Supporting Information

Methylsulfonyl Benzothiazole (MSBT): A Selective Protein Thiol Blocking Reagent

Dehui Zhang[1,a], Nelmi O. Devarie-Baez[1,a], Qian Li[2], Jack R. Lancaster, Jr, [2], and Ming Xian*[,1]

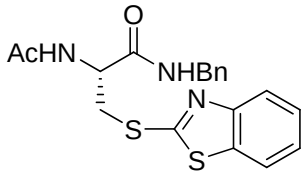
[1]*Department of Chemistry, Washington State University, Pullman, WA 99164.*
[2] *Department of Anesthesiology, Physiology & Biophysics, and Environment Health Sciences, and Center for Free Radical Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294.*

Content:

1. Experimental Procedures and Compound Characterization Data:

All reactions were carried in flame-dried round bottom flasks. All solvents were reagent grade. Anhydrous solvents such as tetrahydrofuran (THF) and methylene chloride (DCM) were obtained by running HPLC grade solvent through Pure Solv MD-3 Solvent Purification System from Innovative Technology under an argon atmosphere. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040 – 0.062mm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Proton and carbon-13 NMR spectra were recorded on 300 MHz spectrometer. Chemical shifts are reported relative to chloroform (δ 7.26) for ^{1}H NMR and chloroform (δ 77.0) ^{13}C NMR.

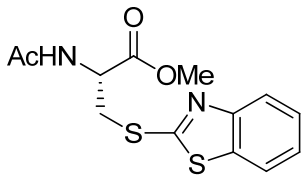
1.1 General procedure for the blocking of free thiol in cysteine derivatives 1a-1g.



2a

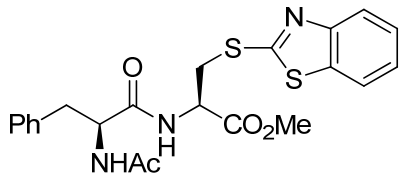
Compound 2a: To a stirring solution of **1a** (50.6 mg, 0.2 mmol) in THF (8 mL) and phosphate buffer (200 mM, pH=7.4, 16 mL) was added methylsulfonyl benzothiazole (MSBT, 86 mg, 0.4 mmol). The reaction was stirred at room temperature for 20 min and then quenched with saturated NaCl. The mixture was extracted with ethyl acetate (EtOAc) two times. The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated. The resulting residue was subjected to flash chromatography (EtOAc : Hexane = 1 : 2 to 2 : 1) to isolate the desired product **2a** in 95 % yield (71 mg, 0.190 mmol). Characterization data: m.p. 192-195°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, *J*= 1.2Hz, 1H), 7.63-7.71 (m, 2H), 7.18-7.35 (m, 7H); 4.76-4.77 (br, 1H); 4.38-4.41 (m, 2H); 3.59-3.79 (m, 2H); 1.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 170.1, 168.3, 152.5, 138.0, 135.6, 128.9, 127.9, 127.7, 126.7, 125.1,

121.5, 121.2, 55.0, 43.8, 35.4, 23.4; IR spectra (cm-1) 3277.6, 3065.4, 2926.8, 1632.0, 1538.5, 1462.3, 1427.8, 1368.9, 990.7; mass spectrum (ESI/MS) m/z 408.1 $[M+Na]^+$ $C_{19}H_{19}N_3NaO_2S_3$: calcd for : 408.1.



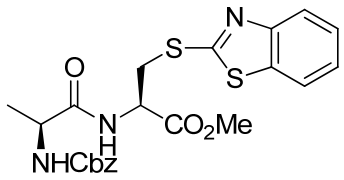
2b

Compound 2b: m.p. 111-113˚C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, *J*= 8.1Hz, 1H), 7.75 (d, *J*= 7.8 Hz, 1H), 7.59-7.61 (br, 1H); 7.43 (t, *J*= 7.8 Hz, 1H), 7.32 (t, *J*= 7.8 Hz, 1H), 4.92-4.95 (m, 1H), 3.80 (d, *J*= 5.7 Hz, 2H), 3.73 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 170.4, 166.7, 152.7, 135.8, 126.6, 125.1, 121.5, 121.4, 53.5, 53.0, 35.0, 23.2; IR spectra (cm-1); 3316.7, 3067.4, 2952.5, 1752.2, 1645.1, 1536.1, 1429.0, 1207.5, 1005.9, 751.6; mass spectrum (ESI/MS) m/z 333.0 $[M+Na]^+$ $C_{13}H_{14}N_2NaO_3S_2$: calcd for 333.0.



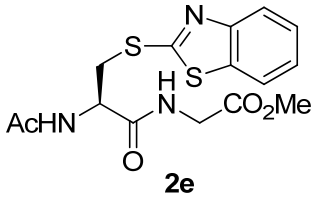
2c

Compound 2c: m.p. 181-183˚C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16-8.18 (br, 1H), 7.87 (d, *J*= 7.8 Hz, 1H), 7.74 (d, *J*= 8.1Hz, 1H), 7.33-7.45 (m, 2H), 7.10-7.13 (m, 5H), 6.31-6.33 (br, 1H), 4.91-4.92 (br, 1H), 4.75-4.77 (br, 1H), 3.73 (br, 5H), 3.01-3.07 (m, 2H), 1.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 170.1, 170.0, 166.6, 152.5, 136.5, 135.7, 129.6, 128.5, 127.0, 126.8, 125.1, 121.8, 121.3, 54.5, 53.5, 52.9, 38.9, 35.0, 23.3; IR spectra (cm-1) 3279.6, 3066.0, 2946.8, 1741.9, 1640.0, 1534.3, 1428.5, 1244.0, 997.9, 751.8; mass spectrum (ESI/MS) m/z 480.10 $[M+Na]^+$ $C_{22}H_{23}N_3NaO_4S_2$: calcd for 480.1.



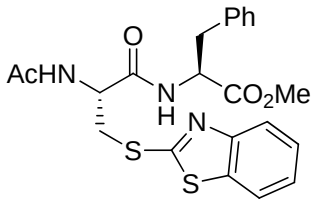
2d

Compound 2d: m.p. 128-130˚C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (br, 1H), 7.90 (d, *J*= 7.8 Hz, 1H), 7.74 (d, *J*= 7.8 Hz, 1H), 7.33-7.43 (m, 7H), 5.37-5.38 (br, 1H), 5.07-5.08 (m, 2H), 4.92-4.95 (m, 1H), 4.21 (br, 1H), 3.79-3.81 (m, 2H), 3.74 (s, 3H), 1.38 (d, *J*= 5.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 170.4, 166.7, 152.6, 136.5, 135.8, 128.8, 128.7, 128.4, 128.3, 126.8, 125.2, 121.6, 121.4, 67.1, 53.5, 53.0, 50.6, 35.0, 19.3; IR spectra (cm-1) 3302.7, 3065.4, 2953.3, 1732.7, 1689.0, 1651.5, 1532.8, 1428.7, 1314.2, 1253.8, 995.6; mass spectrum (ESI/MS) m/z 496.1 $[M+Na]^+$ $C_{22}H_{23}N_3NaO_5S_2$: calcd for 496.1.



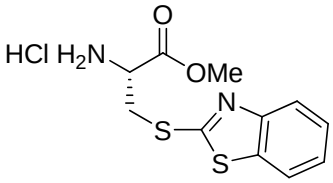
2e

Compound 2e: m.p. 160-163˚C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (br, 1H), 7.76-7.86 (m, 2H), 7.43-7.48 (m, 2H), 7.37 (t, *J*= 7.5 Hz, 1H), 4.82-4.86 (m, 1H), 4.03-4.06 (m, 2H), 3.71-3.75 (m, 5H), 2.03 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 170.6, 170.0, 168.4, 152.5, 135.8, 126.7, 125.1, 121.6, 121.3, 54.8, 52.6, 41.5, 35.0, 23.3; IR spectra (cm-1) 3317.3, 3273.2, 3064.2, 2954.0, 1737.3, 1725.0, 1634.6, 1546.2, 1468.1, 1361.2, 1011.7, 991.2, 746.5; mass spectrum (ESI/MS) m/z 390.1 $[M+Na]^+$ $C_{15}H_{17}N_3NaO_4S_2$: calcd for 390.1.



2f

Compound 2f: m.p. 177-179˚C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, *J*= 6.0 Hz, 1H), 7.71-7.78 (m, 2H), 7.43 (dt, *J*= 7.2 Hz, 1.2 Hz, 1H), 7.34 (dt, *J*= 7.8 Hz, 1.2 Hz, 1H), 7.11-7.23 (m, 5H), 4.79-4.85 (m, 2H), 3.61-3.75 (m, 2H), 3.70 (s, 3H), 3.18 (dd, *J*= 13.8 Hz, 5.7 Hz, 1H), 3.07 (dd, *J*= 13.8 Hz, 6.6 Hz, 1H), 1.96 (s, 3H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 171.2, 169.7, 168.1, 152.2, 135.8, 135.5, 129.3, 128.5, 127.0, 126.4, 124.8, 121.3, 121.0, 54.2, 53.4, 52.4, 37.8, 34.9, 22.9; IR spectra (cm-1) 3296.4, 3064.4, 2963.5, 1736.2, 1635.8, 1537.2, 1461.3, 1271.8, 998.8, 763.7; mass spectrum (ESI/MS) m/z 480.1 $[M+Na]^+$ $C_{22}H_{23}N_3NaO_4S_2$: calcd for 480.1.



2g

Compound 2g: To a stirring solution of **1g** (63 mg, 0.367 mmol) in THF (13 mL) and phosphate buffer (200 mM, pH=7.4, 26 mL) was added MSBT (156 mg, 0.734 mmol). The reaction was stirred at room temperature for 20 min and then acidified with 1N HCl. The mixture was extracted with 10 mL of ethyl acetate (EtOAc) and organic layer was washed three times with 1N HCl (~ 10 mL each time). The combined acidic aqueous layers were neutralized with saturated $NaHCO_3$ and, subsequently adjusted to pH= 8. The neutralized product was then extracted three times with EtOAc (100 mL total volume). The combined organic layers were dried over anhydrous Na_2SO_4 and then filtered and concentrated. The residue was treated with 1.2 M HCl in methanol (3 equiv, 0.9 mL) and re-concentrated. A white solid formed and it was subsequently suspended in anhydrous ether, filtered and dried under *vacuo*. The resulting hydrochloride salt was obtained in 97 % yield (109 mg, 0.358 mmol). m.p. 131-133 ˚C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.82-7.87 (m, 2H), 7.43 (dt, *J*= 7.5 Hz, 1.2 Hz, 1H), 7.33 (dt, *J*= 7.8 Hz, 1.2 Hz, 1H), 4.63 (dd, *J*= 6.6 Hz, 4.5 Hz, 1H), 4.05 (dd, *J*= 15.0 Hz, 4.8 Hz, 1H), 3.80 (dd, *J*= 15.3 Hz, 6.9 Hz, 1H), 3.78 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 169.1, 166.4, 153.9, 136.9, 127.7, 126.2, 122.6,

54.1, 54.0, 33.6; IR spectra (cm-1) 2780.1-3069.9 (br), 2709.0, 2592.9, 2006.8, 1745.2, 1567.3, 1478.6, 1231.5, 989.2, 754.1; mass spectrum (ESI/MS) m/z 269.1 $[M]^+$ $C_{11}H_{13}N_2O_2S_2$: calcd for 269.0.

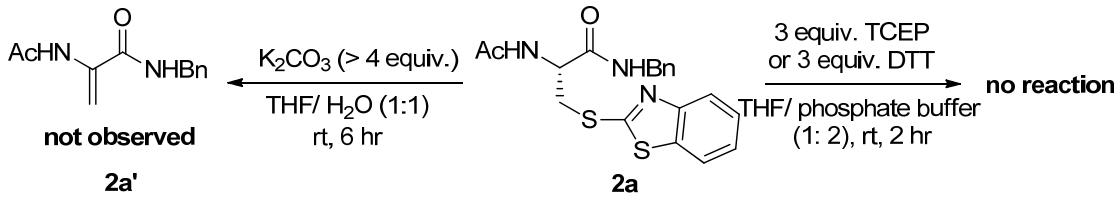
1.2 Control Experiments

General procedure for control experiments with amino acid derivatives **4a-4f**: to a stirring solution of amino acid derivative (0.1 mmol) in THF (4 mL) and 200 mM phosphate buffer (pH=7.4, 8 mL) was added methylsulfonyl benzothiazole (MSBT, 0.5 mmol). The reaction was stirred at room temperature for 4 h and the progress was monitored by TLC. In all cases, we did not observe any reaction.

1.3 Study of the stability of Cys-S-Bt adduct.

Compound **2a** was used as the model to study the stability of S-Bt products in the presence of DTT, TCEP, and K_2CO_3. To a stirring solution of **2a** (29.5 mg, 0.077 mmol) in THF (3 mL) and 200 mM phosphate buffer (pH=7.4, 6 mL) was added DTT or TCEP (3 equiv., 0.231 mmol), respectively. The reaction was stirred at room temperature for 2 hr and monitored by TLC. Compound **2a** was found inert to TCEP and DTT. No degradation of **2a** was observed. Similar concentration of **2a** and experimental procedure was utilized in the experiment performed with K_2CO_3. In this experiment, elimination of the benzothiazole moiety to produce dehydroalanine (**2a'**) was not observed on TLC.

Scheme S1



1.4 Thiol blocking ability of MSBT and MSBT-A on GAPDH

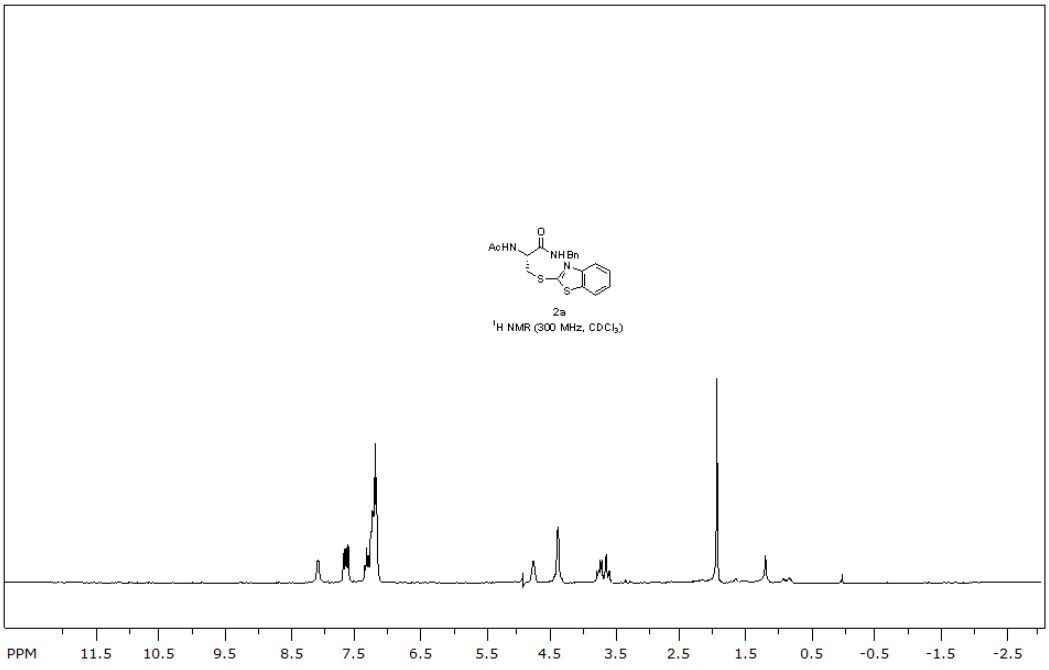
Reagents: glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) from rabbit muscle, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES), Ethylenediaminetetraacetic acid tetrasodium salt dihydrate (EDTA), neocuproine, dimethyl sulphoxide (DMSO) and S-methyl methanethiosulfonate (MMTS) are from Sigma-Aldrich (Saint Louis, MO). Dithiothreitol (DTT) and

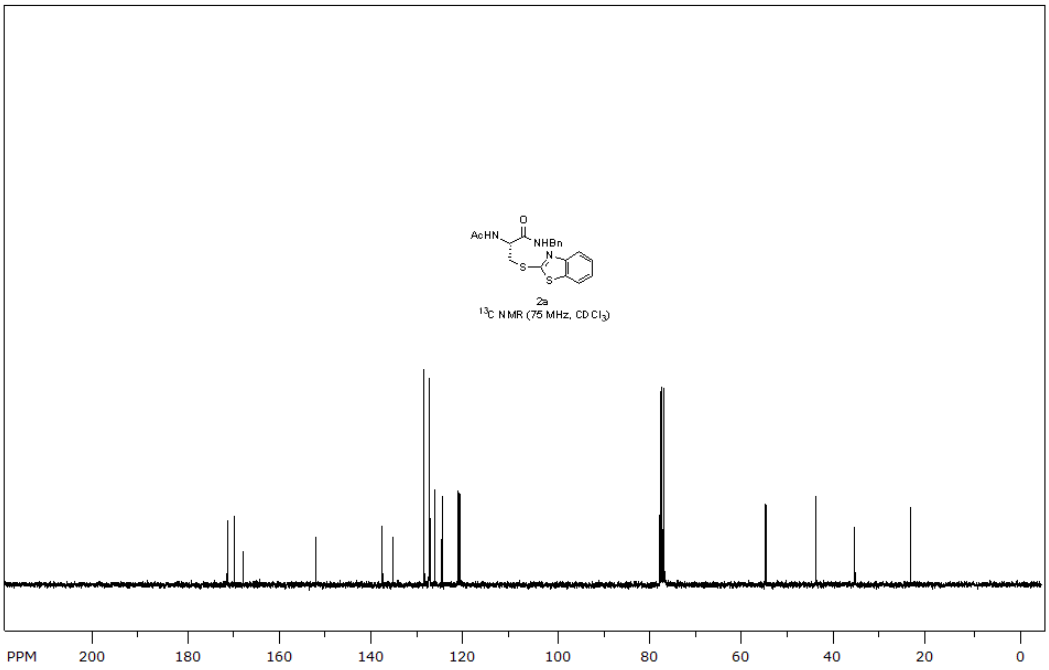
Laemmli sample buffer are from Bio-Rad (Hercules, CA). Sodium Dodecyl Sulfate (SDS) is from Fisher Scientific (Fair Lawn, NJ), N-[6-(Biotinamido)hexyl]-3′-(2′-pyridyldithio)propionamide (EZ-Link HPDP-Biotin) from Pierce Biotechnology (Rockford, IL), polyvinylidene fluoride (PVDF) membrane from Milipore Corporation (Bedford, MA), and anti-biotin goat polyclonal antibody peroxidase conjugate from Calbiochem (Darmstadt, Germany)

The protocol is adapted from the biotin switch assay modified by Wang et al. (Wang et al., Free Rad. Bio. Med., 2008, 1362). 1 mg/ml GAPDH in HEN buffer (250 mM Hepes, 1 mM EDTA and 100 μM neocuproine, pH 7.7) was reduced by 5 mM DTT for 30 min at room temperature, and then desalted by HEN buffer twice by PD-10 column (GE Healthcare, Piscataway, NJ). The purified GAPDH was incubated with DMSO (as vehicle) or 10 mM blocking reagent (MMTS, MSBT or MSBT-A) in HEN buffer containing 1% SDS at 50°C for 30 minutes with occasional vortexing. Following brief desalting by PD MiniTrap G-25 (GE Healthcare, Piscataway, NJ), 0.4 mM EZ-Link HPDP-Biotin was added to the protein sample to label free thiols. After 1 h incubation at room temperature, samples were mixed with Laemmli sample buffer (without reducing agents). Same amounts of protein from each sample were loaded and separated by non-reducing SDS-PAGE, transferred to PVDF membrane. Western blot was performed with anti-biotin antibody.

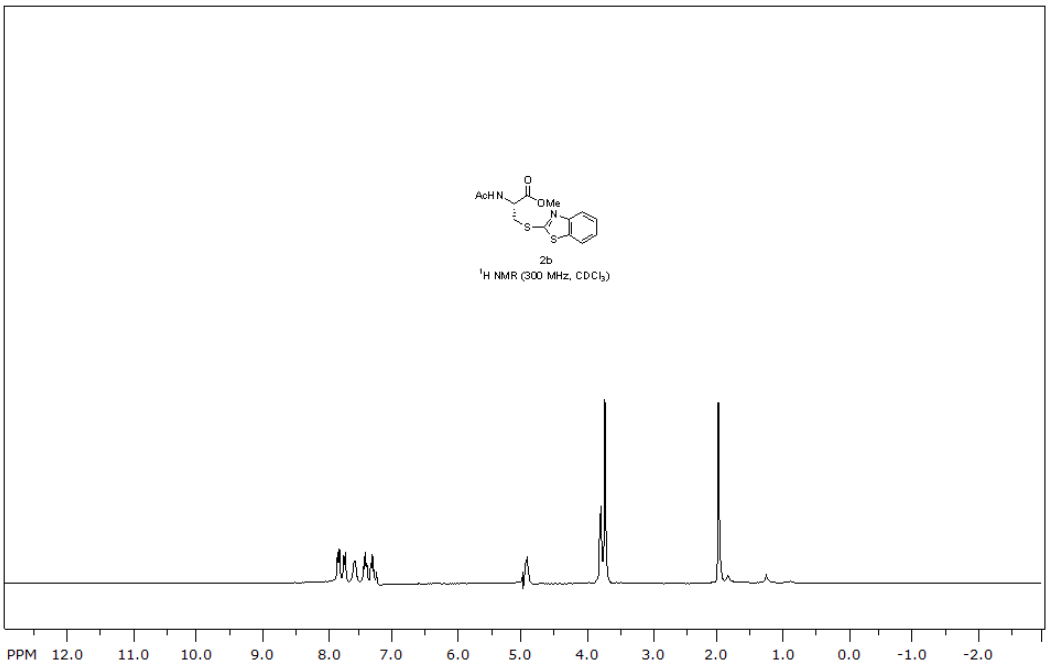
2. ^{1}H NMR and ^{13}C NMR spectrum

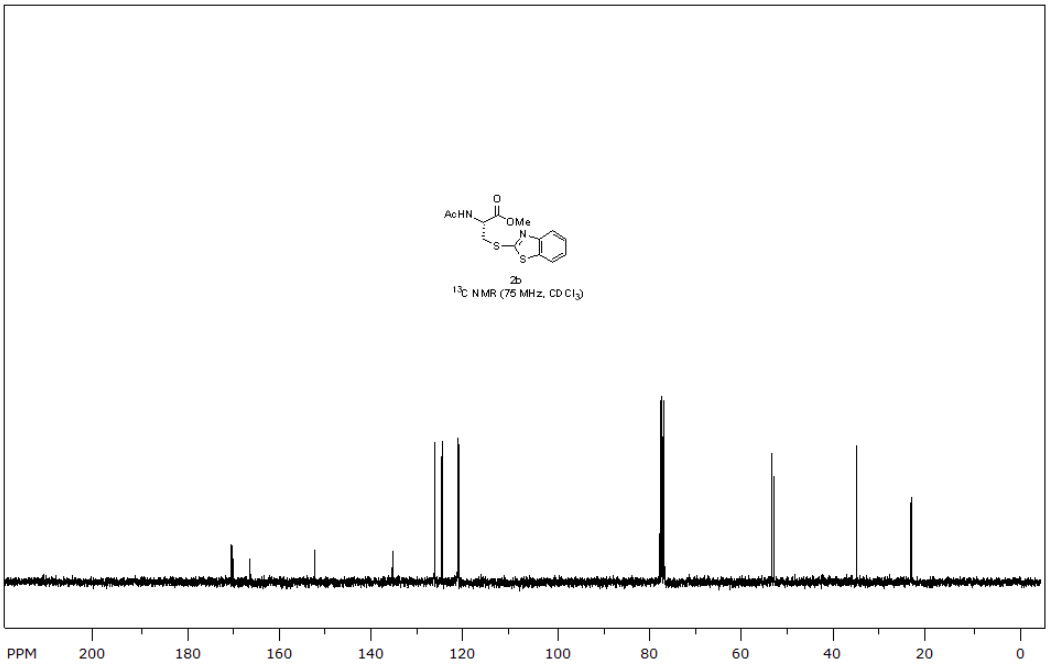
Compound 2a



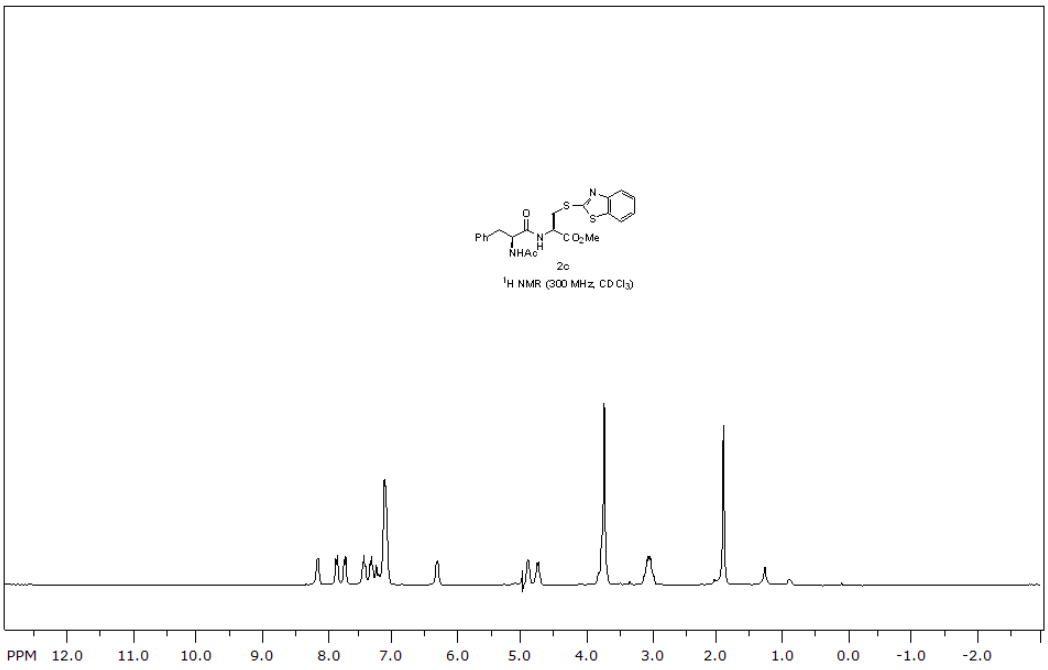


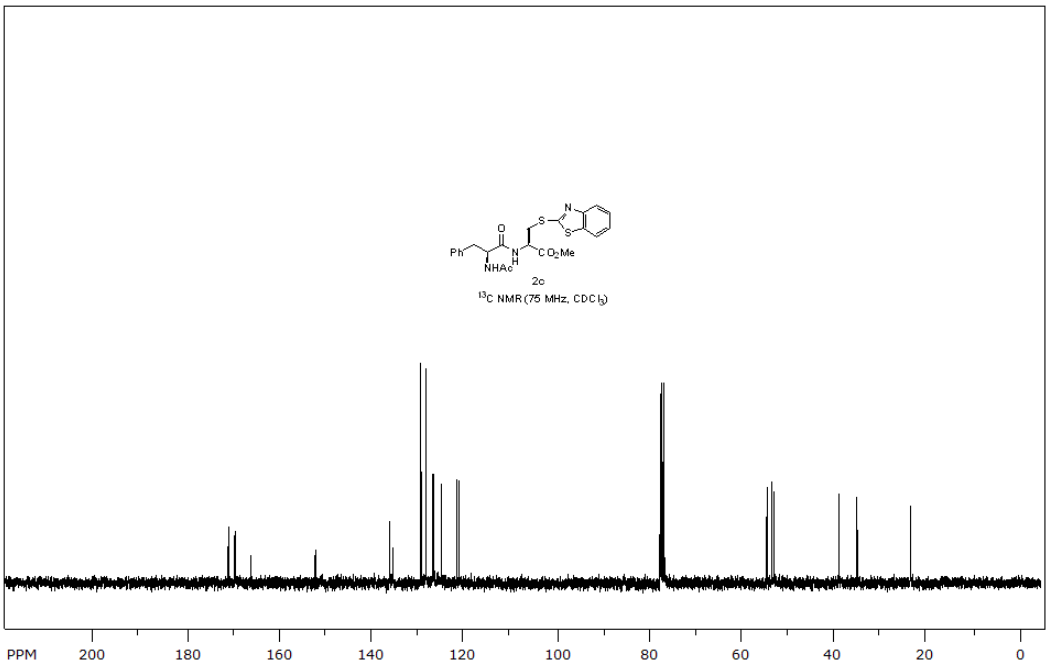
Compound 2b


AcHN
O
OMe
N
S
S
2b
^{1}H NMR (300 MHz, CDCl$_3$)
PPM 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 -1.0 -2.0

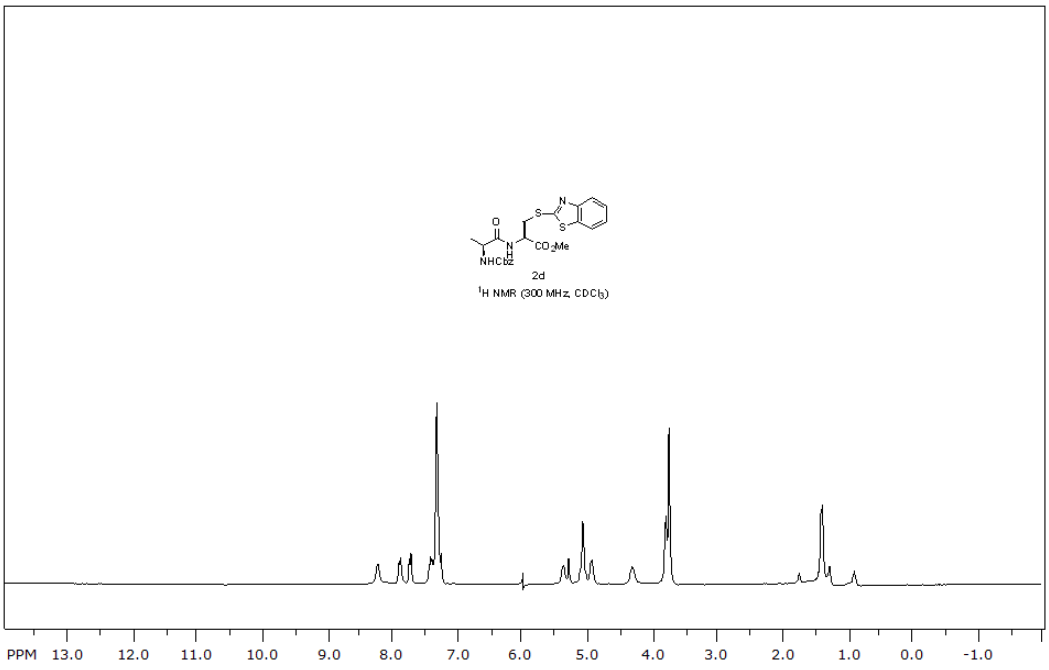

AcHN
O
OMe
N
S
S
2b
^{13}C NMR (75 MHz, CDCl$_3$)
PPM 200 180 160 140 120 100 80 60 40 20 0

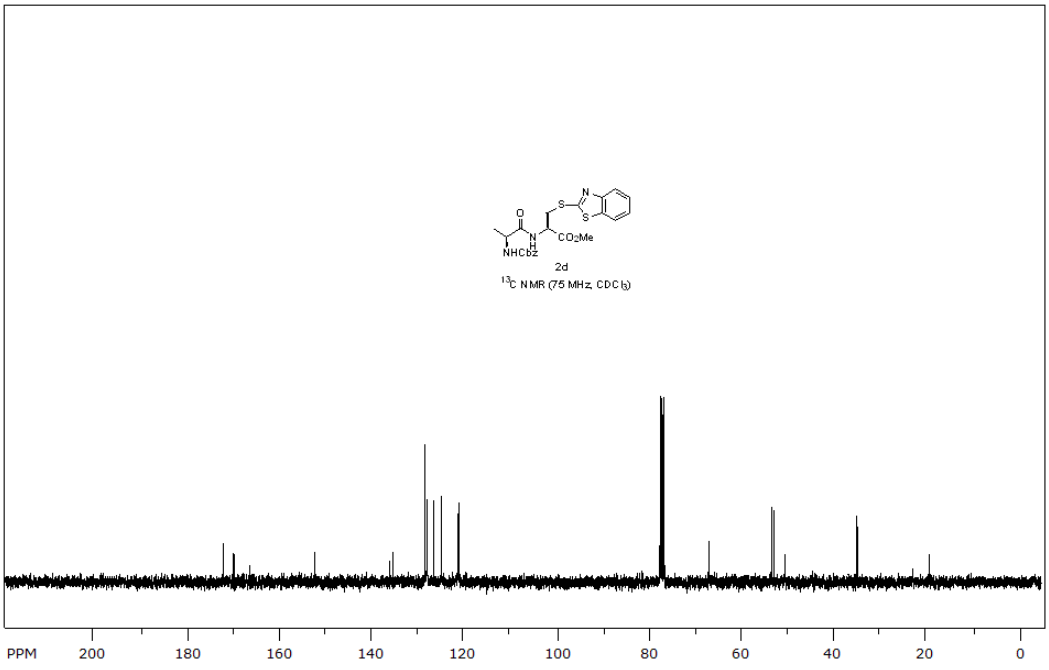
Compound 2c


2c
^{1}H NMR (300 MHz, CDCl$_3$)
PPM 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 -1.0 -2.0

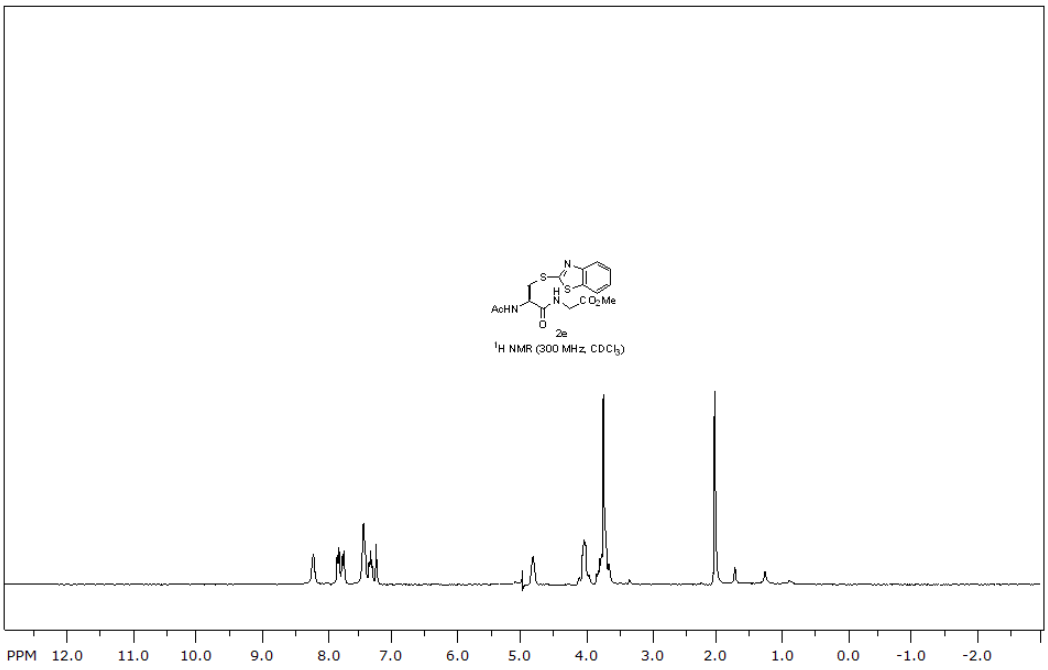

2c
^{13}C NMR (75 MHz, CDCl$_3$)
PPM 200 180 160 140 120 100 80 60 40 20 0

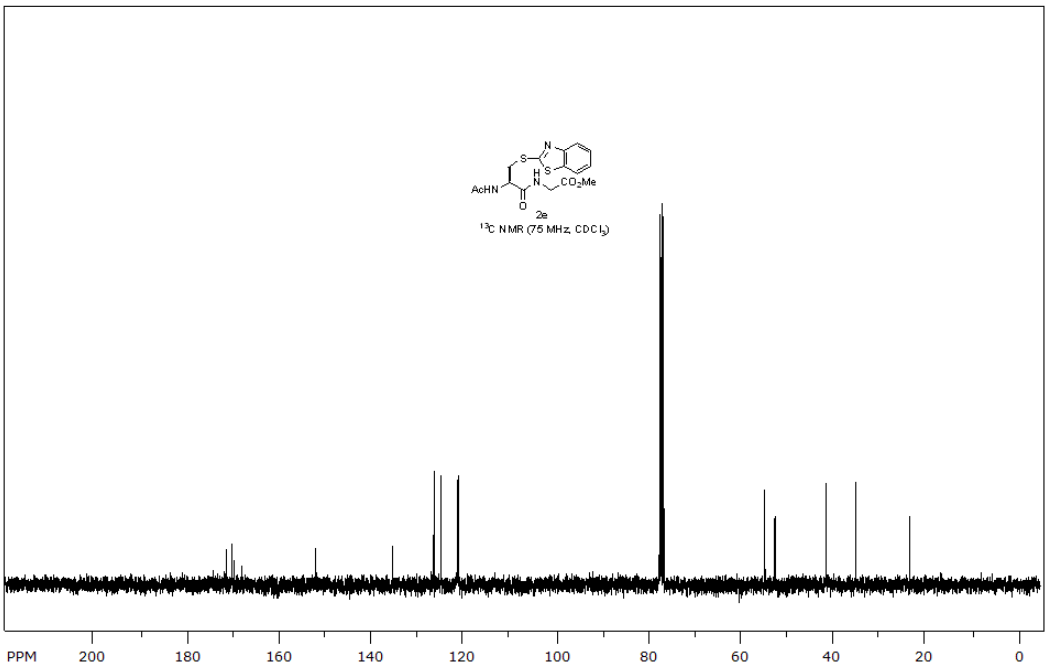
Compound 2d


2d
^{1}H NMR (300 MHz, CDCl$_3$)
PPM 13.0 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 -1.0

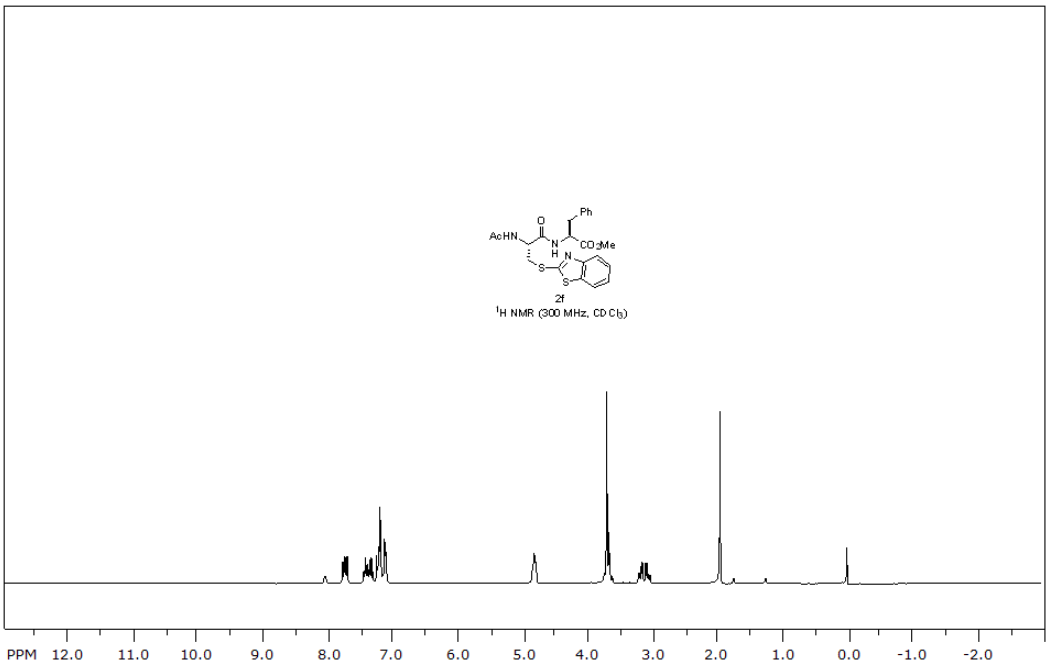

2d
^{13}C NMR (75 MHz, CDCl$_3$)
PPM 200 180 160 140 120 100 80 60 40 20 0

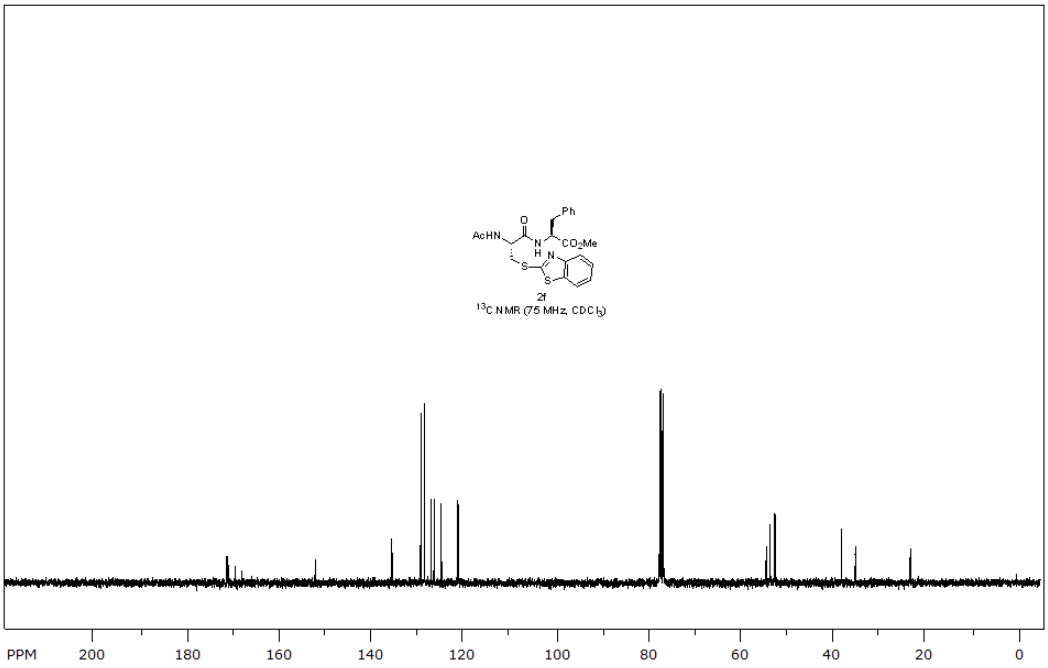
Compound 2e


2e
^{1}H NMR (300 MHz, CDCl$_3$)
PPM 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 -1.0 -2.0

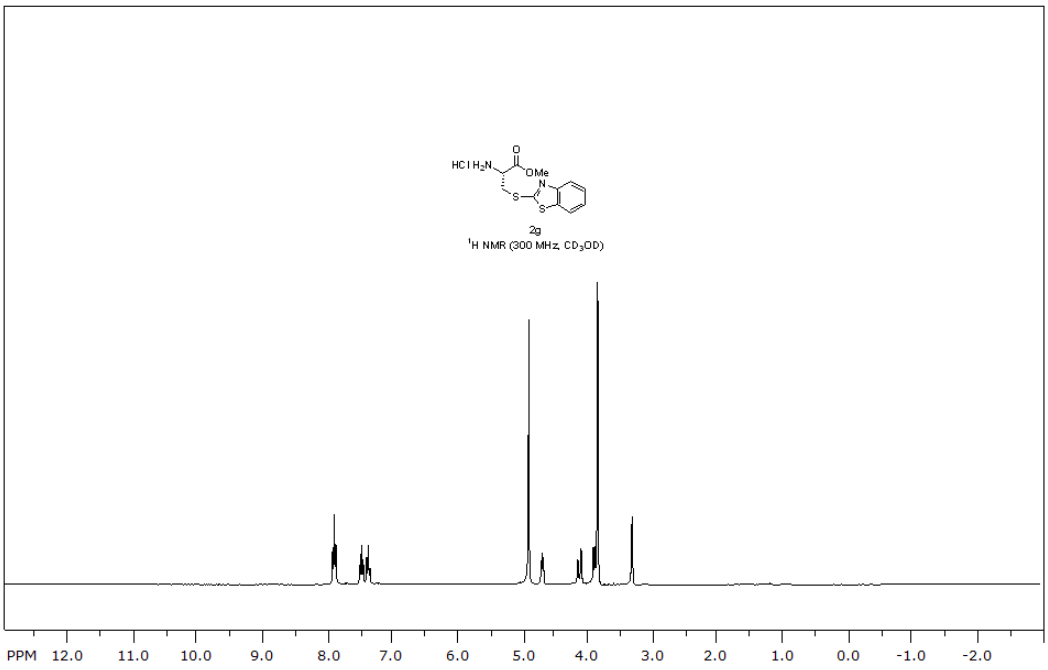

2e
^{13}C NMR (75 MHz, CDCl$_3$)
PPM 200 180 160 140 120 100 80 60 40 20 0

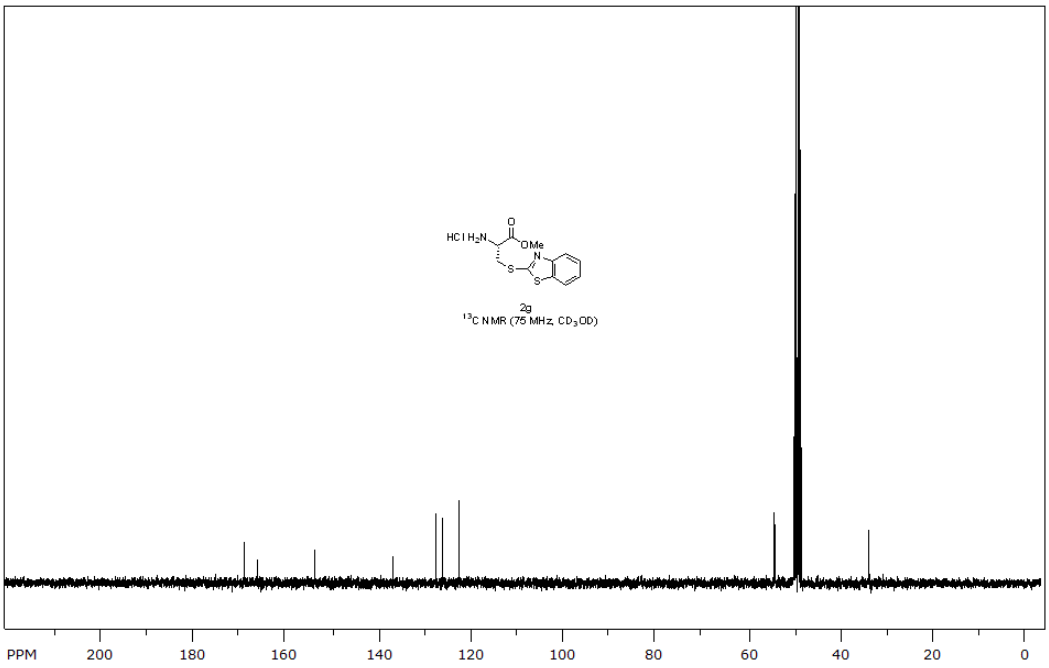
Compound 2f


2f
^{1}H NMR (300 MHz, CDCl$_3$)
PPM 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 -1.0 -2.0


2f
^{13}C NMR (75 MHz, CDCl$_3$)
PPM 200 180 160 140 120 100 80 60 40 20 0

Compound 2g


2g
^{1}H NMR (300 MHz, CD_3OD)
PPM 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 -1.0 -2.0


2g
^{13}C NMR (75 MHz, CD_3OD)
PPM 200 180 160 140 120 100 80 60 40 20 0