Supporting information

Aldol sensors-inspired fluorescent probes for measuring protein citrullination

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- **Supporting data**

Table S1. Quantum yields of Napdialyne and Napdialyne-Cit [a]

Compound	λ_{ex} (nm)	Quantum yield (ΦF)
Napdialyne	342	0.071
Napdialyne-Cit	319	0.916

[a] The quantum yields of tested compounds were recorded in DMSO with 2-aminopyridine in 0.1 N H_2SO_4 as a standard (λ_{ex}=300 nm, ΦF=0.60).

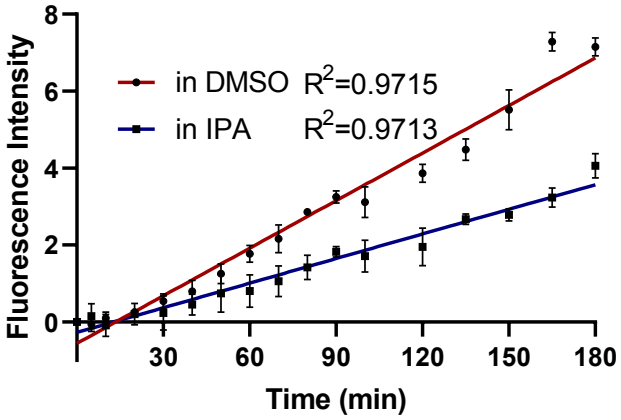


Figure S1. Fluorescence intensity change of reaction mixture over time. Reaction condition: Napdialyne (50 μM), L-citrulline (250 μM), 20% trichloroacetic acid in ddH_2O, 50 °C, 0-3 h. The reaction mixture was diluted 10-times with DMSO or IPA before measurement. For DMSO, λ_{ex} = 320 nm, λ_{em} = 376 nm; For IPA, λ_{ex} = 317 nm, λ_{em} = 360 nm. L-Cit: L-citrulline; IPA: isopropanol. n = 3.

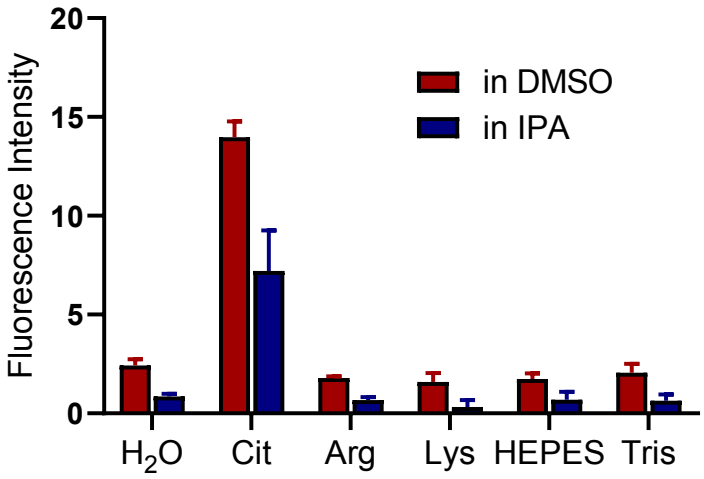


Figure S2. Fluorescence intensity of Napdialyne upon incubation with various analytes. Reaction condition: Napdialyne (50 μM), analyte (250 μM), 20% trichloroacetic acid in ddH_2O, 50 °C, 3 h. The reaction mixture was diluted 10-times with DMSO or IPA before measurement. For DMSO, λ_{ex} = 320 nm, λ_{em} = 376 nm; For IPA, λ_{ex} = 317 nm, λ_{em} = 360 nm. Cit: L-citrulline; Arg: L-arginine; Lys: L-lysine; IPA: isopropanol. n = 2.

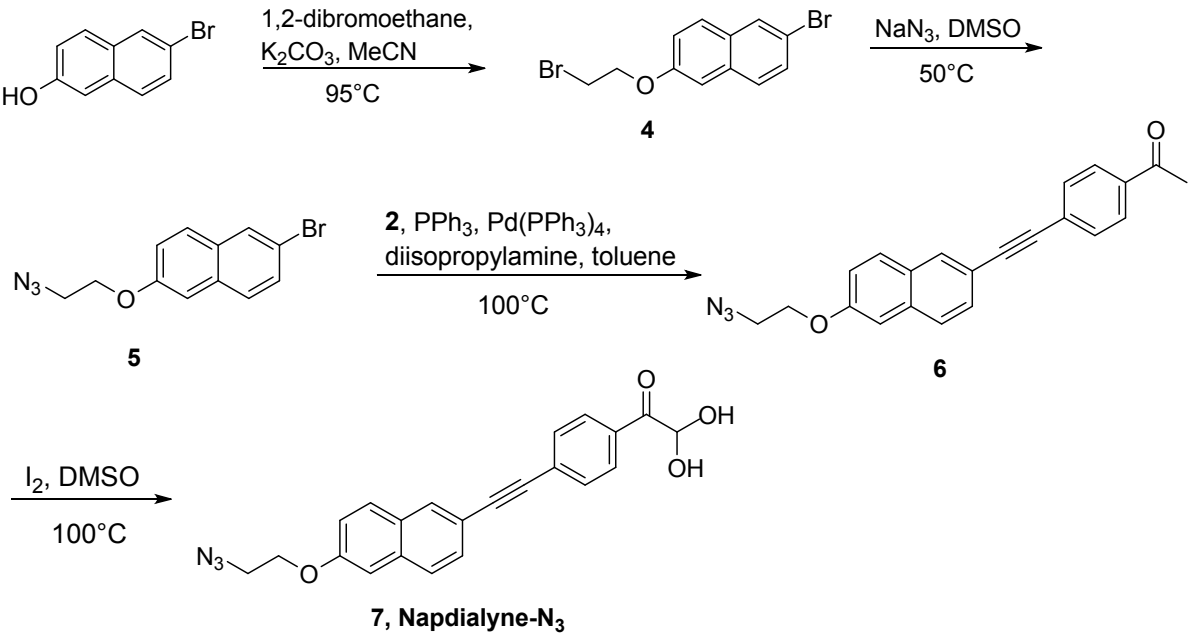


Figure S3. Synthetic routine to probe Napdialyne-N_3.

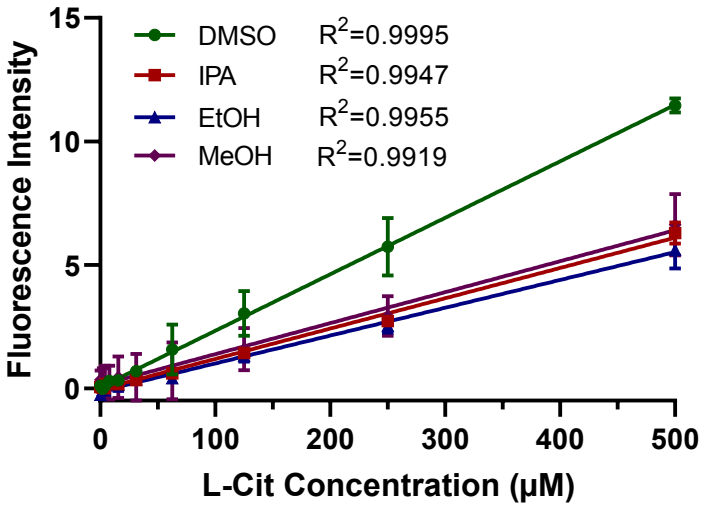


Figure S4. Dose-dependent relationship of fluorescence intensity of Napdialyne-N_3 upon incubation with different concentrations of L-citrulline. Reaction condition: Napdialyne-N_3 (50 μM), L-citrulline (0.5 to 0 mM, 2-fold dilution), 20% trichloroacetic acid in ddH_2O, 50 ^oC, 3 h. The reaction mixture was diluted 10-times with different solvents before measurement. For DMSO, λ_{ex} = 320 nm, λ_{em} = 376 nm; For IPA, λ_{ex} = 317 nm, λ_{em} = 360 nm; For ethanol, λ_{ex} = 315 nm, λ_{em} = 362 nm; For methanol, λ_{ex} = 318 nm, λ_{em} = 360 nm. L-Cit: L-citrulline; IPA: isopropanol. n = 2.

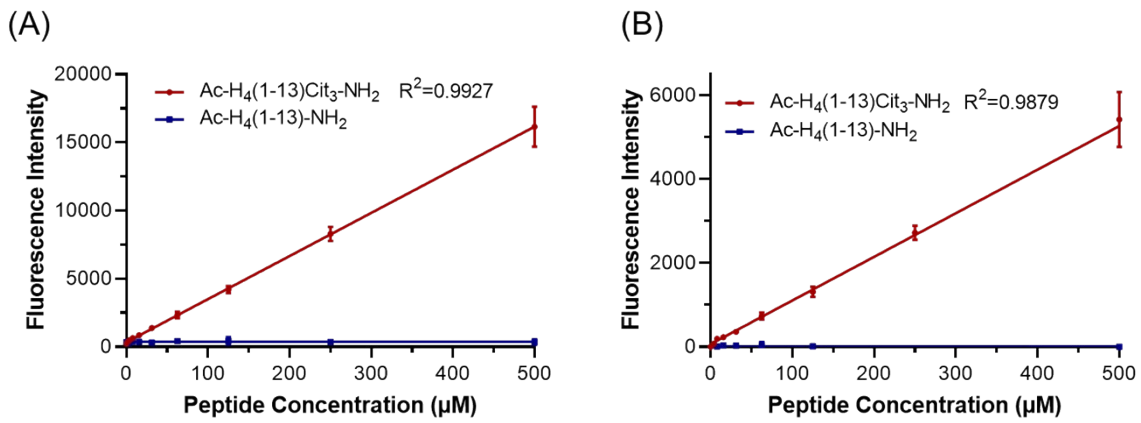


Figure S5. Dose-dependent relationship on fluorescence intensity of probe Napdialyne (A) or probe Napdialyne-N_3 (B) upon incubation with different concentrations of peptides. Reaction condition: probe (50 μM), peptide (0.5 to 0 mM, 2-fold dilution), 20% trichloroacetic acid in ddH_2O, 50 °C, 3 h. The reaction mixture was diluted 10-times with DMSO before measurement. For Napdialyne, λ_{ex} = 320 nm, λ_{em} = 376 nm; For Napdialyne-N_3, λ_{ex} = 317 nm, λ_{em} = 360 nm; n = 3. Note that this data were collected on BioTek Cytation™ 5 imaging reader.

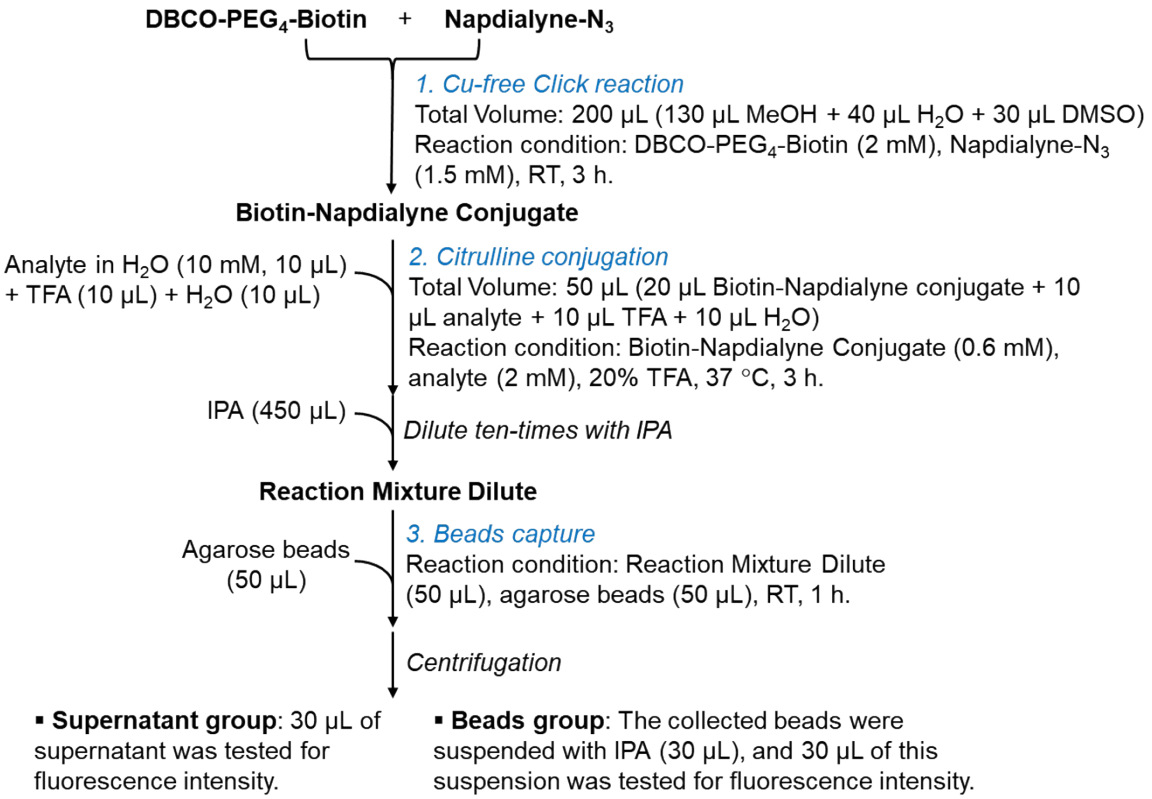


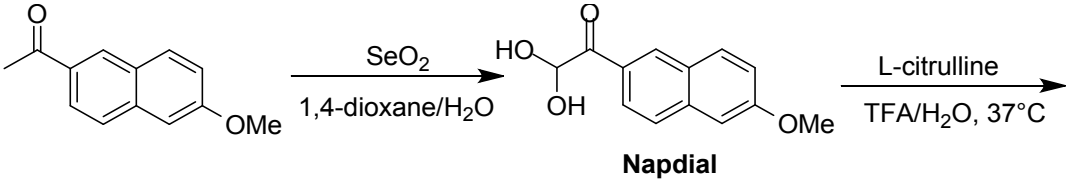
Figure S6. Work-flow of the three-step capture assay by probe Napdialyne-N_3.

- **Experimental procedure**

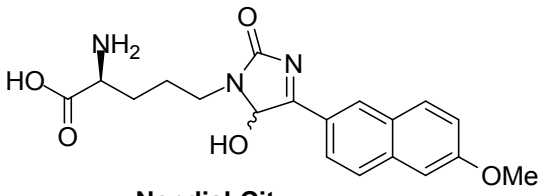
Methods and materials

All chemicals were used as received unless otherwise stated. L-citrulline was obtained from Bide Pharmatech Ltd. (Shanghai, China). The Peptides were custom-synthesized from GL Biochem Ltd. (Shanghai, China). The streptavidin agarose resin (catalog No. 20347) was purchased from Thermo Scientific (USA). The 96-well plate (black, flat, not treated) was purchased from Corning® (USA). ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 400 NMR spectrometer. Proton chemical shifts of NMR spectra were calibrated with TMS as internals reference. ESI-MS and HRMS spectral data were recorded on Quadrupole LC/MS 6120 and TripleTOF™ 5600^{+}. Fluorescence analysis was performed on Thermo Scientific™ Varioskan™ LUX microplate reader unless otherwise stated.

1. Synthesis of probe Napdial



Napdial



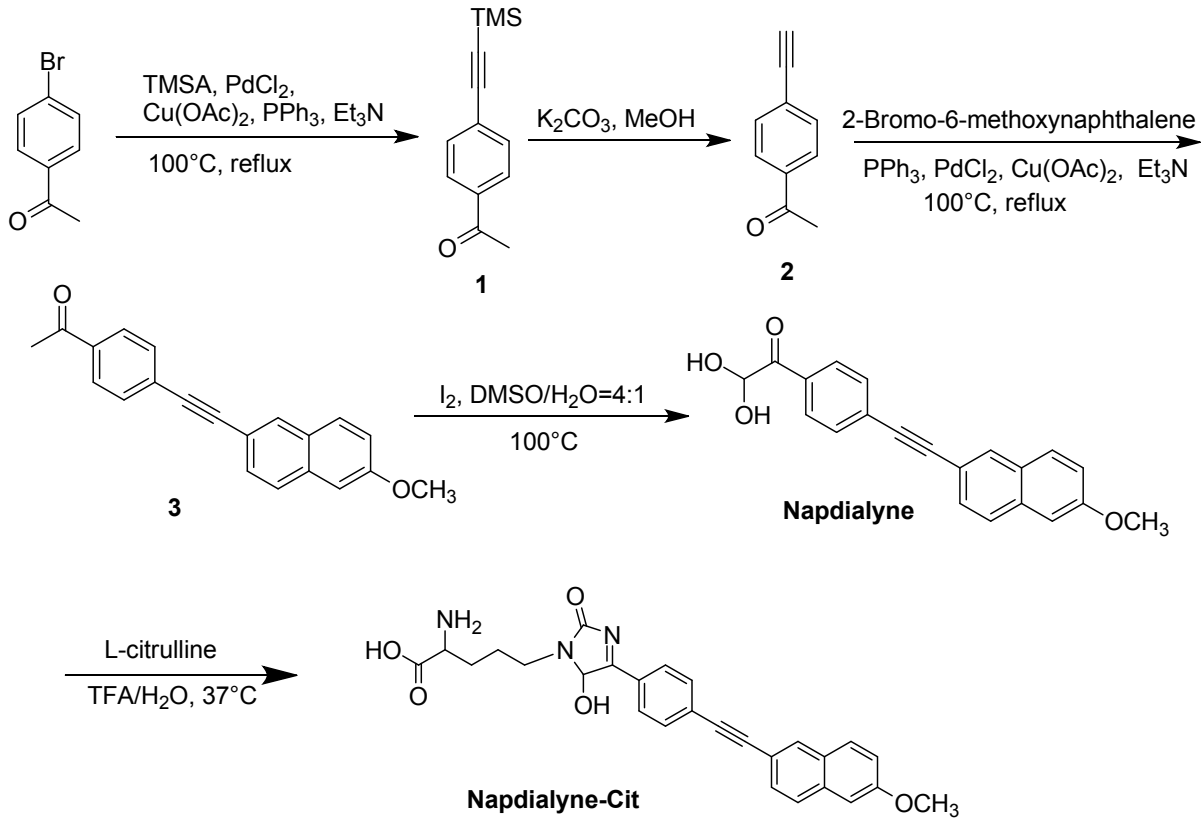
Napdial-Cit

A solution of selenium dioxide (278 mg, 2.5 mmol) in 1,4-dioxane (5 mL) and H_2O (750 μL) was heated to 55°C until the selenium dioxide was dissolved. Then 2-acetyl-6-methoxunaphthalene (500 mg, 2.5 mmol) was added in. The reaction mixture was refluxed overnight. After cooling, the precipitate was filtered off, and the filtrate was concentrated. The crude was purified by silica gel column chromatography (petroleum ether : ethyl acetate = 10 : 1), and compound **Napdial** was obtained as a yellow solid (370 mg, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.75 (s, 0.5H), 8.81 (s, 0.5H), 8.63 (s, 0.5H), 8.11 (t, J = 8.4 Hz, 1H), 7.90 (d, J = 8.8 Hz, 1H), 7.82 (d, J = 8.8 Hz, 1H), 7.24 (dd, J = 2.4, 8.8 Hz, 1H), 7.17 (t, J = 2.5 Hz, 1H), 6.09 (s, 0.5H), 3.97 (s, 3H); ^{13}C NMR (101 MHz, d_6-DMSO) δ 196.33, 193.78, 190.55, 188.11, 160.58, 160.14, 159.94, 138.05, 137.63, 137.45, 133.51, 132.19, 131.92, 131.80, 129.32, 128.98, 127.90, 127.73, 127.66, 127.43, 127.28, 125.84, 125.61, 125.53, 120.28, 119.95, 106.75, 106.53, 91.25, 89.57, 56.00, 55.89; HRMS: (ESI) $[M+H-H_2O]^{+}$ calc. for $C_{13}H_{11}O_3$, 215.0708, observed 215.0707.

To a solution of L-citrulline (41 mg, 0.23 mmol) in H_2O/TFA (1:1, 4 mL) was added Napdial (50 mg, 0.23 mmol). After being stirred under 37 °C for 10 hours, the reaction mixture was concentrated. The obtained crude was purified by reverse-phase silica gel column chromatography (H_2O : MeOH = 2 : 1) to afford **Napdial-Cit** as a white solid (26 mg, 43% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.87 (d, J = 8.4 Hz, 2H), 7.80 (s, 1H), 7.35 (d, J =

2.4 Hz, 1H), 7.28 (d, J = 8.8 Hz, 1H), 7.23 (dd, J = 2.4, 9.2 Hz, 1H), 5.26 (d, J = 3.6 Hz, 1H), 3.88 (s, 3H) , 3.59 (m, 2H), 2.71 (m, 1H), 1.67 (m, 4H); ^{13}C NMR (101 MHz, d_6-DMSO) δ 173.1, 171.2, 158.2, 157.1, 135.0, 129.9, 129.1, 128.7, 128.3, 127.8, 125.4, 119.7, 106.4, 64.3, 64.2, 55.7, 53.1, 52.8, 28.1, 27.9, 23.4, 23.0; HRMS: (ESI) $[M+H]^+$ calc. for $C_{19}H_{22}N_3O_5$, 372.1559, observed 372.1562.

2. Synthesis of probe Napdialyne



The synthesis of probe Napdialyne was based on a combination of previous reports with modifications.[1, 2]

A solution of 4'-bromoacetophenone (1.0 g, 5.02 mmol), triphenylphosphine (65 mg, 0.25 mmol), palladium chloride (9 mg, 0.05 mmol), cupric acetate (9.1 mg, 0.05 mmol), trimethylsilylacetylene (TMSA, 1078 μL, 7.63 mmol) in trimethylamine (25 mL) was refluxed under 100°C for 1.5 h. After cooling, the reaction mixture was mixed with water, and extracted with CH_2Cl_2 twice. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated on a rotary evaporator. The obtained crude was further purified by silica gel column chromatography (petroleum ether : ethyl acetate = 30 : 1) to yield compound **1** as a yellow oil (1.087 g, quantitative yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 2.60 (s, 3H), 0.26 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.32, 136.52, 132.20, 128.27, 128.09, 104.20, 98.22, 26.73, 0.00; HRMS: (ESI) $[M+H]^+$ calc. for $C_{13}H_{17}OSi$, 217.1049, observed 217.0987.

A solution of compound **1** (1.0 g, 5.04 mmol) and K_2CO_3 (690 mg, 5.04 mmol) in MeOH (40 mL) was stirred at room temperature for 15 min. Then the reaction mixture was diluted with water, and extracted with CH_2Cl_2 twice. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated on a rotary evaporator to get

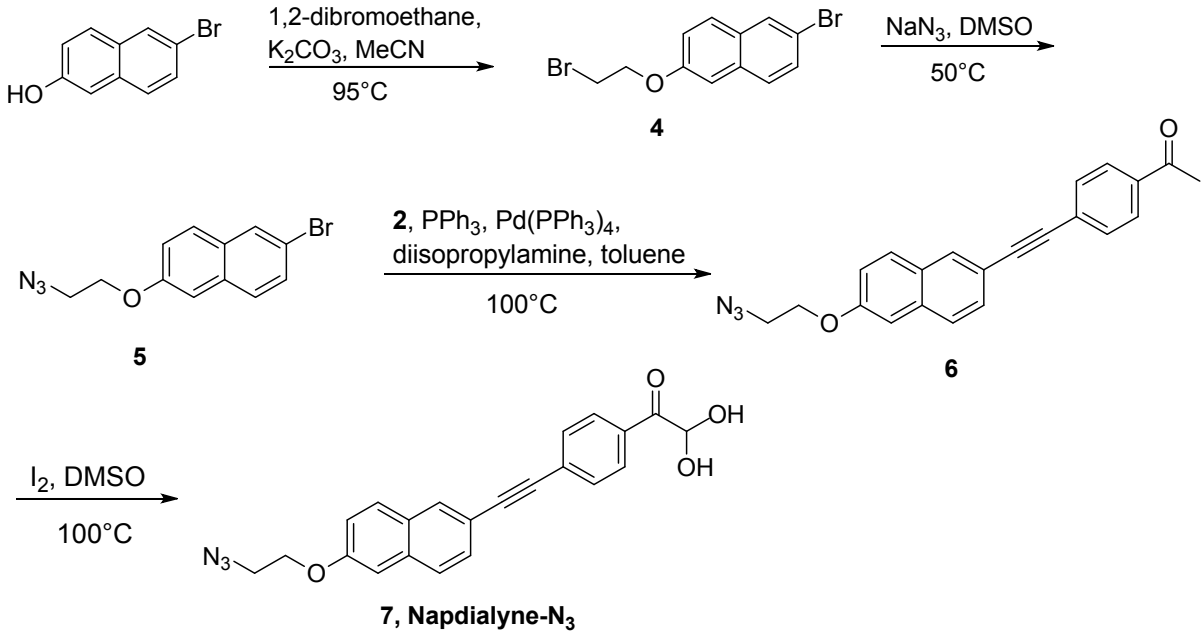
compound **2** as a yellow solid (666 mg, quantitative yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 3.25 (s, 1H), 2.61 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.25, 136.75, 132.28, 128.18, 126.91, 82.76, 80.45, 26.63; HRMS: (ESI) $[M+H]^+$ calc. for $C_{10}H_9O$, 145.0653, observed 145.0602.

A solution of compound **2** (250 mg, 1.74 mmol), triphenylphosphine (23 mg, 0.087 mmol), palladium chloride (15.5 mg, 0.087 mmol), cupric acetate (16 mg, 0.087 mmol), and 2-bromo-6-methoxynaphthalene (1650 mg, 6.96 mmol) in triethylamine (25 mL) was refluxed under 100°C for 2 h. After cooling, the reaction mixture was diluted with water, and extracted with CH_2Cl_2 twice. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated on a rotary evaporator. The obtained crude was purified by Al_2O_3 column chromatography (petroleum ether : ethyl acetate = 20 : 1) to yield compound **3** as a white solid (195 mg, 39% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.97 (d, *J* = 8.4 Hz, 2H), 7.74 (dd, *J* = 4.8, 8.8 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.19 (dd, *J* = 2.4, 8.8 Hz, 1H), 7.14 (d, *J* = 2.0 Hz, 1H), 3.94 (s, 3H), 2.63 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.37, 158.57, 136.02, 134.42, 131.69, 131.63, 129.44, 128.88, 128.45,128.43, 128.30, 126.96, 119.58, 117.47, 105.84, 93.49, 88.38, 55.37, 26.62; HRMS: (ESI) $[M+H]^+$ calc. for $C_{21}H_{17}O_2$, 301.1229, observed 301.1230.

A solution of compound **3** (200 mg, 0.67 mmol) and iodine (338 mg, 1.33 mmol) in a mixture of DMSO and H_2O (20 mL, DMSO/H_2O = 4 /1) was refluxed under 100°C for 2 h. After cooling, the reaction mixture was diluted with H_2O, extracted with CH_2Cl_2 for three times. The combined organic layers were washed sequentially with $Na_2S_2O_3$ aqueous solution (1 mol/L, 20 mL) and brine, dried over anhydrous Na_2SO_4 and concentrated on a rotary evaporator. The crude was stirred with CH_2Cl_2 to give a white slurry, which was filtered to give the final compound **Napdialyne** as a light yellow solid (130 mg, 62% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.17 (s, 1H), 8.13 (d, *J* = 8.4 Hz, 2H), 7.88 (t, *J* = 8.8 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.60 (d, *J* = 9.6 Hz, 1H), 7.38 (d, *J* = 2.0 Hz, 1H), 7.24 (dd, *J* = 2.4, 8.8 Hz, 1H), 6.86 (d, *J* = 7.2 Hz, 2H), 5.68 (t, *J* = 7.2 Hz, 1H), 3.90 (s, 3H); ^{13}C NMR (101 MHz, d_6-DMSO) δ 195.97, 158.94, 134.74, 133.49, 132.01, 131.71, 130.21, 129.98, 128.98, 128.49, 127.71, 127.65, 120.04, 116.94, 106.62, 93.63, 90.03, 88.95, 55.83; HRMS: (ESI) $[M+H-H_2O]^+$ calc. for $C_{21}H_{15}O_3$, 315.1021, observed 315.1005.

To a solution of L-citrulline (56 mg, 0.318 mmol) in H_2O/TFA (1:1, 8 mL) was added Napdialyne (100 mg, 0.318 mmol). After being stirred under 37 °C for 10 hours, the reaction mixture was concentrated. The obtained crude was recrystallized with MeOH, and the obtained solid (unpure) was dissolved in MeOH and further precipitated from petroleum ether. And the obtained solid was further mixed with CH_2Cl_2 to give a white slurry, which was filtered to afford **Napdialyne-Cit** as a white solid (46 mg, 31% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.12 (s, 1H), 7.86 (t, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.58 (dd, *J* = 1.2, 8.0 Hz, 1H), 7.34 (m, 3H), 7.21 (dd, *J* = 2.4, 9.2 Hz, 1H), 5.28 (s, 1H), 3.90 (s, 3H), 3.54 (m, 2H), 2.67 (s, 1H), 1.55 (m, 4H); ^{13}C NMR (101 MHz, d_6-DMSO) δ 173.03, 170.26, 158.71, 157.04, 134.71, 134.66, 134.55, 132.41, 131.68, 129.89, 129.00, 128.51, 127.71, 123.43, 119.97, 106.58, 91.19, 88.89, 63.90, 63.71, 55.82, 54.24, 53.94, 28.44, 23.61, 23.46; HRMS: (ESI) $[M+H]^+$ calc. for $C_{27}H_{26}N_3O_5$, 472.1872, observed 472.1859.

3. Synthesis of probe Napdialyne-N_3



A solution of 6-bromo-2-naphthol (2.0 g, 8.97 mmol), K_2CO_3 (1240 mg, 8.97 mmol) and 1,2-dibromoethane (3863 μL, 44.83 mmol) in acetonitrile (30 mL)was refluxed for 8 h under 95°C. After cooling to room temperature, the reaction mixture was mixed with water, and extracted with CH_2Cl_2 twice. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated on a rotary evaporator. The obtained crude was further purified by silica gel column chromatography (petroleum ether : ethyl acetate = 10 : 1) to yield compound **4** as a yellow oil (1.46 g, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.59 (d, *J* = 8.8 Hz, 1H), 7.51 (dd, *J* = 1.4, 8.8 Hz, 1H), 7.19 (dd, *J* = 2.4, 9.2 Hz, 1H), 7.09 (d, *J* = 2.4 Hz, 1H), 4.40 (t, *J* = 6.4 Hz, 2H), 3.70 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.31, 132.85, 130.27, 129.78, 129.68, 128.75, 128.41, 119.75, 117.44, 107.03, 67.85, 28.89; HRMS: (ESI) $[M+H]^+$ calc. for $C_{12}H_{11}BrO$, 328.9177, observed 328.9129.

A solution of compound **4** (750 mg, 2.29 mmol) and sodium azide (164 mg, 2.52 mmol) in a mixture of DMSO (10 mL) was heated under 50°C for 2 h. After cooling to room temperature, the reaction mixture was diluted with H_2O, and extracted with CH_2Cl_2 for three times. The combined organic layers were washed brine, dried over anhydrous Na_2SO_4 and concentrated on a rotary evaporator to get compound **5** as a white solid (890 mg, quantitative yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.68 (d, *J* = 9.2 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 7.52 (dd, *J* = 2.0, 8.8 Hz, 1H),7.21 (dd, *J* = 2.4, 9.2 Hz, 1H), 7.10 (d, *J* = 2.4 Hz, 1H), 4.24 (t, *J* = 5.2 Hz, 2H), 3.68 (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.45, 132.83, 130.24, 129.75, 129.68, 128.72, 128.40, 119.73, 117.40, 106.79, 66.95, 50.09; HRMS: (ESI) $[M+H]^+$ calc. for $C_{12}H_{11}BrN_3O$, 292.0085, observed 292.0023.

A solution of compound **2** (500 mg, 3.47 mmol), tetrakis(triphenylphosphine) palladium (320 mg, 0.278 mmol), and compound **5** (1210 mg, 4.16 mmol) in toluene (20 mL) and diisopropylamine (20 mL) was refluxed under 100°C for 5 h. After cooling to room temperature, the reaction mixture was diluted with water, and extracted with CH_2Cl_2 twice. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated on a rotary evaporator. The obtained crude was purified by silica gel column chromatography (petroleum ether : DCM = 1 : 1) to yield compound **6** as a yellow solid (440 mg, 36% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.97 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 8.8 Hz, 1H), 7.73(d, *J* = 8.8 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.24 (dd, *J* = 2.4, 2.4 Hz, 1H), 7.14 (d, *J* = 2.0 Hz, 1H), 4.29 (t, *J* = 5.2 Hz, 2H), 3.69 (t, *J* = 4.8 Hz, 2H), 2.63 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.36, 157.13, 136.07, 134.21, 131.69, 131.65, 129.69, 129.01, 128.69, 128.35, 128.31, 127.01, 119.55, 117.86, 106.84, 93.33, 88.54, 66.98, 50.09, 26.63; HRMS: (ESI) $[M+H]^+$ calc. for $C_{22}H_{18}N_3O_2$, 356.1399, observed 356.1394.

A solution of compound **6** (130 mg, 0.366 mmol) and iodine (186 mg, 0.732 mmol) in a mixture of DMSO and H_2O (20 mL, DMSO/H_2O = 4 /1) was refluxed under 100 °C for 4 h. After cooling to room temperature, the reaction mixture was diluted with H_2O, and extracted with CH_2Cl_2 for three times. The combined organic layers were washed sequentially with $Na_2S_2O_3$ aqueous solution (1 mol/L, 20 mL) and brine. Then it was dried over anhydrous Na_2SO_4, and concentrated on a rotary evaporator. The obtained crude was mixed with CH_2Cl_2 to give a white slurry, which was filtered to give the final compound **7** (**Napdialyne-N_3**) as a light yellow solid (130 mg, 65% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.19 (s, 1H), 8.14 (d, *J* = 8.4 Hz, 2H), 7.91 (d, *J* = 9.2 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 8.8 Hz, 2H), 7.62 (dd, *J* = 1.6, 8.4 Hz, 1H), 7.44 (d, *J* = 2.4 Hz, 1H), 7.24 (dd, *J* = 2.4, 8.8 Hz, 1H), 6.87 (d, *J* = 7.2 Hz, 2H), 5.67 (t, *J* = 7.2 Hz, 1H), 4.33 (t, *J* = 4.8 Hz, 2H), 3.75 (t, *J* = 4.8 Hz, 2H); ^{13}C NMR (101 MHz, d_6-DMSO) δ 195.97, 157.49, 134.60, 133.51, 132.03, 131.72, 130.24, 130.19, 129.06, 128.65, 127.85, 127.56, 119.95, 117.17, 107.65, 93.54, 90.04, 89.04, 67.41, 49.97; HRMS: (ESI) $[M+H-H_2O]^+$ calc. for $C_{22}H_{16}N_3O_3$, 370.1192, observed 370.1149.

4. Fluorescence measurement

4.1 Fluorescence measurement using probe Napdial

For a typical experiment, a solution of Napdial, L-citrulline (2-fold dilution) and trichloroacetic acid (40 μL) in ddH_2O (160 μL) was incubated at 50 °C for 3 h in 96-well plates. The final concentration for Napdial was 100 μM. Then 10 μL of reaction mixture was taken out, and diluted with MeOH (90 μL) before measurement.

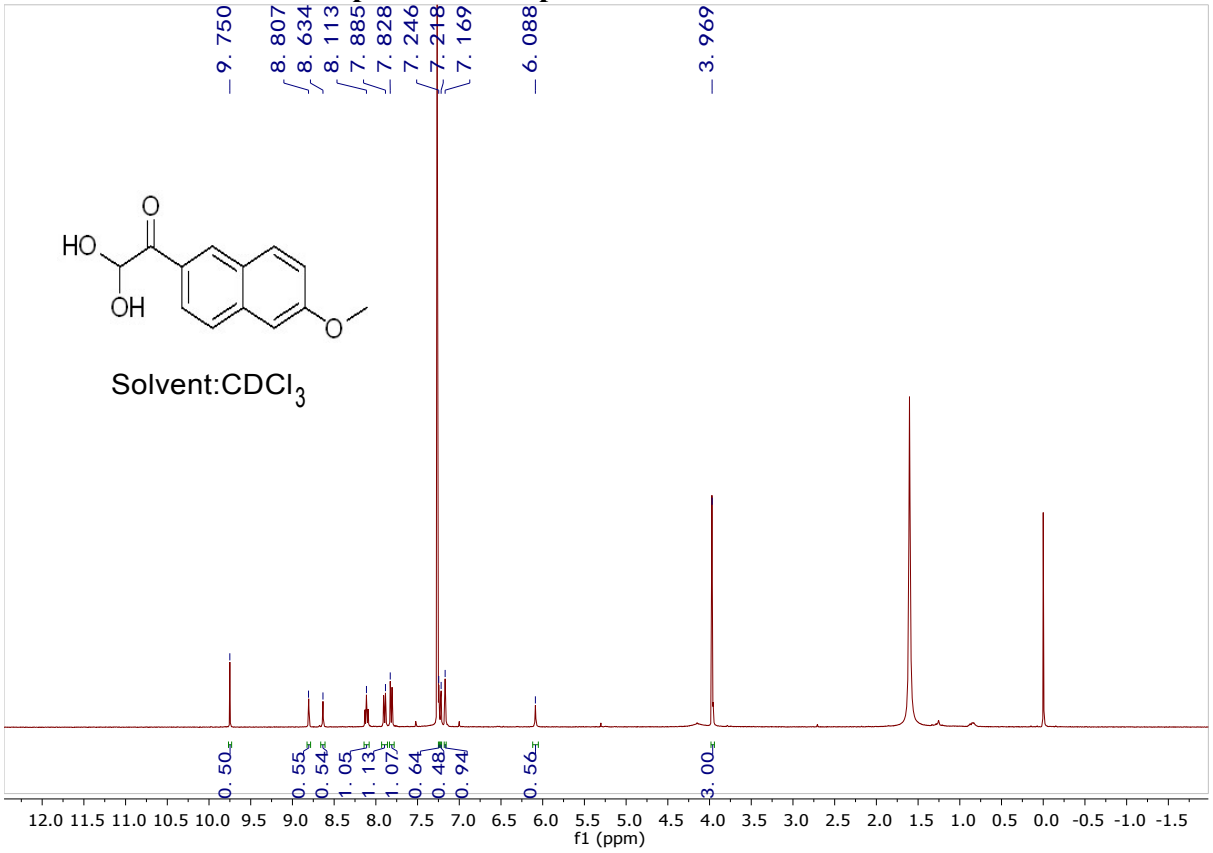
4.2 Fluorescence measurement using probe Napdialyne

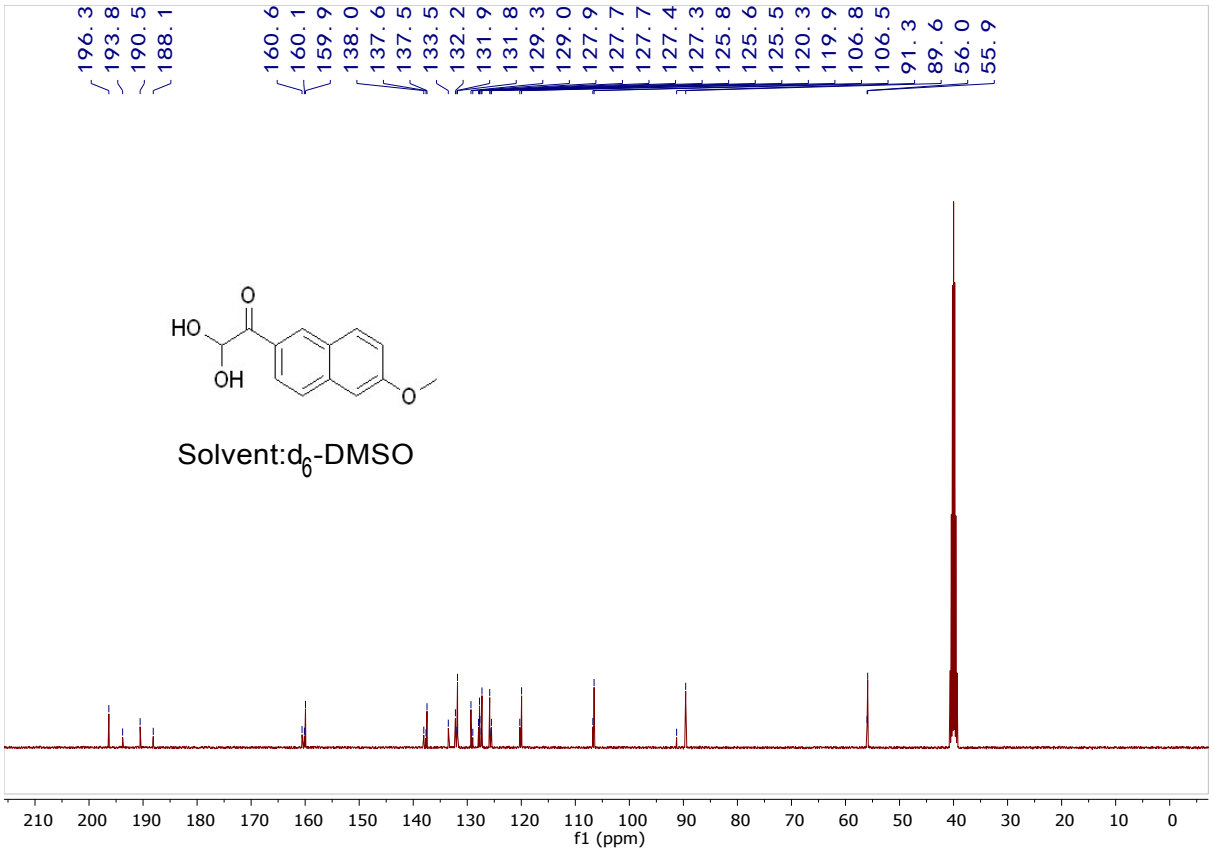
For a typical experiment, a solution of Napdialyne (10 μL), L-citrulline (10 μL, 2-fold dilution) and trichloroacetic acid (40 μL) in ddH_2O (140 μL) to incubated at 50 °C for 3 h in 96-well plates. The final concentration for Napdialyne was 50 μM. Then 10 μL of reaction mixture was taken out, and diluted with different solvents (90 μL) before measurement.

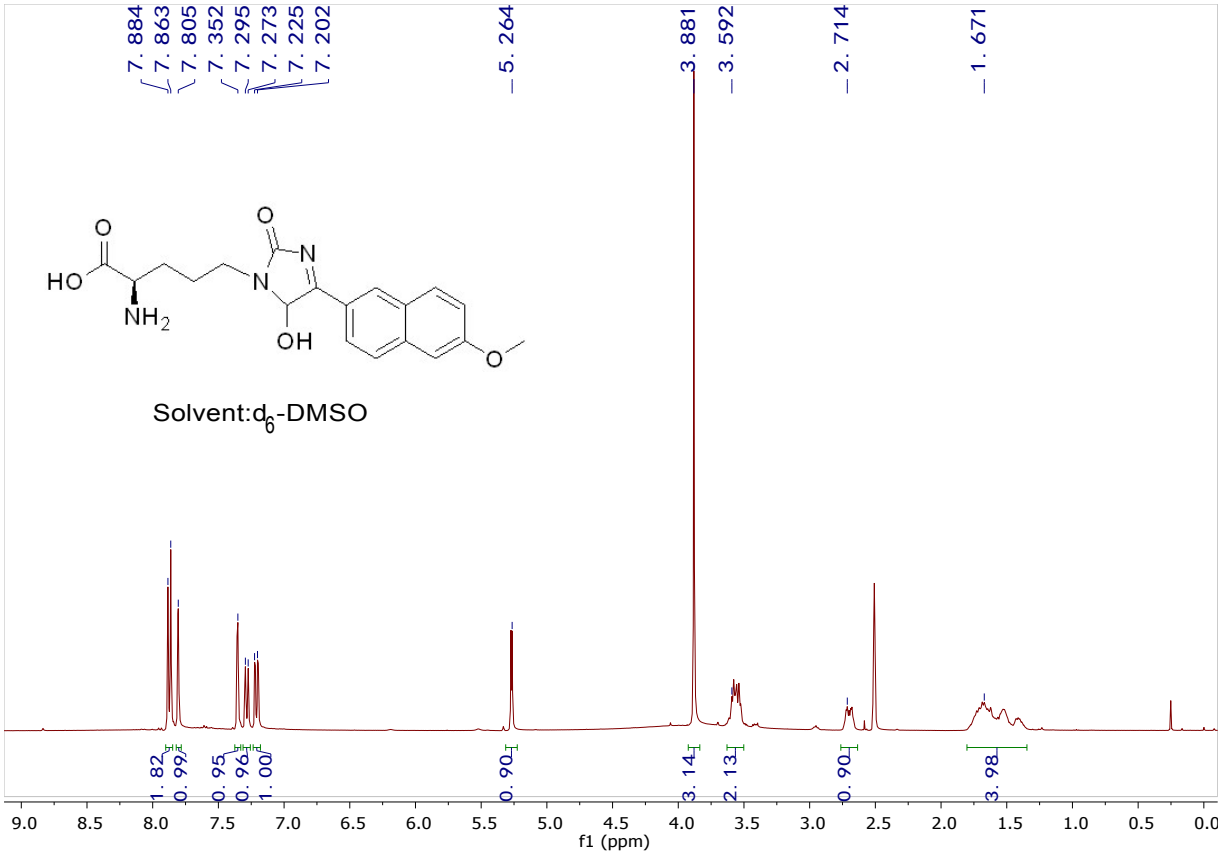
4.3 Fluorescence measurement using Napdialyne-N_3 with DBCO-PEG4-biotin

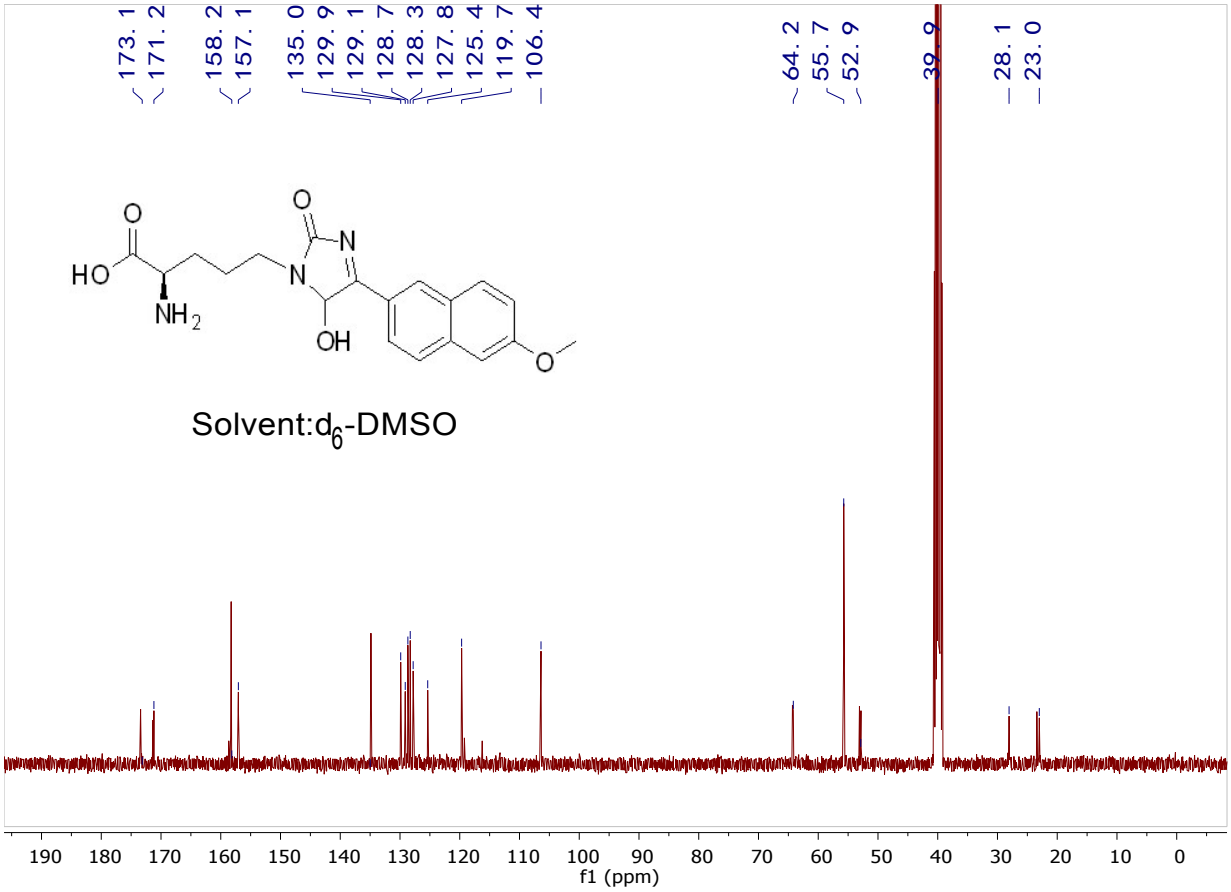
For a typical experiment, a solution of DBCO-PEG_4-Biotin (40 µL, 10 mM in H_2O) and Napdialyne-N_3 (30 µL, 10 mM in DMSO) in MeOH (130 µL) was rotated at room temperature for 3 h in 1.5 mL centrifuge tube. Then 20 µL of this reaction solution was taken out, and mixed with 10 µL of analyte, 10 µL of TFA and 10 µL of H_2O. The mixture was allowed to incubate at 37 °C for 3 h. Then it was diluted 10-times with IPA (450 µL). After that, 50 µL of this dilute was taken out, and mixed with agarose beads (50 µL). The mixture was rotated at room temperature for 1 h, and centrifuged to separate liquid and beads. 30 µL of the supernatant was analyzed by fluorescence measurement, and designed as "supernatant" group. The beads were further diluted with 30 µL of IPA, and 30 µL of this IPA dilute was analyzed by fluorescence measurement, and designed as "beads" group.

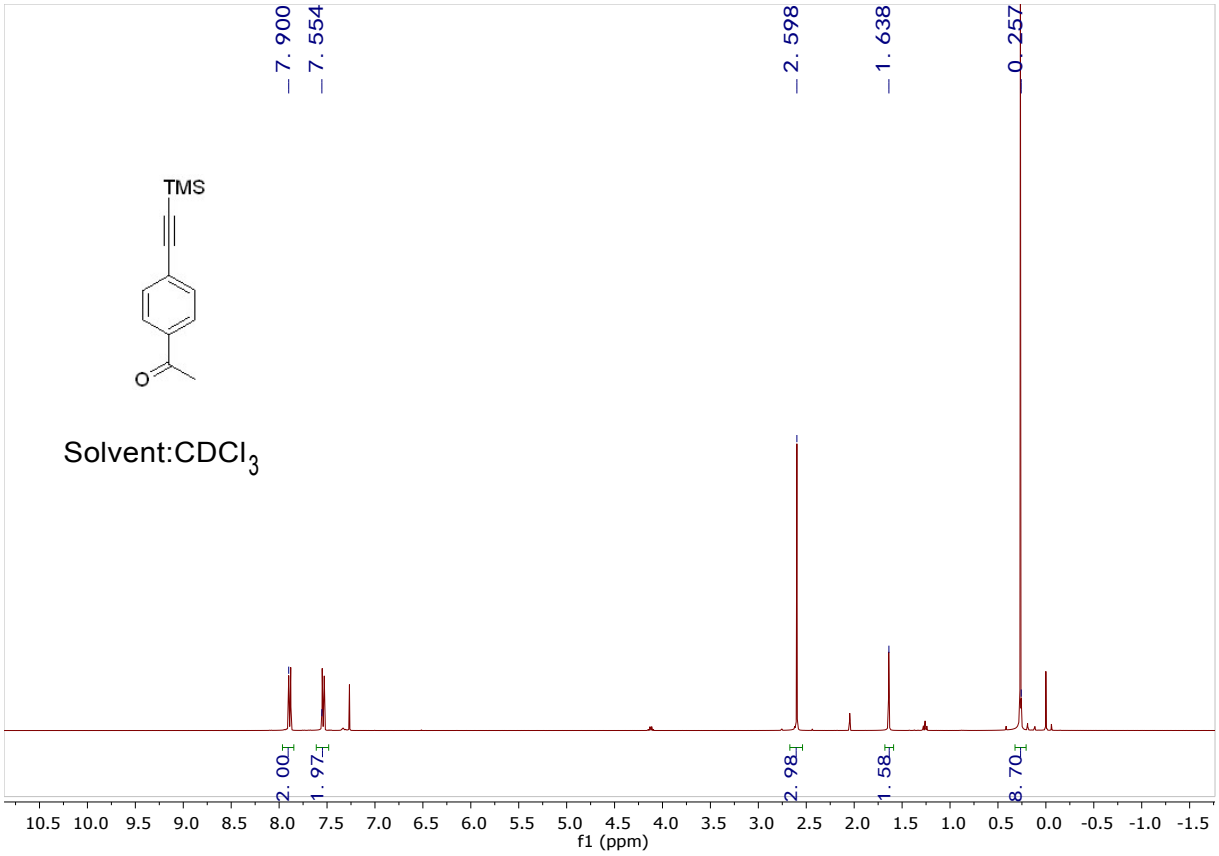
- **^{1}H and ^{13}C NMR spectra of compounds**

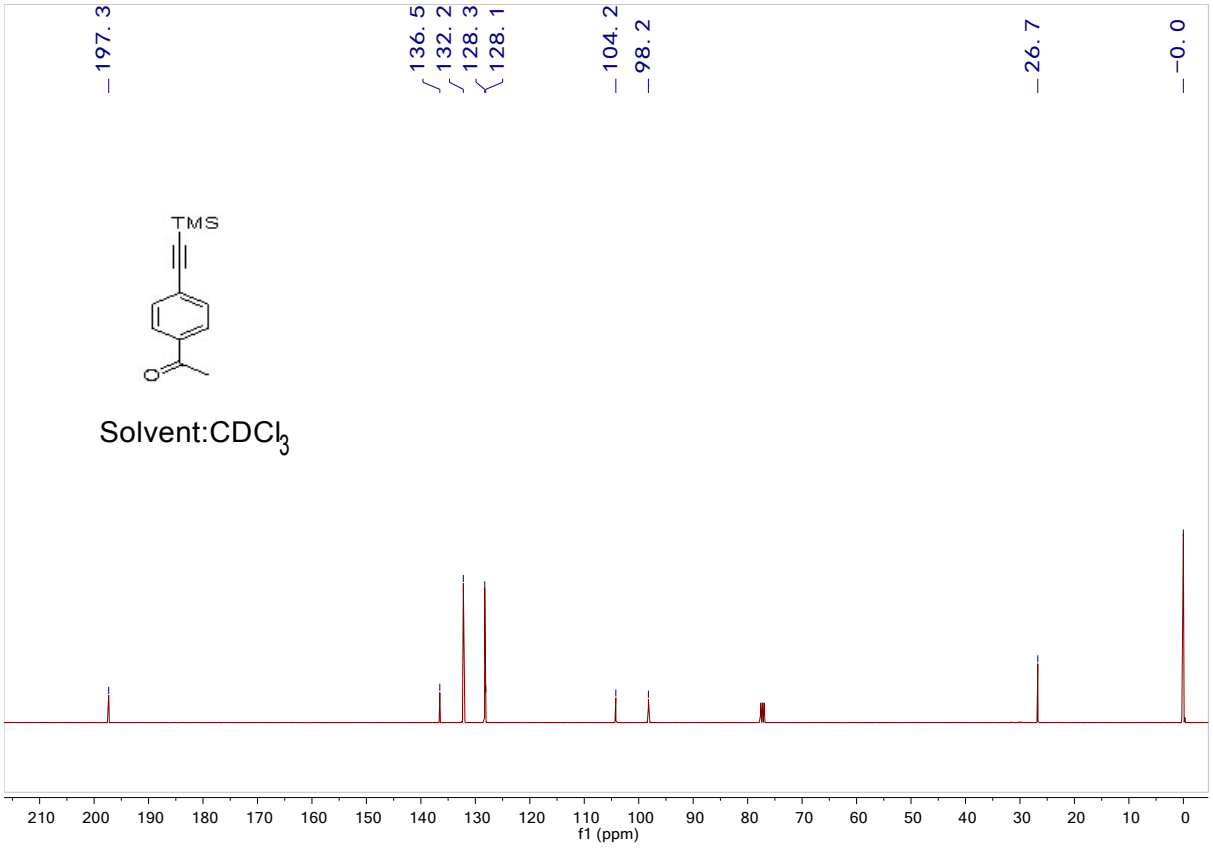


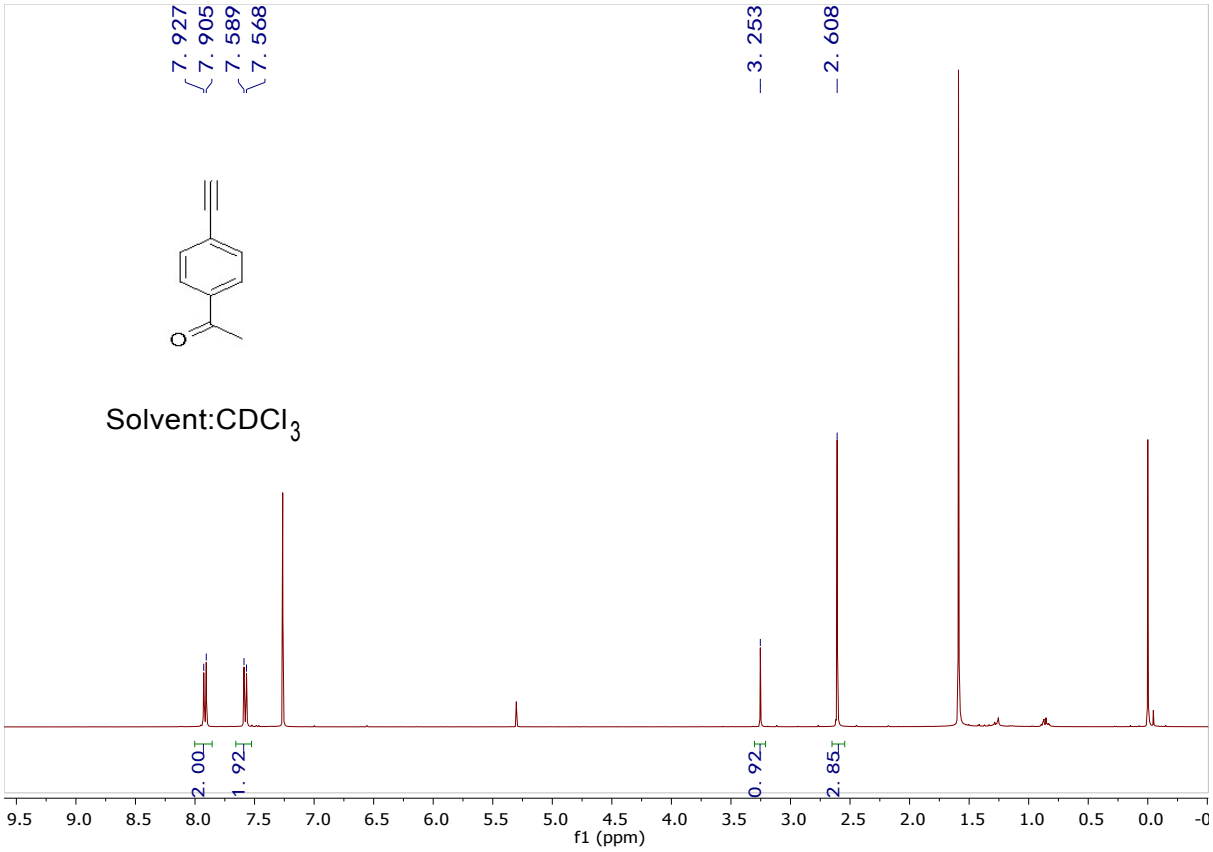


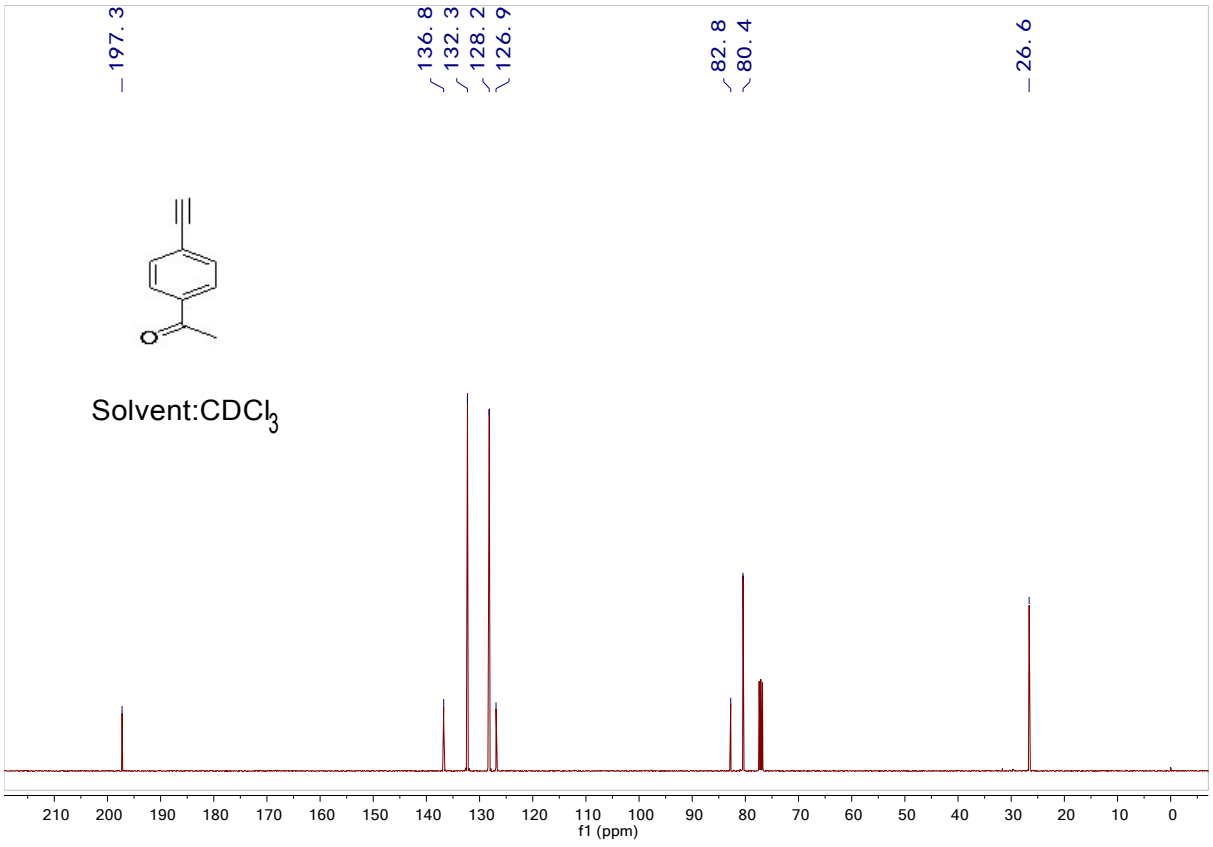

7.884
7.863
7.805
7.352
7.295
7.273
7.225
7.202
5.264
3.881
3.592
2.714
1.671
HO
O
NH2
N
OH
O
Solvent:d6-DMSO
1.82
0.99
0.95
0.96
1.00
0.90
3.14
2.13
0.90
3.98
f1 (ppm)

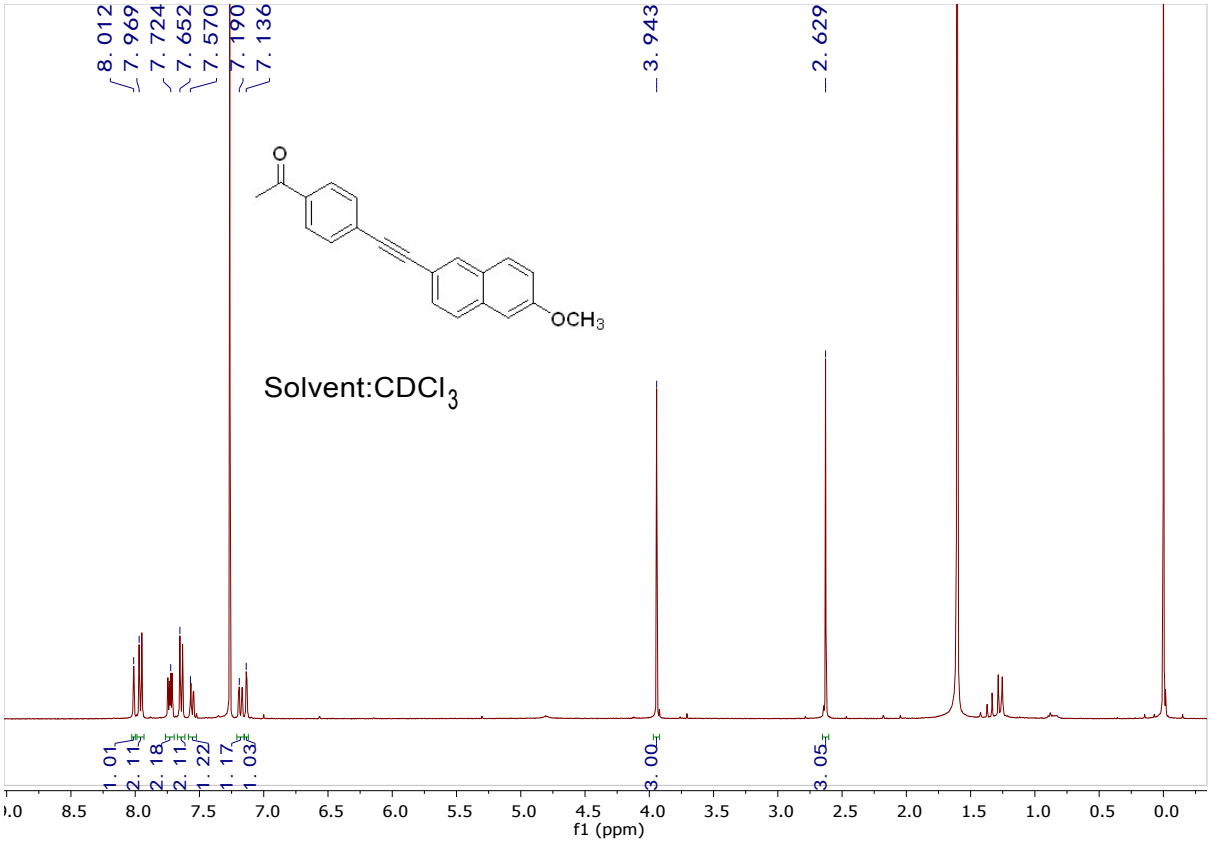

173.1
171.2
158.2
157.1
135.0
129.9
129.1
128.7
128.3
127.8
125.4
119.7
106.4
64.2
55.7
52.9
39.9
28.1
23.0
HO
O
NH2
N
OH
O
Solvent:d6-DMSO
f1 (ppm)

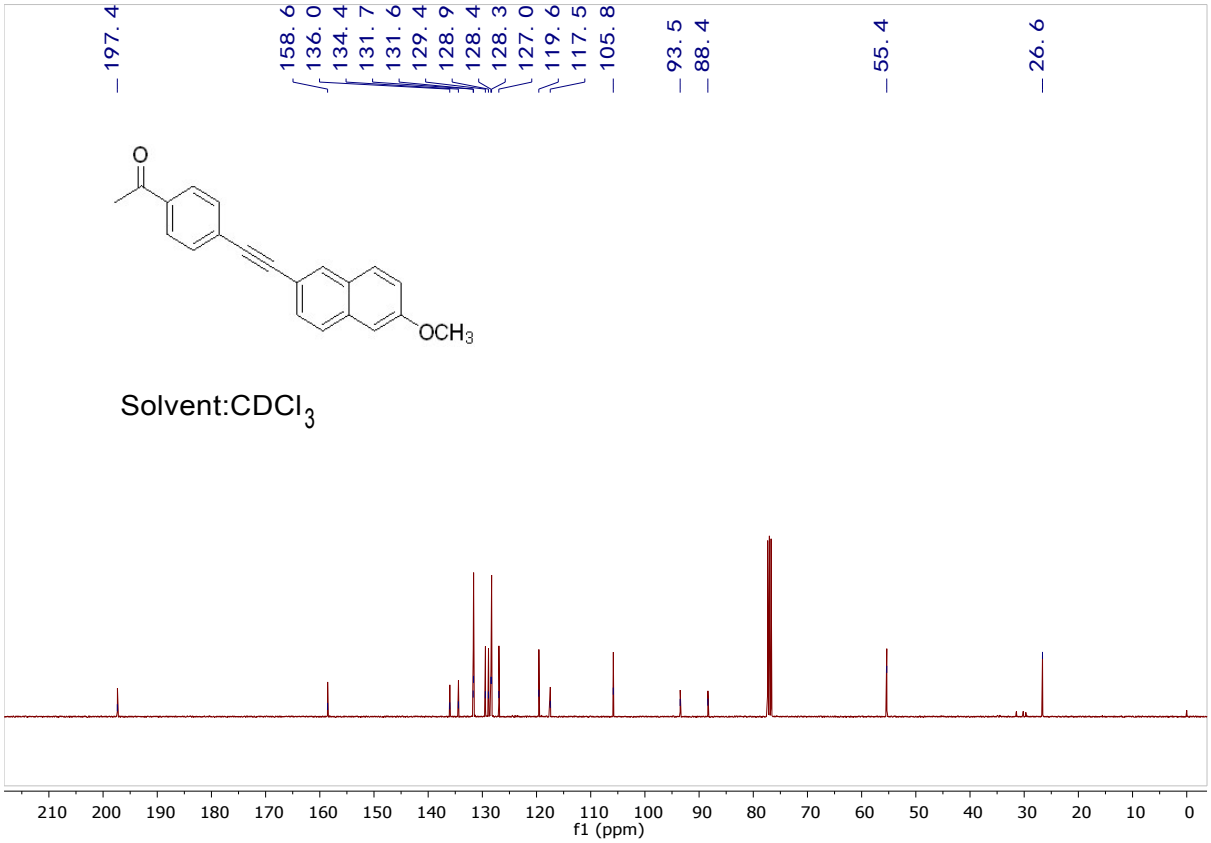

7.900
7.554
2.598
1.638
0.257
TMS
O
Solvent:CDCl3
2.00
1.97
2.98
1.58
8.70
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5
f1 (ppm)

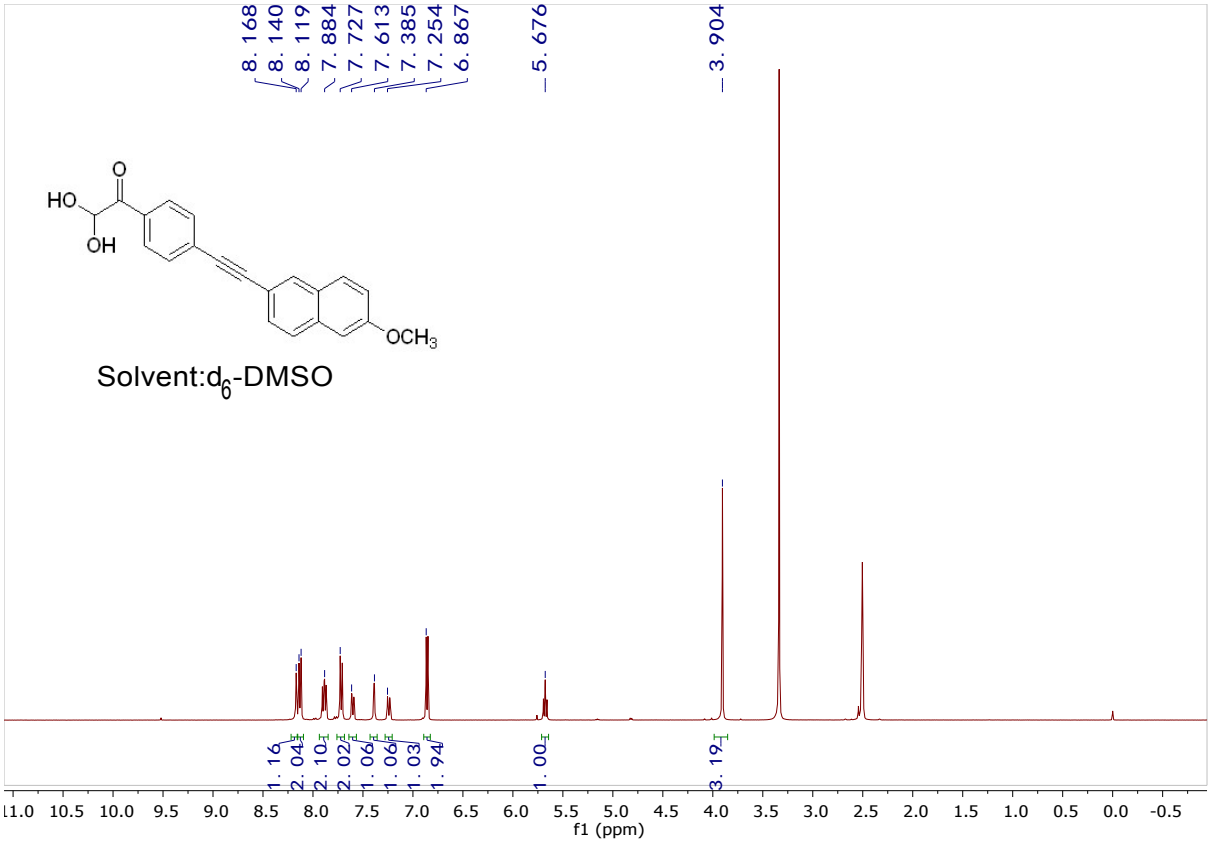

197.3
136.5
132.2
128.3
128.1
104.2
98.2
26.7
0.0
TMS
O
Solvent:CDCl3
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

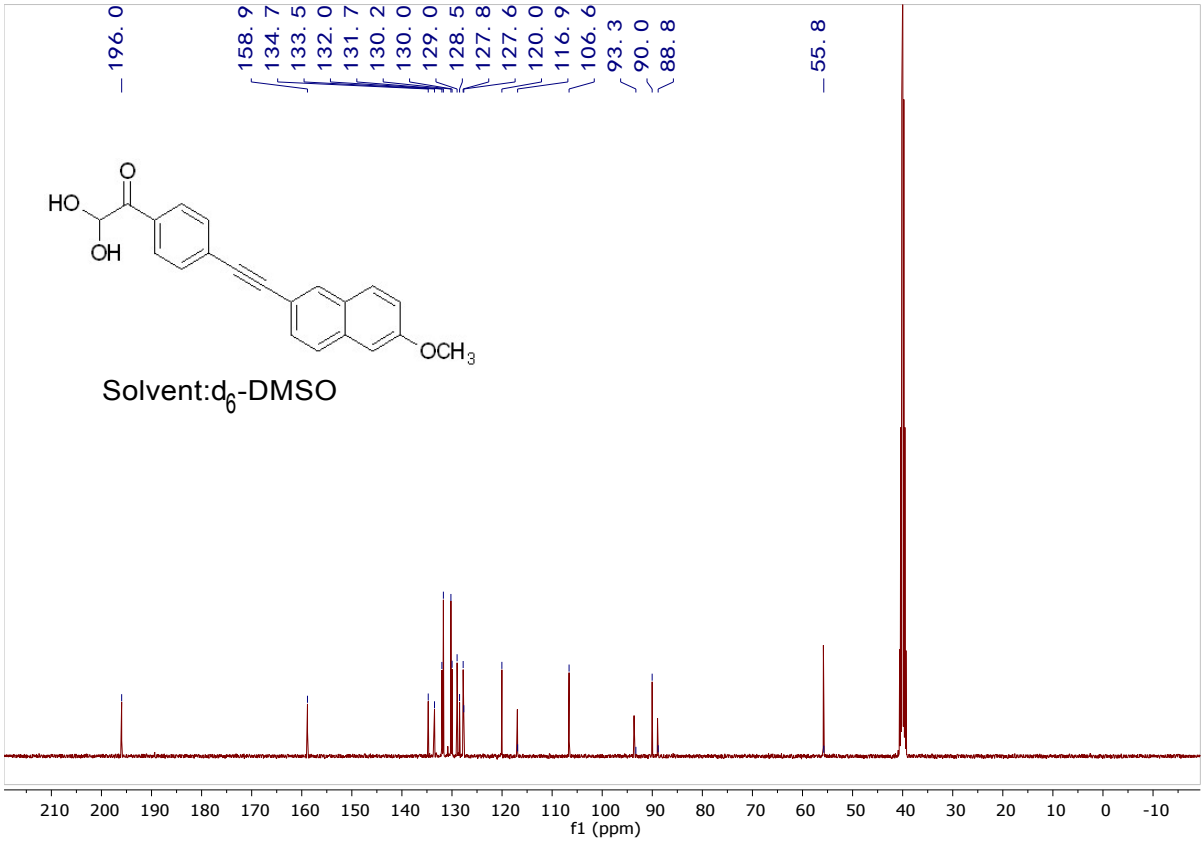

7.927
7.905
7.589
7.568
3.253
2.608
Solvent:CDCl3
2.00
1.92
0.92
2.85
f1 (ppm)

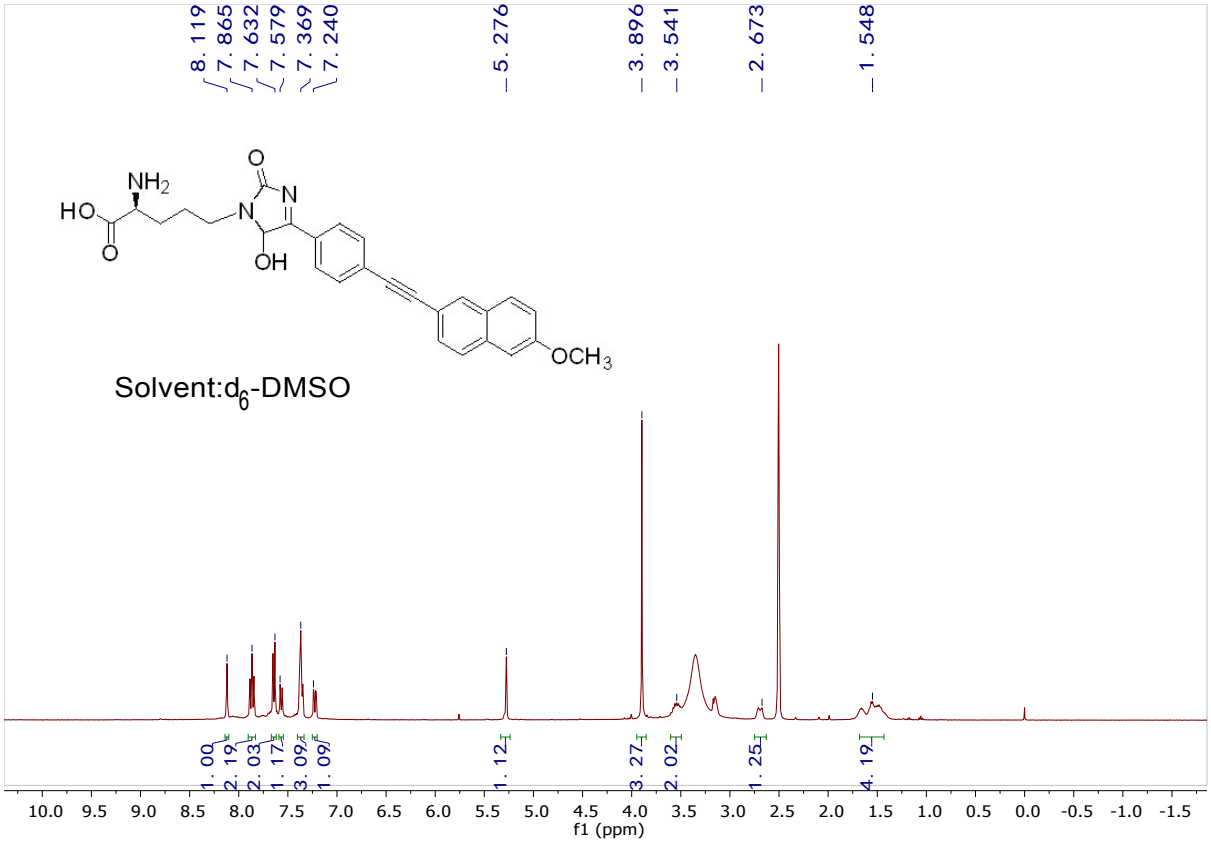

197.3
136.8
132.3
128.2
126.9
82.8
80.4
26.6
Solvent:CDCl3
f1 (ppm)

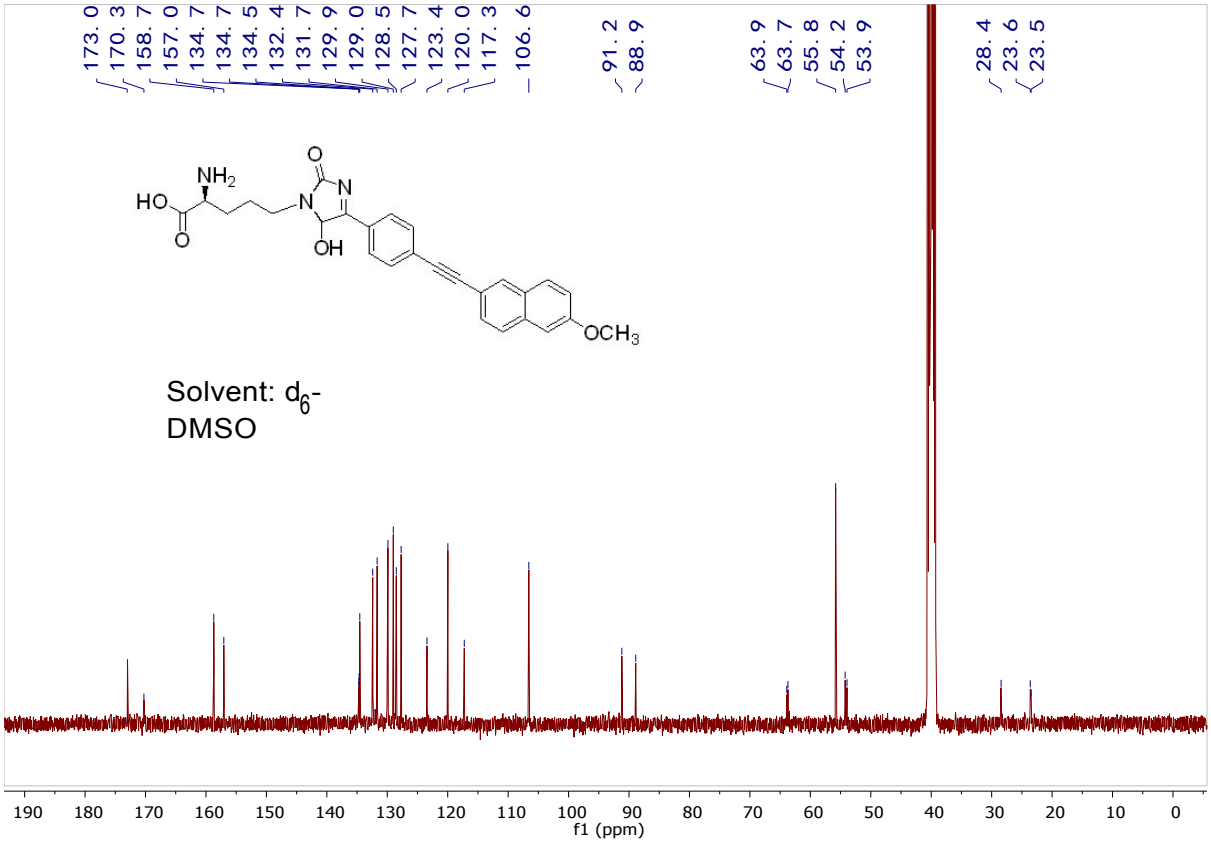

8.012
7.969
7.724
7.652
7.570
7.190
7.136
3.943
2.629
O
OCH3
Solvent:CDCl3
1.01
2.11
2.18
2.11
1.22
1.17
1.03
3.00
3.05
f1 (ppm)

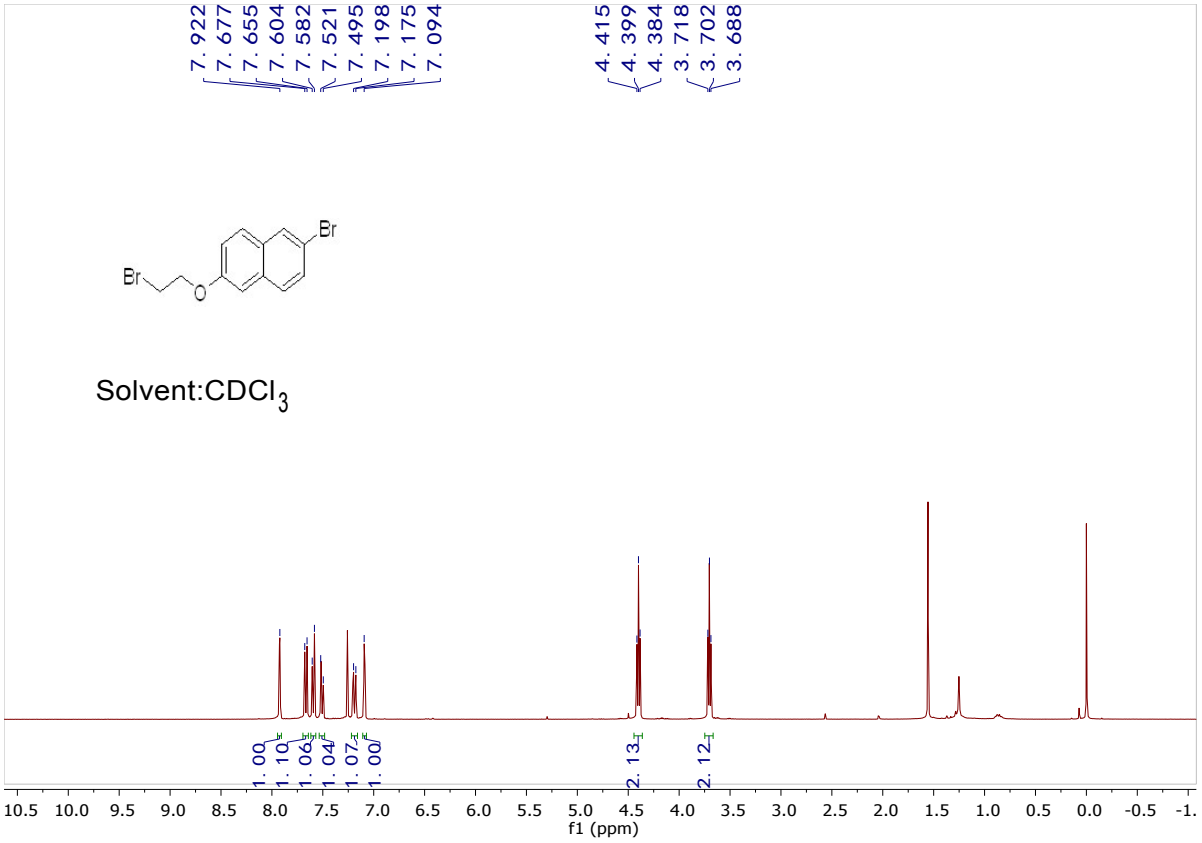

197.4
158.6
136.0
134.4
131.7
131.6
129.4
128.9
128.4
128.3
127.0
119.6
117.5
105.8
93.5
88.4
55.4
26.6
O
OCH3
Solvent:CDCl3
f1 (ppm)

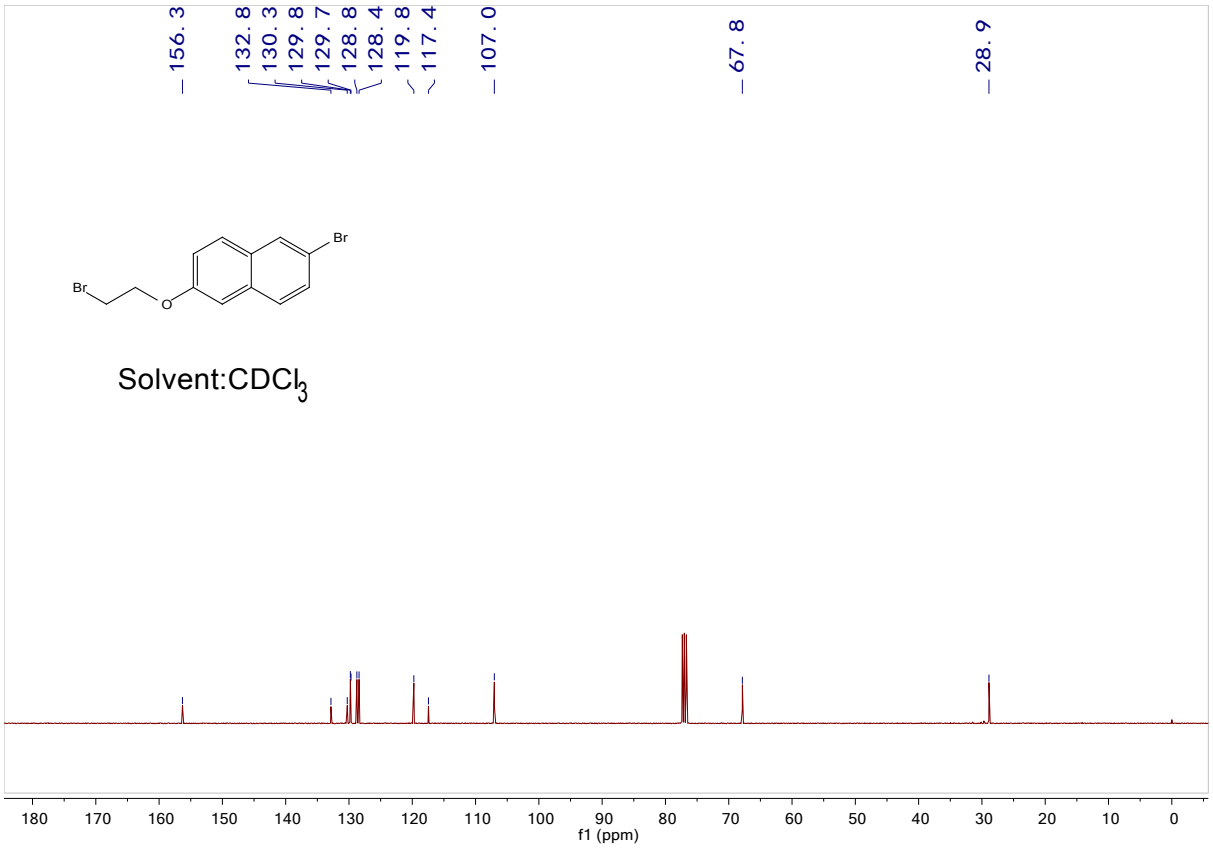

8.168
8.140
8.119
7.884
7.727
7.613
7.385
7.254
6.867
5.676
3.904
HO
O
OH
OCH3
Solvent:d6-DMSO
1.16
2.04
2.10
2.02
1.06
1.06
1.03
1.94
1.00
3.19
f1 (ppm)

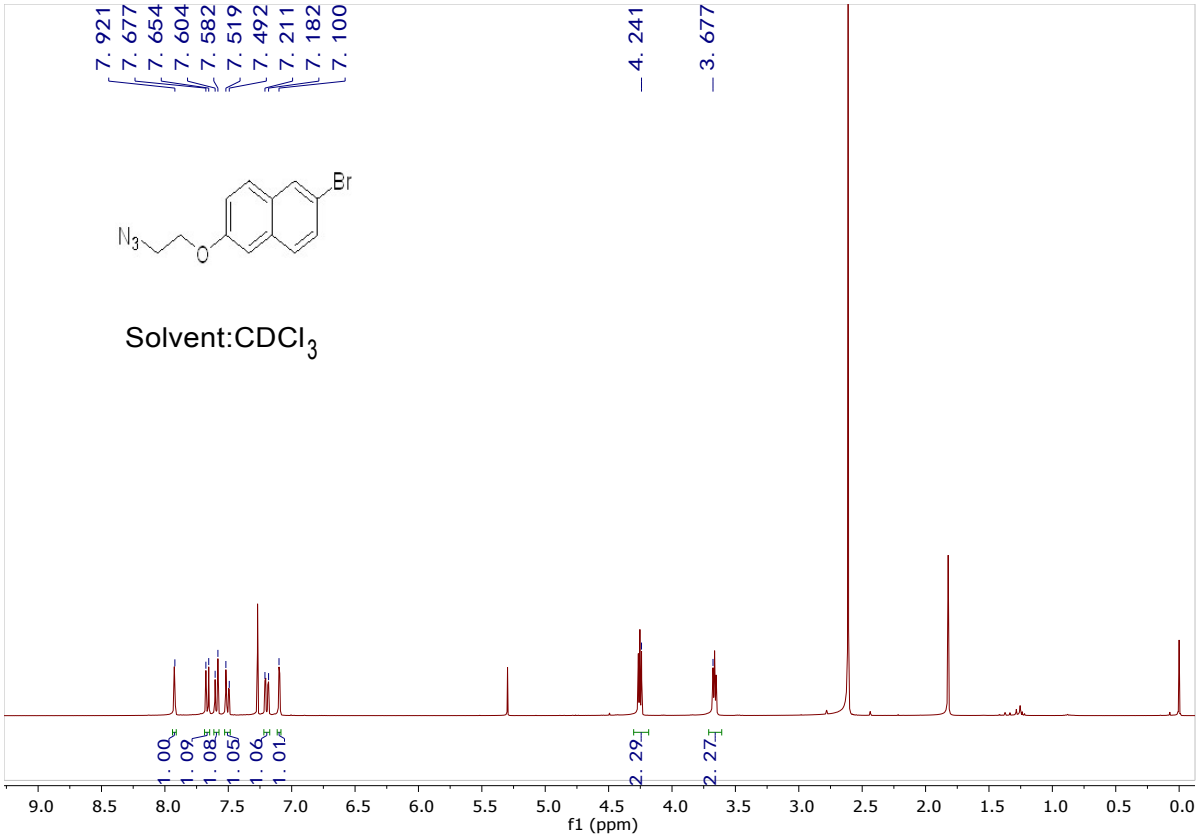

196.0
158.9
134.7
133.5
132.0
131.7
130.2
130.0
129.0
128.5
127.8
127.6
120.0
116.9
106.6
93.3
90.0
88.8
55.8
HO
O
OH
OCH3
Solvent:d6-DMSO
f1 (ppm)

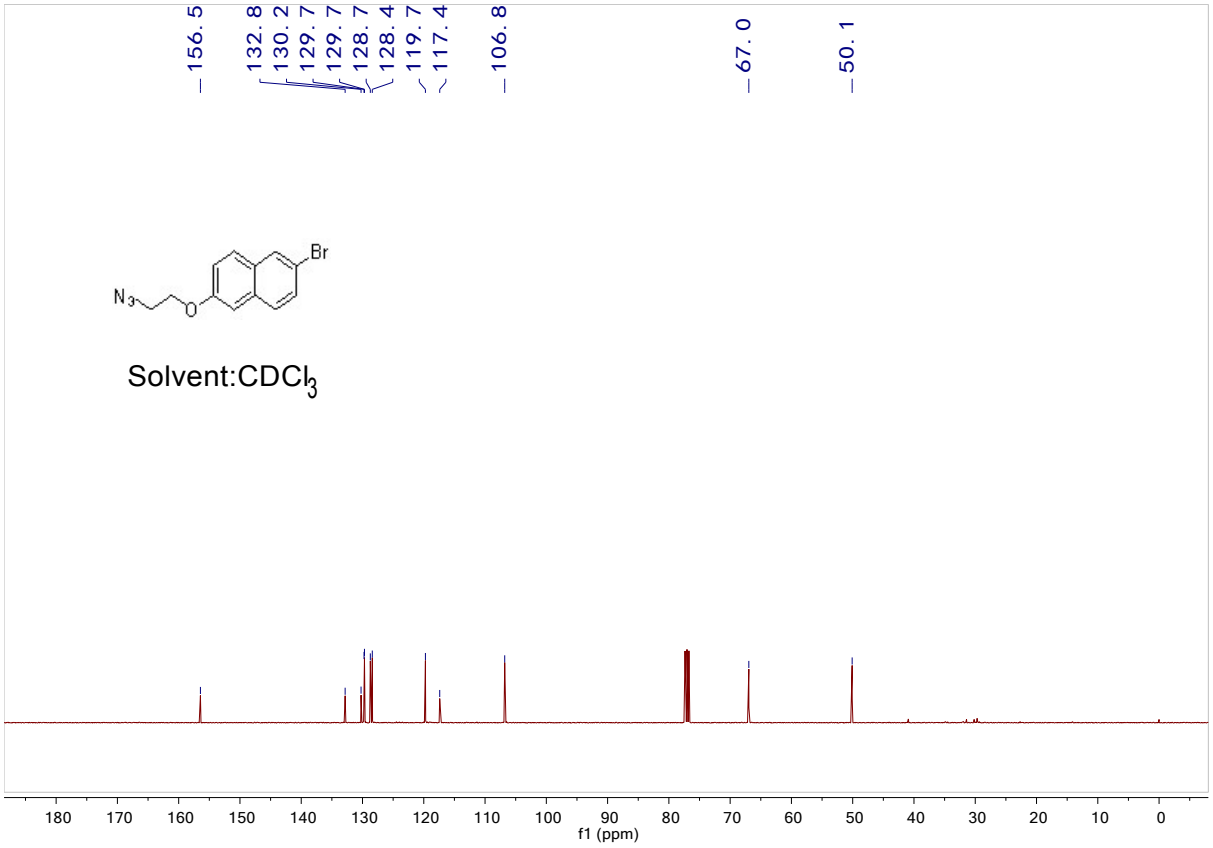

8.119
7.865
7.632
7.579
7.369
7.240
5.276
3.896
3.541
2.673
1.548
NH2
HO
O
N
N
OH
O
OCH3
Solvent:d6-DMSO
1.00
2.19
2.03
1.17
3.09
1.09
1.12
3.27
2.02
1.25
4.19
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5
f1 (ppm)

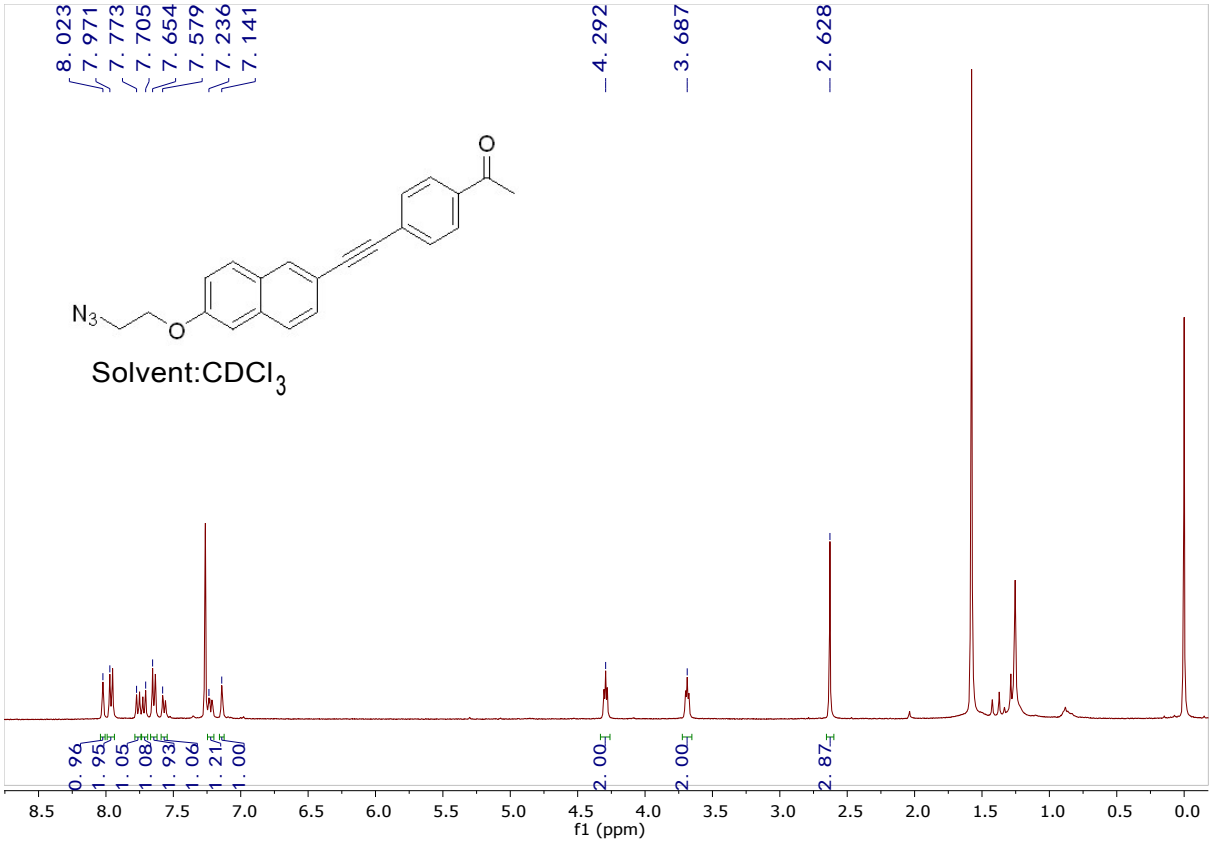

173.0
170.3
158.7
157.0
134.7
134.7
134.5
132.4
131.7
129.9
129.0
128.5
127.7
123.4
120.0
117.3
106.6
91.2
88.9
63.9
63.7
55.8
54.2
53.9
28.4
23.6
23.5
NH2
HO
O
N
N
OH
O
OCH3
Solvent: d6-DMSO
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

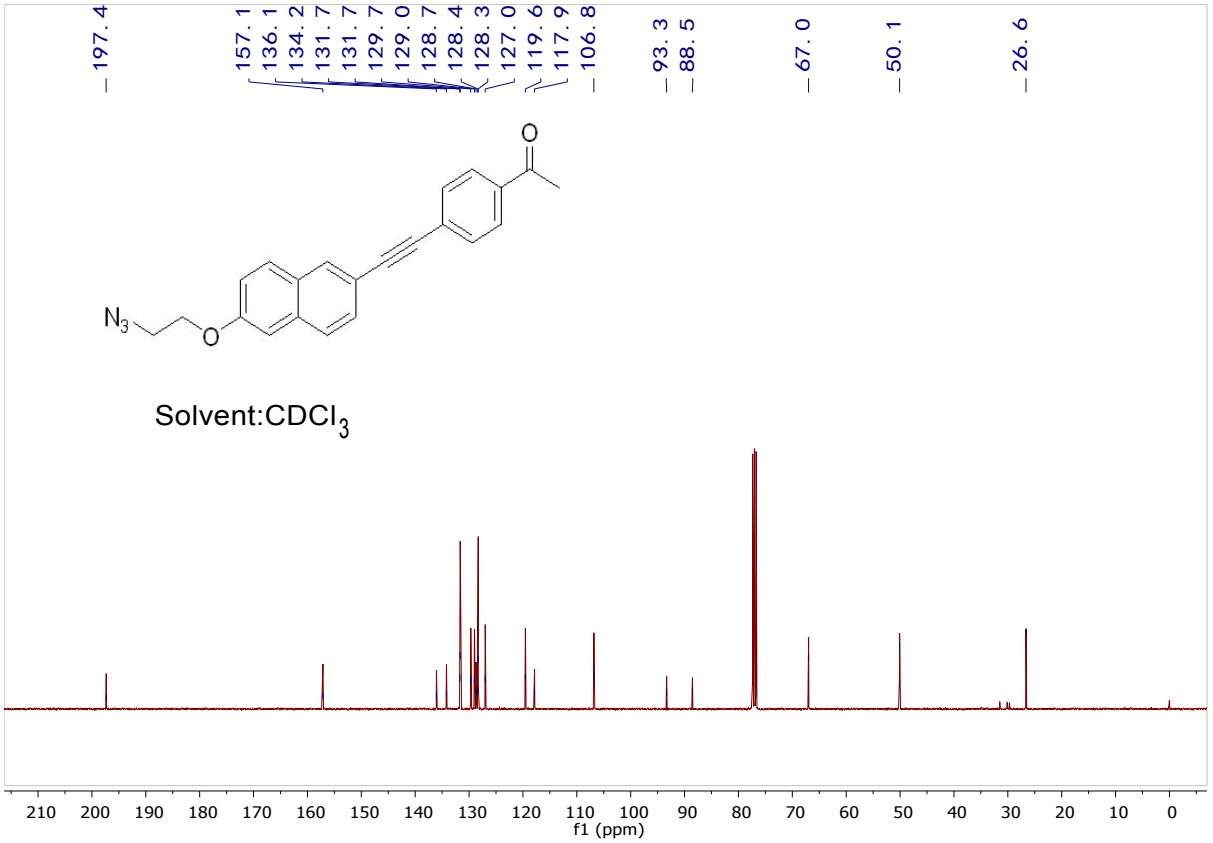

7.922
7.677
7.655
7.604
7.582
7.521
7.495
7.198
7.175
7.094
4.415
4.399
4.384
3.718
3.702
3.688
Br
Br
O
Solvent:CDCl3
1.00
1.10
1.06
1.04
1.07
1.00
2.13
2.12
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.
f1 (ppm)

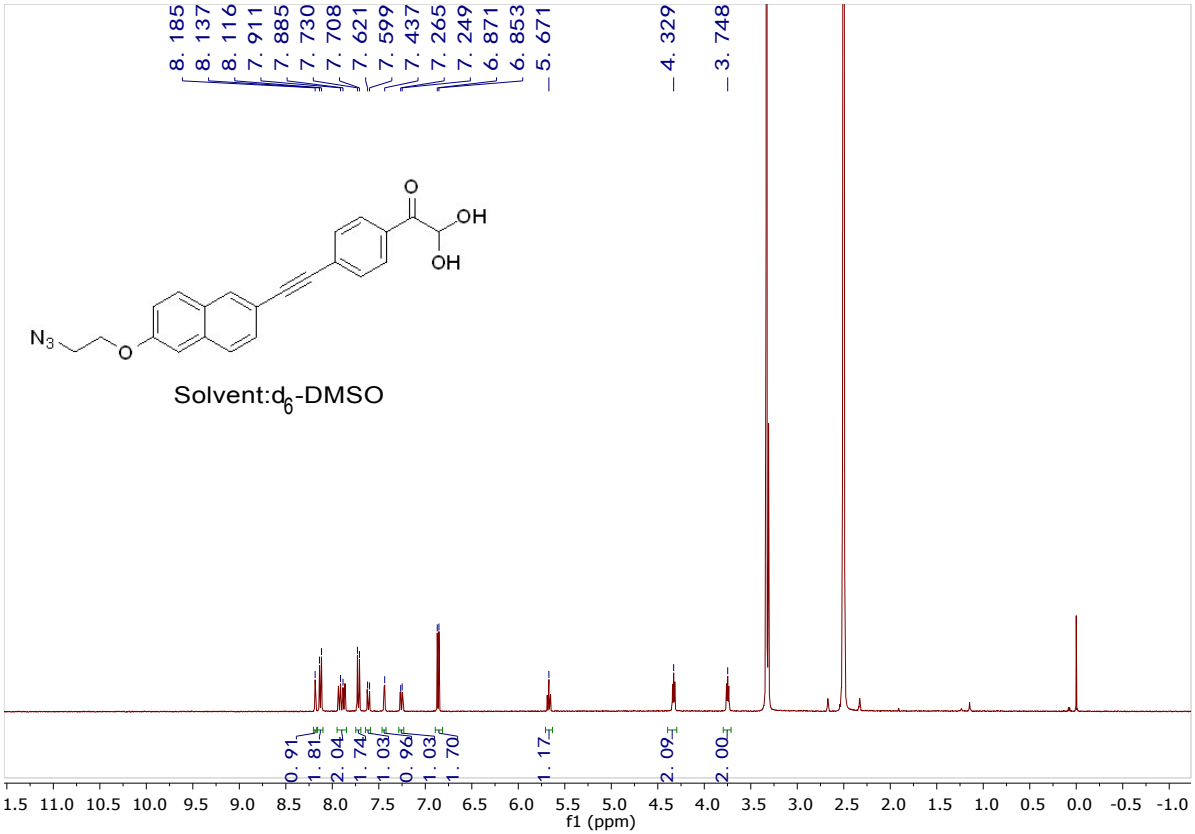

156.3
132.8
130.3
129.8
129.7
128.8
128.4
119.8
117.4
107.0
67.8
28.9
Br
Br
O
Solvent:CDCl3
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

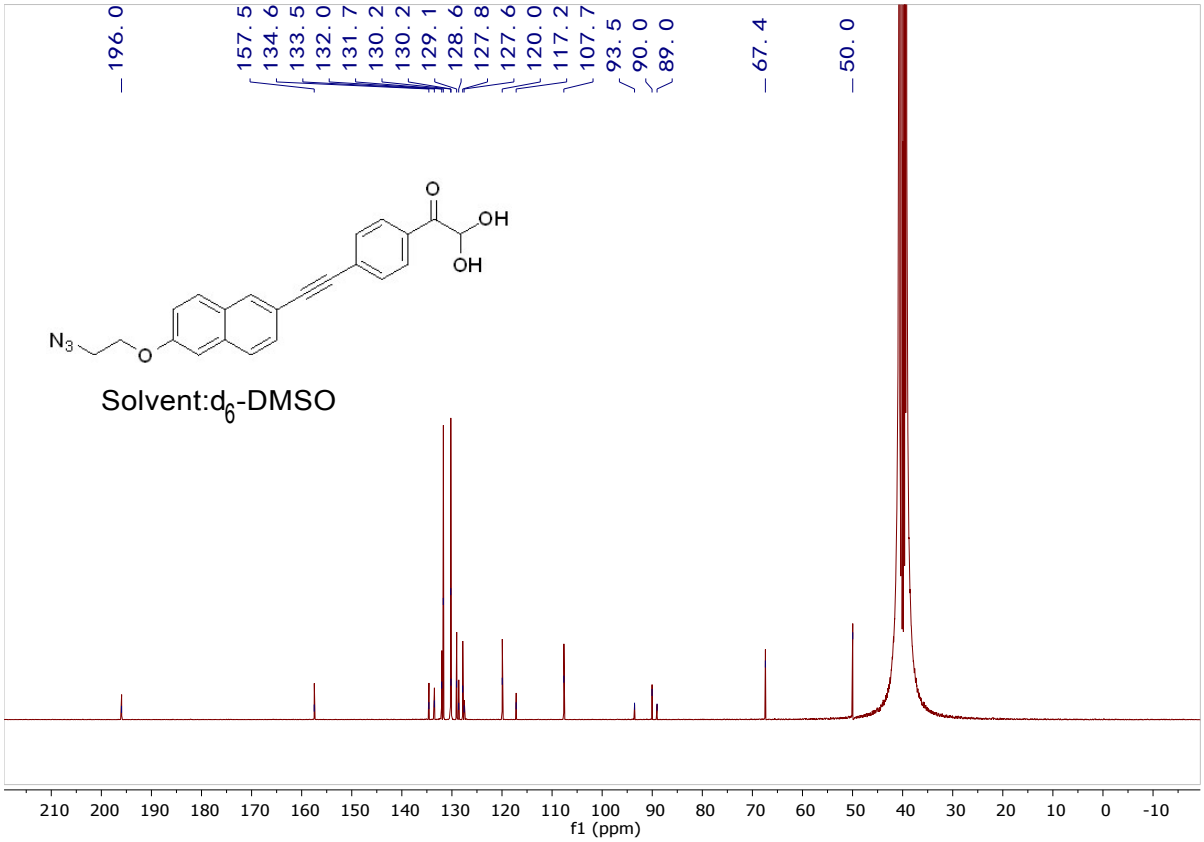

7.921
7.677
7.654
7.604
7.582
7.519
7.492
7.211
7.182
7.100
4.241
3.677
Br
N3
O
Solvent:CDCl3
1.00
1.09
1.08
1.05
1.06
1.01
2.29
2.27
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


156.5
132.8
130.2
129.7
129.7
128.7
128.4
119.7
117.4
106.8
67.0
50.1
Br
N3
O
Solvent:CDCl3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


8.023
7.971
7.773
7.705
7.654
7.579
7.236
7.141
4.292
3.687
2.628
O
N3
O
Solvent:CDCl3
0.96
1.95
1.05
1.08
1.93
1.06
1.21
1.00
2.00
2.00
2.87
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


197.4
157.1
136.1
134.2
131.7
131.7
129.7
129.0
128.7
128.4
128.3
127.0
119.6
117.9
106.8
93.3
88.5
67.0
50.1
26.6
O
N3
O
Solvent:CDCl3
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


8.185
8.137
8.116
7.911
7.885
7.730
7.708
7.621
7.599
7.437
7.265
7.249
6.871
6.853
5.671
4.329
3.748
O
OH
OH
N3
O
Solvent:d6-DMSO
0.91
1.81
2.04
1.74
1.03
0.96
1.03
1.70
1.17
2.09
2.00
1.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)


196.0
157.5
134.6
133.5
132.0
131.7
130.2
130.2
129.1
128.6
127.8
127.6
120.0
117.2
107.7
93.5
90.0
89.0
67.4
50.0
O
OH
OH
N3
O
Solvent:d6-DMSO
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

- **Reference**

1 Q.-H. Gao, Z. Fei, Y.-P. Zhu, M. Lian, F.-C. Jia, M.-C. Liu, N.-F. She and A.-X. Wu, *Tetrahedron*, 2013, **69**, 22-28.

2 N. Mase, T. Ando, F. Shibagaki, A. Sugita, T. Narumi, M. Toda, N. Watanabe and F. Tanaka, *Tetrahedron Lett.*, 2014, **55**, 1946-1948.