Supplementary Information (SI) for Organic & Biomolecular Chemistry.

Heterocyclic Scaffold-Fused Dimethoxy-Dibenzocyclooctynes for Photoactivatable Click Chemistry

N. Alfred Larsson, Taegeun Jo, Ulf Bremberg, Stefano Crespi, Luke R. Odell and Daniel Fürth

Contents

General methods

Chemicals, reagents and solvents were purchased from Sigma-Aldrich, Thermo Fisher Scientific Inc., MedChemExpress LLC, Santa Cruz Biotechnology Inc. or AA Blocks Inc. and used without further purification. NMR analysis was performed using an NMR spectrometer (Ascend 400, Bruker Corporation, USA) and spectra were recorded in $CDCl_3$ at 400 MHz and 101 MHz for ^{1}H NMR and ^{13}C NMR respectively. Preparative liquid chromatography was performed using a liquid chromatograph (1290 Infinity II, Agilent Technologies Inc., USA) with a C18 column. Analytical GC-MS was performed using a gas chromatograph (7890A GC System, Agilent Technologies Inc., USA) with a mass selective detector (5975 Series MSD, Agilent Technologies Inc., USA). Analytical LC-MS was performed using a liquid chromatograph (Dionex UltiMate 3000 UHPLC, Thermo Fisher Scientific Inc., USA). UV-decarbonylation of cyclopropenones was performed with a handheld UV-lamp (UVGL-58, Analytik Jena GmbH+Co. KG, Germany) at 365 nm, as well as with a 308 nm LED (M310L1, Thorlabs Inc., USA) with an LED driver (T-Cube LED Driver, Thorlabs Inc., USA, LEDD1B). Light power was measured using digital optical power and energy meter console (PM100D, Thorlabs Inc., USA) with a photodiode sensor (S120VC, Thorlabs Inc., USA) at 365 nm. Absorption spectra and kinetics were measured using a spectrophotometer (Biochrom Biodrop Duo, Harvard Bioscience Inc., USA). Emission spectra were measured using a fluorescence spectrometer (Fluorolog 1650 0.2 m double spectrometer, Spex Industries Inc., USA).

Synthesis

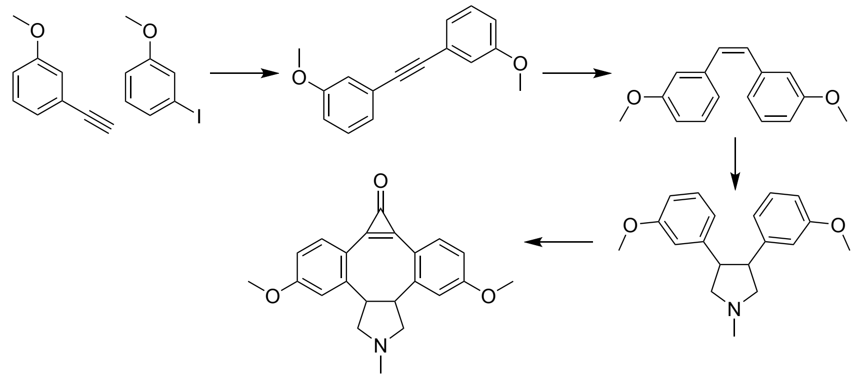


Figure S1: Synthetic route to photo-pyrrolidine-DMBO.

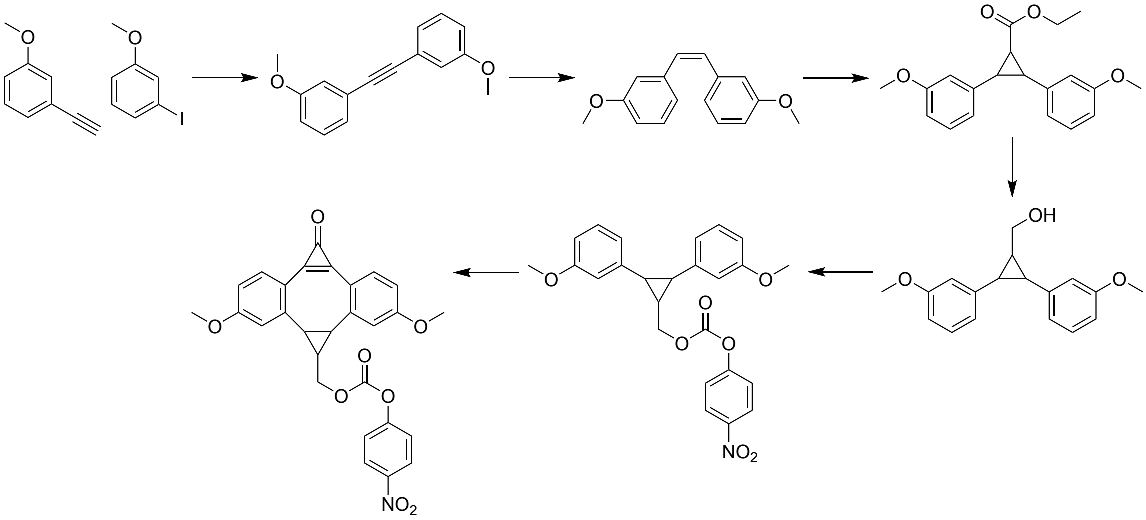
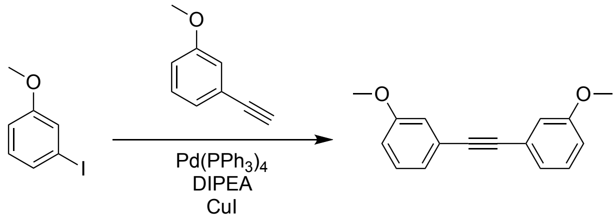


Figure S2: Synthetic route to photo-DMBO for comparison.

Bis(3-methoxyphenyl)acetylene (1)



3-Iodoanisole (6.29 g, 26.9 mmol, 1 eq.), 3-ethynylanisole (3.85 g, 29.1 mmol, 1.1 eq.), $Pd(PPh_3)_4$ (1.56 g, 1.35 mmol, 0.05 eq.), CuI (513 mg, 2.69 mmol, 0.1 eq.) and DIPEA (14 mL, 80.4 mmol, 3 eq.) were refluxed in dry THF (100 mL) overnight. The solvent was evaporated and the crude was purified by column chromatography (petroleum ether:EtOAc = 30:1) yielding a light-yellow solid (5.60 g, 23.5 mmol, 87.5%).

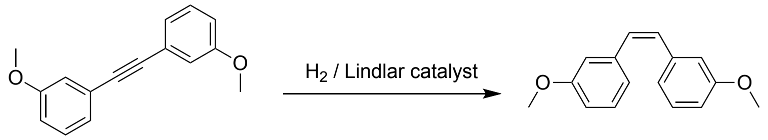
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.31 - 7.23 (m, 1H), 7.15 (dt, *J* = 7.6, 1.2 Hz, 2H), 7.08 (dd, *J* = 2.7, 1.4 Hz, 2H), 6.91 (ddd, *J* = 8.3, 2.6, 1.0 Hz, 2H), 3.84 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 159.49, 129.55, 124.35, 116.48, 115.15, 89.25, 55.44.

MS (EI) *m/z* calculated for $C_{16}H_{14}O_2$: 238.10; found: 238.1.

***R*$_f$** = 0.29 (petroleum ether:EtOAc = 30:1)

Cis-3,3′-dimethoxystilbene (2)

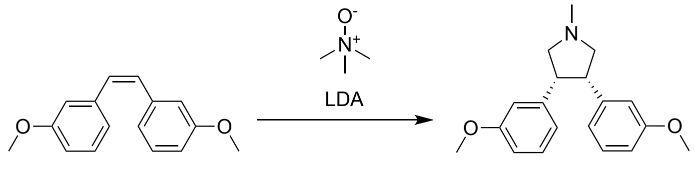


Lindlar catalyst (661 mg) was added to a solution of alkyne **1** (3.31 g, 13.9 mmol, 1 eq.) in DCM (85 mL) and the reaction mixture was stirred under H_2 atmosphere at rt. After 2 h, additional Lindlar catalyst was added (354 mg) and the reaction mixture was stirred for 2 h. The solids were filtered off and the solvent was evaporated leaving a colorless oil (2.99 g, 89.4%, mixture with corresponding alkyne and alkane). The crude product was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.20 - 7.14 (m, 2H), 6.88 (dt, *J* = 7.6, 1.2 Hz, 2H), 6.78 (dd, *J* = 2.6, 1.0 Hz, 2H), 6.77 - 6.73 (m, 2H), 6.60 (s, 2H), 3.68 (s, 6H).

MS (EI) *m/z* calculated for $C_{16}H_{16}O_2$: 240.12; found: 240.1.

(3*S*,4*R*)-3,4-Bis(3-methoxyphenyl)-1-methyl-pyrrolidine (3)



Alkene **2** (1.20 g, 2.5 mmol, 1 eq.) and trimethylamine *N*-oxide (284 mg, 3.78 mmol, 1.5 eq.) were dissolved in dry THF (8 mL) and cooled to 0 °C. LDA (2M, 5.6 mL, 11.2 mmol, 4.5 eq.) was added dropwise and the mixture was stirred at 0 °C for 1 h. The reaction was quenched with H_2O (10 mL) and extracted with EtOAc (2×30 mL), dried over $MgSO_4$ and the solvent was evaporated leaving a yellow oil. The crude product was purified by column chromatography (DCM:MeOH = 16:1) leaving a colorless oil (469 mg, 1.58 mmol, 63.1%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.99 (t, *J* = 7.9 Hz, 2H), 6.60 - 6.51 (m, 4H), 6.43 (t, *J* = 2.1 Hz, 2H), 3.89 - 3.79 (m, 1H), 3.61 (s, 6H), 3.36 (t, *J* = 7.7 Hz, 2H), 3.09 (t, *J* = 7.8 Hz, 2H), 2.66 (s, 3H).

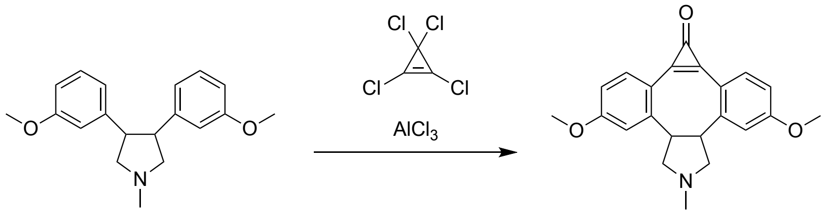
^{13}C NMR (101 MHz, $CDCl_3$) δ = 159.18, 129.62, 128.76, 121.32, 114.60, 112.01, 61.32, 55.23, 49.34, 42.54.

MS (EI) *m/z* calculated for $C_{19}H_{23}NO_2$: 297.17; found: 297.1.

HRMS (ESI) *m/z* calculated for $C_{19}H_{23}NO_2$: 297.17; found: 298.1799 $[M+H]^+$.

***R*f** = 0.26 (DCM:MeOH = 17:1)

Photo-pyrrolidine-DMBO (4a)



Pyrrolidine **3** (469 mg, 1.58 mmol, 1 eq.) was dissolved in dry DCM (9 mL), cooled to −78 °C and $AlCl_3$ (630 mg, 4.73 mmol, 3 eq.) was added. The mixture was stirred for 5 min after which tetrachlorocyclopropene (0.21 mL, 1.71 mmol, 1.1 eq.) was added. The reaction mixture was stirred at −78 °C for 3 h, and then warmed to rt. over 30 min. The reaction was quenched by addition of 1M HCl (20 mL) and the layers were separated. The aq. phase was extracted with $CHCl_3$ (3×20 mL). The organic extracts were combined and washed with brine (50 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated leaving a yellow solid. The crude was purified by preparative HPLC and lyophilized leaving a white solid (30 mg, 86 µmol, 5.5%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.95 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 2.5 Hz, 2H), 6.88 (dd, *J* = 8.5, 2.5 Hz, 2H), 3.89 (s, 6H), 3.24 - 3.17 (m, 2H), 3.13 (dd, *J* = 9.9, 4.3 Hz, 2H), 2.92 (dd, *J* = 9.5, 6.7 Hz, 2H), 2.54 (s, 3H).

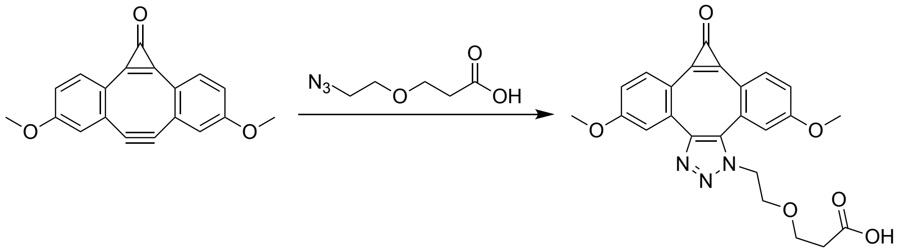
^{13}C NMR (101 MHz, $CDCl_3$) δ = 167.65, 162.71, 159.13, 158.83, 153.64, 148.16, 143.33, 142.05, 136.09, 131.75, 129.27, 117.15, 116.69, 114.04, 110.78, 58.70, 55.63, 55.24, 55.18, 52.63, 50.79, 42.67.

MS (ESI) *m/z* calculated for $C_{22}H_{21}NO_3$: 347.15; found: 348.1 $[M+H]^+$.

HRMS (ESI) *m/z* calculated for $C_{22}H_{21}NO_3$: 347.15; found: 348.1592 $[M+H]^+$.

***R*f** = 0.34 (DCM:MeOH = 17:1)

Photo-triazole-DMBO (6)



FL-DIBO (**5**) (2.8 mg, 9.7 µmol, 1 eq.) was dissolved in DCM/MeOH (4:1, 3 mL). A 524 mM solution of azido-PEG1-acid in MeOH (18.5 µL, 1.5 mg, 9.7 µmol, 1 eq.) was added and the reaction mixture was stirred at rt. for 3 days. The solvent was evaporated and the product was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.64 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 2.6 Hz, 1H), 7.05 – 6.91 (m, 3H), 3.91 – 3.86 (m, 6H), 3.67 – 3.60 (m, 2H), 3.37 (t, *J* = 4.9 Hz, 2H), 2.68 – 2.59 (m, 2H), 2.43 (t, *J* = 5.9 Hz, 2H).

MS (ESI) *m/z* calculated for $C_{24}H_{21}N_3O_6$: 447.14; found: 448.1 $[M+H]^+$.

Reactivity assays

Pyrrolidine-DMBO SPAAC assay (MeOH)

50 µL of a 500 µM solution of photo-pyrrolidine-DMBO (compound **4a**) in MeOH was placed under a UV-lamp (UVGL-58, Analytik Jena GmbH+Co. KG, Germany) at 365 nm and 1.5 mW for 10 min. 2.5 µL of a 100 mM solution of pAz (Santa Cruz Biotechnology Inc., USA, sc-503216) in MeOH was added and the reaction was left for 48 h.

Pyrrolidine-DMBO iEDDAC assay (MeOH)

50 µL of a 500 µM solution of photo-pyrrolidine-DMBO (compound **4a**) in MeOH was placed under a UV-lamp (UVGL-58, Analytik Jena GmbH+Co. KG, Germany) at 365 nm and 1.5 mW for 10 min. 12.5 µL of a 20 mM solution of MeMe-Tet (AA Blocks Inc., USA, AA00B01C) in MeOH was added and the reaction was left for 48 h.

Pyrrolidine-DMBO SPAAC assay (DMSO and DIPEA)

5 µL of a 5 mM solution of photo-pyrrolidine-DMBO (compound **4a**) in MeOH was added to 40 µL DMSO and placed under a UV-lamp (UVGL-58, Analytik Jena GmbH+Co. KG, Germany) at 365 nm and 1.5 mW for 10 min. 2.5 µL of a 100 mM solution of pAz (Santa Cruz Biotechnology Inc., USA, sc-503216) in MeOH and 5 µL of a 5 mM solution of DIPEA in DMSO was added and the reaction was left for 48 h.

Pyrrolidine-DMBO iEDDAC assay (DMSO and DIPEA)

5 µL of a 5 mM solution of photo-pyrrolidine-DMBO (compound **4a**) in MeOH was added to 40 µL DMSO and placed under a UV-lamp (UVGL-58, Analytik Jena GmbH+Co. KG, Germany) at 365 nm and 1.5 mW for 10 min. 12.5 µL of a 20 mM solution of MeMe-Tet (AA Blocks Inc., USA, AA00B01C) in MeOH and 5 µL of a 5 mM solution of DIPEA in DMSO was added and the reaction was left for 48 h.

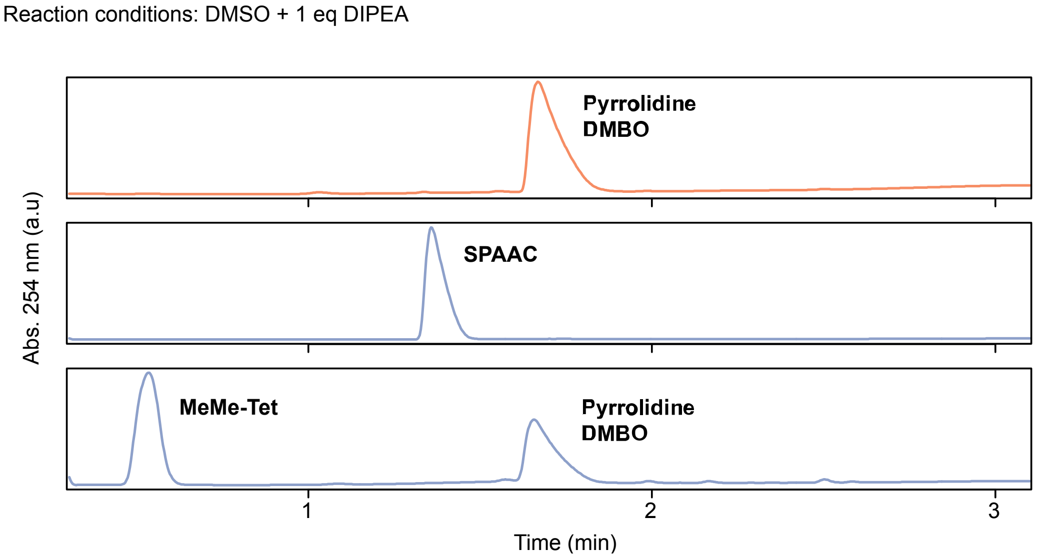


Figure S3: LC-MS chromatograms from SPAAC and iEDDAC assays in DMSO and DIPEA.

Triazole-DMBO SPAAC assay

50 µL of a 500 µM solution of photo-triazole-DMBO (compound **6**) in MeOH was placed under a 308 nm LED (M310L1, Thorlabs Inc., USA) with an LED driver (T-Cube LED Driver, Thorlabs Inc., USA, LEDD1B) at 17 mW for 100 min. 5 µL of a 50 mM solution of pAz (Santa Cruz Biotechnology Inc., USA, sc-503216) in MeOH was added and the reaction was left for 24 h.

Triazole-DMBO iEDDAC assay

50 µL of a 500 µM solution of photo-triazole-DMBO (compound **6**) in MeOH was placed under a 308 nm LED (M310L1, Thorlabs Inc., USA) with an LED driver (T-Cube LED Driver, Thorlabs Inc., USA,

LEDD1B) at 17 mW for 100 min. 12.5 µL of a 20 mM solution of MeMe-Tet (AA Blocks Inc., USA, AA00B01C) in MeOH was added and the reaction was left for 24 h.

Absorption and emission spectra

Absorption

360 µL of photo-triazole-DMBO (compound **6**) at a concentration of 5 µM in MeOH was added to a quartz absorption cuvette (Z600318-1EA, Hellma GmbH & Co. KG, Germany) and absorption was measured on Biochrom BioDrop Duo (7444, Harvard Bioscience Inc., USA).

Emission

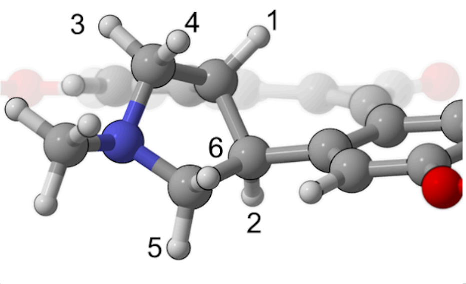
Photo-triazole-DMBO (compound **6**) at a stock concentration of 5 µM was diluted in MeOH to a concentration of 1.8 µM for a total volume of 1.7 mL in a polystyrene disposable cuvette with four clear faces (634-8530, VWR) and emission was measured on a Fluorolog SPEX TCSPC Horiba fluorescence spectrophotometer (ex. wavelength 370 nm, 2 s integration time, 1 nm slit).

Kinetics

In a quartz absorption cuvette (Z600318-1EA, Hellma GmbH & Co. KG, Germany) pAz was diluted in MeOH to the desired concentration and a final volume of 700 µL. A blank measurement of the pAz solution was taken, after which pyrrolidine-DMBO (compound **4b**) was added at a final concentration of 100 µM. Absorbance was measured at 307 nm over 60 min using a Biochrom BioDrop Duo (7444, Harvard Bioscience Inc., USA). Reactions were performed at pAz concentrations of 30, 40, 50, 70 and 100 mM.

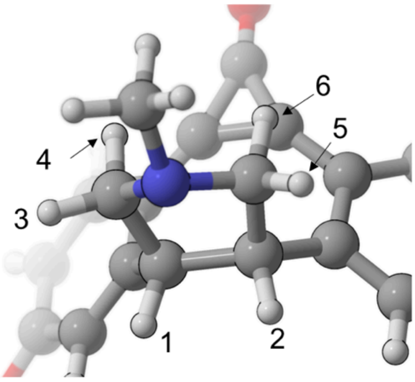
Conformational analysis of *cis* and *trans* isomers

The version 3.0.2 CREST[1] was employed to optimize the geometries of the *cis*- and *trans*-structures at the GFN2-xTB level[2,3]. Conformational analysis of both isomers was performed at the GFN2-xTB level using CREST, and conformer filtering was performed with the CREGEN tool from the CREST package, yielding 26 *trans*- and 10 *cis*-conformers. The resulting conformers were re-optimized using the ORCA 6.0.1 package[4–8] at the r^{2}SCAN-3c level of theory[9]. Solvent effects were modeled implicitly (CPCM)[10], with chloroform specified as the solvent. Electronic energies of the optimized structures were subsequently refined using the ωB97X-V functional[11] with the def2-TZVP basis set[12,13]. Furthermore, chemical shifts and coupling constants were calculated at the ωB97X-V level with pcSseg-2[14] and pcJ-2[15] basis sets, respectively. Final predicted chemical shifts and coupling constants were obtained through Boltzmann averaging of the conformers in accordance with the Gibbs free energies.



coupling (Hz)	1 H	2 H	3 H	4 H	5 H	6 H
1 H	0.0	2.0	8.5	8.7	-0.2	0.6
2 H	2.0	0.0	-0.2	-0.4	6.0	0.4
3 H	8.5	-0.2	0.0	-8.1	0.1	-0.6
4 H	8.7	-0.4	-8.1	0.0	-0.5	1.2
5 H	-0.2	6.0	0.1	-0.5	0.0	-8.6
6 H	0.6	0.4	-0.6	1.2	-8.6	0.0

Figure S4: Calculated ^{1}H NMR coupling constants with Boltzmann averaging for *trans*-isomer.



coupling (Hz)	1 H	2 H	3 H	4 H	5 H	6 H
1 H	0.0	9.6	8.2	7.3	-0.1	-0.2
2 H	9.6	0.0	-0.1	-0.2	7.9	7.0
3 H	8.2	-0.1	0.0	-7.8	0.5	-0.5
4 H	7.3	-0.2	-7.8	0.0	-0.5	0.2
5 H	-0.1	7.9	0.5	-0.5	0.0	-7.8
6 H	-0.2	7.0	-0.5	0.2	-7.8	0.0

Figure S5: Calculated ^{1}H NMR coupling constants with Boltzmann averaging for *cis*-isomer.

The predicted coupling constants between 2H and 6H are 0.4 Hz and 7.0 Hz for the *trans* and *cis*-isomers, respectively. In the recorded ^{1}H NMR spectrum, the corresponding coupling constant is either 4.3 Hz or 6.7 Hz (complete assignment not possible). The large disparity between the measured coupling constant and the predicted coupling constant of the *trans*-isomer suggests that the synthesized compound is the *cis*-isomer of photo-pyrrolidine-DMBO.

Table S1: Calculated ^{1}H NMR coupling constants with Boltzmann averaging for *cis*-isomer.

shift (ppm)	1 H	2 H	3 H	4 H	5 H	6 H
trans	3.04	3.30	3.56	2.42	3.43	3.11
cis	3.96	3.95	2.92	2.68	2.94	2.63

The predicted ^{1}H chemical shifts (1H-6H) showed a clear distinction between the two isomers. The *cis*-isomer displayed three sets of symmetric signals, whereas the *trans*-isomer exhibited six distinct signals due to its asymmetric geometry. The experimental ^{1}H NMR spectrum showed symmetric chemical shifts, indicating that the synthesized compound corresponds to the *cis*-isomer. Thus, both the calculated *J*-coupling constants and chemical shifts support the assignment of the *cis*-isomer.

NMR spectra

Bis(3-methoxyphenyl)acetylene (1)

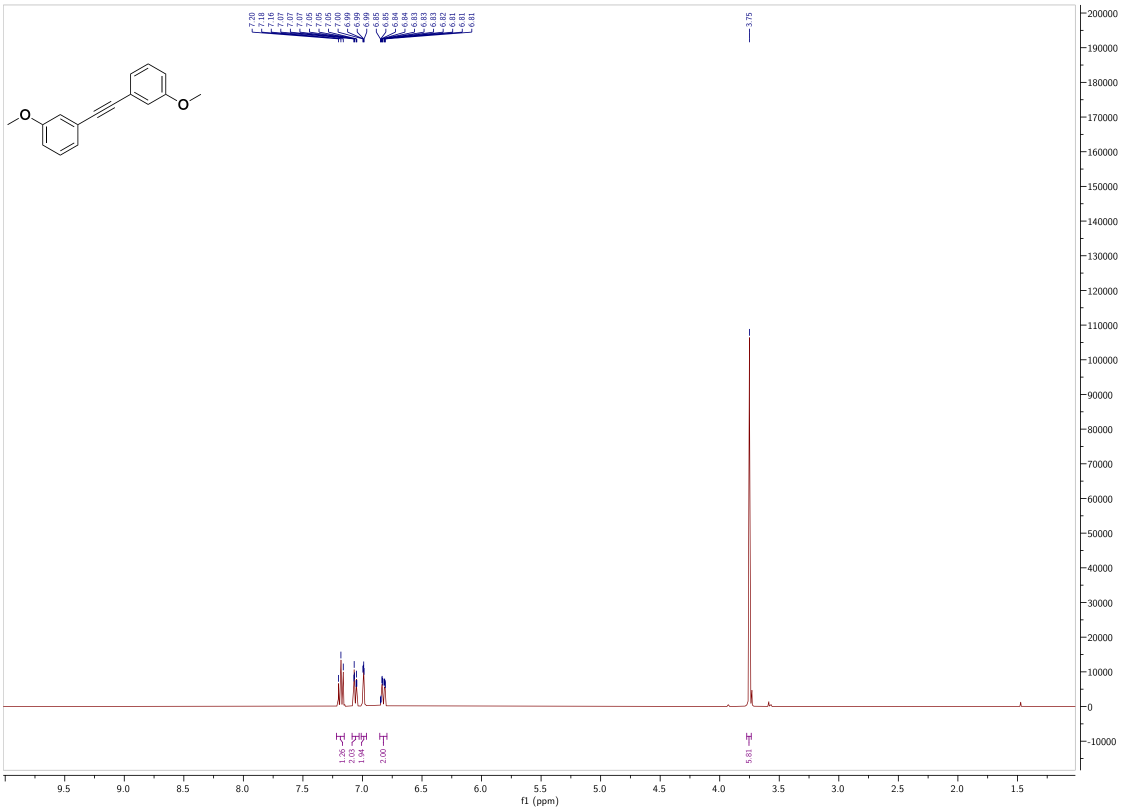


Figure S6: ^{1}H NMR spectrum of compound **1** (400 MHz, $CDCl_3$).

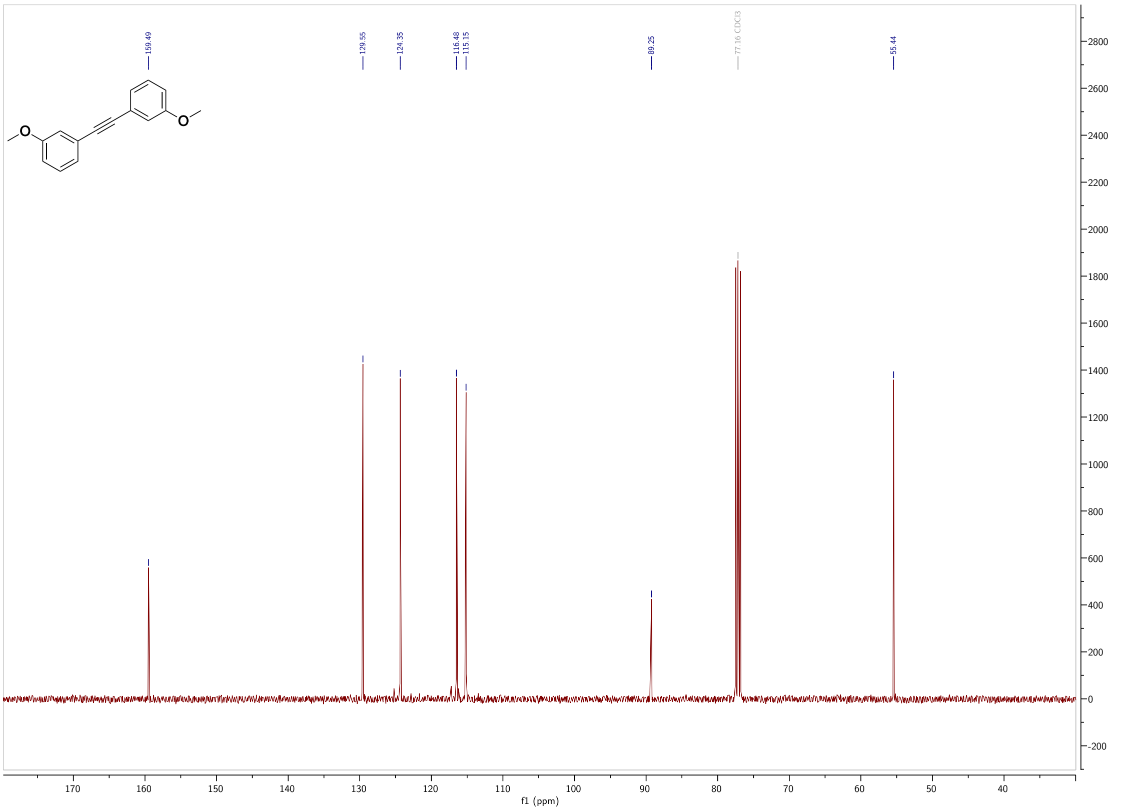


Figure S7: ^{13}C NMR spectrum of compound **1** (101 MHz, $CDCl_3$).

Cis-3,3′-dimethoxystilbene (2)

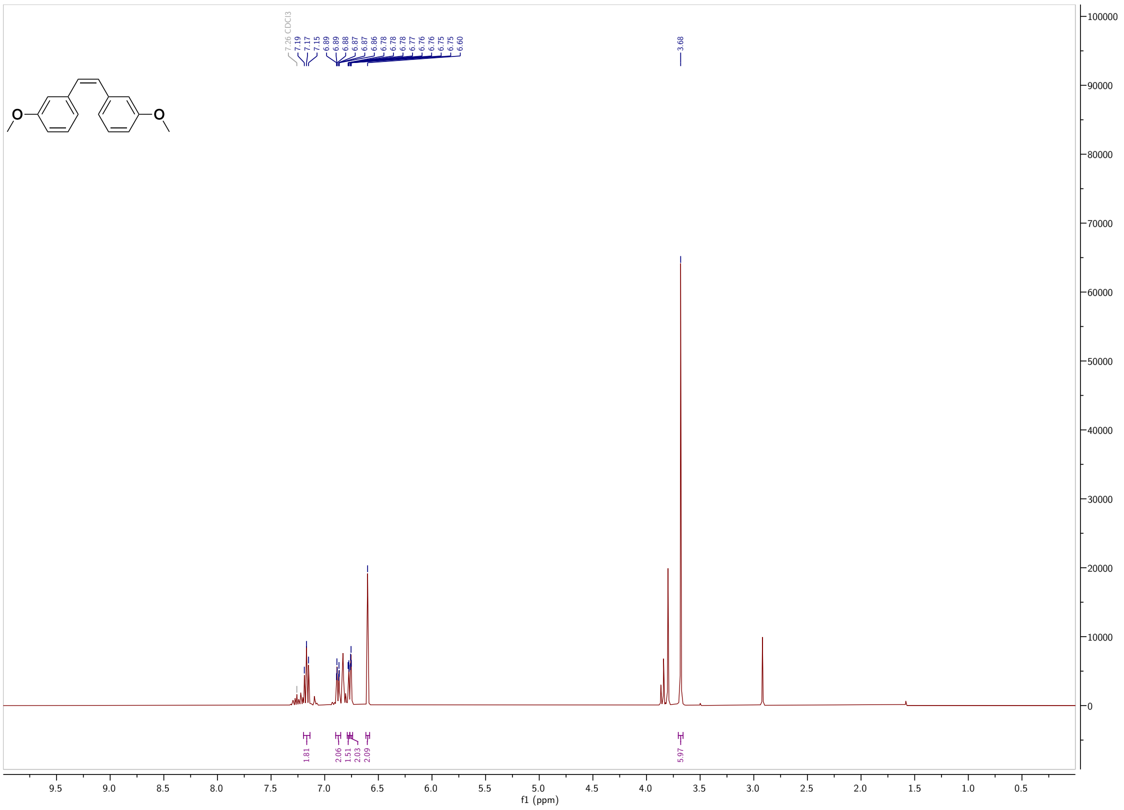


Figure S8: ^{1}H NMR spectrum of compound **2** (400 MHz, $CDCl_3$).

(3*S*,4*R*)-3,4-Bis(3-methoxyphenyl)-1-methyl-pyrrolidine (3)

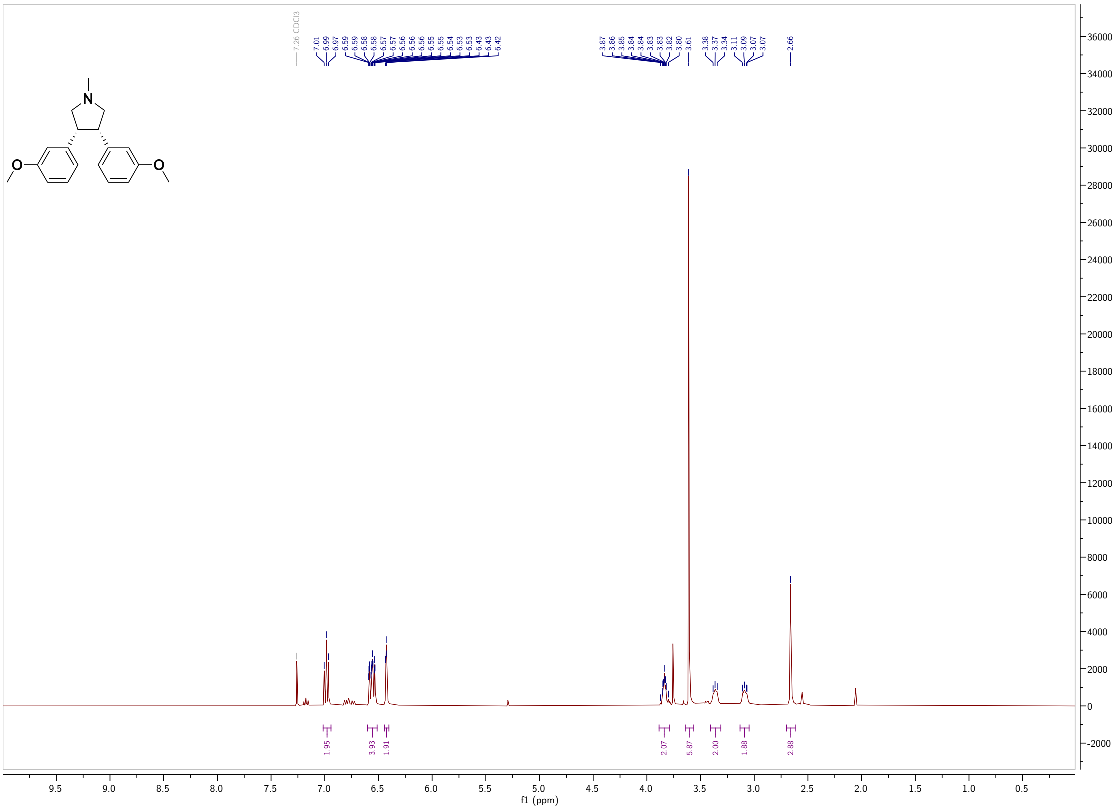


Figure S9: ^{1}H NMR spectrum of compound **3** (400 MHz, $CDCl_3$).

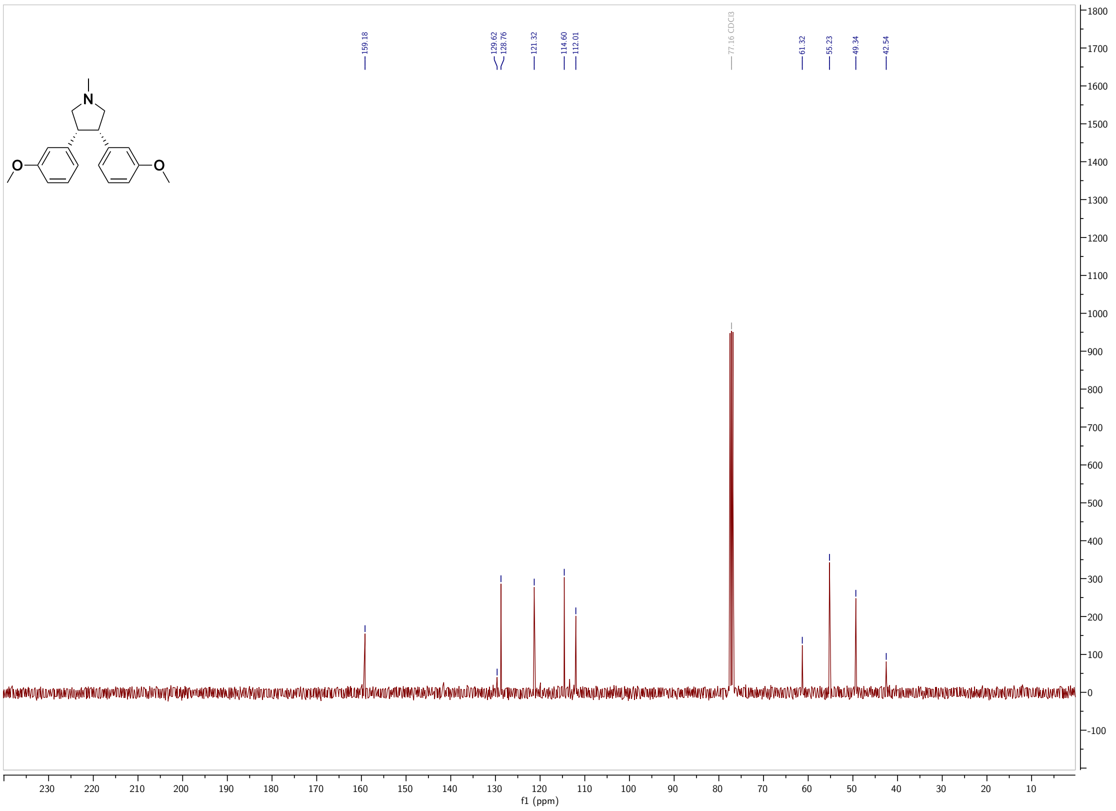


Figure S10: ^{13}C NMR spectrum of compound **3** (101 MHz, $CDCl_3$).

Photo-pyrrolidine-DMBO (4a)

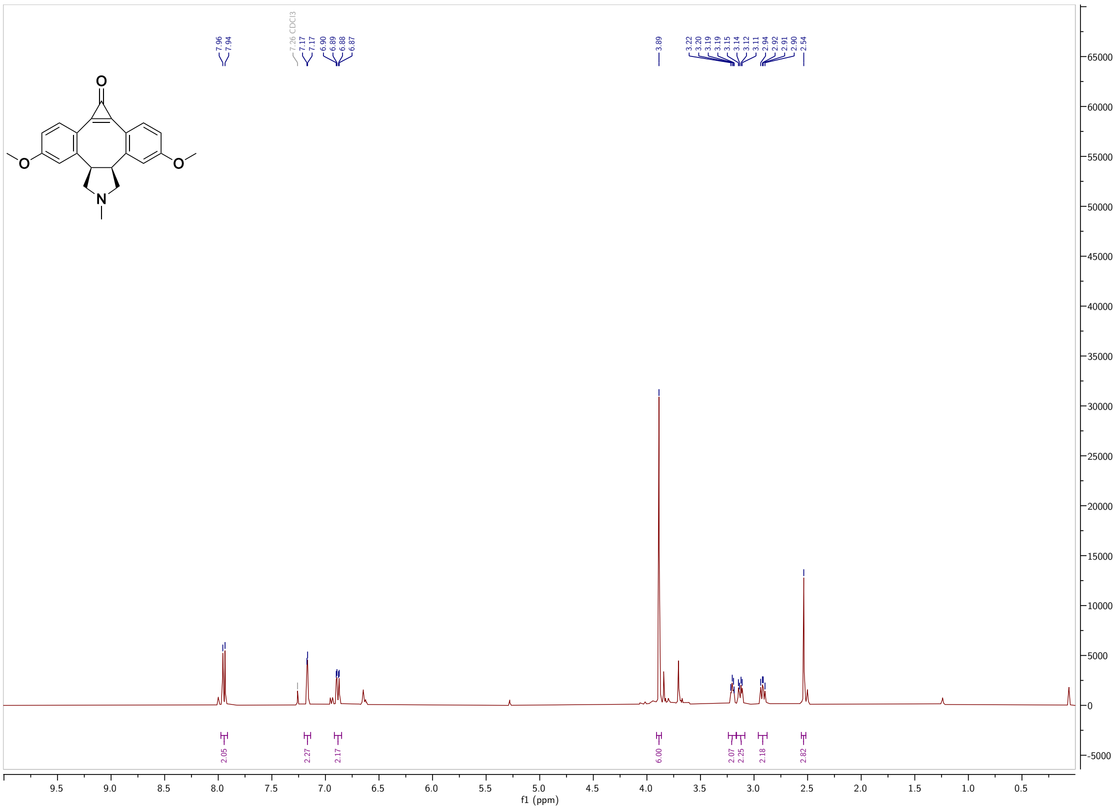


Figure S11: ^{1}H NMR spectrum of compound **4a** (400 MHz, $CDCl_3$).

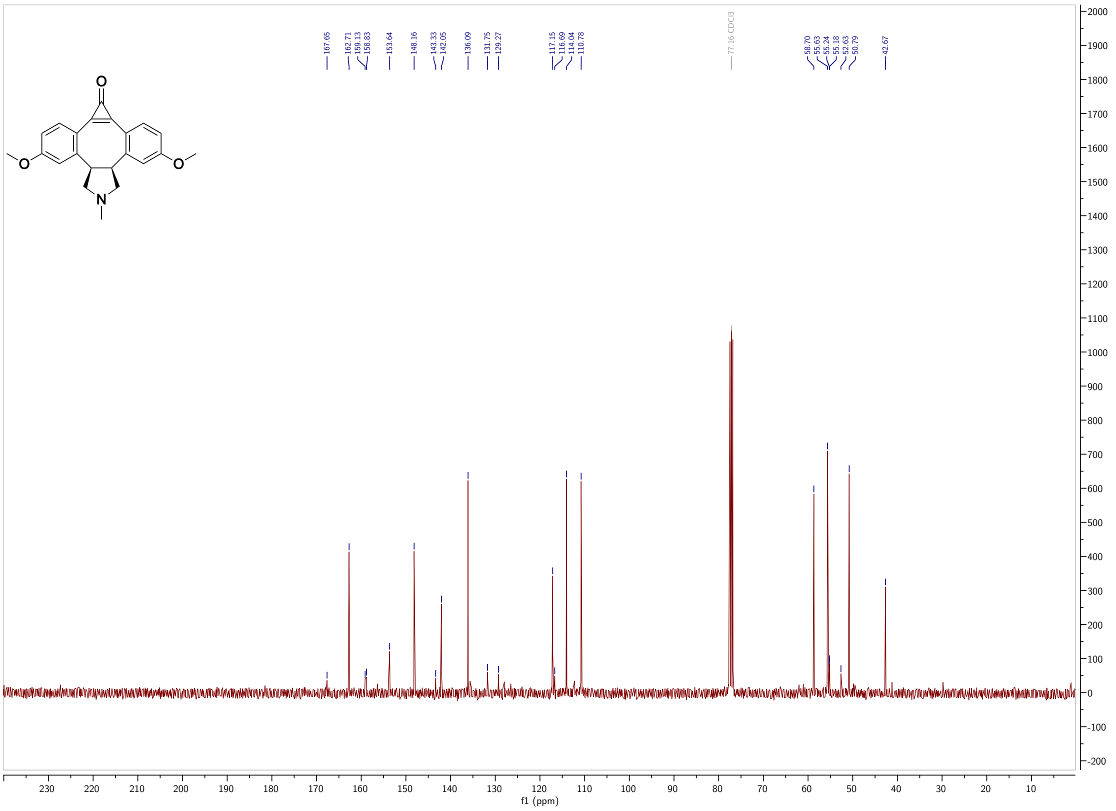


Figure S12: ^{13}C NMR spectrum of compound **4a** (101 MHz, $CDCl_3$).

FL-DIBO (5)

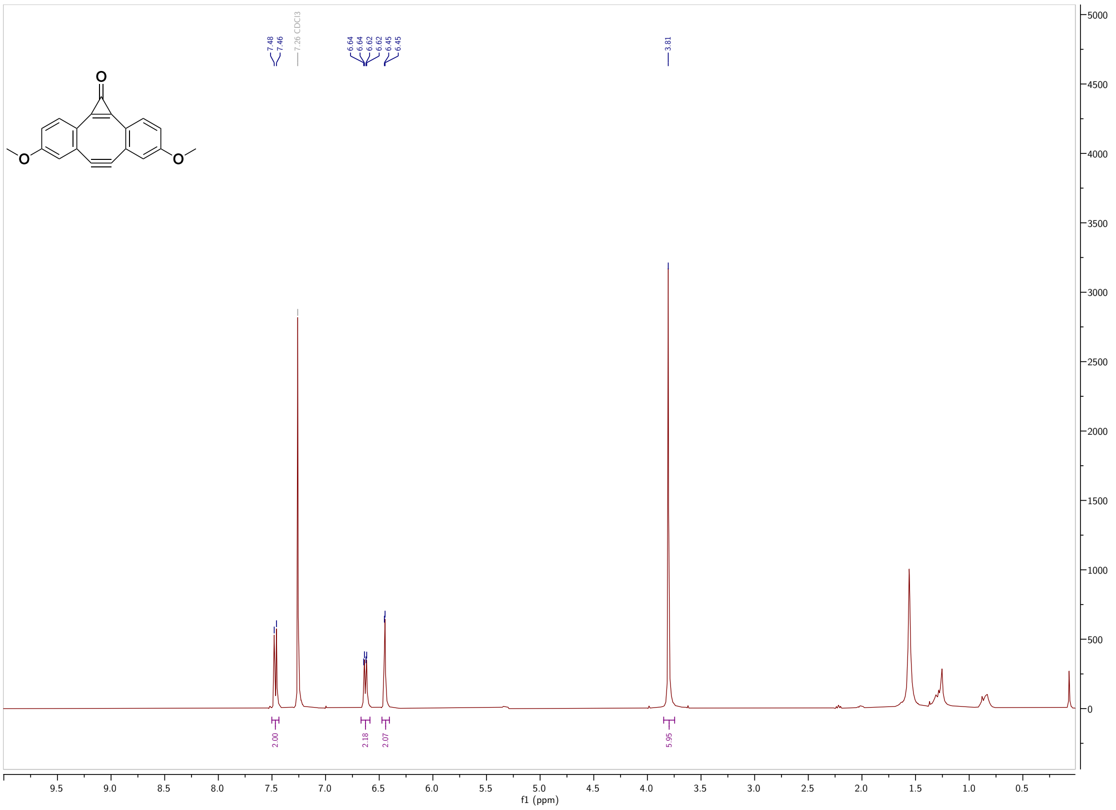


Figure S13: ^{1}H NMR spectrum of compound **5** (400 MHz, $CDCl_3$).

Photo-triazole-DMBO (6)

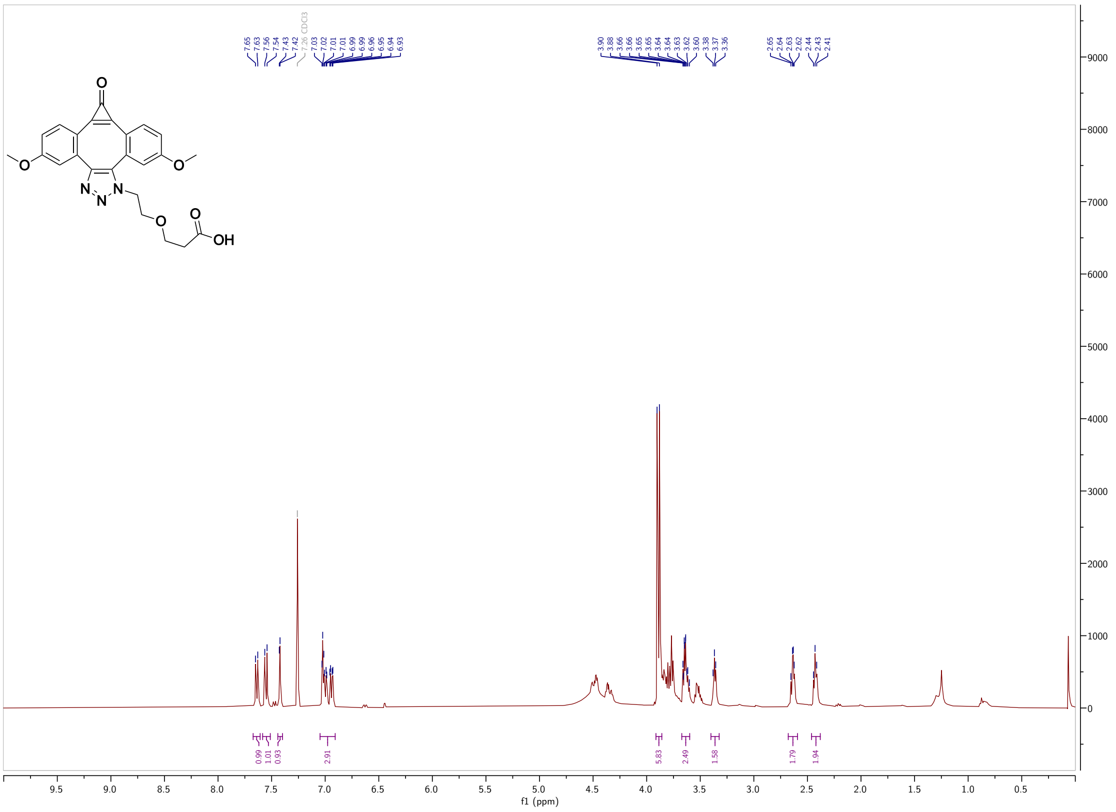


Figure S14: ^{1}H NMR spectrum of compound **6** (400 MHz, $CDCl_3$).

References

1 P. Pracht, S. Grimme, C. Bannwarth, F. Bohle, S. Ehlert, G. Feldmann, J. Gorges, M. Müller, T. Neudecker, C. Plett, S. Spicher, P. Steinbach, P. A. Wesołowski and F. Zeller, *J. Chem. Phys.*, 2024, **160**, 114110.

2 C. Bannwarth, S. Ehlert and S. Grimme, *J. Chem. Theory Comput.*, 2019, **15**, 1652–1671.

3 C. Bannwarth, E. Caldeweyher, S. Ehlert, A. Hansen, P. Pracht, J. Seibert, S. Spicher and S. Grimme, *WIREs Comput. Mol. Sci.*, 2021, **11**, e1493.

4 F. Neese, *J. Comput. Chem.*, 2003, **24**, 1740–1747.

5 S. Grimme, C. Bannwarth, S. Dohm, A. Hansen, J. Pisarek, P. Pracht, J. Seibert and F. Neese, *Angew. Chem. Int. Ed.*, 2017, **56**, 14763–14769.

6 G. L. Stoychev, A. A. Auer, R. Izsák and F. Neese, *J. Chem. Theory Comput.*, 2018, **14**, 619–637.

7 F. Neese, F. Wennmohs, U. Becker and C. Riplinger, *J. Chem. Phys.*, 2020, **152**, 224108.

8 F. Neese, *WIREs Comput. Mol. Sci.*, 2022, **12**, e1606.

9 S. Grimme, A. Hansen, S. Ehlert and J.-M. Mewes, *J. Chem. Phys.*, 2021, **154**, 064103.

10 V. Barone and M. Cossi, *J. Phys. Chem. A*, 1998, **102**, 1995–2001.

11 N. Mardirossian and M. Head-Gordon, *J. Chem. Phys.*, 2016, **144**, 214110.

12 F. Weigend and R. Ahlrichs, *Phys. Chem. Chem. Phys.*, 2005, **7**, 3297–3305.

13 F. Weigend, *Phys. Chem. Chem. Phys.*, 2006, **8**, 1057–1065.

14 F. Jensen, *J. Chem. Theory Comput.*, 2015, **11**, 132–138.

15 F. Jensen, *Theor. Chem. Acc.*, 2010, **126**, 371–382.