Supporting Information

Enabling Universal Access to Rapid and Stable Tetrazine Bioorthogonal Probes Through Triazoly-Tetrazine Formation

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1. General information

Commercially available reagents were purchased from Sigma-Aldrich, *J&K* Scientific, TCI, Energy Chemical, and Bide Pharm, and used without further purification unless otherwise noted. Thin-layer chromatography (TLC) was performed on 0.20 mm silica gel plates and monitored under ultraviolet (UV) light. Flash column chromatography was performed using silica gel (300–400 mesh) from Qingdao Haiyang Chem. Company, Ltd. (Qingdao, China). Differential Scanning Calorimetry was performed on a TA instruments DSC 25 modulated differential scanning calorimeter. NMR spectroscopy was performed on Bruker nuclear magnetic resonance spectrometer at 400 MHz for ^{1}H NMR, 377 MHz for ^{19}F NMR, and 101 MHz for ^{13}C NMR. All ^{13}C NMR spectra were proton decoupled. Chemical shifts were reported as δ in parts per million (ppm) using tetramethylsilane (TMS, 0.00 ppm) or residual deuterated solvent as an internal reference ($CDCl_3$: 7.26 ppm for ^{1}H NMR and 77.2 ppm for ^{13}C NMR; DMSO-d_6: 2.50 ppm for ^{1}H NMR and 39.5 ppm for ^{13}C NMR; CD_3OD: 3.31 ppm for ^{1}H NMR and 49.5 ppm for ^{13}C NMR). Resonance multiplicities were indicated as s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), and m (multiplet). Coupling constants (*J*) were reported in Hertz (Hz), and the number of protons (n) for a given resonance was indicated as nH. High-resolution mass spectra (HRMS) were recorded on a Bruker micro-TOF-QII time-of-flight mass spectrometer with electrospray ionization. UV-Vis absorption data was collected on a Quawell scientific Q6000+ microvolume spectrophotometer.

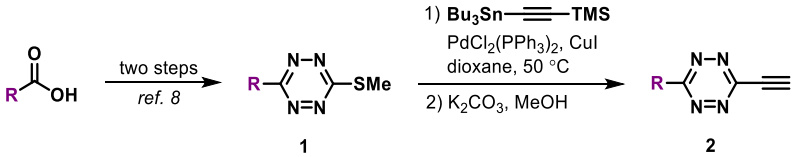
All radiochemical yields (RCY) quoted are decay corrected and are reported as isolated RCY. All RCY were calculated with respect to the starting ^{18}F activity of **^{18}F-N$_3$**. The identities of the ^{18}F-labeled compounds were confirmed by comparison to the corresponding ^{19}F standards. High-performance liquid chromatography-mass spectrometry (HPLC-MS) was performed on an Agilent 1260 Infinity HPLC system equipped with a G7129A 1260 autosampler, a G7111B 1260 Quat Pump, and a G7155A 1260 DAD detector connected to a G6125B single-quadrupole LC/MS system. An Agilent ZORBAX SB-C18 column (5 μm, 4.6×250 mm) was used for HPLC-MS analysis. The solvent composition was controlled by a gradient program. Low-resolution mass spectra were obtained in positive ESI mode in the range 200–2000 m/z.

All commercial azides, **H-Tz-BODIPY**, **Me-Tz-BODIPY**, and **4e-TCO-Cy5** (equatorially configured isomer of TCO) were purchased from Xi'an Dianhua Biotechnology Co., Ltd. The following compounds used in the study were synthesized according to the corresponding references: 1-azido-2-(2-(2-fluoroethoxy)ethoxy)ethane[1], **Ph-Tz**[2], **Me-Tz**[3], **Py-Tz**[4], **H-Tz**[5], *trans*-cyclooctene (axially configured isomer, 4a-TCO)[6], *cis*-dioxolane-fused *trans*-cyclooctene (*d*-TCO)[6] and *d*-TCO-triphenylphosphonium conjugates (***d*-TCO-TPP**)[7].

The predicted octanol/water partition coefficient (predicted log *P*) was calculated using the software Molinspiration Property Calculator (https://www.molinspiration.com/cgi-bin/properties).

2. Preparation of ethynyl-tetrazines

2.1. General procedure for the synthesis of ethynyl-tetrazines



*2.1.1 Synthesis of 3-methylthiotetrazine **1**[8]*

Step I: In an anhydrous DCM (1.0 M), a mixture of 3-methyl-3-oxetanemethanol (1.1 equiv.), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 1.2 equiv.) and DMAP (0.10 equiv.) was prepared under an argon atmosphere. At 0 °C, the carboxylic acid (1.0 equiv.) was added. The reaction mixture was stirred at 0 °C for 10 minutes and then at room temperature for 12 hours. Upon completion of the reaction, as confirmed by TLC, the reaction was quenched with a saturated $NaHCO_3$ solution, followed by extraction with DCM (3×60 mL). The organic phase was then washed with brine. The combined organic extracts were dried using anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (PE:EtOAc = 10:1 to 5:1) to obtain the corresponding oxetane ester.

Step II: In a stirred solution of the corresponding oxetane ester (1.0 equiv.) in DCM (1.0 M) cooled by an ice/brine bath, boron trifluoride etherate (1.2 equiv.) was added dropwise under argon atmosphere. The resulting mixture was stirred at the same temperature until the corresponding oxetane ester was fully consumed, as monitored by TLC. The reaction was quenched with pyridine (3.0 equiv.), and then methyl thiocarbohydrazide iodide salt[8] (0.7 equiv.) and DMF (1.0 M) were added. The mixture was evaporated in vacuo to remove DCM, and then stirred at 80 °C for 60 min under an argon atmosphere. After cooling to 0 °C, phenyliodine diacetate (PIDA, 0.5 equiv.) was added in portions, and the mixture was then stirred at room temperature for 60 min. The reaction was quenched with saturated $NaHCO_3$ solution, extracted with DCM (3×60 mL), and washed with brine. The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was redissolved in EtOAc, washed with H_2O and brine. The organic phase was dried with anhydrous Na_2SO_4, filtered, concentrated under reduced pressure and the residue was purified by silica gel column chromatography to obtain the corresponding 3-methylthiotetrazine **1**.

*2.1.2 Synthesis of ethynyl-tetrazine **2***

To the mixture of $PdCl_2(PPh_3)_2$ (28.0 mg, 0.04 mmol, 20 mol%) and CuI (76.2 mg, 0.4 mmol, 2.0 equiv.) in 1.4-dioxane (2.0 mL), was successively added **1** (0.2 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (146.8 μL, 0.4 mmol, 2.0 equiv.) under argon atmosphere. The reaction mixture was heated at 50 °C for 12 h. After reaction completion, as monitored by TLC, the reaction mixture was filtered and concentrated under reduced pressure and the residue was subjected to silica gel column chromatography (PE:EtOAc = 50:1 to 5:1) to afford the corresponding crude product. The crude product was dissolved in dry MeOH (10 mL), and K_2CO_3 (2.8 mg, 0.1 equiv.) was added. The mixture was stirred at r.t. for 1–5 min. After reaction completion as monitored by TLC, the reaction was immediately extracted with DCM (3×20 mL), and washed with brine. (**Caution!** Dry solvent is necessary and when the reaction is completed, it needs to be worked up immediately.) The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure and the residue was purified by silica gel column chromatography (PE:EtOAc = 50:1 to 5:1) to afford the corresponding product **2.**

2.2. Optimization of Liebeskind−Srogl coupling reaction conditions

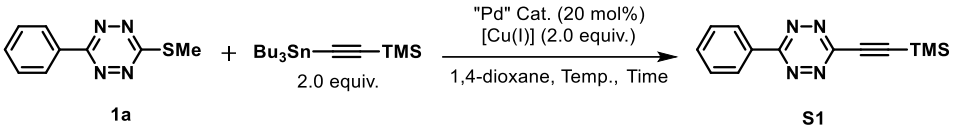


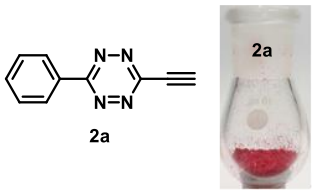
Table S1. Optimization reaction conditions[a]

Entry	"Pd" Cat. (20 mol%)	Cu(I) (2.0 equiv.)	Temperature/Time	Concentration	Yield[b]
1	$PdCl_2(PPh_3)_2$	CuTc	100 °C/1 h	10 mM	61%
2	$PdCl_2(PPh_3)_2$	CuI	100 °C/1 h	10 mM	52%
3	$PdCl_2(PPh_3)_2$	CuBr	100 °C/1 h	10 mM	18%
4	$PdCl_2(PPh_3)_2$	CuCl	100 °C/1 h	10 mM	21%
5	$PdCl_2(PPh_3)_2$	CuTc	75 °C/12 h	10 mM	45%
6	$PdCl_2(PPh_3)_2$	CuTc	50 °C/12 h	10 mM	65%
7	$PdCl_2(PPh_3)_2$	CuTc	r.t./12 h	10 mM	22%
8	$PdCl_2(PPh_3)_2$	CuI	50 °C/12 h	10 mM	87%
9	$PdCl_2(PPh_3)_2$	CuI	50 °C/12 h	20 mM	93%
10	$Pd(PPh_3)_4$	CuI	50 °C/12 h	20 mM	83%
11	$PdCl_2(PPh_3)_2$	CuTc	50 °C/12 h	20 mM	54%
12	$PdCl_2(PPh_3)_2$	CuI	50 °C/12 h	0.1 M	91%
13	$PdCl_2(PPh_3)_2$	CuI	r.t./24 h	0.1 M	77%
14	$PdCl_2(PPh_3)_2$	CuI	100 °C/1 h	0.1 M	81%
15	$Pd(PPh_3)_4$	CuI	50 °C/12 h	0.1 M	77%
16	$Pd_2(dba)_3$/40 mol% PPh_3	CuI	50 °C/12 h	0.1 M	34%
17	$Pd(OAc)_2$/40 mol% PPh_3	CuI	50 °C/12 h	0.1 M	57%
18	$PdCl_2(PPh_3)_2$ (10 mol%)	CuI	50 °C/12 h	0.1 M	79%
19	$PdCl_2(PPh_3)_2$	CuI (1.0 equiv.)	50 °C/12 h	0.1 M	67%
20[c]	$PdCl_2(PPh_3)_2$	CuI	50 °C/12 h	0.1 M	71%

[a]Reaction conditions: unless other wised noted, **1a** (10.2 mg, 0.05 mmol, 1.0 equiv.), trimethylsilylethynyltri-*n*-butyltin (36.7 μL, 0.10 mmol, 2.0 equiv.), "Pd" Cat. (20 mol%), Cu(I) (2.0 equiv.) were used. [b]The yield of **S1** was determined by ^{1}H NMR with mesitylene as an internal standard. [c]trimethylsilylethynyltri-*n*-butyltin (27.5 μL, 0.075 mmol, 1.5 equiv.).

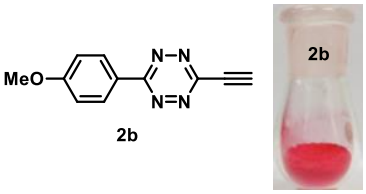
2.3 Preparation and characterization of ethynyl-tetrazines

*2.3.1 Synthesis of tetrazine **2a***



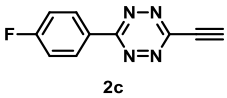
The general procedure described above was followed to prepare compound **2a** from **1a**[8] (1.02 g, 5.0 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (3.67 mL, 10 mmol, 2.0 equiv.). Purification by silica column chromatography afforded **2a** as a red solid (0.63 g, 3.47 mmol, 69%). **^{1}H NMR** (**400 MHz**, **DMSO**) δ 8.51–8.49 (m, 2H), 7.76–7.67 (m, 3H), 5.31 (s, 1H). **^{13}C NMR** (**101 MHz**, **DMSO**) δ 161.3, 155.6, 133.1, 131.4, 129.6, 128.1, 89.2, 77.8. HRMS (DART-TOF) calculated for $C_{10}H_6N_4Na^+$ [M + Na]$^+$ m/z 205.0485, found 205.0491.

*2.3.2 Synthesis of tetrazine **2b***



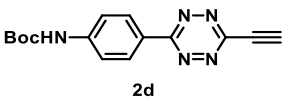
The general procedure described above was followed to prepare compound **2b** from **1b**[8] (1.17 g, 5.0 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (3.67 mL, 10 mmol, 2.0 equiv.). Purification by silica column chromatography afforded **2b** as a red solid (632 mg, 2.98 mmol, 60%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.59 (d, *J* = 9.0 Hz, 2H), 7.10 (d, *J* = 9.0 Hz, 2H), 3.93 (s, 3H), 3.71 (s, 1H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 164.2, 161.8, 155.7, 130.8, 123.6, 115.1, 85.4, 77.4, 55.7. HRMS (DART-TOF) calculated for $C_{11}H_8N_4NaO^+$ [M + Na]$^+$ m/z 235.0590, found 235.0580.

*2.3.3 Synthesis of tetrazine **2c***



The general procedure described above was followed to prepare compound **2c** from **1c**[9] (44.4 mg, 0.2 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (146.8 µL, 0.4 mmol, 2.0 equiv.). Purification by silica column chromatography afforded **2c** as a red solid (29.2 mg, 0.146 mmol, 73%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.66 (dd, *J* = 9.0, 5.3 Hz, 2H), 7.30 (t, *J* = 8.6 Hz, 2H), 3.76 (s, 1H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 166.4 (d, *J* = 256.9 Hz), 161.4, 156.2, 131.2 (d, *J* = 9.3 Hz), 127.5 (d, *J* = 3.1 Hz), 117.0 (d, *J* = 22.2 Hz), 86.2, 77.3. **^{19}F NMR** (**377 MHz**, **$CDCl_3$**) δ: −104.6. HRMS (DART-TOF) calculated for $C_{10}H_6FN_4^+$ [M + H]$^+$ 201.0571, found 201.0572.

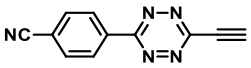
*2.3.4 Synthesis of tetrazine **2d***



The general procedure described above was followed to prepare compound **2d** from **1d**[8] (63.8 mg, 0.2 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (146.8 µL,0.4 mmol, 2.0 equiv.). Purification by silica column

chromatography afforded **2d** as a red solid (36.8 mg, 0.124 mmol, 62%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.57 (d, *J* = 8.9 Hz, 2H), 7.61 (d, *J* = 8.9 Hz, 2H), 6.77 (s, 1H), 3.72 (s, 1H), 1.55 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 161.7, 155.9, 152.2, 143.6, 130.1, 125.3, 118.6, 85.6, 81.7, 77.4, 28.4. HRMS (DART-TOF) calculated for $C_{15}H_{16}N_5O_2^+$ $[M + H]^+$ m/z 298.1299, found 298.1290.

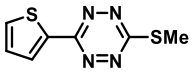
*2.3.5 Synthesis of tetrazine **2e***



2e

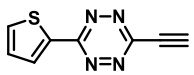
The general procedure described above was followed to prepare compound **2e** from **1e**[8] (45.8 mg, 0.2 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (146.8 µL, 0.4 mmol, 2.0 equiv.). Purification by silica column chromatography afforded **2e** as a red solid (21.5 mg, 0.104 mmol, 52%). **^{1}H NMR** (**400 MHz**, **DMSO**) δ 8.64 (d, *J* = 8.5 Hz, 2H), 8.16 (d, *J* = 8.5 Hz, 2H), 5.40 (s, 1H). **^{13}C NMR** (**101 MHz**, **DMSO**) δ 160.5, 155.6, 135.7, 133.4, 128.7, 118.2, 115.1, 90.0, 77.7. HRMS (DART-TOF) calculated for $C_{11}H_5N_5^-$ $[M]^-$ m/z 207.0550, found 207.0552.

*2.3.6 Synthesis of tetrazine **2f***



1f

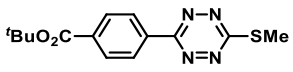
The compound **1f** was prepared according to the general procedure as a red solid (1.26 g, 6.0 mmol, 61%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.16 (dd, *J* = 3.8, 1.2 Hz, 1H), 7.62 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.21 (dd, *J* = 5.0, 3.8 Hz, 1H), 2.75 (s, 3H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 174.5, 160.4, 135.6, 131.9, 130.5, 128.9, 13.5. HRMS (DART-TOF) calculated for $C_7H_6N_4NaS_2^+$ $[M + Na]^+$ m/z 232.9926, found 232.9934.



2f

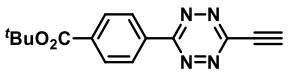
The general procedure described above was followed to prepare compound **2f** from **1f** (42.0 mg, 0.2 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (146.8 µL, 0.4 mmol, 2.0 equiv.). Purification by silica column chromatography afforded **2f** as a red solid (27.4 mg, 0.146 mmol, 73%). **^{1}H NMR** (**400 MHz, $CDCl_3$**) δ 8.34 (dd, *J* = 3.9, 1.2 Hz, 1H), 7.77 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.29 (dd, *J* = 5.0, 3.8 Hz, 1H), 3.73 (s, 1H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 160.1, 155.5, 135.4, 134.5, 133.0, 129.5, 85.8, 77.4. HRMS (DART-TOF) calculated for $C_8H_5N_4S^+$ $[M + H]^+$ m/z 189.0229, found 189.0229.

*2.3.7 Synthesis of tetrazine **2g***



1g

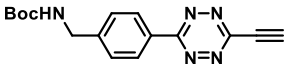
The compound **1g** was prepared according to the general procedure as a red solid (3.0 g, 9.8 mmol, 62%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.58 (d, *J* = 8.6 Hz, 2H), 8.17 (d, *J* = 8.7 Hz, 2H), 2.81 (s, 3H), 1.63 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 175.9, 165.1, 162.0, 135.4, 135.2, 130.3, 127.4, 81.9, 28.3, 13.6. HRMS (DART-TOF) calculated for $C_{14}H_{17}N_4O_2S^+$ $[M + H]^+$ m/z 305.1067, found 305.1063.



2g

The general procedure described above was followed to prepare compound **2g** from **1g** (1.52 g, 5.0 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (3.67 mL, 10 mmol, 2.0 equiv.). Purification by silica column chromatography afforded **2g** as a red solid (0.93 g, 3.3 mmol, 66%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.68 (d, *J* = 8.6 Hz, 2H), 8.20 (d, *J* = 8.6 Hz, 2H), 3.79 (s, 1H), 1.64 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 164.9, 161.7, 156.5, 136.4, 134.6, 130.4, 128.5, 86.6, 82.1, 77.3, 28.3. HRMS (DART-TOF) calculated for $C_{15}H_{15}N_4O_2^+$ [M + H]$^+$ m/z 283.1190, found 283.1187.

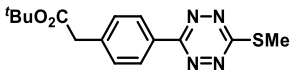
*2.3.8 Synthesis of tetrazine **2h***



2h

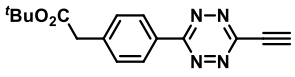
The general procedure described above was followed to prepare compound **2h** from **1h**[8] (66.6 mg, 0.2 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (146.8 µL, 0.4 mmol, 2.0 equiv.). Purification by silica column chromatography afforded **2h** as a red solid (29.8 mg, 0.096 mmol, 48%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.59 (d, *J* = 8.2 Hz, 2H), 7.52 (d, *J* = 8.1 Hz, 2H), 5.00 (s, 1H), 4.44 (d, *J* = 6.2 Hz, 2H), 3.75 (s, 1H), 1.48 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 162.0, 156.3, 156.1, 145.2, 130.2, 129.1, 128.3, 86.0, 80.1, 77.4, 44.5, 28.5. HRMS (DART-TOF) calculated for $C_{16}H_{17}N_5NaO_2^+$ [M + Na]$^+$ m/z 334.1274, found 334.1272.

*2.3.9 Synthesis of tetrazine **2i***



1i

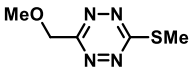
The compound **1i** was prepared according to the general procedure as a red solid (2.18 g, 9.6 mmol, 52%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.47 (d, *J* = 8.3 Hz, 2H), 7.47 (d, *J* = 8.3 Hz, 2H), 3.63 (s, 2H), 2.78 (s, 3H), 1.45 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 175.3, 170.3, 162.3, 139.4, 130.4, 130.3, 127.7, 81.4, 42.8, 28.1, 13.5. HRMS (DART-TOF) calculated for $C_{15}H_{18}N_4NaO_2S^+$ [M + Na]$^+$ m/z 341.1043, found 341.1043.



2i

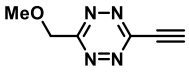
The general procedure described above was followed to prepare compound **2i** from **1i** (63.6 mg, 0.2 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (146.8 µL, 0.4 mmol, 2.0 equiv.). Purification by silica column chromatography afforded **2i** as a red solid (35.5 mg, 0.12 mmol, 60%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.58 (d, *J* = 8.5 Hz, 2H), 7.52 (d, *J* = 8.4 Hz, 2H), 3.75 (s, 1H), 3.65 (s, 2H), 1.45 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 170.1, 162.0, 156.2, 140.8, 130.5, 129.9, 128.9, 86.0, 81.6, 77.4, 42.9, 28.2. HRMS (DART-TOF) calculated for $C_{16}H_{16}N_4NaO_2$ [M + Na]$^+$ m/z 319.1165, found 319.1165.

*2.3.10 Synthesis of tetrazine **2j***



1j

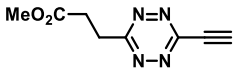
The compound **1j** was prepared according to the general procedure as a red liquid (2.8 g, 16.3 mmol, 65%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 4.96 (s, 2H), 3.57 (s, 3H), 2.75 (s, 3H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 177.0, 164.1, 72.1, 59.6, 13.5. HRMS (DART-TOF) calculated for $C_5H_8N_4NaOS^+$ [M + Na]$^+$ m/z 195.0311, found 195.0310.



2j

The general procedure described above was followed to prepare compound **2j** from **1j** (34.4 mg, 0.2 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (146.8 µL, 0.4 mmol, 2.0 equiv.). Purification by silica column chromatography afforded **2j** as a red liquid (12.0 mg, 0.08 mmol, 40%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 5.09 (s, 2H), 3.75 (s, 1H), 3.62 (s, 3H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 165.0, 157.4, 86.4, 77.4, 72.2, 60.0. HRMS (DART-TOF) calculated for $C_6H_6N_4NaO^+$ [M + Na]$^+$ m/z 173.0434, found 173.0426.

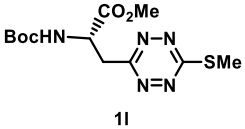
*2.3.11 Synthesis of tetrazine **2k***



2k

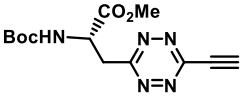
The general procedure described above was followed to prepare compound **2k** from **1k**[8] (42.8 mg, 0.2 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (146.8 µL, 0.4 mmol, 2.0 equiv.). Purification by silica column chromatography afforded **2k** as a red solid (15.7 mg, 0.082 mmol, 41%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 3.73–3.63 (m, 6H), 3.09 (t, *J* = 7.0 Hz, 2H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 172.4, 167.5, 156.6, 85.8, 77.4, 52.2, 30.5, 30.2. HRMS (DART-TOF) calculated for $C_8H_9N_4O_2^+$ [M + H]$^+$ m/z 193.0720, found 193.0721.

*2.3.12 Synthesis of tetrazine **2l***



1l

The compound **1l** was prepared according to the general procedure as a red solid (2.32 g, 7.1 mmol, 42%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 5.52 (d, *J* = 8.3 Hz, 1H), 4.79–4.74 (m, 1H), 3.73 (dd, J = 15.0, 4.8 Hz, 1H), 3.68 (s, 3H), 3.62–3.56 (m, 1H), 2.64 (s, 3H), 1.28 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 175.8, 171.1, 164.3, 155.0, 80.1, 52.7, 52.0, 37.1, 28.1, 13.2. HRMS (DART-TOF) calculated for $C_{12}H_{19}N_5NaO_4S^+$ [M + Na]$^+$ m/z 352.1050, found 352.1050.

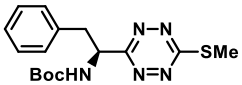


2l

The general procedure described above was followed to prepare compound **2l** from **1l** (65.8 mg, 0.2 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (146.8 µL, 0.4 mmol, 2.0 equiv.). The coupling reaction in the first

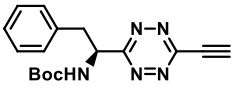
step was carried out at 80 °C for 2 h. Purification by silica column chromatography afforded **2l** as a red liquid (17.8 mg, 0.058 mmol, 29%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 5.46 (d, J = 7.9 Hz, 1H), 4.90–4.85 (m, 1H), 3.95 (dd, *J* = 14.8, 4.8 Hz, 1H), 3.78 (s, 3H), 3.77–3.74 (m, 1H), 1.37 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 171.0, 165.5, 156.6, 155.1, 86.1, 80.7, 77.4, 53.1, 52.2, 38.4, 28.3. HRMS (DART-TOF) calculated for $C_{13}H_{17}N_5NaO_4^+$ [M + Na]$^+$ m/z 330.1173, found 330.1171.

*2.3.13 Synthesis of tetrazine **2m***



1m

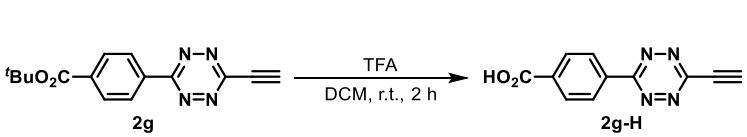
The compound **1m** was prepared according to the general procedure as a red solid (3.33 g, 9.6 mmol, 60%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 7.28–7.20 (m, 3H), 7.06–7.04 (m, 2H), 5.62–5.58 (m, 1H), 5.49–5.37 (m, 1H), 3.43–3.18 (m, 2H), 2.73 (s, 3H), 1.39 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 176.4, 166.7, 155.0, 135.6, 129.4, 128.7, 127.1, 80.2, 54.5, 41.4, 28.3, 13.4. HRMS (DART-TOF) calculated for $C_{16}H_{21}N_5NaO_2S^+$ [M + Na]$^+$ m/z 370.1308, found 370.1308.



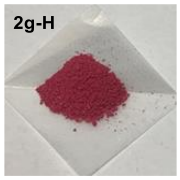
2m

The general procedure described above was followed to prepare compound **2m** from **1m** (69.4 mg, 0.2 mmol, 1.0 equiv.) and trimethylsilylethynyltri-*n*-butyltin (146.8 µL, 0.4 mmol, 2.0 equiv.). Purification by silica column chromatography afforded **2m** as a red liquid (33.1 mg, 0.102 mmol, 51%). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 7.28–7.22 (m, 3H), 7.04–7.02(m, 2H), 5.70–5.65 (m, 1H), 5.49–5.39 (m, 1H), 3.73 (s, 1H), 3.43–3.27 (m, 2H), 1.39 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 167.8, 156.9, 155.2, 135.3, 129.4, 129.0, 127.5, 86.2, 80.6, 77.4, 55.2, 41.3, 28.4. HRMS (DART-TOF) calculated for $C_{17}H_{19}N_5NaO_2^+$ [M + Na]$^+$ m/z 348.1431, found 348.1438.

*2.3.14 Synthesis of tetrazine **2g-H***

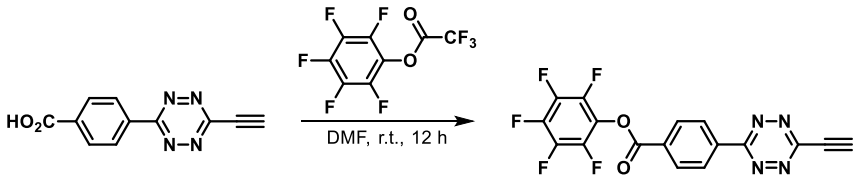


2g **2g-H**



To a solution of **2g** (50.0 mg, 0.177 mmol) in 500 µL of dry DCM was added 50 µL of trifluoroacetic acid (TFA). The mixture was stirred at r.t. for 2 h. Red solids are precipitated, filtered directly, washed with *n*-pentane. The compound **2g-H** (35 mg, 0.155 mmol) was obtained in 87% yield as a red solid. **^{1}H NMR** (**400 MHz**, **DMSO**) δ 8.59 (d, *J* = 8.0 Hz, 2H), 8.22 (d, *J* = 8.1 Hz, 2H), 5.36 (s, 1H). **^{13}C NMR** (**101 MHz**, **DMSO**) δ 166.6, 160.9, 155.6, 135.2, 134.5, 130.3, 128.3, 89.6, 77.8. HRMS (DART-TOF) calculated for $C_{11}H_5N_4O_2^-$ [M - H]$^-$ m/z 225.0418, found 225.0419.

*2.3.15 Synthesis of tetrazine **2g-C_6F_5***



2g-H **2g-C_6F_5**

To a solution of **2g-H** (30.0 mg, 0.13 mmol) in 500 μL of dry DMF was added (34 μL, 0.20 mmol) of pentafluorophenyl trifluoroacetate. The mixture was stirred at r.t. under argon atmosphere for 12 h. The reaction was extracted with EtOAc (3×5 mL), and washed with brine. The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure and the residue was purified by silica gel column chromatography (PE:DCM = 5:1 to 3:1) as a red solid (42.5 mg, 0.11 mmol, 83% yield). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.83 (d, *J* = 8.7 Hz, 2H), 8.44 (d, *J* = 8.7 Hz, 2H), 3.83 (s, 1H). **^{13}C NMR** (**400 MHz**, **$CDCl_3$**) δ 162.0, 161.4, 156.7, 142.9–142.6 (m), 141.04–141.0, 140.4–140.1 (m), 139.7–139.3 (m), 138.7–138.4 (m), 136.7, 131.7, 131.1, 129.0,125.3 (m), 87.1, 77.4. **^{19}F NMR** (**377 MHz**, **$CDCl_3$**) δ −152.2 to −152.3 (m, 2F), −157.2 (t, *J* = 21.9 Hz, 1F), −161.8 to −161.9 (m, 2F). HRMS (DART-TOF) calculated for $C_{17}H_6F_5N_4O_2^+$ [M + H]$^+$ m/z 393.0405, found 393.0487.

2.4 Stability of ethynyl-tetrazines

To evaluate the stability of ethynyl-tetrazines, NMR experiments were conducted. To investigate the long-term storage capability of ethynyl-tetrazines, ethynyl-tetrazines were stored in a 4 °C freezer without the protection of N_2. After 135 or 150 days, samples were taken and NMR experiments were conducted. To investigate the stability of ethynyl-tetrazines in solvent, ethynyl-tetrazines were stored in $CDCl_3$ in NMR tube at room temperature for 15 days. Then, NMR experiments were conducted.

2.4.1 Storage stability of ethynyl-tetrazines at 4 °C

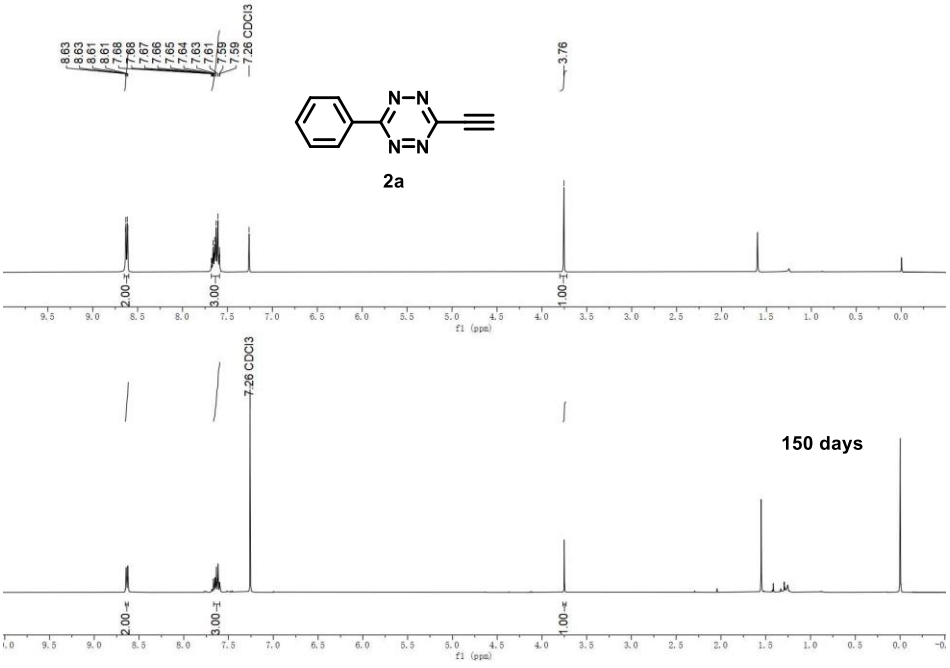


Figure S1. NMR spectra of solid **2a** at the start (*top*) or after 150 days (*bottom*) of storage at 4 °C.

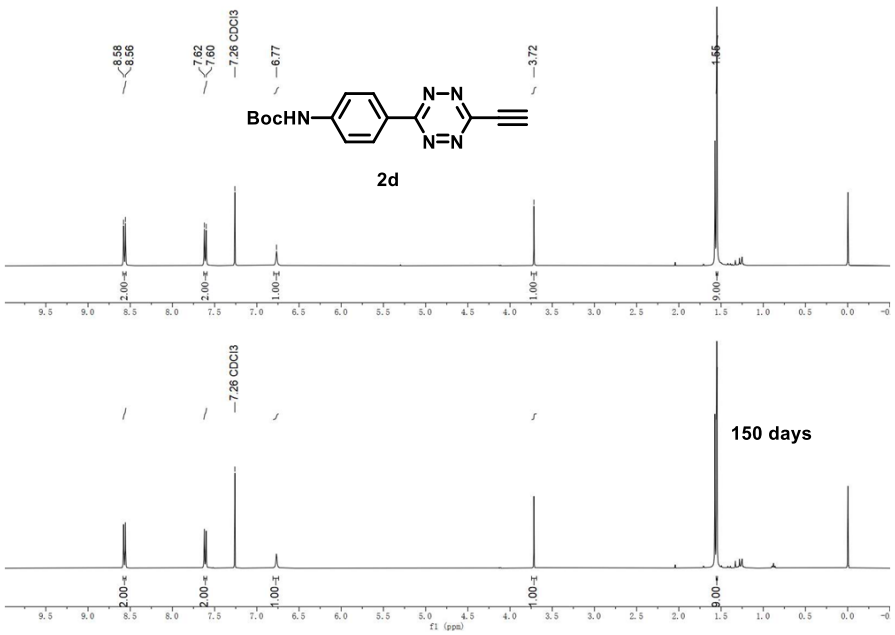


Figure S2. NMR spectra of solid **2d** at the start (*top*) or after 150 days (*bottom*) of storage at 4 °C.

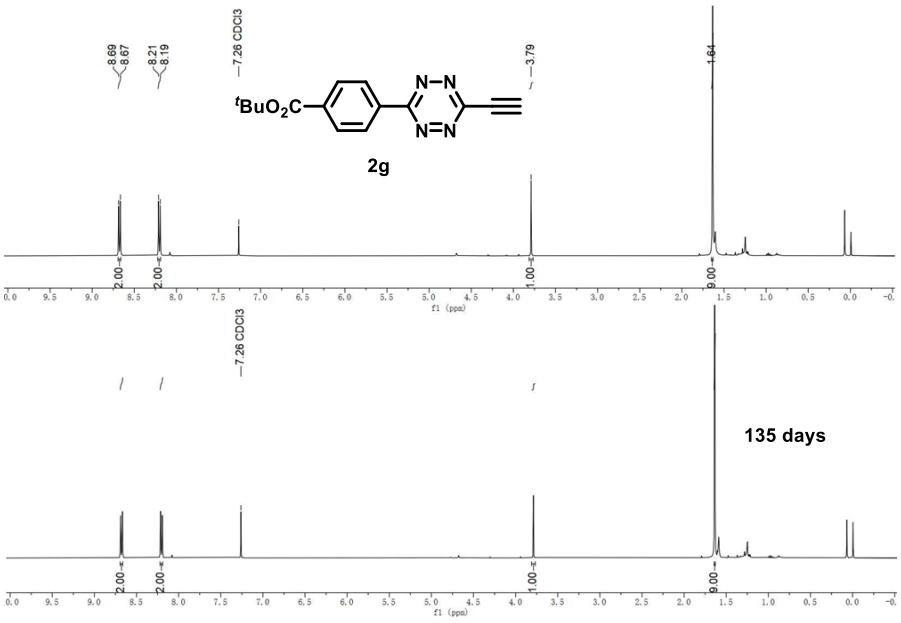


Figure S3. NMR spectra of solid **2g** at the start (*top*) or after 135 days (*bottom*) of storage at 4 °C.

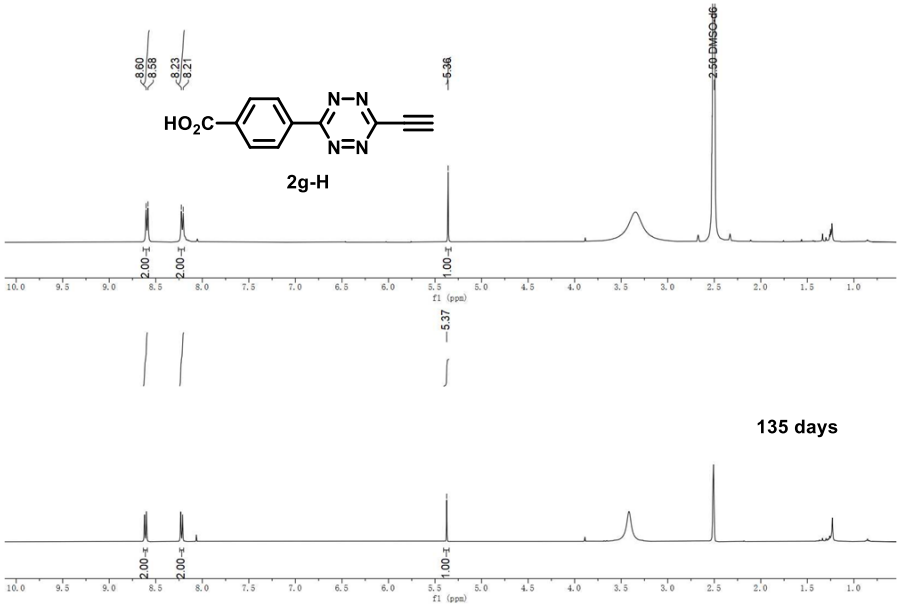


Figure S4. NMR spectra of solid **2g-H** at the start (*top*) or after 135 days (*bottom*) of storage at 4 °C.

2.4.2 Stability of ethynyl-tetrazines in $CDCl_3$ at room temperature

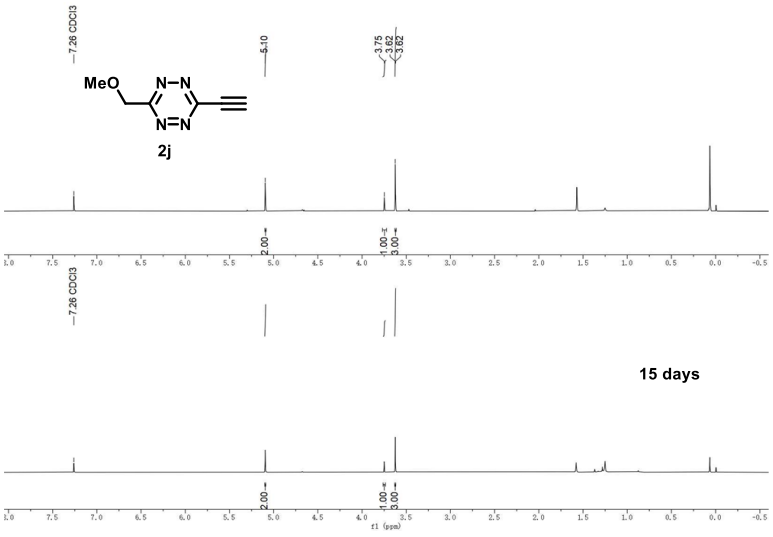


Figure S5. NMR spectra of **2j** at the start (*top*) or after 15 days (*bottom*) of storage in $CDCl_3$ at room temperature.

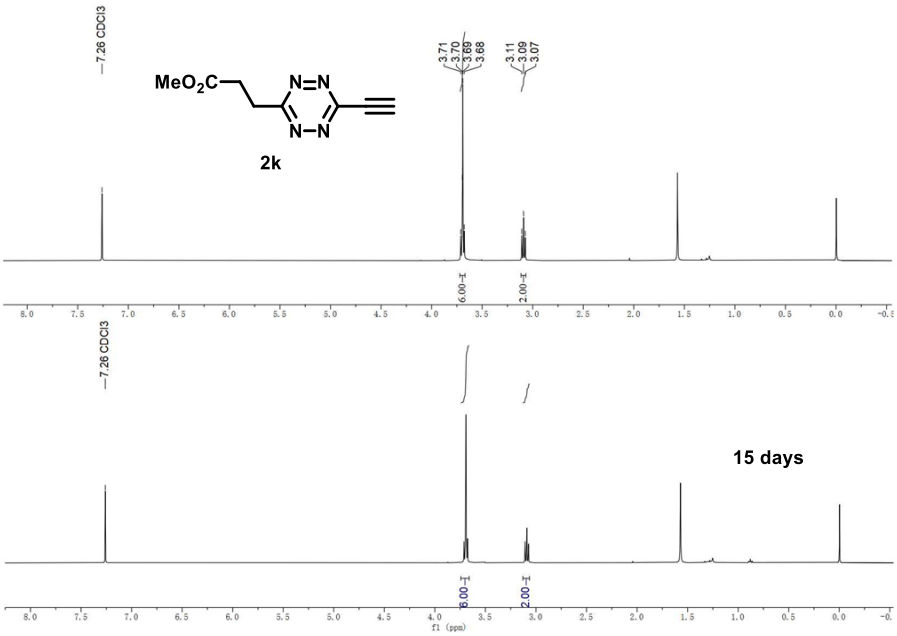


Figure S6. NMR spectra of **2k** at the start (*top*) or after 15 days (*bottom*) of storage in $CDCl_3$ at room temperature

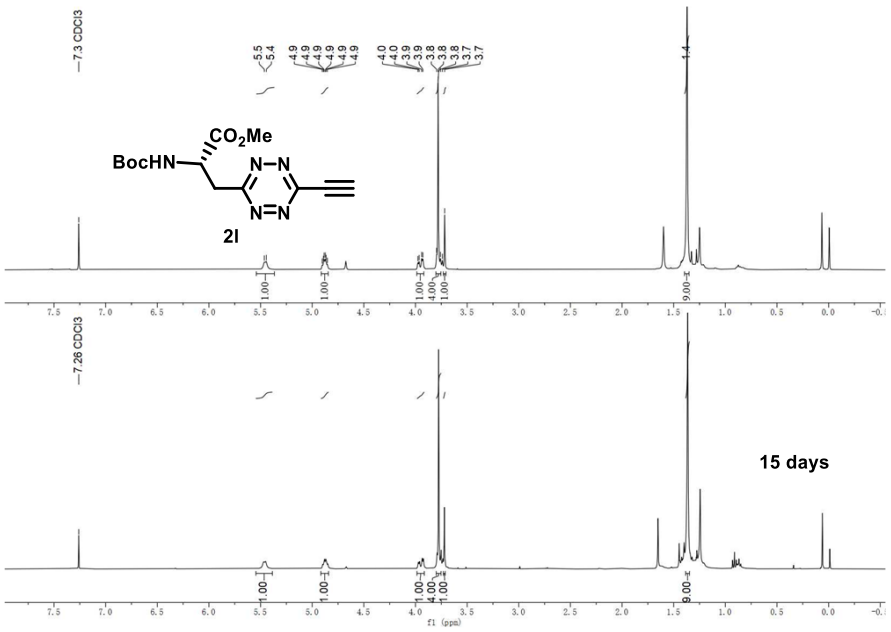
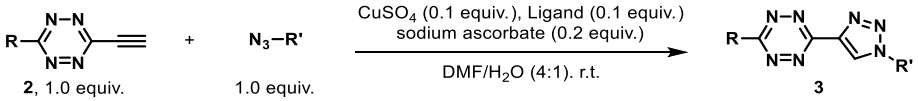
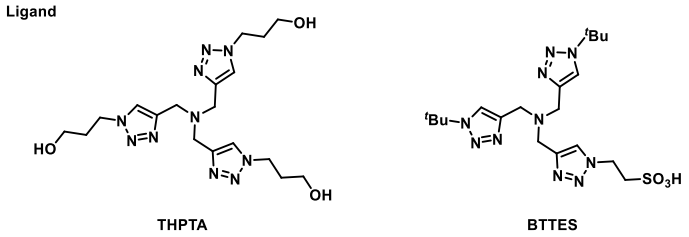


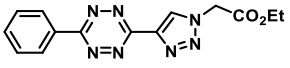
Figure S7. NMR spectra of **2l** at the start (*top*) or after 15 days (*bottom*) of storage in $CDCl_3$ at room temperature.

3. General procedure for the synthesis of triazolyl-tetrazines



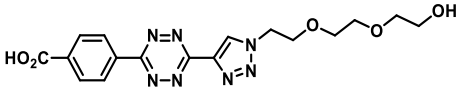


The mixture of $CuSO_4$ (0.1 equiv.), Ligand (0.1 equiv., BTTES[10] was used for **3a, 3d, 3e, 3i**, **3l** and **3p** and THPTA was used for others) and sodium ascorbate (0.2 equiv.) in 2 mL of DMF/H_2O (v/v 4:1) was stirred at r.t. for 10 min. Then, azide (10 mg, 1.0 equiv.) and ethynyl-tetrazine (1.0 equiv.) were added. The reaction mixture was stirred at room temperature. After reaction completion, as monitored by TLC, the reaction was worked up to afford the corresponding product**.**



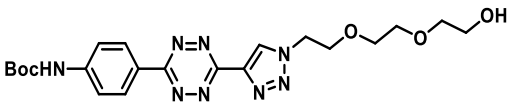
3a

The general procedure was followed to prepare compound **3a** from **2a** (14.1 mg, 0.077 mmol, 1.0 equiv.) and ethyl azidoacetate (10.0 mg, 0.077 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 15 min, then extracted with DCM (3×10 mL) and washed with brine. The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure and the residue was purified by silica gel column chromatography (PE:EtOAc = 5:1 to 2:1) as a red solid (23.4 mg, 97% yield). **^{1}H NMR** (**400 MHz**, **DMSO**) δ 9.20 (s, 1H), 8.54 (d, *J* = 6.2 Hz, 2H), 7.75–7.68 (m, 3H), 5.61 (s, 2H), 4.24 (q, *J* = 7.1 Hz, 2H), 1.26 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR** (**101 MHz**, **DMSO**) δ 167.0, 163.4, 159.5, 141.2, 132.7, 131.9, 129.5, 128.8, 127.6, 61.8, 50.8, 14.0. HRMS (DART-TOF) calculated for $C_{14}H_{13}N_7NaO_2^+$ $[M + Na]^+$ m/z 334.1023, found 334.1019. Predicted log *P* = 1.43.



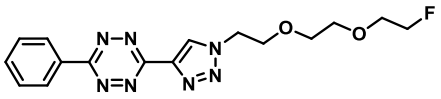
3b

The general procedure was followed to prepare compound **3b** from **2g-H** (12.9 mg, 0.057 mmol, 1.0 equiv.) and 2-[2-(2-azidoethoxy) ethoxy]ethanol (10.0 mg, 0.057 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 60 min, then diluted with water (10 mL), centrifuged (9000 g, 5 min), poured supernatant, added DCM (10 mL), shaken, centrifuged (9000 g, 5 min), poured supernatant, dried and afford the corresponding products as a red solid (20.4 mg, 89% yield).**^{1}H NMR** (**400 MHz**, **DMSO**) δ 9.19 (s, 1H), 8.63 (d, *J* = 8.3 Hz, 2H), 8.23 (d, *J* = 8.3 Hz, 2H), 4.74 (t, *J* = 5.1 Hz, 2H), 4.53 (s, 1H), 3.95 (t, *J* = 5.1 Hz, 2H), 3.60–3.57 (m, 2H), 3.52–3.49 (m, 2H), 3.46 (t, *J* = 5.2 Hz, 2H), 3.38 (d, *J* = 5.2 Hz, 2H). **^{13}C NMR** (**101 MHz**, **DMSO**) δ 166.7, 163.0, 159.6, 141.0, 135.7, 134.1, 130.3, 128.1, 127.7, 72.3, 69.6, 69.6, 68.4, 60.2, 49.9. HRMS (DART-TOF) calculated for $C_{17}H_{19}N_7NaO_5^+$ $[M + Na]^+$ m/z 424.1340, found 424.1339. Predicted log *P* = 0.15.



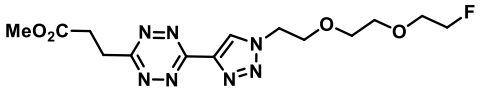
3c

The general procedure was followed to prepare compound **3c** from **2d** (16.9 mg, 0.057 mmol, 1.0 equiv.) and 2-[2-(2-azidoethoxy) ethoxy]ethanol (10.0 mg, 0.057 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 60 min, then extracted with DCM (3×10 mL) and washed with brine. The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure and the residue was purified by silica gel column chromatography (DCM:MeOH = 20:1 to 10:1) as a red solid (23.6 mg, 88% yield). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.83 (s, 1H), 8.57 (d, *J* = 8.6 Hz, 2H), 7.60 (d, *J* = 8.6 Hz, 2H), 6.86 (s, 1H), 4.73 (t, *J* = 4.8 Hz, 2H), 3.97 (t, *J* = 4.8 Hz, 2H), 3.78 (t, *J* = 4.5 Hz, 2H), 3.66 (d, *J* = 2.0 Hz, 4H), 3.62–3.59 (m, 2H), 1.54 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 164.0, 159.6, 152.3, 143.1, 142.0, 129.5, 127.2, 125.9, 118.5, 81.5, 72.8, 70.7, 70.4, 69.2, 61.9, 50.8, 28.4. HRMS (DART-TOF) calculated for $C_{21}H_{28}N_8NaO_5^+$ $[M + Na]^+$ m/z 495.2075, found 495.2079. Predicted log *P* = 1.13.



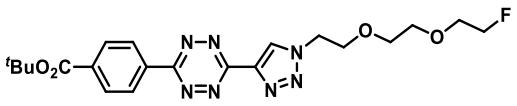
3d

The general procedure was followed to prepare compound **3d** from **2a** (10.0 mg, 0.055 mmol, 1.0 equiv.) and 1-azido-2-(2-(2-fluoroethoxy)ethoxy)ethane[1] (9.6 mg, 0.055 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 20 min, then extracted with DCM (3×10 mL) and washed with brine. The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure and the residue was purified by silica gel column chromatography (DCM:MeOH = 30:1 to 15:1) as a red solid (18.6 mg, 95% yield). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.78 (s, 1H), 8.68–8.63 (m, 2H), 7.66–7.59 (m, 3H), 4.74 (t, *J* = 4.8 Hz, 2H), 4.67–4.61 (m, 1H), 4.55–4.50 (m, 1H), 3.99 (t, *J* = 4.9 Hz, 2H), 3.80–3.76 (m, 1H), 3.71–3.66 (m, 5H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 164.5, 160.1, 142.0, 133.0, 131.9, 129.4, 128.3, 127.3, 83.3 (d, *J* = 169.0 Hz), 70.8 (d, *J* = 9.3 Hz), 70.7, 70.5, 69.3, 50.9. **^{19}F NMR** (**377 MHz**, **$CDCl_3$**) δ 18.6. HRMS (DART-TOF) calculated for $C_{16}H_{18}FN_7NaO_2^+$ $[M + Na]^+$ m/z 382.1398, found 382.1403. Predicted log *P* = 1.26.



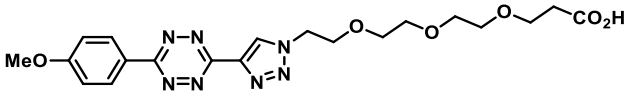
3e

The general procedure was followed to prepare compound **3e** from **2k** (12.0 mg, 0.062 mmol, 1.0 equiv.) and 1-azido-2-(2-(2-fluoroethoxy)ethoxy)ethane[1] (11.0 mg, 0.062 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 20 min, then extracted with DCM (3×10 mL) and washed with brine. The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure and the residue was purified by silica gel column chromatography (DCM:MeOH = 30:1 to 15:1) as a red solid (21.4 mg, 93% yield). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.74 (s, 1H), 4.71 (t, *J* = 4.9 Hz, 2H), 4.64–4.61 (m, 1H), 4.52–4.49 (m, 1H), 3.97 (t, *J* = 4.9 Hz, 2H), 3.77–3.75 (m, 1H), 3.71 (t, *J* = 7.1 Hz, 2H), 3.69 (s, 3H), 3.69–3.65 (m, 5H), 3.10 (t, *J* = 7.1 Hz, 2H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 172.5, 169.1, 160.5, 141.9, 127.4, 83.3 (d, *J* = 169.9 Hz), 70.8 (d, *J* = 9.3 Hz), 70.7, 70.5, 69.3, 52.1, 50.9, 30.8, 30.0. **^{19}F NMR** (**$CDCl_3$, 377 MHz**) δ: 18.6. HRMS (DART-TOF) calculated for $C_{14}H_{21}FN_7O_4^+$ $[M+H]^+$ m/z 370.1634, found 370.1635. Predicted log *P* = –0.74.



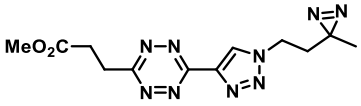
3f

The general procedure was followed to prepare compound **3f** from **2g** (15.9 mg, 0.056 mmol, 1.0 equiv.) and 1-azido-2-(2-(2-fluoroethoxy)ethoxy)ethane[1] (10.0 mg, 0.056 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 60 min, then extracted with DCM (3×10 mL) and washed with brine. The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure and the residue was purified by silica gel column chromatography (DCM:MeOH = 30:1 to 15:1) as a red solid (21.8 mg, 84% yield). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.81 (s, 1H), 8.70 (d, *J* = 8.5 Hz, 2H), 8.21 (d, *J* = 8.5 Hz, 2H), 4.74 (t, *J* = 4.8 Hz, 2H), 4.67–4.62 (m, 1H), 4.55–4.51 (m, 1H), 3.99 (t, *J* = 4.9 Hz, 2H), 3.80–3.76 (m, 1H), 3.72–3.67 (m, 5H), 1.64 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 165.1, 164.0, 160.2, 141.8, 135.8, 135.3, 130.3, 128.0, 127.6, 83.2 (d, *J* = 169.6 Hz), 81.9, 70.8 (d, *J* = 9.8 Hz), 70.7, 70.5, 69.2, 50.9, 28.3. **^{19}F NMR** (**377 MHz**, **$CDCl_3$**) δ 18.6. HRMS (DART-TOF) calculated for $C_{21}H_{26}F_2N_7NaO_4^+$ $[M + Na]^+$ m/z 482.1923, found 482.1923. Predicted log *P* = 2.62.



3g

The general procedure was followed to prepare compound **3g** from **2b** (8.6 mg, 0.04 mmol, 1.0 equiv.) and 3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)propanoic acid (10.0 mg, 0.04 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 2 h, then extracted with DCM (3×10 mL) and washed with brine. The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure and the residue was purified by silica gel column chromatography (DCM:MeOH = 15:1 to 10:1) as a red solid (15.9 mg, 85% yield). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.77 (s, 1H), 8.59 (d, *J* = 9.0 Hz, 2H), 7.09 (d, *J* = 9.0 Hz, 2H), 4.75–4.71 (m, 2H), 3.99–3.96 (m, 2H), 3.92 (s, 3H), 3.77 (t, *J* = 6.1 Hz, 2H), 3.68–3.63 (m, 8H), 2.63 (t, *J* = 6.1 Hz, 2H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 174.7, 164.1, 163.7, 159.5, 142.0, 130.2, 127.1, 124.2, 114.9, 70.8, 70.7, 70.6, 70.5, 69.3, 66.5, 55.7, 50.9, 34.9. HRMS (DART-TOF) calculated for $C_{20}H_{25}N_7NaO_6^+$ $[M + Na]^+$ m/z 482.1759, found 482.1760. Predicted log *P* = 0.34.



3h

The general procedure was followed to prepare compound **3h** from **2k** (15.4 mg, 0.08 mmol, 1.0 equiv.) and 3-(2-Azidoethyl)-3-methyl-3H-diazirine (10.0 mg, 0.08 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 20 min (HPLC yield: 96%), then extracted with DCM (3×10 mL) and washed with brine. The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure and the residue was purified by silica gel column chromatography (DCM:EA = 10:1 to 3:1) as a red solid (23.1 mg, 91% yield). **^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.59 (s, 1H), 4.43 (t, *J* = 7.1 Hz, 2H), 3.70 (t, *J* = 7.1 Hz, 2H), 3.68 (s, 3H), 3.09 (t, *J* = 7.0 Hz, 2H), 2.12 (t, *J* = 7.0 Hz, 2H), 1.03 (s, 3H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 172.5, 169.2, 160.3, 142.1, 126.2, 52.1, 45.7, 35.3, 30.8, 30.0, 23.6, 19.8. HRMS (DART-TOF) calculated for $C_{12}H_{16}N_9O_2^+$ $[M + H]^+$ m/z 318.1421, found 318.1427.

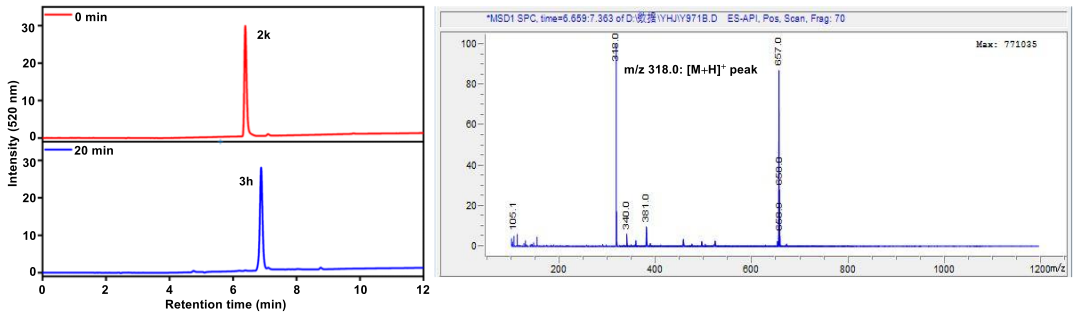
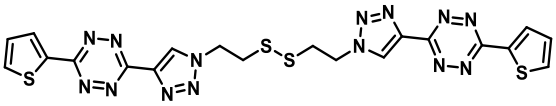
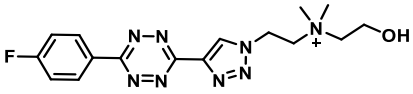


Figure S8. HPLC elution profiles of reaction products **3h** (blue) and **2k** (red) and the mass spectrum of the reaction solution after 20 min. HPLC was conducted with mobile phase of H_2O containing 0.1% formic acid and MeCN flowing at 1 mL/min. The gradient was as follows: 0–7 min, linear increase from 30% to 100% MeCN; 7–10 min, isocratic at 100% MeCN; 10–10.5 min, linear decrease from 100% to 30% MeCN; 10.5–12 min, isocratic at 30% MeCN.



3i

The general procedure was followed to prepare compound **3i** from **2f** (9.9 mg, 0.53 mmol, 1.0 equiv.) and 1,2-bis((2-azidoethyl)thio)ethane (5.4 mg, 0.0264 mmol, 0.5 equiv.). The reaction was stirred at r.t. for 12 h, then diluted with water (10 mL), centrifuged (9000 g, 5 min), poured supernatant, added DCM (10 mL), shaken, centrifuged (9000 g, 5 min), poured supernatant, added DMSO (5 mL), filtered, lyophilized in freeze dryer to afford a red solid (13.2 mg, 86% yield). **^{1}H NMR** (**400 MHz**, **DMSO**) δ 9.20 (s, 2H), 8.27 (dd, *J* = 3.8, 1.2 Hz, 2H), 8.07 (dd, *J* = 5.0, 1.2 Hz, 2H), 7.39 (dd, *J* = 5.0, 3.7 Hz, 2H), 4.86 (t, *J* = 6.5 Hz, 4H), 3.43 (t, *J* = 6.5 Hz, 4H). **^{13}C NMR** (**101 MHz**, **DMSO**) δ 161.4, 159.1, 141.3, 135.7, 133.6, 131.1, 129.4, 127.5, 48.7, 36.8. HRMS (DART-TOF) calculated for $C_{20}H_{16}N_{14}NaS_4^+$ [M + Na]$^+$ m/z 603.0457, found 603.0441.



3j

The general procedure was followed to prepare compound **3j** from **2c** (12.6 mg, 0.063 mmol, 1.0 equiv.) and azido-choline (10.0 mg, 0.063 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 2 h (HPLC yield: 96%), then diluted with water (10 mL), and lyophilized in freeze dryer to afford. Then, the mixture was purified by reversed phase chromatography (H_2O:MeCN = 100:0 to 90:10) as a red solid (21 mg, 93% yield).**^{1}H NMR** (**400 MHz**, **DMSO**) δ 9.35 (s, 1H), 8.67–8.56 (m, 2H), 7.55 (t, *J* = 8.8 Hz, 2H), 5.41 (s, 1H), 5.18 (s, 2H), 4.11 (s, 2H), 3.90 (s, 2H), 3.59 (s, 2H), 3.21 (s, 6H). **^{13}C NMR** (**101 MHz**, **DMSO**) δ 165.0 (d, *J* = 252.5 Hz), 162.8, 159.3, 141.4, 130.3 (d, *J* = 9.1 Hz), 128.4 (d, *J* = 3.1 Hz), 128.2, 116.7 (d, *J* = 21.2 Hz), 65.7, 61.9, 54.9 51.3, 43.8. **^{19}F NMR** (**377 MHz**, **DMSO**) δ -107.0. HRMS (DART-TOF) calculated for $C_{16}H_{20}FN_8O^+$ [M]$^+$ m/z 359.1739, found 359.1738.

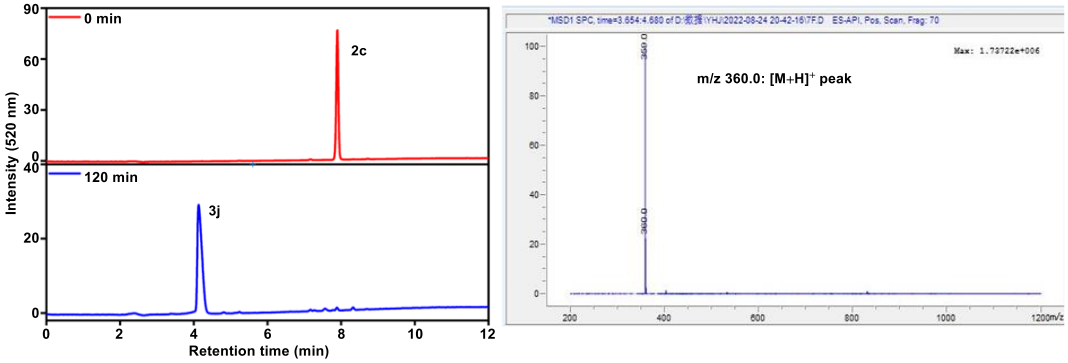
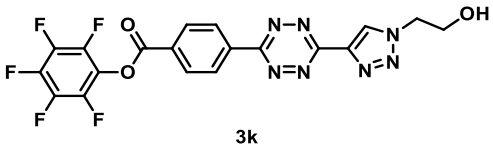
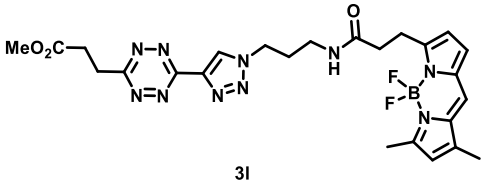


Figure S9. HPLC elution profiles of reaction products **3j** (blue) and **2c** (red) and the mass spectrum of the reaction solution after 20 min. HPLC was conducted with mobile phase of H_2O containing 0.1% formic acid and MeCN flowing at 1 mL/min. The gradient was as follows: 0–7 min, linear increase from 30% to 100% MeCN; 7–10 min, isocratic at 100% MeCN; 10–10.5 min, linear decrease from 100% to 30% MeCN; 10.5–12 min, isocratic at 30% MeCN.



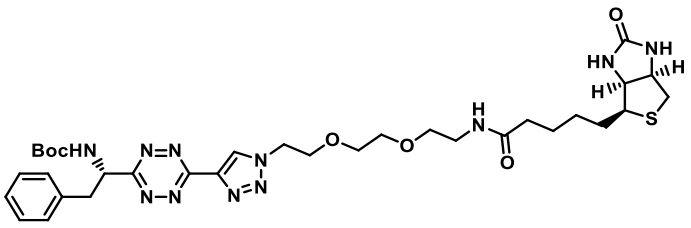
3k

The general procedure was followed to prepare compound **3k** from **2g-C_6F_5**(10.0 mg, 0.026 mmol, 1.0 equiv.) and 2-azidoethanol (2.3 mg, 0.026 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 18 h, then diluted with water (10 mL), centrifuged (9000 g, 5 min), poured supernatant, added DCM (10 mL), shaken, centrifuged (9000 g, 5 min), poured supernatant, filtered, dired and afford the corresponding products as a red solid (10.2 mg, 83% yield). **^{1}H NMR** (**400 MHz**, **DMSO**) δ 9.20 (s, 1H), 8.79 (d, *J* = 8.3 Hz, 2H), 8.49 (d, *J* = 8.3 Hz, 2H), 4.62 (t, *J* = 5.3 Hz, 2H), 3.91 (t, *J* = 5.3 Hz, 2H). **^{19}F NMR** (**377 MHz**, **DMSO**) δ -153.2 to −153.3 (m, 2F), -157.3 (t, *J* = 23.2 Hz, 1F), -162.1 to −162.2 (m, 2F). HRMS (DART-TOF) calculated for $C_{19}H_{10}F_5N_7NaO_3^+$ [M + Na]$^+$ m/z 502.0657, found 502.0652.



3l

The general procedure was followed to prepare compound **3o** from **2k** (1.9 mg, 0.01 mmol, 1.0 equiv.) and BDP FL azide (3.7 mg, 0.01 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 20 min, then the mixture was purified by reversed phase chromatography (H_2O:MeCN = 100:0 to 55:45) as a tawny oli (5.3 mg, 95% yield).**^{1}H NMR** (**400 MHz, $CDCl_3$**) δ 8.54 (s, 1H), 7.07 (s, 1H), 6.87 (d, *J* = 4.0 Hz, 1H), 6.31 (d, *J* = 4.0 Hz, 1H), 6.22 (s, 1H), 6.09 (s, 1H), 4.33 (t, *J* = 7.0 Hz, 2H), 3.72–3.68 (d, *J* = 2.2 Hz, 5H), 3.28 (q, *J* = 7.9, 7.2 Hz, 4H), 3.09 (t, *J* = 7.1 Hz, 2H), 2.71 (t, *J* = 7.3 Hz, 2H), 2.56 (s, 3H), 2.22 (s, 3H), 2.11 (q, *J* = 6.6 Hz, 2H). **^{13}C NMR** (**101 MHz, $CDCl_3$**) δ 172.6, 172.4, 169.1, 161.2, 160.4, 156.5, 144.7, 141.7, 135.5, 133.3, 128.2, 126.5, 124.0, 120.9, 117.4, 52.2, 48.1, 36.2, 35.8, 30.8, 30.4, 30.0, 24.9, 15.1, 11.5. **^{19}F NMR** (**377 MHz, $CDCl_3$**) δ -143.6 to −143.9 (m, 2F). HRMS (DART-TOF) calculated for $C_{25}H_{29}BF_2N_{10}O_3Na^+$ [M + Na]$^+$ m/z 589.2377, found 589.2433.



3m

The general procedure was followed to prepare compound **3m** from **2m** (8.1 mg, 0.025 mmol, 1.0 equiv.) and N-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d] 18 midazole-4-yl)pentanamide (10.0 mg, 0.025 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 2 h (HPLC yield: 92%), then extracted with DCM (3×10 mL) and washed with brine. The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure and the residue was purified by silica gel column chromatography (DCM:MeOH = 20:1 to 10:1) as a red solid (15.7 mg, 87% yield).**^{1}H NMR** (**400 MHz**, **$CDCl_3$**) δ 8.78 (s, 1H), 7.24–7.17 (m, 3H), 7.12–7.06 (m, 2H), 6.55 (t, *J* = 5.7 Hz, 1H), 6.25 (s, 1H), 5.79 (d, *J* = 8.3 Hz, 1H), 5.66 (d, *J* = 7.8 Hz, 1H), 5.40 (s, 1H), 4.75–4.71 (m, 2H), 4.47 (dd, *J* = 7.8, 4.9 Hz, 1H), 4.32–4.27 (m, 1H), 3.97 (t, *J* = 4.9 Hz, 2H), 3.64 (q, *J* = 2.6, 1.9 Hz, 2H), 3.60–3.56 (m, 2H), 3.52 (t, *J* = 5.2 Hz, 2H), 3.45–3.32 (m, 4H), 3.15–3.10 (m, 1H), 2.88 (dd, *J* = 12.8, 4.9 Hz, 1H), 2.70 (d, *J* = 12.8 Hz, 1H), 2.19 (t, *J* = 7.4 Hz, 2H), 1.76–1.70 (m, 1H), 1.68–1.58 (m, 3H), 1.45–1.41 (m, 2H), 1.38 (s, 9H). **^{13}C NMR** (**101 MHz**, **$CDCl_3$**) δ 173.5, 169.6, 163.9, 160.7, 155.4, 141.6, 135.7, 129.5, 128.8, 127.7, 127.3, 80.4, 70.5, 70.2, 69.1, 61.9, 60.3, 55.6, 50.8, 41.3, 40.7, 39.3, 36.0, 28.4, 28.3, 28.2, 25.6. HRMS (DART-TOF) calculated for $C_{33}H_{47}N_{11}NaO_6S^+$ [M + Na]$^+$ m/z 748.3324, found 748.3334.

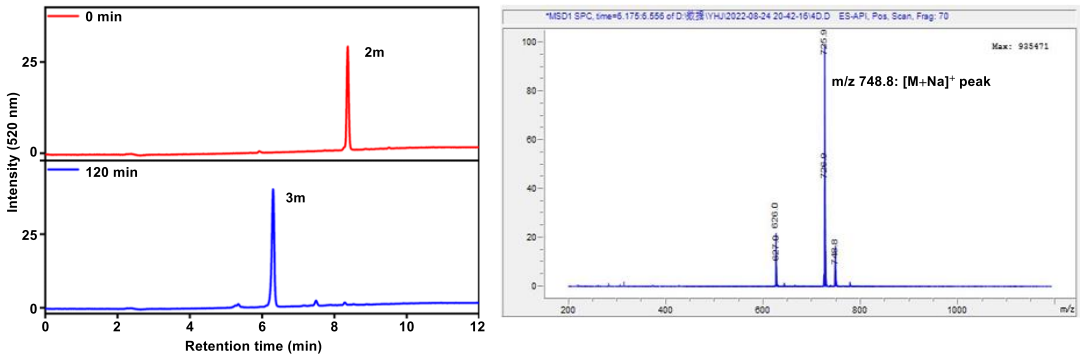
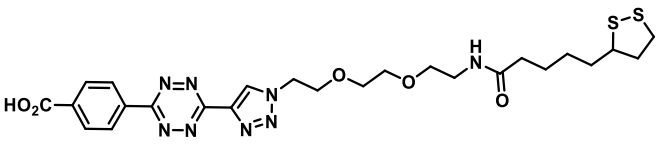


Figure S10. HPLC elution profiles of reaction products **3m** (blue) and **2m** (red) and the mass spectrum of the reaction solution after 20 min. HPLC was conducted with mobile phase of H_2O containing 0.1% formic acid and MeCN flowing at 1 mL/min. The gradient was as follows: 0–7 min, linear increase from 30% to 100% MeCN; 7–10 min, isocratic at 100% MeCN; 10–10.5 min, linear decrease from 100% to 30% MeCN; 10.5–12 min, isocratic at 30% MeCN.



3n

The general procedure was followed to prepare compound **3n** from **2g-H** (6.2 mg, 0.028 mmol, 1.0 equiv.) and N-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-5-(1,2-dithiolan-3-yl)pentanamide (10 mg, 0.028 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 2 h (HPLC yield: 99%), then diluted with water (10 mL), and lyophilized in freeze dryer to afford. The reaction mixture was diluted with water (10 mL), centrifuged (9000 g, 5 min), poured supernatant, added DCM (10 mL), shaken, centrifuged (9000 g, 5 min), poured supernatant, dried and afford the corresponding products as a red solid (14.9 mg, 92% yield).**^{1}H NMR** (**400 MHz**, **DMSO**) δ 9.19 (s, 1H), 8.64 (d, *J* = 7.9 Hz, 2H), 8.23 (d, *J* = 8.1 Hz, 2H), 7.75 (s, 1H), 4.74 (t, *J* = 5.1 Hz, 2H), 3.95 (t, *J* = 5.1 Hz, 2H), 3.62–

3.46 (m, 9H), 3.17–3.11 (m, 2H), 2.00 (t, *J* = 7.3 Hz, 2H), 1.88–1.79 (m, 1H), 1.69–1.53 (m, 2H), 1.49–1.40 (m, 3H), 1.33–1.26 (m, 2H). HRMS (DART-TOF) calculated for $C_{25}H_{32}N_8NaO_5S_2^+$ [M + Na]$^+$ m/z 611.1829, found 611.1824.

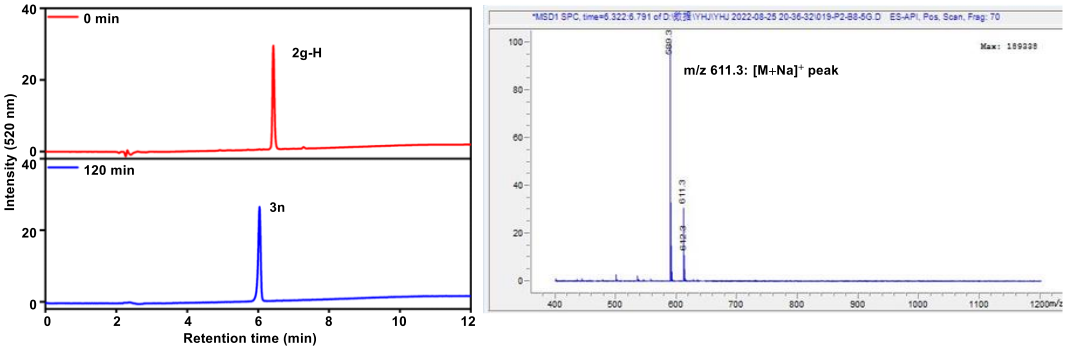
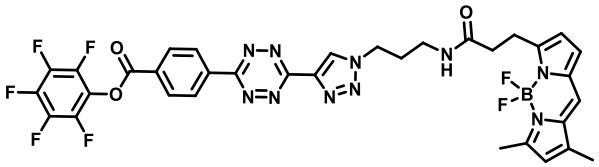
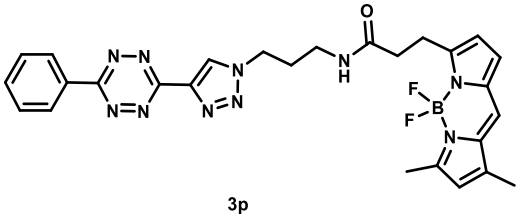


Figure S11. HPLC elution profiles of reaction products **3n** (blue) and **2g-H** (red) and the mass spectrum of the reaction solution after 20 min. HPLC was conducted with mobile phase of H_2O containing 0.1% formic acid and MeCN flowing at 1 mL/min. The gradient was as follows: 0–7 min, linear increase from 30% to 100% MeCN; 7–10 min, isocratic at 100% MeCN; 10–10.5 min, linear decrease from 100% to 30% MeCN; 10.5–12 min, isocratic at 30% MeCN.



3o

The general procedure was followed to prepare compound **3l** from **2g-C_6F_5** (3.0 mg, 0.008 mmol, 1.0 equiv.) and BDP FL azide (2.9 mg, 0.008 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 18 h, then diluted with water (10 mL), centrifuged (9000 g, 5 min), poured supernatant. Then, the reaction was extracted with DCM (3×10 mL), and washed with brine. The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure and the residue was purified by silica gel column chromatography (DCM:MeOH = 20:1 to 15:1) as a orange solid (4.5 mg, 76% yield). **^{1}H NMR** (**400 MHz**, **DMSO**) δ 9.30 (s, 1H), 8.78 (d, *J* = 8.6 Hz, 2H), 8.48 (d, *J* = 8.6 Hz, 2H), 8.09 (t, *J* = 5.6 Hz, 1H), 7.68 (s, 1H), 7.09 (d, *J* = 4.0 Hz, 1H), 6.36 (d, *J* = 4.0 Hz, 1H), 6.28 (s, 1H), 3.18–3.14 (m, 2H), 3.09 (d, *J* = 7.7 Hz, 2H), 2.53–2.50 (m, 4H), 2.46 (s, 3H), 2.25 (s, 3H), 2.14–2.07 (m, 2H). **^{19}F NMR** (**377 MHz**, **DMSO**) δ -143.1 to −143.4 (m, 2F), -153.2 to −153.3 (m, 2F), -157.3 (t, *J* = 23.2 Hz, 1F), -162.1 to −162.2 (m, 2F). HRMS (DART-TOF) calculated for $C_{34}H_{25}BF_7N_{10}O_3^-$ [M - H]$^-$ m/z 765.2104, found 765.2104.



3p

The general procedure was followed to prepare compound **3p** from **2a** (1.8 mg, 0.01 mmol, 1.0 equiv.) and BDP FL azide (3.7 mg, 0.01 mmol, 1.0 equiv.). The reaction was stirred at r.t. for 20 min, then the mixture was purified by reversed phase chromatography (H_2O:MeCN = 100:0 to 50:50) as a tawny oli (4.9 mg, 89% yield).**^{1}H NMR (400 MHz, $CDCl_3$)** 8.68–8.63 (m, 2H), 8.57 (s, 1H), 7.67–7.60 (m, 3H), 7.08 (s, 1H), 6.88 (d, *J* = 4.0 Hz, 1H), 6.33 (d, *J* = 4.0 Hz, 1H), 6.12 (s, 1H), 6.09 (s, 1H), 4.35 (t, *J* = 7.0 Hz, 2H), 3.30 (dt, *J* = 13.0, 6.6 Hz, 4H), 2.73 (t, *J* = 7.3 Hz, 2H), 2.58 (s, 3H), 2.23 (s, 3H), 2.15–2.08 (m, 2H). **^{19}F NMR (377 MHz, $CDCl_3$)** δ -143.6 to −143.8 (m, 2F). HRMS (DART-TOF) calculated for $C_{27}H_{27}BF_2N_{10}ONa^+$ [M + Na]$^+$ m/z 579.2323, found 579.2391.

4. Stability and bioorthogonal reaction kinetics

4.1 Stability test

Solutions of tetrazine compounds in DMSO (20 mM, 10 μL) were diluted in a mixture of DMEM (no phenol red) supplemented with 10% FBS (final concentration: 500 μM for **3b**, **Py-Tz**, **Me-Tz** and **H-Tz**, and 200 μM for **Ph-Tz**) and incubated at 37 °C. At selected time points (0, 3, 9, 12, 24 and 48 h), the samples (30 μL) were treated with an volume of MeCN (60 μL) to precipitate serum proteins. After centrifugation at 15000 g for 3 min, the supernatant was collected and used for further analysis. The peak area of tetrazine compounds at 520 nm was monitored by LC-MS. The stability of the compounds were determined by the decrease of peak area at 520 nm (the peak area at 0 min was defined as 100%). Each stability test was performed in triplicate. The stability of tetrazine probes in FBS was measured by the same method. Our hypothesis is that the likely decomposition products of **3b** include oxadiazole, acylhydrazones, and a trace amount of dihydrotetrazine. These findings are consistent with previous studies.[11]

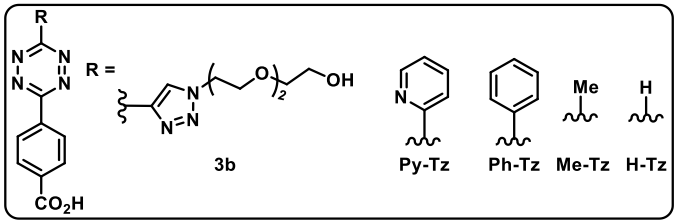


Table S2. Stability of tetrazine probes in DMEM (10% FBS)

	3b	Py-Tz	Ph-Tz	Me-Tz	H-Tz
0 h	100%	100%	100%	100%	100%
3 h	98%	97%	100%	99%	68%
9 h	96%	88%	98%	96%	32%
12 h	92%	80%	97%	95%	22%
24 h	84%	60%	96%	91%	1%
48 h	63%	13%	97%	83%	/

Table S3. Stability of tetrazine probes in FBS

	3b	Py-Tz	Ph-Tz	Me-Tz	H-Tz
0 h	100%	100%	100%	100%	100%
3 h	99%	96%	100%	98%	82%
9 h	95%	90%	100%	97%	58%
12 h	91%	82%	100%	94%	48%
24 h	84%	65%	99%	90%	23%
48 h	65%	38%	99%	84%	/

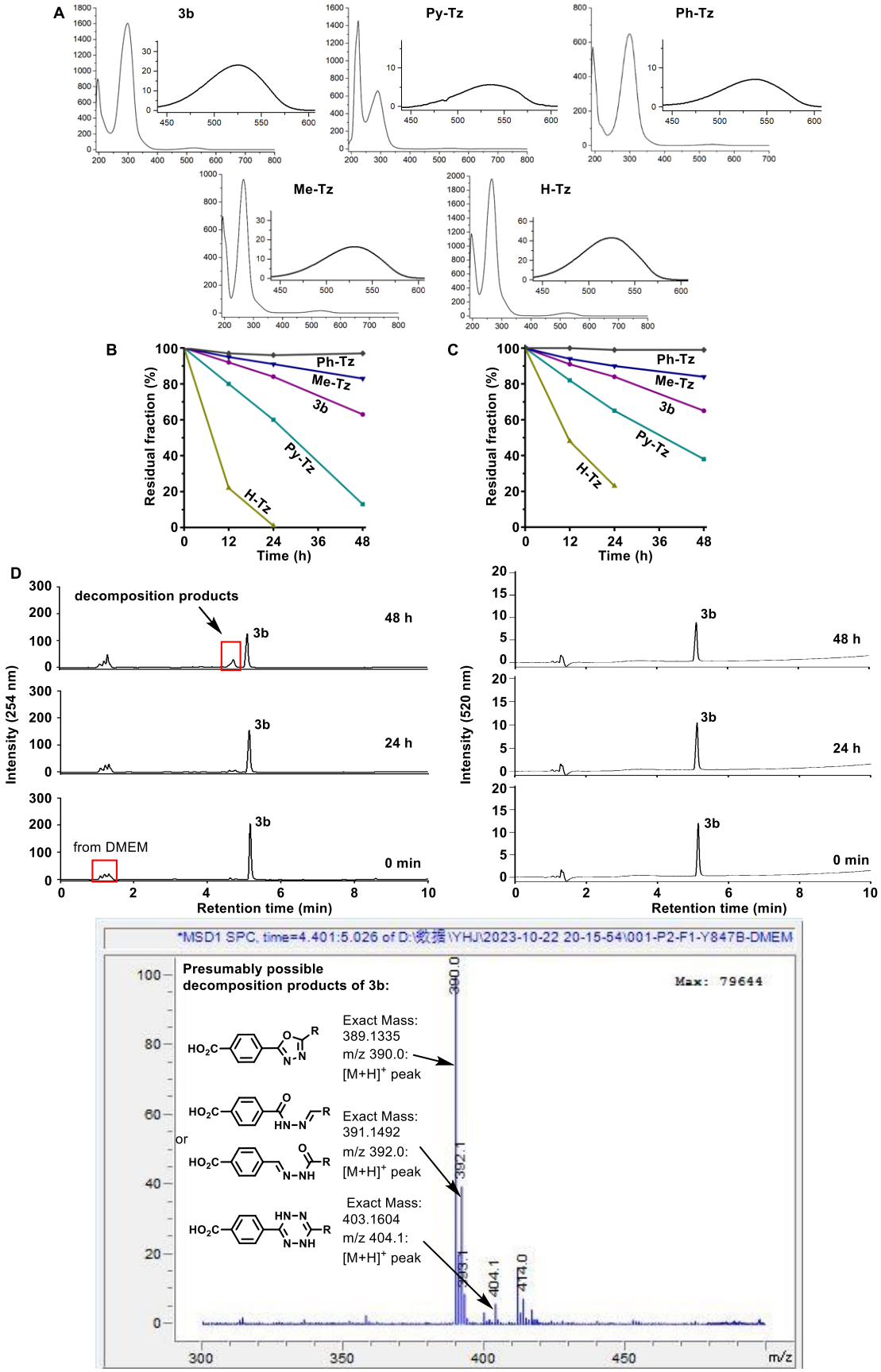


Figure S12. A) UV-vis absorbance spectra of tetrazine compounds, with an inset illustrating the zoomed-in spectra within the wavelength range of 450−600 nm. **B**, **C**) Stability of tetrazine probes in DMEM (**B**) and FBS (**C**) after 12 h, 24 h, and 48 h. **D**) LC-MS characterization of **3b** in DMEM after 24 h and 48 h of incubation in DMEM.

4.2 Bioorthogonal reaction kinetics

*4.2.1 LC-MS characterization of the tetrazine **3b** and the bioorthogonal products*

Stock solutions of **3b** (20 mM in DMSO), 4a-TCO (20 mM in DMSO) were prepared. Then, **3b** (2 μL) and 4a-TCO (2.2 μL) were added to H_2O/MeCN (v/v: 1:1, 195.8 μL), resulting in final concentration of 0.20 mM of **3b** and 0.22 mM 4a-TCO. The mixture was allowed to sit at room temperature for 5 minutes.

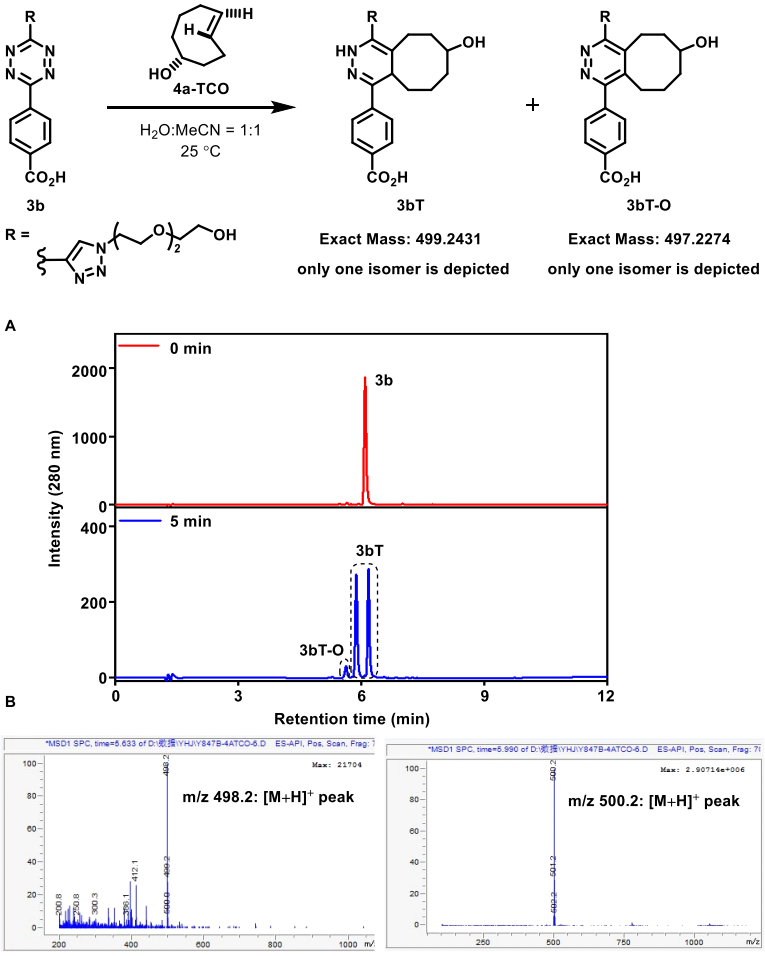


Figure S13. Bioorthogonal reaction between **3b** (0.20 mM) and 4a-TCO (0.22 mM) after 5 min at room temperature in water with 50% MeCN. (A) HPLC traces of **3b** (red) and its bioorthogonal products (blue) at λ = 280 nm. (B) Associated mass traces of the reaction solution.

4.2.2 Bioorthogonal kinetics of tetrazine compounds

The second-order rate constants of the bioorthogonal reactions between tetrazine derivatives (final concentration: 0.1 mM for **Me-Tz** and **Ph-Tz**, and 10 μM for **3b** and **Py-Tz**) and same concentration of 4a-TCO in buffer at 37 °C were determined by UV-Vis spectroscopy under second-order conditions by evaluating the changes in the absorption intensity over time at 520 nm for **Me-Tz** and **Ph-Tz**, and 300 nm for **3b** and **Py-Tz.** Measurements started immediately after the addition of 4a-TCO. The second-order rate constant k_2 was calculated from the slope of the plot of $1/c - 1/c_0$ against time. Each experiment was performed in triplicate, and data were analyzed using GraphPad Prism 6.0. Results are shown as mean ± standard deviation.

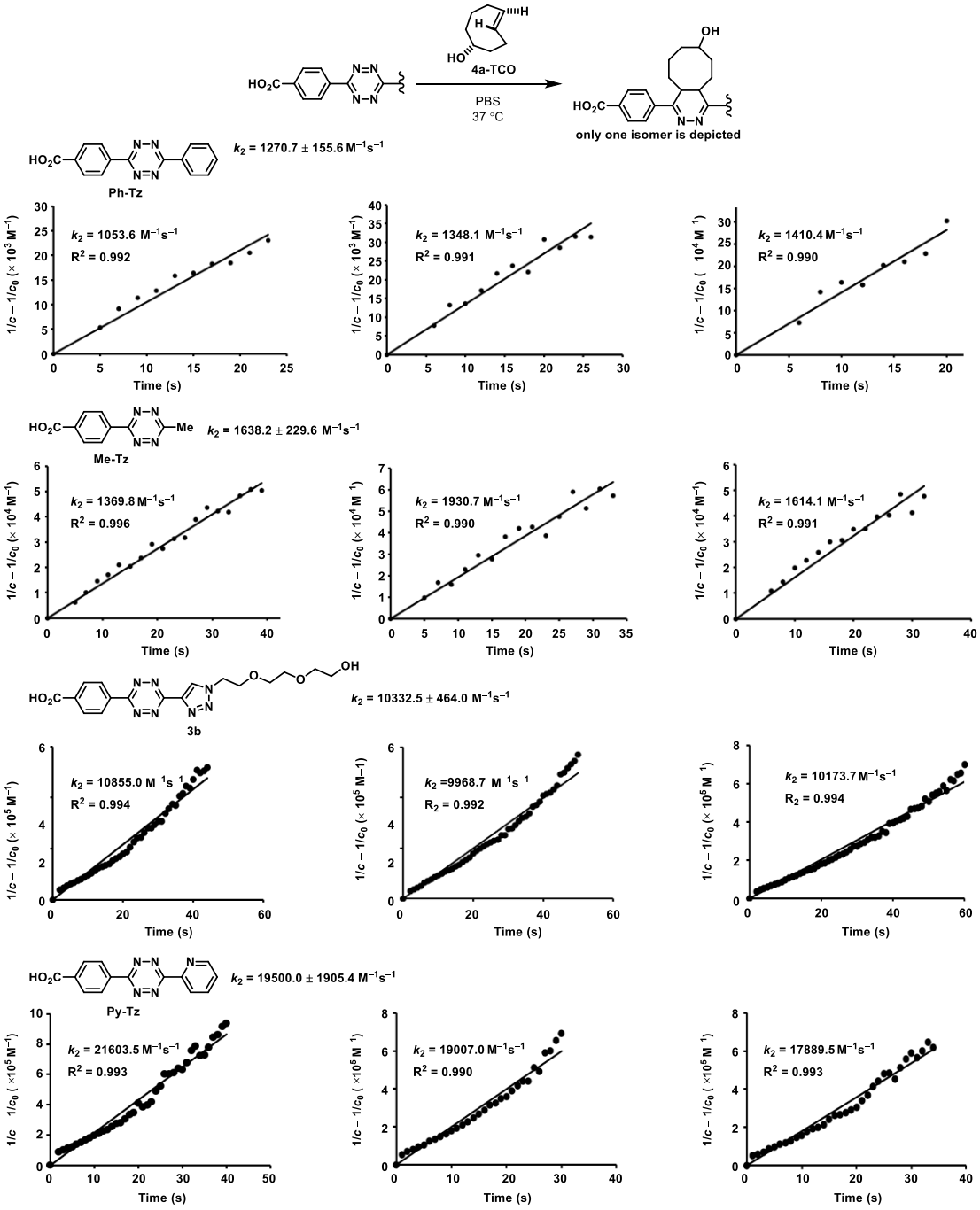


Figure S14. Kinetics plots of the reaction between tetrazines and 4a-TCO in PBS at 37 °C.

Solutions (5 mL) of tetrazine **3b** (80 μM) was prepared in PBS. Solutions (5 mL) of *d*-TCO (1.0 mM) were prepared in PBS. The reaction between tetrazine and *d*-TCO was measured under stopped-flow spectrophotometer (Applied Photophysics Ltd., UK). Tetrazine and *d*-TCO were injected in equal volumes via 5 mL syringes into the stopped-flow instrument at 23 °C, resulting in final concentration of 0.04 mM of tetrazines and 0.50 mM *d*-TCO. The reaction was monitored by the decay of absorbance associated with the tetrazine at 310 nm. Reaction were repeated in triplicate. With Prism software, an observed rate constant (k_{obs} = 19.703 ± 0.081 s^{-1}) was obtained by nonlinear regression. The mean second-order rate constant k_2 was calculated as 39406 ± 162 M^{-1}s^{-1}.

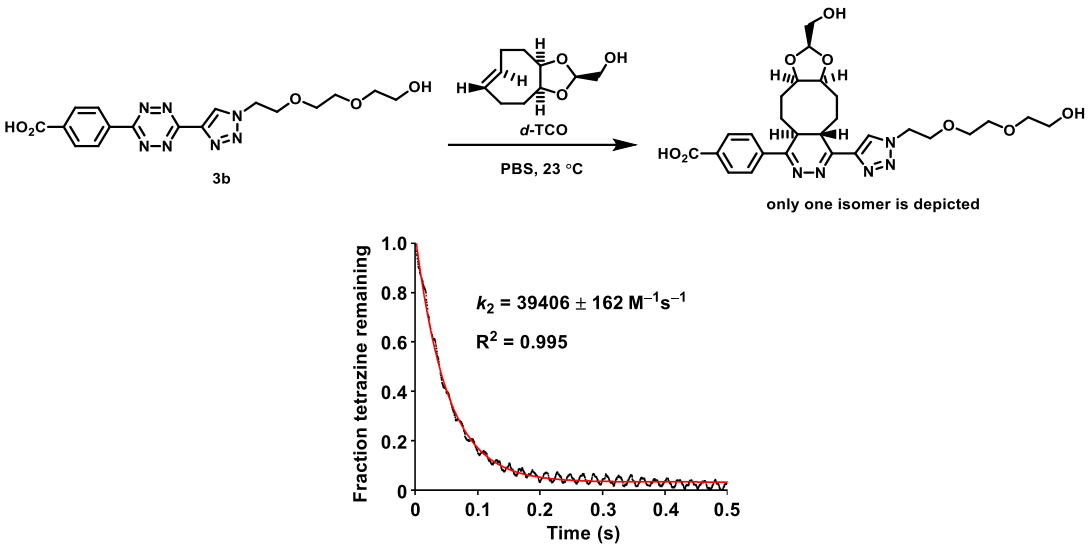


Figure S15. Kinetics plots of the reaction between **3b** (80 μM) and *d*-TCO (1.0 mM) in PBS at 23 °C. The k_2 was determined at 39406 ± 162 M^{-1} s^{-1}.

5. Protein dual-labeling using triazolyl-tetrazine bioorthogonal probe

Stock solutions of Tetrazine-BODIPY (**3o**) (5 mM in DMSO), bovine serum albumin (BSA) (0.4 mM in PBS, pH 7.4) and **4e-TCO-Cy5** (10 mM in DMSO) were prepared. Tetrazine-BODIPY (**3o**) (10 μL) was added to the solution of BSA (10 μL) in PBS (130 μL, pH 7.4) and DMSO (50 μL). The mixture was allowed to sit at 4 °C for 12 h. Two Zeba spin desalting columns (7kDa, 0.5 m, Thermo Scientific) was centrifuged at 1000 g for 1 min. Then, 200 μL of PBS (pH 7.4) was added followed by centrifugation at 1000 g for 1 min and the process was repeated. Protein reaction solution (100 μL) and PBS (30 μL) were added to a Zeba spin desalting column followed by centrifugation at 1500 g for 2 min to remove the excess **3o**. **4e-TCO-Cy5** (0.5 μL) was then added to 50 μL of the solution and the mixture was set at 37 °C for 1 h. Then, the reaction solution and PBS (30 μL) were added to another Zeba spin desalting column followed by centrifugation at 1500 g for 2 min to remove the excess **4e-TCO-Cy5**. Finally, the protein content was quantified by ultraviolet spectrophotometer and the product was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE).

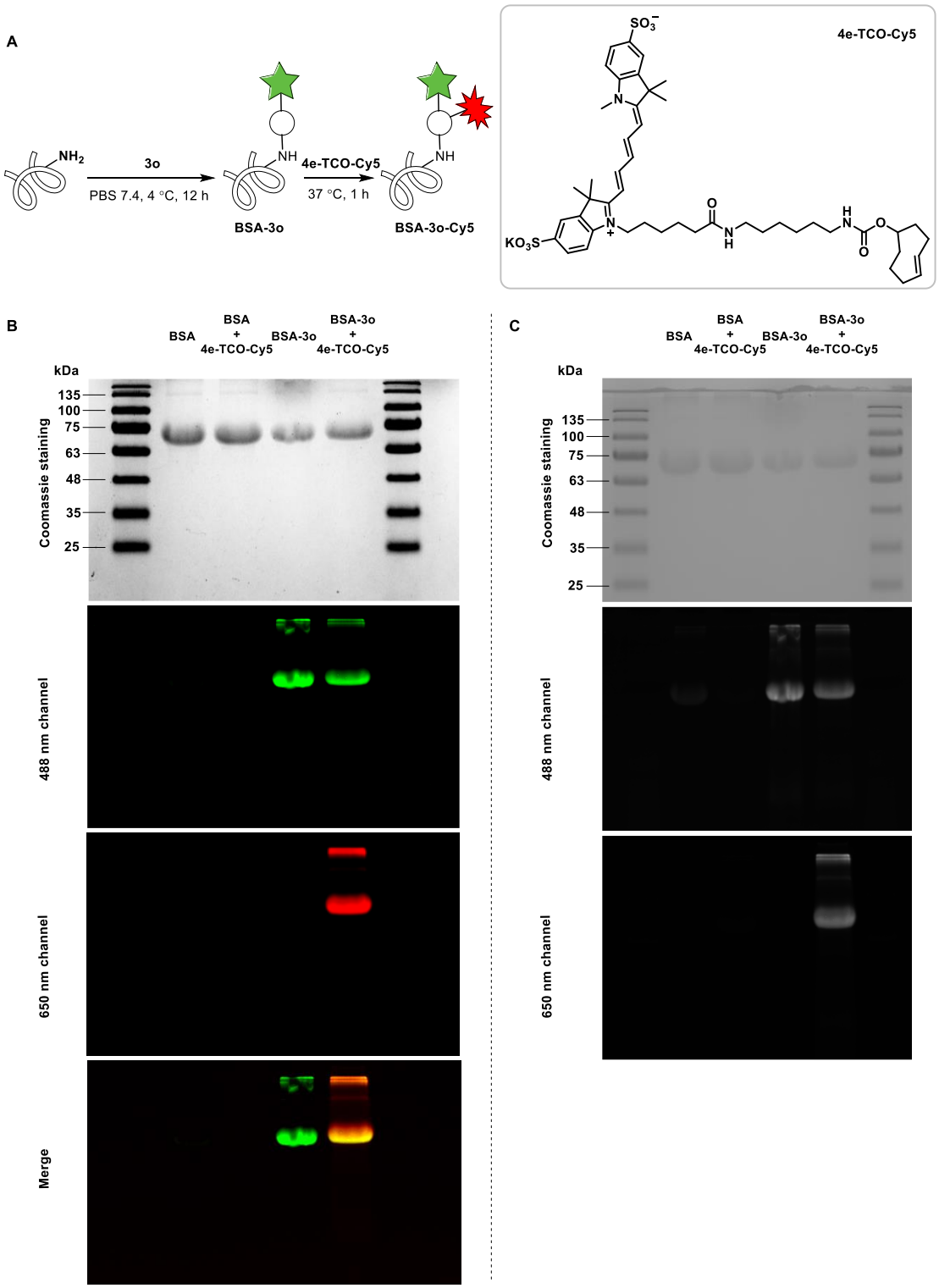


Figure S16. Protein dual-labeling using triazolyl-tetrazine bioorthogonal probe. **A**) Schematic diagram of labeling protein. **B**) Coomassie staining and fluorescence imaging of SDS-PAGE gels of the BSA conjugate at 488 nm and at 650 nm. Gel analysis of BSA (40.0 µM, 7.0 µL), **BSA + 4e-TCO-Cy5** (21.8 µM, 12.8 µL), **BSA-3o** (32.4 µM, 8.6 µL) or **BSA-3o + 4e-TCO-Cy5** (14.5 µM, 19.3 µL). **C**) Original western blot.

6. Live cell imaging

Time course and colocalization imaging of mitochondria with 3p in live SKOV3 cells

SKOV3 cells were initially seeded in 35-mm glass-bottom dishes and cultured for 48 hours. Subsequently, the cells were exposed to ***d*-TCO-TPP** (10 μM) in the culture medium for 90 minutes. Following three PBS washes, the cells were then exposed to probe **3p** (0.1 μM) in the culture medium. Cells were continuously imaged using a confocal laser scanning microscope (Zeiss 880) for 5 minutes.

For the colocalization experiment, the cells were stained with Mito-Tracker Red CMXRos (100 nM) for 15 minutes, subjected to three PBS washes, and treated with Hoechst 33342 (5 μM) in the culture medium for 5 minutes. After another three PBS washes, the cells were incubated in 1X Cell Imaging Solution (Invitrogen) and subsequently imaged using confocal microscopy (Zeiss 880). Cells that were not pre-incubated with ***d*-TCO-TPP** served as the control group.

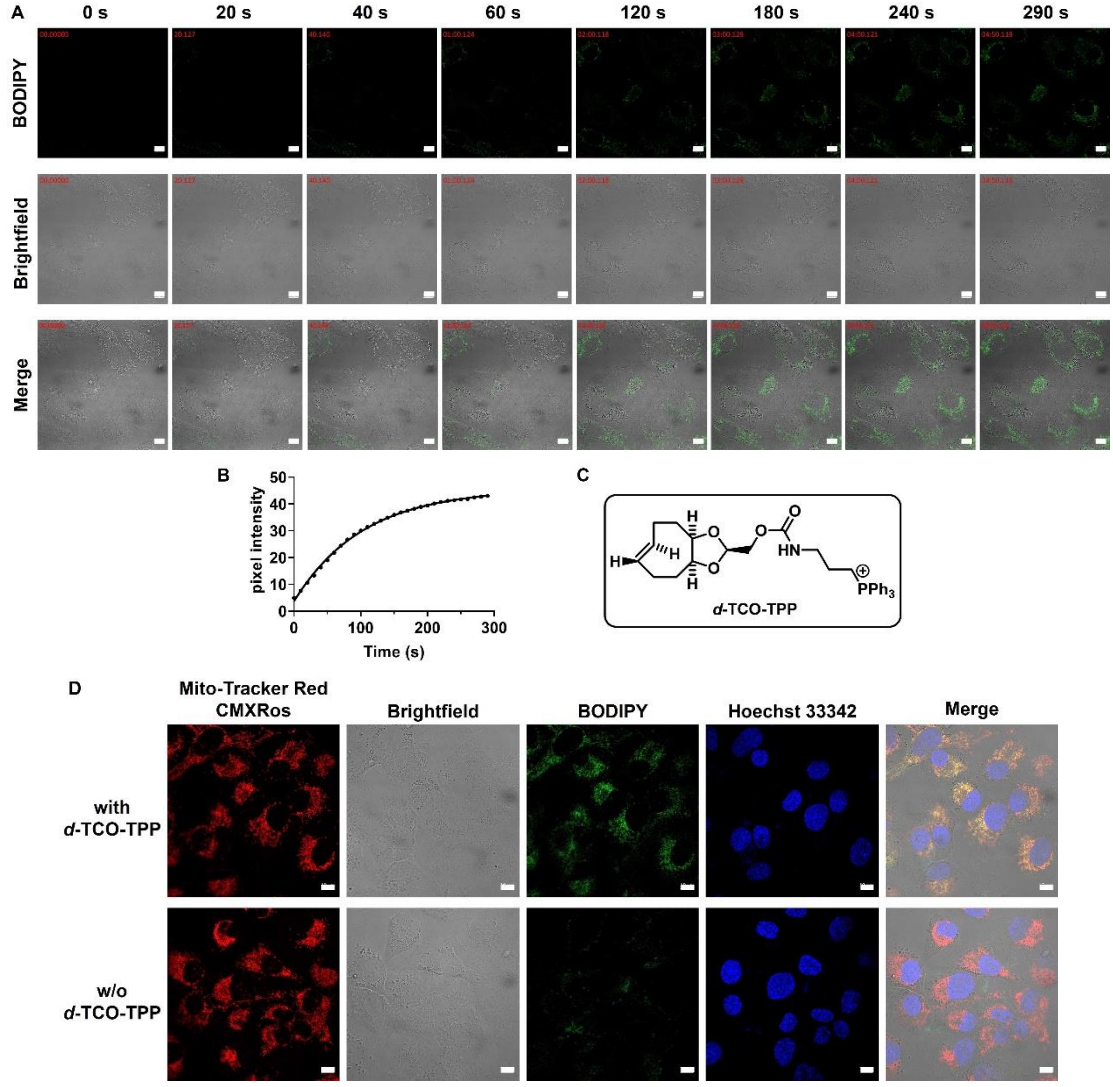


Figure S17. Pre-targeted live cell images of probe **3p**. **A**) Time course imaging of mitochondria with **3p** in live SKOV3 cells. SKOV3 cells incubated with ***d*-TCO-TPP** (10 μM) and probe **3p** (0.1 μM). Fluorescence signals were collected at 483−567 nm with excitation at 488 nm. Scale bar, 10 μm. **B**) Plots of pixel intensity versus time for live cell imaging of mitochondria with **3p**. **C**) Chemical structure of ***d*-TCO-TPP**. **D**) SKOV3 cells incubated with (top) or (bottom) without ***d*-TCO-TPP** (10 μM), probe **3p** (0.1 μM), Mito-Tracker Red (100 nM), and Hoechst 33342 (5 μM). Fluorescence signals were collected at 483−567 nm with excitation at 488 nm for probe **3p**. Scale bar, 10 μm. Pixel intensity comparison for live cell imaging was performed using the built-in tool of ImageJ software.

Comparative analysis of pre-targeted imaging efficiency of tetrazine probes following incubation in FBS

SKOV3 cells were seeded in 35-mm glass-bottom dishes and cultured for 72 h. Prior to cellular treatment, tetrazine-BODIPY derivatives **3p**, **Me-Tz-BODIPY**, and **H-Tz-BODIPY** (each at a concentration of 500 μM), were incubated in FBS to prepare stock solutions. These solutions were maintained at 37 °C for 15 hours. The cells were pre-treated with ***d*-TCO-TPP** (10 μM) in culture medium for 90 min. Following this, the cells underwent three PBS washes before being exposed to the FBS-incubated tetrazine derivatives (**3p**, **Me-Tz-BODIPY**, or **H-Tz-BODIPY**) at a concentration of 100 nM each, in the culture medium for 10 minutes. After another series of three PBS washes, the cells were stained with Mito-Tracker red (100 nM) for 5 min, followed by treatment with Hoechst 33342 (5 μM) in fresh culture medium, after which the cells were imaged using a Zeiss 880 confocal microscope.

Pixel intensity comparisons for live cell imaging were conducted using the built-in tool of the ImageJ software, based on a sample size of 30 cells with 4 images obtained from each experimental iteration. Statistical significance among the three groups was evaluated using an Ordinary one-way ANOVA, where ****P<0.0001 indicated highly significant differences.

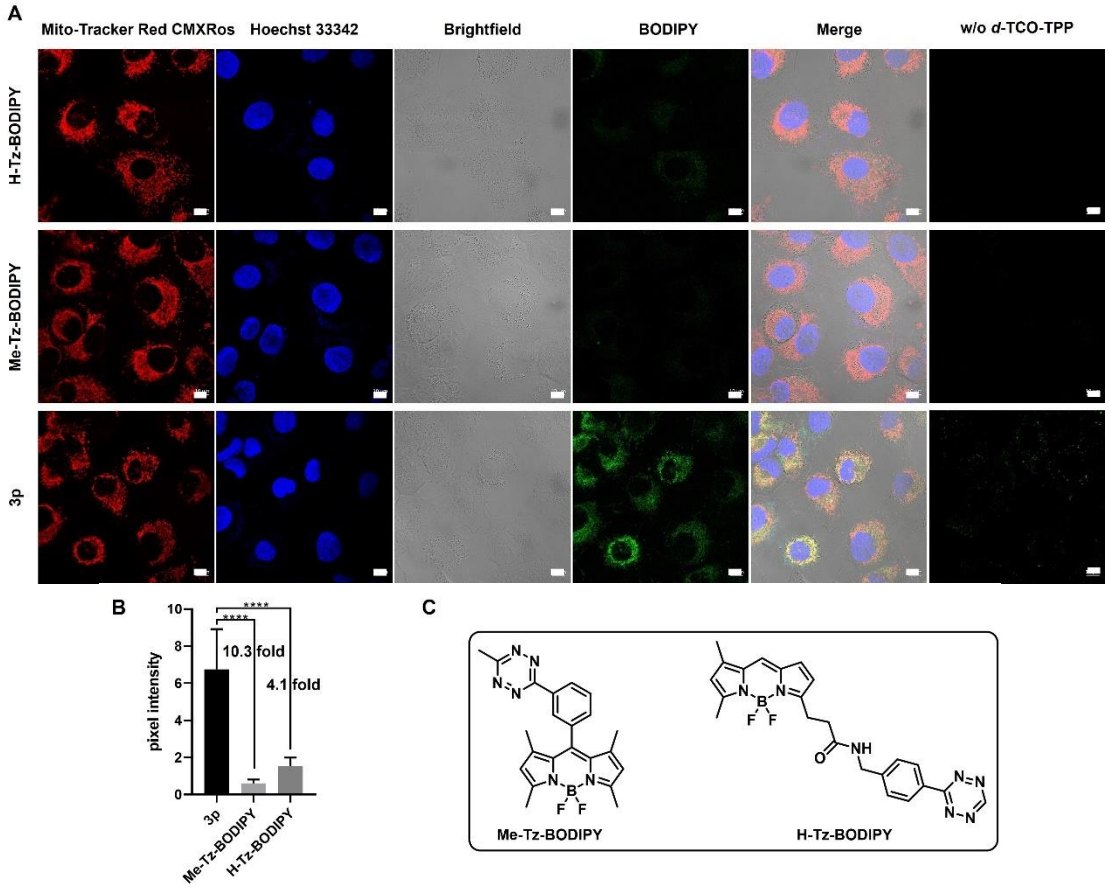


Figure S18. Pre-targeted live cell images by tetrazine probes following incubation in FBS. **A**) SKOV3 cells were incubated with ***d*-TCO-TPP** (10 μM), probes (100 nM), Mito-Tracker Red (100 nM), and Hoechst 33342 (5 μM). Fluorescence signals were captured in the range of 483−567 nm with excitation at 488 nm for probes. Scale bar, 10 μm. **B**) Pixel intensity comparison for live cell imaging was performed using the built-in tool of ImageJ software, analyzing 30 cells (with 4 images from each experiment). Significance differences among the three groups were assesses using Ordinary one-way ANOVA, ****P<0.0001. **C**) Chemical structure of **Me-Tz-BODIPY** and **H-Tz-BODIPY**.

7. Radiosynthesis of ^{18}F-labeled triazolyl-tetrazines

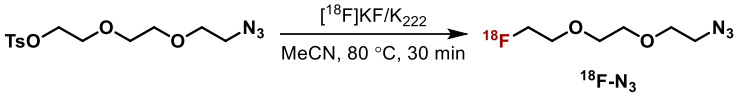
7.1 General information of radiosynthesis

The synthesized ^{19}F standard was used to confirm the identity of the ^{18}F-labeled compound *via* HPLC. An aliquot of the reaction mixture was collected for analysis by radio HPLC on an Agilent ZORBAX SB-C18 column (5 μm, 4.6×250 mm) using H_2O/MeCN mixture as eluent. HPLC gradient A was used for **^{18}F-N$_3$** and**^{18}F-3d**, HPLC gradient B was used for**^{18}F-3e**, HPLC gradient C was used for **^{18}F-3f**. The radiochemical conversion (RCC) refers to HPLC yields and was calculated by crude radio-HPLC traces of the reaction mixture.

The ^{18}F-labeled compounds were purified on semi-preparative HPLC system equipped with an Agilent ZORBAX SB-C18 column (5 μm, 9.4×250 mm) column using H_2O/MeCN mixture as eluent. HPLC gradient D was used for **^{18}F-N$_3$**, **^{18}F-3d** and **^{18}F-3e**, HPLC gradient E was used for **^{18}F-3f**. All radiochemical yields (RCY) are decay-corrected and refer to isolated yields. The radiochemical purity (RCP) of isolated ^{18}F-labeled compound was measured *via* radio HPLC. Molar activity (the measured radioactivity per mole of compound) was calculated using a standard curve of the corresponding ^{19}F standard. The ^{19}F standard curve was created from the UV HPLC trace of standard solution. An aliquot of purified ^{18}F-labeled compound was collected for analysis by radio HPLC; the UV area overlapping with radio peak was then recorded. The standard curve was used to calculate the mass and mole number. Dividing the product activity by the mole number gives the molar activity in Ci/mmol. The total synthesis time refers to the time from the beginning of the reaction between ethynyl-tetrazine **2** and **^{18}F-N$_3$** to obtaining the isolated target ^{18}F-labeled product **^{18}F-3**.

Analytical HPLC gradient A: 0–9 min, linear increase from 30% to 100% MeCN; 9–12 min, isocratic at 100% MeCN; 12–13 min, linear decrease from 100% to 30% MeCN.
Analytical HPLC gradient B: 0–9 min, linear increase from 20% to 100% MeCN; 9–12 min, isocratic at 100% MeCN; 12–13 min, linear decrease from 100% to 20% MeCN.
Analytical HPLC gradient C: 0–9 min, linear increase from 60% to 100% MeCN; 9–12 min, isocratic at 100% MeCN; 12–13 min, linear decrease from 100% to 60% MeCN.
Analytical HPLC gradient D: isocratic at 30% MeCN.
Analytical HPLC gradient E: isocratic at 40% MeCN.

7.2 General procedure for preparation of ^{18}F-labeled triazolyl-tetrazines



^{18}F-N$_3$

The compound **^{18}F-N$_3$** was synthesized through the literature.[1,12] [^{18}F]fluoride was produced in a 10 MeV cyclotron (Sumitomo Heavy Industries, Japan) and trapped in a pre-activated QMA cartidge (Waters, USA) before use. The radioactivity was eluted with 1.0 mL K_{222}/K_2CO_3 solution (33 mg K_2CO_3, 390 mg K_{222}, 24 mL MeCN, 6 mL water) into a V-vial containing a magnetic stirring bar. The solvent was evaporated by azeotropic drying at 100 °C under N_2 atmosphere for 5 min. The evaporation process was repeated three times with the addition of 1.0 mL of dry acetonitrile each time. To the dried residue containing [^{18}F]KF, precursor azide (6.6 mg, 20 μmol) in dry MeCN (400 μL) was added. The mixture was heated at 80 °C for 30 min. Diluted with MeCN/H_2O (1:1, v/v; 2 mL), the crude product was purified on semi-preparative HPLC system equipped with an Agilent ZORBAX SB-C18 column (5 μm, 9.4×250 mm) column using H_2O/MeCN mixture (70/30 v/v) as eluent (5 mL/min). The retention time for **^{18}F-N$_3$** was about 7 min and the HPLC fraction was collected, diluted, and trapped on a Sep-Pak C18 plus light cartridge (Wasters, USA), which was eluted with 0.5 mL of EtOH. An aliquot was collected for analysis by radio HPLC on an Agilent ZORBAX SB-C18 column (5 μm, 4.6×250 mm) using

Gradient A. For all gradients, the mobile phase was H_2O/MeCN flowing at 1 mL/min. The solvent was removed by heating and a stream of nitrogen to afford **^{18}F-N$_3$** (30 mCi, RCY of 60%, RCP of >99%) for the next step.

The following click reactions were performed as follows: A mixture of ethynyl-tetrazine **4** (0.02 mmol), sodium ascorbate (0.79 mg, 0.004 mmol), THPTA (0.87 mg, 0.002 mmol) and $CuSO_4$ (0.32 mg, 0.002 mmol) in 0.4 mL of DMF/H_2O (4:1, v/v) was added to the isolated **^{18}F-N$_3$** *via* a syringe. The sealed vial was then reacted at 35 °C for 30 min. Then, the mixture was diluted with MeCN:H_2O (1:1, v/v; 1.5 mL) and purified by semi-preparative HPLC using water/MeCN mixture (70:30 or 60:40, v/v) as eluent. The collected HPLC fraction was trapped on a Sep-Pak C18 plus light cartridge, which was eluted with EtOH. An aliquot was collected for calculating radiochemical conversion (RCC) by radio-HPLC with an Agilent ZORBAX SB-C18 column (5 μm, 4.6×250 mm). For all gradients, the mobile phase was H_2O/MeCN flowing at 1 mL/min.

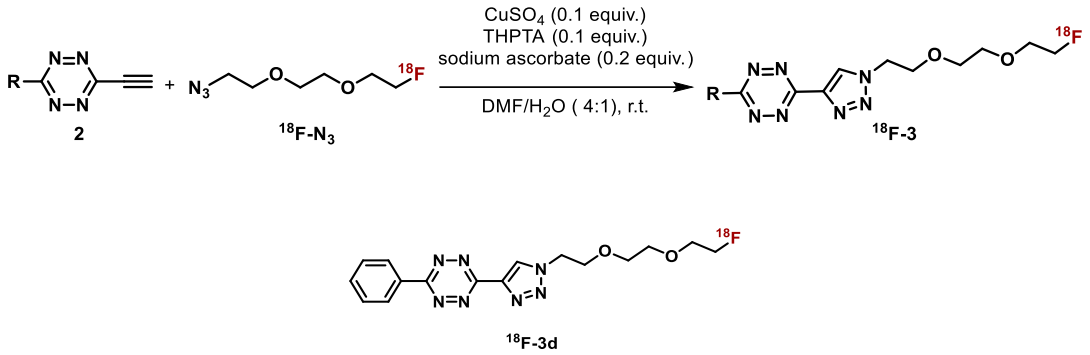


Table S4. Radiosynthesis of **^{18}F-3d**.

Entry	Activity (**^{18}F-N$_3$**)	Radioactivity collected	Decay-corrected radioactivity	Total synthesis time	RCC (HPLC yield)	RCY (Isolated yield)
1	4.1 mCi	2.4 mCi	3.59 mCi	64 min	95%	88%
2	2.0 mCi	1.0 mCi	1.77 mCi	90 min	86%	88%
3	1.1 mCi	0.65 mCi	0.96 mCi	62 min	92%	87%
4	4.4 mCi	2.5 mCi	3.3 mCi	46 min	82%	76%
5	2.9 mCi	1.6 mCi	2.3 mCi	58 min	87%	80%

Average RCY of **^{18}F-3d** from **^{18}F-N$_3$**: 84 ± 5% (the number of replicates, n = 5). Total synthesis time: 64 ± 16 min (the number of replicates, n = 5). RCP: >99%.

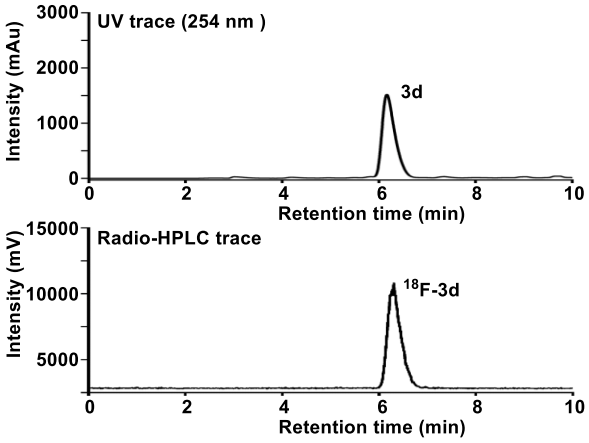
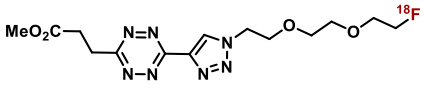


Figure S19. Radio-HPLC trace chromatogram of **^{18}F-3d** after semi-preparative HPLC purification with identical retention time of reference **3d**.



^{18}F-3e

Table S5. Radiosynthesis of **^{18}F-3e**.

Entry	Activity (^{18}F-N_3)	Radioactivity collected	Decay-corrected radioactivity	Total synthesis time	RCC (HPLC yield)	RCY (Isolated yield)
1	7.2 mCi	3.9 mCi	5.2 mCi	46 min	91%	72%
2.	600 µCi	380 µCi	518 µCi	49 min	93%	86%
3	1.5 mCi	716 µCi	1.1 mCi	68 min	95%	76%

Average RCY**^{18}F-3e** from **^{18}F-N_3**: 78 ± 6% (the number of replicates, n = 3). Total synthesis time: 54 ± 12 min (the number of replicates, n = 3). RCP: >99%.

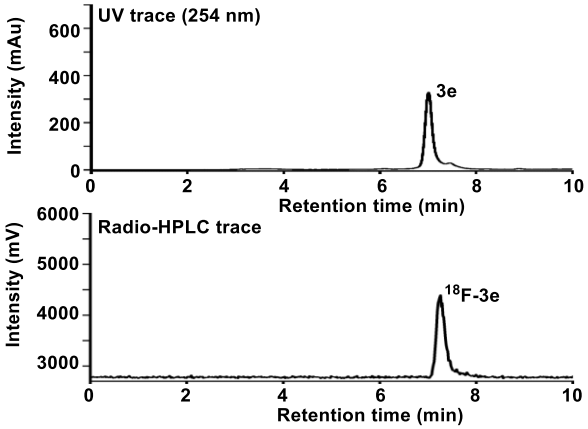
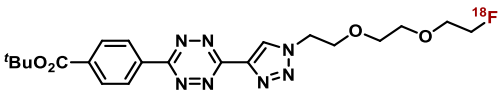


Figure 20. Radio-HPLC trace chromatogram of **^{18}F-3e** after semi-preparative HPLC purification with identical retention time of reference **3e**.



^{18}F-3f

Table S6. Radiosynthesis of **^{18}F-3f**.

Entry	Activity (^{18}F-N_3)	Radioactivity collected	Decay-corrected radioactivity	Total synthesis time	RCC (HPLC yield)	RCY (Isolated yield)
1	7.2 mCi	2.7 mCi	4.1 mCi	67 min	63%	57%
2	1.1 mCi	537 µCi	809 µCi	65 min	91%	74%
3	1.4 mCi	510 µCi	861 µCi	83 min	92%	62%

Average RCY**^{18}F-3f** from **^{18}F-N_3**: 64 ± 9% (the number of replicates, n = 3) Total synthesis time: 72 ± 10 min (the number of replicates, n = 3). RCP: >99%. Molar activity: 20.35 GBq/µmol.

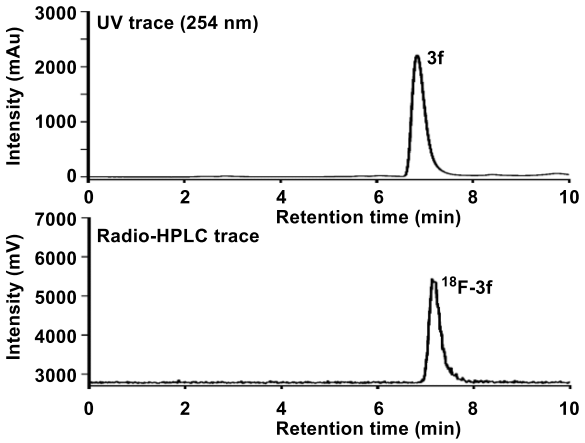


Figure 21. Radio-HPLC trace chromatogram of **^{18}F-3f** after semi-preparative HPLC purification with identical retention time of reference **3f**.

7.3 In vitro stability of ^{18}F-3f

A solution of **^{18}F-3f** in ethanol (0.3 mCi, 50 µL) was added to a solution of DMEM (500 µL) supplemented with 10% FBS and incubated at 25 °C for 120 min. An aliquot (100 µL) was treated with MeCN (200 µL) to precipitate serum proteins, centrifugated at 5000 g for 15 min, and the supernatant was collected for analysis by radio HPLC. Another aliquot (100 µL) was added a solution of ***d*-TCO** in DMSO (10 mM, 0.1 µL), maintained at 25 °C for 1 min, treated with MeCN (200 µL) to precipitate serum proteins, centrifugated at 5000 g for 15 min, and the supernatant was collected for analysis by radio HPLC. Radio HPLC analysis revealed that ^{18}F-3f and the IEDDA pruduct between **^{18}F-3f** and ***d*-TCO** were obtained in >99% radiochemical purity.

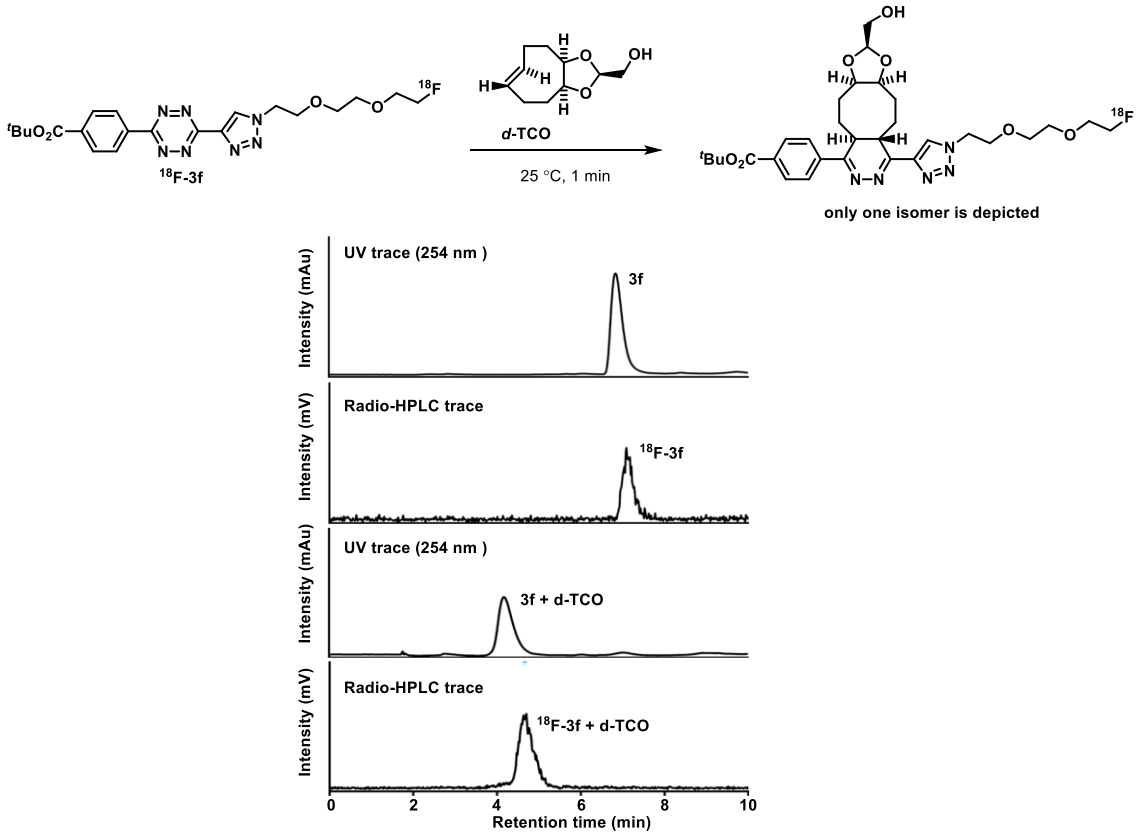


Figure S22. *In vitro* stability of **^{18}F-3f** in DMEM (10% FBS). Compound **^{18}F-3f** was incubated in DMEM (10% FBS) at 25 °C for 120 min. An aliquot was collected for analysis by radio HPLC and another aliquot was treated with *d*-TCO and then analyzed by radio HPLC.

7.4 In vivo PET imaging

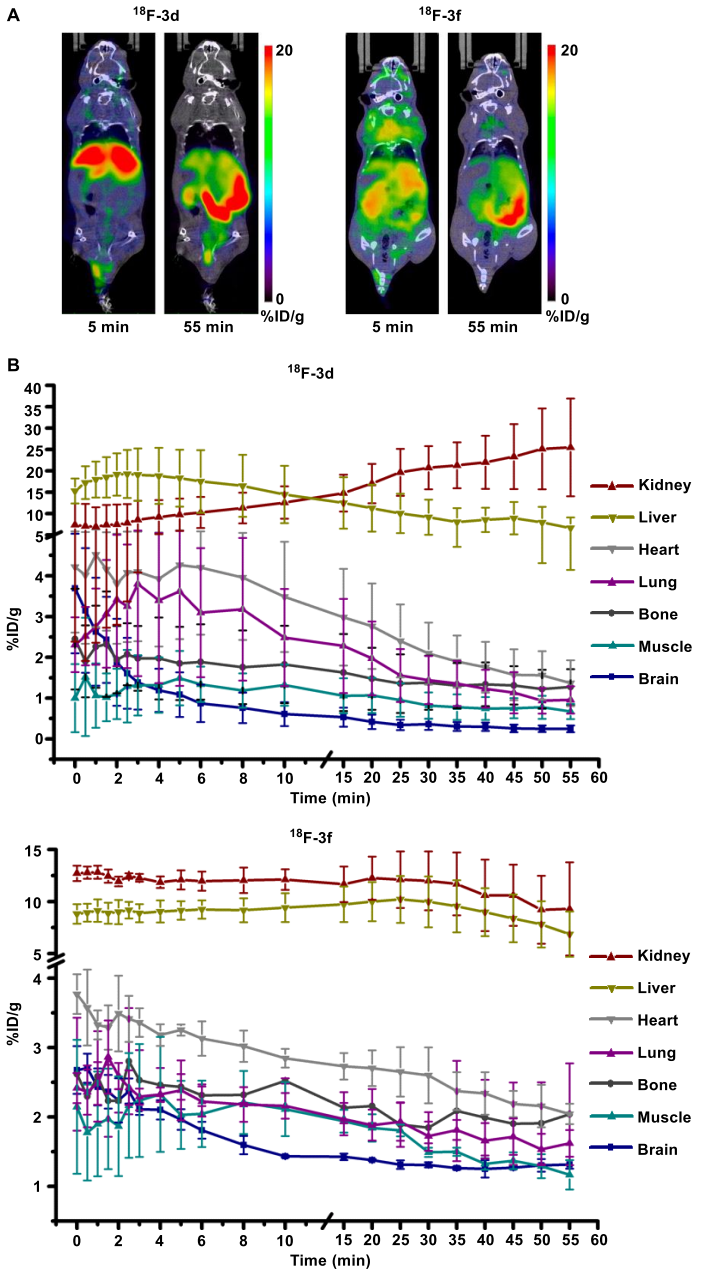


Figure S23. *In vivo* PET imaging of **^{18}F-3d** and **^{18}F-3f**. **A**) PET imagings of **^{18}F-3d** and **^{18}F-3f** in male SPFICR mice at 5 min and 55 min after injection. **B**) Mean organ time–activity curves obtained in ICR mice (n = 3).

Male ICR (Institute of Cancer Research) mice (6-8 weeks old, 27.8 ± 1.2 g) were purchased from Chengdu DOSSY experimental animals Co., LTD (Chengdu, China). All animal experiments were approved by the Committee for Animal Care and Use and the Ethics Committee of West China Hospital, Sichuan University (20221229001). An Micro-PET/CT scanner (Inviscan, France) was used to perform micro-PET dynamic imaging of male ICR mice. Animals were anesthetized under 2.0% isoflurane and randomly divided into two groups. In the first group (n = 3), ICR mice were intravenously injected with 103.2 μCi, 67.6 μCi and 108.1 μCi of **^{18}F-3d** in 125 μL of saline (10% EtOH), respectively. In the second group (n = 3), ICR mice were intravenously injected with 91.9 μCi, 73.0 μCi and 52.7 μCi of **^{18}F-3f** in 125 μL of saline (10% EtOH), respectively. Mice were placed in a prone position in a small animal PET scanner. The dynamic micro-PET scans were then performed for 55 min in tabular mode. All required PET data were reconstructed with a 3D-OSEM algorithm using a a Monte-Carlo

based model, and then handle by osirix software (USA). The regions of interest of major organs were directly drawn in fused dynamic PET/CT images for brain, kidney, bone, muscle, heart, lung and liver. The percentage-injected dose per gram (%ID/g) was calculated and the results were reported as mean ± SD averaged over n (n = 3) mice. The representive PET images were obtained at 5 min and 55 min and presented in Fig. S23A, respectively. Activities in major organs were also directly obtained from dynamic PET images, and decay-corrected and presented as %ID/g in time-activity curves showed in Fig. S23B.

8. Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was performed on a TA instrument DSC 25 modulated differential scanning calorimeter. DSC data were obtained with the software TA Instruments Trios V4.3.0.38388. Samples were loaded into a closed Al container with a gradient of 30 °C to 400 °C at a heating rate of 10 °C/min^{-1}. The exothermic decomposition events for both compounds persisted for a duration exceeding 5 minutes.

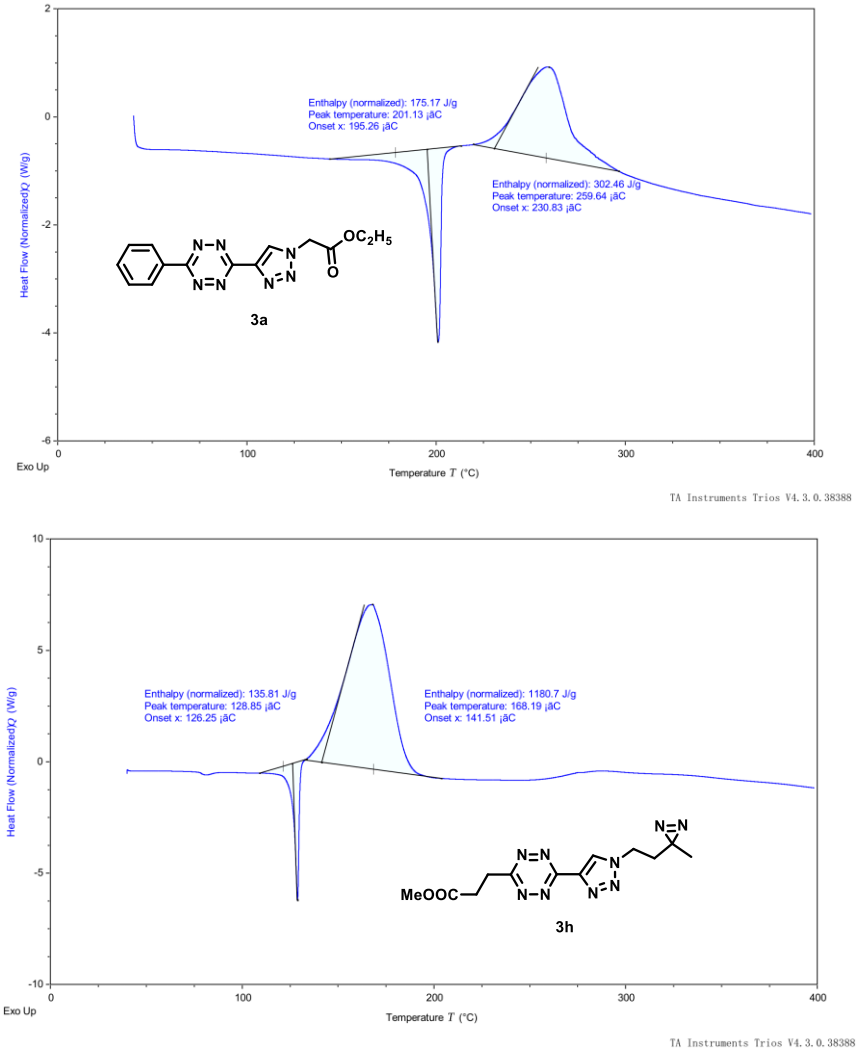
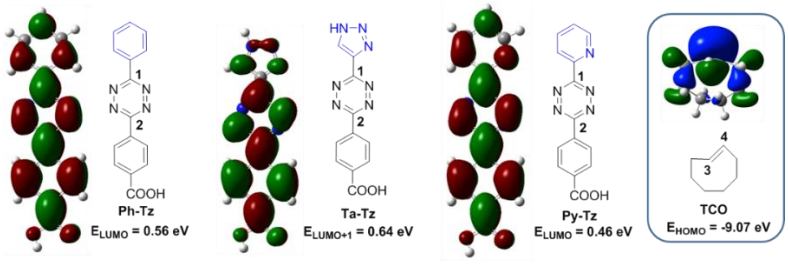


Figure S24 DSC result of **3a** and **3h**

9. Computational analysis

9.1 Computational methods

Theoretical calculations were performed using Gaussian 09 program suite.[13] Geometry optimizations of minima and transition structures, and frequency analyses were carried out at M06-2X level of theory[14] with the 6-31G(d) basis set. A quasi-harmonic correction to entropy was applied by setting all frequencies which are below 100 cm^{-1} to 100 cm^{-1}.[15,16] The single-point energies and solvent effects in water were evaluated at M06-2X/6-311+G(d,p) level with the CPCM model.[17] The computed activation free energy was further corrected by using the equation [$\Delta G^{\neq}_{corr} = (\Delta G^{\neq}_{compt} + 8.4)/1.6$].[18] The frontier molecular orbitals (FMOs) and their energies were computed at the HF/6-311+G(d,p)//M06-2X/6-31G(d) level of theory.[19] Distortion/interaction analyses were conducted according to the reference.[17]



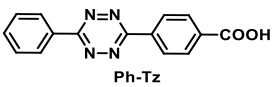
	$\Phi^{C1}_{2P_z}$	$\Phi^{C2}_{2P_z}$
Ph-Tz	0.10024	0.09589
Ta-Tz	0.09729	0.09258
Py-Tz	0.09848	0.09859
	$\Phi^{C3}_{2P_z}$	$\Phi^{C4}_{2P_z}$
TCO	0.14969	0.14969

Figure S25. The orbital coefficients for the reactive atoms on all structures Ph-Tz, Ta-Tz, Py-Tz, and TCO

Tabel S7. The original and empirical corrected activation free energies ($\Delta G^{\neq}$, kcal mol^{-1})

	Calculated $\Delta G^{\neq}$ (kcal mol^{-1})	Empirically corrected $\Delta G^{\neq}$(kcal mol^{-1})
Ph-Tz	15.6	15.0
Ta-Tz	14.9	14.6
Py-Tz	13.8	13.9

9.2 Coordinates and energies of stationary points

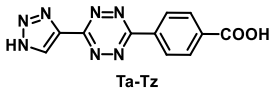


Ph-Tz

G(water) = -946.725282 Hartree

C	-0.130229	0.011920	-0.000091
C	2.442603	0.000882	-0.000049
C	-1.608535	0.018035	-0.000059
C	-2.311743	-1.191553	-0.000144
C	-3.690157	1.234334	0.000095
C	-3.699706	-1.187121	-0.000126
H	-1.761110	-2.125256	-0.000231
C	-4.389466	0.026582	-0.000004
H	-4.252094	2.162487	0.000201
H	-4.252534	-2.119855	-0.000223
C	3.918342.	0.005434	-0.000003
C	4.624201	1.202521	0.000018
C	6.013573	1.192051	0.000057
H	4.075232	2.137550	0.000002
C	6.002940	-1.221303	0.000054
C	6.704871	-0.017679	0.000075
H	6.558679	2.130579	0.000070
H	6.539751.	-2.164619	0.000068
H	7.790578	-0.022463	0.000105
N	0.498314	-1.173820	-0.000029

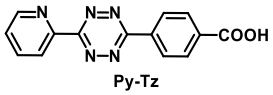
N	1.801651	-1.180097	-0.000012
N	1.811734	1.187341	-0.000050
N	0.508422	1.192335	-0.000070
C	4.613559	-1.219496	0.000017
H	4.056499	-2.149686	-0.000001
C	-2.303407	1.233075	0.000057
H	-1.747096	2.163390	0.000106
C	-5.877415	0.087034	0.000027
O	-6.522311	1.107218	-0.000134
O	-6.451806	-1.131745	0.000278
H	-7.412405	-0.979317	0.000336



Ta-Tz

G(water) = -956.768071 Hartree

C	2.733003	-0.034026	-0.001190
C	0.173239	-0.020239	-0.002425
C	-1.305005	-0.019584	-0.001929
C	-2.004400	1.192005	-0.001839
C	-3.392450	1.191738	-0.000870
H	-1.451892	2.124531	-0.002538
C	-3.390011	-1.229788	-0.000152
C	-4.085695	-0.020140	0.000073
H	-3.943158	2.125850	-0.000463
H	-3.954336	-2.156289	0.000735
N	2.107213	-1.220374	-0.002240
N	0.802543	-1.206343	-0.002848
N	0.808687	1.162729.	-0.002014
N	2.112427	1.156270	-0.001393
C	-2.003430	-1.232966	-0.001194
H	-1.449361	-2.164794	-0.001537
C	4.191405	-0.001209	0.000361
C	5.001048	1.114038	0.001379
N	6.239362	0.591258	0.002838
H	4.785459	2.170190.	0.001254
N	6.215295-	-0.752942.	0.002717
N	4.979456	-1.114615	0.001339
H	7.131741.	1.065643.	0.004008
C	-5.573866	-0.077726	0.001808
O	-6.146224	1.141990	0.002124
H	-7.107101	0.991162	0.003566
O	-6.219933	-1.097089	0.003134



Py-Tz

G(water) = -962.776213 Hartree

C	-2.447341	-0.022475	-0.000027
C	0.118347	-0.015672	-0.000075
C	-3.935167	-0.001285	-0.000002
C	-4.614036	1.219935	0.000146
C	-5.879186	-1.189070	-0.000126
C	-6.003654	1.197645	0.000159
H	-4.056019	2.148344	0.000238
C	-6.654941	-0.029727	0.000026
H	-6.352109	-2.168591	-0.000255
H	-6.566643	2.1257 34	0.000270
H	-7.737599	-0.095632	0.000030
C	1.596619	-0.018819	-0.000046
C	2.292510	-1.233290	0.000097
C	3.679317	-1.232706	0.000125
H	1.736743	-2.163898	0.000172
C	3.685927	1.189111	-0.000150
C	4.376872	-0.024010	-0.000003
H	4.242379	-2.160154	0.000245
H	4.237893	2.122343	-0.000269
N	-1.818461	1.160418	-0.000025
N	-0.513176	1.166864	-0.000036
N	-0.515353	-1.200497	0.000031
N	-1.819079	-1.209389	0.000057
N	-4.549992.	-1.186681	-0.000139
C	2.297981	1.191823	-0.000164
H	1.746073	2.124803	-0.000271
C	5.865097	-0.082788	0.000018
O	6.510963	-1.102258	-0.000024
O	6.438129.	1.1 36616	0.000101
H	7.398893	0.985157	0.000141

TCO

G(water) = -312.998444 Hartree

C	-0.413966	-0.522065	-1.358397
H	-1.490184	-0.336794.	-1.334889
C	0.413966	0.522065	-1.358397
H	1.490184	0.336794	-1.334889
C	0.034981	-1.871510	-0.901221
H	-0.510280	-2.704358	-1.358222
H	1.102540	-2.008137	-1.112700
C	-0.034981	1.871510	-0.901221
H	-1.102540	2.008137	-1.112700
H	0.510280	2.704358	-1.358222
C	0.183001	1.877834	0.635533
H	1.260794	1.924503	0.841500
H	-0.248046	2.798155	1.04827 8

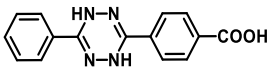
C	-0.413966	0.660993	1.3761 89
H	-0.560669	0.965017	2.418884
H	-1.420918	0.465721	0.984525
C	-0.183001	1.877834	0.635533
H	0.248046	-2.798155	1.048278
H	-1.260794	-1.924503	0.841500
C	0.413966	-0.660993	1.376189
H	0.560669	-0.965017	2.418884
H	1.420918	-0.465721	0.984525

MeSH
G(water) = -438.656116 Hartree

C	1.155518	0.018802	0.000589
H	1.523763	-1.005280	-0.073972
H	1.529379	0.458386	0.926057
H	1.520677	0.582671	-0.858492
S	-0.663354	-0.086550	0.000106
H	-0.893261	1.236215	0.001169

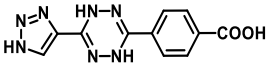
MeSSMe
G(water) = -876.122415Hartree

C	1.802923	0.813826	-0.387497
H	2.799213	0.863745	0.059547
H	1.307224	1.777909-	-0.258681
H	1.891912	0.576977	-1.448222
S	0.907245	-0.506463	0.489207
S	-0.907480	-0.506318	-0.489253
C	-1.802636	0.813993	0.387467
H	-1.309104	1.778579	0.254812
H	-2.800831-	0.861161	-0.055760
H	-1.886370	0.579213	1.449227



Ph-Tz-2H
G(water) = -947.926442Hartree

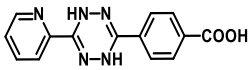
C	-2.450294	-0.055201	-0.510198
C	0.150840	0.134451	-0.682084
C	-3.836425	-0.017508	0.003493
C	-4.335707	1.097448	0.681037
C	-5.969455	-1.118895	0.276397
C	-5.645828	1.103927	1.150506
H	-3.697694	1.954528	0.879939
C	-6.465815	-0.001344	0.946120
H	-6.606211	-1.983077	0.114236
H	-6.02199 1	1.972485	1.681877
H	-7.489009	0.006272	1.308622
C	1.593145	0.048874	0.366274
C	2.173351	-1.141909	0.082456
C	3.530441	-1.192475	0.370121
H	1.564692	-2.027138	0.243913
C	3.744535	1.141071	-0.231260
C	4.318507	-0.053916	0.210353
H	3.996476	-2.105116	0.726992
H	4.364616	2.022655	-0.351351
N	-0.472653	1.239554	-0.524705
C	2.388951	1.189867	-0.519057
H	1.925140	2.105828	-0.868827
C	-4.663137	-1.128425	-0.194106
H	-4.262937	-1.987516	-0.722399
H	-0.024702	-1.857923	-1.093422
N	-0.537067	-0.988454	-1.162727
N	-1.820144	-1.164548	-0.589765
H	-2.312738	1.977102	-0.666836
N	-1.822668	1.130510	-0.923855
C	5.767478	0.164972	0.529579
O	6.435824	0.990613	0.346094
H	7.361295	0.808965	0.584014
O	6.310085	-1.172170	0.914919



Ta-Tz-2H
G(water) = -957.972054Hartree

C	-2.735346	0.062516	0.434404
C	-0.154814	-0.185112	0.604261
C	1.295197	-0.081216	0.331733
C	1.906844	1.152378	0.086212
C	3.270442	1.219024	-0.164802
H	1.319151	2.065388	0.056249
C	3.429637	-1.183810	0.067966
C	4.034540	0.053047	-0.170278
H	3.761168	2.166003	-0.364094
H	4.031315	-2.085814	0.062049
C	2.067311	-1.248859	0.318333
H	1.578788	-2.197965	0.510184
C	-4.094657	0.095413	-0.099584
C	-4.948636	1. 159388	-0.272808
N	-6.053149	0.581884	-0.785794
H	-4.849523	2.214553	-0.077313
N	-5.912528	-0.741444	-0.924967
N	-4.726258	-1.039107	-0.509588
N	-2.106504	1.144666	0.685521
N	-0.790022	-1.243827	0.273883
H	-2.658031	-1.942241	0.195789

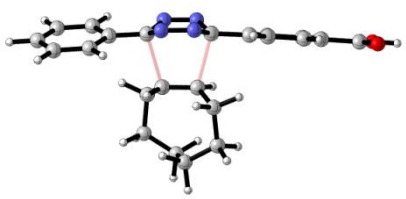
H	-0.306231	1.733742	1.295334
N	-2.148938	-1.189154	0.648224
N	-0.830974	0.871270	1.236592
H	-6.925304	1.01 1394	-1.062067
C	5.492261	0.182291	-0.438290
O	6.060271	1.225263	-0.655645
O	6.137137	-1.000489	-0.415009
H	7.071341	-0.803632	-0.600881



Py-Tz-2H

G(water) = -963.983953Hartree

C	-2.456681	0.039441	0.528236
C	0.129664	-0.193625	0.658271
C	-3.843505	0.065007	0.005899
C	-4.543183	1.268203	-0.109661
C	-5.604302	-1.147206	-0.799722
C	-5.836853	1.224834	-0.607193
H	-4.063770	2.193796	0.187575
C	-6.383521	-0.006676	-0.961364
H	-5.994929	-2.127642	-1.061004
H	-6.413867	2.138106	-0.715479
H	-7.391877	-0.085418	-1.352519
C	1.572991	-0.084589	0.355814
C	2.350017	-1.249047	0.335 106
C	3.706315	-1.180503	0.055269
H	1.869445	-2.198529	0.544432
C	3.532031	1.219624	-0.192480
C	4.300886	0.056808	-0.205517
H	4.311405	-2.080195	0.043577
H	4.014620	2.166963	-0.409271
N	-4.357502	-1.12 1592	-0.327441
C	2.174345	1.149471	0.087826
H	1.582619	2.059981	0.063026
N	-1.825450	1.126277	0.755564
N	-0.507314	-1.258397	0.354346
H	-2.381781	-1.955030	0.311947
H	-0.021651	1.735455	1.326962
N	-1.859045	-1.203309	0.751034
N	-0.542661	0.869588.	1.287222
C	5.751738	0.189932	-0.505900
O	6.401800	-0.990308	-0.489482
H	7.330669	-0.790709	-0.697740
O	6.311108	-1.233459	-0.742279

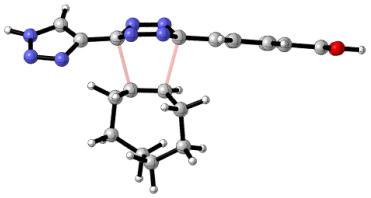


TS-Ph-Tz-TCO

G(water) = -1259.698941Hartree

C	-0.509277	-1.103851	0.019498
C	1.989568	-1.236457	0.041009
C	-1.983980	-0.958012	-0.0106596
C	-2.626015	-0.692572	-1.223853
C	-4.109688	-0.888590	1.128177
C	-4.004201	-0.525157	-1.262213
H	-2.037048	-0.634978	-2.133104
C	-4.747222	-0.619578	-0.083703
H	-4.710843	-0.961109	-2.028736
H	-4.508687	-0.322542	-2.200503
C	3.470901	-1.250504	0.070945
C	4.139771	-1.010450	1.274454
C	5.529328	-1.005869	1.310513
H	3.561353	-0.844352	2.178181
C	5.505804	-1.481431	-1.052268
C	6.260540	-1.240697	0.147822
H	6.042773	-0.821926	2.249117
H	7.161113	-1.671579	-1.959418
H	7.345796	-1.238116	0.177856
N	0.096481	-1.483182	-1.156987
N	1.370397	-1.557598	-1.143924
N	1.345394	-1.532247	1.222840
N	0.070836	-1.471050	1.209604
C	4.205301	-1.485672	-1.095027
H	3.676076	-1.680331	-2.021996
C	1.541476	0.913087	-0.020519
H	2.088892	1.036023	0.915630
C	0.161498	0.991943	0.051095
H	-0.371257	1.161230	-0.886413
C	2.244430	1.408679	-1.251332
H	3.211119	0.918597	-1.405933
H	1.620527	1.208258	-2.131162
C	-0.478538	1.576646	1.277333
H	0.119216	1.313811	2.158795
H	-1.494882	1.202859	1.438295
C	-0.528086	3.111902	1.116795
H	-1.274214	3.363784	0.351601
H	-0.901608	3.533086	2.057360
C	0.807069	3.785909	0.753215
H	0.732313	4.834098	1.061738

H	1.608457	3.358730	1.371305
C	2.469714	2.929370	-1.10413 8
H	2.887360	3.298292	-2.048084
H	3.240082	3.100324	-0.340772
C	1.220565	3.754827	-0.746131
H	1.414269	4.784843	-1.064120
H	0.374303	3.416823	-1.359530
C	-2.732793	-1.058471	1.167273
H	-2.223335	-1.274287	2.100310
C	-6.223657	-0.438290	-0.067031
O	-6.737561	-0.201805	-1.290860
H	-7.696842	-0.100746	-1.166195



TS-Ta-Tz-TCO

G(water) = -1269.742729Hartree

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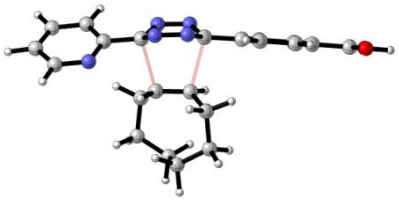
C	0.3676 12.	-1.166988	-0.035061
C	1.831655	-0.953049	0.022841
C	2.442888	-0.682822	1.252050
C	3.809963	-0.458721	1.312597
H	1.837362	-0.665202	2.152321
C	3.971536	-0.774090	-1.081781
C	4.576552	-0.502355	0.146369
H	4.305938	-0.249269	2.254747
H	4.575166	-0.809870	-1.981863
N	-1.459307	-1.599491	-1.285064
N	-0.185369	- 1.505076	-1.244501
N	-0.244431	-1.586665	1.118850
N	-1.519665	-1.692591	1.083947
C	2.601763	-0.997599	-1.144035
H	2.115591	-1.214038	-2.089299
C	-0.399284	0.956788	-0.035411
H	0.117151	1.114714	0.913308
C	1.771294	0.816792	0.008784
H	-2.316359	0.916064	-0.931890
C	0.240098	1.574566	-1.243211
H	1.275993	1.249586	-1.384850
H	-0.328078	1.294250	-2.138519
C	-2.515423	1.249692	1.238909
H	- 1.896300	1.066363	2.125821
H	-3.458989	0.709432	1.370528
C	-2.810621	2.760345	1.107427
H	-3.575836	2.903552	0.333631
H	-3.257491	3.099540	2.049323
C	-1.592716	3.643297	0.777443
H	-1.833819	4.659538	1.106952
H	-0.741580	3.331589	1.397840
C	0.213317	3.110166	-1.066140
H	0.578368	3.559080	-1.997166
H	0.935040	3.390187	-0.287405
C	-1.159722	3.712586	-0.715606
H	-1.133085	4.766721	-1.011344
H	-1.928999	3.252451	-1.350065
C	-3.580214	-1.397211	-0.120245
C	-4.426905	-1.965695	0.80201 8
N	-5.649825	-1.688142	0.305655
H	-4.248512	-2.515301	1.711906
N	-5.579793	-0.989633	-0.834658
N	-4.327682	-0.808944	-1.095488
H	-6.557706	-1.937070	0.672558
C	6.037792	-0.250682	0.267399
O	6.609217	-0.002993	1.301730
O	6.683939	-0.324935	-0.913274
H	7.620649	-0.148518	-0.719977



TS-Py-Tz-TCO

G(water) = -1275.752639Hartree

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C	0.521772	-1.149327	-0.034282
C	-3.456010	-1.253280	-0.122534
C	-4.195651	-1.843815	0.903570
C	-5.339193	-0.604862	-1.233451
C	-5.582667	-1.799652	0.821324
H	-3.681060	-2.320888	1.729277
C	-6.171636	-1.171593	-0.270226
H	-5.762335	-0.096696	-2.097101
H	-6.192602	-2.252408	1.597126
H	-7.249527	-1.113901	-0.376396
C	1.990661	-0.970384	0.015081
C	2.616722	-0.727719	1.242632
C	3.988497	-0.531947	1.295012
H	2.018061	-0.708015	2.147447
C	4.125491	-0.823737	-1.103975
C	4.745127	-0.577478	0.122329

H	4.495383	-0.342876	2.235659	H	-3.241192	0.810767	1. 424344
H	4.721578	-0.861769	-2.008987	C	-2.543428	2.846672	1.175629
N	-1.323891	-1.520744	-1.276070	H	-3.311405	3.016984	0.409746
N	-0.047653	-1.465094	-1.242241	H	-2.974272	3.187278	2.124407
N	-0.093859	-1.562746	1.118739	C	-1.307878	3.703897	0.843270
N	-1.372124	-1.635444	1.086447	H	-1.523051	4.722690	1.183012
N	-4.009836	-0.634725	-1.169193	H	-0.459499	3.368066	1.4547 79
C	2.750992	-1.018666	-1.157980	C	0.471246	3.144691	-1.019062
H	2.253188	-1.214696	-2.101625	H	0.840085	3.594157	-1.948401
C	-0.185056	0.997061	-0.003341	H	1.204803	3.400426	-0.243058
H	0.342289	1.133436	0.942795	C	-0.884565	3.775885	-0.652462
C	-1.559356	0.891036	0.048324	H	-0.835855	4.831677	-0.939649
H	-2.107962	1.005178	-0.888035	H	-1.668948	3.338517	-1.284592
C	0.460798	1.610566	-1.210028	C	6.211683	-0.354775	0.234046
H	1.487891.	1.262973	-1.361632	O	6.796035	-0.127900	1.265955
H	-0.119663	1.351081	-2.103776	O	6.847118	-0.429304	-0.952579
C	-2.285132	1.327823	1.288559	H	7.788448	-0.272761	-0.764846
H	-1.664836	1.119192	2.168944				

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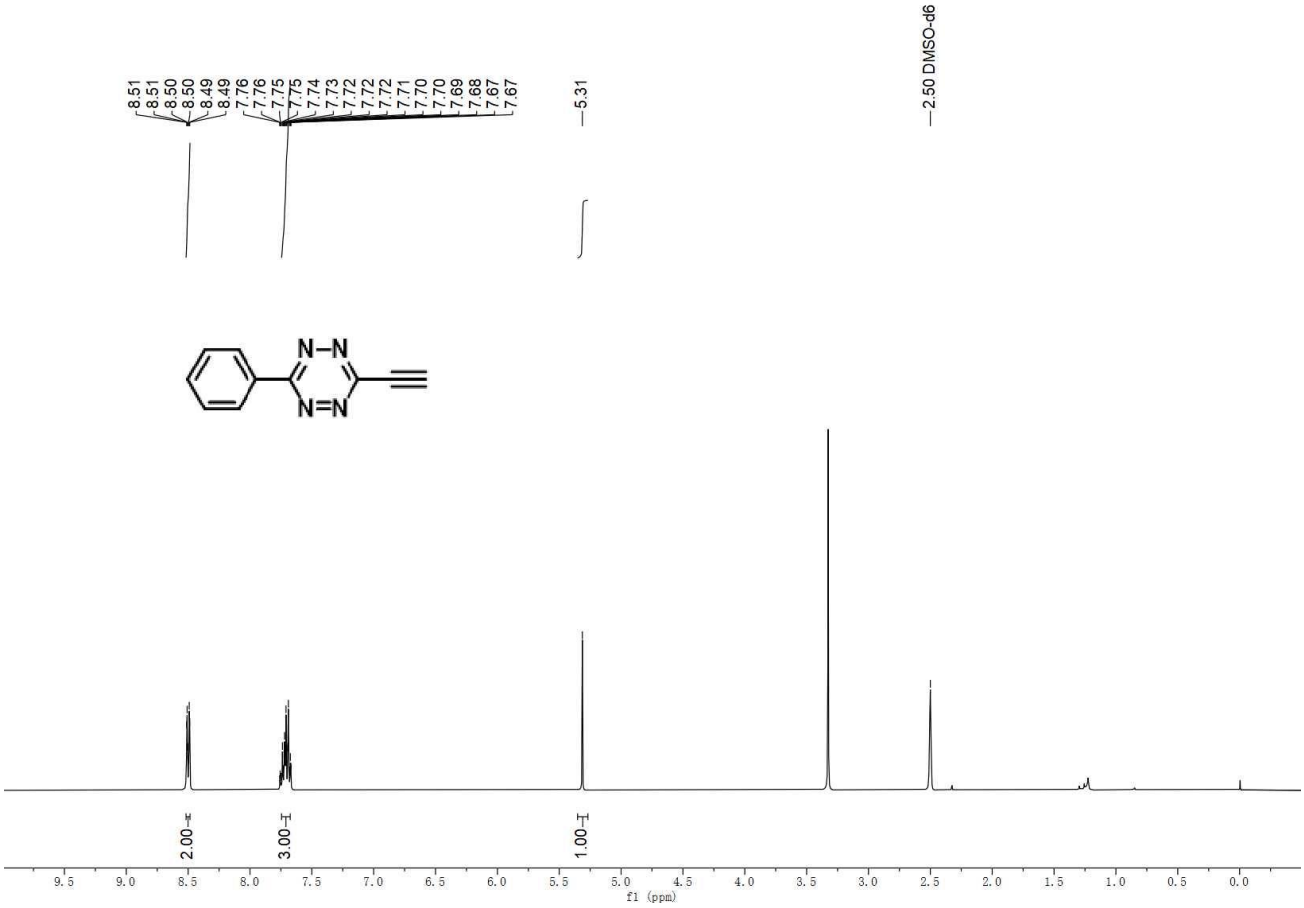
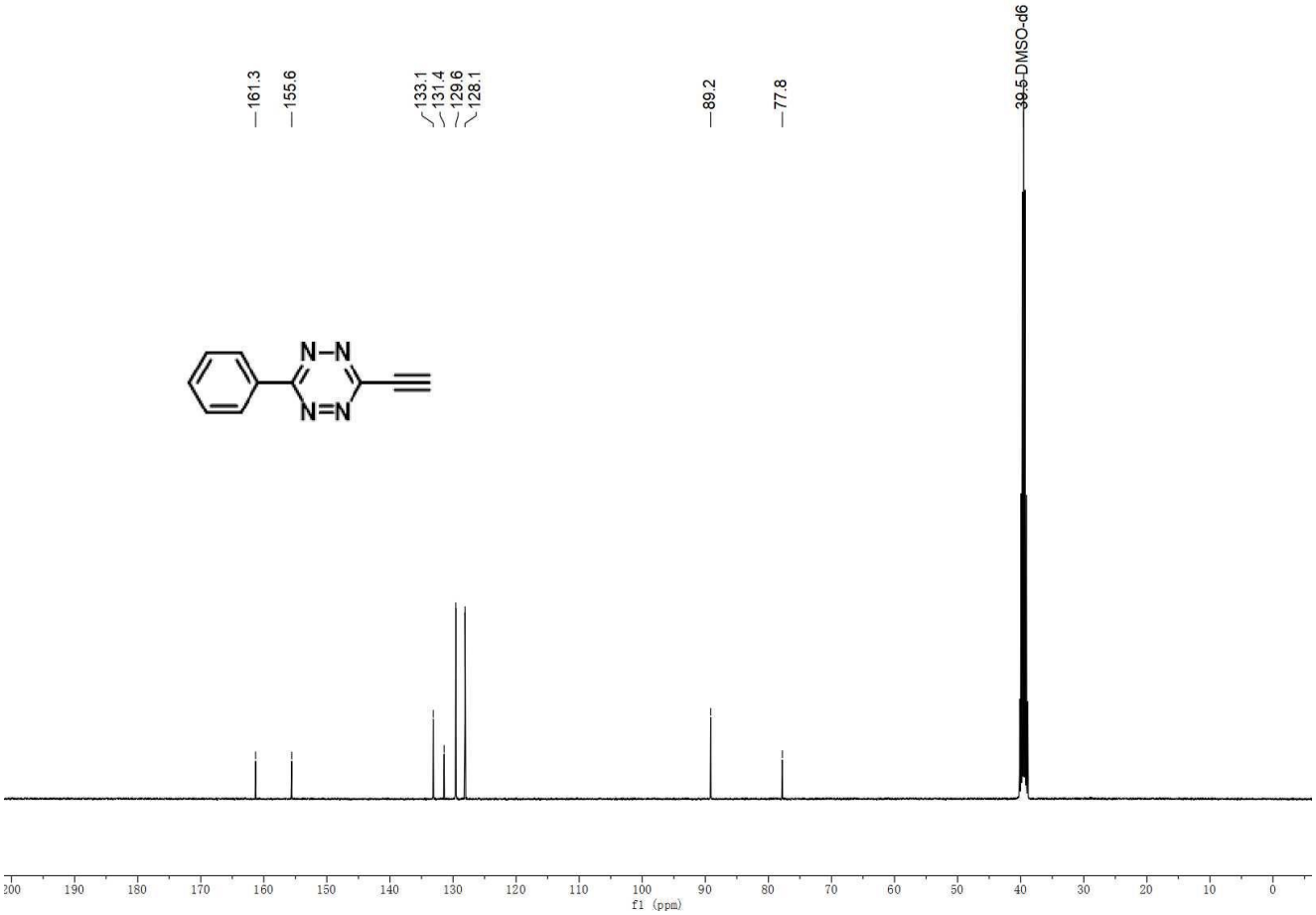
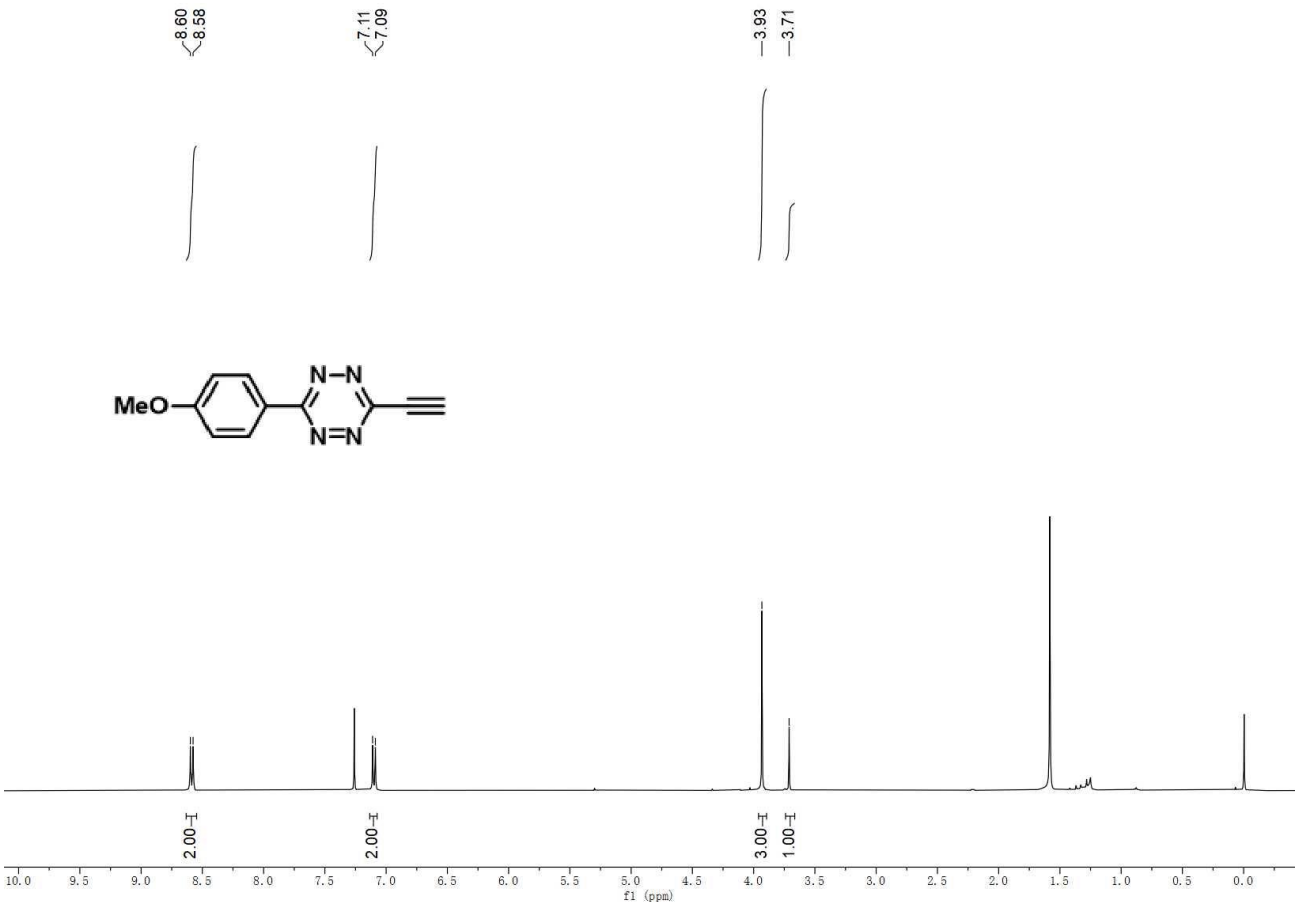
11. NMR spectra and HRMS



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7.11
7.09
3.93
3.71
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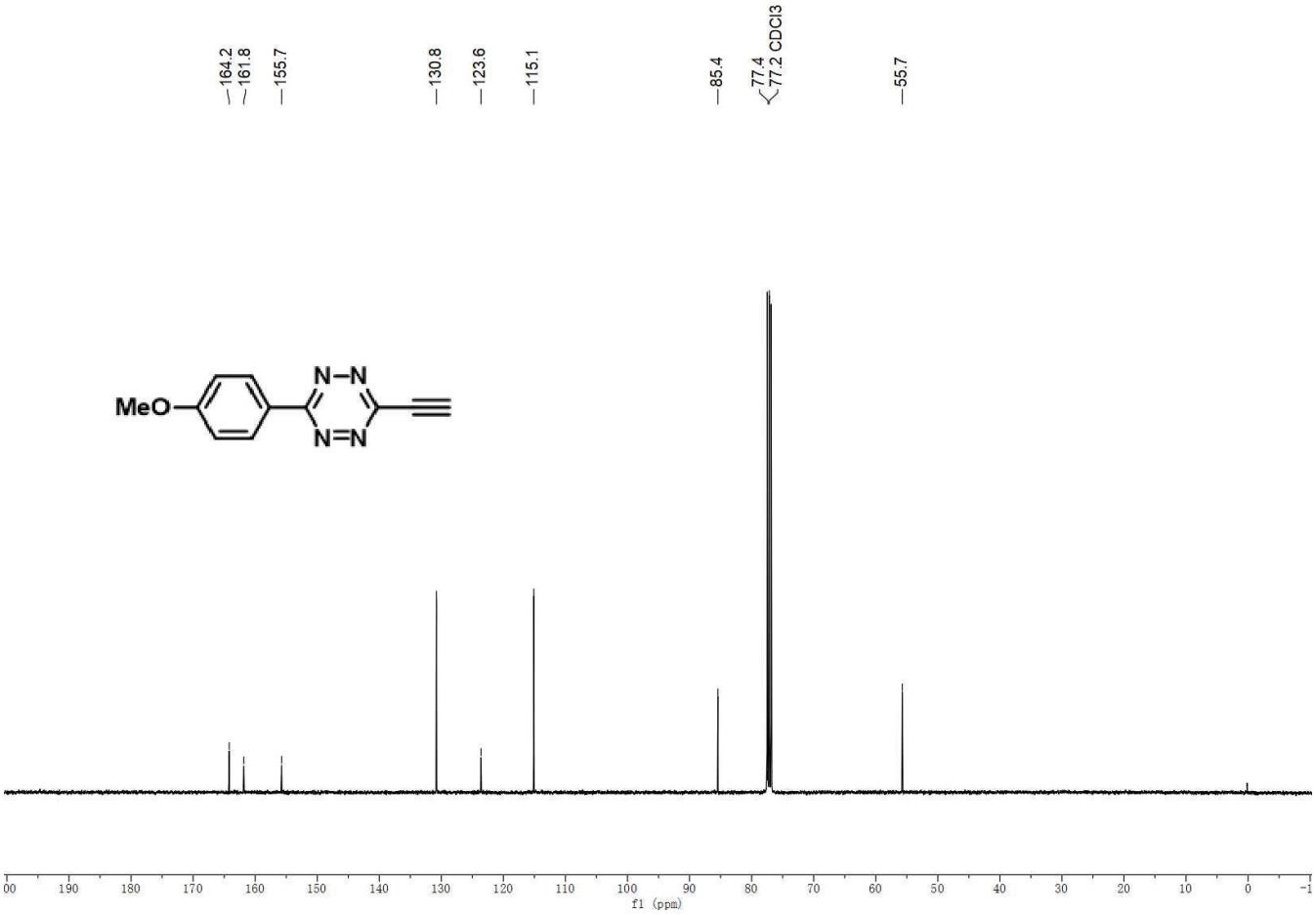
N–N
N=N
2.00
2.00
3.00
1.00
f1 (ppm)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155.7
130.8
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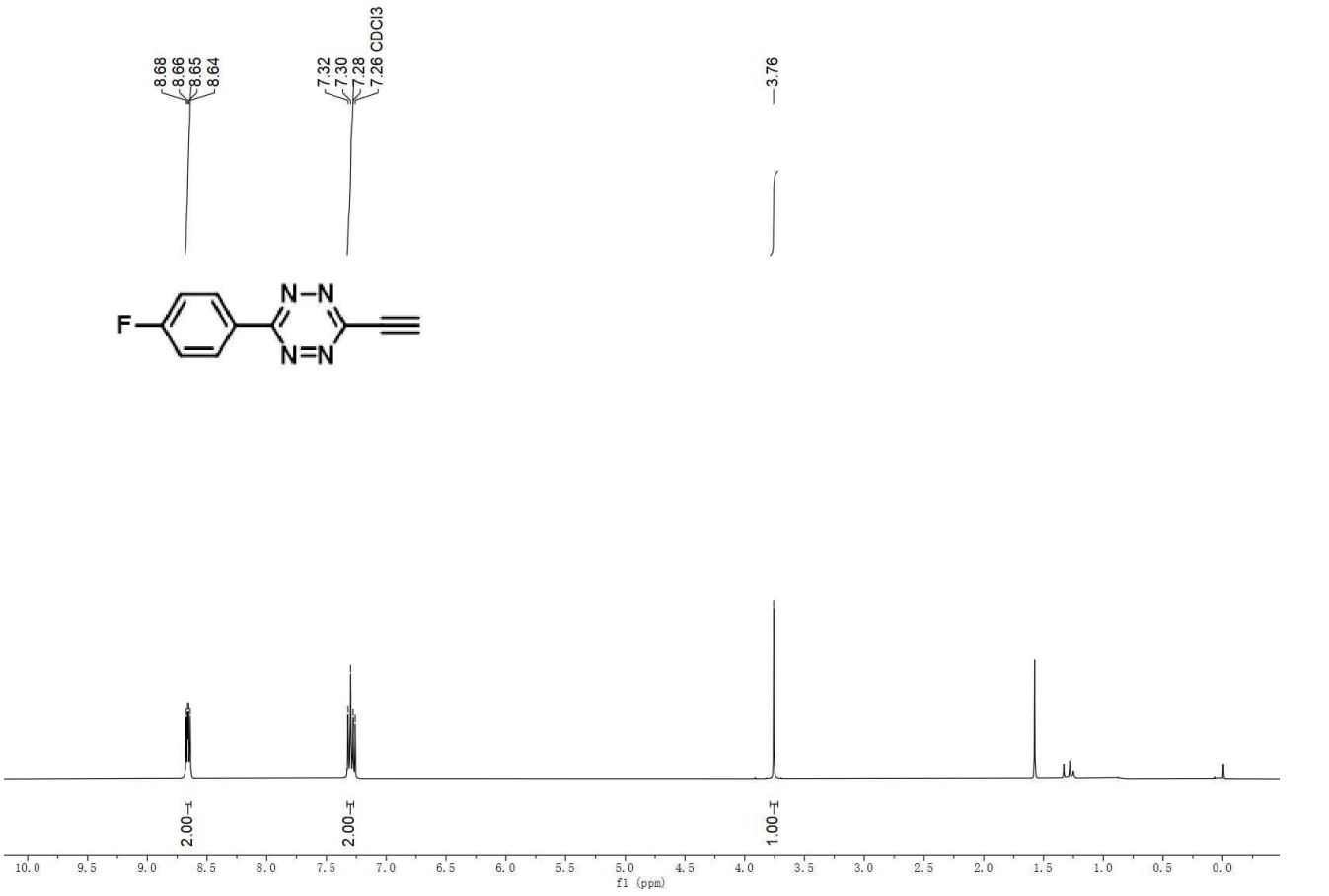
115.1
85.4
77.4
77.2 CDCl3
55.7
MeO
N–N
N=N
f1 (ppm)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3.76
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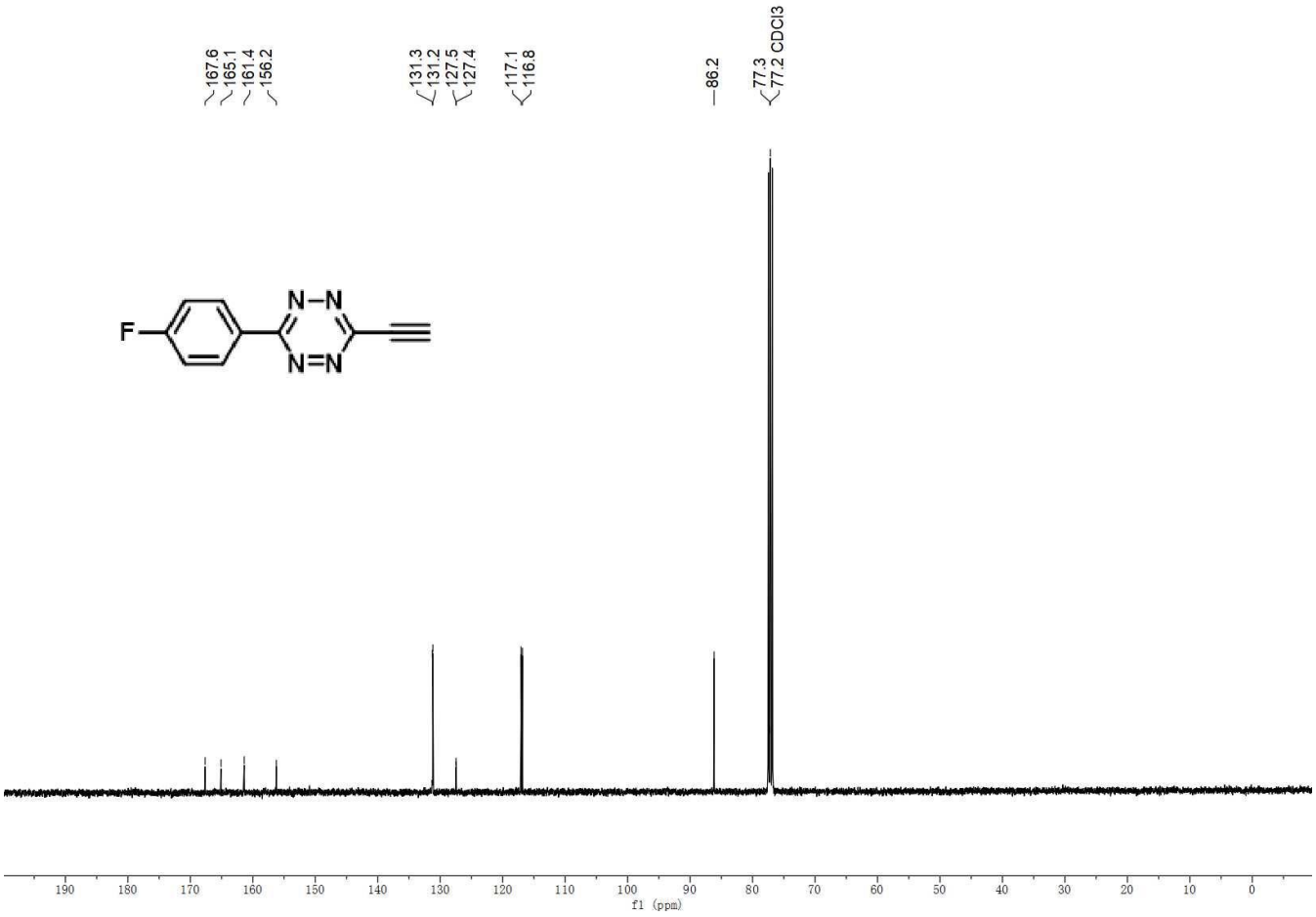
2.00
1.00
f1 (ppm)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131.2
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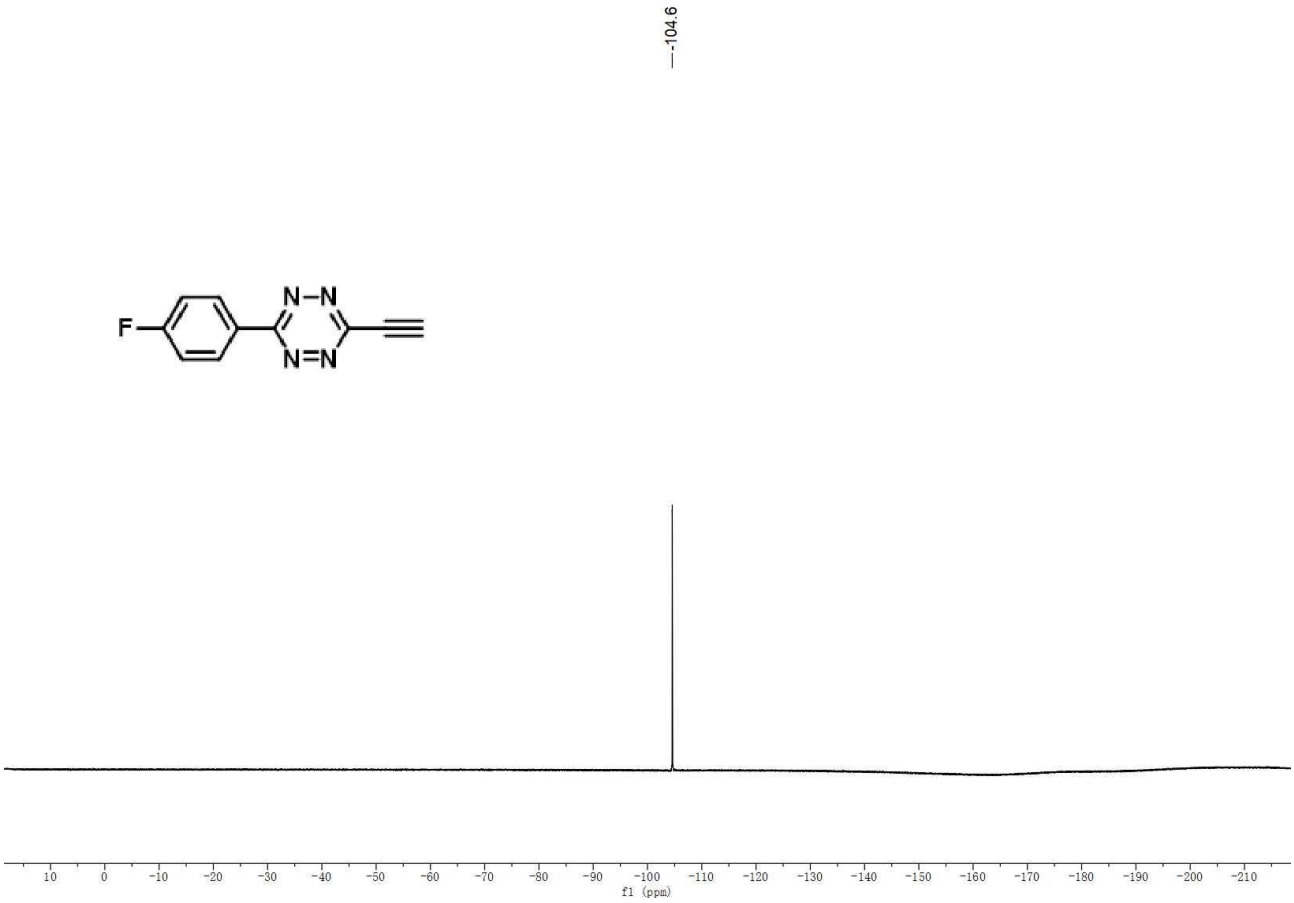
116.8
86.2
77.3
77.2 CDCl3
F
N–N
N=N
f1 (ppm)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F
N–N
N=N
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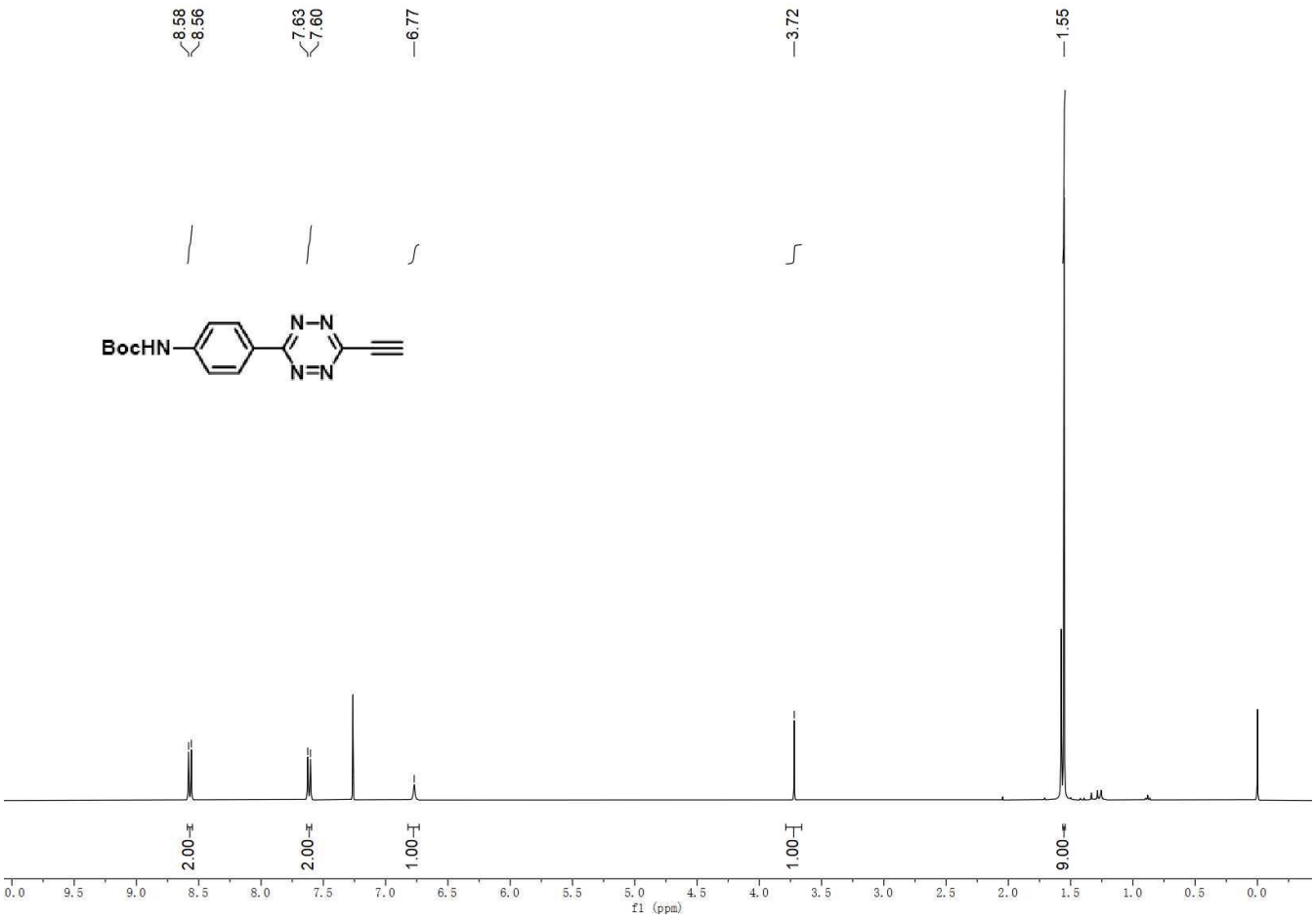
f1 (ppm)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N=N
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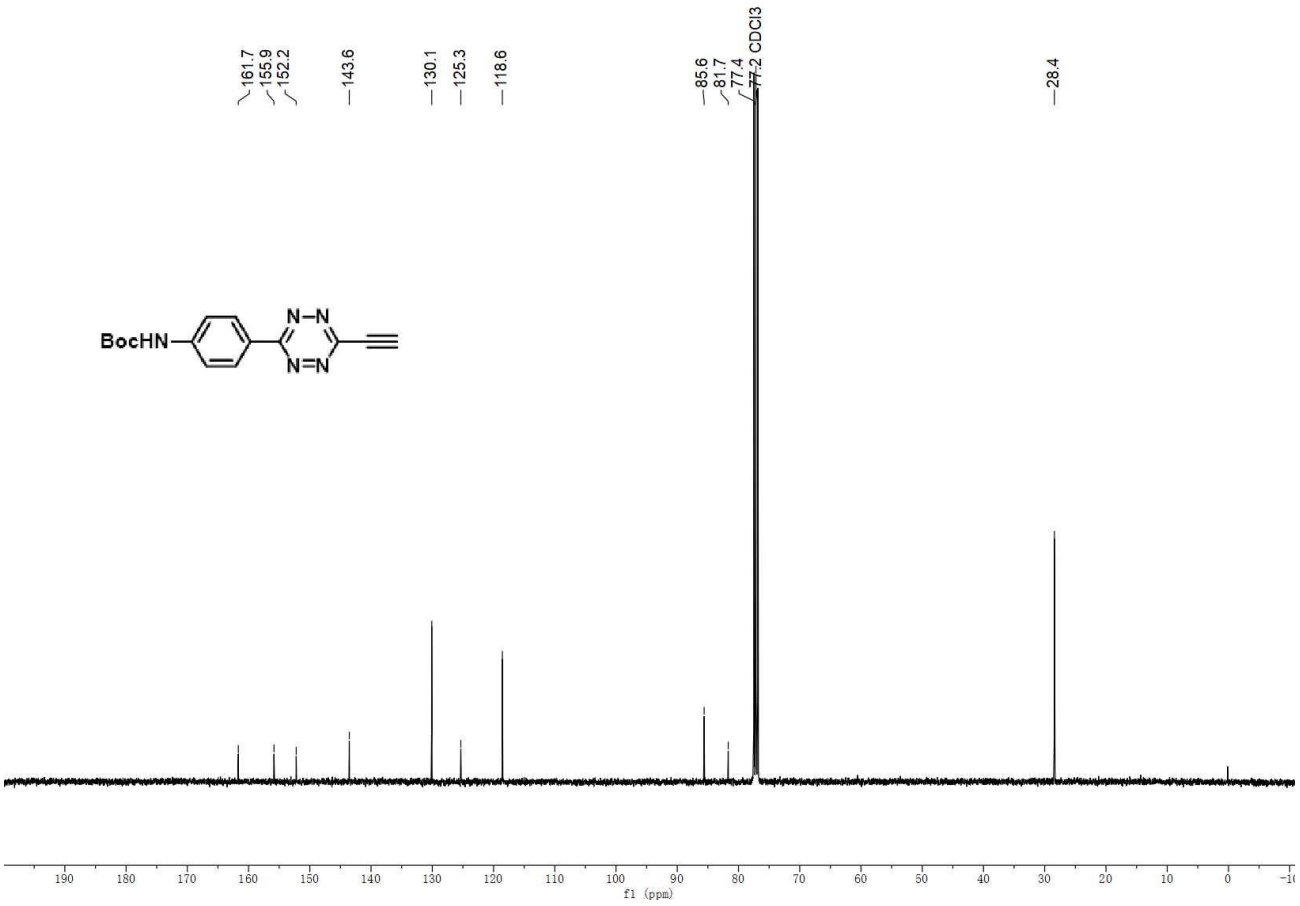
2.00
1.00
1.00
9.00
0.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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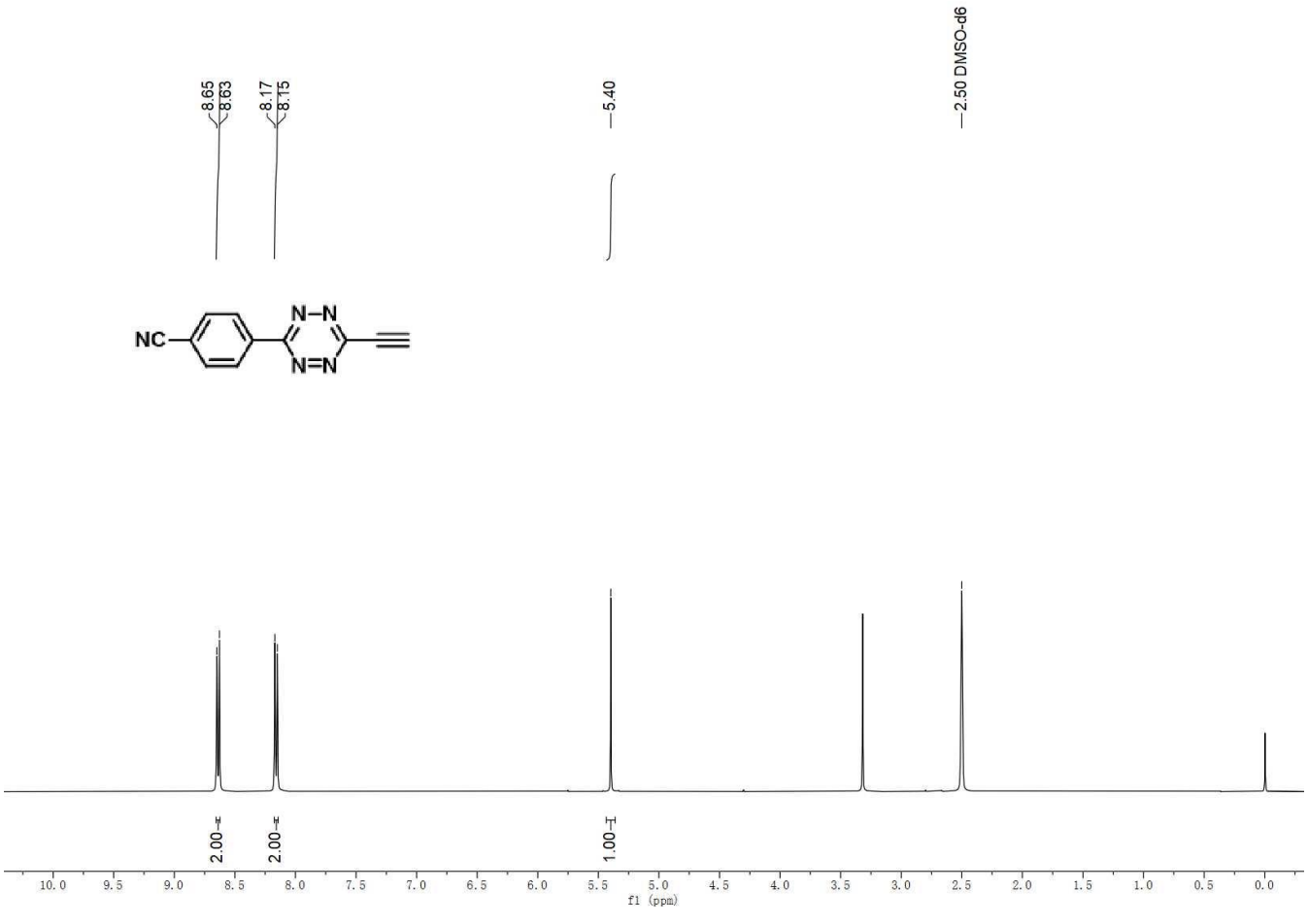
28.4
BocHN
N–N
N=N
f1 (ppm)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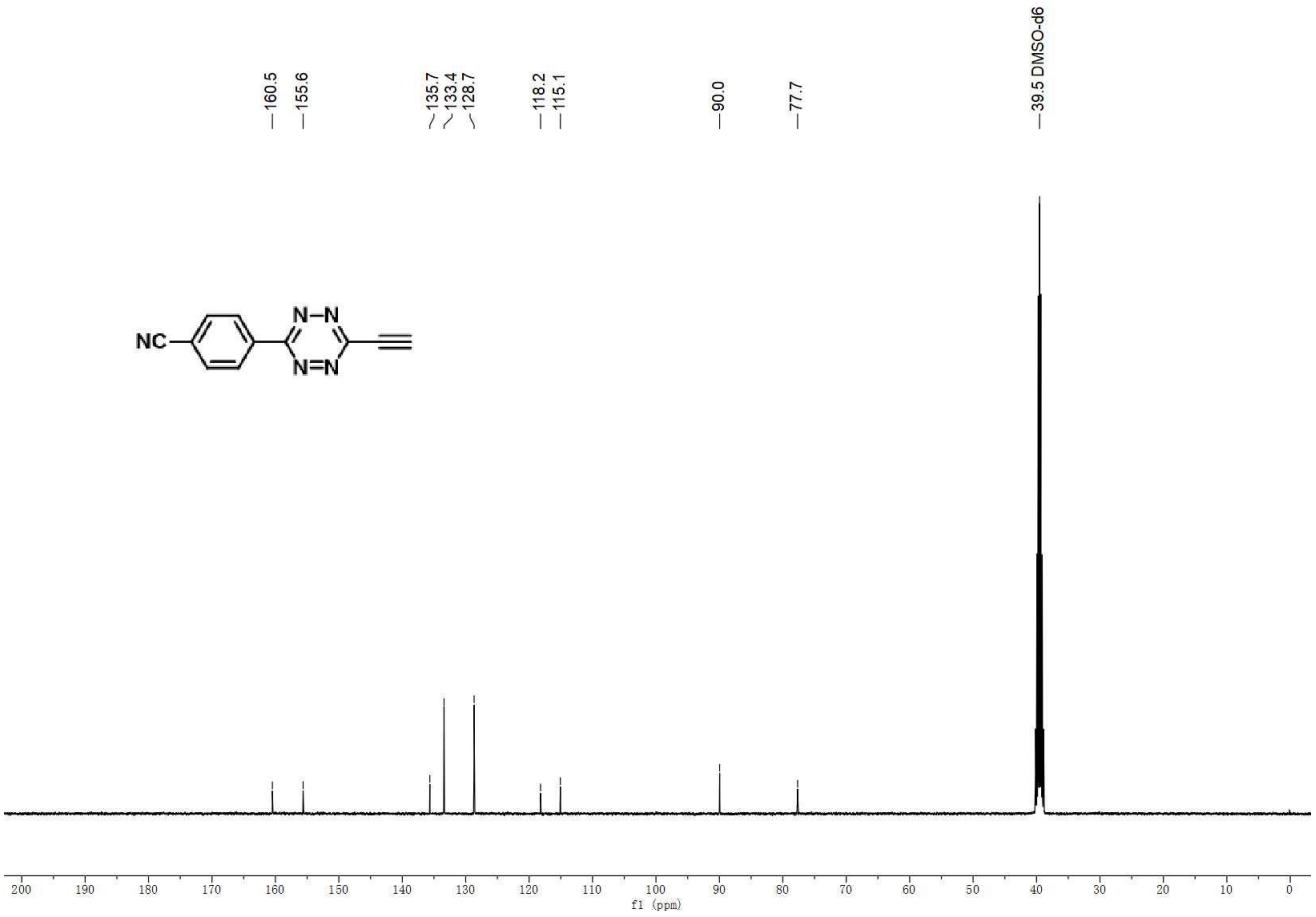
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N–N
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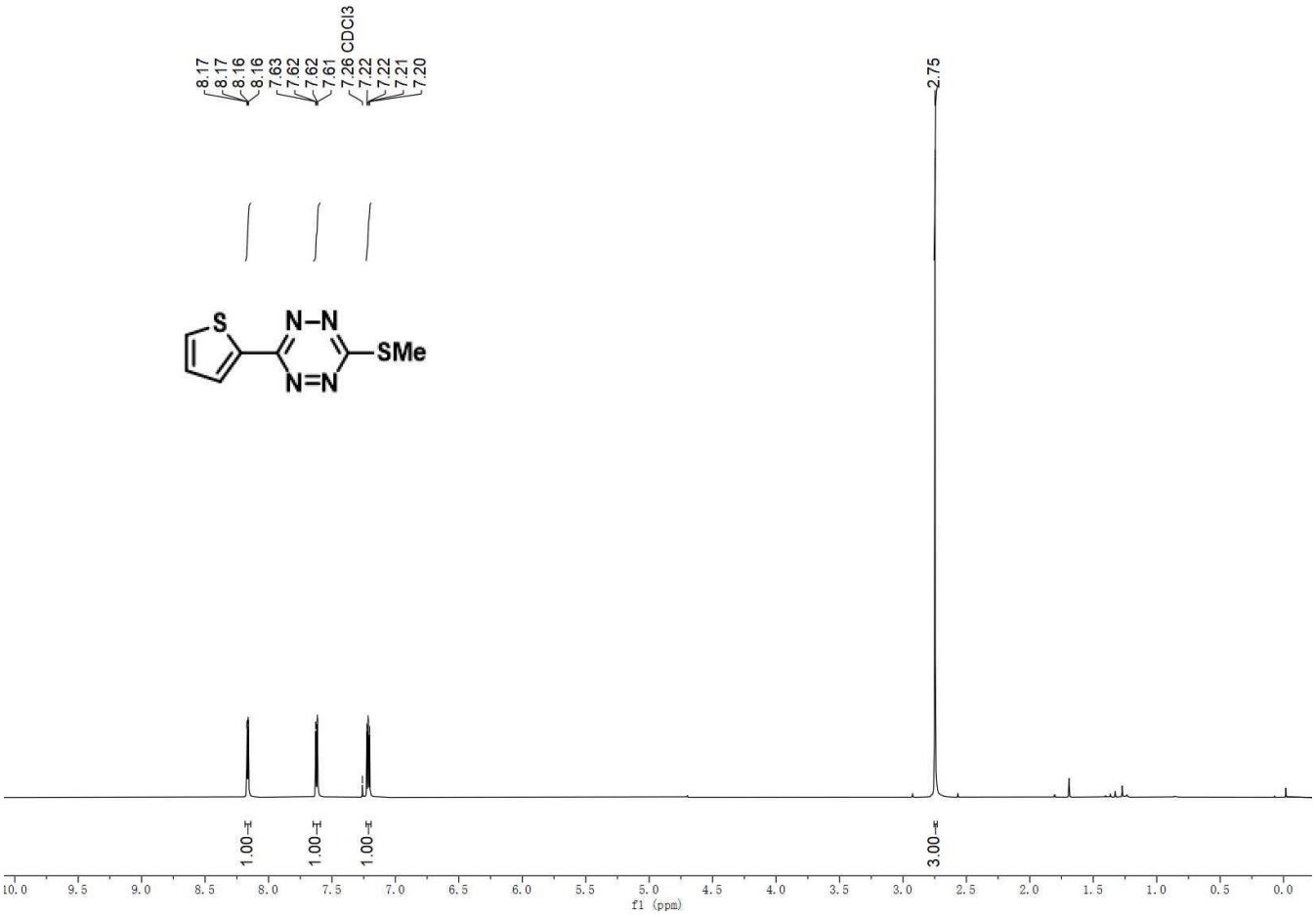
f1 (ppm)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7.62
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7.61
7.26 CDCl3
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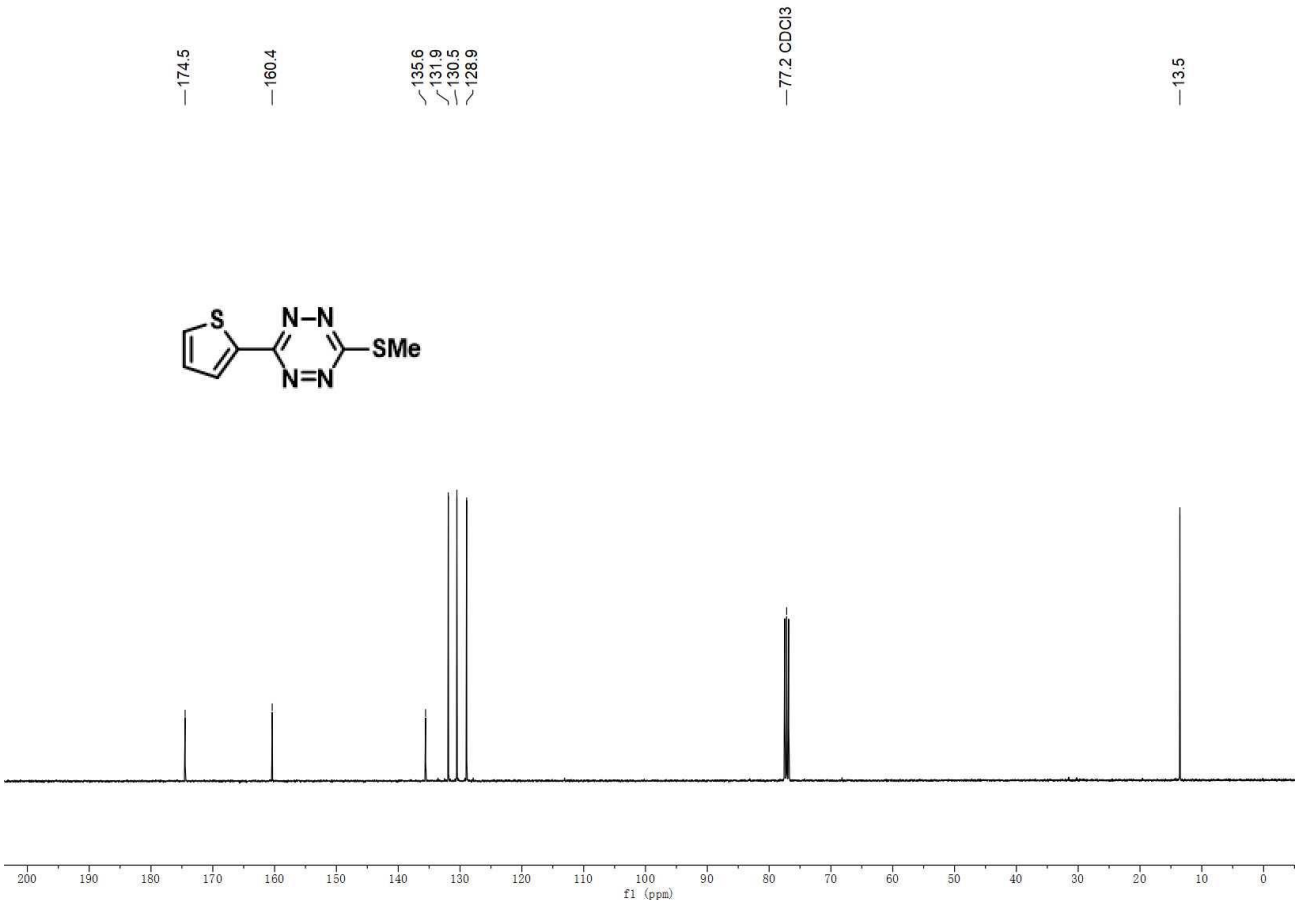
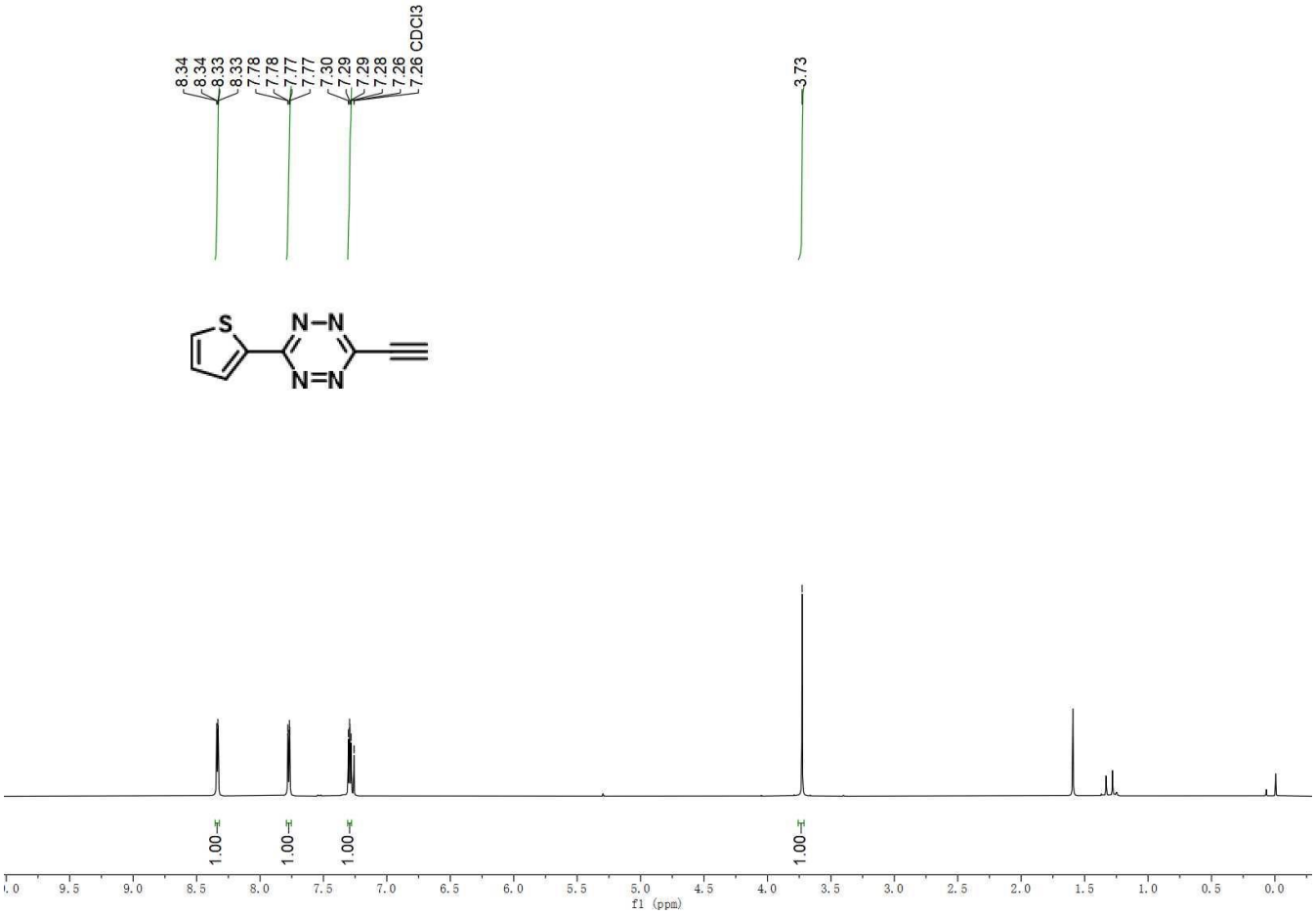
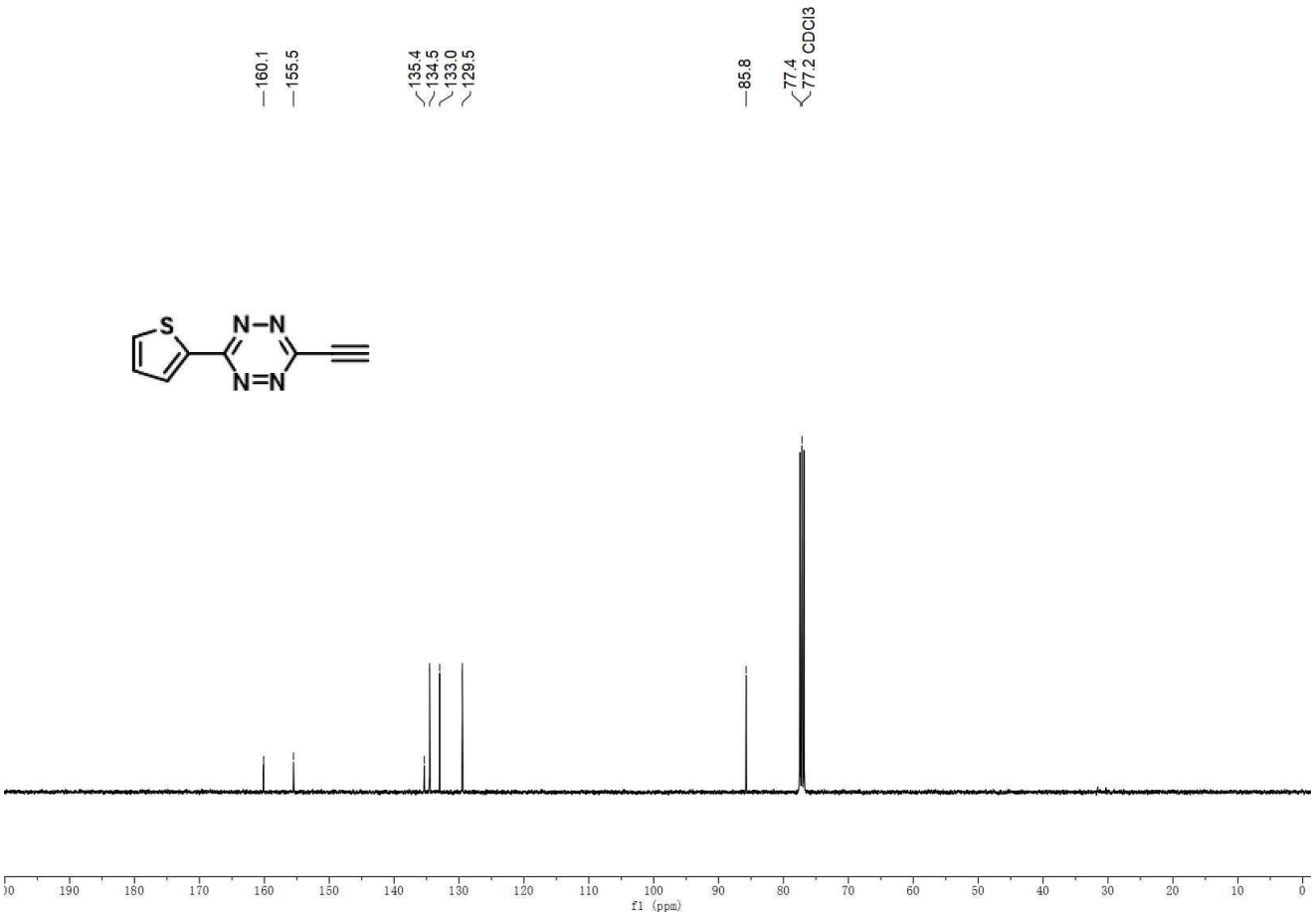
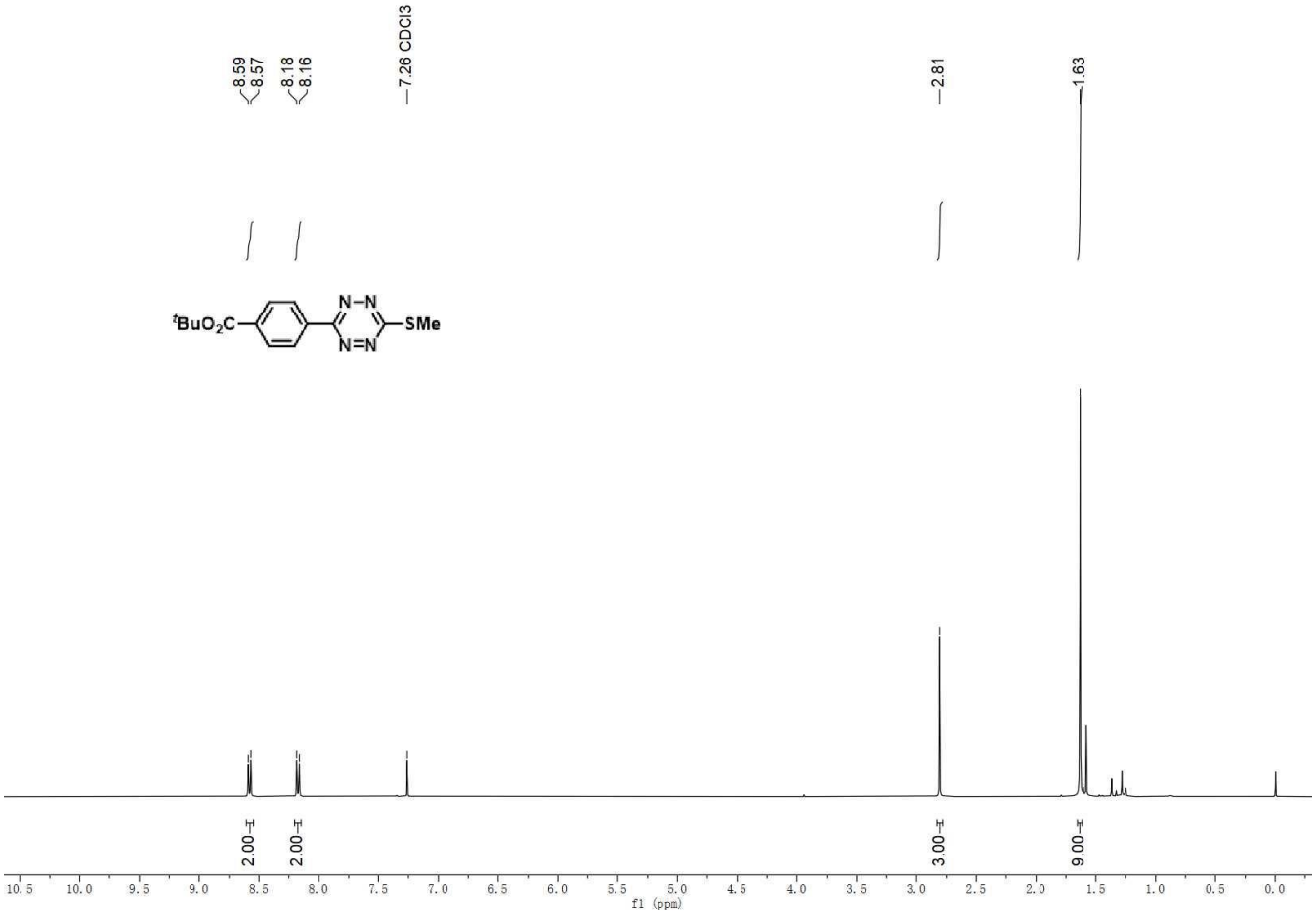
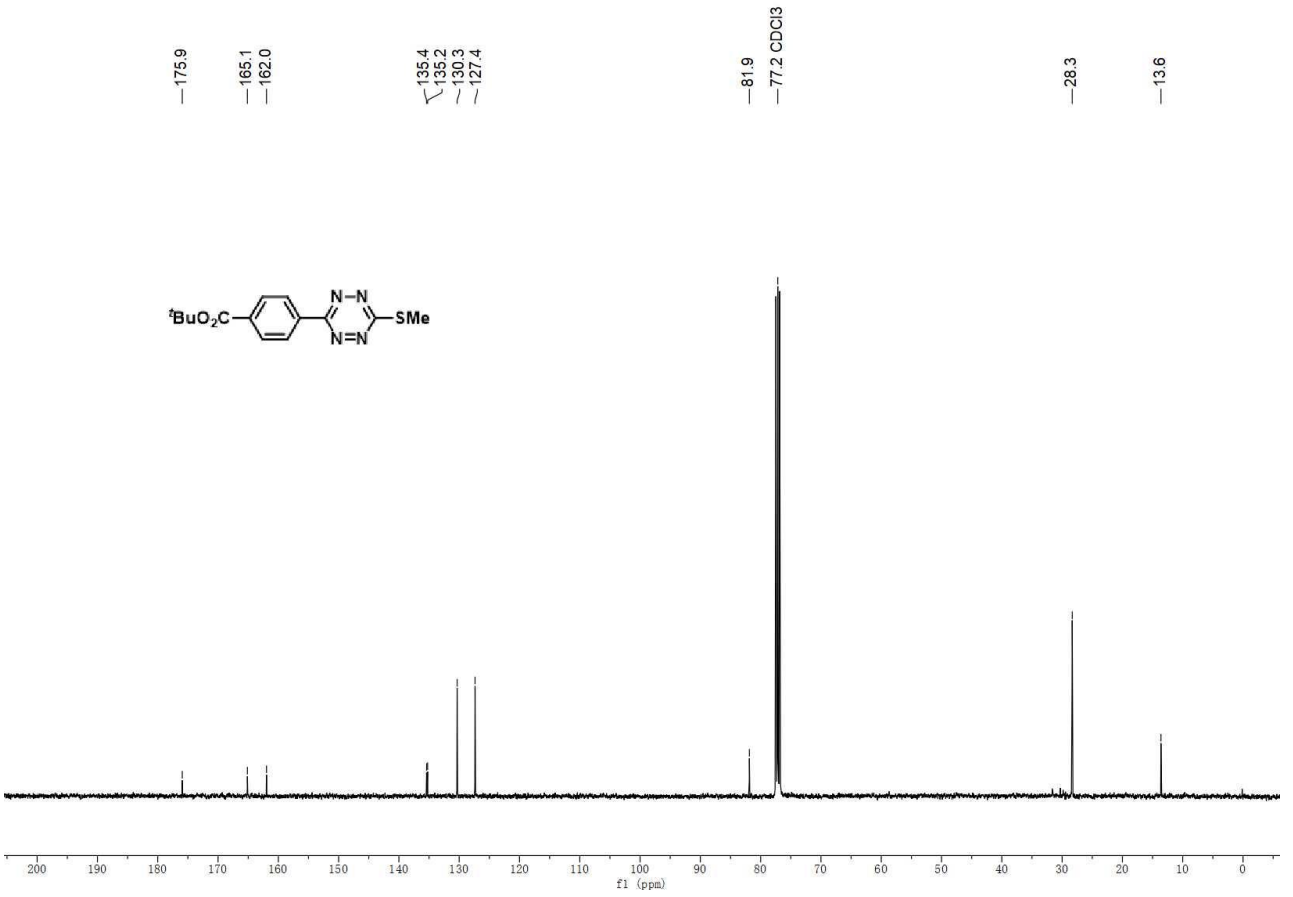
7.22
7.21
7.20
2.75
S
N–N
N=N
SMe
1.00
1.00
1.00
3.00
f1 (ppm)

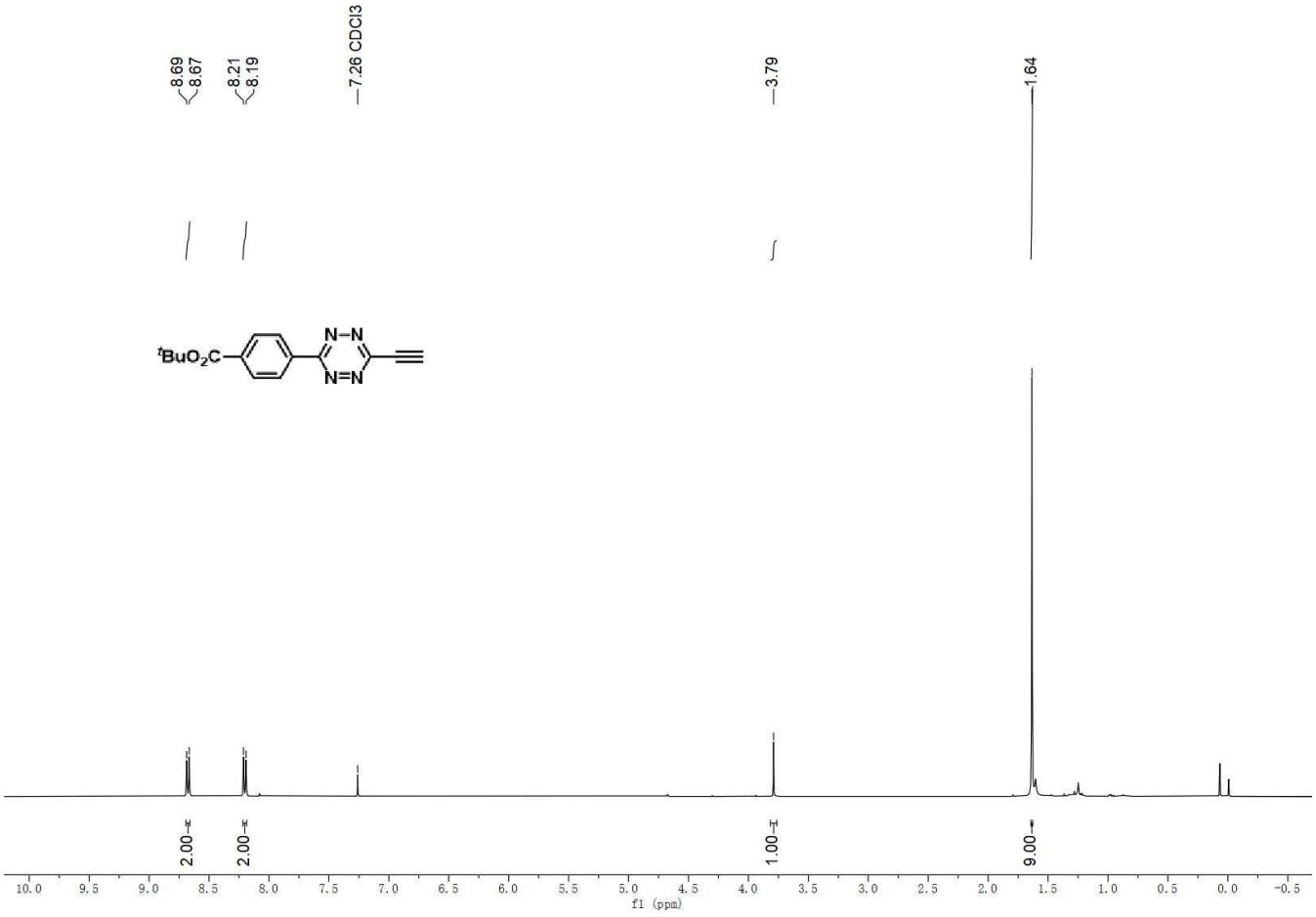

174.5
160.4
135.6
131.9
130.5
128.9
77.2 CDCl3
13.5
S
N–N
SMe
N=N
f1 (ppm)

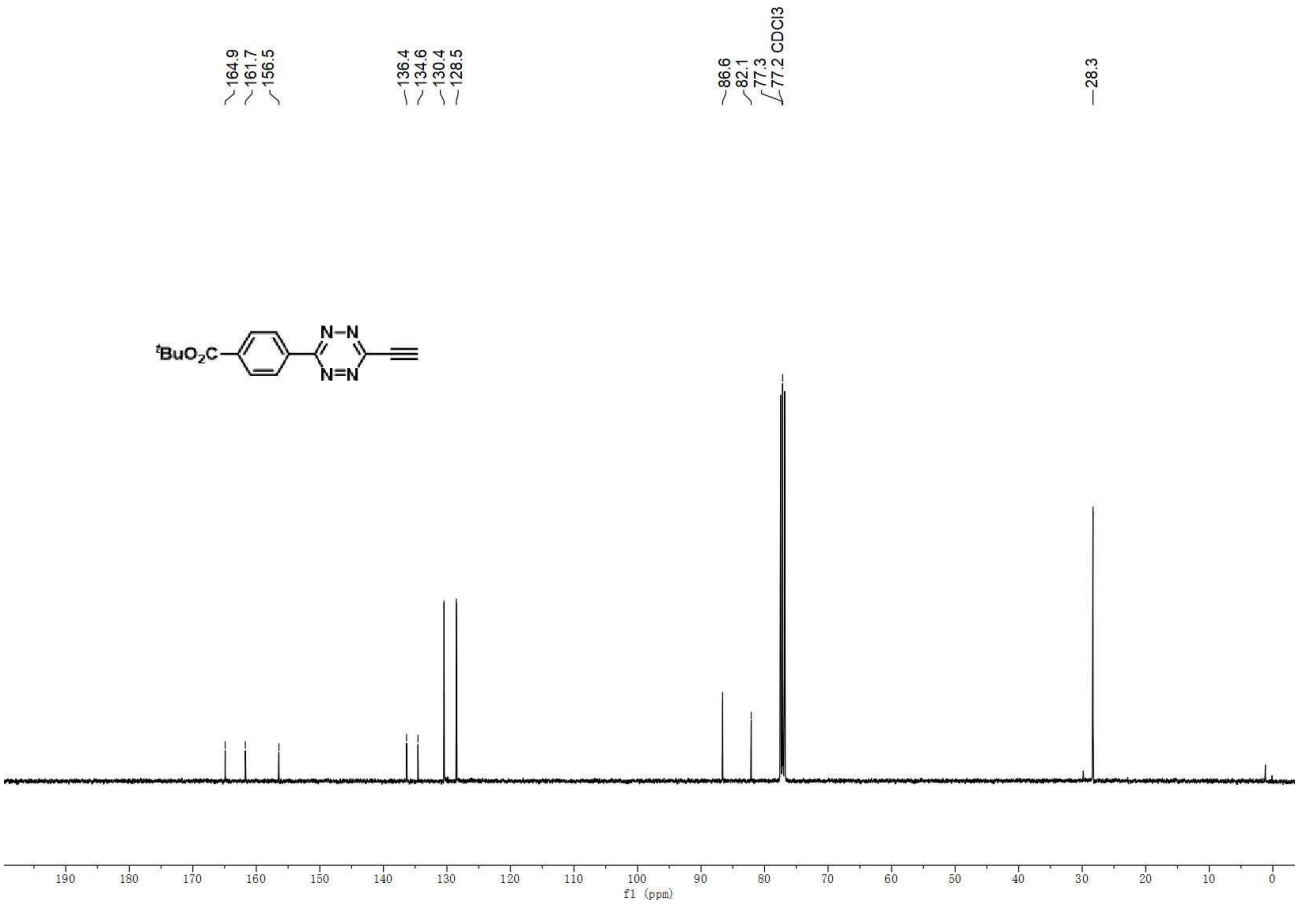

8.34
8.34
8.33
8.33
7.78
7.78
7.77
7.77
7.30
7.29
7.29
7.28
7.26
7.26 CDCl3
3.73
S
N–N
N=N
1.00
1.00
1.00
1.00
f1 (ppm)

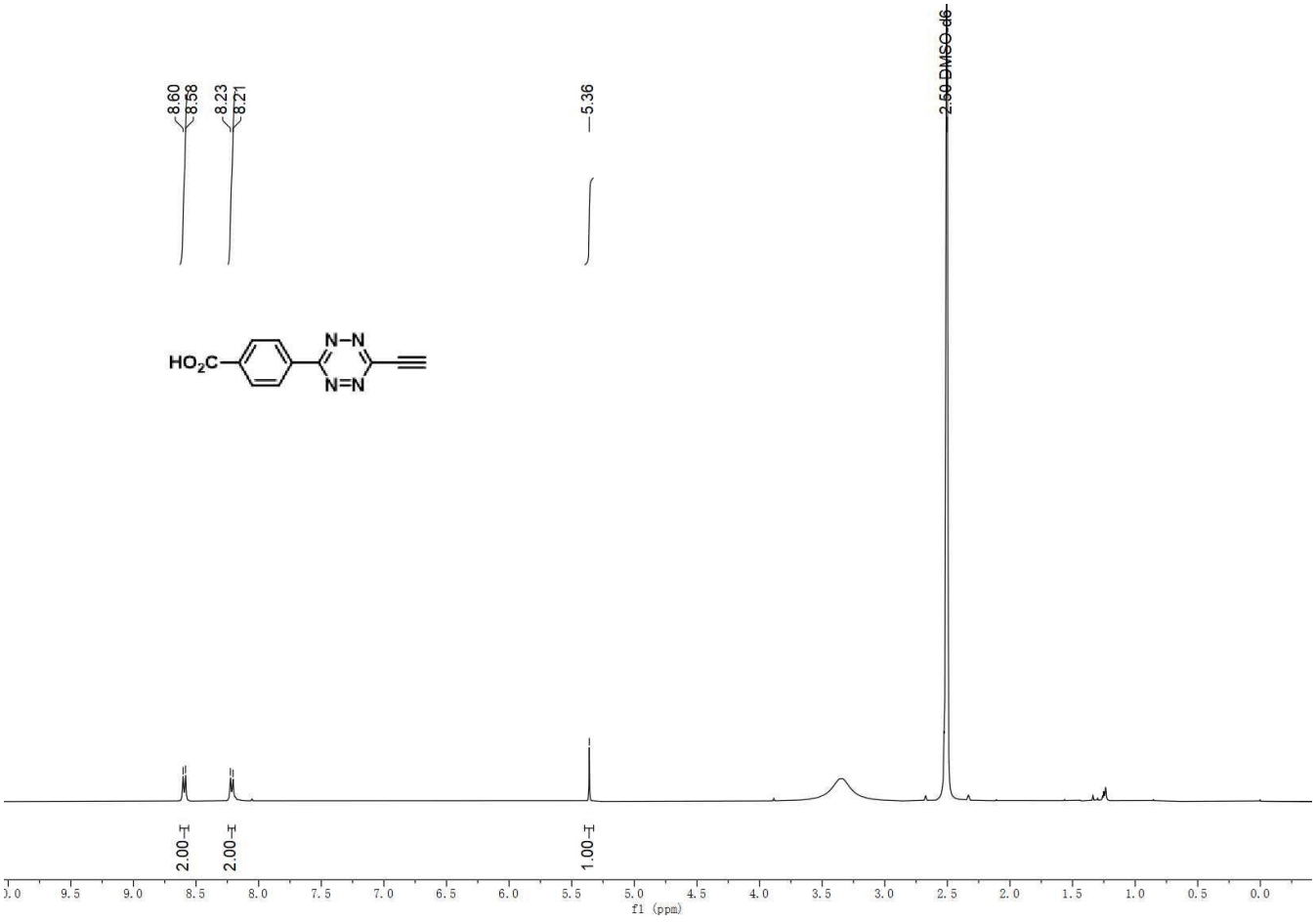

160.1
155.5
135.4
134.5
133.0
129.5
85.8
77.4
77.2 CDCl3
S
N–N
N=N
f1 (ppm)

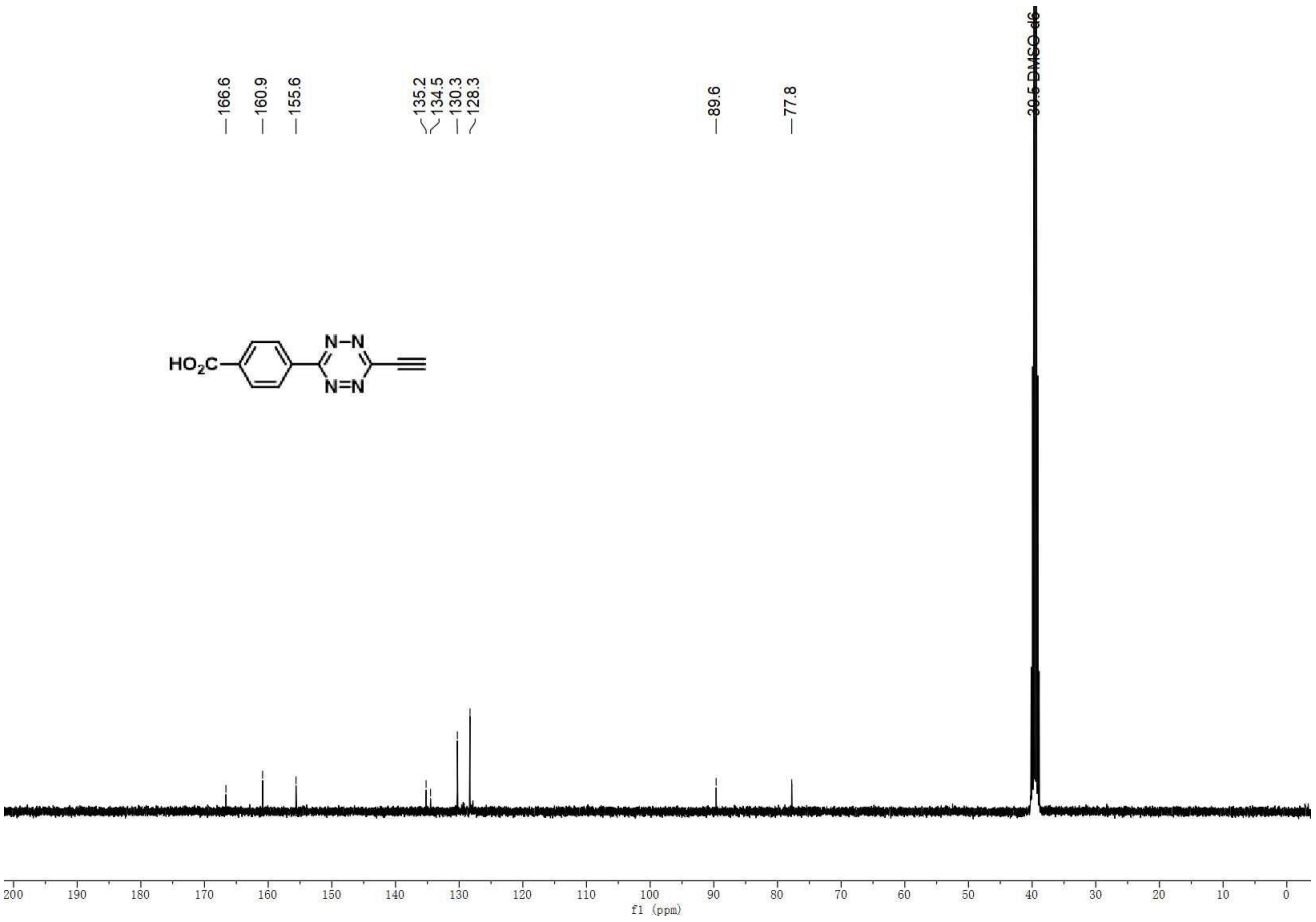

8.59
8.57
8.18
8.16
7.26 CDCl3
2.81
1.63
tBuO2C
N–N
N=N
SMe
2.00
2.00
3.00
9.00
f1 (ppm)

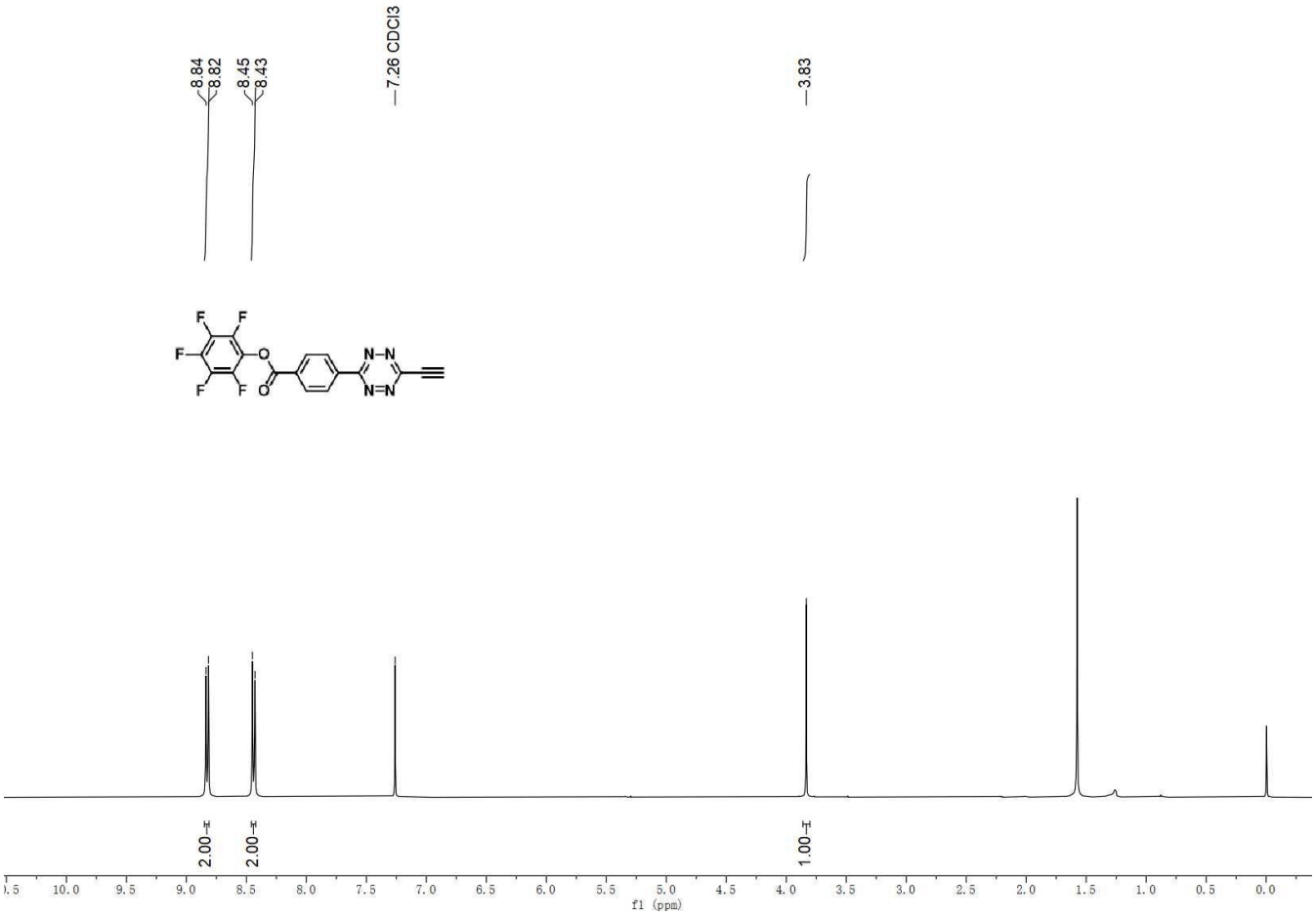

175.9
165.1
162.0
135.4
135.2
130.3
127.4
81.9
77.2 CDCl3
28.3
13.6
tBuO2C
N–N
N=N
SMe
f1 (ppm)

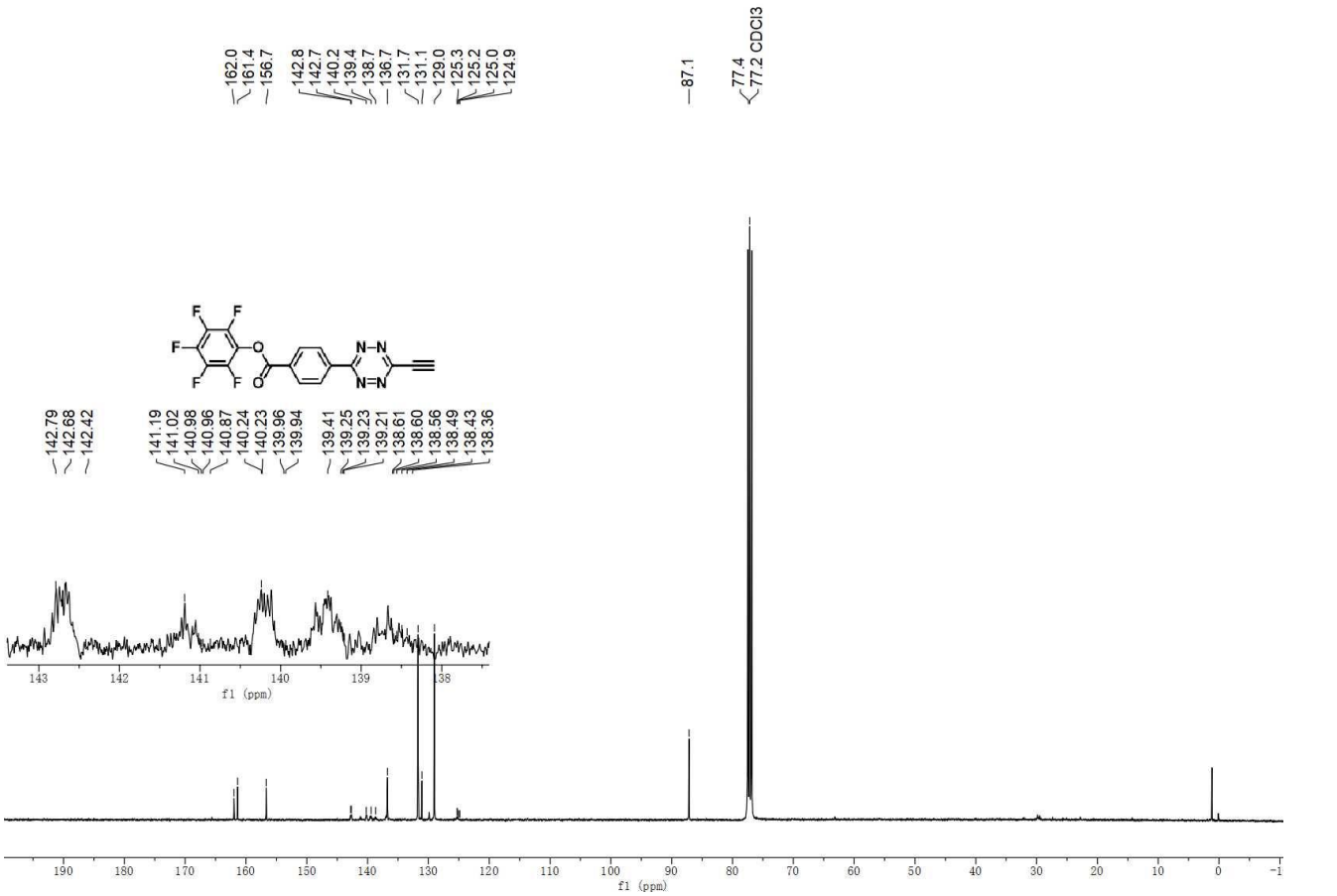

8.69
8.67
8.21
8.19
7.26 CDCl3
3.79
1.64
tBuO2C
N–N
N=N
2.00
2.00
1.00
9.00
f1 (ppm)

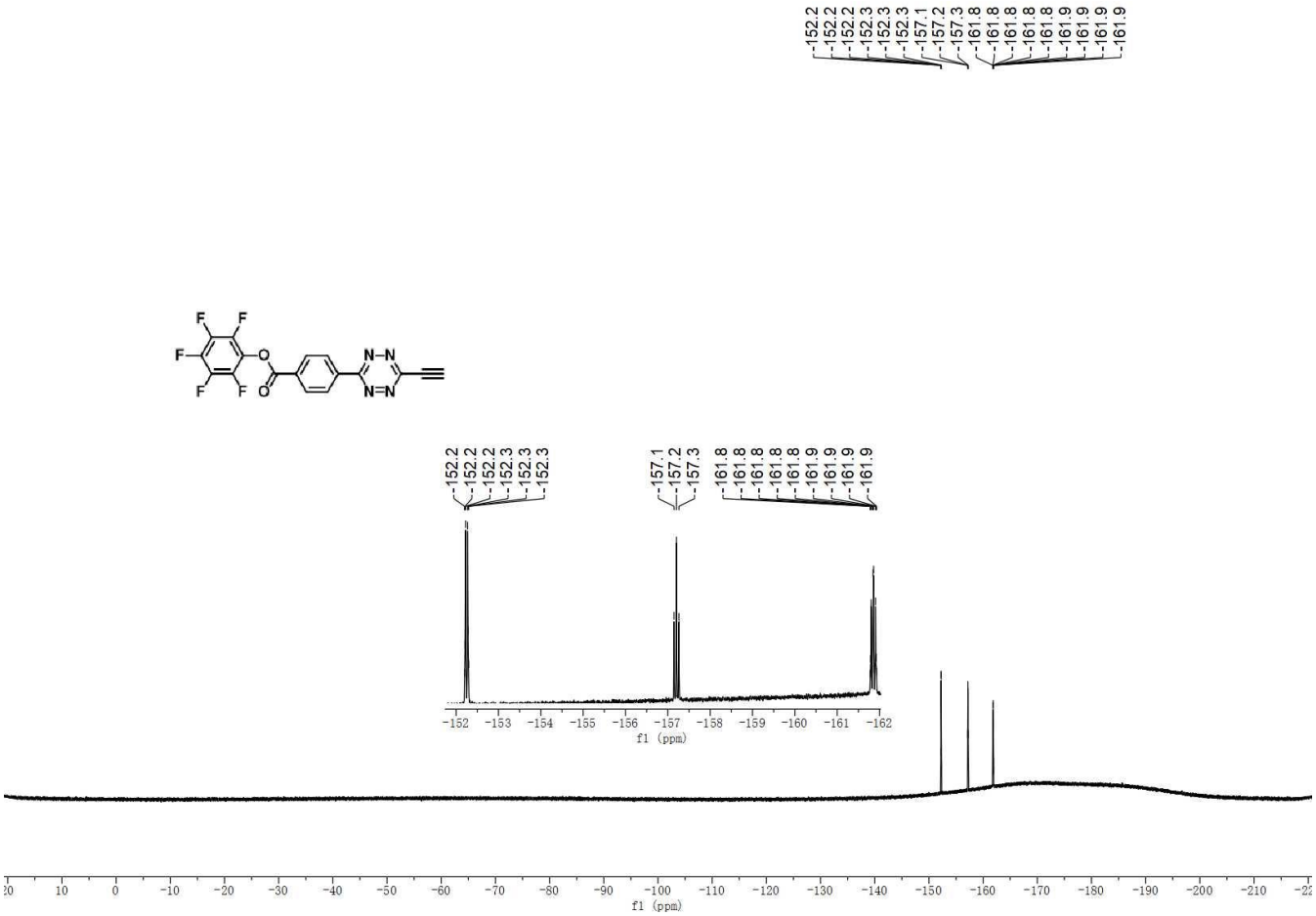

164.9
161.7
156.5
136.4
134.6
130.4
128.5
86.6
82.1
77.3
77.2 CDCl3
28.3
tBuO2C
N–N
N=N
f1 (ppm)

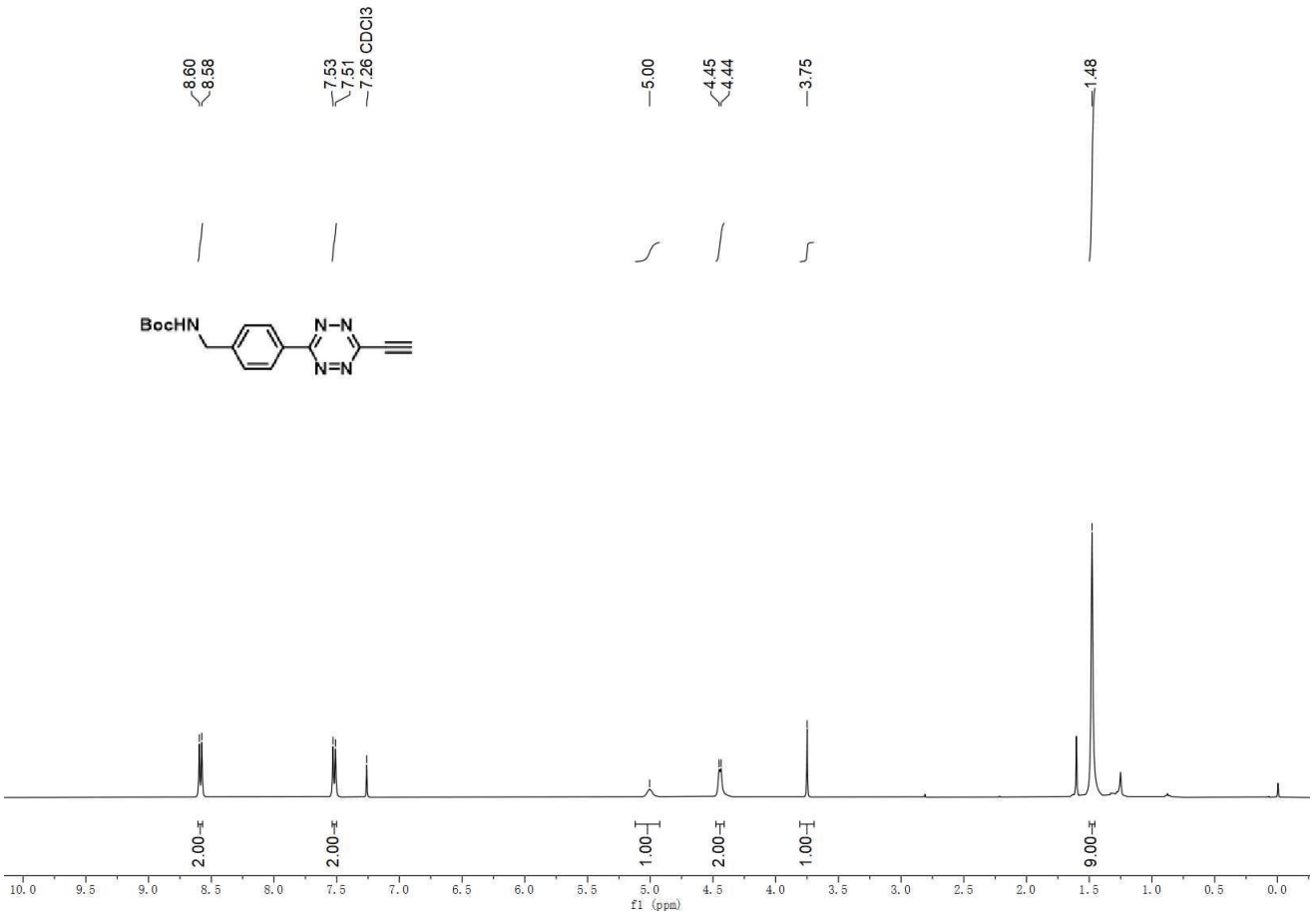

8.60
8.58
8.23
8.21
5.36
2.50 DMSO-d6
HO2C
N–N
N=N
2.00
2.00
1.00
f1 (ppm)

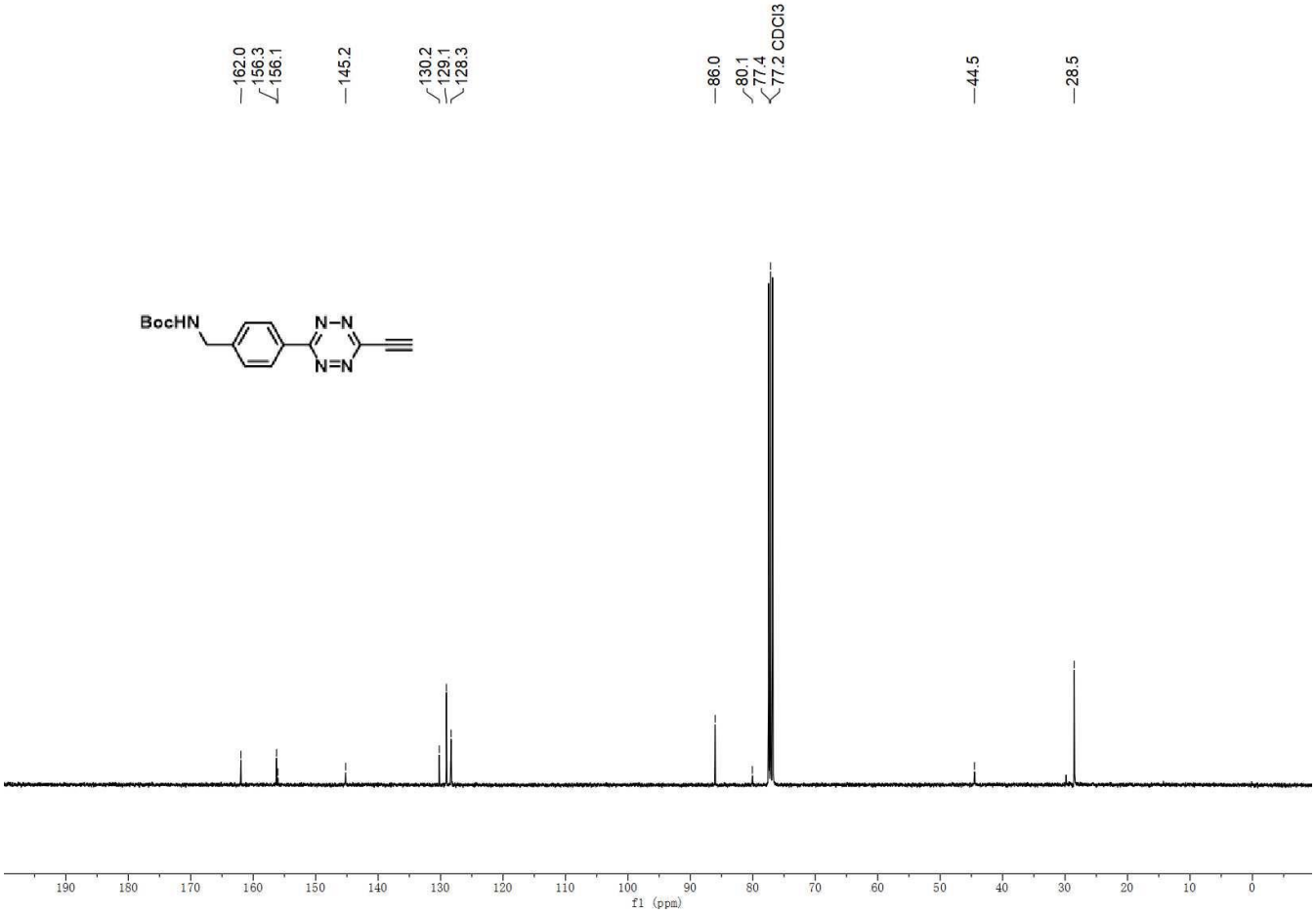

166.6
160.9
155.6
135.2
134.5
130.3
128.3
89.6
77.8
39.5 DMSO-d6
HO2C
N-N
N=N
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

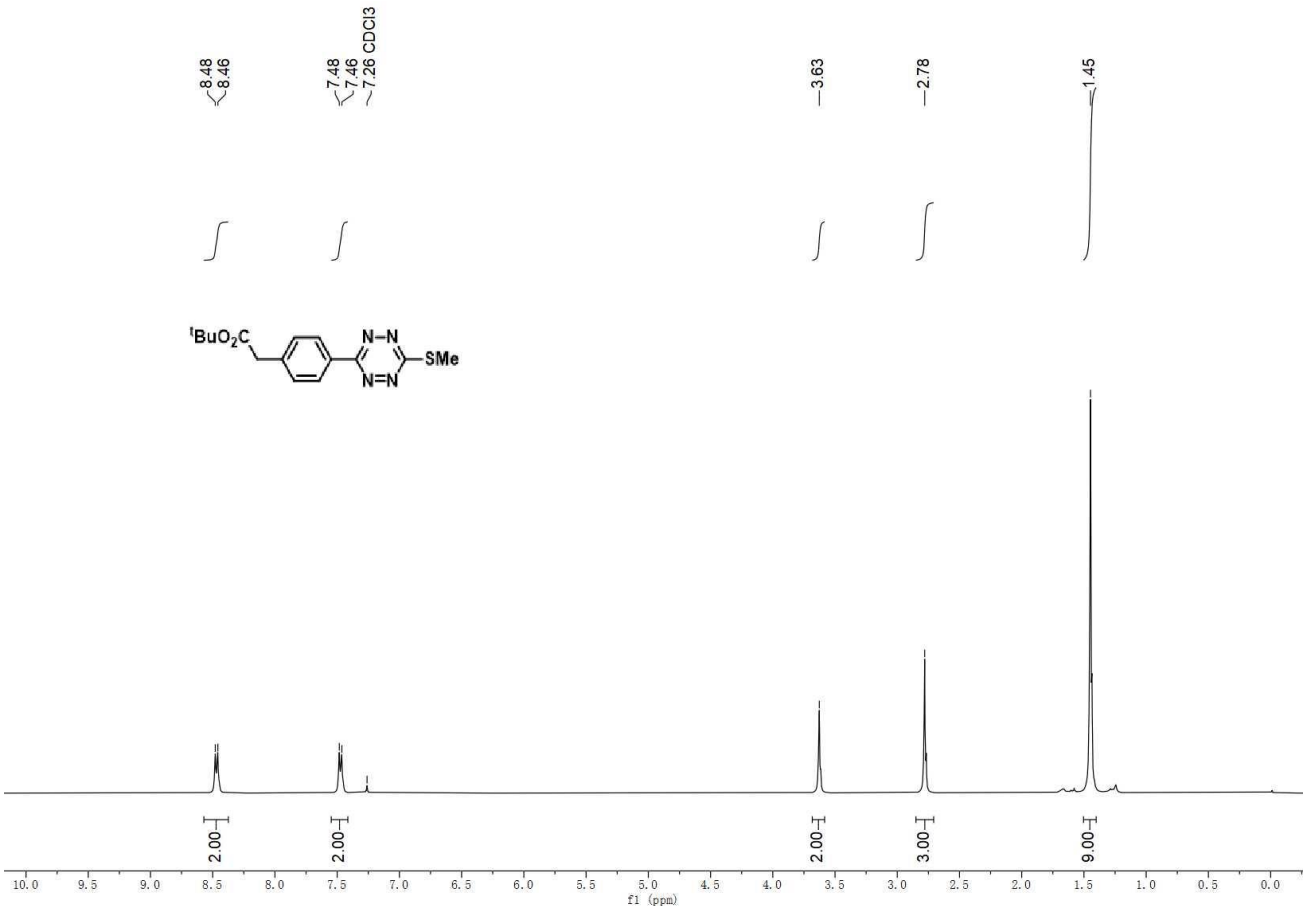

8.84
8.82
8.45
8.43
7.26 CDCl3
3.83
F
O
N-N
N=N
2.00
2.00
1.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

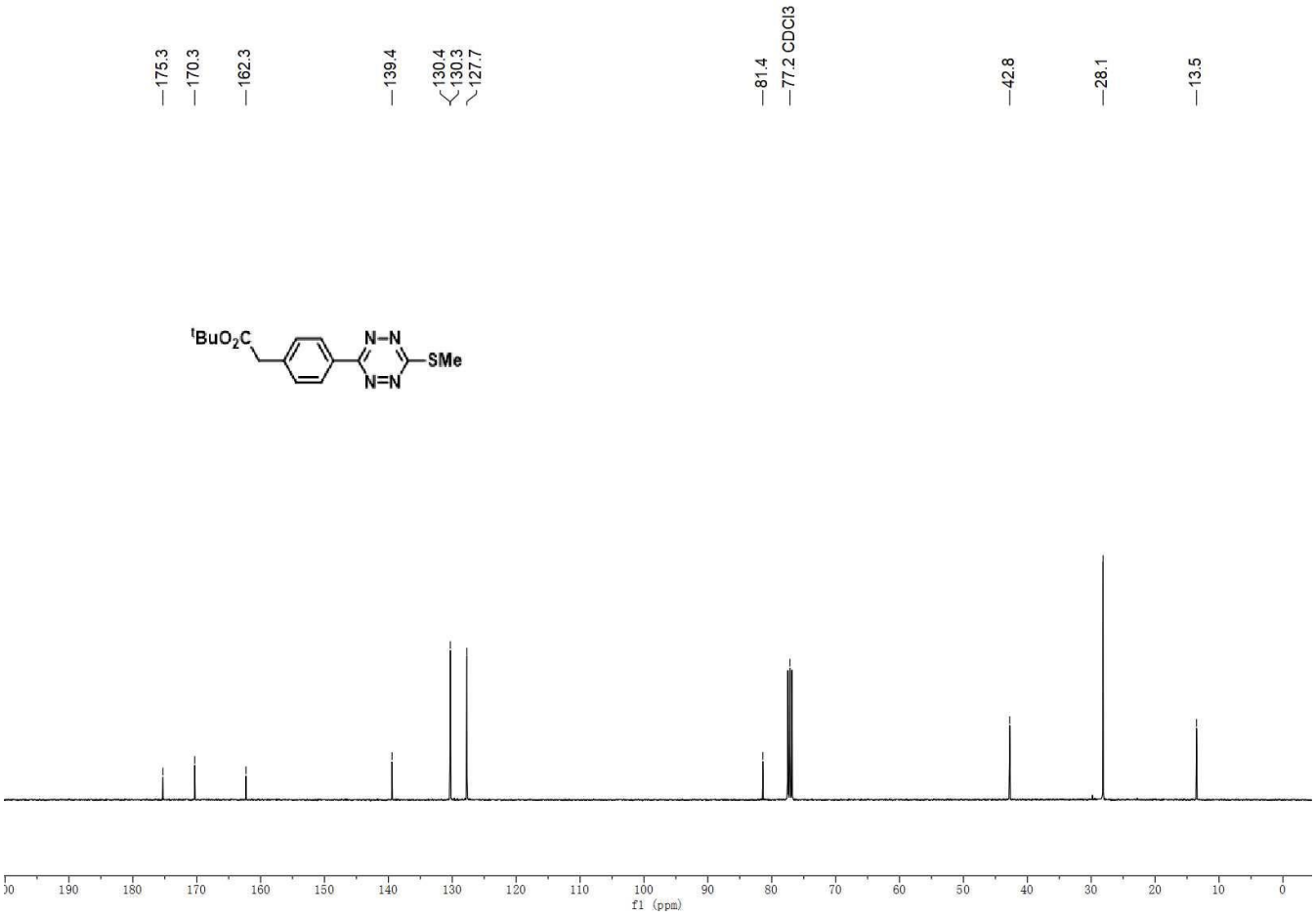

162.0
161.4
156.7
142.8
142.7
140.2
139.4
138.7
136.7
131.7
131.1
129.0
125.3
125.2
125.0
124.9
87.1
77.4
77.2 CDCl3
142.79
142.68
142.42
141.19
141.02
140.98
140.96
140.87
140.24
140.23
139.96
139.94
139.41
139.25
139.23
139.21
138.61
138.60
138.56
138.49
138.43
138.36
f1 (ppm)
f1 (ppm)

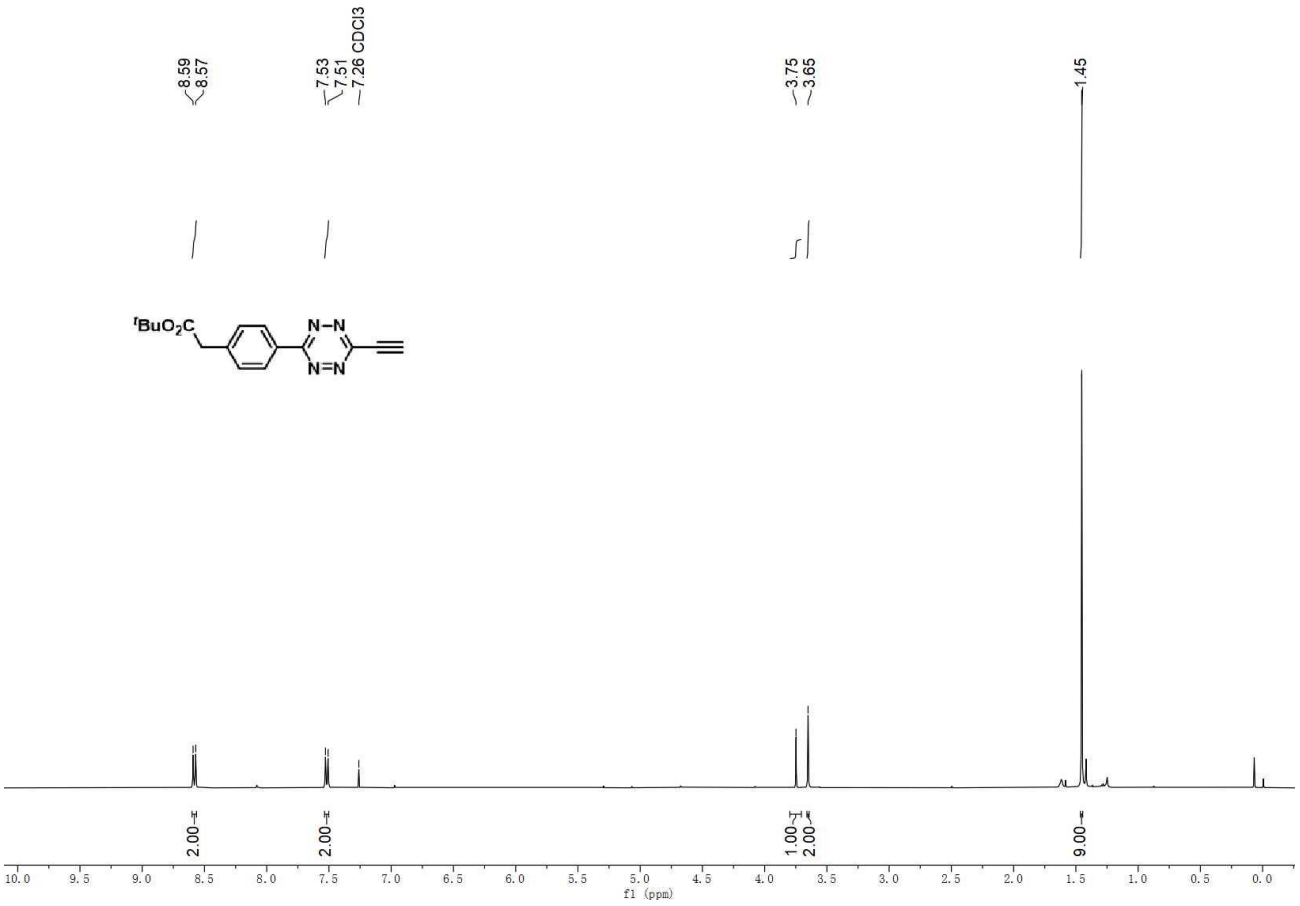

152.2
152.2
152.2
152.3
152.3
152.3
157.1
157.2
157.3
161.8
161.8
161.8
161.8
161.8
161.9
161.9
161.9
161.9
161.9
f1 (ppm)
f1 (ppm)

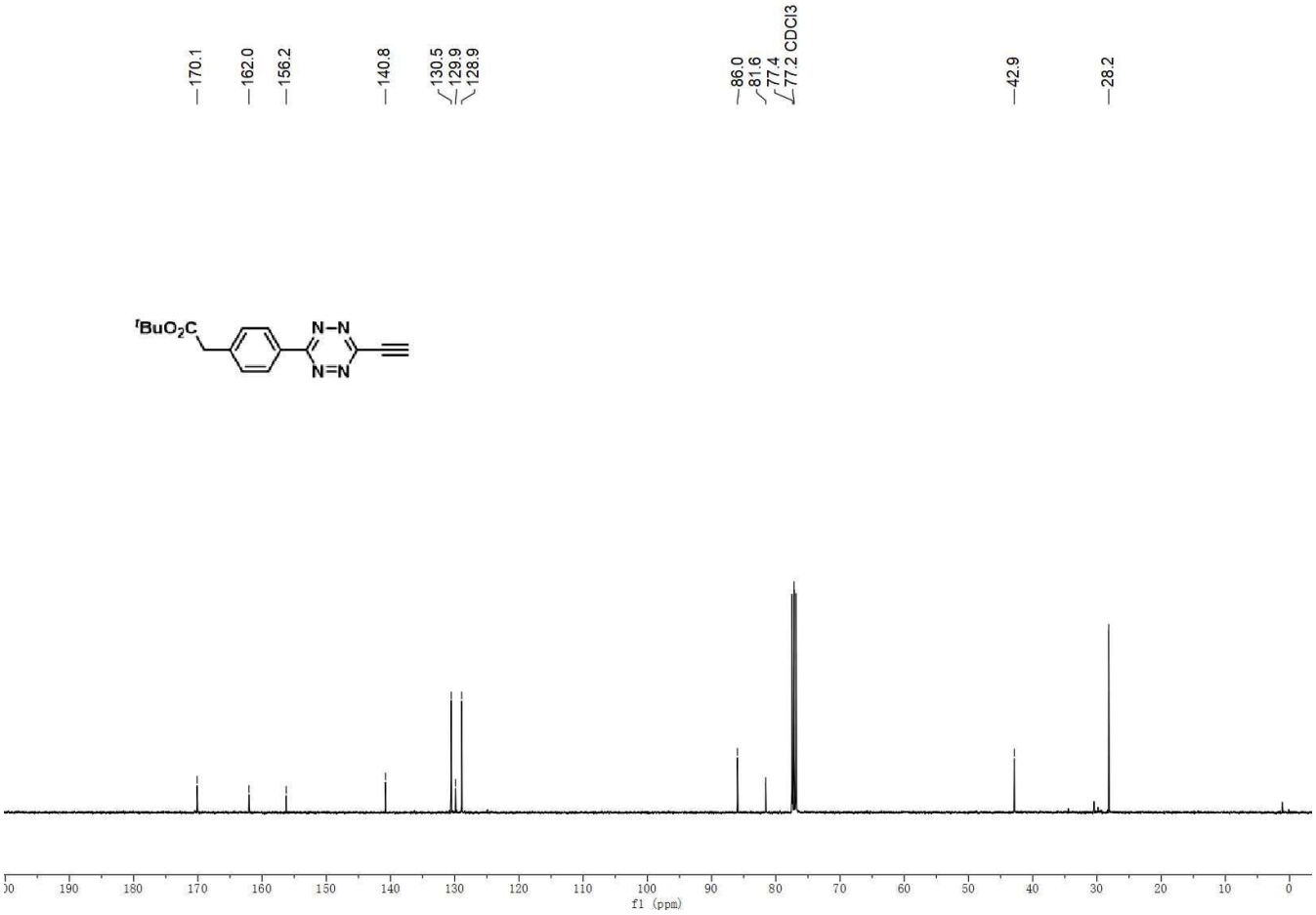

8.60
8.58
7.53
7.51
7.26 CDCl3
5.00
4.45
4.44
3.75
1.48
BocHN
N–N
N=N
2.00
2.00
1.00
2.00
1.00
9.00
f1 (ppm)

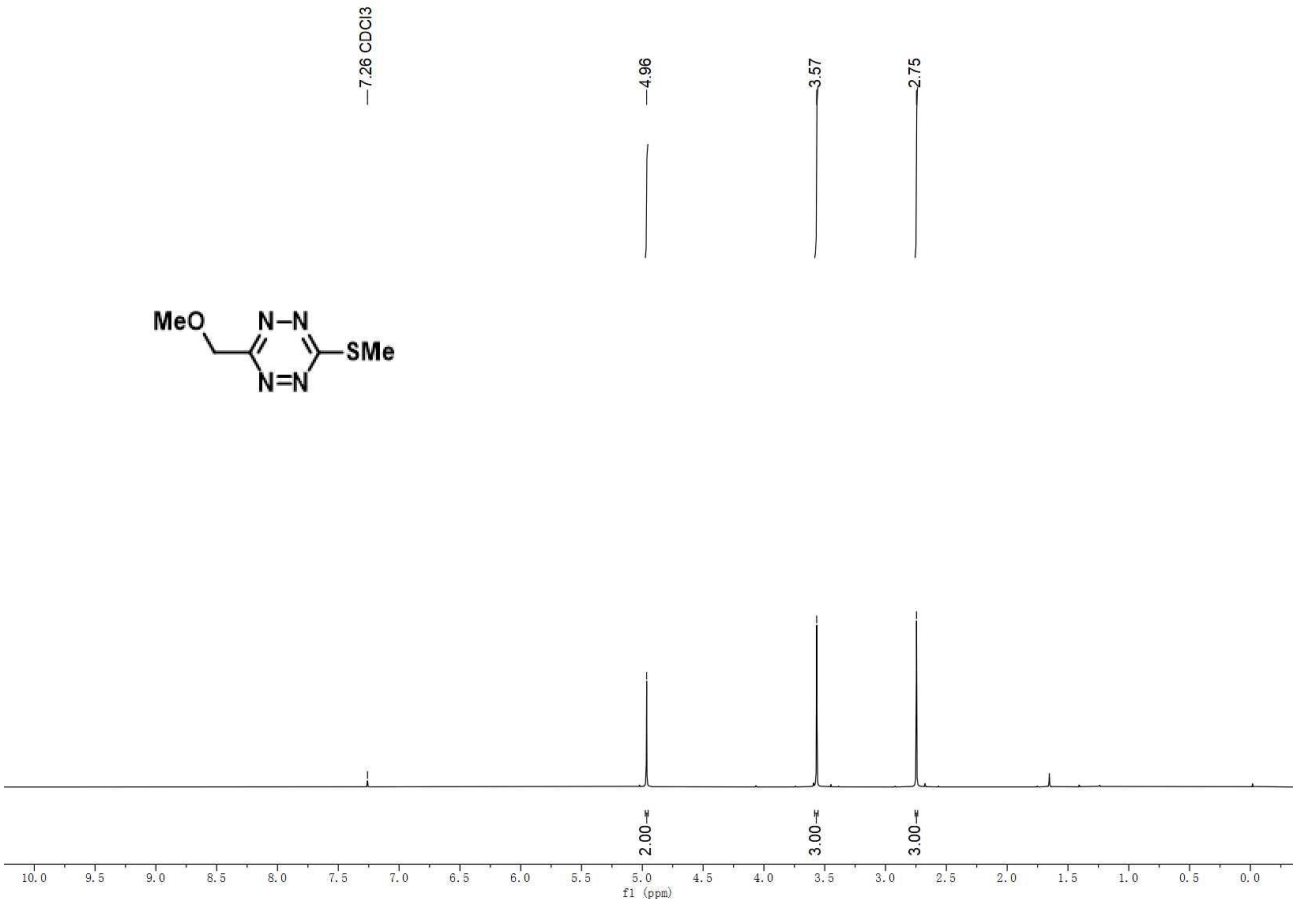

162.0
156.3
156.1
145.2
130.2
129.1
128.3
86.0
80.1
77.4
77.2 CDCl3
44.5
28.5
BocHN
N–N
N=N
f1 (ppm)

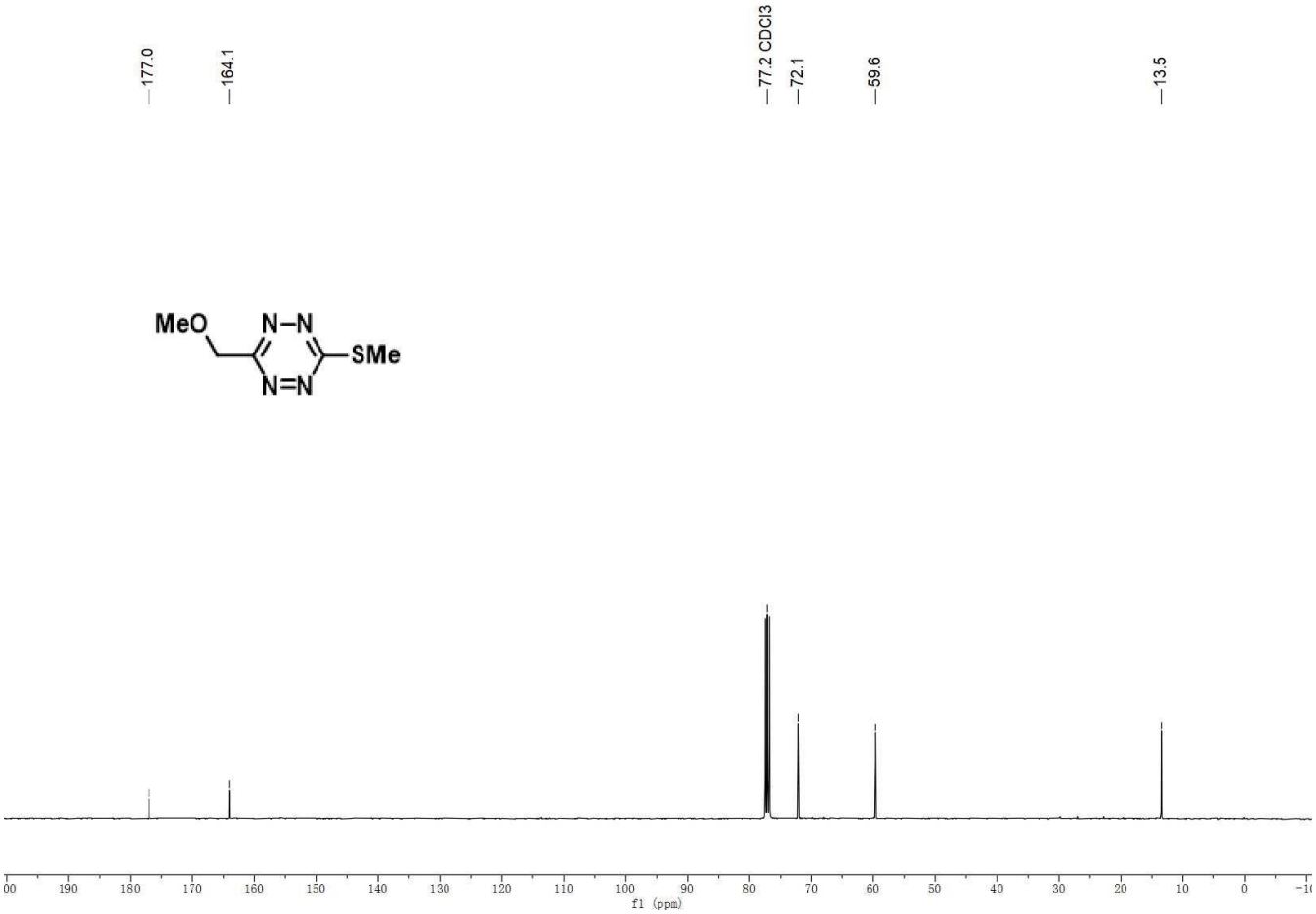

8.48
8.46
7.48
7.46
7.26 CDCl3
3.63
2.78
1.45
tBuO2C
N-N
N=N
SMe
2.00
2.00
2.00
3.00
9.00
f1 (ppm)

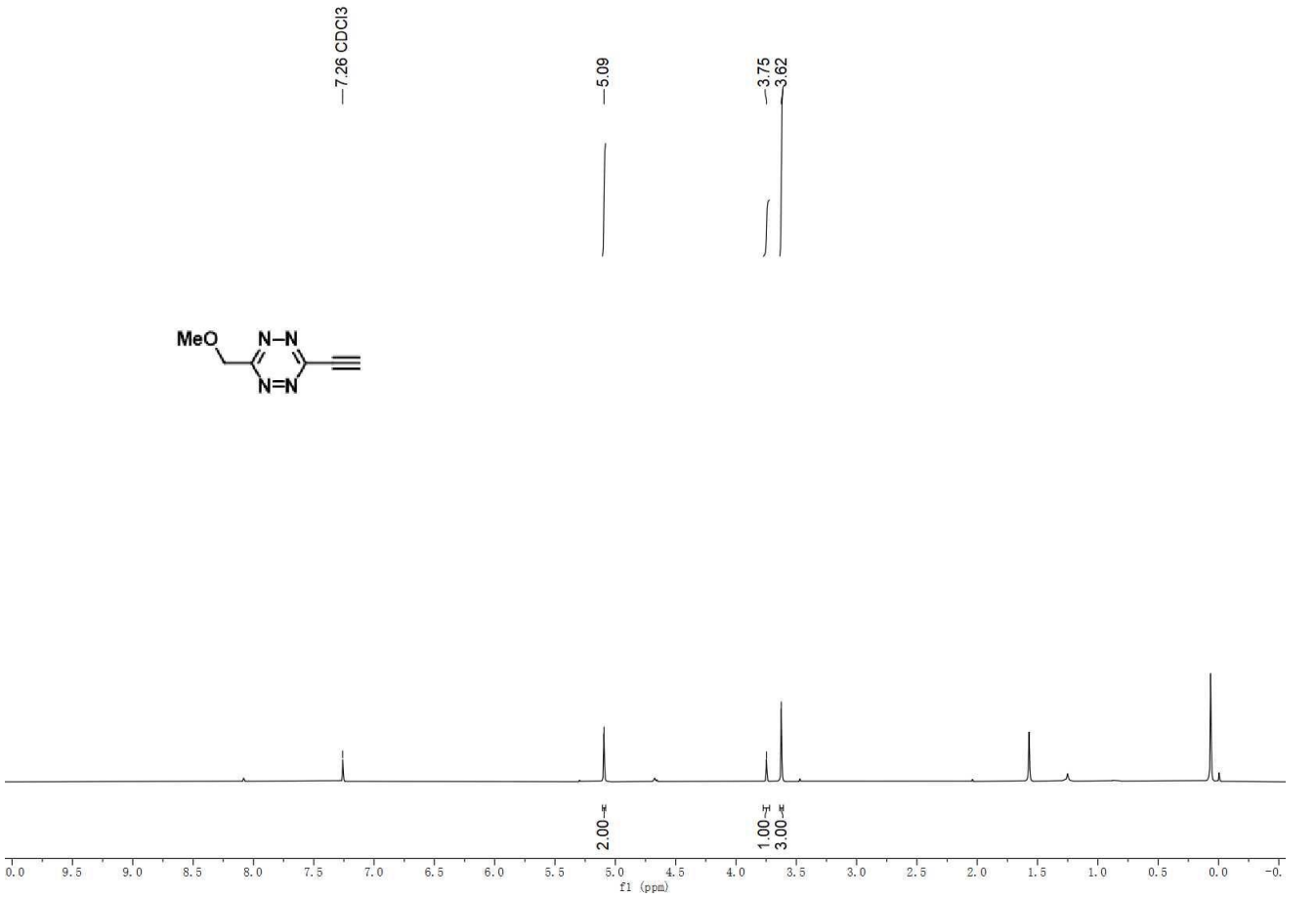

175.3
170.3
162.3
139.4
130.4
130.3
127.7
81.4
77.2 CDCl3
42.8
28.1
13.5
tBuO2C
N-N
N=N
SMe
f1 (ppm)

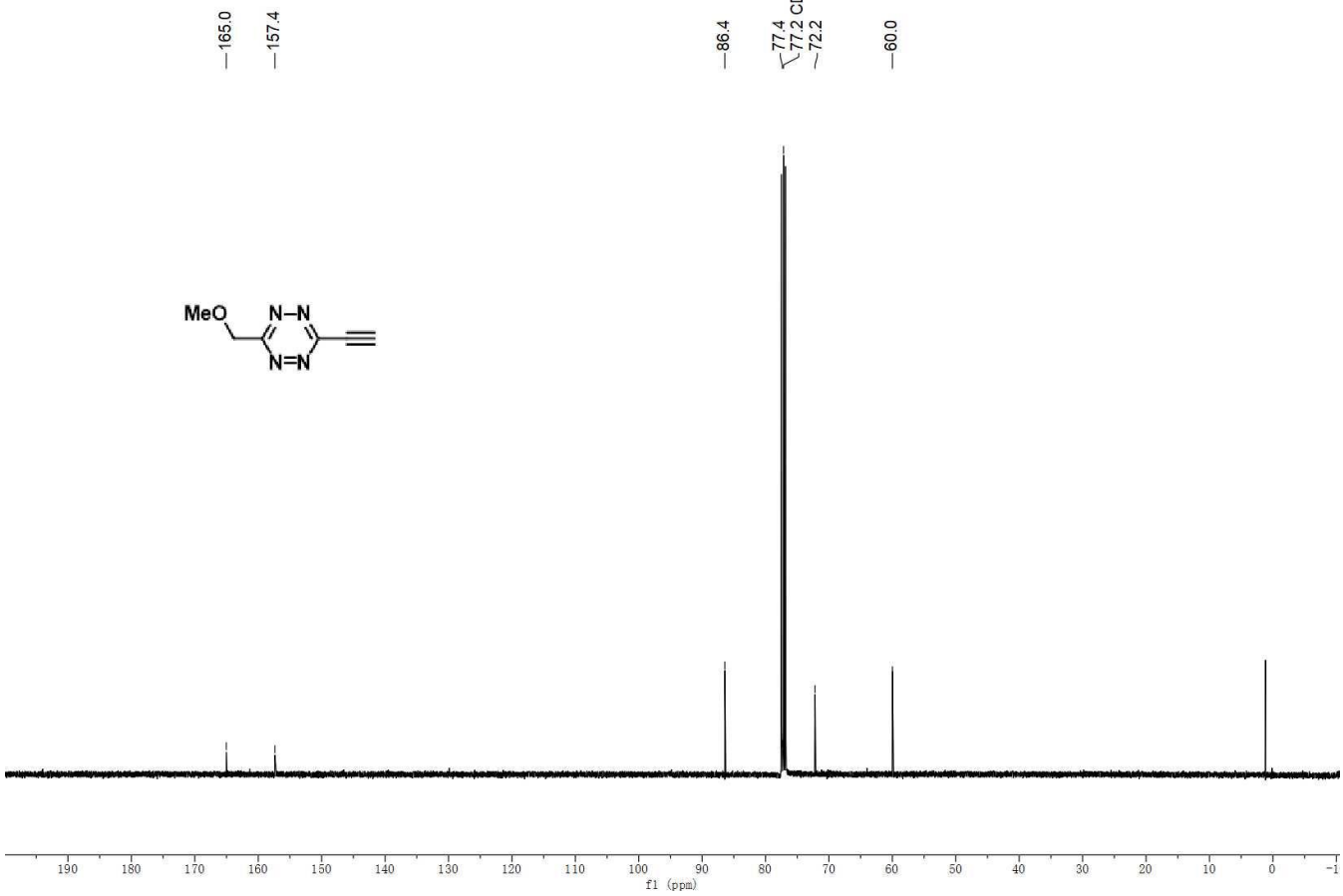

8.59
8.57
7.53
7.51
7.26 CDCl3
3.75
3.65
1.45
tBuO2C
N–N
N=N
2.00
2.00
1.00
2.00
9.00
f1 (ppm)

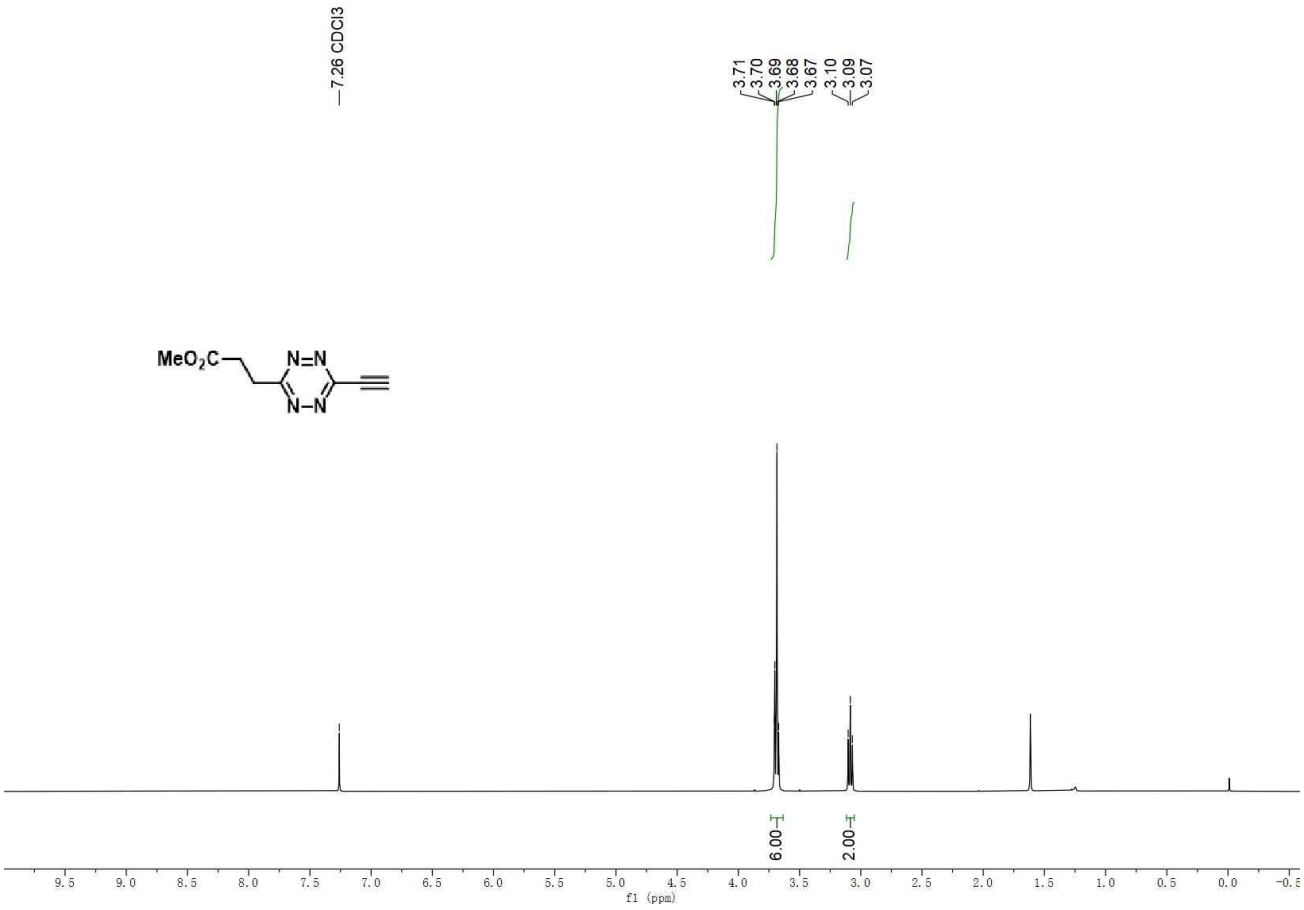

170.1
162.0
156.2
140.8
130.5
129.9
128.9
86.0
81.6
77.4
77.2 CDCl3
42.9
28.2
tBuO2C
N–N
N=N
f1 (ppm)

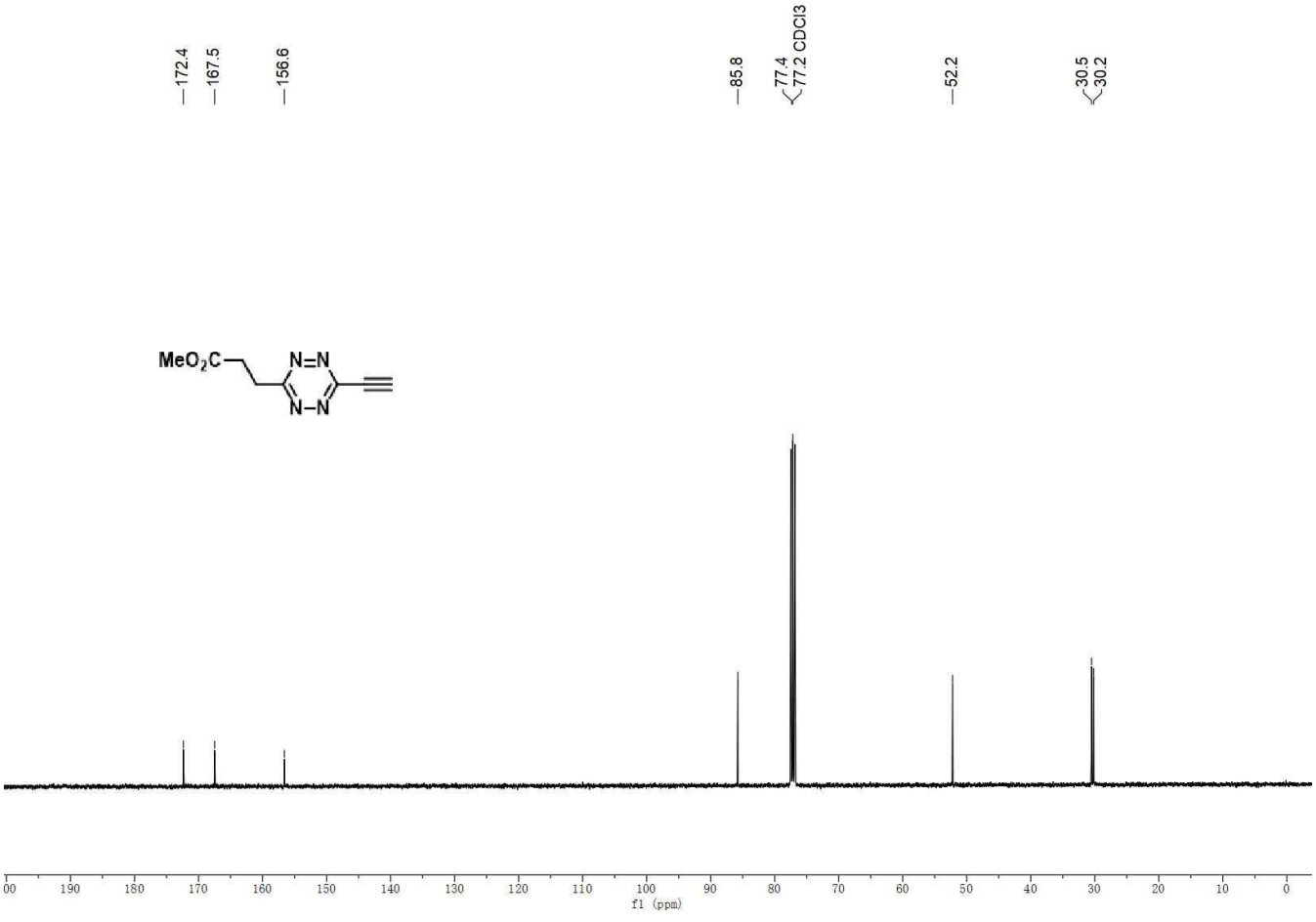

7.26 CDCl3
4.96
3.57
2.75
MeO
N–N
SMe
N=N
2.00
3.00
3.00
f1 (ppm)

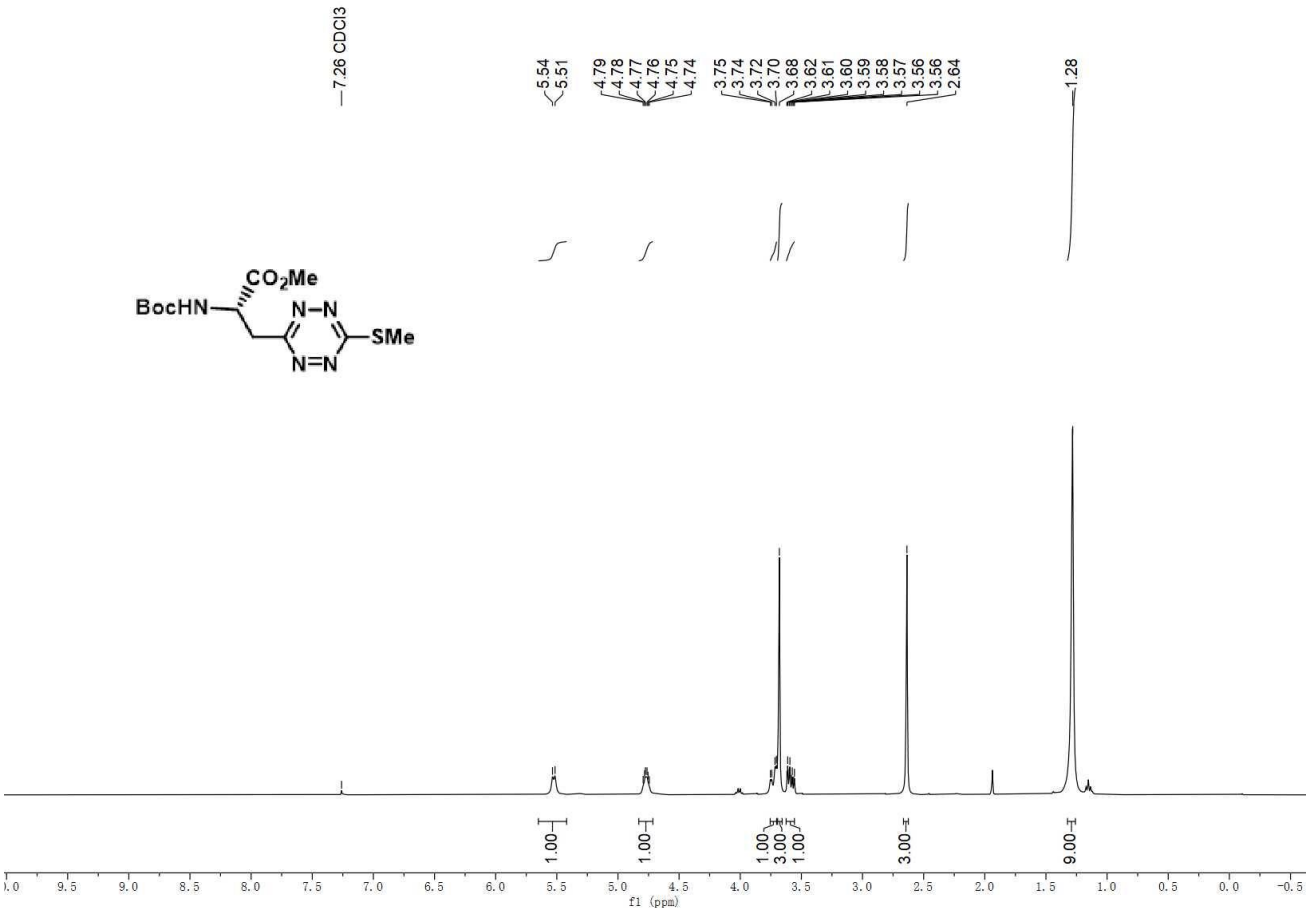

177.0
164.1
77.2 CDCl3
72.1
59.6
13.5
MeO
N–N
SMe
N=N
f1 (ppm)

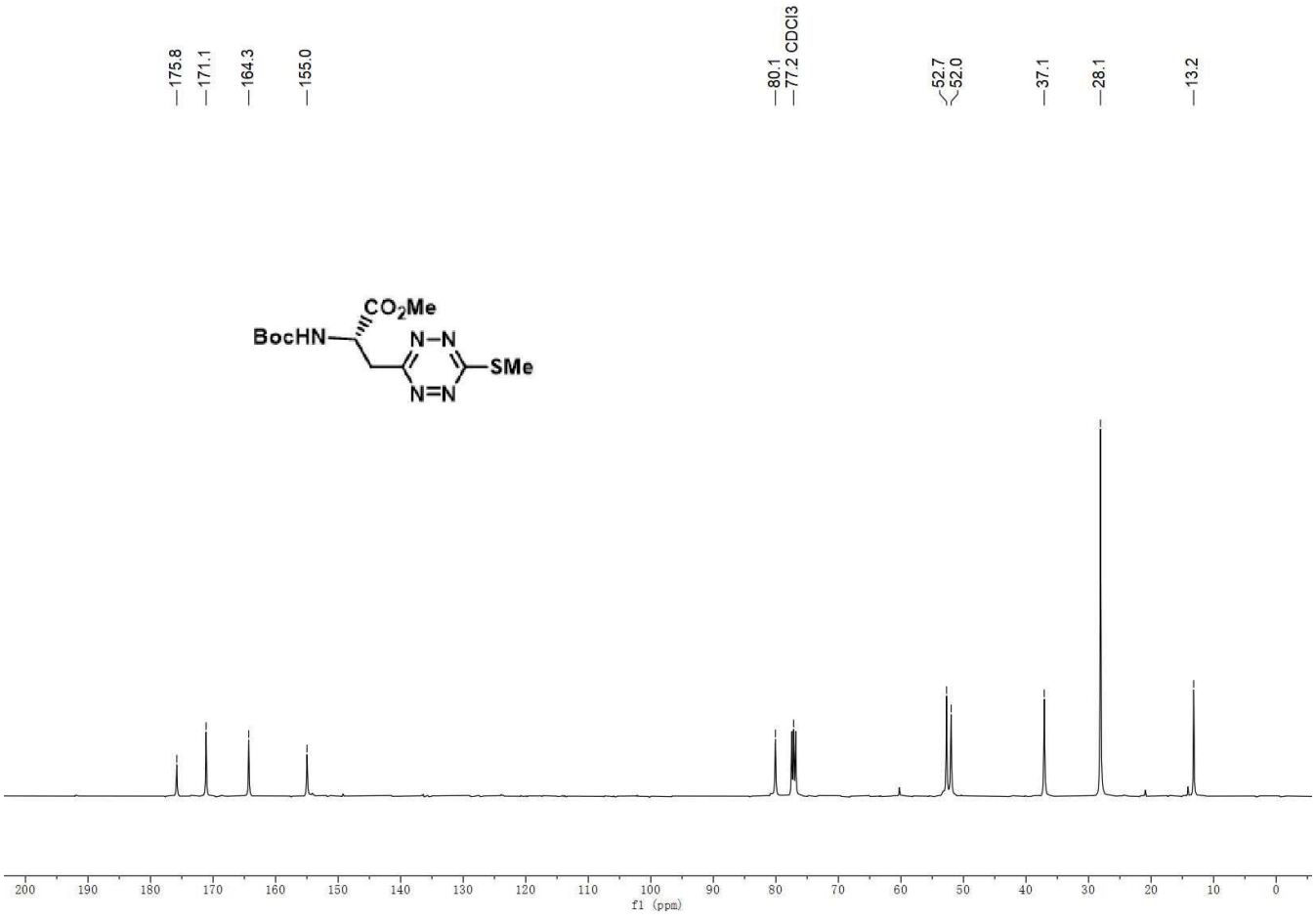

7.26 CDCl3
5.09
3.75
3.62
MeO
N–N
N=N
2.00
1.00
3.00
f1 (ppm)

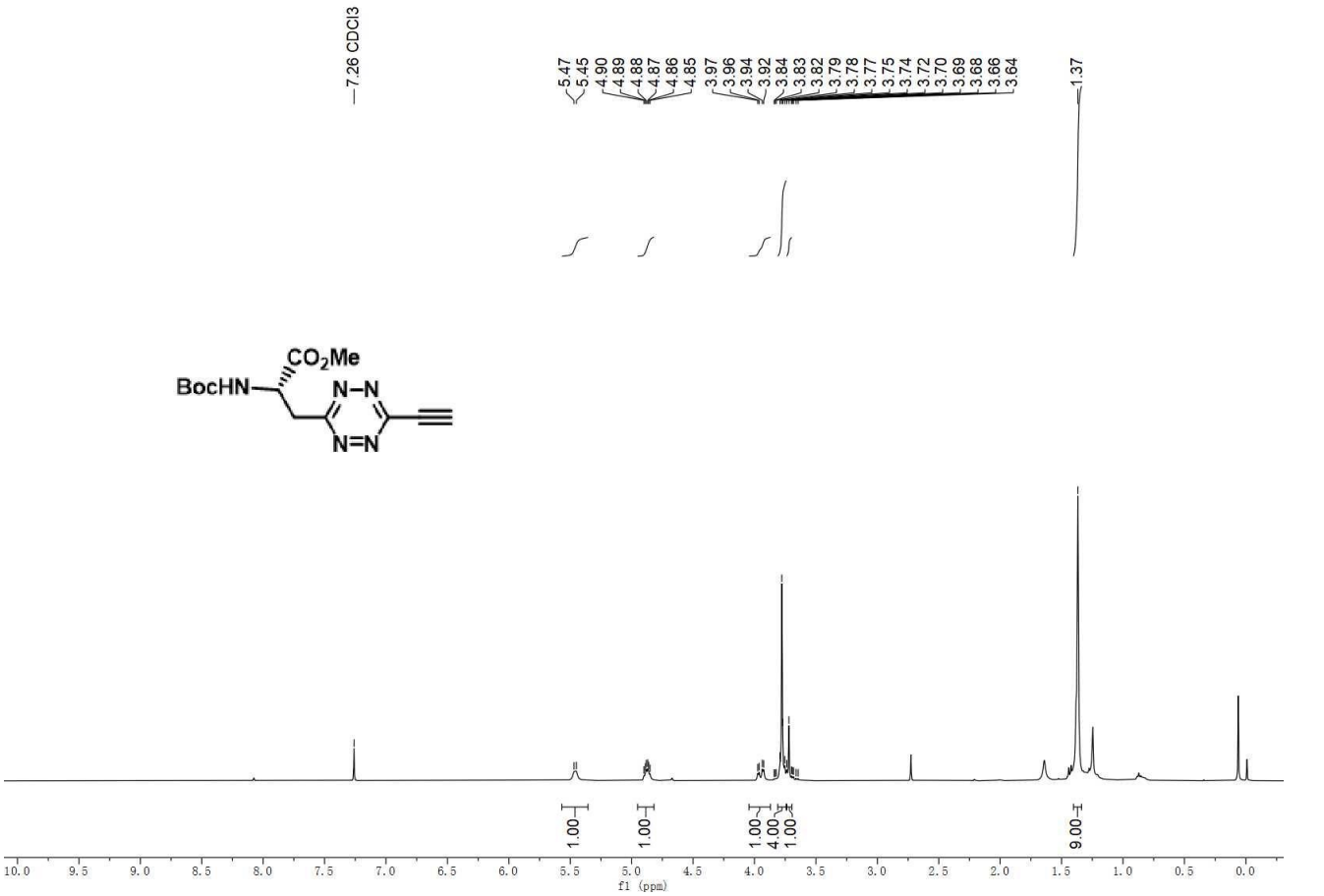

165.0
157.4
86.4
77.4
77.2 CDCl3
72.2
60.0
MeO
N–N
N=N
f1 (ppm)

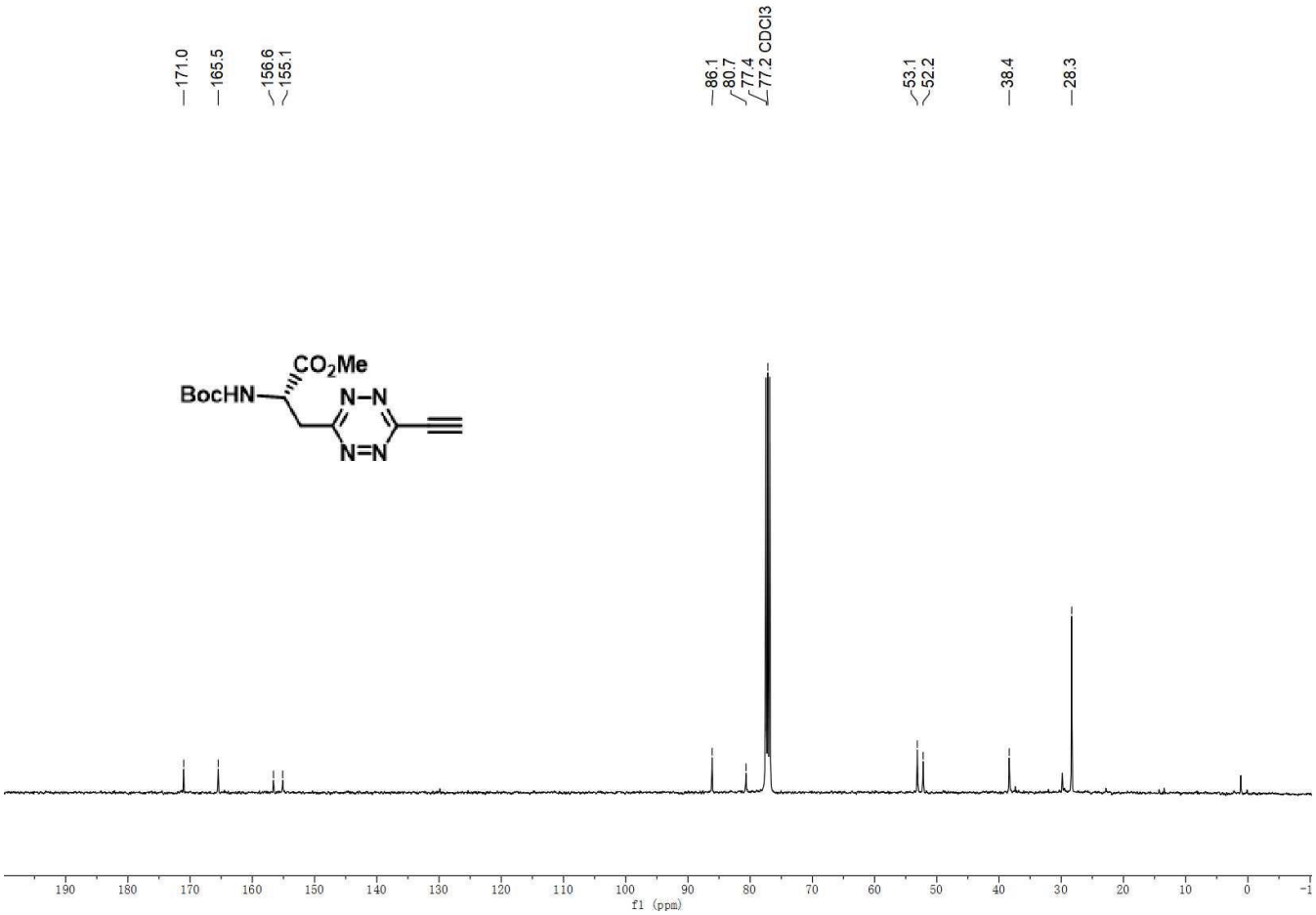

7.26 CDCl3
3.71
3.70
3.69
3.68
3.67
3.10
3.09
3.07
MeO2C
N=N
N-N
6.00
2.00
f1 (ppm)

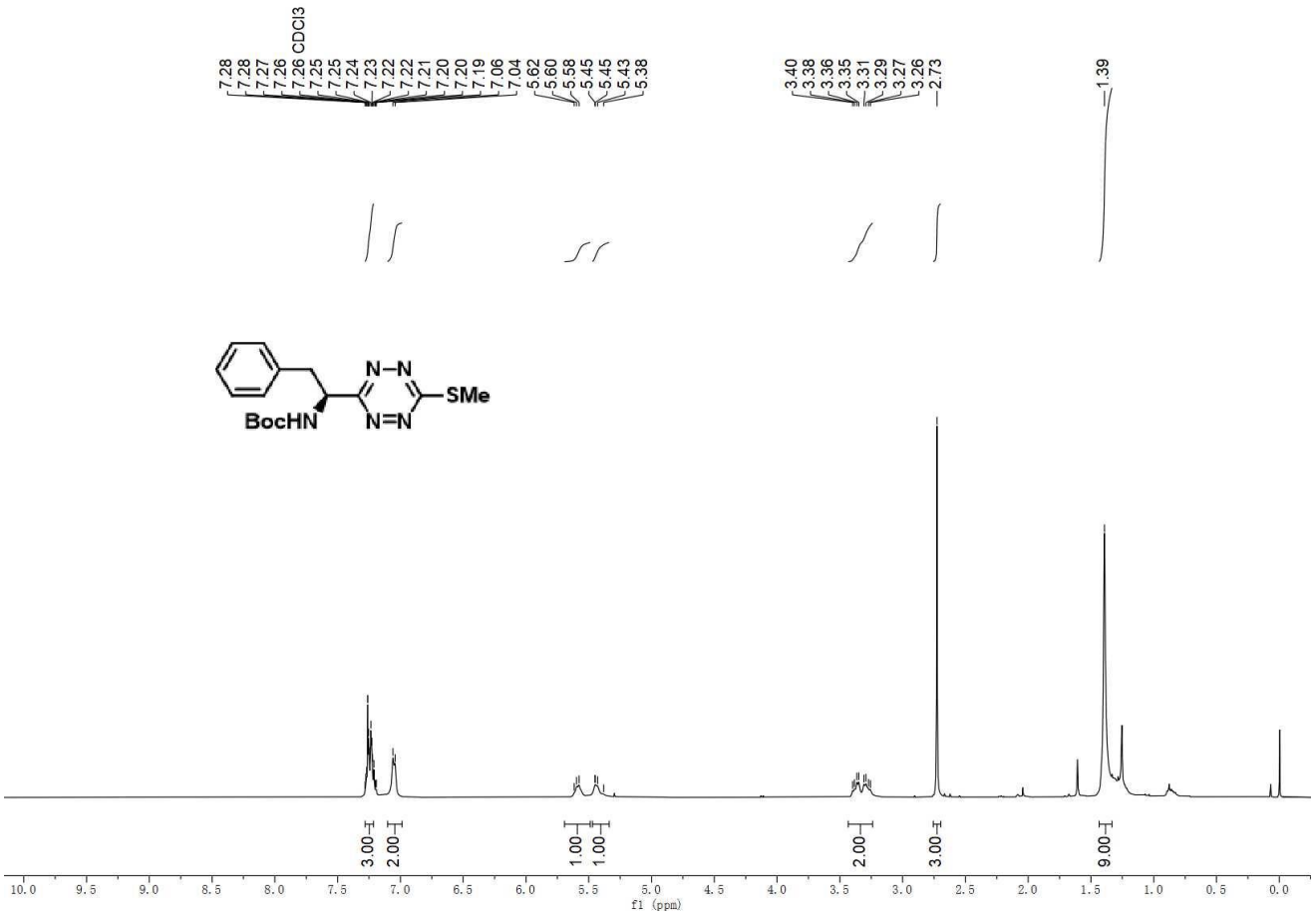

172.4
167.5
156.6
85.8
77.4
77.2 CDCl3
52.2
30.5
30.2
MeO2C
N=N
N-N
f1 (ppm)

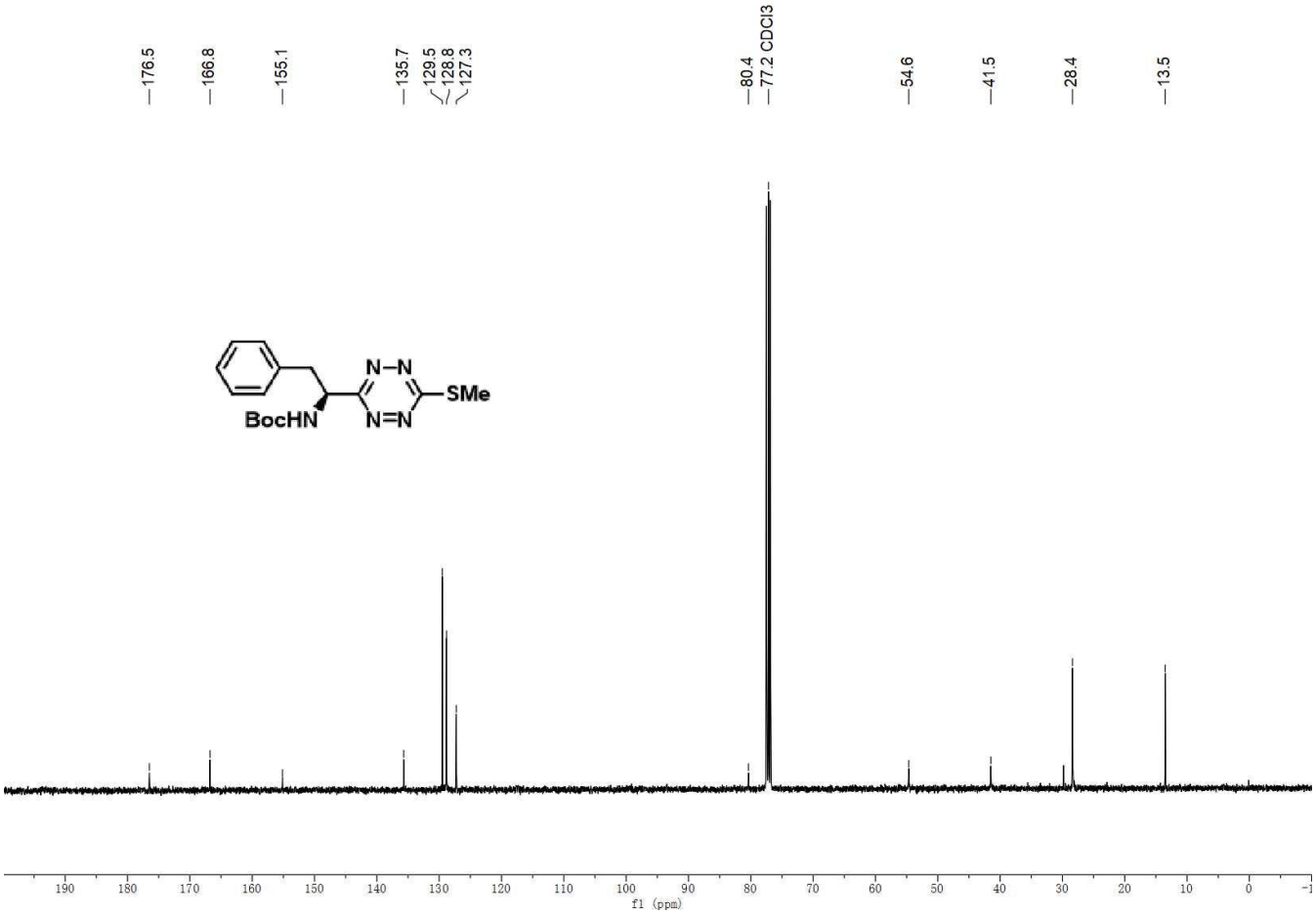

7.26 CDCl3
5.54
5.51
4.79
4.78
4.77
4.76
4.75
4.74
3.75
3.74
3.72
3.70
3.68
3.62
3.61
3.60
3.59
3.58
3.57
3.56
3.56
2.64
1.28
CO2Me
BocHN
N–N
SMe
N=N
1.00
1.00
1.00
3.00
1.00
3.00
9.00
f1 (ppm)

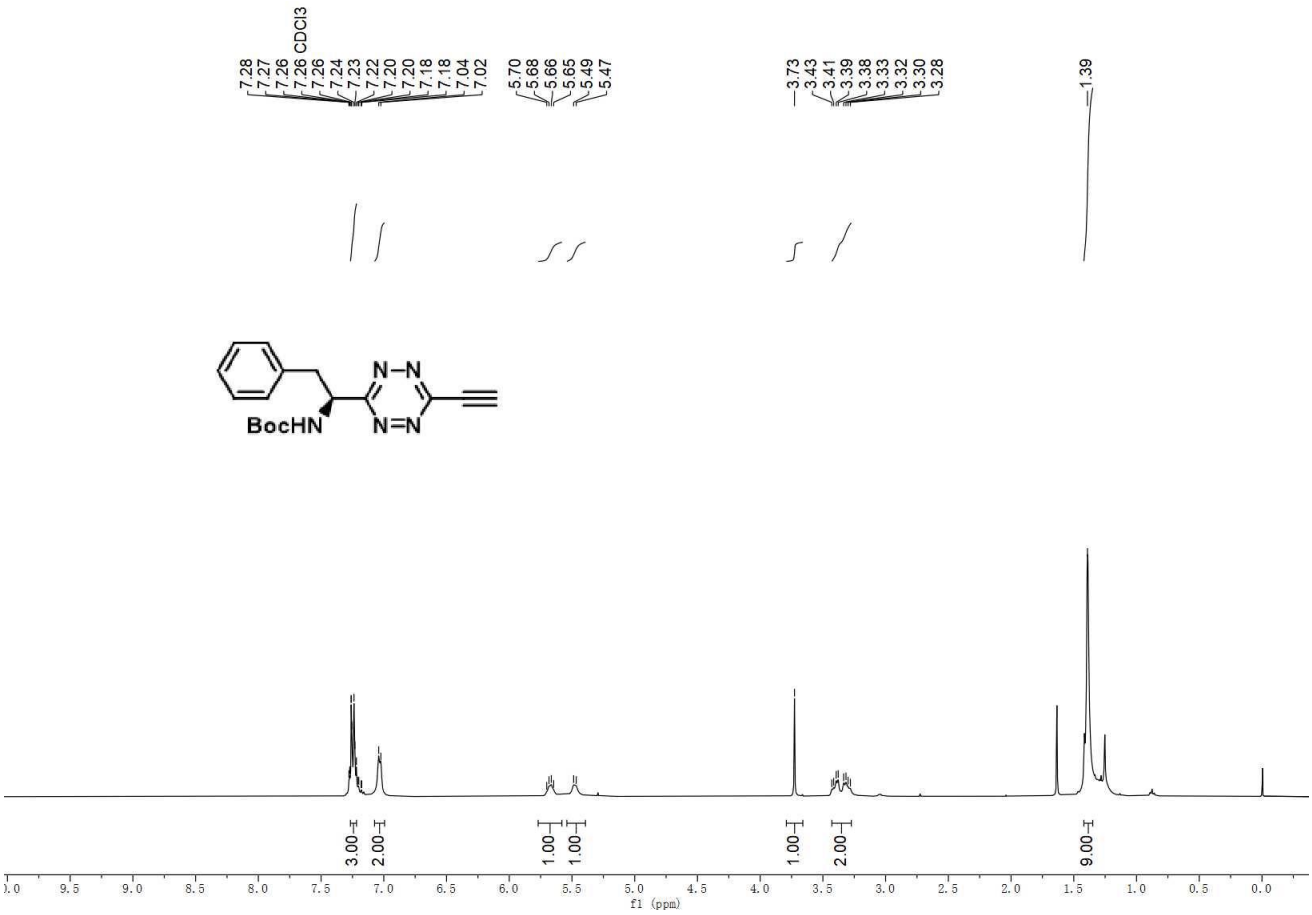

175.8
171.1
164.3
155.0
80.1
77.2 CDCl3
52.7
52.0
37.1
28.1
13.2
CO2Me
BocHN
N–N
SMe
N=N
f1 (ppm)

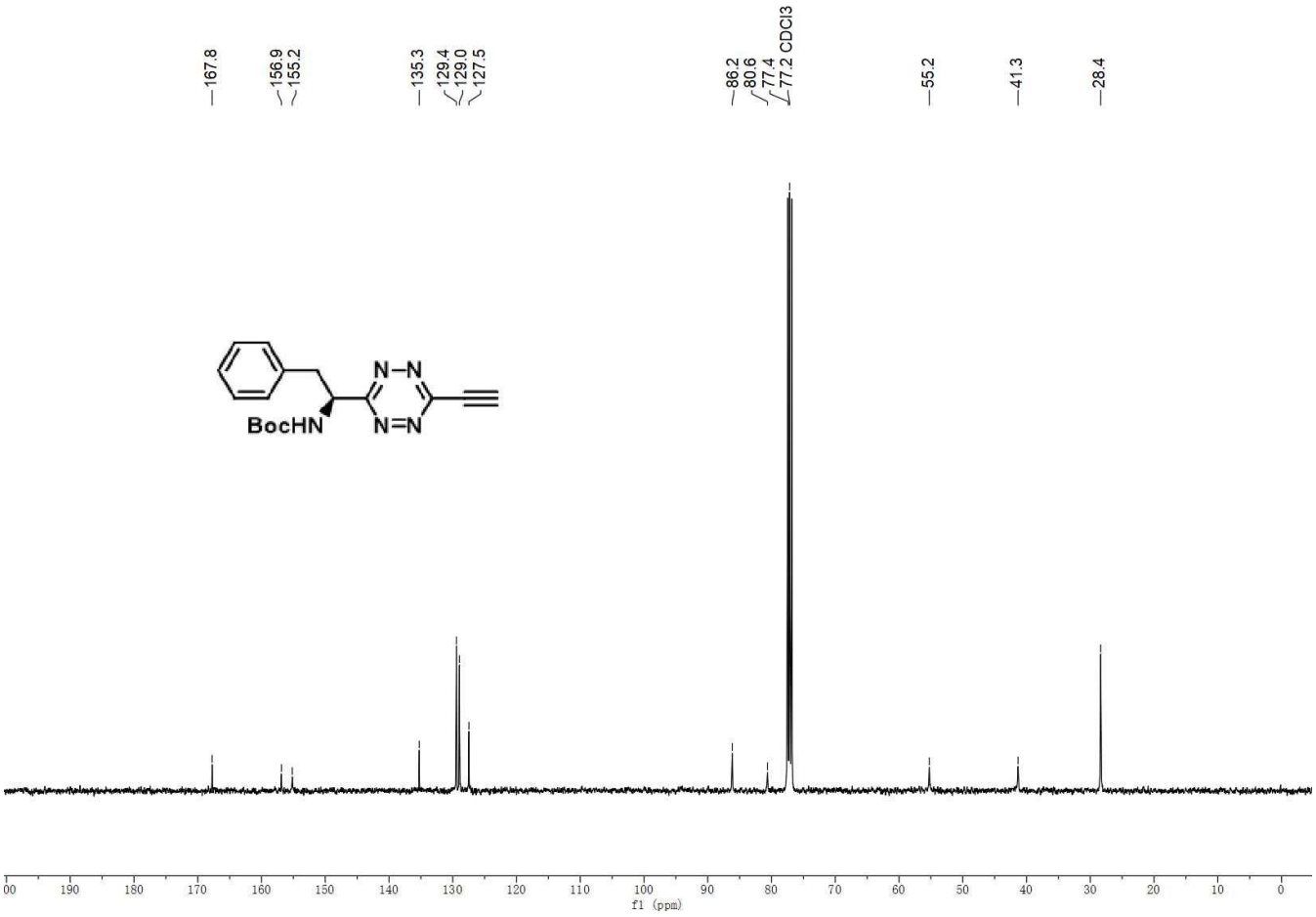

7.26 CDCl3
5.47
5.45
4.90
4.89
4.88
4.87
4.86
4.85
3.97
3.96
3.94
3.92
3.84
3.83
3.82
3.79
3.78
3.77
3.75
3.74
3.72
3.70
3.69
3.68
3.66
3.64
1.37
CO2Me
BocHN
N–N
N=N
1.00
1.00
1.00
4.00
1.00
9.00
f1 (ppm)

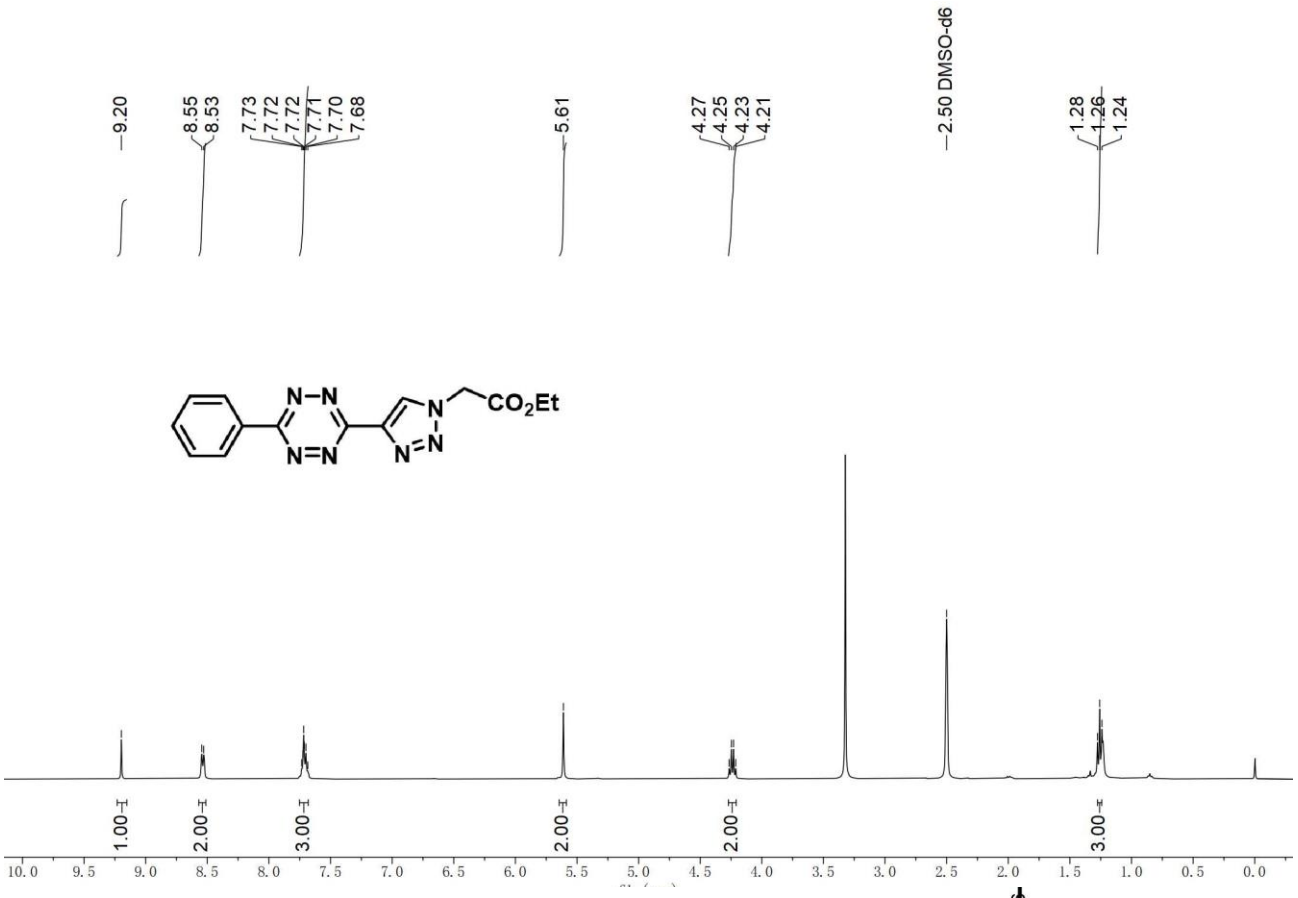

171.0
165.5
156.6
155.1
86.1
80.7
77.4
77.2 CDCl3
53.1
52.2
38.4
28.3
CO2Me
BocHN
N–N
N=N
f1 (ppm)

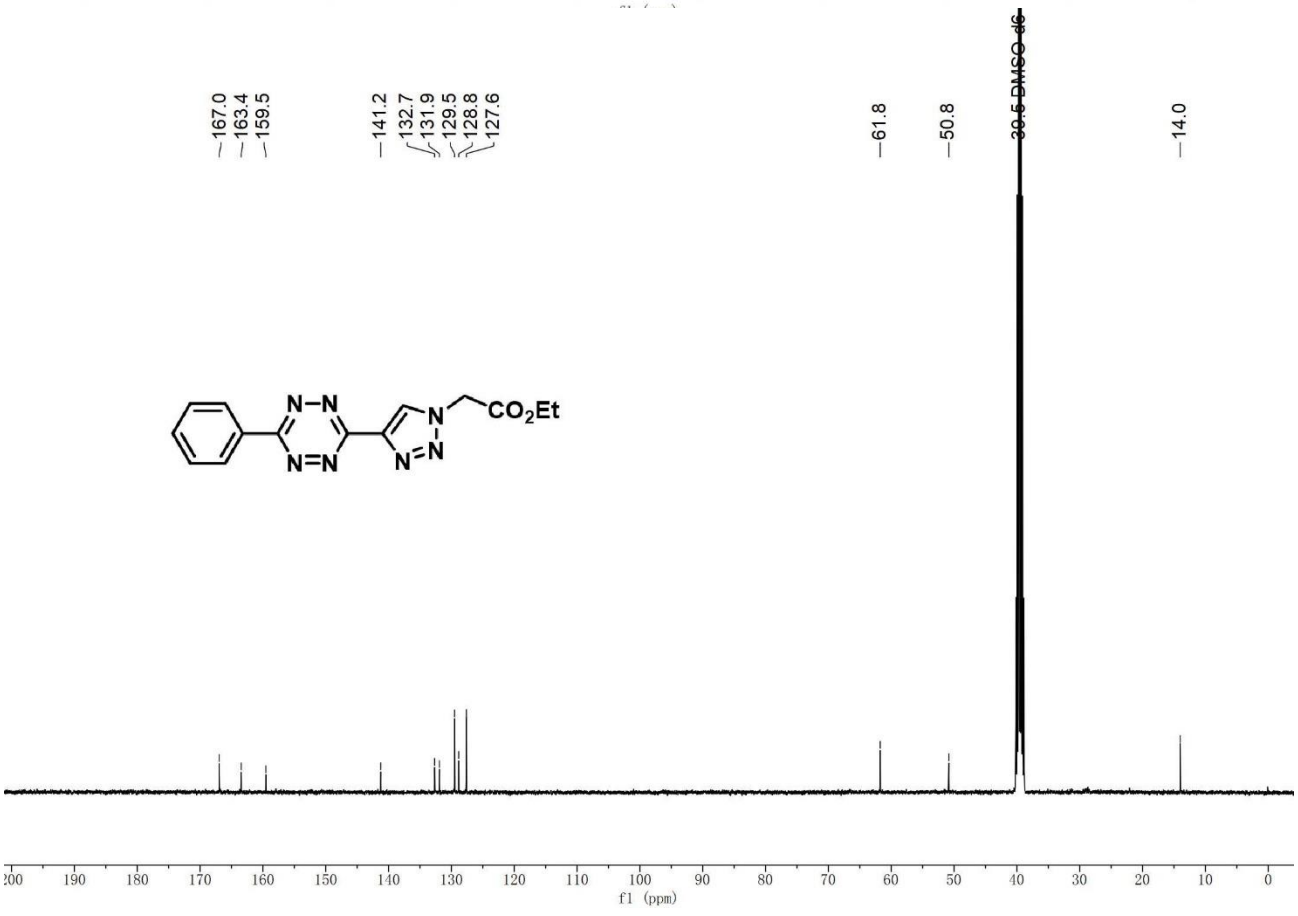

7.28
7.28
7.27
7.26
7.26 CDCl3
7.25
7.25
7.24
7.23
7.22
7.22
7.21
7.20
7.20
7.19
7.06
7.04
5.62
5.60
5.58
5.45
5.45
5.43
5.38
3.40
3.38
3.36
3.35
3.31
3.29
3.27
3.26
2.73
1.39
BocHN
N–N
N=N
SMe
3.00
2.00
1.00
1.00
2.00
3.00
9.00
f1 (ppm)

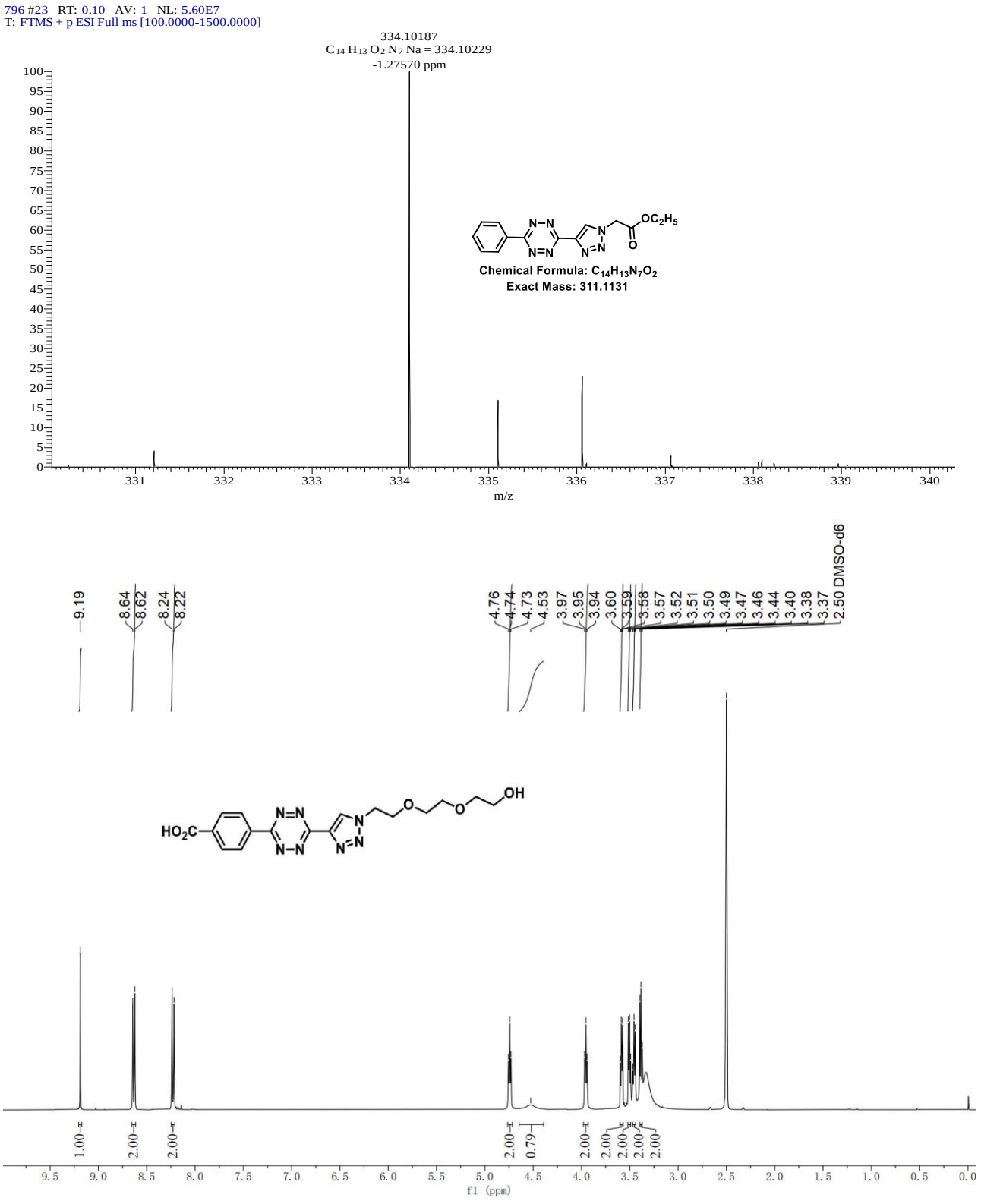

176.5
166.8
155.1
135.7
129.5
128.8
127.3
80.4
77.2 CDCl3
54.6
41.5
28.4
13.5
BocHN
N–N
N=N
SMe
f1 (ppm)

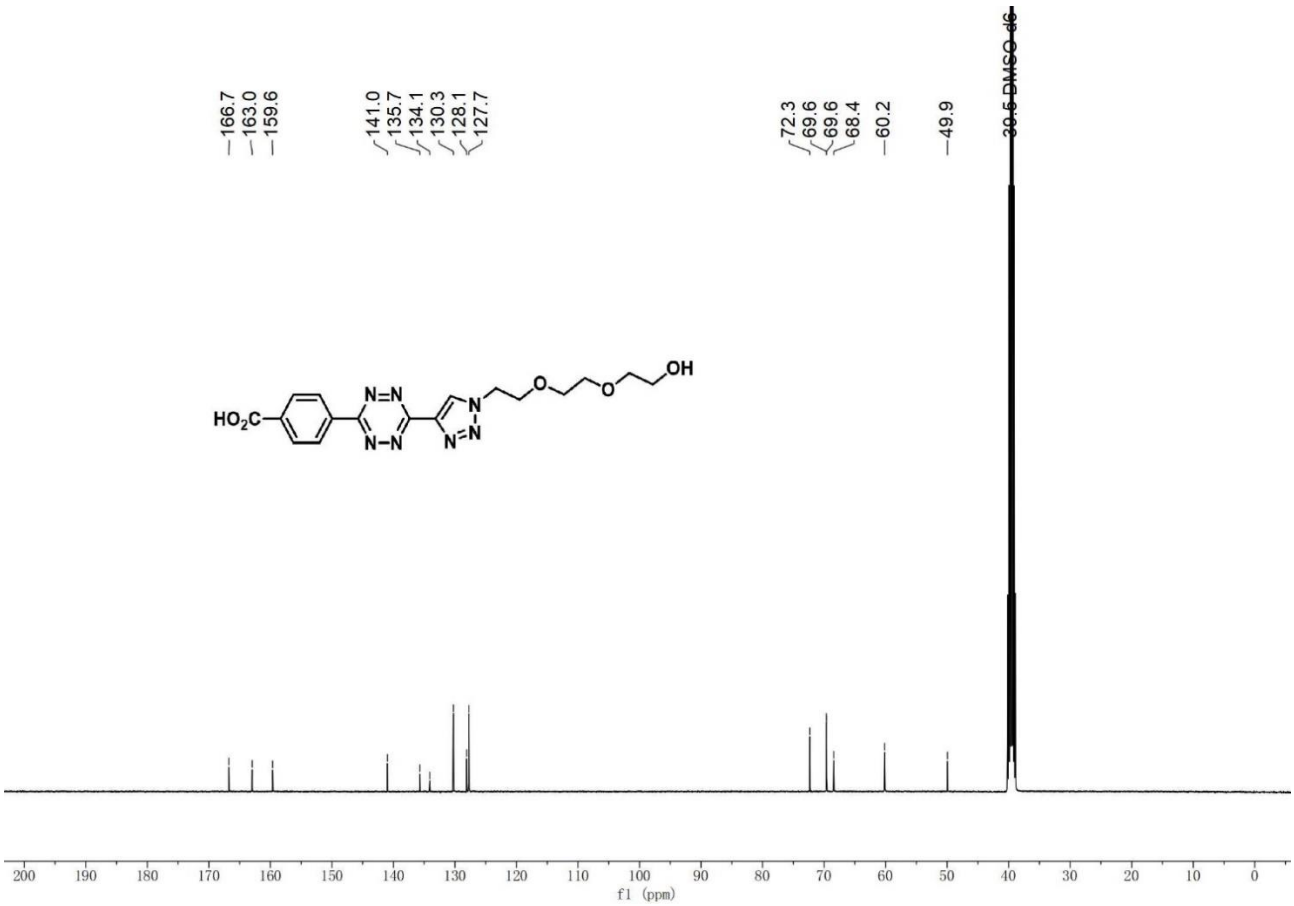

7.28
7.27
7.26
7.26 CDCl3
7.26
7.24
7.23
7.22
7.20
7.20
7.18
7.18
7.04
7.02
5.70
5.68
5.66
5.65
5.49
5.47
3.73
3.43
3.41
3.39
3.38
3.33
3.32
3.30
3.28
1.39
BocHN
N–N
N=N
3.00
2.00
1.00
1.00
1.00
2.00
9.00
f1 (ppm)

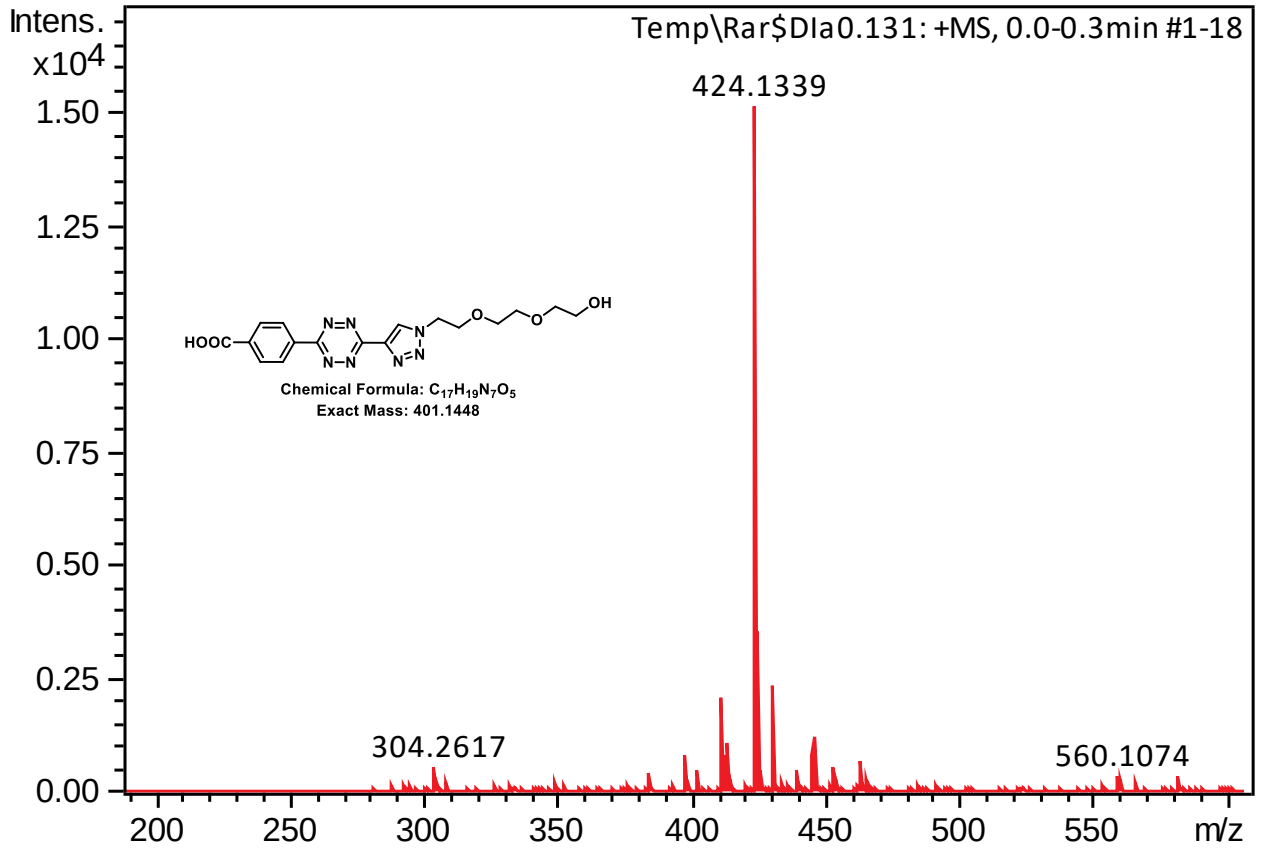

167.8
156.9
155.2
135.3
129.4
129.0
127.5
86.2
80.6
77.4
77.2 CDCl3
55.2
41.3
28.4
BocHN
N–N
N=N
f1 (ppm)

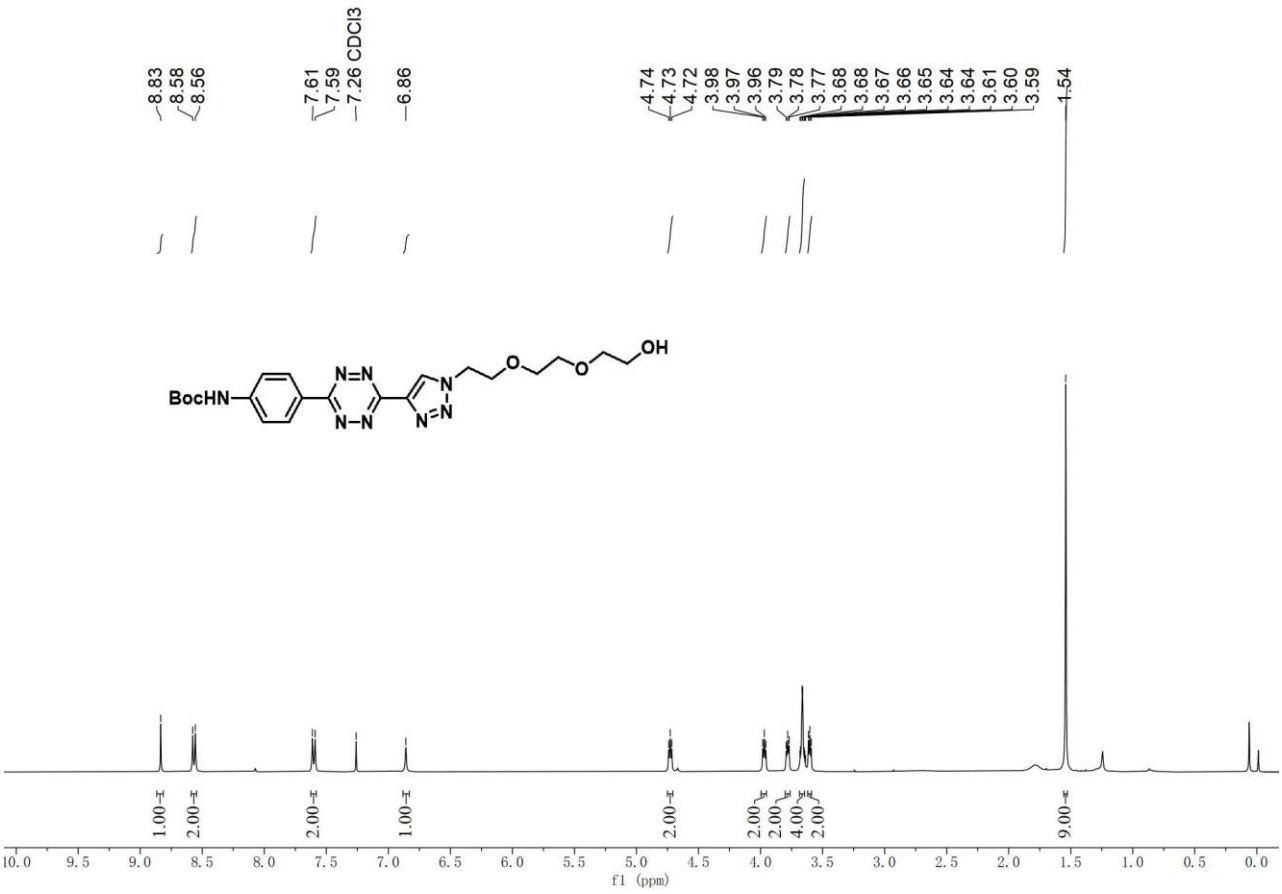

9.20
8.55
8.53
7.73
7.72
7.72
7.71
7.70
7.68
5.61
4.27
4.25
4.23
4.21
2.50 DMSO-d6
1.28
1.26
1.24
CO2Et
1.00
2.00
3.00
2.00
2.00
3.00

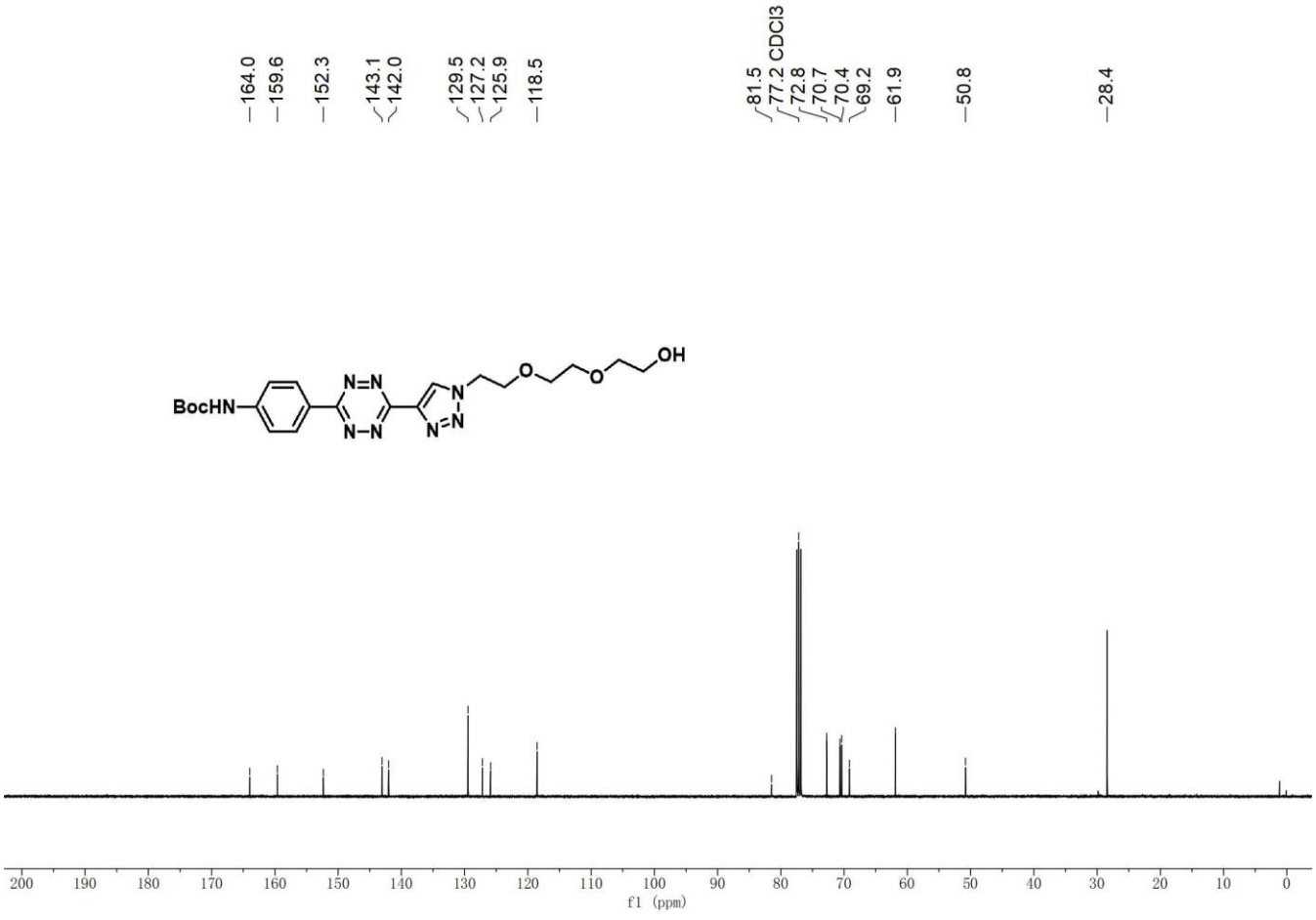

167.0
163.4
159.5
141.2
132.7
131.9
129.5
128.8
127.6
61.8
50.8
39.5 DMSO-d6
14.0
CO2Et
f1 (ppm)

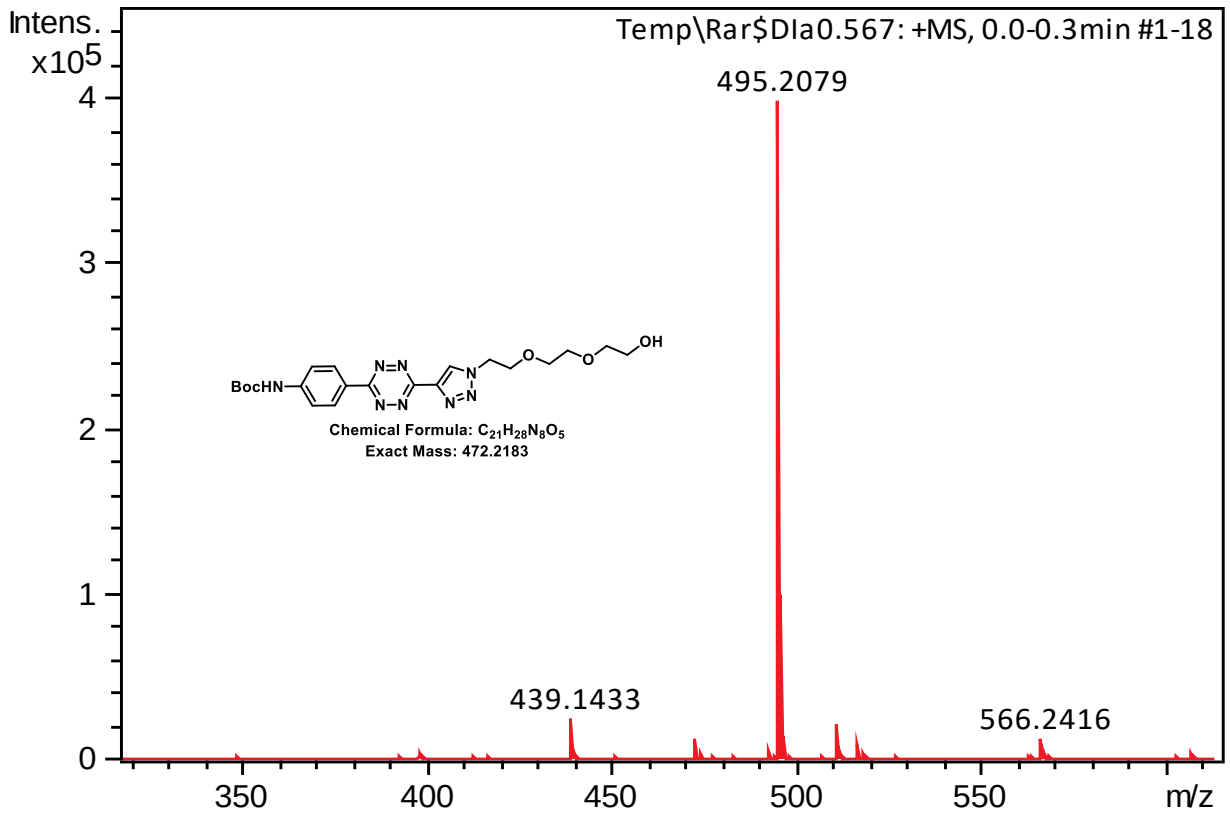

796 #23 RT: 0.10 AV: 1 NL: 5.60E7
T: FTMS + p ESI Full ms [100.0000-1500.0000]
334.10187
C14 H13 O2 N7 Na = 334.10229
-1.27570 ppm
Chemical Formula: C14H13N7O2
Exact Mass: 311.1131
m/z
9.19
8.64
8.62
8.24
8.22
4.76
4.74
4.73
4.53
3.97
3.95
3.94
3.60
3.59
3.58
3.57
3.52
3.51
3.50
3.49
3.47
3.46
3.44
3.40
3.38
3.37
2.50 DMSO-d6
HO2C
OH
1.00
2.00
2.00
2.00
0.79
2.00
2.00
2.00
2.00
2.00
f1 (ppm)

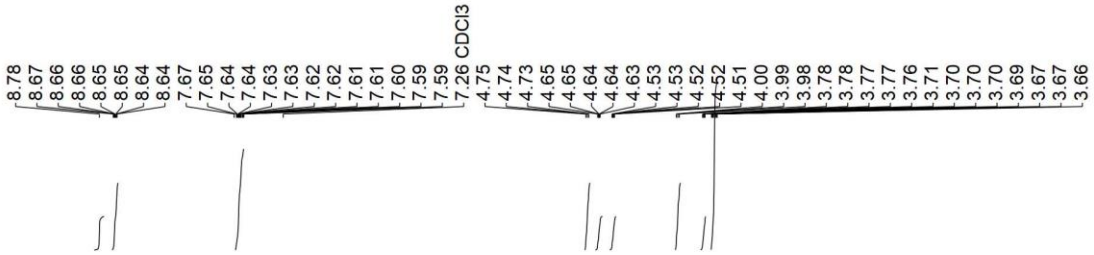

166.7
163.0
159.6
141.0
135.7
134.1
130.3
128.1
127.7
72.3
69.6
69.6
68.4
60.2
49.9
39.5 DMSO-d6
HO2C
N=N
N-N
N=N
N
OH
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

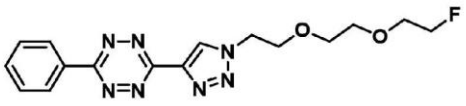

Intens.
x10^4
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Temp\Rar$DIa0.131: +MS, 0.0-0.3min #1-18
424.1339
304.2617
560.1074
HOOC
OH
Chemical Formula: C17H19N7O5
Exact Mass: 401.1448
200 250 300 350 400 450 500 550 m/z

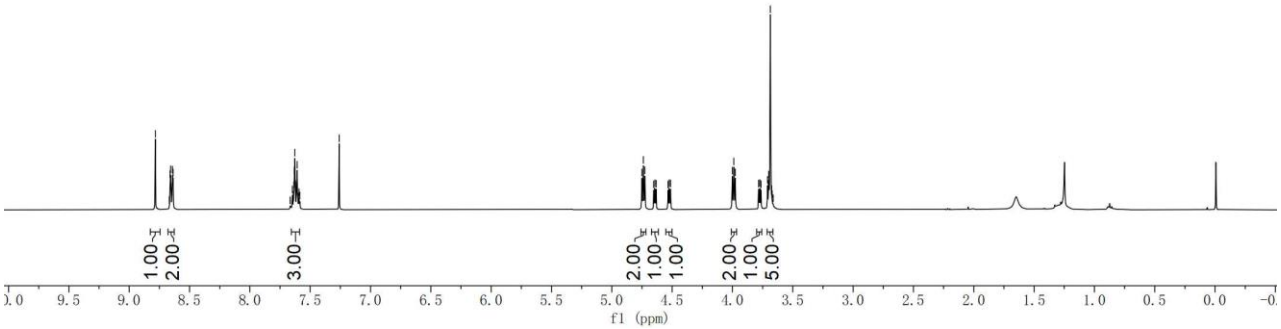

8.83
8.58
8.56
7.61
7.59
7.26 CDCl3
6.86
4.74
4.73
4.72
3.98
3.97
3.96
3.79
3.78
3.77
3.68
3.68
3.67
3.66
3.65
3.64
3.64
3.61
3.60
3.59
1.54
BocHN
OH
1.00
2.00
2.00
1.00
2.00
2.00
2.00
4.00
2.00
9.00
f1 (ppm)

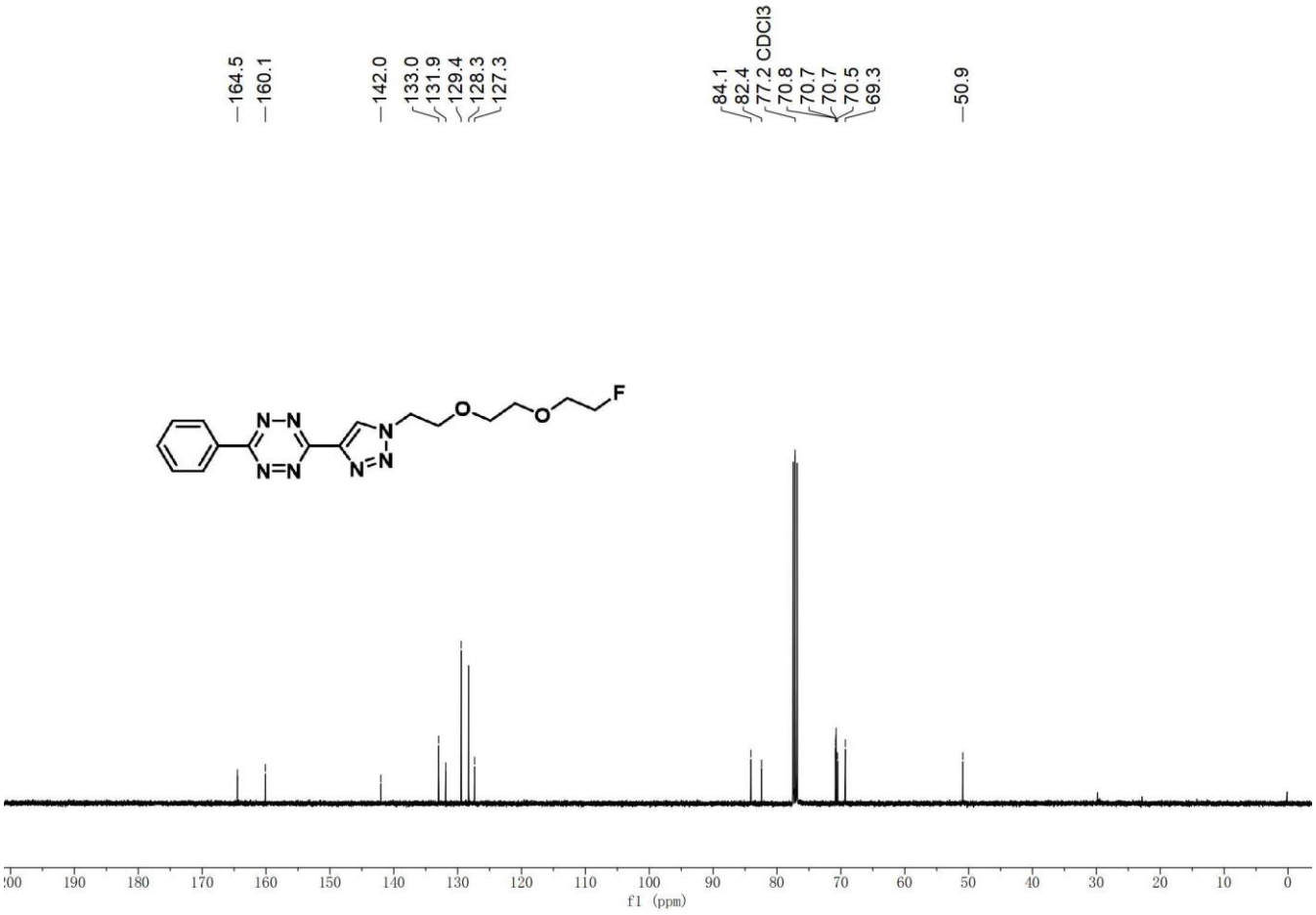

164.0
159.6
152.3
143.1
142.0
129.5
127.2
125.9
118.5
81.5
77.2 CDCl3
72.8
70.7
70.4
69.2
61.9
50.8
28.4
BocHN
OH
f1 (ppm)

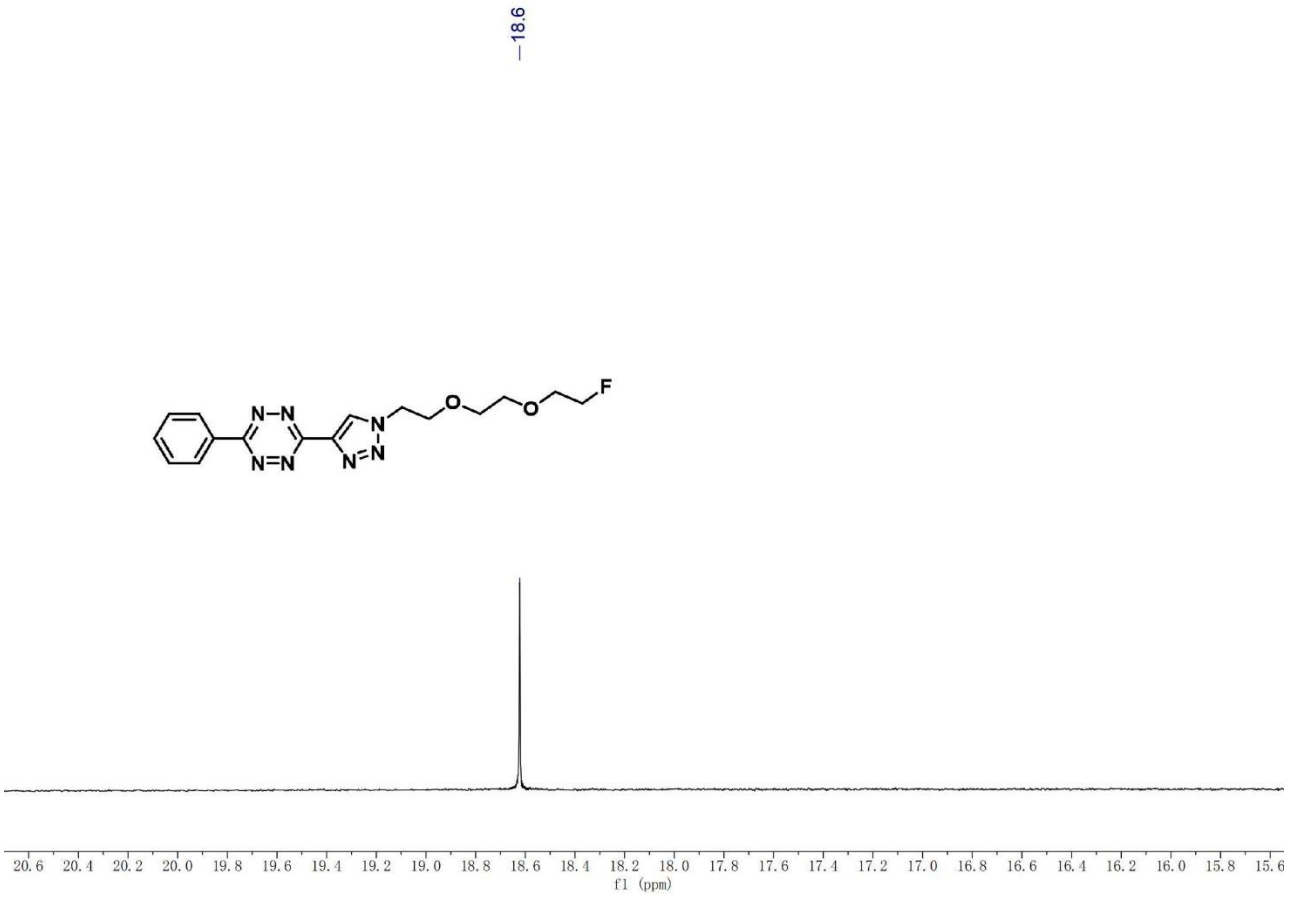

Intens.
x10^5
Temp\Rar$DIa0.567: +MS, 0.0-0.3min #1-18
495.2079
439.1433
566.2416
BocHN
OH
Chemical Formula: C21H28N8O5
Exact Mass: 472.2183
m/z

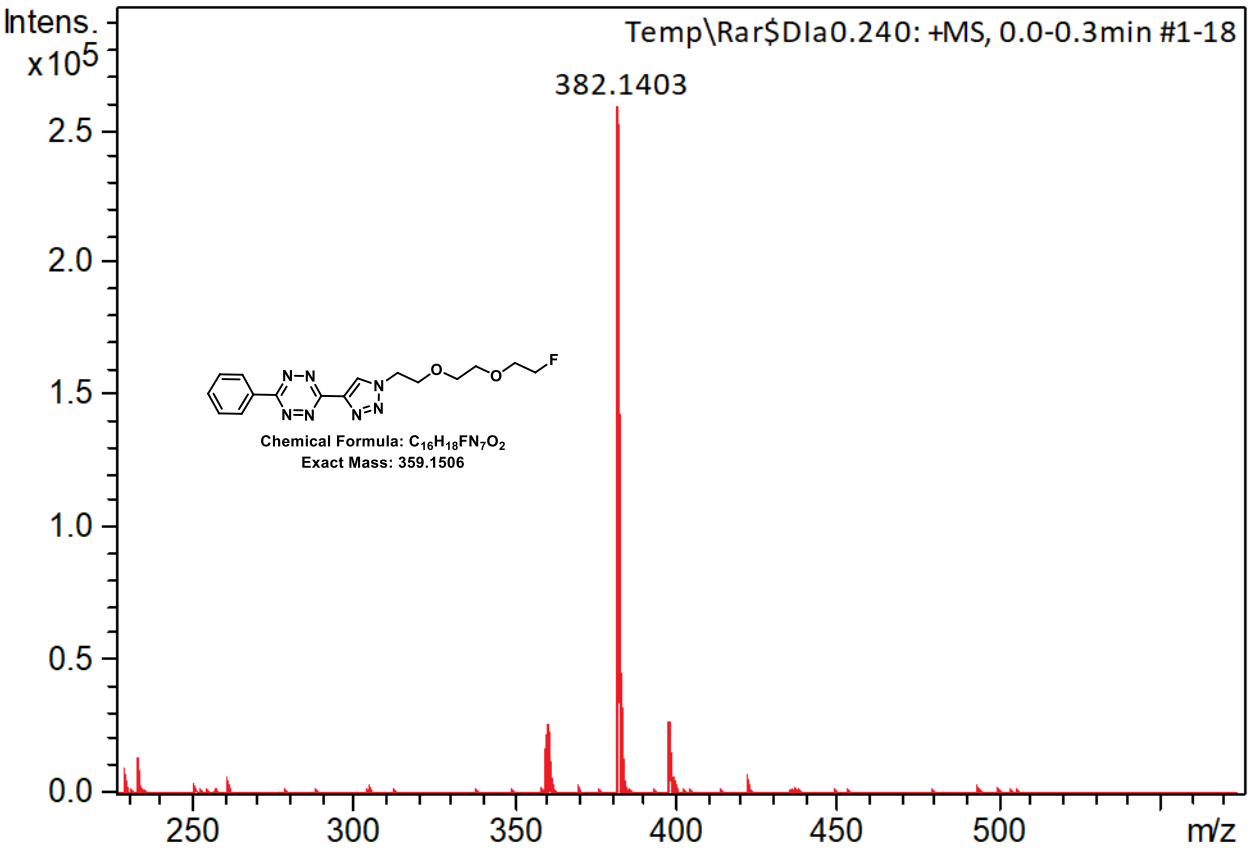

CDCl3

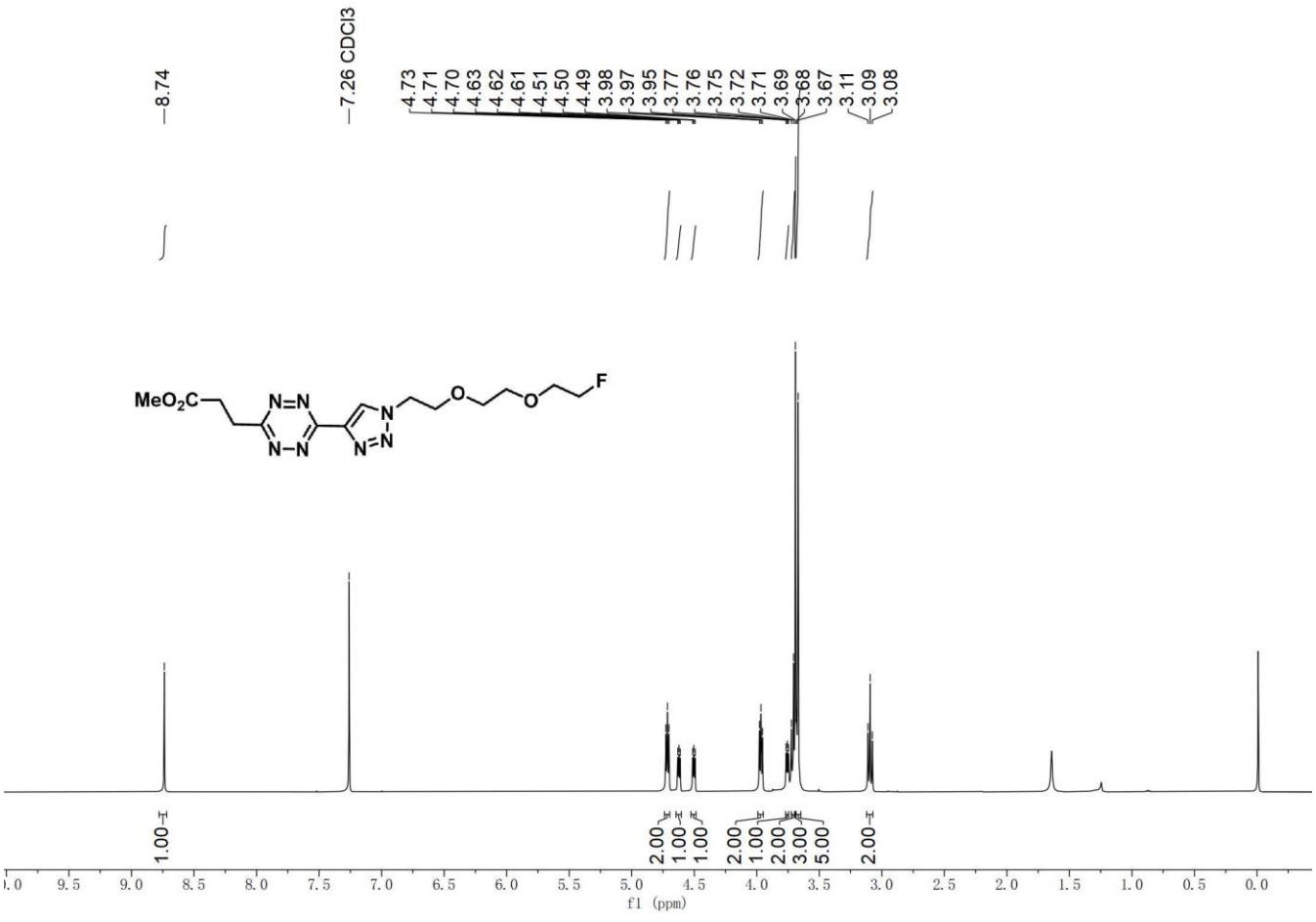

F

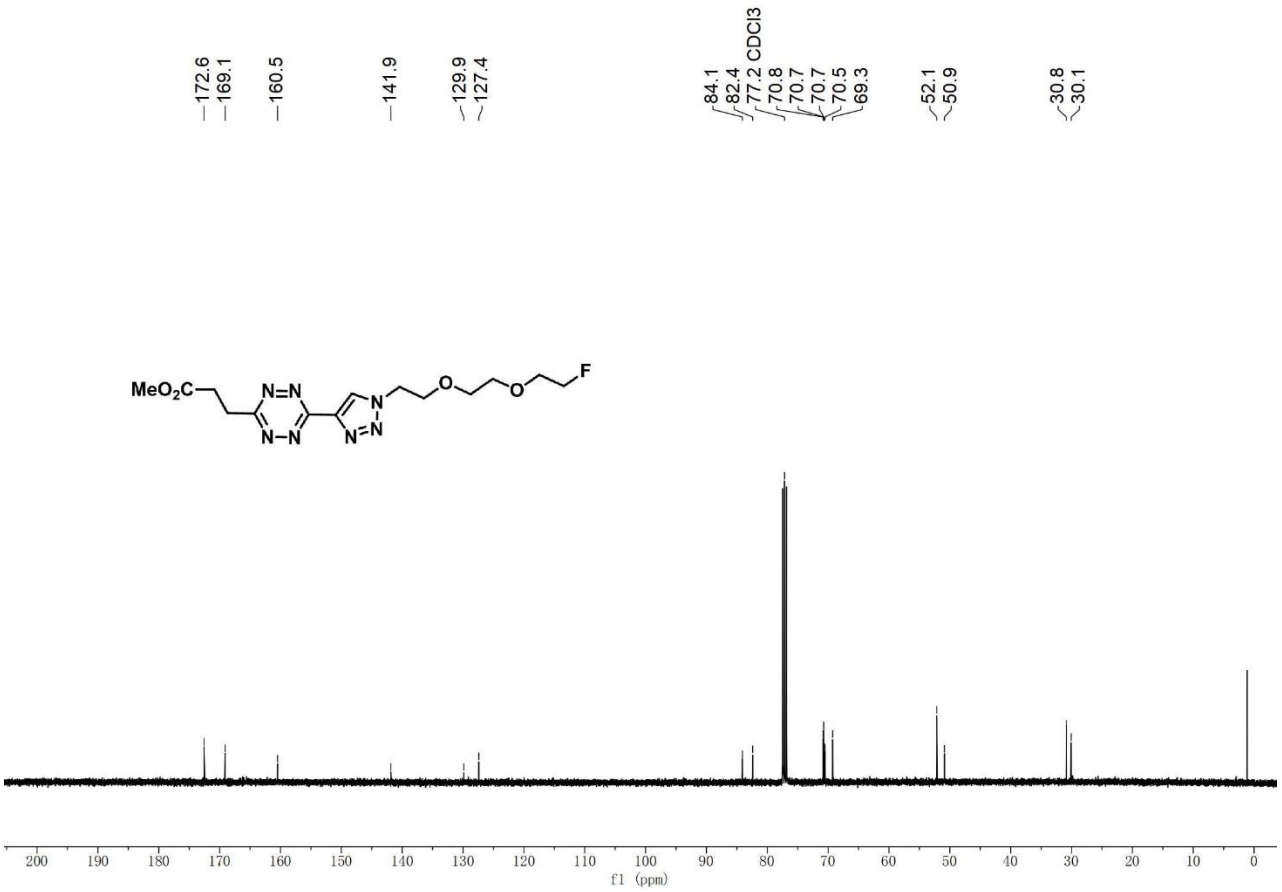

f1 (ppm)

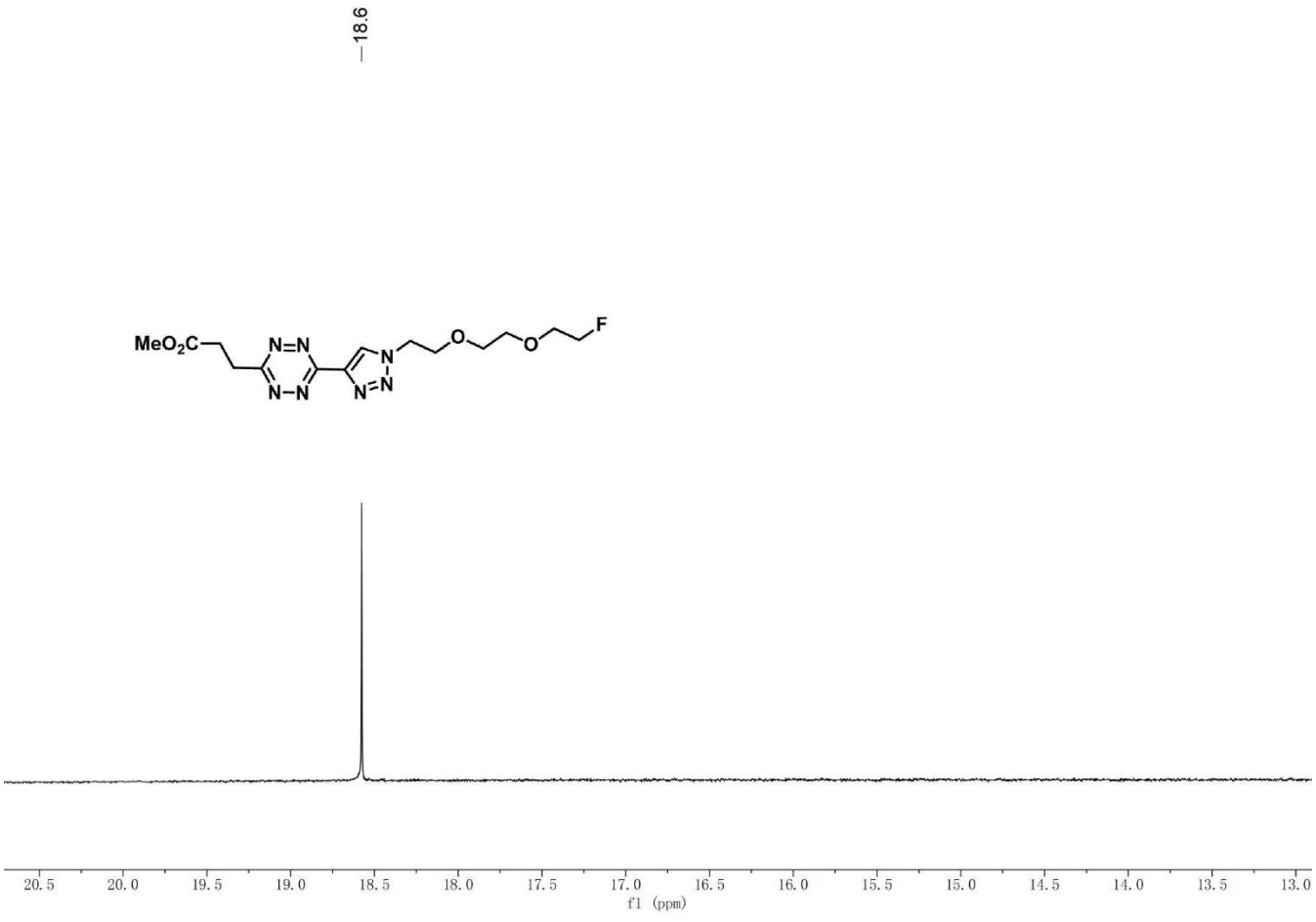

164.5
160.1
142.0
133.0
131.9
129.4
128.3
127.3
84.1
82.4
77.2 CDCl3
70.8
70.7
70.7
70.5
69.3
50.9
f1 (ppm)

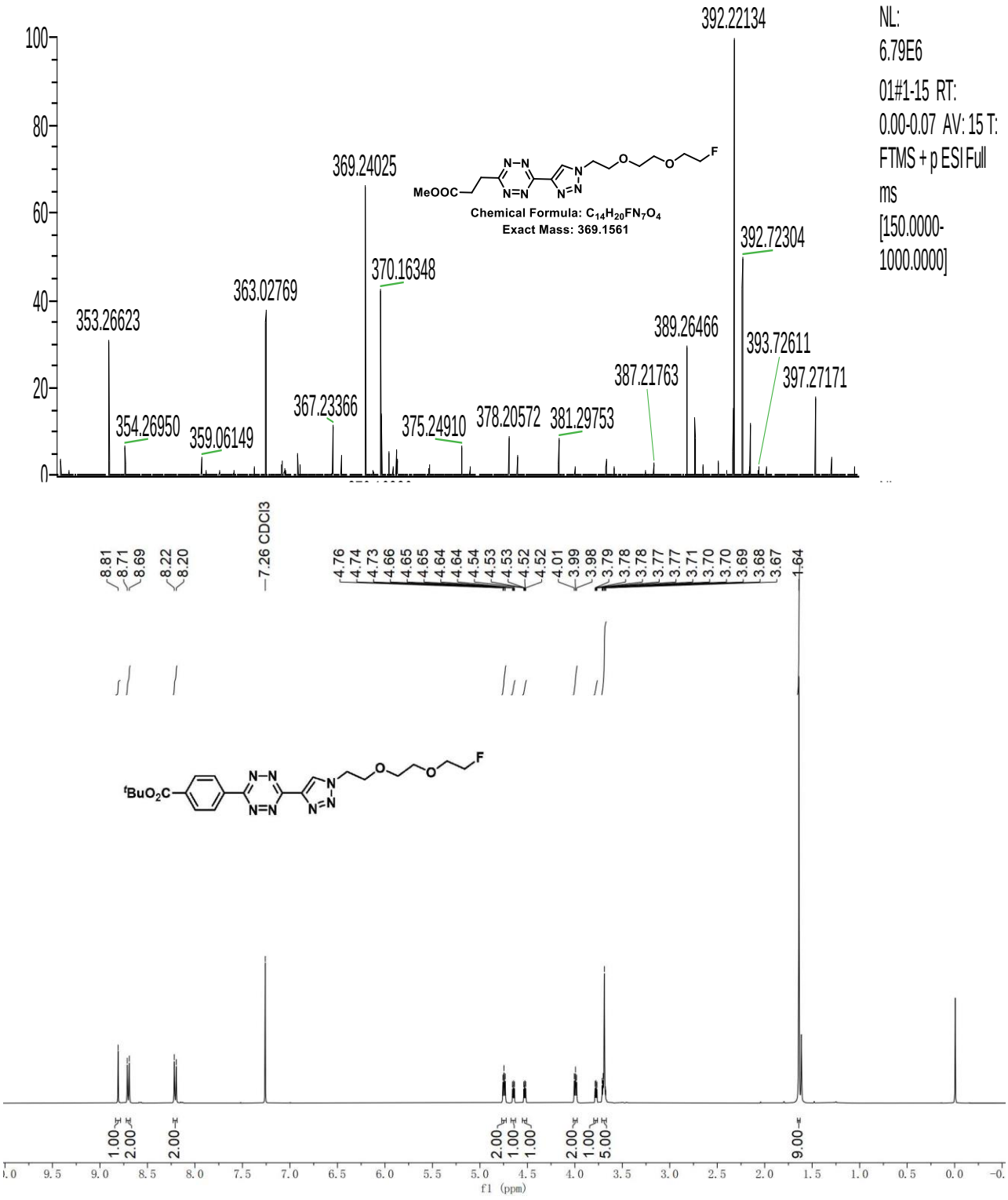

18.6
f1 (ppm)

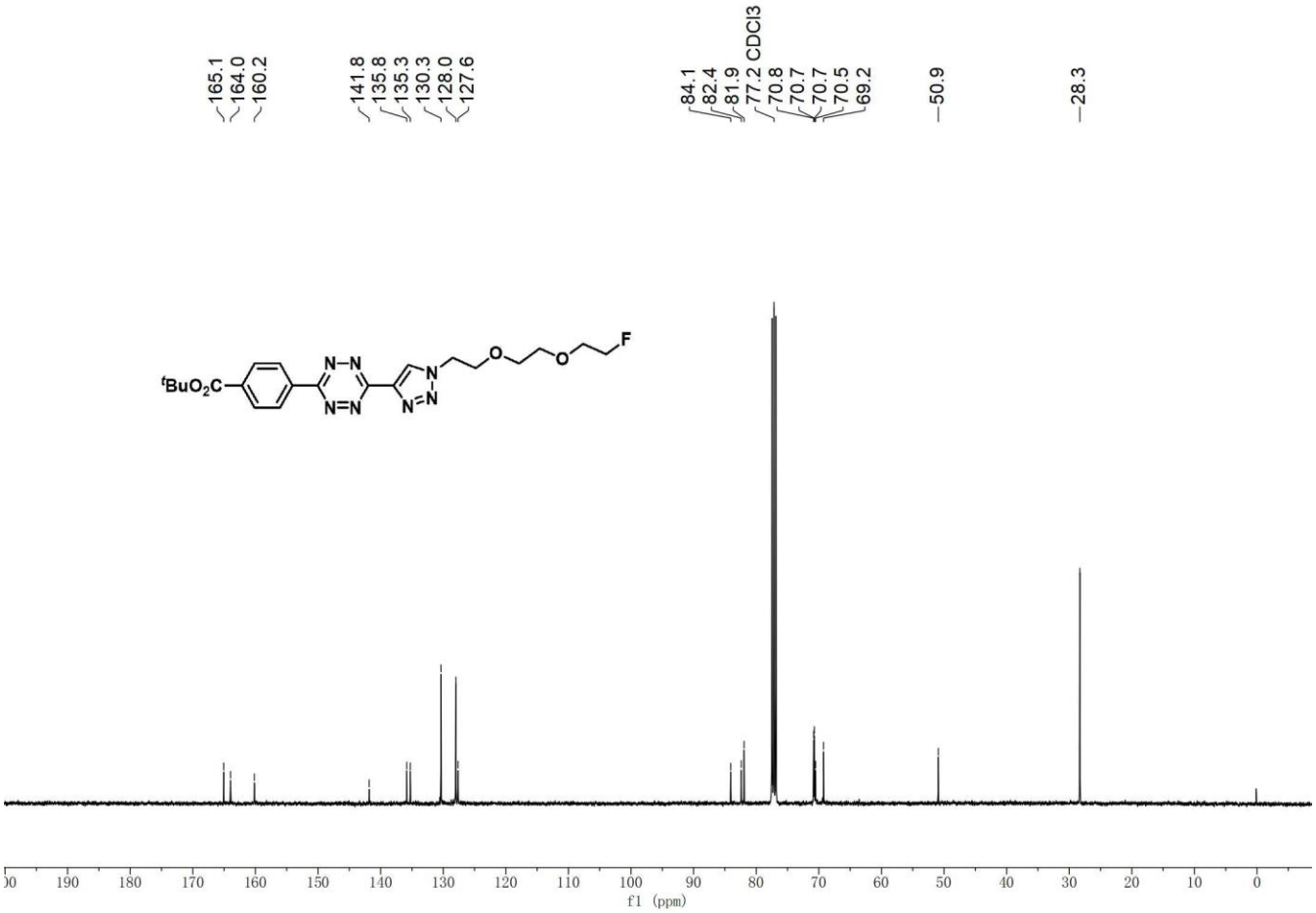

Intens.
x10^5
Temp\Rar$DIa0.240: +MS, 0.0-0.3min #1-18
382.1403
Chemical Formula: C16H18FN7O2
Exact Mass: 359.1506
m/z

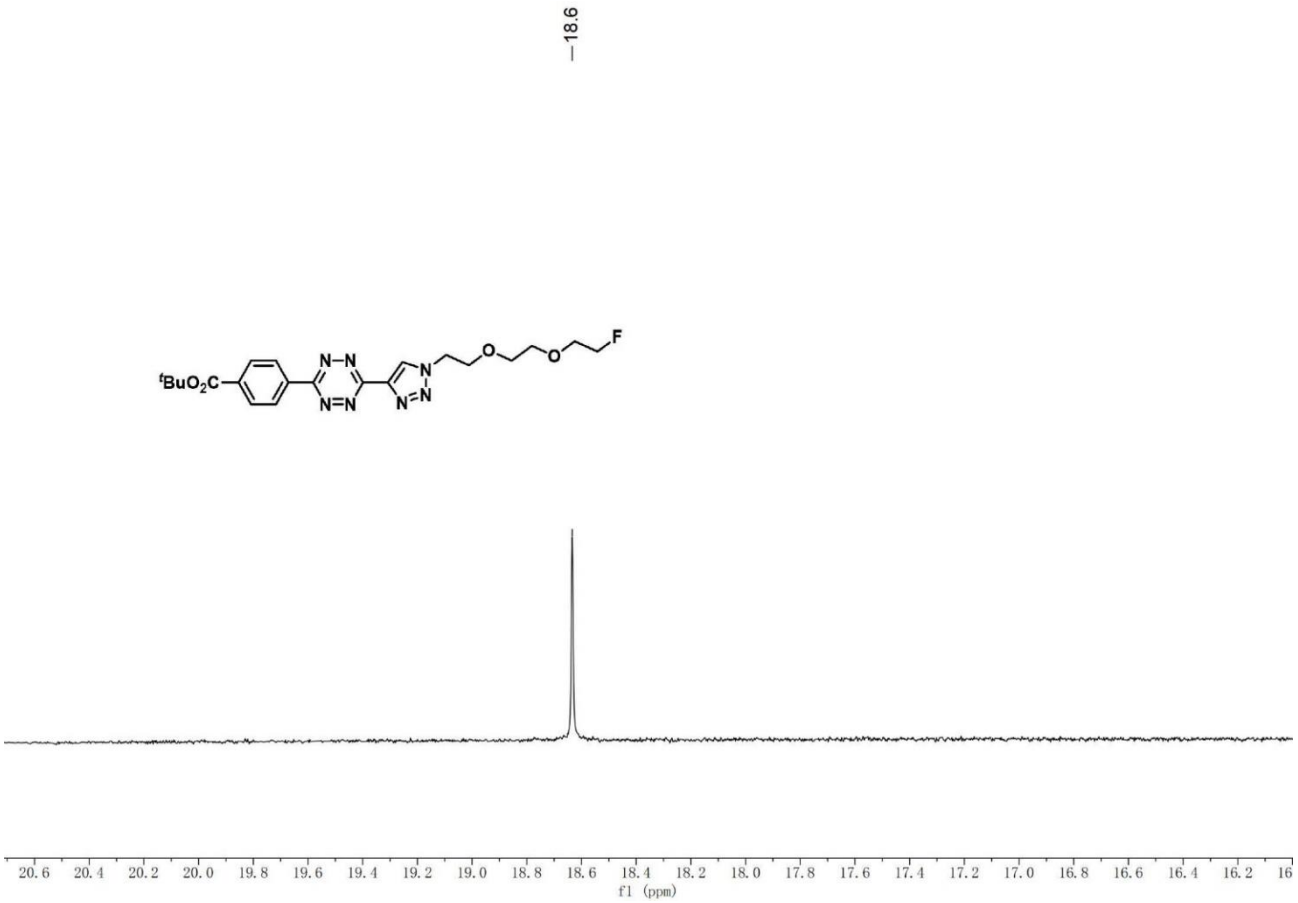

8.74
7.26 CDCl3
4.73
4.71
4.70
4.63
4.62
4.61
4.51
4.50
4.49
3.98
3.97
3.95
3.77
3.76
3.75
3.72
3.71
3.69
3.68
3.67
3.11
3.09
3.08
MeO2C
f1 (ppm)


172.6
169.1
160.5
141.9
129.9
127.4
84.1
82.4
77.2 CDCl3
70.8
70.7
70.7
70.5
69.3
52.1
50.9
30.8
30.1
MeO2C
N=N
N-N
N
N=N
O
O
F
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

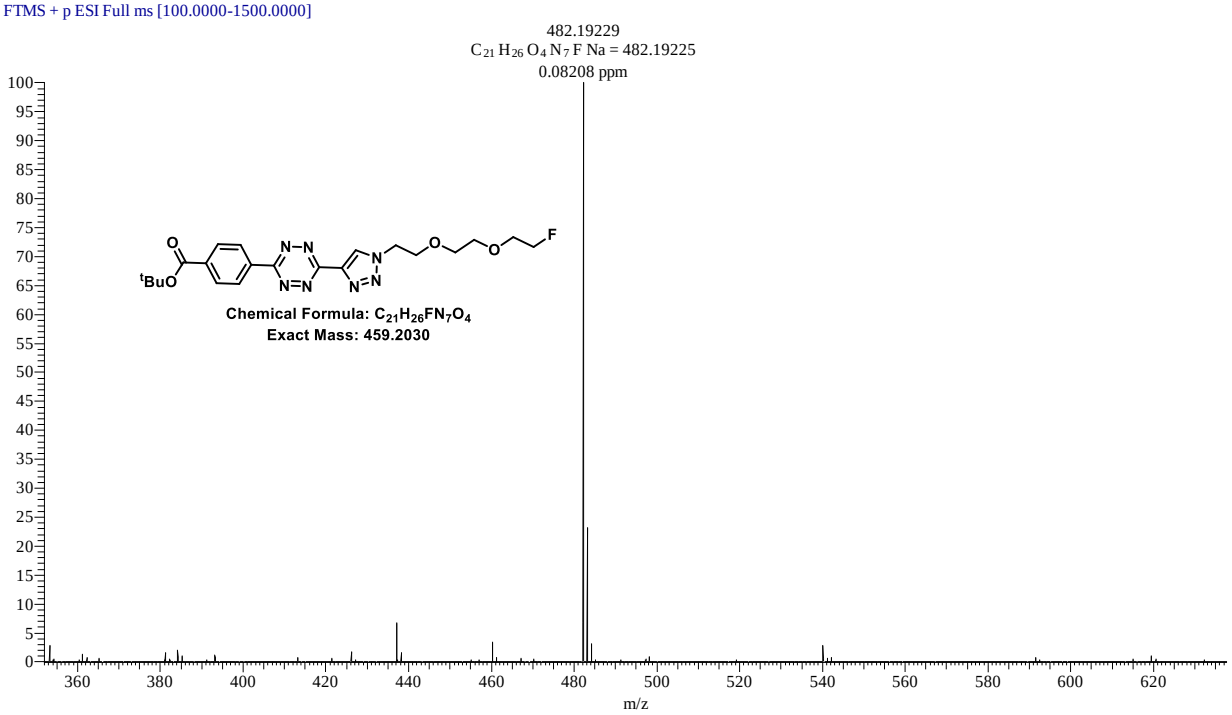

18.6
MeO2C
N=N
N-N
N
N=N
O
O
F
20.5 20.0 19.5 19.0 18.5 18.0 17.5 17.0 16.5 16.0 15.5 15.0 14.5 14.0 13.5 13.0
f1 (ppm)

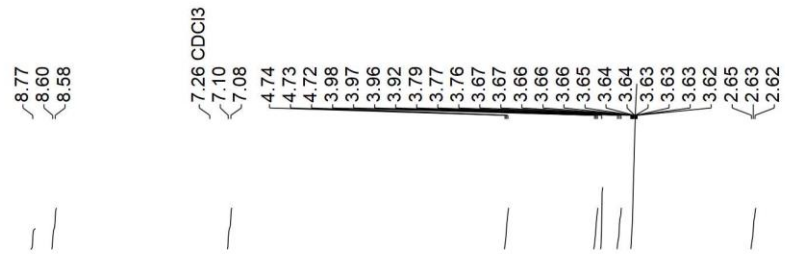

392.22134
NL:
6.79E6
01#1-15 RT:
0.00-0.07 AV: 15 T:
FTMS + p ESI Full
ms
[150.0000-
1000.0000]
369.24025
MeOOC
Chemical Formula: C14H20FN7O4
Exact Mass: 369.1561
392.72304
370.16348
363.02769
353.26623
389.26466
393.72611
387.21763
397.27171
367.23366
354.26950
359.06149
375.24910
378.20572
381.29753
8.81
8.71
8.69
8.22
8.20
7.26 CDCl3
4.76
4.74
4.73
4.66
4.65
4.65
4.64
4.64
4.54
4.53
4.53
4.52
4.52
4.01
3.99
3.98
3.79
3.78
3.78
3.77
3.77
3.71
3.70
3.70
3.69
3.68
3.67
1.64
tBuO2C
1.00
2.00
2.00
2.00
1.00
1.00
2.00
1.00
5.00
9.00
f1 (ppm)

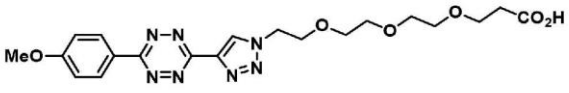

165.1
164.0
160.2
141.8
135.8
135.3
130.3
128.0
127.6
84.1
82.4
81.9
77.2 CDCl3
70.8
70.7
70.7
70.5
69.2
50.9
28.3
tBuO2C
N-N
N=N
N
N=N
O
O
F
f1 (ppm)

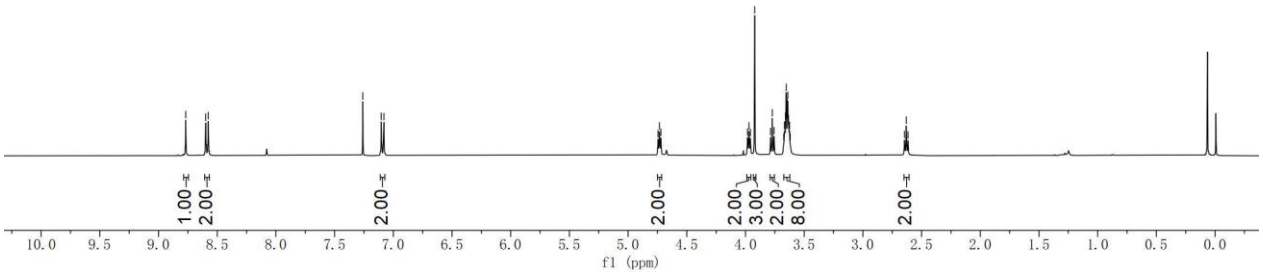

18.6
tBuO2C
N-N
N=N
N
N=N
O
O
F
f1 (ppm)

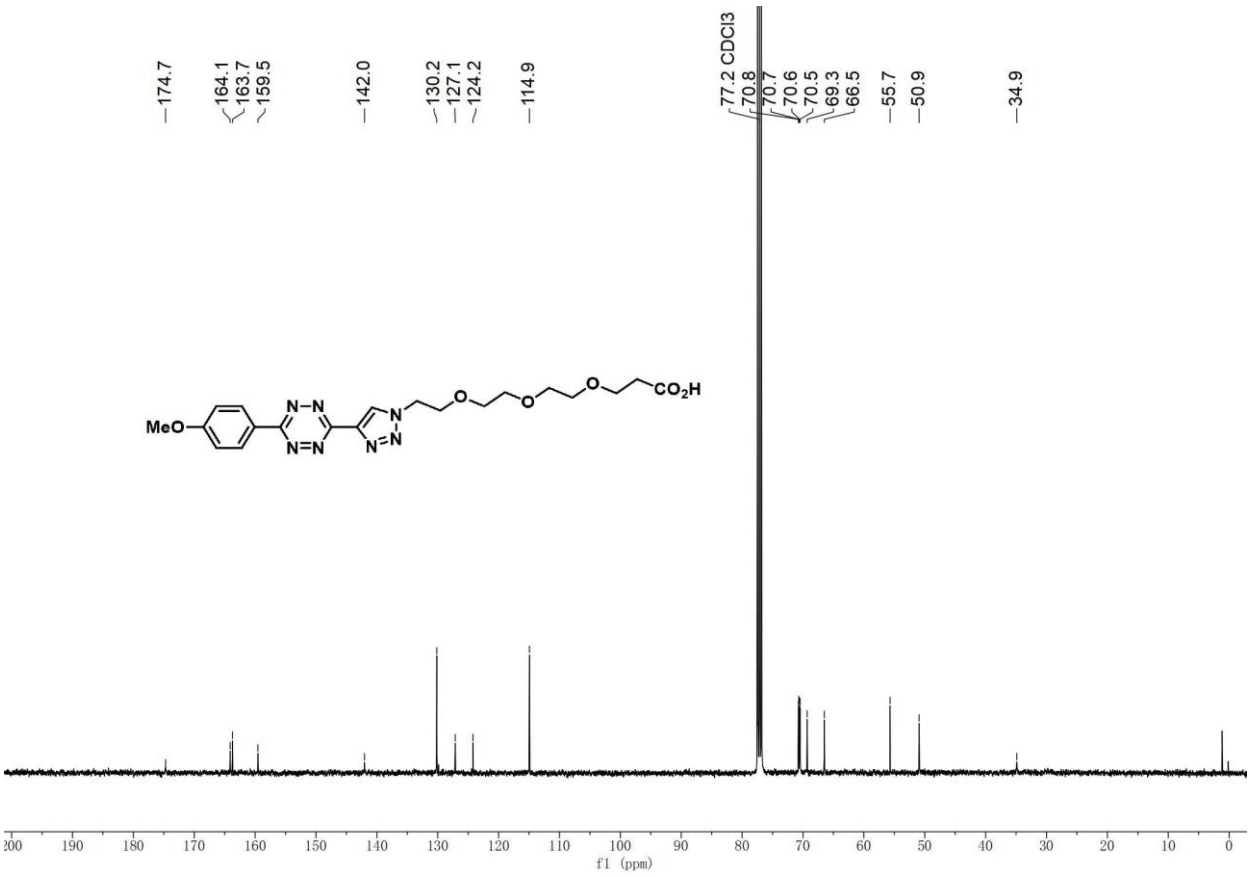
Y1072 #24 RT: 0.10 AV: 1 NL: 1.15E9
T: FTMS + p ESI Full ms [100.0000-1500.0000]



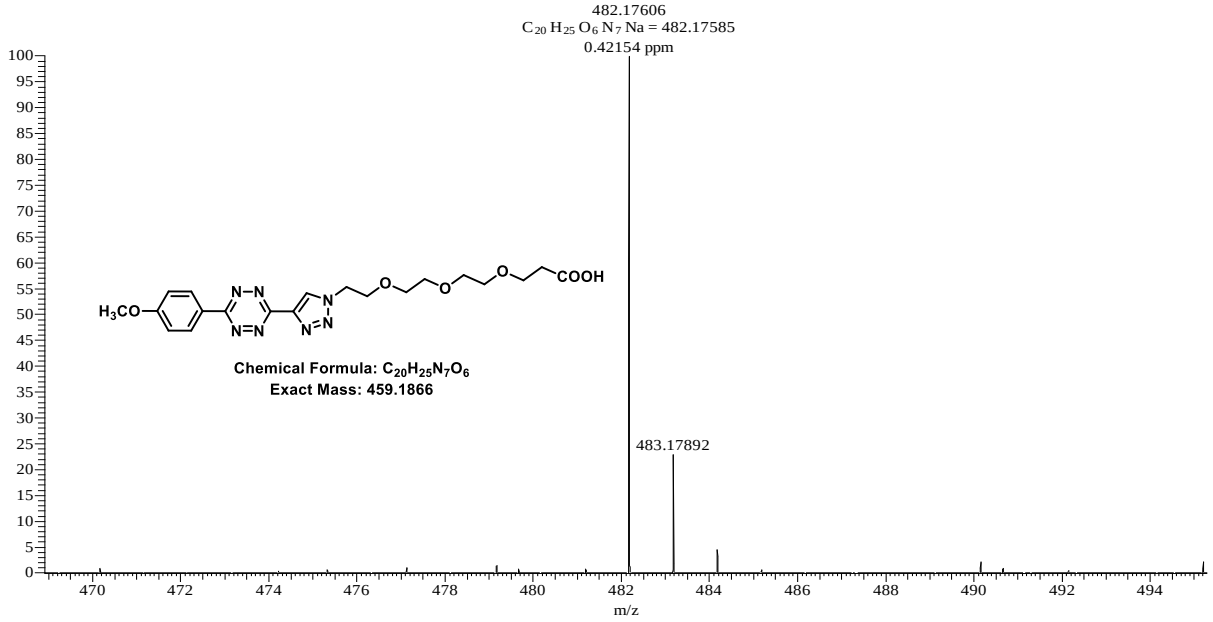


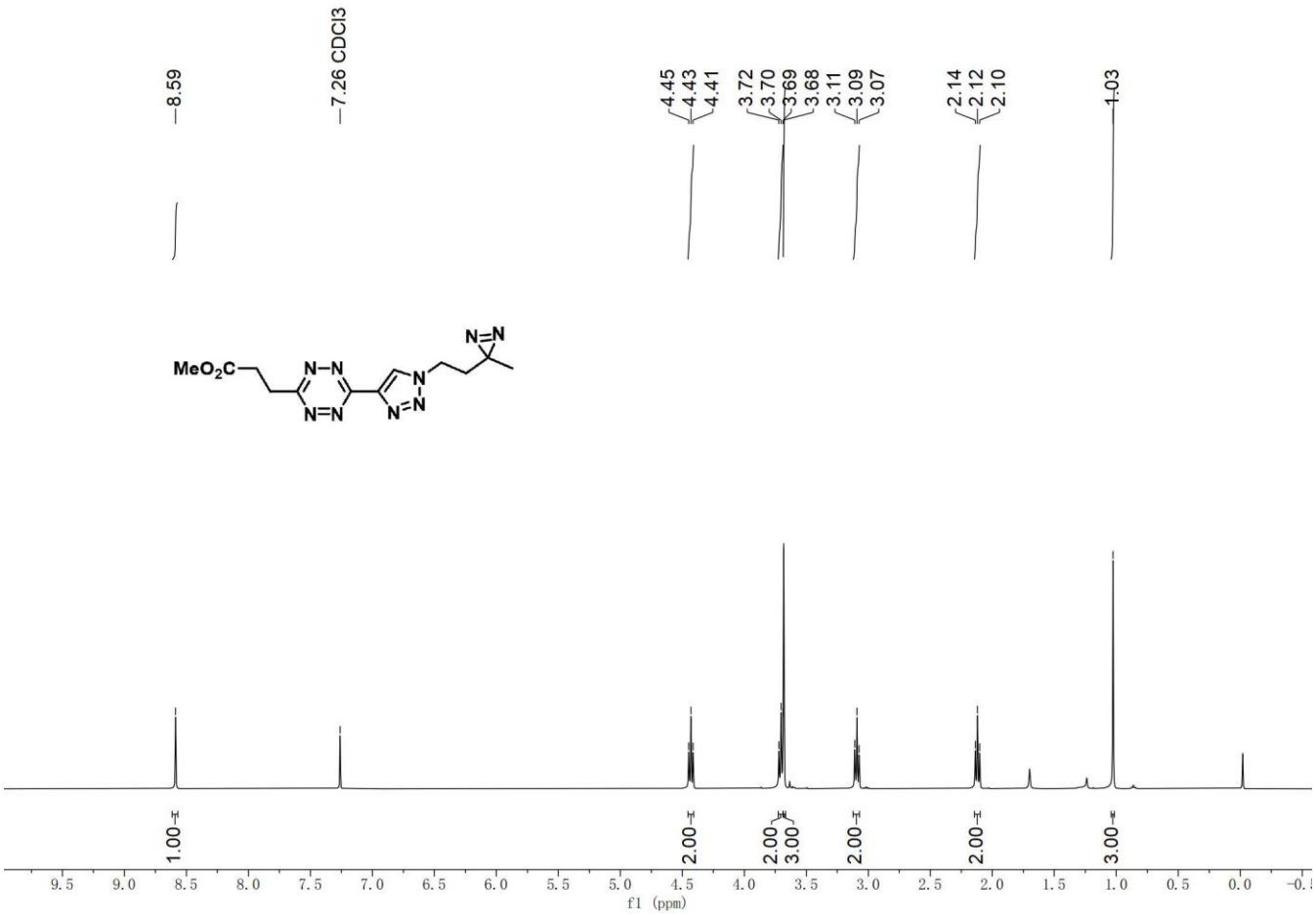


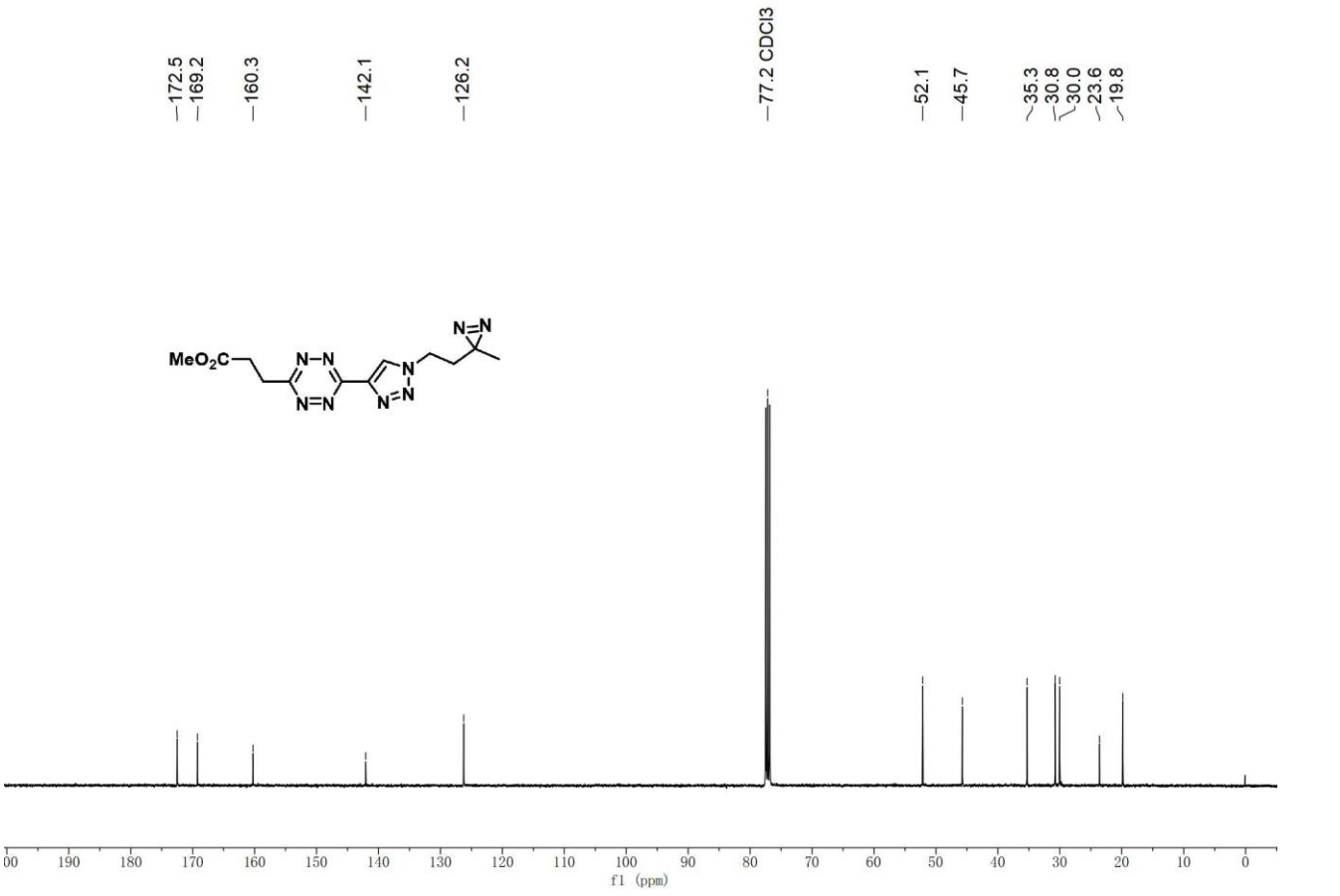


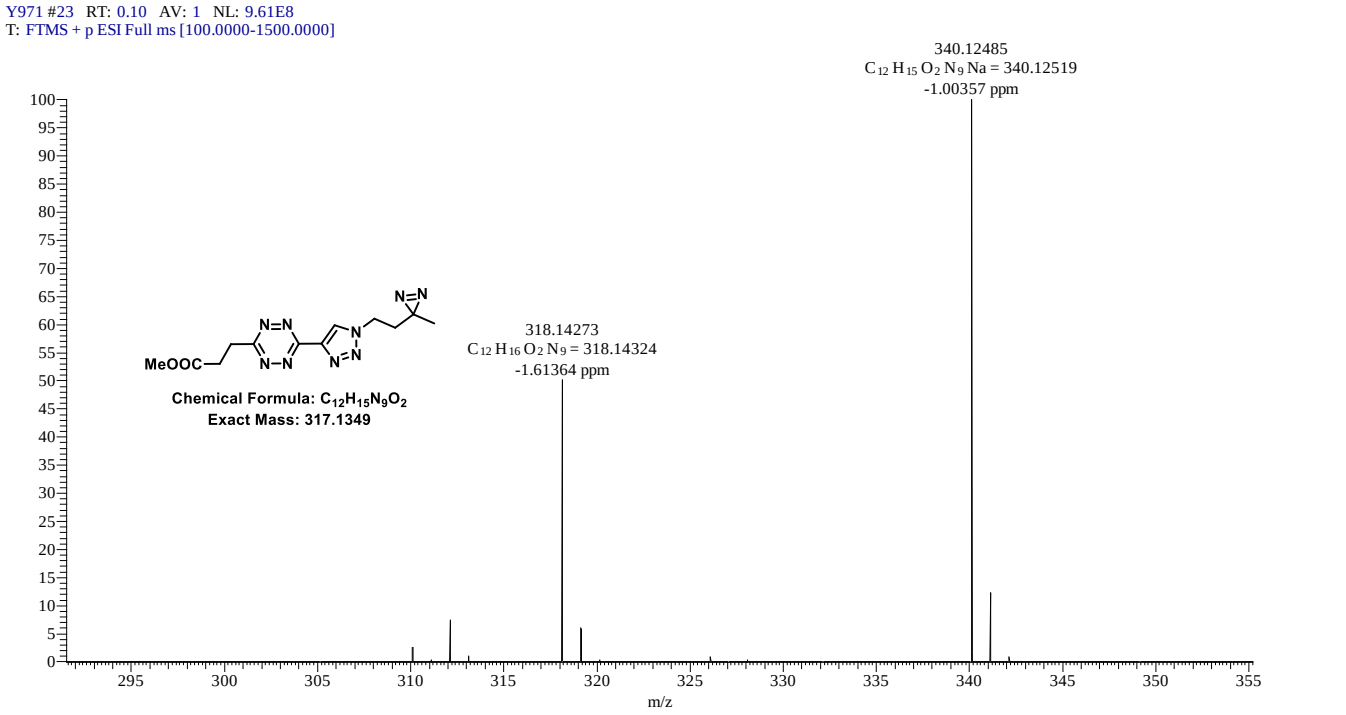


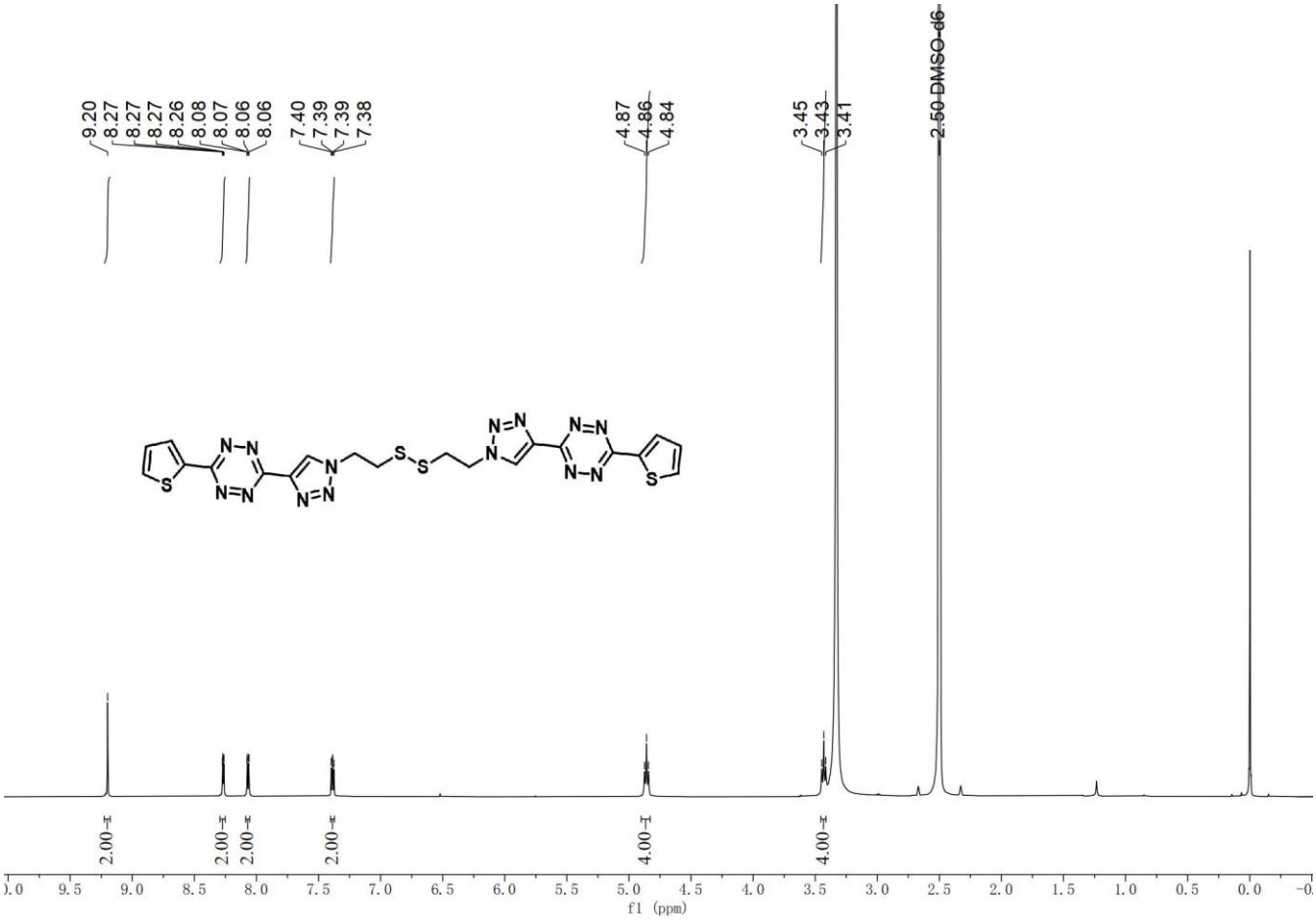
875 #21 RT: 0.09 AV: 1 NL: 3.44E8
T: FTMS + p ESI Full ms [100.0000-1500.0000]

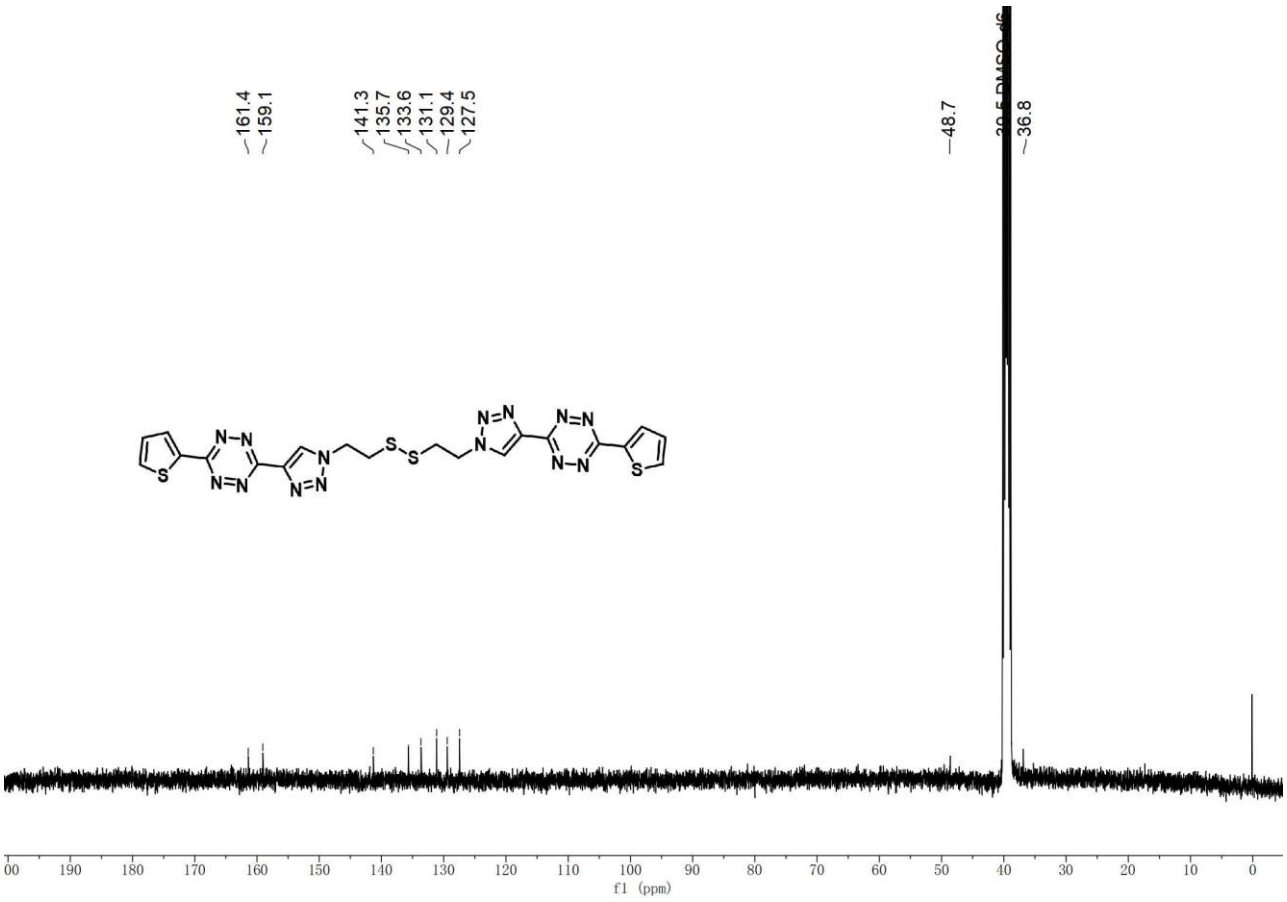


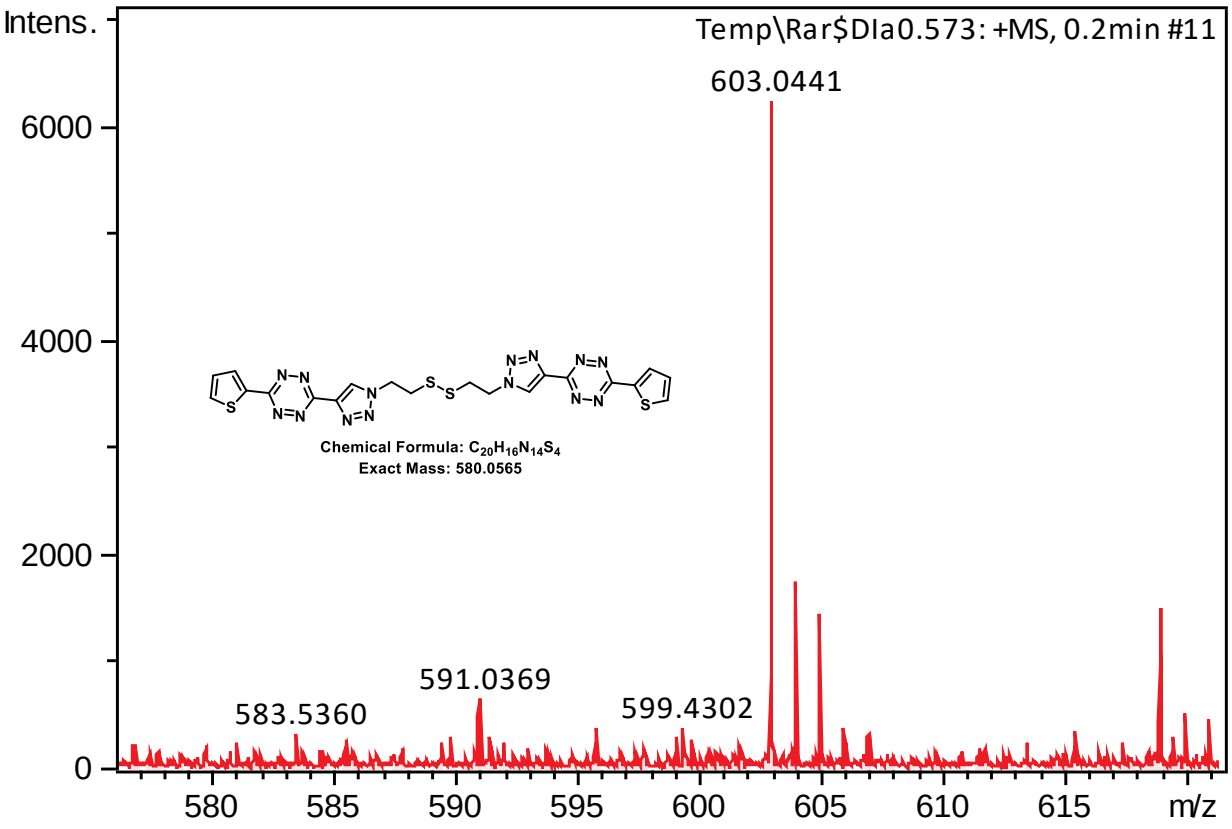

8.59
7.26 CDCl3
4.45
4.43
4.41
3.72
3.70
3.69
3.68
3.11
3.09
3.07
2.14
2.12
2.10
1.03
MeO2C
N=N
1.00
2.00
2.00
3.00
2.00
2.00
3.00
f1 (ppm)

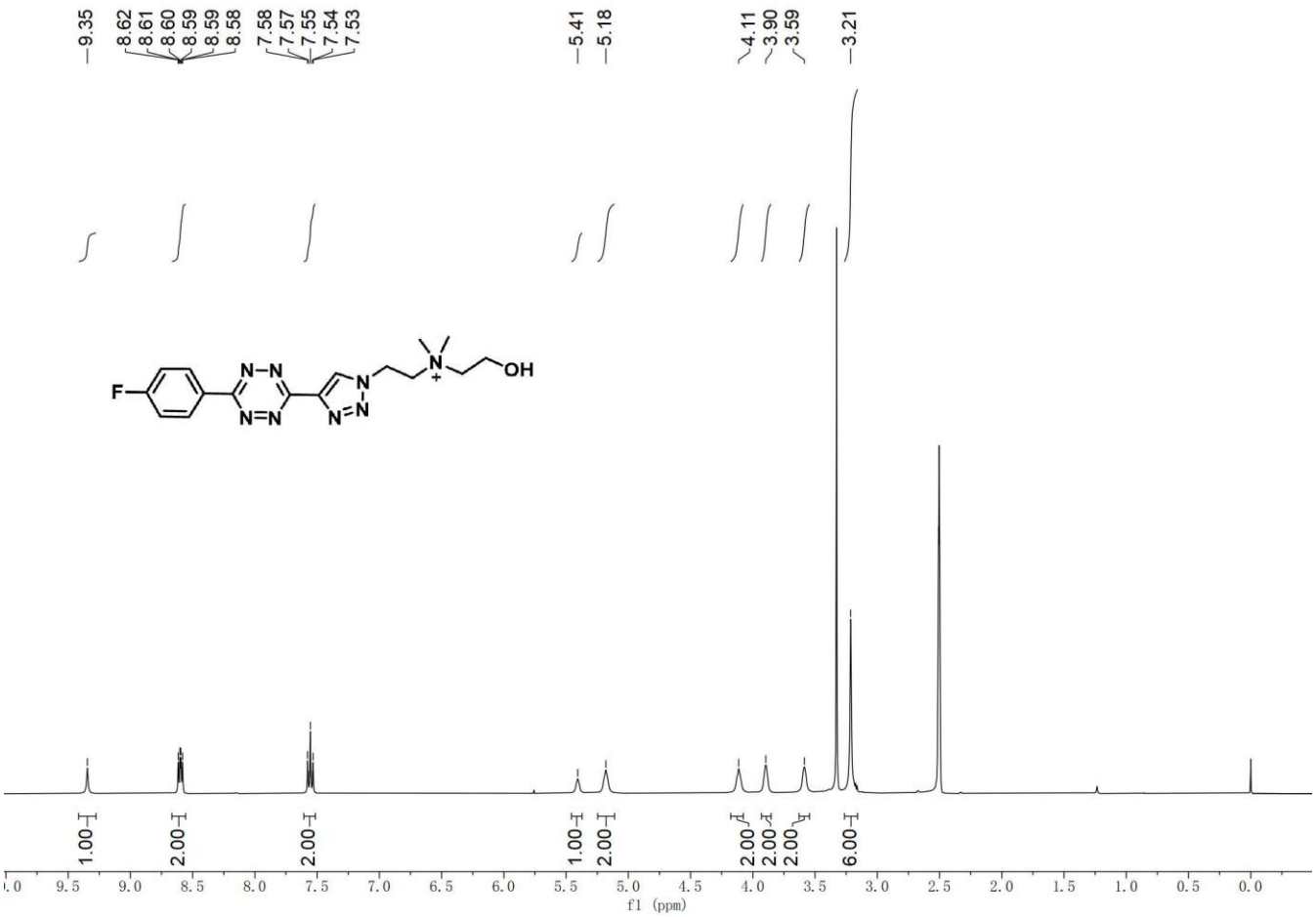

172.5
169.2
160.3
142.1
126.2
77.2 CDCl3
52.1
45.7
35.3
30.8
30.0
23.6
19.8
MeO2C
N=N
f1 (ppm)

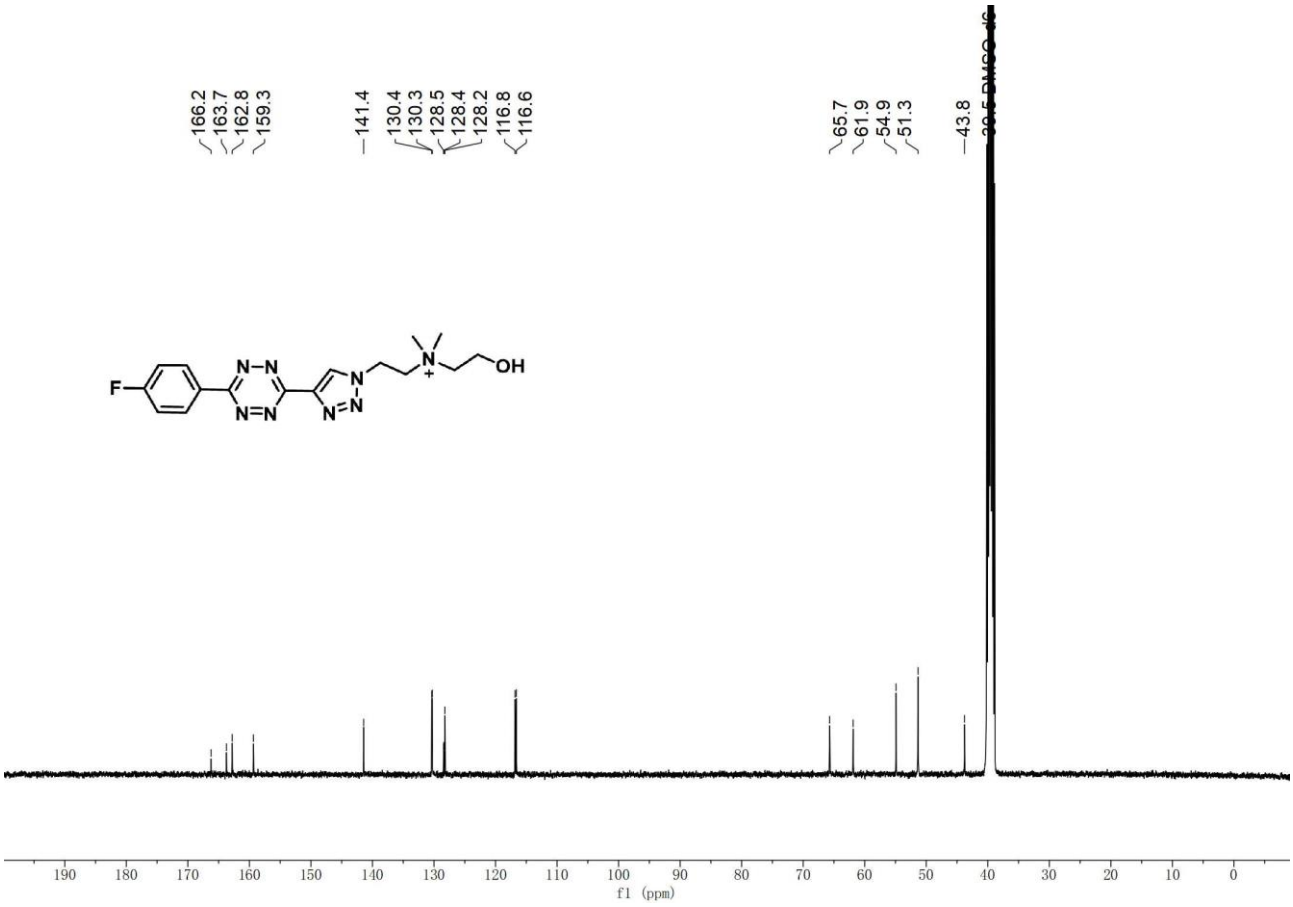

Y971 #23 RT: 0.10 AV: 1 NL: 9.61E8
T: FTMS + p ESI Full ms [100.0000-1500.0000]
340.12485
C12 H15 O2 N9 Na = 340.12519
-1.00357 ppm
318.14273
C12 H16 O2 N9 = 318.14324
-1.61364 ppm
MeOOC
Chemical Formula: C12H15N9O2
Exact Mass: 317.1349
m/z

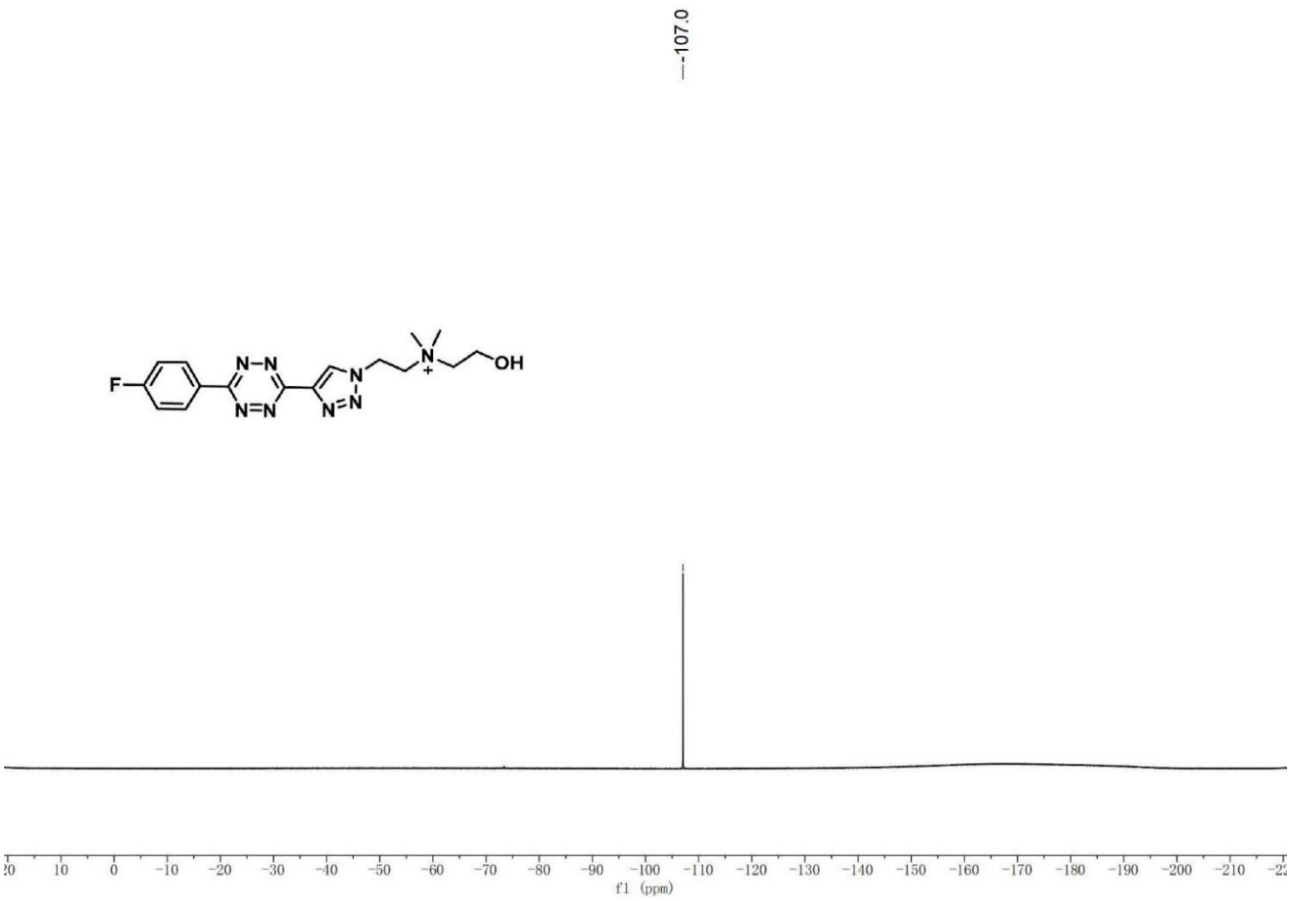

9.20
8.27
8.27
8.27
8.26
8.08
8.07
8.06
8.06
7.40
7.39
7.39
7.38
4.87
4.86
4.84
3.45
3.43
3.41
2.50 DMSO-d6
2.00
2.00
2.00
2.00
4.00
4.00
f1 (ppm)


161.4
159.1
141.3
135.7
133.6
131.1
129.4
127.5
48.7
39.5 DMSO-d6
36.8
f1 (ppm)

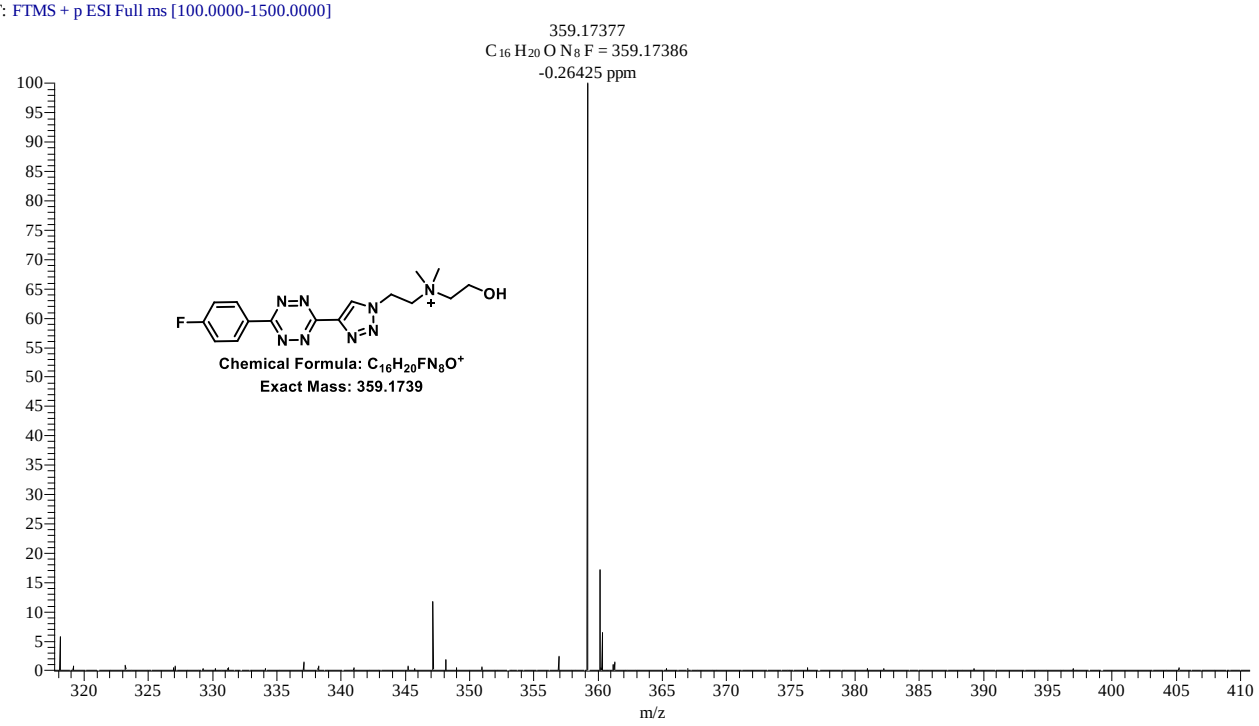

Intens.
Temp\Rar$DIa0.573: +MS, 0.2min #11
603.0441
6000
4000
2000
0
591.0369
583.5360
599.4302
580
585
590
595
600
605
610
615
m/z
Chemical Formula: C20H16N14S4
Exact Mass: 580.0565

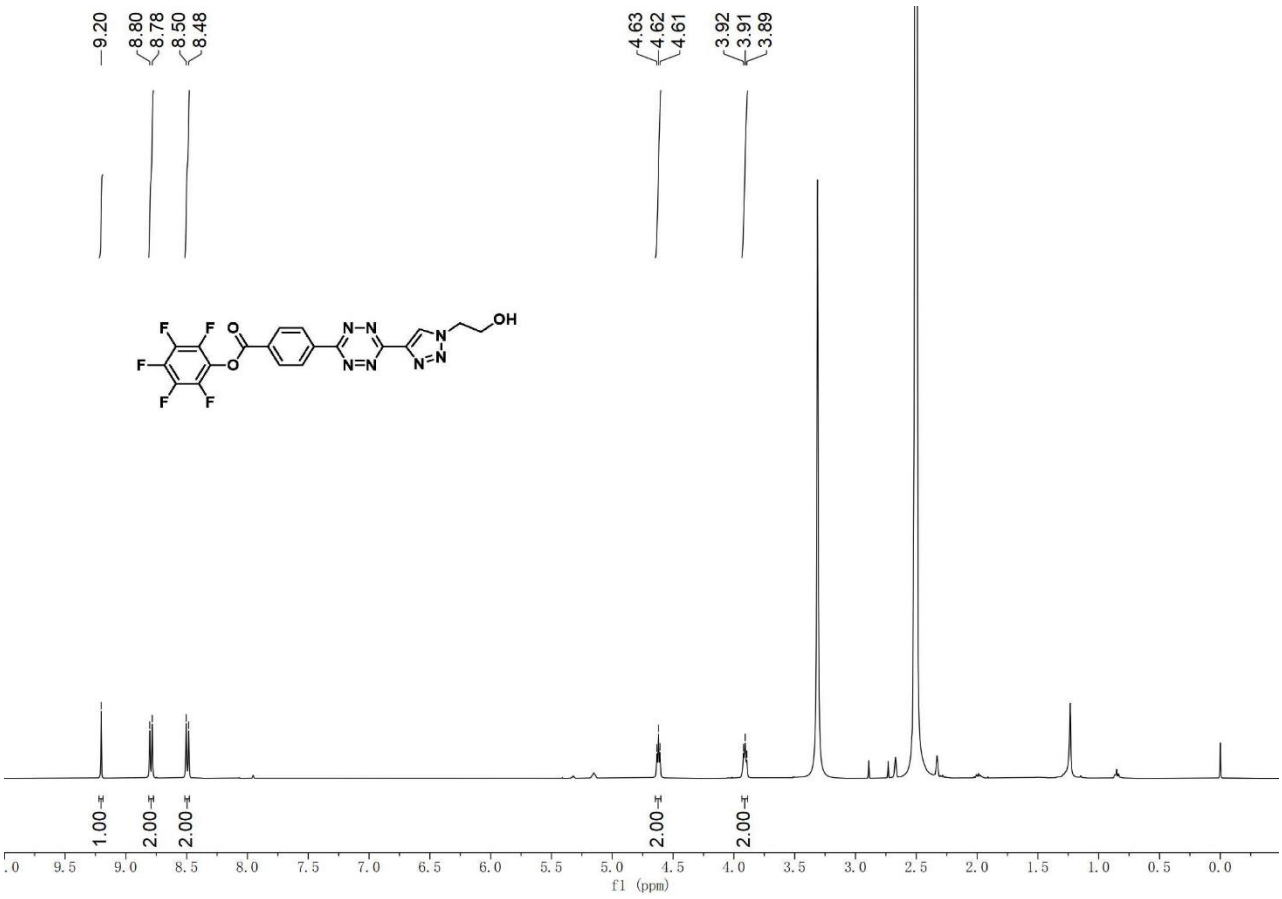

9.35
8.62
8.61
8.60
8.59
8.59
8.58
7.58
7.57
7.55
7.54
7.53
5.41
5.18
4.11
3.90
3.59
3.21
F
N-N
N=N
N
N=N
N
OH
1.00
2.00
2.00
1.00
2.00
2.00
2.00
2.00
6.00
f1 (ppm)

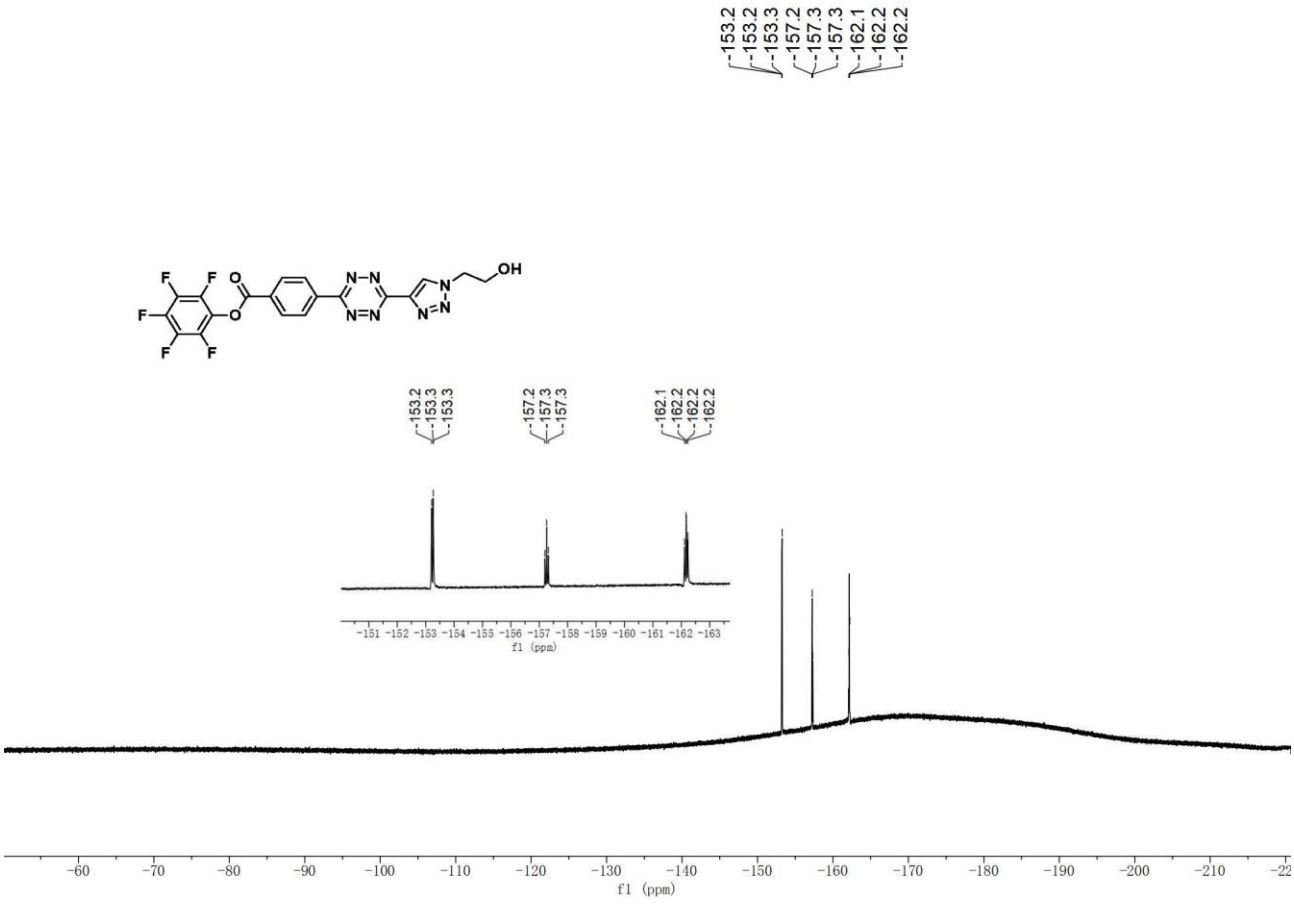

166.2
163.7
162.8
159.3
141.4
130.4
130.3
128.5
128.4
128.2
116.8
116.6
65.7
61.9
54.9
51.3
43.8
F
N-N
N=N
N
N=N
N
OH
f1 (ppm)



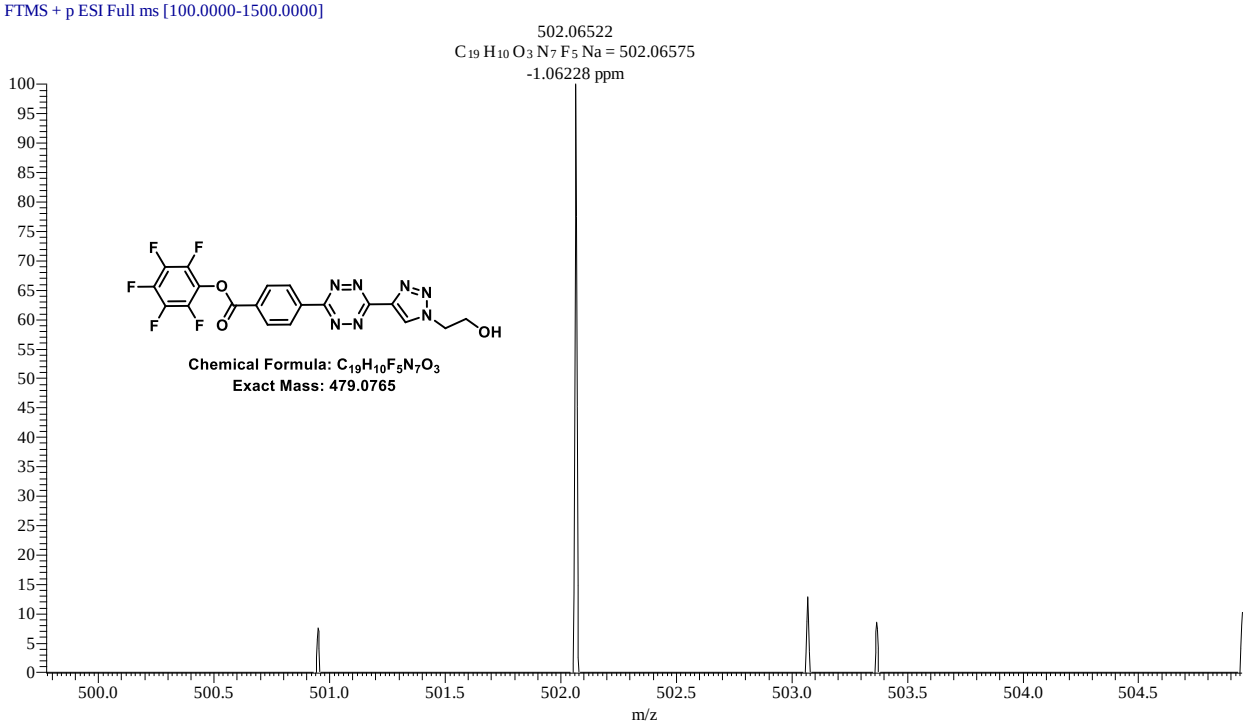
923 #34 RT: 0.15 AV: 1 NL: 1.78E8
T: FTMS + p ESI Full ms [100.0000-1500.0000]

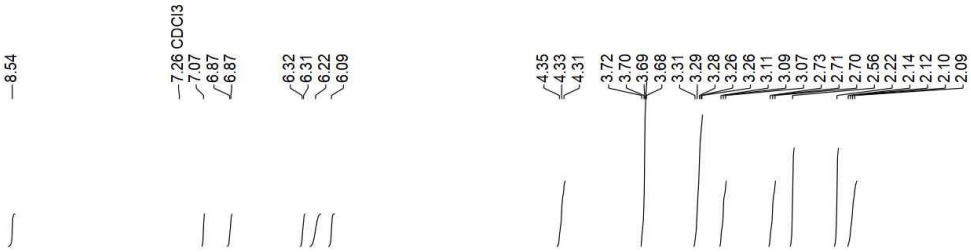


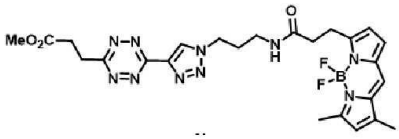

9.20
8.80
8.78
8.50
8.48
4.63
4.62
4.61
3.92
3.91
3.89
1.00
2.00
2.00
2.00
2.00
f1 (ppm)

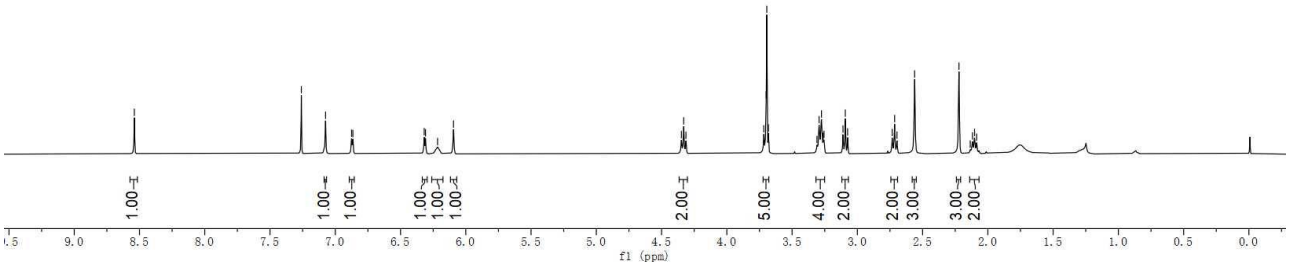

-153.2
-153.2
-153.3
-157.2
-157.3
-157.3
-162.1
-162.2
-162.2
-153.2
-153.3
-153.3
-157.2
-157.3
-157.3
-162.1
-162.2
-162.2
-162.2
f1 (ppm)
f1 (ppm)

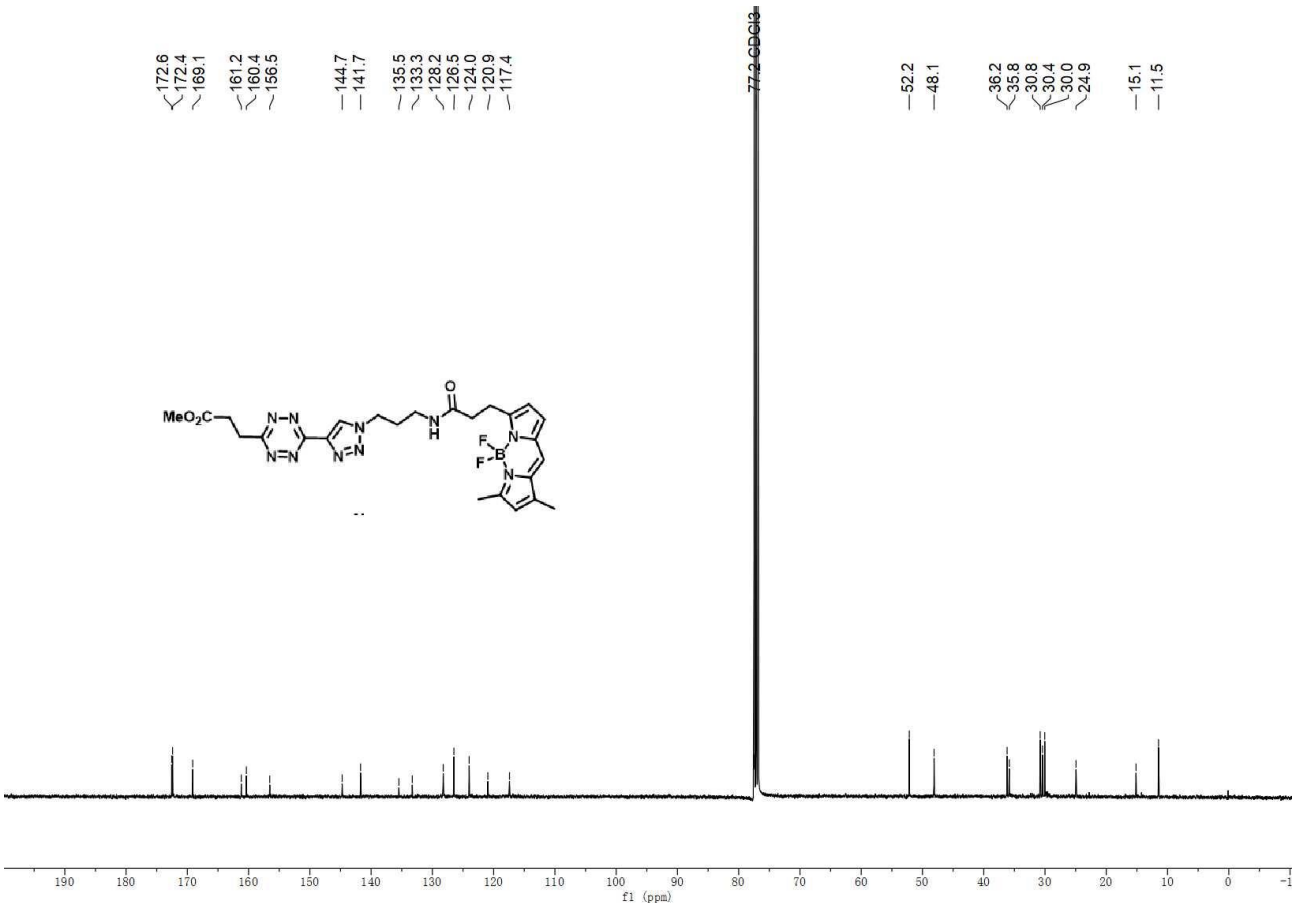
945 #21 RT: 0.09 AV: 1 NL: 9.04E5
T: FTMS + p ESI Full ms [100.0000-1500.0000]

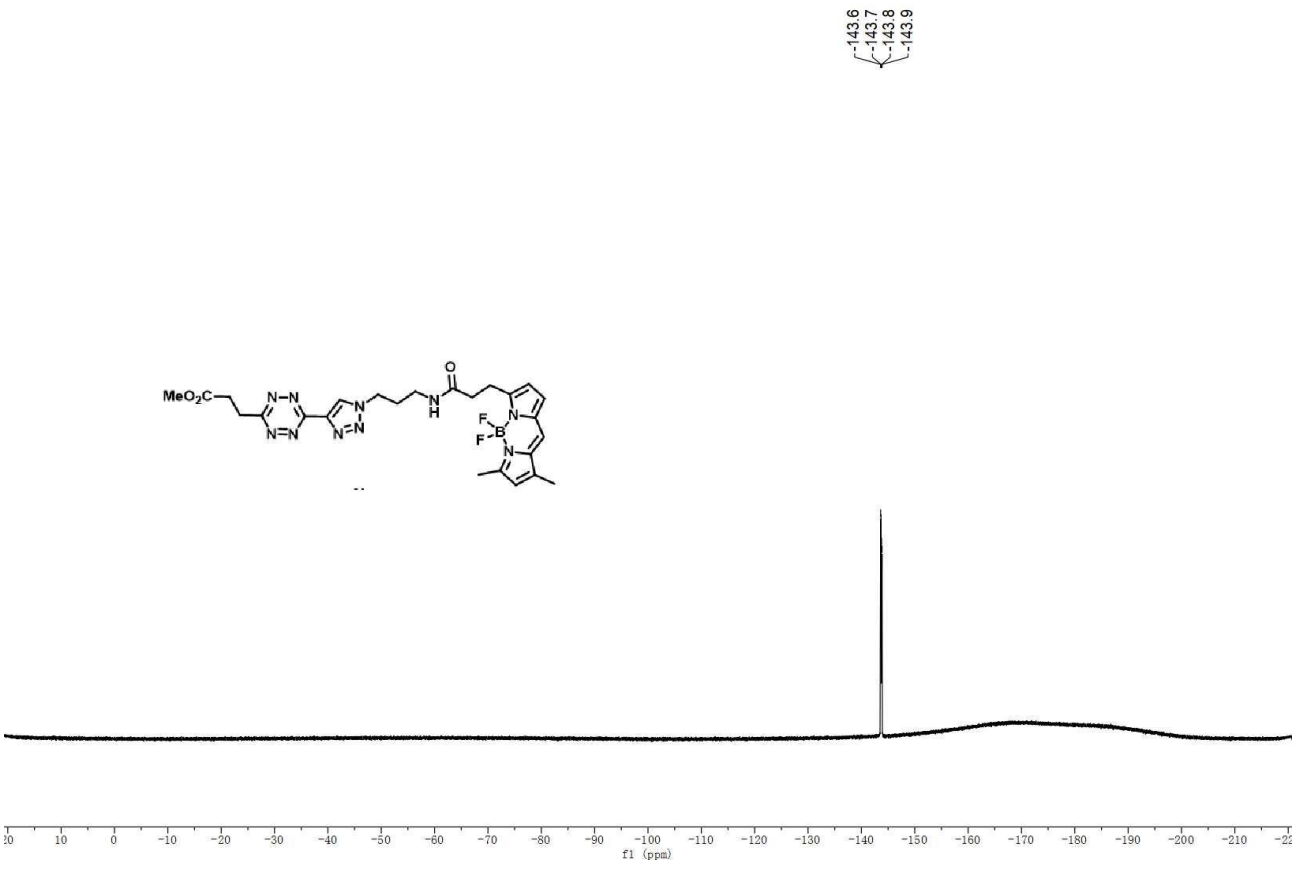


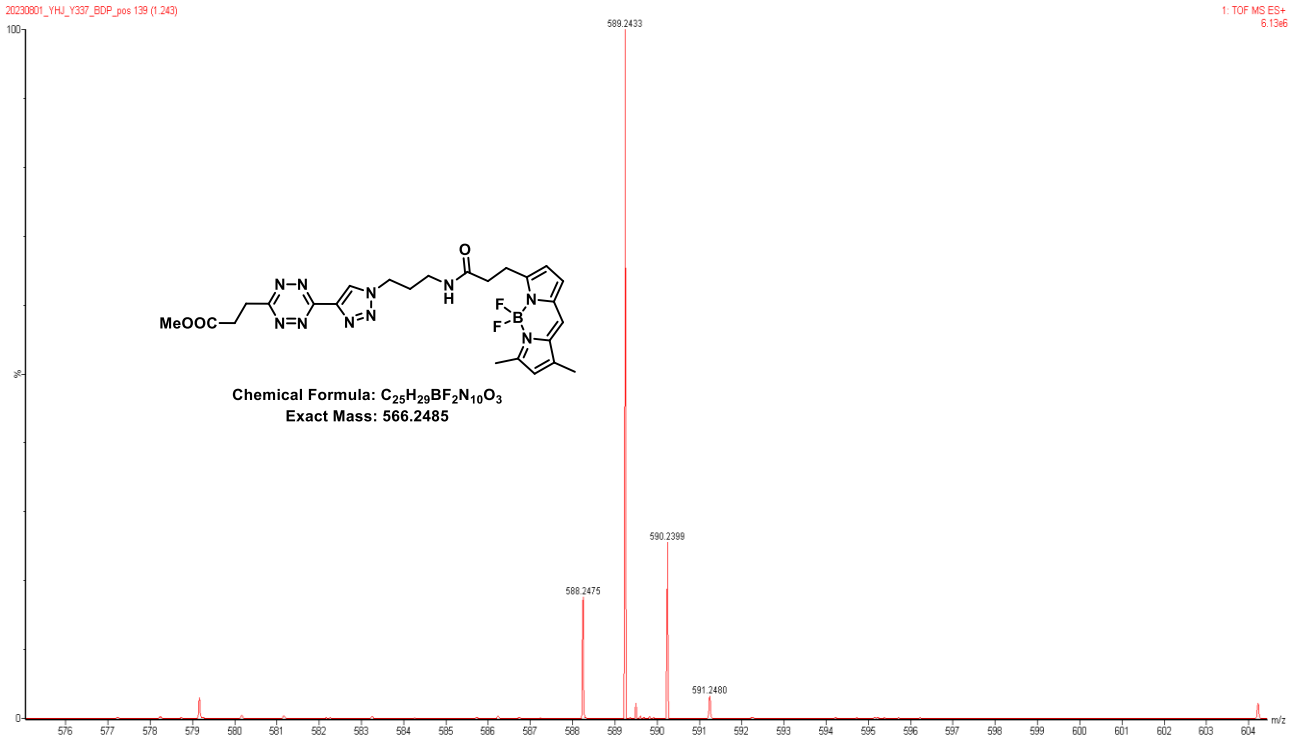


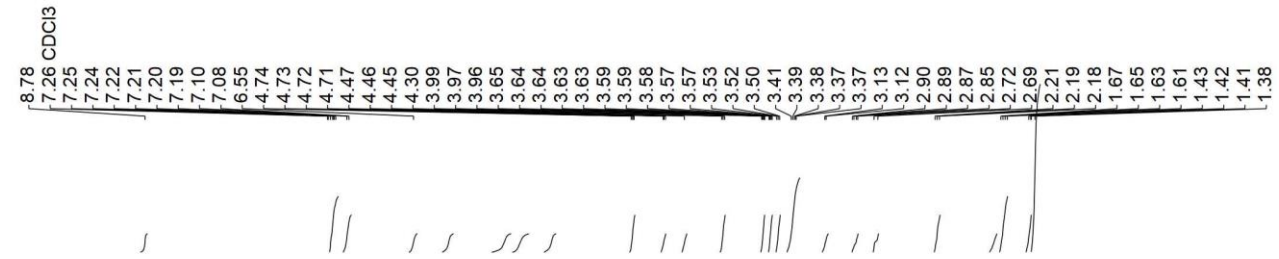





172.6
172.4
169.1
161.2
160.4
156.5
144.7
141.7
135.5
133.3
128.2
126.5
124.0
120.9
117.4
77.2 CDCl3
52.2
48.1
36.2
35.8
30.8
30.4
30.0
24.9
15.1
11.5
MeO2C
O
N
H
F
B
f1 (ppm)


-143.6
-143.7
-143.8
-143.9
MeO2C
O
N
H
F
B
f1 (ppm)


20230801_YHJ_Y337_BDP_pos 139 (1.243)
1: TOF MS ES+
6.13e6
589.2433
590.2399
588.2475
591.2480
MeOOC
Chemical Formula: C25H29BF2N10O3
Exact Mass: 566.2485
m/z


CDCl3
8.78
7.26
7.25
7.24
7.22
7.21
7.20
7.19
7.10
7.08
6.55
4.74
4.73
4.72
4.71
4.47
4.46
4.45
4.30
3.99
3.97
3.96
3.65
3.64
3.64
3.63
3.63
3.59
3.59
3.58
3.57
3.57
3.53
3.52
3.50
3.41
3.39
3.38
3.37
3.37
3.13
3.12
2.90
2.89
2.87
2.85
2.72
2.69
2.21
2.19
2.18
1.67
1.65
1.63
1.61
1.43
1.42
1.41
1.38


BocHN
1.00
3.00
2.00
1.00
1.00
1.00
1.00
1.00
2.00
1.00
1.00
2.00
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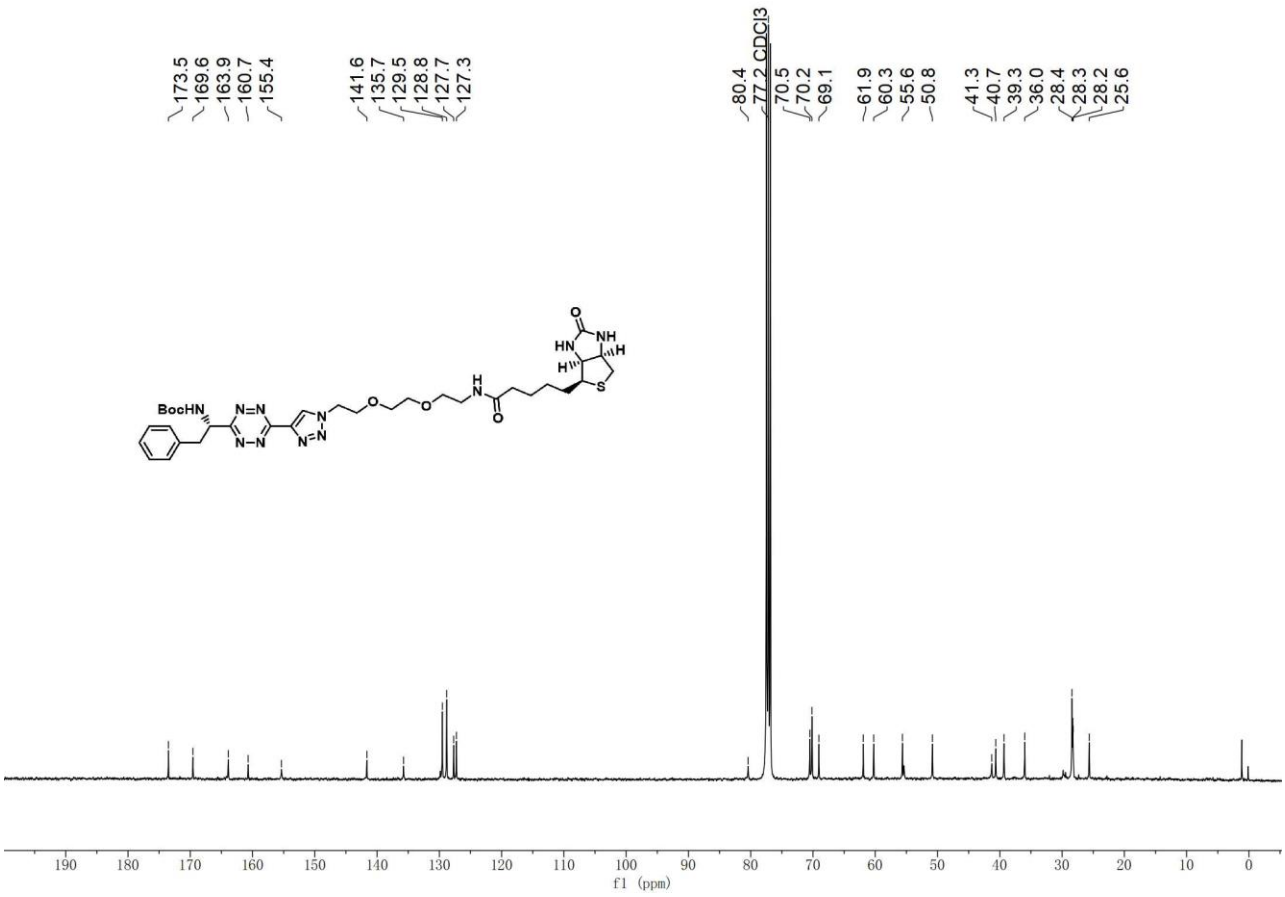
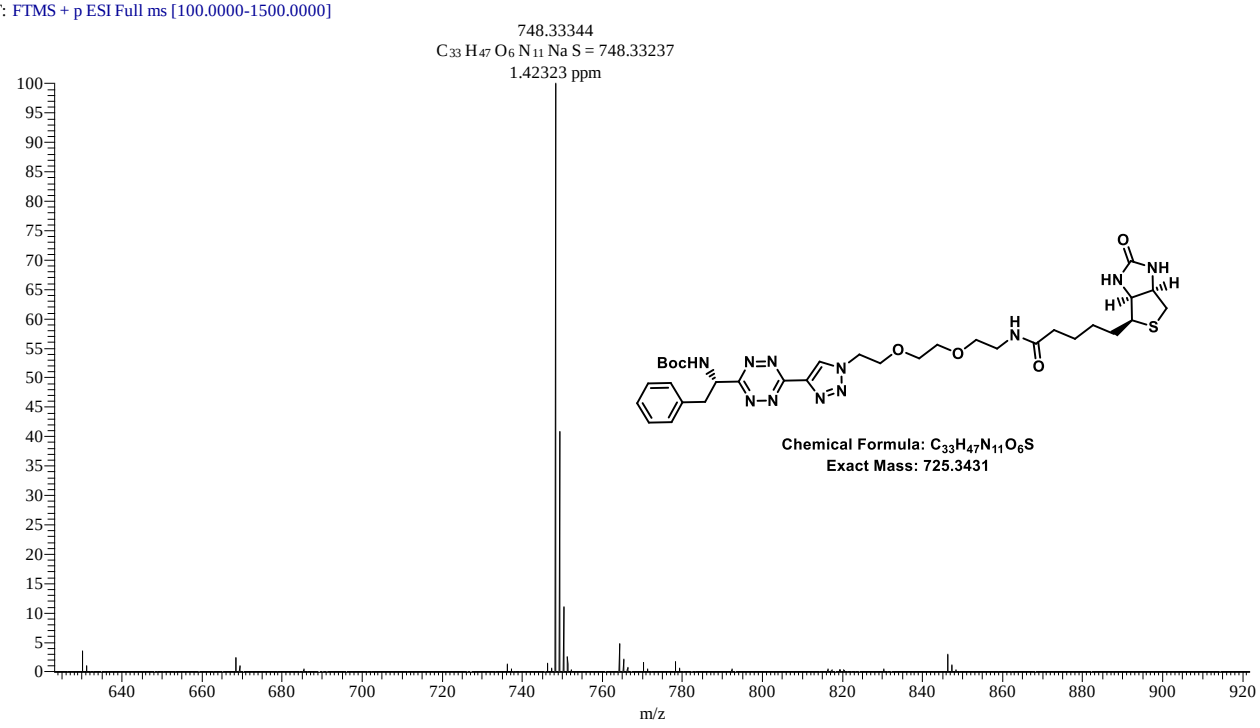
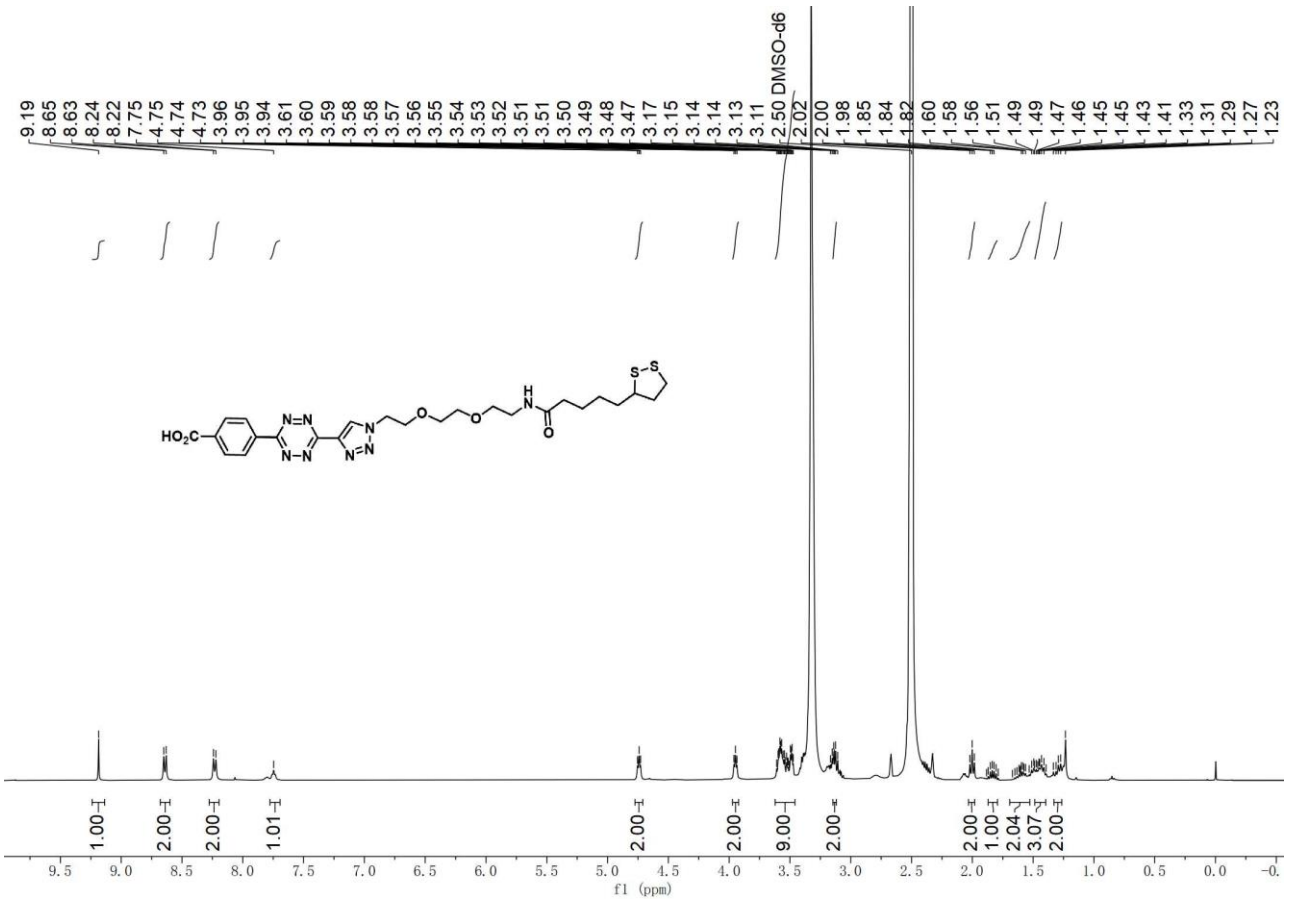
1.00
3.00
2.00
9.00
f1 (ppm)

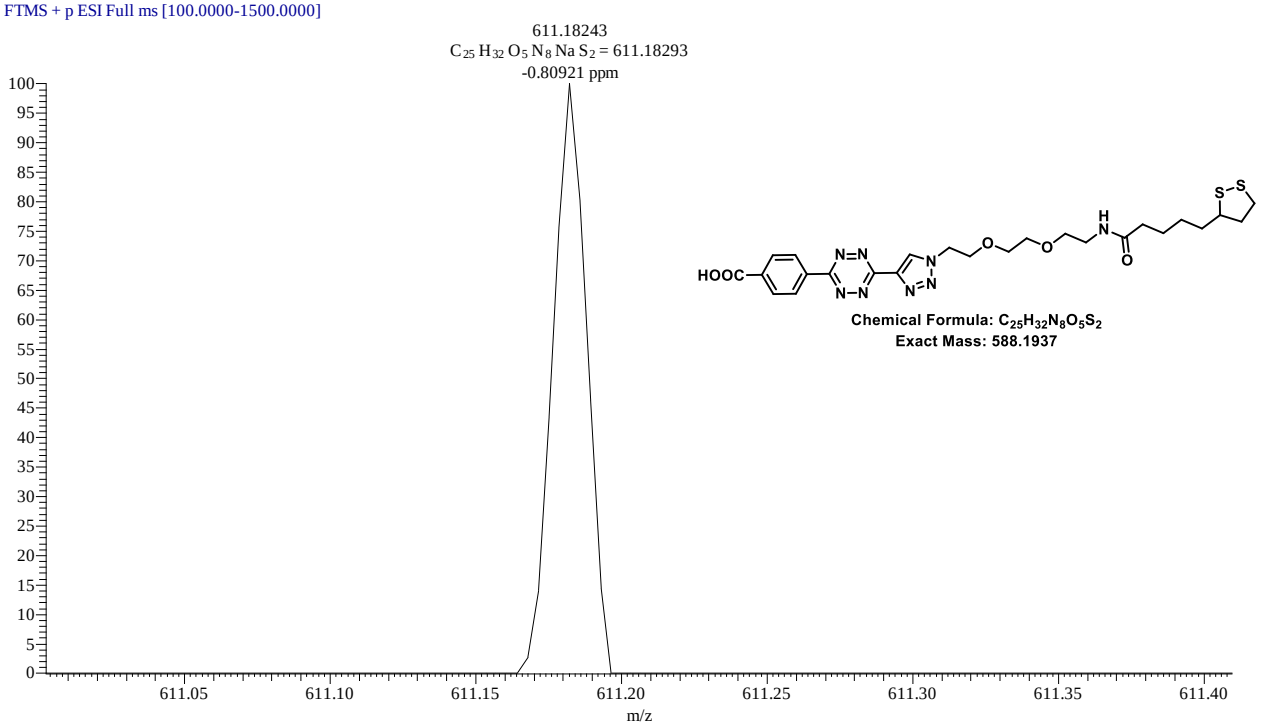


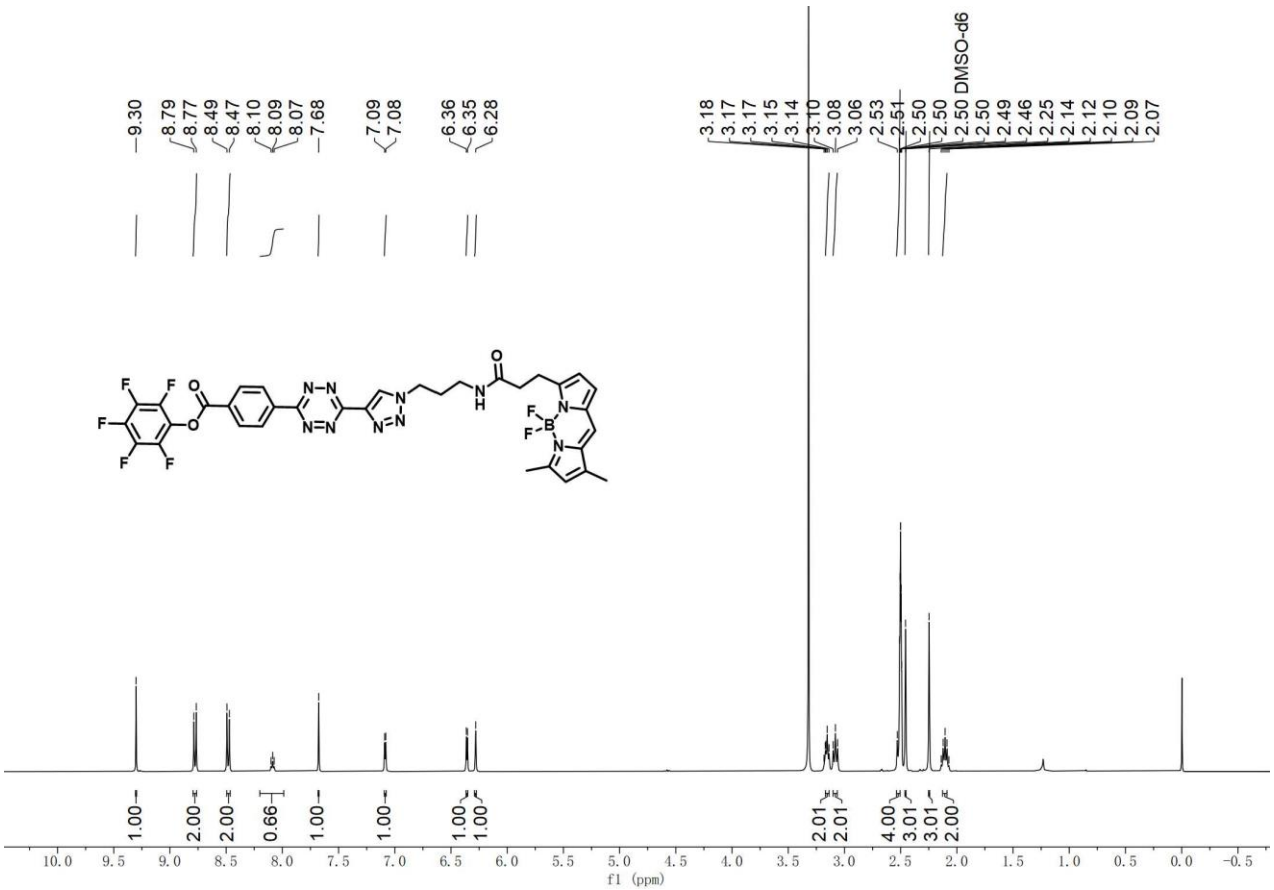
924 #23 RT: 0.10 AV: 1 NL: 2.28E8
T: FTMS + p ESI Full ms [100.0000-1500.0000]



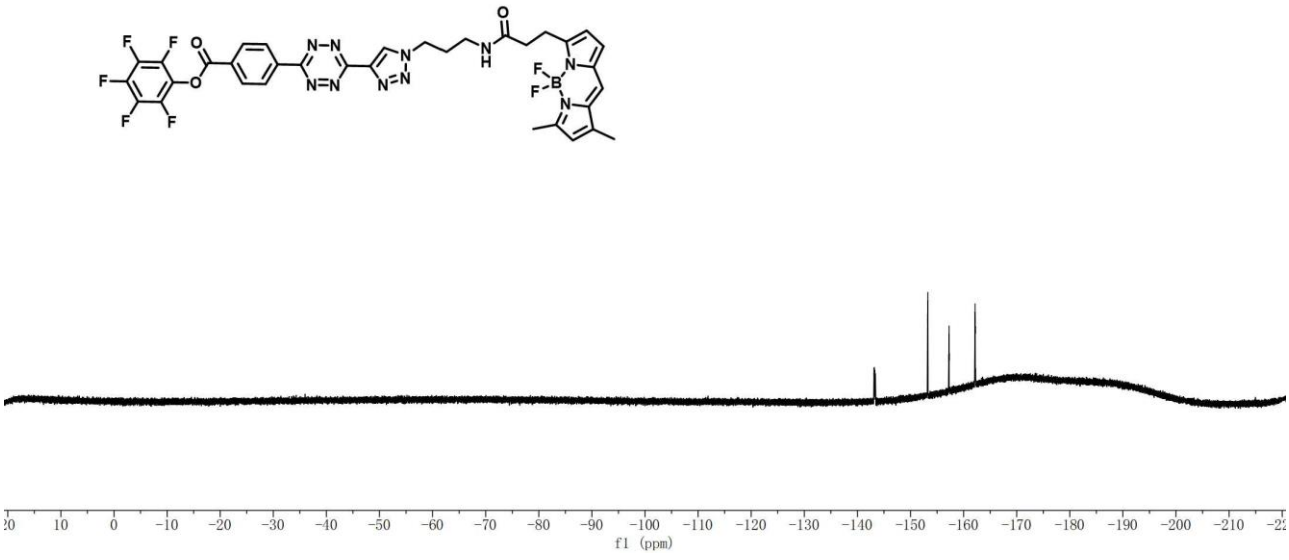


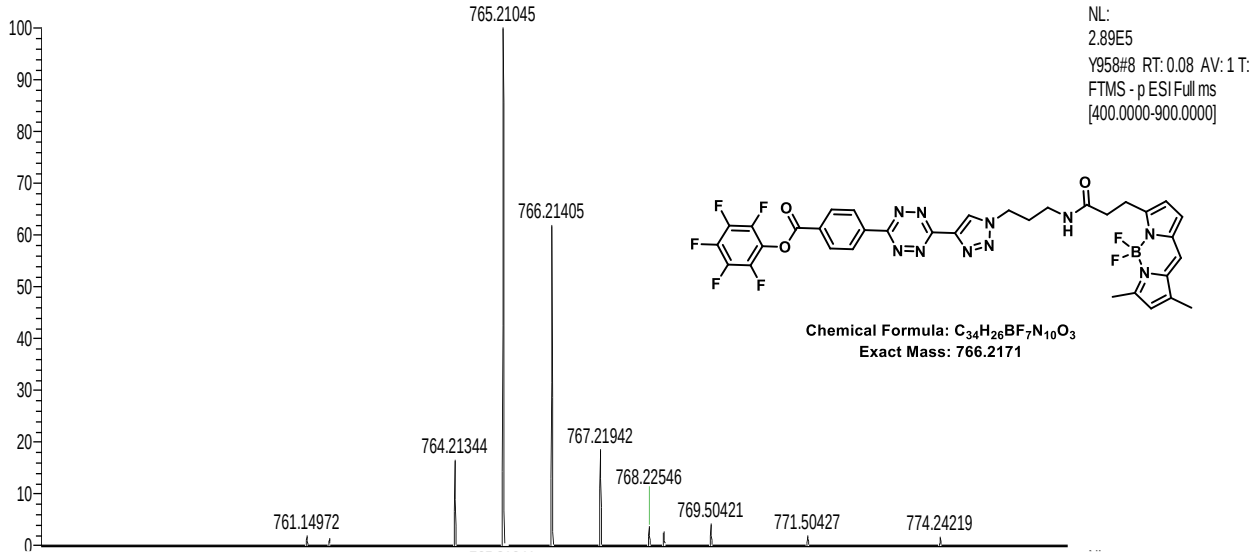
925 #22 RT: 0.10 AV: 1 NL: 1.26E6
T: FTMS + p ESI Full ms [100.0000-1500.0000]

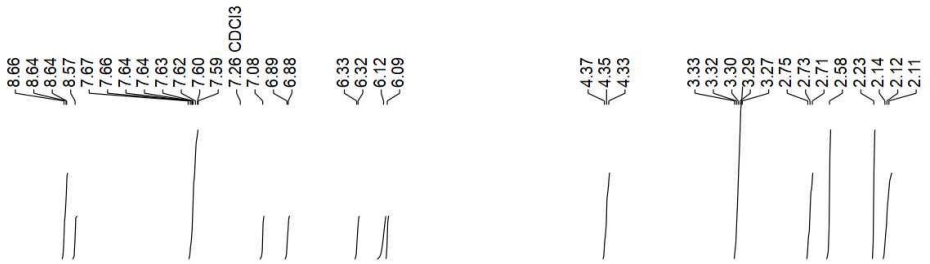


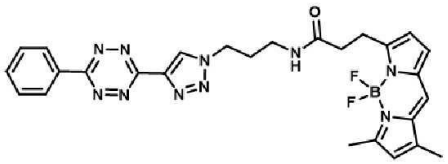

9.30
8.79
8.77
8.49
8.47
8.10
8.09
8.07
7.68
7.09
7.08
6.36
6.35
6.28
3.18
3.17
3.17
3.15
3.14
3.10
3.08
3.06
2.53
2.51
2.50
2.50 DMSO-d6
2.50
2.49
2.46
2.25
2.14
2.12
2.10
2.09
2.07
1.00
2.00
2.00
0.66
1.00
1.00
1.00
1.00
2.01
2.01
4.00
3.01
3.01
2.00
f1 (ppm)

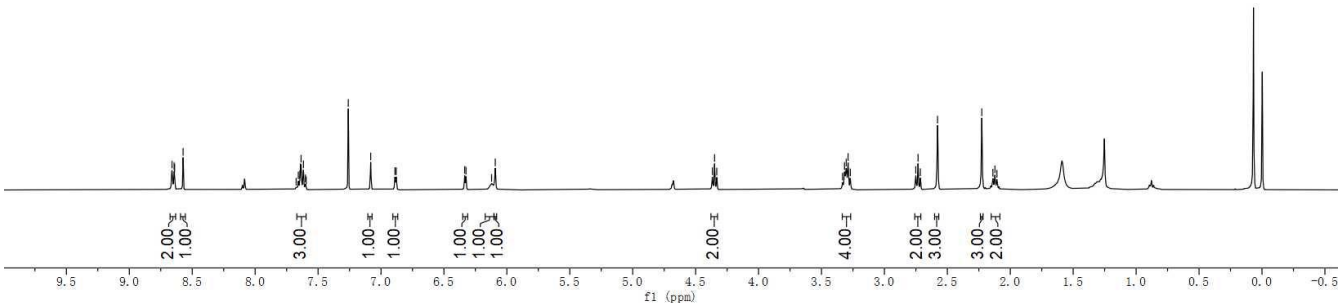

143.1
143.2
143.3
143.4
153.2
153.3
157.2
157.3
157.3
162.1
162.2
162.2
162.2

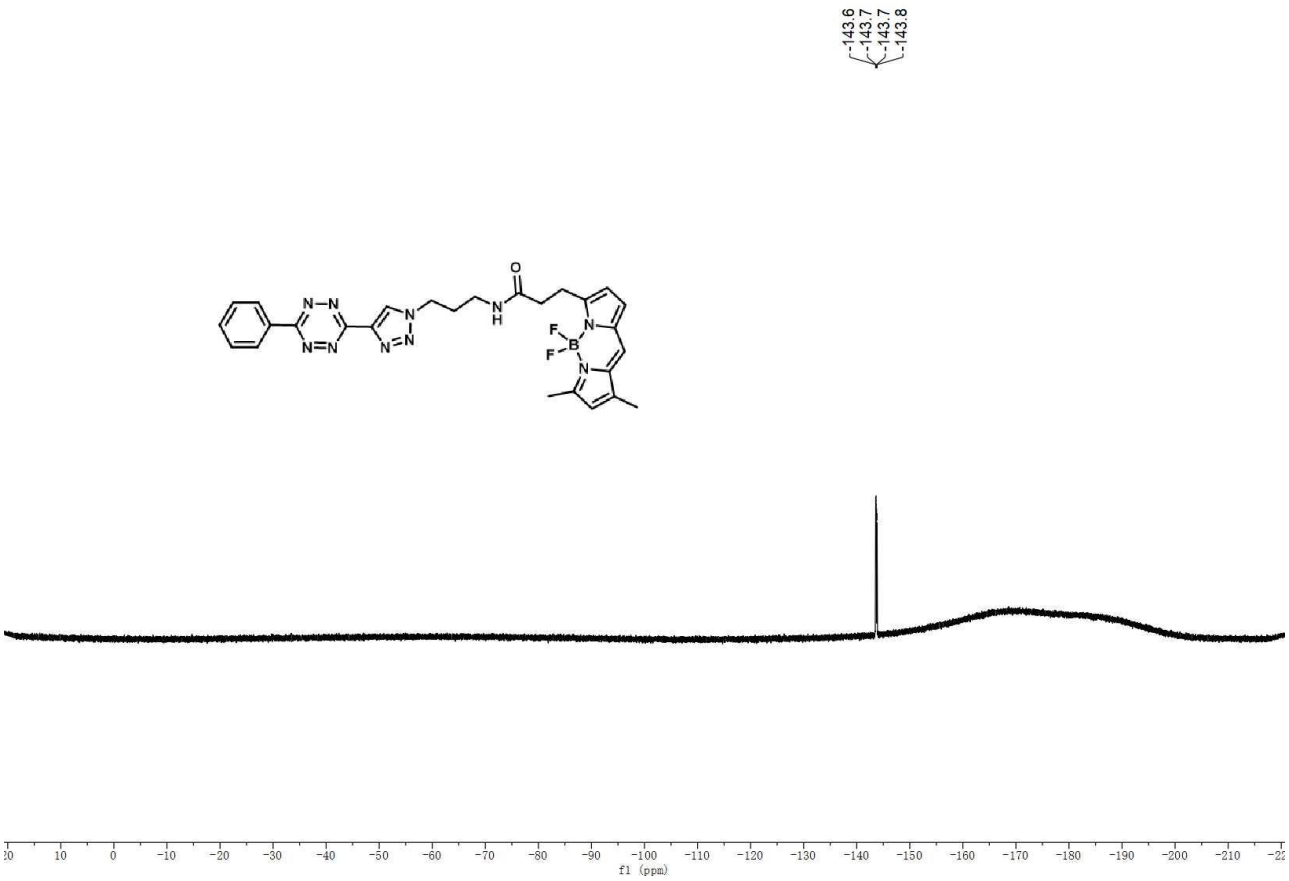

f1 (ppm)

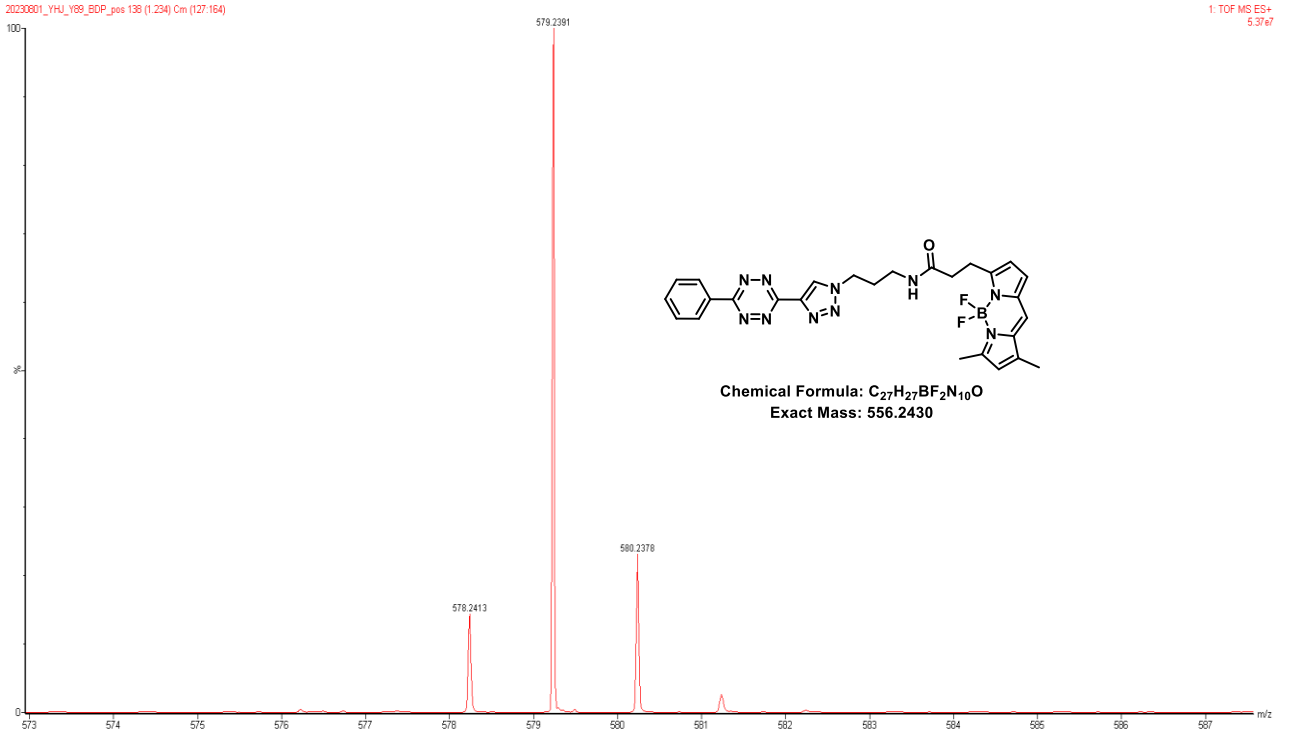

765.21045
766.21405
764.21344
767.21942
768.22546
769.50421
771.50427
774.24219
761.14972
NL:
2.89E5
Y958#8 RT: 0.08 AV: 1 T:
FTMS - p ESI Full ms
[400.0000-900.0000]
Chemical Formula: C34H26BF7N10O3
Exact Mass: 766.2171


CDCl3




f1 (ppm)





Chemical Formula: $C_{27}H_{27}BF_2N_{10}O$
Exact Mass: 556.2430