Supporting Information

Integrated Phenotypic Screening and Activity-based Protein Profiling to Reveal Potential Therapy Targets of Pancreas Cancer

Wenyan Liu, Zhang Zhang, Zhi-Min Zhang, Piliang Hao, Ke Ding, Zhengqiu Li*
School of Pharmacy, Jinan University, Guangzhou, China 510632
*Corresponding author (pharmlzq@jnu.edu.cn)

1. General Information

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. Reaction progress was monitored by TLC on pre-coated silica plates (Qingdao Huanghai $F_{254\ nm}$, 0.25 μm) and spots were visualized by UV, iodine or other suitable stains. Flash column chromatography was carried out using silica gel (Qingdao Huanghai F_{254} nm, 0.040-0.063 μm). All NMR spectra (^{1}H-NMR, ^{13}C-NMR) were recorded on Bruker 300 MHz/400 MHz NMR spectrometers. 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-d_6 = 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). In-gel fluorescence scanning of the SDS-PAGE gels was carried out with Typhoon 9500 fluorescence gel scanner (Amersham Biosciences), the click chemistry ligand tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA), Tris(2-carboxyethyl)phosphine(TCEP) and $CuSO_4$ were purchased from Sigma−Aldrich. TAMRA-Azide (Cat # AZ109) and TAMRA-Biotin-Azide (Cat # 1048) were purchased from Click Chemistry Tools (https://www.clickchemistrytools.com). Water with 0.1% TFA and acetonitrile with 0.1% TFA were used as eluents and the flow rate was 0.5 mL/min. Antibody against GSTO1 (ab129106) was purchased from Abcam and antibody against FAM213A (NBP2-48573) was purchased from Novus Biologicals. Small interfering RNA (siRNA) against GSTO1 (h) (sc-75207) and FAM213A (h) (sc-90705) were purchased from Santa Cruz Biotechnology. BD Pharmingen™ PE Annexin V Apoptosis Detection Kit I (Cat. NO. 559763) and BD Cycletest™ Plus DNA Reagent Kit (Cat. NO. 340242) were purchased from BD company.

2. Cell culture and Western Blot

Cell lines were obtained from the National Cancer Institute Developmental Therapeutics Program (NCI-60). BxPC-3 cells were cultured in RPMI 1640 containing 10% heat-inactivated fetal bovine serum (FBS; Gibco), 100 units/mL penicillin, and 100 μg/mL streptomycin (Thermo Scientific) and maintained in a humidified 37 ℃ incubator with 5% CO_2. To generate protein lysates, cells were washed twice with cold phosphate-buffered saline (PBS), harvested with 1× trypsin or by use of a cell scraper, and collected by centrifugation. Cell pellets were then washed with PBS and lysed with RIPA or sodium dodecyl sulfate (SDS) buffer. Protein concentration was

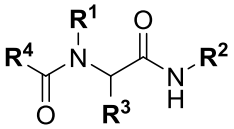
determined by Bradford protein assay. Proteome labeling, in-gel fluorescence scanning and bioimaging experiments were performed as previously reported[1-3].

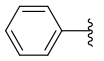
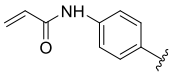
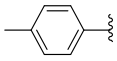
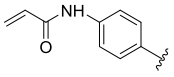
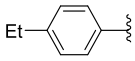
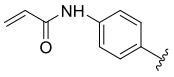
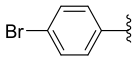
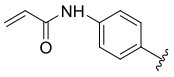
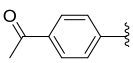
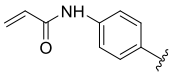
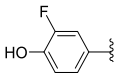
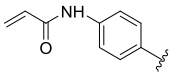
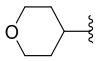
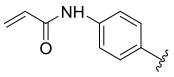
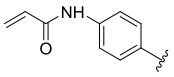
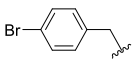
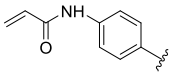
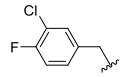
For Western blotting experiments, samples from BxPC-3 cells lysed using 1×SDS sample lysis buffer (CST recommended) with protease and phosphatase inhibitors were resolved and electrophoresed onto 12% by SDS−polyacrylamide gels and transferred to poly(vinylidene difluoride) membranes. Membranes were then blocked with 5% bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO, USA) in TBST (0.1% Tween-20 in Tris-buffered saline) for 1 h at room temperature. After blocking, membranes were incubated with the corresponding primary antibody at 4 ℃ overnight. After incubation, membranes were washed with TBST (3 × 10 min) and then incubated with an appropriate HRP-conjugated secondary antibody for 2 hours at room temperature. Finally, blots were washed again with TBST before being developed with ECL Western Blotting Detection Kit (Thermo Scientific, Grand Island, NY, USA), and detected with Amersham Imager 600 system (GE, Boston, MA, USA).

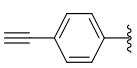
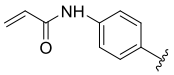
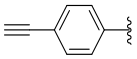
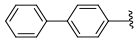
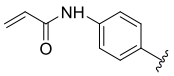
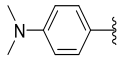
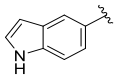
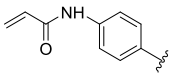
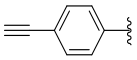
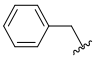
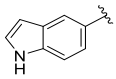
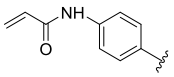
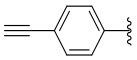
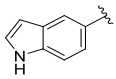
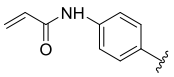
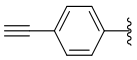
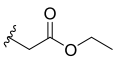
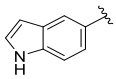
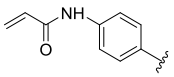
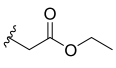
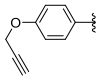
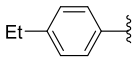
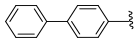
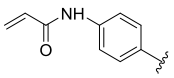
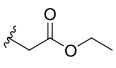
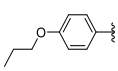
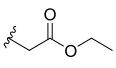
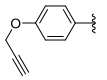
3. Protein preparation

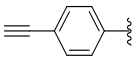
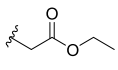
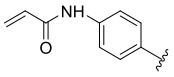
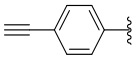
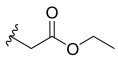
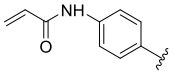
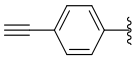
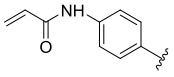
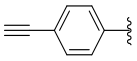
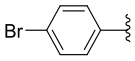
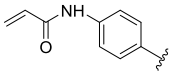
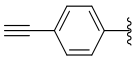
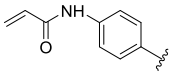
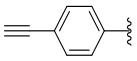
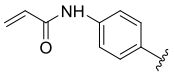
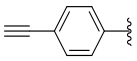
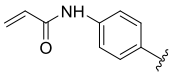
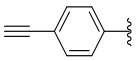
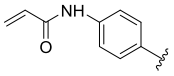
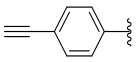
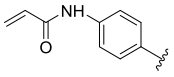
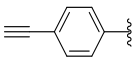
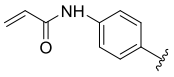
The DNA sequence encoding full-length human GSTO1 was amplified and inserted into a modified pRSFDuet-1 vector, in which the GSTO1 gene was separated from an N-terminal His_6-SUMO tag by a ULP1 (ubiquitin-like-protease 1) cleavage site. The fusion protein was overexpressed in the Rosetta (DE3) cell strain overnight by the addition of 0.4 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) at 16 ℃ when the cell density reached A_{600} of 1.0. The recombinant proteins were purified trough a Nickel Affinity column. The eluted proteins were subsequently cleaved by ULP1, followed by further purification using a second Nickel Affinity column and gel-filtration (Superdex 75, GE). The GSTO1 samples were finally stored at -80 ℃ at a concentration of 160 mg / mL for future use.

Table S1. Chemical structures of the compounds and corresponding IC_{50} values.



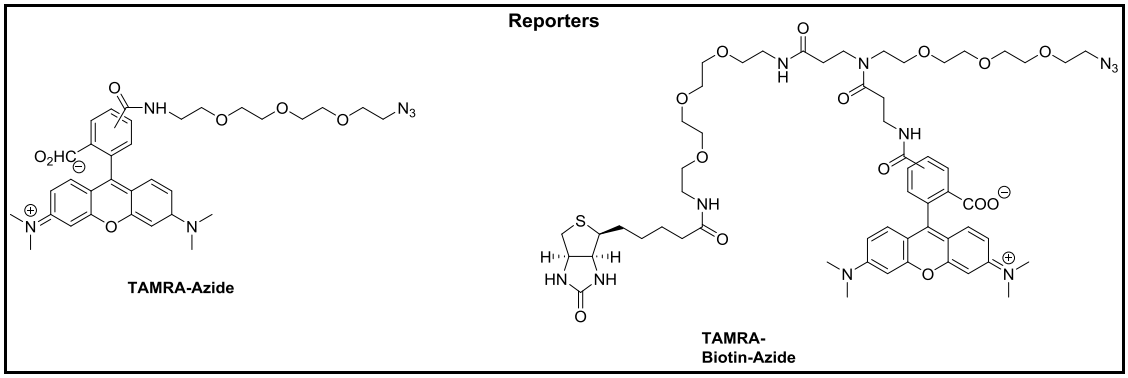
Compounds	R^1	R^2	R^3	R^4	IC_{50} (μM)
P1					**10.65 ±1.23**
P2					**5.54 ±0.92**
P3					**6.55 ±1.25**
P4					**3.40 ±0.40**
P5					**14.31 ±3.04**
P6					**34.26 ±4.37**
P7					**>50**
P8					**7.52 ±0.30**
P9					**5.54 ±2.00**
P10					**5.49 ±1.75**
P11					**3.00 ±0.53**
P12					**5.97 ±0.54**
P13					**6.10 ±1.45**
P14					**9.91 ±1.08**
P15					**15.99 ±3.84**
P16					**24.20 ±0.95**
P17					**12.26 ±2.40**
P18					**12.10 ±0.80**
P19					**11.59 ±1.03**

Compounds	R^1	R^2	R^3	R^4	IC_{50} (μM)
P20					**17.59 ±7.36**
P21					**2.58 ±0.16**
P22					**12.37 ±2.42**
P23					**6.38 ±0.89**
P24					**8.06 ±0.85**
P25					**21.75 ±2.57**
P26					**1.36 ±0.59**
P27					**5.71 ±0.41**
P28					**0.69 ±0.18**
P29					**>50**
P30					**33.43 ±7.68**
P31					**NA**
P32					**25.57 ±7.79**
P33					**29.60 ±4.48**
P34					**20.94 ±5.38**
P35					**11.33 ±1.70**
P36					**22.78 ±3.71**
P37					**5.68 ±0.55**
P38					**11.01 ±1.73**
P39					**2.54 ±0.62**
P40					**5.99 ±1.76**

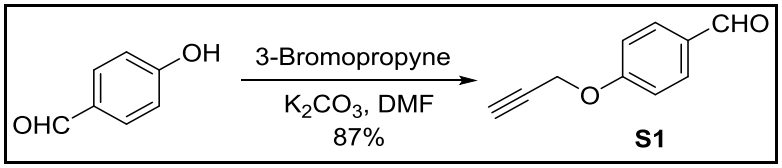
Compounds	R^1	R^2	R^3	R^4	IC_{50} (μM)
P41					**24.92 ±1.03**
P42					**24.96 ±7.34**
P43					**5.73 ±1.89**
P44					**3.57 ±1.38**
P45					**11.31 ±1.62**
P46					**10.68 ±0.80**
P47					**10.12 ±0.36**
P48					**5.76 ±1.79**
P49					**9.73 ±1.87**
P50					**5.65 ±1.02**
P51					**NA**
P52					**NA**
P53					**>50**
P54					**NA**
P55					**NA**
P56					**NA**
P57					**NA**
P58					**>50**
P59					**16.42 ±5.21**
P60					**8.38 ±1.75**
P61					**8.78 ±2.06**

NA: no activity.

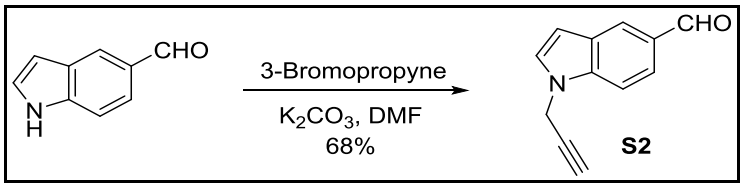
Table S2. Structures of reporters used in the current study.



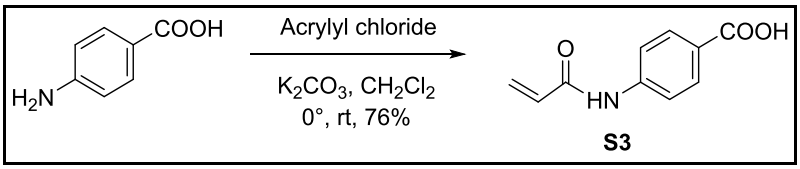
4. Chemical Synthesis



(S1). To a stirred solution of 4-hydroxybenzaldehyde (1.22 g, 10 mmol) in 10 mL DMF was added 3-bromopropyne (958 μL, 11 mmol) and K_2CO_3 (3.45 g, 25 mmol). The resulting mixture was stirred at room temperature for 2 h, followed by addition of water (10 mL) and then extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. **S1** was afforded as a yellow solid (1.4 g, 87%) after solvent evaporation *in vacuo*. ^{1}H NMR (300 MHz, DMSO) δ9.89 (s, 1H), 7.90 (d, *J* = 8.8 Hz, 2H), 7.18 (d, *J* = 8.7 Hz, 2H), 4.95 (d, *J* = 2.4 Hz, 2H), 3.66 (t, *J* = 2.4 Hz, 1H). MS (ESI) *m/z*: 183.1 $[M + Na]^+$.

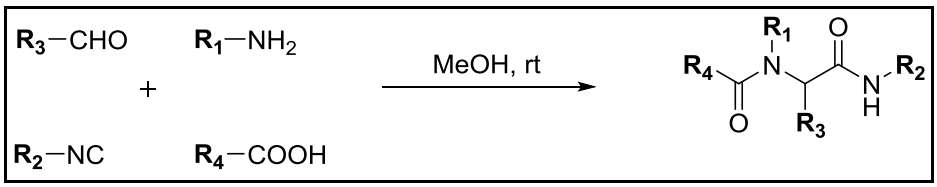


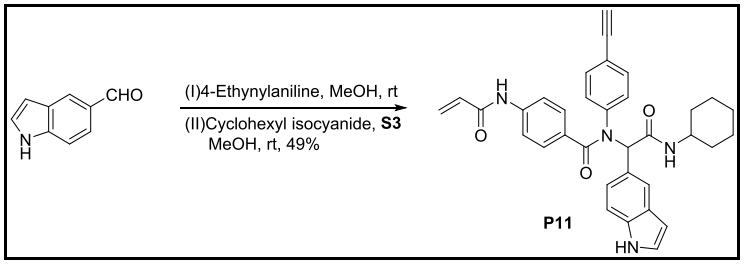
(S2). To a stirred solution of 1*H*-indole-5-carbaldehyde (1.45 g, 10 mmol) in 10 mL DMF was added 3-bromopropyne (958 μL, 11 mmol) and K_2CO_3 (3.45 g, 25 mmol). The resulting mixture was stirred at room temperature for 5 h, followed by addition of water (10 mL) and then extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 5 : 1) to give **S2** as a brown solid (1.24 g, 68%). ^{1}H NMR (400 MHz, DMSO) δ10.01 (s, 1H), 8.34 – 8.14 (m, 1H), 7.80 – 7.66 (m, 2H), 7.59 (d, *J* = 3.2 Hz, 1H), 6.72 (dd, *J* = 3.2, 0.6 Hz, 1H), 5.19 (d, *J* = 2.5 Hz, 2H), 3.47 (t, *J* = 2.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 193.0, 139.1, 131.0, 129.7, 128.6, 126.3, 121.7, 111.3, 103.9, 79.2, 76.4, 35.9. MS (ESI) *m/z*: 206.1 $[M + Na]^+$.



(S3). To a stirred solution of 4-aminobenzoic acid (1.37 g, 10 mmol) and K_2CO_3 (3.45 g，25 mmol) in 10 mL dry CH_2Cl_2 was added acrylyl chloride (975 μL，12 mmol) slowly under argon atmosphere at 0 ℃. The resulting mixture was stirred at 0 ℃ for 1 h and then transfer to room temperature overnight, followed by addition of water (20 mL) and then extracted with ethyl acetate (3 × 10 mL). The aqueous layers were combined and adjust pH with 2N HCl. **S3** was afforded by filtering as a white solid (1.45 g, 76%). ^{1}H NMR (400 MHz, DMSO) δ12.71 (s, 1H), 10.45 (s, 1H), 7.92 (d, *J* = 8.7 Hz, 2H), 7.79 (d, *J* = 8.8 Hz, 2H), 6.47 (dd, *J* = 16.9, 10.1 Hz, 1H), 6.30 (dd, *J* = 17.0, 1.9 Hz, 1H), 5.81 (dd, *J* = 10.0, 2.0 Hz, 1H).^{13}C NMR (101 MHz, DMSO) δ 167.4, 164.0, 143.6, 132.1, 130.8, 128.1, 125.8, 119.1. MS (ESI) *m/z*: 214.1 [M + Na]$^+$.

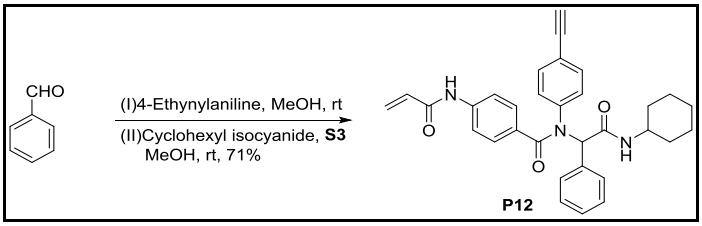
General formula of Ugi reaction[4, 5].





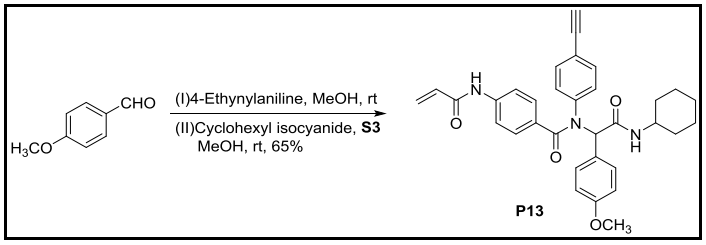
Scheme S1

(P11). To a stirred solution of 1*H*-indole-5-carbaldehyde (145.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 1 : 1) to give it as a white solid (266.3 mg, 49%). ^{1}H NMR (400 MHz, DMSO) δ11.01 (s, 1H), 10.16 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.34 (s, 1H), 7.26 (s, 1H), 7.16 (t, 3H), 7.09 – 6.92 (m, 4H), 6.80 (d, *J* = 8.5 Hz, 1H), 6.37 (q, *J* = 17.1, 10.1 Hz, 3H), 6.23 (d, *J* = 16.9 Hz, 1H), 5.74 (d, *J* =10.2 Hz, 1H), 4.06 (s, 1H), 3.72 – 3.61 (m, 1H), 1.83 – 1.45 (m, 6H), 1.29 – 1.18 (m, 2H), 1.12 – 0.97 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.8, 169.7, 163.7, 142.0, 140.1, 135.5, 132.1, 132.0, 131.6, 131.3, 129.5, 127.7, 127.6, 126.1, 125.8, 123.6, 122.5, 119.8, 118.5, 111.3, 101.7, 83.4, 81.7, 65.0, 48.4, 32.8, 32.7, 25.7, 25.1, 25.0. MS (ESI) *m/z*: 543.2 [M - H]$^-$.



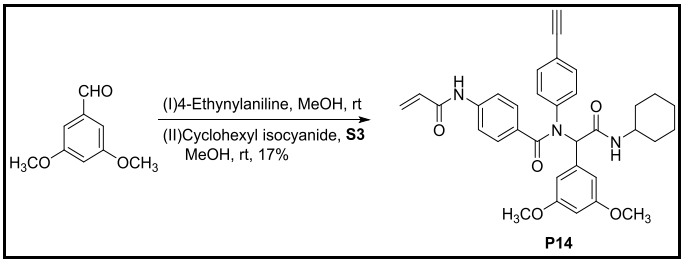
Scheme S2

(P12). To a stirred solution of benzaldehyde (102 μL, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 2 : 1) to give it as a yellow solid (359.8 mg, 71%). ^{1}H NMR (400 MHz, DMSO) δ 10.17 (s, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.26 – 7.09 (m, 8H), 7.05 (s, 3H), 6.37 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.32 (s, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.1, 2.1 Hz, 1H), 4.11 (s, 1H), 3.71 – 3.60 (m, 1H), 1.82 – 1.44 (m, 6H), 1.34 – 1.21 (m, 2H), 1.12 – 0.99 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.7, 168.9, 163.7, 141.7, 140.2, 135.8, 132.1, 131.7, 131.5, 131.5, 130.5, 129.6, 128.4, 128.2, 127.8, 120.0, 118.5, 83.3, 81.8, 64.5, 48.5, 32.7, 32.6, 25.6, 25.1, 25.0. MS (ESI) *m/z*: 528.2 $[M + Na]^+$.



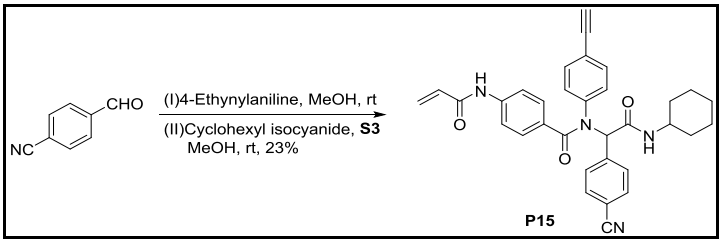
Scheme S3

(P13). To a stirred solution of p-anisaldehyde (121 μL, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 100 : 1) to give it as a yellow solid (345.6 mg, 65%). ^{1}H NMR (400 MHz, DMSO) δ 10.17 (s, 1H), 8.03 (d, *J* = 7.7 Hz, 1H), 7.46 (d, *J* = 8.7 Hz, 2H), 7.16 (d, *J* = 8.7 Hz, 2H), 7.10 – 6.99 (m, 6H), 6.74 (d, *J* = 8.8 Hz, 2H), 6.37 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.27 – 6.19 (m, 2H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.12 (s, 1H), 3.67 (s, 3H), 3.66 – 3.55 (m, 1H), 1.82 – 1.49 (m, 6H), 1.28 – 1.21 (m, 2H), 1.12 – 0.97 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.7, 169.2, 163.7, 159.0, 141.8, 140.2, 132.1, 131.8, 131.6, 131.5, 129.5, 127.7, 127.6, 120.0, 118.5, 113.8, 83.4, 81.8, 64.0, 55.4, 48.4, 32.8, 32.6, 25.7, 25.1, 25.0. MS (ESI) *m/z*: 534.1 $[M - H]^-$.



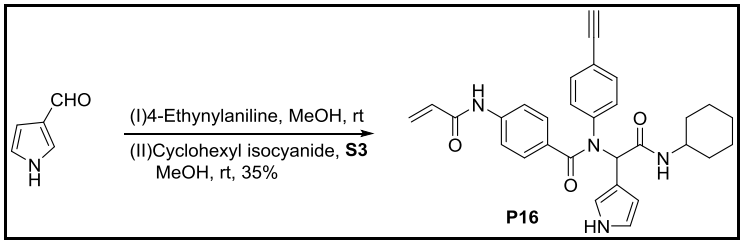
Scheme S4

(P14). To a stirred solution of 3,5-dimethoxybenzaldehyde (166.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 100 : 1) to give it as a yellow solid (96.8 mg, 17%). ^{1}H NMR (400 MHz, DMSO) δ 10.17 (s, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.17 (d, *J* = 8.7 Hz, 2H), 7.15 – 7.04 (m, 4H), 6.37 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.28 (s, 3H), 6.26 – 6.20 (m, 2H), 5.75 (dd, *J* = 10.4, 2.4 Hz, 1H), 4.13 (s, 1H), 3.68 – 3.61 (m, 1H), 3.61 (s, 6H), 1.81 – 1.47 (m, 6H), 1.26 – 1.20 (m, 2H), 1.16 – 1.02 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.7, 168.7, 163.7, 160.3, 141.7, 140.2, 137.8, 132.1, 131.7, 131.5, 131.5, 129.6, 127.8, 120.1, 118.5, 108.7, 100.1, 83.4, 81.8, 64.5, 55.6, 48.5, 32.7, 32.5, 25.6, 25.1, 25.0. MS (ESI) *m/z*: 588.3 $[M + Na]^+$.



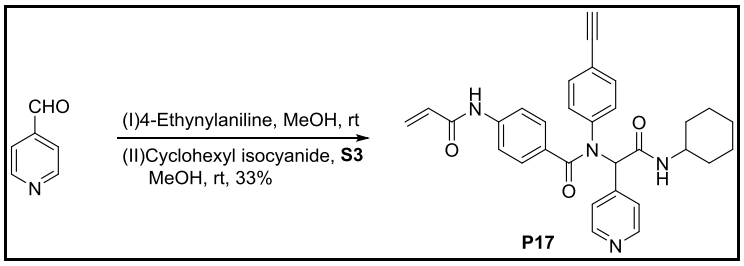
Scheme S5

(P15). To a stirred solution of 4-cyanobenzaldehyde (131.1 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 100 : 1) to give it as a white solid (120.3 mg, 23%). ^{1}H NMR (400 MHz, DMSO) δ 10.19 (s, 1H), 8.22 (d, *J* = 7.7 Hz, 1H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.37 (d, *J* = 8.3 Hz, 2H), 7.20 (d, *J* = 8.7 Hz, 2H), 7.13 (d, *J* = 8.8 Hz, 2H), 7.08 (d, *J* = 7.7 Hz, 2H), 6.42 – 6.33 (m, 2H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.15 (s, 1H), 3.66 – 3.55 (m, 1H), 1.78 – 1.48 (m, 6H), 1.30 – 1.22 (m, 2H), 1.12 – 1.00 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.8, 167.8, 163.8, 141.7, 141.5, 140.4, 132.4, 132.0, 131.8, 131.3, 131.2, 129.6, 127.8, 120.4, 119.0, 118.5, 110.9, 83.2, 82.1, 64.3, 48.5, 32.6, 32.5, 25.6, 25.0, 24.9. MS (ESI) *m/z*: 529.3 $[M - H]^-$.



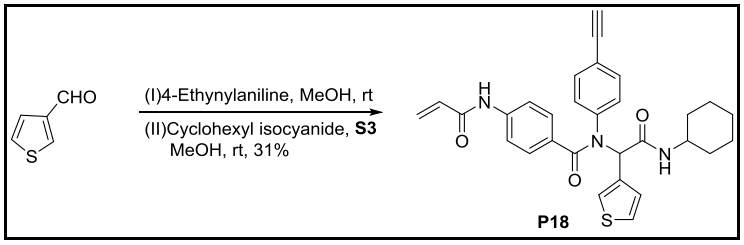
Scheme S6

(P16). To a stirred solution of 3-pyrrolecarboxaldehyde (95.1 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 60 : 1) to give it as a white solid (172.7 mg, 35%). ^{1}H NMR (400 MHz, DMSO) δ 10.55 (d, *J* = 2.2 Hz, 1H), 10.16 (s, 1H), 7.75 (d, *J* = 7.9 Hz, 1H), 7.45 (d, *J* = 8.7 Hz, 2H), 7.14 (d, *J* = 8.7 Hz, 2H), 7.11 – 7.00 (m, 4H), 6.54 – 6.45 (m, 2H), 6.38 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 6.15 (s, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 5.72 – 5.67 (m, 1H), 4.10 (s, 1H), 3.68 – 3.55 (m, 1H), 1.84 – 1.48 (m, 6H), 1.28 – 1.22 (m, 2H), 1.13 – 1.04 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.9, 169.4, 163.7, 142.3, 140.0, 132.2, 132.1, 131.3, 129.5, 127.7, 119.7, 119.0, 118.4, 118.1, 116.8, 109.4, 83.6, 81.5, 60.2, 48.4, 32.8, 32.6, 25.7, 25.2, 25.0. MS (ESI) *m/z*: 517.3 $[M + Na]^+$.



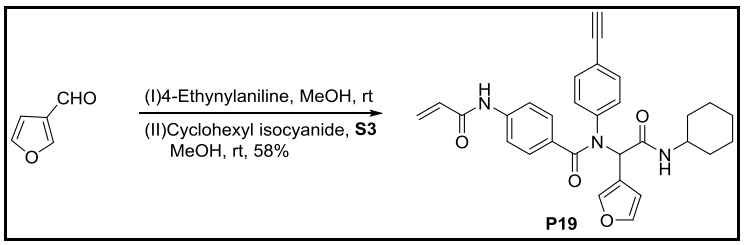
Scheme S7

(P17). To a stirred solution of 4-pyridinecarboxaldehyde (95 μL, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 60 : 1) to give it as a white solid (164.7 mg, 33%). ^{1}H NMR (400 MHz, DMSO) δ 10.20 (s, 1H), 8.41 (dd, *J* = 4.5, 1.5 Hz, 2H), 8.26 (d, *J* = 7.7 Hz, 1H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.24 – 7.20 (m, 1H), 7.19 – 7.16 (m, 1H), 7.13 (s, 4H), 6.38 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.32 (s, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.14 (s, 1H), 3.69 – 3.53 (m, 1H), 1.82 – 1.46 (m, 6H), 1.31 – 1.21 (m, 2H), 1.14 – 1.03 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.8, 167.4, 163.8, 149.8, 145.0, 141.5, 140.4, 132.0, 131.8, 131.2, 131.1, 129.7, 127.8, 125.1, 120.4, 118.5, 83.2, 82.0, 63.8, 48.6, 32.6, 32.5, 25.6, 25.0, 24.9. MS (ESI) *m/z*: 507.3 $[M + H]^+$.



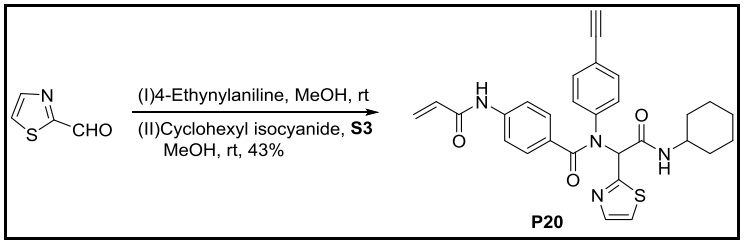
Scheme S8

(P18). To a stirred solution of 3-thiophenecarboxaldehyde (88 μL, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 2 : 1) to give it as a yellow solid (158.8 mg, 31%). ^{1}H NMR (400 MHz, DMSO) δ10.17 (s, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.47 (d, J = 8.4 Hz, 2H), 7.31 (t, 1H), 7.25 (s, 1H), 7.17 (d, J = 8.4 Hz, 2H), 7.13 – 7.01 (m, 4H), 6.78 (d, J = 5.0 Hz, 1H), 6.38 (dd, J = 17.0, 10.0 Hz, 1H), 6.32 (s, 1H), 6.23 (d, J = 16.8 Hz, 1H), 5.75 (d, J = 10.6 Hz, 1H), 4.13 (s, 1H), 3.69 – 3.58 (m, 1H), 1.81 – 1.51 (m, 6H), 1.33 – 1.20 (m, 2H), 1.15 – 1.03 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.5, 168.9, 163.7, 141.8, 140.2, 136.3, 132.1, 131.6, 131.6, 131.2, 129.6, 129.3, 127.8, 126.4, 126.2, 120.1, 118.5, 83.4, 81.8, 60.1, 48.5, 32.7, 32.6, 25.7, 25.1, 25.0. MS (ESI) *m/z*: 510.3 $[M - H]^-$.



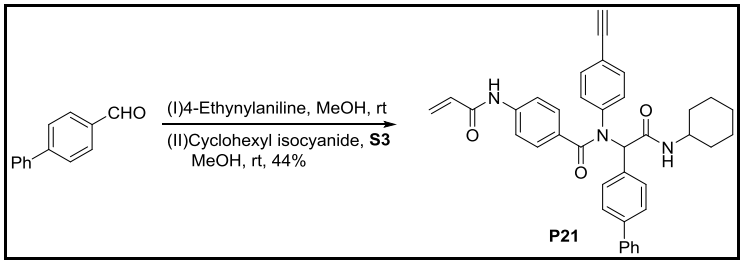
Scheme S9

(P19). To a stirred solution of 3-furaldehyde (86 μL, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 2 : 1) to give it as a yellow solid (288.4 mg, 58%). ^{1}H NMR (400 MHz, DMSO) δ10.18 (s, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 7.46 (t, *J* = 10.6 Hz, 4H), 7.20 – 7.14 (m, 6H), 6.38 (dd, *J* = 16.9, 10.0 Hz, 1H), 6.23 (d, *J* = 16.9 Hz, 1H), 6.18 (s, 1H), 6.11 (s, 1H), 5.75 (d, *J* = 10.5 Hz, 1H), 4.15 (s, 1H), 3.68 – 3.56 (m, 1H), 1.83 – 1.47 (m, 6H), 1.29 – 1.21 (m, 2H), 1.15 – 1.05 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.5, 168.5, 163.8, 143.5, 143.0, 141.7, 140.3, 132.1, 131.8, 131.6, 131.2, 129.6, 127.8, 120.5, 120.3, 118.5, 111.9, 83.4, 81.8, 56.7, 55.4, 48.5, 32.7, 32.6, 25.6, 25.1, 25.0. MS (ESI) *m/z*: 494.1 $[M - H]^-$.



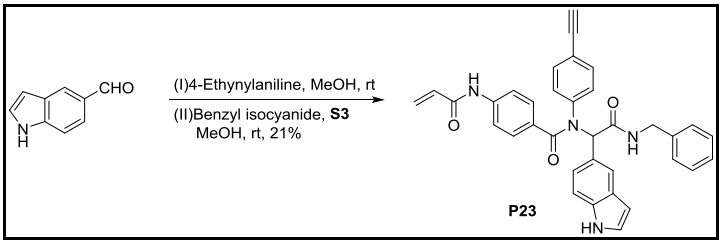
Scheme S10

(P20). To a stirred solution of 1,3-thiazole-2-carbaldehyde (88 μL, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 1 : 1) to give it as a yellow solid (221.4 mg, 43%). ^{1}H NMR (400 MHz, DMSO) δ10.23 (s, 1H), 8.46 (d, *J* = 7.8 Hz, 1H), 7.77 (d, *J* = 3.2 Hz, 1H), 7.69 (d, *J* = 3.2 Hz, 1H), 7.51(d, *J* = 8.4 Hz, 2H), 7.24 (dd, *J* = 8.6, 2.0 Hz, 4H), 7.12 (d, *J* = 8.1 Hz, 2H), 6.49 (s, 1H), 6.39 (dd, *J* = 17.0, 10.0 Hz, 1H), 6.24 (d, *J* = 16.9 Hz, 1H), 5.76 – 5.73 (m, 1H), 4.17 (s, 1H), 3.66 – 3.54 (m, 1H), 1.80 – 1.49 (m, 6H), 1.29 – 1.22 (m, 2H), 1.16 – 1.09 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.6, 166.0, 165.5, 163.8, 142.4, 142.3, 140.8, 132.1, 132.0, 130.6, 129.9, 129.8, 127.9, 122.4, 120.5, 118.6, 83.2, 82.0, 63.1, 48.6, 32.5, 32.4, 25.6, 24.9. MS (ESI) *m/z*: 535.2 $[M + Na]^+$.



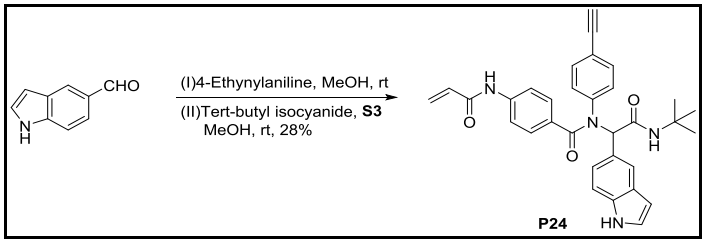
Scheme S11

(P21). To a stirred solution of 4-biphenylcarboxaldehyde (182.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 2 : 1) to give it as a white solid (256.8 mg, 44%). ^{1}H NMR (400 MHz, DMSO) δ 10.18 (s, 1H), 8.15 (d, *J* = 7.7 Hz, 1H), 7.63 – 7.59 (m, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.43 (t, *J* = 7.5 Hz, 2H), 7.34 (t, *J* = 7.3 Hz, 1H), 7.30 – 7.17 (m, 4H), 7.17 – 7.01 (m, 4H), 6.42 – 6.33 (m, 2H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.10 (s, 1H), 3.71 – 3.60 (m, 1H), 1.80 – 1.48 (m, 6H), 1.29 – 1.21 (m, 2H), 1.14 – 1.04 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.8, 168.8, 163.7, 141.8, 140.3, 139.6, 139.6, 135.1, 132.1, 131.6, 131.5, 131.0, 129.6, 129.4, 128.1, 127.8, 126.9, 126.5, 120.1, 118.5, 83.3, 81.8, 64.2, 48.5, 32.7, 32.6, 31.4, 25.1, 25.0. HR-MS (ESI) for $C_{38}H_{35}N_3O_3$ $[M + H]^+$, Calcd: 582.27512, Found: 582.27414.



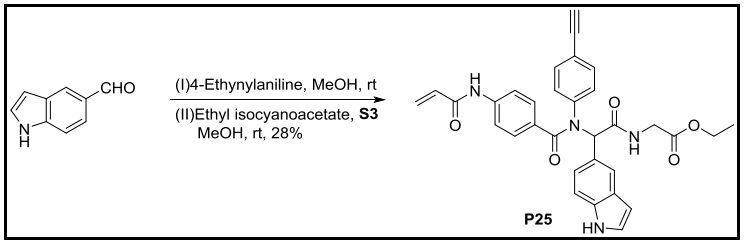
Scheme S12

(P23). To a stirred solution of 1*H*-indole-5-carbaldehyde (145.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Benzyl isocyanide (122 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 50 : 1) to give it as a white solid (116.1 mg, 21%). ^{1}H NMR (400 MHz, DMSO) δ 11.03 (s, 1H), 10.17 (s, 1H), 8.66 (t, *J* = 5.9 Hz, 1H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.34 (s, 1H), 7.30 – 7.14 (m, 10H), 7.12 – 6.93 (m, 4H), 6.83 (dd, *J* = 8.5, 1.1 Hz, 1H), 6.43 (s, 1H), 6.38 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.32 (s, 1H), 6.23 (dd, *J* = 17.0, 1.9 Hz, 1H), 5.75 (dd, *J* = 10.1, 1.9 Hz, 1H), 4.39 (ddd, *J* = 34.0, 15.3, 5.9 Hz, 2H), 4.08 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 171.0, 169.8, 163.7, 141.9, 140.1, 139.9, 135.6, 132.1, 131.9, 131.7, 131.4, 129.5, 128.6, 127.8, 127.7, 127.1, 126.2, 125.4, 123.8, 122.7, 120.0, 118.5, 111.3, 101.7, 83.4, 81.8, 65.4, 60.2, 42.8. MS (ESI) *m/z*: 575.2 $[M + Na]^+$.



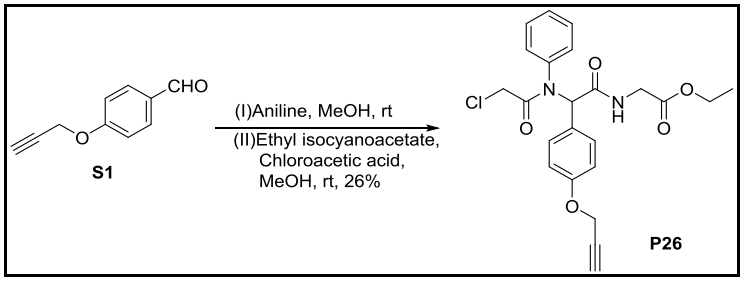
Scheme S13

(P24). To a stirred solution of 1*H*-indole-5-carbaldehyde (145.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Tert-butyl isocyanide (113 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 100 : 1) to give it as a white solid (145.5 mg, 28%). ^{1}H NMR (400 MHz, DMSO) δ 11.00 (s, 1H), 10.16 (s, 1H), 7.73 (s, 1H), 7.46 (d, *J* = 8.7 Hz, 2H), 7.35 (s, 1H), 7.29 – 7.22 (m, 1H), 7.16 (t, *J* = 8.2 Hz, 3H), 7.12 – 6.93 (m, 4H), 6.82 (dd, *J* = 8.5, 1.5 Hz, 1H), 6.42 – 6.32 (m, 3H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.74 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.06 (s, 1H), 1.28 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 170.2, 169.6, 163.7, 142.1, 140.1, 135.4, 132.1, 131.6, 131.3, 129.6, 127.7, 127.6, 126.1, 126.1, 123.7, 122.4, 119.7, 118.5, 111.2, 101.7, 83.4, 81.6, 65.4, 50.8, 29.0. MS (ESI) *m/z*: 517.2 $[M - H]^+$.



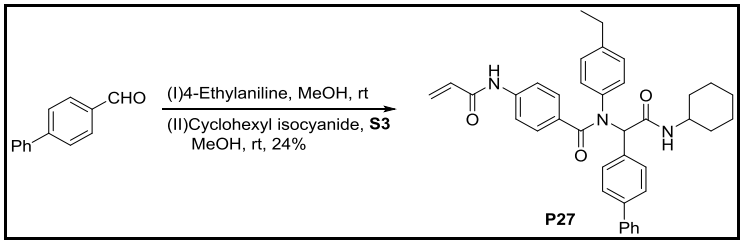
Scheme S14

(P25). To a stirred solution of 1*H*-indole-5-carbaldehyde (145.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Ethyl isocyanoacetate (109.0 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 80 : 1) to give it as a white solid (156.1 mg, 28%). ^{1}H NMR (400 MHz, DMSO) δ 11.02 (s, 1H), 10.17 (s, 1H), 8.55 (t, *J* = 5.7 Hz, 1H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.42 (s, 1H), 7.29 – 7.25 (m, 1H), 7.22 – 7.15 (m, 3H), 7.09 – 6.91 (m, 4H), 6.86 (d, *J* = 8.4 Hz, 1H), 6.53 (s, 1H), 6.41 – 6.30 (m, 2H), 6.22 (dd, *J* = 17.0, 1.9 Hz, 1H), 5.74 (dd, *J* = 10.1, 1.8 Hz, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 4.07 (s, 1H), 4.04 – 3.78 (m, 2H), 1.19 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 171.2, 170.1, 169.7, 163.7, 141.8, 140.2, 135.6, 132.1, 131.8, 131.4, 131.4, 129.6, 127.8, 127.6, 126.1, 125.2, 124.0, 122.9, 119.9, 118.5, 111.2, 101.7, 83.3, 81.8, 64.8, 60.8, 41.7, 14.6. MS (ESI) *m/z*: 571.2 $[M + Na]^+$.



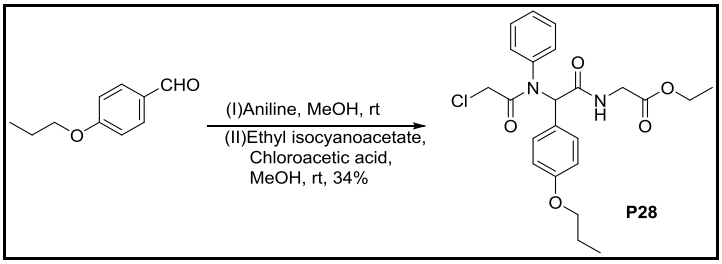
Scheme S15

(P26). To a stirred solution of **S1** (160.2 mg, 1 mmol) in 3 mL MeOH was added aniline (92 μL, 1 mmol) at rt. Ethyl isocyanoacetate (109 μL, 1 mmol) and chloroacetic acid (94.5 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 3 : 1) to give it as a brown solid (115.8 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.23 (m, 4H), 7.10 (d, *J* = 8.7 Hz, 2H), 6.81 (d, *J* = 8.8 Hz, 2H), 6.26 (s, 1H), 6.12 (s, 1H), 4.65 (d, *J* = 2.4 Hz, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 4.09 (d, *J* = 4.9 Hz, 2H), 3.99 – 3.73 (m, 2H), 2.53 (t, *J* = 2.4 Hz, 1H), 1.37 – 1.20 (m, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.3, 170.0, 165.8, 157.1, 138.6, 132.2, 131.2, 129.0, 128.7, 127.3, 114.6, 79.5, 78.6, 63.8, 60.9, 55.7, 55.4, 43.5, 41.5, 14.5. HR-MS (ESI) for $C_{23}H_{23}ClN_2O_5$ $[M + H]^+$, Calcd: 443.13683, Found: 443.13699.



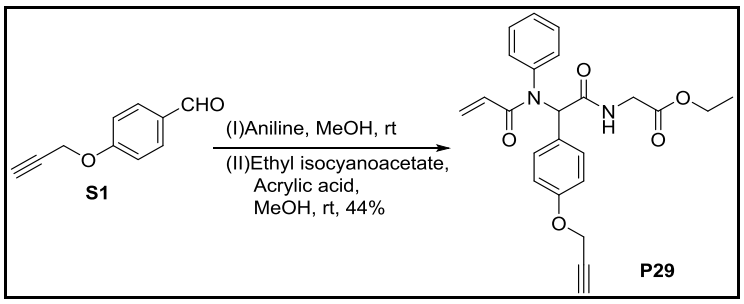
Scheme S16

(P27). To a stirred solution of 4-biphenylcarboxaldehyde (182.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethylaniline (124 μL, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 2 : 1) to give it as a white solid (138.6 mg, 24%). ^{1}H NMR (400 MHz, DMSO) δ 10.16 (s, 1H), 8.05 (d, *J* = 7.7 Hz, 1H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.51 (d, *J* = 8.2 Hz, 2H), 7.44 (q, *J* = 15.2, 8.1 Hz, 4H), 7.34 (t, *J* = 7.3 Hz, 1H), 7.22 (t, *J* = 8.5 Hz, 4H), 6.98 (s, 2H), 6.83 (d, *J* = 8.3 Hz, 2H), 6.38 (q, *J* = 16.9, 10.1 Hz, 1H), 6.27 (s, 1H), 6.23 (dd, *J* = 17.0, 1.8 Hz, 1H), 5.74 (dd, *J* = 10.1, 1.8 Hz, 1H), 3.68 – 3.55 (m, 1H), 2.38 (q, *J* = 7.5 Hz, 2H), 1.77 – 1.51 (m, 6H), 1.27 – 1.16 (m, 4H), 0.98 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.9, 168.8, 163.7, 142.5, 140.1, 139.9, 139.5, 138.8, 135.6, 132.1, 132.0, 131.1, 131.0, 129.6, 129.4, 128.0, 127.7, 127.5, 126.9, 126.4, 118.4, 64.7, 48.4, 32.7, 32.6, 27.9, 25.7, 25.1, 25.0, 15.8. HR-MS (ESI) for $C_{38}H_{39}N_3O_3$ $[M + H]^+$, Calcd: 586.30642, Found: 586.30558.



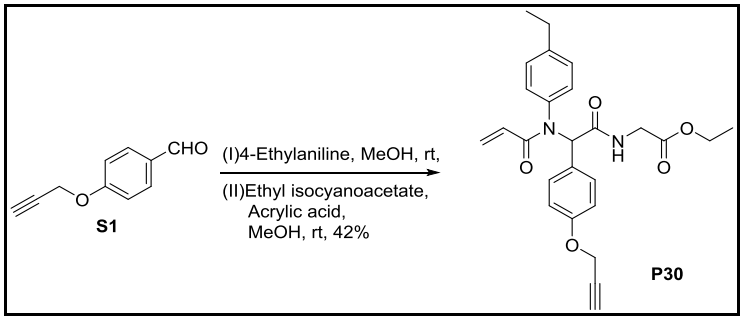
Scheme S17

(P28). To a stirred solution of 4-propoxy-benzaldehyd (158.5 μL, 1 mmol) in 3 mL MeOH was added aniline (92.0 μL, 1 mmol) at rt. Ethyl isocyanoacetate (109.0 μL, 1 mmol) and chloroacetic acid (94.5 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 3 : 1) to give it as a white solid (152.4 mg, 34%). ^{1}H NMR (400 MHz, DMSO) δ 8.53 (t, 1H), 7.94 – 7.01 (m, 5H), 6.96 (d, *J* = 8.7 Hz, 2H), 6.67 (d, *J* = 8.7 Hz, 2H), 6.10 (s, 1H), 4.09 (q, *J* = 7.1 Hz, 2H), 4.00 – 3.91 (m, 2H), 3.90 – 3.73 (m, 4H), 1.65 (q, *J* = 14.0, 6.7 Hz, 2H), 1.18 (t, *J* = 7.1 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.4, 170.0, 165.8, 158.6, 138.6, 132.2, 131.2, 129.0, 128.7, 126.4, 114.2, 69.2, 63.8, 60.8, 43.5, 41.5, 22.4, 14.5, 10.8. HR-MS (ESI) for $C_{23}H_{27}ClN_2O_5$ $[M + H]^+$, Calcd: 447.16813, Found: 447.16945.



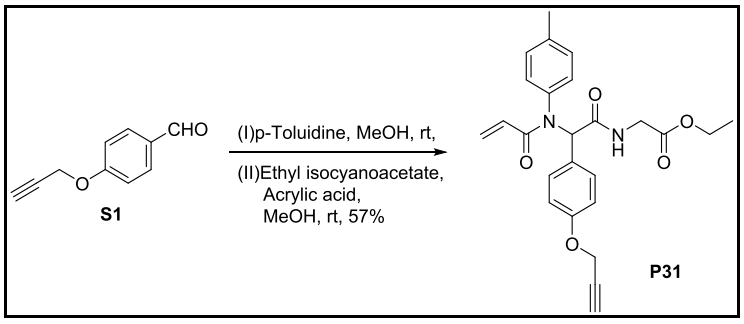
Scheme S18

(P29). To a stirred solution of **S1** (160.2 mg, 1 mmol) in 3 mL MeOH was added aniline (92 μL, 1 mmol) at rt. Ethyl isocyanoacetate (109 μL, 1 mmol) and acrylic acid (69 μL, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 3 : 1) to give it as a brown solid (183.3 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (s, 3H), 7.14 (d, J = 8.3 Hz, 2H), 7.02 (s, 1H), 6.82 (d, J = 8.3 Hz, 2H), 6.51 – 6.39 (m, 2H), 6.17 (s, 1H), 6.01 – 5.92 (m, 1H), 5.58 – 5.53 (m, 1H), 4.67 – 4.62 (m, 2H), 4.21 (q, J = 7.1 Hz, 2H), 4.13 – 4.07 (m, 2H), 2.53 (t, J = 3.8, 2.0 Hz, 1H), 1.28 (t, J = 7.1, 1.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.6, 170.1, 165.0, 157.0, 139.4, 132.2, 131.3, 129.5, 128.9, 128.2, 128.0, 127.8, 114.6, 79.5, 78.6, 63.3, 60.8, 55.7, 41.5, 14.5. MS (ESI) *m/z*: 443.3 $[M + Na]^+$.



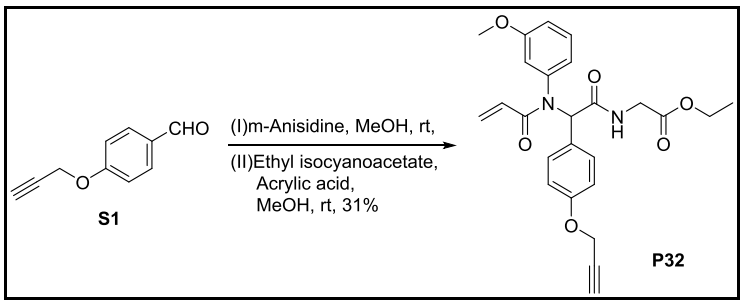
Scheme S19

(P30). To a stirred solution of **S1** (160.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethylaniline (124 μL, 1 mmol) at rt. Ethyl isocyanoacetate (109 μL, 1 mmol) and acrylic acid (69 μL, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 2 : 1) to give it as a white solid (186.4 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.3 Hz, 2H), 7.05 (d, *J* = 7.4 Hz, 2H), 6.96 (d, *J* = 8.0 Hz, 1H), 6.82 (d, *J* = 8.3 Hz, 2H), 6.54 (t, *J* = 5.4 Hz, 1H), 6.44 – 6.36 (m, 1H), 6.12 (s, 1H), 6.03 – 5.94 (m, 1H), 5.56 – 5.50 (m, 1H), 4.68 – 4.62 (m, 2H), 4.23 – 4.11 (m, 4H), 2.61 (q, *J* = 7.6 Hz, 2H), 2.52 (t, *J* = 2.6 Hz, 1H), 1.30 – 1.24 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 170.6, 170.1, 165.1, 157.0, 143.6, 137.0, 132.1, 131.1, 129.6, 128.2, 128.0, 127.9, 114.5, 79.5, 78.6, 63.2, 60.8, 55.7, 41.5, 28.0, 15.7, 14.5. MS (ESI) *m/z*: 471.0 $[M + Na]^+$.



Scheme S20

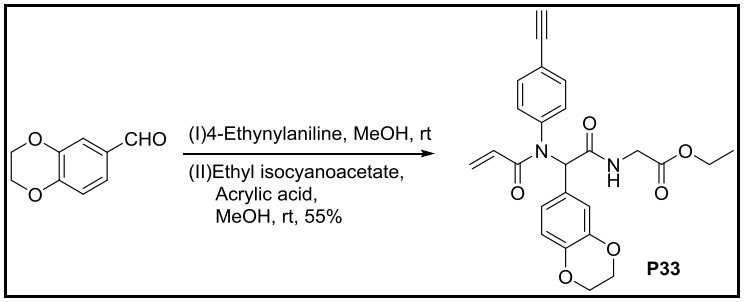
(P31). To a stirred solution of **S1** (160.2 mg, 1 mmol) in 3 mL MeOH was added p-toluidine (107.2 mg, 1 mmol) at rt. Ethyl isocyanoacetate (109 μL, 1 mmol) and acrylic acid (69 μL, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 2 : 1) to give it as a white solid (218.3 mg, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 – 7.12 (m, 2H), 7.08 – 7.00 (m, 2H), 7.00 – 6.87 (m, 1H), 6.85 – 6.80 (m, 2H), 6.51 (t, *J* = 5.4 Hz, 1H), 6.41 (dd, *J* = 16.8, 2.0 Hz, 1H), 6.13 (d, *J* = 7.7 Hz, 1H), 5.98 (dd, *J* = 16.8, 10.4 Hz, 1H), 5.53 (dd, *J* = 10.3, 2.0 Hz, 1H), 4.66 (d, *J* = 2.4 Hz, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 4.12 – 4.08 (m, 2H), 2.53 (t, *J* = 2.4 Hz, 1H), 2.32 (s, 3H), 1.28 (t, *J* = 6.9, 4.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.6, 170.1, 165.1, 157.0, 137.4, 136.8, 132.2, 131.1, 129.6, 129.4, 127.9, 127.8, 114.6, 79.6, 78.6, 63.2, 60.8, 55.7, 41.5, 21.0, 14.5. MS (ESI) *m/z*: 457.1 $[M + Na]^+$.



Scheme S21

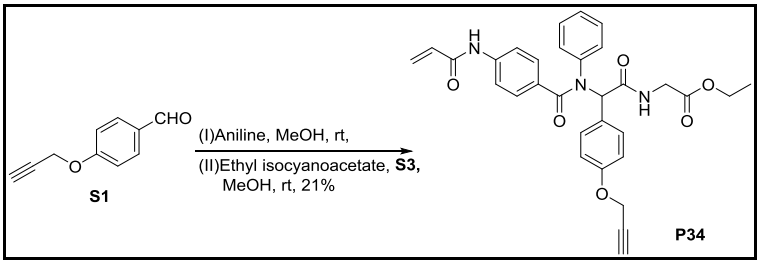
(P32). To a stirred solution of **S1** (160.2mg, 1 mmol) in 3 mL MeOH was added m-anisidine (112 μL, 1 mmol) at rt. Ethyl isocyanoacetate (109 μL, 1 mmol) and acrylic acid (69 μL, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 3 : 1) to give it as a white solid (137.8 mg, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, *J* = 8.7 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 1H), 6.87 – 6.82 (m, 2H), 6.82 – 6.78 (m, 1H), 6.75 – 6.60 (m, 1H), 6.50 (t, *J* = 5.3 Hz, 1H), 6.42 (dd, *J* = 16.8, 2.0 Hz, 1H), 6.13 (s, 1H), 6.06 – 5.97 (m, 1H), 5.58 – 5.54 (m, 1H), 4.65 (d, *J* = 2.4 Hz, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 4.12 – 4.08 (m, 2H), 3.69 (s, 3H), 2.52 (t, *J* = 2.4 Hz, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.8, 169.6, 166.1, 159.9, 157.6, 140.5, 131.8, 129.5, 128.5, 128.5, 127.0, 122.8, 115.4, 114.7, 114.6, 78.2, 75.7, 61.4, 55.7, 55.4,

41.6, 14.1. MS (ESI) *m/z*: 473.2 $[M + Na]^+$.



Scheme S22

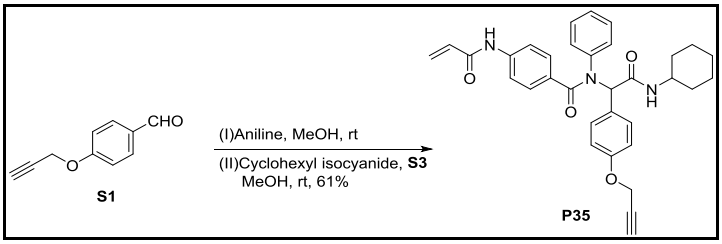
(P33). To a stirred solution of 1,4-Benzodioxin-6-carboxaldehyde (164.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Ethyl isocyanoacetate (109 μL, 1 mmol) and acrylic acid (69 μL, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 3 : 1) to give it as a white solid (246.9 mg, 55%). ^{1}H NMR (400 MHz, DMSO) δ 8.56 (t, *J* = 5.8 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.28 – 6.97 (m, 2H), 6.65 – 6.60 (m, 2H), 6.59 – 6.54 (m, 1H), 6.20 – 6.14 (m, 2H), 5.82 (dd, *J* = 16.7, 10.3 Hz, 1H), 5.57 (dd, *J* = 10.3, 2.4 Hz, 1H), 4.24 (s, 1H), 4.17 – 4.10 (m, 4H), 4.10 – 4.02 (m, 2H), 3.95 (dd, *J* = 17.3, 5.8 Hz, 1H), 3.77 (dd, *J* = 17.3, 5.6 Hz, 1H), 1.19 – 1.15 (m, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.4, 170.0, 164.8, 143.4, 143.0, 140.0, 132.2, 131.6, 129.4, 128.4, 127.6, 124.0, 121.4, 119.7, 116.9, 83.3, 82.2, 64.4, 64.3, 63.3, 60.8, 41.5, 14.5. MS (ESI) *m/z*: 471.1 $[M + Na]^+$.



Scheme S23

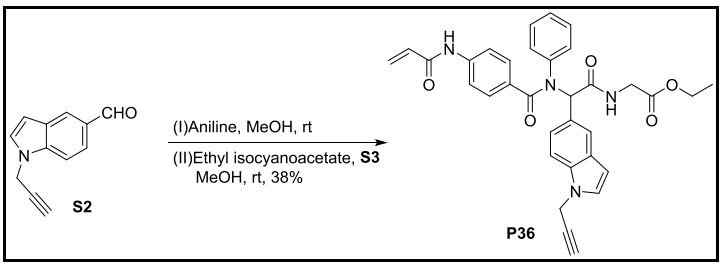
(P34). To a stirred solution of **S1** (160.2 mg, 1 mmol) in 3 mL MeOH was added aniline (92 μL, 1 mmol) at rt. Ethyl isocyanoacetate (109 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 2 : 1) to give it as a white solid (111.7 mg, 21%). ^{1}H NMR (400 MHz, DMSO) δ 10.15(s,1H),8.58(t, *J* = 5.8 Hz,1H), 7.44 (d, *J* = 8.5 Hz, 2H) , 7.19 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.5 Hz, 2H), 7.05 – 6.91 (m, 5H) , 6.77 (d, *J* = 8.4 Hz, 2H) , 6.37 (dd, *J* = 17.0, 10.0 Hz, 2H), 6.22 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.74 (dd, *J* = 17.0, 2.0 Hz, 1H), 4.71 (d, *J* = 2.3 Hz, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 4.02 (dd, *J* = 17.3, 5.8 Hz, 1H), 3.83 (dd, J = 17.2, 5.6 Hz, 1H), 3.53 (t, *J* = 2.3 Hz, 1H), 1.20 (t, *J* = 7.1 Hz, 3H). ^{13}C

NMR (101 MHz, DMSO) δ 170.7, 170.2, 169.8, 163.7, 157.0, 141.0, 140.1, 132.2, 132.1, 131.8, 131.3, 129.6, 128.3, 128.0, 127.7, 127.2, 118.4, 114.6, 79.6, 78.7, 63.9, 60.9, 55.7, 41.6, 14.6. MS (ESI) *m/z*: 538.0 $[M - H]^-$.



Scheme S24

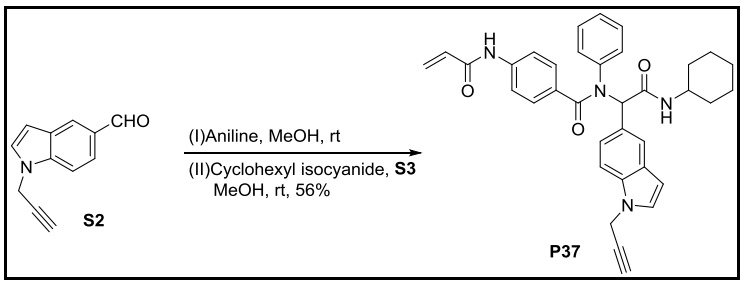
(P35). To a stirred solution of **S1** (160.2 mg, 1 mmol) in 3 mL MeOH was added aniline (92 μL, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 2 : 1) to give it as a white solid (326.0 mg, 61%). ^{1}H NMR (400 MHz, DMSO) δ10.14(s, 1H), 7.99 (d, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.16 (d, *J* = 8.4 Hz, 2H), 7.04 (d, *J* = 8.5 Hz, 2H), 7.00 – 6.90 (m, 5H), 6.76 (d, *J* = 8.3 Hz, 2H), 6.37 (dd, *J* = 16.9, 10.0 Hz, 1H), 6.22 (d, *J* = 15.7 Hz, 1H), 5.74 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.70 (d, *J* = 2.3 Hz, 2H), 3.69 – 3.57 (m, 1H), 3.53 (t, *J* = 2.4 Hz, 1H), 1.78-1.51 (m, 6H), 1.28 – 1.15 (m, 2H), 1.11 – 0.97 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.8, 169.2, 163.7, 156.9, 141.1, 140.0, 132.1, 131.7, 131.4, 129.5, 128.7, 128.1, 127.7, 127.0, 118.4, 114.6, 79.6, 78.7, 64.1, 55.7, 48.4, 32.8, 32.6, 25.7, 25.1, 25.0. MS (ESI) *m/z*: 558.2 $[M + Na]^+$.



Scheme S25

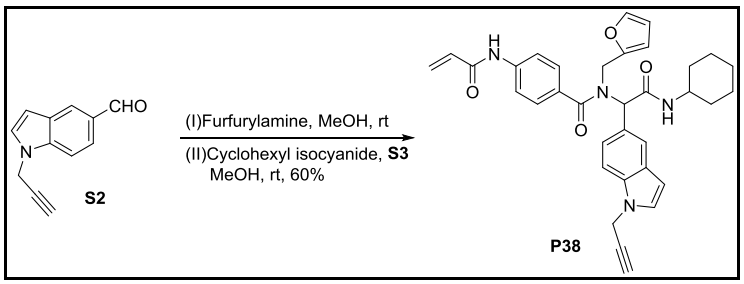
(P36). To a stirred solution of **S2** (182.2 mg, 1 mmol) in 3 mL MeOH was added aniline (92 μL, 1 mmol) at rt. Ethyl isocyanoacetate (109 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 1 : 1) to give it as a yellow solid (214.3 mg, 38%). ^{1}H NMR (400 MHz, DMSO) δ10.14 (s, 1H), 8.47 (t, *J* = 5.6 Hz, 1H), 7.44 (d, *J* = 8.1 Hz, 3H), 7.36 – 7.26 (m, 2H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.08 – 6.85 (m, 6H), 6.52 (s, 1H), 6.41 – 6.32 (m, 2H), 6.22 (d, *J* = 16.7 Hz, 1H), 5.74 (d, *J* = 10.5 Hz, 1H), 5.01 (s, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 4.01 (dd, *J* = 17.5, 6.0 Hz, 1H), 3.83 (dd, *J* = 17.2, 5.6 Hz, 1H), 1.19

(t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 171.2, 170.2, 169.9, 163.7, 141.2, 140.0, 135.3, 132.1, 132.0, 131.2, 129.6, 129.1, 128.2, 128.2, 127.7, 127.0, 126.2, 124.6, 123.4, 118.4, 109.8, 102.0, 79.6, 75.9, 64.9, 60.8, 41.7, 35.5, 14.6. MS (ESI) *m/z*: 561.2 [M - H]$^-$.



Scheme S26

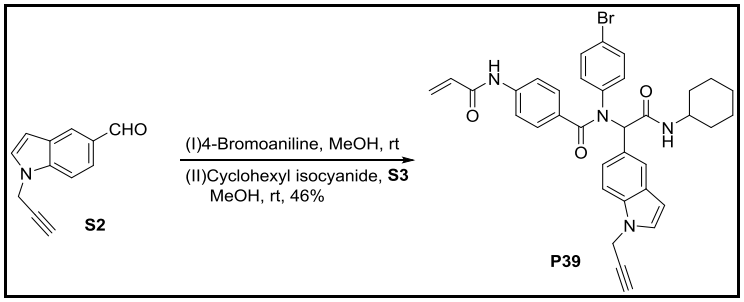
(P37). To a stirred solution of **S2** (182.2 mg, 1 mmol) in 3 mL MeOH was added aniline (92 μL, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 1 : 1) to give it as a white solid (310.0 mg, 56%). ^{1}H NMR (300 MHz, DMSO) δ10.14 (s, 1H), 7.93 (d, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 8.6 Hz, 2H), 7.35 – 7.24 (m, 3H), 7.16 (d, *J* = 8.5 Hz, 2H), 7.12 – 6.97 (m, 2H), 6.95 – 6.82 (m, 4H), 6.42 – 6.30 (m, 3H), 6.21 (dd, *J* = 17.0, 2.2 Hz, 1H), 5.73 (d, *J* = 11.1 Hz, 1H), 5.00 (d, *J* = 2.5 Hz, 2H), 3.73 – 3.57 (m, 1H), 3.36 (t, *J* = 2.6 Hz, 1H), 1.83 – 1.48 (m, 6H), 1.31 – 1.21 (m, 2H), 1.12 – 0.97 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ169.8, 169.7, 163.7, 139.9, 135.2, 132.3, 132.1, 131.4, 129.5, 129.0, 128.3, 128.0, 127.7, 126.7, 124.2, 122.9, 118.4, 109.8, 102.0, 79.6, 76.0, 65.0, 48.4, 35.5, 32.8, 32.7, 25.7, 25.2, 25.0. MS (ESI) *m/z*: 557.3 [M - H]$^-$.



Scheme S27

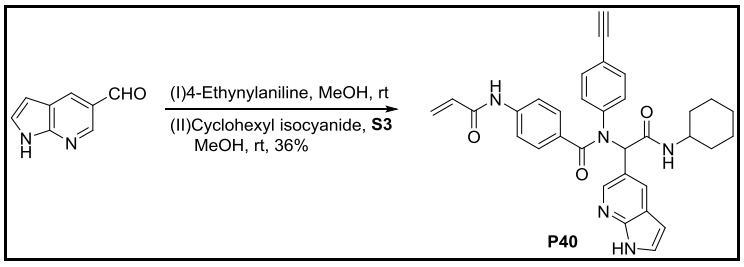
(P38). To a stirred solution of **S2** (182.2 mg, 1 mmol) in 3 mL MeOH was added furfurylamine (88 μL, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 1 : 1) to give it as a white solid (336.6 mg, 60%). ^{1}H NMR (400 MHz, DMSO) δ10.33 (s, 1H), 7.86 (d, *J* = 7.3 Hz, 1H), 7.74 (s, 2H), 7.50 – 7.37 (m, 6H), 7.24 (s, 1H), 7.06 (s, 1H), 6.51 – 6.40 (m, 2H), 6.29 (d, *J* = 17.0 Hz, 1H), 6.10 (s, 1H), 5.79 (d, *J* = 10.2 Hz, 1H), 5.63 (s, 1H), 5.07 (s, 2H), 4.62 (s, 1H), 4.35 (d, *J* = 16.3 Hz, 1H), 3.70 – 3.57 (m, 1H), 3.39 (s, 1H), 1.81 – 1.49 (m, 6H), 1.33 – 1.22 (m, 2H),

1.12 – 1.02 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.2, 163.8, 140.6, 135.5, 132.1, 131.9, 129.4, 128.7, 128.0, 127.8, 127.3, 123.1, 121.7, 119.3, 110.6, 110.4, 102.1, 79.7, 76.0, 48.1, 35.6, 32.7, 32.6, 25.6, 25.0, 25.0. MS (ESI) *m/z*: 561.3 [M - H]$^-$.



Scheme S28

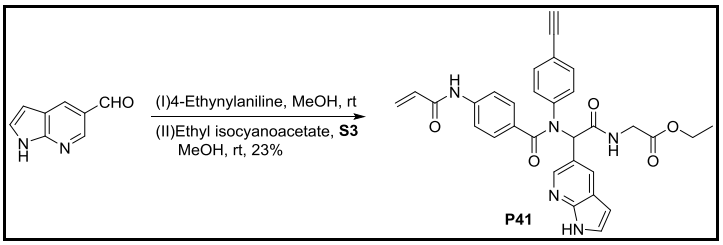
(P39). To a stirred solution of **S2** (182.2 mg, 1 mmol) in 3 mL MeOH was added 4-bromoaniline (172.0 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 1 : 1) to give it as a white solid (295.2 mg, 46%). ^{1}H NMR (400 MHz, DMSO) δ10.18 (s, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.35 (d, *J* = 3.3 Hz, 2H), 7.32 (d, *J* = 8.6 Hz, 1H), 7.18 (d, *J* = 8.7 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.08 – 6.94 (m, 2H), 6.92 (dd, *J* = 8.6, 1.5 Hz, 1H), 6.45 – 6.33 (m, 3H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.3, 2.3 Hz, 1H), 5.02 (d, *J* = 2.4 Hz, 2H), 3.72 – 3.60 (m, 1H), 3.36 (t, *J* = 2.5 Hz, 1H), 1.82 – 1.47 (m, 6H), 1.28 – 1.20 (m, 2H), 1.11 – 0.98 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.8, 169.6, 163.7, 140.7, 140.1, 135.2, 133.5, 132.1, 131.9, 130.9, 129.5, 129.2, 128.4, 127.8, 126.6, 124.1, 122.9, 119.8, 118.5, 110.0, 102.1, 79.6, 76.0, 64.7, 48.5, 35.5, 32.7, 32.6, 25.7, 25.2, 25.0. MS (ESI) *m/z*: 635.3 [M - H]$^-$.



Scheme S29

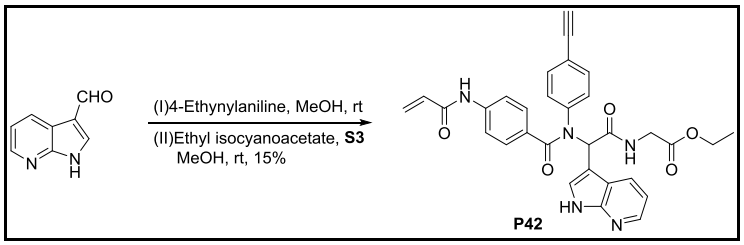
(P40). To a stirred solution of 7-azaindole-5-carbaldehyde (146.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 80 : 1) to give it as a white solid (194.6 mg, 36%). ^{1}H NMR (400 MHz, DMSO) δ 11.55 (s, 1H), 10.17 (s, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 2.0 Hz, 1H), 7.63 (d,

J = 1.8 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.39 (t, 1H), 7.19 (d, *J* = 8.7 Hz, 2H), 7.12 – 6.98 (m, 4H), 6.40 (s, 1H), 6.39 – 6.33 (m, 2H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.09 (s, 1H), 3.76 – 3.57 (m, 1H), 1.87 – 1.48 (m, 6H), 1.28 – 1.20 (m, 2H), 1.11 – 0.96 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.7, 169.2, 163.7, 148.0, 144.9, 141.7, 140.2, 132.1, 131.8, 131.7, 131.6, 130.0, 129.5, 127.8, 127.0, 122.9, 120.2, 119.3, 118.5, 100.5, 83.2, 81.9, 62.9, 48.5, 32.7, 32.6, 25.6, 25.1, 24.9. MS (ESI) *m/z*: 568.3 $[M + Na]^+$.



Scheme S30

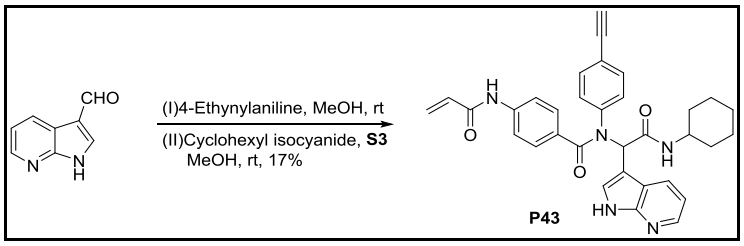
(P41). To a stirred solution of 7-azaindole-5-carbaldehyde (146.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Ethyl isocyanoacetate (109 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 80 : 1) to give it as a yellow solid (125.3 mg, 23%). ^{1}H NMR (400 MHz, DMSO) δ 11.57 (s, 1H), 10.18 (s, 1H), 8.65 (t, *J* = 5.8 Hz, 1H), 8.01 (d, *J* = 2.0 Hz, 1H), 7.72 (d, *J* = 1.7 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.40 (t, 1H), 7.21 (d, *J* = 8.6 Hz, 2H), 7.11 – 6.93 (m, 4H), 6.55 (s, 1H), 6.43 – 6.30 (m, 2H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.16 – 4.06 (m, 3H), 4.05 – 4.00 (m, 1H), 3.84 (dd, *J* = 17.3, 5.6 Hz, 1H), 1.18 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.7, 170.1, 169.7, 163.7, 148.0, 145.2, 141.6, 140.3, 132.0, 131.8, 131.5, 130.5, 129.6, 127.8, 127.0, 122.3, 120.3, 119.2, 118.5, 100.4, 83.2, 82.0, 62.8, 60.9, 41.6, 14.5. MS (ESI) *m/z*: 548.3 $[M - H]^-$.



Scheme S31

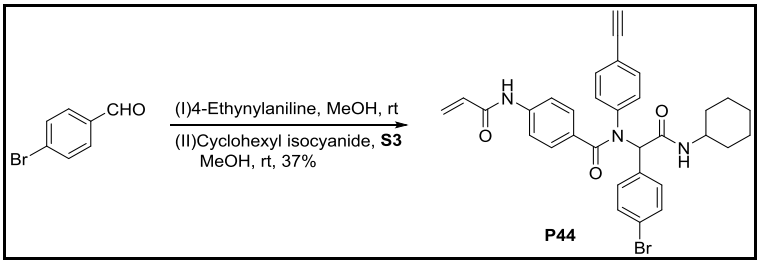
(P42). To a stirred solution of 7-azaindole-3-carbaldehyde (146.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Ethyl isocyanoacetate (109 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 80 : 1) to give it as a white solid (84.2 mg, 15%). ^{1}H NMR (400 MHz, DMSO) δ 11.57 (d, *J* = 2.4 Hz, 1H), 10.18 (s, 1H), 8.63 (t, *J* = 5.8 Hz, 1H), 8.14 (dd, *J* = 4.7, 1.5

Hz, 1H), 7.81 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.33 (d, *J* = 2.5 Hz, 1H), 7.21 (d, *J* = 8.7 Hz, 2H), 7.14 – 6.80 (m, 5H), 6.74 (s, 1H), 6.37 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.74 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.12 (qd, *J* = 7.2, 1.2 Hz, 2H), 4.07 (s, 1H), 4.07 – 4.01 (m, 1H), 3.83 (dd, *J* = 17.3, 5.6 Hz, 1H), 1.19 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.7, 170.3, 169.8, 163.7, 148.5, 143.2, 141.8, 140.3, 132.0, 131.6, 131.4, 130.7, 129.7, 127.9, 127.8, 127.2, 120.0, 119.4, 118.5, 116.0, 107.7, 83.2, 81.8, 61.0, 57.1, 41.6, 14.5. MS (ESI) *m/z*: 572.2 $[M + Na]^+$.



Scheme S32

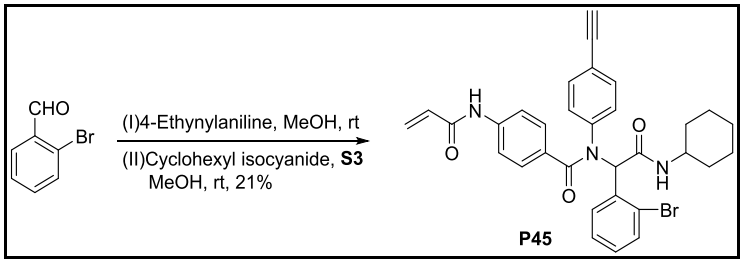
(P43). To a stirred solution of 7-azaindole-3-carbaldehyde (146.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 80 : 1) to give it as a white solid (94.2 mg, 17%). ^{1}H NMR (400 MHz, DMSO) δ 11.52 (d, *J* = 2.3 Hz, 1H), 10.17 (s, 1H), 8.12 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.75 (d, *J* = 6.8 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.19 (d, *J* = 8.5 Hz, 3H), 7.11 – 6.81 (m, 5H), 6.55 (s, 1H), 6.37 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.74 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.06 (s, 1H), 3.73 – 3.60 (m, 1H), 1.84 – 1.47 (m, 6H), 1.32 – 1.19 (m, 2H), 1.12 – 0.96 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.6, 169.1, 163.7, 148.6, 143.2, 141.9, 140.2, 132.1, 131.8, 131.3, 131.0, 129.6, 127.8, 127.4, 127.2, 119.9, 119.4, 118.5, 115.9, 108.3, 83.3, 81.7, 57.5, 48.5, 32.8, 32.6, 25.6, 25.1, 25.0. MS (ESI) *m/z*: 568.0 $[M + Na]^+$.



Scheme S33

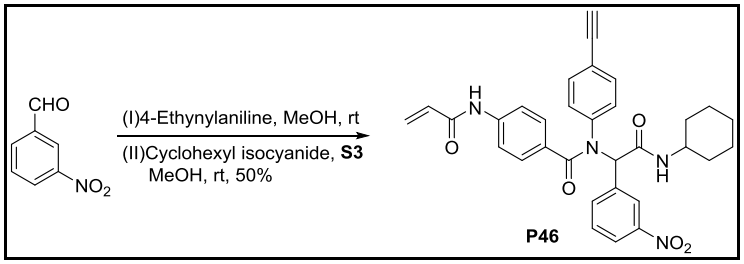
(P44). To a stirred solution of 4-bromobenzaldehyde (185.0 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column

(DCM : MeOH = 60 : 1) to give it as a white solid (214.2 mg, 37%). ^{1}H NMR (400 MHz, DMSO) δ 10.18 (s, 1H), 8.14 (d, *J* = 7.7 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.40 (d, *J* = 8.5 Hz, 2H), 7.18 (d, *J* = 8.7 Hz, 2H), 7.15 – 7.01 (m, 6H), 6.38 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.27 (s, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.14 (s, 1H), 3.72 – 3.52 (m, 1H), 1.82 – 1.47 (m, 6H), 1.30 – 1.20 (m, 2H), 1.12 – 0.98 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.7, 168.5, 163.7, 141.6, 140.3, 135.4, 132.6, 132.0, 131.7, 131.5, 131.4, 129.6, 127.8, 121.5, 120.3, 118.5, 83.3, 82.0, 63.9, 60.2, 48.5, 32.7, 32.6, 25.6, 25.0, 24.9. MS (ESI) *m/z*: 582.2 $[M - H]^-$.



Scheme S34

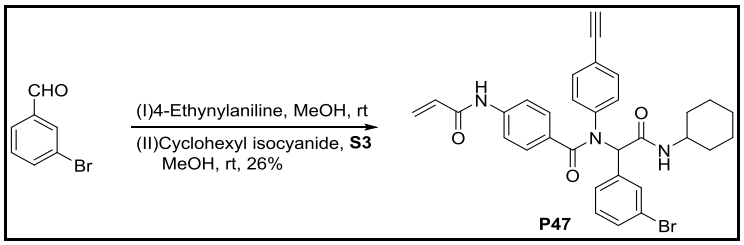
(P45). To a stirred solution of 2-bromobenzaldehyde (185.0 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 60 : 1) to give it as a yellow solid (124.6 mg, 21%). ^{1}H NMR (400 MHz, DMSO) δ 10.19 (s, 1H), 8.34 (d, *J* = 7.6 Hz, 1H), 7.54 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.49 (d, *J* = 8.6 Hz, 2H), 7.22 – 7.03 (m, 9H), 6.50 (s, 1H), 6.38 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 3.72 – 3.62 (m, 1H), 1.82 – 1.50 (m, 6H), 1.27 – 1.19 (m, 2H), 1.13 – 0.99 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.5, 168.4, 163.7, 141.1, 140.3, 135.6, 133.1, 132.0, 131.9, 131.6, 131.5, 130.7, 130.5, 129.4, 127.8, 127.8, 126.3, 120.3, 118.6, 83.2, 81.9, 64.3, 48.6, 32.6, 32.5, 25.6, 25.1, 24.9. MS (ESI) *m/z*: 606.1 $[M + Na]^+$.



Scheme S35

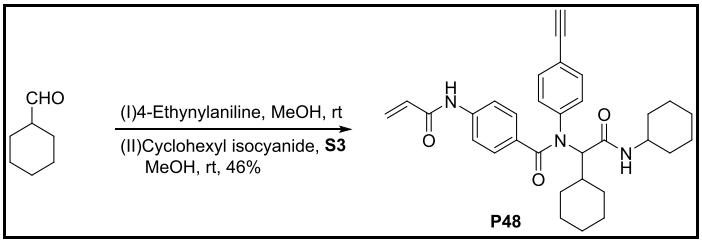
(P46). To a stirred solution of 3-nitrobenzaldehyde (151.1 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (PE : EA = 2 :

1) to give it as a yellow solid (274.8 mg, 50%). ^{1}H NMR (400 MHz, DMSO) δ 10.19 (s, 1H), 8.23 (dd, *J* = 19.0, 7.8 Hz, 1H), 8.07 (ddd, *J* = 8.1, 2.2, 0.9 Hz, 1H), 8.03 (t, *J* = 1.8 Hz, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.51 – 7.46 (m, 2H), 7.25 – 7.19 (m, 2H), 7.14 – 7.07 (m, 4H), 6.41 (s, 1H), 6.39 – 6.33 (m, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.13 (s, 1H), 3.70 – 3.59 (m, 1H), 1.81 – 1.46 (m, 6H), 1.33 – 1.21 (m, 2H), 1.12 – 1.01 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.8, 167.9, 163.8, 147.7, 141.4, 140.4, 138.2, 137.1, 132.0, 131.9, 131.4, 131.2, 130.0, 129.6, 127.8, 125.0, 123.2, 120.5, 118.5, 83.1, 82.0, 63.9, 48.5, 32.6, 32.5, 25.6, 25.0, 24.9. MS (ESI) *m/z*: 549.3 $[M - H]^-$.



Scheme S36

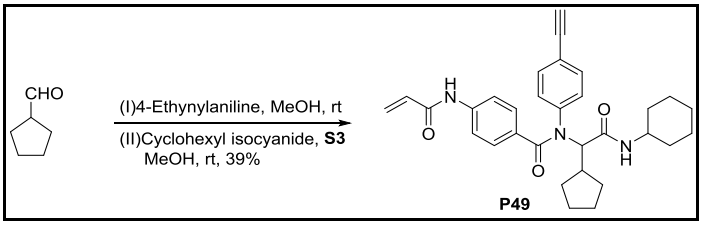
(P47). To a stirred solution of 3-bromobenzaldehyde (117 μL, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 60 : 1) to give it as a white solid (153.8 mg, 26%). ^{1}H NMR (400 MHz, DMSO) δ 10.18 (s, 1H), 8.18 (d, *J* = 7.7 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.37 (dt, *J* = 7.4, 1.7 Hz, 1H), 7.33 (s, 1H), 7.23 – 7.03 (m, 8H), 6.38 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.27 (s, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.14 (s, 1H), 3.69 – 3.58 (m, 1H), 1.83 – 1.44 (m, 6H), 1.28 – 1.19 (m, 2H), 1.15 – 1.05 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.7, 168.3, 163.7, 141.5, 140.3, 138.5, 133.2, 132.0, 131.7, 131.5, 131.0, 130.5, 129.6, 129.4, 127.8, 121.5, 120.3, 118.5, 83.3, 82.0, 64.0, 48.5, 32.6, 32.5, 25.6, 25.0, 24.9. MS (ESI) *m/z*: 606.1 $[M + Na]^+$.



Scheme S37

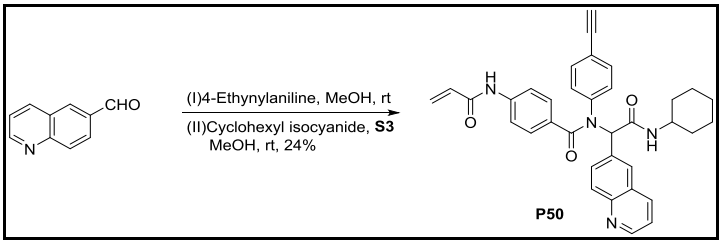
(P48). To a stirred solution of cyclohexanecarboxaldehyde (121 μL, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 100 : 1) to give it as a white solid (231.7 mg, 46%). ^{1}H NMR (400 MHz, DMSO)

δ 10.18 (s, 1H), 8.02 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.30 (d, *J* = 8.7 Hz, 2H), 7.20 (d, *J* = 7.9 Hz, 2H), 7.14 (d, *J* = 8.7 Hz, 2H), 6.38 (dd, *J* = 17.0, 10.0 Hz, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.93 (d, *J* = 10.7 Hz, 1H), 4.20 (s, 1H), 3.51 – 3.40 (m, 1H), 1.86 (s, 1H), 1.71 – 1.49 (m, 10H), 1.23 – 1.04 (m, 10H). ^{13}C NMR (101 MHz, DMSO) δ 170.3, 168.0, 163.7, 141.9, 140.3, 132.1, 132.0, 131.8, 130.3, 129.6, 127.8, 120.3, 118.6, 83.3, 82.0, 65.4, 48.1, 37.2, 32.7, 32.6, 30.2, 29.9, 26.4, 25.8, 25.6, 25.0. MS (ESI) *m/z*: 510.2 [M - H]$^-$.



Scheme S38

(P49). To a stirred solution of cyclopentanecarbaldehyde (107 μL, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 100 : 1) to give it as a yellow solid (196.2 mg, 39%). ^{1}H NMR (400 MHz, DMSO) δ 10.18 (s, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.35 – 7.23 (m, 4H), 7.12 (d, *J* = 8.7 Hz, 2H), 6.38 (dd, *J* = 17.0, 10.0 Hz, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.76 – 5.73 (m, 1H), 5.00 (d, *J* = 11.4 Hz, 1H), 4.20 (s, 1H), 3.59 – 3.48 (m, 1H), 2.27 – 2.15 (m, 1H), 1.75 – 1.51 (m, 8H), 1.47 – 1.14 (m, 10H). ^{13}C NMR (101 MHz, DMSO) δ 170.2, 169.1, 163.7, 141.7, 140.2, 132.1, 132.0, 131.8, 130.6, 129.6, 127.8, 120.4, 118.5, 83.3, 82.0, 64.7, 55.4, 48.2, 32.7, 32.6, 30.5, 30.0, 25.7, 25.6, 25.2, 25.1. MS (ESI) *m/z*: 496.2 [M - H]$^-$.

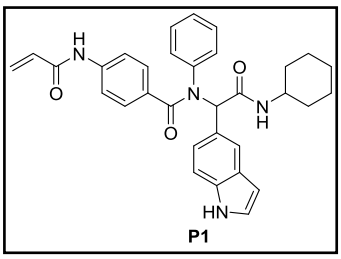


Scheme S39

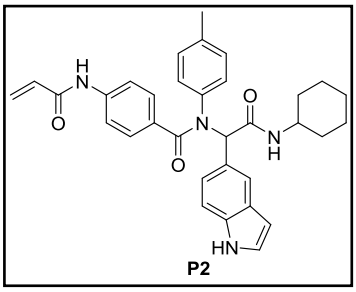
(P50). To a stirred solution of 6-quinolinecarbaldehyde (157.2 mg, 1 mmol) in 3 mL MeOH was added 4-ethynylaniline (117.2 mg, 1 mmol) at rt. Cyclohexyl isocyanide (124.0 μL, 1 mmol) and **S3** (191.2 mg, 1 mmol) were added after 2 h. The resulting mixture was stirred at room temperature for 48 h and then solvent was evaporated, followed by addition of water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and brine. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (DCM : MeOH = 100 : 1) to give it as a white solid (133.6 mg, 24%). ^{1}H NMR (400 MHz, DMSO) δ 10.18 (s, 1H), 8.85 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.31 (d, *J* = 7.5 Hz, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 7.83 – 7.76 (m, 2H), 7.49 (dd, *J* = 8.5, 3.2 Hz, 4H), 7.22 (d, *J* = 8.7 Hz, 2H), 7.18 – 7.06

(m, 2H), 7.02 (d, *J* = 8.6 Hz, 2H), 6.54 (s, 1H), 6.38 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.07 (s, 1H), 3.75 – 3.64 (m, 1H), 1.86 – 1.48 (m, 6H), 1.32 – 1.19 (m, 2H), 1.15 – 1.01 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.8, 168.6, 163.7, 151.4, 147.3, 141.6, 140.3, 136.6, 134.3, 132.0, 131.7, 131.6, 131.5, 131.4, 129.8, 129.6, 128.9, 127.8, 127.7, 122.2, 120.2, 118.5, 83.2, 81.9, 64.3, 48.5, 32.7, 32.6, 25.6, 25.1, 25.0. MS (ESI) *m/z*: 555.2 $[M - H]^-$.

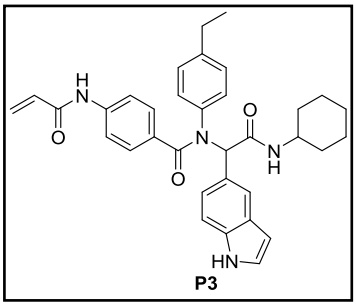
Synthetic procedures were similar to previously reported procedures[4, 5].



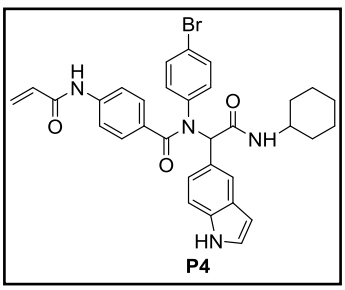
(P1). A white solid (321.1 mg, 62%), R_f = 0.3 (PE : EA = 1 : 1). ^{1}H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 10.13 (s, 1H), 7.90 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 8.7 Hz, 2H), 7.33 (s, 1H), 7.26 – 7.23 (m, 1H), 7.19 – 7.10 (m, 3H), 7.08 – 6.93 (m, 2H), 6.93 – 6.83 (m, 3H), 6.80 (dd, *J* = 8.5, 1.6 Hz, 1H), 6.41 – 6.30 (m, 3H), 6.22 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.74 (dd, *J* = 10.0, 2.0 Hz, 1H), 3.72 – 3.60 (m, 1H), 1.83 – 1.48 (m, 6H), 1.31 – 1.21 (m, 2H), 1.12 – 0.99 (m, 2H). HR-MS (ESI) for $C_{32}H_{32}N_4O_3$ $[M + H]^+$, Calcd: 521.2547, Found: 521.2533.



(P2). A white solid (95.4 mg, 36%), R_f = 0.3 (PE : EA = 1 : 1). ^{1}H NMR (400 MHz, DMSO) δ 10.98 (s, 1H), 10.14 (s, 1H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 8.7 Hz, 2H), 7.34 (s, 1H), 7.28 – 7.23 (m, 1H), 7.19 – 7.11 (m, 3H), 7.06 – 6.83 (m, 2H), 6.81 (dd, *J* = 8.5, 1.4 Hz, 1H), 6.70 (d, *J* = 8.0 Hz, 2H), 6.42 – 6.30 (m, 3H), 6.22 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.74 (dd, *J* = 10.0, 2.0 Hz, 1H), 3.70 – 3.60 (m, 1H), 2.02 (s, 3H), 1.82 – 1.49 (m, 6H), 1.29 – 1.20 (m, 2H), 1.13 – 0.97 (m, 2H). HR-MS (ESI) for $C_{33}H_{34}N_4O_3$ $[M + H]^+$, Calcd: 535.2704, Found: 535.2687.

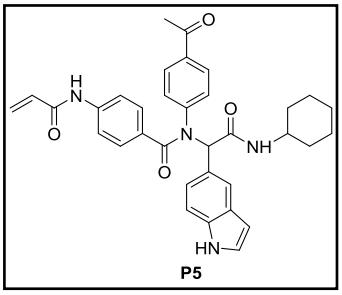


(P3). A white solid (68.4 mg, 25%), R_f = 0.3 (PE : EA = 2 : 1). ^{1}H NMR (400 MHz, DMSO) δ 10.98 (s, 1H), 10.14 (s, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 8.7 Hz, 2H), 7.34 (s, 1H), 7.30 – 7.23 (m, 1H), 7.19 – 7.11 (m, 3H), 7.01 – 6.84 (m, 2H), 6.81 (dd, *J* = 8.5, 1.5 Hz, 1H), 6.74 (d, *J* = 8.2 Hz, 2H), 6.42 – 6.31 (m, 2H), 6.30 (s, 1H), 6.22 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.74 (dd, *J* = 10.0, 2.0 Hz, 1H), 3.70 – 3.58 (m, 1H), 2.34 (q, *J* = 7.5 Hz, 2H), 1.80 – 1.49 (m, 6H), 1.27 – 1.20 (m, 2H), 1.13 – 0.99 (m, 2H), 0.95 (t, *J* = 7.6 Hz, 3H). HR-MS (ESI) for $C_{34}H_{36}N_4O_3$ $[M + H]^+$, Calcd: 549.2860, Found: 549.2845.

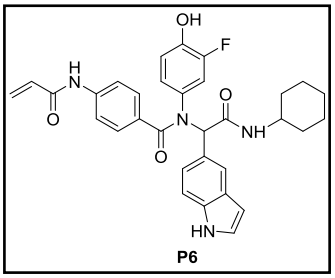


(P4). A white solid (132.9 mg, 22%), R_f = 0.3 (DCM : MeOH = 20 : 1). ^{1}H NMR (400 MHz, DMSO) δ 11.02 (s, 1H), 10.17 (s, 1H), 7.98 (d, *J* = 7.8 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.34 (s, 1H), 7.30 – 7.25 (m, 1H), 7.17 (dd, *J* = 8.8, 2.2 Hz, 3H), 7.13 – 6.86 (m, 4H), 6.80 (dd, *J* = 8.5, 1.5 Hz, 1H), 6.42 – 6.33 (m, 3H),

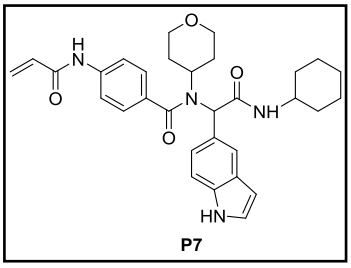
6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.76 – 5.72 (m, 1H), 3.71 – 3.61 (m, 1H), 1.82 – 1.50 (m, 6H), 1.28 – 1.21 (m, 2H), 1.11 – 0.98 (m, 2H). HR-MS (ESI) for $C_{32}H_{31}BrN_4O_3$ $[M + H]^+$, Calcd: 599.1652, Found: 599.1646.



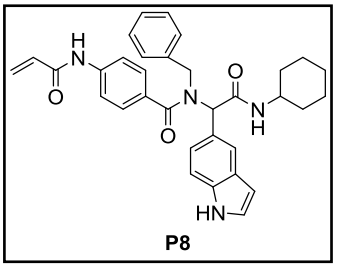
(P5). A white solid (255.8 mg, 45%), R_f = 0.3 (DCM : MeOH = 20 : 1). ^{1}H NMR (400 MHz, DMSO) δ 11.01 (s, 1H), 10.17 (s, 1H), 8.01 (d, *J* = 7.8 Hz, 1H), 7.50 – 7.44 (m, 4H), 7.37 (s, 1H), 7.28 – 7.25 (m, 1H), 7.22 – 7.13 (m, 5H), 6.83 (dd, *J* = 8.5, 1.5 Hz, 1H), 6.45 (s, 1H), 6.41 – 6.32 (m, 2H), 6.22 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.74 (dd, *J* = 10.0, 2.0 Hz, 1H), 3.73 – 3.63 (m, 1H), 2.36 (s, 3H), 1.83 – 1.50 (m, 6H), 1.29 – 1.20 (m, 2H), 1.12 – 1.01 (m, 2H). HR-MS (ESI) for $C_{34}H_{34}N_4O_4$ $[M + H]^+$, Calcd: 563.2653, Found: 563.2637.



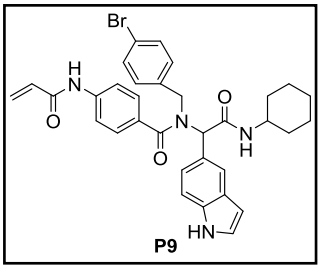
(P6). A white solid (298.3 mg, 54%), R_f = 0.3 (DCM : MeOH = 20 : 1). ^{1}H NMR (400 MHz, DMSO) δ 11.00 (s, 1H), 10.15 (s, 1H), 9.60 (s, 1H), 7.93 (d, *J* = 7.7 Hz, 1H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.32 (s, 1H), 7.28 – 7.24 (m, 1H), 7.20 – 7.14 (m, 3H), 7.08 – 6.78 (m, 2H), 6.43 – 6.25 (m, 5H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.74 (dd, *J* = 10.1, 2.0 Hz, 1H), 3.71 – 3.60 (m, 1H), 1.82 – 1.49 (m, 6H), 1.30 – 1.21 (m, 2H), 1.12 – 0.97 (m, 2H). HR-MS (ESI) for $C_{32}H_{31}FN_4O_4$ $[M + H]^+$, Calcd: 555.2402, Found: 555.2388.



(P7). A white solid (214.6 mg, 40%), R_f = 0.3 (DCM : MeOH = 20 : 1). ^{1}H NMR (400 MHz, DMSO) δ 11.14 (s, 1H), 10.34 (s, 1H), 7.76 (d, *J* = 8.6 Hz, 3H), 7.41 – 7.31 (m, 5H), 6.94 (s, 1H), 6.50 – 6.42 (m, 2H), 6.29 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.80 (dd, *J* = 10.1, 2.0 Hz, 1H), 5.43 (s, 1H), 3.90 – 3.82 (m, 1H), 3.76 – 3.67 (m, 1H), 3.63 – 3.43 (m, 2H), 3.17 (t, *J* = 11.4 Hz, 1H), 2.69 – 2.53 (m, 2H), 2.35 – 2.16 (m, 1H), 1.87 – 1.46 (m, 6H), 1.38 – 1.20 (m, 3H), 1.13 – 1.01 (m, 3H). HR-MS (ESI) for $C_{31}H_{36}N_4O_4$ $[M + H]^+$, Calcd: 529.2809, Found: 529.2802.

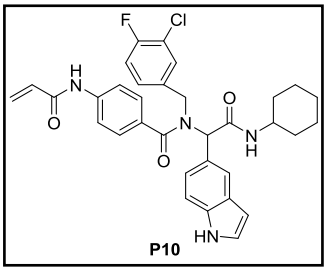


(P8). A white solid (212.4 mg, 40%), R_f = 0.3 (DCM : MeOH = 20 : 1). ^{1}H NMR (400 MHz, DMSO) δ 11.09 (s, 1H), 10.34 (s, 1H), 7.88 (d, *J* = 6.5 Hz, 1H), 7.83 – 7.64 (m, 2H), 7.52 – 7.35 (m, 3H), 7.33 – 7.31 (m, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.13 – 6.88 (m, 6H), 6.49 – 6.39 (m, 2H), 6.29 (d, *J* = 16.8 Hz, 1H), 5.79 (d, *J* = 11.1 Hz, 1H), 5.75 – 5.49 (m, 1H), 4.70 (d, *J* = 15.8 Hz, 1H), 4.40 – 4.22 (m, 1H), 3.74 – 3.59 (m, 1H), 1.82 – 1.50 (m, 6H), 1.32 – 1.23 (m, 2H), 1.11 – 1.00 (m, 2H). HR-MS (ESI) for $C_{33}H_{34}N_4O_3$ $[M + H]^+$, Calcd: 535.2704, Found: 535.2691.

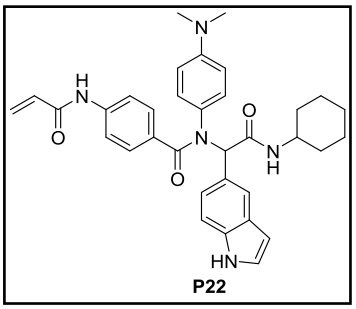


(P9). A white solid (325.6 mg, 53%), R_f = 0.3 (DCM : MeOH = 20 : 1). ^{1}H NMR (400 MHz, DMSO) δ 11.11 (s, 1H), 10.36 (s, 1H), 7.92

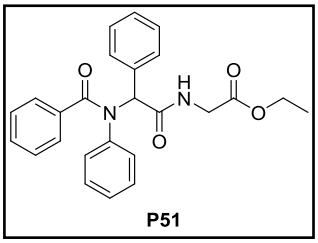
(s, 1H), 7.85 – 7.67 (m, 2H), 7.52 – 7.35 (m, 3H), 7.35 – 7.32 (m, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.26 – 7.18 (m, 2H), 7.12 – 6.82 (m, 3H), 6.50 – 6.38 (m, 2H), 6.29 (d, *J* = 16.7 Hz, 1H), 5.79 (d, *J* = 10.8 Hz, 1H), 5.61 (s, 1H), 4.63 (d, *J* = 15.8 Hz, 1H), 4.32 – 4.14 (m, 1H), 3.76 – 3.62 (m, 1H), 1.84 – 1.49 (m, 6H), 1.33 – 1.23 (m, 2H), 1.12 – 1.00 (m, 2H). HR-MS (ESI) for $C_{33}H_{33}BrN_4O_3$ $[M + H]^+$, Calcd: 613.1809, Found: 613.1798.



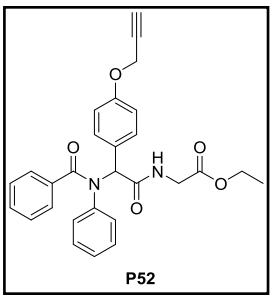
(P10). A white solid (63.9 mg, 22%), R_f = 0.3 (PE : EA = 1 : 1). ^{1}H NMR (400 MHz, DMSO) δ 11.12 (s, 1H), 10.37 (s, 1H), 7.94 (s, 1H), 7.88 – 7.68 (m, 2H), 7.67 – 7.25 (m, 6H), 7.15 – 6.96 (m, 3H), 6.84 (s, 1H), 6.40 (s, 1H), 6.29 (d, *J* = 16.8 Hz, 1H), 5.79 (d, *J* = 10.6 Hz, 1H), 5.59 (s, 1H), 4.68 (d, *J* = 14.3 Hz, 1H), 4.21 (d, *J* = 13.7 Hz, 1H), 3.77 – 3.64 (m, 1H), 1.88 – 1.50 (m, 6H), 1.35 – 1.23 (m, 2H), 1.14 – 1.02 (m, 2H). HR-MS (ESI) for $C_{33}H_{32}ClFN_4O_3$ $[M + H]^+$, Calcd: 587.2220, Found: 587.2207.



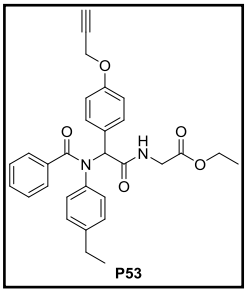
(P22). A white solid (92.5 mg, 33%), R_f = 0.3 (DCM : MeOH = 20 : 1). ^{1}H NMR (400 MHz, DMSO) δ 10.98 (s, 1H), 10.13 (s, 1H), 7.78 (d, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 8.6 Hz, 2H), 7.35 (s, 1H), 7.29 – 7.23 (m, 1H), 7.20 – 7.12 (m, 3H), 6.98 – 6.66 (m, 3H), 6.37 (dd, *J* = 17.0, 10.1 Hz, 2H), 6.27 – 6.17 (m, 4H), 5.74 (dd, *J* = 10.1, 1.9 Hz, 1H), 3.69 – 3.58 (m, 1H), 2.69 (s, 6H), 1.79 – 1.51 (m, 6H), 1.29 – 1.19 (m, 2H), 1.10 – 0.98 (m, 2H). HR-MS (ESI) for $C_{34}H_{37}N_5O_3$ $[M + H]^+$, Calcd: 564.2962, Found: 564.2956.



(P51). A white solid (126.8 mg, 30%), R_f = 0.3 (PE : EA = 1 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (t, *J* = 6.3 Hz, 4H), 7.22 – 7.17 (m, 4H), 7.15 – 7.09 (m, 1H), 7.08 – 7.02 (m, 2H), 7.00 – 6.89 (m, 5H), 6.49 – 6.44 (m, 1H), 6.17 (s, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 4.06 (d, *J* = 5.0 Hz, 2H), 1.20 (t, *J* = 7.0 Hz, 3H). HR-MS (ESI) for $C_{25}H_{24}N_2O_4$ $[M + H]^+$, Calcd:417.1809, Found:417.1795.

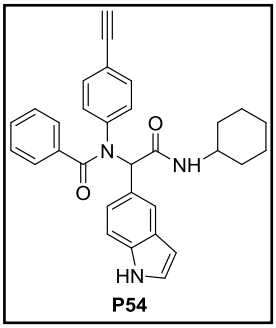


(P52). A white solid (165.2 mg, 35%), R_f = 0.3 (PE : EA = 1 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, *J* = 7.7 Hz, 2H), 7.28 (s, 1H), 7.26 (d, *J* = 8.3 Hz, 2H), 7.20 (t, *J* = 7.0 Hz, 1H), 7.14 (t, *J* = 7.5 Hz, 2H), 7.07 – 7.02 (m, 3H), 7.01 – 6.98 (m, 1H), 6.87 (d, *J* = 8.3 Hz, 2H), 6.55 (t, *J* = 5.3 Hz, 1H), 6.25 (s, 1H), 4.67 (s, 2H), 4.22 (q, *J* = 7.2 Hz, 2H), 4.14 (d, *J* = 6.8 Hz, 2H), 2.53 (s, 1H), 1.32 – 1.27 (m, 3H). HR-MS (ESI) for $C_{28}H_{26}N_2O_5$ $[M + H]^+$, Calcd: 471.1914, Found: 471.1917.



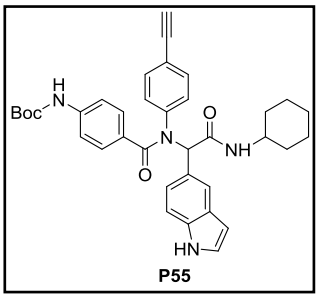
(P53). A white solid (143.9 mg, 29%), R_f = 0.3 (PE : EA = 1 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 7.7 Hz, 2H), 7.27 (d, *J* = 10.0 Hz, 2H), 7.20 (d, *J* = 6.8 Hz, 1H), 7.17 – 7.11 (m, 2H), 6.92 – 6.85 (m, 6H), 6.61 –

6.56 (m, 1H), 6.19 (s, 1H), 4.67 (d, *J* = 2.4 Hz, 2H), 4.22 (q, *J* = 7.2 Hz, 2H), 4.16 – 4.11 (m, 2H), 2.54 – 2.52 (m, 1H), 2.53 – 2.45 (m, 2H), 1.30 (t, *J* = 7.1 Hz, 3H), 1.11 (t, *J* = 7.5 Hz, 3H). HR-MS (ESI) for $C_{30}H_{30}N_2O_5$ $[M + H]^+$, Calcd: 499.2227, Found: 499.2207.



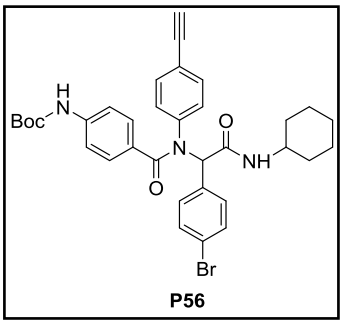
P54

(P54). A white solid (272.8 mg, 57%), R_f = 0.3 (PE : EA = 2 : 1). ^{1}H NMR (400 MHz, DMSO) δ 11.01 (s, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.35 (s, 1H), 7.28 – 7.24 (m, 1H), 7.22 – 7.13 (m, 6H), 7.11 – 6.94 (m, 4H), 6.81 (dd, *J* = 8.5, 1.6 Hz, 1H), 6.39 (s, 1H), 6.35 – 6.32 (m, 1H), 4.05 (s, 1H), 3.73 – 3.62 (m, 1H), 1.82 – 1.50 (m, 6H), 1.30 – 1.22 (m, 2H), 1.12 – 0.98 (m, 2H). HR-MS (ESI) for $C_{31}H_{29}N_3O_2$ $[M + H]^+$, Calcd: 476.2333, Found: 476.2318.



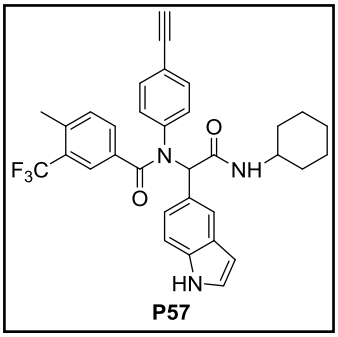
P55

(P55). A white solid (215.2 mg, 36%), R_f = 0.3 (PE : EA = 2 : 1). ^{1}H NMR (400 MHz, DMSO) δ 11.01 (s, 1H), 9.43 (s, 1H), 7.96 (d, *J* = 7.8 Hz, 1H), 7.33 (s, 1H), 7.26 (dd, *J* = 5.8, 3.0 Hz, 2H), 7.22 (s, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 7.09 (d, *J* = 8.8 Hz, 2H), 7.06 – 6.88 (m, 4H), 6.79 (dd, *J* = 8.5, 1.6 Hz, 1H), 6.37 (s, 1H), 6.35 – 6.32 (m, 1H), 4.07 (s, 1H), 3.71 – 3.60 (m, 1H), 1.82 – 1.49 (m, 6H), 1.44 (s, 9H), 1.31 – 1.22 (m, 2H), 1.12 – 0.98 (m, 2H). HR-MS (ESI) for $C_{36}H_{38}N_4O_4$ $[M - H]^-$, Calcd: 589.4124, Found: 589.4131.



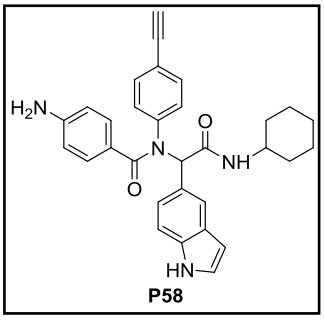
P56

(P56). A white solid (264.1 mg, 42%), R_f = 0.3 (PE : EA = 3 : 1). ^{1}H NMR (400 MHz, DMSO) δ 9.45 (s, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 7.40 (d, *J* = 8.5 Hz, 2H), 7.25 (d, *J* = 8.8 Hz, 2H), 7.15 – 7.00 (m, 8H), 6.25 (s, 1H), 4.15 (s, 1H), 3.67 – 3.55 (m, 1H), 1.79 – 1.50 (m, 6H), 1.44 (s, 9H), 1.29 – 1.21 (m, 2H), 1.14 – 0.99 (m, 2H). HR-MS (ESI) for $C_{34}H_{36}BrN_3O_4$ $[M + H]^+$, Calcd: 630.1962, Found: 630.1935.



P57

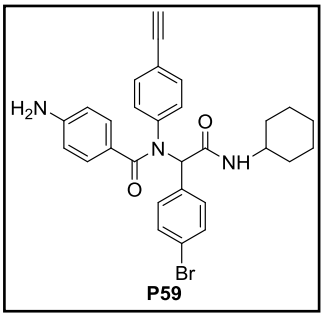
(P57). A white solid (116.8 mg, 21%), R_f = 0.3 (PE : EA = 3 : 1). ^{1}H NMR (400 MHz, DMSO) δ 11.01 (s, 1H), 8.03 (d, *J* = 7.7 Hz, 1H), 7.45 (s, 1H), 7.40 – 7.32 (m, 2H), 7.30 – 7.23 (m, 2H), 7.22 – 6.92 (m, 5H), 6.81 (dd, *J* = 8.5, 1.6 Hz, 1H), 6.38 (s, 1H), 6.36 – 6.30 (m, 1H), 4.07 (s, 1H), 3.73 – 3.62 (m, 1H), 2.34 (s, 3H), 1.81 – 1.50 (m, 6H), 1.28 – 1.23 (m, 2H), 1.12 – 1.00 (m, 2H). HR-MS (ESI) for $C_{33}H_{30}F_3N_3O_2$ $[M + H]^+$, Calcd: 558.2363, Found: 558.2356.



P58

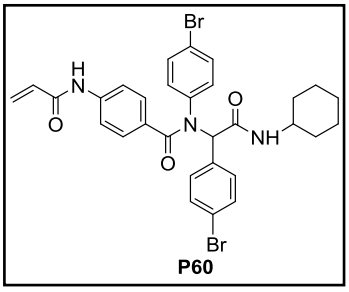
(P58). A white solid (84.1 mg, 17%), R_f = 0.3 (DCM : MeOH = 20 : 1). ^{1}H NMR (400 MHz, DMSO) δ 11.00 (s, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 7.31 (s, 1H), 7.27 – 7.25 (m, 1H), 7.15 (d, *J* = 8.4 Hz, 1H),

7.02 – 6.93 (m, 4H), 6.91 (d, *J* = 8.6 Hz, 2H), 6.79 (dd, *J* = 8.5, 1.6 Hz, 1H), 6.38 (s, 1H), 6.34 – 6.31 (m, 1H), 6.29 – 6.24 (m, 2H), 5.41 (s, 2H), 4.05 (s, 1H), 3.69 – 3.59 (m, 1H), 1.80 – 1.43 (m, 6H), 1.23 – 1.17 (m, 2H), 1.10 – 0.98 (m, 2H). HR-MS (ESI) for $C_{31}H_{30}N_4O_2$ $[M + H]^+$, Calcd: 491.2369, Found: 491.2354.



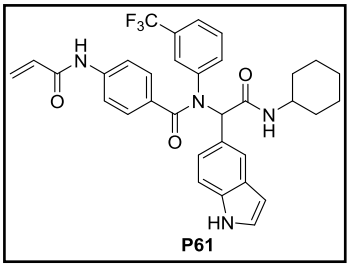
P59

(P59). A white solid (114.5 mg, 22%), R_f = 0.3 (DCM : MeOH = 20 : 1). ^{1}H NMR (400 MHz, DMSO) δ 8.07 (d, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 8.5 Hz, 2H), 7.13 (d, *J* = 8.7 Hz, 2H), 7.07 (d, *J* = 8.5 Hz, 2H), 7.01 (d, *J* = 8.1 Hz, 2H), 6.93 (d, *J* = 8.6 Hz, 2H), 6.31 – 6.24 (m, 3H), 5.46 (s, 2H), 4.13 (s, 1H), 3.65 – 3.54 (m, 1H), 1.79 – 1.44 (m, 6H), 1.25 – 1.14 (m, 2H), 1.12 – 1.01 (m, 2H). HR-MS (ESI) for $C_{29}H_{28}BrN_3O_2$ $[M + H]^+$, Calcd: 530.1438, Found: 530.1432.



P60

(P60). A white solid (124.8 mg, 20%), R_f = 0.3 (PE : EA = 1 : 1). ^{1}H NMR (400 MHz, DMSO) δ 10.19 (s, 1H), 8.14 (d, *J* = 7.7 Hz, 1H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.25 – 7.15 (m, 4H), 7.09 (d, *J* = 8.5 Hz, 2H), 7.06 – 6.92 (m, 2H), 6.38 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.28 – 6.18 (m, 2H), 5.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 3.67 – 3.56 (m, 1H), 1.79 – 1.49 (m, 6H), 1.30 – 1.23 (m, 2H), 1.14 – 0.99 (m, 2H). HR-MS (ESI) for $C_{30}H_{29}Br_2N_3O_3$ $[M + H]^+$, Calcd: 638.0648, Found: 638.0637.



P61

(P61). A white solid (178.9 mg, 30%), R_f = 0.3 (DCM : MeOH = 20 : 1). ^{1}H NMR (400 MHz, DMSO) δ 10.99 (s, 1H), 10.15 (s, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.60 – 7.42 (m, 3H), 7.31 (s, 1H), 7.28 – 7.23 (m, 1H), 7.22 – 7.11 (m, 5H), 7.11 – 7.03 (m, 1H), 6.79 (dd, *J* = 8.5, 1.4 Hz, 1H), 6.40 (s, 1H), 6.38 – 6.32 (m, 1H), 6.32 – 6.29 (m, 1H), 6.22 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.74 (dd, *J* = 10.0, 2.0 Hz, 1H), 3.73 – 3.63 (m, 1H), 1.84 – 1.50 (m, 6H), 1.35 – 1.19 (m, 2H), 1.11 – 0.99 (m, 2H). HR-MS (ESI) for $C_{33}H_{31}F_3N_4O_3$ $[M + H]^+$, Calcd: 589.2421, Found: 589.2412.

5. Cell Growth Inhibition Assay[6]

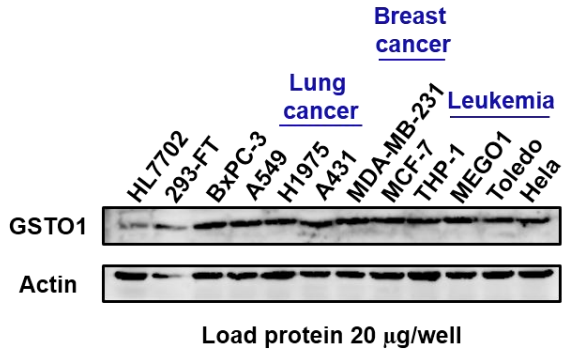
Cell growth inhibition assays were carried out using BxPC-3 cells. Cell viability was determined by cell counting kit 8 (CCK 8, CK04, Dojindo Laboratories, Kumamoto, Japan) assay. The procedures were similar to previously published protocols, 5000 cells per well were seeded in a 96-well plate and incubated for 12 hours in a humidified incubator for adherence. Probes and positive control Gemcitabine were added to cells at different final concentrations and incubated for 72 h. 10 μL of CCK-8 reagent was added to each well and incubated at 37 ℃ with 5% CO_2. Following that, the absorbance was measured at 450 nm and 650 nm on a plate reader (Synergy HI, BioTek Instruments, Inc. Vermont, US). Cell viability rate was determined as VR = (A – A_0) / (As – A_0) × 100%, where A is the absorbance of the experimental group, As is the absorbance of the control group (DMSO was used as the control) and A_0 is the absorbance of the blank group (no

cells). IC_{50} values were calculated using GraphPad Prism.

To test cytotoxicity, probe ***P21/P26*** and corresponding competitors ***P27/P28*** were determined using normal 293-FT and HL7702 cell lines by cell counting kit 8 assay. The procedures were similar to previously published protocols, 10000 cells per well were seeded in a 96-well plate and incubated for adherence. Probes were added to cells at different final concentrations and incubated for 72 h. 10 μL of CCK 8 reagent was added to each well and incubated at 37 ℃ with 5% CO_2. Following that, the absorbance was measured at 450 nm and 650 nm on a plate reader (Synergy HI, BioTek Instruments, Inc. Vermont, US). Cytotoxicity rate was determined as VR = $(A - A_0)/(As - A_0) \times 100\%$, where A is the absorbance of the experimental group, As is the absorbance of the control group (DMSO was used as the control) and A_0 is the absorbance of the blank group (no cells). IC_{50} values were calculated using GraphPad Prism.

The drug combination experiments of ***P28/7rh*** and ***cisplati*** were determined using BxPC-3 cells. 5000 cells per well were seeded in a 96-well plate and incubated for 12 hours in a humidified incubator for adherence. The cells were treated with indicated concentrations of ***P28/7rh*** and ***cisplati*** for 72 h and incubated with CCK 8. Following that, the absorbance was measured at 450 nm and 650 nm on a plate reader (Synergy HI, BioTek Instruments, Inc. Vermont, US). Cell viability rate was determined as VR = $(A - A_0)/(As - A_0) \times 100\%$, where A is the absorbance of the experimental group, As is the absorbance of the control group (DMSO was used as the control) and A_0 is the absorbance of the blank group (no cells). IC_{50} values were calculated using GraphPad Prism and the combination index (CI) was calculated using Calcusyn software. The CI was the ratio of the combination dose to the sum of the single-agent doses at an isoeffective level. Therefore, CI < 1 indicated synergy; CI > 1, antagonism; CI = 1, additive.

(A)



(B)

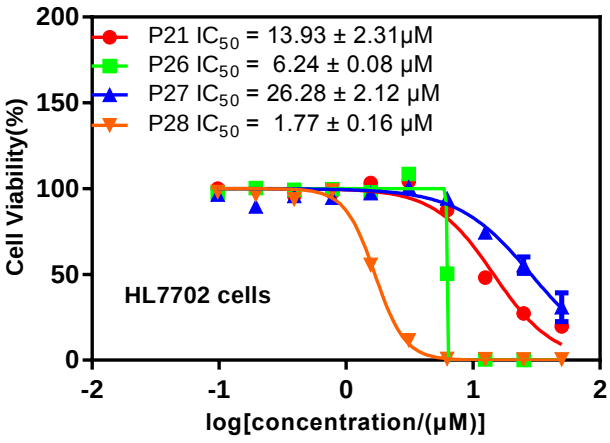


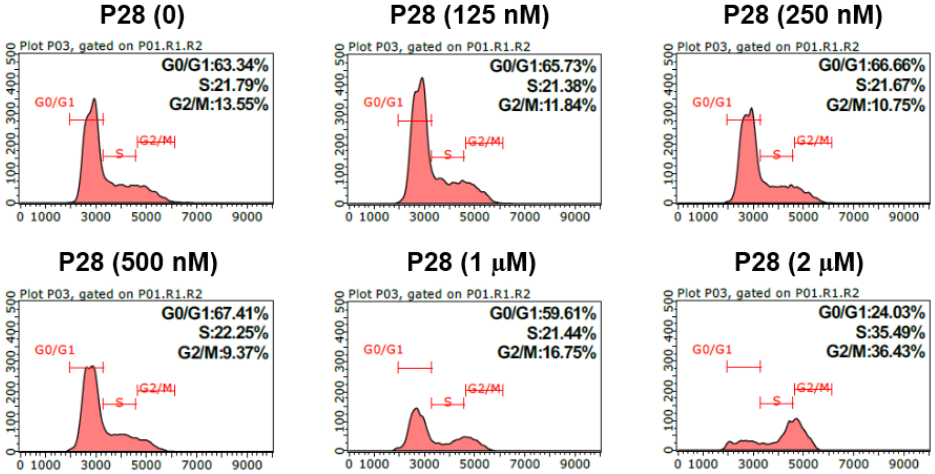
Figure S1. (A) Analysis of GSTO1 expression level in healthy and tumor cells by western blotting. (B) IC_{50} values of ***P21/P26/P27/P28*** against HL7702 healthy cells.

6. Computational Study[7].

All procedures were performed in Maestro 11.7 (Schrodinger LLC). The crystal structure of GSTO1 protein was taken from the PDB (ID 4YQM). The protein was processed using the "Protein Preparation Wizard" workflow in Maestro 11.7 (Schrodinger LLC) to add bond orders and to add hydrogens. All heteroatom residues and crystal water molecules beyond 5 Å from heteroatom group were removed. Inhibitors were built in the LigPrep module using the OPLS3e force field. Covalent module was used as the docking program. Reaction residue numbers and centroid of workspace ligand were selected. The box center was placed on the centroid of the binding ligand in the optimized crystal structure as described above. Nucleophilic substitution was chosed as the reaction Type. Covalent docking was adopted to dock P26 into GSTO1 with the default parameters, and the top-ranking pose was selected for energy minimization using Prime MM-GBSA, under the solvation model of VSGB.

7. Cell Cycle Analysis[8].

Flow cytometry was used to determine cell cycle distribution in BxPC-3 cell line. BxPC-3 cells were cultured in growth medium to 30-40% confluence in 6-well plates. The adherent cells were treated with indicated increasing concentrations of ***P28*** solubilized in DMSO for 24 hours at 37 °C / 5% CO_2 condition. After treatment, cells were trypsinised, collected, washed with PBS and centrifuged (1000 rpm, 3 min at room temperature). According to the kit (BD Cycletest™ Plus DNA Reagent Kit, Cat. NO. 340242) instructions, cell pellets suspended in 1 mL buffer solution were incubated successively by solution A (125 µL), solution B (100 µL) and propidium iodide staining solution C (0.25 mg/mL, 200 µL) for 10 min in dark at room temperature. Cell cycle analysis was performed using flow cytometry (GUAVA easyCyte). The percentages of cells at different phases of the cell cycle were calculated by GuavaSoft.



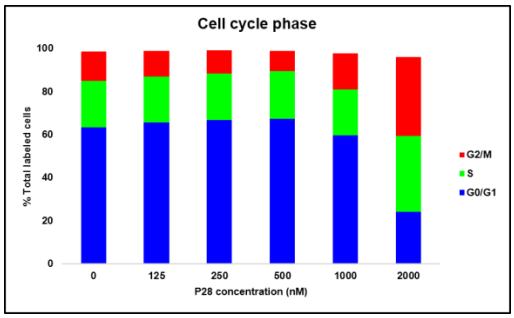


Figure. S2. Cell cycle assay samples were analysed with flow cytometry and GuavaSoft. The BxPC-3 cells were treated with indicated increasing concentrations of ***P28*** for 24 hours.

8. In Vitro and In Situ Proteome Labeling[1-3]

For *in situ* proteome labeling, BxPC-3 cells were grown to 80−90% confluency in 6-well plates under conditions as described above. The medium was removed and the cells were washed twice with PBS and then treated with 2 mL probe-containing medium in the presence or absence of excessive competitors (diluted from DMSO stocks whereby DMSO never exceeded 1% in the final solution). After 2-4 h of incubation, the medium was aspirated and cells were washed twice with PBS to remove excessive probe. The cells were lysed with 200 μL RIPA lysis buffer (Thermo Scientific™ #89900) containing protease and phosphatase inhibitors (Thermo Scientific™ #88669) on ice for 30 min. A soluble protein solution was obtained by centrifugation for 10 min (14000 rpm, 4 ℃). Eventually, the protein concentrations were determined by using the BCA protein assay (Pierce™ BCA protein assay kit) and diluted to 1 mg/mL with RIPA buffer. A freshly pre-mixed click chemistry reaction cocktail (20 μM TAMRA-N_3 from 1 mM stock solution in DMSO, 50 μM TBTA from 2.5 mM freshly prepared stock solution in DMSO, 0.5 mM TCEP from 25 mM freshly prepared stock solution in deionized water, and 0.5 mM $CuSO_4$ from 25 mM freshly prepared stock solution in deionized water) was added to the labeled proteome. The reaction was further incubated for 2 h at rt prior to addition of pre-chilled acetone (-20 ℃). The precipitated proteins were subsequently collected by centrifugation (14000 rpm, 10 min at 4 ℃), and washed with 200 μL of pre-chilled methanol. The samples were dissolved in 1 × SDS loading buffer and heated for 10 min at 95 °C. 20 μg proteins for each lane were loaded on SDS−PAGE (12% gel) and then visualized by in-gel fluorescence scanning (Typhoon FLA 9500).

For recombinant protein labeling, ***P26*** (final concentration of the probe was 1 μM) was incubated with purified GSTO1 protein at different final concentrations in PBS buffer for 1 hour at 37 ℃ with gentle shaking. Subsequently, the labeled proteins were subjected to click reaction with TAMRA azide under standard click chemistry conditions (20 μM TAMRA-N_3 from 1 mM stock solution in DMSO, 50 μM TBTA from 2.5 mM freshly prepared stock solution in DMSO, 0.5 mM TCEP from 25 mM freshly prepared stock solution in deionized water, and 0.5 mM $CuSO_4$ from 25 mM freshly prepared stock solution in deionized water) and dissolved in 1 × SDS loading buffer. All proteins for each sample were loaded on SDS-PAGE (12% gel) and then visualized by in-gel fluorescence scanning (Typhoon FLA 9500) and coomassie brilliant blue staining (CBB).

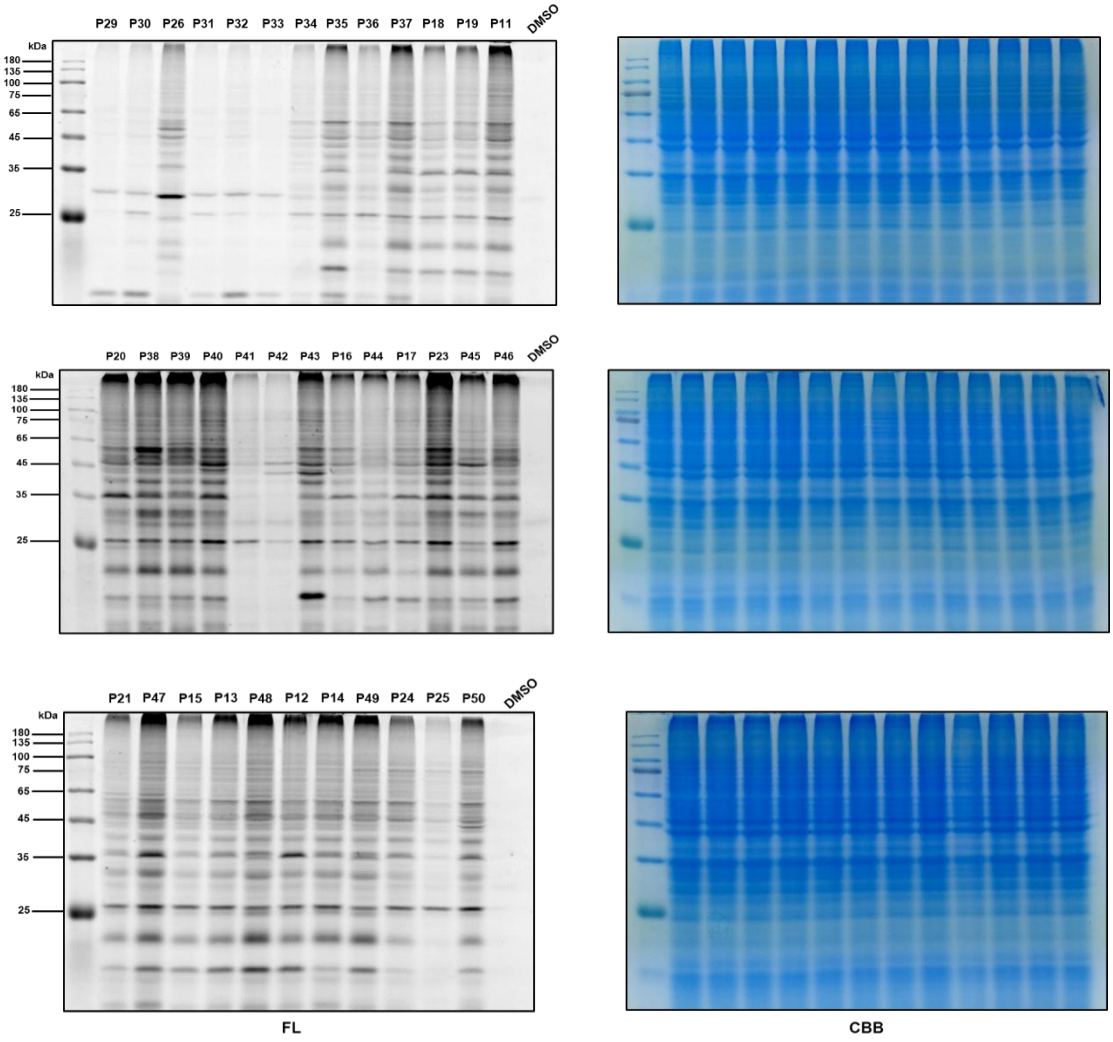


Figure. S3. Proteome profilling in BxPC-3 cells with the alkyne-containing compounds (final concentrations were 1 μM). FL: fluorescence scanning; CBB: coomassie brilliant blue staining.

9. Pull down/LC-MS and Targets Validation

To identify the interacting cellular targets of ***Probe 21 and 26***, pull-down (PD) experiments were carried out, and followed by Western blotting (WB) and LC-MS/MS, where applicable. The general pull-down experiments were based on previously reported procedures[1-3], with the following optimizations. BxPC-3 cells were grown to 90% confluency under the condition of 37 ℃ with 5% CO_2. The medium was removed and the cells were washed 3 times with PBS and treated with probe-containing medium (FBS free) in the presence or absence of corresponding competitors (final concentration of the probe was 1 μM, DMSO never exceeded 1% in the final solution). After 2 h of incubation, the medium was aspirated, and cells were washed twice with PBS to remove excessive probe. The cells were lysed with RIPA buffer including 1 × protease and phosphatase inhibitors on ice for 30 min and centrifuged for 10 min (14000 rpm, 4 ℃) to get a soluble protein solution. Eventually, the protein concentrations were determined by BCA protein assay and then diluted to 1 mg/mL with RIPA buffer. A freshly premixed click chemistry reaction cocktail was added (20 μM Biotin-N_3 from 10 mM stock solution in DMSO, 50 μM TBTA from 25 mM freshly prepared stock solution in DMSO, 0.5 mM TCEP from 250 mM freshly prepared

stock solution in deionized water, and 0.5 mM $CuSO_4$ from 250 mM freshly prepared stock solution in deionized water). The reaction was further incubated for 2 h with gentle mixing prior to precipitation by addition of pre-chilled acetone (−20 ℃). Precipitated proteins were subsequently collected by centrifugation (14000 rpm × 10 min at 4 ℃) and dissolved in PBS containing 1% SDS. Upon incubation with 100 μL streptavidin beads for 4 hours at rt, the beads were washed with PBS containing 1% SDS (2 × 1 mL × 5 min) and 0.1% SDS (2 × 1 mL × 5 min), PBS (2 × 1 mL × 5 min). The enriched proteins was eluted by 1× loading buffer at 95 ℃ for 10 min and separated by SDS-PAGE (12%). Control pull-down experiments using the DMSO were carried out concurrently with live cells. WB experiments were carried out as previously described using the corresponding antibodies.

For on beads digestion, beads were resuspended in 500 μL 6 M urea in PBS, 25 μL of 200 mM DTT in 25 mM NH_4HCO_3 buffer was added and the reaction was incubated for 37 ℃ for 30 min. For alkylation, 25 μL of 500 mM IAA in 25 mM NH_4HCO_3 buffer was added and incubated for 30 min at rt in dark. Then, remove supernatant and wash beads by 1 mL PBS twice. For the digestion, 150 μL 2 M urea in PBS, 150 μL 1 mM $CaCl_2$ in 50 mM NH_4HCO_3 and 1.0 μg of trypsin were added. The reaction was incubated at 37 ^oC overnight. The reaction was quenched by adding TFA, then the resulting peptides supernatant was transferred to a new tube.

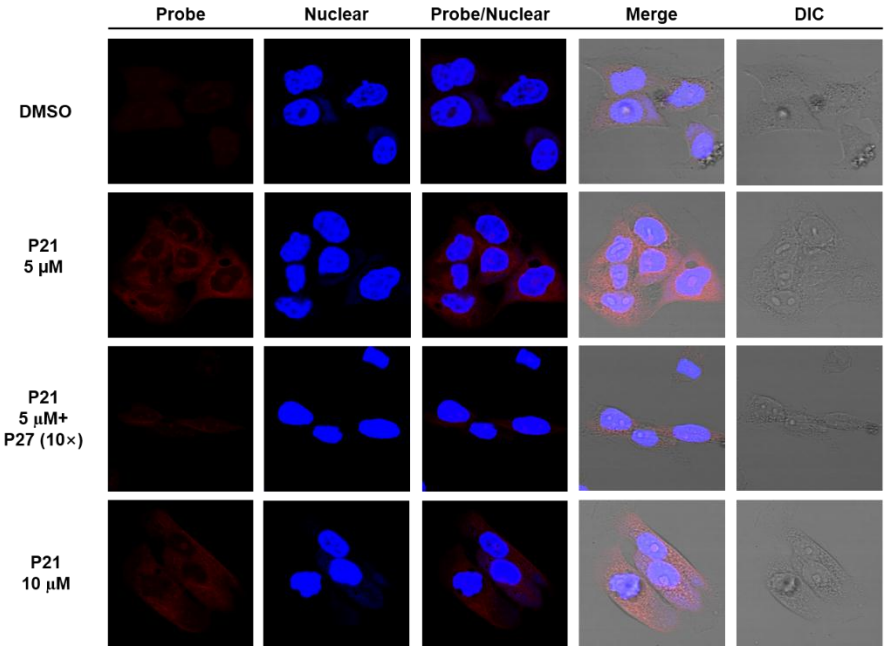
The supernatants containing the digested peptides were collected, desalted with Waters C18 Tips and dried by vacuum centrifugation. The peptides were separated and analyzed on an Easy-nLC 1000 system coupled to a Q Exactive HF (both - Thermo Scientific). About 1 μg of peptides were separated in an home-made column (75 μm × 15 cm) packed with C18 AQ (5 μm, 300Å, Michrom BioResources, Auburn, CA, USA) at a flow rate of 300 nL/min. Mobile phase A (0.1% formic acid in 2% ACN) and mobile phase B (0.1% formic acid in 98% ACN) were used to establish a 60 min gradient comprised of 2 min of 5% B, 40 min of 5-26% B, 5 min of 26-30% B, 1 min of 30-35% B, 2 min of 35-90% B and 10 min of 90% B. Peptides were then ionized by electrospray at 1.9 kV. A full MS spectrum (375-1400 m/z range) was acquired at a resolution of 120,000 at m/z 200 and a maximum ion accumulation time of 20 ms. Dynamic exclusion was set to 30 s. Resolution for HCD MS/MS spectra was set to 30,000 at m/z 200. The AGC setting of MS and MS^2 were set at 3E6 and 1E5, respectively. The 20 most intense ions above a 1.0E3 counts threshold were selected for fragmentation by HCD with a maximum ion accumulation time of 60 ms. Isolation width of 1.6 m/z units was used for MS^2. Single and unassigned charged ions were excluded from MS/MS. For HCD, normalized collision energy was set to 25%.

The raw data were processed and searched with MaxQuant 1.5.4.1 with MS tolerance of 4.5 ppm, and MS/MS tolerance of 20 ppm. The UniProt human protein database (release 2016_07, 70630 sequences) and database for proteomics contaminants from MaxQuant were used for database searches. Reversed database searches were used to evaluate false discovery rate (FDR) of peptide and protein identifications. Two missed cleavage sites of trypsin were allowed. Carbamidomethylation (C) was set as a fixed modification, and oxidation (M), Acetyl (Protein N-term) and deamidation (NQ) were set as variable modifications. The FDR of both peptide identification and protein identification is set to be 1%[9]. The options of "Second peptides", "Match between runs" and "Dependent peptides" were enabled. Label-free quantification was used to quantify the difference of protein abundances between different samples[10, 11].

10. Cellular Imaging

To demonstrate the utility of the probes for imaging of cellular targets, we performed fluorescence microscopy. The general procedures were similar to what was previously reported[1-3]. For fixed cells, BxPC-3 cells seeded in glass bottom dishes and grown until 70−80% confluency were treated with 0.3 mL of RPMI 1640 with probes (***P21*** and ***P26***) at different final concentrations in the presence or absence of corresponding competitors (10×). After incubation for 2-4 h, the medium was removed and cells were gently washed twice with PBS. The cells were fixed for 1 h at room temperature with 3.7% formaldehyde in PBS, washed twice with cold PBS again, and permeabilized with 0.1% Triton X-100 in PBS for 1 h. Cells were then treated with a freshly premixed click chemistry reaction solution in a 200 μL volume (final concentrations of reagents : 20 μM TAMRA-N_3 from 1 mM stock solution in DMSO, 50 μM TBTA from 2.5 mM freshly prepared stock solution in DMSO, 0.5 mM TCEP from 25 mM freshly prepared stock solution in deionized water, and 0.5 mM $CuSO_4$ from 25 mM freshly prepared stock solution in deionized water) for 2 h at room temperature with vigorous shaking. Cells were washed with PBS at least once and 0.1% Tween 20 in PBS for three times. Finally, the cells were stained with Hoechst (1:5000 dilution in PBS) for 10 min at room temperature prior to image.

(A)



(B)

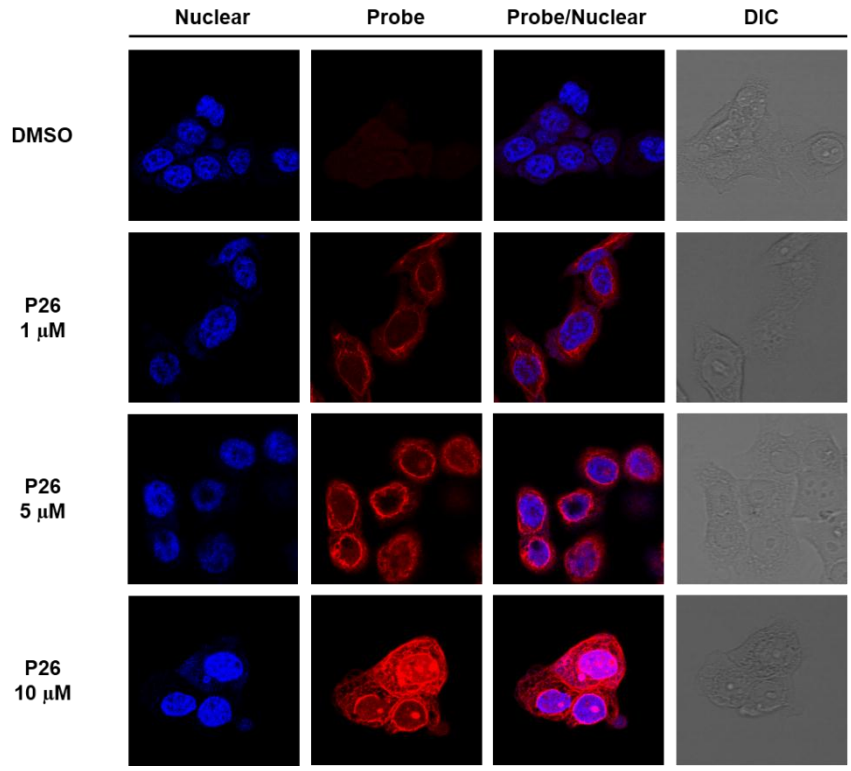


Figure S4. Cellular imaging of BxPC-3 cells with different probes in the presence or absence of competitors(10×); (A) **P21** with different concentrations. (B) **P26** with different concentrations. Scale bar = 10 μm.

11. GSTO1 expression in different cells

To generate protein lysates, different cells (HL7702, 293-FT, BxPC-3, A549, H1975, A431, MDA-MB-231, MCF-7, THP-1, MEGO1, Toledo, Hela) were washed twice with cold phosphate-buffered saline (PBS), harvested with 1× trypsin and collected by centrifugation. Cell pellets were then washed with PBS and lysed using RIPA with protease and phosphatase inhibitors. Protein concentration was determined by Bradford protein assay. For Western blotting experiments, the same amount of cell lysates were resolved and electrophoresed onto 12% by SDS−polyacrylamide gels and transferred to poly(vinylidene difluoride) membranes. Membranes were then blocked with 5% bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO, USA) in TBST (0.1% Tween-20 in Tris-buffered saline) for 1 h at room temperature. After blocking, membranes were incubated with the primary antibody against GSTO1 at 4 ℃ overnight. After incubation, membranes were washed with TBST (3 × 10 min) and then incubated with an appropriate HRP-conjugated secondary antibody for 2 hours at room temperature. Finally, blots were washed again with TBST before being developed with ECL Western Blotting Detection Kit (Thermo Scientific, Grand Island, NY, USA), and detected with Amersham Imager 600 system (GE, Boston, MA, USA). The data were shown in Figure S1A.

13. siRNA Transfection[12, 13]

In order to validate the target authenticity, small interfering RNA (siRNA) duplexes against GSTO1 purchased from Santa Cruz were used to knock down gene expression in BxPC-3 cells. To optimize transfection conditions, different concentrations of siRNAs and transfection reagent were

tested. As negative control, a scrambled oligonucleotide (NControl) was used. 50 nM siRNA resulted as the most active concentration in silencing GSTO1, thus they were selected for the transfection experiments. BxPC-3 cells were seeded in a 6-well plate and transfected with corresponding siRNA from 10 μM stock solution in RNase-free water at 70% confluence using Lipofectamine 2000 (Invitro, USA) according to the manufacturer's recommendations. 48 hours after transfection, cells were washed twice with PBS and lysed. The transfection efficiency was determined by evaluating the expression level of GSTO1 using western blotting.

14. Annexin V / 7-Aminoactinomycin D (7-AAD) Apoptosis Assay

Flow cytometry was used to test cell apoptosis in BxPC-3 cell line[14]. According to the manufacturer's recommendations (BD Pharmingen™ PE Annexin V Apoptosis Detection Kit I, Cat. NO. 559763), BxPC-3 cells (1.0×10^5 cells/mL) were grown in 6-well plates until cells reached 50% confluence. The cells treated with different concentrations of compounds and siRNA duplexes for 48 hours were subsequently detached with EDTA-free trypsin, washed with cold PBS, collected and centrifuged (1000 rpm, 3 min at room temperature). The cell pellets were suspended into 500 μL of 1 × binding buffer containing 2.5 μL Annexin V and 2.5 μL 7-Aminoactinomycin D (7-AAD). After being incubated at room temperature in the dark for 15 min, each sample was analysed with flow cytometry (GUAVA easyCyte) and GuavaSoft.

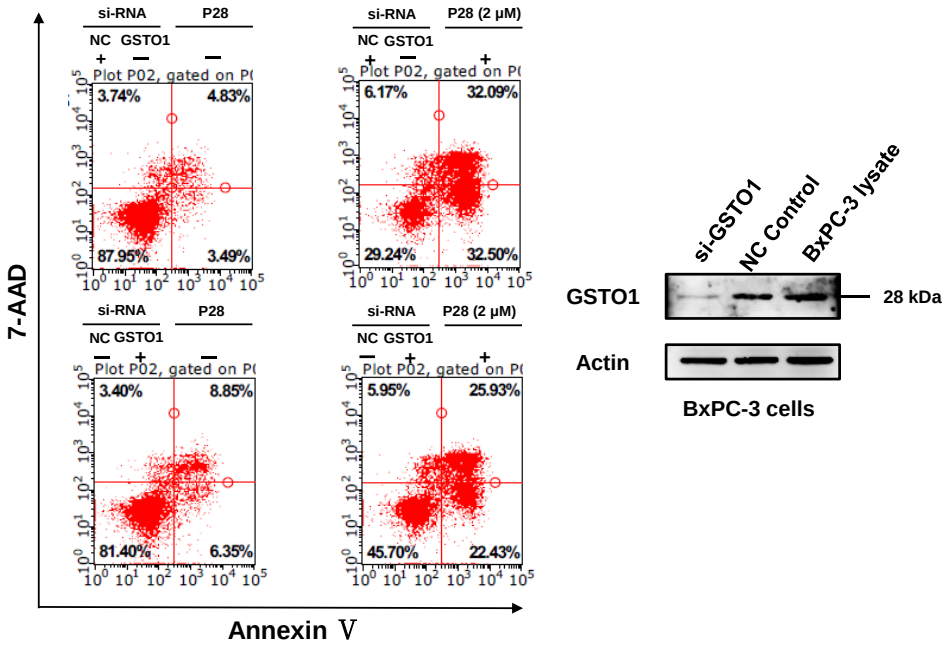


Figure. S5. Apoptosis assay samples were analysed with flow cytometry and GuavaSoft in the presence or absence of siRNA with ***P28***; the apoptosis assay with ***P28*** (2 μM) when si-GSTO1 duplexes were used to knock down gene expression.

15. Cellular Thermal Shift Assay (CETSA) for GSTO1

CETSA was performed as described with some adaptations[15]. BxPC-3 cells were grown to 80−90% confluency in 10 cm dishes under conditions as described above. The medium was removed and cells were washed twice with PBS and then treated with 8 mL probe-containing FBS-free medium (final concentration of the probe was 10 μM). Control cells were incubated with an equal volume of DMSO. After 2 hours of incubation, the medium was aspirated and cells were

washed twice with PBS to remove excessive probe, harvested with trypsin and centrifuged at 800 rpm for 3 min at room temperature. The pellets were resuspended in PBS and the cells were dispensed equally at 100 μL into PCR tubes. The samples were then subjected for 3 minutes to a 8-step temperature gradient (40 – 61 ℃) using BIO-RAD S1000^{TM} Thermal Cycler and lysed by cycling freezing in liquid nitrogen and melting at room temperature three times. After the cell lysates were centrifuged at 14000 rpm for 30 min to remove aggregates at 4 ℃, supernatants were transferred to 1.5 mL Eppendorf tubes and dissolved in 5× SDS loading buffer and heated for 10 min at 95 ℃. The expressed GSTO1 was identified by western blot. Immunoblotting band intensities were quantified using ImageJ software and thermal curves were analyzed by Boltzmann curve fitting using GraphPad Prism software

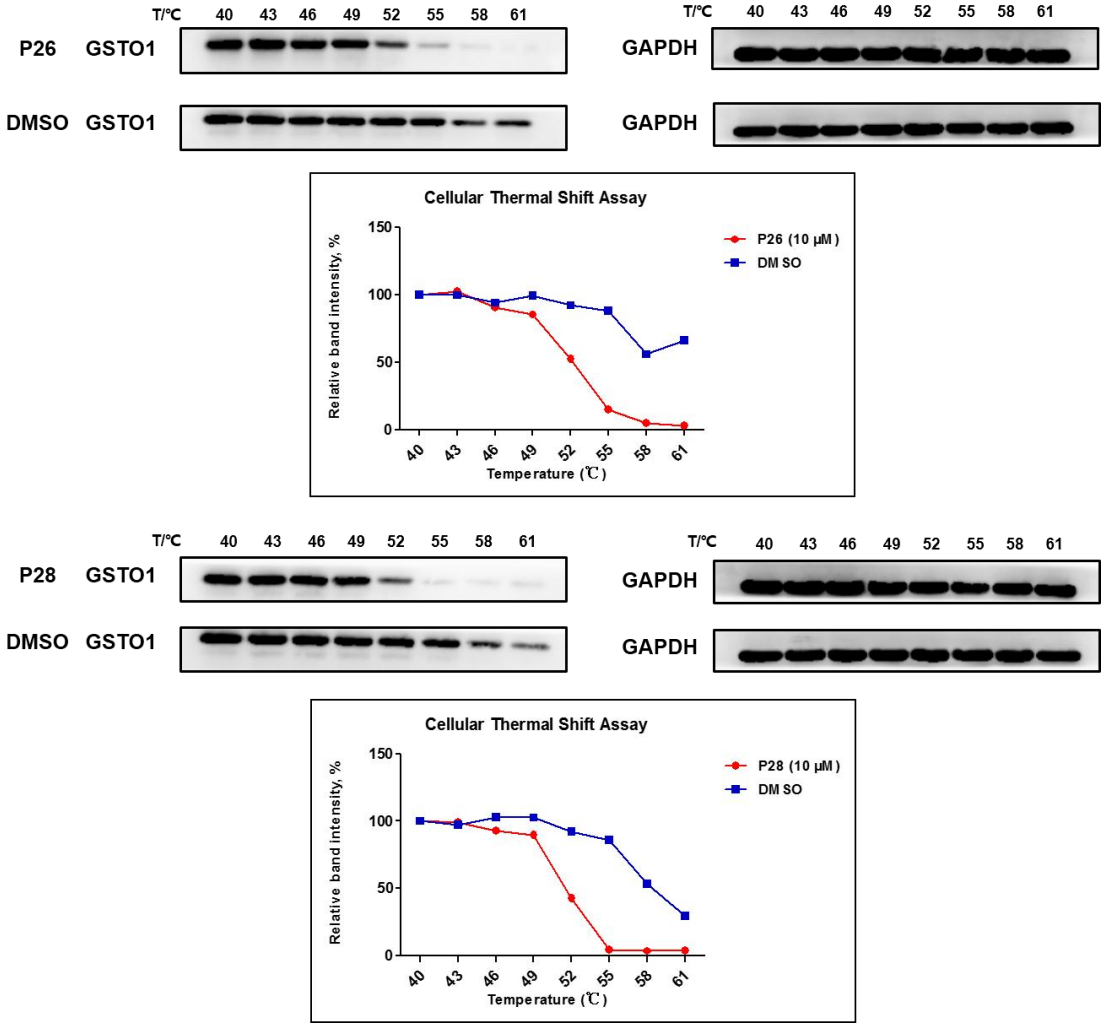
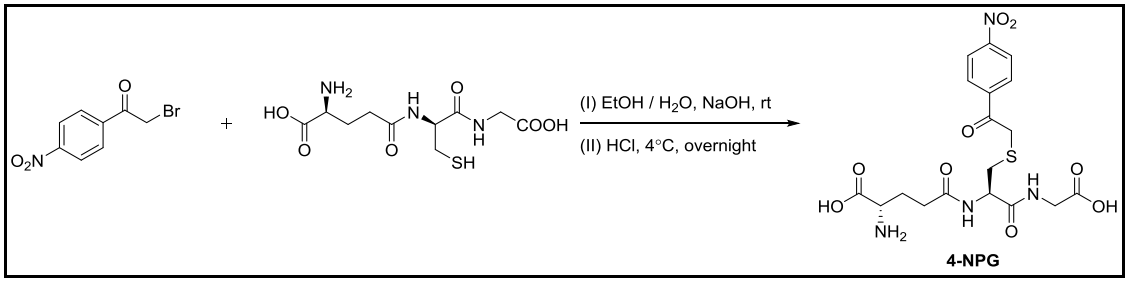


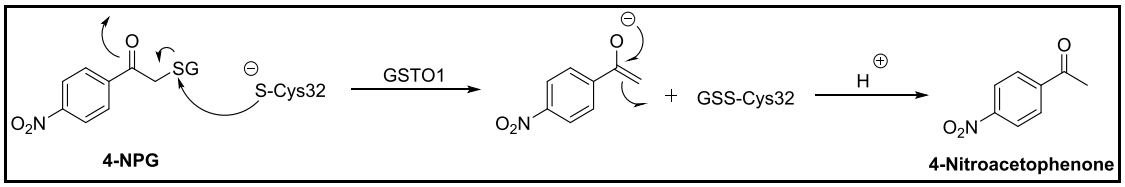
Figure. S6. Compounds ***P26*** and ***P28*** that bind GSTO1 will be unstable compared to control DMSO at indicated increasing temperature (40 – 61 ℃).

12. GSTO1 enzyme activity assay.



Scheme S40

Enzyme activity was measured by monitoring the reduction of S-(4-nitrophenacyl)glutathione (4-NPG) to 4-nitroacetophenone by GSTO1. 4-NPG was prepared by the previously developed method[16]. 2-Bromo-40-nitroacetophenone (2 mmol, 488 mg, Aladdin) was dissolved in 5 mL ethanol and added dropwise to a solution containing glutathione (2 mmol, 610 mg, Aladdin) in a mixture of 5 mL H_2O (pH brought to 10.0 with NaOH) and 10 mL ethanol. After stirring for 3 h at room temperature, the pH was brought to 3.5 with HCl and the reaction mixture was kept at 4 ℃ overnight. The precipitated 4-NPG was collected by filtration and washed thoroughly with ice-cold water. The white product was dried to constant weight and stored at -20 ℃. ^{1}H NMR (400 MHz, DMSO) δ 8.70 (t, *J* = 5.6 Hz, 1H), 8.43 (d, *J* = 8.6 Hz, 1H), 8.35 (d, *J* = 8.9 Hz, 2H), 8.21 (d, *J* = 8.9 Hz, 2H), 4.54 – 4.45 (m, 1H), 4.18 (q, *J* = 36.0, 15.3 Hz, 2H), 3.72 – 3.68 (m, 2H), 3.31 (t, *J* = 6.8 Hz, 2H), 2.93 (dd, *J* = 13.6, 4.5 Hz, 1H), 2.68 (dd, *J* = 13.5, 9.8 Hz, 1H), 2.36 – 2.25 (m, 2H), 1.96 – 1.80 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 194.2, 172.2, 171.4, 171.0, 170.9, 150.4, 140.5, 130.4, 124.3, 53.5, 53.1, 52.3, 41.6, 38.4, 34.0, 31.8, 27.2. HR-MS (ESI) for $C_{18}H_{22}N_4O_9S$ $[M + Na]^+$, Calcd: 493.1014, Found: 493.1003.



Mechanism of the reduction of 4-NPG by GSTO1.

The general procedures of activity assay were similar to what was previously reported[17]. Briefly, in a 200 μL reaction volume, 5 μg ml^{-1} recombinant GSTO1 in reaction buffer (100 mM Tris (pH 8.0), 1.5mM EDTA) was incubated with different concentrations of ***P28*** or DMSO as the negative control for 30 min at 37 ℃. A volume of 4-NPG (final concentration of 1 mM) was added to the reaction and decrease in absorbance at 305 nm was recorded on a plate reader (Synergy HI, BioTek Instruments, Inc. Vermont, US). Assays were repeated at least three times independently, and IC_{50} values were calculated and plotted using GraphPad Prism 5.0 software.

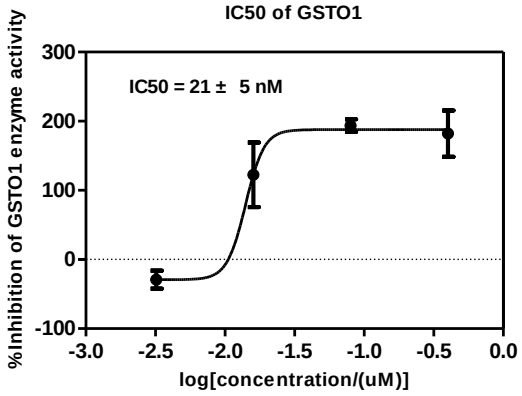


Figure S7. IC_{50} values of ***P28*** against recombinant GSTO1 protein.

Table S3.Protein hits identified by LC-MS/MS with P21 in the presence or absence of its competitor P27.

Protein IDs	Protein names	Gene names	Mol. weight [kDa]	Ratio H/L normalized P21/P21+P26_HL1	Ratio H/L normalized P21/P21+P26_HL2	Ratio H/L normalized P21/P21+P26_HL3	Ratio H/L normalized P21/DMSO_HL1	Ratio H/L normalized P21/DMSO_HL2	Ratio H/L normalized P21/DMSO_HL3	Score
Q9BRX8	Redox-regulatory protein FAM213A	FAM213A	25.764	2.6789	NaN	2.3816	15.365	18.815	16.914	155.45
C9JB90;C9JU14;J	Ras-related protein Rab-6B;Ras-rela	RAB6B;RAB6A	5.8575	7.1915	7.5468	6.1636	10.788	11.154	11.055	59.574
A2A376;O95786	Probable ATP-dependent RNA helic	DDX58	82.612	219.75	633.93	NaN	NaN	NaN	194.44	6.9431
A0A087WTT8;A0	Contactin-5	CNTN5	112.32	18.775	3.5474	NaN	NaN	NaN	NaN	7.1376
P04181	Ornithine aminotransferase, mitochor	OAT	48.534	3.3903	1.1154	1.6399	NaN	NaN	NaN	7.0745
CON__P35527;P3	Keratin, type I cytoskeletal 9	KRT9	62.129	3.3759	2.4246	3.0863	2.3132	1.8578	1.0568	323.31
J3KPP4;O95232;E	Luc7-like protein 3	LUC7L3	58.22	2.2621	1.7893	1.1618	1.6852	1.1102	0.97624	47.548
M0QY96;M0R2I7	Heterogeneous nuclear ribonucleopr	HNRNPM	12.343	2.0711	NaN	1.8008	NaN	NaN	NaN	14.789
CON__P02535-1			57.769	1.9172	1.1893	1.4881	NaN	NaN	NaN	14.555
P05165;Q5JVH2;E	Propionyl-CoA carboxylase alpha ch	PCCA	80.058	1.7576	1.923	2.0251	0.85368	0.95221	0.83847	65.112
P07099	Epoxide hydrolase 1	EPHX1	52.948	1.6173	0.68678	1.4761	1.354	1.1876	1.0106	48.463
P60866;E5RIP1;E	40S ribosomal protein S20	RPS20	13.373	1.4423	2.6149	1.4999	1.7864	1.6313	NaN	18.915

Table S4.Protein hits identified by LC-MS/MS with P26 in the presence or absence of its competitor P28.

Protein IDs	Protein names	Gene names	Mol. weight [kDa]	Ratio H/L normalized P26/P26+P28_HL1	Ratio H/L normalized P26/P26+P28_HL2	Ratio H/L normalized P26/P26+P28_HL3	Ratio H/L normalized P26/DMSO_HL1	Ratio H/L normalized P26/DMSO_HL2	Ratio H/L normalized P26/DMSO_HL3	Score
P78417;Q5TA02;	Glutathione S-transferase omega-1	GSTO1	27.566	1.5654	1.6104	1.5863	3.769	3.8927	4.1438	101.42
Q96IX5	Up-regulated during skeletal muscle g	USMG5	6.4575	2.9748	3.1432	2.5794	4.8598	2.9854	4.6392	7.1432
C9JB90;C9JU14;	Ras-related protein Rab-6B;Ras-relat	RAB6B;RAB6A;	5.8575	2.015	1.99	1.8744	3.3088	2.8898	2.9214	24.748
H0Y653;H0YDU	Nucleolar protein 56	NOP56	24.144	NaN	1.9955	2.6796	NaN	1.2112	1.7994	7.3213
E7ERU0;E9PHM	Dystonin	DST	615.65	4.241	NaN	3.1143	NaN	4.8965	NaN	10.78
O75844	CAAX prenyl protease 1 homolog	ZMPSTE24	54.812	3.5778	NaN	4.7748	NaN	NaN	NaN	12.65
CON__P19013;P	Keratin, type II cytoskeletal 4	KRT4	63.91	2.8135	2.3695	1.4625	1.3169	1.4627	1.7673	77.254
Q5JP53;P07437;C	Tubulin beta chain	TUBB	47.766	2.3425	2.2936	2.1082	0.96647	0.98169	1.0541	81.008
Q3ZCM7;A0A07	Tubulin beta-8 chain	TUBB8	49.775	2.0578	1.7835	1.5857	NaN	1.73	1.147	265.59
E9PFG0;A0A0C4	Alcohol dehydrogenase class 4 mu/sig	ADH7	33.746	1.8615	3.6012	NaN	4.7669	NaN	NaN	14.61
P42126;H3BS70;(	Enoyl-CoA delta isomerase 1, mitoch	ECI1;DCI	32.816	1.8586	1.4291	1.4878	1.2704	1.3291	1.0238	24.351
P62995;H7C2L4;	Transformer-2 protein homolog beta	TRA2B	33.665	1.7902	1.4846	0.59291	NaN	1.2819	1.0384	21.702
P68371;P04350;K	Tubulin beta-4B chain;Tubulin beta-4	TUBB4B;TUBB4	49.83	1.7775	1.6874	1.8196	1.0132	1.0416	1.0504	323.31
P11766	Alcohol dehydrogenase class-3	ADH5	39.724	1.7741	1.8021	0.87269	NaN	2.1519	1.4235	31.244
CON__Q3KNV1	Keratin, type II cytoskeletal 7	KRT7	51.385	1.7343	1.8182	1.7376	1.2948	1.2978	1.3315	249.97
K7ER88;K7EJB1	3-ketoacyl-CoA thiolase, mitochondri	ACAA2	7.5987	1.7186	1.5832	1.5338	2.2201	2.105	1.7365	7.4998
Q71U36;Q13748;	Tubulin alpha-1A chain;Tubulin alpha	TUBA1A;TUBA3	50.135	1.7057	1.7448	1.6938	0.94529	0.95513	0.91536	323.31
K7EM73;A0A0C	Calpain small subunit 1	CAPNS1	15.958	1.705	1.2693	1.6591	0.889	0.83413	0.74342	46.687
Q07065	Cytoskeleton-associated protein 4	CKAP4	66.022	1.6748	1.5914	1.1673	1.7452	1.4649	1.4181	48.087
J3QKT2;Q96KP4	Cytosolic non-specific dipeptidase	CNDP2	27.709	1.4713	2.4299	1.6782	NaN	NaN	NaN	12.417
O95881;V9GY50	Thioredoxin domain-containing protei	TXNDC12	19.206	1.4708	2.5294	2.1107	NaN	11.158	NaN	25.12
P60981;F6RFD5	Destrin	DSTN	18.506	1.3625	1.8512	5.7782	0.66836	1.2594	0.88857	17.499
K7ERE3;CON__	Keratin, type I cytoskeletal 13	KRT13	45.26	1.3016	1.7563	1.9054	1.499	1.5632	1.5418	26.355
P12955	Xaa-Pro dipeptidase	PEPD	54.548	1.1679	2.8382	1.8503	NaN	NaN	NaN	13.155

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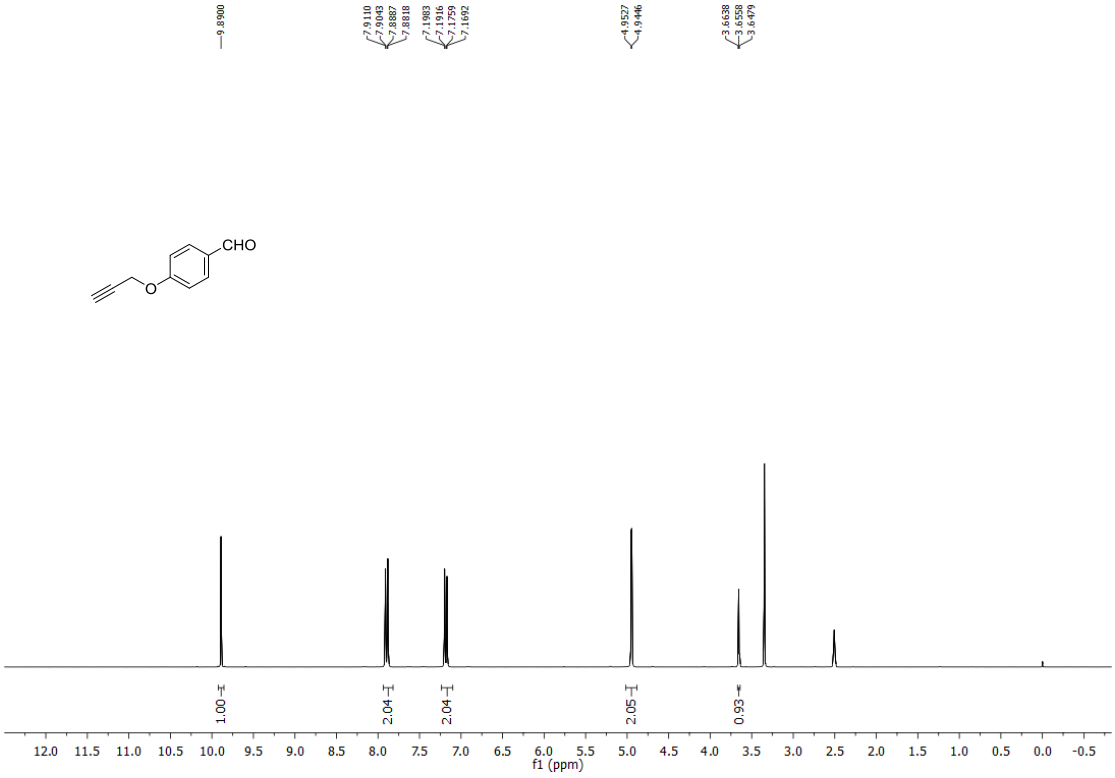
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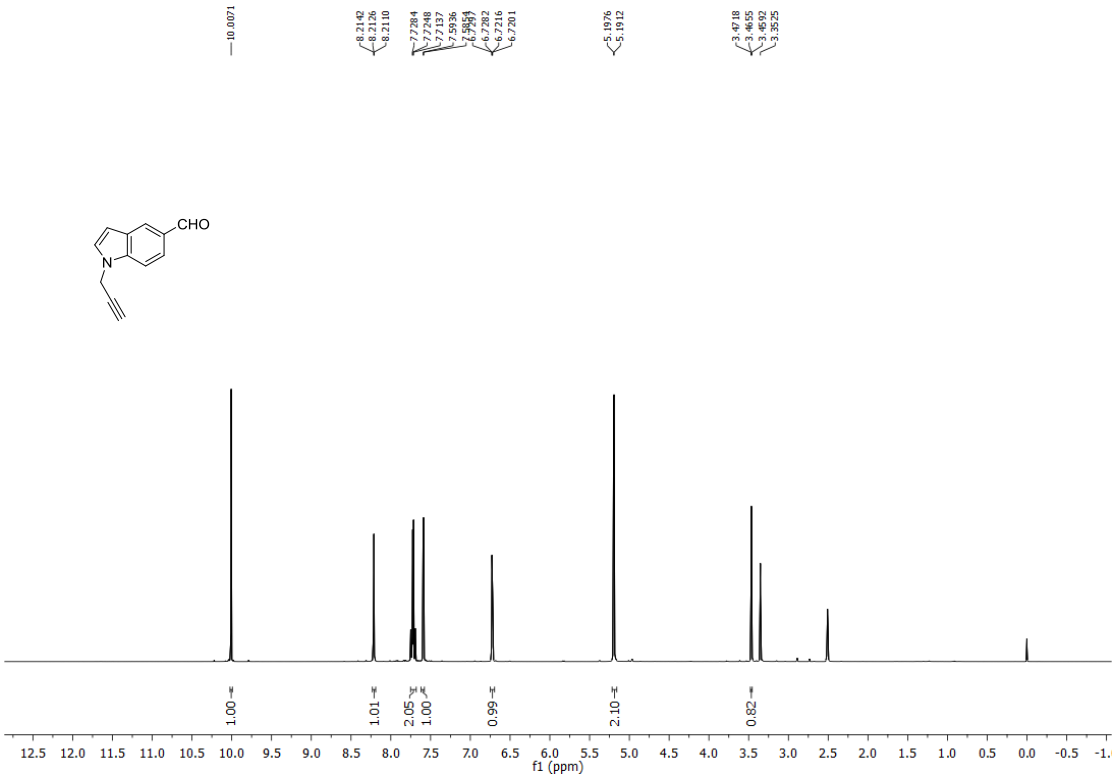
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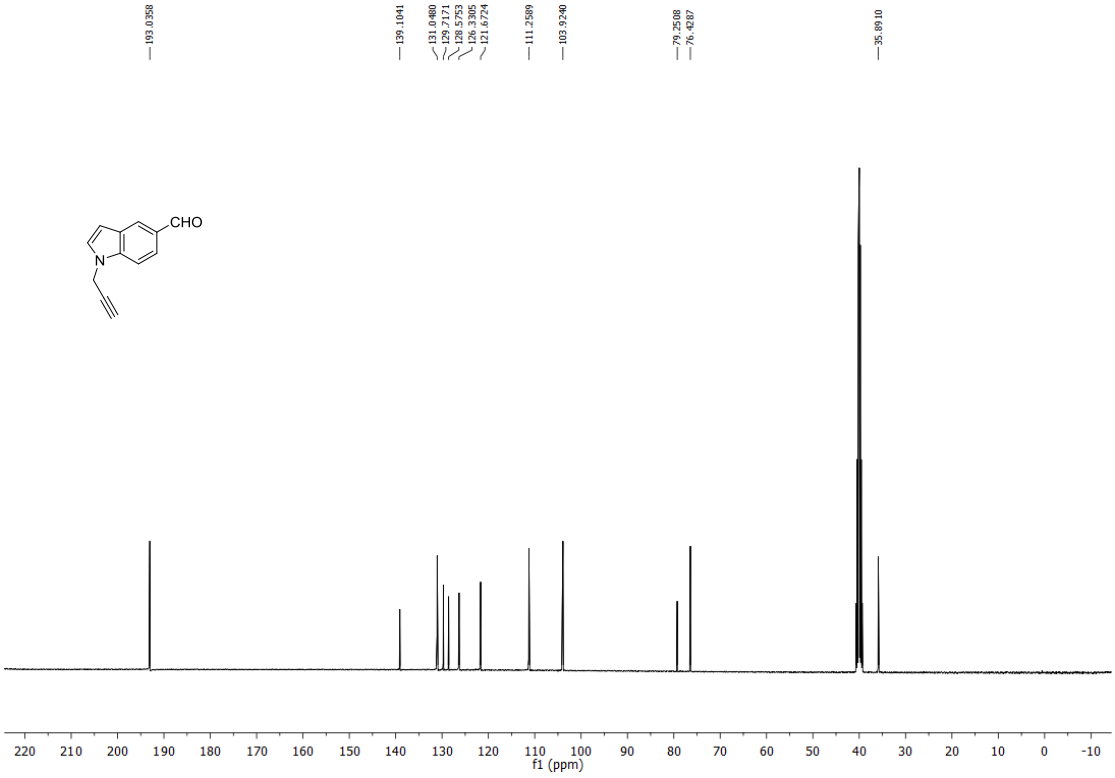
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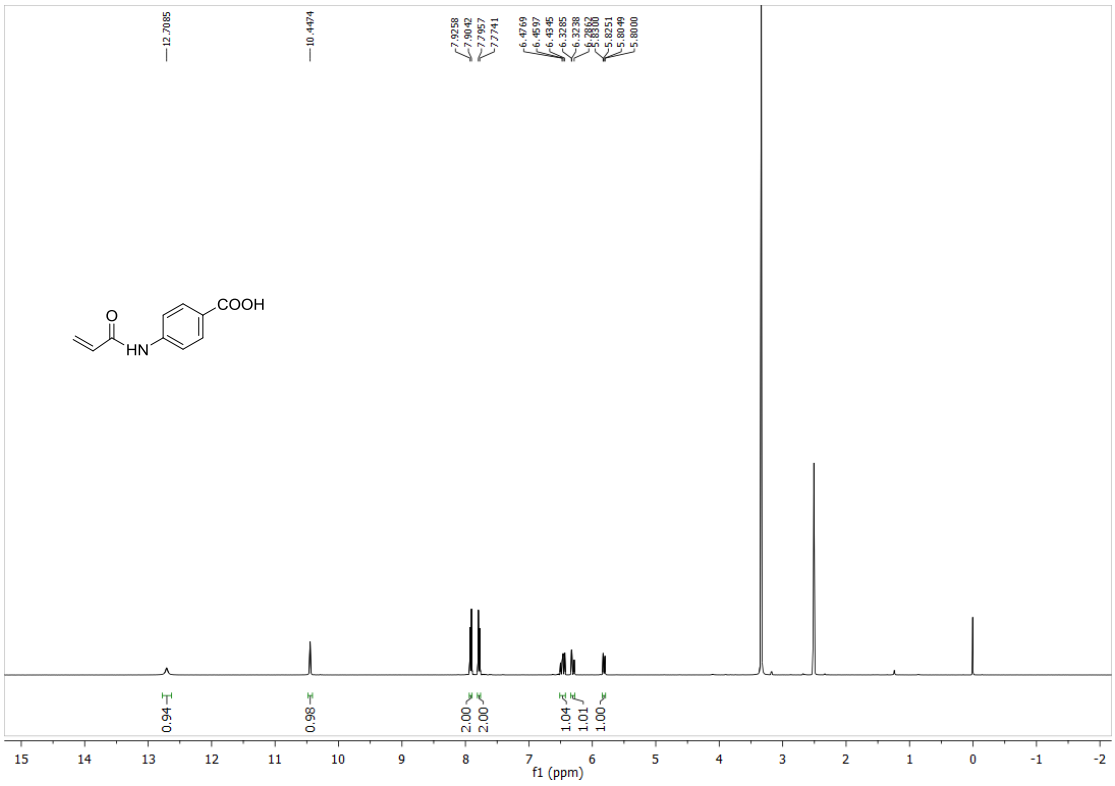
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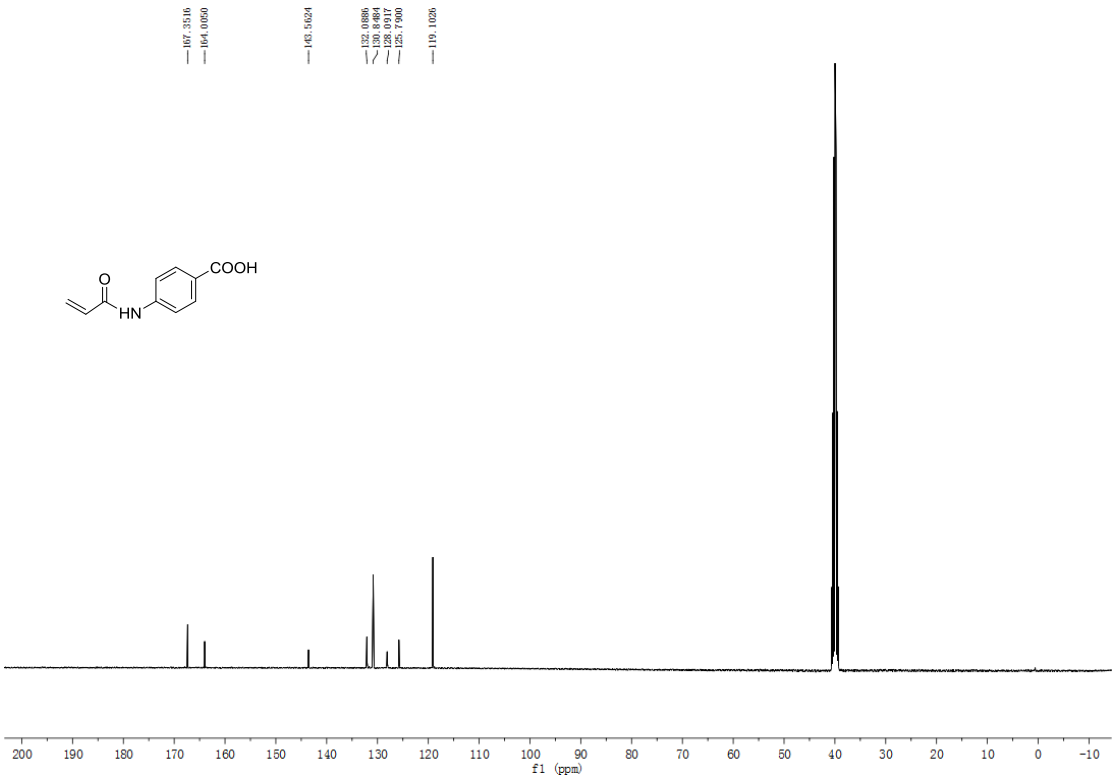
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f1 (ppm)

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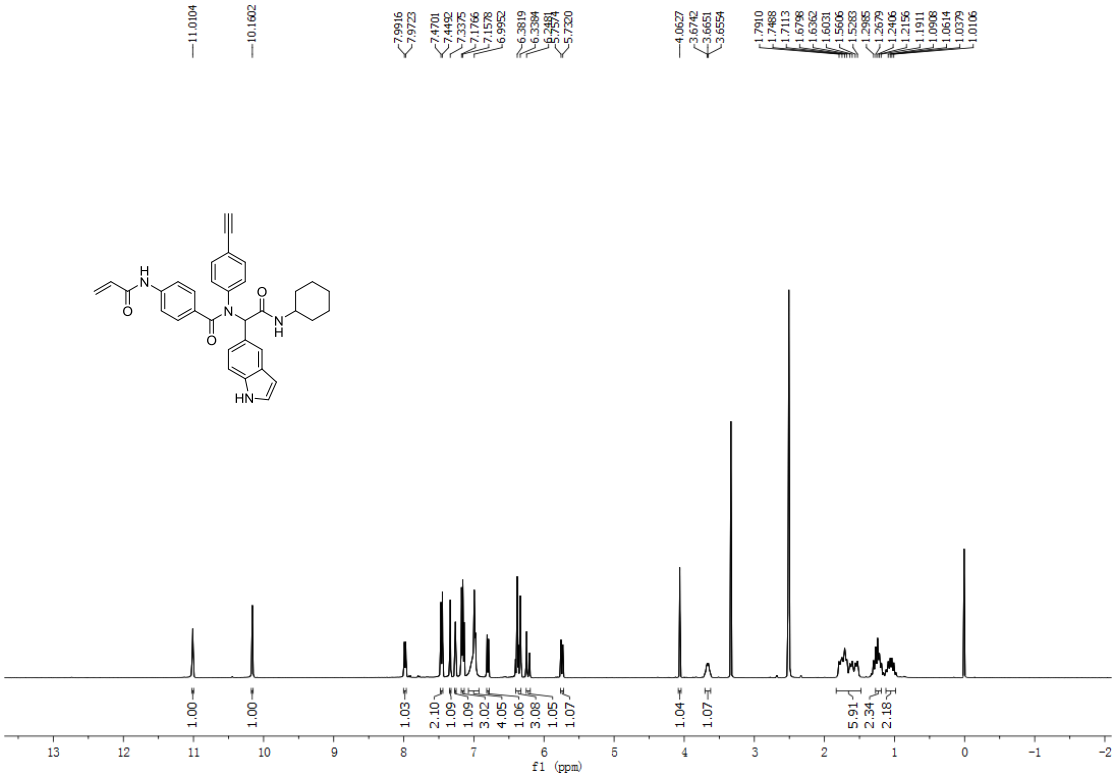
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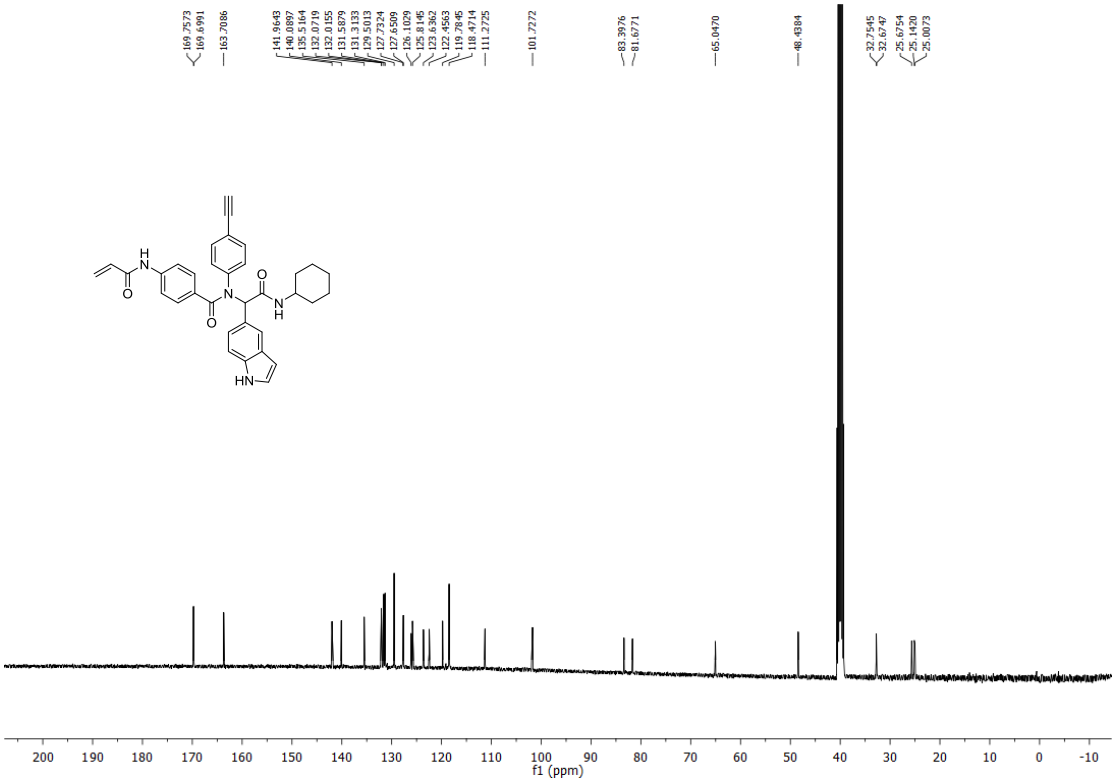
f1 (ppm)

P11 ^{13}C



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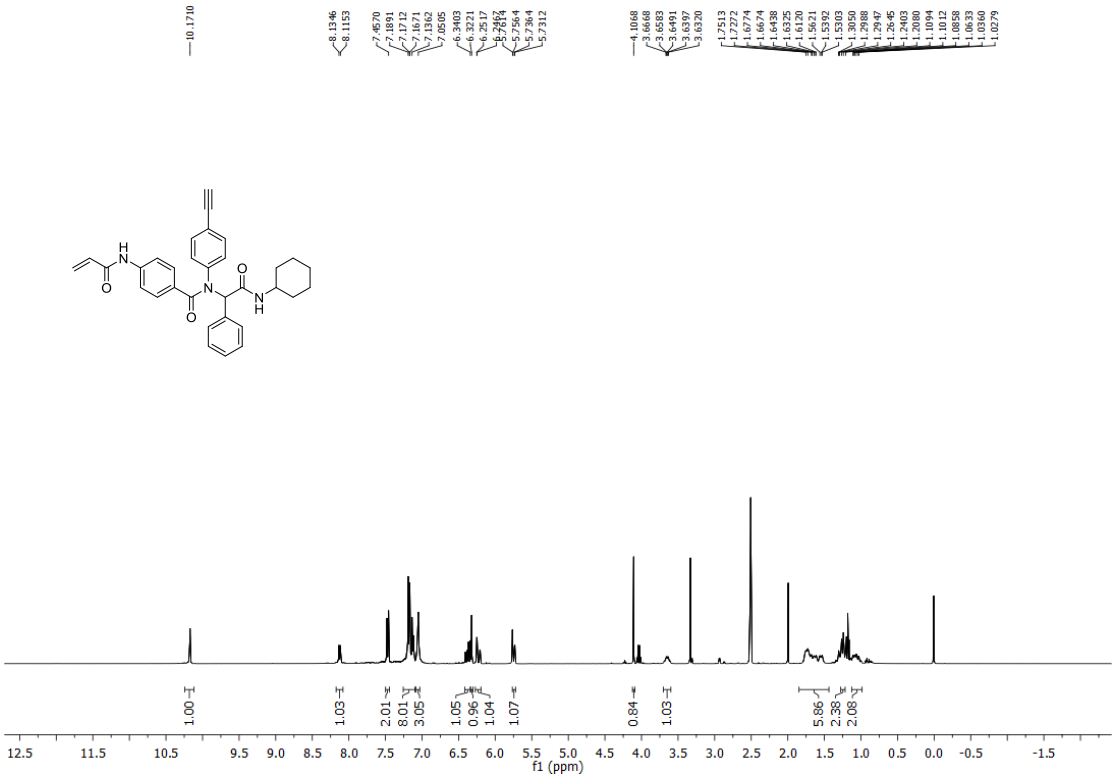

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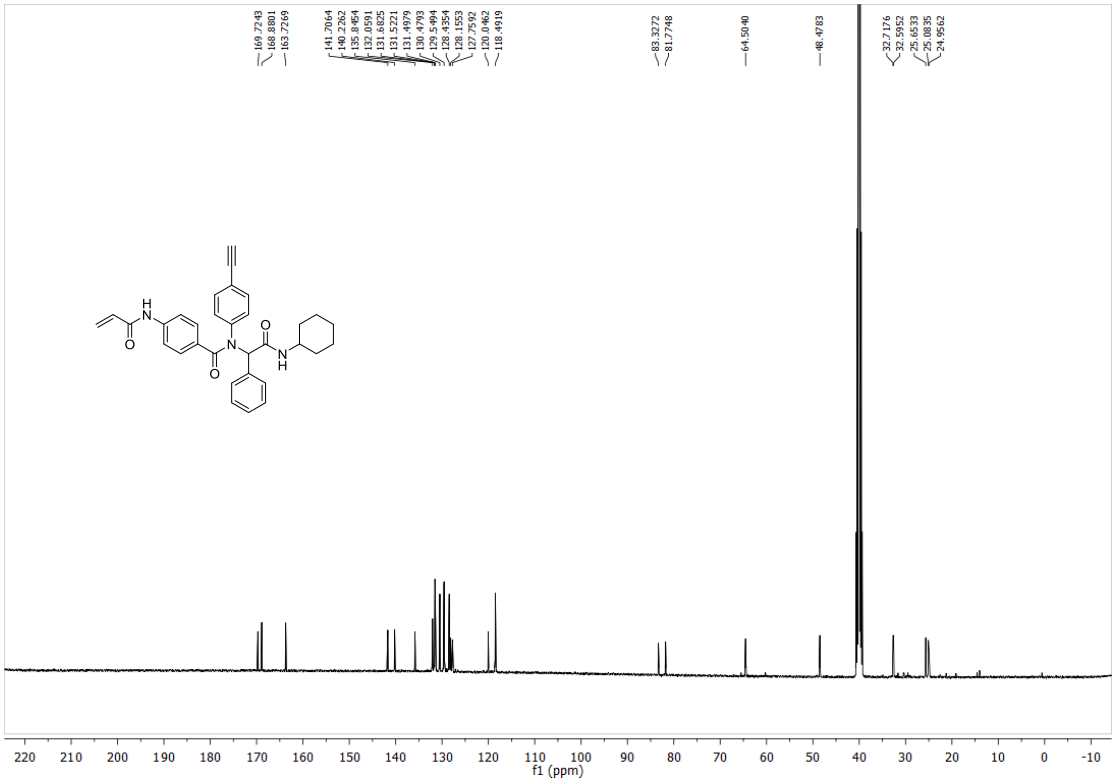

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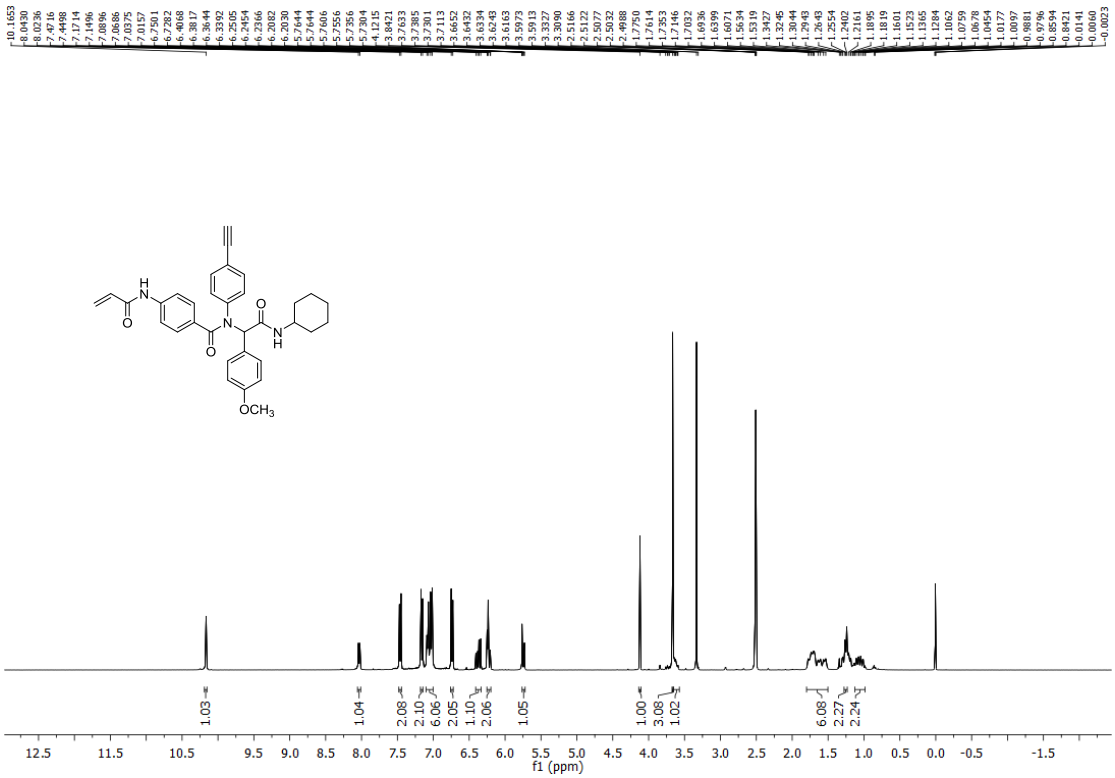

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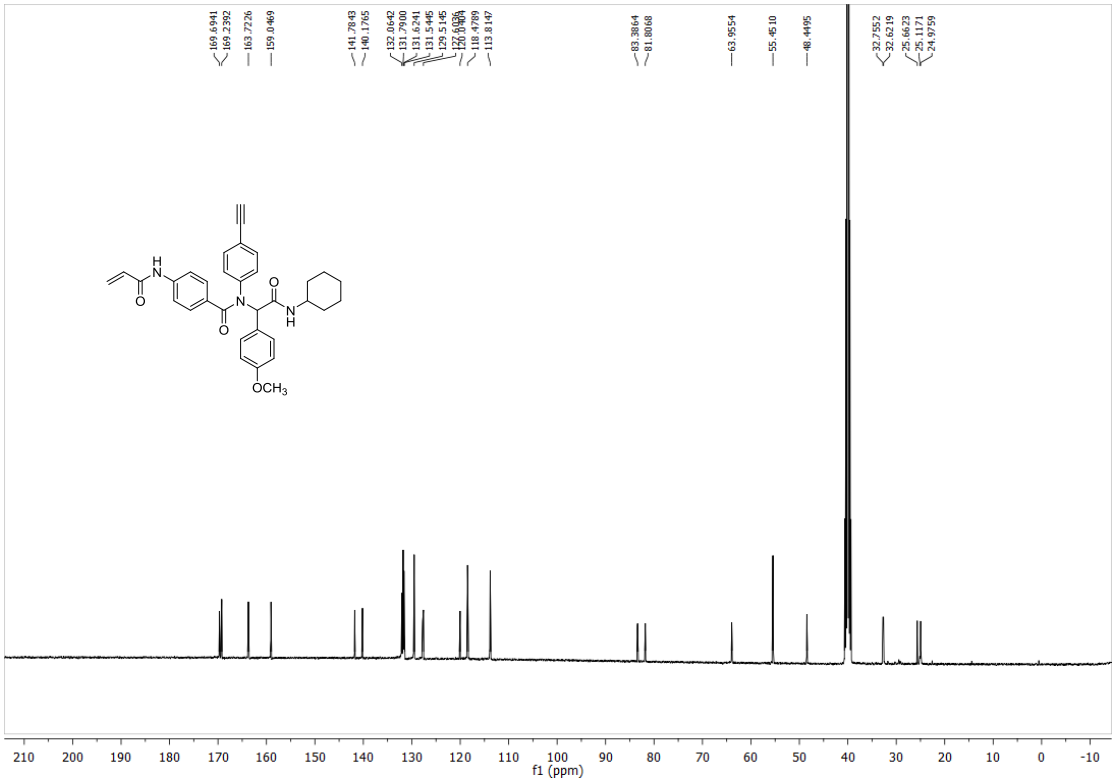

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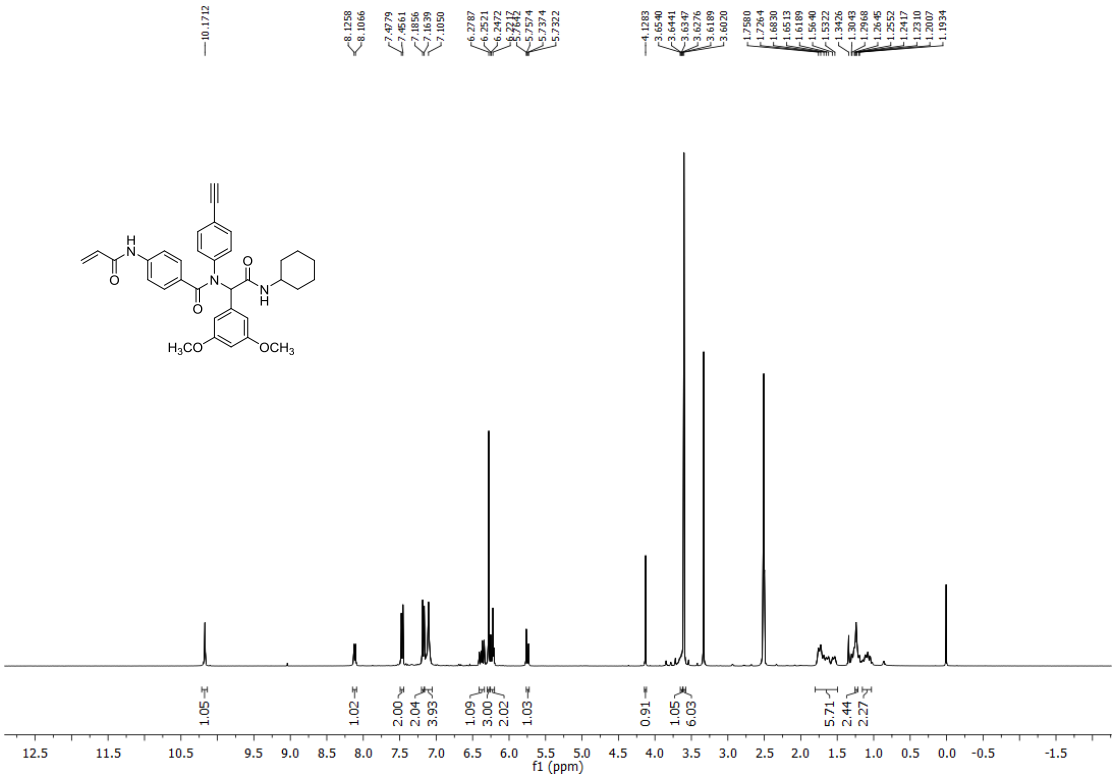

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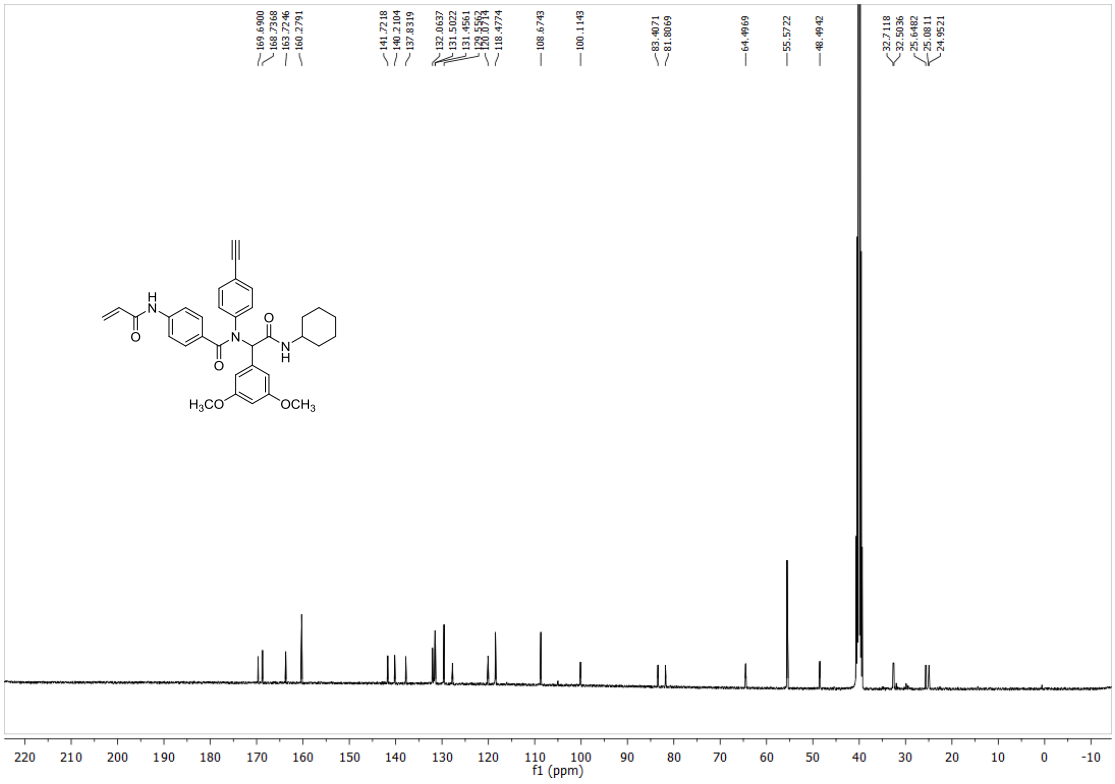

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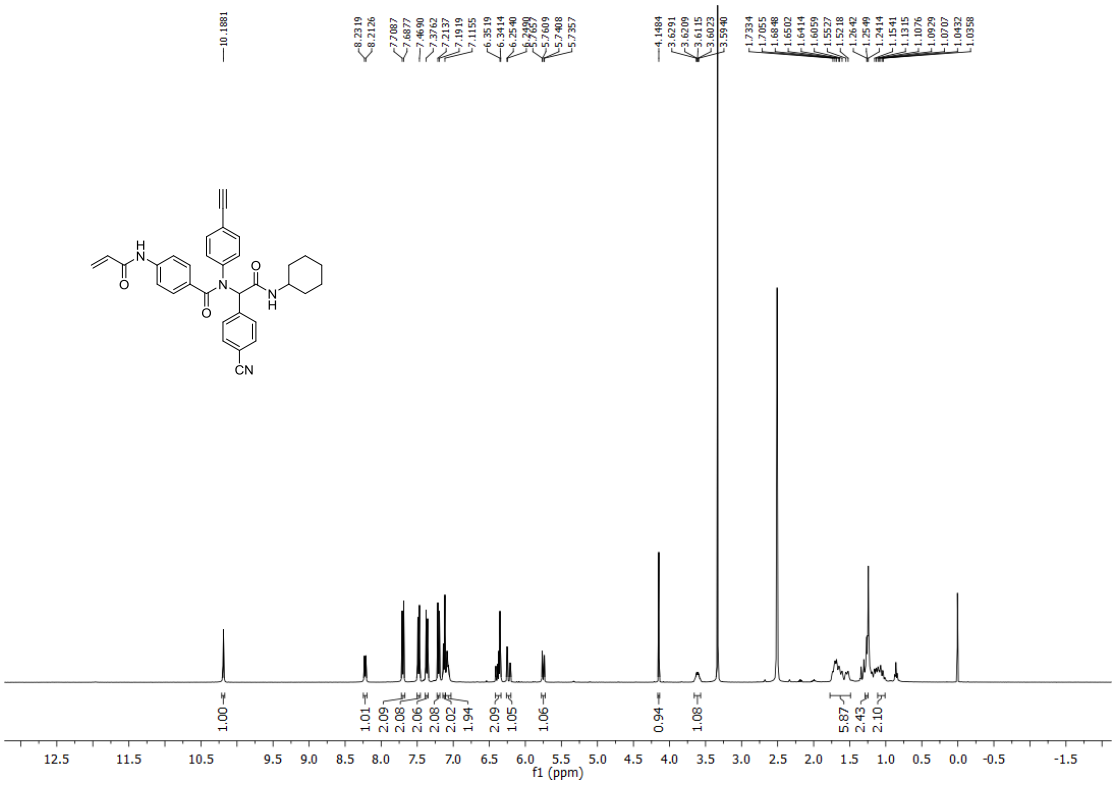

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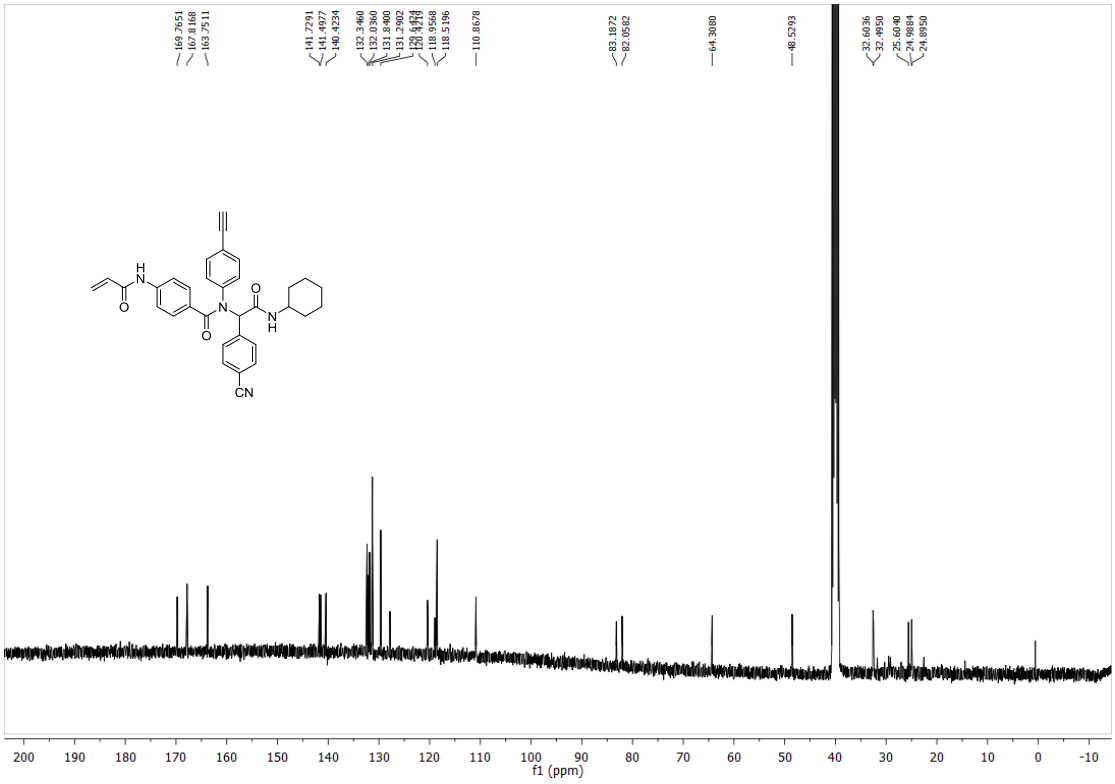

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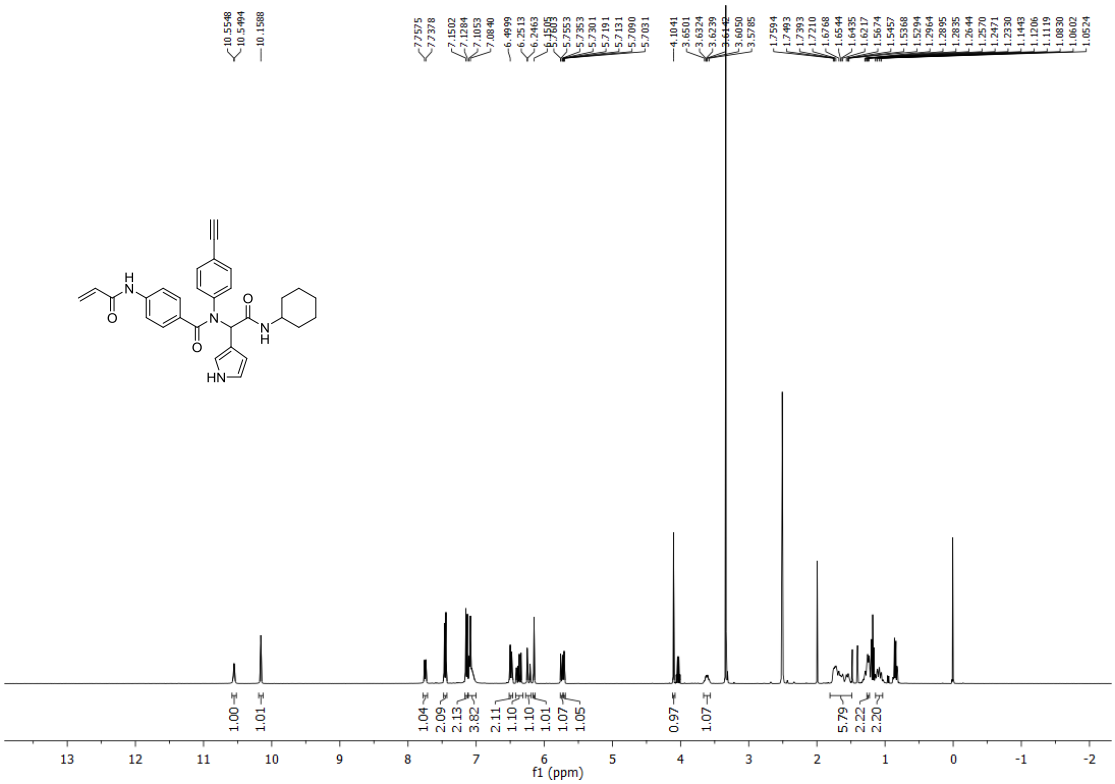
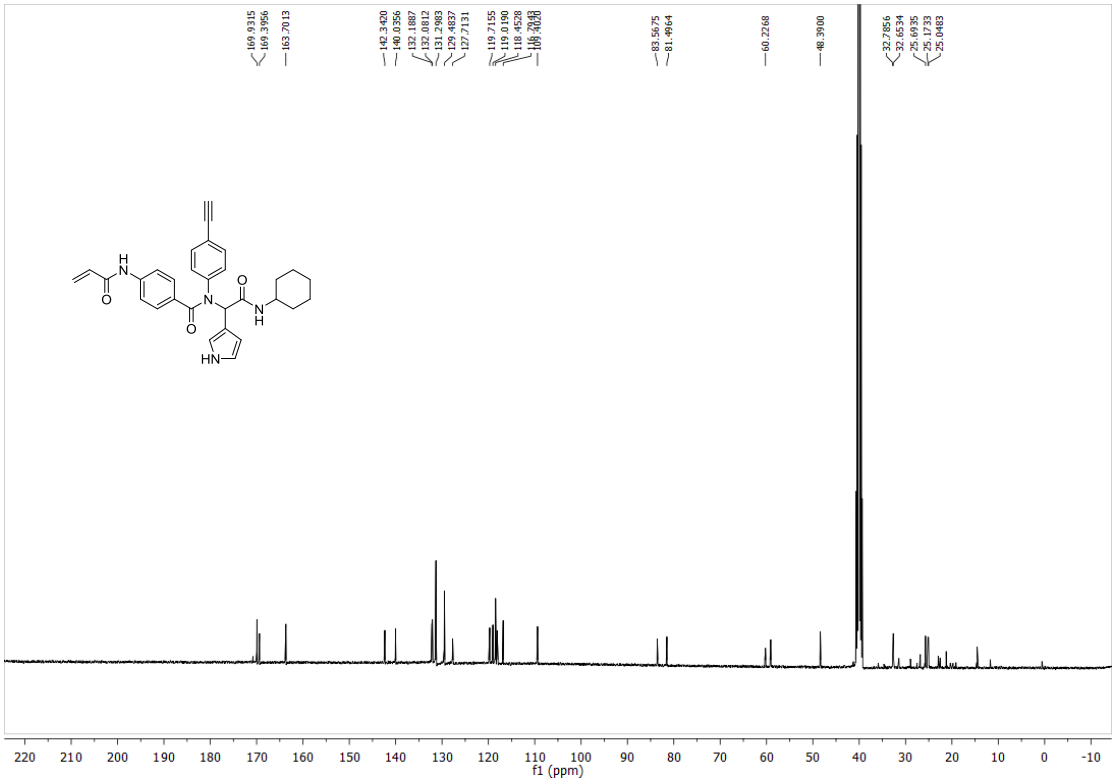
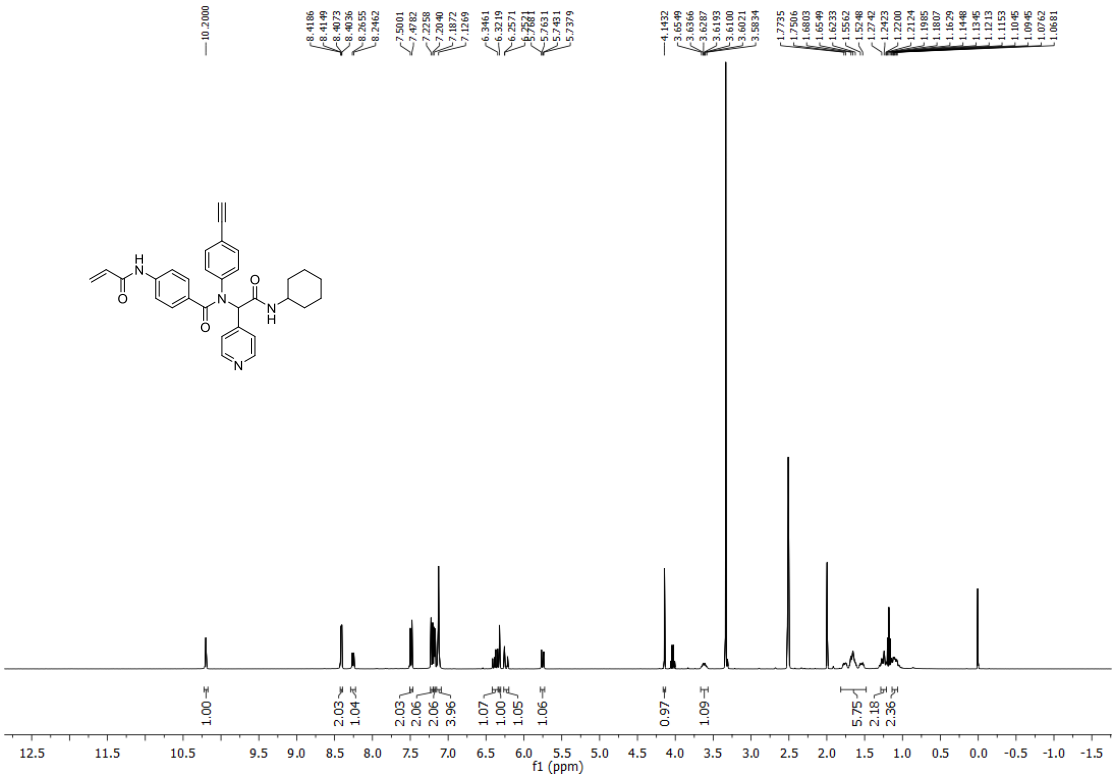


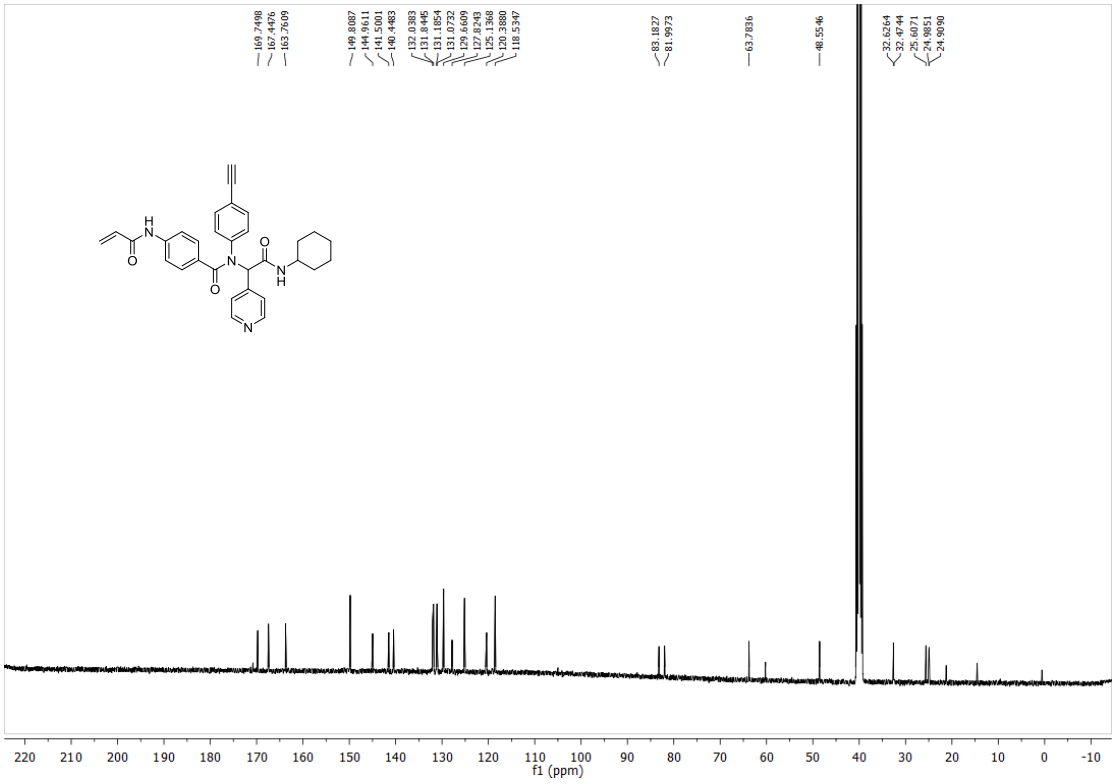
P16 ^{13}C



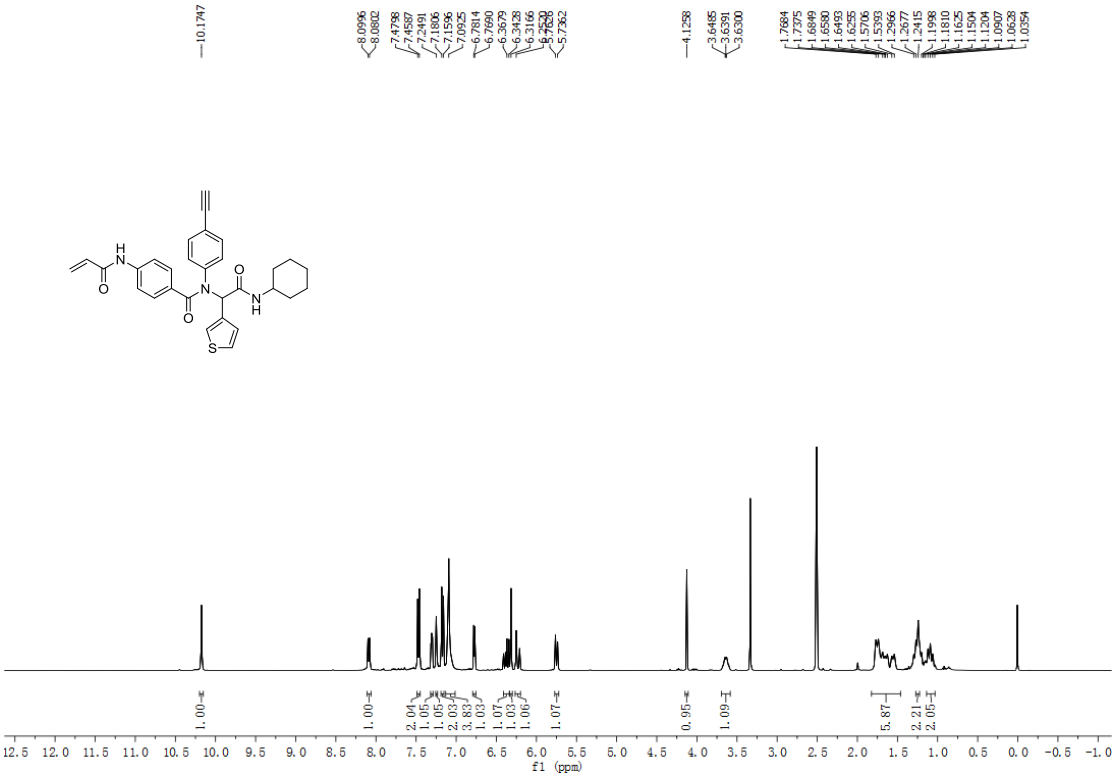
P17 ^{1}H



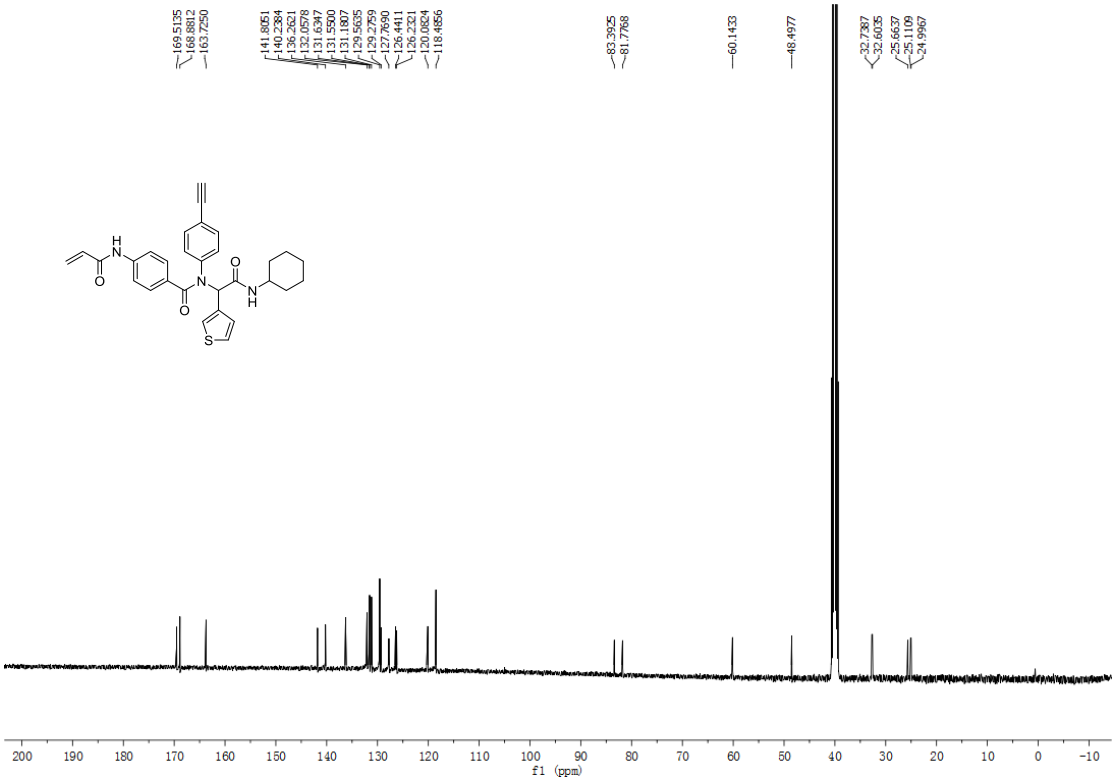
P17 ^{13}C



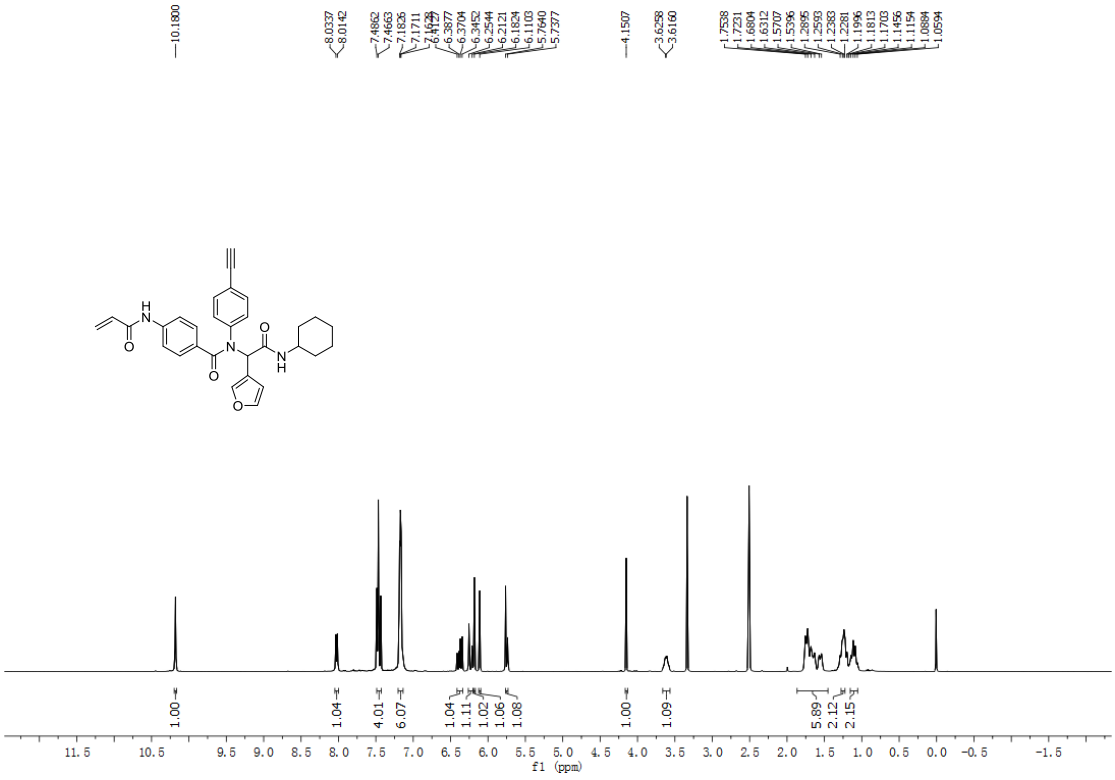
P18 ^{1}H



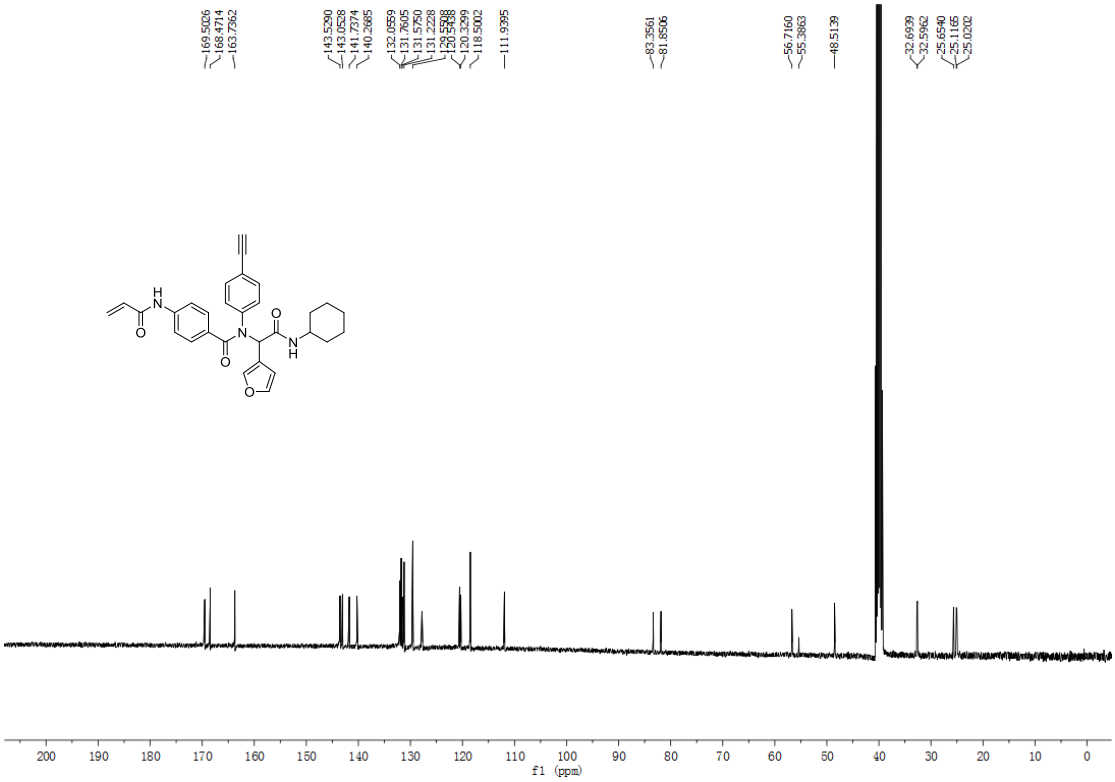
P18 ^{13}C



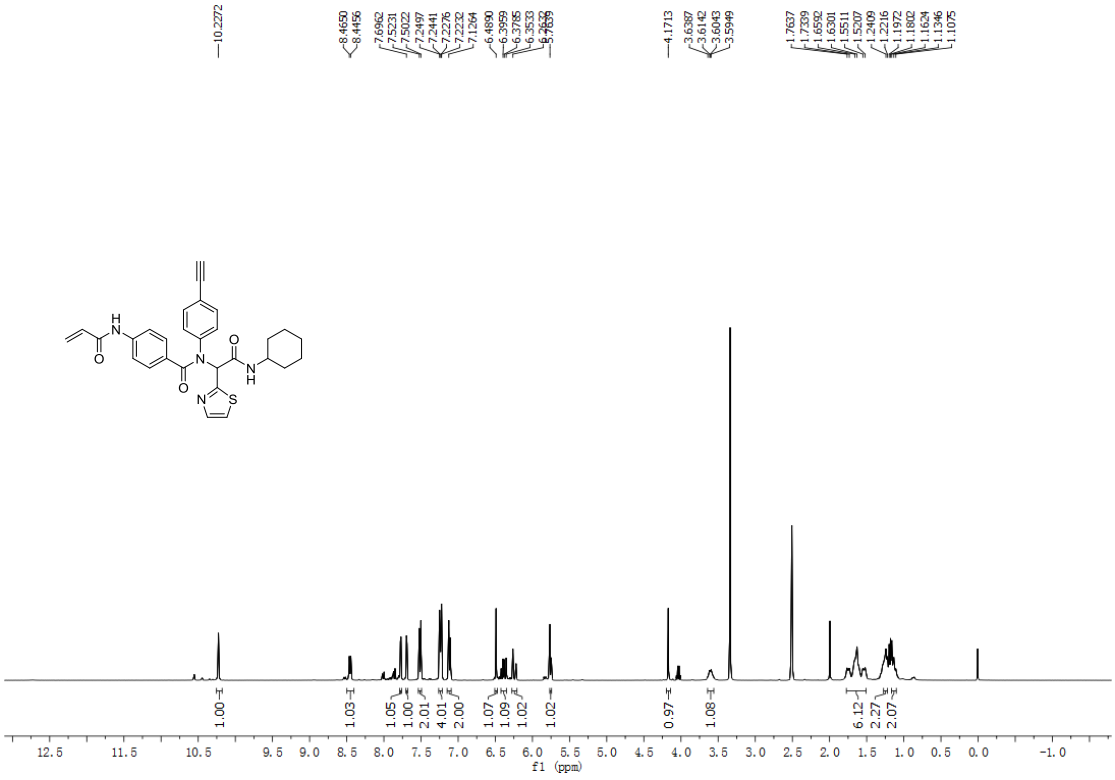
P19 ^{1}H



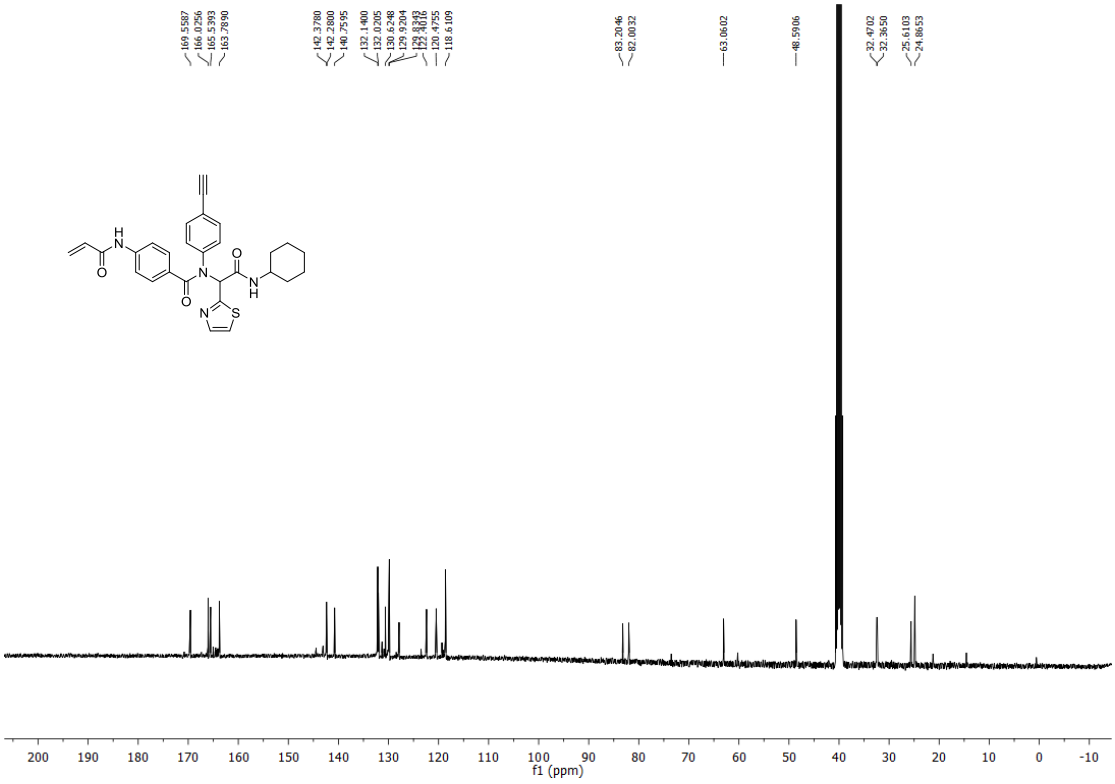
P19 ^{13}C



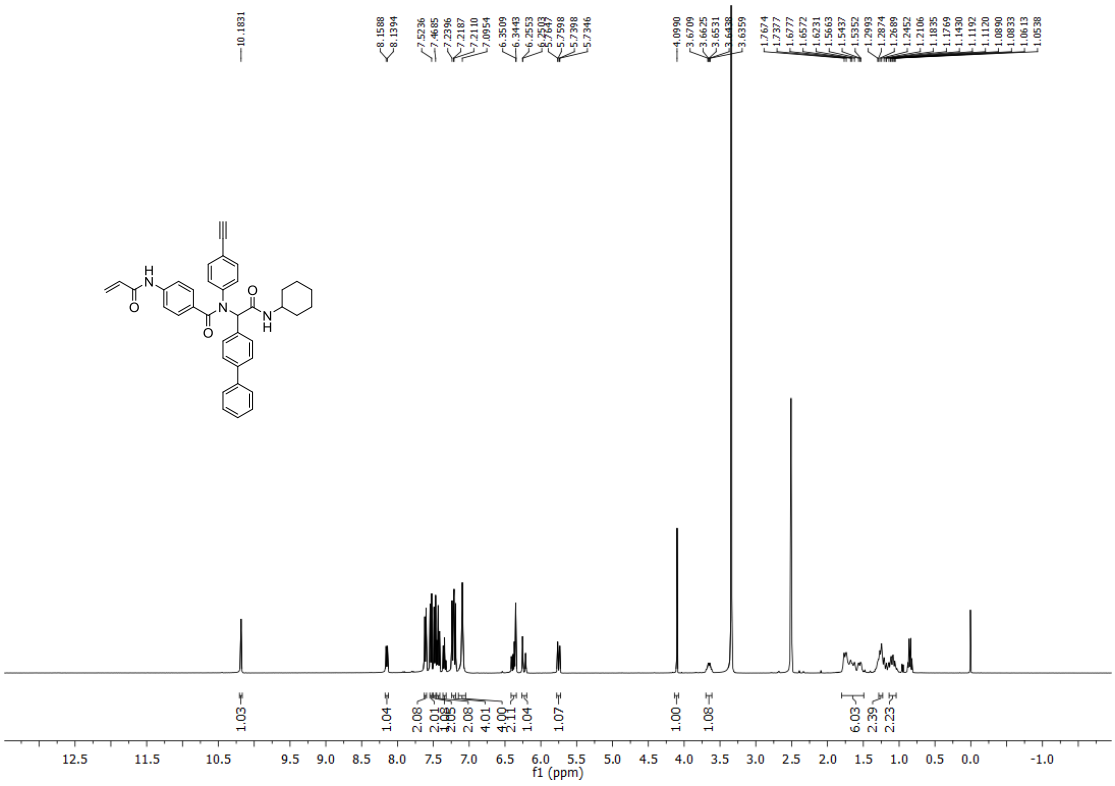
P20 ^{1}H



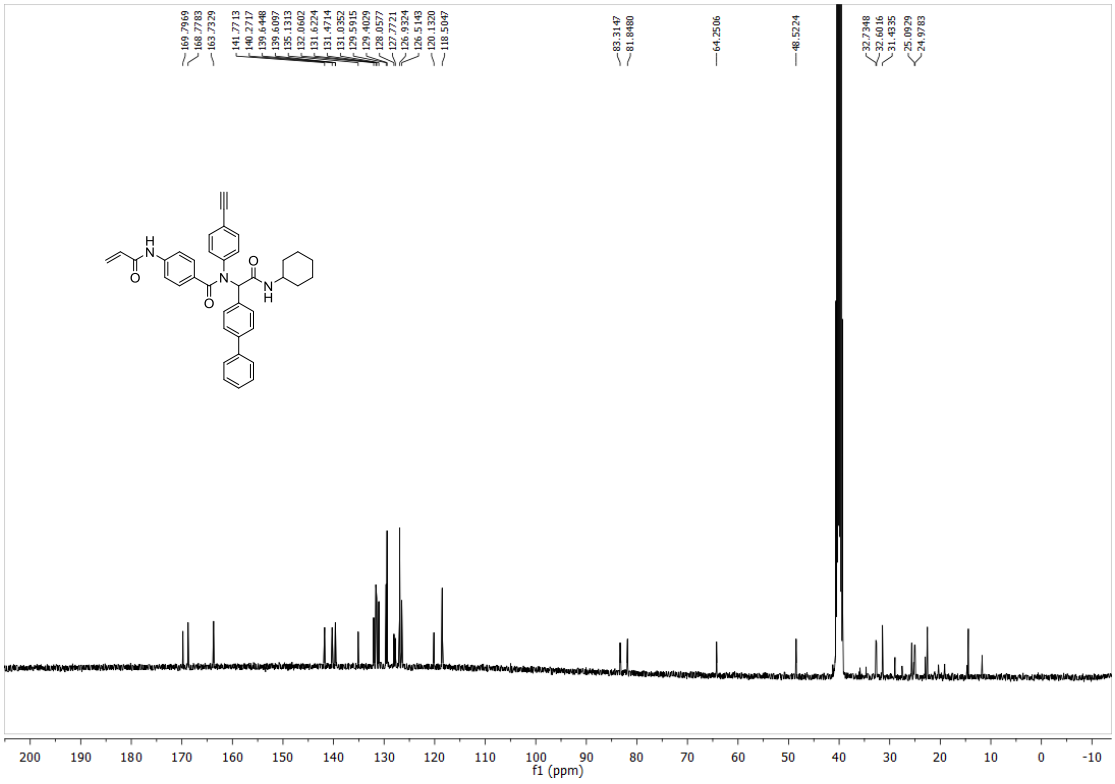
P20 ^{13}C



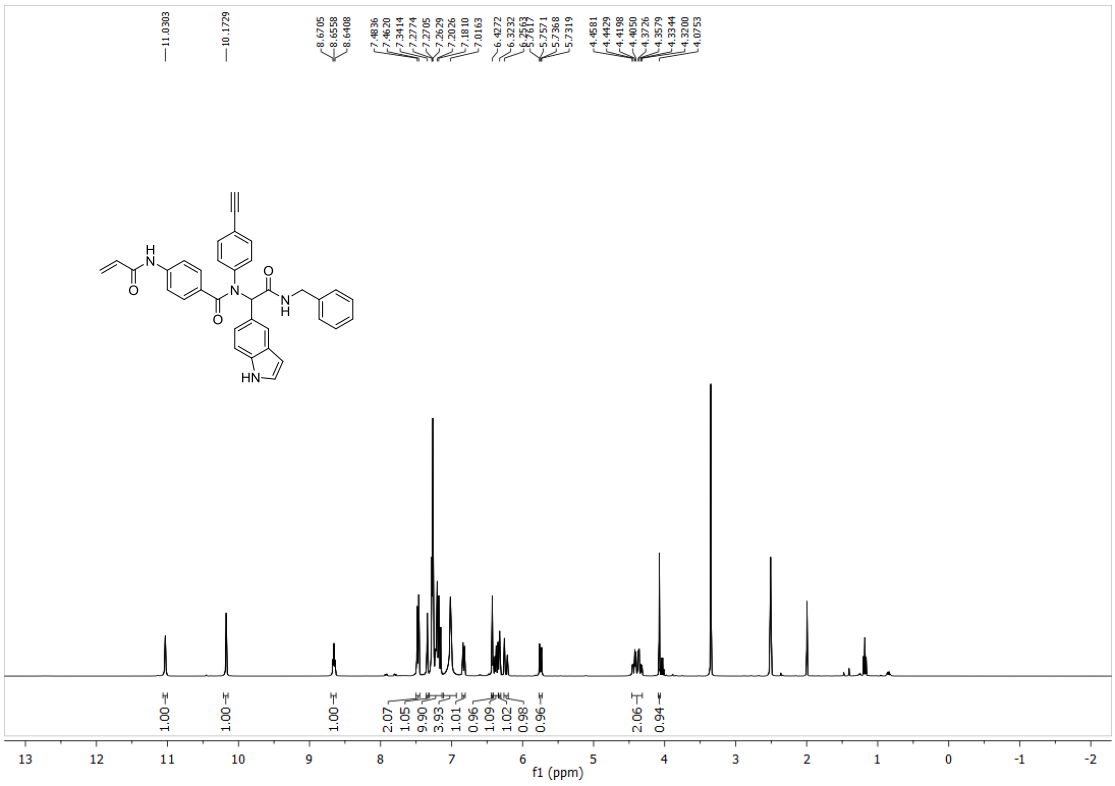
P21 ^{1}H



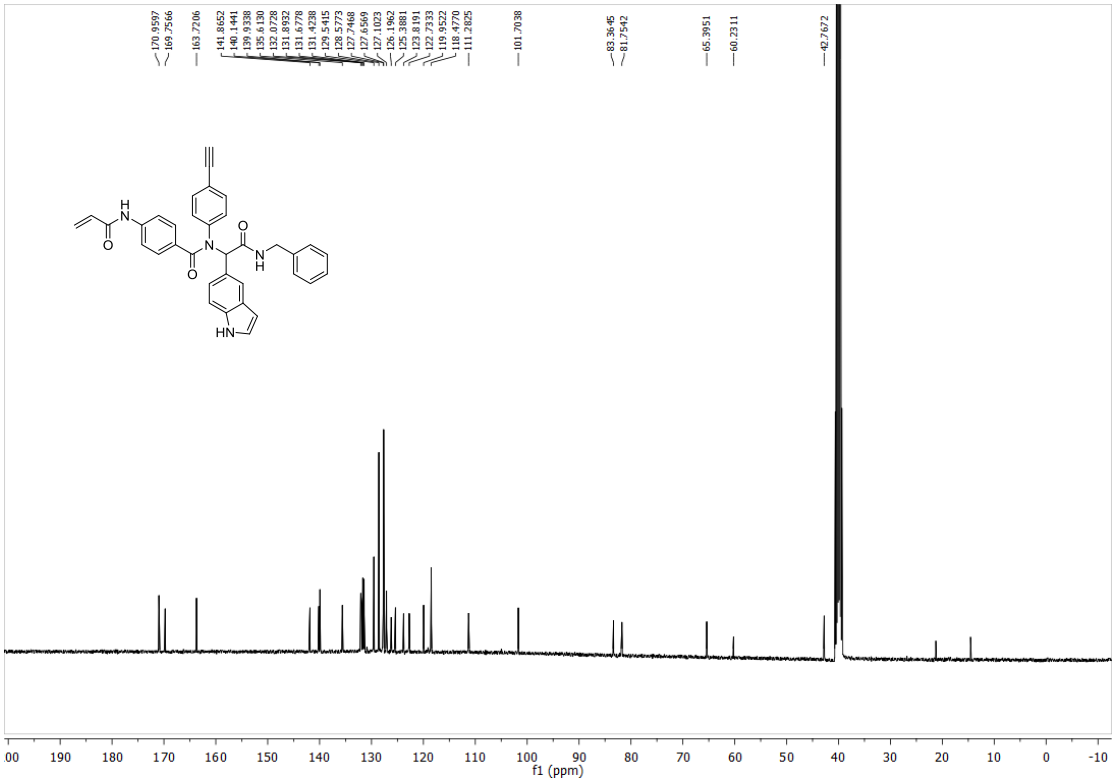
P21 ^{13}C



P23 ^{1}H



P23 ^{13}C



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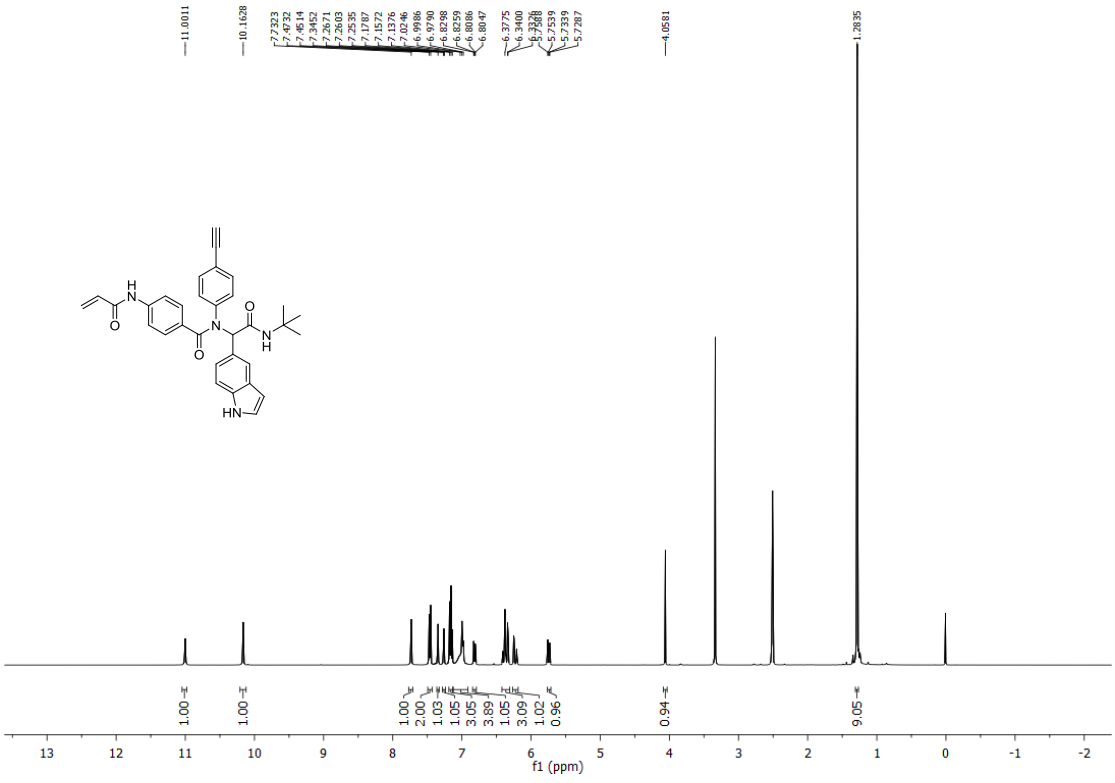

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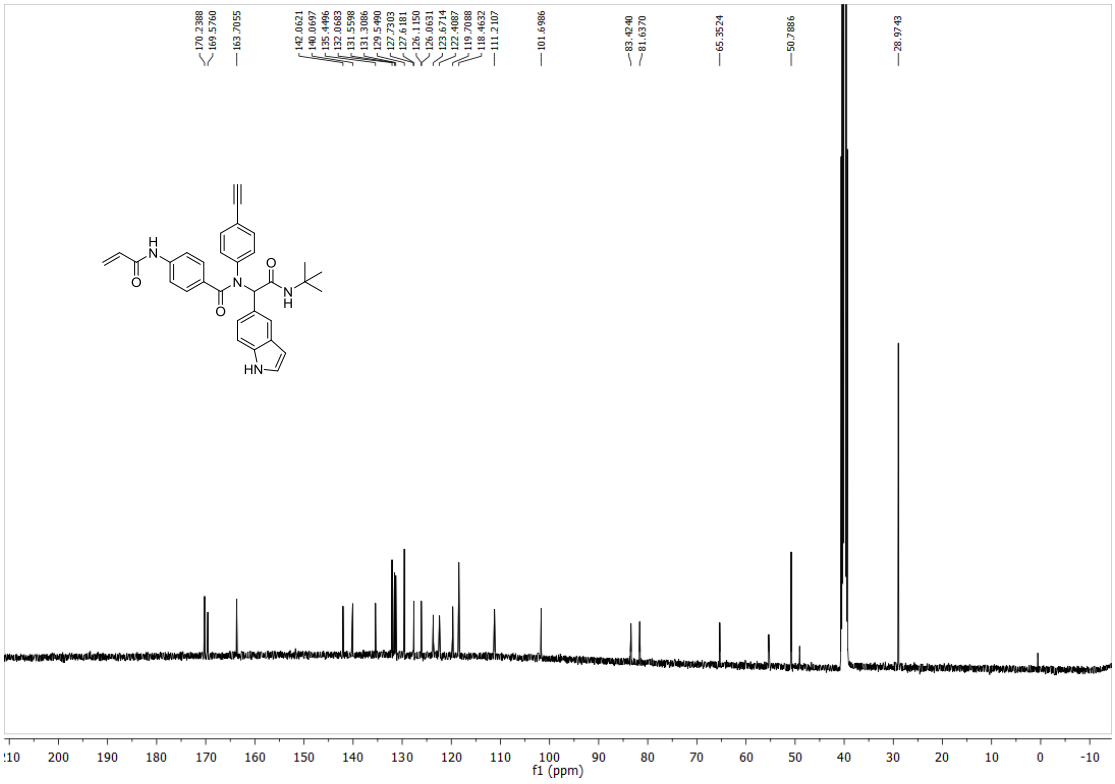
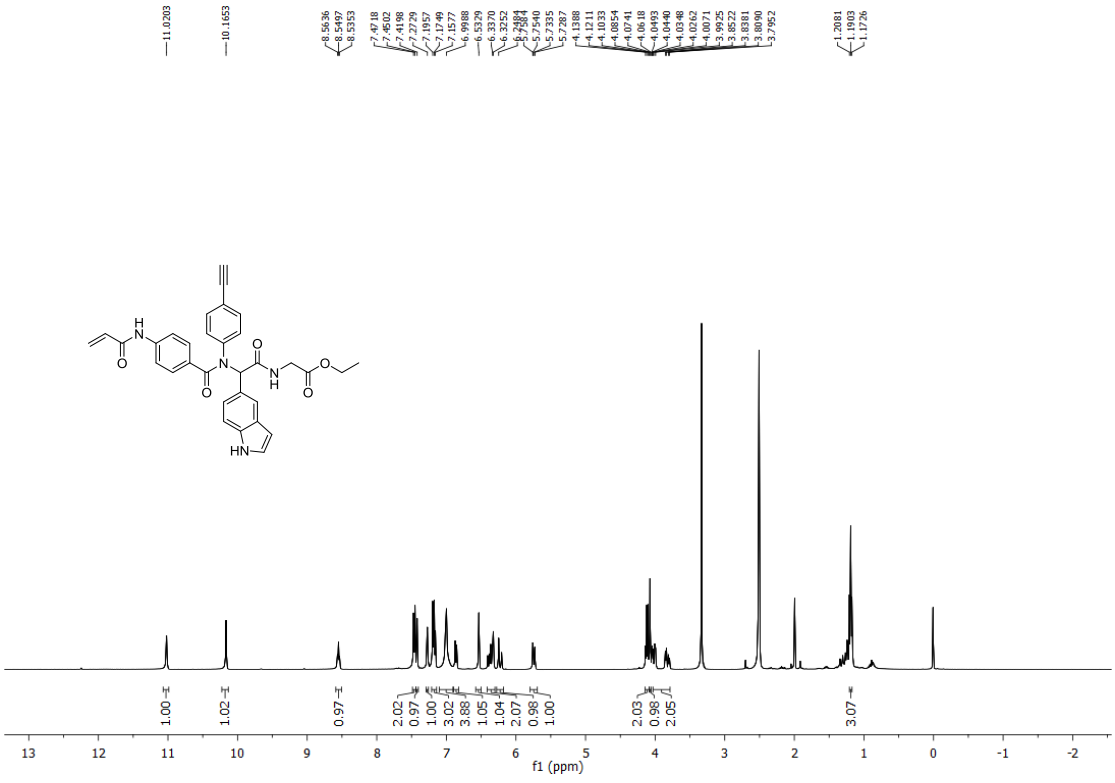
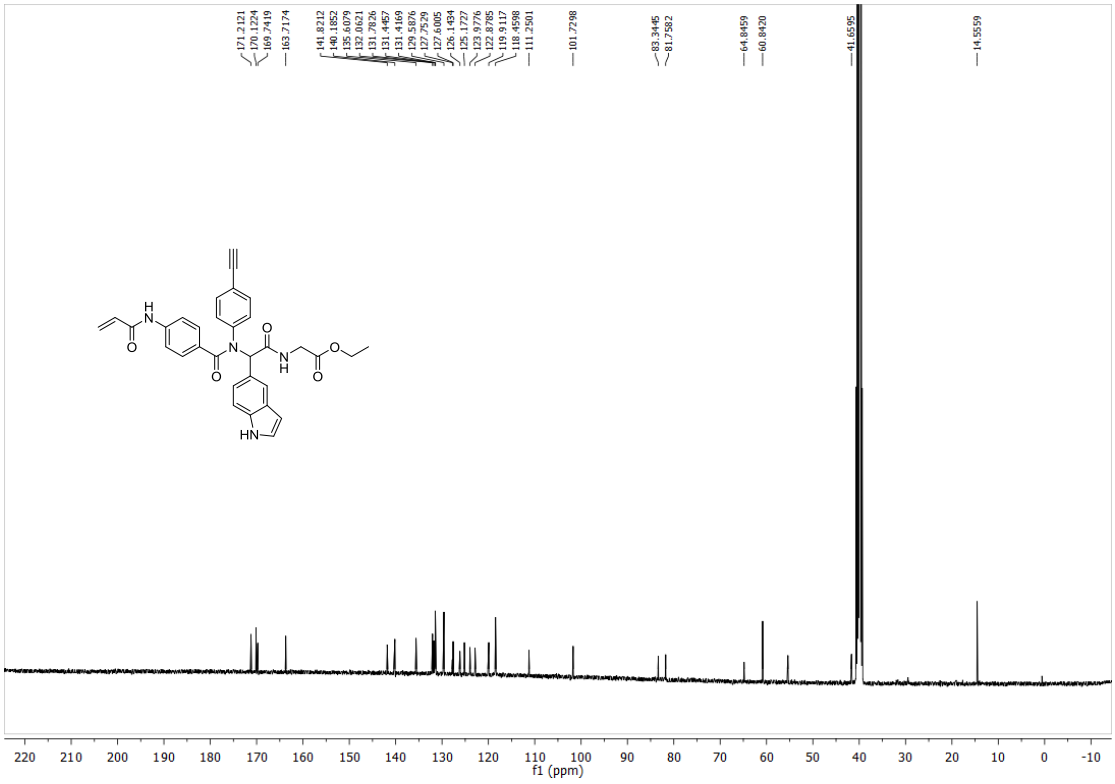


P25 ^{1}H



P25 ^{13}C



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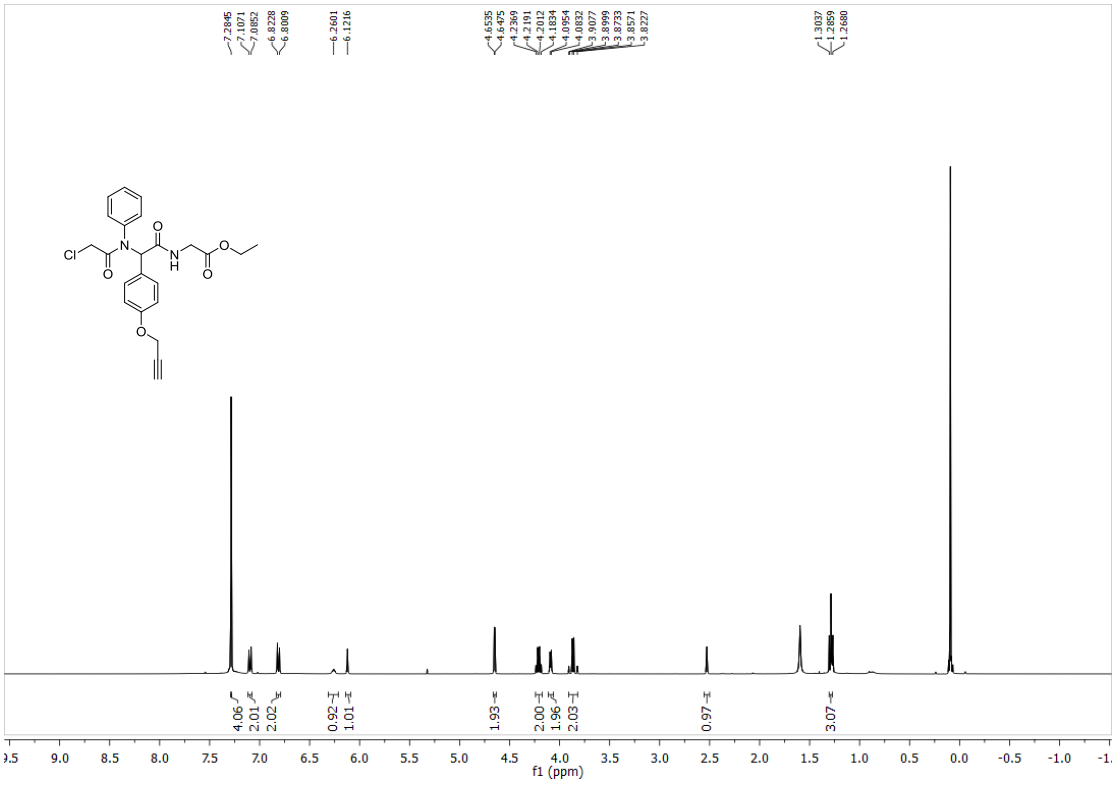
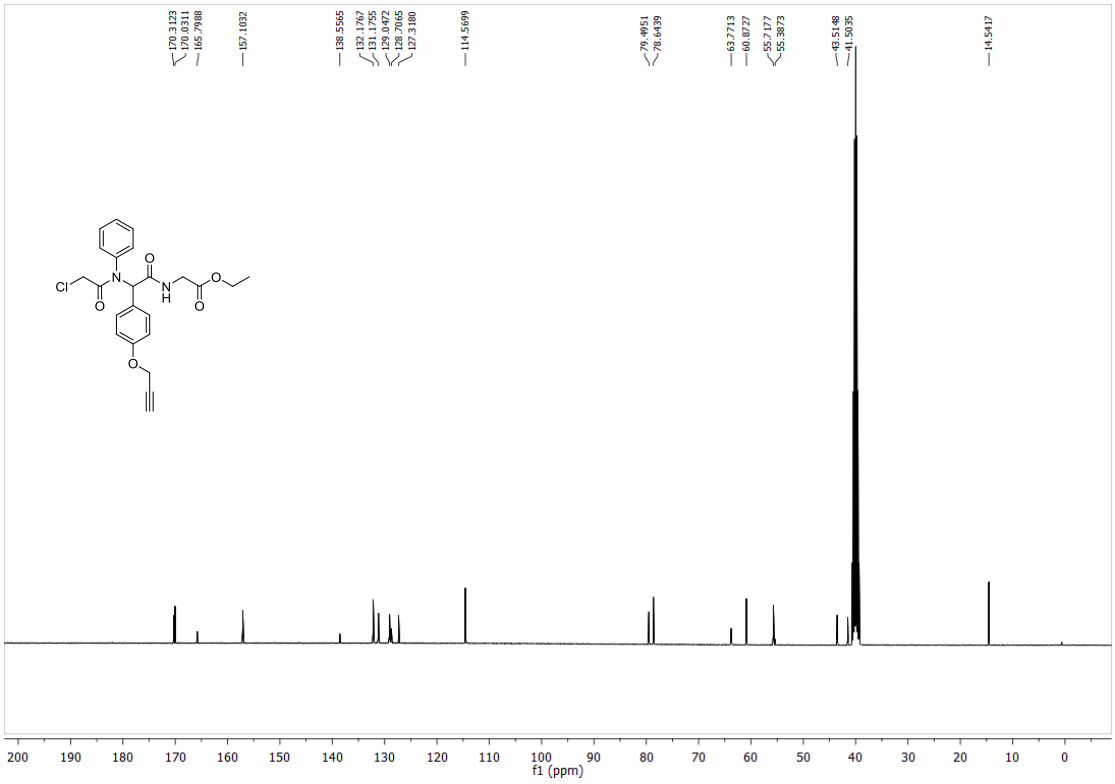
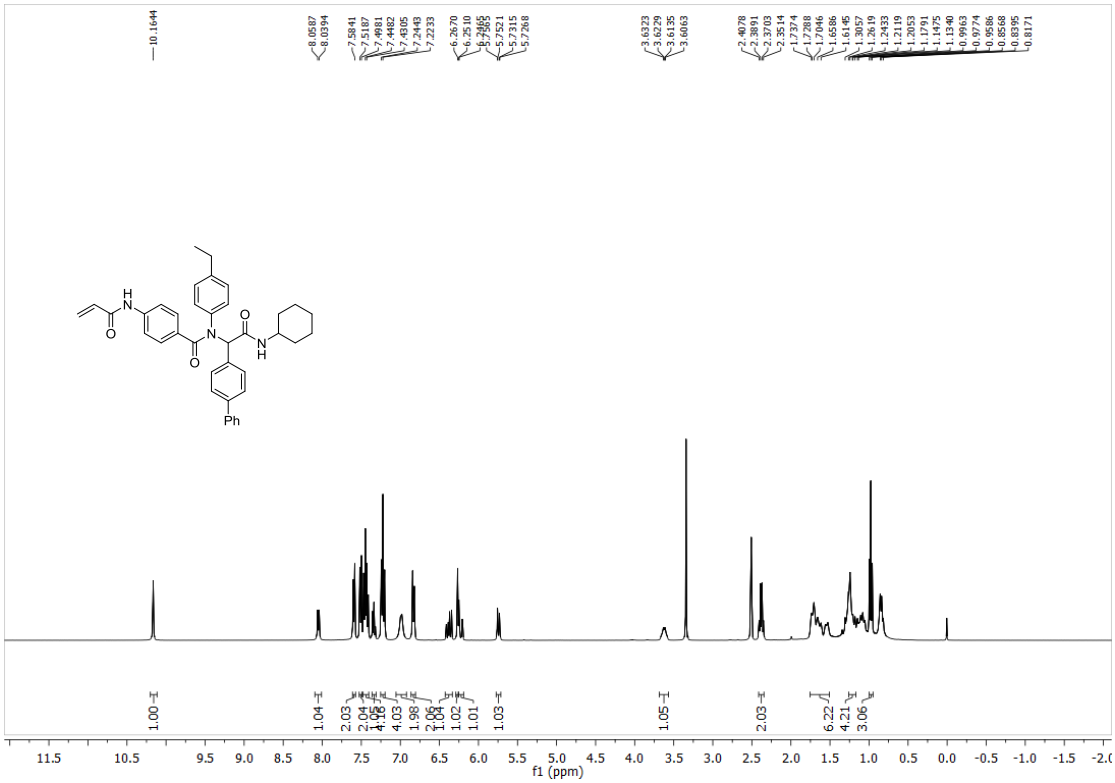


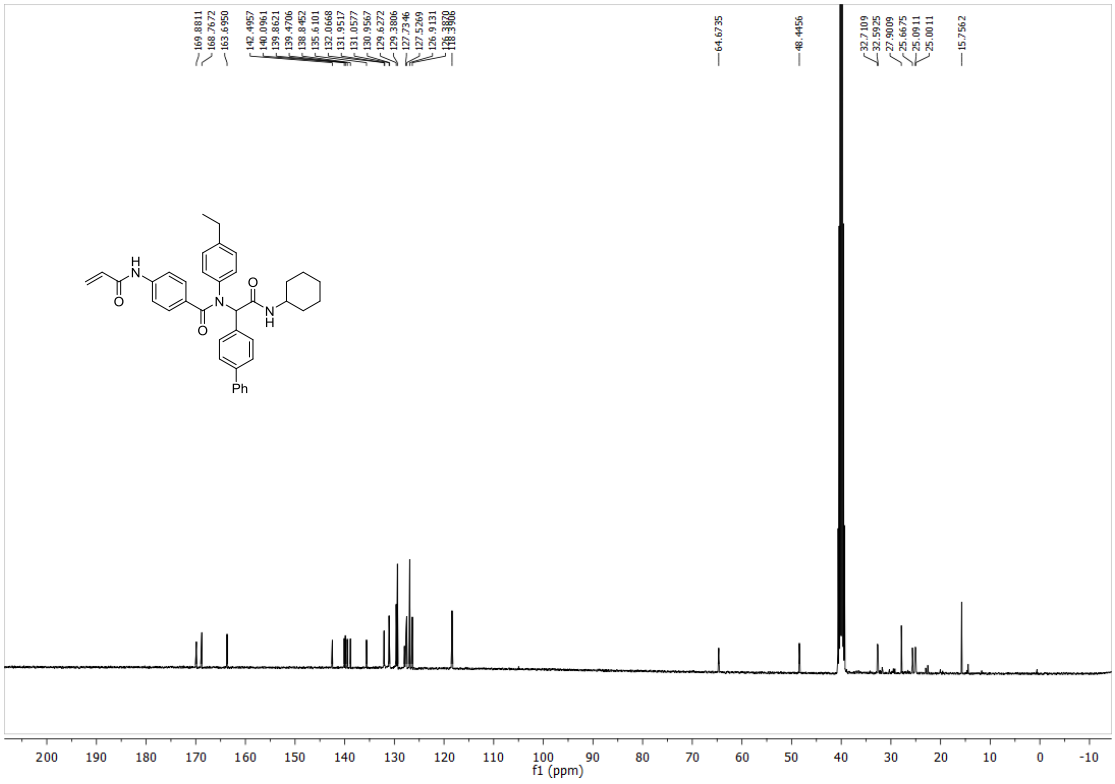
P26 ^{13}C



P27 ^{1}H



P27 ^{13}C



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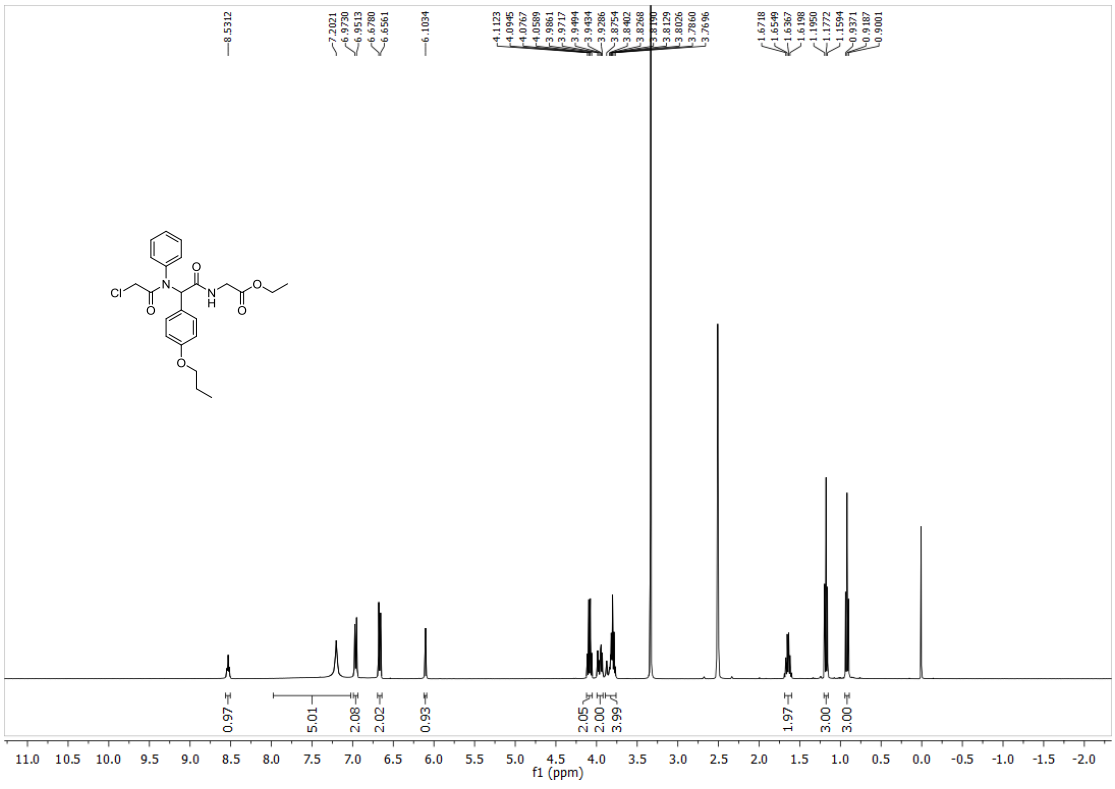

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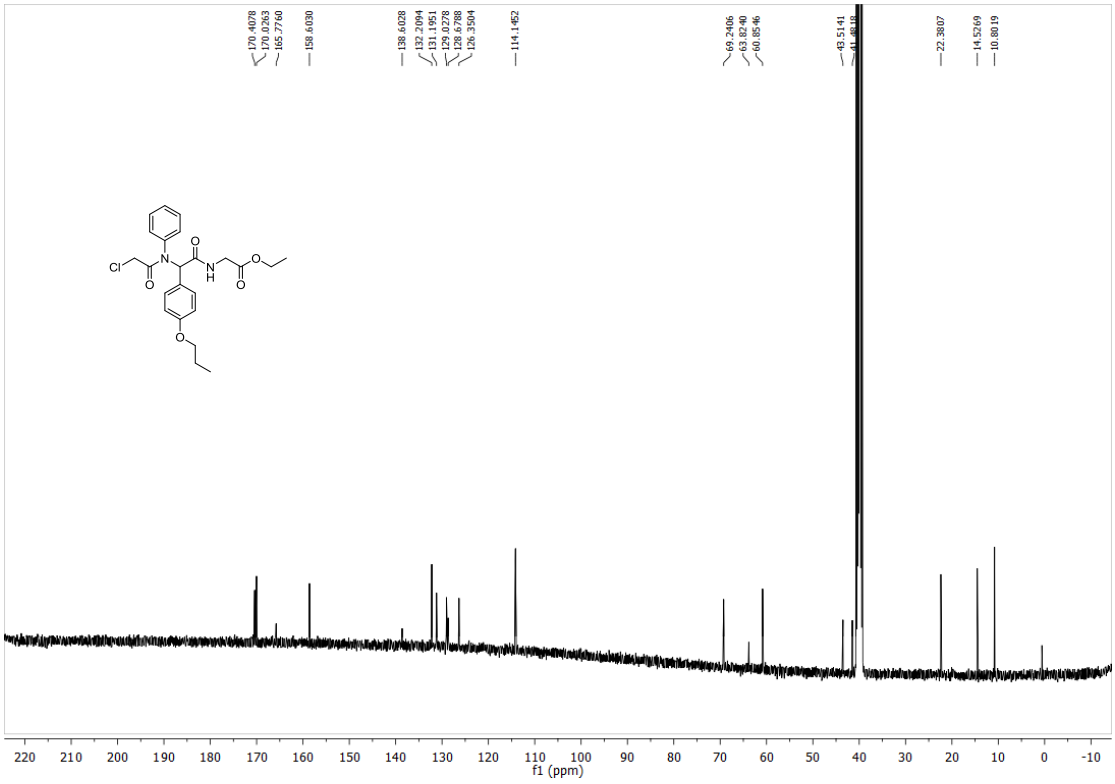

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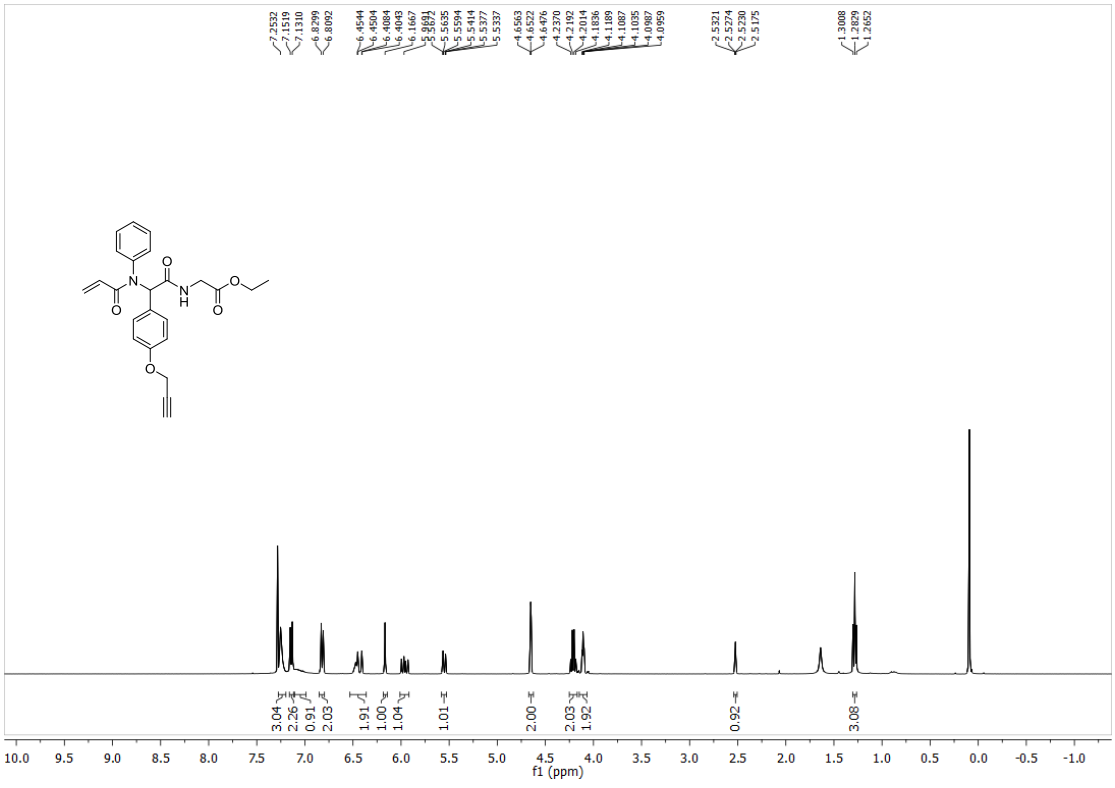
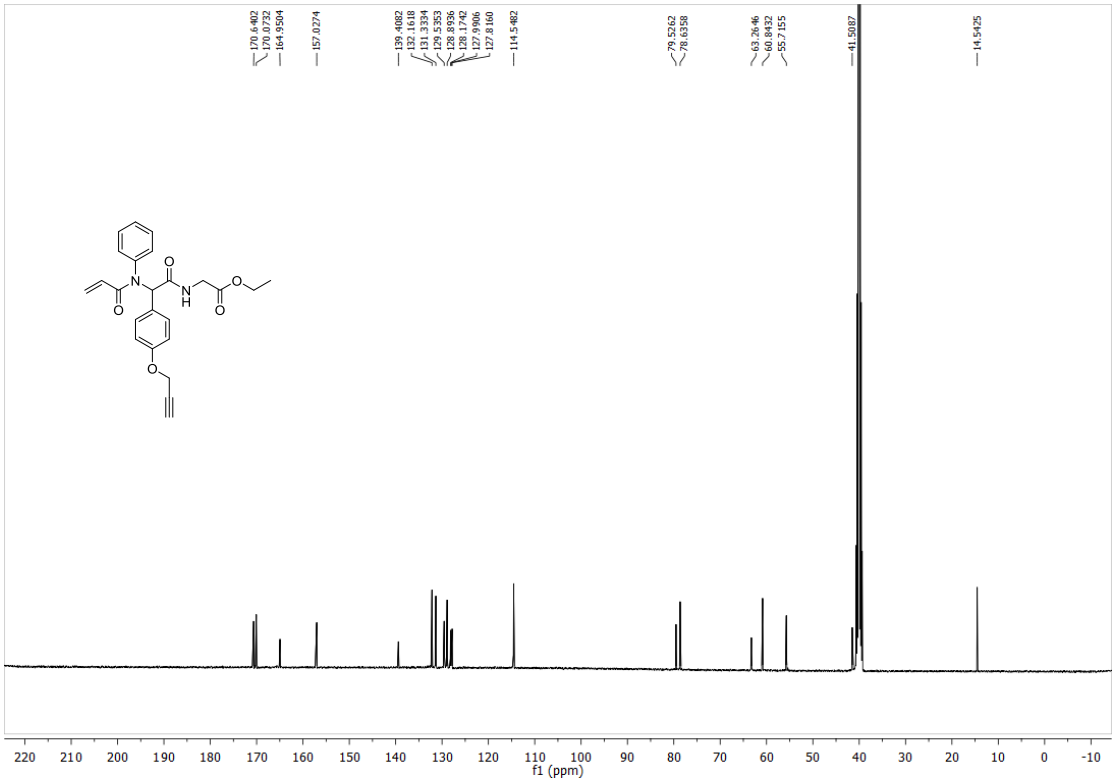


P29 ^{13}C



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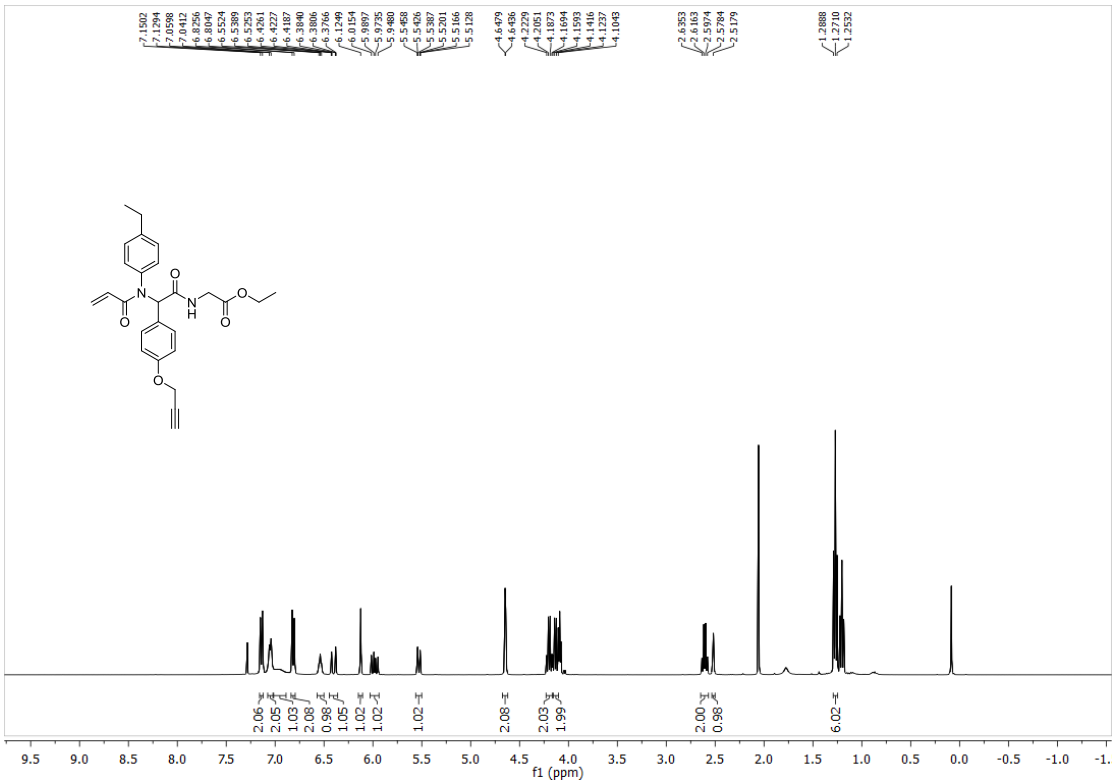

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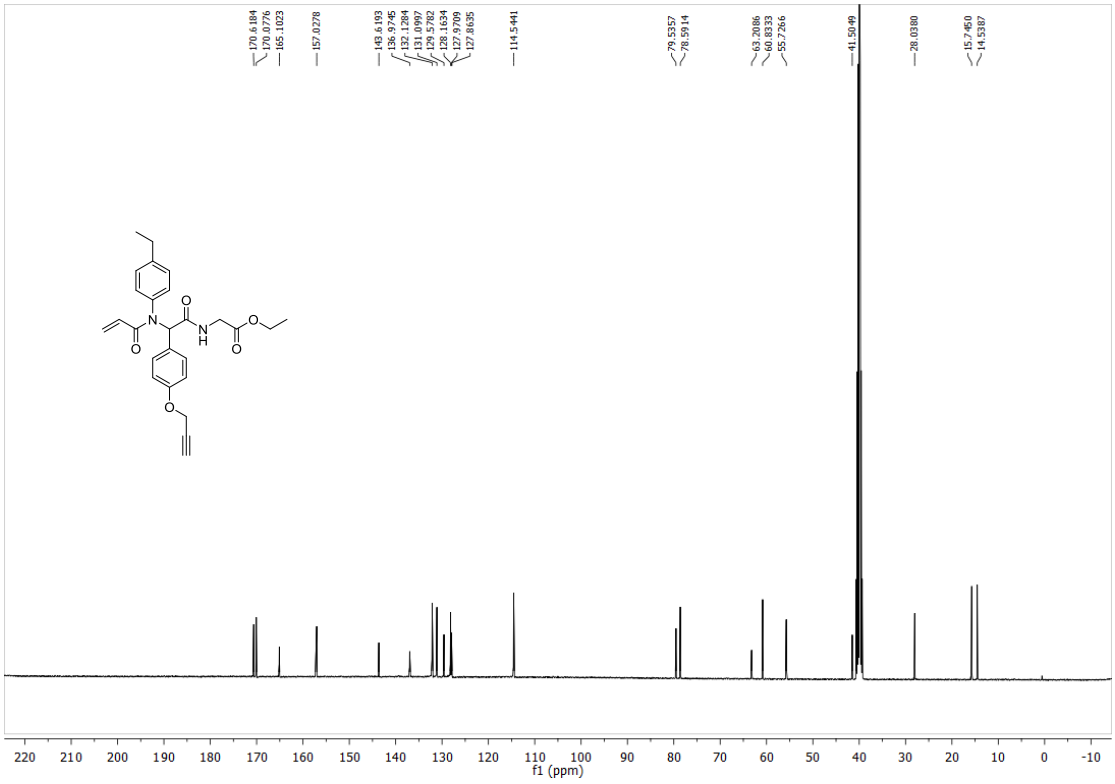
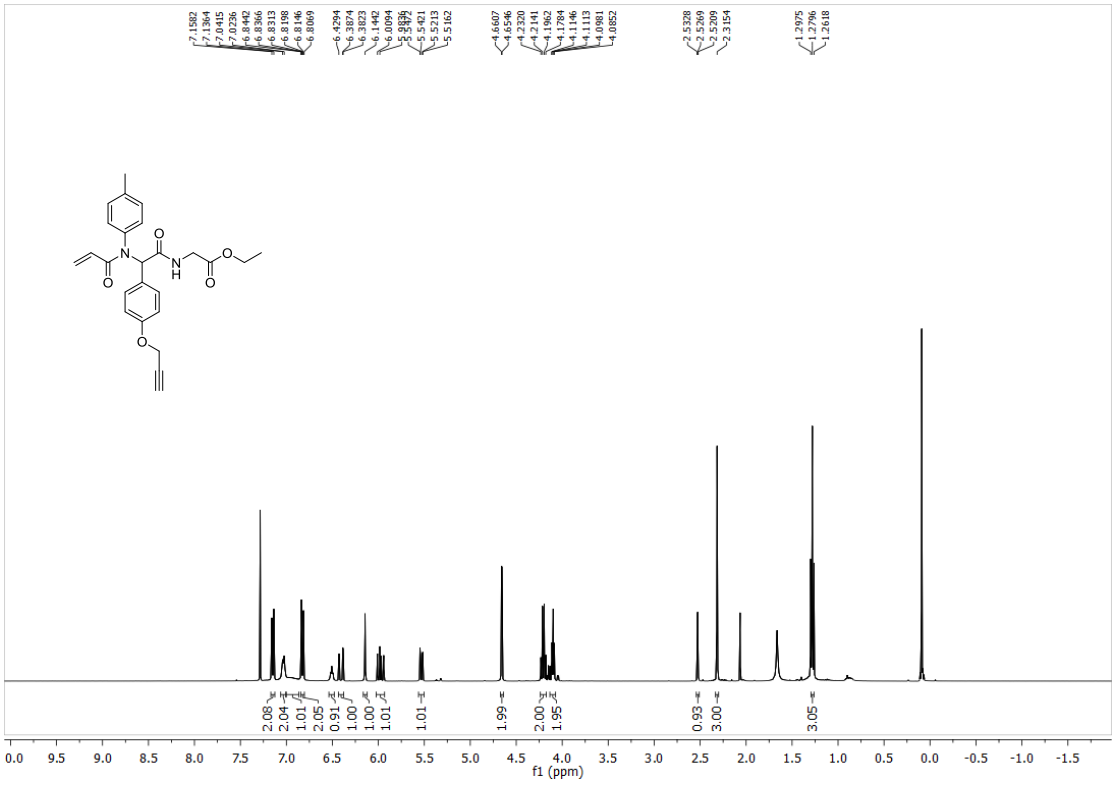
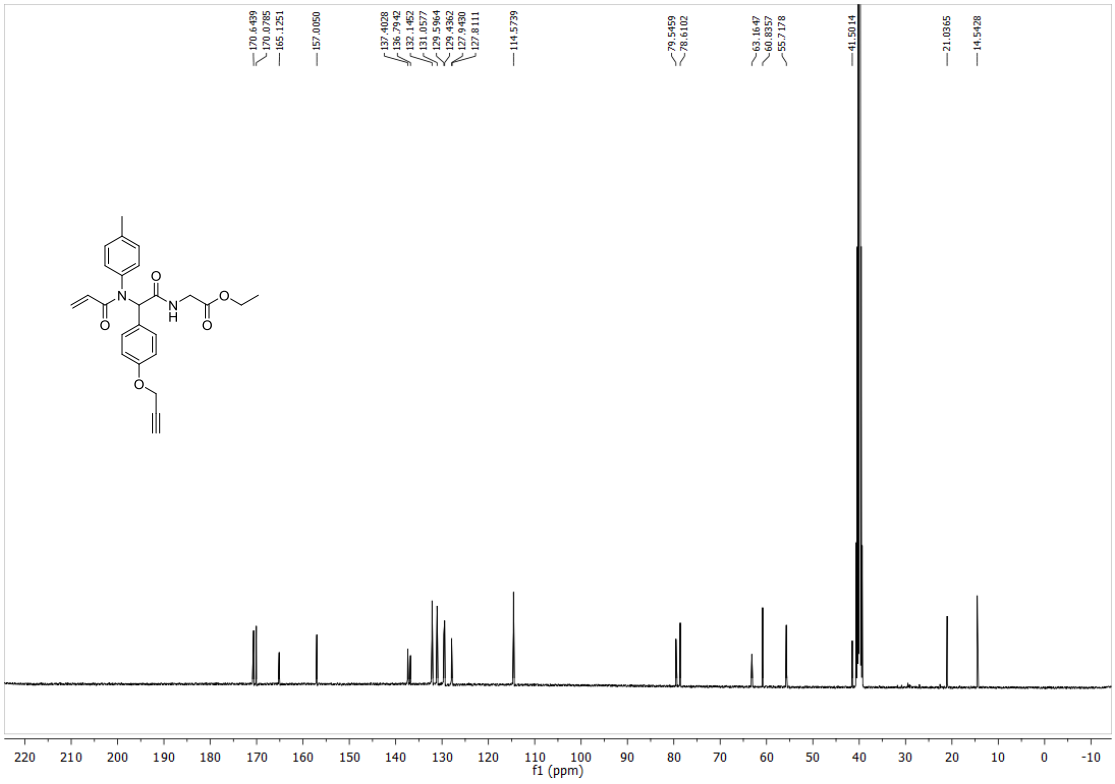


P31 ^{1}H



P31 ^{13}C



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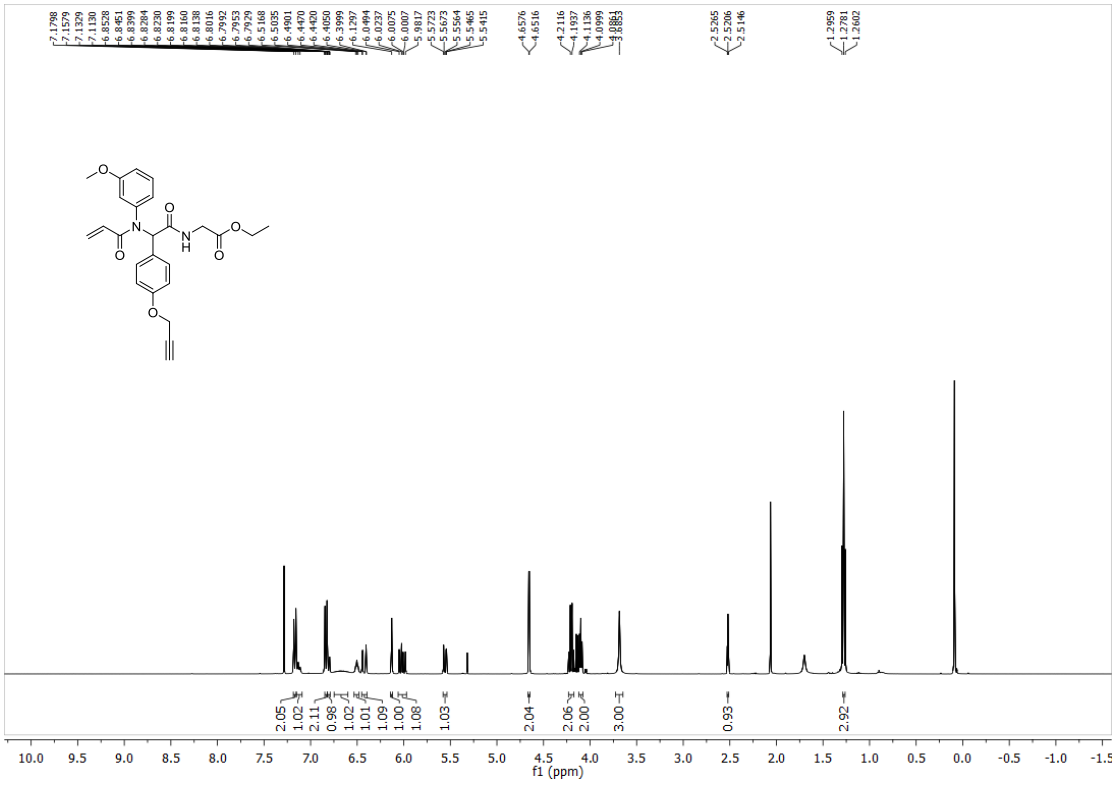

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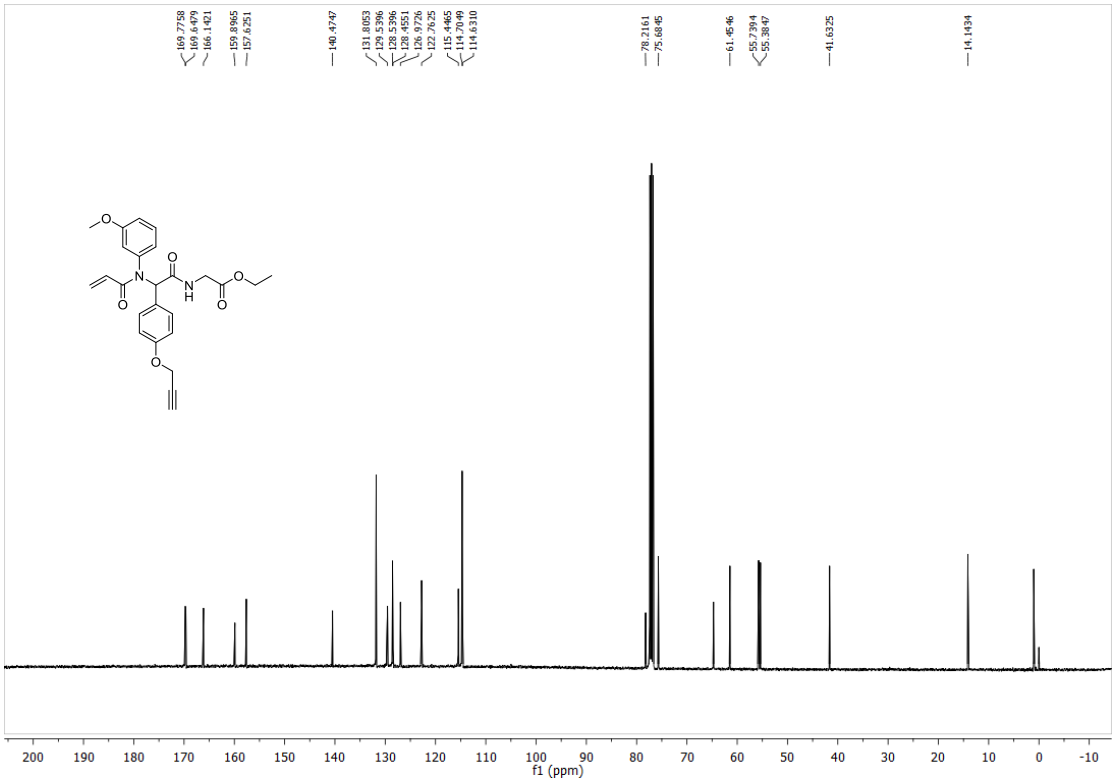

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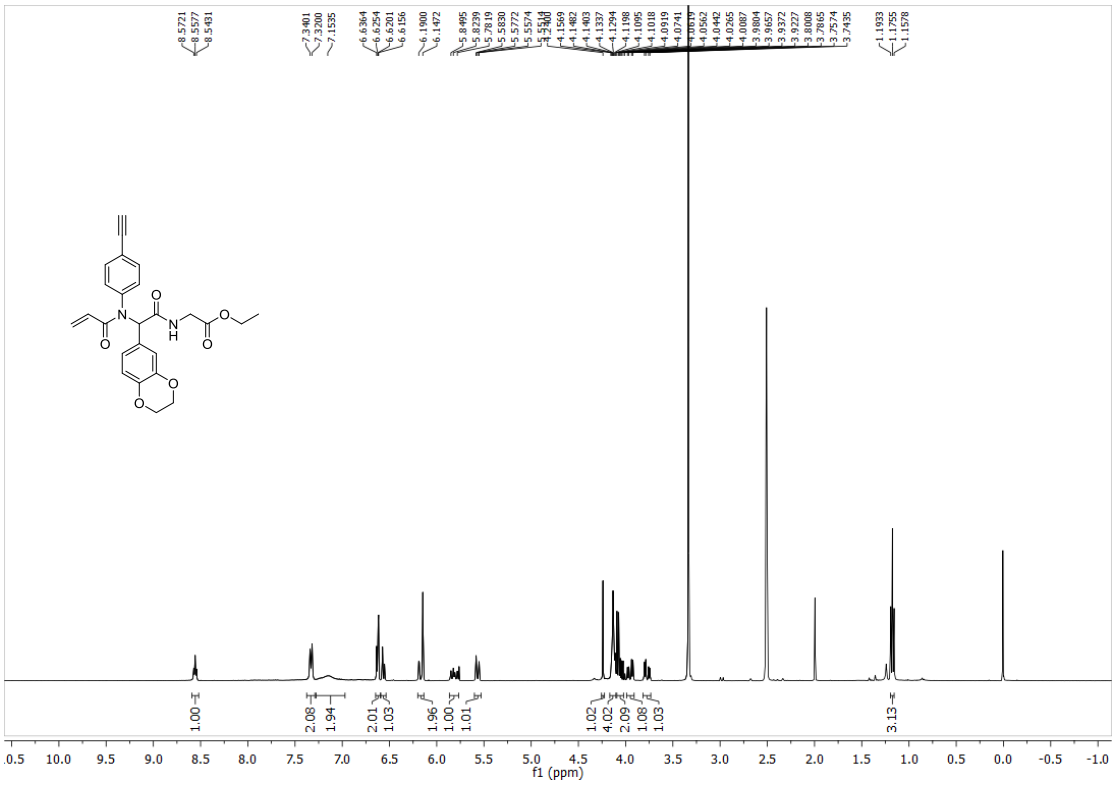
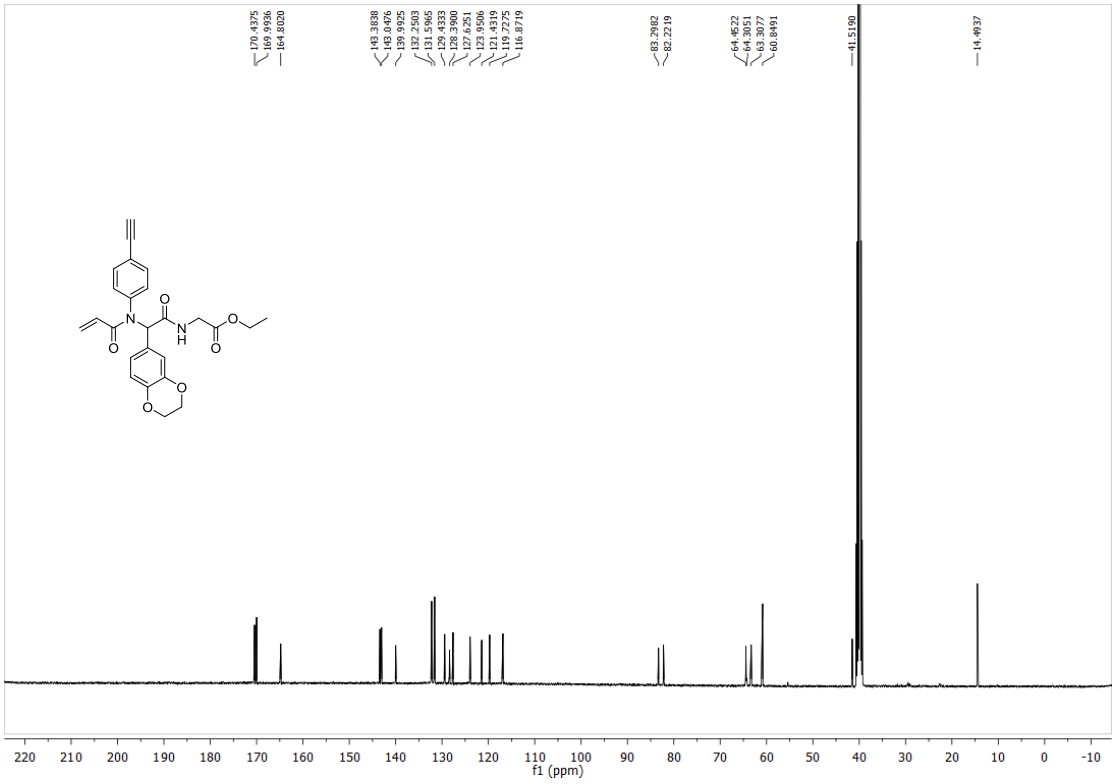
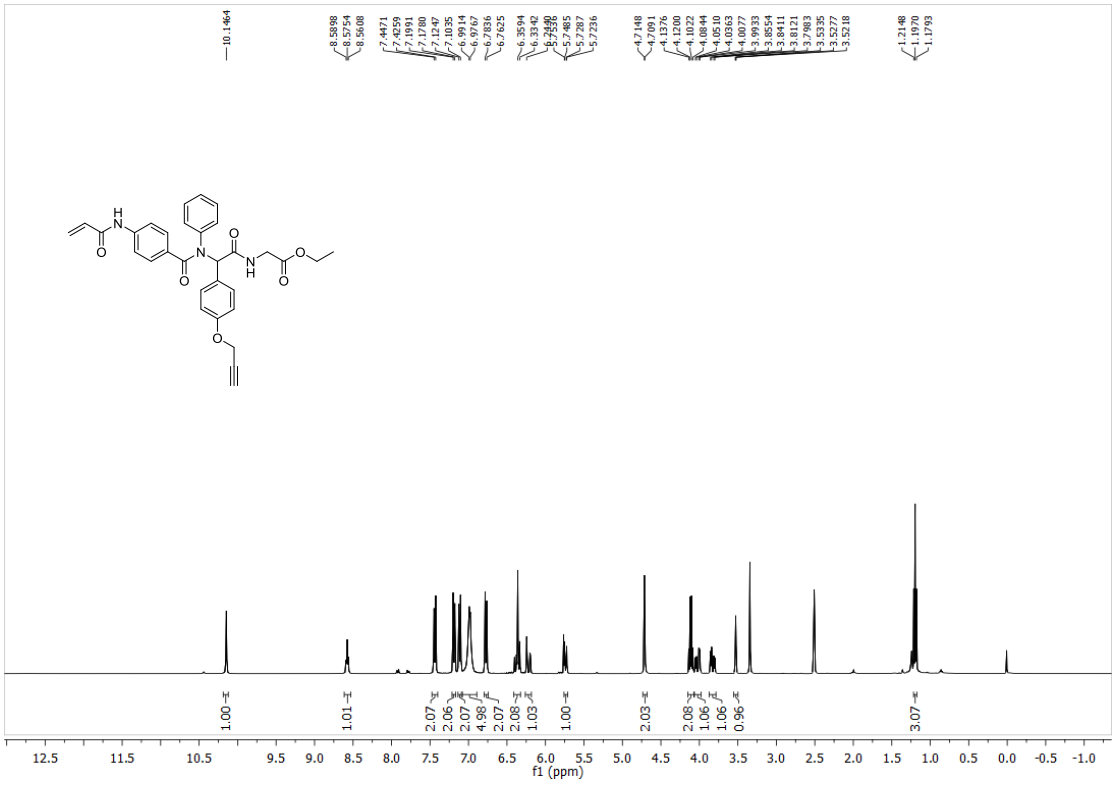


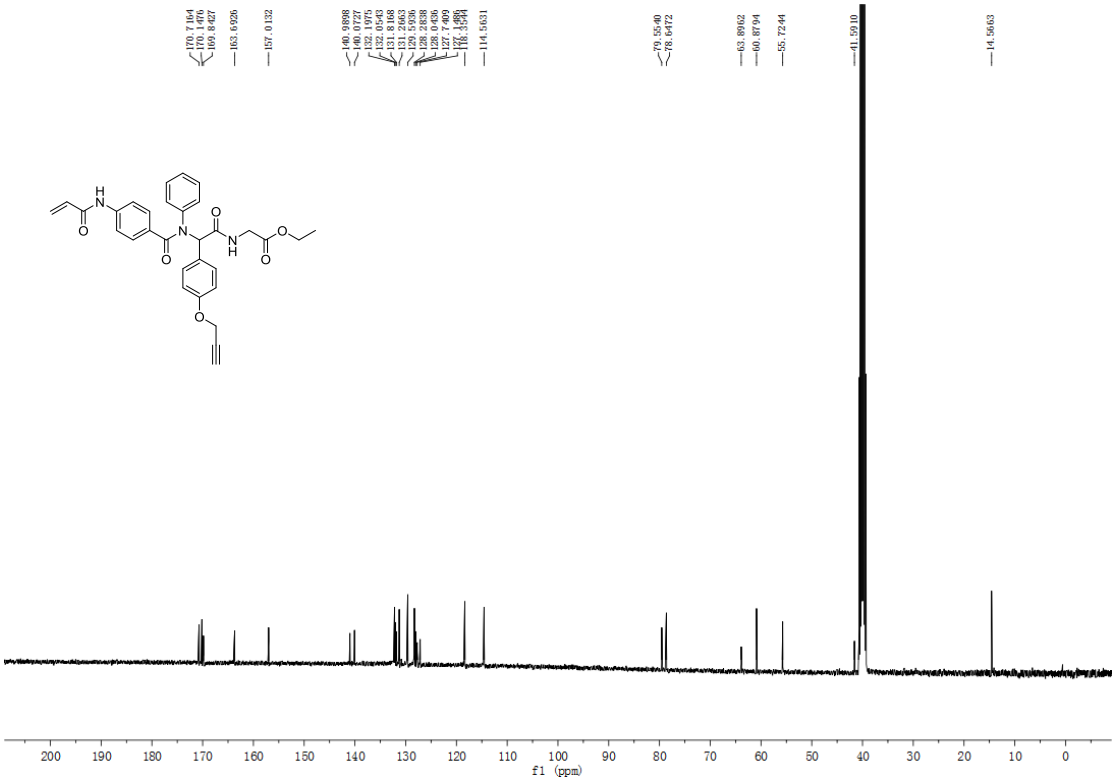
P33 ^{13}C



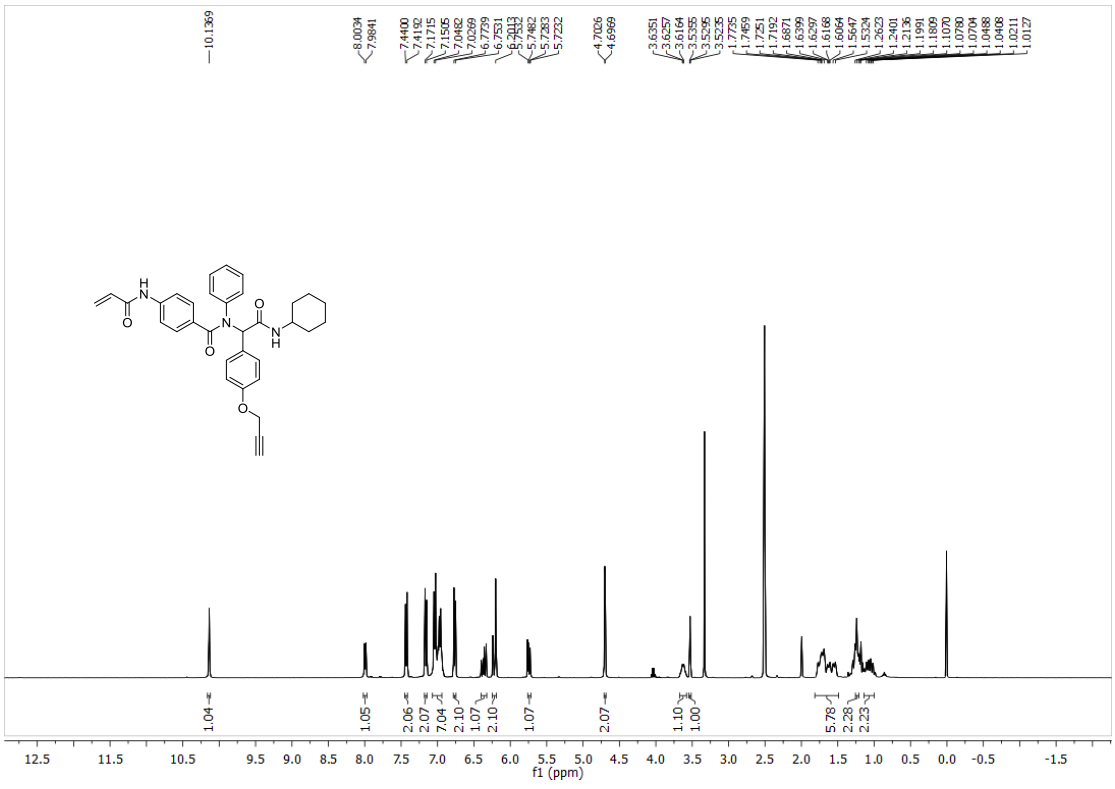
P34 ^{1}H



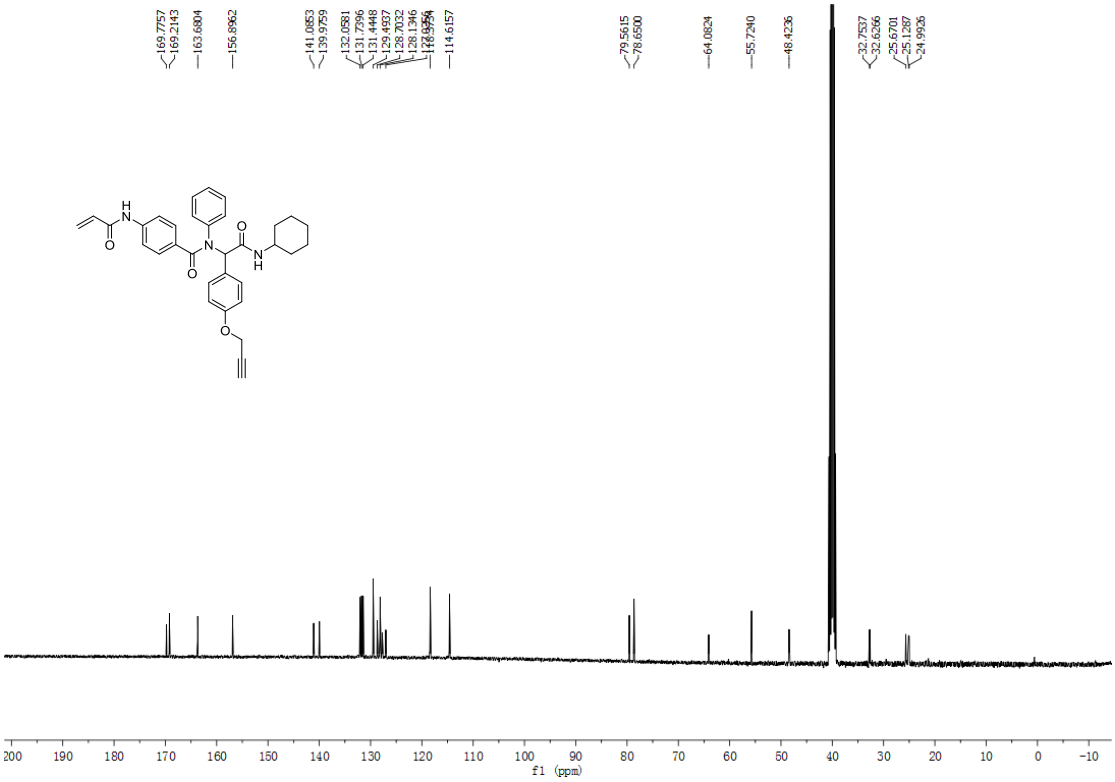
P34 ^{13}C



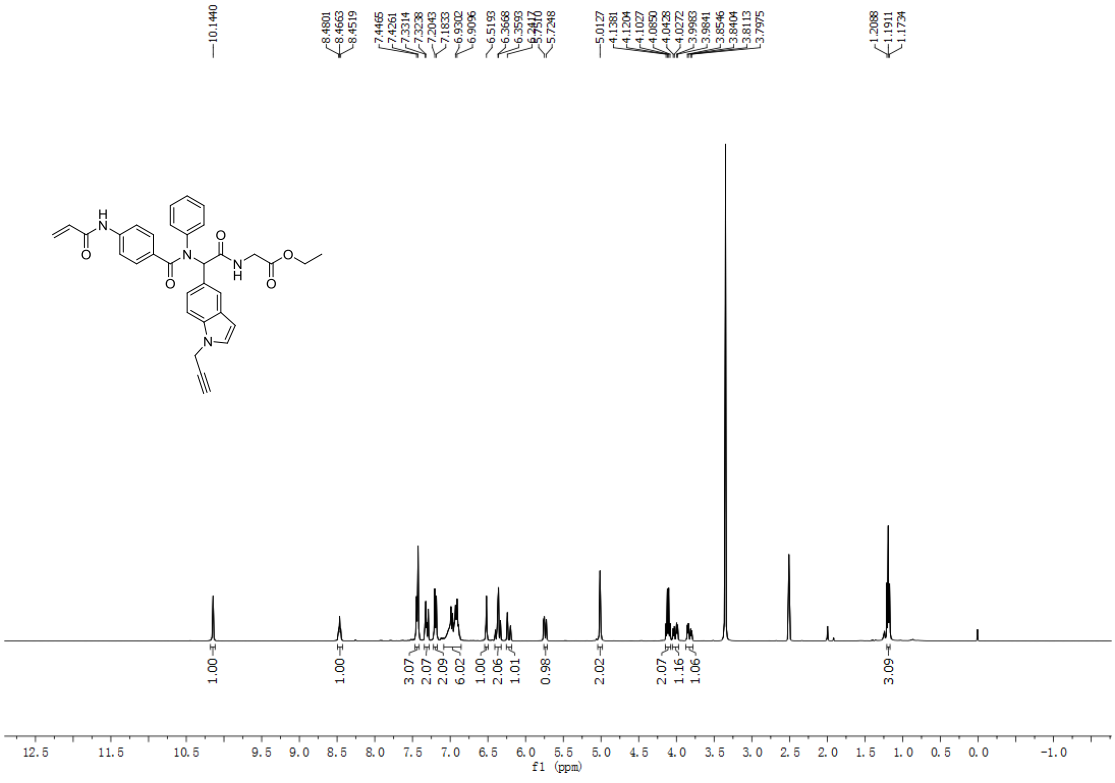
P35 ^{1}H



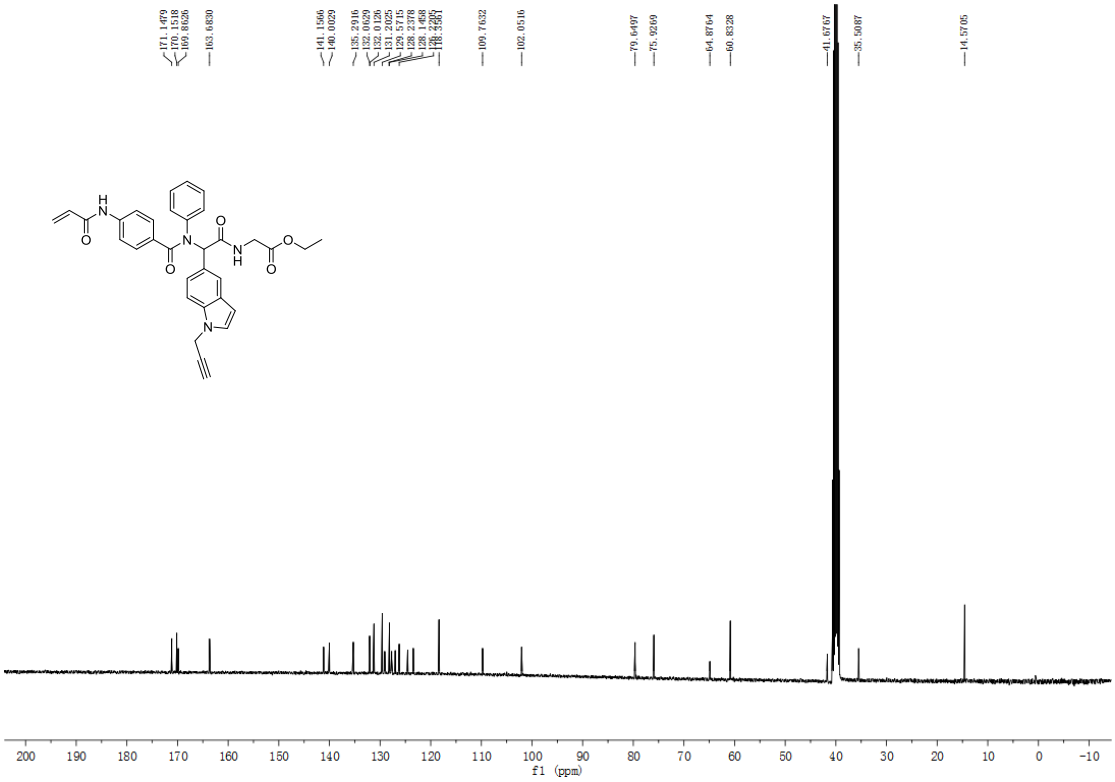
P35 ^{13}C



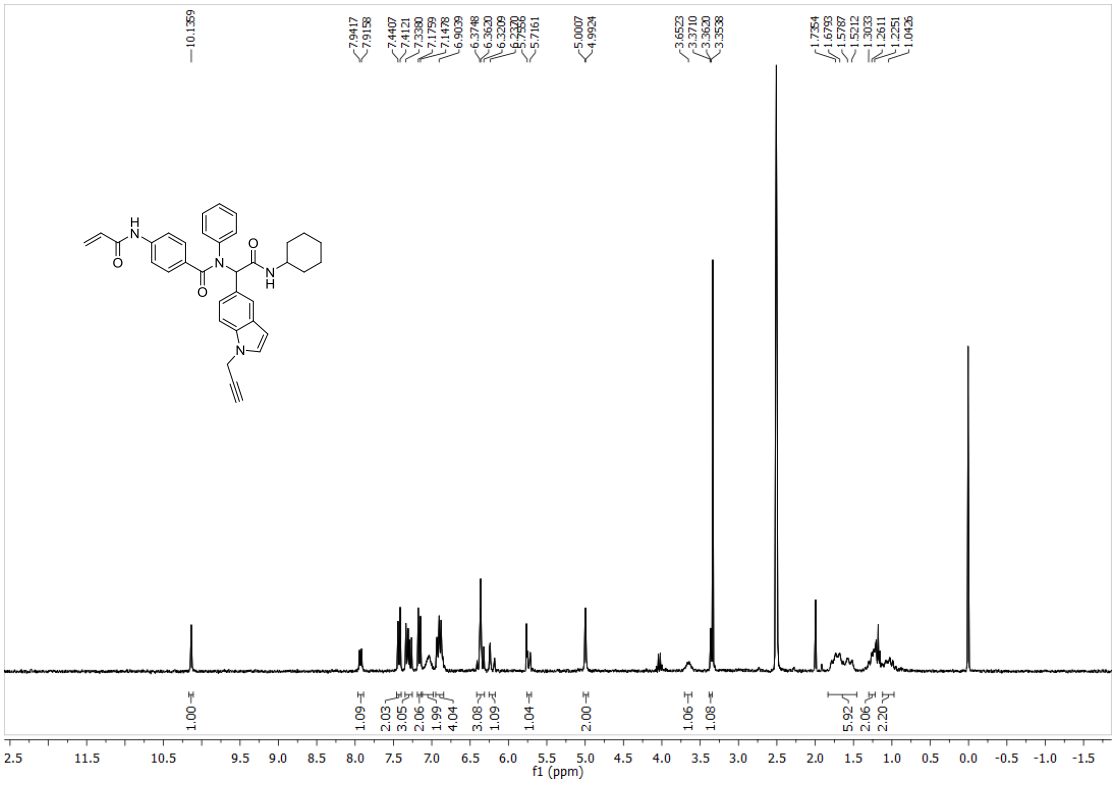
P36 ^{1}H



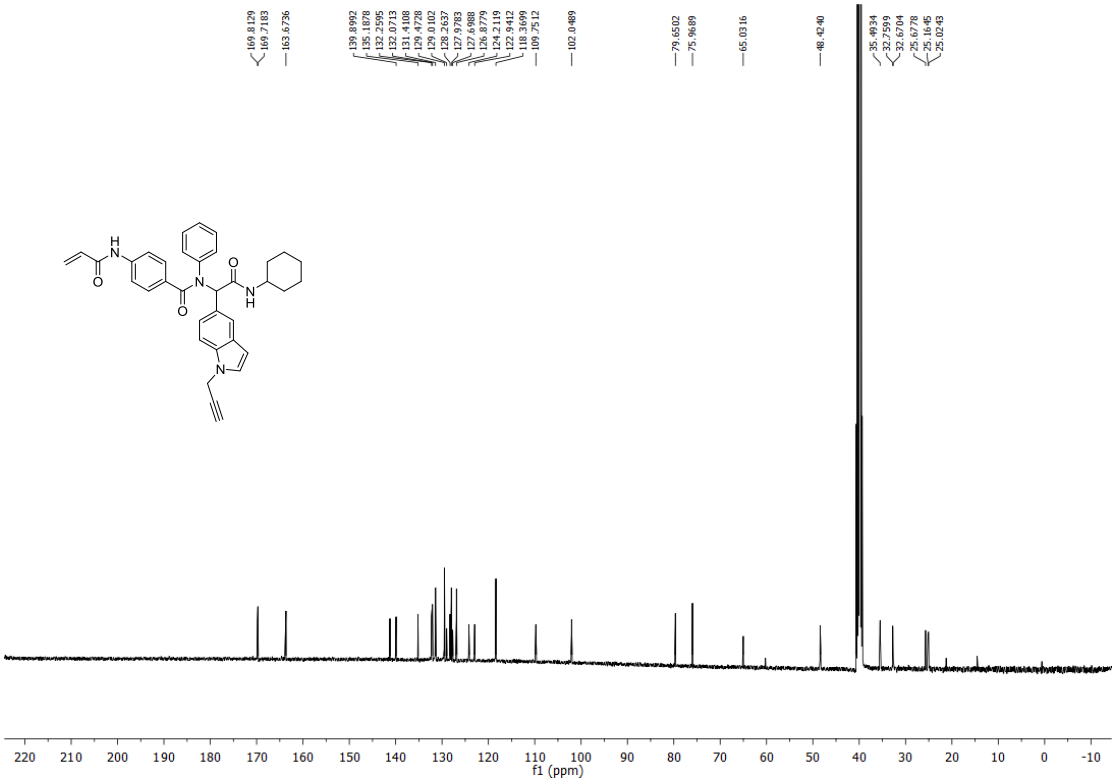
P36 ^{13}C



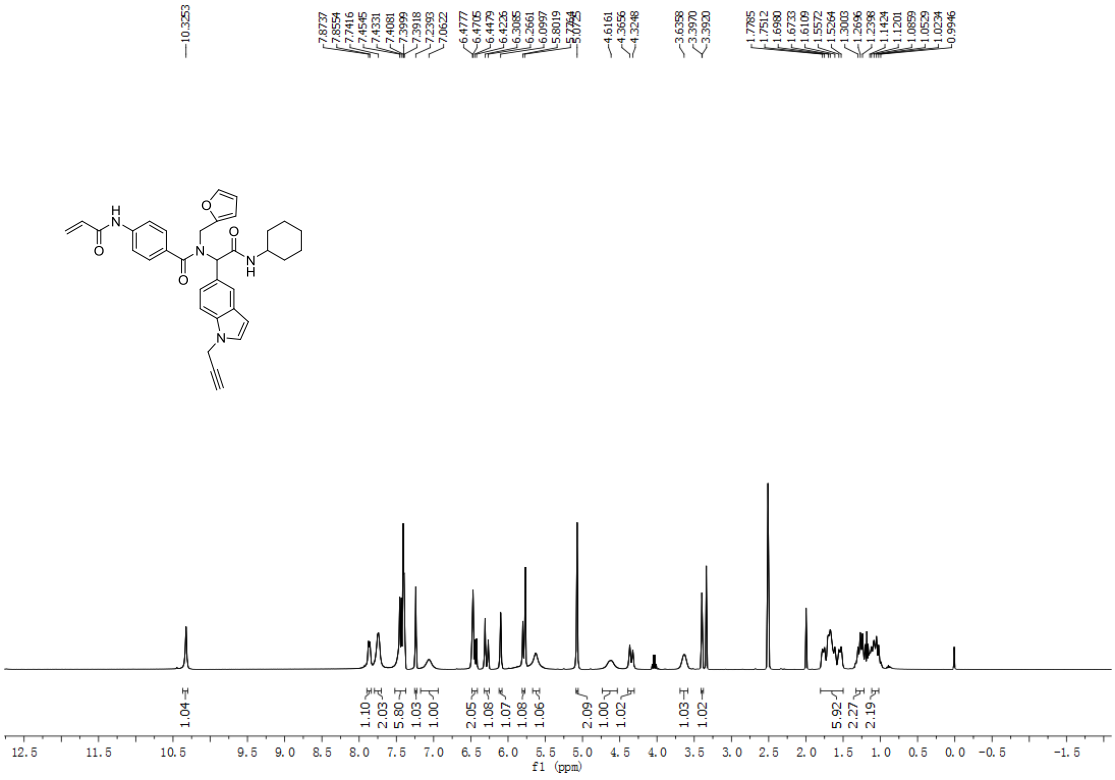
P37 ^{1}H



P37 ^{13}C



P38 ^{1}H



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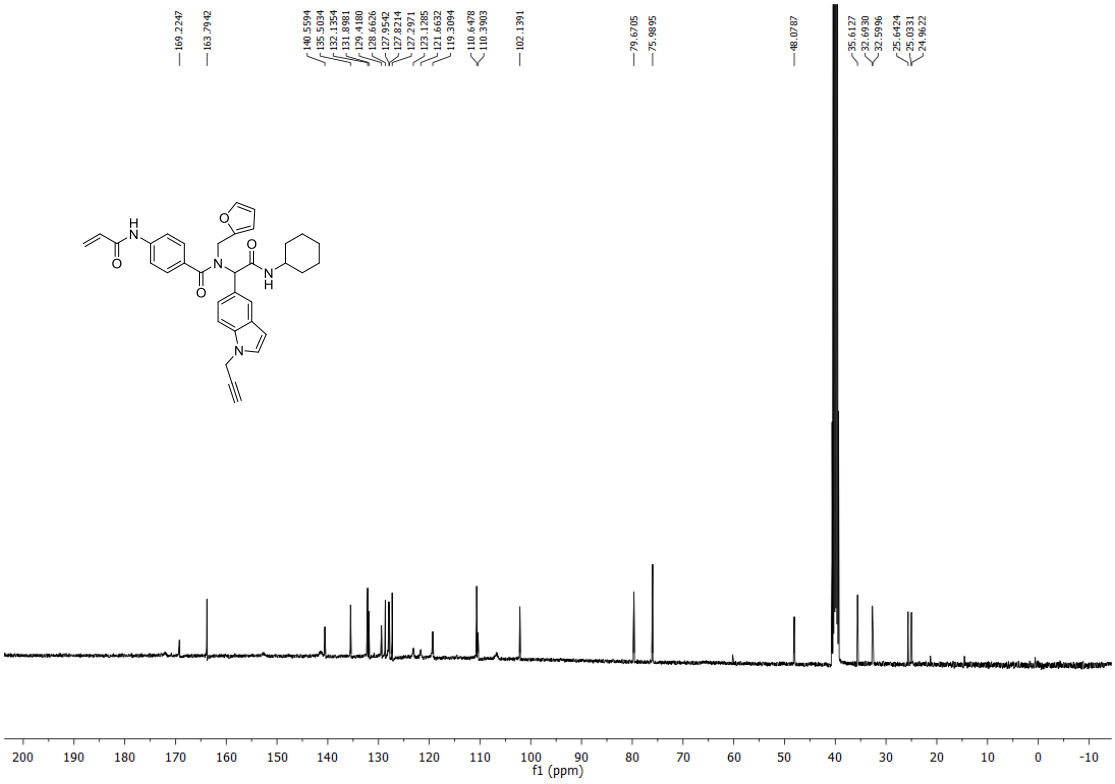

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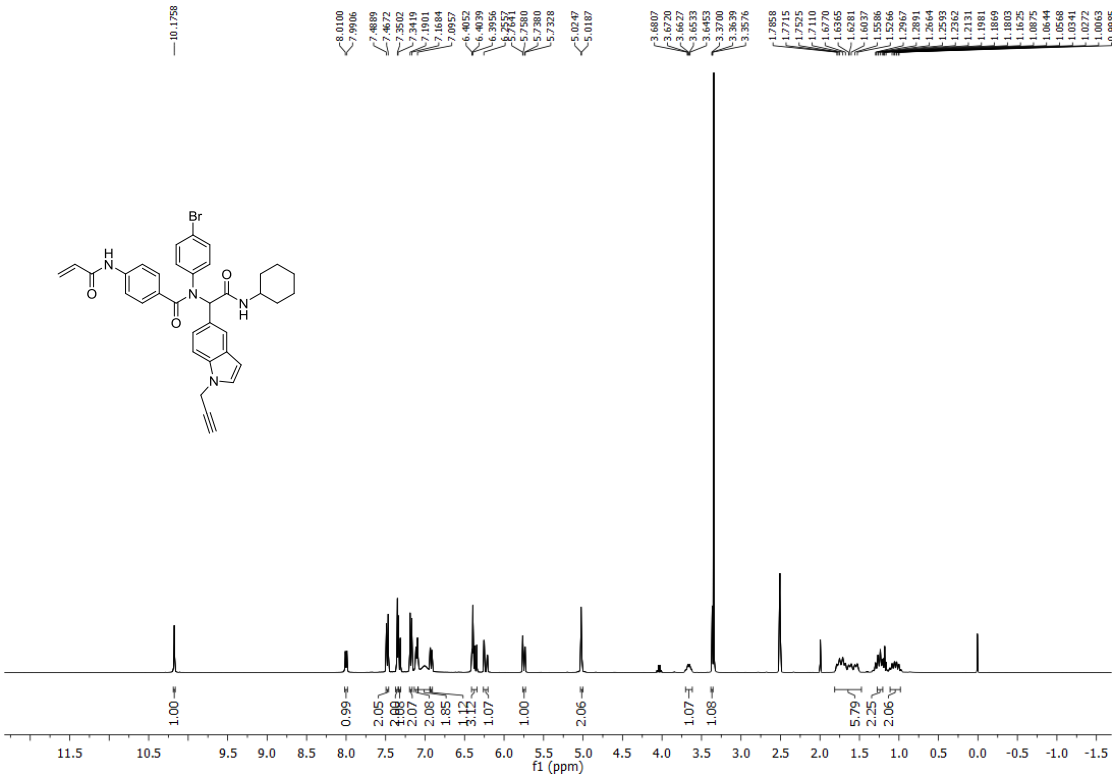
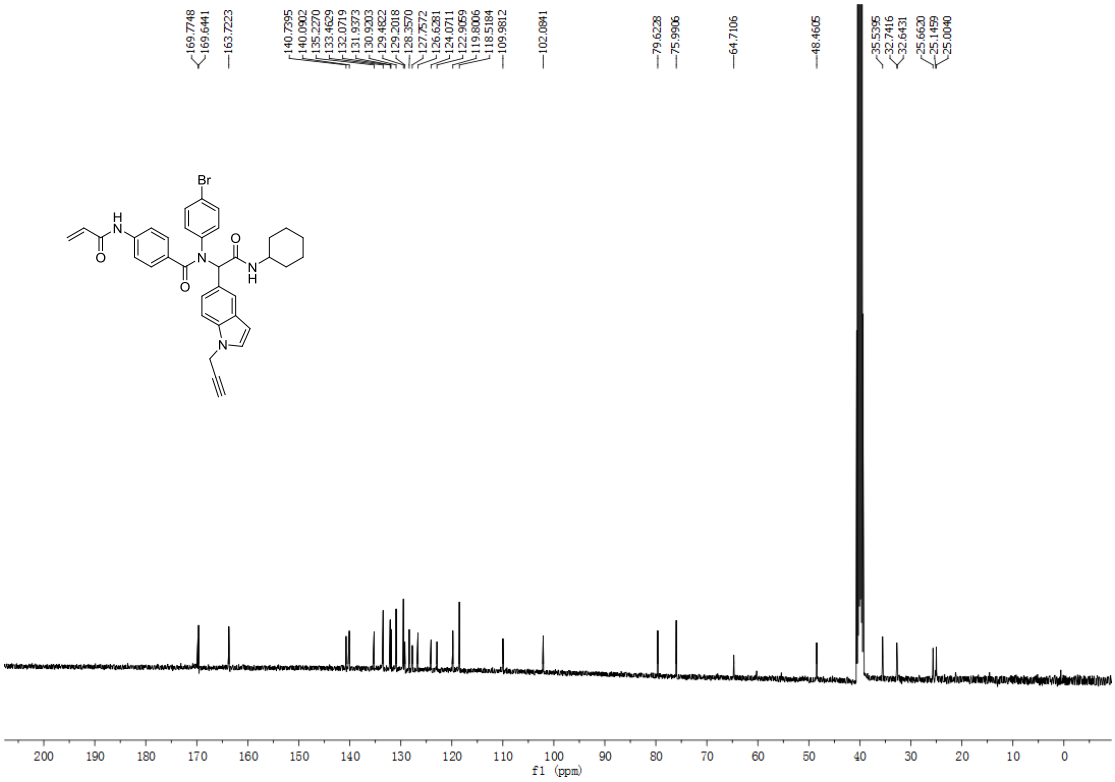
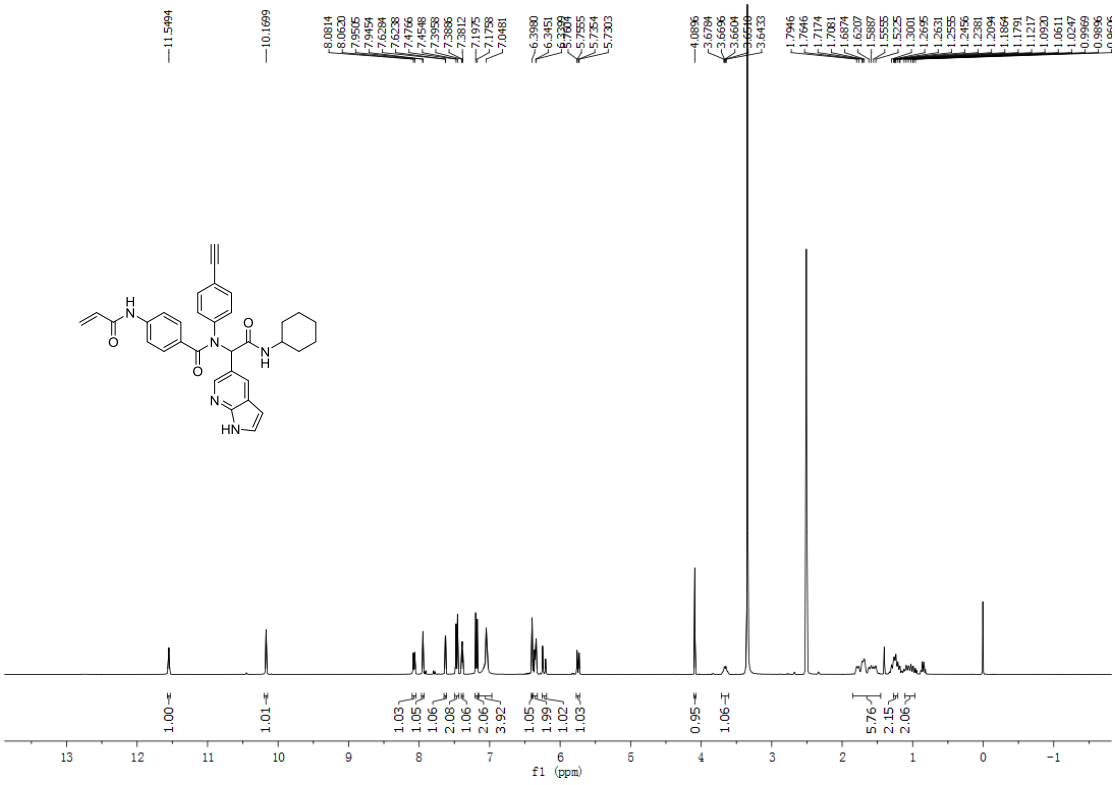


P39 ^{13}C



P40 ^{1}H



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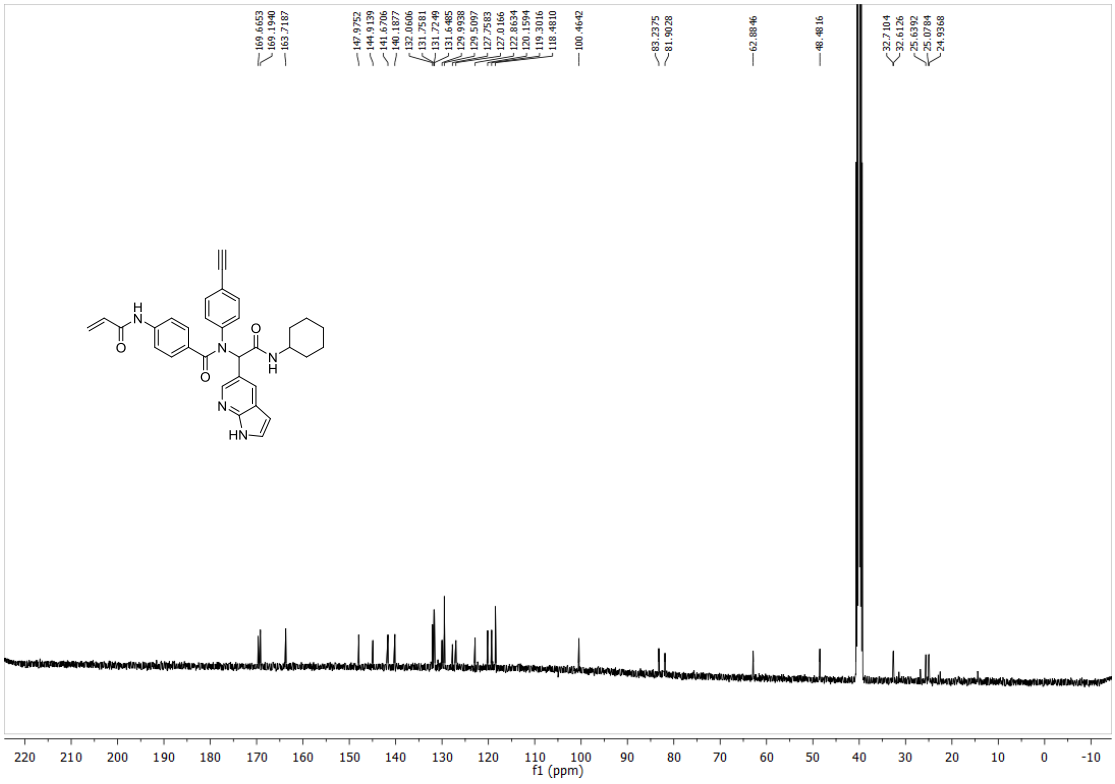
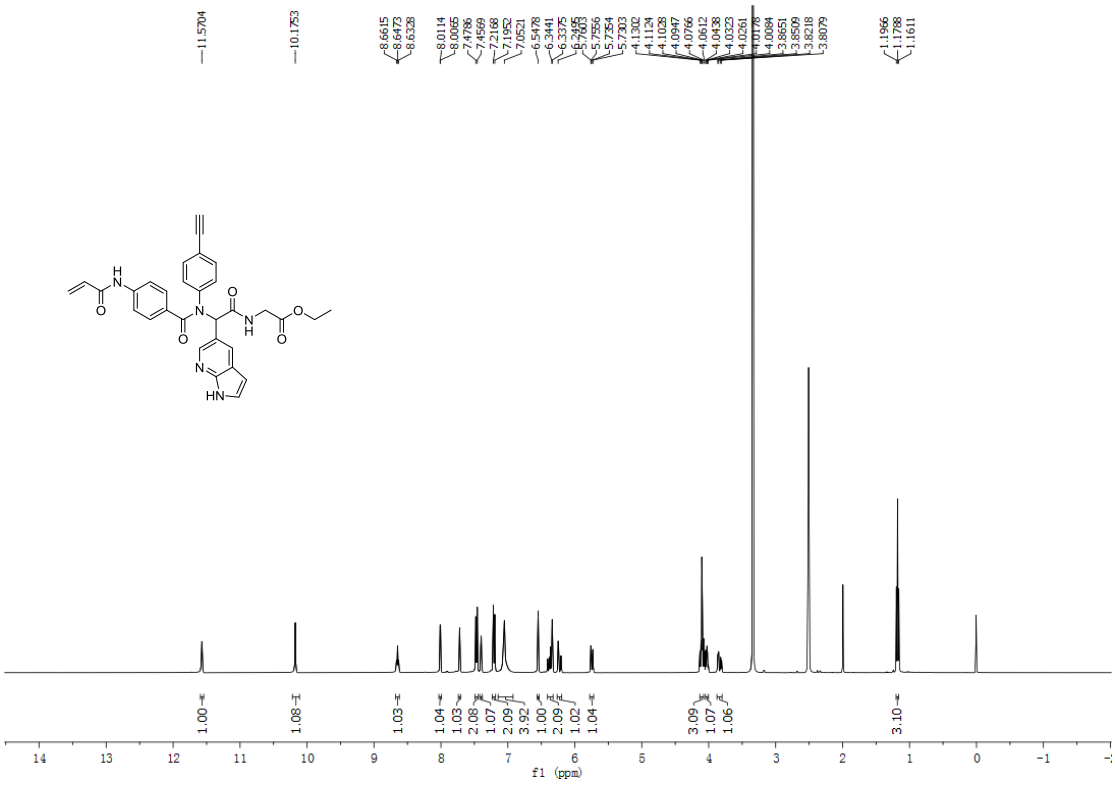


P41 ^{1}H



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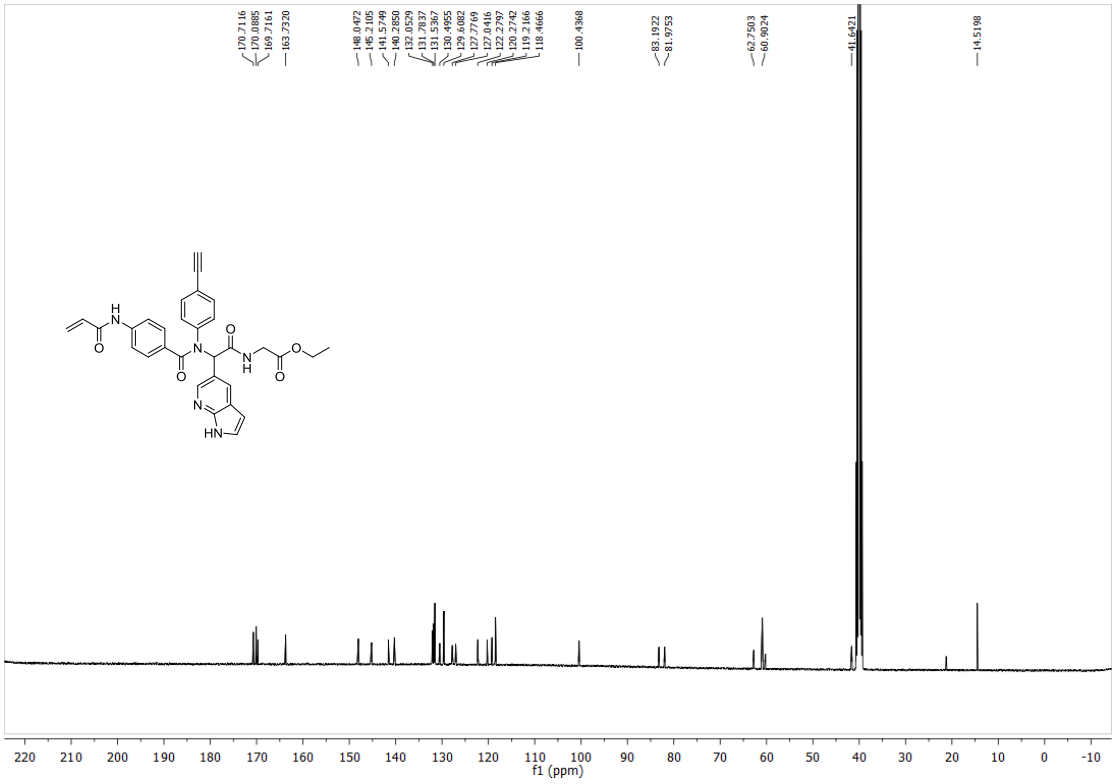
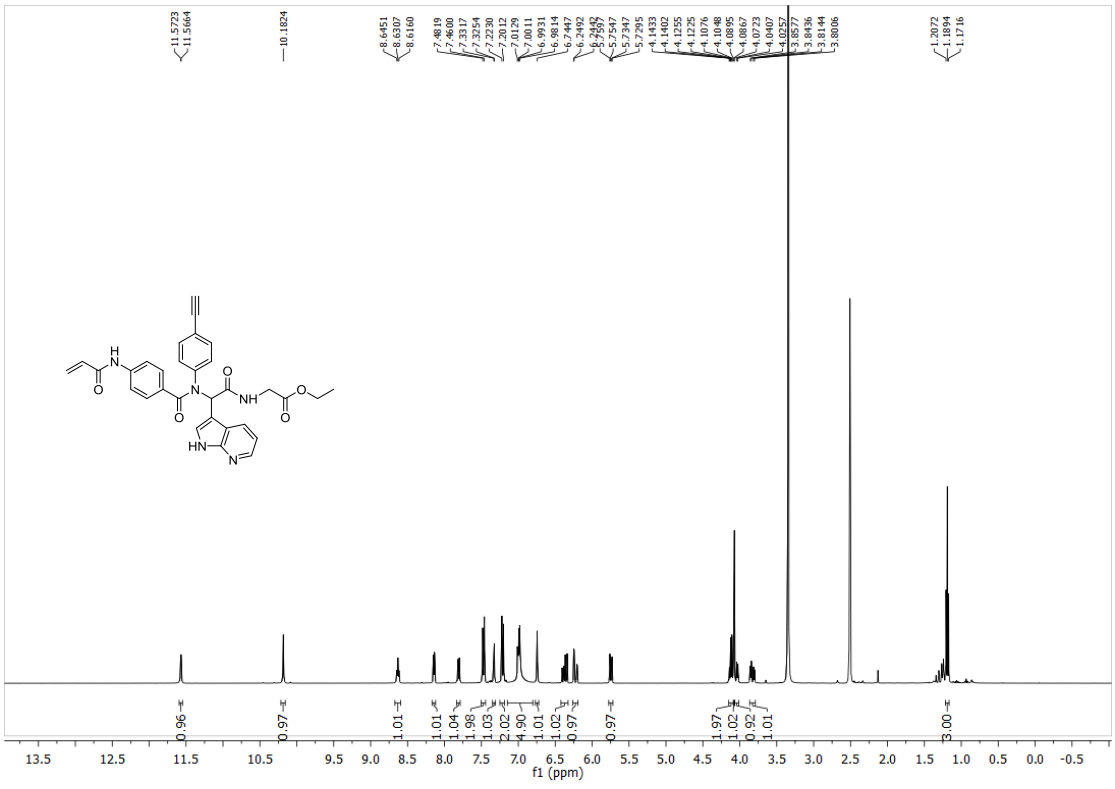


P42 ^{1}H



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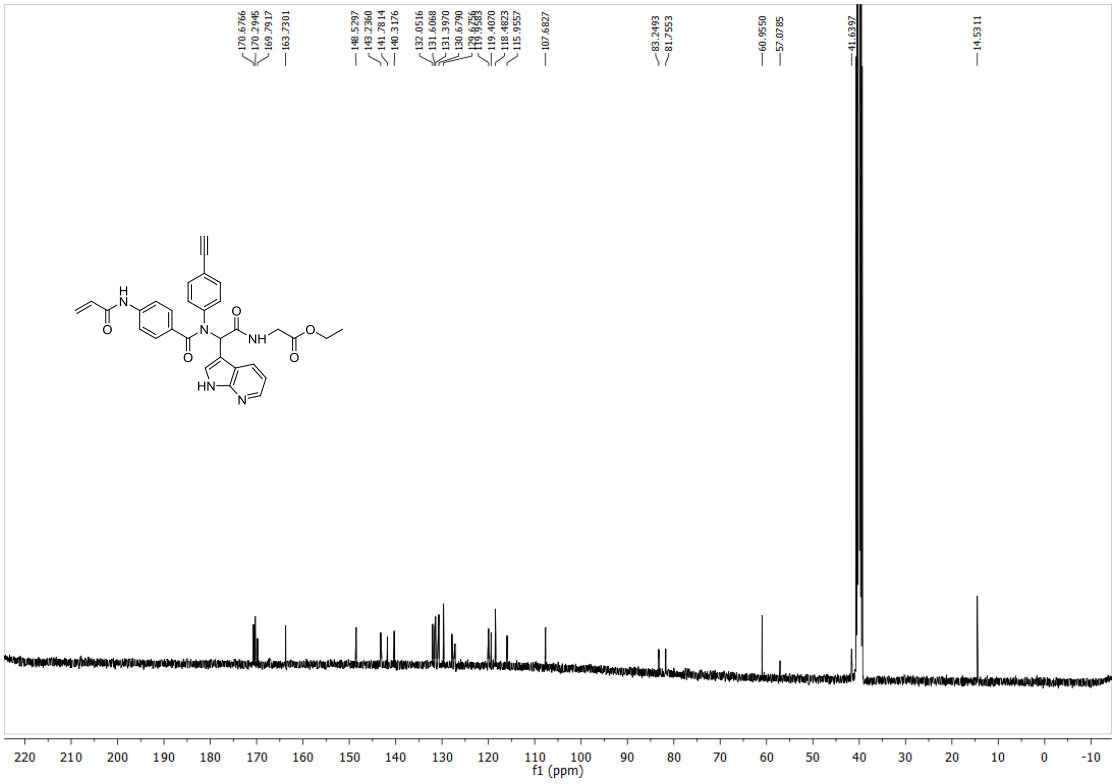
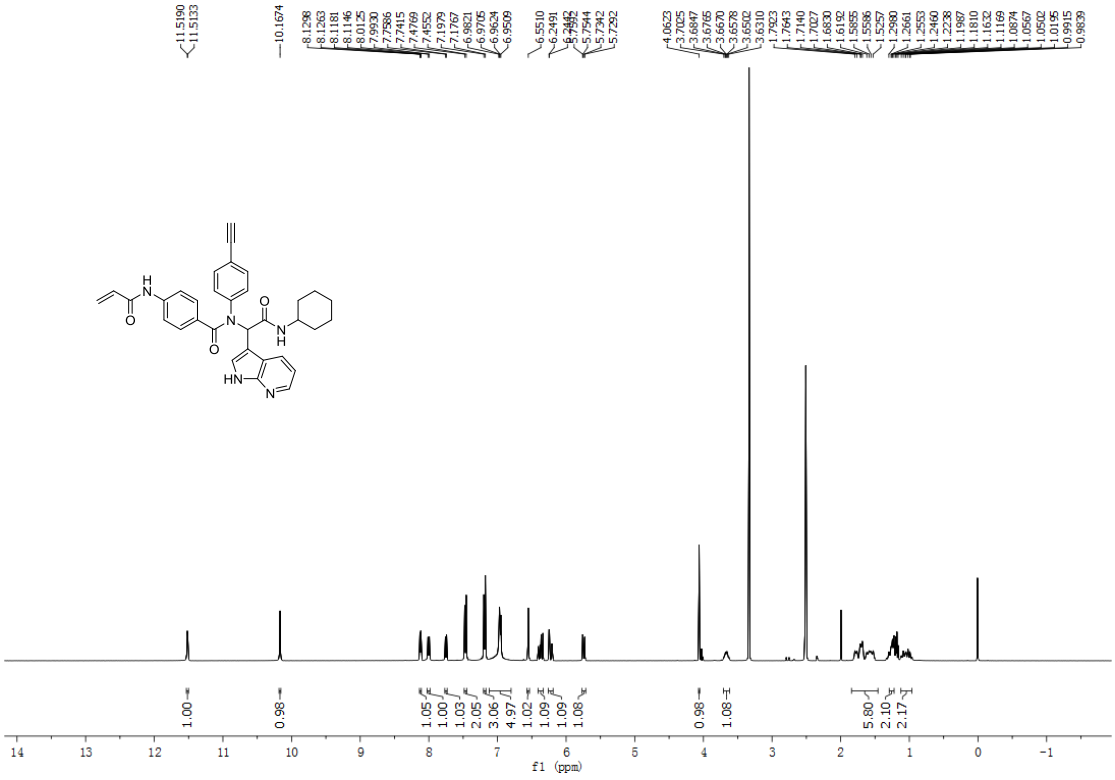
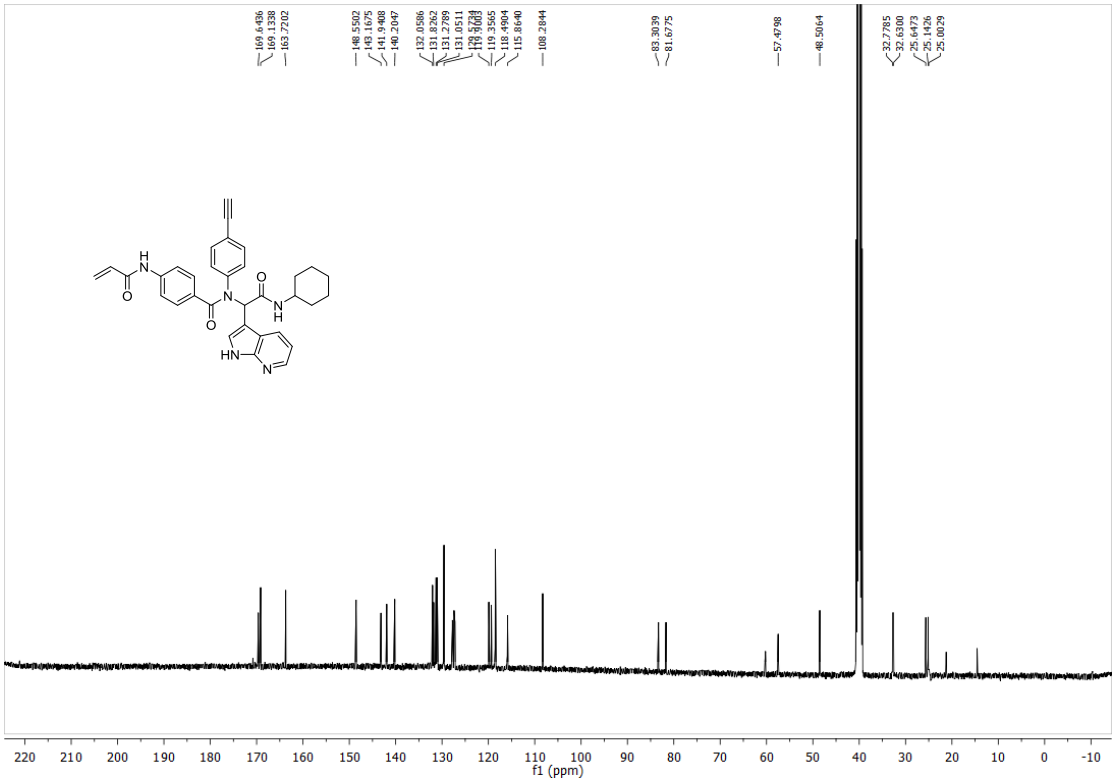


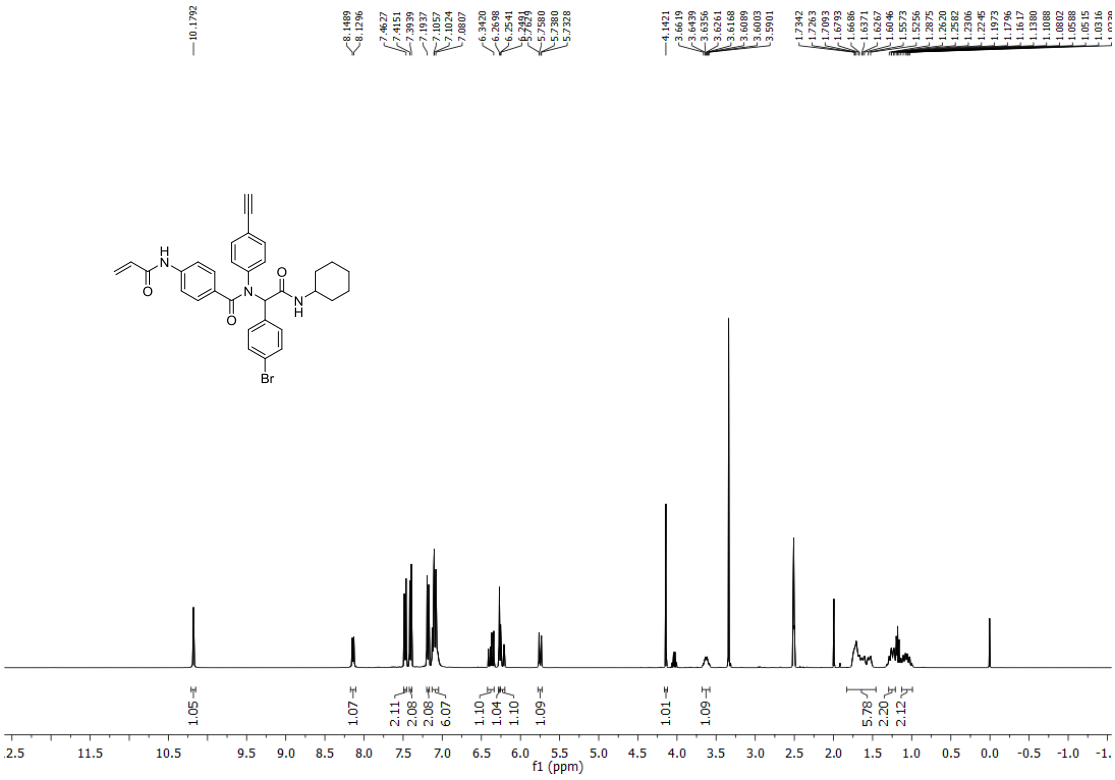
P43 ^{1}H



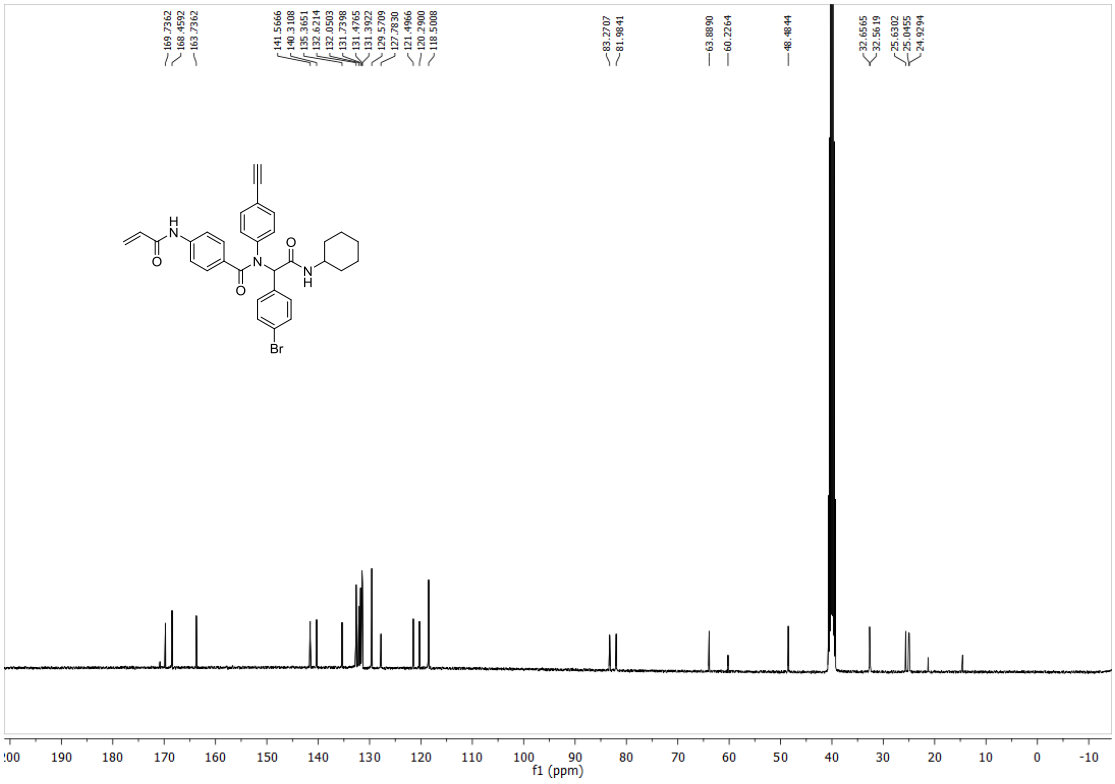
P43 ^{13}C



P44 ^{1}H



P44 ^{13}C



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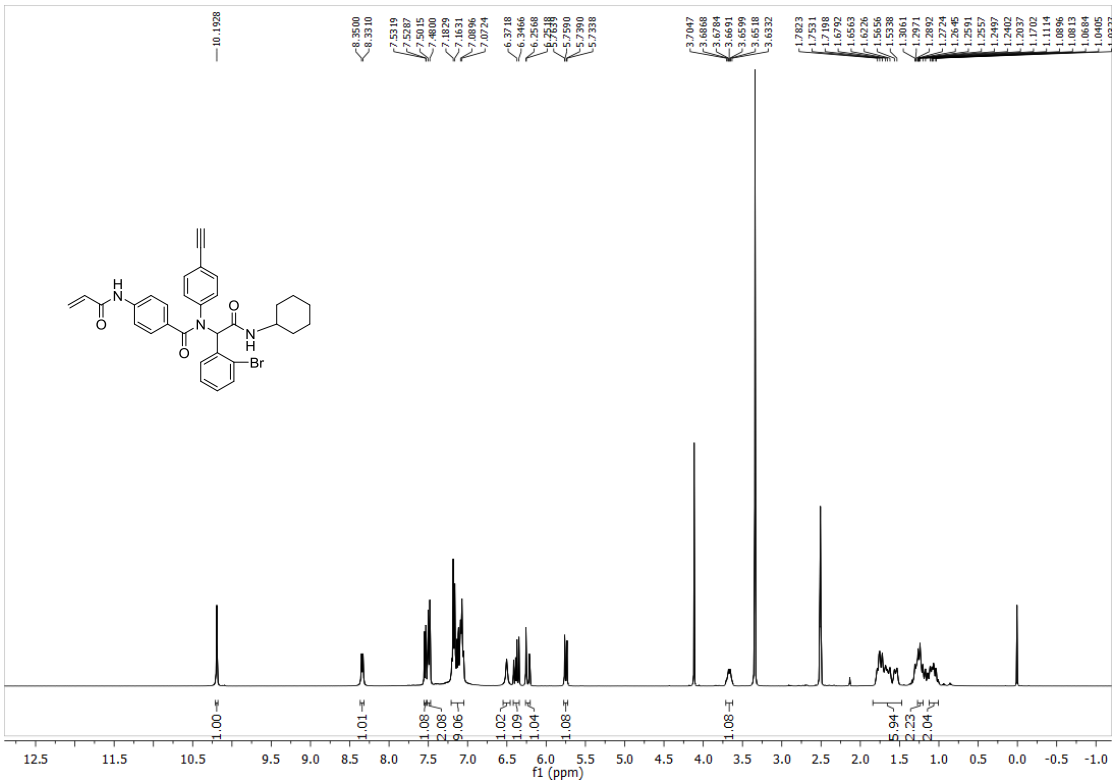

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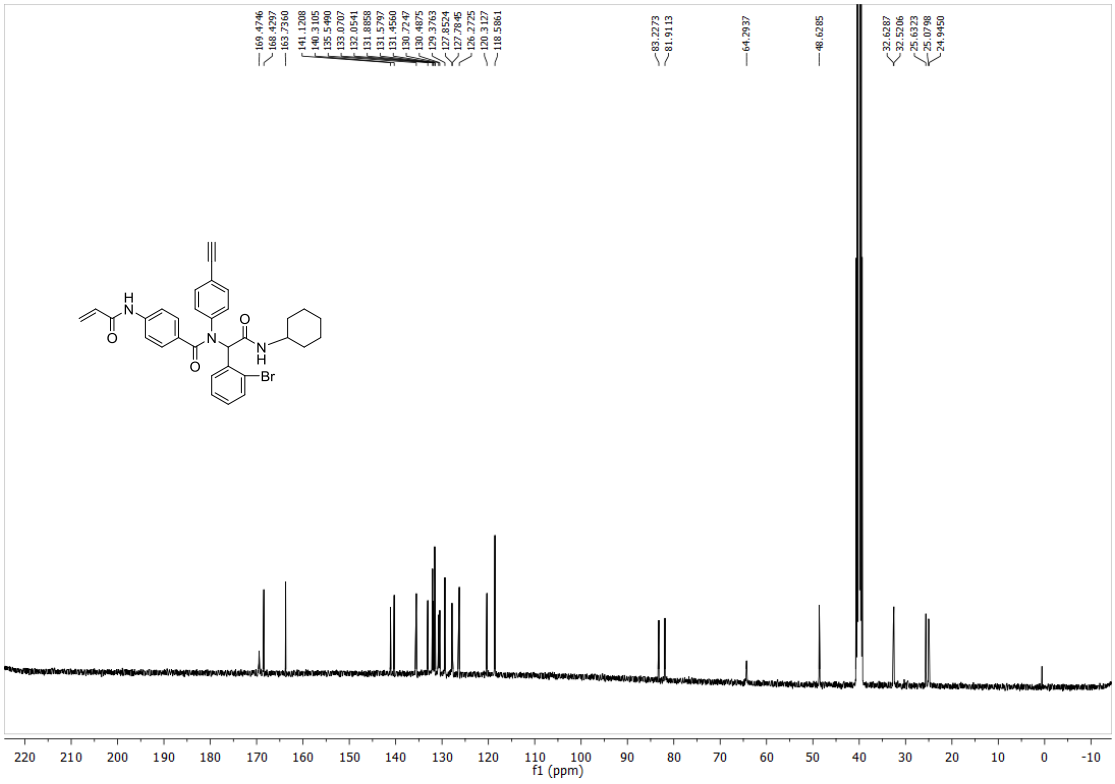
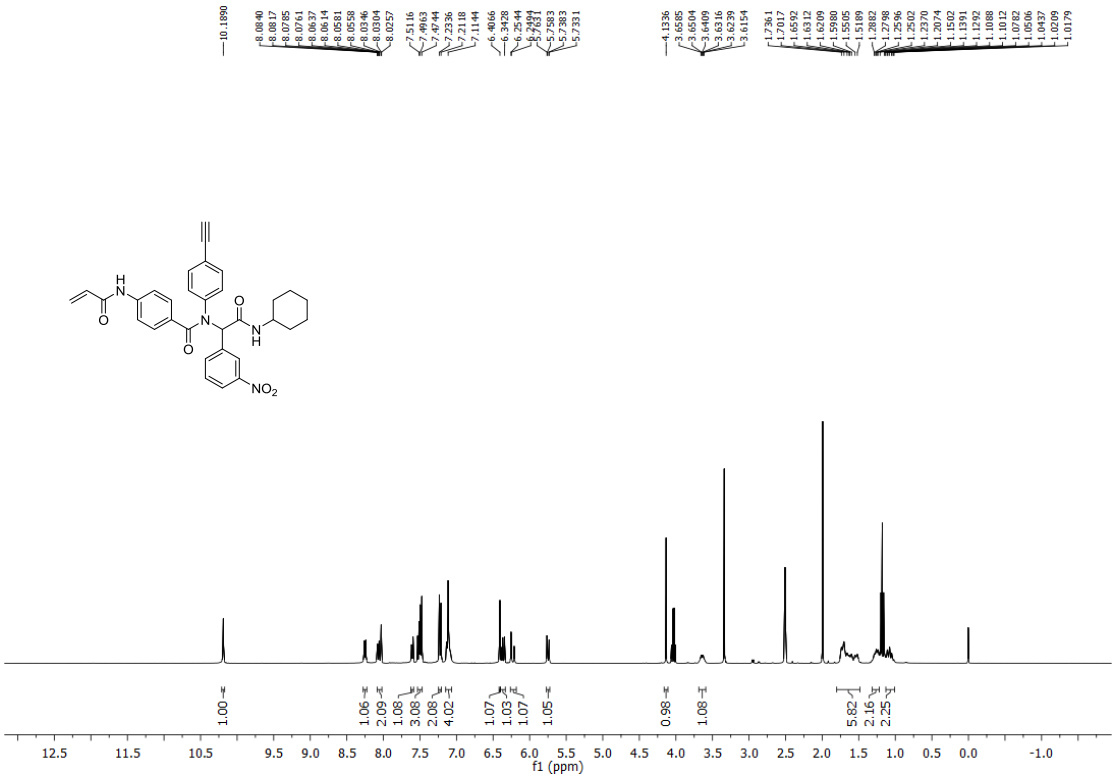
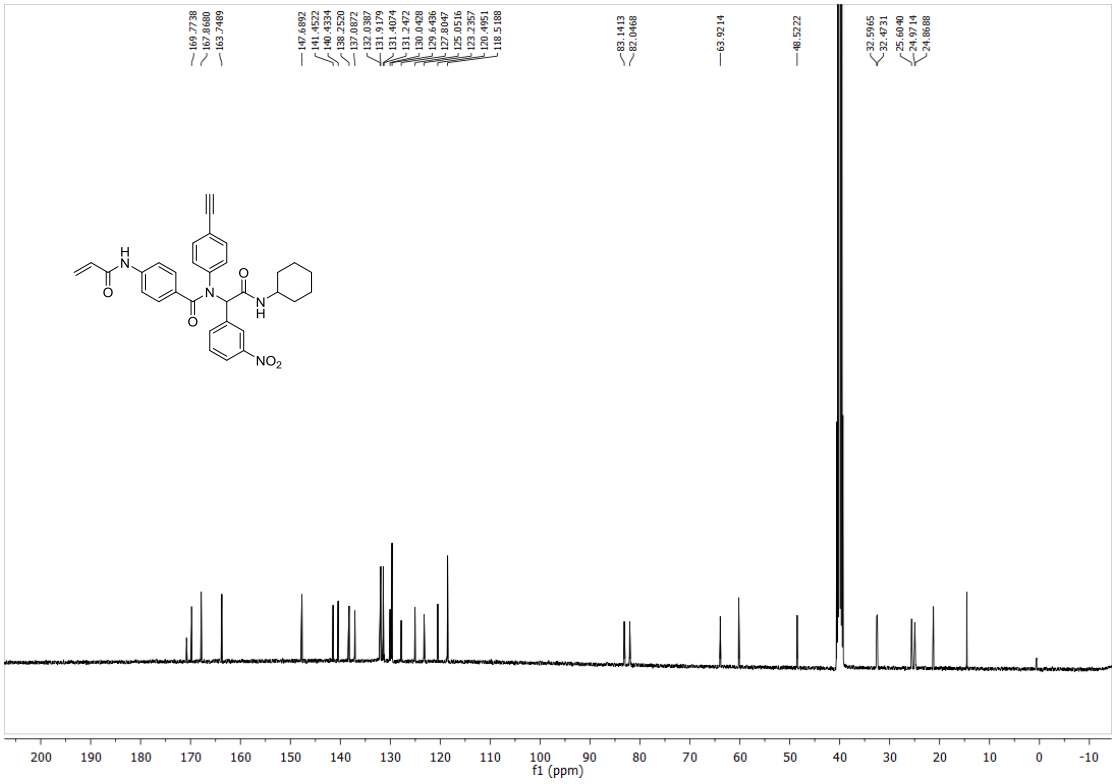


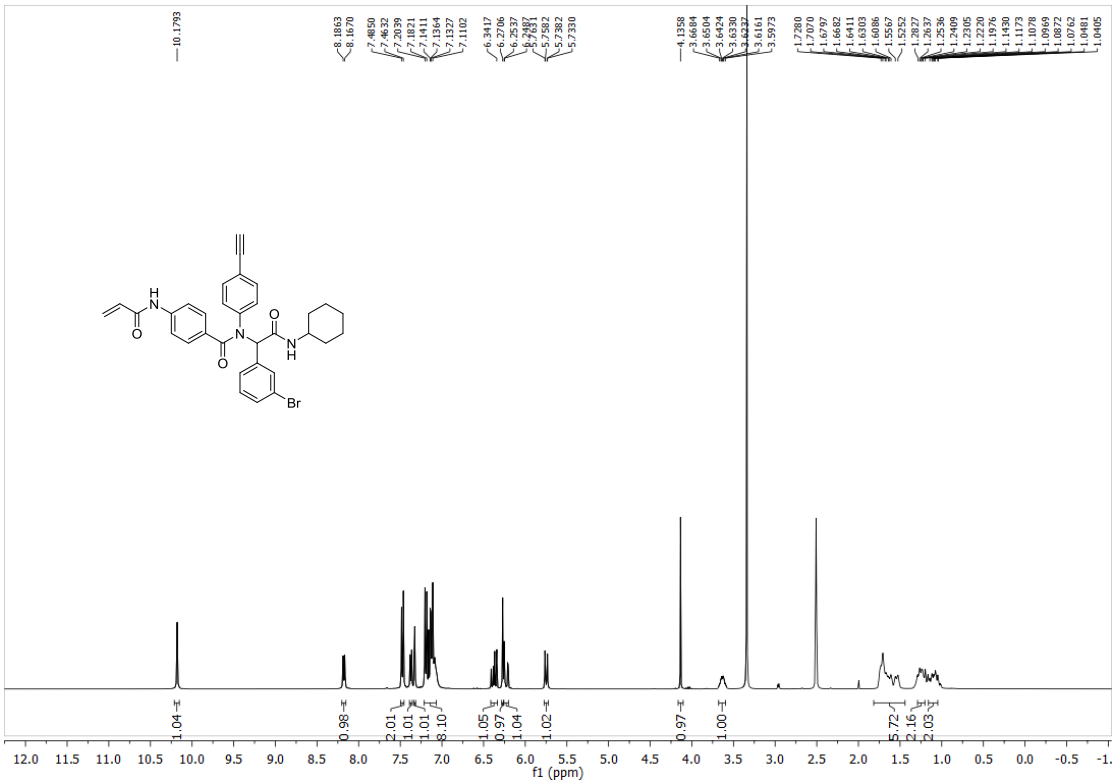
P46 ^{1}H



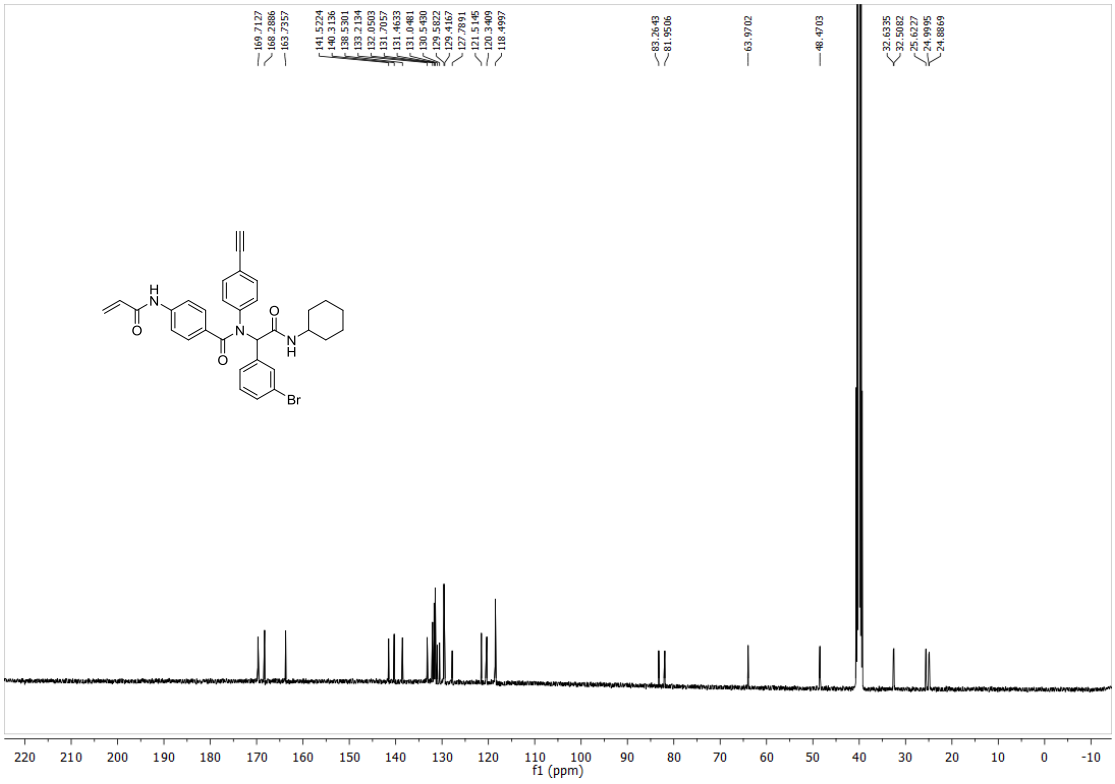
P46 ^{13}C



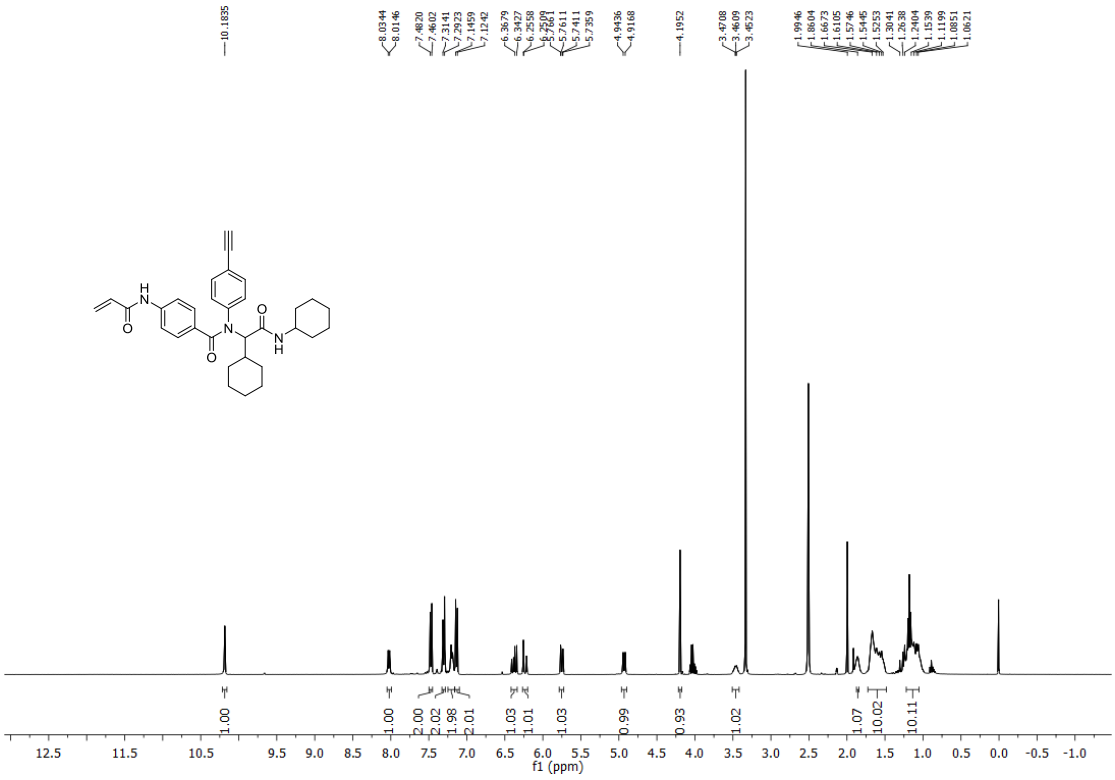
P47 ^{1}H



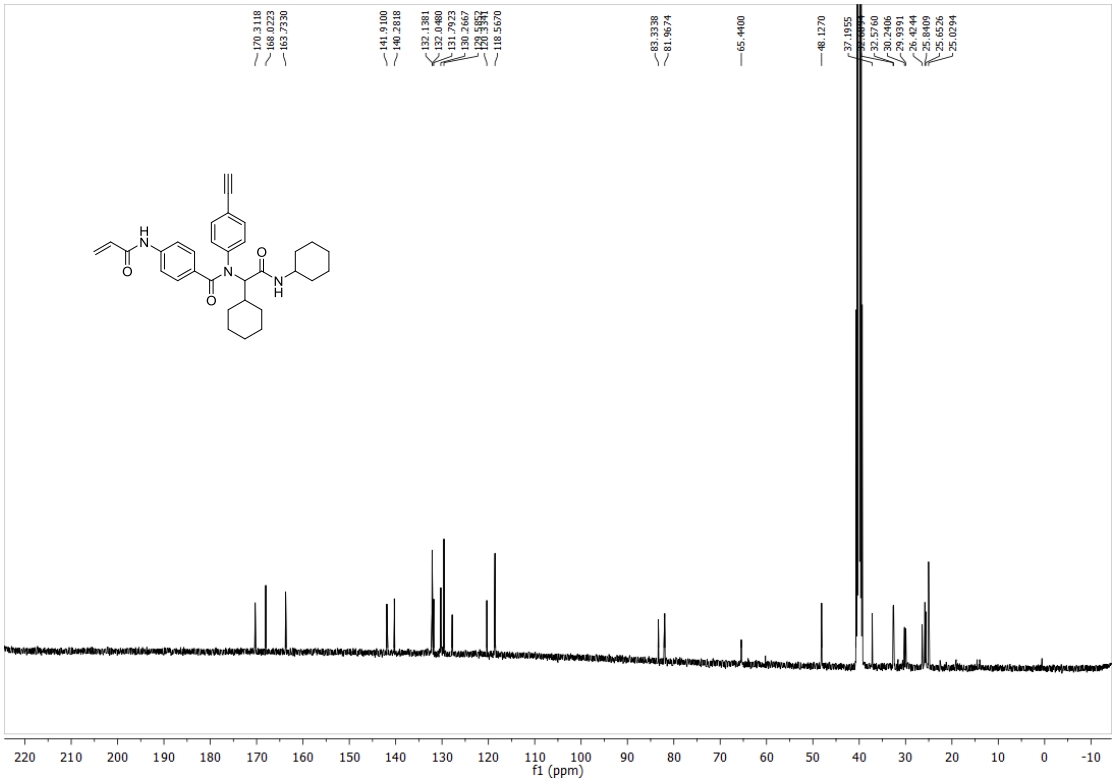
P47 ^{13}C



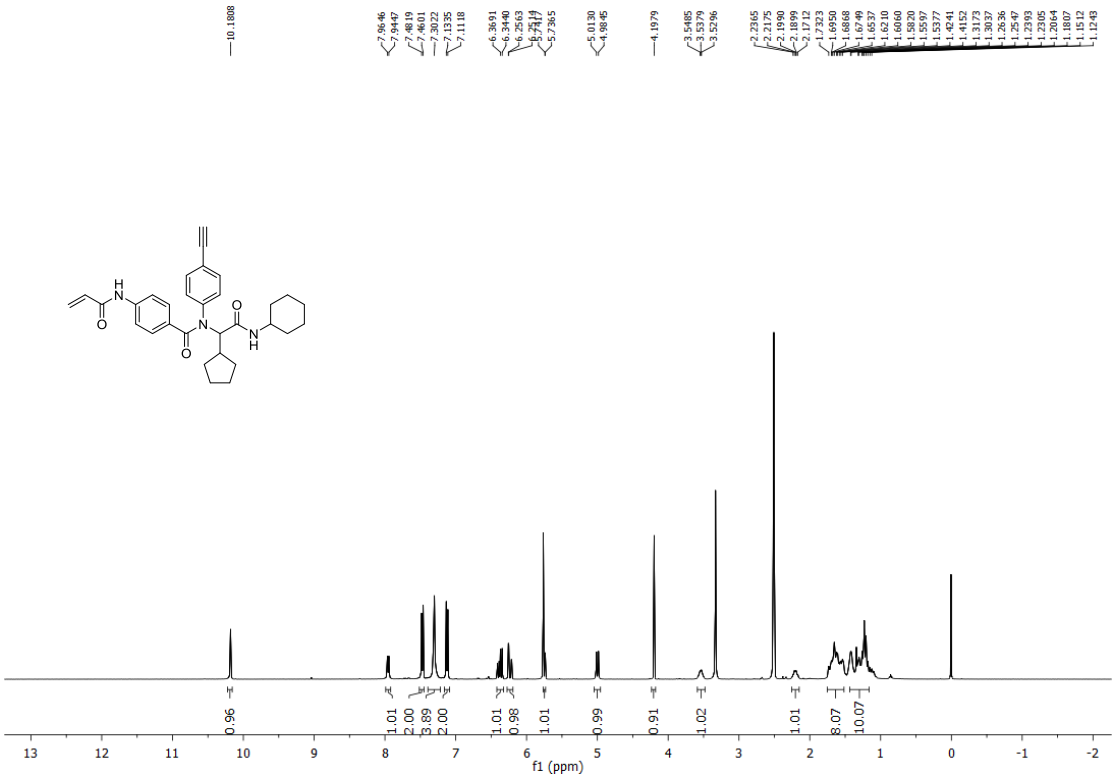
P48 ^{1}H



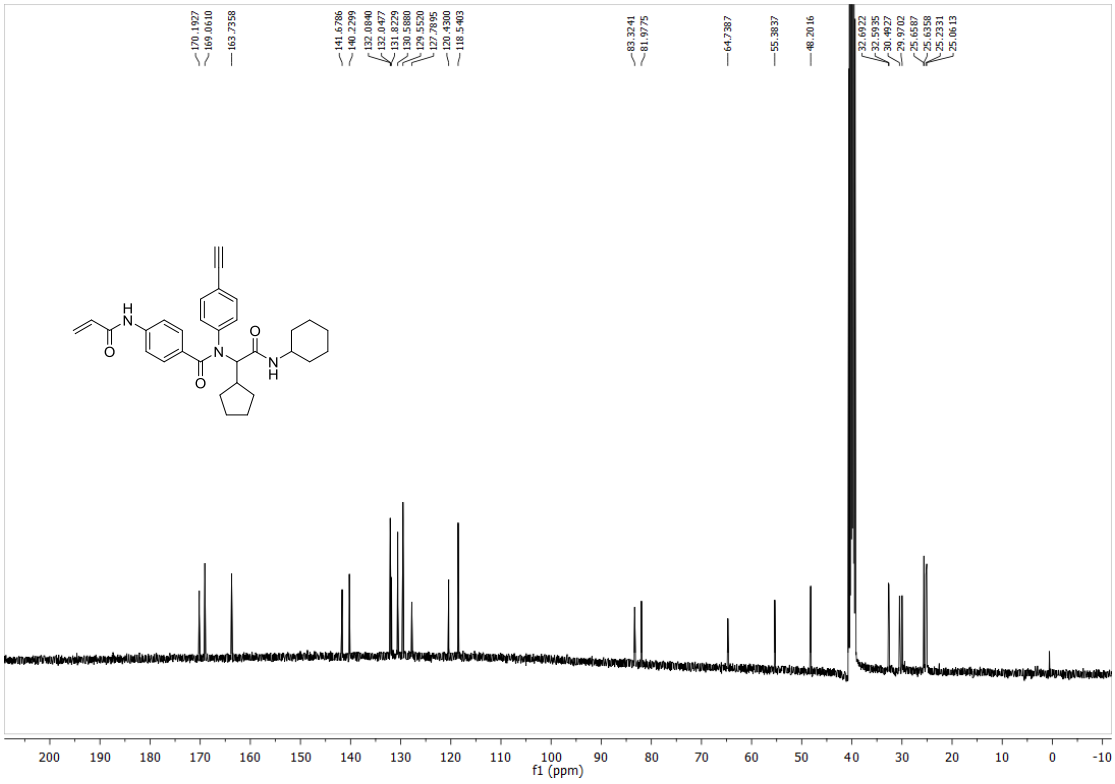
P48 ^{13}C



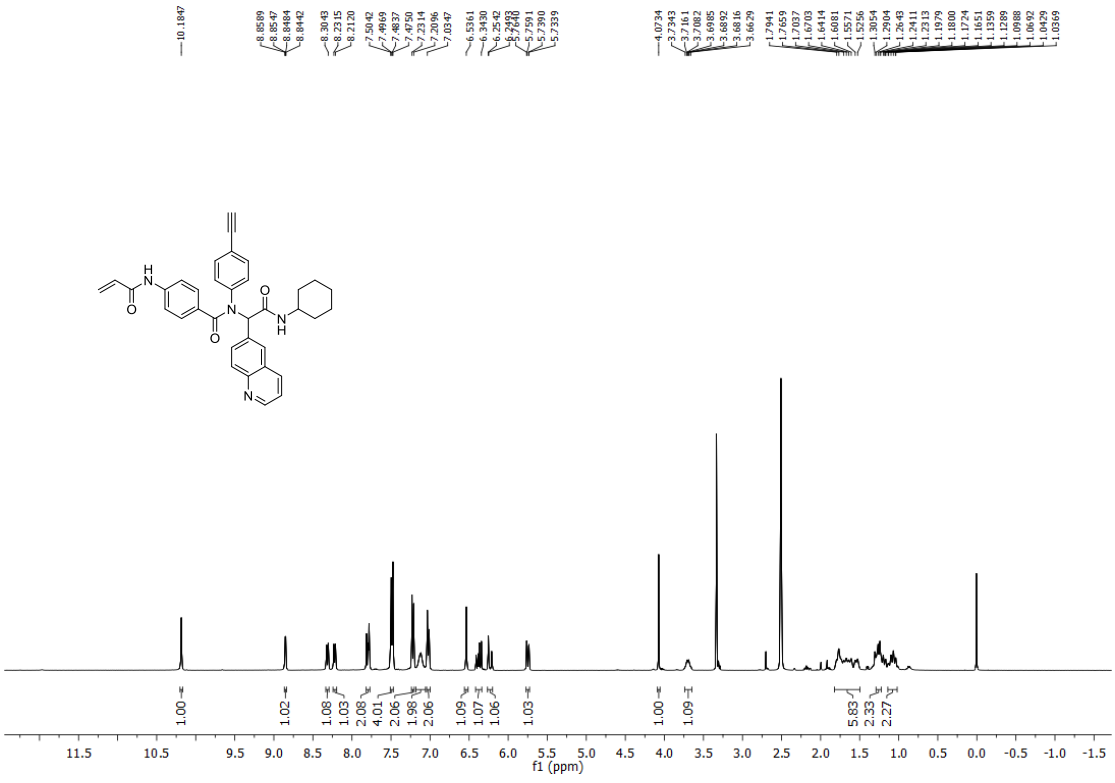
P49 ^{1}H



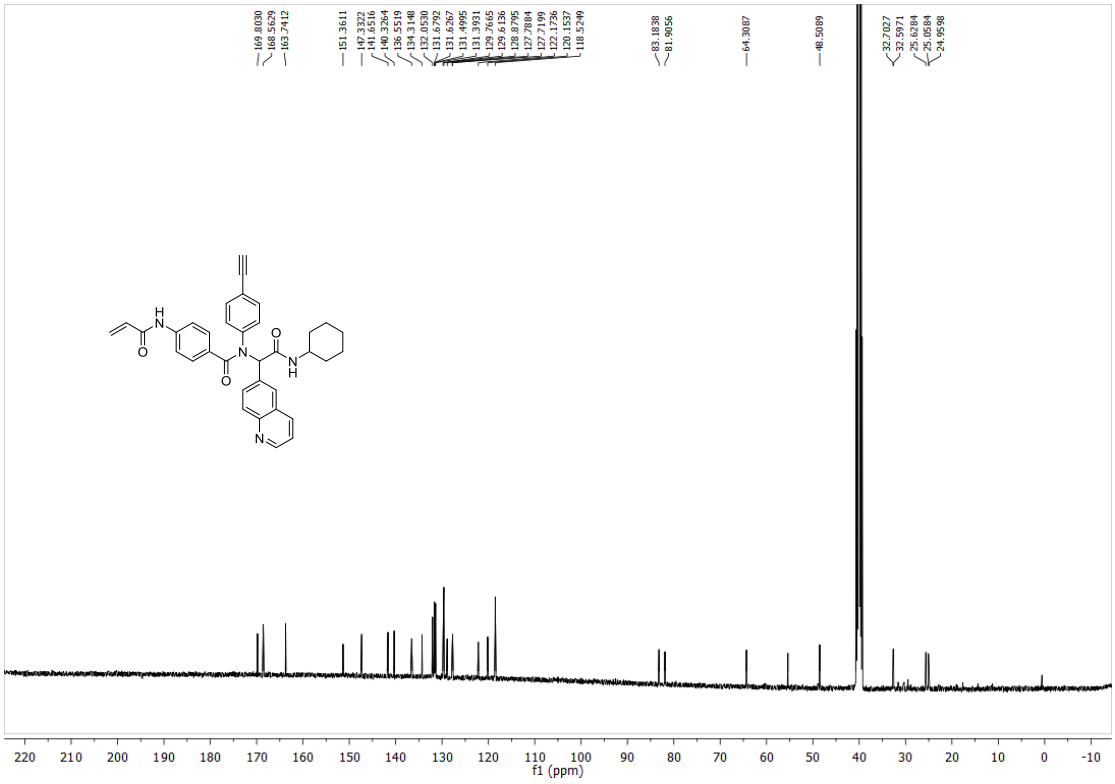
P49 ^{13}C



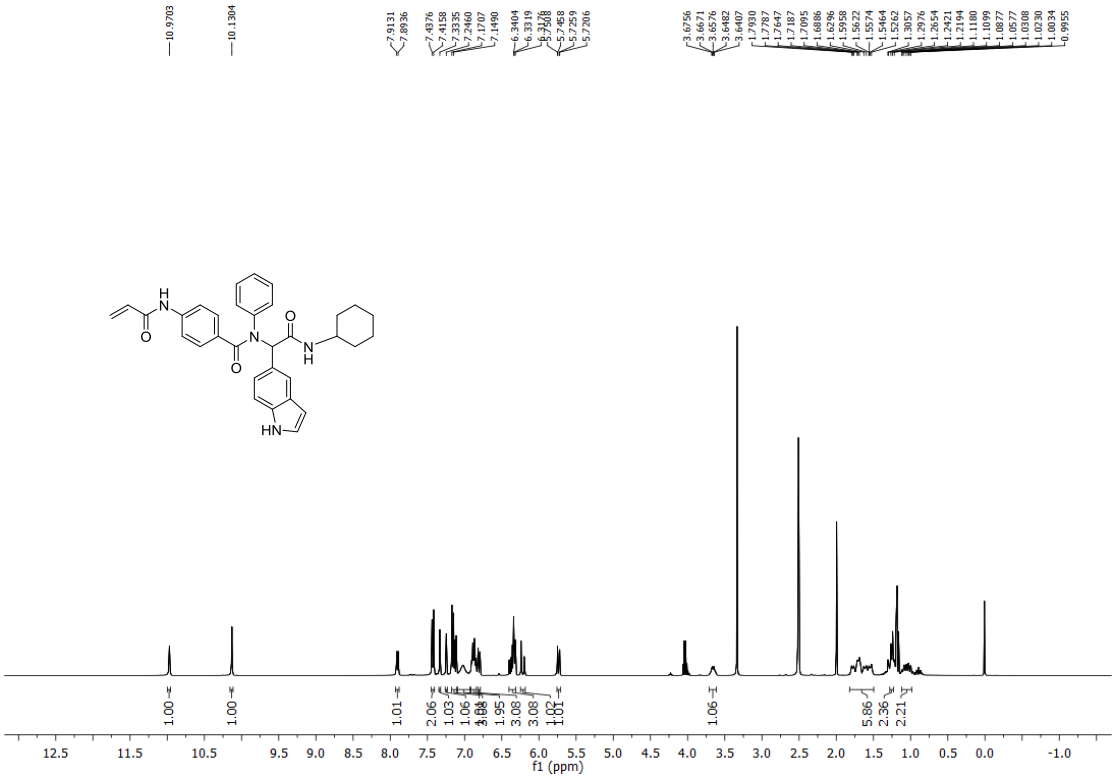
P50 ^{1}H



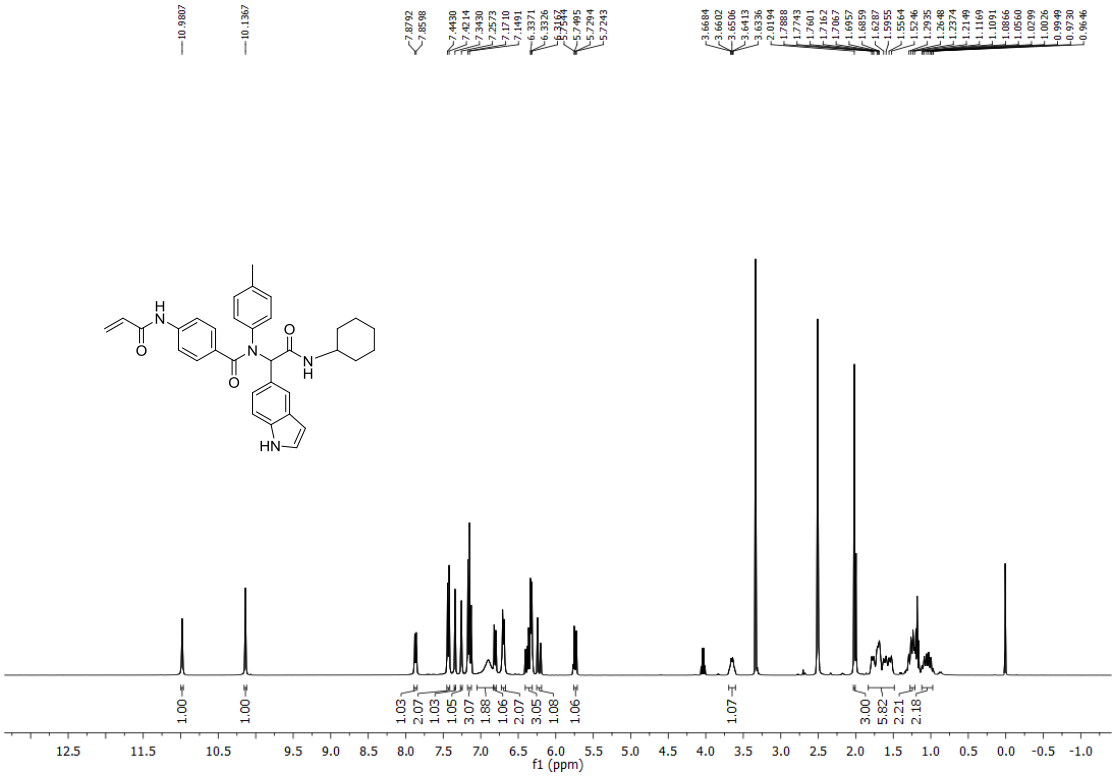
P50 ^{13}C


169.8030
168.5629
163.7412
151.3611
147.3322
141.6516
140.3264
136.5519
134.3148
132.0550
131.6792
131.4995
131.3931
129.7665
129.6136
128.8795
127.7884
127.7199
122.1736
120.1537
118.5249
83.1838
81.9056
64.3087
48.5089
32.7027
32.5971
25.6284
25.0584
24.9598
f1 (ppm)

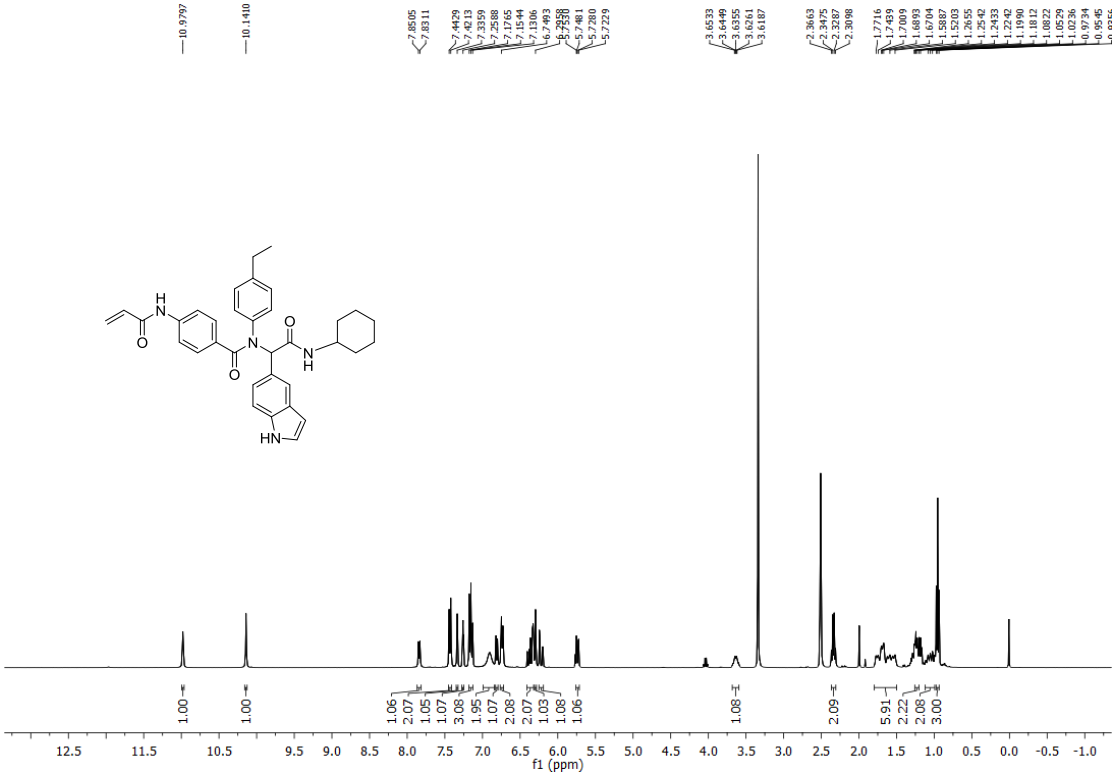
P1 ^{1}H


10.9703
10.1304
7.9131
7.8936
7.4376
7.4158
7.3335
7.2460
7.1707
7.1490
6.3404
6.3319
5.7468
5.7259
5.7206
3.6756
3.6671
3.6576
3.6482
3.6407
1.7930
1.7787
1.7647
1.7187
1.7095
1.6886
1.6296
1.5938
1.5622
1.5514
1.5464
1.5262
1.3057
1.2976
1.2654
1.2421
1.2194
1.1180
1.1099
1.0877
1.0577
1.0308
1.0230
1.0034
0.9955
1.00
1.00
1.01
2.06
1.03
1.06
1.95
3.08
3.08
1.02
1.01
1.06
5.86
2.36
2.21
f1 (ppm)

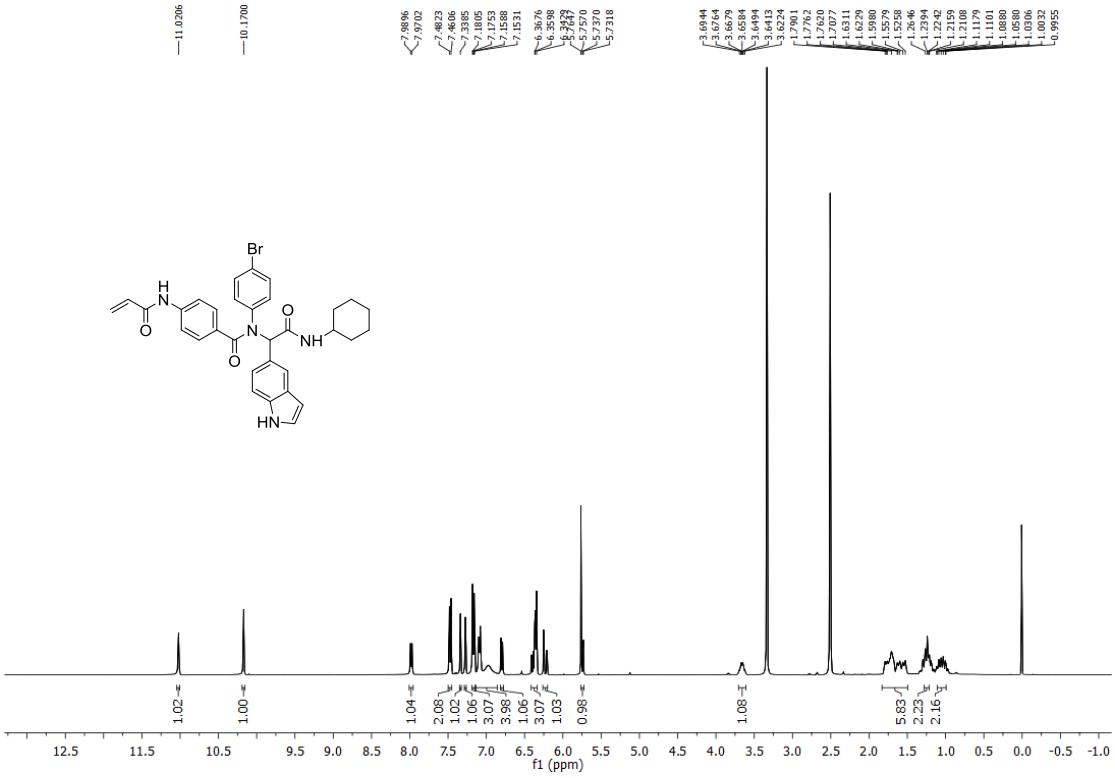
P2 ^{1}H


10.9807
10.1367
7.8792
7.8598
7.4430
7.4214
7.3430
7.2573
7.1710
7.1491
6.3371
6.3326
6.3162
5.7495
5.7294
5.7243
3.6684
3.6602
3.6506
3.6413
3.6336
2.1084
1.7888
1.7743
1.7601
1.7162
1.7067
1.6957
1.6859
1.6287
1.5955
1.5564
1.5246
1.2935
1.2674
1.2149
1.1169
1.1091
1.0866
1.0560
1.0299
1.0026
0.9949
0.9730
0.9646
HN
1.00
1.00
1.03
2.07
1.03
1.05
3.07
1.88
1.06
2.07
3.05
1.08
1.06
1.07
3.00
5.82
2.21
2.18
f1 (ppm)

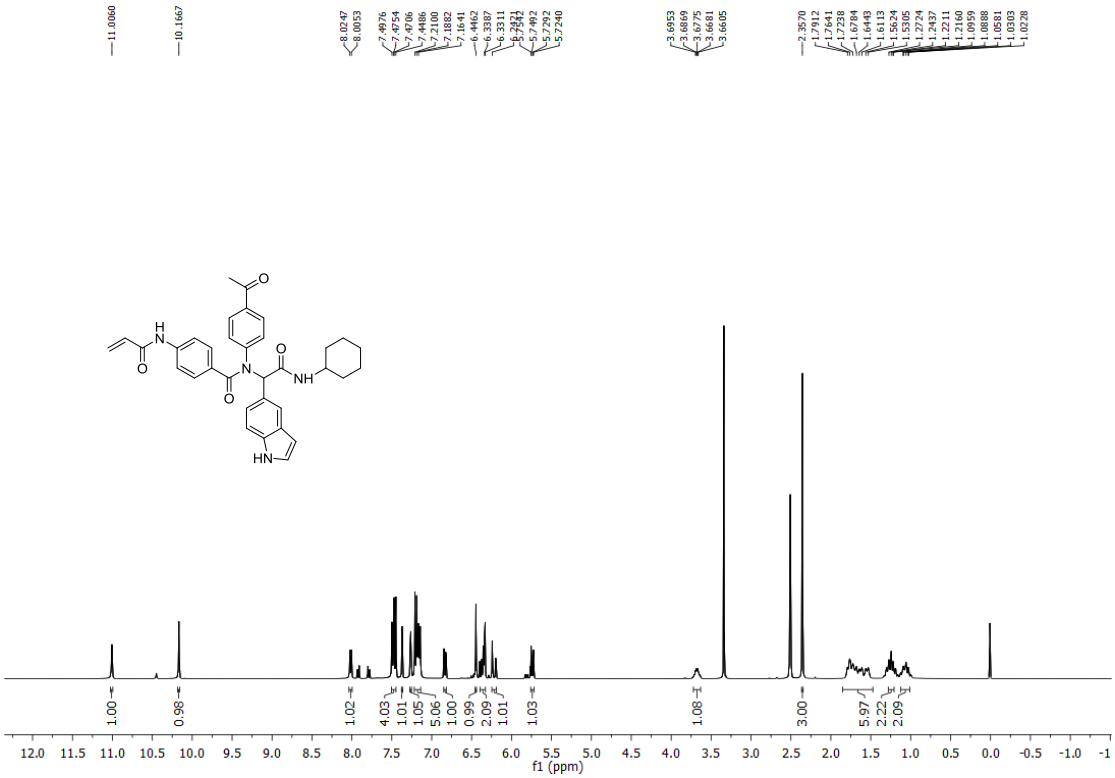
P3 ^{1}H


10.9797
10.1410
7.8505
7.8311
7.4429
7.4213
7.3359
7.2588
7.1765
7.1554
7.1336
6.7493
6.2958
6.2538
5.7481
5.7280
5.7229
3.6533
3.6449
3.6355
3.6261
3.6187
2.3663
2.3475
2.3287
2.3098
1.7716
1.7439
1.7009
1.6893
1.6704
1.5887
1.5203
1.2655
1.2543
1.2433
1.2242
1.1990
1.1812
1.0822
1.0529
1.0236
0.9734
0.9545
HN
1.00
1.00
1.06
2.07
1.05
1.07
3.08
1.95
1.07
2.08
2.07
1.03
1.08
1.06
1.08
2.09
5.91
2.22
2.08
3.00
f1 (ppm)

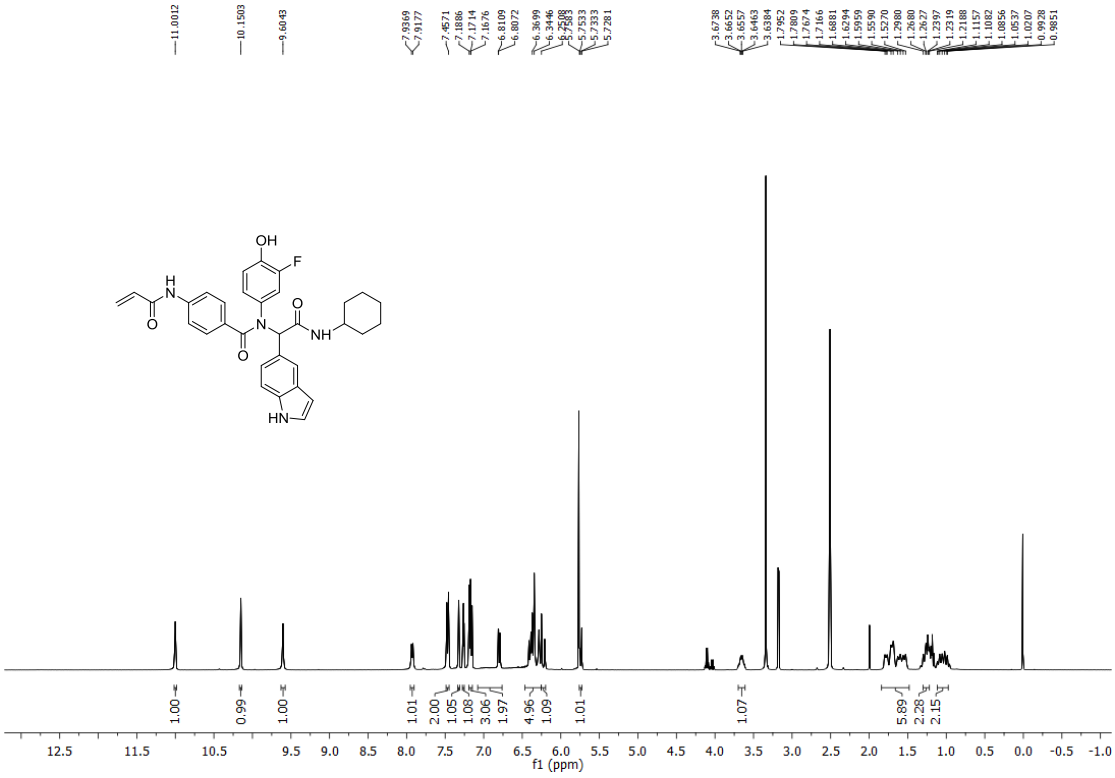
P4 ^{1}H



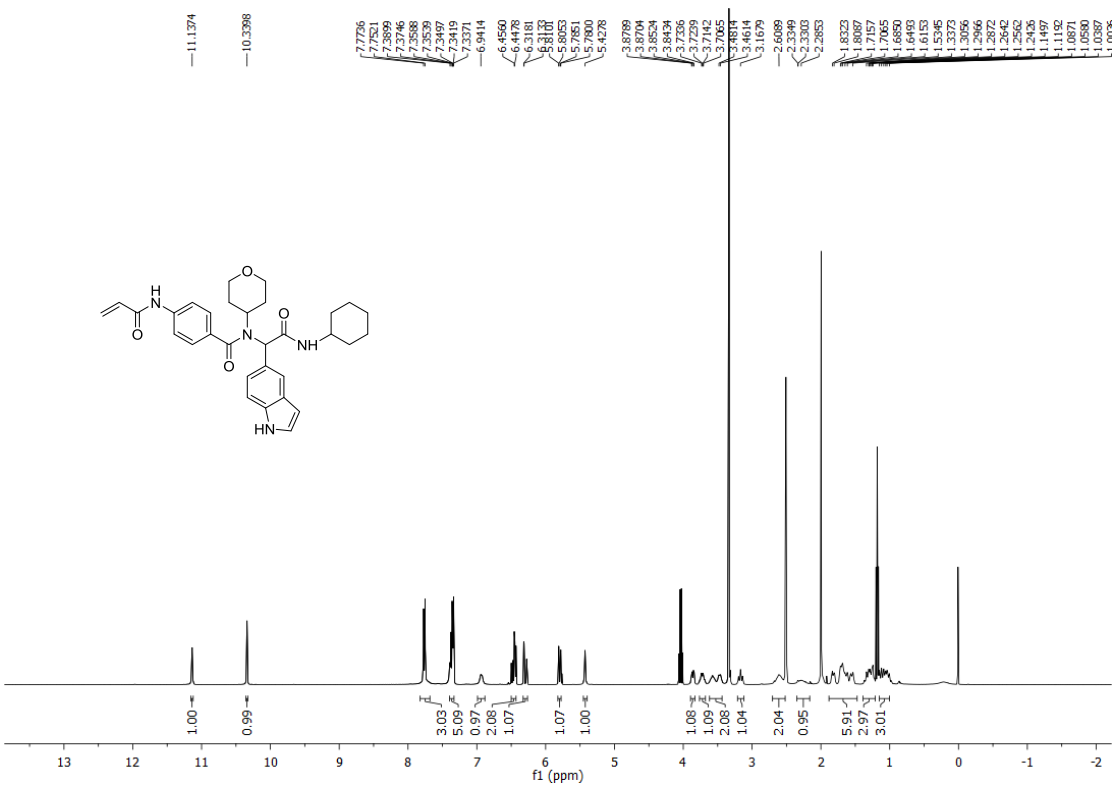
P5 ^{1}H



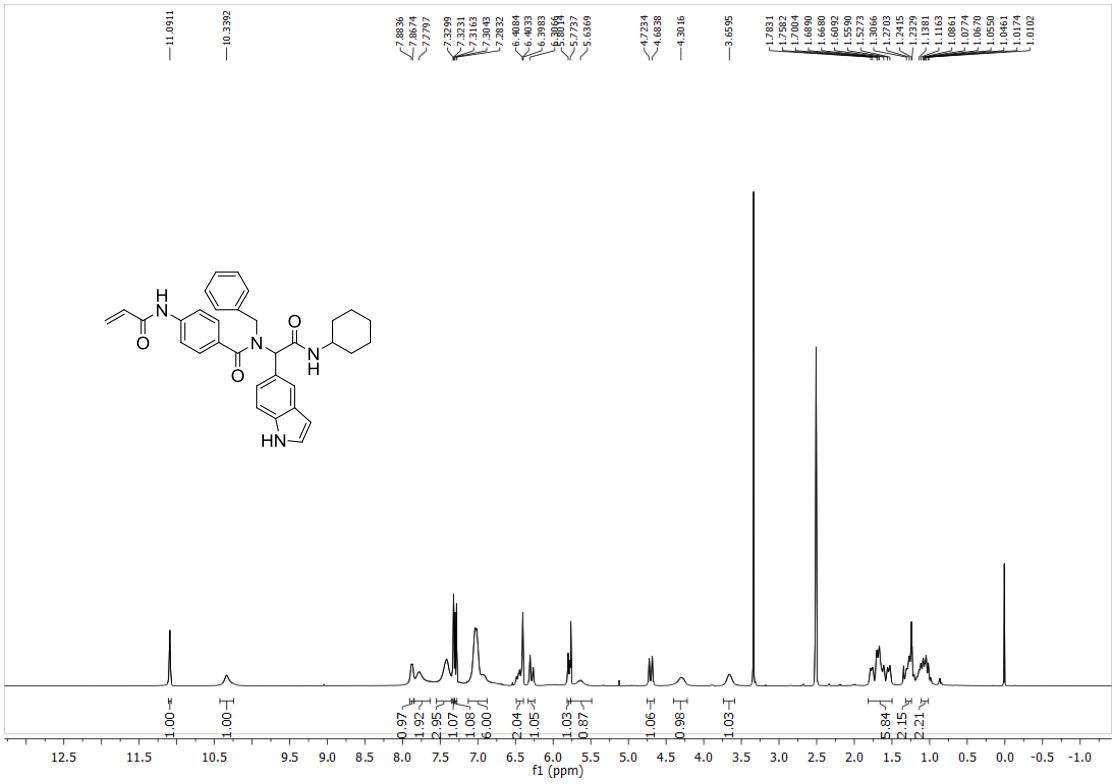
P6 ^{1}H


11.0012
10.1503
9.6043
7.9369
7.9177
7.4571
7.1886
7.1714
7.1676
6.8109
6.8072
6.3699
6.3446
5.7588
5.7533
5.7281
3.6738
3.6652
3.6557
3.6463
3.6384
1.7952
1.7809
1.7674
1.7166
1.6881
1.6294
1.5999
1.5590
1.5270
1.2680
1.2627
1.2397
1.2319
1.2188
1.1157
1.1082
1.0856
1.0537
1.0207
0.9928
0.9851
OH
F
H
N
O
O
N
O
NH
HN
1.00
0.99
1.00
1.01
2.00
1.05
1.08
3.06
1.97
4.96
1.09
1.01
1.07
5.89
2.28
2.15
f1 (ppm)

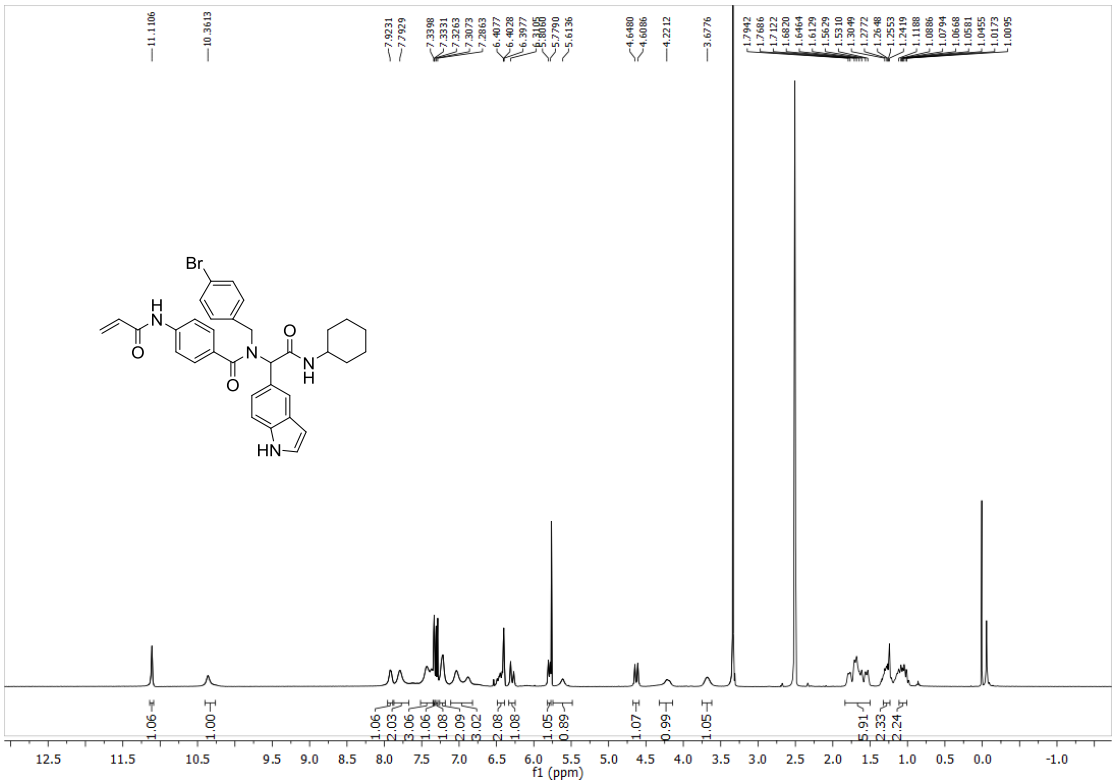
P7 ^{1}H


11.1374
10.3398
7.7736
7.7521
7.3899
7.3746
7.3588
7.3539
7.3497
7.3419
7.3371
6.9414
6.4560
6.4478
6.3181
6.3133
5.8101
5.8053
5.7851
5.7800
5.4278
3.8789
3.8704
3.8524
3.8434
3.7336
3.7239
3.7142
3.7065
3.4814
3.4614
3.1679
2.6089
2.3349
2.3303
2.2853
1.8323
1.8087
1.7757
1.7565
1.6850
1.6493
1.6153
1.5345
1.3373
1.3056
1.2966
1.2872
1.2642
1.2562
1.2426
1.1497
1.1192
1.0871
1.0580
1.0387
O
H
N
O
O
N
O
NH
HN
1.00
0.99
3.03
5.09
0.97
2.08
1.07
1.07
1.00
1.08
1.09
2.08
1.04
2.04
0.95
5.91
2.97
3.01
f1 (ppm)

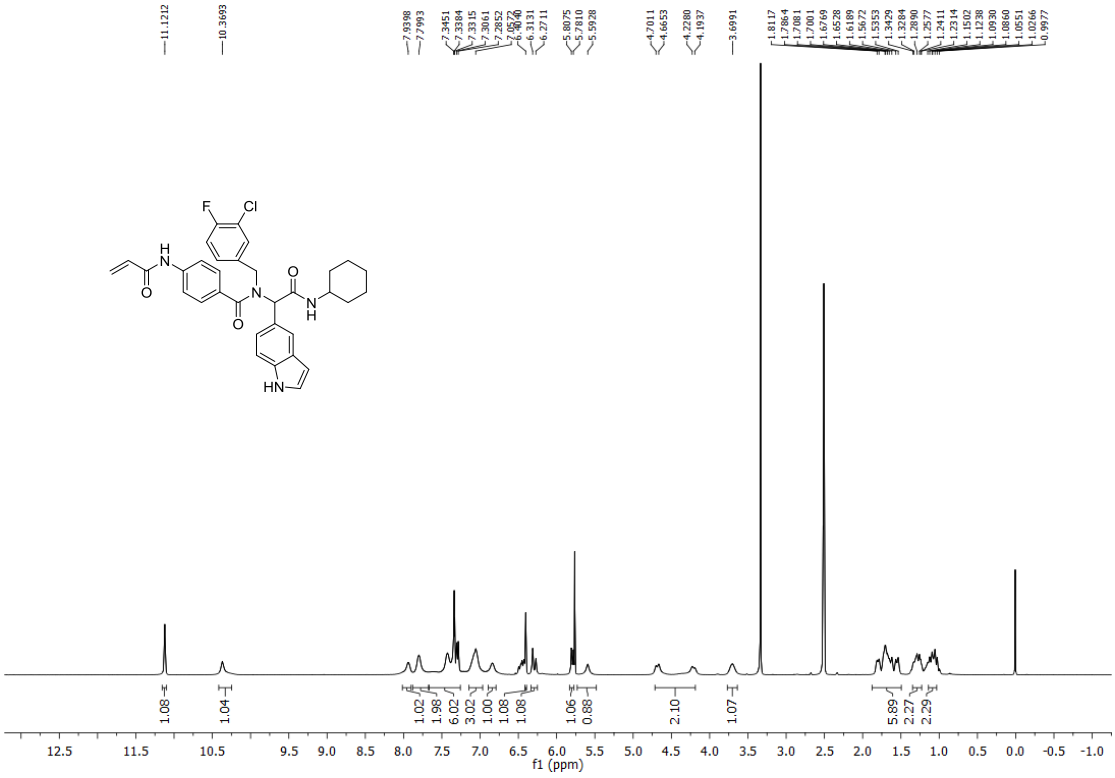
P8 ^{1}H


11.0911
10.3392
7.8836
7.8674
7.7797
7.3299
7.3231
7.3163
7.3043
7.2832
6.4084
6.4033
6.3983
5.7737
5.6369
4.7234
4.6838
4.3016
3.6595
1.7831
1.7582
1.7004
1.6890
1.6680
1.6092
1.5590
1.5273
1.3066
1.2703
1.2415
1.2329
1.1781
1.1163
1.0861
1.0774
1.0670
1.0550
1.0461
1.0174
1.0102
1.00
1.00
0.97
1.92
2.95
1.07
1.08
6.00
2.04
1.05
1.03
0.87
1.06
0.98
1.03
5.84
2.15
2.21
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

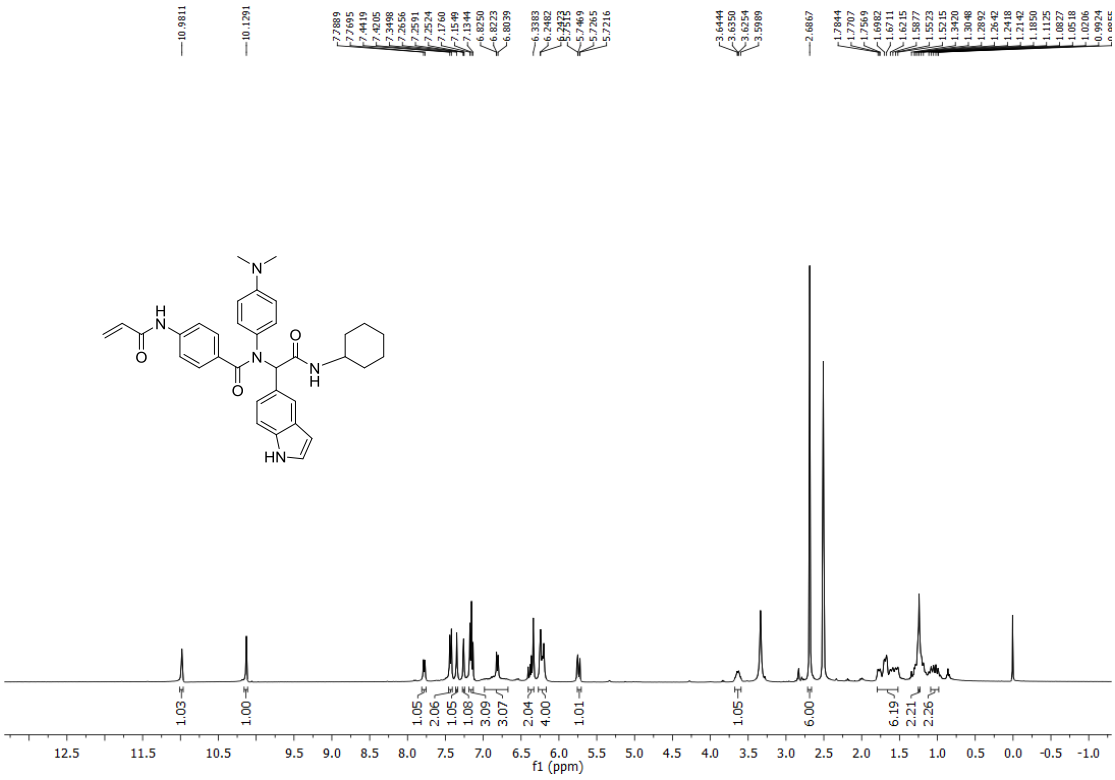
P9 ^{1}H


11.1106
10.3613
7.9231
7.7929
7.3398
7.3331
7.3263
7.3073
7.2863
6.4077
6.4028
6.3977
5.7790
5.6136
4.6480
4.6086
4.2212
3.6776
1.7942
1.7686
1.7122
1.6930
1.6464
1.6129
1.5629
1.5310
1.3049
1.2772
1.2648
1.2553
1.2419
1.1188
1.0886
1.0794
1.0668
1.0561
1.0455
1.0173
1.0095
1.06
1.00
1.06
2.03
3.06
1.08
2.09
3.02
2.08
1.08
1.05
0.89
1.07
0.99
1.05
5.91
2.33
2.24
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-1.0
f1 (ppm)

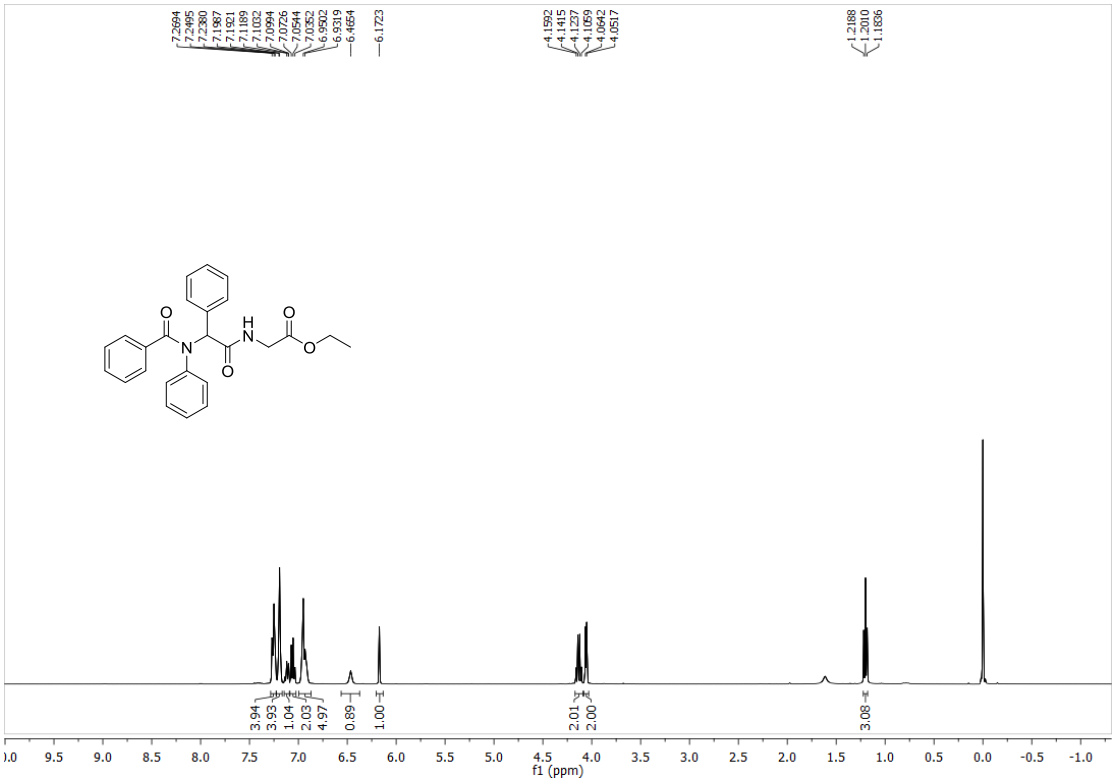
P10 ^{1}H


F
Cl
H
N
O
N
O
O
N
H
HN
11.1212
10.3693
7.9398
7.7993
7.3451
7.3384
7.3315
7.3061
7.2852
6.3131
6.2711
5.8075
5.7810
5.5928
4.7011
4.6653
4.2280
4.1937
3.6991
1.8117
1.7864
1.7681
1.7001
1.6769
1.6528
1.6189
1.5672
1.5353
1.3429
1.3284
1.2890
1.2577
1.2411
1.2314
1.1502
1.1238
1.0930
1.0860
1.0551
1.0266
0.9977
1.08
1.04
1.02
1.98
6.02
3.02
1.00
1.08
1.08
1.06
0.88
2.10
1.07
5.89
2.27
2.29
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

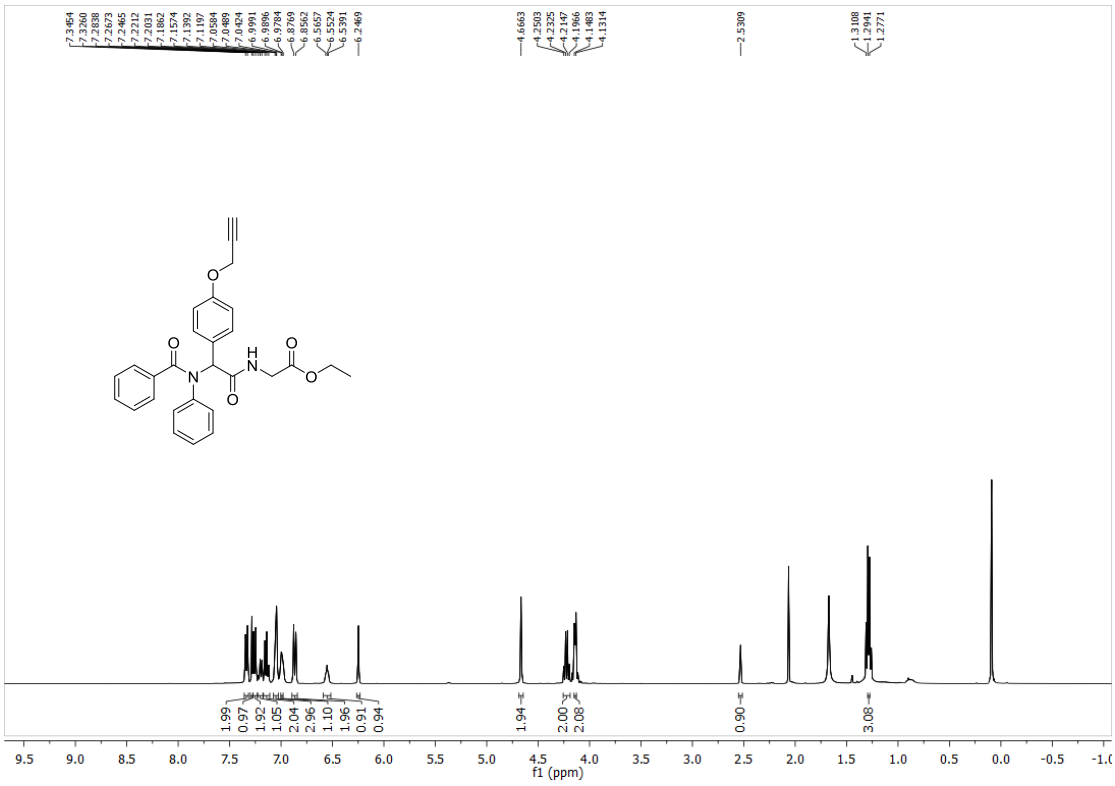
P22 ^{1}H


N
H
N
O
N
O
O
N
H
HN
10.9811
10.1291
7.7889
7.7695
7.4419
7.4205
7.3498
7.2656
7.2591
7.2524
7.1760
7.1549
7.1344
6.8250
6.8223
6.8039
6.3383
6.3262
5.7513
5.7469
5.7265
5.7216
3.6444
3.6350
3.6254
3.5989
2.6867
1.7844
1.7707
1.7569
1.6982
1.6711
1.6215
1.5877
1.5523
1.5215
1.3420
1.3048
1.2808
1.2642
1.2418
1.2142
1.1850
1.1125
1.0827
1.0518
1.0206
0.9924
1.03
1.00
1.05
2.06
1.05
1.08
3.09
3.07
2.04
4.00
1.01
1.05
6.00
6.19
2.21
2.26
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

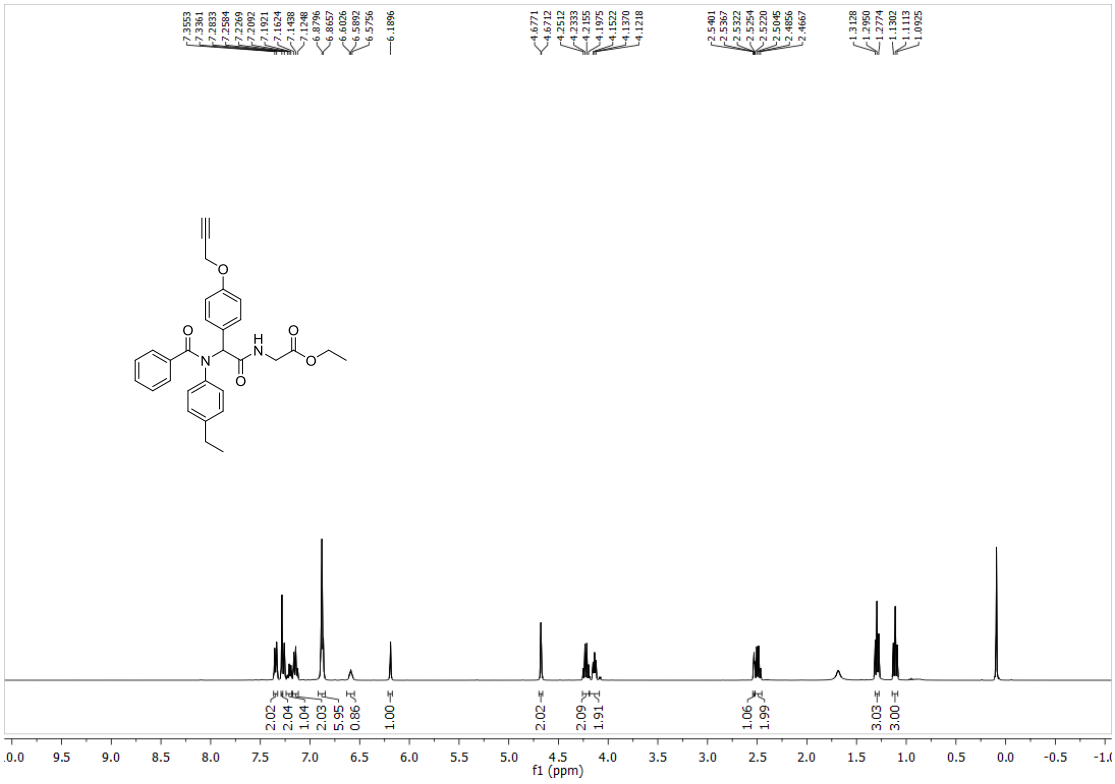
P51 ^{1}H


7.2694
7.2495
7.2380
7.1987
7.1921
7.1189
7.1032
7.0994
7.0726
7.0654
7.0552
6.9902
6.9319
6.4654
6.1723
4.1592
4.1415
4.1237
4.1059
4.0642
4.0517
1.2188
1.2010
1.1836
3.94
3.93
1.04
2.03
4.97
0.89
1.00
2.01
2.00
3.08
f1 (ppm)

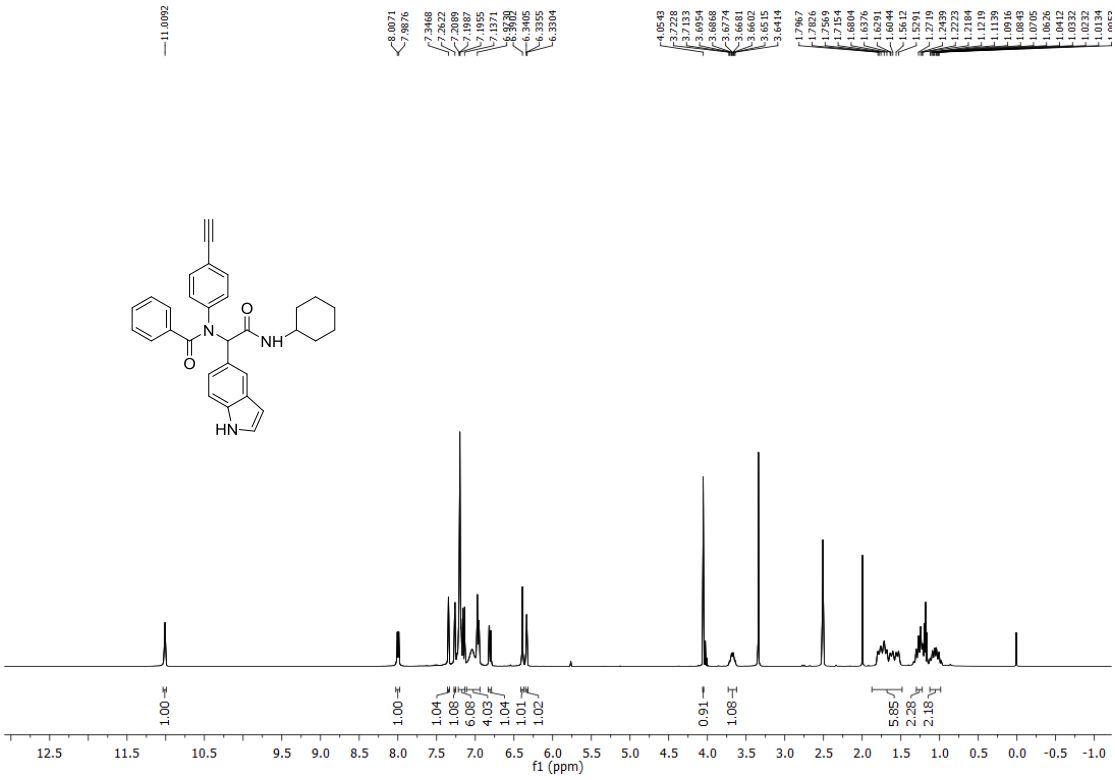
P52 ^{1}H


7.3464
7.3260
7.2838
7.2673
7.2465
7.2212
7.2031
7.1862
7.1574
7.1392
7.1197
7.0584
7.0498
7.0424
6.9991
6.9886
6.9784
6.8769
6.8562
6.5657
6.5524
6.5391
6.2469
4.6663
4.2503
4.2325
4.2147
4.1966
4.1785
4.1314
2.5309
1.3108
1.2941
1.2771
1.99
0.97
1.92
1.05
2.04
2.96
1.10
1.96
0.91
0.94
1.94
2.00
2.08
0.90
3.08
f1 (ppm)

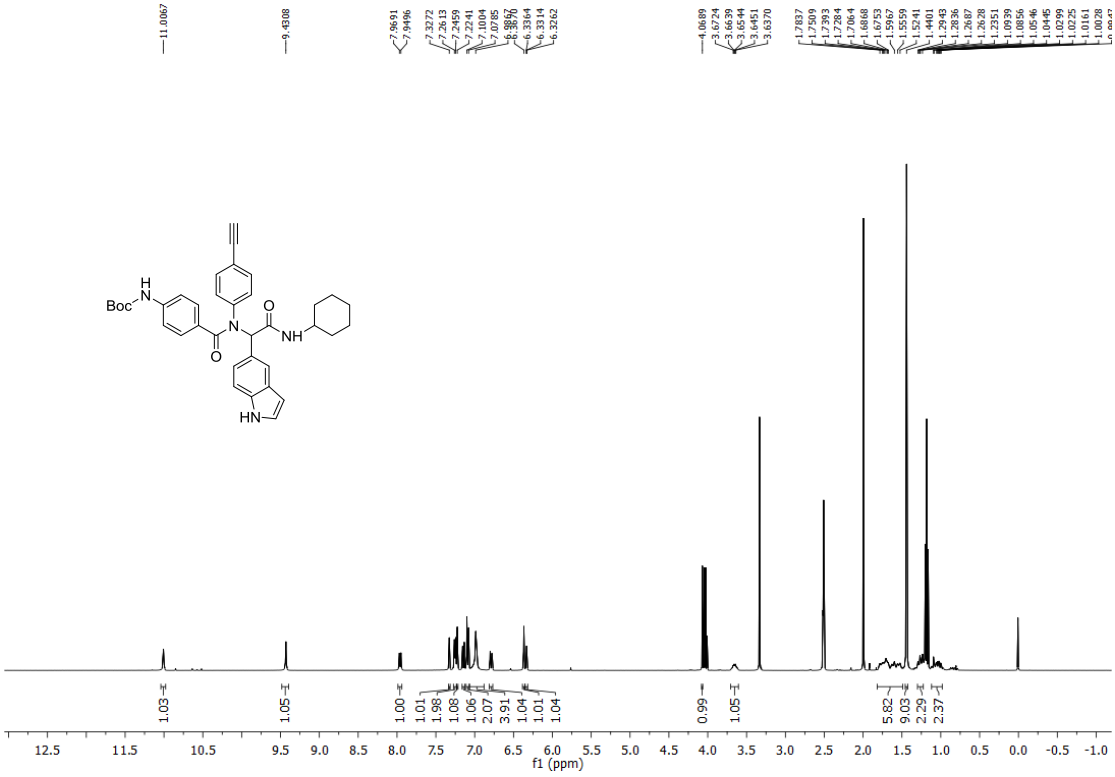
P53 ^{1}H


7.3553
7.3361
7.2833
7.2594
7.2269
7.2092
7.1921
7.1624
7.1438
7.1248
6.8796
6.8657
6.6026
6.5892
6.5756
6.1896
4.6771
4.6712
4.2512
4.2333
4.2155
4.1975
4.1522
4.1370
4.1218
2.5401
2.5367
2.5322
2.5254
2.5220
2.5045
2.4856
2.4667
1.3128
1.2950
1.2774
1.1302
1.1113
1.0925
2.02
2.04
1.04
2.03
5.95
0.86
1.00
2.02
2.09
1.91
1.06
1.99
3.03
3.00
f1 (ppm)

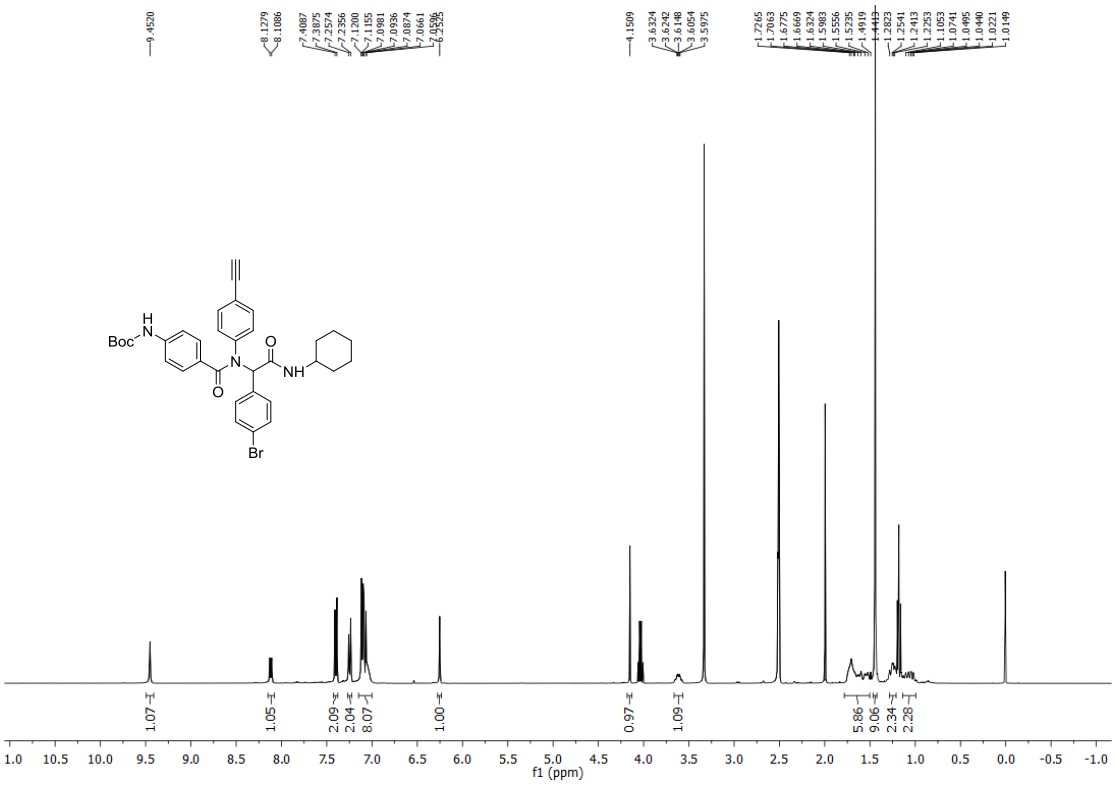
P54 ^{1}H


11.0092
8.0071
7.9876
7.3468
7.2622
7.2089
7.1987
7.1955
7.1371
6.3405
6.3355
6.3304
4.0543
3.7233
3.7133
3.6954
3.6868
3.6774
3.6681
3.6602
3.6515
3.6414
1.7867
1.7826
1.7569
1.7154
1.6804
1.6376
1.6291
1.6044
1.5612
1.5291
1.2719
1.2439
1.2223
1.2104
1.1219
1.1139
1.0916
1.0843
1.0705
1.0626
1.0412
1.0332
1.0232
1.0134
1.00
1.00
1.04
1.08
6.08
4.03
1.04
1.01
1.02
0.91
1.08
5.85
2.28
2.18
f1 (ppm)

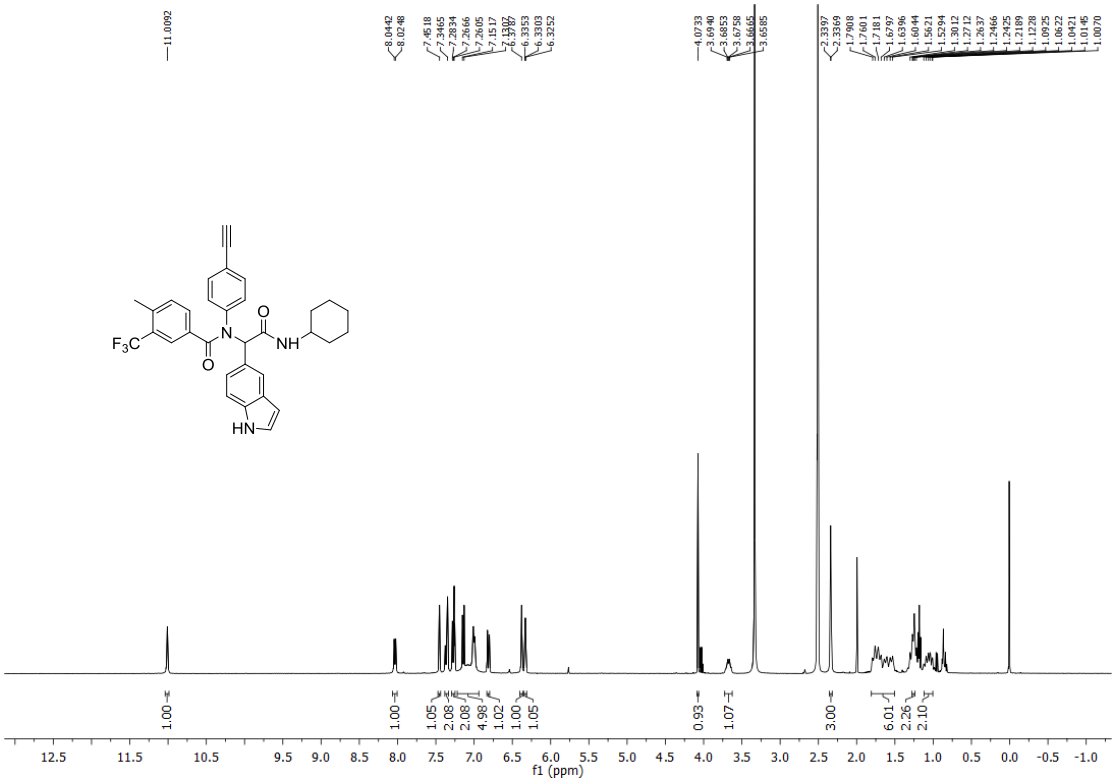
P55 ^{1}H



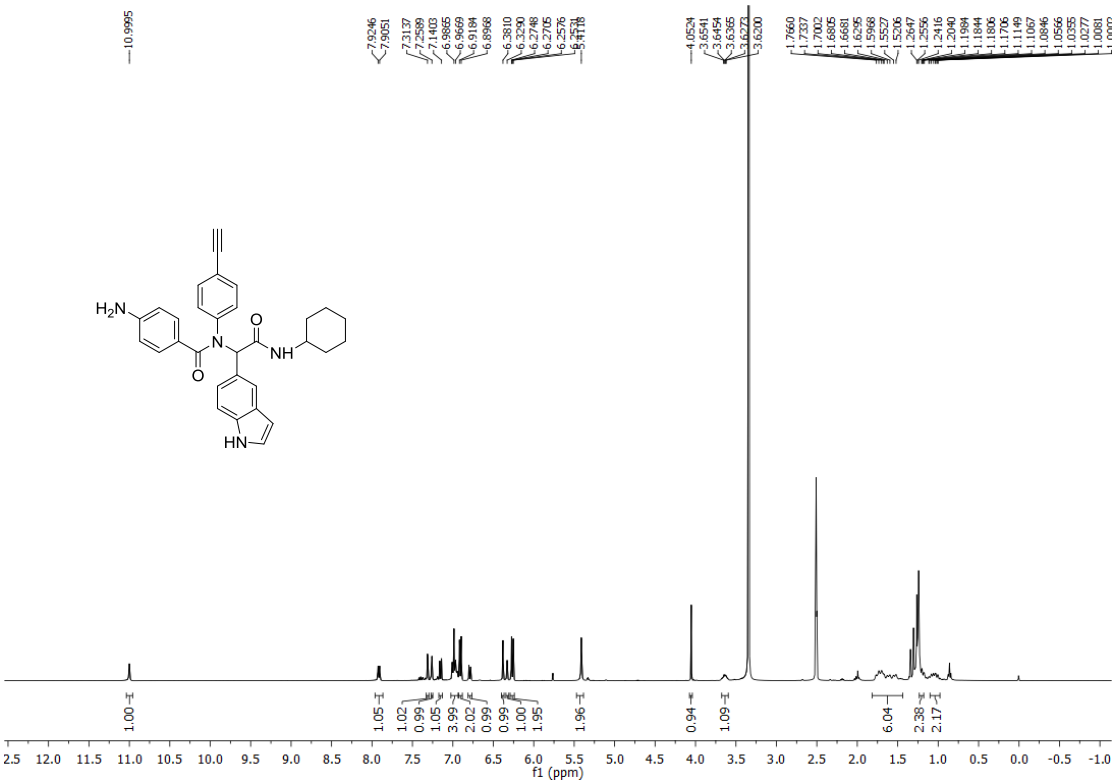
P56 ^{1}H



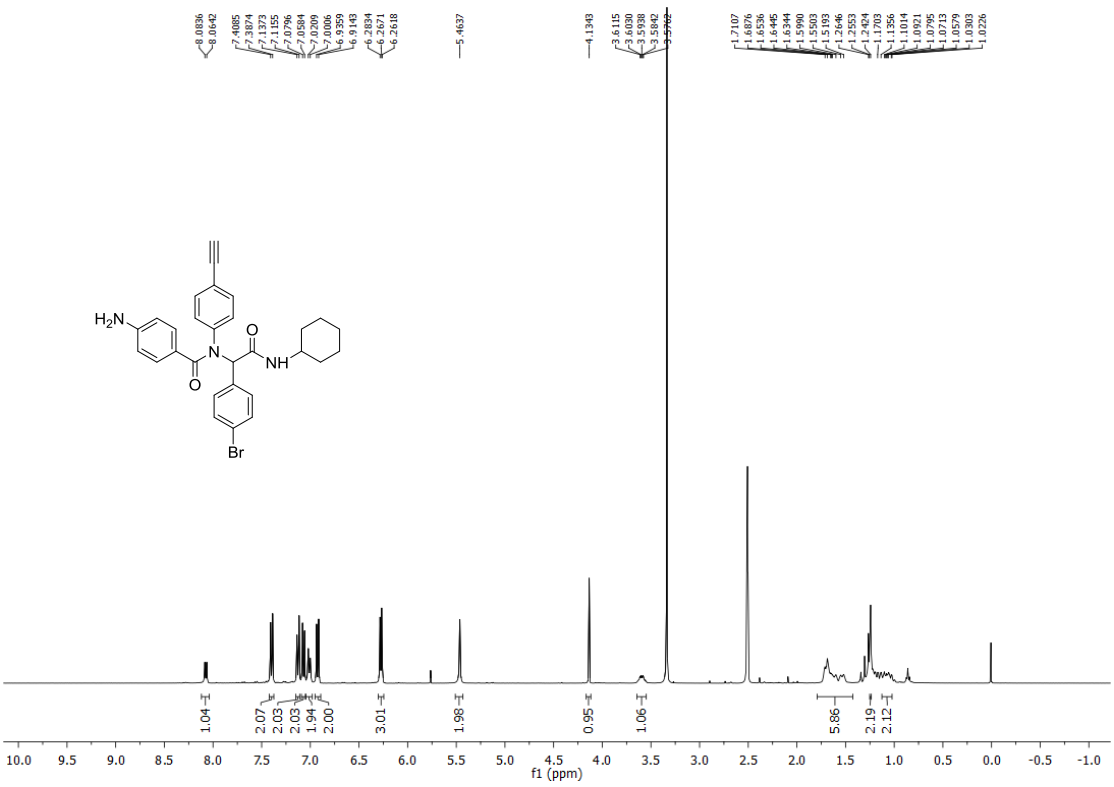
P57 ^{1}H



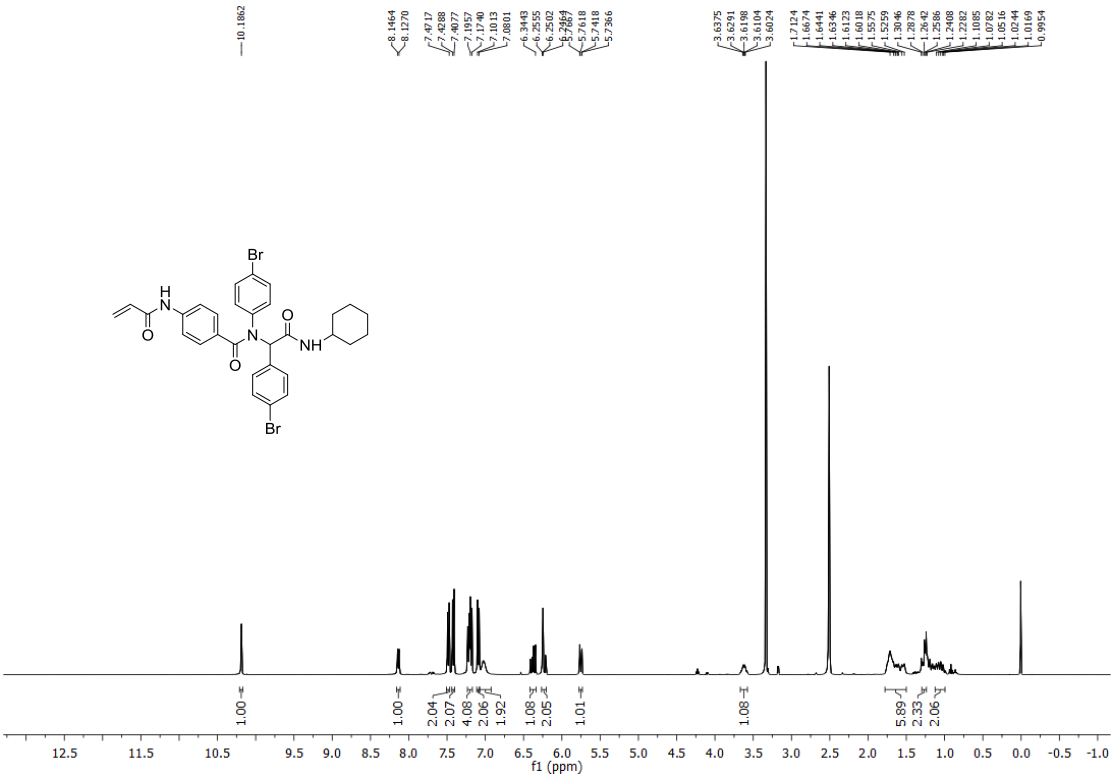
P58 ^{1}H



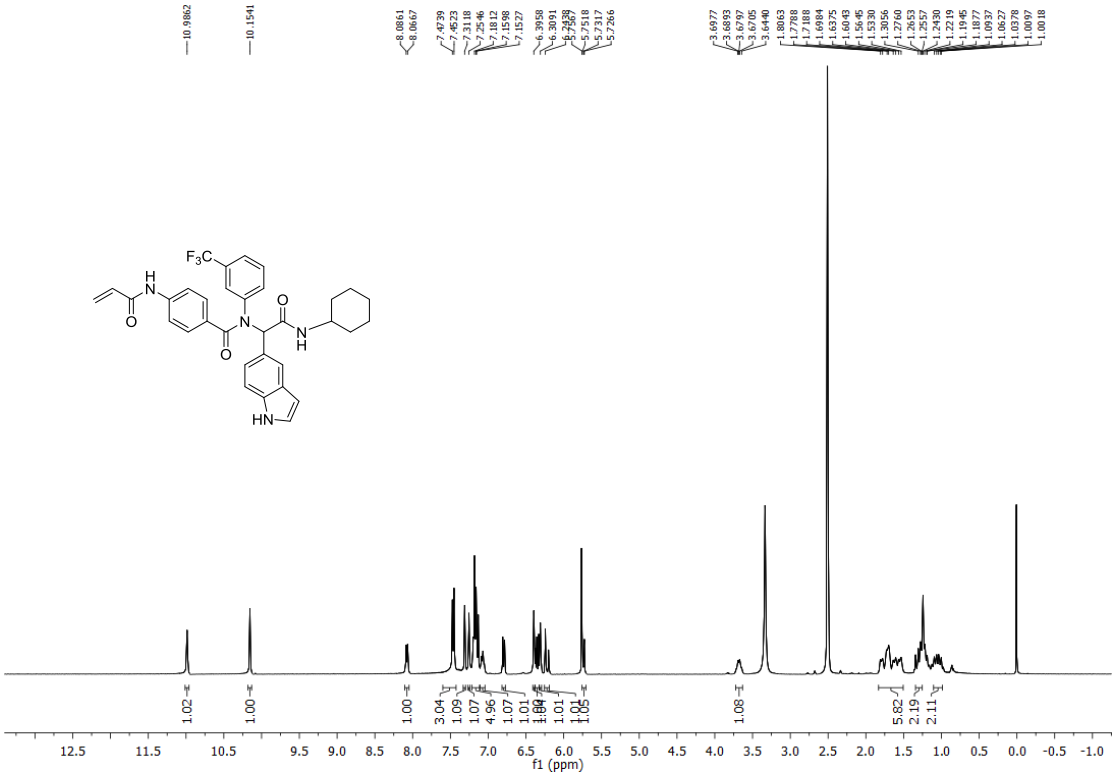
P59 ^{1}H



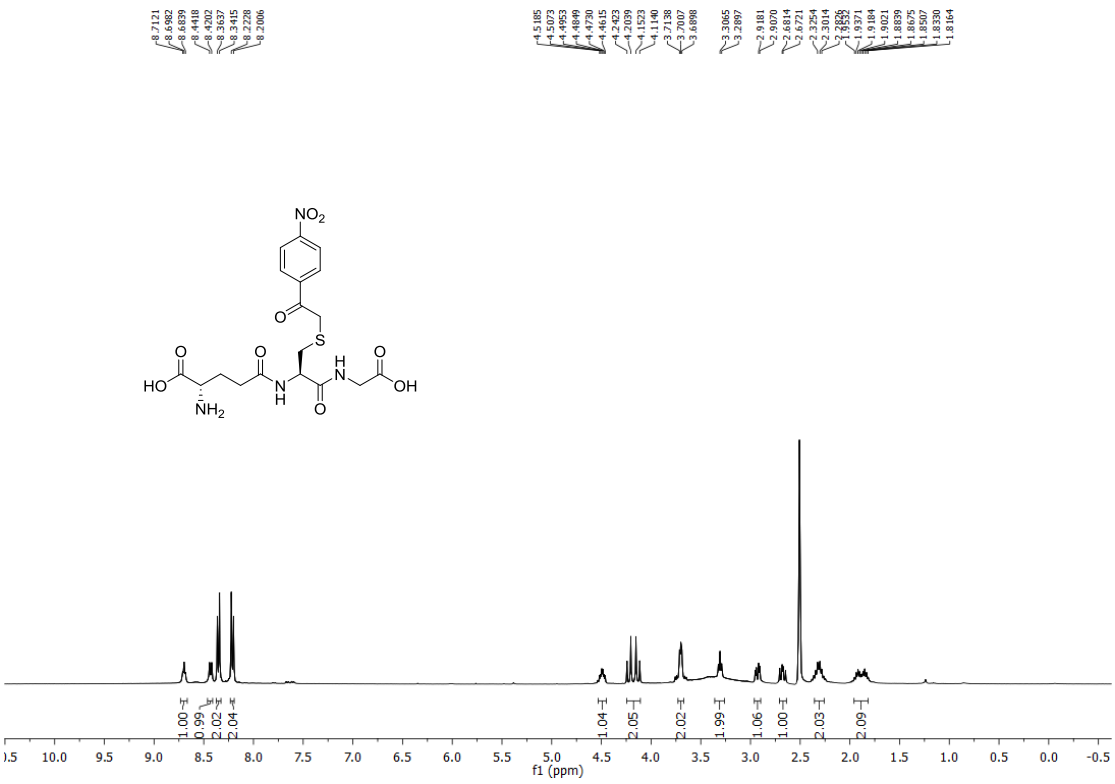
P60 ^{1}H



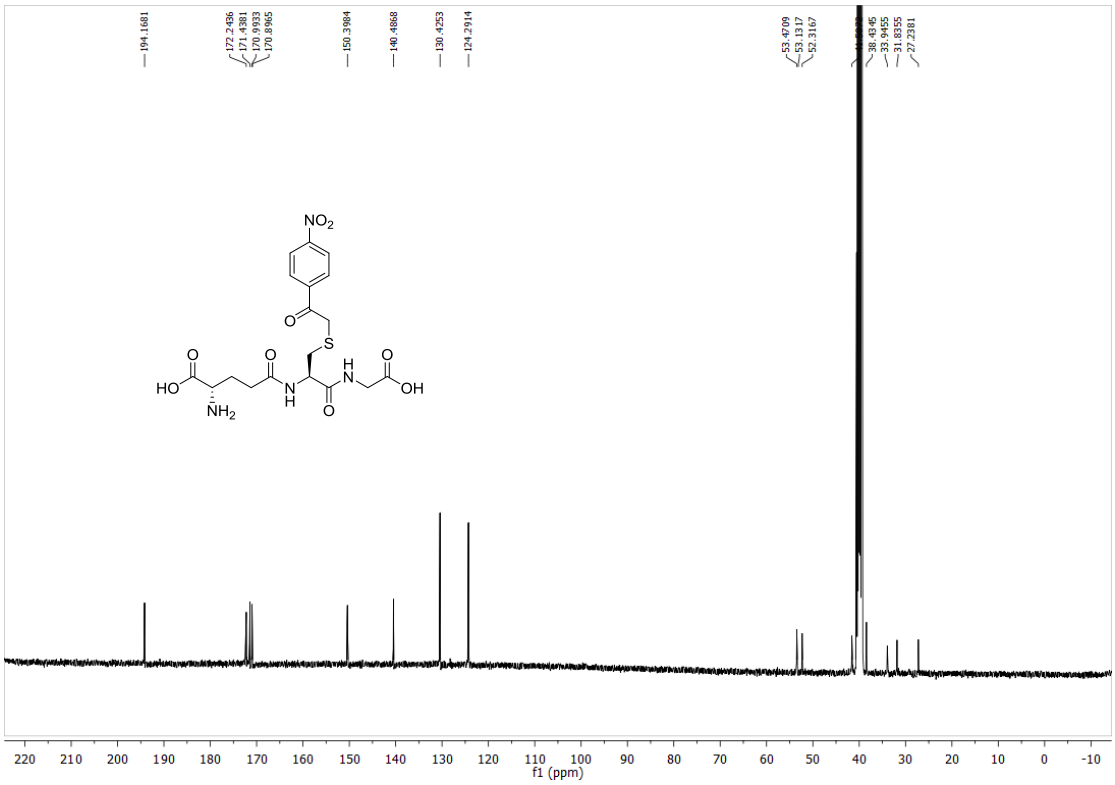
P61 ^{1}H



4-NPG ^{1}H



4-NPG ^{13}C


194.1681
172.2456
171.4381
170.9933
170.8965
150.3984
140.4868
130.4253
124.2914
53.4709
53.1317
52.3167
38.4846
33.9455
31.1855
27.2381
NO2
O
S
HO
O
NH2
O
N
H
O
H
N
O
OH
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

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