Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Microwave-Assisted Chemical Ligation of *S*-Acyl Peptides Containing non-terminal Cysteine Residues

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Supporting Information

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General Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. NMR spectra were recorded in $CDCl_3$, DMSO-d_6 or CD_3OD-d_4 on Gemini or Varian NMR operating at 300 MHz for ^1H and 75 MHz for ^{13}C with TMS as an internal standard. Elemental analyses were performed on a Carlo Erba-1106 instrument. All microwave assisted reactions were carried out with a single mode cavity Discover Microwave Synthesizer (CEM Corporation, NC). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed with a silicon septum and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 sec.; PowerMax-cooling mode). L-Cystine, Boc-Gly-OH and L-Leucine were purchased from Sigma-Aldrich. Cbz-L-Ala-OH and Fmoc-Gly-OH were purchased from Chem-Impex International. L-Phenylalanine and glycine methyl ester hydrochloride were purchased from TCI-US. All commercially available starting materials were used without further purification. Fmoc-Gly-Bt[1] (Bt = benzotriazol-1-yl), Cbz-L-Ala-Bt[2], Boc-Gly-Bt[3] and *N*,*N*'-di-Boc-cystine[4] were prepared according to known procedures. The phosphate buffer (NaH_2PO_4/Na_2HPO_4) (0.4 M, pH 7.8) was degassed by bubbling argon through the buffer. HPLC-MS analyses were performed on reverse phase gradient Phenomenex Synergi Hydro-RP (2.1 x 150 mm; 5 μm) + guard column (2 x 4 mm) or Thermoscientific Hypurity C8 (5μm; 2.1 x 100 mm + guard column) using 0.2% acetic acid in H_2O/methanol as mobile phases; wavelength = 254 nm; and mass spectrometry was done with electro spray ionization (ESI). Product ratios were obtained from HPLC-MS semiquantitation. The area of ion-peak resulting from the sum of the intensities of the $[M+H]^+$ and $[M+Na]^+$ ions of each compound was integrated. Semi-preparative and analytical HPLC were carried out on Phenomenex Luna 10 μm C18(2) columns. Methanol:water (70:30) was used

as eluent for the isolation of compounds **19a**, **20a** and **26**. The mobile phases used for analytical HPLC are given in Figure S10, S12 and S14.

General procedure for the preparation of dimer peptides 8 and 15a,b.

Isobutyl chloroformate (0.6 g, 4.4 mmol) was added to a solution of N-Boc-Gly-OH or the respective Boc-protected dipeptide **14a,b** (4 mmol) and N-methylmorpholine (0.45 g, 4.4 mmol) in dry THF (30 mL) at -10 °C. After 5 min a mixture of {2-amino-3-[2-amino-2-(methoxycarbonylmethyl-carbamoyl)-ethyldisulfanyl]-propionylamino}-acetic acid methyl ester dihydrochloride **7** (0.91 g, 2 mmol) and N-methylmorpholine (0.45 g, 4.4 mmol) in dry DMF (10 mL) was added at -10 °C. The reaction mixture was stirred at rt for 12 h under argon. The THF was removed under reduced pressure, water (30 mL) was added and the resulting solution was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were subsequently washed with 2N HCl (2 x 50 mL), water (30 mL), 5% Na_2CO_3 (2 x 50 mL), brine (30 mL) and dried over $MgSO_4$. Evaporation of the solvent gave the desired products, which were purified by recrystallization from CH_2Cl_2:hexanes.

General procedure for the preparation of Boc-protected dipeptides 14a,b. Boc-Gly-Bt (10 mmol) was added at 25 °C to a solution of the respective amino acid (10 mmol) in MeCN:H_2O (30 mL : 10 mL) in the presence of Et_3N (10 mmol). The reaction mixture was stirred at 25 °C for 2 h. Aq. 4N HCl solution (5 mL) was added and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (50 mL), and the organic extract was washed with 4N HCl (3 × 30 mL), brine (30 mL) and dried over $MgSO_4$. Evaporation of the solvent gave the desired product, which was purified by recrystallization from ethyl acetate:hexanes to yield the desired Boc-protected dipeptides as solid compounds.

Experimental details for compounds 2, 3, 4, 7, 8, 14a,b, 15a,b, 21 and 22

(6*R*,11*R*)-methyl 11-((tert-butoxycarbonyl)amino)-6-((2-methoxy-2-oxoethyl)-carbamoyl)-2,2-dimethyl-4,12-dioxo-3-oxa-8,9-dithia-5,13-diazapentadecan-15-oate (2). A solution of *N*,*N*'- di-Boc-cystine (1.32 g, 3 mmol) in dry THF (10 mL) under argon was cooled to -15 ^{o}C in an ice bath with stirring. N-Methylmorpholine (0.67g, 6.6 mmol), followed by isobutylchloroformate (0.91 g, 6.6 mmol) were added. After 5 min, a solution of glycine methyl ester hydrochloride (0.75 g, 6 mmol) and N-methylmorpholine (0.67 g, 6.6 mmol) in DMF (15 mL) was added. The ice bath was removed after 5 min and the solution was allowed to stir for 24 h at room temperature. The solution was concentrated under vacuum and the residue was dissolved in a mixture of ethyl acetate (20 mL) and water (5 mL). After extraction, the aqueous phase was discarded and the organic phase washed successively with saturated Na_2CO_3 (2 x 10 mL), water (10 mL) and 2N HCl (10 mL). The solution was dried over dry $MgSO_4$, filtered and concentrated under vacuum. The peptide was recrystallized from ethyl acetate:hexanes to give desired product. White microcrystals, 79% yield, mp 140-143 °C; Anal. Calcd for $C_{22}H_{38}N_4O_{10}S_2$: C 45.35; H 6.57; N 9.62. Found: C 45.68; H 6.62; N 9.18; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 18H), 2.89 (dd, *J* = 14.3, 10.2 Hz, 2H), 3.04 (dd, *J* = 14.7, 3.9 Hz, 2H), 3.70 (s, 6H), 3.87 (dd, *J* = 17.6, 5.3 Hz, 2H), 4.11 (dd, *J* = 18.0, 6.3 Hz, 2H), 4.90 (br s, 2H), 5.56 (d, *J* = 9.6 Hz, 2H), 8.13 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.5, 41.1, 46.9, 52.4, 54.7, 80.5, 156.1, 169.9, 171.0.

(*R*)-Methyl 2-(2-((tert-butoxycarbonyl)amino)-3-mercaptopropanamido)-acetate (3). (6*R*,11*R*)-methyl 11-((tert-butoxycarbonyl)amino)-6-((2-methoxy-2-oxoethyl)carbamoyl)-2,2-dimethyl-4,12-dioxo-3-oxa-8,9-dithia-5,13-diazapentadecan-15-oate (400 mg, 0.69 mmol) was treated with PBu_3 (277 mg, 0.34 mL, 1.37 mmol) in 12 mL of MeOH:water (9:1) for 2h at room

temperature. The reaction mixture was concentrated under vacuum and the residue was dissolved in diethylether (15 mL). The solution was dried over magnesium sulfate, and then concentrated under vacuum. The peptide was purified by column chromatography on silica gel (Eluent hexane:AcOEt, 2:1) and recrystallized from Et_2O:hexane to give **3**. White microcrystals, 55% yield, mp 55-57 °C; Anal. Calcd for $C_{11}H_{20}N_2O_5S$: C 45.19; H 6.90; N 9.58. Found: C 45.48; H 7.16; N 9.45; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.70 (dd, *J* = 10.4, 7.6 Hz, 1H), 2.70–2.80 (m, 1H), 3.09-3.18 (m, 1H), 3.76 (s, 3H), 3.98-4.15 (m, 2H), 4.45 (br s, 1H), 5.58 (d, *J* = 8.1 Hz, 1H), 7.0 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.3, 28.5, 41.4, 52.6, 55.7, 80.8, 155.6, 170.2, 170.8.

(*R*)-Methyl 2-(2-((tert-butoxycarbonyl)amino)-3-mercapto-propanamido)acetate (4). Dipeptide Boc-L-Cys-Gly-OMe (0.29 g, 1 mmol) together with an equimolar amount of Fmoc-Gly-Bt (0.40 g, 1 mmol) was suspended in acetonitrile (20 mL) at 25^{o}C. Then, 12 mL of 0.1*N* $KHCO_3$ in water was added dropwise. The solution was stirred at the same temperature and monitored by TLC for starting material consumption. After completion of the reaction, the solution was acidified with 2*N* HCl (15 mL), acetonitrile was removed under reduced pressure. The residue formed was dissolved in ethyl acetate (40 mL), extracted with 2*N* HCl (3 × 15 mL), sat. NaCl solution (20 mL) and dried over $MgSO_4$. Ethyl acetate was removed under reduced pressure and the residue was dissolved in dichloromethane (15 mL), hexanes were added until the solution is turbid and the solution was left to crystallize in the freezer. The solid obtained was filtered, dried to give the corresponding target. White microcrystals, 88% yield, mp 108-112 ^{o}C; Anal. Calcd for $C_{28}H_{33}N_3O_8S$: C 58.83; H 5.82; N 7.35. Found: C 58.93; H 5.87; N 7.38; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9H), 3.25 (dd, *J* = 14.1, 7.6 Hz, 1H), 3.40 (dd, *J* = 14.0, 5.0 Hz, 1H), 3.71 (s, 3H), 4.01 (d, *J* = 5.4 Hz, 2H), 4.14 (d, *J* = 5.5 Hz, 2H), 4.23 (t, *J* = 6.9 Hz, 2H),

4.42 (s, 1H), 4.44 (s, 1H), 5.42 (d, *J* = 7.9 Hz, 1H), 5.63 (br s, 1H), 6.94 (br s, 1H), 7.30 (t, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.1 Hz, 2H), 7.76 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.5, 30.9, 41.4, 47.3, 50.9, 52.6, 54.2, 67.5, 80.9, 120.2, 125.2, 127.3, 128.0, 141.5, 143.9, 155.8, 156.5, 170.1, 170.5, 198.5.

(7*R*,12*R*)-3,6,13,16-Tetraoxo-2,17-dioxa-9,10-dithia-5,14-diazaoctadecane-7,12-diaminium chloride (7). HCl gas was passed through a solution of peptide **2** (4 mmol) in methanol (15 mL) for 30 minutes. The methanol solution was concentrated under vacuum and diethyl ether was added. The turbid solution was left to crystallize in the freezer overnight. The solid formed was filtered and washed with dry ethyl acetate (10 mL) and then with diethyl ether (10 mL) dried to give the corresponding deprotected peptide **7**. White solid, 85% yield, mp 112-116 °C; Anal. Calcd for $C_{12}H_{24}N_4O_6S_2 \cdot 2HCl$: C 31.65; H 5.75; N 12.30. Found: C 31.25; H 5.63; N 10.94; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.18-3.37 (m, 4H), 3.64 (s, 6H), 3.93 (d, *J* = 5.4 Hz, 4H), 4.21-4.22 (m, 2H), 8.63 (br s, 6H), 9.38 (d, *J* = 5.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 38.5, 40.8, 51.1, 52.0, 167.5, 169.7.

(9*R*,9'*R*)-Dimethyl 9,9'-(disulfanediylbis(methylene))bis(2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate) (8). White microcrystals, 62% yield, mp 88-92 °C; Anal. Calcd for $C_{26}H_{44}N_6O_{12}S_2 \cdot H_2O$: C 43.69; H 6.49; N 11.76. Found: C 43.81; H 6.58; N 11.49; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 18H), 2.89-3.08 (m, 4H), 3.76 (s, 6H), 3.82-3.96 (m, 6H), 4.01 (d, *J* = 4.8 Hz, 2H), 4.09-4.17 (m, 2H), 5.43 (br s, 2H), 5.64 (br s, 2H), 7.26 (s, 2H), 8.41 (br s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.5, 41.6, 44.4, 45.2, 52.6, 53.2, 80.4, 156.3, 170.2, 170.5, 170.6.

(*S*)-2-(2-(*tert*-Butoxycarbonylamino)acetamido)-4-methylpentanoic acid (14a). White microcrystals, 65% yield, mp 142-145 °C; Anal. Calcd for $C_{16}H_{22}N_2O_5$: C 54.15; H 8.39; N 9.72.

Found: C 54.11; H 8.53; N 9.65; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80-0.95 (m, 6H), 1.37 (br s, 9H), 1.47-1.54 (m, 2H), 1.55-1.66 (m, 1H), 3.55 (d, *J* = 6.0 Hz, 2H), 4.23 (q, *J* = 6.0 Hz, 1H), 6.91 (t, *J* = 6.0 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.4, 22.9, 24.2, 28.2, 40.2, 43.0, 50.1, 78.0, 155.7, 169.2, 174.0.

(*S*)-2-(2-((*tert*-Butoxycarbonyl)amino)acetamido)-3-phenylpropanoic acid (14b). White microcrystals, 69% yield, mp 147-148 °C; Anal. Calcd for $C_{13}H_{24}N_2O_5$: C 59.62; H 6.88; N 8.69. Found: C 59.58; H 7.02; N 8.64; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (br s, 9H), 2.84-2.95 (m, 1H), 3.00-3.06 (m, 1H), 3.35 (br s, 3H), 3.48-3.60 (m, 2H), 4.41-4.46 (m, 1H), 6.93 (t, *J* = 5.9 Hz, 1H), 7.19-7.26 (m, 5H), 8.02 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.2, 36.8, 43.0, 53.4, 78.0, 78.0, 126.5, 128.2, 129.1, 137.4, 155.7, 169.2, 172.8.

(Boc-Gly-L-Leu-L-Cys-Gly-OCH$_3$)$_2$ (15a). White microcrystals, 75% yield, mp 98-105 °C; Anal. Calcd for $C_{38}H_{66}N_8O_{14}S_2$: C 49.44; H 7.21; N 12.14. Found: C 49.05; H 7.36; N 11.85; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80-0.90 (m, 12H), 1.37 (s, 18H), 1.41-1.50 (m, 4H), 1.53-1.66 (m, 2H), 2.89 (dd, *J* = 12.8, 9.3 Hz, 2H), 3.12 (dd, *J* = 13.7, 3.9 Hz, 2H), 3.56 (d, *J* = 5.8 Hz, 4H), 3.62 (s, 6H), 3.85 (d, *J* = 6.1 Hz, 4H), 4.31 (q, *J* = 7.2 Hz, 2H), 4.44-4.62 (m, 2H), 6.97 (t, *J* = 5.9 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 2H), 8.24 (d, *J* = 8.6 Hz, 2H), 8.32 (t, *J* = 5.6 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.6, 23.2, 24.1, 28.2, 40.8, 41.0, 43.2, 51.1, 51.8, 78.1, 155.8, 169.5, 169.9, 170.3, 172.2.

(Boc-Gly-L-Phe-L-Cys-Gly-OCH$_3$)$_2$ (15b). White microcrystals, 72% yield, mp 155-159 °C; Anal. Calcd for $C_{44}H_{62}N_8O_{14}S_2$: C 53.32; H 6.71; N 11.31. Found: C 52.93; H 6.49; N 10.85; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (br s, 18H), 2.73-2.95 (m, 4H), 3.03-3.16 (m, 4H), 3.45-3.56 (m, 4H), 3.62 (br s, 6H), 3.86 (br s, 4H), 4.58-4.63 (m, 4H), 6.90 (br s, 2H), 7.22 (br s, 10H),

7.95-7.97 (m, 2H), 8.38 (br s, 2H), 8.48 (d, J = 7.3 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.2, 37.6, 40.8, 43.1, 51.8, 51.9, 53.8, 78.1, 126.2, 128.0, 129.3, 137.5, 155.7, 169.3, 169.9, 170.2, 171.1.

(Gly-L-Cys-Gly-OCH$_3$)$_2$ hydrochloride (21). HCl gas was passed through a solution of peptide **8** (4 mmol) in methanol (15 mL) for 30 minutes. The methanol solution was concentrated under vacuum and diethyl ether was added. The turbid solution was left to crystallize in the freezer overnight. The solid formed was filtered and washed with dry ethyl acetate (10 mL) and then with diethyl ether (10 mL) dried to give the corresponding deprotected peptide **21**. White microcrystals, 92% yield, mp 209-212 °C; Anal. Calcd for $C_{16}H_{30}Cl_2N_6O_8S_2.H_2O$: C 32.71; H 5.49; N 14.30. Found: C 32.50; H 5.27; N 13.13; ^{1}H NMR (300 MHz, CD_3OD) δ 2.98 (dd, J = 13.7, 6.5 Hz, 1H), 3.23-3.28 (m, 1H), 3.69 (s, 3H), 3.87 (s, 2H), 3.96 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 41.8, 42.2, 53.0, 54.1, 168.1, 171.8, 172.6.

(Boc-Gly-L-Phe-Gly-L-Cys-Gly-OCH$_3$)$_2$ (22). A solution of 2-(2-((tert-butoxycarbonyl)amino)acetamido)-3-phenylpropanoic acid **14b** (0.97 g, 3 mmol) in dry THF (10 mL) under argon was cooled to -15 °C in a ice bath with stirring. N-Methylmorpholine (0.33g, 3.2 mmol), followed by isobutylchloroformate (0.45 g, 3.2 mmol) were added. After 3 min, a solution of **21** (0.85 g, 1.5 mmol) and N-methylmorpholine (0.7 g, 1.6 mmol) in DMF (10 mL) was added. The ice bath was removed after 5 minutes and the solution was allowed to stir for 12 h at room temperature. The solution was concentrated under vacuum and the residue was dissolved in ethyl acetate (30 mL) and water (5 mL). After extraction, the aqueous phase was discarded and the organic phase washed successively with saturated Na_2CO_3 (2 x 15 mL), water (10 mL), 2N HCl (15 mL) and water (10 mL). The solution was dried over dry $MgSO_4$, filtered and then concentrated under vacuum. The peptide was recrystallized from ethyl acetate/hexane

to give desired (Boc-Gly-L-Phe-Gly-L-Cys-Gly-OCH_3)$_2$. White microcrystals, 74% yield, mp 180-185 °C; Anal. Calcd for $C_{48}H_{68}N_{10}O_{16}S_2$: C 52.16; H 6.20; N 12.67. Found: C 52.44; H 6.60; N 11.83; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (br s, 18H), 2.75-2.89 (m, 4H), 2.99-3.15 (m, 4H), 3.44-3.54 (m, 4H), 3.61 (br s, 6H), 3.67-3.74 (m, 2H), 3.84-3.91 (m, 6H), 4.52 (br s, 2H), 4.62 (br s, 2H), 6.88 (t, *J* = 5.5 Hz, 2H), 7.22 (br s, 10H), 8.03 (d, *J* = 7.8 Hz, 2H), 8.30 (d, *J* = 8.1 Hz, 2H), 8.38 (br s, 2H), 8.52 (br s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.2, 37.6, 40.8, 41.9, 43.1, 51.8, 53.9, 78.1, 126.2, 128.0, 129.2, 137.7, 155.7, 168.9, 169.3, 169.9, 170.4, 171.4.

HPLC chromatograms and MS Spectra

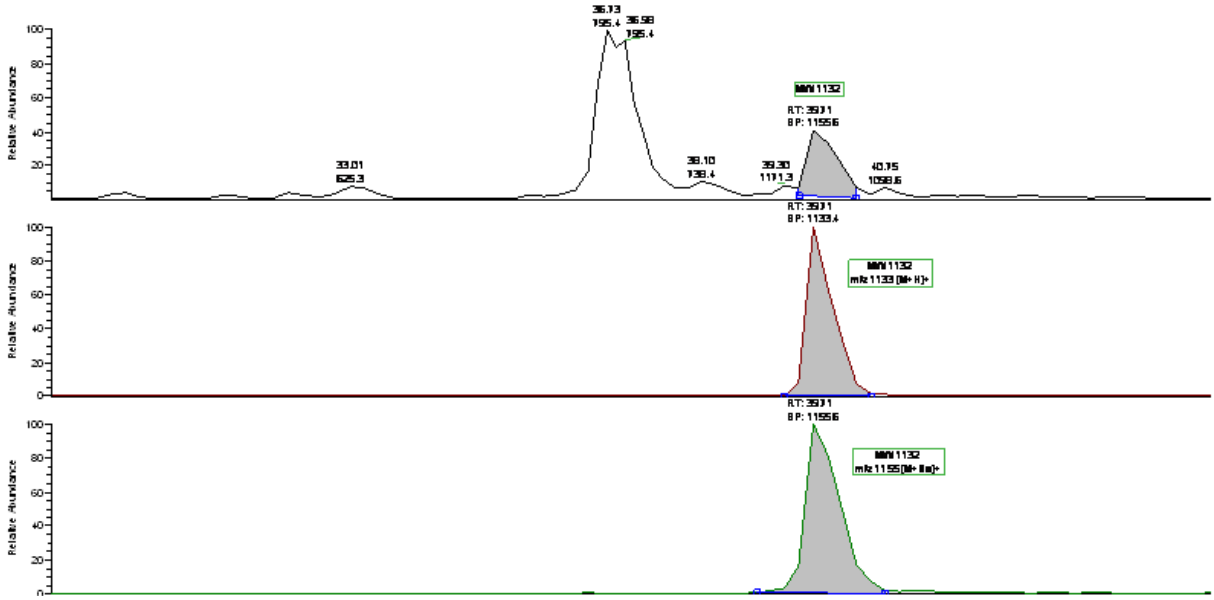

Figure S1. HPLC-MS chromatogram of the chemical ligation experiment of *S*-acyl tetrapeptide **18a.** The desired ligation product **19a** (molecular weight 1132, analyzed as disulfide dimer, shaded peaks) was a major compound in the sample.

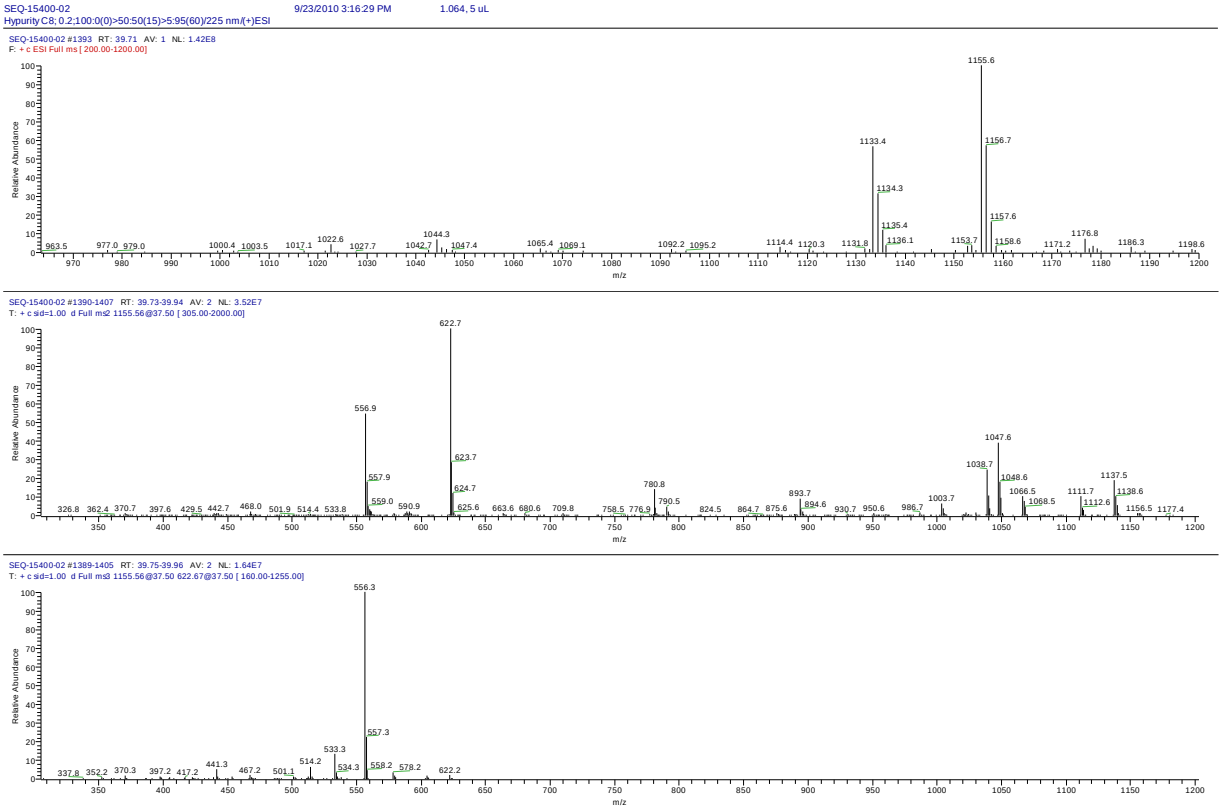

Figure S2. Compound **19a** (MW 1132) produced m/z 1133 $[M+H]^+$ and m/z 1155 $[M+Na]^+$ ions (top). Only the m/z 1155 was selected for MSn scans. The m/z 1155 was dissociated to produce m/z 622 and 556 product ions (middle). The m/z 622 primary product ion was dissociated to yield m/z 556 via loss of HS-SH (66 u) (bottom).

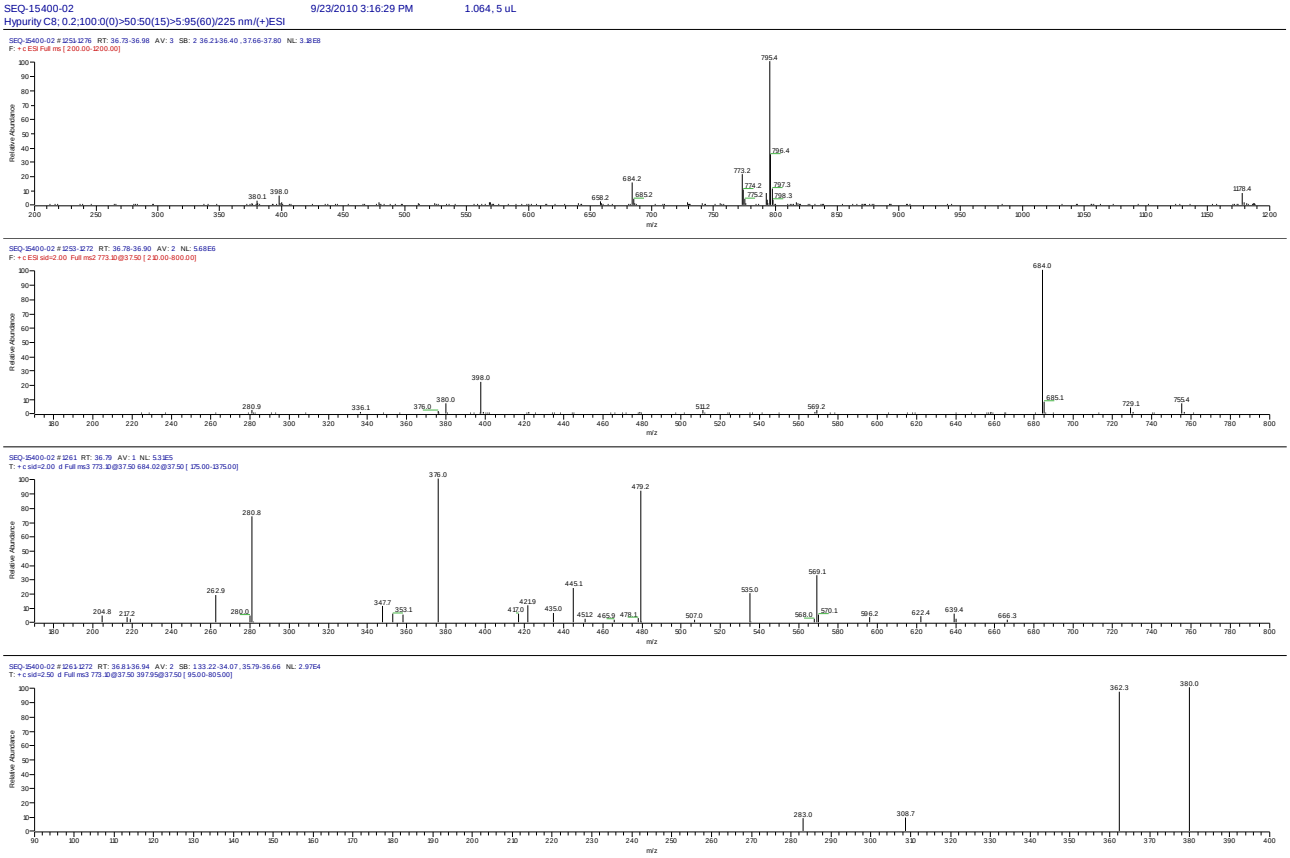

Figure S3. Compound **20a** MW 772 produced m/z 773 $[M+H]^+$ and m/z 795 $[M+Na]^+$ ions and m/z 380, 398 and 684 fragment ions (top). The m/z 773 $[M+H]^+$ ion was dissociated to form m/z 684 and m/z 398 product ions (2^{nd}). The m/z 684 product ion was further dissociated to m/z 479, 376, 281 and other secondary product ions (3^{rd}) while the m/z 398 product ion was dissociated to m/z 380 and 362 (bottom).

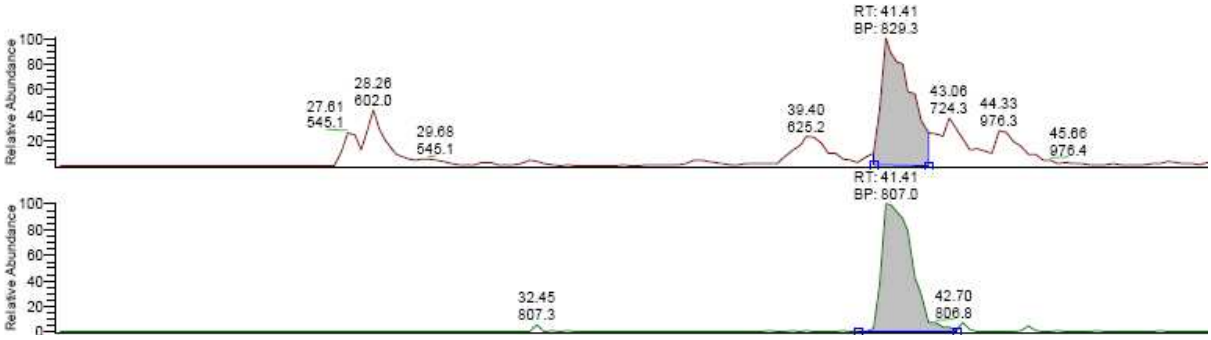

Figure S4. HPLC-MS chromatogram of the chemical ligation experiment of *S*-acyl tetrapeptide **18b.** Compound **20b** (MW 806) was the major compound in the sample and eluted at RT 41.52 min (shaded peaks).

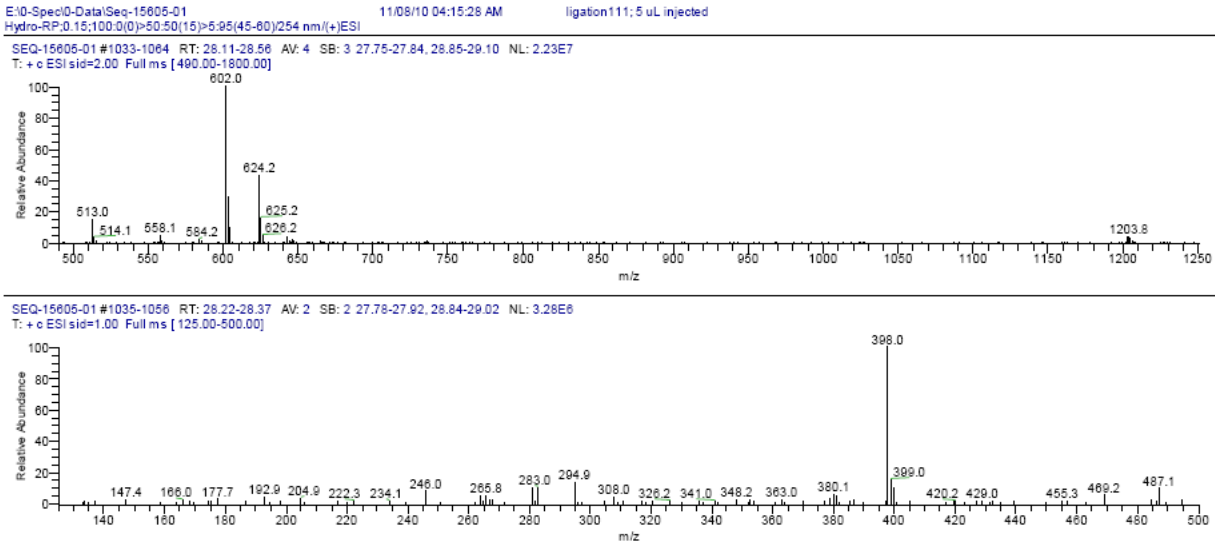

Figure S5. MS data for ligation product **19b** (MW 601, RT 28.26 min). (+)ESI-MS (top) clearly shows the m/z 602 $[M+H]^+$ and m/z 624 $[M+Na]^+$ ions. In addition, the m/z 1203 ion is an $[M+H+M]^+$ ion which helps confirm the MW.

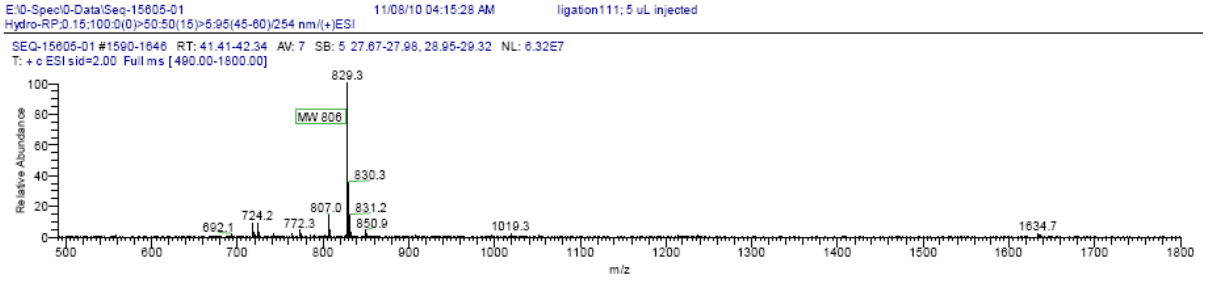

Figure S6. Compound **20b** (MW 806) produced predominantly an m/z 829 $[M+Na]^+$ ion

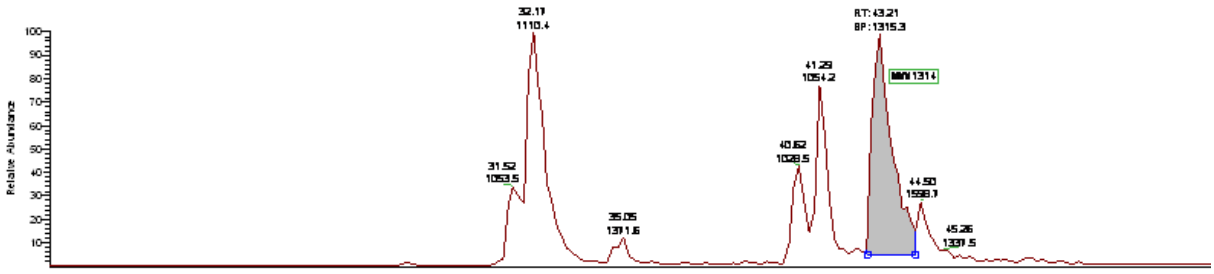

Figure S7. HPLC-MS chromatogram of the chemical ligation experiment of *S*-acyl pentapeptide **25.** The desired ligation product **26** (molecular weight 1314, analyzed as disulfide dimer, shaded peaks) was a major compound in the sample.

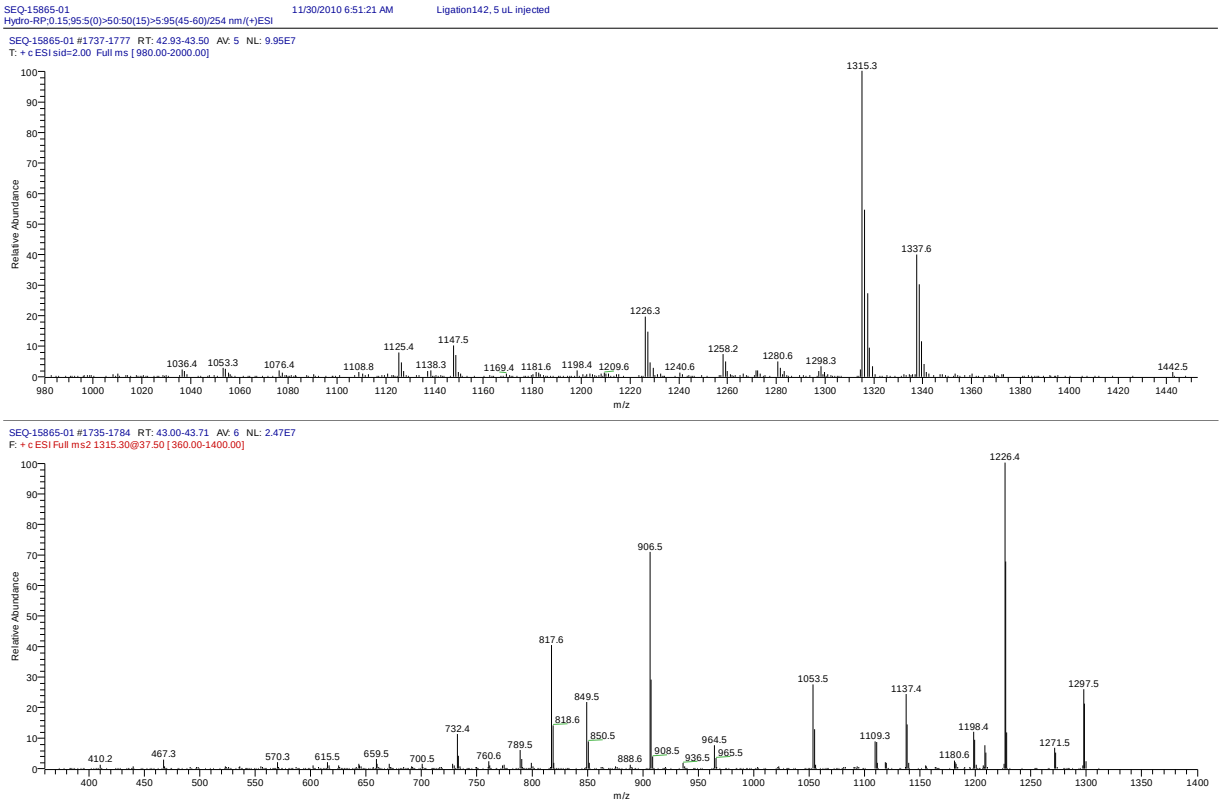

Figure S8. Compound **26** (MW 1314) produced an m/z 1315 $[M+H]^+$ ion and m/z 1337 [M+Na]+ ion (top). The m/z 1315 $[M+H]^+$ ion was dissociated to form a number of product ions (bottom).

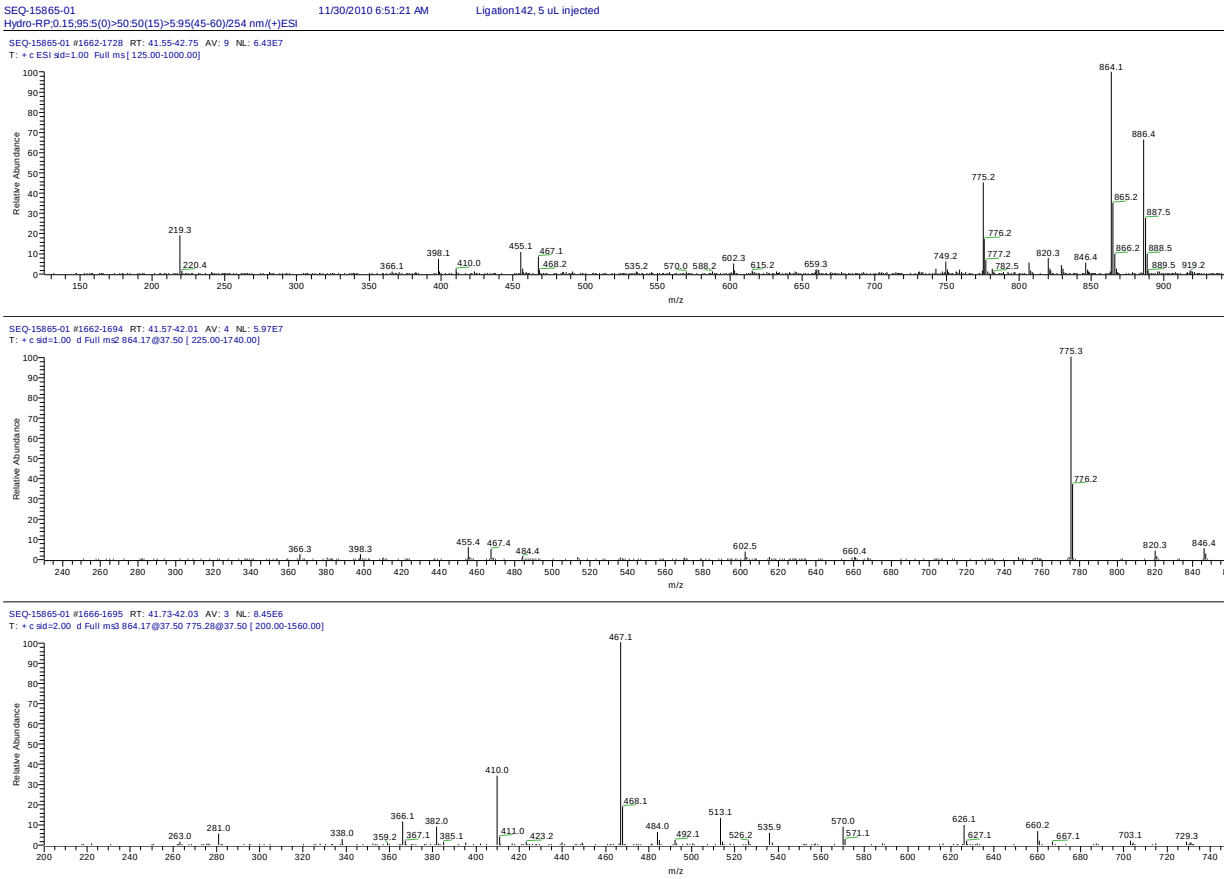

Figure S9. Compound **27** (MW 863): (+)ESI-MS (top) and –MS/MS (middle) and –MS/MS/MS (bottom) of m/z 864 $[M+H]^+$ ion

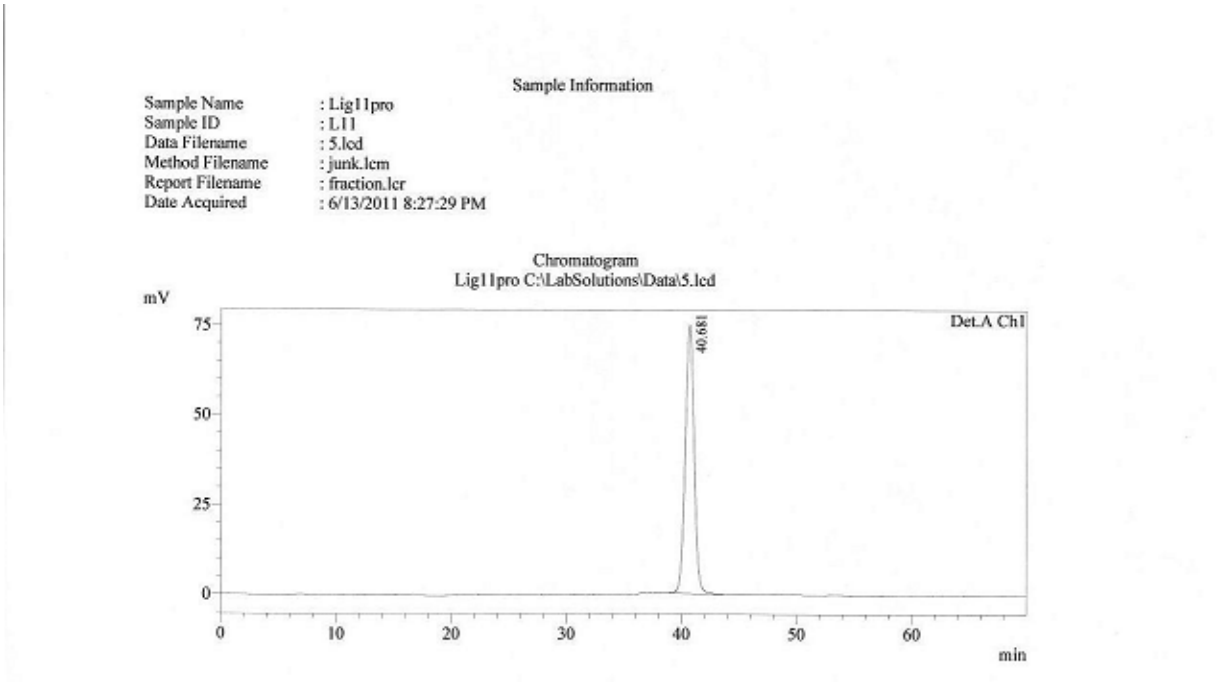

Figure S10. HPLC profile of purified ligation product **19a**. (Eluent = methanol:water (65:35), flow rate = 0.15 mL/min).

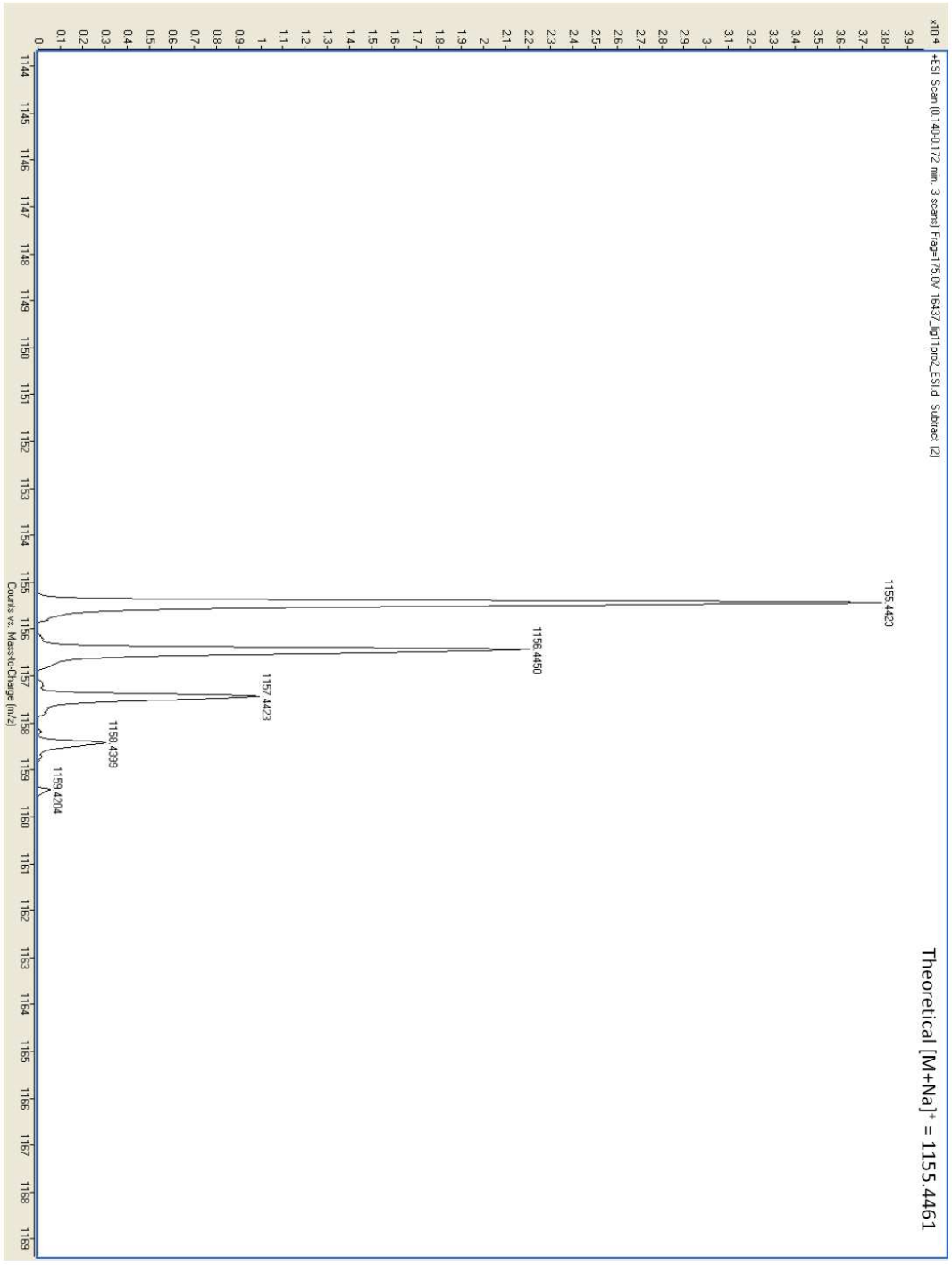

Figure S11. HRMS of ligation product **19a**

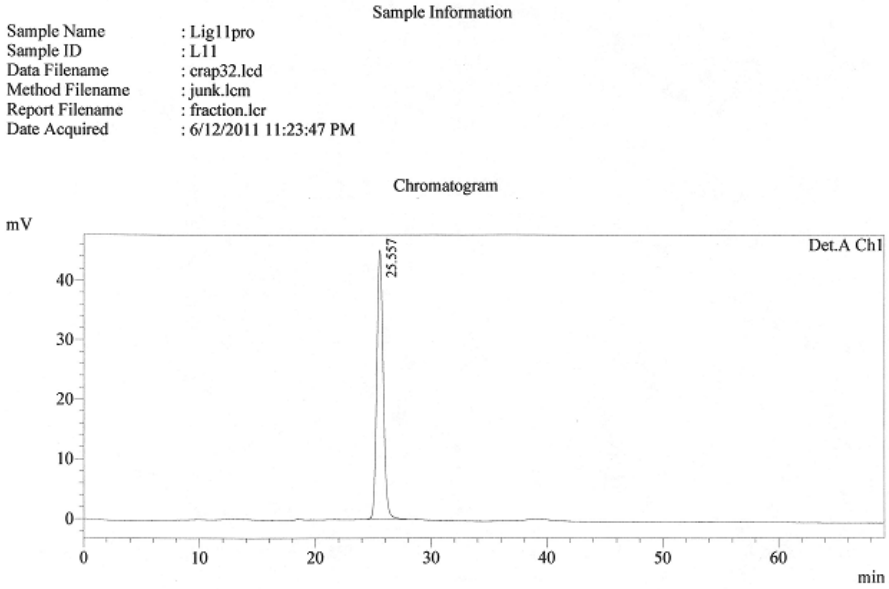

Figure S12. HPLC profile of purified product **20a**. (Eluent = methanol:water (65:35), flow rate = 0.15 mL/min).

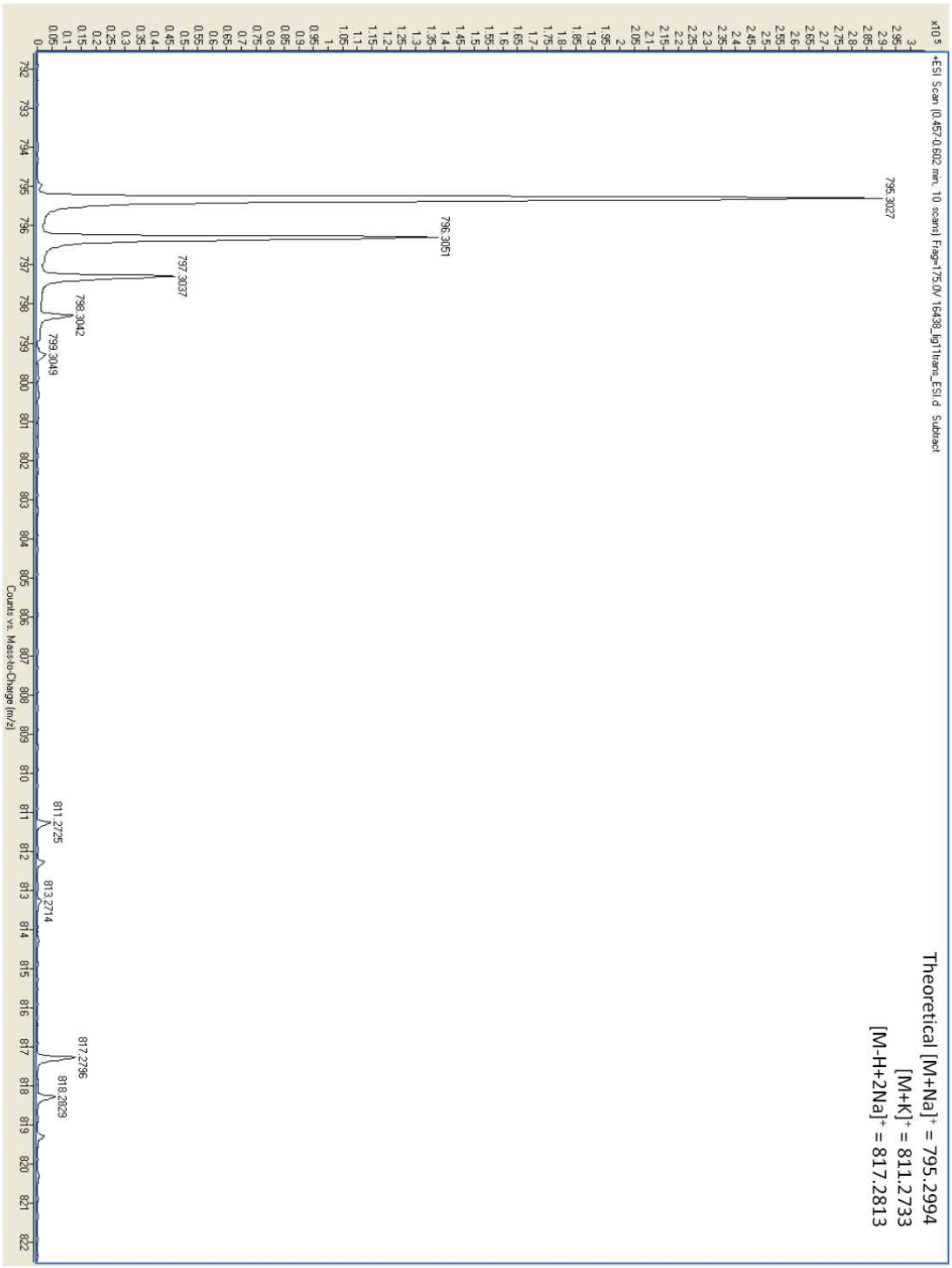

Figure S13. HRMS of product **20a**

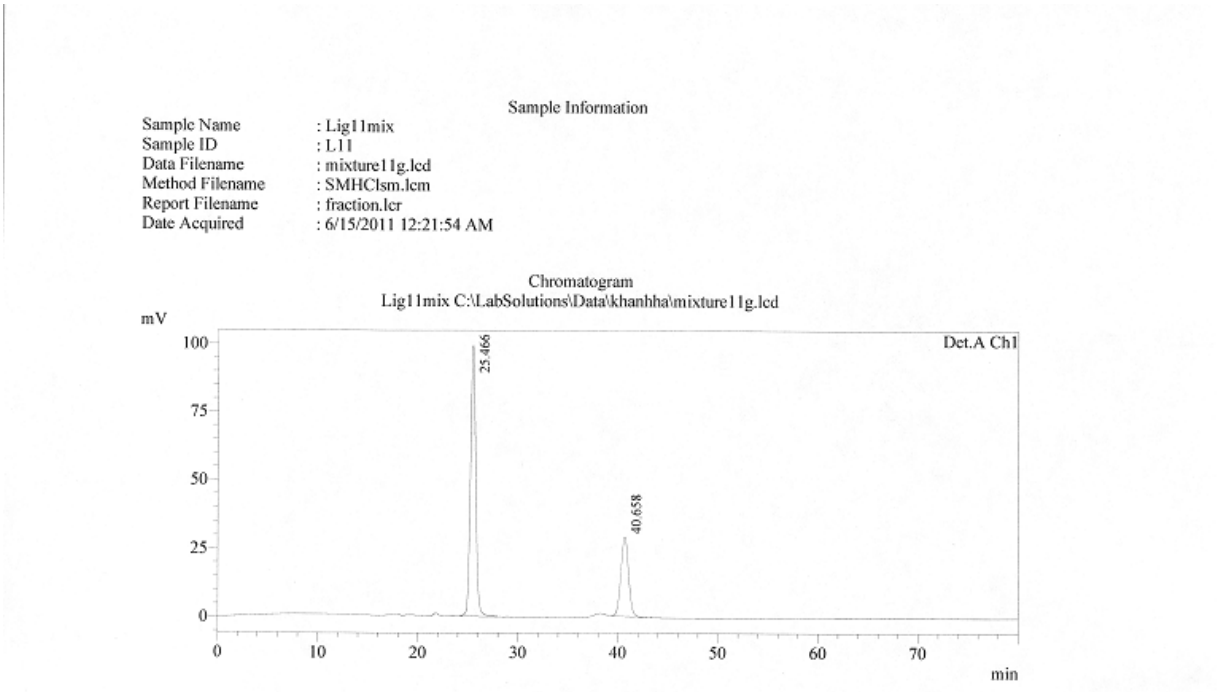

Figure S14. HPLC profile of a mixture of the purified products **19a** and **20a**. (Eluent = methanol:water (65:35), flow rate = 0.15 mL/min).

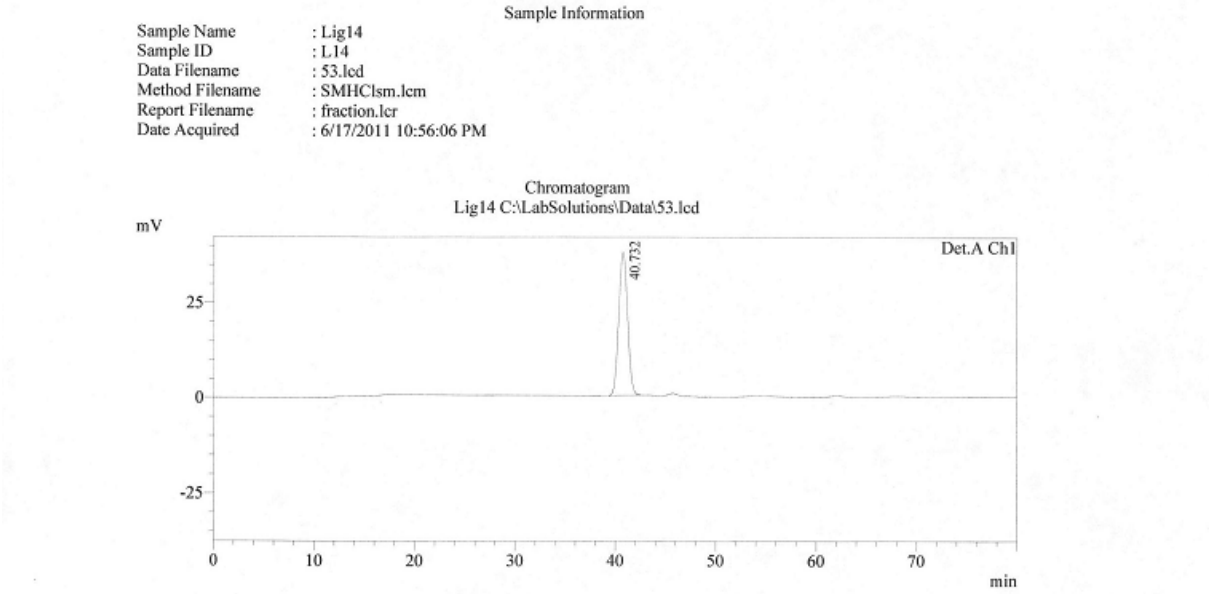

Figure S15. HPLC profile of isolated compound **26**. (Eluent = methanol:water (65:35), flow rate = 0.15 mL/min).

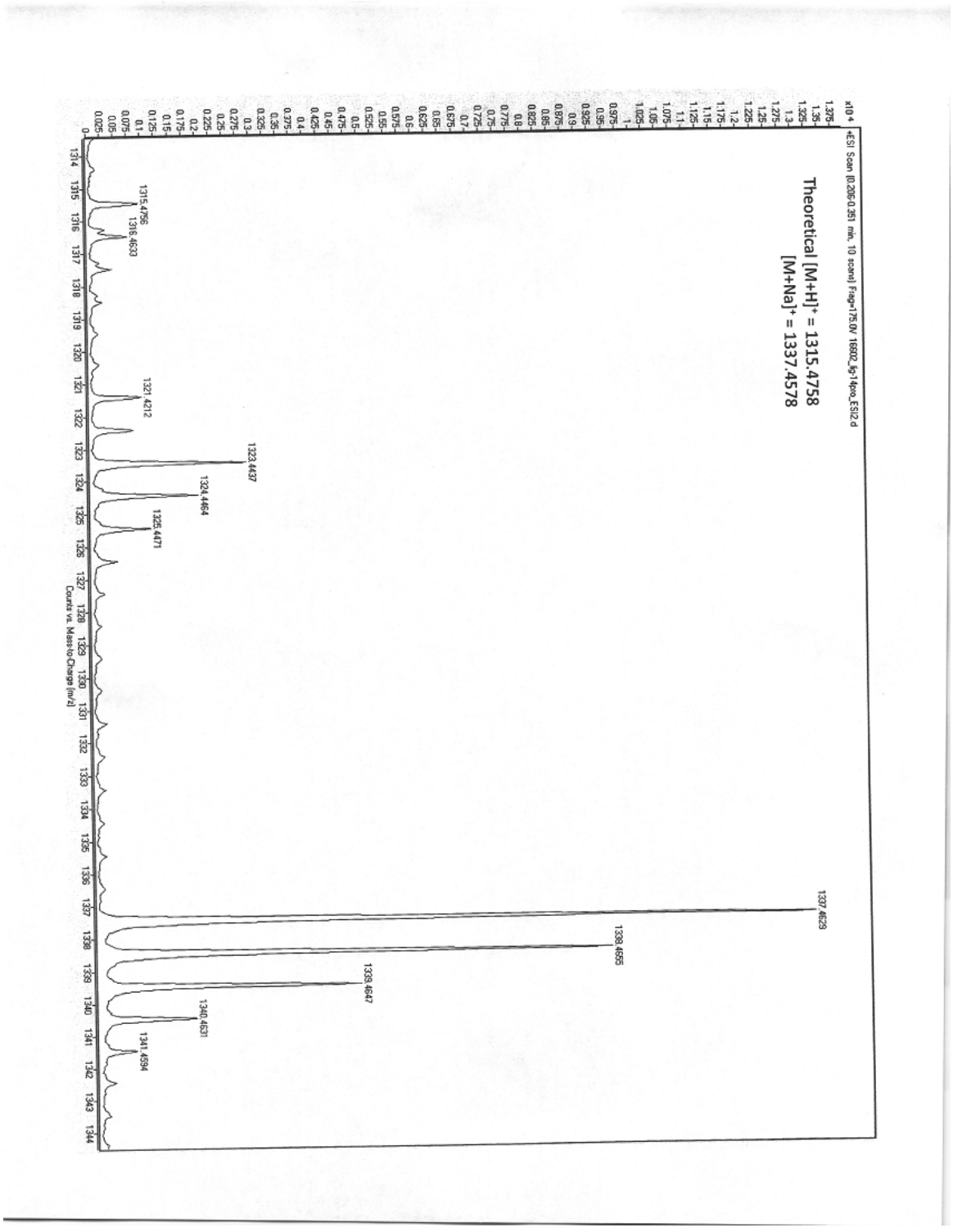

Figure S16. HRMS analysis of compound **26**

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