Electronic Supplementary Information for

Photoactive NO hybrids with pseudo-zero-order release kinetics for antimicrobial applications

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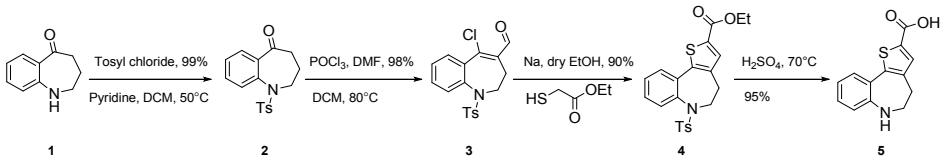
Experimental

1. General information

All chemicals and solvents were purchased from commercial supplier and used without further purification unless otherwise noted. Thin layer chromatography was performed on a 0.25 mm silica gel plate. The silica gel used for column chromatography is 200-300 mesh. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance III 400 MHz NMR spectrometer. High-resolution mass spectrometry (HRMS) was measured using electrospray ionization (ESI). HPLC analysis was performed on Shimadzu LC-20AT system using a Cromasil C18 column (5 μm, 250 mm x 4.6 mm). UV-Vis absorption and fluorescence spectra were recorded on Hitachi U-3900 spectrophotometer and Hitachi F-4600 fluorescence spectrometer, respectively. EPR experiments were carried out on a Bruker EMX-plus X-band spectrometer at room temperature.

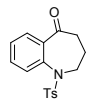
2. Synthetic procedure and structural characterization

2.1 Synthesis of intermediate 5



Scheme S1: The synthetic routes for intermediate 4,5-dihydro-6*H*-thieno[3,2-d]benzazepine (**5**)

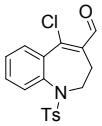
1-tosyl-1,2,3,4-tetrahydro-5H-benzo[b]azepin-5-one (2)[1]



2

To the solution of compound 1 (200 mg, 1.24 mmol) in anhydrous DCM (8 mL) was added pyridine (150 μL, 1.86 mmol). After the solution was cooled to 0°C using an ice-bath, tosyl chloride (709.6 mg, 3.7 mmol) was added dropwise through a syringe. After addition, the resulting mixture was refluxed at 50°C for 24 h. After completion, the mixture was adjusted to neutral pH with 0.5 M HCl (200 μL), and extracted with DCM three times (3 × 30 mL). The combined organic layer was rinsed with saline, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography, eluting with petroleum ether/EtOAc (from 10:1 to 2:1, v/v) to afford the desirable compound as white solid (387 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.61 (d, *J* = 8.4 Hz, 2H), 7.57-7.48 (m, 2H), 7.41 (td, *J* = 7.6, 1.2 Hz, 1H), 7.29 (d, *J* = 7.6 Hz, 2H), 3.88 (t, *J* = 6.8 Hz, 2H), 2.45 (s, 3H), 2.44-2.41 (m, 2H), 2.01-1.95 (m, 2H);

5-chloro-1-tosyl-2,3-dihydro-1H-benzo[b]azepine-4-carbaldehyde (3)[2]

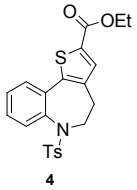


3

A solution of $POCl_3$ (1.172 mL, 12.57 mmol) in DCM (1.108 mL) was added dropwise to an ice-cooled solution of DMF (1.26 mL, 16.35 mmol) in DCM (1.26 mL) in argon atmosphere. Subsequently, the solution of compound 2 (1 g, 3.17 mmol) in anhydrous DCM (5 mL) was added into the prepared solution via a syringe at 0°C. After addition, the resulting mixture was refluxed at 80°C for 2 h. After completion, the mixture was cooled using an ice-bath, and the reaction was quenched by addition of ice-water (20 mL). Then mixture was extracted with DCM three times (3 × 40 mL). The combined organic layer was washed with water (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford the desirable compound as faint yellow solid (1.129 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1H),

7.62-7.48 (m, 4H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 4.14 (s, apparent br, 2H), 2.41 (apparent s, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.6, 147.2, 143.5, 137.3, 137.0, 136.0, 135.2, 133.2, 131.9, 129.8, 129.5, 129.0, 127.1, 57.0, 24.0, 21.5

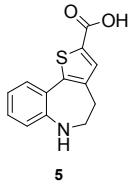
Ethyl 6-tosyl-5,6-dihydro-4H-benzo[b]thieno[2,3-d]azepine-2-carboxylate (4)[2]



4

The solution of EtONa was freshly prepared by adding the sodium metal (25.4 mg, 1.1 mmol) into the dry EtOH (3.048 mL) at 0°C. Subsequently, ethyl 2-mercaptoacetate (60.56 μL, 0.55 mmol) and compound 3 (200 mg, 0.55 mmol) were added sequentially to the solution at 0°C. After addition, the reaction mixture was slowly warmed to room temperature and stirred overnight. After completion indicated by TLC, the mixture was extracted with ethyl acetate three times (3 × 30 mL). The combined organic layer was washed with 0.5 M HCl (20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography, eluting with petroleum ether/EtOAc (from 100:1 to 35:1, v/v) to afford the desirable compound as faint yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.48 (dd, *J* = 7.2, 1.0 Hz, 1H), 7.41-7.33 (m, 2H), 7.28 (s, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 6.94 (d, *J* = 8.0 Hz, 2H), 4.35 (q, *J* = 6.8 Hz, 2H), 4.14 (s, apparent br, 2H), 3.00 (t, *J* = 5.6 Hz, 2H), 2.25 (s, 3H), 1.39 (t, *J* = 7.6 Hz, 3H)

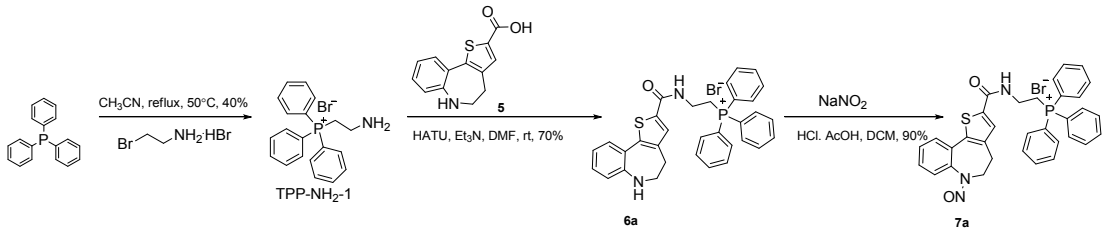
5,6-dihydro-4H-benzo[b]thieno[2,3-d]azepine-2-carboxylic acid (5)[3]



5

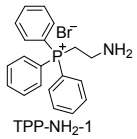
Compound 4 (200 mg, 0.468 mmol) was slowly added to concentrated H_2SO_4 solution (90%, 2 mL) at 0°C using an ice-bath. Then the ice bath was removed, and the mixture was refluxed at 70°C for 2 h. After completion, the reaction mixture was added dropwise to ice-water (5 mL) and extracted with EtOAc three times (3 × 30 mL). The combined organic layer was washed with water, saline, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography with DCM/MeOH (from 100:1 to 20:1, v/v) as eluent to give the desirable compound as yellow needles (109 mg, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.93 (s, br, 1H), 7.53 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.49 (s, 1H), 7.06-7.02 (m, 1H), 6.79 (d, *J* = 7.6 Hz, 1H), 6.69-6.65 (m, 1H), 6.44 (s, 1H), 3.28 (apparent t, *J* = 4.8 Hz, 2H), 2.99 (apparent t, *J* = 4.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.9, 148.6, 144.5, 138.4, 136.9, 128.8, 128.8, 128.7, 118.7, 117.9, 117.4, 43.8, 33.8

2.2 Synthesis of compound 7a



Scheme S2: The synthetic route for compound **7a**

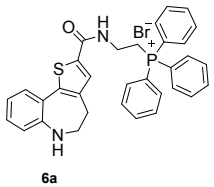
(2-aminoethyl)triphenylphosphonium bromide (TPP-NH_2-1)[4]



To the solution of triphenylphosphane (1 g, 3.812 mmol) in CH_3CN (10 mL) was added 2-bromoethan-1-amine hydrobromide (625 mg, 3.05 mmol). The resulting mixture was refluxed at 110°C for 15 h in argon atmosphere. After completion, the precipitate was filtered, dissolved in H_2O (5 mL), treated with saturated K_2CO_3 until neutral. Then the mixture was extracted with CH_2Cl_2 (3 × 30 mL), dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. After recrystallization from petroleum ether, the desirable compound was obtained as a white solid (640 mg, 40 %). ^{1}H

NMR (400 MHz, DMSO-d_6) δ 7.94-7.75 (m, 15H), 5.42 (s, br, 2H), 3.82-3.74 (m, 2H), 3.01-2.95 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 135.4 (d, *J* = 2.8 Hz), 133.7 (d, *J* = 10.6 Hz), 130.4 (d, *J* = 12.8 Hz), 117.2 (d, *J* = 86.7 Hz), 33.3, 19.6 (d, *J* = 54.6 Hz)

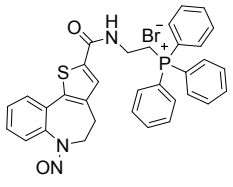
(2-(5,6-dihydro-4*H*-benzo[*b*]thieno[2,3-*d*]azepine-2-carboxamido)ethyl)triphenylphosphonium bromide (6a)



6a

To the solution of compound 5 (100 mg, 0.41 mmol) in anhydrous DMF (5 mL) was added HATU (166 mg, 0.44 mmol) and triethylamine (456 μL, 3.27 mmol). After stirring for 30 min, the intermediate TPP-NH_2-1 (168 mg, 0.4 mmol) was added. The reaction mixture was stirred overnight at room temperature. After completion, the mixture was extracted with ethyl acetate (3 ×20 mL), washed with water (20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography, eluting with DCM/MeOH (100:1,v/v) to afford the desirable compound as a yellow solid (175 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83-7.68 (m, 15H), 7.64 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.31 (s, 1H), 7.08-7.04 (m, 1H), 6.81-6.77 (m, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 3.83-3.76 (m, 2H), 3.56-3.50 (m, 2H), 3.41 (t, *J* = 4.8 Hz, 2H), 3.06 (t, *J* = 4.8 Hz, 2H) (two active protons less); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1, 147.8, 143.7, 138.5, 135.5 (d, *J* = 2.9 Hz), 133.4 (d, *J* = 10.2 Hz), 132.9, 132.5, 130.8 (d, *J* = 12.7 Hz), 129.1 (d, *J* = 81.4 Hz), 119.8, 119.6, 119.1, 118.0, 117.1, 45.2, 34.2, 34.2, 22.6 (d, *J* = 49.3 Hz); HRMS (m/z, ESI), calculated for $C_{33}H_{29}N_2OSP$ $[M\text{-}Br]^+$ 533.1816, found 533.1811.

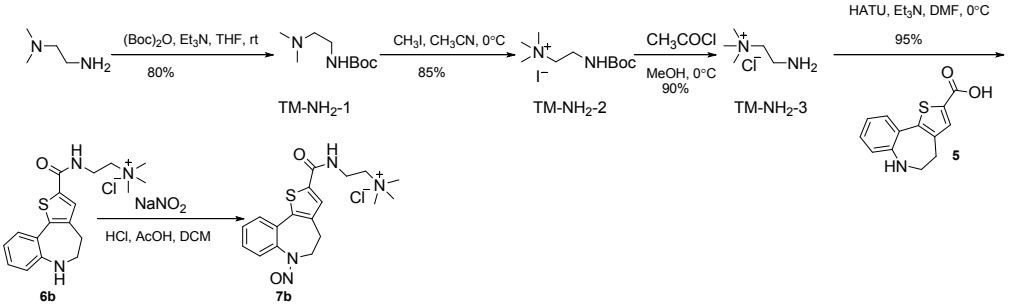
(2-(6-nitroso-5,6-dihydro-4H-benzo[b]thieno[2,3-d]azepine-2-carboxamido)ethyl)triphenylphosphonium bromide (7a)



7a

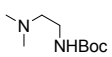
To the mixed solvent of HCl/AcOH/CH_2Cl_2 (1:10:2, v/v, 5 mL) was added compound 6a (150 mg, 0.244 mmol) at 0°C, followed by addition of sodium nitrite (24.33 mg, 0.35 mmol). After stirring for 10 min, the ice bath was removed and the reaction mixture was stirred at room temperature for 2 h. After completion, the reaction was quenched by adding saturated $NaHCO_3$ solution to pH 7. Then the mixture was extracted with CH_2Cl_2 twice (2 ×20 mL), and the combined organic layer was washed with water(20 mL), dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The crude product was purified by silica gel column chromatography, eluting with DCM/MeOH (200:1 v/v) to afford the desirable compound as a faint yellow solid (141 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83-7.72 (m, 16H), 7.61-7.58 (m, 1H), 7.47-7.44 (m, 2H), 7.40 (s, 1H), 4.16 (t, *J* = 5.6 Hz, 2H), 3.85-3.75 (m, 2H), 3.56-3.49 (m, 2H), 3.04 (t, *J* = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 140.5, 139.0, 138.1, 136.2, 135.4 (d, *J* = 3.0 Hz), 133.3 (d, *J* = 10.0 Hz), 131.3, 130.6 (d, *J* = 12 Hz), 129.3, 129.1, 128.8, 127.2, 125.4, 117.3 (d, *J* = 86 Hz), 47.4, 34.0, 27.0, 22.3 (d, *J* = 22.1); HRMS (m/z, ESI), calculated for $C_{33}H_{29}N_3O_2SP$ $[M\text{-}Br]^+$ 562.1713, found 562.1717

2.3 Synthesis of compound 7b



Scheme S3: The synthetic route for compound **7b**

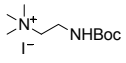
Tert-butyl (2-(dimethylamino)ethyl)carbamate (TM-NH_2-1)[5]


TM-NH_2-1

To the solution of N,N-dimethylethane-1,2-diamine (500 mg, 0.619 mL, 5.545 mmol) in the tetrahydrofuran (THF ,5 mL) was added triethylamine (1.55 mL, 11.12 mmoL) and $(Boc)_2O$ (2.42 g, 2.55 mL, 11.12 mmol) at 0°C. The mixture was

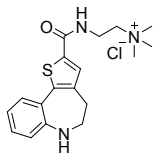
then stirred for 24 h at room temperature. After completion, the solvent was removed under reduced pressure to give the residue, which was re-dissolved in EtOAc (10 mL) and washed with water (20 mL). The aqueous layer was extracted with EtOAc twice (2 x 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude was purified by silica gel column chromatography, eluting with CH_2Cl_2/MeOH (100:1, v/v) to afford the desirable compound as a clear oil (834.8 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.04 (s, br, 1H), 3.20 (apparent q, *J* = 5.6 Hz, 2H), 2.39 (t, *J* = 6.0 Hz, 2H), 2.23 (s, 6H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 78.8, 58.3, 45.1, 37.9, 28.3

2-((tert-butoxycarbonyl)amino)-N,N,N-trimethylethan-1-aminium iodide (TM-NH_2-2)[6]


TM-NH_2-2

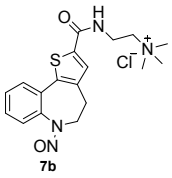
To the solution of compound TM-NH_2-1 (500 mg, 2.657 mmol) in anhydrous CH_3CN (2 mL), CH_3I (0.326 mL, 5.313 mmol) was added dropwise at 0°C. Then the reaction mixture was stirred at room temperature overnight. After completion, the mixture was concentrated under reduced pressure to give the desirable compound as a white solid (745.3 mg, 85%).^{1}H NMR (400 MHz, D_2O) δ 3.54 (t, *J* = 6.4 Hz, 2H), 3.42 (t, *J* = 6.4 Hz, 2H), 3.14 (s, 9H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, D_2O) δ 157.7, 81.7, 64.7, 53.5, 53.5, 53.5, 34.6, 27.7

2-(5,6-dihydro-4H-benzo[b]thieno[2,3-d]azepine-2-carboxamido)-N,N,N-trimethylethan-1-aminium chloride(6b)


6b

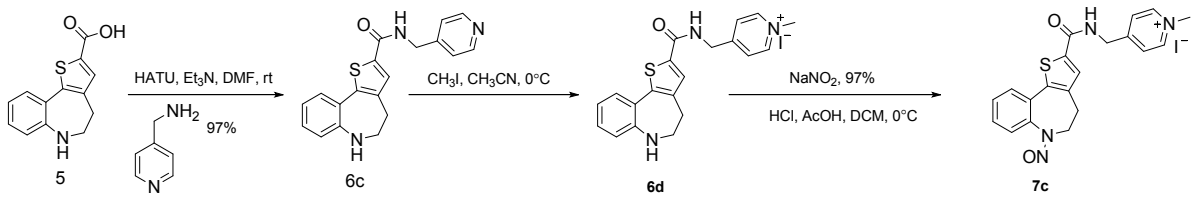
Acetyl chloride (CH_3COCl, 0.471 mL, 6.667 mmol) was added into anhydrous MeOH (1 mL) slowly at ice bath. After stirring for 10 min, a solution of intermediate TM-NH_2-2 (500 mg, 1.515 mmol) in anhydrous MeOH (1 mL) was added, and the resulting mixture was continued to stir at 0 °C for 3 h. After completion, the solvent was reduced under reduced pressure to give the intermediate TM-NH_2-3 as a faint yellow solid, which was used directly into the next step. The compound 6b was synthesized from compound 5 and intermediate TM-NH_2-3 using HATU as coupling reagent according to the procedure for compound 6a. The crude product was purified via C-18 reverse phase chromatography (water/MeOH-5:1, v/v) to give the product as yellow solid in 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.87 (s, 1H), 7.53-7.51 (m, 2H), 7.02 (t, *J* = 8.0 Hz, 1H), 6.79 (d, *J* = 8.4 Hz, 1H), 6.67 (t, *J* = 8.0 Hz, 1H), 6.37 (t, *J* = 4.0 Hz, 1H), 3.70-3.62 (m, 2H), 3.49 (t, *J* = 6.0 Hz, 2H), 3.27 (t, *J* = 4.8 Hz, 2H), 3.14 (s, 9H), 2.98 (t, *J* = 4.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.5, 148.5, 142.5, 138.1, 133.4, 132.4, 128.7, 128.4, 118.7, 118.0, 117.8, 63.8, 52.6, 43.8, 34.1, 33.6; HRMS (m/z, ESI) calculated for $C_{18}H_{23}N_3OS$ [M-Cl]$^+$ 330.1635, found 330.1617.

N,N,N-trimethyl-2-(6-nitroso-5,6-dihydro-4*H*-benzo[*b*]thieno[2,3-d]azepine-2-carboxamido)ethan-1-aminium chloride (7b)


7b

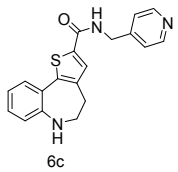
According to the procedure for compound 7a, compound 7b was synthesized from compound 6b. The crude product was purified via C-18 reverse phase chromatography (water/MeOH-2:1, v/v) to give the desirable product as white solid in 95% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, br, 1H), 7.87 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.67-7.64 (m, 2H), 7.62-7.54 (m, 2H), 4.15 (t, *J* = 6.0 Hz, 2H), 3.68 (t, *J* = 4.8 Hz, 2H), 3.50 (t, *J* = 6.4 Hz, 2H), 3.13 (s, 9H), 3.02 (t, *J* = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.0, 138.8, 138.7, 138.3, 137.6, 131.4, 129.6, 129.2, 128.6, 126.8, 125.6, 63.6, 52.6, 46.7, 33.7, 26.9; HRMS (m/z, ESI) calculated for $C_{18}H_{23}N_4O_2S$ [M-Cl]$^+$ 359.1536, found 359.1538.

2.4 Synthesis of compound 7c



Scheme S4: The synthetic route for compound **7c**

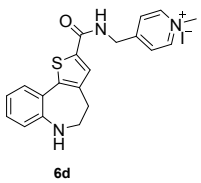
***N*-(pyridin-4-ylmethyl)-5,6-dihydro-4*H*-benzo[*b*]thieno[2,3-d]azepine-2-carboxamide (6c)**


6c

According to the synthetic procedure for compound 6a, compound 6c was synthesized from compound pyridin-4-ylmethanamine and intermediated 5. The crude product was purified by silica gel column chromatography,

eluting with CH_2Cl_2/MeOH (200:1, v/v) to afford the desirable compound as a yellow solid in 97% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J* = 6.0 Hz, 2H), 7.66 (d, *J* = 7.2Hz, 1H), 7.36 (s, 1H), 7.28 (s, 1H), 7.08 (t, *J* = 7.0 Hz, 1H), 6.81 (t, *J* = 7.2 Hz, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 6.36 (t, *J* = 5.6 Hz, 1H), 4.64 (d, *J* = 6.0 Hz, 2H), 3.44 (t, *J* = 5.6 Hz, 2H), 3.08(t, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3, 149.9, 147.8, 147.7, 143.2, 138.0, 132.9, 132.8, 129.4, 128.8, 122.4, 119.8, 119.7, 119.1, 45.1, 42.7, 34.3； HRMS (m/z, ESI) calculated for $C_{19}H_{18}N_3OS$ $[M+H]^+$ 336.1165, found 336.1185

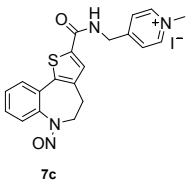
4-((5,6-dihydro-4H-benzo[b]thieno[2,3-d]azepine-2-carboxamido)methyl)-1-methylpyridin-1-ium iodide (6d)



6d

Compound 6d was synthesized from compound 6c. Then the crude product was purified by C-18 reverse phase chromatography, eluting with water/MeOH (9:1, v/v) to afford a yellow solid in 80% yield.^{1}H NMR (400 MHz, MeOD) δ 8.84 (d, *J* = 6.4 Hz, 2H), 8.03 (d, *J* = 6.4 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 3.2 Hz, 1H), 7.08 (t, *J* = 8.0 Hz, 1H), 8.81 (dd, J = 8.0, 0.8 Hz, 1H), 6.77 (apparent t, *J* = 8.0 Hz, 1H), 4.81 (s, 2H), 4.39 (s, 3H), 3.39 (t, *J* = 5.2 Hz, 2H), 3.10 (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, MeOD) δ 165.0, 161.0, 150.2, 146.4, 145.4, 139.6, 134.5, 133.5, 130.1, 129.9, 127.1, 120.6, 120.3, 120.2, 46.0, 35.4 (two carbons less due to overlap with the solvent); HRMS (m/z, ESI) calculated for $C_{20}H_{19}N_3OS$ $[M-I]^+$ 350.1322, found 350.1326.

1-methyl-4-((6-nitroso-5,6-dihydro-4H-benzo[b]thieno[2,3-*d*]azepine-2-carboxamido)methyl)pyridin-1-ium iodide (7c)



7c

According to the synthetic procedure for compound 7a, compound 7c was synthesized from compound 6d. The crude product was purified by silica gel column chromatography, eluting with CH_2Cl_2/MeOH (50:1, v/v) to afford the desirable compound as a white solid in 97% yield.^{1}H NMR (400 MHz, MeOD) δ 8.83 (d, *J* = 6.0 Hz, 1H), 8.02 (d, *J* = 6.0 Hz, 1H), 7.86 (d, *J* = 7.2 Hz, 1H), 7.66 (s, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.57-7.51 (m, 1H), 4.81 (s, 2H), 4.39 (s, 3H), 4.22 (t, *J* = 6.0 Hz, 2H), 3.10 (t, *J* = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, MeOD+$CDCl_3$) δ 164.0, 160.5, 160.5, 146.2, 141.6, 140.4, 139.5, 137.4, 133.1, 130.4, 130.2, 129.7, 128.3, 127.1, 127.0, 126.5, 48.5, 48.3, 47.8, 43.5; HRMS (m/z, ESI) calculated for $C_{20}H_{19}N_4O_2S$ $[M-I]^+$ 379.1233, found 379.1238.

3. Determination of fluorescence quantum yield

Fluorescence quantum yields for compound 5, NO hybrids (7a-b and 7c) and their precursors (6a-b and 6d) were determined in ethanol by using 9,10-Diphenylanthracene (DPA)(Φ_F =0.95 in ethanol) as a fluorescence quantum yield standard. All the samples were dissolved in ethanol with the concentration of 5 μM. The quantum yield was calculated according to the following equation:

$$\Phi_{F(sample)} = \Phi_{F\,(reference)} (A_{reference}/A_{sample}) (F_{sample}/F_{reference}) (n_{sample}/n_{reference})^2$$

where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts sample and reference refer to the standard and to the unknown, respectively. For the synthesized compounds, the excitation wavelength was at 370 nm while keeping the absorption below 0.05.

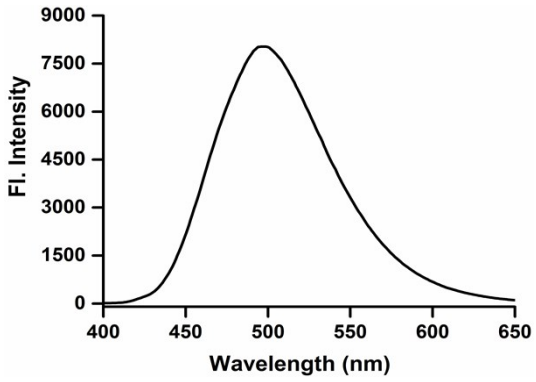


Fig. S1. Fluorescence spectrum of compound 5 (5 μM) in 5% DMSO-PBS (20 mM, pH 7.4); λ_{ex} = 370 nm, λ_{em} = 500 nm.

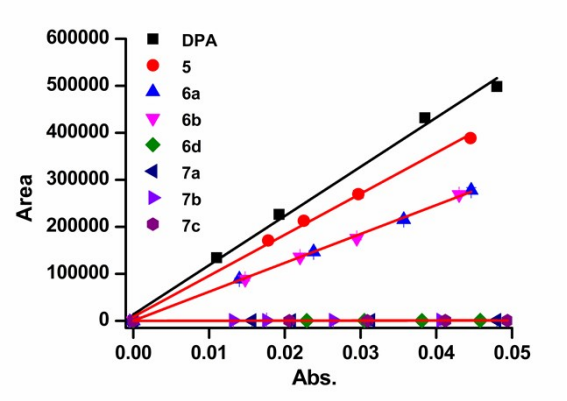


Fig. S2. Fluorescence quantum yields for all compounds were determined by using 9,10-Diphenylanthracene (DPA)(Φ_F =0.95 in ethanol) as a fluorescence standard with excitation at 370 nm, slit = 2.5 nm/5 nm.

4. HPLC analysis

To test the stability of the NO hybrids, the solutions were incubated in CH_3CN at room temperature for 24 h, and analyzed by HPLC. To clarify the product formed in the photolysis, NO hybrids were irradiated with the light at the wavelength of 313 nm for 2 h. Subsequently the reaction solution was analyzed by HPLC. NO hybrids and their precursors were used as the control samples.

All the HPLC analysis including the purities analysis were performed on a Shimadzu LC-20AT system with a Cromasil C18 column (250 mm x 4.6 mm, 5 μm) as the following conditions: UV-vis wavelength = 313 nm, eluted at 1 mL/min with water/CH_3CN (containing 0.1% TFA), gradient eluting from 50% to 90% in 20 min for 7a and 7c, from 10% to 90% in 20 min for 7b.

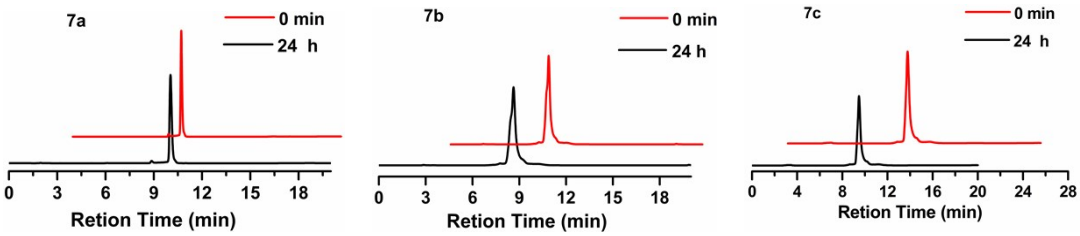


Fig. S3. HPLC analysis of the stability of 7a, 7b and 7c after incubation for 24 h in CH_3CN at room temperature in the dark.

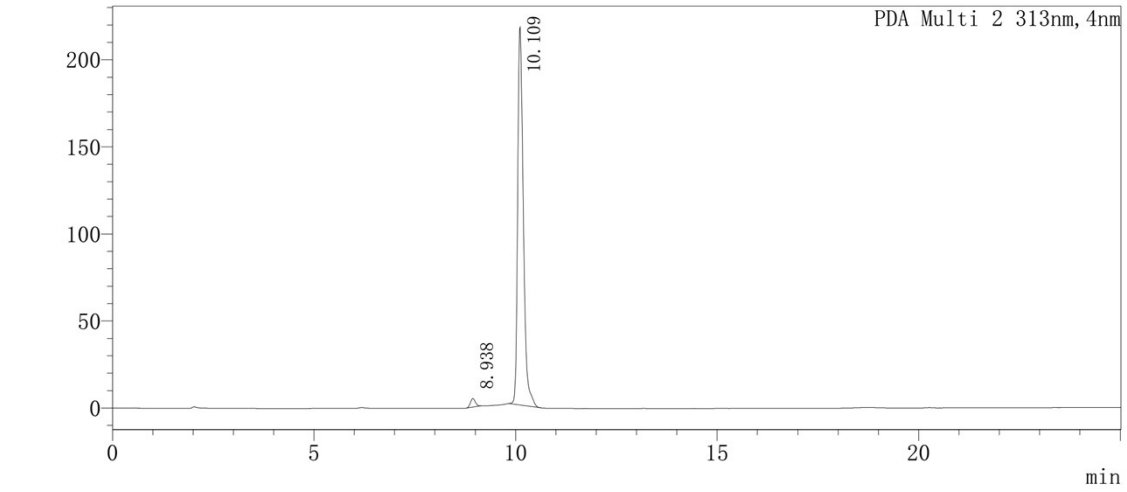
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name : 7a
Sample ID :
Data Filename : NOD.lcd
Method Filename : A.lcm
Batch Filename : 1
Vial# : 1-1 Sample Type :Unknown
Injection Volume : 20 uL
Date Acquired : 2018/7/11 13:36:31 Acquired by : System Administrator
Date Processed : 2018/7/24 20:17:37 Processed by : System Administrator

<Chromatogram>



<Peak Table>

PDA Ch2 313nm

Peak#	Ret.Time	Area	Height	Conc.	Mark
1	8.938	42633	5001	1.975	
2	10.109	2116139	216829	98.025	
总计		2158772	221830		

Analysis of the purity of 7a

SHIMADZU LabSolutions

Analysis Report

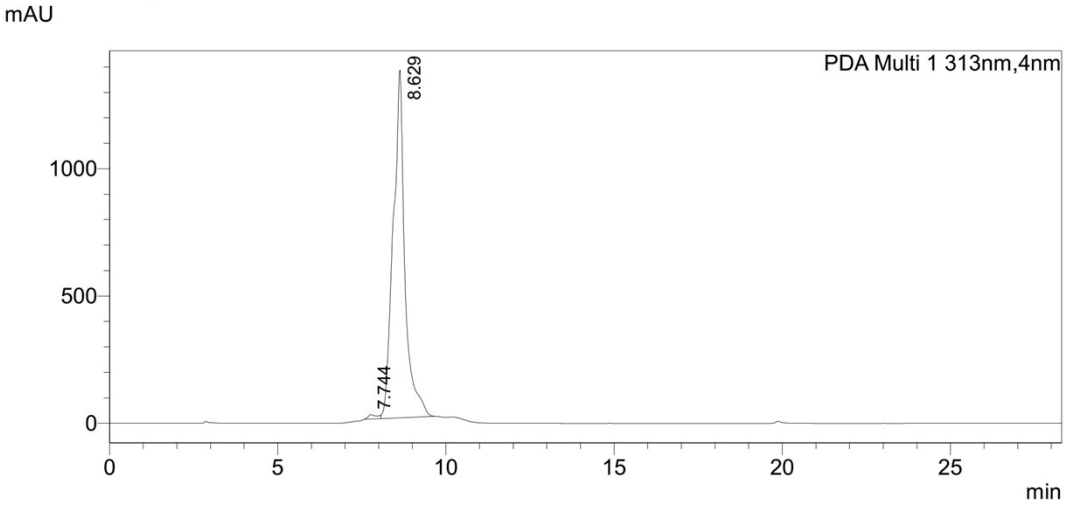
<Sample Information>

Sample Name : 1
Sample ID : 1
Data Filename : 7b.lcd
Method Filename: A.lcm
Batch Filename : B.lcb
Vial# : 1-1
Injection Volume : 20 uL
Date Acquired : 5/1/2019 6:30:35 PM
Date Processed : 1/2/2020 11:18:59 AM

Sample Type : Unknown

Acquired by : System Administrator
Processed by : System Administrator

<Chromatogram>



<Peak Table>

PDA Ch1 313nm

PEAK	Ret.Time	Area	Height	Conc.	Mark
1	7.744	326854	16135	0.980	
2	8.629	33030575	1364307	99.020	V
Total		33357429	1380441		

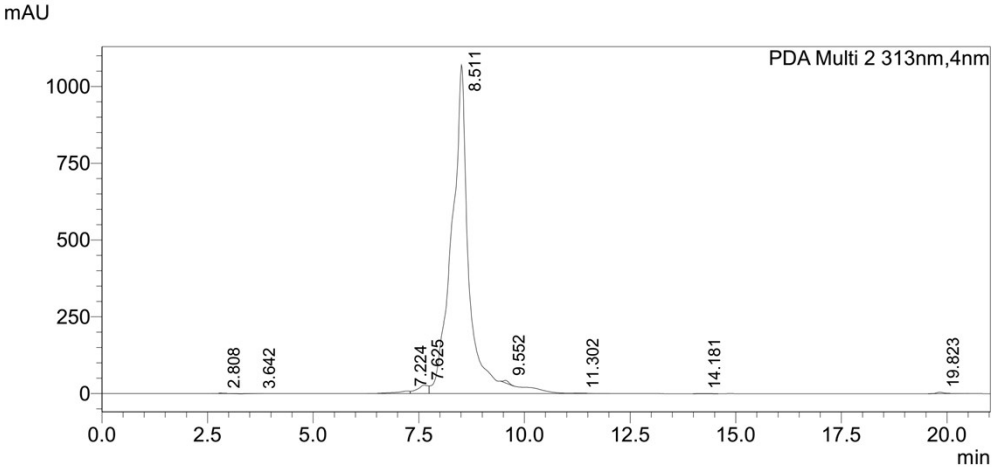
Analysis of the purity of 7b

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name : 8
Sample ID : 2
Data Filename : 8.lcd
Method Filename: A-1.lcm
Batch Filename : B.lcb
Vial# : 1-1 Sample Type : Unknown
Injection Volume : 20 uL
Date Acquired : 5/1/2019 9:12:16 PM Acquired by : System Administrator
Date Processed : 1/2/2020 9:58:44 AM Processed by : System Administrator

<Chromatogram>



<Peak Table>

PDA Ch2 313nm

Peak#	Ret.Time	Area	Height	Conc.	Mark
1	2.808	26113	2291	0.083	
2	3.642	3212	151	0.010	
3	7.224	200383	8627	0.634	
4	7.625	504823	27538	1.597	V
5	8.511	30737180	1069719	97.213	SV
6	9.552	70239	8478	0.222	T
7	11.302	4351	360	0.014	T
8	14.181	8415	725	0.027	
9	19.823	63783	4602	0.202	
Total		31618499	1122491		

Analysis of the purity of 7c

Fig.S4 The purities of of 7a,7b and 7c detected by HPLC method.

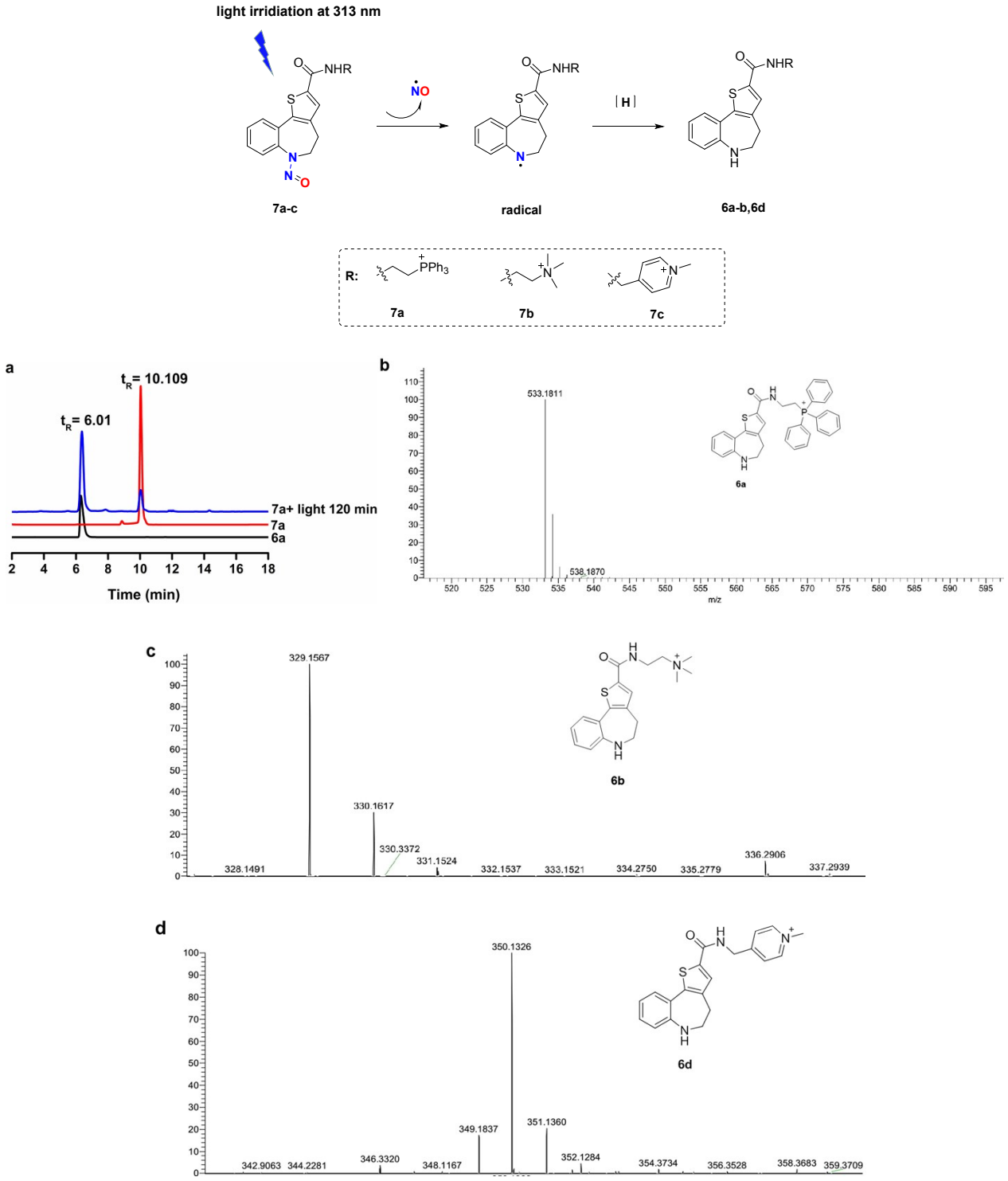


Fig. S5. HPLC (a) and HRMS (b-d) spectra of NO hybrids **(**7a-c) after irradiation at 313 nm for 2 h. The new peak appeared on HPLC column was collected and performed HRMS analysis.

5. Fluorescence stability of NO hybrids and their precursors

To exclude the unexpected fluorescence changes in the photolysis, we detected the fluorescence stability of NO hybrids and their precursors. All of the compounds (5 μM) were incubated in the PBS with different pH values (5.0, 6.0, 7.0, 7.4 and 8.0), or in the presence of ascorbic acid, cations (Na^+, K^+ and Zn^{2+}) and natural amino acids (L-arginine, L-cysteine, L-glycine, GSH, L-proline, L-serine, L-threonine and L-valine). λ_{ex} = 375 nm, λ_{em} = 520 nm, slit = 5 nm/5 nm.

6. Detection of the changes in UV-vis and fluorescence spectra under irradiation

UV-vis absorption: Put the solution of NO hybrids (40 μM, 3.2 mL) in PBS buffer (20 mM, pH = 7.4, containing 5% DMSO) into fluorescence quartz cuvette to be irradiated with the light at 313 nm (6 W LED light) in black box. At the designed time (0,5,15,25⋯150 min), aliquots of 300 μL were transferred to an Uv-cuvette to obtain the UV-vis absorption spectra using PBS with 5% DMSO as the blank. The concentration of the 7a, 7b and 7c was indicated by the maximum absorbance obtained. All the experiment was repeated three times.

Fluorescence spectra： Put the solution of NO hybrids (5 μM, 1.0 mL) in PBS buffer (20 mM, pH = 7.4, containing 5% DMSO) into fluorescence quartz cuvette to be irradiated with the light at 313 nm (6 W LED light) in black box. At the designed time (0,2,4,6,8⋯40 min), the cuvette was taken out to obtain fluorescence spectra with excitation wavelength at 375 nm. Slit = 5 nm/5 nm. The concentration of the generated fluorescent product (6a-b) was related with the fluorescence intensity at the maximum emission wavelength.

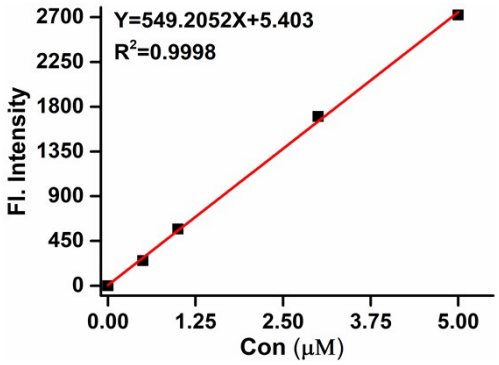


Fig. S6. The relationship between the fluorescence intensity and the concentration of compound 6a. λ_{ex} = 375 nm, λ_{em} = 520 nm, slit = 5 nm/5 nm.

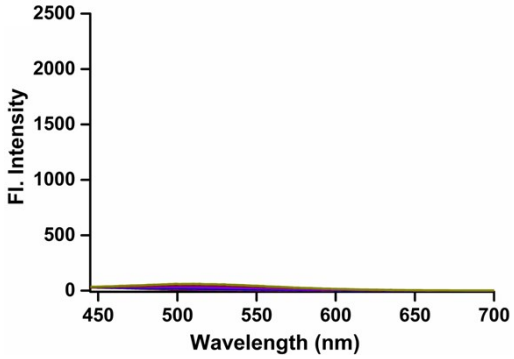


Fig. S7. The time course changes of fluorescent spectra for 7c solution (5 mM) upon irradiation by light; l_{ex} = 375 nm, l_{em} = 520 nm, slit = 5 nm / 5 nm.

7. Detection of nitric oxide release

Griess Method: The solution of 7a-b or 7c (40 μM) in PBS (20 mM, pH = 7.4, containing 5% DMSO) was transferred into fluorescent quartz cuvette and subjected to irradiation with light of wavelength 313 ± 10 nm (6 W LED light) in black box. At the designed time (0.75, 1, 1.5, 2.5, 5 h), 100 μL of the solution was taken out from the cuvette, followed by addition of Griess Reagent A (100 μL) and Griess Reagent B (100 μL). After incubation for 30 min, the UV-vis absorbance of the solution at 540 nm was measured. A standard curve was generated from a dilution series of $NaNO_2$ sample. All experiments were repeated three times.

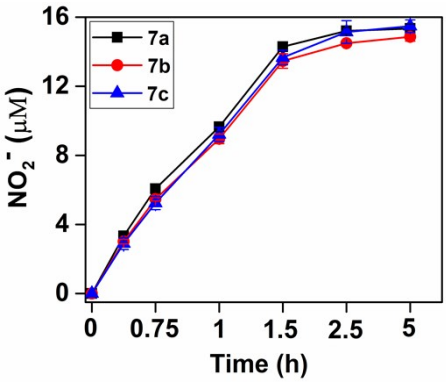


Fig. S8 The NO releases from 7a-c (40 μM) at different time points were detected by Griess Method.

EPR spectroscopy: The following acquisition parameters were used: microwave power, 10 mW; modulation frequency, 100 kHz; modulation amplitude, 1.0 G; scan time, 30 ms,scan width,60 G. Fresh stock solution of (5 mM) was prepared by dissolving MGD sodium salt (5.0 eq.) and ammonium ferrous sulphate hexahydrate (1.0 eq.) in argon-purged DDI water. To the solution of 7a, 7b and 7c (800 μM, 5 μL) in DMSO was added the spin trap Fe^{2+}-$(MGD)_2$ (5 mM, 20 μL)and PBS (20 mM, pH = 7.4,75 μL). After mixing, the solution(40 μM, containing 5% DMSO) was transferred into EPR quartz tube (1.0 mm, id, 50 μL) and subject to the irradiation at 313 ±10 nm (6 W LED light) in black box. At the designed time (0, 2, 4, 6, 8, 10, 15, 20, 30 min), the tube was taken out and performed experiment on EPR spectroscopy.

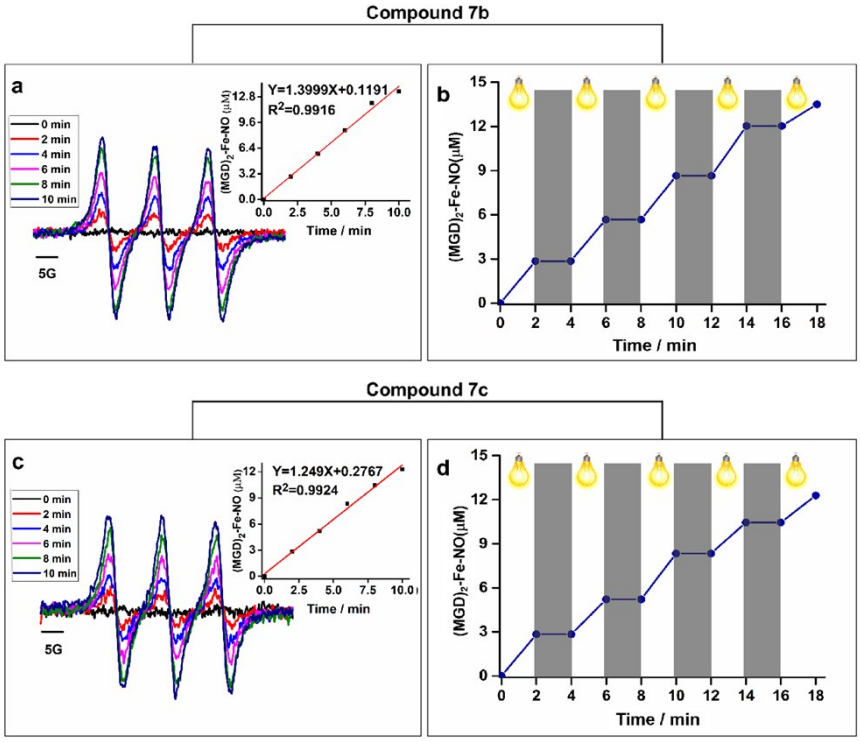


Fig. S9 (a,c) The EPR spectra of 7b and 7c (40 μM) upon irradiation at 313 nm in the presence of spin strap Fe^{2+}-$(MGD)_2$; inset: the relationship between NO generation and irradiation time. (b,d) NO-capture curve of 7b and 7c under light irradiation for different on/off cycles.

8. Antibacterial experiment

Bacteria culture: Gram-positive bacteria Staphylococcus aureus ATCC12600^{GFP} (*S. aureus*) were supplied by Nankai University. Prior to use, *S. aureus* from frozen stocks were subcultured two times in Luria-Bertani broth (16g of Tryptone,10g of Yeast extract,5g of NaCl, per liter of water, pH 7.0) at 37°C in air with shaking at 120 rpm for about 12 h

until the optical density of the culture at 600 nm (OD_{600nm}) reached approximately 0.8. All media and Milli-Q water used for bacterial cultures experiments were sterilized by autoclave.

The antibacterial activity of benzothienoazepine scaffold compound： Antibacterial planktonic activity of compound 4 was evaluated using broth microdilution method. The *S. aureus* bacteria solutions at an initial concentration between 1×10^6 and 1×10^7 CFU/ml were added to 48-well plates. Each wall contained 30 μL of bacteria suspension, 30 μL of compound 4 solution and 240 μL of LB media. The final concentrations of *S. aureus* were between 1×10^5 and 1×10^6 CFU/ml. And the final concentrations for NO hybrids were between 50-250μg/mL. Growth in LB broth and LB media solely were used as two control groups. The mixtures were cultured at 37°C in air for 24 h with shaking at 120 rpm. At 3, 6 ,9 and 24 h intervals, the optical density (OD_{600nm}) was monitored by a microplate reader (Molecular Devices-FilterMax F5).

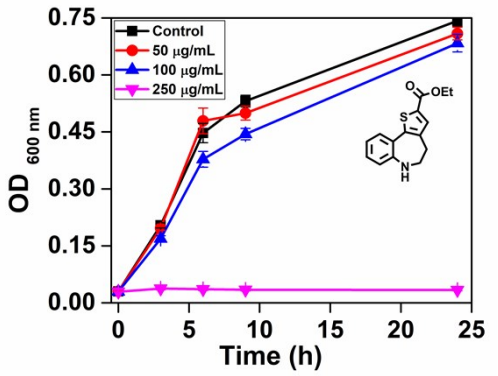


Fig. S10 The antibacterial activity of benzothienoazepine scaffold compound against *S.aureus.*

Determination of MIC and MBC： Antibacterial planktonic activity of the NO hybrids and their precursors were then evaluated *via* minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) using broth microdilution method. Briefly, the NO hybrids were dissolved in sterile Dimethyl sulfoxide (DMSO, 60 mg/mL) and sonicated for 10 min to accelerate the dissolution as the stock solution. Then the stock solution was diluted by LB broth to give work solution of concentration ranged from 75 μg/mL and 1.2 mg/mL in LB broth. The bacteria solutions at an initial concentration between 1×10^6 and 1×10^7 CFU/ml were added to 48-well plates. Each wall contained 30 μL of bacteria suspension, 30 μL of work solution and 240 μL of LB media. The final concentrations of *S. aureus* were between 1×10^5 and 1×10^6 CFU/ml. And the final concentrations for NO hybrids were between 7.5-120 μg/mL. Growth in LB broth and LB media solely were used as two control groups. The mixtures were cultured at 37°C in air for 24 h with shaking at 120 rpm. At 3, 6 ,9 and 24 h intervals, the optical density (OD_{600nm}) was monitored by a microplate reader (Molecular Devices-FilterMax F5). The MIC was the lowest concentration to inhibit the development of visible bacteria growth (no turbidity is visible to the naked eye) in the wells after incubation at 37 °C for 24 h. And then the MBC was determined by taking 20 μL bacterial suspension from the wells, which had no visible bacterial growth and subsequently were coated on the LB plates (60 mm). Then the plates were incubated at 37°C for another 24 h statically. The MBC defined as the lowest concentration, which did not show bacterial growth. The experiments were performed for 3 independent experiments.

Table S1. Minimum inhibitory (MIC) and bactericidal concentrations (MBC) of NO hybrids (7a-b-c) and their NO-released products (6a-b and 6d)

Compound	MIC against *S. aureus* (μg/mL)	MBC against *S. aureus* (μg/mL)
7a	30	60
6a	30	60
7b	>400	>400
6b	>400	>400
7c	>500	>500
6d	>500	>500

Detection of antibiofilm activity: In the 96-well plate, each well contained 200 μL of *S.aureus* bacteria (1 x 10^8 CFUs/mL). The mixture was incubated statically at 37°C in the air for 48 h to form biofilms. Subsequently, the suspensions were discarded and the wells were washed gently three times with 200 μL of sterile PBS to remove the unbound bacteria. After completion, the solution of NO hybrids 7a and 6a was added into the wells with the final concentration as 30, 45, 60, 120 and 180 μg/mL. Subsequently, the samples were subjected to irradiation (313 ± 10 nm) for 4h for the light-exposure groups. After static incubation for 2, 4, 8, 24 h, the suspension was discarded and the wells were washed gently with 200 μL of sterile PBS three times. Then, 200 μL of PBS was added to each well, and the biofilms were resuspended by sonicated for 10 min in the sonication water bath to further detach the biofilms. Then the suspensions were diluted to the appropriate concentration with PBS, and 20 μL of dilution was plated on the LB agar plates (60 mm) and incubated statically at 37°C for 24 h. The number of colony forming units (CFUs) was counted and was expressed as CFUs/mL. Biofilms were treated with PBS as the control group. The CFUs/mL was used to quantify the number of viable bacteria cells in the biofilm. All the experiments are performed for 3 times.

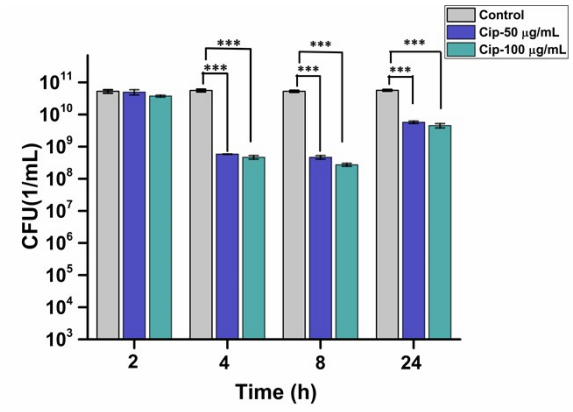


Fig. S11 The antimicrobial efficacy of antibiotic drug ciprofloxacin at various concentrations against 48-h-old *S. aureus* biofilm after incubation for different time (2, 4, 8, 24 h). Data are expressed as means ± S.D. over three independent experiments; *** p<0.0001.

Antibacterial Activity observed by SEM: To further verify the antibacterial mechanism of 6a and 7a against biofilm cells, the morphology of the *S.aureus* bacteria was observed with scanning electron microscopy (SEM). 48 h-old *S. aureus* biofilm was formed as mentioned above. After addition of 60 μg/mL of 6a or 7a to the wells, the samples were exposed to light (313 ± 10 nm) for 4 h (this step is only for light-exposure groups, no-light groups skip this step), then were incubated for 8 h. Subsequently, the samples were washed with PBS and fixed with 2.5% glutaraldehyde (200 μL) overnight at 4°C. After washing 3 times with PBS, the cells were subsequently dehydrated using a graded series of ethanol (30%, 50%, 70%, 90%, 100%, 3 times, each for 10 min). They were then placed in tertiary-butyl alcohol (200 μL, 2 times, each for 15 min) and freeze dried for 24 h. The samples were then coated with gold, and examined by scanning electron microscopy (Quanta 200 Czech).

9. Hemolysis assay

Commercial sheep red blood cells (Shanghai Yuanye Biotechnology) were used to evaluate the blood compatibility of the compounds. Briefly, 50 μL of 4% (v/v) RBC suspension were incubated at 37°C for 2 h with 950 μL of NO hybrids and their precursors at various concentrations (15, 30, 45, 60, 90,120 and 300 μg/mL). The cell suspensions were then centrifuged at room temperature for 10 min at 1500 rpm and the absorbance of the supernatant was measured at 542 nm with a microplate reader (Molecular Devices-FilterMax F5). Water was used as positive control (100% RBC lysis) and saline as negative control (absence of RBC lysis). The values of haemolysis were calculated as a percentage of the positive control according to the following formula. The pictures were obtained by camera. All assays were performed for three times.

Hemolysis (%) = [(OD_{542nm} in the compounds solution-OD_{542nm} in saline)/(OD_{542nm} in water -OD_{542nm} in saline)] x 100.

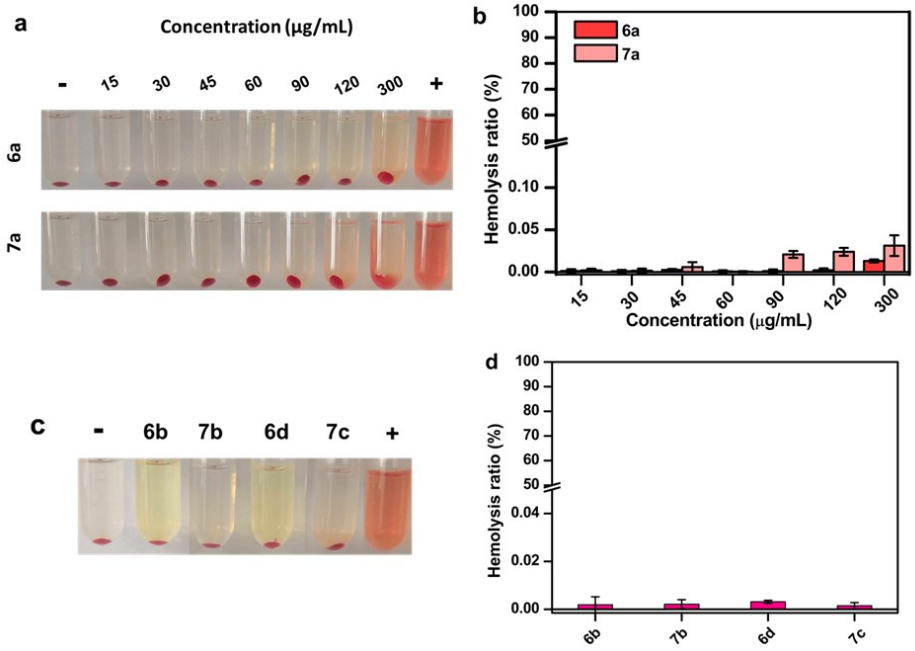


Fig S12. (a,b) Hemolysis ratio of 6a and 7a at different concentrations ranged from 15 μg/mL to 300 μg/mL. (c,d) hemolysis ratio of 6b,7b,6d,7c,at concentration (600 μg/mL).Saline and distilled water were used as negative(-) and positive (+) control, respectively.

Notes and references

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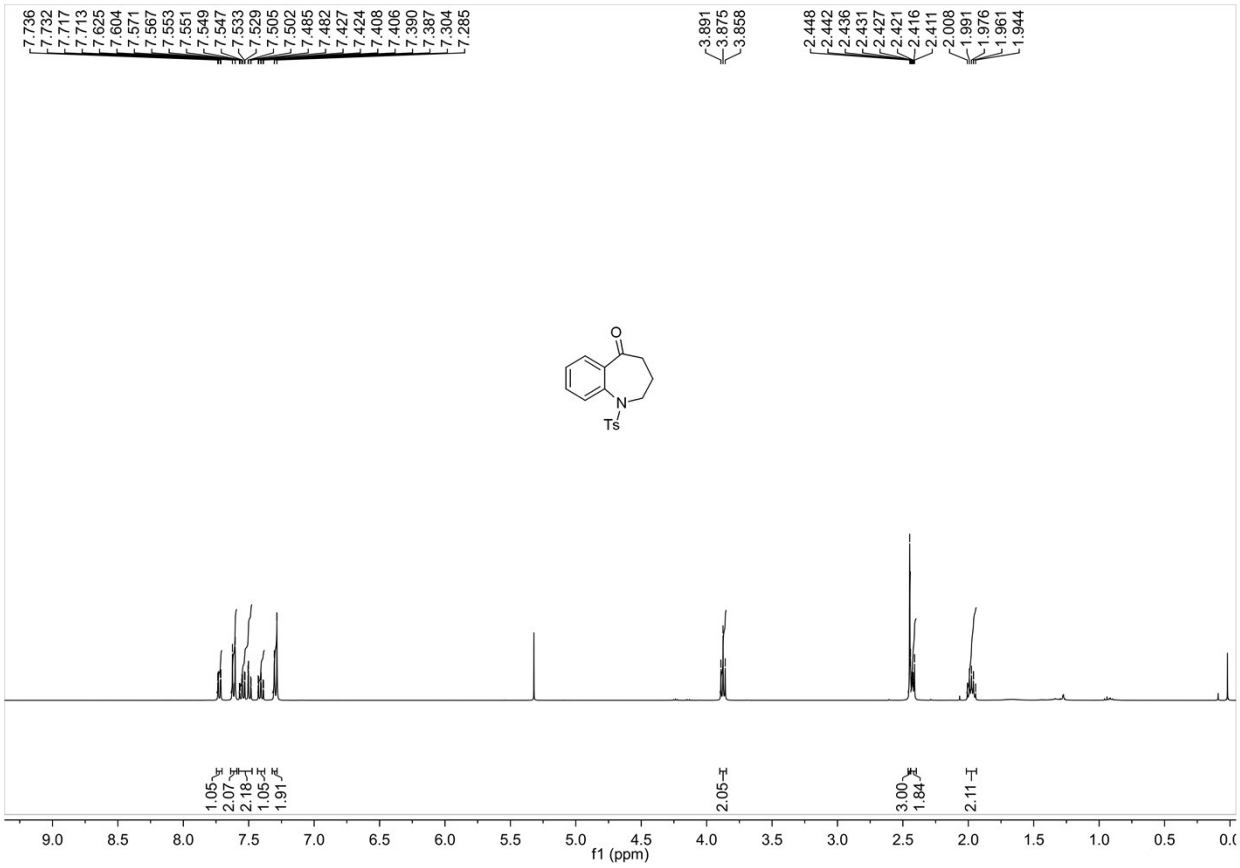
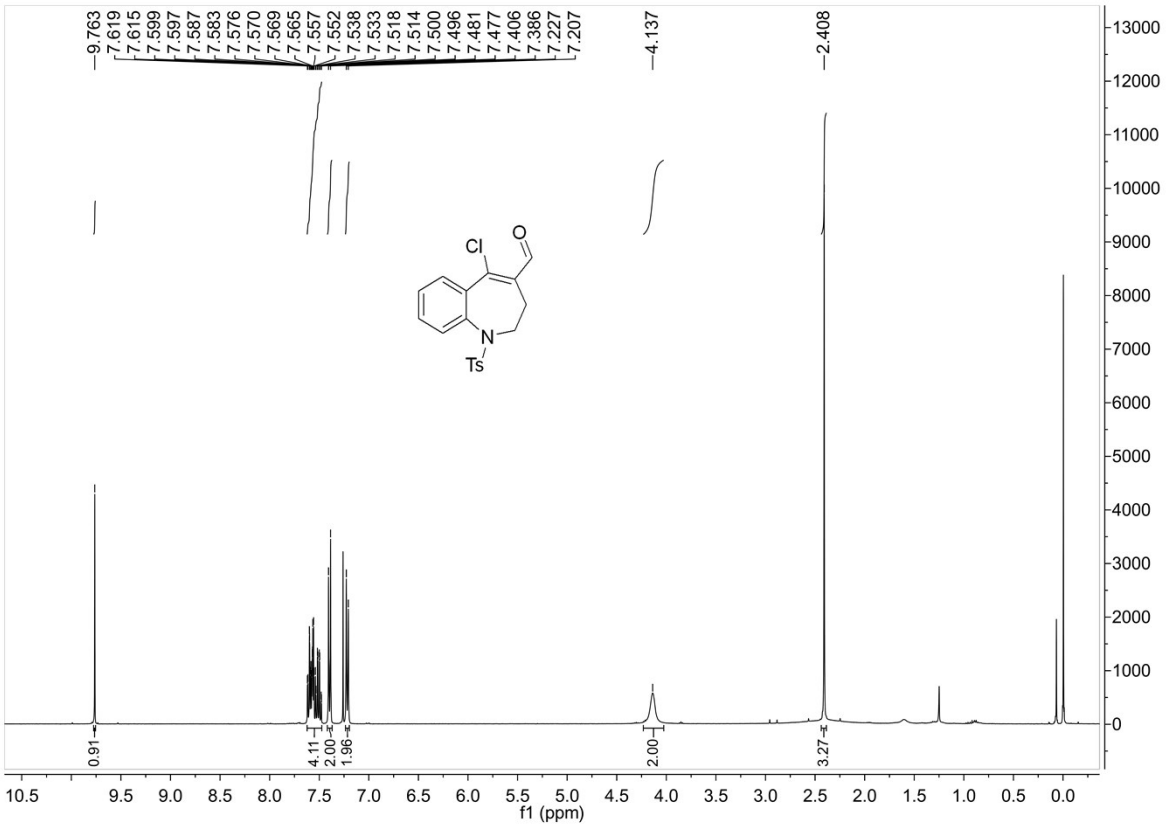
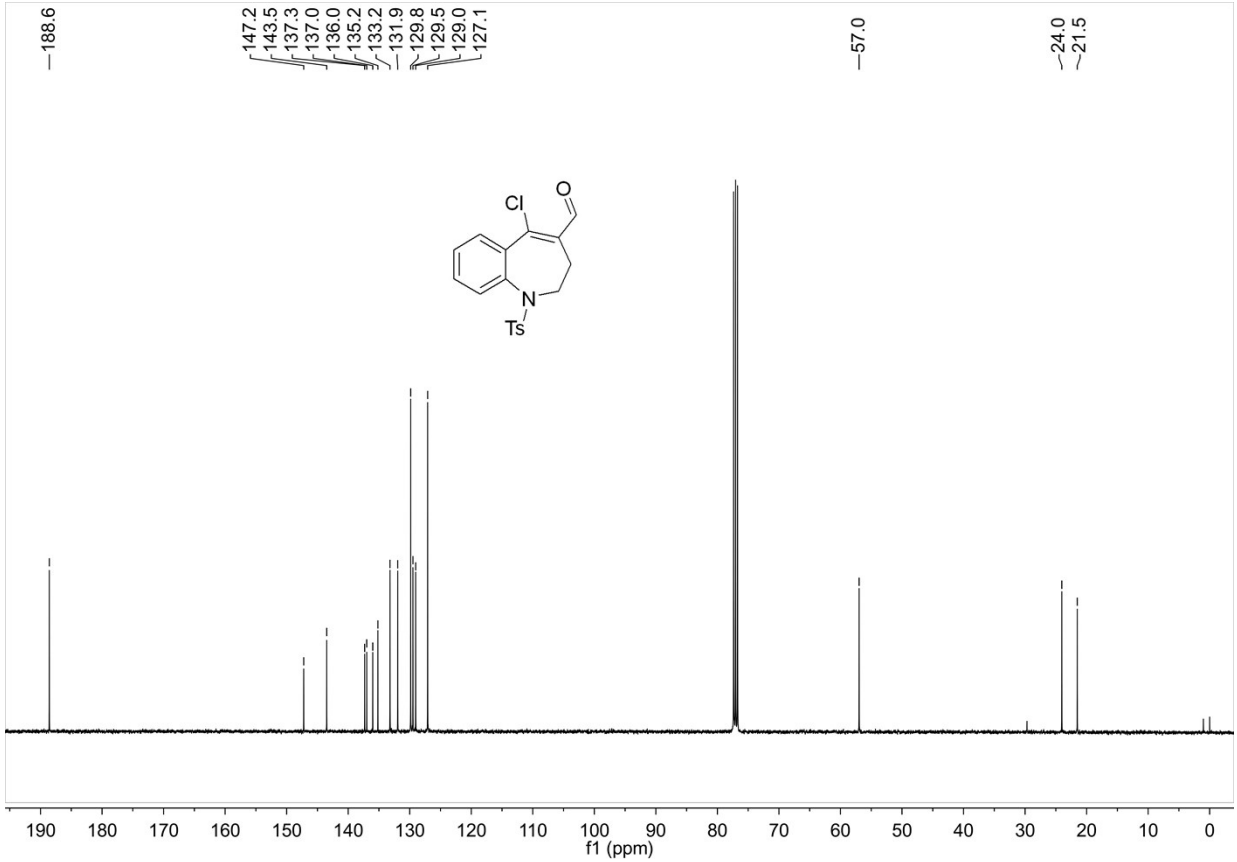
The NMR and HRMS spectra



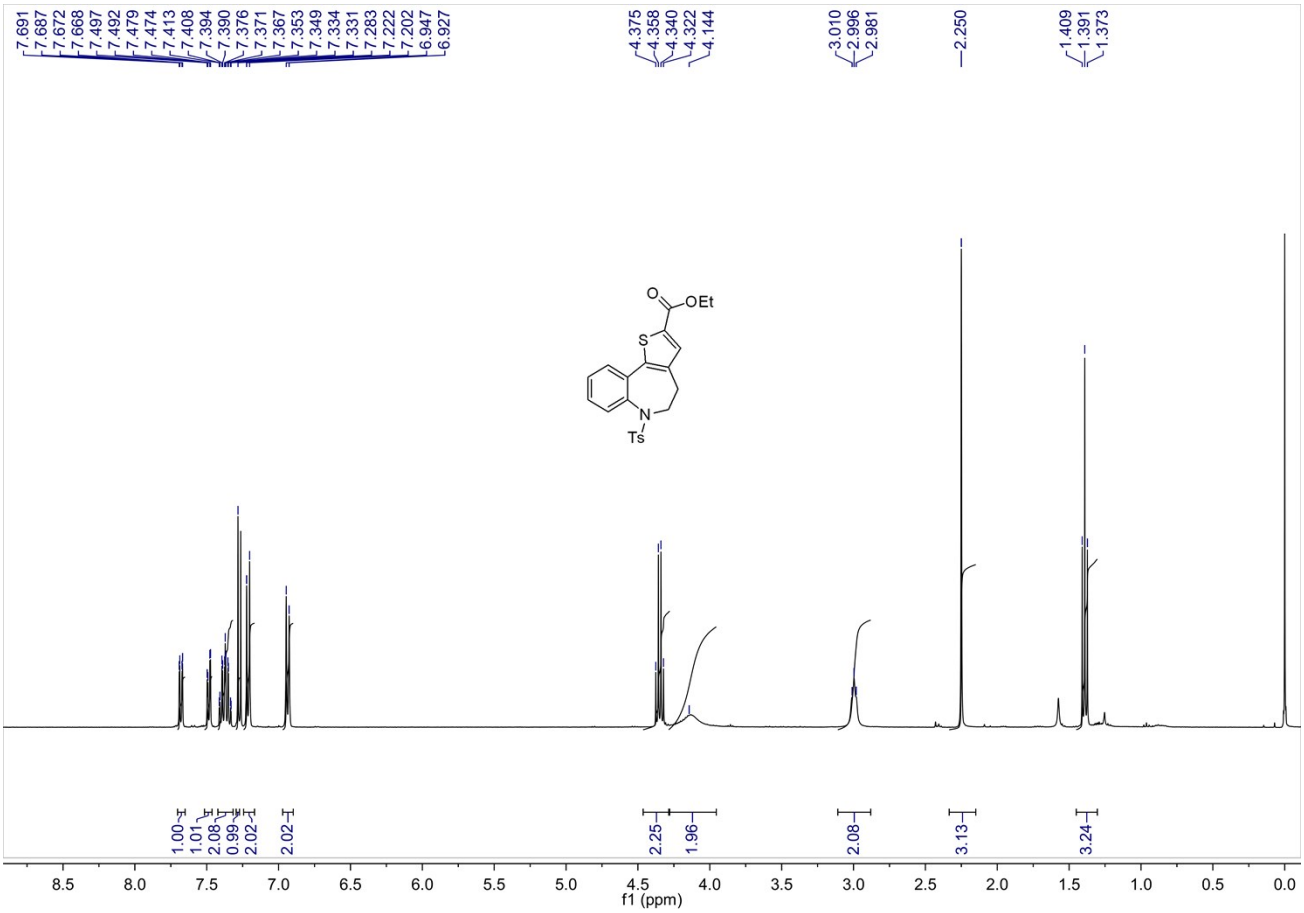
^{1}H NMR of compound 2



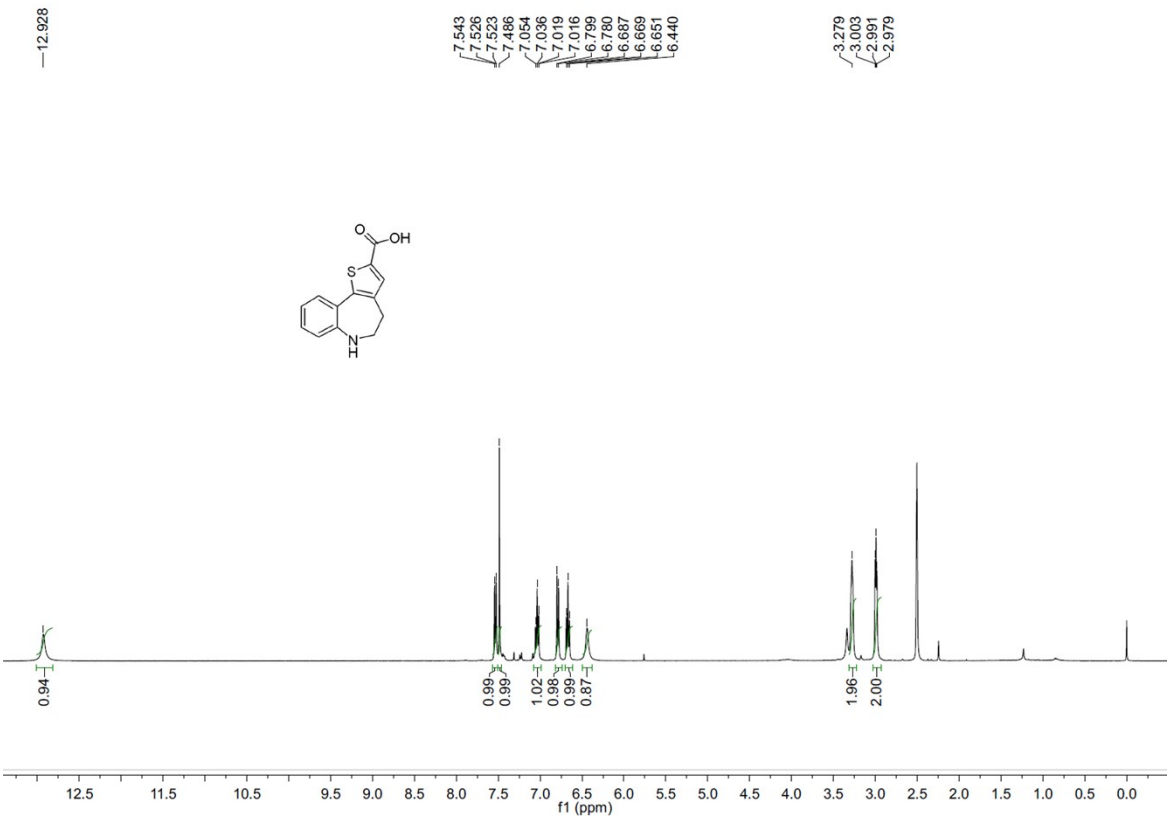
^{1}H NMR of compound 3



^{13}C NMR of compound 3



^{1}H NMR of compound 4



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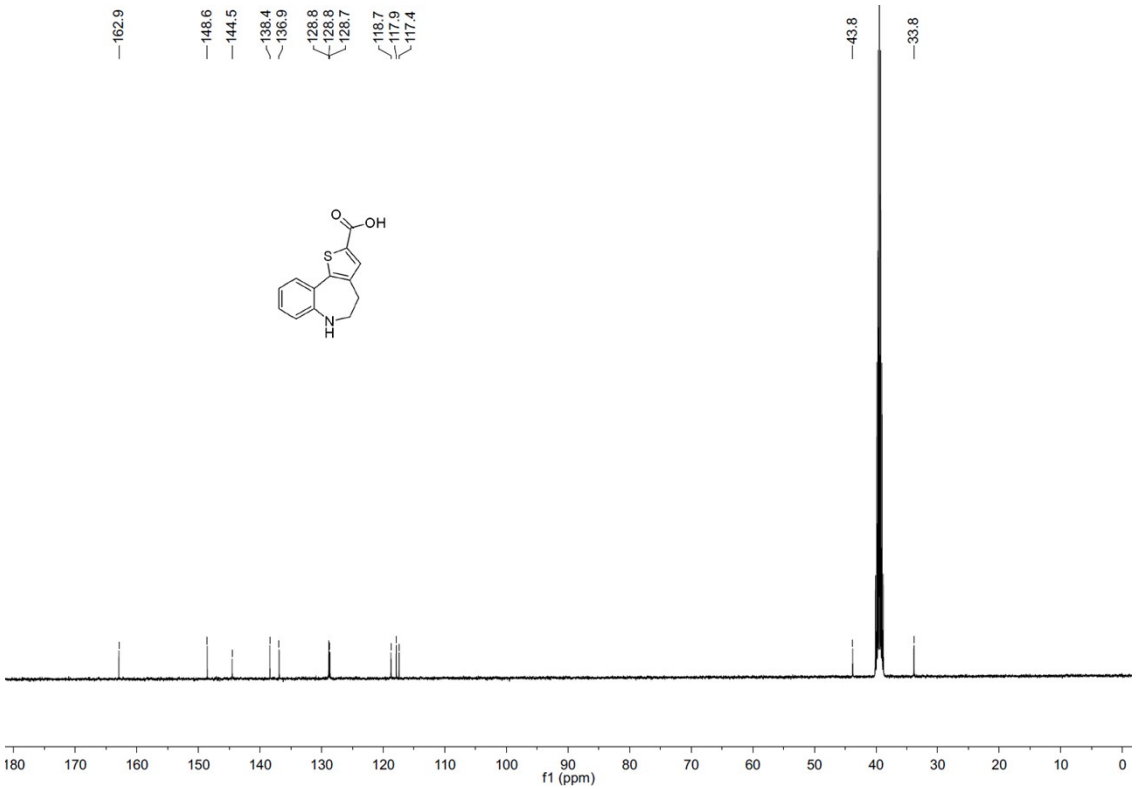

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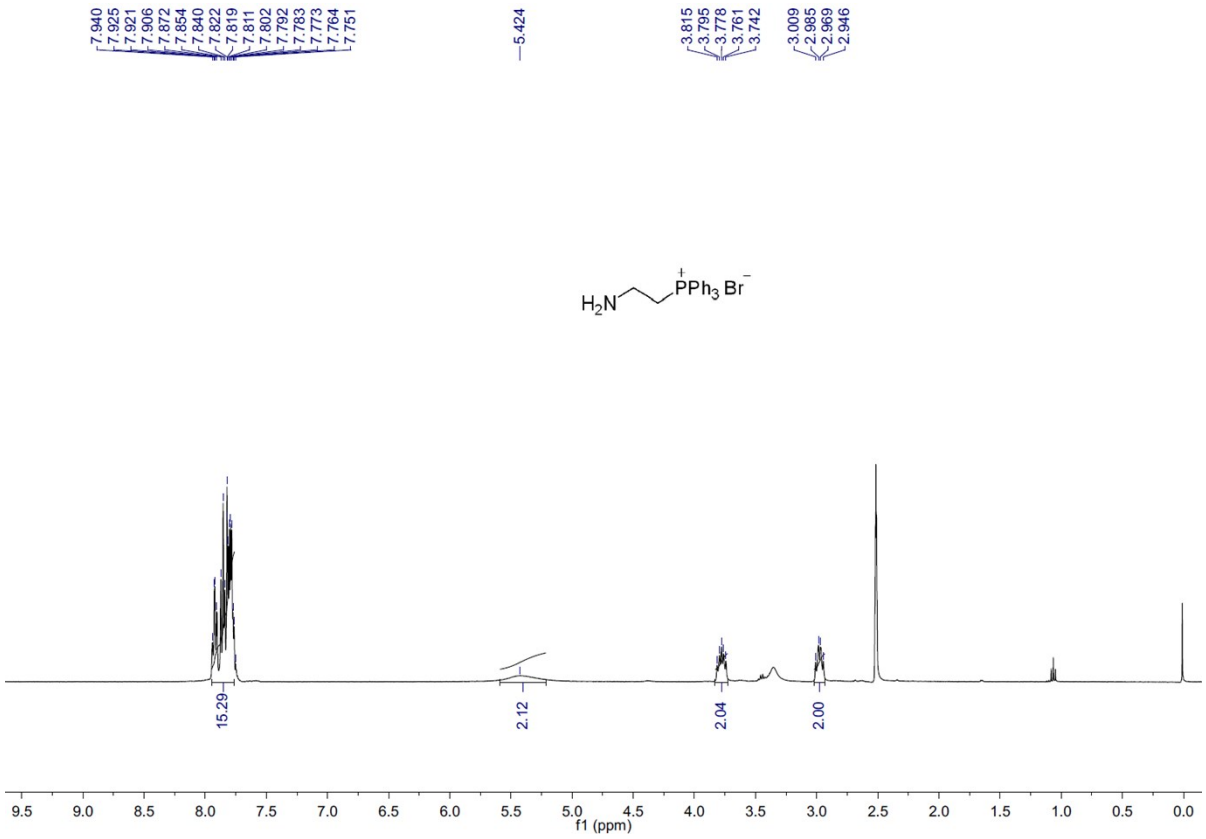
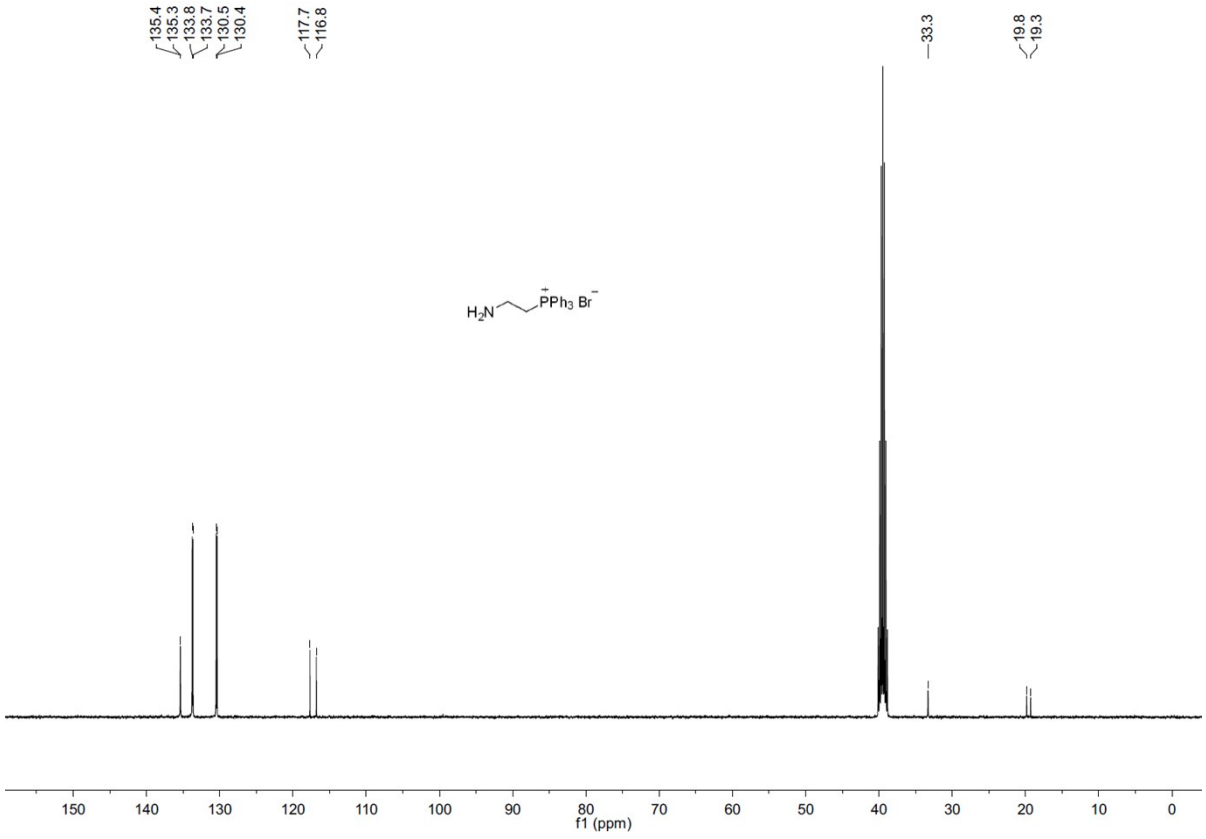


^{1}H NMR of compound TPP-NH_2-1



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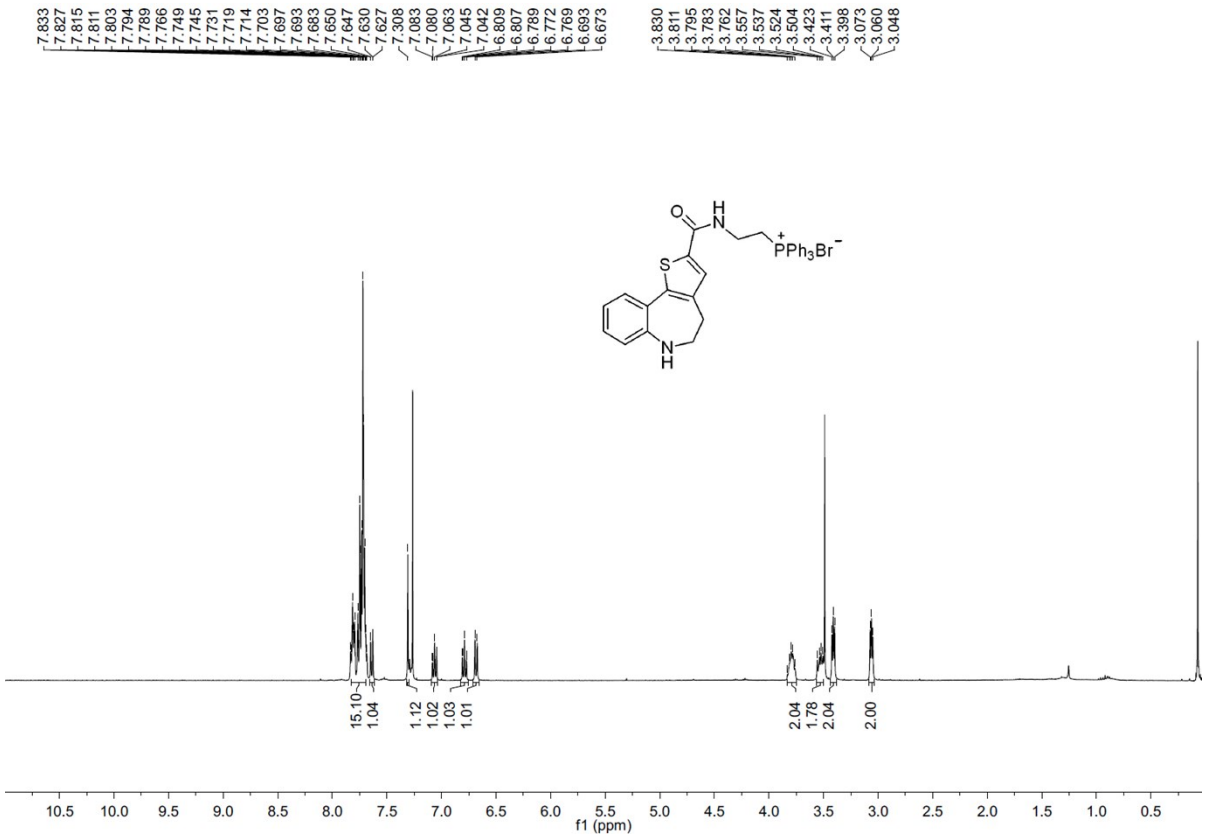
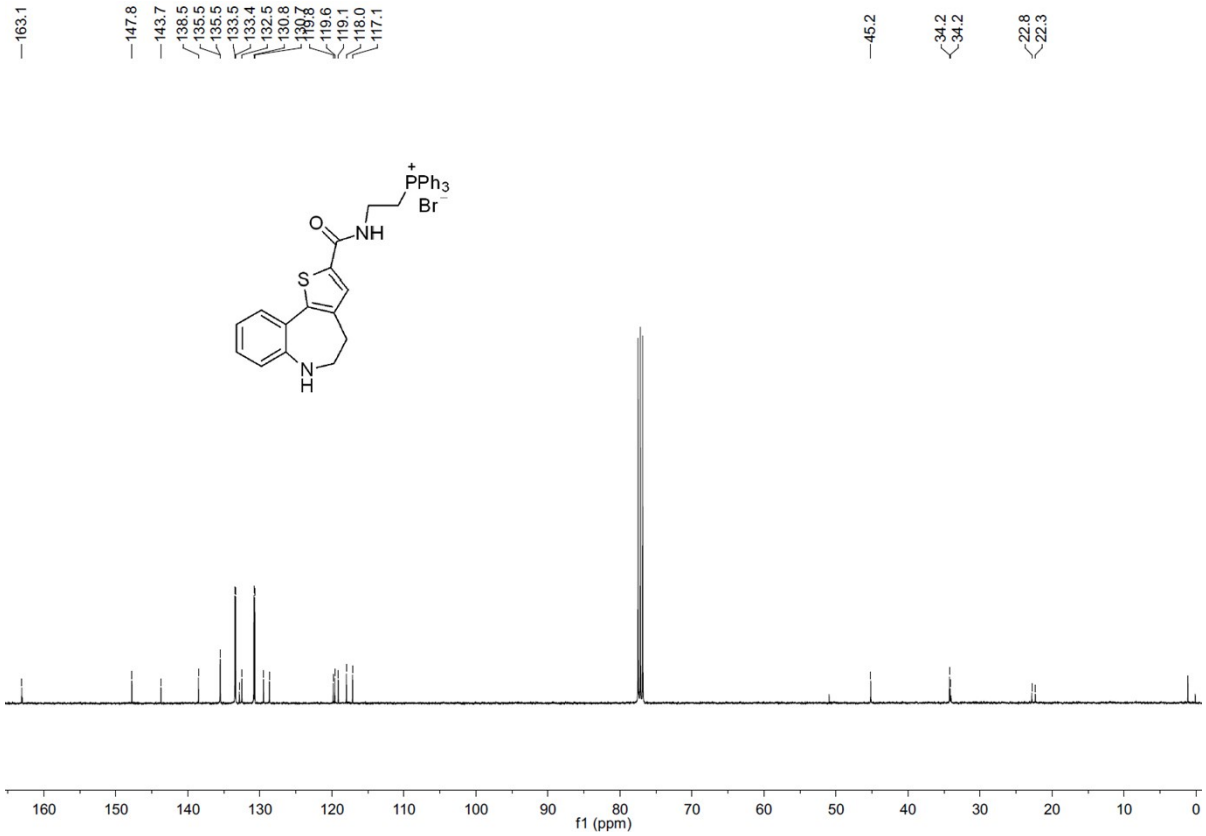


^{1}H NMR of compound 6a



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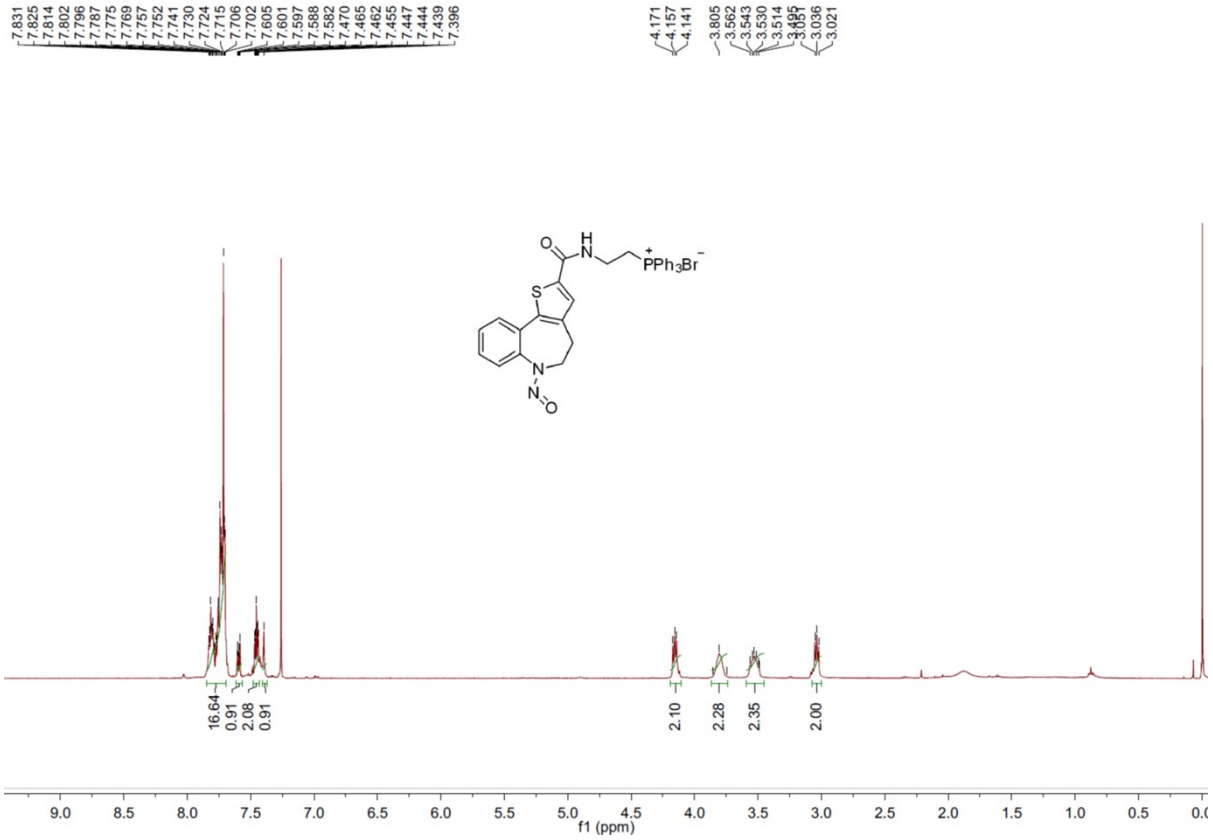

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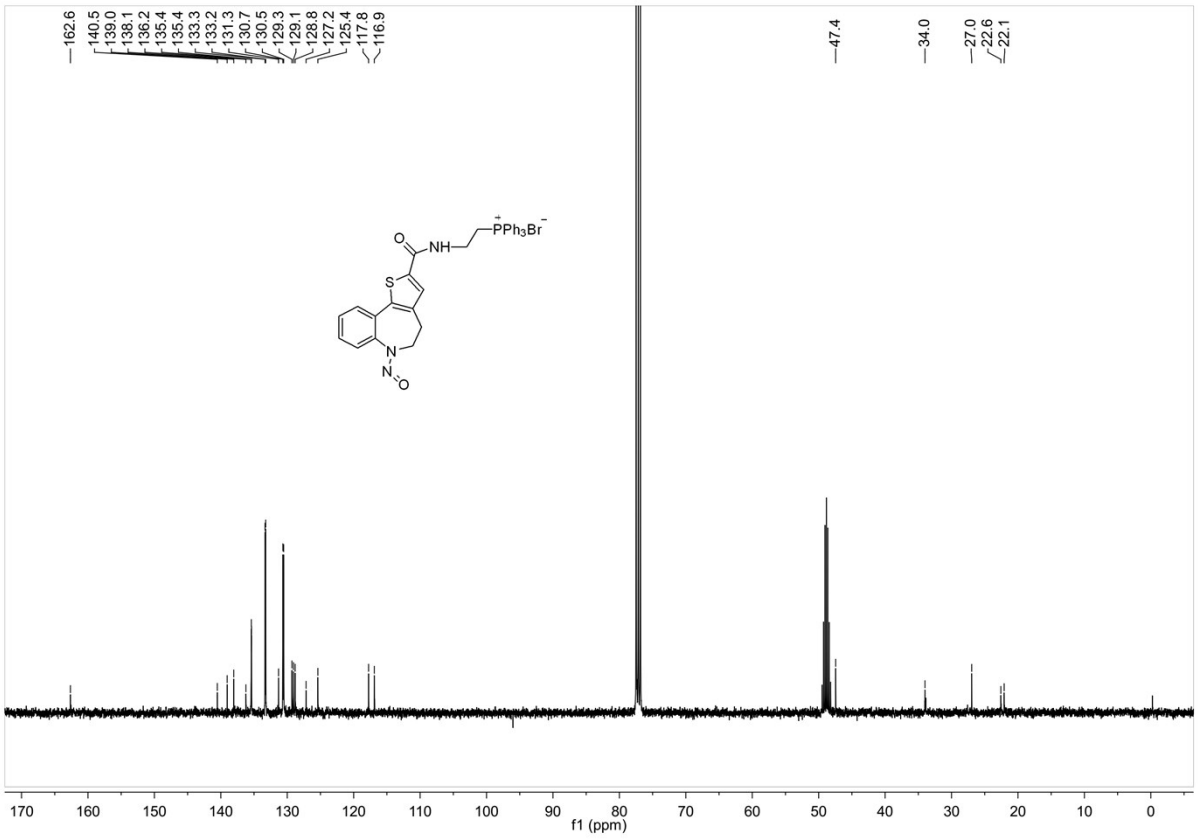

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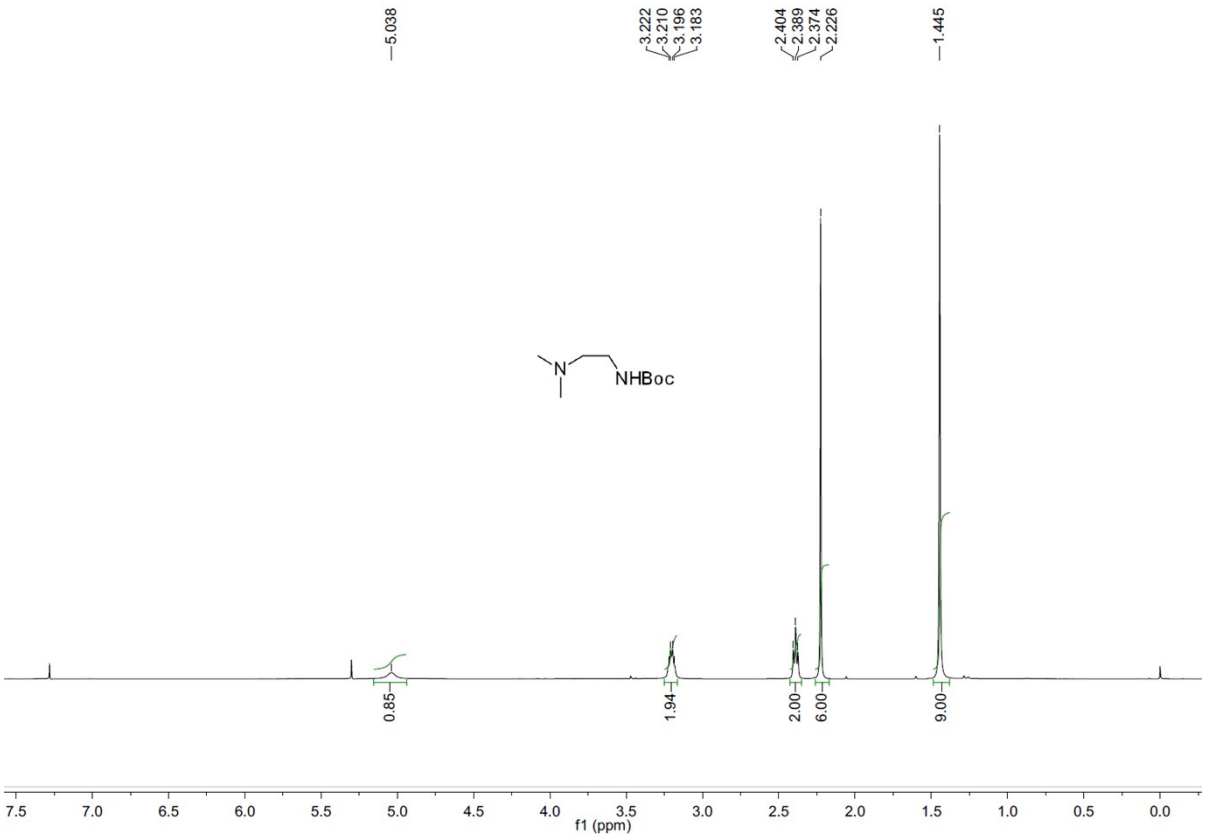

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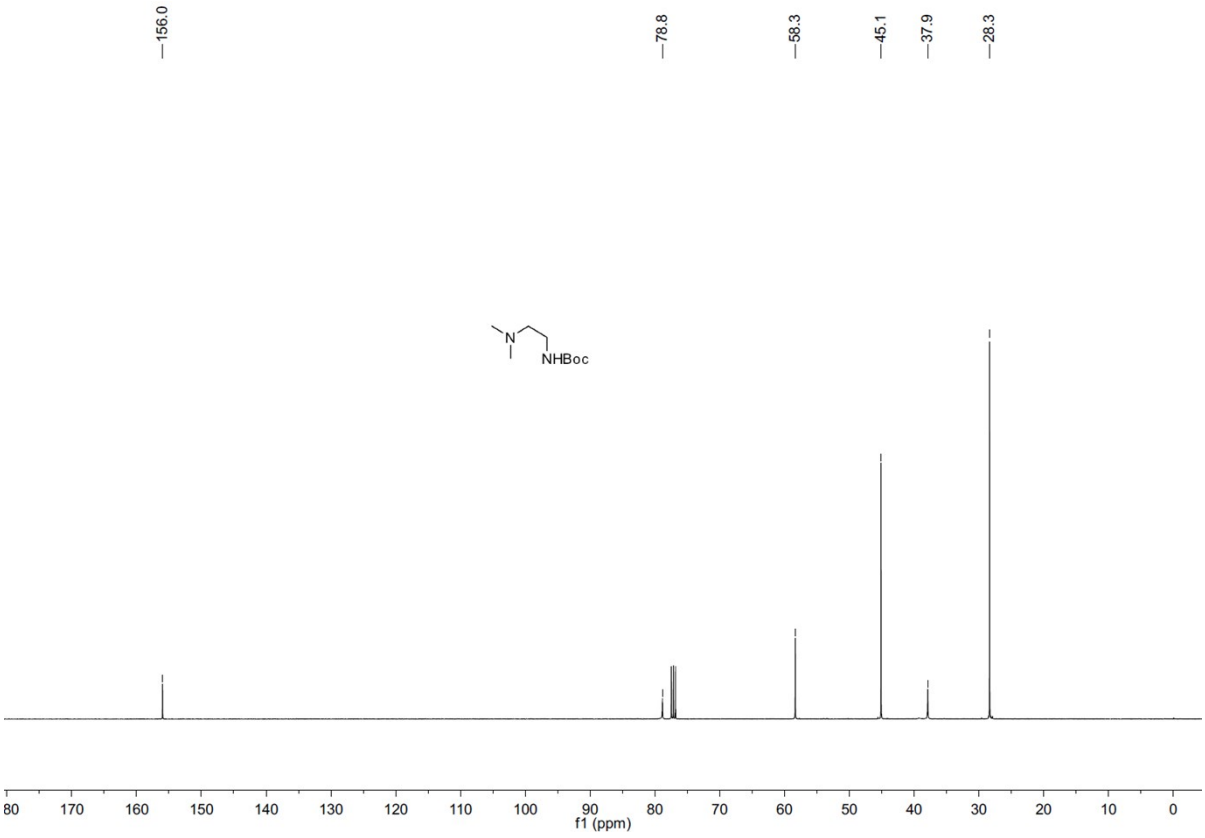

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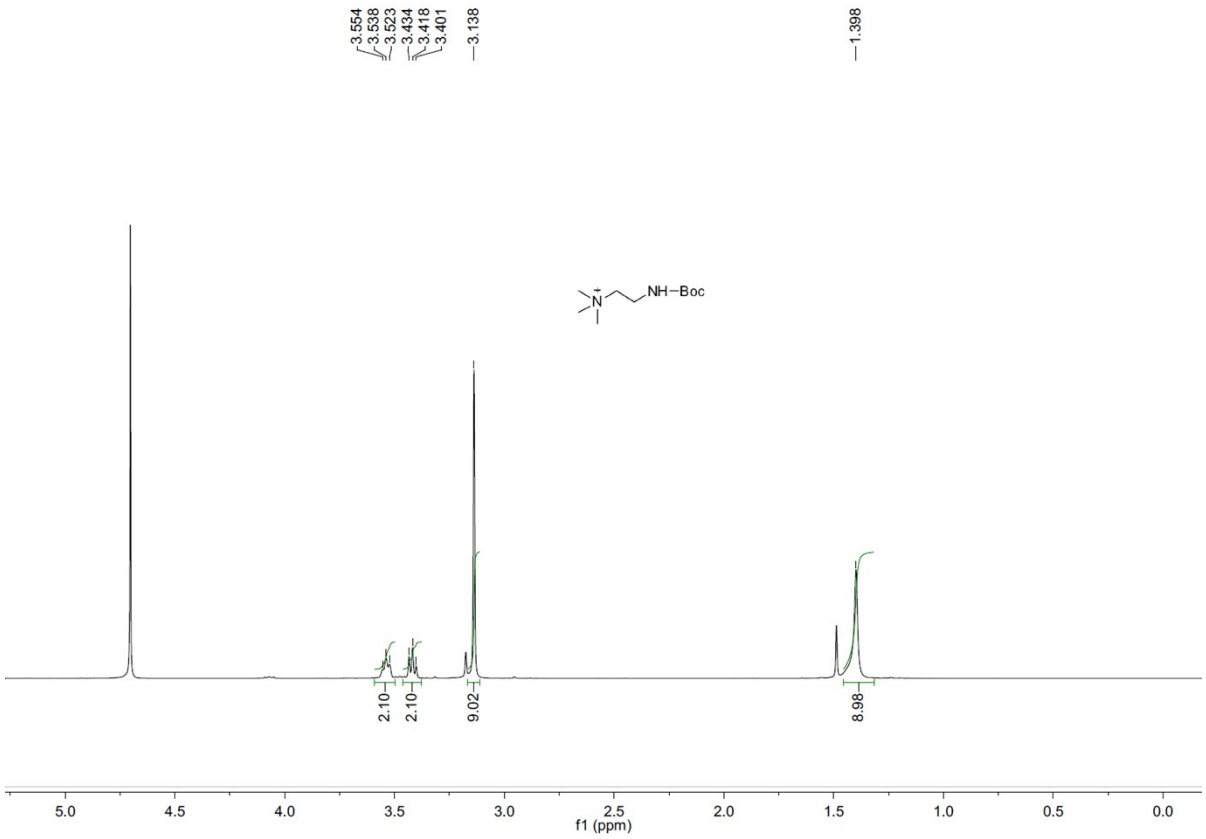

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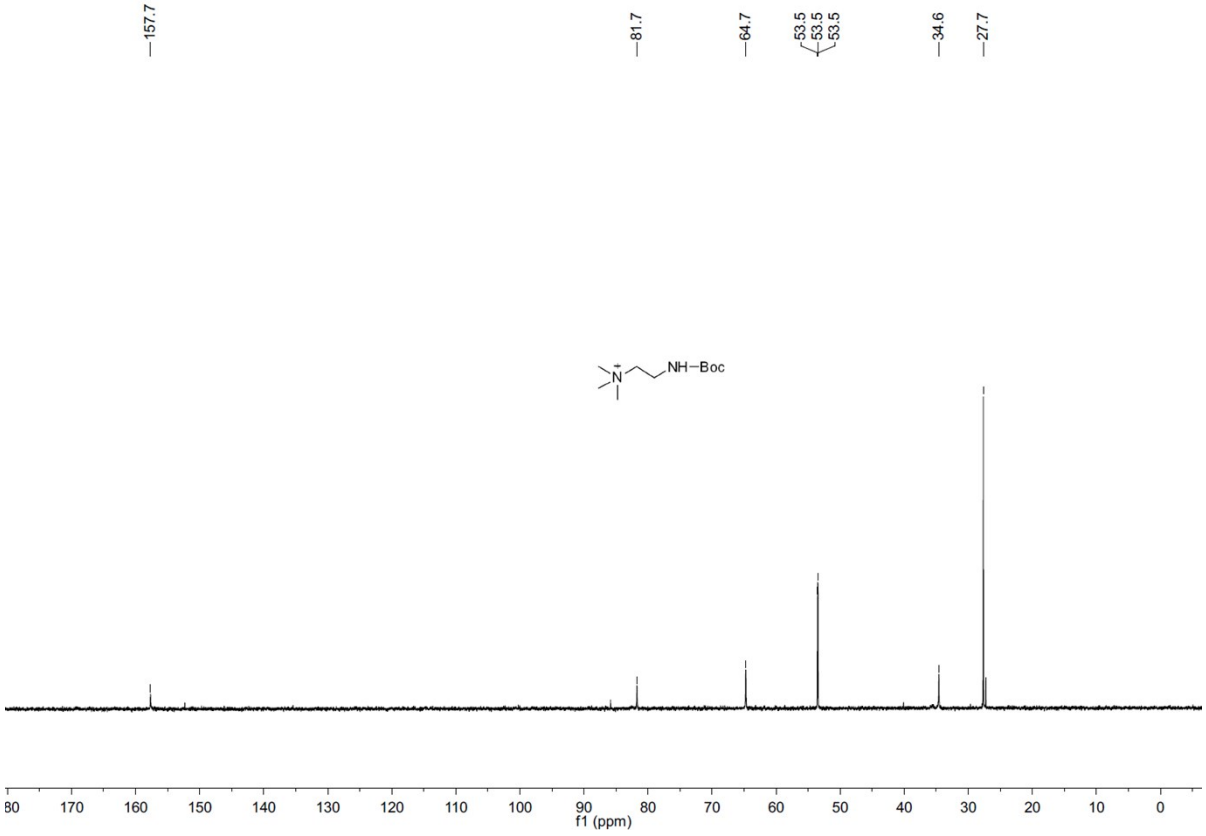

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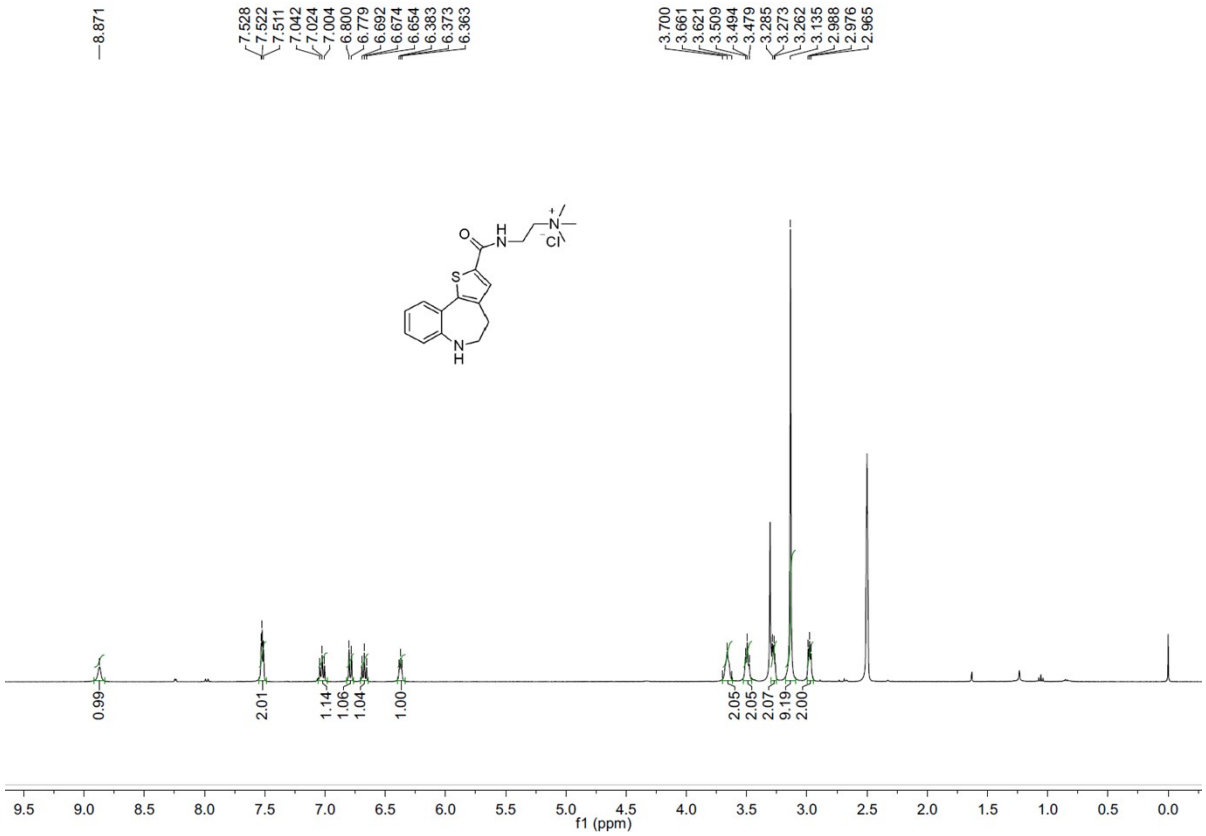
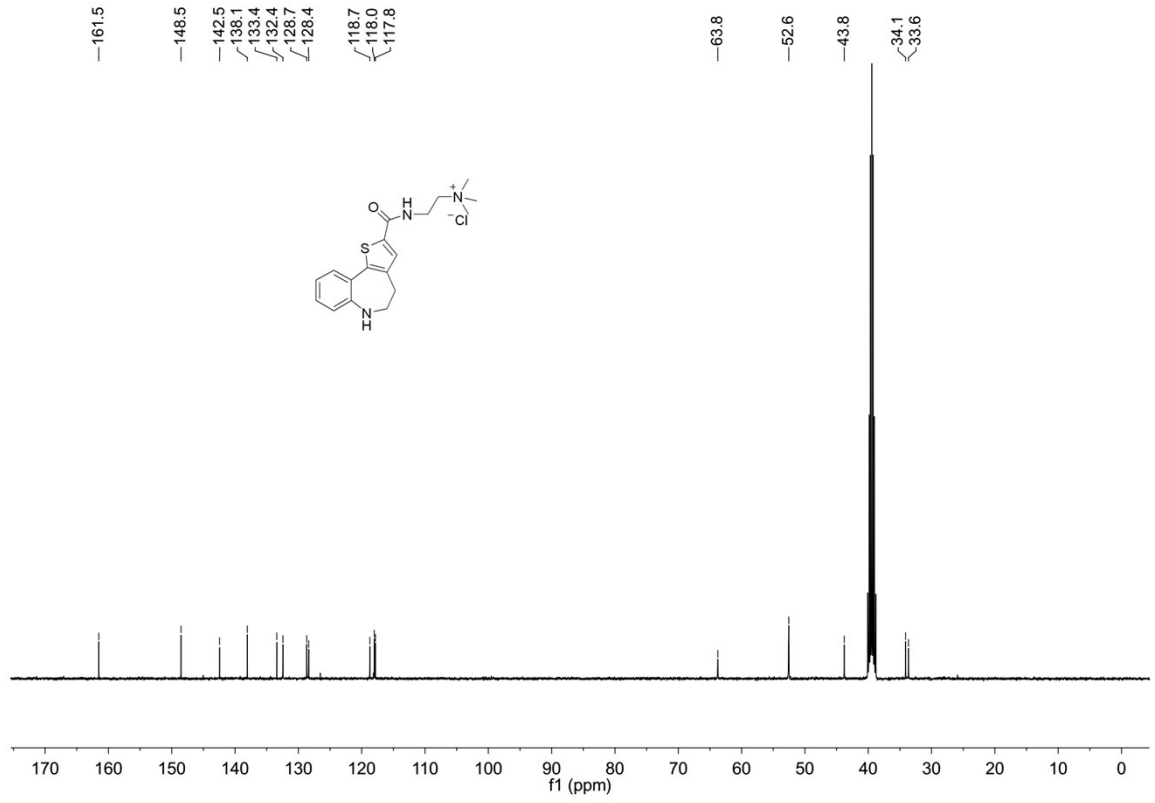


^{1}H NMR of compound 6b



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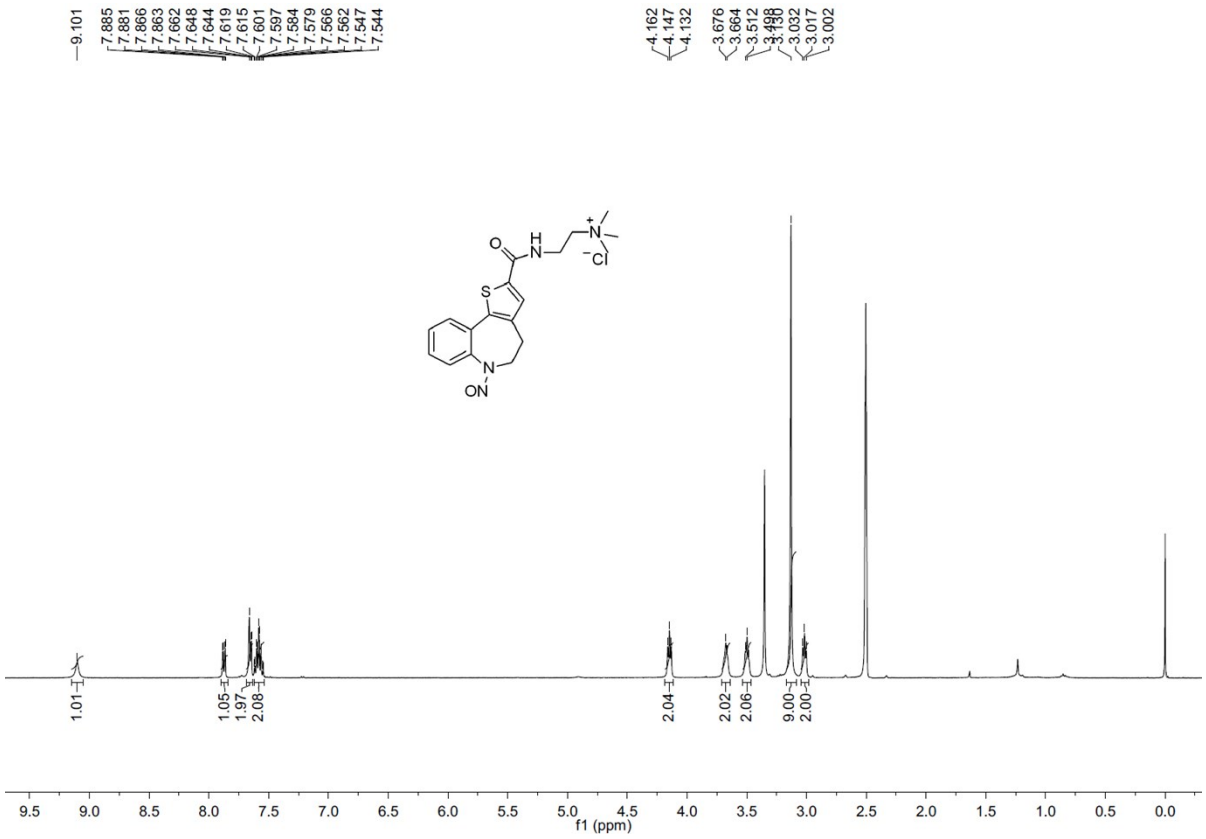

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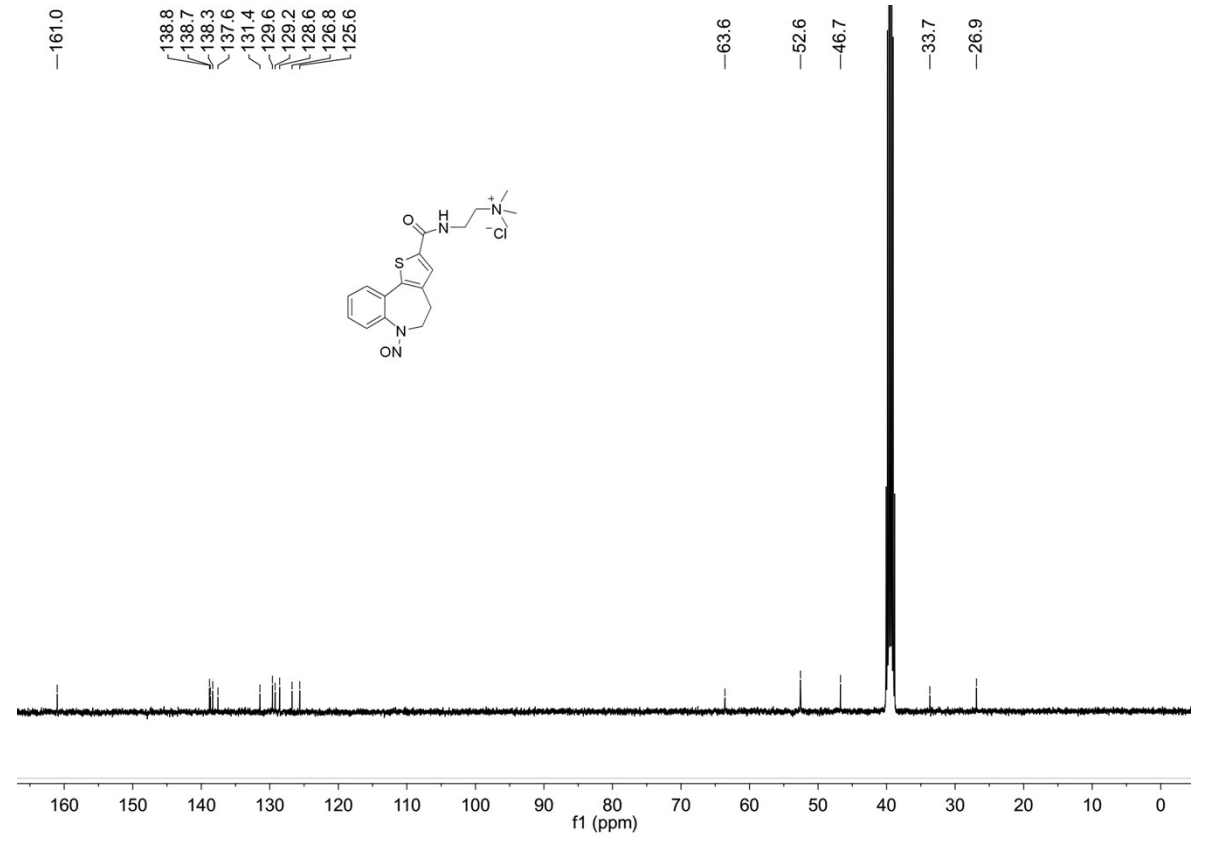

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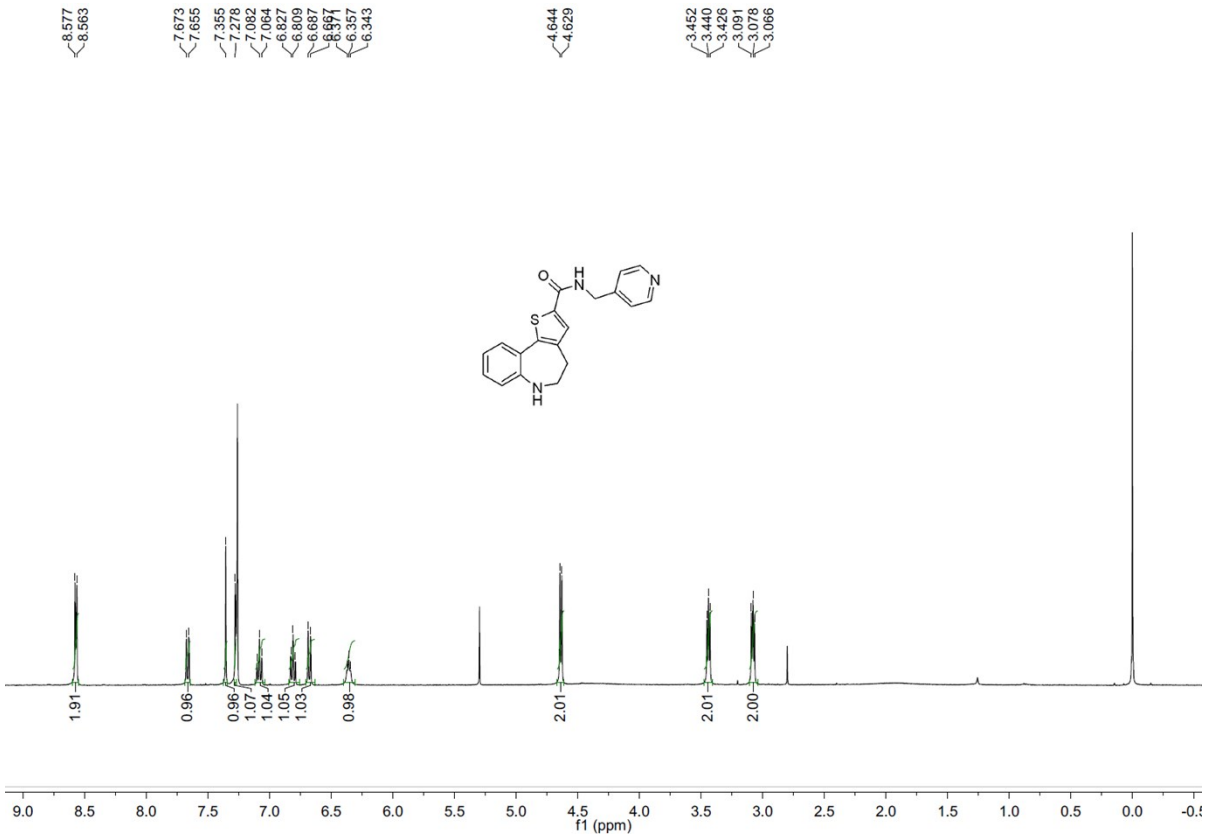

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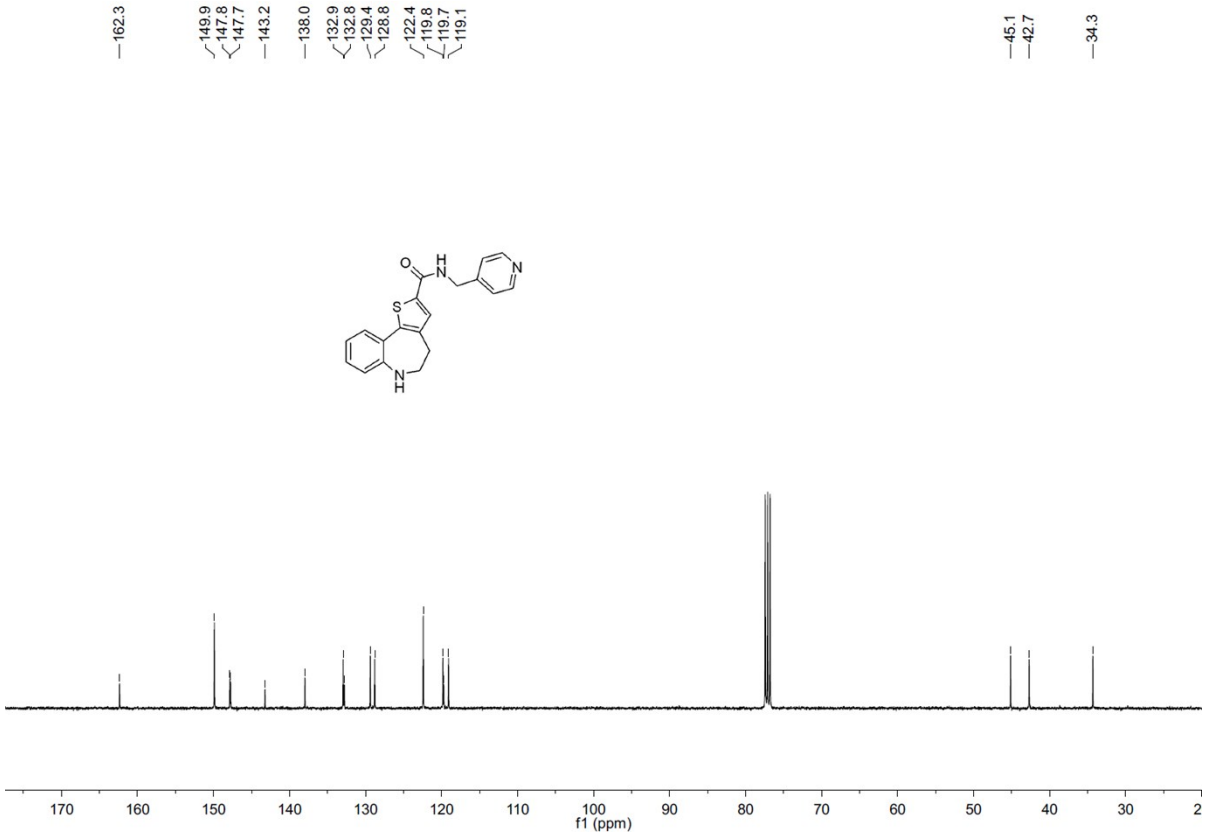

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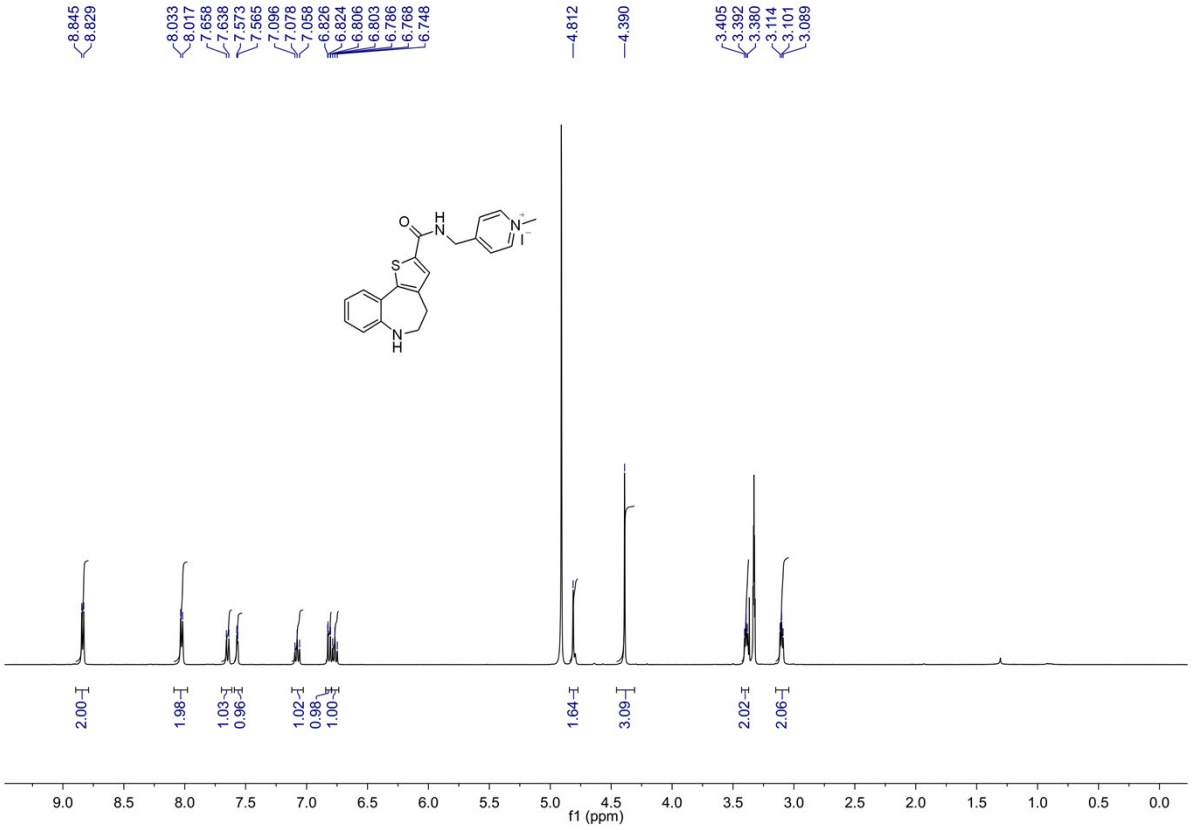

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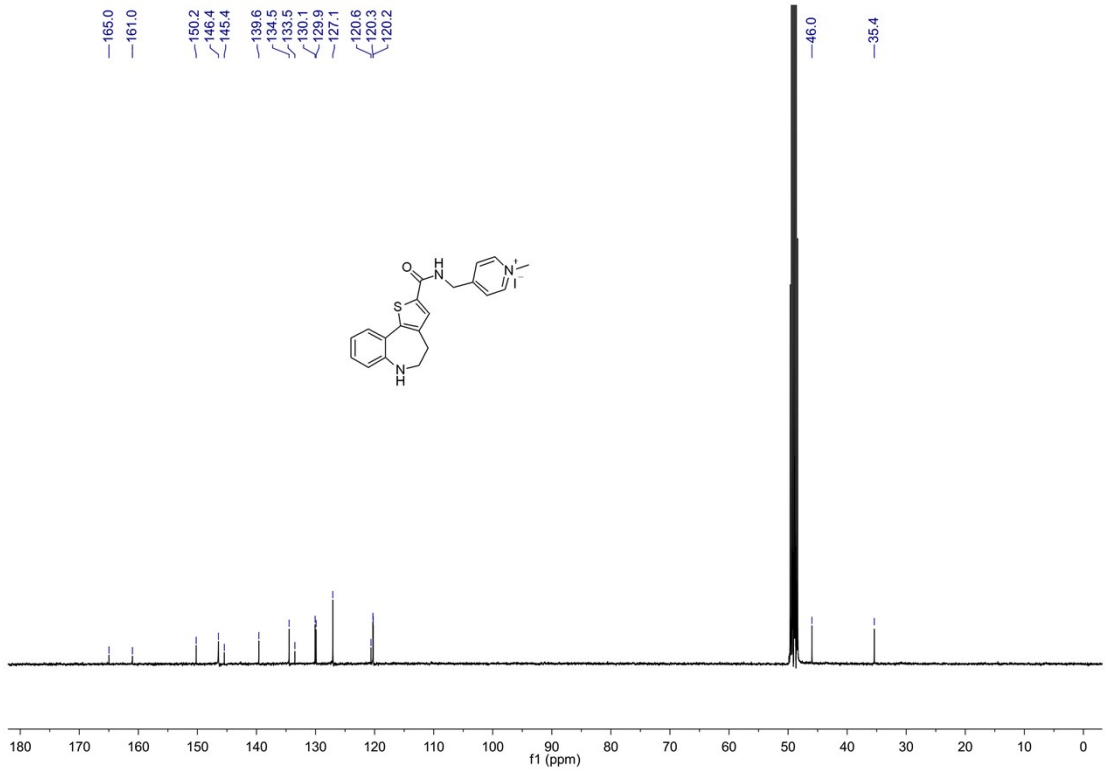

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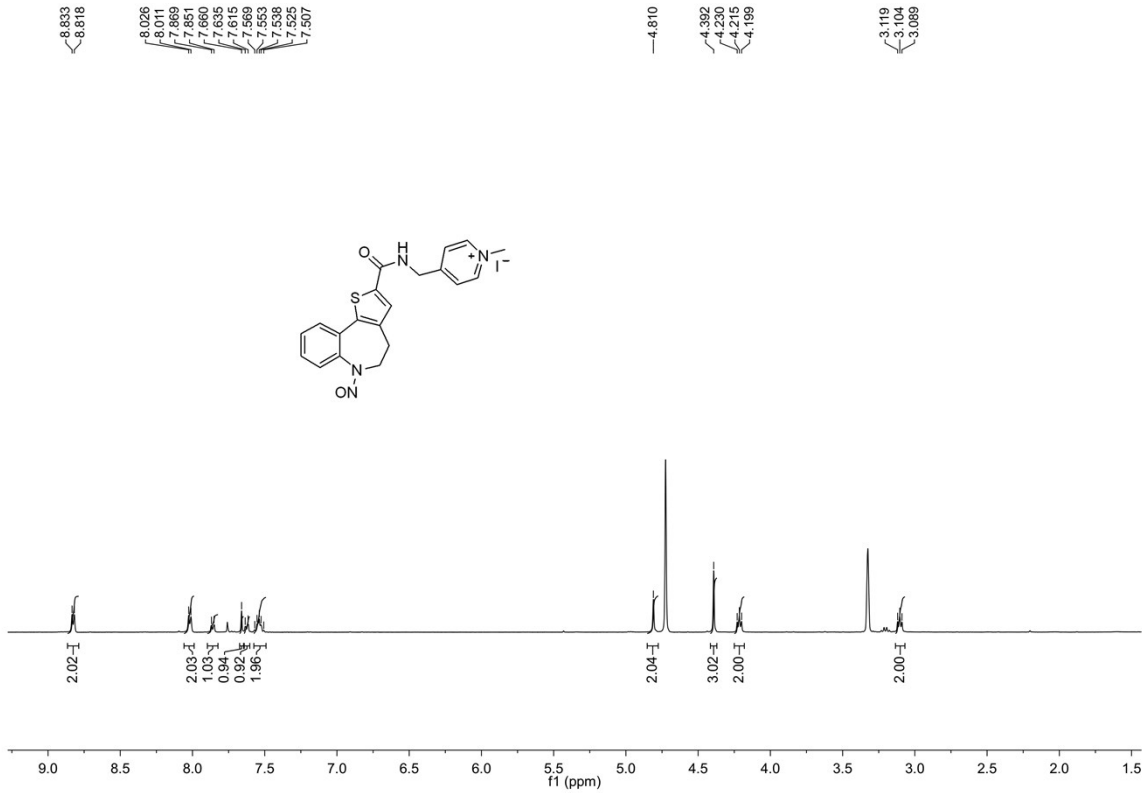

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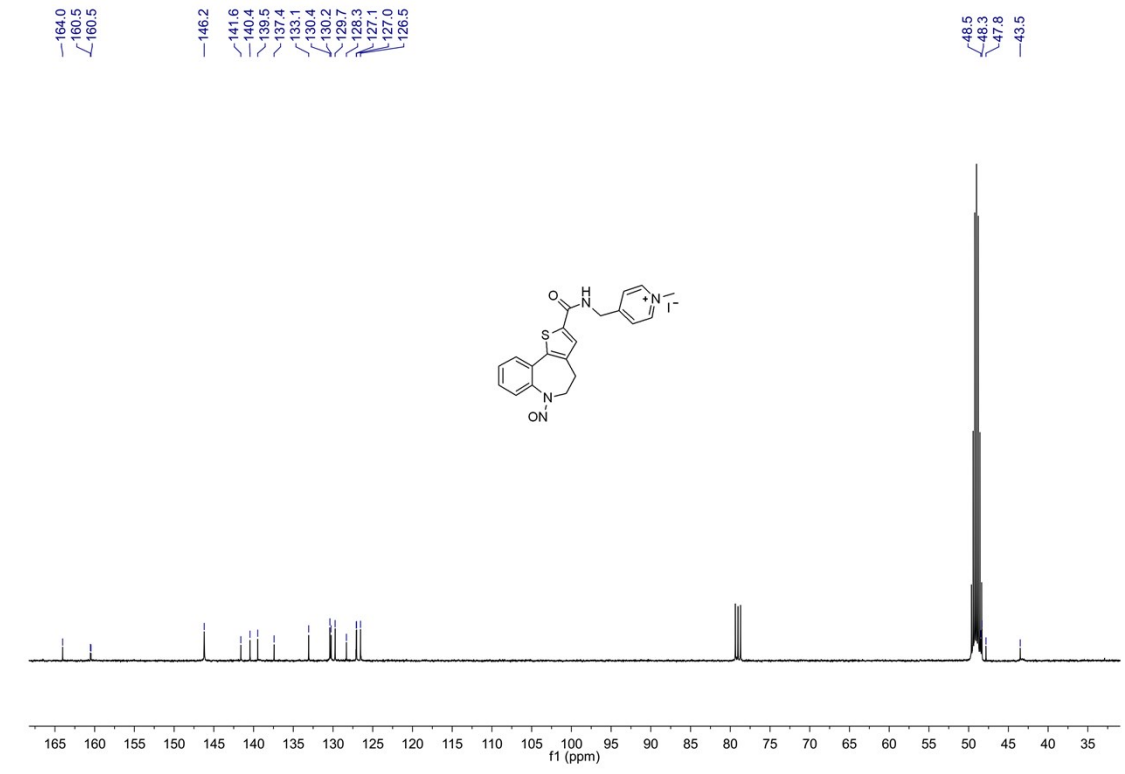

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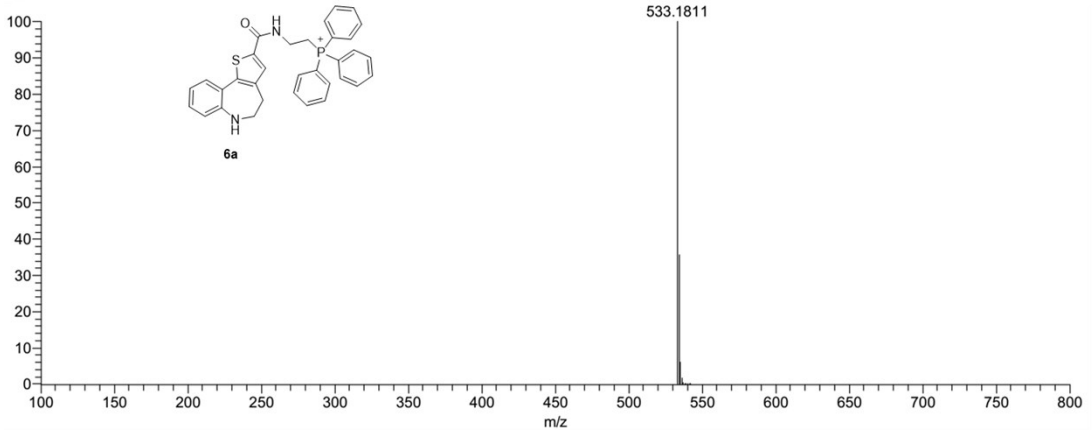
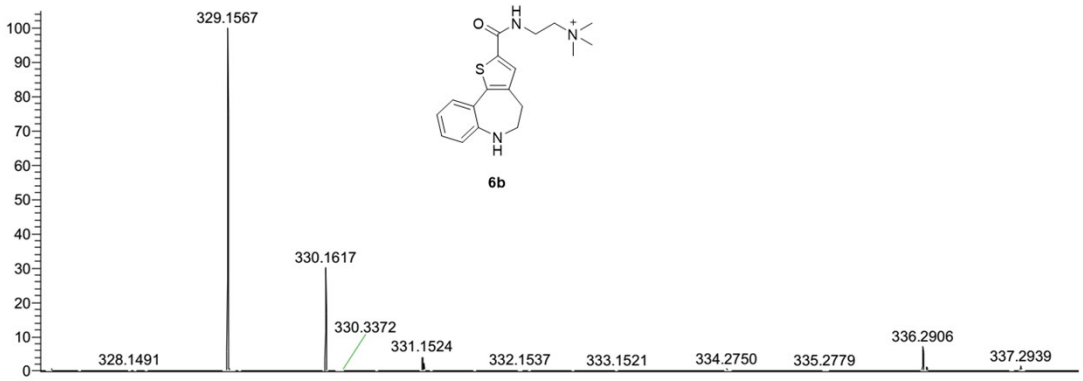


HRMS of compound 6a



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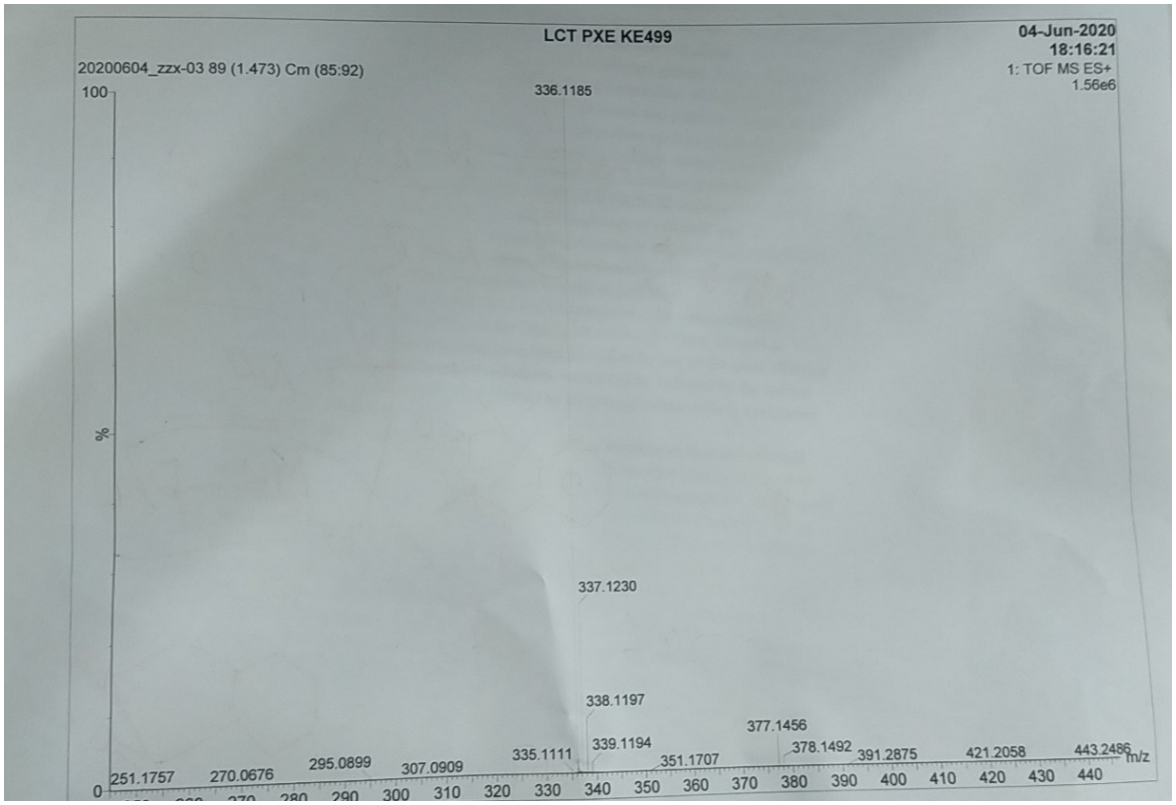

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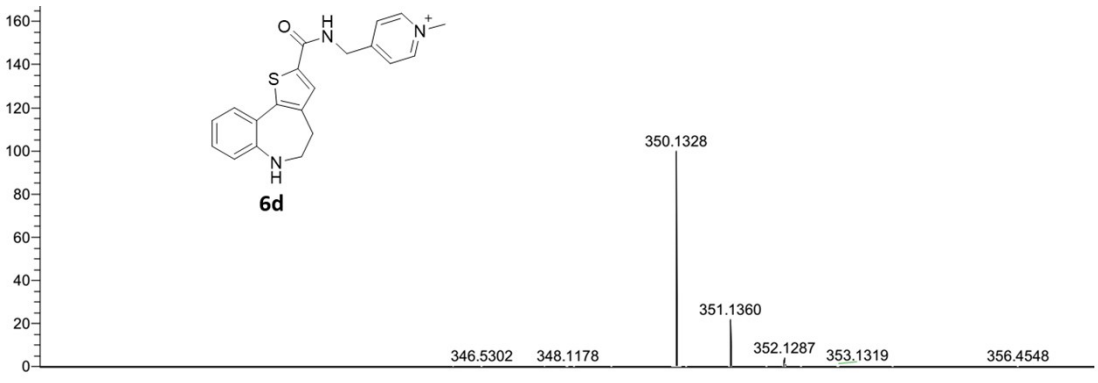

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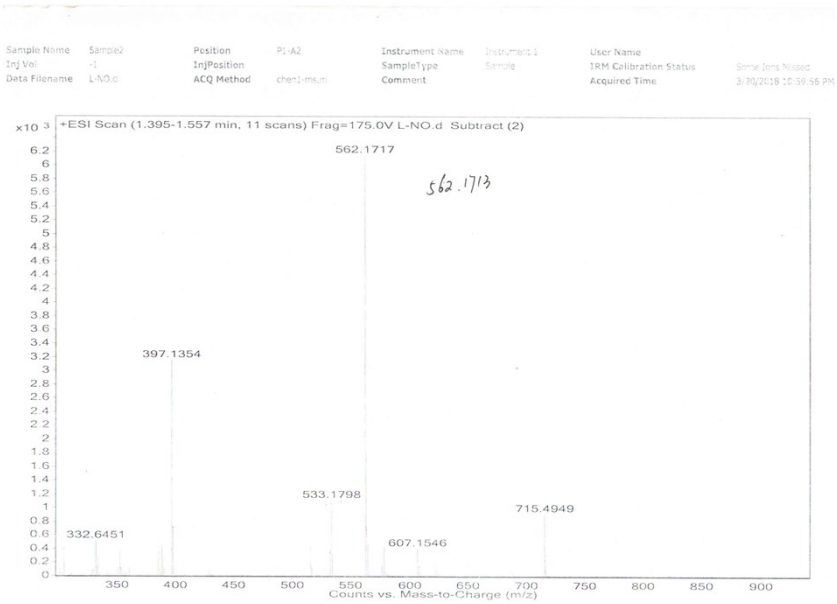

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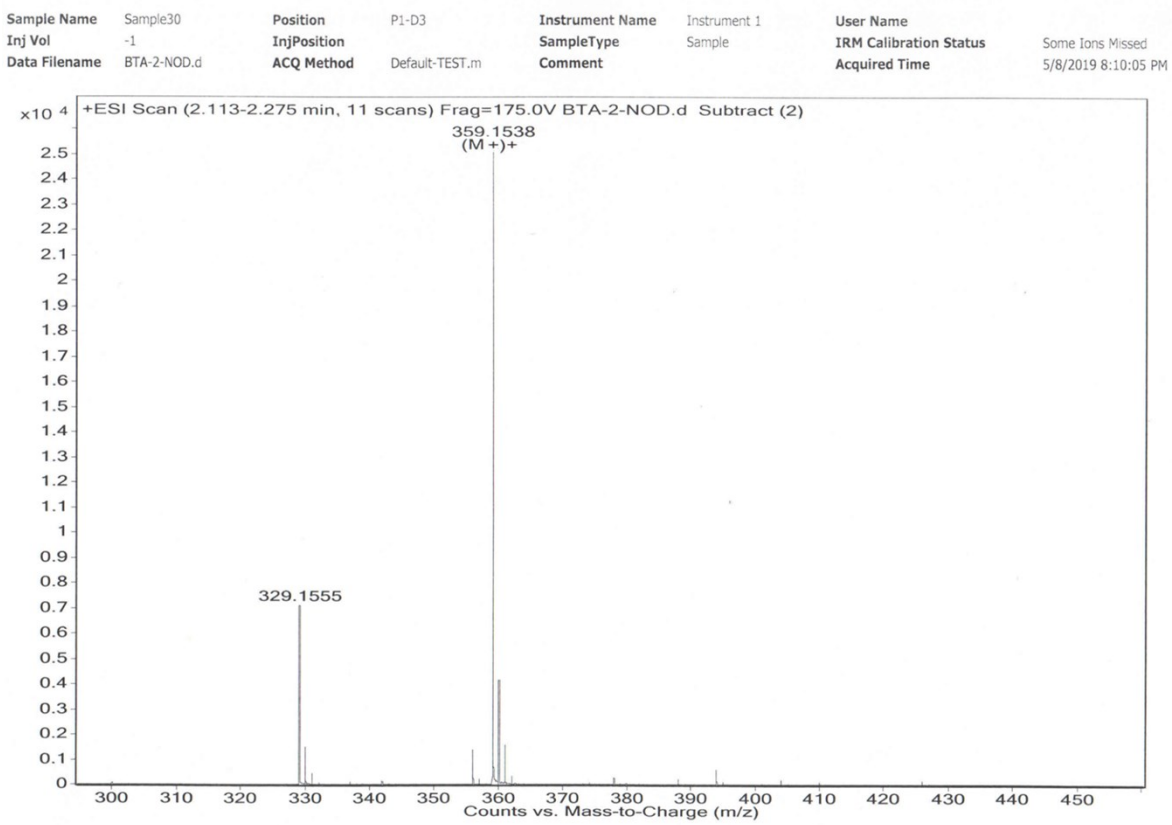

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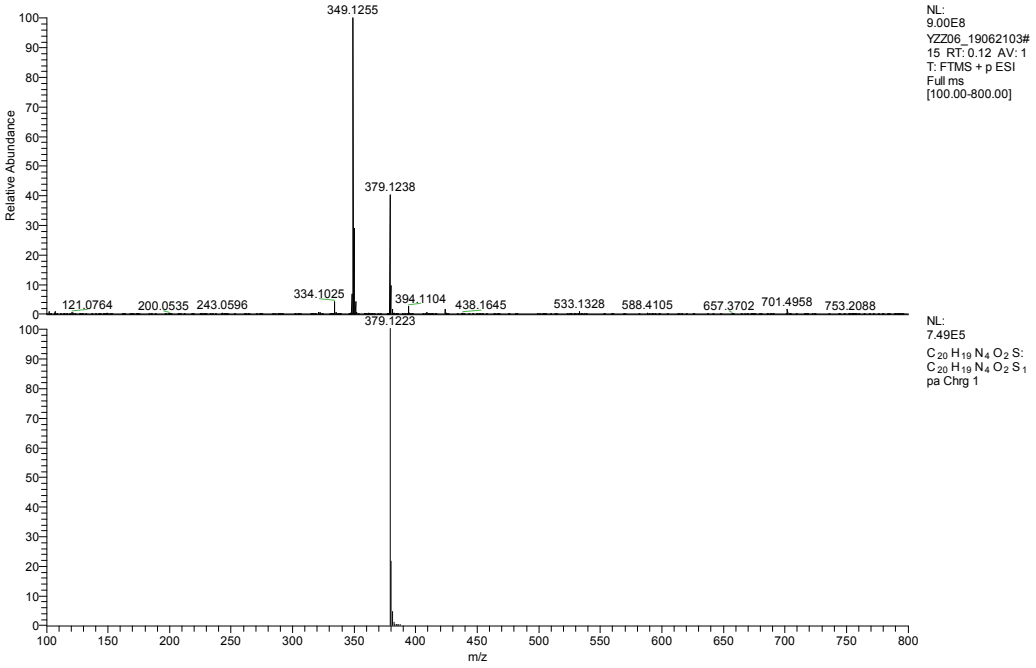

HRMS of compound 7c