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Supporting Information

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Detection of Protein S-Sulfhydration by a Tag-Switch Technique**

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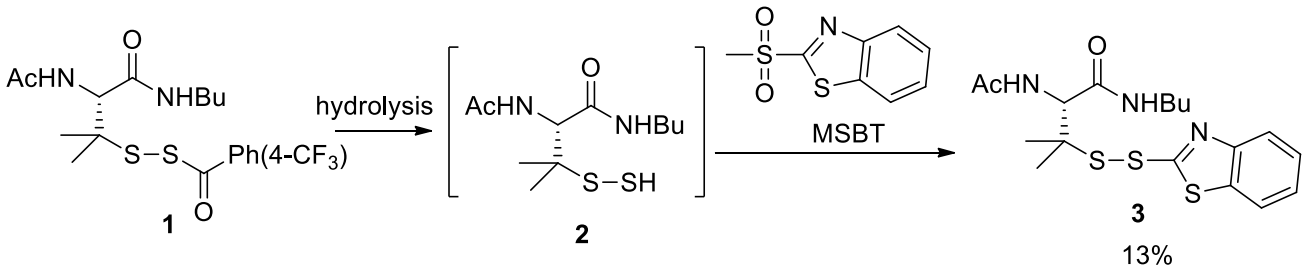
Materials: Reagents and solvents were of the highest grade available. Reagent grade solvents were used for either chromatography or extraction without further purification before use. Dichloromethane (DCM) and tetrahydrofuran (THF) were directly used from a solvent purifier (Pure Solv, Innovative Technology, Inc.). Partially protected amino acids were purchased from Advanced ChemTech and used directly. 2-mercaptobenzothiazole, 2,2'-dibenzothiazolyl disulfide, malononitrile, methyl-2-cyanoacetate, and 1,3-cyclopentadione were purchased from TCI America and used directly. D-(+)-biotin was purchased from Acros and used directly.

Buffers was prepared with nano-pure water, stirred with Chelex-100 resins to remove traces of heavy metals and kept above the resins until used. Sodium sulfide (Na_2S) was purchased as anhydrous, opened and stored in glove box (<2 ppm O_2 and <1 ppm H_2O). 100 mM stock solutions of sodium sulfide were prepared as described previously.[14] Fe^{3+}(P) water-soluble porphyrin was a kind gift from Dr Norbert Jux (Department of Chemistry and Pharmacy, University of Erlangen-Nuremberg). HUVE cells (Human umbilical vein endothelial cells) were obtained from Promo Cell (Heidelberg, Germany). Jurkat cells (T-lymphoblastic cells) were a kind gift from professor Martin Herrmann (Medizinische Klinik 3, Rheumatologie und Immunologie, Erlangen, Germany).

Instrumentation. NMR spectra were recorded on a Varian Vx 300 NMR spectrometer and are reported in parts per million (ppm) on the δ scale relative to residual $CHCl_3$ (δ 7.25 or δ 77.0) and DMSO-d_6 (δ 2.49 or δ 39.5) for ^1H or ^{13}C. 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. FT-IR spectra were recorded on a Thermo Scientific Nicolet iS10 (Thin film) and reported in units of cm^{-1}. Mass spectra were recorded using an electrospray ionization mass spectrometry (ESI, Thermo Finnigan LCQ Advantage). Mass data were reported in units of m/z for $[M+H]^+$ or $[M+Na]^+$.

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), $KMnO_4$ solution (1.5 g of $KMnO_4$, 10 g of K_2CO_3, and 1.25 mL of 10% NaOH), 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.

Scheme S1. The reaction between MSBT and small molecule persulfides



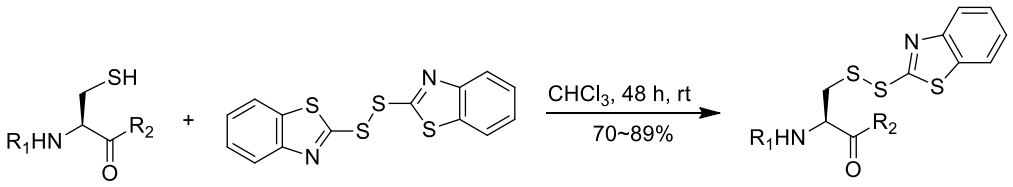
Reaction between MSBT and persulfides (the preparation of **3**): To a solution of S-(4-trifluoromethylbenzothioacidic)-*N*-Ac-penicillamine-NHBu **1** (73.0 mg, 0.163 mmol) in dry THF (12 mL) was added MSBT (139 mg, 0.652 mmol) under argon atmosphere. Then a solution of benzyl amine (52.4 mg, 0.489 mmol) was added dropwise into the mixture at rt. The resulting mixture was stirred overnight and the solvent was removed under vacuum. The crude material was subjected to flash column chromatography (2% MeOH in DCM)

to afford the desired product **3** as a brownish solid in 13 % yield (9 mg). mp 167-168 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.78 (t, *J* = 8.9 Hz, 2H), 7.42 (t, *J* = 7.7 Hz, 1H), 7.32 (t, *J* = 7.5 Hz, 1H), 6.90 (d, *J* = 8.9 Hz, 1H), 4.87 (d, *J* = 9.1 Hz, 1H), 3.43 (m, 1H), 3.27 – 3.09 (m, 1H), 2.02 (s, 3H), 1.52 (m, 5H), 1.39 (m, 5H), 0.89 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 168.9, 153.9, 136.1, 126.6, 125.1, 121.9, 121.5, 58.7, 55.5, 39.7, 31.6, 31.2, 25.2, 23.6, 20.5, 14.0; 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.

Synthesis of R-S-S-BT compounds

Preparation of compound 4: To a solution of 2,2'-dibenzothiazoyl disulfide (523 mg, 1.57 mmol) in THF (50 mL) was added Ac-Cys-NHBn (330 mg, 1.30 mmol) in $CHCl_3$. The reaction mixture was stirred for 48 h at rt then concentrated. The resulting residue was subjected to flash column chromatography (2% MeOH in DCM) to give the desired product **4** as a solid (80% yield). mp 181-182 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (t, *J* = 5.2 Hz, 1H), 7.80 – 7.70 (m, 1H), 7.41 (dt, *J* = 7.3, 3.1 Hz, 1H), 7.36 – 7.20 (m, 7H), 6.99 (d, *J* = 7.5 Hz, 1H), 4.84 (td, *J* = 7.7, 4.7 Hz, 1H), 4.68 – 4.37 (m, 2H), 3.51 (dd, *J* = 14.2, 4.7 Hz, 1H), 3.11 (dd, *J* = 14.2, 8.0 Hz, 1H), 1.98 (s, 3H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 171.6, 169.9, 154.2, 137.6, 135.8, 128.7, 127.7, 127.6, 126.6, 125.1, 121.9, 121.4, 52.4, 43.7, 41.5, 22.6; FT-IR (thin film, cm^{-1}) 3293.1, 3268.6, 3060.2, 3023.4, 1618.5, 1544.2, 1454.3, 1421.6, 1004.6, 735.1; MS (ESI) *m/z* calcd for $C_{19}H_{19}N_3NaO_2S_3$ $[M+Na]^+$ 440.1, found 440.1.

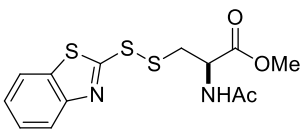
Scheme S2. Preparation of activated-disulfide model substrates



S1: R_1 = Ac, R_2 = OMe
S2: R_1 = Bz, R_2 = OMe
S3: R_1 = Ac-Phe, R_2 = OMe
S4: R_1 = Cbz-Ala, R_2 = OMe
S5: R_1 = Cbz-Pro, R_2 = OMe
S6: R_1 = Ac, R_2 = Phe-OMe

6a: R_1 = Ac, R_2 = OMe
6b: R_1 = Bz, R_2 = OMe
6c: R_1 = Ac-Phe, R_2 = OMe
6d: R_1 = Cbz-Ala, R_2 = OMe
6e: R_1 = Cbz-Pro, R_2 = OMe
6f: R_1 = Ac, R_2 = Phe-OMe

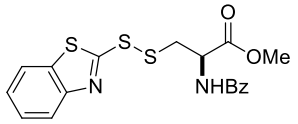
General Procedure for compounds 6. To a solution of 2,2'-dibenzothiazoyl disulfide (1.2 mmol) in THF (50 mL) was added a solution of cysteine derivative (1 mmol) in $CHCl_3$. The reaction mixture was stirred for 48 h at room temperature and then concentrated. The resulting residue was subjected to flash column chromatography to give the desired product.



6a

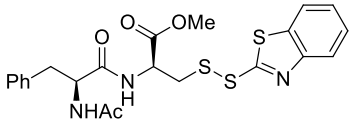
Compound **6a** was obtained as a white solid (70% yield). mp 135-136 °C; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$) δ 8.36 – 8.17 (m, 2H), 7.94 – 7.83 (m, 1H), 7.85 – 7.75 (m, 1H), 5.31 (dd, *J* = 7.4, 4.7 Hz, 1H), 4.18 (s, 3H), 3.92 (dd, *J* = 14.1, 4.8 Hz, 1H), 3.80 (dd, *J* = 14.1, 7.4 Hz, 1H), 2.47 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$) δ 171.6, 170.6, 154.5, 135.6, 126.6, 125.0, 121.8, 121.3, 52.7, 51.9, 40.8, 22.2; FT-IR (thin film, cm^{-1}) 3288.9, 3061.0,

2920.6, 1745.2, 1733.0, 1649.3, 1545.3, 1467.8, 1337.3, 1241.4, 1002.8, 747.8; MS (ESI) *m/z* calcd for $C_{13}H_{14}N_2NaO_3S_3$ $[M+Na]^+$ 365.0, found 365.0.



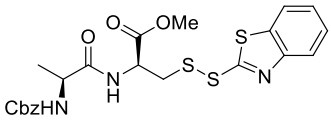
6b

Compound **6b** was obtained as a white solid (77% yield). mp 128-129 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68-7.76 (m, 4H), 7.26-7.53 (m, 6H), 5.05-5.08 (m, 1H), 3.69 (s, 3H), 3.56 (d, *J*= 4.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 167.4, 154.6, 136.1, 133.7, 132.2, 129.0, 128.9, 127.5, 126.7, 125.1, 122.4, 121.6, 53.2, 52.4, 41.7; FT-IR (thin film, cm^{-1}) 3361.3, 2954.4, 2926.1, 1741.0, 1641.1, 1516.1, 1462.0, 1210.0, 1003.9, 764.4; MS (ESI) *m/z* calcd for $C_{18}H_{16}N_2NaO_3S_3$ $[M+Na]^+$ 427.0, found 427.1.



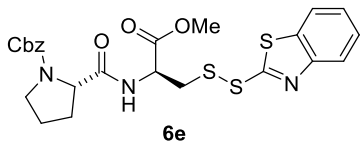
6c

Compound **6c** was obtained as a white solid (75% yield). mp 174-175 °C; ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD) δ 8.47 (d, *J* = 7.8 Hz, 2H), 8.24 – 7.96 (m, 3H), 7.86 (q, *J* = 7.0 Hz, 4H), 5.44 (s, 1H), 5.32 (s, 1H), 4.35 (s, 3H), 4.17 – 4.02 (m, 1H), 3.93 (m, 1H), 3.73 (m, 1H), 3.58 (m, 1H), 2.58 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD) δ 171.9, 171.8, 170.1, 154.6, 136.5, 129.2, 128.5, 126.8, 126.6, 125.0, 121.9, 121.4, 54.4, 54.3, 51.8, 40.7, 37.9, 37.8, 22.4, 22.3; FT-IR (thin film, cm^{-1}) 3301.2, 3260.4, 3068.3, 3027.6, 1732.1, 1670.6, 1642.2, 1527.8, 1425.7, 1000.7, 743.2; MS (ESI) *m/z* calcd for $C_{22}H_{23}N_3NaO_4S_3$ $[M+Na]^+$ 512.1, found 512.2.



6d

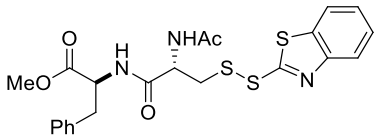
Compound **6d** was obtained as a white solid (72% yield). mp 145-146 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.1 Hz, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.55 – 7.26 (m, 8H), 5.58 (d, *J* = 7.5 Hz, 1H), 5.11 (s, 2H), 4.99 – 4.83 (m, 1H), 4.47 – 4.26 (m, 1H), 3.72 (s, 3H), 3.42 (m, 2H), 1.40 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 170.9, 170.3, 156.2, 154.9, 136.4, 136.1, 128.8, 128.4, 128.3, 126.7, 125.2, 122.5, 121.5, 67.3, 53.2, 52.1, 50.7, 41.2, 18.8; FT-IR (thin film, cm^{-1}) 3269.0, 3093.1, 2987.5, 1736.2, 1646.3, 1683.1, 1531.9, 1258.1, 1000.7, 755.5; MS (ESI) *m/z* calcd for $C_{22}H_{23}N_3NaO_5S_3$ $[M+Na]^+$ 528.1, found 528.1.



6e

Compound **6e** was obtained as a white solid (89% yield). mp 100-101 °C; *The NMR spectra are reported for a dynamic equilibrium between two rotamers*: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 – 7.67 (m, 3H), 7.34 (m, 7H), 5.16

(s, 2H), 4.83 (m, 1H), 4.38 (m, 1H), 3.82 – 3.23 (m, 7H), 2.41 – 1.83 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 171.9, 170.3, 156.1, 155.1, 136.6, 136.1, 128.7, 128.3, 128.2, 126.6, 125.1, 122.4, 121.5, 67.7, 61.0, 60.7, 53.1, 52.2, 51.7, 47.9, 47.3, 41.4, 31.4, 28.5, 24.8; FT-IR (thin film, cm^{-1}) 3329.9, 2974.3, 2945.7, 1740.3, 1703.4, 1695.3, 1654.5, 1519.6, 1417.5, 1352.1, 1205.0, 1111.0, 1090.6, 1000.7, 763.7; MS (ESI) *m/z* calcd for $C_{24}H_{25}N_3NaO_5S_3$ $[M+Na]^+$ 554.1, found 554.1.

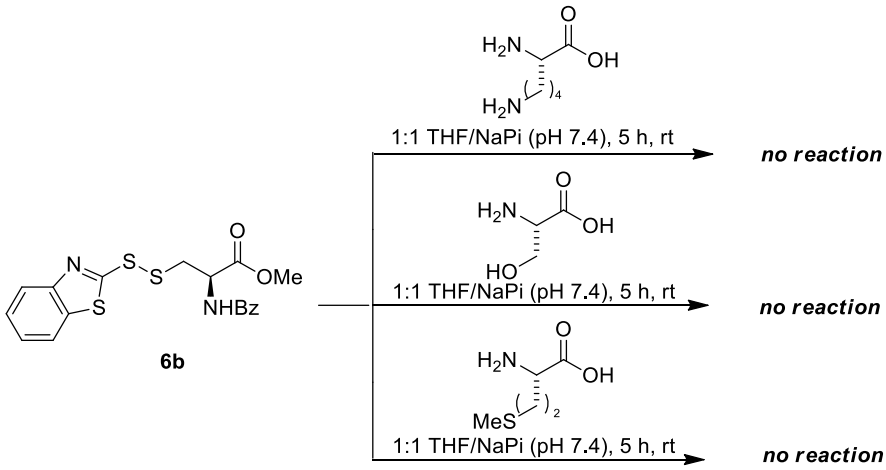


6f

Compound **6f** was obtained as a white solid (79% yield). mp 160-161 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.45 – 7.27 (m, 2H), 7.17 (m, 8.7 Hz, 6H), 5.00 – 4.75 (m, 2H), 3.68 (s, 3H), 3.43 (dd, *J* = 14.1, 6.0 Hz, 1H), 3.19 (dd, *J* = 14.3, 5.8 Hz, 2H), 3.07 (dd, *J* = 13.9, 7.0 Hz, 1H), 1.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 170.7, 169.8, 154.1, 136.4, 129.5, 128.8, 127.3, 126.7, 125.2, 122.2, 121.5, 54.0, 52.7, 52.6, 42.7, 37.8, 23.3; FT-IR (thin film, cm^{-1}) 3288.6, 3059.9, 2926.8, 1736.4, 1642.1, 1537.3, 1458.7, 1376.6, 1214.1, 1008.0, 754.7; MS (ESI) *m/z* calcd for $C_{22}H_{23}N_3NaO_4S_3$ $[M+Na]^+$ 512.1, found 512.1.

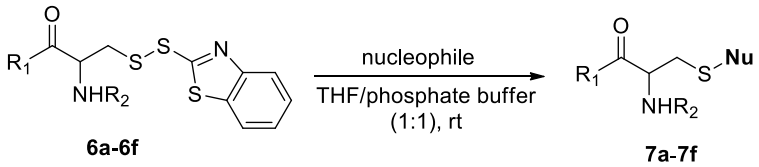
The reaction between 4 and MCA: To a round bottom flask containing compound **4** (93.7 mg, 0.224 mmol) was added 7 mL of THF. The solution was stirred for 5 min and then 7 mL of NaPi buffer (20 mM, pH 7,4) was added into the flask. The mixture was a homogeneous solution. MCA (2 eq, 44.5 mg, 0.449 mmol) was added at rt and the reaction was found to complete within 20 min (monitored by TLC). The aqueous mixture was quenched with 1N HCl (16 mL) and extracted with EtOAc (10 mL × 3). The combined organic layers were dried and concentrated. Flash column chromatography afforded the desired product **5f** (as 1:1 diastereomers) in 98 % as colorless sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (s, 1H), 7.36 – 7.16 (m, 5H), 7.12 – 6.89 (m, 1H), 4.93 – 4.74 (m, 1H), 4.58 (d, *J* = 8.3 Hz, 1H), 4.47 – 4.28 (m, 2H), 3.82 (s, 3H), 3.29 – 2.98 (m, 2H), 1.95 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.1, 164.7, 137.6, 128.9, 127.9, 127.8, 114.4, 114.2, 54.7, 54.6, 52.1, 52.1, 43.9, 36.2, 36.1, 34.9, 34.7, 23.2; FT-IR (thin film, cm^{-1}) 3280.8, 3072.4, 3035.6, 2953.9, 2925.3, 2247.0, 1746.5, 1635.7, 1523.7, 1458.3, 1433.8, 1368.4, 1311.2, 1229.5, 1025.2, 702.4; MS (ESI) *m/z* calcd for $C_{16}H_{19}N_3NaO_4S$ $[M+Na]^+$ 372.1, found 372.1.

Scheme S3. Stability of R-S-S-BT toward potential biological nucleophiles

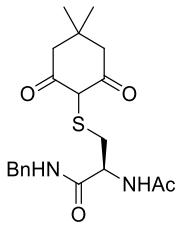


General procedure. To a solution of **6b** (dissolved in 1:1 THF/NaPi (pH 7.4)) was added 5 equiv of corresponding nucleophiles (L-Lysine, L-Serine, and L-methionine). The reaction mixture was stirred for 5 h at room temperature. The reaction was monitored by TLC. We found that the starting material **6b** was always fully recovered with no reactions.

Scheme S4. The reaction of R-S-S-BT substrates with carbon nucleophiles

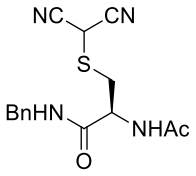


General procedure. To a stirring solution of R-S-S-BT substrate (0.2 mmol) in THF (8 mL) and NaPi (20 mM, 8 mL) was added each carbon nucleophiles (0.4 mmol). The reaction mixture was then stirred for 20 min at room temperature and quenched with 1N HCl (16 mL). The solution was extracted with ethyl acetate (10 mL × 3). The combined organic layers were washed with brine, dried over with anhydrous $MgSO_4$, and concentrated. The resulting residue was subjected to flash column chromatography to isolate the possible products or recovered starting materials.



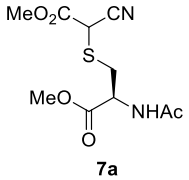
5b

Compound **5b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (t, J = 6.0 Hz, 1H), 7.76 (d, J = 6.9 Hz, 1H), 7.35 – 7.20 (m, 5H), 4.44 (m, 4H), 3.12 (dd, J = 13.6, 4.4 Hz, 1H), 2.43 (m, 5H), 2.02 (s, 3H), 1.09 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.8, 171.9, 171.4, 137.7, 128.6, 127.4, 127.4, 104.5, 53.1, 43.5, 37.7, 31.5, 29.7, 28.2, 22.6; FT-IR (thin film, cm^{-1}) 3268.5, 3066.1, 3029.5, 2952.4, 2923.4, 2854.0, 1645.1, 1543.0, 1475.5, 1369.9, 1264.3, 1144.9, 1025.5, 1009.9, 731.3; MS (ESI) *m/z* calcd for $C_{20}H_{26}N_2NaO_4S$ $[M+Na]^+$ 413.2, found 413.4.



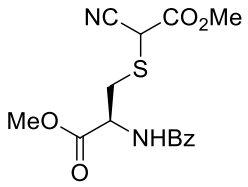
5e

Compound **5e**: 1H NMR (300 MHz, DMSO-*d6*) δ 8.73 (t, *J* = 6.0 Hz, 1H), 8.51 (d, *J* = 8.4 Hz, 1H), 7.90 (s, 1H), 7.32 – 7.23 (m, 5H), 4.73 (m, 1H), 4.29 – 4.25 (m, 2H), 3.32 – 3.21 (m, 2H), 1.91 – 1.81 (m, 3H);^{13}C NMR (75 MHz, $CDCl_3$/MeOH-*d4*) δ 171.7, 169.4, 137.4, 128.8, 127.9, 127.7, 111, 110.9, 51.9, 43.9, 36.1, 29.9, 22.9; FT-IR (thin film, cm^{-1}) 3276.7, 3064.2, 3031.6, 2917.1, 2851.8, 2202.0, 2157.1, 2112.1, 1638.1, 1523.7, 1450.2, 1368.4, 1241.8, 698.3; MS (ESI) *m/z* calcd for $C_{15}H_{16}N_4O_2S$ $[M-H]^-$ 351.1, found 351.5.



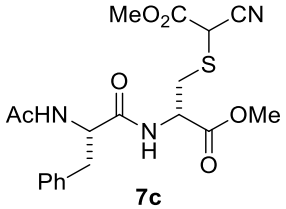
7a

Compound **7a** was obtained as a thick oil (as 1:1 diastereomers, 93% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.38 (s, 1H), 4.90 (q, *J* = 5.9 Hz, 1H), 4.43 (d, *J* = 27.1 Hz, 1H), 3.87 (s, 3H), 3.81 (s, 3H), 3.53 – 3.31 (m, 1H), 3.32 – 3.15 (m, 1H), 2.07 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 170.5, 164.6, 164.3, 114.1, 113.9, 54.6, 53.3, 51.8, 51.7, 36.1, 35.9, 34.7, 23.3; FT-IR (thin film, cm^{-1}) 3374.8, 3280.8, 2963.9, 2917.1, 2843.6, 2247.0, 1740.5, 1654.5, 1531.9, 1429.7, 1368.4, 1213.2, 1008.9; MS (ESI) *m/z* calcd for $C_{10}H_{14}N_2NaO_5S$ $[M+Na]^+$ 297.1, found 297.1.



7b

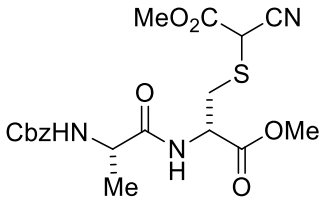
Compound **7b** was obtained as a thick oil (as 1:1 diastereomers, 99% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 – 7.74 (m, 2H), 7.59 – 7.36 (m, 3H), 7.18 – 7.02 (m, 1H), 5.08 (s, 1H), 4.59 – 4.35 (m, 1H), 3.82 (s, 6H), 3.54 (ddd, *J* = 14.9, 10.0, 4.7 Hz, 1H), 3.34 (ddd, *J* = 14.2, 9.2, 6.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 167.5, 164.6, 164.4, 133.4, 132.4, 128.9, 127.5, 114.0, 113.9, 54.6, 53.5, 53.4, 52.2, 52.2, 36.1, 35.9, 34.8, 34.6; FT-IR (thin film, cm^{-1}) 3329.9, 2953.9, 2921.2, 2855.8, 2242.9, 1740.8, 1642.2, 1519.6, 1486.9, 1429.7, 1213.2, 1017.0, 710.6; MS (ESI) *m/z* calcd for $C_{15}H_{16}N_2NaO_5S$ $[M+Na]^+$ 359.1, found 359.1.



7c

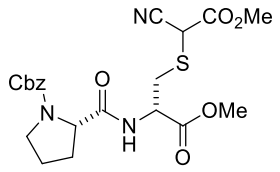
Compound **7c** was obtained as a yellowish solid (as 1:1 diastereomers, 99% yield). mp 113-114 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.04 (m, 6H), 6.53 – 6.30 (m, 1H), 4.87 – 4.65 (m, 2H), 4.55 – 4.28 (m, 1H), 3.77 (s, 3H), 3.67 (s, 3H), 3.36 – 2.97 (m, 4H), 1.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 170.7, 170.1, 169.9, 164.7,

136.5, 129.6, 129.5, 128.8, 127.2, 114.3, 54.6, 54.6, 54.5, 53.3, 52.1, 52.0, 38.2, 36.2, 35.9, 34.6, 34.5, 23.3; FT-IR (thin film, cm^{-1}) 3287.4, 3034.3, 2910.5, 2251.4, 1746.0, 1730.8, 1664.2, 1545.0, 1431.1, 1367.4, 1298.2, 1032.3, 754.2; MS (ESI) *m/z* calcd for $C_{19}H_{23}N_3NaO_6S$ $[M+Na]^+$ 444.1, found 444.2.



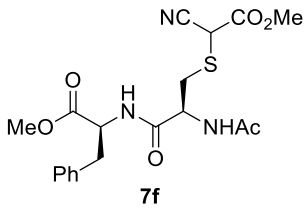
7d

Compound **7d** was obtained as a thick oil (as 1:1 diastereomers, 96% yield): ^1H NMR (300 MHz, $CDCl_3$) δ 7.61 – 6.98 (m, 6H), 5.60 (s, 1H), 5.10 (s, 2H), 4.87 (s, 1H), 4.67 – 4.42 (m, 1H), 4.32 (s, 1H), 3.81 (s, 3H), 3.76 (s, 3H), 3.47 – 3.11 (m, 2H), 1.38 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 170.4, 170.3, 164.7, 164.7, 156.3, 136.4, 128.8, 128.4, 128.3, 114.4, 114.1, 67.3, 67.3, 54.6, 54.6, 53.3, 53.3, 52.0, 50.8, 36.2, 35.9, 34.6, 34.4, 18.6, 18.4; FT-IR spectra (thin film, cm^{-1}) 3313.5, 2953.9, 2921.2, 1851.8, 2247.0, 1740.3, 1738.5, 1670.8, 1515.6, 1429.7, 1319.4, 1209.1, 1017.0, 735.1, 702.4; MS (ESI) *m/z* calcd for $C_{19}H_{23}N_3NaO_7S$ $[M+Na]^+$ 460.1, found 460.2.



7e

Compound **7e** was obtained as a thick oil (as 1:1 diastereomers, 92% yield): *The NMR spectra are reported for a dynamic equilibrium between two rotamers*: ^1H NMR (300 MHz, $CDCl_3$) δ 7.79 – 7.11 (m, 6H), 5.13 (s, 2H), 4.98 – 4.52 (m, 2H), 4.49 – 4.23 (m, 1H), 3.93 – 3.65 (m, 6H), 3.60 – 3.27 (m, 3H), 3.13 (s, 1H), 2.29 – 1.87 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 172.3, 170.3, 164.9, 164.7, 156.2, 155.2, 136.6, 128.7, 128.3, 128.2, 128.1, 114.2, 67.6, 61.0, 60.7, 54.5, 53.3, 53.22, 52.1, 51.9, 51.2, 51.1, 47.8, 47.2, 36.3, 35.7, 35.0, 34.7, 29.9, 28.9, 24.8, 23.9; FT-IR (thin film, cm^{-1}) 3317.1, 2962.1, 2947.5, 2851.8, 2242.9, 1748.5, 1679.0, 1519.6, 1454.3, 1433.8, 1405.2, 1352.1, 1209.1, 111.0, 739.2; MS (ESI) *m/z* calcd for $C_{21}H_{25}N_3NaO_7S$ $[M+Na]^+$ 486.1, found 486.1.

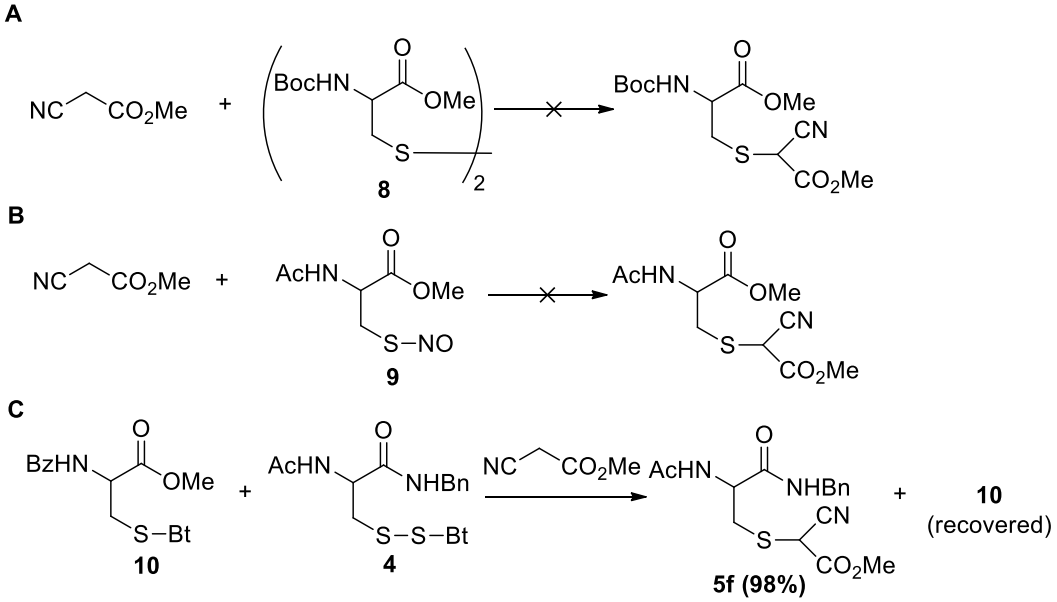


7f

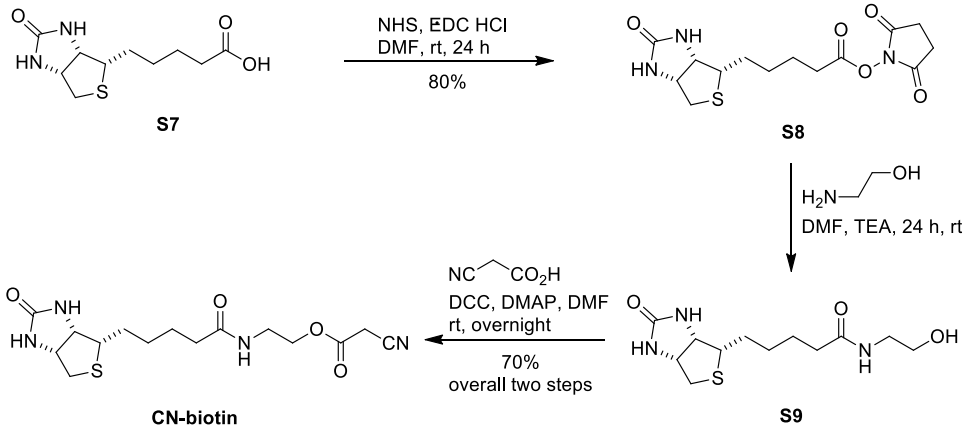
Compound **7f** was obtained as a thick oil (as 1:1 diastereomers, 99% yield): ^1H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.15 (m, 4H), 7.18 – 7.02 (m, 2H), 6.88 – 6.65 (m, 1H), 4.89 – 4.68 (m, 2H), 4.61 (d, *J* = 6.5 Hz, 1H), 3.82 (s, 3H), 3.70 (s, 3H), 3.26 – 2.93 (m, 4H), 1.95 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 171.6, 170.9, 169.9, 165.2, 164.8, 135.9, 129.4, 128.8, 128.8, 127.4, 127.4, 114.6, 114.4, 54.7, 54.6, 53.9, 52.7, 51.9, 37.8, 37.8, 36.3, 36.3,

34.8, 34.5, 23.2; FT-IR (thin film, cm^{-1}) 3289.0, 3060.2, 3027.5, 2958.0, 2940.3, 2851.8, 2247.0, 1744.4, 1650.4, 1519.6, 1429.7, 1972.5, 1245.9, 1209.1, 1017.0, 743.2; MS (ESI) *m/z* calcd for $C_{19}H_{23}N_3NaO_6S$ $[M+Na]^+$ 444.1, found 444.2.

Scheme S5. Control experiments



Scheme S6. Synthesis of biotinylated α-cyano ester (CN-biotin)



Compound S8. To a suspension of biotin **S7** (4.06 mmol, 990 mg) in anhydrous DMF (60 mL) was added 1.1 equiv of *N*-hydroxysuccinimide (4.46 mmol, 514 mg). EDC·HCl (4.87 mmol, 933 mg) was then added and the reaction was stirred for 24 hours (at this time the solution turned clear). The solvent was then removed under reduced pressure to provide a white solid. The solid was washed thoroughly with anhydrous methanol, filtered and dried to provide **S8** as a white solid (1.104 g, 80% yield). The product was used directly in the next step without any further purification.

Compound S9. To a solution of compound **S8** (3.24 mmol, 1.104 g) in anhydrous DMF (66 mL) was added 2-aminoethanol (4.86 mmol, 0.29 mL) dropwise. Then, triethylamine (6.48 mmol, 0.9 mL) was added and the reaction mixture was stirred overnight. The solvent was removed under reduced pressure and the resulting residue

was purified by flash column chromatography (gradient elution: 50:1 DCM/MeOH to 7:1 DCM/MeOH). The product **S9** was used directly in the next step.

CN-Biotin. To a solution of compound **S9** (3.24 mmol) in DMF (50 mL) was added cyanoacetic acid (3.89 mmol, 331 mg). DCC (4.2 mmol, 867 mg) was then added followed by DMAP (0.32 mmol, 40 mg,). The reaction mixture was stirred overnight and the solvent was removed under reduced pressure to give the crude product. Flash column chromatography (gradient elution: 50:1 DCM/MeOH to 7:1 DCM/MeOH) afforded the final product as a white solid (804 mg). Yield: 70% for two steps. mp 132-134 ^{o}C; ^{1}H NMR (300 MHz, DMSO-*d6*) δ 7.96 (t, *J* = 5.3 Hz, 1H), 6.43 (s, 1H), 6.37 (s, 1H), 4.30 (dd, *J* = 7.7, 4.8 Hz, 1H), 4.11 (m, 3H), 3.97 (s, 2H), 3.36 – 3.23 (m, 2H), 3.16 – 3.03 (m, 1H), 2.82 (dd, *J* = 12.4, 5.0 Hz, 1H), 2.57 (m, 1H), 2.06 (t, *J* = 7.3 Hz, 2H), 1.68 – 1.37 (m, 4H), 1.29 (m, 2H); ^{13}C NMR (75 MHz, DMSO-*d6*) δ 172.3, 164.3, 162.6, 114.9, 64.4, 60.9, 59.1, 55.3, 39.7, 37.1, 34.9, 28.1, 27.9, 25.0, 24.5. FT-IR (thin film, cm^{-1}) 3270.6, 3079.7, 2931.3, 2264.3, 2200.7, 1742.4, 1696.7, 1549.2, 1264.7, 1032.1, 726.8. MS (ESI) m/z, calcd for $C_{15}H_{22}N_4NaO_4S$ $[M+Na]^+$ 377.1, found 377.1.

Mass analysis of tag-switch assay with Gpx3-persulfide: A freshly prepared Gpx3 protein persulfide solution[4] (20 μM final concentration) was incubated with (or without) MSBT-A (20 mM final concentration) respectively, followed by the addition of ethyl cyanoacetate (20 mM final concentration) at rt for 1 hour. The protein was then purified through a P-30 spin column. Sample aliquots were then generated and analyzed by LC−MS.

Evaluation of the tag switch assay: Preparation of bovine serum albumin (BSA) sulfenic acid (BSA-SOH) was done following the published protocol.[10] Briefly, 0.98 mM BSA was reduced with 5 mM 2-mercaptoethanol and dialysed over night. Samples were additionally degassed with argon. BSA was then incubated with 10 mM H_2O_2 (15 min, 37 °C) and the reaction was stopped by the addition of bovine heart catalase (1221 U, Sigma Aldrich, USA). Formation of the sulfenic acid was confirmed by treating the freshly prepared BSA-SOH with 50 mM dimedone and detecting the dimedone-labeled peptide (after trypsin digestion) by LC-ESI-TOF-MS. Glutathionylated samples (BSA-SSG) were made by incubation of BSA-SOH with 2 mM glutathione, and S-sulfhydrated samples (BSA-SSH) by incubation with 2 mM H_2S (15 min, room temperature). Each sample was desalted on biospin column, treated with 10 mM MSBTA (15 min, 37 °C) and 2 mM CN-biotin (with desalting on biospin column after each step). Prepared samples were then analyzed by ultra-high resolution ESI-TOF-MS (maXis, Bruker Daltonics). One part of the samples was mixed with streptavidin agarose resin (Thermo Fischer Scientific), incubated for 2 h at room temperature and the concentration of remaining proteins in the supernatant quantified by Roti^{R} Nanoquant (Karl Roth, Karlsruhe, Germany). Resins were washed, the bound proteins eluted following the manufacturer's instruction and the absorbance at 280 nm measured. In a separate experiment, 10 μL of BSA-SH, BSA-SOH, BSA-SSG and BSA-SSH (treated with tag switch assay) were loaded on nitrocellulose membrane. After drying, membrane was blocked with 5 % non-fat milk for 1 h at room temperature, and incubated 2 h with HRP-labelled mouse monoclonal anti-biotin antibody (Sigma Aldrich, St. Louis, MO) according to the manufacture recommendation. Signal was visualized using Pierce ECL reagent (Thermo Scientific, IL, USA) and exposing membrane to x-ray films.

Mechanistic studies of protein S-sulfhydration: 2 mg/mL GAPDH (from rabbit muscles, Sigma Aldrich, USA) was incubated with either 100 μM H_2S, 100 μM H_2S with rigorous vortexing, 100 μM Angeli's salt and 100 μM H_2O_2 for 30 min at rom temperature. GAPDH was also treated with 100 μM Angeli's salt and 100 μM H_2O_2 for 15

min followed by additional 15 min with 100 μM H_2S. Additionally, 2 mg/mL GAPDH was mixed with 100 μM H_2S and 20 μM water-soluble iron prophyrin for 30 min. Samples were then desalted in biospin columns and further labeled by tag switch assay.

Cell culture: Jurkat cells were cultured in RPMI supplemented with 10% FCS, 1% nonessential amino acid mix and 1% Streptomycin-Penicillin at 37 °C, and 5% CO_2. Human umbilical vein endothelial cells (HUVECs, passage 2-3) were cultured in 35-mm μ-Dishes (ibidi, Martinsried, Germany) in endothelial cell growth medium 2 (PromoCell GmbH) at 37 °C and 5% CO_2.

Jurkat cell extracts: JURKAT cells (5 x 10^6) were incubated without or with 200 μM Na_2S (30 min). Cells were centrifuged and lysed in HEN buffer (250 mM HEPES, 50 mM NaCl, 1mM EDTA, 0.1 mM neocuproine, 1 % protease inhibitors, pH 7.4) containing 1 % NP40 detergent using ultrasonicator. Total cell lysates were then split into two parts, one kept as it was and the other one was additionally mixed with 200 μM Na_2S and vortexed for 30 min.

Tag-switch assay for *S*-sulfhydration: Protein samples were treated with 50 mM MSBT-A (2.5% SDS, 1 h, 37 °C) and desalted on biospin columns (BioRad). Proteins were then treated with CN-biotin (1h, 37 °C). Prior the electrophoresis, samples were additionally desalted, mixed with 5 x Laemmli buffer, and then resolved on 8% or 10% SDS polyacrylamide gel. Proteins were transferred on nitrocellulose membrane using semi dry system (Bio Rad, USA), blocked with 5% non-fat milk for 1 h at room temperature, and incubated over night with HRP-labelled mouse monoclonal anti-biotin antibody (Sigma Aldrich, St. Louis, MO) according to the manufacture recommendation. Signal was visualized using Pierce ECL reagent (Thermo Scientific, IL, USA) and exposing membrane to x-ray films.

Pull-down assay: Biotinylated whole cell lysates were additionally incubated with streptavidin agarose resins (1-3 mg of biotin/mL binding capacity) for 30 min at room temperature with constant mixing and unbound proteins analysed by SDS-PAGE followed by Western Blot.

MeOH fixation and *S*-Sulfhydration detection: HUVECs were grown in ibidi dishes and then treated with 100 μM Na_2S for 30 min, 2 mM PG (propargylglycine, CSE inhibitor) for 2 h or pre-treated with 2 mM PG (2 h) and then exposed to Na_2S for 30 min. After the treatment cells were fixed with ice cold MeOH and kept at -20 °C for 15 min. Cells were washed with 80 % MeOH/20 % HEN buffer (250 mM HEPES, 50 mM NaCl, 1mM EDTA, 0.1 mM neocuproine) (v/v) and then exposed to 50 mM MSBT-A over night in the same solvent (MeOH/HEN buffer, 80/20). After thorough washing (5 x 10 min) the cells were exposed to 2 mM CN-biotin for 2 h in the same solvent. Finally, cells were incubated 1 h with fluorescein-labelled streptavidin (Thermo Fischer Scientific, IL, USA) in PBS.

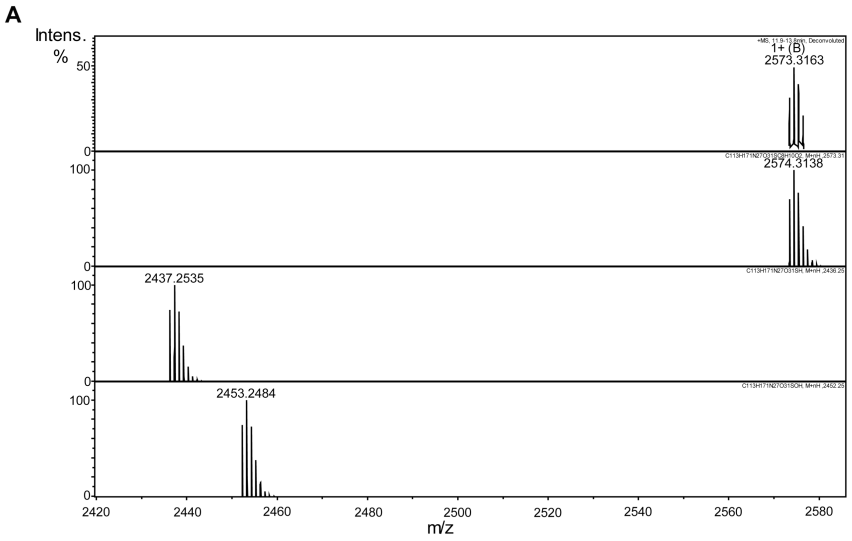
Paraformaldehyde fixation and *S*-sulfhydration: HUVECs were grown in ibidi dishes and treated with 100 μM Na_2S (30 min), and 2 mM 2-ketobutiric acid (2 h). Cells were fixed with 4 % paraformaldehyde for 20 min. After washing with PBS cells were exposed to PBS containing 1 % Triton X-100 (v/w) for 2 h to permeabilize the cells and increase availability of thiols to MSBT-A. 50 mM MSBTA was made in the same solvent and cells kept in this solution at room temperature over night. Cells were then washed (5 x 10 min) with cold PBS and treated with 2

mM CN-biotin in PBS for 2 h. This was followed by incubation with fluorescein-labelled streptavidin (Thermo Fischer Scientific, IL, USA) for 1 h.

Colocalization studies: For visualization of endoplasmic reticulum HUVECs were transfected with CellLight^{TM} ER-GFP following the instruction of the manufacturer (Molecular Probes, Invitrogen, Germany). Mitochondria were visualized by treating the cell with 500 nM MitoTracker^{R} Red CMXRos (Molecular Probes, Invitrogen) for 45 min prior the fixation. Cells were treated with 100 μM Na_2S (30 min), washed and fixed with ice cold methanol for 20 min at -20 °C. Cell permabilization was performed with 5 min exposure to ice cold acetone. After that cells were washed and treated with 50 mM MSBT-A, then 5 mM CN-biotin and finally with DyLight^{TM}405 conjugated streptavidin (Thermo Fischer Scientific, IL, USA), 45 min at 37 °C for each step. Between each step samples were washed extensively (5 x 5 min).

Evaluation of the *in situ* detection of *S*-sulfhydrated proteins: Paraformaldehyde fixed HUVEC cells were exposed to either CN-biotin alone or the whole tag switch assay. Additionally, after fixation and permeabliziation HUVECs were pre-incubated with 50 mM dimedone and protein persulfides labelled by tag switch assay. This was followed by incubation with fluorescein-labelled streptavidin (Thermo Fischer Scientific, IL, USA) for 1 h. Nuclei were stained with DAPI.

Fluorescent microscopy: Fluorescent microscopy was carried out using Carl Zeiss Axiovert 40 CLF inverted microscope, equipped with monochromatic RGB CoolLed light source (Andover, UK) and monochromatic AxioCam Icm1 camera. All experiments were performed at least in triplicate. Images were post-processed in ImageJ software (NIH, USA).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Intens.
%
1+ (B)
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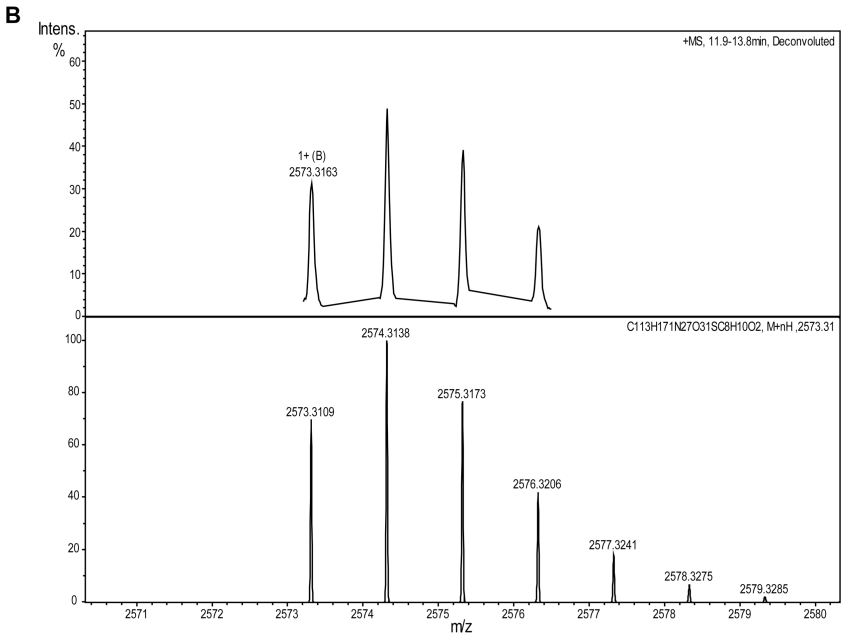
m/z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%
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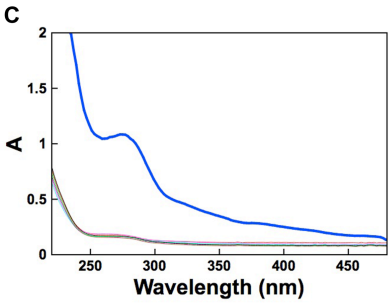

C
A
Wavelength (nm)

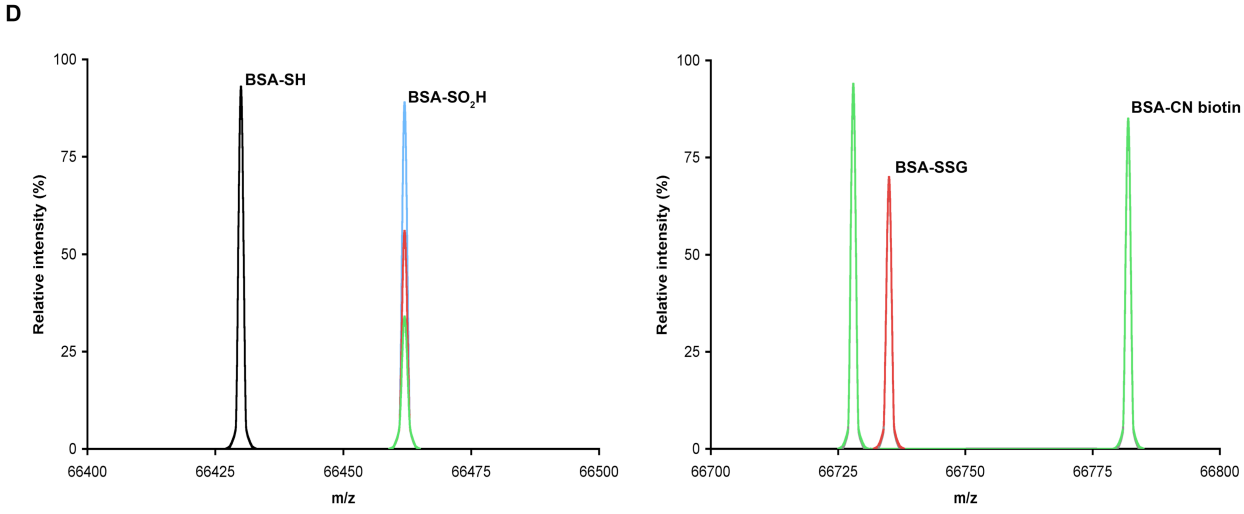

D
BSA-SH
BSA-SO2H
BSA-SSG
BSA-CN biotin
Relative intensity (%)
m/z

Figure S1. Evaluation of the tag switch assay. A) Preparation of bovine serum albumin (BSA) sulfenic acid (BSA-SOH) was done following the published protocol.[10] Formation of the sulfenic acid was confirmed by treating the freshly prepared BSA-SOH with 50 mM dimedone and detecting the dimedone-labeled peptide (after trypsin digestion) by LC-ESI-TOF-MS. Original MS data from DataAnalysis software shown in **A**) represent the measured, deconvoluted spectrum of GLVLIAFSQYLQQ^{34}CPFDEHVK peptide. Spectrum bellow is simulated isotopic distribution for the peptide labeled with dimedone. The third spectrum represents the simulation of the isotopic distribution of the peptide only, and the last spectrum is the simulation of isotopic distribution for the peptide containing cysteine in the form of sulfenic acid. B) Isotopic distribution of the measured (upper spectrum) and simulated (lower spectrum) of dimedone-labeled GLVLIAFSQYLQQ^{34}CPFDEHVK confirming that the used synthetic protocol indeed gives mainly BSA-SOH. **C**) UV-vis detection of the proteins eluted from streptavidin agarose resins. BSA-SH (black line), BSA-SOH (blue line), BSA-SSG (red line) and BSA-SSH (green line) were treated with tag switch assay and mixed with streptavidin agarose resins. Resins were washed and the eluted proteins analyzed by UV-vis spectroscopy for the presence of the proteins (280 nm). **D**) ESI-TOF MS analysis of the BSA-SOH, BSA-SSG and BSA-SSH treated with tag switch assay. Figure shows re-drown deconvoluted MS spectra obtained using DataAnalysis software (Bruker Daltonics). Untreated BSA served as a control giving the peak at m/z 66430±2 Da. BSA-SOH sample gave only the peak at m/z 66462±2 Da which we assigned to BSA-SO_2H (mass change is 32 when compared to the control spectrum). BSA-SO_2H was inevitable end product after treating the samples of BSA-SOH for few hours with all the reagents of tag switch assay. The same peak could be seen in BSA-SSG and BSA-SSH samples. However, BSA-SSG sample clearly shows the peak at m/z 66735±2 Da (addition of a glutathione moiety) while the BSA-SSH sample has a peak at m/z 66782±2 Da, which is assigned to the BSA labeled with CN-biotin. ~50 % yield of the BSA-SSH is in good agreement with the data obtained by the analysis of the proteins bound after exposure to streptavidin agarose resins (Figure 2). The peak at m/z 66728 could be the decomposition product of BSA-CN biotin derivative, suggesting the loss of NC(O)N moiety of biotin structure.

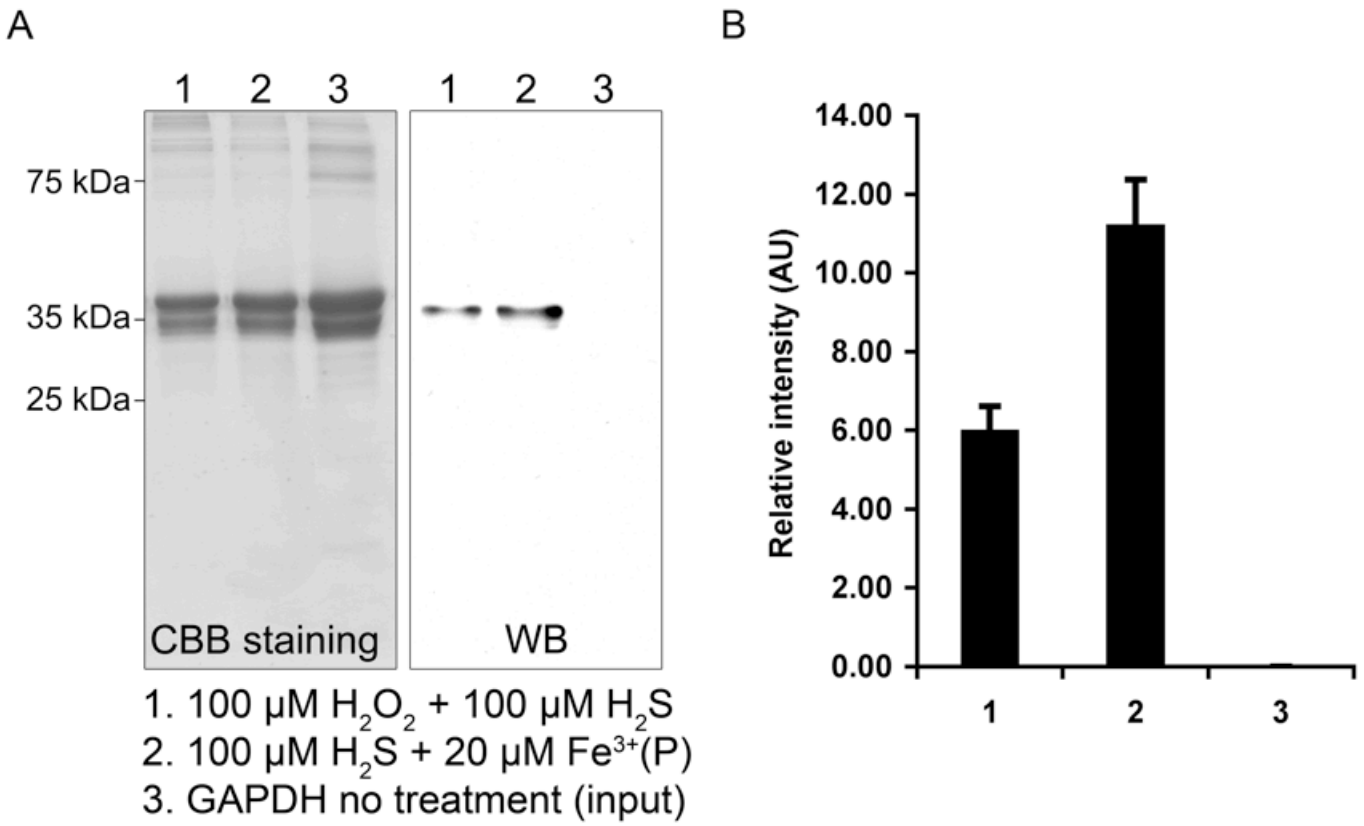


Figure S2. Possible reaction pathways for S-sulfhydration. A) Coomassie blue-staining for the protein load (left) and Western blot of *S*-sulfhydrated GAPDH. B) Quantification of the *S*-sulfhydration yield, compared to the control. GAPDH (2 mg/mL) was used as a model protein. Untreated sample served as a control. Samples were treated as marked in the figure.

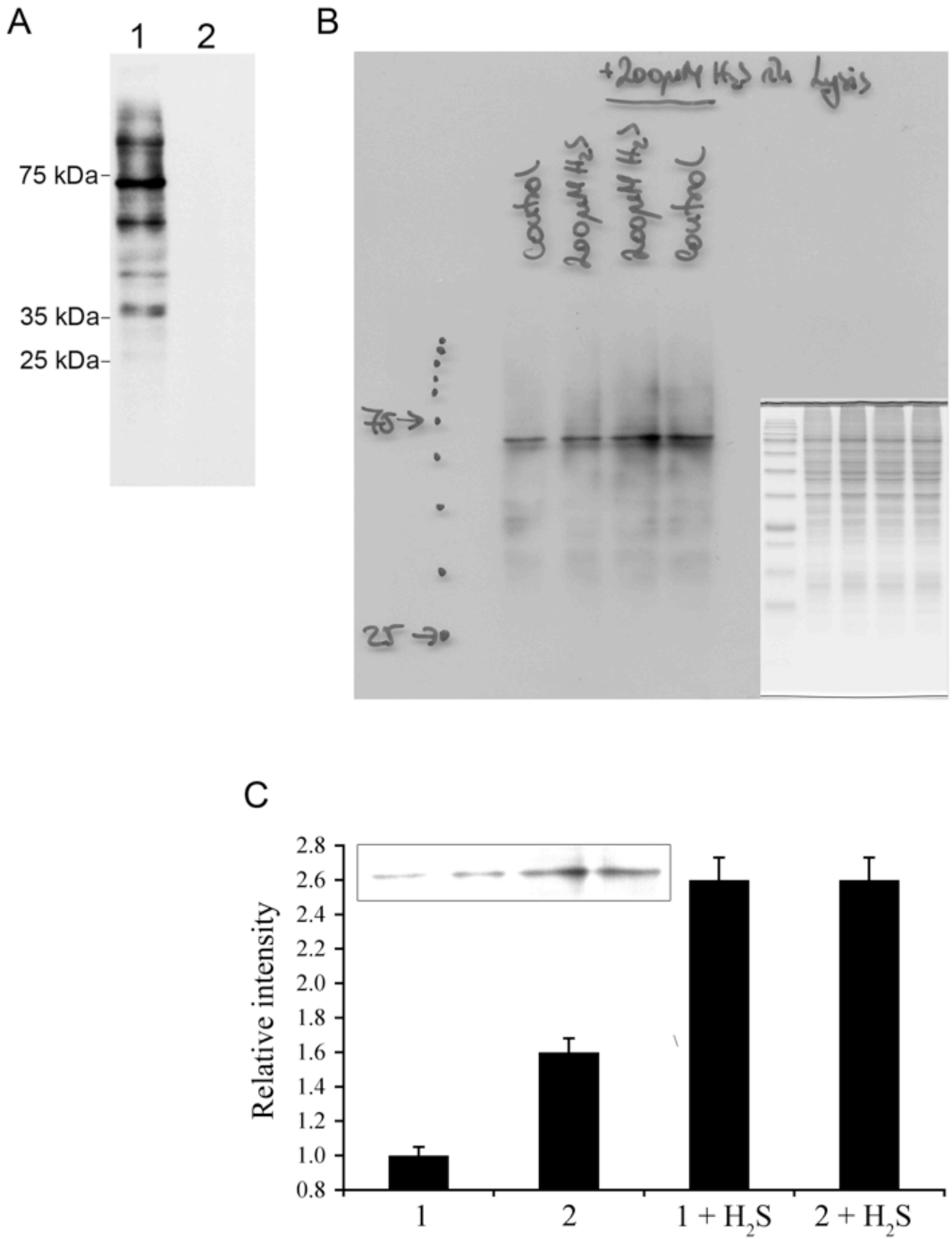


Figure S3. Tag-switch assay for protein *S*-sulfhydration. Jurkat cells (5 x 10^6) were incubated without or with 200 μM Na_2S (30 min) and then centrifuged and lysed in HEN buffer (250 mM HEPES, 50 mM NaCl, 1mM EDTA, 0.1 mM neocuproine, 10 % protease inhibitors, pH 7.4) containing 1 % NP40 detergent. Total cell lysates were then split into two parts, one kept as it was and the other one was additionally mixed with 200 μM Na_2S and vortexed for 30 min. **A**) Western blot of the whole cell lysate before (1) and after (2) it has been mixed with streptavidin agarose resins. **B**) Original, unprocessed scan of the nitrocellulose membrane. Inset: Coomasie blue staining for the protein load. **C**) Quantification of the *S*-sulfhydration based on the intensity of the 70 kDa band. 1: control, 2: H_2S-treated cells, 1 + H_2S: control cell lysates treated with H_2S, 2 + H_2S: cell lysates from H_2S-treated cells additionally treated with 200 μM H_2S.

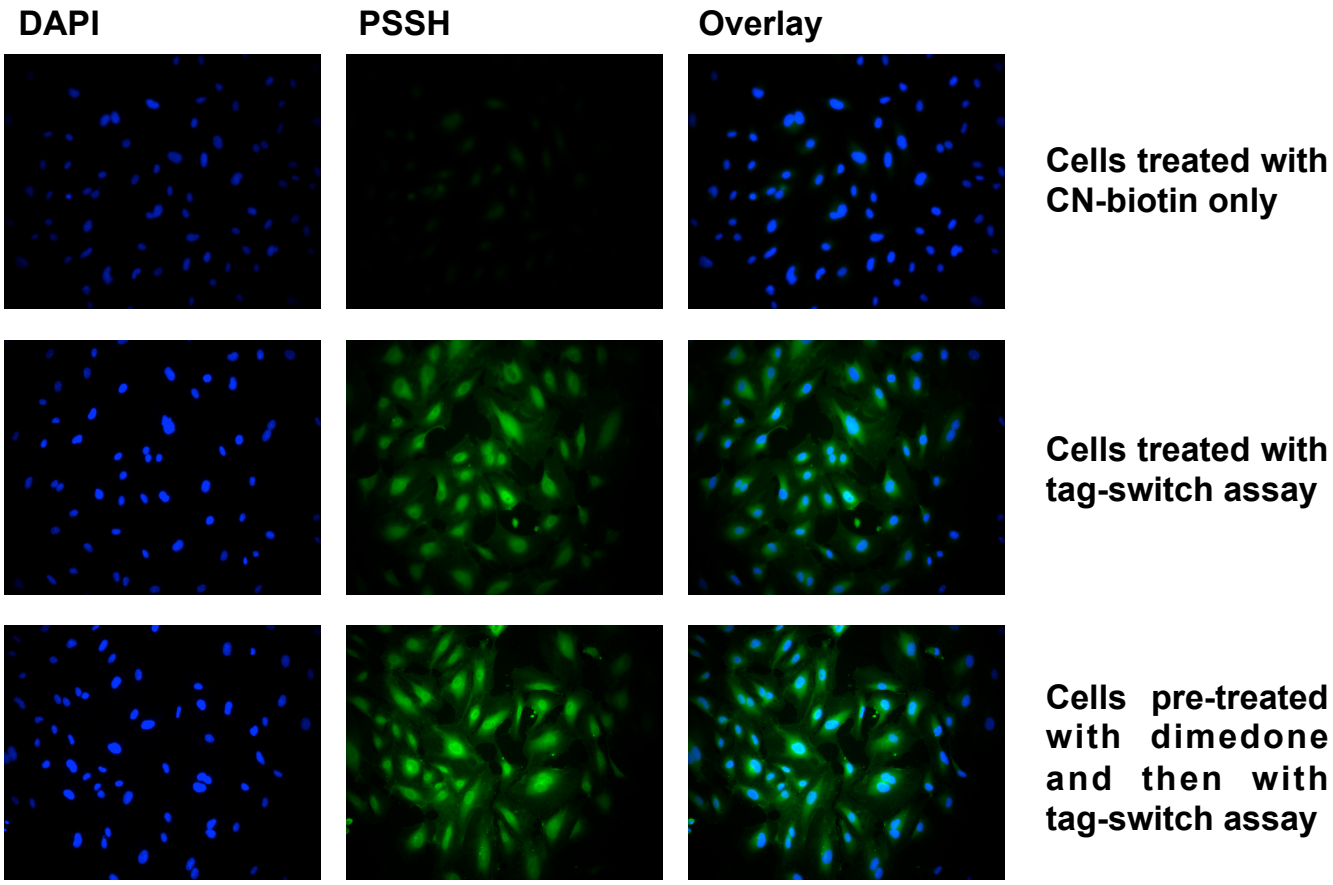


Figure S4. Evaluation of the in situ method for the intracellular detection of protein persulfides. Paraformaldehyde fixed HUVEC cells (treated with 200 µM Na_2S) were exposed to either CN-biotin alone or the whole tag switch assay. Additionally, after fixation and permeablization HUVECs were pre-incubated with 50 mM dimedone (to block all intracellular sulfenic acids) and protein persulfides labelled by tag switch assay. Biotinylated proteins were visualized with fluorescein-labelled streptavidin (Thermo Fischer Scientific, IL, USA) for 1 h. Nuclei were stained with DAPI.

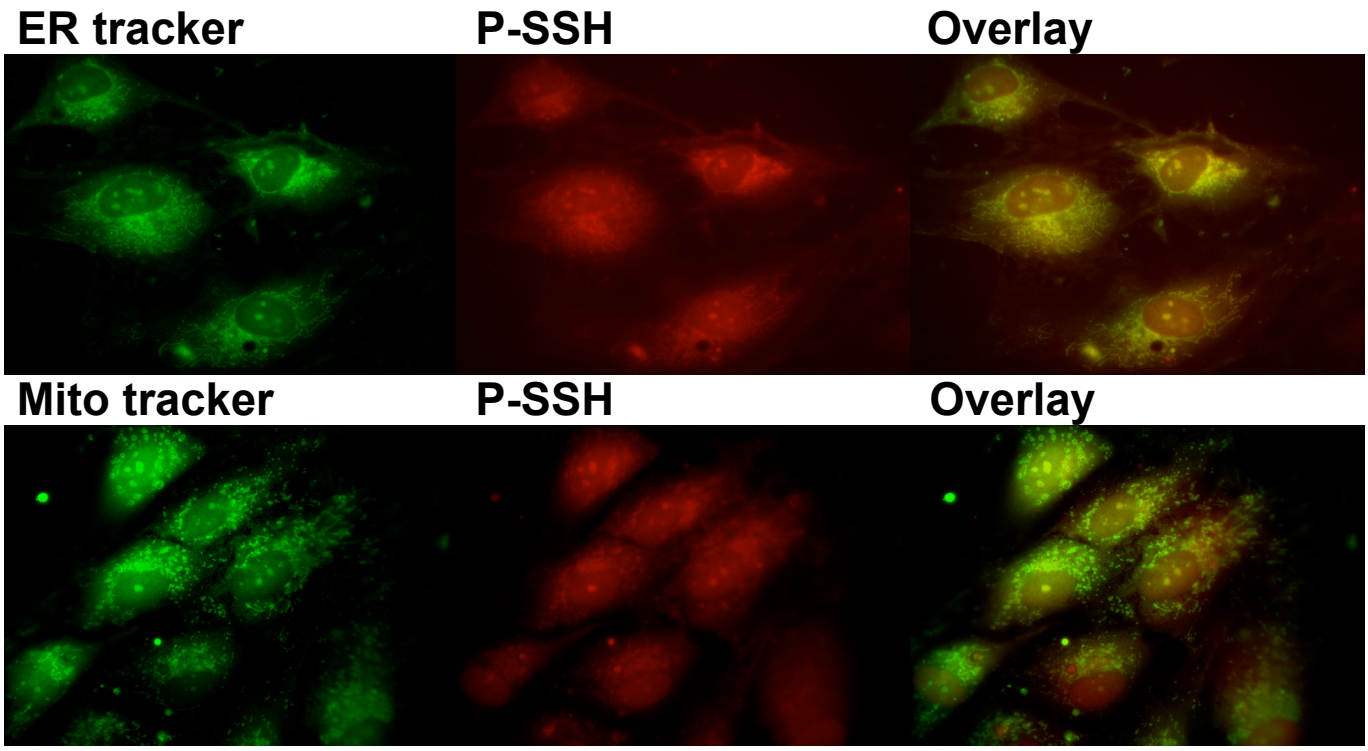
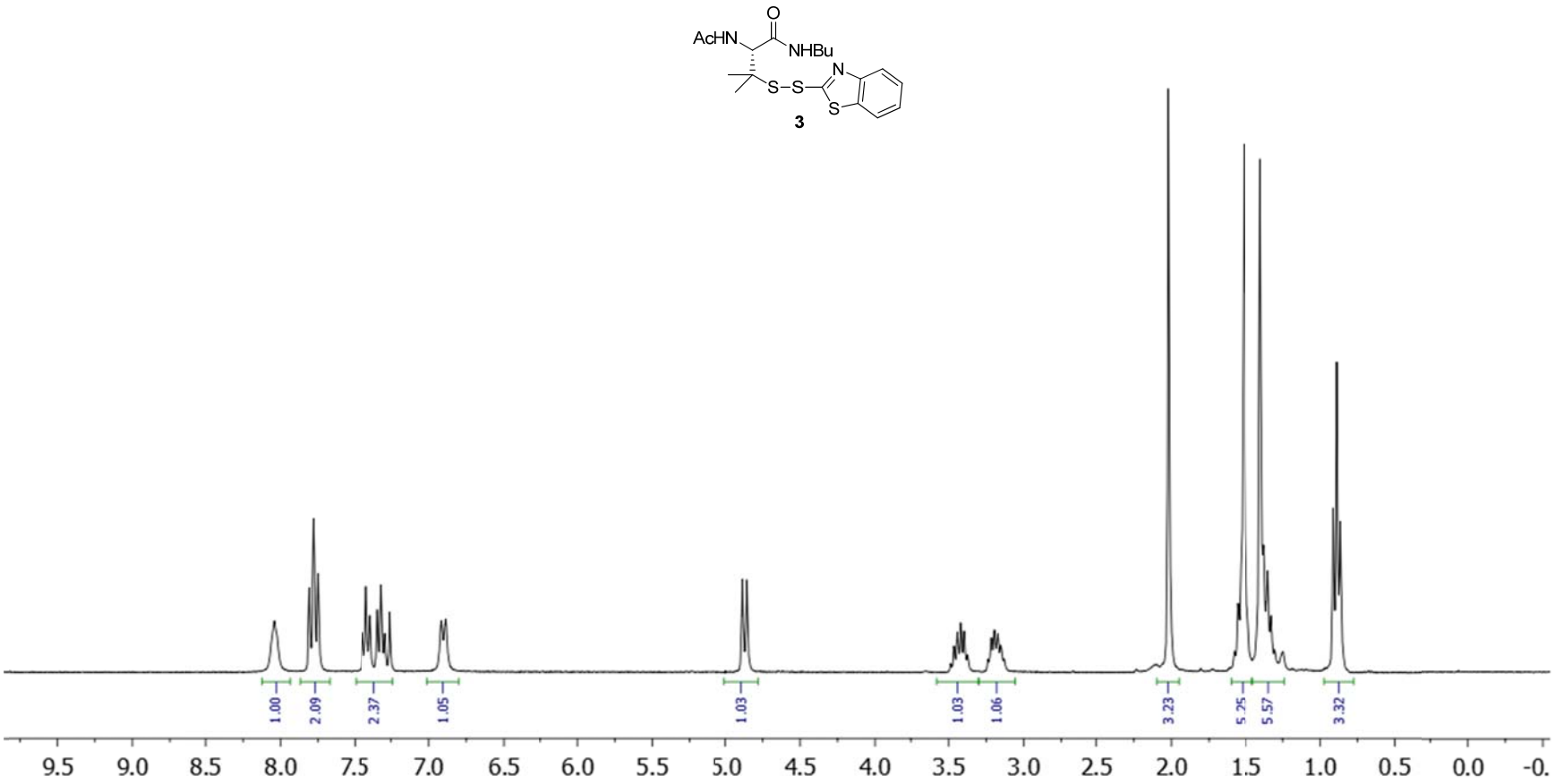
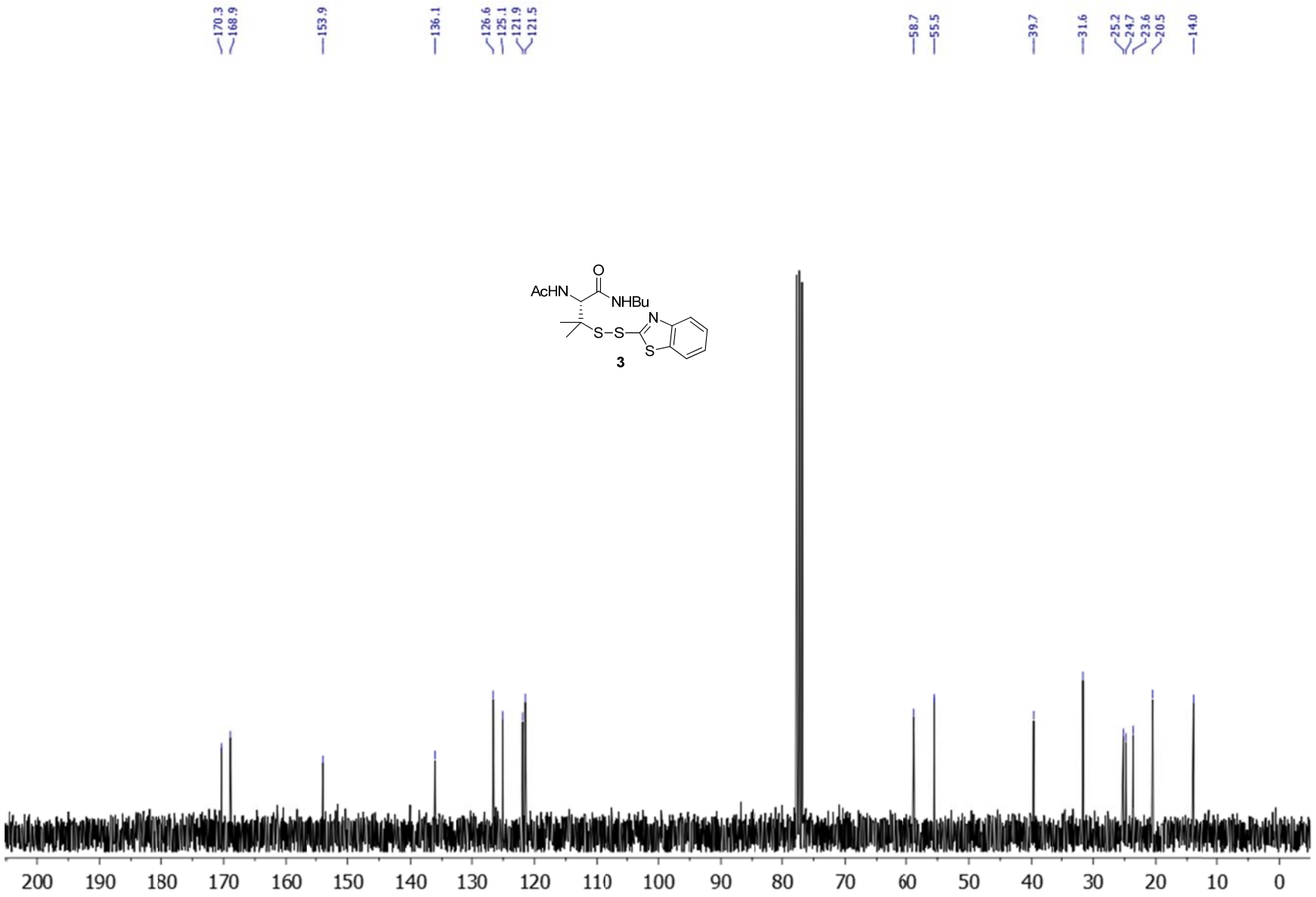


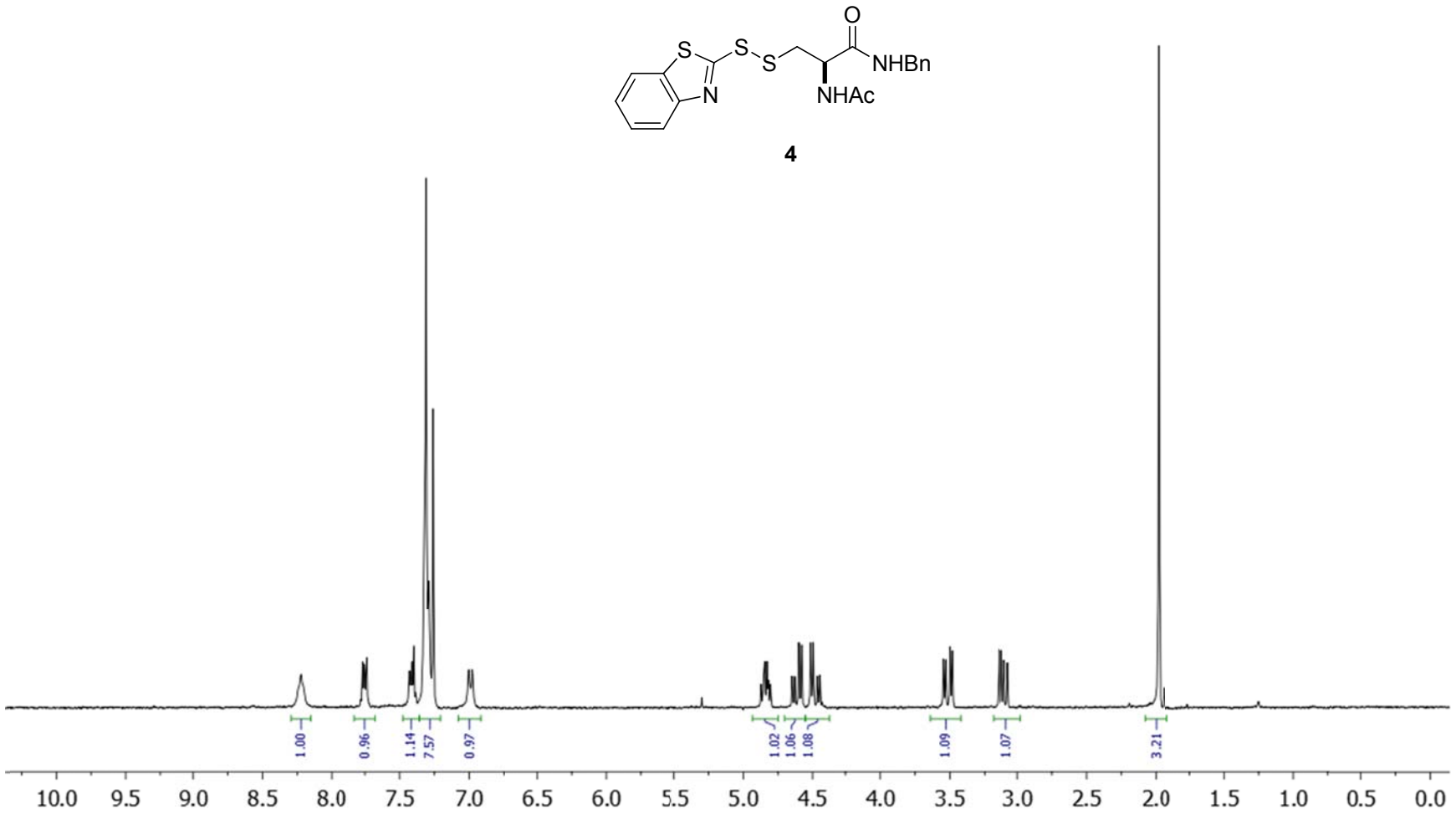
Figure S5. Colocalization of intracellular persulfides with endoplasmic reticulum and mitochondria. For visualization of endoplasmic reticulum HUVECs were transfected with CellLight^{TM} ER-GFP (green fluorescence) following the instruction of the manufacturer (Molecular Probes, Invitrogen, Germany). Mitochondria were visualized by treating the cell with 500 nM MitoTracker^{R} Red CMXRos (red fluorescence, Molecular Probes, Invitrogen) for 45 min prior the fixation. Cells were treated with 100 μM Na_2S (30 min), washed and fixed with ice cold methanol for 20 min at -20 °C. Cell permabilization was performed with 5 min exposure to ice cold acetone. After that cells were washed and treated with 50 mM MSBT-A, then 5 mM CN-biotin and finally with DyLight^{TM}405 conjugated streptavidin (blue fluorescence, Thermo Fischer Scientific, IL, USA), 45 min at 37 °C for each step. For better presentation pictured were artificially colored green and red.



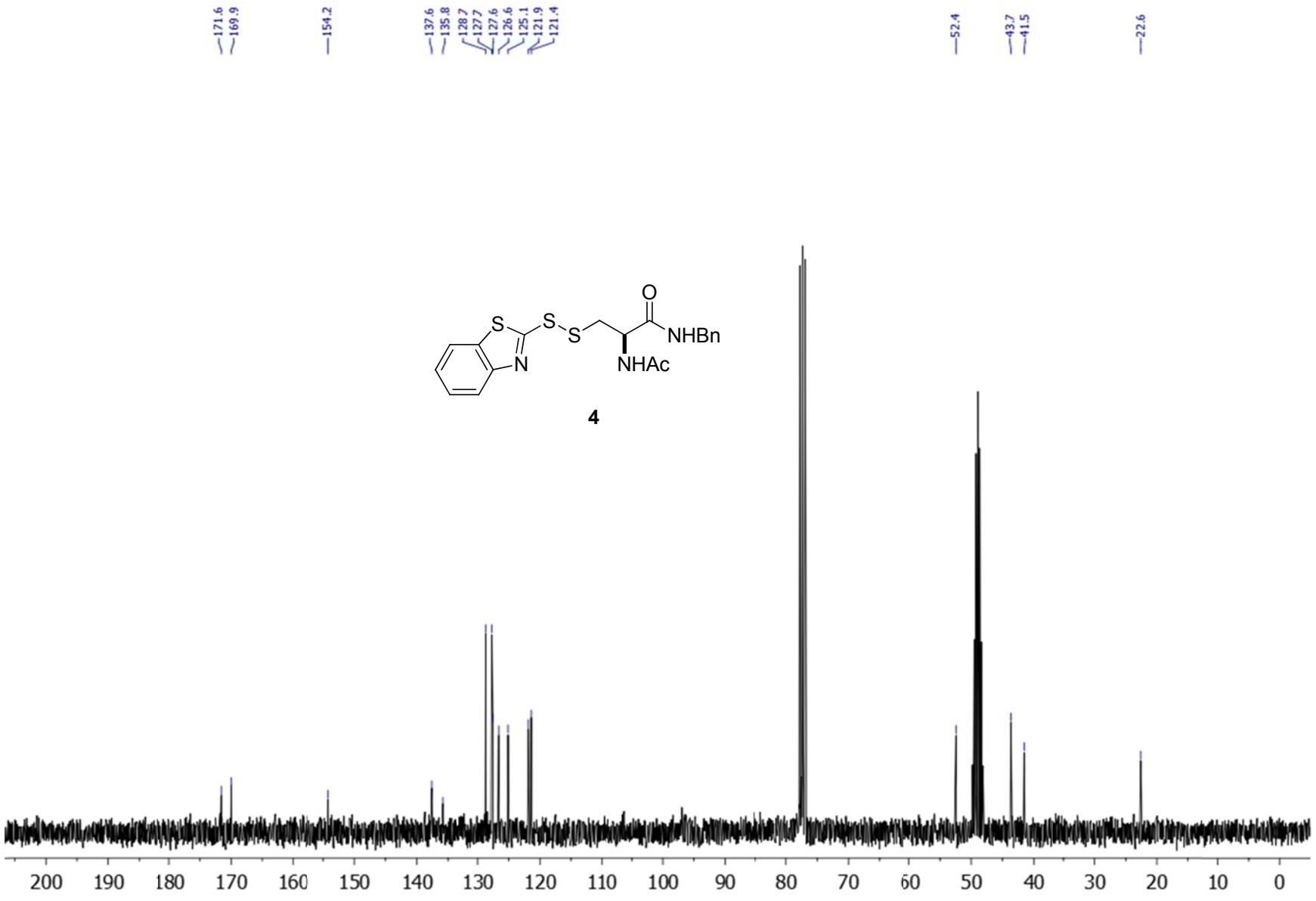
300MHz ^{1}H NMR for compound **3** in $CDCl_3$ (ppm)



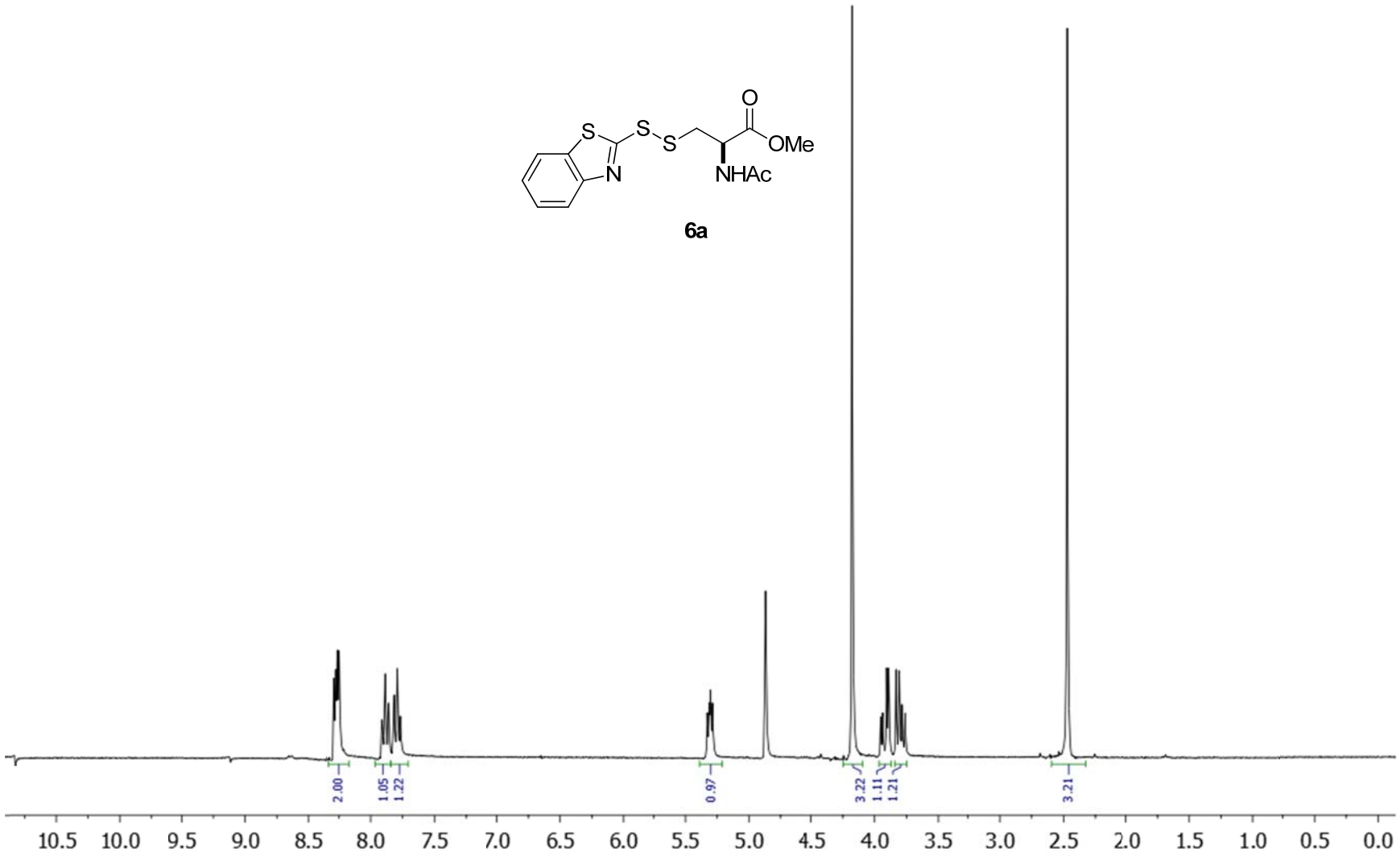
75MHz ^{13}C NMR for compound **3** in $CDCl_3$/MeOH-*d4* (**p**pm)



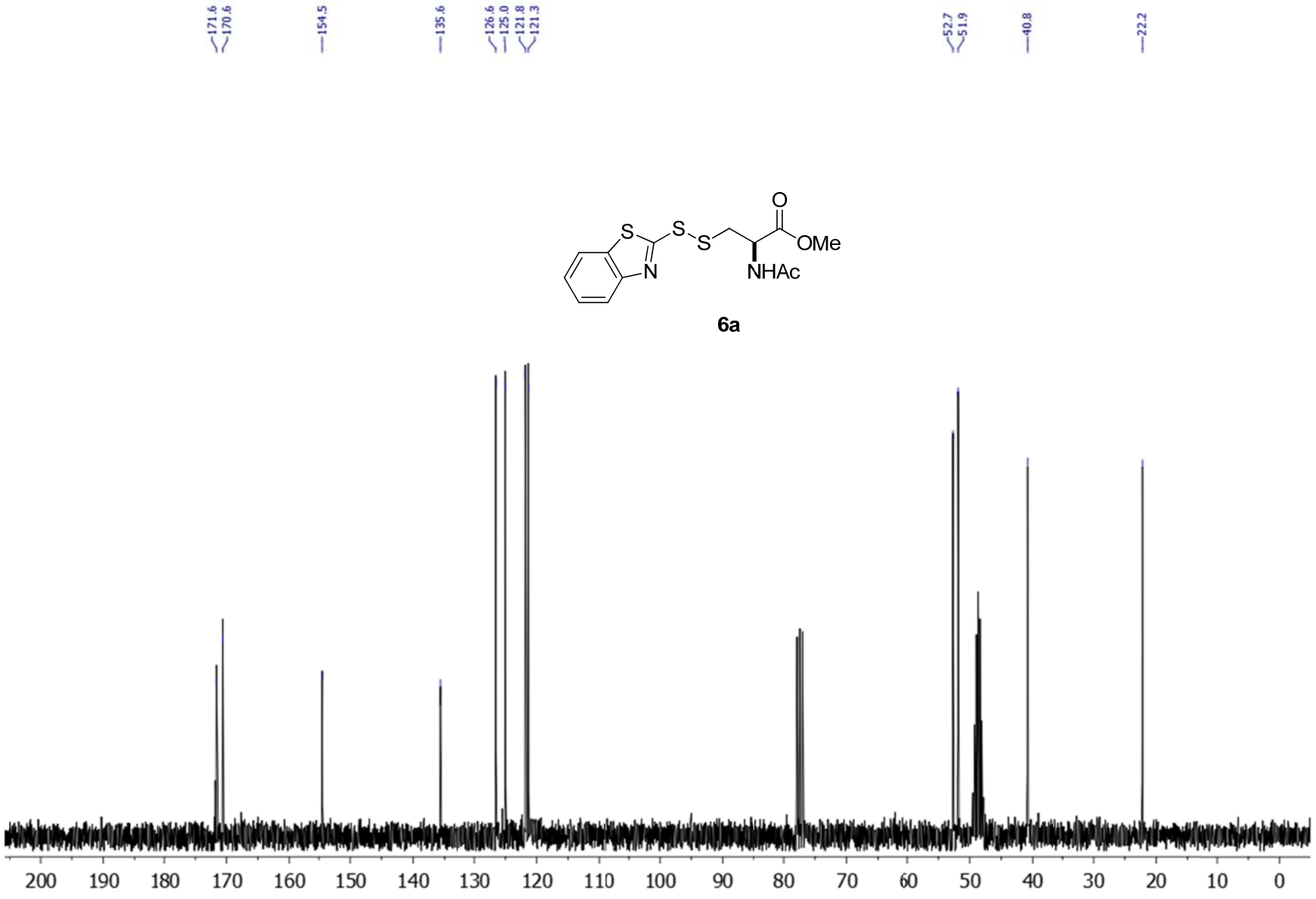
300MHz ^{1}H NMR for compound **4** in $CDCl_3$ (ppm)



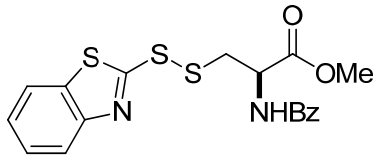
75MHz ^{13}C NMR for compound **4** in $CDCl_3$/MeOH-*d4* (ppm)



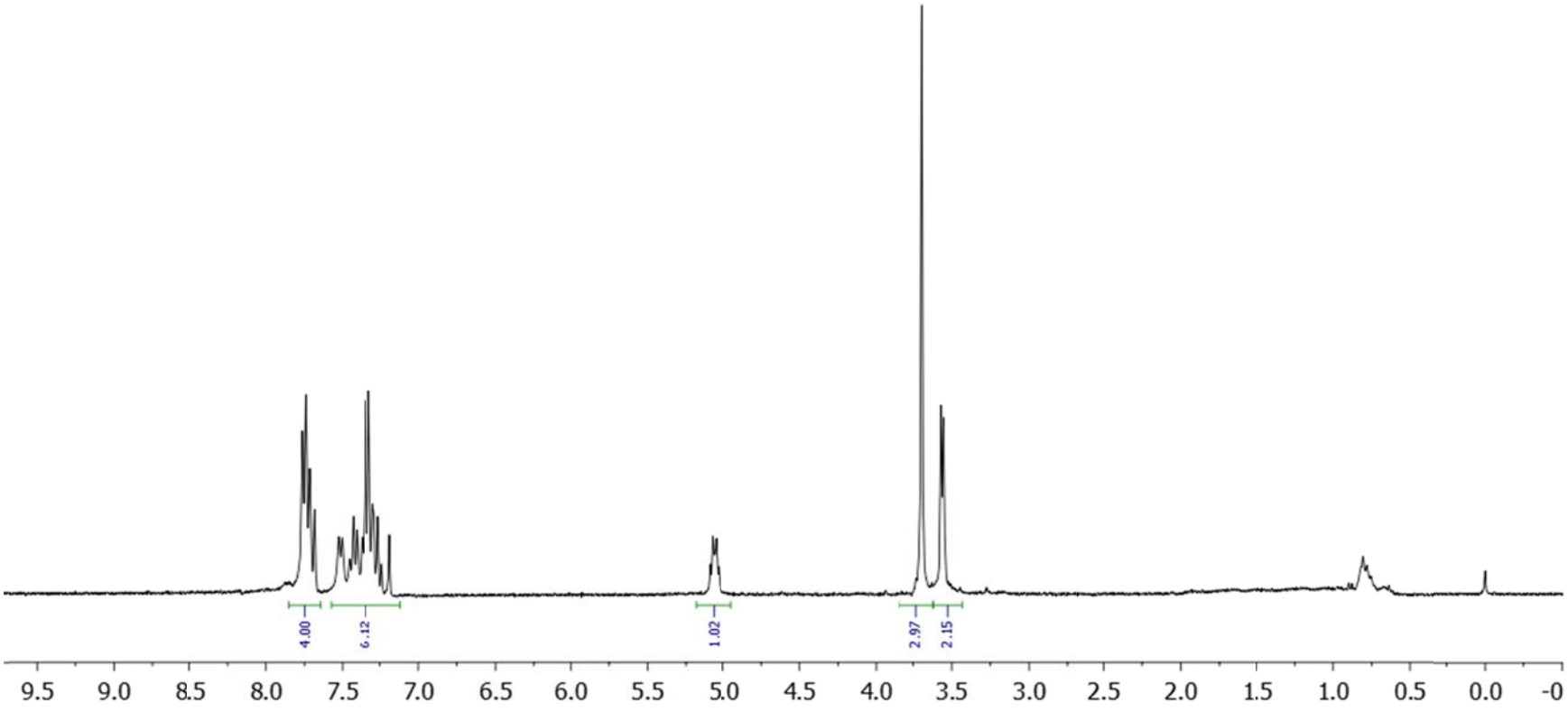
300MHz ^{1}H NMR for compound **6a** in $CDCl_3$/MeOH-*d4* (ppm)



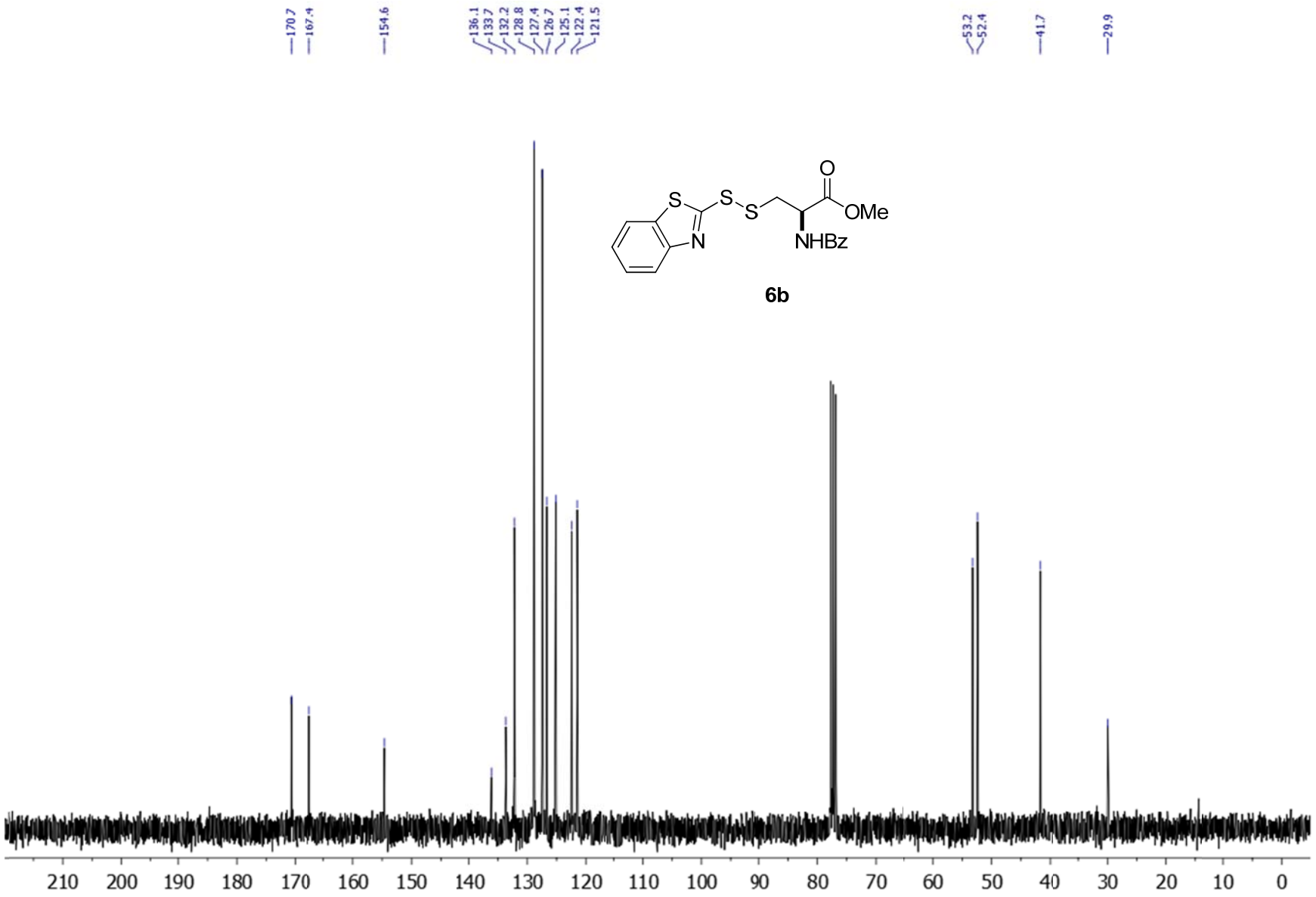
75MHz ^{13}C NMR for compound **6a** in $CDCl_3$/MeOH-*d4* (ppm)



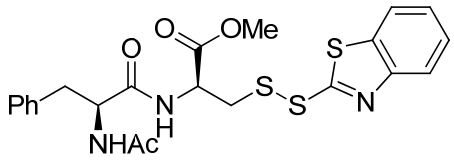
6b



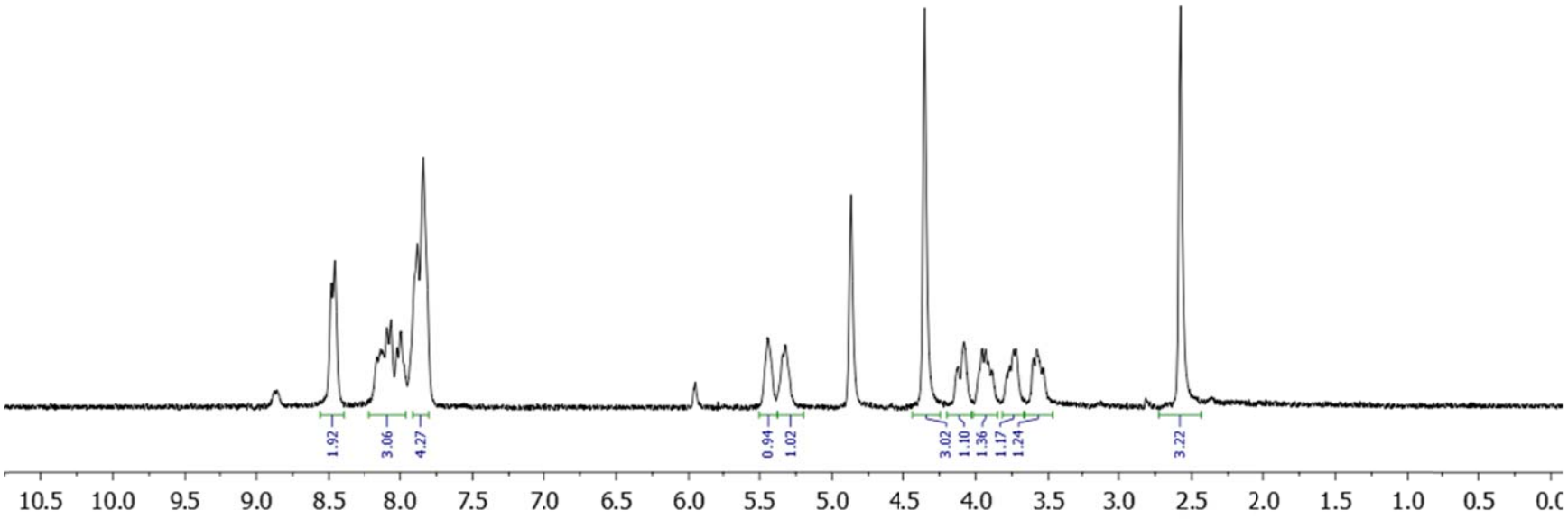
300MHz ^{1}H NMR for compound **6b** in $CDCl_3$ (ppm)



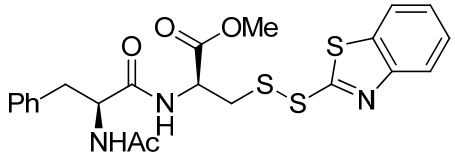
75MHz ^{13}C NMR for compound **6b** in $CDCl_3$ (ppm)



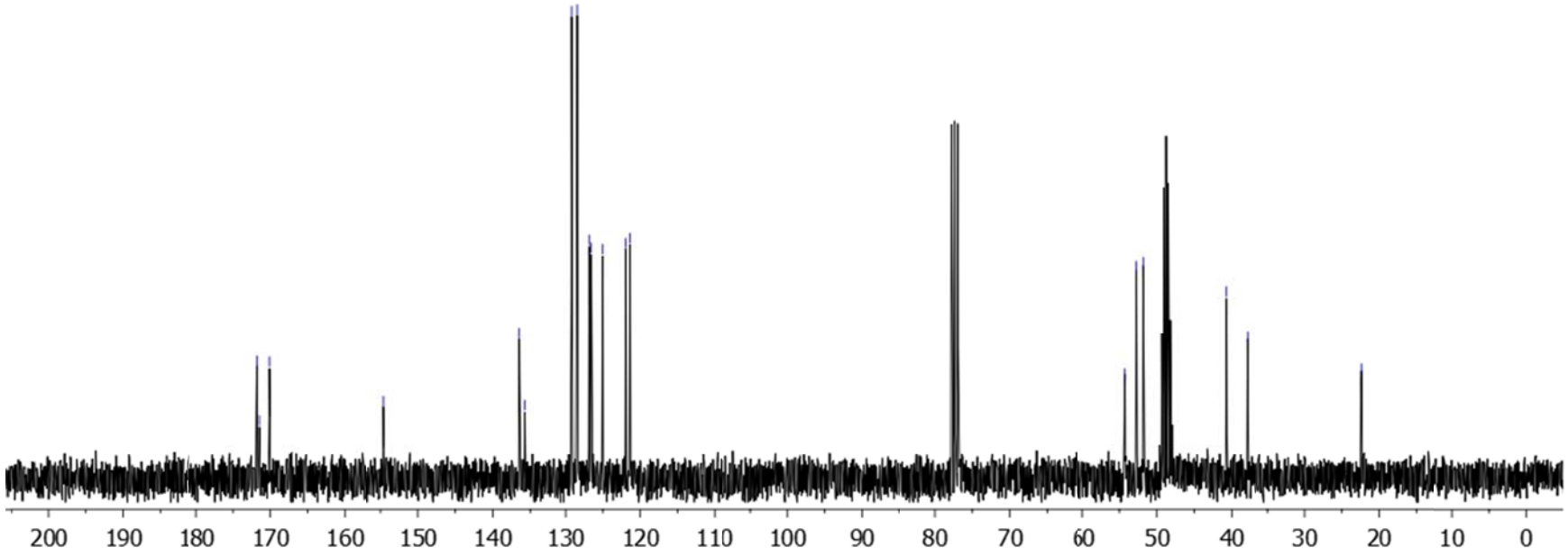
6c



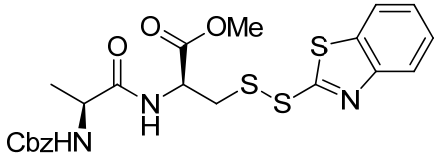
300MHz ^{1}H NMR for compound **6c** in $CDCl_3$/MeOH-*d4* (ppm)



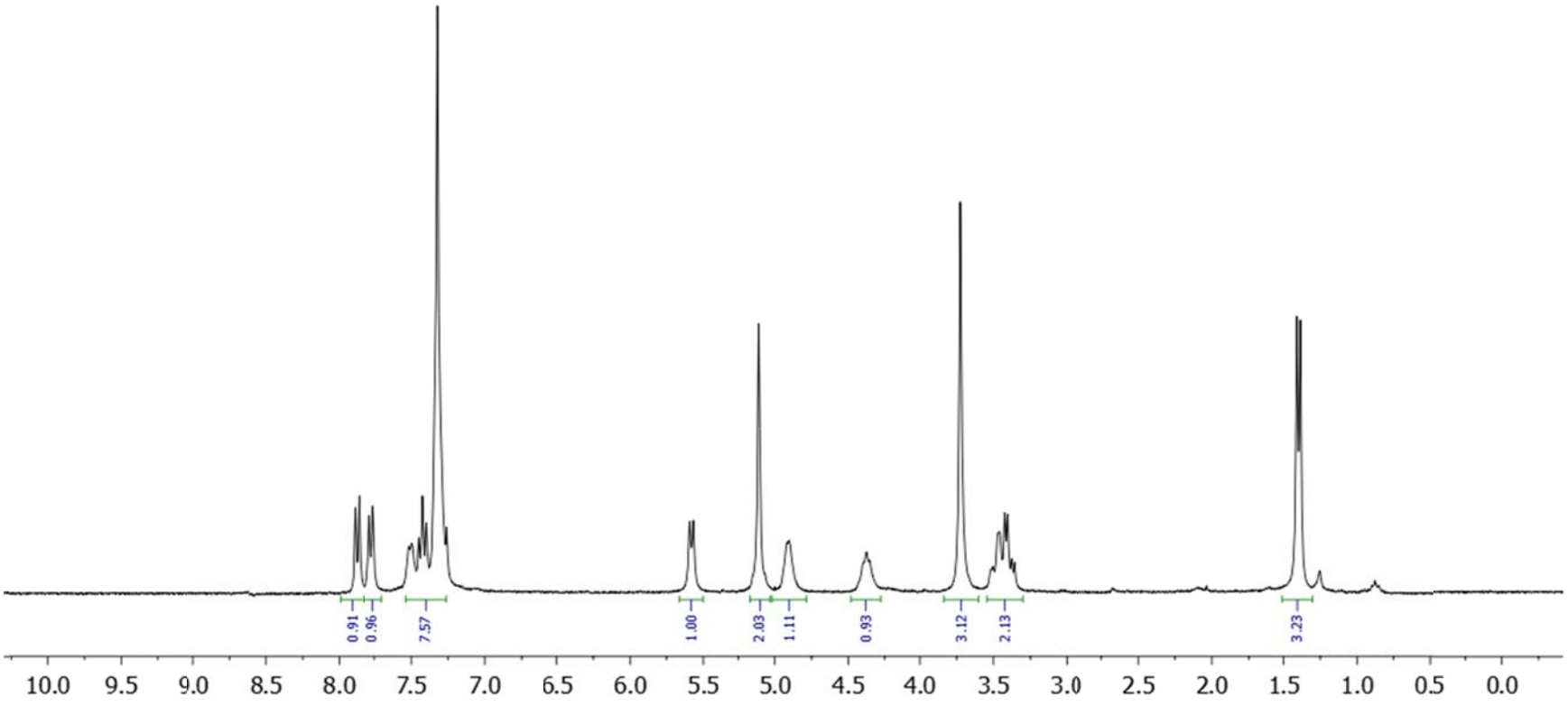
6c



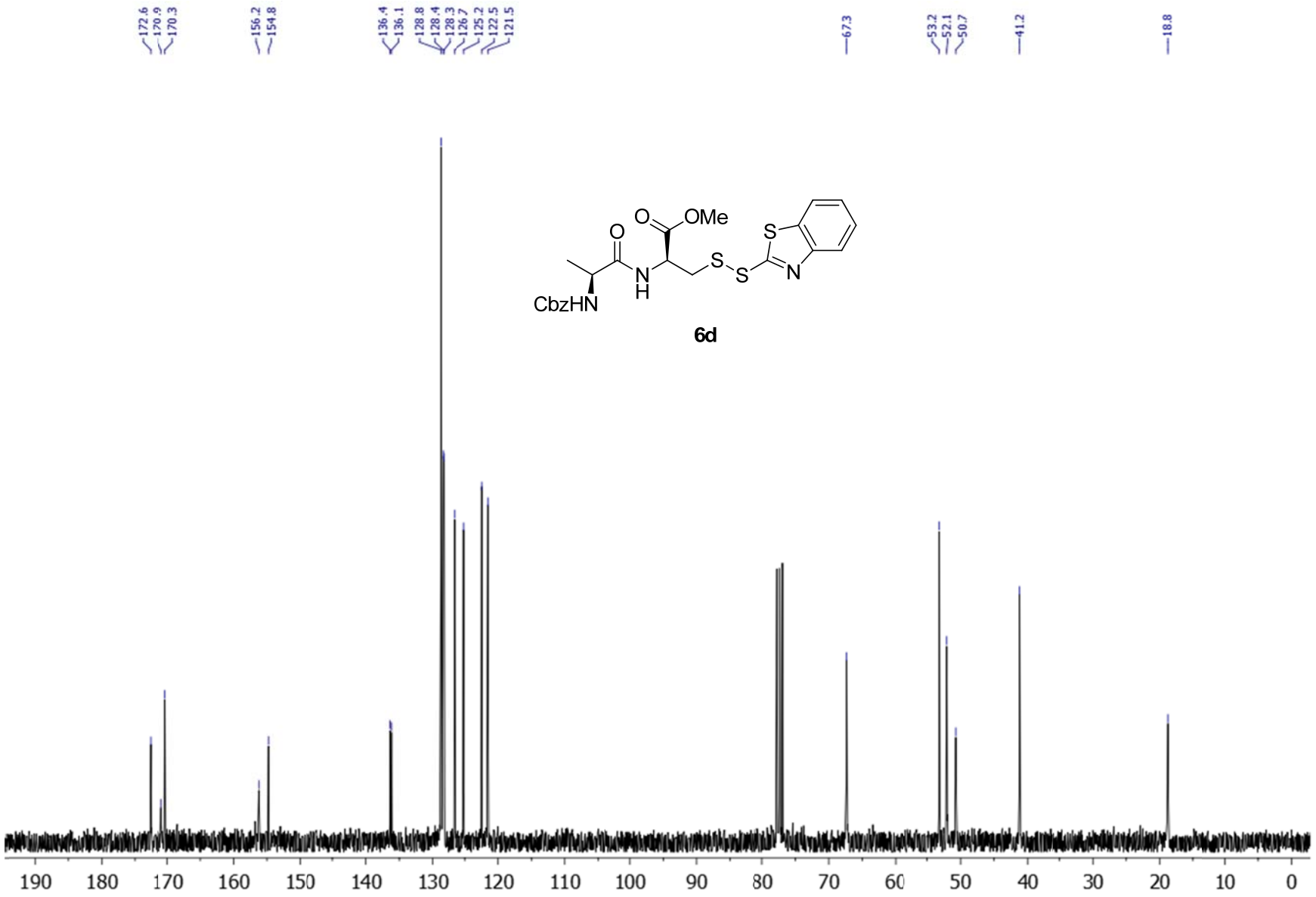
75MHz ^{13}C NMR for compound **6c** in $CDCl_3$/MeOH-*d4* (ppm)



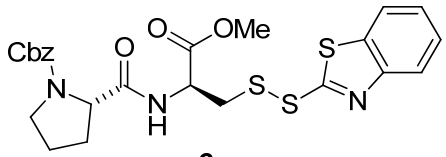
6d



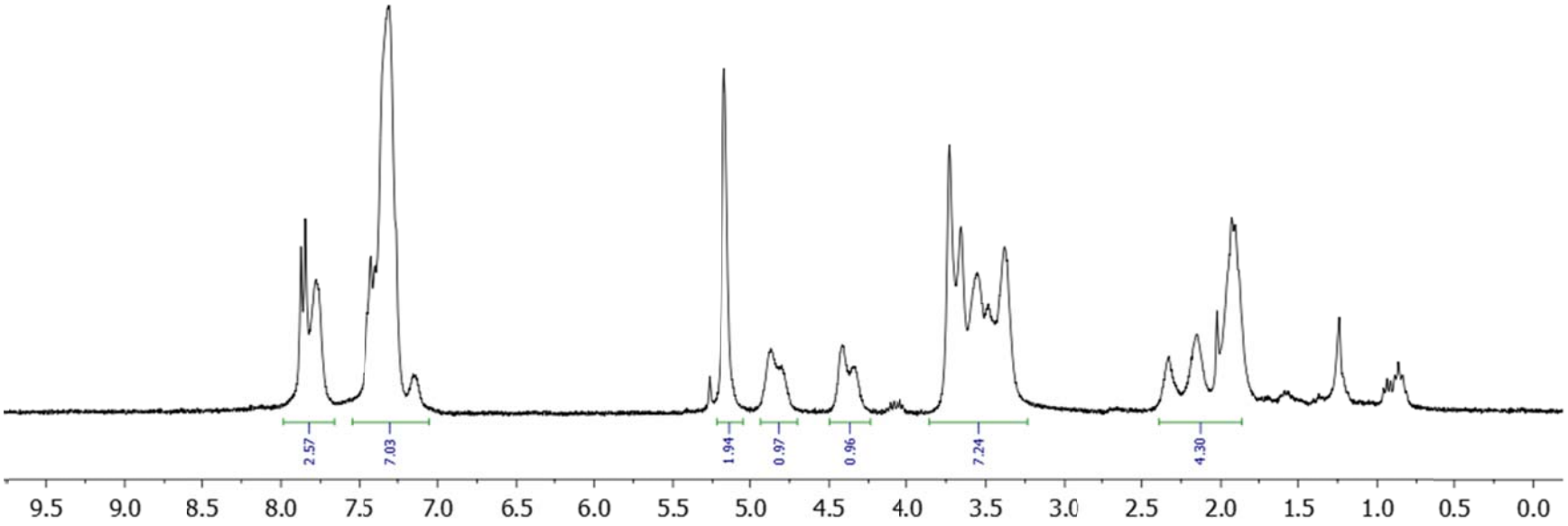
300MHz ^{1}H NMR for compound **6d** in $CDCl_3$ (ppm)



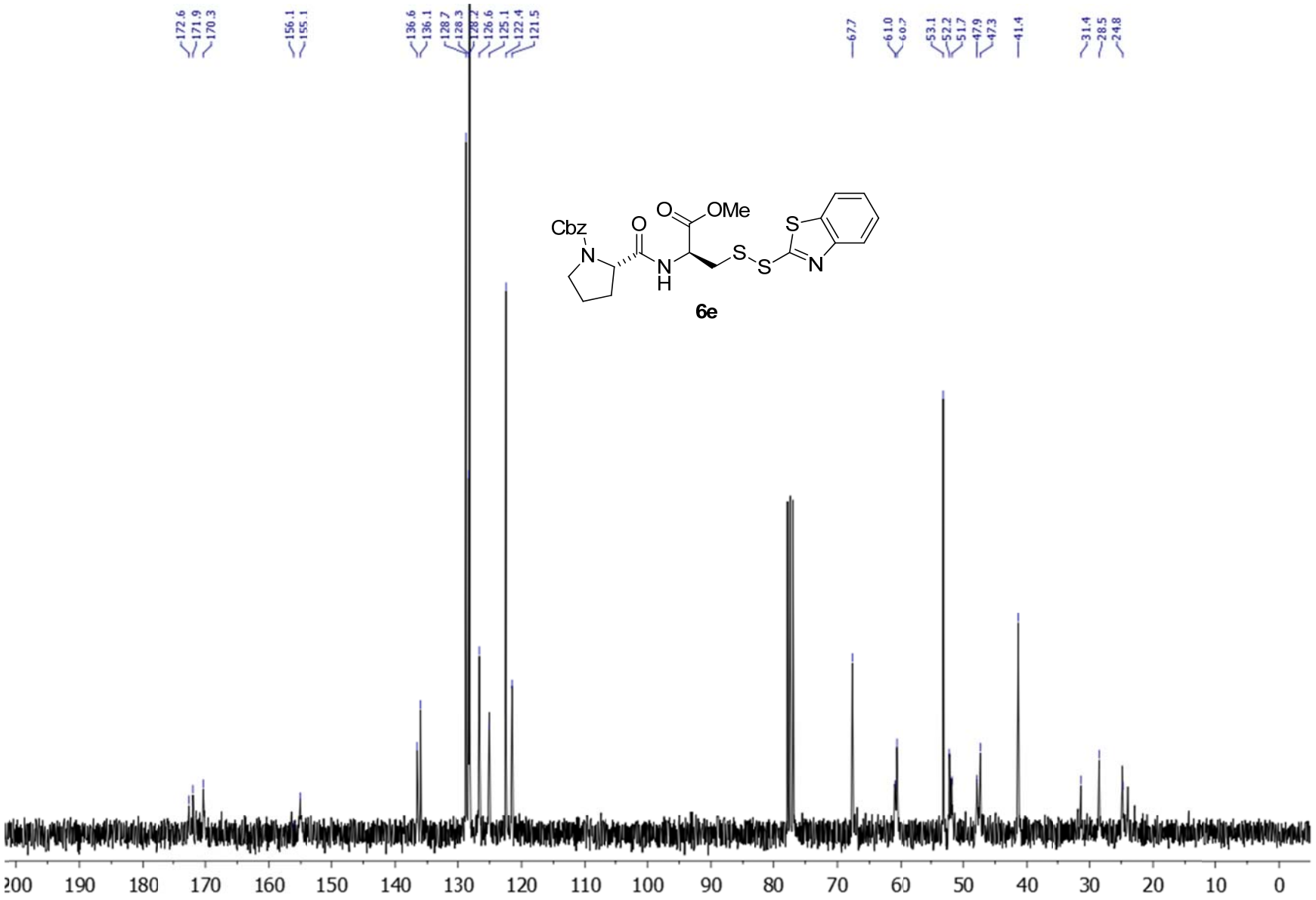
75MHz ^{13}C NMR for compound **6d** in $CDCl_3$ (ppm)



6e



300MHz ^{1}H NMR for compound **6e** in $CDCl_3$ (ppm)



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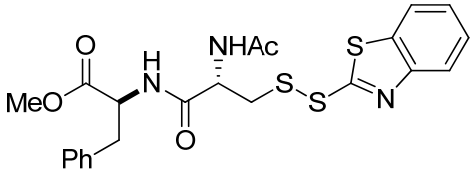
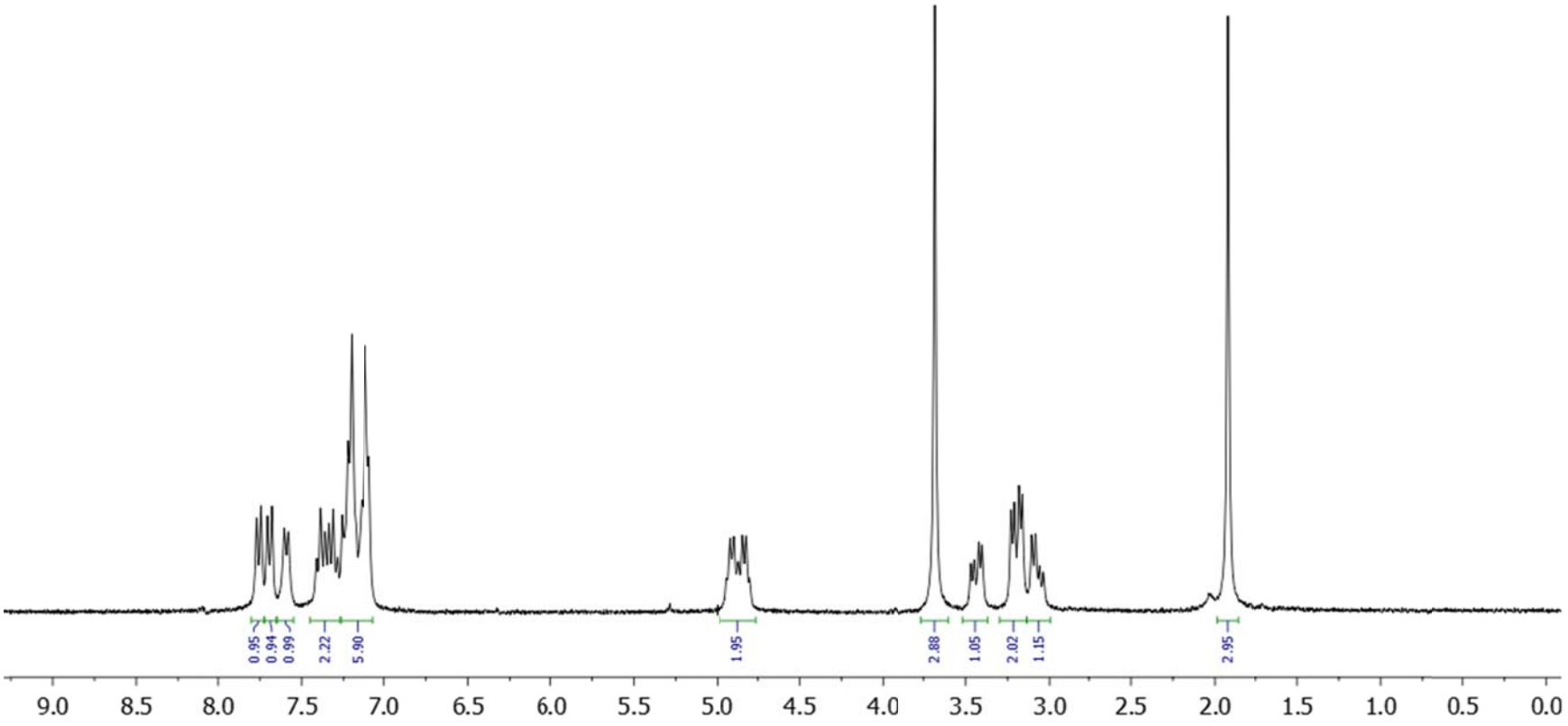
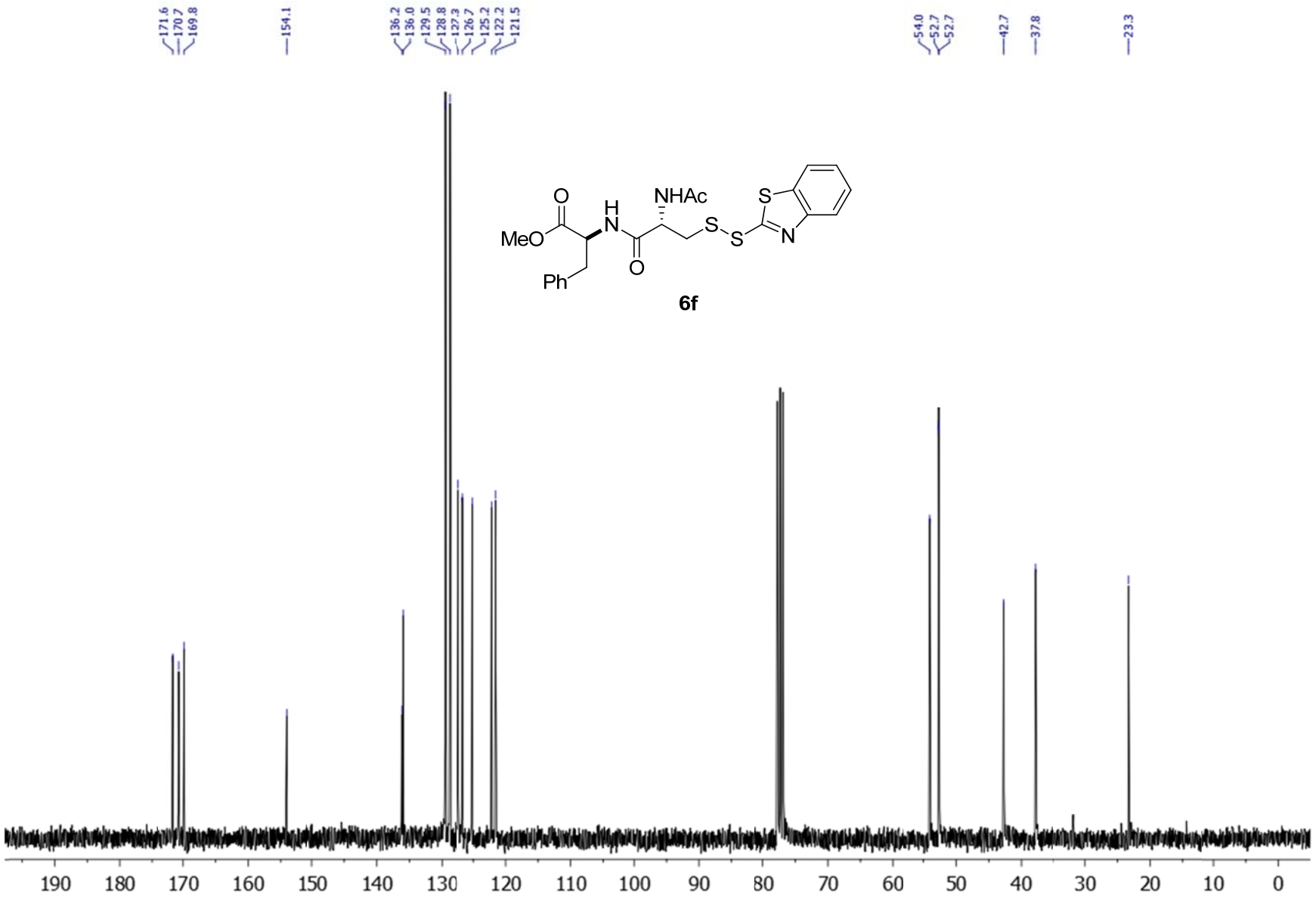


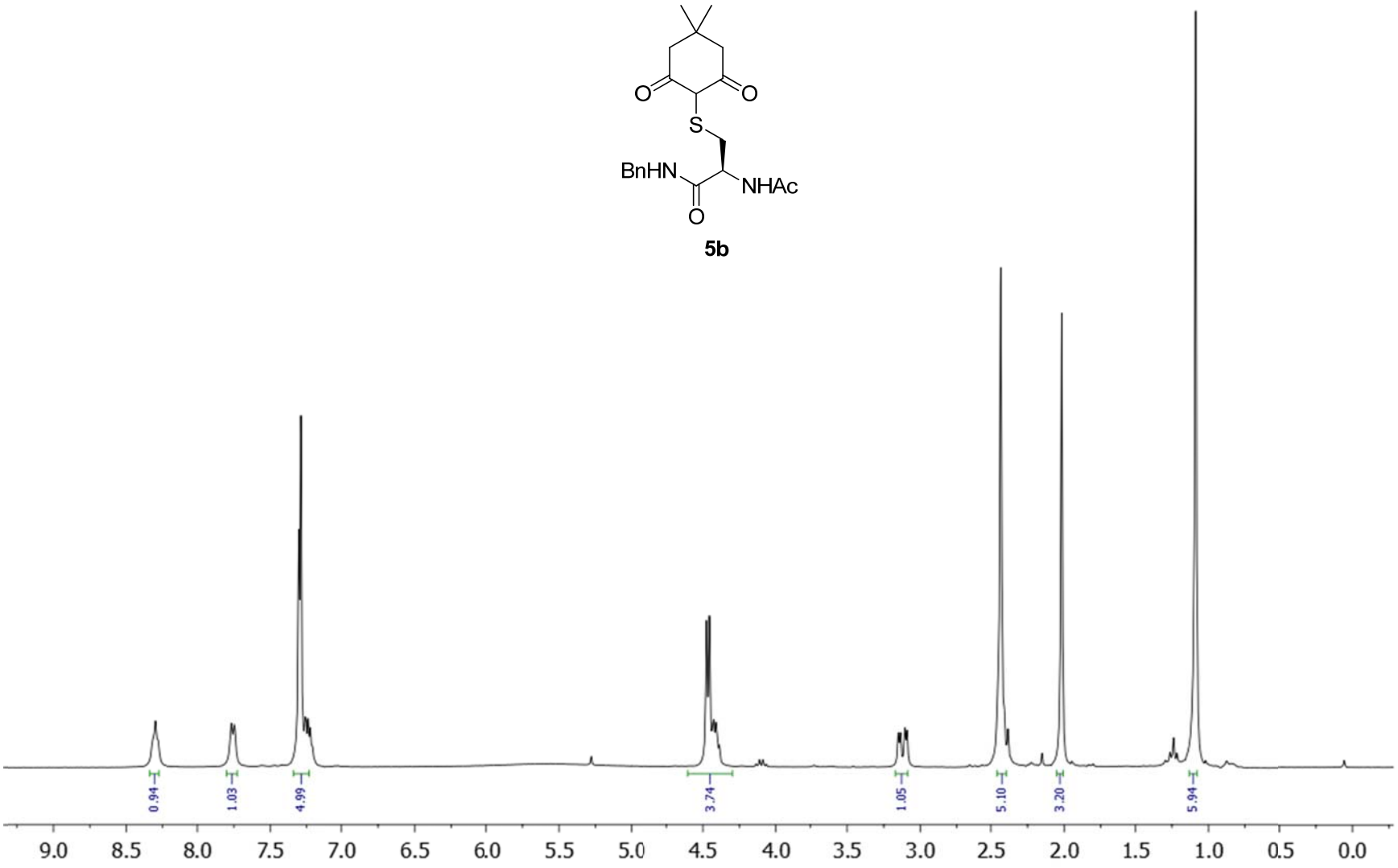
6f



300MHz ^{1}H NMR for compound **6f** in $CDCl_3$ (ppm)

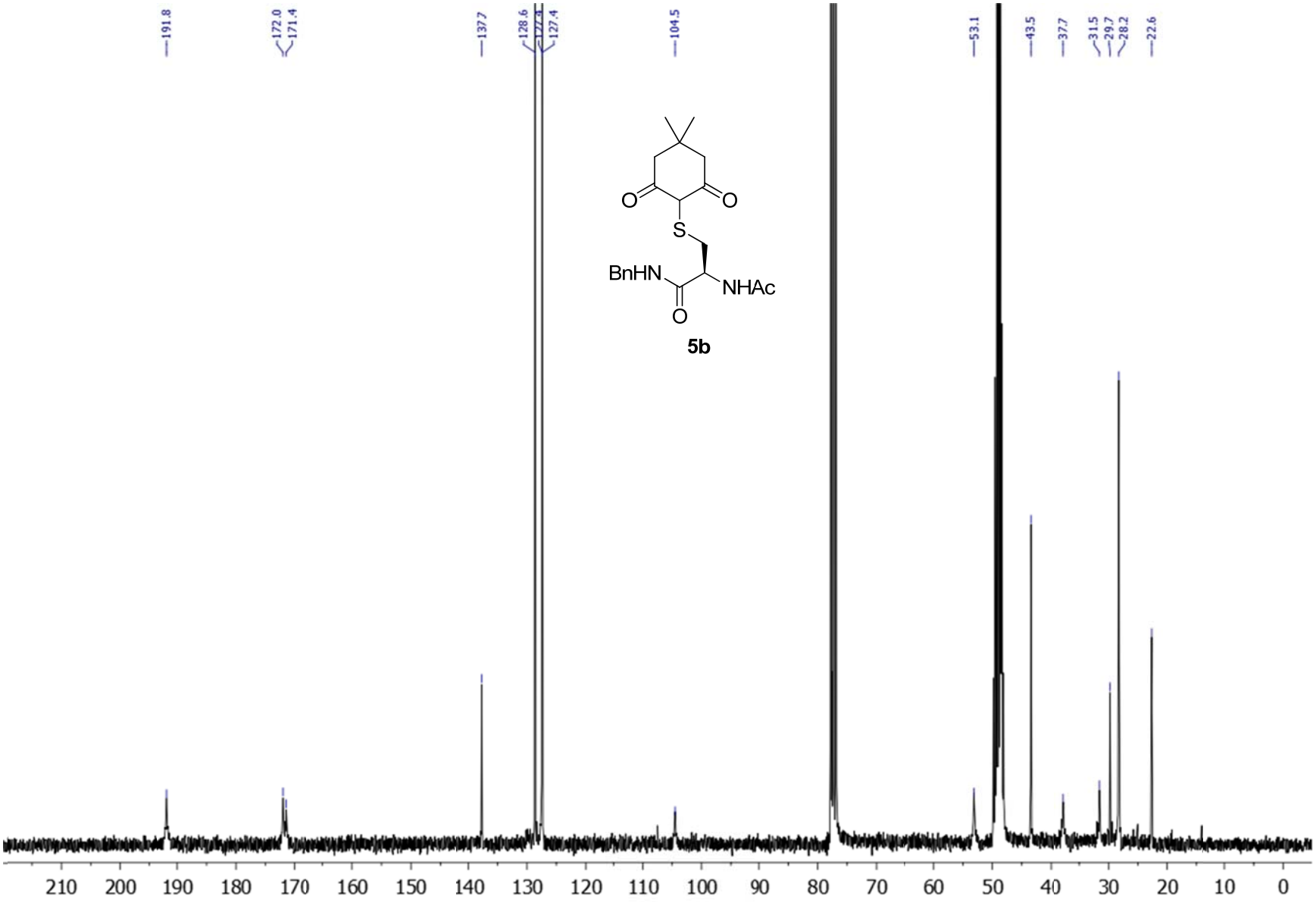


75MHz ^{13}C NMR for compound **6f** in $CDCl_3$ (ppm)

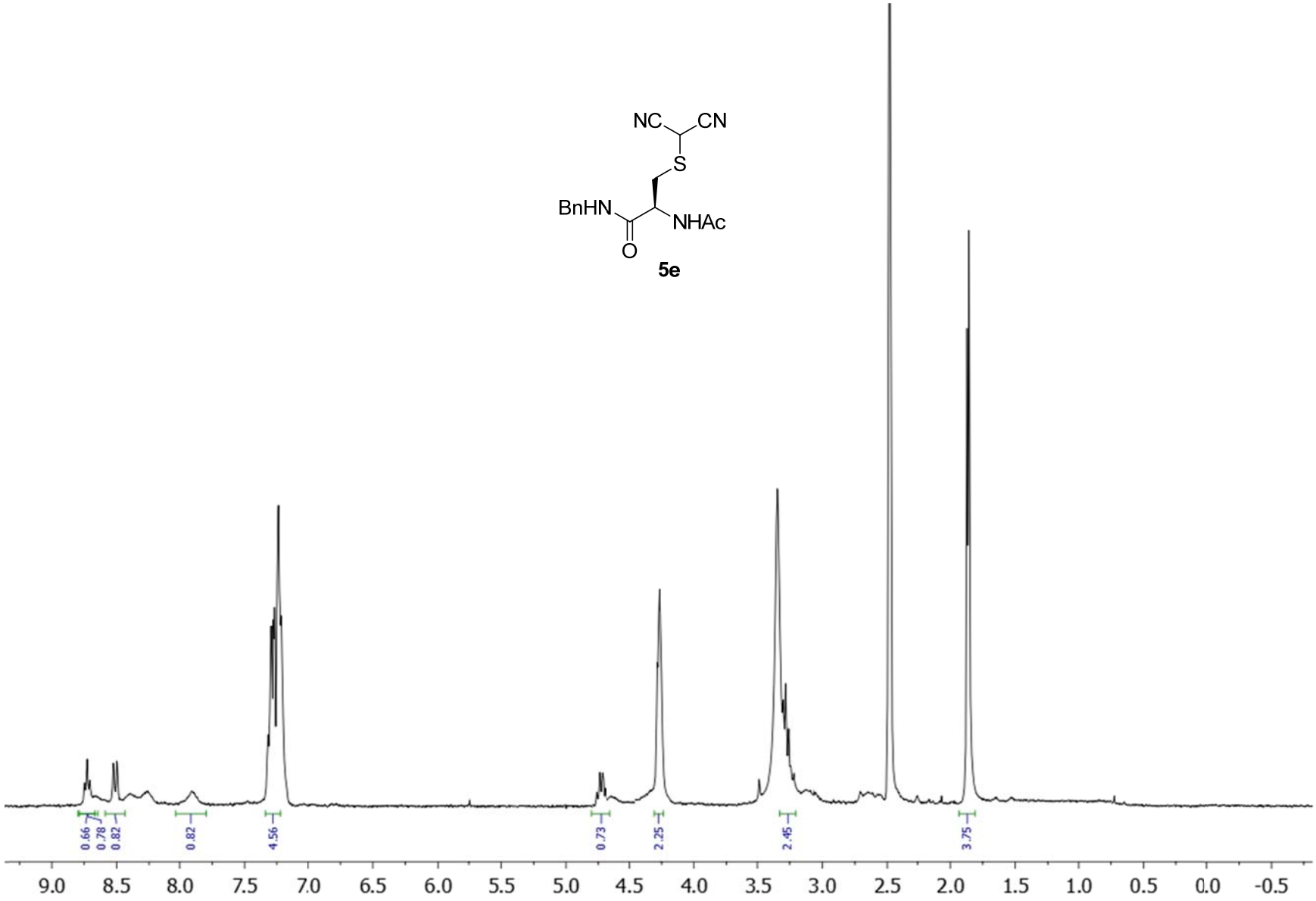


5b

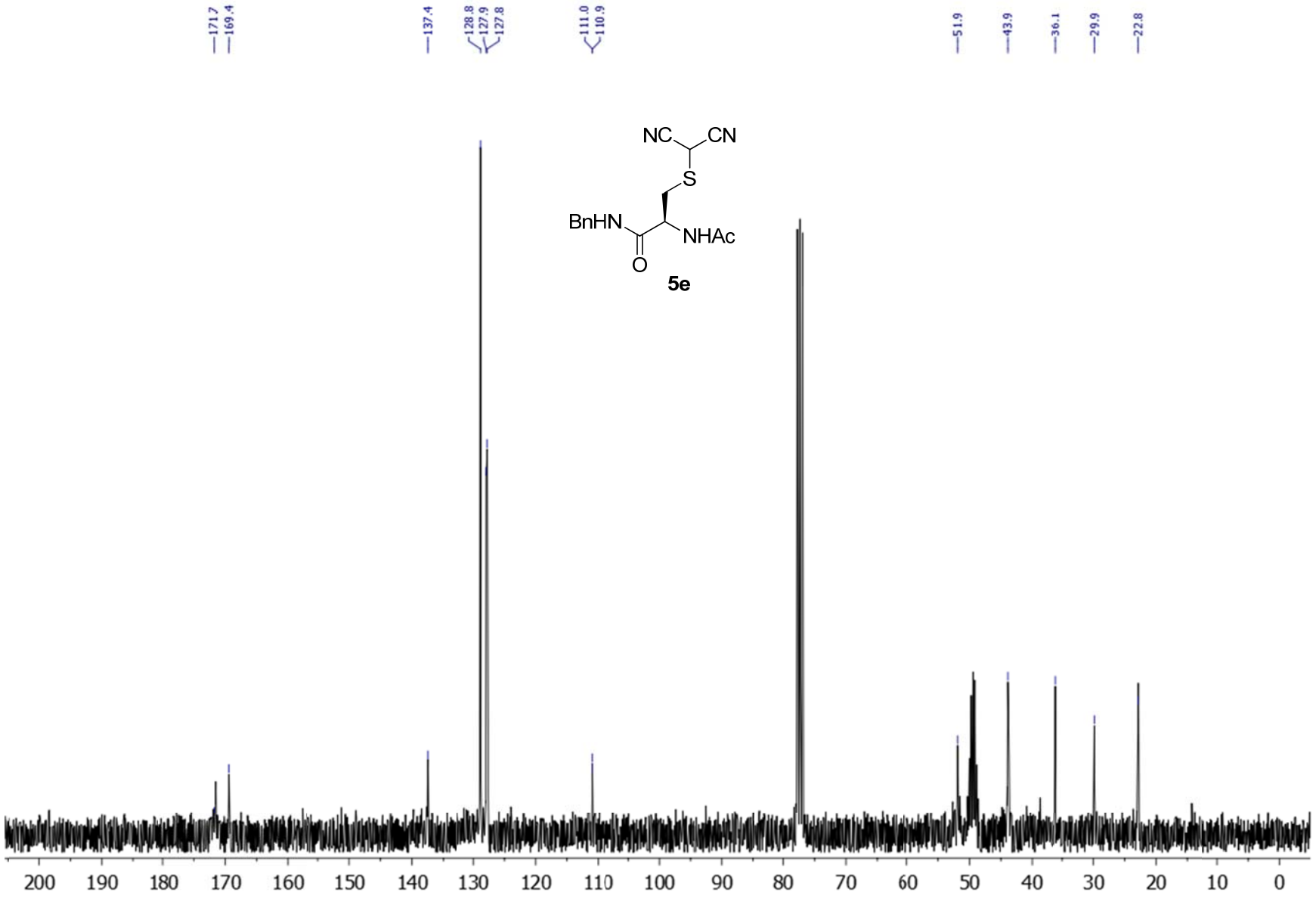
300MHz ^{1}H NMR for compound **5b** in $CDCl_3$ (ppm)



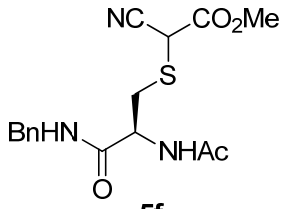
75MHz ^{13}C NMR for compound **5b** in $CDCl_3$/MeOH-*d4* (ppm)



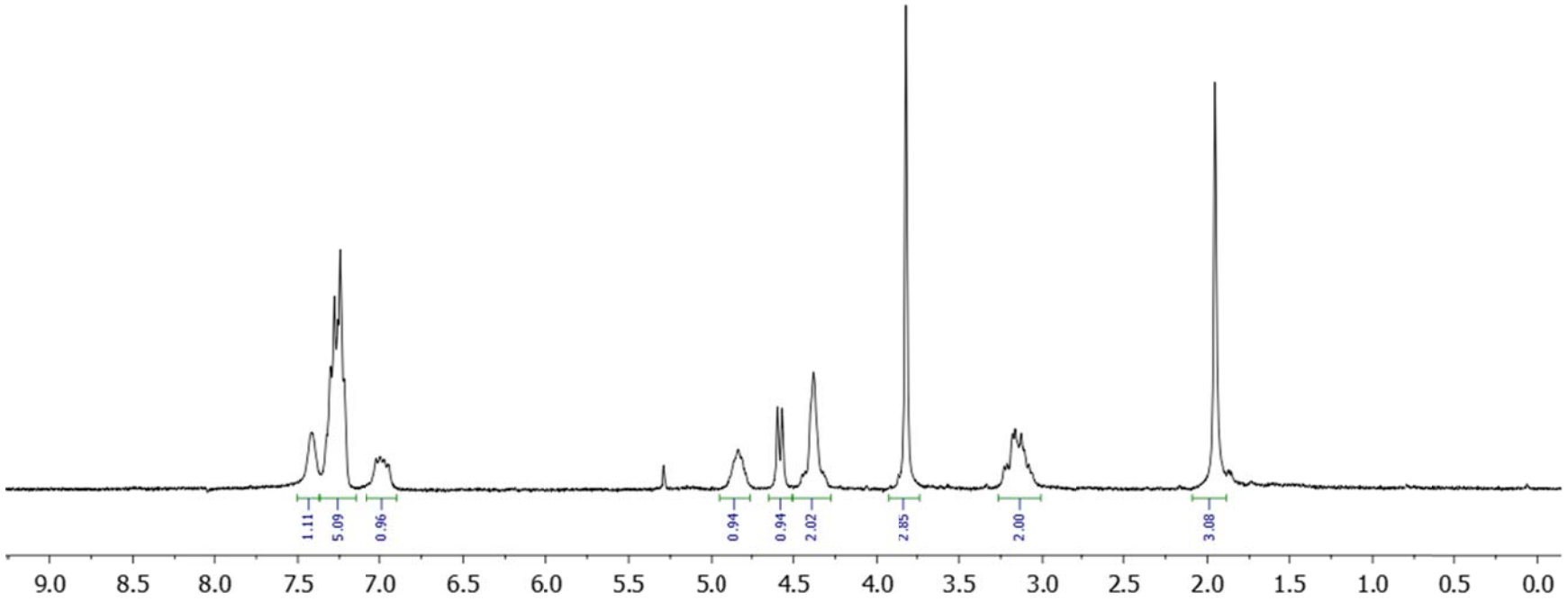
300MHz ^{1}H NMR for compound **5e** in DMSO-*d6* (ppm)



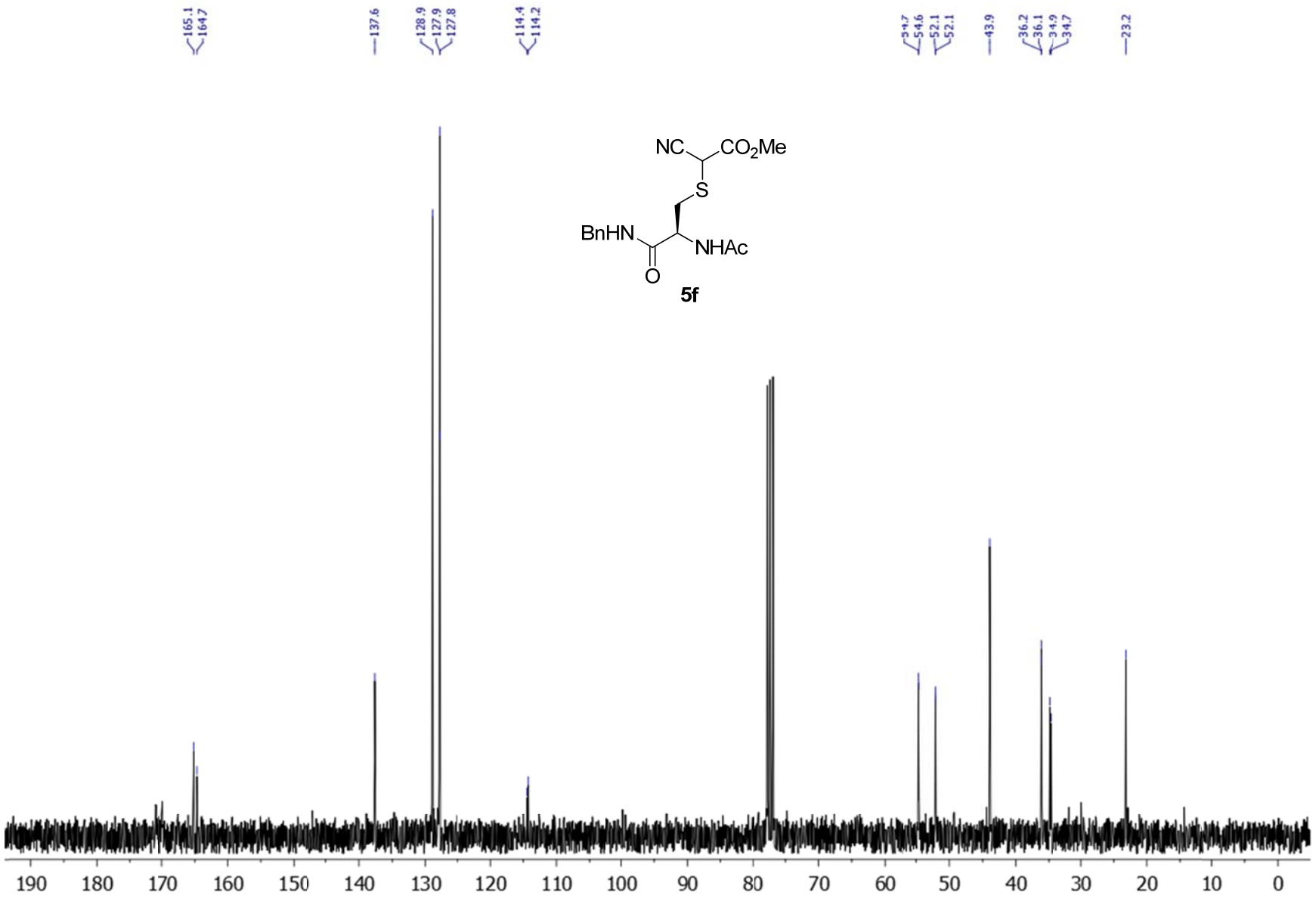
75MHz ^{13}C NMR for compound **5e** in $CDCl_3$/MeOH-*d4* (ppm)



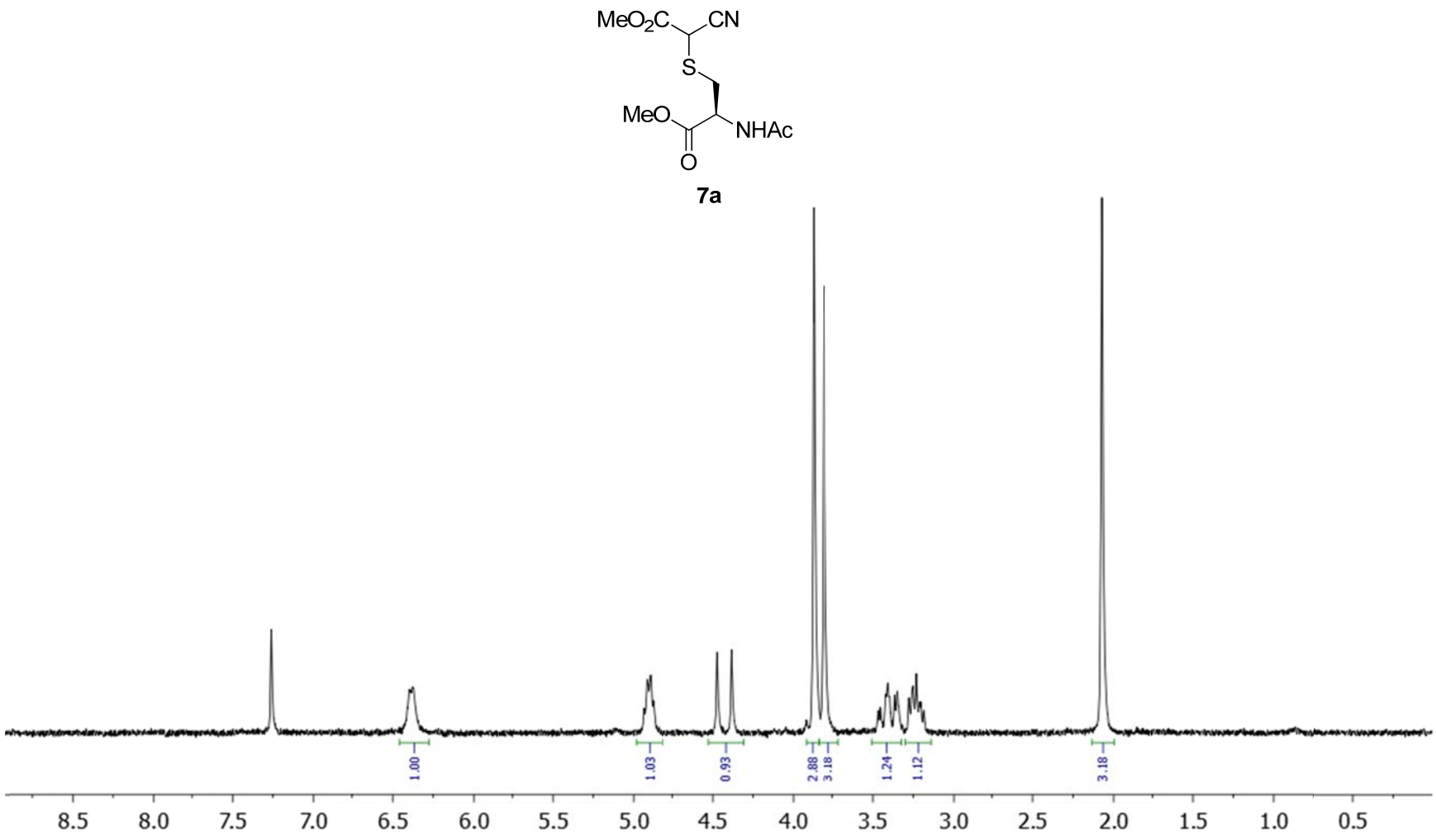
5f



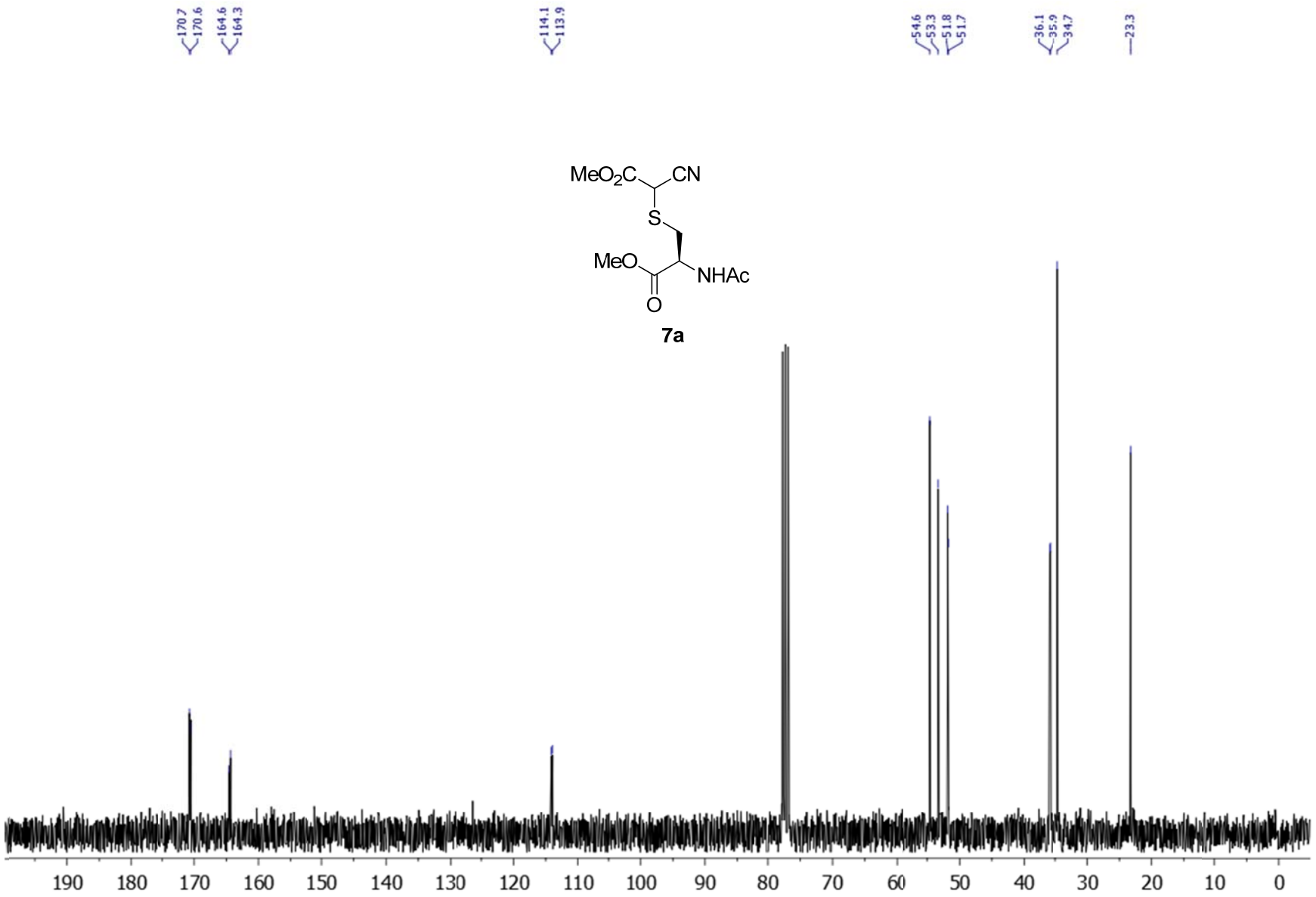
300MHz ^{1}H NMR for compound **5f** in $CDCl_3$ (ppm)



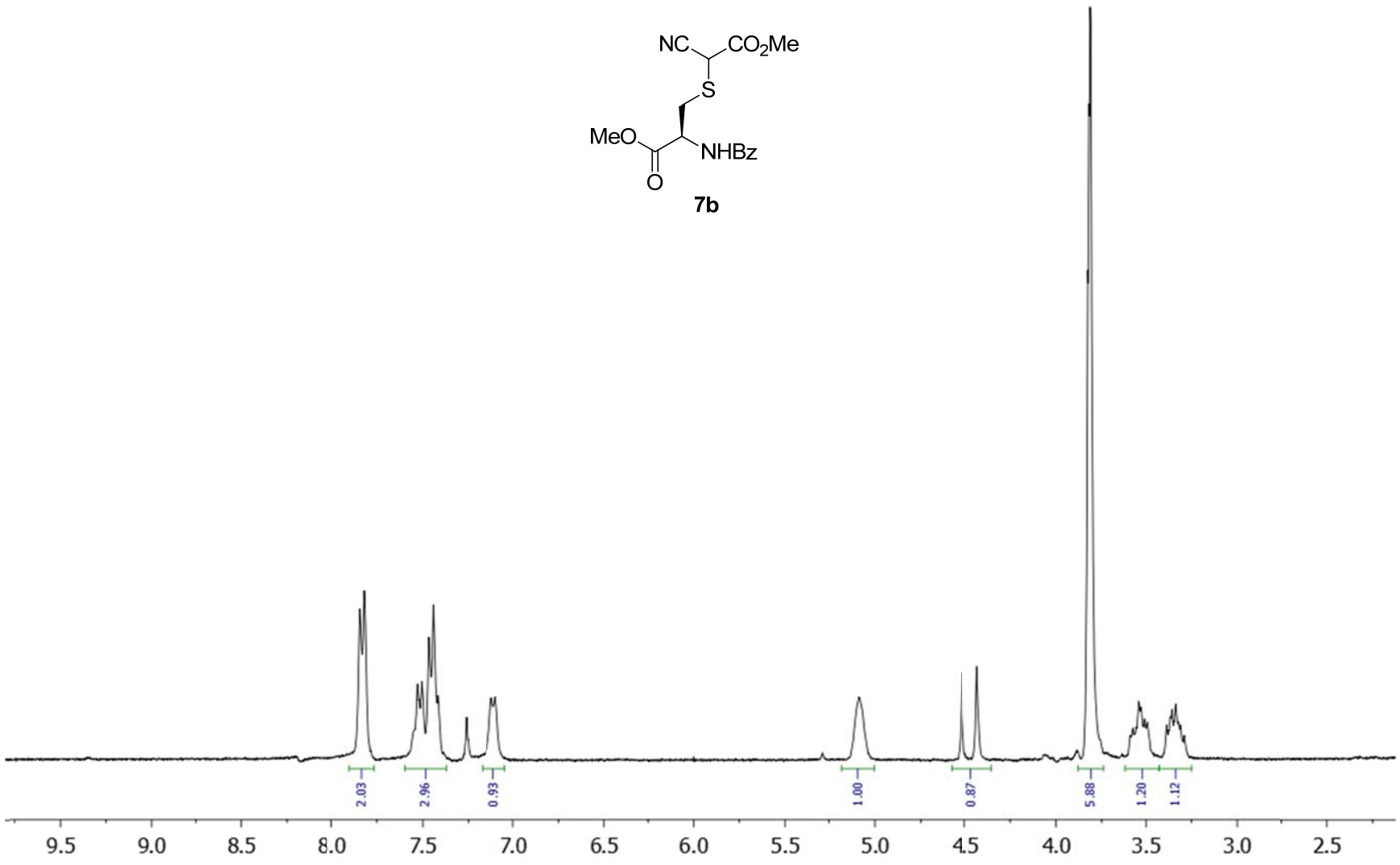
75MHz ^{13}C NMR for compound **5f** in $CDCl_3$ (ppm)



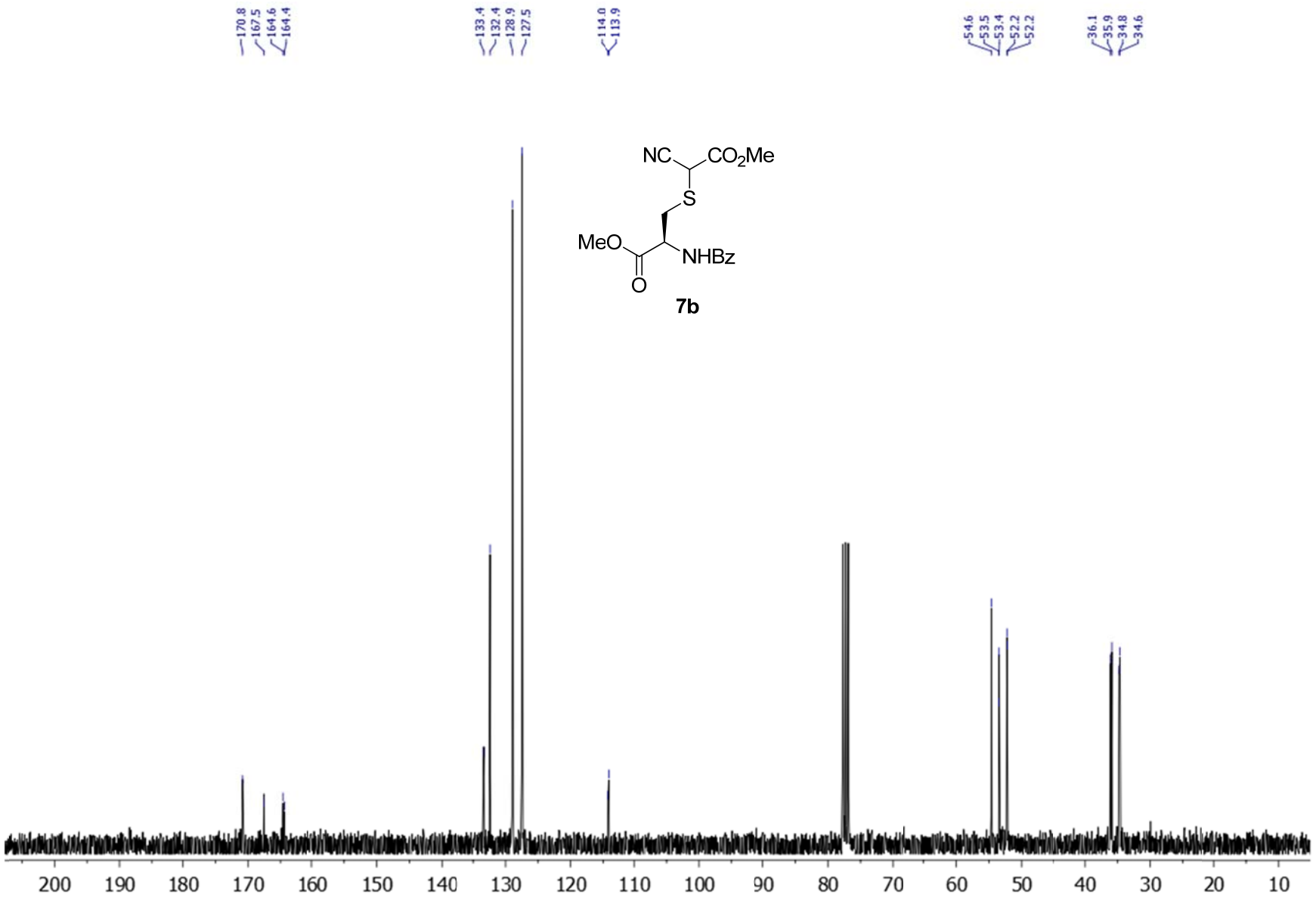
300MHz ^{1}H NMR for compound **7a** in $CDCl_3$ (ppm)



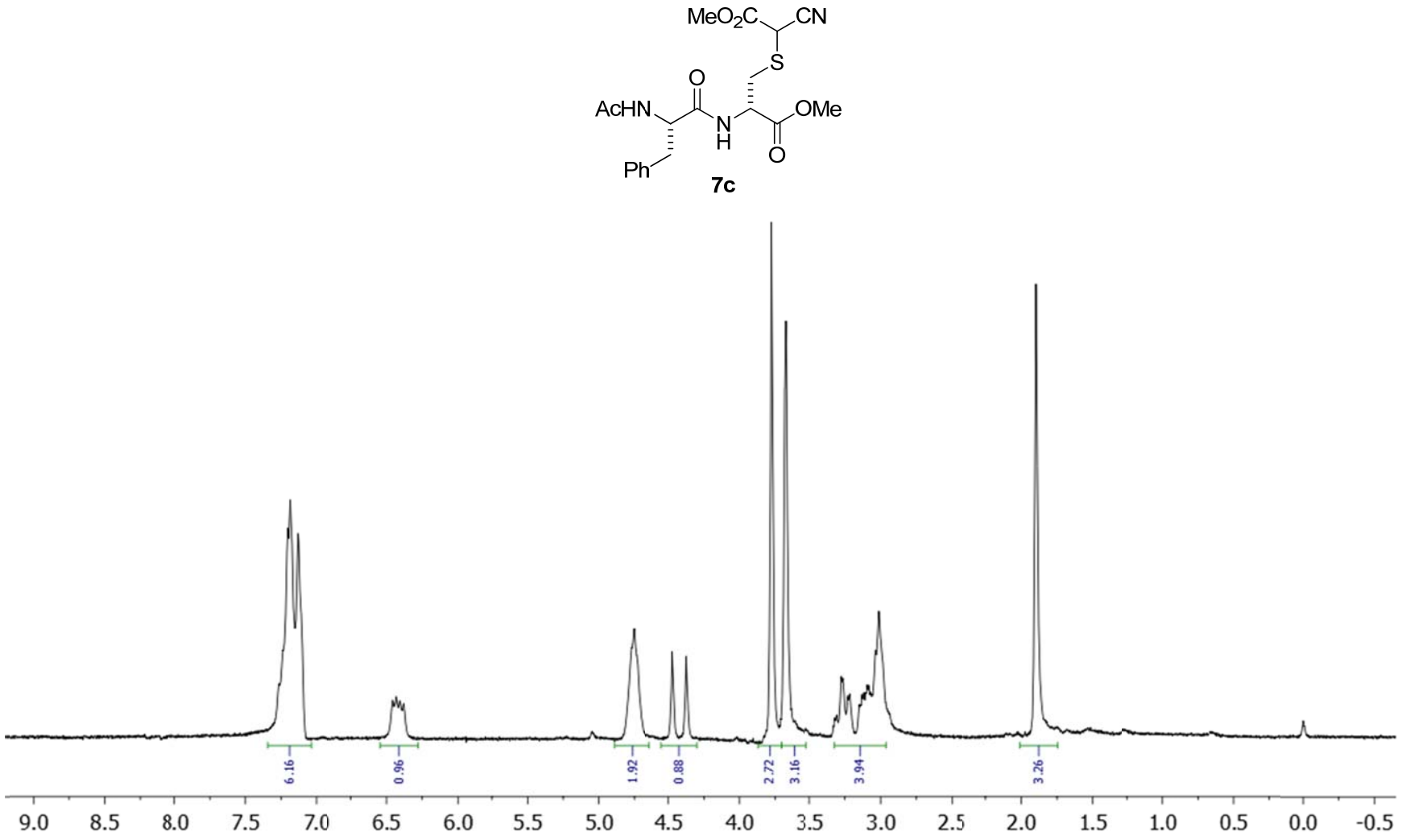
75MHz ^{13}C NMR for compound **7a** in $CDCl_3$ (**p**pm)



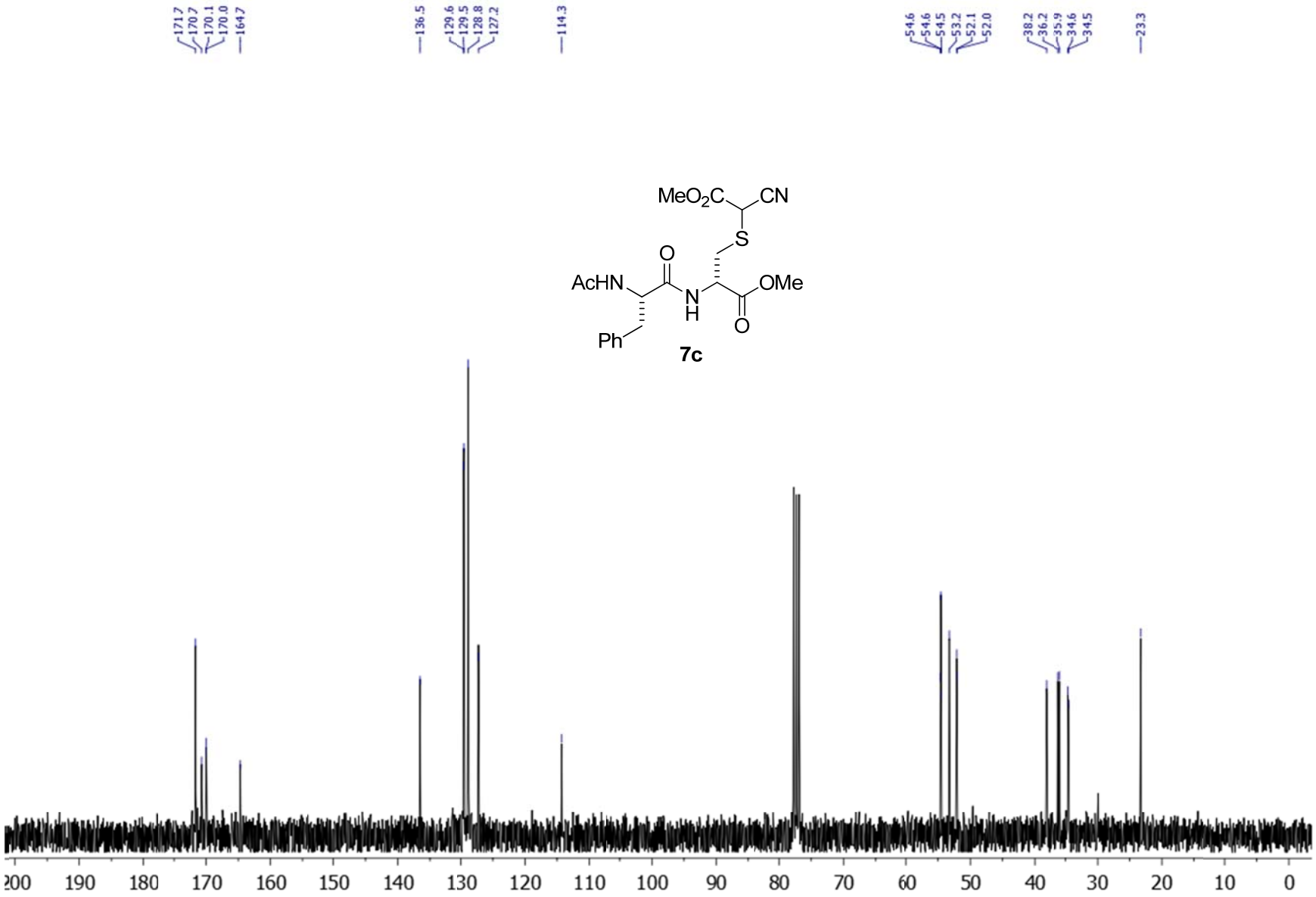
300MHz ^{1}H NMR for compound **7b** in $CDCl_3$ (ppm)



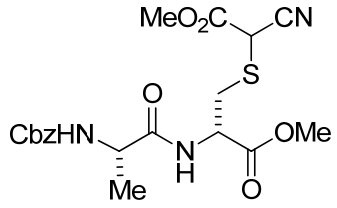
75MHz ^{13}C NMR for compound **7b** in $CDCl_3$ (ppm)



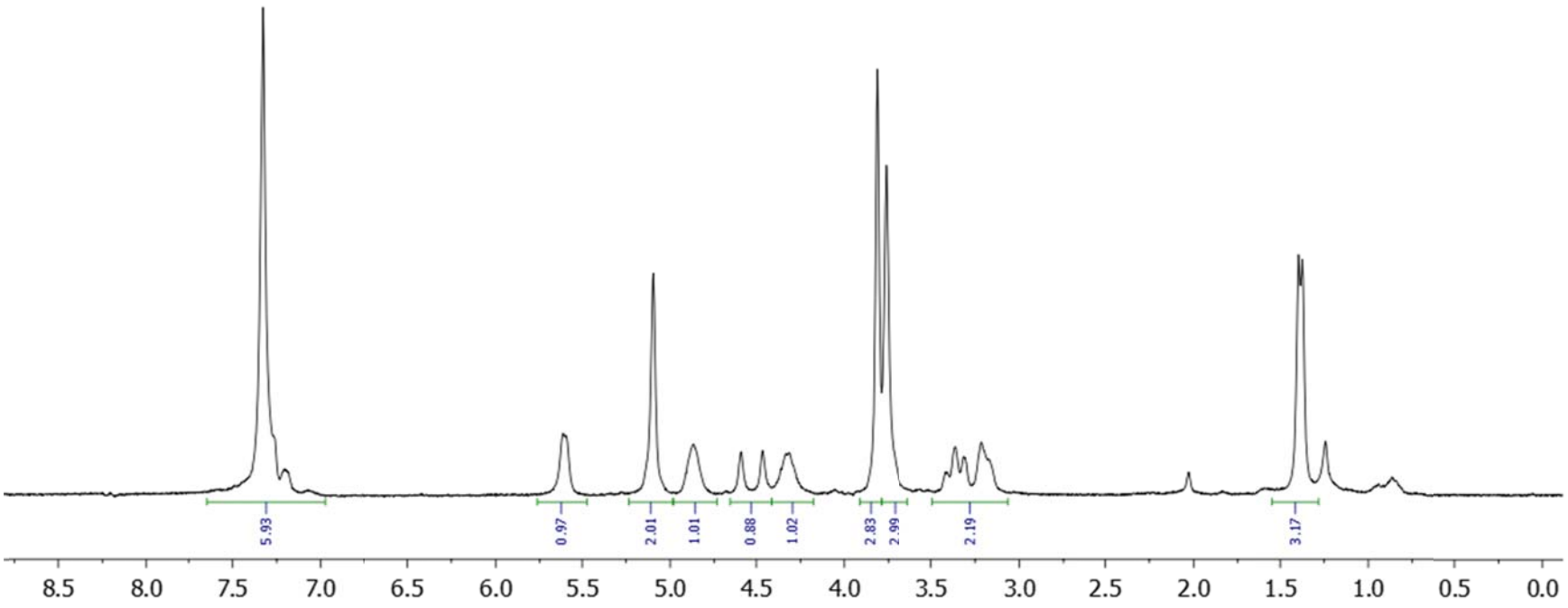
300MHz ^{1}H NMR for compound **7c** in $CDCl_3$ (ppm)



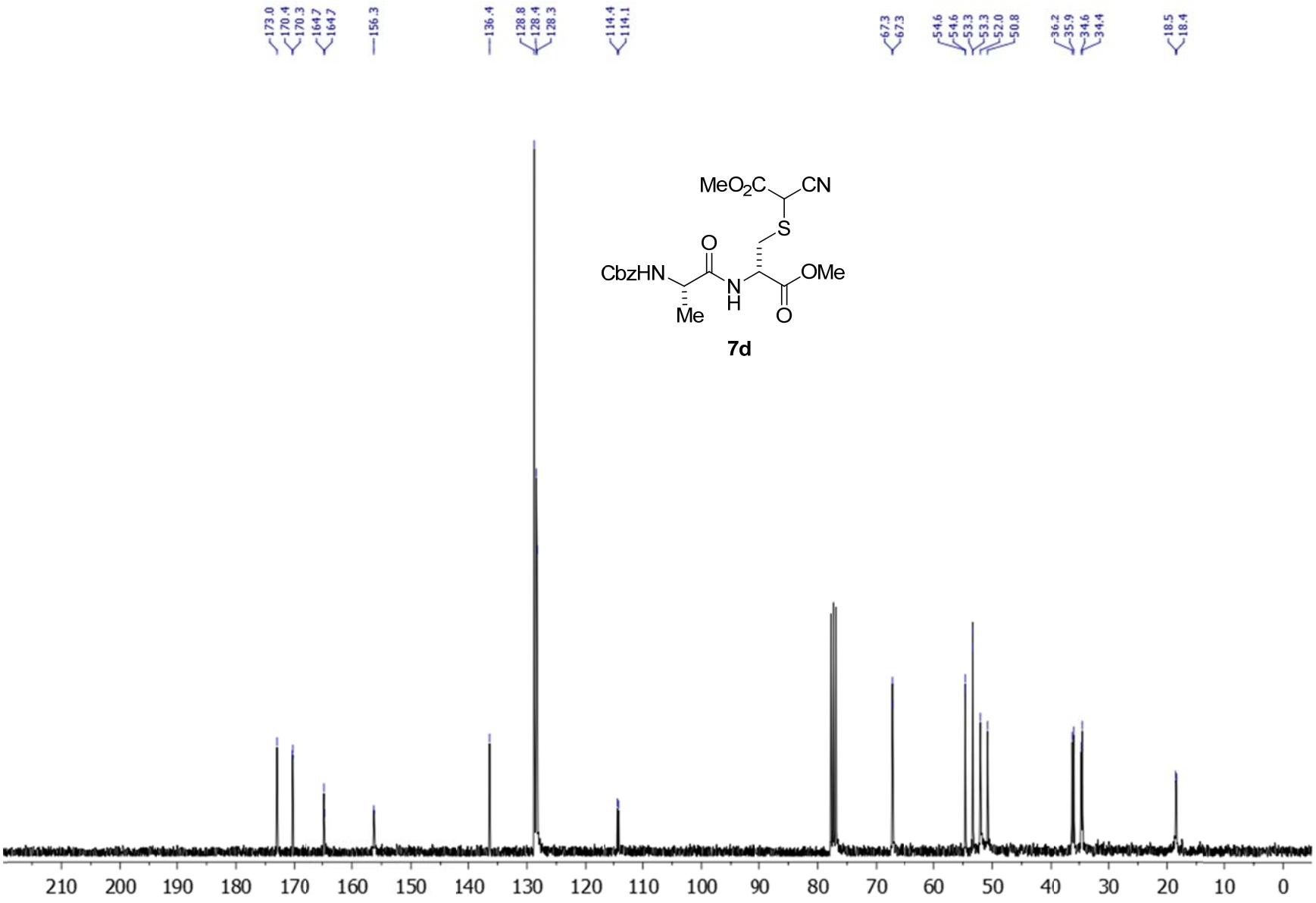
75MHz ^{13}C NMR for compound **7c** in $CDCl_3$ (ppm)



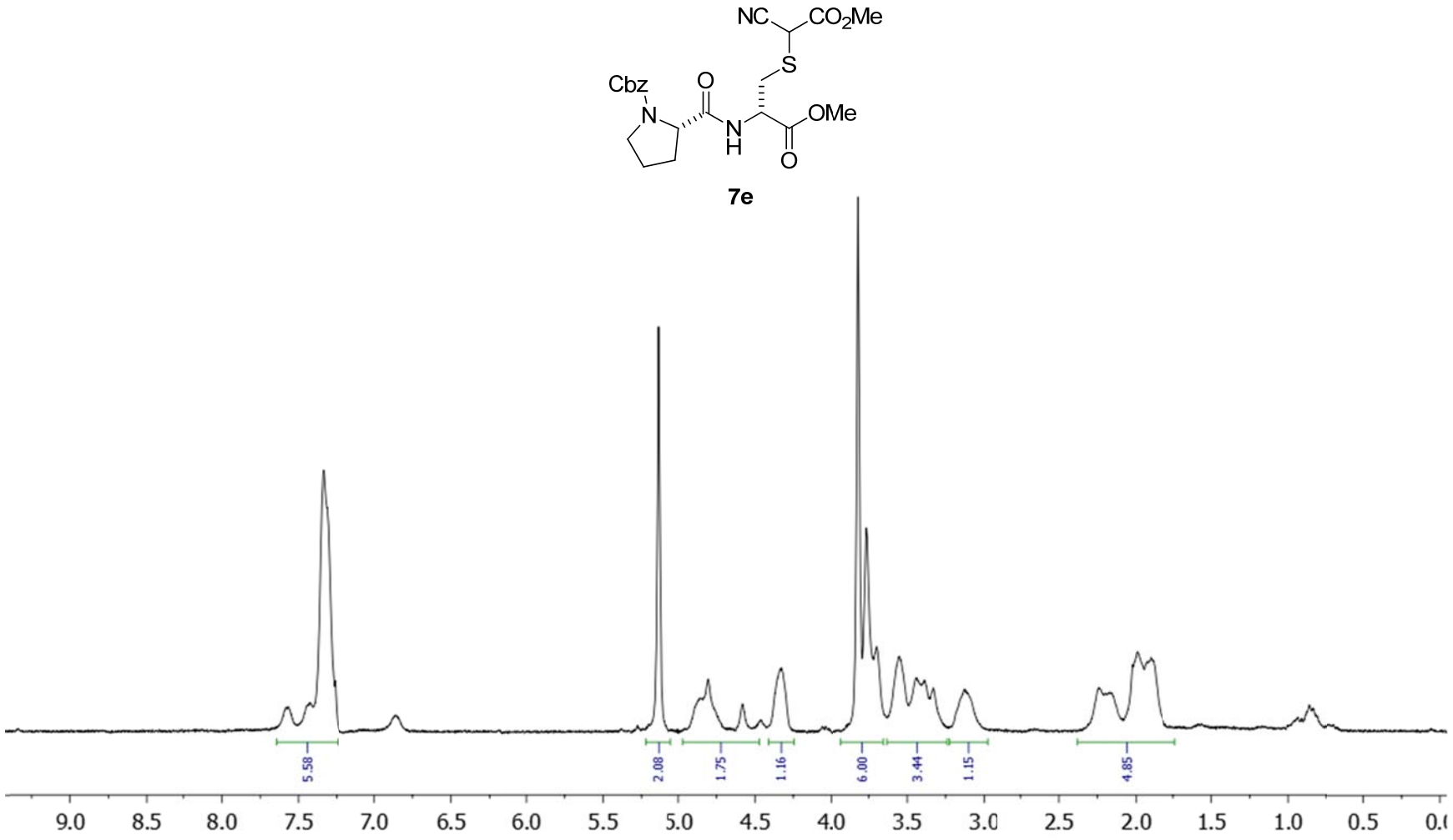
7d



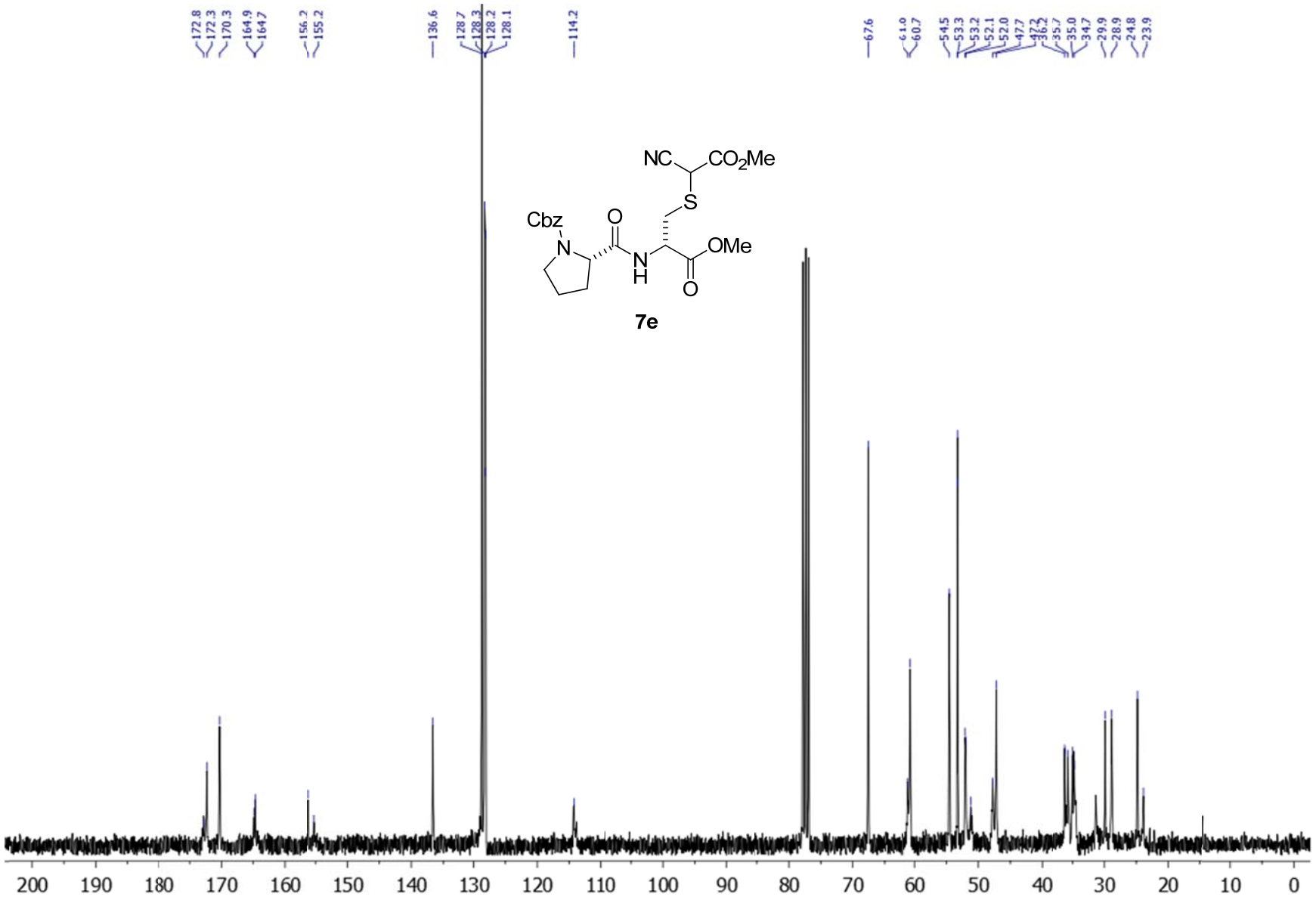
300MHz ^{1}H NMR for compound **7d** in $CDCl_3$ (ppm)



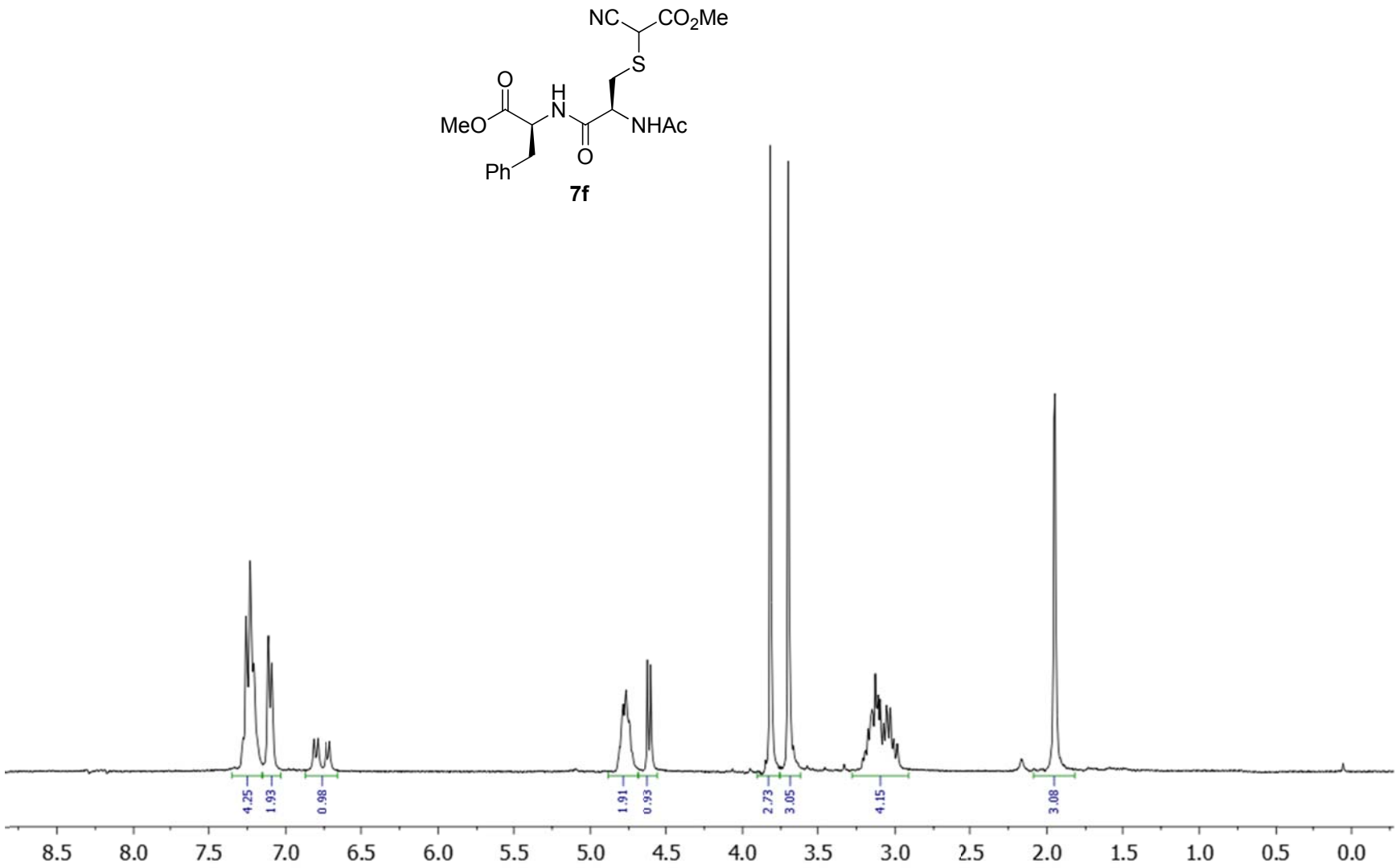
75MHz ^{13}C NMR for compound **7d** in $CDCl_3$ (ppm)



300MHz ^{1}H NMR for compound **7e** in $CDCl_3$ (ppm)



75MHz ^{13}C NMR for compound **7e** in $CDCl_3$ (ppm)



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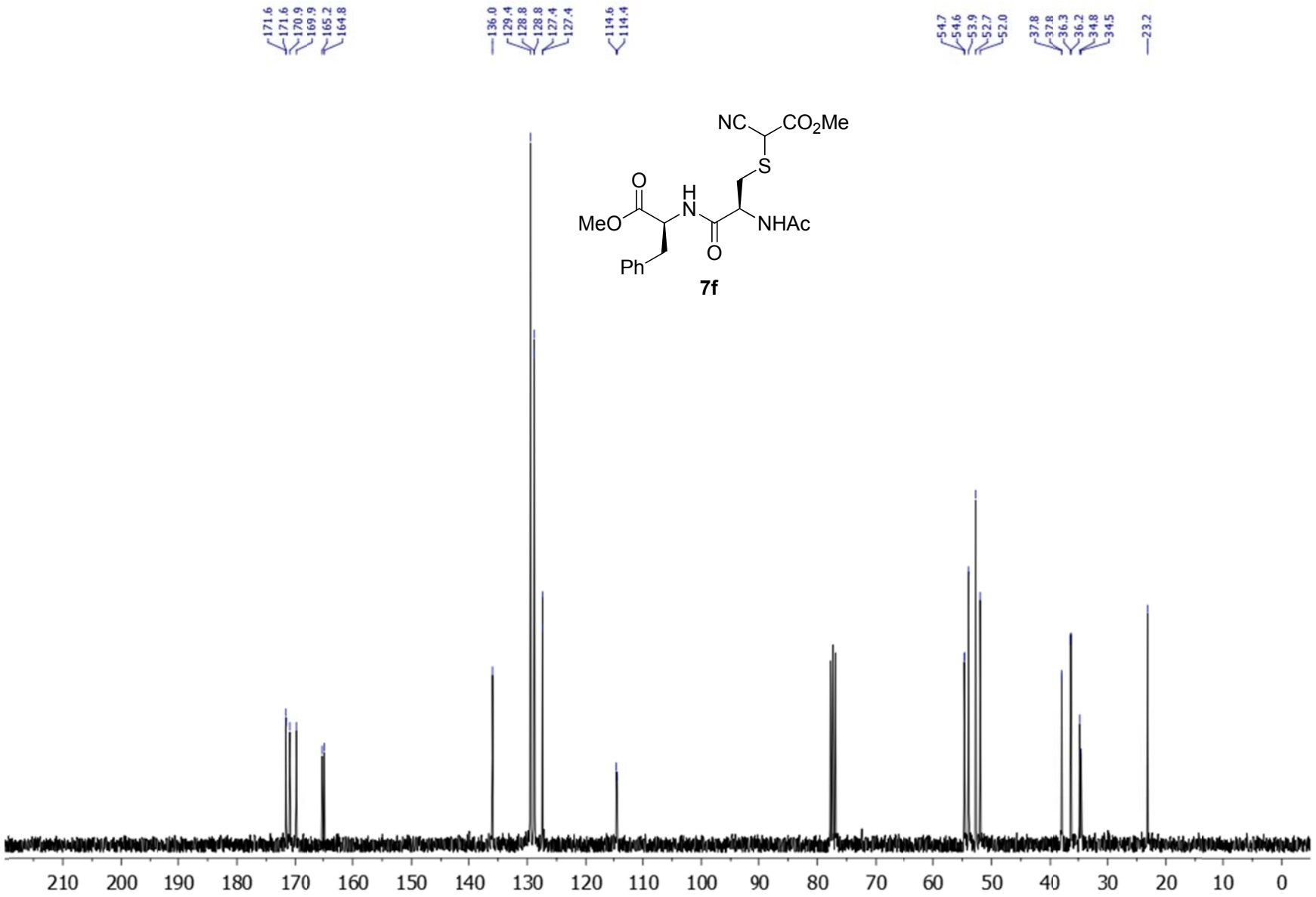
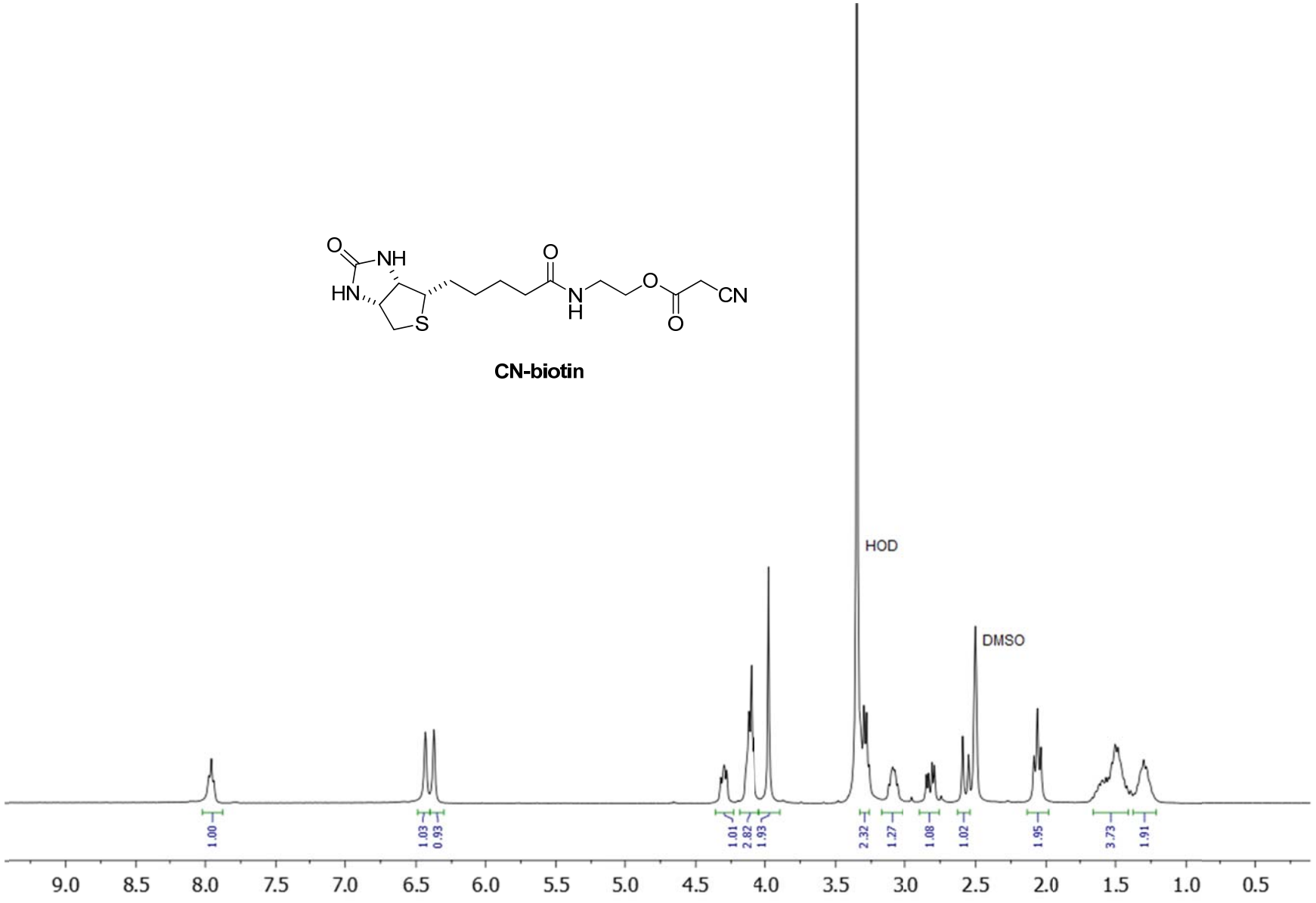
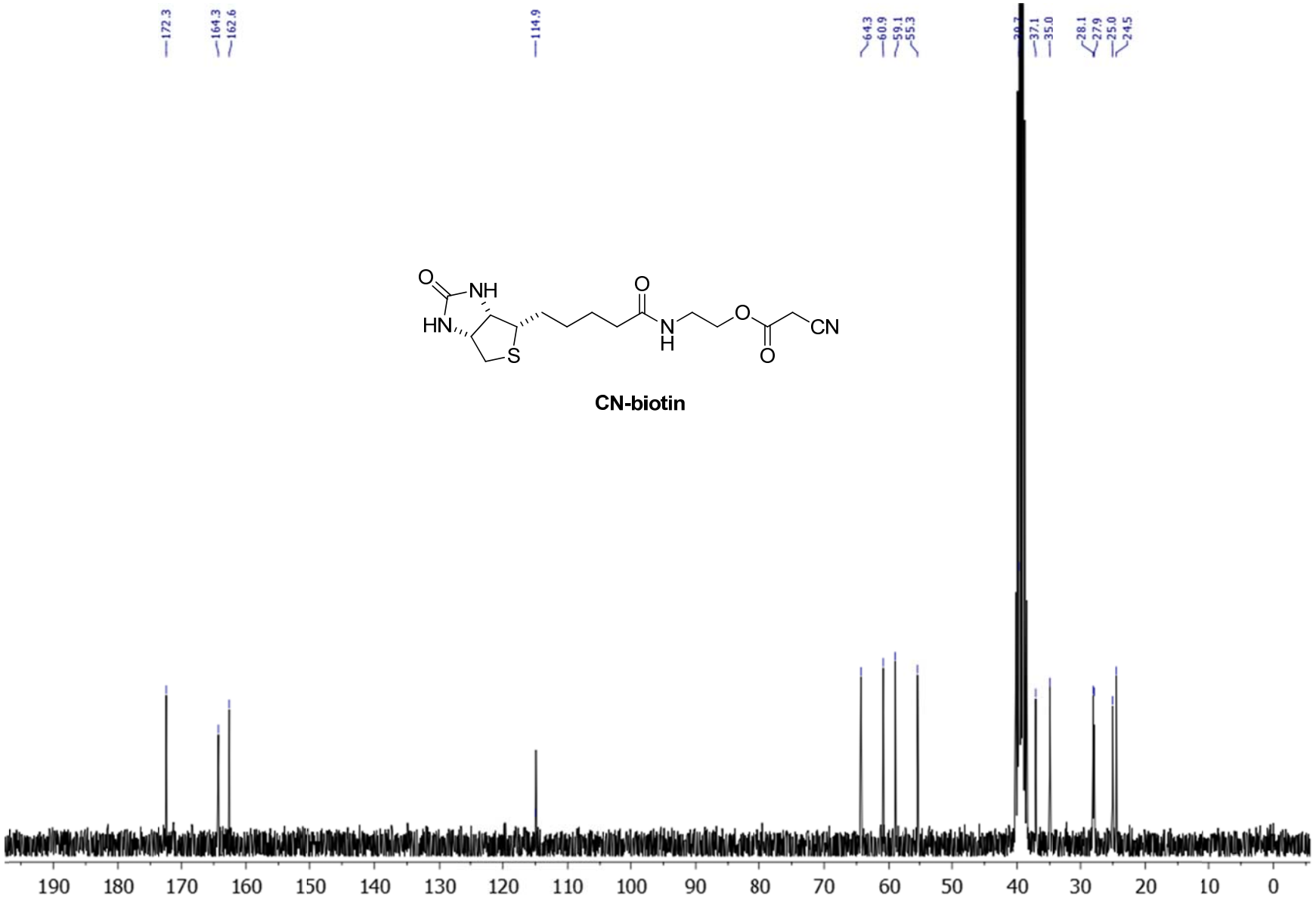


75MHz ^{13}C NMR for compound **7f** in $CDCl_3$ (ppm)



300MHz ^{1}H NMR for compound CN-biotin in DMSO-*d6* (ppm)



75MHz ^{13}C NMR for compound CN-biotin in DMSO-*d6* (ppm)