Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A Cell-Permeable and Triazole-Forming Fluorescence Probe for Glycoconjugate Imaging in Live Cells

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Experimental Section

Materials and Methods.

All the reagents were commercially available and used without further purification unless indicated otherwise. All solvents were anhydrous grade unless indicated otherwise. All non-aqueous reactions were carried out in oven-dried glassware under a slightly positive pressure of argon unless otherwise noted. Reactions were magnetically stirred and monitored by thin-layer chromatography on silica gel. Column chromatography was performed on silica gel of 40–63 μm particle size. Yields are reported for spectroscopically pure compounds. Melting points were recorded on an Electrothermal MEL-TEMP® 1101D melting point apparatus and were not corrected. NMR spectra were recorded on a Bruker AVANCE 600 spectrometer (600 MHz). Chemical shifts are given in δ values relative to tetramethylsilane (TMS); coupling constants *J* are given in Hz. Internal standards were $CDCl_3$ (δ_H = 7.24) or DMSO-d_6 (δ_H = 2.49) for ^{1}H-NMR spectra, $CDCl_3$ (δ_c = 77.0) or DMSO-d_6 (δ_C = 39.5) for ^{13}C-NMR spectra. The splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad) and dd (double of doublets). High resolution ESI mass spectra were recorded on a Bruker Daltonics spectrometer.

Kinetics Measurements of Compound (1) by ^{1}H NMR. (Experiment I): Compound **1** and benzyl azide were predissolved in CD_3CN, and then mixed at an equimolar concentration of 20 mM. The reaction was monitored by ^{1}H-NMR analysis over a period of 1 h. The concentration of each component was determined, based on the concentration of initial compound **1**, by integration at multiple chemical shifts in the

^{1}H-NMR spectrum. By plotting 1/[**1**] (M^{-1}) vs. time (sec), a second order rate constant in units of $M^{-1}s^{-1}$ was determined using linear regression analysis. This procedure was repeated 3 times with a concentration of 20 mM to afford a rate constant of 0.012 $M^{-1}s^{-1}$ at 25 °C. The results are shown in Figure S1. (**Experiment II):** Compound **1** and *N*-azidoacetylmannosamine were predissolved in CD_3OD/D_2O (5:1, v/v), and then mixed at equimolar concentration of 20 mM. The reaction was monitored by ^{1}H-NMR analysis over a period of 1 h. The concentration of each component was determined, based on the concentration of initial compound **1**, by integration at multiple chemical shifts in the ^{1}H-NMR spectrum. By plotting 1/[**1**] (M^{-1}) vs. time (sec), a second order rate constant in unit of $M^{-1}s^{-1}$ was determined using linear regression analysis. This procedure was repeated 3 times with a concentrated of 20 mM to afford a rate constant of 0.010 $M^{-1}s^{-1}$ at 25 °C. The results are shown in Figure S2

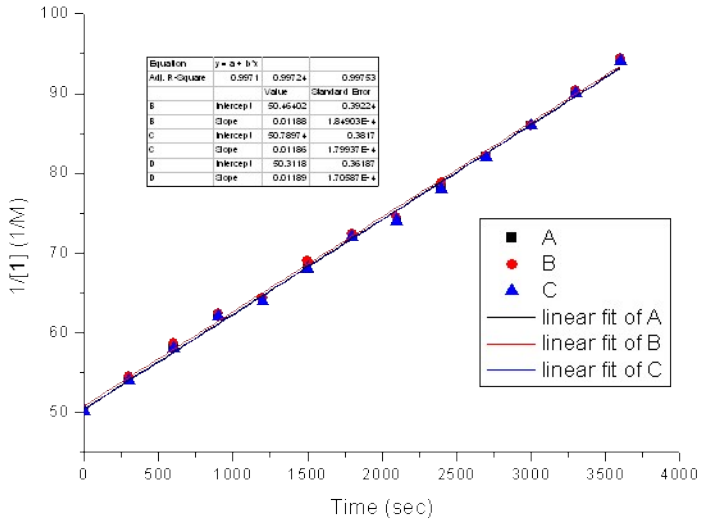


Figure S1. Plot of 1/[**1**] vs. time for the reaction of compound **1** and benzyl azide in CD_3CN as monitored by ^{1}H-NMR.

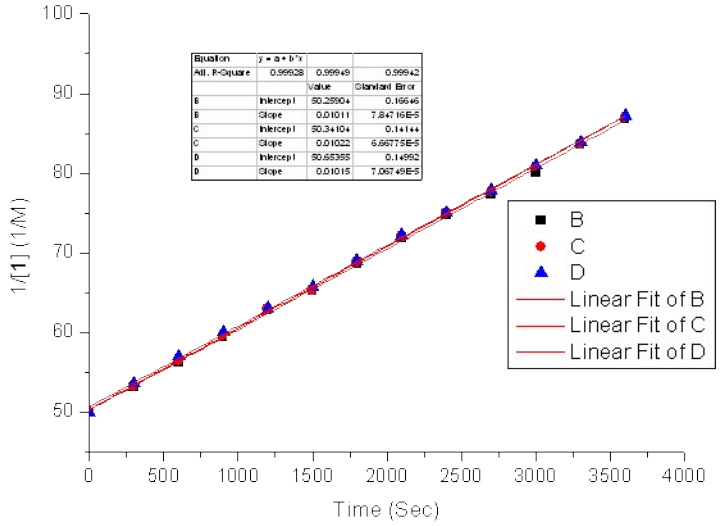


Figure S2. Plot of 1/[**1**] vs. time for the reaction of compound **1** and *N*-azidoacetylmannosamine in a solution of $CD_3OD–D_2O$ (5:1, v/v) as monitored by ^{1}H-NMR.

Spectroscopic Materials and Methods. All spectroscopic measurements of compound **1** and the corresponding triazole products **9** and **10** were performed in a mixture of 10% DMSO in PBS buffer. UV-vis spectra and fluorescence spectra were recorded on a Molecular Devices Spectramax M5 spectrometer. For each experiment, the absorption spectra were measured within an absorbance range of 0.07 to 0.7 (l = 10 cm). Quantum yields were determined from the slope of the integrated fluorescence emission between 360 and 550 nm (excitation at 330 nm) versus absorbance using quinine sulfate (Φ_f = 0.54 ± 0.03)[S1] as fluorescence standard. The quantum yield was calculated as an average of 4 points according to the following equation:

$$\Phi_{sample} = \Phi_{standard} (A_{standard} / A_{sample}) (F_{sample}/F_{standard}) (n_{sample} / n_{standard})^2$$

where "Φ" is the quantum yield, "A" is the absorbance at the excitation frequency, "F" is the area under the emission curve, and "n" is the refractive index of the solvent.

The results are shown in Table S1.

Table S1. Spectroscopic properties of probe **1** and triazoles **9**, **10**.

Compound	Absorption (λ_{max}, nm)	ε ($M^{-1}cm^{-1}$)[a]	Emission (λ_{max}, nm)	Stokes shift (cm^{-1})	Φ_f[b]
1	336	7800	405	5070	0.011
9	328	12200	435	7500	0.23
10	330	10800	435	7320	0.21

[a] Extinction coefficient; measured at 340 nm for **1**, and at 330 nm for **9** and **10**.

[b] Fluorescence quantum yield; using quinine sulfate ($\Phi_f = 0.54 \pm 0.03$) as a standard.

Time Course Measurements by Fluorescence Spectroscopy. A solution of probe **1** (0.075 μmol) and *N*-azidoacetylmannosamine (0.075 μmol) in a mixture of 10% DMSO in PBS buffer (2.5 mL) was incubated at 37 °C in the presence or absence of NaOCl (5 equiv.). The fluorescence emission intensity at 435 nm upon excitation at 330 nm was monitored in 5 min intervals. For each point the fluorescence intensity was measured over a period of 5 sec and averaged over a total of 3 points. In a control experiment the same conditions were used except that *N*-acetylmannosamine (0.075 μmol) was added to the solution. The results are shown in Figure S3.

(a) (b)

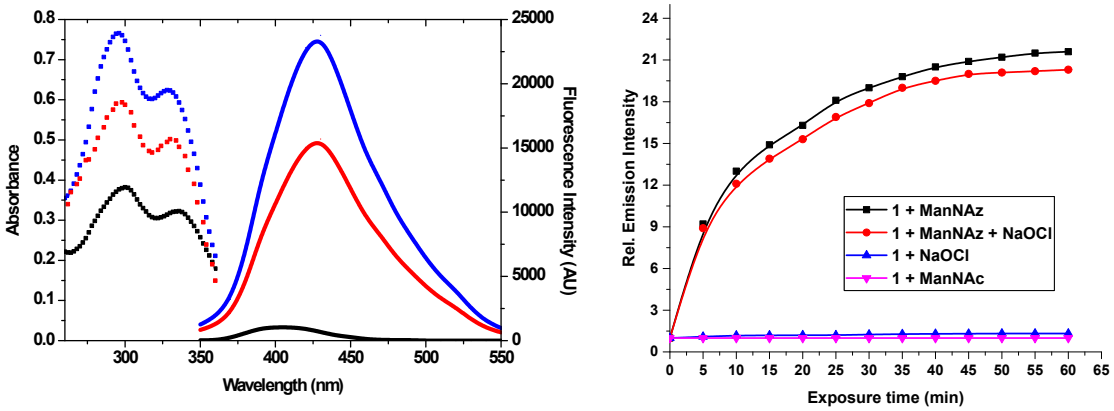


Figure S3. (a) Absorption (dashed lines) and fluorescence emission (solid lines) spectra (λ_{ex} = 330 nm) of **1** (black), **9** (blue) and **10** (red) (45 μM, PBS buffer containing 10% DMSO, pH 7.4). (b) Time course of normalized fluorescence intensity at 435 nm (λ_{ex} = 330 nm) for the triazole-forming reaction of **1** (30 μM) with *N*-azidoacetylmannosamine (30 μM) in PBS buffer containing 10% DMSO in the prescence or absence of NaOCl (5equiv.).

Microscopic Analysis of Fluorescence Labeling in Live Cells. HeLa cells were seeded onto coverslips (4×10^4 cells/1 mL per well) and incubated in culture medium (DMEM supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 1 mM sodium pyruvate) with either 100 μM of control sugar (peracetylated *N*-acetylmannosamine, Ac_4ManNAc; *N*-acetylgalactosamine, Ac_4GalNAc and *N*-acetylglucosamine, Ac_4GluNAc) or azido-sugar (peracetylated *N*-azidoacetylmannosamine, Ac_4ManNAz; *N*-azidoacetylgalactosamine, Ac_4GalNAc and *N*-azidoacetylglucosamine, Ac_4GluNAc) for 3 days, respectively. The cells were then washed three times with PBS to remove the unbound sugars. Prewashed cells were incubated with 2.5 μM of coumOCT (**1**) in Opti-MEM with 2 % DMSO for 30 min at 37 °C. The probe-labeled cells were fixed with 3% paraformaldehyde in PBS at room temperature for 20 min. The fluorescence imaging were collected using a LSM780 confocal laser scanning microscope equipped with a 63× objective lens.

To identify the location of azido-glycoconjugates, the probe-labeled cells were washed with PBS, fixed with 3% paraformaldehyde in PBS at room temperature for 20

min, permeablized with 0.2% Triton X-100 in PBS at room temperature for 20 min, and blocked with 3% bovine serum albumin in PBS at room temperature for 30 min. The cells were stained with anti-GM130 (Santa Cruz, sc-16268) followed by Cy3-conjugated antibodies for Golgi apparatus. The results are shown in Figures S4 and S5.

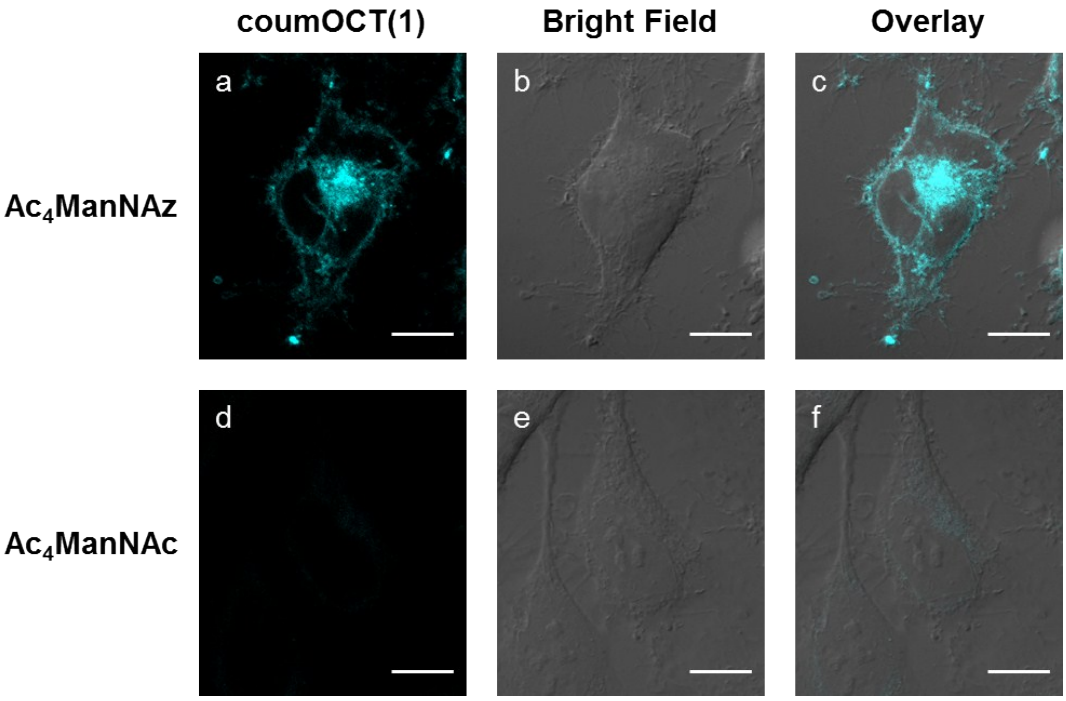


Figure S4. Fluorescence images of sialylated glycoconjugate using coumOCT (**1**) for SPAAC in live cells by confocal microscopy. HeLa cells were incubated with 100 μM of Ac_4ManNAz or Ac_4ManNAc for 3 days, and then labeled with 2.5 μM of **1** for 30 min at 37 ^{o}C under no-wash condition: fluorescence (a, d) and bright field (b, e) and overlaid image of cells (c, f). Control: cells incubated with Ac_4ManNAc. (Scale bar: 10 μm)

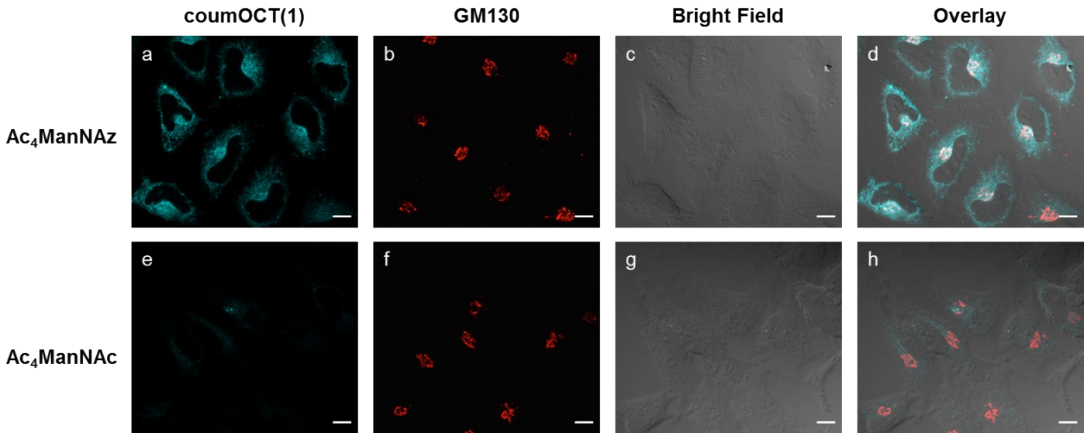


Figure S5. Localization of probe-labeled sialylated glycoconjugates in HeLa cells under confocal microscopy. HeLa cells were incubated with 100 μM of Ac_4ManNAz or Ac_4ManNAc for 3 days, and then treated with 2.5 μM of **1** (cyan) for 30 min at 37 °C. The labeled cells were stained with anti-GM130 followed by Cy3-conjugated antibody (for Golgi, red). (Scale bar: 10 μm)

Time-Lapse Microscopic Analysis of Fluorescence Labeling in Live Cells. HeLa cells were seeded on chamber slide (4×10^4 cells/1 mL per well) and incubated in culture medium (DMEM supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 1 mM sodium pyruvate) with either 100 μM of control sugar (peracetylated *N*-acetylmannosamine, Ac_4ManNAc) or azido-sugar (peracetylated *N*-azidoacetylmannosamine, Ac_4ManNAz) for one hour, and then the cells were washed with PBS to remove the unbound sugars. For time-lapse imaging of live cells, the experiments were carried out using a confocal microscope (Inverted Microscope Eclipse Ti-E with PFS, Nikon) equipped with an incubator to keep the cells in culture conditions. The cells were incubated with 5 μM of DRAQ5™ (ab108410) for 15 min at 37°C for nucleus straining, and then washed three times with PBS. Prewashed cells were incubated with 2.5 μM of coumOCT(**1**) in Opti-MEM with 2 % DMSO and fluorescence imaging of live cells from the previous experiment over 1.5 h. The images

were acquired at 450 nm emission and in 30-min intervals. The results are shown in Figures S7 and S8.

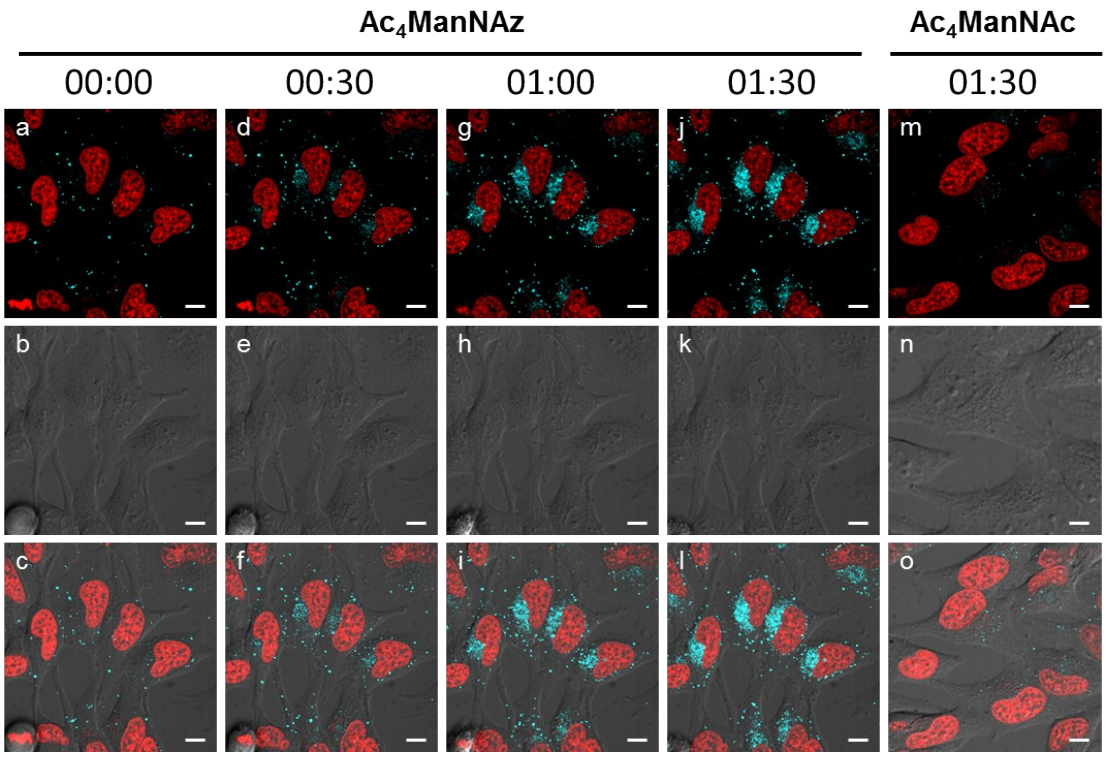


Figure S6. Time-lapse intracellular fluorescence imaging of sialylated glycoconjugate trafficking using coumOCT (**1**) for SPAAC in live cells by confocal microscopy. HeLa cells were incubated with 100 μM of Ac_4ManNAz for 1 h and subsequently washed with PBS buffer to remove excess Ac_4ManNAz. The sugar-treated cells were treated with DRAQ5™ for nucleus staining (red), and then labeled with 2.5 μM of **1** (cyan) under no-wash and no-fixation as well as SPAAC-based conditions. The images were acquired at 450 nm emission and in 30-min intervals: fluorescence (upper row) and bright field (middle row) and overlay images of cell (bottom row). Control: cells incubated with Ac_4ManNAc. (Scale bar: 10 μm)

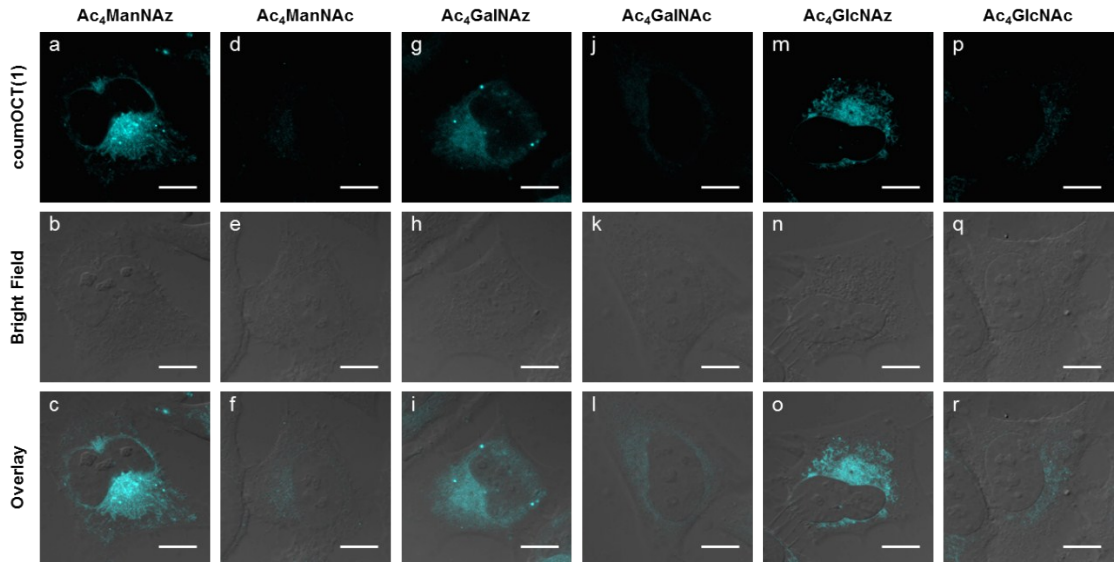


Figure S7. A comparison of fluorescence images upon treatment with different azido-sugars in live cells using coumOCT (**1**) for SPAAC by confocal microscopy. HeLa cells were incubated with 100 μM of azido-sugars (Ac_4ManNAz, Ac_4GalNAz and Ac_4GlcNAz), or control sugars (Ac_4ManNAc, Ac_4GalNAc and Ac_4GlcNAc) for 3 days, respectively, and then treated with 2.5 μM of coumOCT for 30 min at 37°C. (Scale bar: 10 μm)

Stability of Compound (1) and Compound (9) in the Presence of 3-Mercaptopropionic Acid by ^{1}H NMR. A solution of compound **1** and compound **9** (25 mM in $CDCl_3$) was incubated with a solution of 3-mercaptopropionic acid (32 mM in $CDCl_3$) at room temperature (25 ^{o}C). The reaction was monitored by ^{1}H-NMR analysis over a period of 24 h. Compounds **1** and **9** are inert to thiols as there is no reaction of compound **1** or compound **9** with 3-mercaptopropionic acid according to the ^{1}H-NMR analysis. The results are shown in Figures S8 and S9.

Triazole Formation of Compound (1) with Benzyl Azide in the Presence of 3-Mercaptopropionic Acid by ^{1}H NMR. A solution of compound **1** (10 mM in $CDCl_3$)

was incubated with a solution of 3-mercaptopropionic acid (30 mM in $CDCl_3$) at room temperature (25 ^oC) for 1 h, and subsequently added a solution of benzyl azide (30 mM in $CDCl_3$). The mixture was monitored over a period of 1 h. ^1H-NMR analysis indicated that the triazole-forming reaction was complete in 1 h at room temperature without interference by the presence of thiol. Furthermore, both compound **1** and the formed triazole product are stable in the presence of thiols during the reaction. The results are shown in Figure S10.

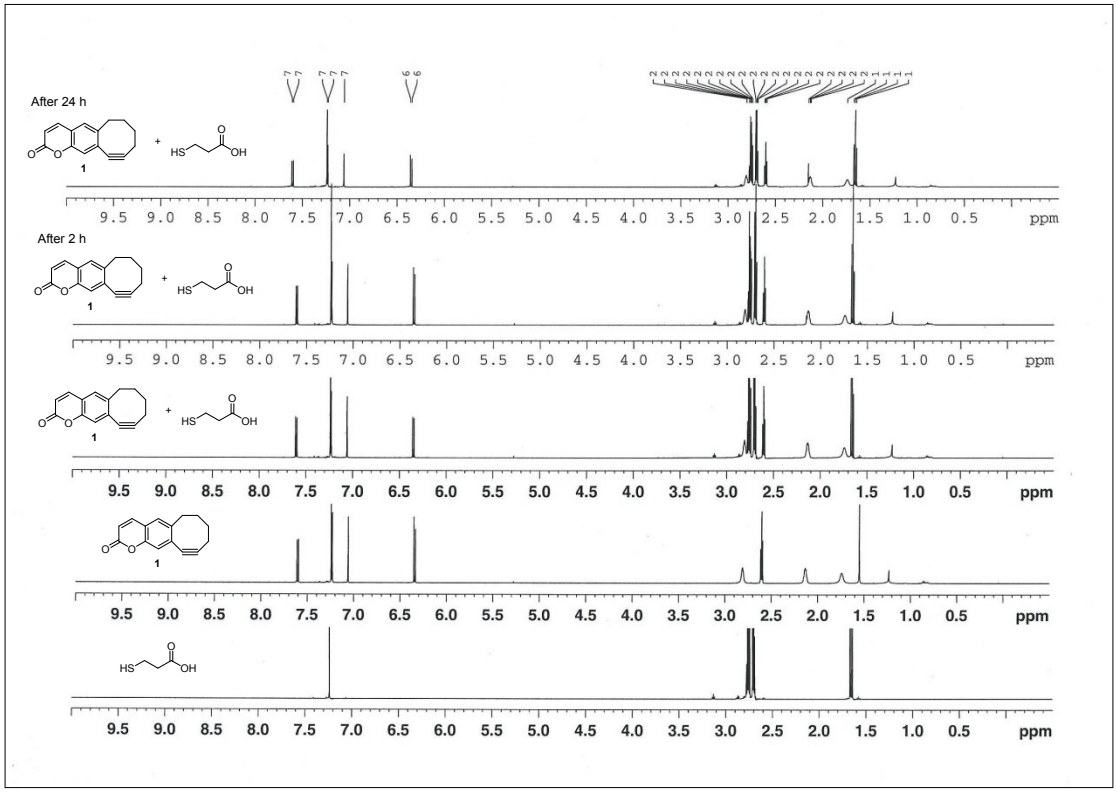


Figure S8. ^1H-NMR spectrum in $CDCl_3$ of compound **1** and 3-mercaptopropionic acid alone, and ^1H-NMR spectrum of compound **1** treated with 3-mercaptopropionic acid at 25 ^oC for 0 h, 2 h and 24 h.

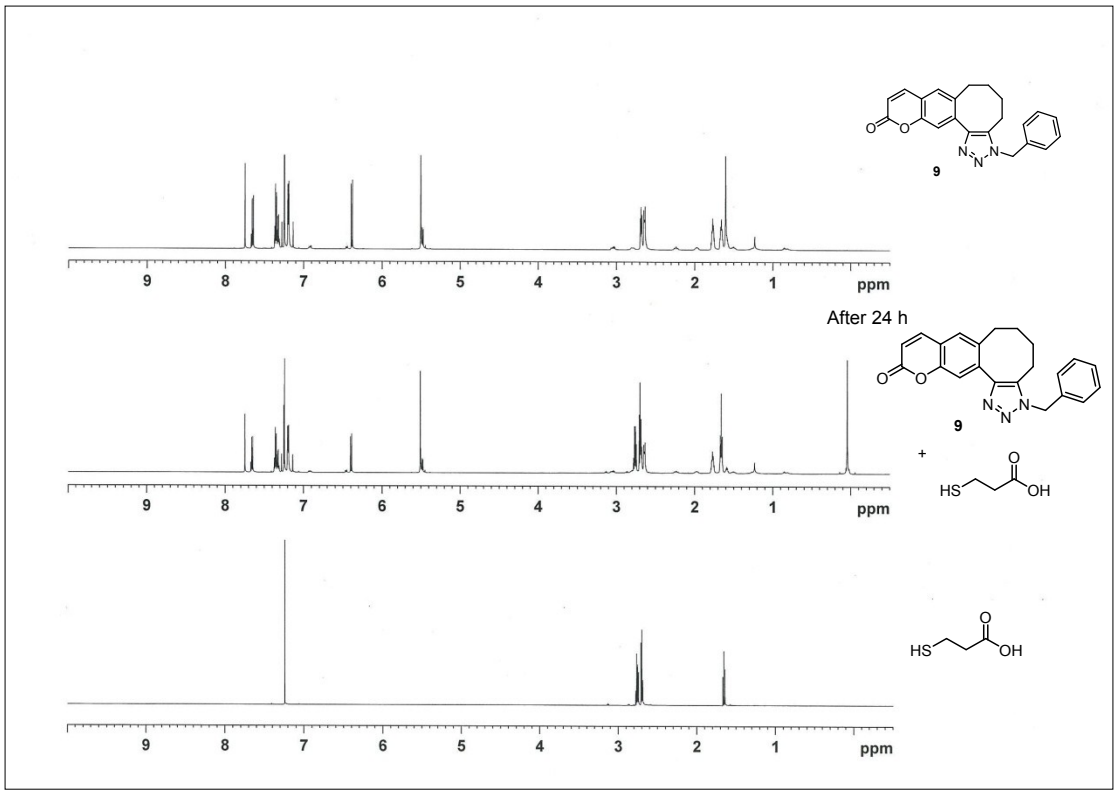


Figure S9. ^{1}H-NMR spectrum in $CDCl_3$ of compound **9** and 3-mercaptopropionic acid alone, and ^{1}H-NMR spectrum of compound **9** treated with 3-mercaptopropionic acid at 25 ^{o}C for 24 h.

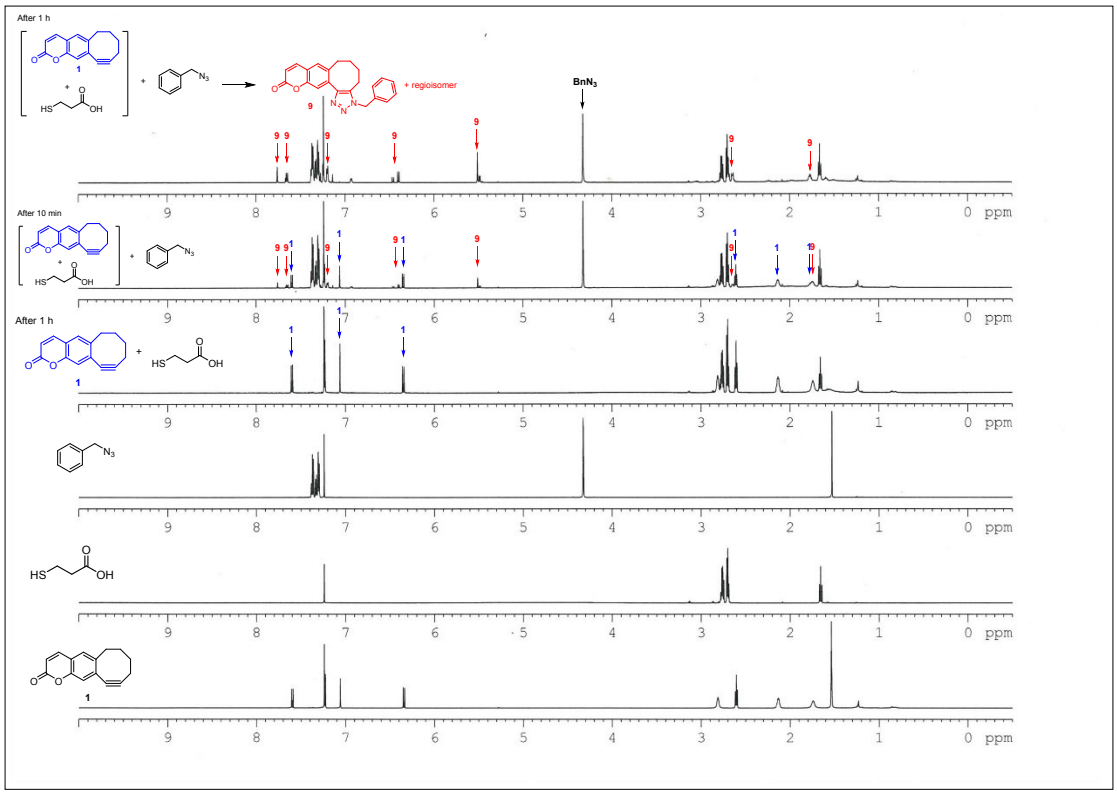
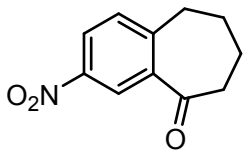


Figure S10. ^{1}H-NMR spectrum in $CDCl_3$ of compound **1**, 3-mercaptopropionic acid and benzyl azide alone, and ^{1}H-NMR spectrum of compound **1** treated with 3-mercaptopropionic acid at 25 ^{o}C for 1 h and subsequently added with benzyl azide for another 1 h.

Synthetic Procedures and Product Characterization.

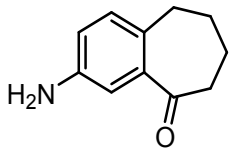
3-Nitro-6,7,8,9-tetrahydrobenzocyclohepten-5-one (A).[S2]



A solution of 1-benzosuberone (4.0 g, 25 mmol) in concentrated H_2SO_4 (28 mL) was cooled at 0 ^{o}C, and a solution of KNO_3 (2.8 g, 27.7 mmol) in concentrated H_2SO_4 (7.5 mL) was added dropwise over a period of 30 min. The mixture was stirred for additional 1 h at 0 ^{o}C, and then poured into crushed ice. The precipitate was filtered, washed with

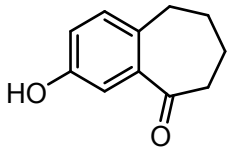
water and air-dried to yield a yellow solid. The crude product was purified by column chromatography on silica gel (EtOAc/hexane, 1:4) to afford the pure nitro product **A** (3.69 g, 72%). $C_{11}H_{11}NO_3$, white needles, mp 90–92 °C (lit.[S2] mp 89–90 °C); TLC (EtOAc/hexane, 1:4) R_f = 0.31; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.53 (1 H, d, J = 2.5 Hz), 8.22 (1 H, dd, J = 8.3, 2.5 Hz), 7.37 (1 H, d, J = 8.3 Hz), 3.01 (2 H, t, J = 6.4 Hz), 2.77 (2 H, t, J = 6.1 Hz), 1.94–1.90 (2 H, m), 1.85–1.81 (2 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.4, 148.0, 147.0, 139.8, 131.0, 126.2, 123.9, 40.4, 32.4, 24.7, 20.5; HRMS calcd for $C_{11}H_{12}NO_3$: 206.0812, found: m/z 206.0814 $[M + H]^+$.

3-Amino-6,7,8,9-tetrahydrobenzocyclohepten-5-one (B).[S2]



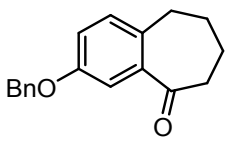
A mixture of nitro compound **A** (2.05 g, 10 mmol) and Sn (8.31 g, 70 mmol) in concentrated HCl (45 mL) and ethanol (25 mL) was heated at reflux for 50 min. The mixture was cooled to room temperature, and basified with 30% NaOH aqueous solution. The mixture was filtered through a pad of Celite, and washed with ethanol. The filtrate was extracted with EtOAc (5 × 50 mL). The combined organic extracts were washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the analytically pure amino product **B** (1.44 g, 82%). $C_{11}H_{13}NO$, yellowish solid, mp 102–104 °C (lit.[S2] mp 103–105 °C); TLC (EtOAc/hexane, 3:7) R_f = 0.29; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.02 (1 H, d, J = 2.6 Hz), 6.96 (1 H, d, J = 8.0 Hz), 6.72 (1 H, dd, J = 8.0, 2.6 Hz), 3.65 (2 H, br s, NH), 2.79 (2 H, t, J = 5.5 Hz), 2.67 (2 H, t, J = 6.6 Hz), 1.81–1.74 (4 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.2, 144.9, 139.3, 131.6, 130.7, 118.8, 114.5, 40.8, 31.5, 25.4, 20.9; HRMS calcd for $C_{11}H_{14}NO$: 176.1070, found: m/z 176.1069 $[M + H]^+$.

3-Hydroxy-6,7,8,9-tetrahydrobenzocyclohepten-5-one (2).[S3]



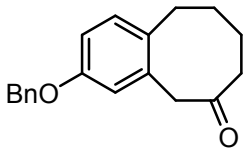
A cold (0 °C) solution of amino compound **B** (1.45 g, 8.3 mmol) in H_2SO_4 (40 mL of 10% aqueous solution) was cautiously added an aqueous solution of $NaNO_2$ (687 mg, 9.96 mmol) in water (3 mL). The reaction mixture was stirred for 30 min at 0 °C, and then sulfamic acid was added to destroy excess nitrous acid. The suspension was filtered and the filtrate was poured into a 10% aqueous solution of H_2SO_4 (100 mL) and toluene (50 mL). The mixture was stirred for 3 days at room temperature. The layers were then separated and the aqueous layer was extracted with EtoAc (5 × 30 mL). The combined organic extracts were washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexane, 1:4) to afford the analytically pure alcohol product **2** (1.11 g, 76%). $C_{11}H_{12}O_2$, yellow solid, mp 98–100 °C (lit.[S3] mp 96–99 °C); TLC (EtOAc/hexane, 3:7) R_f = 0.37; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.33 (1 H, d, J = 2.8 Hz), 7.07 (1 H, d, J = 8.2 Hz), 6.94 (1 H, dd, J = 8.2, 2.8 Hz), 6.27 (1 H, s, OH), 2.84 (2 H, t, J = 5.7 Hz), 2.72 (2 H, t, J = 6.4 Hz), 1.86–1.76 (4 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.7, 154.7, 139.2, 133.9, 131.3, 119.8, 114.9, 40.8, 31.6, 25.3, 20.8; HRMS calcd for $C_{11}H_{13}O_2$: 177.0910, found: m/z 177.0911 $[M + H]^+$.

3-Benzyloxy-6,7,8,9-tetrahydrobenzocyclohepten-5-one (3).



A solution of alcohol compound **2** (1.25 g, 7.1 mmol) in anhydrous DMF (10 mL) was treated with benzyl bromide (1 mL, 8.4 mmol) and potassium carbonate (2.1 g, 15.2 mmol). The suspension was vigorously stirred for 24 h at room temperature. The mixture was poured into water (20 mL) and extracted with Et_2O (4 × 30 mL), The combined organic extracts were washed with water (3 × 20 mL) and brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexane, 1:9) to afford the pure benzyloxy product **3** (1.85 g, 98%). $C_{18}H_{18}O_2$, pale yellow oil; TLC (EtOAc/hexane, 1:9) R_f = 0.37; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (2 H, d, J = 7.2 Hz), 7.37–7.35 (3 H, m), 7.31 (1 H, d, J = 7.4 Hz), 7.10 (1 H, d, J = 8.3 Hz), 7.02 (1 H, dd, J = 8.3, 2.9 Hz), 5.06 (2 H, s), 2.86 (2 H, t, J = 5.8 Hz), 2.71 (2 H, t, J = 6.2 Hz), 1.84–1.78 (4 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 205.6, 157.4, 139.5, 136.7, 134.2, 131.0, 128.5 (2×), 127.9, 127.5 (2×), 119.7, 113.2, 70.1, 40.7, 31.6, 25.3, 20.8; HRMS calcd for $C_{18}H_{19}O_2$: 267.1380, found: *m*/*z* 267.1383 $[M + H]^+$.

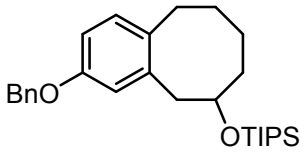
3-Benzyloxy-7,8,9,10-tetrahydro-5*H*-benzocycloocten-6-one (4).



A stirred solution of (trimethylsilyl)diazomethane (5 mL, ca. 2 M solution in hexane, 10 mmol) in CH_2Cl_2 (10 mL) was added dropwise over a period of 1 h to a stirred solution of compound **3** (1.6 g, 6 mmol) and $BF_3{\cdot}OEt_2$ (820 μL, 10 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The mixture was stirred for 12 h at 0 °C, and then poured into crushed ice. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with brine (50 mL), dried over $MgSO_4$, and concentrated to give an orange oil that was purified by column chromatography on silica gel (EtOAc/hexane,

1:19) to afford pure cyclooctanone product **4** (1.23 g, 73%). $C_{19}H_{20}O_2$, colorless oil; TLC (EtOAc/hexane, 1:9) R_f = 0.29; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 (2 H, d, J = 7.4 Hz), 7.37–7.34 (2 H, m), 7.31–7.29 (1 H, m), 7.09 (1 H, d, J = 8.4 Hz), 6.84 (1 H, dd, J = 8.4, 2.7 Hz), 6.75 (1 H, d, J = 2.7 Hz), 5.01 (2 H, s), 3.72 (2 H, s), 2.74 (2 H, t, J = 5.8 Hz), 2.31 (2 H, t, J = 5.3 Hz), 1.81–1.77 (2 H, m), 1.72–1.68 (2 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.8, 157.4, 136.9, 134.7, 133.4, 131.2, 128.5 (2 ×), 127.9, 127.5 (2 ×), 116.0, 114.4, 70.0, 48.8, 41.0, 32.3, 31.5, 24.7; HRMS calcd for $C_{19}H_{21}O_2$: 281.1536, found: m/z 281.1539 $[M + H]^+$.

3-Benzyloxy-6-triisopropylsilyloxy-5,6,7,8,9,10-hexahydrobenzocyclooctene (5).

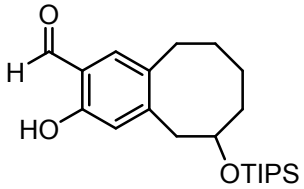


A cold (0 °C) solution of compound **4** (4.8 g, 17.1 mmol) in methanol (40 mL) was treated with $NaBH_4$ (970 mg, 25.7 mmol). The mixture was stirred for 1 h at 0 °C, and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (80 mL), and washed with 1 M HCl aqueous solution (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to provide a crude alcohol product as colorless foam (4.8 g), which was used in the next step without further purification.

The above-prepared alcohol (4.8 g, 17.0 mmol) and 2,6-lutidine (8 mL, 68.7 mmol) were dissolved in anhydrous CH_2Cl_2 (50 mL), and cooled to 0 °C. Triisopropylsilyl trifluoromethanesulfonate (9.2 mL, 34.2 mmol) was added dropwise over a period of 3 min to the mixture. The mixture was stirred for 1 h at room temperature, and then diluted with CH_2Cl_2 (100 mL). The solution was washed with saturated aqueous $NaHCO_3$ (50 mL), 1 M HCl aqueous solution (50 mL), and brine (50 mL). The organic

layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:9) to afford the pure silyl ether product **5** (7.2 g, 96% for two steps). $C_{28}H_{42}O_2Si$, colorless syrup; TLC (EtOAc/hexane, 1:9) R_f = 0.51; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (2 H, d, J = 7.4 Hz), 7.37–7.34 (2 H, m), 7.31–7.28 (1 H, m), 6.99 (1 H, dd, J = 6.6, 2.5 Hz), 6.75–6.74 (2 H, m), 5.01 (2 H, s), 3.96–3.93 (1 H, m), 2.91–2.83 (2 H, m), 2.77–2.72 (1 H, m), 2.65–2.61 (1 H, m), 1.76–1.72 (1 H, m), 1.71–1.64 (1 H, m), 1.50–1.41 (3 H, m), 1.18–1.15 (1 H, m), 1.07–1.05 (21 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.8, 138.5, 137.3, 134.1, 130.1, 128.5 (2 ×), 127.8, 127.5 (2 ×), 116.7, 112.5, 73.8, 70.0, 40.9, 34.5, 32.3, 32.0, 20.8, 18.2 (6 ×), 12.4 (3 ×); HRMS calcd for $C_{28}H_{43}O_2Si$: 439.3032, found: m/z 439.3022 $[M + H]^+$.

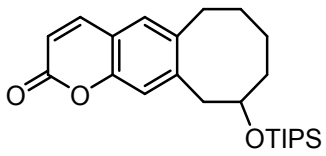
3-Hydroxy-6-triisopropylsilyloxy-5,6,7,8,9,10-hexahydrobenzocycloocten-2-carboxaldehyde (6).



A solution of compound **5** (7.1 g, 16.2 mmol) in methanol (50 mL) and EtOAc (20 mL) was treated with Pd/C (100 mg) under an atmosphere of hydrogen. After stirring for 1 h, the mixture was filtered through Celite, and rinsed with EtOAc. The filtrate was concentrated under reduced pressure to give a light brown syrup (5.6 g), which was dissolved in anhydrous acetonitrile (150 mL) and treated with anhydrous $MgCl_2$ (4.64 g, 48.6 mmol), triethylamine (13.5 mL, 97.2 mmol) and paraformaldehyde (4.86 g, 162 mmol). The suspension was heated at reflux for 12 h. The mixture was cooled to room temperature, and the deep-yellow suspension was acidified with a 1 M HCl aqueous

solution (200 mL). The solution was extracted with EtOAc (5 × 150 mL). The combined organic extracts were washed with brine (200 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexane, 1:19) to afford the pure salicylaldehyde product **6** (5.3 g, 87% for two steps). $C_{22}H_{36}O_3Si$, pale yellow syrup; TLC (EtOAc/hexane, 1:9) R_f = 0.71; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.77 (1 H, s), 9.79 (1 H, s), 7.24 (1 H, s), 6.76 (1 H, s), 4.03–3.99 (1 H, m), 2.94–2.88 (2 H, m), 2.81–2.76 (1 H, m), 2.71–2.67 (1 H, m), 1.80–1.75 (1 H, m), 1.72–1.66 (1 H, m), 1.52–1.46 (2 H, m), 1.43–1.38 (1 H, m), 1.24–1.16 (1 H, m), 1.07–1.05 (21 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.9, 159.5, 148.2, 133.8, 133.7, 119.5, 118.8, 73.2, 41.0, 34.4, 32.2, 32.1, 20.6, 18.1 (6×), 12.4 (3×); HRMS calcd for $C_{22}H_{37}O_3Si$: 377.2506, found: *m*/*z* 377.2511 $[M + H]^+$.

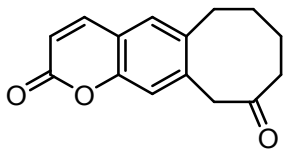
6,7,8,9,10,11-Hexahydro-10-triisopropylsilyloxy-cycloocta[g]chromen-2(2*H*)-one (7)



Preparation of ketenylidenetriphenylphosphorane: A stirred solution of carbethoxymethylenetriphenylphosphorane (10 g, 30 mmol) in anhydrous toluene (200 mL) was added dropwise to a solution of sodium hexamethyldisilazide (17.5 mL, 2 M solution in THF, 35 mmol) at 0 °C. Once the addition was complete, the mixture was heated at 60 °C for 24 h. The reaction was then allowed to cool to room temperature and filtered out. The filtrate was concentrated under reduced pressure, and then poured into ether (200 mL). The precipitate was filtered, washed with ether, and air-dried to afford ketenylidenetriphenylphosphorane (5.8 g, 64%) as pale yellow solids.

A stirred solution of salicylaldehyde **6** (4.3 g, 11.42 mmol) in anhydrous toluene (100 mL) was added to the fresh prepared ketenylidenetriphenylphosphosphorane (5.2 g, 17.2 mmol) at room temperature. The mixture was heated at 90 °C for 1.5 h, and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:9) to afford the pure coumarin product **7** (3.8 g, 83%). $C_{24}H_{36}O_3Si$, colorless solid, mp 103–105 °C; TLC (EtOAc/hexane, 1:9) R_f = 0.25; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.61 (1 H, d, J = 9.5 Hz), 7.18 (1 H, s), 7.10 (1 H, s), 6.33 (1 H, d, J = 9.5 Hz), 4.02 (1 H, dd, J = 11.9, 5.5 Hz), 3.00–2.94 (2 H, m), 2.86–2.81 (1 H, m), 2.76–2.72 (1 H, m), 1.80–1.73 (1 H, m), 1.69–1.64 (1 H, m), 1.60–1.55 (1 H, m), 1.47–1.43 (2 H, m), 1.20–1.13 (1 H, m), 1.11–1.03 (21 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.3, 152.3, 143.3, 142.4, 138.4, 127.8, 118.1, 117.3, 115.7, 73.3, 40.8, 34.5, 32.4, 32.0, 20.6, 18.1 (6×), 12.4 (3×); HRMS calcd for $C_{24}H_{37}O_3Si$: 401.2506, found: m/z 401.2511 $[M + H]^+$.

6,7,8,9-Tetrahydro-11*H*-10-oxo-cycloocta[g]chromen-2(2*H*)-one (8)

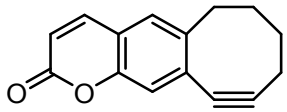


A cold (0 °C) solution of compound **7** (3.0 g, 7.5 mmol) in THF (20 mL) was treated with a solution of tetrabutylammonium fluoride (10 mL, 1 M solution in THF, 10 mmol). After stirring for 1 h at room temperature, the mixture was concentrated under reduced pressure. The residual oil was filtered through a short pad of silica gel (EtOAc/hexane, 1:4) and the filtrate was concentrated to give a colorless solid (1.67 g).

A solution of DMSO (1.5 mL, 21.2 mmol) in anhydrous CH_2Cl_2 (10 mL) was added dropwise to a stirred solution of oxalyl chloride (0.89 mL, 10.3 mmol) in anhydrous CH_2Cl_2 (10 mL) at –78 °C under an atmosphere of nitrogen. The mixture was stirred

for 30 min at −78 °C, and the above-prepared alcohol (1.67 g) in anhydrous CH_2Cl_2 (10 mL) was added dropwise. The mixture was stirred for an additional 30 min at –78 °C, and triethylamine (7.1 mL, 50.4 mmol) was added. The mixture was allowed to warm to 0 °C for 30 min, and then poured into water (40 mL). The aqueous layer was extracted with CH_2Cl_2 (5 × 50 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexane, 1:4) to afford the desired product **8** (1.45 g, 78% for two steps). $C_{15}H_{14}O_3$, colorless solid, mp 127–129 °C; TLC (EtOAc/hexane, 3:7) R_f = 0.32; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.63 (1 H, d, J = 9.5 Hz), 7.29 (1 H, s), 7.09 (1 H, s), 6.34 (1 H, d, J = 9.5 Hz), 3.82 (2 H, s), 2.84 (2 H, t, J = 5.7 Hz), 2.31 (2 H, t, J = 5.6 Hz), 1.86–1.84 (2 H, m), 1.73–1.71 (2 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 209.9, 160.7, 152.6, 142.8, 138.2, 137.7, 129.0, 118.4, 117.7, 116.6, 48.8, 41.1, 32.3, 31.4, 24.5; HRMS calcd for $C_{15}H_{15}O_3$: 243.1016, found: m/z 243.1016 $[M + H]^+$.

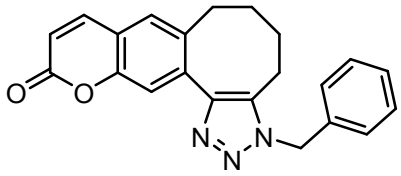
6,7,8,9-Tetrahydro-10,11-didehydro-cycloocta[g]chromen-2(2*H*)-one (1)



A cold (–78 °C) solution of compound **8** (245 mg, 1 mmol) and *N*-phenyl bis(trifluoromethanesulfonimide) (393 mg, 1.1 mmol) in anhydrous THF (10 mL) was added to a solution of sodium hexamethyldisilazide (0.55 mL, 2 M solution in THF, 1.1 mmol) via syringe over a period of 5 min. The mixture was stirred at –78 °C for 1 h, and another batch of sodium hexamethyldisilazide (0.55 mL, 2 M solution in THF, 1.1 mmol) was added. The mixture was allowed to warm to 0 °C, stirred for an additional 1 h, and then quenched with methanol (1 mL). The mixture was concentrated under

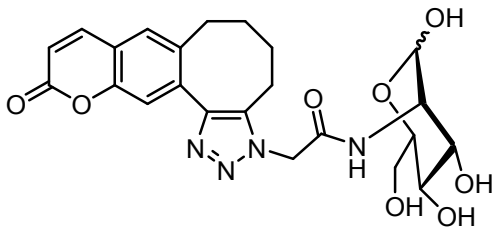
reduced pressure to give a yellow syrup, which was purified by column chromatography on silica gel (EtOAc/hexane, 1:9) to afford the target product **1** (72 mg, 32%). $C_{15}H_{12}O_2$, light-yellow solid, mp 98–100 °C; TLC (EtOAc/hexane, 3:7) R_f = 0.42; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60 (1 H, d, J = 9.5 Hz), 7.23 (1 H, s), 7.06 (1 H, s), 6.35 (1 H, d, J = 9.5 Hz), 2.81 (2 H, br s), 2.61 (2 H, t, J = 6.7 Hz), 2.13 (2 H, br s), 1.74 (2 H, br s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.1, 152.6, 147.4, 143.3, 128.6, 127.1, 117.5, 117.4, 116.1, 113.9, 92.5, 38.3, 33.5, 25.5, 20.5; HRMS calcd for $C_{15}H_{13}O_2$: 225.0910, found: m/z 225.0910 $[M + H]^+$.

10-Benzyl-6,7,8,9-tetrahydro-cyclooctatriazolo[5,4-*g*]chromen-2(2*H*)-one (9)



A solution of compound **1** (50 mg, 0.22 mmol) in CH_3CN (5 mL) was treated with benzyl azide (44 μL, 0.33 mmol). After stirring for 2 h at room temperature, the mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 3:7) to afford the desired triazole product **9** (75 mg, 95%). $C_{22}H_{19}N_3O_2$, colorless solid, mp 60–62 °C; TLC (EtOAc/hexane, 1:1) R_f = 0.35; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75 (1 H, s), 7.65 (1 H, d, J = 9.4 Hz), 7.37–7.32 (3 H, m), 7.24 (1 H, s), 7.20–7.19 (2 H, m), 6.39 (1 H, d, J = 9.4 Hz), 5.50 (2 H, s), 2.69–2.67 (2 H, m), 2.65–2.63 (2 H, m), 1.79–1.75 (2 H, m), 1.67–1.64 (2 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.0, 152.4, 143.1, 136.1, 135.3, 135.0, 134.4, 129.0 (2 ×), 128.7, 128.4, 128.1, 127.1 (2 ×), 126.9, 116.7, 116.4, 51.9, 30.8, 30.6, 23.9, 20.0; HRMS calcd for $C_{22}H_{20}N_3O_2$: 358.1550, found: m/z 358.1548 $[M + H]^+$.

***N*-[2-(11-Oxo-4,6,7,11-tetrahydrochromeno[7',6':3,4]cycloocta[1,2-*d*][1,2,3]triazol-3(5*H*)-yl)]acetamido-2-deoxy-α,β-D-mannopyranose (10)**



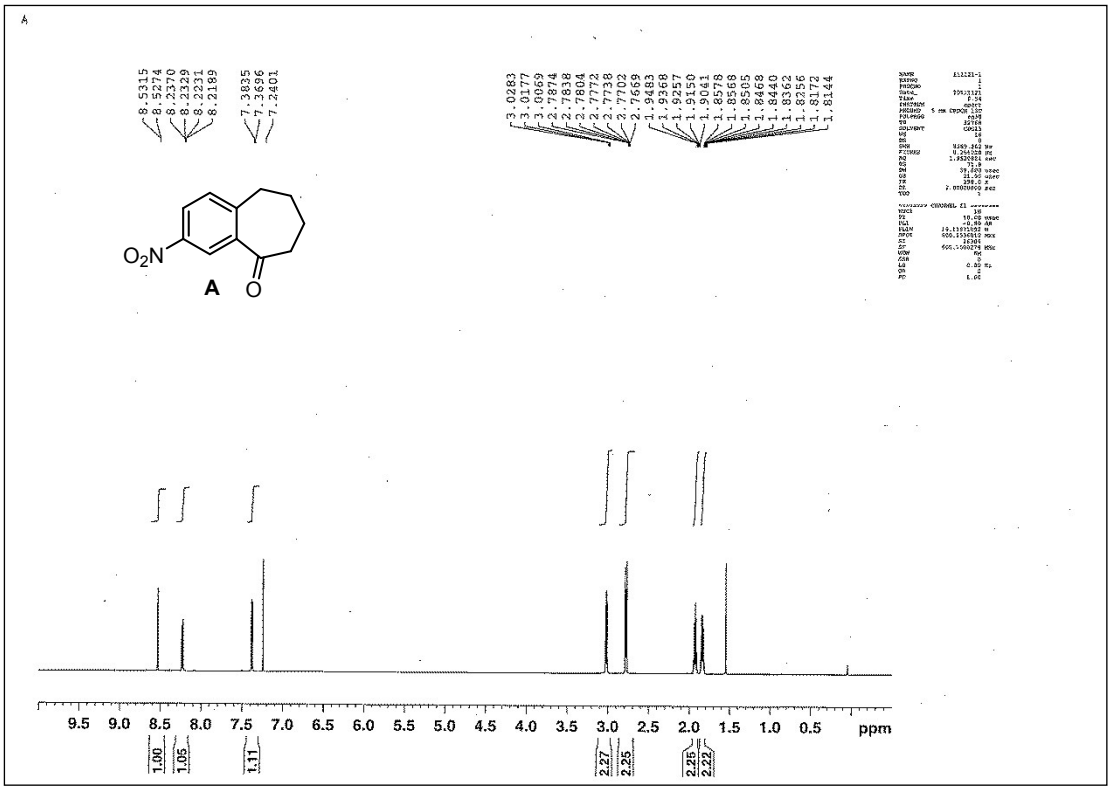
A solution of compound **1** (50 mg, 0.22 mmol) in MeOH (5 mL) and water (1 mL) was treated with *N*-azidoacetylmannosamine (142 mg, 0.33 mmol). After stirring for 2 h at room temperature, the mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel ($MeOH/CH_2Cl_2$, 1:9) to afford the desired triazole product **10** (98 mg, 92%). $C_{23}H_{26}N_4O_8$, colorless solid, mp 170–172 °C (dec.); TLC ($MeOH/CH_2Cl_2$, 1:8) R_f = 0.25; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.04 (1 H, d, J = 9.5 Hz),7.59 (1 H, s), 7.52 (1 H, s), 6.48 (1 H, d, J = 9.5 Hz), 5.15 (1 H, d, J = 2.9 Hz), 5.11 (1 H, d, J = 6.9 Hz), 4.98–4.85 (1 H, m), 4.89–4.81 (2 H, m), 4.44–4.38 (1 H, m), 3.61–3.59 (3 H, m), 3.53–3.45 (2 H, m), 3.42–3.37 (1 H, m), 3.16–3.14 (2 H, m), 2.86–2.82 (2 H, m), 2.72–2.70 (2 H, m), 1.78 (2 H, br s), 1.61 (2 H, br t, J = 5.5 Hz); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.2, 160.1, 151.9, 143.8, 140.6, 137.0, 135.9, 134.7, 128.8, 118.6, 116.1, 115.2, 92.5, 90.4, 72.9, 72.1, 71.0, 70.5, 68.2, 67.5, 61.5, 61.0, 54.7, 54.2, 50.0, 49.9, 30.6, 29.9, 22.8, 19.6; HRMS calcd for $C_{23}H_{27}N_4O_8$: 487.1829, found: m/z 487.1827 $[M + H]^+$.

References:

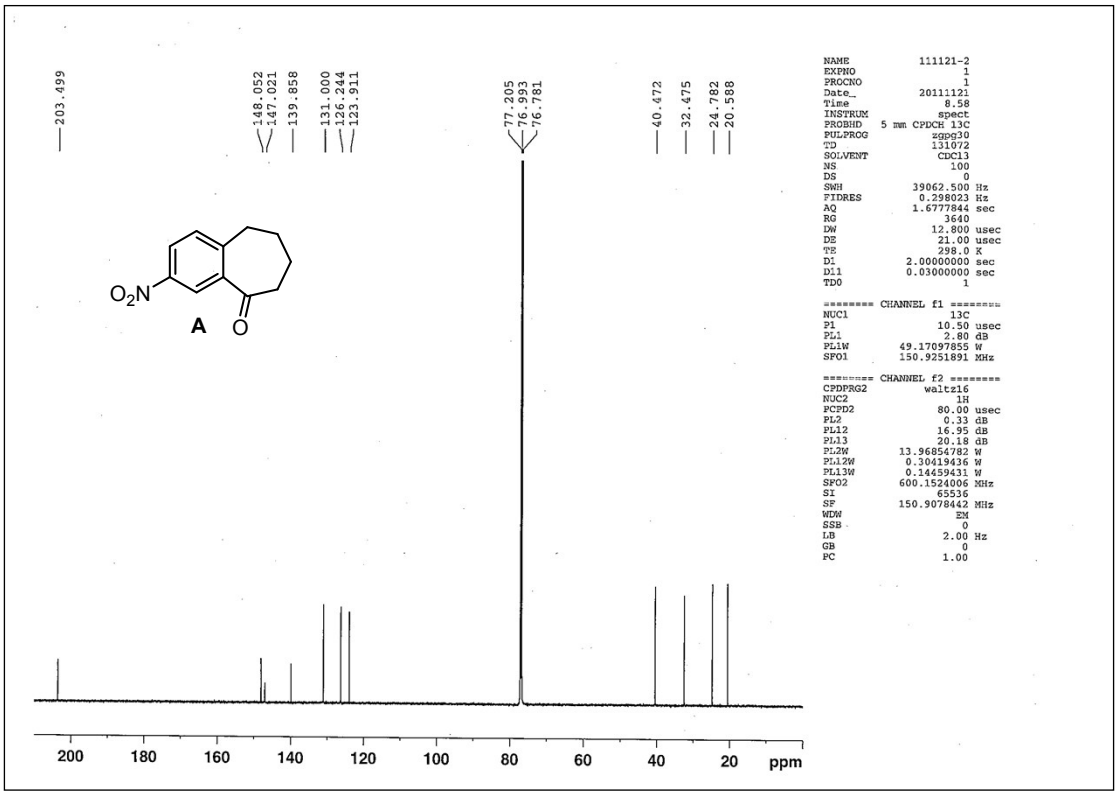
S1. J. N. Demas and G. A. Crosby, *J. Phys. Chem.*, 1971, **75**, 991.

S2. G. Murineddu, S. Ruiu, G. Loriga, I. Manca, P. Lazzari, R. Reali, L. Pani, L. Toma and G. A. Pinna, *J. Med. Chem.*, 2005, **48**, 7351.

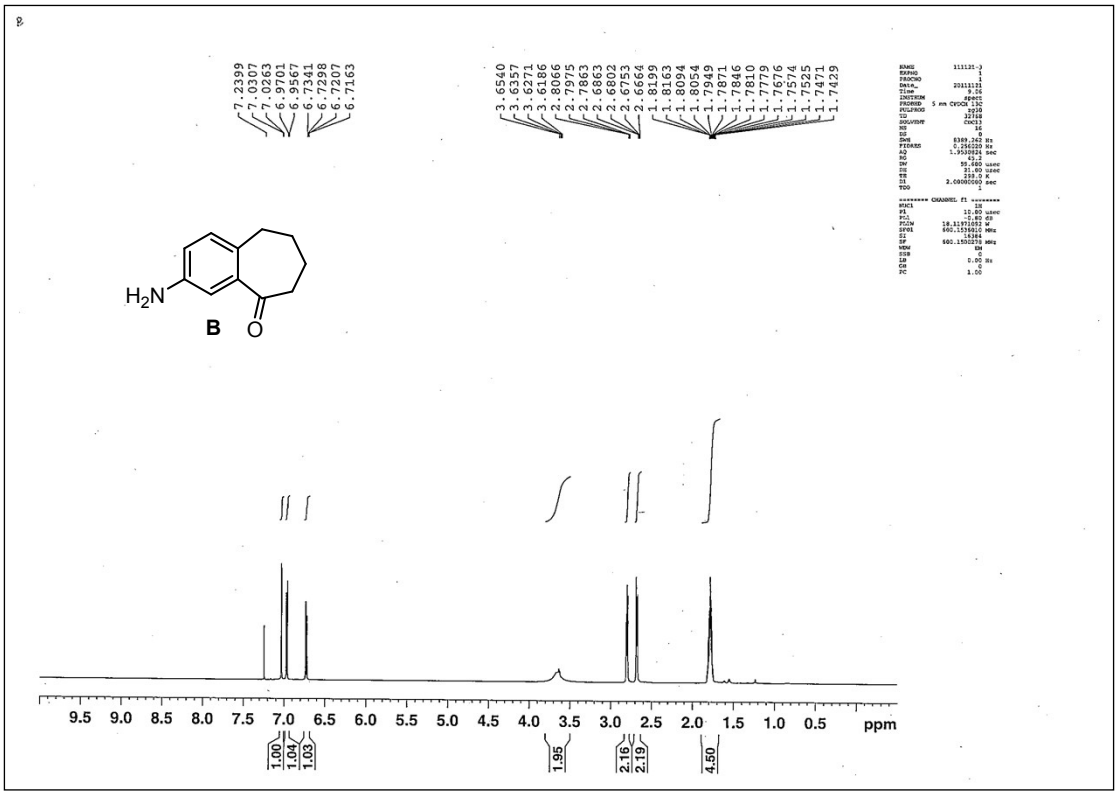
S3. P. A. S. Smith and W. L. Berry, *J. Org. Chem*., 1961, **26***,* 27.



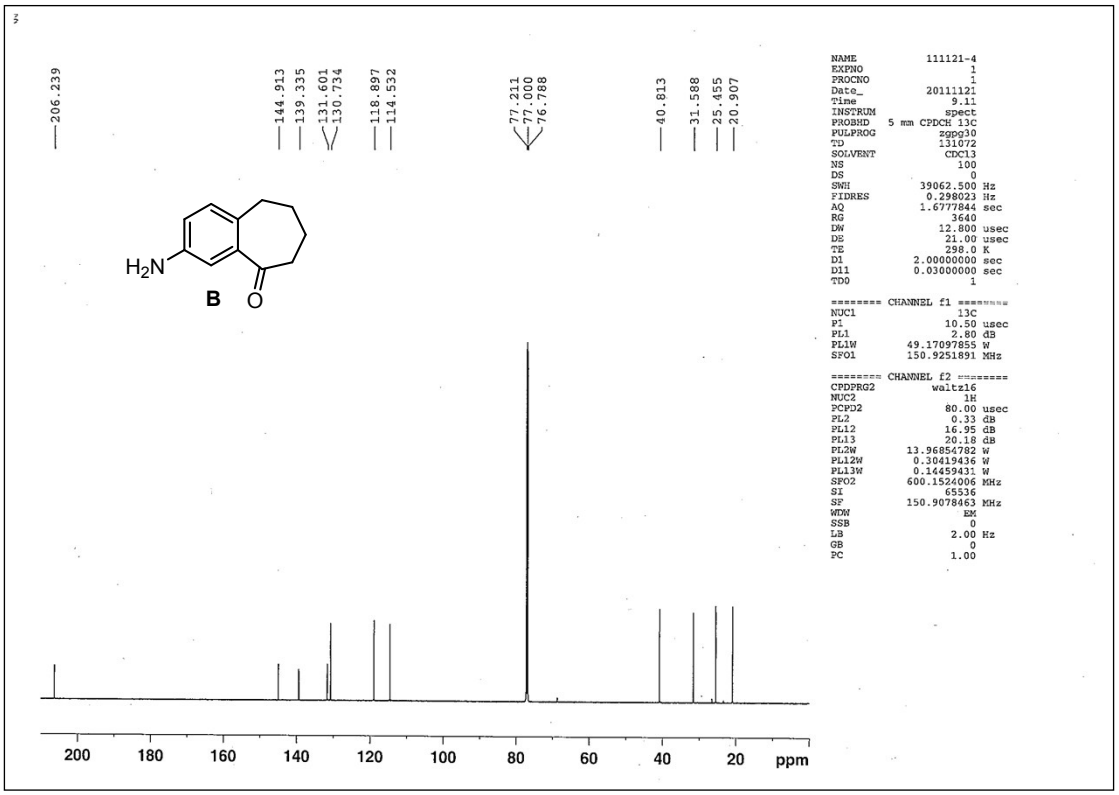
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of Compound **A**.



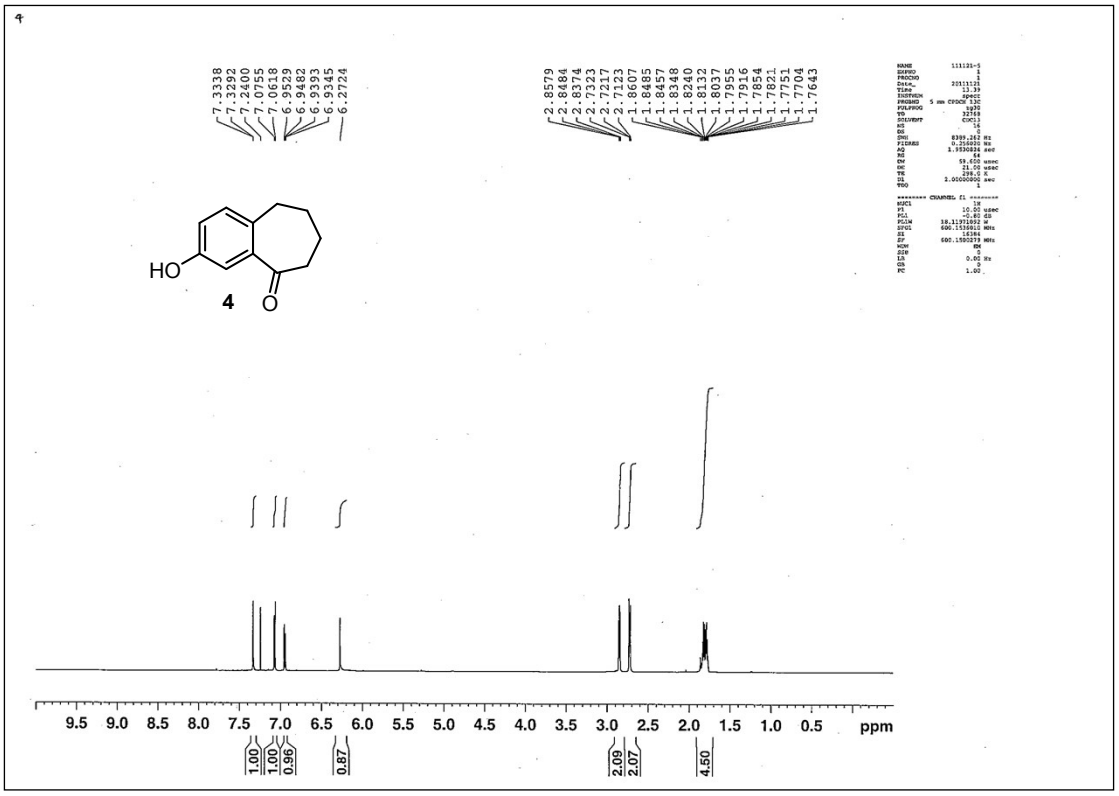
^{13}C NMR Spectrum (150 MHz, $CDCl_3$) of Compound **A**.



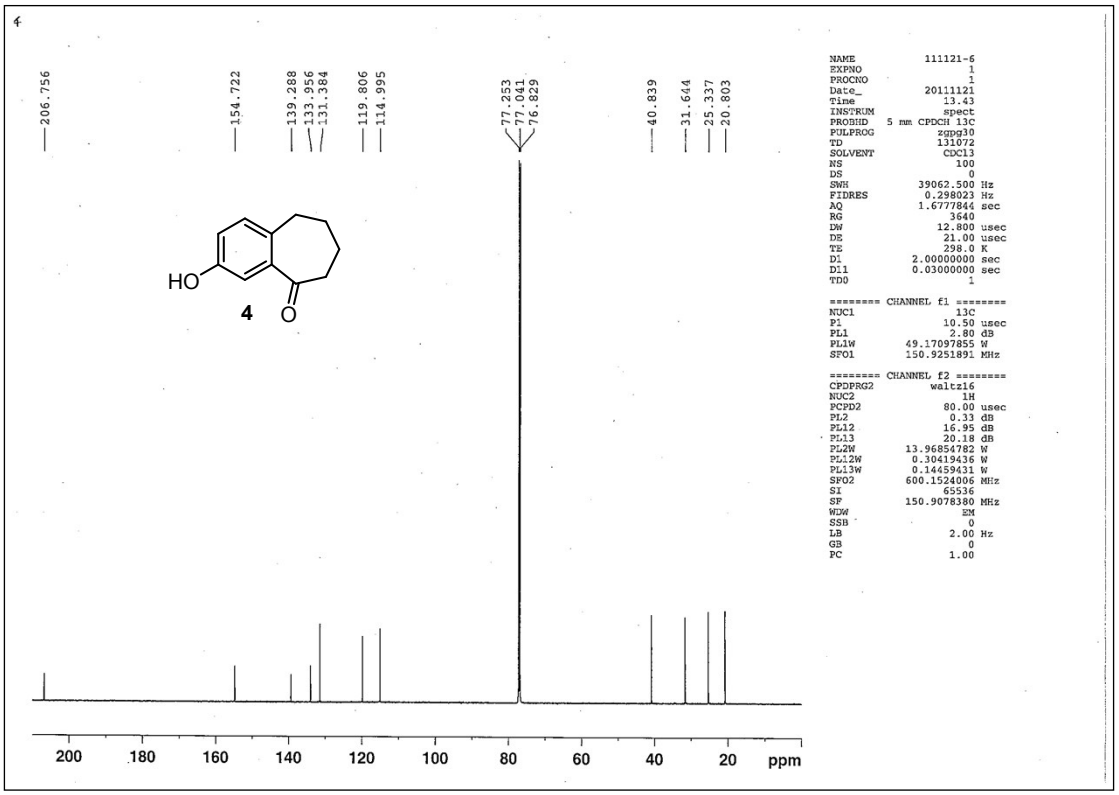
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of Compound **B**.



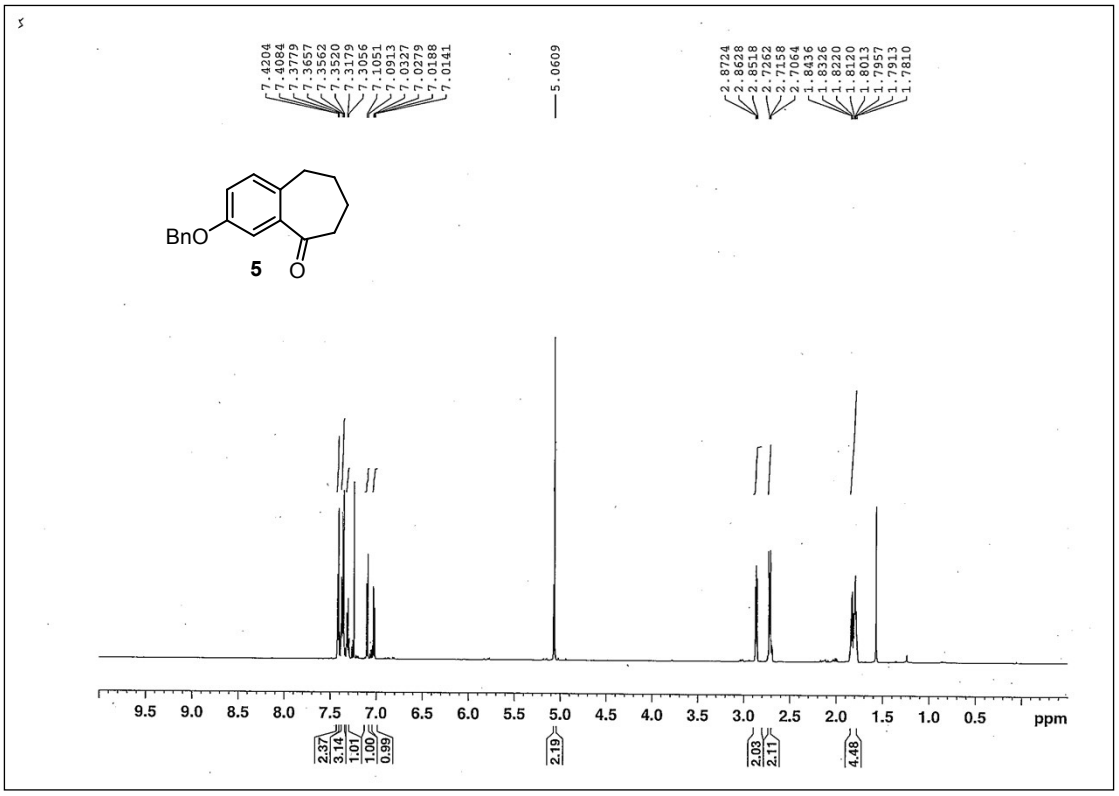
^{13}C NMR Spectrum (150 MHz, $CDCl_3$) of Compound **B**.



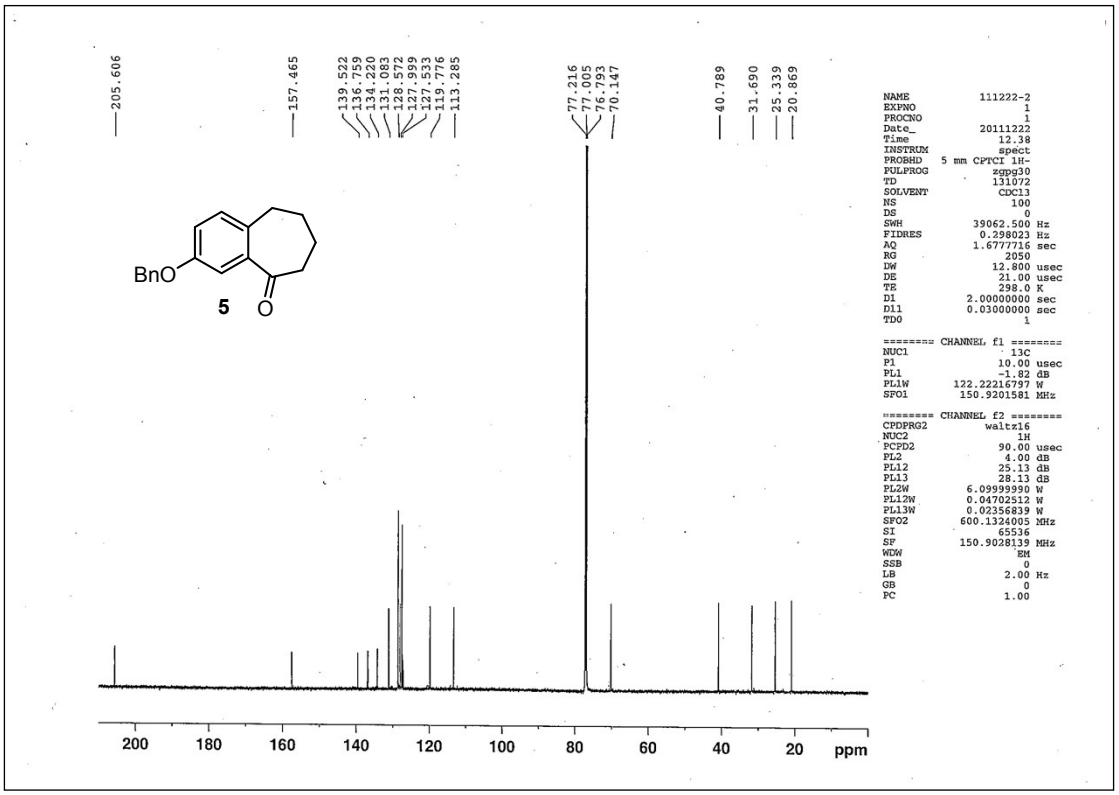
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of Compound **4**.



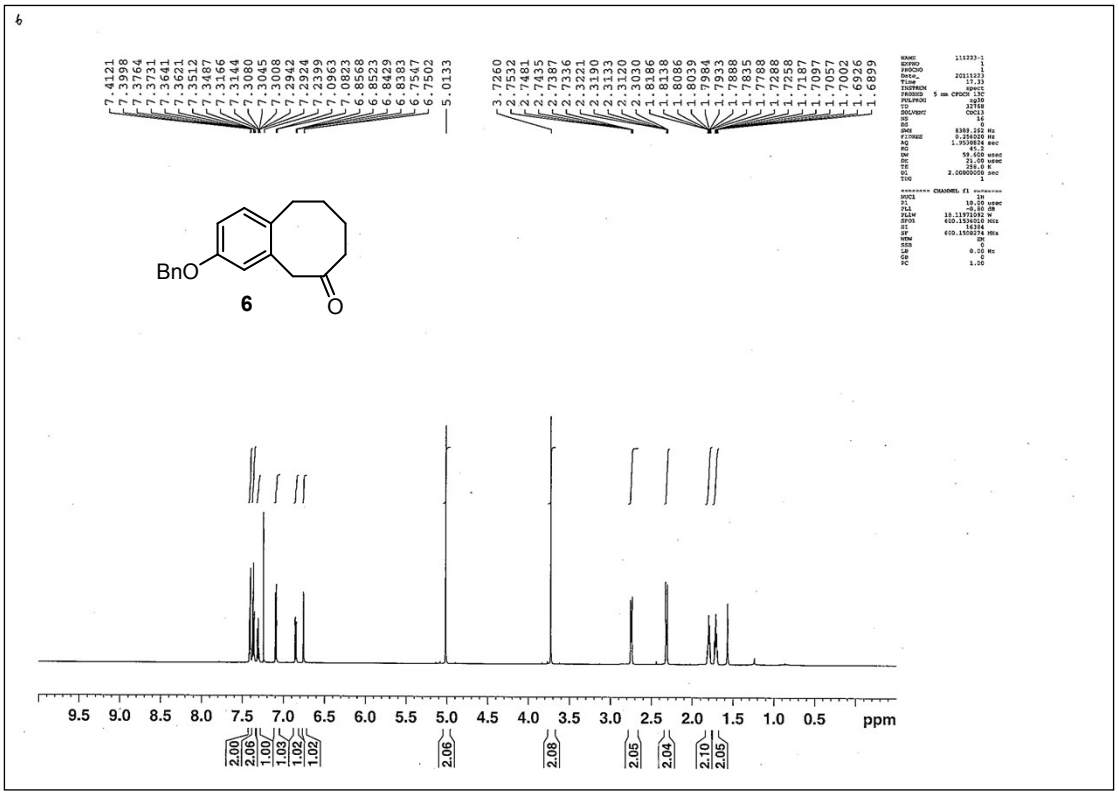
^{13}C NMR Spectrum (150 MHz, $CDCl_3$) of Compound **4**.



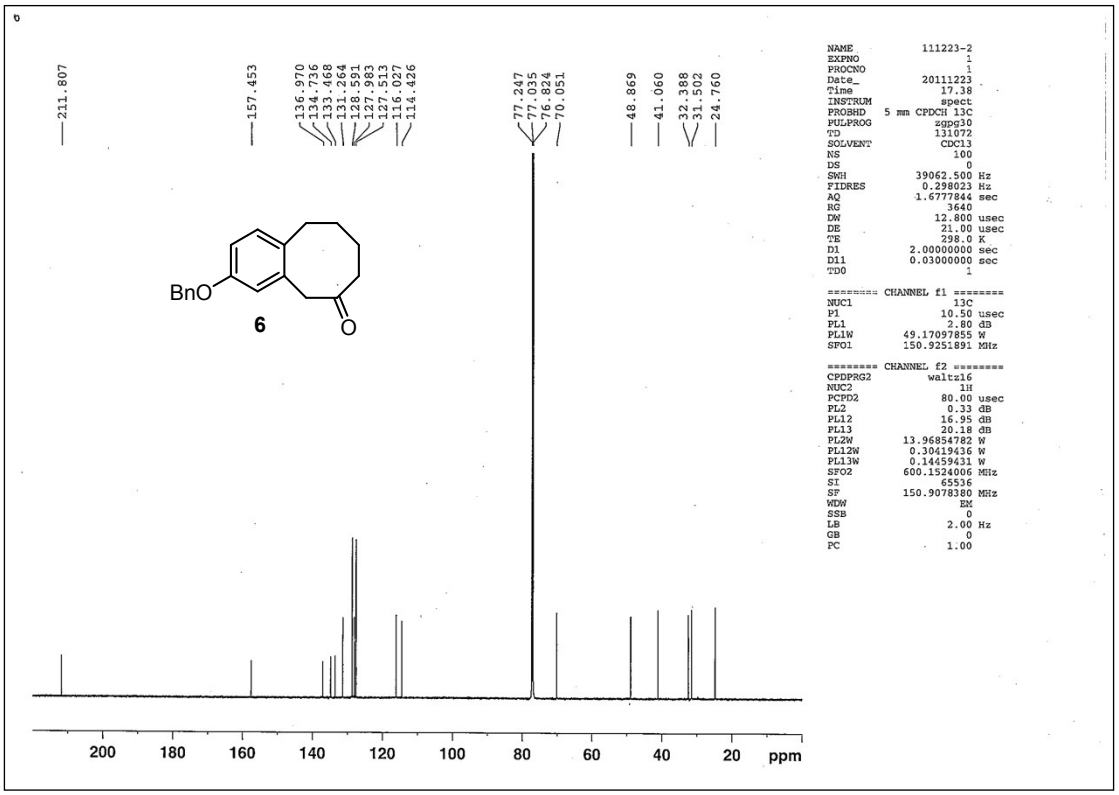
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of Compound **5**.



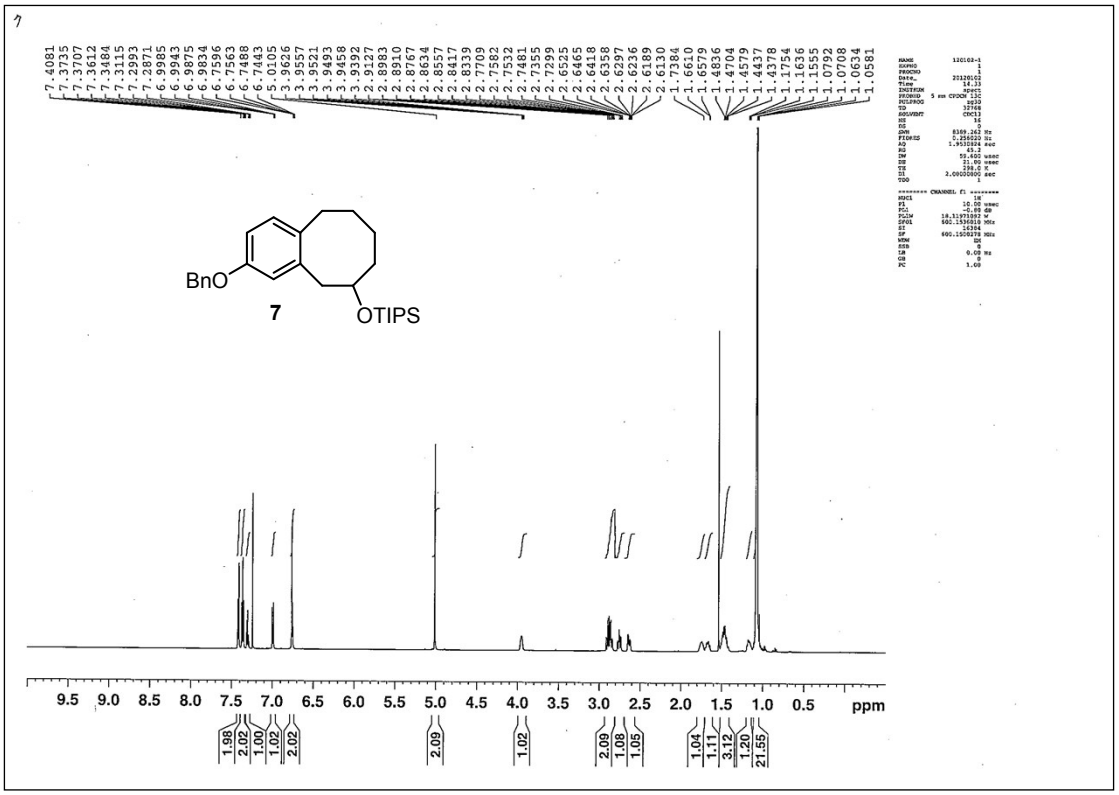
^{13}C NMR Spectrum (150 MHz, $CDCl_3$) of Compound **5**.



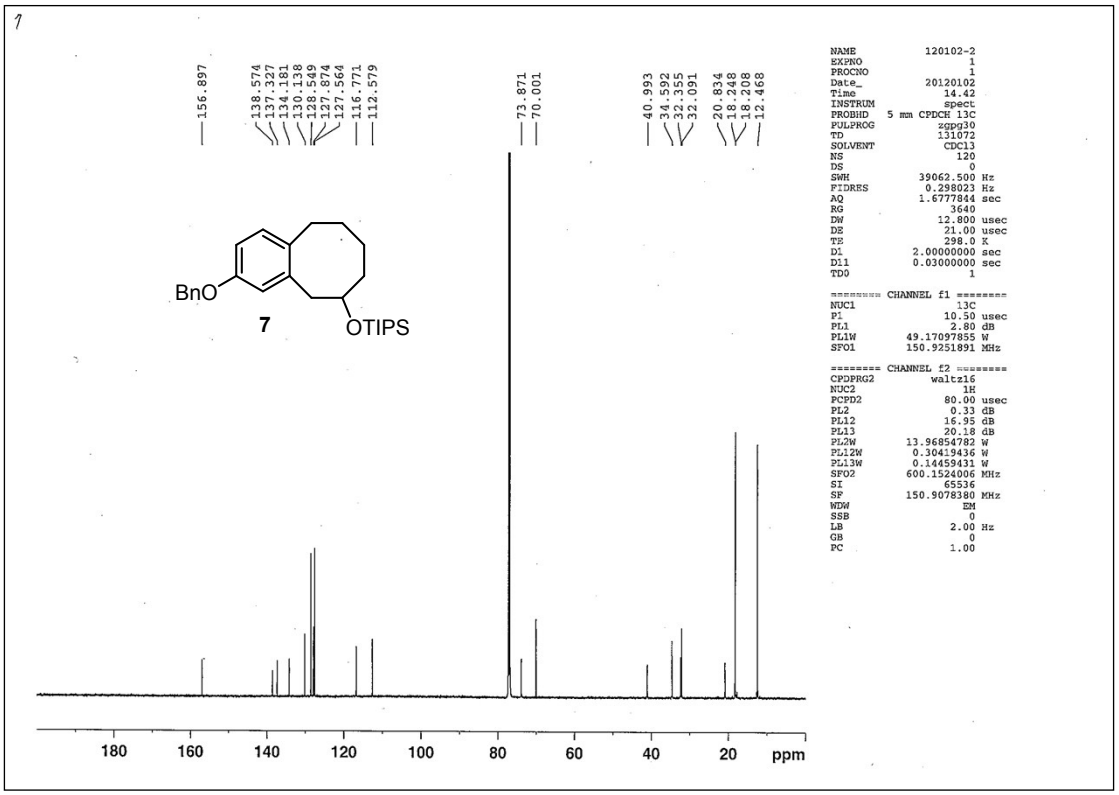
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of Compound **6**.



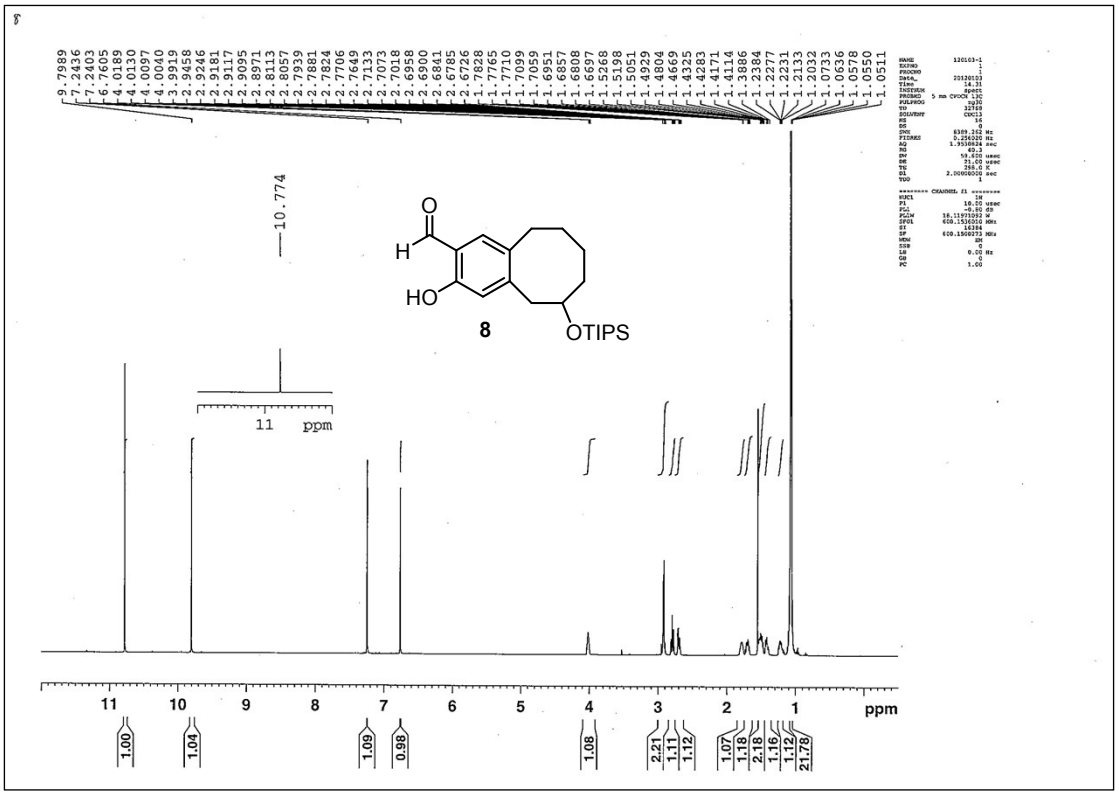
^{13}C NMR Spectrum (150 MHz, $CDCl_3$) of Compound **6**.



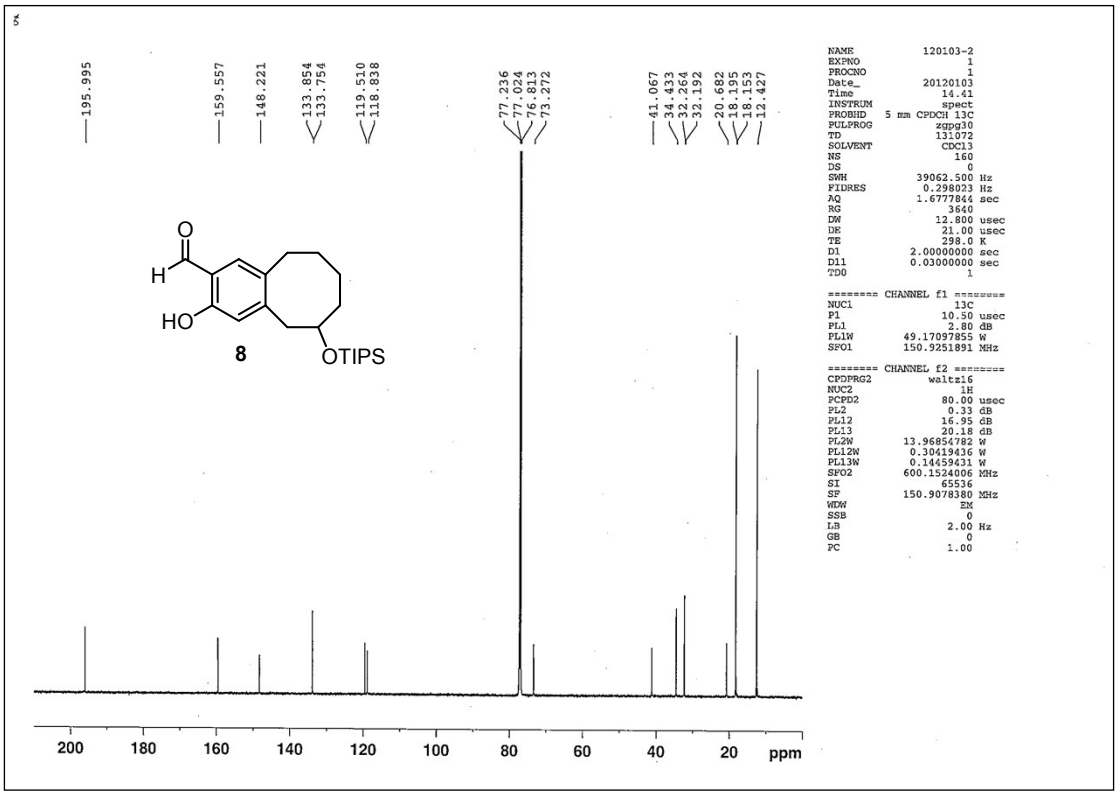
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of Compound **7**.



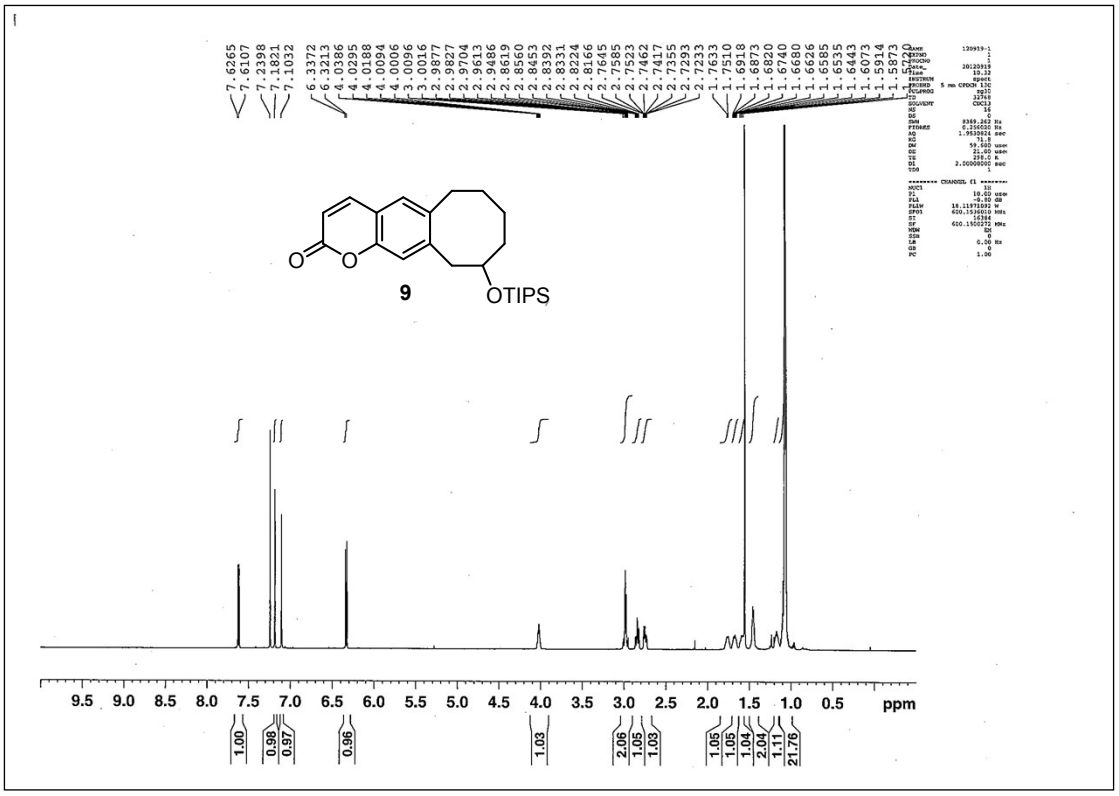
^{13}C NMR Spectrum (150 MHz, $CDCl_3$) of Compound **7**.



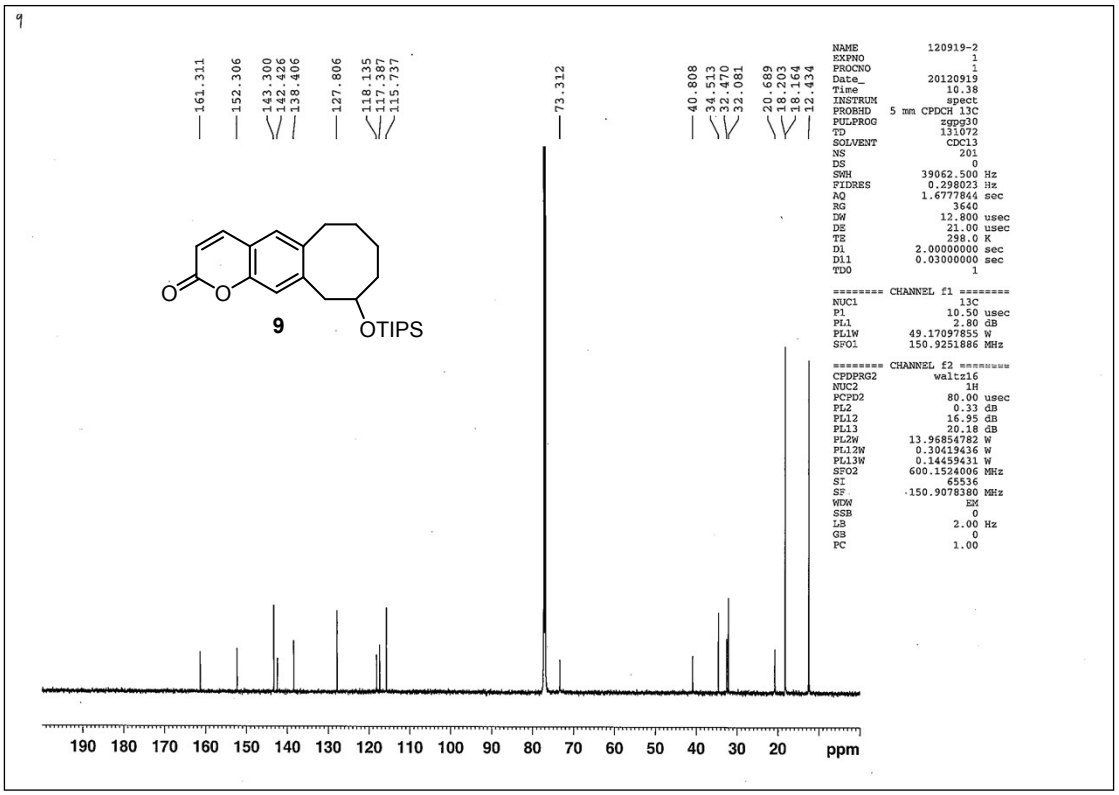
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of Compound **8**.



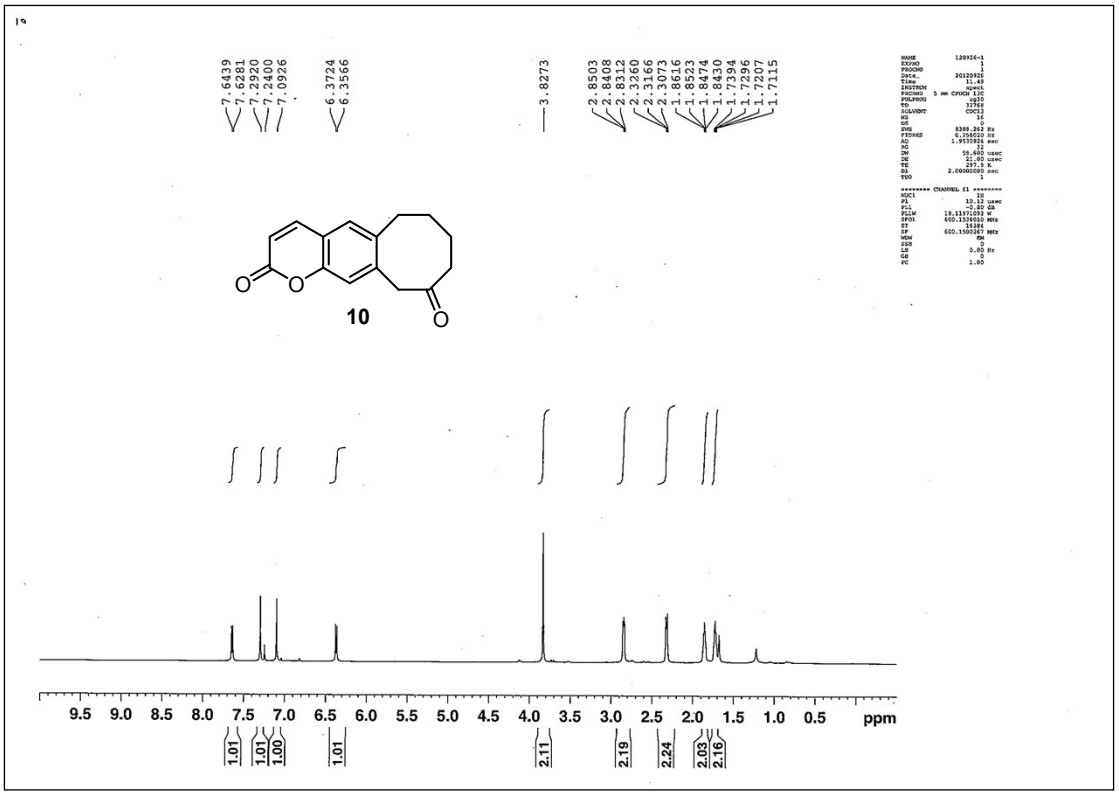
^{13}C NMR Spectrum (150 MHz, $CDCl_3$) of Compound **8**.



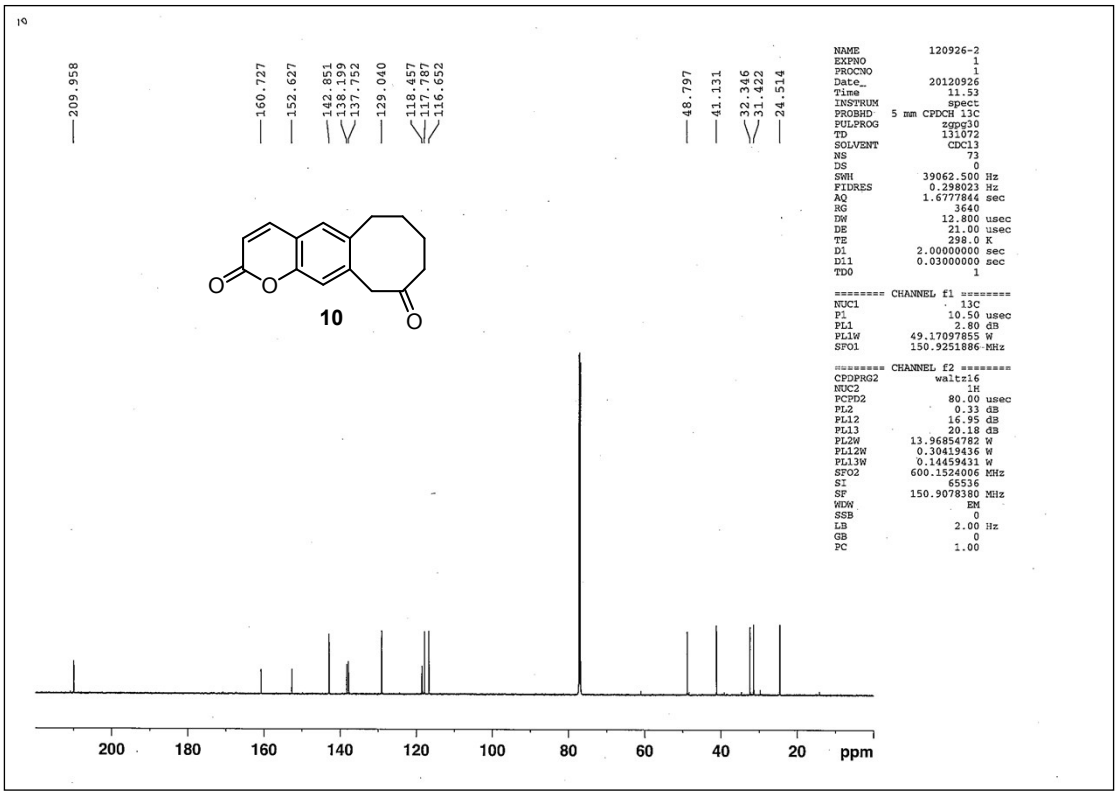
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of Compound **9**.



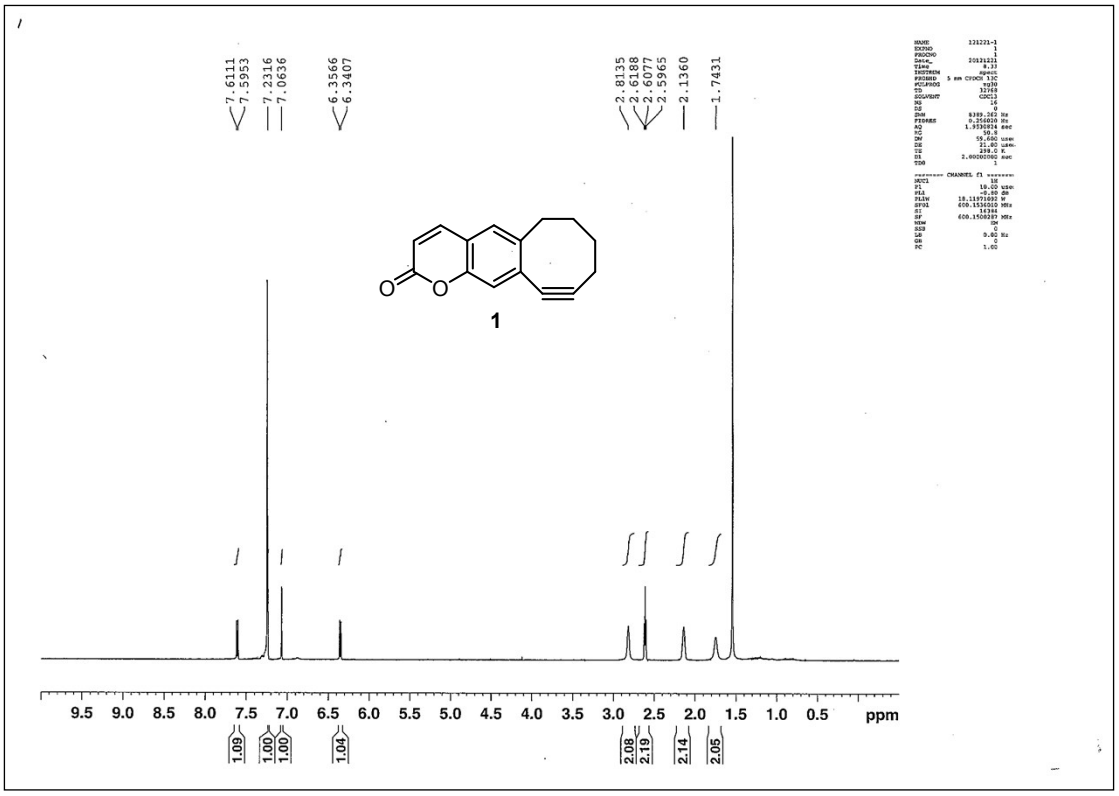
^{13}C NMR Spectrum (150 MHz, $CDCl_3$) of Compound **9**.



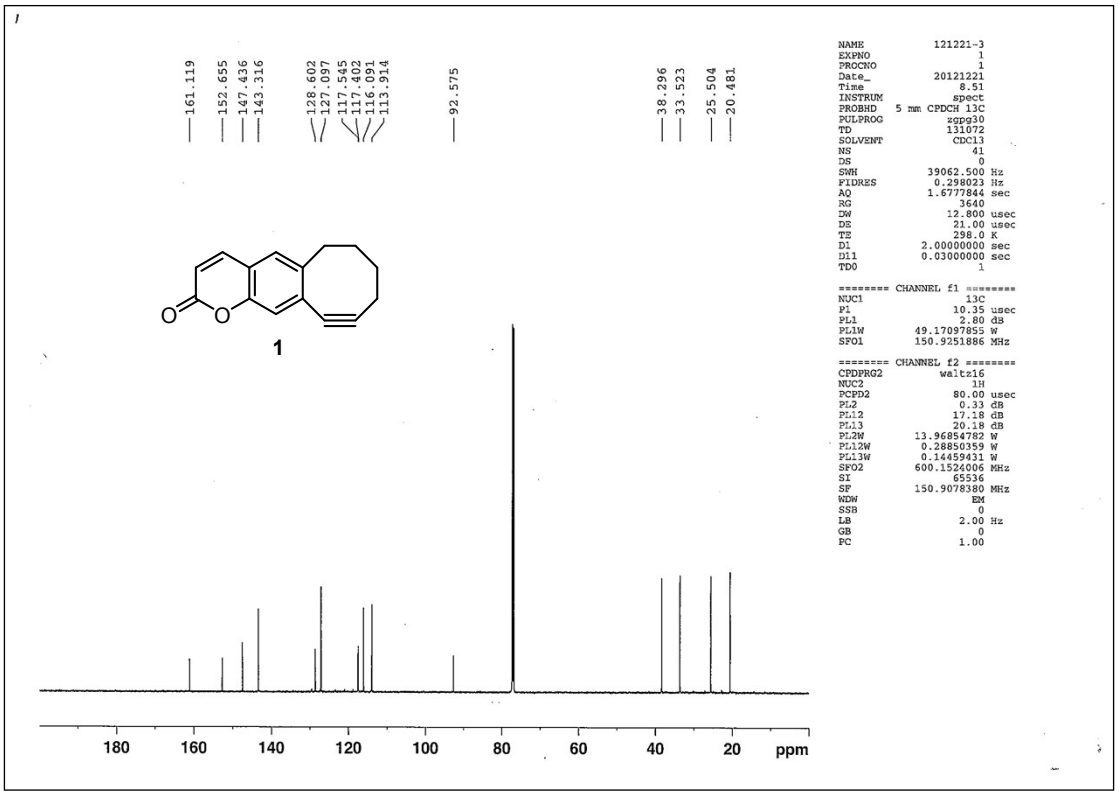
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of Compound **10**.



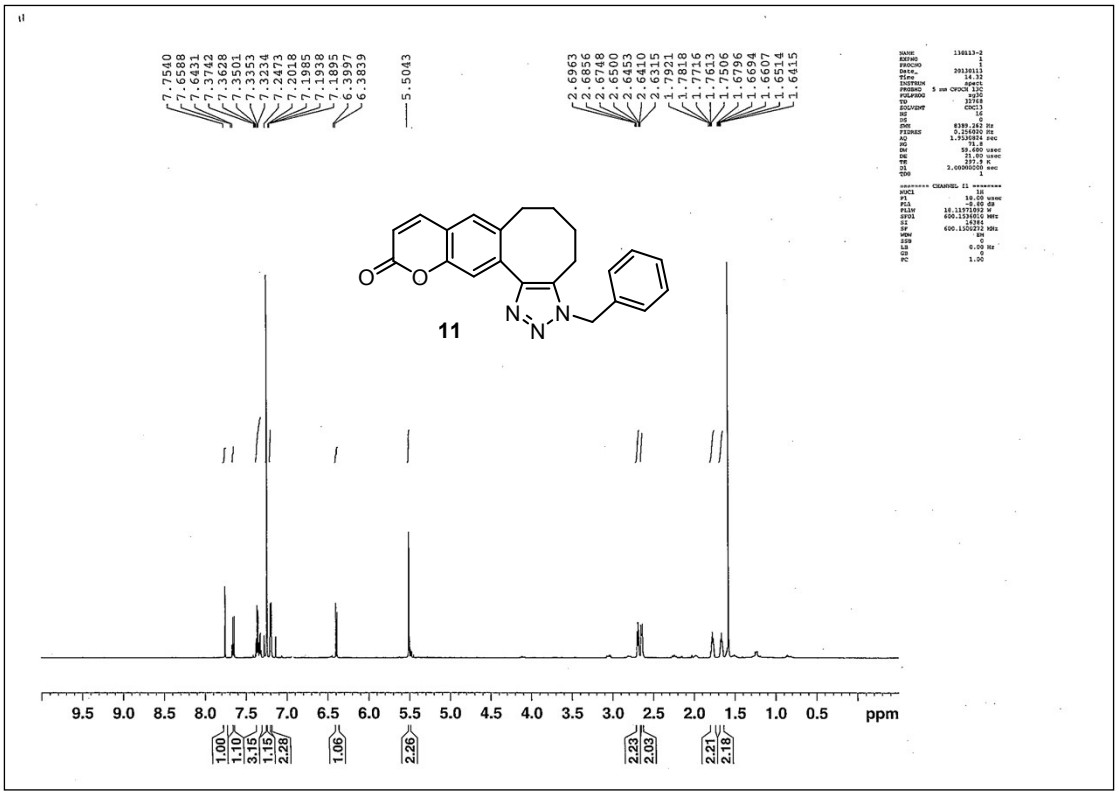
^{13}C NMR Spectrum (150 MHz, $CDCl_3$) of Compound **10**.



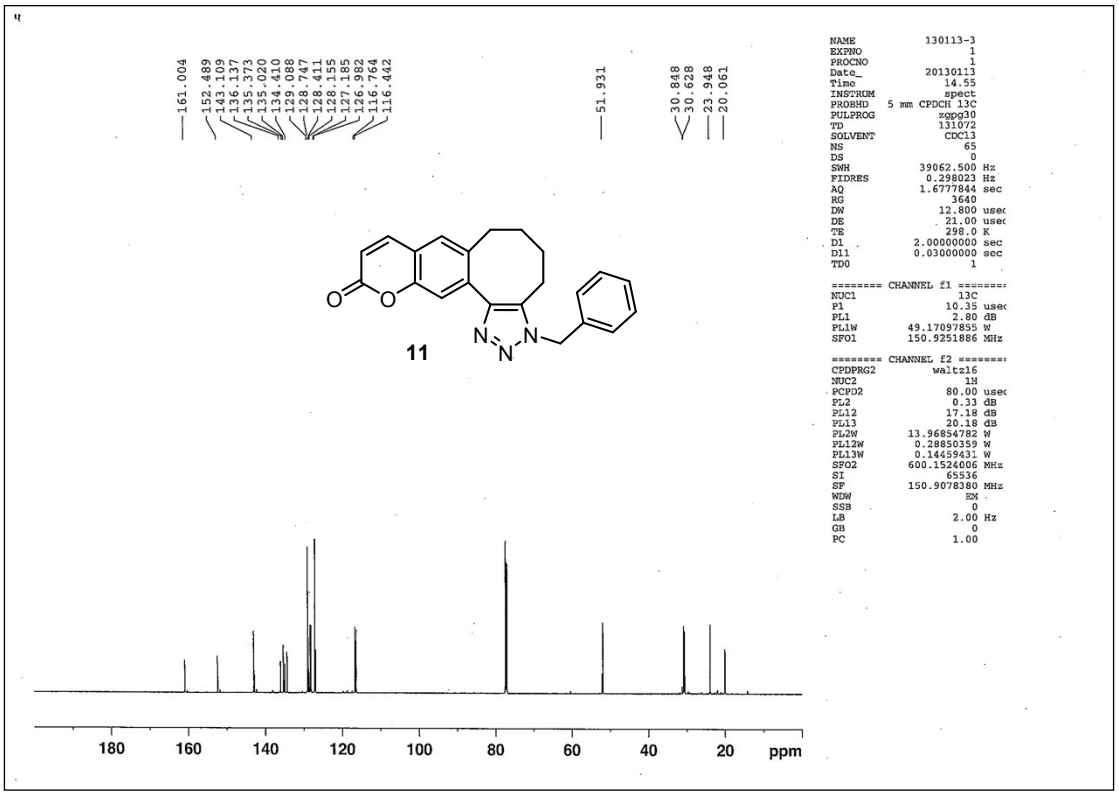
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of Compound **1**.



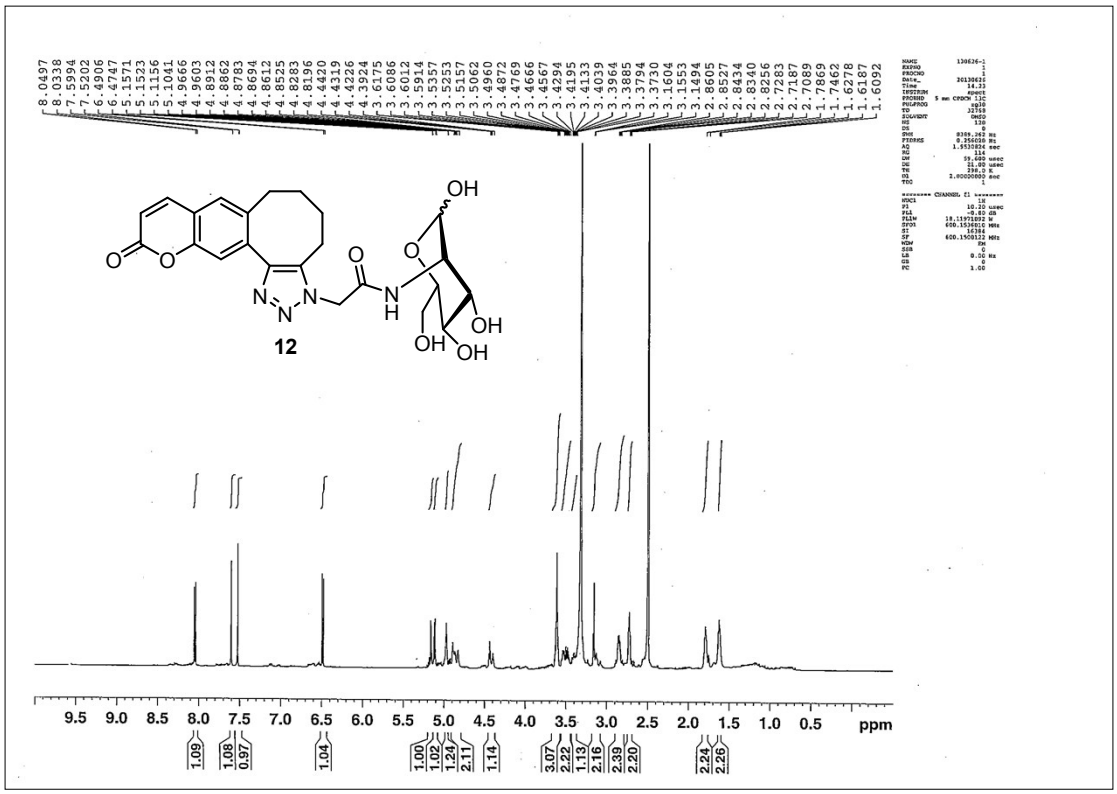
^{13}C NMR Spectrum (150 MHz, $CDCl_3$) of Compound **1**.



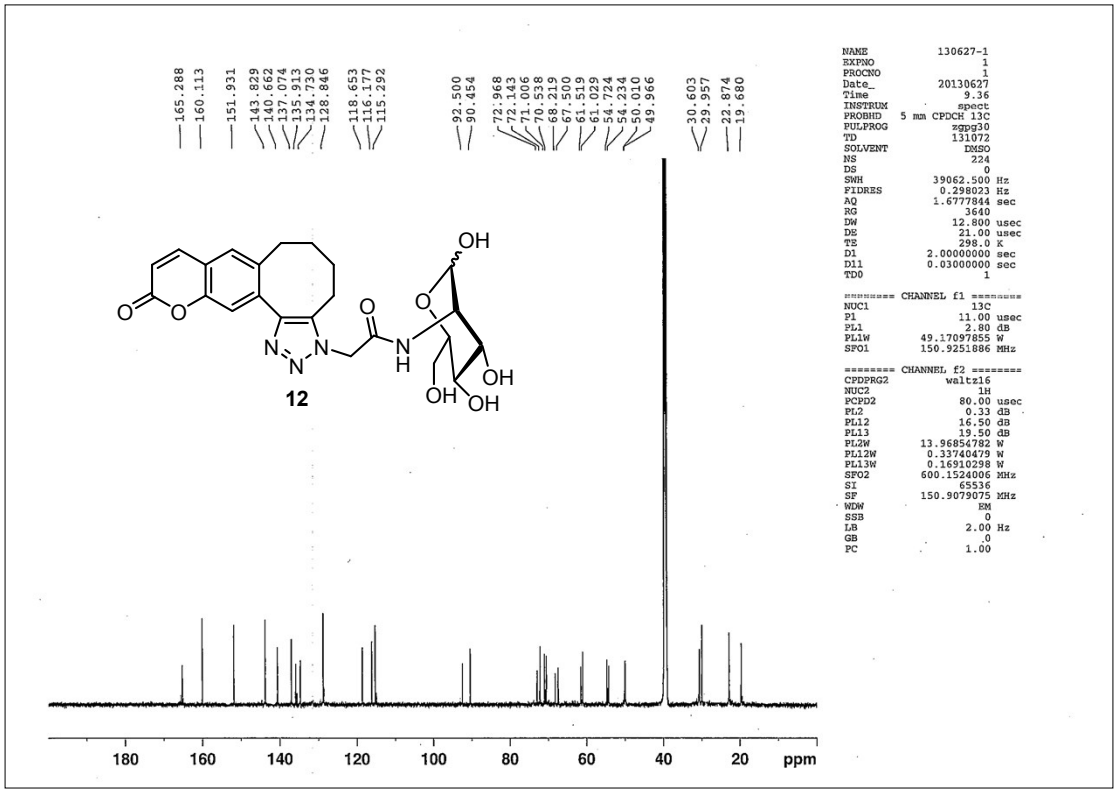
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of Compound **11**.



^{13}C NMR Spectrum (150 MHz, $CDCl_3$) of Compound **11**.



^{1}H NMR Spectrum (600 MHz, DMSO-d_6) of Compound **12**.



^{13}C NMR Spectrum (150 MHz, DMSO-d_6) of Compound **12**.