Supporting Information

9-Fluorenylmethyl (Fm) Disulfides: Biomimetic Precursors for Persulfides

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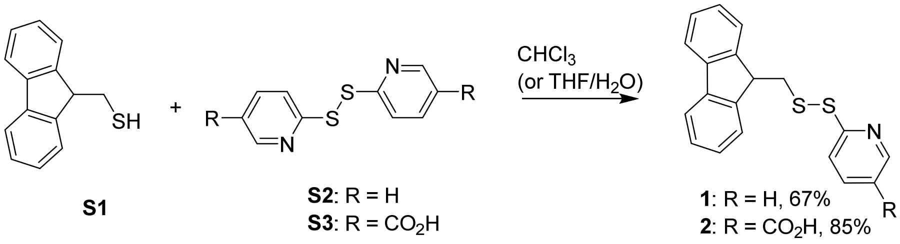
***Instrumentation*:** ^{1}H NMR spectra and ^{13}C NMR were recorded at 300 MHz (Varian, VX 300) and are reported in parts per million (ppm) on the δ scale relative to residual $CDCl_3$ (δ 7.25), DMSO-d6 (δ 2.50), $CHCl_3$ (δ 77.0), and DMSO (δ 39.5) respectively. NMR experiments were performed at room temperature. All reported melting points for solid materials were measured by Fisher-Johns melting point apparatus and not corrected. Mass spectra were recorded using a matrix-assisted laser desorption ionization (MALDI, Bruker Solarix 9.4T), matrix-assisted laser desorption ionization-time of flight (Maldi-Tof/Tof, Bruker UltrafleXtreme), and an electrospray ionization (ESI, Orbitrap Fusion Nano LC-MS/MS). Mass data were reported in units of m/z for $[M+H]^+$or $[M+Na]^+$. Infrared spectra were recorded on a Thermo Scientific Nicolet iS10 (Thin film) and reported in units of cm^{-1}.

***Solvents and Reagents*:** Reagents and solvents employed were of the highest grade available. Reagent grade solvents were used for either chromatography or extraction without further purification before use. Tetrahydrofuran (THF) and dichloromethane (DCM) were directly used from a solvent purifier (Pure Solv, Innovative Technology, inc.). *N,N'*-Dicyclohexyldicarbodiimide (DCC) and all protected and unprotected natural amino acids were purchased from Advanced ChemTech and used as received. 9-Fluorenemethanol (FmOH), S-methyl methanethiosulfonate (MMTS), Iodoacetamide (IAM), 4,4'-dithiodipyridine (PySSPy), and 6,6'-dithiodinicotinic acid (PySSPy-A) were purchased from TIC. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) and triethylamine (TEA) were purchased from Aldrich and TEA was freshly distilled before use.

***Chromatography*:** The progress of the reactions was monitored by analytical thin layer chromatography (VWR, TLC 60 F_{254} plates). Plates were visualized first with UV (254 nm) and then illuminated by CAM stain (2.5 g of ammonium molybdate tetrahydrate and 1 g of cerium ammonium sulfate in a solution of 10% sulfuric acid in water) or ninhydrin solution (0.3 % ninhydrin in a solution of 3 % acetic acid in ethanol). Flash column chromatography was performed using silica gel (230-400 mesh). The solvent compositions for all separations are on a volume/volume (v/v) basis.

Synthesis of FmSSPy and FmSSPy-A



S1

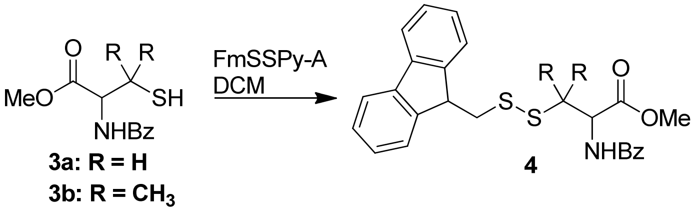
S2: R = H
S3: R = CO_2H

1: R = H, 67%
2: R = CO_2H, 85%

2-(((9H-Fluoren-9-yl)methyl)disulfanyl)pyridine (FmSSPy, 1): To a stirred solution of FmSH (202.6 mg, 0.954 mmol) in $CHCl_3$ (5 mL) was added PySSPy (252.3 mg, 1.145 mmol) followed by TEA (0.26 mL, 1.90 mmol) at room temperature. The reaction mixture was stirred overnight. Solvents were removed and the remained residue was extracted with EtOAc. The organic layers were washed with water and brine. Flash column chromatography using 1:5 EtOAc/Hex gave a yellow oil as the product (207 mg) in 67% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 8.47 (m, 1H), 7.79 – 7.09 (m, 11H), 4.32 (t, *J* = 6.2 Hz, 1H), 3.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.17, 149.93, 145.65, 141.27, 137.30, 127.95, 127.34, 125.15, 120.91, 120.23, 119.93, 46.56, 44.01; IR (thin film) 3045.4, 2912.3, 1714.6, 1608.1, 1570.1, 1554.9, 1444.6, 1410.4, 1117.6, 756.4, 737.4 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{19}H_{16}NS_2$ $[M+H]^+$ 322.0724, found 322.0728.

6-(((9H-Fluoren-9-yl)methyl)disulfanyl)nicotinic acid (FmSSPy-A, 2): To a stirred solution of FmSH (60.0 mg, 0.283 mmol) in 18 mL of 1:1 THF/buffer (pH 8.5) was added 6,6'-dithiodinicotinic acid (136 mg, 0.424 mmol) at room temperature. The reaction mixture was stirred overnight. Organic solvent was removed and the pH of the remained solution was adjusted to 5 with 1N HCl. The mixture was then extracted with EtOAc. The organic layers were washed with water and brine. A flash column chromatography using 10% MeOH in DCM gave a yellow solid as the product (88 mg) in 85% yield. mp 190-191 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 11.7 (br-s, 1H), 8.84 (s, 1H), 8.17 – 8.04 (m, 1H), 7.88 (d, *J* = 7.4 Hz, 2H), 7.71 (d, *J* = 7.3 Hz, 2H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.36 (dt, *J* = 23.5, 7.3 Hz, 4H), 4.36 (t, *J* = 4.5 Hz, 1H), 3.70 (d, *J* = 5.2 Hz, 2H); ^{13}C NMR (75 MHz) δ 166.71, 165.43, 150.54, 144.83, 141.21, 138.49, 127.83, 127.13, 124.71, 120.00, 119.02, 46.44, 43.88; IR (thin film) 3064.4, 2910.7, 1707.0, 1585.3, 1551.1, 1444.6, 1364.8, 1292.5, 1273.5, 1254.5, 1144..2, 1129.0, 1091.0, 1022.6, 847.6, 817.2, 767.8, 737.0 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{20}H_{16}NO_2S_2$ $[M+H]^+$ 366.0622, found 366.0623.

Experimental Procedures and Characterization of Compounds 4a-4d:



General procedure: To a solution of thiol in DCM or DMSO (*c* = 0.07~0.10 M) was added 1.1 equiv of FmSSPy-A at room temperature. The color of the reaction immediately became yellow and the reaction was completed in 30 min at room temperature. The mixture was diluted with DCM and washed with water and brine. A flash column chromatography using a mixture of EtOAc and hexanes on silica gel gave the corresponding products.

Bz-Cys[SFm]-OMe (4a): This compound was obtained as a white solid (155 mg) in 95% yield. mp 114-116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 – 7.70 (m, 4H), 7.63 (d, *J* = 7.4 Hz, 2H), 7.56 – 7.24 (m, 7H), 7.05 (d, *J* = 7.5 Hz, 1H), 5.06 (dt, *J* = 7.4, 5.0 Hz, 1H), 4.26 (t, *J* = 6.1 Hz, 1H), 3.71 (s, 3H), 3.41 – 3.26 (m, 2H), 3.21 (dd, *J* = 14.3, 4.6 Hz, 1H), 3.07 (dd, *J* = 14.3, 5.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.65, 205.14, 191.32, 171.15, 169.97, 167.20, 145.58, 145.56, 141.35, 133.77, 132.15, 128.89, 127.94, 127.43, 127.36, 127.34, 125.11, 125.08, 120.26, 52.99, 52.72, 46.52, 44.22, 40.65; IR (thin film) 3310.1, 2925.3, 2909.0, 2855.8, 1748.5, 1638.1, 1523.7, 1446.1, 1209.1, 1160.0, 743.2, 726.9, 707.7, .687.9 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{25}H_{24}NO_3S_2$ $[M+H]^+$ 450.1198, found 450.1207.

Bz-D,L-penicillamine[SFm]-OMe (4b): This compound was obtained as a sticky solid (880 mg) in 92% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 – 7.78 (m, 2H), 7.78 – 7.71 (m, 2H), 7.69 – 7.60 (m, 2H), 7.58 – 7.24 (m, 7H), 6.97 (d, *J* = 8.6 Hz, 1H), 4.94 (d, *J* = 8.7 Hz, 1H), 4.25 (t, *J* = 6.5 Hz, 1H), 3.75 (s, 3H), 3.30 (ddd, *J* = 6.7, 3.4, 0.9 Hz, 2H), 1.57 (s, 3H), 1.49 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.71, 167.24, 164.95, 145.61, 145.57, 141.20, 133.90, 132.20, 128.94, 127.92, 127.89, 127.38, 127.34, 127.29, 125.12, 125.08, 120.20, 59.84, 53.02, 52.66, 46.75, 45.41, 26.82, 25.39; IR (thin film) 3323.1, 2954.2, 2919.9, 1733.6, 1649.9, 1513.1, 1482.6, 1448.4, 1345.8, 1208.9, 741.2, 707.0, 691.7 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{27}H_{28}NO_3S_2$ $[M+H]^+$ 478.1511, found 478.1516.

Ac-Cys[SFm]-Gly-Phe-OMe (4c): This compound was obtained as a sticky solid (147 mg) in 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 7.73 (t, *J* = 5.7 Hz, 1H), 7.59 – 7.41 (m, 4H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.24 – 6.87 (m, 10H), 4.53 (dd, *J* = 7.5, 5.7 Hz, 1H), 4.43 (dd, *J* = 8.1, 5.4 Hz, 1H), 4.08 (t, *J* = 6.4 Hz, 1H), 3.79 (d, *J* = 16.9 Hz, 1H), 3.54 (d, *J* = 16.9 Hz, 1H), 3.45 (s, 3H), 3.14 – 3.04 (m, 2H), 2.97 – 2.69 (m, 4H), 1.78 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6) δ 171.92, 170.75, 170.50, 168.80, 145.59, 141.02, 136.50, 129.30, 128.53, 127.71, 127.18, 126.95, 125.08, 119.99, 53.66, 53.57, 52.99, 52.23, 46.24, 43.45, 42.84, 37.70, 23.04; IR (thin film) 3326.8, 3292.6, 3030.2, 2973.2, 2950.4, 1729.8, 1646.1, 1634.7, 1528.3, 1444.6, 1364.8, 1227.9, 1053.0, 1026.4, 999.7, 821.0, 771.6, 741.0, 699.4 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{31}H_{34}N_3O_5S_2$ $[M+H]^+$ 592.1940, found 592.1953.

Ac-Cys[SFm]-Pro-Phe-OMe (4d): This compound was obtained as a white solid (113 mg) in 95% yield. mp 73-75 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 8.44 (d, *J* = 4.8 Hz, 1H), 7.76 – 7.50 (m, 4H), 7.40 – 6.95 (m, 9H), 6.29 (d, *J* = 7.3 Hz, 1H), 4.88 – 4.65 (m, 2H), 4.44 – 4.28 (m, 1H), 4.20 (t, *J* = 6.1 Hz, 1H), 3.64 (s, 3H), 3.61 – 3.50 (m, 1H), 3.28 (m, 3H), 3.12 (dd, *J* = 13.9, 5.4 Hz, 1H), 3.01 – 2.79 (m, 2H), 2.69 (dd, *J* = 13.9, 6.4 Hz, 1H), 2.01 (m, 1H), 1.90 (s, 3H), 1.72 (m, 2H), 1.56 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.23, 170.73, 170.70, 169.96, 149.80, 145.54, 145.51, 141.32, 141.30, 137.65, 136.72, 129.51, 128.60, 127.96, 127.39, 127.36, 127.12, 125.10, 121.35, 120.20, 119.86, 60.70, 53.43, 52.55, 51.04, 47.45, 46.54, 43.73, 39.81, 37.91, 28.57, 24.31, 23.20; IR (thin film) 3315.4, 3285.0, 3049.2, 2946.6, 1741.2, 1634.7, 1543.5, 1444.6, 1414.2, 1281.1, 1208.9, 1117.6, 1026.4, 980.7, 764.0, 741.2, 699.4 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{34}H_{38}N_3O_5S_2$ $[M+H]^+$ 632.2253, found 632.2271.

Generation of Persulfides and Their Trapping with Various Electrophiles:

General procedure: In a round bottom flask, RSSFm and 2 equiv of an electrophile were combined and dissolved in THF or DCM (total concentration = 0.03~0.05M). To the solution was slowly added 2 equiv of DBU in 1-2 mL of DCM at room temperature. The resulting mixture was stirred for 15~20 min at room temperature. Upon completion of the reaction (monitored by TLC), the solvent was removed under reduced pressure. The residue was dissolved in DCM and washed with $NaHCO_3$, water and brine. The solution was dried over $MgSO_4$ and concentrated under reduce pressure. Purification by flash column chromatography using a mixture of EtOAc and hexanes afforded the corresponding products.

Methyl S-((2-amino-2-oxoethyl)thio)-N-benzoyl-D-cysteinate (6a): This compound was obtained as a solid (39 mg) in 80% yield. mp 150-151 °C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.22 (d, *J* = 7.7 Hz, 1H), 7.58 – 7.44 (m, 2H), 7.04 (m, 3H), 6.93 (s, 1H), 6.27 (s, 1H), 4.49 (ddd, *J* = 9.2, 7.7, 4.4 Hz, 1H), 3.33 (s, 3H), 3.09 – 2.79 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6) δ 171.04, 170.89, 167.39, 133.67, 131.57, 128.23, 127.58, 78.14, 52.49, 52.39, 42.41; IR (thin film) 3353.4, 3296.4, 3193.7, 2987.3, 1726.0, 1661.4, 1627.1, 1532.1, 1334.3, 1239.3, 1159.4, 1026.4, 840.0, 703.2, 687.0, 672.7 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{13}H_{17}N_2O_4S_2$ $[M+H]^+$ 329.0630, found 329.0631.

Methyl (S)-3-((2-amino-2-oxoethyl)disulfanyl)-2-benzamido-3-methylbutanoate (6b): This compound was obtained as a white solid (41 mg) in 95% yield. mp 104-105 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 – 7.73 (m, 2H), 7.57 – 7.38 (m, 3H), 6.98 (d, *J* = 9.1 Hz, 1H), 6.57 (s, 1H), 6.02 (s, 1H), 4.97 (d, *J* = 9.2 Hz, 1H), 3.77 (s, 3H), 3.61 – 3.32 (m, 2H), 1.47 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.93, 170.80, 167.57, 133.71, 132.38, 128.98, 127.44, 59.26, 53.20, 52.79, 43.42, 26.34, 24.55; IR (thin film) 3402.8, 3300.2, 3246.9, 3205.1, 2931.3, 1718.4, 1638.5, 1509.3, 1482.6, 1368.6, 1220.3, 1148.3, 1110.0, 691.7 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{15}H_{21}N_2O_4S_2$ $[M+H]^+$ 357.0943, found 357.0946.

Methyl N-benzoyl-S-((3-methoxy-3-oxopropyl)thio)-D-cysteinate (6c): This compound was obtained as a sticky oil (56 mg) in 64% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 – 7.73 (m, 2H), 7.58 – 7.32 (m, 3H), 7.10 (d, *J* = 7.5 Hz, 1H), 5.01 (dt, *J* = 7.5, 5.0 Hz, 1H), 3.78 (s, 3H), 3.65 (s, 3H), 3.20 – 3.04 (m, 2H), 2.83 – 2.75 (m, 2H), 2.57 (td, *J* = 7.2, 0.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.30, 171.47, 167.27, 133.77, 132.16, 128.84, 127.47, 127.41, 53.04, 52.63, 52.09, 34.67, 34.52, 27.81; IR (thin film) 3321.3, 2950.4, 2923.7, 1737.4, 1649.9, 1520.7, 1482.6, 1433.2, 1353.4, 1208.9, 1174.6, 908.6, 729.8, 684.1 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{15}H_{20}NO_5S_2$ $[M+H]^+$ 358.0783, found 358.0784.

Methyl *N*-benzoyl-S-(((2,5,6-trimethyl-1,7-dioxo-1H,7H-pyrazolo[1,2-a]pyrazol-3-yl)methyl)thio)-D-cysteinate (6d): This compound was obtained as an yellow solid (38 mg) in 74% yield. mp 175-176 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (m, 2H), 7.63 – 7.33 (m, 3H), 7.02 (d, *J* = 7.4 Hz, 1H), 5.09 (q, *J* = 6.1 Hz, 1H), 3.98 – 3.82 (m, 2H), 3.79 (s, 3H), 3.25 (ddd, *J* = 55.3, 14.1, 5.4 Hz, 2H), 2.33 (s, 3H), 1.90 (s, 3H), 1.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.96, 167.32, 160.88, 160.28, 146.24, 144.77, 133.51, 132.34, 128.92, 127.40, 115.82, 113.07, 53.31, 52.28, 40.61, 32.22, 12.14, 7.64, 7.09; IR (thin film) 3307.8, 2954.2, 2916.1, 1729.8, 1653.7, 1524.5, 1402.8, 1418.0, 1292.5, 1227.9, 1170.8, 1144.2, 988.3, 745.0, 718.4, 695.5 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{21}H_{24}N_3O_5S_2$ $[M+H]^+$ 462.1157, found 462.1159 .

Methyl *N*-benzoyl-S-(methyldisulfanyl)-D-cysteinate (6e): This compound was obtained as a white solid (23 mg) in 53% yield. mp 64-65 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (dt, *J* = 6.9, 1.5 Hz, 2H), 7.62 – 7.38 (m, 3H), 7.07 (d, *J* = 7.4 Hz, 1H), 5.16 (dt, *J* = 7.3, 4.8 Hz, 1H), 3.82 (s, 3H), 3.61 – 3.44 (m, 2H), 2.54 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.03, 167.24, 133.80, 132.20, 128.87, 127.43, 52.32, 49.12, 40.60, 22.67; IR (thin film) 3353.4, 2946.6, 2916.1, 1741.2, 1638.5, 1513.1, 1486.4, 1429.4, 1296.3, 1258.3, 1231.7, 1208.9, 1163.2, 1007.3, 718.4, 684.1 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{12}H_{16}NO_3S_3$ $[M+H]^+$ 318.0287, found 318.0293.

Methyl-2-benzamido-3-((3-methoxy-3-oxopropyl)disulfanyl)-3-methylbutanoate (6f): This compound was obtained as a sticky oil (43 mg) in 82% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 – 7.73 (m, 2H), 7.59 – 7.37 (m, 3H), 6.93 (d, *J* = 8.8 Hz, 1H), 4.91 (d, *J* = 8.7 Hz, 1H), 3.77 (s, 3H), 3.67 (s, 3H), 3.05 – 2.88 (m, 2H), 2.69 (t, *J* = 7.1 Hz, 2H), 1.52 (s, 3H), 1.46 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.19, 170.68, 167.23, 133.88, 132.19, 128.91, 127.37, 59.70, 52.89, 52.67, 52.15, 35.03, 34.13, 26.48, 25.21; IR (thin film) 3334.1, 2950.4, 2927.5, 1729.8, 1659.7, 1513.1, 1478.4, 1342.0, 1299.3, 1205.1, 1167.0, 1117.6, 1014.9, 710.8, 691.7 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{17}H_{24}NO_5S_2$ $[M+H]^+$ 386.1096, found 386.1099.

Methyl-2-benzamido-3-methyl-3-(((2,5,6-trimethyl-1,7-dioxo-1H,7H-pyrazolo[1,2-a]pyrazol-3-yl)methyl)disulfanyl)butanoate (6g): This compound was obtained as an yellow solid (48 mg) in 92% yield. mp 72-75 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 – 7.71 (m, 2H), 7.60 – 7.40 (m, 3H), 6.82 (d, *J* = 9.3 Hz, 1H), 5.01 (d, *J* = 9.3 Hz, 1H), 4.04 – 3.82 (m, 2H), 3.79 (s, 3H), 2.38 (s, 3H), 1.89 (s, 3H), 1.81 (s, 3H), 1.51 (s, 3H), 1.47 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.60, 167.33, 160.86, 160.33, 146.11, 144.64, 133.67, 132.45, 129.02, 127.31, 115.44, 113.14, 58.88, 53.81, 52.87, 33.92, 26.24, 24.59, 12.08, 7.79, 7.11; IR (thin film) 3227.9, 3197.5, 2931.3, 2916.1, 1737.4, 1663.7, 1433.2, 1406.6, 1216.5, 1151.8, 1079.6, 1030.2, 718.4, 695.5 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{23}H_{28}N_3O_5S_2$ $[M+H]^+$ 490.1470, found 490.1465.

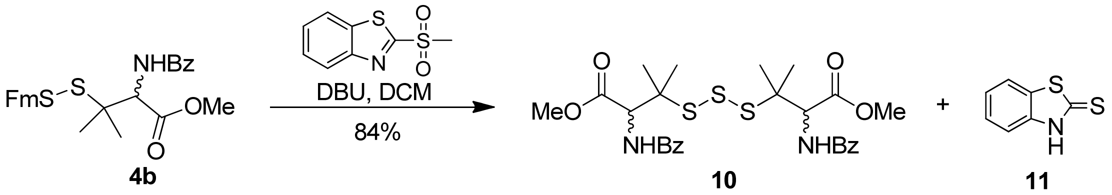
Methyl 2-benzamido-3-(benzyldisulfanyl)-3-methylbutanoate (6h): This compound was obtained as a sticky solid (39 mg) in 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 – 7.77 (m, 2H), 7.58 – 7.39 (m, 3H), 7.34 – 7.21 (m, 5H), 6.98 (d, *J* = 8.7 Hz, 1H), 4.93 (d, *J* = 8.7 Hz, 1H), 4.06 – 3.87 (m, 2H), 3.77 (s, 3H), 1.51 (s, 3H), 1.46 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.69, 167.23, 136.85, 133.93, 132.18, 129.53, 128.91, 128.85, 127.88, 127.39, 59.95, 52.92, 52.64, 45.47, 26.71, 25.32; IR (thin film) 3364.8, 2965.6, 2946.6, 2923.7, 1741.2, 1653.7, 1509.3, 1478.8, 1452.2, 1429.4, 1334.3, 1205.1, 1163.2, 1022.6, 764.0, 695.5 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{20}H_{24}NO_3S_2$ $[M+H]^+$ 390.1198, found 390.1201.

Methyl-2-benzamido-3-methyl-3-(methyltrisulfanyl)butanoate (6i): This compound was obtained as a white solid (39 mg) in 90% yield. mp 63-64 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 – 7.72 (m, 2H), 7.61 – 7.34 (m, 3H), 6.95 (d, J = 8.7 Hz, 1H), 4.93 (m, 1H), 3.78 (m, 3H), 2.47 (m, 3H), 1.66 – 1.40 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.62, 167.36, 133.97, 132.17, 128.91, 128.88, 127.43, 127.37, 59.79, 53.56, 52.70, 26.59, 25.54, 22.82; IR (thin film) 3380.0, 2977.0, 2942.8, 2916.1, 1726.0, 1665.2, 1509.3, 1482.6, 1429.4, 1361.0, 1307.7, 1262.1, 1220.3, 1132.8, 1072.0, 1014.9, 756.4, 703.2, 691.7 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{14}H_{20}NO_3S_3$ $[M+H]^+$ 346.0600, found 346.0600.

Ac-Cys[SCH_2CONH_2]-Gly-Phe-OMe (6j) This compound was obtained as a white solid (22 mg) in 69% yield. mp 135-138 °C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 8.01 (br-s, 1H, N*H*), 7.60 (br-s, 1H, N*H*), 7.53 – 7.41 (br-s, 1H, N*H*), 7.32 – 7.14 (m, 6H, Ph+ N*H*), 6.67 (br-s, 1H, N*H*), 4.78 (m, 2H), 3.67 (s, 3H), 3.56 (m, 2H), 3.49 (s, 2H), 3.24 (m, 1H), 3.11 (m, 3H), 2.03 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-*d*$_6$) 171.54, 170.60, 170.46, 170.30, 168.67, 136.64, 128.90, 128.09, 126.42, 53.46, 51.71, 48.16, 42.14, 37.79, 37.04, 28.40, 26.14, 23.46, 22.57; IR (thin film) 3307.8, 3300.2, 3007.4, 2942.8, 1729.8, 1657.5, 1630.9, 1532.1, 1452.2, 1414.2, 1220.3, 1030.2, 737.4, 703.2, 668.9 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{19}H_{27}N_4O_6S_2$ $[M+H]^+$ 471.1372, found 471.1378.

Ac-Cys[SCH_2CONH_2]-Pro-Phe-OMe (6k): This compound was obtained as a white solid (23 mg) in 64% yield. mp 59 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 6.5 Hz, 1H, N*H*), 7.16 (m, 6H, Ph+N*H*), 6.99 (br-s, 1H, N*H*), 6.29 (br-s, 1H, N*H*), 4.73 (m, 2H), 4.41 (dd, *J* = 7.3, 3.6 Hz, 1H), 3.63 (s, 3H), 3.55 – 3.39 (m, 4H), 3.19 – 3.08 (m, 2H), 3.03 – 2.86 (m, 2H), 2.03 (m, 1H), 1.93 (s, 3H), 1.79 – 1.66 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.33, 171.57, 171.02, 170.55, 166.57, 136.87, 129.46, 128.63, 127.12, 61.04, 55.08, 53.57, 52.57, 49.15, 42.97, 38.38, 29.18, 26.96, 24.16, 23.01; IR (thin film) 3296.4, 3277.4, 2950.4, 2927.5, 1737.4, 1620.1, 1535.9, 1524.5, 1440.8, 1368.6, 1224.1, 1212.7, 737.4, 691.7, 668.9 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{22}H_{31}N_4O_6S_2$ $[M+H]^+$ 511.1685, found 511.1691.

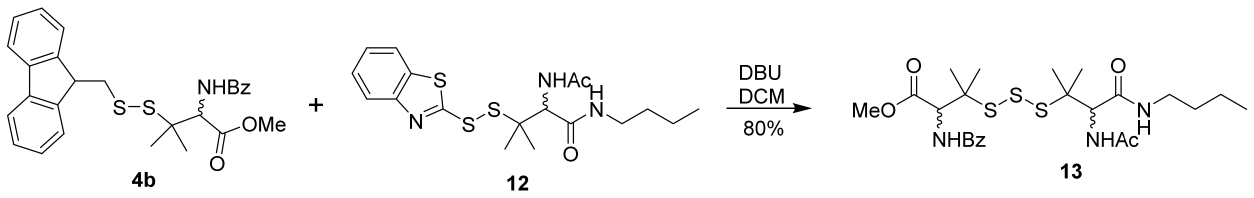
Reaction between 4b and MSBT



Compound **10**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 – 7.72 (m, 4H), 7.57 – 7.35 (m, 6H), 6.90 (m, 2H), 4.94 (m, 2H), 3.77 (s, 3H), 3.76 (s, 3H), 1.50 (m, 12H); IR (thin film) 3364.8, 2973.2, 2950.4, 2923.7, 1745.0, 1653.7, 1505.5, 1482.6, 1429.5, 1338.2, 1205.1, 1117.6, 1014.9, 714.6, 687.9 cm^{-1}; ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.59, 167.33, 133.88, 132.20, 128.88, 127.44, 59.56, 54.24, 52.73, 26.61, 26.51, 25.50, 25.18; MS (ESI) m/z calcd for $C_{26}H_{32}N_2NaO_6S_3$ $[M+Na]^+$ 587.1, found 587.2.

Compound **11**: ^{1}H NMR (300 MHz, DMSO-*d*$_6$ + $CDCl_3$) δ 13.35 (br-s, 1H, *NH*), 7.41 – 7.05 (m, 4H). ^{13}C NMR (75 MHz, DMSO) δ 190.69, 141.71, 130.16, 127.03, 124.32, 121.27, 112.88. MS (ESI) m/z calcd for $C_7H_5NNaS_2$ $[M+Na]^+$ 190.0, found 190.1.

Crossover experiment between Bz-DL-penicillamine[SFm]-OMe and Ac-DL-penicillamine[SSBt]-ONH(CH_2)$_4$.



Preparation of compound **12**: 2,2'-Dibenzothiazolyl disulfide (2.16 g, 6.50 mmol) was dissolved into 250 mL of $CHCl_3$. To this solution was added a DL-penicillamine derivative (1.18 g, 4.80 mmol). The reaction mixture was stirred at room temperature for 48 h. Excess solvent was removed under reduced pressure and then remaining 2,2'-dibenzothiazolyl disulfide was filtered using DCM. The crude product was then purified by flash column chromatography using 3% MeOH in DCM to give the product **12** (2.54 g) in 95% yield as white solid. mp 167-168 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (br-s, 1H), 7.72 (ddd, *J* = 9.4, 8.2, 1.2 Hz, 2H), 7.31 (m, 2H), 6.80 (br-s, 1H), 4.78 (d, *J* = 9.0 Hz, 1H), 3.37 (m, 1H), 3.13 (m, 1H), 1.95 (s, 3H), 1.54 – 1.39 (m, 5H), 1.39 – 1.23 (m, 5H), 0.83 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.21, 170.34, 168.92, 136.08, 126.65, 125.14, 121.82, 121.52, 58.77, 55.41, 39.75, 31.63, 25.30, 24.65, 23.59, 20.48, 13.96; IR (thin film, cm^{-1}) 3273, 3080, 2951, 2864, 1683, 1634, 1564, 1455, 1424, 1380, 1363, 1002, 749, 721; MS (ESI) *m/z* calcd for $C_{18}H_{25}N_3NaO_2S_3$ $[M+Na]^+$ 434.0, found 434.1.

Compound **13** (diastereomers): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (m, 2H), 7.41 (m, 3H), 6.89 (m, 1H), 6.52 (dd, *J* = 24.0, 8.6 Hz, 2H), 4.91 (m, 1H), 4.59 (d, *J* = 8.7 Hz, 1H), 3.72 (s, 3H), 3.32 – 2.99 (m, 2H), 1.94 (m, 3H), 1.60 – 1.16 (m, 16H), 0.83 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.75, 170.66, 170.37, 170.30, 168.98, 168.95, 167.37, 167.35, 133.86, 133.82, 132.23, 128.91, 127.51, 127.46, 59.72, 59.56, 58.91, 58.54, 54.39, 54.29, 54.18, 54.12, 52.78, 52.75, 39.70, 39.66, 31.60, 26.72, 26.62, 25.62, 25.45, 25.25, 25.18, 24.97, 23.55, 20.36, 13.94; IR (thin film) 3289.0, 2966.2, 2953.9, 2945.7, 1744.4, 1642.2, 1515.6, 1478.8, 1450.2, 1433.8, 1360.3, 1339.8, 1205.0, 1115.1, 710.6, 690.1 cm^{-1}; HRMS (Maldi) m/z calcd for $C_{24}H_{38}N_3O_5S_3$ $[M+H]^+$ 544.1968, found 544.1970.

Labeling of all free thiols on reduced BSA

Freshly prepared 50 mM HEPES buffer (pH 7.7) was used. 10 mg of BSA was dissolved in HEPES buffer to obtain a 150 µM stock solution. A 200 mM FmSSPy-A solution was prepared in DMF. A 10 mM DTNB solution was prepared in PBS buffer (pH 8.0). A 100 mM monobromobimane stock solution in DMF was freshly prepared.

Procedure:

1. **Reduction of BSA**: To a 700 µL of BSA solution (10 mg/mL, 0.150 mM) was added 28.0 µL of 500 mM DTT at room temperature. The mixture was stirred at 37 °C for 1h in dark. The excess DTT was removed by Zeba desalting column with PBS buffer (2×). 120 µL of aliquot was taken and the

concentration of free thiols was determined by Ellman's reagent (DTNB). Zeba desalting column can efficiently remove unreacted DTT. One time desalting removed >99.9% of DTT, while two time desalting can remove DTT completely. The Ellman's assay showed the conversion of BSA disulfide to free thiol is about 52.2% after DTT reduction (Figure S1).

2. **FmSSPy-A treatment:** To a 550 µL of purified and reduced BSA solution was slowly added 50.0 µL of 200 mM FmSSPy-A. The mixture was incubated at 37 ^{o}C for 1h in dark. The proteins were purified by Zeba desalting column (2 mL). 120 µL of aliquot was taken and the concentration of free thiols on BSA was determined by Ellman's reagent (DTNB). **<u>Measurement</u>**: 5 µL of Ellman's Reagent solution (10 mM) and 250 uL of Ellman's reaction buffer were combined in a test tube. To the test tube was added 25 µL of BSA aliquots (treated and untreated separately). The mixture was gently swirled and incubated at room temperature for 15 min. UV intensity of the solution was measured at 410 nm and recorded (Figure S1).

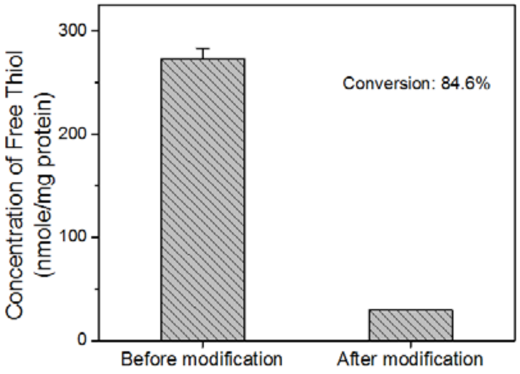


Figure S1. Determination of the concentration of free thiols before/after treatment of FmSSPy-A.

3. **Monobromobimane treatment:** To 225 µL of FmSSPy-A treated-BSA solution was added 25 uL of 100 mM monobromobimane (final conc. = 10 mM) then the mixture was vortexed for 10 min. To the mixture was added 50 uL of 1 M DBU in DMF (final conc. = 200 mM) and stirred for 1 h at room temperature in dark. The labeled BSA was purified by Zeba column.

<u>Protein digestion</u>: The concentrations of protein samples (DTT-treated BSA, FmSSPy-A-treated BSA, monobromobimane-treated BSA) were measured by BCA. Each protein sample (200 µL from 1 mg/mL of a protein) was digested by 5 µg of trypsin for overnight (about 16 h) at 37 ^{o}C. Without further purification, tryptic peptides were subjected to LC-MS/MS analysis (Figure S2). The peak area of peptide ion in the extracted ion chromatogram was used to calculate the conversion of reaction at different cysteine residues in BSA. The results were summarized in Table S1.

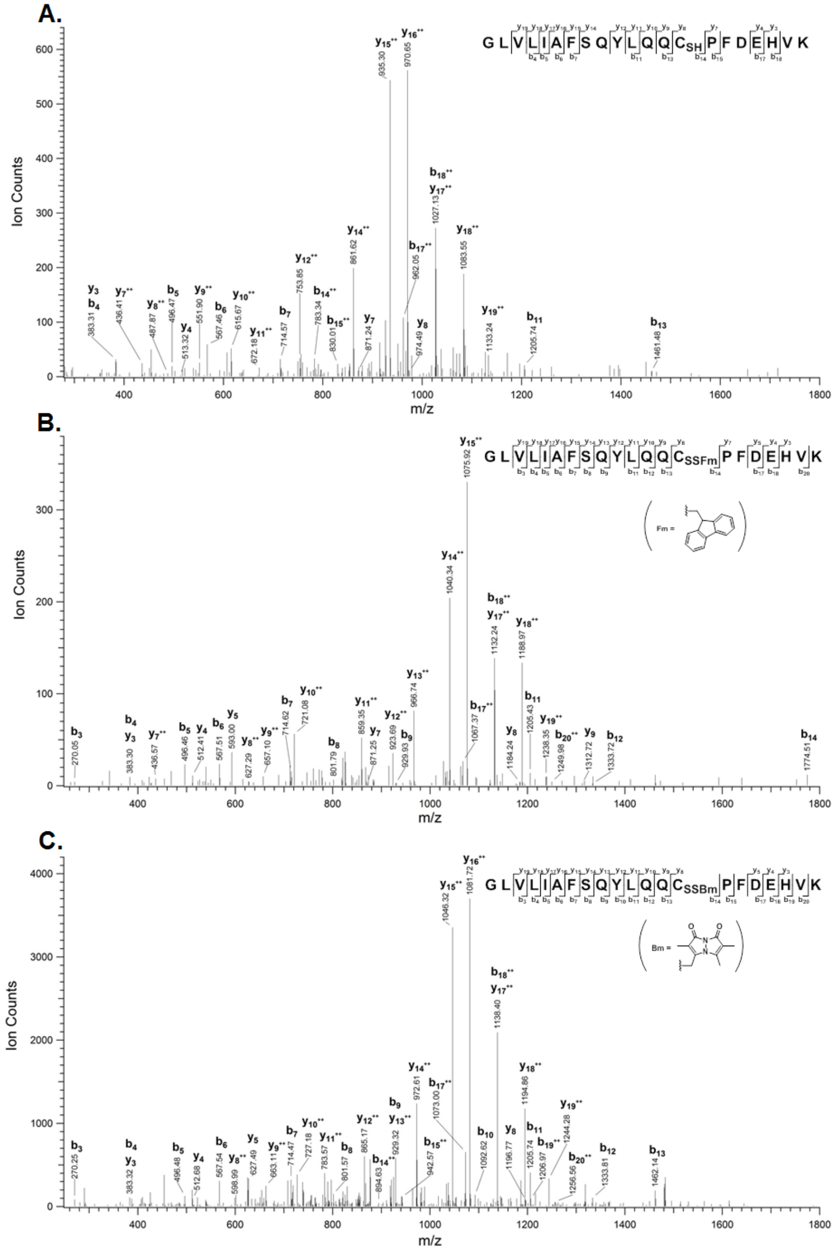


Figure S2. MS/MS analysis for selected tryptic-digested peptides from unlabeled and labeled BSA samples. (A) An unlabeled peptide: GLVLIAFSQYLQQCPFDEHVK. (B) A peptide labeled with FmSSPy-A: GLVLIAFSQYLQQC[SFm]PFDEHVK. (C) A peptide labeled with monobromobimane: GLVLIAFSQYLQQC[SSBm]PFDEHVK.

Table S1. Conversion of BSA cysteines from PSSFm to PSSBm.

group	peptide[a]	Cys site	m/z[b]	e	peak area[c]	conversion (%)[d]
1	SLHTLFGDELC+222.046K	Cys99	792.86	2	2.07E+09	4.8
	SLHTLFGDELC+210.050K	Cys99	786.86	2	4.08E+10	
2	GLVLIAFSQYLQQC+222.046PFDEHVK	Cys58	886.43	3	1.35E+09	71.2
	GLVLIAFSQYLQQC+210.050PFDEHVK	Cys58	882.44	3	5.46E+08	
3	LFTFHADIC+222.046TLPDTEK	Cys537	1036.98	2	2.90E+08	2.4
	LFTFHADIC+210.050TLPDTEK	Cys537	1030.98	2	1.16E+10	
4	HADIC+222.046TLPDTEK	Cys537	782.84	2	7.22E+07	9.2
	HADIC+210.050TLPDTEK	Cys537	776.84	2	7.16E+08	
5	MPC+222.046TEDYLSLILNR	Cys471	945.43	2	4.93E+08	22.9
	MPC+210.050TEDYLSLILNR	Cys471	939.44	2	1.66E+09	
6	YIC+222.046DNQDTISSK	Cys288	804.84	2	1.68E+09	10.3
	YIC+210.050DNQDTISSK	Cys288	798.84	2	1.46E+10	
7	LKPDPNTLC+222.046DEFK	Cys147	871.40	2	1.98E+09	4.8
	LKPDPNTLC+210.050DEFK	Cys147	865.40	2	3.96E+10	
8	QEPERNEC+222.046FLSHK	Cys125	919.90	2	1.51E+07	10.7
	QEPERNEC+210.050FLSHK	Cys125	913.90	2	1.26E+08	
9	DDPHAC+222.046YSTVFDK	Cys392	860.34	2	4.81E+08	7.3
	DDPHAC+210.050YSTVFDK	Cys392	854.34	2	6.14E+09	
10	RPC+222.046FSALTPDETYVPK	Cys510	1023.48	2	4.87E+08	3.4
	RPC+210.050FSALTPDETYVPK	Cys510	1017.48	2	1.38E+10	
11	SHC+222.046IAEVEK	Cys312	619.27	2	1.39E+09	6.2
	SHC+210.050IAEVEK	Cys312	613.27	2	2.11E+10	
12	C+222.046C+210.050TESLVNR	Cys499	728.78	2	5.85E+07	20.6
	C+210.050C+210.050TESLVNR	Cys499	722.78	2	2.25E+08	
13	PC+222.046FSALTPDETYVPK	Cys510	945.43	2	4.93E+08	48.0
	PC+210.050FSALTPDETYVPK	Cys510	939.42	2	5.35E+08	

a. Labeled with monobromobimane [SSBm]: +222.046; labeled with FmSSPy-A [SFm]: +210.050.

b. The m/z value of parent ion for peptides.

c. Peak area of peptide ions in selective extracted ion chromatogram.

d. Conversion (%) = Peak area (Peptide[SSBm])/(Peak area (Peptide[SSBm])+ Peak area (Peptide[SFm]).

The labeling reaction was carried out at protein level, followed by tryptic digestion and LC-MS/MS analysis. The peak area of peptide in exacted ion chromatogram was used for quantification. BSA sequence includes a 24-AA length signal fragment. Therefore, Cys34 mentioned in the text is Cys58 in this table.

Labeling of a specific site (Cys34) on non-reduced BSA

Procedure of BSA gel-filtrations:

Bovine serum albumin (BSA) was purchased from Aldrich and used as received. For the gel-filtration (size exclusion), Sephadex G25 pre-packed in PD-10 columns was purchased from General Electronics. Before loading of samples, the column was first equilibrated with 20 mM PBS (pH 7.4, 3.5 mL × 4). 2.5 mL of reaction solution was loaded on the column, eluted by gravity and discarded. Then 3.5 mL of buffer solution was added and samples were collected in separate new vials. The collected solutions were directly used for MS analysis.

BSA[Cys34] modification with FmSSPy-A and IAM:

To 5.05 mg of BSA dissolved in 2 mL of sodium phosphate buffer (100 mM, pH 7.1) was added 5 equiv (19 µL) of FmSSPy-A in DMF (20 mM stock solution) at room temperature. The mixture was stirred for 1 h. The volume of the solution was adjusted to 2.5 mL with the same buffer solution then it was directly loaded to an equilibrated PD-10 column. After draining the solution by gravity, 3.5 mL of buffer was added to elute the desired protein in a new vial. To the eluent was added 152 µL of iodoacetamide (IAM, 50 mM stock solution in DMF) followed by 304 µL of DBU (in 25 mM stock solution in DMF). The mixture was stirred for 1 h. Then 2.5 mL of the solution was loaded to a pre-equilibrated PD-10 column and drained. The protein sample was collected by elution of 3.5 mL of buffer. The eluent was directly used for the trypsin digestion and subjected to LC-MS/MS analysis.

High resolution nano-HPLC tandem mass spectrometry analysis:

The peptide samples were subjected to Thermo Scientific^{TM} Orbitrap Fusion^{TM} Tribrid^{TM} with an Easy-nLC^{TM} 1000 ultra-high pressure LC on a Thermo Scientific^{TM} PepMap 100 C18 column (2 µm, 50 µm x 15 cm). The peptides were separated over 115 min gradient eluted at 300 nL/min with 0.1% formic acid (FA) in water (solvent A) and 0.1% FA in acetonitrile (solvent B) (5-35% B in 85 min, followed by 35-50% B over 10 min and 50-95% B over 10 min). The run was completed by holding a 95% B for 10 min. MS1 data was acquired on an Orbitrap Fusion mass spectrometry using a full scan method according to the following parameters: scan range 400-1500 m/z, Orbitrap resolution 120,000; AGC target 400,000; and maximum injection time of 50 ms. MS2 data were collected using the following parameters: rapid scan rate, HCD collision energy 35%, 1.6 m/z isolation window, AGC 2,000 and maximum injection time of 50 ms. MS2 precursors were selected for a 3 s cycle. The precursors with an assigned monoisotopic m/z and a charge state of 2-7 were interrogated. The precursors were filtered using a 60 s dynamic exclusion window.

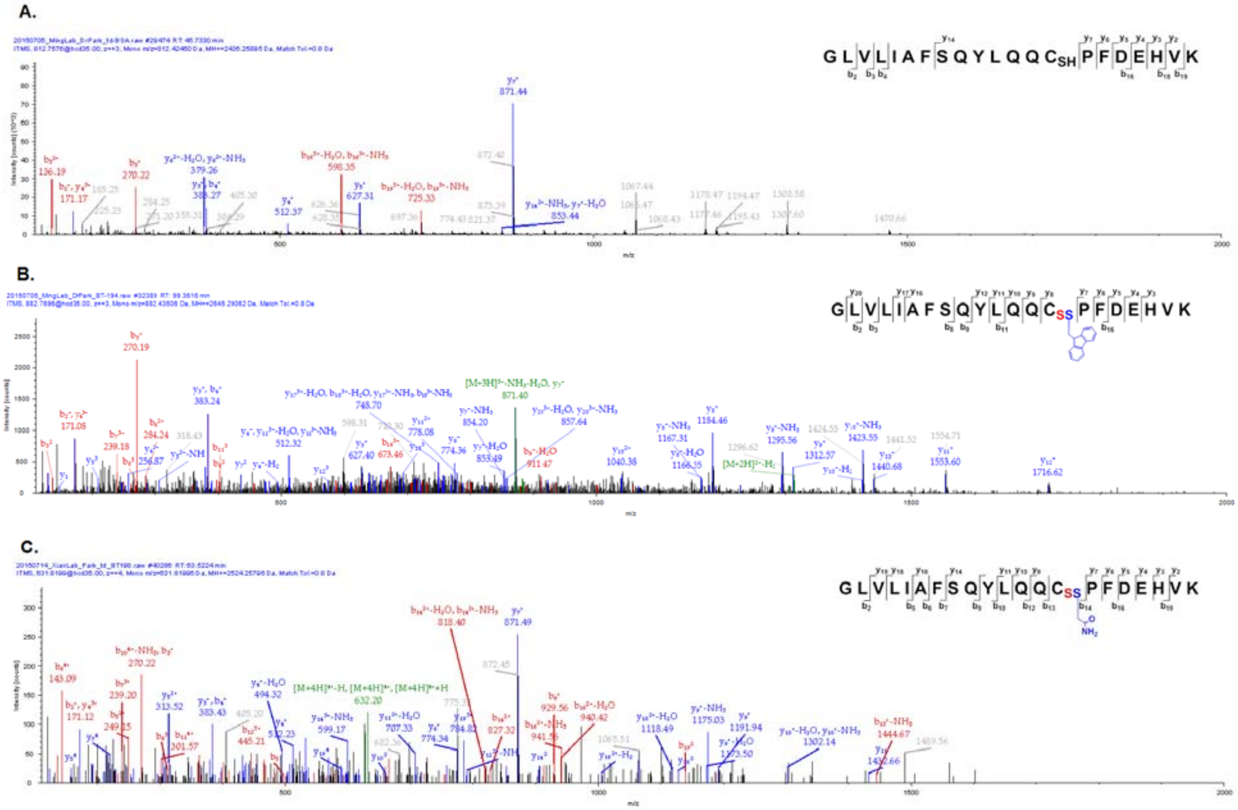


Figure S3. MS/MS analysis by Proteome Discoverer^{TM} 2.0 of (A) peptide **14** (GLVLIAFSQYLQQCPFDEHVK), (B) peptide **16** (GLVLIAFSQYLQQC[SFm]PFDEHVK), (C) peptide **19** (GLVLIAFSQYLQQC[SS(CH_2CONH_2)]PFDEHVK).

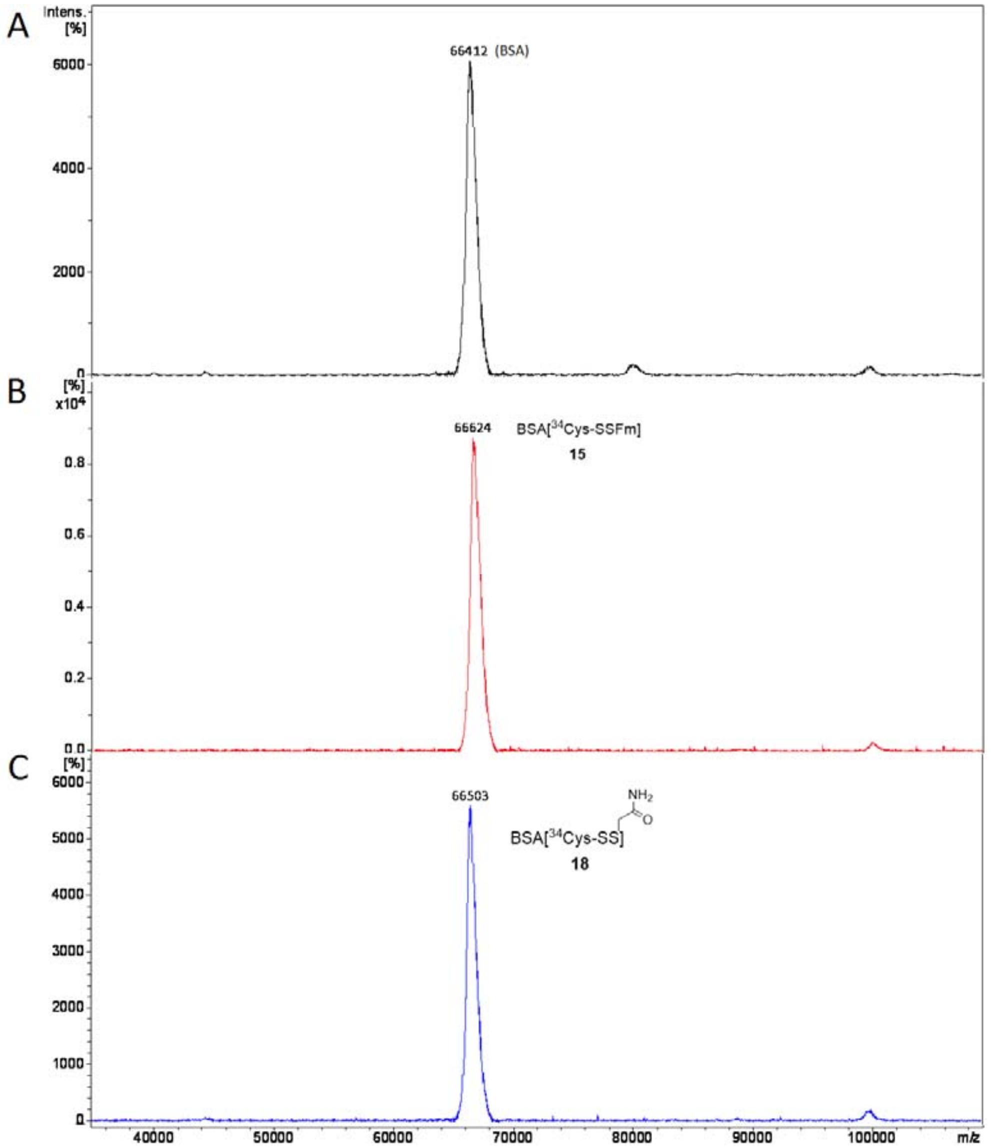
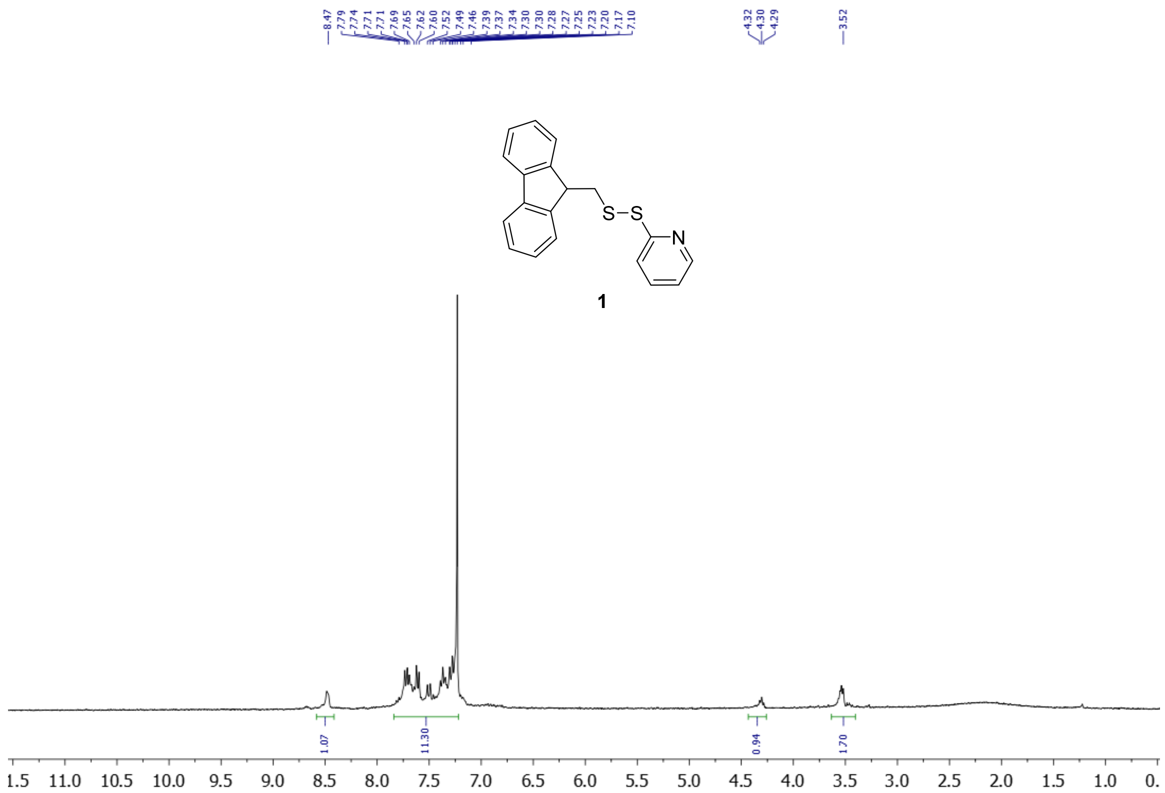
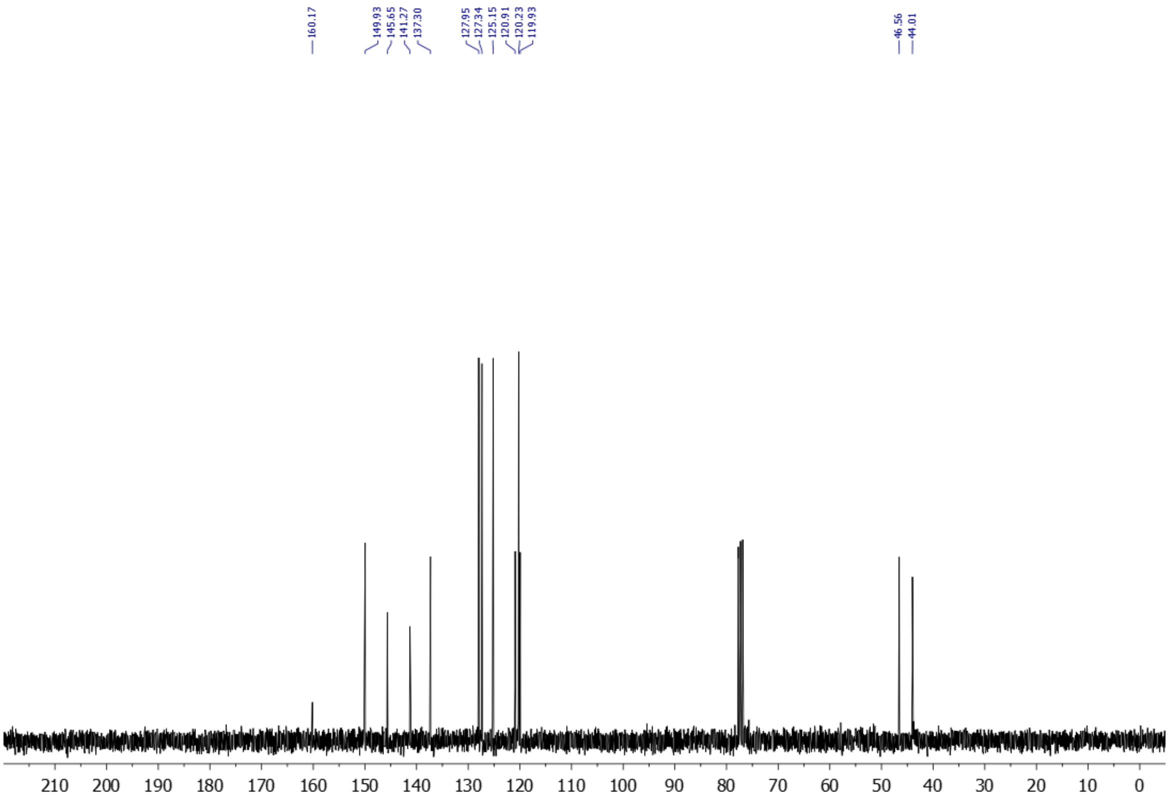


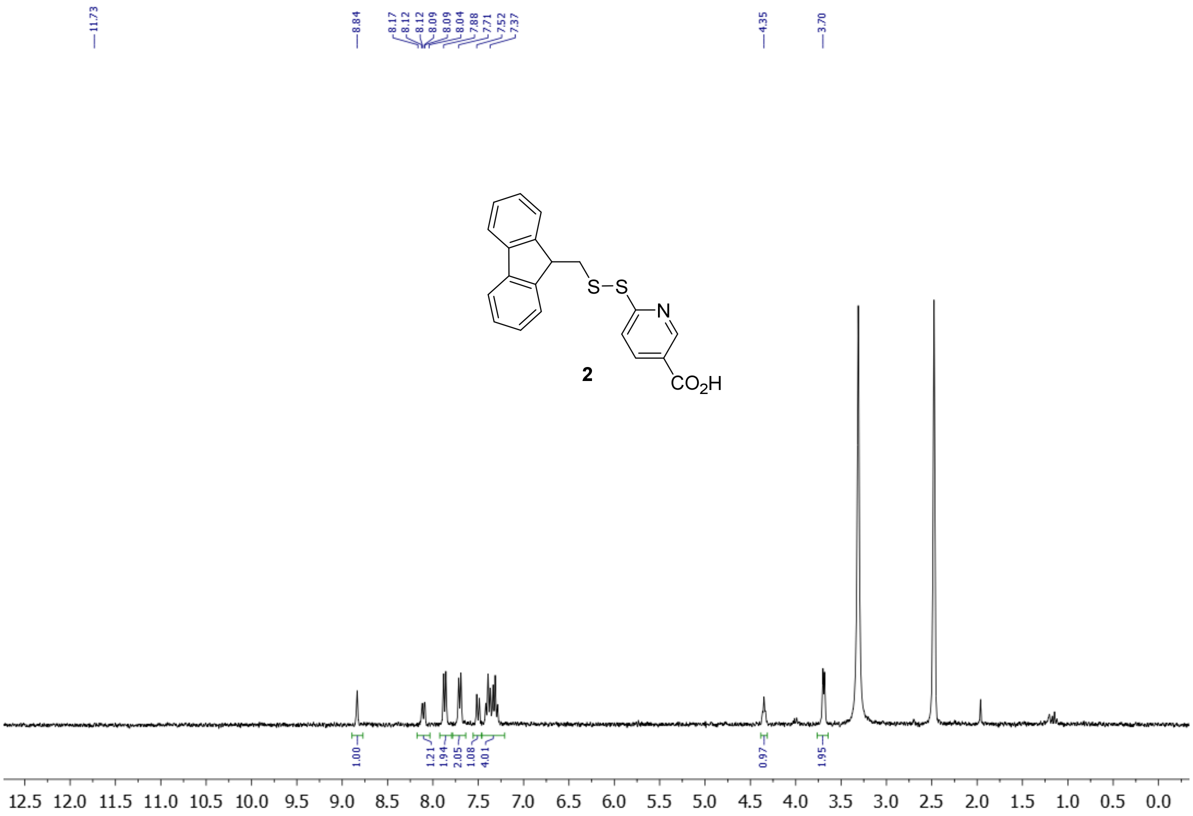
Figure S4. Intact BSA analysis by Maldi-TOF/TOF (Bruker UltrafleXtreme, Billerica, MA) of (A) non-reduced BSA, (B) BSA-SFm **15**, (C) BSA-S(CH_2CONH_2) **18**.



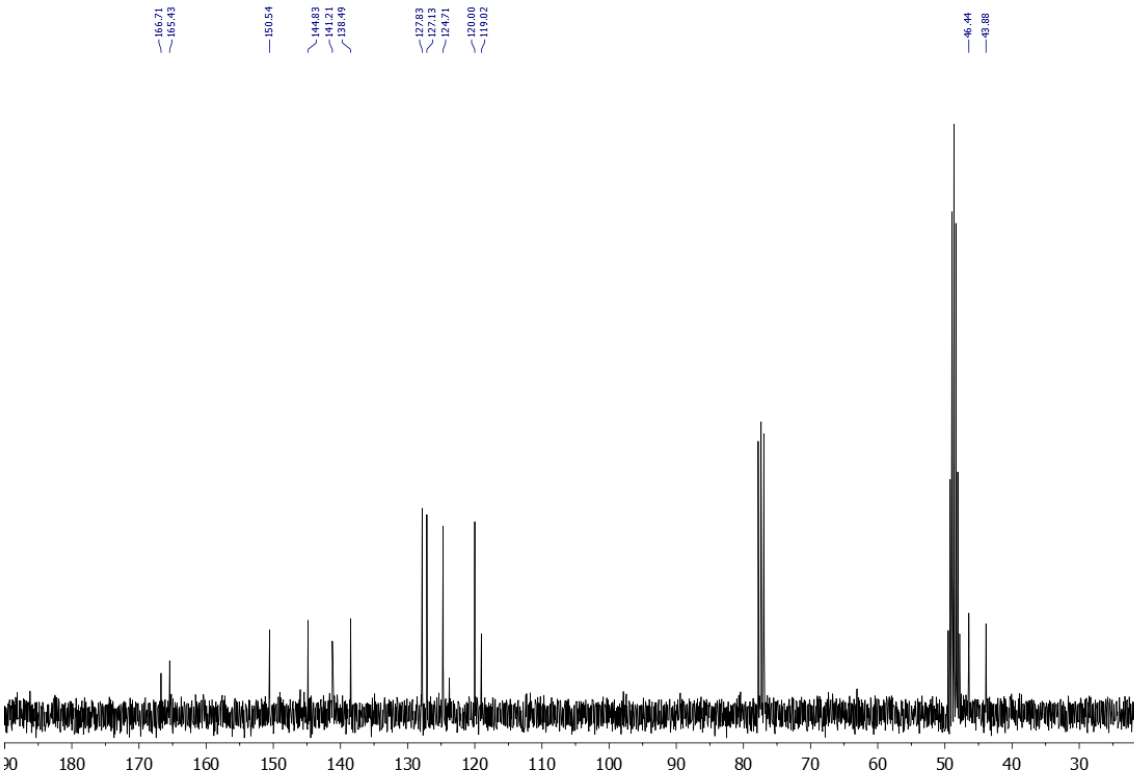
300MHz ^{1}H NMR for FmSSPy **1** in $CDCl_3$ (ppm)



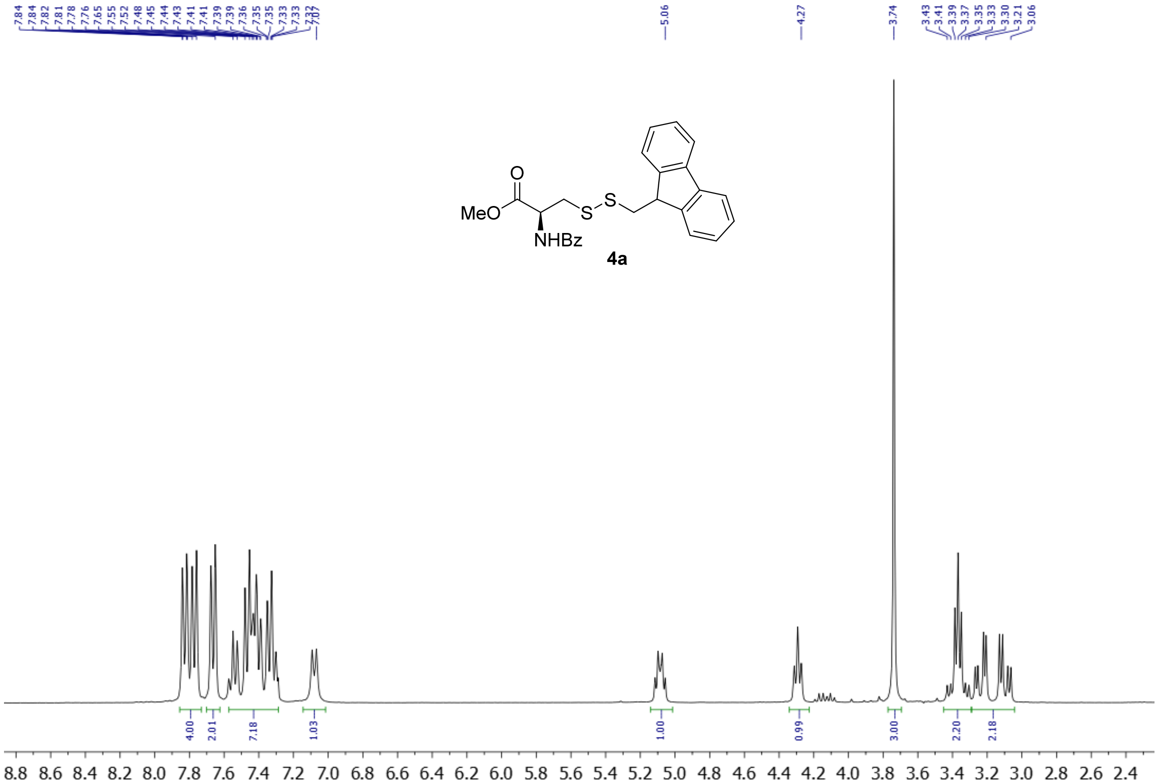
75MHz ^{13}C NMR for FmSSPy **1** in $CDCl_3$ (ppm)



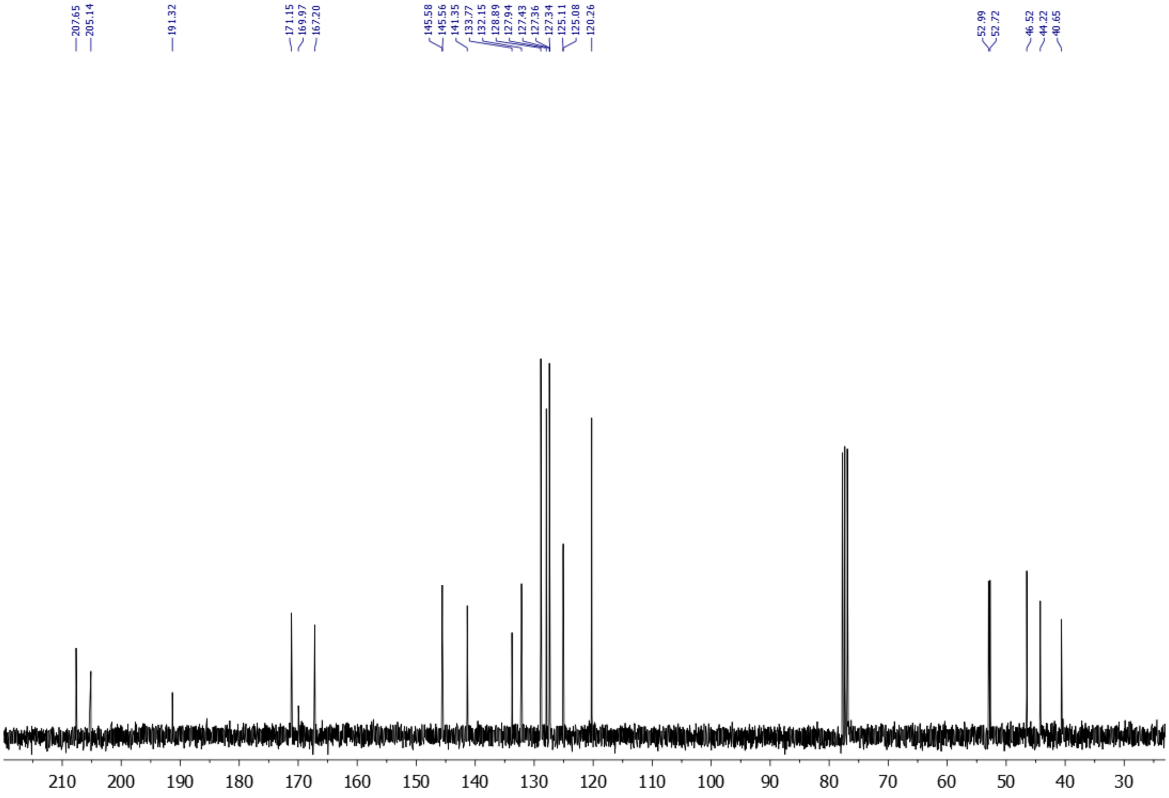
300MHz ^{1}H NMR for FmSSPy-A **2** in DMSO-d_6 (ppm)



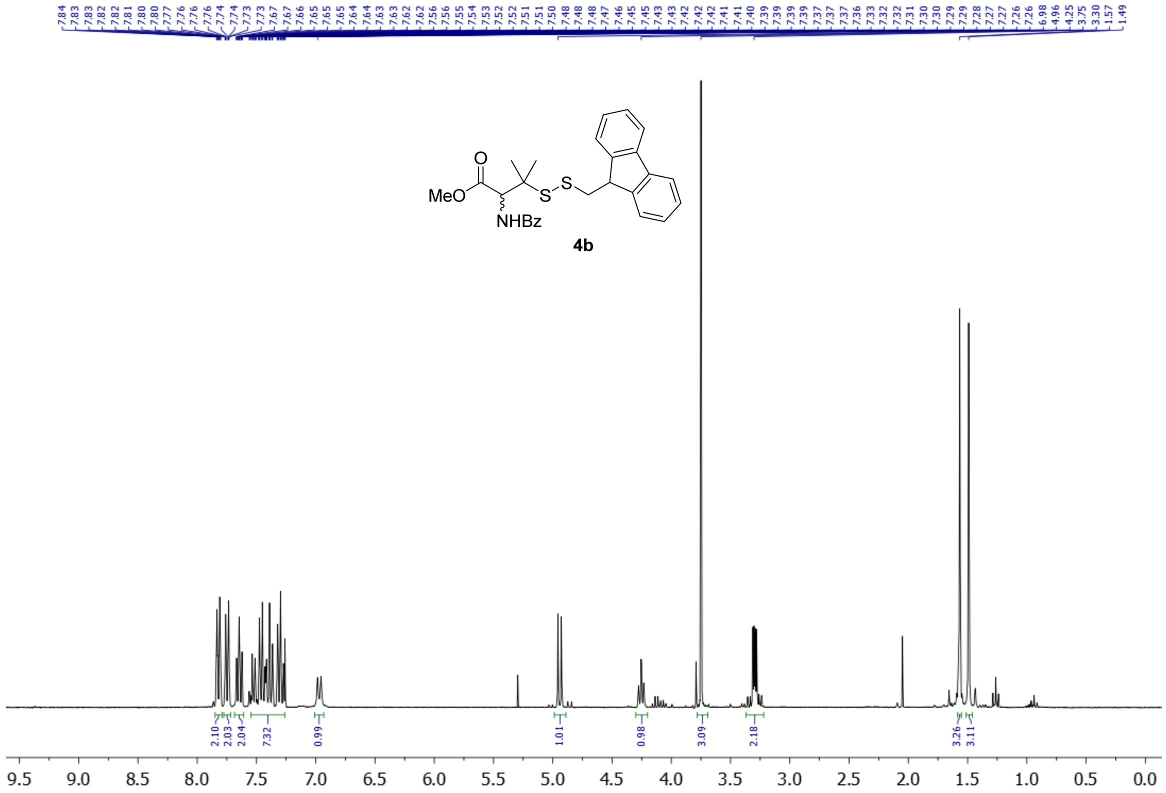
75MHz ^{13}C NMR for FmSSPy-A **2** in $CDCl_3$ + MeOH-d_3 (ppm)



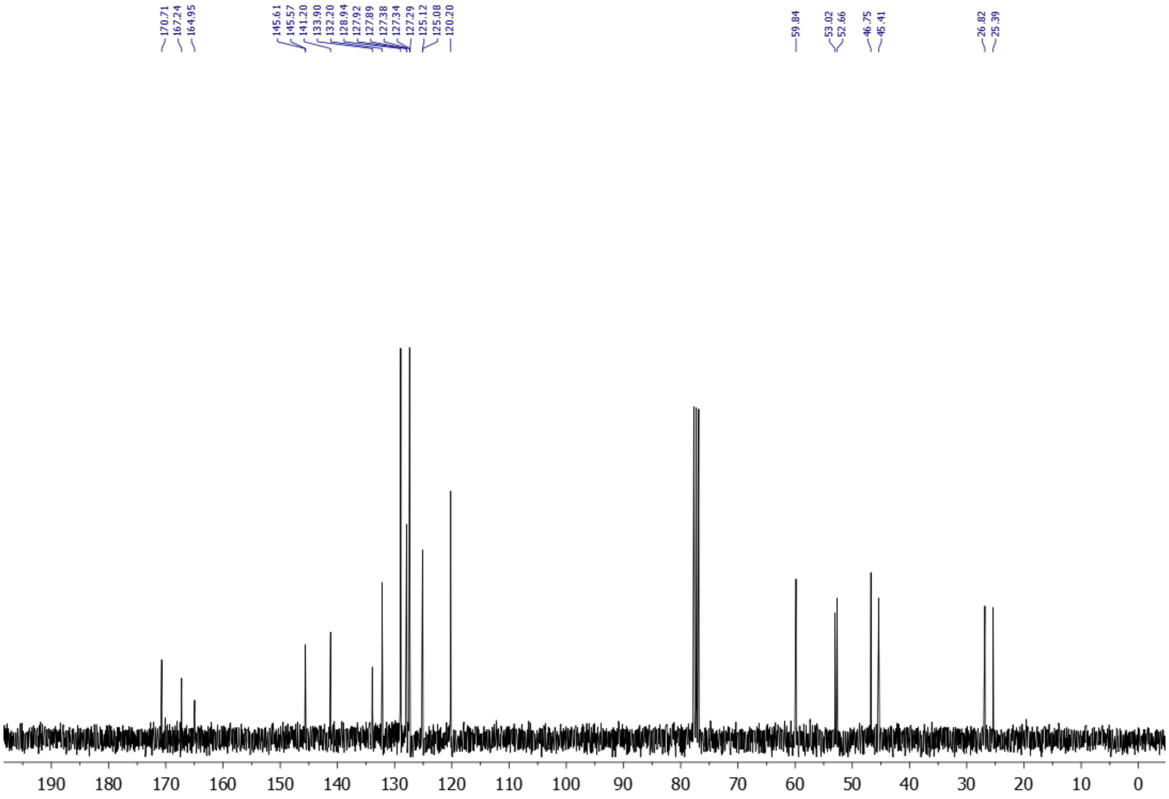
300MHz ^{1}H NMR for Bz-Cys[SSFm]-OMe **4a** in CDCl$_3$ (ppm)



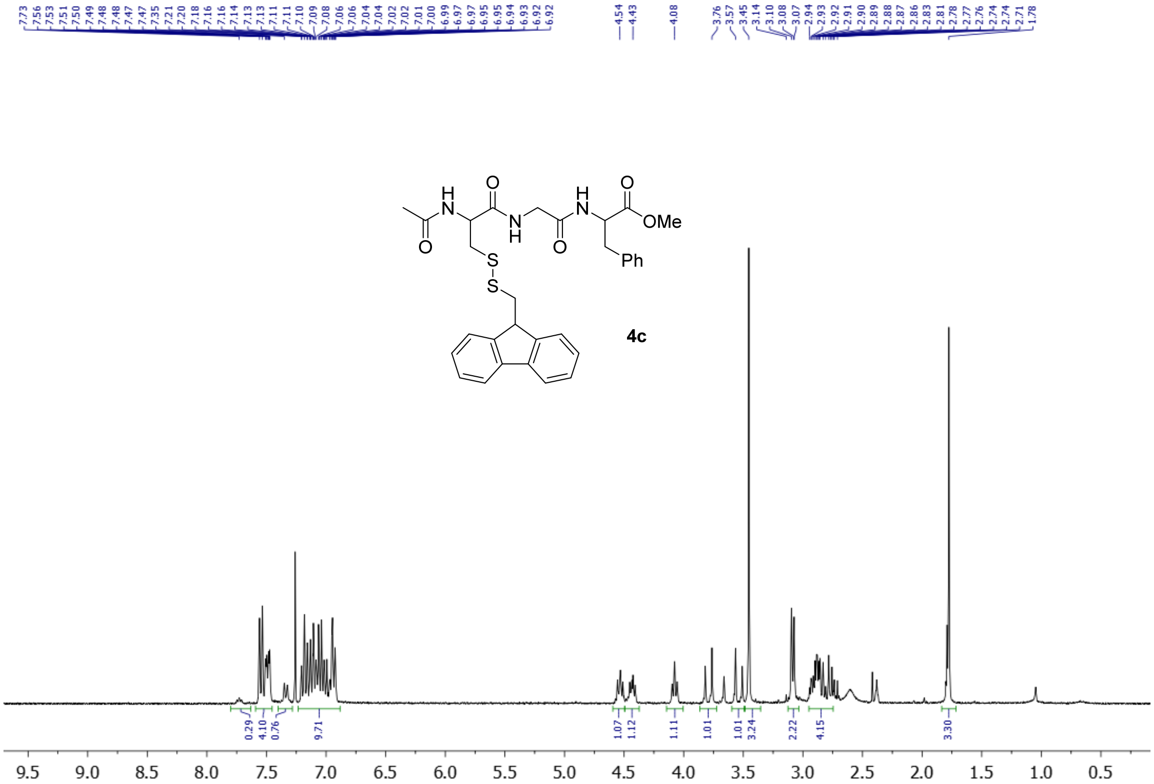
75MHz ^{13}C NMR for Bz-Cys[SSFm]-OMe **4a** in CDCl$_3$ (ppm)



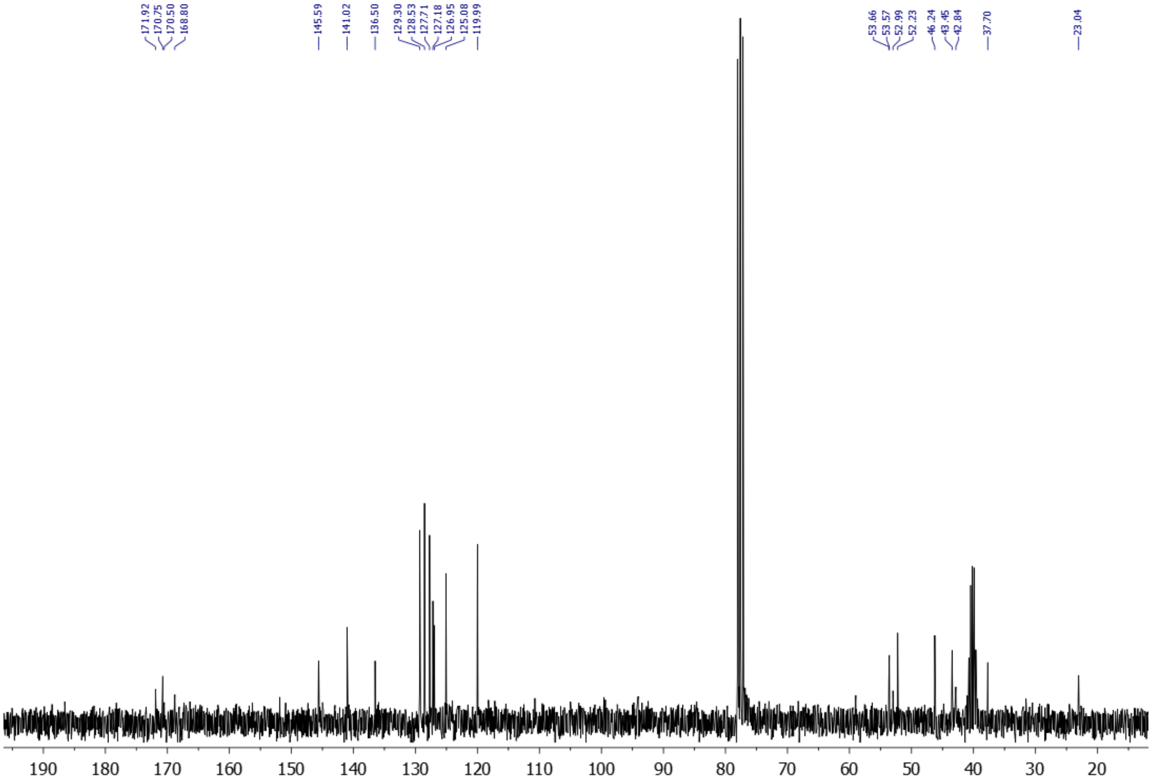
300MHz ^{1}H NMR for Bz-DL-penicillamine[SSFm]-OMe **4b** in CDCl$_3$ (ppm)



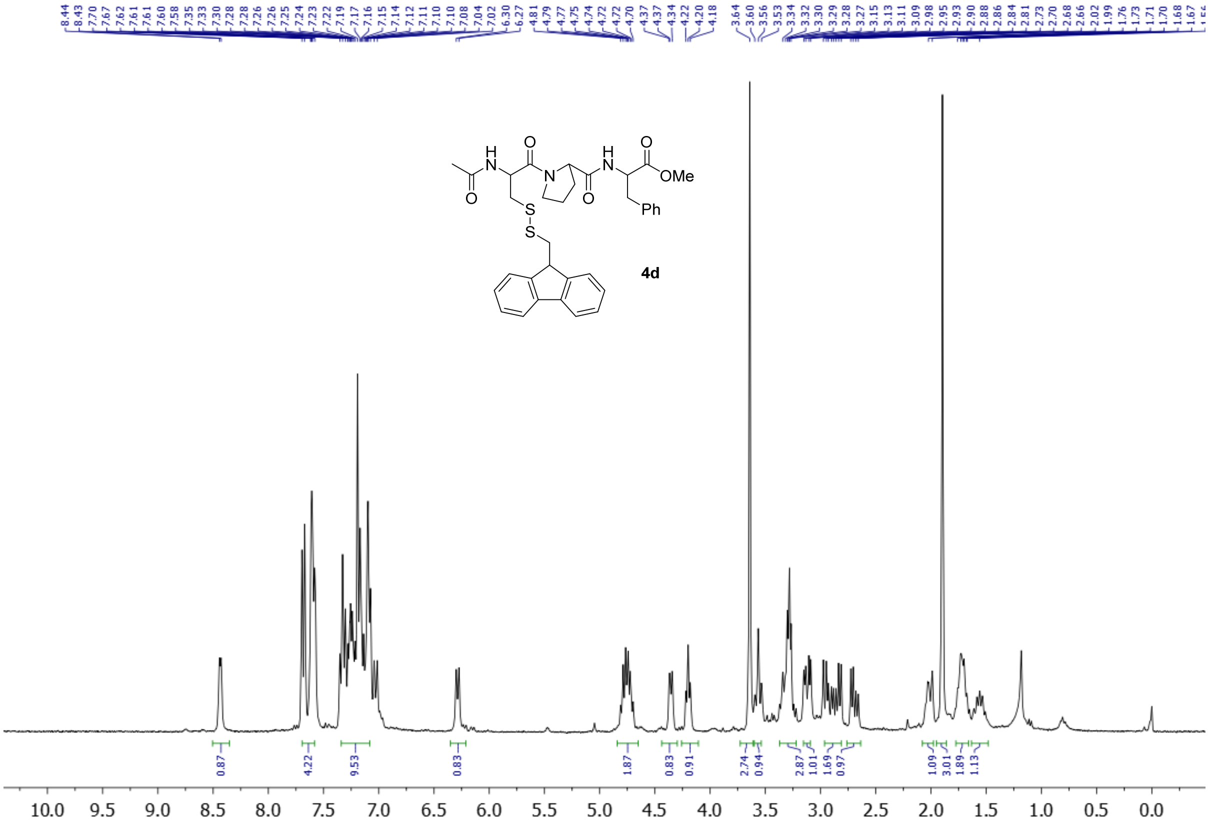
75MHz ^{13}C NMR for Bz-DL-penicillamine[SSFm]-OMe **4b** in CDCl$_3$ (ppm)



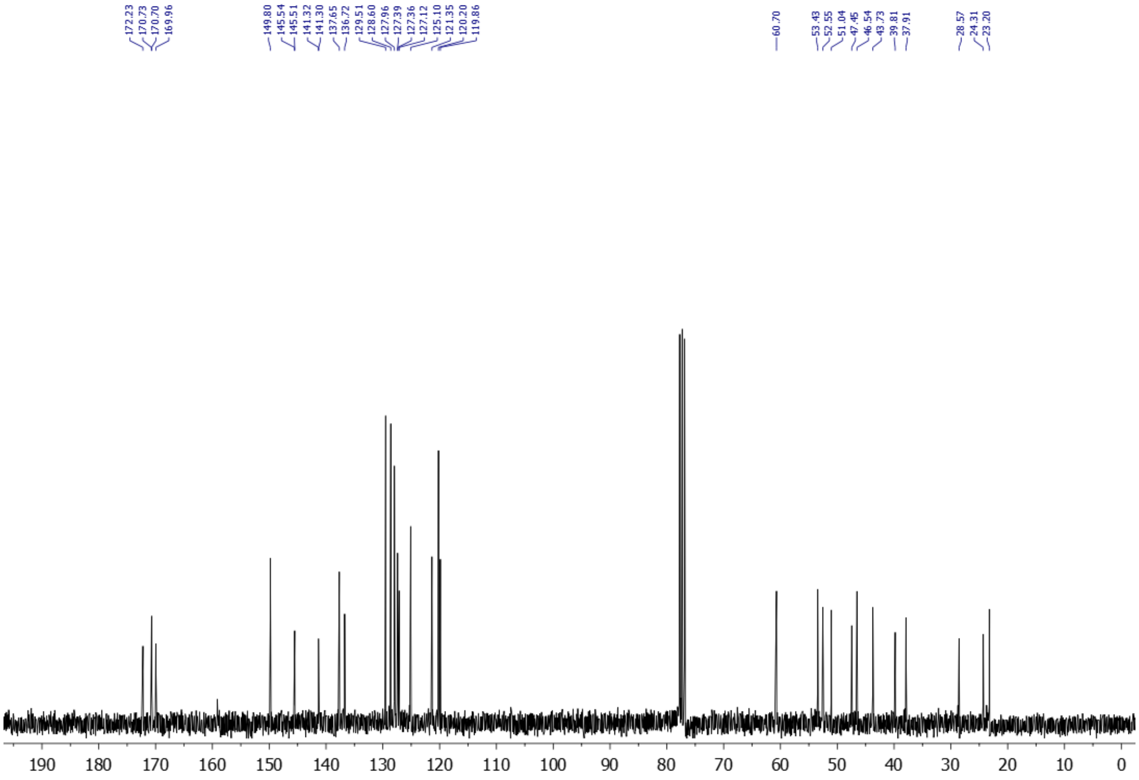
300MHz ^{1}H NMR for Ac-Cys[SSFm]-Gly-Phe-OMe **4c** in $CDCl_3$ (ppm)



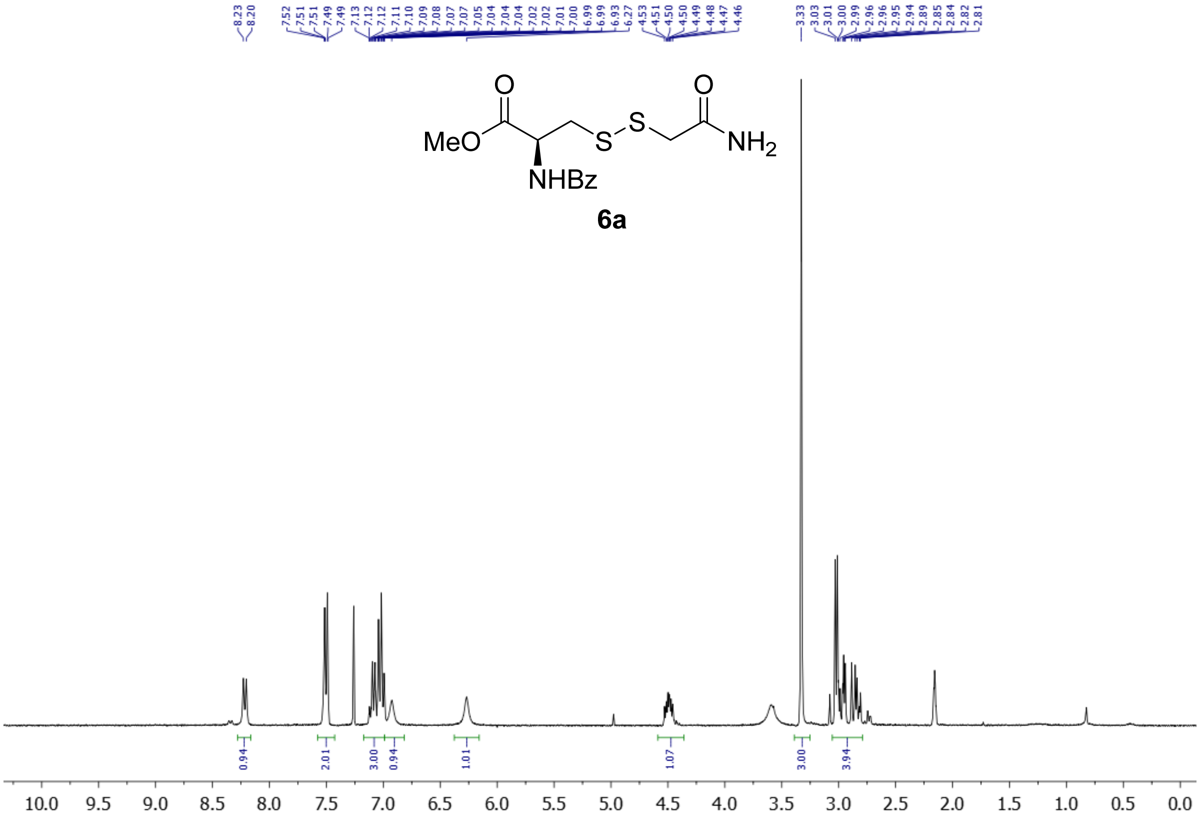
75MHz ^{13}C NMR for Ac-Cys[SSFm]-Gly-Phe-OMe **4c** in $CDCl_3$ + MeOH-d_3 (ppm)



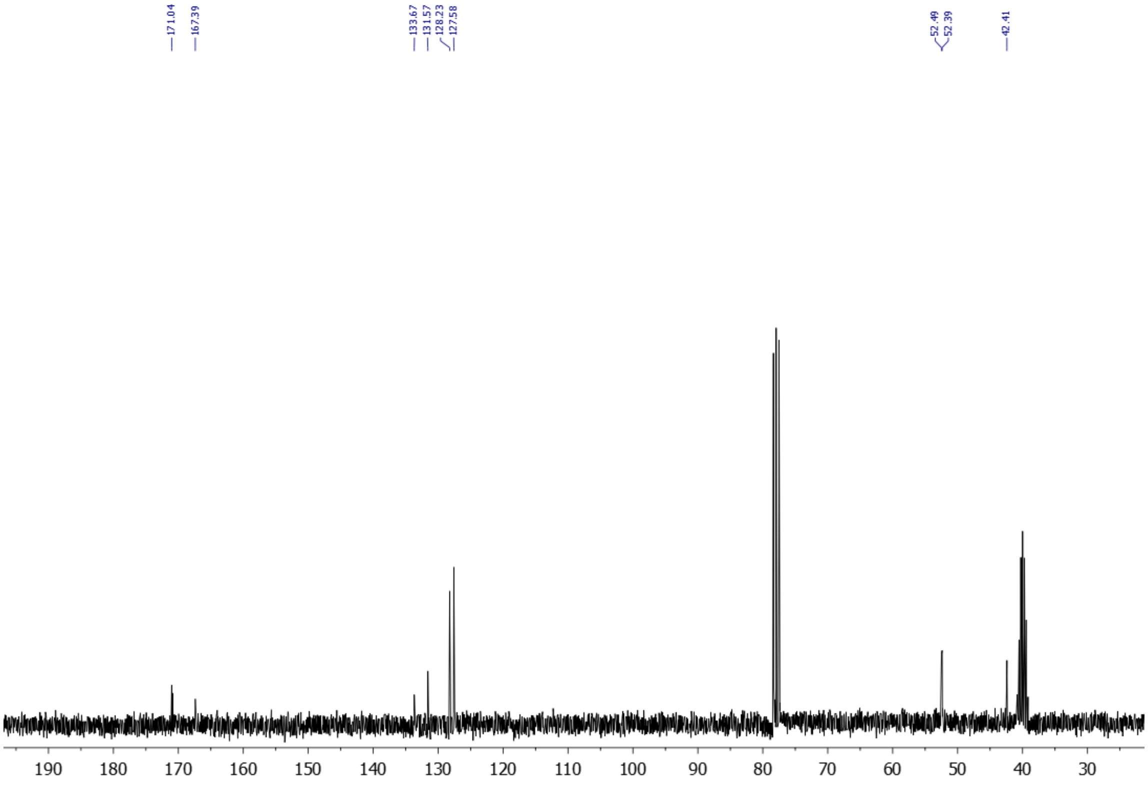
300MHz ^{1}H NMR for Ac-Cys[SSFm]-Pro-Phe-OMe **4d** in $CDCl_3$ (ppm)



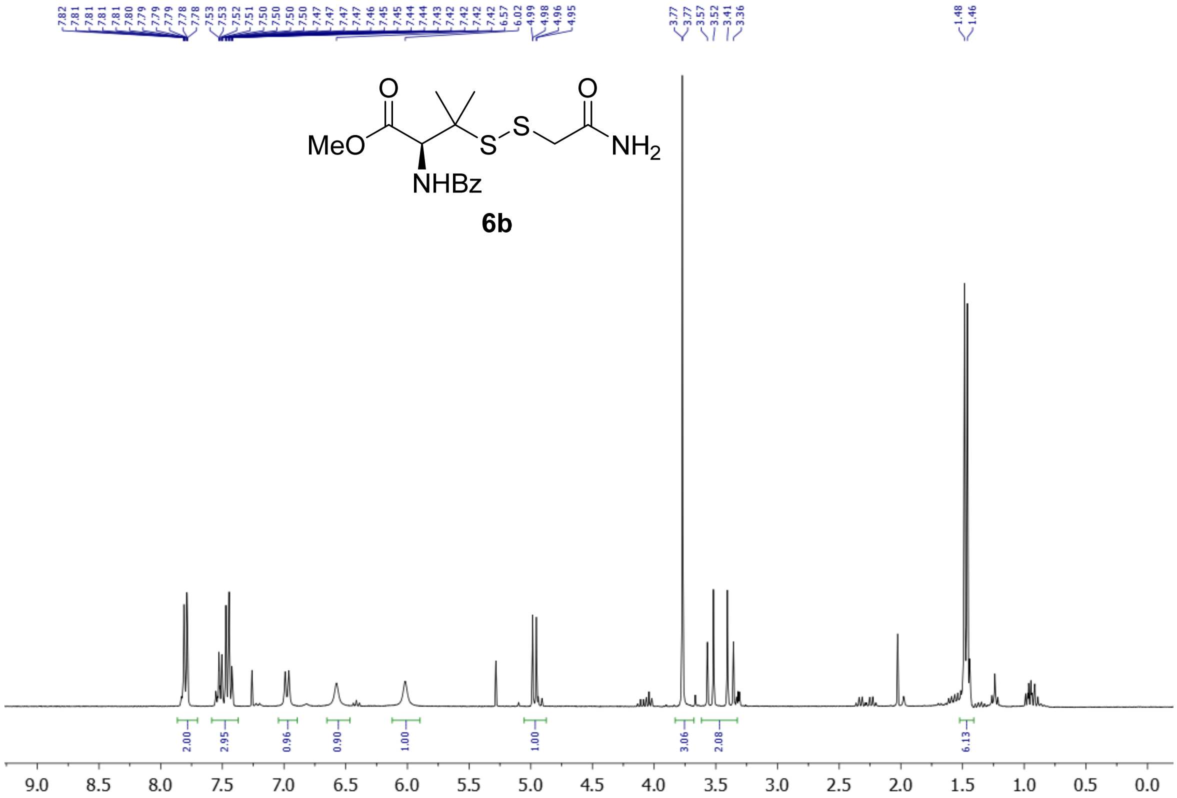
75MHz ^{13}C NMR for Ac-Cys[SSFm]-Gly-Phe-OMe **4d** in $CDCl_3$ (ppm)



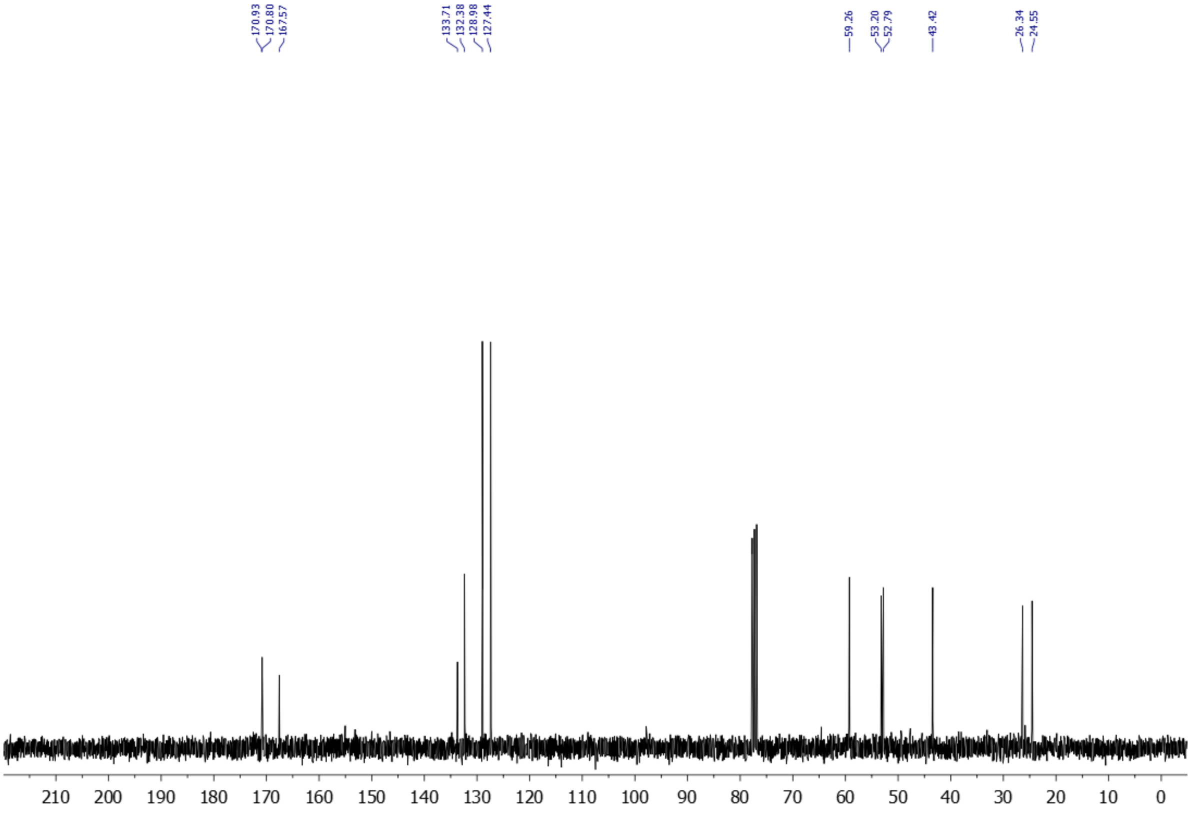
300MHz ^{1}H NMR for Bz-Cys[SS(CH$_2$CONH$_2$)]-OMe **6a** in CDCl$_3$ + DMSO-*d*$_6$ (ppm)



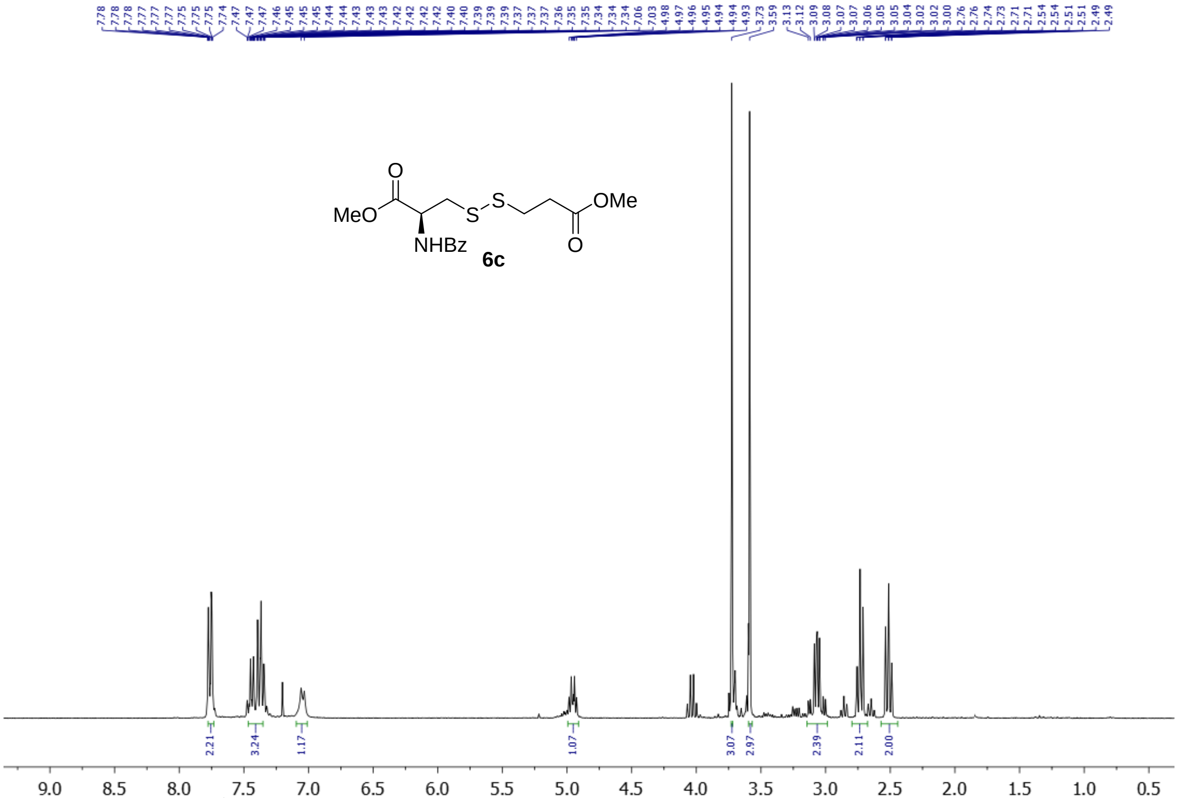
75MHz ^{13}C NMR for Bz-Cys[SS(CH$_2$CONH$_2$)]-OMe **6a** in CDCl$_3$ + DMSO-*d*$_6$ (ppm)



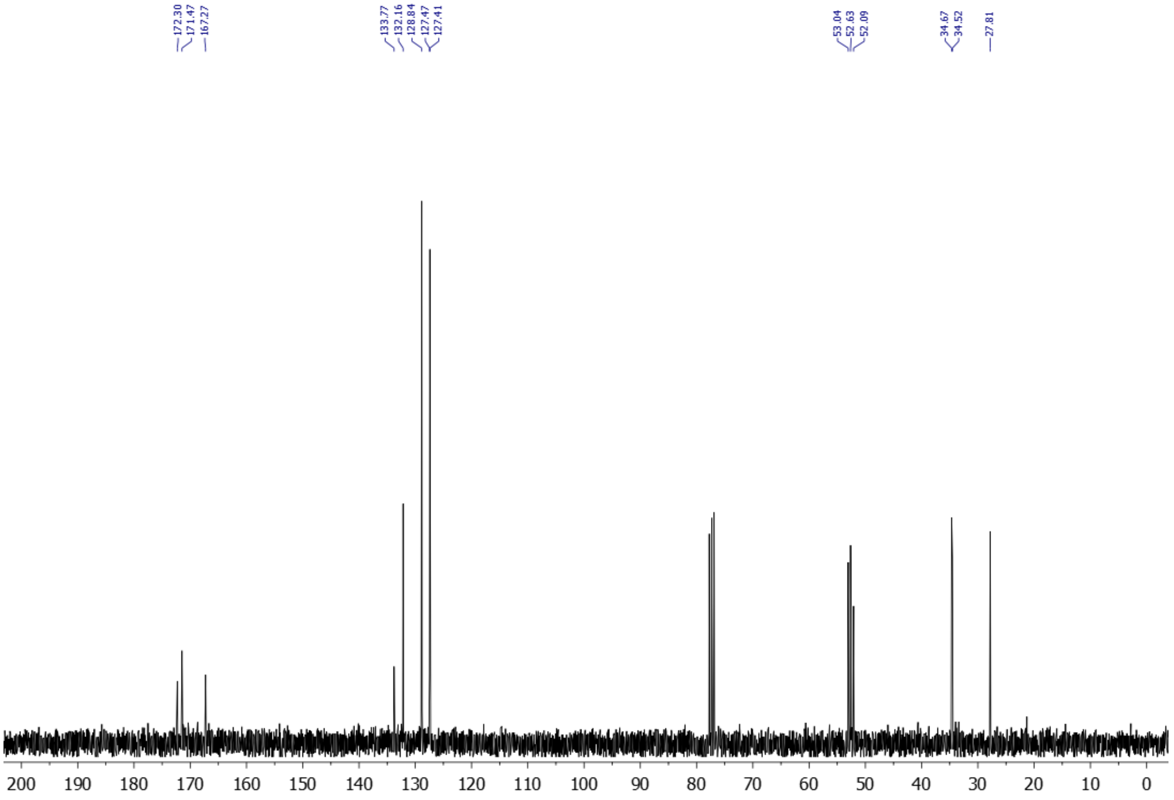
300MHz ^{1}H NMR for Bz-DL-penicillamine[SS(CH_2CONH_2)]-OMe **6b** in $CDCl_3$ (ppm)



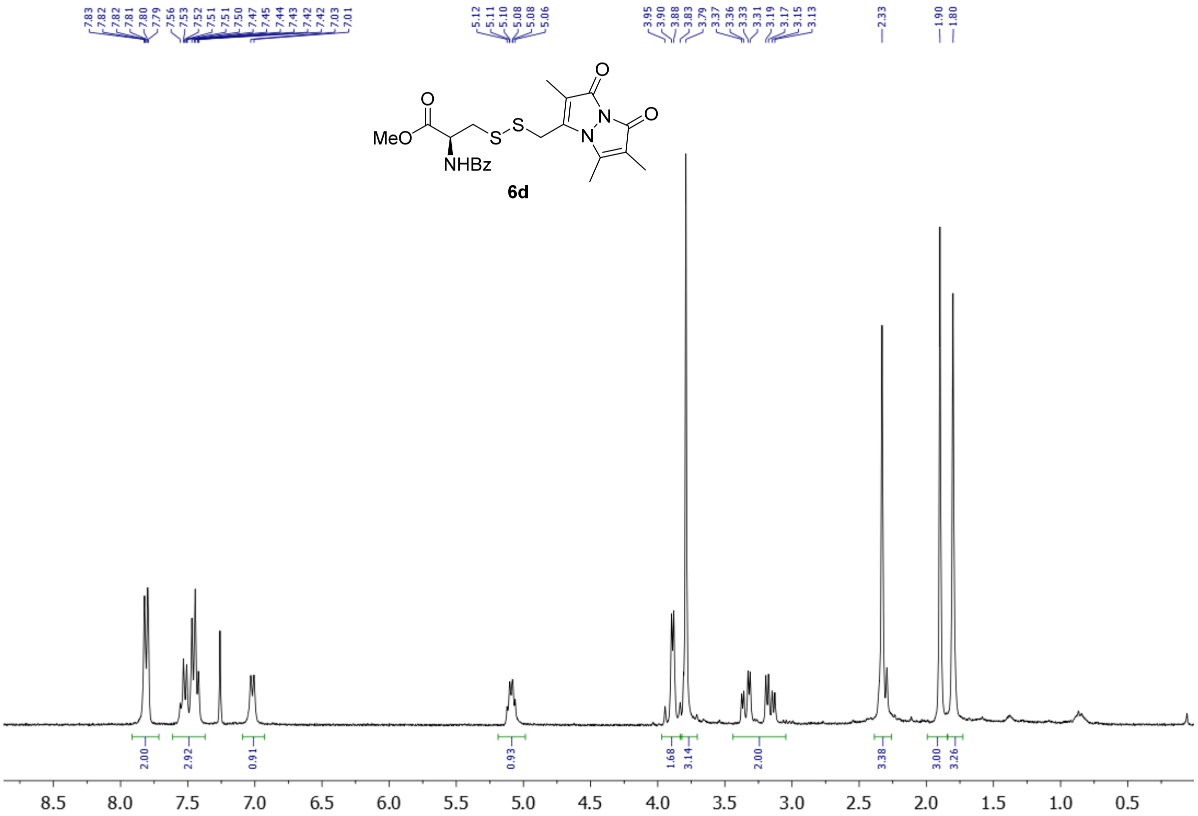
75MHz ^{13}C NMR for Bz-DL-penicillamine[SS(CH_2CONH_2)]-OMe **6b** in $CDCl_3$ (ppm)



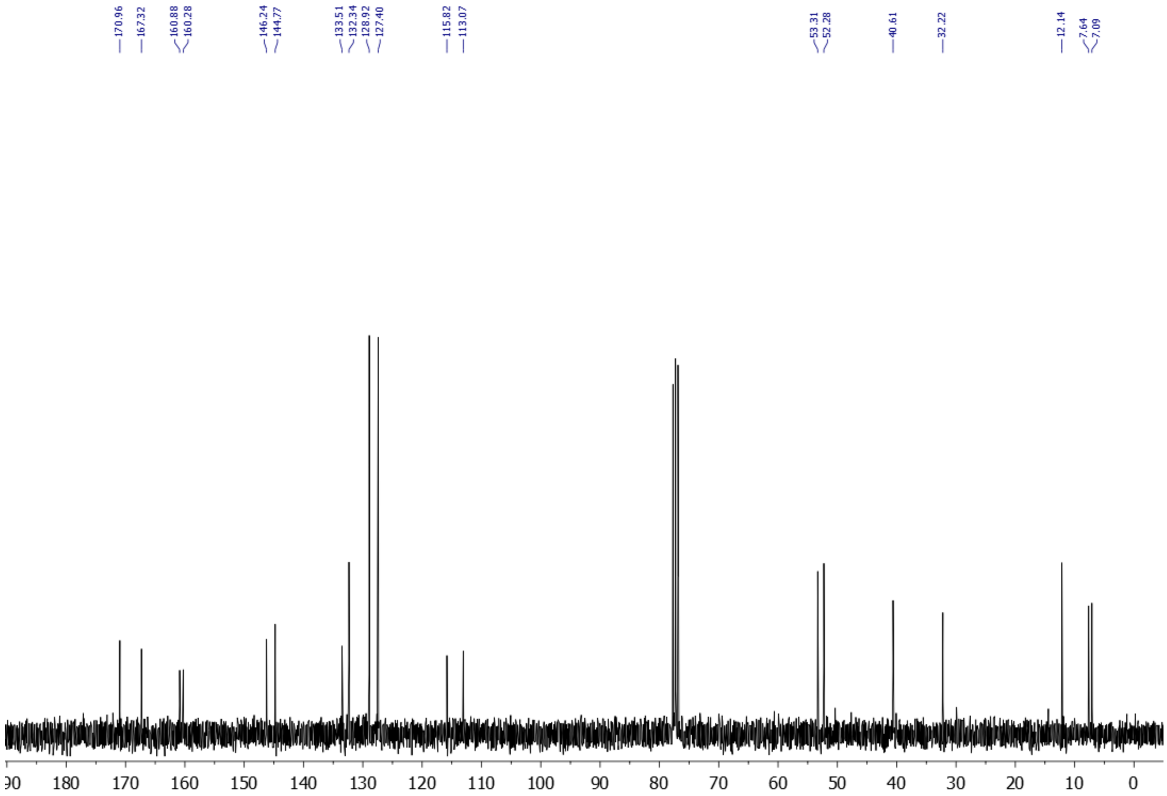
300MHz ^{1}H NMR for Bz-Cys[SS(CH$_2$CH$_2$CO$_2$Me)]-OMe **6c** in CDCl$_3$ (ppm)



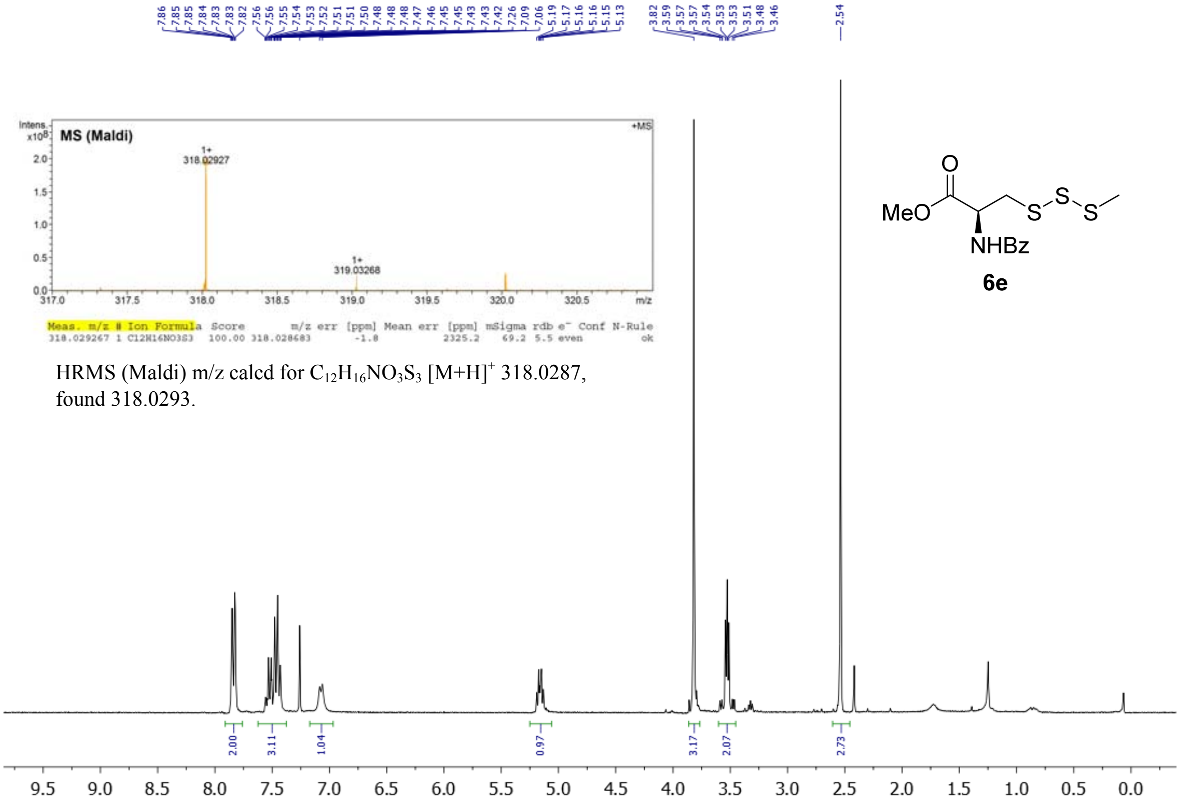
75MHz ^{13}C NMR for Bz-Cys[SS(CH$_2$CH$_2$CO$_2$Me)]-OMe **6c** in CDCl$_3$ (ppm)



300MHz ^{1}H NMR for Bz-Cys[SS(mB)]-OMe **6d** in $CDCl_3$ (ppm)



75MHz ^{13}C NMR for Bz-Cys[SS(mB)]-OMe **6d** in $CDCl_3$ (ppm)

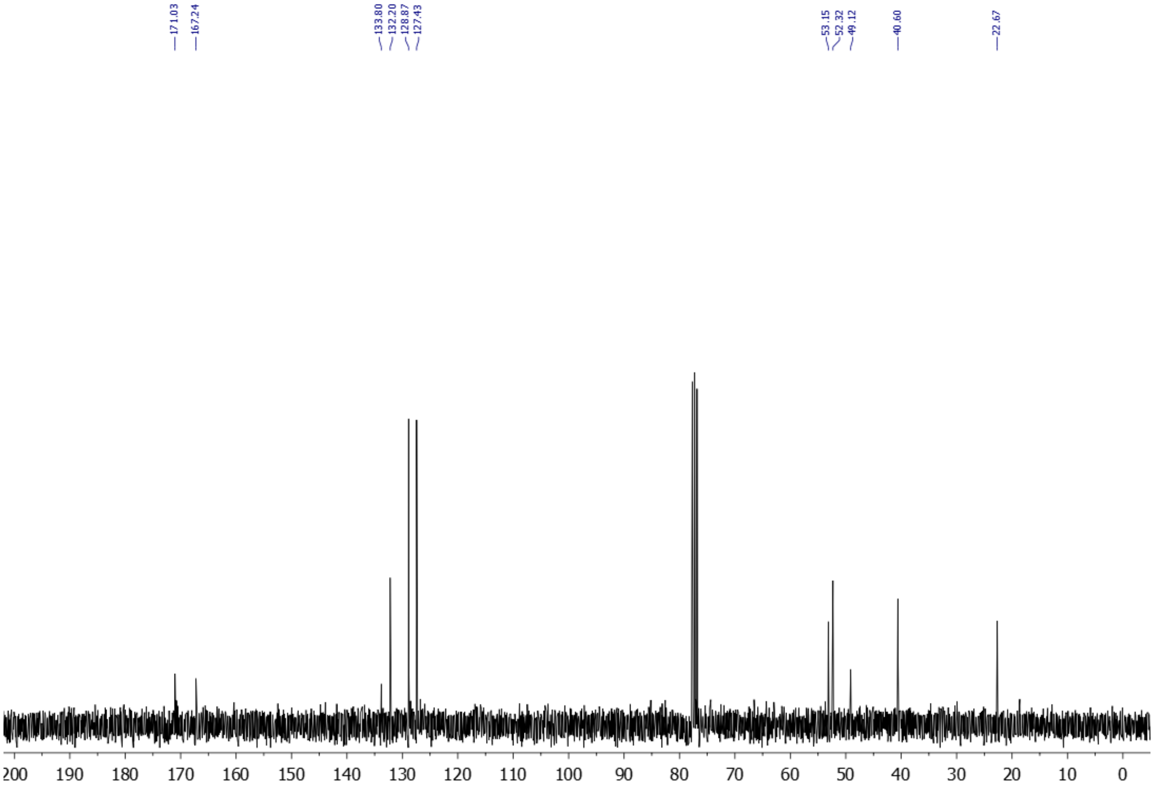


6e

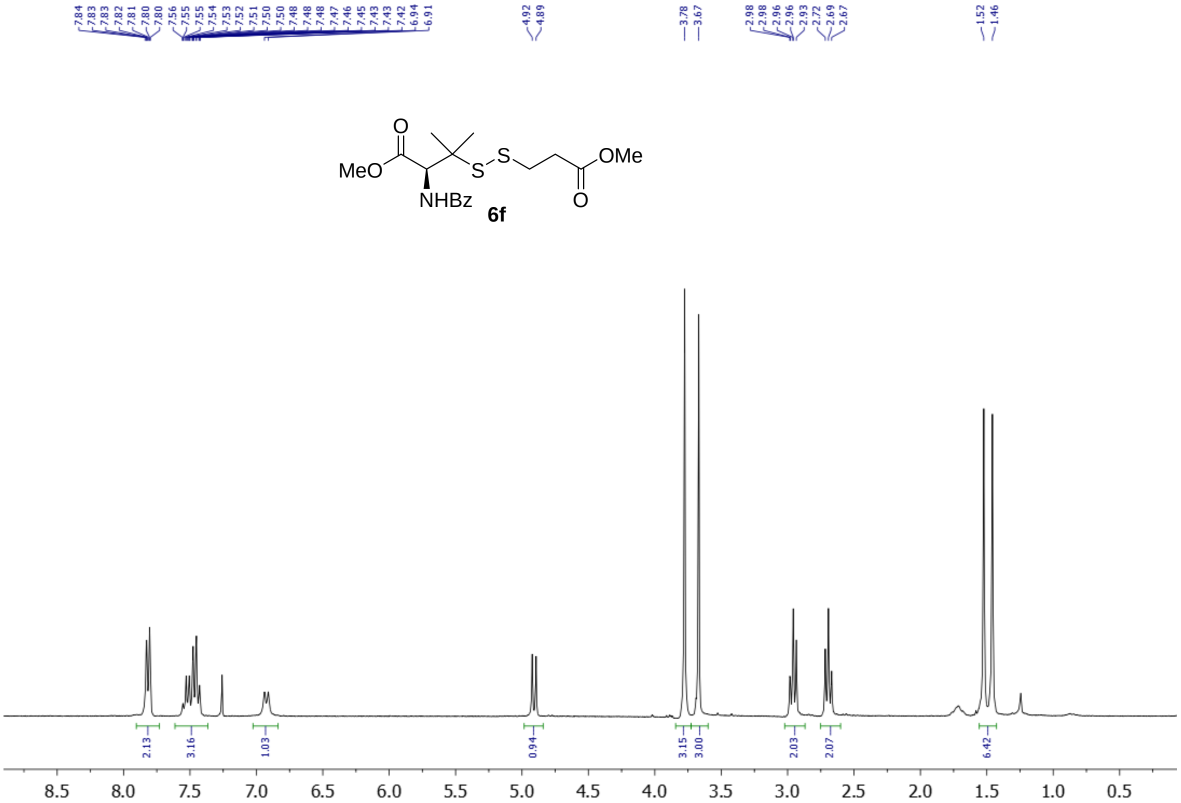
```
Meas. m/z # Ion Formula  Score       m/z err [ppm] Mean err [ppm] mSigma rdb e⁻ Conf N-Rule
318.029267 1 C12H16NO3S3  100.00 318.028683     -1.8           2325.2    69.2 5.5 even        ok
```

HRMS (Maldi) m/z calcd for $C_{12}H_{16}NO_3S_3$ $[M+H]^+$ 318.0287, found 318.0293.

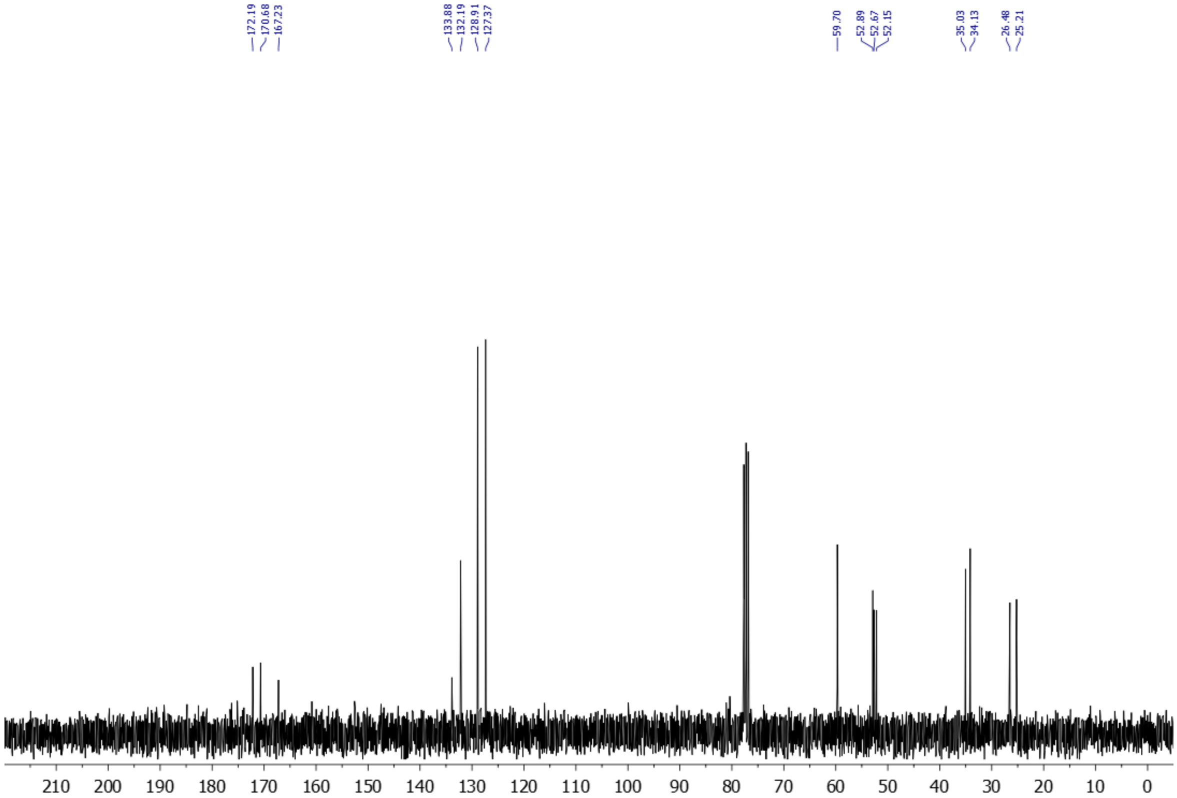
300MHz ^1H NMR for Bz-Cys[SSSMe]-OMe **6e** in $CDCl_3$ (ppm)



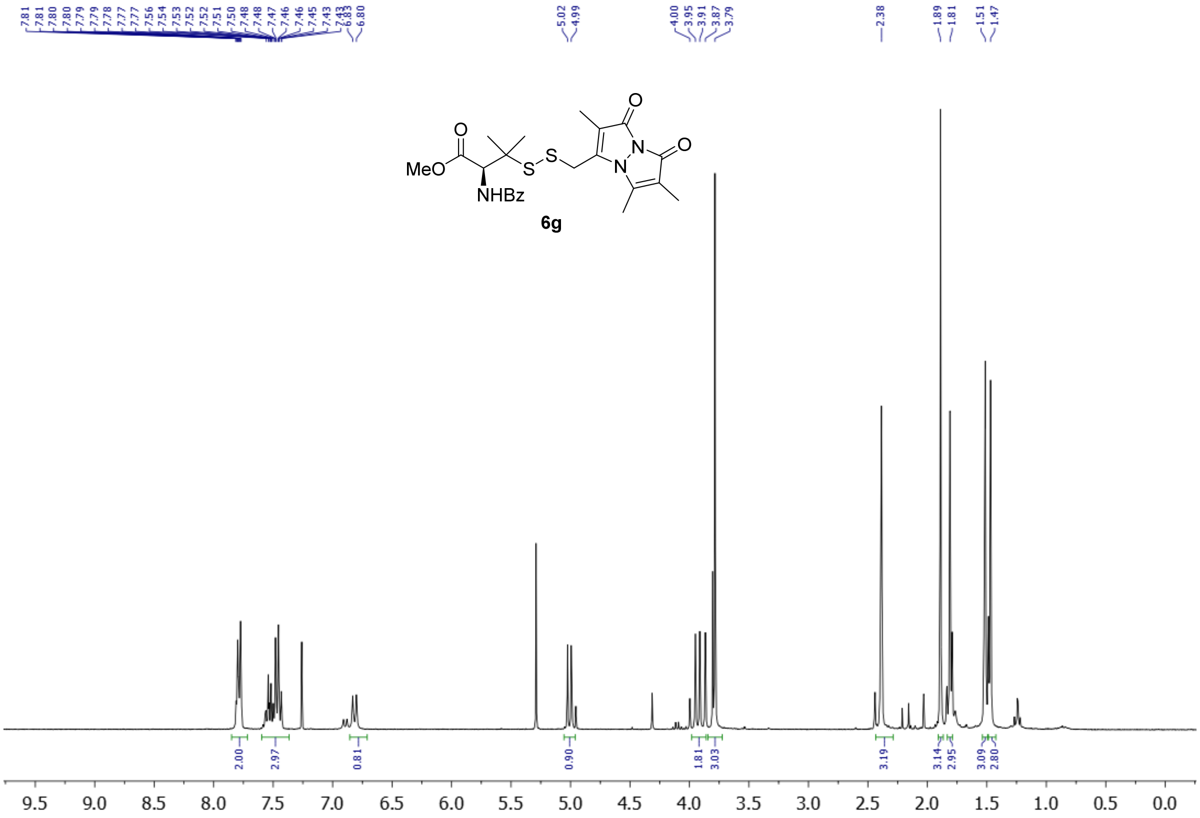
75MHz ^{13}C NMR for Bz-Cys[SSSMe]-OMe **6e** in $CDCl_3$ (ppm)



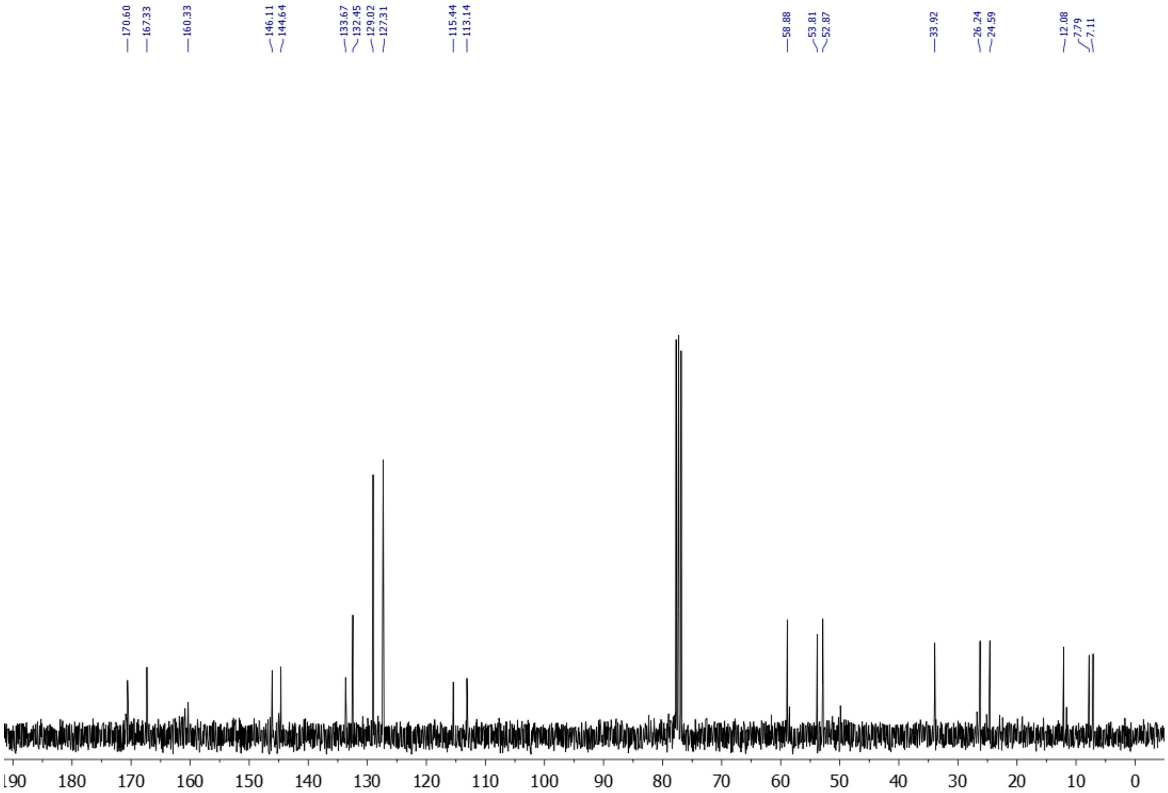
300MHz ^{1}H NMR for Bz-DL-penicillamine[SS($CH_2CH_2CO_2Me$)]-OMe **6f** in $CDCl_3$ (ppm)



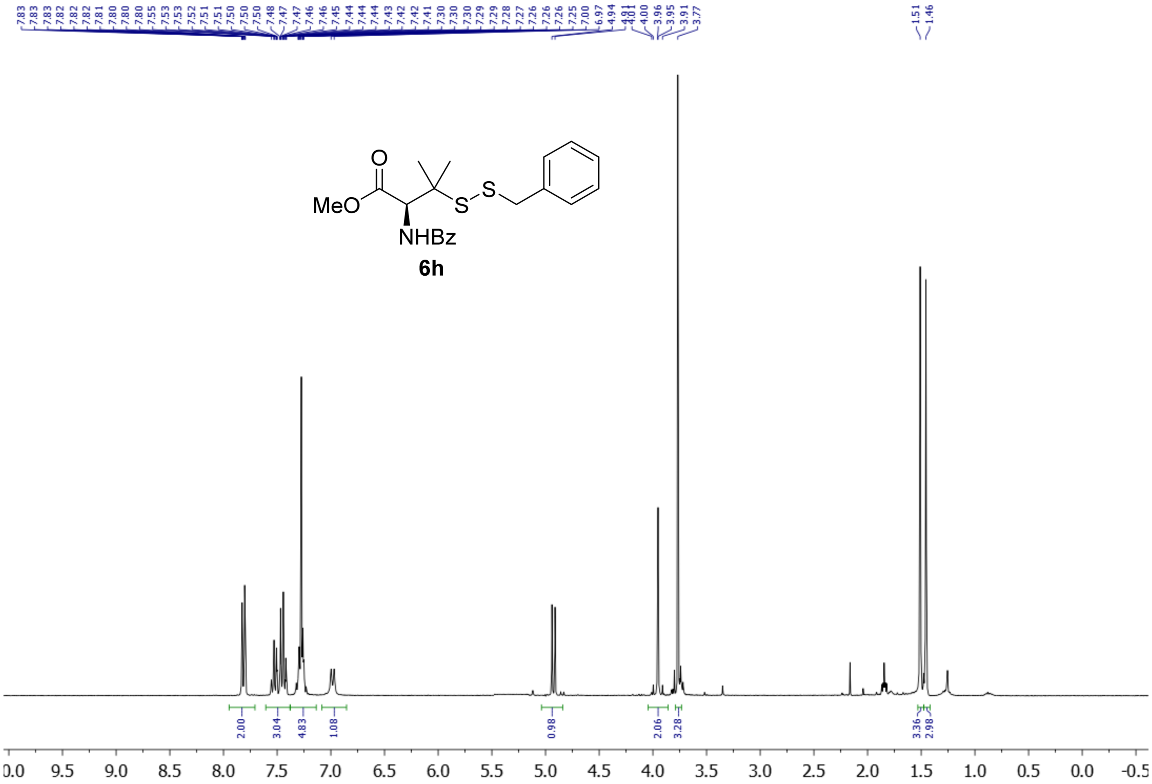
75MHz ^{13}C NMR for Bz-DL-penicillamine[SS($CH_2CH_2CO_2Me$)]-OMe **6f** in $CDCl_3$ (ppm)



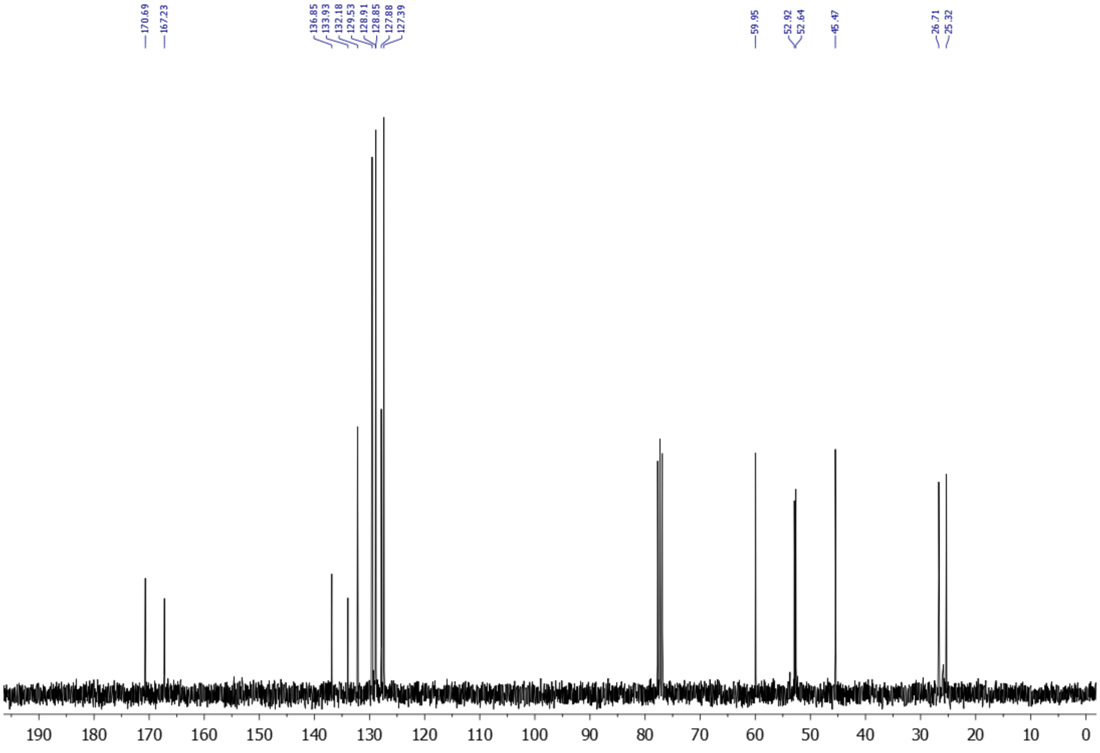
300MHz ^{1}H NMR for Bz-DL-penicillamine[SS(mB)]-OMe **6g** in $CDCl_3$ (ppm)



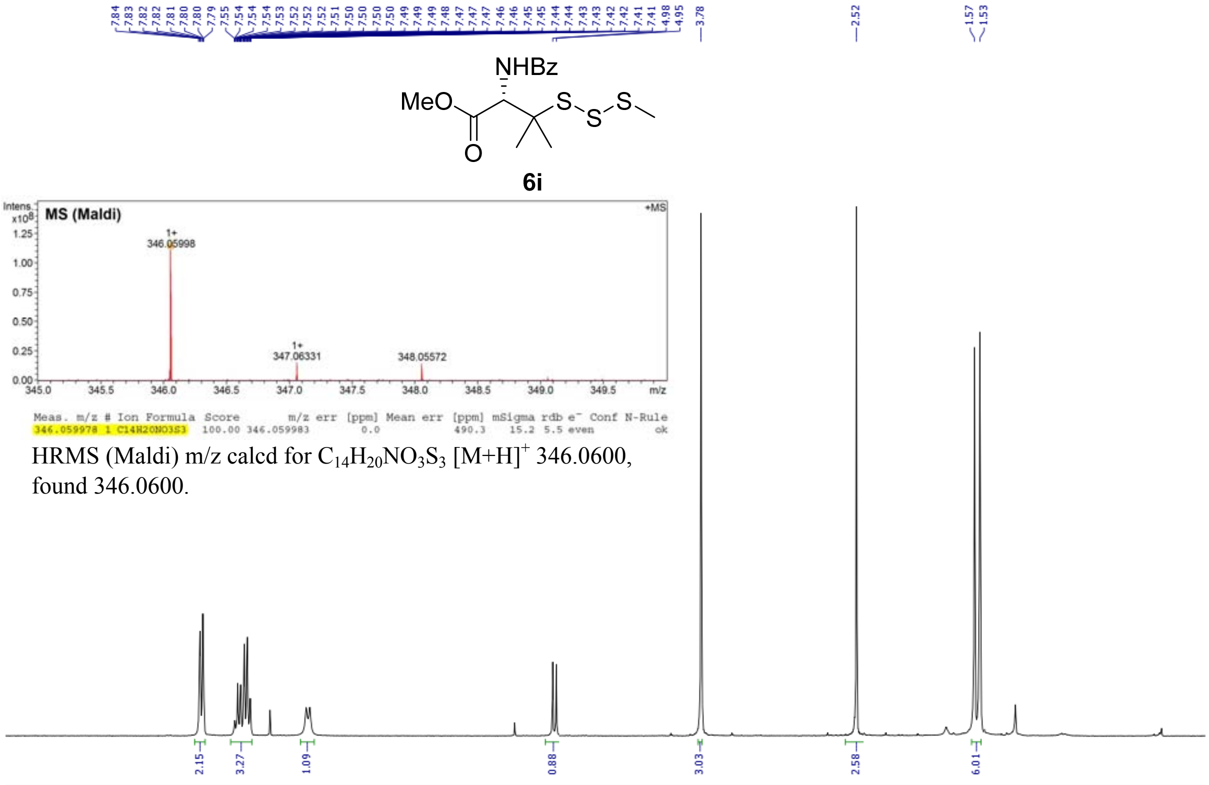
75MHz ^{13}C NMR for Bz-DL-penicillamine[SS(mB)]-OMe **6g** in $CDCl_3$ (ppm)



300MHz ^{1}H NMR for Bz-DL-penicillamine[SSBn]-OMe **6h** in $CDCl_3$ (ppm)



75MHz ^{13}C NMR for Bz-DL-penicillamine[SSBn]-OMe **6h** in $CDCl_3$ (ppm)

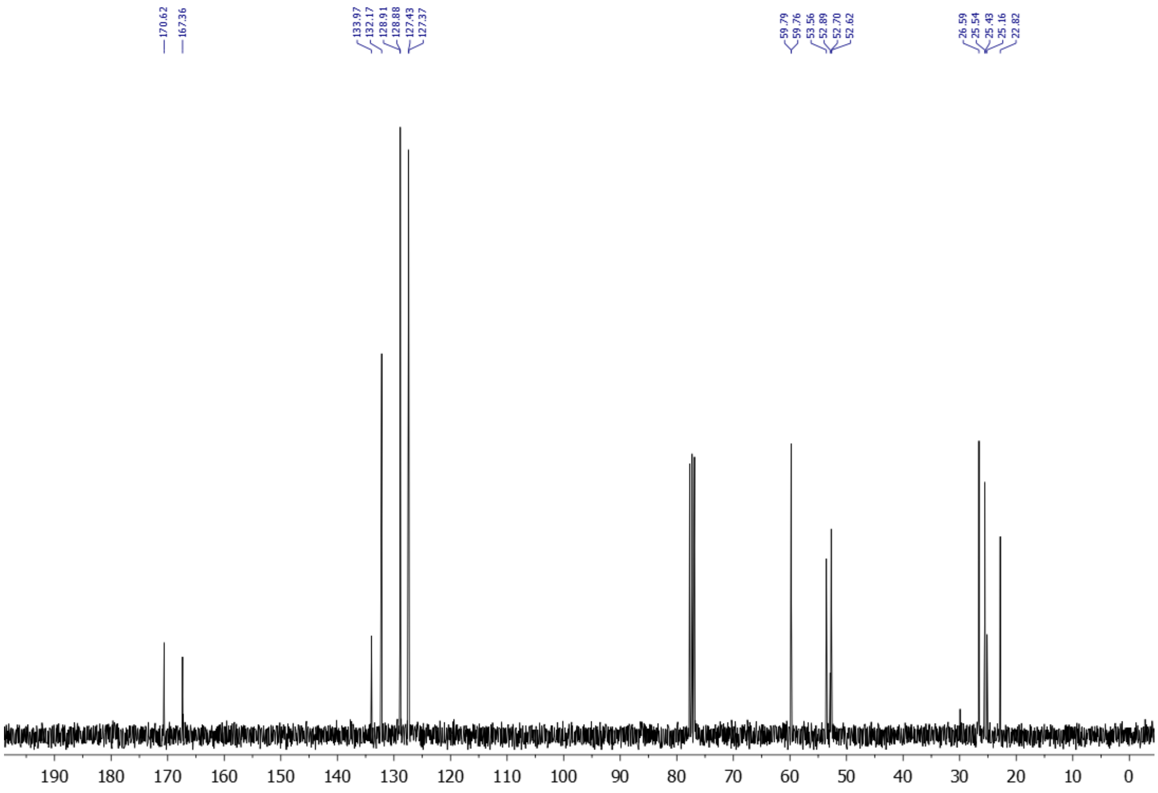


6i

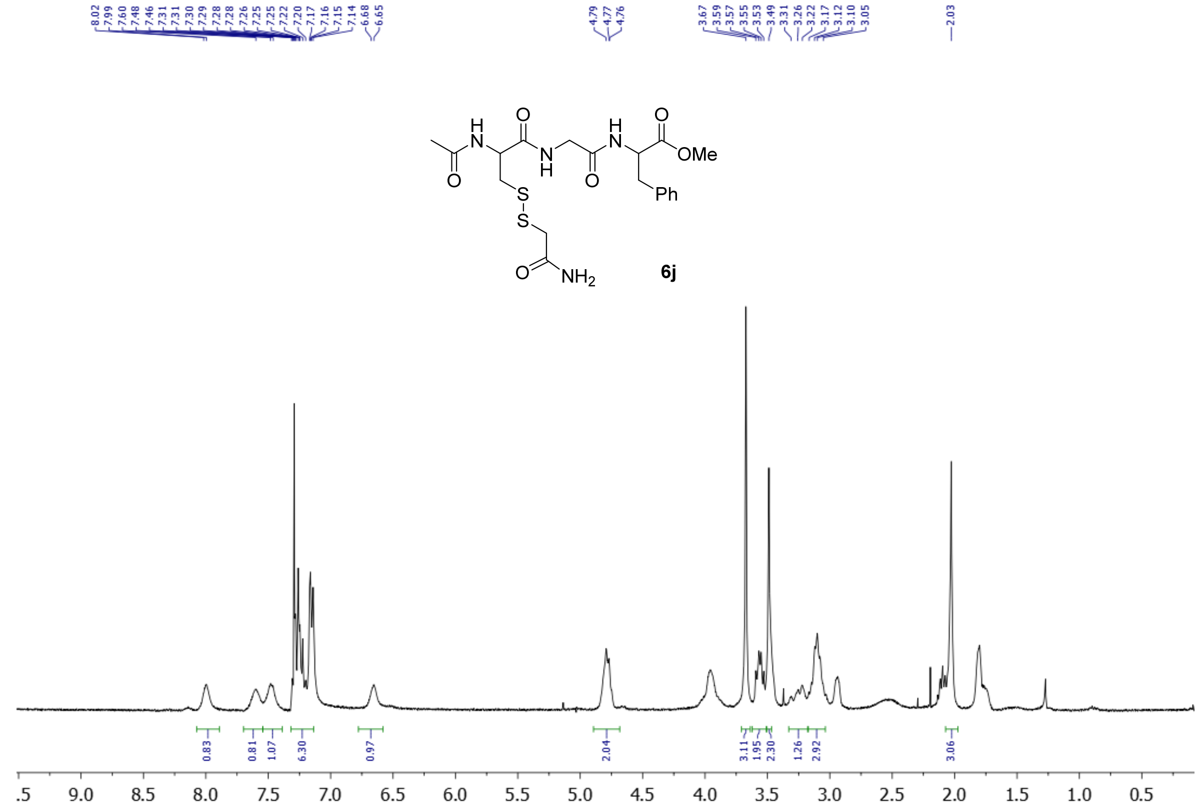
```
Meas. m/z # Ion Formula  Score        m/z err [ppm] Mean err [ppm] mSigma rdb e¯ Conf N-Rule
346.059978 1 C14H20NO3S3  100.00 346.059983    0.0                  490.3   15.2  5.5 even      ok
```

HRMS (Maldi) m/z calcd for $C_{14}H_{20}NO_3S_3$ $[M+H]^+$ 346.0600, found 346.0600.

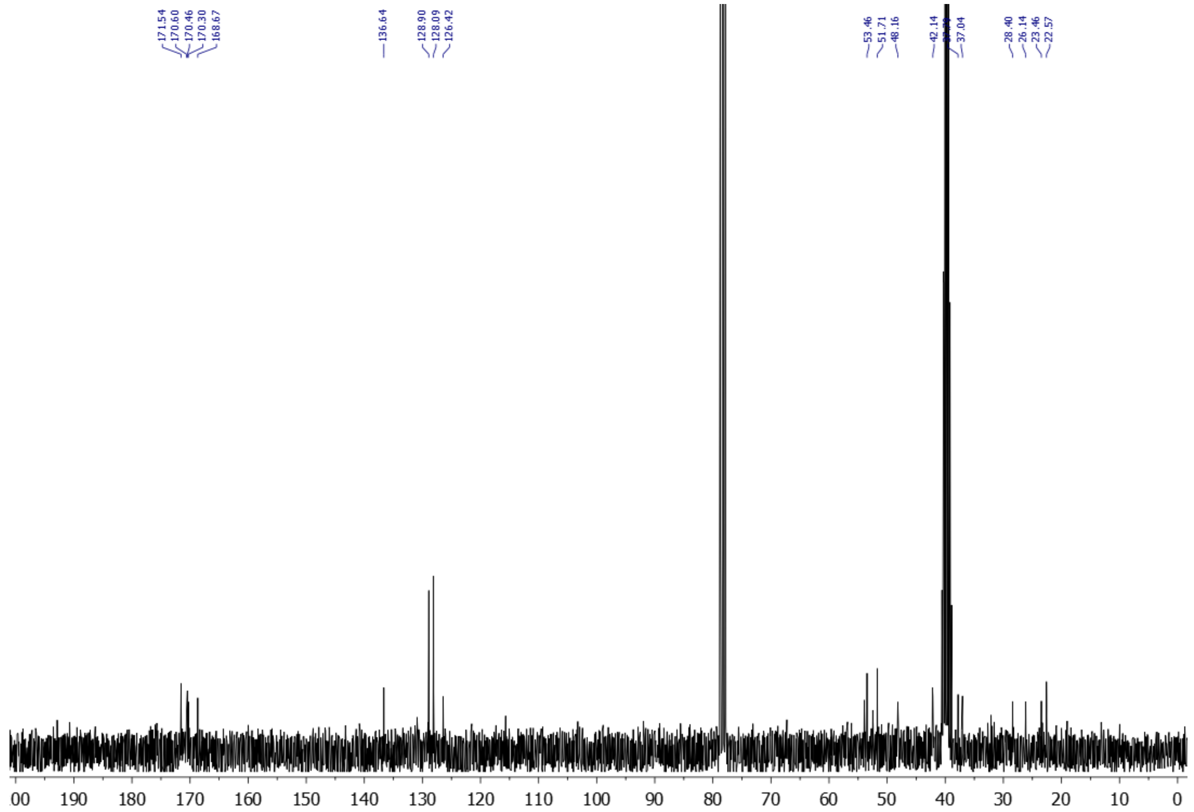
300MHz ^{1}H NMR for Bz-DL-penicillamine[SSSMe]-OMe **6i** in $CDCl_3$



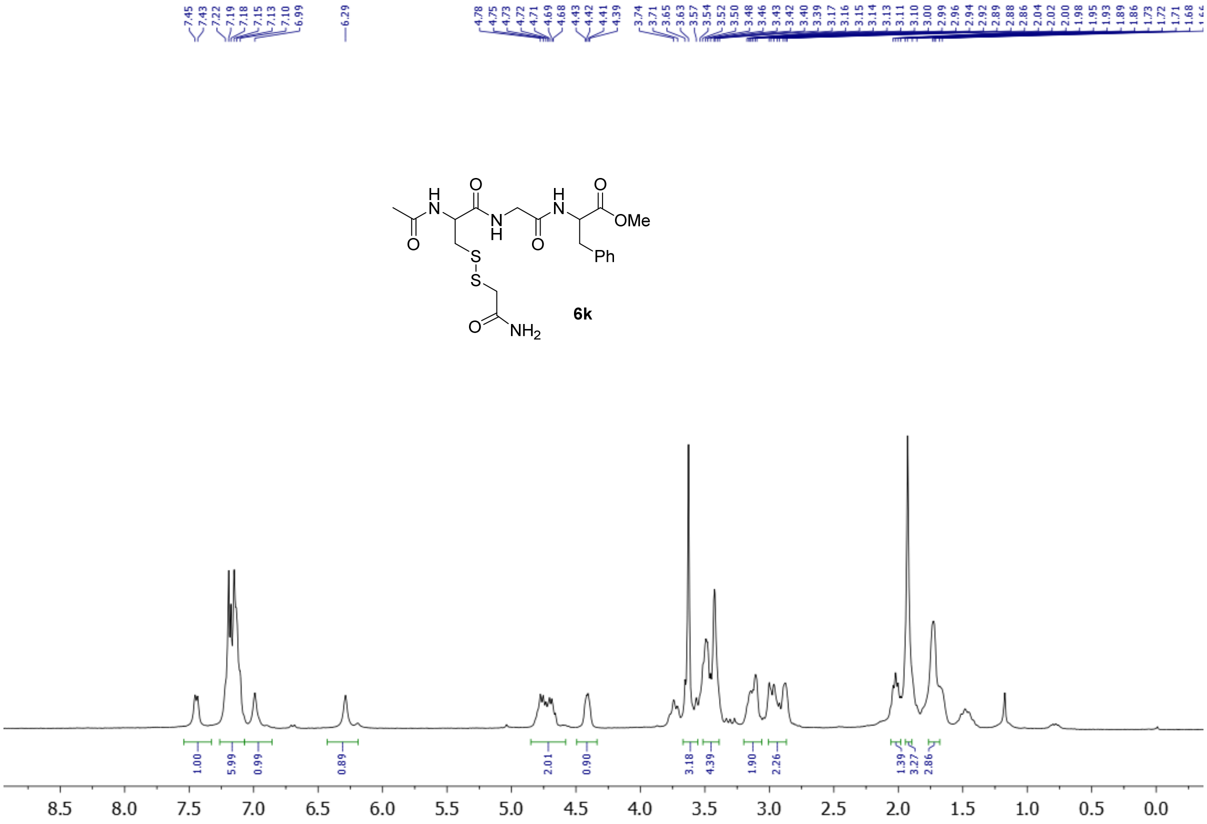
75MHz ^{13}C NMR for Bz-DL-penicillamine[SSSMe]-OMe **6i** in $CDCl_3$ (ppm)



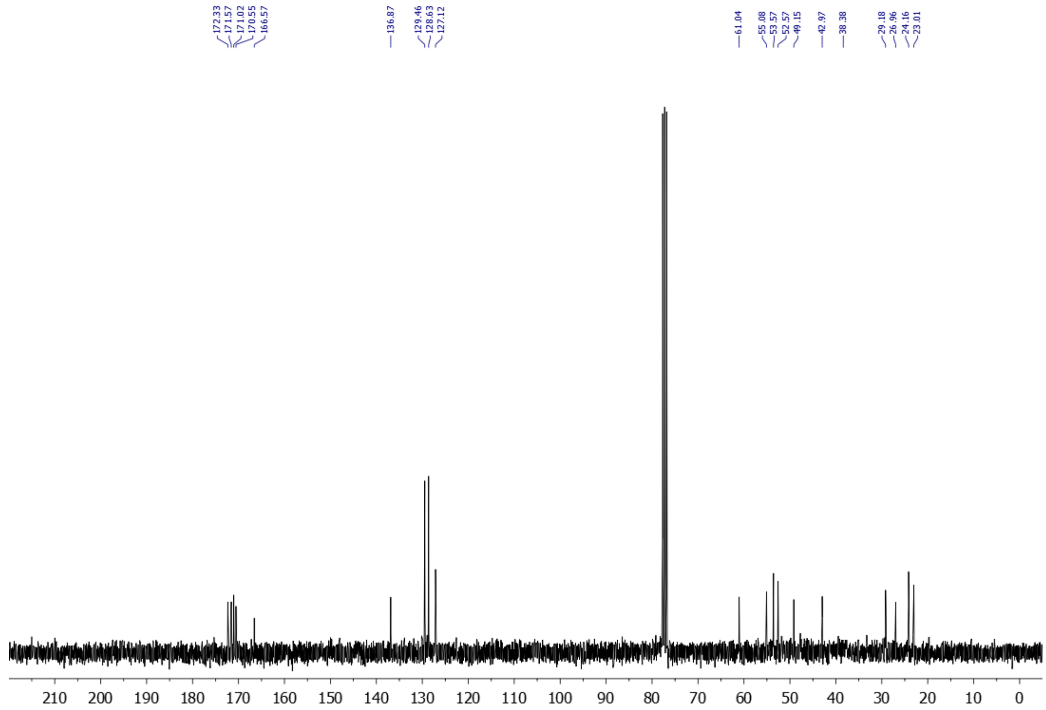
300MHz ^{1}H NMR for Ac-Cys[SS(CH$_2$CONH$_2$)]-Gly-Phe-OMe **6j** in CDCl$_3$ + DMSO-d_6 (ppm)



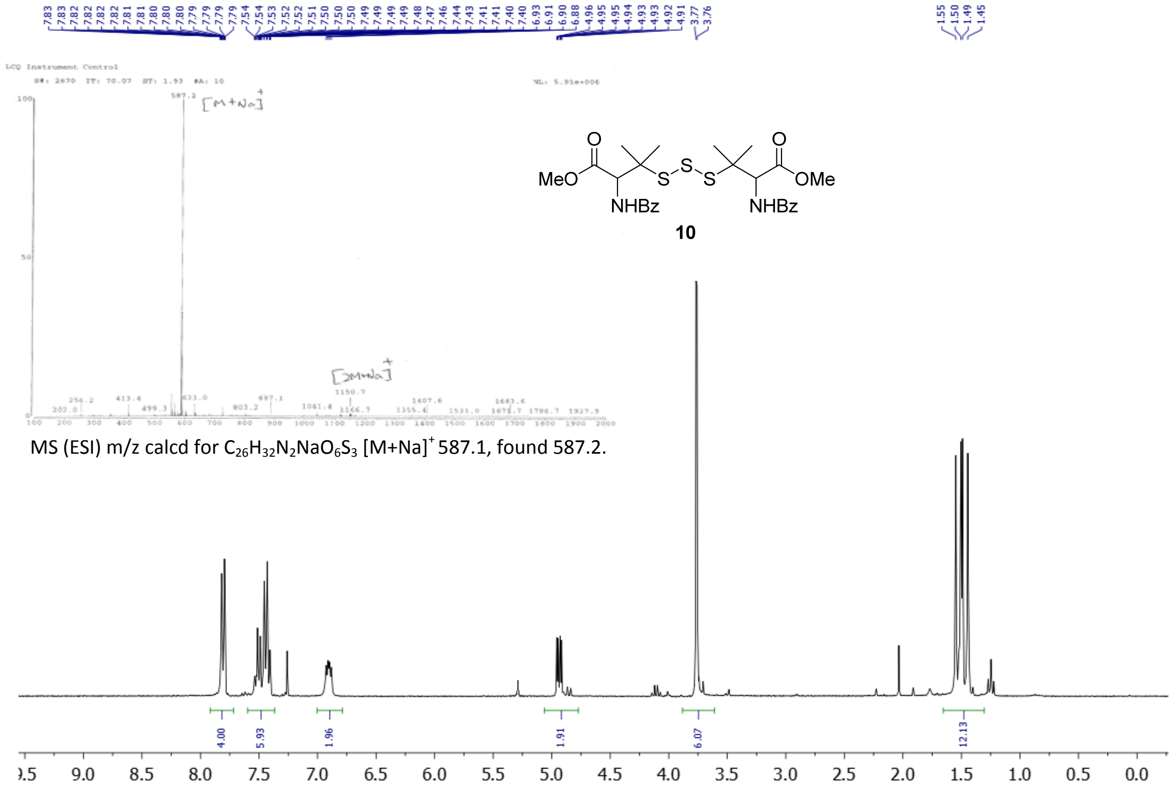
75MHz ^{13}C NMR for Ac-Cys[SS(CH$_2$CONH$_2$)]-Gly-Phe-OMe **6j** in CDCl$_3$ + DMSO-d_6 (ppm)



300MHz ^{1}H NMR for Ac-Cys[SS(CH_2CONH_2)]-Pro-Phe-OMe **6k** in $CDCl_3$ (ppm)



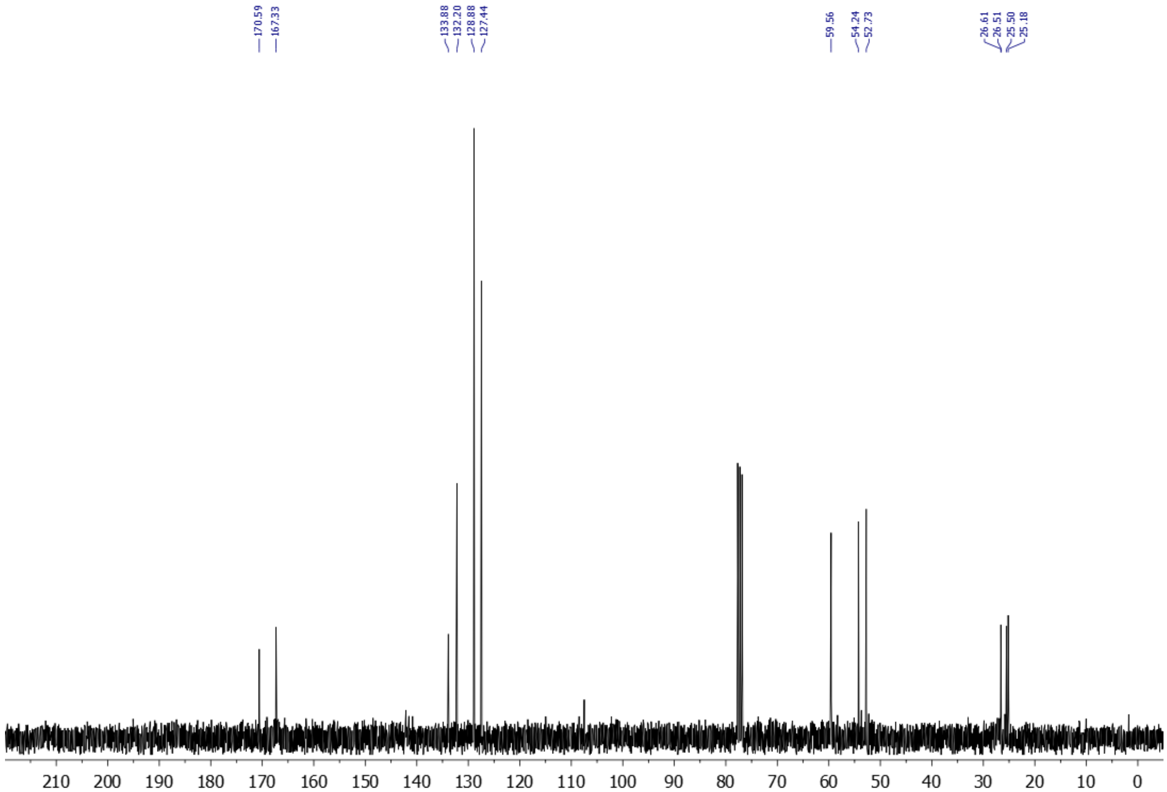
75MHz ^{13}C NMR for Ac-Cys[SS(CH_2CONH_2)]-Pro-Phe-OMe **6k** in $CDCl_3$ (ppm)



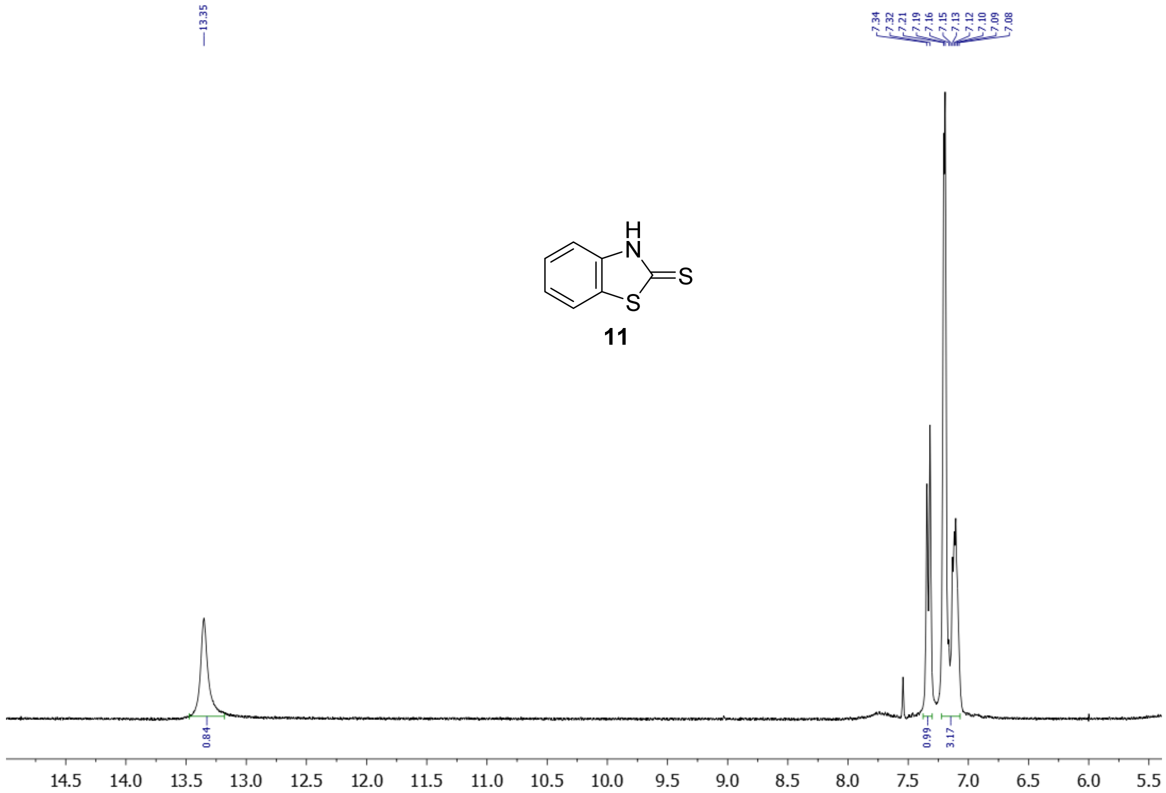
10

MS (ESI) m/z calcd for $C_{26}H_{32}N_2NaO_6S_3$ $[M+Na]^+$ 587.1, found 587.2.

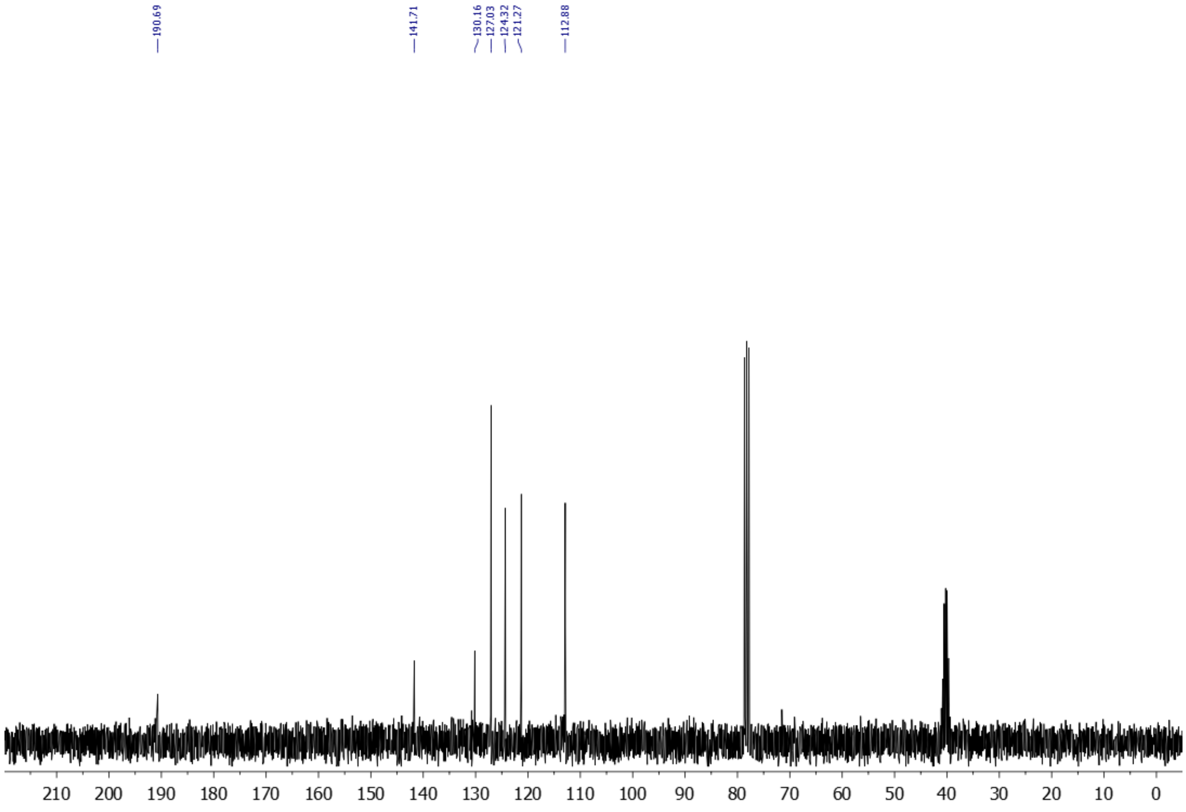
300MHz ^{1}H NMR for Bz-DL-enicillamine[SSS-(Bz)-PL-penicillamine-OMe]-OMe **10** in $CDCl_3$ (ppm)



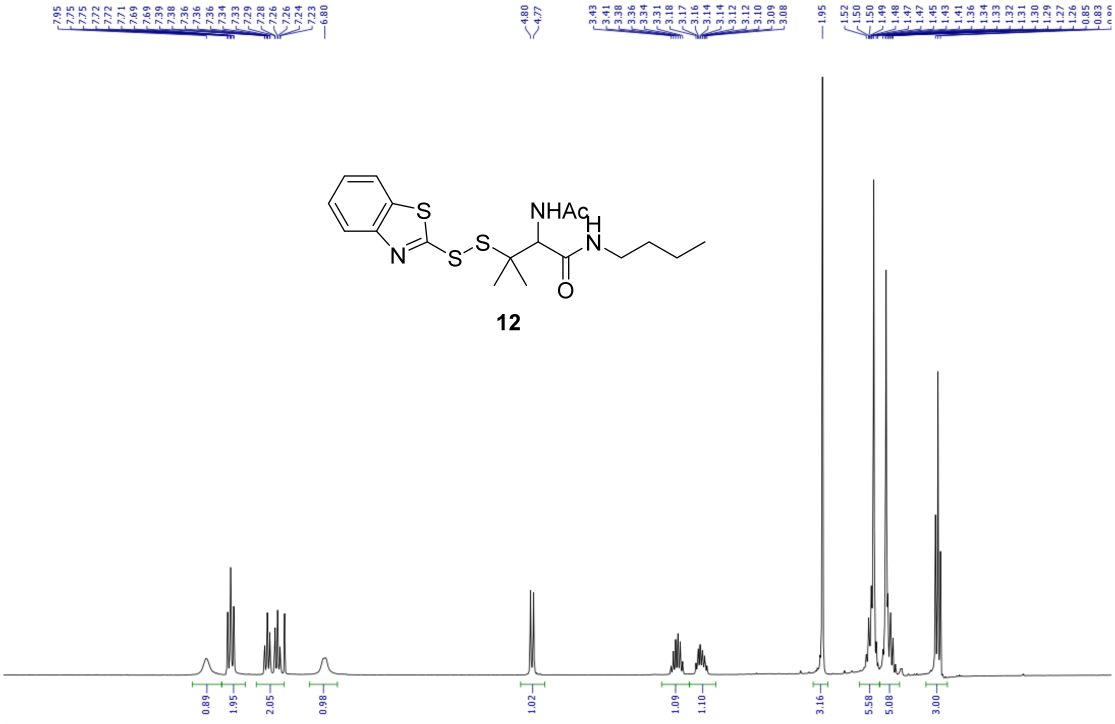
75MHz ^{13}C NMR for Bz-DL-penicillamine[SSS-(Bz)-DL-penicillamine-OMe]-OMe **10** in $CDCl_3$ (ppm)



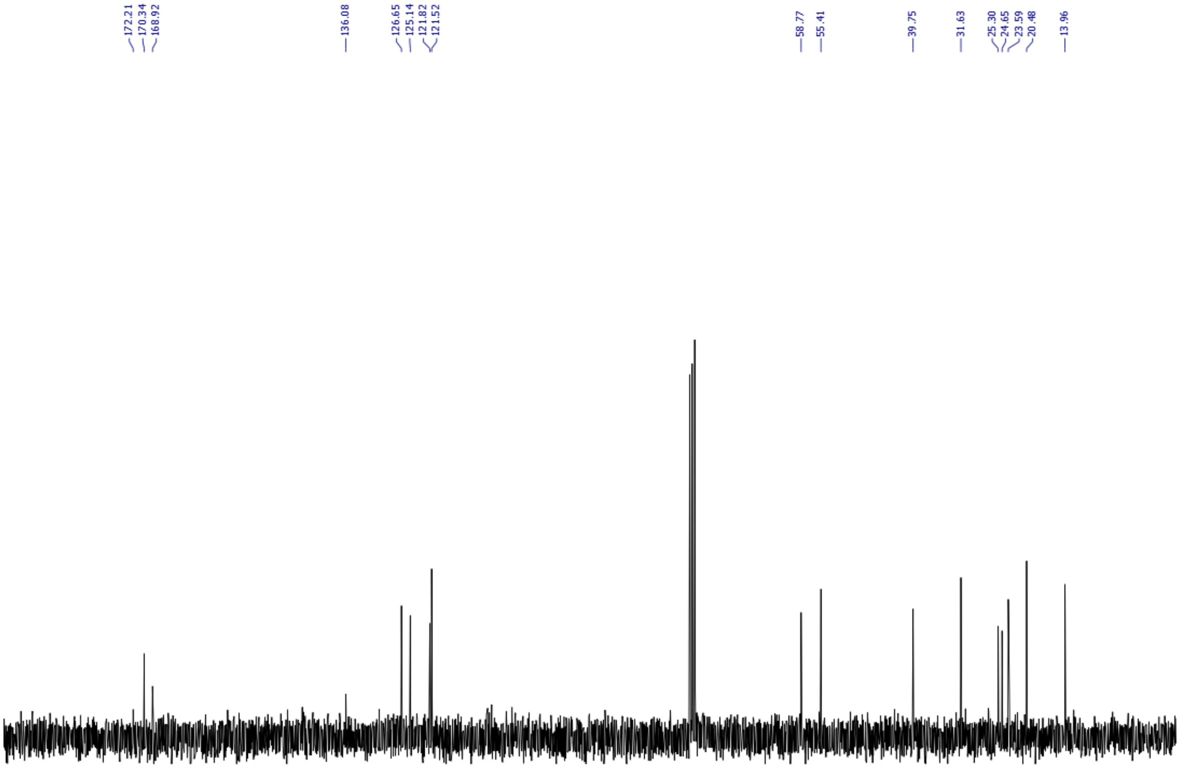
300MHz ^{1}H NMR for benzo[d]thiazole-2(3H)-thione **11** in $CDCl_3$ + DMSO-d_6 (ppm)



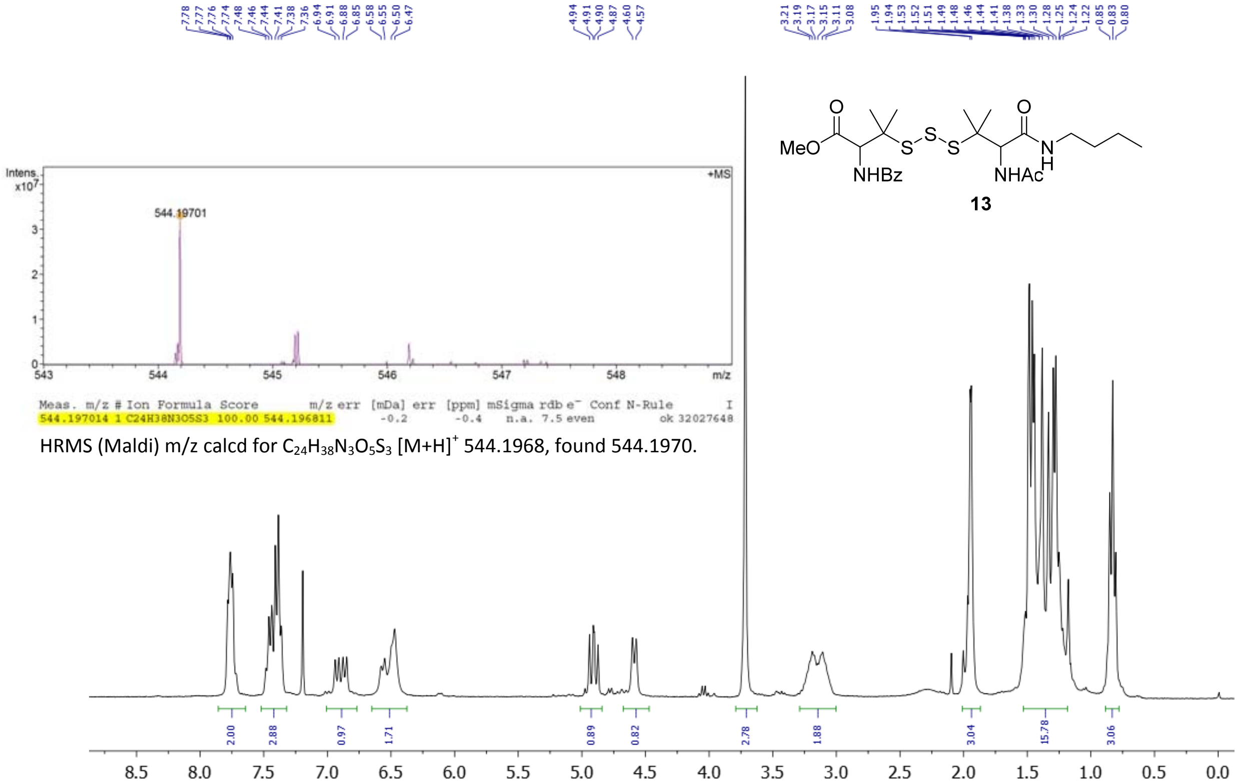
75MHz ^{13}C NMR for benzo[d]thiazole-2(3H)-thione **11** in $CDCl_3$ + DMSO-d_6 (ppm)



300MHz ^{1}H NMR for **12** in $CDCl_3$ (ppm)



75MHz ^{13}C NMR for **12** in $CDCl_3$ (ppm)

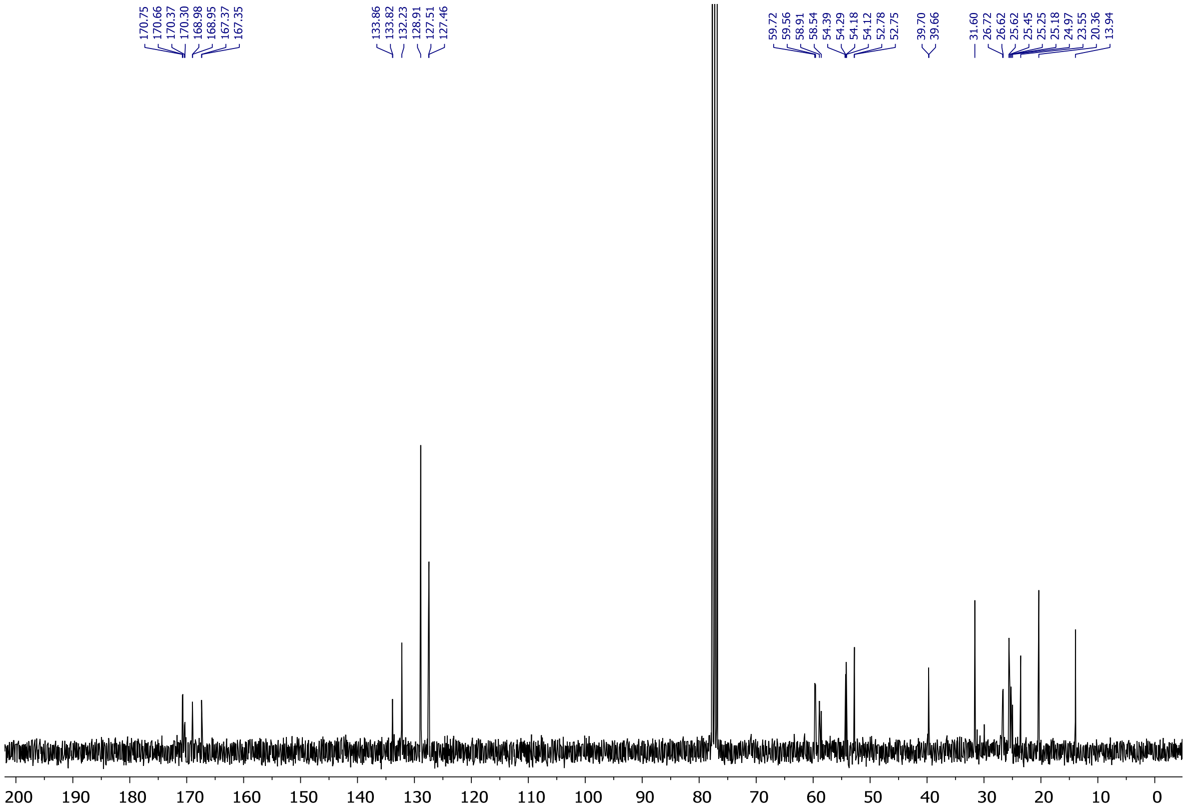


13

```
Meas. m/z # Ion Formula Score     m/z err  [mDa] err  [ppm] mSigma rdb e⁻  Conf N-Rule        I
544.197014 1 C24H38N3O5S3 100.00 544.196811  -0.2       -0.4  n.a.  7.5 even         ok 32027648
```

HRMS (Maldi) m/z calcd for $C_{24}H_{38}N_3O_5S_3$ $[M+H]^+$ 544.1968, found 544.1970.

300MHz ^{1}H NMR for **13** (diastereomer mixture) in $CDCl_3$



75MHz ^{13}C NMR for **13** (diastereomer mixture) in $CDCl_3$ (ppm)