# Supplementary Information

# Reimagining proteome-wide evaluation of residue-specific bioconjugation chemistries

Youlong Fan[1,7], Yaping Peng[2,7], Jixiang He[3,7], Jun Xiong[4,7], Kang Duan[1], Keke Liu[3], Caiping Tian[3], Ling Fu[3], Hao Chi[2]*, Zhengqiu Li[1]*, Jing Yang[3,5,6]*

[1] College of Pharmacy, Jinan University, Guangzhou, China

[2] Key Laboratory of Intelligent Information Processing of Chinese Academy of Sciences (CAS); Institute of Computing Technology, CAS; University of Chinese Academy of Sciences, Beijing, China.

[3] State Key Laboratory of Proteomics; Beijing Proteome Research Center; National Center for Protein Sciences • Beijing; Beijing Institute of Lifeomics, Beijing, China

[4] School of Pharmacy, Xianning Medical College, Hubei University of Science and Technology, Hubei, China

[5] Guangzhou National Laboratory, Guangzhou International Bio Island, Guangzhou, China

[6] Guangzhou Medical University, Guangzhou, China

[7] These authors contribute equally to this work.

*Correspondence should be addressed to H.C. (Email: chihao@ict.ac.cn ORCID: 0000-0002-7898-2599), Z.L (Email: pharmlzq@jnu.edu.cn ORCID: 0000-0002-0433-2147) or J.Y. (Email: yang_jing@gzlab.ac.cn ORCID: 0000-0001-8486-273X)

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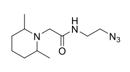
## 1. General methods

All reactions were carried out under an atmosphere of argon in oven-dried glassware with a magnetic stirring bar**,** all chemicals were purchased from commercial vendors and used without further purification, unless indicated otherwise. All reactions requiring anhydrous conditions were carried out under argon or nitrogen atmosphere using oven-dried glassware. AR-grade solvents were used for all reactions. Reaction progress was monitored by TLC on pre-coated silica plates (HSG F254 nm, 0.25 μm) and spots were visualized by UV, iodine or other suitable stains. Flash column chromatography was carried out using silica gel (HSG F254 nm, 0.040-0.063 μm). All NMR spectra ($^1$H-NMR, $^{13}$C-NMR) were recorded on Bruker Vance ARX- 400. Chemical shifts were reported in parts per million (ppm) referenced with respect to appropriate internal standards or residual solvent peaks ($CDCl_3$ = 7.26 ppm, DMSO-*d6* = 2.50 ppm). The following abbreviations were used in reporting spectra, br s (broad singlet), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets). Mass spectra were obtained on Agilent LC-ESI-MS system. All analytical HPLC were carried out on Agilent system.

## 2. Chemical structures of DMP tags and covalent probes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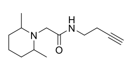
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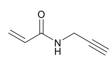
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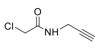
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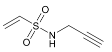
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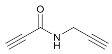
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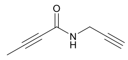
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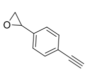
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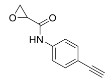
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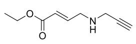
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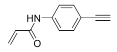
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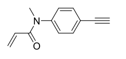
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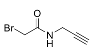
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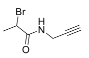
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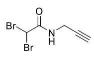
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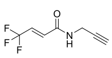
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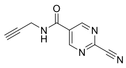
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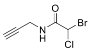
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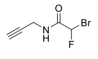
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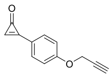
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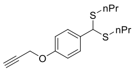
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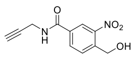
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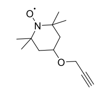
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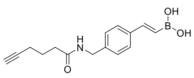
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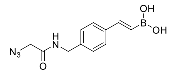
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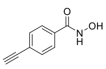
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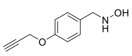
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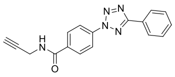
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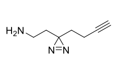
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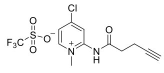
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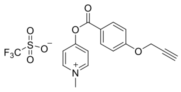
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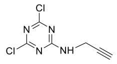
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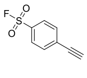
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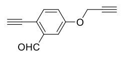
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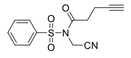
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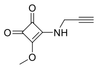
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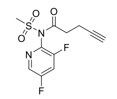
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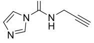
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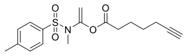
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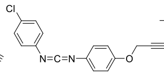
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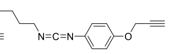
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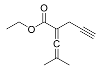
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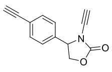
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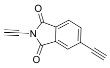
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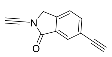
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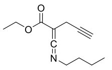
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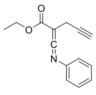
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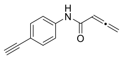
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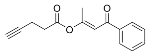
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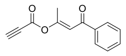
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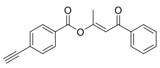
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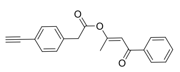
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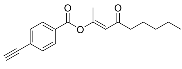
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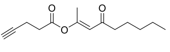
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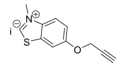
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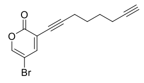
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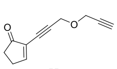
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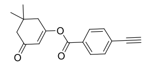
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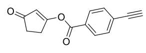
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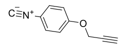
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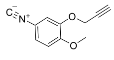
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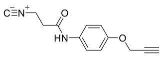
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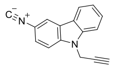
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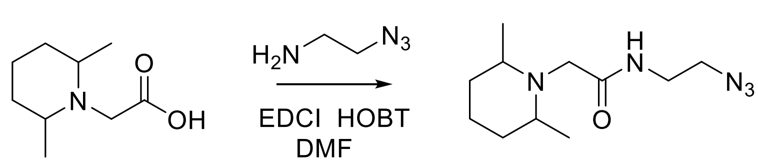
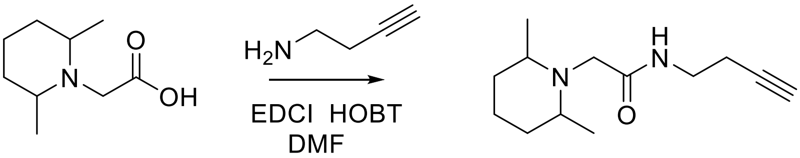
## 3. Synthetic procedures of DMP tags

### DMP-tag1



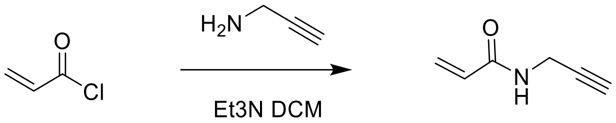
**N-(2-azidoethyl)-2-(2,6-dimethylpiperidin-1-yl)acetamide (DMP-tag1)** DMP-tag1 was reported and synthesis of DMP-tag1 was carried out similar to literature procedure[1] 2-(2,6-dimethylpiperidin-1-yl)acetic acid (171 mg, 1 mmol), HOBT (148 mg, 1.1 mmol), EDC.HCl (210 mg, 1.1 mmol) and DIPEA (387 mg, 3 mmol) were dissolved in anhydrous DMF (15 mL). The mixture was stirred for 3 h at r.t. The mixture of azide (75.9 mg, 1.1mmol) was added to the mixture. The mixture was stirred under rt for 72 h at r.t. The mixture was washed with $H_2O$ (15 mL) and brine (1 × 15 mL). The organic phase was dried over anhydrous $Mg_2SO_4$ and the solvent was removed in vacuo. The product was purified by column chromatography using 5% MeOH in DCM as eluent. Product was obtained as a yellow liquid (0.16 g, 70%),purified 97.8% .$^1$H NMR (400 MHz, DMSO) δ 7.94 (s, 1H), 3.38 (t, *J* = 5.7 Hz, 2H), 3.31 (dd, *J* = 10.5, 4.5 Hz, 3H), 2.98 (s, 2H), 2.48 – 2.39 (m, 2H), 1.64 – 1.57 (m, 1H), 1.57 – 1.45 (m, 2H), 1.26 (dt, *J* = 21.3, 12.0 Hz, 3H), 0.98 (d, *J* = 6.3 Hz, 7H). HRMS (ESI) calcd.for $C_{13}H_{22}N_2O$ 239.1750 $[M + H]^+$, found 240,1720.



**N-(but-3-yn-1-yl)-2-(2,6-dimethylpiperidin-1-yl)acetamide (DMP-tag2)** DMP-tag2 was reported and synthesis of DMP-tag2 was carried out similar to literature procedure[1]. 2-(2,6-dimethylpiperidin-1-yl)acetic acid (171 mg, 1 mmol), HOBT (148 mg, 1.1 mmol), EDC.HCl (210 mg, 1.1 mmol) and DIPEA (387 mg, 3 mmol) were dissolved in anhydrous DMF (15 mL). The mixture was stirred for 3 h at r.t. The mixture of but-3-yn-1-amine (94.6 g, 1.1mmol) was added to the mixture. The mixture was stirred under rt for 72 h at r.t. The mixture was washed with $H_2O$ (15 mL) and brine (1 × 15 mL). The organic phase was dried over anhydrous $Mg_2SO_4$ and the solvent was removed in vacuo. The product was purified by column chromatography using 5% MeOH in DCM as eluent. Product was obtained as a yellow liquid (0.13 g, 60%) purified 97.8% .$^1$H NMR (400 MHz, DMSO).δ 7.91 (s, 1H), 3.23 (q, J = 6.7 Hz, 2H), 2.96 (s, 2H), 2.84 (t, J = 2.6 Hz, 1H), 2.47 – 2.40 (m, 2H), 2.30 (td, J = 6.8, 2.6 Hz, 2H), 1.66 – 1.44 (m, 3H), 1.27 (dddd, J = 23.8, 14.6, 9.7, 3.3 Hz, 3H), 0.96 (t, J = 9.2 Hz, 6H) HRMS (ESI) calcd.for $C_{13}H_{22}N_2O$ 222.1730 $[M + H]^+$, found 223,1700.

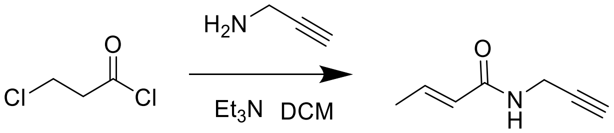
## 4. Synthetic procedures of covalent probes

**1**



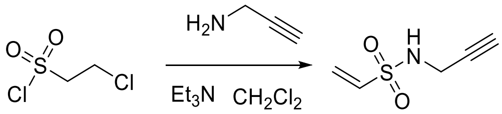
**N-(prop-2-yn-1-yl)acrylamide (1)** To a stirred solution of propargylamine (40 mg, 0.709 mmol) in dichloromethane (5 mL) was added triethylamine (0.1 mL, 1.06 mmol) followed by acryloyl chloride (70 mg, 0.779 mmol), and the resultant mixture was stirred at rt for 4 h under nitrogen atmosphere. The reaction mixture was diluted with dichloromethane (5 mL) white powder (96 mg, yield 80%)$^1$H NMR (400 MHz, DMSO-*d6*) δ 8.49 (d, *J* = 35.0 Hz, 1H), 6.22 (dd, *J* = 17.3, 10.0 Hz, 1H), 6.14 (d, *J* = 2.6 Hz, 1H), 5.63 (dd, *J* = 10.1, 2.6 Hz, 1H), 3.91 (ddd, *J* = 23.1, 5.7, 2.7 Hz, 2H), 3.13 (t, *J* = 2.8 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO) δ 164.75 (s), 131.55 (s), 126.35 (s), 81.38 (s), 73.59 (s), 28.32 (s). HRMS (ESI) calcd.for $C_6H_7NO$ 110.0600 [M + H]$^+$, found 110.0597.

**2**



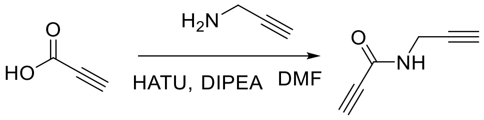
**(E)-N-(prop-2-yn-1-yl)but-2-enamide (2)** To a stirred solution of propargylamine (38 mg, 0.709 mmol) in dichloromethane (5 mL) was added triethylamine (0.1 mL, 1.06 mmol) followed by 3-chloropropanoyl chloride (97 mg, 0.779 mmol), and the resultant mixture was stirred at rt for 4 h under nitrogen atmosphere. The reaction mixture was diluted with dichloromethane (10 mL) white powder (126 mg, yield 78%) $^1$H NMR (400 MHz, DMSO) δ 8.68 (s, 1H), 4.08 (s, 2H), 3.90 (dd, *J* = 5.8, 2.7 Hz, 2H), 3.13 (t, *J* = 2.7 Hz, 1H).$^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.85 (s), 78.66 (s), 72.14 (s), 42.39 (s), 29.56 (s). HRMS (ESI) calcd.for $C_5H_6ClNO$ 132.0210 [M + H]$^+$, found 132.0211.

**3**



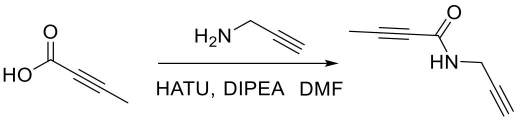
**N-(prop-2-yn-1-yl)ethenesulfonamide (3)** A solution of 2-chloroethanesulfonyl chloride (3.125 g, 19.3 mmol, 1.0 eq.) in $CH_2Cl_2$ (15 mL) was treated with propargylamine (1.118 g, 20.2 mmol, 1.04 eq.) and $Et_3N$ (2.174 g, 21.5 mmol, 1.1 eq.), and stirred at 0 °C for 20 min. Concentration and chromatography gaied the N-(prop-2-yn-1-yl)ethenesulfonamide (510 mg, yield 11.9%) as an orange oil. $R_f = 0.30$ (DCM). $^1$H NMR (400 MHz, DMSO) : δ 7.82 (s, 1H), 6.73 (dd, *J* = 16.5, 10.0 Hz, 1H), 6.12 – 5.96 (m, 2H), 3.72 (d, *J* = 2.5 Hz, 2H), 3.26 (t, *J* = 2.6 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO) : δ 137.41, 126.24, 80.50, 75.19, 32.16. HRMS (ESI) calcd. for $C_6H_5NO$ 146.0275 $[M+H]^+$, found 146.0270.

**4**



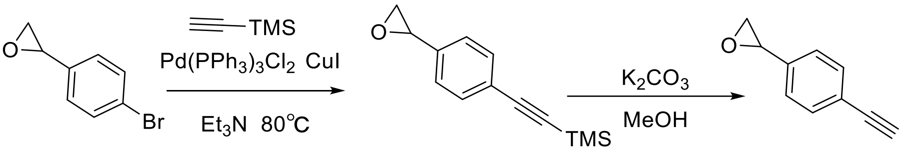
**N-(prop-2-yn-1-yl)propiolamide (4)** Under a Ar atmosphere, propiolic acid (210.0 mg, 3.0 mmol, 1.0 eq.) was dissolved in dry DMF (10.0 mL) and HATU (1.425 g, 3.75 mmol, 1.25 eq.) was added. propargylamine (198.0 mg, 3.6 mmol, 1.2 eq.) and DIPEA (1.163 g, 9.0 mmol, 3.0 eq.) were added and the mixture was stirred at room temperature for 1h. The mixture was diluted with $H_2O$ (15 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with $H_2O$ (2 x 15 mL) and brine (15 mL). The organic layers were concentrated in vacuo and purified by flash chromatography (hexane/EtOAc=5:1) to yield the desired product as slightly yellow solid (216 mg, yield 53.3%). TLC (5:1 hexane/EtOAc) $R_f = 0.20$. Analytical data was in agreement with reported data. $^1$H NMR (400 MHz, $CDCl_3$): δ 6.20 (s, 1H), 4.12 (dd, *J* = 5.4, 2.5 Hz, 2H), 2.86 (s, 1H), 2.30 (t, J = 2.6 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO) : δ 151.85, 80.55, 78.11, 76.90, 73.71, 28.55. HRMS (ESI) calcd. for $C_6H_5NO$ 108.0444 $[M+H]^+$, found 108.0448.

**5**



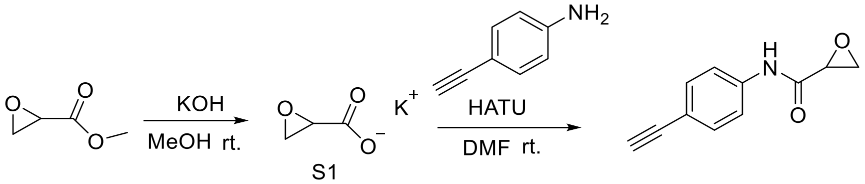
**N-(prop-2-yn-1-yl)but-2-ynamide (5)** Under a Ar atmosphere, but-2-ynoic acid (252.1 mg, 3.0 mmol, 1.0 eq.) was dissolved in dry DMF (10.0 mL) and HATU (1.425 g, 3.75 mmol, 1.25 eq.) was added. propargylamine (198.0 mg, 3.6 mmol, 1.2 eq.) and DIPEA (1.163 g, 9.0 mmol, 3.0 eq.) were added and the mixture was stirred at room temperature for 1 h. The mixture was diluted with $H_2O$ (15 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with $H_2O$ (2 x 15 mL) and brine (15 mL). The organic layers were concentrated in vacuo and purified by flash chromatography (DCM → DCM/MeOH=100:1) to yield the desired product as slightly yellow solid (153 mg, yield 34.0%). TLC (1:1 hexane/EtOAc) $R_f = 0.40$. Analytical data was in agreement with reported data. $^1$H NMR (400 MHz, DMSO) δ 7.82 (s, 1H), 6.73 (dd, J = 16.5, 10.0 Hz, 1H), 6.12 – 5.96 (m, 2H), 3.72 (d, J = 2.5 Hz, 2H), 3.26 (t, J = 2.6 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO) δ 137.41, 126.24, 80.50, 75.19, 32.16. HRMS (ESI) calcd. for $C_7H_7NO$ 122.0600 $[M+H]^+$, found 122.0601.

**6**



**2-(4-ethynylphenyl)oxirane (6)** A solution of (4-bromophenoxy)triisopropylsilane (0.99 g, 5.0 mmol), $PdCl_2(PPh_3)_2$ (70 mg, 0.1 mmol), and CuI (15.6 mg, 0.2 mmol) in $NEt_3$ (8 mL) was added to the trimethylsilane acetylene (0.877 g, 15 mmol). The mixture was stirred at 80°C under $N_2$ atmosphere for 2 h. After the complete consumption of 4-bromophenoxy)triisopropylsilane, the reaction mixture was quenched with water and extracted with $CH_2Cl_2$ three times. The combined organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to afford the desired produc The crude product was not purified and was directly used in the next reaction.A mixture of triisopropyl(4-((trimethylsilyl)ethynyl)phenoxy)silane (216 mg, 1.0 mmol), anhydrous potassium carbonate (12.4 mg, 0.09 mmol) in anhydrous MeOH (3 mL) was stirred at 25°C under argon for 1 h. The solvent was evaporated under reduced pressure, and the residue was mixed with 2 mL of aqueous sodium bicarbonate and extracted with ethyl acetate (10 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography on silica gel to afford the desired product(194 mg ,yield 90%) $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.2 Hz, 1H), 7.25 (d, *J* = 8.2 Hz, 1H), 3.87 (dd, *J* = 4.0, 2.6 Hz, 1H), 3.18 (dd, *J* = 5.5, 4.1 Hz, 1H), 3.12 (s, 1H), 2.79 (dd, *J* = 5.5, 2.5 Hz, 1H). $^{13}C$ NMR (151 MHz, DMSO) δ 139.19 (s), 132.26 (s), 126.45 (s), 121.83 (s), 83.69 (s), 81.59 (s), 51.62 (s), 51.08 (s). HRMS (ESI) calcd.for $C_{10}H_9O$ 145.0647 $[M + H]^+$, found 145.0645.

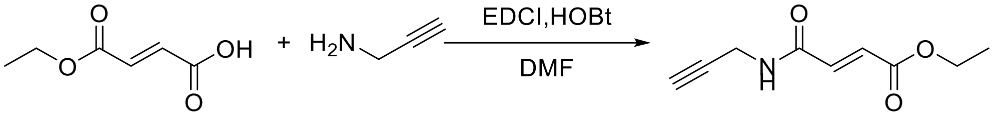
**7**



**Oxirane-2-carboxylate** (**S1**). Methyl oxirane-2-carboxylate (163 μL, 2.0 mmol) was dissolved in methanol (4 mL). The KOH (135 mg, 2.4 mmol) was added to the solution and the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and washed with anhydrous ether to obtain S1 as a white solid, which can be directly used for the next reaction without purification.

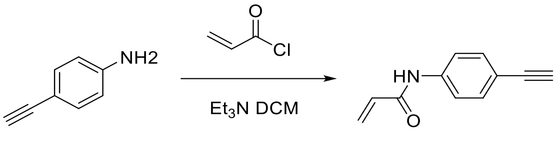
**N-(4-ethynylphenyl) oxirane-2-carboxamide** (7). S1 (147 mg, 1.2 mmol) and HATU (445 mg, 1.2 mmol) were dissolved in 8 mL of dimethylformamide (DMF), N,N-Diisopropylethylamine(DIEPA) (100 μL, 0.6 mmol) was added to the reaction mixture, which was stirred under room temperature for 3 min. And then the 4-ethynylaniline (151 mg, 1.3 mmol) was added to the reaction mixture and stirred at room temperature for 3 h. The reaction mixture was quenched by addition of water and extracted with ethyl acetate (EA) three times. The combined organic layer was then washed with brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was purified by column chromatography (eluting with EA:PE=1:5) on silica gel to afford the desired product as a white solid (36 mg, 0.19 mmol, yield 17%). 1H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.54 – 7.42 (m, 4H), 3.56 (dd, J = 4.7, 2.6 Hz, 1H), 3.09 (t, J = 5.1 Hz, 1H), 3.05 (s, 1H), 2.91 (dd, J = 5.3, 2.6 Hz, 1H). 13C NMR (151 MHz, $CDCl_3$) δ 166.49, 137.12, 133.04, 119.35, 118.29, 83.17, 77.11, 49.93, 47.69. HRMS (ESI) calcd.for $C_{11}H_9N_2O$ 188.0706 [M + H]+, found 188.0707.

**8**



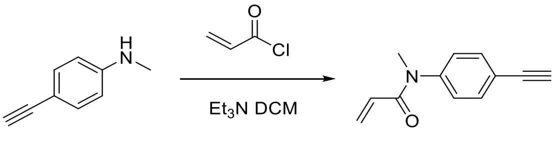
**Ethyl(E)-4-oxo-4-(prop-2-yn-1-ylamino)but-2-enoate (8)** Fumaric acid monoethylester (1.366 g, 9.48 mmol, 1.1 eq) was solved in DMF (30 mL). To this solution, EDCI·HCl (2 g, 10.43 mmol, 1 eq) and HOBt (1.4 g, 10.43 mmol, 1 eq) were added. The solution was incubated for 30-40 min at 0°C to form the reactive ester. Finally, propargylamine (547 mg, 10.43 mmol, 1.1 eq) was added and stirred at ambient temperature overnight. While the solution turned brown, a white precipitate formed. After completion of the reaction the solvent was evaporated and the crude was purified by flash-chromatography (hexane/EtOAc = 3:1). Slightly yellow solid, (960 mg, yield 55.94%).$^1$H NMR (400 MHz, DMSO) δ 8.97 (s, 1H), 6.99 (d, *J* = 18.8 Hz, 1H), 6.62 (d, *J* = 1.1 Hz, 1H), 4.18 (dd, *J* = 17.1, 7.8 Hz, 2H), 4.09 – 3.85 (m, 2H), 3.17 (d, *J* = 2.6 Hz, 1H), 1.47 – 1.01 (m, 3H). $^{13}$C NMR (151 MHz, DMSO) δ 165.36 (s), 163.02 (s), 137.12 (s), 129.51 (s), 80.76 (s), 74.19 (s), 61.24 (s), 28.69 (s), 14.46 (s). HRMS (ESI) calcd. for $C_9H_{11}NO_3$ 182.0812 $[M+H]^+$, found 182.0804.

**9**



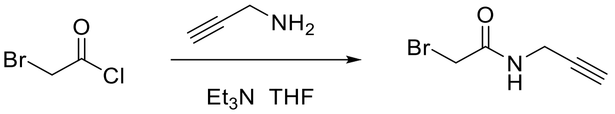
**N-(4-ethynylphenyl)acrylamide (9)** The compound was purchased from Bide Pharmaceuticals (CAS: 1233382-94-9, Cat. No BD01086297).

**10**



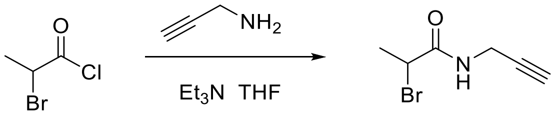
**(N-(4-ethynylphenyl)-N-methylacrylamide (10)** A solution of methacryloyl chloride ( 2 g, 22 mmol, 1.0 eq.) in $CH_2Cl_2$ (15 mL) was treated with 4-ethynylaniline (2.3 g, 17.5 mmol, 1.04 eq.) and $Et_3N$ (2.174 g, 21.5 mmol, 1.1 eq.), and stirred at 0 °C for 20 min. Concentration and chromatography gave N-(4-ethynylphenyl)-N-methylacrylamide (2.1 mg , yield 46 %) as an white solid. $^1$H NMR (400 MHz, DMSO) δ 7.53 (d, *J* = 6.7 Hz, 2H), 7.29 (d, *J* = 8.5 Hz, 2H), 6.13 (s, 1H), 5.58 (d, *J* = 9.4 Hz, 1H), 4.25 (s, 1H), 3.24 (s, 3H), 2.49 (s, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 164.80 (s), 143.96 (s), 133.33 (s), 129.15 (s), 127.99 (s), 127.85 (s), 121.09 (s), 83.19 (s), 82.01 (s), 37.18 (s). HRMS (ESI) calcd. for $C_{12}H_{11}NO$ 186.0913 $[M+H]^+$, found 186.0906.

**11**



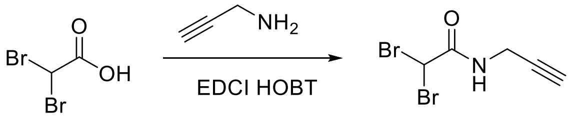
**2-bromo-N-(prop-2-yn-1-yl) acetamid (11)** To a solution of acetylenamine (55 mg, 1 mmol) and triethylamine (151 mg, 1.5 mmol) in anhydrous THF (200 mL) and cooled to 0°C under nitrogen, 2-bromopropionyl bromide was added (170 mg,1.1 mmol), Under a nitrogen atmosphere, a solution of anhydrous THF (1 mL) was added dropwise over 10 min. The reaction mixture was warmed to room temperature and stirred overnight. The precipitate was filtered off and the filtrate was evaporated to dryness. Crude oil products are dissolved in DCM (10 mL) and wash sequentially with 5% (w/v) $NaHCO_3$ aqueous solution (5 mL), saturated $NH_4Cl$ solution (5 mL), and 5% brine solution (50 mL) Finally use distilled water (5 mL). The organic layer was dried over anhydrous $NaSO_4$, filtered and concentrated, then purified by column chromatography (methanol/methanol). A colored oily product (146 mg, yield 87%). $^1$H NMR (400 MHz, $CDCl_3$) δ 6.69 (s, 1H), 4.09 (d, *J* = 2.3 Hz, 2H), 3.90 (d, *J* = 2.7 Hz, 2H), 2.28 (s, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.57 (s), 78.67 (s), 72.16 (s), 29.94 (s), 28.65 (s). HRMS (ESI) calcd. for $C_5H_6BrNO$ 175.9697 $[M+H]^+$, found 175.9705.

**12**



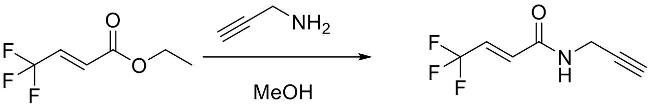
**2-bromo-N-(prop-2-yn-1-yl)propanamide (12)** To a solution of acetylenamine (55 mg, 1 mmol) and triethylamine (151 mg, 1.5 mmol) in anhydrous THF (200 mL) and cooled to 0°C under nitrogen, 2 bromo-propanoyl chloride was added(185 mg, 1.1 mmol) Under a nitrogen atmosphere, a solution of anhydrous THF (1 mL) was added dropwise over 10 min. The reaction mixture was warmed to room temperature and stirred overnight. The precipitate was filtered off and the filtrate was evaporated to dryness. Crude oil products are dissolved in DCM (10 mL) and wash sequentially with 5% (w/v) $NaHCO_3$ aqueous solution (5 mL), saturated $NH_4Cl$ solution (5 mL), and 5% brine solution (50 mL) Finally use distilled water ( 5 mL). The organic layer was dried over anhydrous $NaSO_4$, filtered and concentrated, then purified by column chromatography (methanol/methanol). Pale A colored oily product(190 mg, yield 80%). $^1$H NMR (400 MHz, $CDCl_3$) δ 6.57 (s, 1H), 4.42 (d, *J* = 6.4 Hz, 1H), 4.08 (s, 1H), 2.28 (s, 2H), 1.89 (d, *J* = 5.6 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 169.16 (s), 78.77 (s), 72.12 (s), 44.30 (s), 29.96 (s), 22.88 (s). HRMS (ESI) calcd. for $C_6H_8BrNO$ 189.9851 $[M+H]^+$, found 189.9862.

**13**



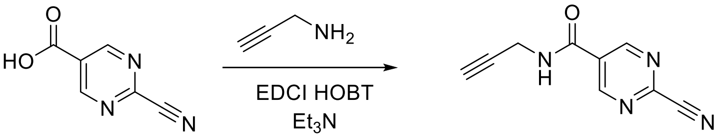
**2,2-dibromo-N-(prop-2-yn-1-yl)acetamide (13)** To a stirred solution of 2-bromo-2-fluoroacetic acid (122 mg, 0.57 mmol) was added in the in DCM (5.0 mL),then EDCI(163 mg, 0.85 mmol) and HOBT(115 mg, 0.85 mmol) were added into the mixture. After 5 min, followed by the propargylamine(38 mg, 0.69 mmol) were added. The mixture was stirred at room temperature overnight, followed by addition of water (10 mL). The mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and dried over anhydrous $Na_2SO_4$. After evaporation of the solvent, the residue was purified by flash chromatography to gain a white solid (96 mg, yelid 70%) $^1$H NMR (400 MHz, $CDCl_3$) δ 6.67 (s, 1H), 6.67 (s, 1H), 5.83 (s, 1H), 4.13 (dd, J = 5.5, 2.7 Hz, 2H), 2.57 – 2.13 (m, 1H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 164.02 (s), 77.89 (s), 72.83 (s), 35.45 (s), 30.53 (s). HRMS (ESI) calcd. for $C_5H_5Br_2NO$ 253.8810 $[M+H]^+$, found 253.8808.

**14**



**(E)-4,4,4-trifluoro-N-(prop-2-yn-1-yl)but-2-enamide (14)** Propargylamine (55 mg, 1 mmol) and ethyl (E)-4,4,4-trifluorobut-2-enoate (218 mg, 1.3 mmol) were dissolved in methanol (5 mL) and stirred for 24 h in the dark at room temperature. After removal of the solvent under reduced pressure saturated $NaHCO_3$ solution (5 mL) was added to the residue and the aqueous phase was extracted with DCM (10 mL). The combined organic layers were dried through $Na_2SO_4$, filtered and the solvent was removed under reduced pressure. Desired product was obtained as yellow oil (177 mg, yield 75%).$^1$H NMR (400 MHz, $CDCl_3$) δ 6.79 (d, J = 9.4 Hz, 1H), 6.55 (d, J = 14.9 Hz, 2H), 4.16 (s, 2H), 2.28 (s, 1H). $^{13}$C NMR (101 MHz, CDCl3) δ 162.69 (s), 130.17 (q, J = 5.8 Hz), 129.07 (q, J = 35.4 Hz), 123.69 (s), 78.37 (s), 72.29 (s), 29.65 (s). HRMS (ESI) calcd. for $C_7H_6F_3NO$ 178.0474 $[M+H]^+$ found 178.0465.

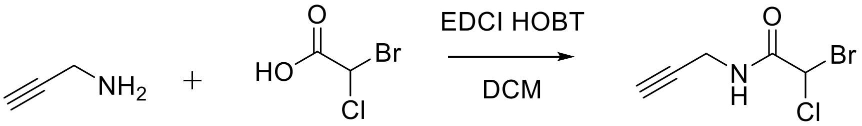
**15**



**2-cyano-N-(prop-2-yn-1-yl)pyrimidine-5-carboxamide (15)** To a solution of acetylenamine (55 mg, 1 mmol) and triethylamine(151 mg, 1.5 mmol), EDCI(229mg, 1.2 mmol), DMAP(12 mg, 0.01 mmol) in anhydrous DCM (2 mL) , 2-cyanopyrimidine-5-carboxylic acid was added (163 mg, 1.1 mmol), The reaction mixture was warmed to room temperature and stirred overnight. The precipitate was filtered off and the filtrate was evaporated to dryness. Crude oil products are dissolve in DCM (10 mL) and wash sequentially with 5% (w/v) $NaHCO_3$ aqueous solution (5 mL), saturated $NH_4Cl$ solution (5 mL), and 5% brine solution (5 mL) Finally use distilled water ( 5 mL). The organic layer was dried over anhydrous $NaSO_4$, filtered and concentrated, then purified by column chromatography (methanol/methanol). A colored oily product(90.4 mg, yield 45%). $^1$H NMR (400 MHz, DMSO) δ 9.59 – 9.49 (m, 1H), 9.50 (dd, J = 28.4, 22.8 Hz, 2H), 9.32 (s, 3H), 4.13 (dd, J = 5.8, 2.7 Hz, 2H), 3.23 – 3.15 (m, 1H). $^{13}$C NMR

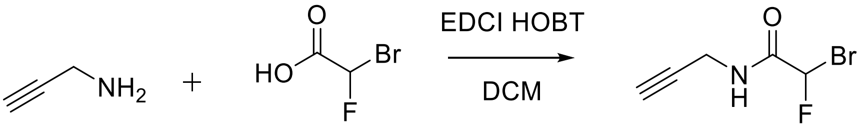
(151 MHz, DMSO) δ 162.00 (s), 157.93 (s), 145.53 (s), 129.47 (s), 116.25 (s), 80.62 (s), 74.23 (s), 29.22 (s). HRMS (ESI) calcd. for $C_9H_6N_4O$ 187.0614 $[M+H]^+$, found 187.0613.

**16**



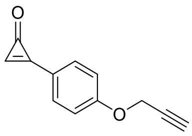
**2-bromo-2-fluoro-N-(prop-2-yn-1-yl)acetamide (16)** To a stirred solution of 2-bromo-2-fluoroacetic acid (100 mg, 0.57 mmol) was added in the in DCM(5.0 mL),then EDCI(163 mg, 0.85 mmol) and HOBT(115 mg, 0.85 mmol) were added into the mixture. After 5 min, followed by the Propargylamine(38 mg, 0.69 mmol) were added. The mixture was stirred at room temperature overnight, followed by addition of water (10 mL). The mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and dried over anhydrous $Na_2SO_4$. After evaporation of the solvent, the residue was purified by flash chromatography to gain a white solid (96 mg, yelid 70%) $^1$H NMR (400 MHz, DMSO) δ 9.13 (s, 1H), 7.06 (d, J = 49.2 Hz, 1H), 3.94 (dd, J = 5.6, 2.5 Hz, 2H), 3.18 (t, J = 2.5 Hz, 1H), $^{13}$C NMR (151 MHz, DMSO) δ 171.10 (s), 87.81 (s), 74.06 (s), 73.17 (s), 28.11 (s).. HRMS (ESI) calcd. for $C_5H_5BrClNO$ 209.9315 $[M+H]^+$, found 209.9314.

**17**



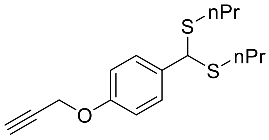
**2-bromo-2-chloro-N-(prop-2-yn-1-yl)acetamide (17)** To a stirred solution of 2-bromo-2-chloroacetic acid (100 mg, 0.64 mmol) was added in the in DCM (5.0 mL),then EDCI(163 mg, 0.85 mmol) and HOBT(115 mg, 0.85 mmol) were added into the mixture. After 5 min, followed by the Propargylamine(38 mg, 0.69 mmol) were added. The mixture was stirred at room temperature overnight, followed by addition of water (10.0 mL). The mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and dried over anhydrous $Na_2SO_4$. After evaporation of the solvent, the residue was purified by flash chromatography to gain a white solid (89 mg, yelid 65%). $^1$H NMR (400 MHz, DMSO) δ 8.98 (s, 1H), 6.43 (s, 1H), 4.11 – 3.82 (m, 2H), 3.21 (t, J = 2.5 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO) δ 171.10 (s), 87.81 (s), 74.06 (s), 73.17 (s), 28.11 HRMS (ESI) calcd. for $C_5H_5BrFNO$ 193.9611 [M+H]$^+$, found 193.9610.

**18**



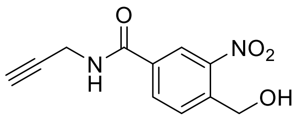
**2-(4-(prop-2-yn-1-yloxy)phenyl)cycloprop-2-en-1-one (18)** Synthesis of **18** was carried out as previously described[2] to yield a white solid (109.1 mg, yield 90%) HPLC purity: 96.78% (MeOH:$H_2O$ = 80:20) . $^1$H NMR (400 MHz, DMSO-*d*$_6$) δ 8.94 (s, 1H), 7.87 (d, *J* = 8.9 Hz, 2H), 7.23 (d, *J* = 8.8 Hz, 2H), 4.96 (d, *J* = 2.4 Hz, 2H), 3.67 (s, 1H). $^{13}$C NMR (101 MHz, DMSO-*d*$_6$) δ 161.49, 160.36, 154.69, 141.12, 133.53, 116.93, 116.29, 79.40, 79.00, 56.31. HRMS (ESI) calcd. for $C_{12}H_8O_2$ 185.0597 [M+H]$^+$, found 185.0592.

**19**



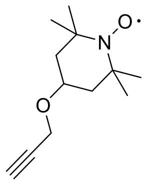
**((4-(prop-2-yn-1-yloxy)phenyl)methylene)bis(propylsulfane) (19)** The compound was kindly provided by the Dr. Zigang Li [3].

**20**



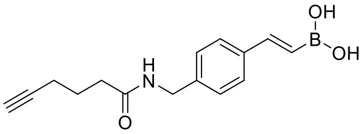
**4-(hydroxymethyl)-3-nitro-N-(prop-2-yn-1-yl)benzamide (20)** The compound 20 was synthesized as previously described [4] $^1$H NMR (400 MHz, DMSO) δ 9.33 – 9.20 (m, 1H), 8.53 (d, J = 2.0 Hz, 1H), 8.24 (dd, J = 8.5, 1.8 Hz, 1H), 7.95 (d, J = 8.7 Hz, 1H), 5.68 (t, J = 5.9 Hz, 1H), 4.88 (d, J = 5.9 Hz, 2H), 4.10 (dd, J = 5.9, 2.7 Hz, 2H), 3.20 – 3.01 (m, 2H). 13C NMR (151 MHz, DMSO) δ 164.29 (s), 147.03 (s), 142.15 (s), 133.62 (s), 132.64 (s), 129.03 (s), 123.69 (s), 81.34 (s), 73.67 (s), 60.37 (s), 29.16 (s).

**21**



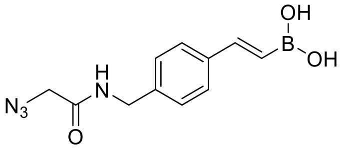
**(21)** The compound was first reported in ref [5] and can be purchased from Bide Pharmaceuticals purify 95% (CAS: 147045-24-7, Cat. No BD01096089).

**22**



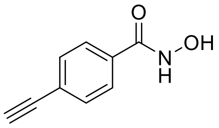
**(E)-(4-(hex-5-ynamidomethyl)styryl)boronic acid (22)** The compound was kindly provided by Dr. Bobo Dang [6].

**23**



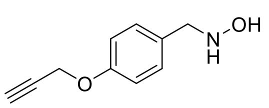
**(E)-(4-((2-azidoacetamido)methyl)styryl)boronic acid (23)** The compound was a was kindly provided by Dr. Bobo Dang [6].

**24**



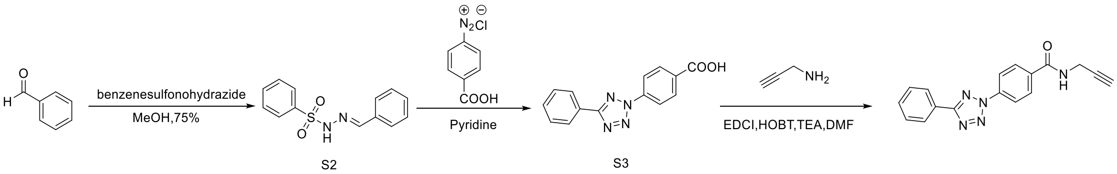
**4-ethynyl-N-hydroxybenzamide (24)** The compound was synthsized as previously described [7] $^1$H NMR (400 MHz, DMSO) δ 11.31 (s, 1H), 9.12 (s, 1H), 7.75 (d, J = 7.8 Hz, 2H), 7.55 (d, J = 7.8 Hz, 2H), 4.35 (s, 1H). $^{13}$C NMR (151 MHz, DMSO) δ 163.86, 133.33, 132.20, 127.63, 124.85, 83.30, 83.23. ESI-MS calcd for $C_9H_7NO_2$ $[M+H]^+$ 162.0550; Found 162.0551.

**25**



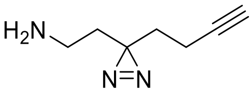
**N-(4-(prop-2-yn-1-yloxy)benzyl)hydroxylamine (25)** The compound was synthesized as previously described [7] .

**26**



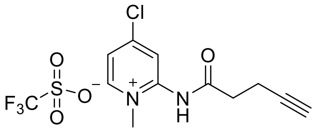
**4-(5-phenyl-2H-tetrazol-2-yl)-N-(prop-2-yn-1-yl)benzamide (26)** The compound was synthesized as previously described [8]. To a suspension of 4-aminobenzoic acid (121 mg, 1 mmol) in 5 mL $H_2O$ and 5 mL EtOH was added 0.5 mL concentrated HCl, followed by addition of a solution of $NaNO_2$ (69 mg, 1 mmol) in 1 mL $H_2O$ at 0 °C. After stirring for 10 min, the solution was added dropwise to the solution of **S2** (1 mmol) in 5 mL pyridine over 50 min at -10 °C. The mixture was warmed up to rt. prior to addition of 30 mL $H_2O$. The solution was extracted with 2 × 10 mL of ethyl acetate, the combined organic layers were washed successively with 3×100 mL 2 N HCl and 70 mL brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE: EA = 1:1, $R_f$ = 0.2) to give **S3** as a brown solid (200 mg ,60%). To a stirred solution of S3 (40 mg, 0.15 mmol) in 5 mL of DMF was added HOBT (41 mg, 0.3 mmol), EDCI (58 mg, 0.3 mmol) and TEA (30 mg, 0.3 mmol). The mixture was stirred for 30 min at r.t followed by addition of propargylamine (9 mg, 0.3 mmol). The reaction was then stirred at room temperature overnight in the dark. Subsequently, it was quenched by addition of 5 mL water and extracted with 2 × 10 mL of ethyl acetate. The organic layers were washed with 2×10 mL brine and dried over anhydrous $Na_2SO_4$ Upon solvent evaporation in vacuo, the residue was purified by flash column (PE : EA = 5:1) to give 26 as a white solid (38 mg, 83%), $R_f$ = 0.6 (PE:EA = 1:1)$^1$H NMR (400 MHz, DMSO-*d*$_6$) δ 9.24 (d, *J* = 5.5 Hz, 1H), 8.32 – 8.24 (m, 3H), 8.22 – 8.13 (m, 5H), 7.66 – 7.55 (m, 4H), 4.11 (dd, *J* = 5.5, 2.5 Hz, 2H), 3.20 (t, *J* = 2.5 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO-*d*$_6$) δ 165.12 (d, *J* = 2.7 Hz), 138.35, 135.36, 131.58, 129.81 (d, $J$ = 10.8 Hz), 127.17, 126.70, 120.17, 81.53, 73.62, 29.12. HR-MS (ESI) Calcd $C_{17}H_{13}N_5O$ for $[M+H]^+$ 304.1198; found 304.1193.

**27**



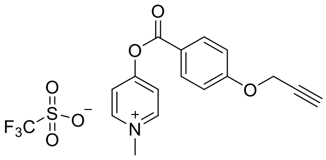
**2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethan-1-amine (27)** The compound was purchased from Bide Pharmaceuticals purify 95% (CAS: 1450752-97-2, Cat.No BD627887) [9].

**28**



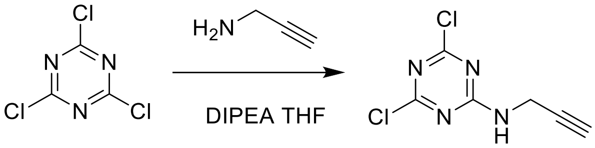
**4-chloro-1-methyl-2-(pent-4-ynamido)pyridin-1-ium-trifluoromethanesulfonate salt (28)** The compound was kindly provided by Dr. Zigang Li [10].

**29**



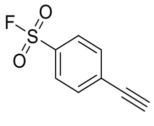
**1-methyl-4-((4-(prop-2-yn-1-yloxy)benzoyl)oxy)pyridin-1-ium-trifluoromethane -sulfonate salt (29)** The compound was kindly provided by Dr. Zigang Li [11].

**30**



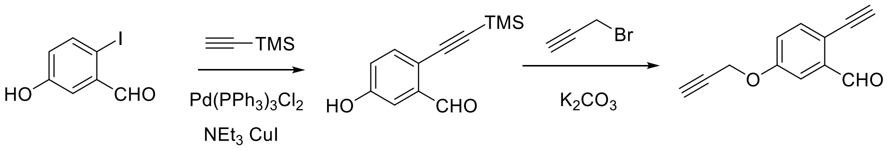
**4,6-dichloro-N-(prop-2-yn-1-yl)-1,3,5-triazin-2-amine (30)** Propargylamine (0.59 g, 0.69 mL, 10.76 mmol) was added to a stirred solution of cyanuric chloride (2.00 g, 10.85 mmol) in dry THF (25 mL) cooled to -20 °C. DIPEA (1.54 g, 2.08 mL, 11.94 mmol) in dry THF (5 mL) was then added dropwise over a period of 2 h with the help of a syringe pump. Thereafter, reaction mixture was stirred for another 3 h maintaining the reaction temperature between -20 and 0 °C. After this time, THF was removed under vacuum to yield a residue that was dissolved in EtOAc (50 mL) in a separating funnel and washed with water (3 × 15 mL), followed by brine solution (30 mL) and finally dried over solid anhydrous $Na_2SO_4$ to yield a pure product (white precipitate) in quantitative yield (2.18 g, yield 99%) under reduced pressure $^1$H NMR (400 MHz, $CDCl_3$) δ 6.74 (s, 1H), 4.30 (dd, *J* = 5.8, 2.5 Hz, 2H), 2.31 (t, *J* = 2.5 Hz, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.73 (s), 169.65 (s), 165.00 (s), 77.18 (s), 72.31 (s), 30.76 (s). HRMS (ESI) calcd.for $C_6H_4Cl_2N_4$ 202.9875 $[M + H]^+$, found 202.9885.

**31**



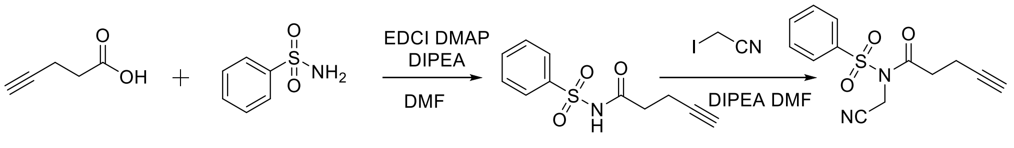
**4-ethynylbenzenesulfonyl fluoride (31)** The compound was purchased from Bide Pharmaceuticals（NO.BD01353772）, purity 95% $^1$H NMR (400 MHz, DMSO) δ 8.13 (d, *J* = 2.0 Hz, 2H), 7.86 (d, *J* = 10.3 Hz, 2H), 4.75 (d, *J* = 2.9 Hz, 1H).$^{13}$C NMR (101 MHz, $CDCl_3$) δ 133.12 (s), 132.81 (s), 132.55 (s), 129.98 (s), 128.41 (s), 82.75 (s), 81.27 (s).

**32**



**2-ethynyl-5-(prop-2-yn-1-yloxy)benzaldehyde (32) 32** was synthsised according the reference reported[8] $^1$H NMR (400 MHz, $CDCl_3$) δ 9.51 (s, 1H), 6.81 (d, J = 12.3 Hz, 1H), 6.56 (d, J = 3.1 Hz, 1H), 6.48 (dd, J = 9.1, 3.0 Hz, 1H), 4.10 (s, 2H), 2.80 (s, 1H), 1.67 (s, 1H). $^{13}$C NMR (151 MHz, DMSO) δ 191.16 (s), 158.08 (s), 137.87 (s), 135.83 (s), 122.30 (s), 118.25 (s), 112.39 (s), 86.78 (s), 79.46 (d, J = 2.2 Hz), 78.94 (s), 56.37 (s). HRMS (ESI) calcd. for $C_{12}H_8O_2$ 185.0597 $[M+H]^+$, found 185.0587.

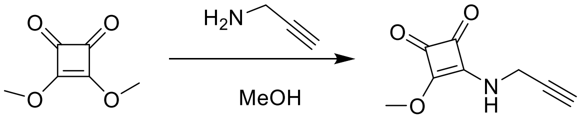
**33**



**N-(cyanomethyl)-N-(phenylsulfonyl)pent-4-ynamide (33) 33** was synthsised as previously described [9]. $^1$H NMR (400 MHz, DMSO) δ 8.05 (d, J = 10.0 Hz, 1H), 7.86

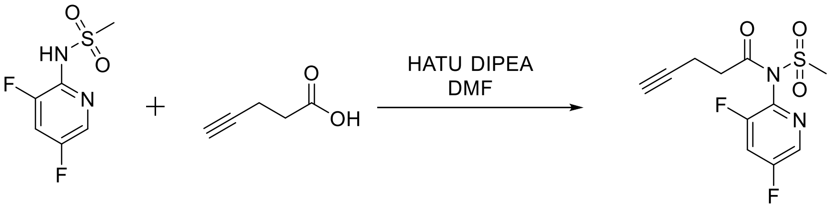
– 7.79 (m, 1H), 7.72 (d, J = 9.4 Hz, 1H), 4.96 (s, 2H), 2.93 (d, J = 8.5 Hz, 1H), 2.76 (s, 1H), 2.42 – 2.28 (m, 2H). $^{13}$C NMR (151 MHz, DMSO) δ 170.91 (s), 138.20 (s), 135.38 (s), 130.27 (s), 128.19 (s), 116.67 (s), 83.27 (s), 72.13 (s), 35.10 (s), 34.31 (s), 13.70 (s). HRMS (ESI) calcd. for $C_{13}H_{12}N_2O_3S$ 299.0460 [M+Na]$^+$, found 299.0444.

**34**



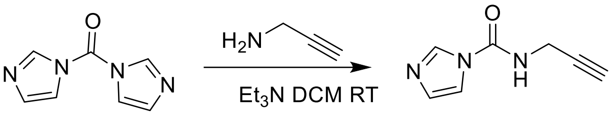
**3-methoxy-4-(prop-2-yn-1-ylamino)cyclobut-3-ene-1,2-dione (34)** To a stirred solution of 3,4-dimethoxycyclobut-3-ene-1,2-dione (300 mg, 2.1 mmol) was added in the in MeOH (5.0 mL), followed by the Propargylamine(104 mg, 1.9 mmol) were added. The mixture was stirred at room temperature at 2 h, The product precipitates directly in methanol, filtrated from MeOH to gain a white solid (303 mg, yelid 75%) $^1$H NMR (400 MHz, DMSO) δ 9.06 (s, 1H), 4.29 (s, 3H), 4.07 – 3.81 (m, 1H), 3.35 (d, J = 20.7 Hz, 2H). $^{13}$C NMR (151 MHz, DMSO) δ 189.62 (s), 183.35 (s), 178.52 (s), 172.72 (s), 80.46 (s), 76.02 (s), 60.79 (s), 33.57 (s). HRMS (ESI) calcd. for $C_8H_7NO_3$ 166.0498 [M+H]$^+$, found 166.0489.

**35**



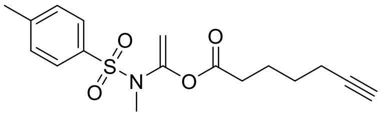
**N-(3,5-difluoropyridin-2-yl)-N-(methylsulfonyl)pent-4-ynamide (35)** To a stirred solution of N-(3,5-difluoropyridin-2-yl)methanesulfonamide (50 mg, 0.24 mmol) was added in the in DMF(5.0 mL),then HATU(91 mg, 0.24 mmol) and DIPEA(38.7 mg, 0.3 mmol) were added into the mixture. After 5min, followed by the Propargylamine(19.6 mg, 0.2 mmol) were added. The mixture was stirred at room temperature overnight, followed by addition of water (10.0 mL). The mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and dried over anhydrous $Na_2SO_4$. After evaporation of the solvent, the residue was purified by flash chromatography to gain a white solid (31.8 mg, yelid 45%). $^1$H NMR (400 MHz, DMSO) δ 8.05 (d, J = 10.0 Hz, 1H), 7.81 (d, J = 8.8 Hz, 1H), 7.71 (t, J = 9.1 Hz, 1H), 4.96 (s, 2H), 3.03 – 2.85 (m, 1H), 2.76 (s, 1H), 2.44 – 2.25 (m, 2H). $^{13}$C NMR (151 MHz, DMSO) δ 173.30 (s), 137.78 (s), 131.44 (s), 114.38 (s), 114.25 (s), 114.09 (s), 83.92 (s), 71.82 (s), 43.01 (s), 33.35 (s), 14.28 (s). HRMS (ESI) calcd. for $C_{11}H_{10}F_2N_2O_3S$ 311.0272 [M+Na]$^+$, found 311.0251.

**36**



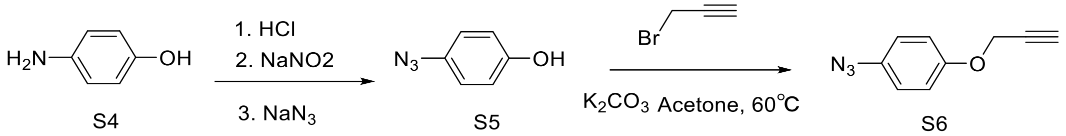
**N-(prop-2-yn-1-yl)-1H-imidazole-1-carboxamide (36)** CDI (1.7 g, 11 mmol, 1.10 eq) and propargyl amine (0.55 g, 10 mmol, 1.0 eq) were dissolved in DMF (5 mL) and acetonitrile (6 mL). The solution was stirred at room temperature for 2 h give a white solid (1.59 g, yield 71%) $^1$H NMR (600 MHz, DMSO) δ 9.02 (s, 1H), 8.26 (s, 1H), 7.68 (s, 1H), 7.04 (s, 1H), 4.08 (s, 2H), 3.25 (s, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 148.80 (s), 135.92 (s), 129.88 (s), 116.70 (s), 78.54 (s), 72.47 (s), 30.57 (s). HRMS (ESI) calcd $C_7H_7N_3O$ 150.0661 for [M+H]$^+$ found 150.0654.

**37**

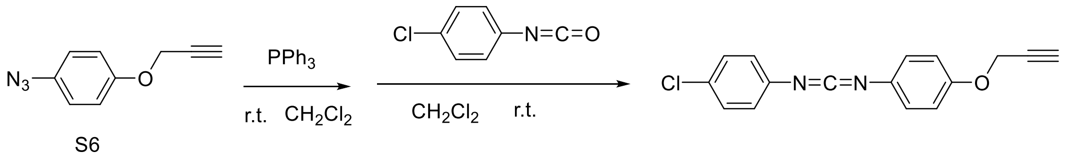


**1-((N,4-dimethylphenyl)sulfonamido)vinyl hept-6-ynoate (37)** The compound was synthesized as previously described [12]. $^1$H NMR (400 MHz, CDCl3) δ 7.78 – 7.65 (m, 2H), 7.33 (d, J = 8.1 Hz, 2H), 4.81 (d, J = 2.6 Hz, 1H), 4.62 (d, J = 2.6 Hz, 1H), 3.01 (s, 3H), 2.44 (s, 3H), 2.40 – 2.29 (m, 2H), 2.20 (m, 2H), 1.96 (t, J = 2.6 Hz, 1H), 1.78 – 1.63 (m, 2H), 1.63 – 1.47 (m, 2H). 13C NMR (151 MHz, CDCl3) δ 170.8, 147.0, 144.1, 133.8, 130.0, 129.6, 129.6, 129.5, 128.0, 128.0, 128.0, 127.3, 100.4, 83.8, 68.8, 37.3, 33.4, 27.6, 23.5, 21.6, 18.1. ESI-MS calcd for C17H21NO4S [M+Na]+ m/z = 358.1084; Found 358.1074.

**38**



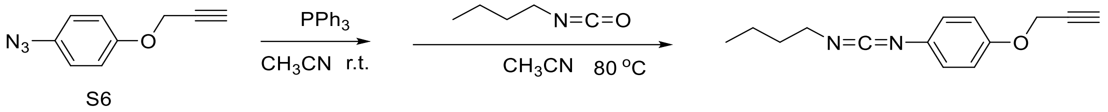
**1-azido-4-(prop-2-yn-1-yloxy)benzene (S6)** To a mixture of 4-aminophenol **S4** (0.01mol) and 15% HCl (10mL), NaNO$_2$ (0.012mol) in $H_2O$ (30mL) was added dropwise at 0°C. After the completion of addition, the reaction mixture was stirred at this temperature for 30min. A solution of sodium azide (0.02mol in 10mL $H_2O$) was added dropwise to the reaction mixture at 0°C. After addition the reaction mixture was maintained at 0°C for 4h. The product was extracted by using ethyl acetate followed by washing with water up to neutral pH. Organic layer was dried with anhydrous sodium sulfate, filtered and concentrated under vacuum to afford product **5**, which was used as the starting material in the next step without further purification and characterization. To a solution of crude azide **S5** in dry acetone (30 mL) was added the propargyl bromide (11 mmol) and $K_2CO_3$ (40 mmol) at r.t., then the mixture was stirred at 60 °C for 12 h, when the reaction was complete, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, EtOAc/petroleum ether, 1:10) to give product **S6,** the synthesized compound **S6** using the next step for starting material.



***N*-(4-chlorophenyl)-*N*-(4-(prop-2-yn-1-yloxy)phenyl)methanediimine (38):**To a solution of azide **S6** (2 mmol) in $CH_2Cl_2$ (10 mL) was added $PPh_3$ (2 mmol), the mixture was stirred at room temperature for 1 h (TLC monitoring). Then *p*-chlorophenyl isocyanate (2 mmol) was added at rt., and the reaction mixture was stirred at room temperature for 6 h. when the reaction was complete, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, EtOAc/petroleum ether = 1:10) to give product **38**. yellow solid (0.475g, 84% yield), mp 155-157°C; $^1$H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.28-7.26 (m, 2H), 7.13-7.08 (m, 4H), 6.95-6.93 (m, 2H), 4.68 (d, *J* = 2.4 Hz, 2H), 2.53 (t, *J* = 2.4 Hz, 1H); $^{13}$C NMR

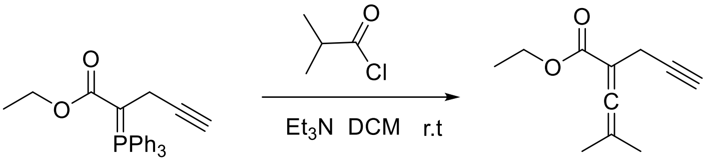
(CDCl3, 100 MHz) δ (ppm) 155.5, 137.5, 135.3, 131.1, 130.8, 129.5, 125.3, 125.2, 115.9, 78.2, 75.8, 56.1. HRMS (ESI) calcd.for $C_{14}H_{16}N_2O$ 283.0614 $[M + H]^+$, found. 283.0617.

**39**



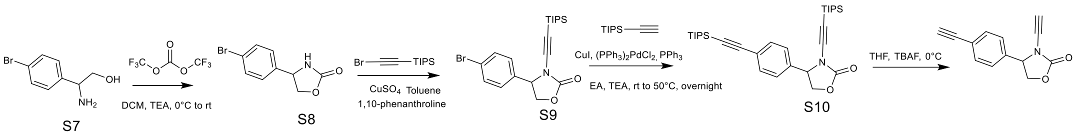
***N*-butyl-*N*-(4-(prop-2-yn-1-yloxy)phenyl)methanediimine (39):** To a solution of azide **S6** (2 mmol) in $CH_3CN$ (10 mL) was added $PPh_3$ (2 mmol), the mixture was stirred at room temperature for 1 h (TLC monitoring). Then *n*-butyl isocyanate (2 mmol) was added at r.t, and the reaction mixture was stirred at 80 °C for 8 h. when the reaction was complete, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, EtOAc/petroleum ether, 1:10) to give product **39**. yellow oil (0.365 g, 80% yield); $^1H$ NMR (CDCl3, 400 MHz) δ (ppm) 7.04-7.01 (m, 2H), 6.92-6.89 (m, 2H), 4.66 (d, $J$ = 2.4 Hz, 2H), 3.39 (t, $J$ = 6.8 Hz, 1H), 3.39 (t, $J$ = 6.8 Hz, 1H), 2.52 (t, $J$ = 6.8 Hz, 1H), 1.69-1.62 (m, 2H), 1.47-1.42 (m, 2H), 0.95 (t, $J$ = 7.6 Hz, 1H); $^{13}C$ NMR (CDCl3, 100 MHz) δ (ppm) 154.6, 136.7, 134.2, 124.3, 115.8, 78.5, 75.5, 56.1, 46.6, 33.4, 19.9, 13.5. HRMS (ESI) calcd.for $C_{14}H_{16}N_2O$ 229.1335 $[M + H]^+$, found. 229.1331.

**40**



**Ethyl 4-methyl-2-(prop-2-yn-1-yl)penta-2,3-dienoate (40):**To a solution of phosphorane (2 mmol) in dry $CH_2Cl_2$ (10 mL) was added $Et_3N$ (3 mmol), then isobutyryl chloride (2 mmol) was added at 0 °C. After the addition, the mixture was stirred for 24 h at room temperature. when the reaction was complete, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, EtOAc/petroleum ether, 1:10) to give product **40**. yellow oil (0.278g, 78% yield); $^1H$ NMR (CDCl3, 400 MHz) δ (ppm) 4.22-4.16 (m, 4H), 3.14 (d, $J$ = 2.4 Hz, 2H), 2.04 (t, $J$ = 2.4 Hz, 1H), 1.82 (s, 6H), 1.27 (t, $J$ = 7.2 Hz, 3H); $^{13}C$ NMR (CDCl3, 100 MHz) δ (ppm) 207.7, 166.8, 100.7, 94.7, 81.1, 69.5, 60.8, 19.5, 19.4, 14.2. HRMS (ESI) calcd.for $C_{11}H_{14}O_2$ 179.1063 [M + H] $^+$, found. 179.1065.

**41**



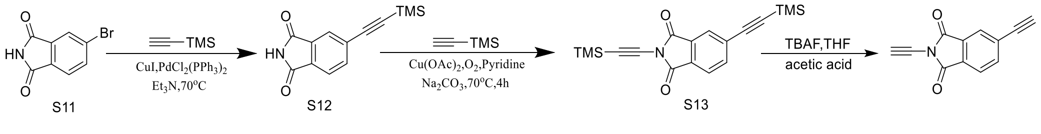
**4-(4-bromophenyl)oxazolidin-2-one (S7)** In the first step, 2-amino-1-(4-bromophenyl) ethanol (300 mg, 1.39 mmol) was added to a dry round-bottomed flask, and then ultra-dry DCM (5 mL) and $Et_3N$( 310 mg, 3.05 mmol). After stirring at room temperature for 10 min, triphosgene (110 mg, 5.55 mmol) was slowly added in portions, and stirred in an ice-water bath for 8 h. The reaction process was monitored by thin-layer chromatography until the reaction was completed, extracted with 20 mL ×3 ethyl acetate, the organic phase was washed with saturated brine and dried over anhydrous sodium sulfate, and crude product of compound **S8** (310 mg) was obtained after distillation under reduced pressure, which was directly applied to the next step of reaction.

**4-(4-bromophenyl)-3-((triisopropylsilyl)ethynyl)oxazolidin-2-one (S9)** In the second step, the crude product of compound **S8** (200 mg) was added under the protection of $N_2$ anhydrous, then $CuSO_4$ (26.4 mg, 0.2 eq), $K_3PO_4$ (350.8 mg, 2.0eq), 1, 10-phenanthroline (74.4 mg, 0.5 eq) were added into the toluene(5 mL) which mixture was stirred at room temperature for 10 min. And then (2-bromoethynyl) triisopropylsilane (260.0 mg, 1.2 eq) was added dropwise to the system. The reaction mixture was stirred at reflux for 4 h. Monitor the reaction process with thin-layer chromatography until the reaction is complete, extracted with 20 mL× 3 ethyl acetate, washed the organic phase with saturated brine and dried over anhydrous sodium sulfate,. Fianlly we removed the solvent by distillation under reduced pressure and used PE: EA=7: 1 to purified by silica gel column chromatography to obtain compound **S9**(272.6 mg, yield 78%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 8.5 Hz, 2H), 7.26 (d, J = 8.4 Hz, 2H), 5.06 (dd, J = 8.7, 7.4 Hz, 1H), 4.75 (t , J = 8.9 Hz, 1H), 4.25 (dd, J = 9.0, 7.4 Hz, 1H), 0.94 (d, J = 2.2 Hz, 21H).

**3-((triisopropylsilyl)ethynyl)-4-(4-((triisopropylsilyl)ethynyl)phenyl)oxazolidin-2-one (S10)** In the third step, compound **S9** (140.0 mg, 0.33 mmol), cuprous iodide (1.3 mg, 0.006 mmol), triphenylphosphine palladium dichloride (4.7 mg, 0.006 mmol) were added to anhydrous ethyl acetate(3 mL) under the protection of $N_2$, DIPEA (642.5 mg, 4.9 mmol) and triisopropylsilylacetylene (90.7 mg, 0.3 mmol) were added sequentially under stirring, stirred at room temperature for 10 min, and the reaction system was refluxed at 50°C for 6 h. The reaction process was monitored by thin-layer chromatography until the reaction was completed, extracted with 20 mL ×3 ethyl acetate, the organic phase was washed with saturated brine and dried over anhydrous sodium sulfate, and the solvent was distilled off under reduced pressure to obtain crude compound **S10** (160 mg ), which was directly carried out next step of reaction.

**3-ethynyl-4-(4-ethynylphenyl)oxazolidin-2-one (41)** In the fourth step, crude compound **S10** (50 mg )was added anhydrous tetrahydrofuran(3 mL) under the protection of $N_2$, stired in an ice-water bath for 10 min, then added a solution of tetrabutylammonium fluoride in tetrahydrofuran (4 M, 57 μL), and continue stirring for 10 min in an ice-water bath. Monitor the reaction process with thin-layer chromatography until the reaction is complete, extract with 10 mL× 3 ethyl acetate, washed the organic phase with saturated brine and dry over anhydrous sodium sulfate, and evaporated the solvent under reduced pressure. And PE:EA=3:1 was appied to purify the reaction production by silica gel column chromatography under the elution conditions , finally obtained compound **41** (32 mg, yield 65%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 8.2 Hz, 2H), 7.37 – 7.33 (m, 2H), 5.12 – 5.07 (m, 1H), 4.80 – 4.75 (m, 1H), 4.28 – 4.23 (m, 1H), 3.17 (s, 1H), 2.76 (s, 1H). HRMS (ESI) calcd. for $C_{13}H_9NO_2$ 212.0633 $[M + H]^+$, found 212.0706.

**42**

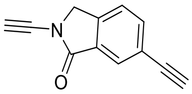


**5-((trimethylsilyl)ethynyl)isoindoline-1,3-dione (S11)** The first step, compound **S11** (904 mg, 4 mmol), $PdCl_2(PPh_3)_2$ (56 mg, 0.08 mmol), CuI (30 mg, 0.16 mmol), $Et_3N$ (860 uL, 6.2 mmol) were added in a dry round bottom flask, and $N_2$ Ultra-dry THF (20 mL) was added under protection, heated to 70°C, and then ethynyltrimethylsilane (706 uL, 5 mmol) was added slowly. The progress of the reaction was monitored by thin layer chromatography until the reaction completed. After cooling to room temperature, the mixture was diluted with 5 mL of ethyl acetate and filtered through celite. The filtrate was evaporated under reduced pressure, and the crude product was purified by silica gel column chromatography to obtain compound **S12** (900 mg, yield 92.6%)。 $^1$H NMR (400 MHz, DMSO-*d6*) δ 11.50 (s, 1H), 7.88 – 7.79 (m, 3H), 0.27 (s, 9H). $^{13}$C NMR (400 MHz, DMSO-*d6*) δ 168.98, 168.83, 137.77, 133.64, 132.69, 128.22, 125.98, 123.79, 103.84, 99.27, 90.14.

**2,5-bis((trimethylsilyl)ethynyl)isoindoline-1,3-dione (S13)** In the second step, compound **S12** (243 mg, 1 mmol), $Na_2CO_3$ (212 mg, 2 mmol), $Cu(OAc)_2$ (40 mg, 0.2 mmol), and 4 A molecular sieves (400 mg) were added to a dry round bottom flask. A solution of pyridine (161 uL, 2 mmol) in toluene (10 mL) was added to the reaction vial, which was then purged with the volume of 3-fold $O_2$. The reaction flask was stirred in an oil bath for 1 h (70°C.), and a solution of ethynyltrimethylsilane (216 uL, 1.2 mmol) was added to dry toluene (1 mL), and then slowly added to the reaction systems. The reaction mixture was stirred for 4 h. The reaction production was filtered through celite, concentrated under reduced pressure, and purified by silica gel column chromatography to obtain compound **S13** (100 mg, yield 29.5%). $^1$H NMR (400 MHz, DMSO-*d6*) δ 7.94 – 7.92 (m, 3H), 0.27 (s, 9H), 0.25 (s, 9H). $^{13}$C NMR (400 MHz, DMSO-*d6*) δ 164.91, 164.83, 138.57, 132.09, 130.93, 129.22, 126.98, 124.80, 103.37, 100.56, 86.60, 82.37, 0.33, 0.09.

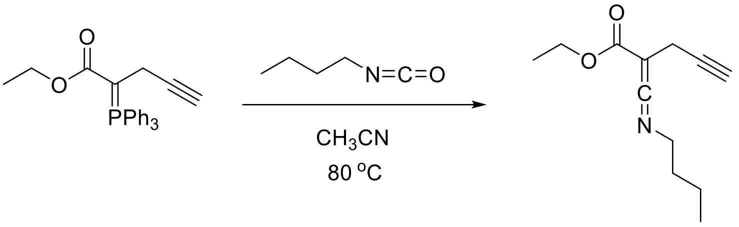
**2,5-diethynylisoindoline-1,3-dione (42)** In the third step, compound **S13** (50 mg, 0.147 mmol) was dissolved in 2 mL dry THF by adding a magnetic stirring bar in a single-neck round bottom flask and cooled in an ice bath to 4°C. Then acetic acid (16 uL, 0.294 mmol) was added to the reaction medium and stirred for 5 min. TBAF (1 M in THF) (48 uL, 0.354 mmol) was added dropwise to this stirred reaction medium over 30 min. The reaction continued to stir at 4°C until complete consumption of starting material and judged the reaction producs by thin layer chromatography analysis. The reaction was then returned to room temperature, then transferred to a separatory funnel, quenched with cold water, and extracted three times with dichloromethane. Dry over anhydrous sodium sulfate and evaporate under vacuum. And carried out silica gel column chromatography purification subsequently to obtain desired product **42** (17 mg, yield 59.3%)。 $^1$H NMR (400 MHz, DMSO-*d6*) δ 8.03 – 7.94 (m, 3H), 4.72 (s, 1H), 4.59 (s,1H). $^{13}$C NMR (400 MHz, DMSO-*d6*) δ 165.18, 165.09, 138.86, 132.09, 131.09, 128.90, 127.12, 124.77, 86.36, 82.20, 70.92, 67.71. HRMS (ESI) calcd. for $C_{12}H_5NO_2$ 196.0320 [M + H]$^+$, found 196.0393.

**43**



**2,6-diethynylisoindolin-1-one (43)** The synthetic route of 43 is similar to that of 42 (210 mg, yield 53%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 7.9 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.59 (s, 1H), 4.73 (s, 2H), 3.39 – 3.30 (m, 1H), 3.13 (s, 1H), 1.07 (s, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 168.38 (s), 140.81 (s), 132.73 (s), 129.47 (s), 127.29 (s), 126.63 (s), 124.65 (s), 82.43 (s), 80.69 (s), 73.63 (s), 62.36 (s), 51.82 (s). HRMS (ESI) calcd. for $C_{12}H_7NO$ 182.0600 [M + H]$^+$, found 182.0598.

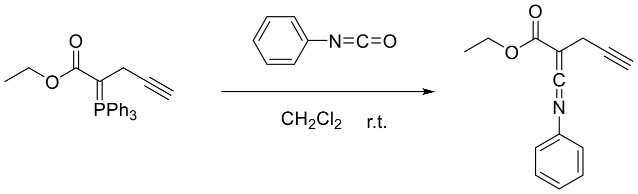
**44**



**Ethyl 2-((butylimino)methylene)pent-4-ynoate (44)**:To a solution of phosphorane (2 mmol) in dry $CH_3CN$ (10 mL) was added the *n*-butyl isocyanate (2 mmol) at r.t., then the mixture was stirred at 80 °C for 12 h, when the reaction was complete, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, EtOAc/petroleum ether, 1:10) to give product **44** yellow oil (0.286g, 69% yield); $^1$H NMR ($CDCl_3$, 400 MHz) δ (ppm) 4.26-4.21 (m, 4H), 3.39 (t, J = 6.0 Hz, 1H), 3.32-3.26 (m, 2H), 2.82-2.80 (m, 2H), 1.53-1.47 (m, 2H), 1.38-1.33 (m, 2H), 1.29 (t, J = 8.0 Hz, 3H), 0.92 (t, J = 6.0 Hz, 3H); 13C NMR (CDCl3, 100 MHz) δ (ppm) 169.8, 166.4, 80.4, 70.4, 61.8, 51.6, 39.5, 31.4, 19.9, 19.0, 14.0, 13.6.

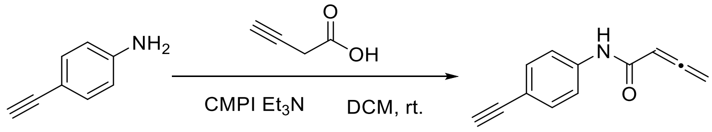
HRMS (ESI) calcd.for $C_{12}H_{17}NO_2$ 208.1332 [M + H]$^+$, found. 208.1322.

**45**



**Ethyl 2-((phenylimino)methylene)pent-4-ynoate (45)**To a solution of phosphorane (2 mmol) in dry $CH_2Cl_2$ (10 mL) was added the phenyl isocyanate (2 mmol) at r.t., the mixture was stirred for 4-6 h, when the reaction was complete, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, EtOAc/petroleum ether, 1:6) to give product **45**.: yellow oil (0.332g, 73% yield); 1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.43-7.32 (m, 5H), 4.25-4.18 (m, 4H), 3.21 (d, J = 2.8 Hz, 1H), 2.65-2.63 (m, 2H), 1.28-1.24 (m, 3H); 13C NMR ($CDCl_3$, 100 MHz) δ (ppm) 179.8, 172.2, 168.3, 137.5, 129.6, 128.4(minor), 124.3, 80.4, 80.3(minor), 70.4, 70.1(minor), 64.13, 61.0(minor), 60.8, 42.9, 19.8. HRMS (ESI) calcd.for $C_{14}H_{13}NO_2$ 228.1019 [M + H] $^+$, found.228.1013.

**46**

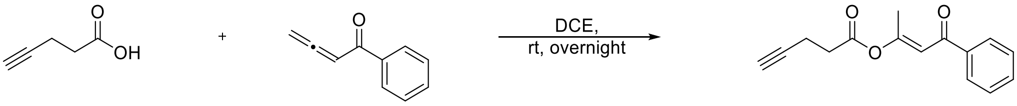


**N-(4-ethynylphenyl)buta-2,3-dienamide (46)** 3-butynoic acid (232 mmol, 2.76 mmol) and 2-chloro-1-methyl pyridinium iodide(CMPI) (1057 mg, 4.14 mmol) were charged in a 25 mL two neck oven dried RBF fitted with a magnetic stirrer bar. 8 mL dry dichloromethane was added to it and the mixture was stirred under nitrogen for 1 h. A solution of 4-ethynylaniline (291 mg, 2.48 mmol) and triethylamine (376.43 mg, 3.72 mmol) in 6 mL dry dichloromethane was added to it drop wise after which the reaction mixture was stirred for another half an hour. After the work up as described above, product was purified by flash column chromatography (PE:EA=8:1) to get a light brown solid. Finally, it was readily isomerized to allenamide by treating with 1 eq. of triethylamine in dichloromethane(261 mg, 50%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.50 (d, *J* = 8.3 Hz, 2H), 7.45 (d, *J* = 7.5 Hz, 2H), 5.76 (t, *J* = 6.7 Hz, 1H), 5.38

(d, $J$ = 6.6 Hz, 2H), 3.05 (s, 1H). $^{13}$C NMR (151 MHz, DMSO) δ 207.24, 133.41, 128.23, 114.41, 87.07, 78.62, 76.82, 72.14. HRMS (ESI) calcd.for $C_{12}H_9NO$ 184.0757 [M + H]$^+$, found 184.0754.

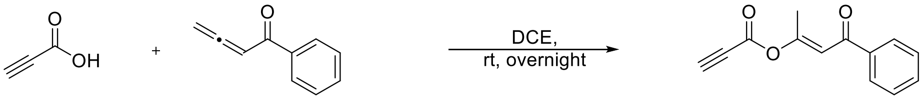
**47-52 General Procedure:** Alkynyl acid (0.2 mmol) and allenone (0.2 mmol) were dissolved in 4 mL of DCE. The mixture was stirred at rt overnight until no starting material monitoring by TLC. The reaction mixture was concentrated under vacuum and the residues purified by column chromatography on silica gel to afford the desired products.

**47**



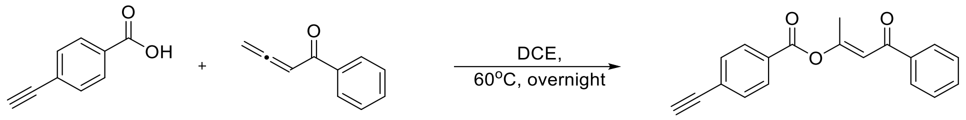
**(E)-4-oxo-4-phenylbut-2-en-2-yl pent-4-ynoate (47)** was synthesized by the general procedure as a white solid (20.8 mg, 43 % yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.01 – 7.81 (m, 2H), 7.70 – 7.54 (m, 1H), 7.54 – 7.43 (m, 2H), 6.83 (d, $J$ = 1.2 Hz, 1H), 2.75 (t, $J$ = 7.0 Hz, 2H), 2.68 – 2.55 (m, 2H), 2.43 (d, $J$ = 1.0 Hz, 3H), 2.06 (t, $J$ = 2.6 Hz, 1H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 190.3, 169.2, 163.5, 138.6, 132.9, 128.6, 128.2, 113.7, 81.8, 69.6, 33.6, 19.0, 14.4. HRMS (ESI) calcd. for $C_{15}H_{15}O_3$ [M+H]$^+$ 243.1021, found 243.1025.

**48**



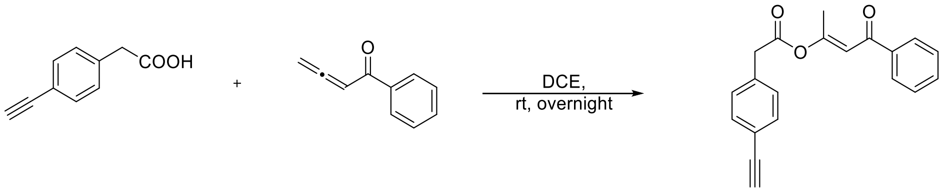
**(E)-4-oxo-4-phenylbut-2-en-2-yl propiolate (48)** was synthesized by the general procedure as a white solid ( 21.7 mg, 51 % yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.94 (dd, $J$ = 7.2, 1.7 Hz, 2H), 7.59 (td, $J$ = 7.3, 1.5 Hz, 1H), 7.55 – 7.42 (m, 2H), 6.93 – 6.84 (m, 1H), 3.10 (s, 1H), 2.45 (d, $J$ = 0.8 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 190.0, 162.3, 149.6, 138.3, 133.2, 128.7, 128.2, 128.2, 114.4, 74.0, 18.6. HRMS (ESI) calcd. for $C_{13}H_{11}O_3$ [M+H]$^+$ 215.0703, found 215.0702.

**49**



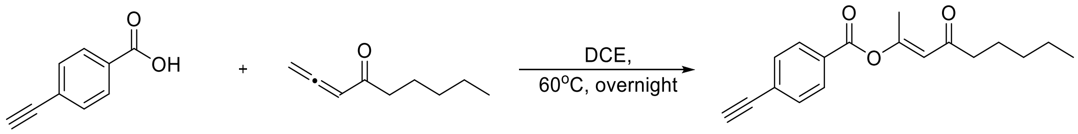
**(E)-4-oxo-4-phenylbut-2-en-2-yl 4-ethynylbenzoate (49)** was synthesized by the general procedure as a white solid (49.9 mg, 86 % yield). $^{1}$H NMR (400 MHz, CDCl$_3$) δ 8.16 – 8.05 (m, 2H), 8.02 – 7.93 (m, 2H), 7.66 – 7.61 (m, 2H), 7.61 – 7.54 (m, 1H), 7.49 (dd, *J* = 8.2, 6.9 Hz, 2H), 6.96 (d, *J* = 1.2 Hz, 1H), 3.32 (s, 1H), 2.55 (d, *J* = 1.0 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 190.3, 163.9, 163.4, 138.6, 133.0, 132.3, 130.0, 129.1, 128.6, 128.2, 127.8, 114.0, 82.6, 80.9, 19.1. HRMS (ESI) calcd. for $C_{19}H_{15}O_3$ $[M+H]^+$ 291.1021, found 291.1017.

**50**



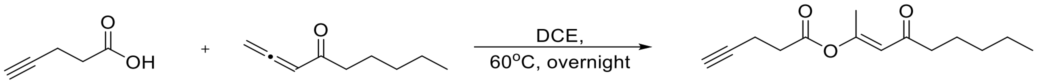
**(E)-4-oxo-4-phenylbut-2-en-2-yl2-(4-ethynylphenyl)acetate (50**) was synthesized by the general procedure as a white solid (44.4 mg, 73 % yield). $^{1}$H NMR (400 MHz, CDCl$_3$) δ 7.95 – 7.87 (m, 2H), 7.61 – 7.44 (m, 5H), 7.37 – 7.30 (m, 2H), 6.79 (d, *J* = 1.1 Hz, 1H), 3.81 (s, 2H), 3.12 (s, 1H), 2.38 (d, *J* = 1.0 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 190.3, 168.5, 168.1, 163.5, 138.5, 134.0, 132.9, 132.6, 132.4, 129.8, 129.3, 128.6, 128.2, 121.5, 113.7, 83.4, 77.7, 41.4, 18.8. HRMS (ESI) calcd. for $C_{20}H_{17}O_3$ $[M+H]^+$ 305.1178, found 305.1176.

**51**



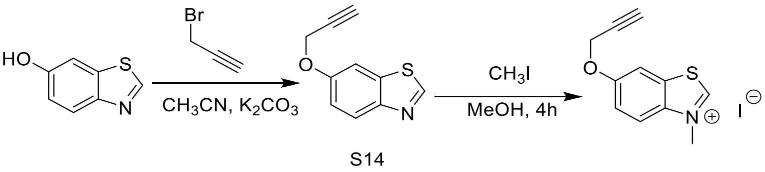
**(E)-4-oxonon-2-en-2-yl 4-ethynylbenzoate (51**) was synthesized by the general procedure as a white solid (47.8 mg, 84 % yield). $^{1}$H NMR (400 MHz, CDCl$_3$) δ 8.12 – 7.98 (m, 2H), 7.72 – 7.52 (m, 2H), 6.23 (d, *J* = 1.2 Hz, 1H), 3.30 (s, 1H), 2.55 – 2.40 (m, 5H), 1.65 (dd, *J* = 14.7, 7.4 Hz, 3H), 1.41 – 1.29 (m, 5H), 0.92 (t, *J* = 6.8 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 200.1, 163.4, 162.5, 132.3, 129.9, 129.9, 116.3, 82.6, 80.8, 45.0, 31.4, 23.7, 22.5, 18.7, 13.9. HRMS (ESI) calcd. for $C_{18}H_{21}O_3$ [M+H]$^+$ 285.1491, found 285.1489.

**52**



**(E)-4-oxonon-2-en-2-yl pent-4-ynoate (52)** was synthesized by the general procedure as a white solid (41.1 mg, 87 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 6.09 (d, *J* = 1.1 Hz, 1H), 2.69 (t, *J* = 7.0 Hz, 2H), 2.57 (td, *J* = 8.0, 7.4, 1.8 Hz, 2H), 2.46 (t, *J* = 7.4 Hz, 2H), 2.34 (d, *J* = 1.0 Hz, 3H), 2.04 (t, *J* = 2.6 Hz, 1H), 1.68 – 1.55 (m, 3H), 1.31 (dtd, *J* = 11.7, 6.8, 4.0 Hz, 5H), 0.90 (t, *J* = 6.9 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 200.1, 169.2, 168.7, 162.1, 116.1, 82.3, 81.8, 69.6, 69.2, 44.9, 33.5, 31.4, 31.3, 23.7, 22.5, 18.6, 14.3, 14.1, 13.9, 13.9. HRMS (ESI) calcd. for $C_{14}H_{21}O_3$ [M+H]$^+$ 237.1491, found 237.1492.

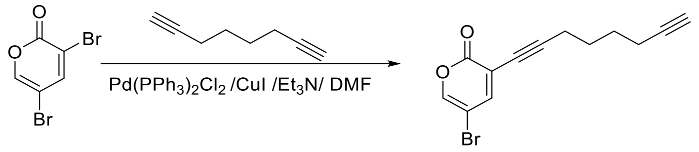
**53**



**6-(prop-2-yn-1-yloxy)benzo[d]thiazole(S14)**: To a stirred solution of benzo[d]thiazol-6-ol (1.0 eq) in MeCN was added 3-bromoprop-1-yne (1.5 eq) and potassium carbonate (2 eq). The resulting mixture was stirred overnight. After the reaction was completed, the solution was concentrated under reduced pressure, and the reaction production was purified by flash column chromatography to get compound **S14**.

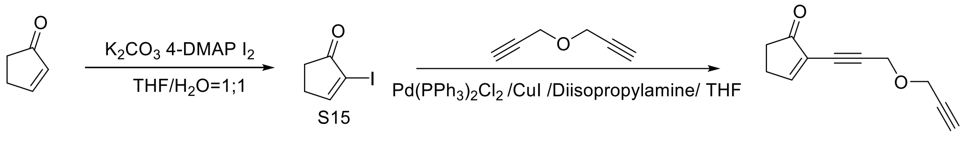
**3-methyl-6-(prop-2-yn-1-yloxy)benzo[d]thiazol-3-ium iodide (53)**: To a stirred solution of **S14** (1eq) in methanol systems was added iodomethane (1.5 eq). The resulting mixture was stirred for 4 h. After the reaction was completed, the solution was concentrated under reduced pressure, and the residue was washed with EA and DCM to produce pure **53** (320 mg, yield 80%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.36 (s, 1H), 8.24 (d, *J* = 9.3 Hz, 1H), 8.10 (d, *J* = 2.5 Hz, 1H), 7.59 (dd, *J* = 9.3, 2.5 Hz, 1H), 4.99 (d, $J$ = 2.4 Hz, 2H), 4.36 (s, 3H), 3.68 (s, 1H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 163.23, 157.53, 136.39, 133.42, 119.80, 118.67, 108.85, 79.65, 78.78, 56.92.

**54**



**5-bromo-3-(octa-1,7-diyn-1-yl)-2H-pyran-2-one (54)** To a mixture of 580 mg of 3,5-dibromo- 2-pyrone (2.28 mmol), 90 mg of $Pd(PPh_3)_2Cl_2$ (0.11 mmol), and CuI (44 mg, 0.24 mmol) in 1,4-dioxane (30 mL), Then mixture was added $Et_3N$ (0.38 mL, 2.74 mmol) and octa-1,7-diyne (290 mg , 2.74 mmol). After stirring for 0.5 h under $N_2$, the reaction mixture was filtered through a plug of Celite and the filtered material was washed with ether. The filtrate was washed with $H_2O$, dried over $MgSO_4$, concentrated, and chromatographed (PE/EA=10:1) to obtain **54** (820 mg, 83% yield). $^1$H NMR (400 MHz, Chloroform-$d$) δ 7.55 (d, $J$ = 2.5 Hz, 1H), 7.45 (d, $J$ = 2.6 Hz, 1H), 2.51 (t, $J$ = 6.7 Hz, 2H), 2.26 (td, $J$ = 6.7, 2.7 Hz, 2H), 1.98 (t, $J$ = 2.6 Hz, 1H), 1.78 – 1.67 (m, 4H).$^{13}$C NMR (101 MHz, $CDCl_3$) δ 159.16, 148.22, 146.53, 115.03, 100.46, 99.62, 83.94, 74.03, 68.73, 27.49, 27.13, 19.32, 17.96. HRMS (ESI) calcd. for $C_{13}H_{11}BrO_2$ 279.0015 $[M+H]^+$, found 279.0007.

**55**

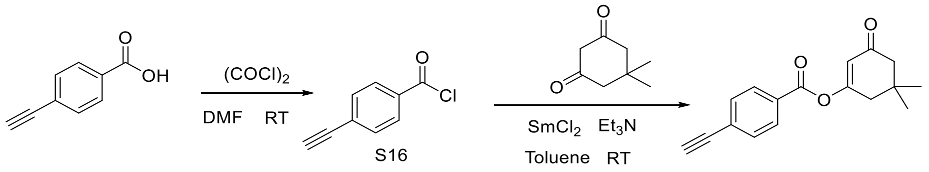


**2-iodocyclopent-2-en-1-one (S15)** Cyclopent-2-en-1-one (1eq) was added to the system THF:$H_2O$=1:1, then $K_2CO_3$ (2eq) added. Followed by ,the compounds DMAP (1eq) and $I_2$ (1.5eq) were added the mixture reaction systems . The mixed system was stirred for 1 h at ambient temperature, and then **S15** was obtained directly for the next step of the reaction without silica gel column purification.

**2-(3-(prop-2-yn-1-yloxy)prop-1-yn-1-yl)cyclopent-2-en-1-one (55**) To a mixture of 2-iodocyclopent-2-en-1-one (207 mg , 1 mmol), $Pd(PPh_3)_2Cl_2$ (90 mg , 0.11 mmol), and CuI (44 mg , 0.24 mmol) in THF (30 mL). Then miture was added

Diisopropylamine(1 mL) and 3-(prop-2-yn-1-yloxy)prop-1-yne (257 mg , 2.74 mmol). After stirring for 2 h under $N_2$ atmosphere, the reaction mixture was filtered through a plug of Celite. The filtrate was washed with $H_2O$, then dried with $MgSO_4$, concentrated and chromatographed (PE /EA=5:1) to gain **55** (348 mg, yield 75%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.83 (t, $J$ = 3.0 Hz, 1H), 4.47 (s, 2H), 4.29 (t, $J$ = 4.1 Hz, 2H), 2.82 – 2.67 (m, 2H), 2.53 – 2.45 (m, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 205.58 (s), 166.00 (s), 129.49 (s), 91.00 (s), 78.83 (s), 77.53 (s), 75.15 (s), 57.21 (s), 56.67 (s), 33.93 (s), 27.40 (s). HRMS (ESI) calcd. for $C_{11}H_{10}O_2$ 175.0754 $[M+H]^+$found 175.0755.

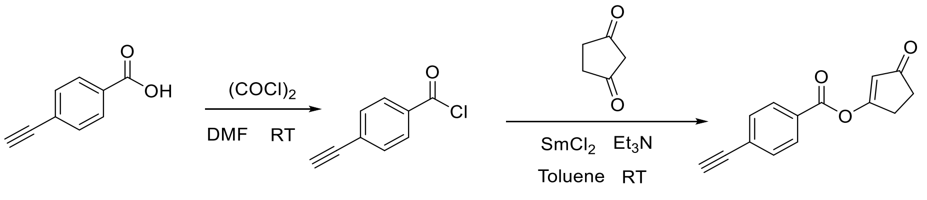
**56**



**4-ethynylbenzoyl chloride (S16)** Under the protection of argon, 4-ethynylbenzoyl chloride (**S16**)., 4-ethynylbenzoic acid (293 mg, 2.0 mmol) were dissolved in ultra-dry DCM solvent, and then oxalyl chloride (514.9 μL, 6.0 mmol) was added dropwise. The mixture was stirred overnight at room temperature, and the solvent was removed under reduced pressure. The obtained crude product was added to anhydrous $Na_2SO_4$ dried DCM to remove excessive oxalyl chloride to obtain a red solid **S16**, which could be directly used for the next reaction without purification.

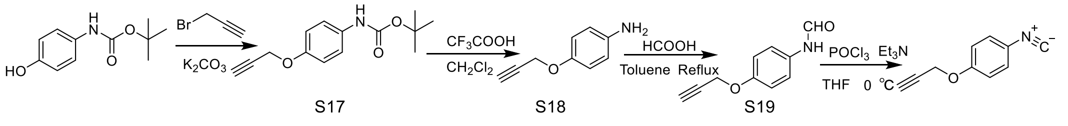
**5,5-dimethyl-3-oxocyclohex-1-en-1-yl 4-ethynylbenzoate (56)**. **S16** (328 mg, 2.0 mmol) and 5,5-dimethylcyclohexane-1,3-dione (255 mg, 1.82 mmol), $SmCl_3$ (25.6 mg, 0.1 mmol), $Et_3N$ (303.6 μL, 2.18 mmol) were dissolved in toluene solution (20 mL) under argon protection. The reaction was placed at room temperature for 4 h. The reaction mixture was quenched with water and extracted three times with ethyl acetate (EA). The combined organic layer was washed with salt water, dried with anhydrous $Na_2SO_4$, and concentrated in vacuum. The residue was purified by silica gel column chromatography (eluting with EA:PE=1:6) to obtain a yellow solid (410.3 mg, yield 84.1 %).$^1$H NMR (400 MHz, Chloroform-*d*) δ 8.05 – 8.00 (m, 2H), 7.62 – 7.57 (m, 2H), 6.04 (t, $J$ = 1.3 Hz, 1H), 3.29 (s, 1H), 2.54 (d, $J$ = 1.4 Hz, 2H), 2.32 (s, 2H), 1.15 (s, 6H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 199.11, 168.91, 162.72, 132.71, 130.56, 127.95, 116.66, 85.19, 82.92, 50.70, 41.69, 33.36, 28.05. HRMS (ESI) calcd.for $C_{17}H_{16}O_3$ 291.0992 [M + H]$^+$, found 291.0981.

**57**



**3-oxocyclopent-1-en-1-yl 4-ethynylbenzoate**(**57**). **S16** (328 mg, 2.0 mmol) and cyclopentane-1,3-dione (180 mg, 1.82 mmol), $SmCl_3$ (25.6 mg, 0.1 mmol), $Et_3N$ (303.6 μL, 2.18 mmol) were dissolved in toluene solution (20 mL) under argon protection. The reaction was placed at room temperature for 4 h. The reaction mixture was quenched with water and extracted three times with ethyl acetate (EA). The combined organic layer was washed with salt water, dried with anhydrous $Na_2SO_4$, and concentrated in vacuum. The residue was purified by silica gel column chromatography (eluting with EA: DCM =1:60) to obtain a white solid (89 mg, yield 19.7 %).$^1$H NMR (400 MHz, Chloroform-$d$) δ 8.13 – 8.07 (m, 2H), 7.67 – 7.59 (m, 2H), 6.41 (d, $J$ = 1.7 Hz, 1H), 2.96 – 2.91 (m, 2H), 2.58 – 2.53 (m, 2H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 201.84, 174.87, 156.78, 127.75, 125.50, 123.72, 123.24, 112.27, 77.63, 76.65, 28.76, 24.07. HRMS (ESI) calcd.for $C_{14}H_{10}O_3$ 249.0522 [M + H]$^+$, found 249.0518.

**58**



**tert-butyl (4-(prop-2-yn-1-yloxy)phenyl)carbamate(S17)** Synthesis of **58** was carried out similar to literature procedure [13](**S17**). a 100 mL round-bottom flask was added tert-butyl (4-hydroxyphenyl) carbamate (1.045 g, 5 mmol), 3-bromopropyne (900 mg, 7.5 mmol), potassium carbonate (1.38 g, 10 mmol) and 15 mL DMF. The reaction mixture was stirred at room temperature for 2 h, and then was quenched by addition of water and extracted with ethyl acetate 2-3 times. The organic phase was washed twice with saturated brine, and dried over anhydrous $Na_2SO_4$. The organic solvent was evaporated under reduced pressure, the residue was purified by silica gel column (PE:EA=8:1) to afford **S17**. $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 9.18 (s, 1H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.03 – 6.65 (m, 2H), 4.72 (d, *J* = 2.4 Hz, 2H), 3.54 (s, 1H), 1.47 (s, 9H).

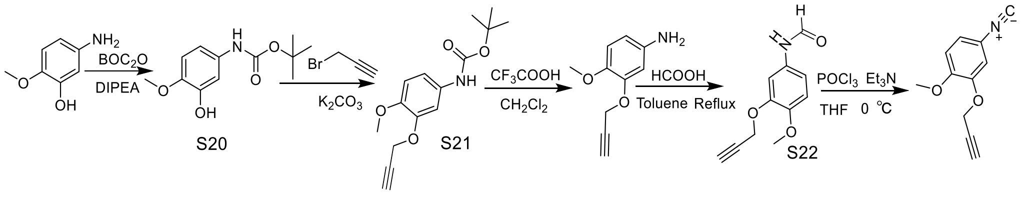
**4-(prop-2-yn-1-yloxy)aniline (S18)** To a 50 mL-round bottom flask, add **S17** (1 g, 4 mmol), 4 mL $CH_2Cl_2$ and 4 mL trifluoroacetic acid. After later, the extra $CH_2Cl_2$ and TFA were evaporated under reduced pressure. then, 100 mL saturated sodium bicarbonate was added to neutralize the extra acid and extracted with ethyl acetate, the organic phase was washed twice with saturated brine, and dried over anhydrous $Na_2SO_4$. Upon evaporation of the solvent in vacuo and the crude product was purified by flash column (PE:EA=3:1) to afford **S18** (500 mg, 84%). $^1H$ NMR (400 MHz, DMSO-*d6*) δ 6.80 – 6.65 (m, 2H), 6.59 – 6.45 (m, 2H), 4.69 (s, 2H), 4.61 (s, 2H), 3.48 (t, J = 2.4 Hz, 1H).

**N-(4-(prop-2-yn-1-yloxy)phenyl)formamide (S19)** To a 50 mL-two round bottom flask, add **S18** (441 mg, 3 mmol), 8 mL toluene and formic acid (230 μL, 6 mmol). The reaction mixture was stirred at 110°C for 1 h. Then the mixture was evaporated under reduced pressure and the crude product was purified by flash column (PE:EA=2:1) to afford **S19** (420 mg, 80%). $^1H$ NMR (400 MHz, DMSO-*d6*) δ 10.08 (s, 1H), 8.62 (d, J = 11.1 Hz, 1H), 8.22 (d, J = 2.0 Hz, 1H), 7.60 – 7.47 (m, 2H), 7.14 (d, J = 9.0 Hz, 1H), 7.01 – 6.87 (m, 3H), 4.76 (d, J = 2.4 Hz, 2H), 3.56 (t, J = 2.4 Hz, 1H).

**1-isocyano-4-(prop-2-yn-1-yloxy)benzene (58)** To a 100 mL-two round bottom flask, add **S19** (525 mg, 3 mmol), 6 mL $CH_2Cl_2$ and triethylamine (1.25 mL, 9 mmol), the reaction mixture was stirred in ice bath and $POCl_3$ (335 μL, 3.6 mmol) was added slowly. After stirring for 1 h in room temperature, the reaction mixture was cooled to 0 °C, then poured to 20 mL saturated sodium bicarbonate solution and stirred for 30 min in 0 °C to neutralize the extra acid. The mixture was extracted with ethyl acetate, washed with brine and dried over by anhydrous $Na_2SO_4$. evaporated the reaction solvent in vacuo, the crude product was purified by flash column (PE:EA=15:1) to afford **58** (400 mg, 90%).$^1H$ NMR (400 MHz, DMSO-$d_6$) δ 7.57 – 7.47 (m, 2H), 7.15

– 6.99 (m, 2H), 4.88 (d, *J* = 2.4 Hz, 2H), 3.62 (t, *J* = 2.4 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO) δ 163.39, 158.07, 128.30, 119.60, 116.34, 79.22, 56.30. HRMS (ESI) calcd. for $C_{10}H_7NO$ 158.0600, [M + H]$^+$, found 158.0603.

**59**



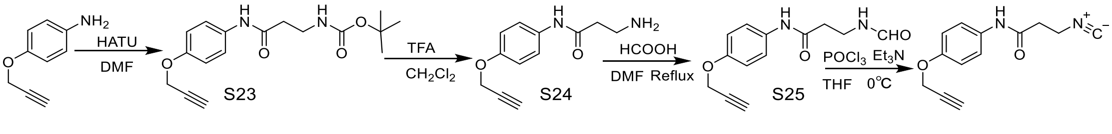
**tert-butyl (3-hydroxy-4-methoxyphenyl)carbamate (S20)** To a 100 mL round-bottom flask, add 5-amino-2-methoxyphenol (1.39 g, 10 mmol), di-tert-butyl dicarbonate (4.5 mL, 20 mmol), DIPEA (5 mL, 30 mmol) and 15 mL THF. The reaction mixture was stirred at 60°C for 1 h, quenched by addition of water and extracted with ethyl acetate 2-3 times. The organic phase was washed twice with saturated brine, and dried over anhydrous $Na_2SO_4$. Upon evaporation of the solvent in vacuo, the crude product was purified by silica gel column (PE:EA=6:1) to afford **S20** (1.4 g, 60%). $^1$H NMR (400 MHz, DMSO-*d6*) δ 9.03 (s, 1H), 8.94 (s, 1H), 7.01 (s, 1H), 6.78 (d, J = 2.6 Hz, 2H), 3.69 (s, 3H), 1.45 (s, 9H).

**tert-butyl (4-methoxy-3-(prop-2-yn-1-yloxy)phenyl)carbamate (S21)** To a 100 mL round-bottom flask, add **S20** (1.2 g, 5 mmol), 3-bromopropyne (900 mg, 7.5 mmol), potassium carbonate (1.38 g, 10 mmol) and 15 mL DMF. The reaction mixture was stirred at 60°C for 1 h, quenched by addition of water and extracted with ethyl acetate 2-3 times. The organic phase was washed twice with saturated brine, and dried over anhydrous $Na_2SO_4$. After evaporation of the solvent, the crude product was purified by silica gel column to afford **S21** (1.24 g, 90%).$^1$H NMR (400 MHz, DMSO-*d6*) δ 9.17 (s, 1H), 7.26 – 7.19 (m, 1H), 7.00 (d, J = 8.8 Hz, 1H), 6.87 (d, J = 8.8 Hz, 1H), 4.69 (d, J = 2.4 Hz, 2H), 3.73 (s, 3H), 3.56 (t, J = 2.4 Hz, 1H), 1.46 (s, 9H).

**N-(4-methoxy-3-(prop-2-yn-1-yloxy)phenyl)formamide (S22)** To a 50 mL round bottom flask, add **S21** (1.38 g, 5 mmol), 5 mL $CH_2Cl_2$ and 5 mL trifluoroacetic acid, after later, the extra $CH_2Cl_2$ and TFA were evaporated under reduced pressure. Then 100 mL saturated sodium bicarbonate was added to neutralize the remaining acid and extracted with ethyl acetate, the organic phase was washed twice with saturated brine, and dried over anhydrous $Na_2SO_4$. Upon evaporation of the solvent in vacuo, the crude product was purified by flash column (PE:EA=3:1) to afford the compound (708 mg, 80%). The reaction product was added to two round bottom flask (50 mL) with 10 mL toluene and formic acid (377 μL, 10 mmol) and the reaction was stirred at 110 °C for 1 h. The mixture was evaporated under reduced pressure and the crude product was purified by flash column(PE:EA = 2:1) to afford **S22** (492 mg, yield 60%).$^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.02 (d, *J* = 34.7 Hz, 1H), 8.63 (d, *J* = 11.0 Hz, 1H), 8.20 (d, *J* = 2.0 Hz, 1H), 7.34 (d, *J* = 2.4 Hz, 1H), 7.19 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.94 (d, *J* = 8.9 Hz, 1H), 6.74 (dd, *J* = 8.6, 2.5 Hz, 1H), 4.76 (dd, *J* = 19.0, 2.4 Hz, 2H), 3.73 (d, *J* = 1.7 Hz, 3H), 3.57 (t, *J* = 2.5 Hz, 1H).

**4-isocyano-1-methoxy-2-(prop-2-yn-1-yloxy)benzene (59)** To a 100 mL two round bottom flask, add **S22**(308 mg, 1.5 mmol), 4 mL $CH_2Cl_2$ and triethylamine (0.7 mL, 4.5 mmol). After that, the reaction mixture was stirred in ice bath and $POCl_3$ (118 μL, 1.8 mmol) was added slowly. After stirring for 1 h in room temperature, the reaction mixture was cooled to 0°C then poured to 20 mL saturated sodium bicarbonate solution and stirred for 30 min in 0°C to neutralize the remaining acid. The mixture was extracted with ethyl acetate and washed with brine. Upon evaporation of the solvent in vacuo, the crude product was purified by flash column (PE:EA=10:1) to afford **59** (200 mg, yield 71 %). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.25 (d, *J* = 2.4 Hz, 1H), 7.19 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.05 (d, *J* = 8.6 Hz, 1H), 4.86 (d, *J* = 2.4 Hz, 2H), 3.81 (s, 3H), 3.64 (t, *J* = 2.4 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 162.91, 150.58, 146.94, 120.49, 118.51, 112.57, 112.17, 79.33, 79.09, 56.68, 56.30 HRMS (ESI) calcd. for $C_{11}H_9NO_2$ 188.0706 [M + H]$^+$, found 188.0703.

**60**



**tert-butyl (3-oxo-3-((4-(prop-2-yn-1-yloxy)phenyl)amino)propyl)carbamate (S23)** To a 100 mL round-bottom flask, add 4-(prop-2-yn-1-yloxy)aniline (1.47 g, 10 mmol), HATU ( 4.182 g, 11 mmol) and 20 mL DMF, the mixture stirred for 5 min. then DIPEA

(3.5 mL, 20 mmol) and 3-((tert-butoxycarbonyl)amino)propanoic acid (2.08 g, 11 mmol) was added. The reaction was stirred at room temperature for 2 h, quenched by addition of water and extracted with ethyl acetate 2-3 times. The organic phase was washed twice by saturated sodium chloride, dried by anhydrous $Na_2SO_4$ and purified by flash column to afford **S23** (2.87 g, yield 90%).$^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.85 (s, 1H), 7.50 (d, *J* = 9.0 Hz, 2H), 6.92 (d, *J* = 9.0 Hz, 3H), 4.74 (d, *J* = 2.4 Hz, 2H), 3.57 (t, *J* = 2.4 Hz, 1H), 3.23 – 3.17 (m, 2H), 2.43 (t, *J* = 7.2 Hz, 2H), 1.38 (s, 9H).

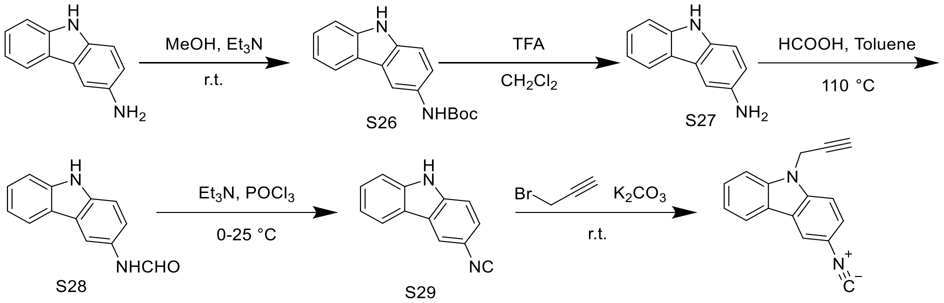
**3-amino-N-(4-(prop-2-yn-1-yloxy)phenyl)propanamide (S24)** To a 50 mL round bottom flask, add **S23** (0.954 g, 3 mmol), $CH_2Cl_2$ (5 mL )and trifluoroacetic acid(5 mL). The reaction was stirred for 0.5 h, then was evaporated under reduced pressure and the crude product was purified by flash column (DCM:MeOH=20:1) to afford **S24** (410 mg, yield 63 %).$^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.10 (s, 1H), 7.82 (s, 3H), 7.51 (d, *J* = 9.1 Hz, 2H), 6.94 (d, *J* = 9.0 Hz, 2H), 4.75 (d, *J* = 2.4 Hz, 2H), 3.58 (t, *J* = 2.3 Hz, 1H), 3.08 (t, *J* = 6.8 Hz, 2H), 2.67 (t, *J* = 6.8 Hz, 2H).

**3-formamido-N-(4-(prop-2-yn-1-yloxy)phenyl)propanamide (S25)** To a 50 mL round bottom flask, add **S24** (654 mg, 3 mmol), 10 mL DMF and 3 mL formic acid. The mixture was stirred at 110 °C for 3 h, then was evaporated under reduced pressure and the crude product was purified by flash column (DCM:MeOH=5:1) to afford **S25** (221 mg, yield 30 %). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.88 (s, 1H), 8.15 (d, *J* = 6.0 Hz, 1H), 8.02 – 7.98 (m, 1H), 7.57 – 7.47 (m, 2H), 6.92 (d, *J* = 9.0 Hz, 2H), 4.75 (d, *J* = 2.4 Hz, 2H), 3.56 (d, *J* = 2.4 Hz, 1H), 3.36 (d, *J* = 7.6 Hz, 2H), 2.47 (d, *J* = 6.6 Hz, 2H).

**2-isocyano-N-(4-(prop-2-yn-1-yloxy)phenyl)propanamide (60)** To a 50 mL two round bottom flask, add **S25** (147.6 mg, 0.6 mmol), THF (3 mL) and triethylamine (0.24 mL, 1.8 mmol), the resulting mixture was stirred in ice bath and $POCl_3$ (66 μL, 0.7 mmol) was added slowly. After stirring for 1 h in room temperature, the reaction mixture was cooled to 0°C then poured to 20 mL saturated sodium bicarbonate solution and stirred for 30 min in 0°C to neutralize the remaining acid. The mixture was extracted with ethyl acetate, washed with brine and dried by anhydrous $Na_2SO_4$. Upon evaporation of the solvent in vacuo, the crude product was purified by flash column

(PE:EA=2:1) to afford **60** (103 mg, yield 76 %). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.00 (s, 1H), 7.51 (d, *J* = 9.1 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 4.75 (d, *J* = 2.4 Hz, 2H), 3.75 (d, *J* = 5.3 Hz, 2H), 3.54 (t, *J* = 2.4 Hz, 1H), 2.70 (dt, *J* = 6.8, 3.7 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 167.54, 156.27, 153.58, 133.14, 120.97, 115.46, 79.83, 78.63, 55.98, 38.13, 36.00. HRMS (ESI) calcd. for $C_{26}H_{19}N_3O_4S$ 228.0899 $[M + H]^+$, found 229.0972.

**61**



**Tert-butyl (9H-carbazol-3-yl)carbamate** (**S26**). Add Boc$_2$O (598.86mg, 2.74mmol) slowly to a solution of 9H-carbazol-3-amine(500 mg, 2.74 mmol) and triethylamine (286 µL, 2.06 mmol) in methanol (10 mL). Stir the reaction mixture at room temperature for 24 h. Remove the solvent by rotary evaporation and added water to the residue. Collect the precipitate by filtration. Wash the precipitate with water and dry under vacuum to obtain **S26** (774 mg, 99%) as a white solid, which can be directly used for the next reaction without purification.

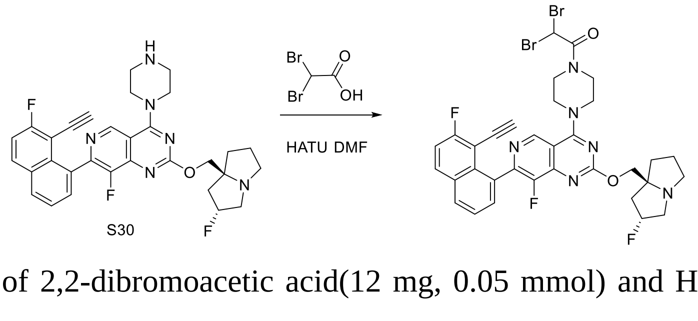
**9H-carbazol-3-amine** (**S27**). To a 50 mL round bottom flask, add **S26** (700 mg, 2.48 mmol), 4 mL $CH_2Cl_2$ and 4 mL trifluoroacetic acid. A few minutes later, the extra $CH_2Cl_2$ and TFA were evaporated under reduced pressure to obtain **S27**(450 mg, 99%), which can be directly used for the next reaction without purification.

**N-(9H-carbazol-3-yl) formamide** (**S28**). To a 50 mL two round bottom flask, add **S27**(400 mg, 2.2 mmol), 8 mL toluene and formic acid (166 µL, 4.4 mmol). The reaction mixture was stirred at 110°C for 1 h, then the mixture was evaporated under reduced pressure and then poured to 20 mL saturated sodium bicarbonate solution to neutralize the remaining acid. The mixture was extracted with dichloromethane and washed with brine. Upon evaporation of the solvent in vacuo to obtain **S28** (450 mg, 97%), the crude product can be directly used for the next reaction without purification.

**3-isocyano-9H-carbazole** (**S29**). To a 50 mL two round bottom flask, add **S28** (400 mg, 1.9 mmol), 6 mL $CH_2Cl_2$ and triethylamine (794 μL, 5.7 mmol), the reaction mixture was stirred in ice bath and $POCl_3$ (212.5 μL, 2.28 mmol) was added slowly. After stirring for 1 h in room temperature, the reaction mixture was cooled to 0 °C then poured to 20 mL saturated sodium bicarbonate solution and stirred for 30 min in 0°C to neutralize the remaining acid. The mixture was extracted with ethyl acetate and washed with brine. Upon evaporation of the solvent in vacuo to obtain **S29** (346 mg, 95%), the crude product can be directly used for the next reaction without purification.

**N-methylidyne-9-(prop-2-yn-1-yl)-9H-carbazol-3-aminium** (**61**). To a 50 mL two round bottom flask, add **S29** (307 mg, 1.56 mmol), 3-bromopropyne (202 μL, 2.34 mmol), potassium carbonate (431 mg, 3.12 mmol) and 15 mL DMF. Upon evaporation of the solvent in vacuo, the crude product was purified by flash column (PE:EA = 100:1) to afford **61**(306 mg, yield 85 %).$^1$H NMR (400 MHz, DMSO-*d6*) δ 8.48 (d, J = 2.0 Hz, 1H), 8.25 (dt, J = 7.9, 1.0 Hz, 1H), 7.78 (d, J = 8.7 Hz, 1H), 7.74 (d, J = 8.3 Hz, 1H), 7.66 (dd, J = 8.7, 2.0 Hz, 1H), 7.57 (m, J = 8.3, 7.1, 1.2 Hz, 1H), 7.33 – 7.28 (m, 1H), 5.37 (d, J = 2.5 Hz, 2H), 3.29 (t, J = 2.4 Hz, 1H).$^{13}$C NMR (151 MHz, DMSO-*d6*) δ 140.87, 139.58, 127.57, 124.35, 123.11, 122.19, 121.58, 120.74, 119.41, 111.05, 110.61, 79.14, 75.38, 32.58. HRMS (ESI) calcd.for $C_{16}H_{10}N_2$ 231.0917 $[M + H]^+$, found 231.0919.

**DB1**



The compound of 2,2-dibromoacetic acid(12 mg, 0.05 mmol) and HATU (19.0 mg, 0.05 mmol) were dissolved in DMF(2 mL). Then DIEPA (8.9 μL, 0.054 mmol) was added to the reaction mixture, which was stirred under room temperature for 5 min. And then S30 (25 mg, 0.04 mmol) was added to the reaction mixture and stirred at room temperature for 3 h. The reaction mixture was quenched by addition of water and extracted with ethyl acetate (EA) three times. The combined organic layer was then washed with brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluting with MeOH: DCM = 20:1) to afford DB1 as a white solid (14.8 mg, 40% yield). $^1H$ NMR (400 MHz, $CDCl_3$) δ 9.09 (d, J = 4.1 Hz, 1H), 8.01 (dd, J = 8.0, 1.6 Hz, 2H), 7.64 – 7.59 (m, 2H), 7.36 (dd, J = 11.6, 5.8 Hz, 1H), 6.15 (s, 1H), 5.52 (d, J = 52.7 Hz,1H), 4.89 (d, J = 13.3 Hz, 1H), 4.55 (s, 1H), 4.39 – 3.90 (m, 8H), 3.41 (d, J = 32.6 Hz, 3H), 3.02 (s, 2H), 2.34 (dd, J = 15.4, 8.0 Hz, 2H), 2.25 – 2.12 (m, 2H), 2.04 (s, 2H). $^{13}C$ NMR (151 MHz, $CDCl_3$) δ 164.03 (s), 162.75 (s), 161.51 (s), 146.06 (s), 142.23 (s), 131.00 (s), 130.60 (s), 130.08 (s), 129.96 (s), 129.73 (s), 128.28 (s), 124.40 (s), 124.09 (s), 123.93 (s), 119.17 (s), 115.14 (s), 114.97 (s), 110.18 (s), 74.31 (s), 73.96 (s), 70.26 (s), 59.13 (s), 56.09 (s), 47.45 (s), 44.77 (s), 41.52 (s), 34.51 (s), 33.75 (s), 30.91 (s), 28.68 (s), 24.11 (s), 21.68 (s), 13.11 (s). HR-MS (ESI) Calcd $C_{33}H_{29}Br_2F_3N_6O_2$ for $[M+H]^+$ 757.0743; found 757.0721

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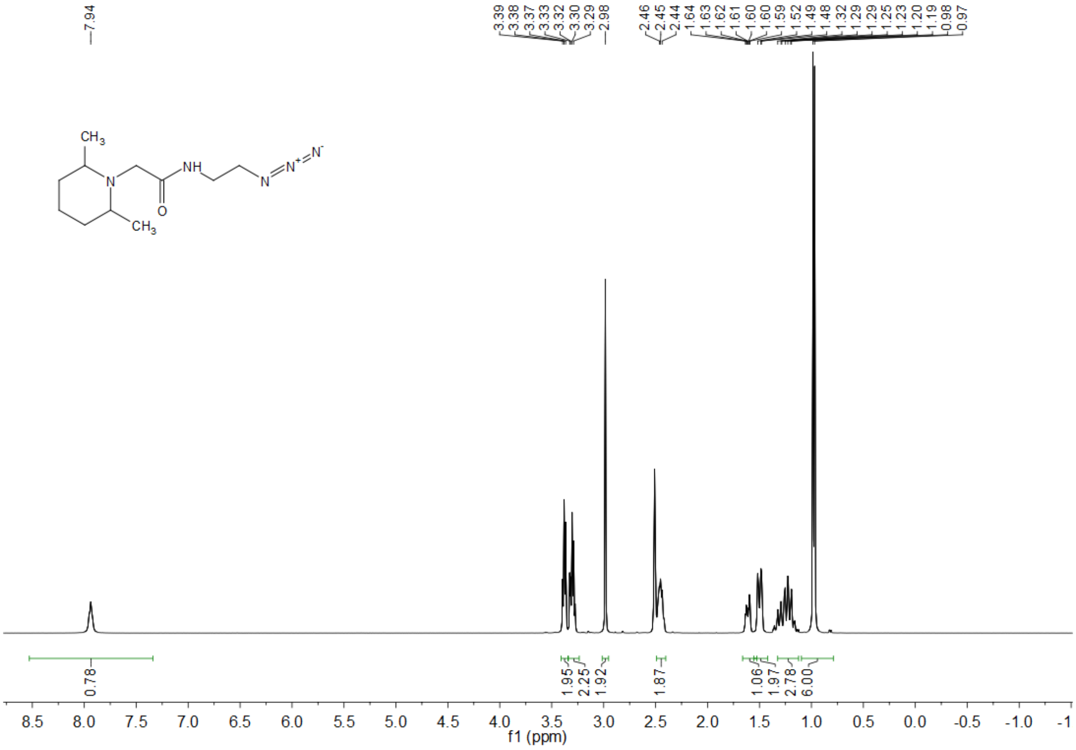
## 6. NMR

### DMP tag1 $^1$H NMR



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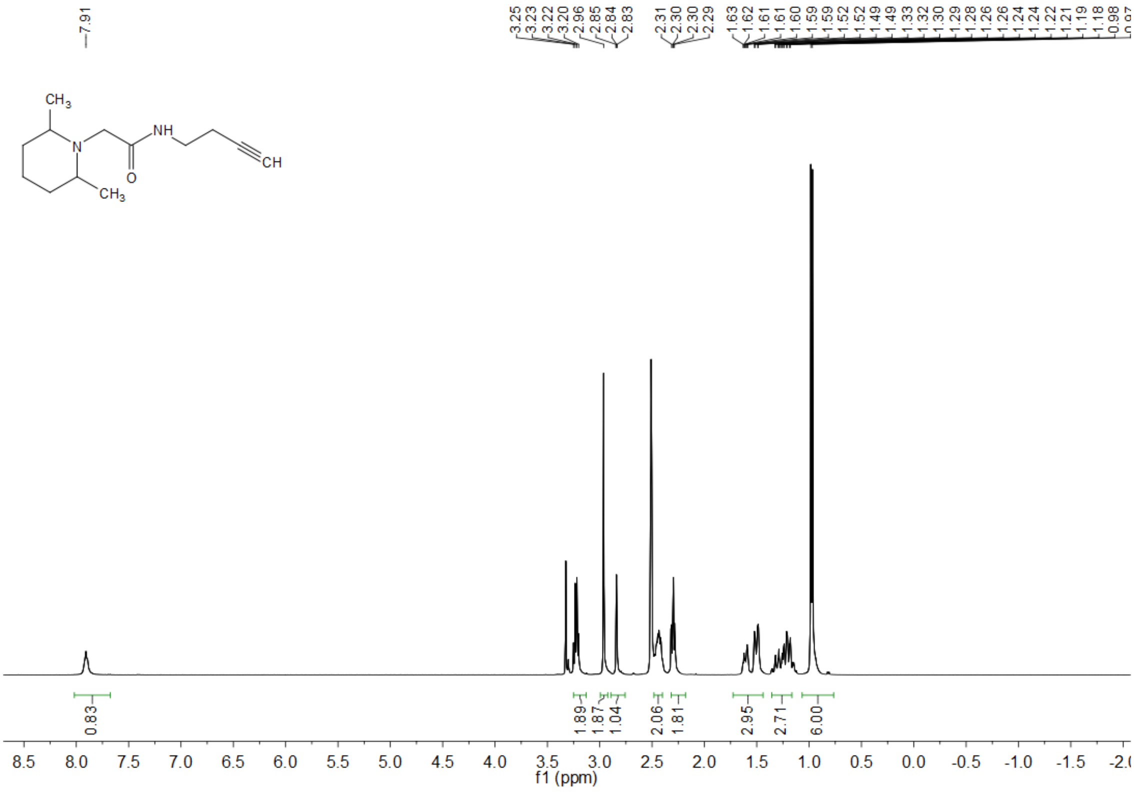

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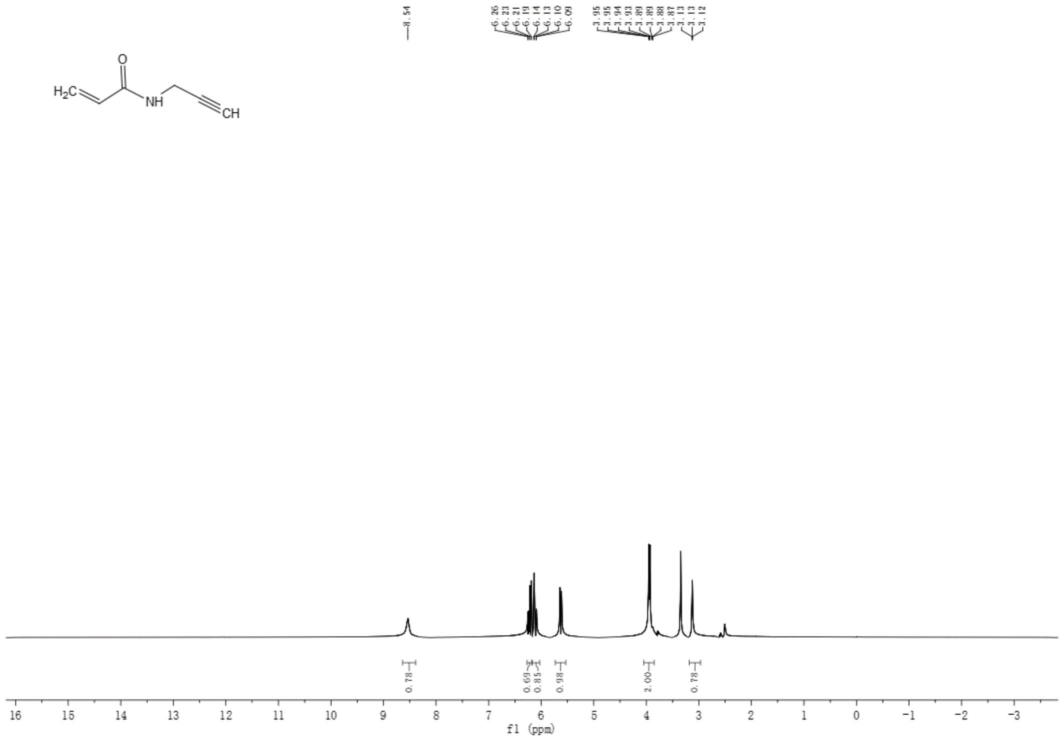

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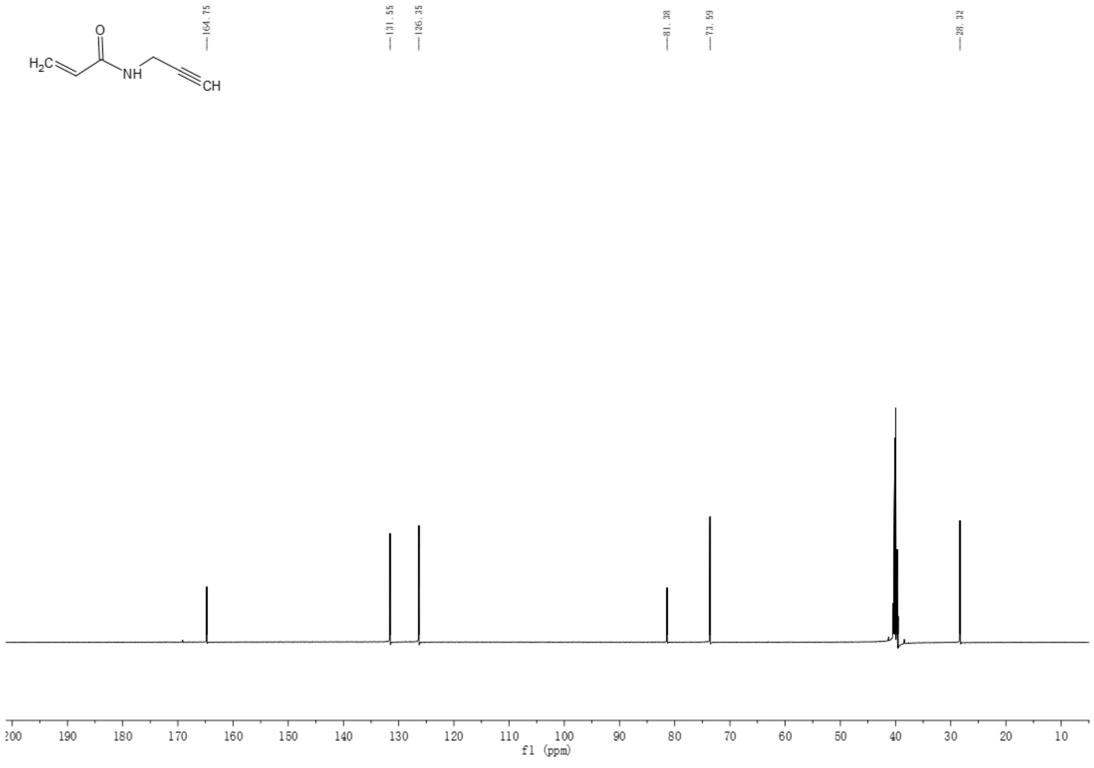

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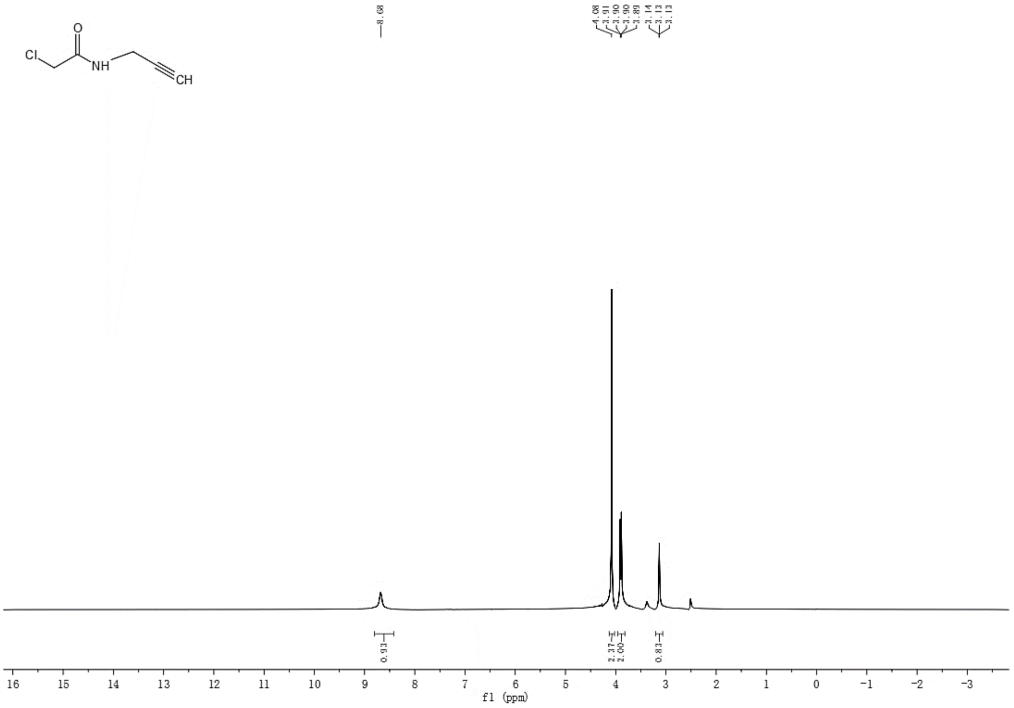

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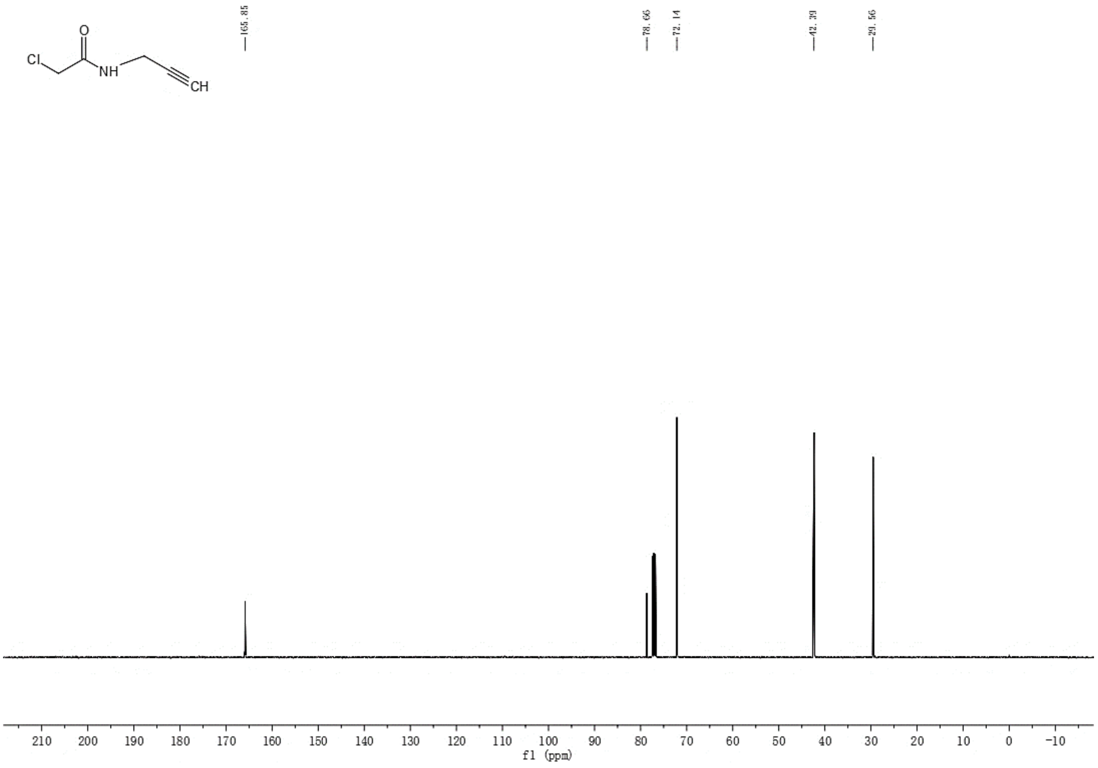

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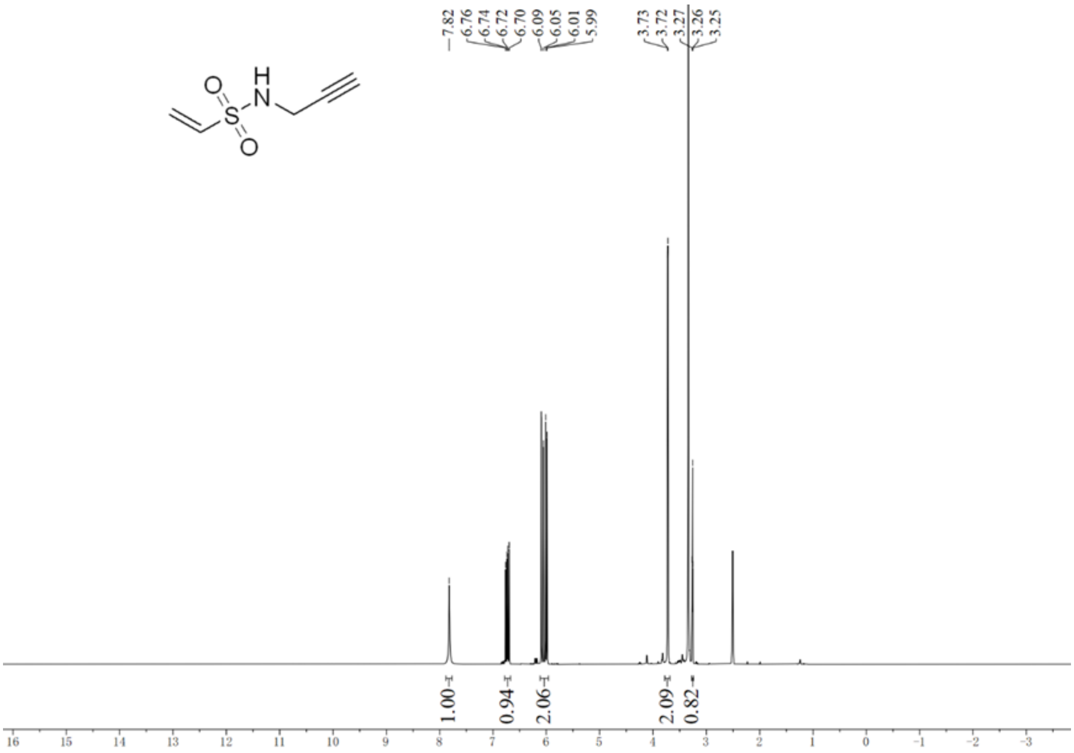

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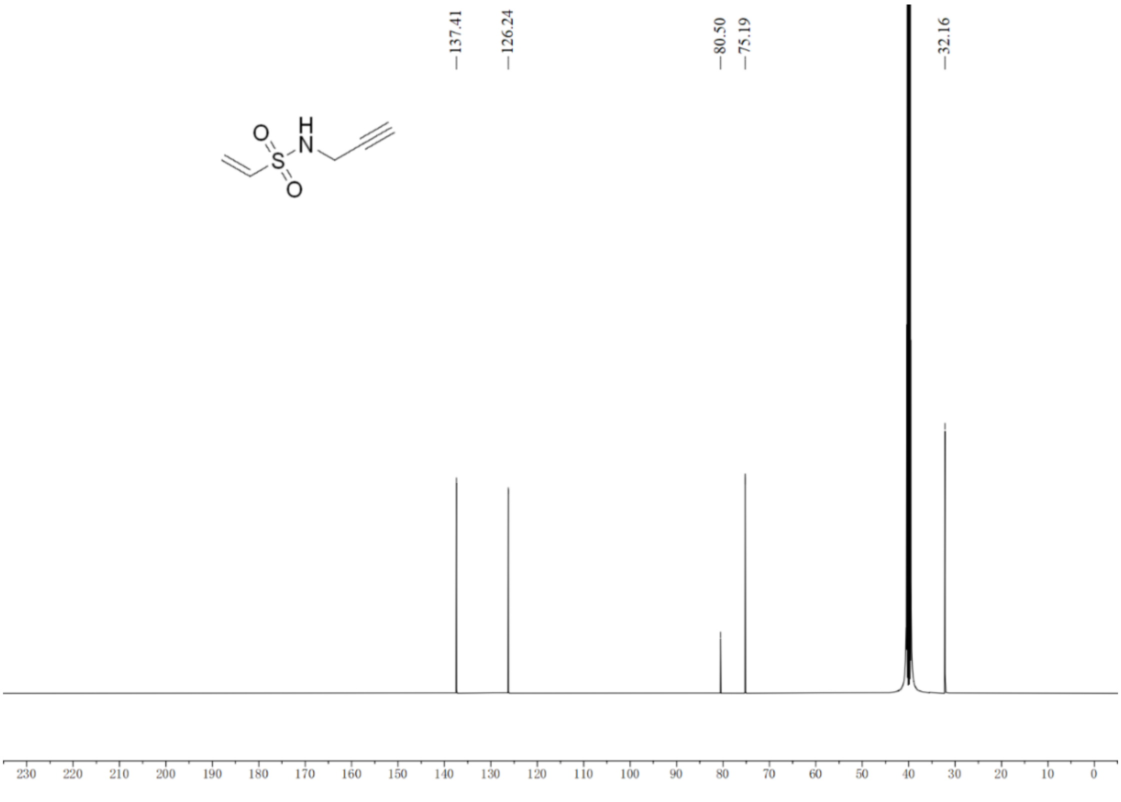

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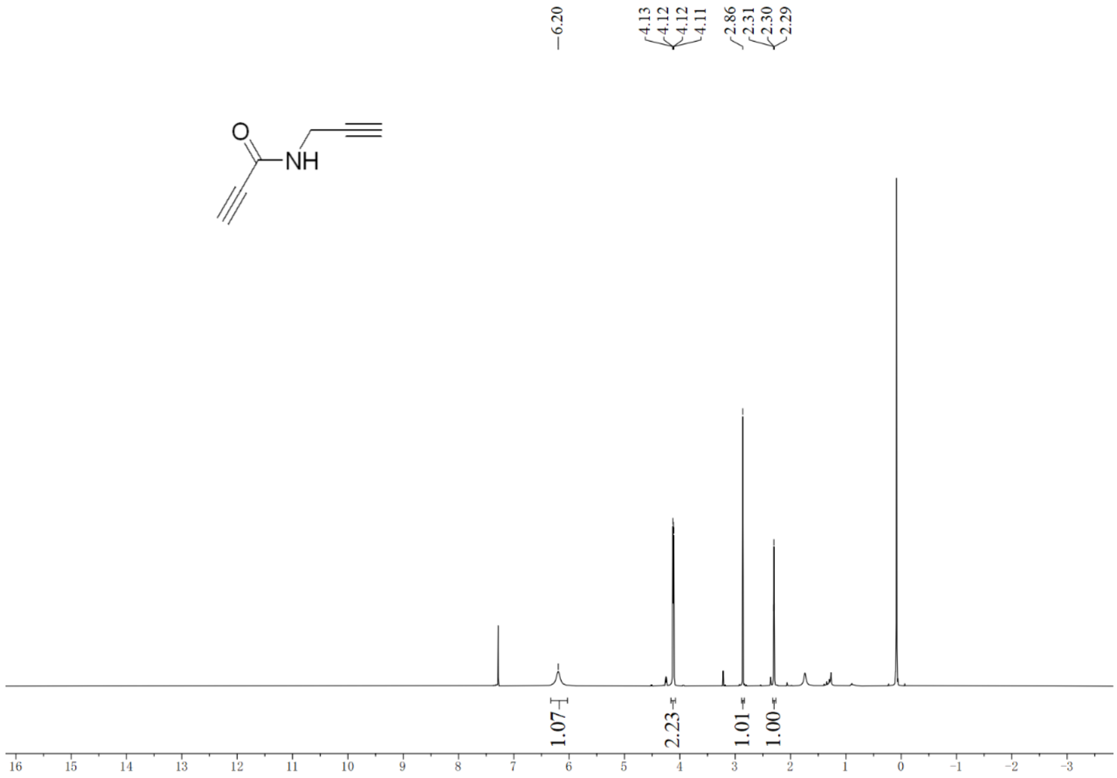

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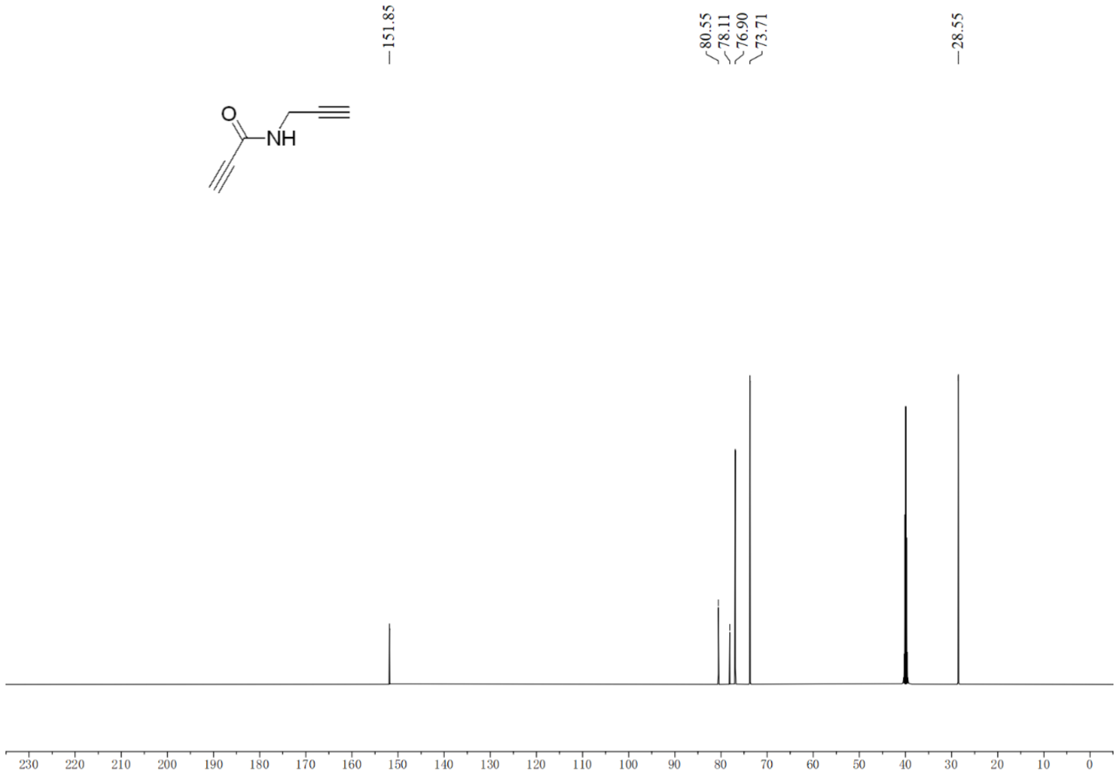

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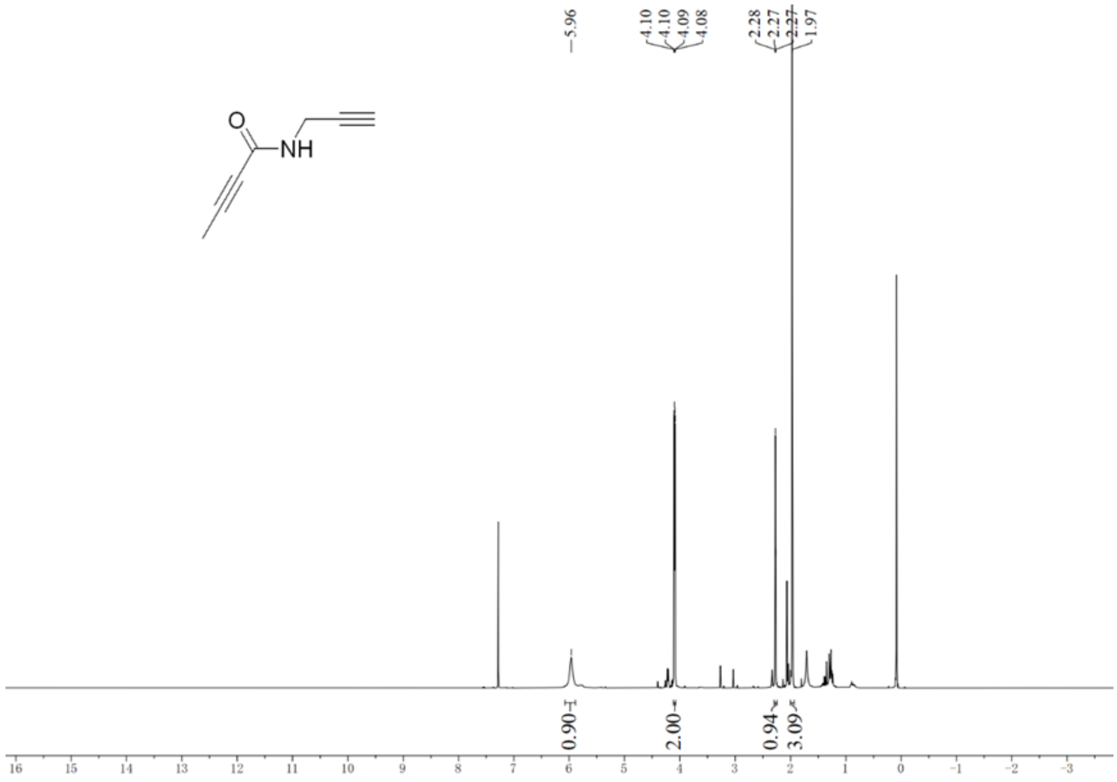

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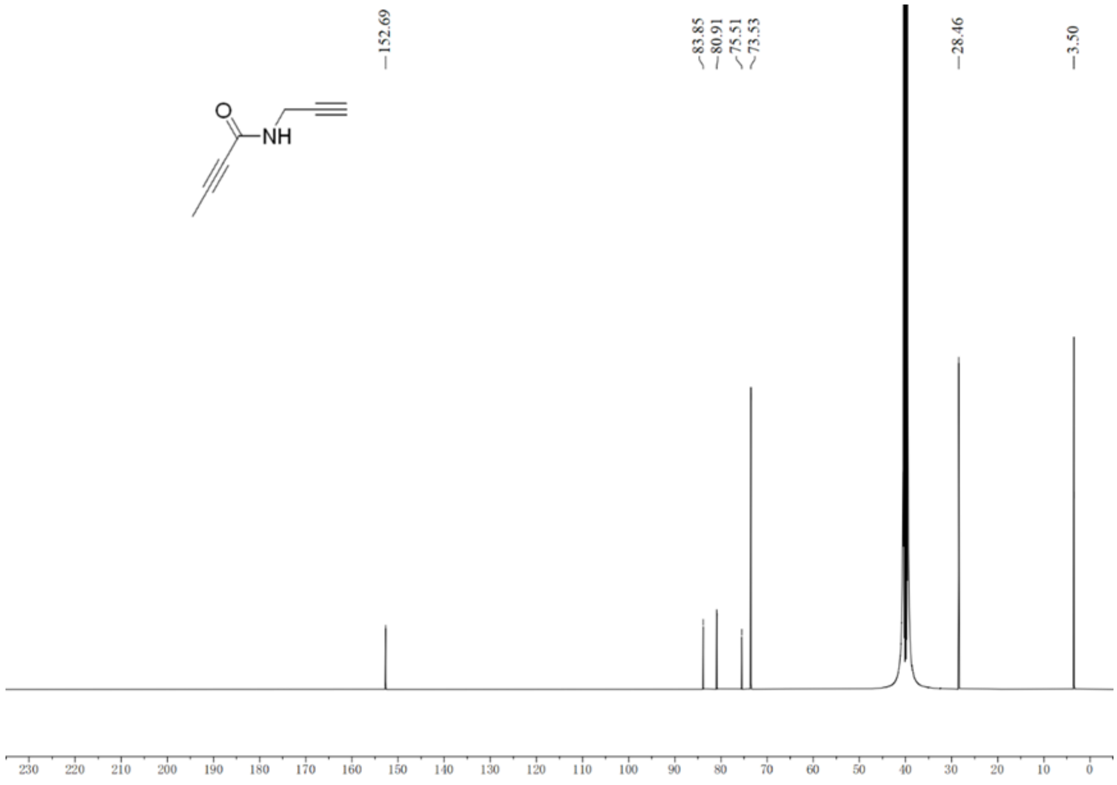

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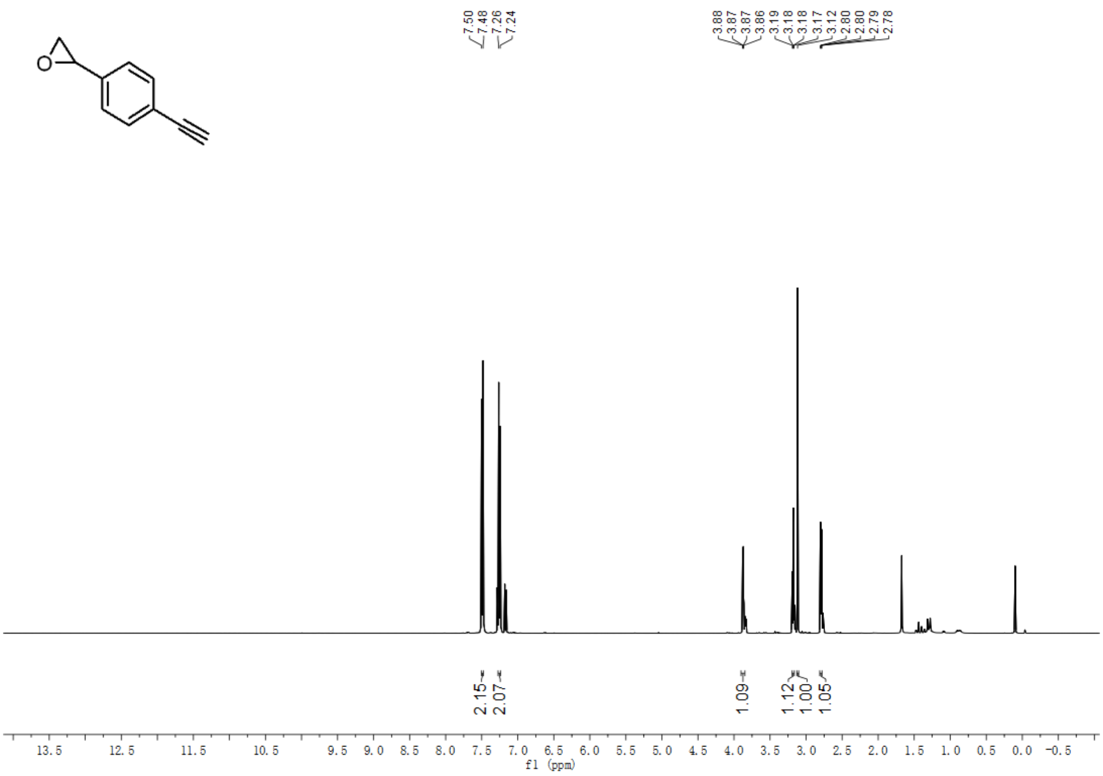

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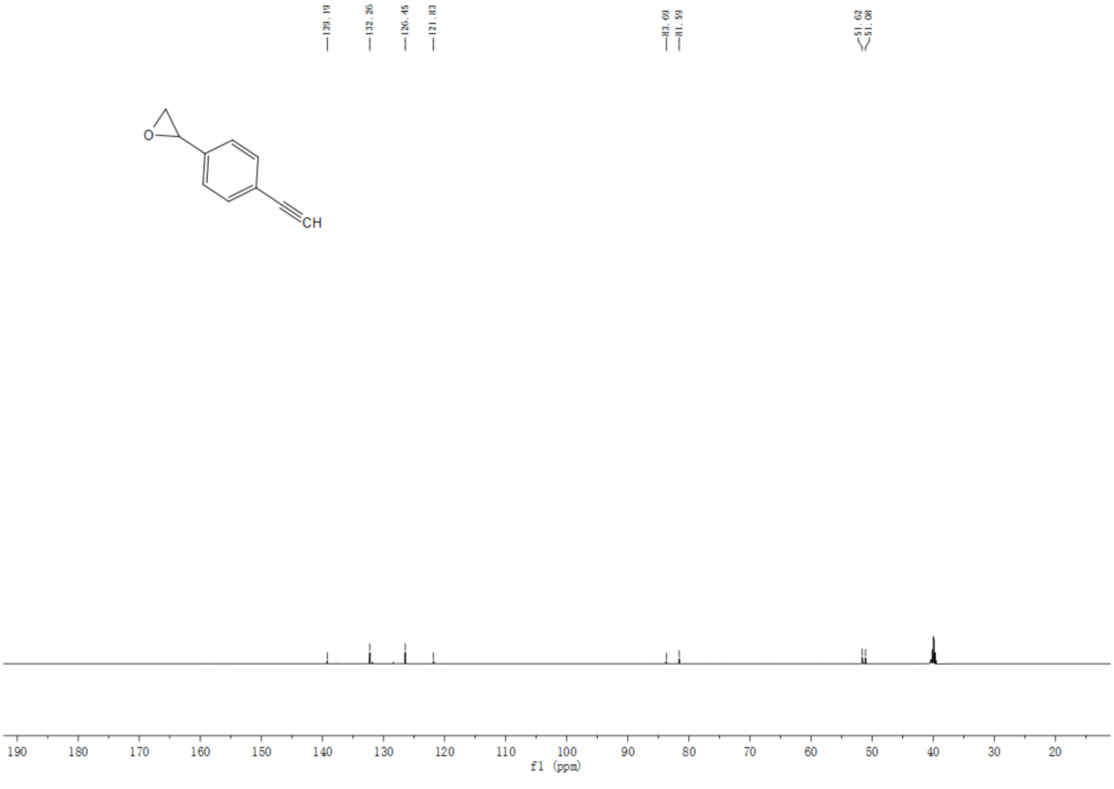

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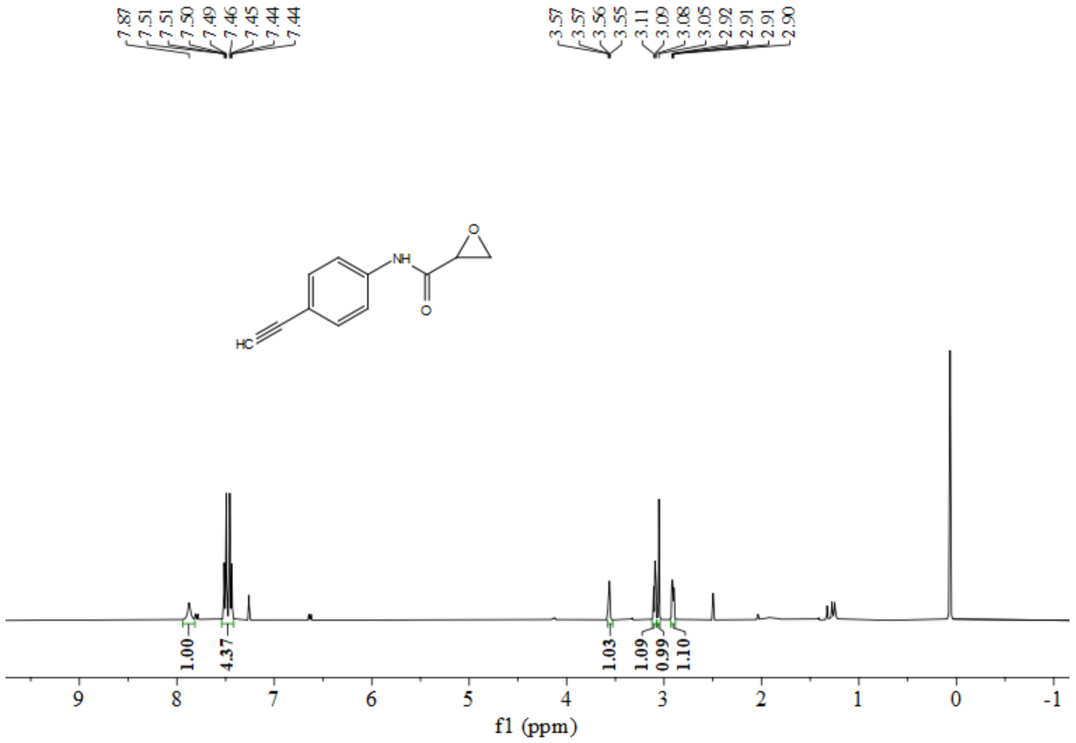

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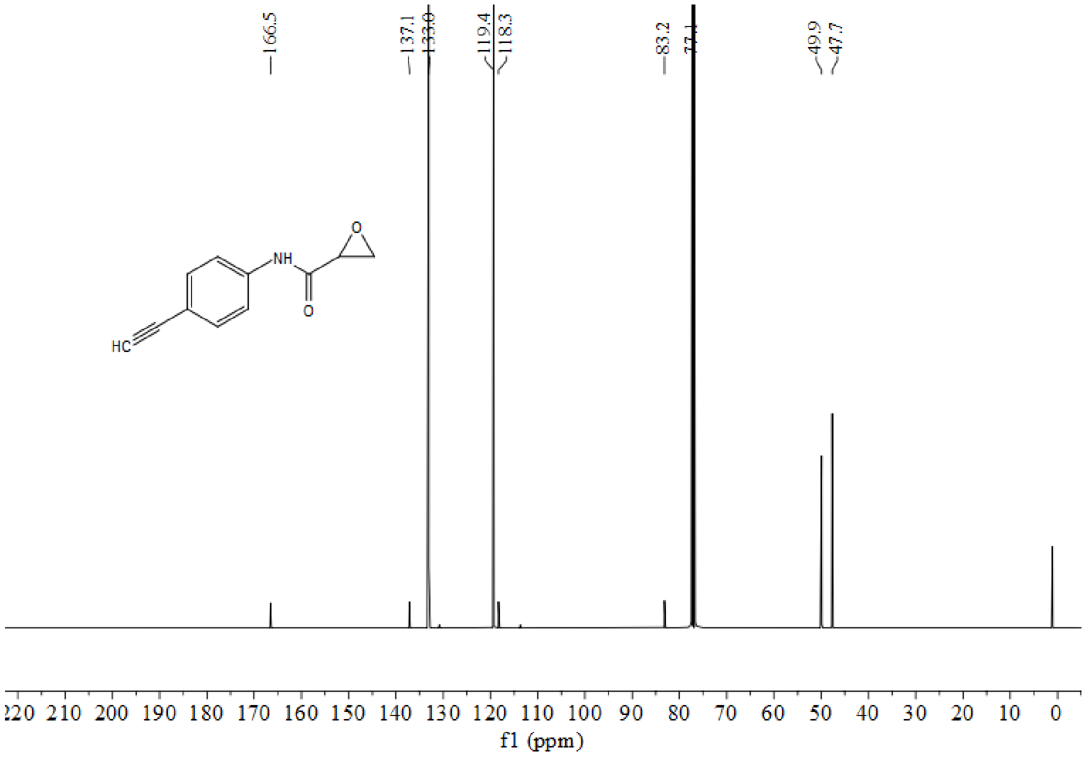

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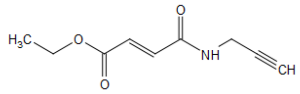

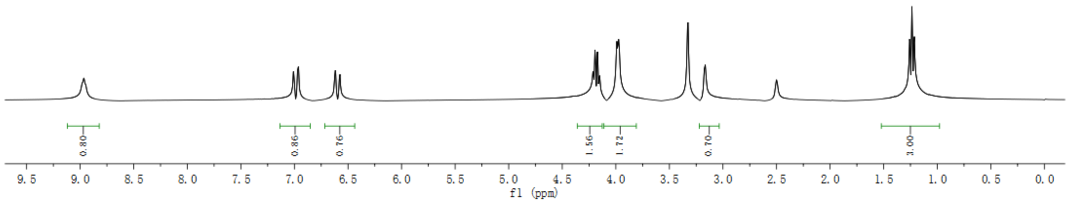

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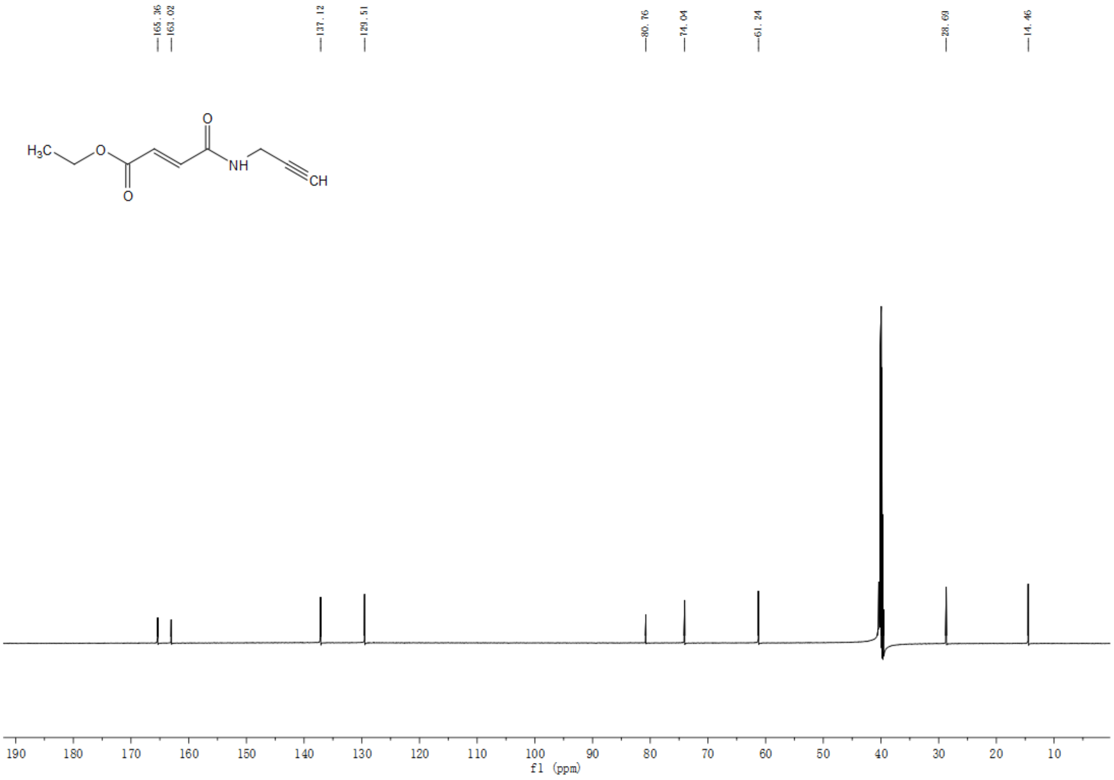

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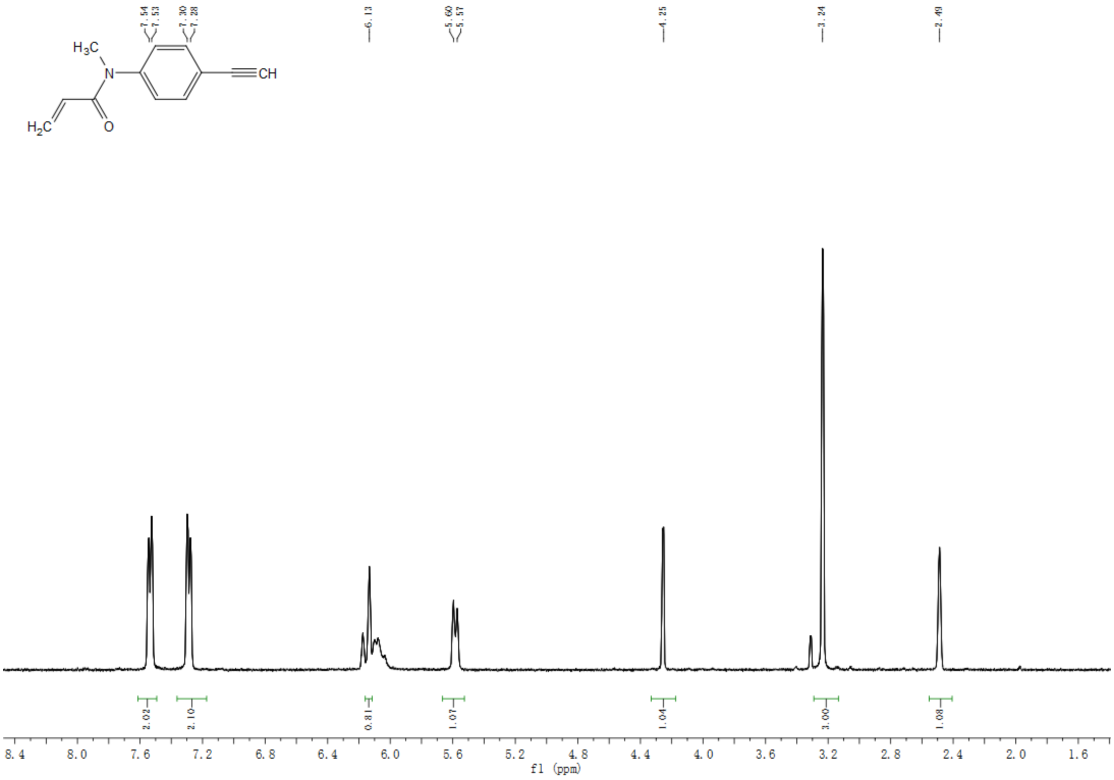

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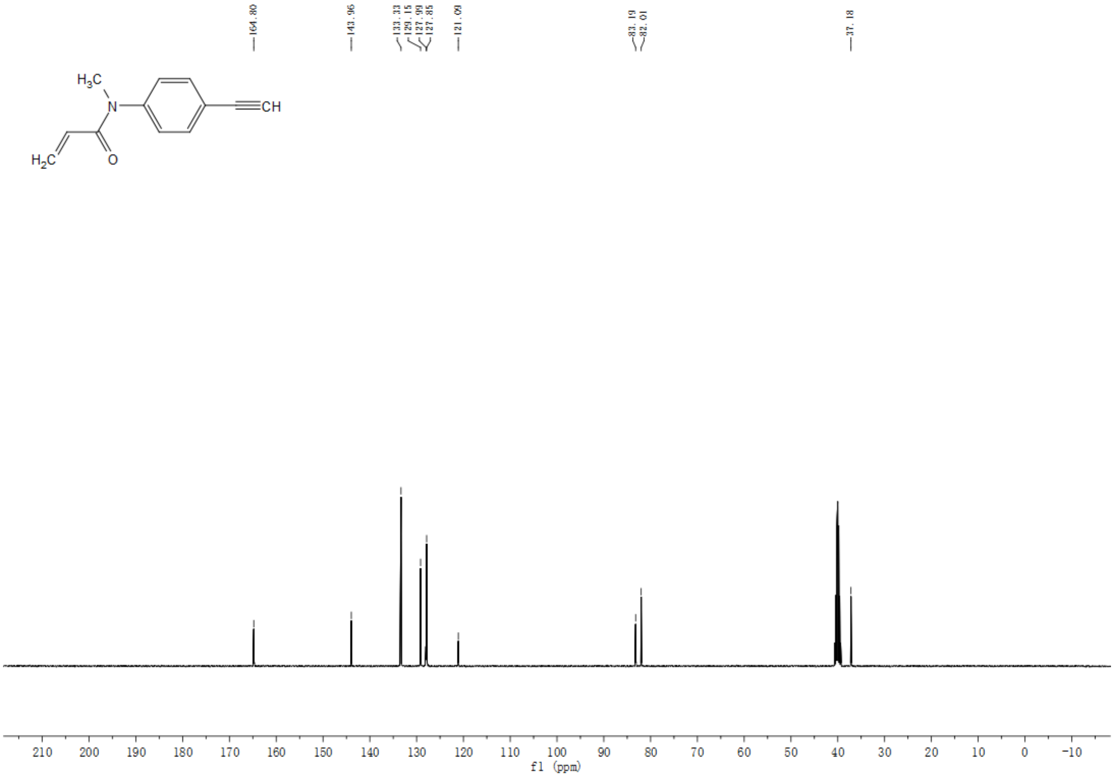

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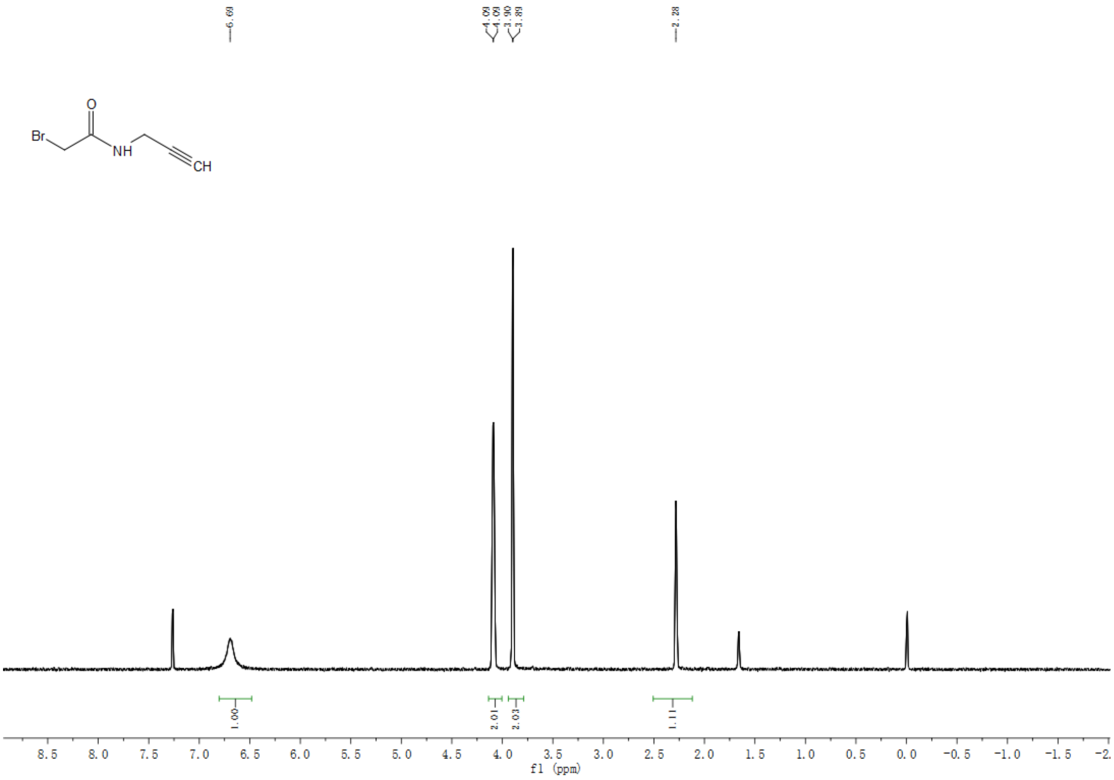

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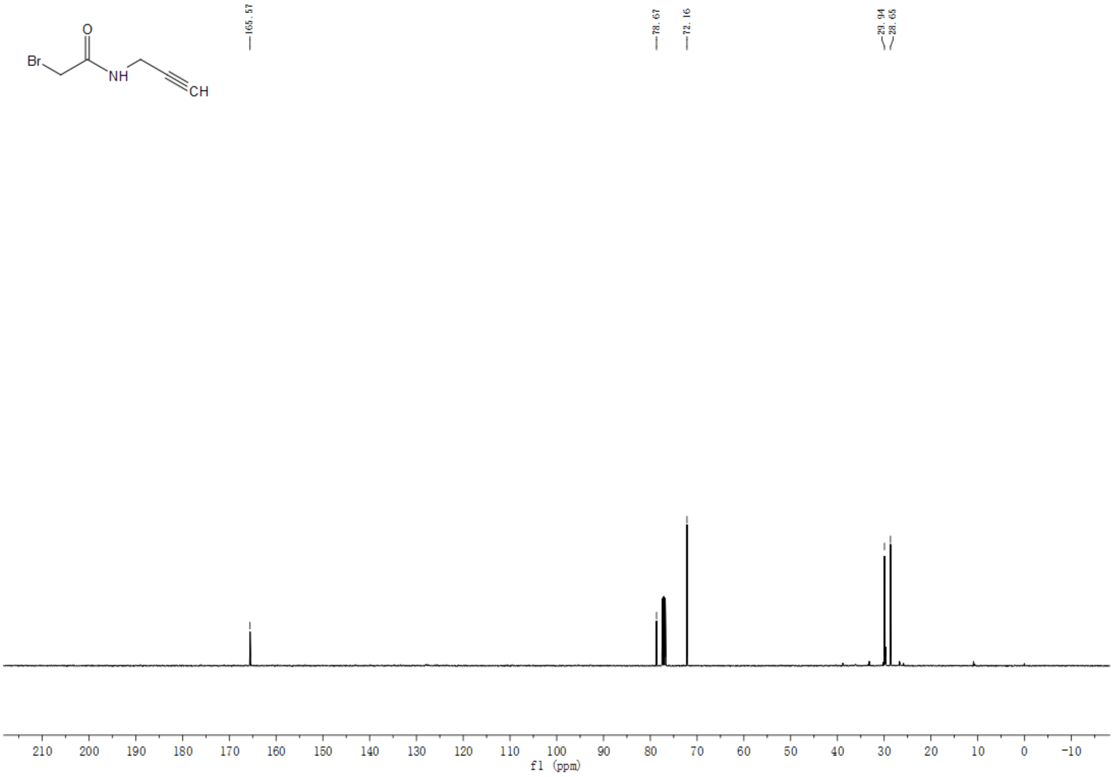

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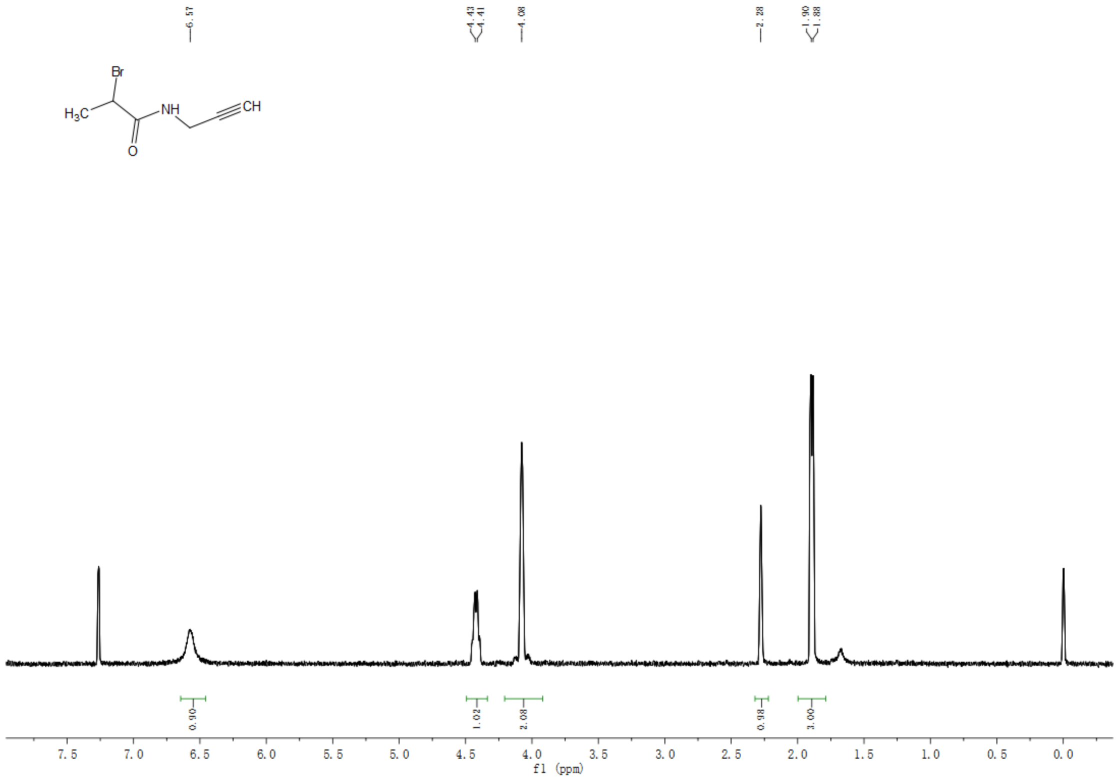

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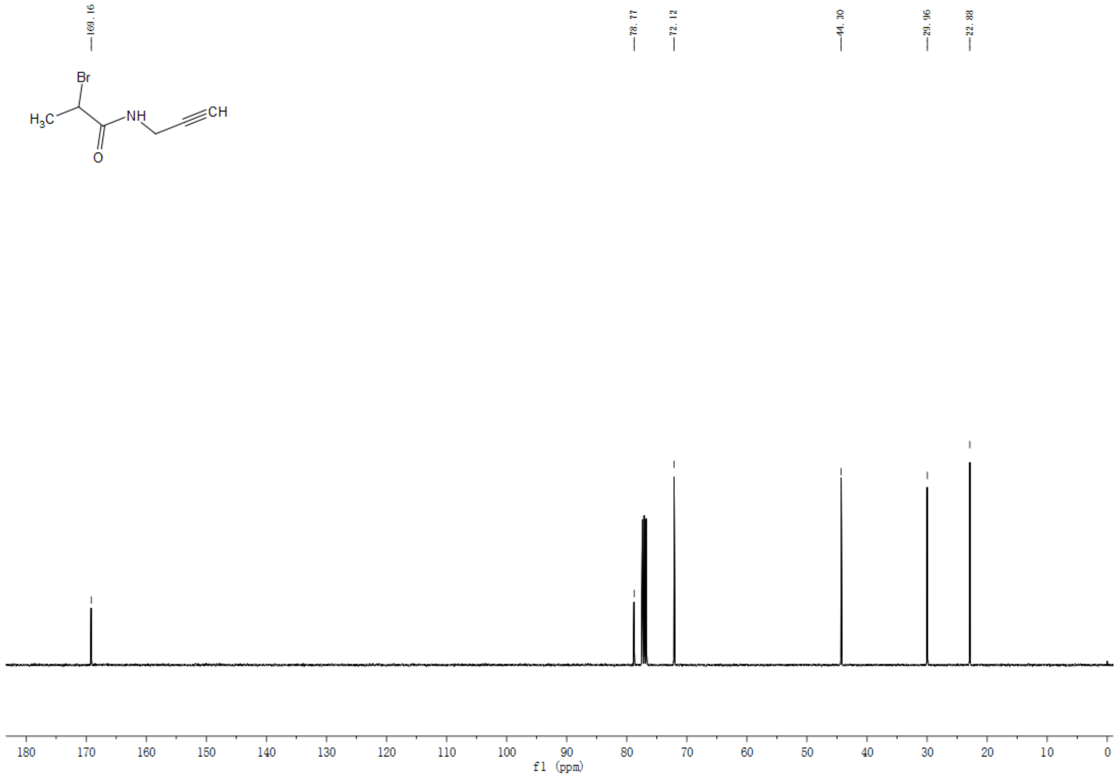

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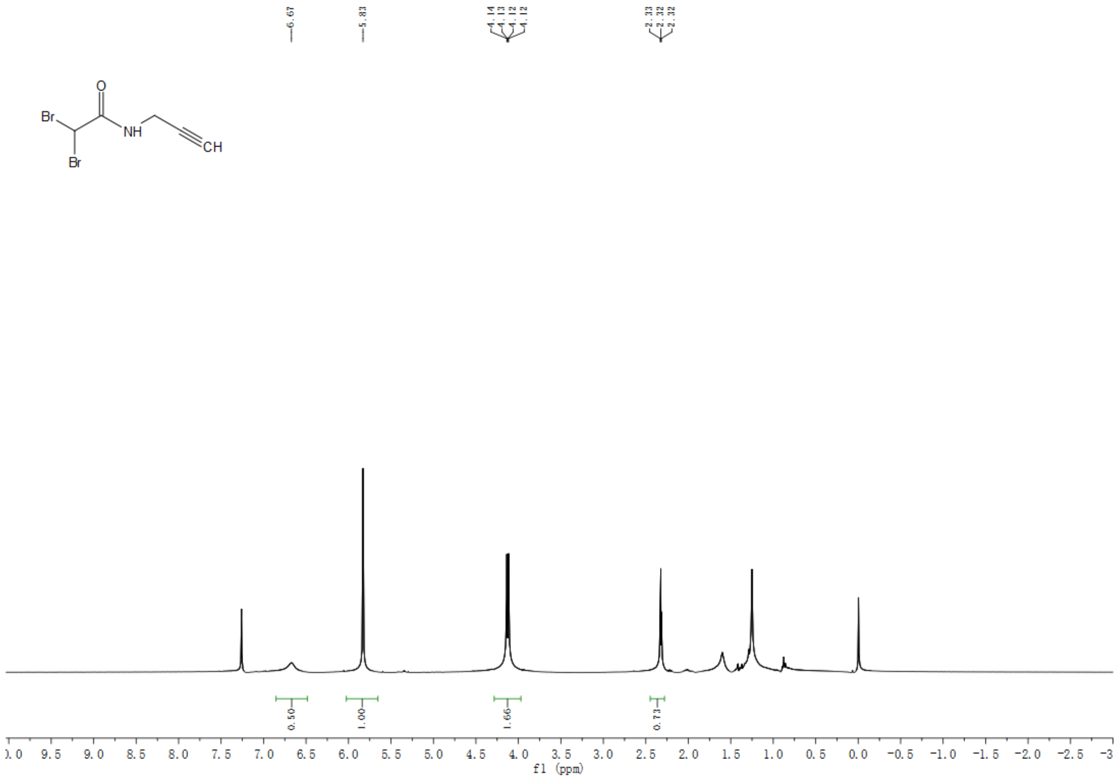

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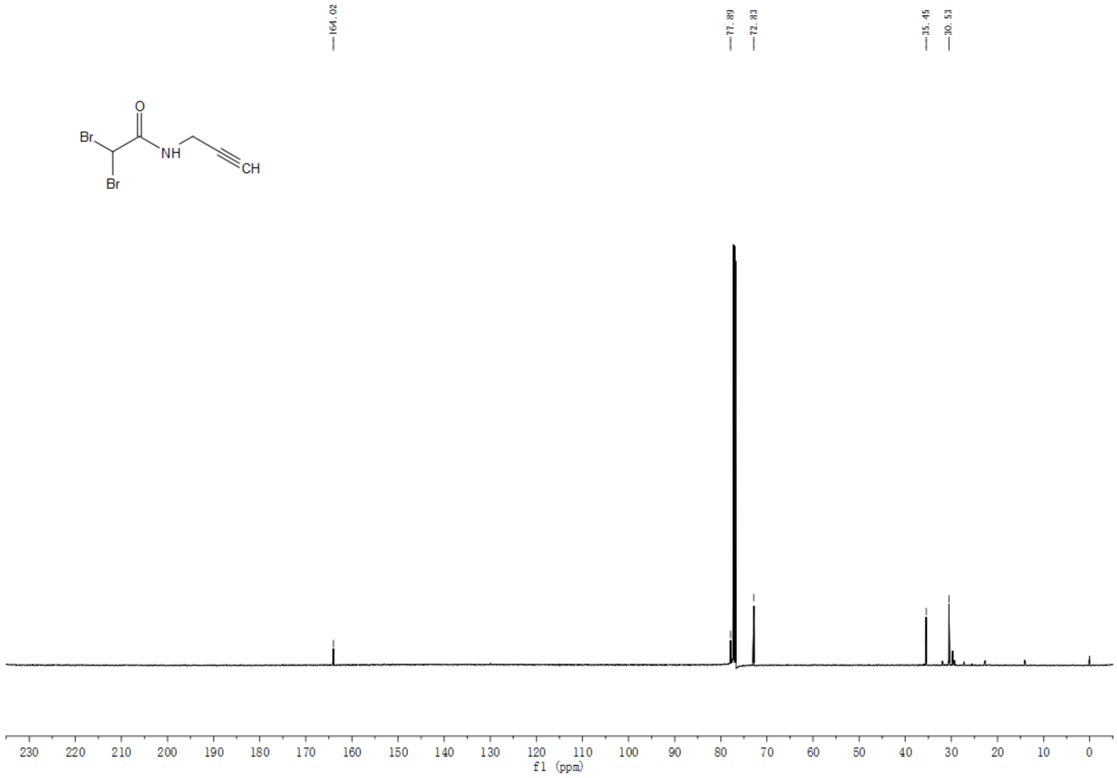

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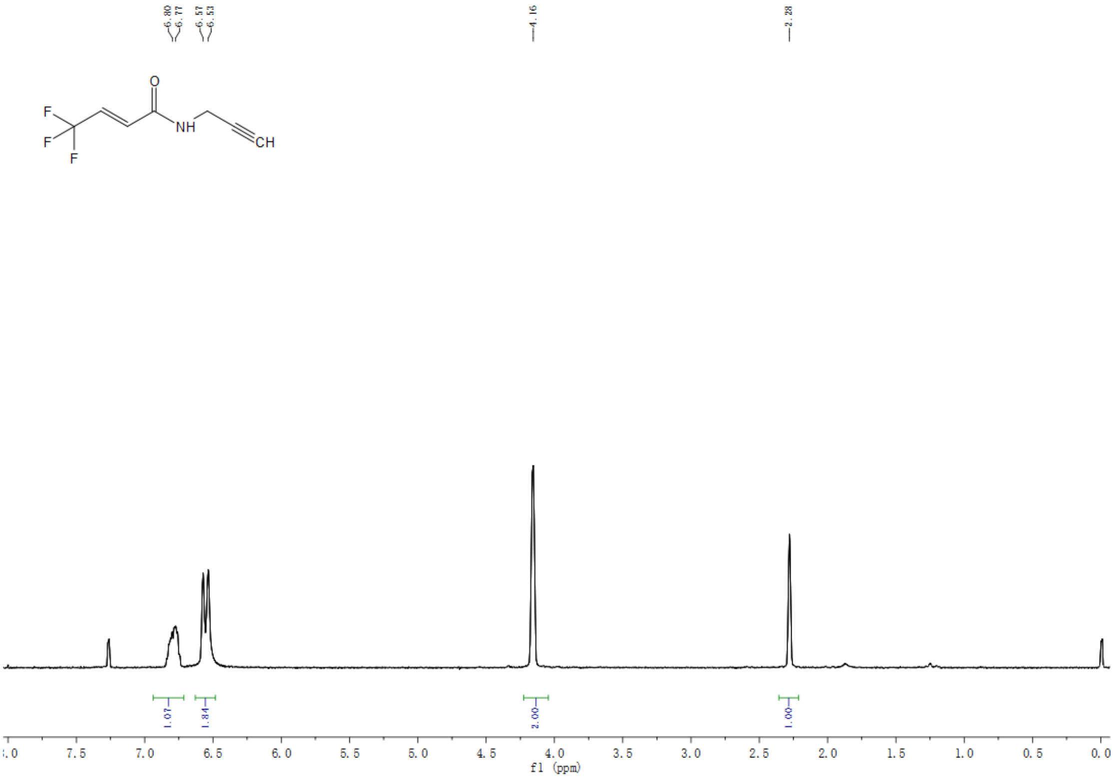

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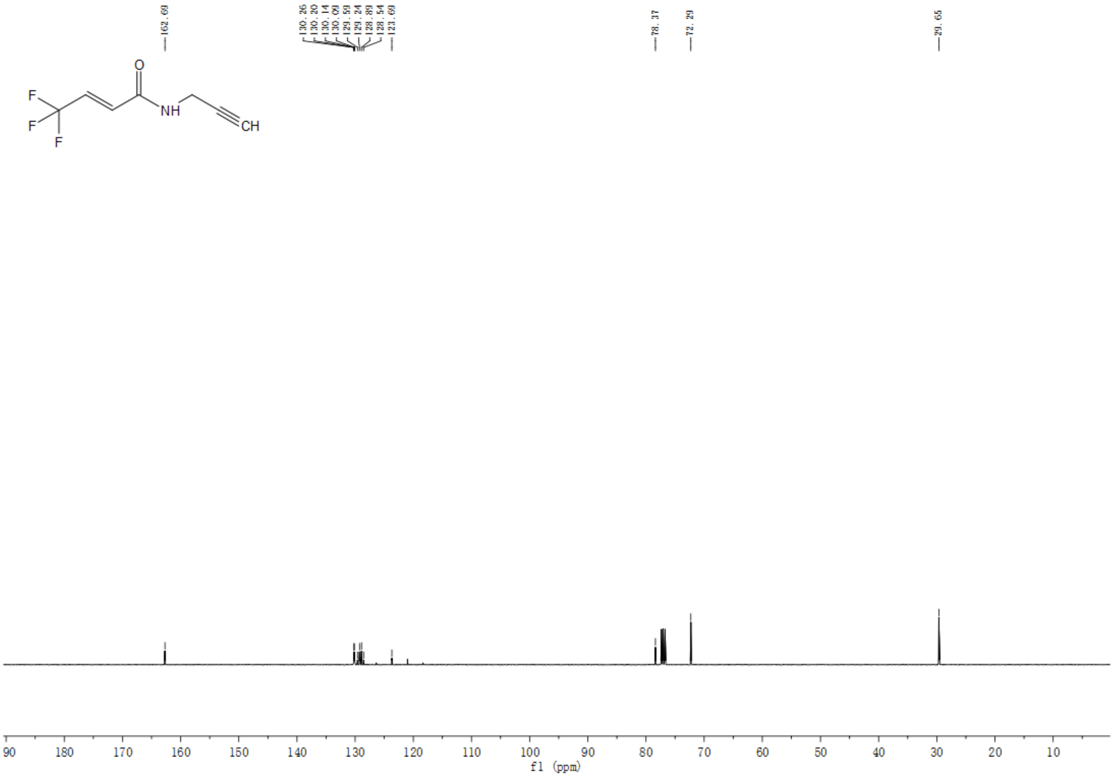

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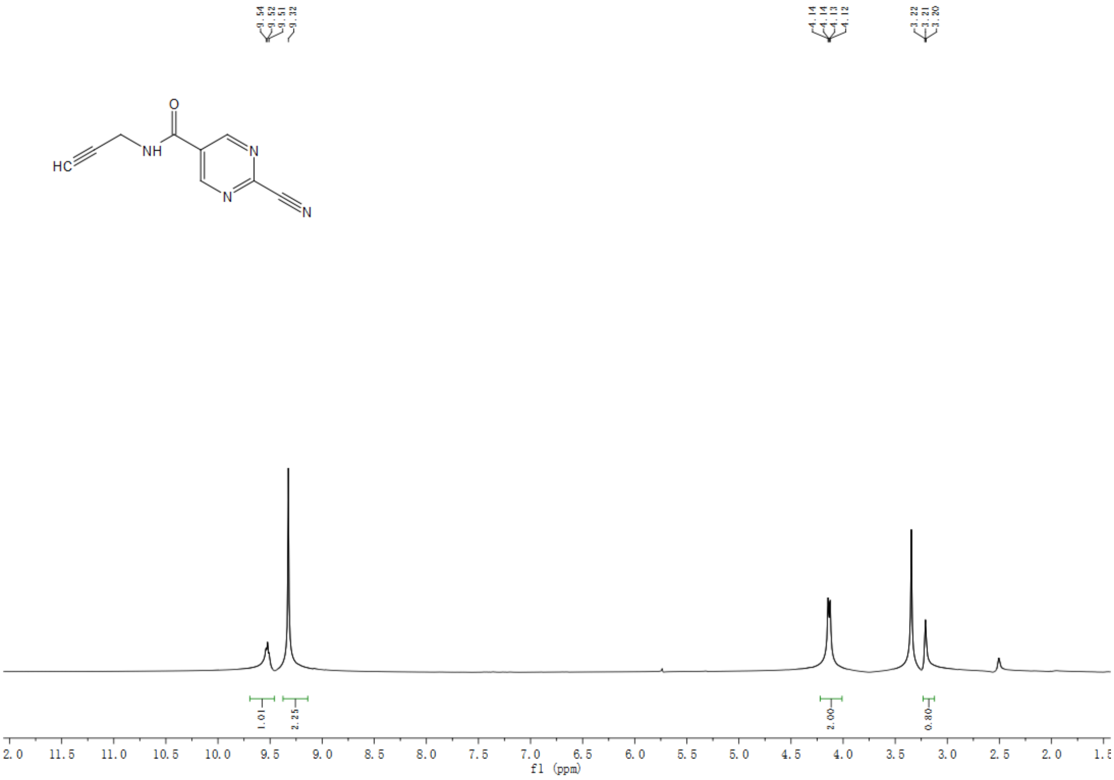

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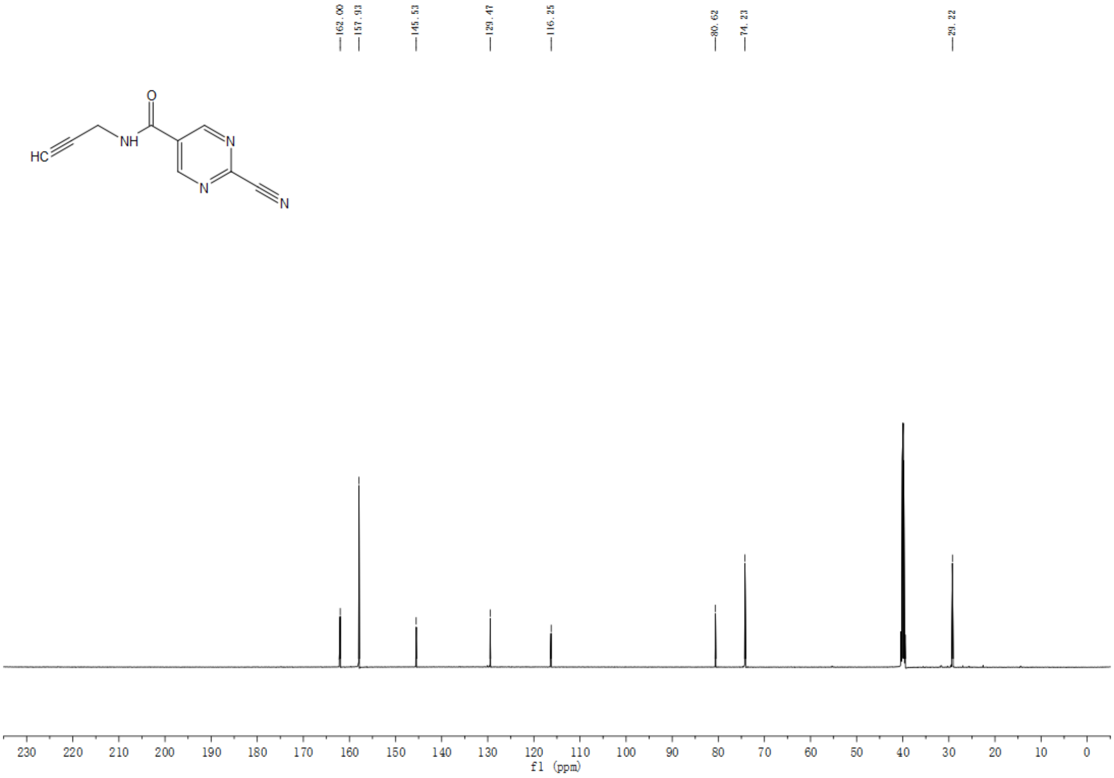

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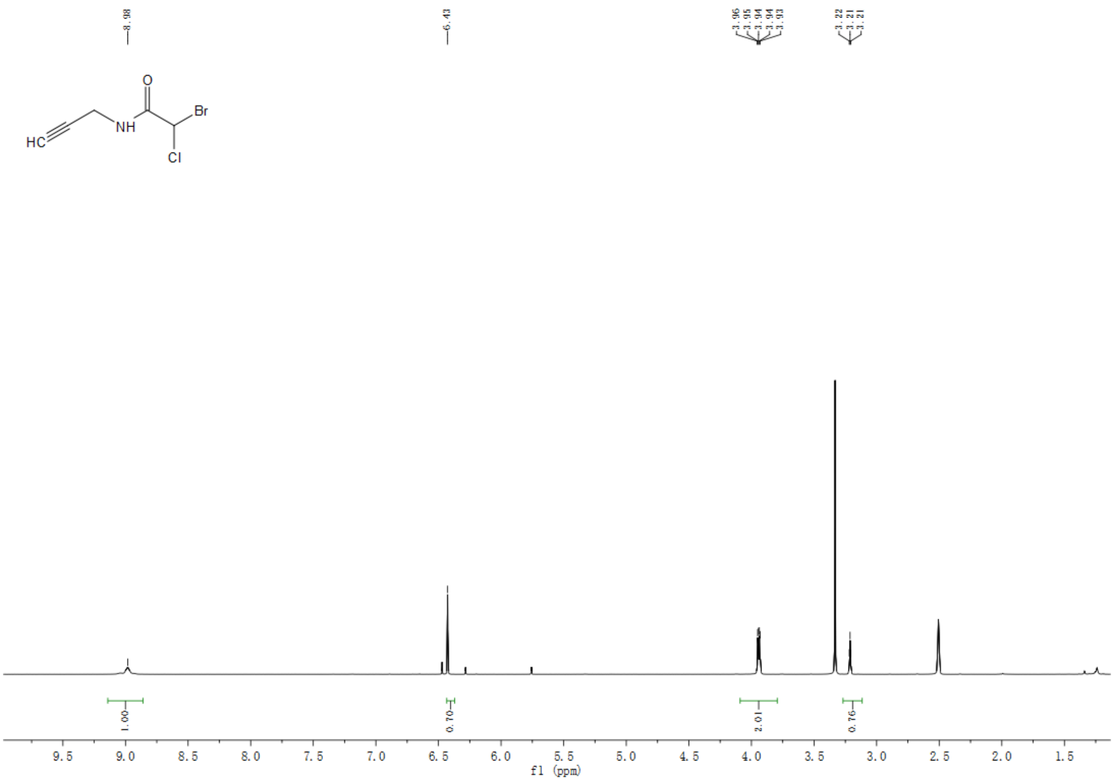

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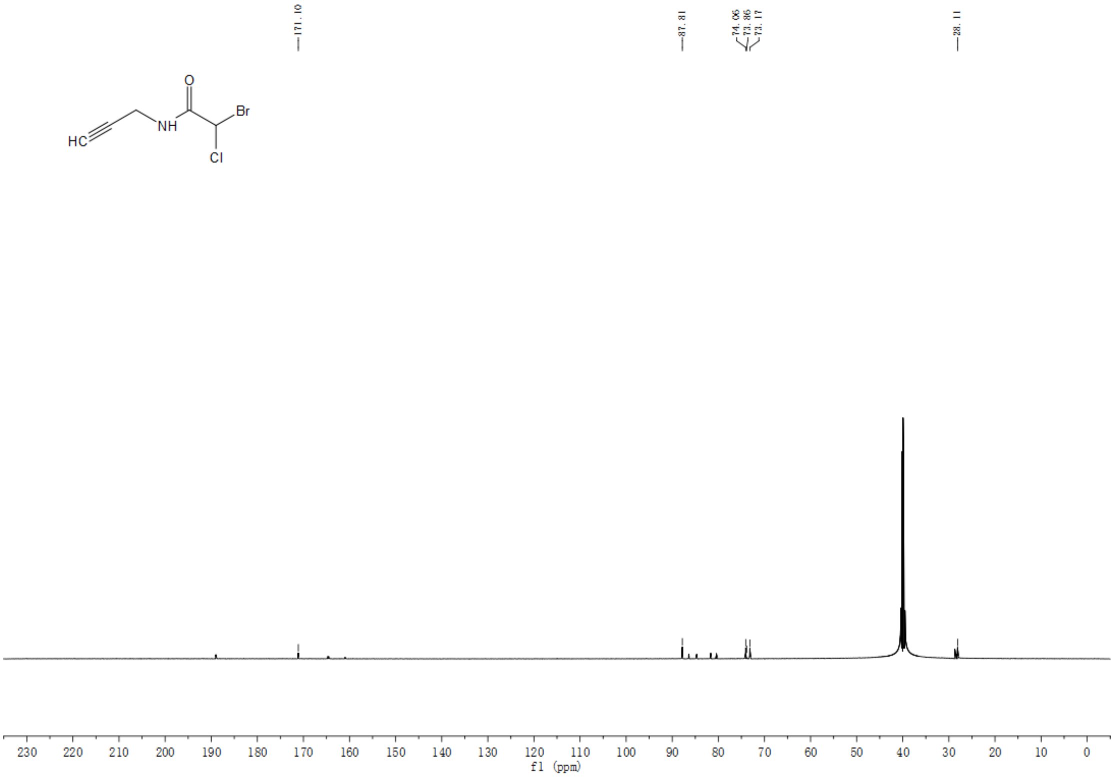

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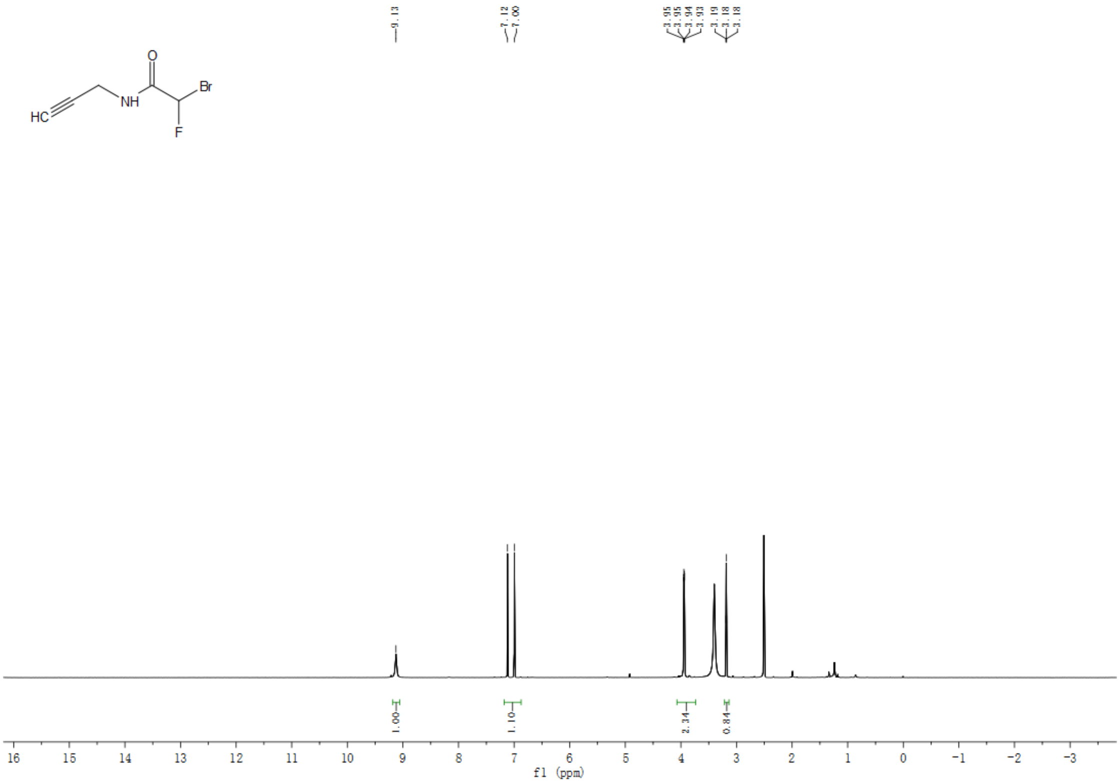

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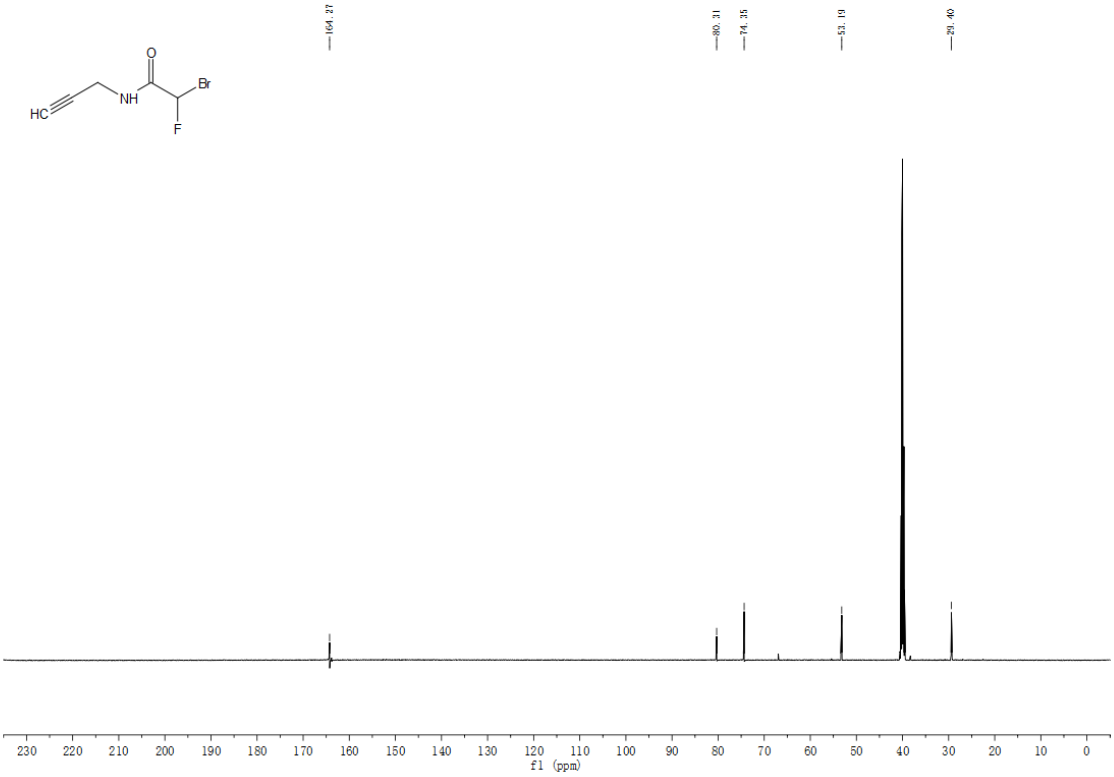

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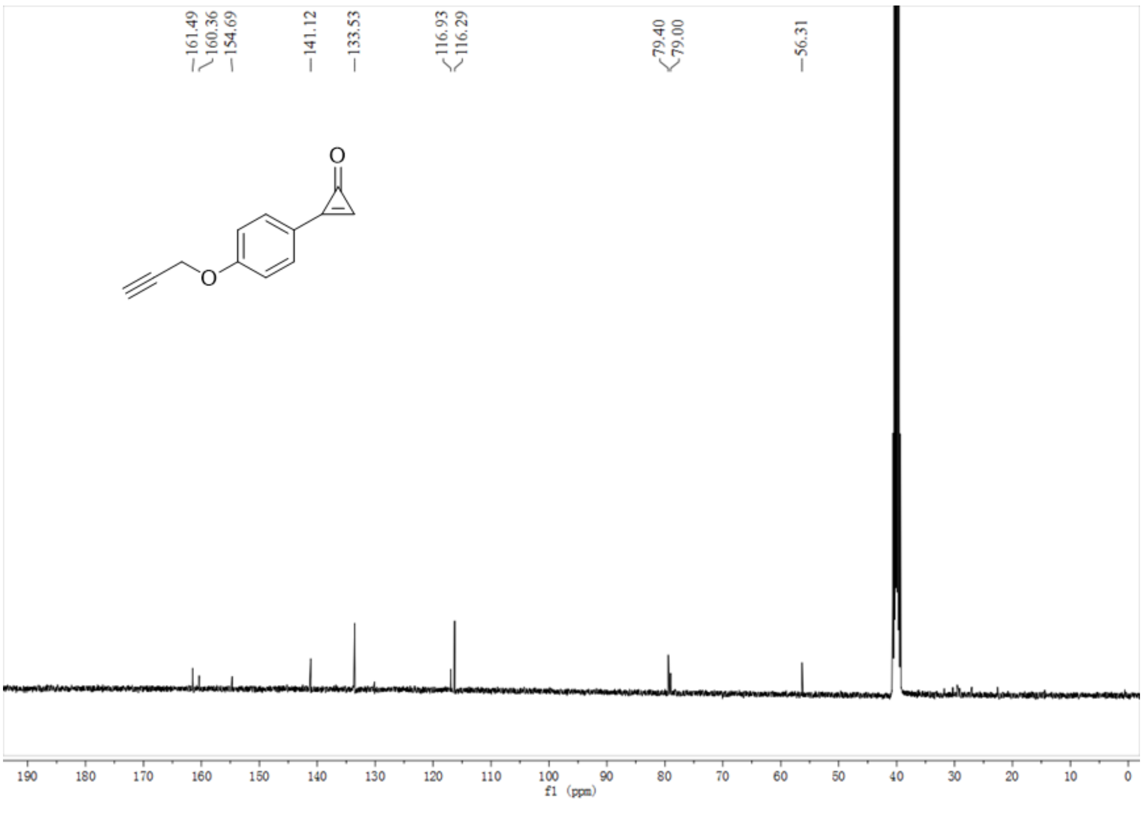
## 18 $^{13}$C NMR



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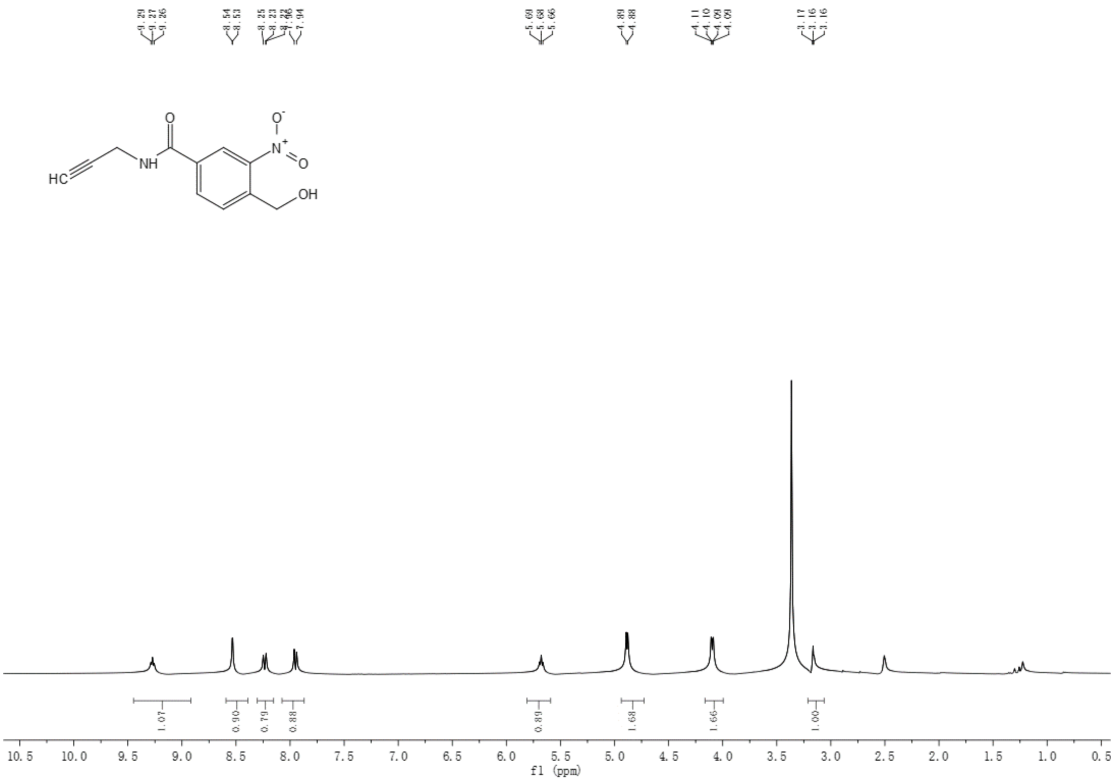

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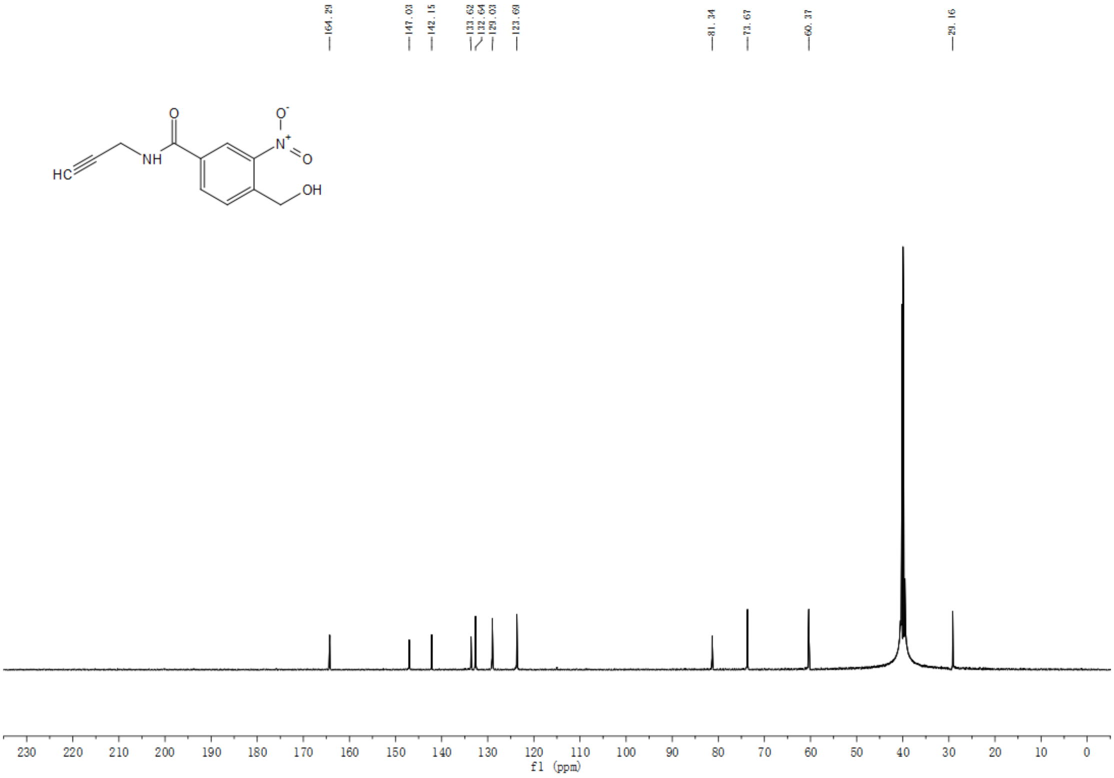
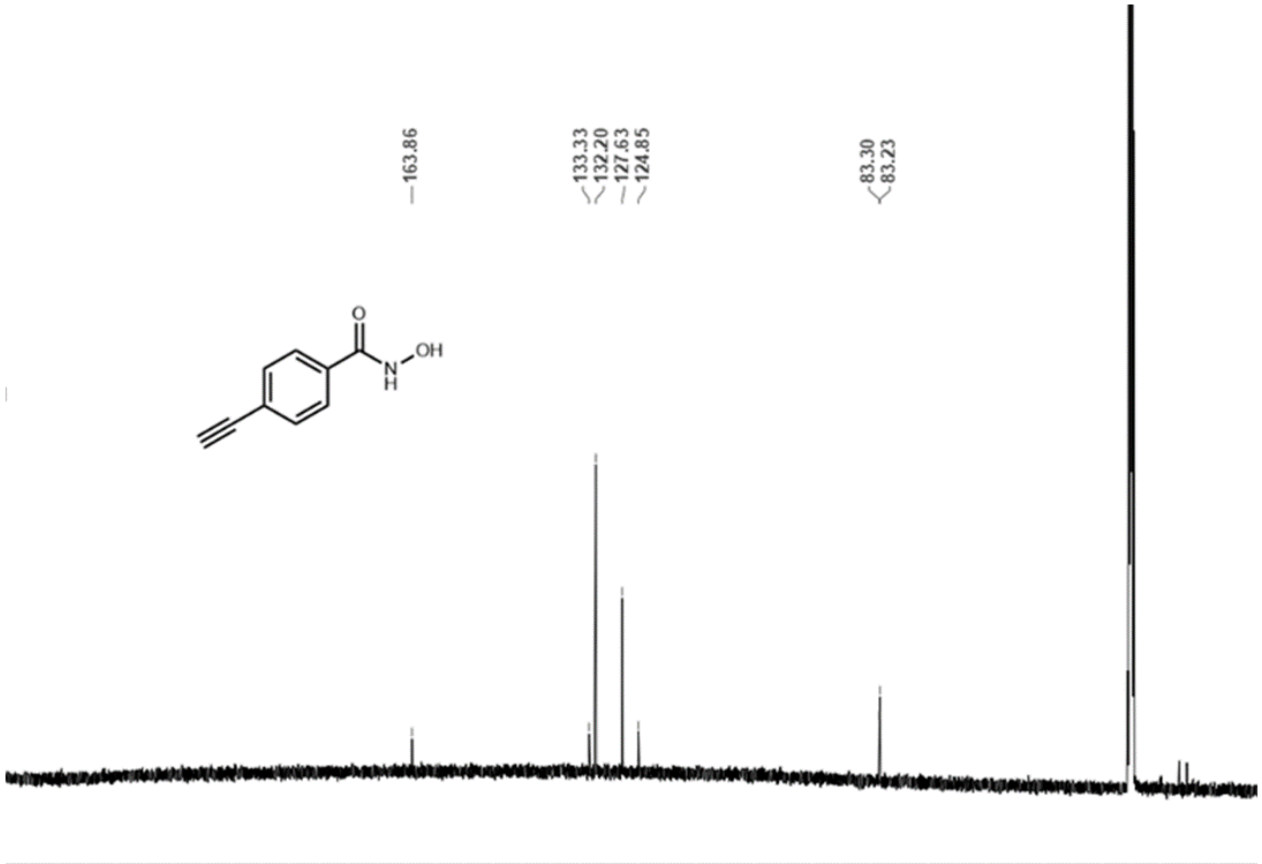


**24 $^{1}$H NMR**

**24 $^{13}$C NMR**



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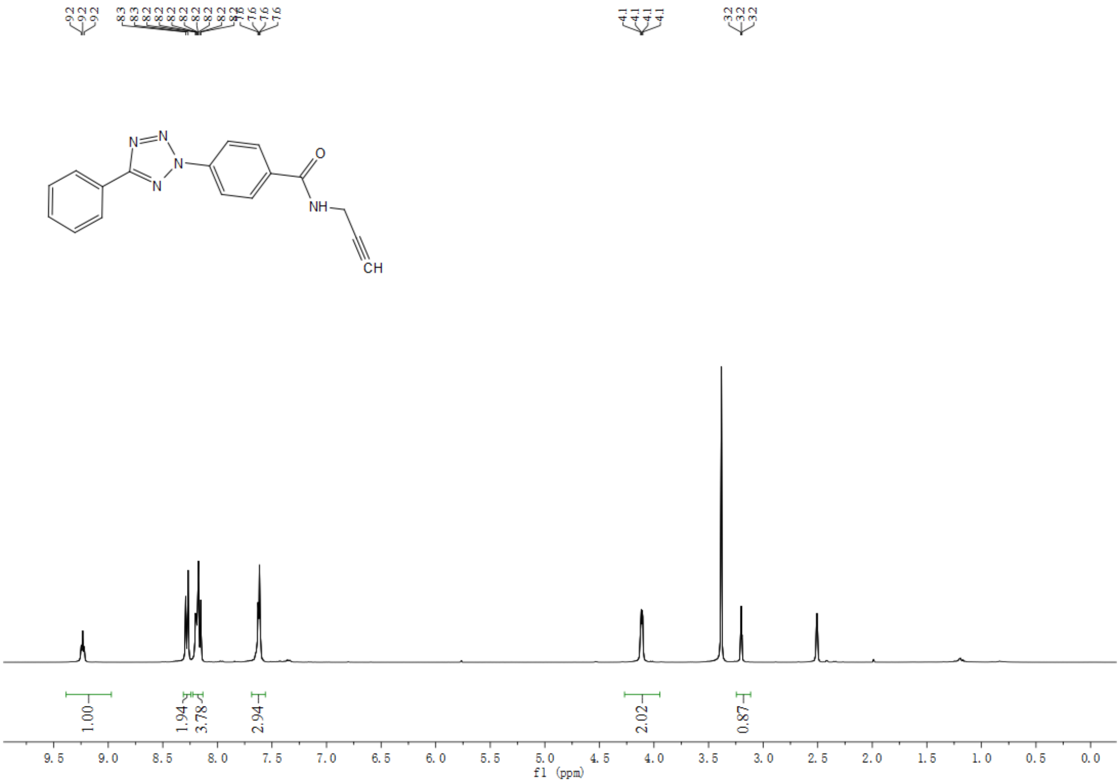

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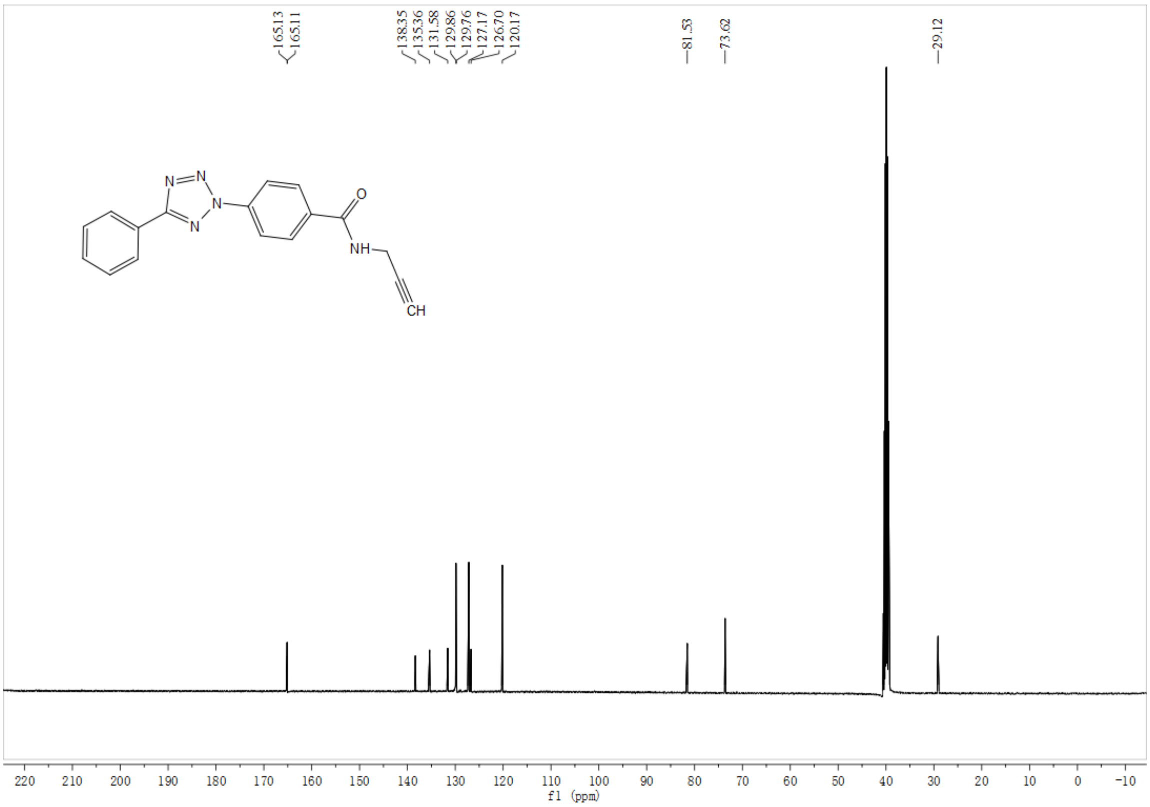

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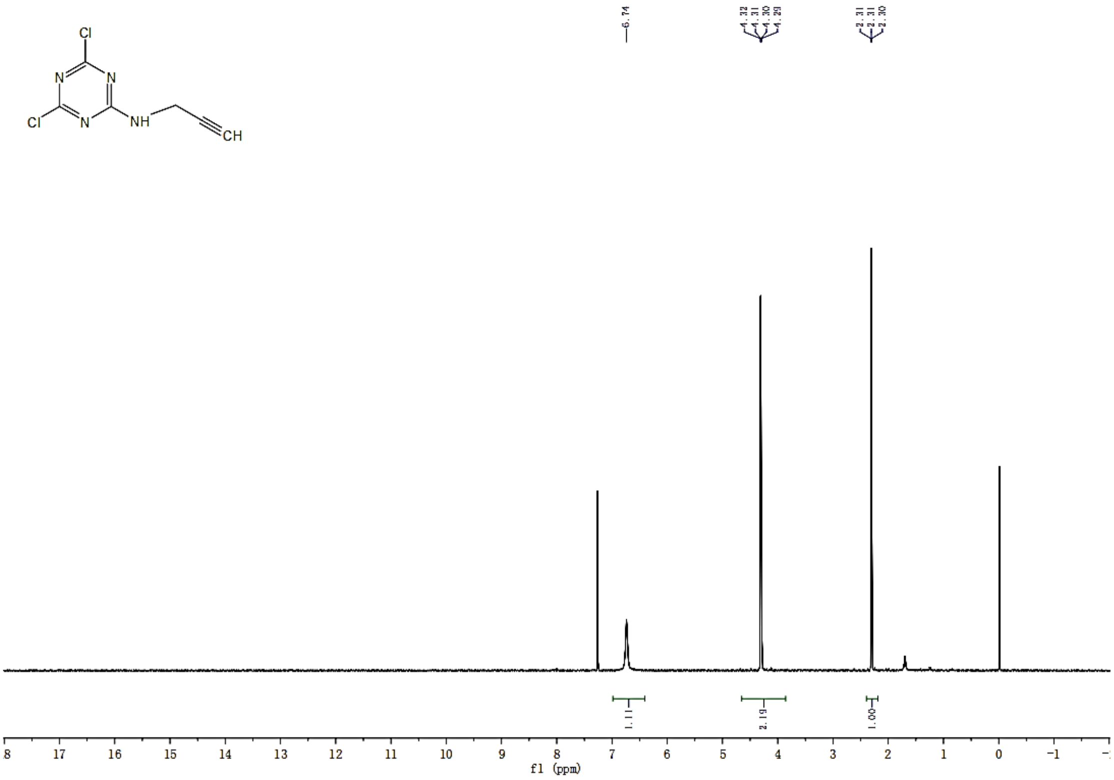

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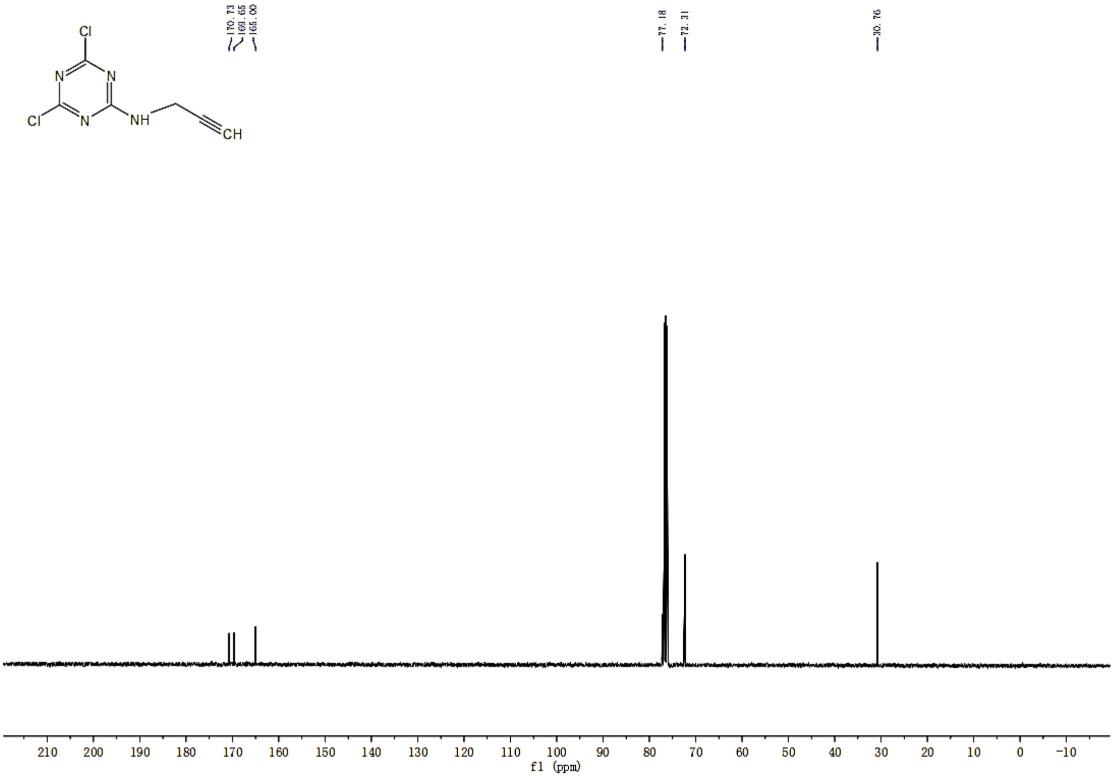

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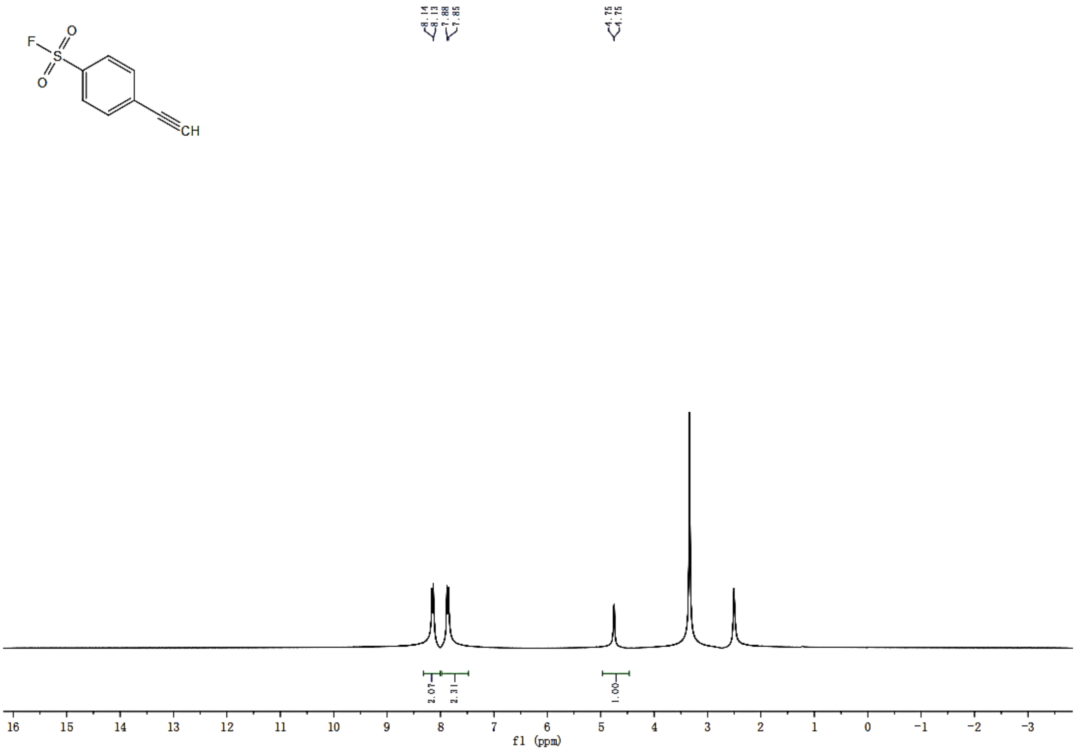

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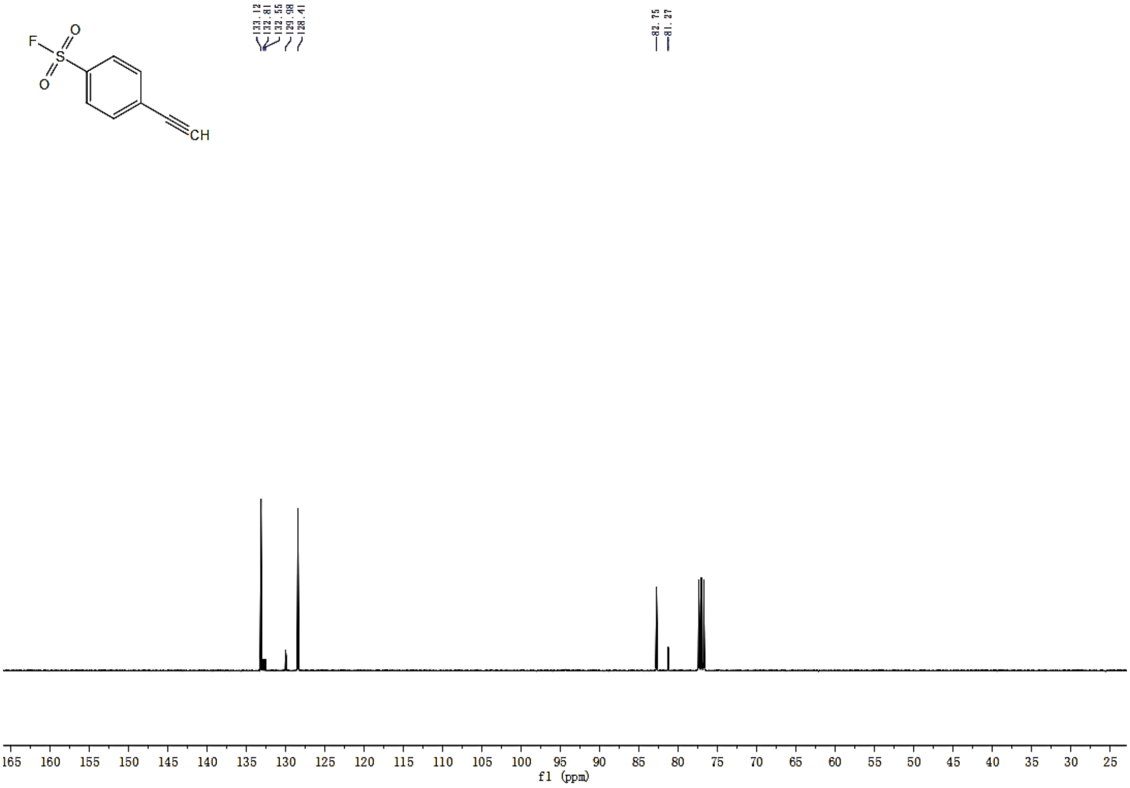

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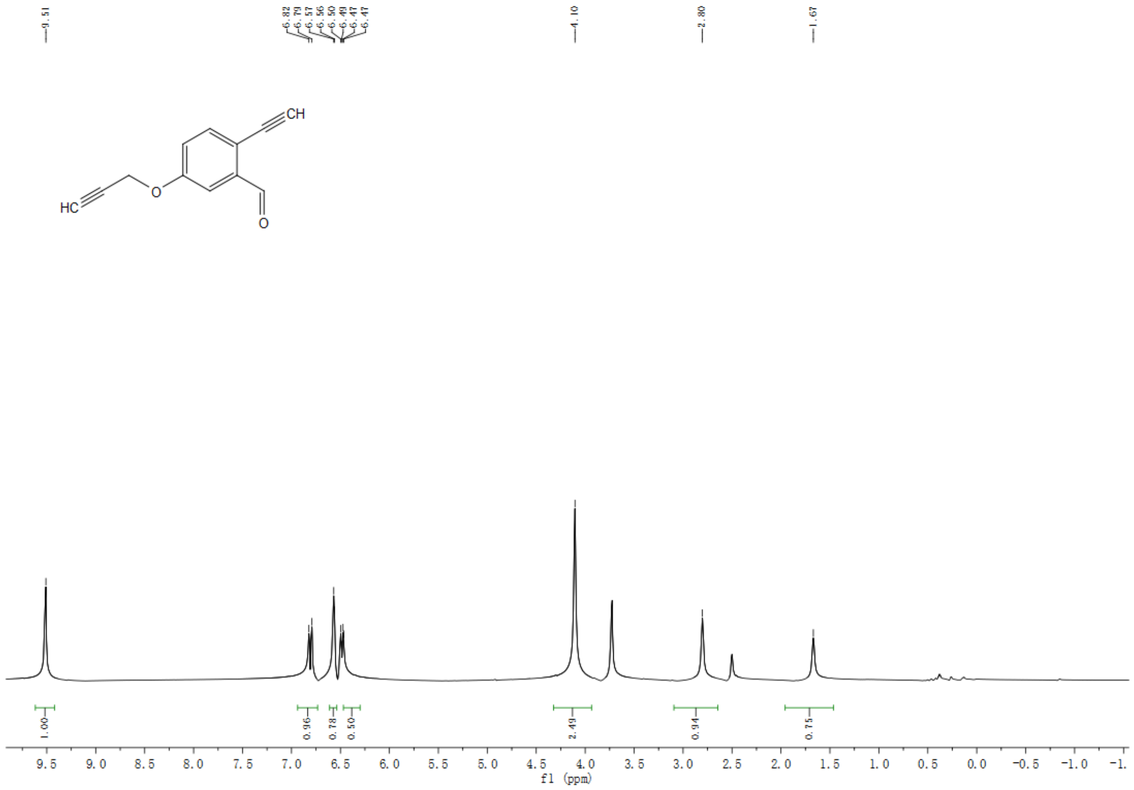

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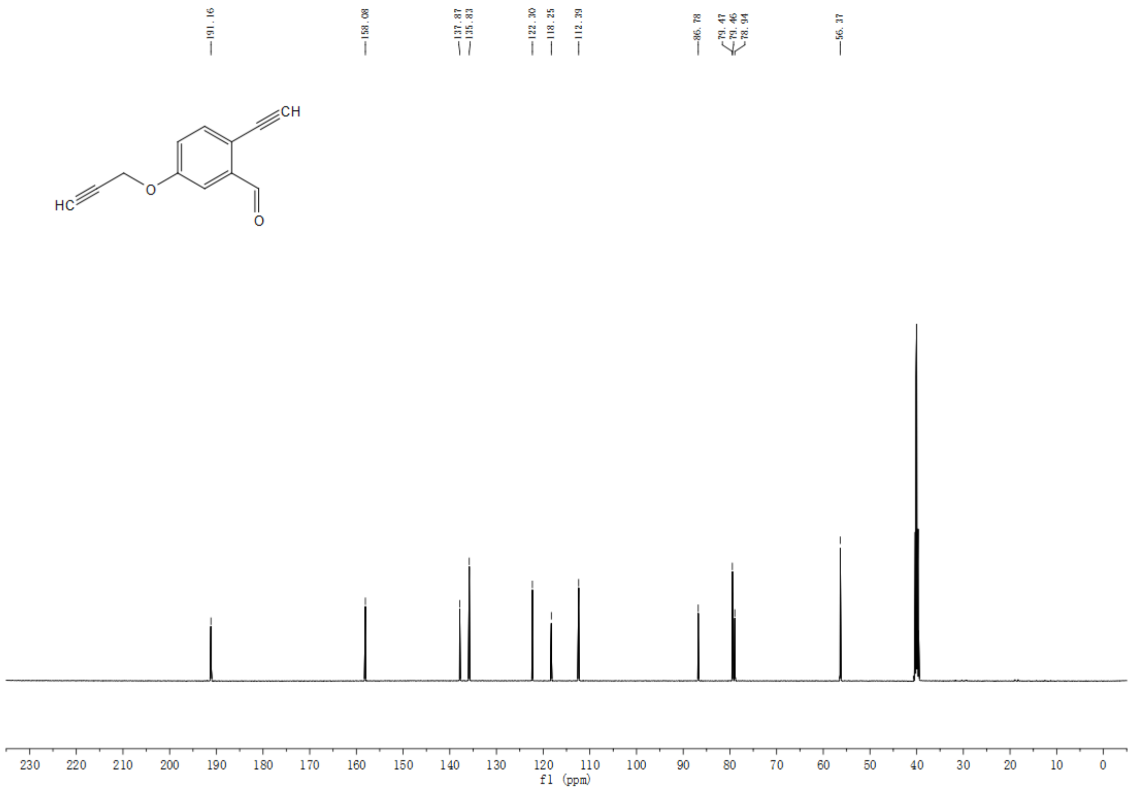
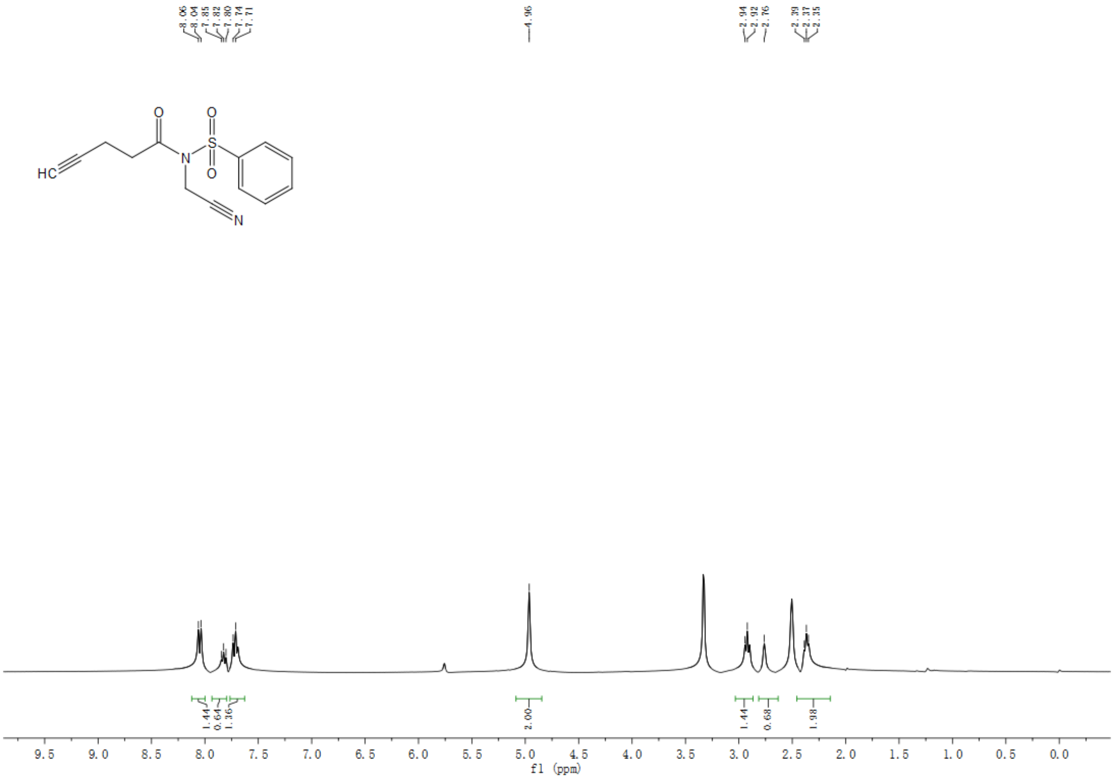


# 33 $^1$H NMR



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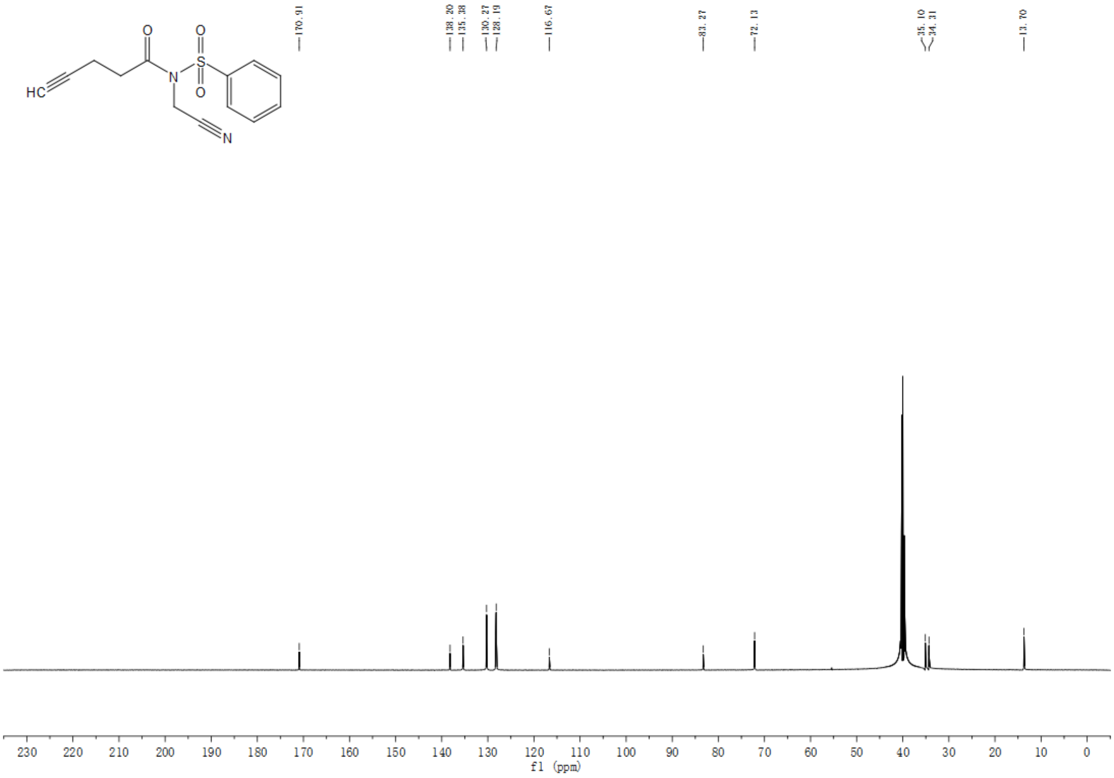

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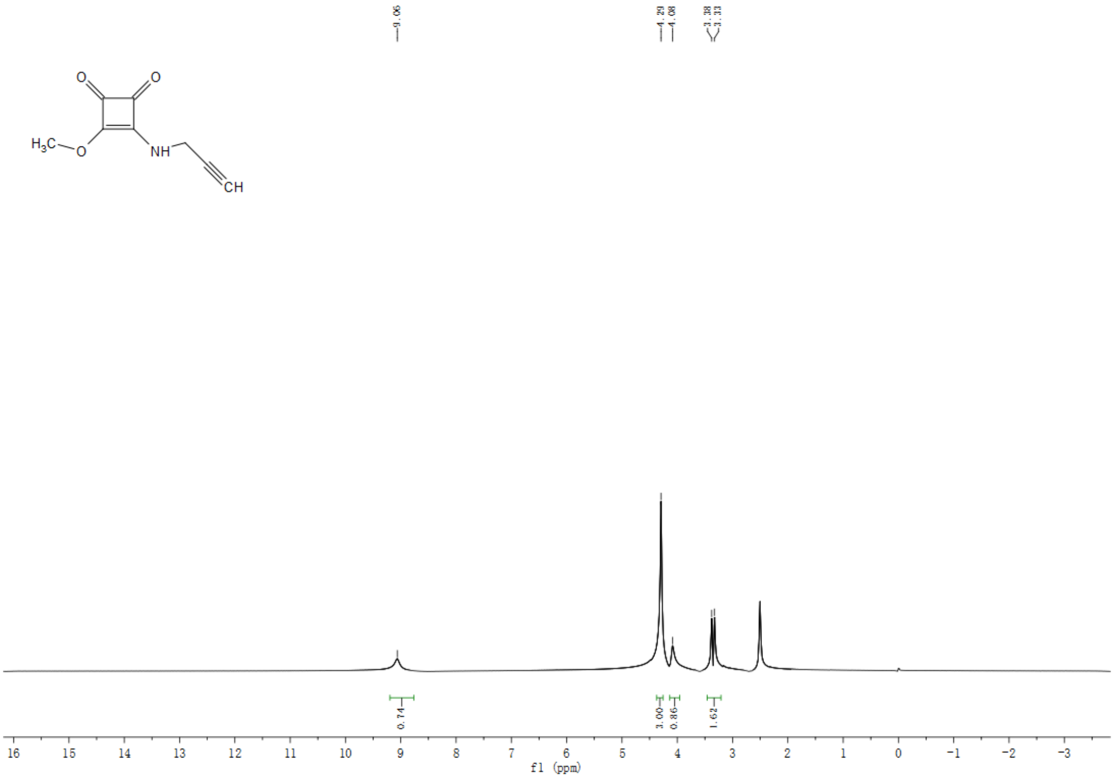

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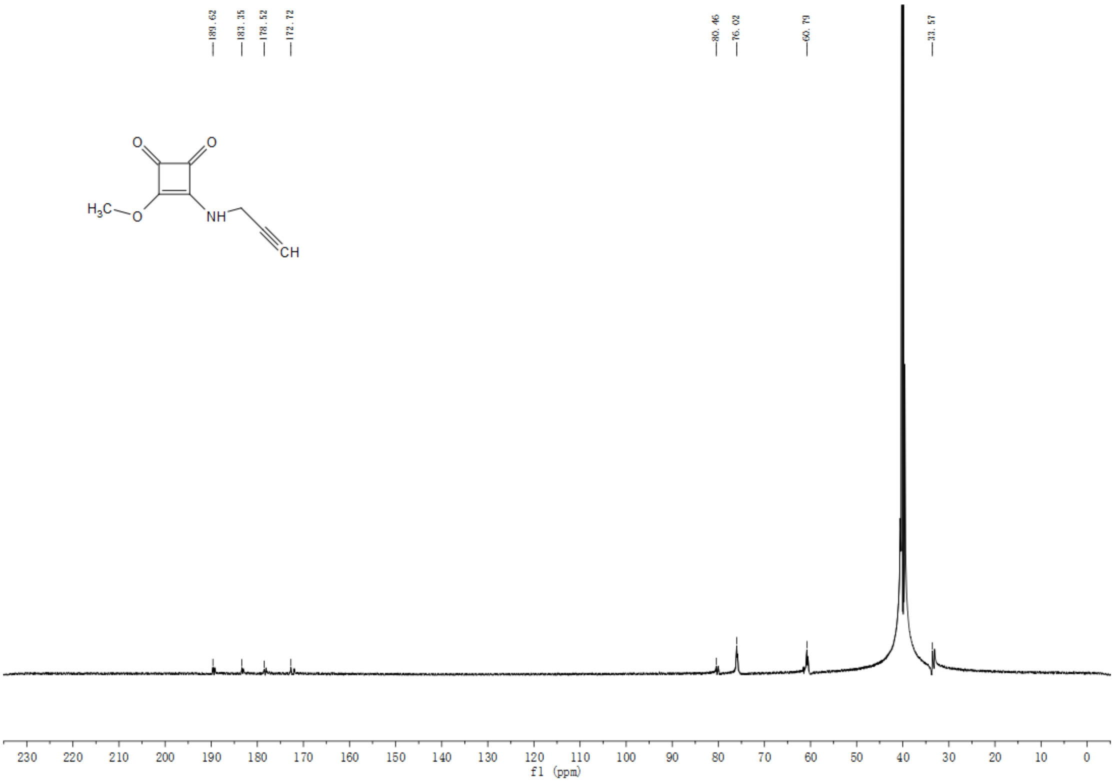

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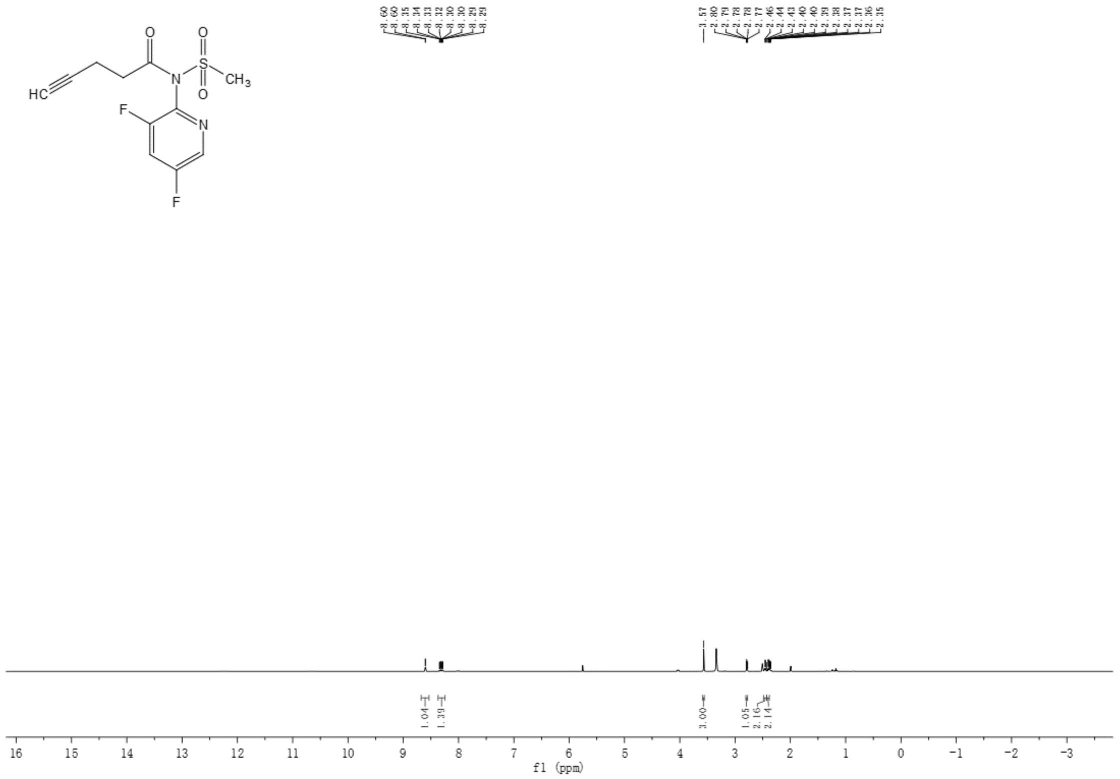

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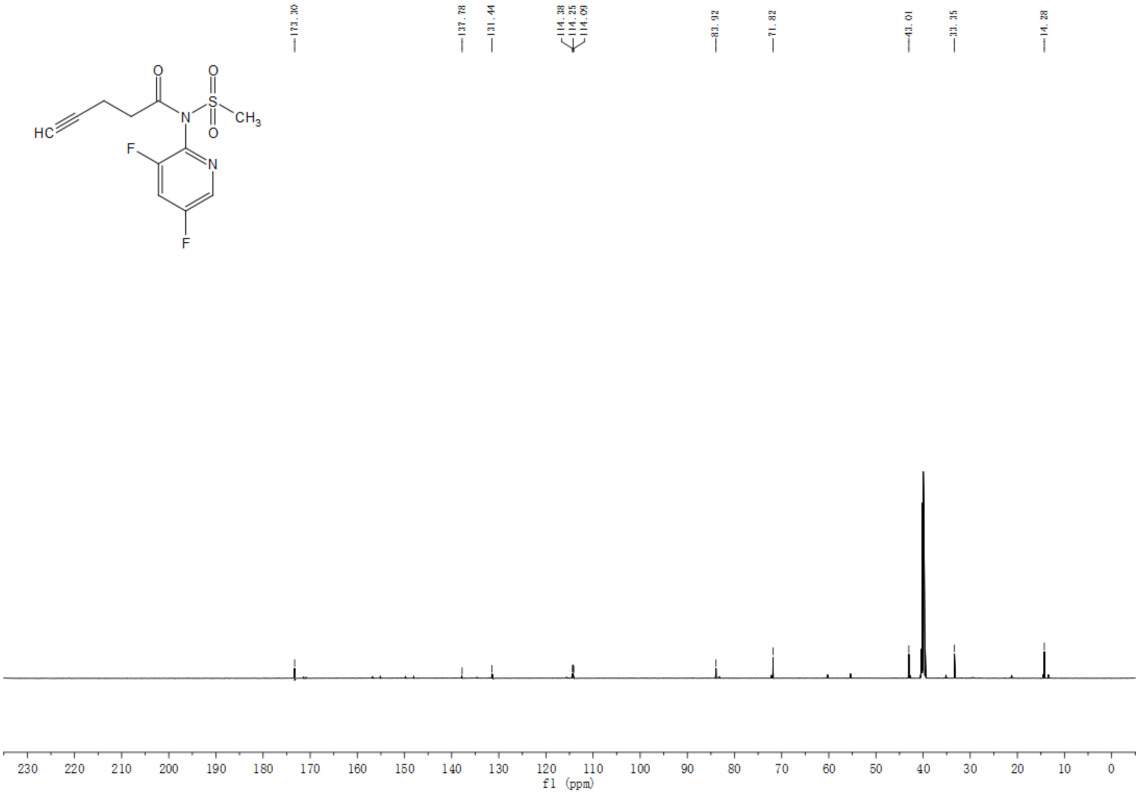

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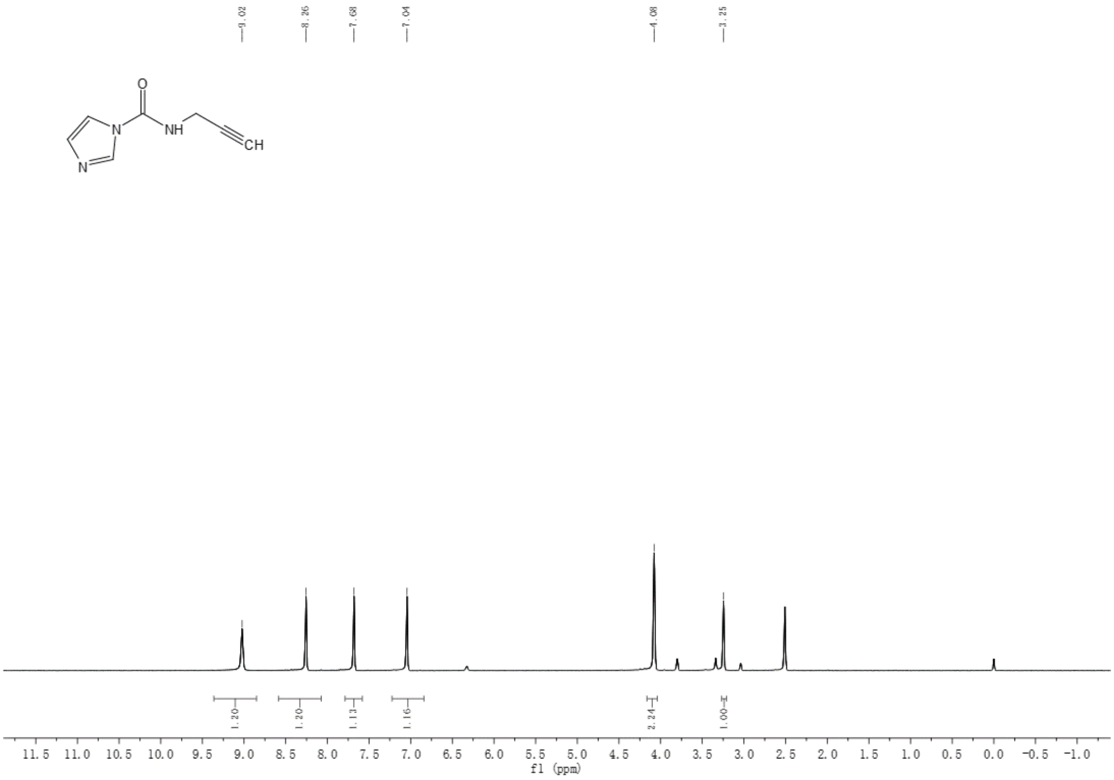

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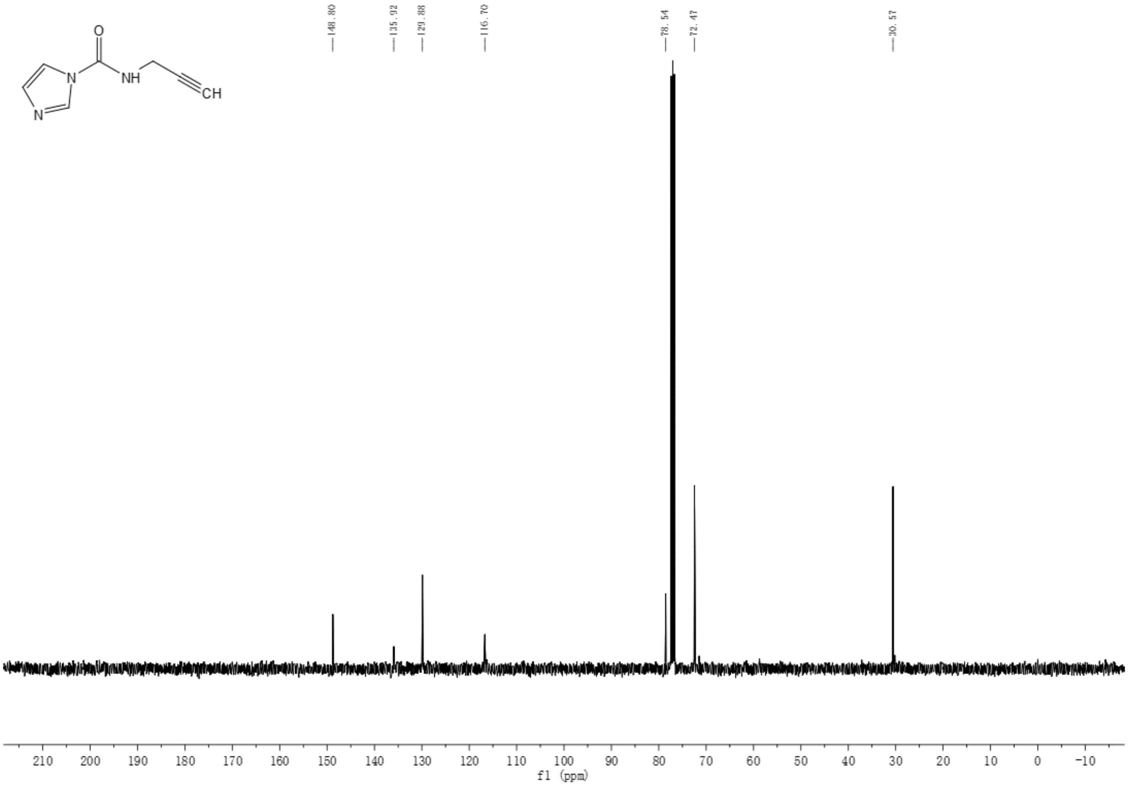

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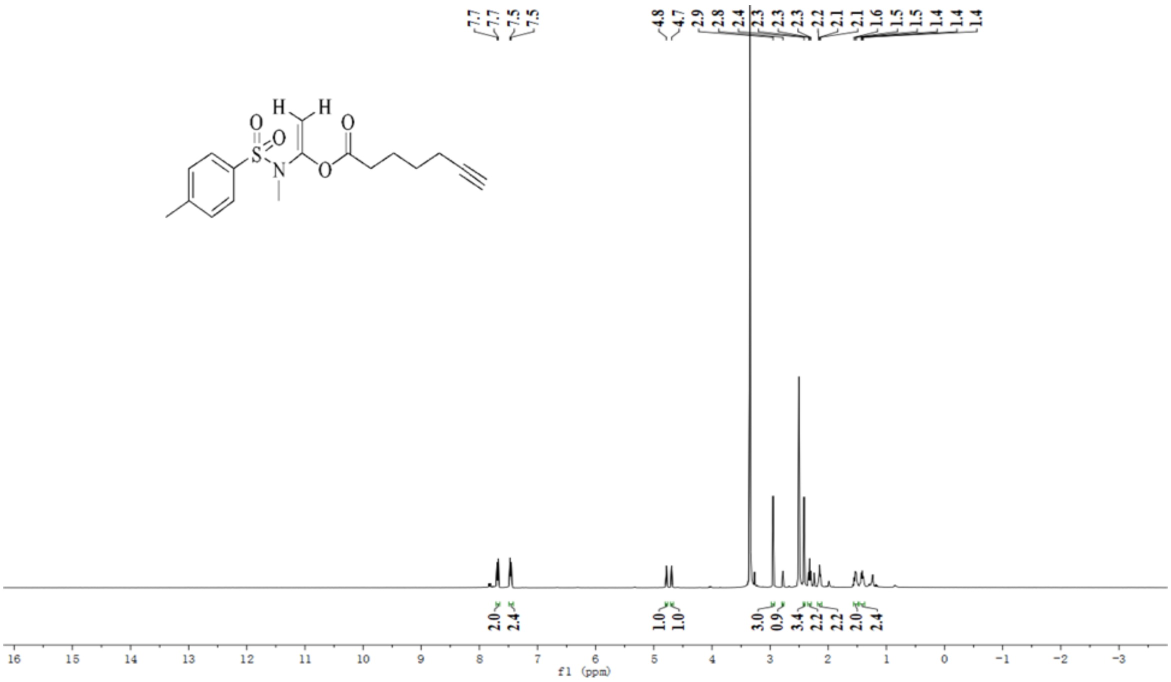

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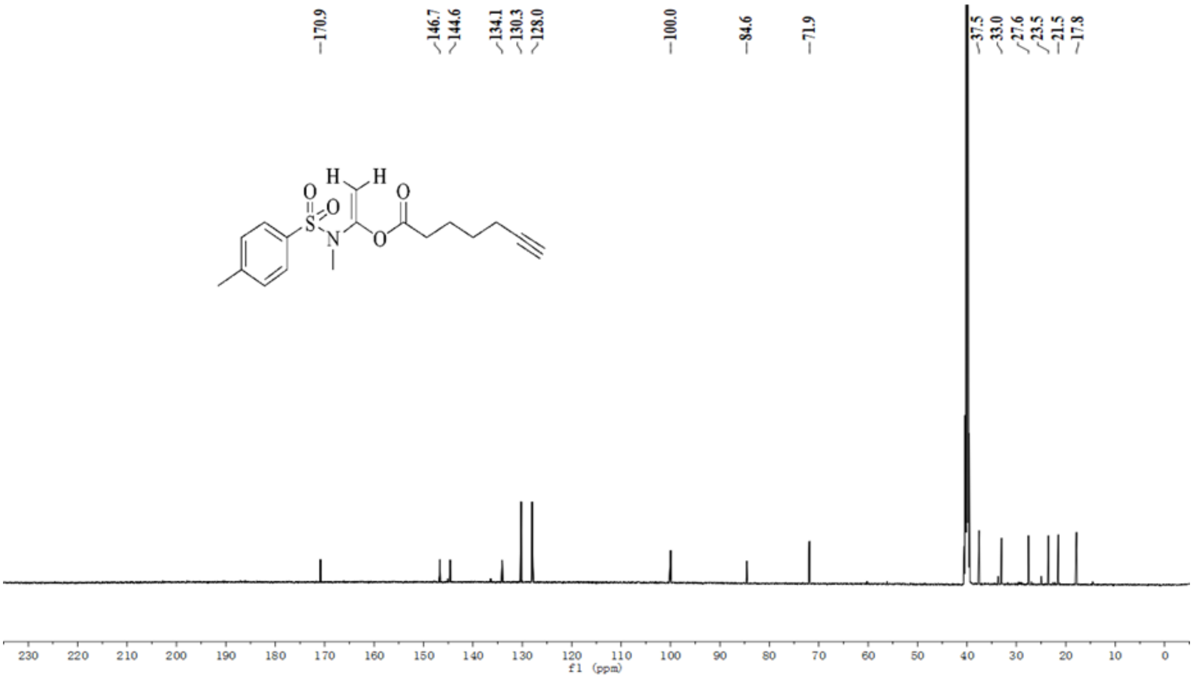

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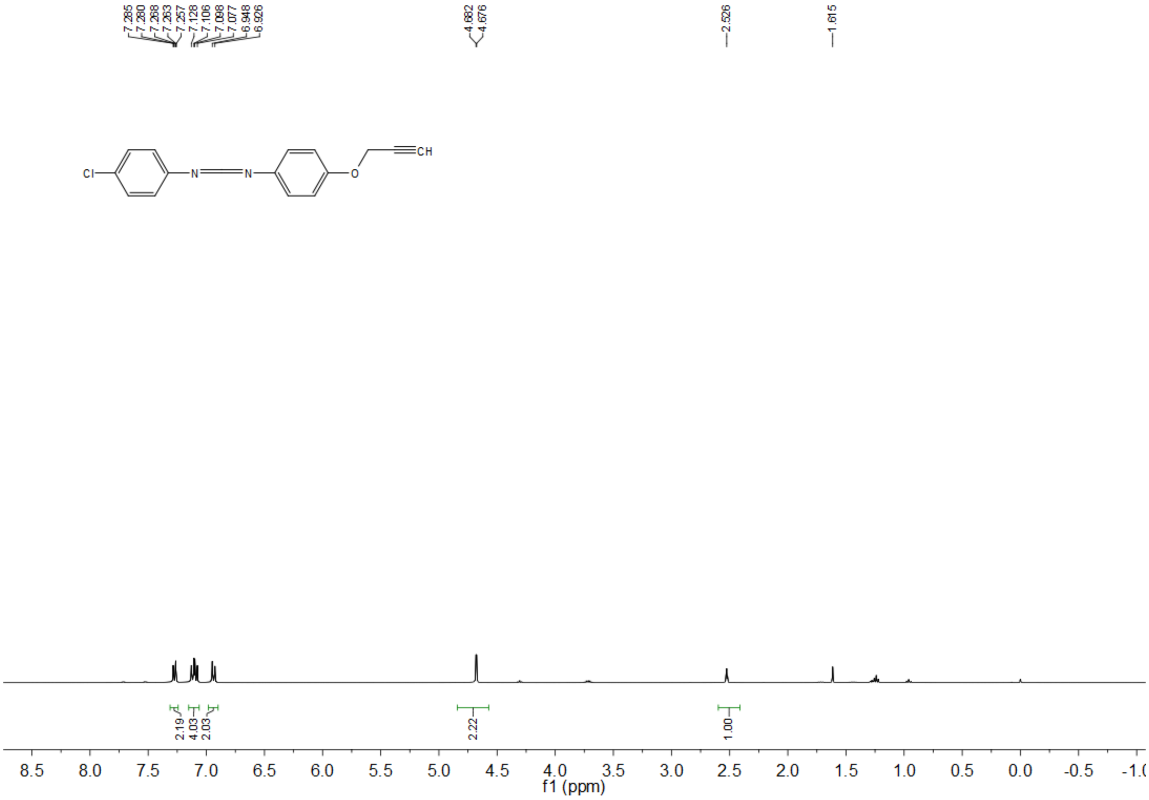

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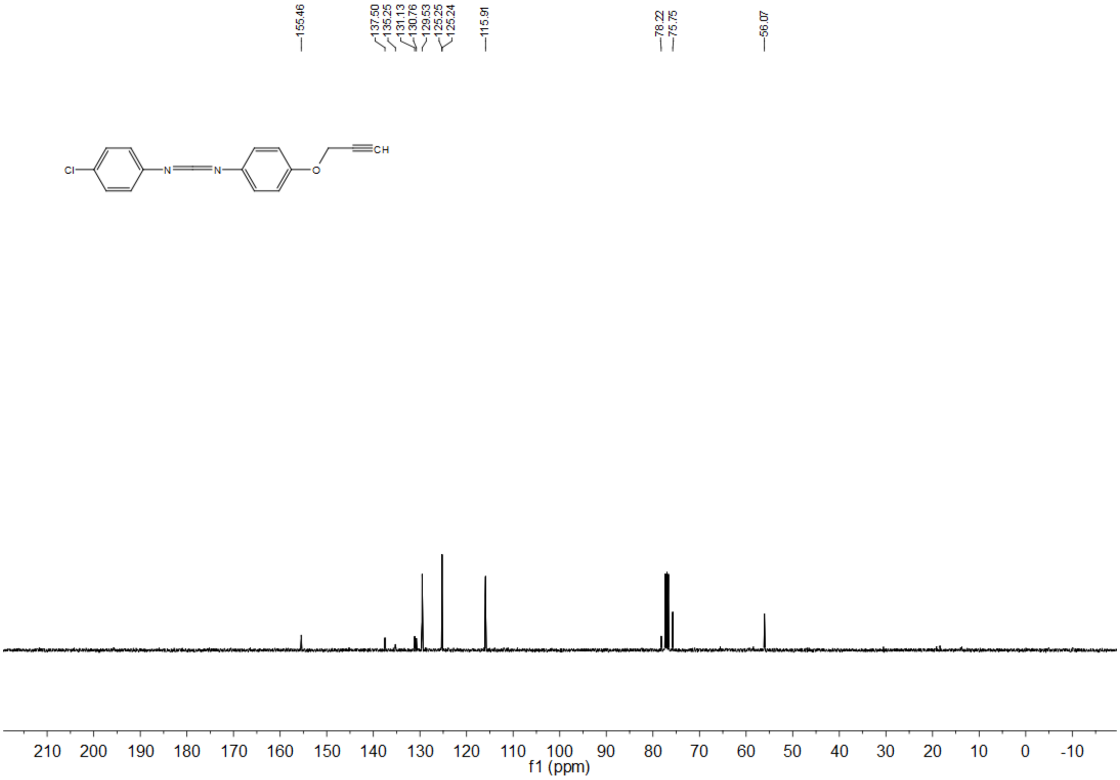

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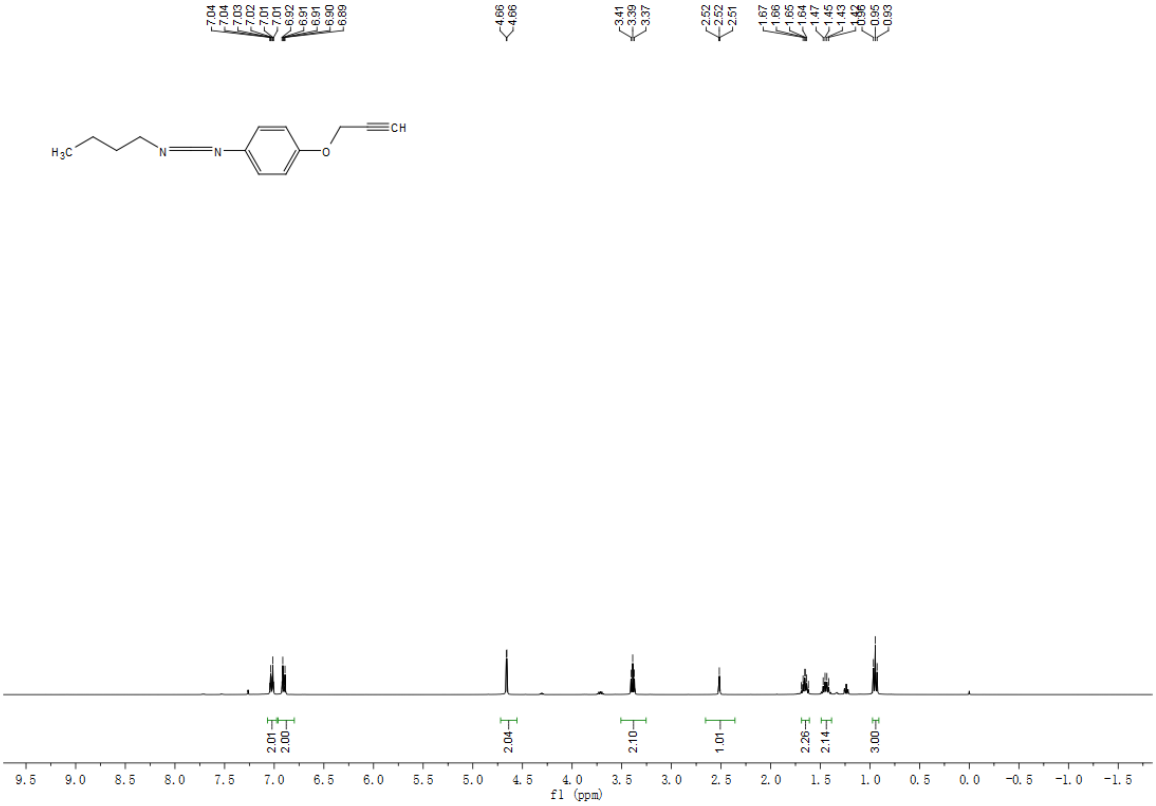

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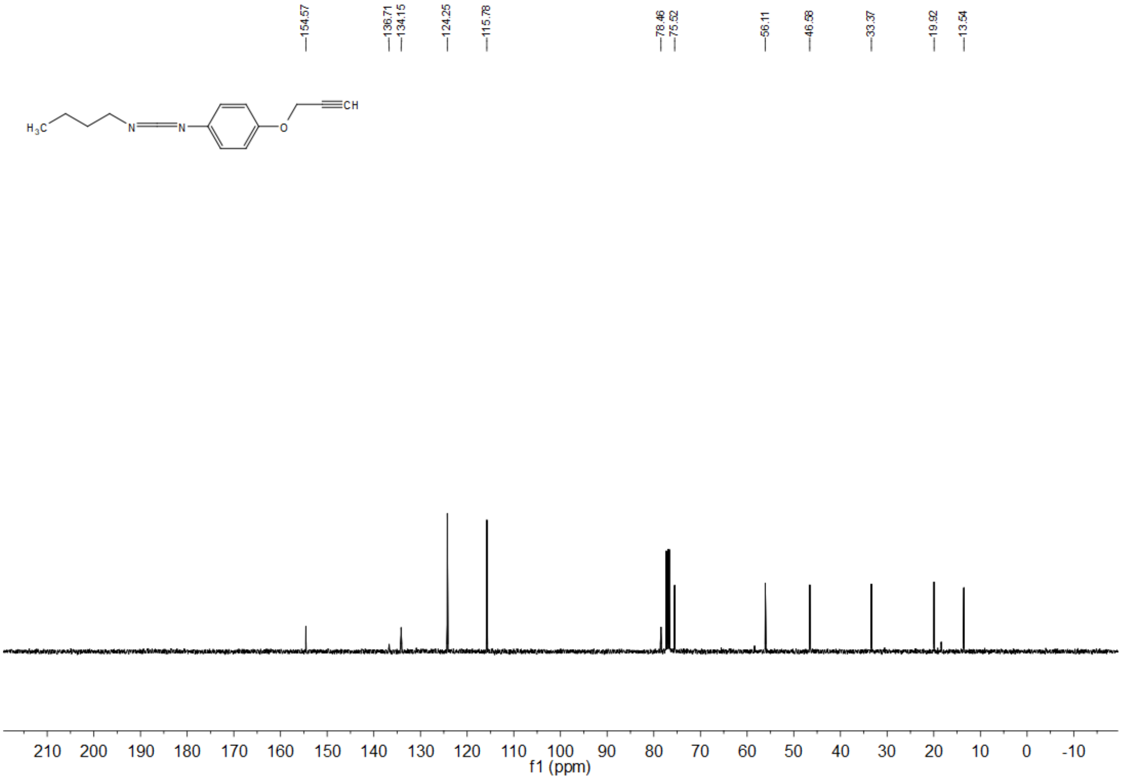

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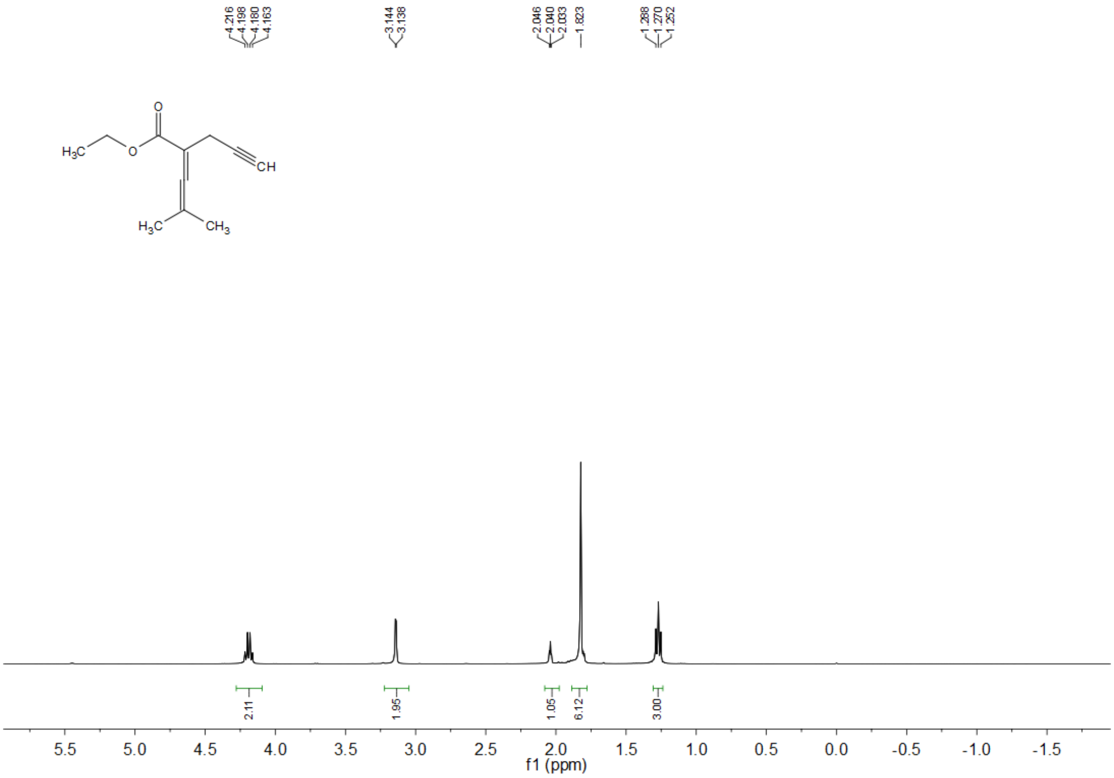

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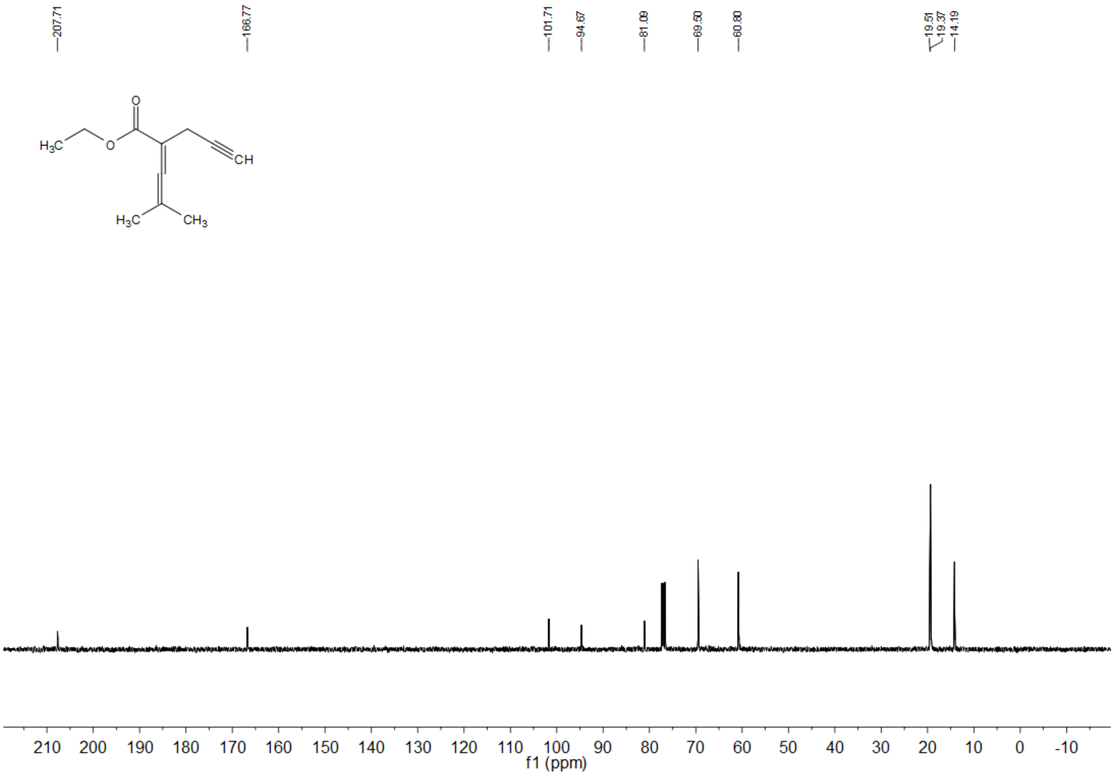

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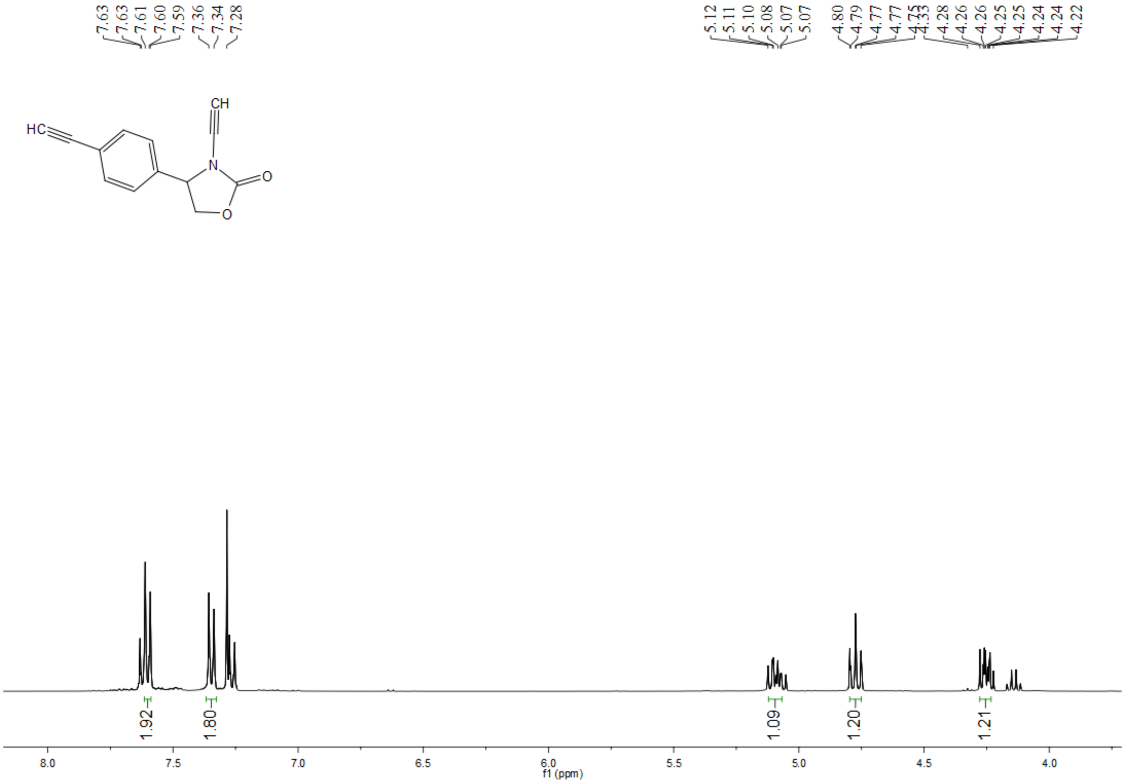

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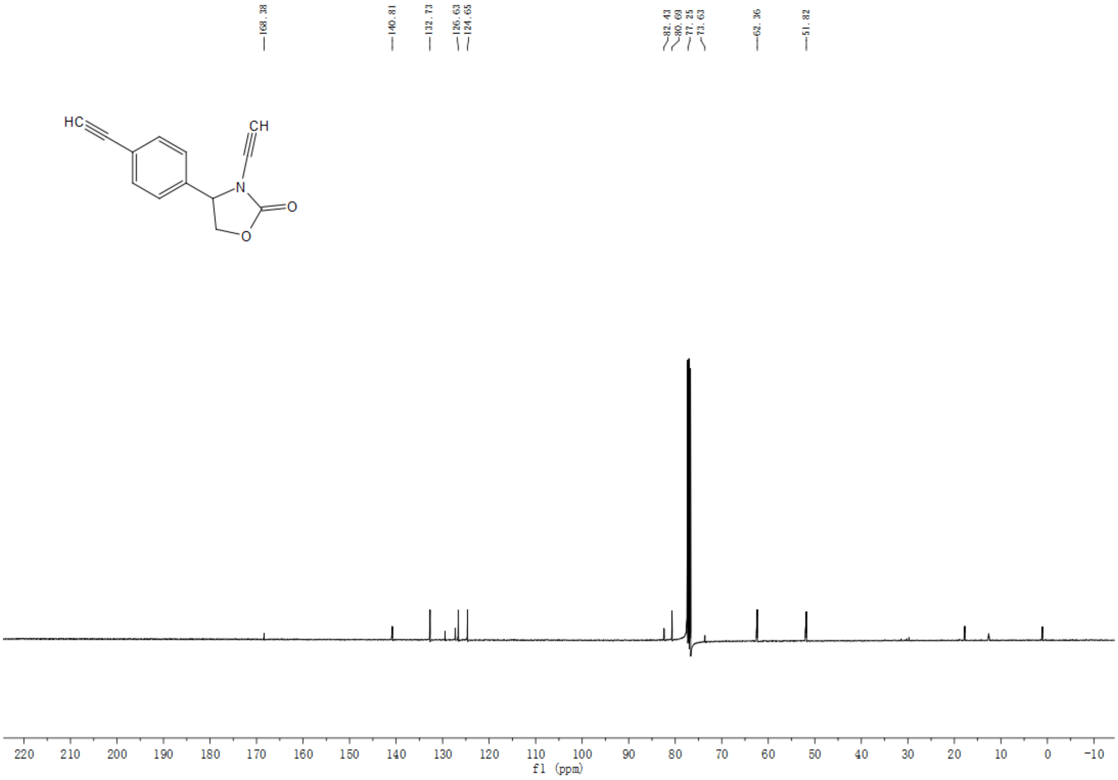

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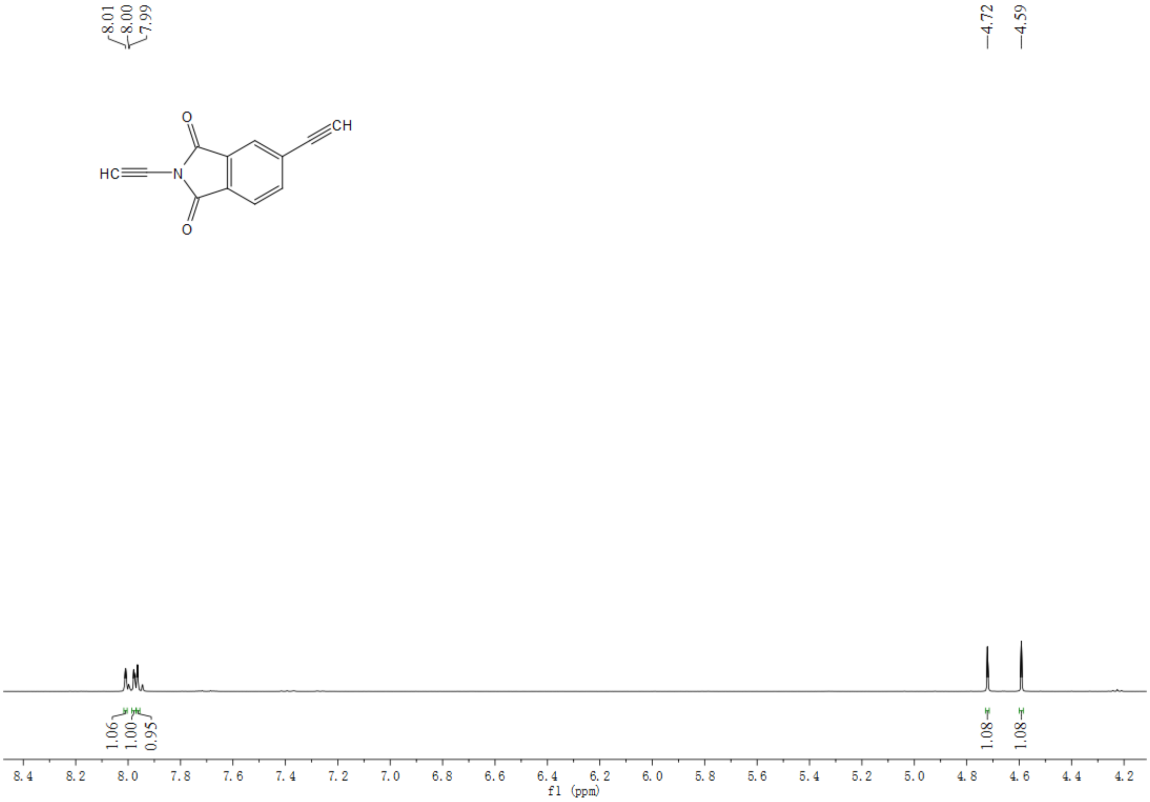

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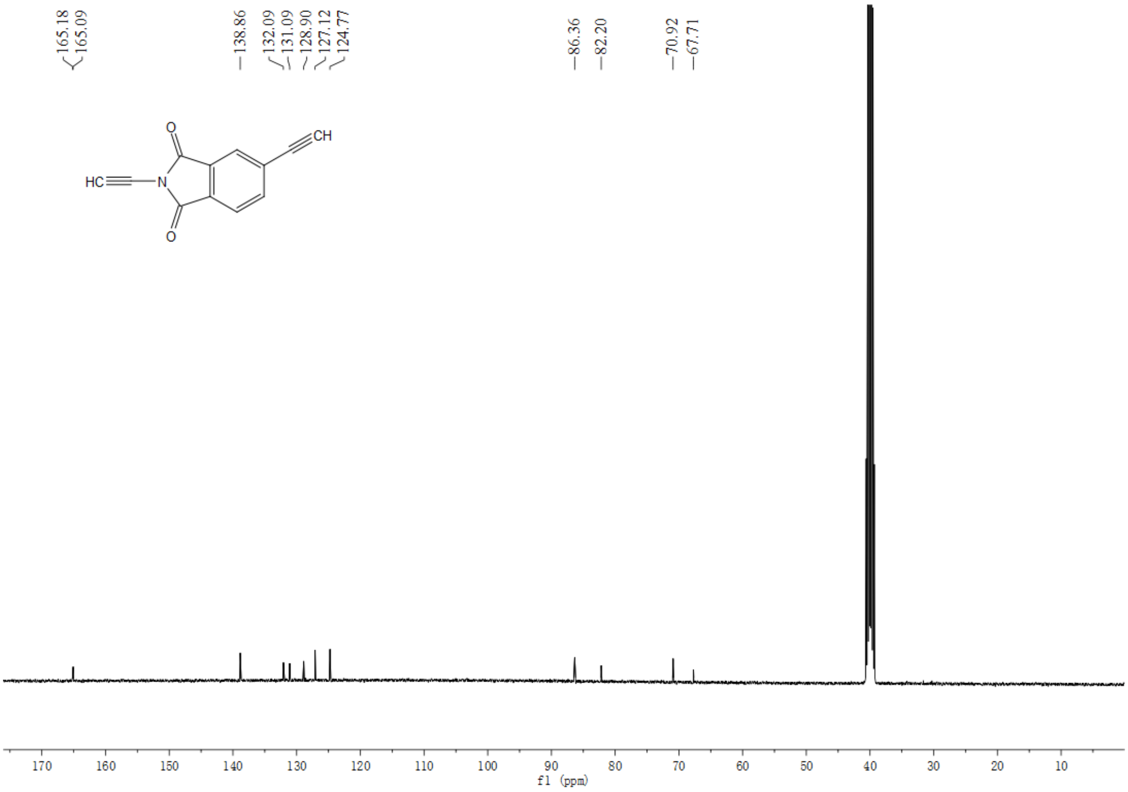

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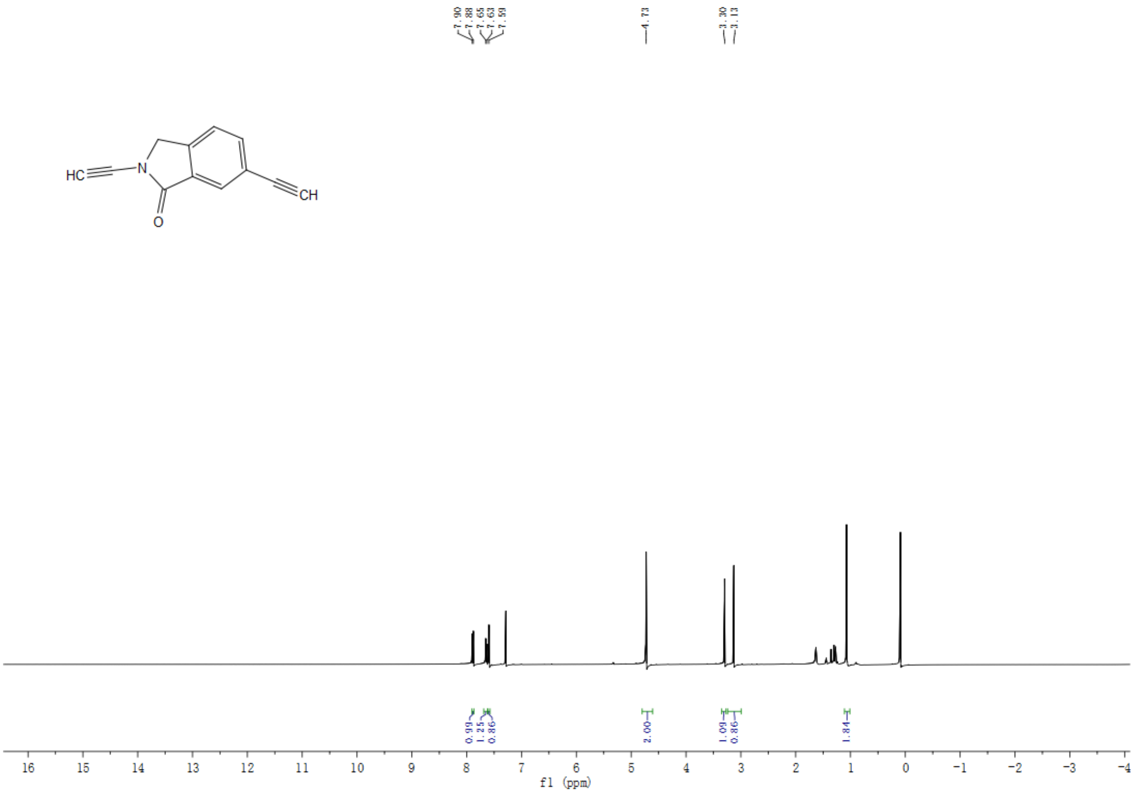

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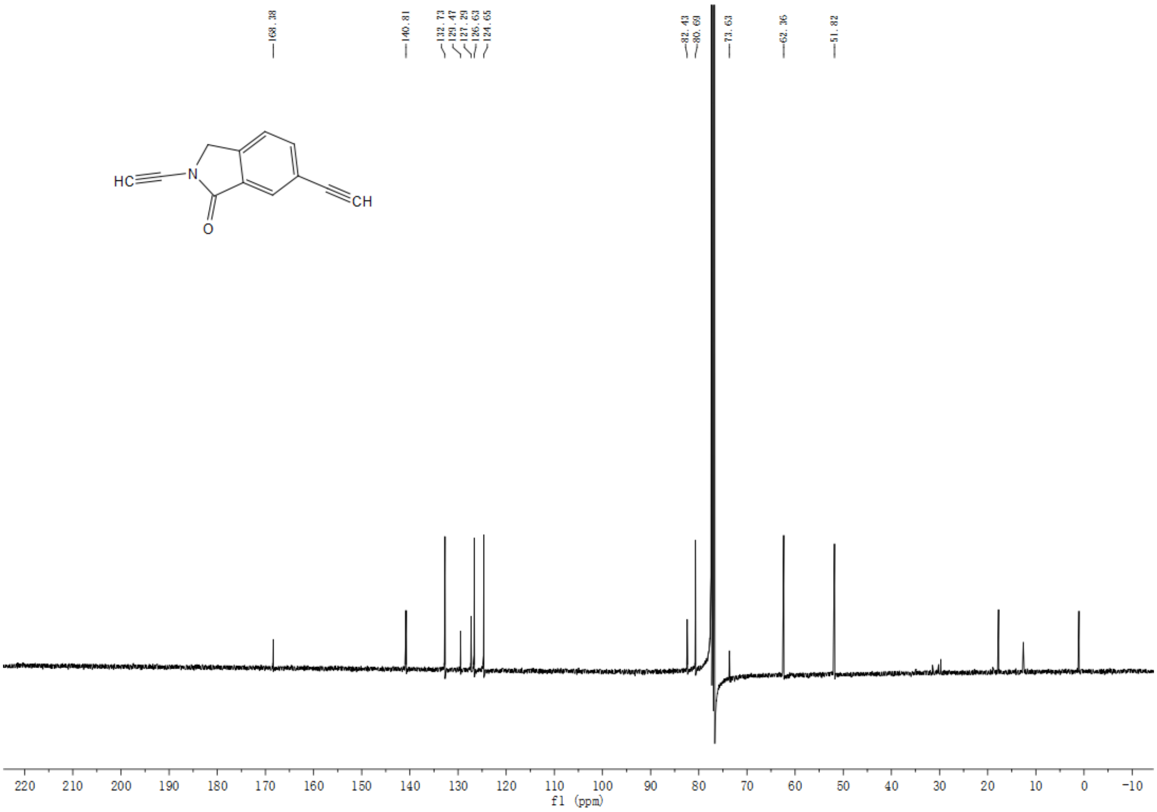

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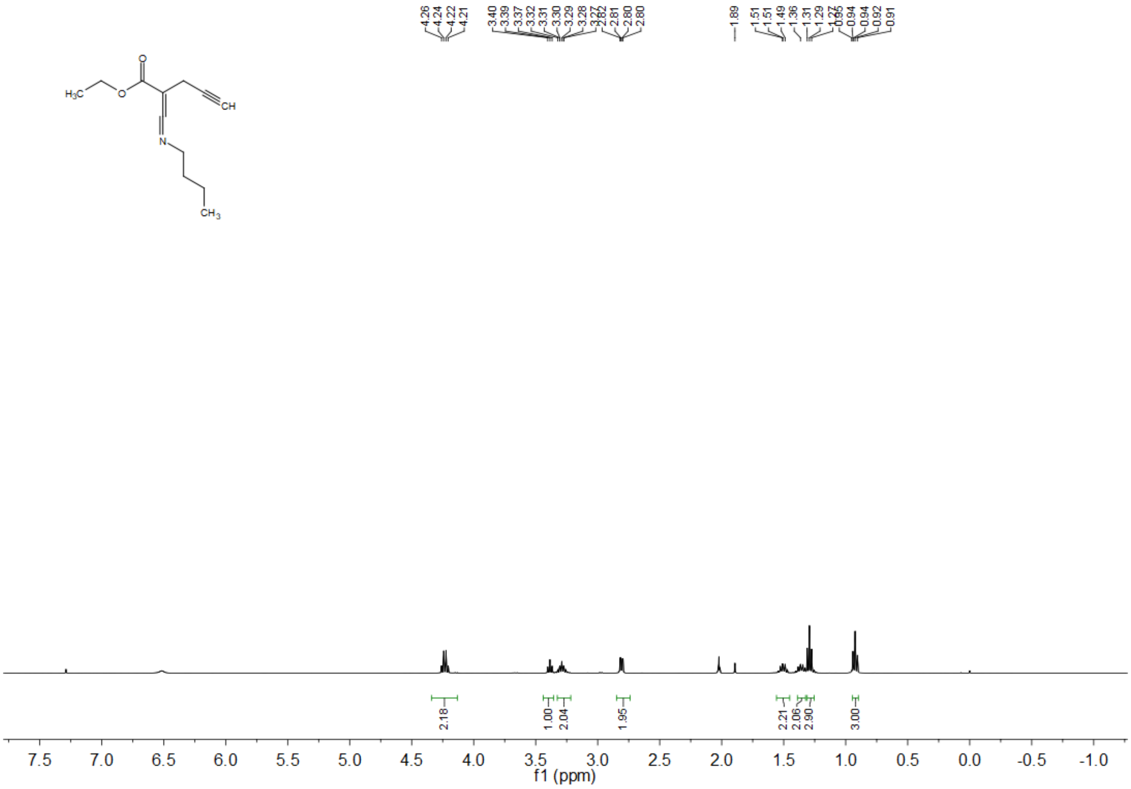

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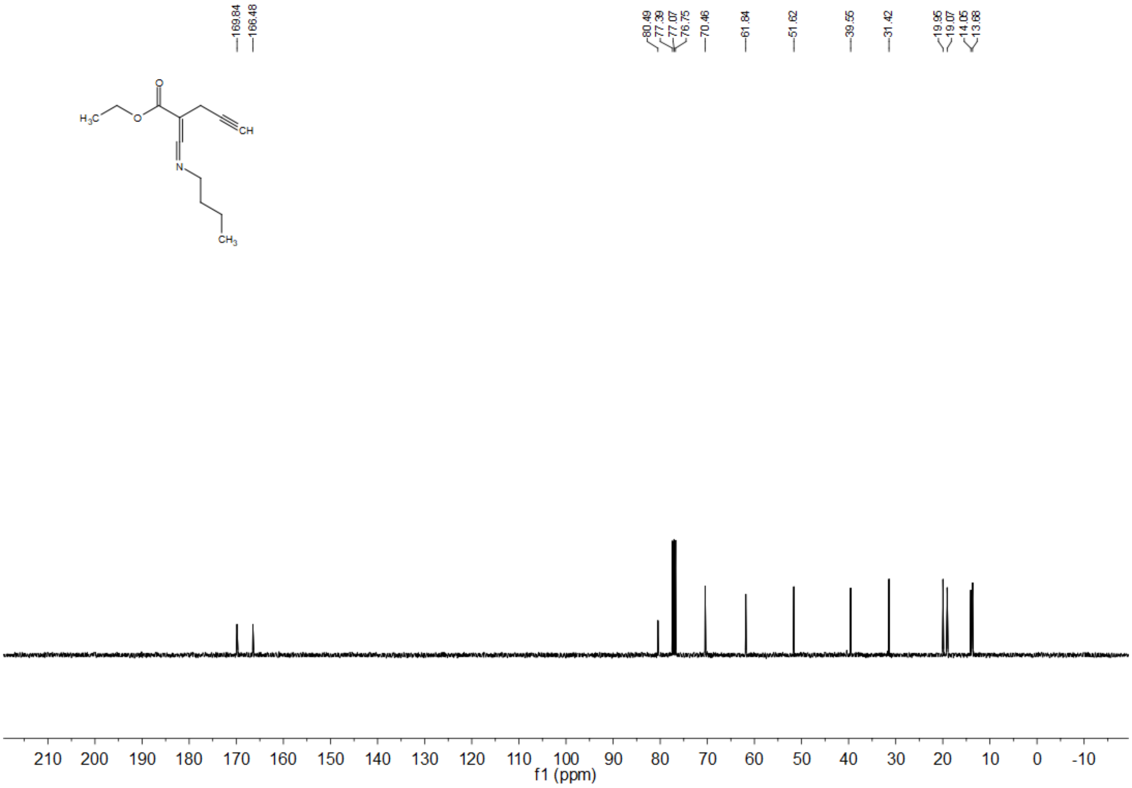

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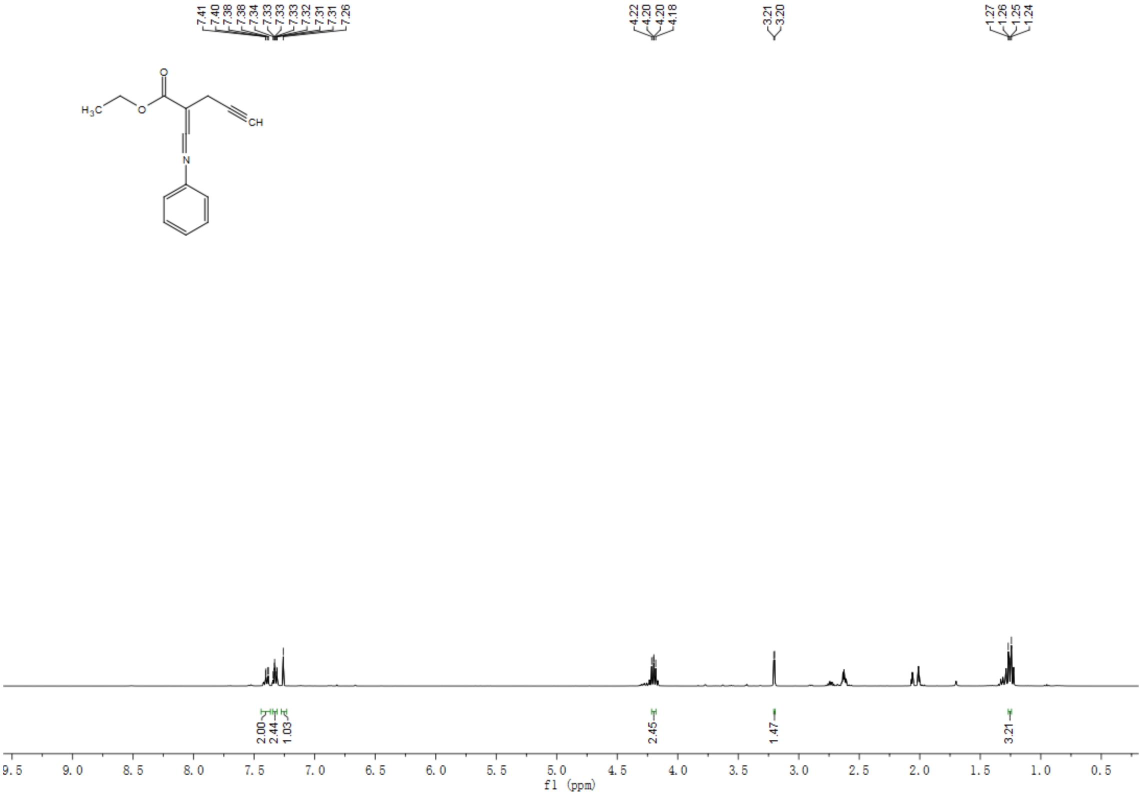

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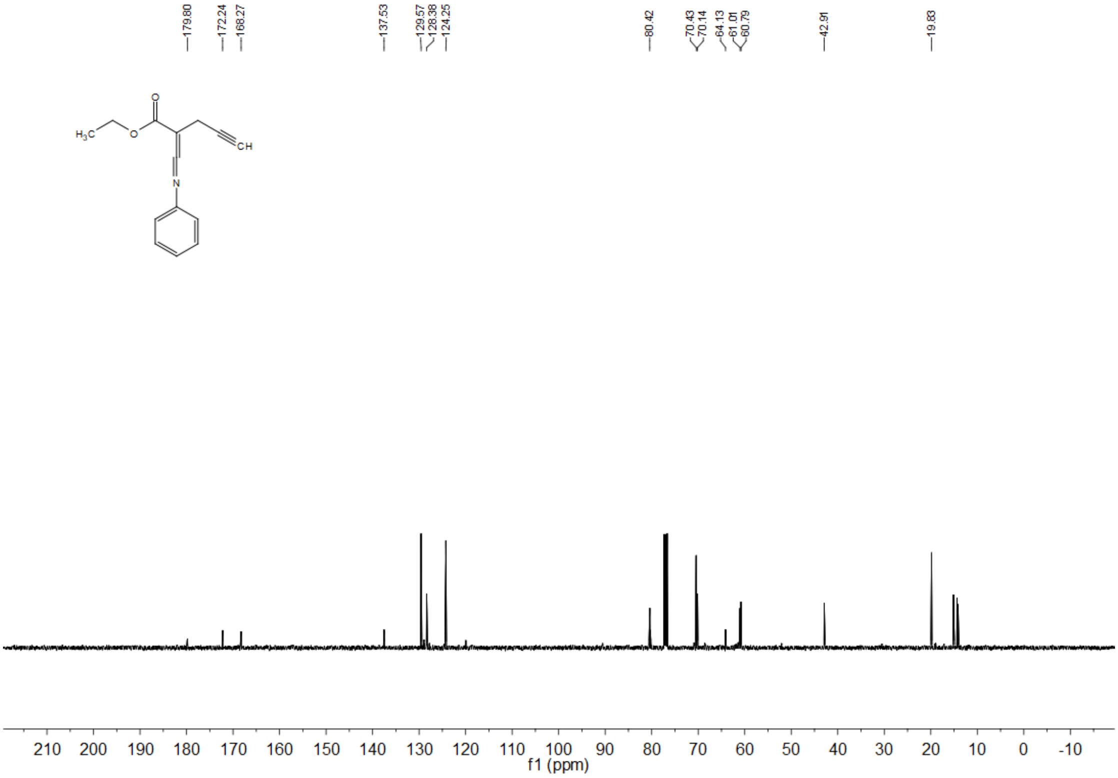

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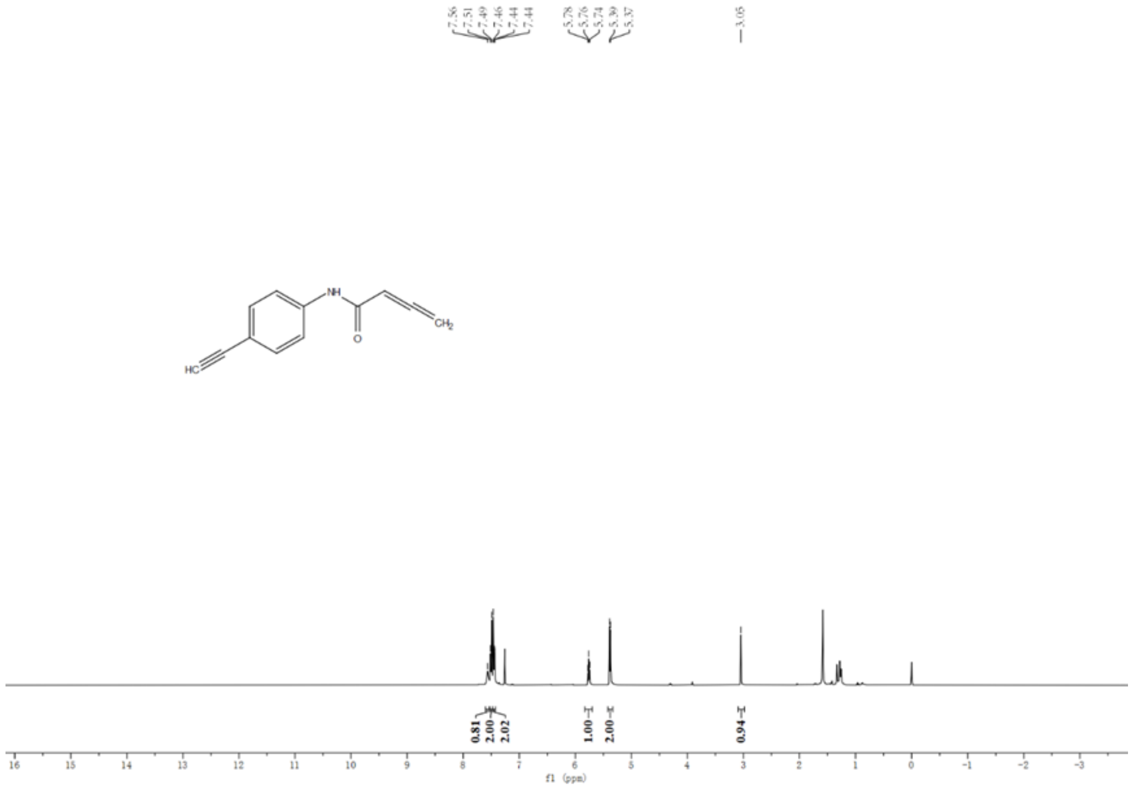

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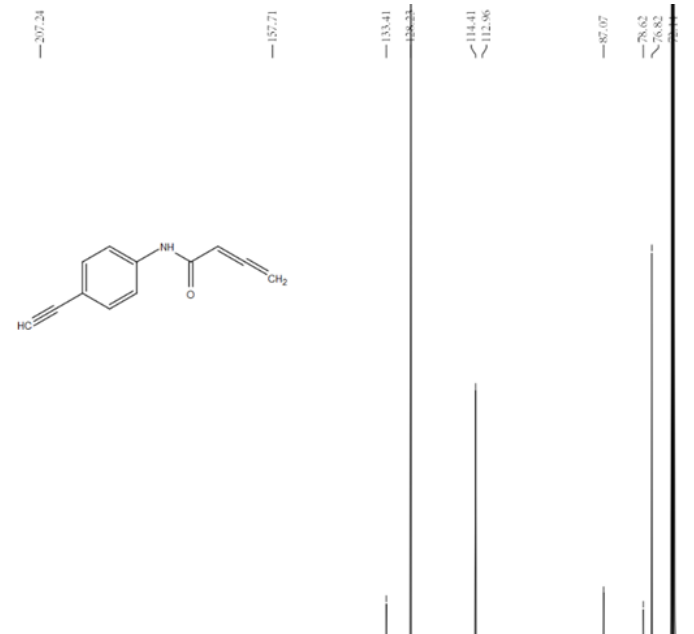

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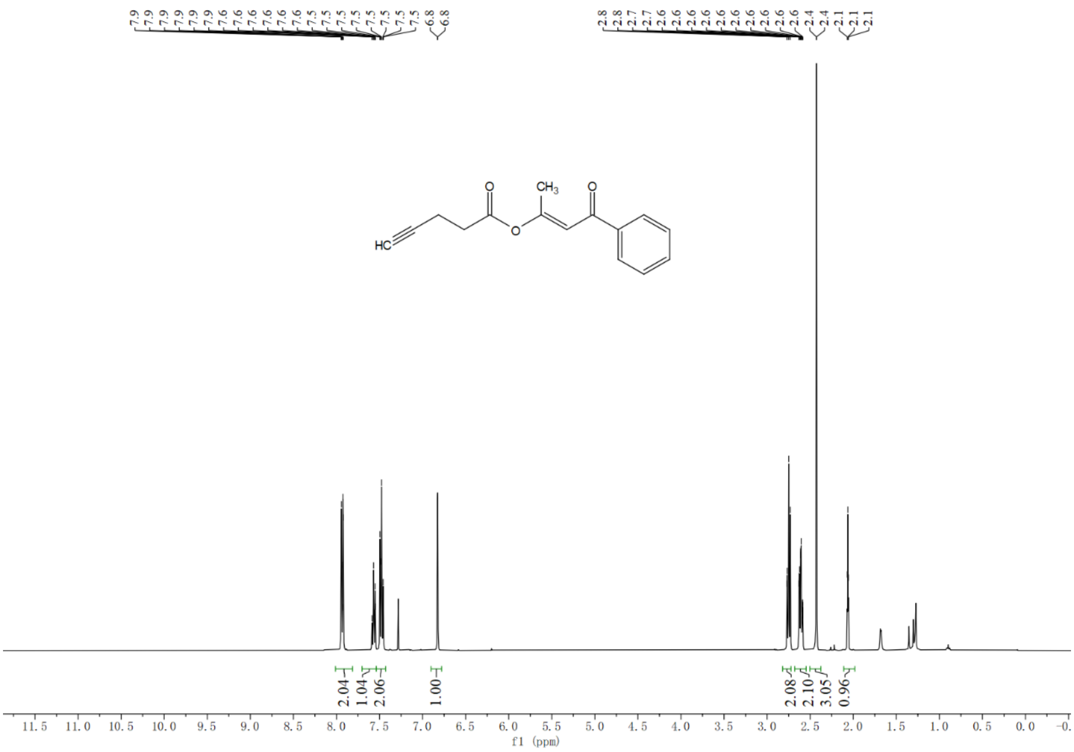
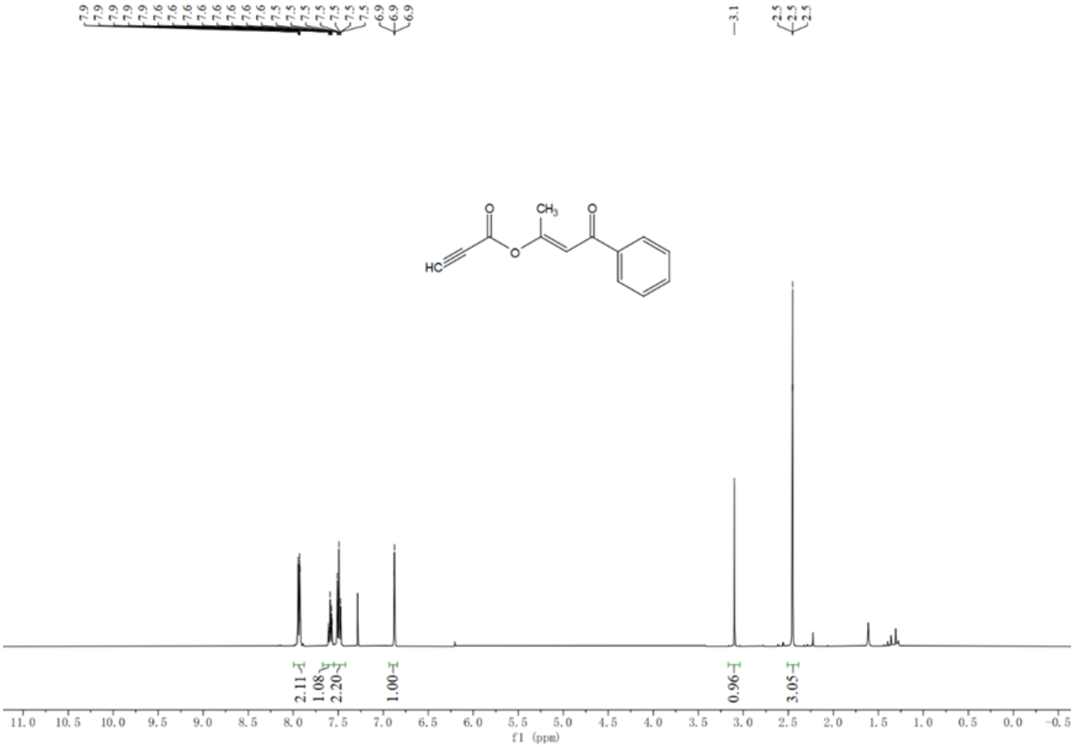


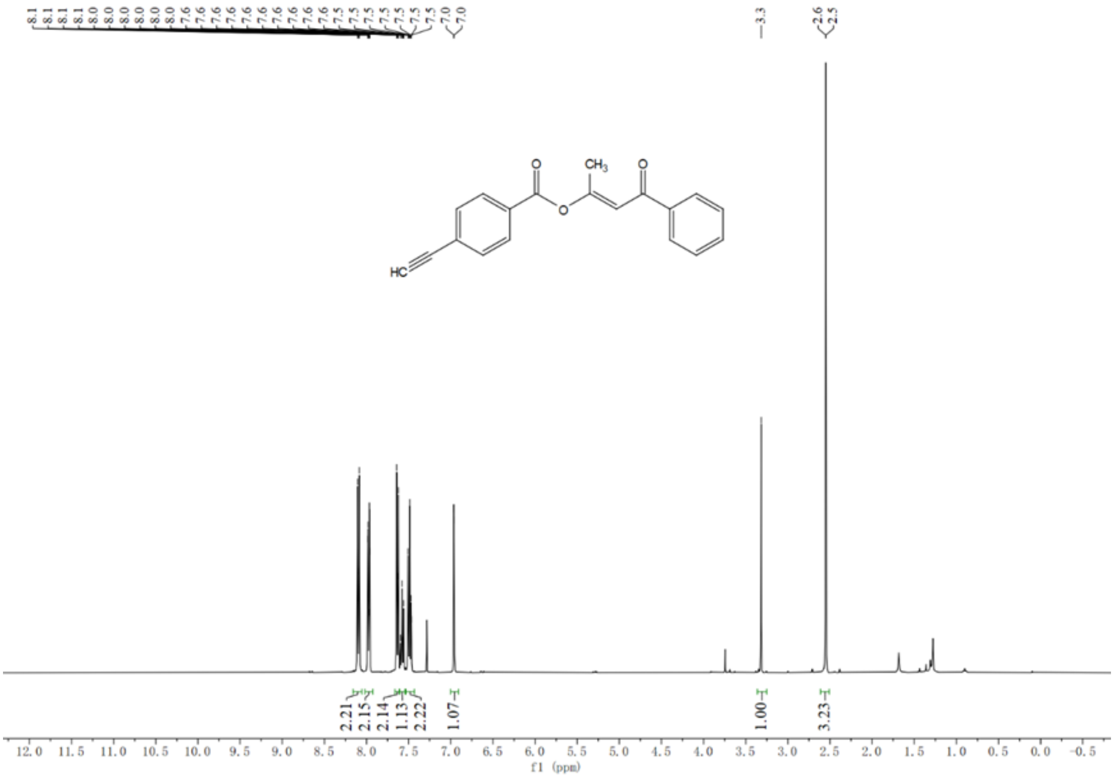
## 47 $^{13}$C NMR

## 48 $^1$H NMR



## 48 $^{13}$C NMR

## 49 $^{1}$H NMR



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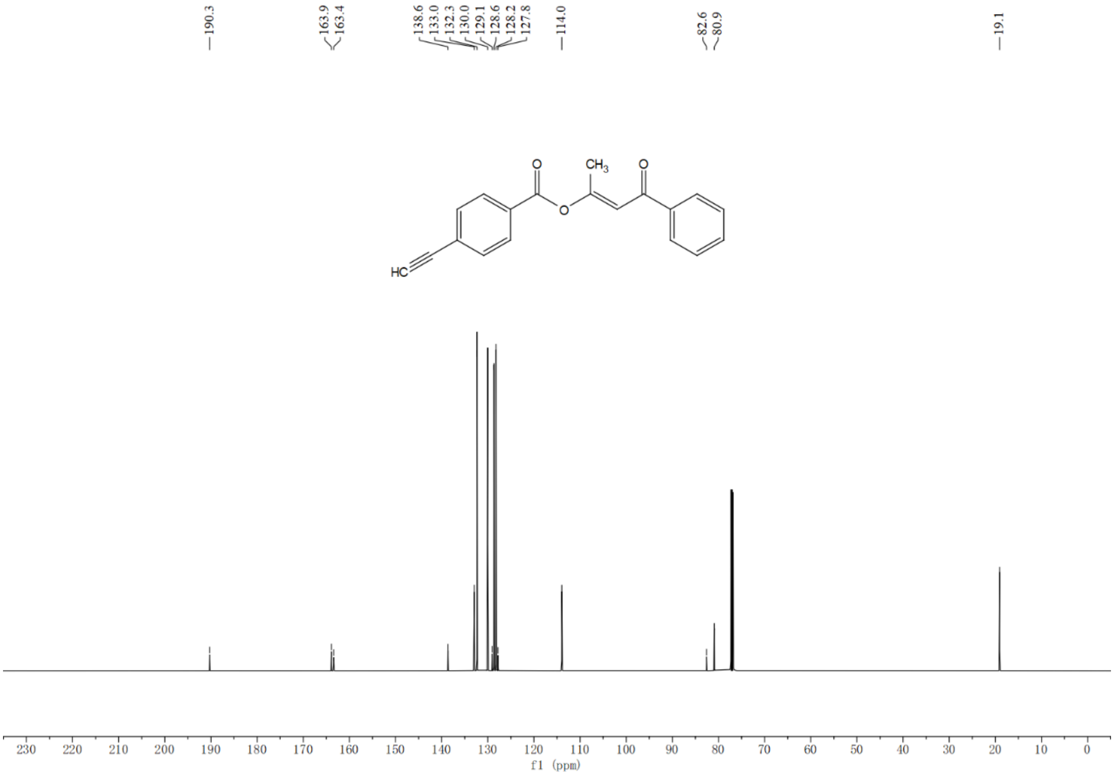

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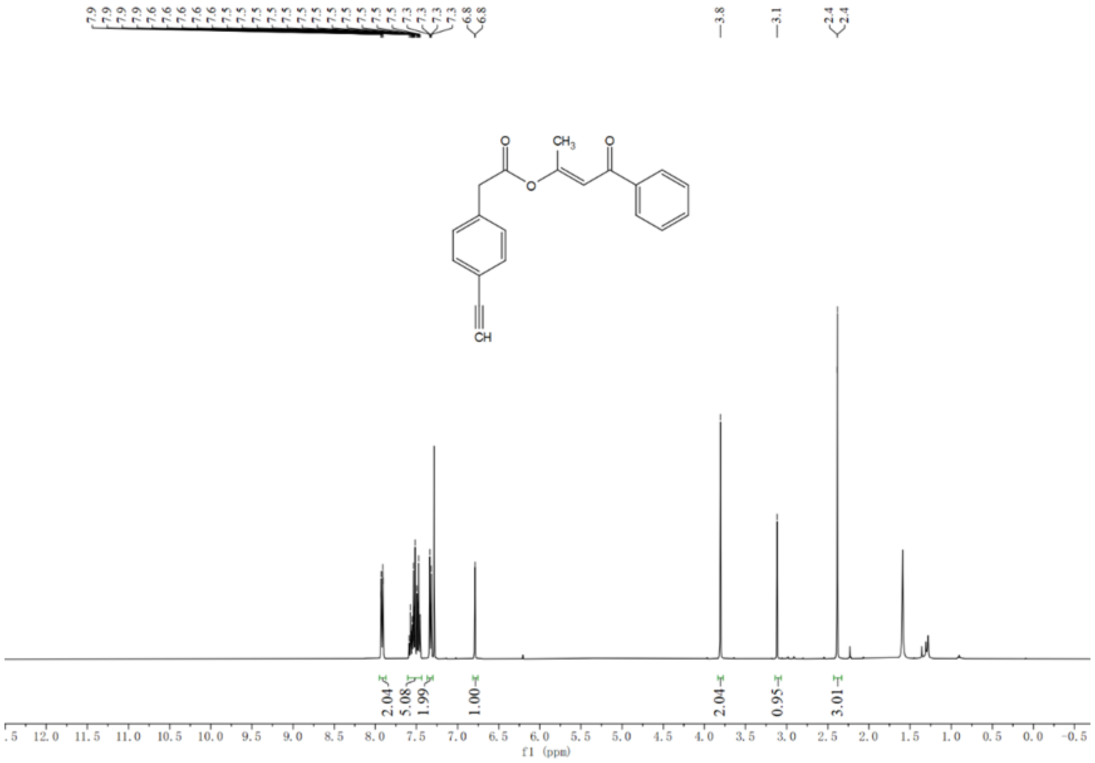

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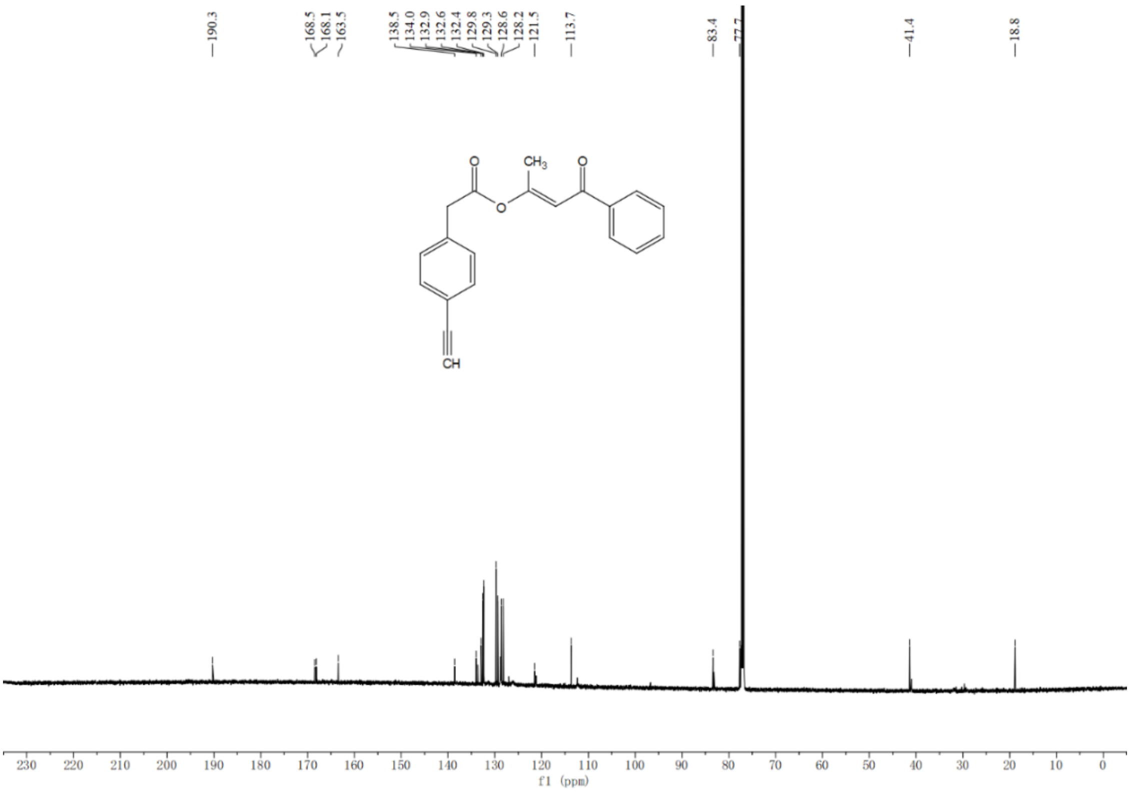

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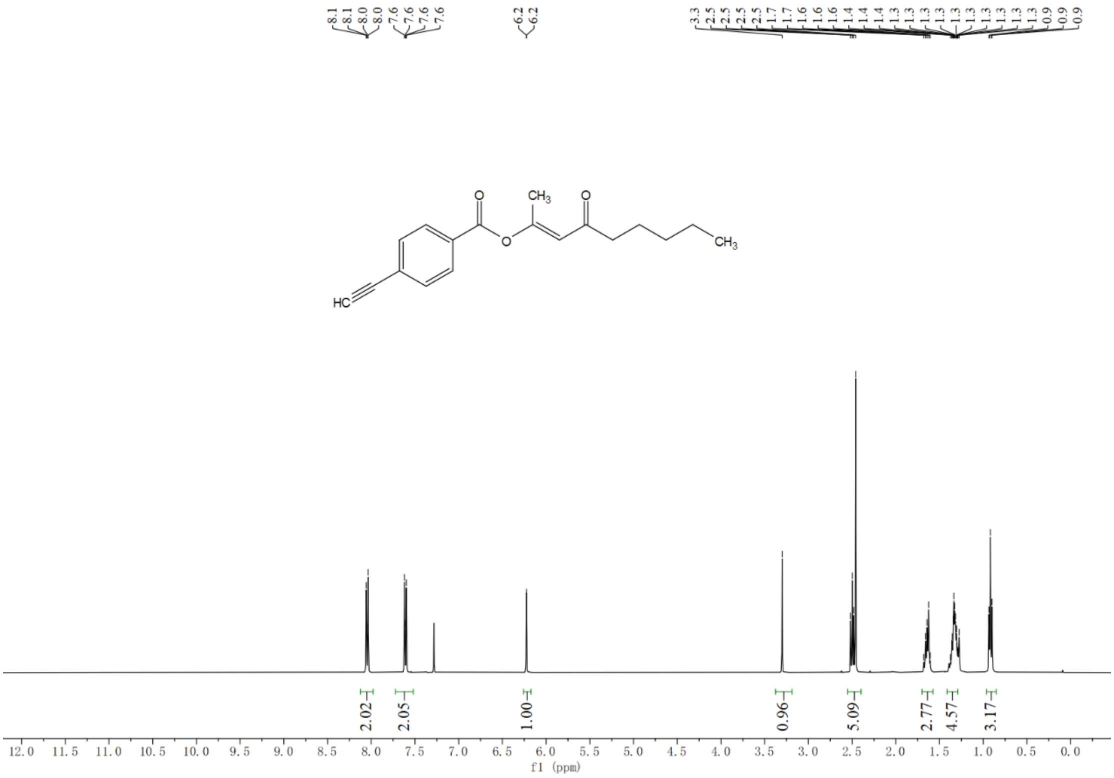

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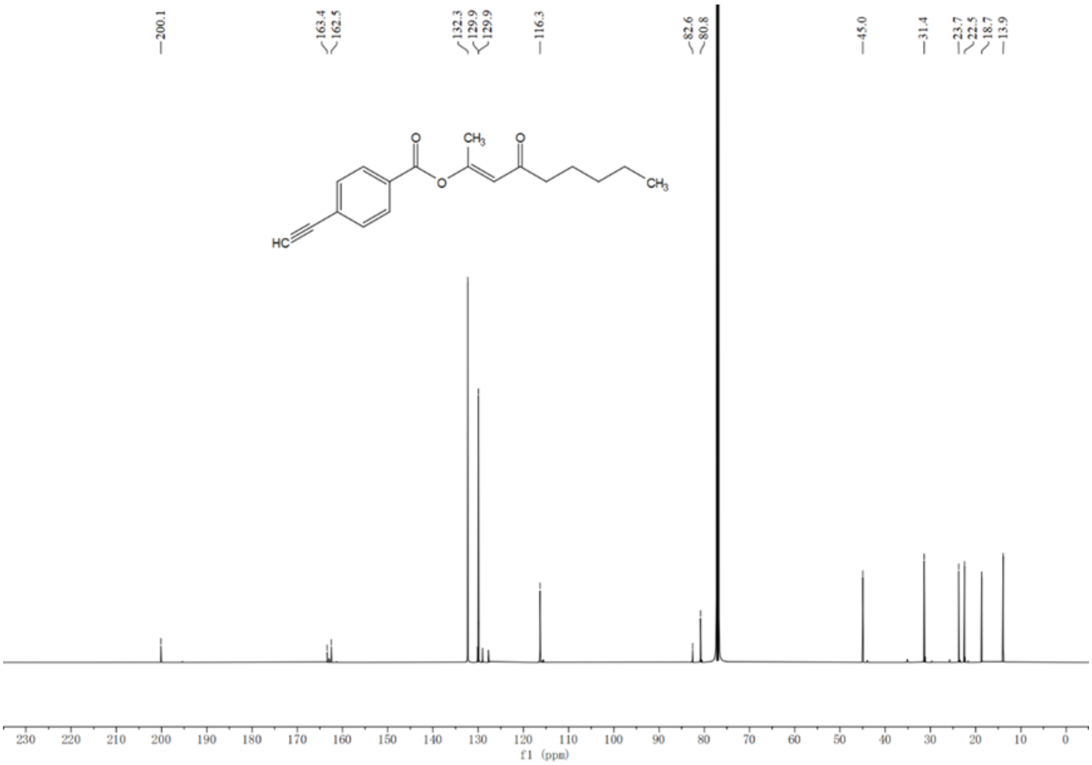

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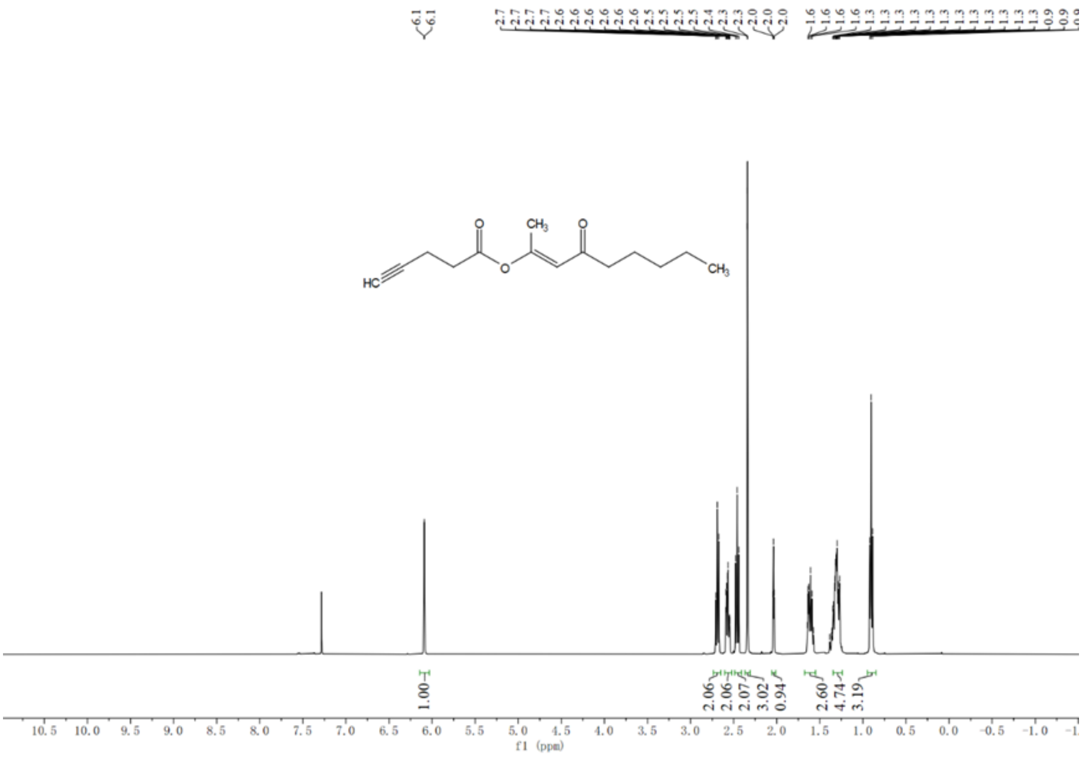

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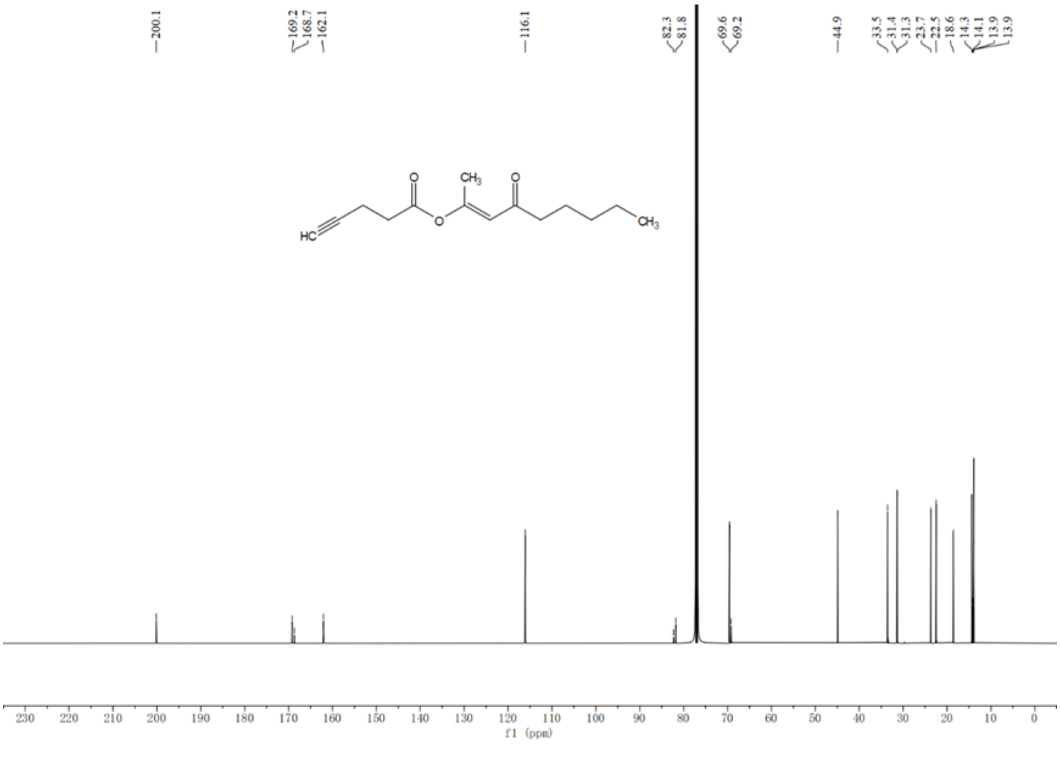

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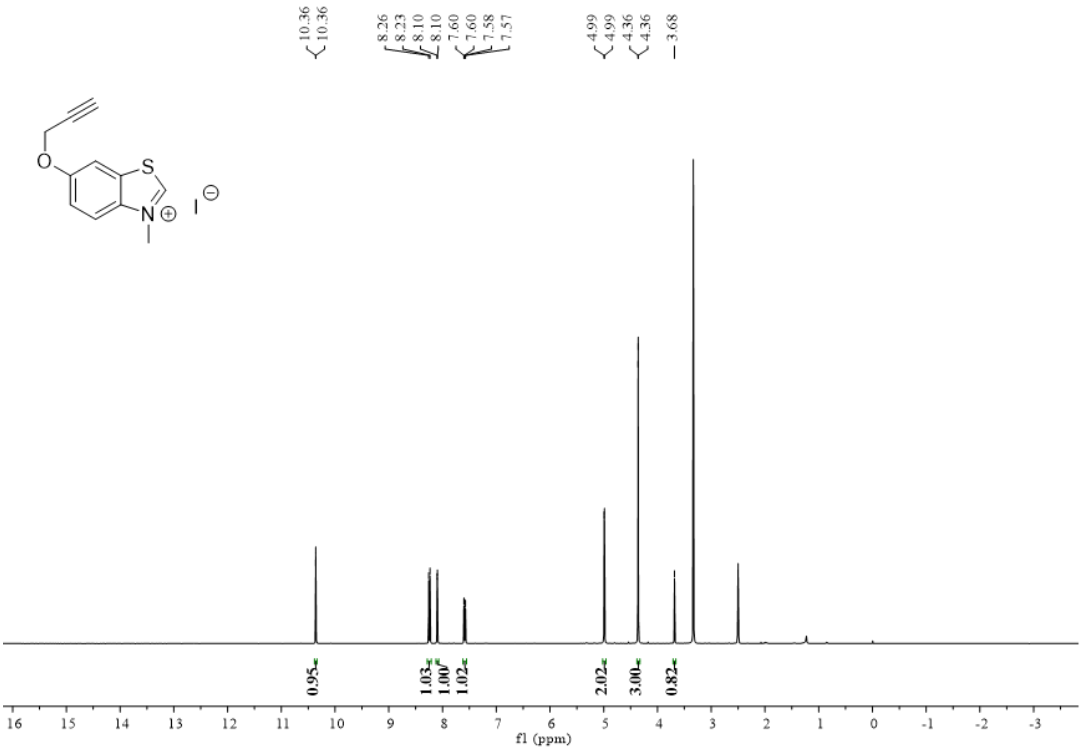

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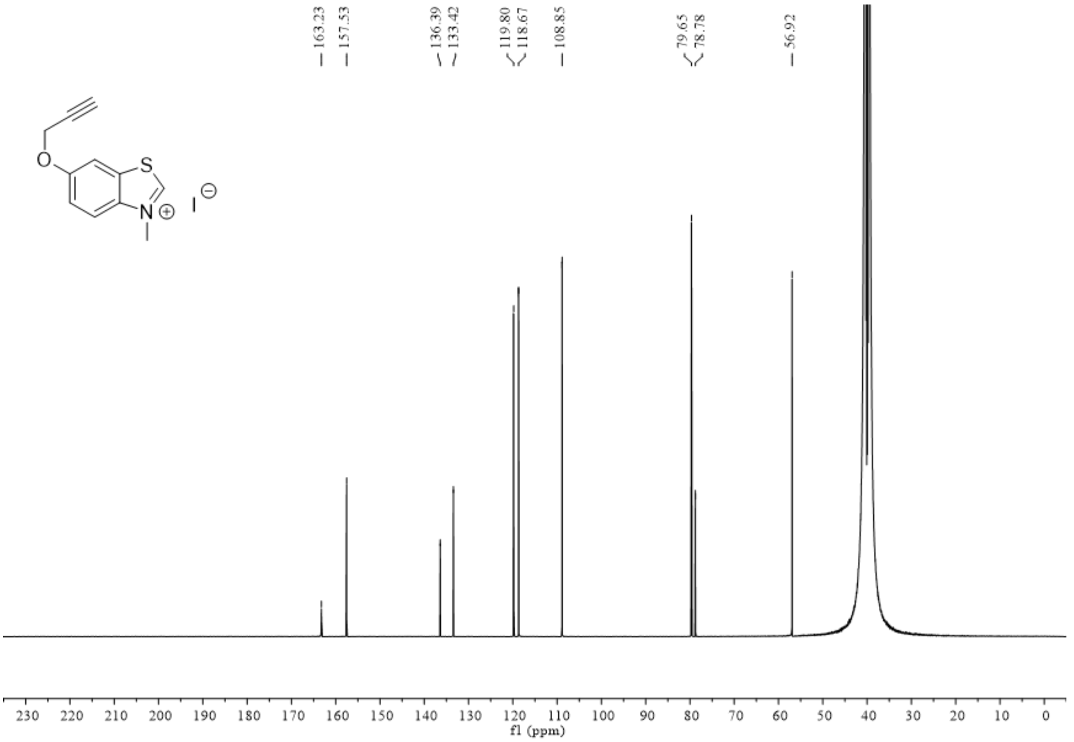

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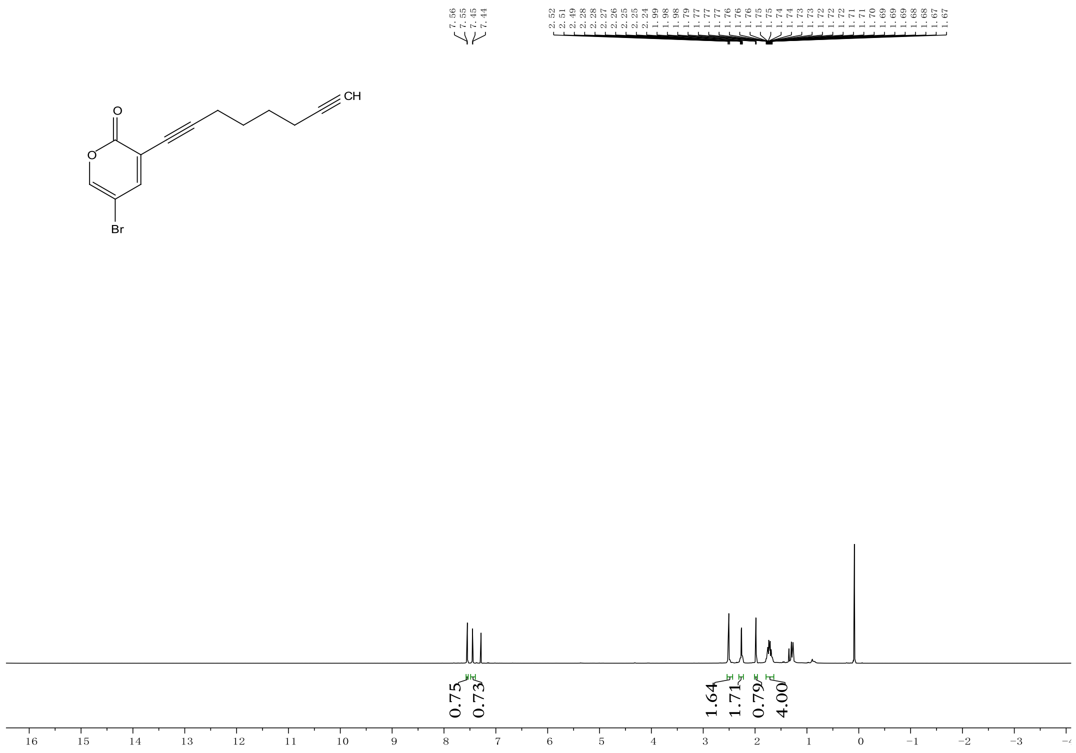

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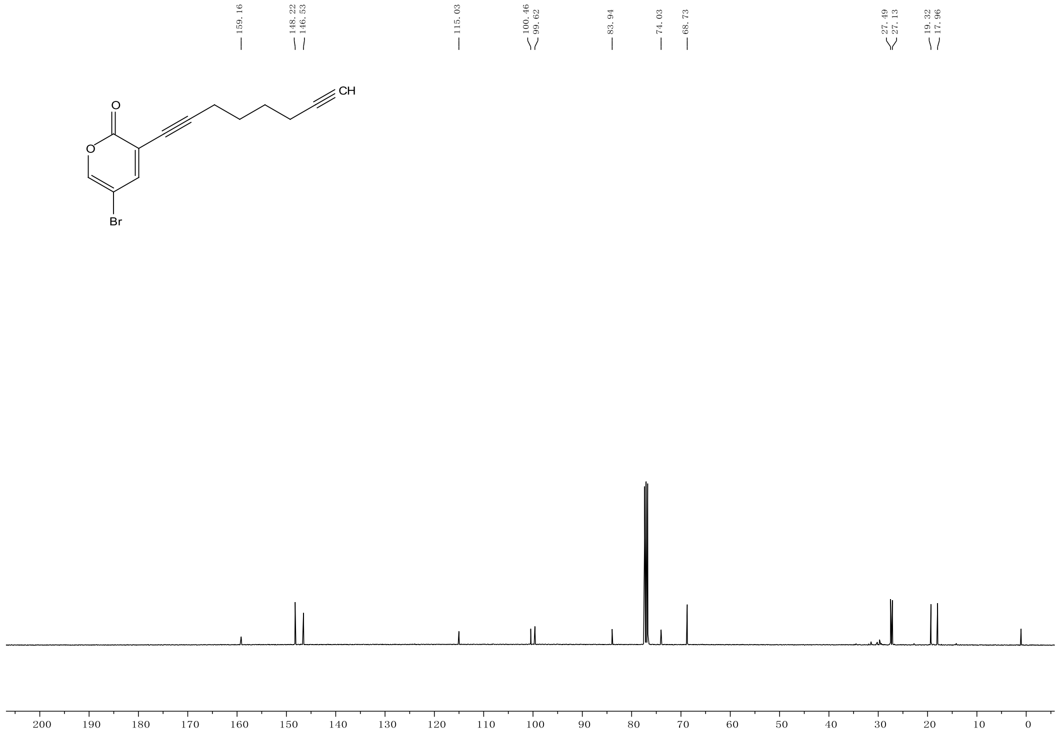

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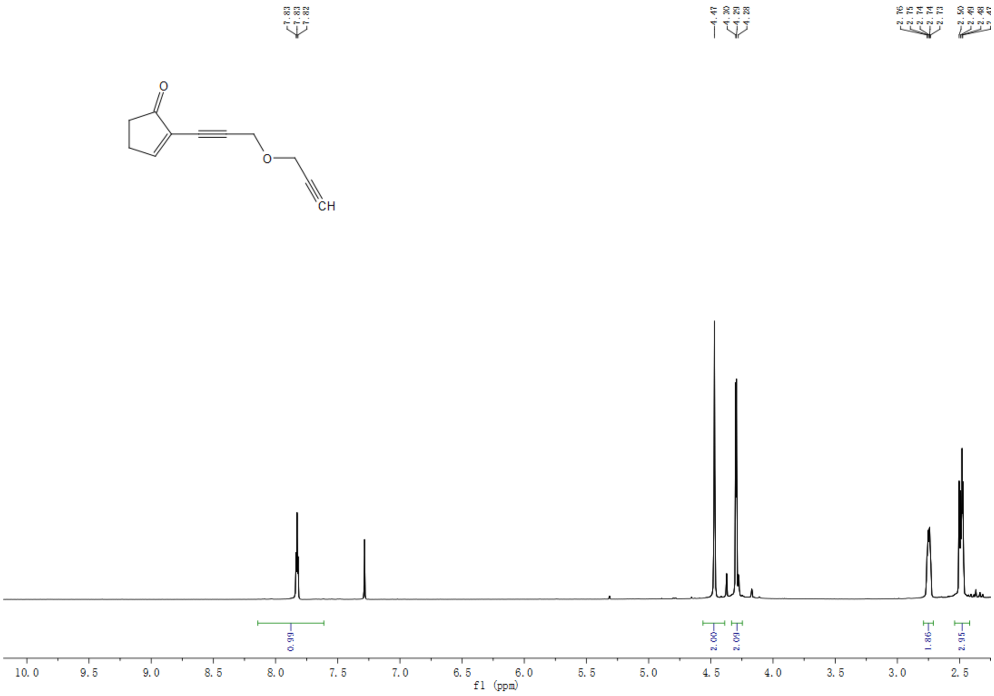

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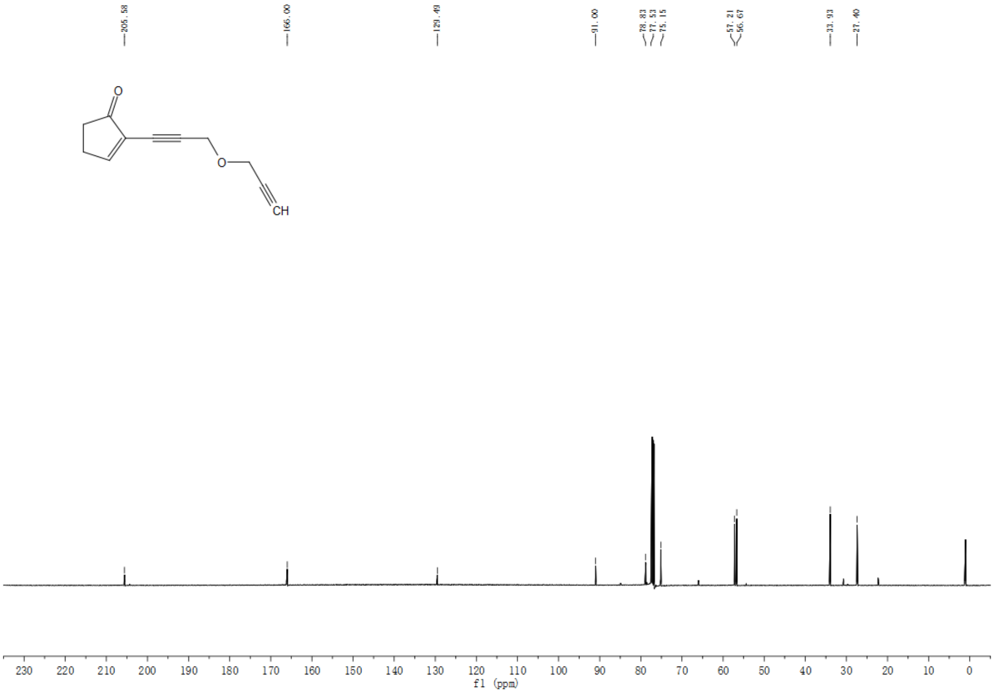

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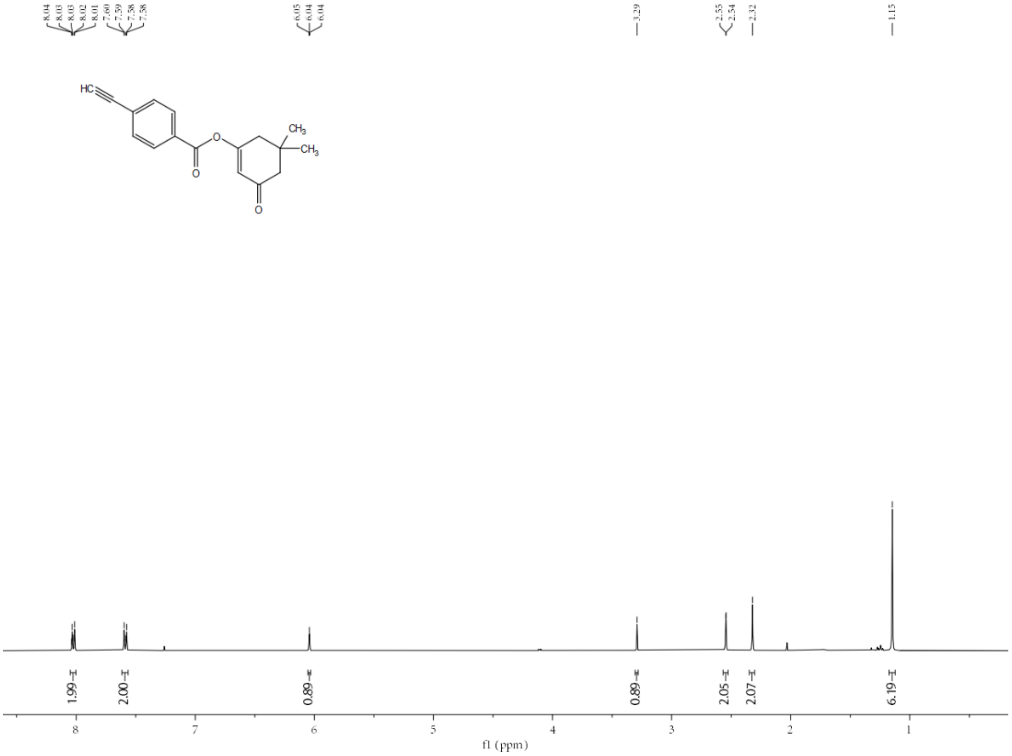

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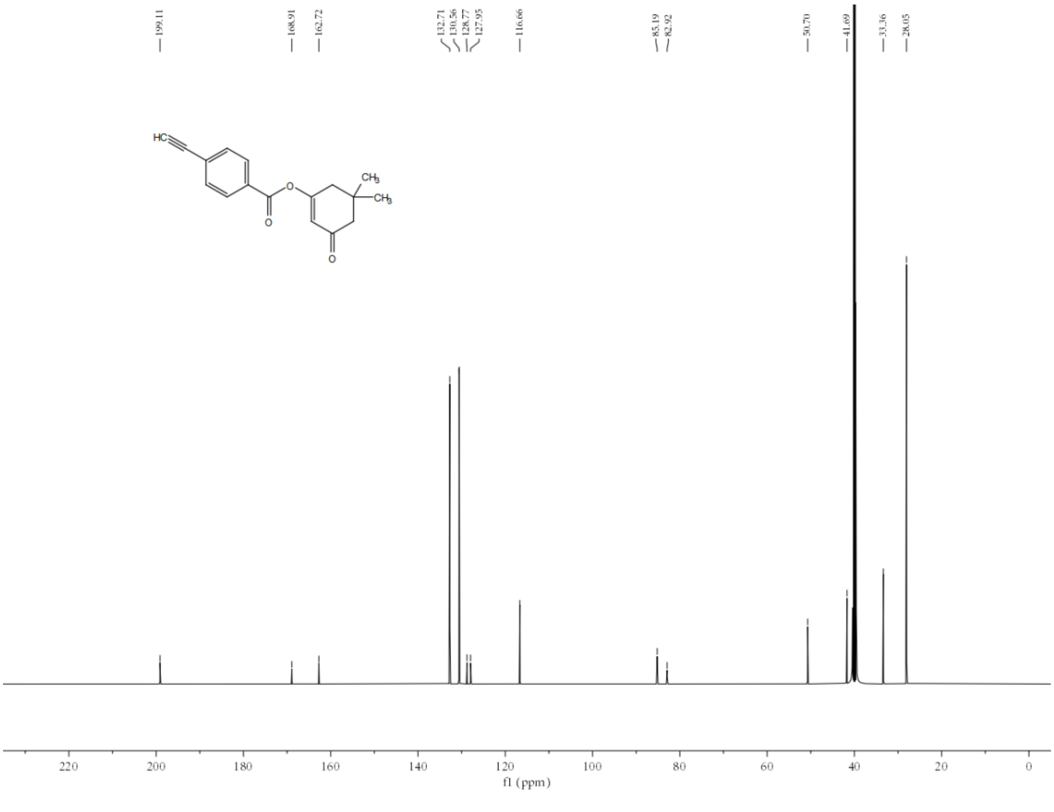

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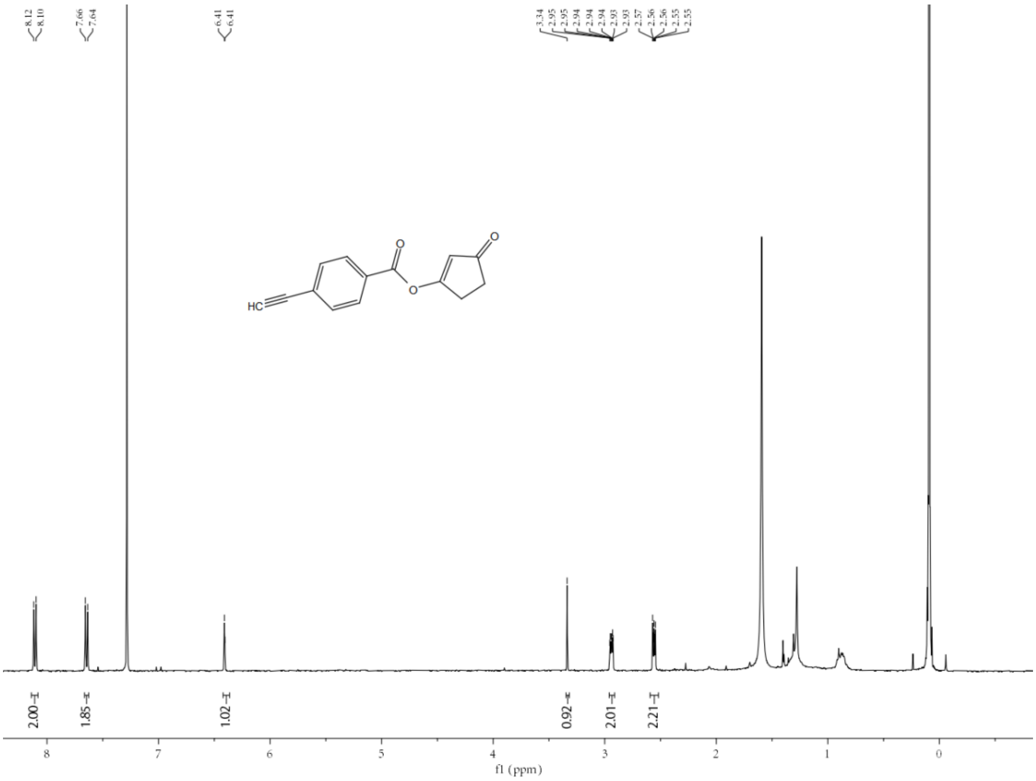

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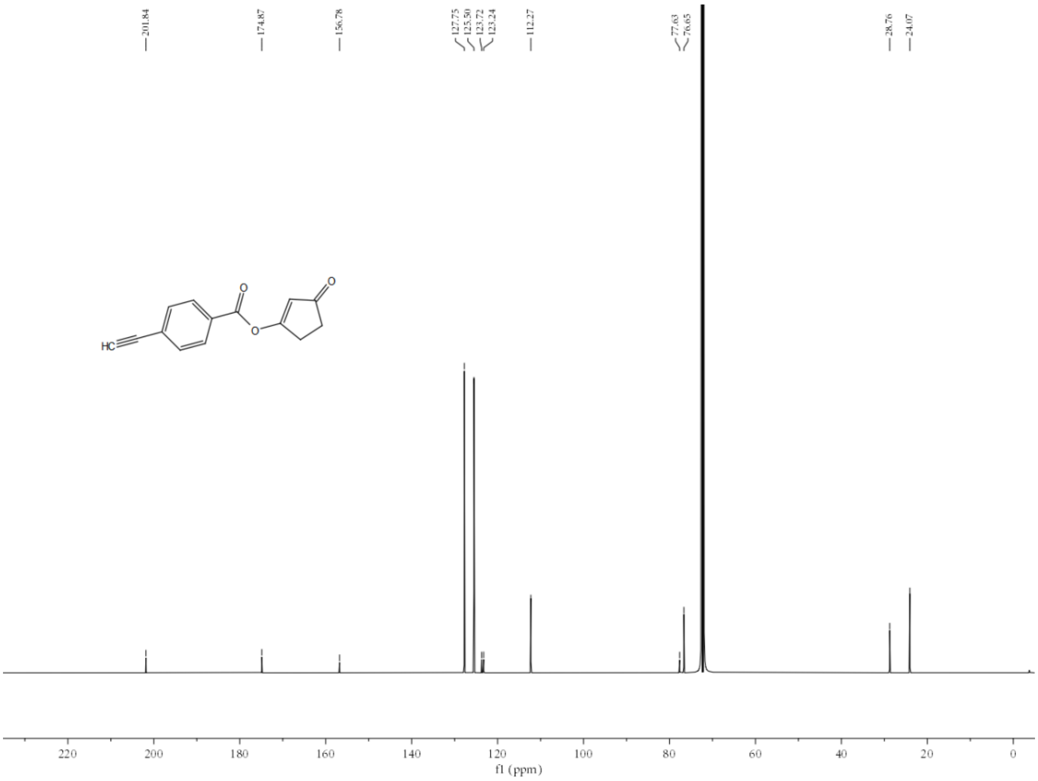

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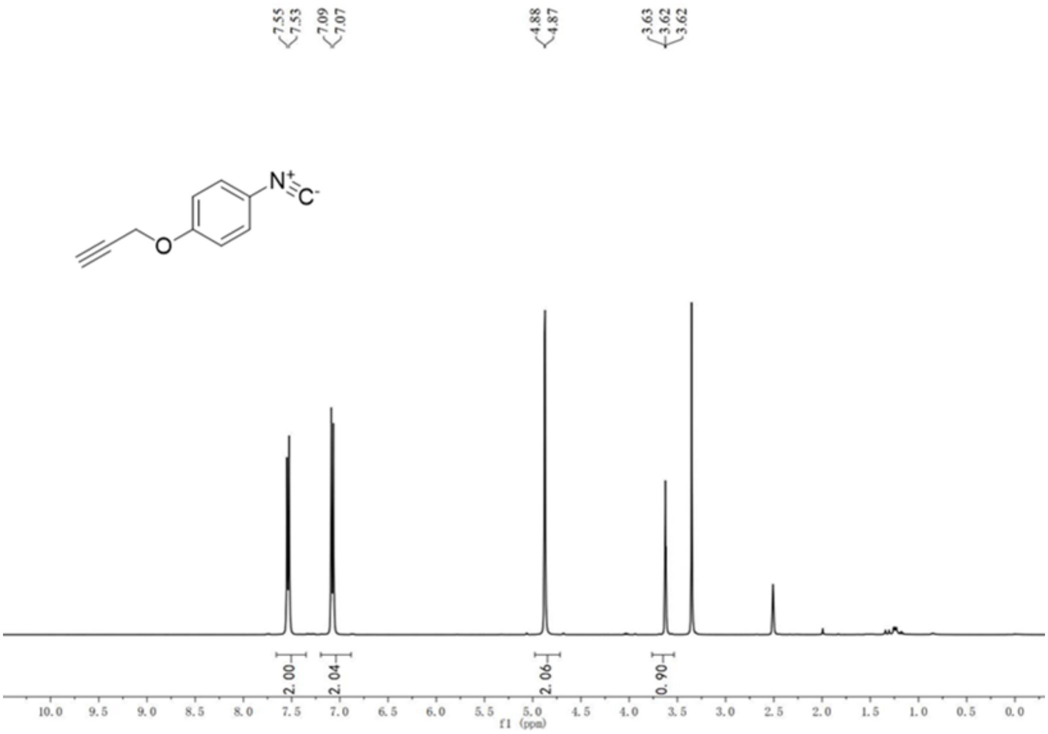

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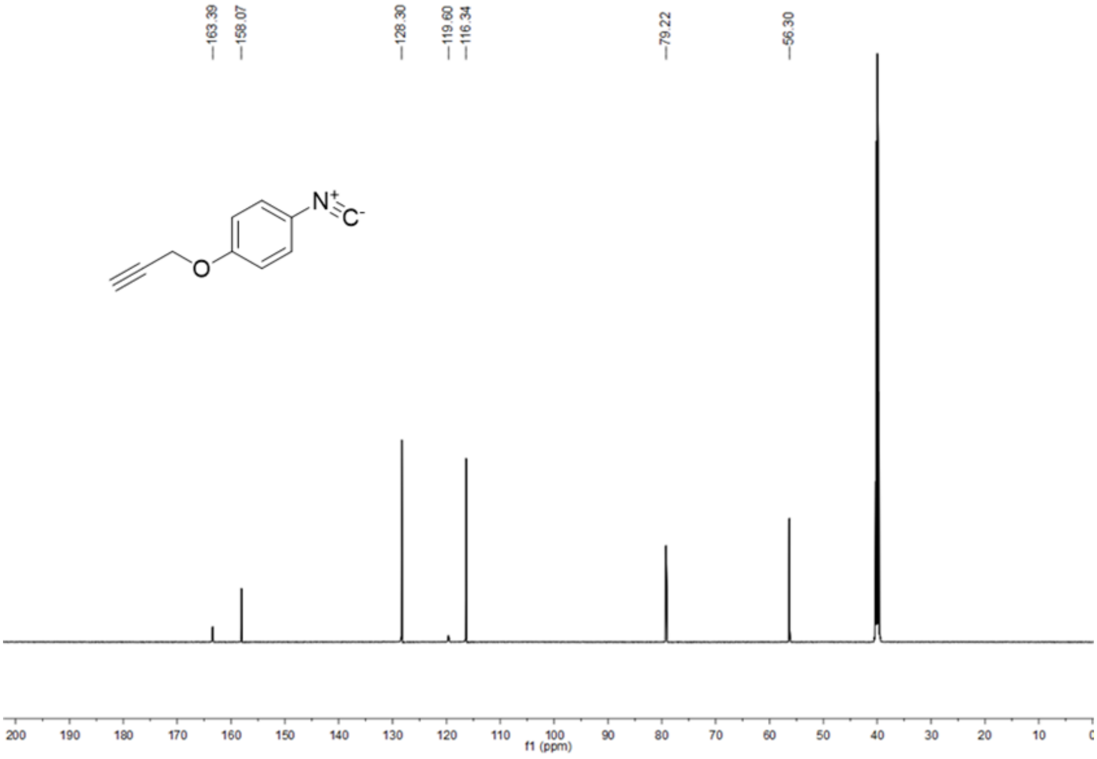

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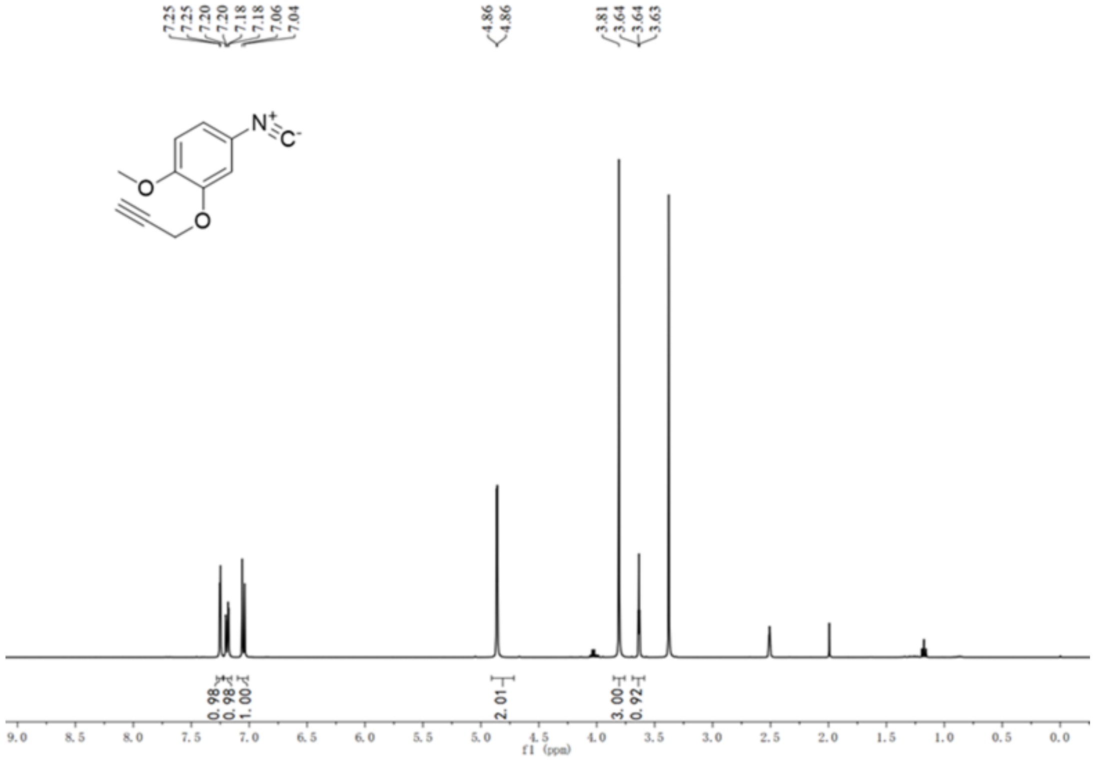

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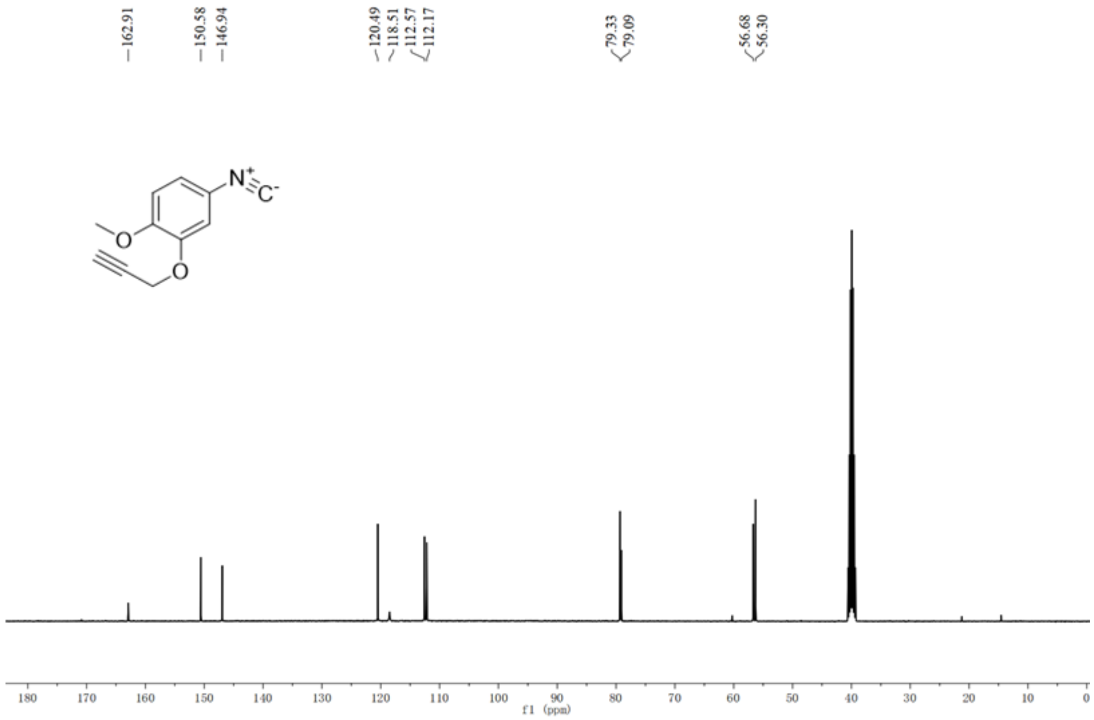

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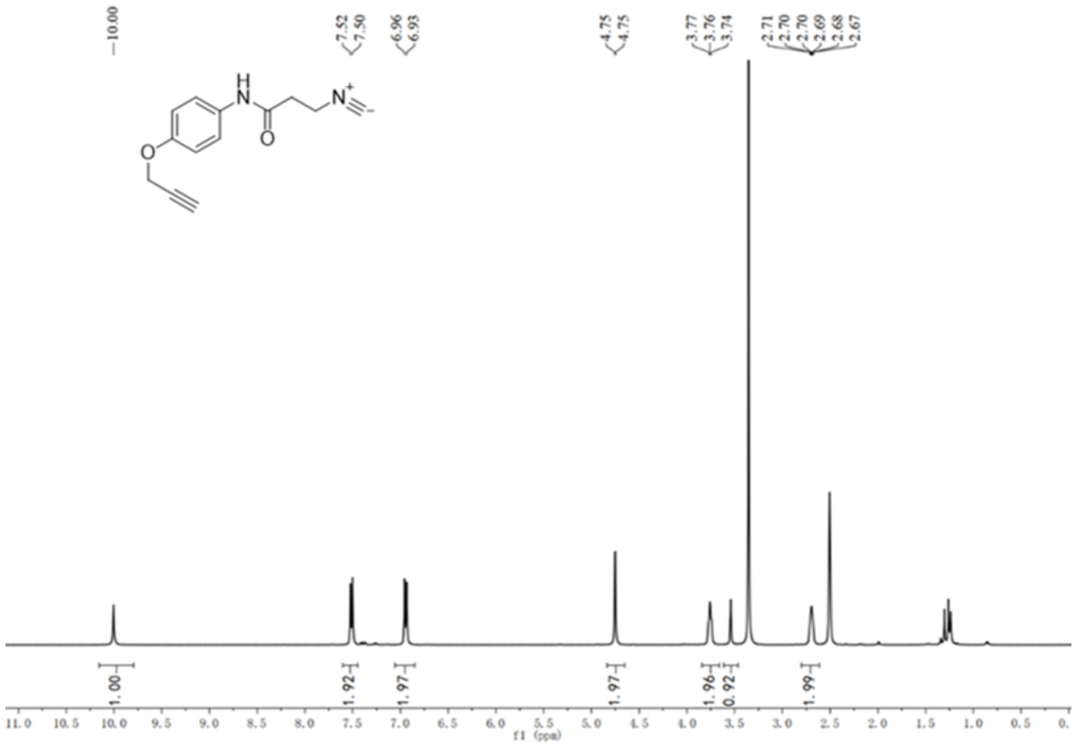

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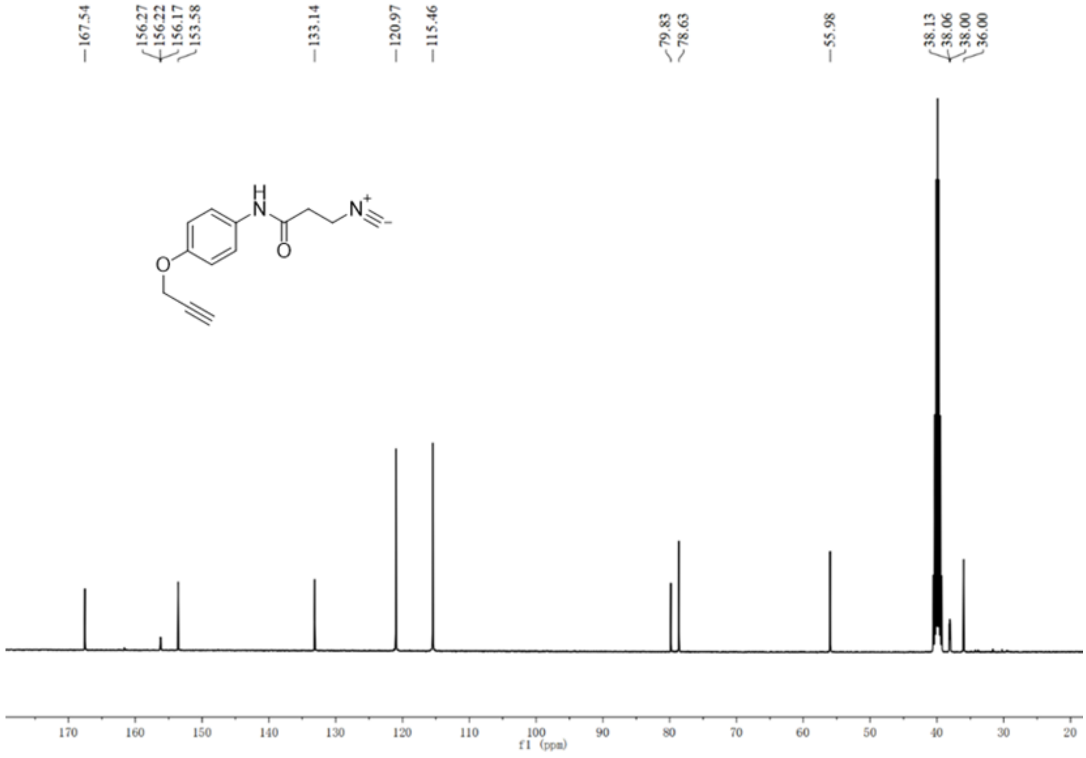

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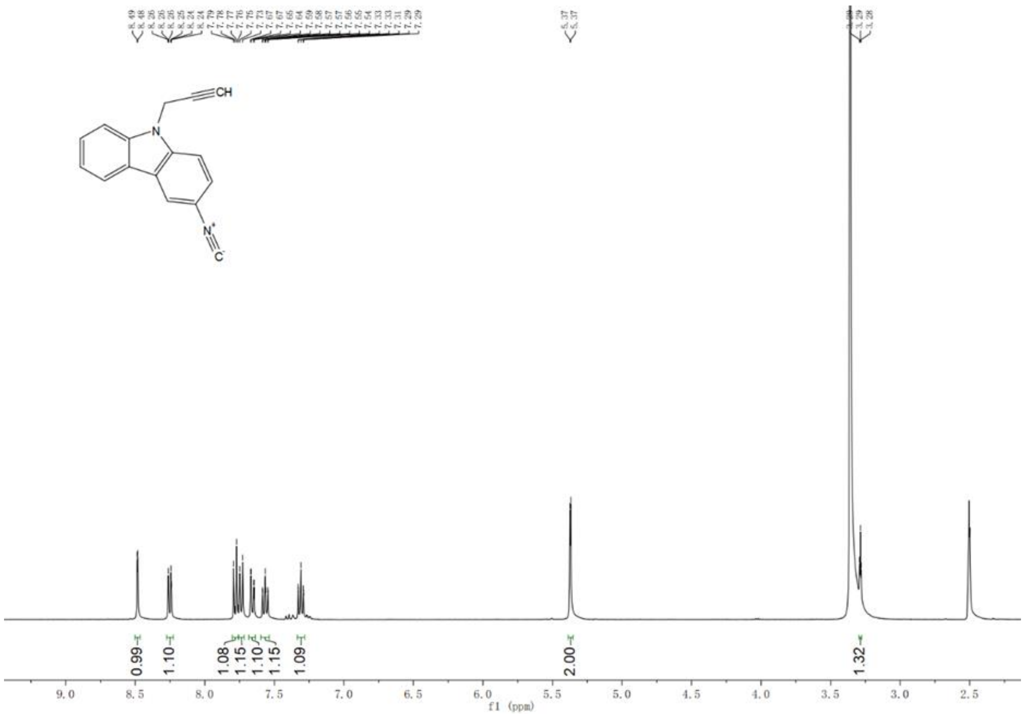

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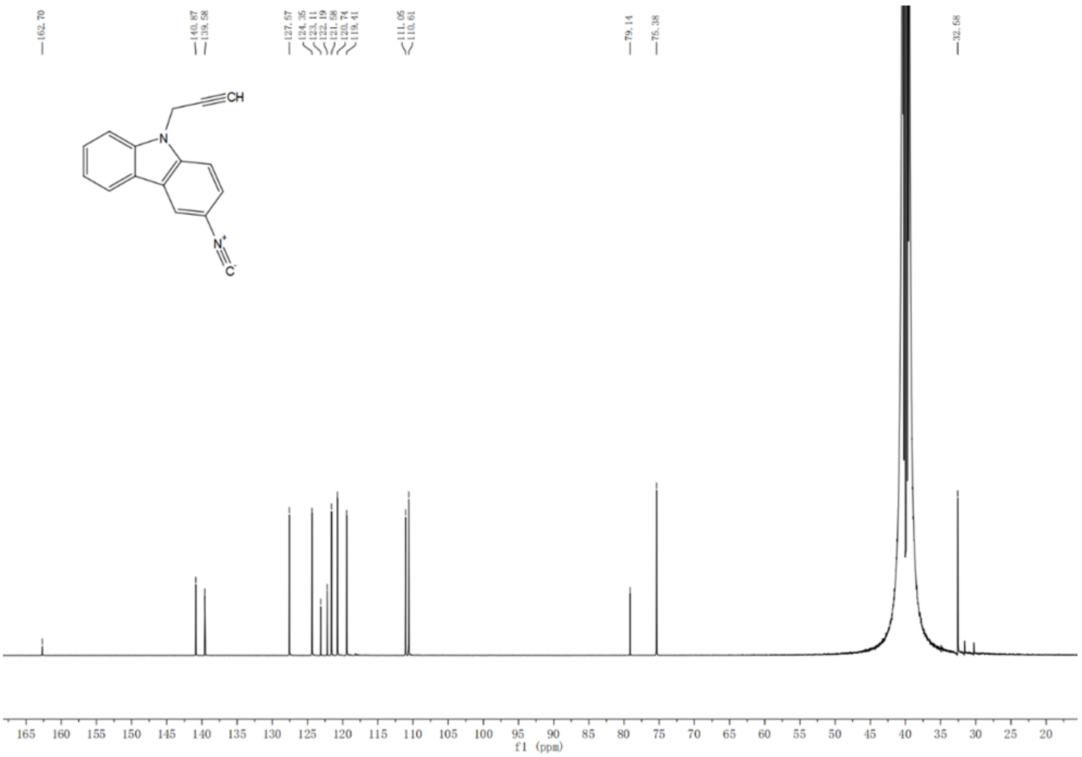

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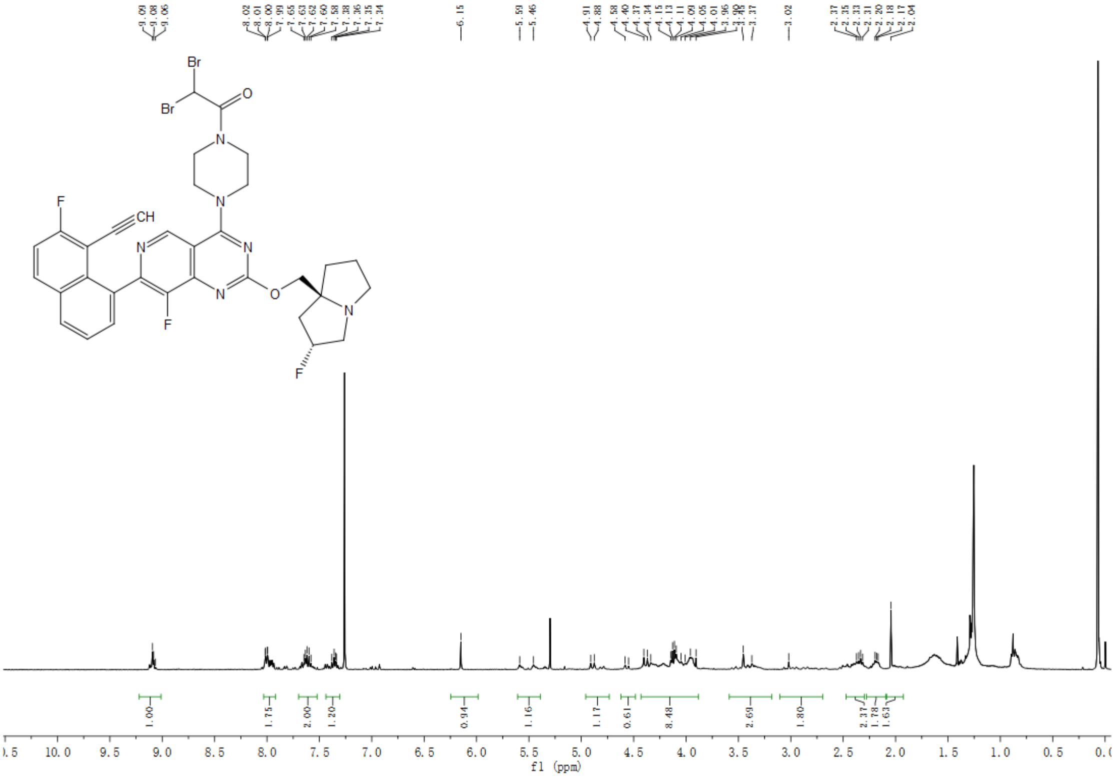

## DB1 $^{13}$C NMR