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Supporting Information for
Affinity Selection of Double-Click Triazole Libraries for Rapid Discovery of Allosteric Modulators for GLP-1 Receptor

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This PDF file includes:

Contents

SI Materials and Methods

Materials. GLP-1(7-36)NH_2, GLP-1(9-36)NH_2, mutant GLP-1(9-36)NH_2 (V16A or L20A) and glucagon were synthesized and purified to >95% purity at GenScript (Nanjing, China). Chemical materials were purchased from Aladdin (Shanghai, China), TCI (Tokyo, Japan), Macklin (Shanghai, China), Energy (Shanghai, China), Alfa Aesar (Shanghai, China), Adamas (Shanghai, China), Tianlian (Shanghai, China), Shuya (Shanghai, China), and Bide (Shanghai, China). Solvents were purchased from Macklin, Adamas, Tianlian. All purchased reagents and solvents were used as received, without further purification or special handling practice.

Protein expression and purification. The GLP-1R TMD plasmid was constructed as previously described (1). Briefly, the modified construct comprised residues 128-431 and contained 10 thermostabilizing mutations. Nine residues in ECL1 (205-213) were replaced by a GSG linker. To increase the protein stability, three residues (258-260) at intracellular loop 2 (ICL2) were replaced with the T4 lysozyme (T4L) as a fusion partner. In addition, a 10× histidine tag and thermostabilized *Escherichia coli* apocytochrome B_{562}RIL (BRIL) were inserted before the protein N-terminus to facilitate protein expression and purification.

The protein expression and membrane isolation were performed with the same procedure as described before (1). The isolated membranes were re-suspended in the buffer containing 10 mM HEPEs (pH 7.5), 1 M NaCl, 10 mM $MgCl_2$, 20 mM KCl, 30% (v/v) glycerol and an EDTA-free protease inhibitor cock tail, and stored at -80°C for further usage. Before solubilization, the membranes were incubated with 2 mg ml^{-1} iodoacetamide for 45 min at 4 °C. The GLP-1R TMD was extracted from the membrane by adding a 2× solubilization buffer [1 M NaCl, 100 mM HEPEs (pH 7.5), 1% (w/v) n-dodecyl-beta-D-maltopyranoside (DDM, Affymetrix) and 0.2% (w/v) cholesteryl hemisuccinate (CHS, Sigma)] and stirring for 3 h at 4 °C. Then the solubilized mixture was ultra-centrifuged at 160,000 g for 30 min, and the supernatant was incubated with TALON IMAC resin (Clontech) at 4°C, overnight. The resin was packed into a purification column and washed with 20 column volumes wash buffer 1 [500 mM NaCl, 50 mM HEPEs (pH 7.5), 5% (v/v) glycerol, 30 mM Imidazole, 0.05% (w/v) DDM and 0.01% (w/v) CHS] and 10 column volumes wash buffer 2 [500 mM NaCl, 25 mM HEPEs (pH = 7.5), 5% (v/v) glycerol, 30 mM Imidazole, 0.01% DDM (w/v) and 0.002% (w/v) CHS] before bound receptors was harvested with an elution buffer [500 mM NaCl, 25 mM HEPEs (pH = 7.5), 5% (v/v) glycerol, 300 mM Imidazole, 0.01% DDM (w/v) and 0.002% (w/v) CHS].

Preparation of an amine library in microplates. Because most free amines (primary or otherwise) are unstable during long-term storage in air, the majority of the primary aliphatic amines were purchased as their acid salts (hydrochloride, tosylate, tartrate, mesylate or hydrobromide salts). The aliphatic primary amines purchased with naked $–NH_2$ groups were treated with methanesulfonic acid to form mesylate salts. All the primary amines in this library were stored as solutions in DMSO in microplates. Each well of the microplate contains the solution of a single amine compound, with the molar concentration of reactive $–NH_2$ groups at 100 mM.

Synthesis of the azide library in microplates. To a 96-well deepwell microplate, 96 amine solutions (100 mM in DMSO, 200 µl each, containing 20 µmol of each amine) were transferred. Aqueous $KHCO_3$ solution (3.0 M, 26.7 µl each well, containing 80 µmol $KHCO_3$) was added, followed by the FSO_2N_3 solution (200 mM in DMSO/MTBE 1:1, 100 µl each well, containing 20 µmol FSO_2N_3). Additional DMSO (73 µl) was added so that the total volume of the mixture in each well was approximately 400 µl. The microplate was then sealed and incubated in a microplate shaker at 800 r.p.m. and 30 °C for 1 h to yield azides. A total of 3840 azides were synthesized and stored at 4 °C. The library was found to be stable for at least 6 months.

Synthesis of triazole libraries with azide pools for the AS-MS screen. We first prepared four azide pools (each containing 960 azides) with each compound at 50 mM. Each azide pool (20 µl) was added to an empty tube, and supplemented with an alkyne backbone solution (20 µl), an acidic sodium ascorbate/Na_2HPO_4/citric acid buffer (20 µl), an aqueous $CuSO_4$/THPTA solution (20 µl,

0.2 μmol each) and DMSO (20 μl). The mixtures were swirled at 800 rpm and 40 °C for 18 hours to afford the corresponding triazole pools, each composed of 960 expected products. Ten resulting triazole libraries were stored at 4 °C. For each library, two triazole pools were randomly selected and mixed to generate a 1,920-mix pool for subsequent AS-MS screen. A detailed procedure is provided in Supplementary Note.

Synthesis of triazole library N3 for the functional screen. The 96 azide solutions (20 μl) in each microplate were transferred from azide library plates 1-40 to the corresponding empty 96-well microplate. A sodium ascorbate solution (20 μl) was then added to each well of the newly loaded plate. The microplate was sealed and swirled at 800 rpm and 30 °C for 15 min. A solution of alkyne **1-3** (20 μl) was added to each well, followed by an aqueous $CuSO_4$/THPTA solution (20 μl) and DMSO (20 μl). The microplate was sealed again and swirled at 800 rpm and 40 °C for 18 hours to afford the corresponding triazole product in each well. The resulting library N3 was used for the functional screening. A detailed procedure is provided in Supplementary Note.

Synthesis of focused triazole libraries for the functional screen. The 96 alkyne solutions (10 μl) in each microplate were transferred from alkyne library plates 1-5 to the corresponding empty 96-well microplate. An acidic sodium ascorbate/Na_2HPO_4/citric acid buffer (20 μl), an azide backbone solution (20 μl), an aqueous $CuSO_4$/THPTA solution (20 μl) and DMSO (30 μl) were added to each well of the newly loaded plate. The microplate was sealed and swirled at 800 rpm and 40 °C for 18 hours to afford the corresponding triazole product in each well. The resulting five focused libraries composed of 2400 expected products were used for the functional screening. A detailed procedure is provided in Supplementary Notes.

Affinity selection. 3~5 μg of DDM-solubilized purified GLP-1R TMD or HCA_2 (hydroxyl carboxylic acid receptor 2, the control protein) was incubated with Ni-Charged MagBeads (GenScript) overnight in the binding buffer [500 mM NaCl, 25 mM HEPEs (pH = 7.5), 0.02% DDM (w/v) and 0.004% (w/v) CHS]. The immobilized proteins were then incubated with individual 1,920-mix pools of triazole products in binding buffer for 1 h at 4 °C, with the protein target and compound at a final concentration of 500 nM and 100 nM, respectively. After removing the supernatant, the microbeads were washed six times with ice-cold 150 mM ammonium acetate. The ligands that bind to the receptor were extracted by adding 200 μl of methanol and incubation for 20 min. The supernatant was obtained, evaporated in a speed vacuum machine, and dissolved in 50% methanol before LC-HRMS analysis.

LC-HRMS analysis. Samples were analyzed on a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with an electrospray ion source and an ACQUITY UPLC system (Waters). Chromatographic separation was achieved via an ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 mm × 100 mm, Waters) with mobile phases A of water with 0.1% formic acid and B of acetonitrile with 0.1% formic acid. The LC gradient was as follows: 0-2 min, 5% B; 2-4 min, 5-20% B; 4-30 min, 20-50% B; 30-40 min, 50-90% B; 40-47 min, 90% B. The flow rate was 300 μl min^{-1} and the column was heated to 40 °C. Full MS scans were acquired in the range of 210-1200 m/z at a resolution of 140,000, an automatic gain control (AGC) target of 3×10^6 and a maximum injection time of 250 ms. The ESI source parameter were as follow: positive ionization mode, sheath gas flow rate, 35; aux gas flow rate, 10; spray voltage, 3.5 kV; capillary temperature, 320°C; aux gas heater temperature, 350°C.

AS-MS data processing. Compounds in the target (GLP-1R TMD) and control (HCA_2) samples were identified by extracting selected ion chromatograms (EICs) using TraceFinder 4.1 (Thermo Fisher Scientific) based on the restraints of accurate mass (<5 ppm deviation), isotope envelop (<40% deviation) and retention time (<0.2 min shift). The binding index (BI) is defined to be the ratio of the compound's EIC peak area detected in the target vs control. Statistical analysis was performed on the EIC peak areas from the target and control for each compound using a two-tailed *t*-test. Hits were selected based on a mean BI >2 and P <0.05 from three experimental replicates.

AS-MS-based ligand binding and competition assays. In the ligand binding assay, compounds **3-24**, **3-20** and **3-29** were mixed and incubated with purified GLP-1R or HCA_2 protein under the same condition of the AS-MS screen. In the competition assay, purified GLP-1R was incubated with a mixture of **3-24**, **3-20** and **3-29** (each at 500 nM) in the absence or presence of a competitor (PF-06372222 or **3-29** at 100 μM) at 4°C for 1 h. After incubation, the compounds were dissociated from the GLP-1R, reconstituted, and analyzed by LC-HRMS analysis as previously described. A short gradient was applied for compound separation: 0-2 min, 5% B; 2-7 min, 5-95% B; 7-8 min, 95% B, then re-equilibrate for 2 min at 5% B.

Cell culture and transfection. Chinese hamster ovary (CHO)-K1 cells stably expressing GLP-1R or GCGR were cultured in Ham's F12 medium (Cellfr) supplemented with 10% (v/v) fetal bovine serum and 800 μg ml^{-1} G418 (Invitrogen). In mutagenesis experiments, the mutations were introduced into Flag-tagged human GLP-1R in the pcDNA3.1/V5-His-TOPO vector (Invitrogen). The HEK293 cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum and then transfected transiently with GLP-1R wild-type, GLP-1R mutants or the empty vector using Lipo8000 transfection reagents (Beyotime, Shanghai, China). Cells were collected at 24 h post-transfection for different assays. The rat pancreatic INS-1E *β*-cells endogenously expressing GLP-1R were cultured in Roswell Park Memorial Institute (RPMI) 1640 (Gibco) containing 2 mM L-Glutamine, supplemented with 5% fetal bovine serum, 10 mM HEPEs, 100 IU ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, 50 μM β-mercaptoethanol and 1 mM sodium pyruvate. All the above cells were maintained at an incubator at 37 °C in 5% CO_2.

cAMP accumulation assay. Intracellular cAMP accumulation was measured using the cAMP-Gs dynamic kit (Cisbio, 62AM4PEC) based on the homogeneous time-resolved fluorescence (HTRF) technology. Briefly, CHO-K1 cells stably expressing GLP-1R or GCGR were lifted and seeded into a 384-shallow well plate at a density of 2,500 cells per well filled with Ham's F12 and 1% dialyzed FBS. For the mutant activity measurement, HEK293 cells transfected with GLP-1R wild-type or mutants were lifted and seeded into a 384-shallow well plate at a density of 3,000 cells per well filled with DMEM and 1% dialyzed FBS. INS-1E *β*-cells endogenously expressing GLP-1R were lifted and seeded into a 384-shallow well plate at a density of 6,000 cells per well filled with RPMI 1640 and 1% dialyzed FBS.

For functional screens of triazole libraries in microplates, we implicated two assay formats: (a) a single-dose cAMP accumulation assay using an EC_{50} of GLP-1(9-36) (1.12 μM) and each CuAAC reaction product at 50 μM; (b) a PAM titration assay using an EC_{20} of GLP-1(9-36) (0.6 μM) and varying doses of each triazole product (crude or purified). For pharmacological characterization of new PAMs, we performed a peptide agonist titration assay using varying concentrations of GLP-1(7-36), GLP-1(9-36) or glucagon in combination with a fix concentration of a purified triazole product.

For cAMP accumulation assays in CHO-K1 cells or HEK293 cells, PAMs and agonists were diluted at a 4× final concentration in an assay buffer containing 1× Hank's Balanced Salt Solution (HBSS) and 0.1% bovine serum albumin, and were incubated with cells for 20 min at room temperature. For cAMP accumulation assays in INS-1E cells, the compounds were diluted with assay buffer supplemented with 500 μM isobutylmethylxanthine (IBMX), and were incubated with cells for 30 min at room temperature. Time-resolved FRET signals were measured on an EnVision (PerkinElmer) at 665 nm and 620 nm. All the dose-response curve fits were analyzed with GraphPad Prism 7.0 using an equation of log(agonist) vs response (4-parameter logical model) and normalized to the percentage of GLP-1(7-36), glucagon or BETP stimulation.

BRET β-arrestin recruitment assay. HEK293 cells (3×10^6 cells/10 cm plate) were grown for 24 h before transiently transfected with plasmids encoding GLP-1R-RLuc8 and β-arrestin 2-GFP2 at a ratio of 1:4. Transfected cells were seeded into 96-well culture plates at a density of 50,000 cells per well and incubated for 24 h in DMEM containing 1% dialyzed FBS at 37 °C in 5% CO_2. Cells were rinsed once with 1× HBSS and then incubated with fresh HBSS for 20 min. After incubation with varying concentrations of GLP-1(9-36) or GLP-1(7-36) in combination with a fixed

concentration of **3-24** or **3-20** for 10 min, 4 μM coelenterazine 400a (Nanolight Technologies) was added. Bioluminescence resonance energy transfer (BRET) responses were collected immediately with 410 nm (RLuc8-coelenterazine 400a) and 510 nm (GFP2) emission filters using a LB940 Mithras plate reader (Berthold Technologies). The BRET signal was calculated by subtracting the ratio of the GFP2 emission over RLuc8 emission for the vehicle control from the same ratio for the ligand treated sample. Data were normalized to the percent GLP-1(7-36) stimulation. All the dose-response curve fits were analyzed with GraphPad Prism 7.0 using an equation of log(agonist) vs response (4-parameter logical model).

Insulin secretion assay. Islets were isolated from male C57BL/6 mice aged 8 to 12 weeks using the collagenase digestion procedure described elsewhere (2), and cultured overnight in RPMI1640 media with 10% fetal bovine serum, 100 IU ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin (Invitrogen). The next day, islets were incubated at 37°C for 30 minutes in Krebs-Ringer Bicarbonate HEPES (KRBH) buffer containing 2.8 mM glucose. Then, groups of three islets of a similar size were hand-selected and incubated at 37 °C for 90 minutes in 0.25 ml of KRBH buffer containing the indicated concentrations of glucose and ligands. Following 5 minutes of shaking and 3 minutes of centrifugation at 1,000 g, the supernatants were collected and stored at -20 °C until insulin levels were determined using an Enzyme-Linked Immuno Sorbent Assay Kit (Mercodia, Sweden). The data were calibrated against external standards and represented as nanograms per milliliter of insulin in the culture medium.

Molecular docking simulation. The cryo-EM structure of GLP-1R bound with GLP-1(7-37) and LSN3160440 (PDB code: 6VCB) was prepared using Protein Preparation Wizard in Maestro 2021-4. The grid box covered the entire LSN3160440 molecule was generated using Receptor Grid Generation module. Docking calculation was performed using Glide with XP precision and expanded sampling. MMGBSA was used to calculate every docking pose. Protein-ligand interactions were analyzed with Maestro Ligand Interaction Diagram Panel and Protein-Ligand Interaction Profiler (PLIP). Figures were made using Pymol and Chimera.

The crystal structure of human GLP-1R TMD bound with PF-06372222 (PDB code: 5VEW) was prepared using Protein Preparation Wizard. Considering the lipid environment of ligand binding pocket, the receptor was placed in a POPC membrane with a 10 × 10 × 10 Å boundary barrier aligned with placement in the OPM database. A model system was built using the OPLS3e protein force field and TIP3P water. Desmond simulations were then performed with a 100 ps minimization step followed by a 5 ns NPT restrained molecular dynamics run at 297 K. The final equilibrated model was used for similar docking calculation with Glide.

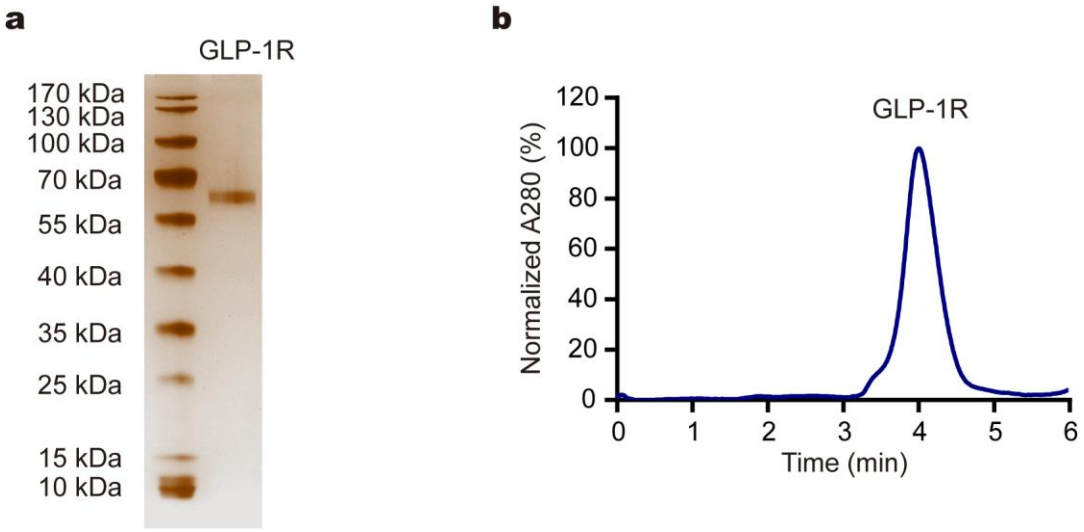


Fig. S1. Purification of the apo GLP-1R transmembrane domain. SDS-PAGE image (**a**) and size-exclusion chromatography profile (**b**) of the purified GLP-1R transmembrane domain.

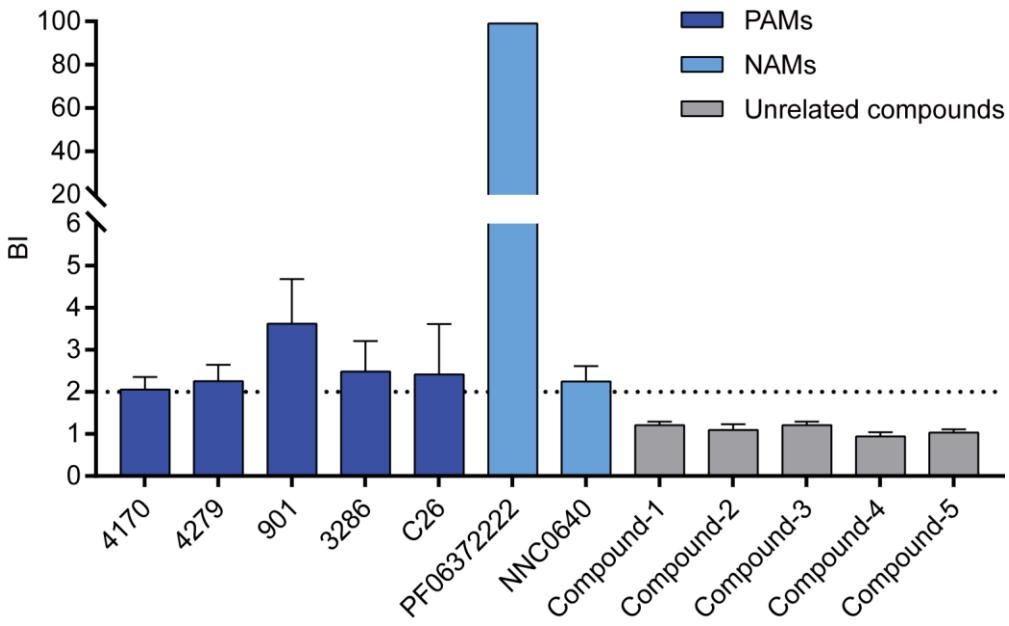


Fig. S2. AS-MS analysis of known allosteric modulators of the GLP-1R. AS-MS analysis of a simple mixture of known GLP-1R PAMs (dark blues bars), NAMs (light blue bars) and unrelated compounds (grey bars). The binding index (BI) is defined as the ratio of the compound's MS response detected in the target *vs* control. Data were obtained from three independent experiments. Error bars represent SEM.

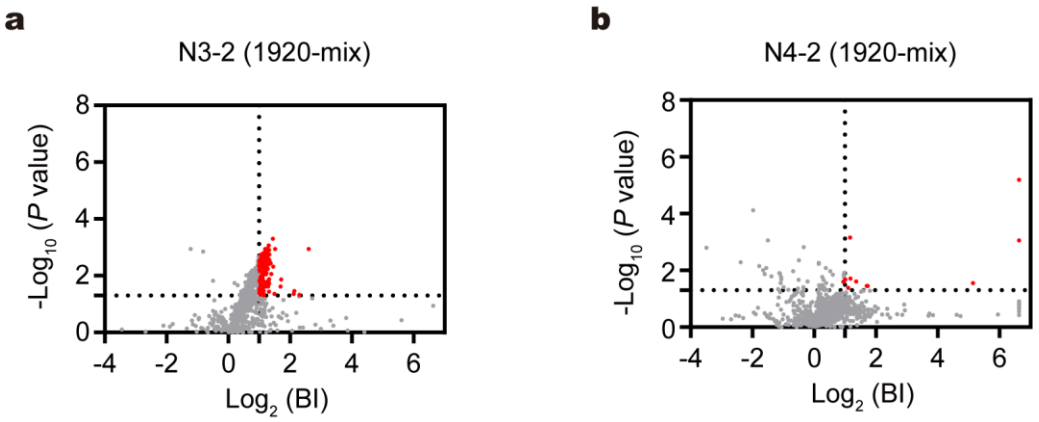


Fig. S3. AS-MS results for screening 1,920-mix pools from the other half of the libraries N3 (**a**) and N4 (**b**). Hits of individual screens are indicated by red dots. The binding index (BI) is defined as the ratio of the compound's MS response detected in the target *vs* control. BI data represent means of three independent experiments.

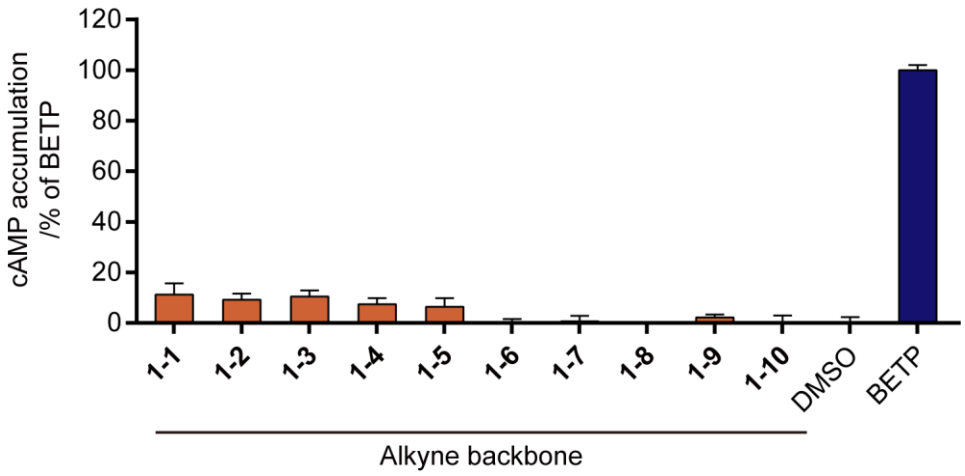


Fig. S4. Bioactivity of 10 alkyne backbones used in the triazole library synthesis. cAMP accumulation was measured in CHO-K1 cells expressing human GLP-1R treated by different alkyne backbones (50 μM) in the presence of EC_{20} of GLP-1(9-36) (0.6 μM). cAMP accumulation was normalized to the full response of BETP. Data represent means ± SEM of three independent experiments performed in triplicate.

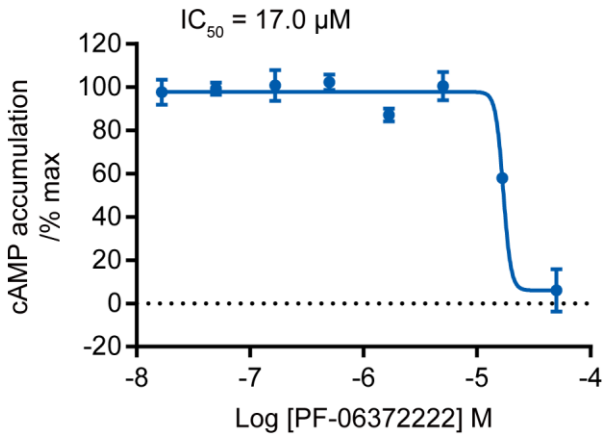


Fig. S5. Dose response curve of PF-06372222 measured in CHO-K1 cells expressing human GLP-1R in the presence of EC_{80} of GLP-1(9-36) (2 µM). Data represent means ± SEM of three independent experiments performed in triplicate.

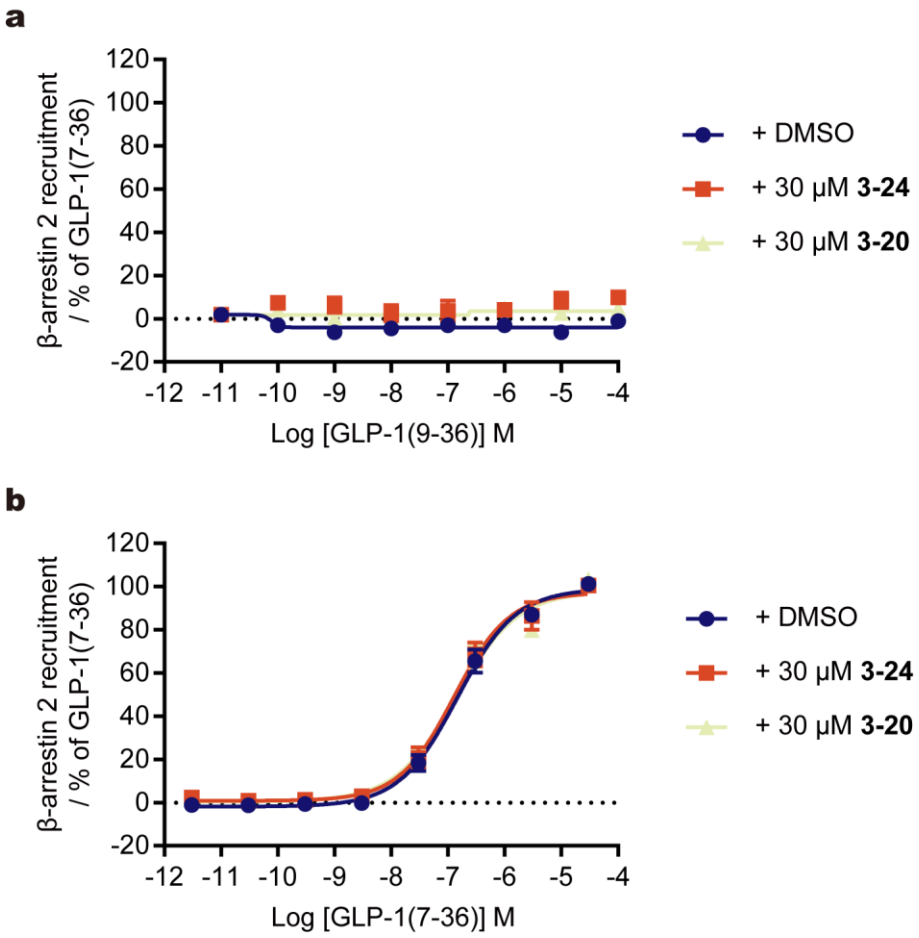


Fig. S6. β-arrestin 2 recruitment measured in HEK293 cells expressing human GLP-1R in the presence of 30 μM **3-24** or **3-20** in combination of GLP-1(9-36) (**a**) or GLP-1(7-36) (**b**) using a BRET assay. Data represent means ± SEM of three independent experiments performed in triplicate.

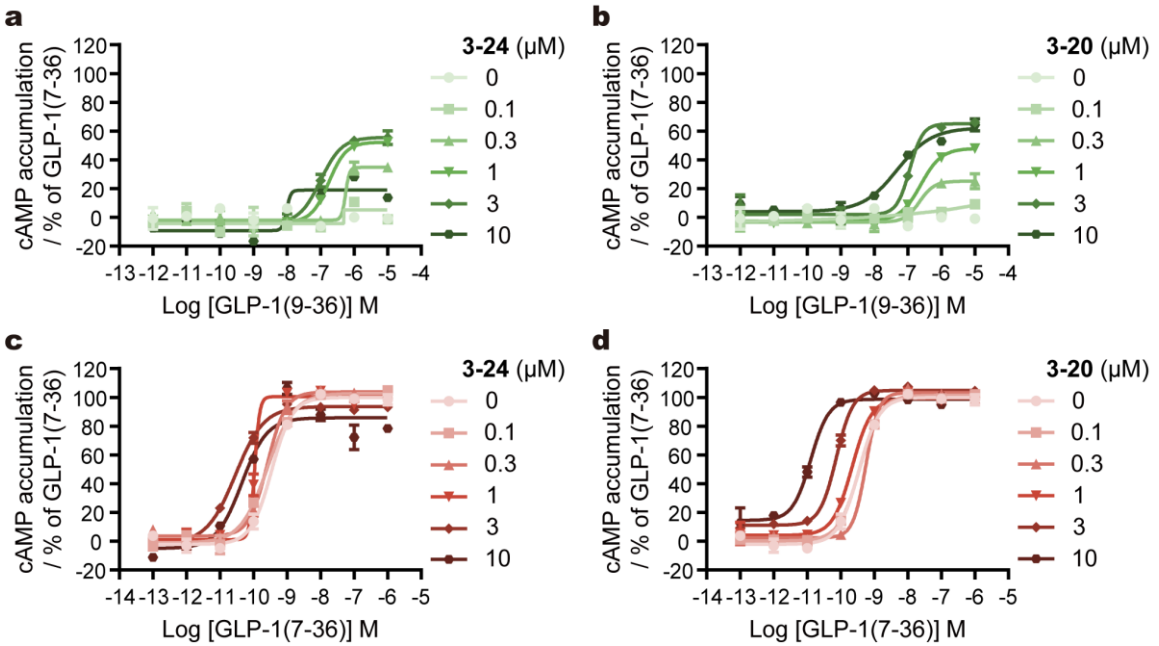


Fig. S7. The pharmacological profiles of new PAMs with different peptide agonists in INS-1E *β*-cells. a-b, Potentiation of the cAMP accumulation in INS-1E *β*-cells produced by GLP-1(9-36) at the GLP-1R in combination with different fixed concentrations of **3-24** (**a**) or **3-20** (**b**). **c-d**, Potentiation of the cAMP accumulation in INS-1E *β*-cells produced by GLP-1(7-36) at the GLP-1R in combination with fixed concentrations of **3-24** (**c**) or **3-20** (**d**). Data represent means ± SEM of three independent experiments performed in triplicate.

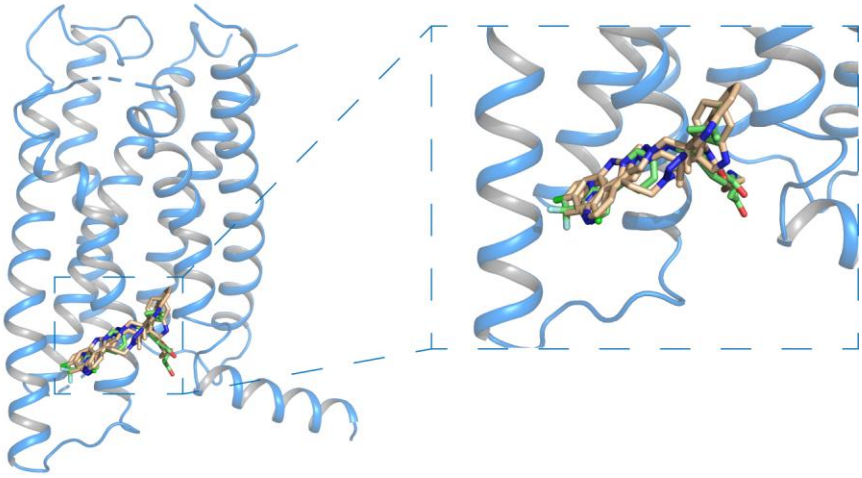


Fig. S8. Docking models of **3-24**, **3-20** and **3-29** (wheat) in the PF-06372222 pocket based on the crystal structure of PF-06372222 (lime) bound to GLP-1R transmembrane domain (marine) (PDB code: 5VEW).

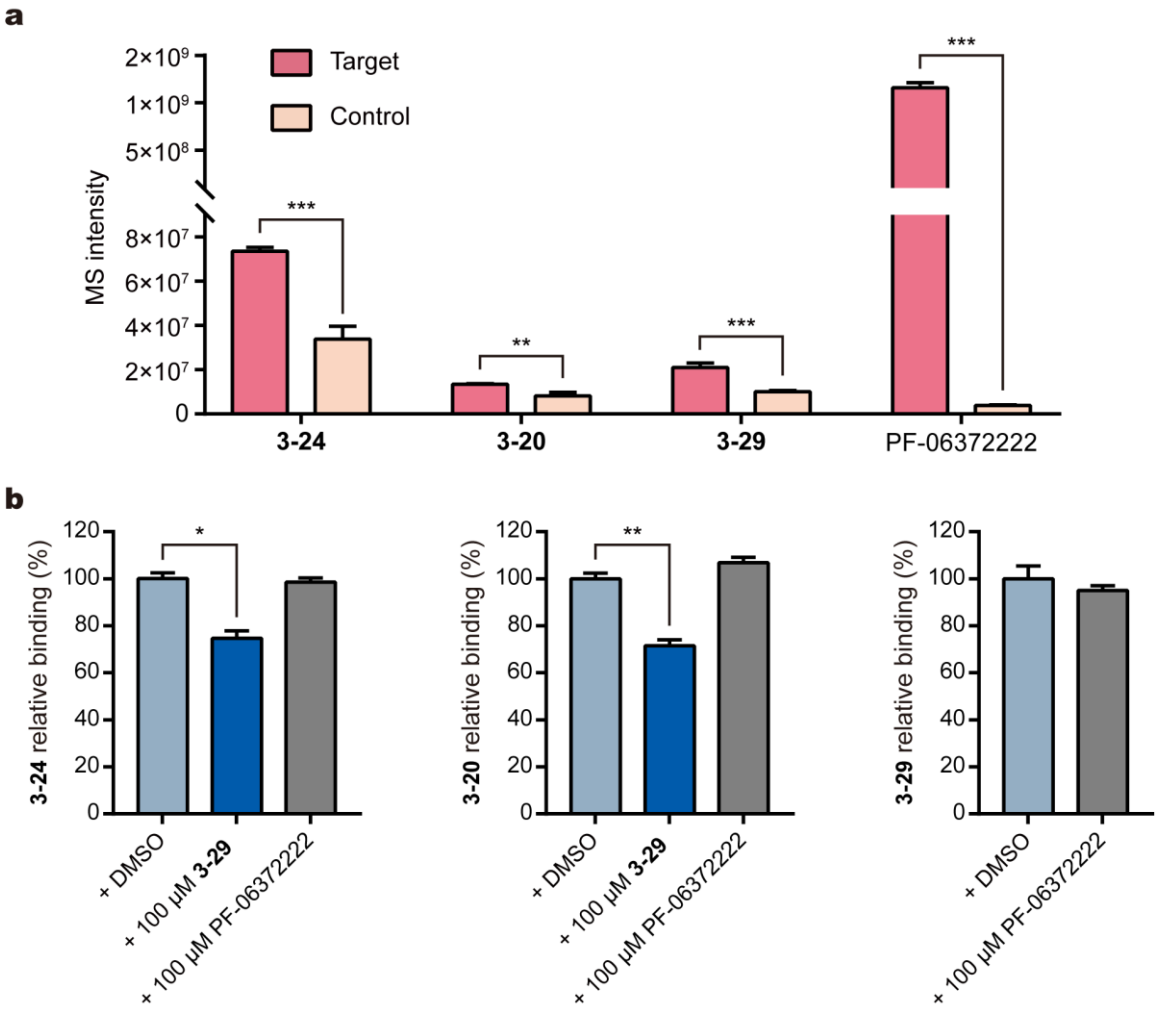
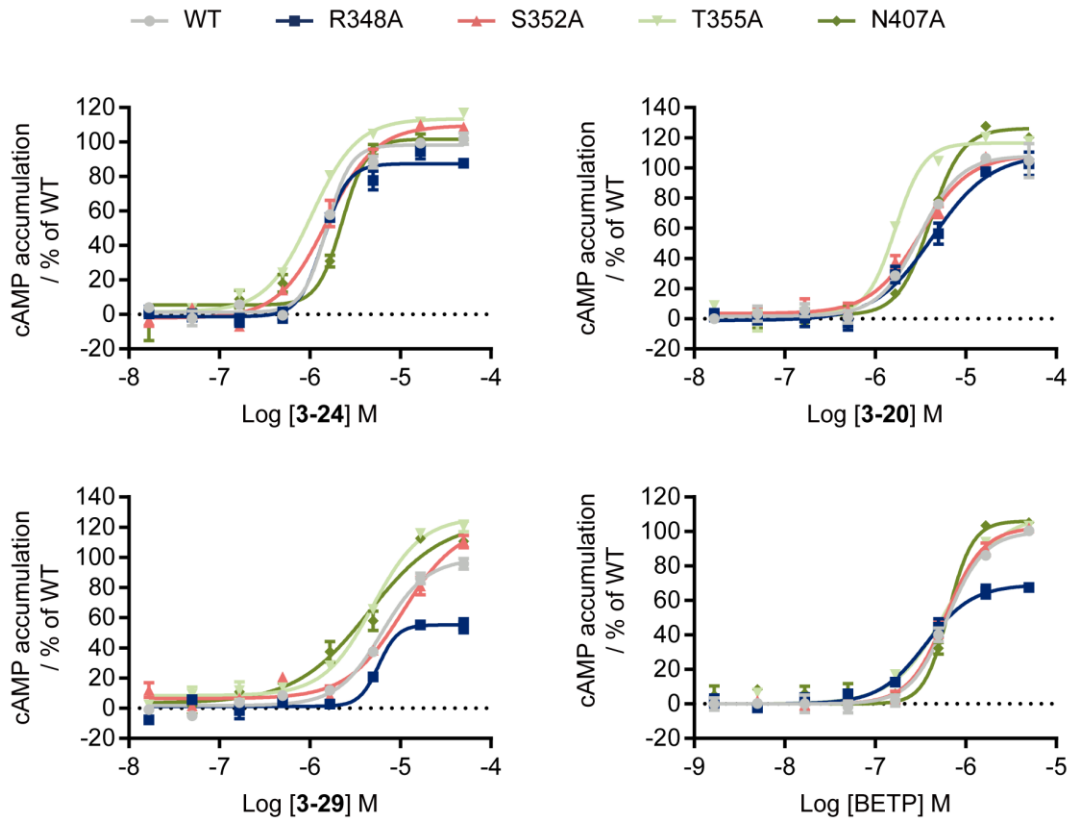


Fig. S9. Binding analysis of 3-24, 3-20 and 3-29 with the AS-MS assay. a, Binding of **3-24**, **3-20**, **3-29** and PF-06372222 to GLP-1R TMD in the AS-MS assay. The MS intensity of each compound was significantly higher in the target sample (GLP-1R TMD) than control, indicating specific association of each compound with the target. **b**, Binding of **3-24** or **3-20** to GLP-1R TMD was significantly competed off in the presence of 100 μM **3-29** while not affected in the presence of 100 μM PF-06372222 relative to DMSO. Relative binding% was derived from the MS intensity of each compound associated with GLP-1R in the presence of **3-29** normalized to that in DMSO from the AS-MS assay. Data represent means ± SEM of three independent experiments. Statistical analyses were performed using a two-tailed *t*-test. **P* <0.05; ***P* <0.01; ****P* <0.001.

a



b

	WT	R348A	S352A	T355A	N407A
Expression (%)	100.0 ± 2.7	107.8 ± 1.7	131.8 ± 5.6	84.0 ± 11.2	110.1 ± 2.3

Fig. S10. a, Potentiation of cAMP accumulation produced by various concentrations of **3-24**, **3-20**, **3-29** and BETP in combination with GLP-1(9-36) (2 μM) using wild-type (WT) or different mutant GLP-1R-transfected cells. **b,** Cell surface expression levels of GLP-1R mutants relative to WT measured by flow cytometry. Data represent means ± SEM of three independent experiments performed in triplicate.

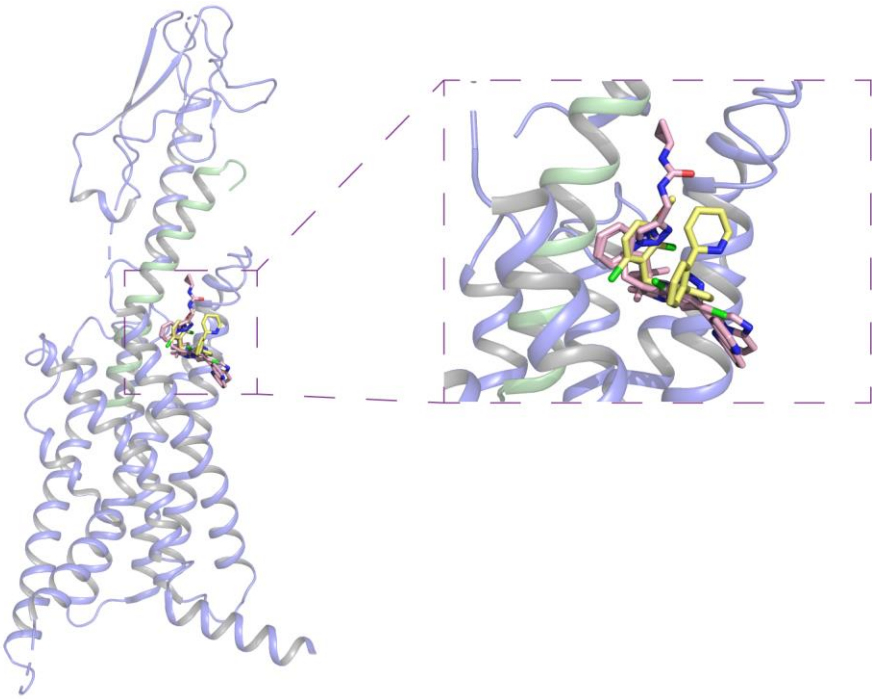
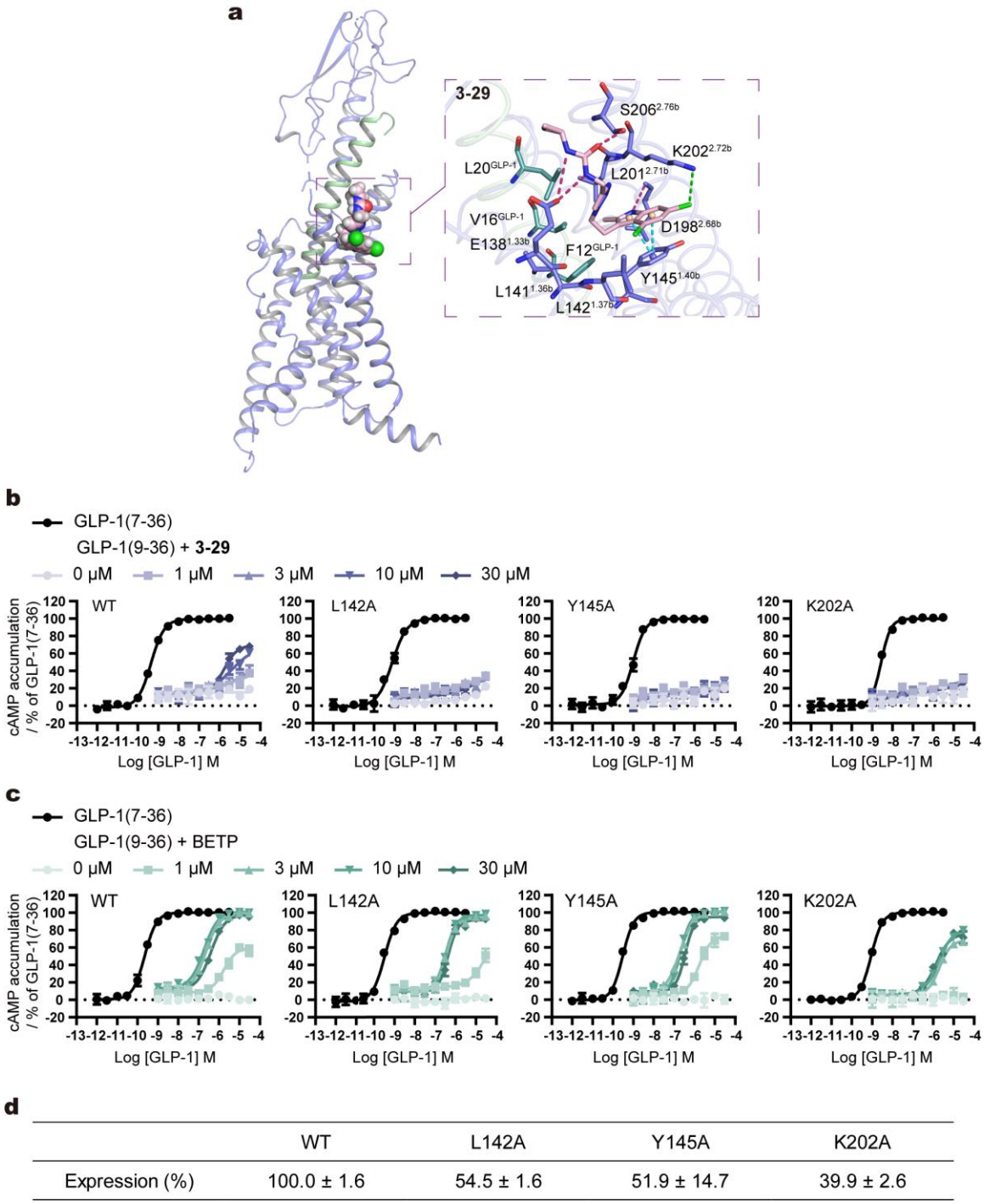


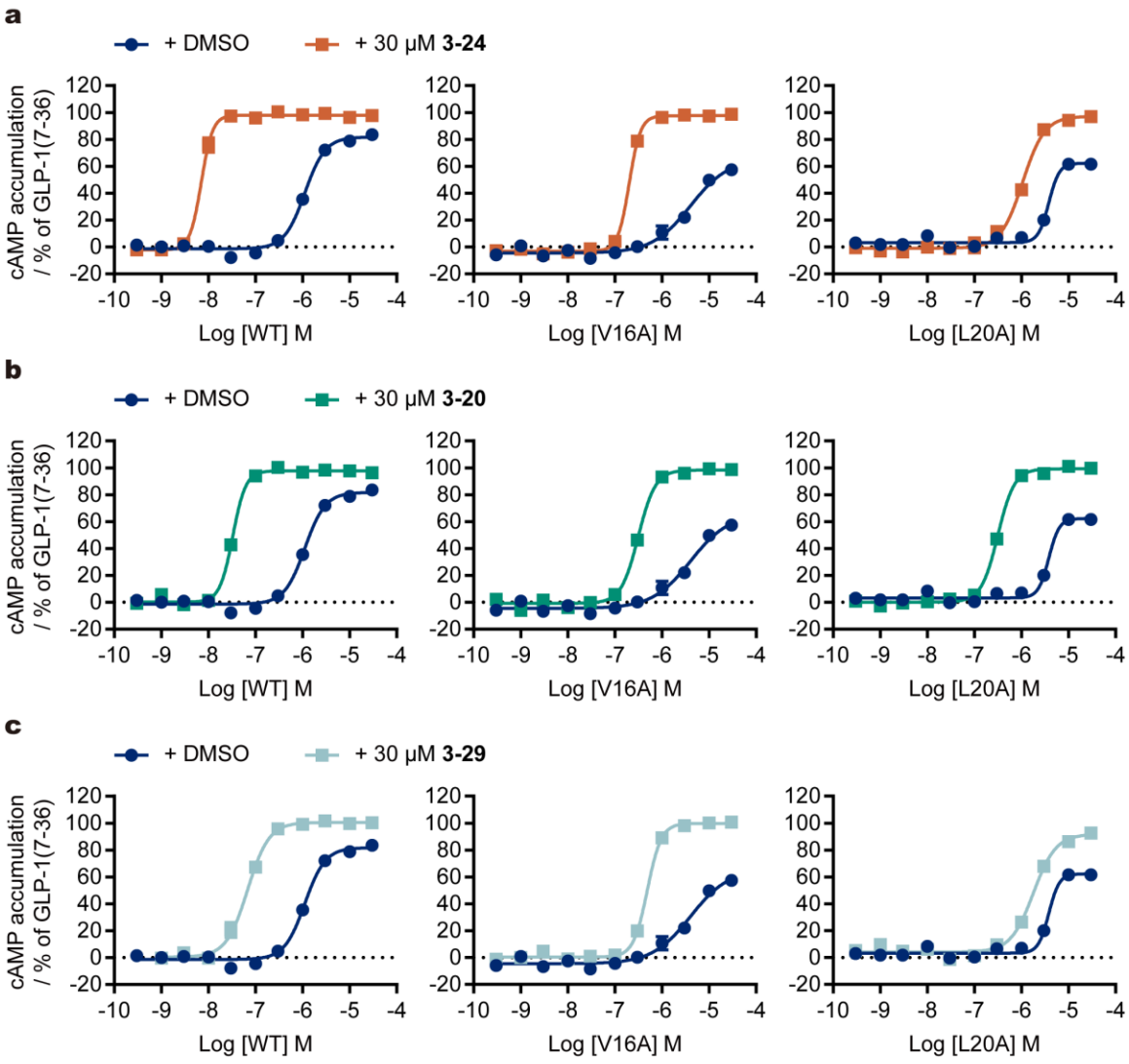
Fig. S11. Docking models of **3-24**, **3-20** and **3-29** (lightpink) in the LSN3160440 pocket based on the cryo-EM structure of GLP-1R (slate) in complex with LSN3160440 (paleyellow), and GLP-1(7-37) (palegreen) (PDB code: 6VCB).



d

	WT	L142A	Y145A	K202A
Expression (%)	100.0 ± 1.6	54.5 ± 1.6	51.9 ± 14.7	39.9 ± 2.6

Fig. S12. Characterization of the binding pocket of 3-29. a, Molecular docking model showing the binding mode of **3-29** in the extracellular allosteric pocket of the GLP-1R. Hydrogen bonding, halogen bonding and π-π interactions are indicated by dashed lines. **b-c**, Potentiation of the cAMP accumulation produced by GLP-1(9-36) in combination with different fixed concentrations of **3-29** (**b**) or BETP (**c**) using wild-type (WT) or different mutant GLP-1R-transfected cells. **d,** Cell surface expression levels of GLP-1R mutants relative to WT measured by flow cytometry. Data represent means ± SEM of three independent experiments performed in triplicate.



d

Functional potency (pEC_{50})

	WT	V16A	L20A
+ DMSO	5.96 ± 0.01	5.42 ± 0.00	5.41 ± 0.01
+ 30 μM 3-24	7.96 ± 0.17	6.69 ± 0.12	6.09 ± 0.09
+ 30 μM 3-20	7.50 ± 0.05	6.50 ± 0.01	6.30 ± 0.20
+ 30 μM 3-29	7.14 ± 0.06	6.33 ± 0.01	5.72 ± 0.06

Fig. S13. Effects of GLP-1 peptide mutants on the potency of new PAMs. a-c, Potentiation of the cAMP accumulation produced by wild-type (WT) or different mutant GLP-1(9-36) in combination with a fixed concentration of **3-24** (a), **3-20** (b) and **3-29** (c). **d**, Summary of the pEC_{50} values of specific PAMs in their potentiation capability for wild-type or mutant GLP-1(9-36), as determined from the cAMP accumulation assays. Data represent means ± SEM of three independent experiments performed in triplicate.

Supplementary Note 1. General experimental

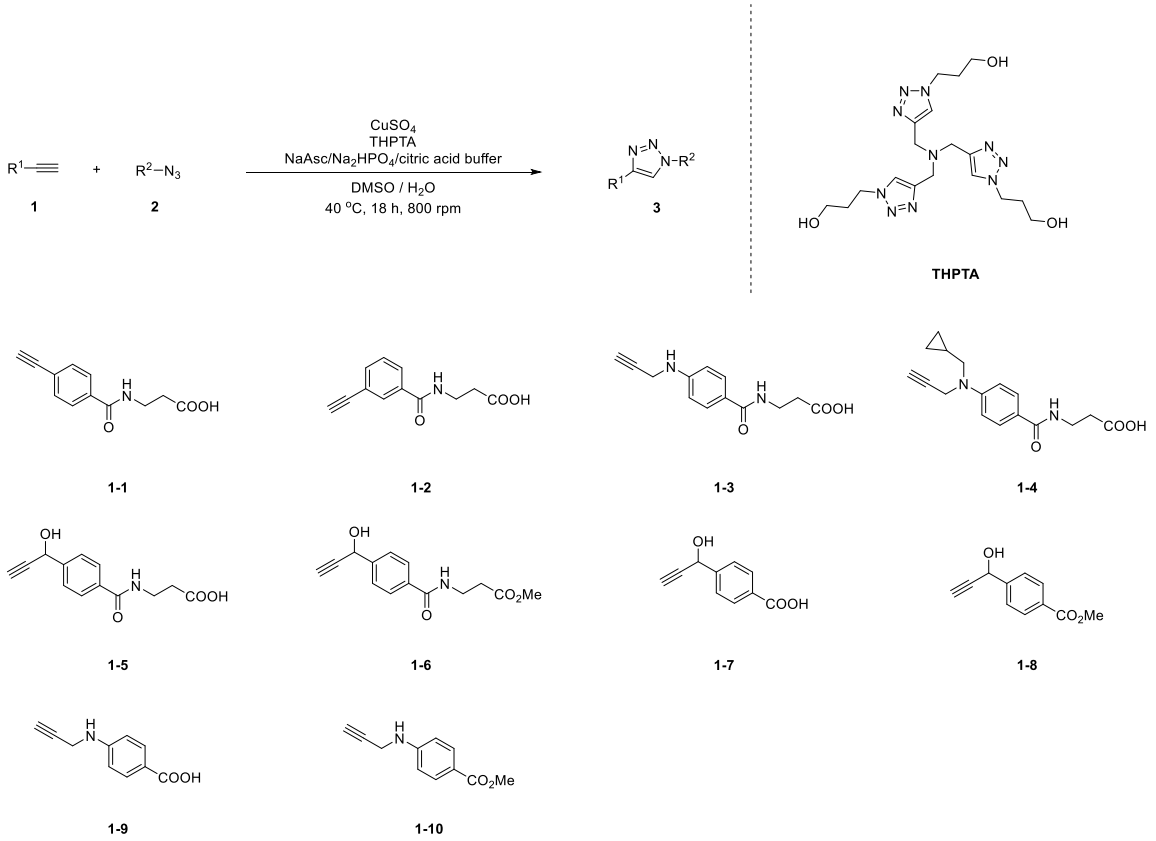
^{1}H, ^{19}F NMR and ^{13}C NMR spectra were recorded on an Agilent-400 instrument or Bruker AM-500 instrument. Proton magnetic resonance (^{1}H NMR) spectra were recorded at 400 MHz or 500 MHz. Carbon magnetic resonance (^{13}C NMR) spectra were recorded at 101 MHz or 126 MHz. Fluorine magnetic resonance (^{19}F NMR) spectra were recorded at 376 MHz. The ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded at 297K in $CDCl_3$, CD_3OD or $(CD_3)_2SO$, and the chemical shifts (δ) are presented in parts per million (ppm). Data for ^{1}H NMR were reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, sept = septet, m = multiplet, br = broad), coupling constant (J) in Hertz (Hz), and integration. Data for ^{13}C and ^{19}F NMR spectra were reported in a similar pattern if applicable, with the exception that singlet peaks in the ^{13}C NMR spectra were reported only with their chemical shifts. For ^{1}H NMR, the chemical shifts were calibrated in reference to the tetramethylsilane (0 ppm), or the residual undeuterated solvents ($CDCl_3$, 7.26 ppm; CD_3OD, 3.34 ppm; $(CD_3)_2SO$, 2.50 ppm). Data of the ^{13}C NMR spectra were calibrated in reference to the deuterated solvents ($CDCl_3$, 77.16 ppm; CD_3OD, 49.86 ppm; $(CD_3)_2SO$, 39.52 ppm). Data for ^{19}F NMR spectra were calibrated in referenced to $CFCl_3$ (0 ppm).

High resolution mass spectrometry (HRMS) was recorded on a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with an electrospray ion source operating in the positive ion mode.

Analytical LC-MS data were recorded on a Waters ACQUITY UPLC H-Class system with a Waters ACQUITY QDa system operating in the electrospray ionization (ESI) mode eluting with H-O (with 0.1% trifluoroacetic acid) and CH_3CN. [Method: 7000 psi, flow rate = 0.6 mL/min. Eluent: t = 0, 95% H_2O; t = 0.10, 95% H_2O; t = 1.20, 5% H_2O; t = 2.00, 5% H_2O; t = 2.50, 95% H_2O. Total acquisition time = 2.5 min.]. The figures of LC-MS data in this supporting information show the plot of total UV S3 absorbance (wavelength range 210-400 nm) along the vertical axis with respect to time along the horizontal axis, and the peak annotations include the retention time (in min) and the wavelength (in nm) at maximal absorbance.

Thin layer chromatography (TLC) was performed using TLC silica gel plates HSG F254 (Jiangyou) and visualized using UV light, iodine, ninhydrin or potassium permanganate. Silica gel column chromatography was carried out using 300-400 mesh silica gel (Jiangyou). Reagents were purchased from Aladdin, TCI, Macklin, Energy, Alfa Aesar, Adamas, Tianlian, Shuya, and Bide. Solvents were purchased from Macklin, Adamas, Tianlian. All purchased reagents and solvents were used as received, without further purification or special handling practice.

Supplementary Note 2. Triazole library preparation



Reagents:

- Azide solutions from the azide libray (Plate 1-40, prepared as previously described (3)): individual azide solutions at a concentration of ca. 50 mM.

- Sodium ascorbate/Na_2HPO_4/citric acid buffer: an aqueous acidic buffer (pH ca. 5), was made with sodium ascorbate (2.48 g, 12.5 mmol), Na_2HPO_4 (7.0 g, 49.3 mmol) citric acid (4.87 g, 25.4 mmol) and water (to make a total volume of 100 ml).

- Solutions of alkyne (compound **1-1** to **1-10** shown in the scheme above): individual alkyne solutions at a 50 mM in DMSO.

- Aqueous $CuSO_4$/THPTA solution: 10 mM concentration for both $CuSO_4$ and THPTA; made by dissolving $CuSO_4$(16 mg, 100 μmol) and THPTA (43.5 mg, 100 μmol) in water (10 ml).

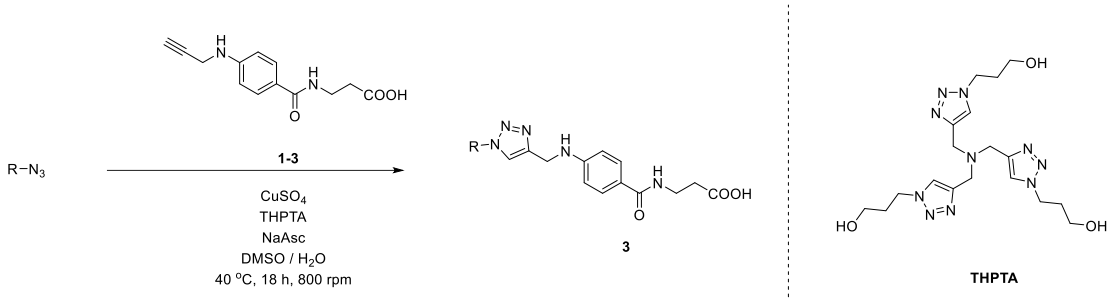
- DMSO as solvents.

Procedure:

The azide solutions (10 μl from each well, containing 1 μmol of an azide) from the azide library (Plate 1-40, prepared as previously described (3), 10 plates as a group) were transferred to the corresponding plastic bottles and mixed homogeneously to afford azide mixture pools (4 bottles of solution, 960 azides each, overall azide solutions at a concentration of ca. 50 mM, individual azide concentration at approximately 50 μM).

To an empty plastic tube was added the alkyne solution (20 µl, containing 1 µmol alkyne), the sodium ascorbate/Na_2HPO_4/citric acid buffer (20 µl), azide mixture (20 µl, containing 1 µmol azide overall), the aqueous $CuSO_4$/THPTA solution (20 µl, 0.2 µmol each) and DMSO (20 µl) sequentially. The mixture was swirled at 800 rpm and 40 °C for 18 hours to afford the corresponding triazole mixture library. The mixture was stored at 4 °C. Each well contained 960 triazoles in theory, further analysis was measured by AS-MS.

For the alkyne **1-1** to **1-4**, the corresponding triazole mixture library was constructed with aqueous sodium ascorbate solution (200 mM, 20 µl) instead of the sodium ascorbate/Na_2HPO_4/citric acid buffer (20 µl), the other components remain unchanged.

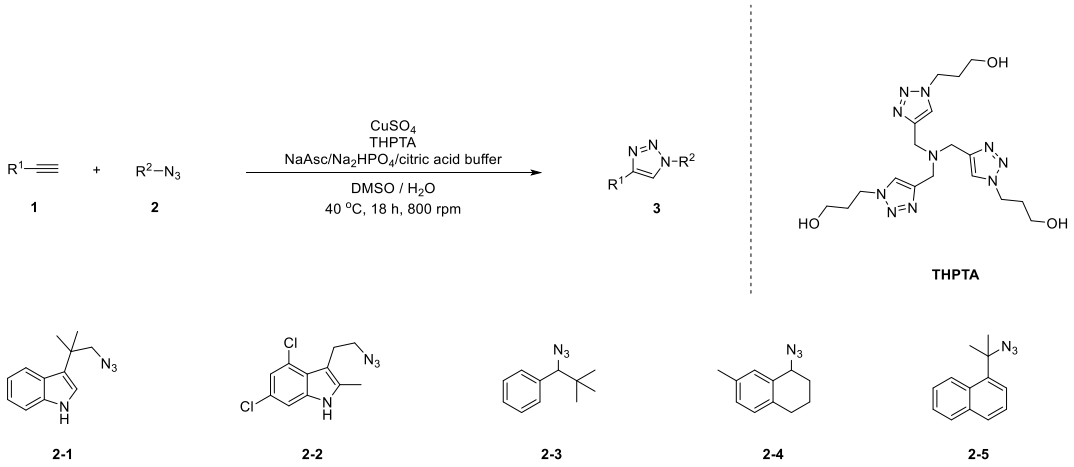


Reagents:

- Azide solutions from the azide libray (Plate 1-48, prepared as previously described (3)): individual azide solutions at a concentration of ca. 50 mM.
- Aqueous sodium ascorbate solution: concentration at 200 mM in water.
- A solution of 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (compound **1-3** shown in the scheme above): concentration at 50 mM in DMSO.
- Aqueous $CuSO_4$/THPTA solution: 10 mM concentration for both $CuSO_4$ and THPTA; made by dissolving $CuSO_4$(16 mg, 100 µmol) and THPTA (43.5 mg, 100 µmol) in water (10 ml).
- DMSO as solvents.

Procedure:

From azide library Plate 1-48, the 96 azide solutions (20 µl from each well, containing 1 µmol of an azide) in each microplate were transferred to the corresponding empty 96-well microplate. To each well of this newly loaded plate was added this sodium ascorbate solution (20 µl). The microplate was sealed and swirled at 800 rpm and 30 °C for 15 min. The solution of alkyne **1-3** (20 µl, containing 1 µmol **1-3**) was added to each well, followed by an aqueous $CuSO_4$/THPTA solution (20 µl, 0.2 µmol each) and DMSO (20 µl). The microplate was sealed and swirled at 800 rpm and 40 °C for 18 hours to afford the corresponding triazole product in each well. Each well contained a single triazole at a concentration of approximately 10 mM in DMSO/H_2O (3:2).



Reagents:

- Azide solutions (compound **2-1** to **2-5** shown in the scheme above): concentration at 50 mM in DMSO.
- Sodium ascorbate/Na_2HPO_4/citric acid buffer: an aqueous acidic buffer (pH ca. 5), was made with sodium ascorbate (2.48 g, 12.5 mmol), Na_2HPO_4 (7.0 g, 49.3 mmol) citric acid (4.87 g, 25.4 mmol) and water (to make a total volume of 100 ml).
- Alkyne solutions from alkyne library (Plate 1-5, prepared from commercially available alkynes): individual concentration at 100 mM in DMSO.
- Aqueous $CuSO_4$/THPTA solution: 10 mM concentration for both $CuSO_4$ and THPTA; made by dissolving $CuSO_4$(16 mg, 100 μmol) and THPTA (43.5 mg, 100 μmol) in water (10 ml).
- DMSO as solvents.

Procedure:

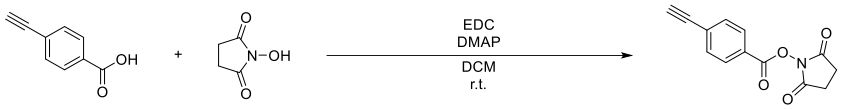
From alkyne library plate 1-5, the 96 alkyne solutions (10 μl from each well, containing 1 μmol of an zlkyne) in each microplate were transferred to the corresponding empty 96-well microplate. To each well of this newly loaded plate was added the acidic sodium ascorbate/Na_2HPO_4/citric acid buffer (20 μl), a solution of azide (20 μl, containing 1 μmol azide), an aqueous CuSO4/THPTA solution (20 μl, 0.2 μmol each) and DMSO (30 μl). The microplate was sealed and swirled at 800 rpm and 40 °C for 18 hours to afford the corresponding triazole product in each well. Each well contained a single triazole at a concentration of approximately 10 mM in DMSO/H_2O (3:2).

Supplementary Note 3. Compounds synthesis

Section 1. Synthesis of alkynes.

Synthesis of alkyne 1-1:

*3-(4-ethynylbenzamido)propanoic acid (**1-1**).*

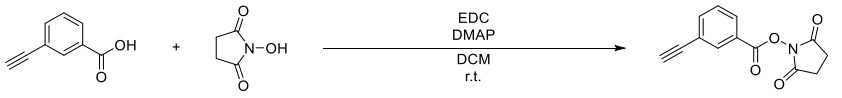


The first step: To a solution of 4-ethynylbenzoic acid (1 mmol, 1 eq.) and NHS (1.5mmol, 1.5 eq.) in anhydrous DCM (5 mL) were added EDC (1.5 mmol, 1.5 eq.) and DMAP (2 mmol, 2.00 eq.). The mixture was stirred for 16 h at room temperature. The organic layer was successively washed twice with an aqueous solution of 1 M HCl and brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to form a crude product of 2,5-dioxopyrrolidin-1-yl 4-ethynylbenzoate. The crude product was used directly for the second step without further purification.

The second step: Crude product of 2,5-dioxopyrrolidin-1-yl 4-ethynylbenzoate (1 mmol, see the first step) was dissolved in 5 mL of DMF and degassed with N_2 for 5 min. 3-Aminopropanoic acid (2 mmol, 2 eq.) and triethylamine (5 mmol, 5 eq.) were added. The reaction was stirred at room temperature for 18 h at room temperature, monitored by LC-MS. After completion, EtOAc was added and the mixture was washed sequentially with aqueous LiCl solution, water and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel chromatography (eluting with DCM and MeOH, ratio from 100 : 0 to 95 : 5) to afford the alkyne product **1-1**. The physic properties of **1-1** are as follows: pale yellow solid (167 mg, 77% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 12.33 (s, 1H), 8.62 (t, J = 5.5 Hz, 1H), 7.83 (d, J = 8.0 Hz, 2H), 7.56 (d, J = 8.0 Hz, 2H), 4.36 (s, 1H), 3.46 (q, J = 6.6 Hz, 2H), 2.55 – 2.50 (m, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.9, 165.4, 134.4, 131.6, 127.5, 124.4, 99.5, 82.9, 35.7, 33.7. HRMS(ESI, m/z): calcd for $C_{12}H_{12}NO_3$: 218.0812 $[M+H]^+$, Found: 218.0811.

Synthesis of alkyne 1-2:

*3-(3-ethynylbenzamido)propanoic acid (**1-2**).*

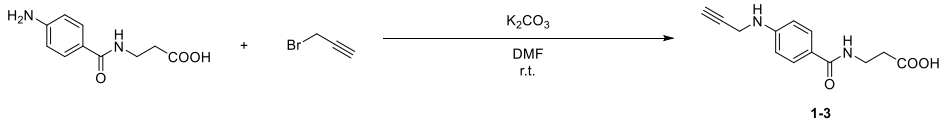


The first step: Similar to the synthesis of alkyne 1-1, to a solution of 3-ethynylbenzoic acid (1 mmol, 1 eq.) and NHS (1.5mmol, 1.5 eq.) in anhydrous DCM (5 mL) at 0 °C were added EDC (1.5 mmol, 1.5 eq.) and DMAP (2 mmol, 2.00 eq.). The mixture was stirred for 16 h at room temperature. The organic layer was successively washed twice with an aqueous solution of 1 M HCl and brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to form a crude product of 2,5-dioxopyrrolidin-1-yl 3-ethynylbenzoate. The crude product was used directly for the second step without further purification.

The second step: Crude product of 2,5-dioxopyrrolidin-1-yl 3-ethynylbenzoate (1 mmol, see the first step) was dissolved in 5 mL of DMF and degassed with N_2 for 5 min. 3-Aminopropanoic acid (2 mmol, 2 eq.) and triethylamine (5 mmol, 5 eq.) were added. The reaction was stirred at room temperature for 18 h at room temperature, monitored by LC-MS. After completion, EtOAc was added and the mixture was washed sequentially with aqueous LiCl solution, water and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel chromatography (eluting with DCM and MeOH, ratio from 100 : 0 to 95 : 5) to afford the alkyne product **1-2**. The physic properties of **1-2** are as follows: pale yellow solid (182 mg, 84% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 12.40 (s, 1H), 8.64 (t, *J* = 5.5 Hz, 1H), 7.93 (s, 1H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.48 (t, *J* = 7.7 Hz, 1H), 4.27 (s, 1H), 3.45 (q, *J* = 6.6 Hz, 2H), 2.51 (t, *J* = 7.1 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.9, 165.3, 134.8, 134.2, 130.2, 128.9, 127.8, 121.8, 82.9, 81.4, 35.7, 33.6. HRMS(ESI, m/z): calcd for $C_{12}H_{12}NO_3$: 218.0812 $[M+H]^+$, Found: 218.0811.

Synthesis of alkyne 1-3:

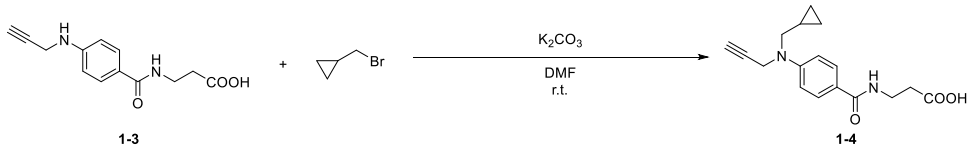
*3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (**1-3**).*



To a 100 ml glass round-bottom flask was added sequentially the 3-(4-aminobenzamido)propanoic acid (10.0 mmol), 3-bromoprop-1-yne (10 mmol, 1 eq.) and K_2CO_3 (12 mmol, 1.2 eq.). The reaction mixture was stirred for 12 h at room temperature, monitored by LC-MS. After completion, EtOAc was added and the aqueous phase was acidified by 1 M HCl, the organic phase was washed sequentially with aqueous LiCl solution, water and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel chromatography (eluting with DCM and MeOH, ratio from 100 : 0 to 95 : 5) to afford the alkyne product **1-3**. The physic properties of **1-3** are as follows: white solid (1.97 g, 80% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.10 (t, *J* = 5.5 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 2H), 6.56 (d, *J* = 8.2 Hz, 2H), 4.69 (d, *J* = 2.4 Hz, 2H), 3.54 (d, *J* = 2.5 Hz, 1H), 3.43 (q, *J* = 6.6 Hz, 2H), 2.59 (t, *J* = 7.0 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 170.8, 166.3, 150.9, 128.7, 121.5, 113.0, 78.5, 77.7, 51.7, 35.2, 33.7. HRMS(ESI, m/z): calcd for $C_{13}H_{15}N_2O_3$: 247.1077 $[M+H]^+$, Found: 247.1076.

Synthesis of alkyne 1-4:

*3-(4-((cyclopropylmethyl)(prop-2-yn-1-yl)amino)benzamido)propanoic acid (**1-4**).*

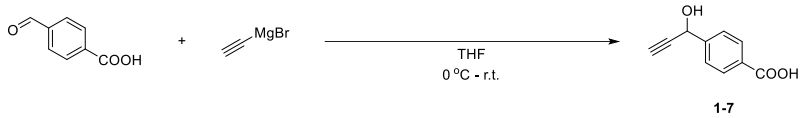


To a 25 ml glass round-bottom flask was added sequentially the 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1.0 mmol), (bromomethyl)cyclopropane (1.2 mmol, 1.2 eq.) and K_2CO_3 (1.2 mmol, 1.2 eq.). The reaction mixture was stirred for 12 h at room temperature, monitored by LC-MS. After completion, EtOAc was added and the aqueous phase was acidified by 1 M HCl, the organic phase was washed sequentially with aqueous LiCl solution, water and brine,

dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel chromatography (eluting with DCM and MeOH, ratio from 100 : 0 to 95 : 5) to afford the alkyne product **1-4**. The physic properties of **1-4** are as follows: white solid (237 mg, 79% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.15 (t, *J* = 5.5 Hz, 1H), 7.63 (d, *J* = 8.3 Hz, 2H), 6.63 (d, *J* = 8.3 Hz, 2H), 6.51 (t, *J* = 6.0 Hz, 1H), 3.91 (dd, *J* = 6.1, 2.4 Hz, 2H), 3.86 (d, *J* = 7.3 Hz, 2H), 3.45 (q, *J* = 6.6 Hz, 2H), 3.09 (s, 1H), 2.56 (t, *J* = 7.1 Hz, 2H), 1.13 – 1.01 (m, 1H), 0.52 – 0.43 (m, 2H), 0.25 (t, *J* = 4.6 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.5, 166.2, 150.2, 128.5, 122.1, 111.5, 81.7, 73.2, 68.5, 35.4, 34.1, 31.8, 9.7, 3.1. HRMS(ESI, m/z): calcd for $C_{17}H_{21}N_2O_3$: 301.1547 $[M+H]^+$, Found: 301.1545.

Synthesis of alkyne 1-7:

*4-(1-hydroxyprop-2-yn-1-yl)benzoic acid (**1-7**).*

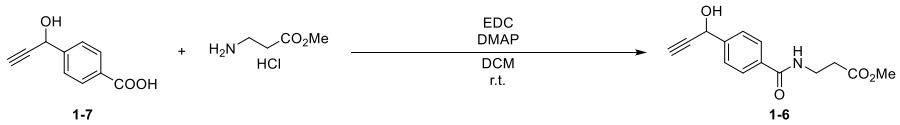


1-7

A solution of 4-carboxybenzaldehyde (3 mmol, 1 eq.) and ethynlmagnesium bromide solution (21 mL, 10.5 mmol) in 20 mL of anhydrous THF was stirred at 0 °C for 12 h. After completion, H_2O was carefully added to the reaction mixture to quench the reaction. After evaporation of THF, the resulting solution was acidified with 1 M HCl. The reaction mixture was extracted with EtOAc and dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (eluting with DCM and MeOH, ratio from 100 : 0 to 95 : 5) to afford the alkyne product **1-7**. The physic properties of **1-7** are as follows: pale yellow solid (438 mg, 83% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 12.95 (s, 1H), 7.94 (d, *J* = 8.1 Hz, 2H), 7.57 (d, *J* = 8.1 Hz, 2H), 6.22 (d, *J* = 6.0 Hz, 1H), 5.47 – 5.41 (m, 1H), 3.54 (d, *J* = 2.2 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 167.1, 146.6, 130.0, 129.4, 126.4, 85.0, 76.3, 62.0, 38.9. HRMS(ESI, m/z): calcd for $C_{10}H_9O_3$: 177.0546 $[M+H]^+$, Found: 177.0547.

Synthesis of alkyne 1-6:

*methyl 3-(4-(1-hydroxyprop-2-yn-1-yl)benzamido)propanoate (**1-6**).*

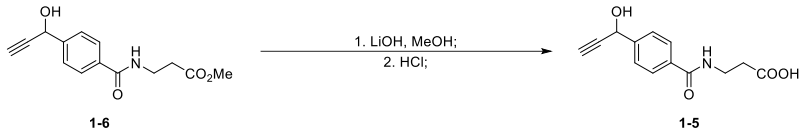


1-7 **1-6**

Similar to the synthesis of alkyne 1-1, to a solution of 4-(1-hydroxyprop-2-yn-1-yl)benzoic acid (**1-7**) (1 mmol, 1 eq.) and methyl 3-aminopropanoate hydrochloride (1.5mmol, 1.5 eq.) in anhydrous DCM (5 mL) at 0 °C were added EDC (1.5 mmol, 1.5 eq.) and DMAP (2 mmol, 2.00 eq.). The mixture was stirred for 16 h at room temperature. The organic layer was successively washed twice with an aqueous solution of 1 M HCl and brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (eluting with DCM and MeOH, ratio from 100 : 0 to 95 : 5) to afford the alkyne product **1-6**. The physic properties of **1-6** are as follows: white solid (222 mg, 85% yield). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.21 (t, *J* = 6.0 Hz, 1H), 5.43 – 5.38 (m, 1H), 4.69 (d, *J* = 5.9 Hz, 1H), 3.63 (s, 3H), 3.60 (q, *J* = 6.1 Hz, 2H), 2.62 (d, *J* = 2.2 Hz, 1H), 2.58 (t, *J* = 6.2 Hz, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.3, 167.6, 144.0, 133.7, 127.2, 126.7, 83.5, 74.8, 63.5, 52.0, 35.5, 33.7. HRMS(ESI, m/z): calcd for $C_{14}H_{16}NO_4$: 262.1074 $[M+H]^+$, Found: 262.1074.

Synthesis of alkyne 1-5:

*3-(4-(1-hydroxyprop-2-yn-1-yl)benzamido)propanoic acid (**1-5**).*

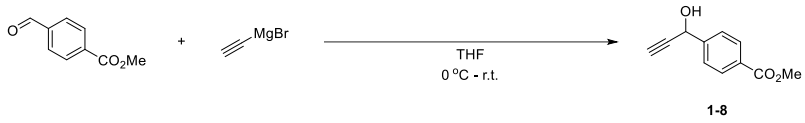


1-6 **1-5**

A solution of methyl 3-(4-(1-hydroxyprop-2-yn-1-yl)benzamido)propanoate (**1-6**) (1 mmol, 1 eq.) and LiOH monohydrate 0.5 M in methanol were stirred at room temperature for 24h. The reaction was monitored by LC-MS. After completion, the mixture was then acidified by 1 M HCl and extracted with EtOAc. The organic layer was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (eluting with DCM and MeOH, ratio from 100 : 0 to 95 : 5) to afford the alkyne product **1-6**. The physic properties of **1-6** are as follows: white solid (215 mg, 87% yield). **^{1}H NMR** (500 MHz, DMSO-*d*$_6$) δ 12.29 (s, 1H), 8.54 (t, *J* = 5.4 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.53 (d, *J* = 8.0 Hz, 2H), 6.21 – 6.16 (m, 1H), 5.42 (s, 1H), 3.51 (d, *J* = 2.2 Hz, 1H), 3.49 – 3.44 (m, 2H), 2.53 (t, *J* = 7.0 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO-*d*$_6$) δ 173.0, 166.1, 144.9, 133.8, 127.3, 126.2, 85.2, 76.2, 62.1, 35.7, 33.9. HRMS(ESI, m/z): calcd for $C_{13}H_{14}NO_4$: 248.0917 $[M+H]^+$, Found: 248.0917.

Synthesis of alkyne 1-8:

*methyl 4-(1-hydroxyprop-2-yn-1-yl)benzoate (**1-8**).*

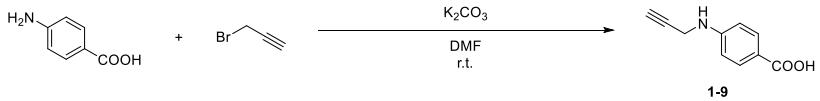


1-8

Similar to the synthesis of alkyne 1-7, a solution of methyl 4-formylbenzoate (3 mmol, 1 eq.) and ethynlmagnesium bromide solution (21 mL, 10.5 mmol) in 20 mL of anhydrous THF was stirred at 0 °C for 12 h. After completion, H_2O was carefully added to the reaction mixture to quench the reaction. The reaction mixture was extracted with EtOAc and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (eluting with DCM and MeOH, ratio from 100 : 0 to 98 : 2) to afford the alkyne product **1-8**. The physic properties of **1-8** are as follows: white solid (428 mg, 75% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 2.0 Hz, 1H), 7.62 (d, *J* = 2.0 Hz, 1H), 5.51 (dd, J_1 = 6.1 Hz, J_2 = 2.3 Hz, 1H), 3.91 (s, 3H), 2.69 (d, *J* = 2.3 Hz, 1H), 2.64 (m, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 167.0, 144.9, 130.2, 130.1, 126.6, 83.1, 75.4, 64.0, 52.4.

Synthesis of alkyne 1-9:

*4-(prop-2-yn-1-ylamino)benzoic acid (**1-9**).*



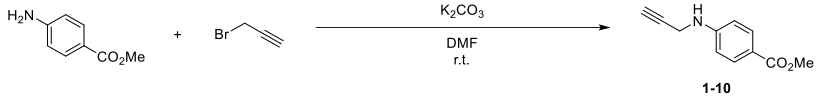
1-9

Similar to the synthesis of 1-3, to a 100 ml glass round-bottom flask was added sequentially the 4-aminobenzoic acid (10.0 mmol), 3-bromoprop-1-yne (10 mmol, 1 eq.) and K_2CO_3 (12 mmol, 1.2 eq.). The reaction mixture was stirred for 12 h at room temperature, monitored by LC-MS. After completion, EtOAc was added and the aqueous phase was acidified by 1 M HCl, the organic phase was washed sequentially with aqueous LiCl solution, water and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel chromatography (eluting with DCM and MeOH, ratio from 100 : 0 to 95 : 5) to afford the alkyne product **1-9**. The physic properties of **1-9** are as follows: yellow solid (1.35 g, 77% yield). **^{1}H NMR**

(400 MHz, DMSO-d_6) δ 7.64 (d, J = 8.6 Hz, 2H), 6.57 (d, J = 8.6 Hz, 2H), 6.06 (s, 1H), 4.82 (d, J = 2.5 Hz, 2H), 3.53 (t, J = 2.5 Hz, 1H), 3.34 (s, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.0, 153.9, 153.8, 131.3, 114.8, 112.7, 112.7, 79.1, 77.3, 51.3. HRMS(ESI, m/z): calcd for $C_{10}H_{10}NO_2$: 176.0706 $[M+H]^+$, Found: 176.0706.

Synthesis of alkyne 1-10:

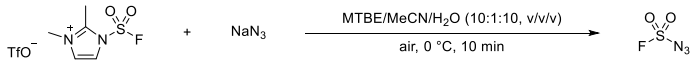
*methyl 4-(prop-2-yn-1-ylamino)benzoate (**1-10**).*



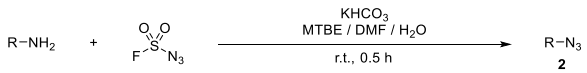
1-10

Similar to the synthesis of 1-3, to a 100 ml glass round-bottom flask was added sequentially the methyl 4-aminobenzoate (10.0 mmol), 3-bromoprop-1-yne (10 mmol, 1 eq.) and K_2CO_3 (12 mmol, 1.2 eq.). The reaction mixture was stirred for 12 h at room temperature, monitored by LC-MS. After completion, EtOAc was added, the organic phase was washed sequentially with aqueous LiCl solution, water and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel chromatography (eluting with DCM and MeOH, ratio from 100 : 0 to 95 : 5) to afford the alkyne product **1-10**. The physic properties of **1-10** are as follows: yellow solid (1.97 g, 80% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.90 (d, J = 7.8 Hz, 2H), 6.64 (d, J = 7.8 Hz, 2H), 4.39 (m, 1H), 3.98 (dd, J_1 = 6.2 Hz, J_2 = 2.5 Hz, 2H), 3.85 (s, 3H), 2.25 (t, J = 2.5 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 167.3, 150.7, 131.6, 119.8, 112.3, 80.1, 71.9, 51.8, 33.2. HRMS(ESI, m/z): calcd for $C_{11}H_{12}NO_2$: 190.0863 $[M+H]^+$, Found: 190.0863.

Section 2. Synthesis of azides



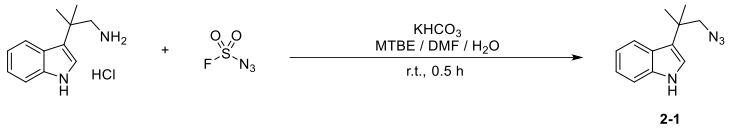
Preparation of FSO_2N_3: following the previous reference (3), a 100 ml cylindrical plastic bottle was charged with aqueous NaN_3 solution (0.50 M in 40 ml H_2O, containing 1.3 g (20 mmol) NaN_3) and methyl *tert*-butyl ether (MTBE, 40 ml). Compound 1 (7.9 g, 24 mmol) was dissolved in MeCN (2 ml), and the resultant viscous solution was added rapidly to the stirred NaN_3/H_2O/MTBE mixture in an ice-water bath. This was followed by a rinse of the vial used for preparing the solution of 1 with additional MeCN (2 ml), which was also added to the reaction mixture. The reaction mixture was stirred vigorously (approximately 600 r.p.m.) in an ice-water bath for 10 min in the loosely sealed plastic bottle, then the mixture was poured into a glass separating funnel. The mixture was kept in the funnel at room temperature for 30 min for phase separation. The organic phase was separated from the aqueous phase, and this organic phase—containing FSO_2N_3—was kept in a loosely sealed plastic bottle at room temperature for at least 12 h. The orange-red residual aqueous phase (approximately 1 ml in volume), which developed during the 12-hour resting period, was removed with a plastic pipette. The colorless organic phase could be used as a solution of FSO_2N_3 in MTBE without further purification. The concentration and yield of the FSO_2N_3 solution was measured by ^{19}F NMR with a known amount of TsF or $PhCF_3$ added as an internal standard. We have repeated this procedure many times, with a consistent yield of FSO_2N_3 in the range of 86%–93%, and concentration in the range of 420–470 mM.



2

General procedure 1: To a 20 ml glass round-bottom flask was added sequentially the primary amine (1.0 mmol), FSO_2N_3 solution (1 mmol, 1 eq., approximately 200 mM in DMF/MTBE 1:1, approximately 5 ml, volume adjusted according to the concentration) and aqueous $KHCO_3$ solution (3 M, 1.33 ml, 4 eq.). The reaction mixture was stirred for 0.5 h at room temperature, monitored by

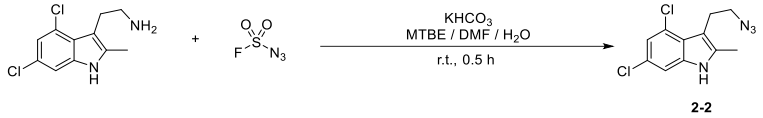
LC-MS. After completion, EtOAc was added and the mixture was washed sequentially with aqueous LiCl solution, water and brine, dried over Na_2SO_4, concentrated by rotary evaporation and dried *in vacuo* to afford the azide product **2**.



2-1

*3-(1-azido-2-methylpropan-2-yl)-1H-indole (**2-1**).*

Following the **General procedure 1**, the title compound **2-1** was prepared from 2-(1H-indol-3-yl)-2-methylpropan-1-amine hydrochloride (1 mmol) and FSO_2N_3 solution. The physic properties of **2-1** are as follows: colorless liquid (197 mg, 92% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.79 (d, *J* = 7.4 Hz, 1H), 7.38 (d, *J* = 8.2 Hz, 1H), 7.26-7.21 (m, 1H), 7.18-7.12 (m, 1H), 6.99 (d, *J* = 1.5 Hz, 1H), 3.64 (s, 2H), 1.53 (s, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.2, 125.6, 121.9, 121.8, 121.3, 120.8, 119.4, 111.7, 62.1, 37.0, 26.3.

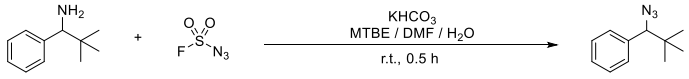


2-2

*3-(2-azidoethyl)-4,6-dichloro-2-methyl-1H-indole (**2-2**).*

Following the **General procedure 1**, the title compound **2-2** was prepared from 2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethan-1-amine (1 mmol) and FSO_2N_3 solution. The physic properties of **2-2** are as follows: white solid (198 mg, 74% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.14 (s, 1H), 7.06 (s, 0H), 3.51 (t, *J* = 7.1 Hz, 2H), 3.16 (t, *J* = 7.1 Hz, 2H), 2.39 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 136.6, 134.7, 126.8, 125.4, 123.8, 121.0, 109.3, 108.5, 53.0, 24.9, 11.7.

*(1-azido-2,2-dimethylpropyl)benzene (**2-3**).*



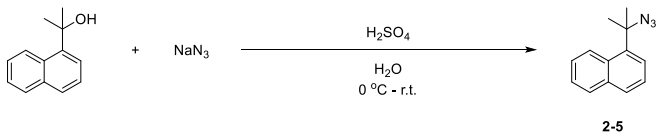
2-3

Following the **General procedure 1**, the title compound **2-3** was prepared from 2,2-dimethyl-1-phenylpropan-1-amine (1 mmol) and FSO_2N_3 solution. The physic properties of **2-3** are as follows: colorless liquid (157 mg, 83% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.44-7.31 (m, 4H), 4.35 (s, 1H), 0.99 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.7, 128.6, 128.0, 127.9, 76.7, 35.8, 26.5.

2-4

*1-azido-7-methyl-1,2,3,4-tetrahydronaphthalene (**2-4**).*

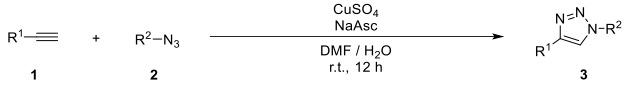
Following the **General procedure 1**, the title compound **2-4** was prepared from 7-methyl-1,2,3,4-tetrahydronaphthalen-1-amine hydrochloride (1 mmol) and FSO_2N_3 solution. The physic properties of **2-4** are as follows: colorless liquid (174 mg, 93% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.16 (s, 1H), 7.12-7.05 (m, 2H), 4.57 (t, *J* = 4.4 Hz, 1H), 2.90-2.69 (m, 2H), 2.39 (s, 3H), 2.07-1.79 (m, 4H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 135.7, 134.2, 133.6, 129.6, 129.4, 129.1, 59.6, 29.3, 28.5, 21.1, 19.2.



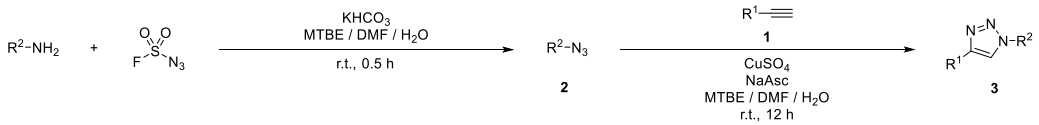
*1-(2-azidopropan-2-yl)naphthalene (**2-5**).*

To a 20 ml glass round-bottom flask was added H_2O (2 ml) and H_2SO_4 (2 ml) sequentially, with strong stirring in an ice-cooled bath. The solution was cooled to 0 °C and slowly treated with sodium azide (156mg, 2.4 mmol, 1.2 eq.), maintain the temprerature of <10 °C in order to preclude accidental volatilization of HN_3.When all of the NaN_3 has dissolved, 2-(naphthalen-1-yl)propan-2-ol was added to the mixture. The reaction mixture was stirred and allowed to stand at room temperature for 2 h, monitored by LC-MS. After completion, EtOAc (20 ml) and H_2O (20 ml) were added and transferred to separatory funnel. The organic phase was washed by NaOH (20 ml, 2 M) to remove all traces of HN_3. The aqueous phase was collected and quenched by aqueous NaClO solution. The organic phase was dried over Na_2SO_4 and concentrated by rotary evaporation. The crude product was further purified by silica gel chromatography (eluting with PE and DCM, ratio from 100:0 to 90:10) to afford the title product **2-5** (341 mg, 81% yield) as colorless liquid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.74 (d, *J* = 9.3 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 7.60-7.50 (m, 3H), 7.44 (t, *J* = 7.8 Hz, 1H), 1.89 (s, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 138.8, 134.9, 130.7, 129.6, 129.4, 126.4, 126.0, 125.6, 124.7, 124.1, 64.6, 28.6.

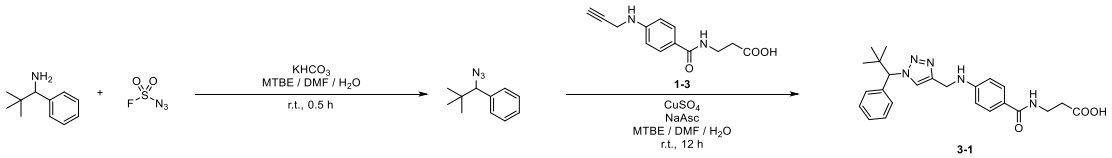
Section 3. Synthesis of triazoles



General procedure 2: In a 20 ml glass round-bottom flask, the alkyne **1** (0.5 mmol, 1 eq.) and azide **2** (0.5 mmol, 1 eq.) were dissolved in DMF (5 ml), then the yellow mixture of aqueous $CuSO_4$ solution (50 μl, 1M concentration, 0.05 mmol, 0.1 eq.) and sodium ascorbate (198 mg, 1 mmol, 1 eq.) was added to the mixture above. The reaction mixture was stirred for 12 h, while monitored by LC-MS. After completion, EtOAc (30 ml) was added and the mixture was washed sequentially with aqueous LiCl solution (30 ml × 3, 1 M concentration), water (30 ml× 2) and brine (50 ml), dried over Na_2SO_4, concentrated by rotary evaporation. Purification by silica gel chromatography afforded the title compound **3**.

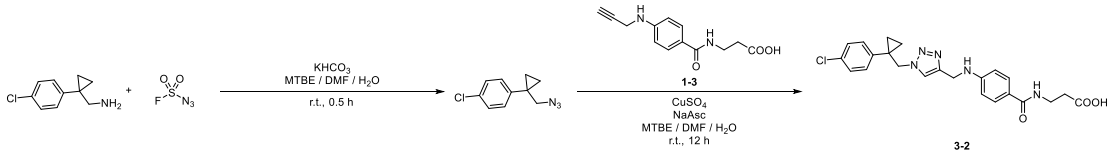


General procedure 3: To a 20 ml glass round-bottom flask was added sequentially the primary amine (0.5 mmol), FSO_2N_3 solution (0.5 mmol, 1 eq., approximately 200 mM in DMF/MTBE 1:1, approximately 2.5 ml, volume adjusted according to the concentration; prepared according to previous reference (3)) and aqueous $KHCO_3$ solution (3 M, 0.67 ml, 4 eq.). The reaction mixture was stirred for 0.5 h at room temperature, monitored by LC-MS. After completion, alkyne **1**, azide **2** and yellow mixture of aqueous $CuSO_4$ solution (50 μl, 1M concentration, 0.05 mmol, 0.1 eq.) and sodium ascorbate (198 mg, 1 mmol, 1 eq.) were added sequentially to the flask. The reaction mixture was then stirred for 12 h, while monitored by LC-MS. After completion, EtOAc (30 ml) was added and the mixture was washed sequentially with aqueous LiCl solution (30 ml × 3, 1 M concentration), water (30 ml × 2) and brine (50 ml), dried over Na_2SO_4, concentrated by rotary evaporation. Purification by silica gel chromatography afforded the title compound **3**.



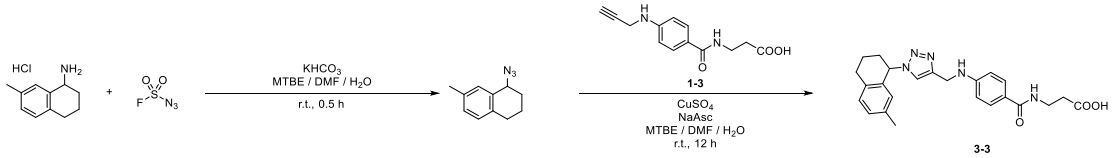
*3-(4-(((1-(2,2-dimethyl-1-phenylpropyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-1**).*

Following the **General procedure 3**, the title compound **3-1** was prepared from 2,2-dimethyl-1-phenylpropan-1-amine (0.5 mmol), FSO_2N_3 solution and 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1-3). Recrystallization from DCM and PE giving **3-1** as white solid (150 mg, 69% yield). **^{1}H NMR**(400MHz, DMSO-d_6) δ: 8.25 (s, 1H), 8.15 (t, *J* = 5.5 Hz, 1H), 7.60 (td, *J* = 5.6, 2.6 Hz, 4H), 7.41-7.26 (m, 3H), 6.61 (d, *J* = 8.4 Hz, 2H), 5.74 (s, 1H), 5.58 (s, 1H), 5.17 (d, *J* = 2.1 Hz, 2H), 3.48 (q, *J* = 6.5 Hz, 2H), 2.59 (t, *J* = 6.9 Hz, 2H), 0.94 (s, 9H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ: 171.3, 166.4, 151.1, 140.9, 136.4, 129.4, 128.8, 128.0, 126.2, 121.5, 113.0, 73.4, 57.3, 54.9, 35.9, 35.3, 34.0, 26.8. HRMS(ESI, m/z): calcd for $C_{24}H_{29}N_5O_3$: 436.2343 $[M+H]^+$, Found: 436.2339.



*3-(4-(((1-((1-(4-chlorophenyl)cyclopropyl)methyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-2**).*

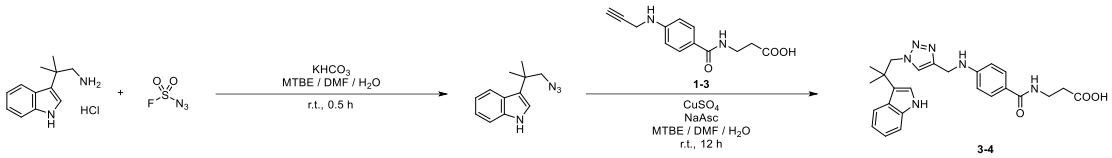
Following the **General procedure 3**, the title compound **3-2** was prepared from (1-(4-chlorophenyl)cyclopropyl)methanamine (0.5 mmol), FSO_2N_3 solution and 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1-3). The physic properties of **3-2** are as follows: pale yellow solid (185 mg, 82% yield). ^{1}H NMR(400MHz, DMSO-d_6) δ: 8.10 (t, *J* = 5.5 Hz, 1H), 7.91 (s, 1H), 7.57 (d, *J* = 8.7 Hz, 2H), 7.26 (d, *J* = 8.4 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H), 6.56 (d, *J* = 8.7 Hz, 2H), 5.11 (s, 2H), 4.54 (s, 2H), 3.66-3.21 (m, 2H), 2.58 (t, *J* = 7.0 Hz, 2H), 1.20-1.12 (m, 2H), 1.03-0.82 (m, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ: 171.2, 166.4, 151.7, 141.7, 140.5, 131.3, 130.4, 128.8, 128.2, 124.6, 121.0, 112.6, 57.6, 57.2, 54.9, 35.3, 34.0, 25.5, 12.4. HRMS (ESI, m/z): calcd for $C_{23}H_{24}ClN_5O_3$: 454.1640 $[M+H]^+$, Found:454.1638.



*3-(4-(((1-(7-methyl-1,2,3,4-tetrahydronaphthalen-1-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-3**).*

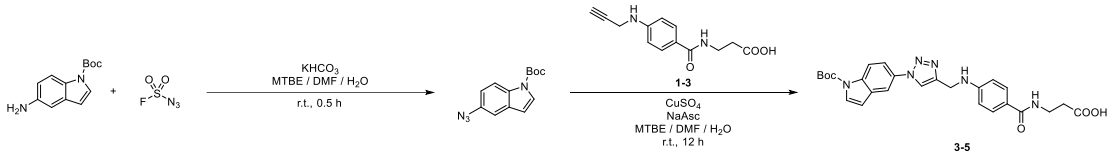
Following the **General procedure 3**, the title compound **3-3** was prepared from 7-methyl-1,2,3,4-tetrahydronaphthalen-1-amine hydrochloride (0.5 mmol), FSO_2N_3 solution and 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1-3). Recrystallization from DCM and PE giving **3-3** as white solid (143 mg, 66% yield). ^{1}H NMR(400MHz, CD_3OD) δ 7.71 (s, 1H), 7.68 (d, *J* = 8.6 Hz, 2H), 7.17-7.07 (m, 1H), 6.91 (d, *J* = 8.6 Hz, 2H), 6.65 (s, 1H), 5.90 (t, *J* = 6.0 Hz, 1H), 5.21 (s, 2H), 3.63 (t, *J* = 6.7 Hz, 2H), 3.02-2.77 (m, 2H), 2.68 (t, *J* = 6.7 Hz, 2H), 2.34-2.23 (m, 2H), 2.21 (s, 3H), 1.87 (p, *J* = 6.3 Hz, 2H). **^{13}C NMR** (126 MHz, CD_3OD) δ 174.0, 171.1, 138.1, 136.9, 134.6, 131.4, 131.3,

130.8, 130.7, 126.1, 125.3, 116.6, 61.7, 59.3, 37.6, 35.8, 33.0, 30.2, 21.8, 21.6. HRMS (ESI, m/z): calcd for $C_{24}H_{28}N_5O_3$: 434.2187 $[M+H]^+$, Found:434.2183.



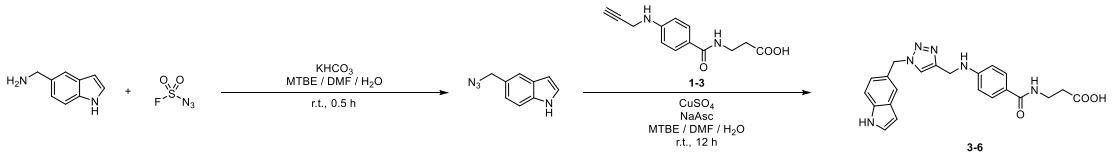
*3-(4-(((1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-4**).*

Following the **General procedure 3**, the title compound **3-4** was prepared from 2-(1H-indol-3-yl)-2-methylpropan-1-amine hydrochloride (1 mmol), FSO_2N_3 solution and 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1-3). Recrystallization from DCM and PE giving **3-4** as white solid (330 mg, 72% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.80 (d, *J* = 8.4 Hz, 1H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 9.2 Hz, 1H), 7.16 (t, *J* = 7.5 Hz, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 11.0 Hz, 2H), 5.02 (s, 2H), 4.73 (s, 2H), 3.58 (t, *J* = 6.8 Hz, 2H), 2.60 (t, *J* = 6.8 Hz, 2H), 1.46 (s, 6H). **^{13}C NMR** (126 MHz, CD_3OD) δ 173.7, 171.1, 152.1, 143.9, 139.8, 130.8, 127.3, 126.9, 125.2, 124.4, 123.3, 121.9, 121.3, 120.9, 116.6, 113.8, 61.9, 59.0, 38.7, 37.6, 35.7, 27.7. HRMS (ESI, m/z): calcd for $C_{25}H_{29}N_6O_3$: 461.2296 $[M+H]^+$, Found:461.2293.



*3-(4-(((1-(1-(tert-butoxycarbonyl)-1H-indol-5-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-5**).*

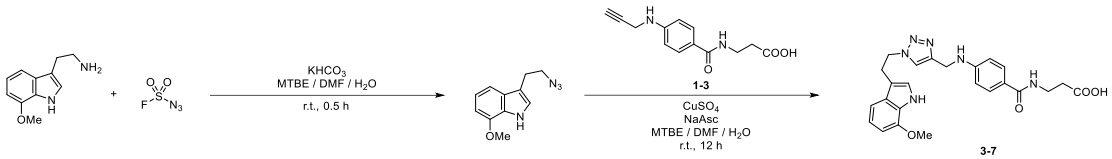
Following the **General procedure 3**, the title compound **3-5** was prepared from tert-butyl 5-amino-1*H*-indole-1-carboxylate (0.5 mmol), FSO_2N_3 solution and 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1-3). The physic properties of **3-5** are as follows: brown solid (187 mg, 74% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.20 (d, *J* = 8.7 Hz, 1H), 8.14-8.10 (m, 2H), 7.82 (dd, J_1 = 8.9 Hz, J_2 = 2.2 Hz, 1H), 7.79 (d, *J* = 3.8 Hz, 1H), 7.55 (d, *J* = 8.7 Hz, 2H), 6.81 (d, *J* = 3.8 Hz, 1H), 6.53 (d, *J* = 8.7 Hz, 2H), 5.62 (s, 1H), 5.26 (s, 2H), 3.49 (q, *J* = 6.6 Hz, 2H), 2.63 (t, *J* = 6.6 Hz, 2H), 1.63 (s, 9H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.3, 166.5, 151.7, 148.8, 143.1, 134.1, 132.0, 130.8, 128.7, 128.2, 123.1, 121.0, 116.8, 115.6, 112.8, 112.6, 107.6, 84.4, 57.2, 54.9, 35.3, 34.0, 27.6. HRMS (ESI, m/z): calcd for $C_{26}H_{29}N_6O_5$: 505.2194 $[M+H]^+$, Found:505.2195.



*3-(4-(((1-((1H-indol-5-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-6**).*

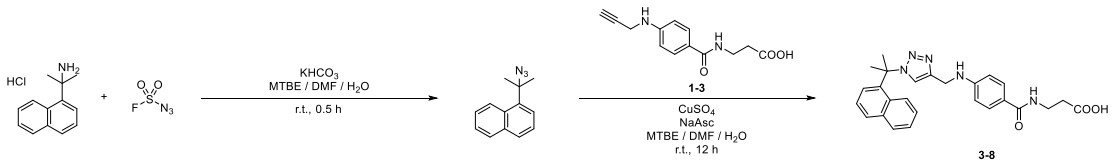
Following the **General procedure 3**, the title compound **3-6** was prepared from (1H-indol-5-yl)methanamine (1 mmol), FSO_2N_3 solution and 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1-3). Recrystallization from DCM and PE giving **3-6** as yellow solid (191 mg, 46% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.18 (s, 1H), 8.10 (s, 1H), 8.08 (t, *J* = 5.6 Hz, 1H), 7.58-7.53 (m, 3H),

7.41-7.32 (m, 2H), 7.10 (dd, J_1 = 8.4 Hz, J_2 = 1.7 Hz, 1H), 6.55 (d, J = 8.6 Hz, 2H), 6.44 (t, J = 2.5 Hz, 1H), 5.64 (s, 2H), 5.59 (s, 2H), 5.12 (s, 2H), 3.54-3.37 (m, 2H), 2.56 (t, J = 7.0 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ: 171.3, 166.4, 151.7, 142.1, 135.6, 128.8, 127.7, 126.3, 126.2, 124.4, 121.5, 121.0, 120.3, 112.6, 111.8, 101.3, 57.3, 53.8, 35.3, 33.9, 30.7. HRMS (ESI, m/z): calcd for $C_{22}H_{23}N_6O_3$: 419.1826 $[M+H]^+$, Found:419.1823.



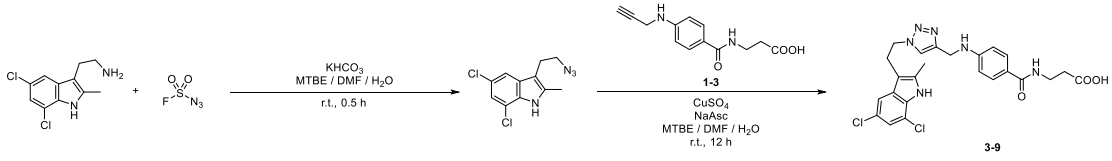
*3-(4-(((1-(2-(7-methoxy-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-7**).*

Following the **General procedure 3**, the title compound **3-7** was prepared from 2-(7-methoxy-1H-indol-3-yl)ethan-1-amine (0.2 mmol), FSO_2N_3 solution and 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1-3). Recrystallization from DCM and PE giving **3-7** as white solid (62 mg, 66% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.97 (s, 1H), 8.10-8.06 (m, 2H), 7.54 (d, J = 87 Hz, 2H), 7.11 (d, J = 8.0 Hz, 1H), 6.99 (d, J = 2.5 Hz, 1H), 6.91 (t, J = 7.7 Hz, 1H), 6.64 (d, J = 7.7 Hz, 1H), 6.53 (d, J = 8.7 Hz, 2H), 5.62 (s, 2H), 5.11 (s, 2H), 4.60 (t, J = 7.3 Hz, 2H), 3.88 (s, 3H), 3.44 (q, J = 6.7 Hz, 2H), 3.23 (t, J = 7.3 Hz, 2H), 2.56 (t, J = 7.0 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.2, 166.4, 151.7, 146.2, 141.7, 128.7, 128.4, 126.2, 124.7, 122.8, 121.0, 119.1, 112.5, 111.0, 110.5, 101.6, 57.3, 55.0, 50.0, 35.3, 33.9, 26.1. HRMS (ESI, m/z): calcd for $C_{24}H_{27}N_6O_4$: 463.2088 $[M+H]^+$, Found:463.2086.



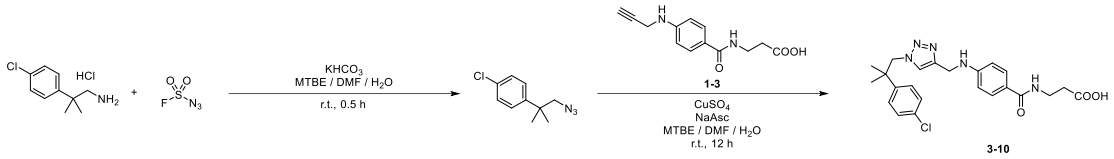
*3-(4-(((1-(2-(naphthalen-1-yl)propan-2-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-8**).*

Following the **General procedure 3**, the title compound **3-8** was prepared from 2-(naphthalen-1-yl)propan-2-amine hydrochloride (0.2 mmol), FSO_2N_3 solution and 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1-3). Recrystallization from DCM and PE giving **3-8** as pale yellow solid (44 mg, 48% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.95-7.87 (m, 2H), 7.84 (d, J = 6.2 Hz, 1H), 7.64-7.54 (m, 4H), 7.40 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 7.09 (d, J = 8.5 Hz, 1H), 6.67 (d, J = 8.5 Hz, 2H), 5.12 (s, 2H), 3.54 (t, J = 6.7 Hz, 2H), 2.59 (t, J = 6.7 Hz, 2H), 2.23 (s, 6H). **^{13}C NMR** (126 MHz, CD_3OD) δ 173.9, 171.2, 154.1, 145.4, 140.1, 137.3, 132.4, 132.1, 131.6, 130.8, 128.1, 127.4, 126.9, 126.6, 125.9, 125.7, 123.8, 115.6, 67.5, 59.2, 37.5, 35.8, 31.5. HRMS (ESI, m/z): calcd for $C_{26}H_{28}N_5O_3$: 458.2187 $[M+H]^+$, Found:458.2181.



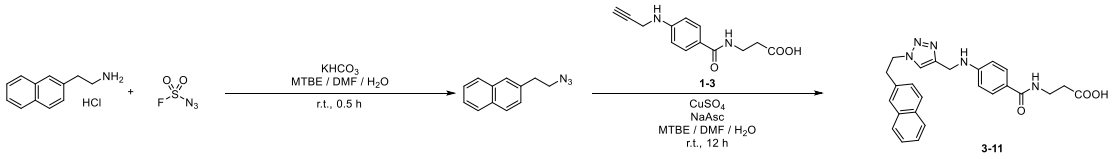
*3-(4-(((1-(2-(5,7-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-9**).*

Following the **General procedure 3**, the title compound **3-9** was prepared from 2-(5,7-dichloro-2-methyl-1H-indol-3-yl)ethan-1-amine (1 mmol), FSO_2N_3 solution and 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1-3). The physic properties of **3-9** are as follows: white solid (245 mg, 95% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.53 (d, *J* = 8.6 Hz, 2H), 7.39 (s, 1H), 7.12 (d, *J* = 1.8 Hz, 1H), 6.91 (d, *J* = 1.8 Hz, 1H), 6.61 (d, *J* = 8.6 Hz, 2H), 5.08 (s, 2H), 4.53 (t, *J* = 6.5 Hz, 2H), 3.55 (t, *J* = 6.8 Hz, 2H), 3.28 (t, *J* = 6.5 Hz, 2H), 2.59 (t, *J* = 6.8 Hz, 2H), 1.81 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 173.8, 171.2, 154.0, 144.5, 139.3, 138.1, 130.8, 130.7, 127.8, 127.0, 126.6, 125.3, 123.8, 121.5, 115.6, 111.4, 108.0, 59.1, 54.1, 37.6, 35.8, 27.9, 11.5. HRMS (ESI, m/z): calcd for $C_{24}H_{25}Cl_2N_6O_3$: 515.1360 $[M+H]^+$, Found:515.1360.



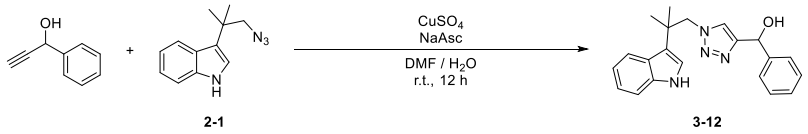
*3-(4-(((1-(2-(4-chlorophenyl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido) propanoic acid (**3-10**).*

Following the **General procedure 3**, the title compound **3-10** was prepared from 2-(4-chlorophenyl)-2-methylpropan-1-amine hydrochloride (0.2 mmol), FSO_2N_3 solution and 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1-3). Recrystallization from DCM and PE giving **3-10** as white solid (54 mg, 59% yield). **^{1}H NMR** (500 MHz, DMSO-*d*$_6$) δ 8.05 (t, *J* = 5.6 Hz, 1H), 7.55 (s, 1H), 7.52 (d, *J* = 8.7 Hz, 2H), 7.36 (s, 4H), 6.52 (d, *J* = 8.7 Hz, 2H), 5.63 (s, 1H), 5.05 (s, 2H), 4.51 (s, 2H), 3.41 (q, *J* = 7.0 Hz, 2H), 2.53 (t, *J* = 7.0 Hz, 2H), 1.27 (s, 6H). **^{13}C NMR** (126 MHz, DMSO-*d*$_6$) δ 171.1, 166.3, 151.6, 144.6, 141.2, 131.1, 128.7, 128.2, 128.1, 125.6, 121.0, 112.5, 60.1, 57.1, 35.2, 33.9, 26.2. HRMS (ESI, m/z): calcd for $C_{23}H_{26}ClN_5O_3$: 456.1797 $[M+H]^+$, Found:456.1793.



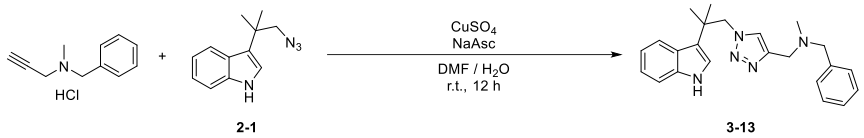
*3-(4-(((1-(2-(naphthalen-2-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-11**).*

Following the **General procedure 3**, the title compound **3-11** was prepared from tert-butyl 5-amino-1*H*-indole-1-carboxylate (0.2 mmol), FSO_2N_3 solution and 3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (1-3). The physic properties of **3-11** are as follows: white solid (77 mg, 87% yield). **^{1}H NMR** (500 MHz, DMSO-*d*$_6$) δ 8.07 (s, 2H), 7.86 (d, *J* = 7.5 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.80 (d, *J* = 7.5 Hz, 1H), 7.66 (s, 1H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.50-7.43 (m, 2H), 7.37 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H), 6.53 (d, *J* = 8.6 Hz, 2H), 5.64 (s, 1H), 5.09 (s, 2H), 4.69 (t, *J* = 7.3 Hz, 2H), 3.42 (q, *J* = 7.0 Hz, 2H), 3.31 (t, *J* = 7.3 Hz, 2H), 2.53 (t, *J* = 7.0 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO-*d*$_6$) δ 171.2, 166.3, 151.6, 141.8, 135.2, 133.0, 131.9, 128.7, 127.9, 127.5, 127.4, 127.2, 127.0, 126.1, 125.6, 124.7, 121.0, 112.5, 57.2, 50.4, 35.8, 35.3, 33.9. HRMS (ESI, m/z): calcd for $C_{25}H_{26}N_5O_3$: 444.2030 $[M+H]^+$, Found:444.2027.



*(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)(phenyl)methanol (**3-12**).*

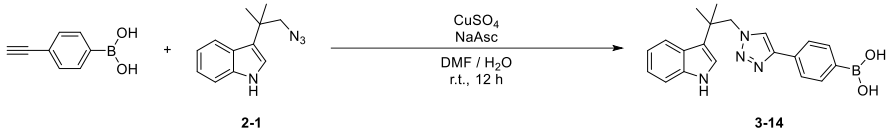
Following the **General procedure 2**, the title compound **3-12** was prepared from 1-phenylprop-2-yn-1-ol (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-12** are as follows: white solid (127 mg, 74% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.76 (d, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.32-7.21 (m, 3H), 7.21-7.12 (m, 3H), 7.09-7.04 (m, 1H), 6.81 (s, 1H), 6.56 (s, 1H), 5.76 (s, 1H), 4.66 (q, *J* = 13.6 Hz, 2H), 3.38 (s, 1H), 1.43 (d, *J* = 16.1 Hz, 6H). **^{13}C NMR** (126 MHz, CD_3OD) δ 152.4, 144.7, 139.7, 130.2, 129.5, 128.3, 127.2, 124.8, 124.3, 123.2, 121.8, 121.3, 120.8, 113.9, 70.6, 61.7, 38.6, 27.8, 27.7. HRMS (ESI, m/z): calcd for $C_{21}H_{23}N_4O$: 347.1866 $[M+H]^+$, Found:347.1863.



2-1 3-13

*1-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)-N-benzyl-N-methylmethanamine (**3-13**).*

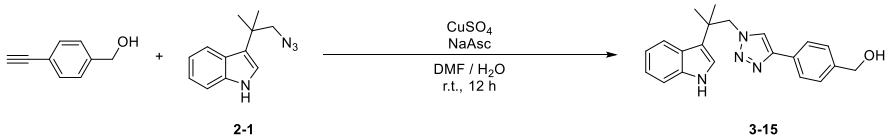
Following the **General procedure 2**, the title compound **3-13** was prepared from N-benzyl-N-methylprop-2-yn-1-amine hydrochloride (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-13** are as follows: white solid (160 mg, 86% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.88 (d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.32-7.25 (m, 3H), 7.22-7.17 (m, 1H), 7.16-7.11 (m, 3H), 6.89 (s, 1H), 6.72 (s, 1H), 4.81 (s, 2H), 3.50 (s, 2H), 3.29 (s, 2H), 2.01 (s, 3H), 1.52 (s, 6H). **^{13}C NMR** (126 MHz, CD_3OD) δ 144.2, 139.9, 139.8, 131.3, 130.2, 129.2, 127.4, 126.5, 124.4, 123.4, 121.9, 121.5, 121.0, 113.9, 62.3, 61.8, 59.2, 52.3, 42.6, 38.9, 27.9. HRMS (ESI, m/z): calcd for $C_{23}H_{28}N_5$: 374.2339 $[M+H]^+$, Found:374.2337.



2-1 3-14

*(4-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)phenyl)boronic acid (**3-14**).*

Following the **General procedure 2**, the title compound **3-14** was prepared from (4-ethynylphenyl)boronic acid (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-14** are as follows: white solid (72 mg, 40% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.83 (d, *J* = 7.9 Hz, 1H), 7.74 (d, *J* = 7.4 Hz, 1H), 7.48 (d, *J* = 7.9 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 7.08 (s, 1H), 6.89 (s, 1H), 4.74 (s, 2H), 1.49 (s, 6H). **^{13}C NMR** (126 MHz, CD_3OD) δ 148.5, 139.8, 136.3, 136.0, 130.6, 127.3, 126.4, 124.4, 123.6, 123.4, 122.0, 121.5, 120.9, 113.8, 62.0, 38.7, 27.7. HRMS (ESI, m/z): calcd for $C_{20}H_{22}BN_4O_2$: 361.1830 $[M+H]^+$, Found:361.1828.

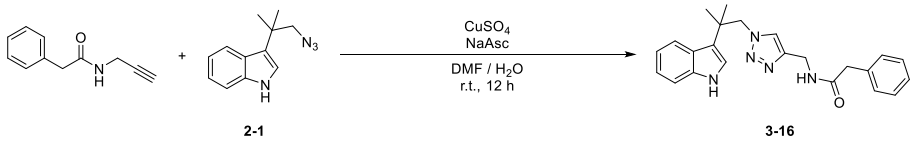


2-1 3-15

*(4-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)phenyl)methanol (**3-15**).*

Following the **General procedure 2**, the title compound **3-15** was prepared from (4-ethynylphenyl)methanol (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). Recrystallization from DCM and PE giving **3-15** as white solid (110 mg, 64% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.87 (d, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.46 (d, *J* = 8.2 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.19 (t, *J* = 7.7 Hz, 1H), 7.15-7.10 (m, 2H), 6.94 (s, 1H), 4.82 (s, 2H), 4.61 (s, 2H),

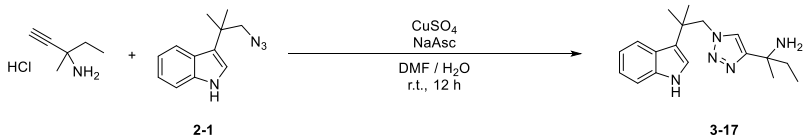
1.54 (s, 6H). **^{13}C NMR** (126 MHz, CD3OD) δ 148.5, 143.7, 139.9, 131.4, 129.3, 127.4, 127.3, 124.4, 123.4, 123.3, 122.0, 121.6, 120.9, 113.8, 65.7, 62.0, 38.8, 27.8. HRMS (ESI, m/z): calcd for $C_{21}H_{23}N_4O$: 347.1866 $[M+H]^+$, Found:347.1864.



2-1 3-16

*N-((1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)methyl)-2-phenylacetamide (**3-16**).*

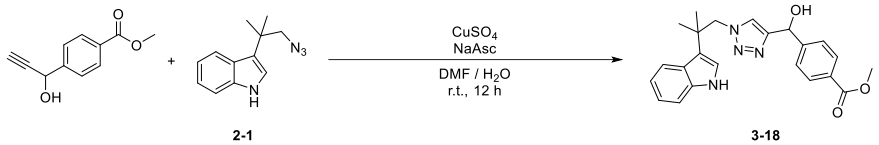
Following the **General procedure 2**, the title compound **3-16** was prepared from 2-phenyl-*N*-(prop-2-yn-1-yl)acetamide (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-16** are as follows: white solid (163 mg, 84% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.80 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.32-7.20 (m, 5H), 7.17 (M, 1H), 7.10 (m, 1H), 6.86 (s, 1H), 6.79 (s, 1H), 4.72 (s, 2H), 4.26 (s, 2H), 3.45 (s, 2H), 1.44 (s, 6H). **^{13}C NMR** (126 MHz, CD_3OD) δ 174.6, 146.1, 139.9, 137.5, 130.9, 130.4, 128.8, 127.3, 125.4, 124.3, 123.3, 121.9, 121.5, 120.8, 113.8, 61.9, 44.5, 38.6, 36.4, 27.7. HRMS (ESI, m/z): calcd for $C_{23}H_{26}N_5O$: 388.2132 $[M+H]^+$, Found:388.2127.



2-1 3-17

*2-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)butan-2-amine (**3-17**).*

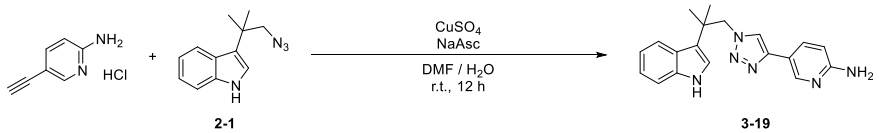
Following the **General procedure 2**, the title compound **3-17** was prepared from 3-methylpent-1-yn-3-aminium chloride (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-17** are as follows: white solid (122 mg, 78% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.81 (d, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.19-7.14 (td, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 1H), 7.10 (td, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 1H), 6.89 (s, 1H), 6.71 (s, 1H), 4.74 (s, 2H), 1.65 (q, *J* = 7.4 Hz, 2H), 1.51 (s, 6H), 1.28 (s, 3H), 0.64 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 155.1, 139.8, 127.5, 124.3, 123.8, 123.3, 122.0, 121.6, 120.8, 113.8, 62.0, 53.5, 38.8, 37.8, 28.3, 27.8, 9.6. HRMS (ESI, m/z): calcd for $C_{18}H_{26}N_5$: 312.2183 $[M+H]^+$, Found:312.2181.



2-1 3-18

*methyl 4-((1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)(hydroxy)methyl)benzoate (**3-18**).*

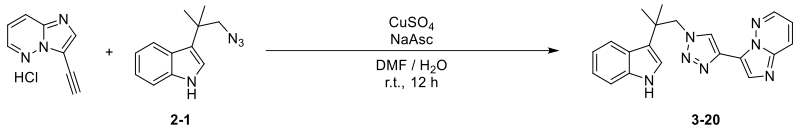
Following the **General procedure 2**, the title compound **3-18** was prepared from 4-(1-hydroxyprop-2-yn-1-yl)benzoate (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-18** are as follows: white solid (122 mg, 78% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.95 (d, *J* = 2.6 Hz, 1H), 7.92 (d, *J* = 8.1 Hz, 2H), 7.82 (d, *J* = 8.1 Hz, 1H), 7.39 (d, *J* = 8.3 Hz, 3H), 7.13-7.05 (m, 2H), 7.03-6.95 (m, 2H), 5.83-5.78 (m, 1H), 4.64 (s, 2H), 3.85 (s, 3H), 1.35 (d, *J* = 12.3 Hz, 6H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 166.2, 149.9, 149.3, 137.1, 129.1, 128.3, 126.6, 125.0, 122.8, 122.4, 120.8, 120.1, 119.5, 118.5, 111.9, 67.4, 67.3, 59.0, 52.1, 36.1, 26.2, 26.2. HRMS (ESI, m/z): calcd for $C_{23}H_{25}N_4O_3$: 405.1921 $[M+H]^+$, Found:405.1915.



2-1 3-19

*5-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)pyridin-2-amine (**3-19**).*

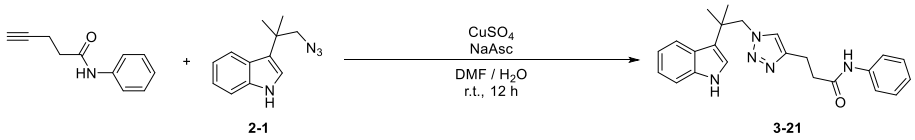
Following the **General procedure 2**, the title compound **3-19** was prepared from 5-ethynylpyridin-2-amine hydrochloride (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-19** are as follows: pale yellow solid (138 mg, 83% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.94 (d, *J* = 2.0 Hz, 1H), 8.26 (s, 1H), 7.84 (m, 2H), 7.69 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.13-7.07 (m, 2H), 7.03 (t, *J* = 7.0 Hz, 1H), 6.49 (s, 1H), 6.08 (s, 2H), 4.66 (s, 2H), 1.40 (s, 6H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 159.8, 145.2, 144.5, 137.6, 134.6, 125.5, 122.7, 121.3, 120.8, 120.6, 120.1, 118.9, 112.4, 59.8, 36.5, 26.7. HRMS (ESI, m/z): calcd for $C_{19}H_{21}N_6$: 333.1822 $[M+H]^+$, Found:333.1816.



2-1 3-20

*3-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)imidazo[1,2-b]pyridazine (**3-20**).*

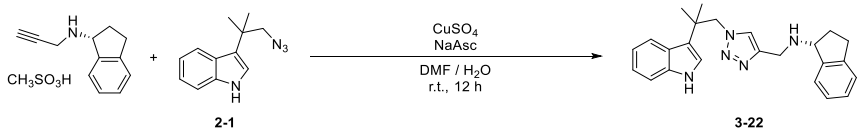
Following the **General procedure 2**, the title compound **3-20** was prepared from 3-ethynylimidazo[1,2-b]pyridazine hydrochloride (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-20** are as follows: brown solid (159 mg, 89% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 10.92 (s, 1H), 8.49 (d, *J* = 4.4 Hz, 1H), 8.28-8.19 (m, 1H), 7.97-7.89 (m, 2H), 7.43 (d, *J* = 8.1 Hz, 1H), 7.26 (dd, J_1 = 9.0 Hz, J_2 = 4.4 Hz, 1H), 7.14 (t, *J* = 7.5 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 7.00 (d, *J* = 2.4 Hz, 1H), 4.83 (s, 2H), 1.43 (s, 6H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 144.1, 137.3, 135.0, 131.2, 126.0, 125.1, 122.6, 122.1, 120.9, 120.2, 119.4, 118.6, 117.0, 111.9, 59.2, 36.3, 26.2. HRMS (ESI, m/z): calcd for $C_{20}H_{20}N_7$: 358.1775 $[M+H]^+$, Found:358.1771.



2-1 3-21

*3-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)-N-phenylpropanamide (**3-21**).*

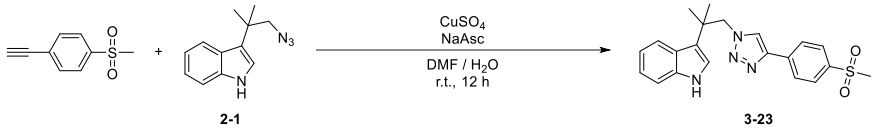
Following the **General procedure 2**, the title compound **3-21** was prepared from *N*-phenylpent-4-ynamide (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-21** are as follows: brown solid (180 mg, 93% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.90 (s, 1H), 9.90 (s, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.30-7.23 (m, 3H), 7.09 (t, *J* = 7.6 Hz, 1H), 7.04-6.98 (m, 3H), 4.60 (s, 2H), 2.85 (t, *J* = 7.5 Hz, 2H), 2.59 (t, *J* = 7.5 Hz, 2H), 1.31 (s, 6H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 170.1, 145.0, 139.2, 137.1, 128.7, 125.0, 123.0, 122.8, 122.2, 120.8, 120.1, 119.8, 119.0, 118.4, 111.9, 59.0, 36.0, 26.2, 21.0. HRMS (ESI, m/z): calcd for $C_{23}H_{26}N_5O$: 388.2132 $[M+H]^+$, Found:388.2128.



2-1 3-22

*(R)-N-((1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)methyl)-2,3-dihydro-1H-inden-1-amine (**3-22**).*

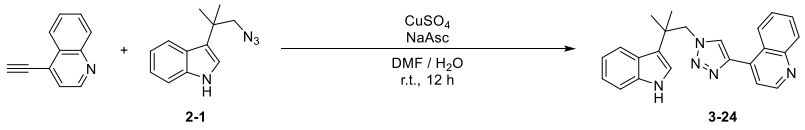
Following the **General procedure 2**, the title compound **3-22** was prepared from *(R)-N*-(prop-2-yn-1-yl)-2,3-dihydro-1H-inden-1-amine methanesulfonate (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-22** are as follows: pale yellow solid (176 mg, 91% yield). **^{1}H NMR** (500 MHz, CD_3OD) δ 7.80 (d, *J* = 8.0 Hz, 1H), 7.39-7.36 (m, 1H), 7.25-7.20 (m, 3H), 7.19-7.16 (m, 1H), 7.14 (t, *J* = 7.6 Hz, 1H), 7.09 (t, *J* = 7.6 Hz, 1H), 6.88 (d, *J* = 5.6 Hz, 2H), 4.75 (s, 2H), 4.03-3.98 (m, 1H), 3.82-3.71 (m, 2H), 3.05-2.96 (m, 1H), 2.83-2.73 (m, 1H), 2.28-2.19 (m, 1H), 2.16 (s, 1H), 1.84-1.74 (m, 1H), 1.48 (d, *J* = 3.4 Hz, 6H). **^{13}C NMR** (126 MHz, CD_3OD) δ 146.0, 139.8, 129.7, 128.2, 127.4, 126.6, 126.2, 125.8, 124.3, 123.3, 121.9, 121.5, 120.8, 113.8, 63.6, 61.9, 38.7, 33.9, 32.0, 27.9, 27.9. HRMS (ESI, m/z): calcd for $C_{24}H_{27}N_5$: 386.2339 $[M+H]^+$, Found:386.2333.



2-1 3-23

*3-(2-methyl-1-(4-(4-(methylsulfonyl)phenyl)-1H-1,2,3-triazol-1-yl)propan-2-yl)-1H-indole (**3-23**).*

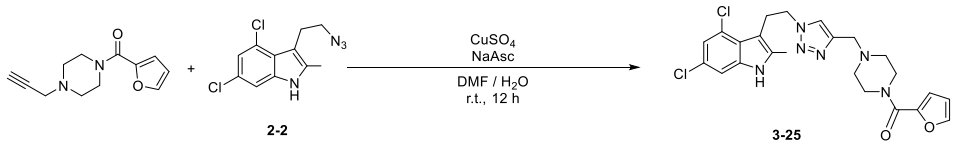
Following the **General procedure 2**, the title compound **3-23** was prepared from 1-ethynyl-4-(methylsulfonyl)benzene (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-23** are as follows: yellow solid (112 mg, 57% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.89 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.32 (s, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.92 (s, 1H), 4.80 (s, 2H), 3.11 (s, 3H), 1.52 (s, 6H). **^{13}C NMR** (126 MHz, CD_3OD) δ 146.8, 141.9, 139.8, 137.9, 129.9, 127.9, 127.4, 124.9, 124.4, 123.4, 122.0, 121.5, 120.9, 113.8, 62.2, 45.2, 38.7, 27.8. HRMS (ESI, m/z): calcd for $C_{21}H_{23}N_4O_2S$: 395.1536 $[M+H]^+$, Found:395.1530.



2-1 3-24

*4-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)quinoline (**3-24**).*

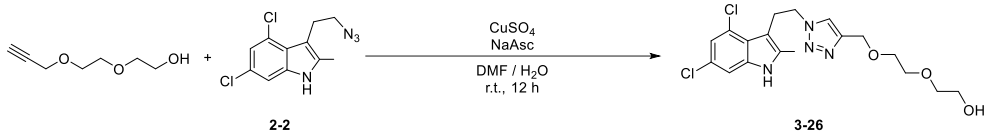
Following the **General procedure 2**, the title compound **3-24** was prepared from 4-ethynylquinoline (0.5 mmol) and 3-(1-azido-2-methylpropan-2-yl)-1*H*-indole (2-1). The physic properties of **3-24** are as follows: white solid (155 mg, 84% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.03 (s, 1H), 8.90 (d, *J* = 4.5 Hz, 1H), 8.04 (dd, J_1 = 14.4 Hz, J_2 = 8.5 Hz, 2H), 7.90-7.84 (m, 2H), 7.79 (t, *J* = 7.6 Hz, 1H), 7.67 (d, *J* = 4.5 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.46 (d, *J* = 8.1 Hz, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.09 (d, *J* = 2.5 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 4.82 (s, 2H), 1.49 (s, 6H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 1150.2, 148.3, 142.1, 137.2, 135.7, 129.6, 129.5, 127.1, 125.9, 125.1, 125.0, 124.6, 122.7, 121.0, 120.1, 119.9, 119.3, 118.6, 112.0, 59.5, 36.4, 26.3. HRMS (ESI, m/z): calcd for $C_{23}H_{22}N_5$: 368.1870 $[M+H]^+$, Found:368.1865.



2-2 3-25

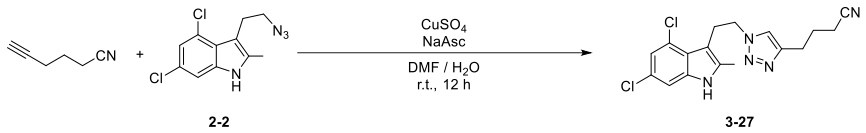
*(4-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)(furan-2-yl)methanone (**3-25**).*

Following the **General procedure 2**, the title compound **3-25** was prepared from furan-2-yl(4-(prop-2-yn-1-yl)piperazin-1-yl)methanone (0.5 mmol) and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-25** are as follows: white solid (178 mg, 73% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.72 (dd, J_1 = 1.8 Hz, J_2 = 0.8 Hz, 1H), 7.43 (s, 1H), 7.21 (d, J = 1.8 Hz, 1H), 7.06 (dd, J_1 = 3.5 Hz, J_2 = 0.8 Hz, 1H), 7.01 (d, J = 1.8 Hz, 1H), 6.62 (dd, J_1 = 3.5 Hz, J_2 = 1.8 Hz, 1H), 4.72 (t, J = 6.5 Hz, 2H), 3.79 (s, 4H), 3.65 (s, 2H), 3.48 (t, J = 6.5 Hz, 2H), 2.45 (t, J = 5.1 Hz, 4H), 2.02 (s, 3H). **^{13}C NMR** (126 MHz, CD_3OD) δ 161.8, 149.1, 146.8, 144.7, 139.3, 138.1, 127.8, 126.7, 126.6, 125.5, 121.6, 118.6, 113.3, 111.4, 108.1, 54.1, 54.0, 31.5, 27.7, 25.1, 11.8. HRMS (ESI, m/z): calcd for $C_{23}H_{25}Cl_2N_6O_2$: 487.1411 $[M+H]^+$, Found:487.1406.



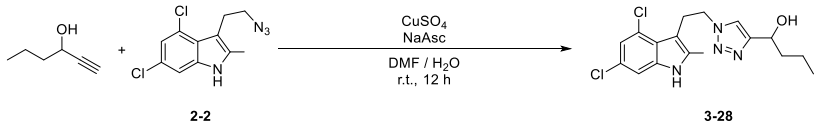
*2-(2-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)ethoxy)ethan-1-ol (**3-26**).*

Following the **General procedure 2**, the title compound **3-26** was prepared from 2-(2-(prop-2-yn-1-yloxy)ethoxy)ethan-1-ol (0.5 mmol) and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-26** are as follows: yellow oil (85 mg, 41% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.57 (s, 1H), 7.14 (s, 1H), 7.01 (s, 1H), 6.92 (s, 1H), 4.62 (t, J = 6.3 Hz, 2H), 4.53 (s, 2H), 3.74 (s, 2H), 3.62-3.55 (m, 4H), 3.53 (d, J = 4.5 Hz, 2H), 3.35 (t, J = 6.3 Hz, 2H), 1.78 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 137.0, 136.2, 126.2, 124.7, 123.9, 123.1, 120.3, 109.7, 106.2, 72.5, 70.3, 69.3, 64.1, 61.5, 52.3, 26.2, 10.6. HRMS (ESI, m/z): calcd for $C_{18}H_{23}Cl_2N_4O_3$: 413.1142 $[M+H]^+$, Found:413.1136.



*4-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)butanenitrile (**3-27**).*

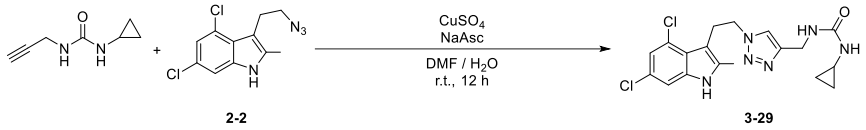
Following the **General procedure 2**, the title compound **3-27** was prepared from hex-5-ynenitrile (0.5 mmol) and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-27** are as follows: white solid (160 mg, 88% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.29 (s, 1H), 7.68 (s, 1H), 7.25 (s, 1H), 7.00 (s, 1H), 4.47 (t, J = 6.9 Hz, 2H), 3.27 (t, J = 6.9 Hz, 2H), 2.65 (t, J = 7.4 Hz, 2H), 2.47 (t, J = 7.1 Hz, 2H), 1.96 (s, 3H), 1.82 (p, J = 7.4 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 145.1, 136.7, 136.4, 124.5, 124.0, 123.1, 122.3, 120.4, 119.0, 109.6, 106.0, 51.1, 25.7, 24.9, 23.9, 15.8, 10.5. HRMS (ESI, m/z): calcd for $C_{17}H_{18}Cl_2N_5$: 362.0934 $[M+H]^+$, Found:362.0931.



*1-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)propan-1-ol (**3-28**).*

Following the **General procedure 2**, the title compound **3-28** was prepared from hex-1-yn-3-ol (0.5 mmol) and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-28** are as follows: white solid (167 mg, 91% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.37 (s, 1H), 7.21 (d, J = 1.7 Hz, 1H), 7.00 (d, J = 1.7 Hz, 1H), 4.64 (t, J = 6.7 Hz, 2H), 3.69 (td, J_1 = 7.4 Hz, J_2 = 3.8 Hz, 1H), 3.44 (t, J = 6.7 Hz, 2H), 2.77 (qd, J_1 = 7.4 Hz, J_2 = 3.8 Hz, 2H), 2.01 (s, 3H), 1.52-1.32 (m,

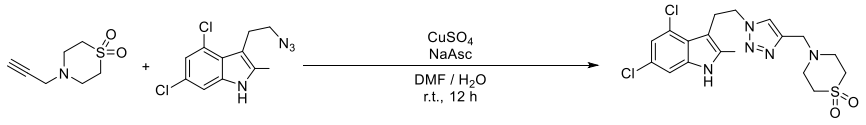
2H). 0.97 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 146.8, 139.4, 138.2, 127.9, 126.6, 125.5, 125.5, 121.6, 111.4, 108.2, 74.1, 54.0, 34.7, 31.2, 27.9, 11.6, 11.1. HRMS (ESI, m/z): calcd for $C_{17}H_{21}Cl_2N_4O$: 367.1687 $[M+H]^+$, Found:367.1084.



2-2 3-29

*1-cyclopropyl-3-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)urea (**3-29**).*

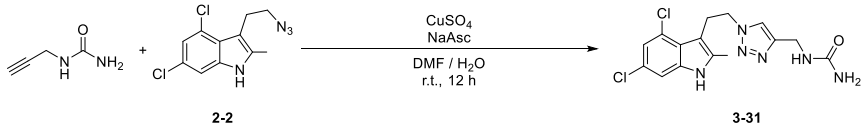
Following the **General procedure 2**, the title compound **3-29** was prepared from 1-cyclopropyl-3-(prop-2-yn-1-yl)urea (0.5 mmol) and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-29** are as follows: yellow solid (171 mg, 84% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.45 (s, 1H), 7.22 (d, *J* = 1.8 Hz, 1H), 7.01 (d, *J* = 1.8 Hz, 1H), 4.66 (t, *J* = 6.7 Hz, 2H), 4.36 (s, 2H), 3.44 (t, *J* = 6.6 Hz, 2H), 2.45 (tt, J_1 = 7.0 Hz, J_2 = 3.6 Hz, 1H), 1.98 (s, 3H), 0.71 (td, J_1 =7.0 Hz, J_2 = 4.8 Hz, 2H), 0.49-0.42 (m, 2H). **^{13}C NMR** (126 MHz, CD_3OD) δ 162.6, 139.4, 138.3, 127.9, 126.6, 125.5, 125.2, 121.6, 111.4, 108.2, 59.2, 54.1, 37.0, 27.9, 24.0, 19.2, 11.6, 8.3. HRMS (ESI, m/z): calcd for $C_{18}H_{21}Cl_2N_6O$: 407.1148 $[M+H]^+$, Found:407.1844.



2-2 3-30

*4-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)thiomorpholine 1,1-dioxide (**3-30**).*

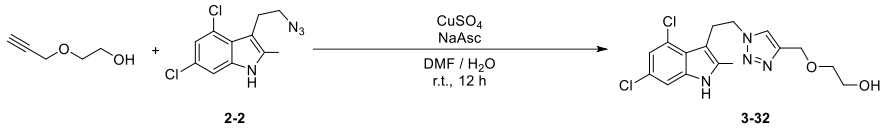
Following the **General procedure 2**, the title compound **3-30** was prepared from 4-(prop-2-yn-1-yl)thiomorpholine 1,1-dioxide and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-30** are as follows: white solid (190 mg, 90% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.41 (s, 1H), 7.20 (d, *J* = 1.7 Hz, 1H), 6.99 (d, *J* = 1.7 Hz, 1H), 4.70 (t, *J* = 6.5 Hz, 2H), 3.74 (s, 2H), 3.45 (t, *J* = 6.5 Hz, 2H), 3.08-3.03 (m, 4H), 2.88-2.84 (m, 4H), 1.99 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 139.4, 138.2, 128.0, 126.7, 125.5, 121.7, 111.5, 108.2, 54.2, 53.0, 52.1, 50.7, 27.7, 11.7. HRMS (ESI, m/z): calcd for $C_{18}H_{22}Cl_2N_5O_2S$: 442.0866 $[M+H]^+$, Found:442.0860.



2-2 3-31

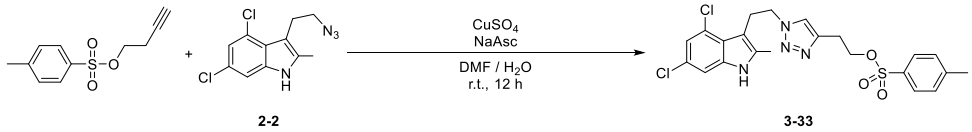
*1-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)urea (**3-31**).*

Following the **General procedure 2**, the title compound **3-31** was prepared from 1-(prop-2-yn-1-yl)urea and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-31** are as follows: white solid (119 mg, 65% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 7.77 (s, 1H), 7.28 (d, *J* = 1.8 Hz, 1H), 7.06 (d, *J* = 1.8 Hz, 1H), 6.33 (s, 1H), 5.51 (s, 1H), 4.51 (t, *J* = 7.1 Hz, 2H), 4.17 (d, *J* = 5.1 Hz, 2H), 3.33-3.28 (m, 2H), 2.03 (s, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 158.6, 146.0, 136.7, 136.6, 124.5, 124.0, 123.2, 122.5, 119.0, 109.7, 106.0, 51.1, 34.9, 25.8, 10.6. HRMS (ESI, m/z): calcd for $C_{15}H_{17}Cl_2N_6O$: 367.0835 $[M+H]^+$, Found:367.0832.



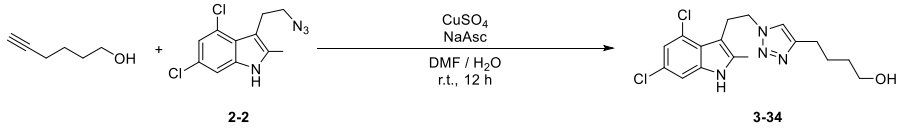
*2-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)ethan-1-ol (**3-32**).*

Following the **General procedure 2**, the title compound **3-32** was prepared from 2-(prop-2-yn-1-yloxy)ethan-1-ol and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-32** are as follows: pale yellow oil (127 mg, 69% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.56 (s, 1H), 7.17 (d, *J* = 1.7 Hz, 1H), 6.96 (d, *J* = 1.7 Hz, 1H), 4.63 (t, *J* = 6.7 Hz, 2H), 4.55 (s, 2H), 3.67-3.62 (m, 2H), 3.52-3.48 (m, 2H), 3.40 (t, *J* = 6.7 Hz, 2H), 1.96 (s, 3H). **^{13}C NMR** (126 MHz, CD_3OD) δ 139.2, 138.1, 127.8, 126.5, 126.2, 125.3, 121.5, 111.4, 108.0, 73.5, 65.6, 62.9, 54.0, 27.9, 11.6. HRMS (ESI, m/z): calcd for $C_{16}H_{18}Cl_2N_4O_2$: 369.0880 $[M+H]^+$, Found:369.0874.



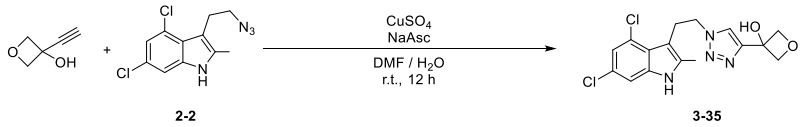
*2-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl 4-methylbenzenesulfonate (**3-33**).*

Following the **General procedure 2**, the title compound **3-33** was prepared from but-3-yn-1-yl 4-methylbenzenesulfonate and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-33** are as follows: white solid (199 mg, 81% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 11.34 (s, 1H), 7.75-7.70 (m, 3H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 1.7 Hz, 1H), 7.06 (d, *J* = 1.7 Hz, 1H), 4.48 (t, *J* = 7.0 Hz, 2H), 4.21 (t, *J* = 6.5 Hz, 2H), 3.27 (t, *J* = 7.0 Hz, 2H), 2.92 (t, *J* = 6.5 Hz, 2H), 2.41 (s, 3H), 1.99 (s, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 145.0, 141.9, 136.7, 136.5, 132.2, 130.2, 127.6, 124.5, 123.9, 123.1, 122.9, 119.0, 109.6, 105.9, 69.6, 51.2, 25.7, 25.1, 21.1, 10.6. HRMS (ESI, m/z): calcd for $C_{22}H_{23}Cl_2N_4O_3S$: 493.0862 $[M+H]^+$, Found:493.0863.



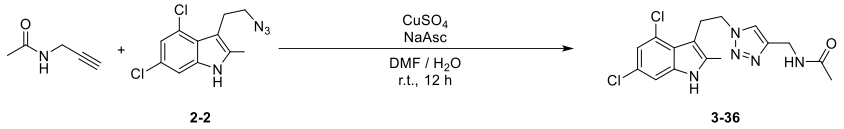
*4-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)butan-1-ol (**3-34**).*

Following the **General procedure 2**, the title compound **3-34** was prepared from hex-5-yn-1-ol and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-34** are as follows: colorless oil (146 mg, 80% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.30 (s, 1H), 7.22 (d, *J* = 1.7 Hz, 1H), 7.01 (d, *J* = 1.7 Hz, 1H), 4.65 (t, *J* = 6.6 Hz, 2H), 3.58 (t, *J* = 6.6 Hz, 2H), 3.44 (t, *J* = 6.6 Hz, 2H), 2.66 (t, *J* = 7.5 Hz, 2H), 1.98 (s, 3H), 1.72-1.61 (m, 2H), 1.59-1.49 (m, 2H). **^{13}C NMR** (126 MHz, CD_3OD) δ 139.4, 138.2, 127.8, 126.6, 125.4, 124.7, 121.6, 111.4, 108.2, 63.4, 54.0, 33.8, 27.9, 27.8, 26.8, 11.6. HRMS (ESI, m/z): calcd for $C_{17}H_{21}Cl_2N_4O$: 367.1087 $[M+H]^+$, Found:367.1084.



*3-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)oxetan-3-ol (**3-35**).*

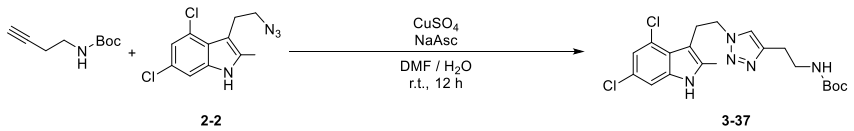
Following the **General procedure 2**, the title compound **3-35** was prepared from 3-ethynyloxetan-3-ol and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-35** are as follows: colorless oil (82 mg, 45% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.34 (s, 1H), 7.89 (s, 1H), 7.28 (d, *J* = 1.7 Hz, 1H), 7.06 (d, *J* = 1.7 Hz, 1H), 6.39 (s, 1H), 4.80 (d, *J* = 6.2 Hz, 2H), 4.67 (d, *J* = 6.2 Hz, 2H), 4.54 (t, *J* = 7.0 Hz, 2H), 3.36-3.27 (m, 2H), 2.00 (s, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 150.2, 136.7, 136.5, 124.5, 124.0, 123.1, 122.0, 119.0, 109.6, 105.9, 83.7, 70.4, 51.2, 25.7, 10.5. **HRMS** (ESI, m/z): calcd for $C_{16}H_{17}Cl_2N_4O_2$: 367.0723 $[M+H]^+$, Found:367.0721.



2-2 3-36

*N-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)acetamide (**3-36**).*

Following the **General procedure 2**, the title compound **3-36** was prepared from *N*-(prop-2-yn-1-yl)acetamide and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-36** are as follows: white solid (153 mg, 84% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.48 (s, 1H), 7.22 (d, *J* = 1.7 Hz, 1H), 7.01 (d, *J* = 1.7 Hz, 1H), 4.66 (t, *J* = 6.7 Hz, 2H), 4.37 (s, 2H), 3.44 (t, *J* = 6.7 Hz, 2H), 1.99 (s, 3H), 1.96 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 174.0, 139.4, 138.3, 127.9, 126.7, 125.5, 121.6, 111.4, 108.2, 54.1, 36.4, 27.9, 23.3, 11.5. HRMS (ESI, m/z): calcd for $C_{16}H_{18}Cl_2N_5O$: 366.0883 $[M+H]^+$, Found:366.0880.

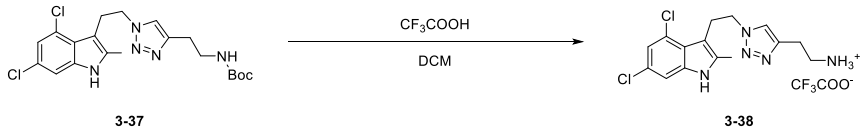


2-2 3-37

*tert-butyl (2-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)carbamate (**3-37**).*

Following the **General procedure 2**, the title compound **3-37** was prepared from *tert*-butyl but-3-yn-1-ylcarbamate and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-37** are as follows: white solid (162 mg, 74% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.42 (s, 1H), 7.22 (d, *J* = 1.7 Hz, 1H), 7.02 (d, *J* = 1.7 Hz, 1H), 4.65 (t, *J* = 6.7 Hz, 2H), 3.44 (t, *J* = 6.7 Hz, 2H), 3.27 (t, *J* = 6.8 Hz, 2H), 2.82 (t, *J* = 6.8 Hz, 2H), 2.00 (s, 3H), 1.44 (s, 9H). **^{13}C NMR** (126 MHz, CD_3OD) δ 139.4, 138.2, 127.9, 126.7, 125.5, 121.6, 111.4, 108.3, 80.9, 54.1, 42.0, 29.6, 27.9, 27.8, 11.6.

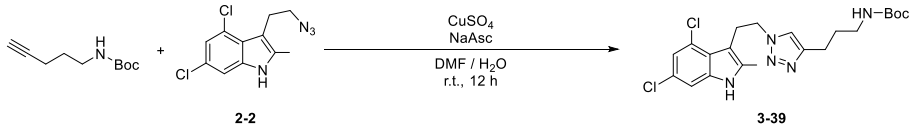
*2-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethan-1-aminium 2,2,2-trifluoroacetate (**3-38**).*



3-37 3-38

In a 20 ml glass round-bottom bottle, tert-butyl (2-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)ethyl)carbamate (**3-37**) (0.3 mmol) was dissolved in DCM (2 ml) at 0 °C, CF_3COOH was added dropwise . The mixture was stirred at room temperature for 1 h, monitored by LC-MS. After completion, the solvent and excess CF_3COOH were evaporated by rotary evaporation to afford the title product (pale yellow solid, 129 mg, 95% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.48 (s, 1H), 8.01 (s, 2H), 7.88 (s, 1H), 7.30 (d, *J* = 1.8 Hz, 1H), 7.05 (d, *J* = 1.8 Hz, 1H), 4.51 (t, *J* = 7.1 Hz, 2H), 3.31 (t, *J* = 7.1 Hz, 2H), 3.10-3.03 (m, 2H), 2.91 (t, *J* = 7.7 Hz, 2H), 2.05 (s, 3H). **^{19}F NMR** (376 MHz, DMSO-d_6) δ -73.70. **^{13}C NMR** (126 MHz, DMSO-d_6) δ 142.5,

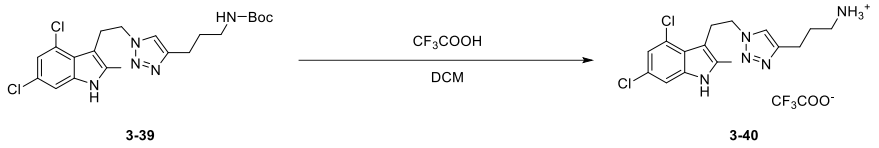
136.8, 136.5, 124.5, 123.9, 123.2, 122.9, 119.0, 109.7, 105.9, 51.2, 38.5, 25.8, 23.4, 10.6. HRMS (ESI, m/z): calcd for $C_{15}H_{18}Cl_2N_5$: 338.0934 $[M\text{-}CF_3COO^-]^+$, Found:338.0930.



*tert-butyl (3-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)propyl)carbamate (**3-39**).*

Following the **General procedure 2**, the title compound **3-39** was prepared from *tert*-butyl but-4-yn-1-ylcarbamate and 3-(2-azidoethyl)-4,6-dichloro-2-methyl-1*H*-indole (2-2). The physic properties of **3-39** are as follows: white solid (196 mg, 87% yield). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.33 (s, 1H), 7.22 (d, *J* = 1.7 Hz, 1H), 7.02 (d, *J* = 1.7 Hz, 1H), 4.65 (t, *J* = 6.6 Hz, 2H), 3.45 (t, *J* = 6.6 Hz, 2H), 3.04 (t, *J* = 6.9 Hz, 2H), 2.66 (t, *J* = 7.6 Hz, 2H), 1.98 (s, 3H), 1.75 (p, *J* = 7.1 Hz, 2H), 1.46 (s, 9H). **^{13}C NMR** (126 MHz, CD_3OD) δ 138.2, 127.9, 126.7, 125.5, 124.7, 121.6, 111.4, 108.3, 80.8, 54.1, 41.4, 31.7, 29.6, 27.9, 24.2, 11.6.

*3-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)propan-1-aminium 2,2,2-trifluoroacetate (**3-40**).*



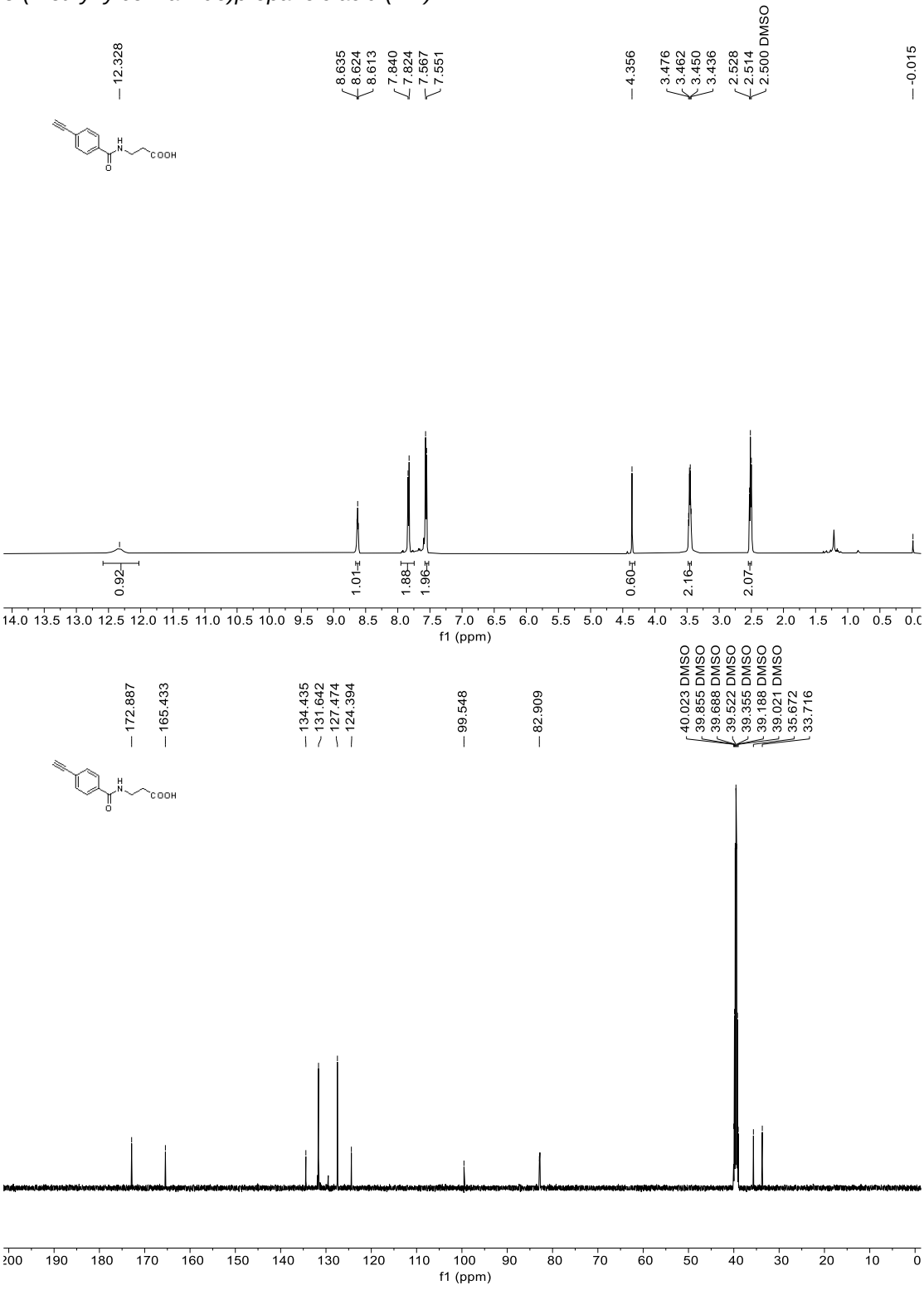
Similar to the preparation of 2-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethan-1-aminium 2,2,2-trifluoroacetate (**3-38**), tert-butyl (3-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)propyl)carbamate (**3-39**) (0.3 mmol) was dissolved in DCM (2 ml) at 0 °C in a glass round-bottom bottle, CF_3COOH was added dropwise . The mixture was stirred at room temperature for 1 h, monitored by LC-MS. After completion, the solvent and excess CF_3COOH were evaporated by rotary evaporation to afford the title product (pale yellow solid, 135 mg, 97% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.47 (s, 1H), 7.95 (s, 2H), 7.72 (s, 1H), 7.29 (d, *J* = 1.8 Hz, 1H), 7.04 (d, *J* = 1.8 Hz, 1H), 4.50 (t, *J* = 6.9 Hz, 2H), 3.30 (t, *J* = 6.9 Hz, 2H), 2.83 (m, 2H), 2.66 (t, *J* = 7.5 Hz, 2H), 2.00 (s, 3H), 1.86 (p, *J* = 7.6 Hz, 2H). **^{19}F NMR** (376 MHz, DMSO-d_6) δ -73.87. **^{13}C NMR** (126 MHz, DMSO-d_6) δ 145.5, 136.8, 136.5, 124.5, 124.0, 123.2, 122.2, 119.0, 109.7, 106.0, 51.2, 38.4, 27.0, 25.8, 22.0, 10.6. HRMS (ESI, m/z): calcd for $C_{16}H_{20}Cl_2N_5$: 352.1090 $[M\text{-}CF_3COO^-]^+$, Found:352.1086.

SI References

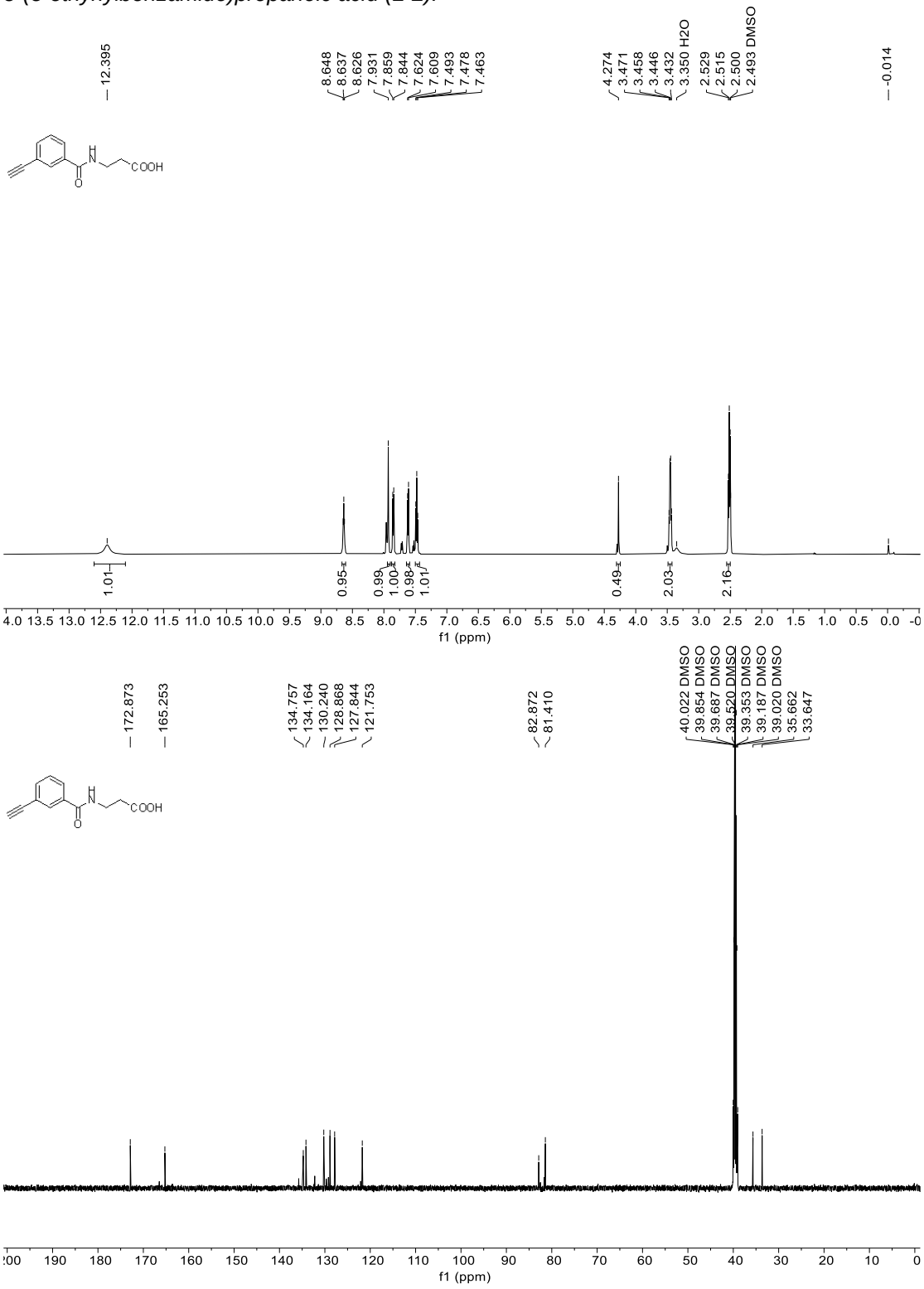
1. G. Song *et al.*, Human GLP-1 receptor transmembrane domain structure in complex with allosteric modulators. *Nature* **546**, 312-315 (2017).
2. J. F. Rivera, S. Costes, T. Gurlo, C. G. Glabe, P. C. Butler, Autophagy defends pancreatic beta cells from human islet amyloid polypeptide-induced toxicity. *J. Clin. Invest.* **124**, 3489-3500 (2014).
3. G. Meng *et al.*, Modular click chemistry libraries for functional screens using a diazotizing reagent. *Nature* **574**, 86-89 (2019).

NMR Spectra for Compounds

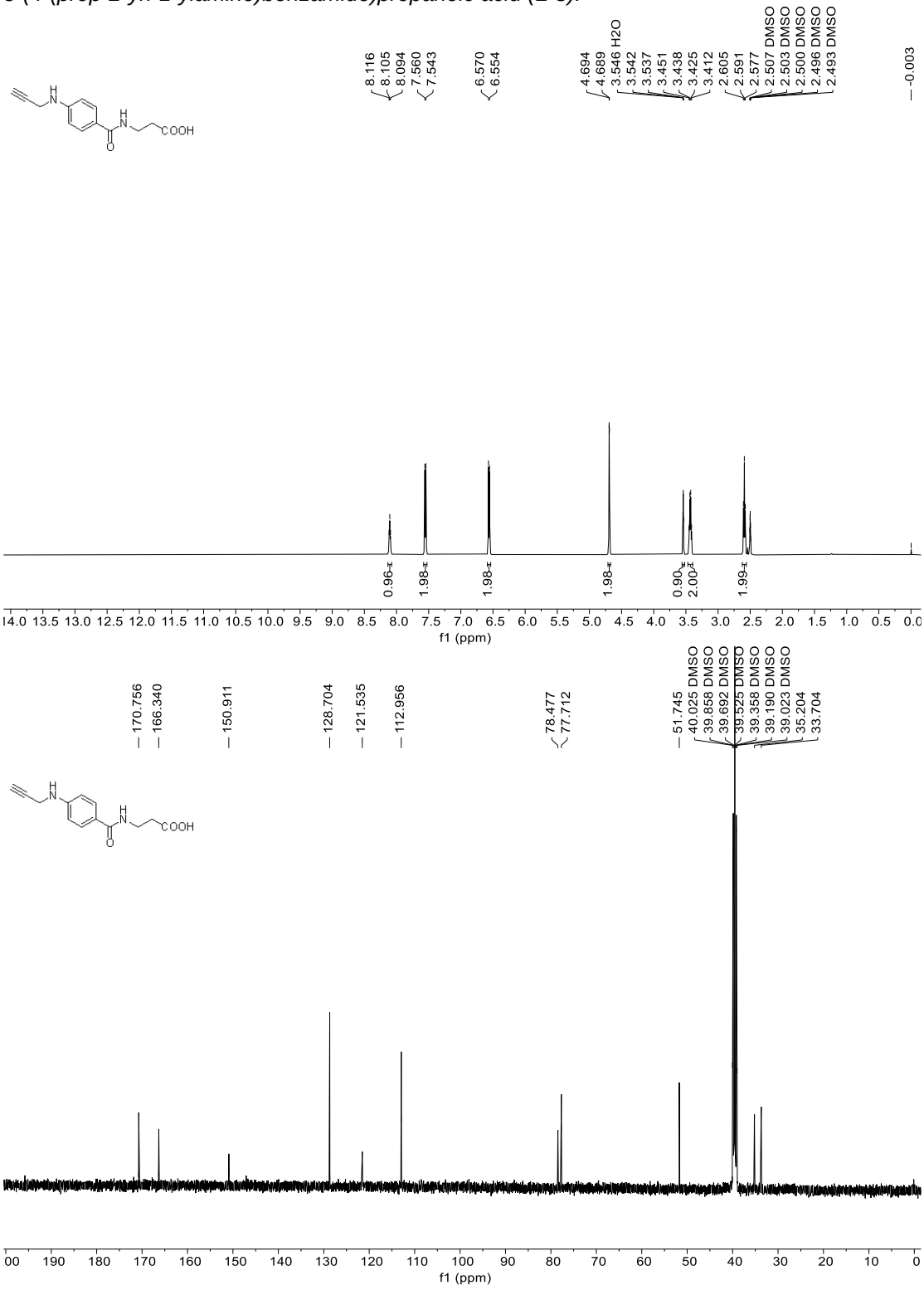
*3-(4-ethynylbenzamido)propanoic acid (**1-1**).*



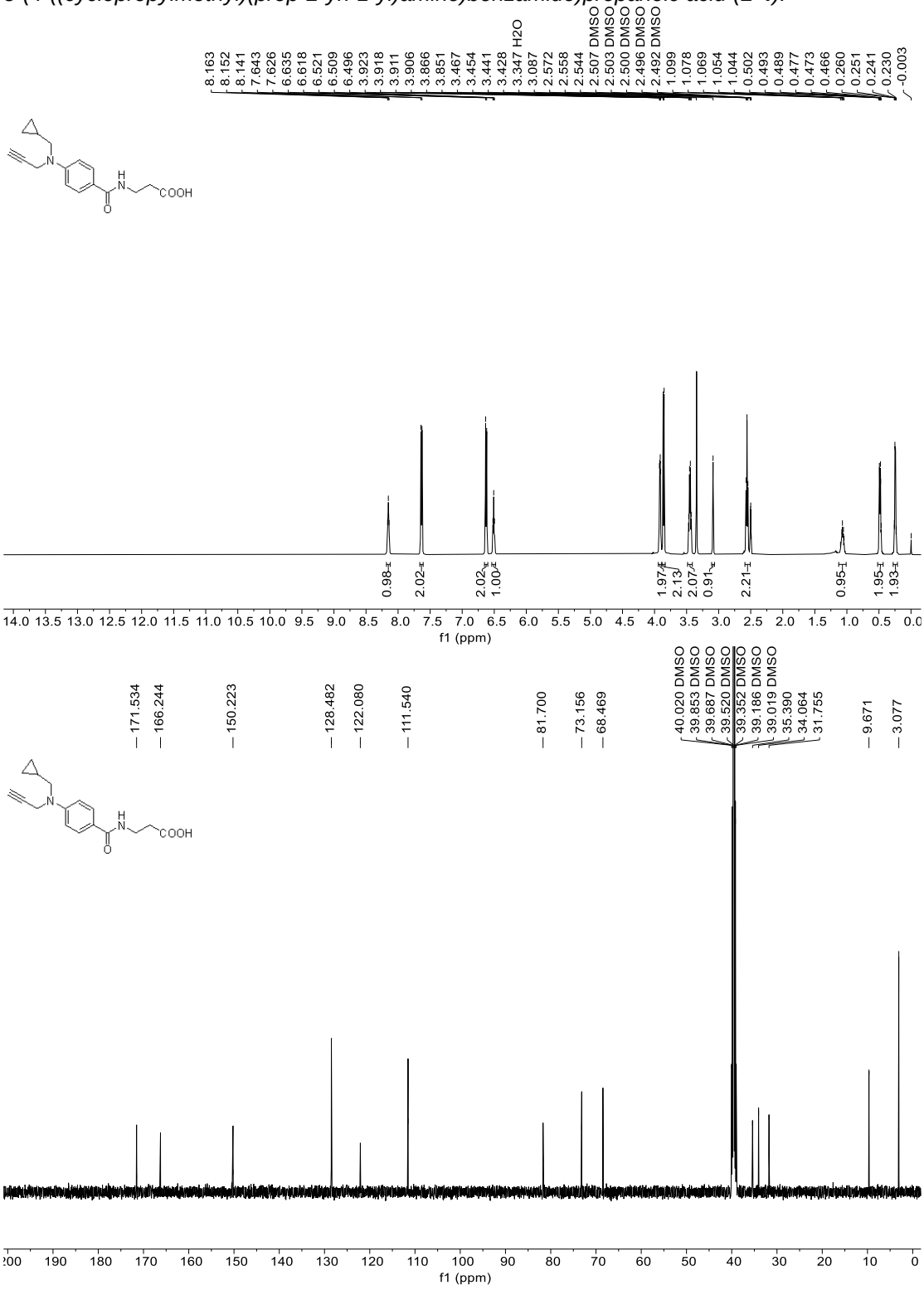
*3-(3-ethynylbenzamido)propanoic acid (**1-2**).*



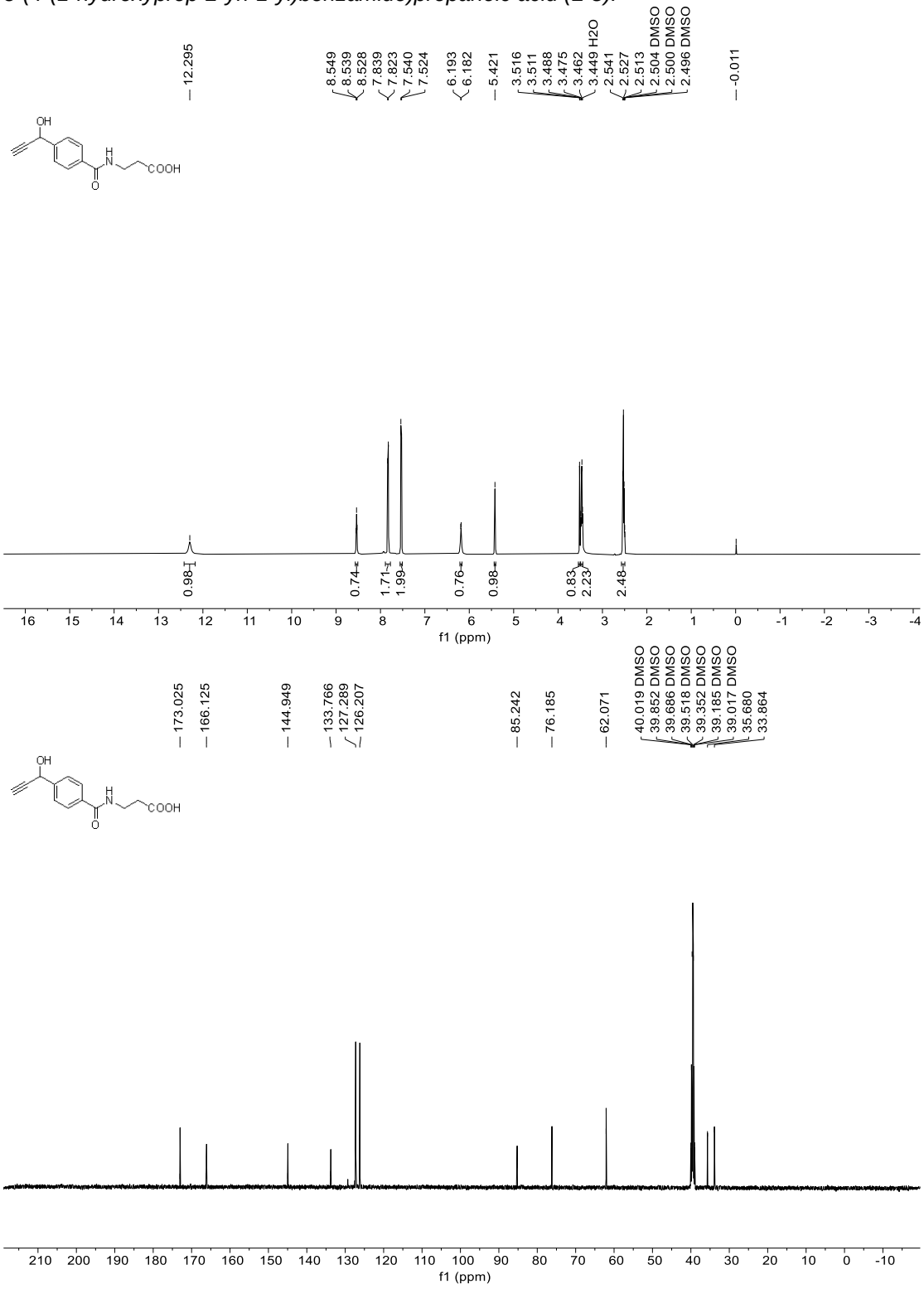
*3-(4-(prop-2-yn-1-ylamino)benzamido)propanoic acid (**1-3**).*



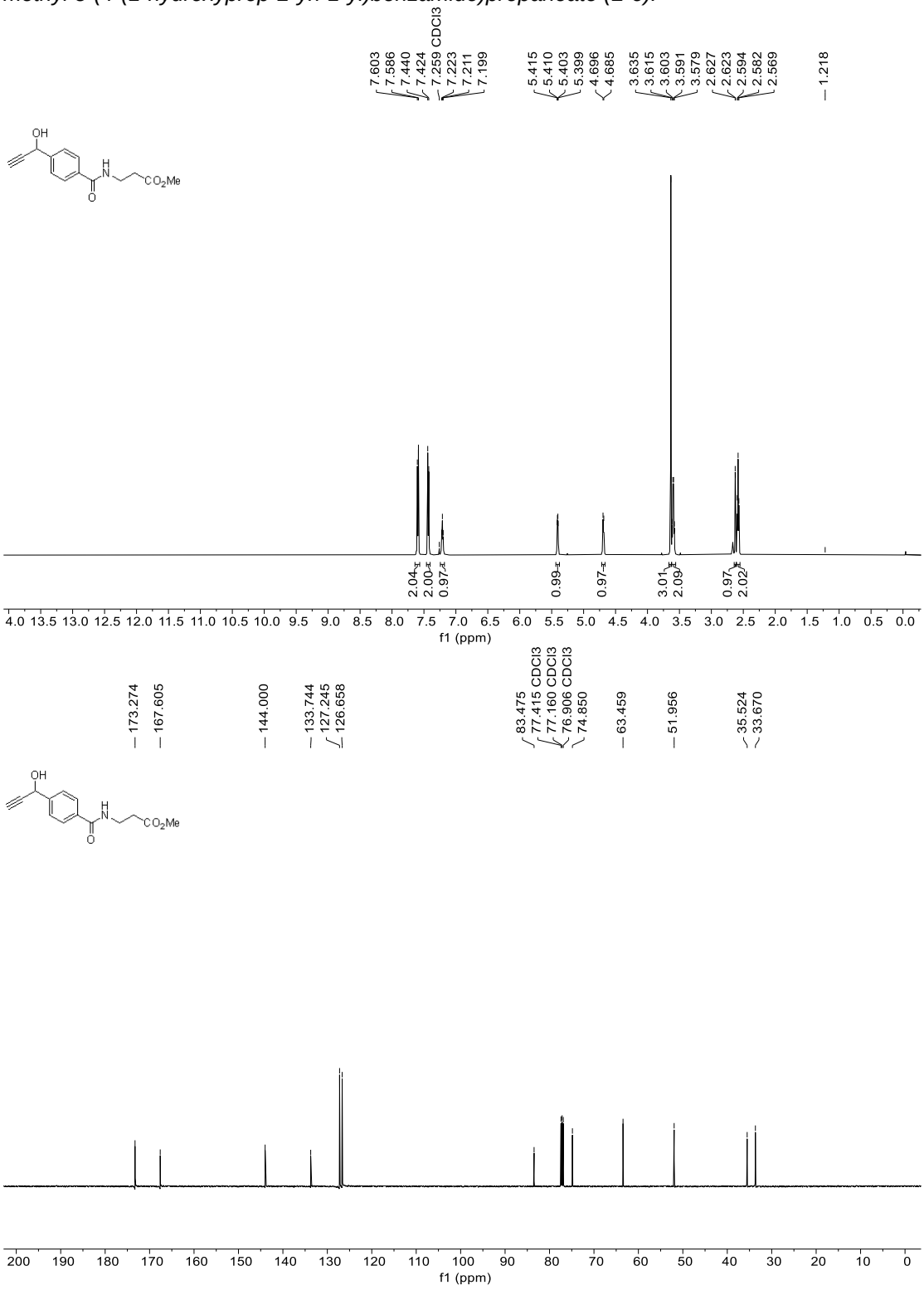
*3-(4-((cyclopropylmethyl)(prop-2-yn-1-yl)amino)benzamido)propanoic acid (**1-4**).*



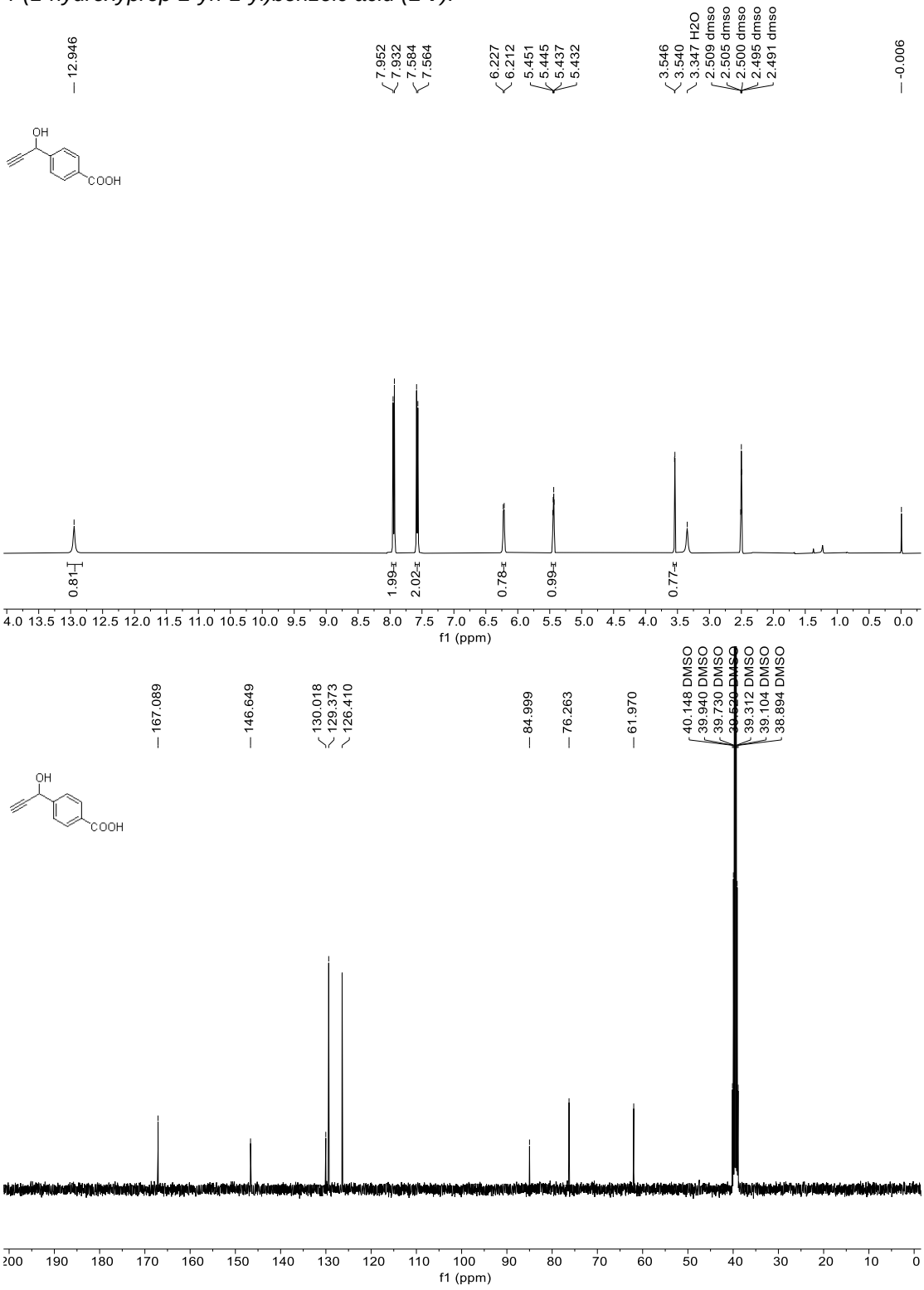
*3-(4-(1-hydroxyprop-2-yn-1-yl)benzamido)propanoic acid (**1-5**).*



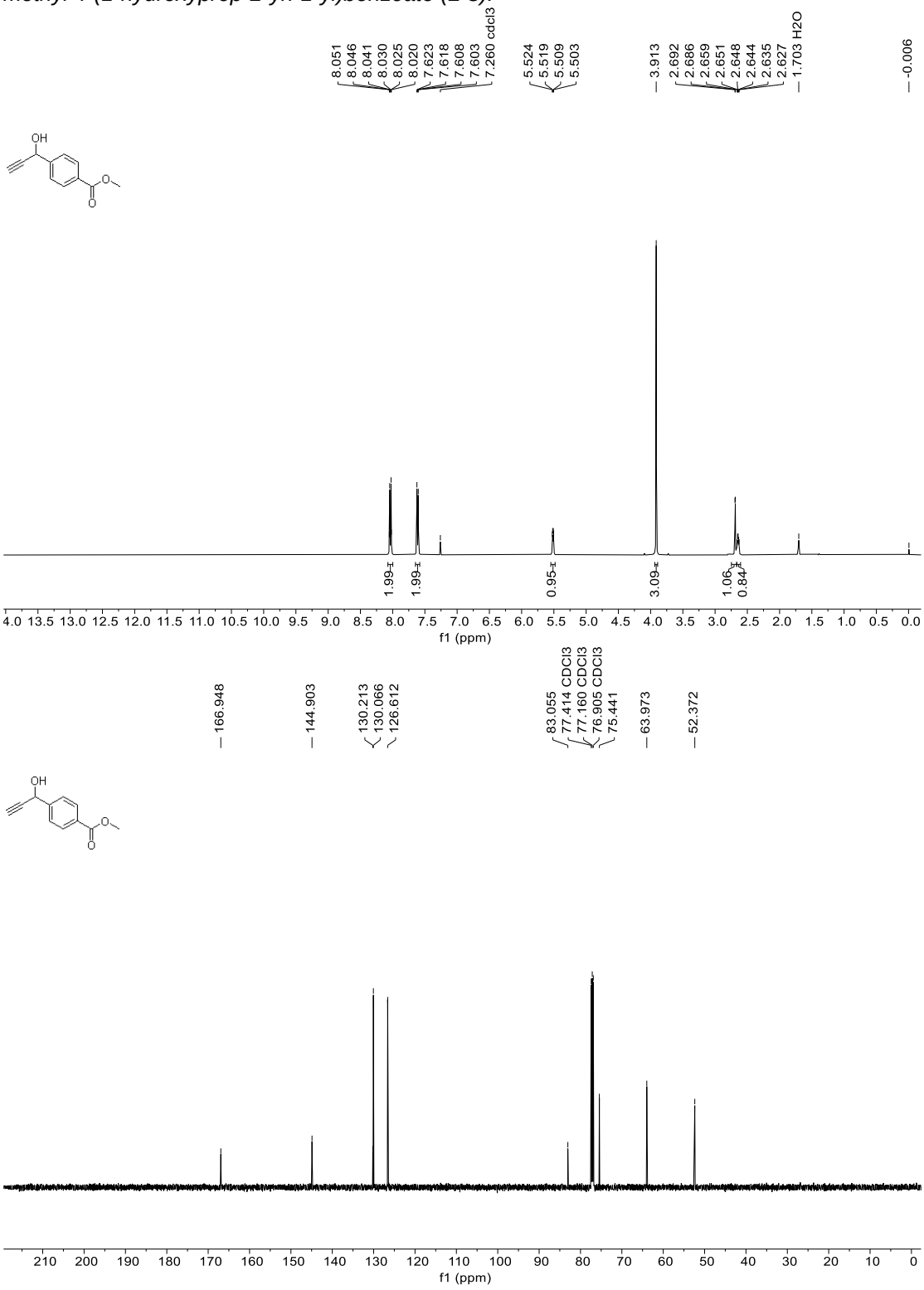
*methyl 3-(4-(1-hydroxyprop-2-yn-1-yl)benzamido)propanoate (**1-6**).*



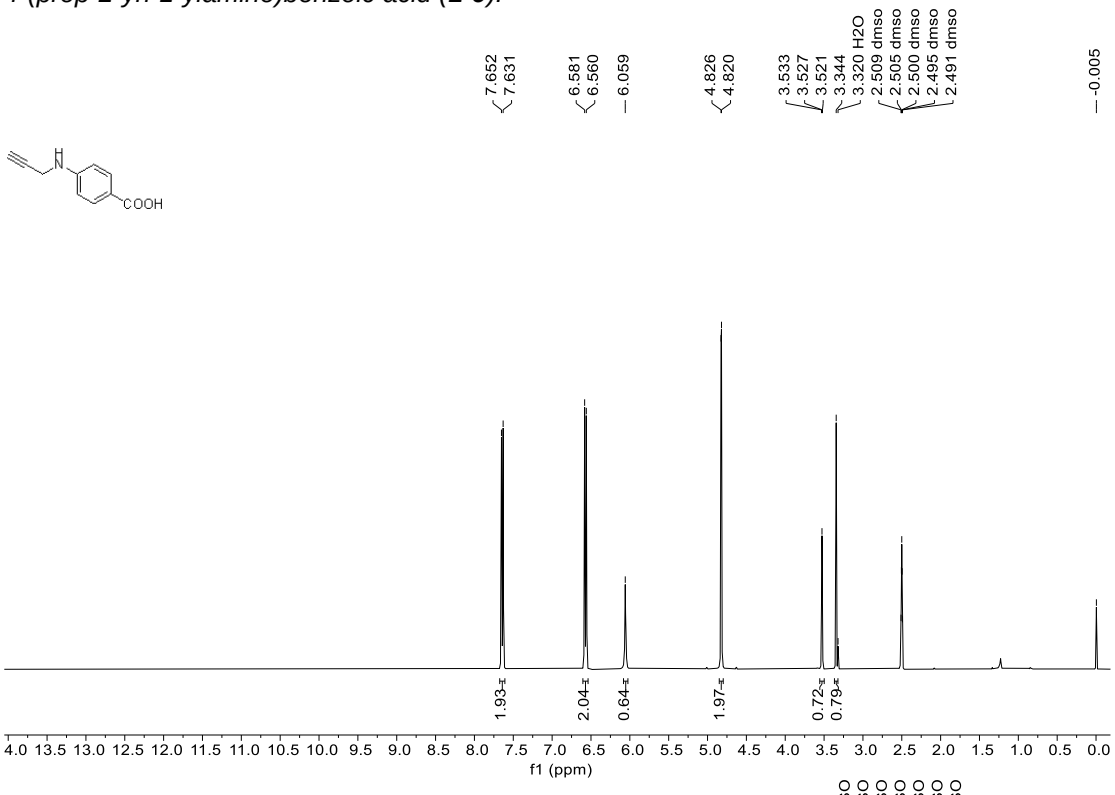
*4-(1-hydroxyprop-2-yn-1-yl)benzoic acid (**1-7**).*

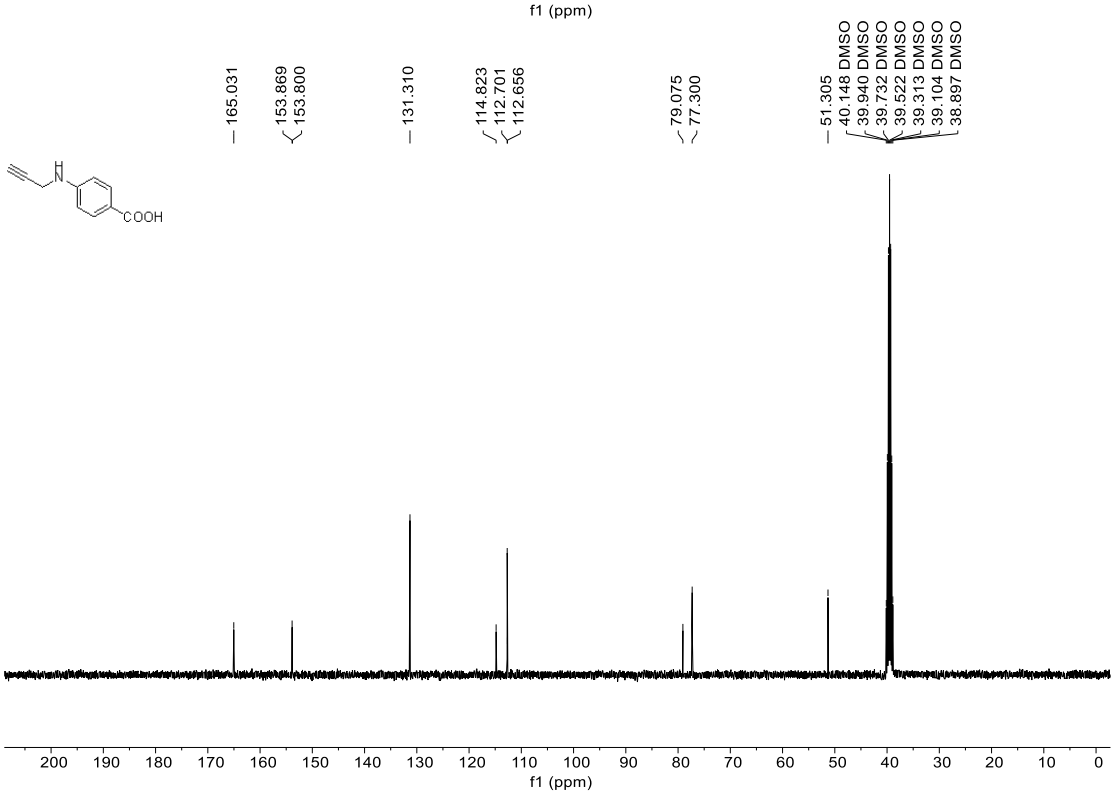


*methyl 4-(1-hydroxyprop-2-yn-1-yl)benzoate (**1-8**).*

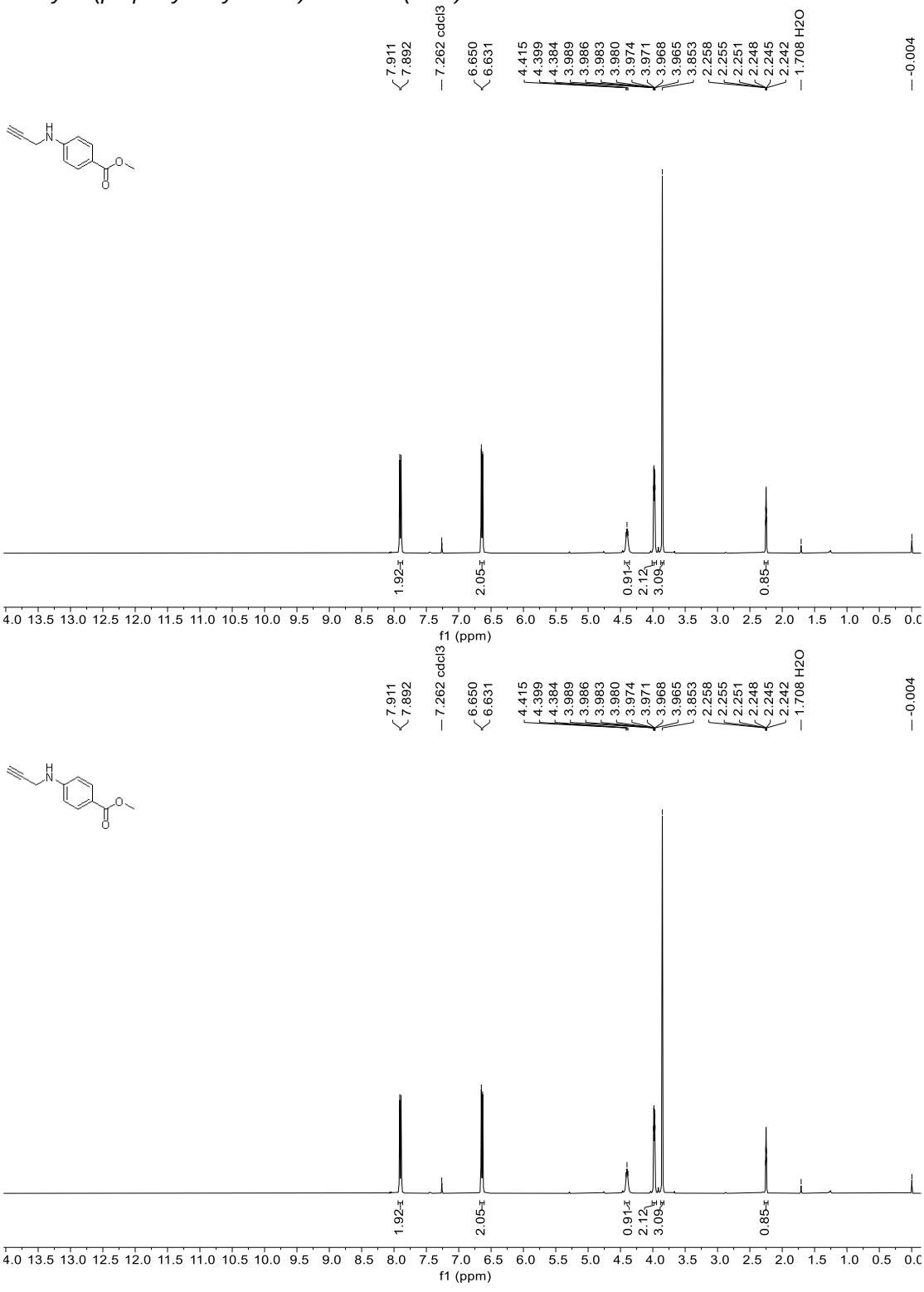


*4-(prop-2-yn-1-ylamino)benzoic acid (**1-9**).*

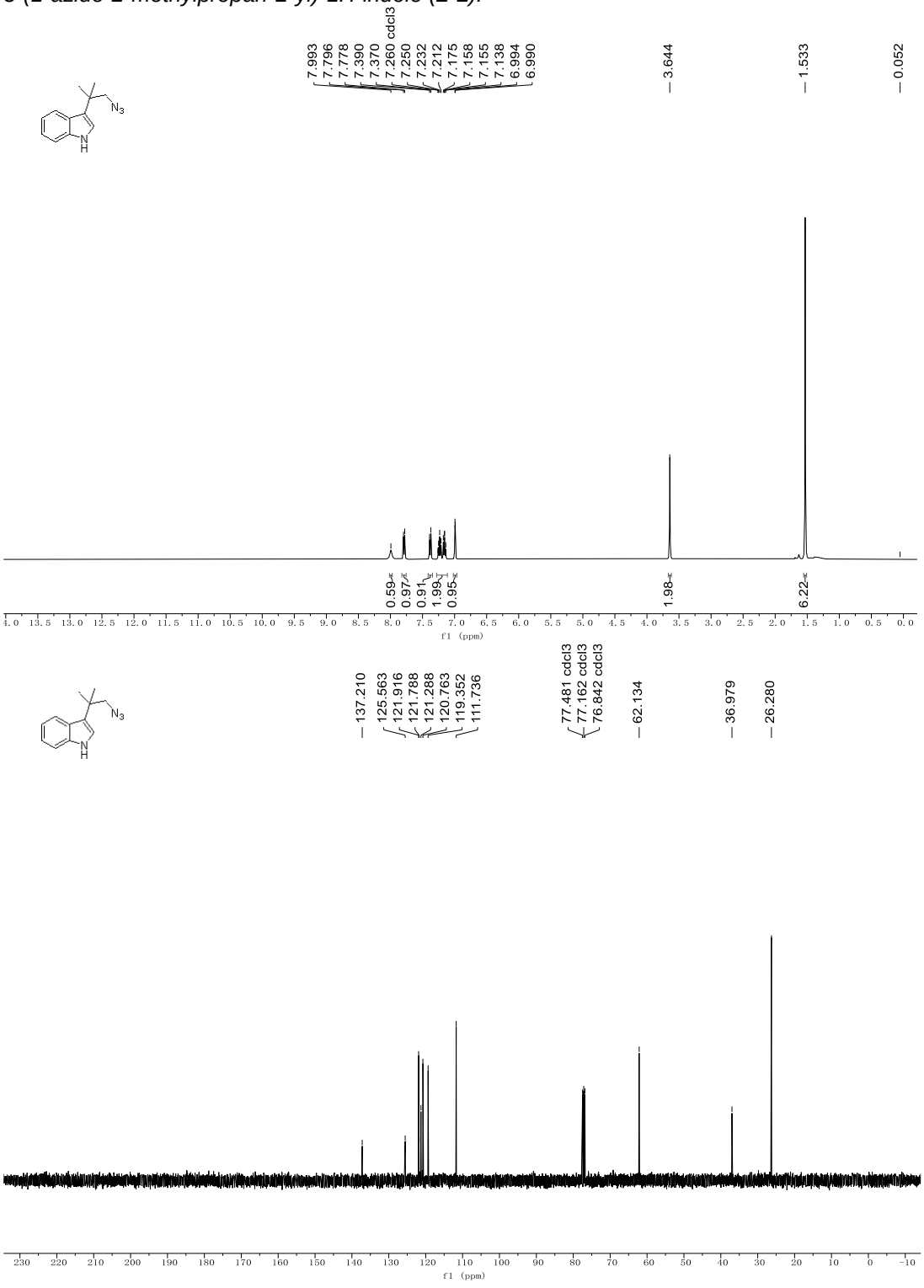




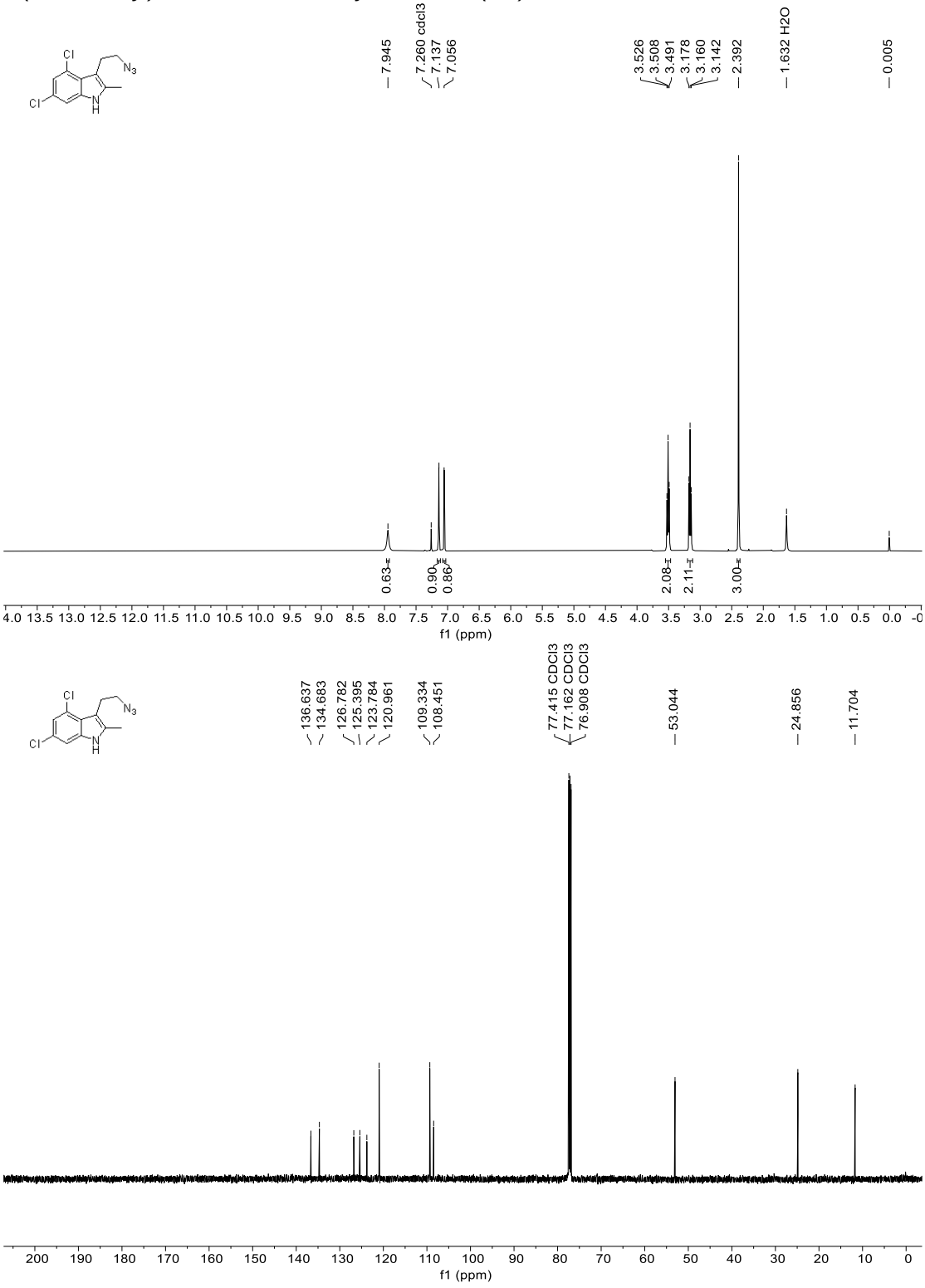
*methyl 4-(prop-2-yn-1-ylamino)benzoate (**1-10**).*



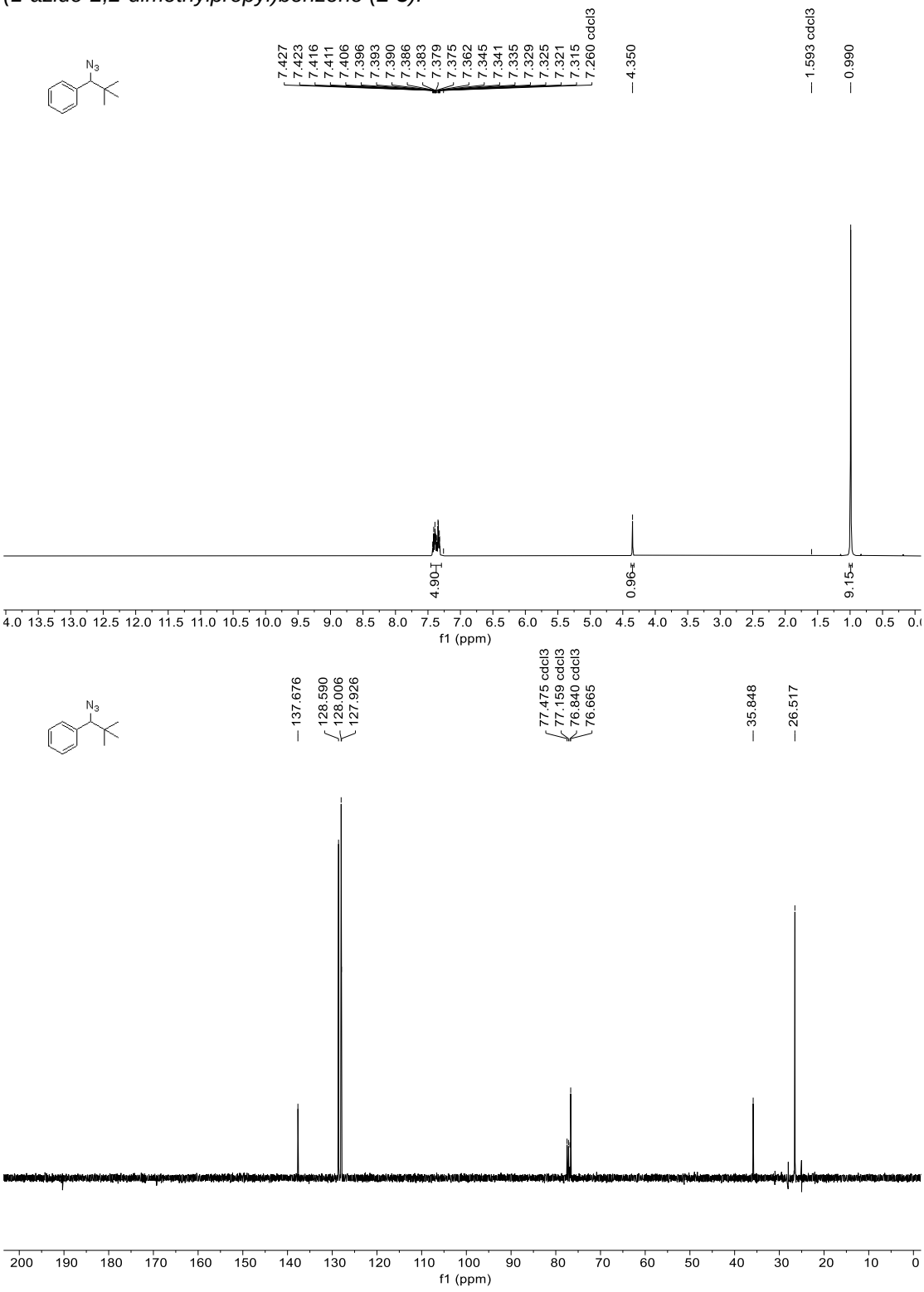
*3-(1-azido-2-methylpropan-2-yl)-1H-indole (**2-1**).*



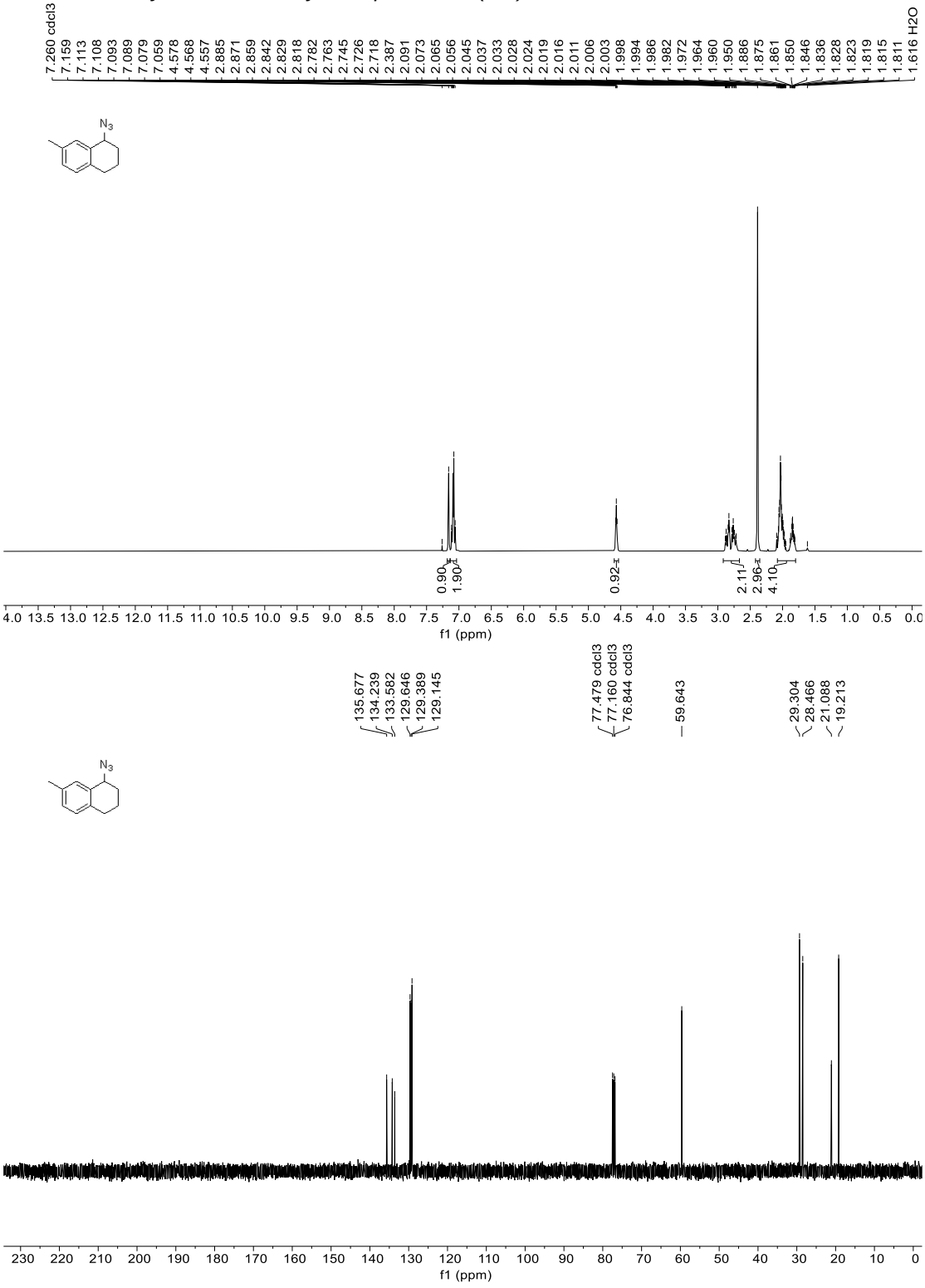
*3-(2-azidoethyl)-4,6-dichloro-2-methyl-1H-indole (**2-2**).*



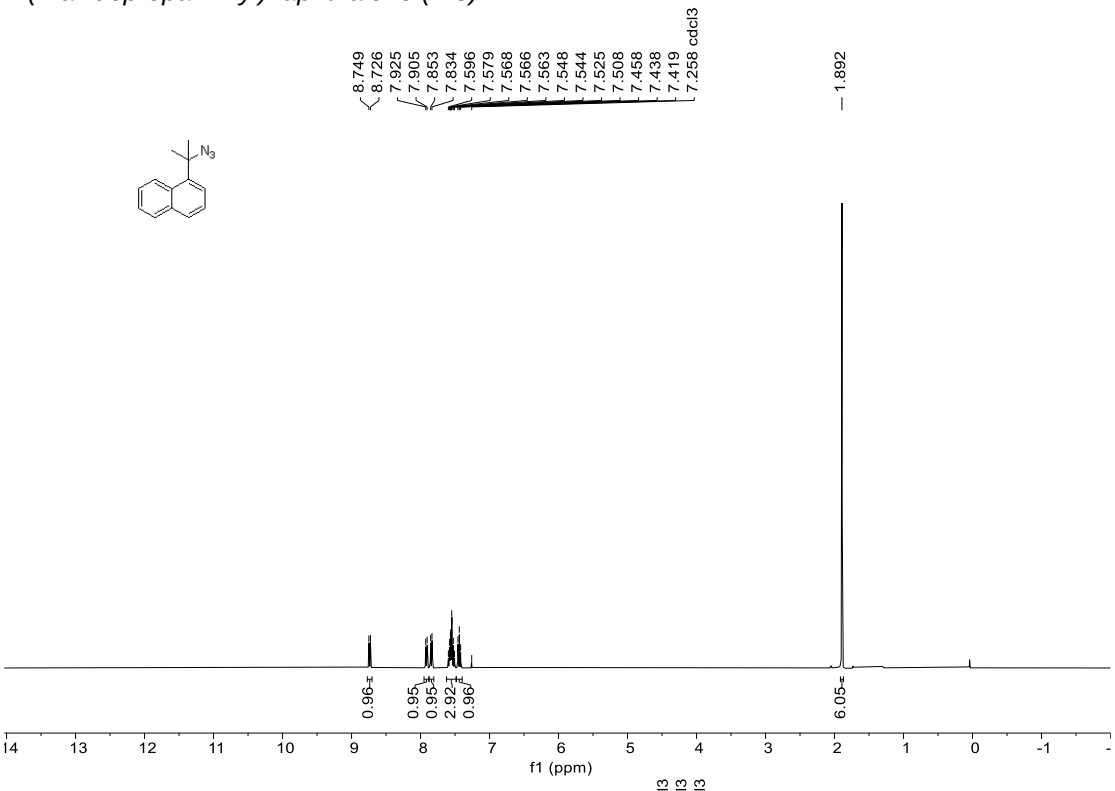
*(1-azido-2,2-dimethylpropyl)benzene (**2-3**).*

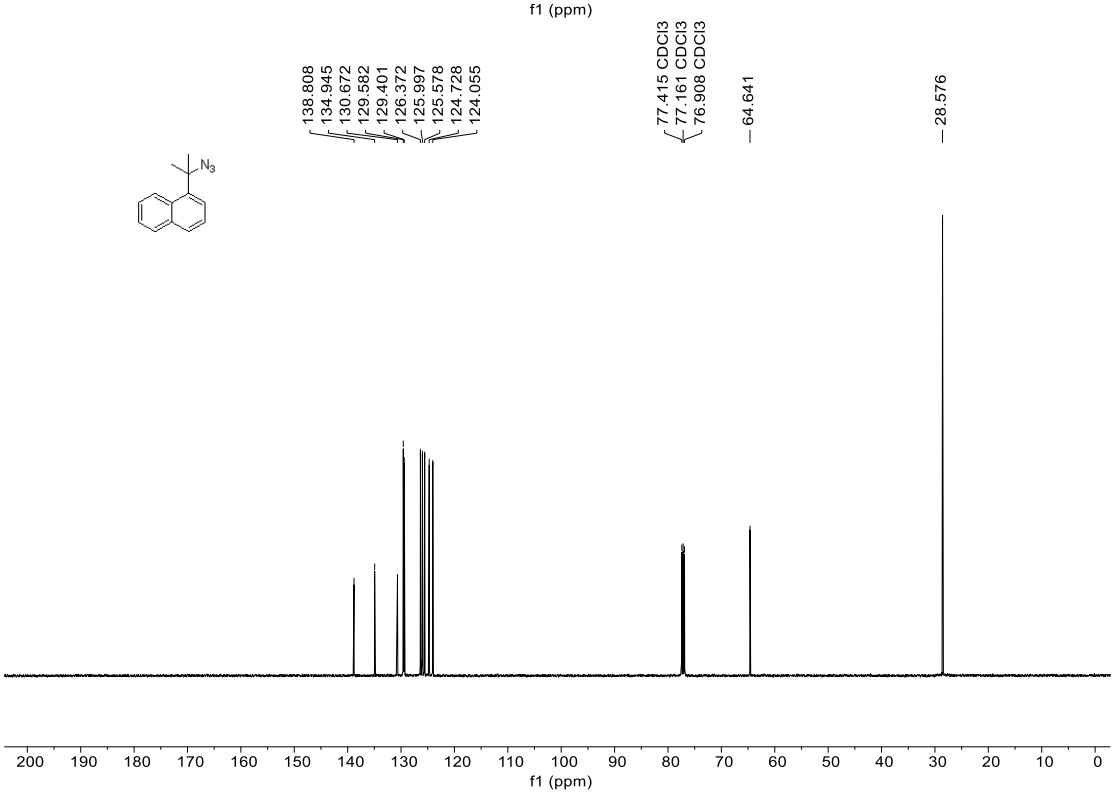


*1-azido-7-methyl-1,2,3,4-tetrahydronaphthalene (**2-4**).*

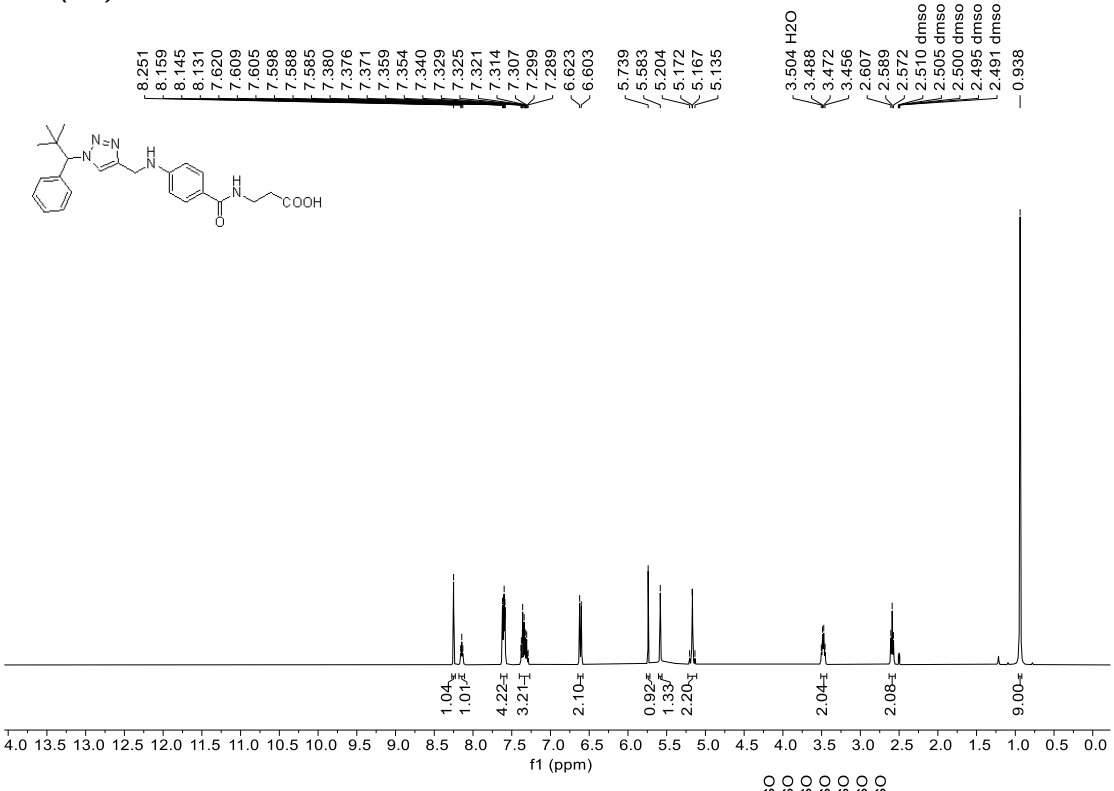


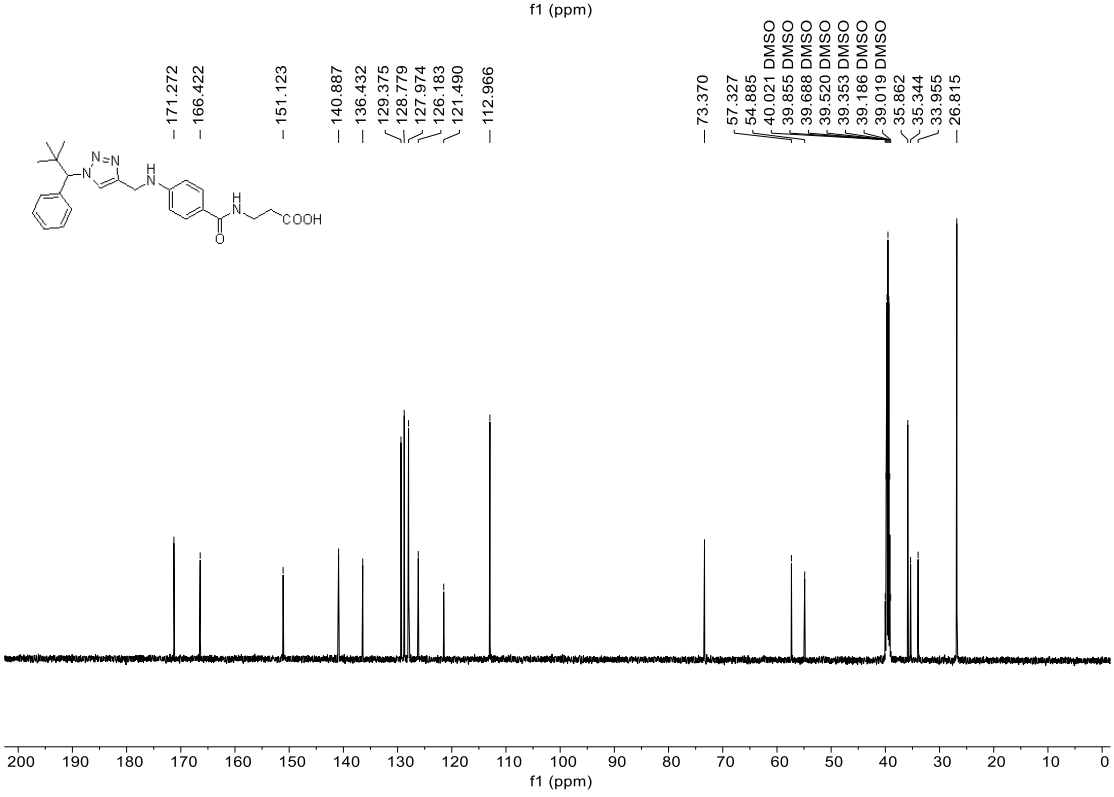
*1-(2-azidopropan-2-yl)naphthalene (**2-5**).*



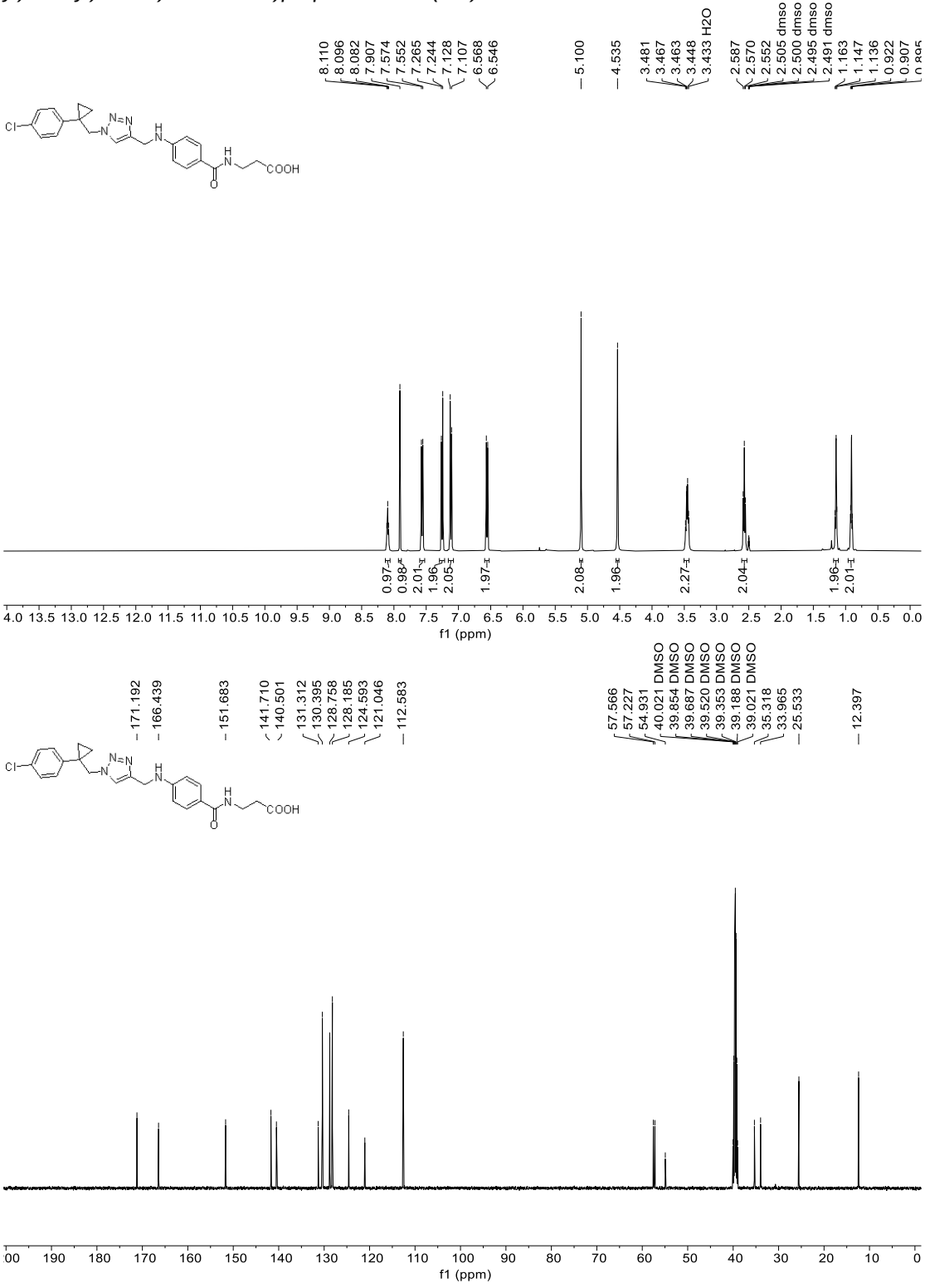


*3-(4-(((1-(2,2-dimethyl-1-phenylpropyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-1**).*

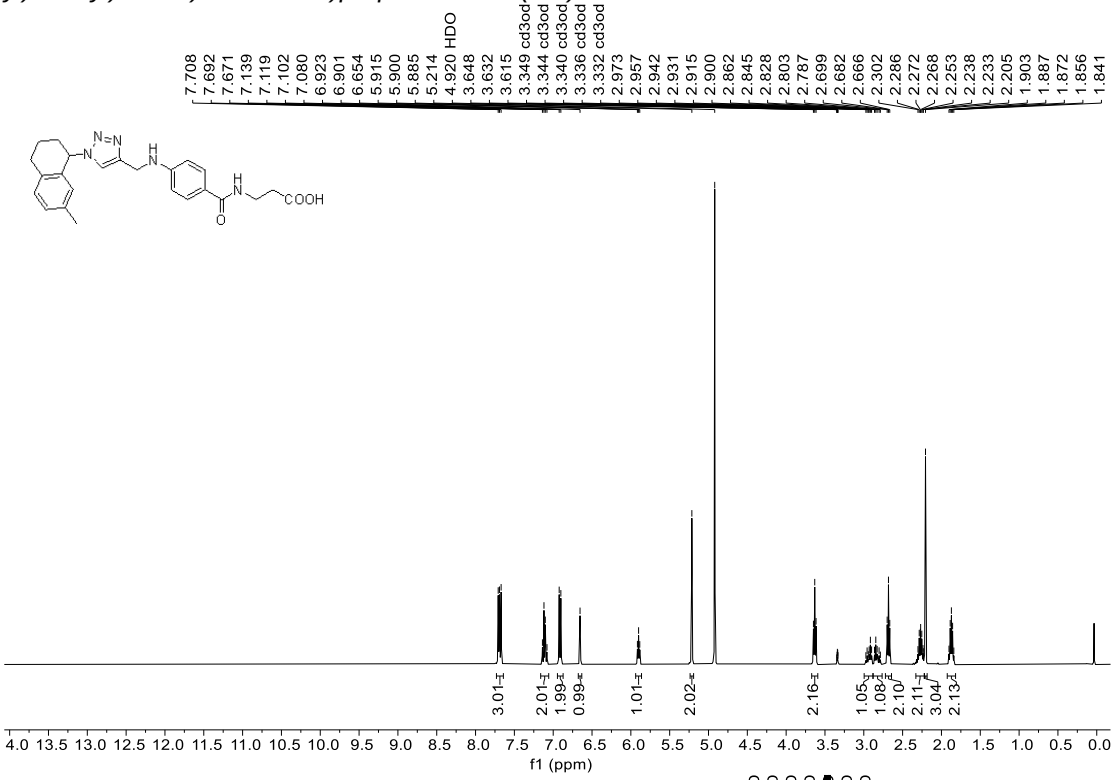


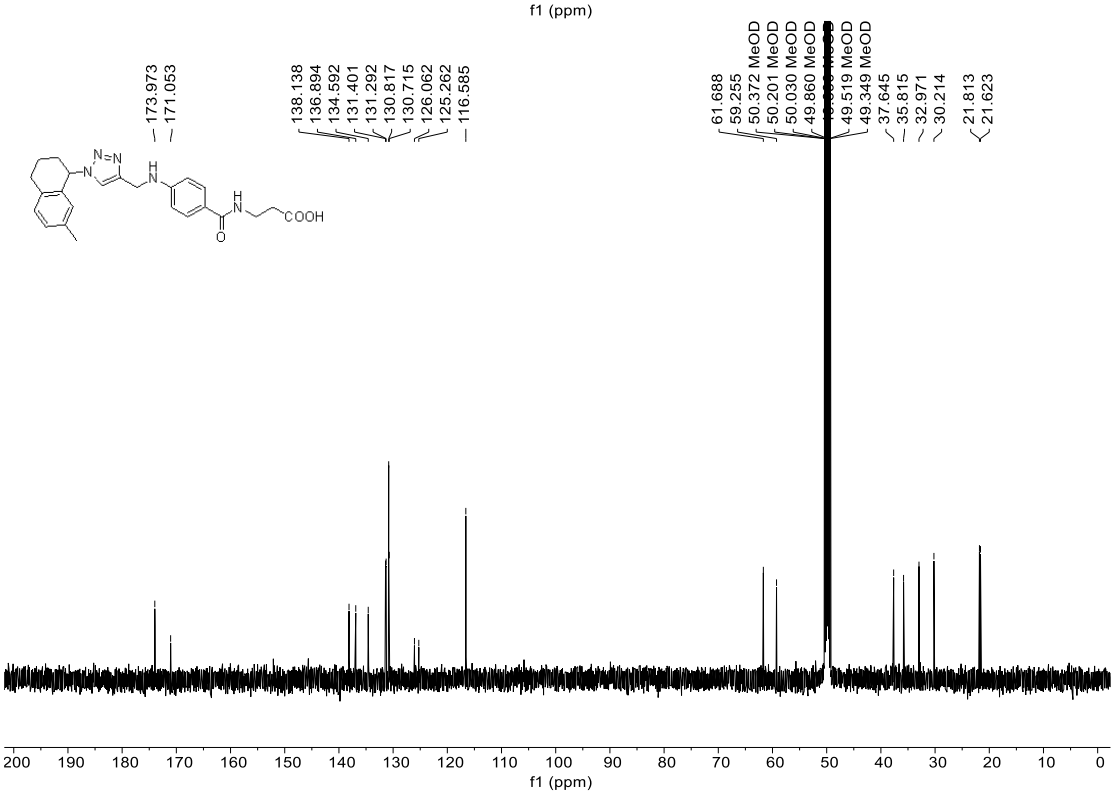


*3-(4-(((1-((1-(4-chlorophenyl)cyclopropyl)methyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-2**).*

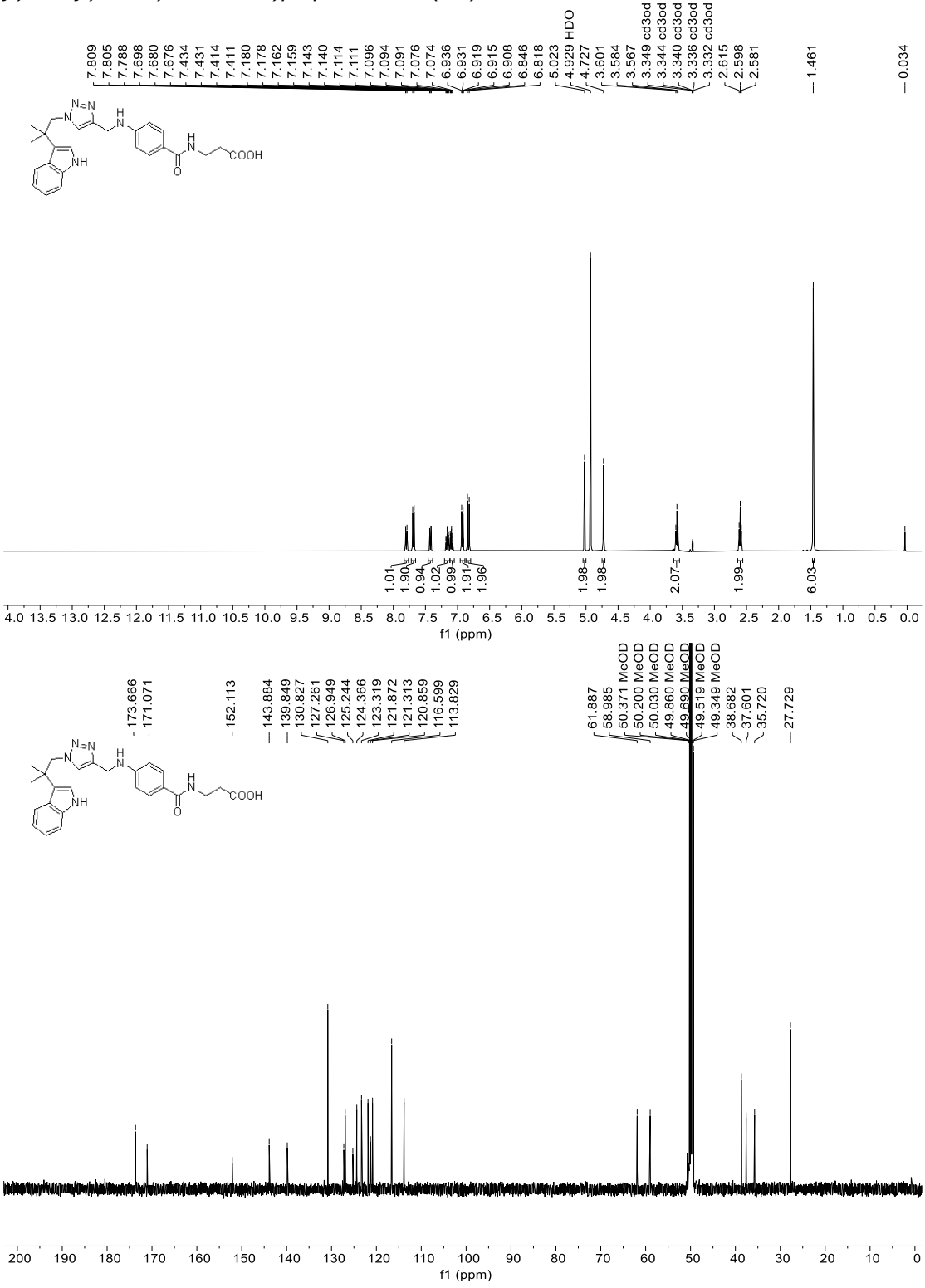


*3-(4-(((1-(7-methyl-1,2,3,4-tetrahydronaphthalen-1-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-3**).*

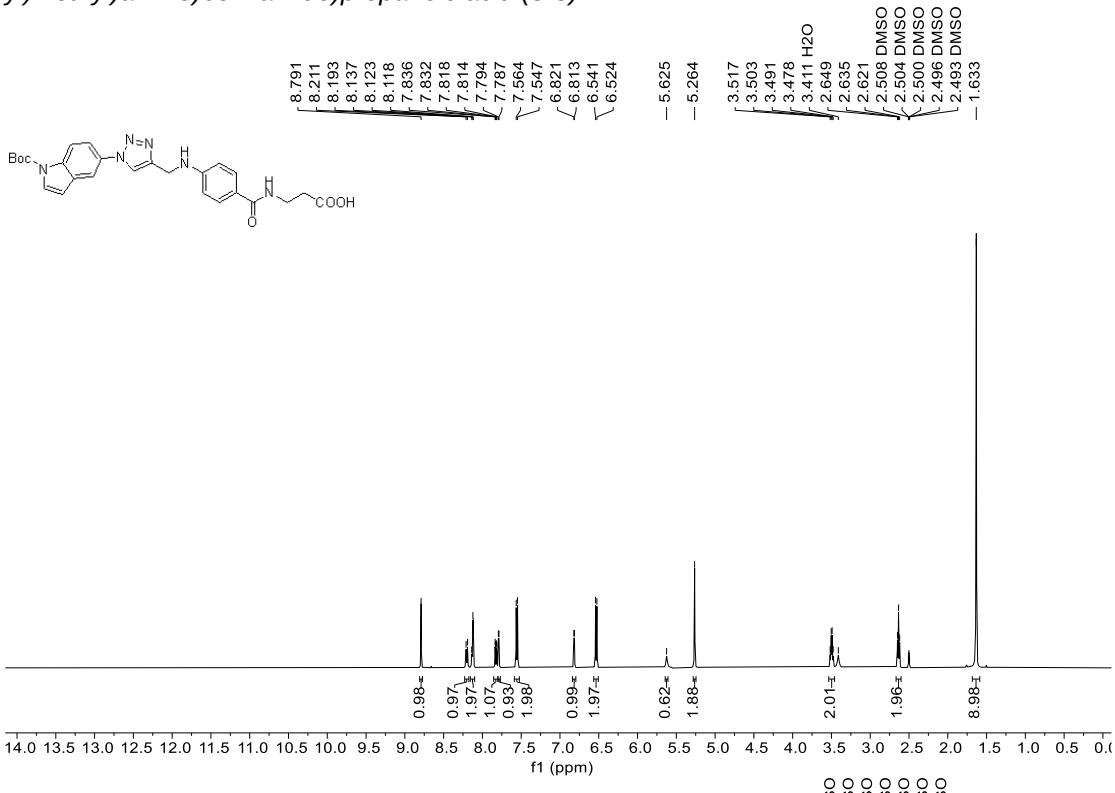


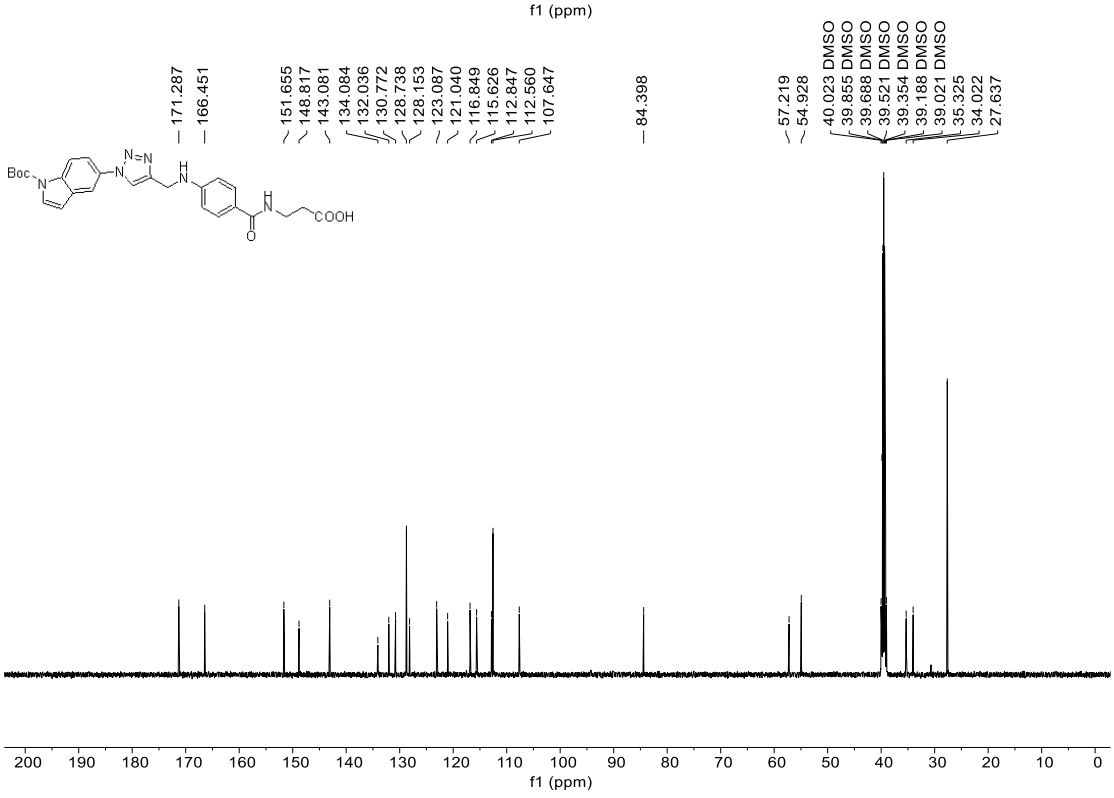


*3-(4-(((1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-4**).*

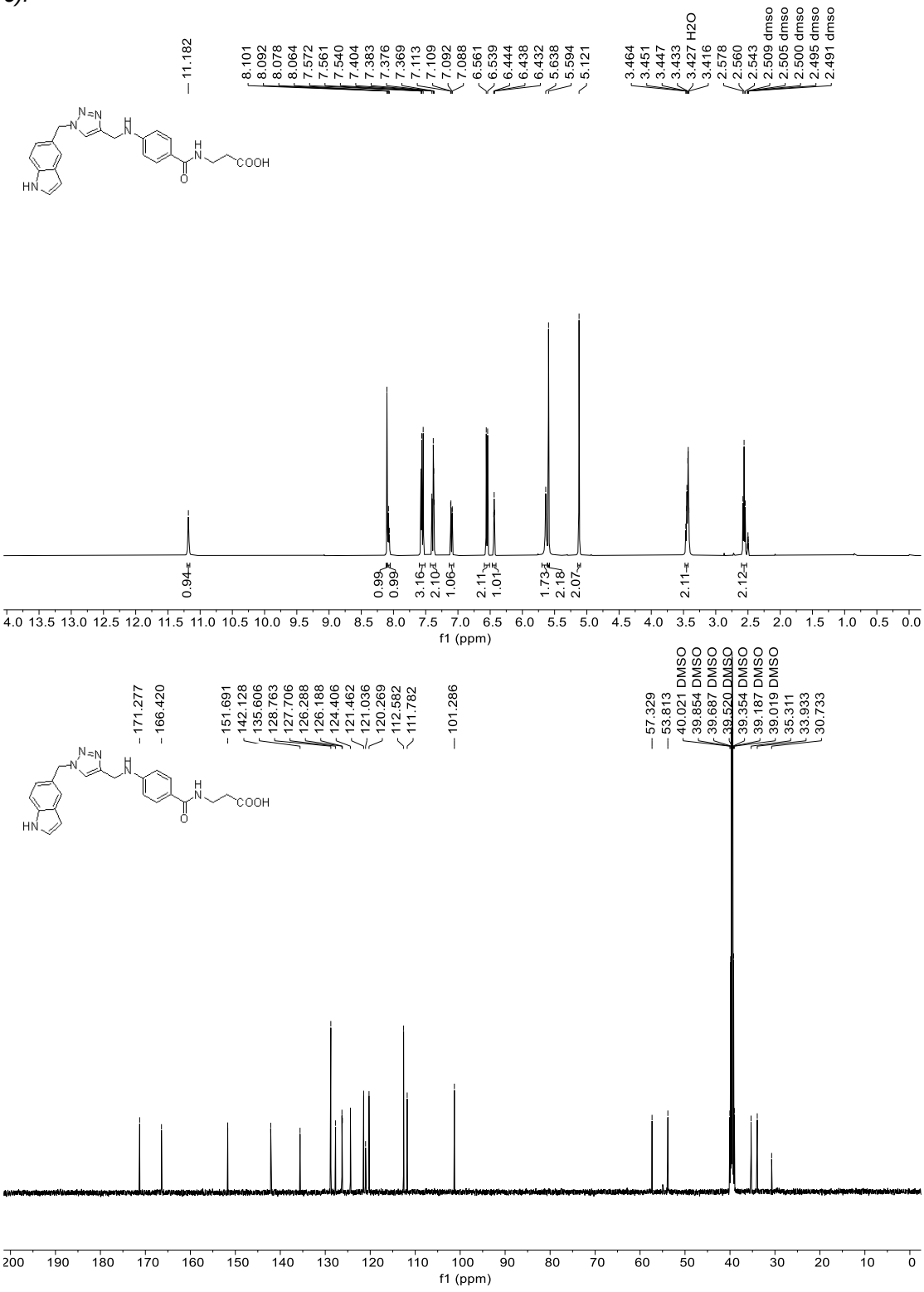


*3-(4-(((1-(1-(tert-butoxycarbonyl)-1H-indol-5-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-5**).*

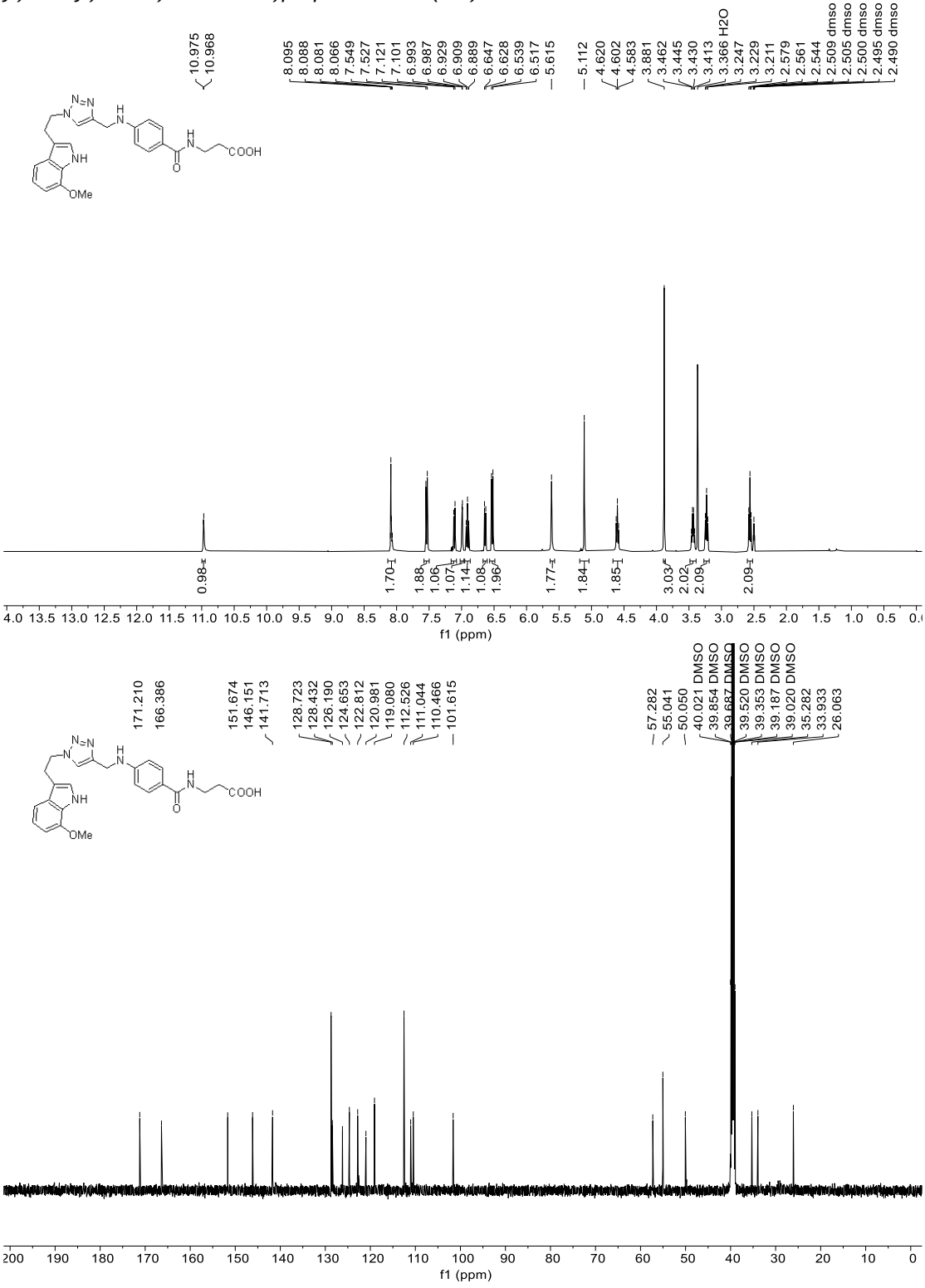




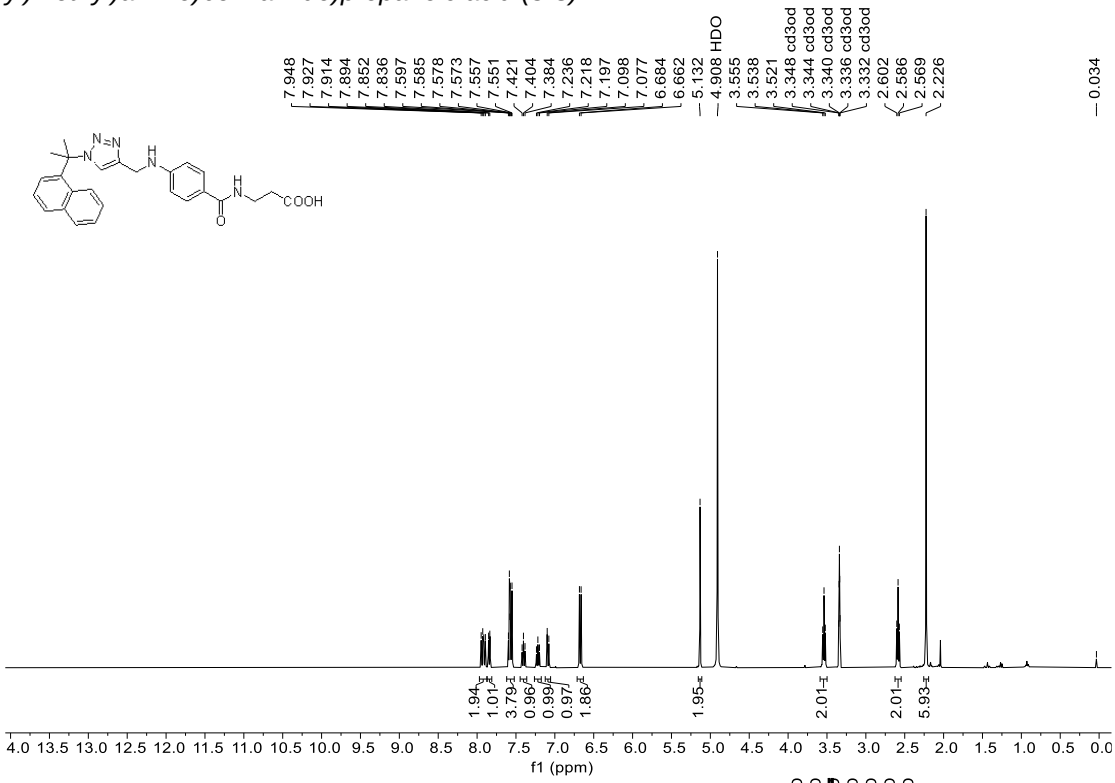
*3-(4-(((1-((1H-indol-5-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-6**).*

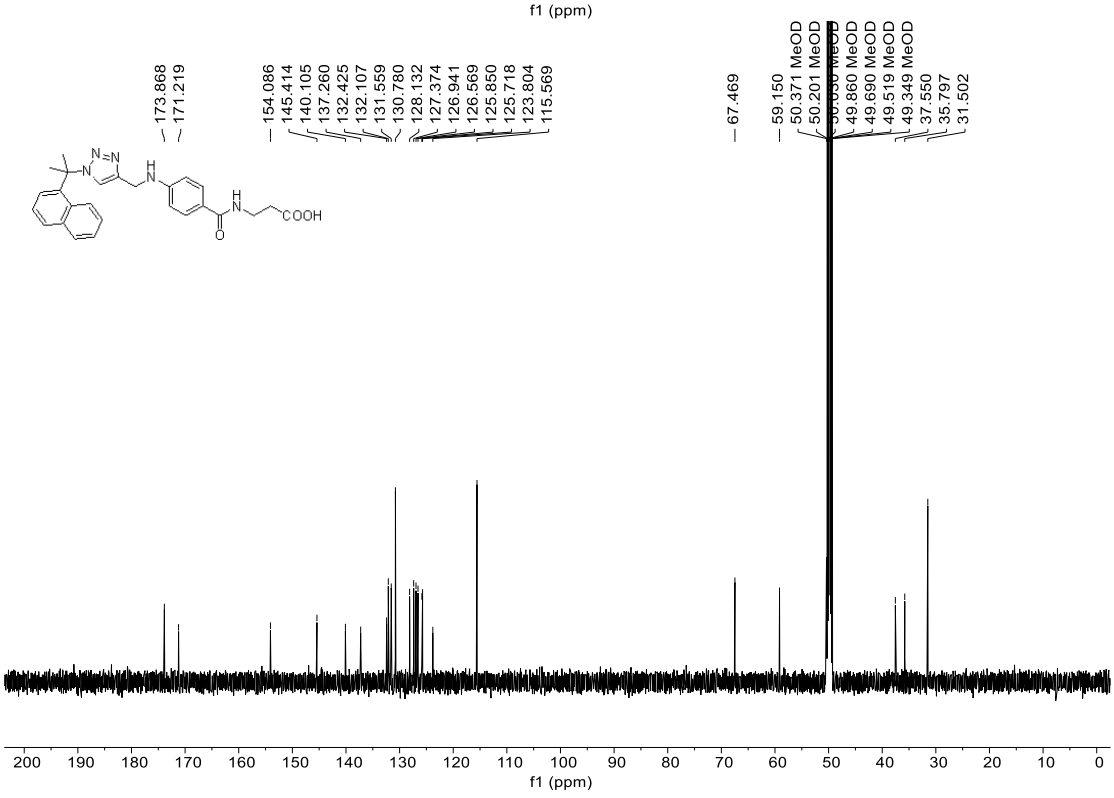


*3-(4-(((1-(2-(7-methoxy-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-7**).*

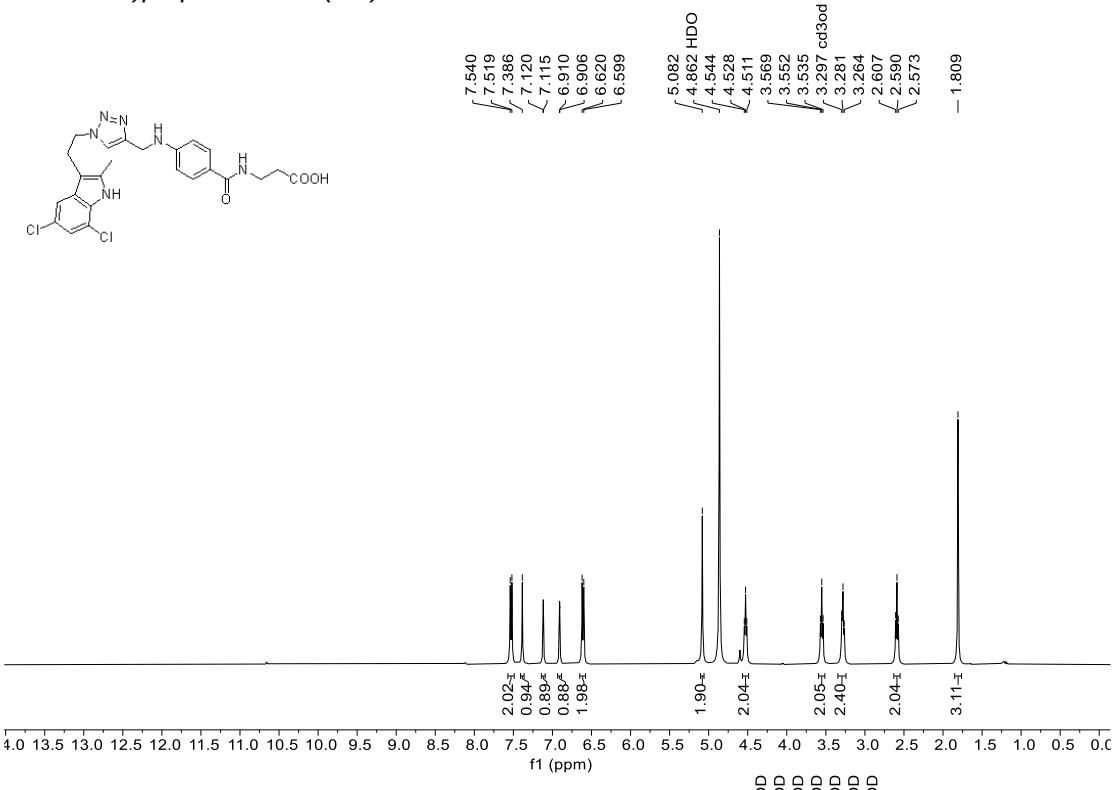


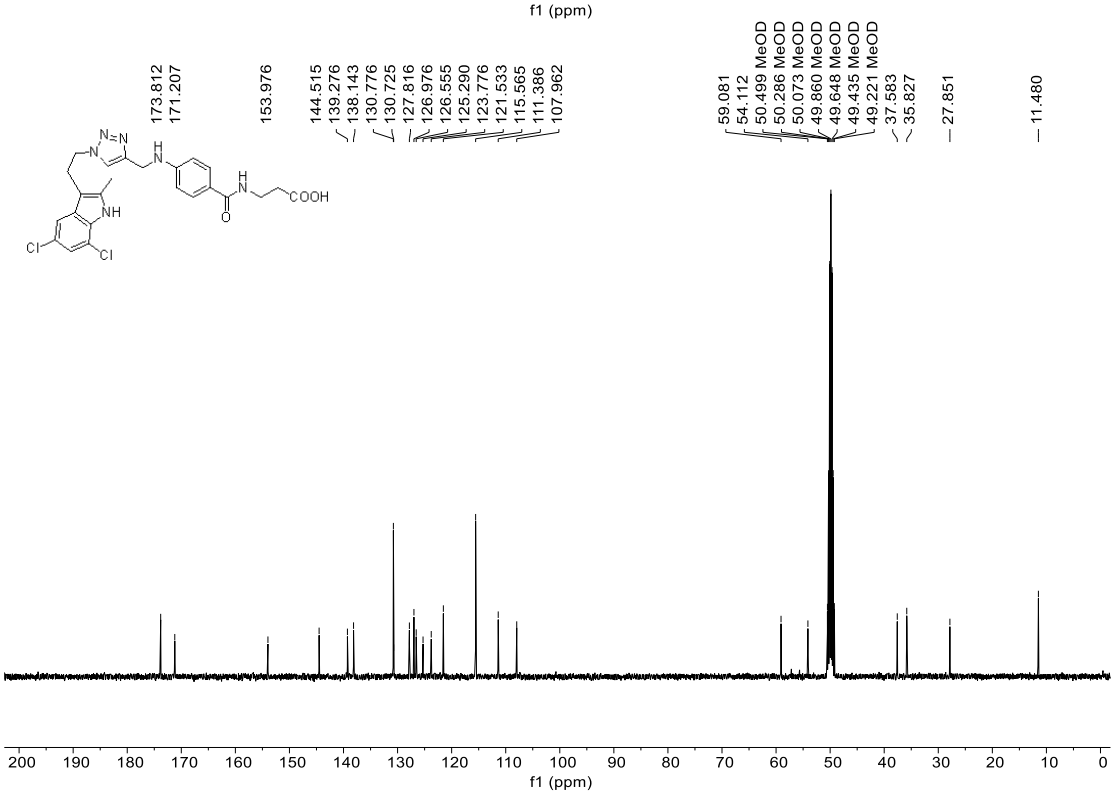
*3-(4-(((1-(2-(naphthalen-1-yl)propan-2-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-8**).*



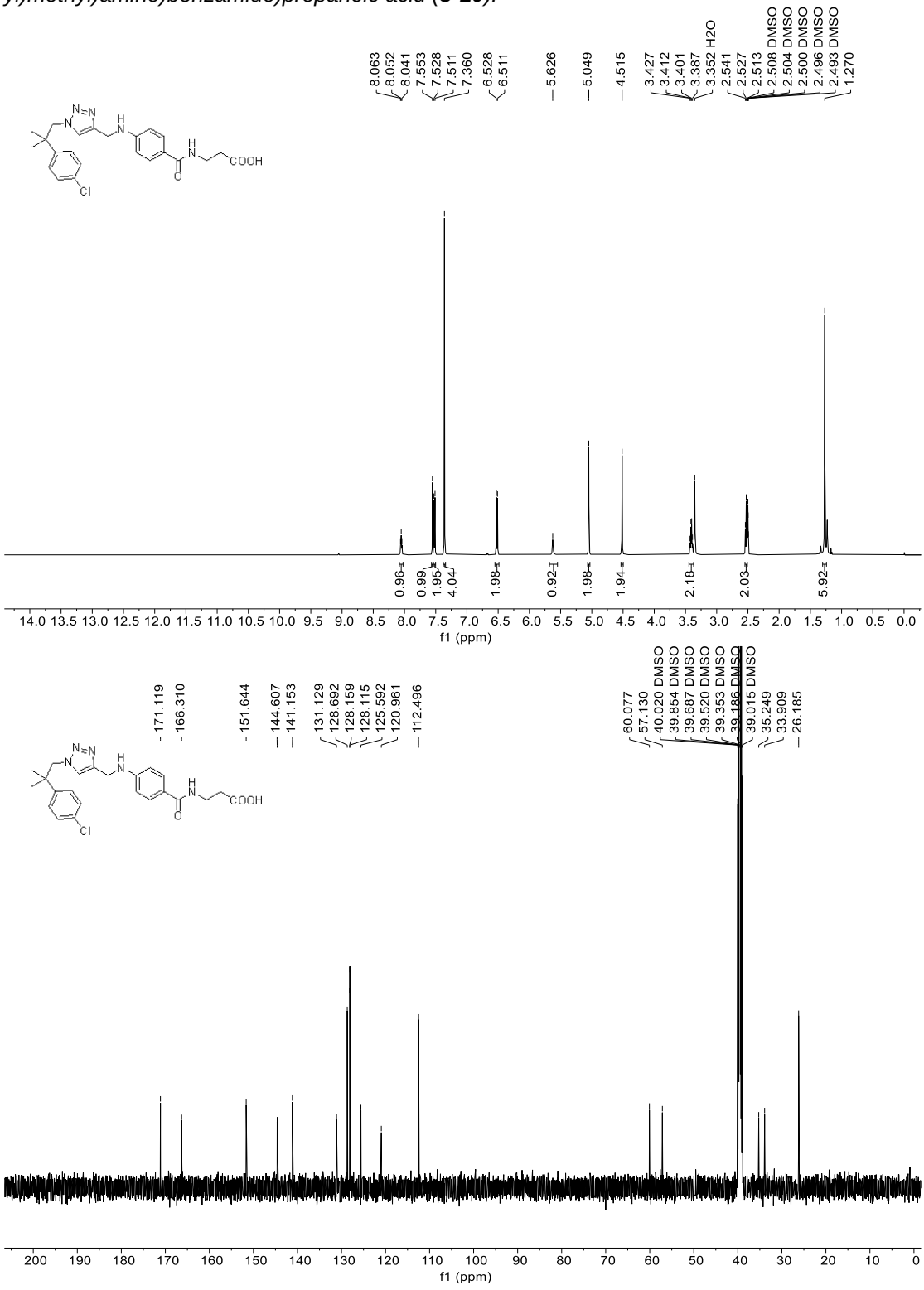


*3-(4-(((1-(2-(5,7-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino) benzamido)propanoic acid (**3-9**).*

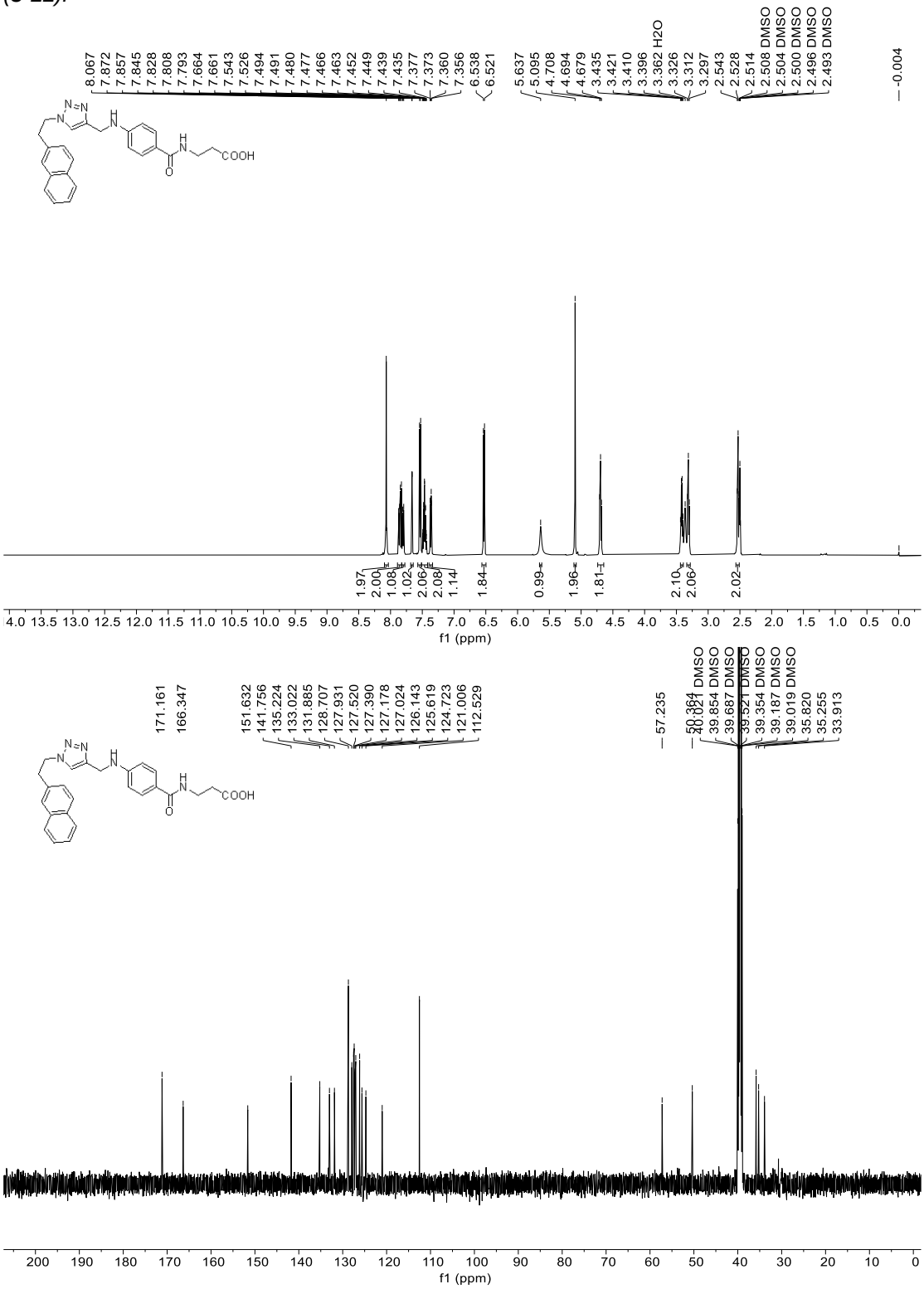




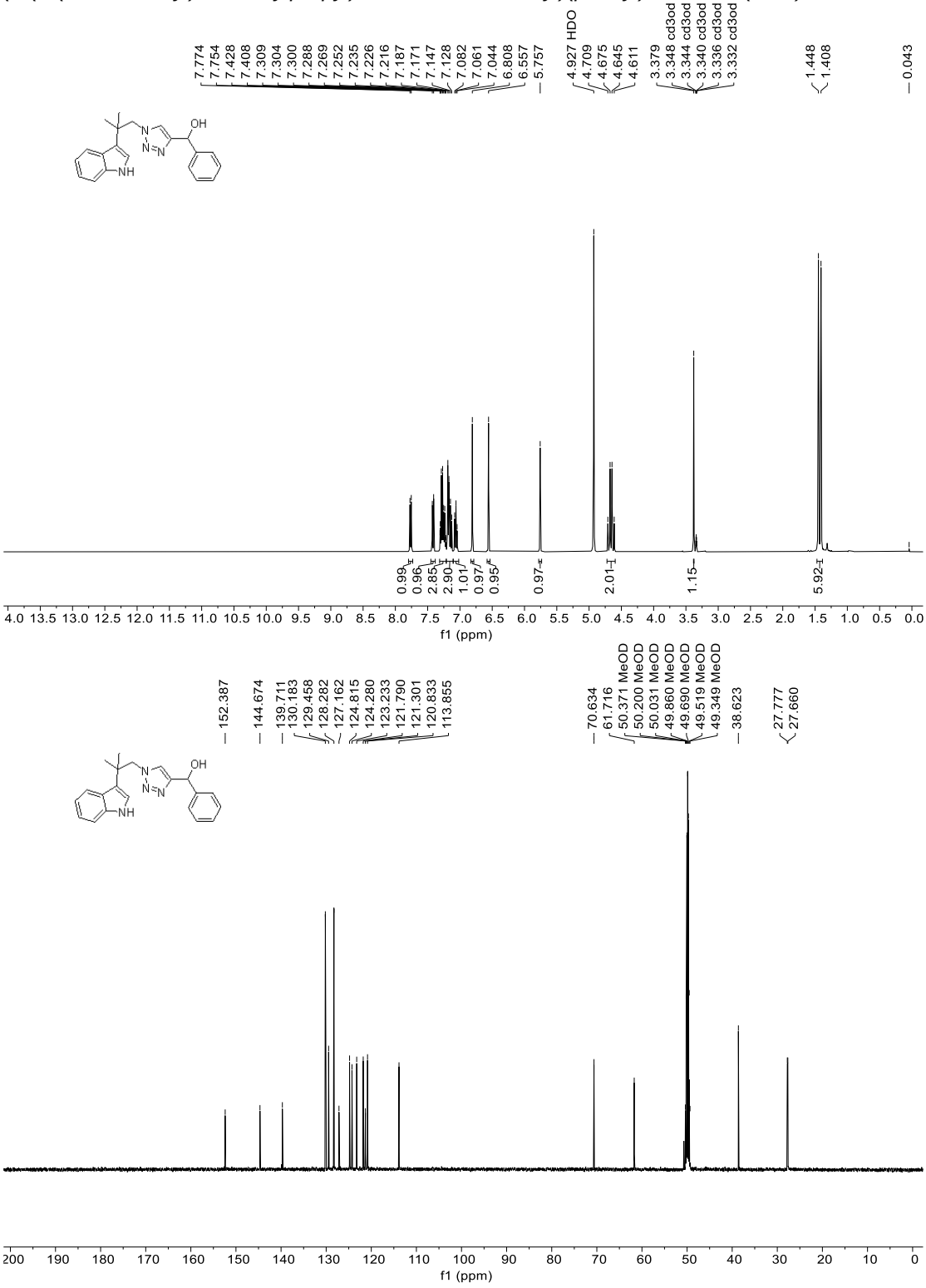
*3-(4-(((1-(2-(4-chlorophenyl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-10**).*



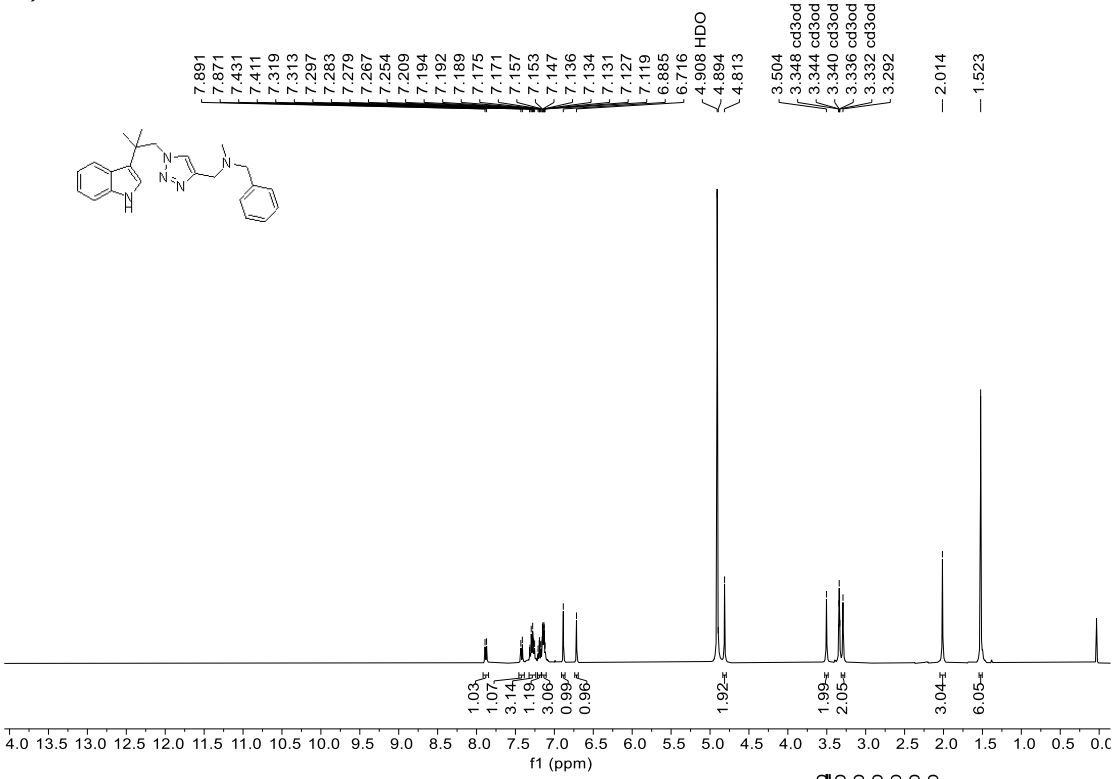
*3-(4-(((1-(2-(naphthalen-2-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzamido)propanoic acid (**3-11**).*

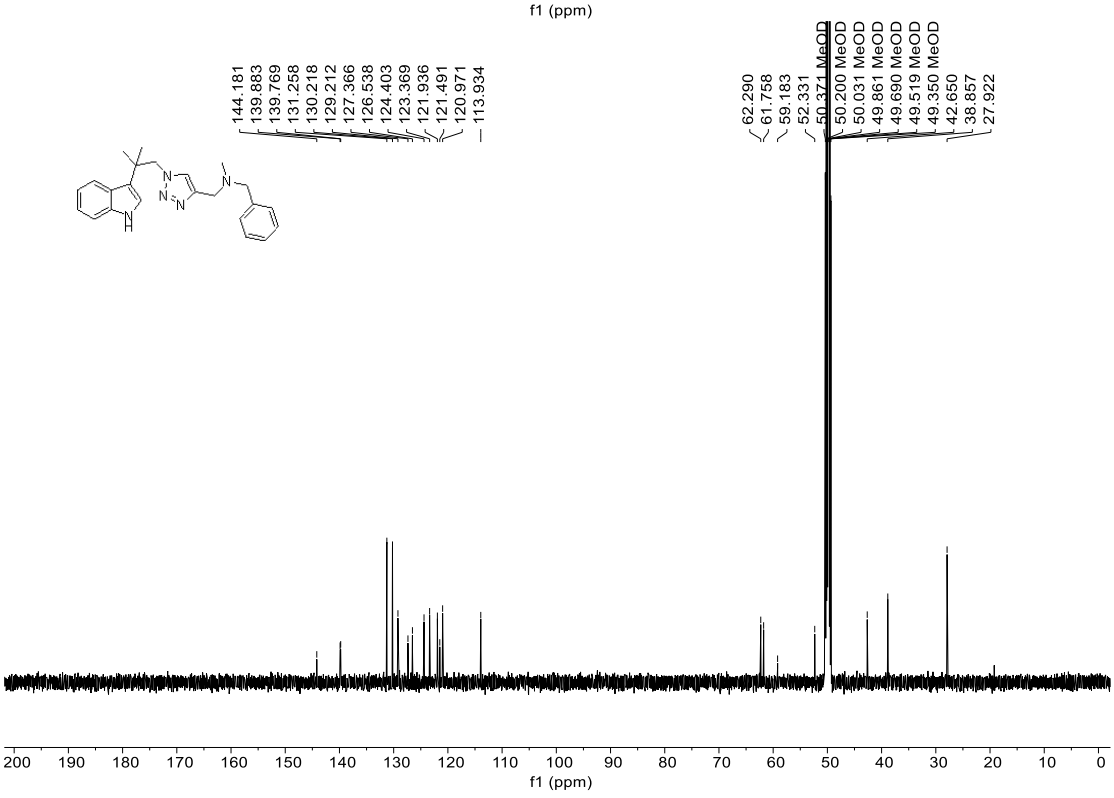


*(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)(phenyl)methanol (**3-12**).*

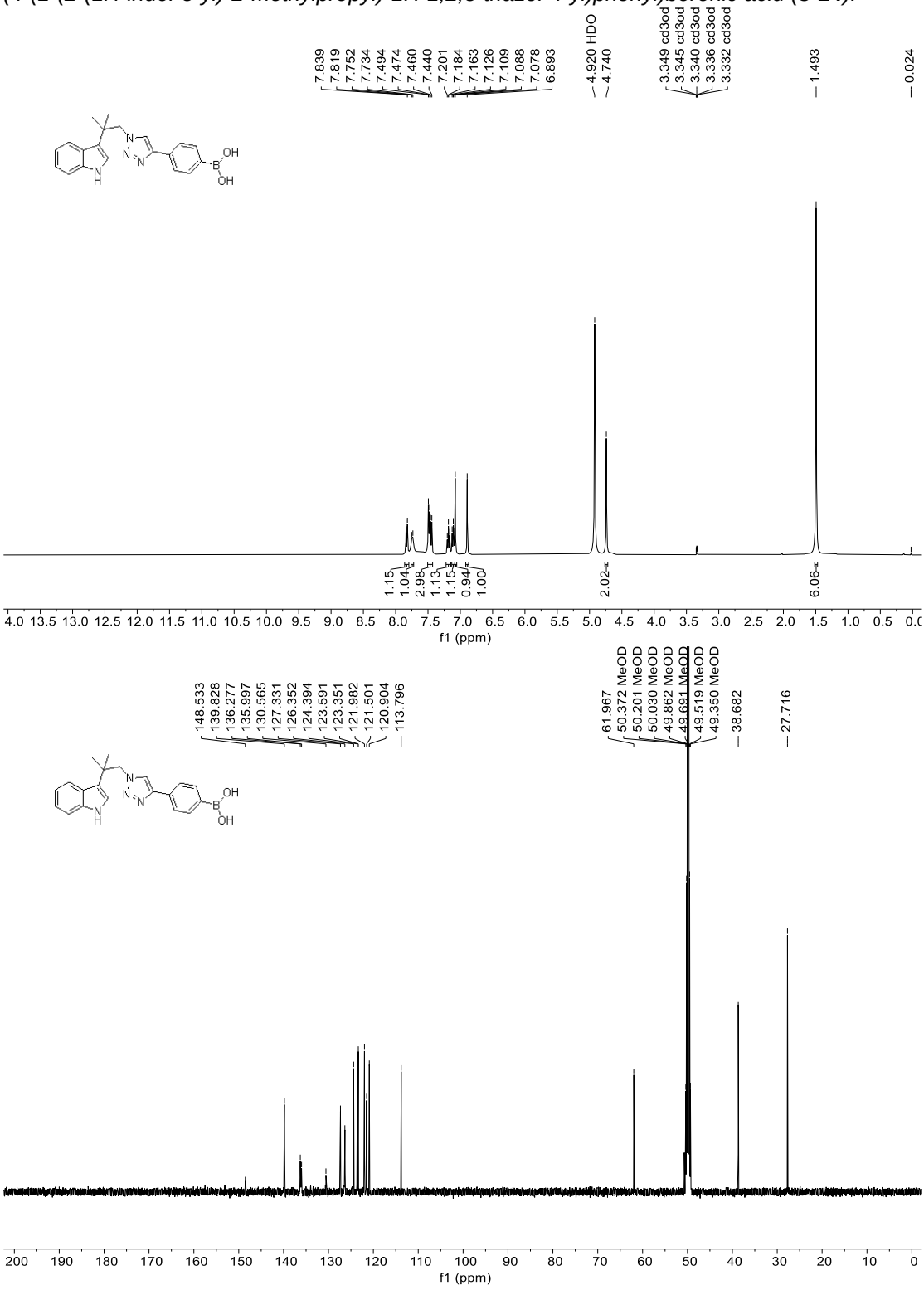


*1-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)-N-benzyl-N-methylmethanamine (**3-13**).*

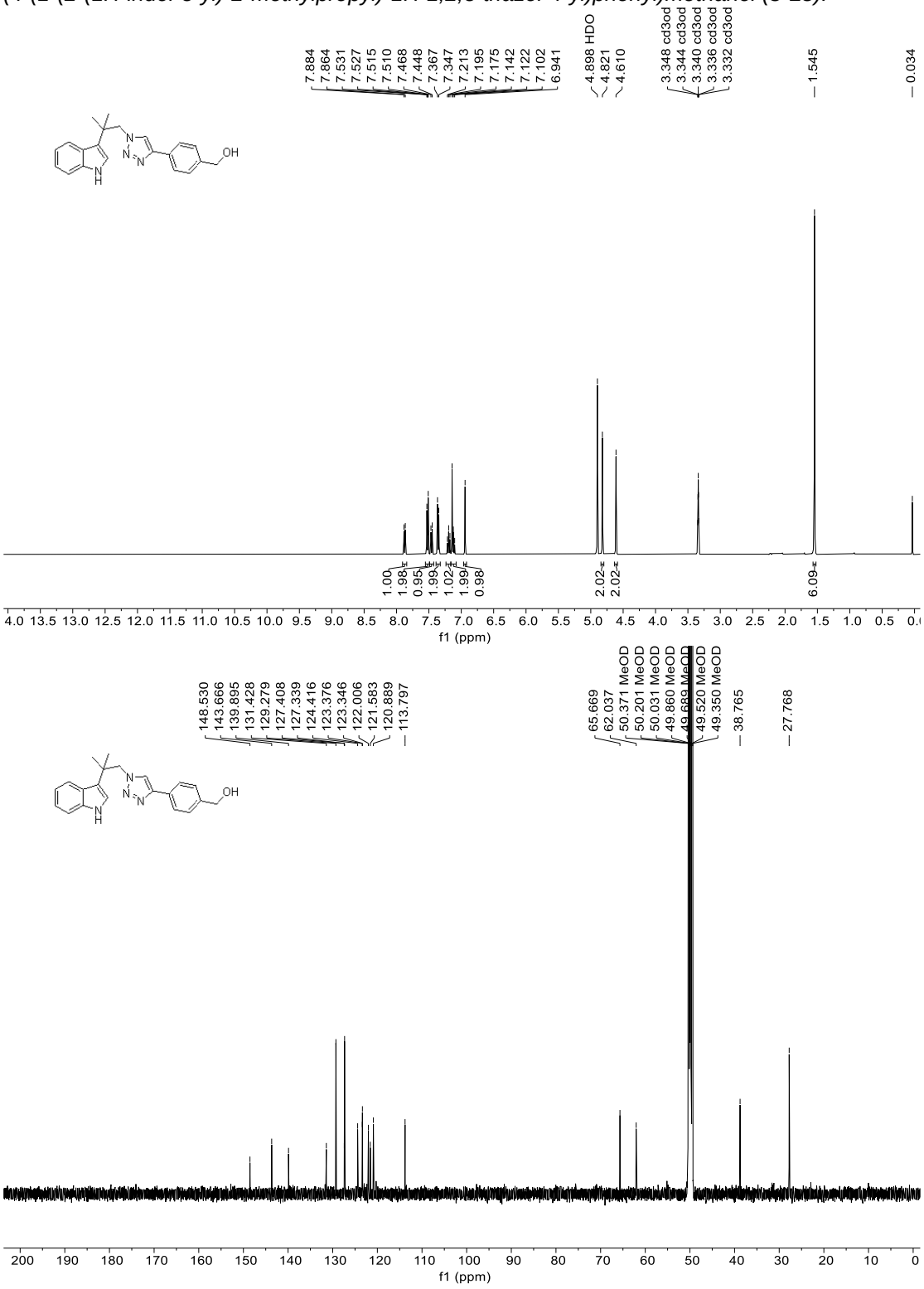




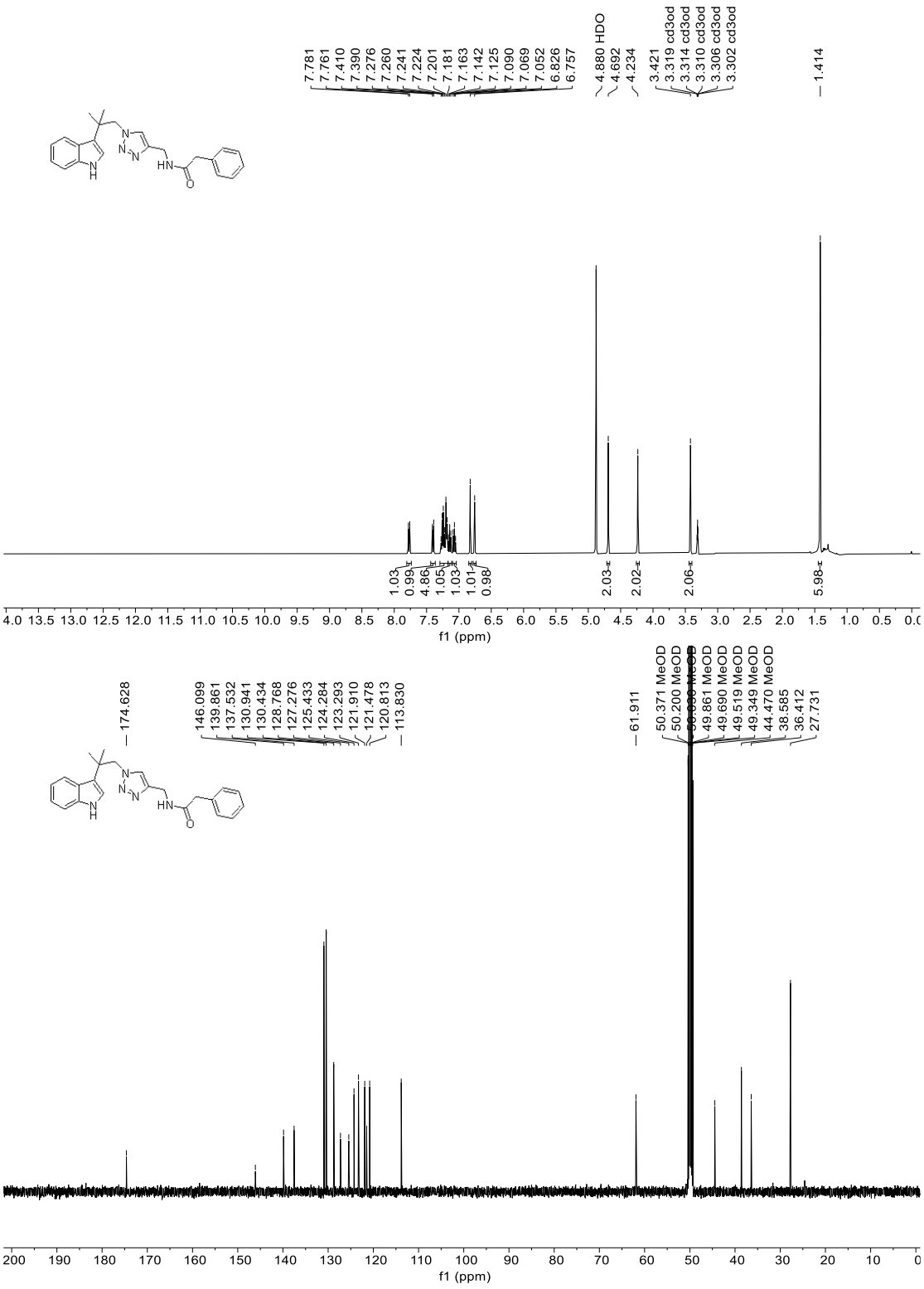
*(4-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)phenyl)boronic acid (**3-14**).*



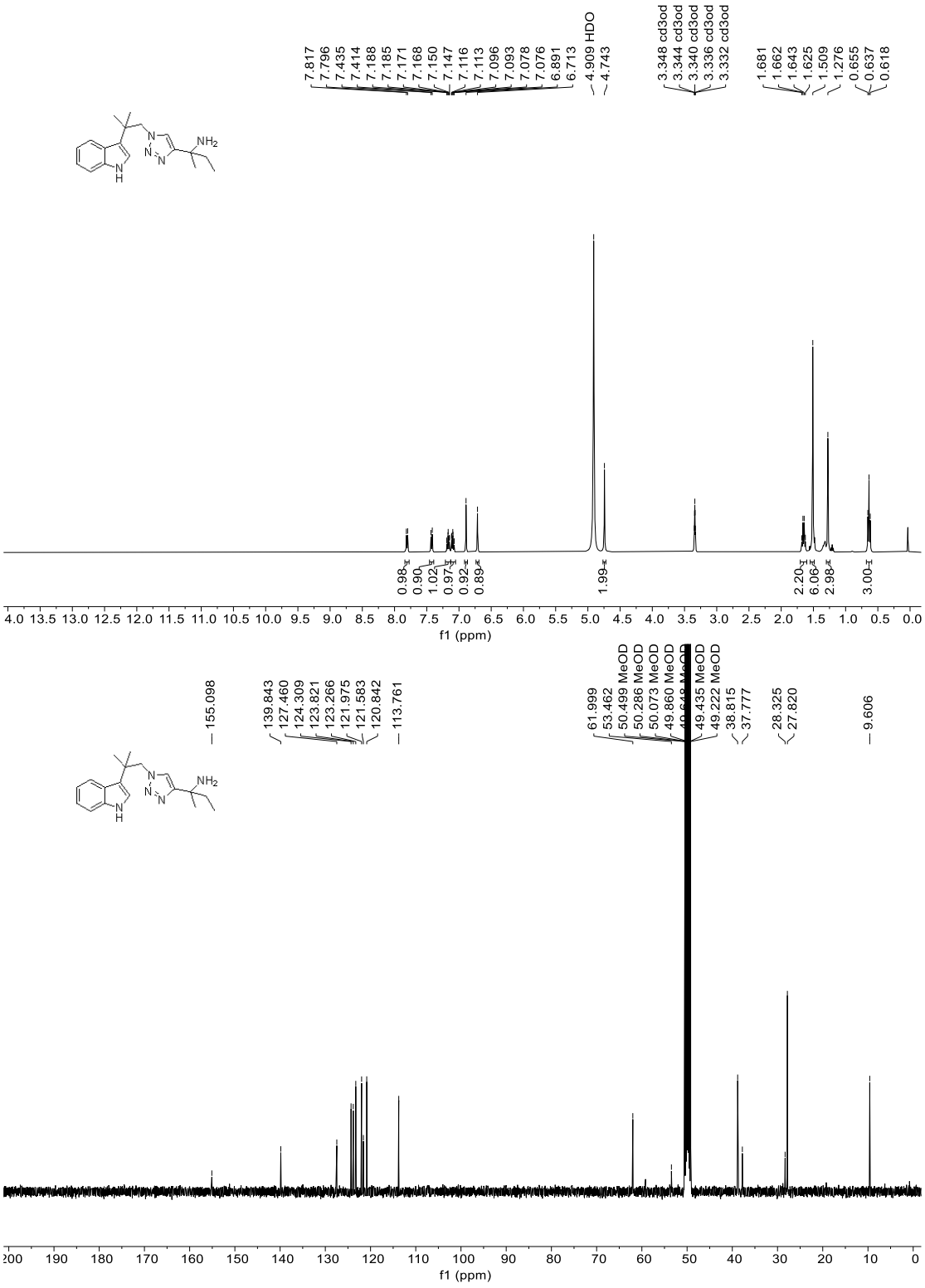
*(4-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)phenyl)methanol (**3-15**).*



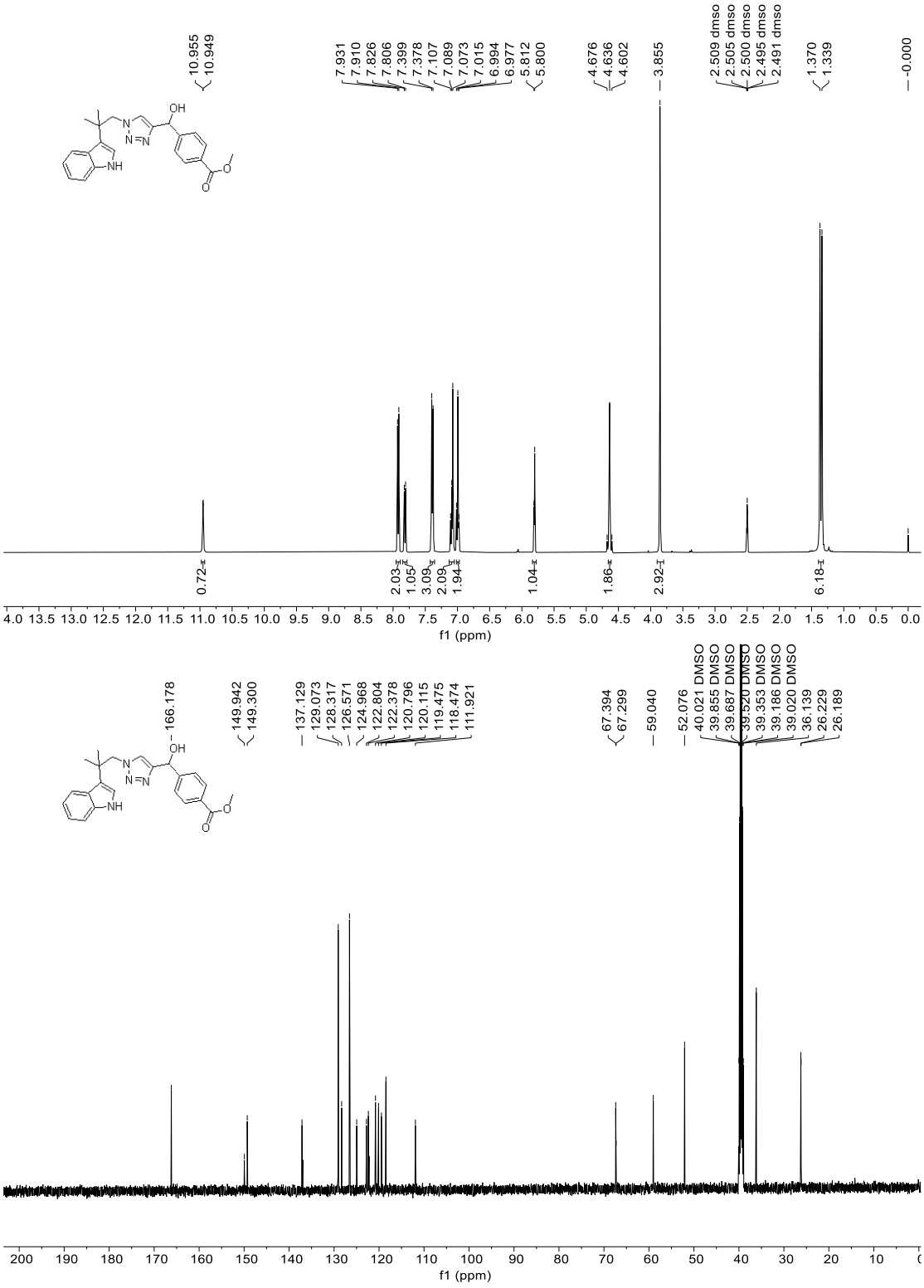
*N-((1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)methyl)-2-phenylacetamide (**3-16**).*



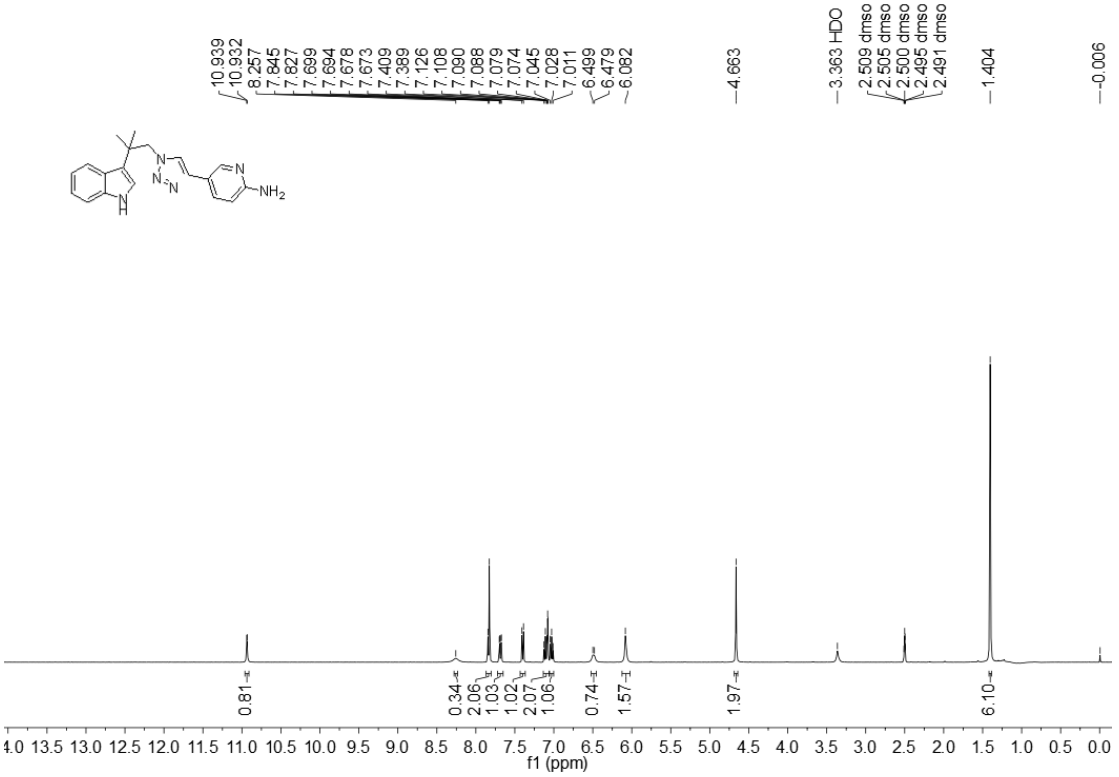
*2-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)butan-2-amine (**3-17**).*

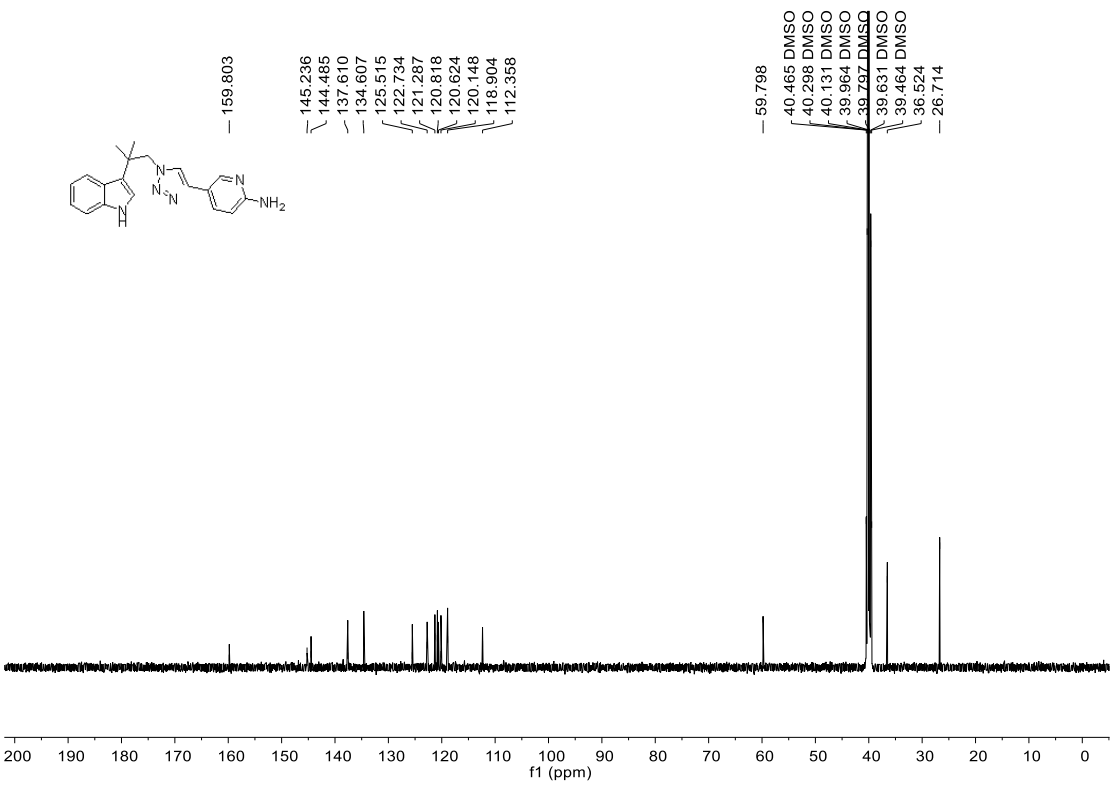


*methyl 4-((1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)(hydroxy)methyl)benzoate (**3-18**).*

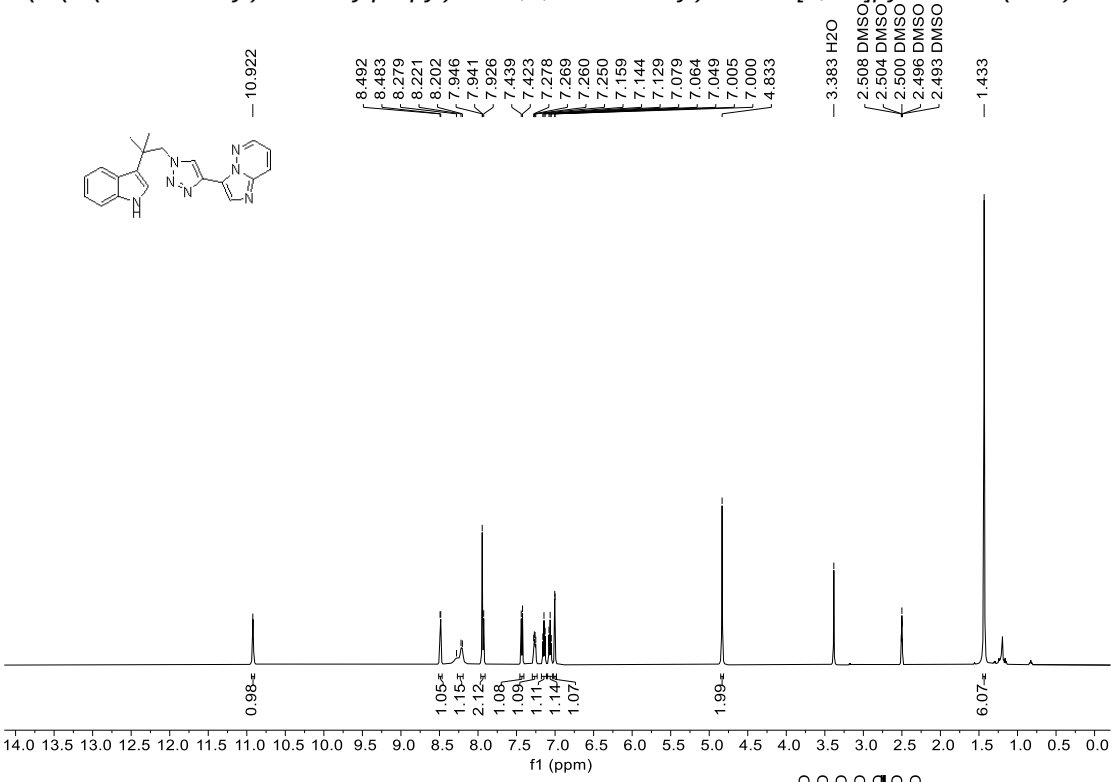


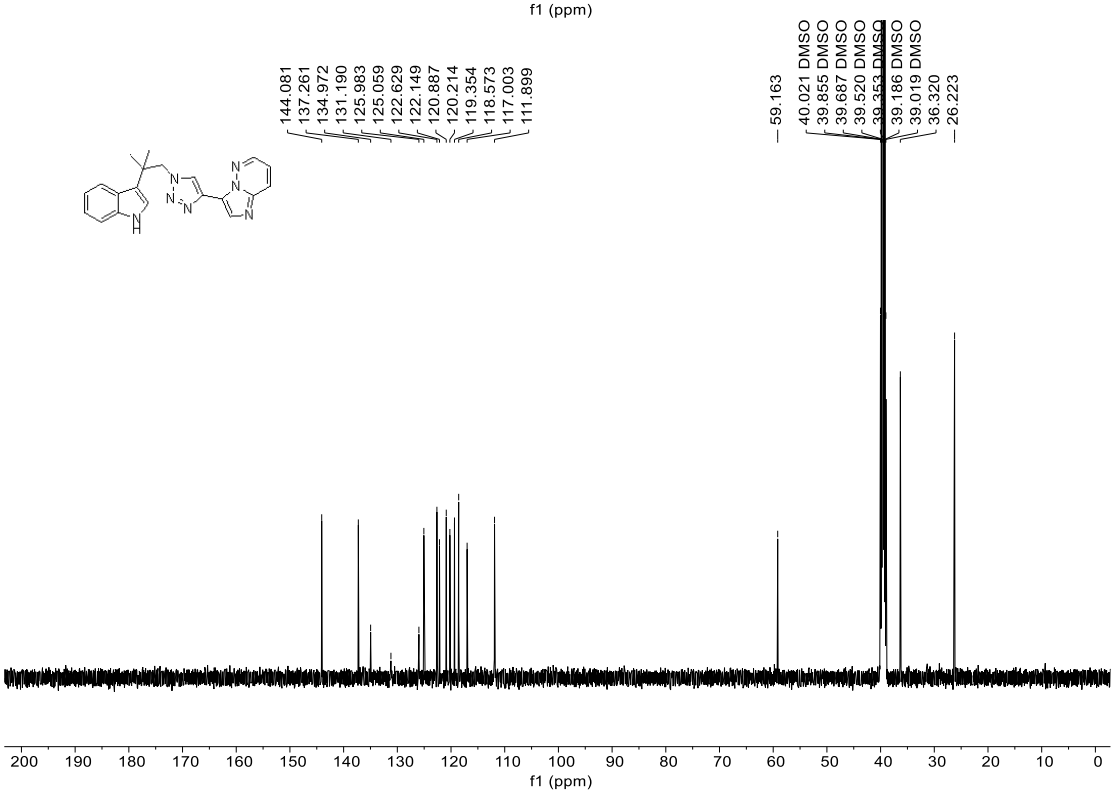
*6-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)pyridin-3-amine (**3-19**).*


10.939
10.932
8.257
7.845
7.827
7.699
7.694
7.678
7.673
7.409
7.389
7.126
7.108
7.090
7.088
7.079
7.074
7.045
7.028
7.011
6.499
6.479
6.082
4.663
3.363 HDO
2.509 dmso
2.505 dmso
2.500 dmso
2.495 dmso
2.491 dmso
1.404
-0.006
0.81
0.34
2.06
1.03
1.02
2.07
1.06
0.74
1.57
1.97
6.10
f1 (ppm)

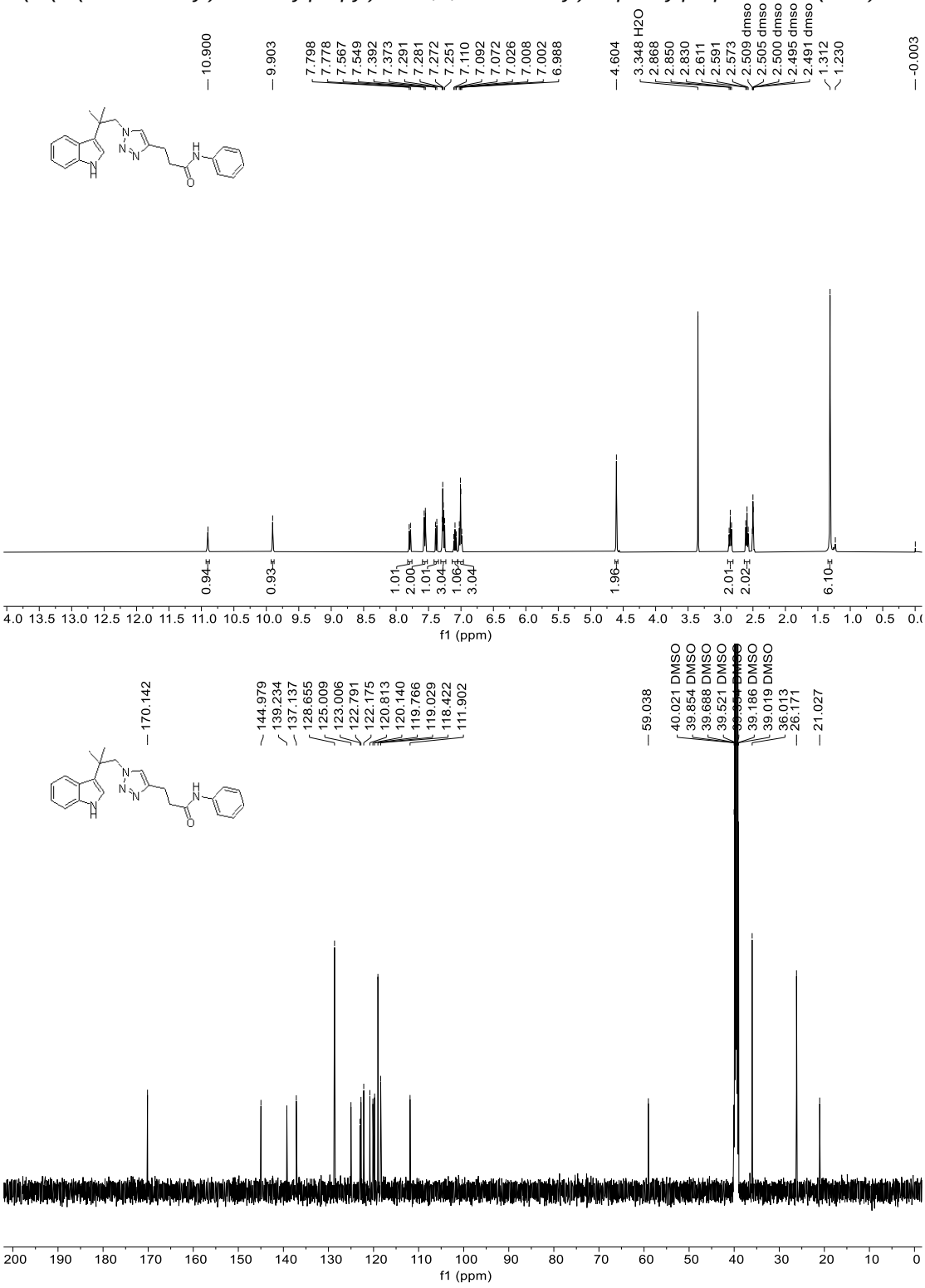

159.803
145.236
144.485
137.610
134.607
125.515
122.734
121.287
120.818
120.624
120.148
118.904
112.358
59.798
40.465 DMSO
40.298 DMSO
40.131 DMSO
39.964 DMSO
39.797 DMSO
39.631 DMSO
39.464 DMSO
36.524
26.714
f1 (ppm)

*3-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)imidazo[1,2-b]pyridazine (**3-20**).*

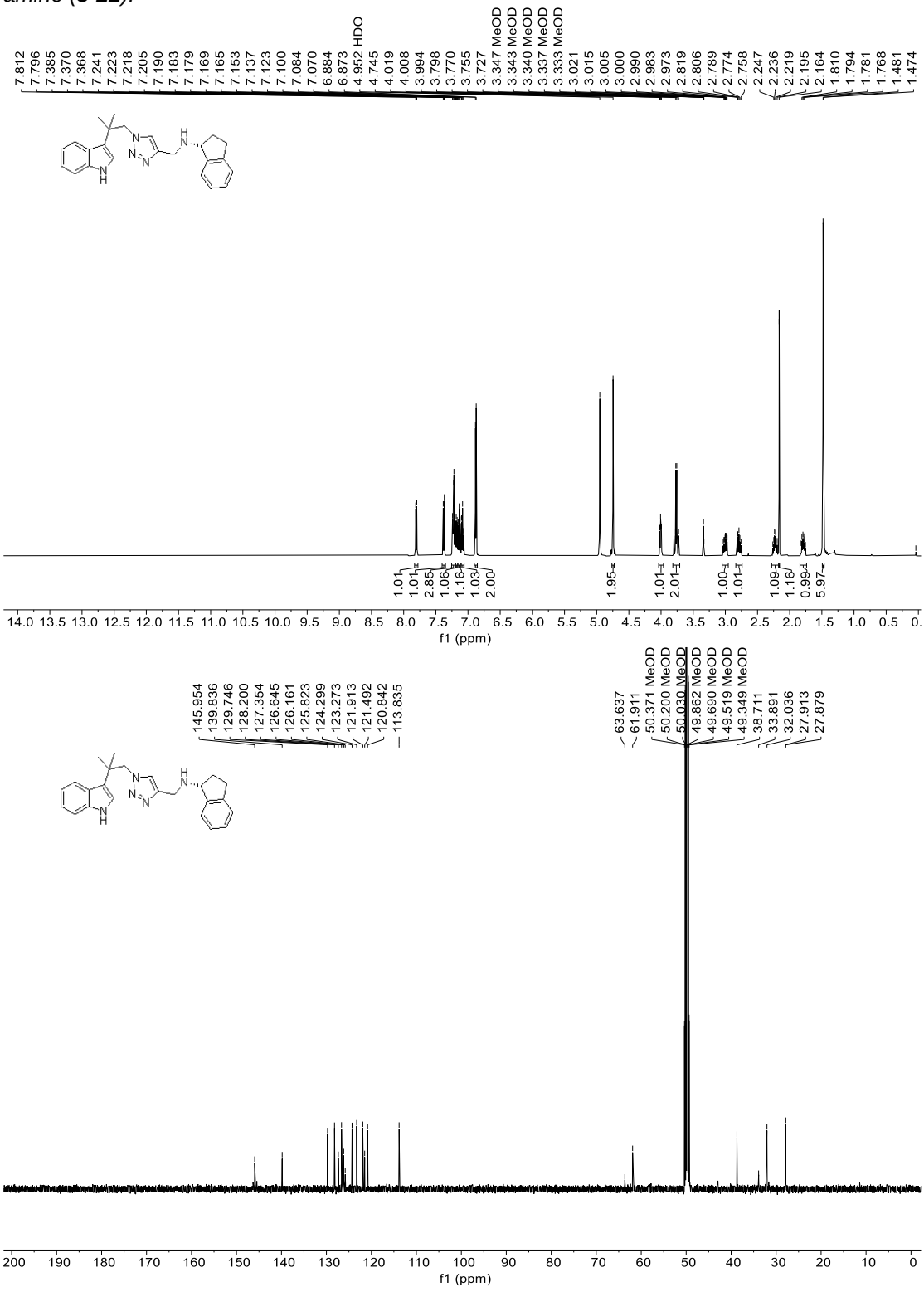




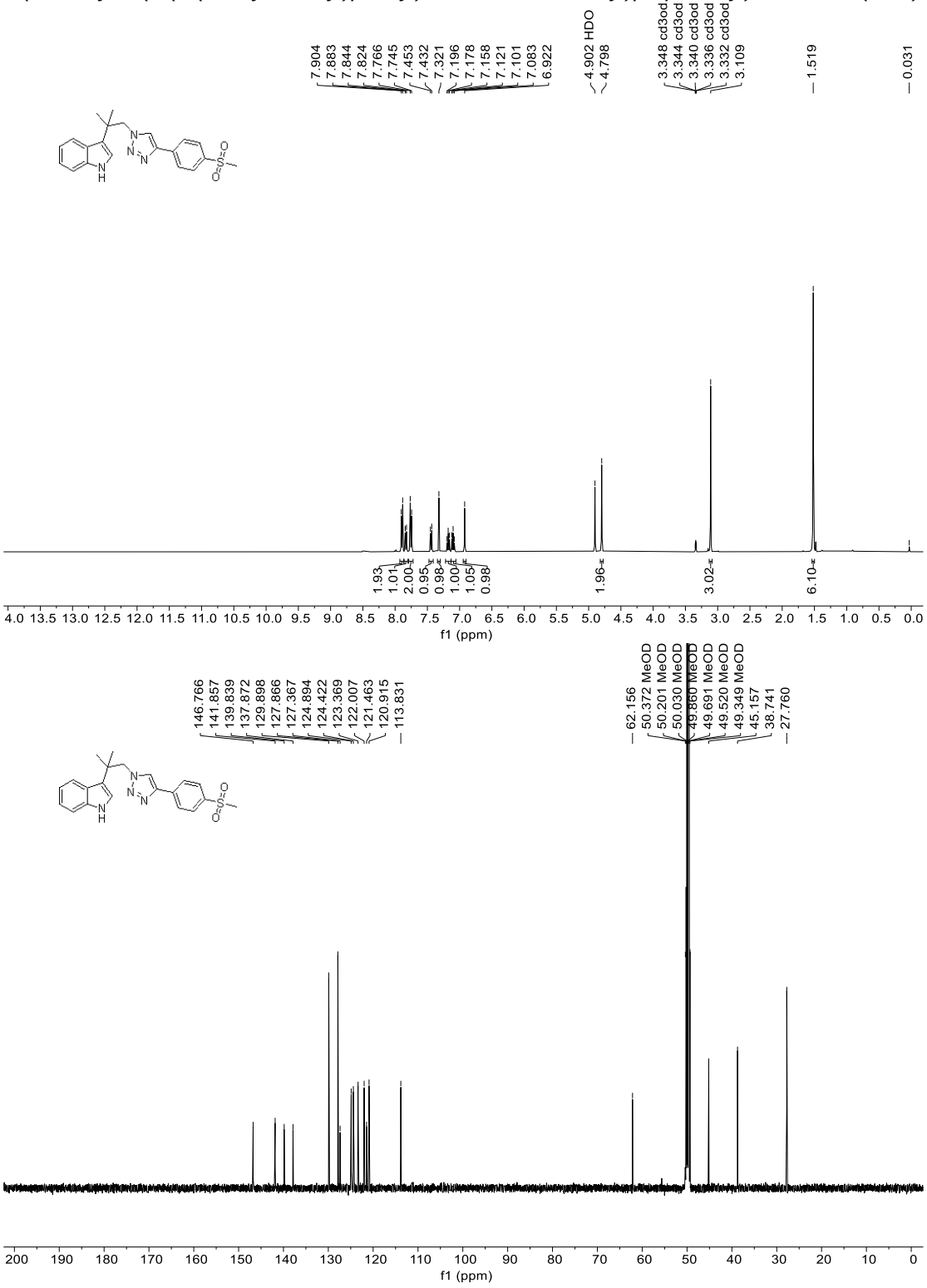
*3-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)-N-phenylpropanamide (**3-21**).*



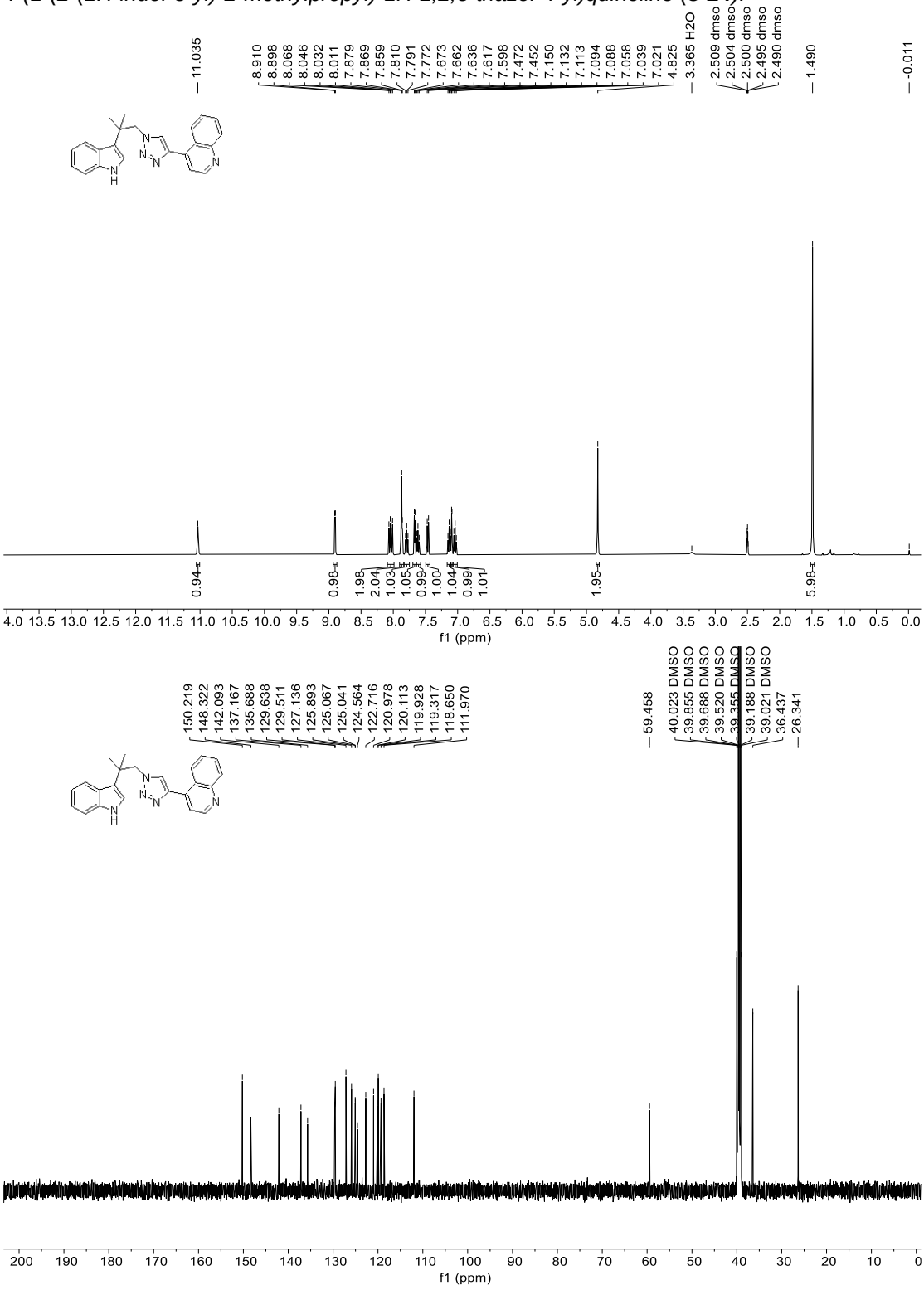
*(R)-N-((1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)methyl)-2,3-dihydro-1H-inden-1-amine (**3-22**).*



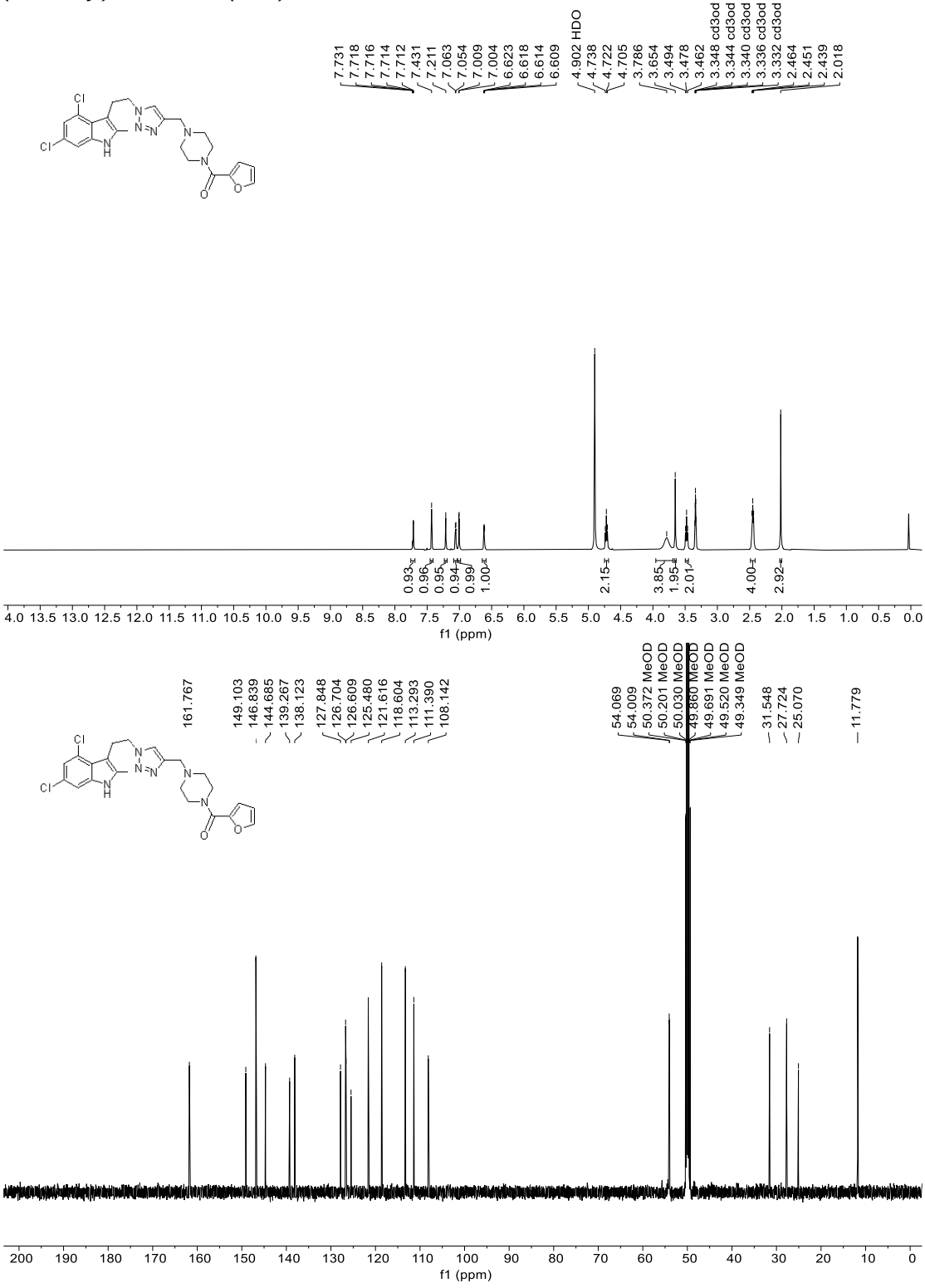
*3-(2-methyl-1-(4-(4-(methylsulfonyl)phenyl)-1H-1,2,3-triazol-1-yl)propan-2-yl)-1H-indole (**3-23**).*



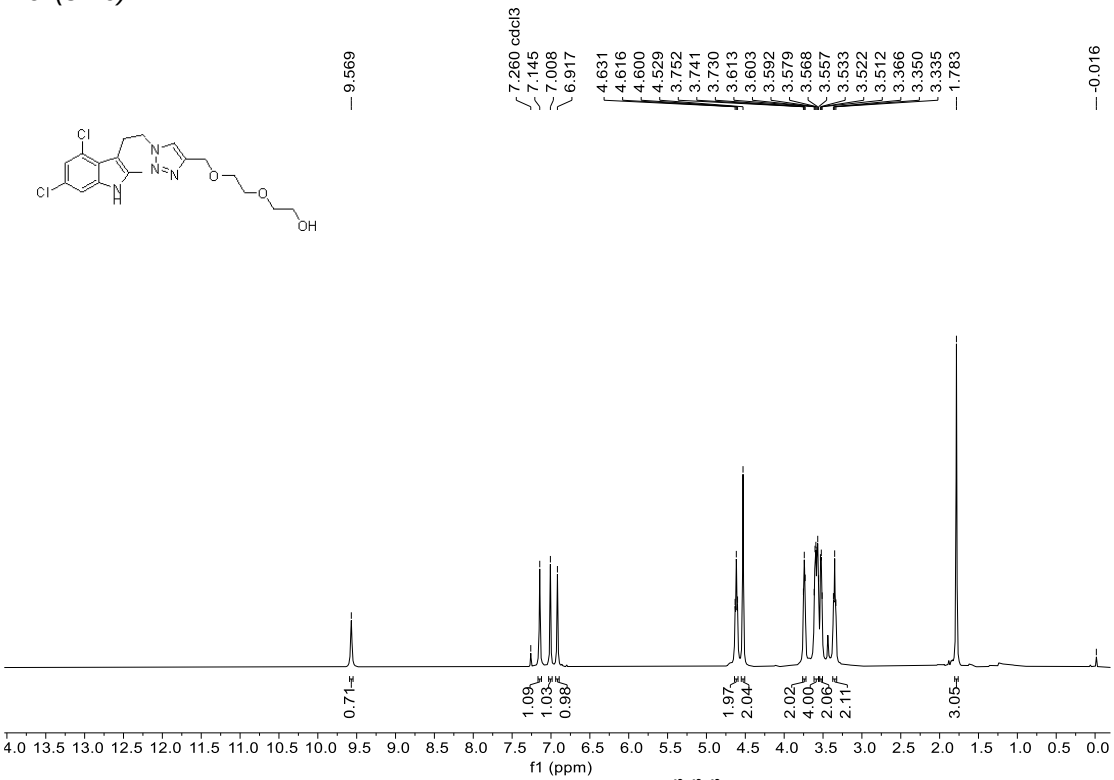
*4-(1-(2-(1H-indol-3-yl)-2-methylpropyl)-1H-1,2,3-triazol-4-yl)quinoline (**3-24**).*

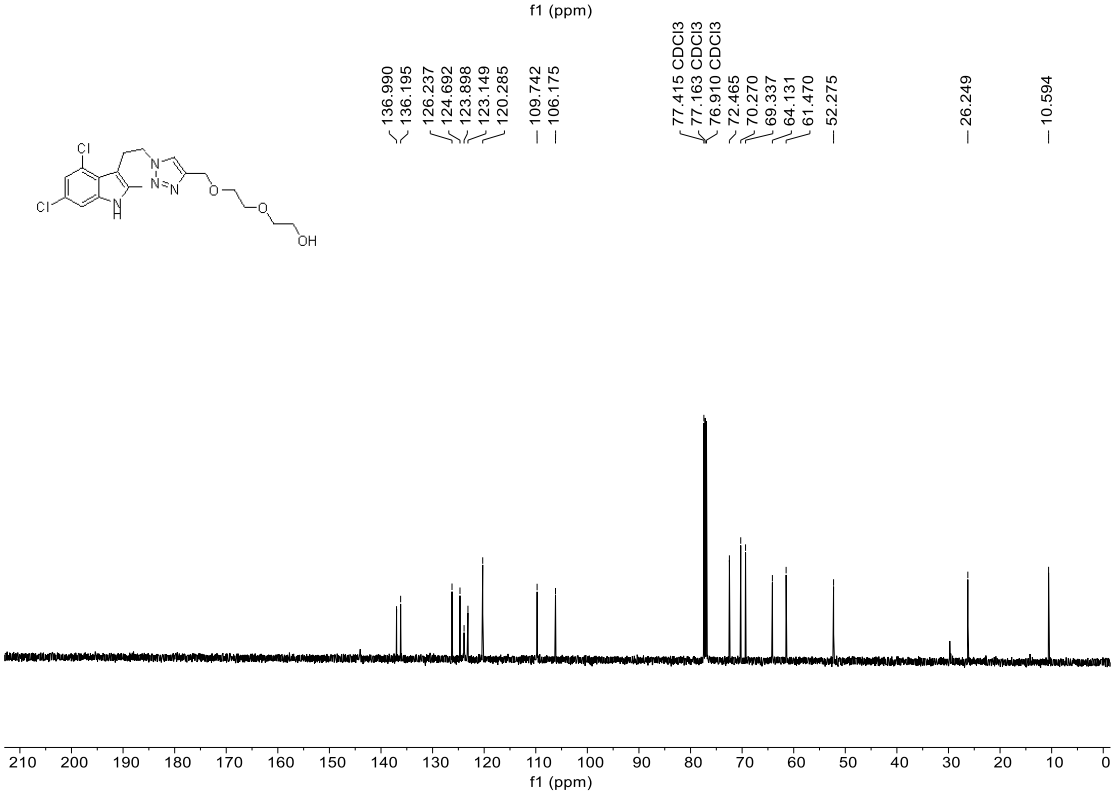


*(4-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)(furan-2-yl)methanone (**3-25**).*

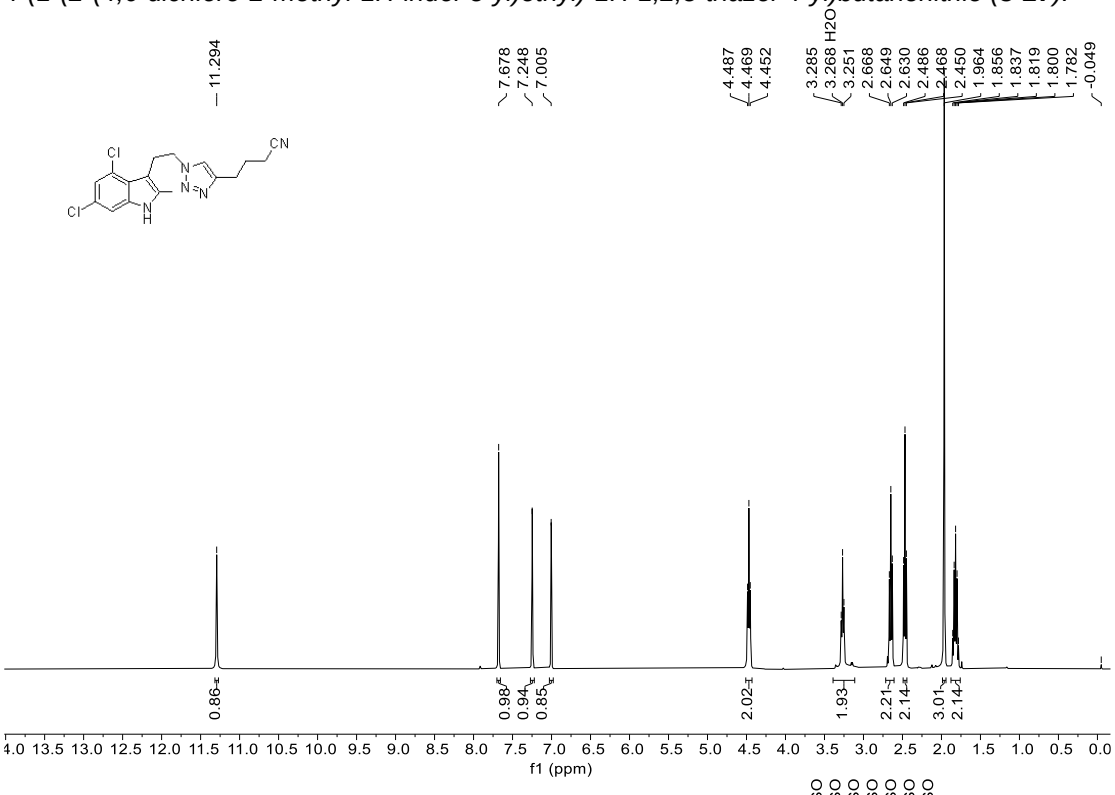


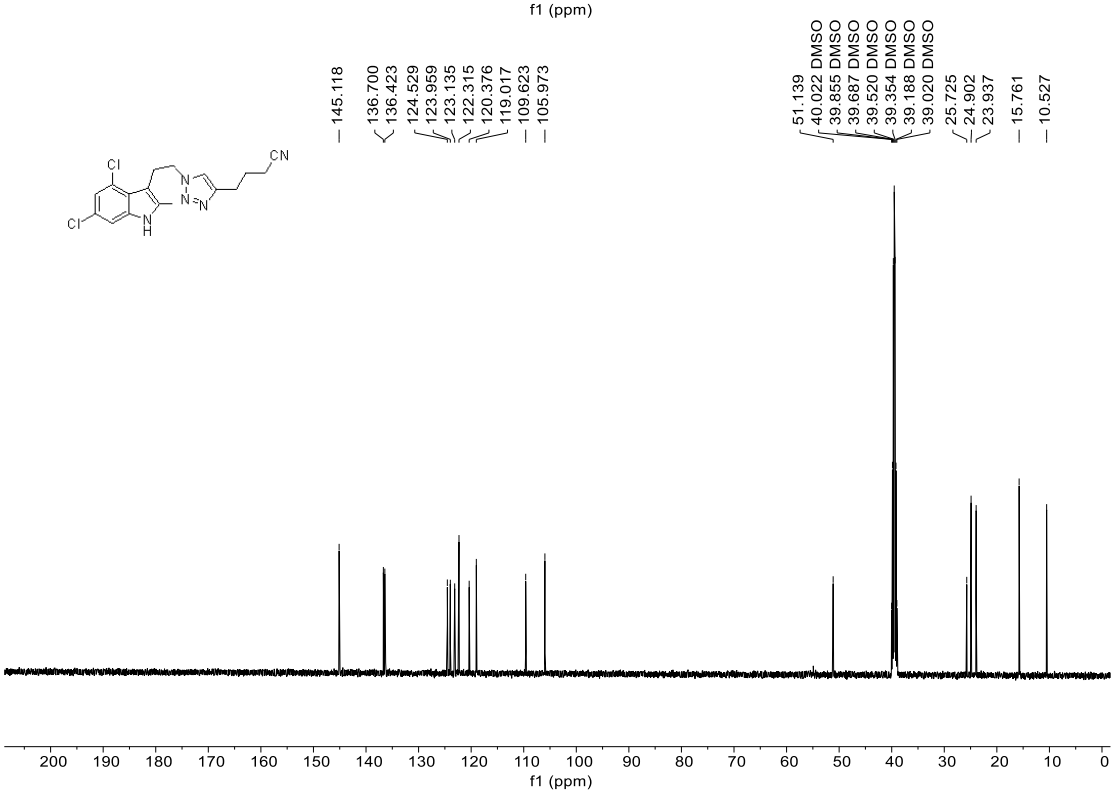
*2-(2-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)ethoxy)ethan-1-ol (**3-26**).*



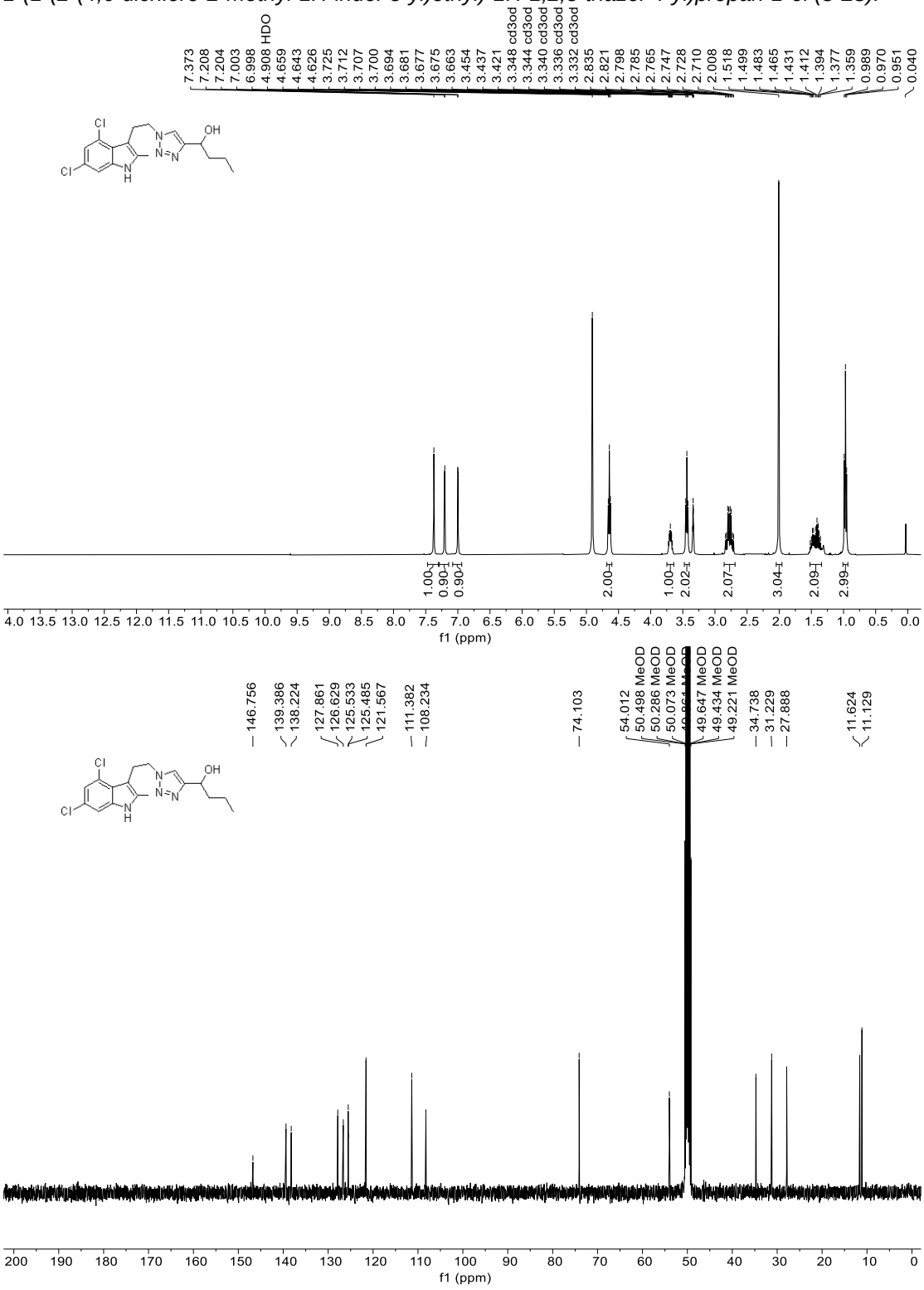


*4-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)butanenitrile (**3-27**).*

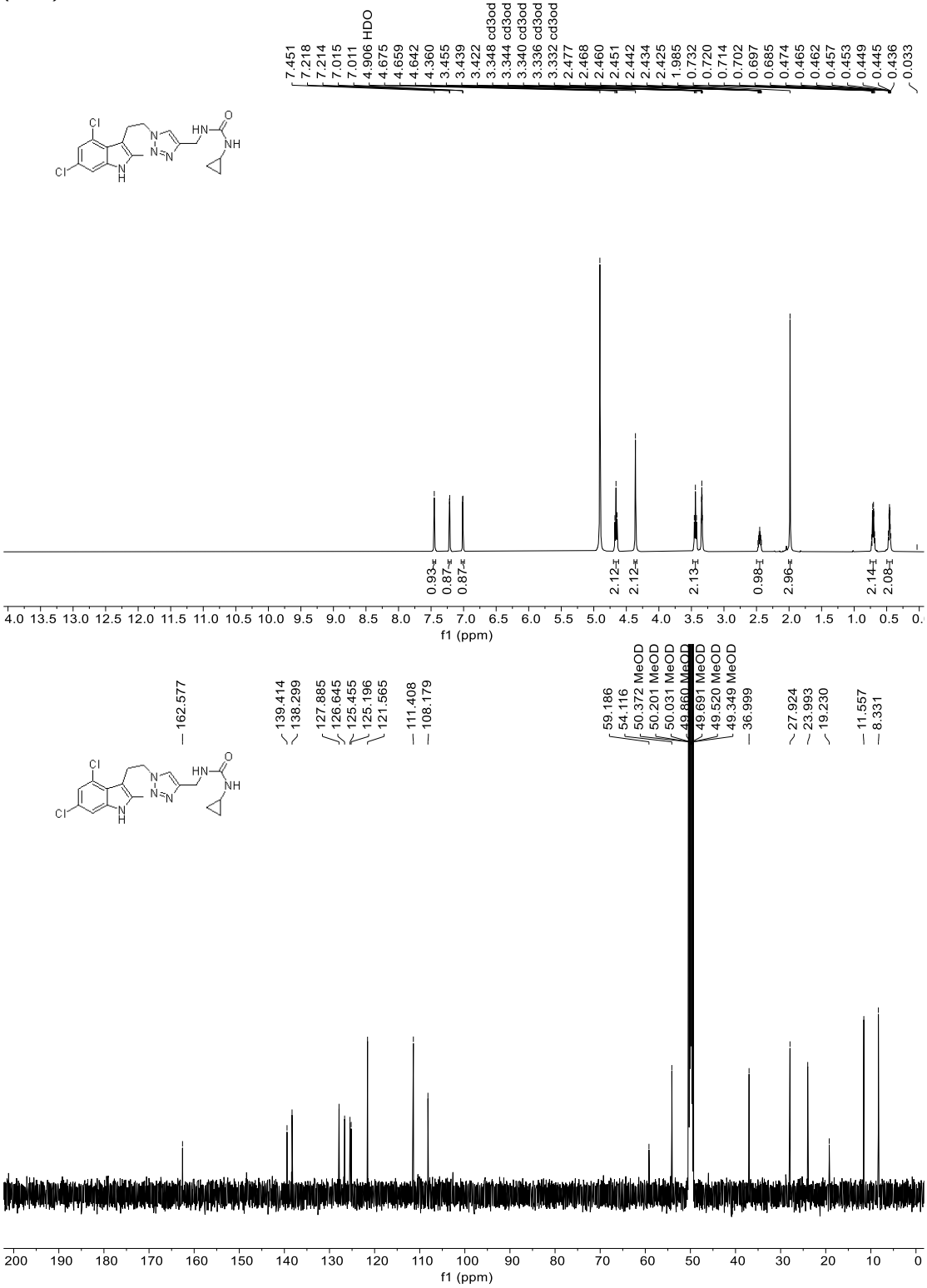




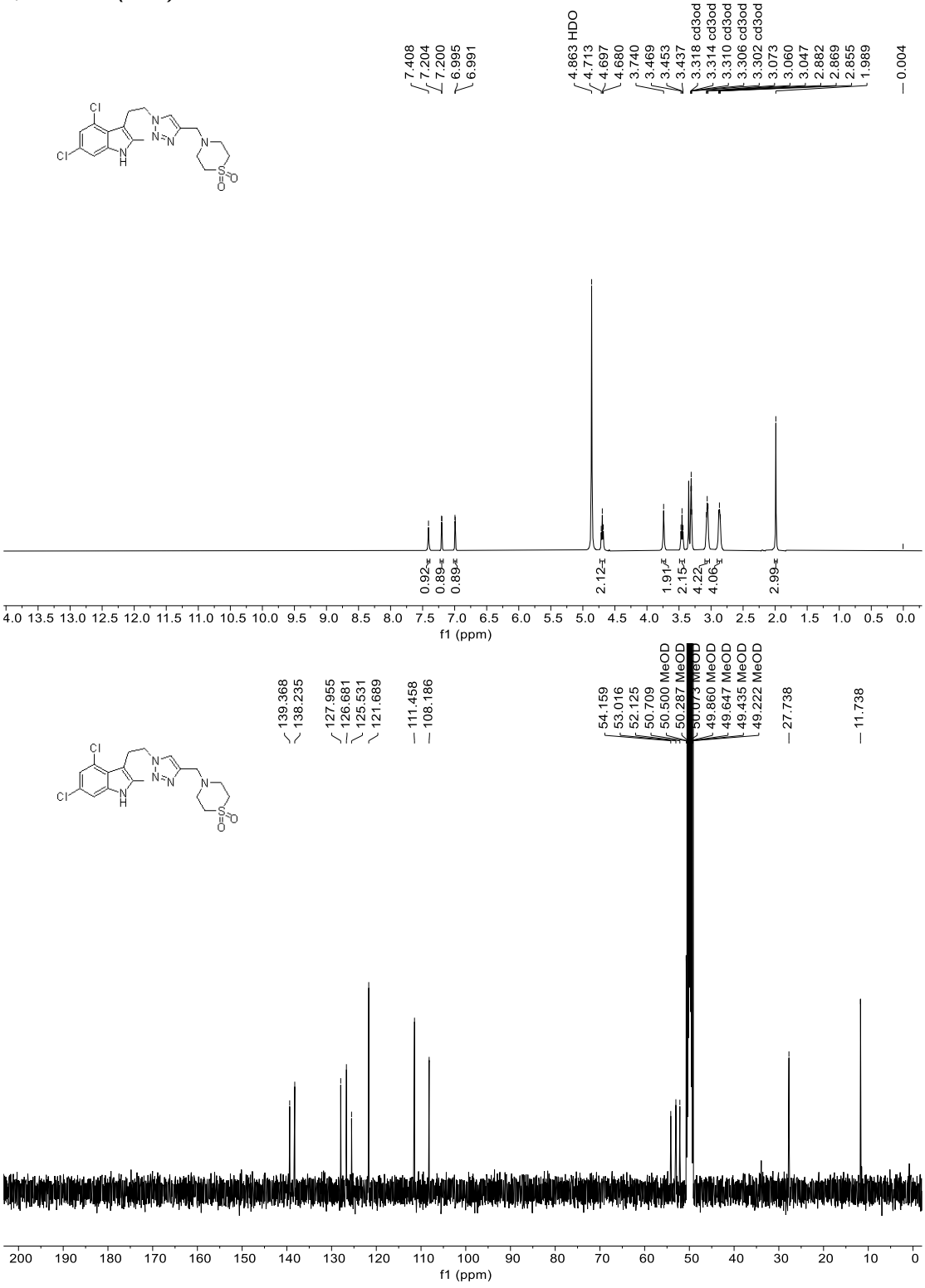
*1-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)propan-1-ol (**3-28**).*



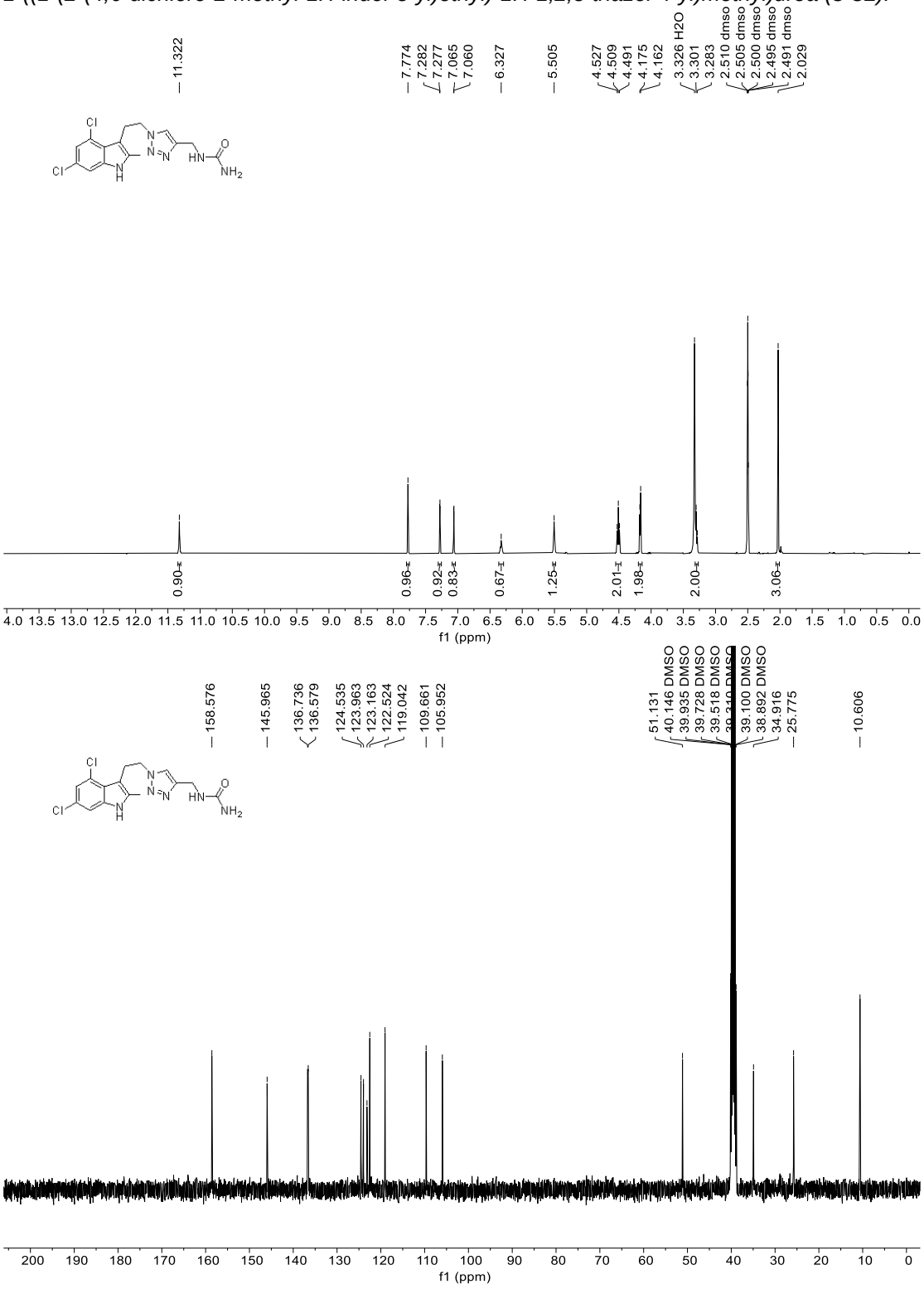
*1-cyclopropyl-3-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)urea (**3-29**).*



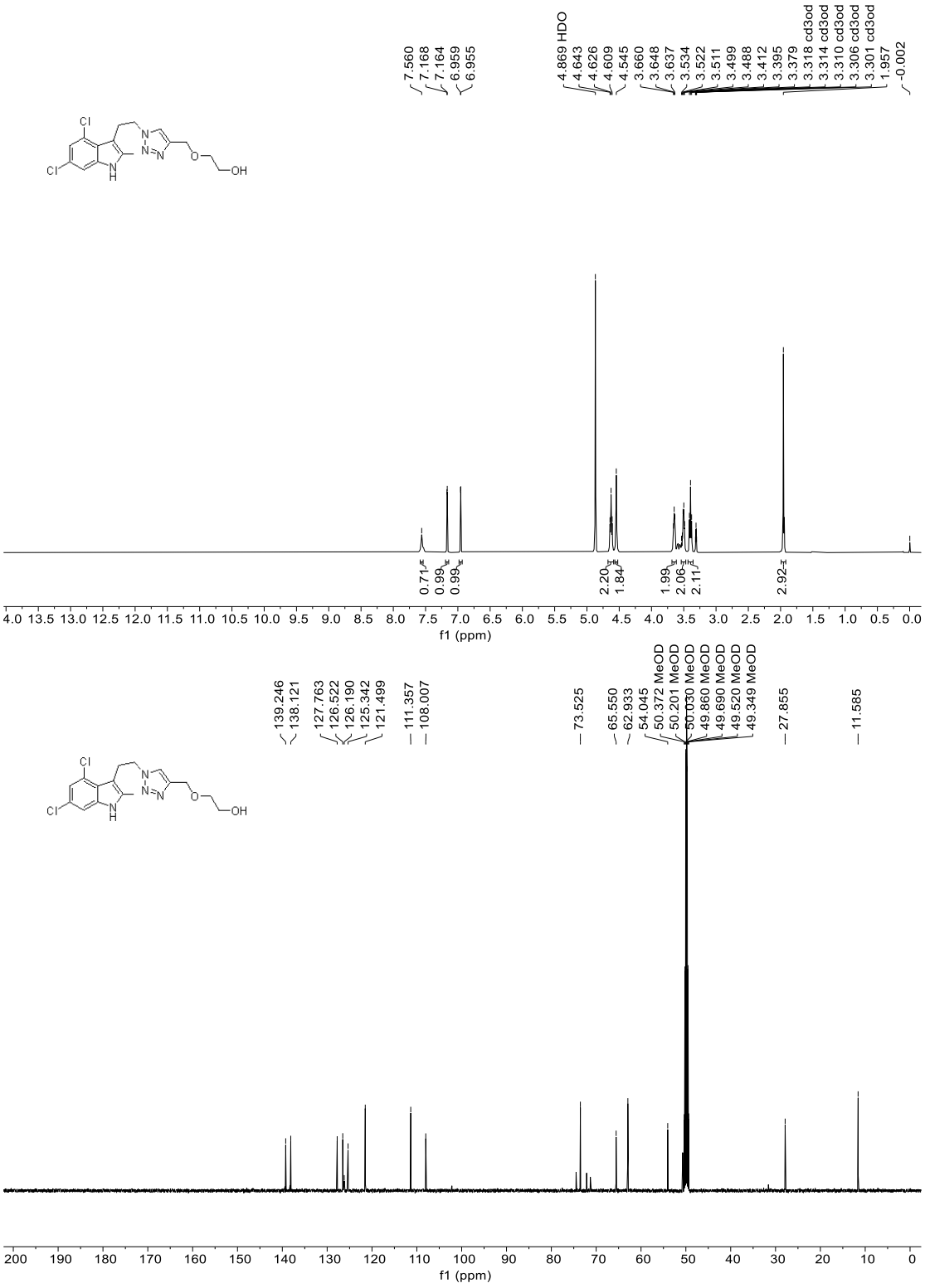
*4-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)thiomorpholine 1,1-dioxide (**3-30**).*



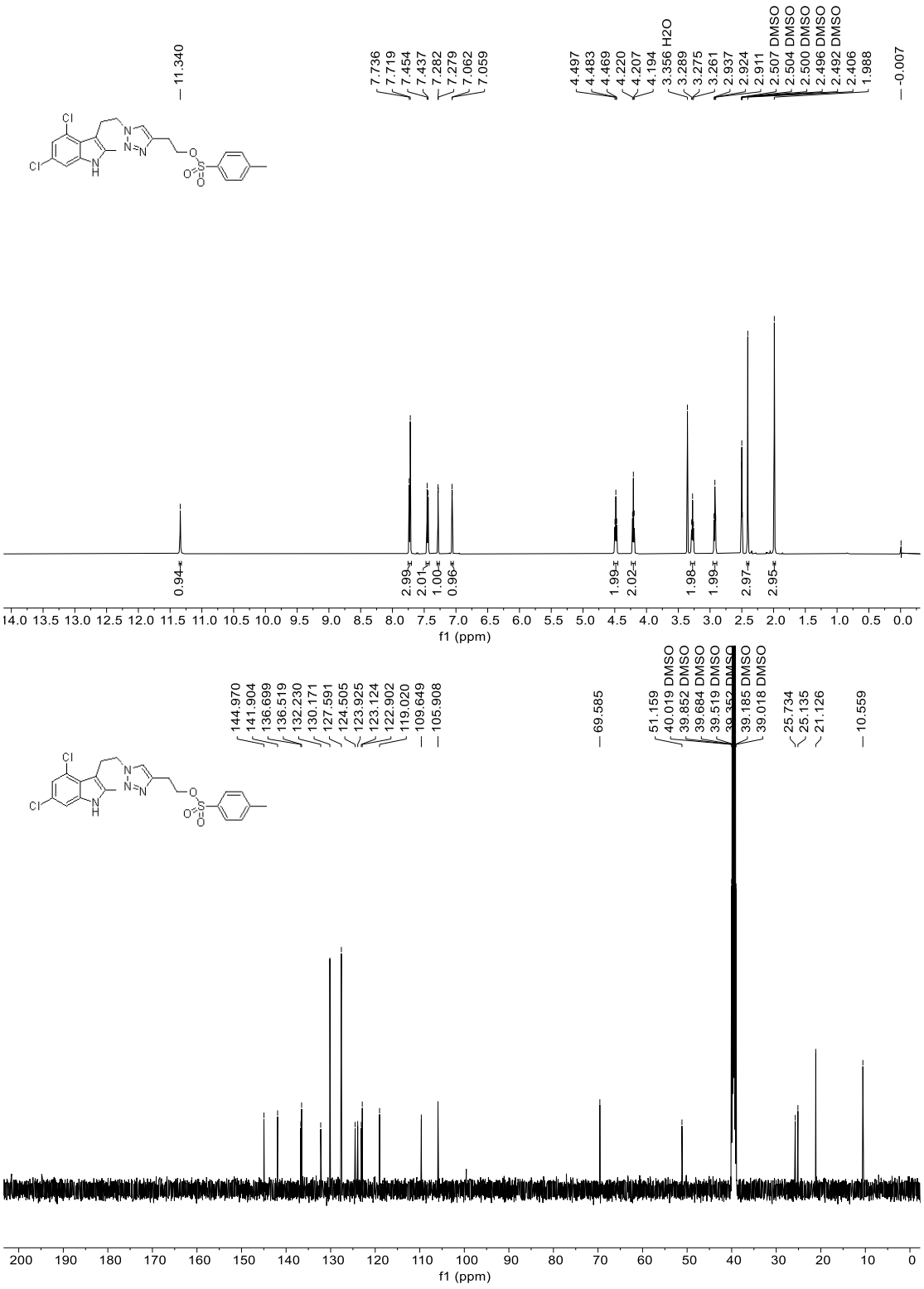
*1-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)urea (**3-31**).*



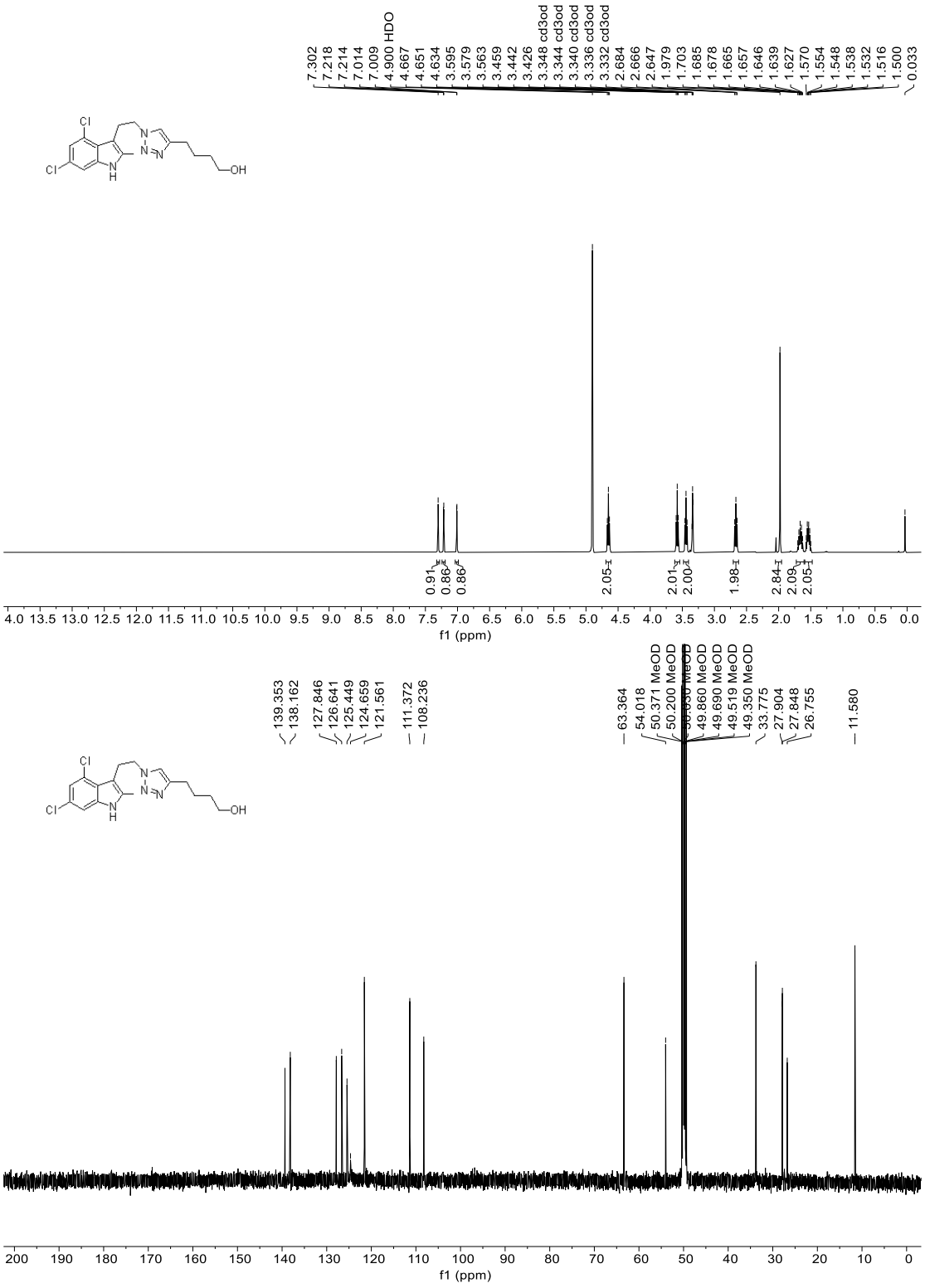
*2-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)ethan-1-ol (**3-32**).*



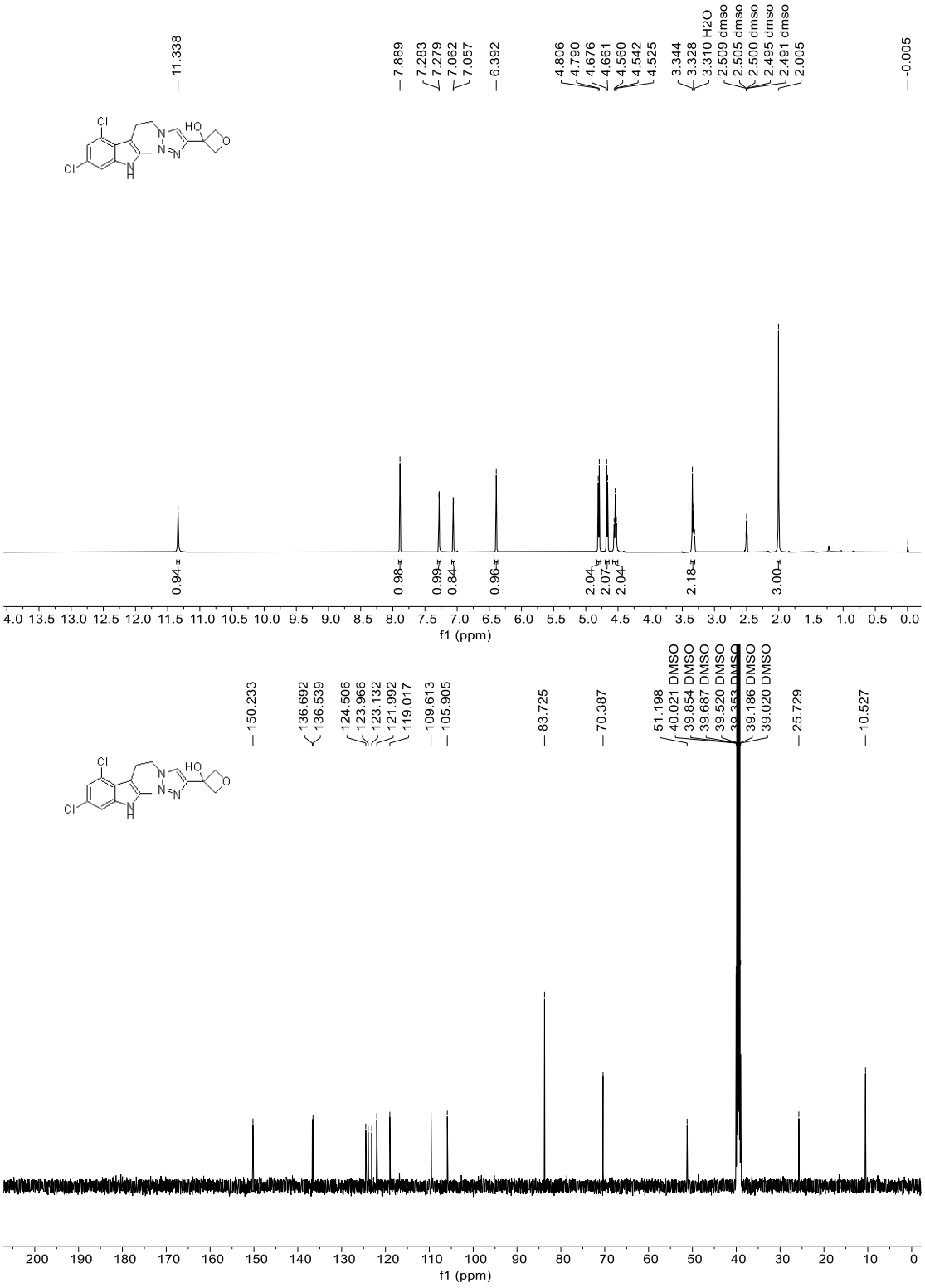
*2-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl 4-methylbenzenesulfonate (**3-33**).*



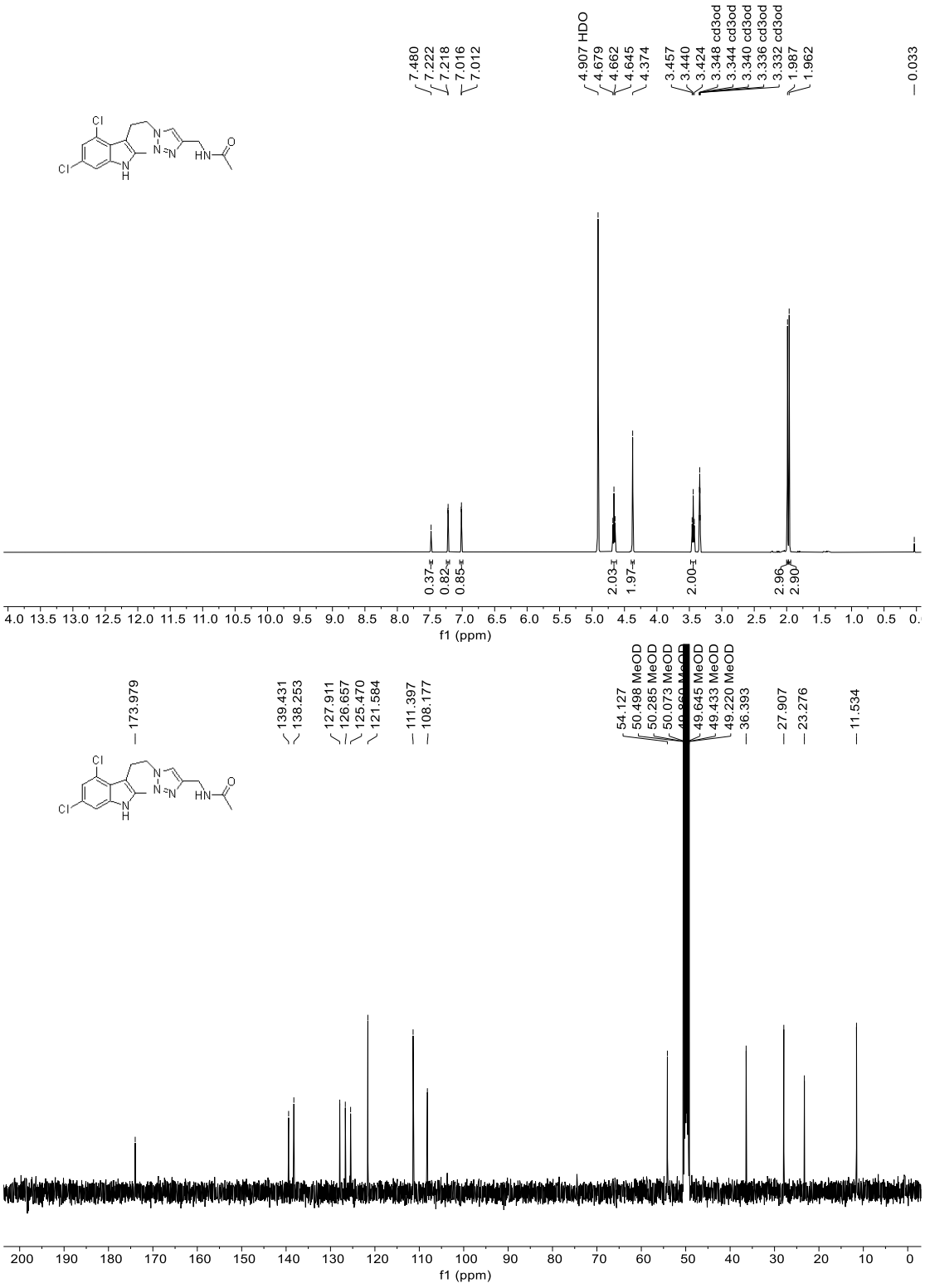
*4-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)butan-1-ol (**3-34**).*



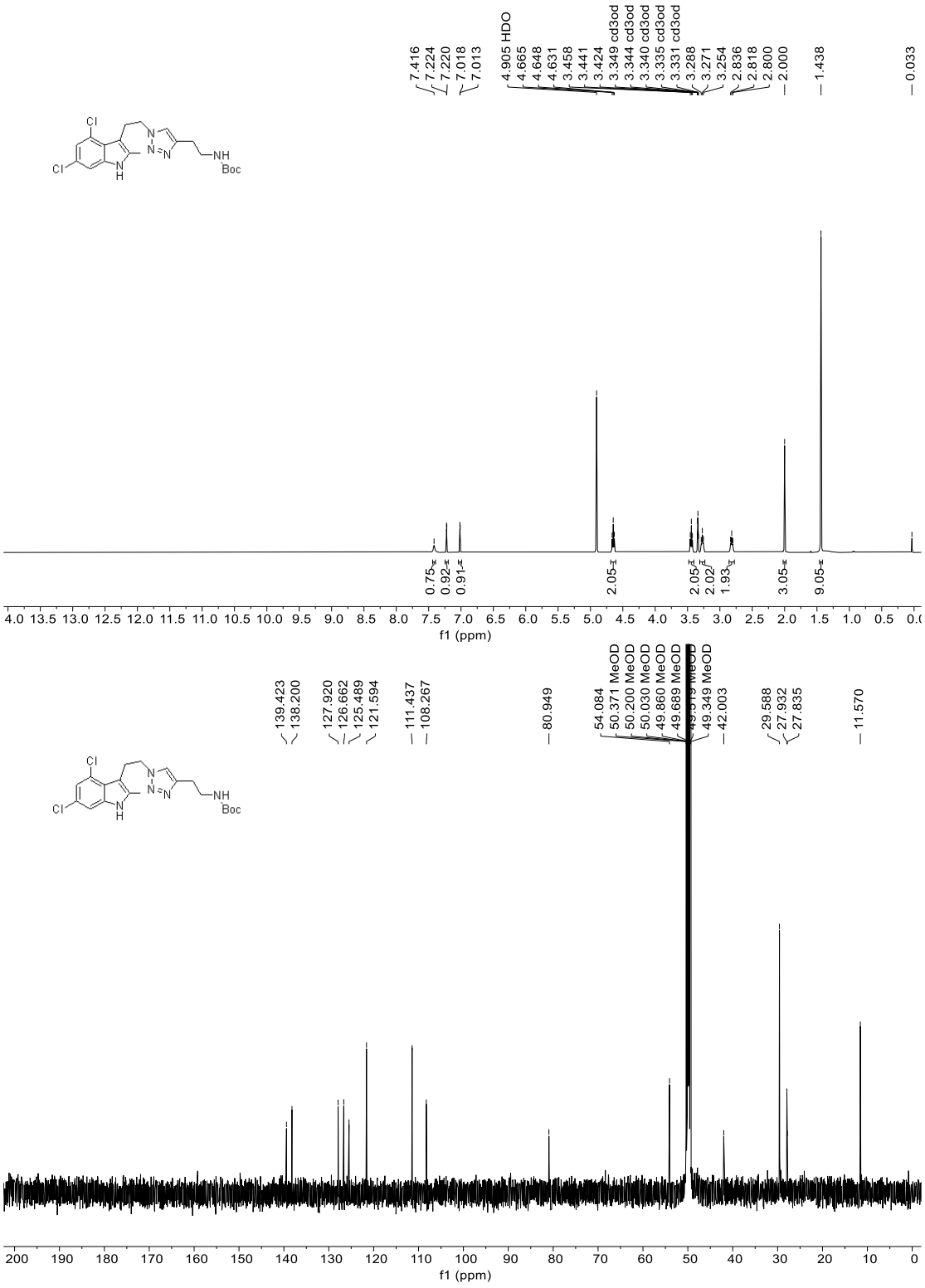
*3-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)oxetan-3-ol (**3-35**).*



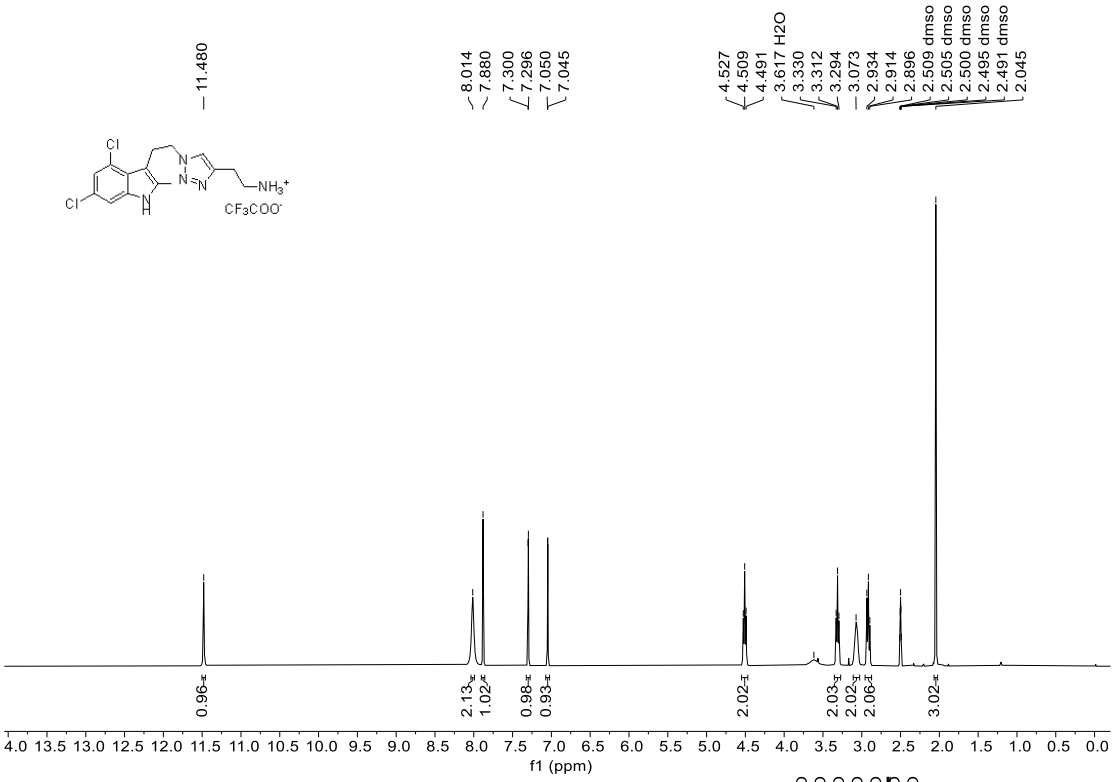
*N-((1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)acetamide (**3-36**).*

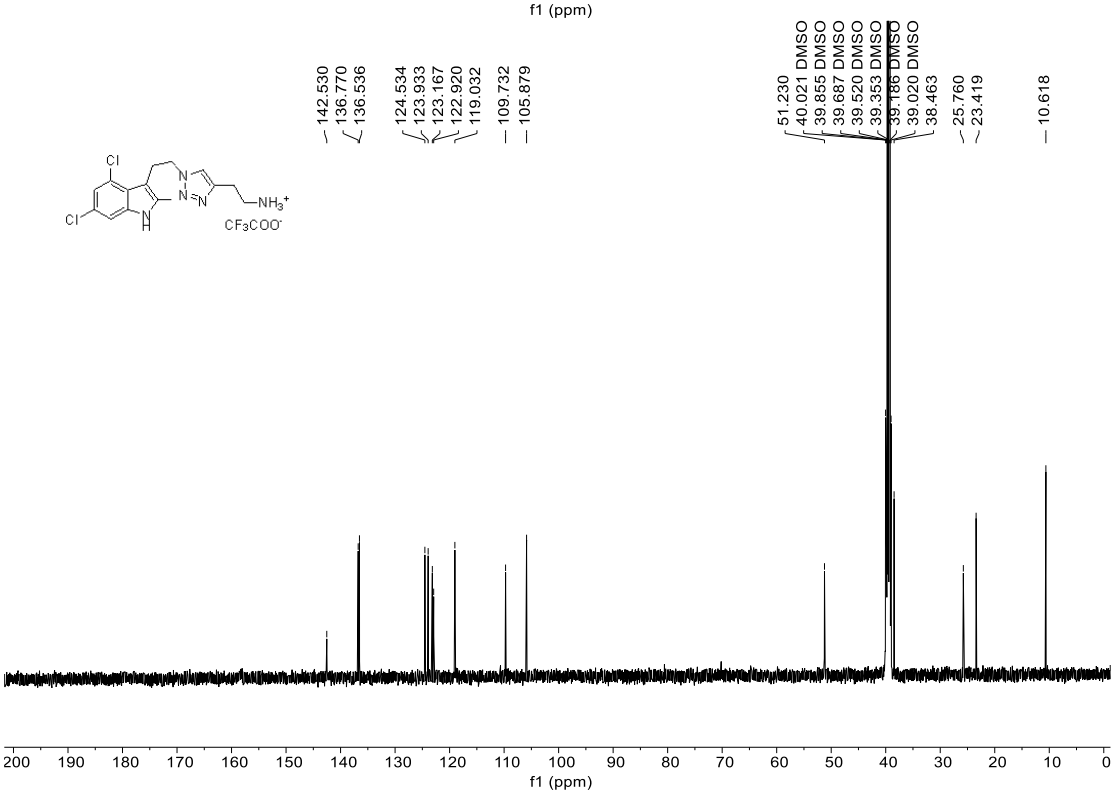


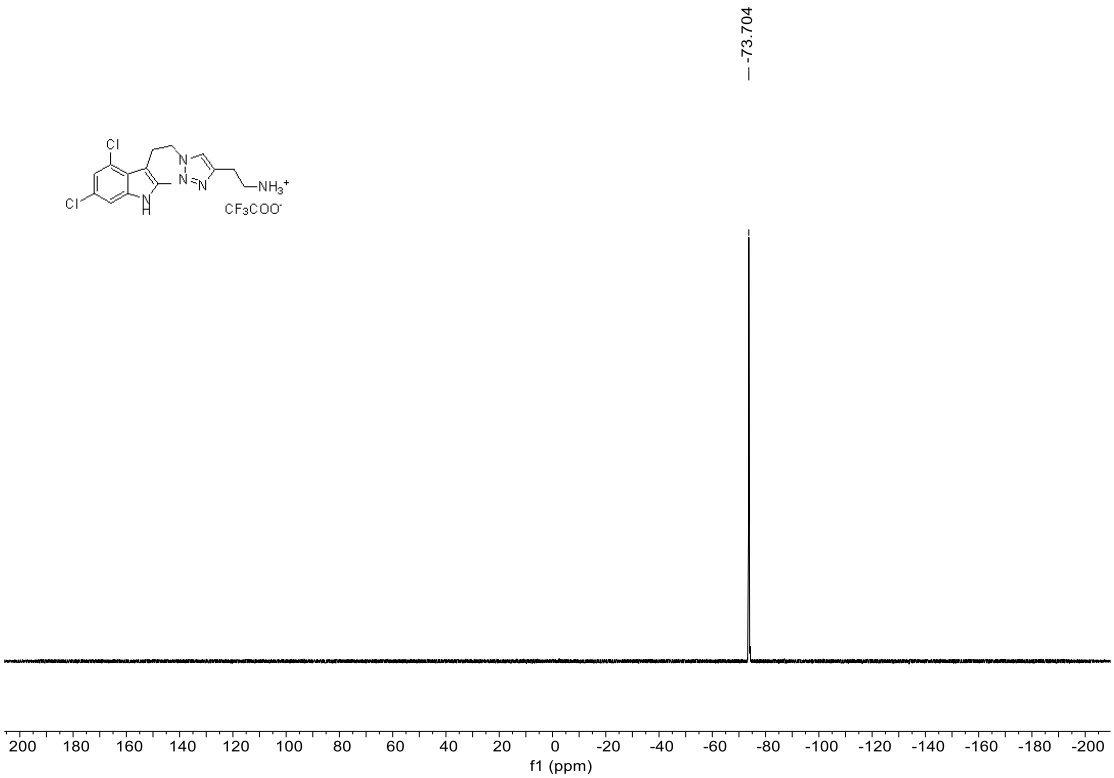
*tert-butyl (2-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)carbamate (**3-37**).*



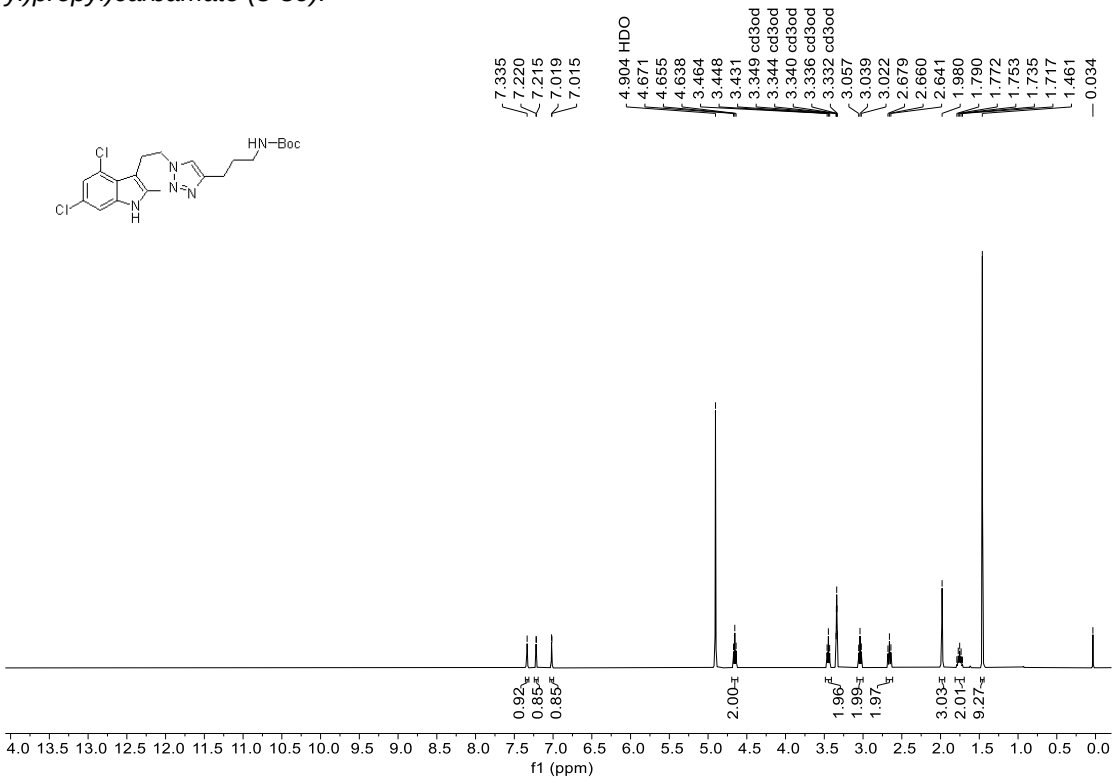
*2-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethan-1-aminium 2,2,2-trifluoroacetate (**3-38**).*

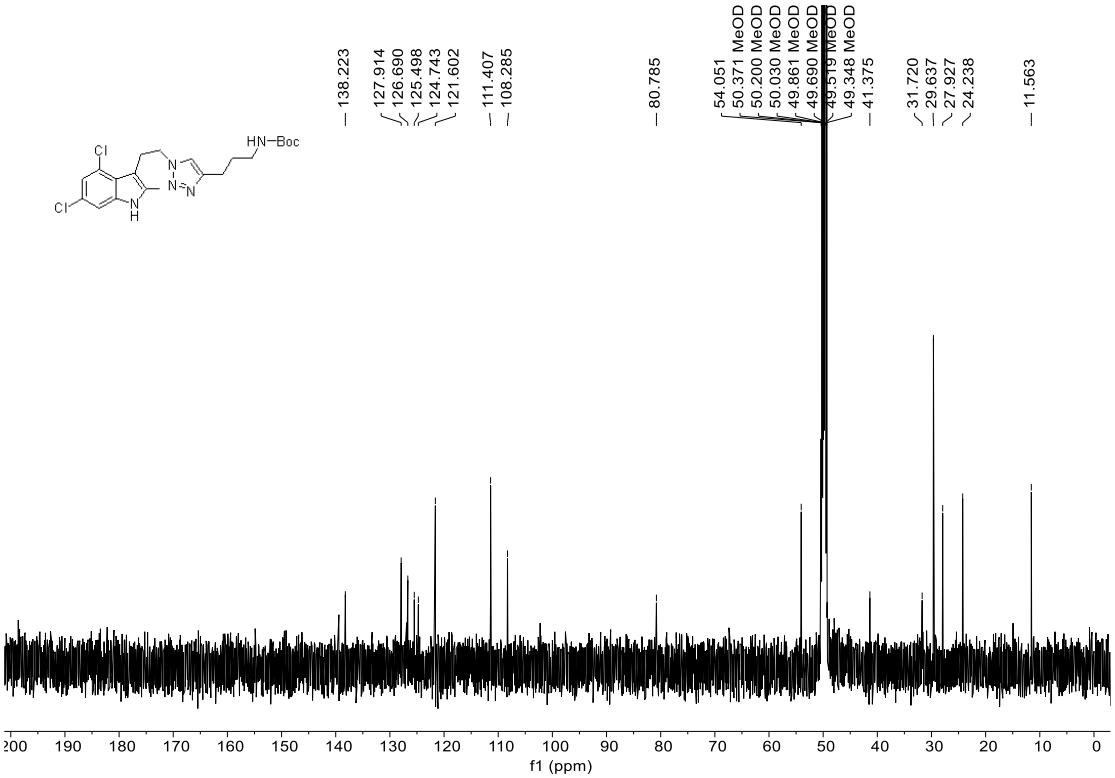

11.480
8.014
7.880
7.300
7.296
7.050
7.045
4.527
4.509
4.491
3.617 H2O
3.330
3.312
3.294
3.073
2.934
2.914
2.896
2.509 dmso
2.505 dmso
2.500 dmso
2.495 dmso
2.491 dmso
2.045
Cl
Cl
N
N
N
NH
NH3+
CF3COO-
0.96
2.13
1.02
0.98
0.93
2.02
2.03
2.02
2.06
3.02
f1 (ppm)


142.530
136.770
136.536
124.534
123.933
123.167
122.920
119.032
109.732
105.879
51.230
40.021 DMSO
39.855 DMSO
39.687 DMSO
39.520 DMSO
39.353 DMSO
39.186 DMSO
39.020 DMSO
38.463
25.760
23.419
10.618
Cl
Cl
N
N
N
NH
NH3+
CF3COO-
f1 (ppm)

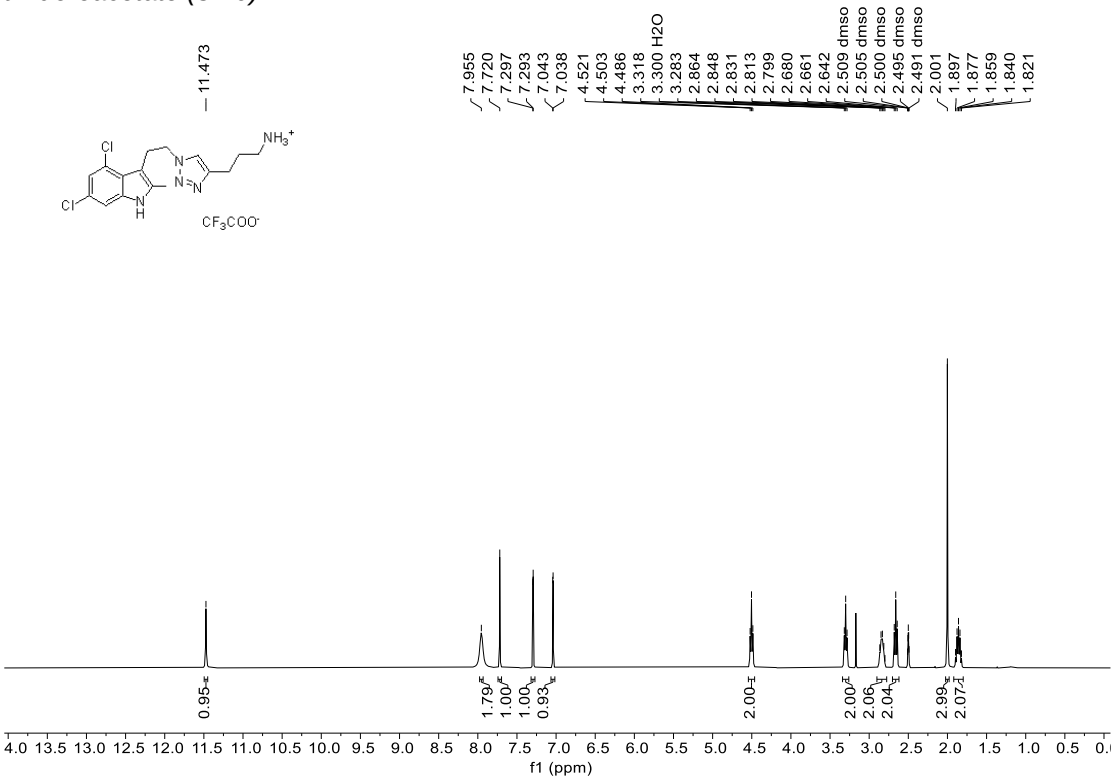


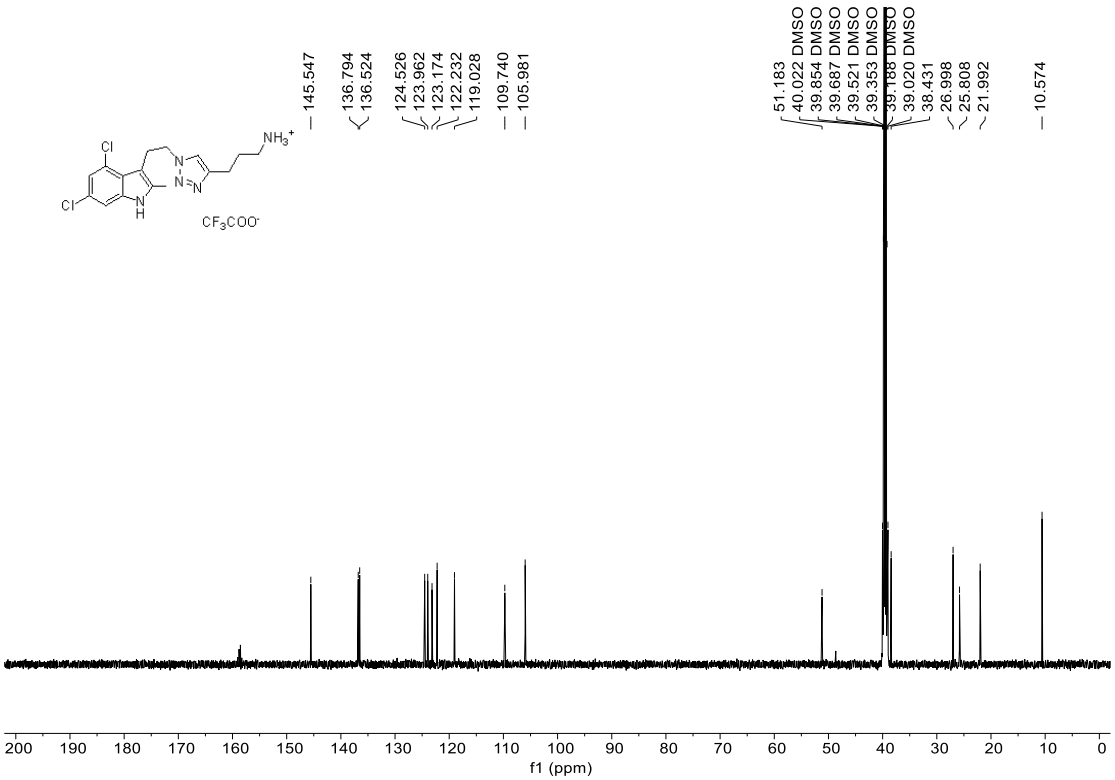
*tert-butyl (3-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)propyl)carbamate (**3-39**).*

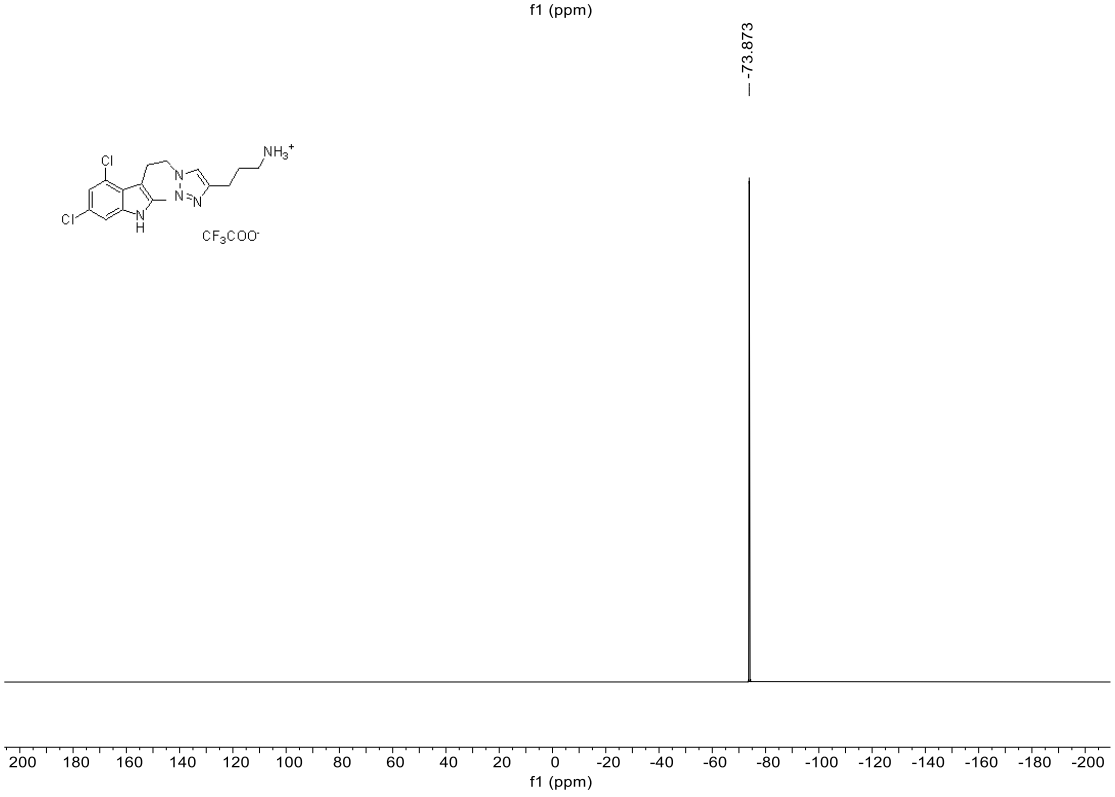




*3-(1-(2-(4,6-dichloro-2-methyl-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)propan-1-aminium 2,2,2-trifluoroacetate (**3-40**).*




145.547
136.794
136.524
124.526
123.962
123.174
122.232
119.028
109.740
105.981
51.183
40.022 DMSO
39.854 DMSO
39.687 DMSO
39.521 DMSO
39.353 DMSO
39.188 DMSO
39.020 DMSO
38.431
26.998
25.808
21.992
10.574
NH3+
Cl
Cl
N
N
N
H
CF3COO-
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


-73.873
NH3+
Cl
Cl
N
N
N
H
CF3COO-
200 180 160 140 120 100 80 60 40 20 0 -20 -40 -60 -80 -100 -120 -140 -160 -180 -200
f1 (ppm)