Supporting information

ReACT (Redox-Activated Chemical Tagging) chemistry enables direct derivatization and fluorescent detection of *S*-adenosyl-*L*-homocysteine (SAH)

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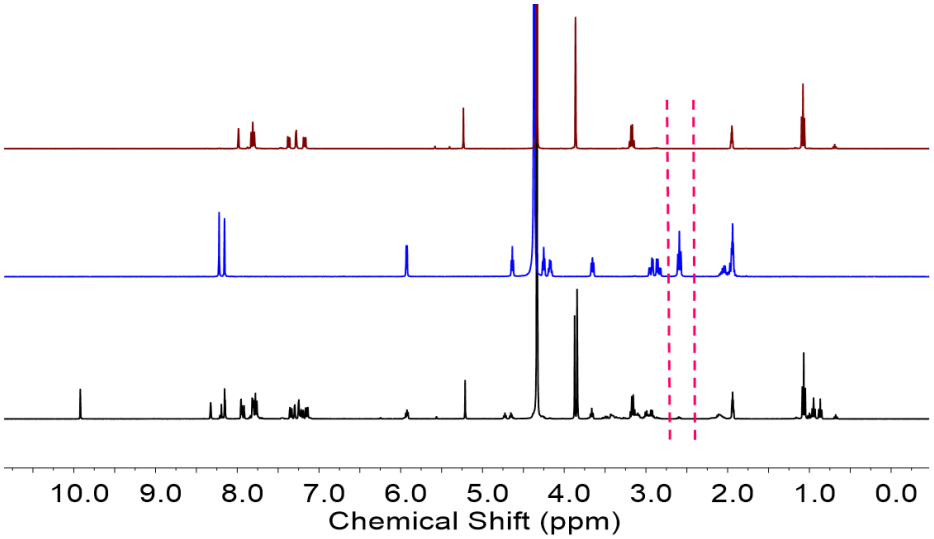


Figure S1. ^{1}H NMR comparison of probe **NapOx** (top), SAH (middle), and their reaction mixture (bottom). Reaction condition: **NapOx** (2.0 equiv), SAH (1.0 equiv), D_2O/CD_3CN (5:3), r.t., 20 min.

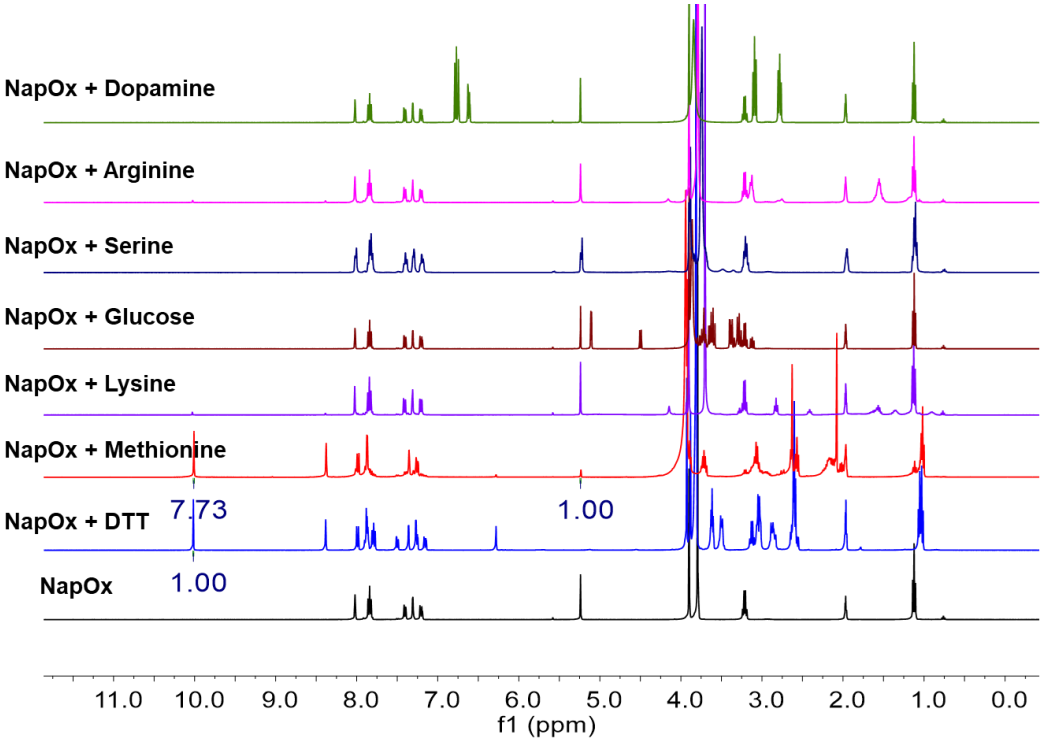


Figure S2. ^{1}H NMR analysis of the reaction mixtures of **NapOx** with different biomolecules. Reaction condition: **NapOx** (1.0 equiv), biomolecule (2.0 equiv), CD_3CN/D_2O (3:1), 22 °C, 10 min. For the reaction of methionine, CD_3CN/D_2O (7:3) was applied since methionine showed poor solubility in CD_3CN/D_2O (3:1).

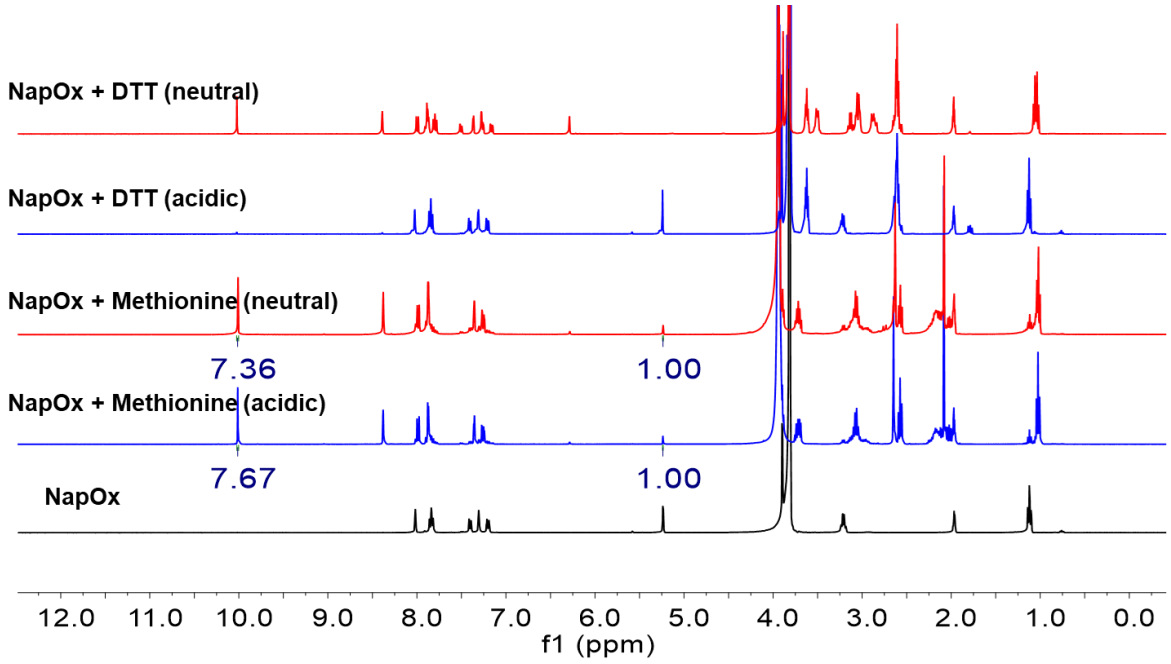


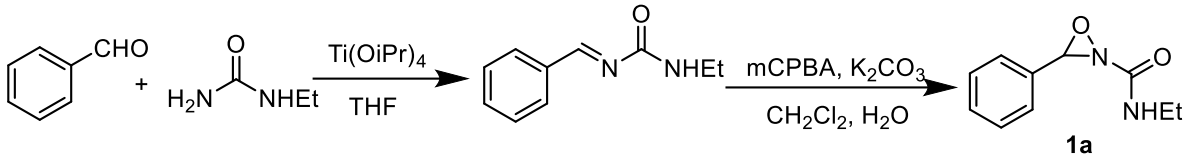
Figure S3. ^{1}H NMR analysis of the reaction mixtures of **NapOx** with DTT and methionine under neutral and acidic condition. For DTT reaction under neutral condition: **NapOx** (1.0 equiv), DTT (2.0 equiv), CD_3CN/D_2O (3:1), 22 °C, 10 min. For acidic reaction condition, 1 mM deuterated HCl solution was used to replace D_2O. For methionine reaction, CD_3CN/D_2O (7:3) or CD_3CN/1 mM deuterated HCl solution (7:3) were used to replace CD_3CN/D_2O (3:1) or CD_3CN/1 mM deuterated HCl solution (3:1).

1. Methods and materials

All chemicals were used as received unless otherwise stated. ^{1}H NMR and ^{13}C NMR spectra were collected on Bruker AVANCE III HD400 spectrometer. Proton chemical shifts of NMR spectra were calibrated with TMS as internals reference. HR-MS spectral data were recorded on Aglient 7250 and JEOL-JMS-T100LPAccuTOF devices. Analytical thin-layer chromatography (TLC) analysis was performed on TLC silica gel plates (0.2 ± 0.03 mm) and visualized with ultraviolet light (254 nm) to monitor reaction progression. The 96-well plate (black, flat, not treated) was purchased from Corning® (USA). Fluorescence analysis was performed on Thermo Scientific™ Varioskan™ LUX microplate reader. The analytic HPLC was run on Agilent 1260-workstation equipped with a Luna C18 column, 250 x 4.6 mm, particle size 5 µm, pore size 110 Å (Phenomenex) with a flow rate of 1 mL/min. Solvent A: H_2O with 0.1% trifluoroacetic acid; Solvent B: MeCN with 0.1% trifluoroacetic acid.

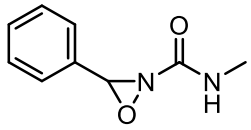
2. Compound Synthesis

2.1 Synthesis of oxaziridines 1a-1g



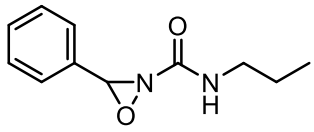
1a

The compound **1a** was synthesized following reported procedure.[1] To a mixture of ethylurea (0.62 g, 5.84 mmol) in anhydrous THF (14 mL) was added benzaldehyde (0.8 mL, 8.16 mmol) and Ti(O*i*Pr)$_4$ (2.20 mL, 7.48 mmol) sequentially under N_2. The white suspension was allowed to stir overnight at r.t. The reaction mixture was dried over evaporator, and the obtained crude was dissolved in DCM (5 mL). Then this mixture was added slowly to a suspension of mCPBA (4.60 g, 75% purity, 20 mmol) in saturated K_2CO_3 aqueous solution (20 mL) and DCM (20 mL), and the resulted mixture was stirred at r.t. for 6 h. After that, the mixture was diluted with 20 mL water, and extracted with DCM for three times. The combined organic was washed with brine, and dried over anhydrous Na_2SO_4. The crude mixture was filtered, and the filtrate was concentrated under vacuum. The obtained residue was purified over silica gel chromatography (PE/ EA = 4:1) to yield the desired compound **1a** as a white solid (150 mg, 13% yield for two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (m, 5H), 6.04 (br, 1H), 5.00 (s, 1H), 3.33 (m, 2H), 1.22 (t, *J* = 7.2 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 162.1, 132.4, 131.0, 128.6, 128.0, 79.3, 35.4, 15.2; The data are consistent with those reported in previous literature.[1]



1b

1b was synthesized with the same procedure as compound **1a**. The obtained residue was purified over Al_2O_3 column chromatography (DCM) to yield the desired compound **1b** as a yellow liquid (100 mg, 17% yield for two steps).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 5H), 6.13 (br, 1H), 5.00 (s, 1H), 2.87 (d, *J* = 4.8 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 162.9, 132.4, 131.0, 128.6, 128.0, 79.4, 27.0; HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_9H_{11}N_2O_2$ 179.0815; found 179.0815.

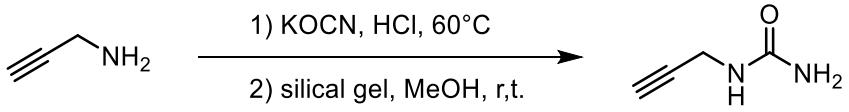


1c

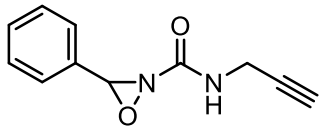
1c was synthesized with the same procedure as compound **1a**. The obtained residue was purified over Al_2O_3 column chromatography (PE/ EA = 6:1) to yield the desired compound **1c** as a white solid (102 mg, 15% yield for two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (m, 5H), 6.08 (br, 1H), 5.00 (s, 1H), 3.25 (m, 2H), 1.59 (m, 2H), 0.96 (t, *J* = 7.6 Hz, 3H). Please note that the integration of the hydrogens around δ 1.59 ppm is ca. 3.7 in supplementary NMR figure, which

is higher than 2. This is due to interference of extra H_2O signal at ca. 1.6 ppm. $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 162.2, 132.4, 131.0, 128.6, 128.0, 79.4, 42.1, 22.7, 11.2; HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{11}H_{15}N_2O_2$ 207.1128; found 207.1124.

Synthesis of 1-(Prop-2-yn-1-yl)urea[1]

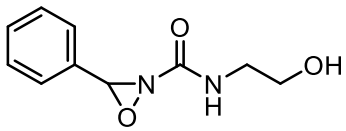


This compound was synthesized following reported procedure[1]. To a solution of propargylamine (1.6 mL, 25 mmol) in aqueous HCl solution (1.0 mol, 25 mL) was added KOCN (8 g, 100 mmol). After being heated at 60 °C for 40 h, the mixture was placed in the -20 °C refrigerator to obtain a white precipitate. After vacuum filtration, the obtained solid was dissolved in MeOH (75 mL) and stirred with silica gel (10 g) for 6 h. The mixture was then filtered, and the residue was washed with MeOH. The combined filtrate was concentrated under vacuum to attain the desired urea as a white solid without further purification (1.9 g, 78%). ^1H NMR (400 MHz, d_6-DMSO) δ 6.29 (t, J = 6.0 Hz, 1H), 5.60 (s, 2H), 3.75 (dd, J = 5.6, 2.4 Hz, 2H), 3.03 (t, J = 2.4 Hz, 1H). $^{13}C\{^1H\}$ NMR (101 MHz, d_6-DMSO) δ 158.6, 83.0, 72.9, 29.1; The data are consistent with those reported in previous literature.[1]



1d

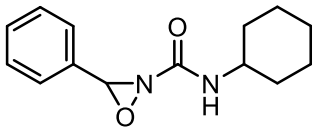
1d was synthesized with the same procedure as compound **1a**. The obtained residue was purified over Al_2O_3 column chromatography (DCM) to yield the desired compound **1d** as a yellow liquid (111 mg, 17% yield for two steps). ^1H NMR (400 MHz, $CDCl_3$) δ 7.45 (m, 5H), 6.37 (br, 1H), 5.03 (s, 1H), 4.07 (m, 2H), 2.30 (t, J = 2.4 Hz, 1H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 162.0, 132.0, 131.1, 128.7, 128.0, 79.5, 78.3, 72.6, 30.2; The data are consistent with those reported in previous literature.[1]



1e

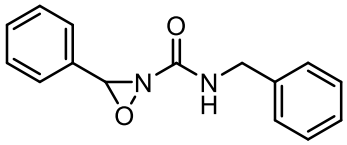
1e was synthesized with the same procedure as compound **1a**. The obtained residue was purified over Al_2O_3 column chromatography (EA) to yield the desired compound **1e** as a white solid (40 mg, 4% yield for two steps). ^1H NMR (400 MHz, $CDCl_3$) δ 7.45 (m, 5H), 6.52 (br, 1H), 5.04 (s,

1H), 3.77 (q, *J* = 5.2 Hz, 2H), 3.46 (m, 2H), 2.19 (t, *J* = 5.2 Hz, 1H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 163.1, 132.2, 131.1, 128.7, 128.0, 79.5, 61.5, 42.7; HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{10}H_{13}N_2O_3$ 209.0926; found 209.0907.



1f

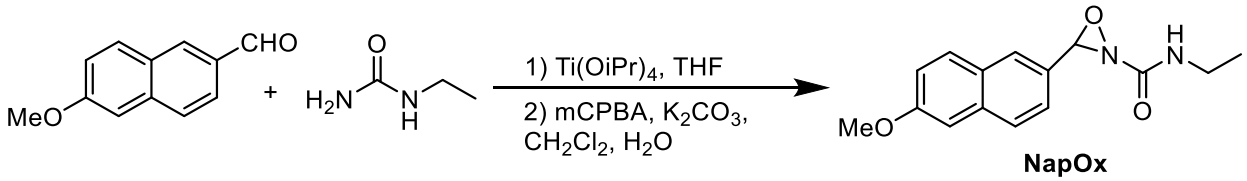
1f was synthesized with the same procedure as compound **1a**. The obtained residue was purified over Al_2O_3 column chromatography (PE/ EA = 6:1) to yield the desired compound **1f** as a white solid (29 mg, 6% yield for two steps). ^1H NMR (400 MHz, $CDCl_3$) δ 7.44 (m, 5H), 5.96 (br, 1H), 4.98 (s, 1H), 3.66 (m, 1H), 1.96 (m, 2H), 1.74 (m, 2H), 1.63 (m, 1H), 1.37 (m, 2H), 1.21 (m, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 161.3, 132.5, 131.0, 128.6, 128.0, 79.4, 49.4, 32.9, 32.8, 25.3, 24.7; The data are consistent with those reported in previous literature.[2]



1g

1g was synthesized with the same procedure as compound **1a**. The obtained residue was purified over Al_2O_3 column chromatography (PE/ EA = 6:1) to yield the desired compound **1g** as a white solid (35 mg, 4% yield for two steps). ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (m, 10H), 6.36 (br, 1H), 5.04 (s, 1H), 4.47 (m, 2H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 162.2, 137.0, 132.3, 131.1, 128.9, 128.7, 128.0, 127.8, 79.5, 44.4; The data are consistent with those reported in previous literature.[2]

2.2 Synthesis of compound NapOx

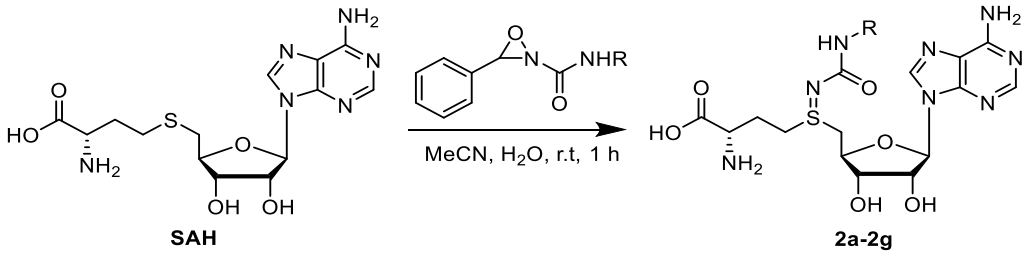


NapOx

The compound **NapOx** was synthesized with the same procedure as compound **1a** on page S3. The obtained crude was purified over Al_2O_3 chromatography with DCM as eluent to yield crude as a light yellow solid, which was further recrystallized from PE/DCM to provide the desired product **NapOx** as a white solid (140 mg, 24% yield for two steps). ^1H NMR (400 MHz, $CDCl_3$)

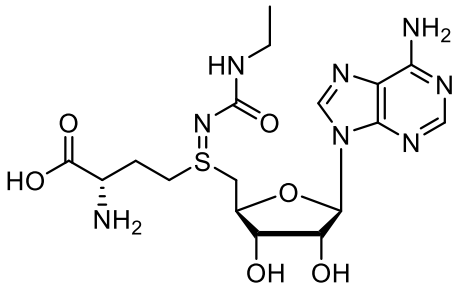
δ 7.93 (s, 1H), 7.76 (d, *J* = 8.8 Hz, 2H), 7.43 (dd, *J* = 8.8, 1.6 Hz, 1H), 7.16 (m, 2H), 6.09 (br, 1H), 5.13 (s, 1H), 3.93 (s, 3H), 3.35 (m, 2H), 1.23 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.2, 158.8, 136.1, 129.8, 129.0, 128.1, 127.5, 127.4, 124.1, 119.5, 105.8, 79.9, 55.4, 35.4, 14.8; HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{15}H_{17}N_2O_3$ 273.1234; found 273.1230.

2.3 Synthesis of compounds 2a-2g



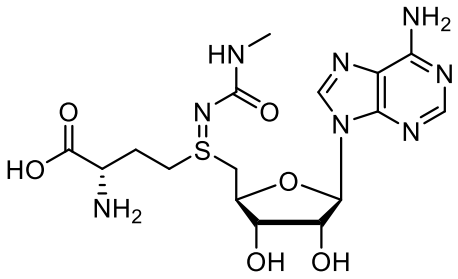
SAH **2a-2g**

General synthetic procedure for compounds 2a-2g: A solution of oxaziridine **1a-1g** (0.26 mmol, 2.0 equiv), SAH (0.13 mmol, 1.0 equiv) in ca. 20% MeCN aqueous solution was stirred at room temperature for approximately 1 h. The reaction mixture was washed with CH_2Cl_2 for three times. Then the aqueous layer was concentrated under vacuum, and the obtained crude was purified by column chromatography over reverse silica gel to afford the target compound.



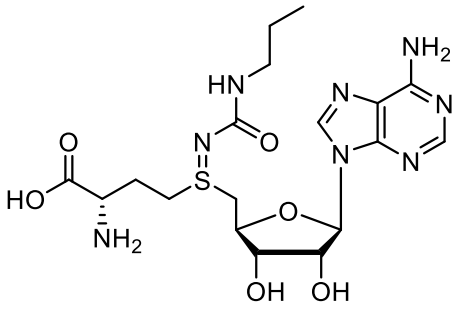
2a

Compound 2a was synthesized using the procedure described above. The obtained residue was purified over reverse phase silica gel column chromatography (10% MeOH in H_2O) to yield the desired compound **2a** (50 mg, 83% yield) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ [8.12] 7.98 (s, 1H), [8.06] 7.96 (s, 1H), [5.89] 5.86 (d, *J* = 5.2 Hz, 1H), 4.73 (q, *J* = 5.2 Hz, 1H), 4.38 (m, 2H), 3.70 (q, *J* = 6.8 Hz, 1H), 3.47 (m, 2H), 3.12 (m, 2H), [2.96] 2.82 (q, *J* = 7.2 Hz, 2H), 2.09 (m, 2H), [0.91] 0.78 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ [173.1] 173.0, [167.7] 161.2, [155.2] 155.2, [152.6] 152.6, [148.6] 148.4, [140.2] 140.1, [118.6] 118.6, [88.3] 88.1, 79.0, 77.8, [72.9] 72.7, [72.8] 72.5, [53.3] 52.9, 49.5, [43.4] 43.0, [24.6] 24.5, [14.4] 14.3; HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{17}H_{27}N_8O_6S$ 471.1769; found 471.1780.



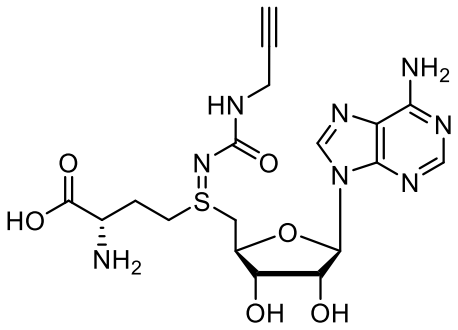
2b

Compound 2b was synthesized using the procedure described above. The obtained residue was purified over reverse phase silica gel column chromatography (10% MeOH in H_2O) to yield the desired compound **2b** (30 mg, 51% yield) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ [8.14] 8.03 (s, 1H), [8.09] 8.02 (s, 1H), [5.92] 5.90 (d, *J* = 5.2 Hz, 1H), 4.77 (m, 1H), 4.37 (m, 2H), 3.69 (q, *J* = 6.4 Hz, 1H), 3.47 (m, 2H), 3.08 (m, 2H), [2.54] 2.39 (s, 3H), 2.09 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 173.3, 168.4, [155.3] 155.3, [152.7] 152.7, [148.7] 148.5, [140.3] 140.1, [118.8] 118.7, [88.4] 88.1, 78.9, 77.8, [72.8] 72.6, [72.8], 72.5, [53.3] 52.9, 49.6, [43.5] 43.1, [24.7] 24.6; HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{16}H_{25}N_8O_6S$ 457.1612; found 457.1622.



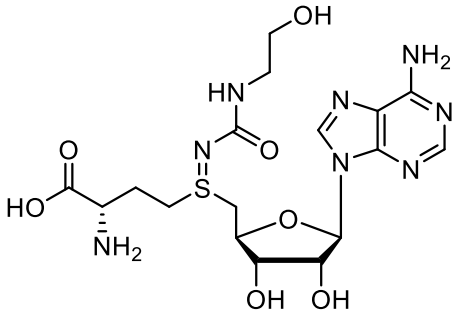
2c

Compound 2c was synthesized using the procedure described above. The obtained residue was purified over reverse phase silica gel column chromatography (30% MeOH in H_2O) to yield the desired compound **2c** (28 mg, 45% yield) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ [8.19] 8.09 (s, 1H), [8.13] 8.08 (s, 1H), [5.94] 5.92 (d, *J* = 5.2 Hz, 1H), 4.78 (m, 1H), 4.38 (m, 2H), 3.65 (t, *J* = 6.4 Hz, 1H), 3.48 (m, 2H), 3.06 (m, 2H), [2.89] 2.74 (t, *J* = 7.2 Hz, 2H), 2.08 (m, 2H), [1.29] 1.11 (q, *J* = 7.2 Hz, 2H), [0.71] 0.58 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ 173.9, 167.8, 155.5, [152.8] 152.8, [148.8] 148.7, [140.4] 140.2, [118.9] 118.8, [88.3] 88.0, 79.3, 77.9, [72.9] 72.7, [72.8] 72.5, [53.4] 53.1, 49.5, [43.4] 43.1, [25.0] 24.9, [22.7] 22.6, [10.5] 10.3; HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{18}H_{29}N_8O_6S$ 485.1925; found 485.1936.



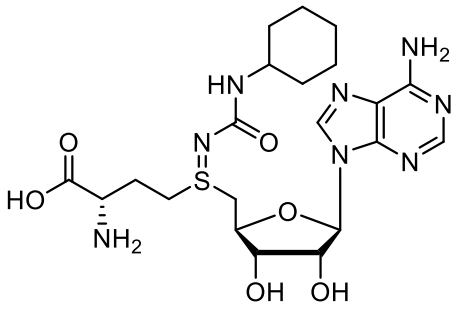
2d

Compound 2d was synthesized using the procedure described above. The obtained residue was purified over reverse phase silica gel column chromatography (10% MeOH in H_2O) to yield the desired compound **2d** (33 mg, 53% yield) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ [8.17] 8.07 (s, 1H), [8.12] 8.06 (s, 1H), [5.94] 5.91 (d, *J* = 5.2 Hz, 1H), 4.77 (q, *J* = 5.2 Hz, 1H), 4.40 (m, 2H), 3.72 (m, 2H), 3.61 (s, 1H), 3.50 (m, 2H), 3.13 (m, 2H), [2.45] 2.32 (t, *J* = 2.4 Hz, 1H), 2.12 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 173.2, 167.1, [155.4] 155.4, [152.8] 152.7, [148.7] 148.6, [140.4] 140.2, [118.9] 118.8, [88.4] 88.1, [81.4] 81.3, 79.0, 77.8, [72.8] 72.8, [72.8] 72.5, [71.1] 70.9, [53.2] 52.9, 49.4, [43.3] 43.0, [24.7] 24.6; HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{18}H_{25}N_8O_6S$ 481.1612; found 481.1643.



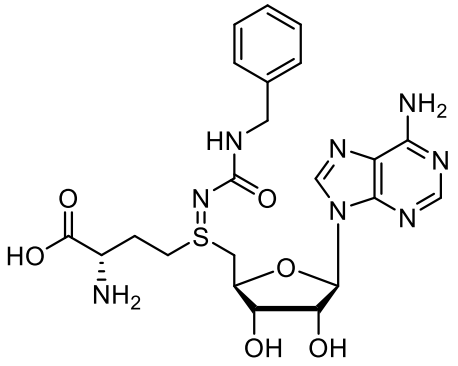
2e

Compound 2e was synthesized using the procedure described above. The obtained residue was purified over reverse phase silica gel column chromatography (5% MeOH in H_2O) to yield the desired compound **2e** (36 mg, 57% yield) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ [8.14] 8.02 (s, 1H), [8.09] 8.01 (s, 1H), [5.92] 5.90 (d, *J* = 4.8 Hz, 1H), 4.76 (t, *J* = 5.2 Hz, 1H), 4.38 (m, 2H), 3.71 (q, *J* = 6.8 Hz, 1H), 3.48 (m, 4H), 3.13 (m, 2H), 3.02 (m, 2H), 2.11 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 173.1, 167.5, [155.3] 155.2, [152.7] 152.6, [148.6] 148.4, [140.3] 140.1, [118.7] 118.6, [88.4] 88.1, 78.8, 77.8, [72.8] 72.6, 72.7, [60.8] 60.8, [53.2] 52.9, [49.6] 49.3, [43.4] 43.0, [24.6] 24.5; HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{17}H_{27}N_8O_7S$ 487.1718; found 487.1739.



2f

Compound 2f was synthesized using the procedure described above. The obtained residue was purified over reverse phase silica gel column chromatography (40% MeOH in H_2O) to yield the desired compound **2f** (42 mg, 69% yield) as a light yellow solid. ^{1}H NMR (400 MHz, D_2O) δ [8.15] 8.00 (s, 1H), [8.07] 7.97 (s, 1H), [5.89] 5.86 (d, *J* = 4.8 Hz, 1H), 4.72 (br, 1H), 4.36 (m, 2H), 3.72 (t, *J* = 6.4 Hz, 1H), 3.48 (m, 2H), 3.15 (m, 2H), 3.01 (m,1H), 2.12 (m,2H), [1.45] 0.97 (m, 10H). ^{13}C NMR (101 MHz, D_2O) δ [173.0] 173.0, [166.9] 166.6, [155.3] 155.2, [152.7] 152.6, [148.6] 148.4, [140.2] 140.1, [118.6] 118.5, [88.2] 87.9, 79.5, 77.8, [72.9] 72.5, 72.9, [53.3] 52.9, [50.0] 49.9, 49.3, [43.2] 43.0, [32.8] 32.8, [32.8] 32.6, [25.0] 24.9, [24.6] 24.5, 24.4; HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{21}H_{33}N_8O_6S$ 525.2238; found 525.2248.



2g

Compound 2g was synthesized using the procedure described above. The obtained residue was purified over reverse phase silica gel column chromatography (30% MeOH in H_2O) to yield the desired compound **2g** (30 mg, 44% yield) as a light yellow solid. ^{1}H NMR (400 MHz, D_2O) δ [8.01] 7.96 (s, 1H), [8.99] 7.95 (s, 1H), [7.08] 6.89 (m,5H), 5.80 (t, *J* = 4.4 Hz, 1H), 4.64 (t, *J* = 5.2 Hz, 1H), [4.48] 4.22 (t, *J* = 5.2 Hz, 1H), [4.42] 4.29 (m, 1H), 4.04 (m, 2H), 3.71 (q, *J* = 6.4 Hz, 1H), 3.46 (m, 2H), 3.10 (m, 2H), 2.12 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ [172.9] 172.9, 167.3, 155.2, [152.5] 152.5, [148.3] 148.2, [140.1] 139.5, [139.6] 139.5, [129.1] 127.1, [128.6] 126.7, [128.4] 128.1, [126.8] 126.6, 126.5, 118.7, [88.5] 88.2, 79.3, 77.8, [73.3] 72.4, 72.9, [53.2] 52.9, [49.3] 48.8, 43.3, [24.6] 24.5; HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{22}H_{29}N_8O_6S$ 533.1925; found 533.1943.

3. Fluorescence measurement

3.1 SAH concentration measurement

A solution of **NapOx** in IPA (20 μL, 100 μM) and SAH in ddH_2O (20 μL, 0 - 2 mM, 2-fold dilution) was added to a mixture of IPA/ddH_2O (7:1, 160 μL) in 96-well plate, with a final concentration of 10 μM for **NapOx** and 0-200 μM for SAH. The mixture was incubated at 37 ºC for 30 min. Then it was directly used for fluorescence measurement.

3.2 Fluorescence profiling of NapOx reaction with thiol or thioether compound

A solution of **NapOx** in IPA (20 μL, 2 mM) and thiol or thioether compound in 1X PBS (20 μL, 8 mM) was added to a mixture of IPA/1XPBS (7:1, 160 μL) in 96-well plate, with a final concentration of 200 μM for **NapOx** and 800 μM for thiol or thioether compound. The mixture was allowed to incubate at room temperature for 3 h. Then it was directly analyzed by fluorescence measurement.

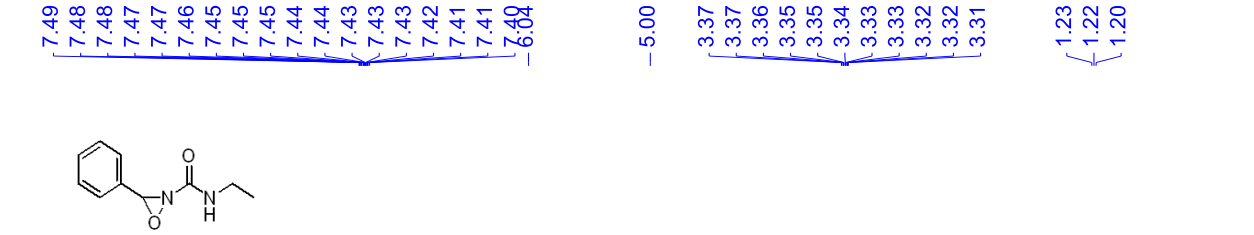
3.3 NapOx selectivity under neutral or acidic condition

For the reaction performed under neutral condition, a solution of **NapOx** in IPA (20 μL, 100 μM) and analyte in ddH_2O (20 μL, 2 mM) was incubated in a mixture of IPA/ddH_2O (7:1, 160 μL) in 96-well plate at 37 ºC for 30 min, then it was directly used for fluorescence measurement. For the reaction under acidic condition, the procedure was the same except that 1 mM HCl aqueous solution was used to replace ddH_2O.

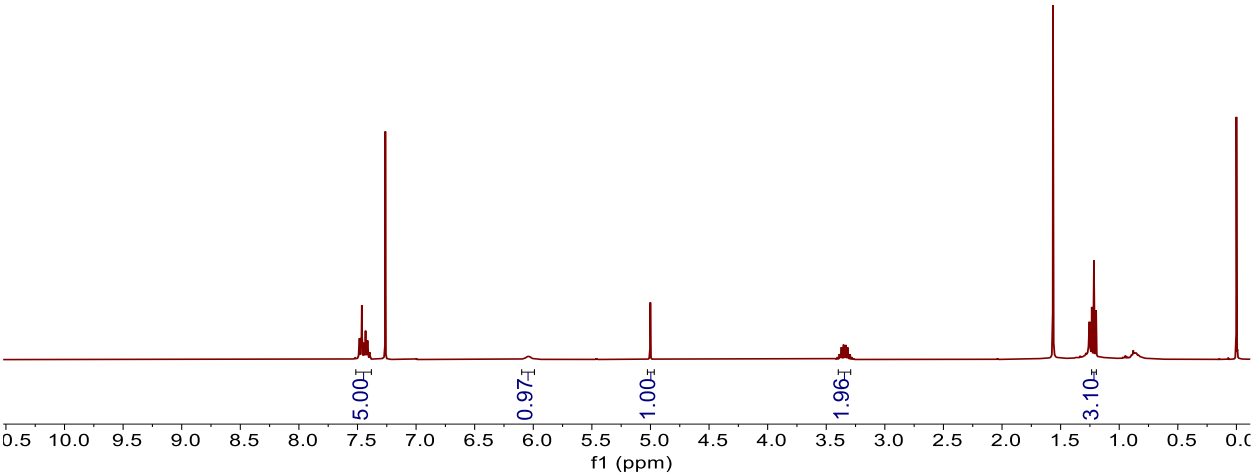
4. HPLC experiment

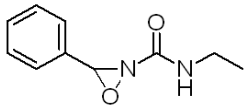
Compound **1a** (2.0 equiv) was mixed with SAH (1.0 equiv) in a mixture of H_2O/CH_3OH (1:1), and the mixture was allowed to incubate at room temperature for 10 min. Then, 100 μL of reaction mixture was taken, and analyzed by HPLC.

5. NMR spectra of compounds

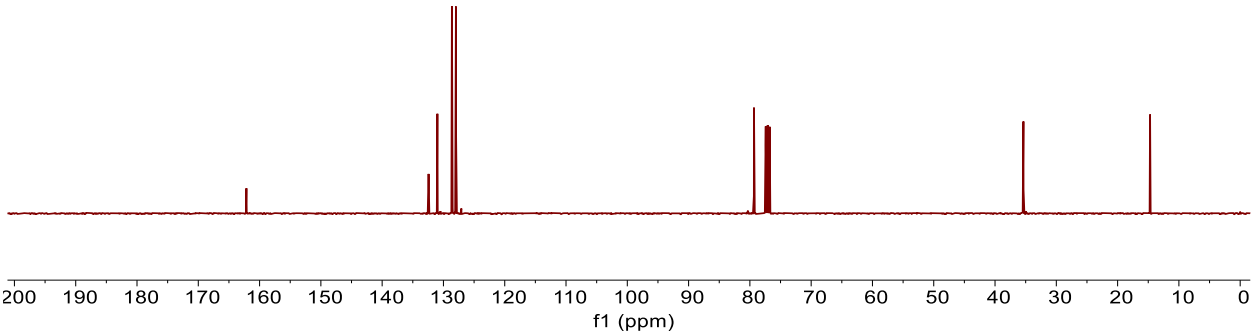


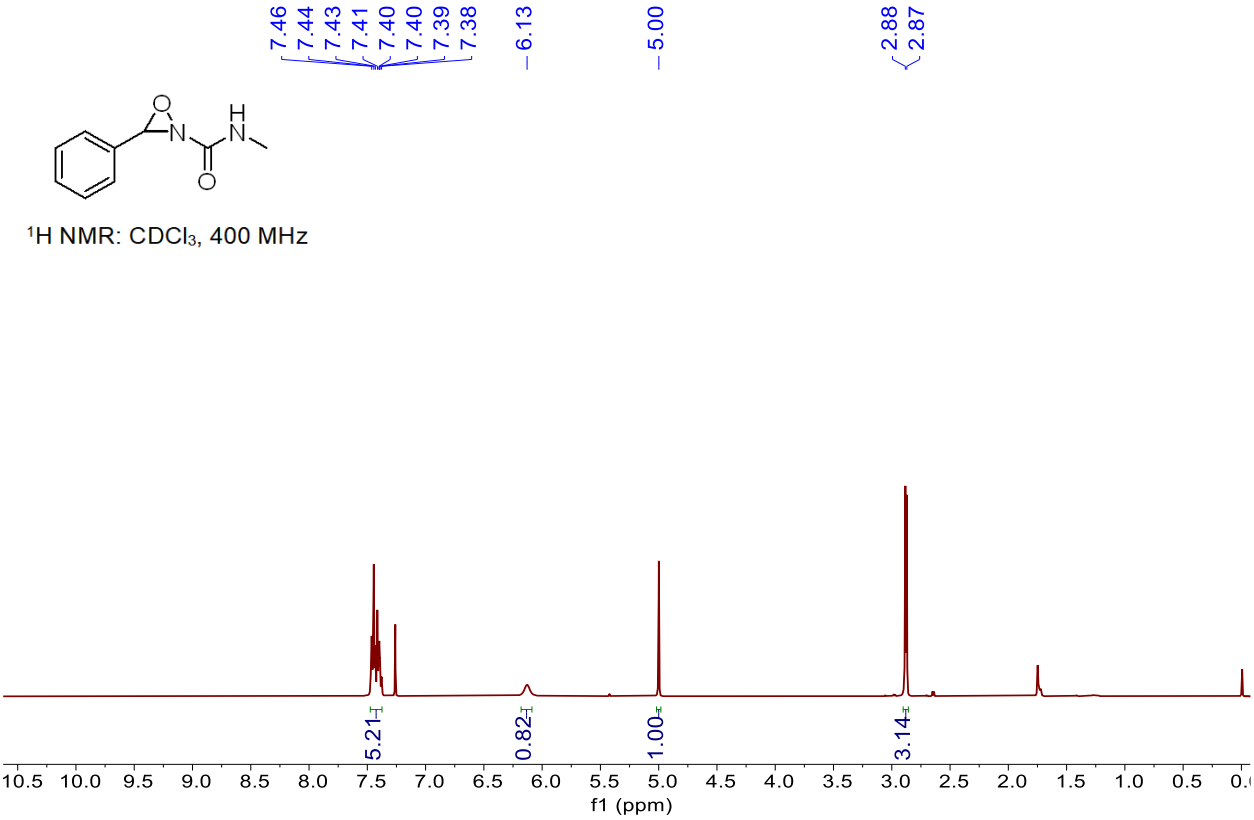
^{1}H NMR: $CDCl_3$, 400 MHz



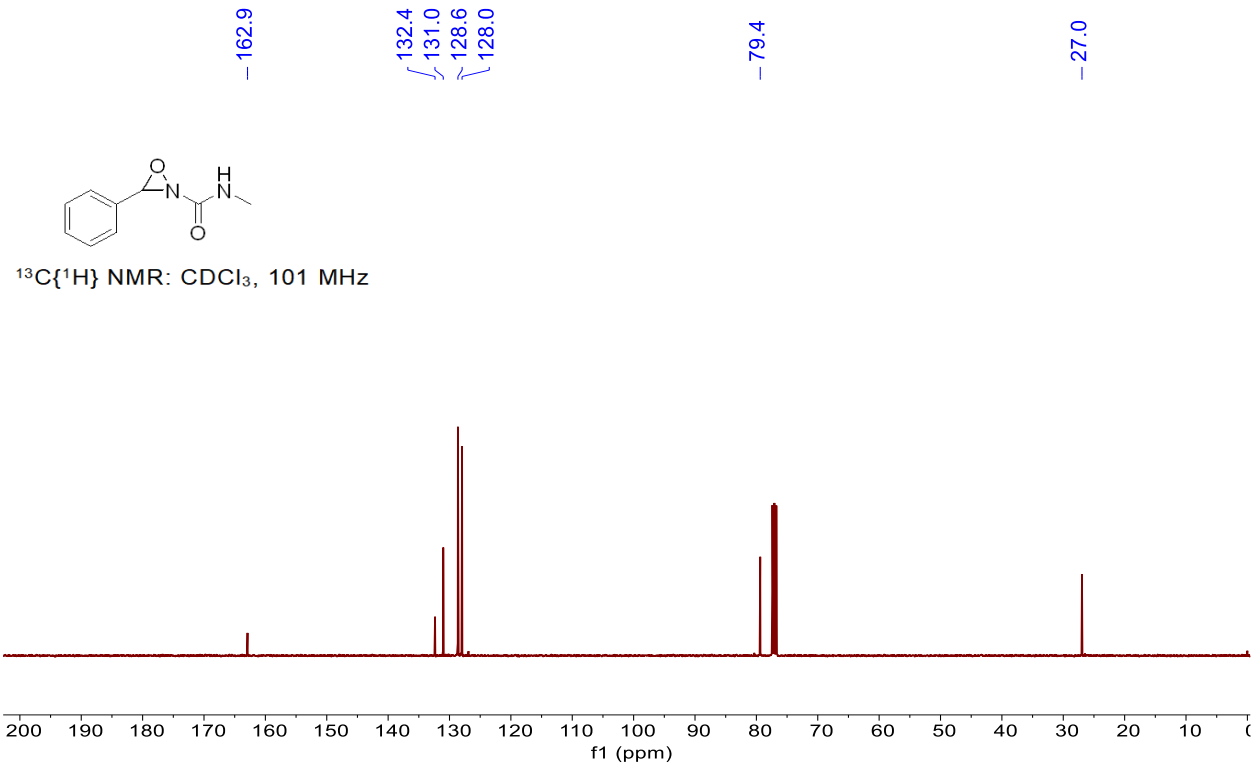


^{13}C{^{1}H} NMR: $CDCl_3$, 101 MHz

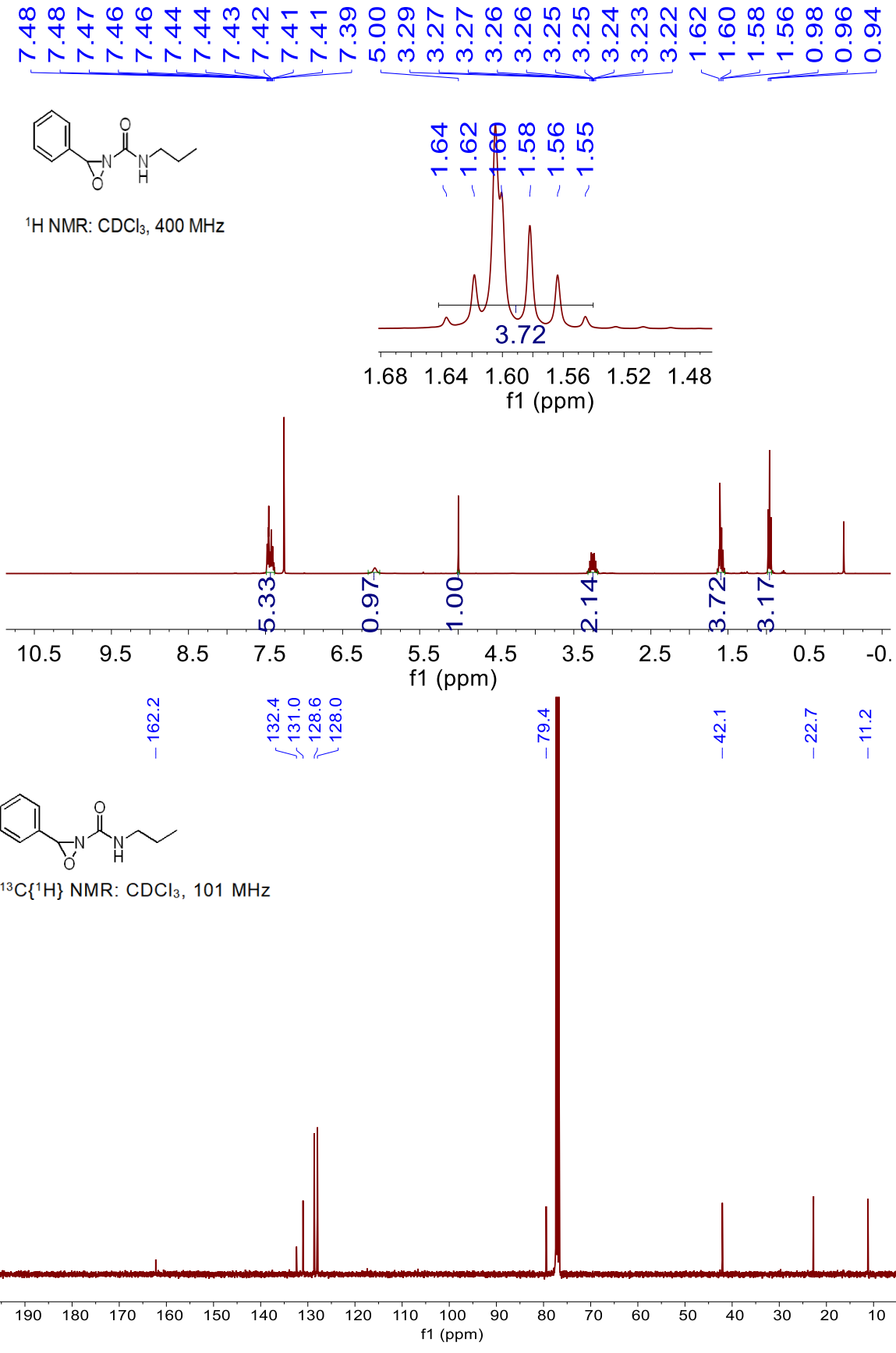


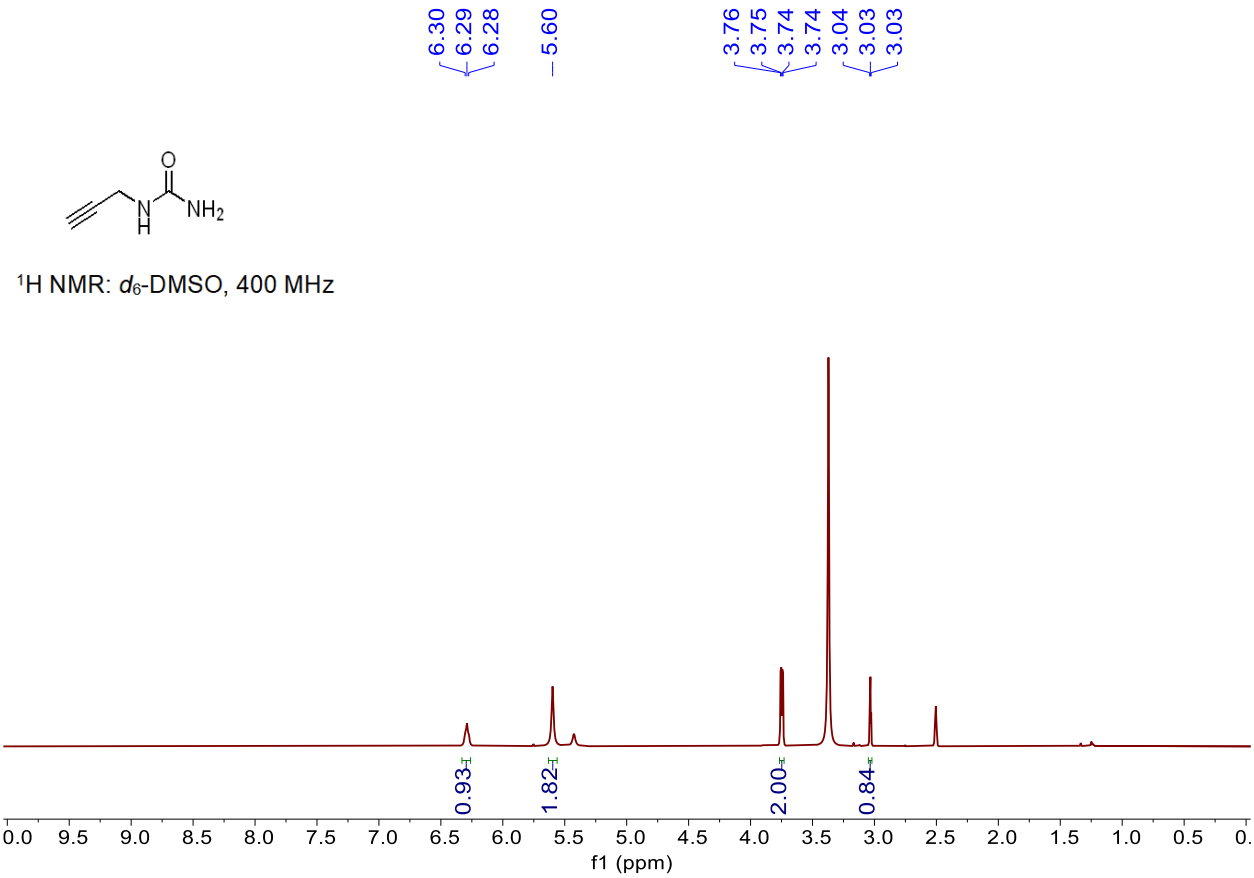


^{1}H NMR: $CDCl_3$, 400 MHz

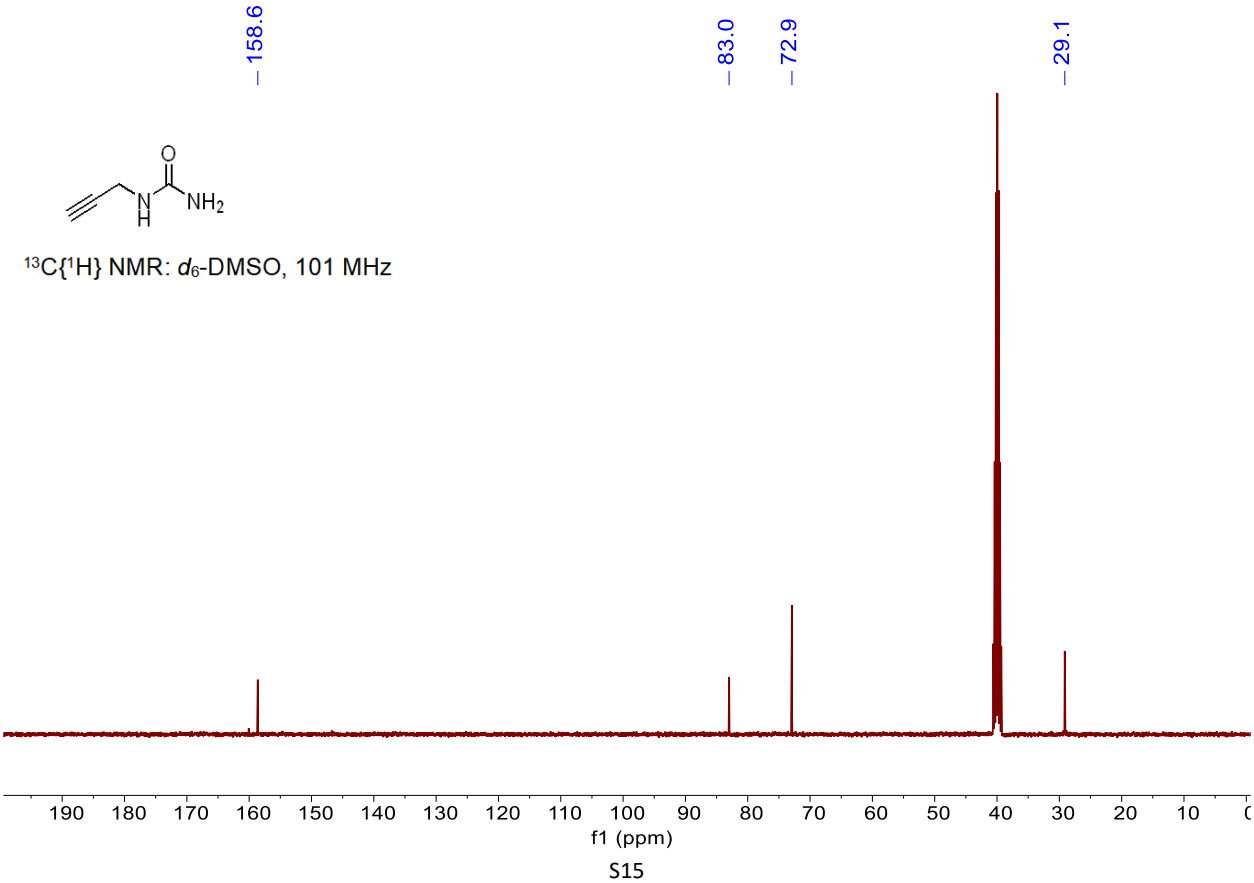


$^{13}C\{^{1}H\}$ NMR: $CDCl_3$, 101 MHz

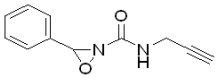

7.48 7.48 7.47 7.46 7.46 7.44 7.44 7.43 7.42 7.41 7.41 7.39 5.00 3.29 3.27 3.27 3.26 3.26 3.25 3.25 3.24 3.23 3.22 1.62 1.60 1.58 1.56 0.98 0.96 0.94
1H NMR: CDCl3, 400 MHz
1.64 1.62 1.60 1.58 1.56 1.55
3.72
1.68 1.64 1.60 1.56 1.52 1.48
f1 (ppm)
5.33 0.97 1.00 2.14 3.72 3.17
10.5 9.5 8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5 -0.
f1 (ppm)
162.2 132.4 131.0 128.6 128.0 79.4 42.1 22.7 11.2
13C{1H} NMR: CDCl3, 101 MHz
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



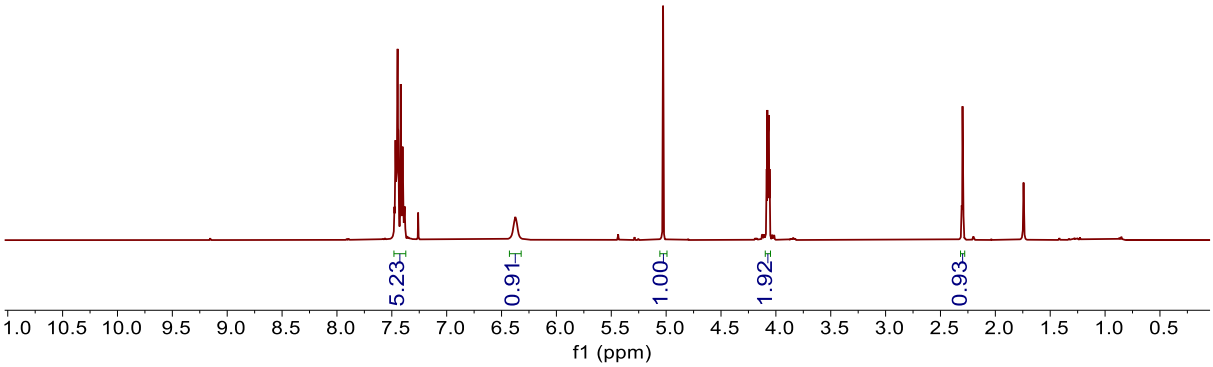
^{1}H NMR: d_6-DMSO, 400 MHz

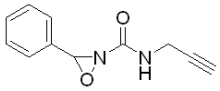


^{13}C{^{1}H} NMR: d_6-DMSO, 101 MHz

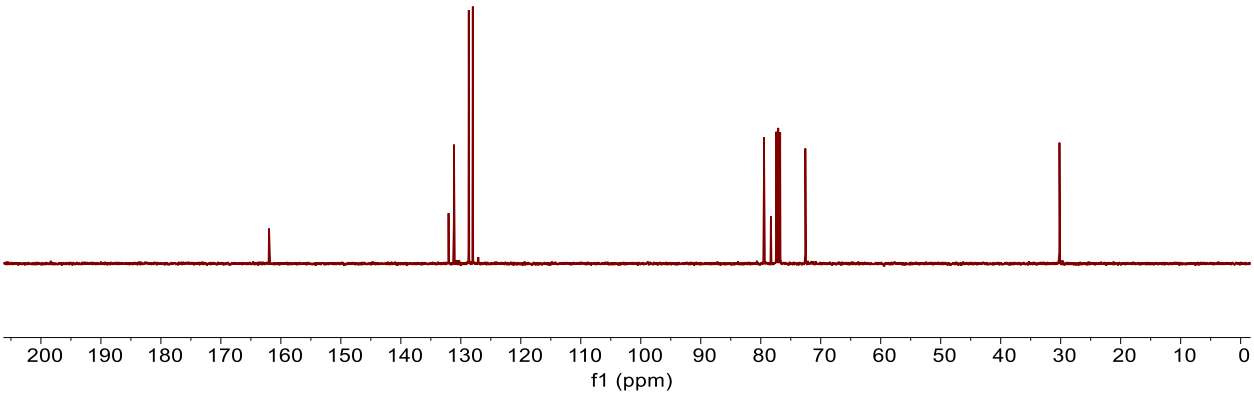


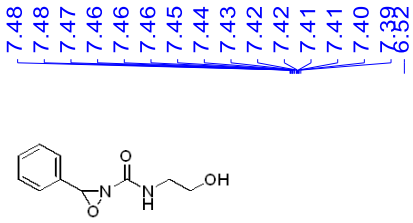
^{1}H NMR: $CDCl_3$, 400 MHz



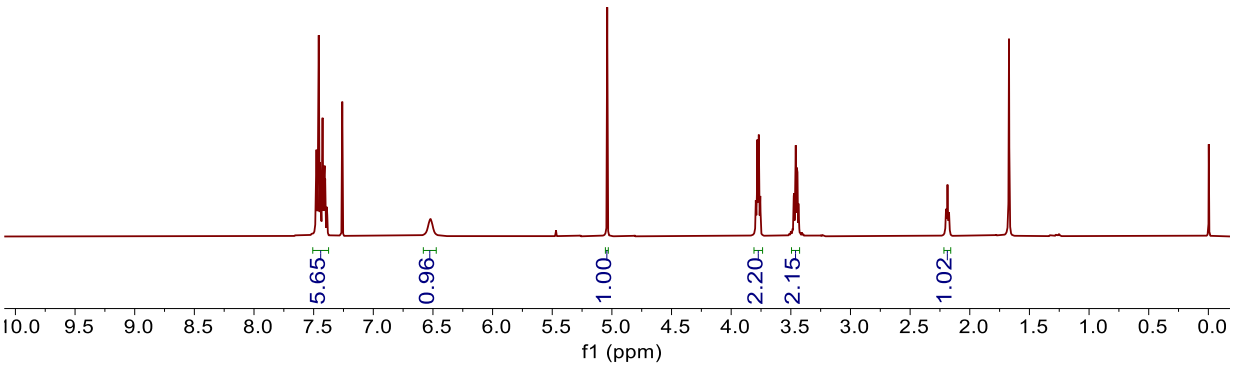


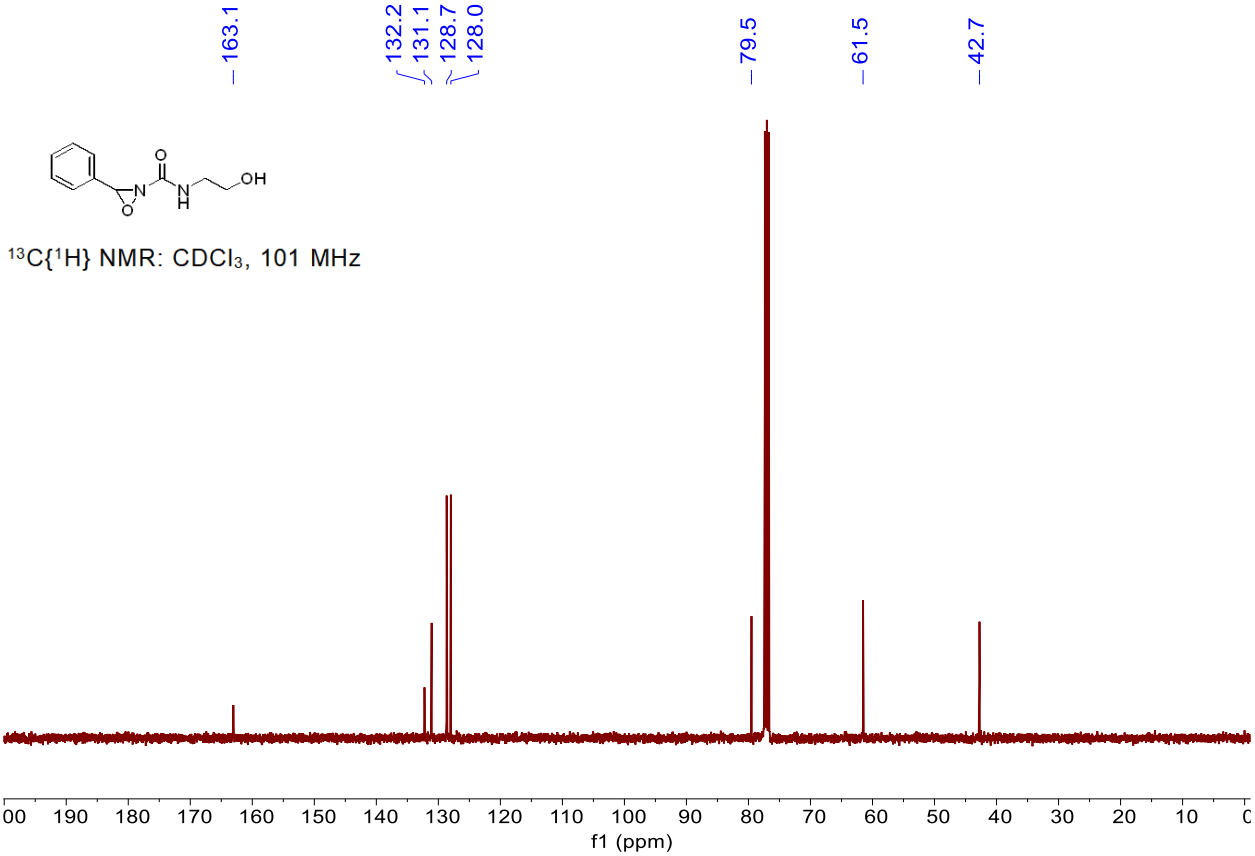
^{13}C{^{1}H} NMR: $CDCl_3$, 101 MHz



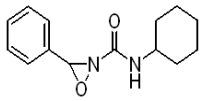


^{1}H NMR: $CDCl_3$, 400 MHz





^{13}C{^{1}H} NMR: $CDCl_3$, 101 MHz



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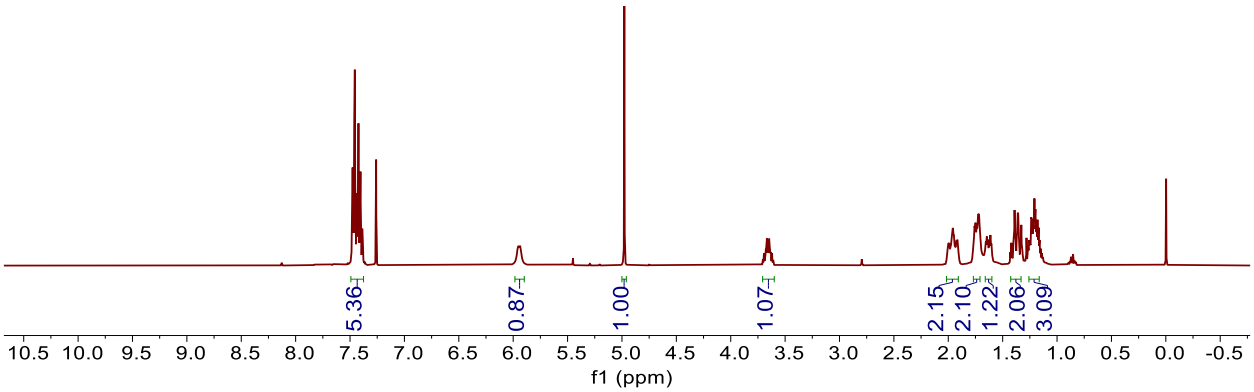
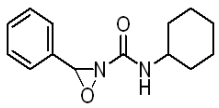
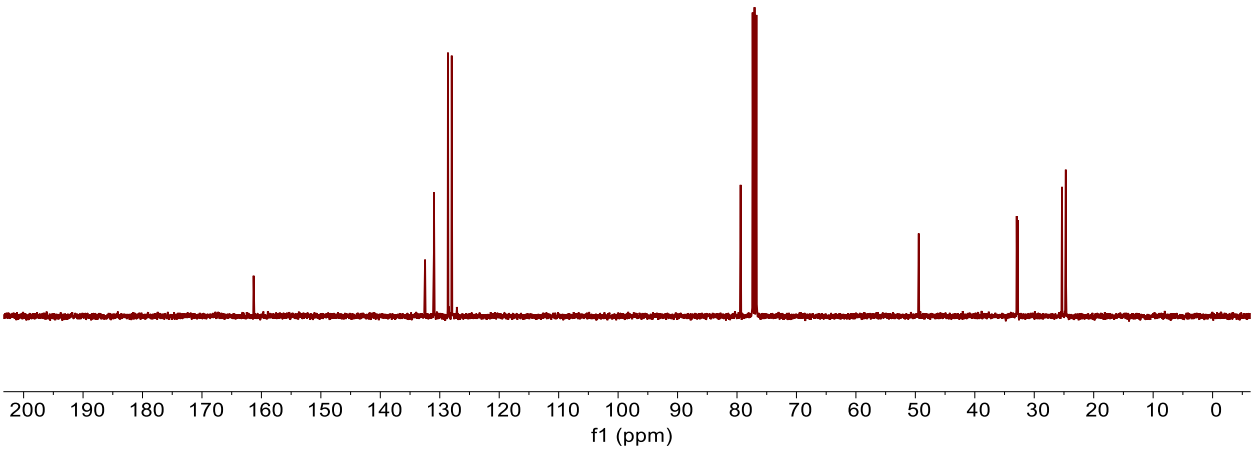
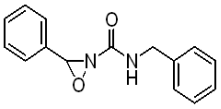


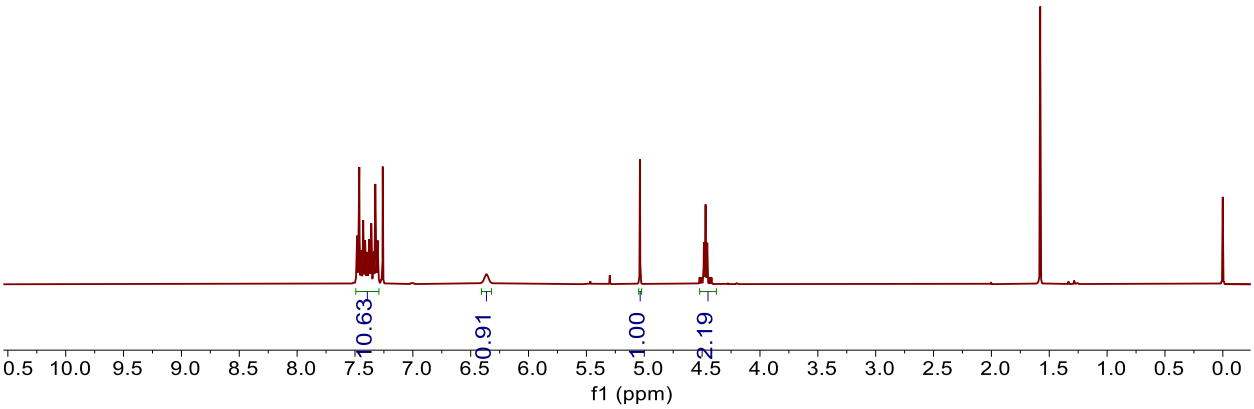


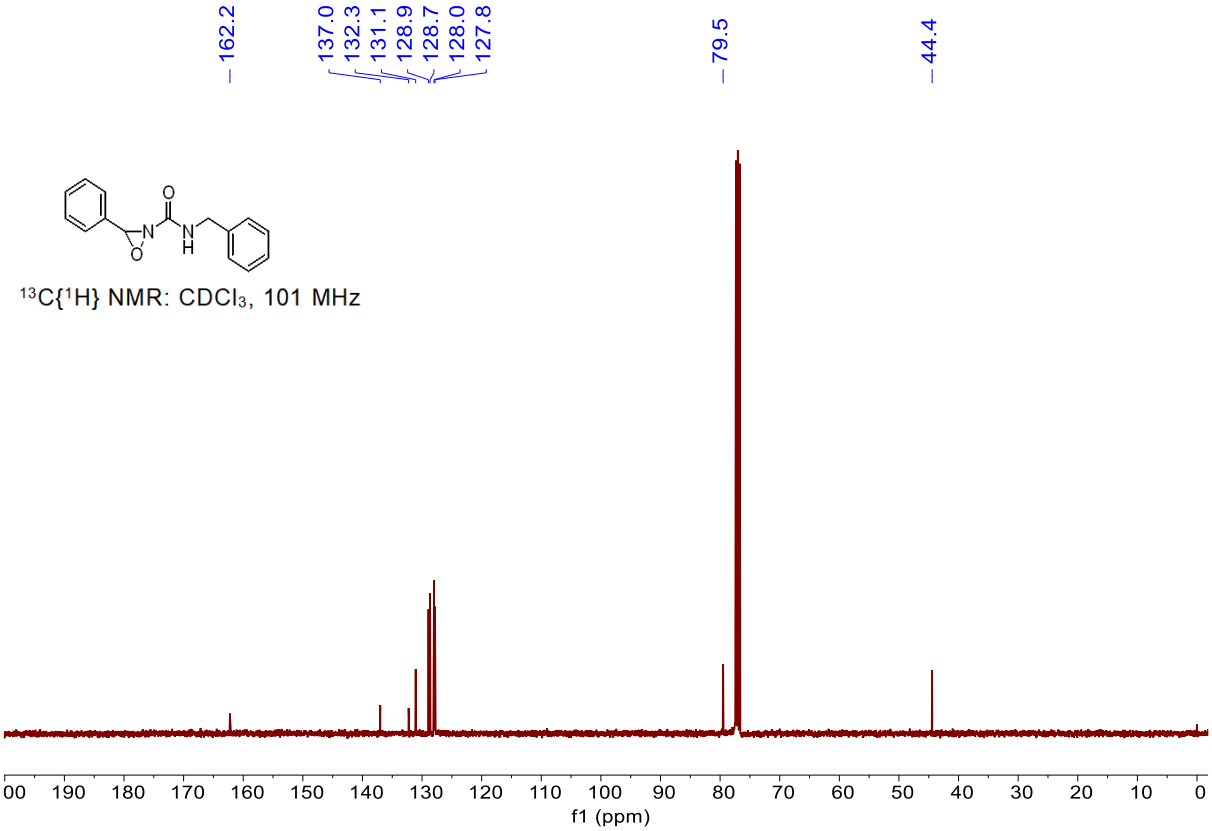
^{13}C{^{1}H} NMR: $CDCl_3$, 101 MHz



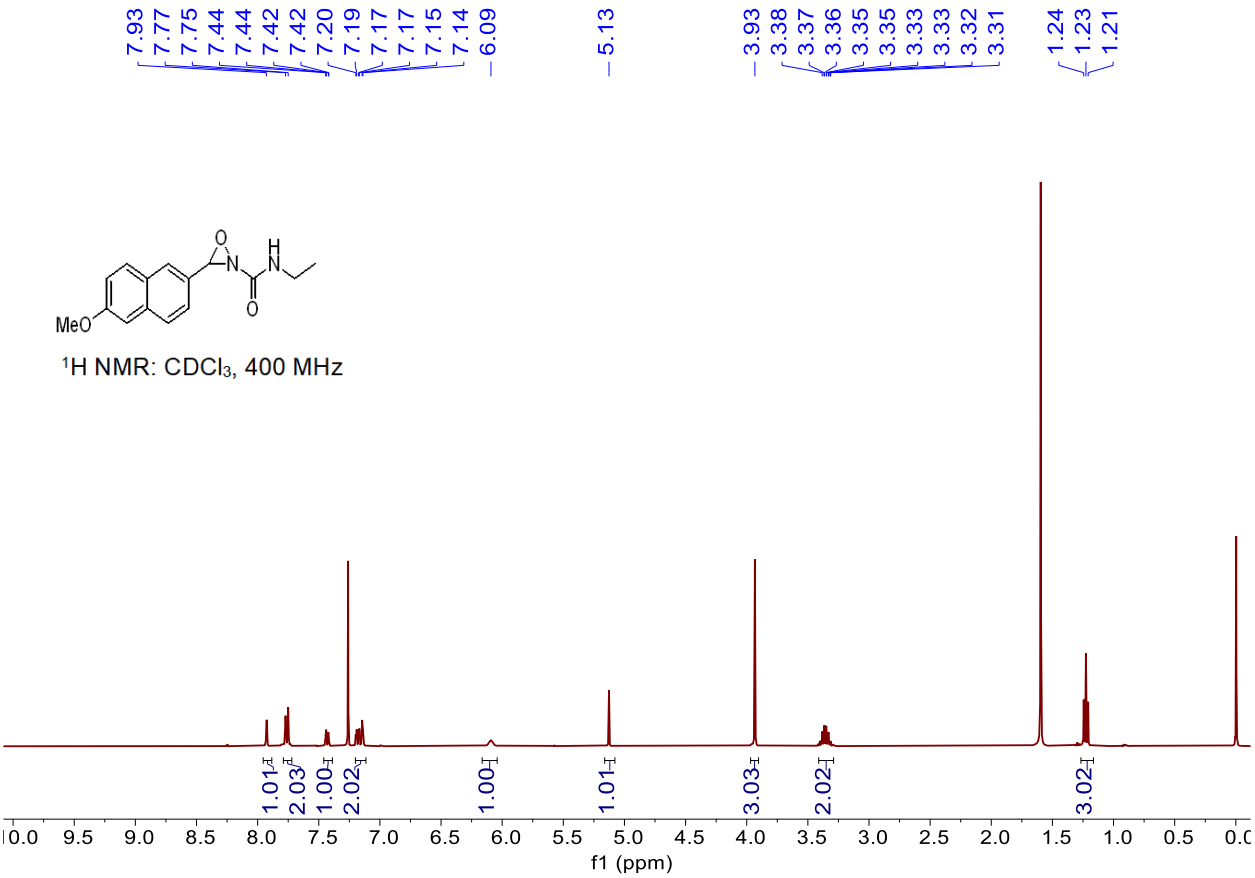


^{1}H NMR: $CDCl_3$, 400 MHz

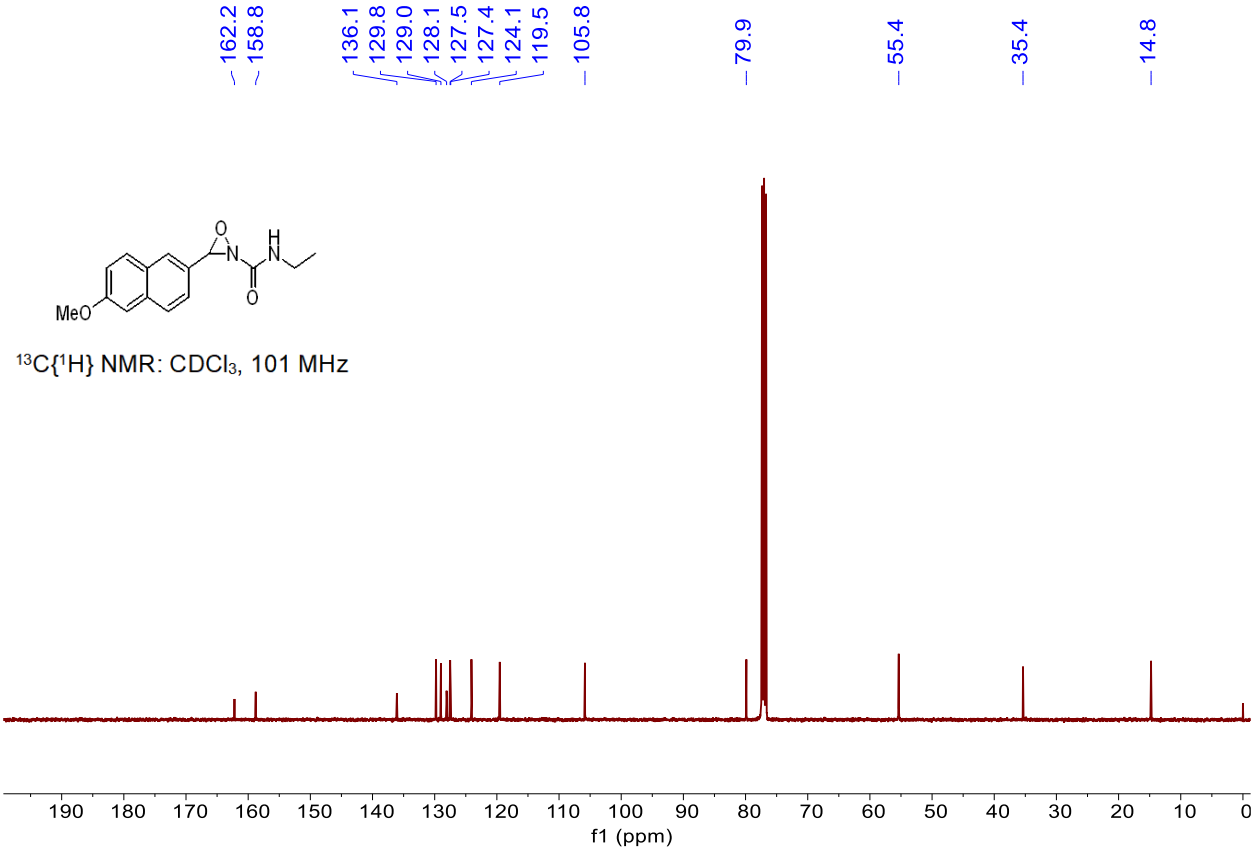




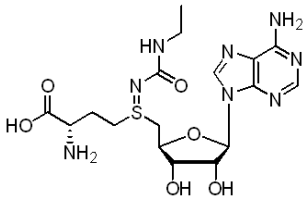
^{13}C{^{1}H} NMR: $CDCl_3$, 101 MHz



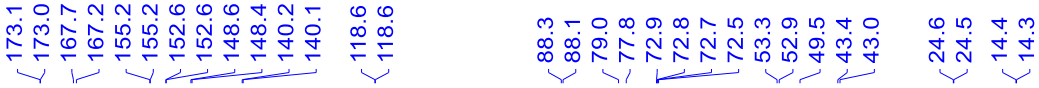
^{1}H NMR: $CDCl_3$, 400 MHz

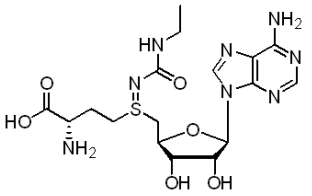


^{13}C{^{1}H} NMR: $CDCl_3$, 101 MHz



^{1}H NMR: D_2O, 400 MHz





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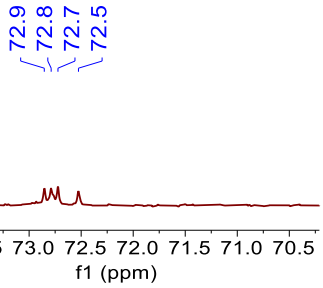
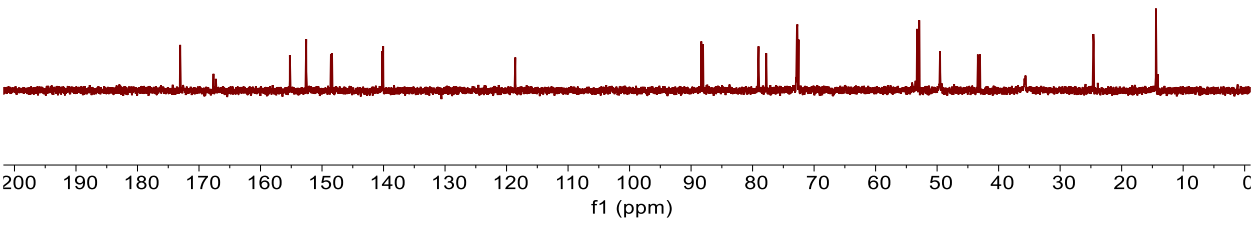
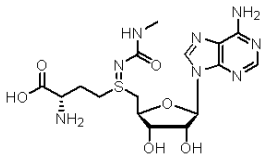
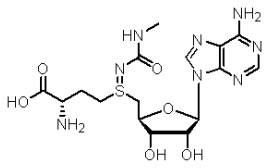


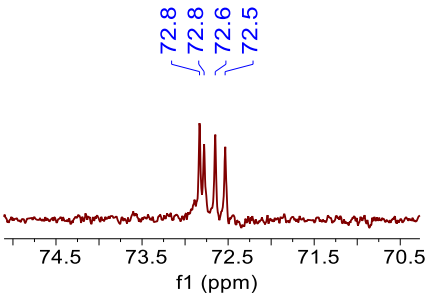


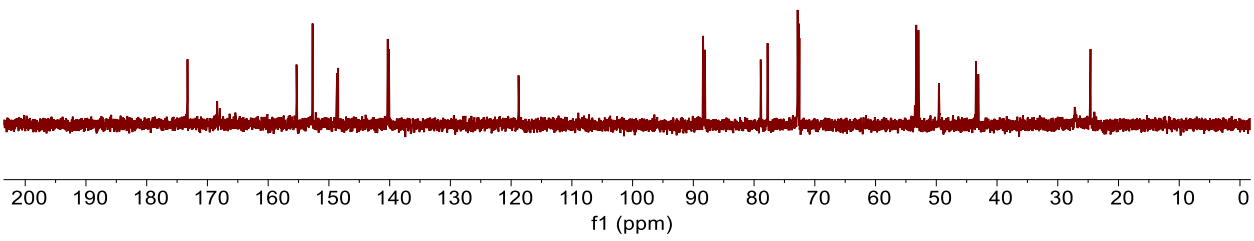


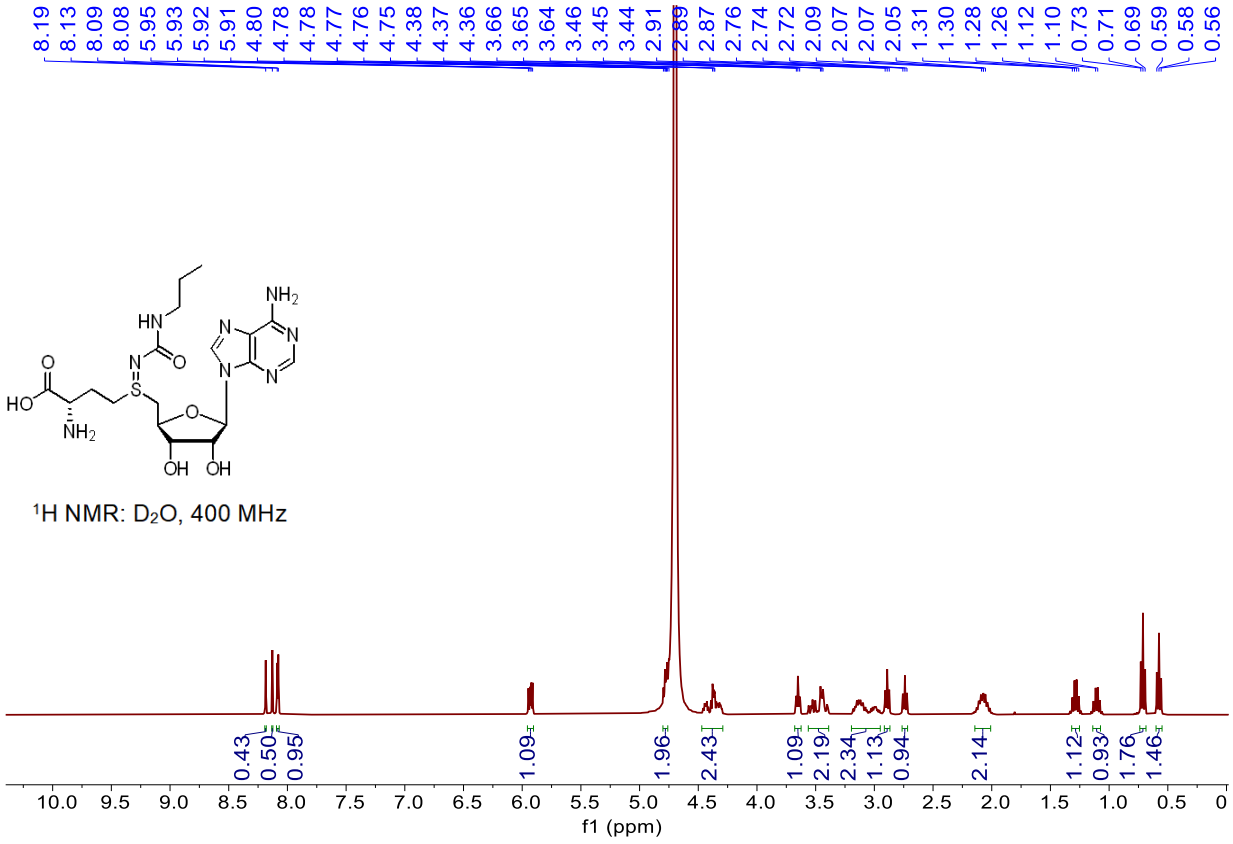
^{1}H NMR: D_2O, 400 MHz



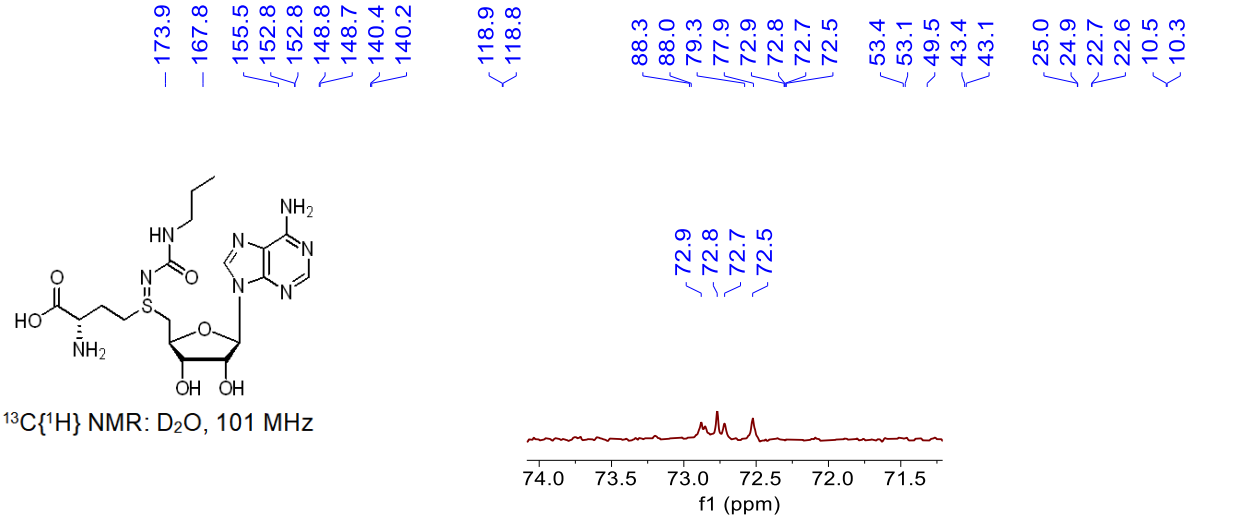
^{13}C{^{1}H} NMR: D_2O, 101 MHz



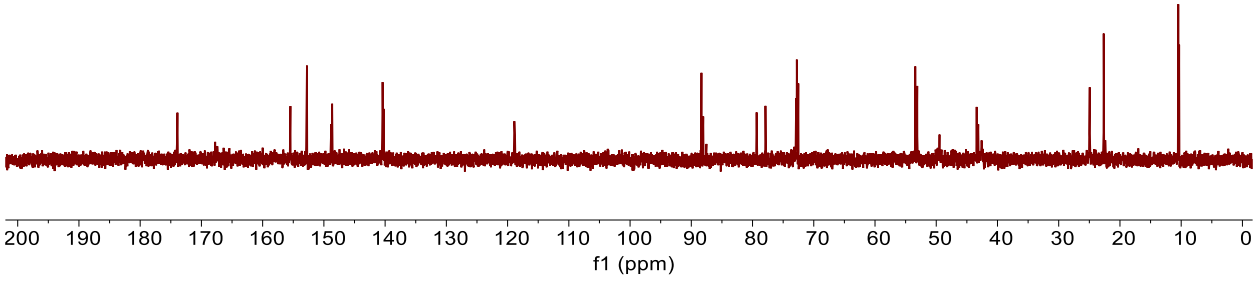


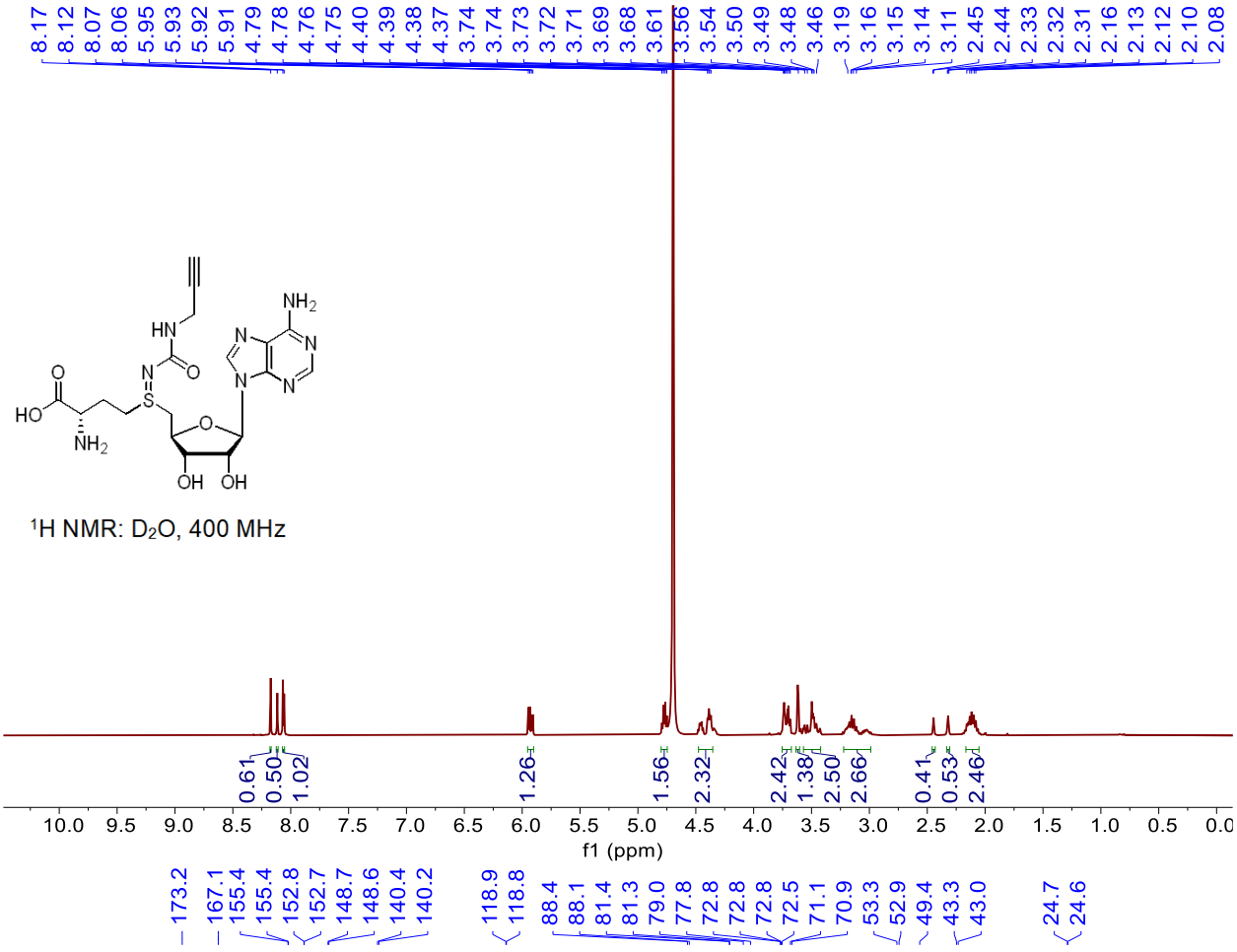


^{1}H NMR: D_2O, 400 MHz

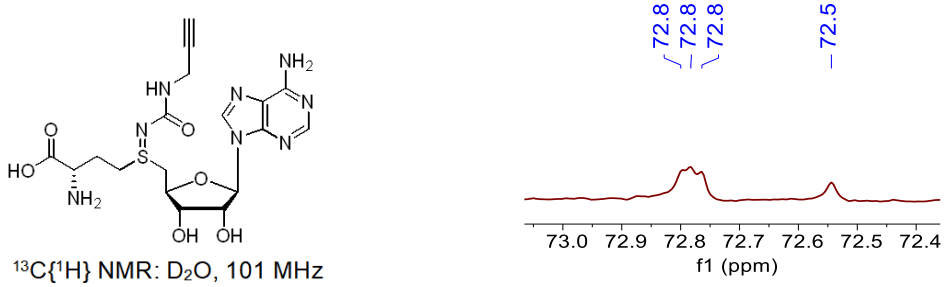


^{13}C{^{1}H} NMR: D_2O, 101 MHz

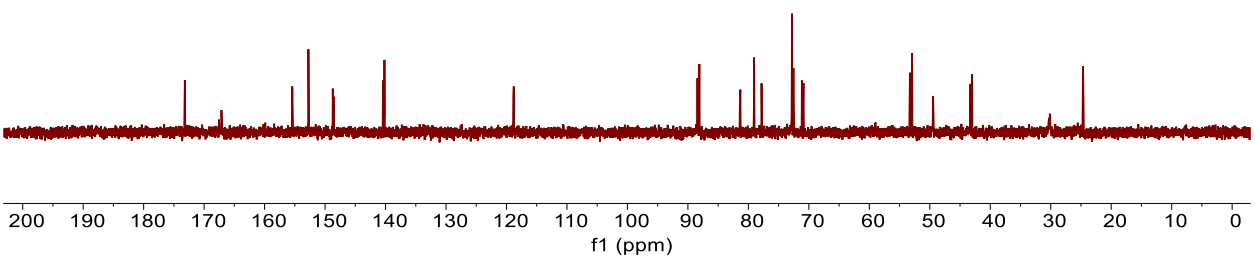


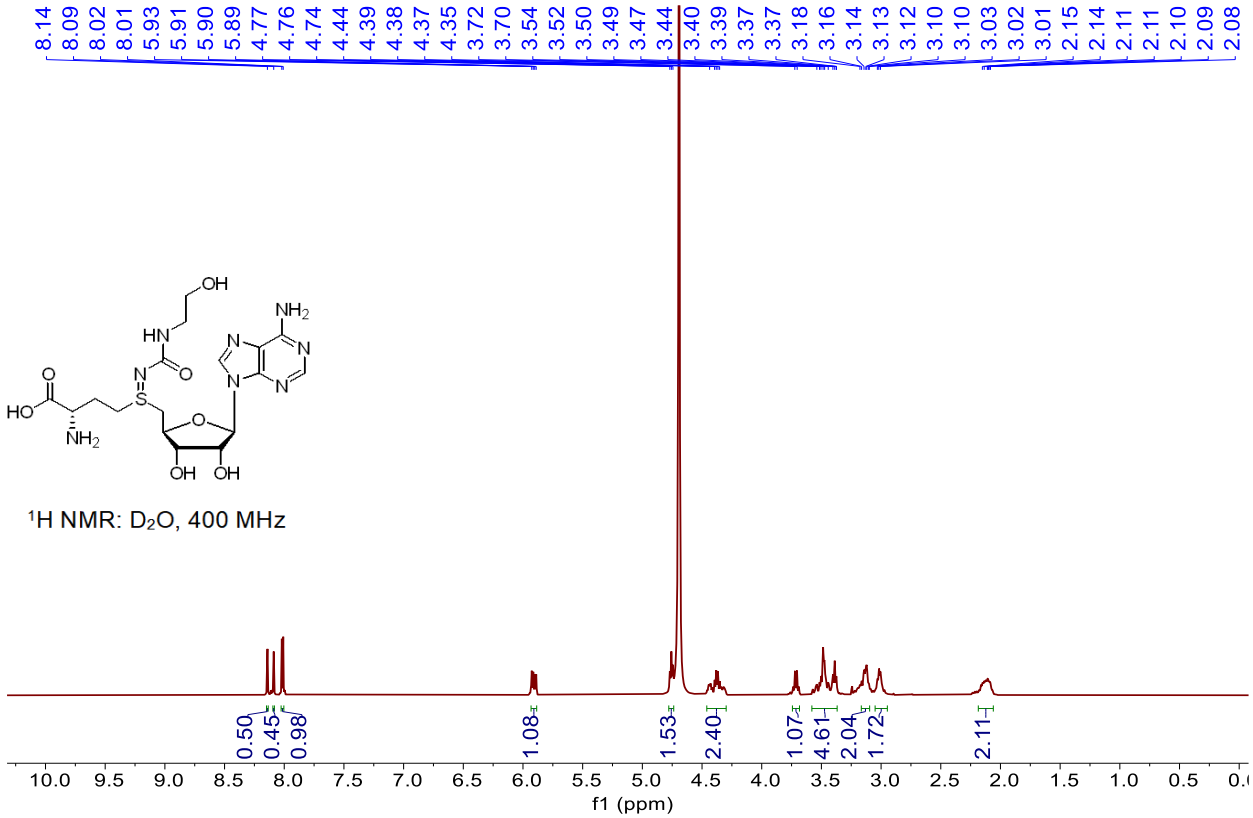


^{1}H NMR: D_2O, 400 MHz

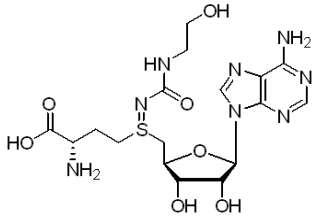


^{13}C{^{1}H} NMR: D_2O, 101 MHz

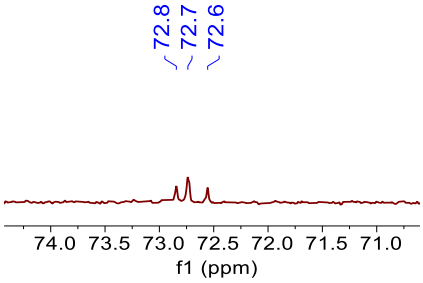


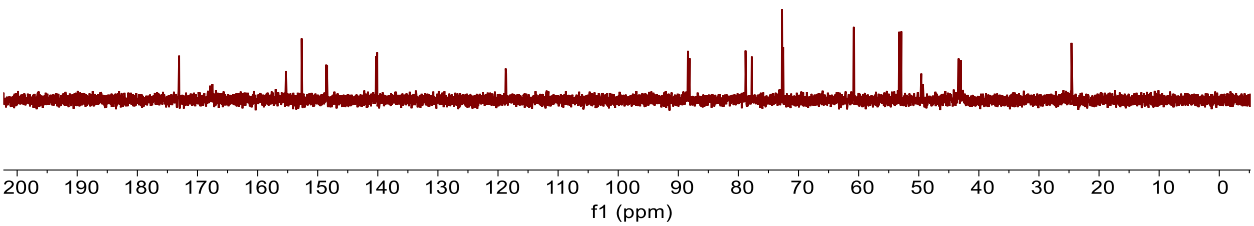


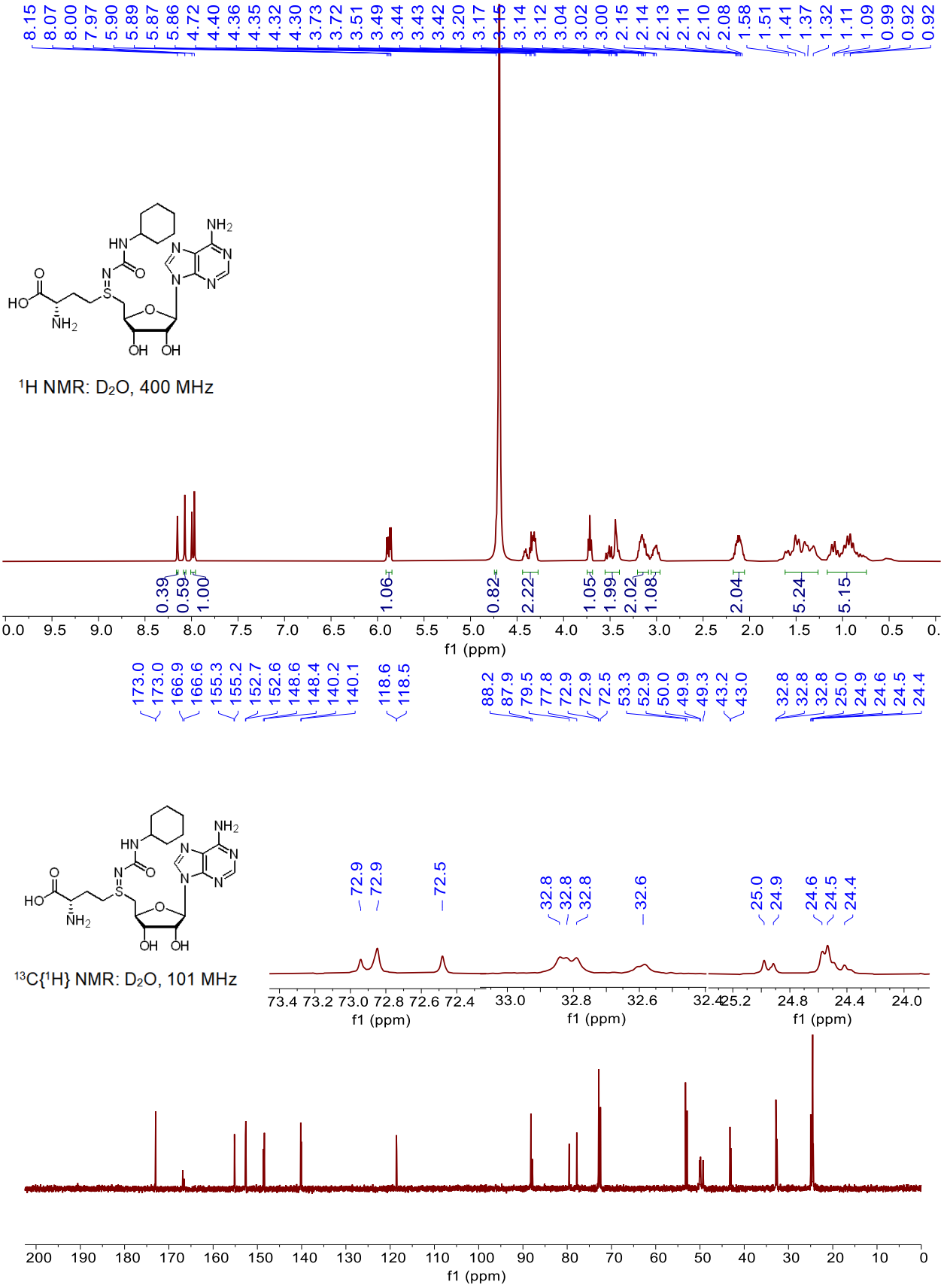
^{1}H NMR: D_2O, 400 MHz

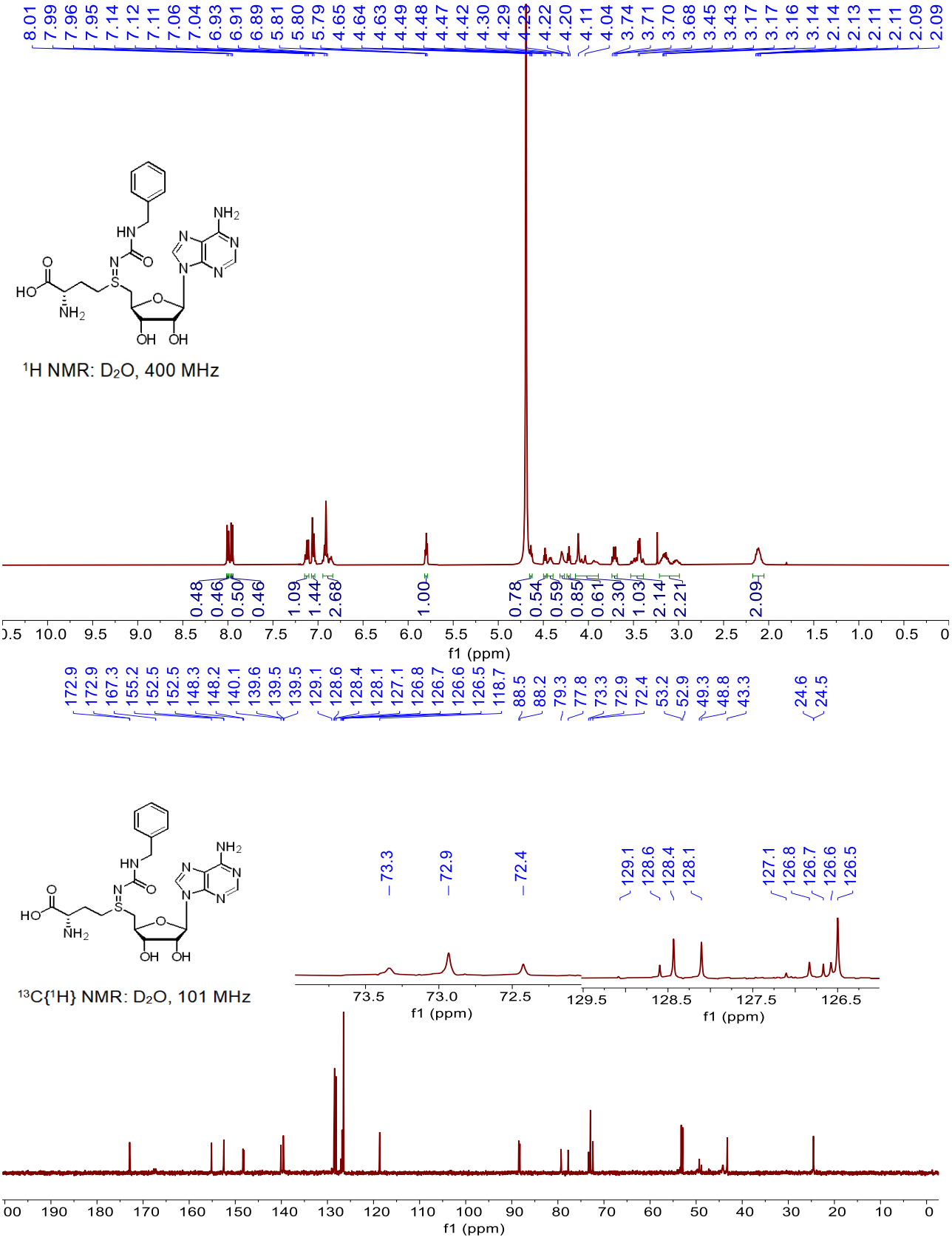


^{13}C{^{1}H} NMR: D_2O, 101 MHz

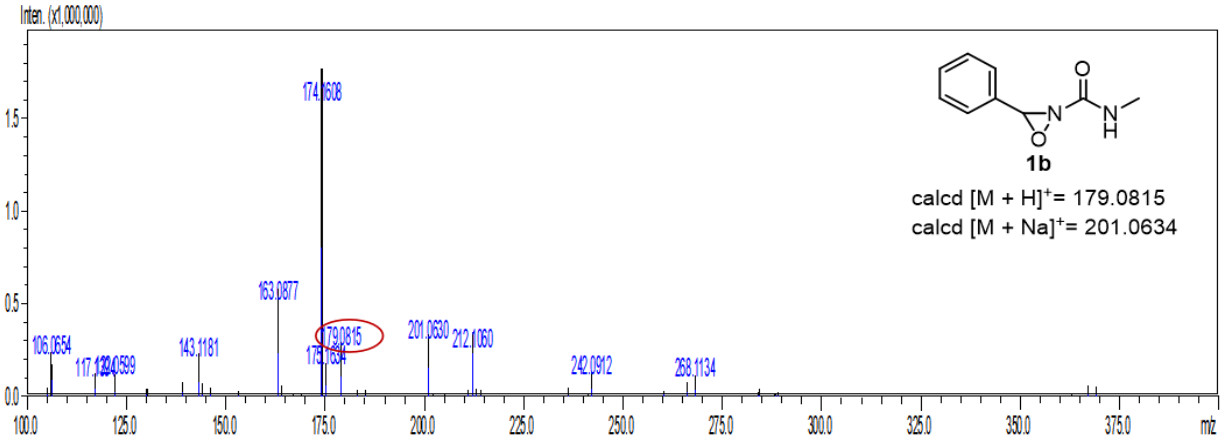


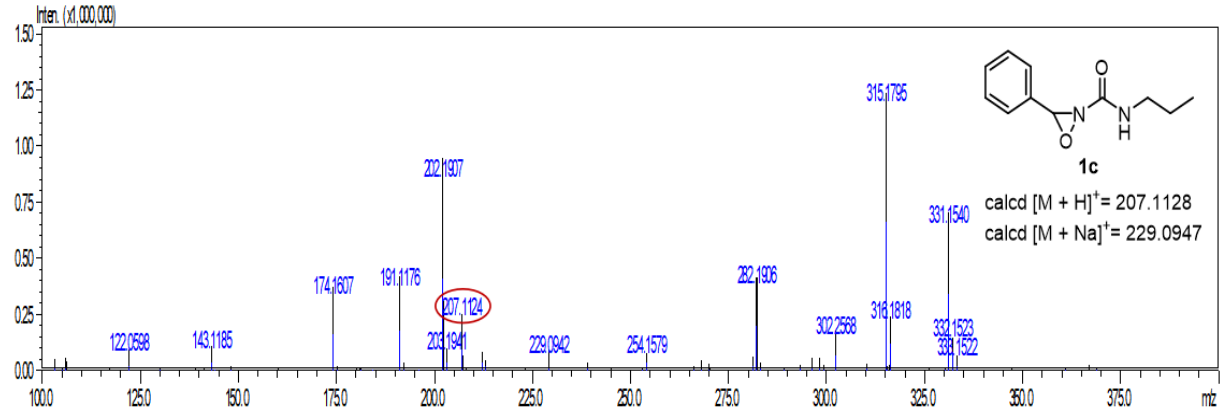


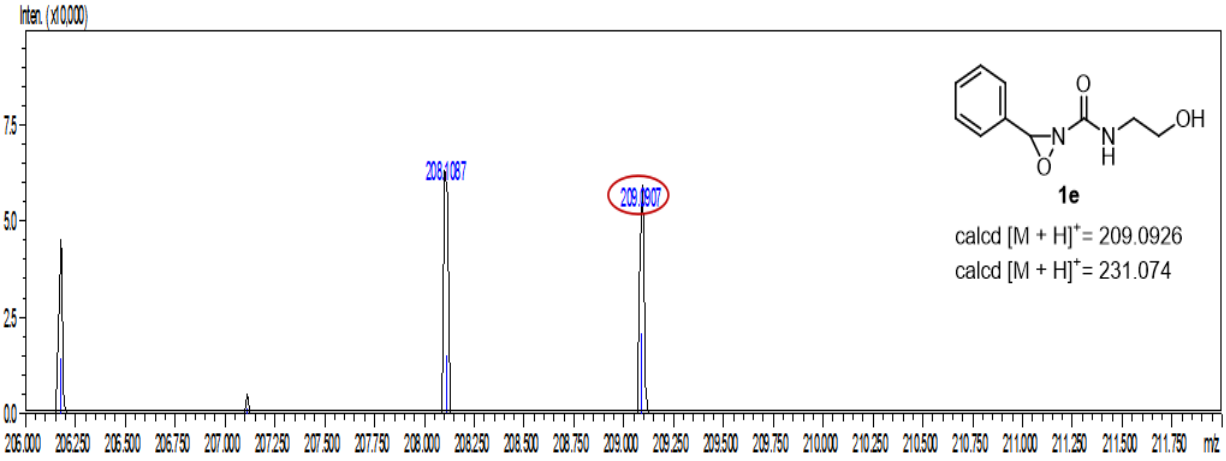

8.15 8.07 8.00 7.97 5.90 5.89 5.87 5.86 4.72 4.40 4.36 4.35 4.32 4.30 3.73 3.72 3.51 3.49 3.44 3.43 3.42 3.20 3.17 3.15 3.14 3.12 3.04 3.02 3.00 2.15 2.14 2.13 2.11 2.10 2.08 1.58 1.51 1.41 1.37 1.32 1.11 1.09 0.99 0.92 0.92
NH2
HN
N
O
HO
S
NH2
OH OH
1H NMR: D2O, 400 MHz
0.39 0.59 1.00 1.06 0.82 2.22 1.05 1.99 2.02 1.08 2.04 5.24 5.15
f1 (ppm)
173.0 173.0 166.9 166.6 155.3 155.2 152.7 152.6 148.6 148.4 140.2 140.1 118.6 118.5
88.2 87.9 79.5 77.8 72.9 72.9 72.5 53.3 52.9 50.0 49.9 49.3 43.2 43.0
32.8 32.8 32.8 25.0 24.9 24.6 24.5 24.4
13C{1H} NMR: D2O, 101 MHz
72.9 72.9 72.5 32.8 32.8 32.8 32.6 25.0 24.9 24.6 24.5 24.4
f1 (ppm)

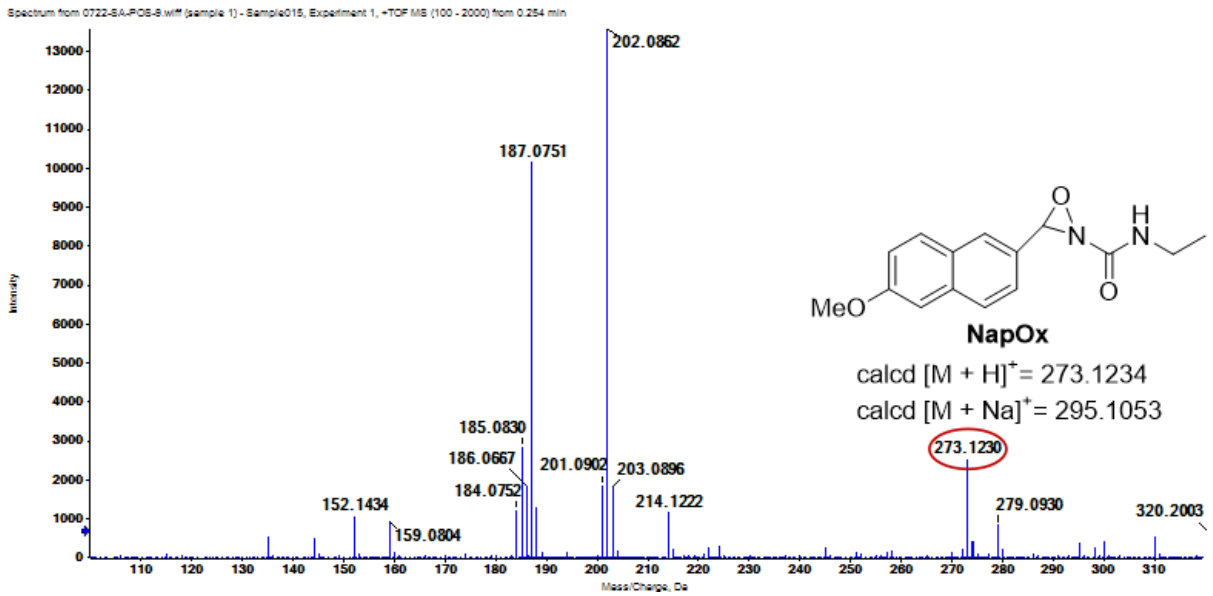

8.01 7.99 7.96 7.95 7.14 7.12 7.11 7.06 7.04 6.93 6.91 6.89 5.81 5.80 5.79 4.65 4.64 4.63 4.49 4.48 4.47 4.42 4.30 4.29 4.23 4.22 4.20 4.11 4.04 3.74 3.71 3.70 3.68 3.45 3.43 3.17 3.17 3.16 3.14 2.14 2.13 2.11 2.11 2.09 2.09
HO
NH2
HN
O
N
S
O
OH OH
NH2
N
N
N
N
1H NMR: D2O, 400 MHz
0.48 0.46 0.50 0.46 1.09 1.44 2.68 1.00 0.78 0.54 0.59 0.85 0.61 2.30 1.03 2.14 2.21 2.09
f1 (ppm)
172.9 172.9 167.3 155.2 152.5 152.5 148.3 148.2 140.1 139.6 139.5 139.5 129.1 128.6 128.4 128.1 127.1 126.8 126.7 126.6 126.5 118.7 88.5 88.2 79.3 77.8 73.3 72.9 72.4 53.2 52.9 49.3 48.8 43.3 24.6 24.5
13C{1H} NMR: D2O, 101 MHz
73.3 72.9 72.4 129.1 128.6 128.4 128.1 127.1 126.8 126.7 126.6 126.5
f1 (ppm)

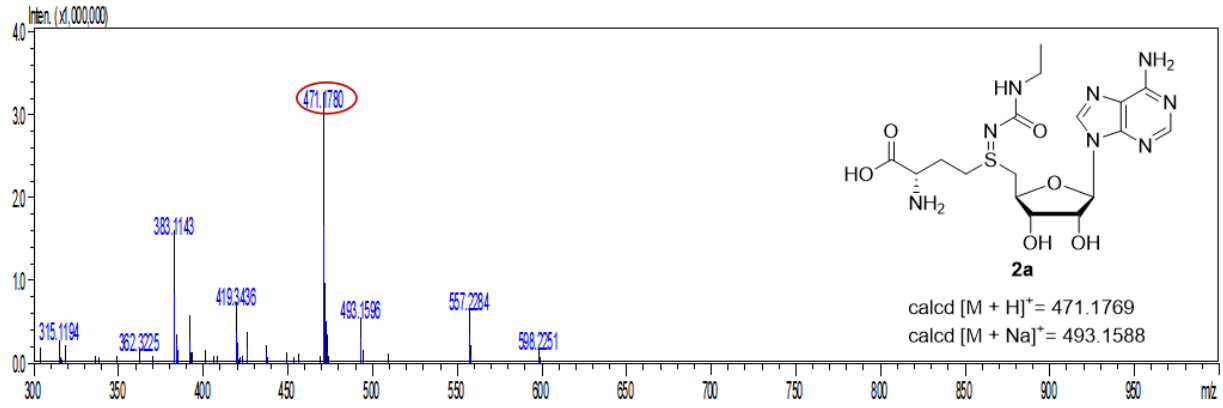
6. HRMS spectra of compounds

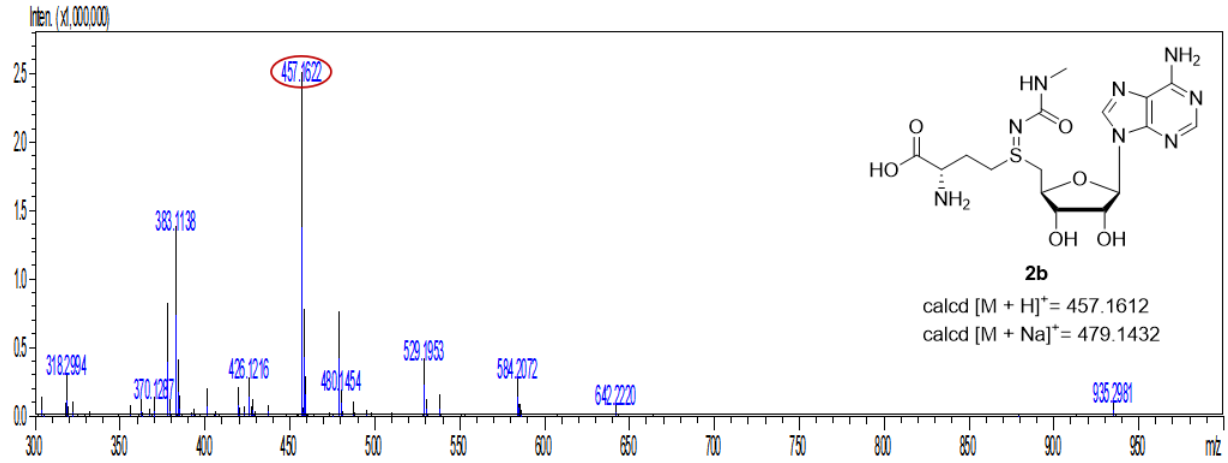


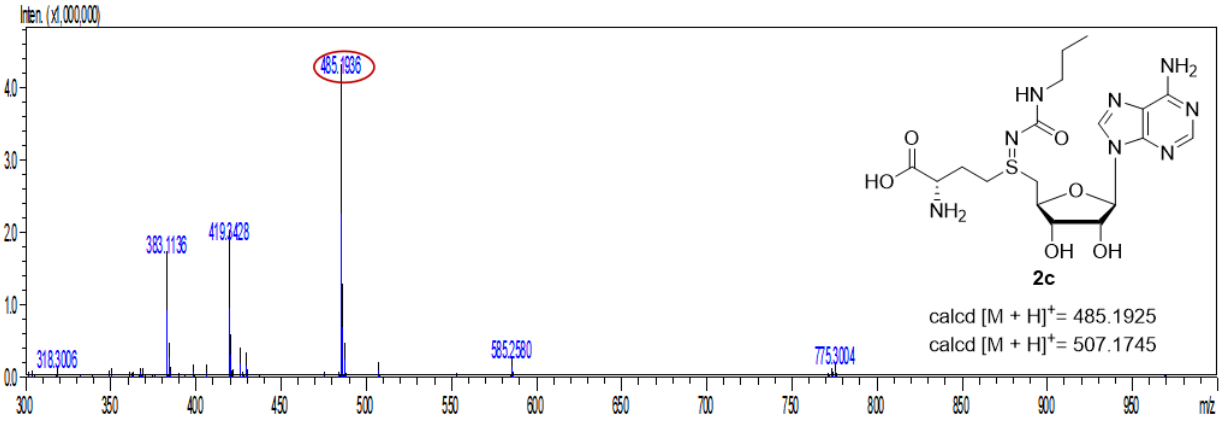


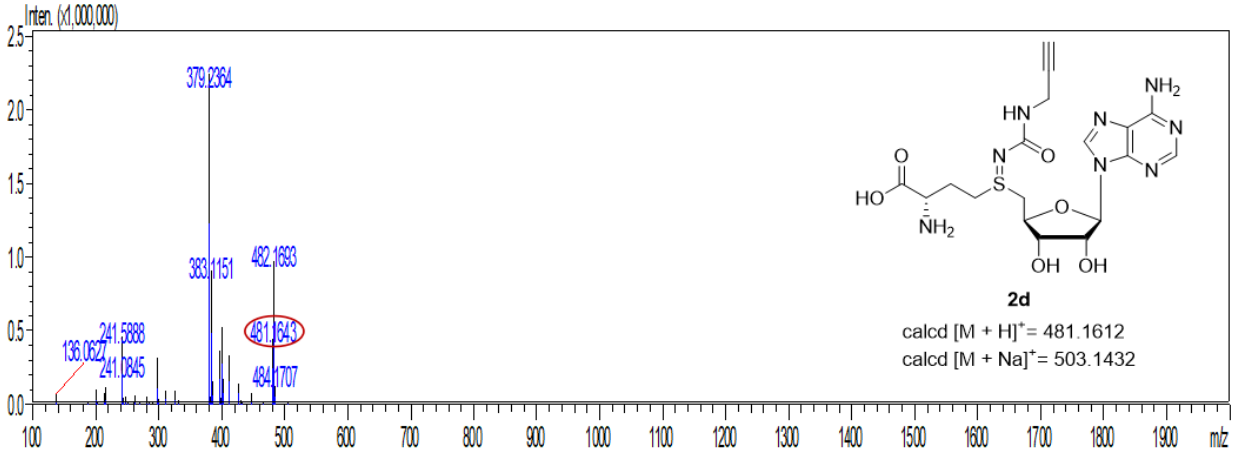


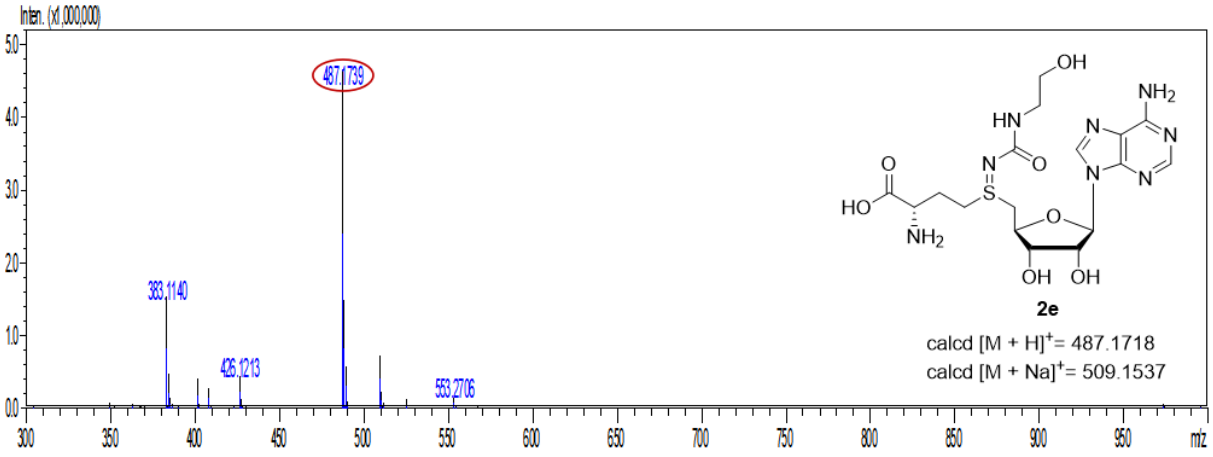

Spectrum from 0722-SA-POS-9.wiff (sample 1) - Sample015, Experiment 1, +TOF MS (100 - 2000) from 0.254 min
Intensity
Mass/Charge, Da
202.0862
187.0751
185.0830
186.0667
184.0752
201.0902
203.0896
214.1222
152.1434
159.0804
273.1230
279.0930
320.2003
MeO
NapOx
calcd [M + H]+= 273.1234
calcd [M + Na]+= 295.1053

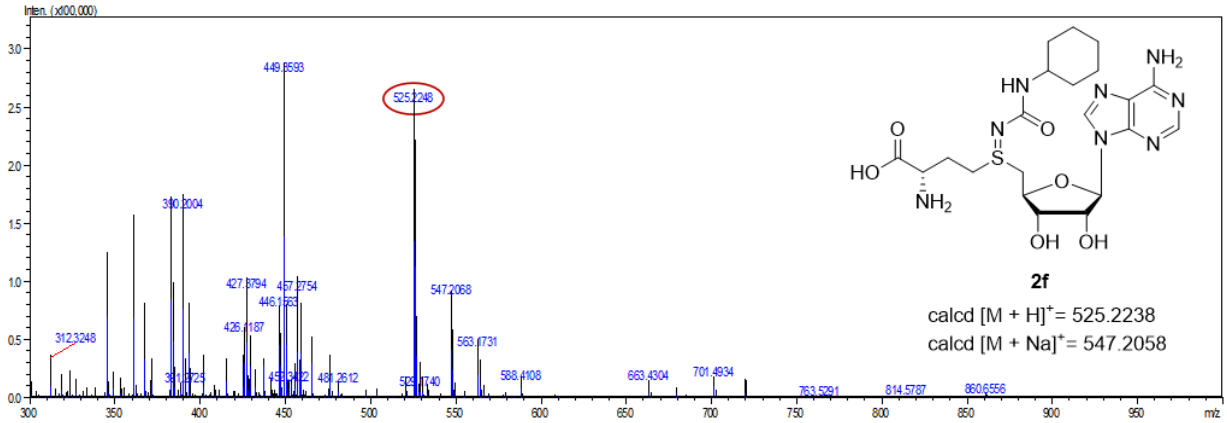

Inten. (x1,000,000)
471.1780
383.1143
419.3436
493.1596
557.2284
315.1194
362.2225
598.2251
m/z
2a
calcd [M + H]+= 471.1769
calcd [M + Na]+= 493.1588

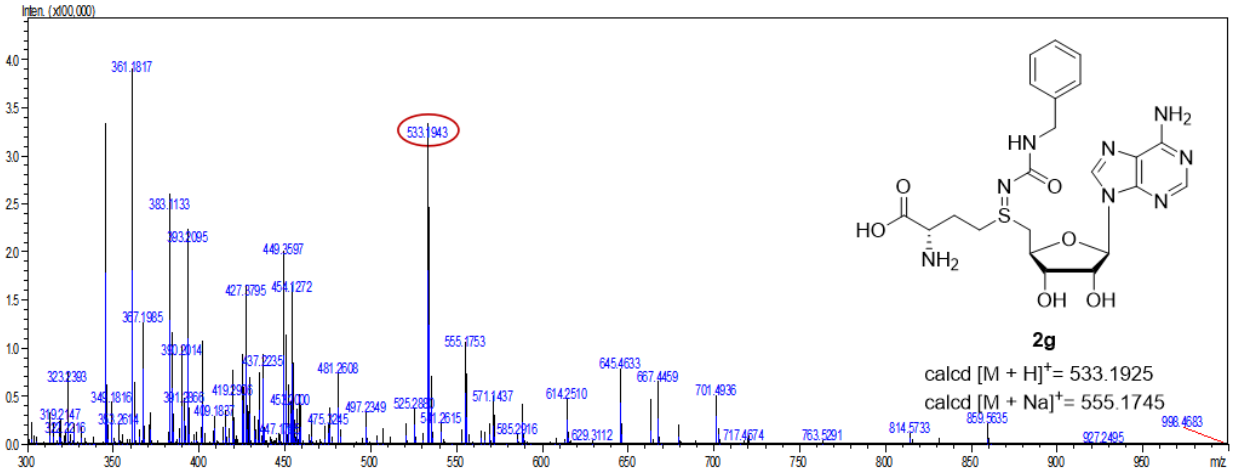

Inten. (x1,000,000)
457.1622
383.1138
318.2994
370.1287
426.1216
480.1454
529.1953
584.2072
642.2220
935.2981
m/z
2b
calcd [M + H]+= 457.1612
calcd [M + Na]+= 479.1432


Inten. (x1,000,000)
485.1936
383.1136
419.1428
318.3006
585.2580
775.3004
m/z
2c
calcd [M + H]+= 485.1925
calcd [M + H]+= 507.1745


Inten. (x1,000,000)
379.2364
383.1151
482.1693
481.1643
241.5888
136.0627
241.0845
484.1707
m/z
2d
calcd [M + H]+= 481.1612
calcd [M + Na]+= 503.1432


Inten. (x1,000,000)
487.1739
383.1140
426.1213
553.2706
m/z
2e
calcd [M + H]+= 487.1718
calcd [M + Na]+= 509.1537





7. Reference

[1] S. Lin, X. Yang, S. Jia, A. M. Weeks, M. Hornsby, P. S. Lee, R. V. Nichiporuk, A. T. Iavarone, J. A. Wells, F. D. Toste, C. J. Chang, *Science* **2017**, *355*, 597-602.

[2] A. H Christian, S. Jia, W. Cao, P. Zhang, A. T Meza, M. S Sigman, C. J Chang, F. D Toste. *J. Am. Chem. Soc.* **2019**, *141*, 12657-12662.