.24 (m, 1H, CH), 4.01 - 3.96 (m, 2H, CH), 3.09 - 3.21 (m, 3H, CH_2), 3.03 (d, *J*=13.37 Hz, 3H, CH_2), 2.97 – 2.94 (m, 1H, CH_2), 2.90 -2.88 (m, 1H, CH_2), 2.73 (d, *J*=7.34 Hz, 2H, CH_2), 2.27 - 2.35 (m, 3H, CH_2), 2.14 - 2.26 (m, 4H, CH_2), 1.85 (d, *J*=7.67 Hz, 1H, CH_2), 1.65 (d, *J*=6.96 Hz, 4H, CH_2), 1.43 - 1.55 (m, 5H, CH_2), 1.34 - 1.39 (m, 28H, CH_2+CH_3), 1.27 - 1.24 (m, 3H, CH_2).

HRMS (m/z, ESI): calculated for $C_{62}H_{90}ClN_8O_{12}$ – $[M+H]^+$ 1255.5602, found: 1255.5610

Synthesis of compound **4d**

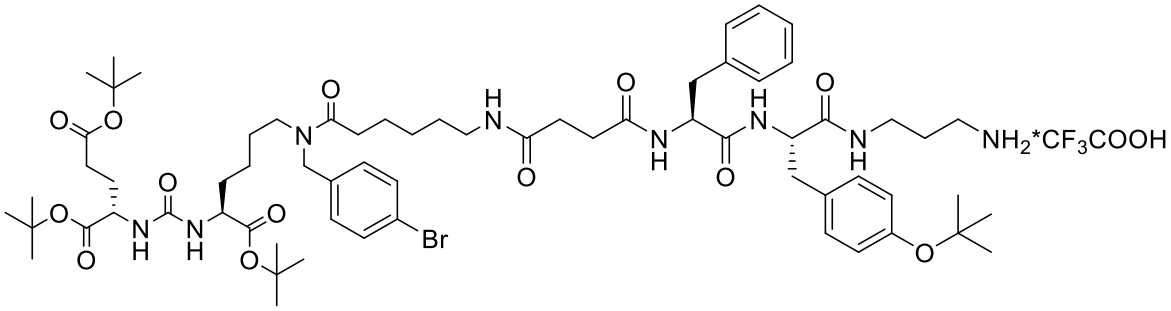


From 490 mg 2-CTC resin (0.5 mmol) with immobilized 1,3-diaminopropane fragment by successive steps 4) 459 mg Fmoc-(L)-Tyr(OtBu)-OH, 256 µL DIPEA, 379 mg HBTU, 34 mg HOBt, 3) 4 mL 20% 4-methylpiperidine in DMF, 4) 459 mg Fmoc-(L)-Tyr(OtBu)-OH, 256 µl DIPEA, 379 mg HBTU, 34 mg HOBt, 3) 6 mL 20% 4-methylpiperidine in DMF, 5) 495 mg of compound **1a**, 256 µl DIPEA, 379 mg HBTU, 34 mg HOBt and 6) 131 mg (18% yield) compound **4d** as trifluoroacetate was obtained.

^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.24 - 7.37 (m, 2H, Ar) 7.15 - 7.20 (m, 1H, Ar) 7.12 (dd, *J*=8.44, 2.85 Hz, 3H, Ar) 6.99 (d, *J*=7.29 Hz, 2H, Ar) 6.85 (d, *J*=8.55 Hz, 2H, Ar) 6.78 (d, *J*=8.33 Hz, 2H, Ar) 4.51 (m, 1H, CH) 4.44 (m, *J*=2.69 Hz, 1H, CH) 4.28 (dd, *J*=9.48, 4.44 Hz, 1H, CH) 4.14 - 4.21 (m, 1H, CH) 3.99 (dd, *J*=7.86, 5.29 Hz, 1H, CH) 3.88 - 3.96 (m, 1H, CH) 3.10 - 3.19 (m, 3H, CH_2) 2.94 - 3.05 (m, 4H, CH_2) 2.80 - 2.89 (m, 1H, CH_2) 2.67 - 2.76 (m, 3H, CH_2) 2.52 - 2.63 (m, 1H, CH_2) 2.28 - 2.38 (m, 4H, CH_2) 2.13 - 2.22 (m, 4H, CH_2) 1.83 (dd, *J*=12.99, 5.97 Hz, 1H, CH_2) 1.63 (s, 3H, CH_2) 1.41 - 1.52 (m, 5H, CH_2) 1.31 - 1.38 (m, 31H, $C(CH_3)_3$) 1.27 (s, 2H, CH_2) 1.18 - 1.24 (m, 24H, $C(CH_3)_3$) 1.13 (s, 2H, CH_2).

HRMS (m/z, ESI): calculated for $C_{70}H_{107}ClN_8O_{14}$ – $[M+H]^+$ 1319.7668, found: 1319.7669

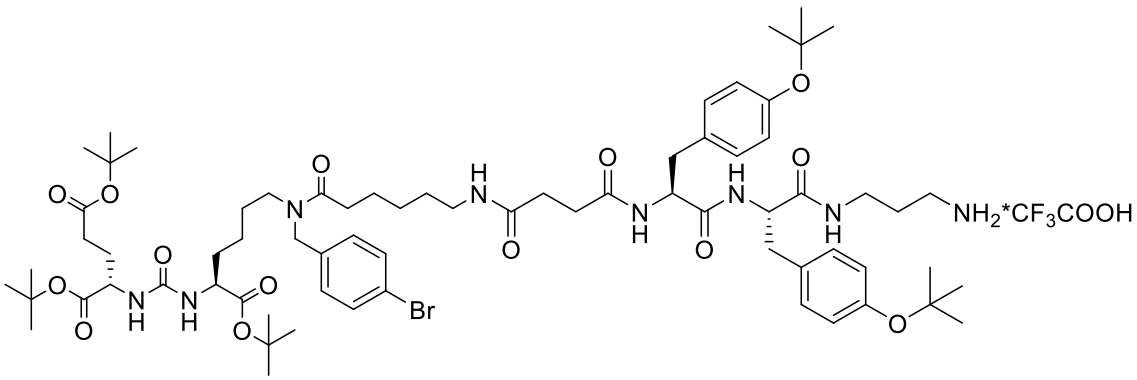
Synthesis of compound **4e**



From 251 mg 2-CTC resin (0.328 mmol) with immobilized 1,3-diaminopropane fragment by successive steps 4) 302 mg Fmoc-(L)-Tyr(OtBu)-OH, 171 μL DIPEA, 249 mg HBTU, 22 mg HOBt, 3) 4 mL 20% 4-methylpiperidine in DMF, 4) 254 mg Fmoc-(L)-Phe-OH, 171 μl DIPEA, 249 mg HBTU, 22 mg HOBt, 3) 6 mL 20% 4-methylpiperidine in DMF, 5) 342 mg of compound **1b**, 171 μl DIPEA, 249 mg HBTU, 22 mg HOBt and 6) 228 mg (49% yield) compound **4e** as trifluoroacetate was obtained.

^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.34 (d, *J*=7.34 Гц, 1H, NH), 8.14 (d, *J*=8.22 Гц, 1H, NH), 7.97-7.87 (m, 1H, NH), 7.67 (br, s., 4H, NH_3^+), 7.43-7.56 (m, 2H, Ar), 7.26-7.17 (m, 3H, Ar), 7.17-7.08 (m, 7H, Ar), 6.87 (d, *J*=7.62 Гц, 2H, Ar), 6.34-6.21 (m, 2H, NH), 4.52-4.41 (m, 2H, CH_2), 4.37-4.30 (m, 1H, CH), 4.30-4.21 (m, 1H, CH), 4.05-3.98 (m, 1H, CH), 3.98-3.89 (m, 1H, CH), 3.21-3.08 (m, 4H, CH_2), 3.07-2.93 (m, 5H, CH_2), 2.90-2.80 (m, 2H, CH_2), 2.79-2.68 (m, 3H, CH_2), 2.68-2.57 (m, 1H, CH_2), 2.39-2.28 (m, 4H, CH_2), 2.28-2.11 (m, 6H, CH_2), 1.89-1.80 (m, 1H, CH_2), 1.70-1.58 (m, 4H, CH_2), 1.58-1.41 (m, 7H, CH_2), 1.41-1.30 (m, 36H, CH_2+CH_3), 1.30-1.12 (m, 18H, CH_2+CH_3).

Synthesis of compound **4f**

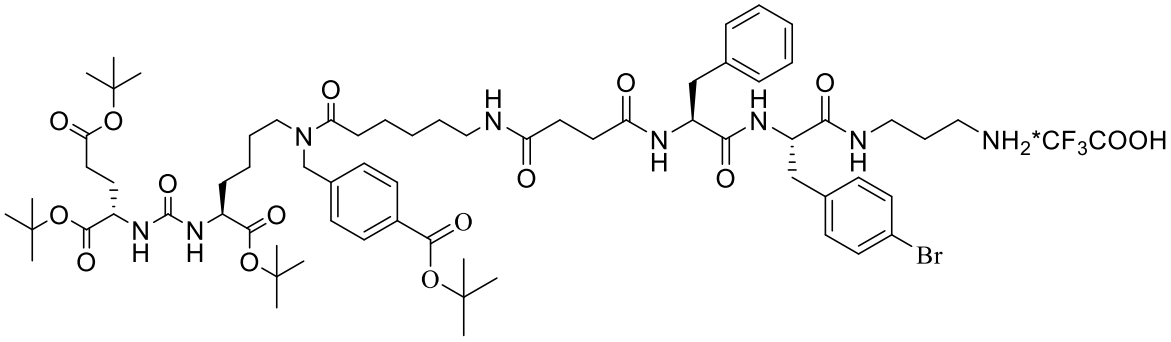


From 250 mg 2-CTC resin (0.25 mmol) with immobilized 1,3-diaminopropane fragment by successive steps 4) 235 mg Fmoc-(L)-Tyr(OtBu)-OH, 132 μL DIPEA, 195 mg HBTU, 17 mg HOBt, 3) 4 mL 20% 4-methylpiperidine in DMF, 4) 235 mg Fmoc-(L)-Tyr(OtBu)-OH, 132 μl DIPEA, 195 mg HBTU, 17 mg HOBt, 3) 6 mL 20% 4-methylpiperidine in DMF, 5) 261 mg of compound **1b**, 132 μl DIPEA, 195 mg HBTU, 17 mg HOBt and 6) 107 mg (29% yield) compound **4f** as trifluoroacetate was obtained.

^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.24 - 7.37 (m, 2H, Ar) 7.15 - 7.20 (m, 1H, Ar) 7.12 (dd, *J*=8.44, 2.85 Hz, 3H, Ar) 6.99 (d, *J*=7.29 Hz, 2H, Ar) 6.85 (d, *J*=8.55 Hz, 2H, Ar) 6.78 (d, *J*=8.33 Hz, 2H, Ar) 4.51 (m, 1H, CH) 4.44 (m, *J*=2.69 Hz, 1H, CH) 4.28 (dd, *J*=9.48, 4.44 Hz, 1H, CH) 4.14 - 4.21 (m, 1H, CH) 3.99 (dd, *J*=7.86, 5.29 Hz, 1H, CH) 3.88 - 3.96 (m, 1H, CH) 3.10 - 3.19 (m, 3H, CH_2) 2.94 - 3.05 (m, 4H, CH_2) 2.80 - 2.89 (m, 1H, CH_2) 2.67 - 2.76 (m, 3H, CH_2) 2.52 - 2.63 (m, 1H, CH_2) 2.28 - 2.38 (m, 4H, CH_2) 2.13 - 2.22 (m, 4H, CH_2) 1.83 (dd, *J*=12.99, 5.97 Hz, 1H, CH_2) 1.63 (s, 3H, CH_2) 1.41 - 1.52 (m, 5H, CH_2) 1.31 - 1.38 (m, 31H, $C(CH_3)_3$) 1.27 (s, 2H, CH_2) 1.18 - 1.24 (m, 24H, $C(CH_3)_3$) 1.13 (s, 2H, CH_2).

HRMS (m/z, ESI): calculated for $C_{70}H_{107}BrN_8O_{14}$ – $[M+H]^+$ 1363.7163, found: 1363.7157

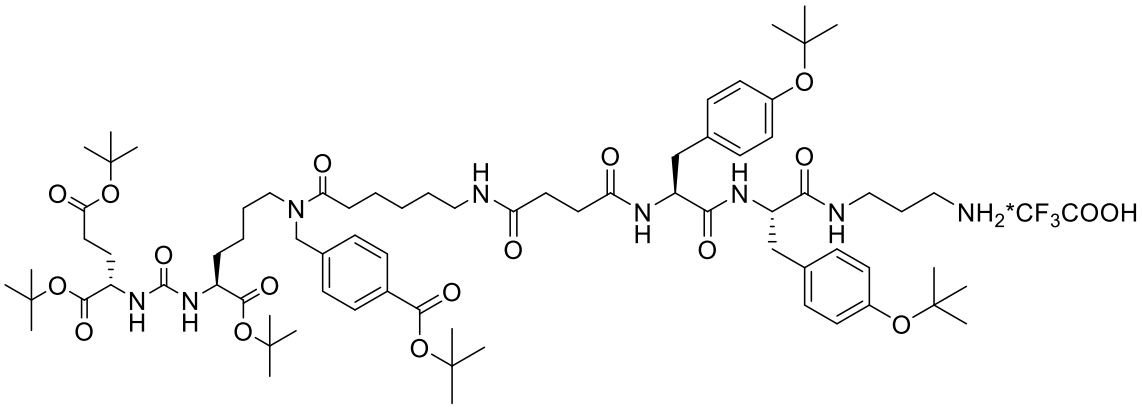
Synthesis of compound **4g**



From 222 mg 2-CTC resin (0.25 mmol) with immobilized 1,3-diaminopropane fragment by successive steps 4) 140 mg Fmoc-(L)-Phe(4-Br)-OH, 128 μL DIPEA, 190 mg HBTU, 17 mg HOBt, 3) 4 mL 20% 4-methylpiperidine in DMF, 4) 193 mg Fmoc-(L)-Phe-OH, 128 μl DIPEA, 190 mg HBTU, 17 mg HOBt, 3) 6 mL 20% 4-methylpiperidine in DMF, 5) 267 mg of compound **1c**, 128 μl DIPEA, 190 mg HBTU, 17 mg HOBt and 6) 70 mg (20% yield) compound **4g** as trifluoroacetate was obtained.

^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.31 (d, *J*=6.41 Hz, 1H, NH), 8.17 (d, *J*=6.85 Hz, 1 H), 7.94 (d, *J*=5.21 Hz, 1H, NH), 7.87 (d, *J*=8.00 Hz, 1H, NH), 7.82 (d, *J*=8.06 Hz, 1 H), 7.74 (br.s., 4H, NH_3^+), 7.45 (d, *J*=8.28 Hz, 2H, Ar), 7.24 - 7.31 (m, 3H, Ar), 7.12 - 7.24 (m, 8H, Ar), 6.24 - 6.34 (m, 2H, NH), 4.60 (s, 2H, CH_2), 4.53 (s, 3H, CH_2), 3.89 - 4.06 (m, 5H, CH), 3.20 – 3.18 (m, 1H, CH_2), 3.07 - 3.17 (m, 3H, CH_2), 2.98 - 3.07 (m, 3H, CH_2), 2.95 – 2.90 (m, 2H, CH_2), 2.88 – 2.85 (m, 2H, CH_2), 2.75 - 2.73 (m, 2H, CH_2), 2.65 – 2.63 (m, 1H, CH_2), 2.26 - 2.38 (m, 4H, CH_2), 2.13 - 2.24 (m, 5H, CH_2), 1.60 - 1.70 (m, 3H, CH_2), 1.52 (s, 11H, CH_3), 1.40 - 1.48 (m, 4H, CH_2), 1.33 - 1.39 (m, 31H, CH_3), 1.29 (m, 3H, CH_2), 1.20 - 1.24 (m, 6H, CH_3).

Synthesis of compound **4h**

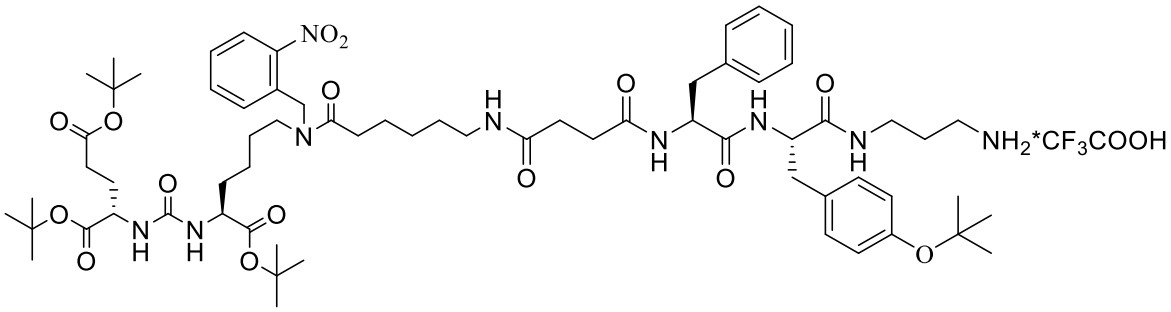


From 250 mg 2-CTC resin (0.25 mmol) with immobilized 1,3-diaminopropane fragment by successive steps 4) 235 mg Fmoc-(L)-Tyr(OtBu)-OH, 132 μL DIPEA, 195 mg HBTU, 17 mg HOBt, 3) 4 mL 20% 4-methylpiperidine in DMF, 4) 235 mg Fmoc-(L)-Tyr(OtBu)-OH, 132 μl DIPEA, 195 mg HBTU, 17 mg HOBt, 3) 6 mL 20% 4-methylpiperidine in DMF, 5) 267 mg of compound **1c**, 132 μl DIPEA, 195 mg HBTU, 17 mg HOBt and 6) 85 mg (23% yield) compound **4h** as trifluoroacetate was obtained.

^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.33 (d, *J*=2.74 Hz, 1H, NHC(O)) 8.12 (d, *J*=9.76 Hz, 1H, NHC(O))) 7.85 - 7.96 (m, 2H, Ar) 7.82 (d, *J*=8.33 Hz, 1H, Ar) 7.65 (br, s., 4H, NH_3^+) 7.21 - 7.30 (m, 2H, Ar) 7.09 - 7.16 (m, 2H, Ar) 7.02 (d, *J*=8.77 Hz, 2H, Ar) 6.83 - 6.90 (m, 2H, Ar) 6.80 (d, *J*=8.50 Hz, 2H, Ar) 6.22 - 6.31 (m, 2H, NH) 4.60 (br, s., 2H, CH) 4.53 (d, *J*=5.81 Hz, 2H, CH) 4.32 (br, s., 3H, CH) 4.22 (br, s., 2H, CH) 3.89 - 4.05 (m, 3H, CH) 3.15 (t, *J*=7.26 Hz, 3H, CH_2) 2.93 - 3.06 (m, 4H, CH_2) 2.85 (dd, *J*=15.10, 9.13 Hz, 2H, CH_2) 2.68 - 2.80 (m, 3H, CH_2) 2.55 - 2.68 (m, 1H, CH_2) 2.33 (d, *J*=8.11 Hz, 4H, CH_2) 2.12 - 2.28 (m, 5H, CH_2) 1.76 - 1.90 (m, 1H, CH_2) 1.58 - 1.69 (m, 3H, CH_2) 1.52 (d, *J*=1.48 Hz, 11H, $C(CH_3)_3$) 1.33 - 1.39 (m, 30H, $C(CH_3)_3$) 1.23 (dd, *J*=1.59, 1.48 Hz, 22H, CH_3).

HRMS (m/z, ESI): calculated for $C_{70}H_{107}BrN_8O_{14}$ – $[M+H]^+$ 1385.8582, found: 1385.8575

Synthesis of compound **4i**

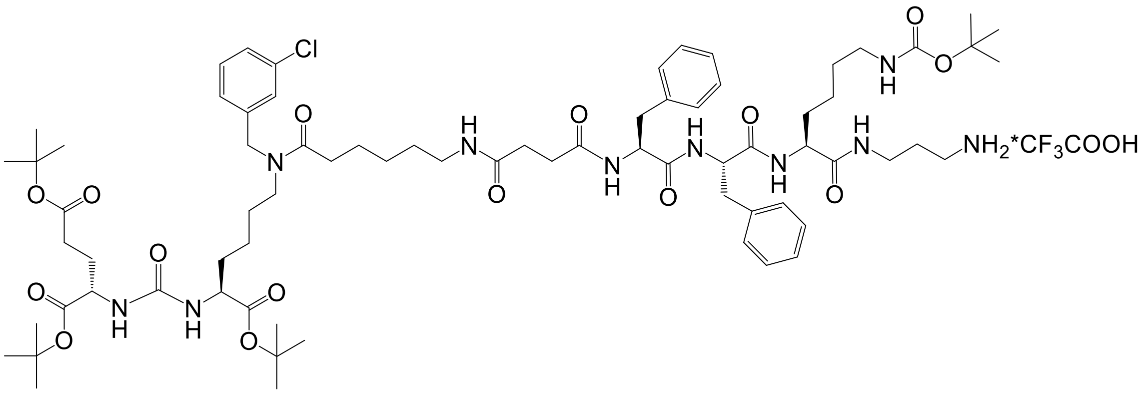


From 783 mg 2-CTC resin (1.024 mmol) with immobilized 1,3-diaminopropane fragment by steps 4) 941 mg Fmoc-(L)-Tyr(OtBu)-OH, 535 μL DIPEA, 777 mg HBTU, 78 mg HOBt, were obtained 1,173 g of 2-CTC resin with immobilized fragment. Then from 586 mg of that resin by

successive steps 3) 6 mL 20% 4-methylpiperidine in DMF, 4) 397 mg Fmoc-(L)-Phe-OH, 268 μl DIPEA, 389 mg HBTU, 39 mg HOBt, 3) 8 mL 20% 4-methylpiperidine in DMF were obtained 549 mg of 2-CTC resin with immobilized fragment **5b**. After this by successive steps 5) 144 mg of compound **1d**, 171 μl DIPEA, 249 mg HBTU, 22 mg HOBt and 6) 108 mg (55% yield) compound **4i** as trifluoroacetate was obtained.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ): 8.35 (d, *J*=7.13 Hz, 1H, NH), 8.18-8.00 (m, 2H, NH+Ar), 7.98-7.86 (m, 1H, NH), 7.80-7.60 (m, 5H, NH+Ar+NH_3^+), 7.60-7.47 (m, 1H, Ar), 7.30-7.08 (m, 8H, Ar), 6.92-6.82 (m, 2H, Ar), 6.35-6.20 (m, 2H, NH), 4.96-4.70 (m, 2H, CH_2), 4.39-4.29 (m, 1H, CH), 4.29-4.21 (m, 1H, CH), 4.07-3.88 (m, 2H, CH+CH), 3.32-3.09 (m, 3H, CH_2), 3.08-2.90 (m, 4H, CH_2), 2.89-2.81 (m, 2H, CH_2), 2.80-2.69 (m, 2H, CH_2), 2.68-2.60 (m, 1H, CH_2), 2.45-2.10 (m, 8H, CH_2), 1.91-1.79 (m, 1H, CH_2), 1.71-1.57 (m, 4H, CH_2), 1.56-1.40 (m, 7H, CH_2), 1.40-1.32 (m, 29H, CH_2+CH_3), 1.32-1.11 (m, 20H, CH_2+CH_3).

Synthesis of compound **8**

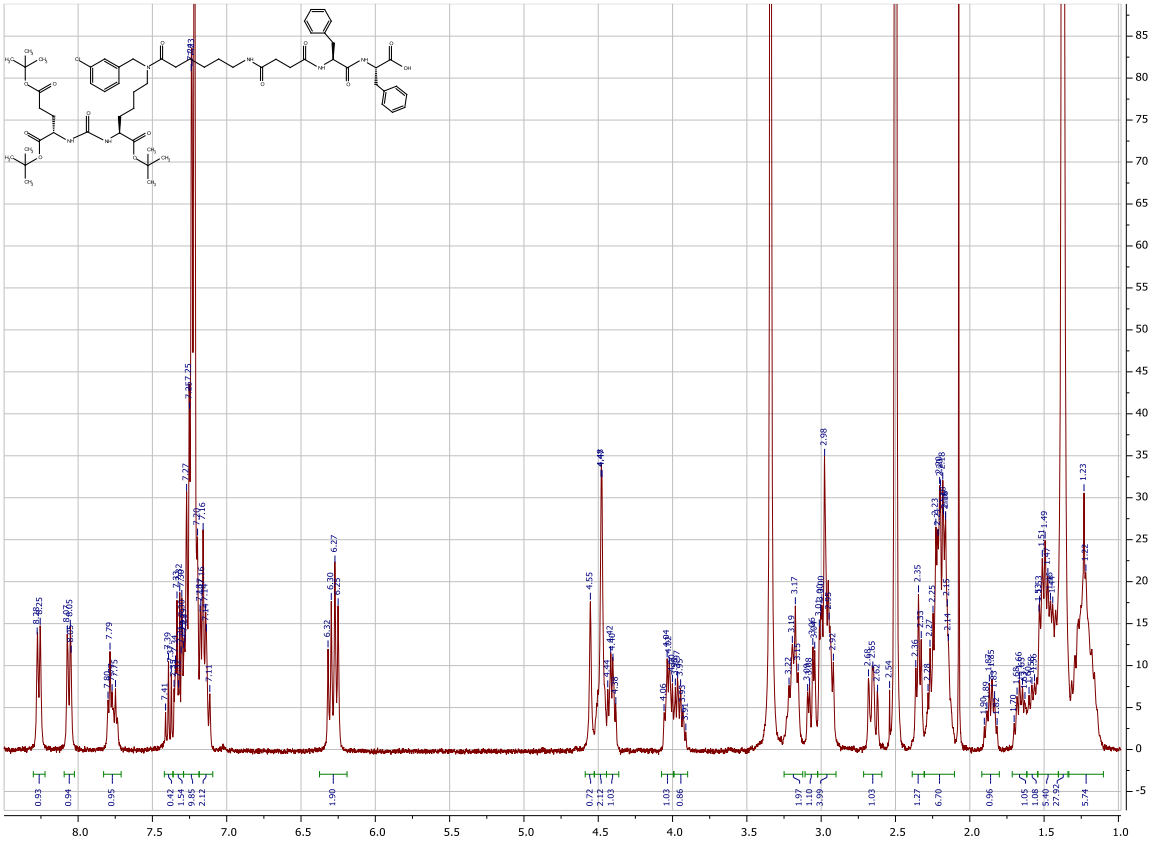


From 195 mg 2-CTC resin (0.255 mmol) with immobilized 1,3-diaminopropane fragment by successive steps 4) 239 mg Fmoc-(L)-Lys(ε-NHBoc)-OH, 134 μL DIPEA, 193 mg HBTU, 17 mg HOBt, 3) 4 mL 20% 4-methylpiperidine in DMF, 4) 198 mg Fmoc-(L)-Phe-OH, 134 μL DIPEA, 193 mg HBTU, 17 mg HOBt, 3) 6 mL 20% 4-methylpiperidine in DMF, 4) 198 mg Fmoc-(L)-Phe-OH, 134 μl DIPEA, 193 mg HBTU, 17 mg HOBt, 3) 6 mL 20% 4-methylpiperidine in DMF, 5) 253 mg of compound **1a**, 134 μl DIPEA, 193 mg HBTU, 17 mg HOBt and 6) 111 mg (31% yield) compound **8** as trifluoroacetate was obtained. HPLC data showed that the purity of the product obtained is 21%

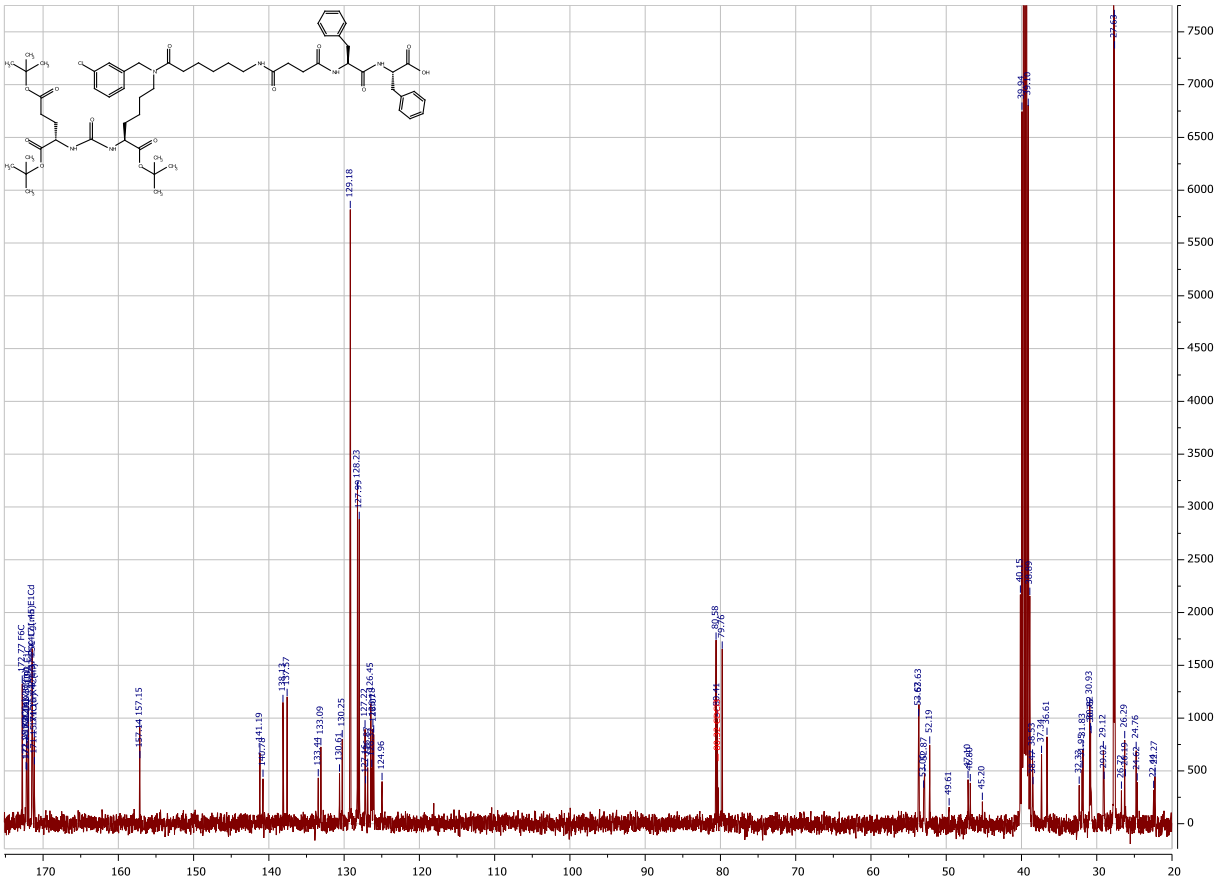
^{1}H NMR (400 MHz, DMSO-d$_6$, δ): 8.36 (d, *J*=6.91 Гц, 1H, NH), 8.20 (d, *J*=7.40 Гц, 1H, NH), 8.03-7.93 (m, 1H, NH), 7.75-7.60 (m, 5H, NH+NH_3^+), 7.40-7.08 (m, 15H, Ar), 6.78 (t, *J*=4.52 Гц, 1H, NH), 6.34-6.21 (m, 2H, NH), 4.57-4.45 (m, 2H, CH_2), 4.44-4.39 (m, 1H, CH), 4.32-4.24 (m, 1H, CH), 3.23-3.07 (m, 5H, CH_2), 3.07-2.90 (m, 5H, CH_2), 2.89-2.82 (m, 3H, CH_2), 2.80-2.71 (m, 2H, CH_2), 2.69-2.60 (m, 1H, CH_2), 2.39-2.27 (m, 4H, CH_2), 2.26-2.11 (m, 5H, CH_2), 1.90-1.80 (m, 1H, CH_2), 1.71-1.61 (m, 4H, CH_2), 1.60-1.40 (m, 9H, CH_2), 1.39-1.29 (m, 43H, CH_2+CH_3), 1.29-1.10 (m, 9H, CH_2+CH_3).

Spectral data for synthesized compounds

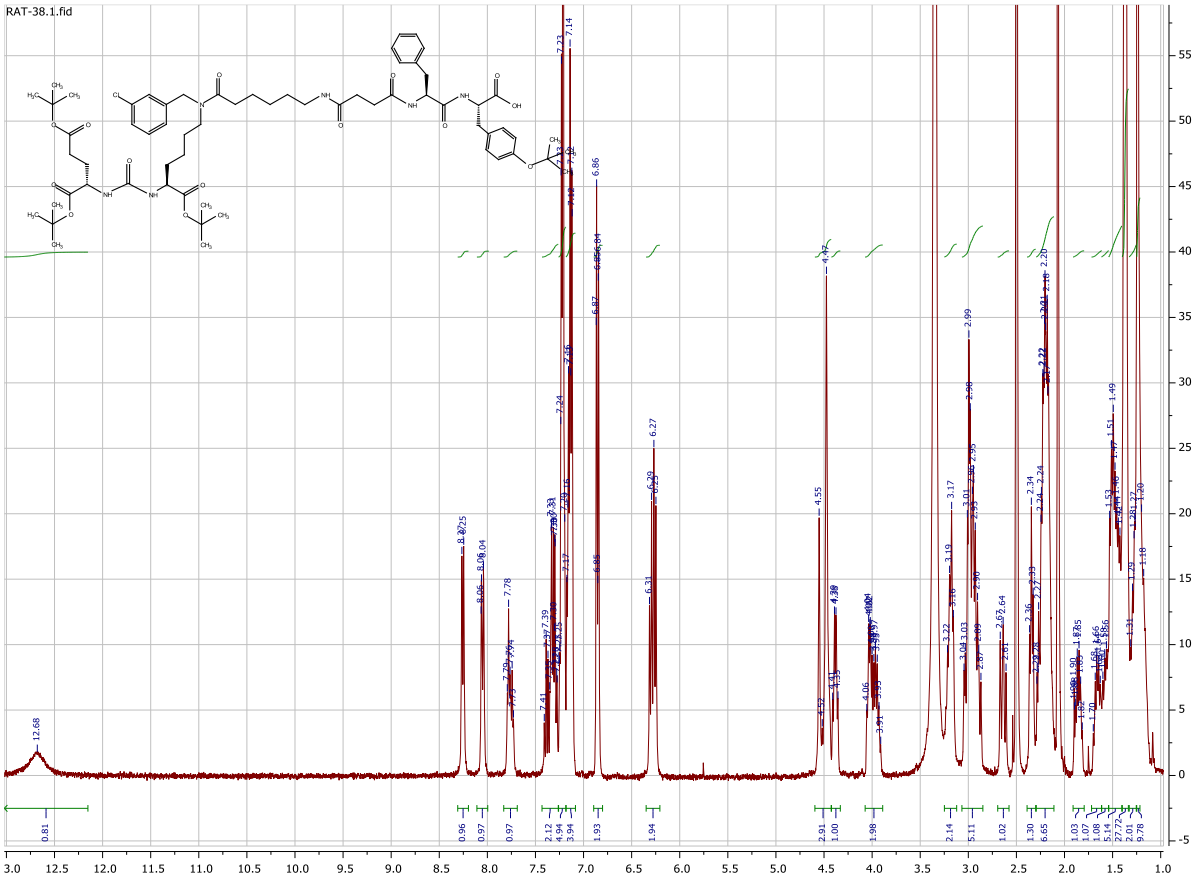
^{1}H NMR spectra for compound **2a**



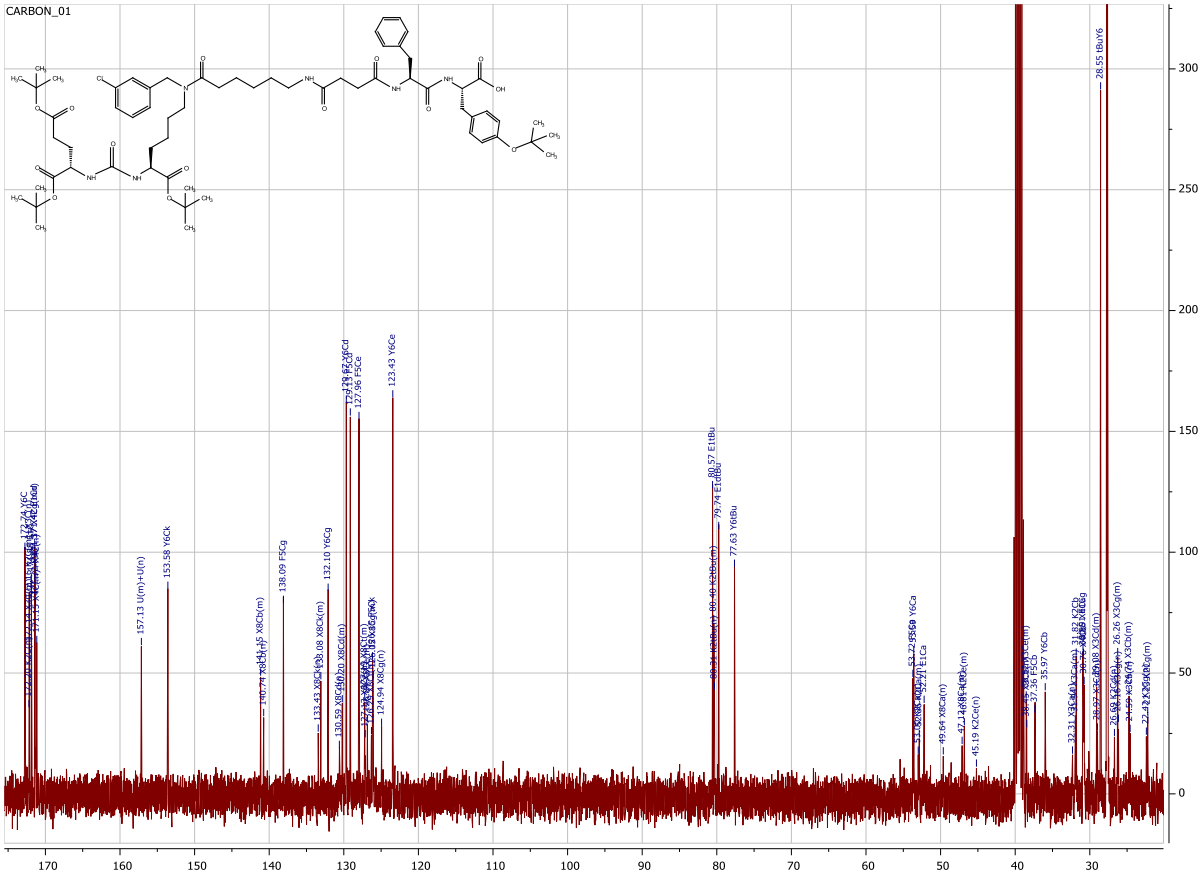
^{13}C NMR spectra for compound **2a**



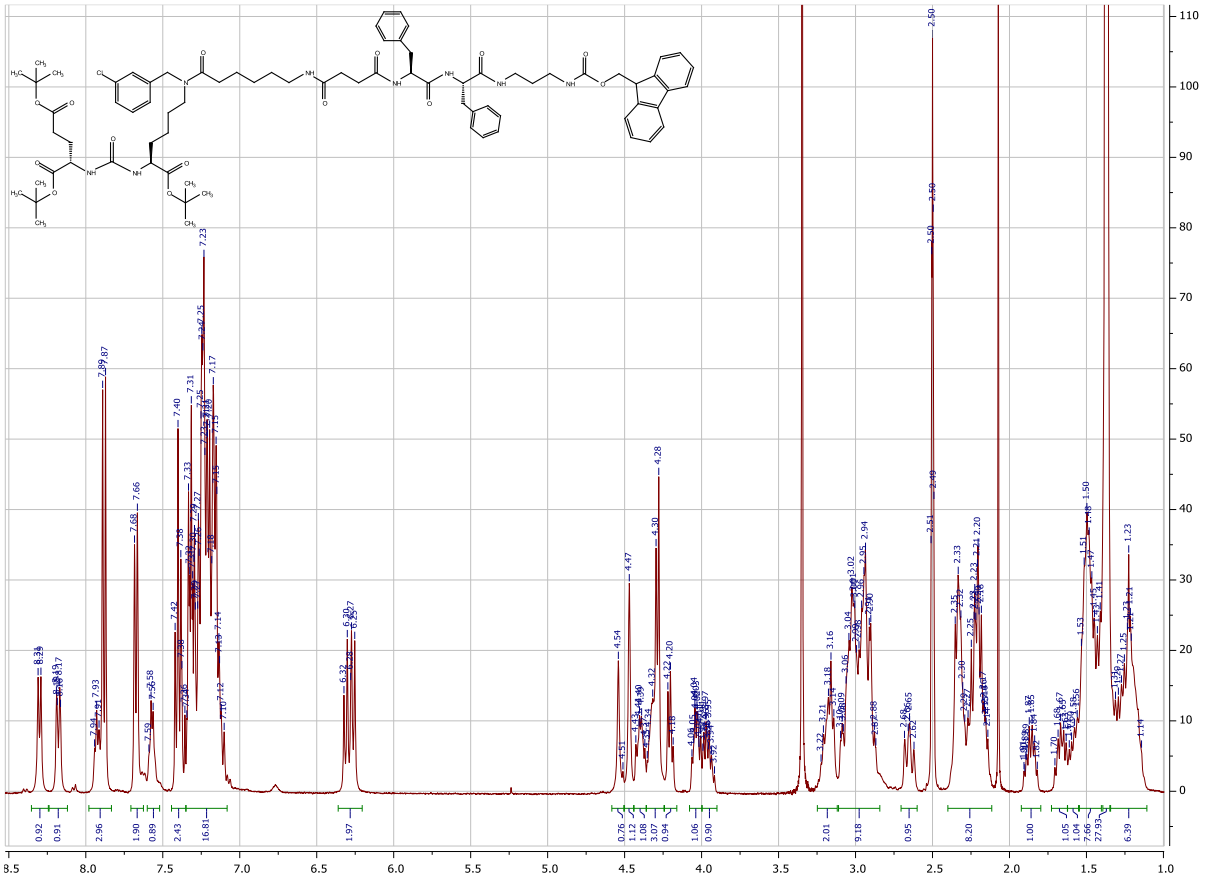
^{1}H NMR spectra for compound **2b**



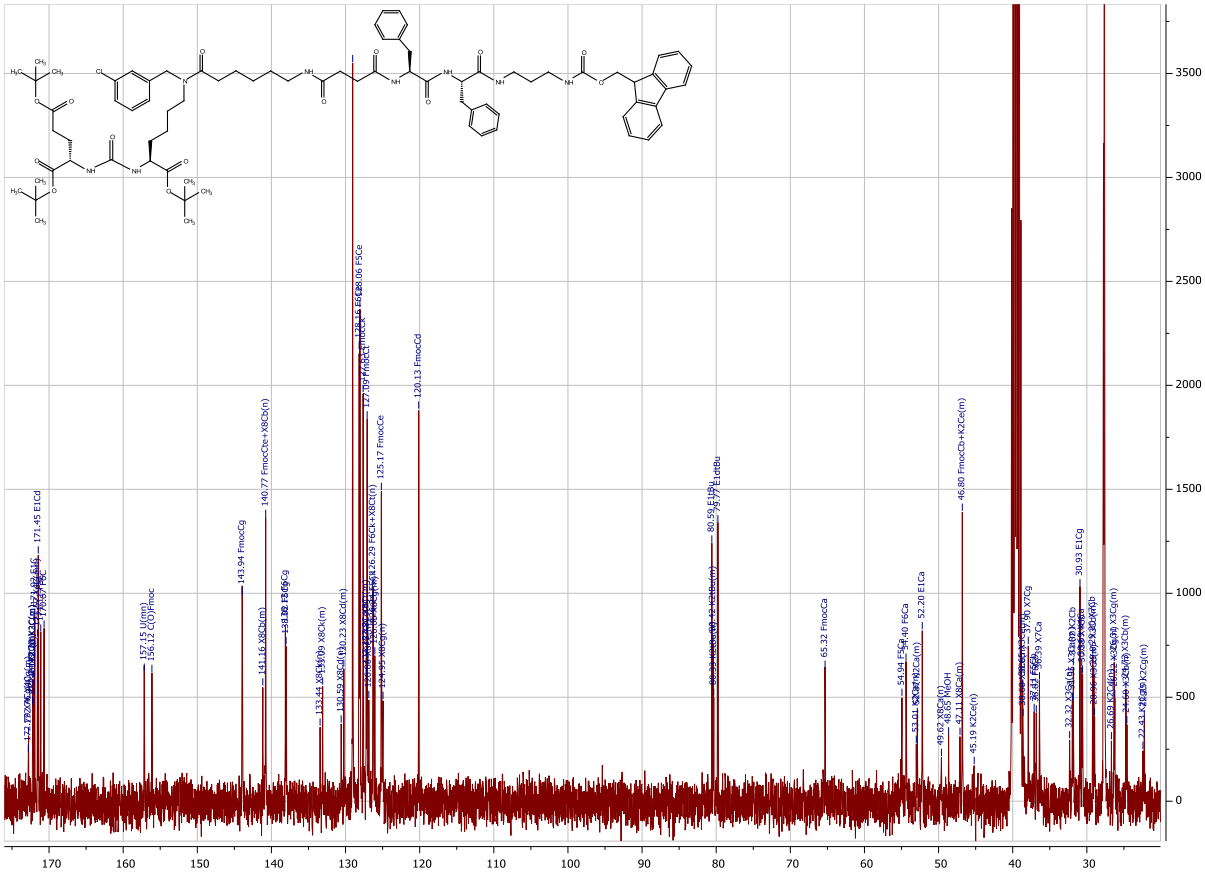
^{13}C NMR spectra for compound **2b**



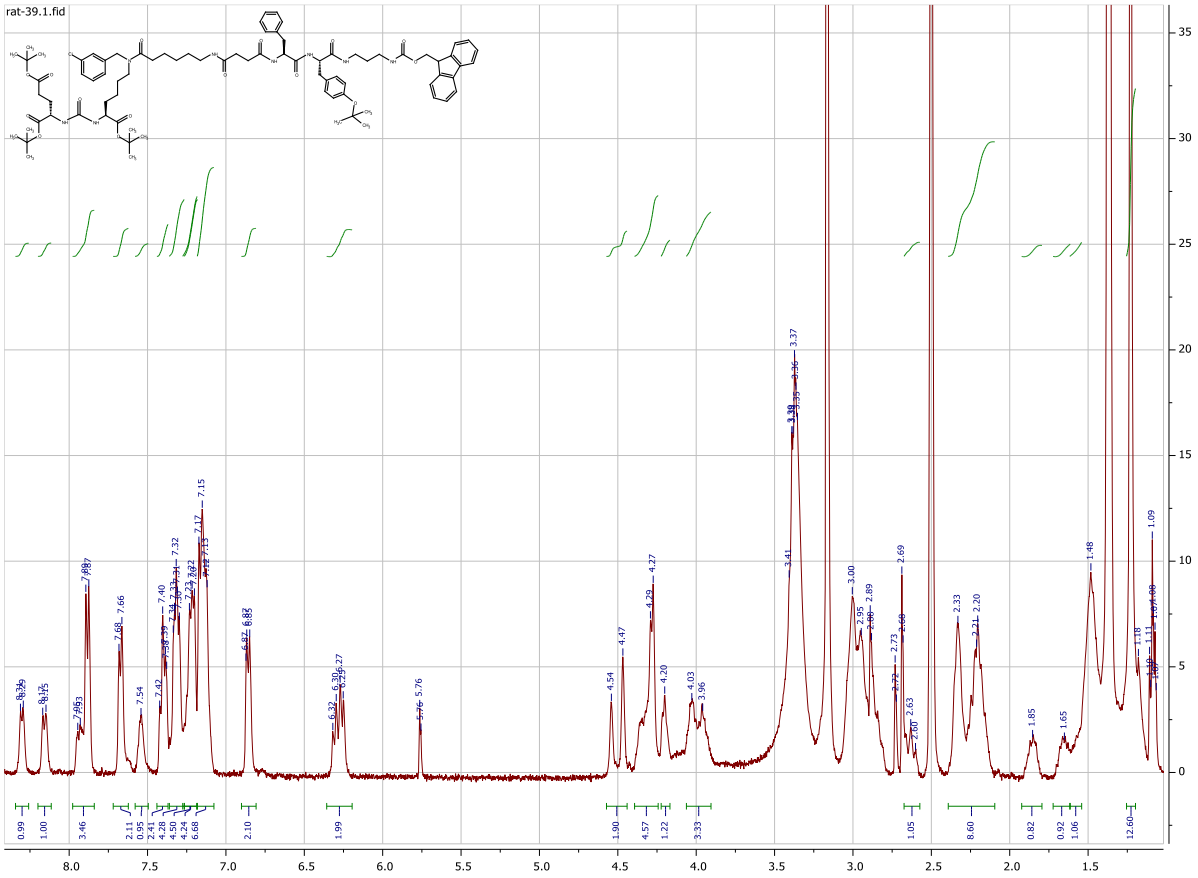
^{1}H NMR spectra for compound **3a**



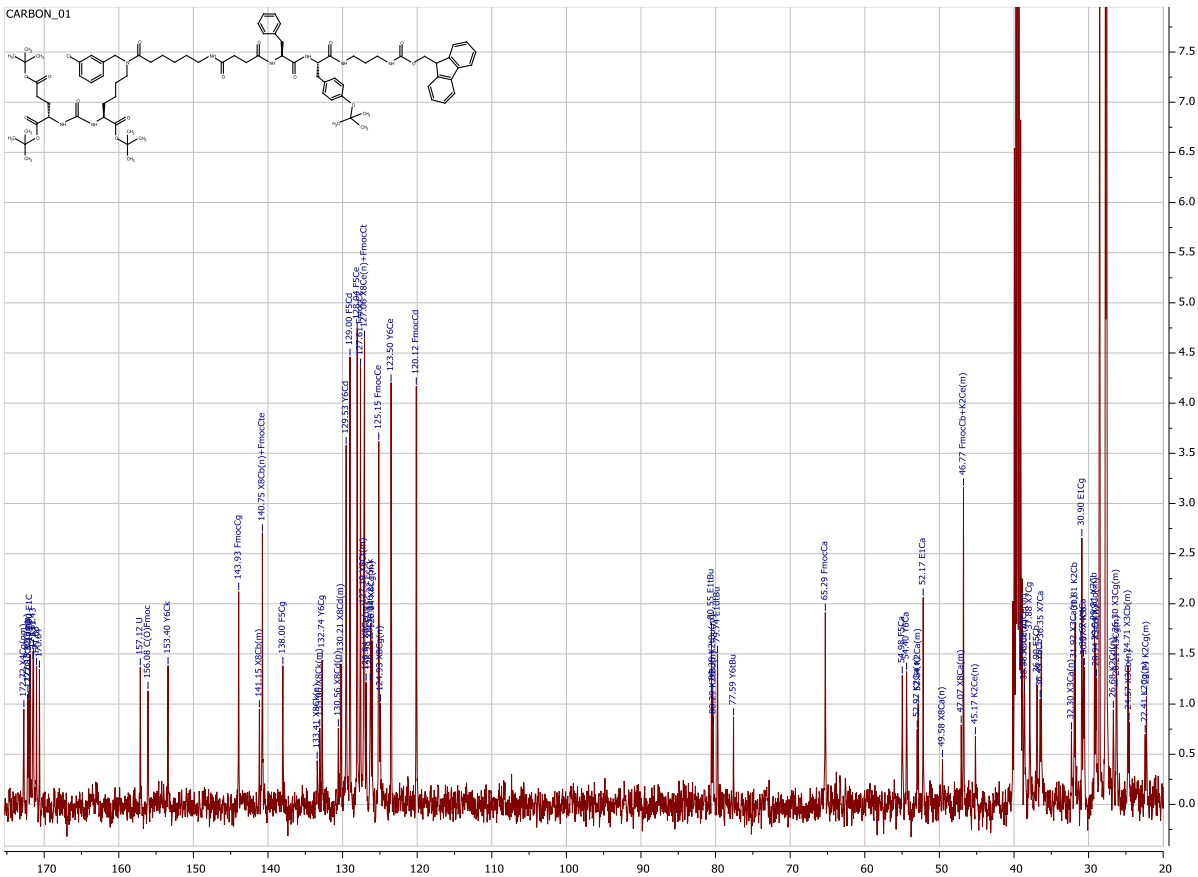
^{13}C NMR spectra for compound **3a**



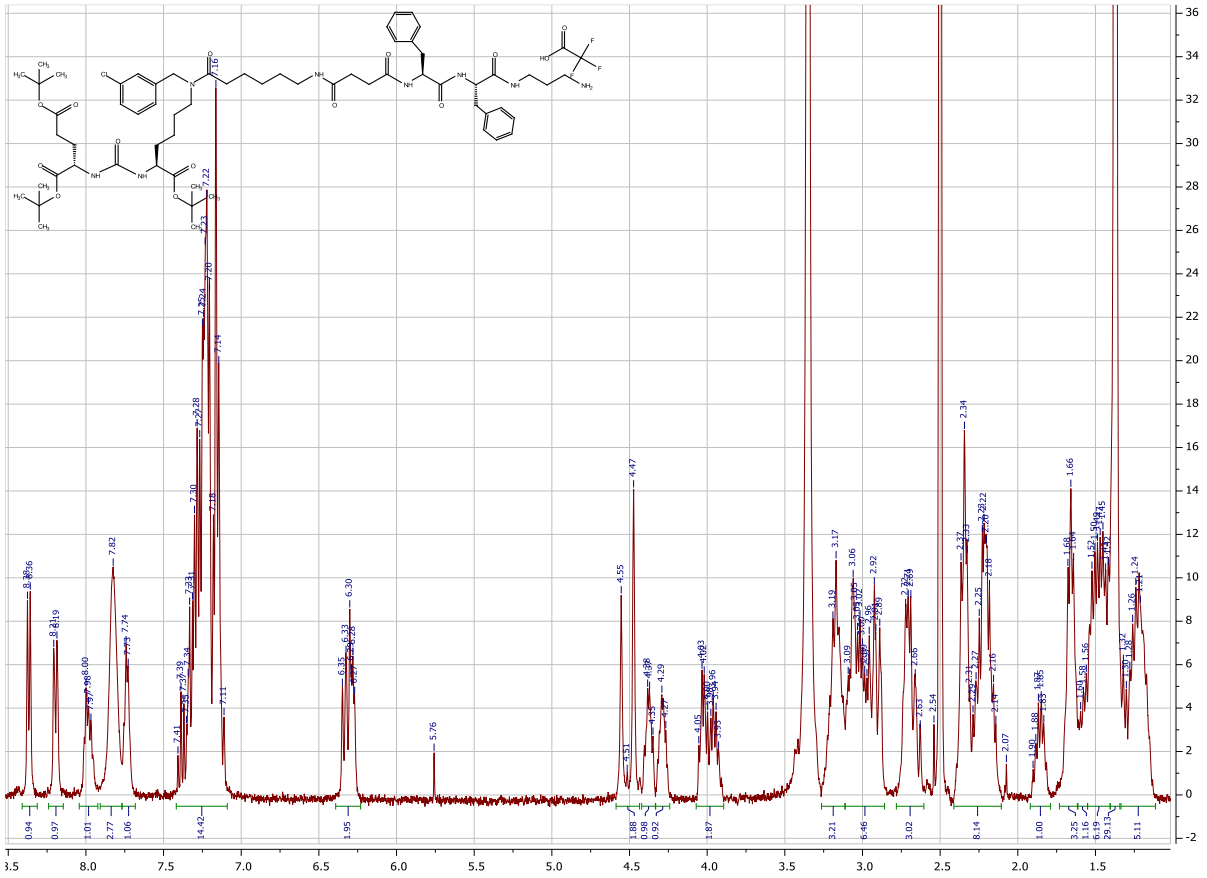
^{1}H NMR spectra for compound **3b**



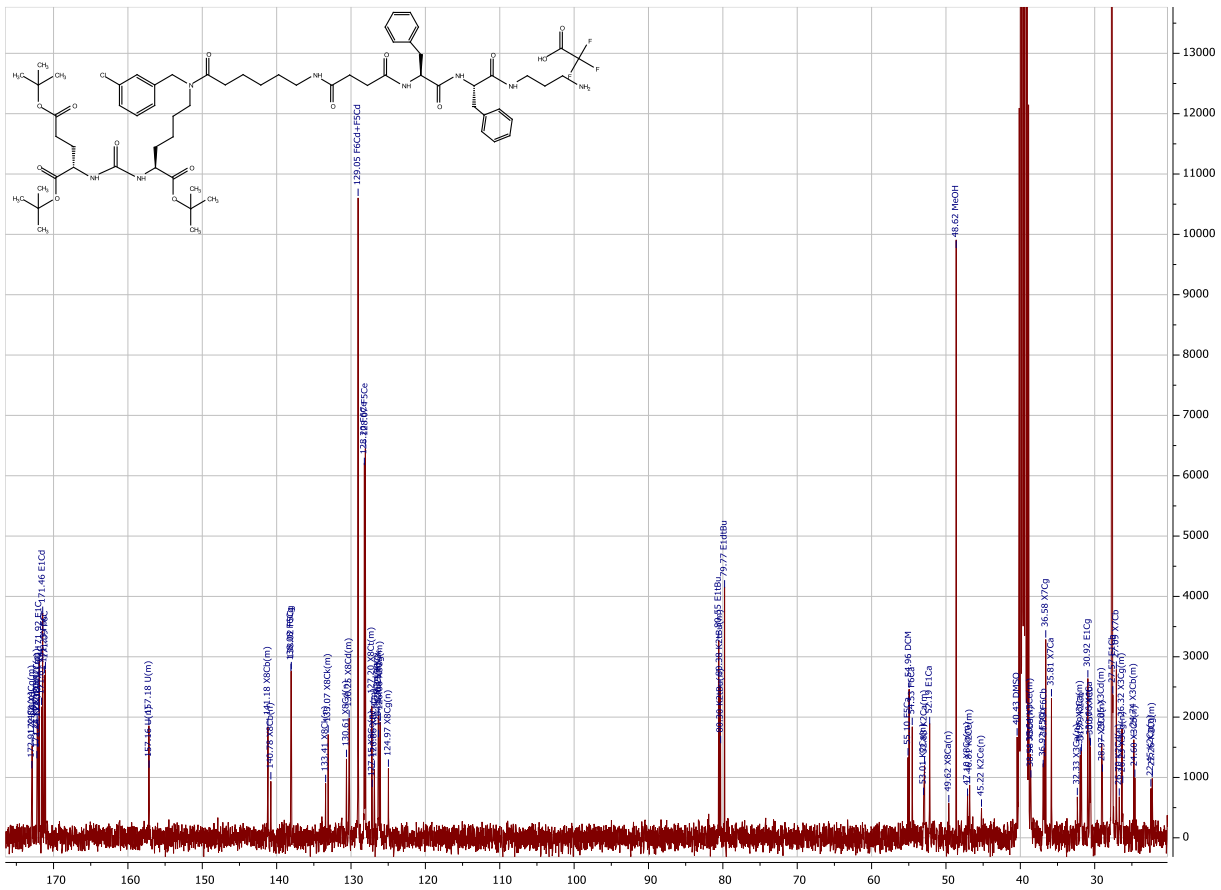
^{13}C NMR spectra for compound **3b**



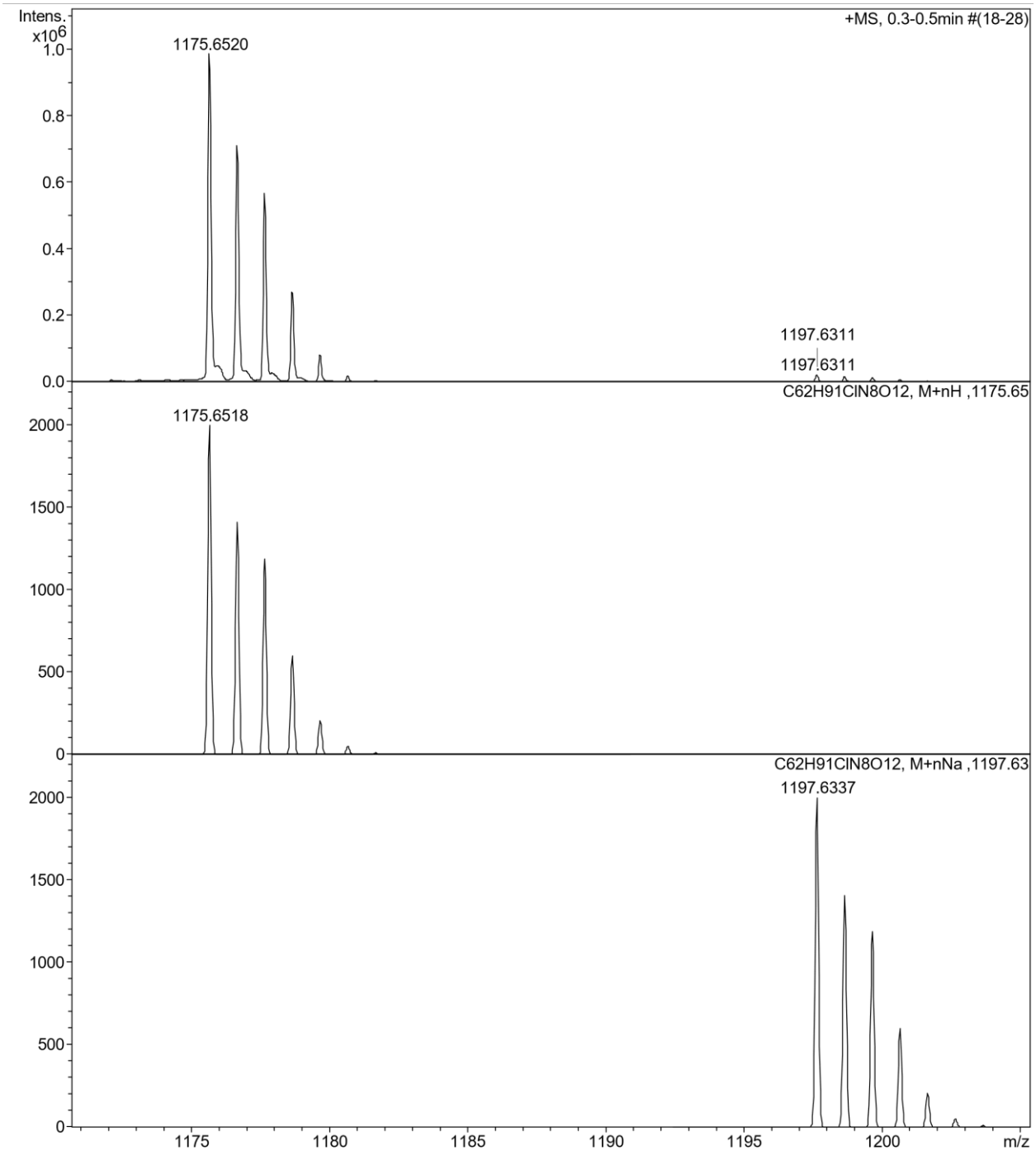
^{1}H NMR spectra for compound **4a**



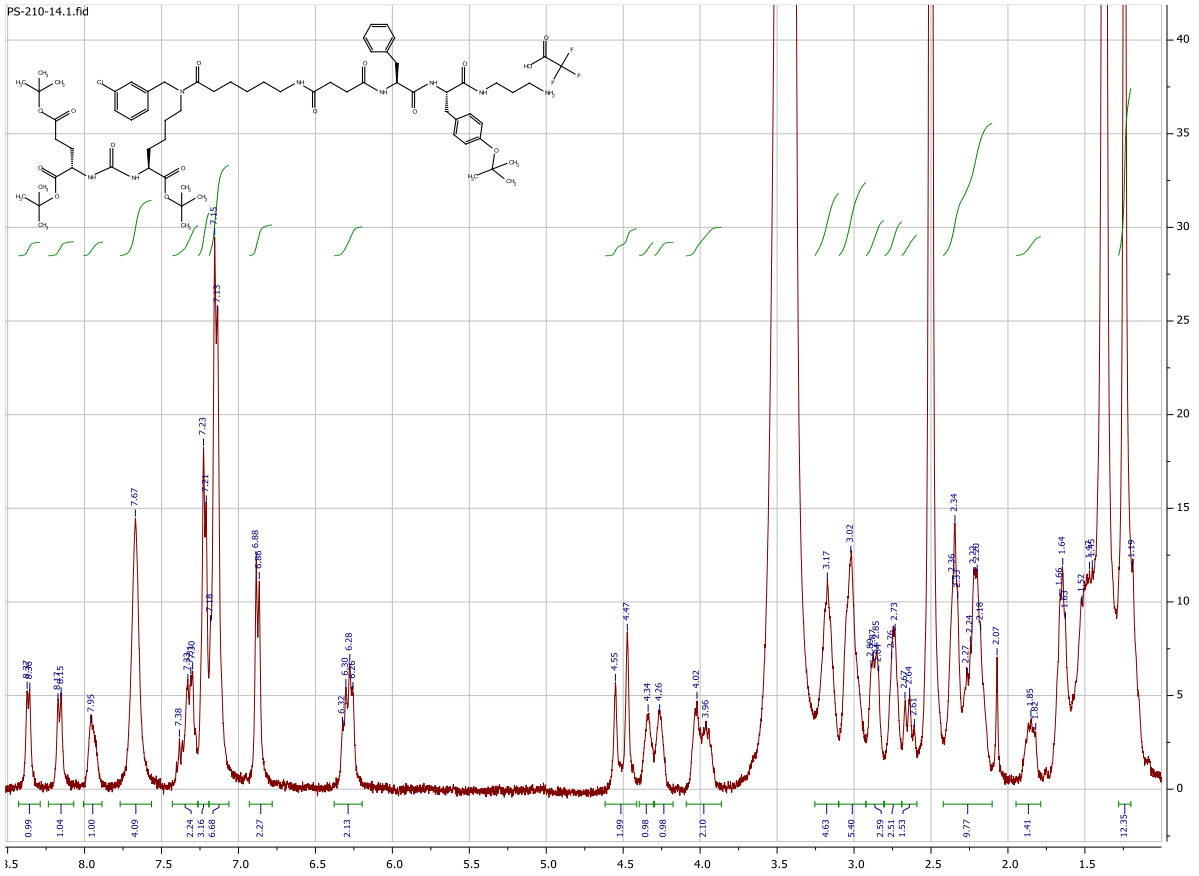
^{13}C NMR spectra for compound **4a**



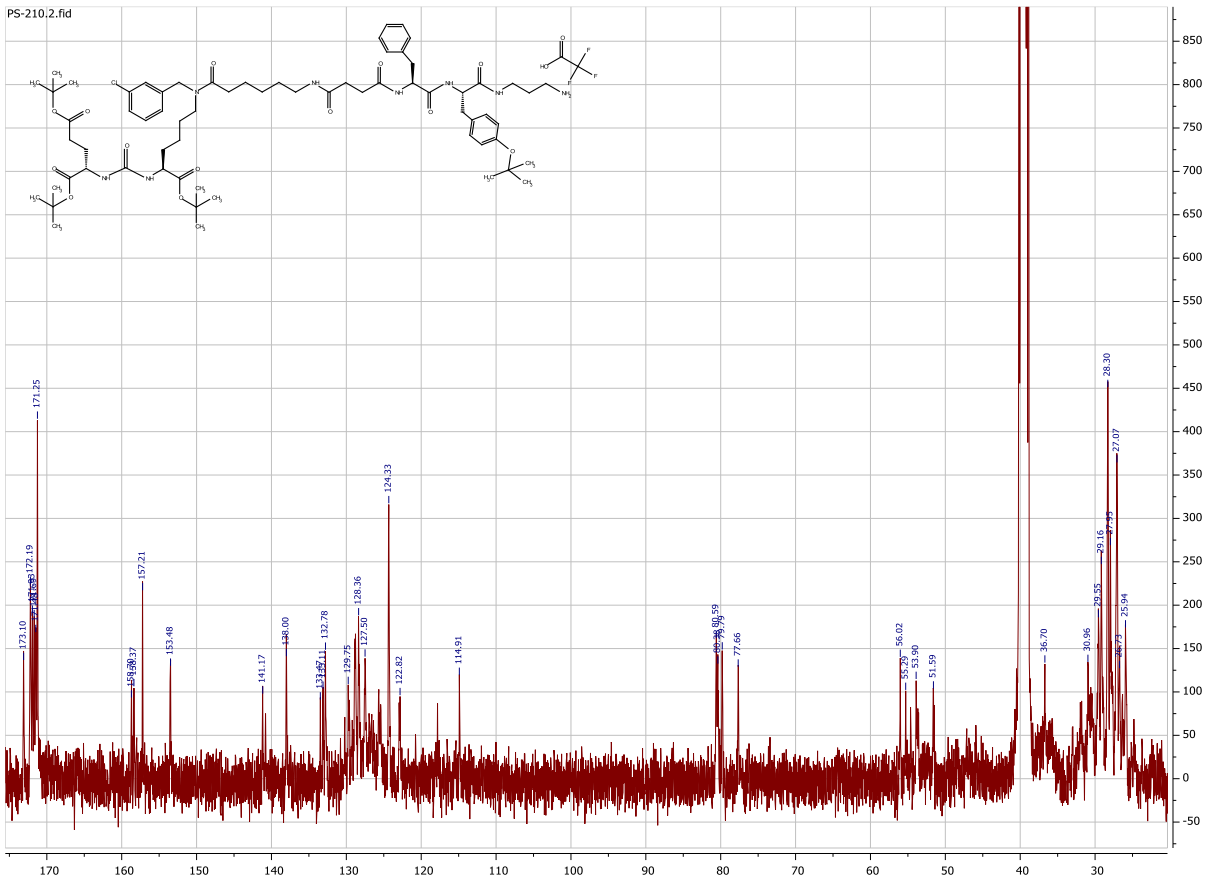
HRMS spectra for compound **4a**



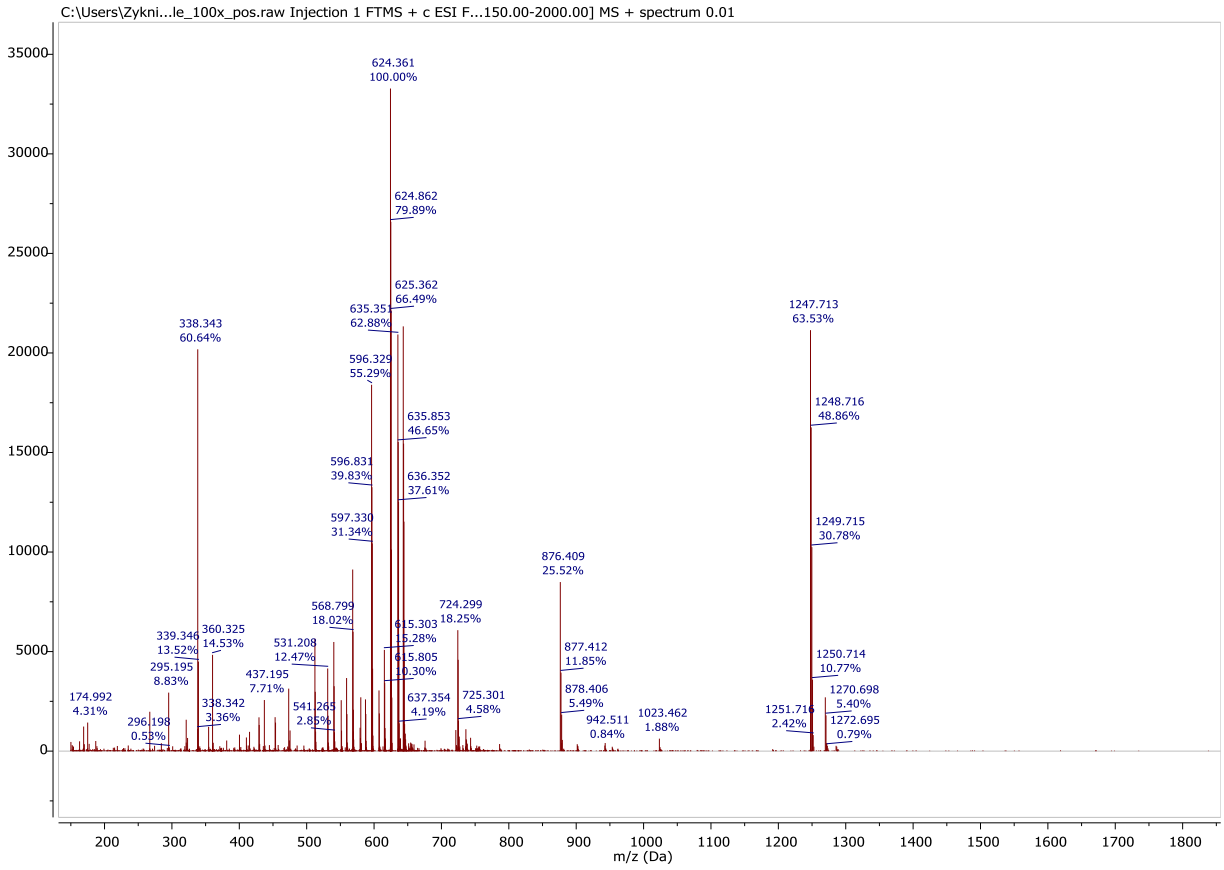
^{1}H NMR spectra for compound **4b**



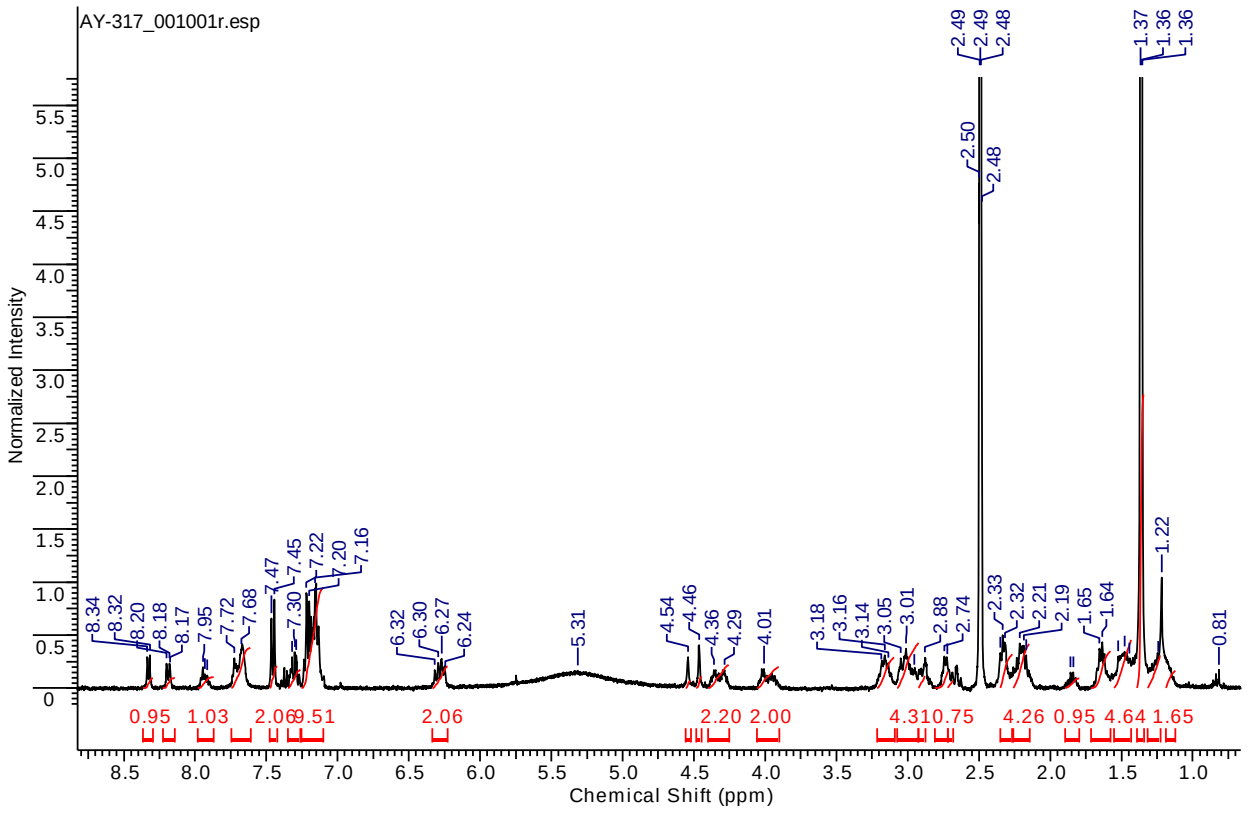
^{13}C NMR spectra for compound **4b**



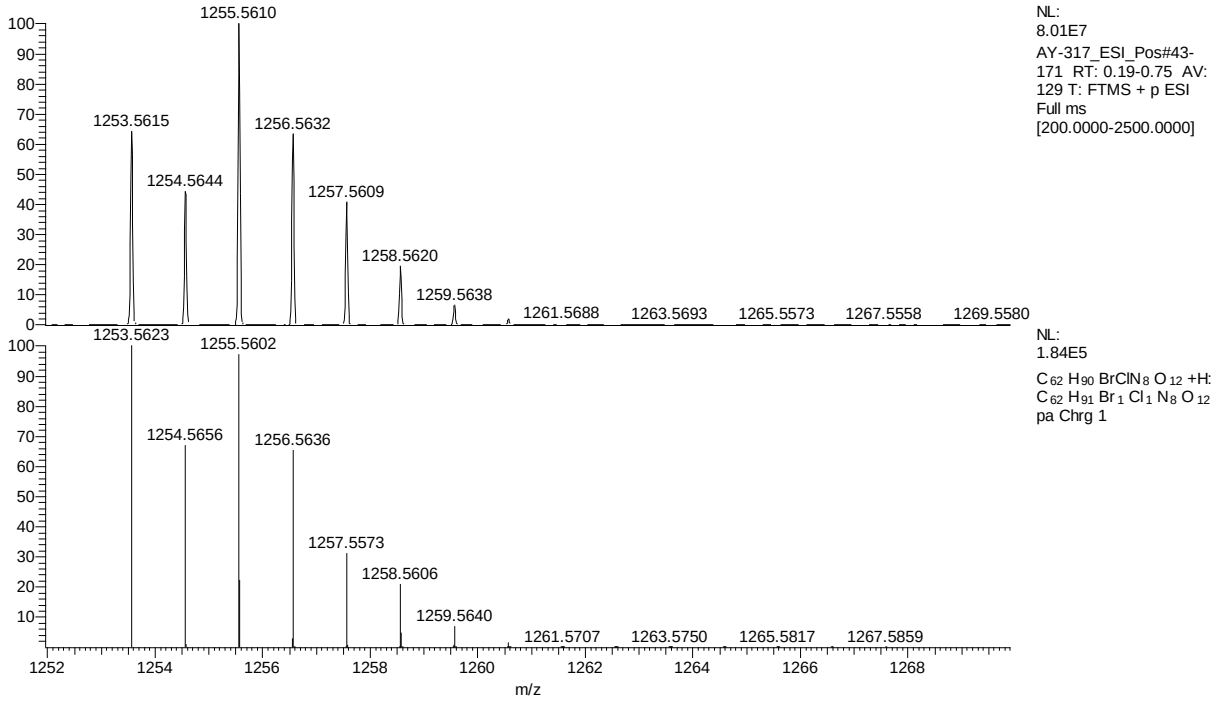
HRMS spectra for compound **4b**



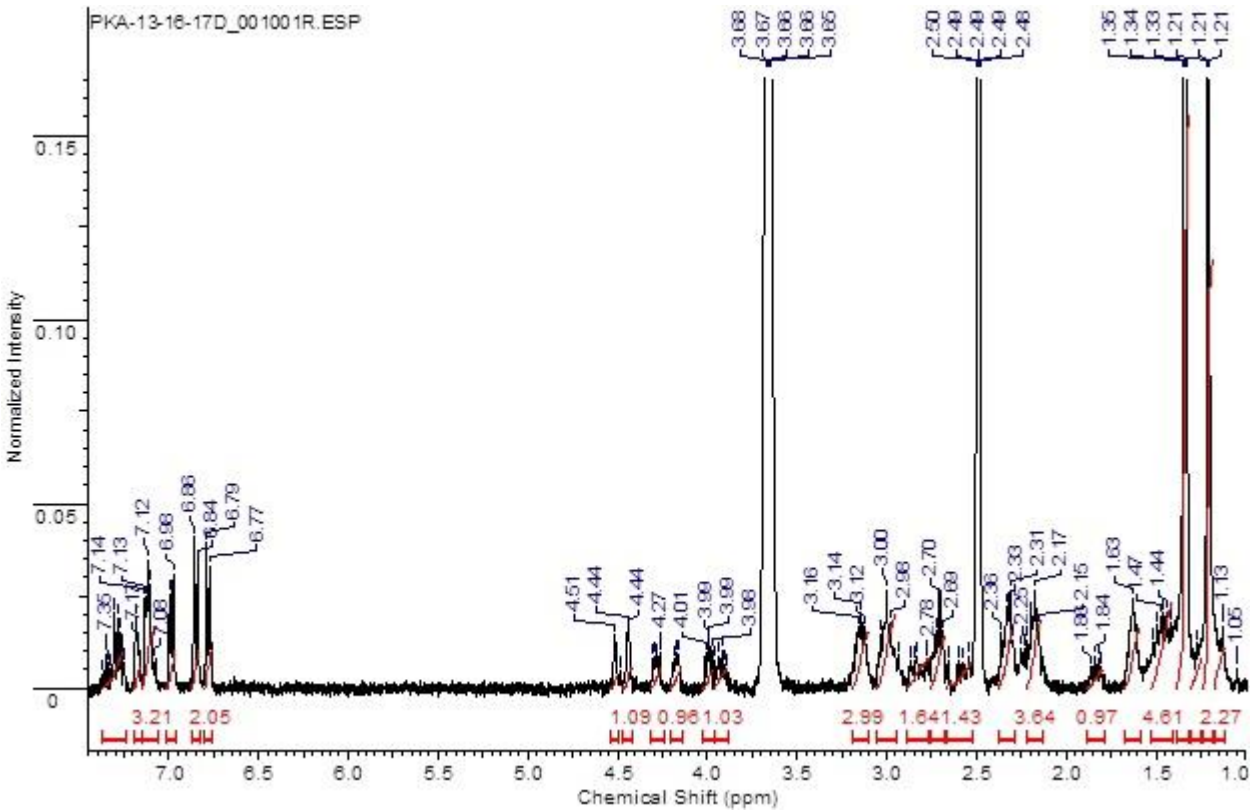
^{1}H NMR spectra for compound **4c**



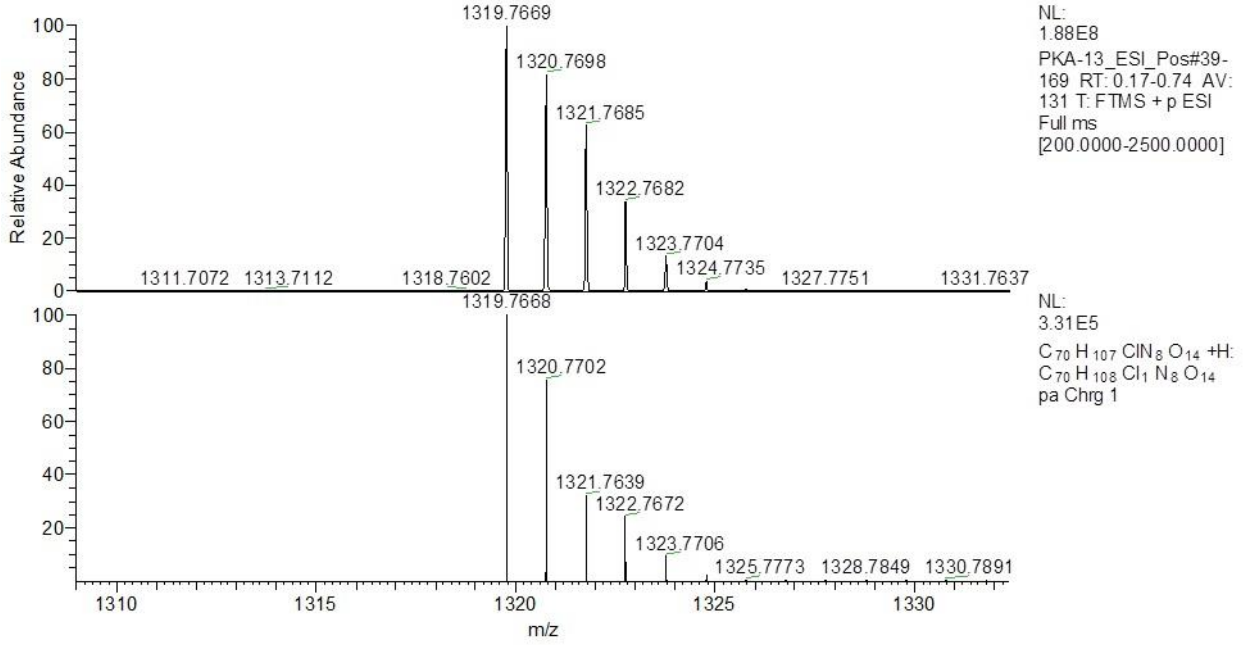
HRMS spectra for compound **4c**



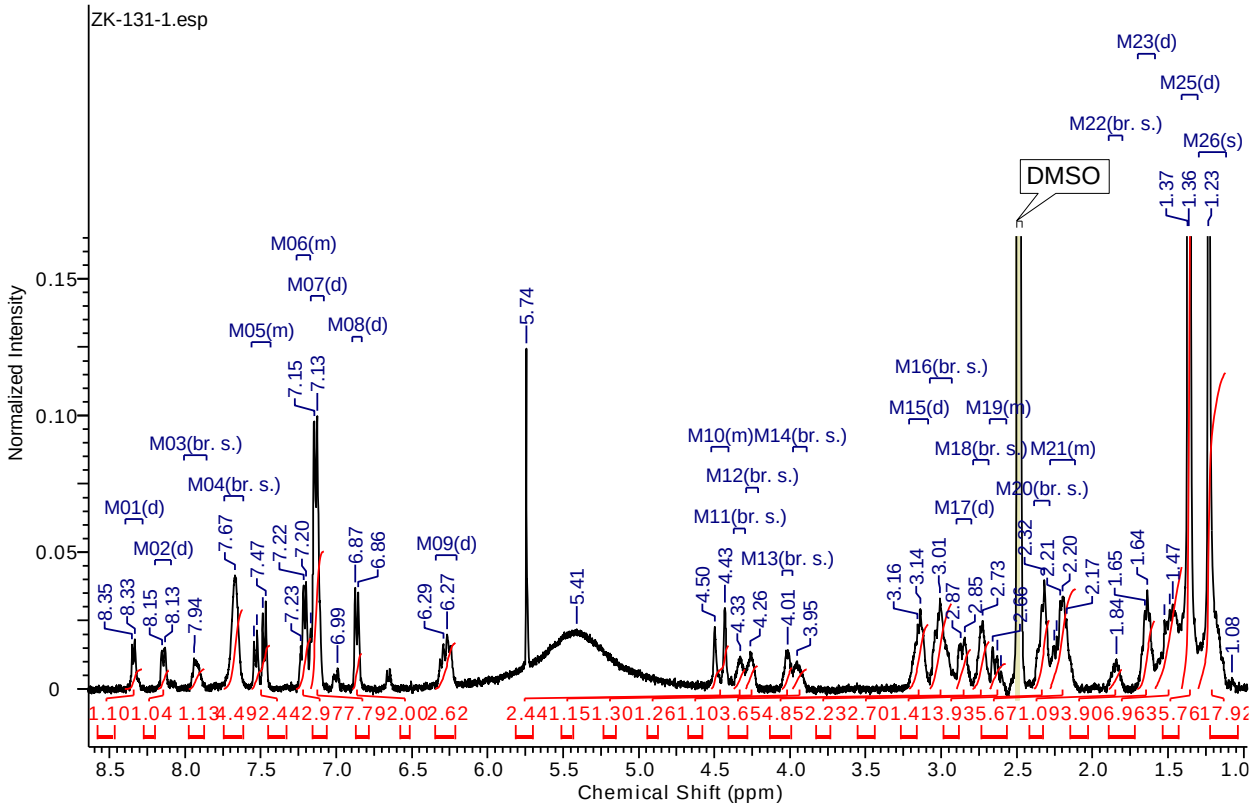
^{1}H NMR spectra for compound **4d**



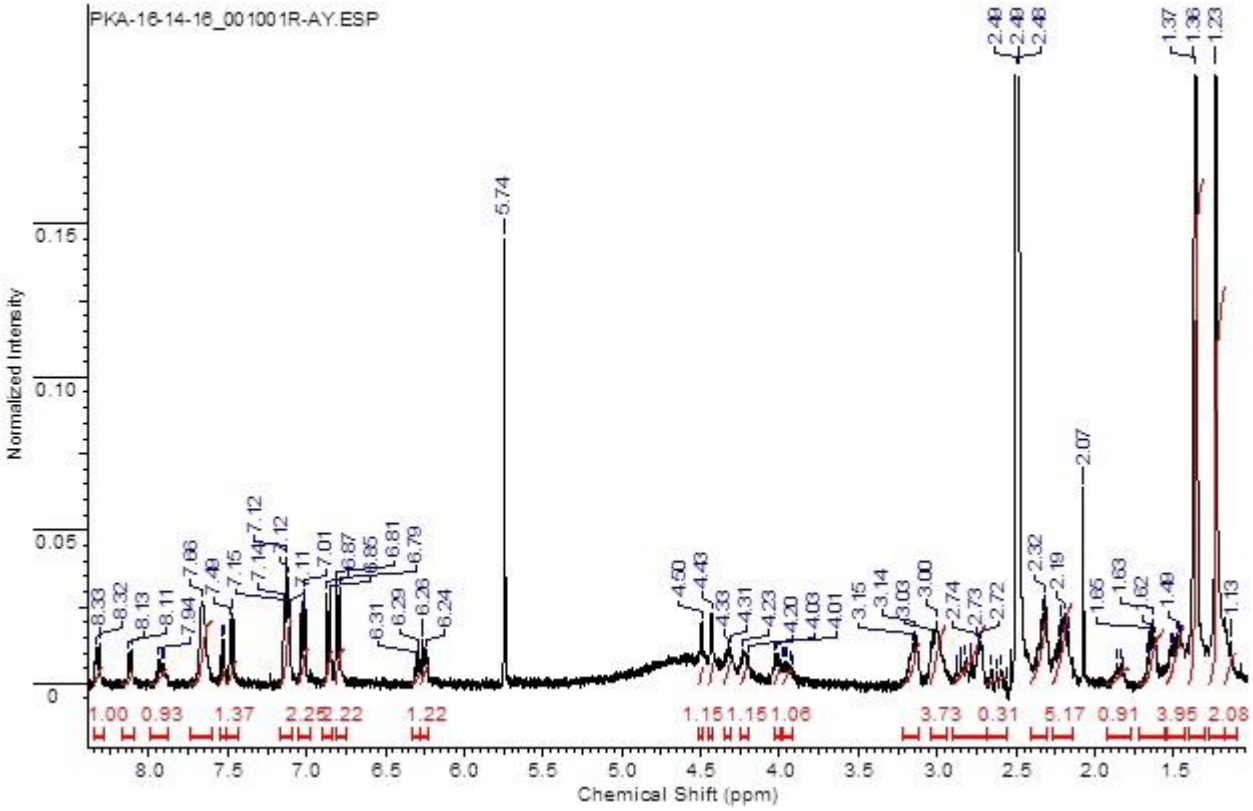
HRMS spectra for compound **4d**



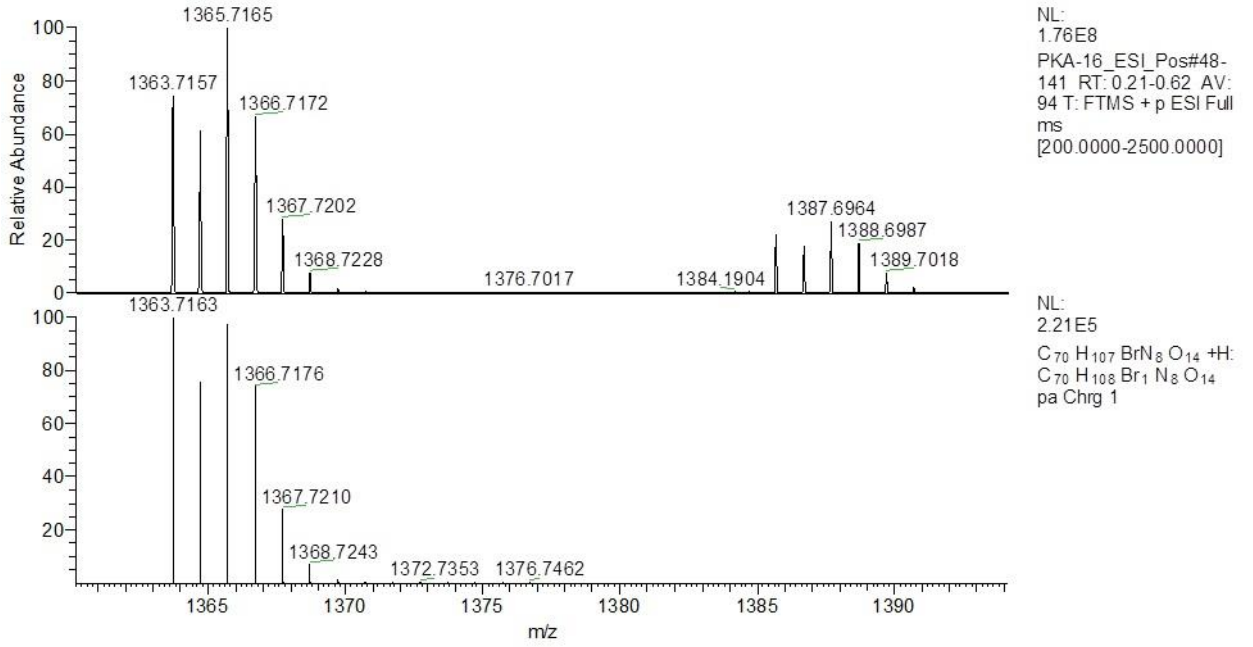
^{1}H NMR spectra for compound **4e**



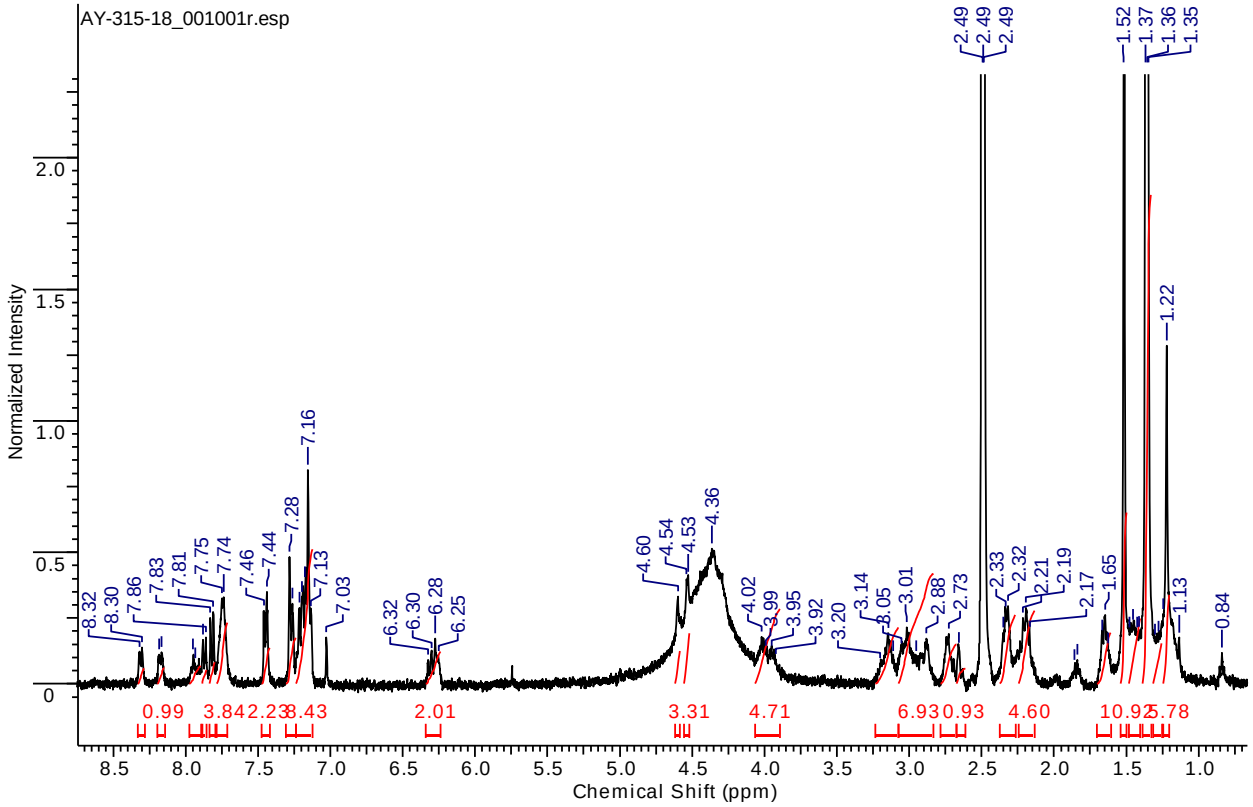
^{1}H NMR spectra for compound **4f**



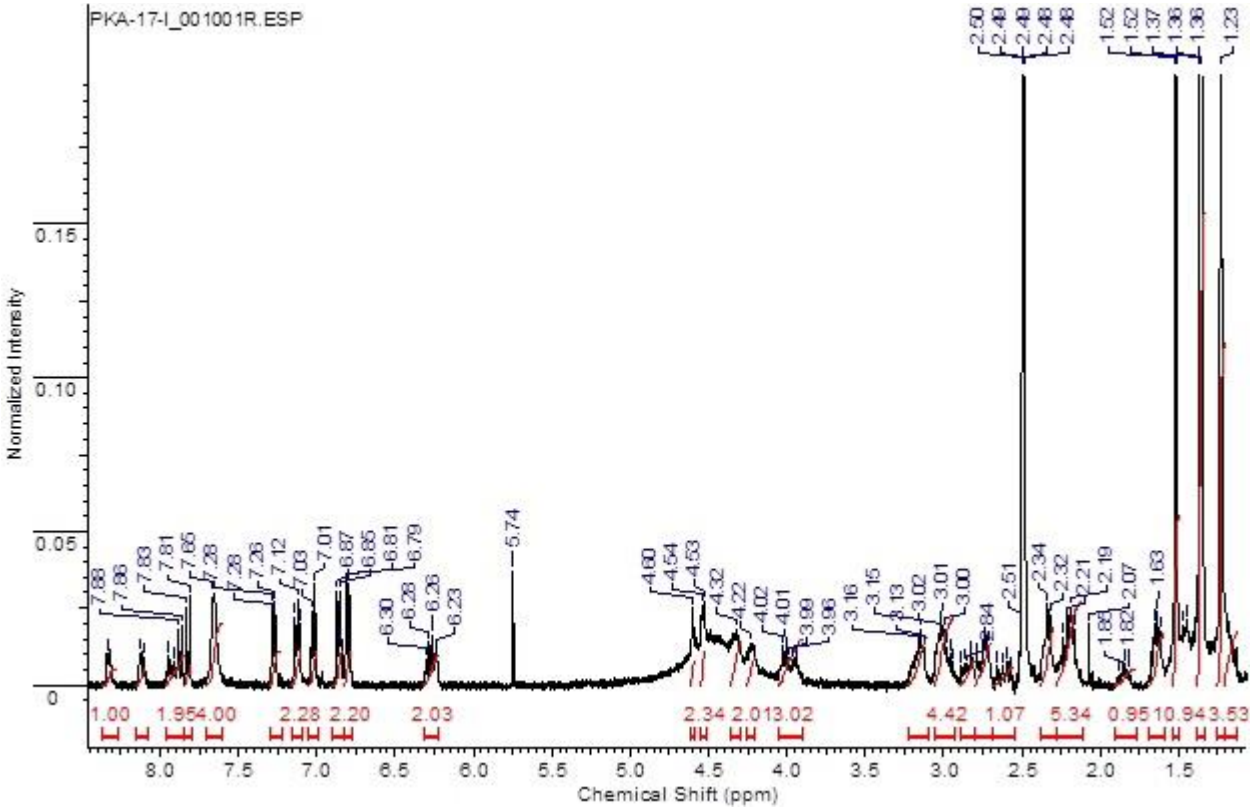
HRMS spectra for compound **4f**



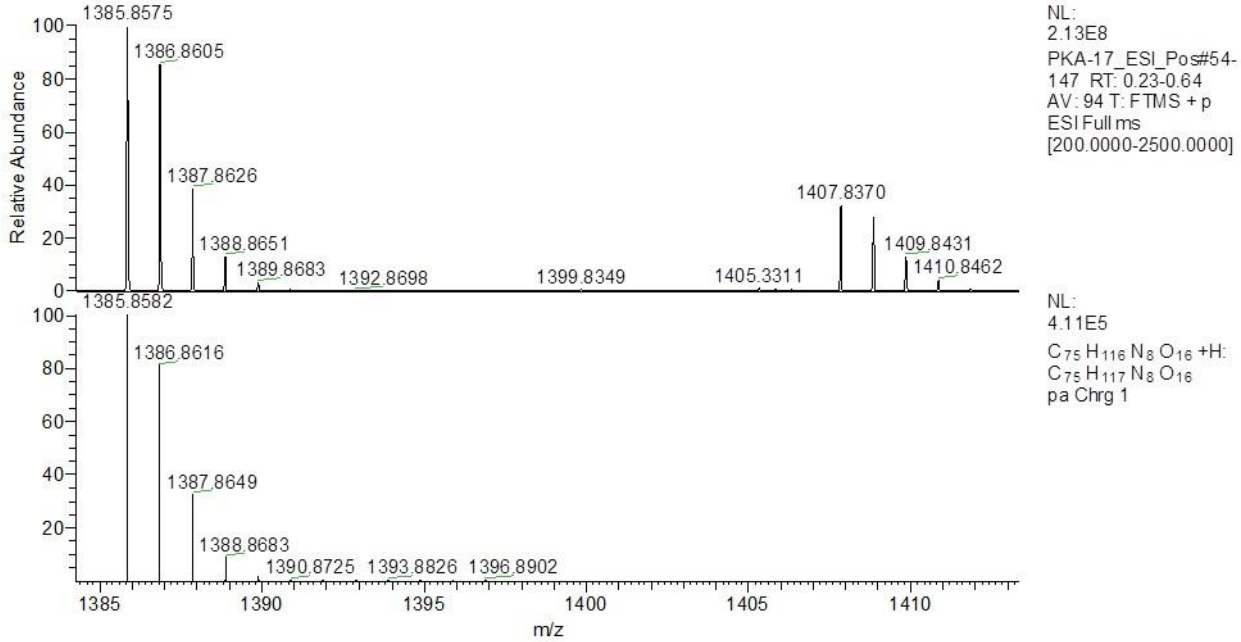
^{1}H NMR spectra for compound **4g**



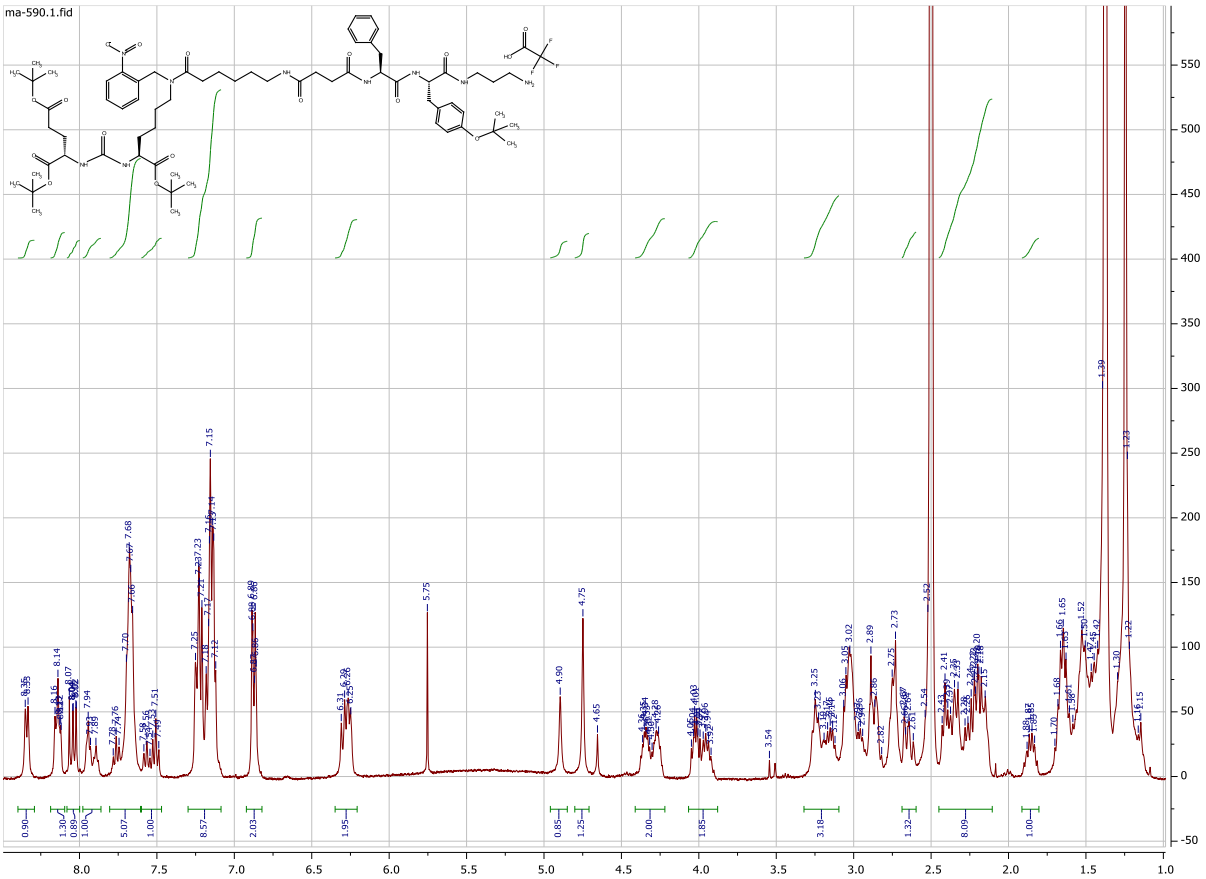
^{1}H NMR spectra for compound **4h**



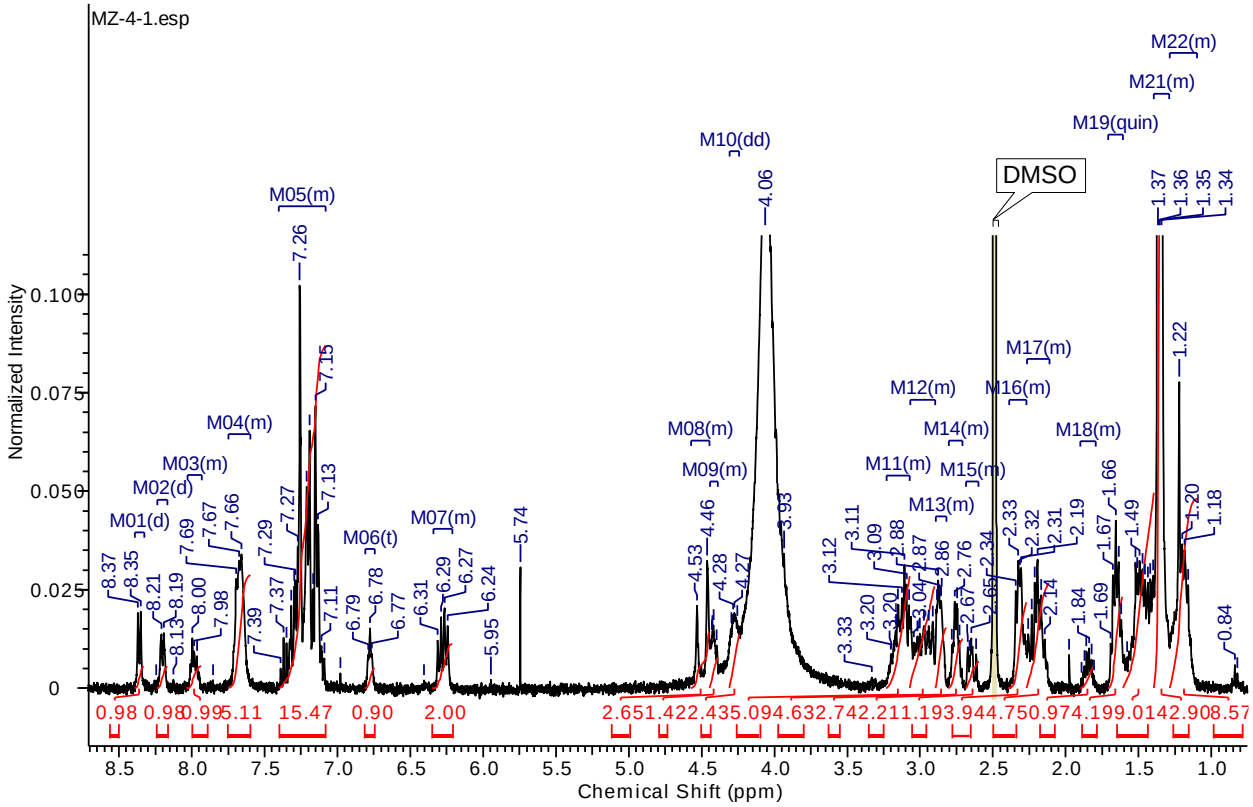
HRMS spectra for compound **4d**



^{1}H NMR spectra for compound **4i**

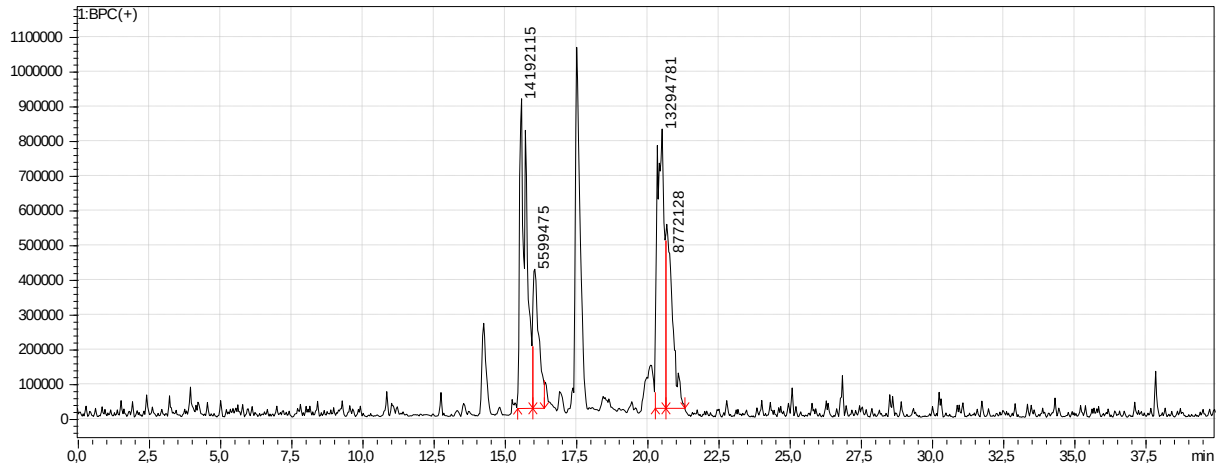


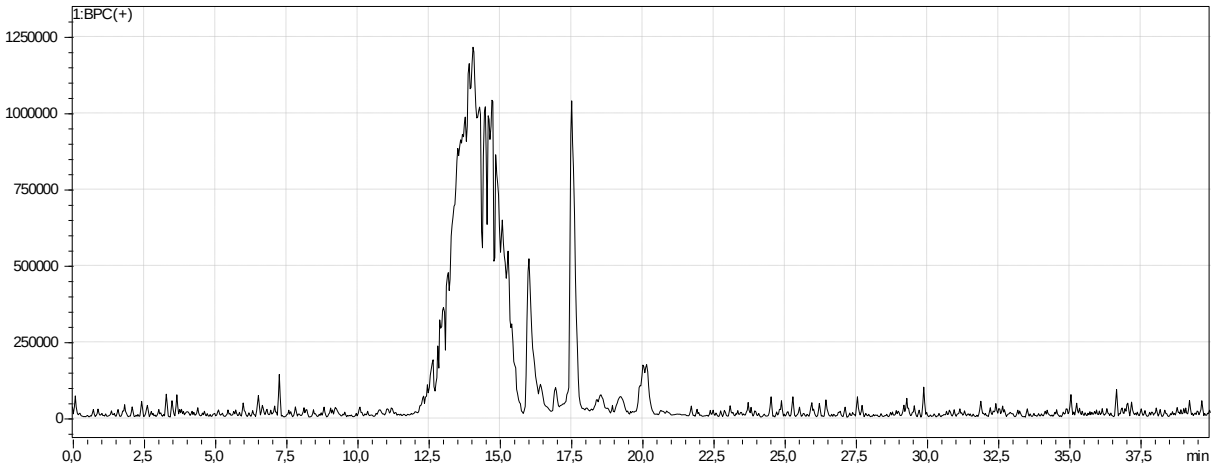
^{1}H NMR spectra for compound **8**



HPLC chromatogram for compound **8**

Positive ions (chromatogram and blank)

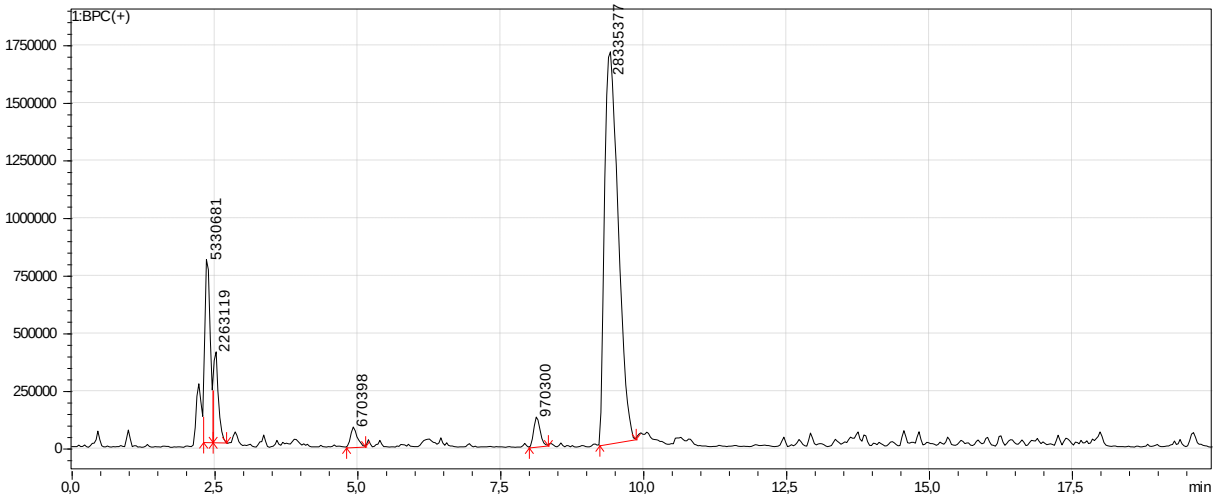


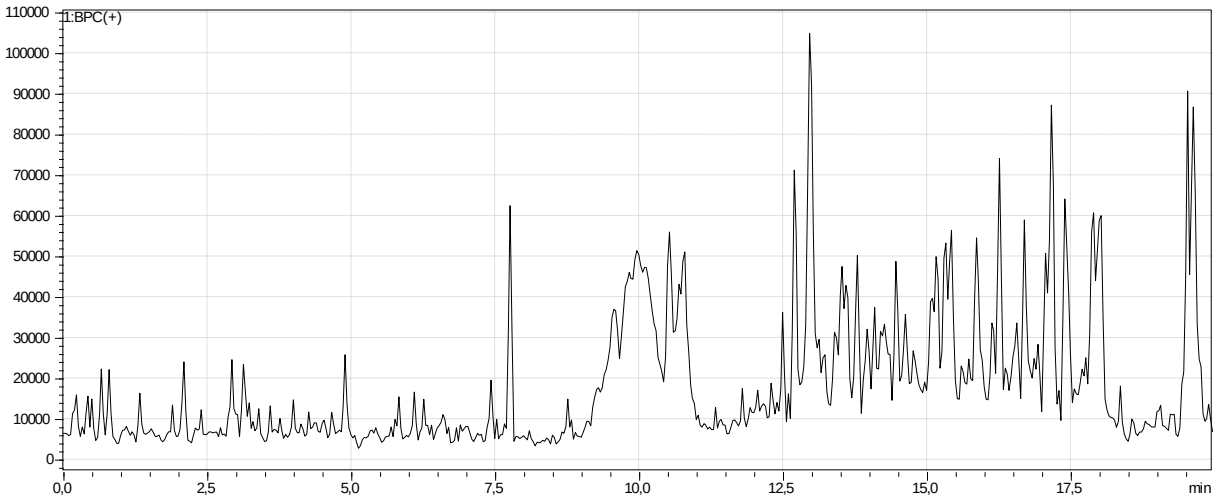


HPLC data for the reaction mixtures, and for the compound 8

HPLC chromatogram for reaction mixture of compound **4a**

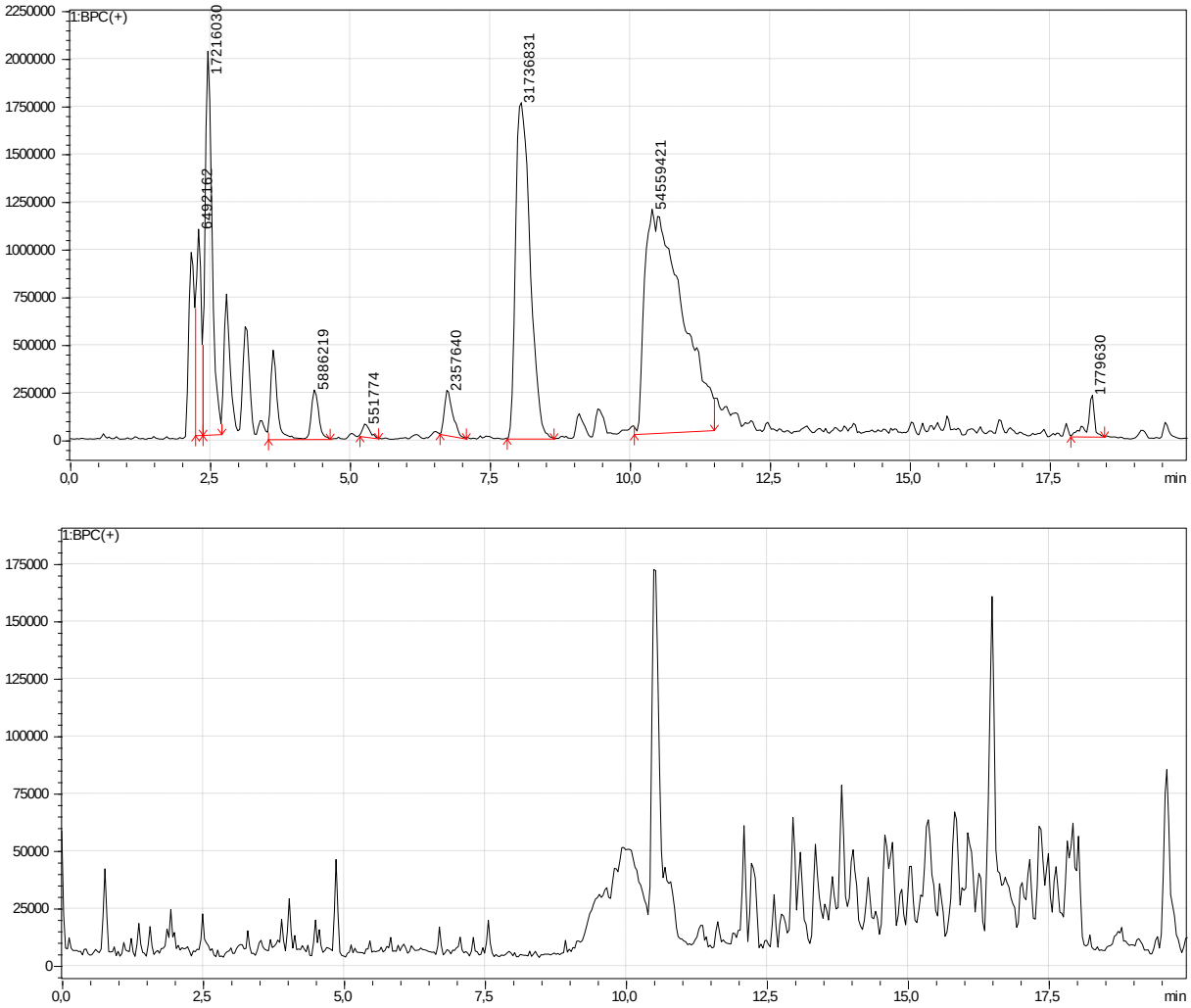
Positive ions (chromatogram and blank)





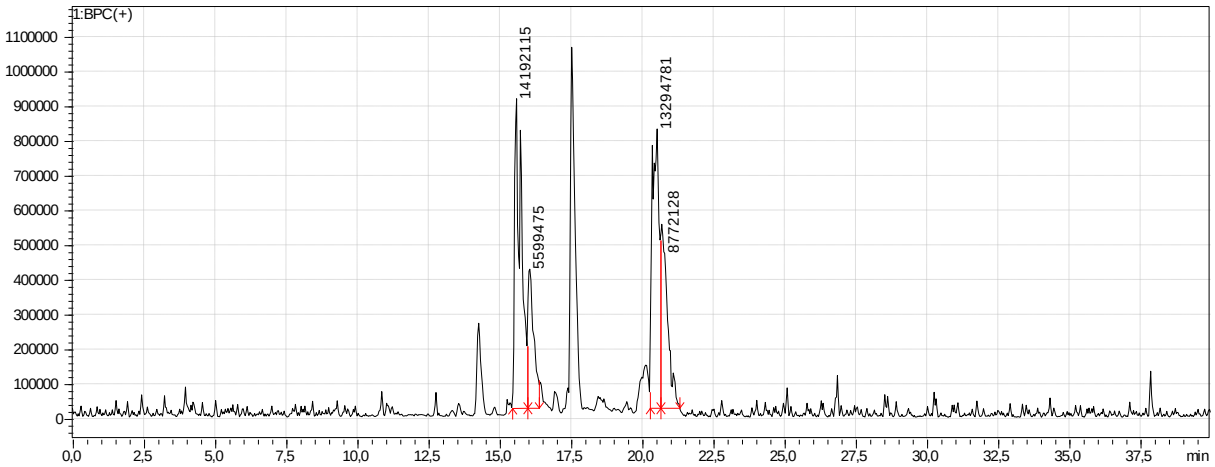
HPLC chromatogram for reaction mixture of compound **4b**

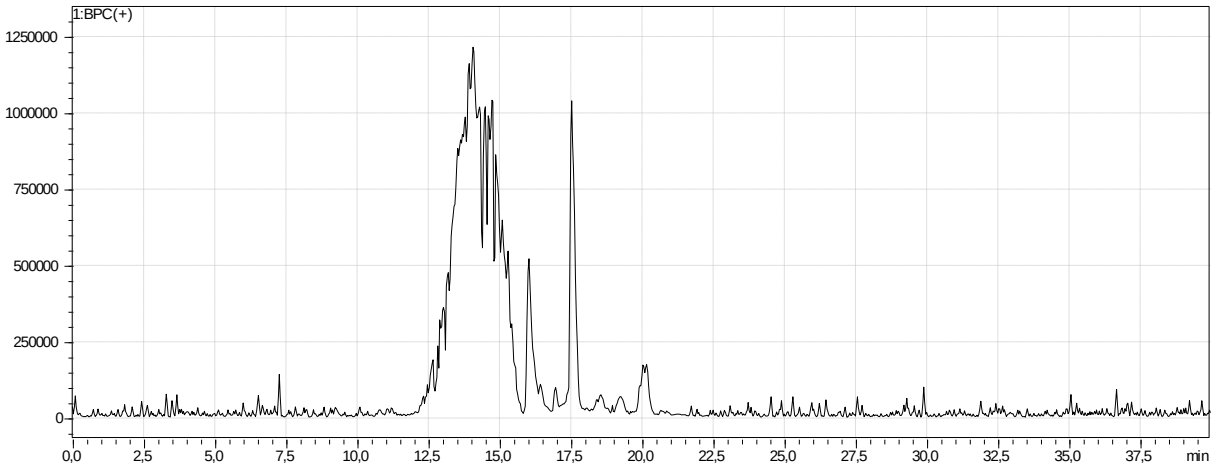
Positive ions (chromatogram and blank)



HPLC chromatogram for compound **8**

Positive ions (chromatogram and blank)

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