Supporting Information

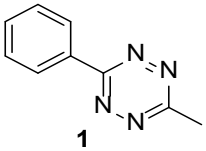
Intramolecular Tetrazine-Acryloyl Cycloaddition: Chemistry and Applications

Akil Hamsath, Oren L. Lederberg, Qi Cui, Meg Shieh, Yannie Lam, Brock J. Brummett, Shi Xu, Jerome R. Robinson, and Ming Xian*

Materials and Methods: All solvents were reagent grade. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.062 mm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Proton and carbon-NMR spectra were recorded on a 400 MHz spectrometer unless otherwise stated. Chemical shifts are reported relative to chloroform (δ 7.26) for ^{1}H NMR and chloroform (δ 77.0) for ^{13}C NMR. Absorption spectra were recorded on a Thermo Scientific Evolution 300 UV-Vis Spectrophotometer using 1 cm quartz cells. Fluorescence excitation and emission spectra were measured on a Cary Eclipse fluorescence spectrophotometer. Cell imaging was performed using the Keyence All-in-One Fluorescence Microscope (BZ-X810).

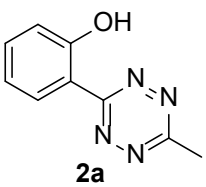
Experimental Procedures

Tetrazine Synthesis:



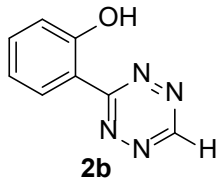
1

Compound **1**: To a 100 mL round bottom flask was added benzonitrile (516 mg, 5.0 mmol), anhydrous-hydrazine (1.6 mL, 50.0 mmol), N-acetylcysteine (1.6 g, 10.0 mmol), and 10 mL of acetonitrile. The resulting mixture was refluxed at 82 °C overnight under argon. The mixture was then cooled to room temperature and transferred to an Erlenmeyer flask. The flask was placed in an ice bath and a solution of sodium nitrite (2.8 g, 40.0 mmol dissolved in 20 mL H_2O) was added to the stirring reaction mixture. 1N HCl was slowly added into the reaction mixture until there was no more gas evolution. The resulting red mixture was left to stir for 15 minutes and then extracted 3 times with CH_2Cl_2 (10 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and absorbed onto silica in vaccuo. 119 mg (0.69 mmol, 14%) of **1** was obtained as a red powder by column chromatography (hexanes : ethyl acetate = 4 : 1, R_f = 0.4). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 8.52 – 8.48 (m, 2H), 7.57 – 7.48 (m, 3H), 3.03 (s, 3H); The characterization data was consistent with those previously reported.[1]



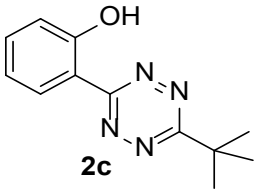
2a

Compound **2a** was prepared from 2-hydroxybenzonitrile using the same procedure as **1**. 94 mg (0.5 mmol, 10%) of **2a** was obtained as a red powder by column chromatography (hexanes : ethyl acetate = 5 : 1, R_f = 0.5). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 11.12 (s, 1H), 8.66 – 8.64 (m, 1H), 7.54 – 7.50 (m, 1H), 7.15 – 7.05 (m, 2H), 3.12 (s, 3H); The characterization data was consistent with those previously reported.[2]



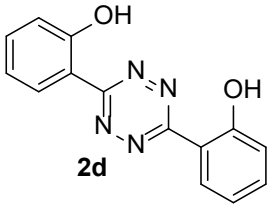
2b

Compound **2b**: To a 20 mL microwave vial was added 2-hydroxybenzonitrile (476 mg, 4.0 mmol), elemental sulfur (256 mg, 8.0 mmol), dichloromethane (256 µL, 4.0 mmol), anhydrous-hydrazine (1.0 mL, 32.0 mmol), and 2.0 mL of ethanol. The reaction was sealed and heated to 50°C in an oil bath overnight. The reaction was taken off the oil bath and was allowed to cool to room temperature. A syringe needle was used to pierce the microwave cap to release the pressure. Caution should be taken as the H_2S gas produced is toxic. The mixture was transferred to an Erlenmeyer flask and was placed in an ice bath. A solution of sodium nitrite (2.8 g, 40.0 mmol dissolved in 20 mL H_2O) was added to the stirring reaction mixture. 1N HCl was slowly pipetted into the reaction mixture until there was no more gas evolution. The resulting red mixture was left to stir for 15 minutes and then extracted 3 times with CH_2Cl_2 (10 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and absorbed onto silica in vaccuo. 169 mg (0.97 mmol, 24%) of **2b** was obtained as a red viscous powder by column chromatography (hexanes : ethyl acetate = 4 : 1, R_f = 0.5). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 11.06 (s, 1H), 10.21 (s, 1H), 8.65 – 8.62 (m, 1H), 7.55 – 7.50 (m, 1H), 7.13 – 7.05 (m, 2H); **^{13}C NMR** (101 MHz, chloroform-*d*) δ 167.3, 160.4, 156.8, 135.8, 128.8, 120.3, 118.9, 113.8; **GCMS (9.40 min):** m/z $M^{+\cdot}$ calcd. for $[C_8H_6N_4O]^{+\cdot}$ 174.05 found: 174.1.



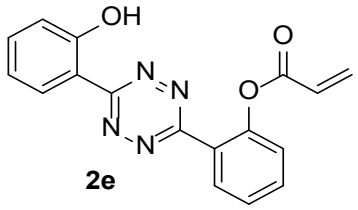
2c

Compound **2c** was prepared from 2-hydroxybenzonitrile and trimethyl acetonitrile using the same procedure as **1**. 45 mg (0.19 mmol, 4%) of **2c** was obtained as a dark red viscous oil by column chromatography (hexanes : ethyl acetate = 20 : 1, R_f = 0.6). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 11.18 (s, 1H), 8.66 – 8.63 (m, 1H), 7.52 – 7.48 (m, 1H), 7.11 – 7.04 (m, 2H), 1.57 (s, 9H); **^{13}C NMR** (101 MHz, chloroform-*d*) δ 174.9, 164.4, 160.0, 135.0, 128.5, 120.2, 118.7, 114.1, 38.1, 29.1; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{12}H_{15}N_4O]^+$ 231.1246 found: 231.1232.



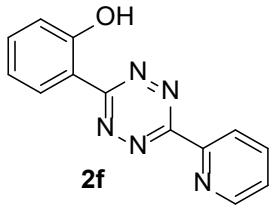
2d

Compound **2d**: To a 100 mL bomb flask was added, 2-hydroxybenzonitrile (2.0 g, 16.8 mmol), anhydrous-hydrazine (3.2 mL, 100 mmol), and 30 mL of ethanol. The reaction was sealed and heated to 80°C in an oil bath overnight. The reaction was taken off the oil bath and was allowed to cool to room temperature. The mixture was then transferred to a round bottom flask and stirred for 1 hr under an O_2 atmosphere (balloon). The resulting red precipitate (**2d**) was filtered and washed with cold ethanol. 784 mg (2.9 mmol, 35%) of **2d** was obtained as a red powder. **^{1}H NMR** (400 MHz, chloroform-*d*) δ 10.89 (s, 2H), 8.71 – 8.62 (m, 2H), 7.62 – 7.45 (m, 2H), 7.21 – 7.09 (m, 4H). The characterization data was consistent with those previously reported.[3]



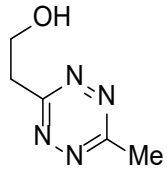
2e

Compound **2e**: To a 20 mL vial was added **2d** (53 mg, 0.2 mmol), triethylamine (42 μL, 0.3 mmol), acryloyl chloride (100 μL, 0.1 mmol, from 1.0 M stock solution), and 1.9 mL acetonitrile. The mixture was stirred for 1 hr, absorbed onto silica in vaccuo and subject to flash column chromatography. 26 mg (0.081 mmol, 40%) of **2e** was obtained as a red viscous oil by column chromatography (hexanes : ethyl acetate = 4 : 1). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 11.18 (s, 1H), 8.70 – 8.66 (m, 1H), 8.47 – 8.44 (m, 1H), 7.72 – 7.68 (m, 1H), 7.57 – 7.51 (m, 2H), 7.37 – 7.34 (m, 1H), 7.16 – 7.07 (m, 2H), 6.63 – 6.58 (m, 1H), 6.40 – 6.33 (m, 1H), 6.09 – 6.06 (m, 1H); **^{13}C NMR** (101 MHz, chloroform-*d*) δ 164.7, 164.0, 163.3, 160.3, 149.5, 135.4, 133.6, 133.3, 131.1, 128.8, 127.5, 126.9, 124.9, 124.6, 120.4, 118.8, 114.0; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{17}H_{13}N_4O_3]^+$ 321.0988, found: 321.0997.



2f

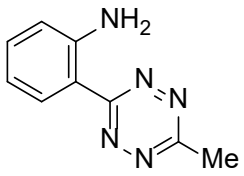
Compound **2f** was synthesized using literature procedures.[2] 56 mg (0.22 mmol, 7%) of **2f** was obtained as a red powder by column chromatography (ethylacetate : hexanes = 1 : 1, R_f = 0.3). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 11.23 (s, 1H), 8.97 – 8.93 (m, 1H), 8.73 – 8.66 (m, 2H), 8.04 – 7.98 (m, 1H), 7.59 – 7.51 (m, 2H), 7.15 – 7.07 (m, 2H); This compound showed identical spectroscopic properties to those previously reported.[2]



2g

Compound **2g** was synthesized using literature procedures.[2] 87.4 mg (0.62 mmol, 12%) of **2g** was obtained as a red powder by column chromatography (ethylacetate : hexanes = 3 : 2, R_f = 0.5). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 4.20 (t, *J* = 5.9 Hz, 2H), 3.51 (t, *J* = 5.9 Hz, 2H), 3.01 (s, 3H),

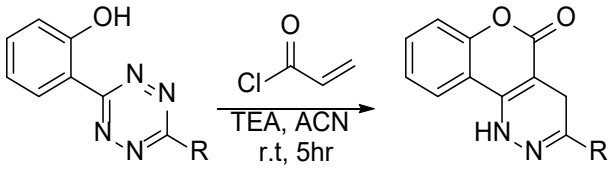
2.93 (br s, 1H); This compound showed identical spectroscopic properties to those previously reported.[2]



2h

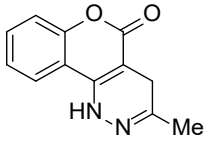
Compound **2h**: To a 100 mL round bottom flask was added 2-aminobenzonitrile (1.0 g, 8.5 mmol), elemental sulfur (272 mg, 8.5 mmol), acetonitrile (4.4 mL, 85.0 mmol), anhydrous-hydrazine (1.4 mL, 42.5 mmol), and 3.0 mL of ethanol. The mixture was refluxed overnight under argon. The resulting yellow solution was concentrated in vaccuo and the residue was washed with brine and extracted 3x with ethyl acetate (20mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated in vaccuo. The residue was redissolved in ethanol and stirred overnight under an O_2 atmosphere (balloon). The resulting dark red solution was concentrated in vaccuo, redissolved in ethyl acetate, and absorbed onto silica gel. 78 mg (0.42 mmol, 5%) of **2h** was obtained as a dark red powder by column chromatography (dichloromethane : hexanes = 4 : 1, R_f = 0.3). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$) δ 8.36 – 8.32 (m, 1H), 7.34 – 7.28 (m, 1H), 6.97 (s, 2H), 6.94 – 6.90 (m, 1H), 6.75 – 6.71 (m, 1H), 2.96 (s, 3H); **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 165.5, 165.0, 150.1, 133.6, 129.7, 117.6, 116.3, 111.9, 21.2; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_9H_{10}N_5]^+$ 188.0936 found: 188.0924.

General procedure for cycloaddition products (4a-h):



2a~h **4a~h**

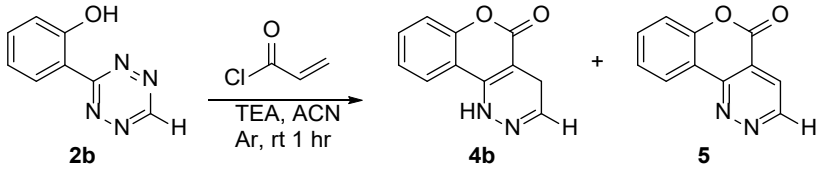
To a 20 mL vial was added tetrazine (**2a-h**, 0.1 mmol), triethyl amine (28 µL, 0.2 mmol), acryloyl chloride (16 µL, 0.2 mmol), and 1.0 mL of acetonitrile. The mixture was stirred at room temperature for 5 hours then directly absorbed onto silica gel in vaccuo and subjected to flash column chromatography.



4a

Compound **4a** was synthesized using the general procedures. 20.5 mg (0.096 mmol, 96%) of **4a** was obtained as a pale yellow oil by column chromatography (ethyl acetate : hexanes = 4 : 1, R_f = 0.4). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$) δ 10.17 (s, 1H), 8.00 – 7.98 (m, 1H), 7.61 – 7.58 (m, 1H), 7.36 – 7.32 (m, 2H), 3.10 (s, 2H), 1.95 (s, 3H); **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 161.0, 152.6, 149.4, 142.8, 132.2, 124.3, 122.5, 117.4, 111.9, 87.2, 24.8, 23.6; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{12}H_{11}N_2O_2]^+$ 215.0821, found: 215.0811; **UV/Vis (ACN):** λ_{max} (ε) = 300 nm (6,748 M^{-1} cm^{-1}), 350 nm (4,320 M^{-1} cm^{-1}); **Fluorescence (ACN):** λ_{ex} = 350 nm, λ_{em} = 450 nm.

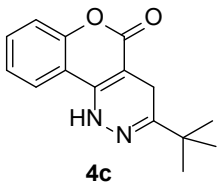
Scheme S1



To a 20 mL vial was added **2b** (17.4 mg, 0.1 mmol), triethylamine (28 µL, 0.2 mmol), and 1.0 mL of acetonitrile. The mixture was flushed with argon for 5 min and acryloyl chloride (16 µL, 0.2 mmol) was added via syringe in one portion. The mixture was stirred at room temperature for 1 hour then directly absorbed onto silica gel in vaccuo and subjected to flash column chromatography.

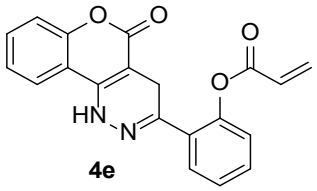
17 mg (0.087 mmol, 87%) of **4b** was obtained as a pale yellow oil by column chromatography (ethyl acetate : hexanes = 1 : 1, R_f = 0.2). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$) δ 10.91 (s, 1H), 8.02 – 8.01 (m, 1H), 7.62 – 7.60 (m, 1H), 7.37 – 7.34 (m, 2H), 7.04 (t, *J* = 2.7 Hz, 1H), 3.13 (d, *J* = 2.7 Hz, 2H); **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 160.8, 152.7, 143.7, 142.1, 132.3, 124.4, 122.5, 117.4, 111.9, 87.4, 21.7; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{11}H_9N_2O_2]^+$ 201.0664, found: 201.0654.

2.2 mg (0.011 mmol, 11%) of **5** was obtained as a pale yellow oil by column chromatography (ethyl acetate : hexanes = 4 : 1, R_f = 0.2). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$) δ 9.65 (d, *J* = 5.2, 1H), 8.78 – 8.76 (m, 1H), 8.33 (d, *J* = 5.2 Hz, 1H), 7.78 – 7.75 (m, 1H), 7.56 – 7.52 (m, 2H); **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 160.2, 152.8, 152.6, 151.1, 133.7, 125.8, 125.7, 124.2, 120.2, 117.9, 117.3; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{11}H_7N_2O_2]^+$ 199.0508, found: 199.0503.



4c

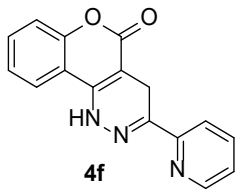
Compound **4c** was synthesized using the general procedures. 9.7 mg (0.038 mmol, 38%) of **4c** was obtained as a pale yellow oil by column chromatography (ethyl acetate : hexanes = 4 : 1, R_f = 0.5). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 8.10 (s, 1H), 7.57 – 7.53 (m, 1H), 7.41 – 7.29 (m, 3H), 3.27 (s, 2H), 1.21 (s, 9H); **^{13}C NMR** (101 MHz, chloroform-*d*) δ 161.6, 158.0, 152.7, 143.3, 131.7, 124.0, 119.5, 117.9, 111.5, 90.3, 37.7, 27.2, 19.6; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{15}H_{17}N_2O_2]^+$ 257.1290, found: 257.1304.



4e

Compound **4e** was synthesized using the general procedures. 11 mg (0.03 mmol, 30%) of **4a** was obtained as a pale yellow oil by column chromatography (ethyl acetate : hexanes = 4 : 1, R_f = 0.2). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$) δ 11.32 (s, 1H), 8.11 – 8.08 (m, 1H), 7.70 – 7.69 (m, 1H), 7.65 – 7.61 (m, 1H), 7.54 – 7.50 (m, 1H), 7.41 – 7.36 (m, 3H), 7.28 – 7.26 (m, 1H), 6.54 – 6.49 (m, 1H),

6.42 – 6.36 (m, 1H), 6.15 – 6.12 (m, 1H), 3.44 (s, 2H); **^{13}C NMR** (101 MHz, DMSO-*d*$_6$): δ 164.0, 160.3, 152.1, 147.9, 144.1, 142.3, 133.9, 131.9, 130.4, 130.1, 129.1, 127.4, 126.3, 124.0, 123.5, 122.1, 117.0, 111.4, 87.8, 23.4; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{20}H_{15}N_2O_4]^+$ 347.1032, found: 347.1039.

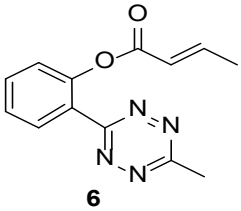


4f

Compound **4f** was synthesized using the general procedures. 7.8 mg (0.028 mmol, 28%) of **4f** was obtained as a pale yellow oil by column chromatography (ethyl acetate : hexanes = 4 : 1, R_f = 0.2). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$) δ 11.42 (s, 1H), 8.68 – 8.67 (m, 1H), 8.12 – 8.07 (m, 2H), 7.92 – 7.88 (m, 1H), 7.65 – 7.61 (m, 1H), 7.47 – 7.44 (m, 1H), 7.42 – 7.37 (m, 2H), 3.67 (s, 2H); **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 161.1, 154.0, 152.7, 149.4, 146.5, 142.5, 137.1, 132.4, 124.8, 124.5, 122.6, 120.3, 117.5, 111.9, 90.3, 20.5; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{16}H_{12}N_3O_2]^+$ 278.0930, found: 278.0942.

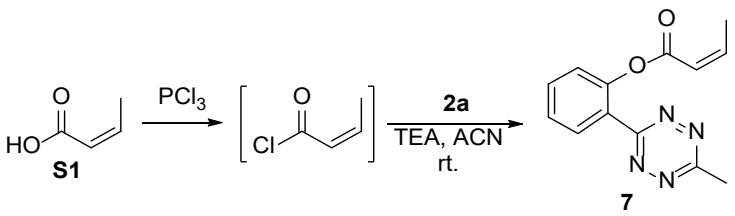
4h

Compound **4h** was synthesized using the general procedures. 18 mg (0.085 mmol, 85%) of **4h** was obtained as a pale yellow oil by column chromatography (acetone : ethyl acetate = 4 : 1, R_f = 0.4). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$) δ 11.20 (s, 1H), 10.18 (s, 1H), 7.95 – 7.91 (m, 1H), 7.46 – 7.42 (m, 1H), 7.26 – 7.24 (m, 1H), 7.14 – 7.11 (m, 1H), 3.10 (s, 2H), 1.94 (s, 3H); **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 161.9, 146.4, 141.6, 138.2, 130.3, 121.9, 121.5, 116.0, 110.9, 95.3, 24.8, 23.4; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{12}H_{12}N_3O]^+$ 214.0980; found: 214.0975; **UV/Vis (ACN):** λ_{max} (ε) = 320 nm (7,653 mol^{-1} cm^{-1}); **fluorescence (ACN):** λ_{ex} = 320 nm, λ_{em} = 425 nm.

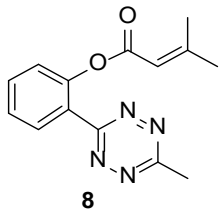


6

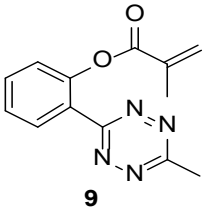
Compound **6** was synthesized from **2a** and crotonoyl chloride using the same procedure as **4a-h**. 21 mg (0.082 mmol, 82%) of **6** was obtained as a violet oil by column chromatography (hexanes : ethyl acetate = 4 : 1, R_f = 0.4). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 8.37 – 8.35 (m, 1H), 7.66 – 7.61 (m, 1H), 7.50 – 7.46 (m, 1H), 7.31 – 7.28 (m, 1H), 7.20 – 7.11 (m, 1H), 6.08 – 6.03 (m, 1H), 3.08 (s, 3H), 1.96 (dd, *J* = 6.9, 1.7 Hz, 3H); **^{13}C NMR** (101 MHz, chloroform-*d*) δ 166.6, 164.9, 164.4, 149.6, 147.6, 133.2, 131.2, 126.6, 125.4, 124.5, 121.7, 21.2, 18.4; **HRMS (ESI):** m/z $[M+Na]^+$ calcd. for $[C_{13}H_{12}N_4NaO_2]^+$ 279.0858; found: 279.0886.



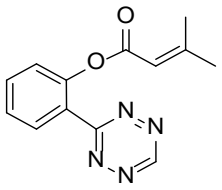
To a 5 mL microwave vial was added **S1**[4] (43 mg, 0.5 mmol), phosphorus trichloride (15 µL, 0.17 mmol) and 600 µL acetonitrile. This mixture was stirred at 60°C for 30 min. After 30 min, 300 µL (0.25 mmol **S1**) of this mixture was added to a premade solution of **2a** (18.8 mg, 0.1 mmol) and triethyl amine (28 µL, 0.2 mmol) dissolved with 700 µL acetonitrile. The reaction was stirred for 1 hr then absorbed onto silica gel in vaccou and subjected to flash column chromatography. 12.8 mg (0.05 mmol, 50%) of **7** was obtained as a violet oil by column chromatography (hexanes : ethyl acetate = 4 : 1, R_f = 0.4). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 8.43 – 8.41 (m, 1H), 7.69 – 7.66 (m, 1H), 7.53 – 7.50 (m, 1H), 7.35 – 7.33 (m, 1H), 6.62 – 6.56 (m, 1H), 6.10 – 6.06 (m, 1H), 3.11 (s, 3H), 2.20 (dd, *J* = 7.3, 1.8 Hz, 3H); **^{13}C NMR** (101 MHz, chloroform-*d*) δ 166.5, 164.8, 164.3, 149.5, 148.3, 133.1, 131.1, 126.6, 125.3, 124.6, 119.5, 21.2, 15.6; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{13}H_{13}N_4O_2]^+$ 257.1039; found: 257.1048.



Compound **8** was synthesized from **2a** and 3,3-dimethylacryloyl chloride using the same procedure as **4a-h**. 26 mg (0.096 mmol, 96%) of **8** was obtained as a violet oil by column chromatography (hexanes : ethyl acetate = 4 : 1, R_f = 0.4). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 8.36 – 8.33 (m, 1H), 7.65 – 7.61 (m, 1H), 7.49 – 7.45 (m, 1H), 7.30 – 7.27 (m, 1H), 5.92 (s, 1H), 3.08 (s, 3H), 2.17 (s, 3H), 1.98 (s, 3H); **^{13}C NMR** (101 MHz, chloroform-*d*) δ 166.5, 164.9, 164.5, 160.6, 149.6, 133.1, 131.1, 126.4, 125.5, 124.7, 114.9, 27.8, 21.2, 20.5; **HRMS (ESI):** m/z $[M+Na]^+$ calcd. for $[C_{14}H_{14}N_4NaO_2]^+$ 293.1014; found: 293.1021.

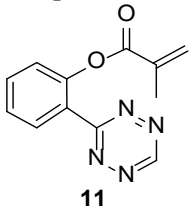


Compound **9** was synthesized from **2a** and methacryloyl chloride using the same procedure as **4a-h**. 24 mg (0.094 mmol, 94%) of **9** was obtained as a violet oil by column chromatography (hexanes : ethyl acetate = 4 : 1, R_f = 0.4). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 8.43 – 8.39 (m, 1H), 7.67 – 7.62 (m, 1H), 7.51 – 7.47 (m, 1H), 7.33 – 7.30 (m, 1H), 6.31 – 6.28 (m, 1H), 5.77 – 5.75 (m, 1H), 3.07 (s, 3H), 2.06 – 2.05 (m, 3H); **^{13}C NMR** (101 MHz, chloroform-*d*) δ 166.6, 166.1, 164.3, 149.9, 135.6, 133.2, 131.2, 127.6, 126.7, 125.2, 124.5, 21.2, 18.4; **HRMS (ESI):** m/z $[M+Na]^+$ calcd. for $[C_{13}H_{12}N_4NaO_2]^+$ 279.0858; found: 279.0886.



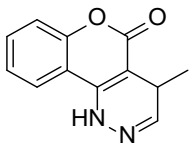
10

Compound **10** was synthesized from **2b** and 3,3-dimethylacryloyl chloride using the same procedure as **4a-h**. 21 mg (0.082 mmol, 82%) of **10** was obtained as a violet oil by column chromatography (hexanes : ethyl acetate = 4 : 1, R_f = 0.4). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 10.19 (s, 1H), 8.40 – 8.35 (m, 1H), 7.69 – 7.64 (m, 1H), 7.51 – 7.47 (m, 1H), 7.33 – 7.29 (m, 1H), 5.94 – 5.91 (m, 1H), 2.17 (d, *J* = 1.1 Hz, 3H), 1.99 (d, *J* = 1.1 Hz, 3H); **^{13}C NMR** (101 MHz, chloroform-*d*) δ 167.0, 164.8, 160.7, 156.9, 149.8, 133.5, 131.3, 126.5, 125.3, 124.7, 114.8, 27.7, 20.5; **HRMS (ESI):** m/z $[M+Na]^+$ calcd. for $[C_{13}H_{12}N_4NaO_2]^+$ 279.0858; found: 279.0825.



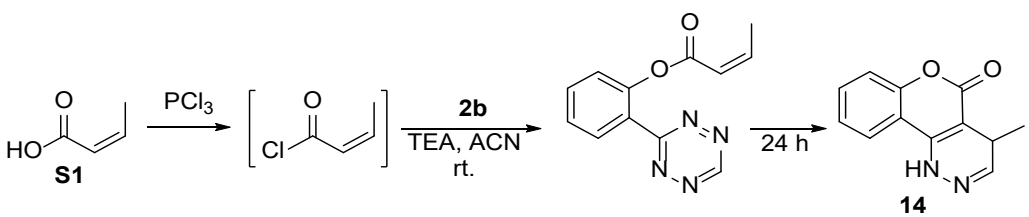
11

Compound **11** was synthesized from **2b** and methacryloyl chloride using the same procedure as **4a-h**. 22 mg (0.091 mmol, 91%) of **11** was obtained as a violet oil by column chromatography (hexanes : ethyl acetate = 4 : 1, R_f = 0.4). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 10.18 (s, 1H), 8.45 – 8.43 (m, 1H), 7.71 – 7.67 (m, 1H), 7.54 – 7.50 (m, 1H), 7.35 – 7.33 (m, 1H), 6.31 – 6.29 (m, 1H), 5.77 – 5.76 (m, 1H), 2.07 – 2.05 (m, 3H); **^{13}C NMR** (101 MHz, chloroform-*d*) δ 166.8, 166.1, 157.0, 150.1, 135.6, 133.7, 131.4, 127.7, 126.7, 125.1, 124.6, 18.4; **HRMS (ESI):** m/z $[M+Na]^+$ calcd. for $[C_{12}H_{10}N_4NaO_2]^+$ 265.0701; found: 265.0709.

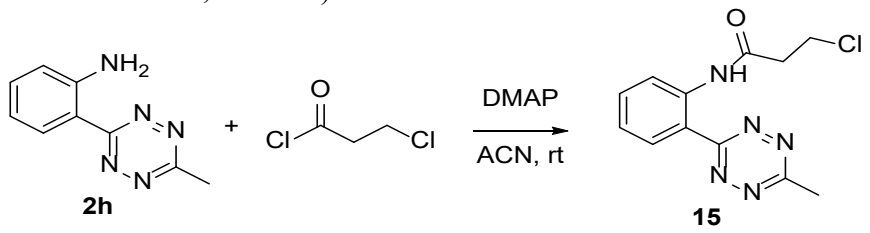


14

Compound **14** (via E-acryloyl group) was synthesized from **2b** and crotonyl chloride using the same procedures as **4a-h**. 15 mg (0.069 mmol, 69%) of **14** was obtained as a pale yellow powder by column chromatography (ethyl acetate : hexanes = 4 : 1, R_f = 0.5). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 8.06 – 8.03 (m, 1H), 7.64 – 7.60 (m, 1H), 7.39 – 7.35 (m, 2H), 7.06 – 7.04 (m, 1H), 3.48 – 3.44 (m, 1H), 1.10 (d, *J* = 6.9 Hz, 3H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 160.8, 152.7, 145.2, 142.9, 132.3, 124.4, 122.7, 117.4, 112.1, 93.1, 25.8, 19.2; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{12}H_{11}N_2O_2]^+$ 215.0821; found: 215.0827.

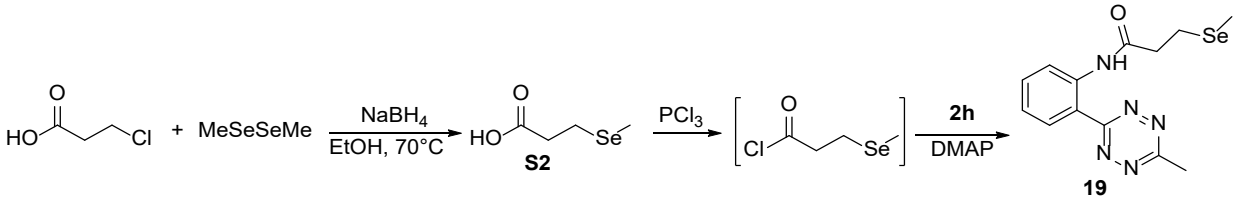


To a 5 mL microwave vial was added **S1**[4] (43 mg, 0.5 mmol), phosphorus trichloride (15 µL, 0.17 mmol) and acetonitrile (0.6 mL). This mixture was stirred at 60 °C for 30 min. After 30 min, 300 µL (0.25 mmol **S1**) of this mixture was added to a premade solution of **2a** (18.8 mg, 0.1 mmol) and triethyl amine (28 µL, 0.2 mmol) dissolved with 700 µL acetonitrile. The reaction was stirred for 24 hrs then absorbed onto silica gel in vaccou and subjected to flash column chromatography. 11 mg (0.051 mmol, 51%) of **14** was obtained as a pale yellow powder by column chromatography (hexanes : ethyl acetate = 4 : 1, R_f = 0.4).



Compound **15** was synthesized from **2h** (70 mg, 0.37 mmol), 3-chloropropionyl chloride (71 µL, 0.74 mmol), and DMAP (45 mg, 0.37 mmol) using the same procedures as **4a-h**. 83 mg (0.3 mmol, 81%) of **15** was obtained as a violet oil by column chromatography (ethyl acetate : hexanes = 1 : 10, R_f = 0.2). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 11.44 (s, 1H), 8.76 – 8.70 (m, 2H), 7.68 – 7.60 (m, 1H), 7.36 – 7.29 (m, 1H), 3.91 (t, *J* = 6.5 Hz, 2H), 3.14 (s, 3H), 2.95 (t, *J* = 6.5 Hz, 2H); **^{13}C NMR** (101 MHz, chloroform-*d*): δ 168.2, 166.1, 165.1, 139.1, 133.8, 130.1, 124.1, 122.0, 117.9, 41.3, 39.6, 21.1; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{12}H_{13}ClN_5O]^+$ 278.0809, found: 278.0805.

Synthesis of Compound 19:

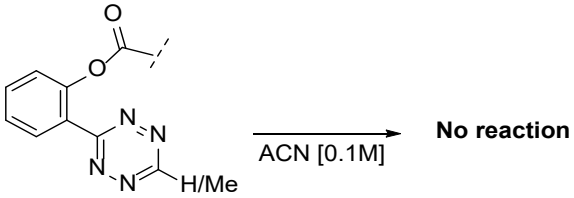


A three-necked 100 mL round bottom flask was equipped with reflux condenser and two septa. The flask was evacuated and filled with argon (3x). Dimethyl-diselenide (287 µL, 3.0 mmol) and ethanol (10 mL) was added via syringe and stirred. Solution A: In a separate vial was added sodium borohydride (400 mg) and ethanol (5 mL). Solution B: In another separate vial was added 3-chloropropionic acid (814 mg, 7.5 mmol), sodium carbonate (400 mg, 3.8 mmol) and H_2O (2 mL). Solution A was added slowly via syringe into the orange dimethyl-diselenide mixture until the reaction mixture turned white. Immediately after, solution B was added in one portion. The resulting white mixture was refluxed for 2.5 hours. The reaction mixture was then diluted with H_2O and extracted with DCM (10 mL, 3x) to separate any unreacted dimethyl diselenide. The aqueous layer was acidified with conc HCl forming a white precipitate (this is the product). The aqueous layer was extracted once more with DCM (10 mL, 3x). These organic layers were combined, dried with $MgSO_4$ and concentrated in vaccuo. Crude **S2** was further reacted without purification.

To a 10 mL microwave vial was added **S2** (84 mg, 0.5 mmol), phosphorous trichloride (15 µL, 0.17 mmol) and acetonitrile (0.6 mL). The clear solution was sealed and stirred at 60°C for 30 minutes. In a separate 20 mL scintillation vial was added **2h** (47 mg, 0.25 mmol), DMAP (31 mg,

0.25 mmol) and acetonitrile (1.4 mL). After 30 minutes, the **S2** solution was transferred into the **2h** solution in one portion and the resulting mixture was stirred at room temperature for 15 minutes. The resulting violet solution was diluted with sat. $NaHCO_3$ and extracted with ethyl acetate (10 mL, 3x). The organic layers were combined, dried with $MgSO_4$ and filtered. The resulting violet solution was absorbed onto silica gel in vaccuo and was subjected to column chromatography. 37 mg (0.11 mmol, 44%) of **19** was obtained as a violet viscous oil by column chromatography (ethyl acetate : hexanes = 1 : 5, R_f = 0.2). **^{1}H NMR** (400 MHz, chloroform-*d*) δ 11.36 (s, 1H), 8.73 – 8.65 (m, 2H), 7.63 – 7.57 (m, 1H), 7.32 – 7.25 (m, 1H), 3.12 (s, 3H), 2.92 – 2.86 (m, 4H), 2.05 (s, 3H); **^{13}C NMR** (101 MHz, chloroform-*d*) δ 170.4, 166.0, 165.2, 139.4, 133.9, 130.1, 123.9, 122.0, 117.9, 39.4, 21.2, 19.6, 4.6; **HRMS (ESI):** m/z $[M+H]^+$ calcd. for $[C_{13}H_{16}N_5OSe]^+$ 338.0520; found: 338.0539.

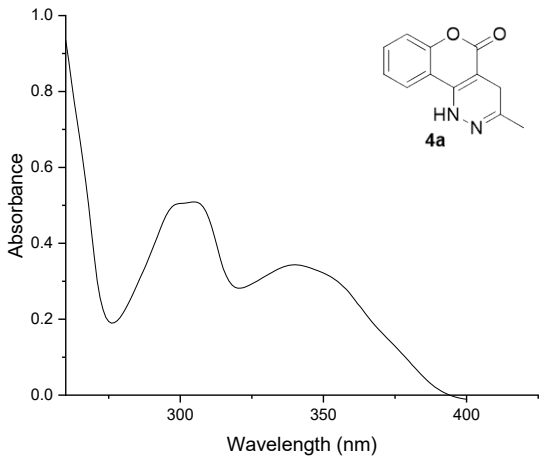
Test cycloaddition of compounds 6 – 11.

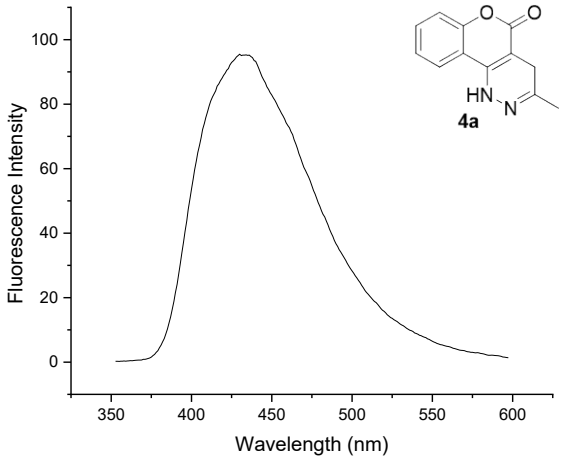


6 - 11

To a 20 mL scintillation vial was added **6 – 11** (0.1 mmol) and acetonitrile (1.0 mL). The reaction was stirred at room temperature for 24 hrs.

Photochemical properties of 4a and 4h





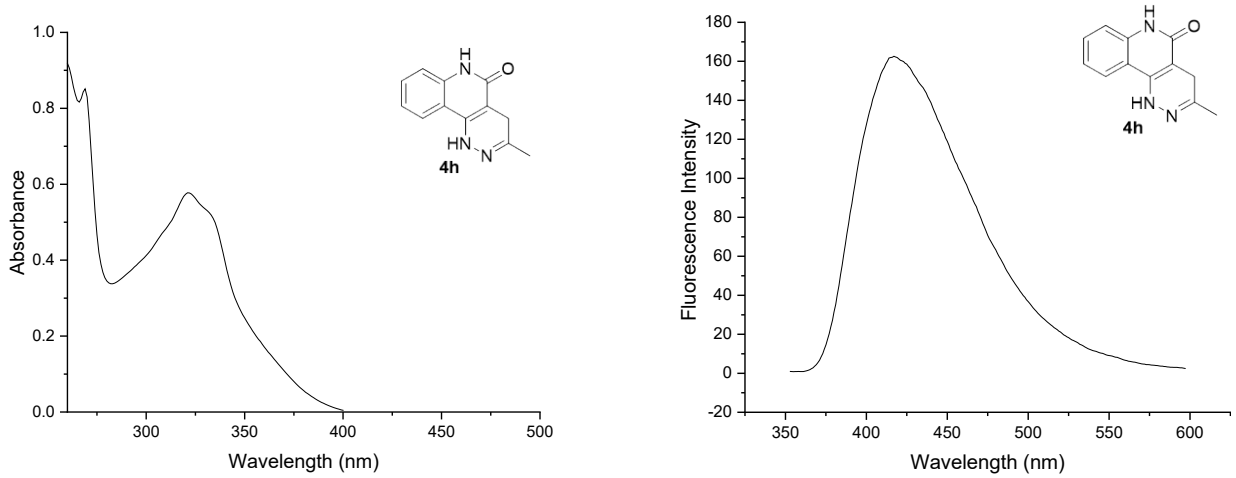


Figure S1. Stock solutions (7.5 mM) of compounds **4a** and **4h** were prepared in DMSO and diluted with H_2O to afford 50 µM in 1% DMSO in H_2O. The same solutions were used to obtain both the absorbance and fluorescence spectra. Coumarin and 1H-quinolin-2-one was analyzed using these same conditions.

Compound	λ_{ex} (nm)	λ_{em} (nm)	ε ($M^{-1}cm^{-1}$)	Stokes shift (nm)	Φ
4a	350	450	4,320	100	0.25
4h	320	425	7,653	105	0.34

Table S1. Photochemical properties of compounds **4a** and **4h** from the data shown in Figure S1.

Quantum Yields

The quantum yields of **4a** and **4h** were calculated according to the equation:

$$\mathbf{\Phi}_{sample} = \mathbf{\Phi}_{standard} \times (I_{sample} / I_{standard}) \times (A_{standard} / A_{sample}) \times (n_{sample} / n_{standard})^2$$

Φ denotes the quantum yield; *I* denotes the area under the fluorescence band; *A* denotes the absorbance at the excitation wavelength; *n* denotes the refractive index of the solvent. Quantum yield was determined using fluorescein as a standard by comparing the area under the corrected emission spectrum of the test sample with that of a solution of quinine excited at 490 nm in 0.1 N $HClO_4$, which has a quantum efficiency ($\mathbf{\Phi}_{standard}$) of 0.60 according to the literature.[5]

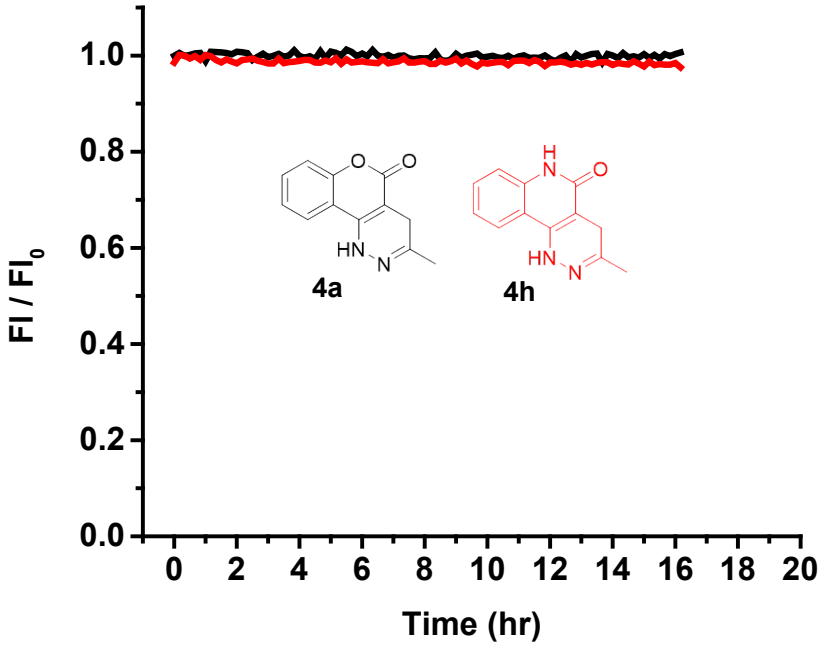


Figure S2. Photostability experiment. Compounds **4a** and **4h** were dissolved in 1% DMSO/H_2O at a concentration of 50 μM. The resulting solutions were placed in the fluorometer separately and were exited at their corresponding absorption wavelength every 5 min for 16 hours. The fluorescent intensities were recorded and normalized.

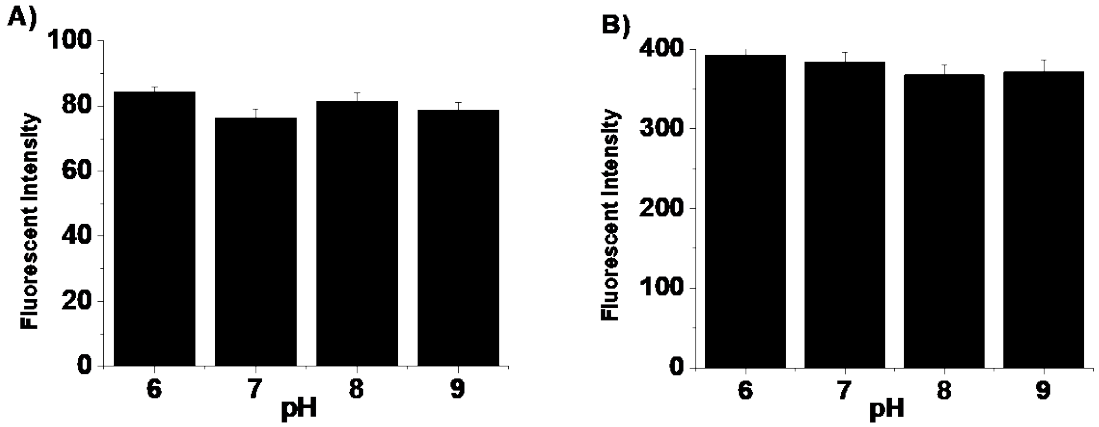
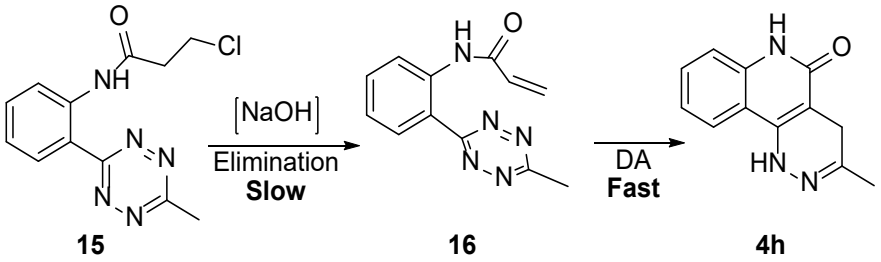
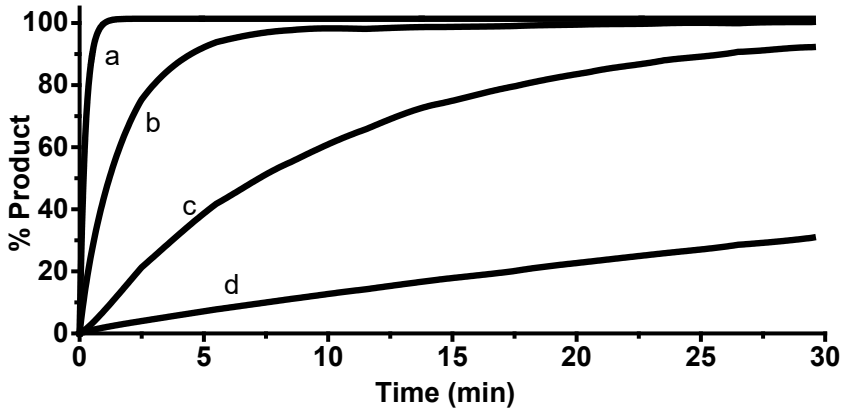


Figure S3. pH stability studies. Compounds **4a** (A) and **4h** (B) were dissolved in 1% DMSO/buffer (pH 6 to 9) at a concentration of 50 μM. Their fluorescence intensities were measured using a fluorescence spectrophotometer.

Procedure for fluorescent monitoring of 4h by reacting 15 with NaOH:

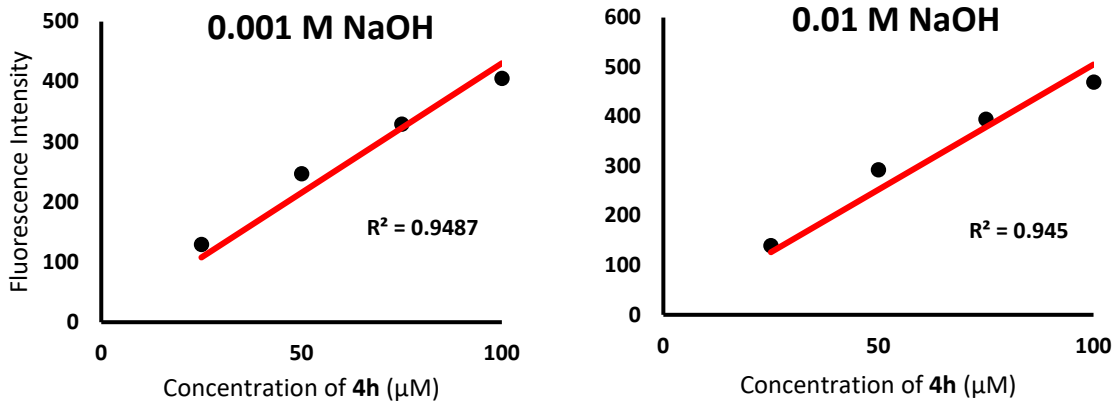


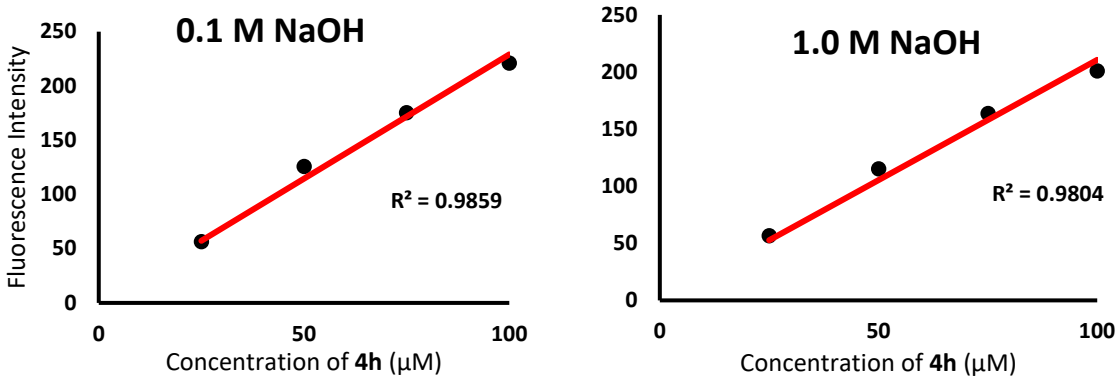


15 stock solution (Solution A): **15** was dissolved in DMSO to afford a 4.0 mM stock solution.

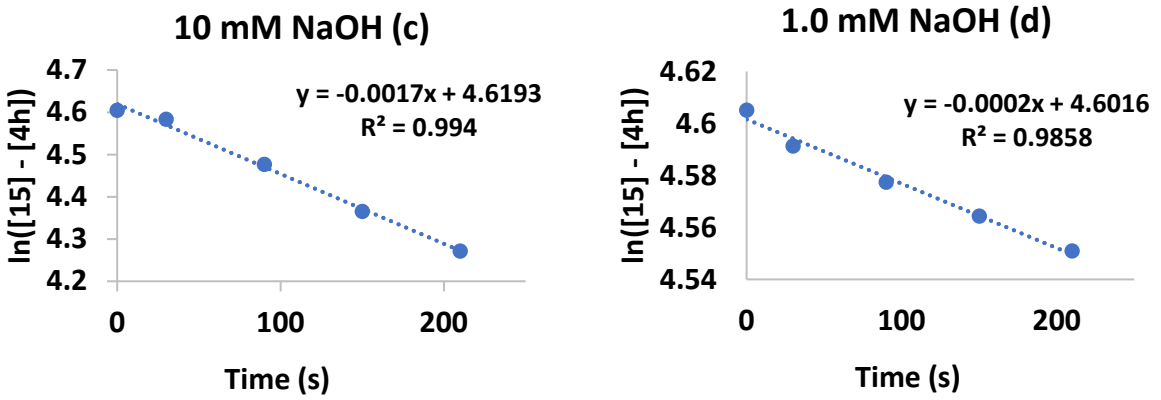
Fluorescent measurement: To a 20 mL glass scintillation vial was added 100 µL of solution A and 300 µL of DMSO. 3.6 mL of aq NaOH solution (0.001 M) was then added to the vial to afford a total volume of 4 mL yielding a final **15** concentration of 100 µM in 10% DMSO/aq NaOH. The fluorescence intensity of the solution was recorded every minute for 1 hour (λ_{ex} = 320 nm, λ_{em} = 425 nm). This same procedure was duplicated with equal amounts **15** and varying concentrations of NaOH (0.01, 0.1, and 1.0 M). This data was converted into % product via calibration curves specific to each condition using pure **4h** and plotted in Figure 1.

Calibration Curves:





Experimental Rate Constant Calculations:



$$[\mathbf{4h}] = [\mathbf{15}]_o(1-e^{-kt})$$

$$[\mathbf{4h}] = [\mathbf{15}]_o - [\mathbf{15}]_o e^{-kt}$$

$$[\mathbf{15}]_o - [\mathbf{4h}] = [\mathbf{15}]_o e^{-kt}$$

$$\ln([\mathbf{15}]_o - [\mathbf{4h}]) = \ln[\mathbf{15}]_o - kt$$

$$\ln([\mathbf{15}]_o - [\mathbf{4h}]) = -k_{obs}t + \ln[\mathbf{15}]_o$$

$$y = mx + b$$

$$\text{Rate} = k_{rxn}[\mathbf{15}][\text{NaOH}]$$

$$[\text{NaOH}] \approx [\text{NaOH}]_o$$

$$k_{obs} = k_{rxn}[\text{NaOH}]_o$$

$$k_{obs\ (10\ mM\ NaOH)} = 0.0017\ s^{-1}$$

$$k_{obs\ (1.0\ mM\ NaOH)} = 0.0002\ s^{-1}$$

$$k_{rxn\ (10\ mM\ NaOH)} = 0.17\ M^{-1}\ s^{-1}$$

$$k_{rxn\ (1.0\ mM\ NaOH)} = 0.2\ M^{-1}\ s^{-1}$$

$$k_{rxn} \approx 0.18\ M^{-1}\ s^{-1}$$

Kinetic Modeling (COPASI):

Using a kinetic modeling software (COPASI), kinetic data collected at various reagent concentrations were fit to the following model to calculate the rate constant of the cycloaddition step:

Model

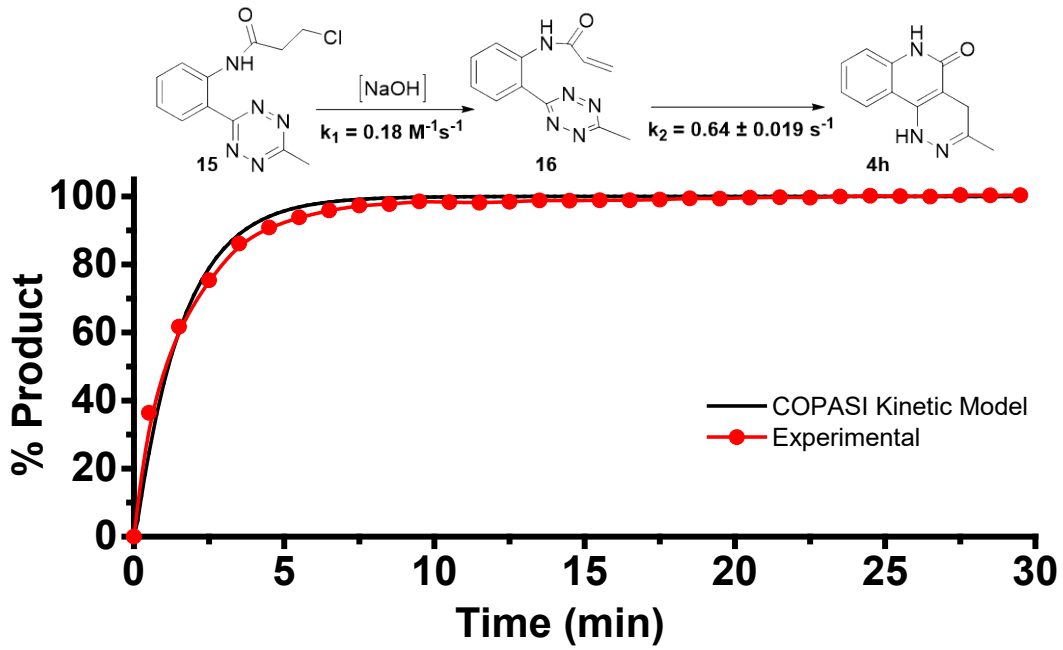


Figure S4: Kinetic model used to obtain rate constant k_2 through parameter estimation of k_1. [**15**] = 0.1 mM, [NaOH] = 100 mM.

The conversion from **15** to **16** was set to be irreversible due to the saturation conditions employed. Only k_2 was fit using the parameter estimation function. The k_1 value was set at 0.18 $M^{-1}s^{-1}$ to reflect the experimental second-order rate constant observed under saturated NaOH. The results of the kinetic modeling are shown in Figure S4 and are overlayed with experimental data shown in Figure 2.

Computational methods: All calculations were performed with the Gaussian 09 program1. Geometry optimizations of all minima and transition states involved were carried out using M06-2X functional2 and SMD3 solvation model in H_2O solvent and the basis set was 6-311G(d).[6-9] It was labelled as SMD(H2O)/M06-2X/6-311G(d) level. Frequency calculations at the same level were performed to validate each structure as either a minimum or a transition state and to evaluate its zero-point energy and thermal corrections at 298 K. Standard states are the hypothetical states at 1 mol/L.

Computed energies of all the stationary points:
Thermal correction to Enthalpy ***TCH***, Thermal correction to Gibbs Free Energy ***TCG***, Sum of electronic and thermal Enthalpies ***H***, Sum of electronic and thermal Free Energies ***G***, delta Gibbs free energy ***ΔG.***

SMD(H2O)/M06-2X/6-311G(d) level:

	TCH	***TCG***	***H***	***G***	***ΔG (kcal/mol)***
16	0.23914	0.178368	-812.445206	-812.505978	**0.0**
TS1	0.237789	0.184233	-812.425969	-812.479525	**16.6**

17	0.241099	0.188637	-812.488661	-812.541123	**-22.1**
TS2	0.238675	0.185464	-812.476579	-812.52979	**-14.9**
18	0.229745	0.177951	-703.039001	-703.090795	**-71.7**
4h	0.230519	0.177973	-703.059477	-703.112023	**-85.0**
N2	0.009073	-0.012654	-109.5107	-109.532427	
12	0.226002	0.16636	-832.31502	-832.374662	
12TS1	0.224987	0.17183	-832.28837	-832.341527	**20.8**
13	0.226055	0.167838	-832.312761	-832.370979	
13TS1	0.225099	0.17151	-832.284148	-832.337737	**20.9**

Cartesian coordinates of all computed structures

16
C 0.78129400 3.58516700 -0.02451900
C 2.07058500 3.07644400 -0.14426900
C 2.28353600 1.70917100 -0.23399500
C 1.20950600 0.81385000 -0.19820500
C -0.10336200 1.32312400 -0.07368900
C -0.28972400 2.70850000 0.00691800
H 0.61055500 4.65303700 0.03338700
H 2.92141700 3.74673600 -0.18323300
H 3.28618500 1.32588900 -0.34606900
H -1.29804700 3.09337500 0.08812600
C 2.53249200 -1.26068300 0.11232500
O 3.51066200 -0.74320900 0.63600200
C 2.43214700 -2.72768300 -0.13074000
H 1.52559100 -3.10862200 -0.59045500
C 3.43113900 -3.53511200 0.20505000
H 3.37502900 -4.60339800 0.03231000
H 4.33440300 -3.14598600 0.66333300
C -1.31126100 0.47506500 -0.01961400
C -3.48090000 -0.92786500 0.02199900
N -2.39767500 0.98732100 0.59156800
N -3.48780600 0.28567900 0.60449500

N -1.30535000 -0.73582600 -0.57637700
N -2.40155900 -1.45458400 -0.55018200
C -4.74569800 -1.70727100 0.03803400
H -4.59121400 -2.68963600 -0.40252700
H -5.10244100 -1.81880400 1.06308900
H -5.51456900 -1.17614800 -0.52678400
N 1.43046900 -0.57002300 -0.30386800
H 0.64271800 -1.11498600 -0.63440900

TS1
C 3.51588800 -1.63401900 -0.10720300
C 4.11052200 -0.37742700 -0.04387900
C 3.32724700 0.76531100 0.04896900
C 1.93886100 0.65960100 0.09559000
C 1.33501200 -0.60395500 0.06474600
C 2.13167100 -1.74110200 -0.05532700
H 4.12466300 -2.52613100 -0.18796700
H 5.18937800 -0.28166400 -0.07681400
H 3.78160000 1.74933200 0.07863600
H 1.65554600 -2.71365800 -0.08849800
C -0.12294900 2.01252700 -0.15912900
O -0.75197100 2.99995000 0.19261800
C -0.73586500 0.96268200 -1.04170600
H -0.11414200 0.56802100 -1.83811000
C -2.10260400 0.87982800 -1.11150400
H -2.57434700 0.40263600 -1.96151400
H -2.70916400 1.57170600 -0.53753800
C -0.13729100 -0.73168800 0.17055500
C -2.63763500 -0.74680000 0.19320500
N -0.74380100 -1.73132700 -0.53924200
N -2.02124000 -1.74525400 -0.51898600
N -0.73705400 -0.31189700 1.33411200
N -2.01563600 -0.31318200 1.33655500
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H -4.48410000 0.25759300 0.54856600
H -4.48376700 -0.81591600 -0.86970600
H -4.53719600 -1.50097300 0.76294600
N 1.17542600 1.83812000 0.20139500
H 1.59737100 2.61956300 0.69081100

17
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C 4.08269200 -0.52062100 0.07892900
C 3.38045100 0.67668600 0.13695100
C 1.98975600 0.66096300 0.06518700
C 1.30345100 -0.55044800 -0.05968700

C 2.01796400 -1.74124700 -0.11648900
H 3.95843000 -2.66270500 -0.09651300
H 5.16490600 -0.50473900 0.13365000
H 3.89886500 1.62432000 0.23585000
H 1.48061900 -2.67772400 -0.20946900
C -0.03859800 2.02440800 -0.17113600
O -0.61132800 3.09527200 -0.05418000
C -0.71641700 0.79351200 -0.74822600
H -0.39588100 0.74086600 -1.79350400
C -2.23146500 0.79118400 -0.62769500
H -2.72146300 0.82090300 -1.59914200
H -2.59222100 1.62677700 -0.02810000
C -0.18702400 -0.49281900 -0.06468100
C -2.63373400 -0.52252200 0.08258600
N -0.81576900 -1.61878800 -0.77053500
N -2.04746300 -1.62861500 -0.69911600
N -0.71741300 -0.46194300 1.32934600
N -1.94648400 -0.47845000 1.39088100
C -4.11349100 -0.70846800 0.25654900
H -4.52813100 0.12305100 0.82733000
H -4.59965700 -0.74143400 -0.71902800
H -4.32193900 -1.63893500 0.78658800
N 1.27140800 1.86640600 0.14651700
H 1.77194000 2.70199300 0.42844800

TS2

C 3.47090900 -1.68497400 -0.05858100
C 4.10627500 -0.45774700 0.12595100
C 3.36832600 0.71658300 0.17642000
C 1.98254000 0.66454400 0.04006900
C 1.33424000 -0.56293400 -0.14140300
C 2.08910200 -1.73339500 -0.18574100
H 4.05067800 -2.59901800 -0.09880300
H 5.18391100 -0.41359600 0.23160900
H 3.85270600 1.67645500 0.32002800
H 1.58104800 -2.68007400 -0.32138100
C -0.07683900 1.97749200 -0.21175000
O -0.67989900 3.02911100 -0.07189300
C -0.71507000 0.74176100 -0.82522400
H -0.39554100 0.74286300 -1.87348200
C -2.23574400 0.71721300 -0.72930600
H -2.69348000 0.72820900 -1.71837100
H -2.61995400 1.57067600 -0.16924300
C -0.13926400 -0.54279400 -0.23280900
C -2.67434100 -0.57803200 -0.05476500
N -0.76433600 -1.71036800 -0.55146800

N -2.05411600 -1.73235100 -0.46767500
N -0.76550300 -0.25801600 1.46150200
N -1.92012400 -0.29016100 1.49184700
C -4.14232800 -0.71204600 0.20170800
H -4.50894100 0.14934300 0.76099800
H -4.67351800 -0.75342400 -0.75161000
H -4.35375000 -1.62286400 0.76165500
N 1.23338400 1.84871400 0.11891900
H 1.70407200 2.68980100 0.43445500

18
C 3.34183300 -1.70842800 0.03572900
C 3.97718800 -0.46760800 0.11551000
C 3.23642400 0.70375700 0.09956100
C 1.84590500 0.64166900 0.00566800
C 1.19461700 -0.59541000 -0.06637400
C 1.96087800 -1.76527100 -0.05388400
H 3.92444800 -2.62145600 0.04116400
H 5.05720500 -0.41283300 0.18638000
H 3.72087100 1.67235900 0.16141300
H 1.45075000 -2.71841800 -0.12022800
C -0.23256500 1.94228300 -0.12074300
O -0.79731900 3.02443700 -0.05494900
C -1.00784400 0.67142000 -0.39032200
H -1.25900000 0.70617700 -1.46168000
C -2.29821200 0.62184500 0.41786400
H -2.95550500 1.45323900 0.17042600
H -2.07680300 0.67352900 1.49116000
C -0.27162000 -0.61737900 -0.13750100
C -2.96469100 -0.68662200 0.10421300
N -0.89696600 -1.72334500 0.03013700
N -2.29669200 -1.75475400 -0.12929100
C -4.45361400 -0.73295800 0.04599300
H -4.86925900 -0.38698000 0.99727100
H -4.82051900 -0.05312500 -0.72737400
H -4.80705000 -1.74265400 -0.15683600
N 1.11150700 1.83383100 0.00951700
H 1.61868500 2.70076000 0.15106800

4h
C -3.32816100 -1.69225300 -0.01912900
C -3.97823700 -0.45828700 -0.16098900
C -3.25416500 0.71537900 -0.18781900
C -1.85735100 0.67329200 -0.07310000
C -1.19043700 -0.55673700 0.06482300
C -1.95312900 -1.73751600 0.09159000

H -3.90270900 -2.60994400 0.00438700
H -5.05770800 -0.42239600 -0.24947500
H -3.74428800 1.67707000 -0.29543600
H -1.47154700 -2.70119200 0.20813900
C 0.23467800 1.92723900 0.03363700
O 0.79403400 3.03225100 0.02686200
C 0.92845700 0.67412700 0.18344900
C 2.41174400 0.67280900 0.39686600
H 2.66570400 0.81992700 1.45564400
H 2.88708700 1.48995300 -0.14977000
C 0.25591900 -0.51300900 0.18075500
C 2.97936100 -0.64624300 -0.05952600
N 0.97603800 -1.67861400 0.30426800
N 2.31092800 -1.73455100 -0.08131100
C 4.41088700 -0.67558400 -0.48177200
H 4.55485200 -0.03750000 -1.35799000
H 5.04194400 -0.27073400 0.31499000
H 4.73237100 -1.68896600 -0.71786700
N -1.12945500 1.84289500 -0.09808000
H -1.62734100 2.72142100 -0.18963300
H 0.50937800 -2.54306200 0.07528200

N_2
N 0.00000000 0.00000000 0.54483800
N 0.00000000 0.00000000 -0.54483800

12
C 3.84529700 -0.91469600 0.16122800
C 3.32850000 -2.19222900 -0.02743600
C 1.96735500 -2.36507800 -0.24610100
C 1.12892900 -1.26260100 -0.28962200
C 1.62808500 0.03301000 -0.10823200
C 2.99850200 0.18364400 0.12554500
H 4.90516500 -0.77157600 0.33188300
H 3.98059100 -3.05704400 -0.00196000
H 1.53685100 -3.34915200 -0.38666400
H 3.39610200 1.18100100 0.26673700
C -1.16615500 -1.10621300 0.31102500
O -0.89575200 -0.58296300 1.36365700
C -2.50355200 -1.38439300 -0.22378600
H -2.56222100 -1.89457700 -1.17830600
C -3.58859300 -0.99420000 0.44755700
H -3.45119000 -0.47990100 1.39637300
C 0.78095100 1.24074600 -0.15115200
C -0.66217900 3.34315300 -0.18975200
N 1.14146100 2.26603400 0.63124600

N 0.40514400 3.34576200 0.60508100
N -0.27379700 1.26559900 -0.97603000
N -1.02168400 2.33846400 -0.98427800
H -1.28095600 4.22904900 -0.19466100
C -4.98725900 -1.20812900 -0.01049000
H -5.54568600 -1.77338900 0.74098400
H -5.49774700 -0.24670400 -0.11690800
H -5.02898500 -1.74072600 -0.96062100
O -0.20788300 -1.52279800 -0.56636900

12TS1
C -3.52682800 -1.36221000 0.35384800
C -3.98306000 -0.05029700 0.44334000
C -3.10878500 1.01206900 0.24750400
C -1.78024800 0.74963200 -0.04570500
C -1.30472100 -0.55755800 -0.14797000
C -2.19129500 -1.61204300 0.06582800
H -4.20871900 -2.18943700 0.50717100
H -5.02315200 0.15158200 0.66948000
H -3.43799500 2.04161900 0.31653600
H -1.82348900 -2.62846000 -0.00434900
C 0.37194200 1.85327800 -0.08288700
O 1.01277100 2.72634200 -0.60071100
C 0.95059400 0.78194500 0.77258700
H 0.35883800 0.44904100 1.61893400
C 2.32353000 0.61898800 0.78324400
C 0.12466000 -0.80224400 -0.42519800
C 2.57651100 -0.95053500 -0.65692100
N 0.71789900 -1.88241800 0.17941500
N 1.98560500 -1.96218700 0.05154000
N 0.63906500 -0.38006600 -1.63199800
N 1.91354700 -0.44251600 -1.73627000
H 3.65573600 -0.95444000 -0.70483100
O -0.97486000 1.85715700 -0.28296400
H 2.90704500 1.27643500 0.14542800
C 3.02444700 0.01765600 1.96687800
H 3.96044400 -0.46447600 1.68450800
H 3.26518100 0.82722300 2.66191900
H 2.38953400 -0.69884700 2.48850300

13
C 3.84760300 0.60899500 0.25638000
C 3.93866200 -0.76782800 0.08098000
C 2.79570300 -1.51740100 -0.17606300
C 1.57083100 -0.88061200 -0.27799700
C 1.45889700 0.50503000 -0.11831300

C 2.61267000 1.23760700 0.16373900
H 4.73542400 1.19391000 0.46258400
H 4.89797200 -1.26591400 0.15355200
H 2.83797000 -2.59274200 -0.29950400
H 2.53239600 2.30988600 0.29701800
C -0.60767700 -1.63121700 0.27020400
O -0.55339600 -1.08939900 1.34508800
C -1.76477300 -2.30046400 -0.33719100
H -1.54553100 -3.07285900 -1.06440100
C -3.02413000 -1.92327500 -0.09332600
C 0.16098900 1.20148500 -0.21756500
C -2.11254300 2.34456500 -0.30758600
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N -1.25612900 2.75606600 0.62502300
N -0.67861500 0.85027100 -1.20015900
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H -2.70633500 -0.16555000 1.13750100
H -4.24806500 -0.22383600 0.24999700
H -3.80242200 -2.46371900 -0.62638000
O 0.46514100 -1.66899500 -0.57797400

13TS1
C 4.03722300 0.30259200 -0.18525100
C 3.03682900 1.25573200 -0.04747900
C 1.72023900 0.84417000 0.09696200
C 1.38151700 -0.50683300 0.10472200
C 2.39678100 -1.45157300 -0.05309500
H 4.49814500 -1.79720700 -0.29982100
H 5.06641200 0.62296300 -0.29371800
H 3.25707700 2.31623000 -0.04609700
H 2.13624000 -2.50284700 -0.05921700
C -0.52844500 1.76848300 -0.03734100
O -1.24351900 2.64060000 0.37317000
C -0.97255400 0.63822700 -0.90125900
H -0.30508500 0.38554000 -1.71777000
C -2.31812700 0.31277600 -1.01054900
H -2.57517000 -0.26039500 -1.89589700
C -0.02744600 -0.93146900 0.22869200
C -2.44132800 -1.44608500 0.22443000
N -0.40799300 -2.02229600 -0.51340400
N -1.65687300 -2.28615500 -0.51417000
N -0.69222700 -0.69230500 1.41161600
N -1.94529600 -0.95191900 1.39766100

H -3.50749700 -1.61232400 0.17632600
O 0.79175600 1.85774600 0.28647100
C -3.46454300 1.05880300 -0.37911300
H -3.59330400 2.00833400 -0.90525900
H -4.38442300 0.48499200 -0.48449100
H -3.29778500 1.28488800 0.67182600

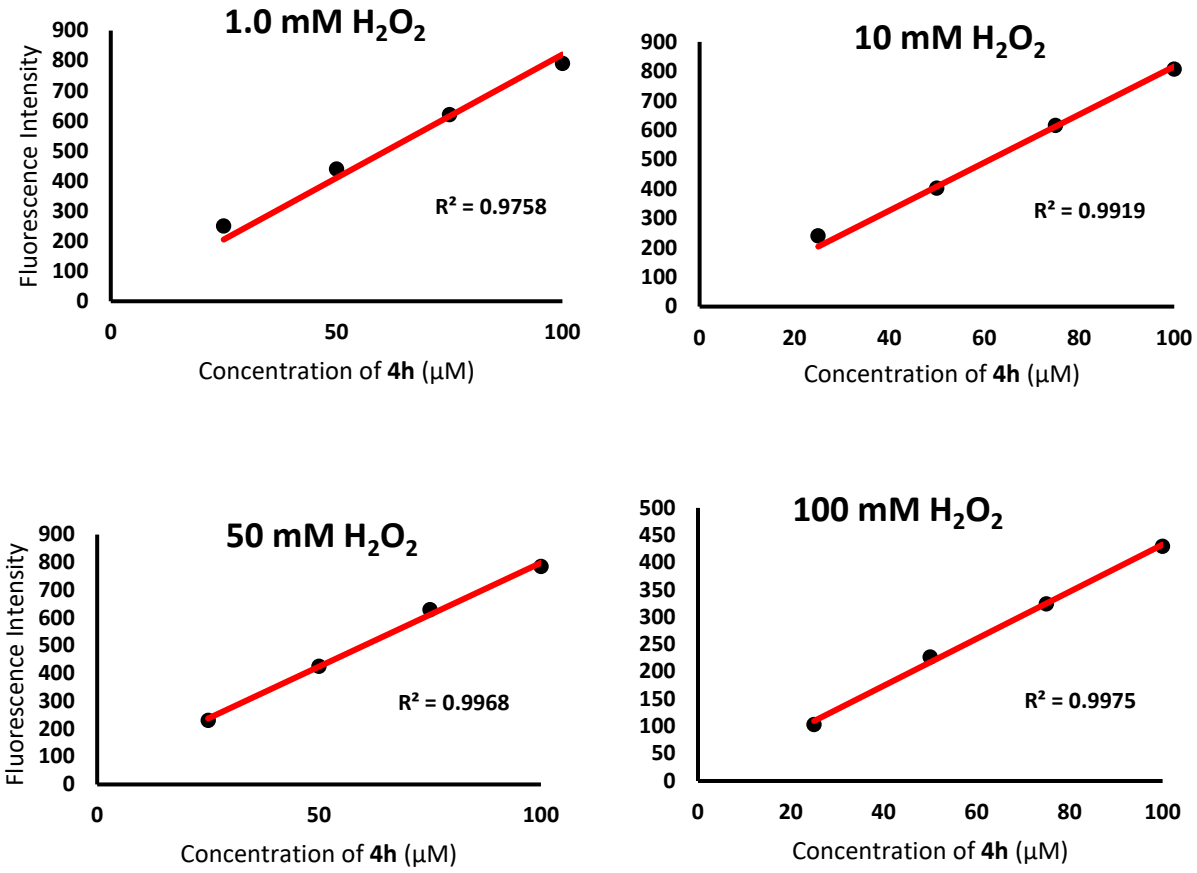
Compound 19 with various ROS:
Stock solutions for hydrogen peroxide (H_2O_2), t-butyl hydroperoxide (TBuOOH), sodium hypochlorite (NaOCl), and Sodium nitroprusside (SNP) were prepared in deionized water. H_2O_2 was diluted from a commercially available solution (30%). The concentration of H_2O_2 was determined using the absorption at 240 nm (ε= 43.6 M^{-1} cm^{-1}). NaOCl was diluted from commercially available solution containing 11-15% available chlorine. The concentration of HOCl was determined using the absorption at 292 nm (ε = 391 M^{-1} cm^{-1}).[10] TBuOOH was diluted from commercially available solutions (70%). SNP (26.2 mg) was dissolved in DI water (10 mL) to afford a 10 mM stock solution. Superoxide solution (O_2^-) was prepared by adding KO_2 (7.0 mg) to dry dimethyl sulfoxide (10 mL) and stirring vigorously for 10 min to afford a 10 mM stock solution. $ONOO^-$ was generated from $NaNO_2$ and H_2O_2, and quantified according to literature.[10] Compound **19** was dissolved in DMSO to afford a 4.0 mM stock solution.

Fluorescent measurement: To a 20 mL scintillation vial was added compound **19** (100 µL, 4.0 mM in DMSO) and DMSO (300 µL). PBS buffer (10 mM, pH = 7.4) was added along with the appropriate amount of freshly prepared ROS stock solution to afford a total volume of 4 mL, a total ROS concentration of 200 µM, and a final **19** concentration 100 µM. The mixture was incubated for 1 hour at 37°C and the solution fluorescence intensity was measured (λ_{ex} = 320 nm, λ_{em} = 425 nm). Positive controls were measured by following this exact procedure replacing **19** with **4h**. The experiments were done in triplicate and the results were reported in Figure 4.

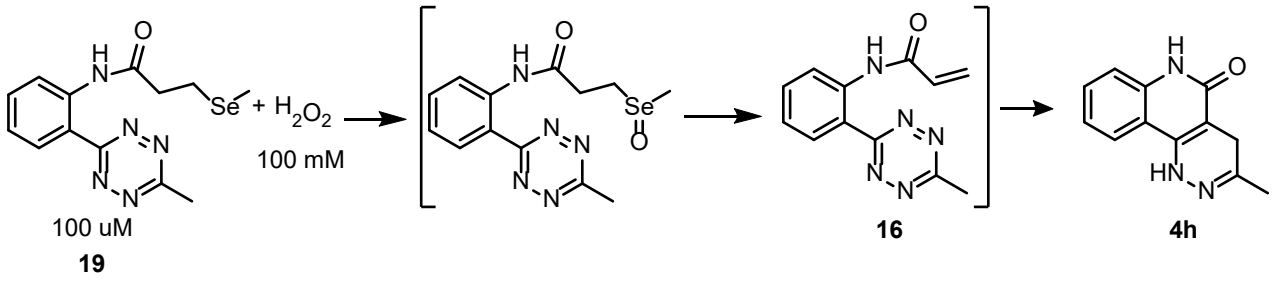
Procedure for fluorescent monitoring of 4h by reacting 19 with H_2O_2:
19 stock solution (Solution A): **19** was dissolved in DMSO to afford a 4.0 mM stock solution.

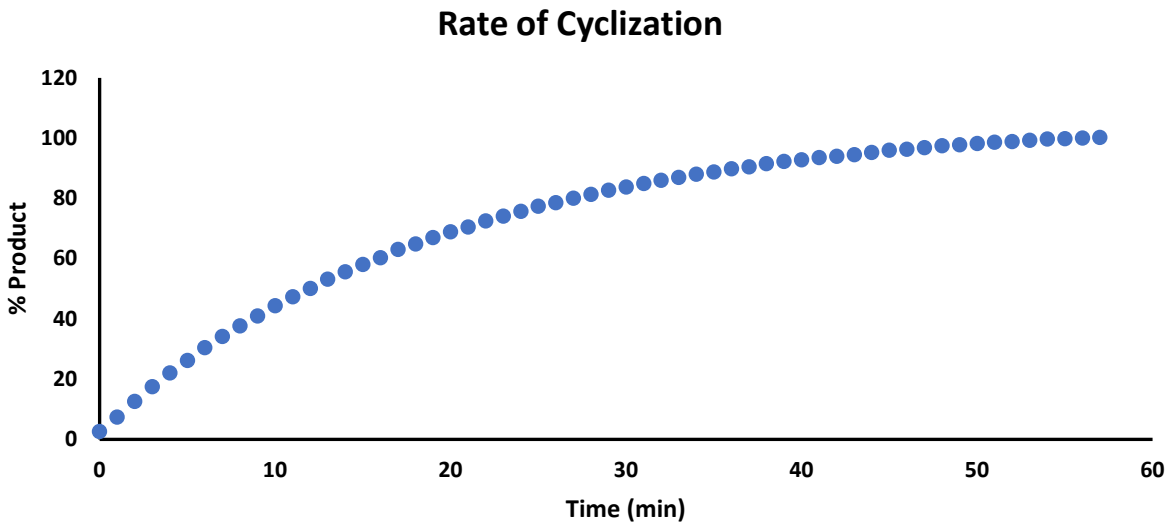
Fluorescent measurement: To a 20 mL glass scintillation vial was added 100 µL of solution A and 300 µL of DMSO. PBS buffer (10 mM, pH = 7.4) was added along with the appropriate amount of freshly prepared H_2O_2 stock solution to afford a total volume of 4 mL, a total H_2O_2 concentration of 1.0 mM, and a final **19** concentration 100 µM. The fluorescence emission intensity of the solution was recorded every minute for 1 hour (λ_{ex} = 320 nm, λ_{em} = 425 nm). This same procedure was used with equal amounts **19** and varying total concentrations of H_2O_2 (10, 50, and 100 mM). This data was converted into % product via calibration curves specific to each condition using pure **4h** (λ_{ex} = 320 nm, λ_{em} = 425 nm) and plotted in Figure 4.

Calibration Curves:



Rate Constant Calculation for Reaction of 19 with H_2O_2:





$[\mathbf{4h}] = [\mathbf{19}]_o(1-e^{-kt})$

$[\mathbf{4h}] = [\mathbf{19}]_o - [\mathbf{19}]_o e^{-kt}$

$[\mathbf{19}]_o - [\mathbf{4h}] = [\mathbf{19}]_o e^{-kt}$

$\ln([\mathbf{19}]_o - [\mathbf{4h}]) = \ln[\mathbf{19}]_o - kt$

$\ln([\mathbf{19}]_o - [\mathbf{4h}]) = -k_{obs}t + \ln[\mathbf{19}]_o$

$y = mx + b$

$\text{Rate} = k_2[\mathbf{19}][H_2O_2]$

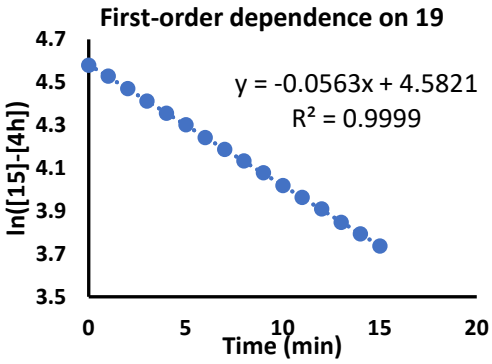
$[H_2O_2] \approx [H_2O_2]_o = 0.1\text{M}$

$k_{obs} = k_2[H_2O_2]_o$

$k_{obs} = 0.0563\ s^{-1}$

$k_2 = 0.0563\ s^{-1} / 0.1\ M$

$k_2 = \mathbf{0.563\ M^{-1}\ s^{-1}}$



Cell Imaging:

HeLa cells were seeded in 48-well plates and cultured in DMEM supplemented with 10% FBS at 37°C under 5% CO_2 for 24 h. Live cells were then washed three times with PBS buffer. N-acetyl cysteine (NAC) was dissolved in PBS buffer and added to cells for pre-treatment at a final concentration of 100 µM. After pre-treated cells were incubated for 15 min at 37°C under 5% CO_2, NAC was removed, and cells were washed with PBS buffer (2x). CTAB (500 µM) and **19** (200 µM) were added to all cells. After 1 h of incubation at 37°C under 5% CO_2, the cells were washed again with PBS buffer (2x) and H_2O_2 or HOCl was added at a final concentration of 200 µM. After a 30 min incubation at 37°C under 5% CO_2, the cells were taken for fluorescence imaging (λ_{ex}: 340 - 360nm ; λ_{em}: 450 - 460nm). The obtained results were summarized in Figure 5.

Cell viability and cytotoxicity assay:

HeLa cells were cultured in DMEM supplemented with 10% FBS at 37°C, 5% CO_2 for 24 hrs. Cells were then inoculated in a 96-well black, clear flat-bottomed plate at 37°C, 5% CO_2 overnight.

After incubation, media was aspirated from wells, and cells were washed twice with 1X PBS. Compounds 1, 2, and 3 were then added to separate wells at different concentrations (0, 50, 100, 200, 300 μM), and cells were cultured for 22 hrs. WST-8 from the CCK-8 assay was then added to the cells and incubated for 2 hrs at 37°C, 5% CO_2 for a total incubation time of 24 hrs. The absorbance at 450 nm was measured with a microplate reader. The optical density (OD) of the wells (3 wells per condition) was used to calculate the relative cell viability (%) according to the following formula:

$$\text{Cell Viability (\%)} = (OD_{\text{treatment group}} / OD_{\text{control group}}) * 100$$

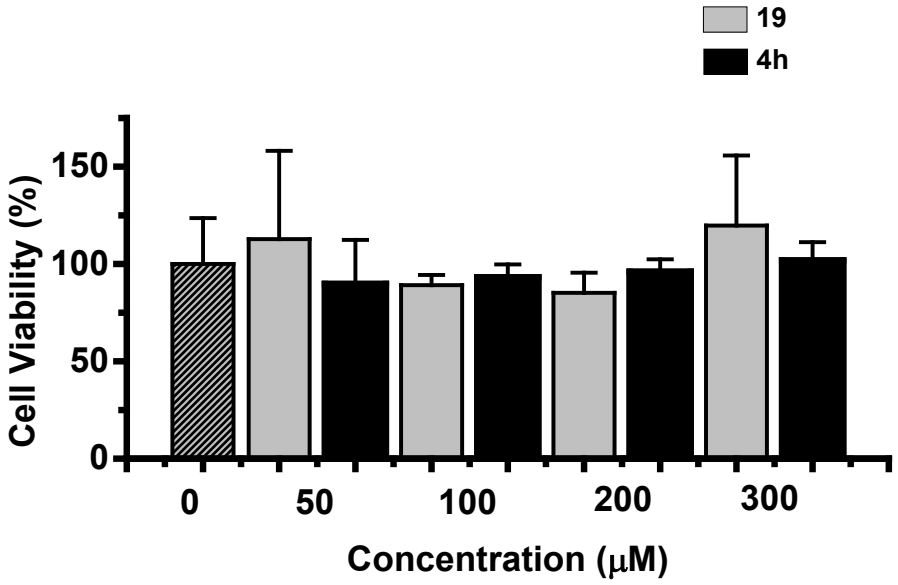


Figure S5. Cell viability with varying concentrations of **19** and **4h**.

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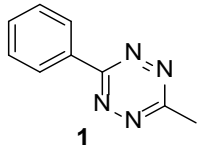
$^{1}H/^{13}CNMR$ Spectra



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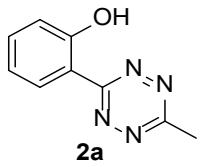

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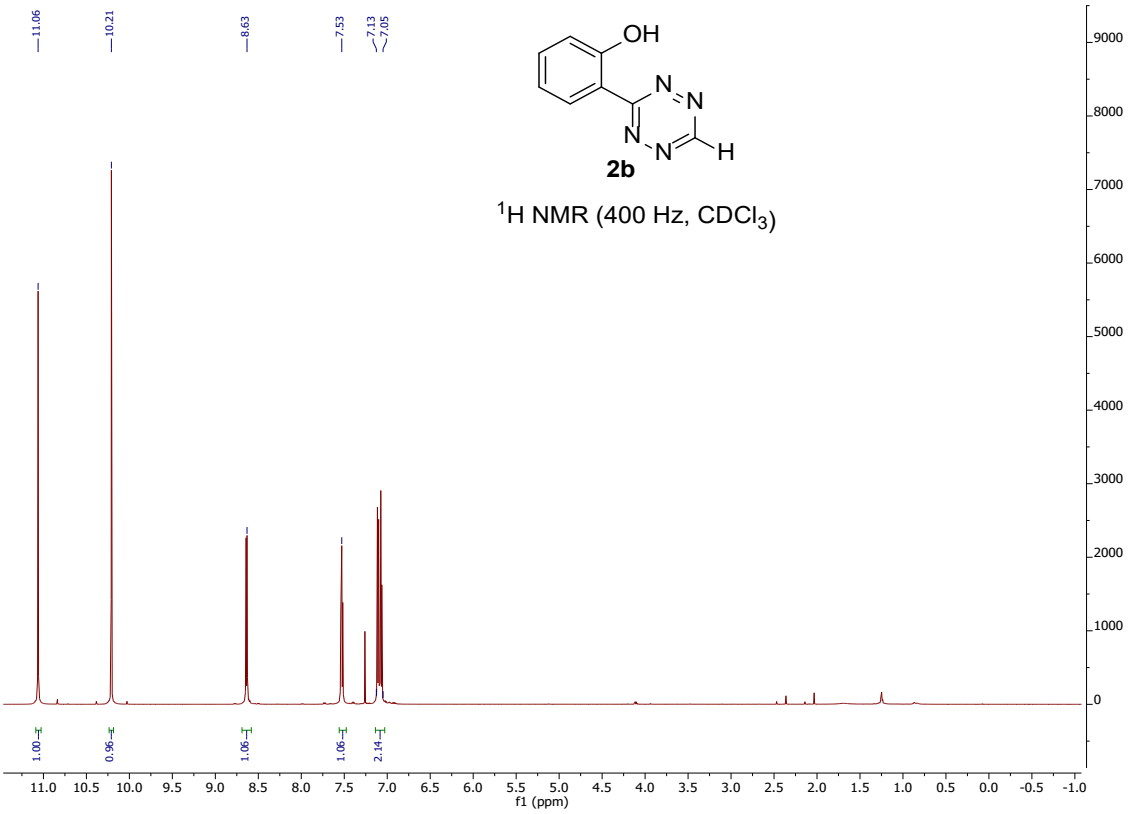

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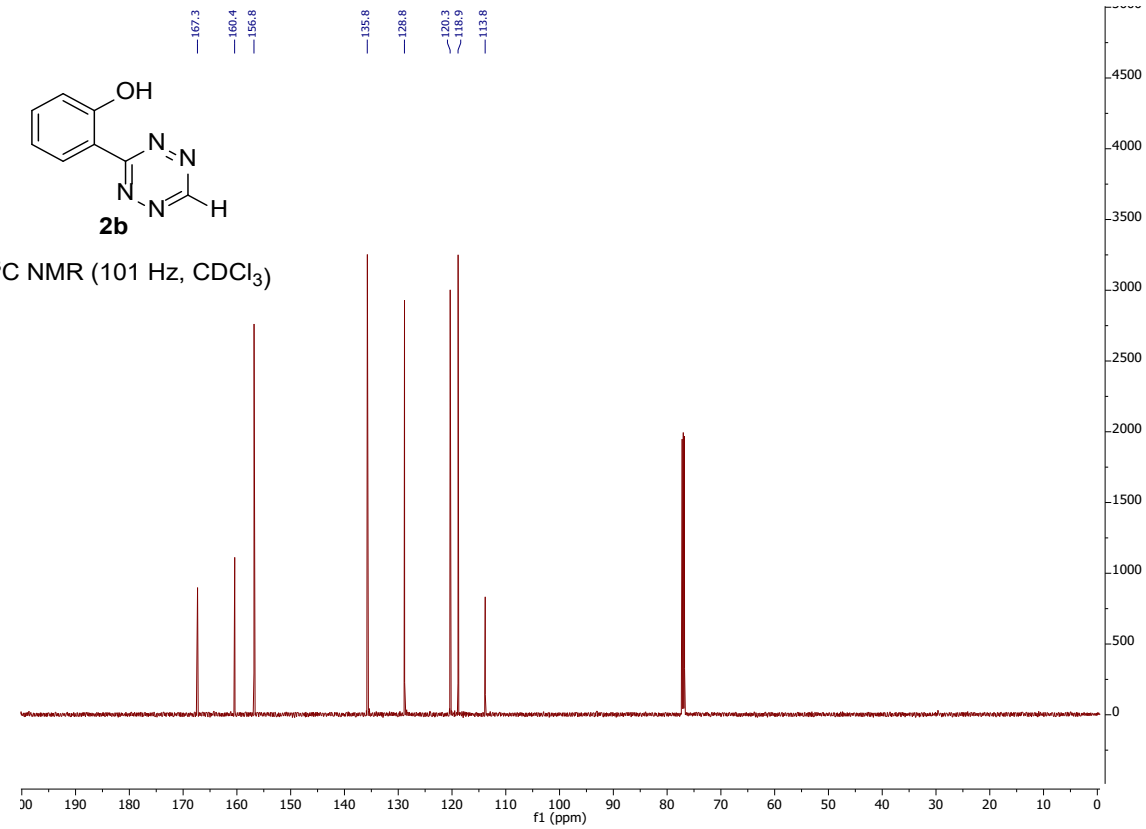

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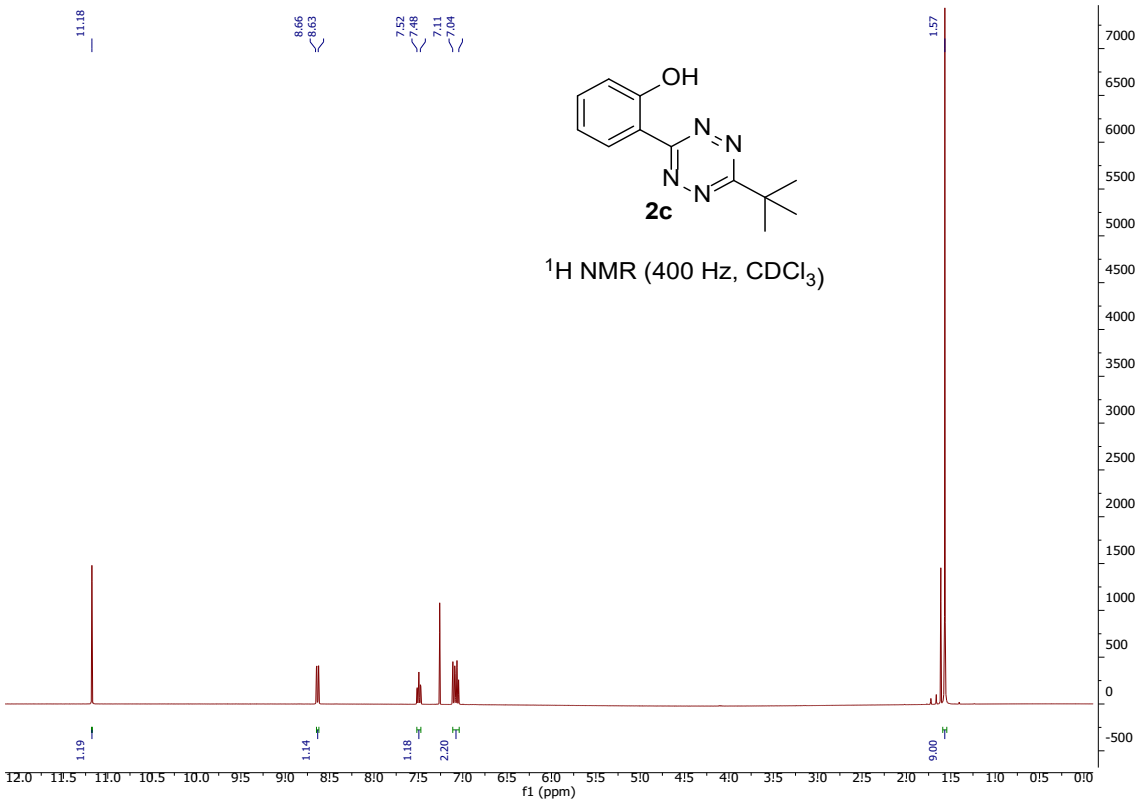

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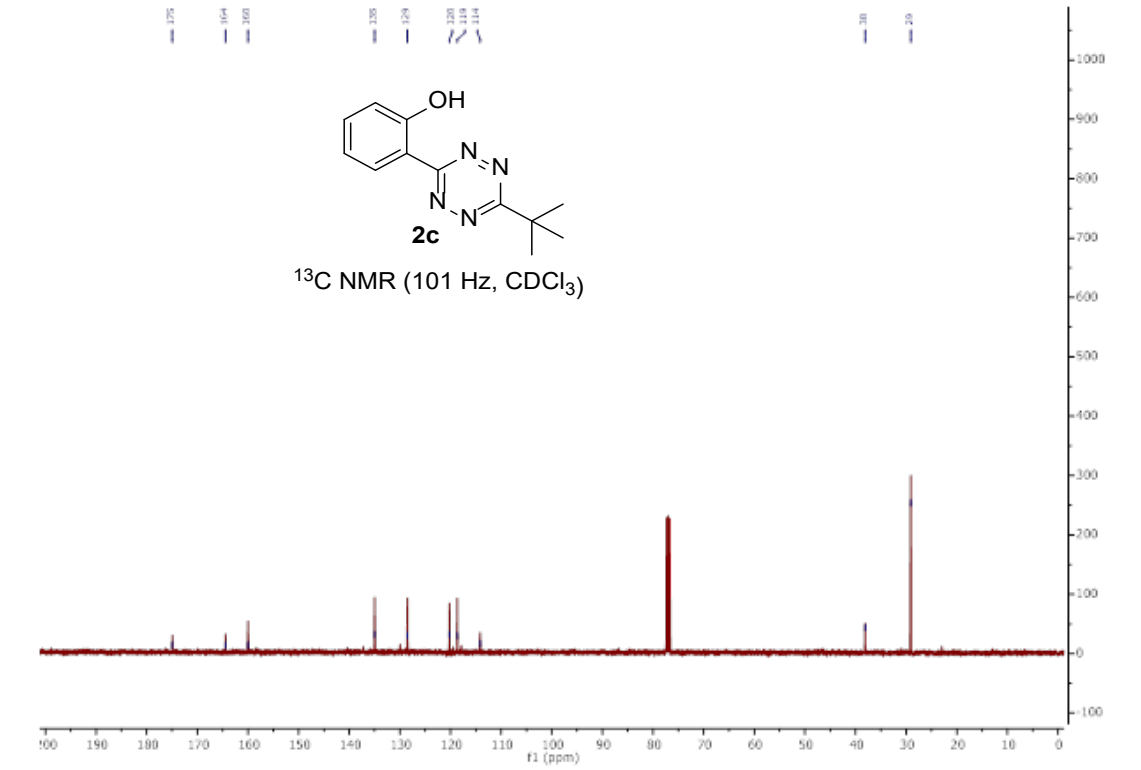

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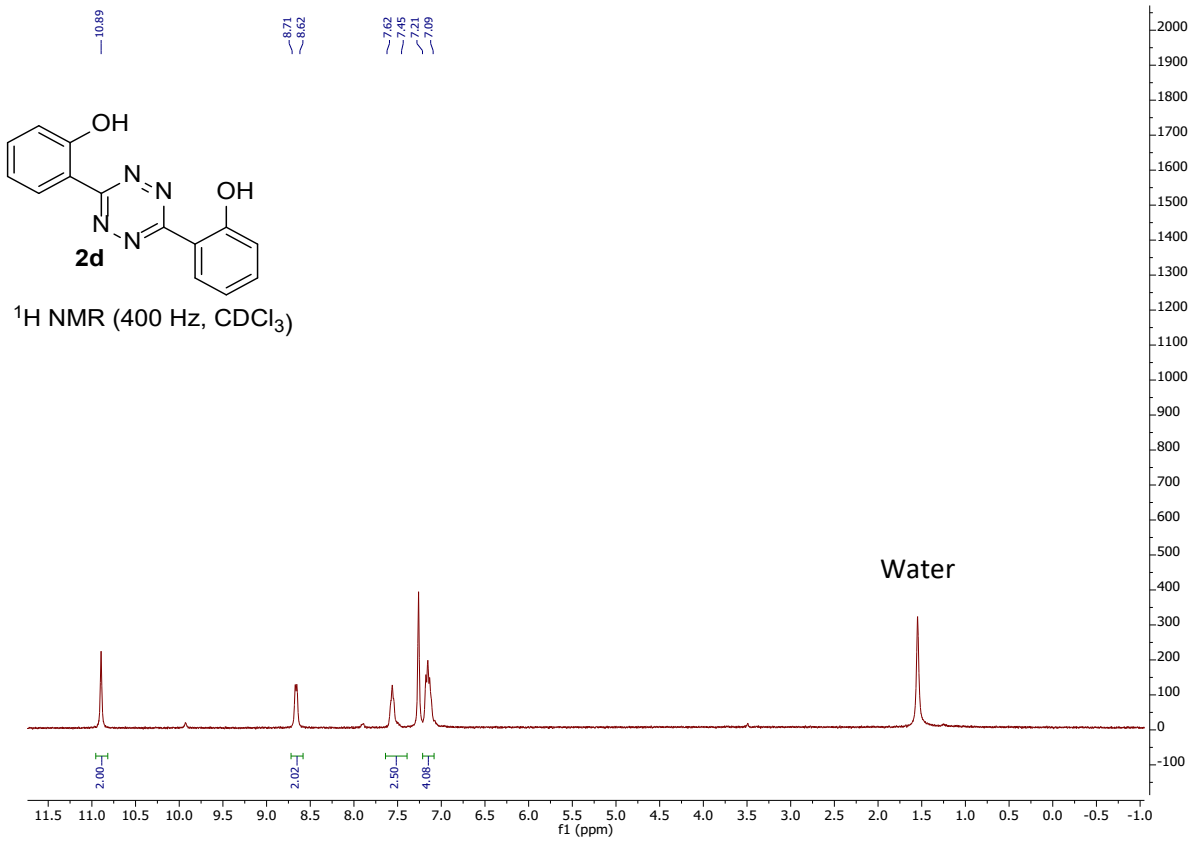

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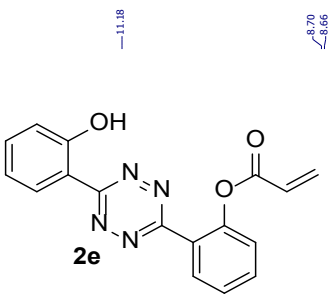

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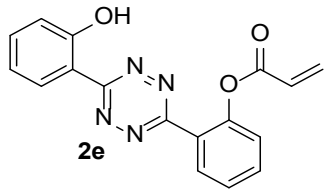

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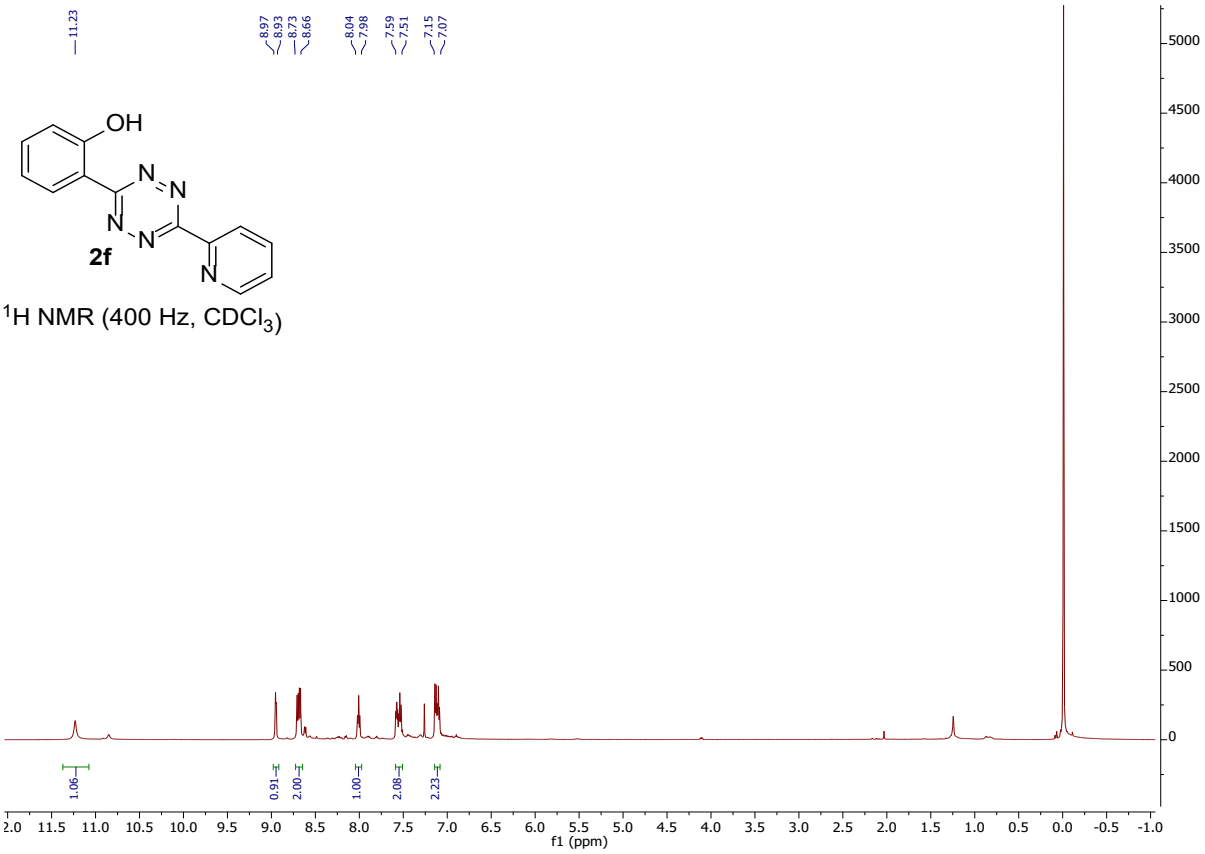

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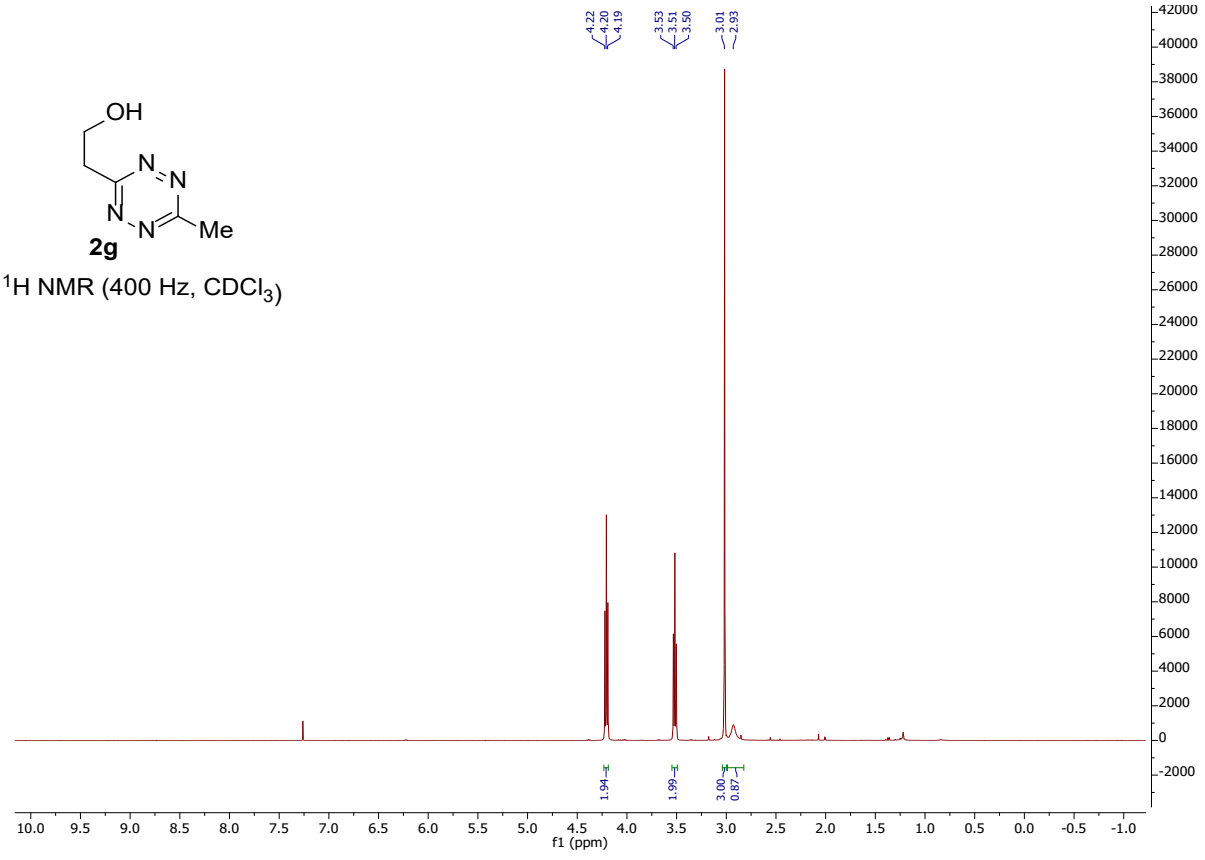

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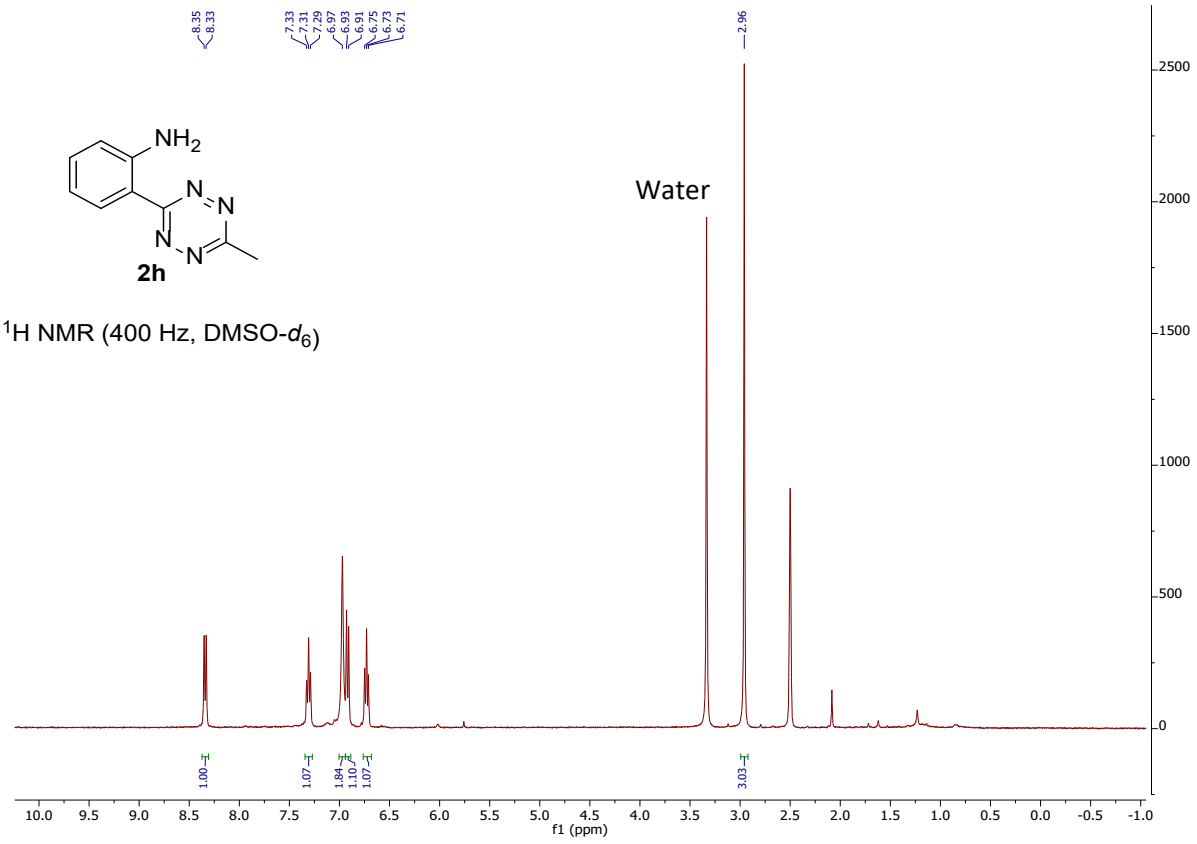

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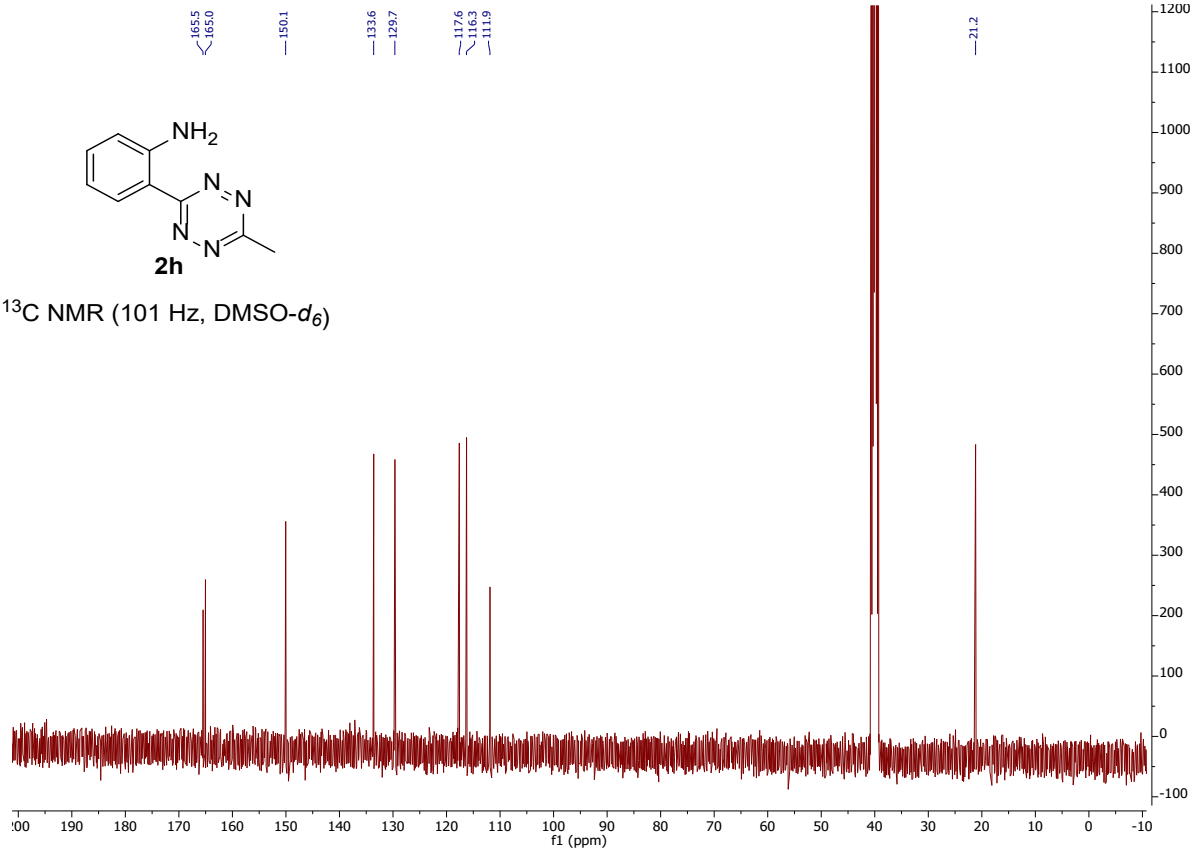

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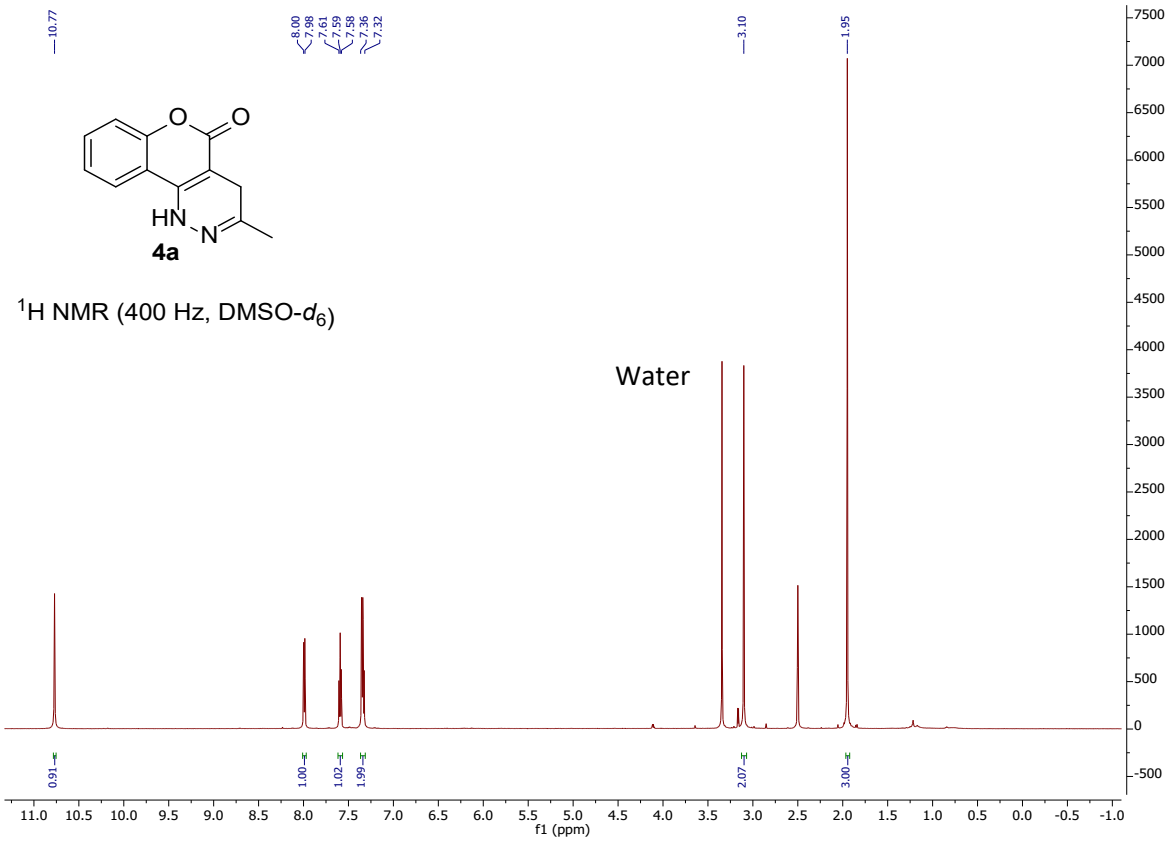

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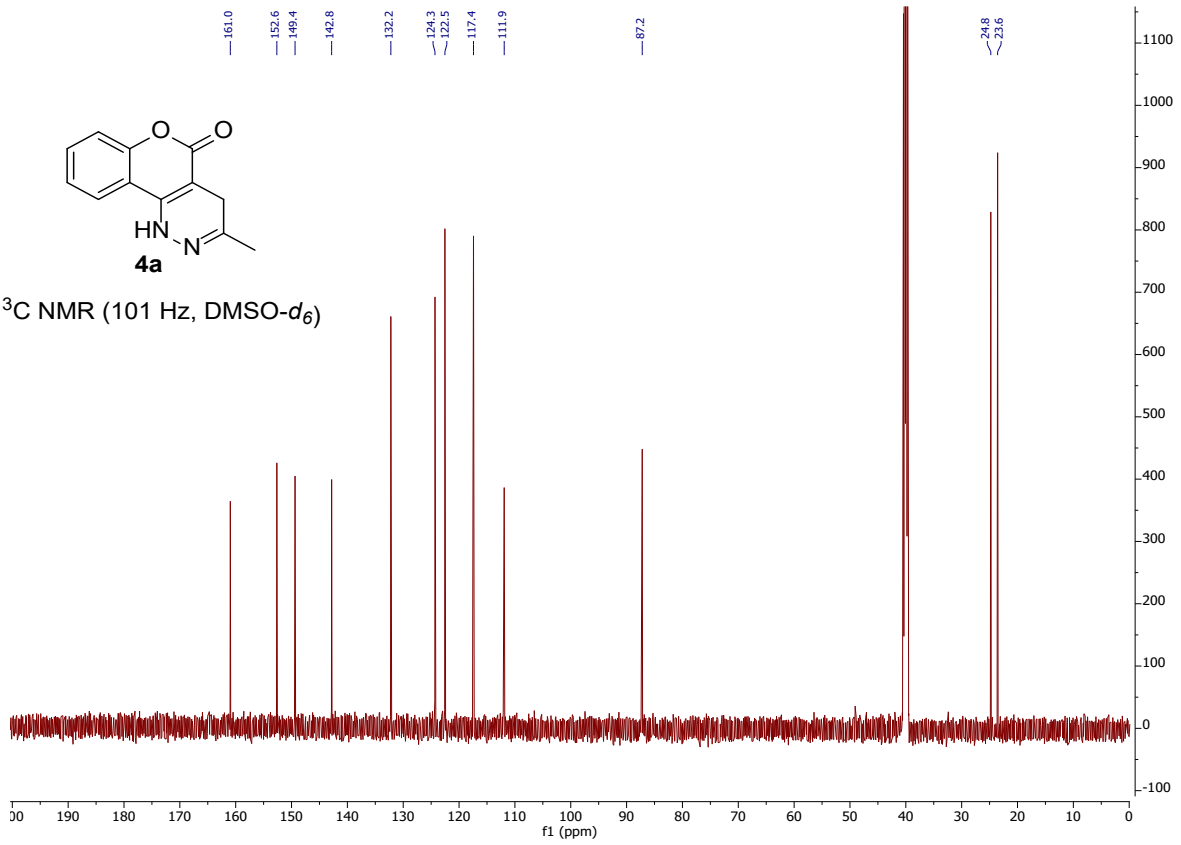

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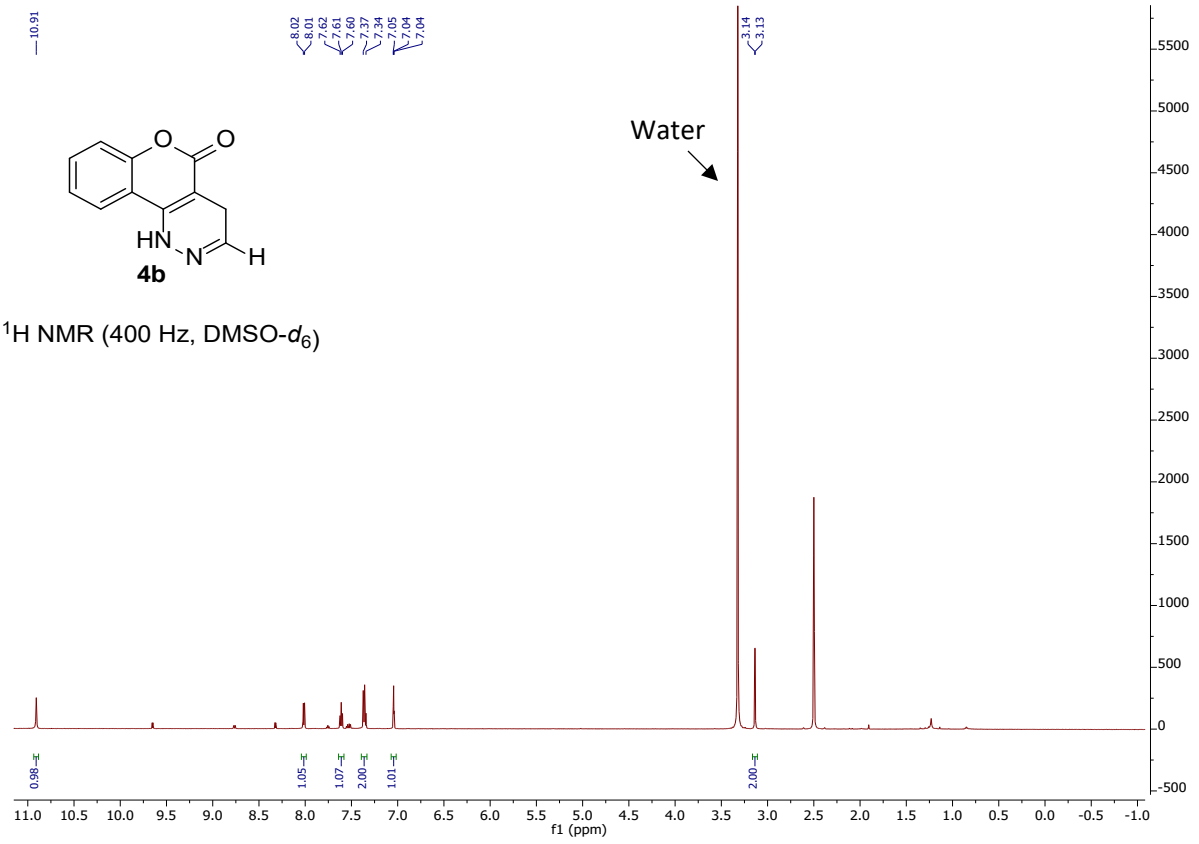

4b

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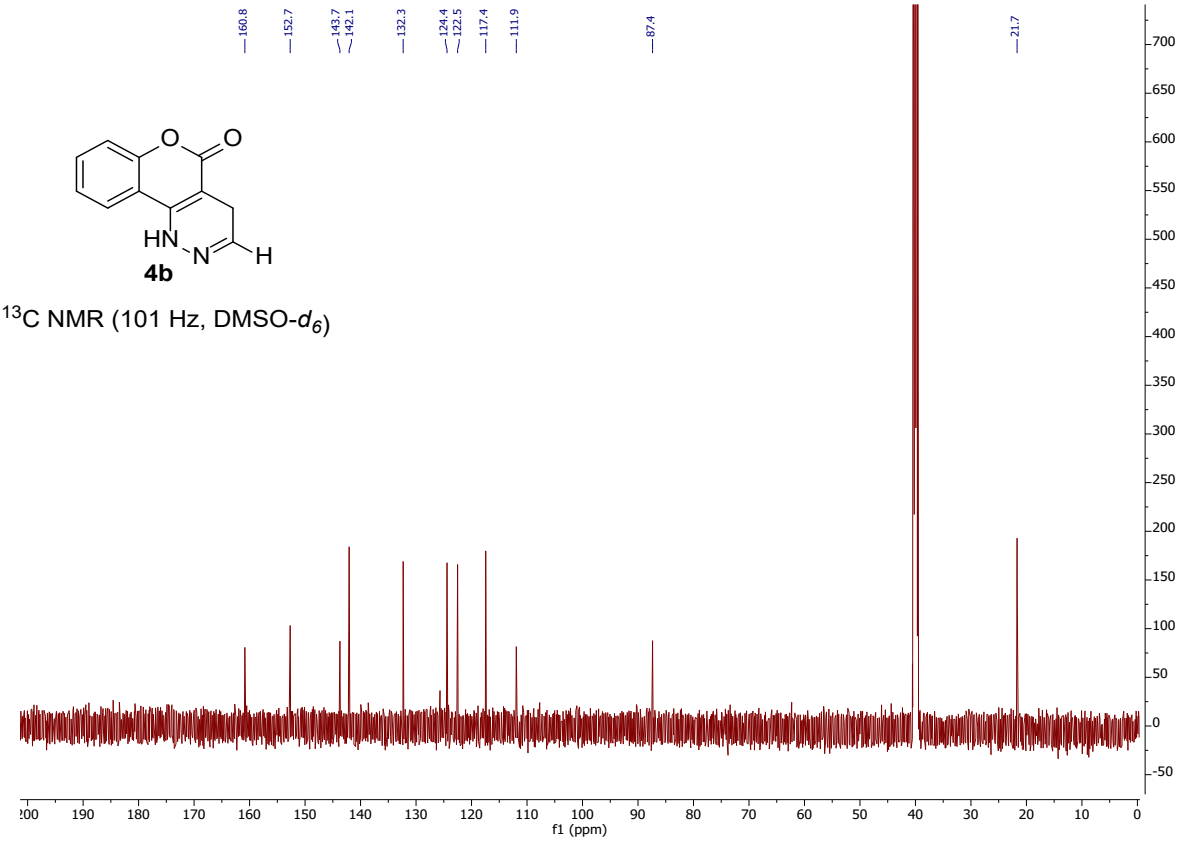

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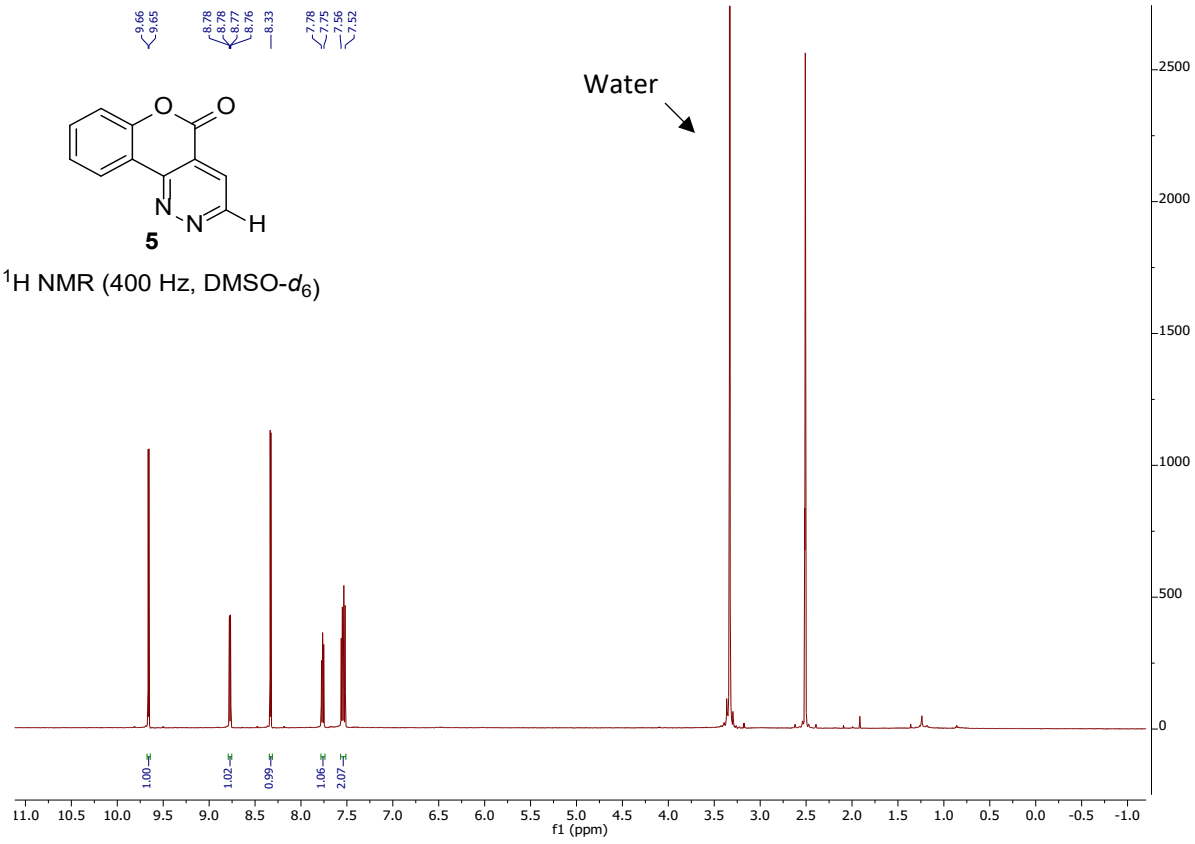

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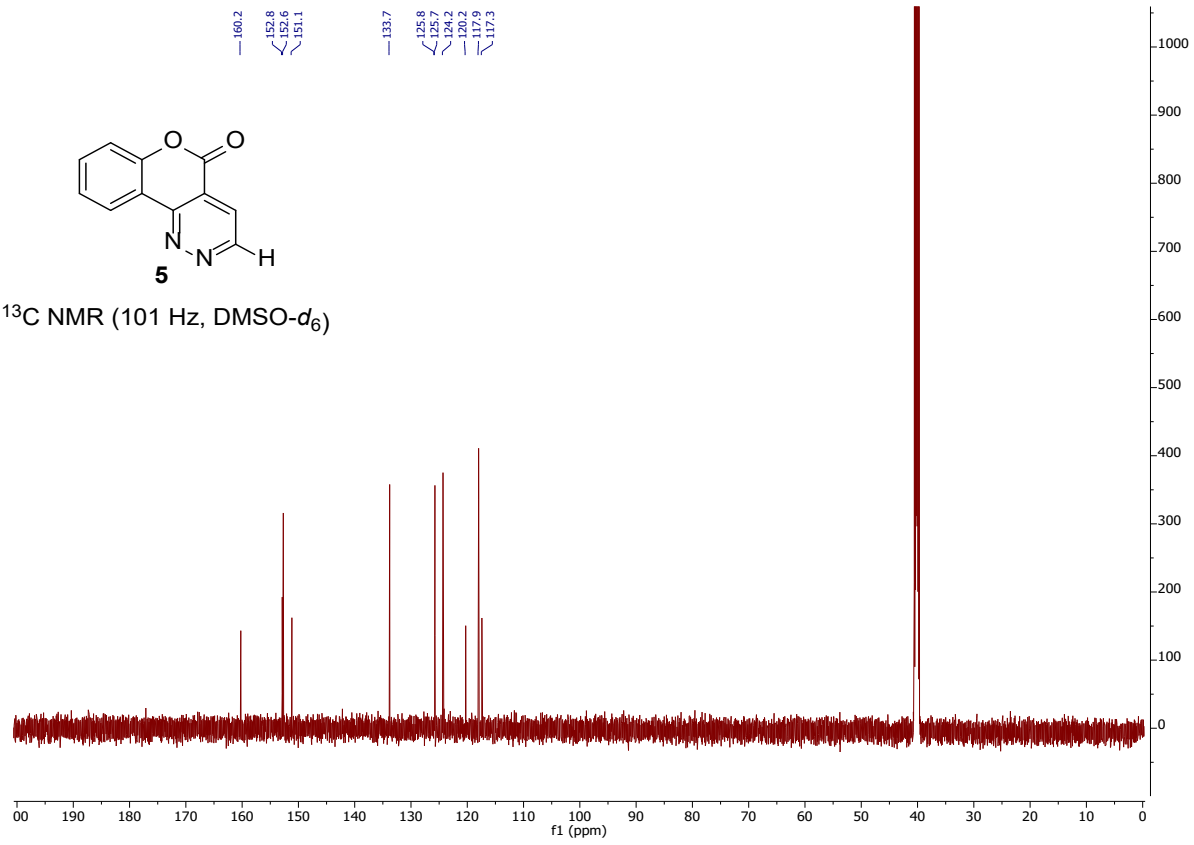

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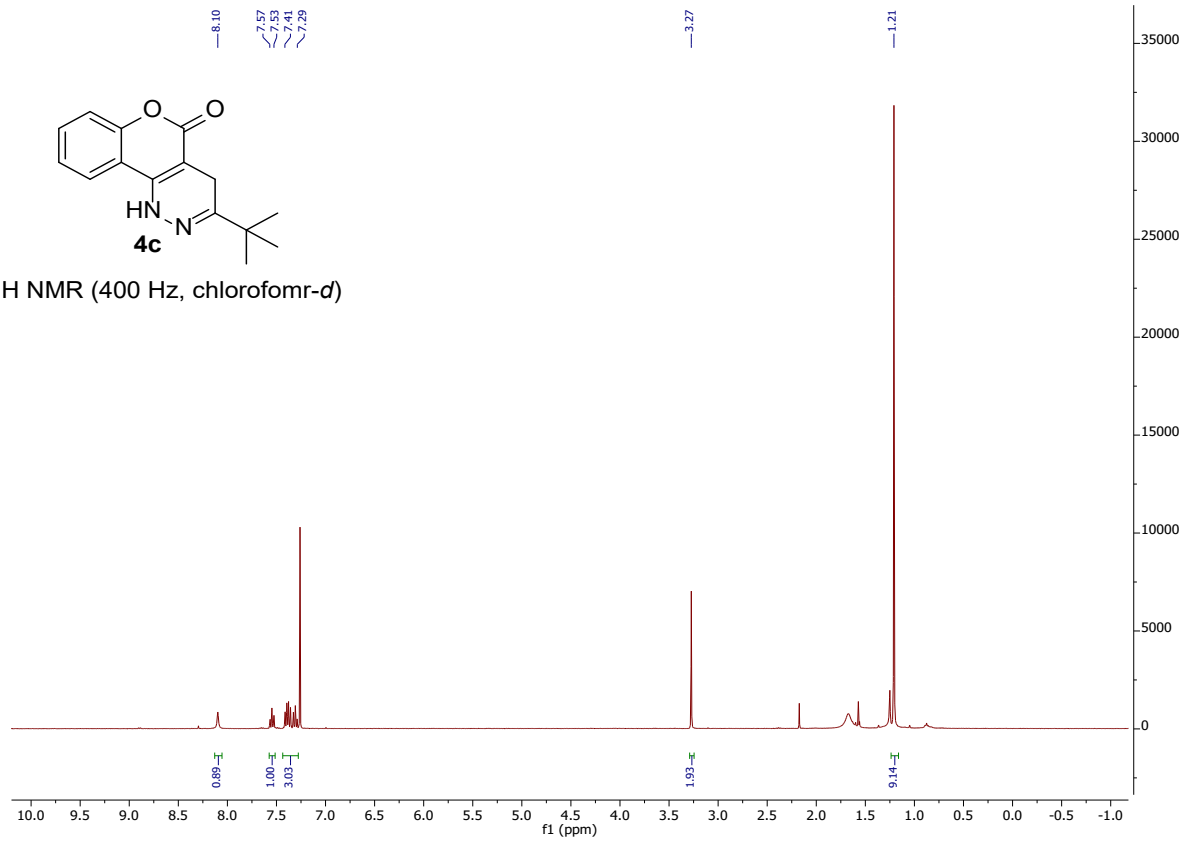

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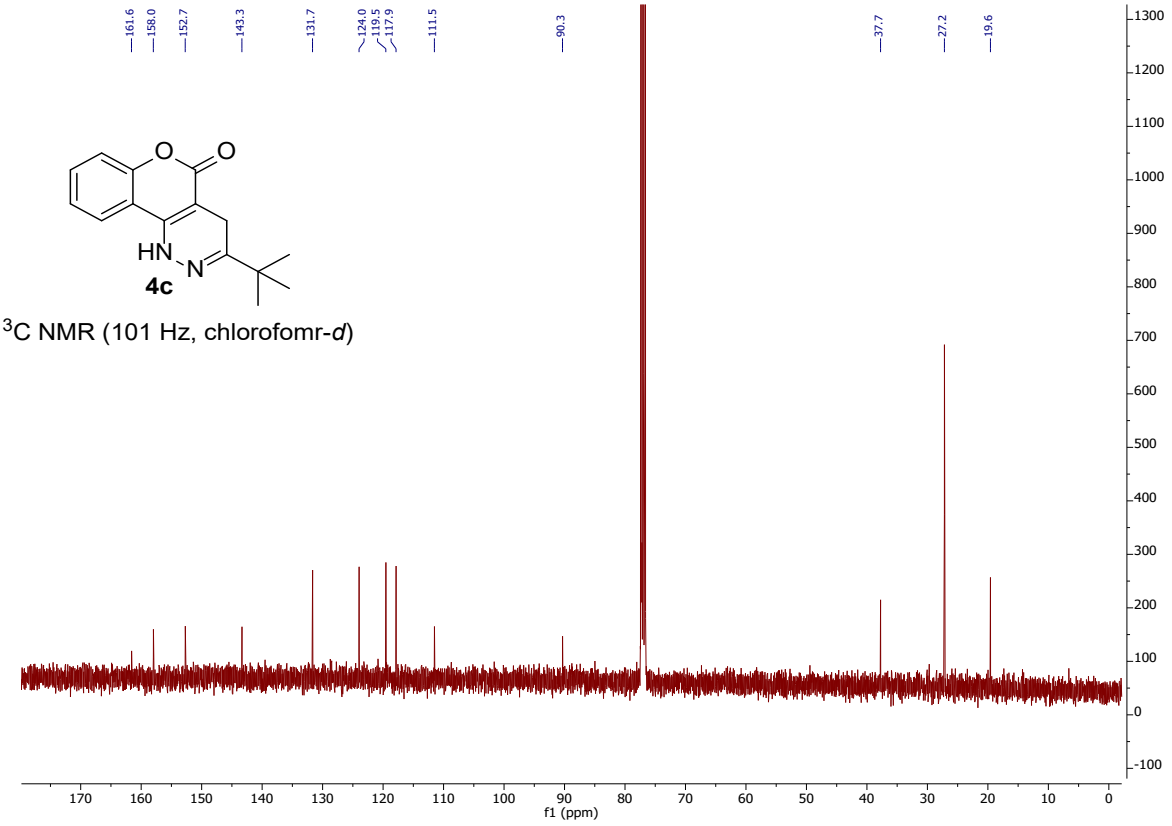

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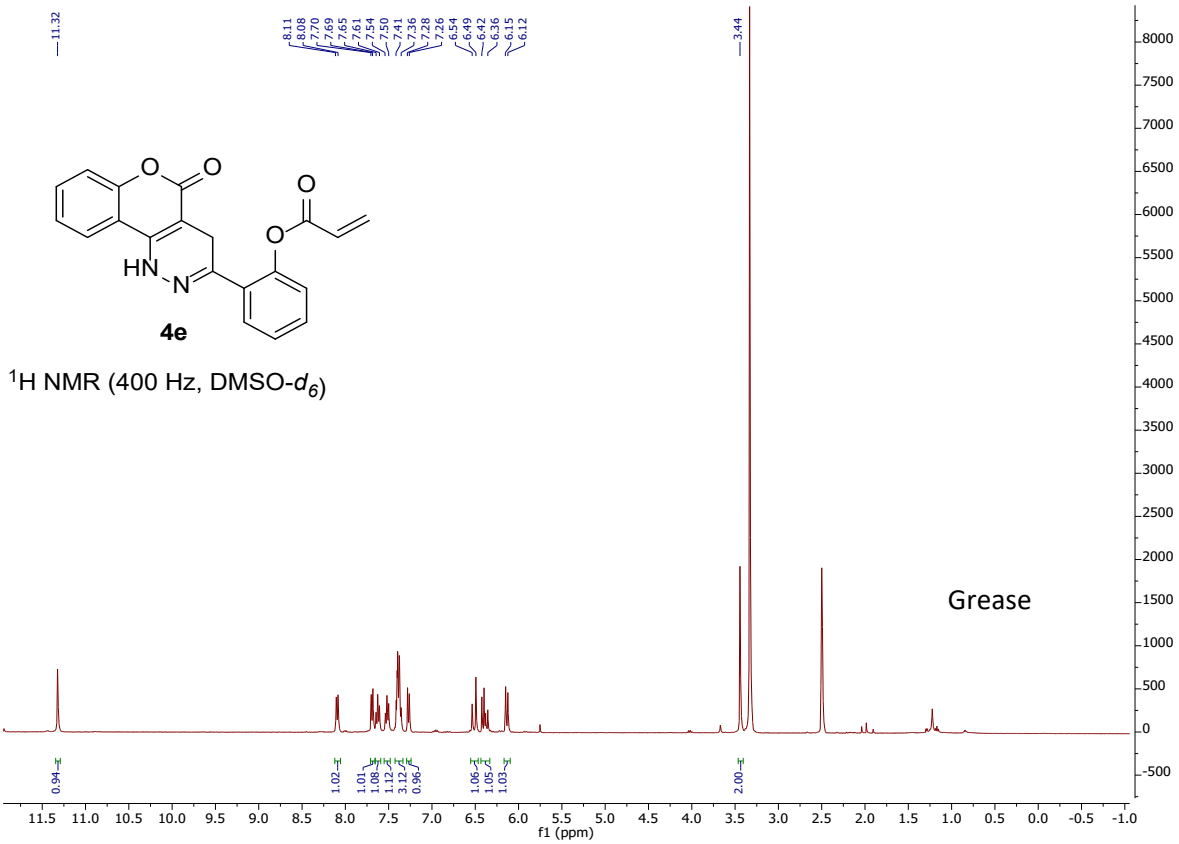

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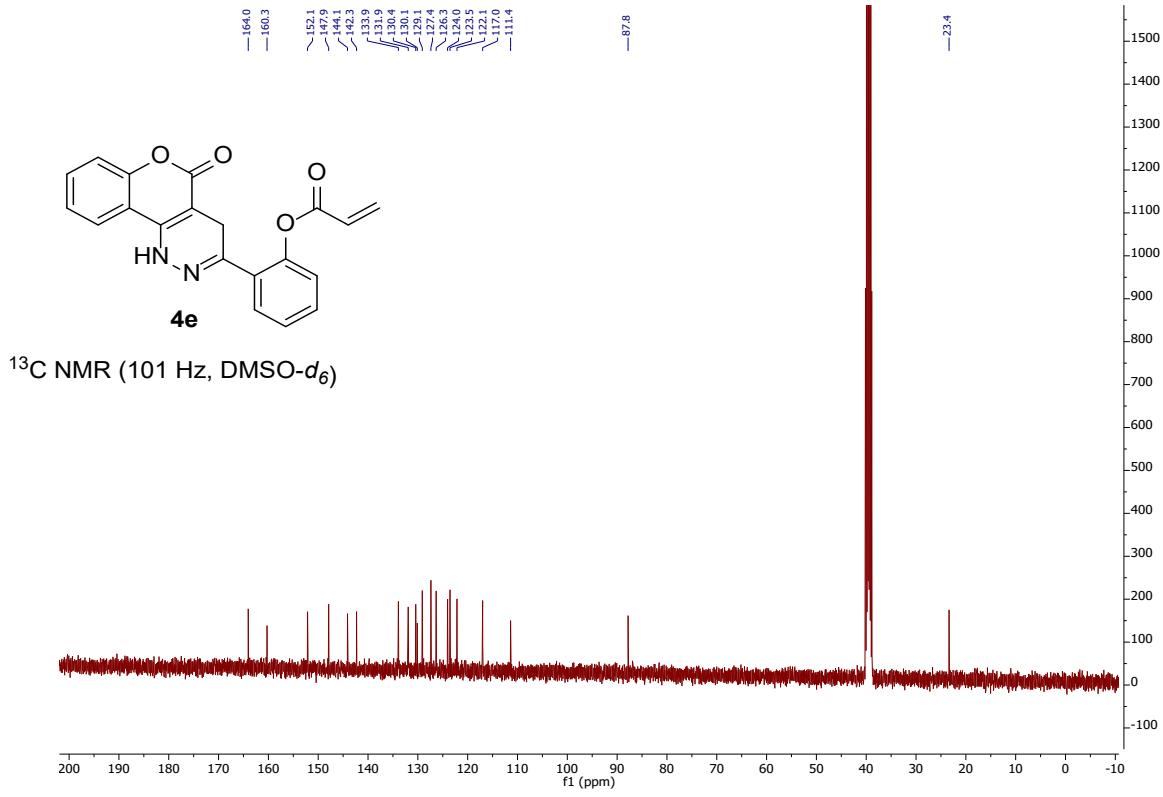

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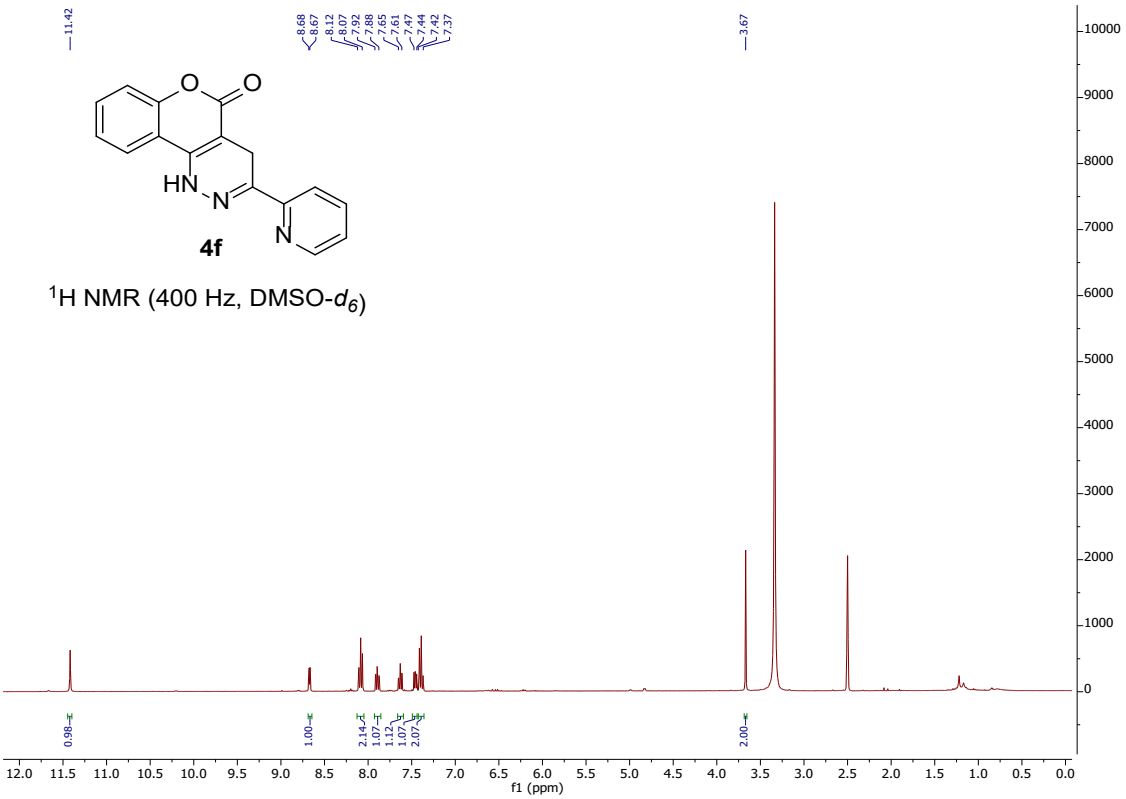

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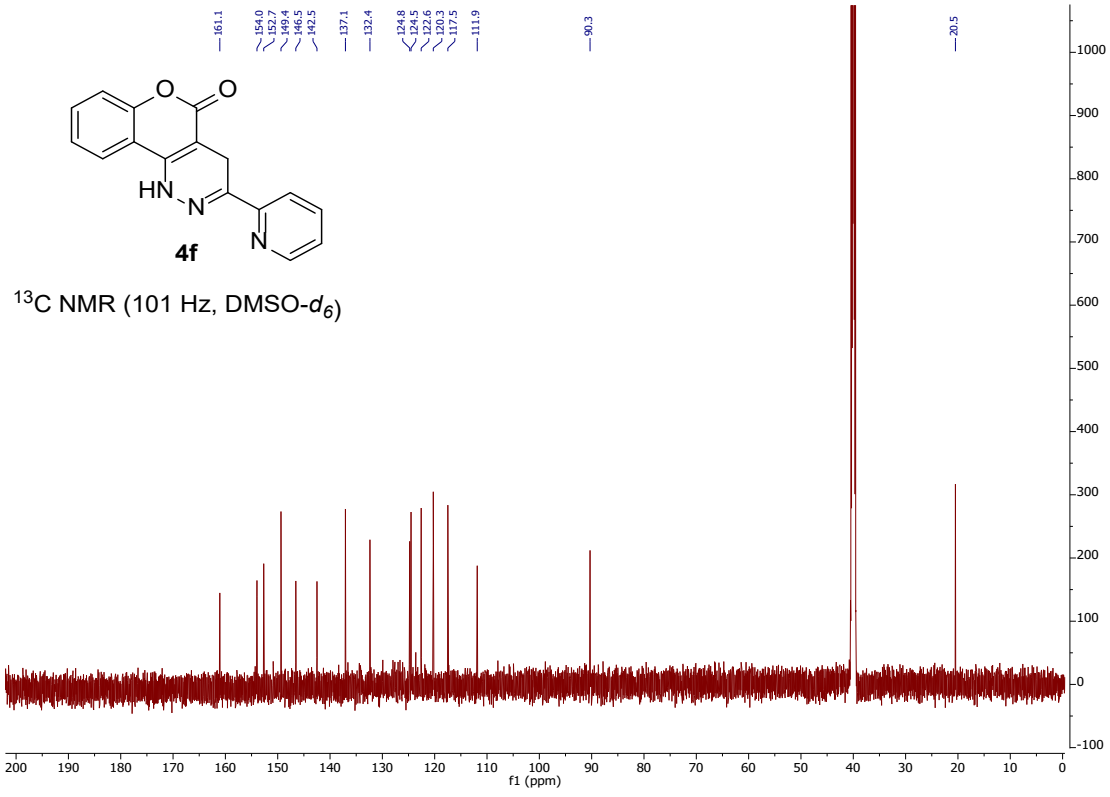

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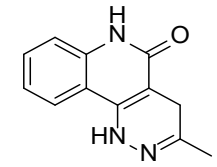

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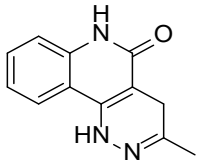

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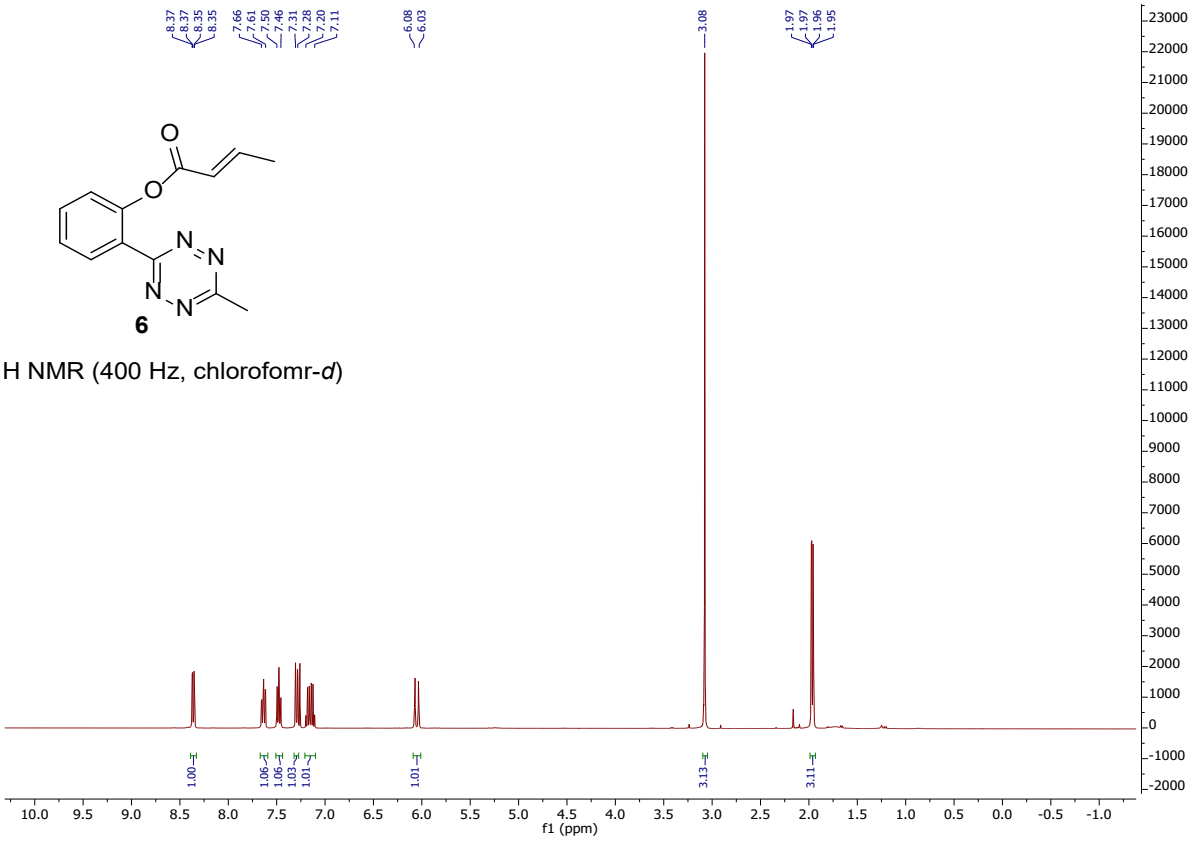

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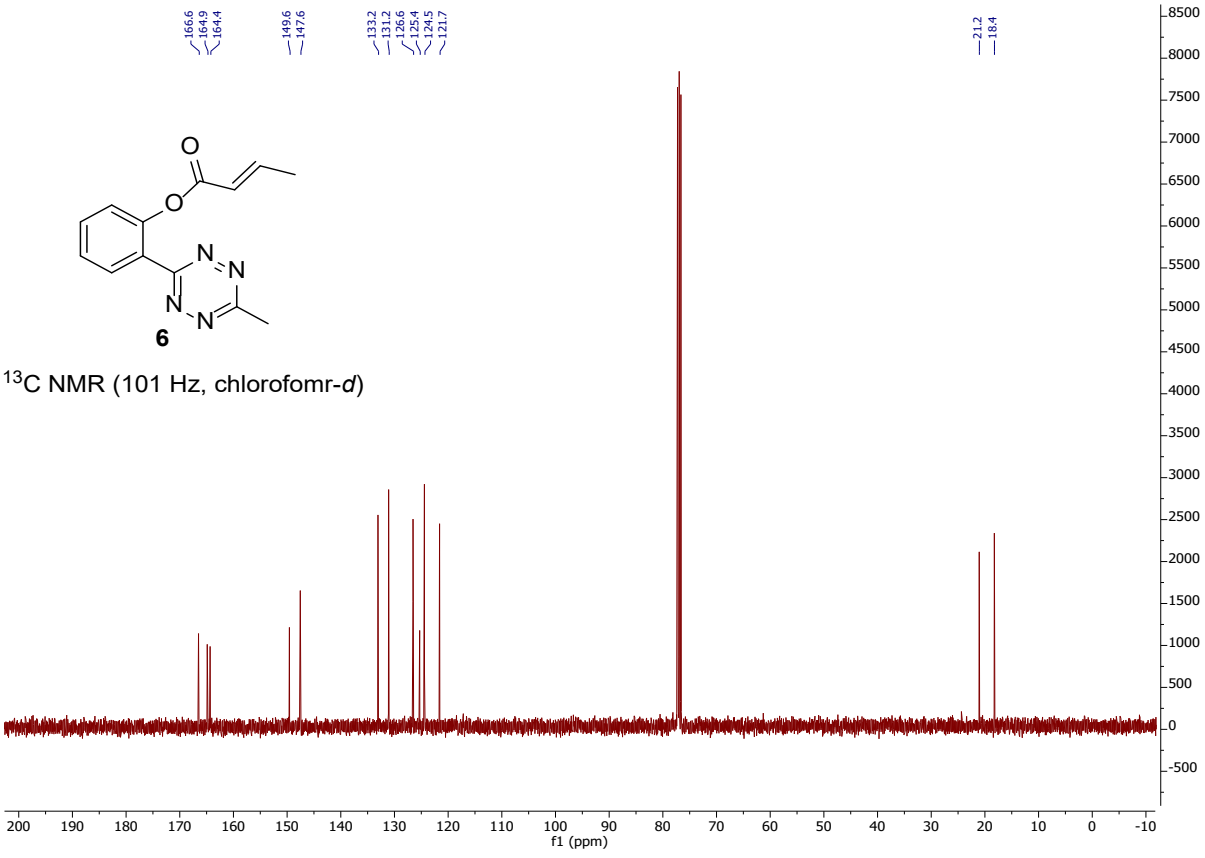

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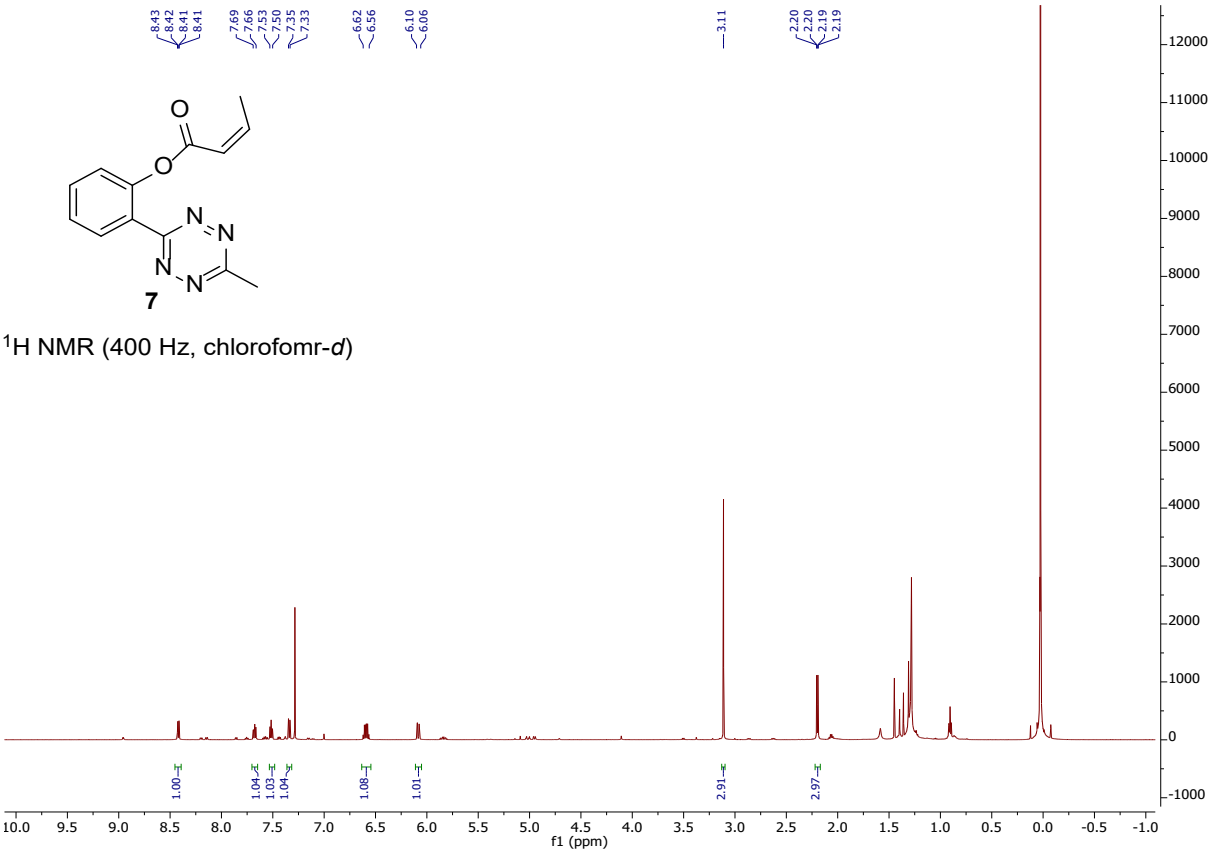

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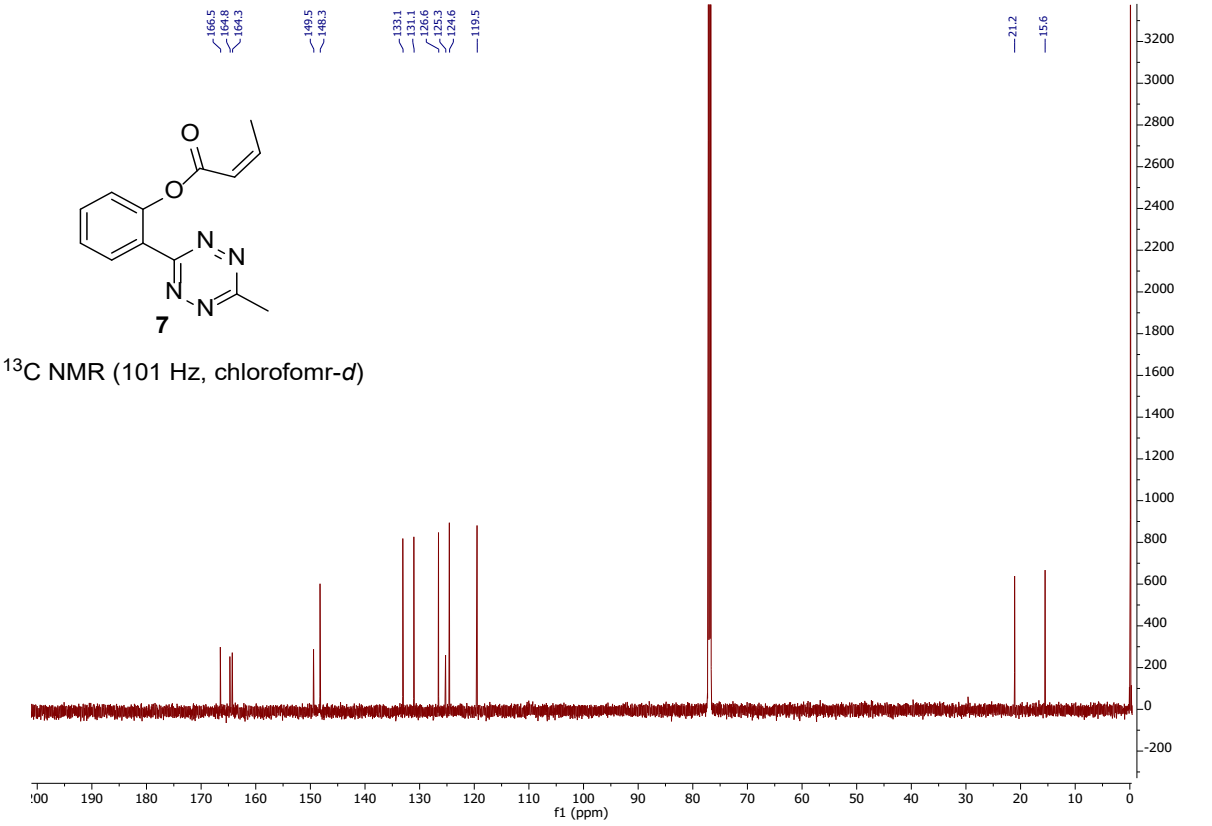

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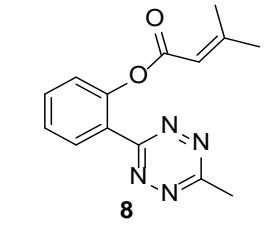

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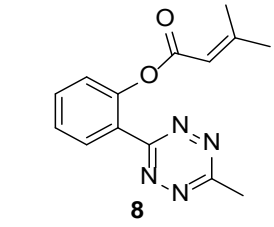

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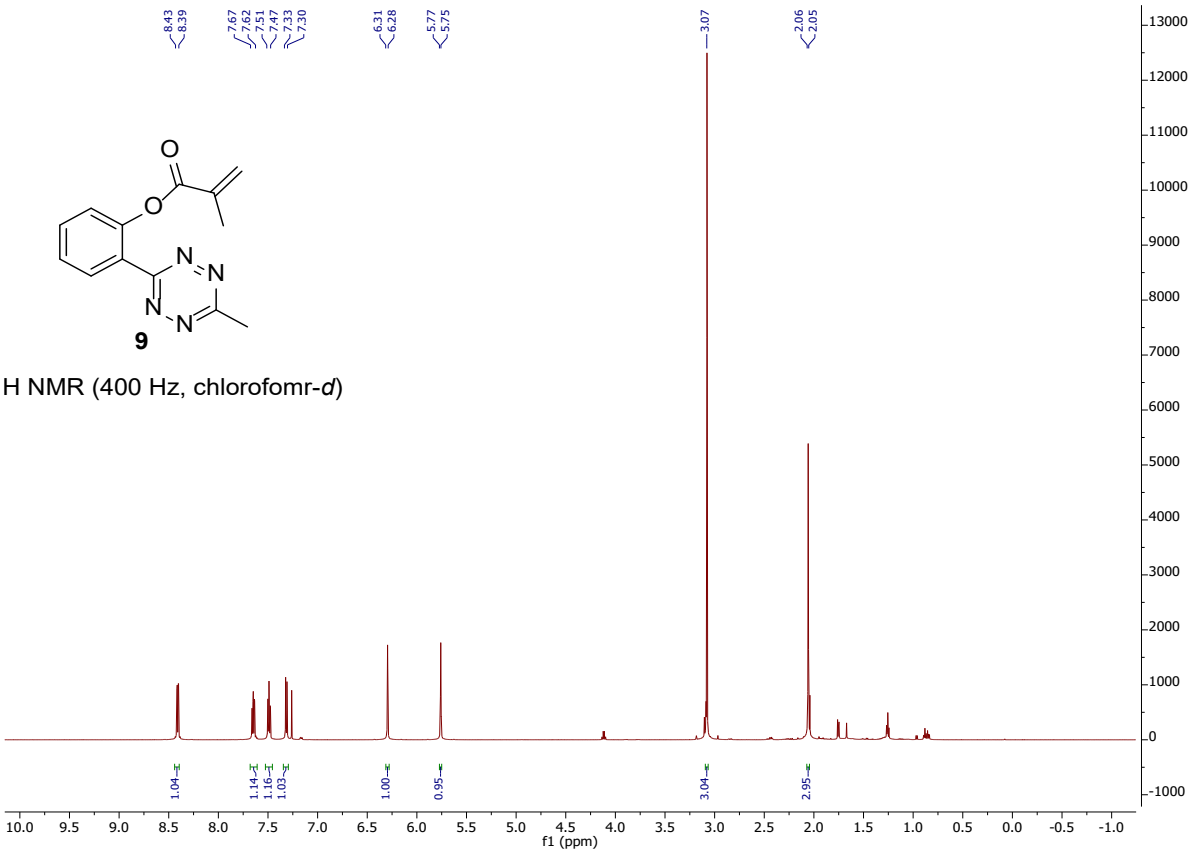

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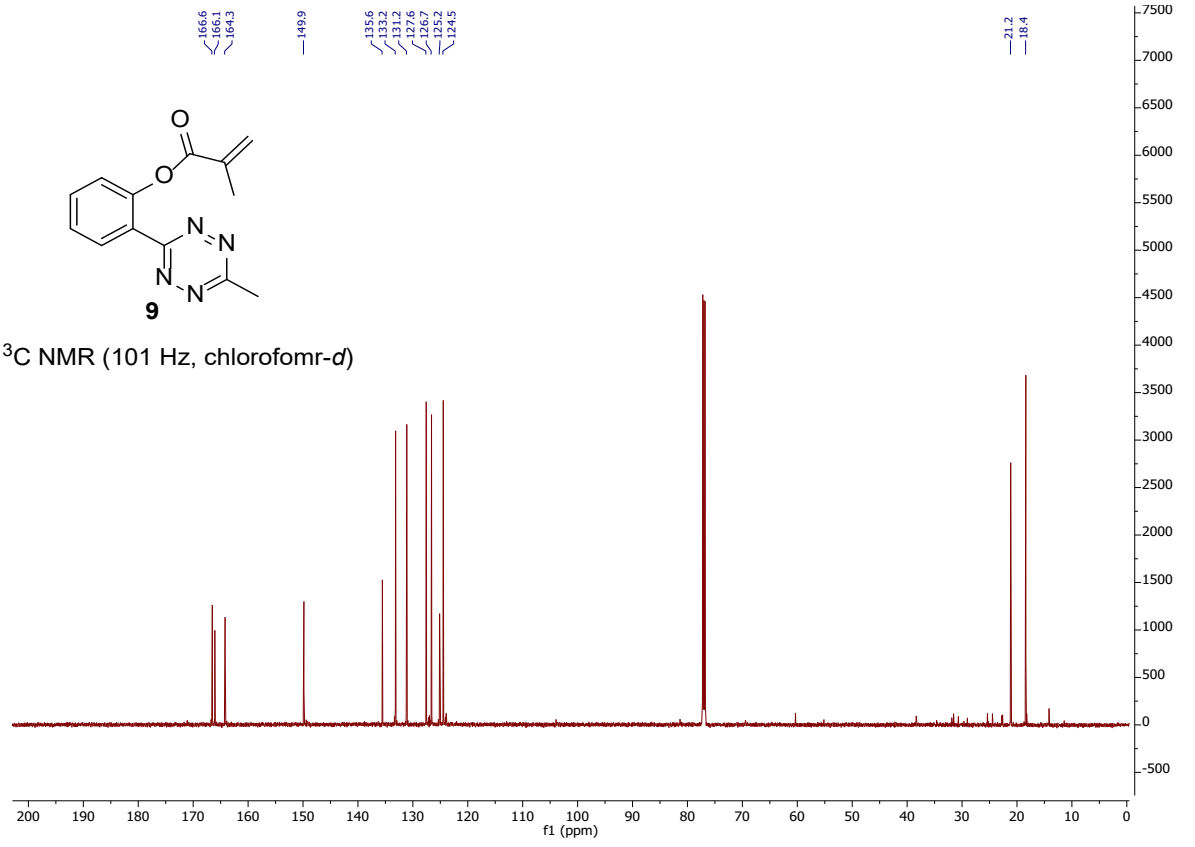

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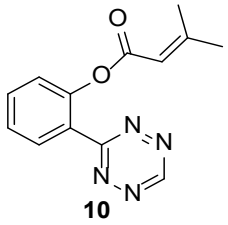

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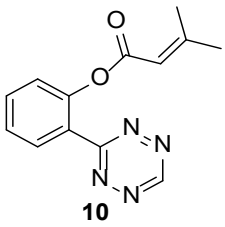

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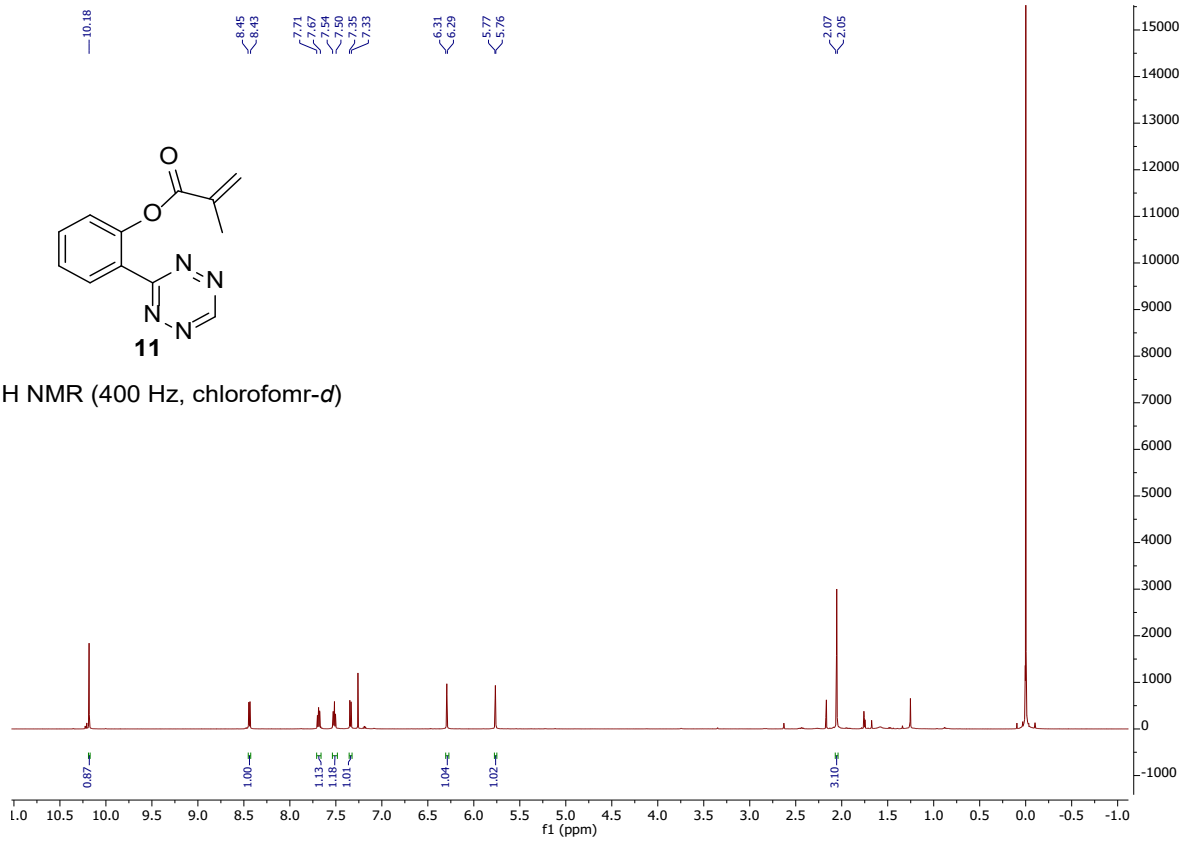

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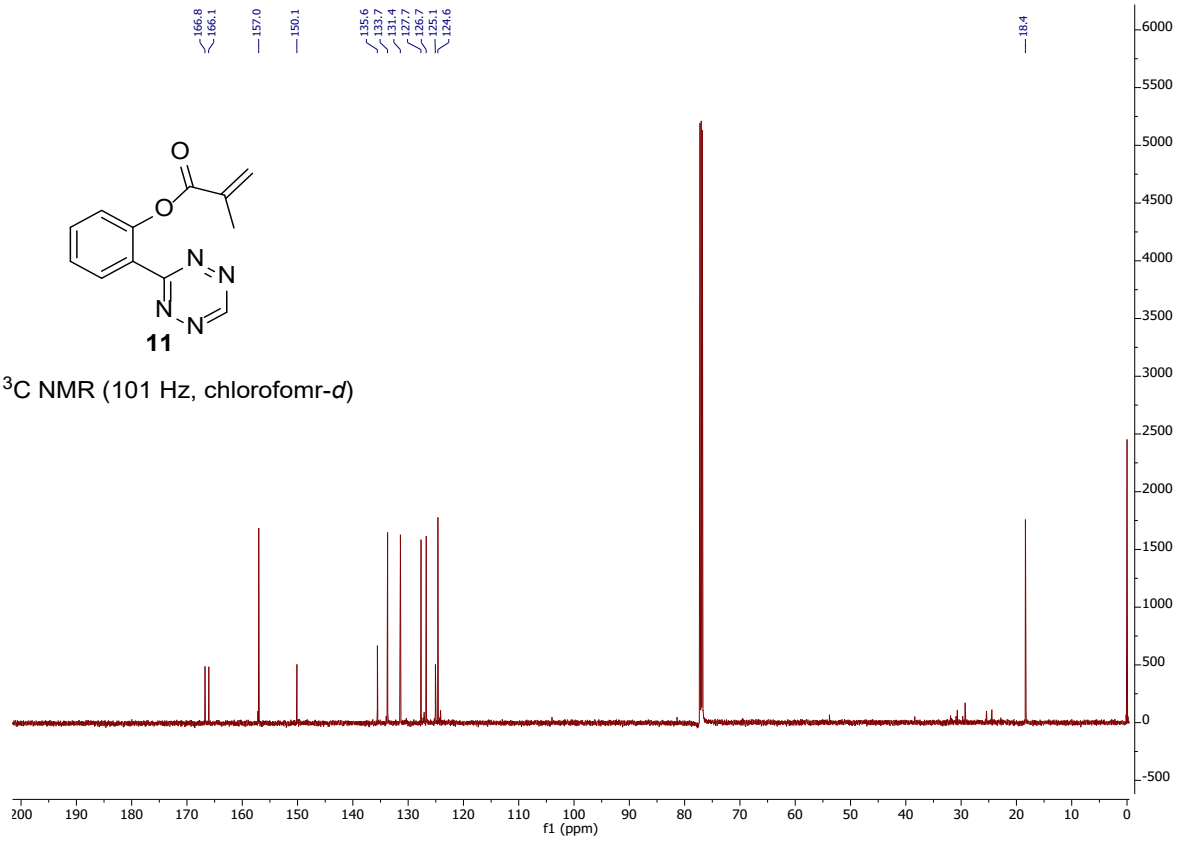

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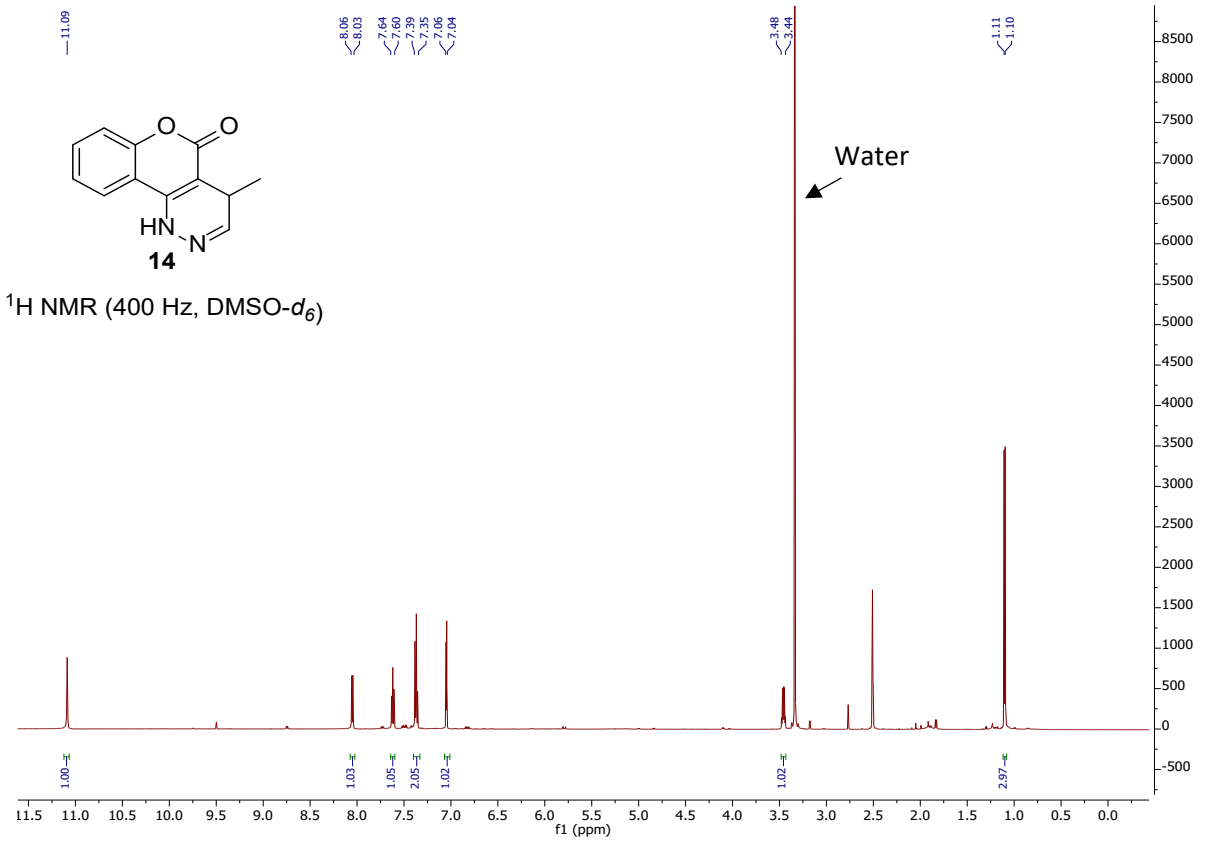

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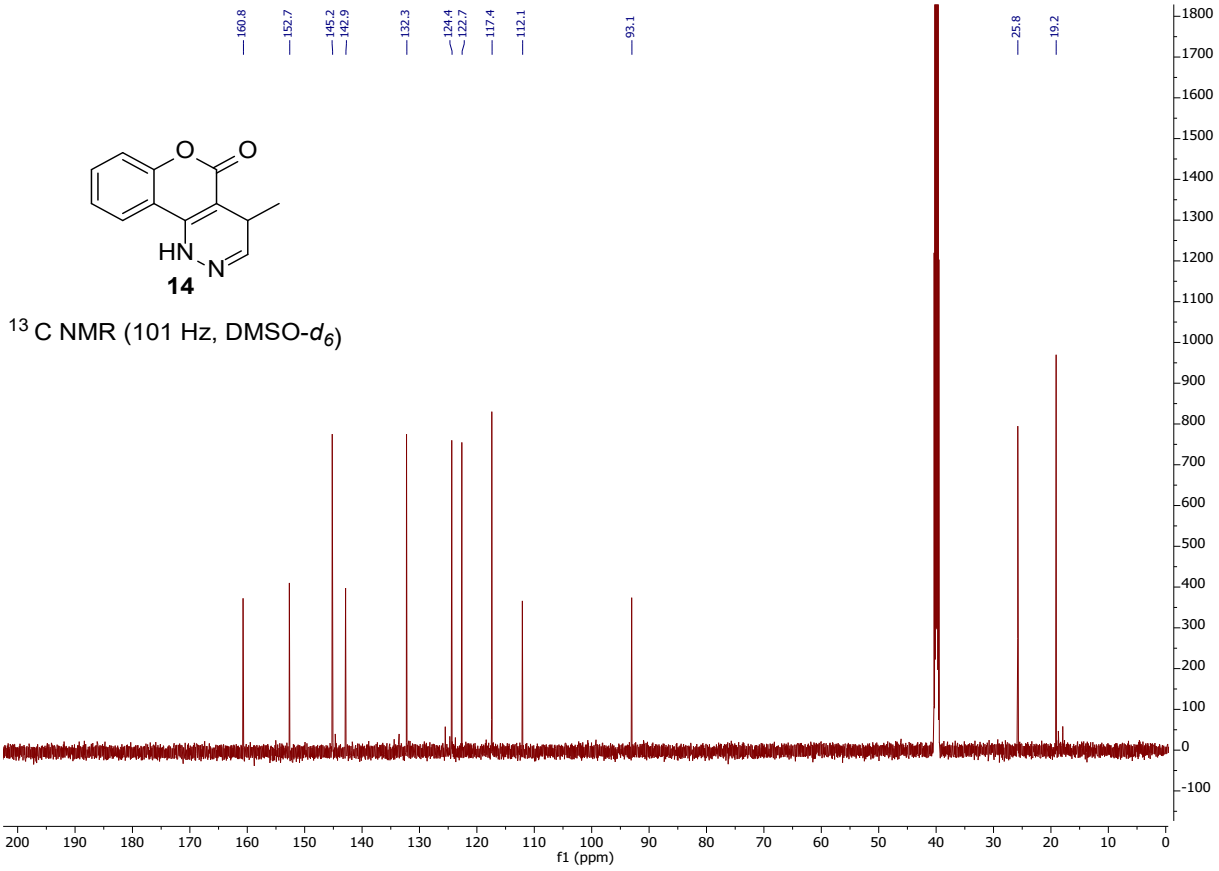

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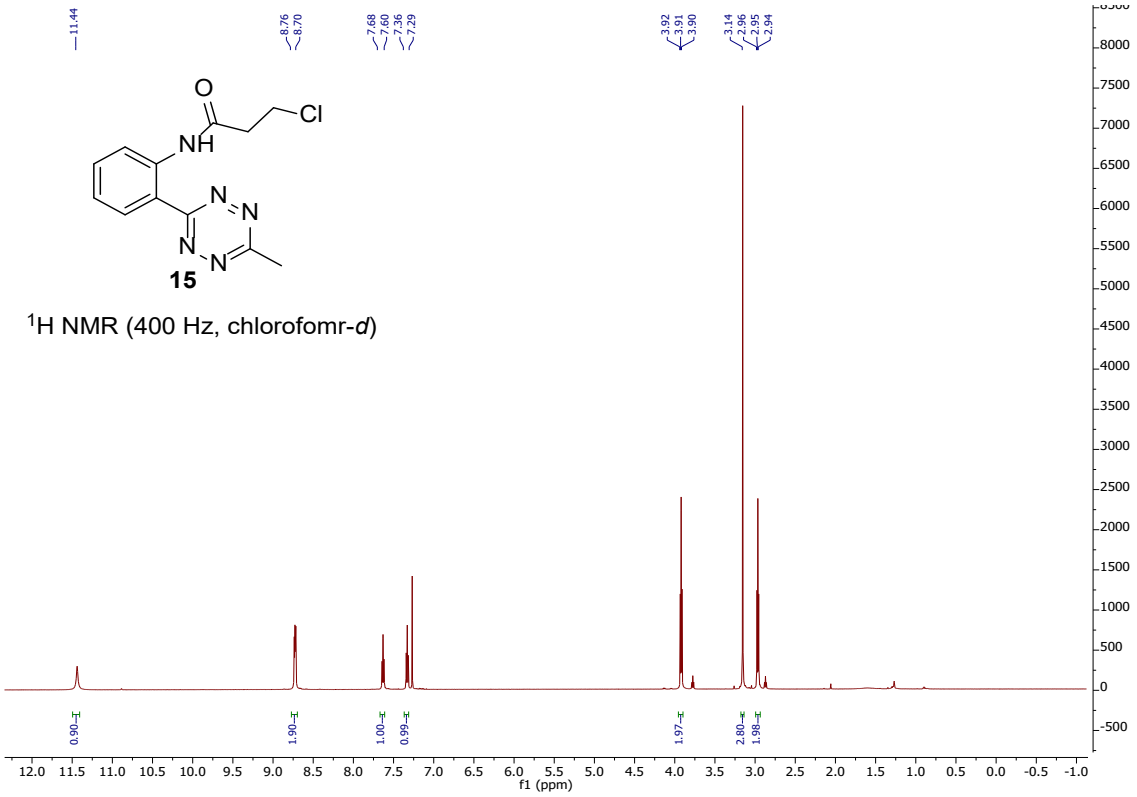

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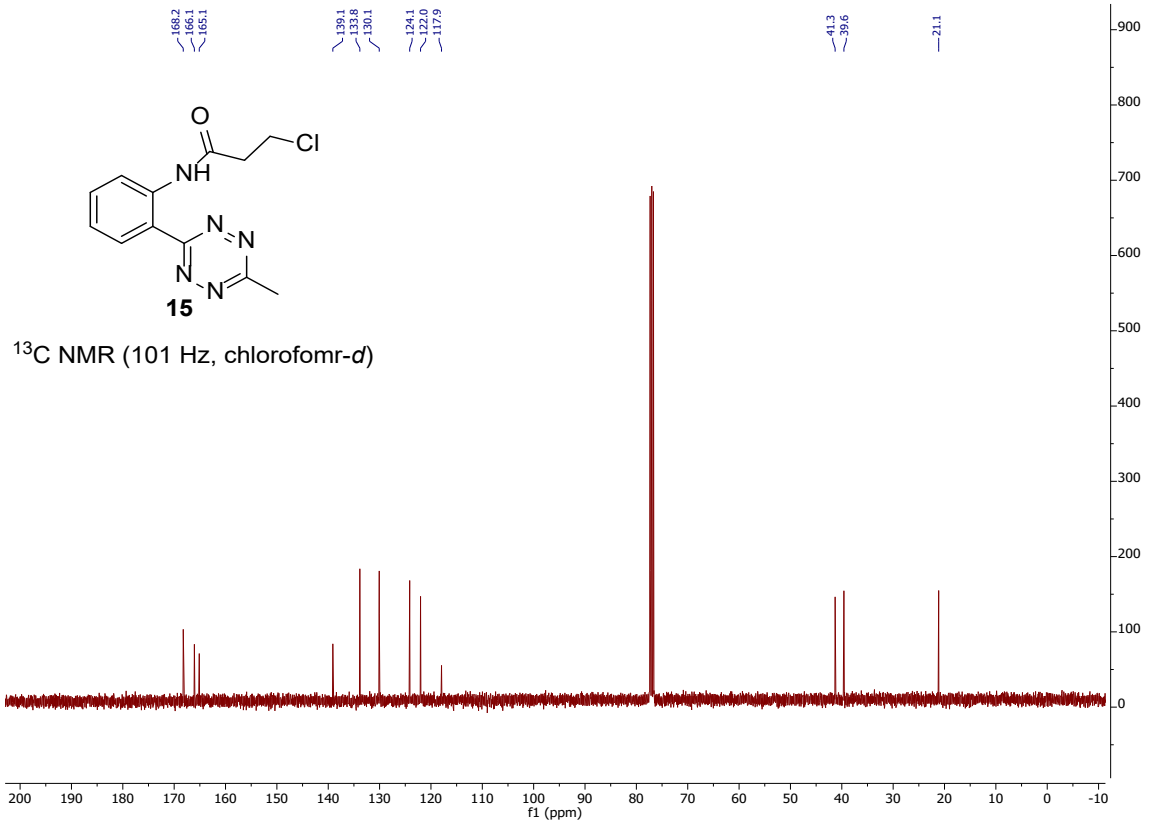

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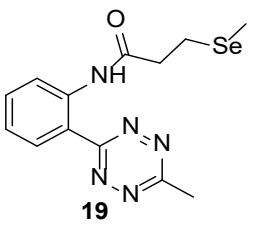

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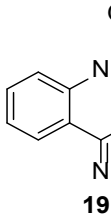

19

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