Supplementary Data

In Pursuit of Feedback Activation: New Insights into Redox-Responsive Hydropersulfide Prodrug Combating Oxidative Stress

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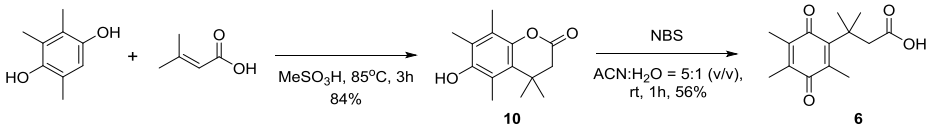
1. Chemical Synthesis

1.1 Synthesis of starting materials



Scheme S1. Synthesis of acid **6**

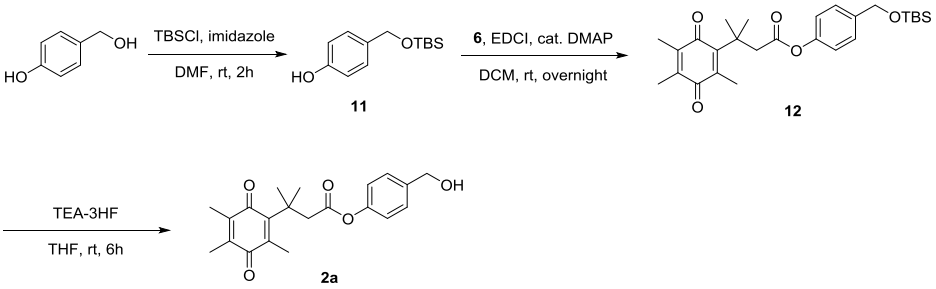
6-hydroxy-4,4,5,7,8-pentamethylchroman-2-one (10)

A 100 mL two-neck flask was charged with *trimethylhydroquinone* (4.86 g, 32 mmol) and *3,3-dimethylacrylic acid* (3.68 g, 36.8 mmol) and equipped with a nitrogen balloon. After the Ar replacement, to the flask was added *methanesulfonic acid* (50 mL) with stirring. The resulting mixture was then heated to 85°C for 3 hours. The reaction was monitored by TLC until complete consumption. The solution was quenched with ice water (100 g) and extracted by ethyl acetate (5 × 50 mL). The organic layers were combined, washed with $NaHCO_3$ solution (2 × 50 mL) and brine, dried over Na_2SO_4, and concentrated. The residue was purified by trituration with hot solution (petroleum ether/ethyl acetate = 2:1) to give compound **10** as a brown solid (6.3 g, 84% yield). This compound can also be purified by column chromatography (dichloromethane/methanol = 60/1). ^{1}H NMR (300 MHz, DMSO-*d6*) δ 7.98 (s, 1H), 2.57 (s, 2H), 2.27 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 1.35 (s, 6H). Analytical data agree with literature values [3].

3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid (6)

A solution of **10** (6.27 g, 26.8 mmol) in a mixed solvent of acetonitrile and water (75 mL, acetonitrile/water = 5/1) in a 200 mL flask was cooled to 0°C, and then NBS (5 g, 28.4 mmol) was added in portions over a few minutes. The reaction was stirred at room temperature for 1 hour, and the TLC indicated the consumption of **10**. The ice water (100 g) was added and the mixture extracted by ethyl acetate (5 × 50 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by trituration with diethyl ether to obtain acid **6** as a yellow

powder (3.72g, 56%). ^{1}H NMR (300 MHz, Chloroform-*d*) δ 3.04 (s, 2H), 2.16 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.45 (s, 6H). Analytical data agree with literature values [3].



Scheme S2. Synthesis of **2a**

4-(((tert-butyldimethylsilyl)oxy)methyl)phenol (11)

A solution of *p-hydroxybenzyl alcohol* (744 mg, 6 mmol) and imidazole (490 mg, 7.2 mmol) in dry DMF (10 mL) in a 50 mL two-neck flask was cooled to 0°C, and then a solution of TBSCl (1.1 g, 7.2 mmol) in dry DMF (10 mL) was added dropwise over 10 minutes. The resulting slurry was stirred for 2 hours. The mixture was quenched by ice water (100 g) and extracted by ethyl acetate (5 × 20 mL). The organic layers were combined, washed with 10% LiCl solution (20 mL), $NaHCO_3$ solution (20 mL) and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 20:1) to give **11** as a golden oil (1.2 g, 84%). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.15 (d, J = 8.1 Hz, 2H), 6.75 (d, J = 8.6 Hz, 2H), 4.64 (s, 2H), 0.94 (s, 9H), 0.10 (s, 6H). Analytical data agree with literature values [4].

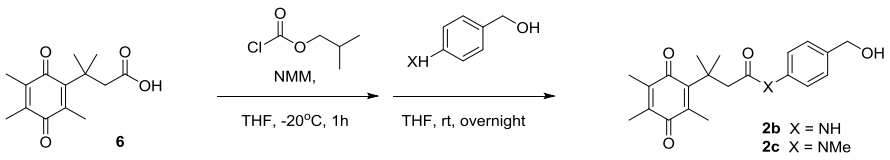
4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxo-cyclohexa-1,4-dien-1-yl)butanoate (12)

A 50 mL two-neck flask was charged with acid **6** (600 mg, 2.4 mmol), phenol **11** (476 mg, 2 mmol) and DMAP (36 mg, 0.3 mmol). To the flask was added dry DCM (20 mL), and EDCI (768 mg, 4 mmol) was added in portions. The resulting mixture was stirred overnight until the TLC indicated the consumption of **11**. The mixture was quenched by ice water (10 g) and extracted with DCM (2 × 20 mL). The organic layers

were combined, washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 15:1) to give **12** as a golden oil (838 mg, 89%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.30 (d, J = 8.3 Hz, 2H), 6.96 (d, J = 8.5 Hz, 2H), 4.68 (s, 2H), 3.16 (s, 2H), 2.12 (s, 3H), 1.87 (s, 6H), 1.47 (s, 6H), 0.89 (s, 9H), 0.07 (s, 6H). Analytical data agree with literature values [5].

4-(hydroxymethyl)phenyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoate (2a)

A 50 mL flask was charged with ester **12** (838 mg, 1.8 mmol) in THF (18 mL). To the flask was added *triethylamine trihydrofluoride* (1.2 g, 7.1 mmol), and the resulting solution was stirred for 6 hours. Following water added, the reaction was extracted by ethyl acetate (5 × 20 mL). The organic layers were combined, washed with $NaHCO_3$ solution (20 mL) and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 10:1) to give **2a** as a golden oil (370 mg, 59%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.31 (d, J = 8.4 Hz, 2H), 6.94 (d, J = 8.4 Hz, 2H), 5.21 (s, 1H), 4.47 (s, 2H), 3.16 (s, 2H), 2.11 (s, 3H), 1.87 (s, 6H), 1.47 (s, 6H). Analytical data agree with literature values [5].



Scheme S3. Synthesis of **2b** and **2c**

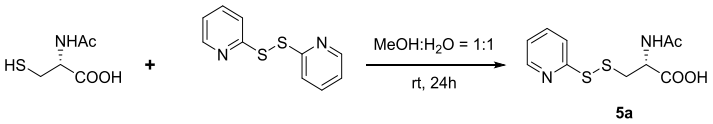
N-(4-(hydroxymethyl)phenyl)-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanamide (2b)

A 250 mL two-neck flask was charged with acid **6** (3.5 g, 14 mmol) and equipped with a nitrogen balloon. After the gas replacement, to the flask was added dry THF (40 mL) and *N-Methylmorpholine* (1.5 g, 15.2 mmol) with stirring. The resulting solution was cooled to -20°C, and then a solution of *isobutyl chlorocarbonate* (2.1 g, 15.2 mmol) in dry THF (20 mL) was added dropwise. After 1 hour, *4-aminobenzyl alcohol* (2.58 g,

21 mmol) in dry THF (10 mL) was added. The reaction solution was slowly warmed to room temperature and stirred overnight. The reaction solution was quenched with ice water (100 g) and extracted by ethyl acetate (5 × 50 mL). The organic layers were combined, washed with $NaHCO_3$ solution (2 × 50 mL) and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 5:1) to give a brown solid, which was further purified by trituration with diethyl ether to obtain **2b** as a yellow powder (3.1 g, 62% yield). ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.40 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 5.2 Hz, 2H), 4.64 (s, 2H), 3.02 (s, 2H), 2.17 (s, 3H), 1.96 (s, 6H), 1.68 (s, 3H), 1.51 (s, 6H). Analytical data agree with literature values [3].

N-(4-(hydroxymethyl)phenyl)-N,3-dimethyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanamide (2c)

Compound **2c** was prepared according to the above procedure for **2b**. Yellow solid, 802 mg, 82.4%. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.46 (d, *J* = 7.8 Hz, 2H), 7.21 (d, *J* = 6.6 Hz, 2H), 4.78 (s, 2H), 3.17 (s, 3H), 2.76 (s, 2H), 2.12 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.32 (s, 6H). Analytical data agree with literature values [3].

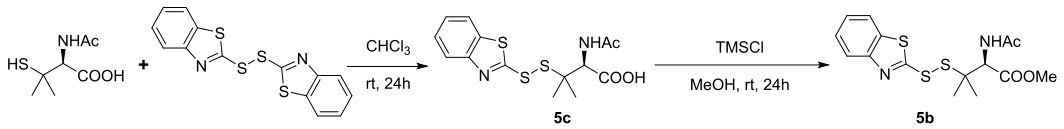


Scheme S4. Synthesis of **5a**

N-acetyl-S-(pyridin-2-ylthio)-L-cysteine (5a)

A round bottom flask was charged with *N*-acetylcysteine (NAC) (2.0 g, 12.3 mmol) and H_2O (17 mL). A clear solution was obtained after stirred at room temperature. A solution of 2, 2'-dipyridyl disulfide (5.40 g, 24.5 mmol) in MeOH (17 mL) was added in one portion resulting in a clear, yellow solution. The reaction mixture was stirred at room temperature for 24 hours. The resulting yellow solution was concentrated via rotary evaporation and then extracted with DCM (3 x 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by

trituration with acetone, yielding white powder (1.53 g, 46%). ^{1}H NMR (300 MHz, DMSO) δ 8.44 (d, *J* = 4.2 Hz, 1H), 8.32 (d, *J* = 7.6 Hz, 1H), 7.86 – 7.71 (m, 2H), 7.29 – 7.18 (m, 1H), 4.44 (q, *J* = 8.1 Hz, 1H), 3.22 (dd, *J* = 13.5, 4.4 Hz, 1H), 3.08 (dd, *J* = 13.3, 8.9 Hz, 1H), 1.85 (s, 3H). Analytical data agree with literature values [6].



Scheme S5. Synthesis of **5b**

2-acetamido-3-(benzo[d]thiazol-2-yldisulfanyl)-3-methylbutanoic acid (5c)

To a solution of 2, 2'-dibenzothiazolyl disulfide (2.61 g, 7.84 mmol) in $CHCl_3$ (100 mL), *N*-acetyl-*D*-penicillamine (1 g, 5.23 mmol) was added. The reaction was stirred at room temperature for 24 hours. The solvent was concentrated via rotary evaporation, and the residue was purified by trituration with acetone for two times to obtain **5c** as a white solid (913 mg, 49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, *J* = 8.1 Hz, 1H), 7.78 (d, *J* = 7.9 Hz, 1H), 7.47 (t, *J* = 7.3 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 6.68 (d, *J* = 8.3 Hz, 1H), 4.89 (d, *J* = 8.5 Hz, 1H), 2.02 (s, 3H), 1.57 (s, 3H), 1.40 (s, 3H). Analytical data agree with literature values [2].

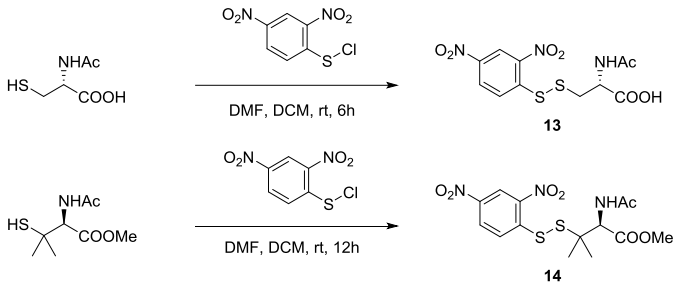
methyl 2-acetamido-3-(benzo[d]thiazol-2-yldisulfanyl)-3-methylbutanoate (5b)

A two-necked bottom flask was charged with **13** (713 mg, 2.0 mmol) and anhydrous methanol (15 mL). Under nitrogen atmosphere, chlorotrimethylsilane (0.76 mL, 6.0 mmol) was added dropwise. The mixture was stirred at room temperature for 24 hours, and then concentrated under vacuum. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 5/1 ~ 1/1) to afford **5b** as a yellow oil (296 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, *J* = 8.1 Hz, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.46 (t, *J* = 7.2 Hz, 1H), 7.36 (t, *J* = 7.6 Hz, 1H), 6.64 (d, *J* = 9.2 Hz, 1H), 4.85 (d, *J* = 9.2 Hz, 1H), 3.77 (s, 3H), 2.06 (s, 3H), 1.55 (s, 3H), 1.49 (s, 3H). Analytical data agree with literature values [2].

N-acetyl-penicillamine methyl ester (NAP-Me)

A 100 mL two-neck flask was charged with a solution of *N-acetyl-D-penicillamine* (1.91 g, 10 mmol) in anhydrous methanol (50 mL) at 0°C. TMSCl (2 mL, 20 mmol) was added dropwise. The reaction solution was slowly warmed to room temperature and stirred overnight. The mixture was directly evaporated under vacuum, and the resulting residue was purified by silica gel chromatography (petroleum ether/ethyl acetate = 10/1 ~ 1/1) to give a white solid (0.96 g, 47% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.36 (s, 1H), 4.64 (d, J = 9.4 Hz, 1H), 3.75 (s, 3H), 2.06 (s, 3H), 1.98 (s, 1H), 1.49 (s, 3H), 1.35 (s, 3H). Analytical data agree with literature values [2].

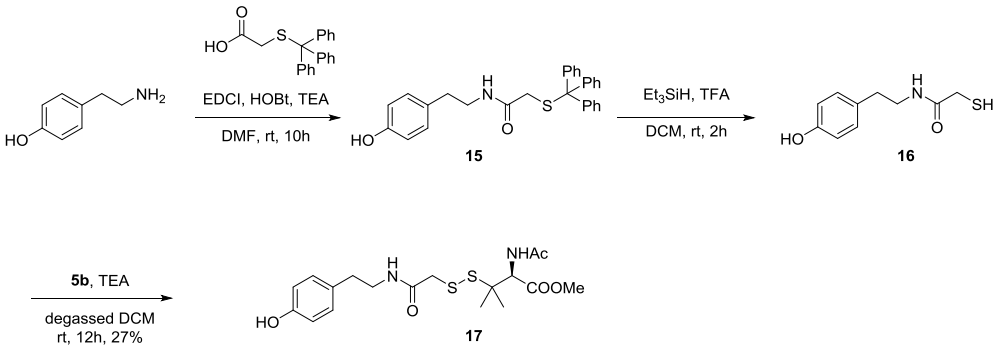
1.2 Synthesis of reference compounds and derivatization reagent



Scheme S6. Synthesis of reference compounds **13** and **14**

A 10 mL flask was charged with *N-Acetyl-L-cysteine* (80 mg, 0.49 mmol) and DMF (1 mL). To the flask was added a solution of *2,4-dinitrophenylsulfenyl chloride* (115 mg, 0.49 mmol) in dry DCM (4 mL) at 0°C under a nitrogen atmosphere. The resulting mixture was stirred for 6 hours, quenched by ice water (10 mL) and extracted with DCM (3 × 30 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (dichloromethane/methanol = 100/1 ~ 30/1) to give **13** as a yellow solid (107 mg, 60%). ^{1}H NMR (300 MHz, Methanol-d_4) δ 9.08 (s, 1H), 8.59 (d, J = 2.5 Hz, 1H), 8.56 (d, J = 7.2 Hz, 1H), 4.71-4.66 (m, 1H), 3.42-3.37 (m, 1H), 3.24-3.10 (m, 1H), 2.03 (s, 3H). Analytical data agree with literature values [1].

Compound **14** was prepared according to the above procedure. Light yellow solid, (48 mg, 24%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.12 (d, *J* = 2.4 Hz, 1H), 8.55 (d, *J* = 9.0 Hz, 1H), 8.48 (dd, *J* = 9.0, 2.4 Hz, 1H), 4.87 (d, *J* = 9.3 Hz, 1H), 3.84 (s, 3H), 2.12 (s, 3H), 1.45 (s, 3H), 1.43 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.2, 169.9, 146.6, 145.7, 145.3, 129.5, 127.1, 121.5, 58.9, 55.6, 52.7, 26.0, 25.0, 23.3. HRMS (ESI) for $C_{14}H_{18}N_3O_7S_2$ $[M+H]^+$ calcd 404.0581, found 404.0577.



Scheme S7. Synthesis of reference compound **17**

Compound **17** and HPE-IAM was prepared according to reported methods with appropriate modification [2].

N-(4-hydroxyphenethyl)-2-(tritylthio)acetamide (15)

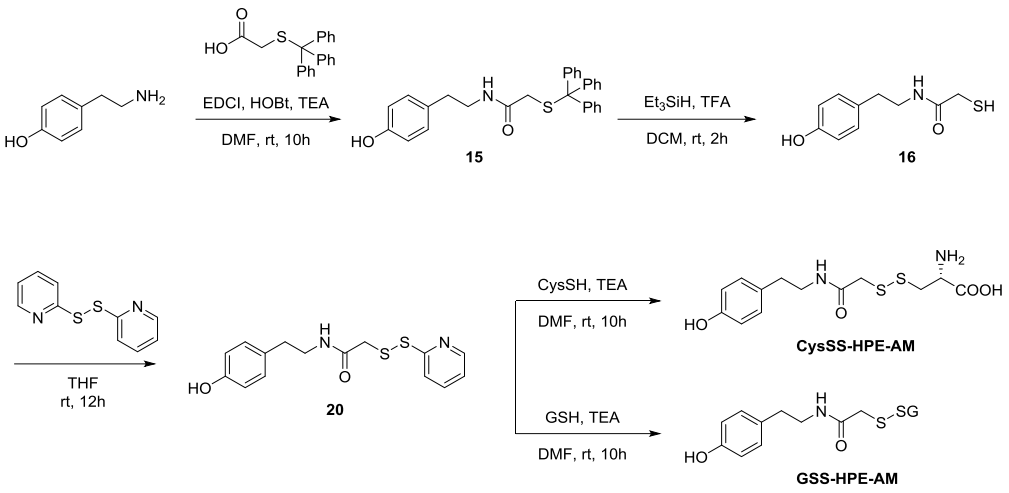
To a 25mL double-neck flask was charged with *4-(2-aminoethyl)phenol* (137 mg, 1 mmol), *2-(Tritylthio)acetic acid* (334 mg, 1 mmol), triethylamine (0.28 mL, 2 mmol) and DMAP (61 mg, 0.5 mmol) in DMF (10 mL), and then HOBt (135 mg, 1 mmol) and EDCI (288 mg, 1.5 mmol) was added portionwise. The solution was stirred at room temperature for 10 hours, and then 1N HCl (2 mL) was added for quench. The resulting mixture was extracted by ethyl acetate (5 × 10 mL). The organic layers were combined, washed with 10% LiCl solution (2 × 20 mL), dried over Na_2SO_4, and concentrated. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate = 20/1 ~ 5/1) to afford a white solid **15** (430 mg, 95%). ^{1}H NMR (500 MHz, DMSO) δ 9.14 (s, 1H), 7.86 (t, *J* = 5.6 Hz, 1H), 7.33 (m, *J* = 5.7 Hz, 12H), 7.28 – 7.23 (m, 3H), 6.95 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 8.4 Hz, 2H), 3.11 (q, *J* = 7.1 Hz, 2H), 2.76 (s, 2H), 2.53 (m, 2H). Analytical data agree with literature values.

N-(4-hydroxyphenethyl)-2-mercaptoacetamide (16)

To a solution of **15** (226 mg, 0.5 mmol) in DCM (10 mL) at 0°C, Et_3SiH (116 mg, 1 mmol) and TFA (1 mL) was subsequently added dropwise. The reaction was stirred for 2 hours, and the solvent was evaporated under vacuum. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate = 20/1 ~ 5/1), giving **16** as a yellow oil (96 mg, 91% yield). ^{1}H NMR (500 MHz, DMSO) δ 9.14 (s, 1H), 8.01 (t, *J* = 5.7 Hz, 1H), 6.99 (d, *J* = 8.5 Hz, 2H), 6.68 (d, *J* = 8.4 Hz, 2H), 3.22 (dt, *J* = 13.3, 6.9 Hz, 2H), 3.07 (d, *J* = 7.1 Hz, 2H), 2.70 – 2.64 (m, 1H), 2.59 (t, *J* = 7.4 Hz, 2H). Analytical data agree with literature values.

(S)-2-acetamido-3-((2-((4-hydroxyphenethyl)amino)-2-oxoethyl)disulfanyl)-3-methylbutanoic acid (17)

A double-neck round-bottom flask was charged with thiol **16** (106 mg, 0.5 mmol), degassed DCM (2.5 mL), and a stir bar. Compound **5b** (185 mg, 0.5 mmol) in degassed DCM (2.5 mL) was added dropwise. The reaction was stirred at room temperature overnight, and quenched by the addition of 1N HCl (2 mL). The residue was extracted with DCM (3 × 10 mL), the combined organic layers were washed with brine and dried over Na_2SO_4, filtered, and concentrated on rotary evaporator. The crude product was purified by silica gel chromatography (DCM/MeOH = 100/1 ~ 10/1) to obtain **17** as a white solid (108 mg, 54% yield). ^{1}H NMR (500 MHz, DMSO) δ 9.12 (s, 1H), 8.24 (d, *J* = 8.9 Hz, 1H), 8.04 (t, *J* = 5.7 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 2H), 6.65 (d, *J* = 8.5 Hz, 2H), 4.56 (d, *J* = 8.9 Hz, 1H), 3.45 – 3.32 (m, 2H), 3.26 – 3.09 (m, 1H), 2.57 (t, *J* = 7.4 Hz, 2H), 1.88 (s, 3H), 1.29 (d, *J* = 9.7 Hz, 5H). Analytical data agree with literature values.

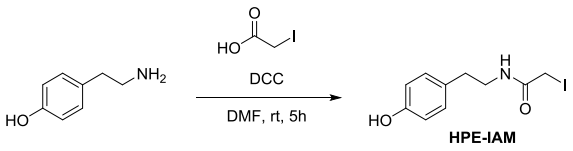


Scheme S8. Synthesis of **CysSS-HPE-AM** and **GSS-HPE-AM**

Compound **16** (192 mg, 0.9 mmol) was dissolved in THF (20 mL). To the solution was added 2, 2'-dithiodipyridine (400 mg, 1.82 mmol), and then the reaction was stirred at room temperature for 12 hours. Upon completion of the reaction, the solvent was removed and the obtained compound **20** was used for the next step without further purification.

Compound **20** (25 mg, 0.11 mmol) was dissolved in DMF (1 mL). To the solution was added *L*-cysteine (16 mg, 0.13 mmol), and then the reaction was stirred at room temperature for 10 hours. Upon completion of the reaction, the solvent was directly evaporated under vacuum, and the residue was purified by silica gel chromatography (DCM/MeOH = 10/1 ~ 5/1) to give a yellowish solid (26 mg, 76%). ^{1}H NMR (500 MHz, DMSO) δ 7.01 (d, J = 8.2 Hz, 2H), 6.69 (d, J = 8.5 Hz, 2H), 4.11 (s, 1H), 3.49 – 3.39 (m, 3H), 3.25 (t, J = 6.5 Hz, 3H), 2.62 (t, J = 7.6 Hz, 2H), 1.40 (s, 9H).

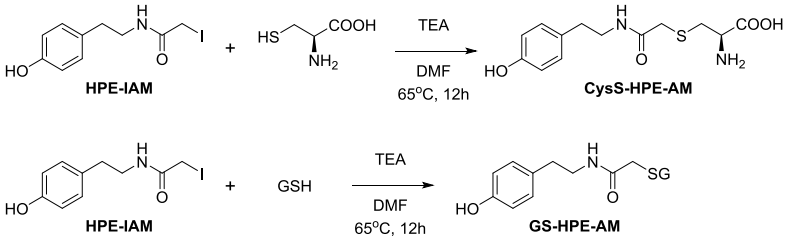
GSS-HPE-AM was prepared according to the above procedure described for **CysSS-HPE-AM**. A yellow solid was obtained.



Scheme S9. Synthesis of **HPE-IAM**

N-(4-hydroxyphenethyl)-2-iodoacetamide (HPE-IAM)

A 100 mL two-neck flask was charged with a solution of *4-(2-aminoethyl)phenol* (548 mg, 4 mmol) and DCC (824 mg, 4 mmol) in anhydrous DCM (50 mL) at 0°C. *iodoacetic acid* (744 mg, 4 mmol) was added dropwise. The reaction solution was slowly warmed to room temperature and stirred for 5 hours. The mixture was directly evaporated under vacuum, and the resulting residue was purified by silica gel chromatography (DCM/MeOH = 100/1 ~ 30/1) to give a white solid (230 mg, 19% yield). ^{1}H NMR (500 MHz, MeOD) δ 7.91 (s, 1H), 7.07 (d, *J* = 8.4 Hz, 2H), 6.74 (d, *J* = 8.4 Hz, 2H), 3.69 (s, 2H), 3.38 (t, *J* = 7.3 Hz, 2H), 3.34 (br s, 1H), 2.72 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 167.9, 156.2, 130.0, 129.7, 115.6, 41.6, 34.5, 1.4. HRMS (ESI) for $C_{10}H_{13}NO_2I$ $[M+H]^+$ calcd 305.9985, found 305.9979.



Scheme S10. Synthesis of **CysS-HPE-AM** and **GS-HPE-AM**

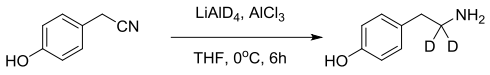
L-cysteine (18.2 mg, 0.15 mmol) was dissolved in DMF (1 mL). To the solution was added ***HPE-IAM*** (50.3 mg, 0.165 mmol) and TEA (30.4 mg, 0.3 mmol). Then the reaction was stirred at 65°C for 12 hours. Upon completion of the reaction, the solvent was removed using a rotary evaporator and the crude product was triturated with ethyl acetate. Filtered to give the pure product as a light yellow solid (15 mg, 33%). ^{1}H NMR (300 MHz, DMSO) δ 9.38 (br s, 2H), 8.41 (t, *J* = 5.8 Hz, 1H), 7.92 (br s, 2H), 7.00 (d, *J* = 8.4 Hz, 2H), 6.69 (d, *J* = 8.4 Hz, 2H), 3.42 (dd, *J* = 8.0, 3.9 Hz, 1H), 3.30 – 2.99 (m, 5H), 2.87 (dd, *J* = 14.5, 7.9 Hz, 1H), 2.61 (t, *J* = 7.5 Hz, 2H).

GS-HPE-AM was prepared according to the above procedure described for **CysS-HPE-AM**. A white solid was obtained (45 mg, 62%) as a triethylamine salt form. ^{1}H NMR (300 MHz, DMSO) δ 6.98 (d, *J* = 8.4 Hz, 2H), 6.68 (d, *J* = 8.4 Hz, 2H), 4.55 –

4.42 (m, 1H), 3.66 – 3.53 (m, 2H), 3.44 (d, *J* = 7.0 Hz, 1H), 3.28 – 3.10 (m, 4H), 2.91 (d, *J* = 4.6 Hz, 1H), 2.76 – 2.65 (m, 1H), 2.59 (t, *J* = 7.4 Hz, 2H), 2.34 (t, *J* = 8.1 Hz, 2H), 2.05 – 1.84 (m, 2H).

1.3 Synthesis of deuterium reference compounds

4-(2-aminoethyl-2,2-d2)phenol



Scheme S11. Synthesis of *4-(2-aminoethyl-2,2-d2)phenol*

To a double-neck flask was charged with lithium tetradeuterioaluminate ($LiAlD_4$, 200 mg, 4.8 mmol), aluminum chloride (266 mg, 2 mmol) and equipped with a nitrogen balloon. The flask was cooled to 0°C, followed by a dropwise addition of the solution of 4-hydroxyphenylacetonitrile in anhydrous THF (10 mL) within 0.5 hour. The mixture was stirred at room temperature for 6 h. Upon completion of the reaction, the mixture was cooled to 0°C, and several drops of water were added to quench the reaction, and then anhydrous Na_2SO_4 was added under stirring. Filter and evaporate the organic solvent, and the residue was purified by silica gel chromatography (DCM/MeOH = 100/1 ~ 20/1) to give a light yellow solid (150 mg, 54% yield). ^{1}H NMR (500 MHz, DMSO) δ 7.02 (d, *J* = 8.4 Hz, 2H), 6.73 (d, *J* = 8.5 Hz, 2H), 2.71 (s, 2H).

Five deuterium reference compounds

The deuterium reference compounds: **17-d2**, **CysSS-HPE-AM-d2**, **GSS-HPE-AM-d2**, **CysS-HPE-AM-d2**, and **GS-HPE-AM-d2** were prepared according to the above procedure.

17-d2: ^{1}H NMR (300 MHz, DMSO) δ 9.16 (s, 1H), 8.29 (d, *J* = 8.8 Hz, 1H), 8.07 (s, 1H), 7.00 (d, *J* = 8.6 Hz, 2H), 6.67 (d, *J* = 8.5 Hz, 2H), 4.59 (d, *J* = 8.7 Hz, 1H), 3.65 (s, 3H), 3.41 (d, *J* = 2.4 Hz, 2H), 2.58 (s, 2H), 1.91 (s, 3H), 1.32 (d, *J* = 6.1 Hz, 6H).

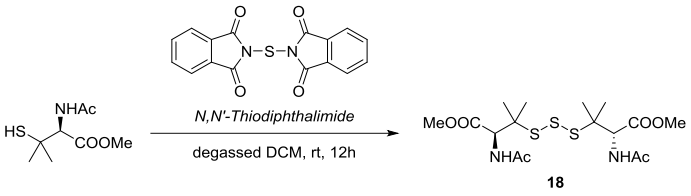
CysSS-HPE-AM-d2: ^{1}H NMR (300 MHz, DMSO) δ 7.00 (d, *J* = 8.6 Hz, 2H), 6.67 (d, *J* = 8.5 Hz, 2H), 3.95 (d, *J* = 12.7 Hz, 1H), 3.57 – 3.31 (m, 4H), 2.59 (s, 2H).

GSS-HPE-AM-d2: ^{1}H NMR (300 MHz, DMSO) δ 7.00 (d, *J* = 8.5 Hz, 2H), 6.68 (d, *J* = 8.3 Hz, 2H), 4.55 (s, 1H), 3.71 (s, 2H), 3.53 – 3.34 (m, 4H), 2.88 (s, 1H), 2.59 (s, 2H), 2.35 (s, 2H), 1.95 (s, 2H).

CysS-HPE-AM-d2: ^{1}H NMR (300 MHz, DMSO) δ 7.00 (d, *J* = 8.5 Hz, 2H), 6.69 (d, *J* = 8.5 Hz, 2H), 3.54 – 3.42 (m, 1H), 3.28 – 3.12 (m, 2H), 3.09 (d, *J* = 5.5 Hz, 1H), 2.90 (dd, *J* = 14.5, 7.8 Hz, 1H), 2.59 (s, 2H).

GS-HPE-AM-d2: ^{1}H NMR (300 MHz, DMSO) δ 6.99 (d, *J* = 8.5 Hz, 2H), 6.68 (d, *J* = 8.4 Hz, 2H), 4.54 – 4.42 (m, 1H), 3.73 (d, *J* = 6.4 Hz, 2H), 3.41 (t, *J* = 6.7 Hz, 1H), 3.13 (s, 2H), 2.99 – 2.87 (m, 1H), 2.76 – 2.67 (m, 1H), 2.58 (s, 2H), 2.33 (d, *J* = 7.8 Hz, 2H), 1.92 (s, 2H).

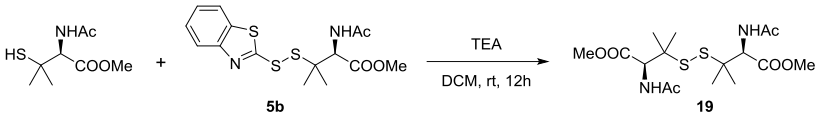
1.4 Synthesis of trisulfide 18 and disulfide 19



Scheme S12. Synthesis of trisulfide **18**

A double-neck round-bottom flask was charged with **NAP-Me** (102 mg, 0.5 mmol), degassed DCM (2 mL), and a stir bar. *N*, *N*'-Thiodiphthalimide (81 mg, 0.25 mmol) in degassed DCM (2 mL) was added under Ar atmosphere. The reaction was stirred at room temperature for 12 hours, monitoring reaction progress by TLC. The solvent was removed under vacuum, and the residue was extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine and dried over Na_2SO_4, filtered, and concentrated on rotary evaporator. The crude product was purified by silica gel chromatography (DCM/MeOH = 100/1 ~ 10/1) to obtain **18** (64 mg, 58%) as a white solid, m.p. 102–103°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.51 (d, *J* = 8.9 Hz, 1H), 4.78 (d, *J* = 9.1 Hz, 2H), 3.75 (s, 6H), 2.06 (s, 6H), 1.46 (d, *J* = 11.6 Hz, 12H). ^{13}C NMR (126

MHz, $CDCl_3$) δ 170.6, 170.0, 59.1, 53.8, 52.4, 26.3, 25.8, 23.3. HRMS (ESI) for $C_{16}H_{29}N_2O_6S_3$ $[M+H]^+$ calcd 441.1182, found 441.1172.

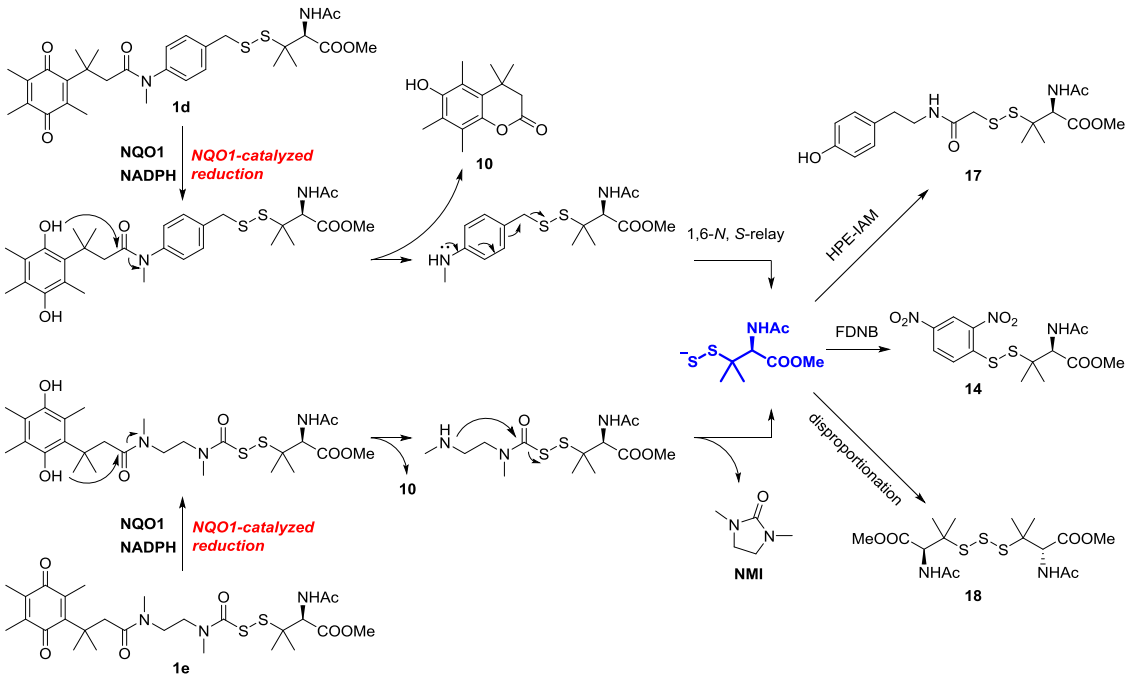


Scheme S13. Synthesis of disulfide **19**

NAP-Me (88 mg, 0.24 mmol) and compound **5b** (40 mg, 0.2 mmol) were dissolved in DCM (4 mL). To the solution was added TEA (44 mg, 0.44 mmol), and then the reaction was stirred at room temperature for 12 hours. Upon completion of the reaction, the solvent was removed using a rotary evaporator, and the residue was purified by silica gel chromatography (DCM/MeOH = 100/1 ~ 20/1) to give **19** (40 mg, 49%) as a white solid, m.p. 109–110°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.27 (d, J = 9.0 Hz, 2H), 4.67 (d, J = 9.1 Hz, 2H), 3.78 (s, 6H), 2.07 (s, 6H), 1.46 (s, 6H), 1.38 (s, 6H).

2. Validating RSSH release

2.1 Proposed pathway for prodrug activation and RSSH release



Scheme S14. Proposed degradation pathway for prodrugs to RSSH.

Proposed mechanism of RSSH release: NQO1 is one of the two major quinone reductases in mammalian systems for quinone detoxification, using NADPH/NADH as hydride donors. The two series of prodrugs both contain a 1,4-benzoquinone group, a specific moiety for enzymatic recognition, which can be transformed into a hydroquinone structure after NQO1-catalyzed reduction. The resulting phenols can realize spontaneous self-lactonization (producing **10**), and the leaving amino group can drive a 1, 6-*N*, *S*-relay, or a spontaneous ring closure (producing **NMI**) to squeeze out a molecule of RSSH (Scheme S14).

The direct evidence for the generation of RSSH comes from the production of trapping agent-dependent derivatives **14** and **17**. Here, two trapping agents ***HPE-IAM*** and ***FDNB*** were used. In addition, trapping agent-free condition was considered, in which RSSH generated trisulfide **18**. The NQO1-related degradation product **10** was not suitable for LC-MS detection, prompting a HPLC-UV assay to realize the observation of **10**.

2.2 LC-MS/MS assay for validation of RSSH release by recombinant enzyme (Analytical Method A and Analytical Method B)

(1) HPLC conditions

Analytical Method A and Analytical Method B shared the same liquid phase conditions described below. A gradient elution program was conducted with mobile phase A (0.1% formic acid in water) and mobile phase B (methanol) shown in the following Table S1.

Table S1. Gradient elution program

Time (min)	Phase B (%)
0.01	40
2.00	40
5.00	85
9.00	85
10.00	95
12.00	95
13.00	40
15.00	40
15.01	Stop

(2) MS conditions of Analytical Method A

The RSSH generation **in enzyme assays with trapping agents** were measured by a LC-MS/MS system containing a Shimadzu HPLC system (Kyoto, Japan) coupled with a SCIEX API 4000 triple-quadrupole mass spectrometer (Foster City, CA, USA) equipped with an electrospray ionization (ESI) interface in positive or negative ionization mode. The MRM parameters were optimized as listed in above Table S2.

Table S2. MRM parameters for LC/MS/MS

prodrug	Q1 Mass (m/z)	Q3 Mass (m/z)	Declustering Potential (V)	Collision Energy (eV)
negative ESI mode				
1a	532.0	161.9	-27	-24
1b	531.2	161.8	-85	-30
1c	545.1	161.9	-77	-20
13	360.0	199.0	-43	-19
Warfarin[a]	307.1	250.0	-80	-30
positive ESI mode				
1d	589.2	205.5[b]	112	47
	589.2	119.9[c]	97	24
1e	584.1	205.3[b]	92	52
	584.1	233.1[c]	119	31
14	404.2	71.0[b]	74	51
	404.2	113.2[c]	74	30
17	415.2	206.1[b]	89	19
	415.2	244.3[c]	89	22
18	441.1	172.2[b]	85	33

NMI	441.1	381.3 [c]	85	22
	115.3	58.0 [b]	64	25
	115.3	99.1 [c]	64	36
NAP-Me	206.0	87.1	55	34
Warfarin[a]	309.0	163.1	77	22

[a] internal standard (IS); [b] for quantification; [c] for qualification

Calibration curves

A working solution of the above analytes was using a serial dilution procedure at 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01 μg/mL to make calibration standards in acetonitrile. 10 μL of each dilution was added to 90 μL of PBS buffer, and the resulting mixture was diluted by 500 μL IS working solution. The acetonitrile solution was centrifuged at 18,000×g at 4℃ for 5 min to remove invisible impurities. The supernatant was analyzed by LC-MS/MS directly.

Calibration curves for each sample or standard were obtained as plots of relative intensities (ratios of analytes and IS peak areas) by linear regression using a weighting factor of the reciprocal of the relative concentration (1/x) versus concentration over the range from 0.1-500 μg/mL of reaction solution. See Table S3.

Table S3. Linearity of representative calibration curves

Compound	Mean linear relationship	Regression coefficient (r)
1a	y = 0.000841 x + 0.000815	0.9990
1b	y = 0.00183 x + 0.00359	0.9990
1c	y = 0.00376 x + 0.0105	0.9992
1d	y = 0.0183 x + 0.00637	0.9970
1e	y = 0.047 x + 0.0264	0.9927
14	y = 0.000942 x + 0.00028	0.9957
15	y = 0.00944 x + 0.00933	0.9984
17	y = 0.00834 x + 0.0021	0.9943
18	y = 0.0201 x + 0.0542	0.9944
NMI	y = 0.0706 x + 0.201	0.9979
NAP-Me	y = 0.0304 x + 0.0279	0.9979

(3) MS conditions of Analytical Method B

The RSSH generation **in enzyme assays without trapping agents** were measured by a LC-MS/MS system containing a Shimadzu HPLC system (Kyoto, Japan) coupled with a SCIEX API 6500 triple-quadrupole mass spectrometer (Foster City, CA, USA) equipped with an electrospray ionization (ESI) interface in positive or negative ionization mode. The MRM parameters were optimized as listed in above Table S4.

Table S4. MRM parameters for LC/MS/MS

prodrug	Q1 Mass (m/z)	Q3 Mass (m/z)	Declustering Potential (V)	Collision Energy (eV)
positive ESI mode				
1e	584.4	233.0	132	28
17	415.3	354.8	23	22
18	441.3	172.0	91	29
19	409.3	204.2	95	22
NMI	115.4	58.1	45	21
NAP-Me	206.4	103.9	29	26
Warfarin[a]	309.4	251.1	101	22

Calibration curves

The operations are the same as in **Analytical Method A**. The results are shown in Table S5.

Table S5. Linearity of representative calibration curves

Compound	Mean linear relationship	Regression coefficient (r)
1e	y = 0.00647 x + 0.0115	0.9960
17	y = 0.00157 x + 0.00109	0.9979
18	y = 0.00127 x - 0.0032	0.9930
19	y = 0.00384 x + 0.00351	0.9975
NMI	y = 0.00219 x + 0.00264	0.9963
NAP-Me	y = 0.00127 x + 0.0000958	0.9974

Results

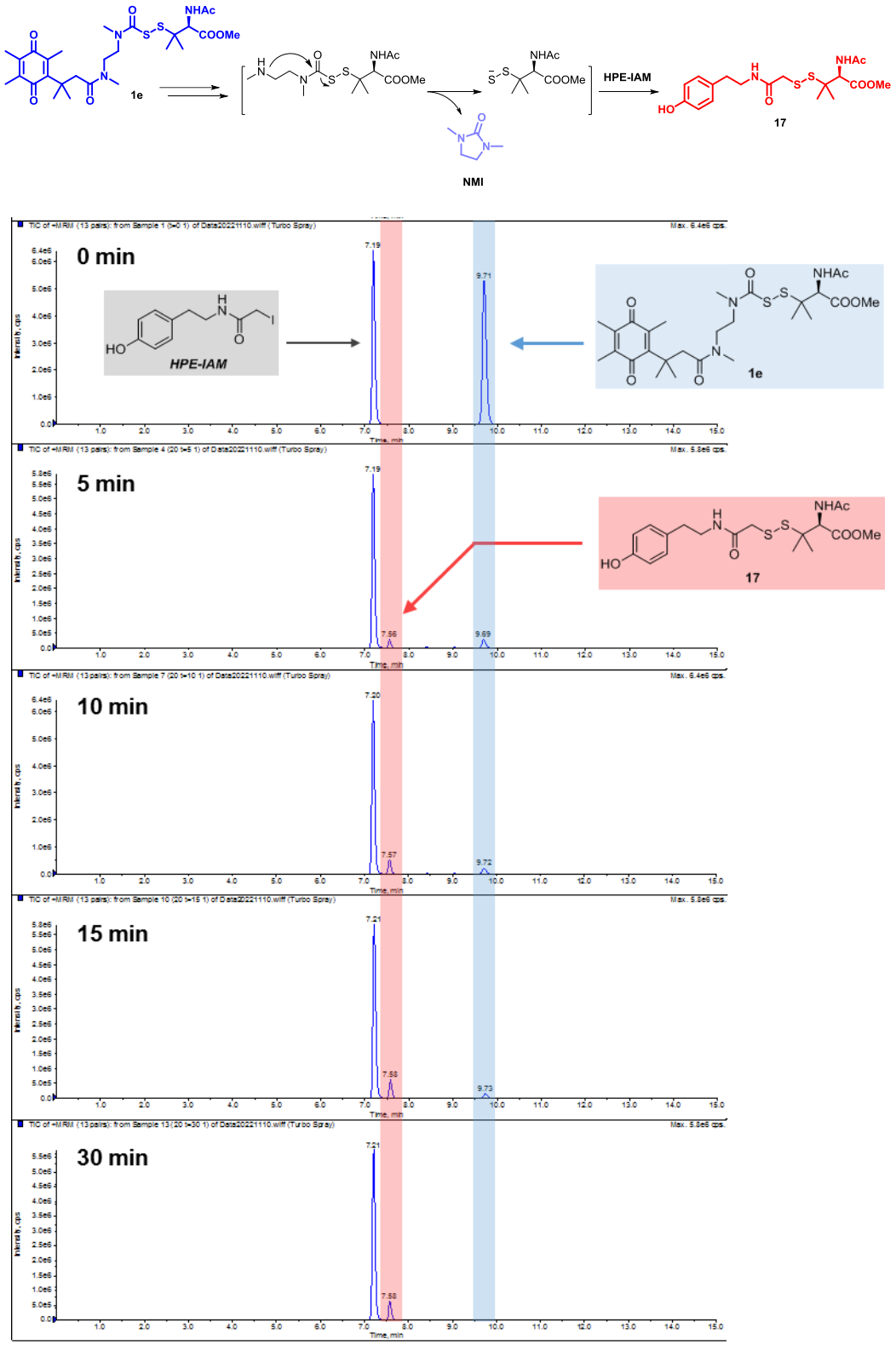


Figure S1. LC-MS/MS chromatogram of **1e**, **17** and **HPE-IAM** during trapping reaction by LC-MS/MS. **1e**: t_R = 9.71 min; **17**: t_R = 7.56 min; HPE-IAM: t_R = 7.19 min.

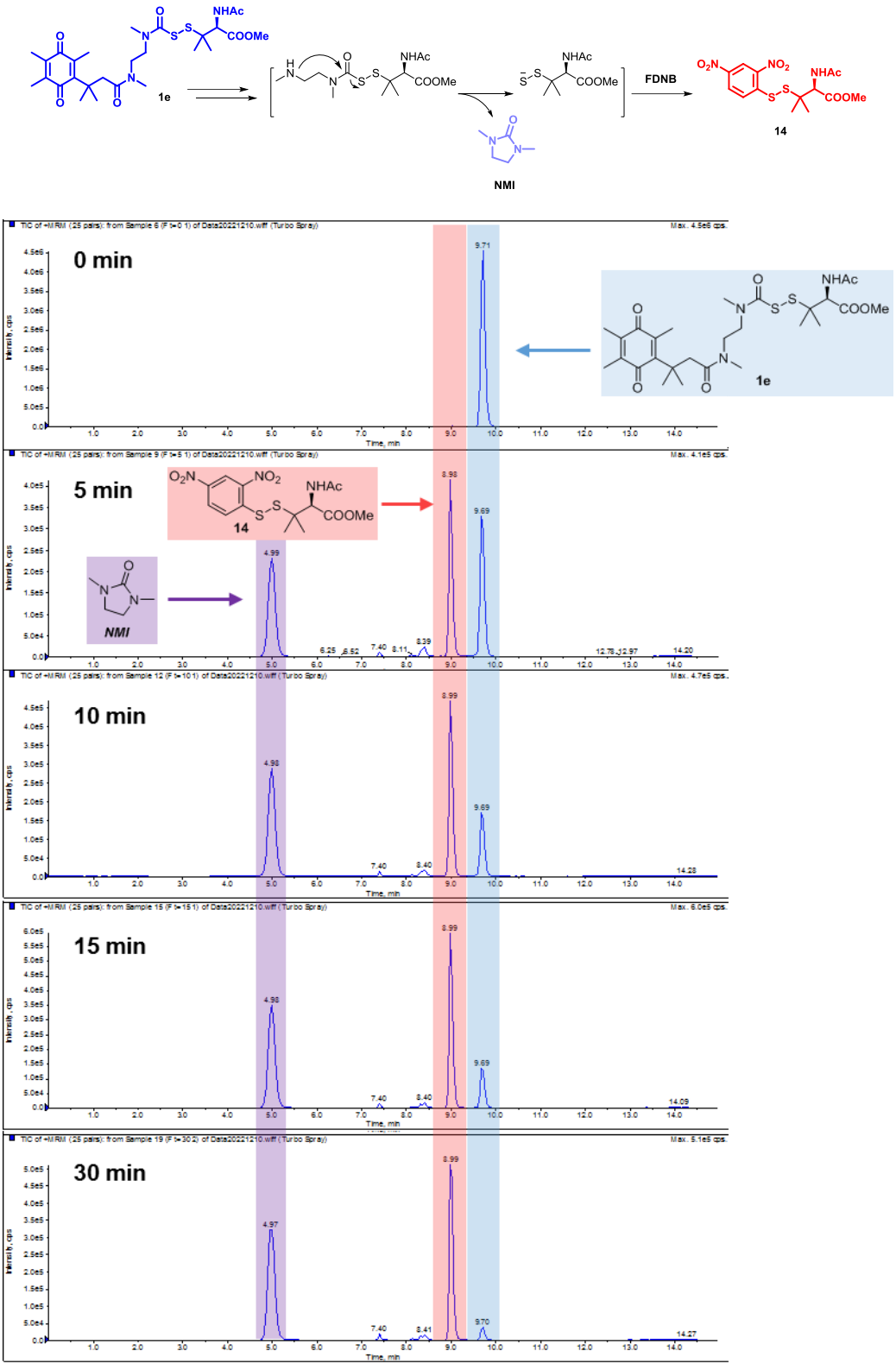


Figure S2. LC-MS/MS chromatogram of **1e**, **14** and **NMI** during trapping reaction by LC-MS/MS. **1e**: t_R = 9.71 min; **14**: t_R = 8.98 min; **NMI**: t_R = 4.99 min.

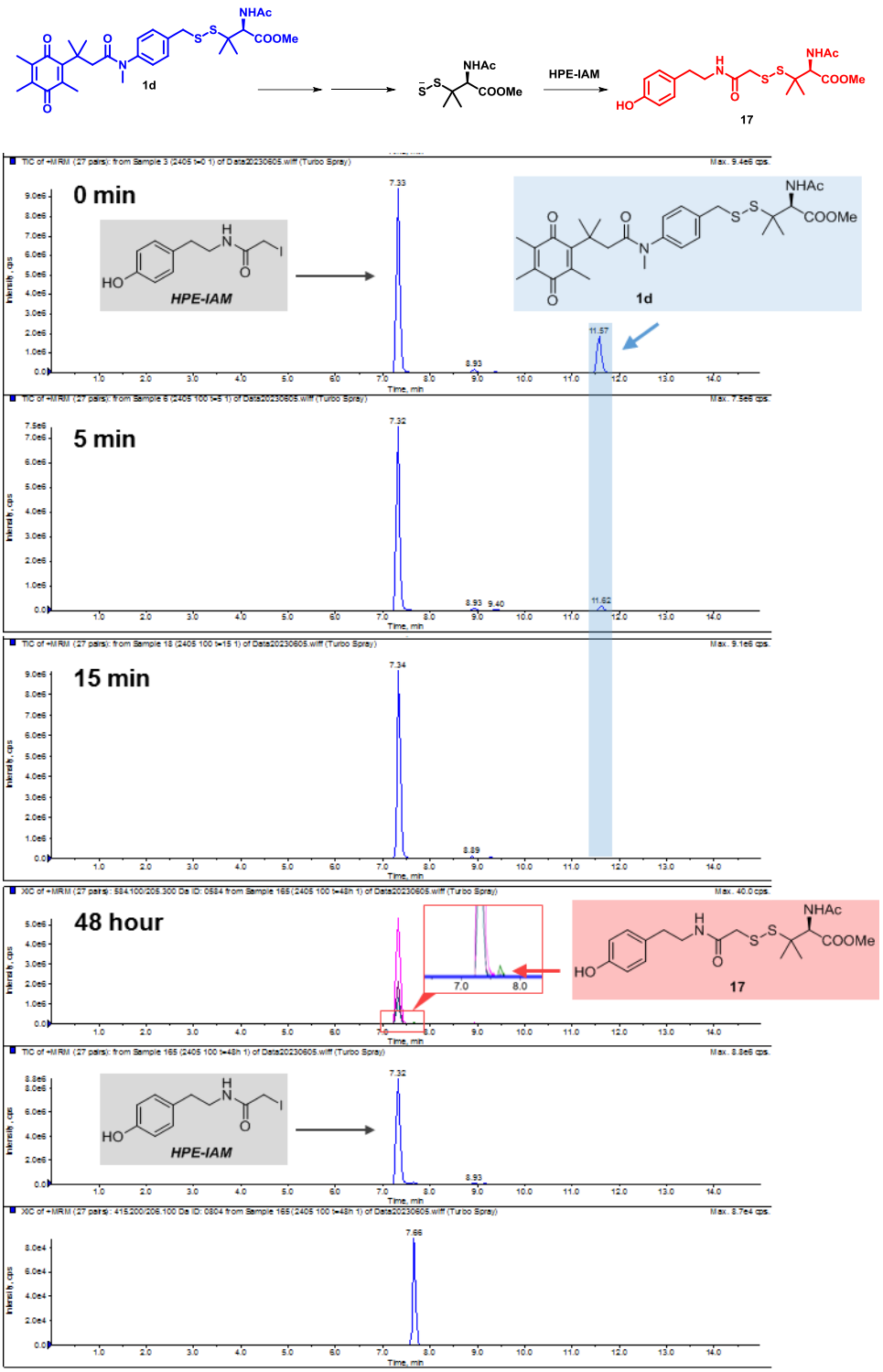


Figure S3. LC-MS/MS chromatogram of **1d**, **17** and **HPE-IAM** during trapping reaction by LC-MS/MS. **1d**: t_R = 11.57 min; **17**: t_R = 7.66 min; **HPE-IAM**: t_R = 7.33 min.

The results of the experiment using FDNB as the trapping agent were similar to those described above. The LC-MS/MS chromatogram of prodrugs **1a**, **1c** and **1d** is not shown.

2.3 HPLC-UV assay

HPLC condition: The HPLC condition in this assay were the same as that mentioned in LC-MS/MS assay with appropriate modification. A gradient elution program was conducted with mobile phase A (0.1% formic acid in water) and mobile phase B (methanol).

Reaction solutions: 4 μL of each prodrug solution (50 mM) was transferred into a 1.5 mL EP tube, followed by the addition of 388 μL of PBS buffer solution. The mixture was vortexed for approximately 30 s prior to addition of 4 μL of NADPH solution (400 mM in PBS), and then 4 μL of human NQO1 solution (4 mg/mL in PBS). After a second 30 s vortex, the reaction was incubated at 37°C. At a series of time points (0, 5, 15, 30, and 60 min), 50 μL of the reaction solution was diluted by 250 μL working solution containing IS (warfarin). The solution was centrifuged twice at 18,000×g at 4°C for 5 min to remove invisible impurities. 80 μL of the resulting supernatant was analyzed by HPLC-UV directly. In each control group, we replaced NQO1 with PBS. The IS working solution was made by diluting IS stock solution with acetonitrile, resulting in a 50 μg/mL solution, storing at 4°C. Above assays were repeated in triplicate and recorded as the mean ± SD from three experiments.

Results

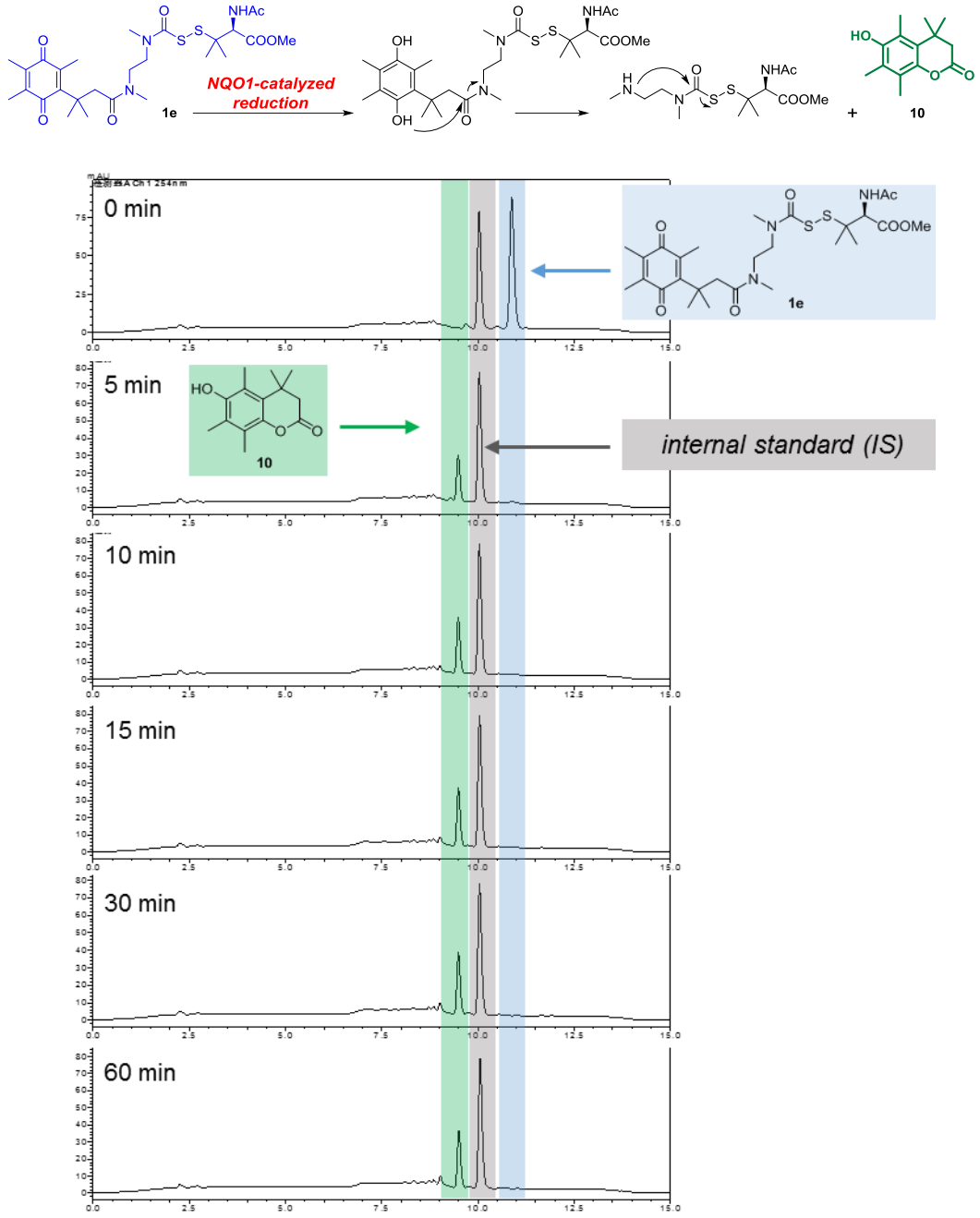


Figure S4. HPLC-UV chromatogram of **1e**, **10** and **IS**. **1e**: t_R = 9.490 min; **10**: t_R = 10.881 min; **IS**: t_R = 10.033 min.

2.4 LC-Q-TOF/MS assay

LC-Q-TOF/MS system: The HPLC system consisted of a Shimadzu DGU-20A5 online degasser, two Shimadzu LC-30AD pumps with a high-pressure mixer, a Shimadzu CTO-20A column oven and a Shimadzu SIL-30AC autosampler (Shimadzu, Kyoto, Japan). Chromatographic separation was carried out at 40 °C HPLC XSelect HSS T3 Column (150 × 4.6 mm; Waters) with the mobile phase of water containing 0.1% formic acid (A) and methanol (B). Gradient elution started from 9% B for 1 min; increased linearly to 98% B over 15 min; was maintained at 98% B for 8 min; decreased linearly to 9% B over the next 2 min; and was maintained for 5 min to reequilibrate the column. Mass spectrometric analysis was performed with a LC-Q-TOF/MS high-resolution mass spectrometer (Triple-TOF 5600, Sciex) operating in positive mode using a DuoSpray ion source. High-resolution MS and MS/MS data were acquired by the information-dependent acquisition (IDA) method. The IDA method was composed of a TOF MS survey scan (accumulation time 250 ms) and 4 dependent product ion scans (accumulation time 100 ms). The mass ranges of the TOF MS and product ion scans were both m/z 100–1300. The parameters were set as follows: ion source gas 1, 33 psi; ion source gas 2, 33 psi; temperature, 500°C; curtain gas, 25 psi; ion spray voltage, 4500 V; declustering potential, 93 V; and collision energy, 5 eV in TOF MS and 40 eV in the product ion scans.

Reaction solutions: The reaction condition in this assay was the same as that mentioned in LC-MS/MS assay.

Results

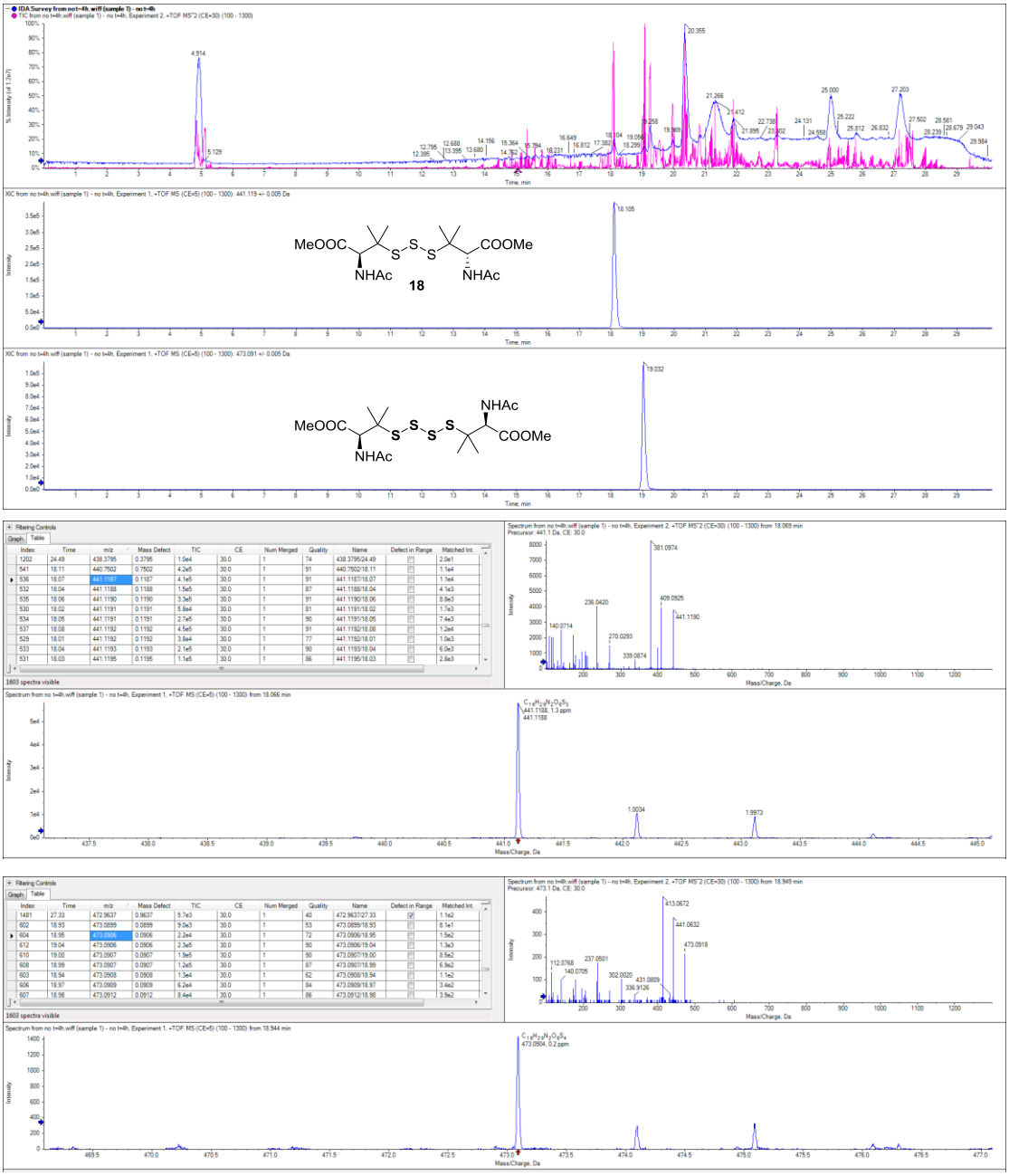


Figure S5. The MS^2 total ion chromatographs and MS^2 spectrum of **18** and its tetrasulfide analog. The information about the polysulfide identified on LC-Q-TOF/MS system: **18 (*NAPSSSNAP*)**: HRMS (ESI) for $C_{16}H_{29}N_2O_6S_3$ $[M+H]^+$ calcd 441.1182, found 441.1188; t_R = 18.105 min. ***tetrasulfide* (*NAPSSSSNAP*)**: HRMS (ESI) for $C_{16}H_{29}N_2O_6S_4$ $[M+H]^+$ calcd 473.0903, found 473.0902; t_R = 19.032 min.

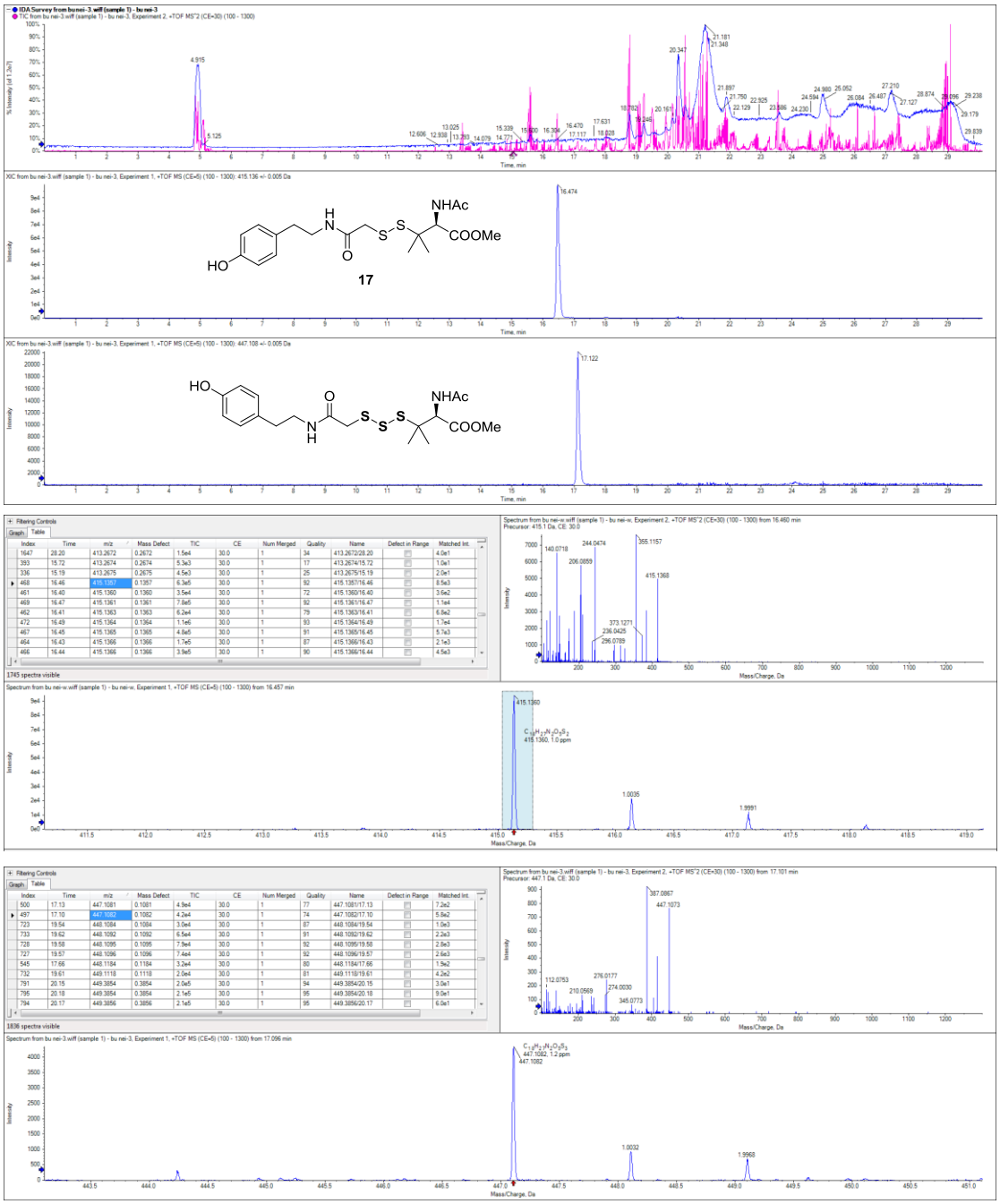


Figure S6. The MS^2 total ion chromatographs and MS^2 spectrum of **17** and its trisulfide analog. The information about the polysulfide identified on LC-Q-TOF/MS system: **17 (*HPE-SSR*)**: HRMS (ESI) for $C_{18}H_{27}N_2O_5S_2$ $[M+H]^+$ calcd 415.1356, found 415.1360; t_R = 16.474 min. ***trisulfide* (*HPE-SSSR*)**: HRMS (ESI) for $C_{18}H_{27}N_2O_5S_3$ $[M+H]^+$ calcd 447.1077, found 447.1082; t_R = 17.122 min.

We observed related polysulfide species that could demonstrate the RSSH release and the subsequent polymerization in our reaction system with or without the trapping agents.

2.5 LC-MS/MS assay for validation of RSSH release in cells (Analytical Method C)

(1) HPLC conditions of Analytical Method C

The RSSH generation **in cells** were measured by a LC-MS/MS system containing a Shimadzu HPLC system (Kyoto, Japan) coupled with a SCIEX API 6500 triple-quadrupole mass spectrometer (Foster City, CA, USA) equipped with an electrospray ionization (ESI) interface in positive or negative ionization mode.

A gradient elution program was conducted with mobile phase A (0.1% formic acid and 2 mM ammonium formate in water) and mobile phase B (methanol) shown in the following Table S6.

Table S6. Gradient elution program

Time (min)	Phase B (%)
0.01	10
1.00	10
1.50	70
3.00	70
3.10	10
6.01	Stop

(2) MS conditions of Analytical Method C

Ion source parameters: The pressure of curtain gas, collision gas, ion source gas 1 and ion source gas 2 were 12, 30, 60 and 70 psi, respectively. The ion source temperature was maintained at 550°C. The optimized ion spray voltage was 4500 V and dwell time of 100 ms for the analytes and IS applied.

The MRM parameters were optimized as listed in above Table S7.

Table S7. MRM parameters for LC/MS/MS

analyte	Q1 Mass (m/z)	Q3 Mass (m/z)	Declustering Potential (V)	Collision Energy (eV)
positive ESI mode				
17	415.3	354.8	23	22
17-d2	417.2	357.0	46	22
CysS-HPE-AM	299.2	121.0	102	36
CysS-HPE-AM-d2	301.2	123.0	102	36
CysSS-HPE-AM	331.4	121.0	110	35
CysSS-HPE-AM-d2	333.3	123.1	110	35
GS-HPE-AM	485.4	355.9	114	22
GS-HPE-AM-d2	487.4	357.9	114	22
GSS-HPE-AM	517.3	390.0	103	18
GSS-HPE-AM-d2	519.3	388.0	103	22

Calibration curves for LC-MS/MS assay

A working solution of the above analytes was using a serial dilution procedure at 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01 μg/mL to make calibration standards in acetonitrile. 10 μL of each dilution was added to 90 μL of PBS buffer, and the resulting mixture was diluted by 500 μL IS working solution. The acetonitrile solution was centrifuged at 18,000×g at 4℃ for 5 min to remove invisible impurities. The supernatant was analyzed by LC-MS/MS directly.

The calculation is the same as in **Analytical Method A**. The results are shown in Table S8.

Table S8. Linearity of representative calibration curves

Analyte	Mean linear relationship	Regression coefficient (r)
17	y = 0.00746 x - 0.00104	0.9921
CysS-HPE-AM	y = 453 x - 160	0.9986
CysSS-HPE-AM	y = 0.0113 x + 0.0968	0.9960
GS-HPE-AM	y = 0.00902 x + 0.0417	0.9969
GSS-HPE-AM	y = 0.0524 x + 0.643	0.9952

3. Cellular protection against cisplatin-induced oxidative stress

3.1 Cell viability assay (CCK-8)

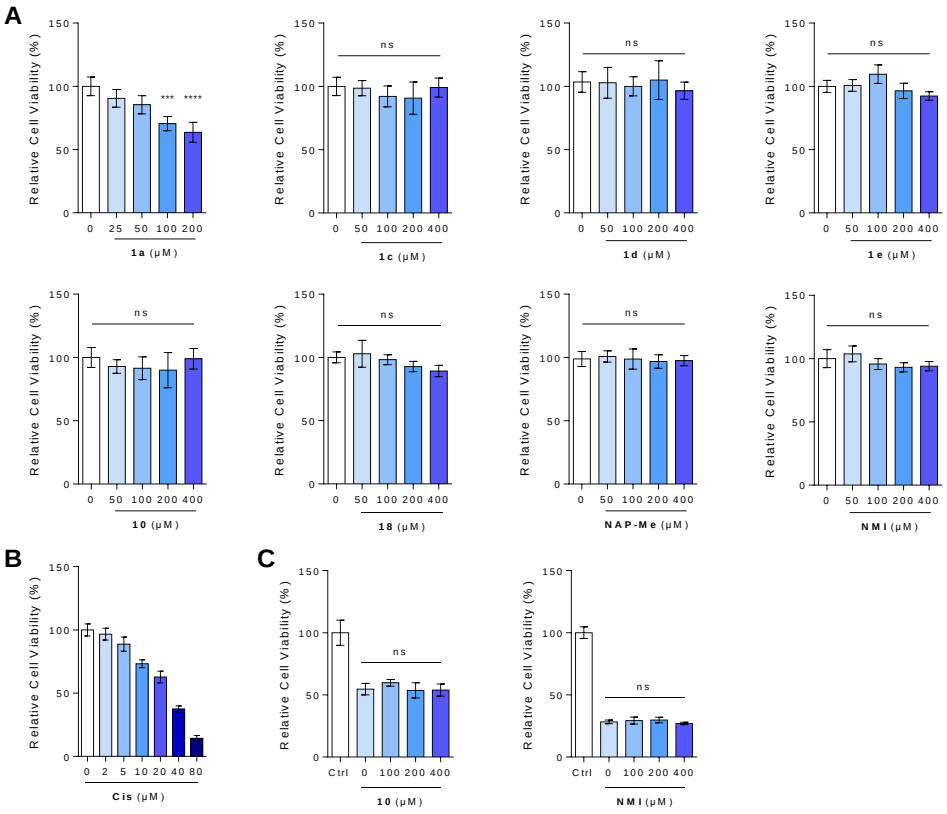


Figure S7. Most prodrugs were not cytotoxic; by-products during prodrug activation do not exhibit antioxidant activity. (A-B) Cytotoxicity for HK-2 cells treated with **1a**, **1c**, **1d**, **1e**, **10**, **18**, **NAP-Me**, and **NMI** (0, 50, 100, 200, 400 μM) and cisplatin (**Cis**, 0, 2, 5, 10, 20, 40, 80 μM) for 24 h. (C) Protection for HK-2 cells pretreated with **10** (100, 200, 400 μM) and **NMI** (100, 200, 400 μM) for 0.5 h followed by exposure to **Cis** (40 μM) for 24 h. The results are expressed as the mean ± SD (n = 3). ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ versus cisplatin group; $^{\#}$ $p < 0.05$ versus control group.

Cell viability was evaluated using CCK-8 (US Everbright® Inc., Suzhou, China), according to the manufacturer's protocol. Briefly, cells were incubated in 96-well plates (5×10^3 cells/well) for 24 h at 37°C. Subsequently, a portion of cells was treated with various concentrations of **1e** and **18** (50, 100, 200, and 400 μM) for 24 h at 37°C. Subsequently, CCK-8 reagent was added to the cells, which were incubated for 1 h at 37°C. The absorbance was measured at 450 nm using a microplate (Synergy H1 Hybrid Multi-Mode Reader, BioTek Instruments, Inc., Winooski, Vermont, USA). The results

were expressed as the percentage of cell viability (%) with respect to the control (medium treated cells). The results are expressed as the mean ± SD (n = 6). See Figure 5A and Figure S7.

Except for **1a**, other prodrugs and metabolites showed no cytotoxicity. The cytotoxicity of cisplatin is dose-dependent, and two doses, 10 and 40 μM, were selected for subsequent experiments. We also verified the protective effects of the above prodrugs and metabolites on cisplatin-induced oxidative damage. Compounds **10** and **NMI** did not appear to contribute to antioxidant activity.

3.2 Flow-cytometry analysis

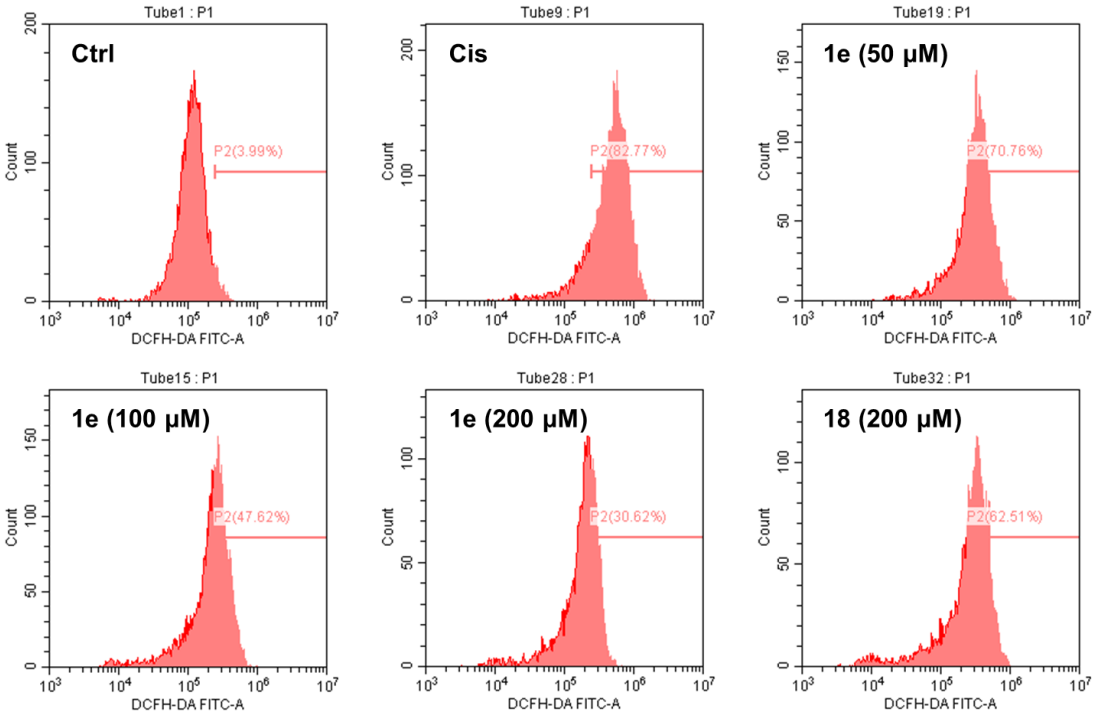


Figure S8. ROS detection in HK-2 cells by flow cytometry using a DCFH-DA assay. Cells were pretreated with **1e** (50, 100, 200 μM), and **18** (200 μM) for 1 h and exposed to cisplatin (40 μM) for 24 h, after addition of DCFH-DA, ROS levels were measured by flow cytometry. Upper middle panel: exposure to cisplatin leads to increased ROS levels. Upper right panel, Lower left and middle panel: exposure to **1e** leads to dosage-dependent decrease of ROS levels. Lower right panel: exposure to **18** leads to dosage-dependent decrease of ROS levels. Each experiment was repeated three times, and representative results are shown.

From the level of intracellular ROS, it can be found that both compounds **1e** and **18** can reduce the accumulation of ROS induced by cisplatin.

3.3 Gene Expression Analysis (qPCR assay)

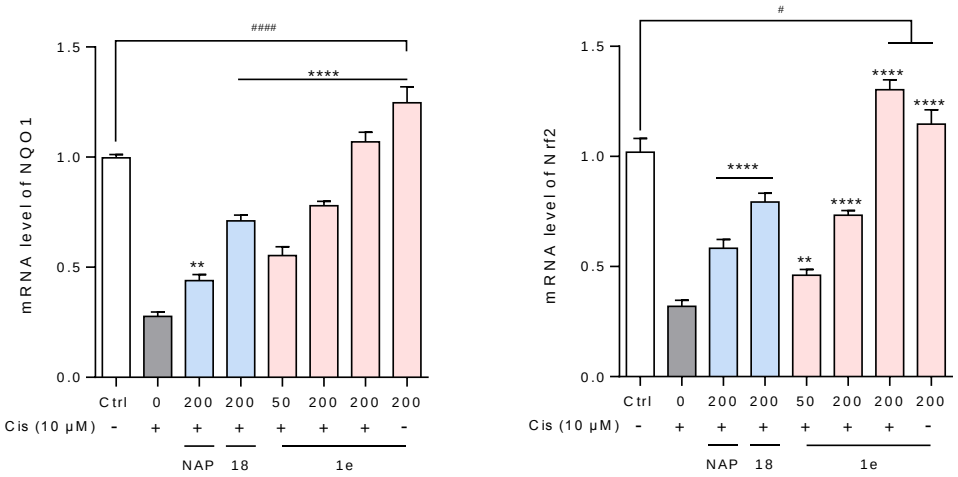


Figure S9. Quantification of Nrf2 and NQO1 mRNA expression levels in different conditions-treated HK-2 cells by realtime PCR; cisplatin (10 μM), **NAP-Me** (200 μM), **18** (200 μM), **1e** (50, 100, 200 μM). The results are expressed as the mean ± SD (n = 3). ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ versus cisplatin group; #### $p < 0.0001$, # $p < 0.05$ versus control group.

While measuring the expression of the target protein in HK-2 cells, we also quantified the mRNA of the corresponding protein. Compared with the cisplatin model group, the compound **1e** and **18** showed more obvious improvement in the mRNA level of NQO1 and Nrf2 than the protein level. Compound **1e** can significantly increase the mRNA expression of NQO1 in both the damaged state and the normal state.

Table S5 Primer sequences used in qPCR assay

Gene		Primer sequence (5'-3')
human NQO1	forward	GCCATGAACTTCAATCCCATCA
	reverse	CTGGAATATCACAAGGTCTGCG
human Nrf2	forward	AGTGGAAGAGCTAGATAGTGCC
	reverse	GACCAGGACTTACAGGCAATTC
GAPDH	forward	CAAATTCCATGGCACCGTCA
	reverse	AGCATCGCCCCACTTGATTT

Nrf2, nuclear factor erythroid 2−related factor 2; NQO1, NAD(P)H quinone dehydrogenase 1.

Reference

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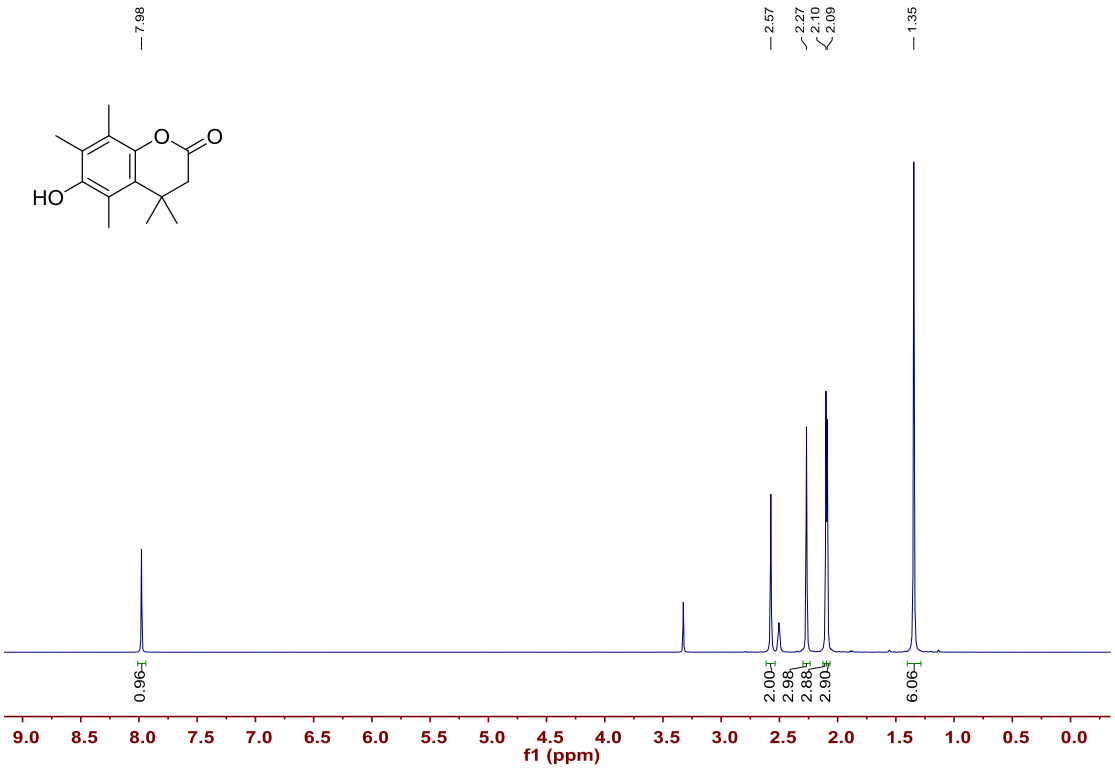
NMR spectra of synthesized compounds

10 ^{1}H NMR



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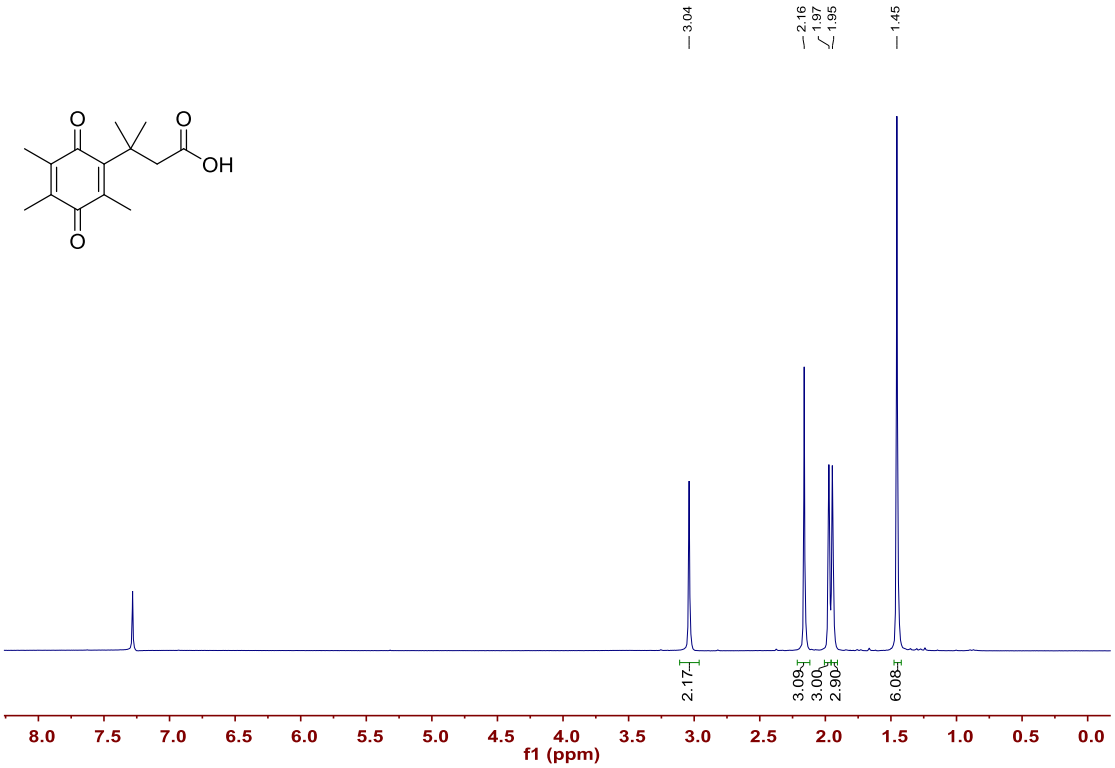
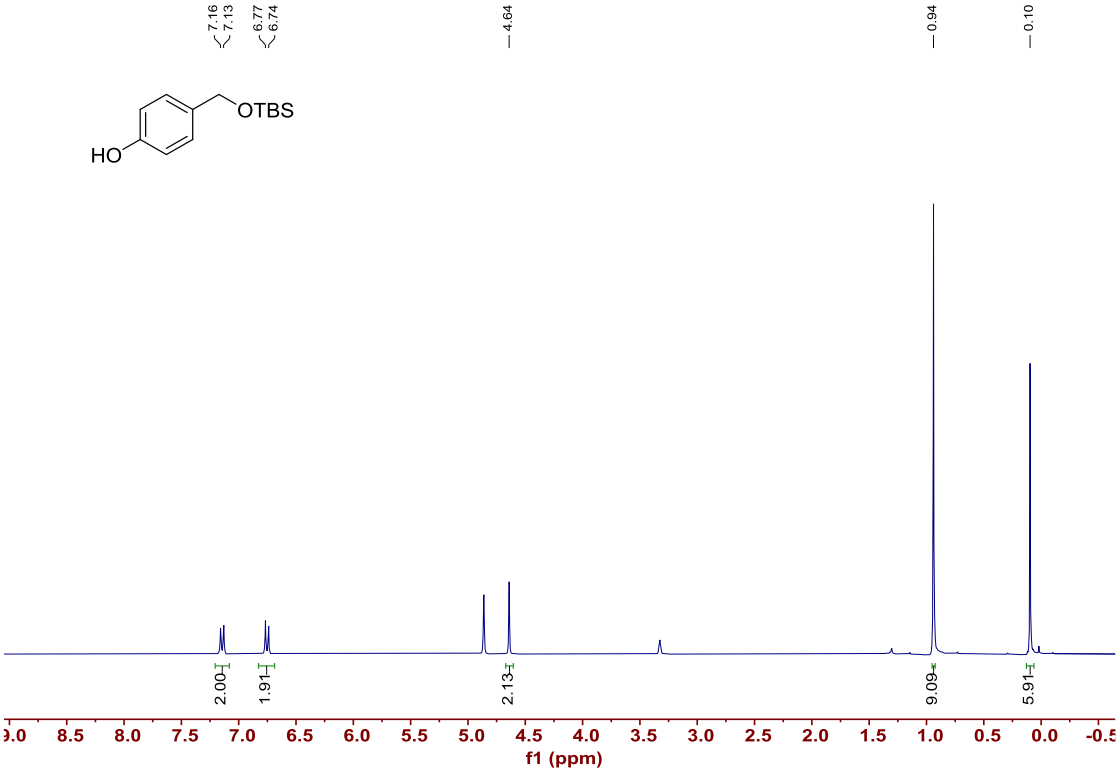
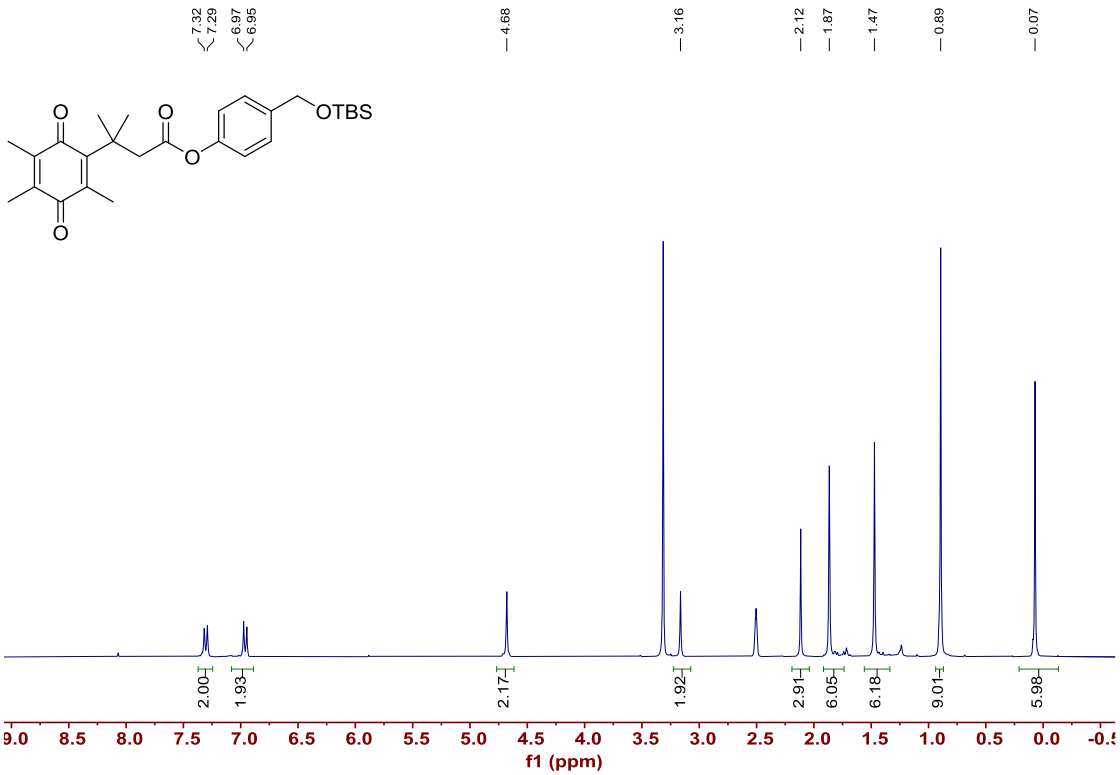


11 ^{1}H NMR



12 ^{1}H NMR



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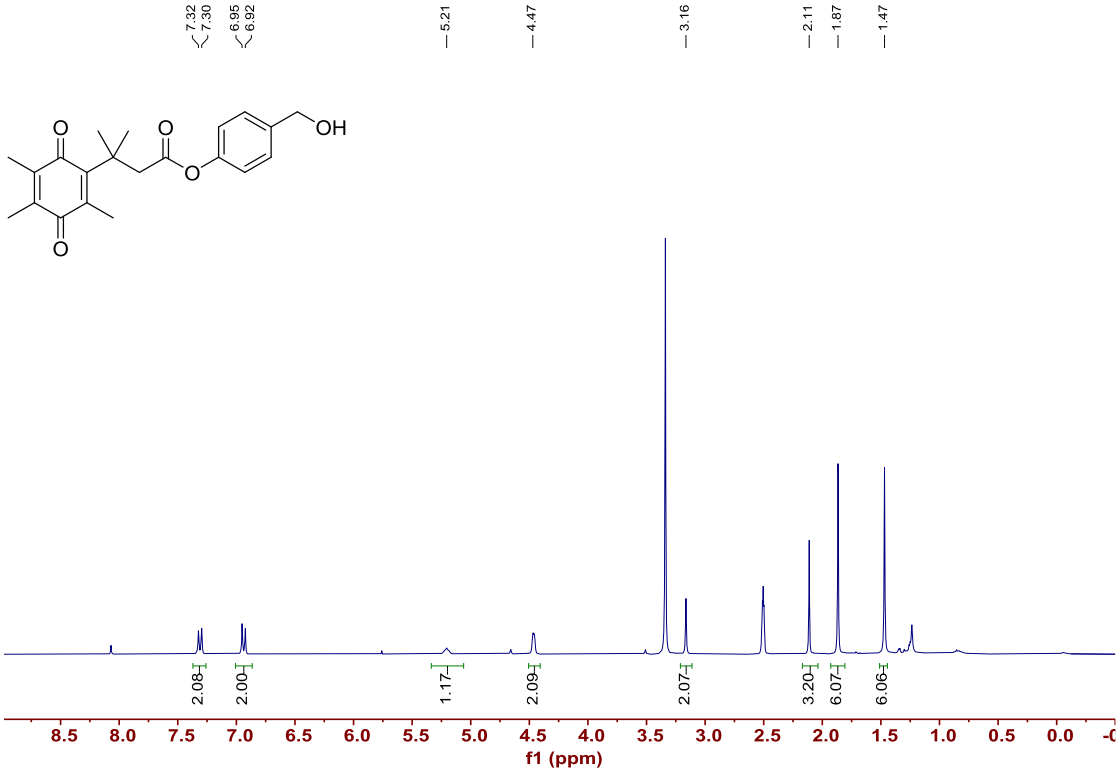

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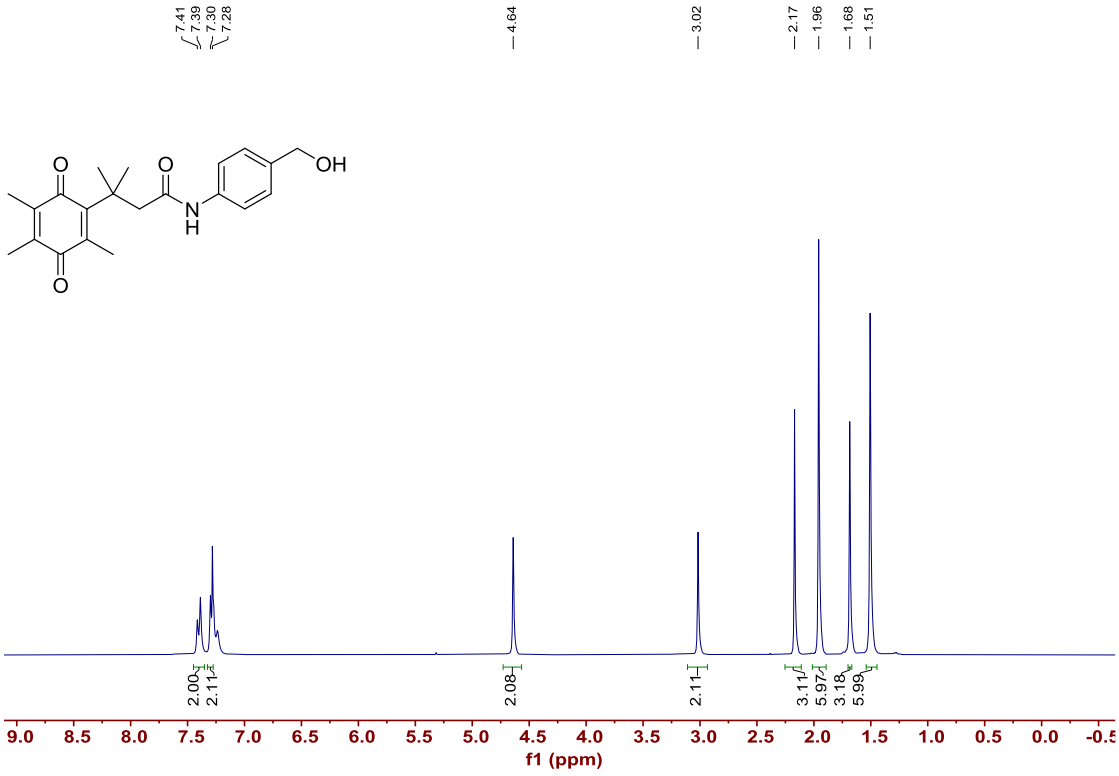

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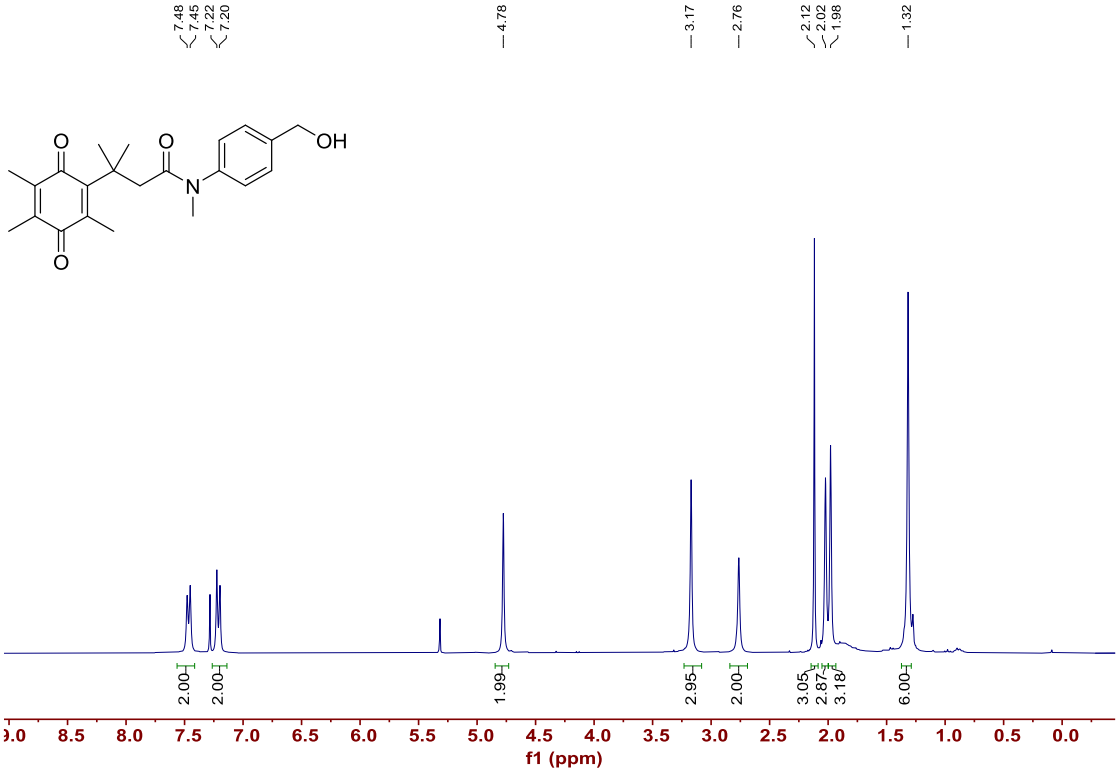

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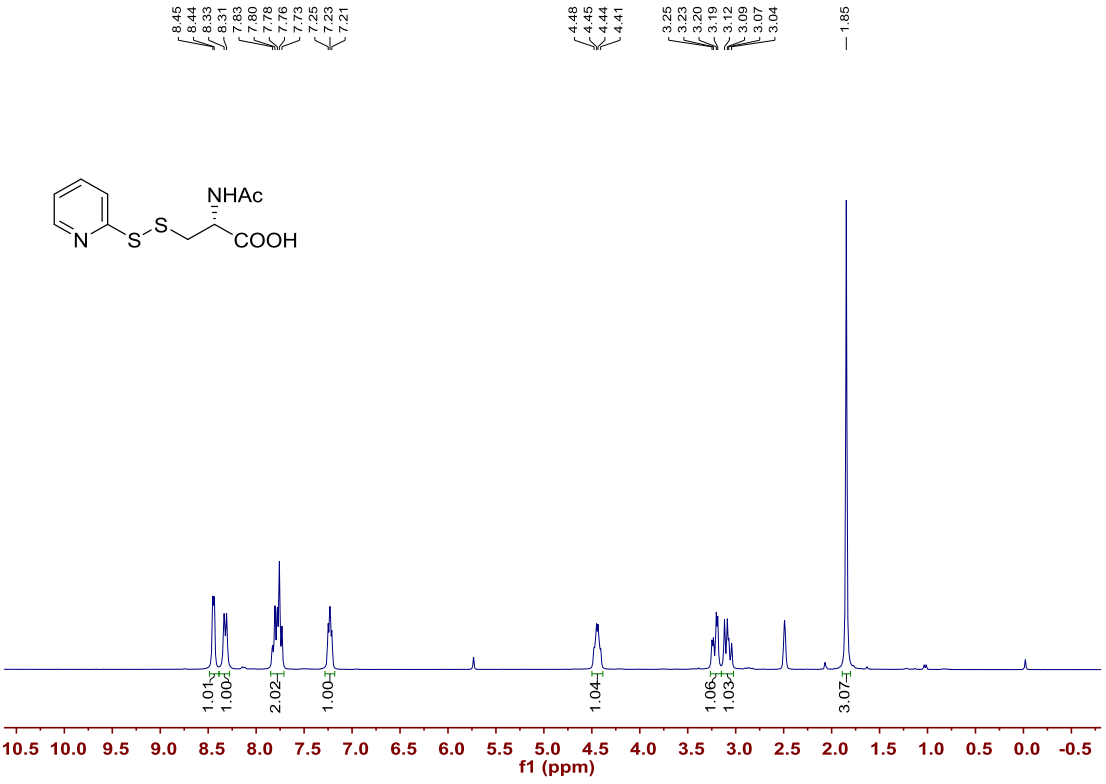

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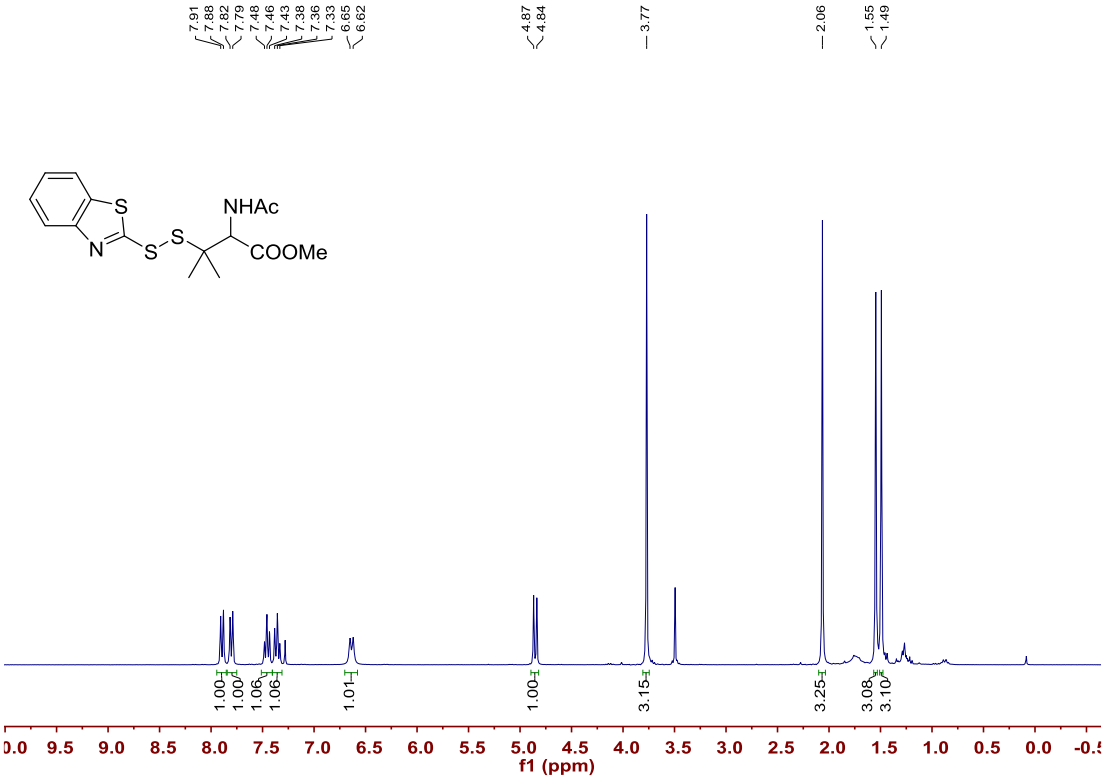

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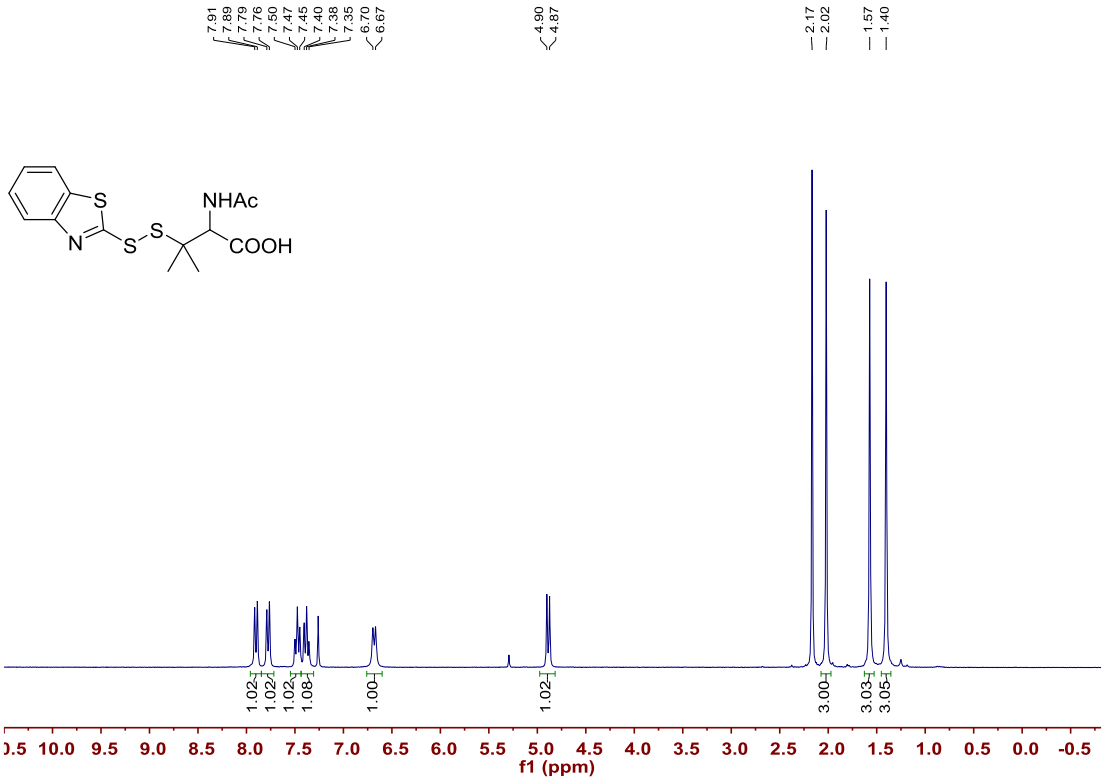

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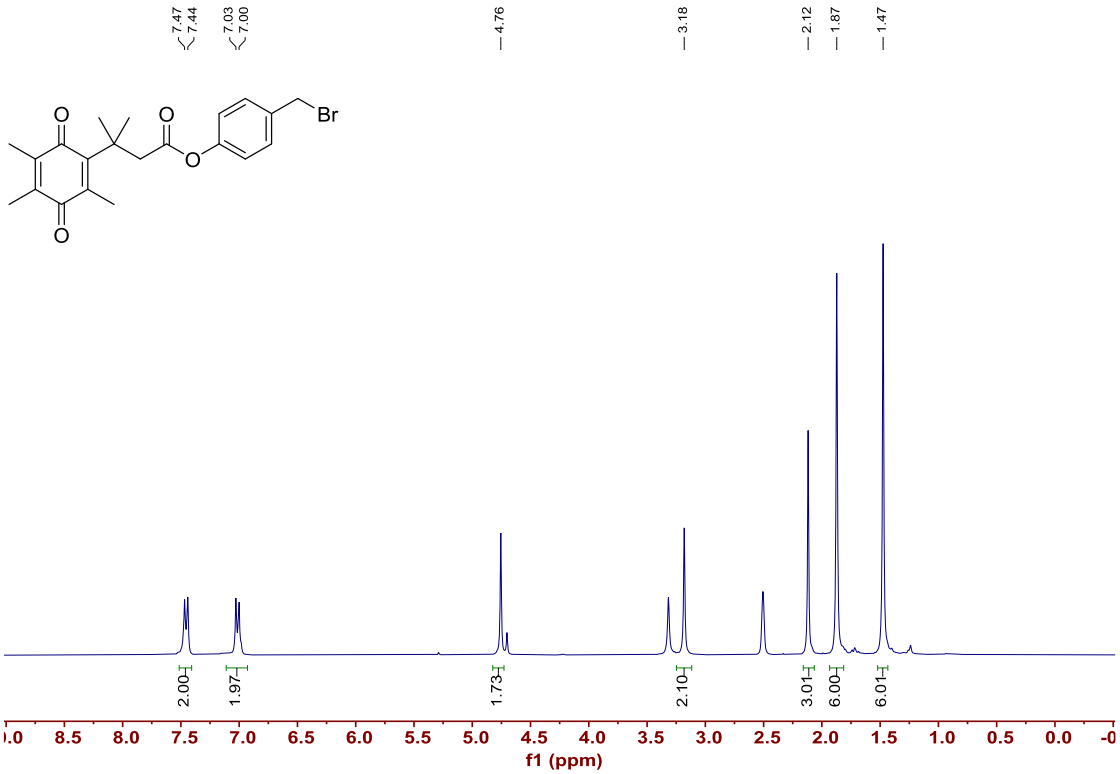

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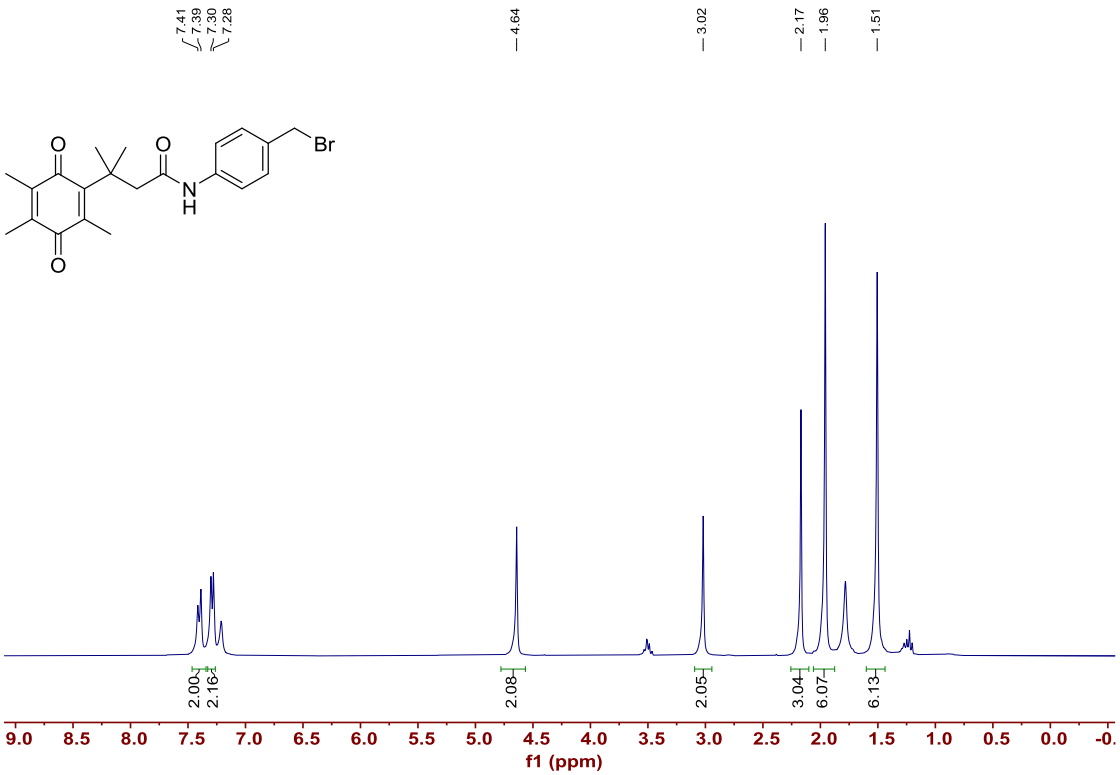
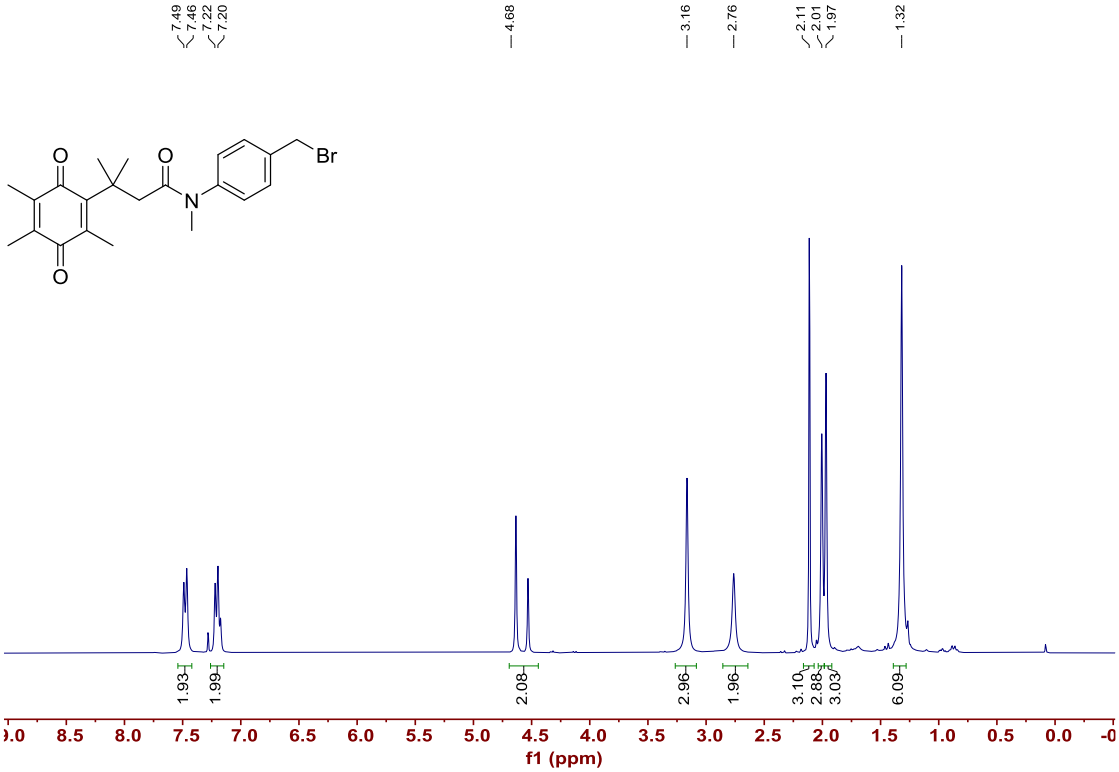


3c ^{1}H NMR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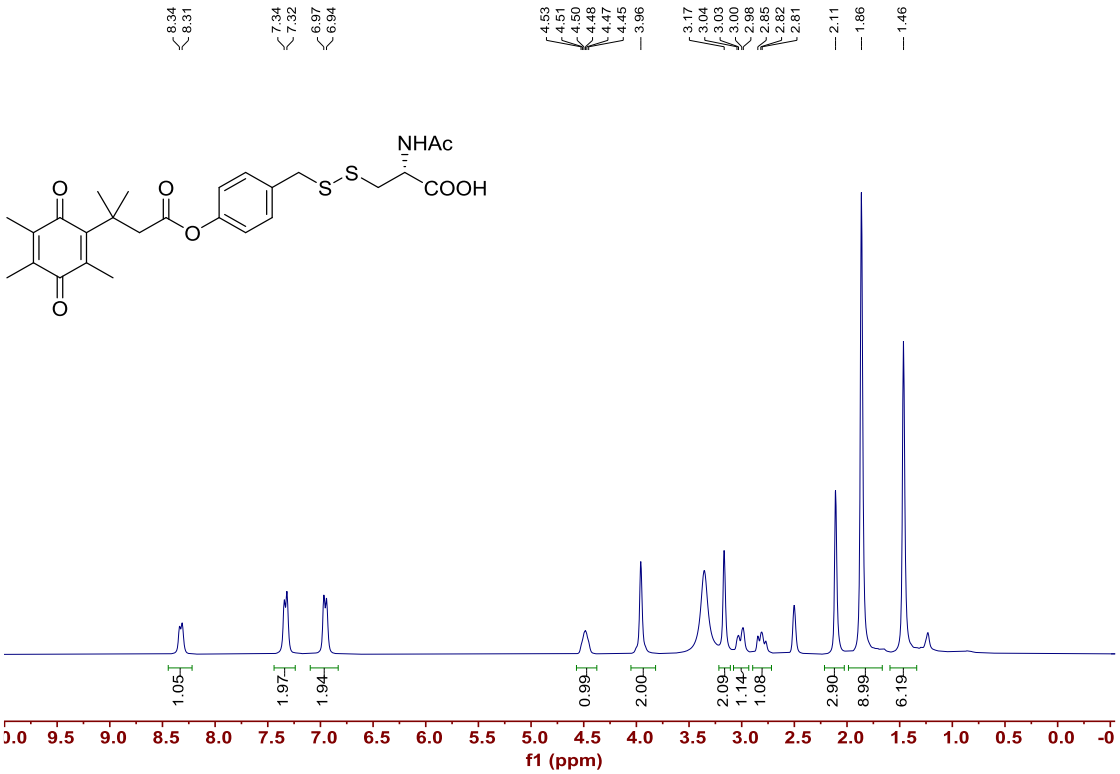
7.46
7.22
7.20
4.68
3.16
2.76
2.11
2.01
1.97
1.32
O
O
N
Br
O
1.93
1.99
2.08
2.96
1.96
3.10
2.88
3.03
6.09
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

1a ^{1}H NMR



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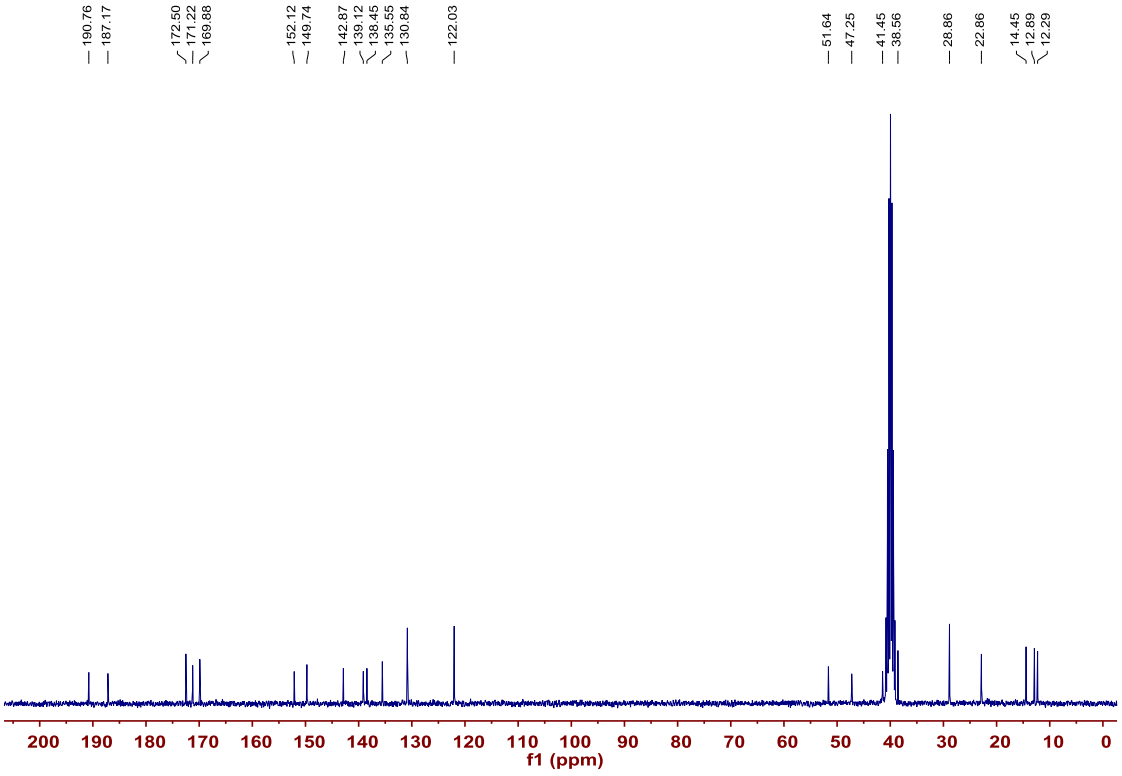

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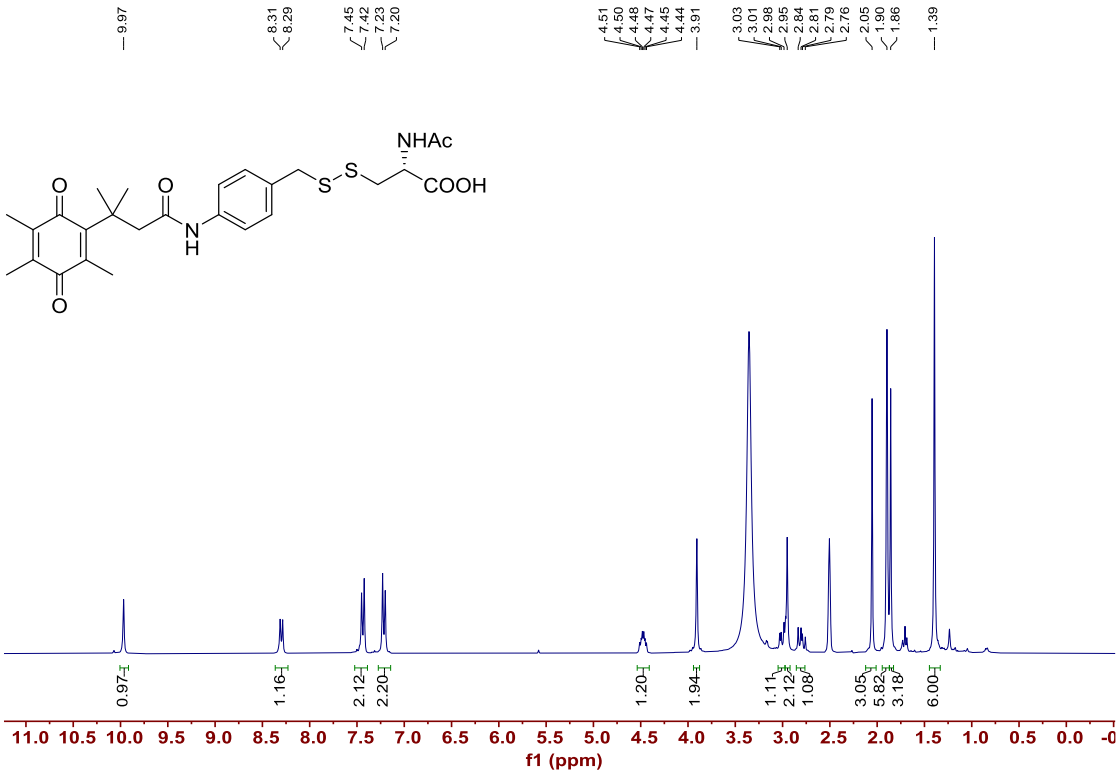

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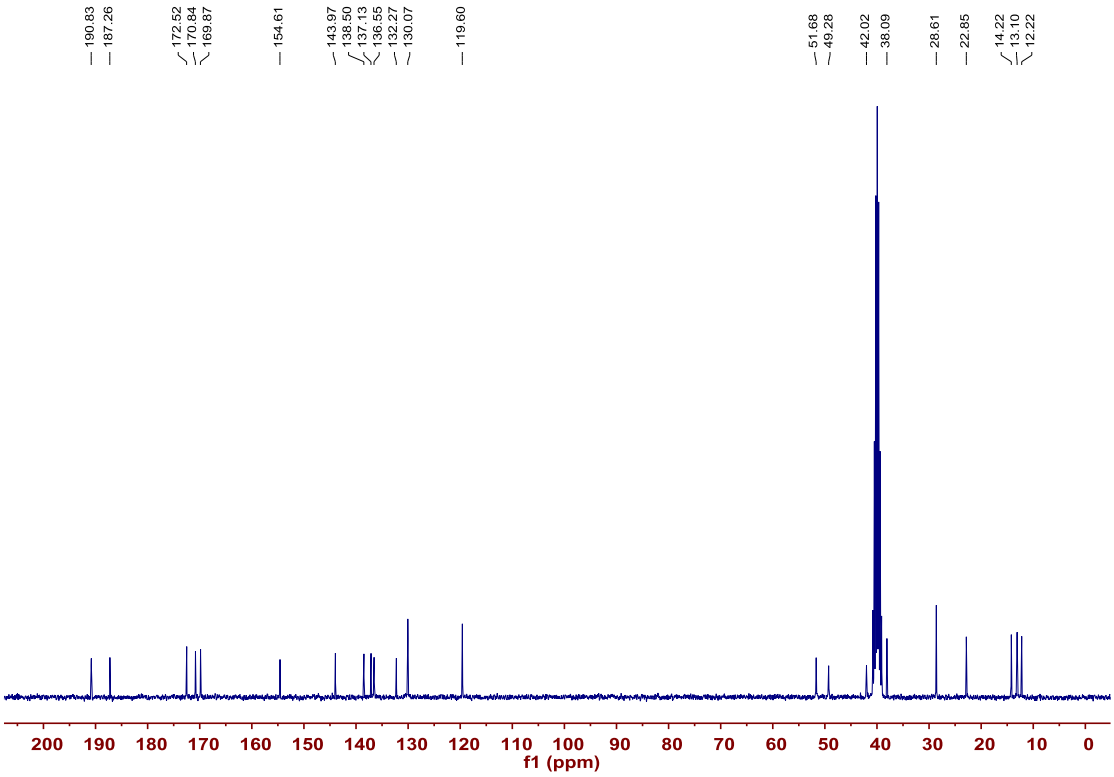

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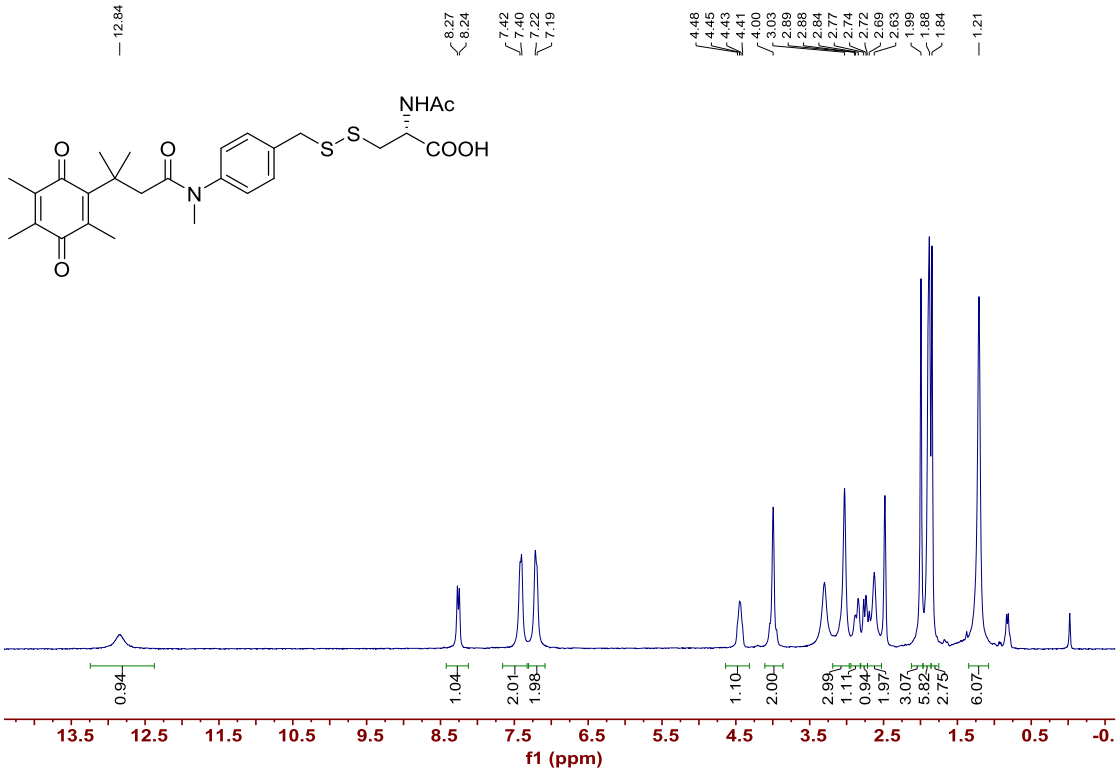

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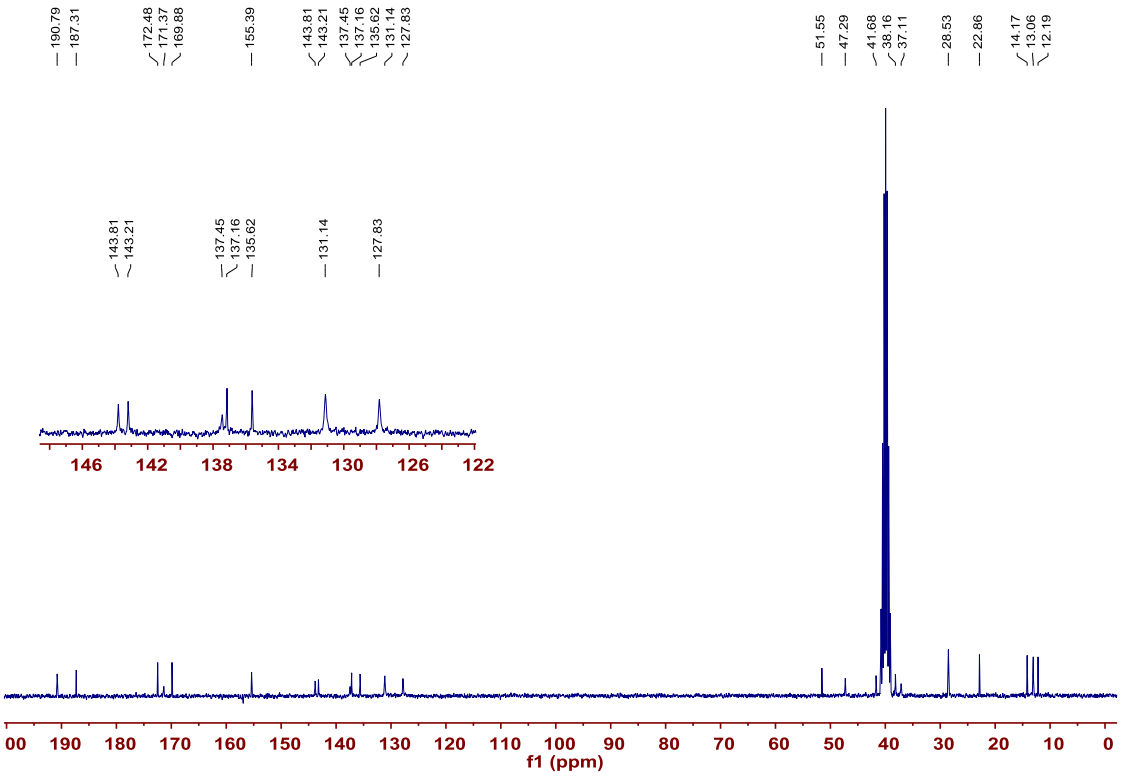

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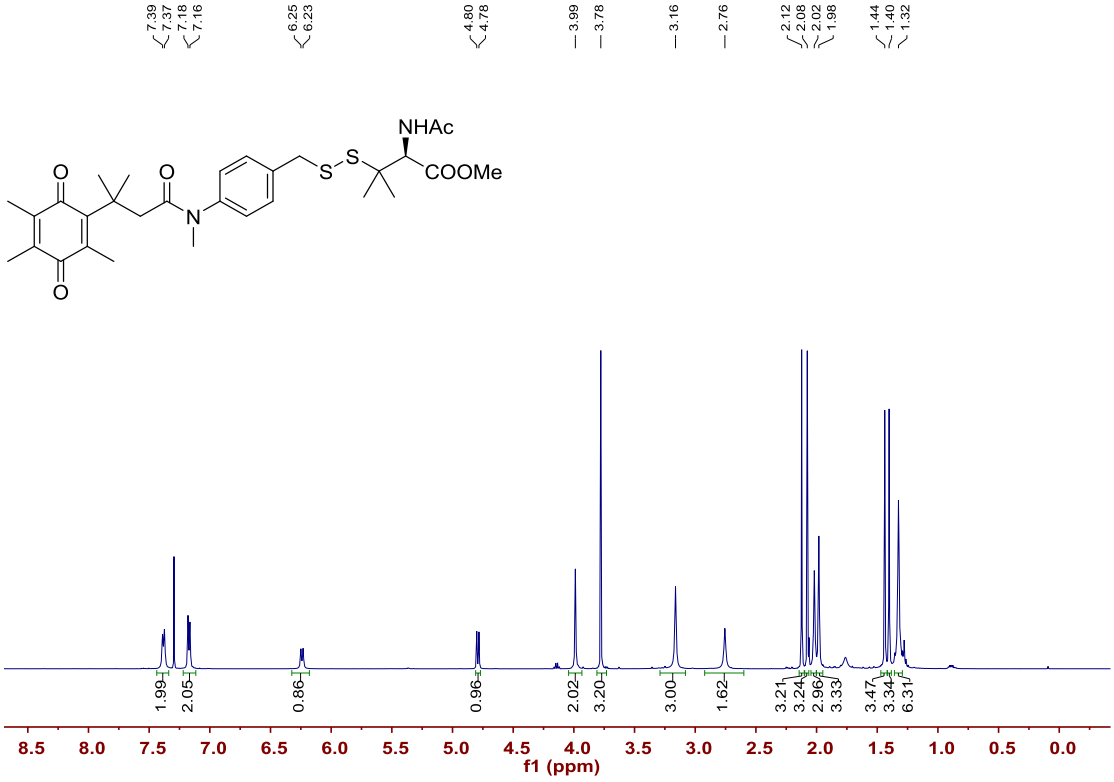

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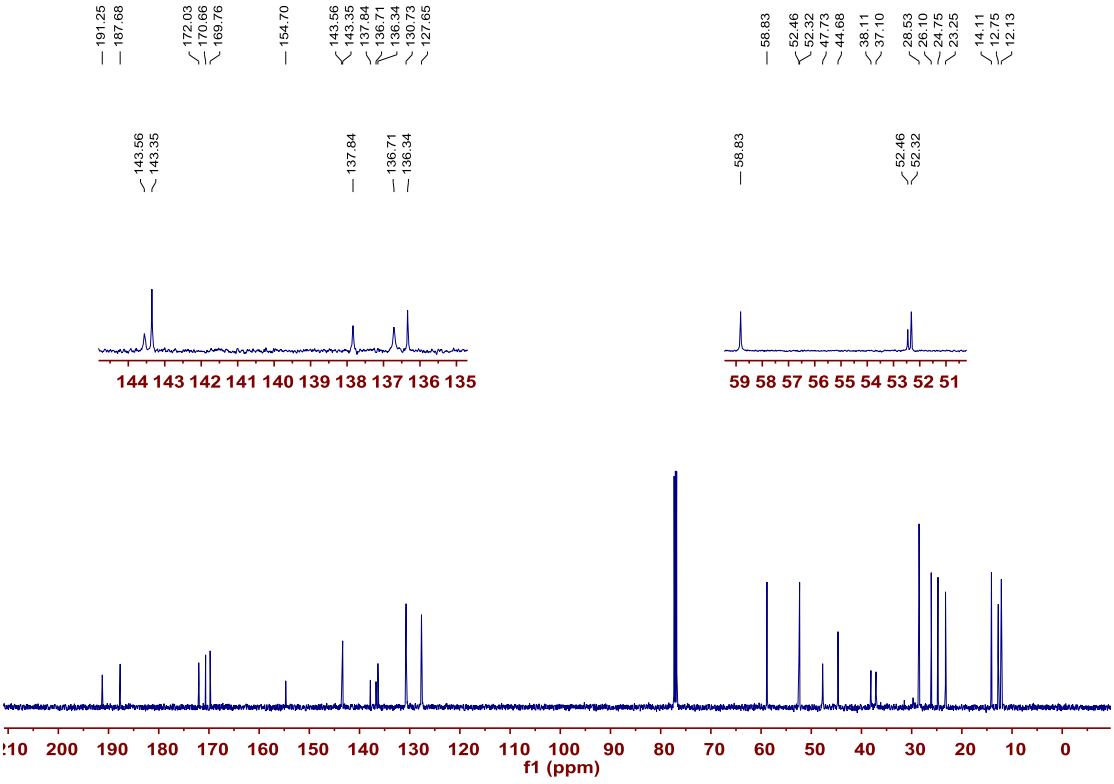
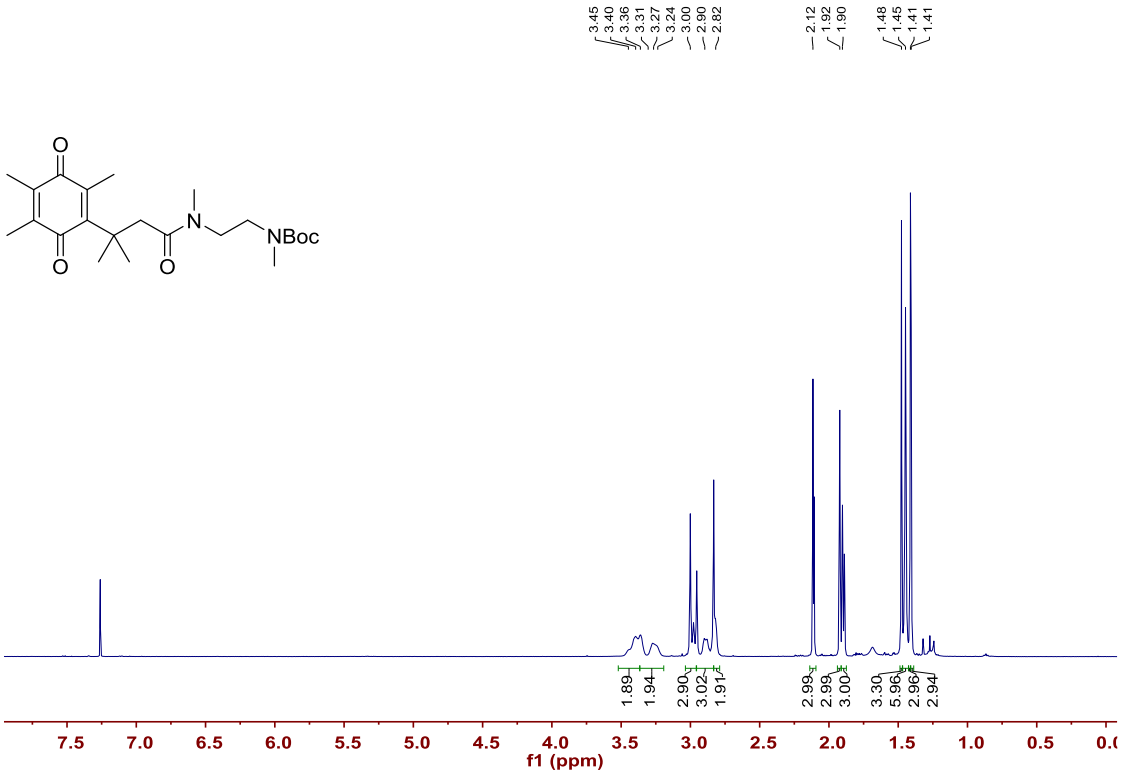
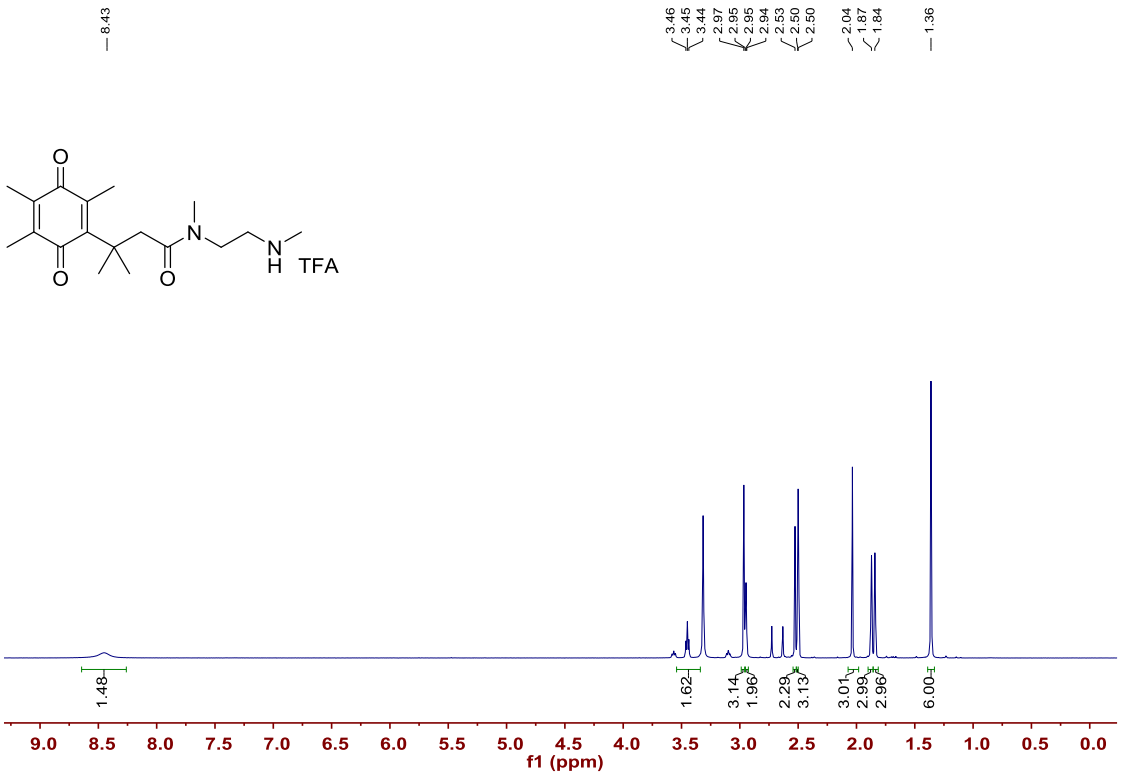


7 ^{1}H NMR



8 ^{1}H NMR



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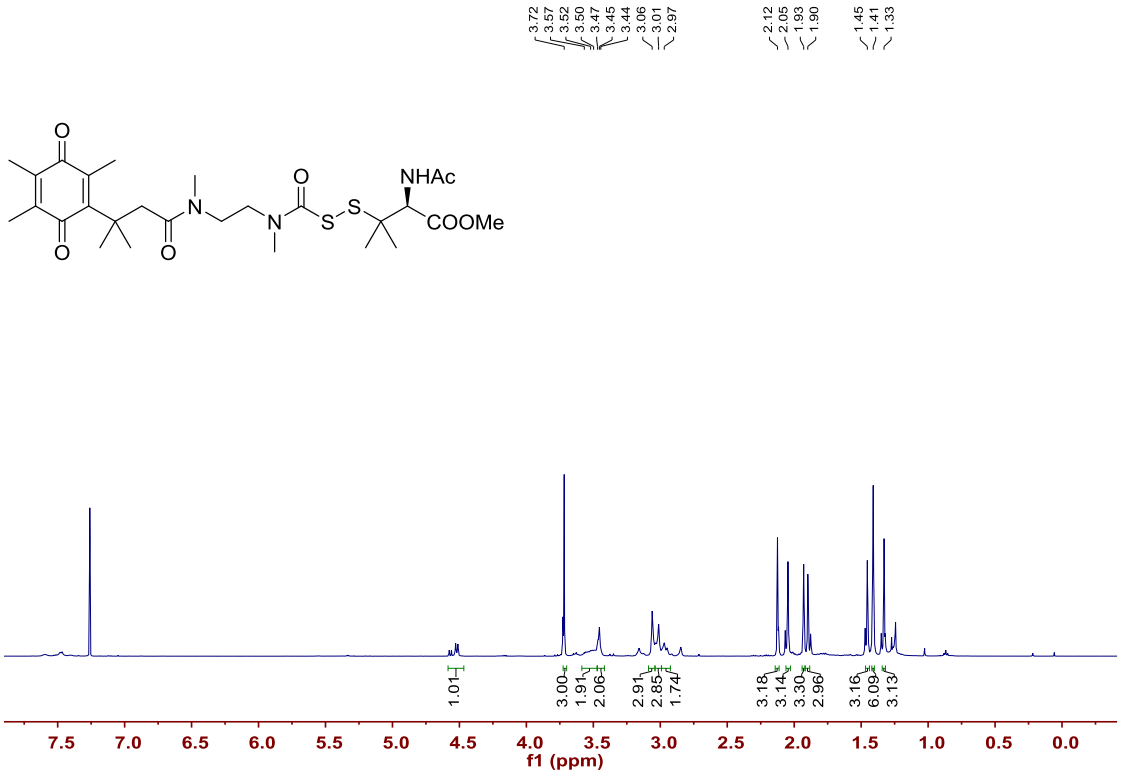

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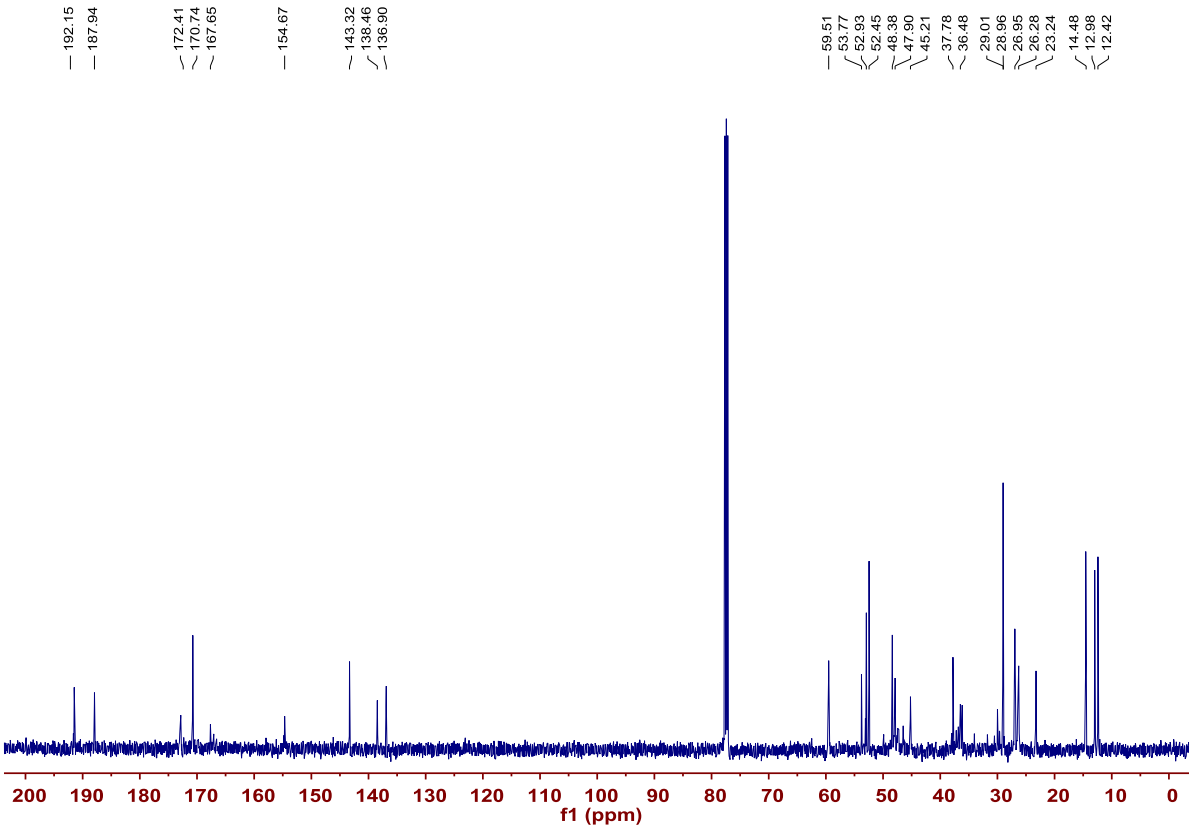

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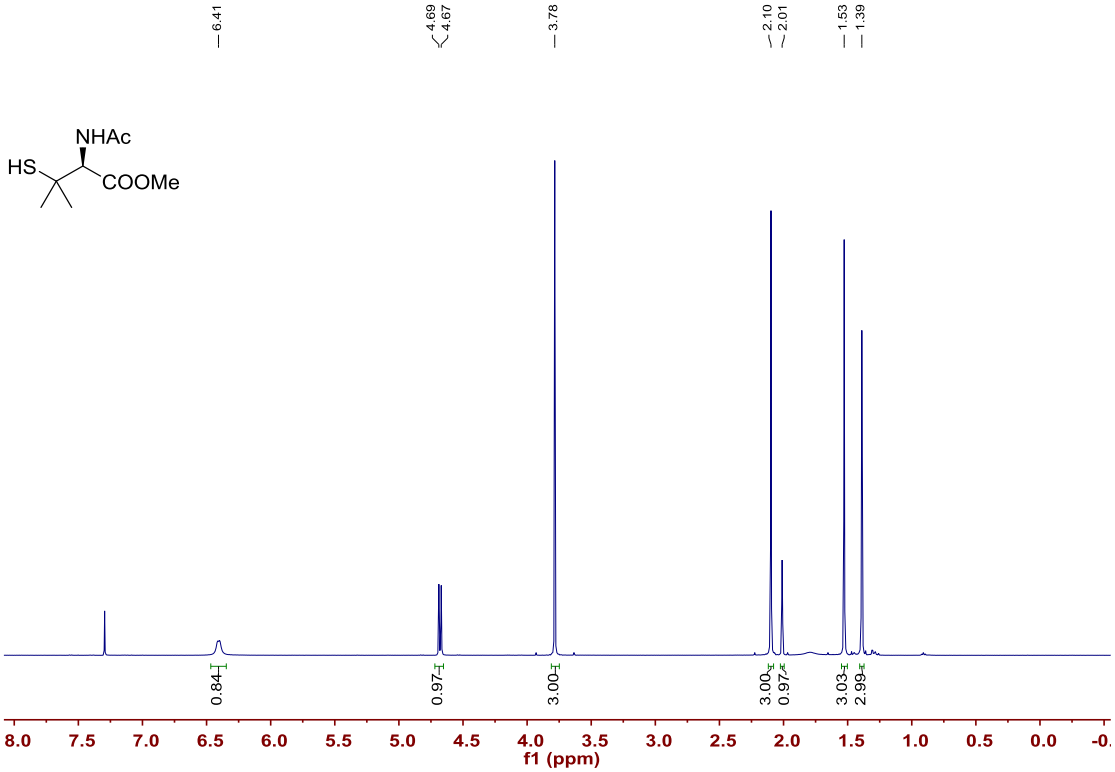

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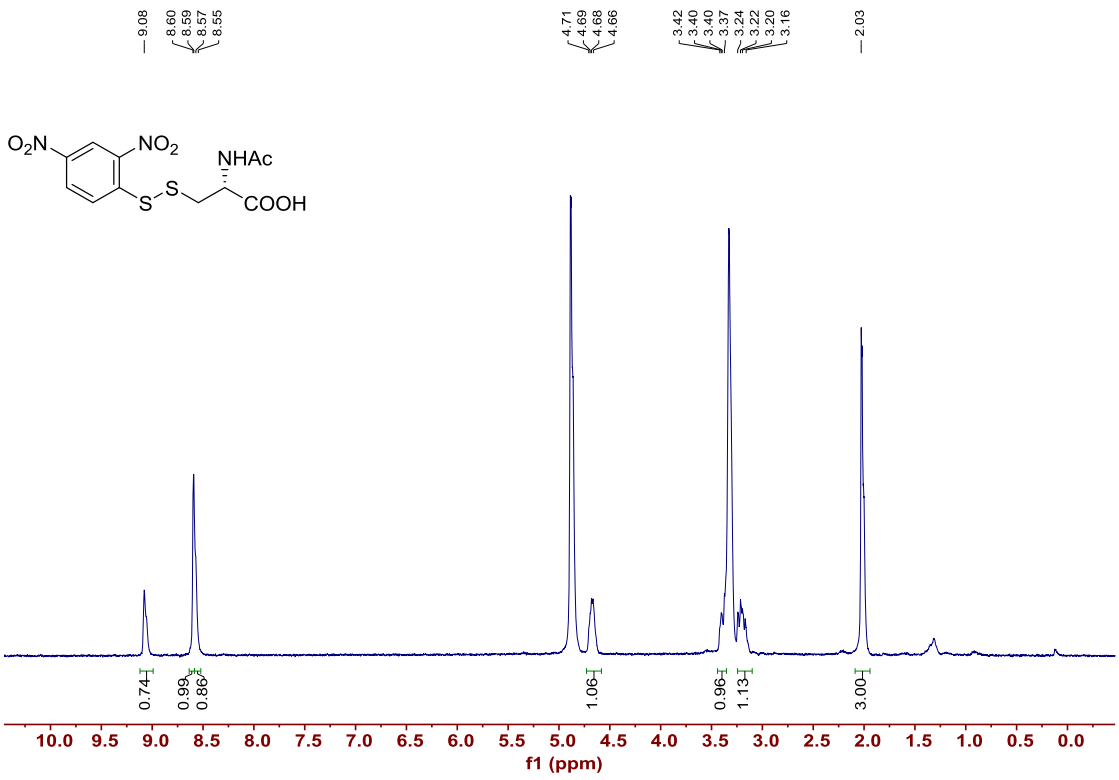

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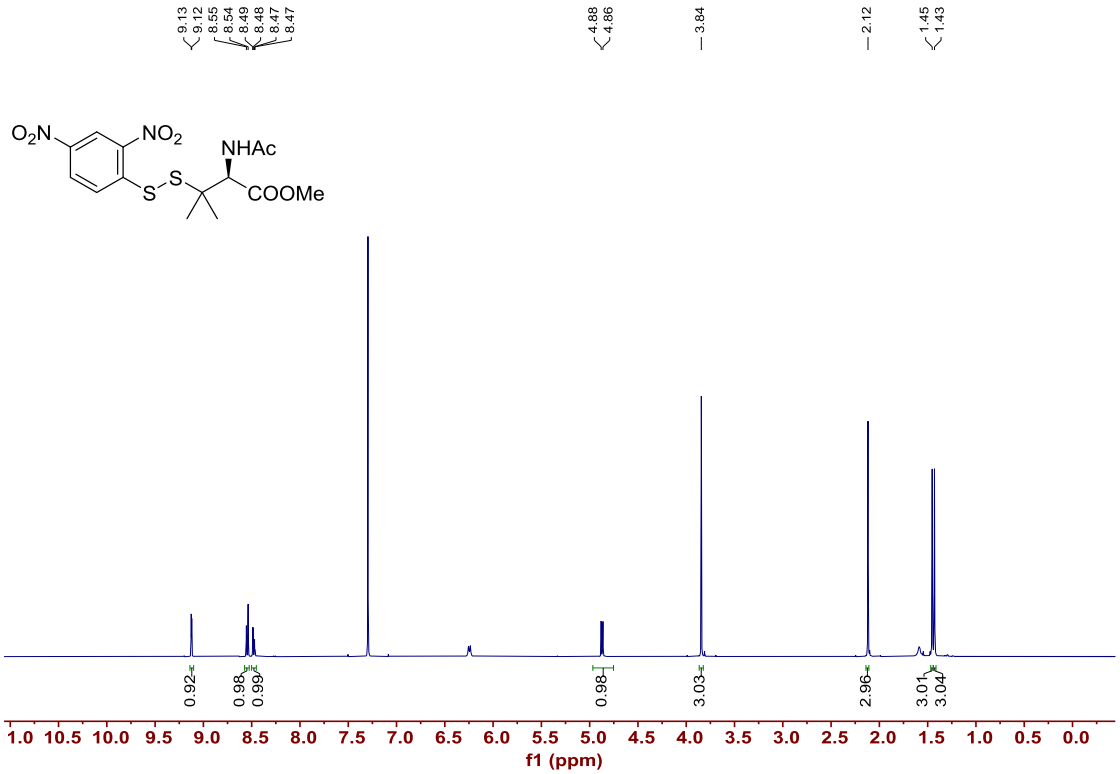

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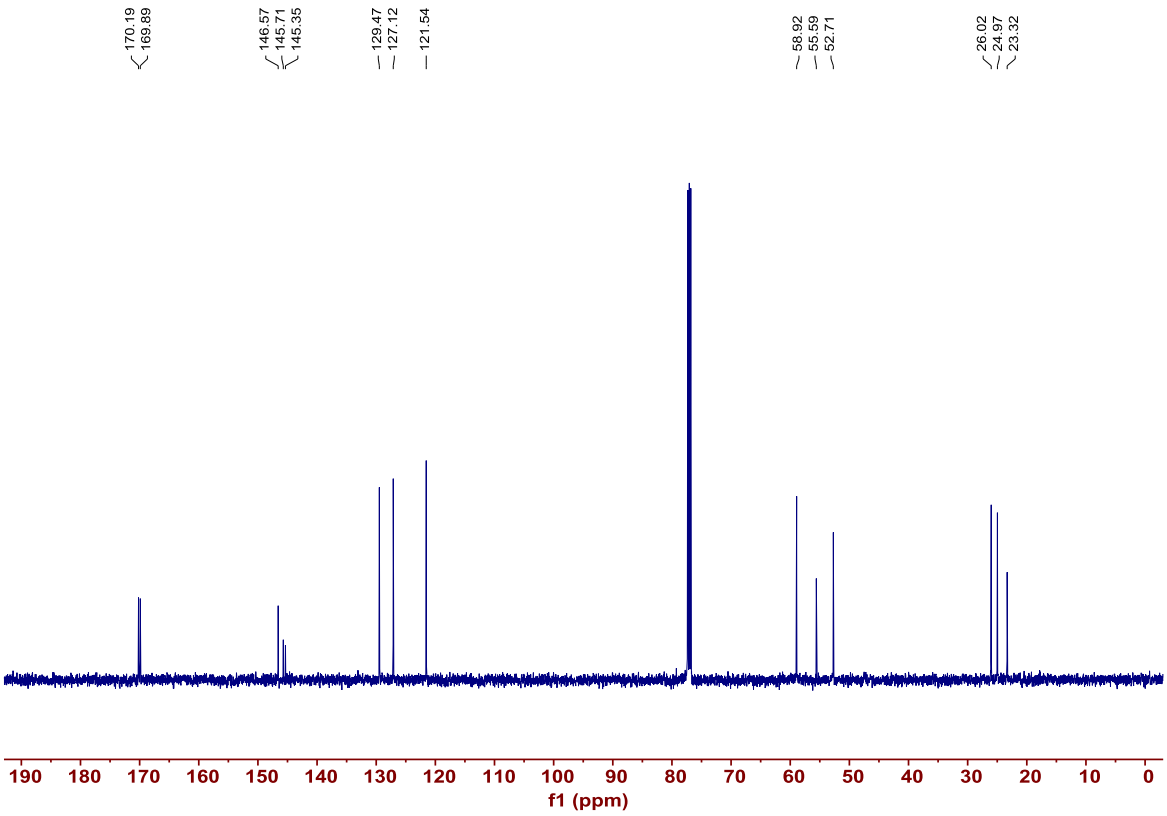
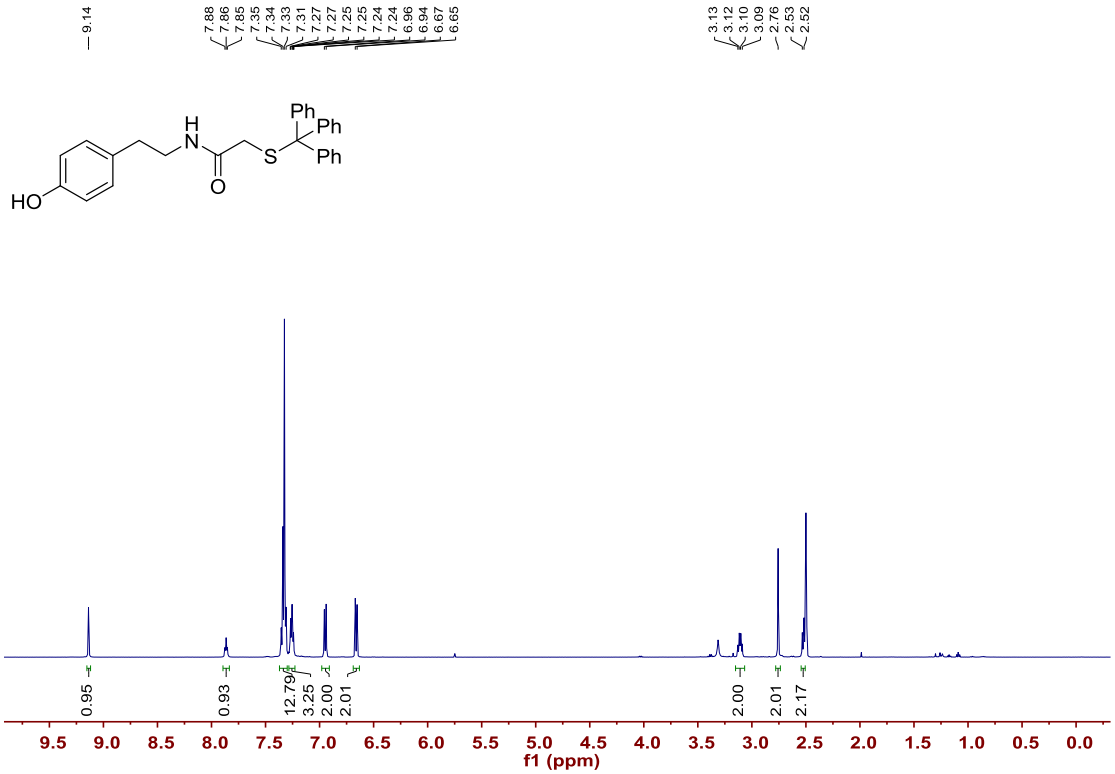


15 ^{1}H NMR



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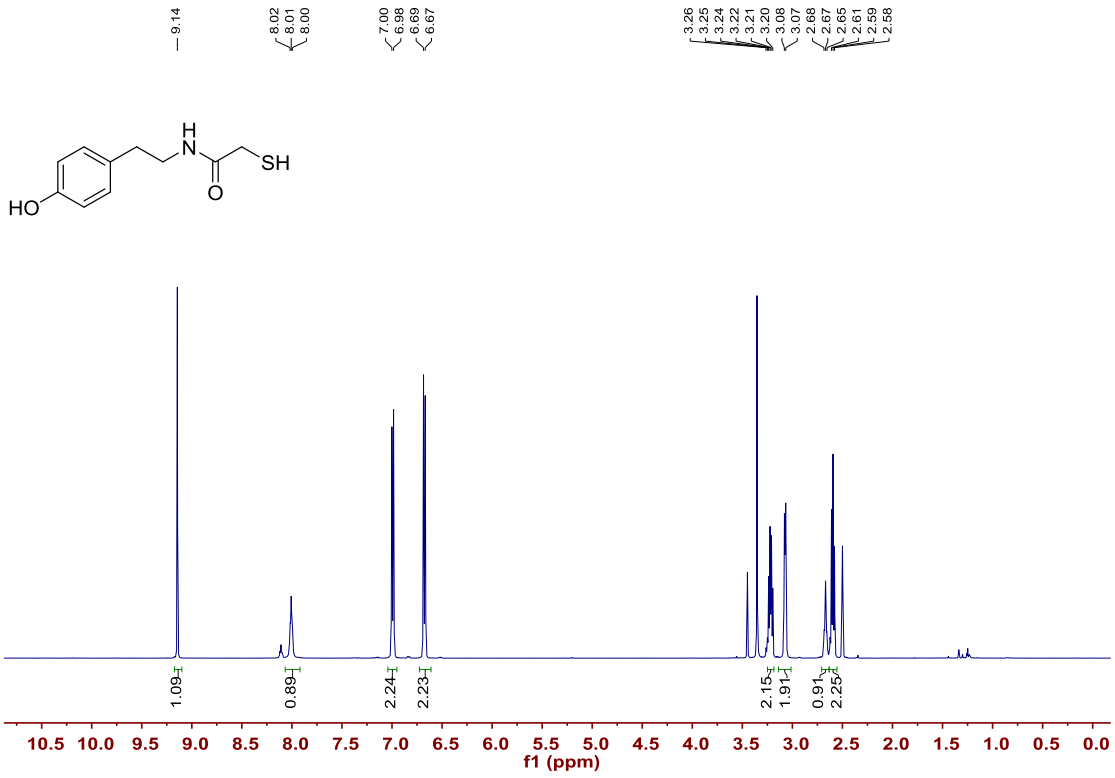
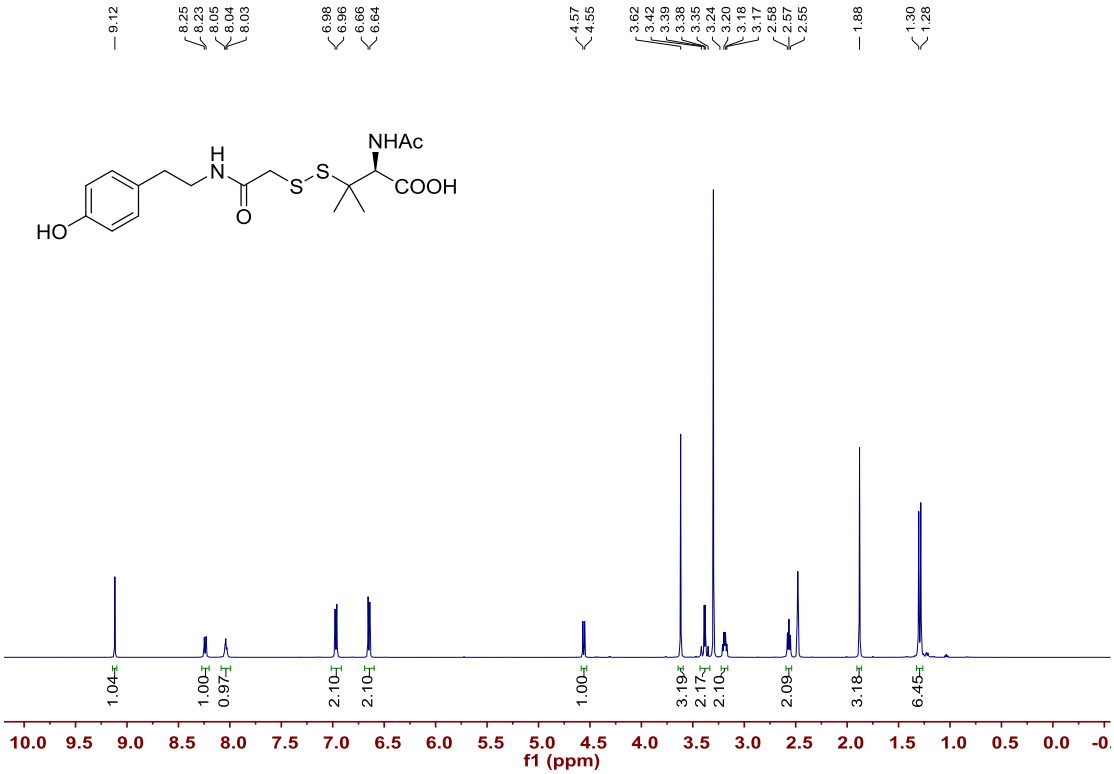


17 ^{1}H NMR



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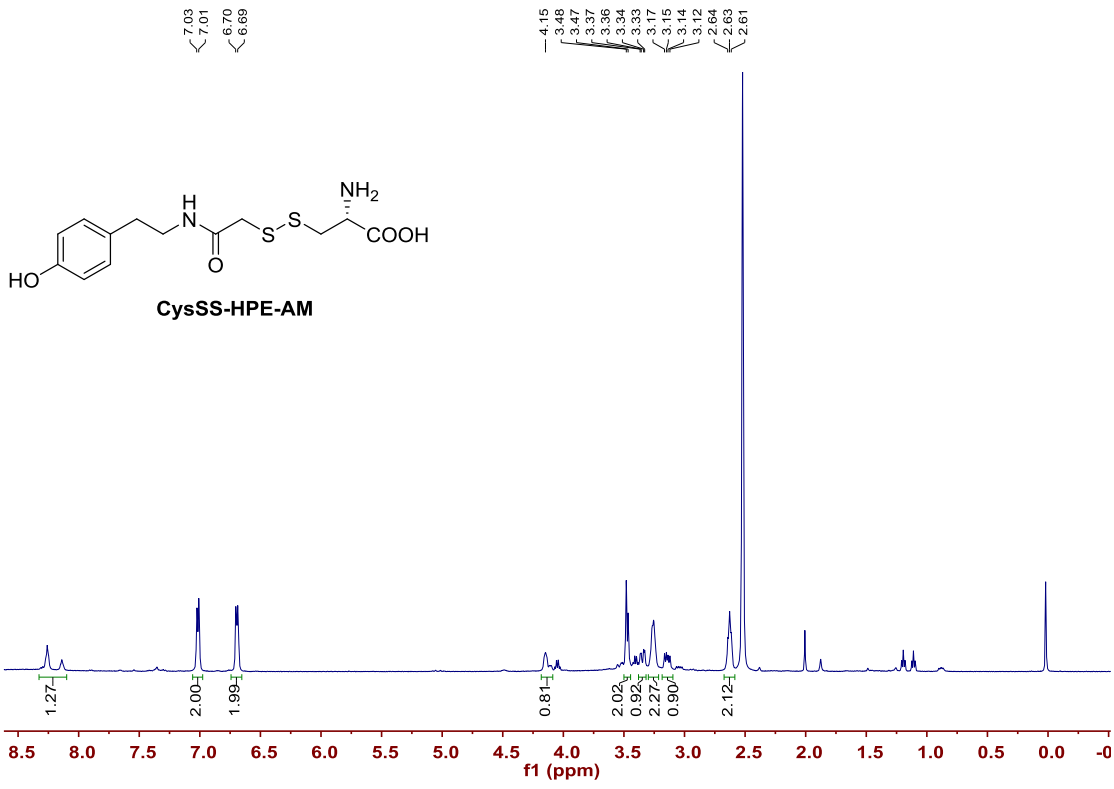

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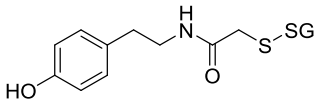

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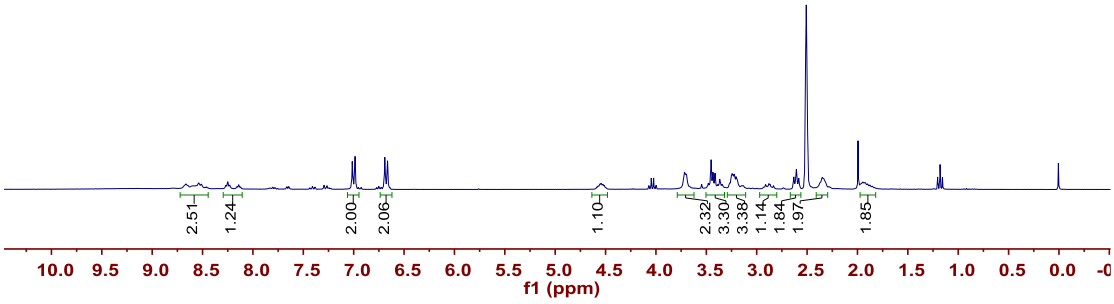

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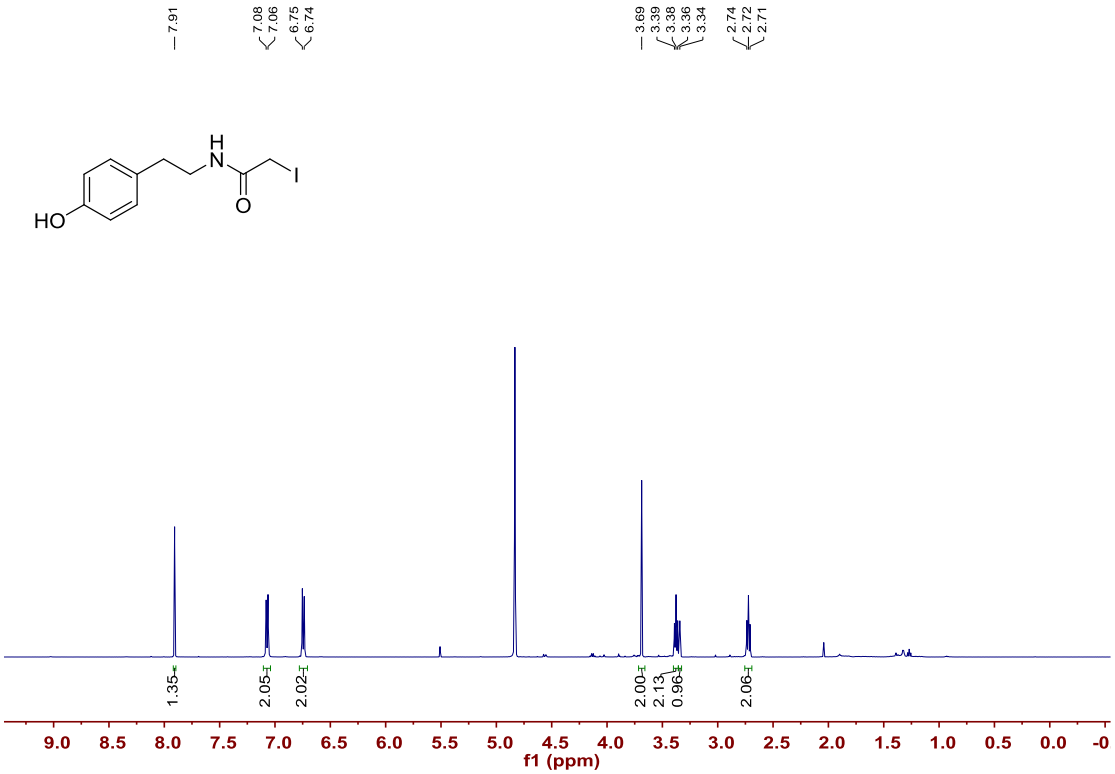

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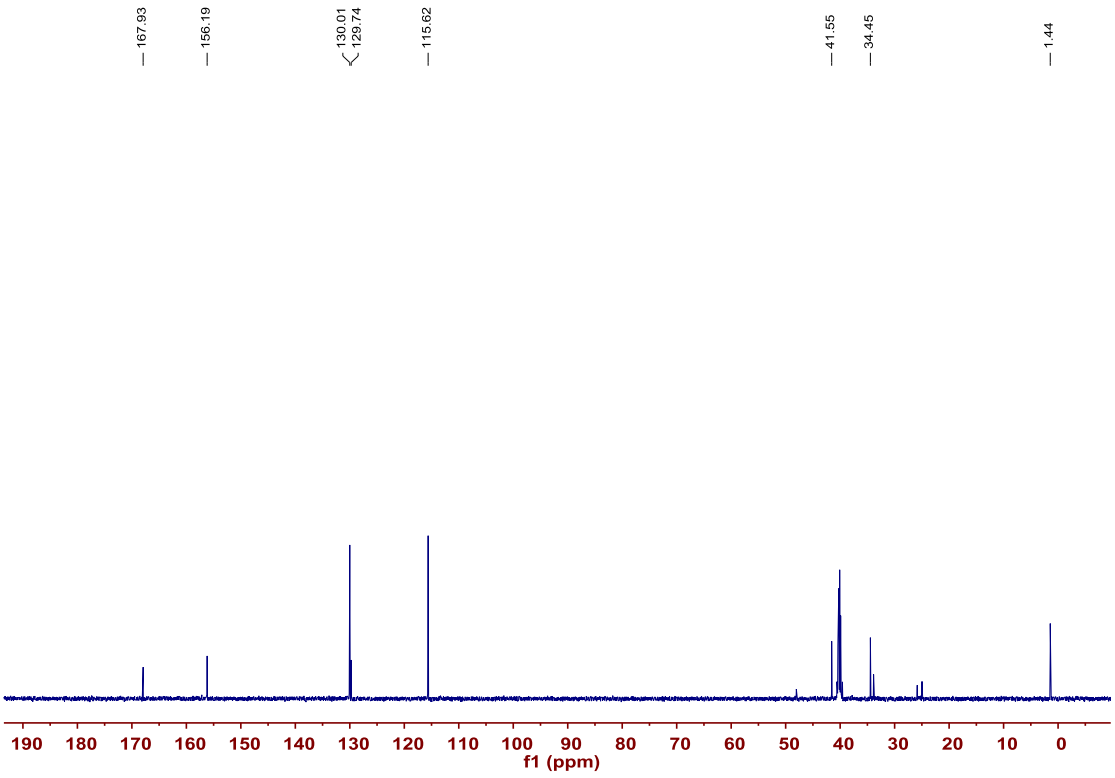
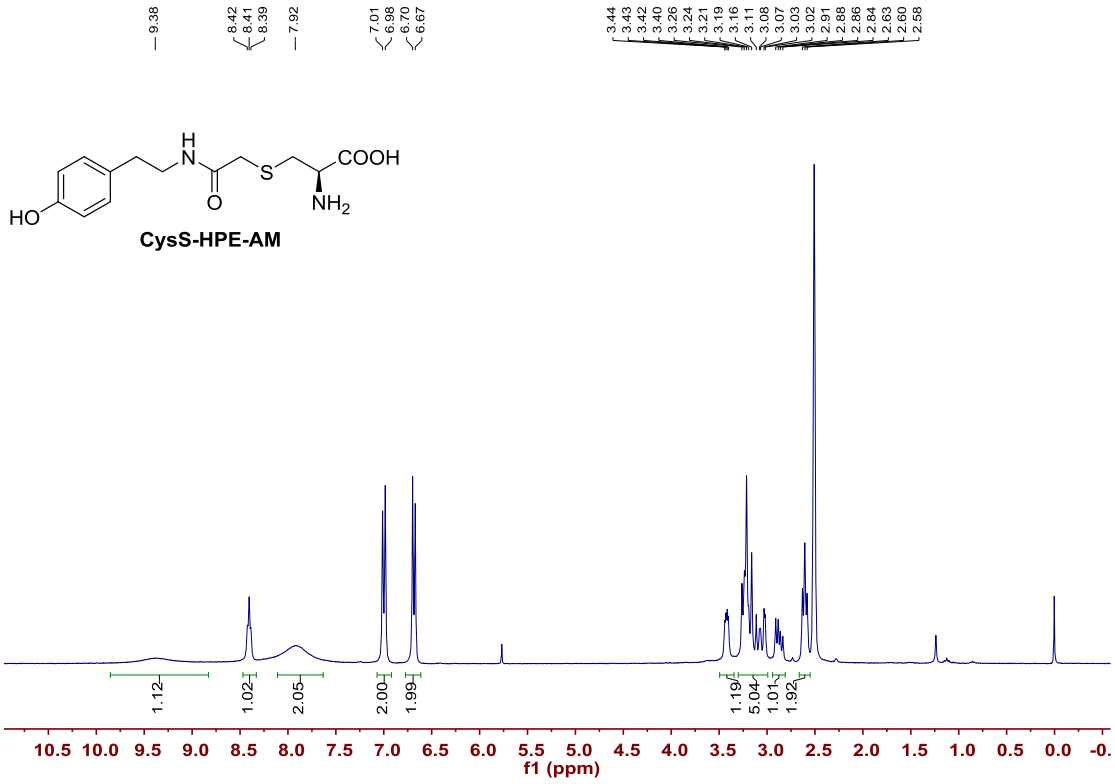


CysS-HPE-AM ^{1}H NMR



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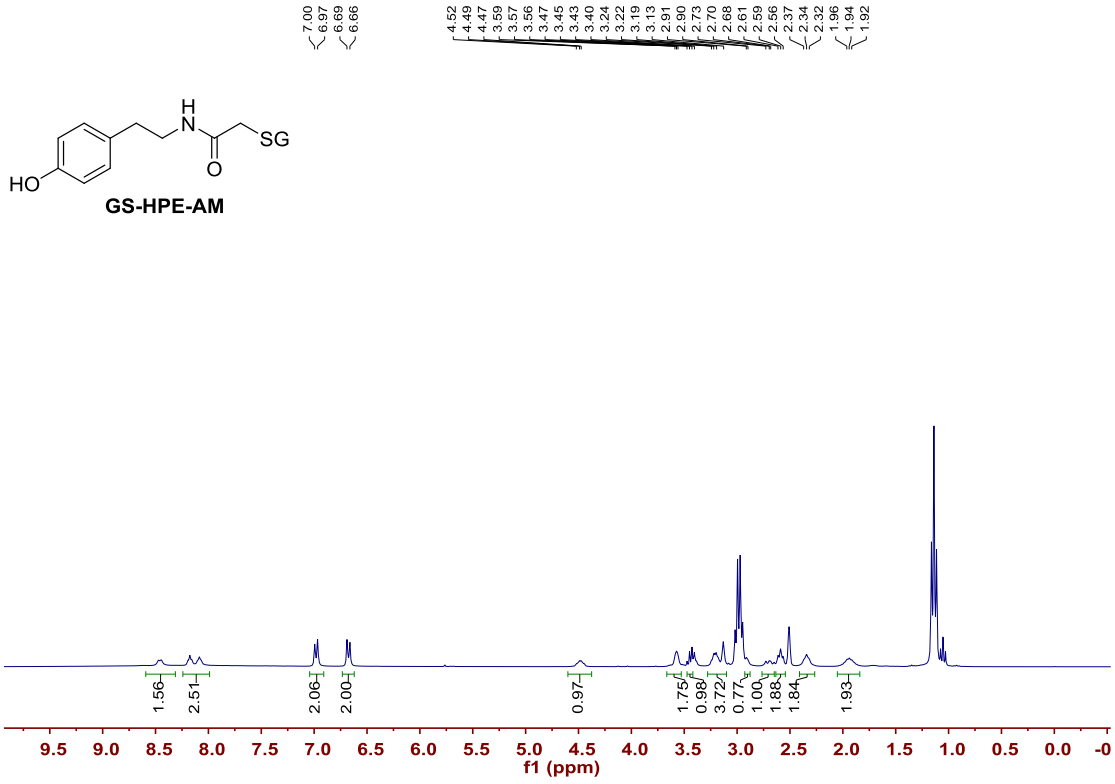

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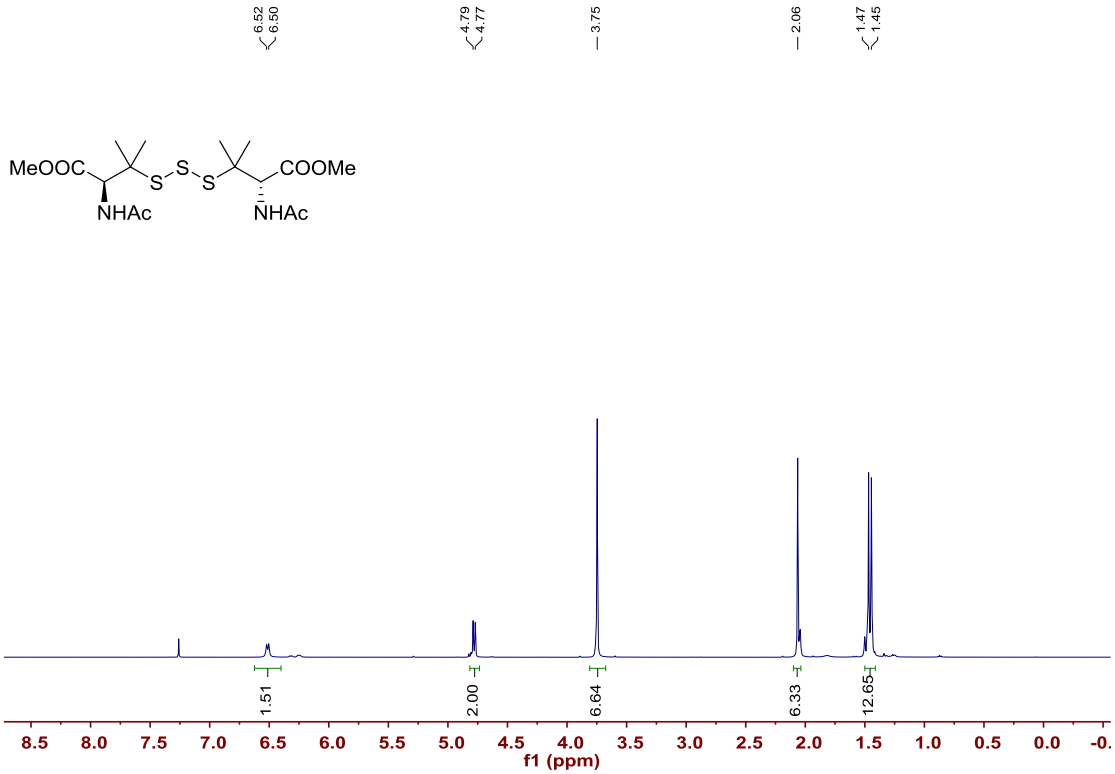

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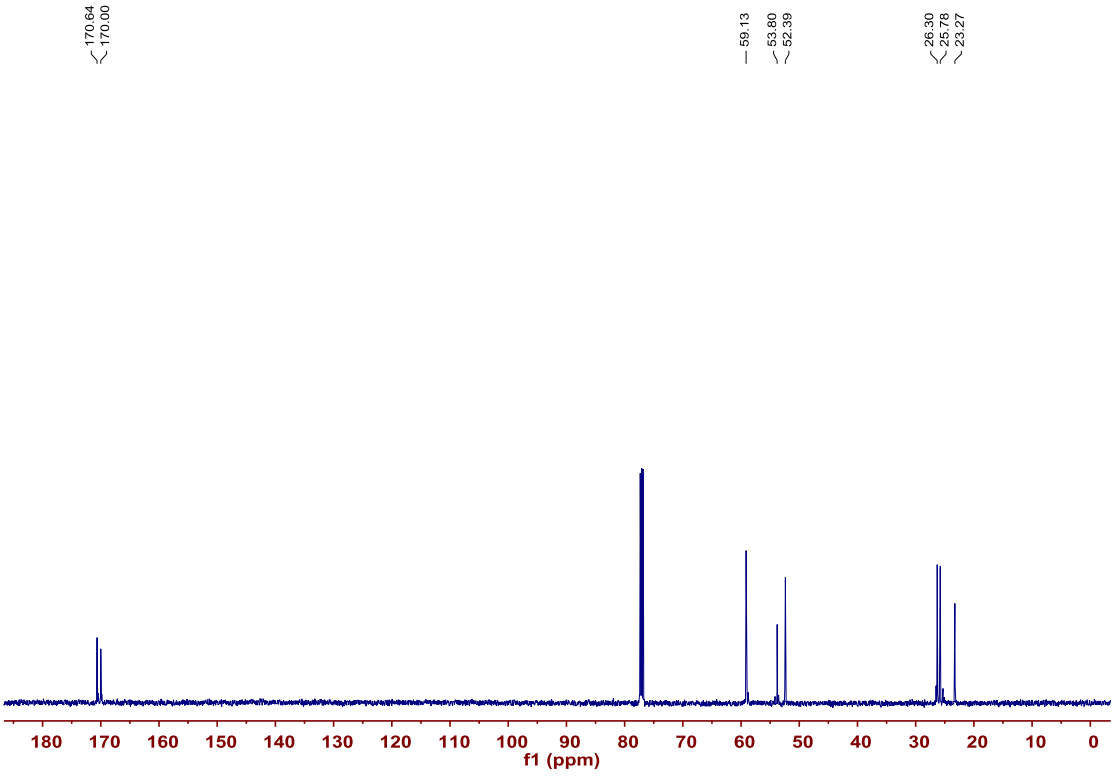
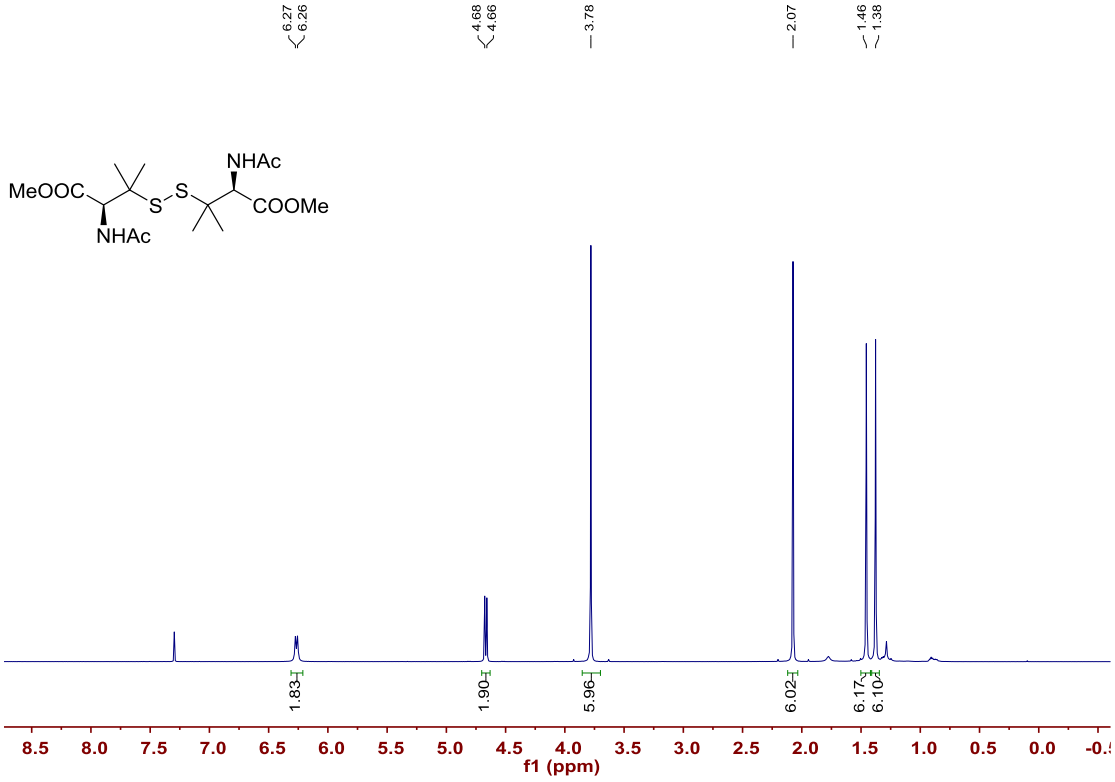


19 ^{1}H NMR



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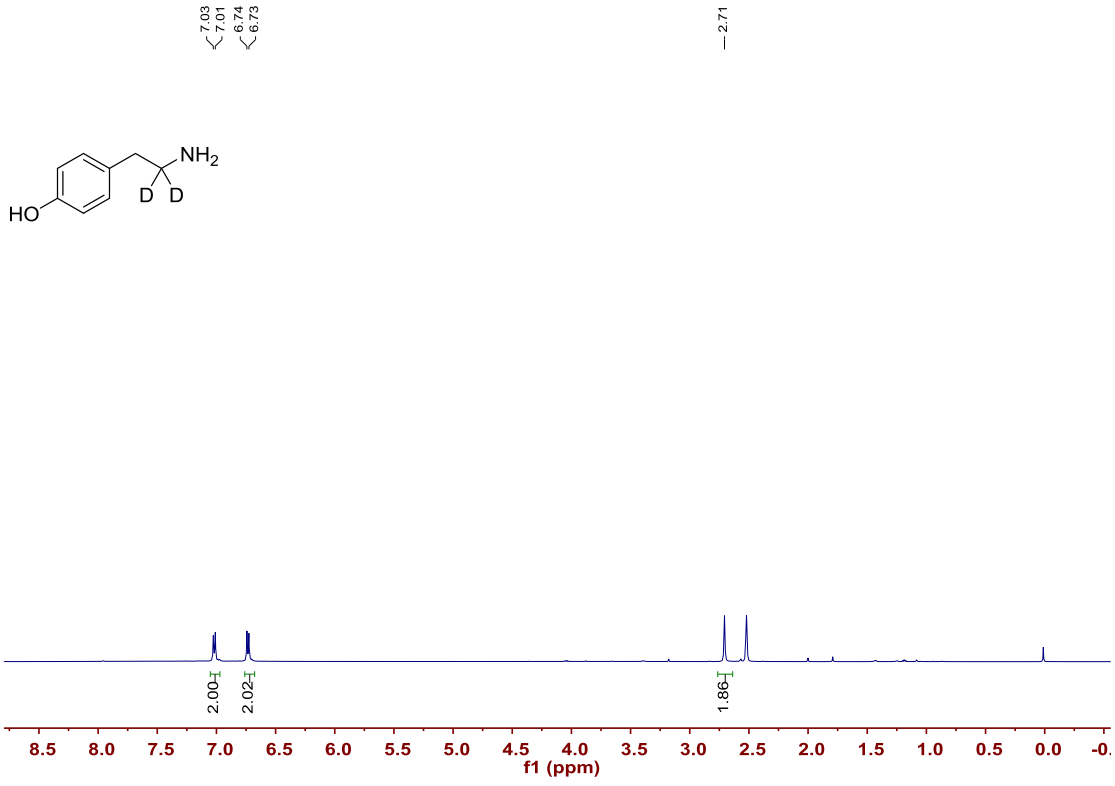

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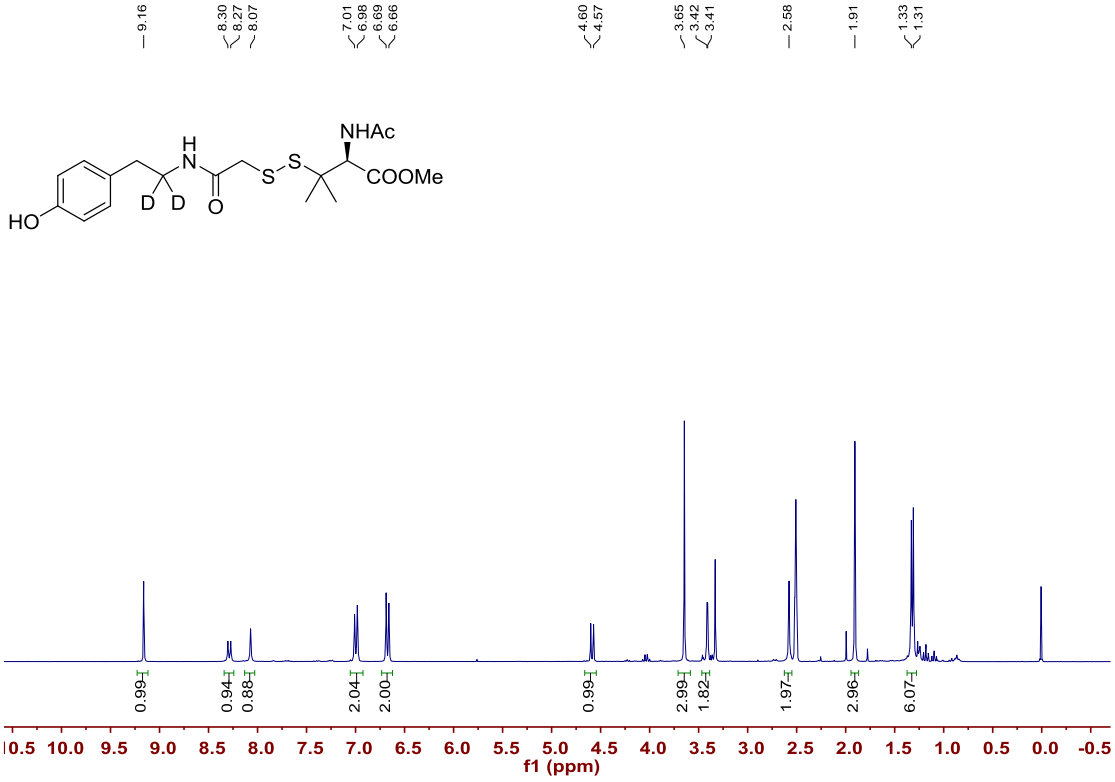

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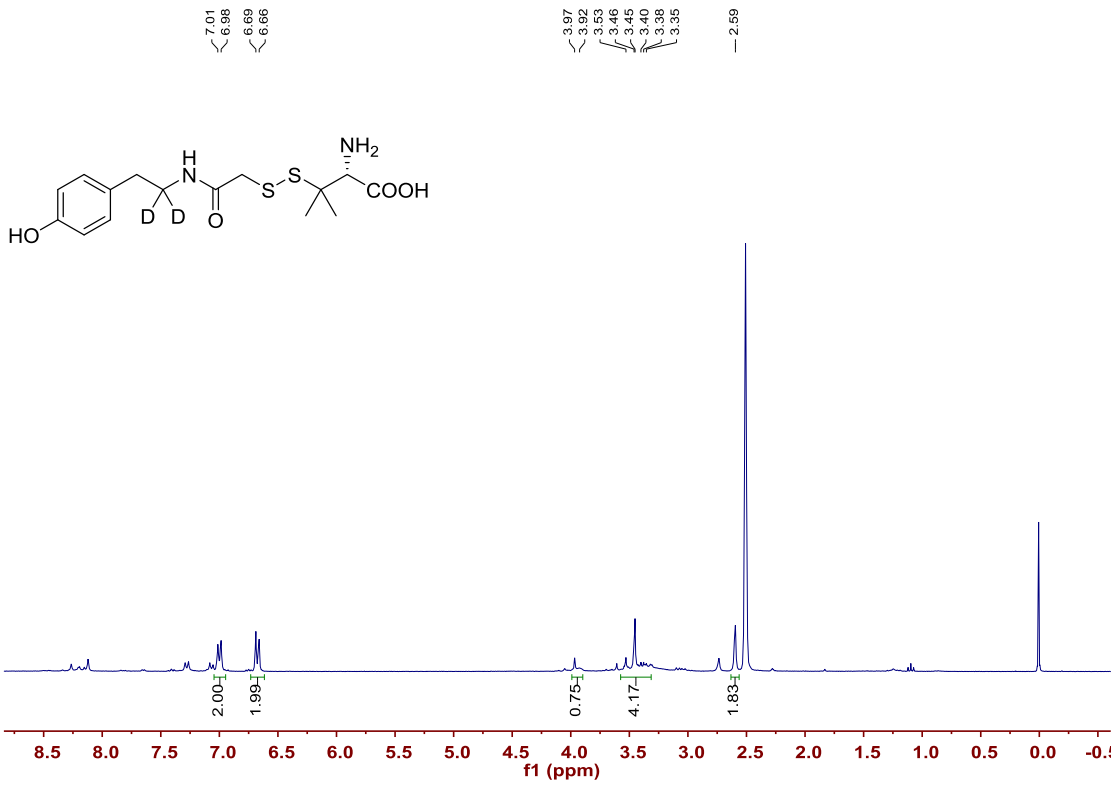
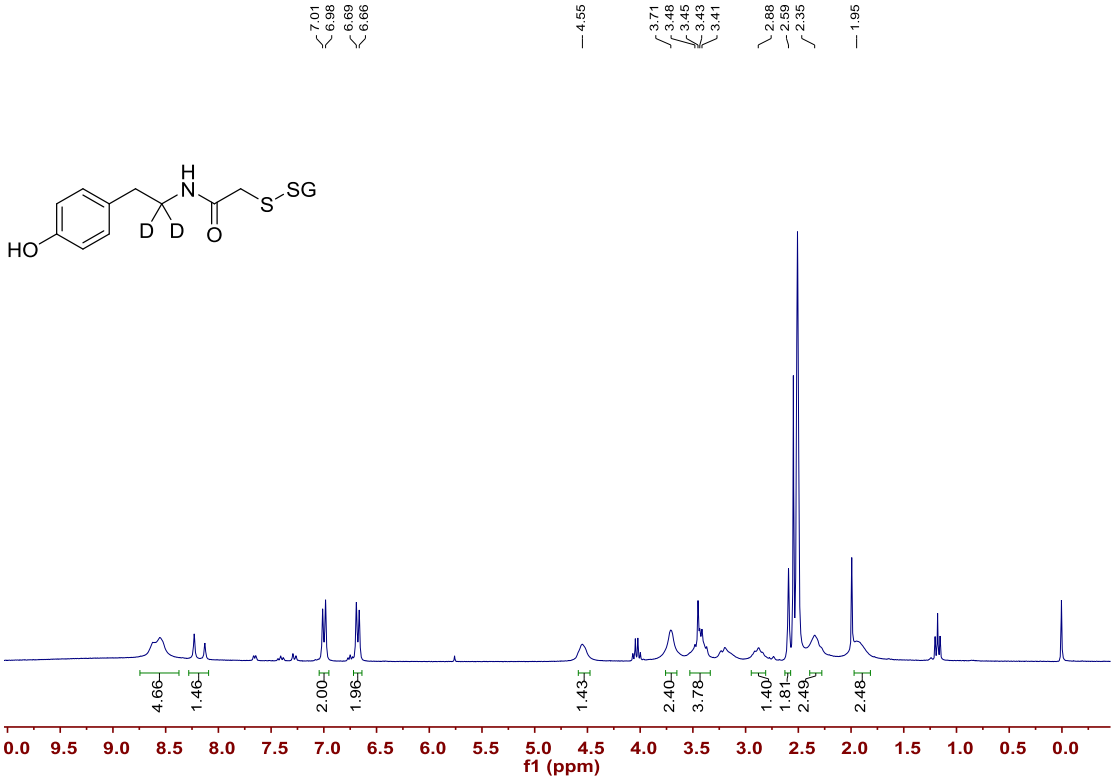
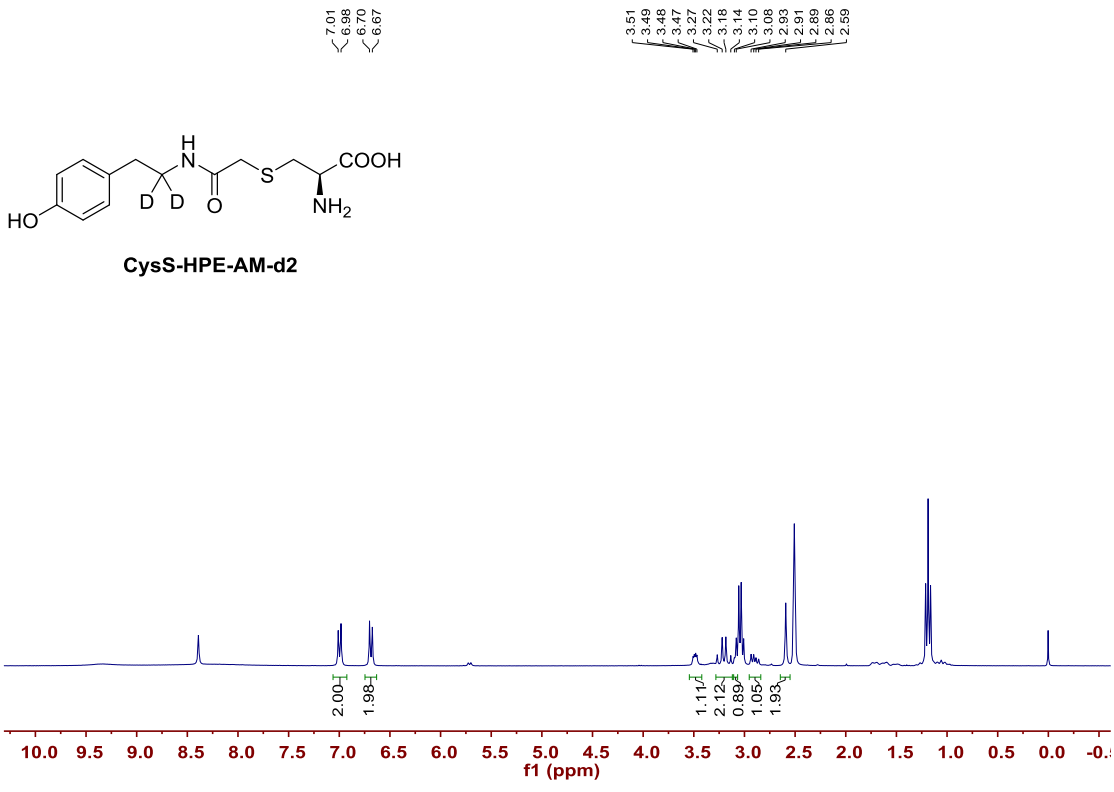


GSS-HPE-AM-d2 ^{1}H NMR

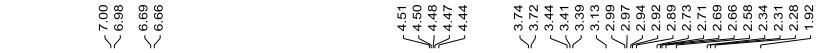


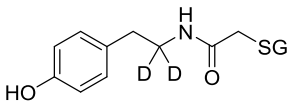
CysS-HPE-AM-d2 ^{1}H NMR



CysS-HPE-AM-d2

GS-HPE-AM-d2 ^{1}H NMR





GS-HPE-AM-d2