Electronic Supplementary Material (ESI) for ChemComm.


# Supporting Information:

## Bio-orthogonal "Click and Release" Donation of Caged Carbonyl Sulfide (COS) and Hydrogen Sulfide ($H_2S$)

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## Experimental Details

**Materials and Methods**. All organic chemicals were purchased from Krackeler Scientific and used without further purification. Chromatographic purifications were conducted using SiliaSphere™ spherical silica gel 5μm, 60 Å silica gel (Silicycle). Thin layer chromatography (TLC) was performed on SiliaPlate™ silica gel TLC plates (250 μm thickness) purchased from Silicycle. $^{1}$H and $^{13}$C{$^{1}$H} NMR spectra were acquired a Bruker NMR instrument at 400 MHz ($^{1}$H) and 100 ($^{13}$C) MHz. Mass spectra were acquired using a DART-SVP ion source (IonSense, Saugus, MA, USA) coupled to a JEOL AccuTOF time-of-flight mass spectrometer (JEOL USA, Peabody, MA, USA) in positive ion mode. The DART ion source parameters were: grid voltage, 250 V; gas heater temperature, 350 °C. The mass spectrometer settings were: ring lens voltage, 5 V; orifice 1 voltage, 20 V; orifice 2 voltage, 5 V; peak voltage 600 V. Spectra were obtained at 1 scan per second. The helium flow rate for the DART source was 2.0 L $s^{-1}$.

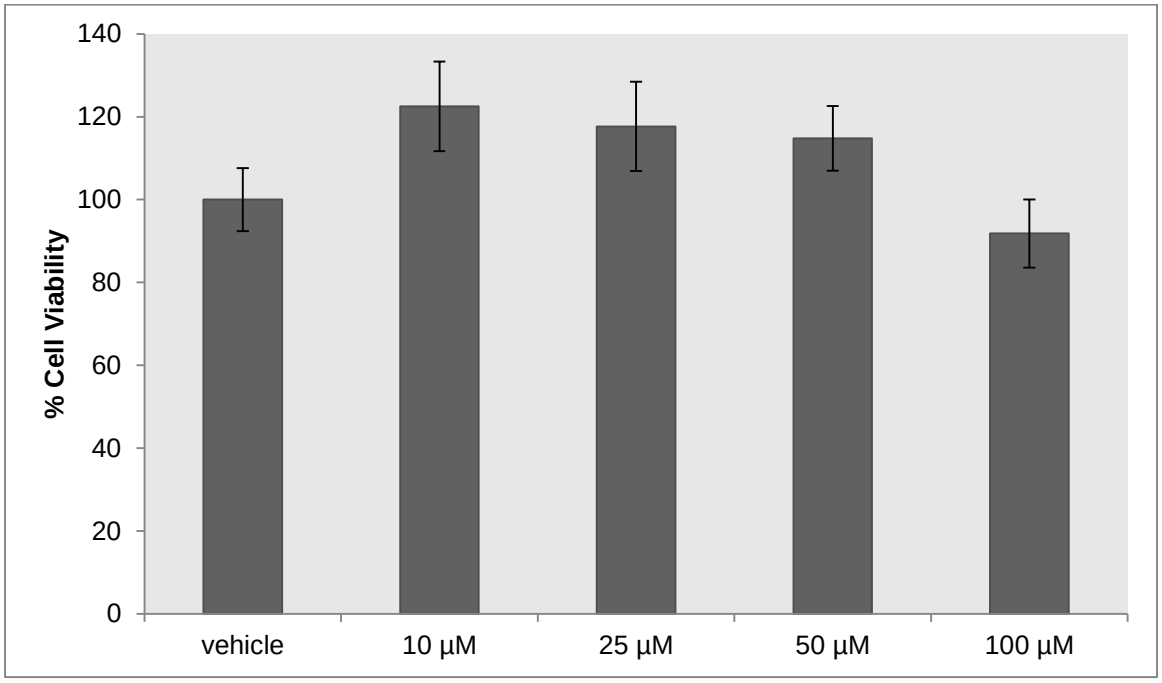
**$H_2S$ Electrode Materials and Methods.** Phosphate buffered saline (PBS) tablets (1X, CalBioChem) were used to make buffered solutions (PBS, 140 mM NaCl, 3 mM KCl, 10 mM phosphate, pH 7.4) in Millipore water. Buffer solutions were sparged with $N_2$ to remove dissolved oxygen and stored in an $N_2$-filled glovebox. Whole bovine blood, whole sheep blood, and sheep serum were obtained from Carolina Biological Supply Company, stored at 4 °C, and warmed to room temperature immediately before use. Carbonic anhydrase (CA) from bovine erythrocytes (≥3,500 W/A units/mg) was obtained from Sigma Aldrich and a 1% CA stock solution was prepared in deoxygenated buffer (PBS, 140 mM NaCl, 3 mM KCl, 10 mM phosphate, pH 7.4) in a glovebox, and stored under nitrogen at 4 °C and warmed to room temperature immediately before use. Stock solutions of TCO **1** (10 mM) and tetrazine **2** (100 mM) were prepared in the dark in an $N_2$-filled glovebox in DMSO and stored, shielded from light, at -25 °C. Stock solutions were thawed at room temperature immediately before use.

**General Procedure for $H_2S$ Electrode Experiments.** Scintillation vials containing 20.00 mL of degassed phosphate buffer (PBS, 140 mM NaCl, 3 mM KCl, 10 mM phosphate, pH 7.4) were prepared in an $N_2$-filled glovebox. A split-top septum cap was placed on the vial after probe insertion and the headspace was sparged with Ar. For electrode experiments in complex media,

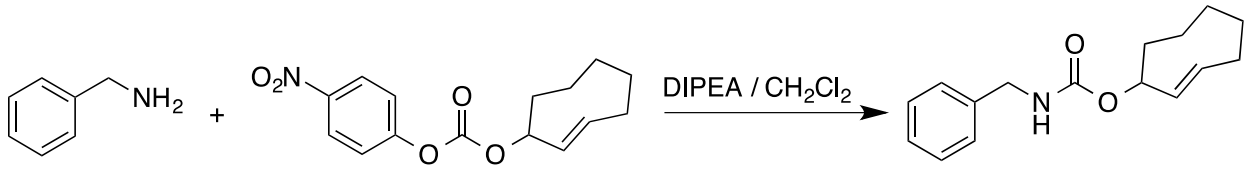
whole blood or serum (2.50 mL) was pipetted into a 10 mL vial with phosphate buffer (PBS, 140 mM NaCl, 3 mM KCl, 10 mM phosphate, pH 7.4) and stirred prior to the start of the experiment. The WPI electrode was then inserted into the vial and the measured voltage was allowed to equilibrate before starting the experiment, and the vial was wrapped in foil to shield the reaction from light. With moderate stirring, CA stock solution (50 µL, 2.5 µg/mL) was injected when applicable, followed by subsequent injections of acetazolamide (10 µL, 2.5 µM, 10 mM stock solution in PBS buffer), TCO stock (10 mM in DMSO), or tetrazine stock solutions (100 mM in DMSO).

**Cytotoxicity of TCO 1**

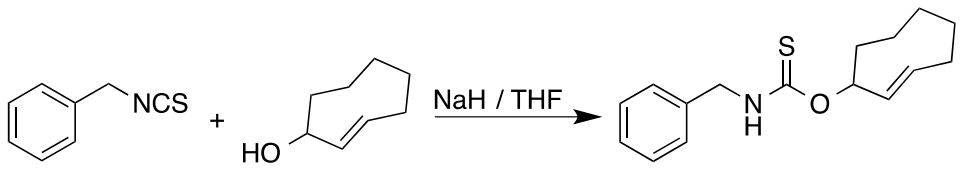
N2A cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C under 5% $CO_2$. Once confluent, cells were incubated in FBS-free DMEM containing vehicle (0.5% DMSO) or TCO **1** (10 – 100 μM) for 3 hours in a 96-well plate. CCK-8 solution (10% in FBS-free DMEM) was added to each well, and cells were incubated for 3 hours at 37 °C. The absorbance at 450 nm was measured by using a microplate reader. The cell viability was measured and normalized to the vehicle group. Results are expressed as mean ± SEM (n = 6).



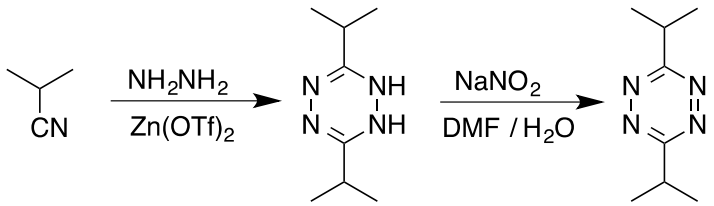
**Synthesis**




**(*E*)-Cyclooct-2-enyl benzylcarbamate (1).** A solution of (*E*)-cyclooct-2-enyl 4-nitrophenyl carbonate (500 mg, 1.7 mmol) in $CH_2Cl_2$ (5 mL) was added to a solution containing benzylamine (370 μL, 3.4 mmol) and *N*,*N*-diisopropylethylamine (880 μL, 5.1 mmol) in $CH_2Cl_2$ (5 mL). The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 18 h. The reaction mixture was diluted with $CH_2Cl_2$ (50 mL) and washed with water (20 mL). The organic layer was dried over $Na_2SO_4$ and concentrated. The title product was purified by flash chromatography using a 9:1 mixture of hexanes:ethyl acetate to provide the product as a white powder (260 mg, 58%). $^1$H NMR ($CDCl_3$, 400 MHz) δ: 7.36-7.26 (m, 5H), 5.83 (t, *J* = 12.3 *Hz*, 1H), 5.54 (d, *J* = 17.8 *Hz*, 1H), 5.37 (s, 1H), 5.16 (bs, 1H), 4.38 (d, *J* = 5.5 *Hz*, 2H), 2.47 (d, *J* = 10.9 *Hz*, 1H), 2.09-1.82 (m, 5H), 1.72-1.45 (m, 3H), 1.11-1.03 (m, 1H), 0.84-0.76 (m, 1H), which matches the previously-reported $^1$H NMR spectrum: Versteegen, R. M.; Rossin, R.; ten Hoeve, W.; Janssen, H. M.; Robillard, M. S. *Angew. Chem. Int. Ed.* **2013**, *52*, 14112-14116.

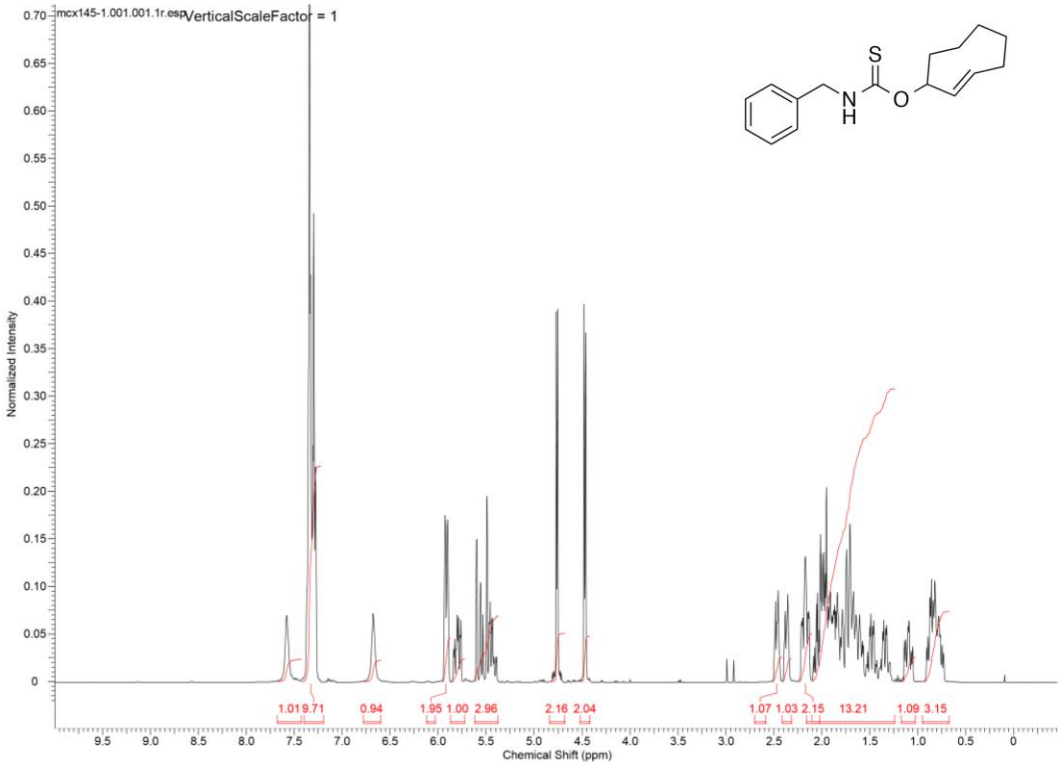



***O*-(*E*)-Cyclooct-2-enyl *N*-benzylcarbamothioate (2).** A solution of (*E*)-cyclooct-2-enol (120 mg, 0.95 mmol) in 1 mL THF was added dropwise to a suspension of NaH (45 mg, 1.13 mmol, 60% in mineral oil) in 1 mL THF. The resulting suspension was stirred at 0 ºC for 1 h under a nitrogen atmosphere. A solution of benzyl isothiocyanate (150 mg, 1.0 mmol) in 1 mL THF was added dropwise, and the mixture was stirred for an additional hour at 0 ºC. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ (2 mL). The aqueous layer was extracted with ethyl acetate (3 x 10 mL), and the combined organic phases were dried with $MgSO_4$. The title product was isolated as a white powder by flash chromatography using a 3:1 solution of hexanes:ether as a mobile phase (199 mg, 76%). *Note*: Slow rotation around the thiocarbamate group results in the observation of rotational isomers on the NMR timescale, which results in a doubling of peaks in the NMR spectrum. This phenomenon has been observed previously in for thiocarbmates. $^{1}$H NMR ($CDCl_3$, 400 MHz) δ: 7.58 (br s, 1H), 7.34-7.28 (m, 10H), 6.68 (br s, 1H), 5.92 (d, *J* = 10.9 *Hz*, 2H), 5.83-5.78 (m, 1H), 5.60-5.43 (m, 3H), 4.76 (d, *J* = 5.5 *Hz*, 2H), 4.47 (d, *J* = 6.8 *Hz*, 2H), 2.47 (d, *J* = 5.5 *Hz*, 1H), 2.37 (d, *J* = 9.6 *Hz*, 1H), 2.20-2.06 (m, 2H), 2.05-1.32 (m, 13H), 1.11-1.09 (m, 1H), 0.87-0.77 (m, 3H). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz) δ: 189.45, 188.63, 136.88, 136.74, 132.61, 132.18, 130.92, 130.19, 128.61, 128.58, 127.86, 127.68, 127.52, 127.20, 80.81, 79.17, 49.05, 47.02, 40.38, 40.37, 35.84, 35.83, 35.79, 35.67, 28.90, 28.80, 24.34, 24.20. HRMS (ESI) *m/z*: calcd. for $C_{16}H_{22}NOS$ $[M+H]^+$ 276.1422; found 276.1444




Isobutyronitrile (691 mg, 10 mmol) and zinc triflate (182 mg, 0.5 mmol) were combined with anhydrous hydrazine (1.6 mL) and stirred at 60 ºC for 24 h under nitrogen atmosphere. The reaction mixture was diluted with DMF (2 mL). An aqueous solution of $NaNO_2$ (3.5 g in 50 mL) was slowly added. Inside a thoroughly ventilated fume hood, an aqueous 2M solution of HCl was added slowly until reaching pH~3. (Caution! The last step generates highly toxic fumes, containing reactive nitrogen species.) The product was extracted with $CH_2Cl_2$ (3x100 mL), dried with $Na_2SO_4$ and concentrated. The title product was obtained by chromatography using 1% $Et_2O$ in pentane (1.2 g, 72%). $^1$H NMR ($CDCl_3$, 400 MHz) δ: 3.62 (sep, *J* = 6.8 *Hz*, 2H), 1.51 (d, *J* = 6.9 *Hz*, 12H). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) δ: 173.69, 34.14, 21.22. HRMS (DART) *m/z*: calcd. for $C_8H_{15}N_4$ $[M+1]^+$ 167.1297; found 167.1306

## NMR Spectra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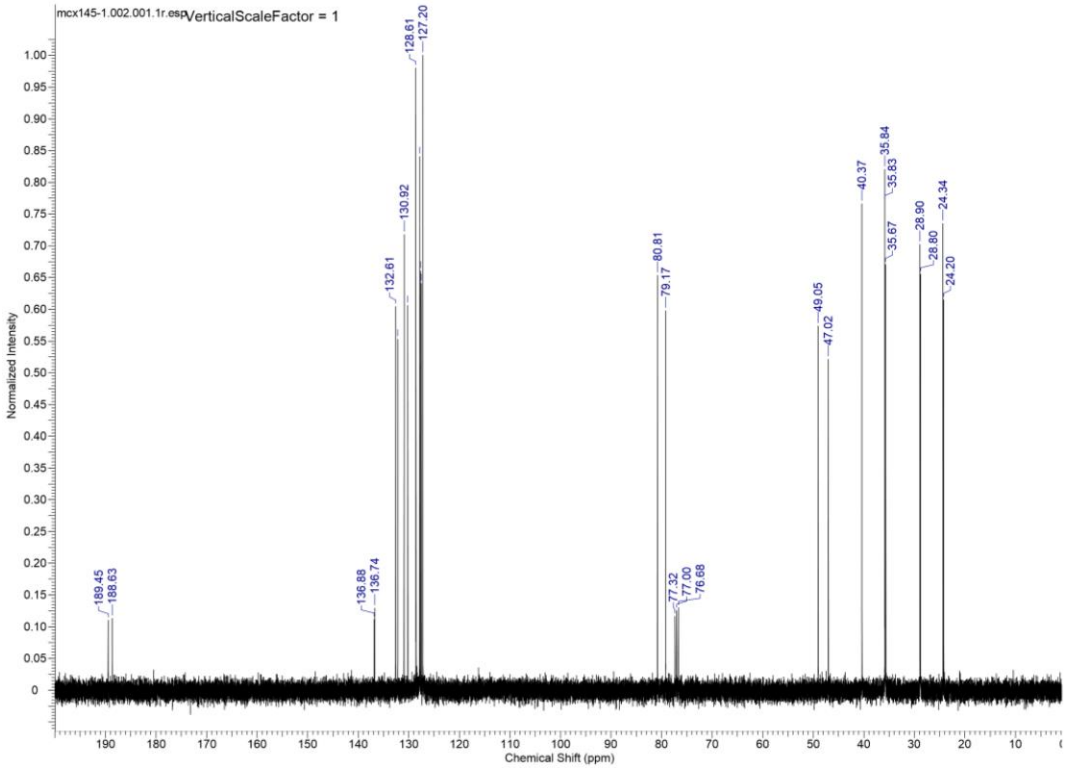
N
H
O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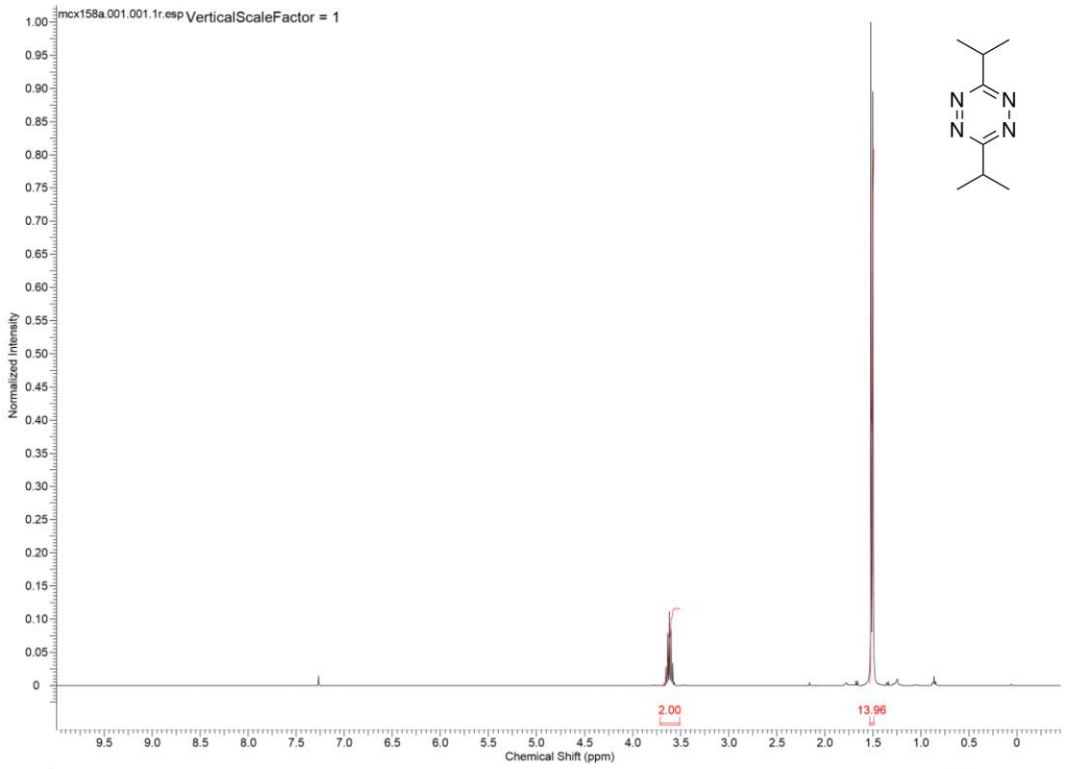
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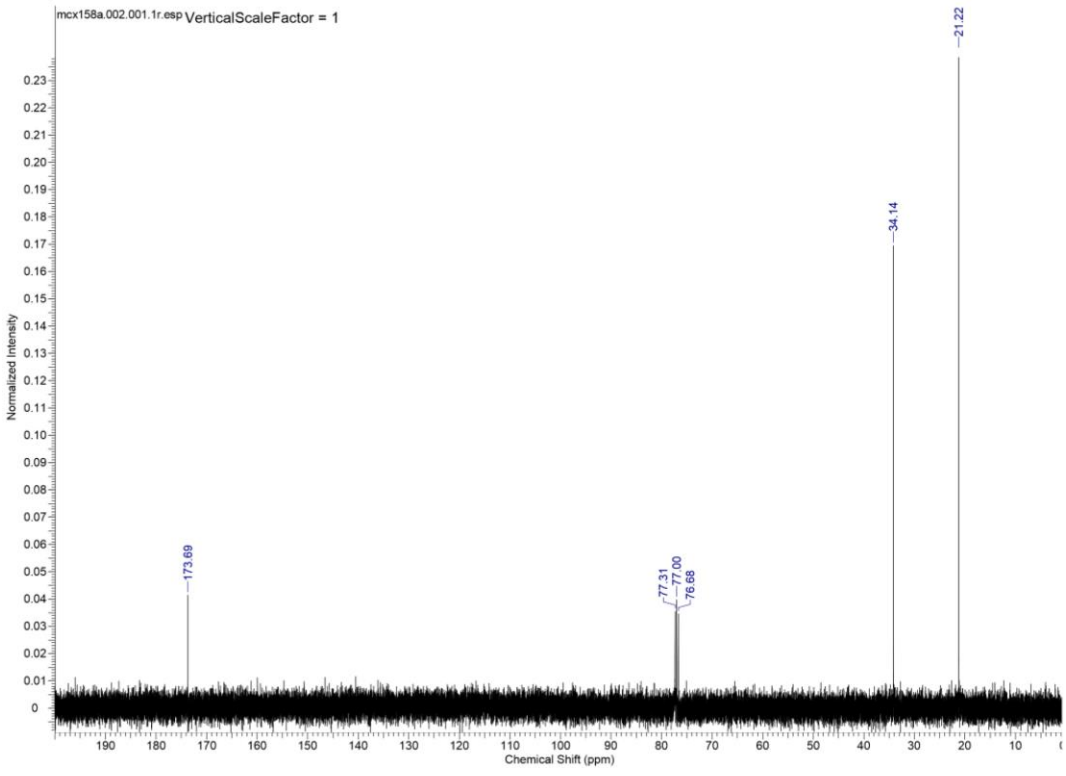
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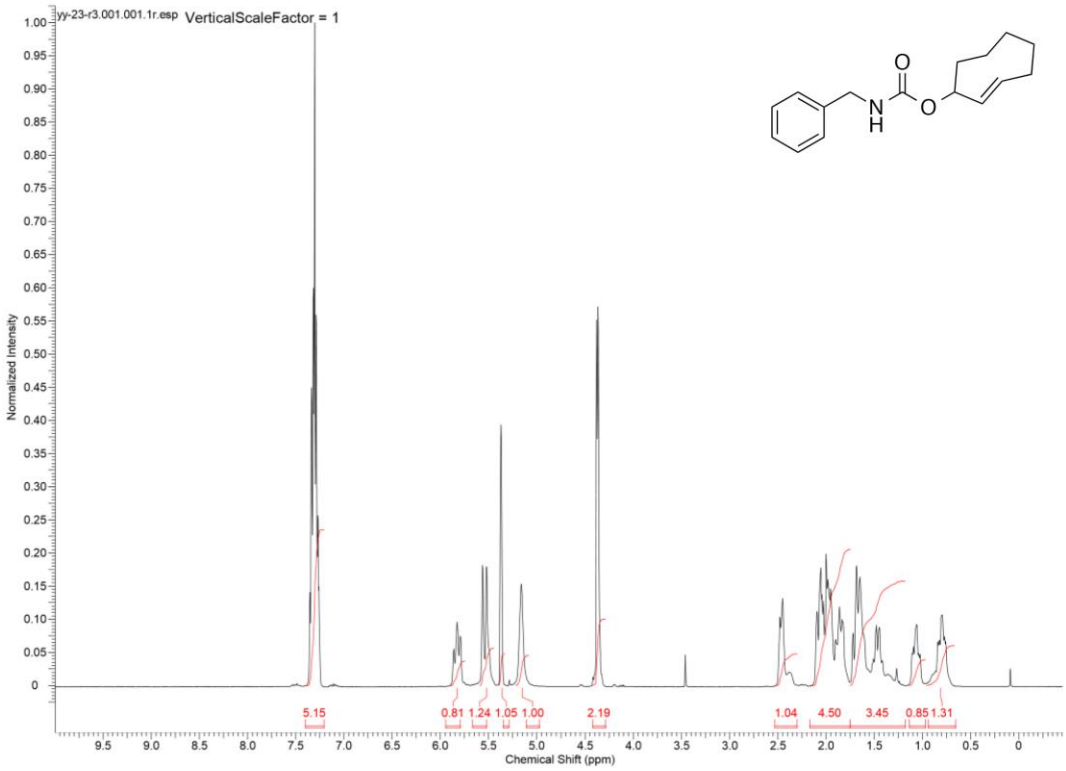
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O
N
H
O