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Materials and Methods

All solvents were of reagent grade and used as received. Dichloromethane was distilled over calcium hydride. All reagents were purchased from Sigma-Aldrich® unless stated otherwise. Room temperature refers to ambient temperature (20-22 °C). Reactions were monitored by Thin Layer Chromatography (TLC) using aluminum backed silica gel 60 (F254) plates, visualized using UV254 nm and potassium permanganate, ninhydrin and cerium molibdate dips as appropriate. Column chromatography was carried out using silica gel G60 (SiliCycle, 60-200μm, 60 Å) as the stationary phase.

NMR Spectroscopy

All NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer. Chemical shifts are reported in δ units, parts per million (ppm) downfield from TMS. Coupling constants (J) are reported in Hertz (Hz) without adjustments; therefore, due to limits in digital resolution, in some cases there are small differences (<1 Hz) in the measured J value of the same coupling constant determined from different signals. Splitting patterns are designed as follows: s – singlet, d – doublet, t – triplet, dd – doublet of doublets, dt – doublet of triplets, td – triplet of doublets, ddd – doublet of doublet of doublets, tt – triplet of triplets, sp – septet, m – multiplet, br – broad. Various 2D techniques and DEPT experiments were used to establish the structures and to assign the signals.

Mass Analysis

High-resolution mass spectra were obtained by using MALDI-ToF (Applied Biosystems SciEx 5800 instrument) with 2,5-dihydroxybenzoic acid as matrix.

Absorption and Fluorescence Measurements

UV-Vis spectra were recorded at 25 ± 0.1 °C using a Varian Cary Bio50 UV-Vis spectrophotometer. Fluorescence spectra were recorded with a PTI fluorimeter using a cuvette with 1 cm path length. All spectra were corrected for the spectral response of the detection system and for the spectral irradiance of the excitation source (via a calibrated photodiode).

Quantum Yield Determination

Quantum yields were determined from the slope of the integrated fluorescence emission between 360 and 700 nm (excitation at 346 nm) versus absorbance using quinine sulfate in 1.0 N H_2SO_4 (Φ_f = 0.54 ± 0.03) as fluorescence standard.[1] For each compound, four data points were acquired with

absorbances ranging between 0.05 and 0.5 (l = 10 cm).

In-gel Visualization

Following reaction with FL-DIBO, some protein samples were analyzed via SDS-PAGE (10% gradient) and visualized by in-gel fluorescence scanning (BioRad, GelDoc XR PC, 365 nm excitation / 480 nm emission). The gels were then stained with Coomassie Blue to reveal total protein content.

Computational Studies

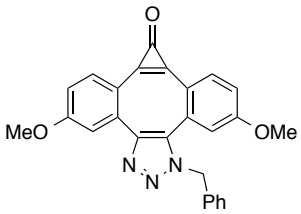
All quantum chemical studies were performed with the Gaussian 09 (Rev. C01) software package.[2] Molecular structures for compounds **10b** and **11b** were obtained by optimizing their gas-phase geometries by DFT with the B3LYP hybrid functional and Pople's 6-31G(d) split valence basis set. To account for bulk solvent effects, the gas-phase geometries were further geometry-optimized with inclusion of the polarizable continuum model (PCM), which creates a solute cavity based on a set of overlapping spheres.[3] Coordinates for the final geometries are listed in Tables S1 and S2. Both structures were validated based on vibrational frequency analysis to ensure a stationary point on the ground state potential surface. Vertical excitation energies were computed based on the solvent-equilibrated ground-state geometries (B3LYP/6-31G(d)) using TD-DFT with the CAM-B3LYP functional[4] and the 6-31+G(d) basis set with added diffuse functions. Electron density differences between the ground and respective excited states were determined from the corresponding Gaussian cube output files and visualized with the VMD software package.[5]

Synthetic Procedures

General procedure for the cycloaddition of Fl-DIBO with various 1,3-dipoles

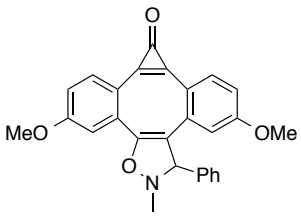
A solution of Fl- DIBO (14.4 mg, 0.05 mmol) and the respective 1,3-dipole (0.1 mmol) in a mixture of CH_2Cl_2 and methanol (4:1, 5 mL) was stirred at room temperature for 2 h. All volatiles were removed under reduced pressure and the residue was purified by flash chromatography on silica gel (7 g) using an appropriate mixture of methanol and CH_2Cl_2 affording the desired pure cycloadducts **5**, **6**, **7**, **10a-d** and **11a-b**, respectively.

1-Benzyl-5,11-dimethoxydibenzo[3,4:7,8]cyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol- 8(1*H*)-one (5).



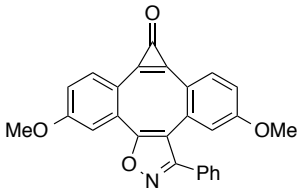
(19.6 mg, 93%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.66 (s, 3H), 3.92 (s, 3H), 5.33 (d, J = 15.5 Hz, 1H), 5.57 (d, J = 15.5 Hz, 1H), 6.77 (d, J = 2.5 Hz, 1H), 6.95 (m, 2H), 7.01 (m, 2H), 7.29 (m, 3H), 7.45 (d, J = 2.6 Hz, 1H), 7.53 (d, J = 2.8 Hz, 1H), 7.56 (d, J = 2.7 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 53.25 (CH_2), 55.70 (CH_3), 55.86 (CH_3), 115.37 (2×CH), 117.19 (C), 117.41 (CH), 118.23 (CH), 119.22 (C), 126.96 (2×CH), 128.55 (CH), 129.14 (2×CH), 130.46 (C), 133.44 (C), 133.57 (CH), 133.63 (CH), 135.23 (C), 135.88 (C), 143.35 (C), 149.82 (C), 151.75 (C), 153.35 (C=O), 162.45 (C), 163.35 (C); HRMS (*m/z*): [M-CO+H+] calcd. for $C_{25}H_{20}N_3O_2$, 394.1550; found, 394.1579.

5,11-Dimethoxy-2-methyl-3-phenyl-2,3-dihydro- 8*H*-dibenzo[3,4:7,8]cyclopropa[5,6]cyclo-octa[1,2-d]isoxazol-8-one (6).



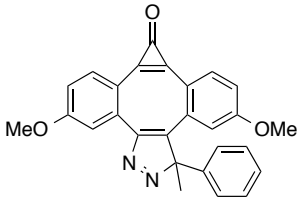
(19.3 mg, 91%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.01 (s, 3H), 3.68 (s, 3H), 3.84 (s, 3H), 4.95 (s, 1H), 6.62-6.69 (m, 2H), 6.90 (dd, J = 8.6, 2.6 Hz, 1H), 7.11 (d, J = 2.6 Hz, 1H), 7.23-7.31 (m, 5H), 7.38 (d, J = 8.6 Hz, 1H), 7.51 (d, J = 8.6 Hz, 1H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 46.78 (CH_3), 55.60 (CH_3), 55.89 (CH_3), 83.61 (CH), 109.96 (C), 112.41 (CH), 115.48 (CH), 117.57 (CH), 117.79 (C), 118.40 (C), 118.55 (CH), 127.37 (CH), 128.54 (CH), 129.11 (3×CH), 133.30 (C), 133.75 (CH), 134.24 (CH), 136.77 (C), 137.08 (C), 145.92 (C), 150.64 (C), 151.86 (C), 152.06 (C=O), 162.60 (C), 163.05 (C); HRMS (m/z): [M-CO+Na^+] calcd. for $C_{26}H_{21}NO_3Na$, 418.1414; found, 418.1422.

5,11-Dimethoxy-3-phenyl-8*H*-dibenzo[3,4:7,8]cyclopropa[5,6]cycloocta[1,2-d]isoxazol-8-one (7).



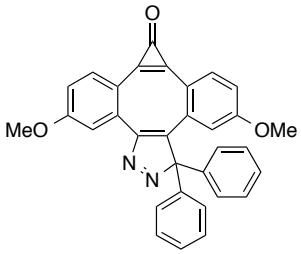
(19.1 mg, 94%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.37 (s, 3H), 3.90 (s, 3H), 6.29 (d, J = 2.5 Hz, 1H), 6.80 (dd, J = 8.5, 2.5 Hz, 1H), 7.01 (dd, J = 8.5, 2.5 Hz, 1H), 7.18 (d, J = 6.7 Hz, 1H), 7.29-7.36 (m, 3H), 7.43 (d, J = 7.5 Hz, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.81 (d, J = 7.5 Hz, 1H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 55.44 (CH_3), 56.03 (CH_3), 115.29 (CH), 116.77 (CH), 116.99 (C), 117.63 (CH), 118.24 (C), 119.45 (CH), 127.18 (C), 127.72 (CH), 128.84 (CH), 128.97 (CH), 129.34 (CH), 129.80 (CH), 132.08 (C), 132.89 (C), 133.00 (C), 133.67 (CH), 134.31 (CH), 150.17 (C), 150.85 (C), 151.74 (C), 152.03 (C=O), 162.76 (C), 164.32 (C), 164.78 (C); HRMS (m/z): [M-CO+Na$^+$] calcd. for $C_{25}H_{17}NO_3Na$, 402.1101; found, 402.1115.

5,11-Dimethoxy-3-methyl-3-phenyldibenzo[3,4:7,8]cyclopropa[5,6]cycloocta[1,2-c]pyrazol-8(3*H*)-one (10b).



(17.6 mg, 84%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.72 (s, 3H), 3.33 (s, 3H), 3.93 (s, 3H), 5.92 (d, J = 2.5 Hz, 1H), 6.78 (dd, J = 8.6, 2.5 Hz, 1H), 6.99 (dd, J = 8.6, 2.6 Hz, 1H), 7.04-7.13 (m, 2H), 7.36-7.44 (m, 3H), 7.47 (d, J = 8.6 Hz, 1H), 7.52 (d, J = 2.6 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 19.03 (CH_3), 55.13 (CH_3), 56.03 (CH_3), 105.77 (C), 115.34 (CH), 115.82 (CH), 116.15 (CH), 117.45 (C), 118.01 (C), 118.09 (CH), 126.68 (2×CH), 129.29 (CH), 129.59 (2×CH), 131.43 (C), 133.29 (CH), 133.86 (CH), 134.81 (C), 136.53 (C), 149.72 (C), 151.56 (C), 151.72 (C), 152.47 (C), 154.31 (C=O), 162.22 (C), 163.31 (C); HRMS (m/z): [M-CO+Na$^+$] calcd. for $C_{26}H_{20}N_2O_2Na$, 415.1417; found, 415.1428.

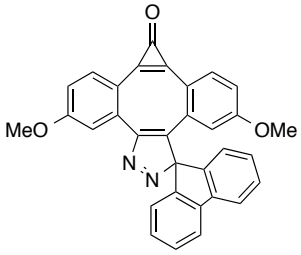
5,11-Dimethoxy-3,3-diphenyldibenzo[3,4:7,8]cyclopropa[5,6]cycloocta[1,2-c]pyrazol-8(3*H*)-one (10c).



(17.8 mg, 74%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.34 (s, 3H), 3.93 (s, 3H), 6.14 (d, J = 2.5 Hz, 1H), 6.79 (dd, J = 8.6, 2.5 Hz, 1H), 6.99 (dd, J = 8.6, 2.5 Hz, 1H), 7.08-7.15 (m, 4H), 7.27-7.38 (m, 6H), 7.45 (d, J = 8.6 Hz, 1H), 7.53 (d, J = 8.6 Hz, 1H), 7.56 (d, J = 2.5 Hz, 1H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 55.21 (CH_3), 56.05 (CH_3), 112.59 (C), 115.89 (CH), 115.95 (CH), 116.27 (CH), 117.97 (C), 118.10 (C), 118.45 (CH), 128.72 (4×CH), 129.07 (2×CH), 129.16 (4×CH), 133.12 (CH), 133.78 (CH), 133.93 (2×C), 135.64 (C), 136.38 (C), 151.35

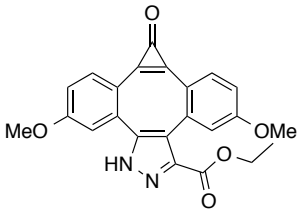
(C), 151.53 (C), 151.66 (C), 152.11 (C), 153.25 (C=O), 162.19 (C), 163.26 (C); HRMS (m/z): [M-CO+Na$^+$] calcd. for $C_{31}H_{22}N_2O_2Na$, 477.1573; found, 477.1581.

5,11-Dimethoxy-3,3-9'-fluorenyldibenzo[3,4:7,8]cyclopropa[5,6]cycloocta[1,2-c]pyrazol-8(3*H*)-one (10d).



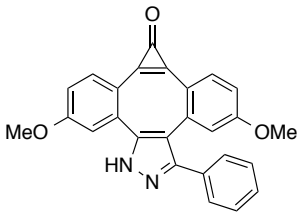
(23.1 mg, 96%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.15 (s, 3H), 3.94 (s, 3H), 6.11 (d, J = 2.5 Hz, 1H), 6.56 (dd, J = 8.6, 2.5 Hz, 1H), 6.98-7.05 (m, 3H), 7.25-7.32 (m, 3H), 7.41 (td, J = 7.5, 1.0 Hz, 2H), 7.58 (d, J = 2.6 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.71 (d, J = 7.5 Hz, 2H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 54.94 (CH_3), 56.04 (CH_3), 113.73 (C), 113.89 (CH), 115.98 (CH), 116.66 (CH), 116.88 (C), 117.93 (C), 118.37 (CH), 121.29 (2×CH), 123.80 (2×CH), 128.86 (2×CH), 130.36 (2×CH), 133.49 (CH), 133.89 (CH), 134.16 (C), 135.35 (2×C), 136.29 (C), 143.62 (2×C), 146.35 (C), 151.55 (C), 151.88 (C), 152.03 (C), 153.14 (C=O), 162.05 (C), 163.38 (C); HRMS (m/z): [M-CO+Na$^+$] calcd. for $C_{31}H_{20}N_2O_2Na$, 475.1417; found, 475.1426.

Ethyl 5,11-dimethoxy-8-oxo-1,8-dihydrodibenzo[3,4:7,8]cyclopropa[5,6]cycloocta[1,2-c]pyrazole-3-carboxylate (11a).



(18.5 mg, 92%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.22 (t, J = 7.0 Hz, 3H), 3.81 (s, 3H), 3.82 (s, 3H), 4.25 (q, J = 7.0 Hz, 2H), 6.72 (dd, J = 8.5, 2.4 Hz, 1H), 6.86 (dd, J = 8.5, 2.4 Hz, 1H), 6.89-6.95 (m, 2H), 7.10 (d, J = 2.4 Hz, 1H), 7.46 (d, J = 8.5 Hz, 1H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 14.19 (CH_3), 55.79 (CH_3), 55.99 (CH_3), 61.53 (CH_2), 113.48 (CH), 115.30 (CH), 117.32 (C), 117.73 (CH), 117.75 (C), 120.40 (C), 120.91 (CH), 132.54 (CH), 132.97 (CH), 134.31 (C), 135.39 (C), 141.68 (C), 142.72 (C), 150.84 (C), 151.22 (C), 152.07 (C=O), 161.66 (C=O), 162.40 (C), 162.75 (C); HRMS (m/z): [M-CO+Na$^+$] calcd. for $C_{22}H_{18}N_2O_4Na$, 397.1159; found, 397.1173.

5,11-Dimethoxy-3-phenyldibenzo[3,4:7,8]cyclopropa[5,6]cycloocta[1,2-c]pyrazol-8(1*H*)-one (11b).



(15.1 mg, 74%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.39 (s, 3H), 3.89 (s, 3H), 6.44 (d, J = 2.5 Hz, 1H), 6.77 (dd, J = 8.5, 2.5 Hz, 1H), 6.81 (dd, J = 8.5, 2.5 Hz, 1H), 7.11-7.17 (m, 2H), 7.18-7.22 (m, 2H), 7.28-7.33 (m, 3H), 7.52 (d, J = 8.5 Hz, 1H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 55.32 (CH_3), 55.88 (CH_3), 114.41 (CH), 114.88 (CH), 116.96 (CH), 117.70 (C), 118.41 (C), 119.66 (CH),

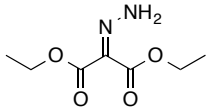
120.61 (C), 128.42 (CH), 128.74 (2×CH), 129.09 (2×CH), 130.29 (C), 131.84 (C), 132.85 (CH), 133.48 (CH), 134.58 (C), 142.60 (C), 144.25 (C), 146.30 (C), 147.30 (C), 152.37 (C=O), 162.26 (C), 162.66 (C); HRMS (m/z): [M-CO+Na^+] calcd. for $C_{25}H_{18}N_2O_2Na$, 401.1260; found, 401.1277.

Synthesis of diazo compounds 8a-d and 9b.

General procedure for the synthesis of hydrazones 13a-c and 14b.

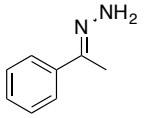
A solution of aldehyde or ketone (13.3 mmol) and hydrazine hydrate (1g, 20.0 mmol) in ethanol (20 mL) was refluxed overnight. After being cooled to room temperature, the reaction mixture was diluted with water (15 mL) and the water phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The remaining residue was then purified by flash chromatography on silica gel (24 g) using an appropriate mixture of hexane and ethyl acetate affording pure hydrazones **13a-c** and **14b**.

Diethyl 2-hydrazonomalonate (13a).



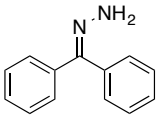
(1.16g, 46%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.1 Hz, 3H), 1.33 (t, J = 7.1 Hz, 3H), 4.27 (q, J = 7.1 Hz, 2H), 4.28 (q, J = 7.1 Hz, 2H), 7.22-7.32 (m, 5H), 7.40-7.56 (m, 5H), 9.38 (brs, 2H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 14.16 (CH_3), 14.31 (CH_3), 61.05 (CH_2), 61.14 (CH_2), 121.37 (C=N), 162.66 (C=O), 163.39 (C=O).

Acetophenone hydrazone (13b).



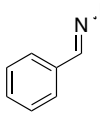
(1.44 g, 81%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.09 (s, 3H, CH_3), 5.40 (brs, 2H), 7.25-7.40 (m, 3H), 7.61-7.69 (m, 2H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 11.56 (CH_3), 125.43 (2×CH), 127.93 (CH), 128.21 (2×CH), 139.36 (C), 147.04 (C=N) in agreement with the literature data.[6]

Benzophenone hydrazone (13c).



(1.11g, 45%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.42 (brs, 2H), 7.22-7.32 (m, 5H), 7.40-7.56 (m, 5H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 126.61 (2×CH), 128.25 (CH), 128.29 (2×CH), 128.98 (2×CH), 129.06 (CH), 129.57 (2×CH), 133.15 (C), 138.59 (C), 149.33 (C=N) in agreement with the literature data.[7]

Benzaldehyde hydrazone (14b).

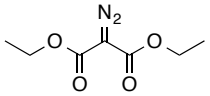


(630 mg, 39%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.52 (brs, 2H), 7.28-7.40 (m, 3H), 7.52-7.60 (m, 2H), 7.72 (s, 1H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 126.27 (2×CH), 128.68 (2×CH), 128.75 (CH), 135.26 (C), 143.17 (CH=N) in agreement with the literature data.[8]

General procedure for the synthesis of diazo compounds 8a-c and 9b from the respective hydrazones 13a-c and 14b.

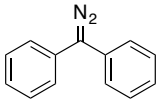
Activated manganese dioxide (345 mg, 4.0 mmol) was added to a cold solution (0 °C) of hydrazone (1.0 mmol) and $MgSO_4$ (482 mg, 4.0 mmol) in CH_2Cl_2 (3 mL). The reaction mixture was stirred for 2 h at 0 °C and 1 h at room temperature, filtered, and concentrated under reduced pressure affording pure diazo compounds. Compounds **8b** and **9b** were used without further purification nor NMR analysis due to their facile decomposition.

Diethyl 2-diazomalonate (8a).



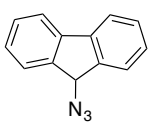
Crude diazo was purified by flash chromatography on alumina (14 g) using a mixture of hexane, ethylacetate and triethylamine (90:9:1) affording pure diethyl-2-diazomalonate **8a** (103 mg, 56%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.29 (t, J = 7.1 Hz, 6H), 4.27 (q, J = 7.1 Hz, 4H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 14.39 (2×CH_3), 61.66 (2×CH_2), 161.11 (2×C=O) in agreement with the literature data.[9]

Diphenyldiazomethane (8c).



Crude diazo was purified by flash chromatography on alumina (14 g) using a mixture of hexane and triethylamine (99:1) affording pure diphenyldiazomethane **8c** (133 mg, 68%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.75-6.90 (m, 2H), 6.90-7.10 (m, 8H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 125.39 (4×CH), 125.82 (2×CH), 129.33 (4×CH), 129.76 (2×C) in agreement with the literature data.[10]

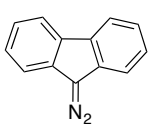
9-Azido-9*H*-fluorene (15).



A solution of sodium azide (1.33 g, 20.4 mmol) in water (3 mL) was added dropwise to a solution of 9-bromofluorene (1.0 g, 4.08 mmol) in acetone (7 mL). The reaction mixture was stirred overnight at room temperature and the aqueous phase was then extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The remaining residue was purified by flash chromatography

on silica gel (20 g) using a mixture of hexane and ethyl acetate (8:1) affording pure 9-azido-9*H*-fluorene (**15**) (564 mg, 67%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.20 (s, 1H), 7.36 (td, *J* = 7.4, 1.2 Hz, 2H), 7.44 (t, *J* = 7.4 Hz, 2H), 7.63 (d, *J* = 7.4 Hz, 2H), 7.70 (d, *J* = 7.4 Hz, 2H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): *δ* 64.42 (CH), 120.44 (2×CH), 125.41 (2×CH), 128.10 (2×CH), 129.58 (2×CH), 140.88 (2×C), 141.79 (2×C) in agreement with the literature data.[11]

9-Diazo-fluorene (8d).



A solution of *N*-succinimidyl 3-(diphenylphosphino)propionate (118 mg, 0.33 mmol) in dry toluene (1 mL) was added dropwise to a cold (0 °C) solution of 9-azido-9*H*-fluorene **15** (62 mg, 0.3 mmol) in toluene (1.5 mL). After stirring at 0 °C for 5 h, the reaction mixture was allowed to warm to room temperature and then further stirred overnight. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on alumina (7 g) using a mixture of hexane and triethylamine (99:1) affording pure 9-diazo-fluorene (**8d**) (38 mg, 66%): ^{1}H-NMR (300 MHz, $CDCl_3$): *δ* 7.32 (td, *J* = 7.4, 1.3 Hz, 2H), 7.38 (td, *J* = 7.4, 1.3 Hz, 2H), 7.50 (d, *J* = 7.4 Hz, 2H), 7.94 (d, *J* = 7.4 Hz, 2H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): *δ* 119.50 (2×CH), 121.16 (2×CH), 124.72 (2×CH), 126.52 (2×CH), 131.65 (2×C), 133.18 (2×C) in agreement with the literature data.[11]

Protein labeling experiments

Diazo-BSA.

A solution of BSA (400 μL, 20 mg/mL) in PBS (pH 7.4) was incubated with a solution of NHS-activated diazo ester **12**[12] (100 μL, 25 mM) in DMSO overnight at room temperature. The excess of low molecular weight NHS-activated diazo ester was removed by spin-filtration (MWCO = 10 kDa). Diazo-BSA was then dissolved in PBS (pH 7.4) and stored at 4 °C.

SPAAC with Diazo-BSA.

Diazo-labeled BSA (180 μL, 50 μM) was incubated with Fl-DIBO **1** (20 μL, 2.5 mM) at 37 °C for 18 h and analyzed by in-gel fluorescence imaging.

Absorption and Fluorescence Spectra

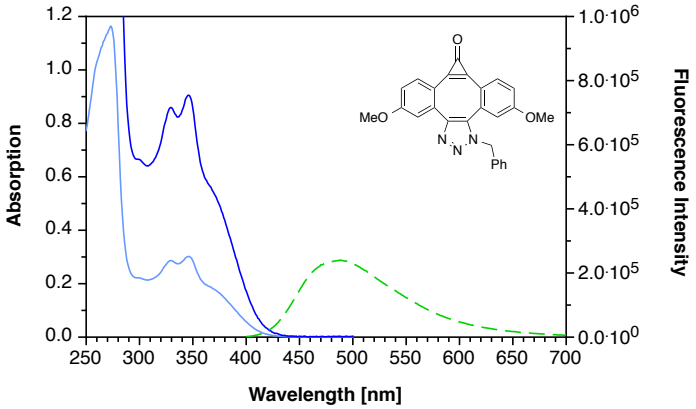


Figure S1. Absorption (blue traces (40 and 120 μM in MeOH at 25 °C)) and fluorescence emission (dashed green trace (λ_{exc}=370 nm, 160 μM in MeOH at 25 °C)) spectra of triazole **5**.

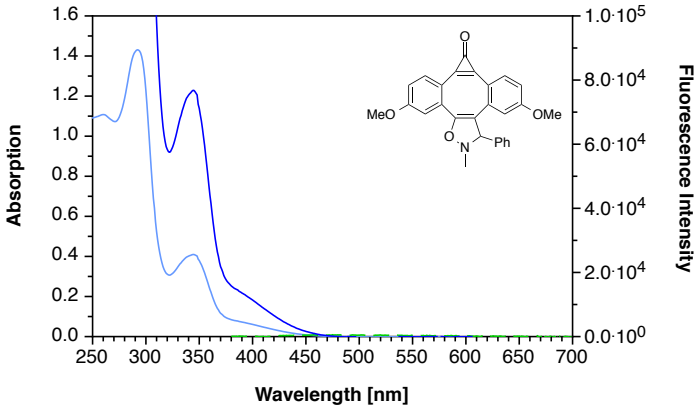


Figure S2. Absorption (blue traces (80 and 240 μM in MeOH at 25 °C)) and fluorescence emission (dashed green trace (λ_{exc}=370 nm, 560 μM in MeOH at 25 °C)) spectra of isoxazoline **6**.

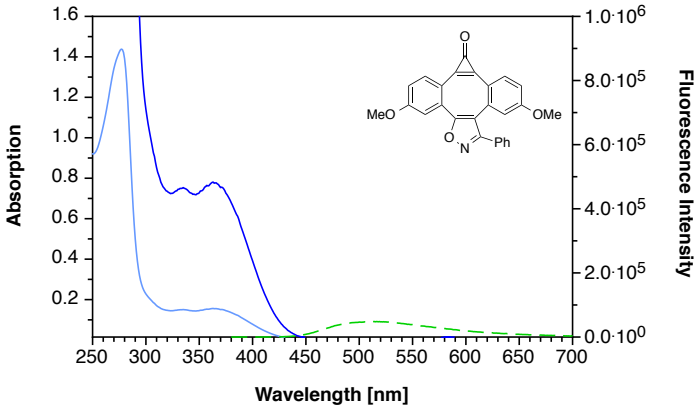


Figure S3. Absorption (blue traces (10 and 50 μM in MeOH at 25 °C)) and fluorescence emission (dashed green trace (λ_{exc}=370 nm, 40 μM in MeOH at 25 °C)) spectra of isoxazole **7**.

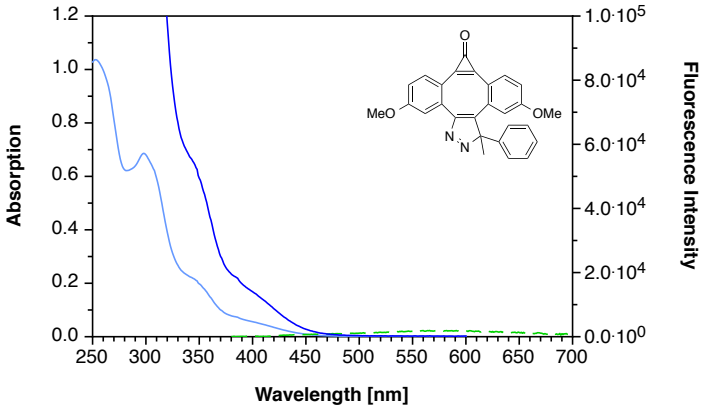


Figure S4. Absorption (blue traces (80 and 240 μM in MeOH at 25 °C)) and fluorescence emission (dashed green trace (λ_{exc}=370 nm, 550 μM in MeOH at 25 °C)) spectra of pyrazole **10b**.

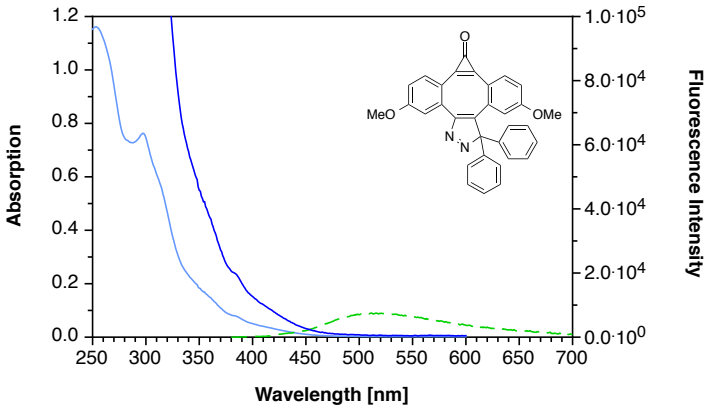


Figure S5. Absorption (blue traces (40 and 120 μM in MeOH at 25 °C)) and fluorescence emission (dashed green trace (λ_{exc}=370 nm, 300 μM in MeOH at 25 °C)) spectra of pyrazole **10c**.

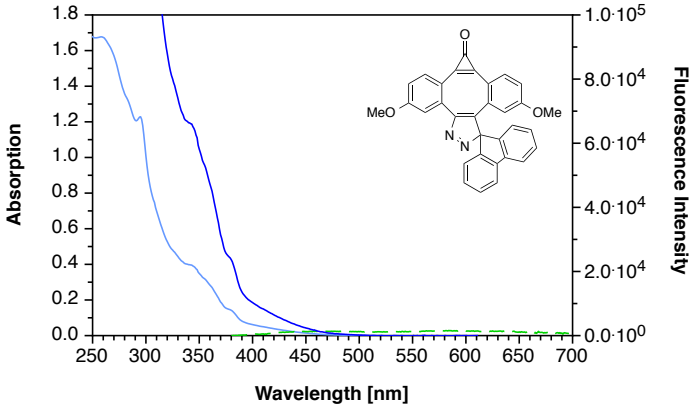


Figure S6. Absorption (blue traces (90 and 270 μM in MeOH at 25 °C)) and fluorescence emission (dashed green trace (λ_{exc}=370 nm, 350 μM in MeOH at 25 °C)) spectra of pyrazole **10d**.

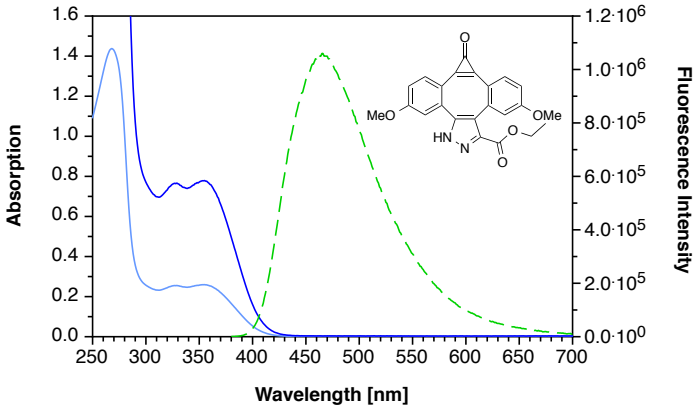


Figure S7. Absorption (blue traces (20 and 60 μM in MeOH at 25 °C)) and fluorescence emission (dashed green trace (λ_{exc}=370 nm, 60 μM in MeOH at 25 °C)) spectra of pyrazole **11a**.

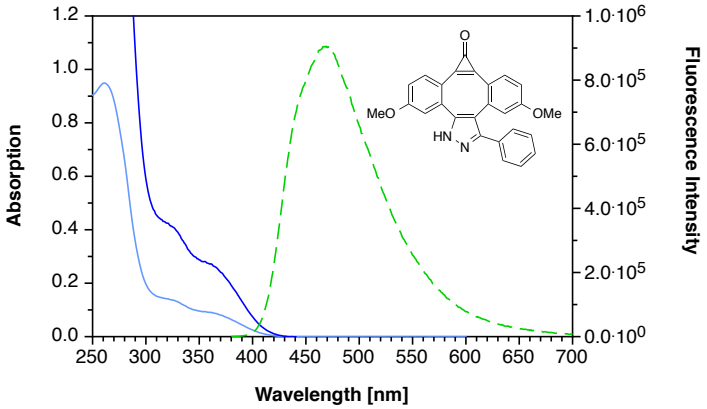


Figure S8. Absorption (blue traces (10 and 30 μM in MeOH at 25 °C)) and fluorescence emission (dashed green trace (λ_{exc}=370 nm, 50 μM in MeOH at 25 °C)) spectra of pyrazole **11b**.

Reaction Kinetics

Rate measurements for the cycloaddition of Fl-DIBO (**1**) with diazo ester **9a** were conducted by using ^{1}H NMR spectroscopy (Varian Mercury 300 MHz) at 25 °C. A 20 mM solution of diazo ester **9a** (0.2 mL) in $CDCl_3$/MeOD (4:1) was added to a thermally equilibrated solution of Fl-DIBO (**1**) (10 mM, 0.4 mL) in a mixture of $CDCl_3$/MeOD (4:1), leading to a mixture of both reactants in 1:1 ratio with a respective concentration of 6.66 mM. Reactions were monitored by following the decay of characteristic resonances of cyclooctyne **1** (δ = 3.78 (2 × OCH_3), 6.43 (2 × CH_{Ar}) and 6.63 (2 × CH_{Ar}) ppm) as well as the formation of characteristic pyrazole **11a** resonances (δ = 3.81 (OCH_3), 3.82 (OCH_3) and 1.22 (CH_3) ppm). Consumption of starting materials followed a second-order equation and the second-order rate constants were obtained by fitting the data to a linear equation (Figure S9). The reported rate constant of the cycloaddition reaction was calculated as the average of the observed second-order rate constants of two independent measurements.

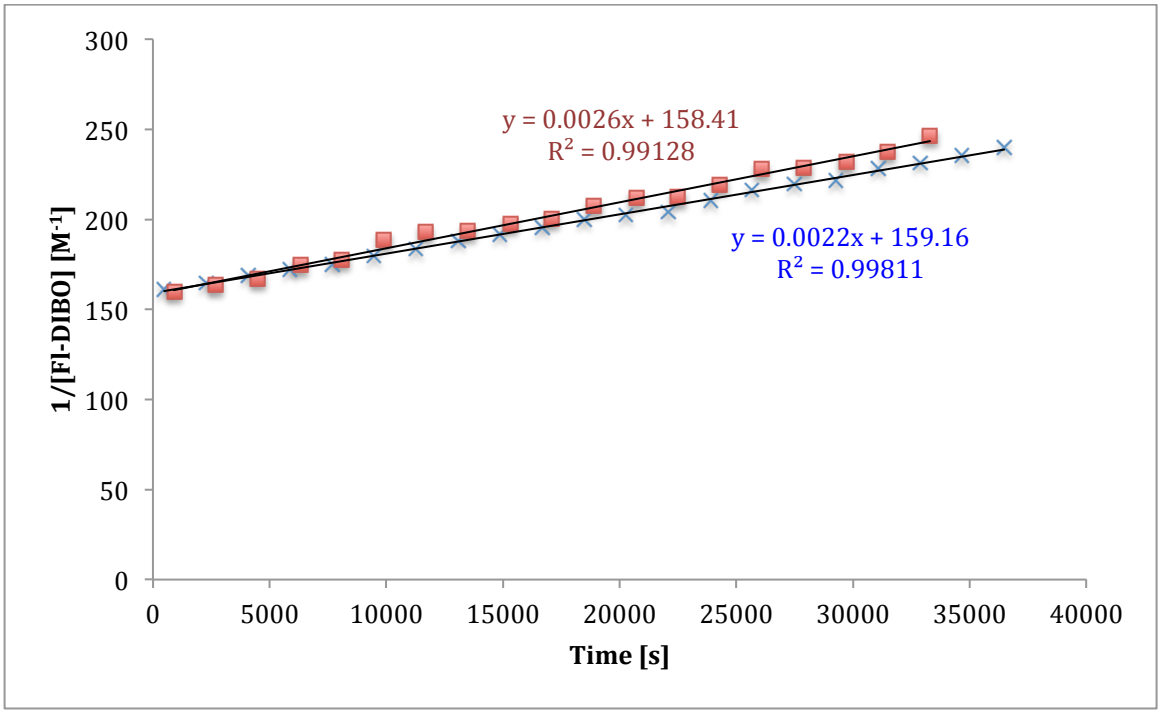


Figure S9. Kinetic measurements plots of Fl-DIBO **1** (6.66 mM) with diazo ester **9a** (6.66 mM) in $CDCl_3$/MeOD (4:1) at 298 K (two independent experiments).

Table S1: Cartesian Atomic Coordinates for the Geometry-Optimized Structure of Compound **10b** (B3LYP/6-31G(d), PCM with methanol as solvent)

Atom	x/Å	y/Å	z/Å
H	-3.859908	-0.898059	2.575782
C	-3.736981	-0.605893	1.537249
C	-3.408384	0.169040	-1.108710
C	-2.456546	-0.272174	1.074516
C	-4.845553	-0.593583	0.694952
C	-4.678789	-0.214049	-0.643964
C	-2.292181	0.133409	-0.277675
H	-5.816833	-0.872548	1.084702
H	-3.318612	0.508441	-2.134997
C	-1.320287	-0.405911	1.951107
C	0.004464	-0.744275	1.932127
C	-0.670335	-0.693028	3.188567
C	1.013861	-1.225181	1.020445
C	2.686726	-2.569140	-0.786464
C	1.901048	-2.218010	1.457468
C	1.017162	-0.836247	-0.348988
C	1.844359	-1.532047	-1.228239
C	2.738846	-2.893954	0.574396
H	1.906257	-2.488085	2.509151
H	1.855202	-1.300463	-2.285919
H	3.399616	-3.667130	0.946499
C	-1.028643	0.715125	-0.790261
C	0.280539	0.361909	-0.825967
O	-0.691700	-0.845873	4.402343
N	-1.191827	2.017768	-1.399628
N	-0.087772	2.507314	-1.735112
C	1.014678	1.561121	-1.441695
O	3.417351	-3.171765	-1.755194
O	-5.673830	-0.161653	-1.562413
C	-6.998832	-0.511851	-1.156320
H	-7.618506	-0.400385	-2.046458
H	-7.362228	0.159512	-0.370201
H	-7.044352	-1.548928	-0.805388
C	4.313485	-4.221403	-1.381780
H	3.773203	-5.059206	-0.926944
H	5.081311	-3.858140	-0.689619
H	4.783837	-4.550226	-2.308762
C	1.674342	1.314373	-2.820857
H	0.961764	0.844162	-3.504898
H	2.556346	0.679781	-2.742535
H	1.980316	2.277026	-3.238348
C	1.987902	2.192313	-0.428221
C	3.735253	3.302448	1.481681
C	1.553064	3.207964	0.436412
C	3.307979	1.735193	-0.315280
C	4.173974	2.286247	0.631837
C	2.420020	3.759827	1.380446
H	0.535537	3.577802	0.367431
H	3.673970	0.940741	-0.956680
H	5.193601	1.917322	0.701200
H	2.064191	4.550545	2.035261
H	4.410887	3.733253	2.215321

Table S2: Cartesian Atomic Coordinates for the Geometry Optimized Structure of Compound **11b** (B3LYP/6-31G(d), PCM with methanol as solvent)

Atom	x/Å	y/Å	z/Å
H	-4.487651	1.869133	-1.236181
C	-4.164806	0.997968	-0.674156
C	-3.320549	-1.221787	0.758719
C	-2.808841	0.643819	-0.700119
C	-5.099227	0.280585	0.068213
C	-4.671378	-0.832271	0.803673
C	-2.381583	-0.508551	0.014876
H	-6.135739	0.594640	0.072233
H	-3.025796	-2.095648	1.330871
C	-1.866713	1.501597	-1.374462
C	-0.582030	1.963180	-1.283047
C	-1.495775	2.644414	-2.142221
C	0.588064	1.866956	-0.447566
C	2.655212	1.866147	1.447411
C	1.347584	3.021006	-0.205571
C	0.907989	0.659518	0.233730
C	1.929383	0.692130	1.180605
C	2.375804	3.038333	0.732766
H	1.104290	3.930834	-0.746399
H	2.199592	-0.205979	1.725137
H	2.932263	3.951646	0.903898
C	-1.027853	-1.090374	-0.138983
C	0.304376	-0.654171	-0.107550
O	-1.771019	3.559012	-2.908107
N	-0.962020	-2.428970	-0.393637
N	0.280768	-2.899536	-0.561030
C	1.071449	-1.834463	-0.386730
O	3.610815	1.752896	2.403542
O	-5.472033	-1.602836	1.577731
C	-6.859600	-1.269981	1.668824
H	-7.295400	-2.007647	2.343010
H	-7.346663	-1.334893	0.689450
H	-6.997988	-0.265478	2.083986
C	4.394100	2.903674	2.725252
H	3.764593	3.720382	3.096583
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C	3.156667	-3.157922	-0.031240
C	3.313902	-1.072471	-1.242102
C	4.681949	-1.280287	-1.417859
C	4.524541	-3.365556	-0.209946
H	2.559560	-3.886753	0.508331
H	2.845496	-0.187845	-1.662003
H	5.269793	-0.549094	-1.966184
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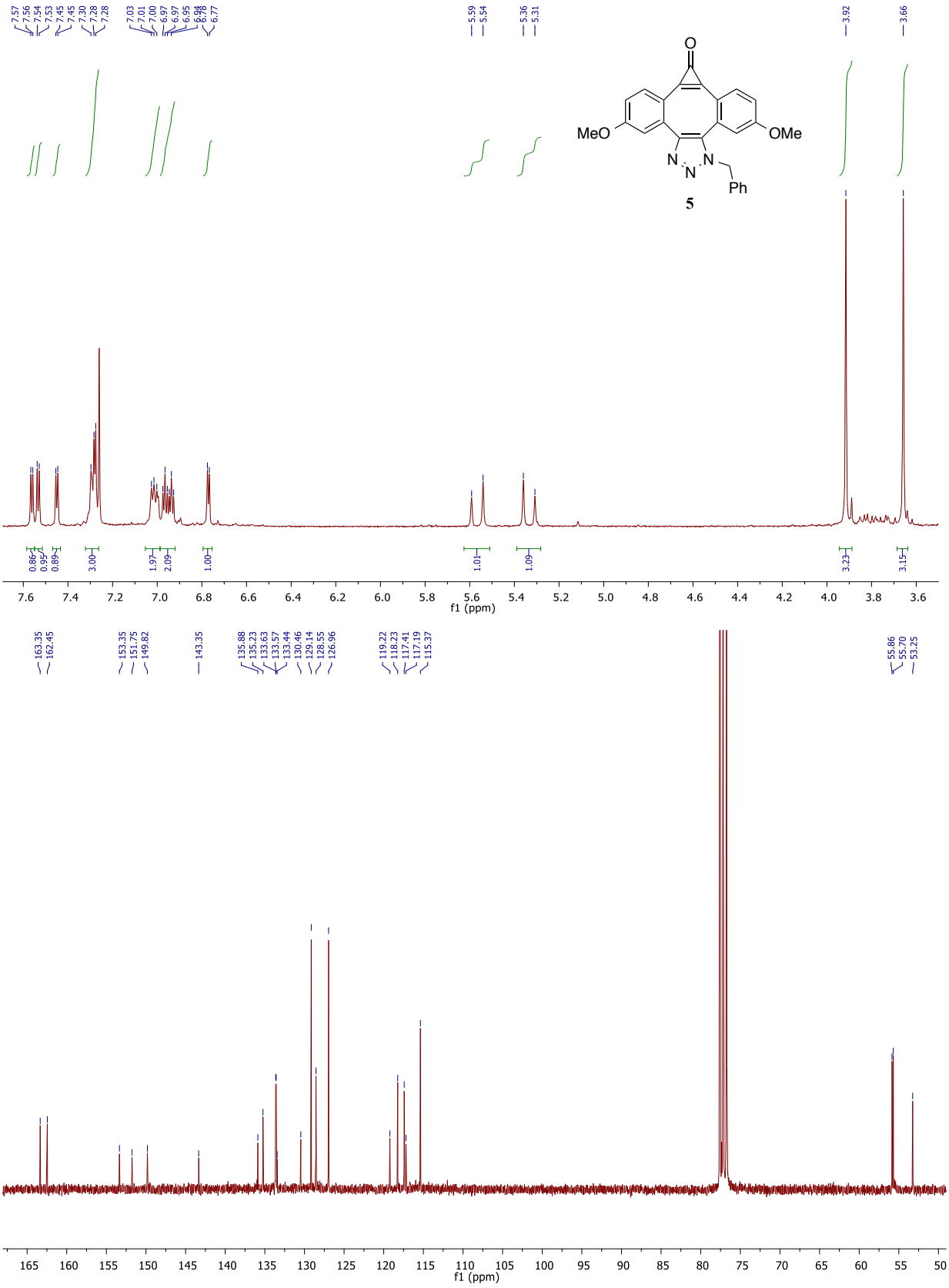
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5.31
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3.66
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OMe
N
N
N
Ph
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1.01
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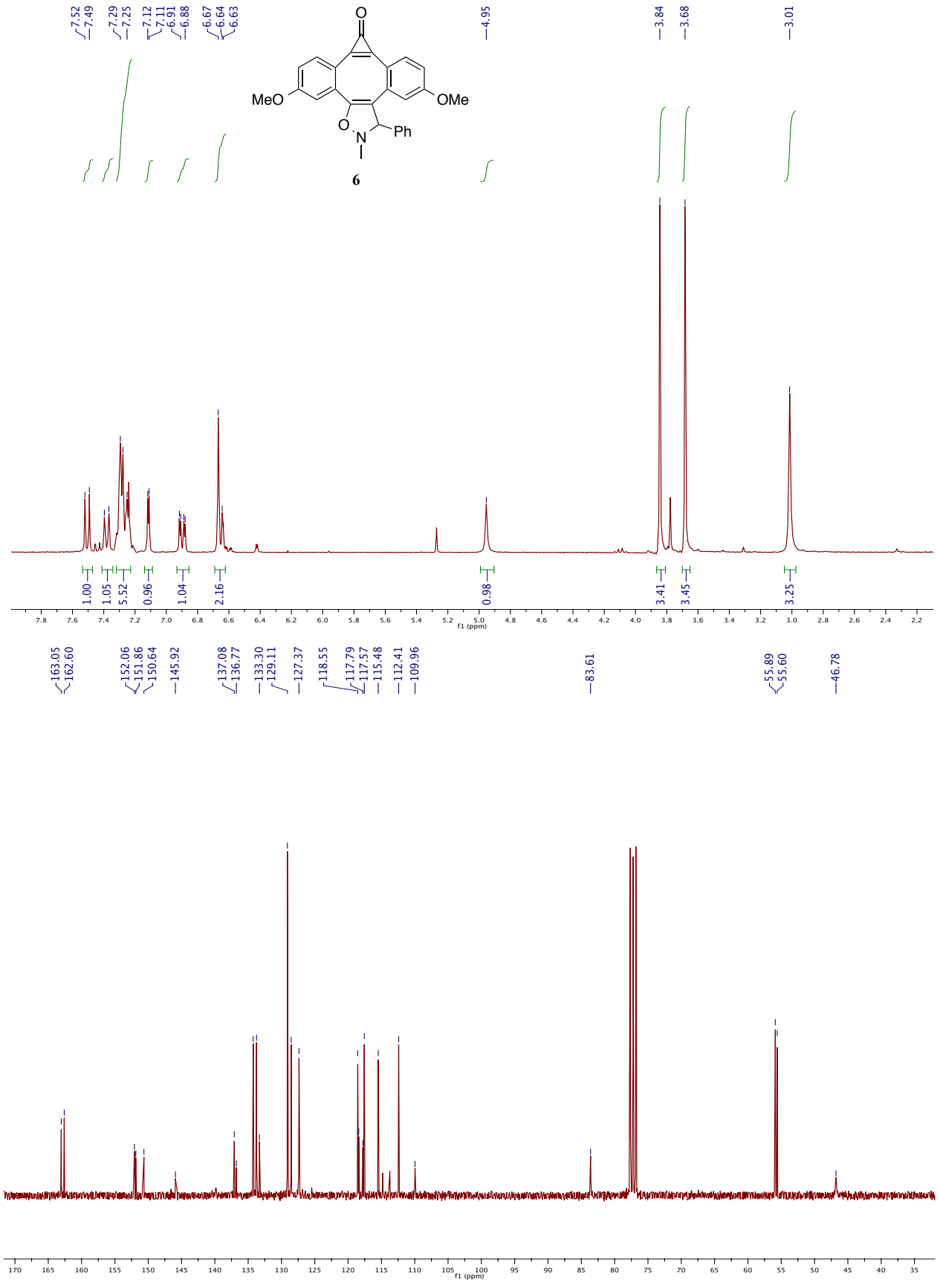
f1 (ppm)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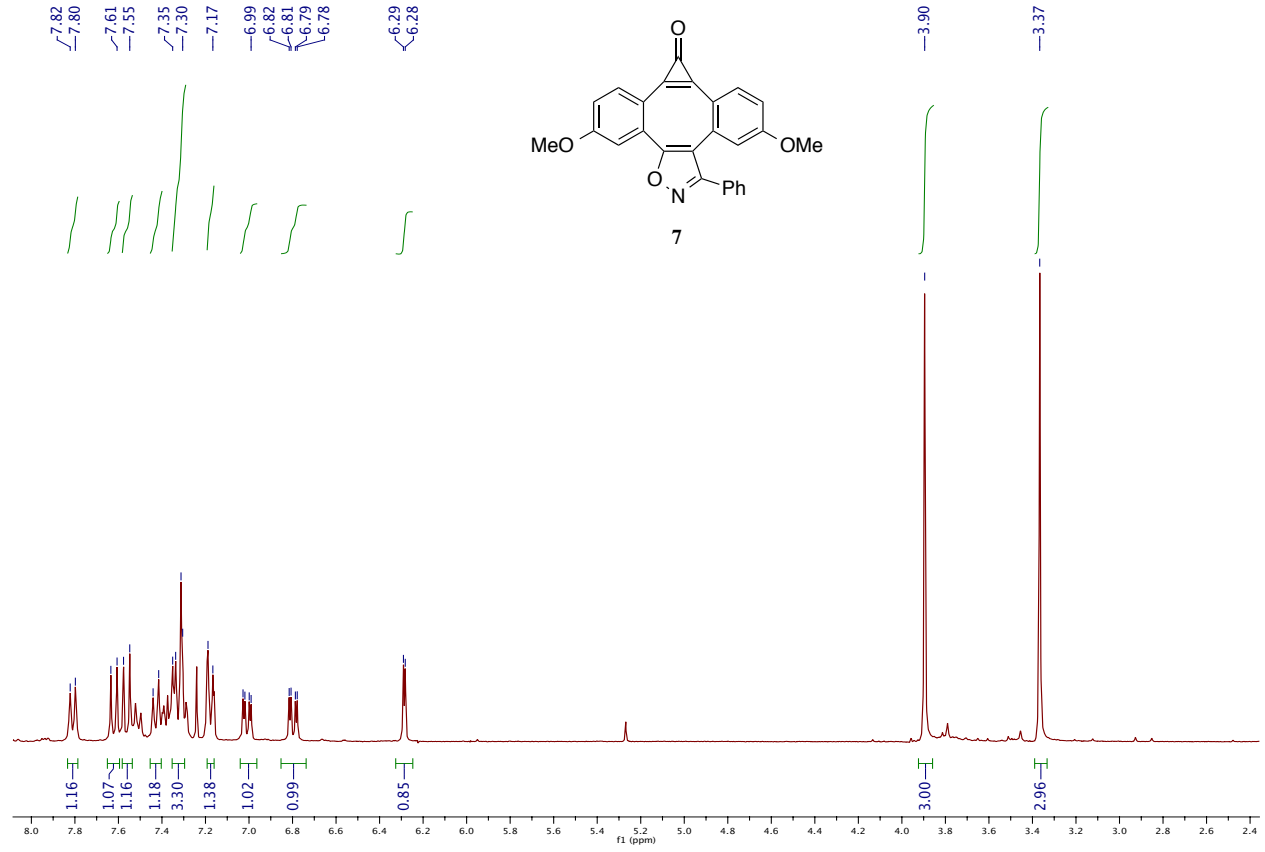
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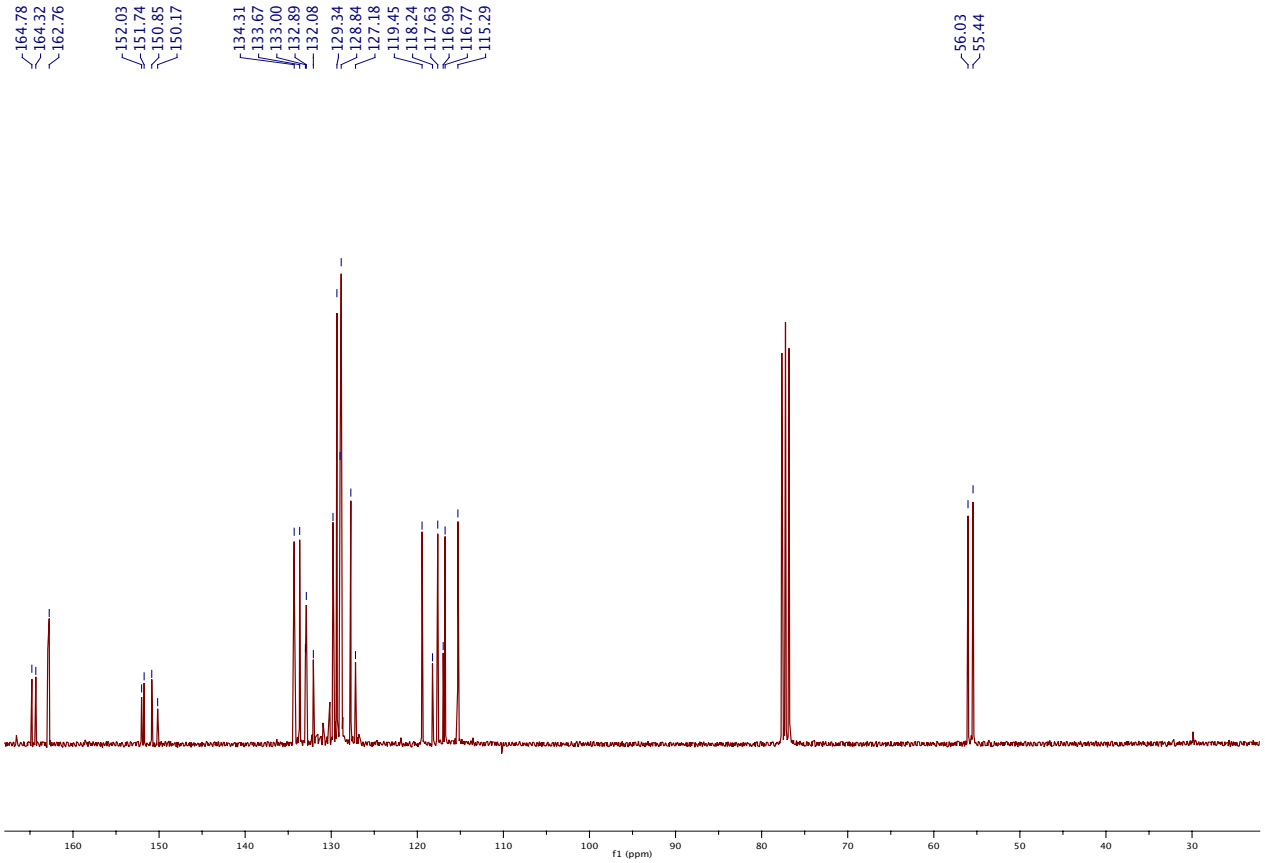
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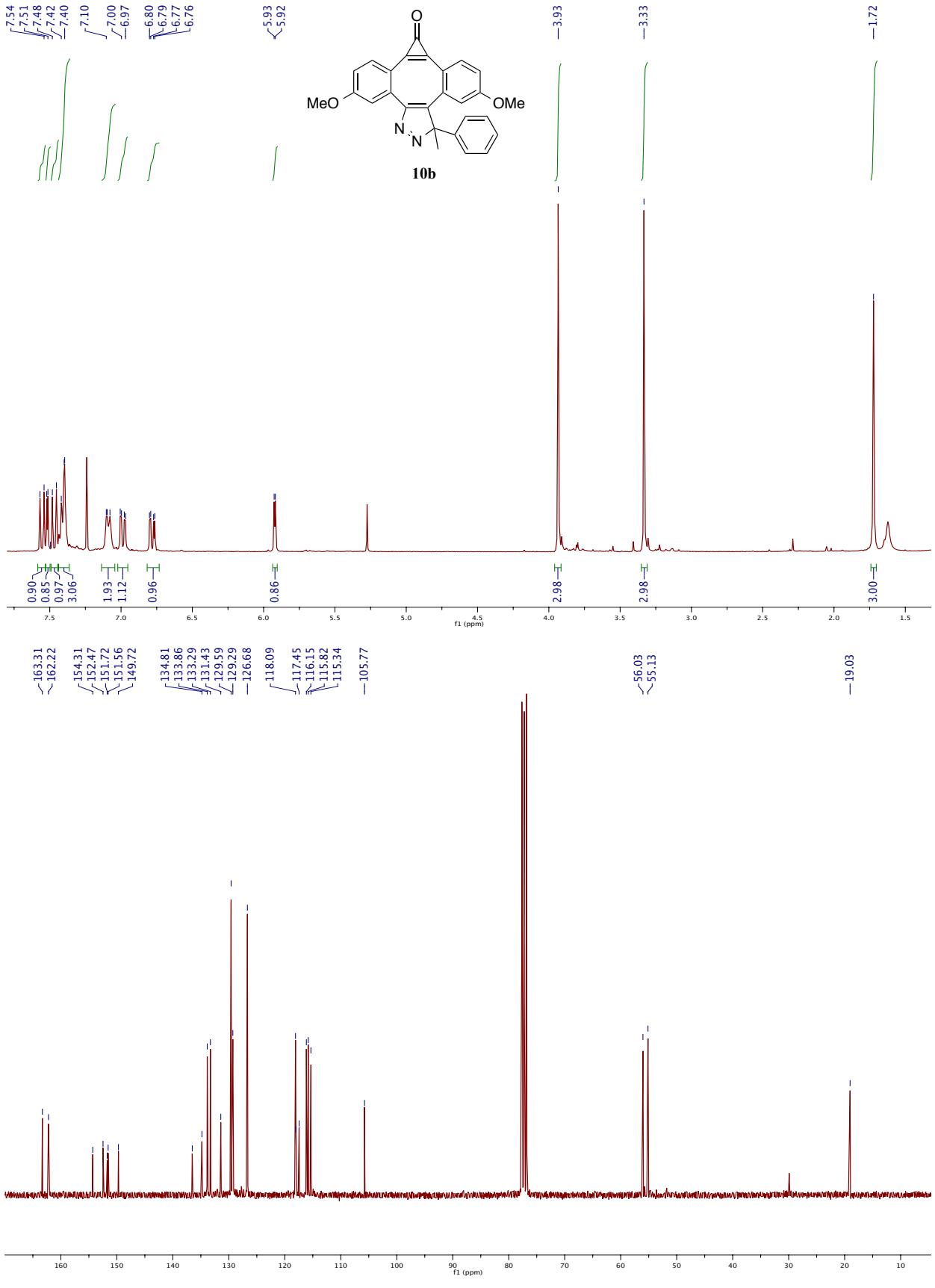
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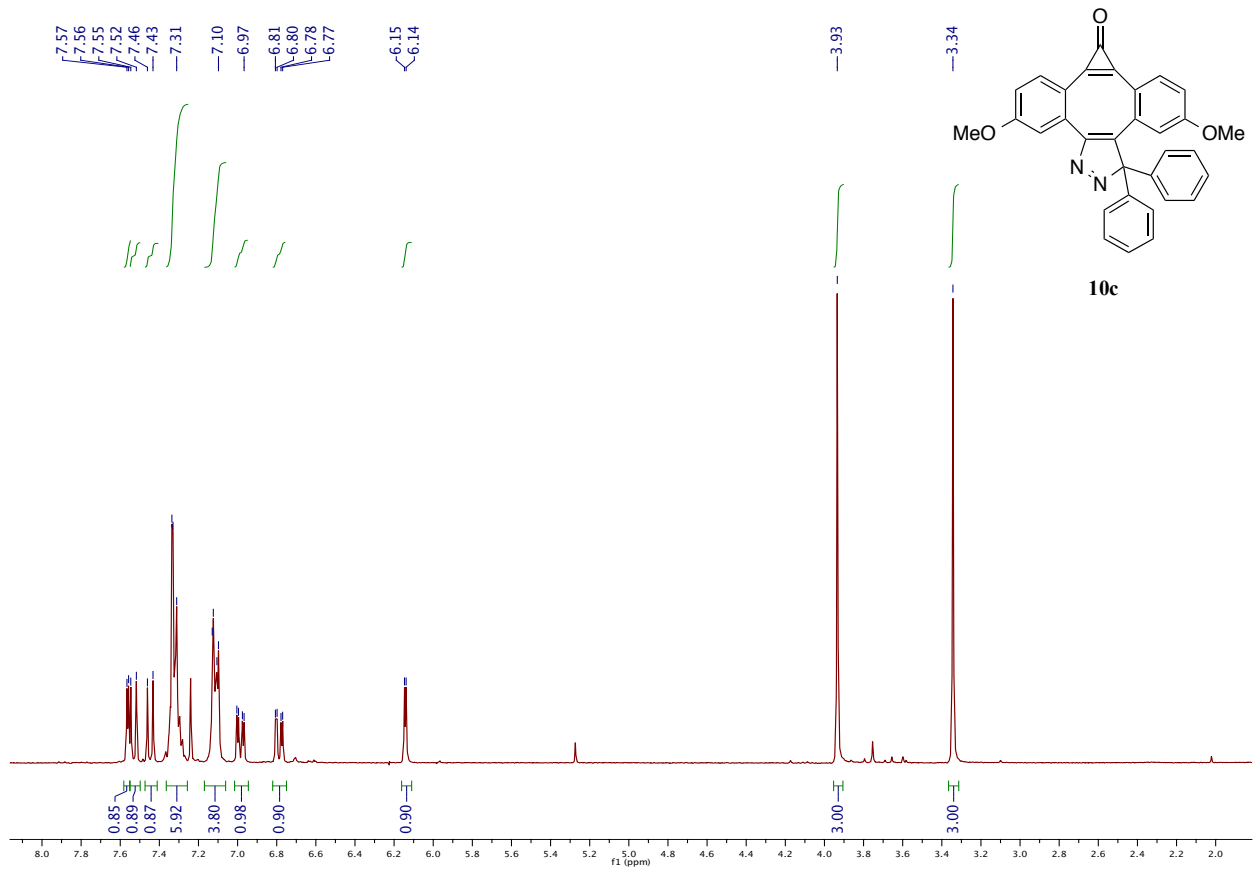
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0.89
0.87
5.92
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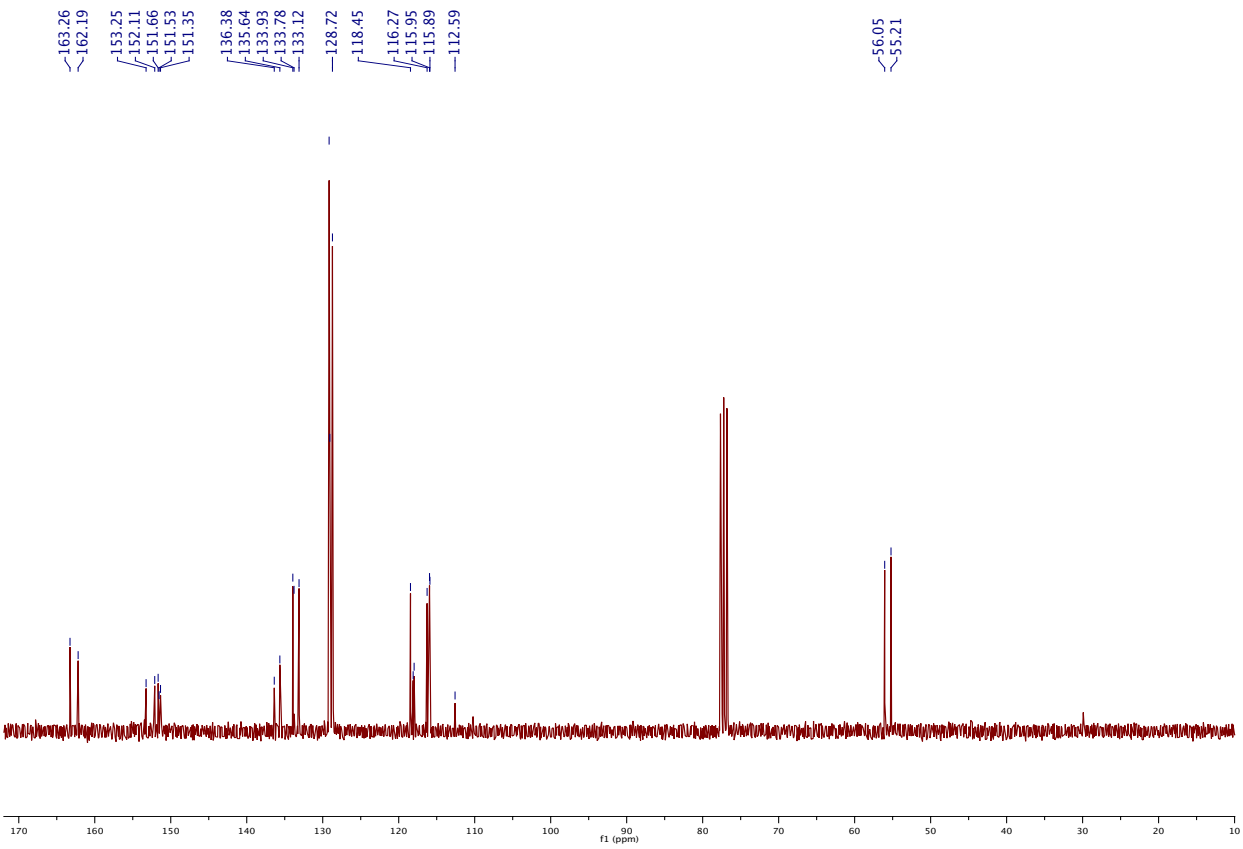
f1 (ppm)

O
MeO
OMe
N
N

10c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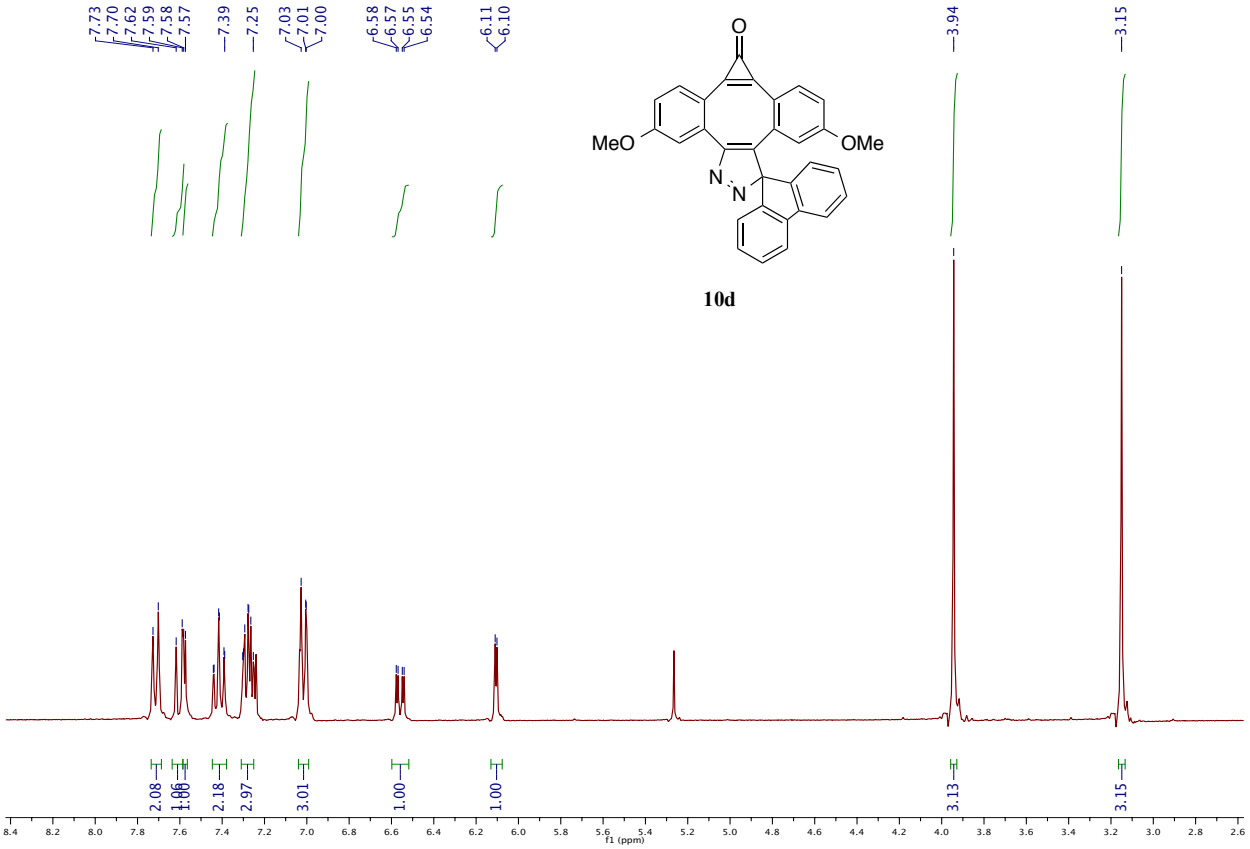
f1 (ppm)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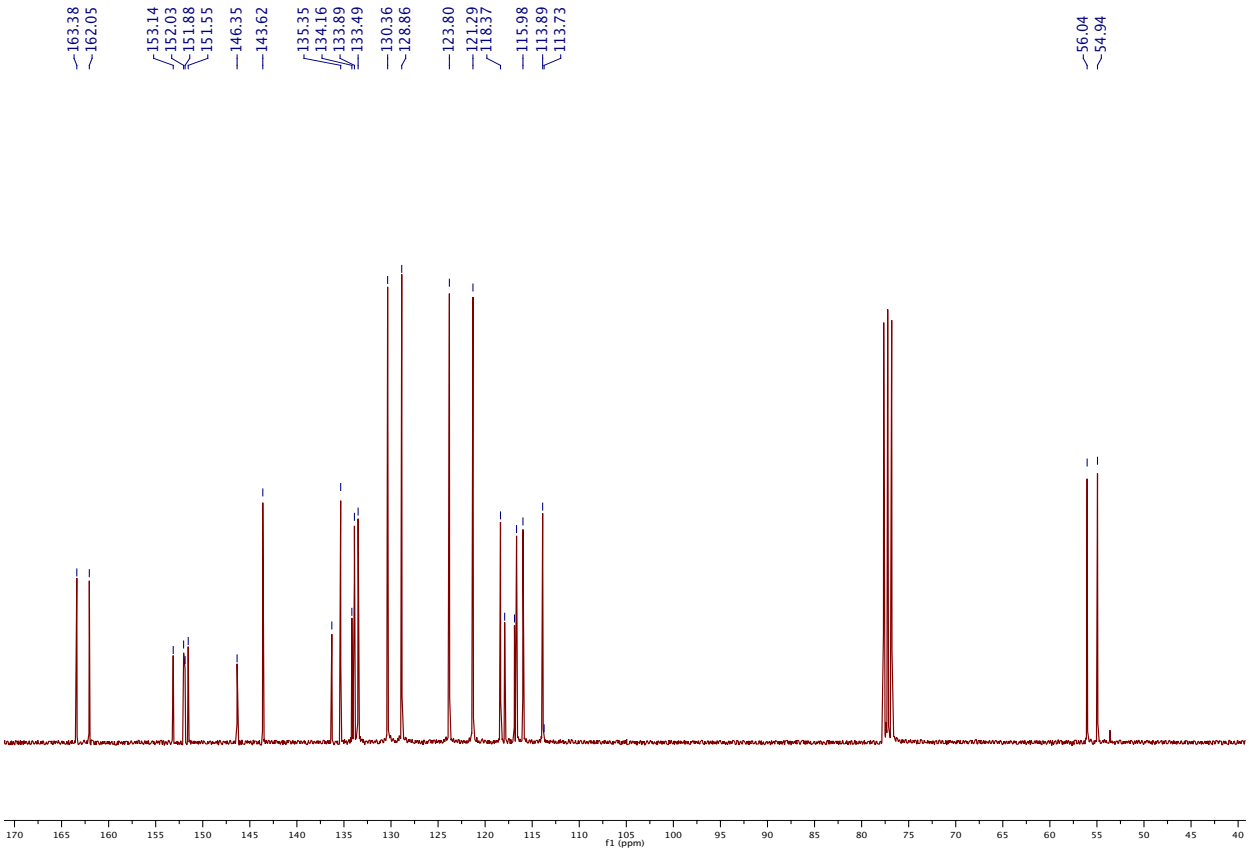
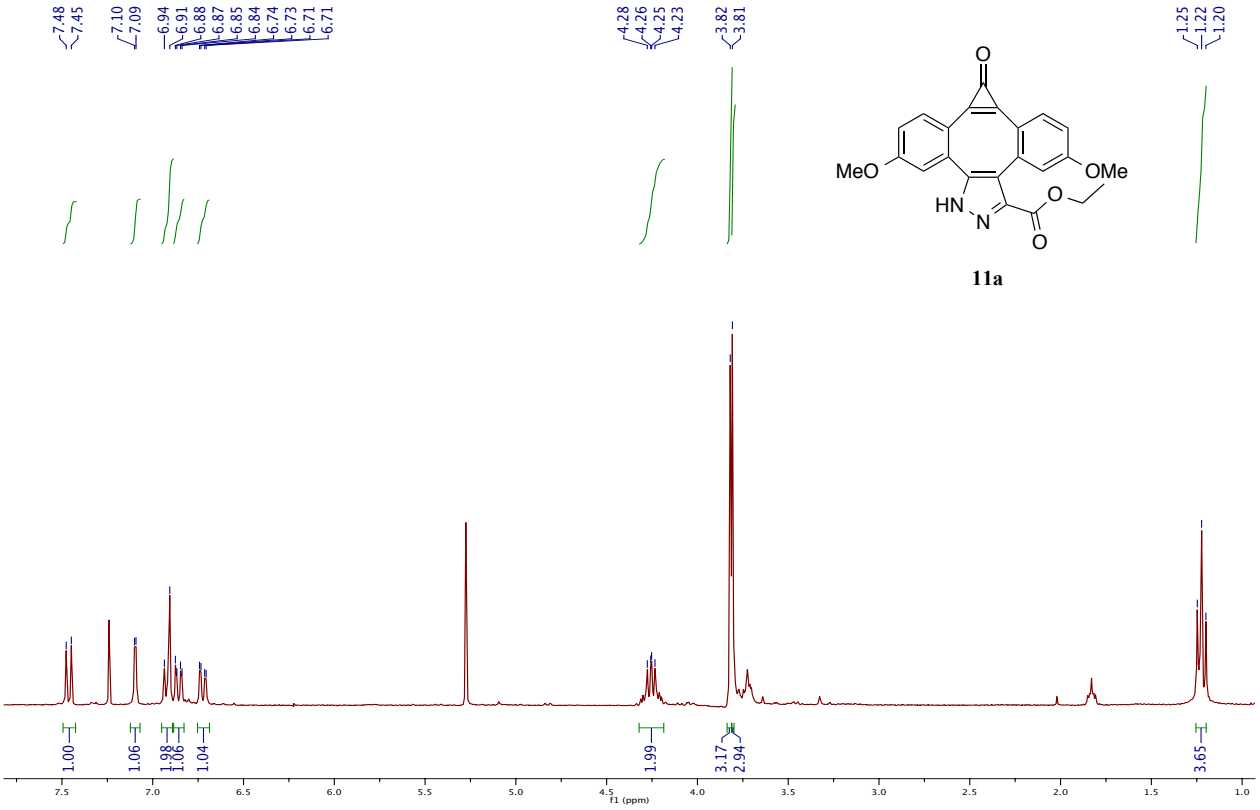
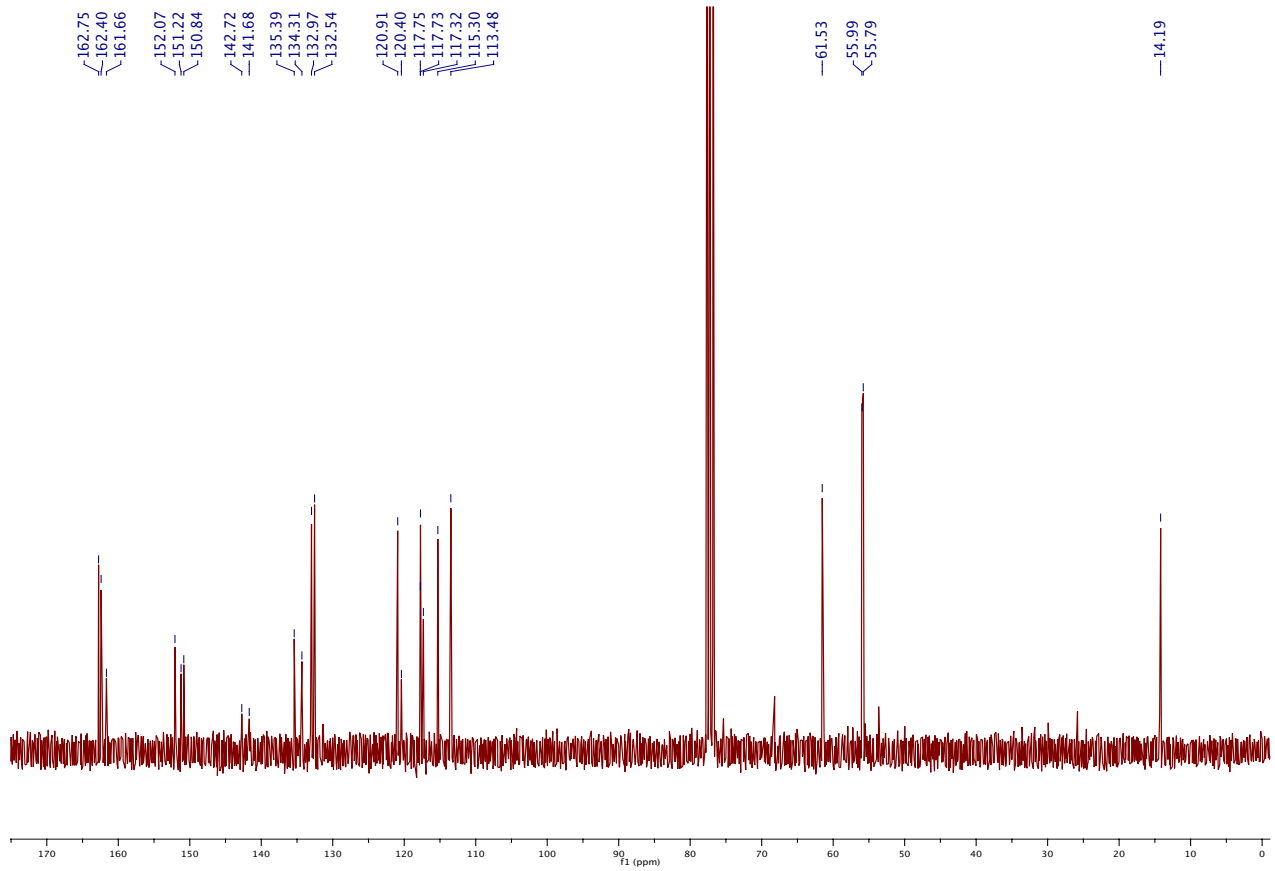
7.62
7.59
7.58
7.57
7.39
7.25
7.03
7.01
7.00
6.58
6.57
6.55
6.54
6.11
6.10
3.94
3.15
O
MeO
OMe
N
N
2.08
1.06
1.00
2.18
2.97
3.01
1.00
1.00
3.13
3.15
f1 (ppm)

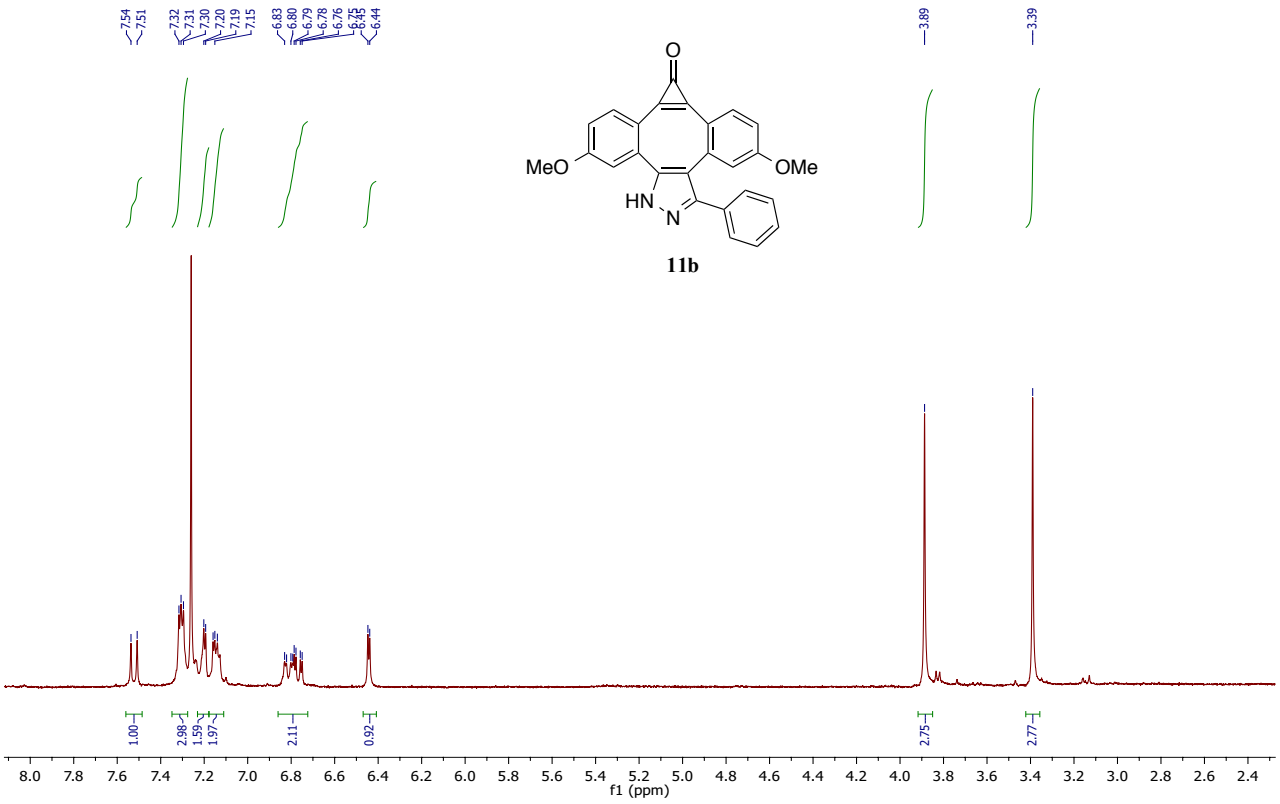
10d


163.38
162.05
153.14
152.03
151.88
151.55
146.35
143.62
135.35
134.16
133.89
133.49
130.36
128.86
123.80
121.29
118.37
115.98
113.89
113.73
56.04
54.94
f1 (ppm)

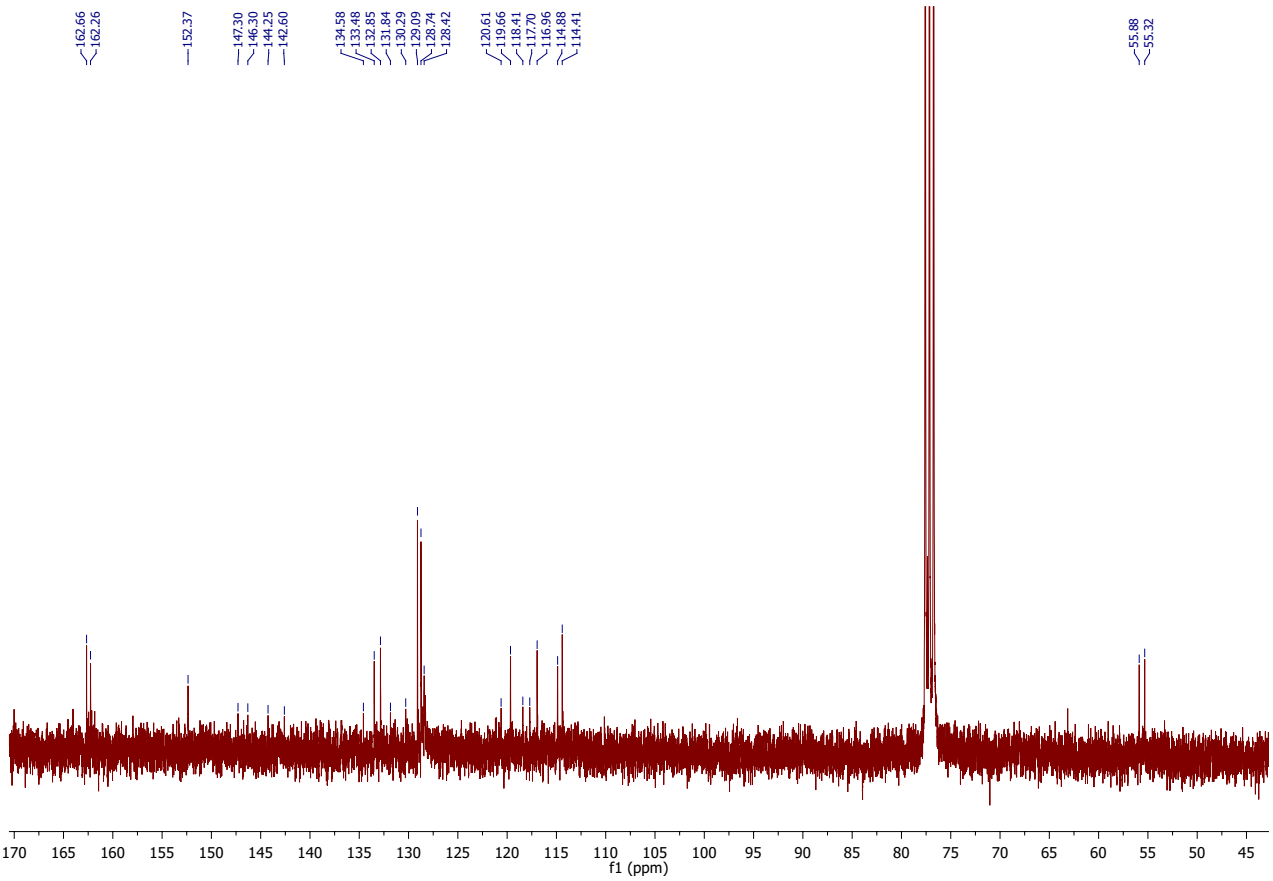

7.48
7.45
7.10
7.09
6.94
6.91
6.88
6.87
6.85
6.84
6.74
6.73
6.71
6.71
4.28
4.26
4.25
4.23
3.82
3.81
1.25
1.22
1.20
O
MeO
OMe
HN
N
O
O
O
1.00
1.06
1.98
1.06
1.04
1.99
3.17
2.94
3.65
f1 (ppm)

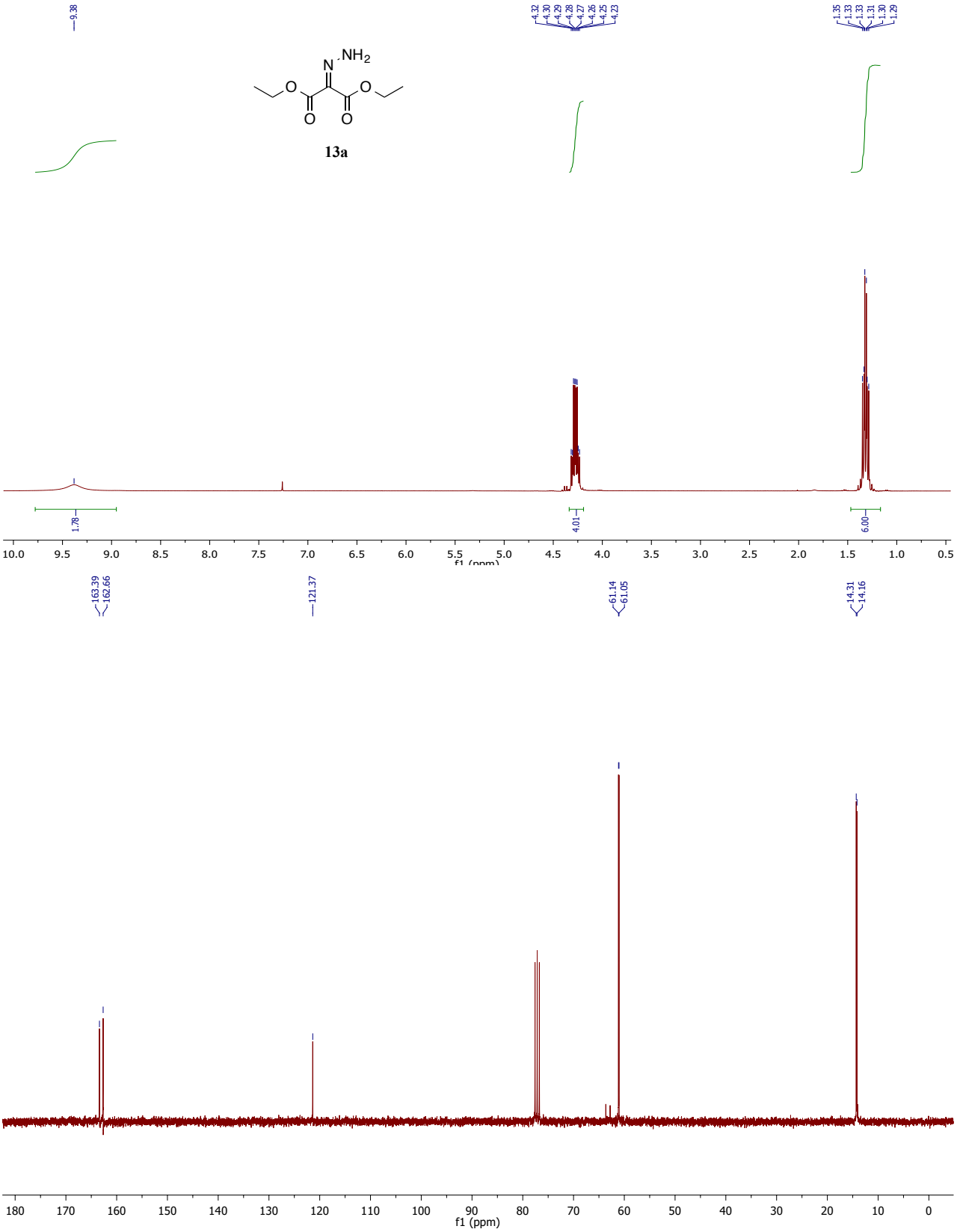
11a

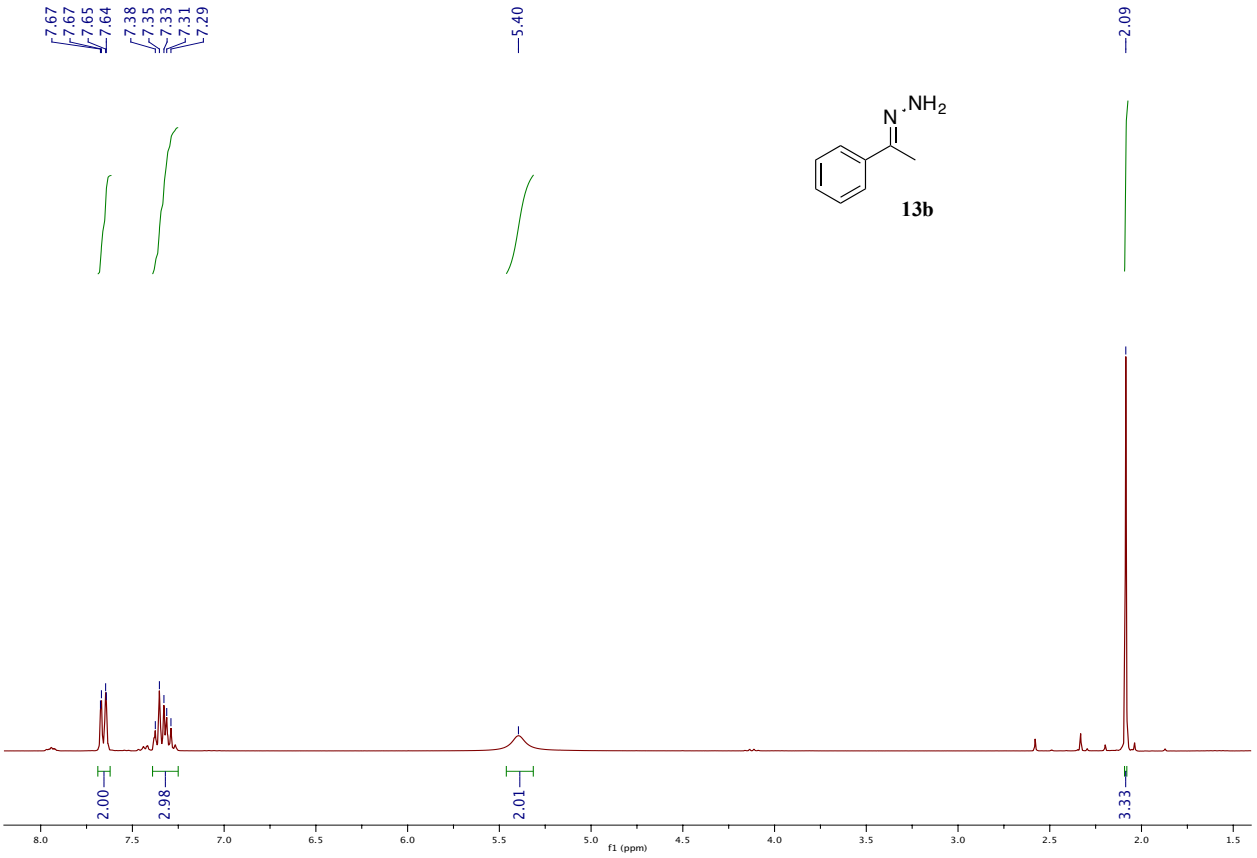

162.75
162.40
161.66
152.07
151.22
150.84
142.72
141.68
135.39
134.31
132.97
132.54
120.91
120.40
117.75
117.73
117.32
115.30
113.48
61.53
55.99
55.79
14.19
f1 (ppm)

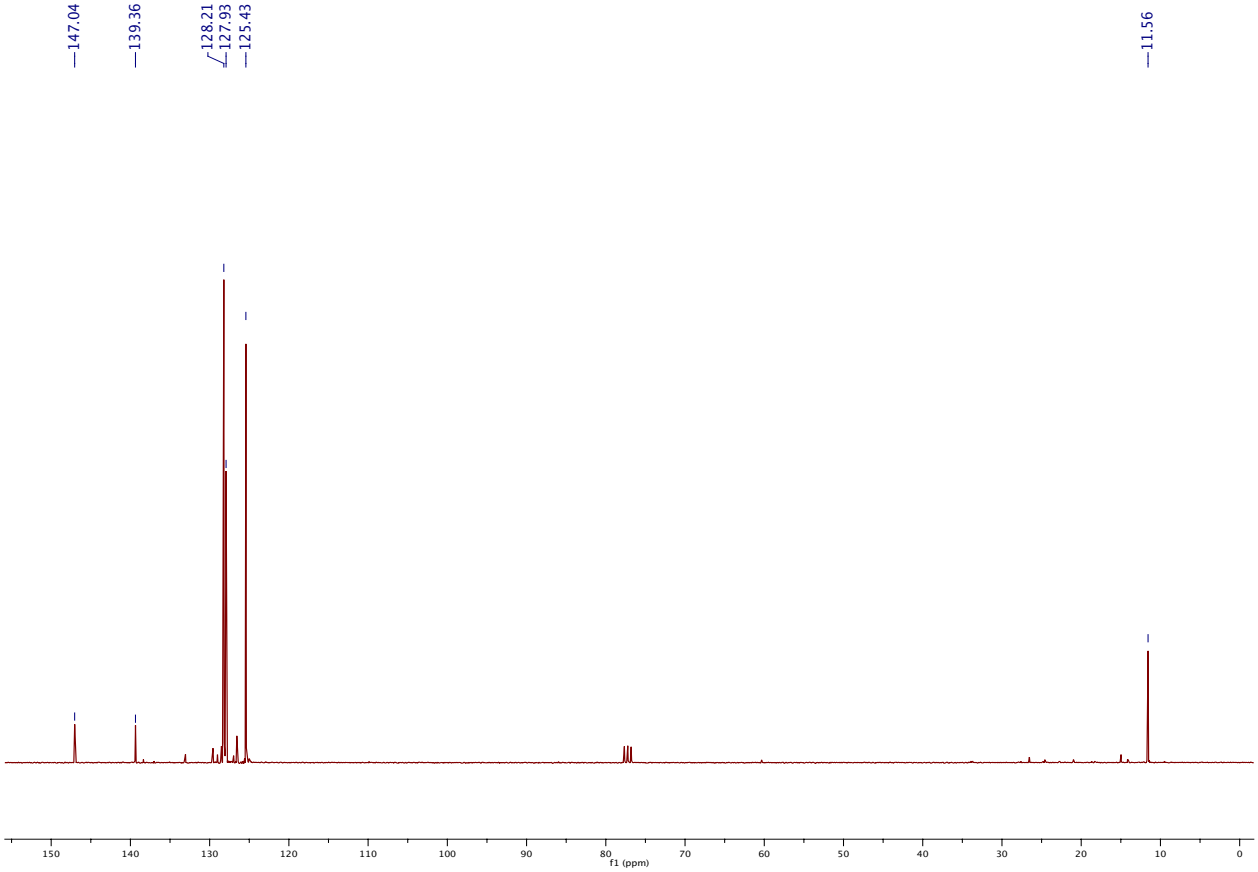

7.54
7.51
7.32
7.31
7.30
7.20
7.19
7.15
6.83
6.80
6.79
6.78
6.76
6.75
6.45
6.44
3.89
3.39
O
MeO
OMe
HN
N
1.00
2.98
1.59
1.97
2.11
0.92
2.75
2.77
f1 (ppm)

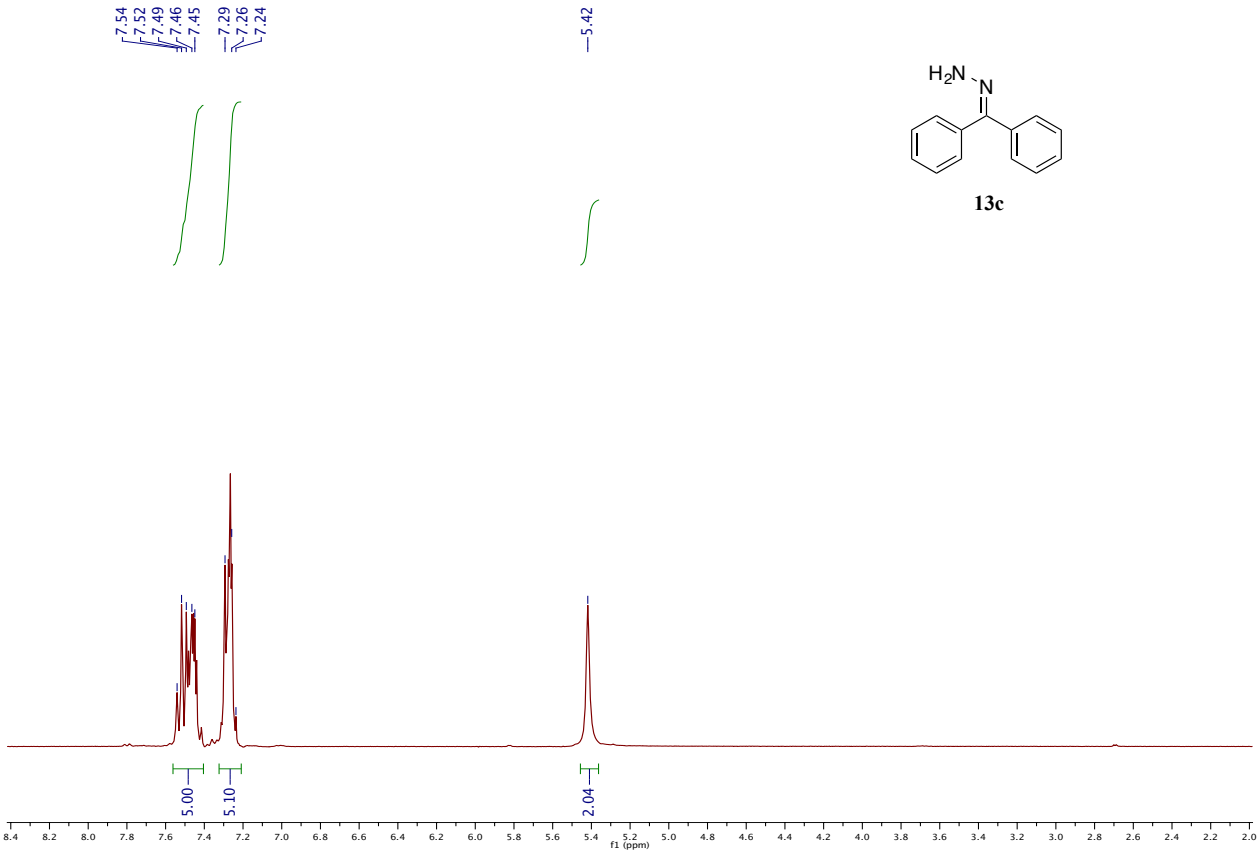
11b


162.66
162.26
152.37
147.30
146.30
144.25
142.60
134.58
133.48
132.85
131.84
130.29
129.09
128.74
128.42
120.61
119.66
118.41
117.70
116.96
114.88
114.41
55.88
55.32
f1 (ppm)

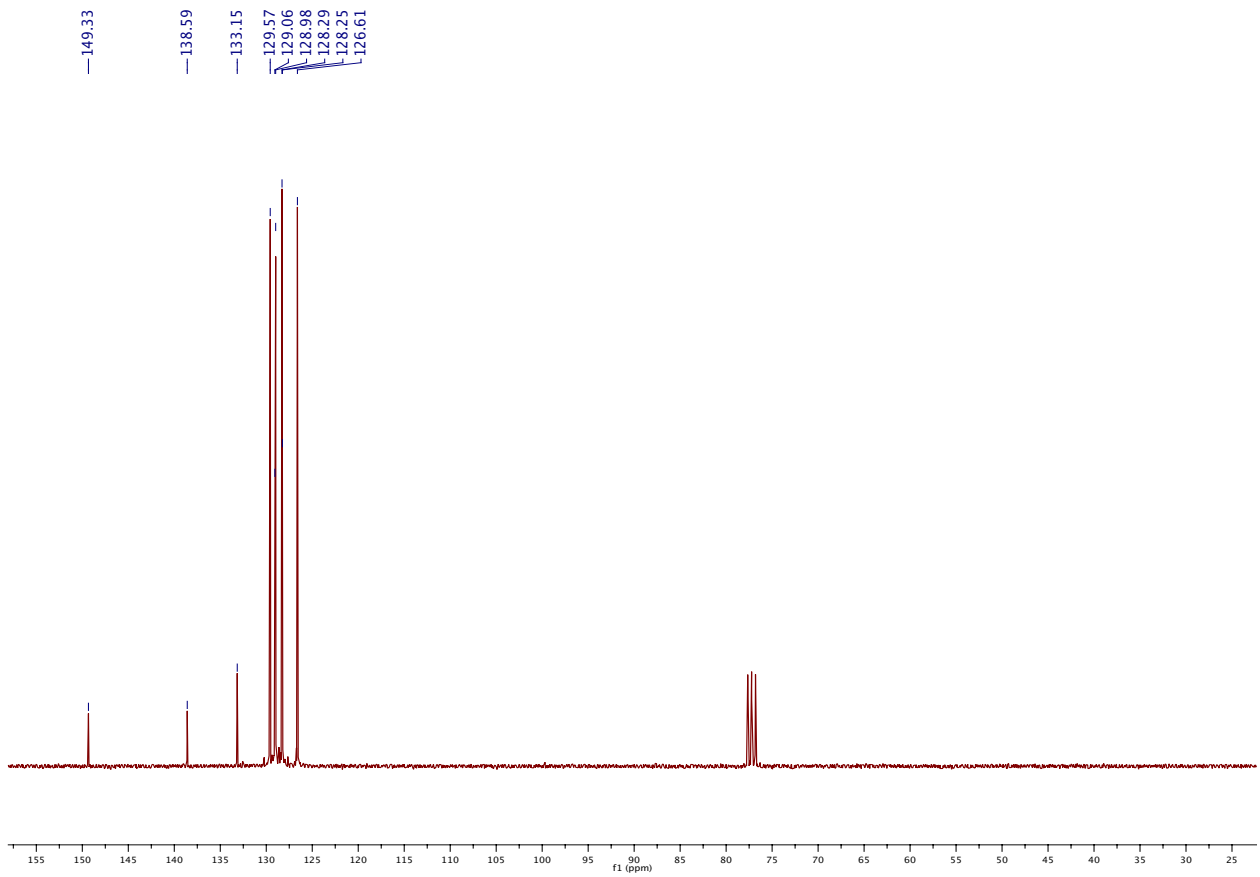

9.38
4.32
4.30
4.29
4.28
4.27
4.26
4.25
4.23
1.35
1.33
1.33
1.31
1.30
1.29
NH2
N
O
O
O
O
13a
1.78
4.01
6.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)
163.39
162.66
121.37
61.14
61.05
14.31
14.16
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

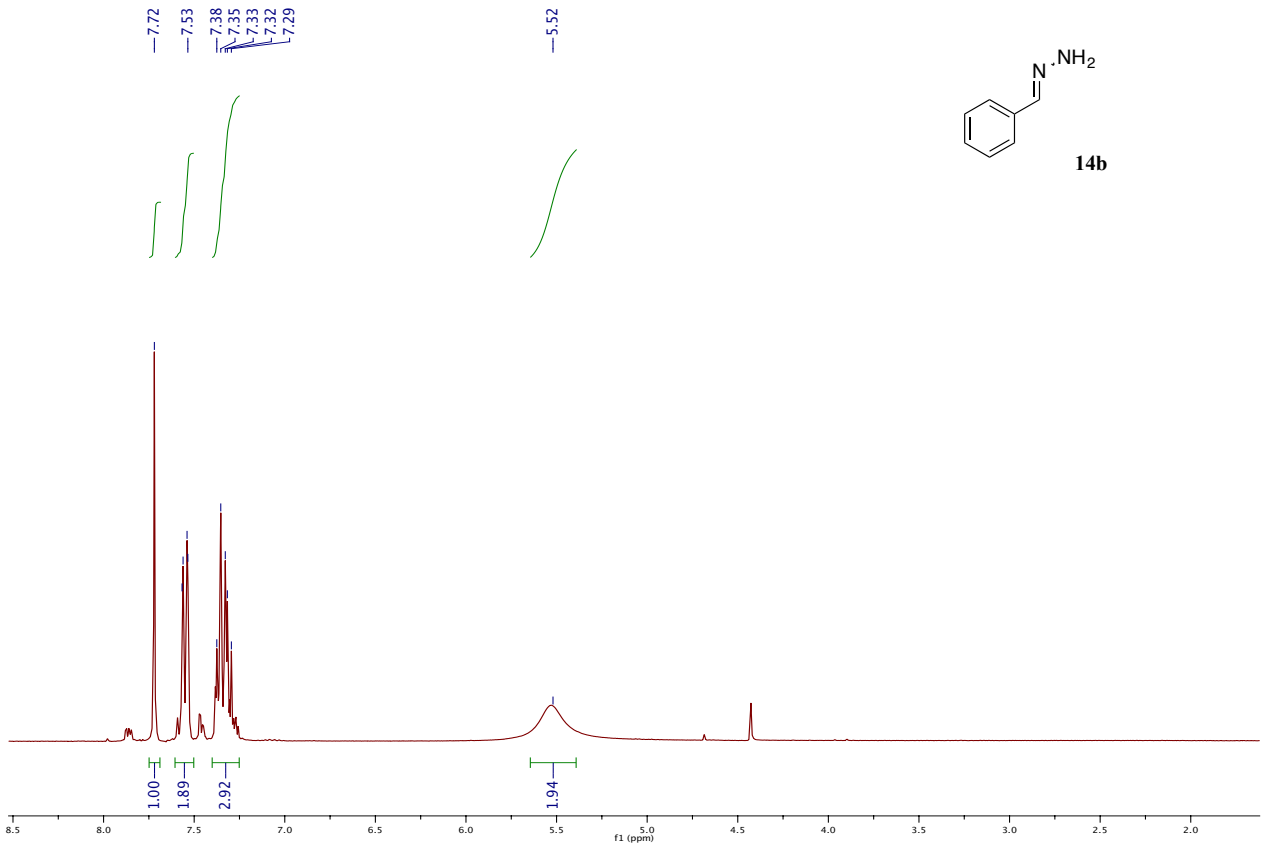

7.67
7.67
7.65
7.64
7.38
7.35
7.33
7.31
7.29
5.40
2.09
N
NH2
13b
2.00
2.98
2.01
3.33
f1 (ppm)


147.04
139.36
128.21
127.93
125.43
11.56
f1 (ppm)

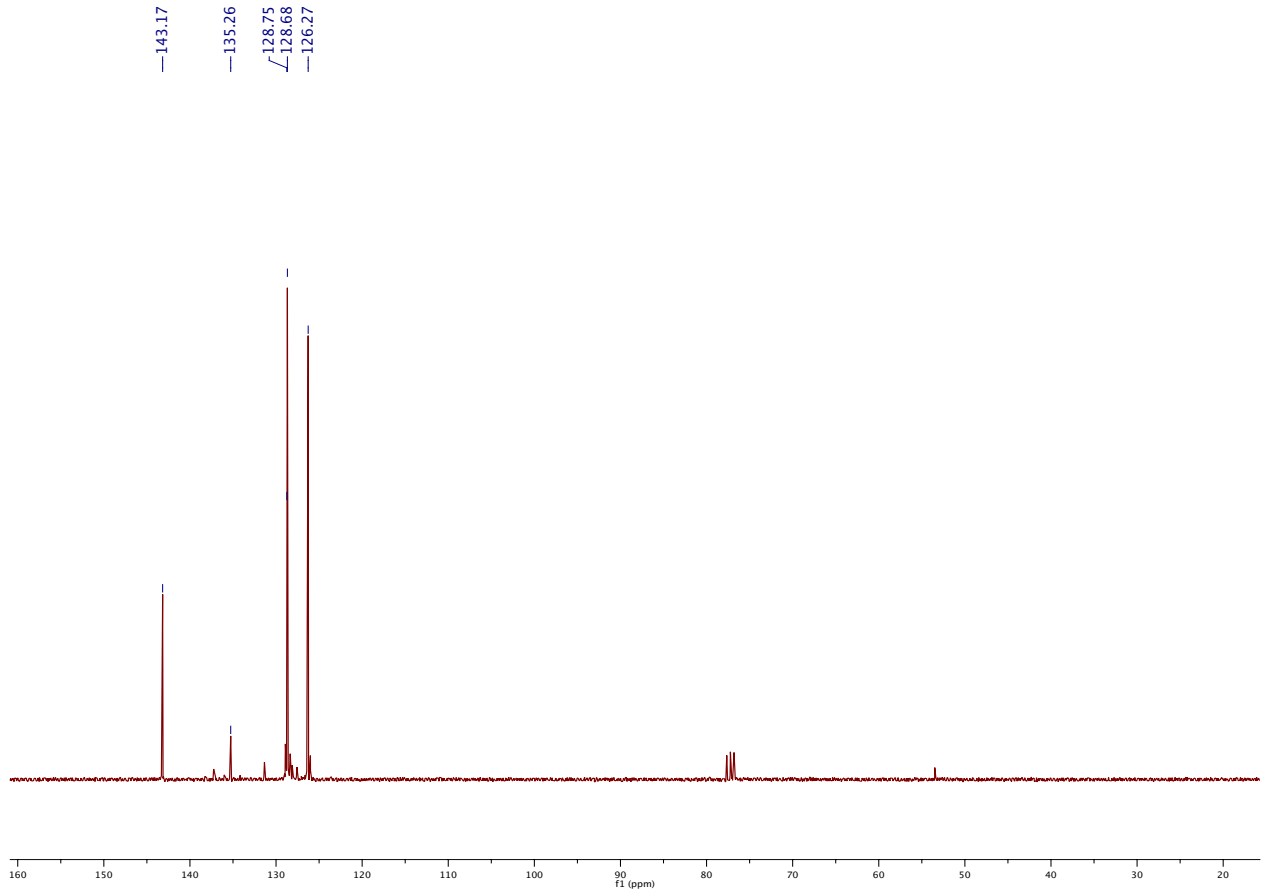

7.54
7.52
7.49
7.46
7.45
7.29
7.26
7.24
5.42
5.00
5.10
2.04
f1 (ppm)

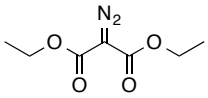
13c


149.33
138.59
133.15
129.57
129.06
128.98
128.29
128.25
126.61
f1 (ppm)


7.72
7.53
7.38
7.35
7.33
7.32
7.29
5.52
1.00
1.89
2.92
1.94
f1 (ppm)

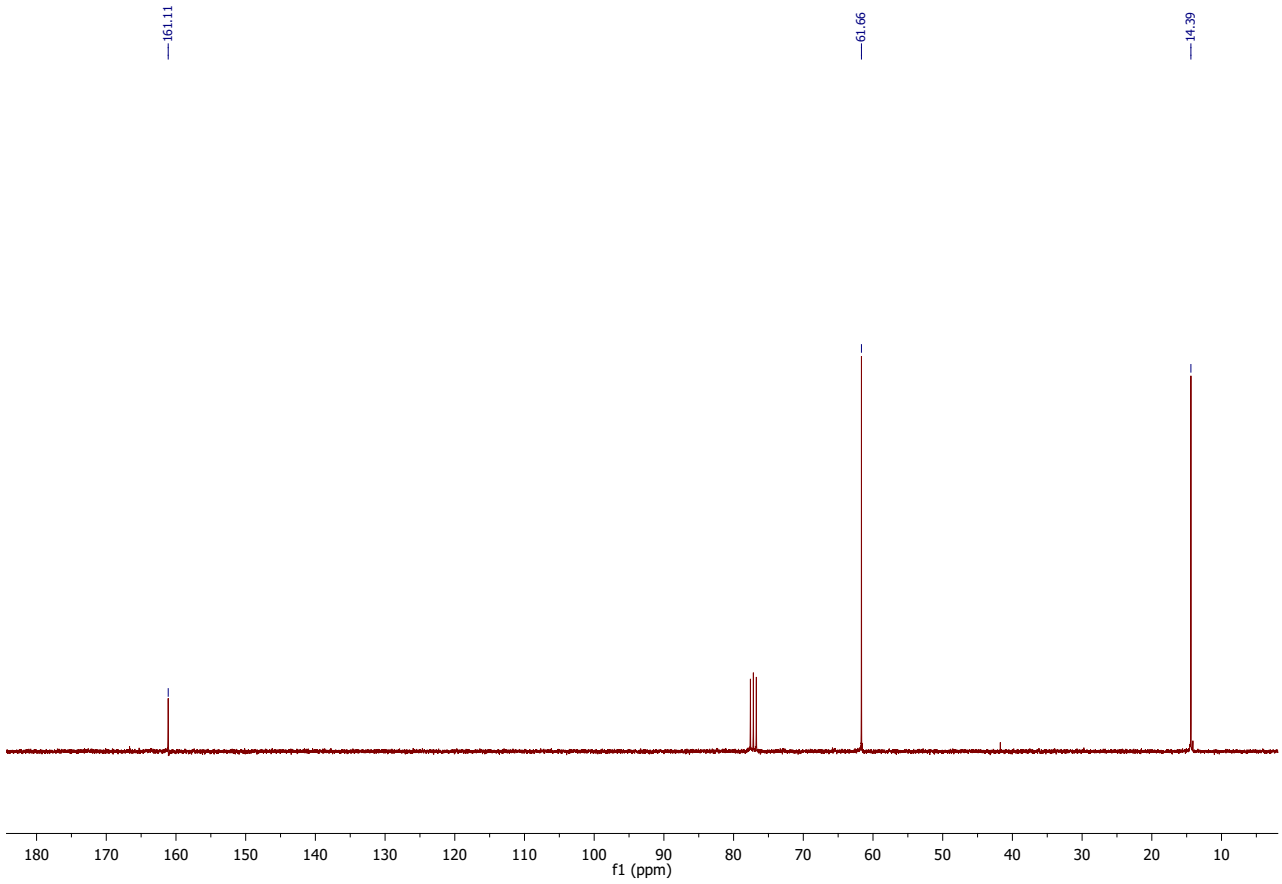
14b

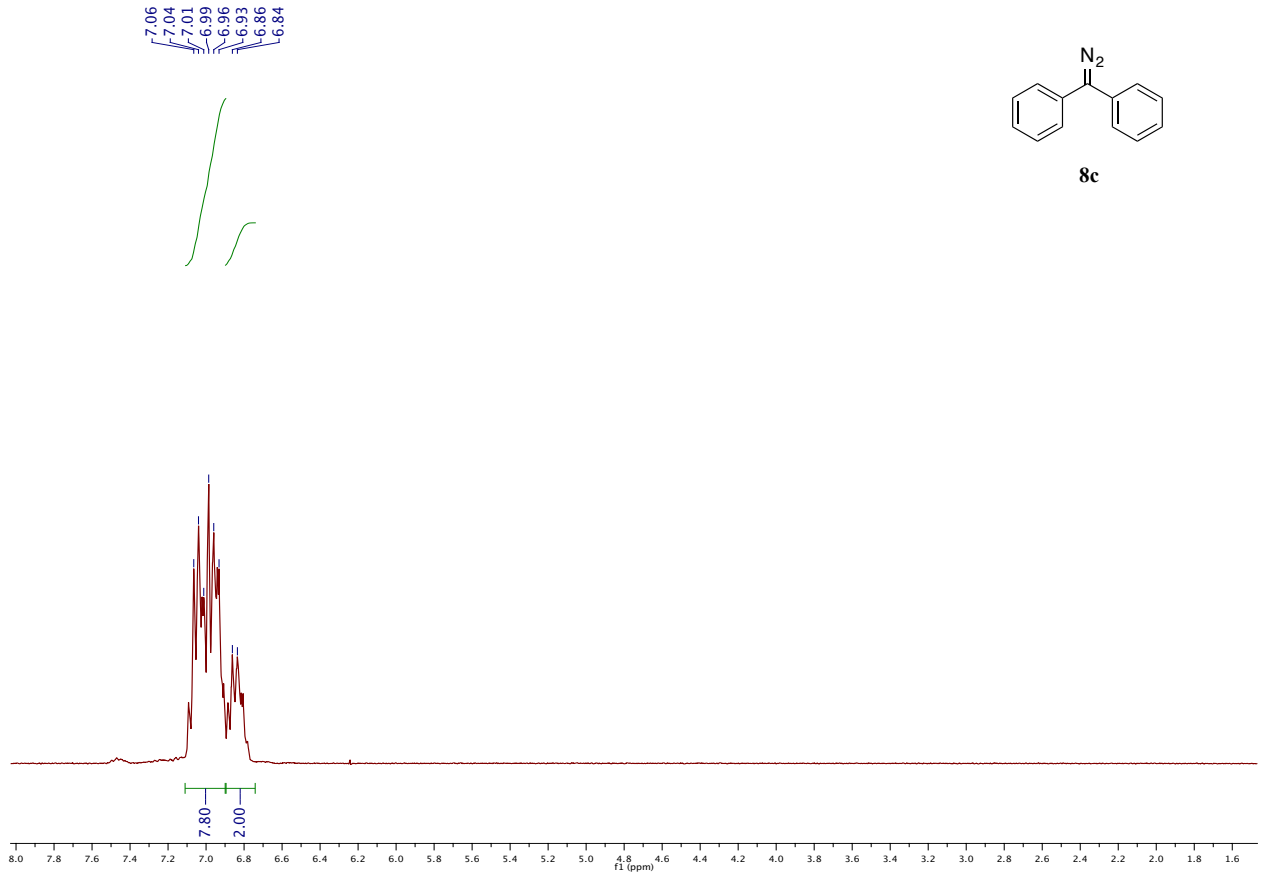

143.17
135.26
128.75
128.68
126.27
f1 (ppm)


N2
O
O
O
O

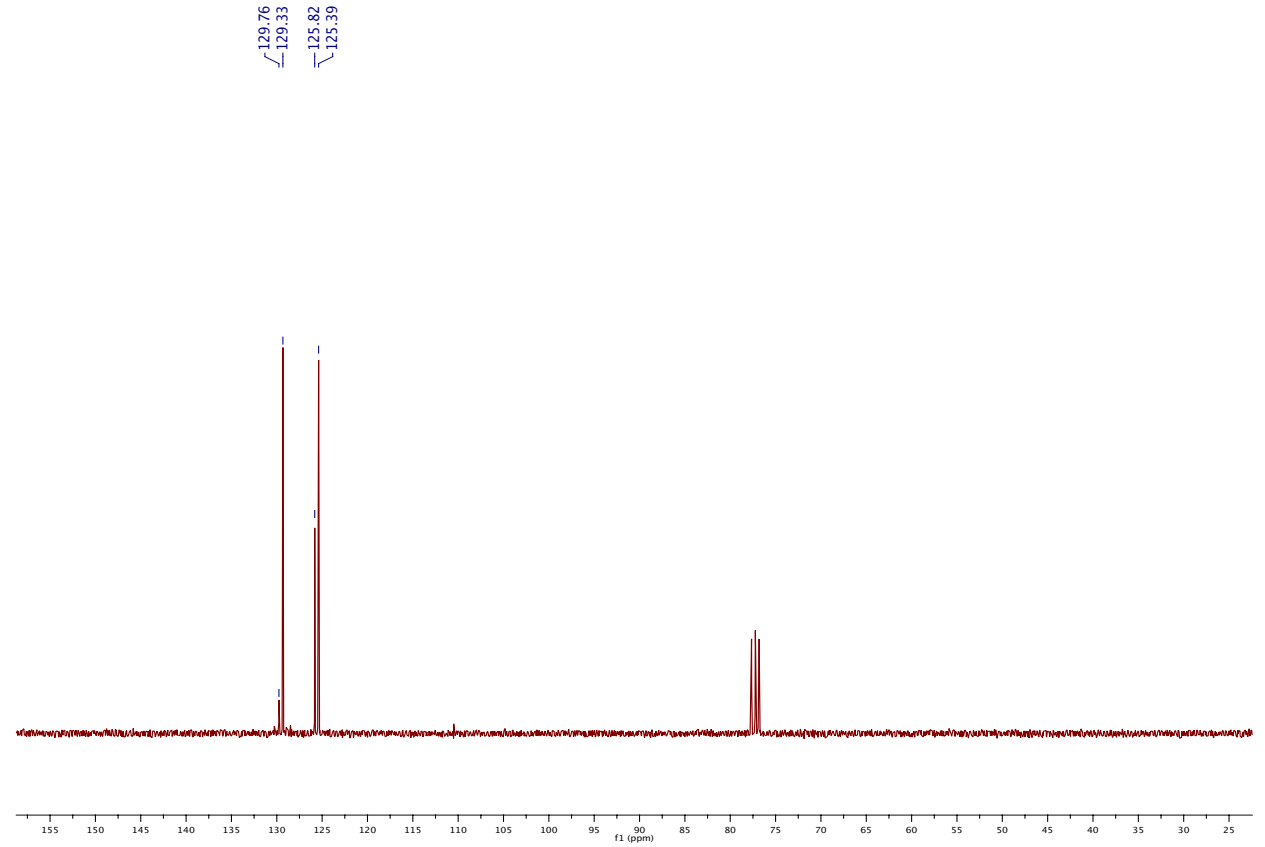
8a

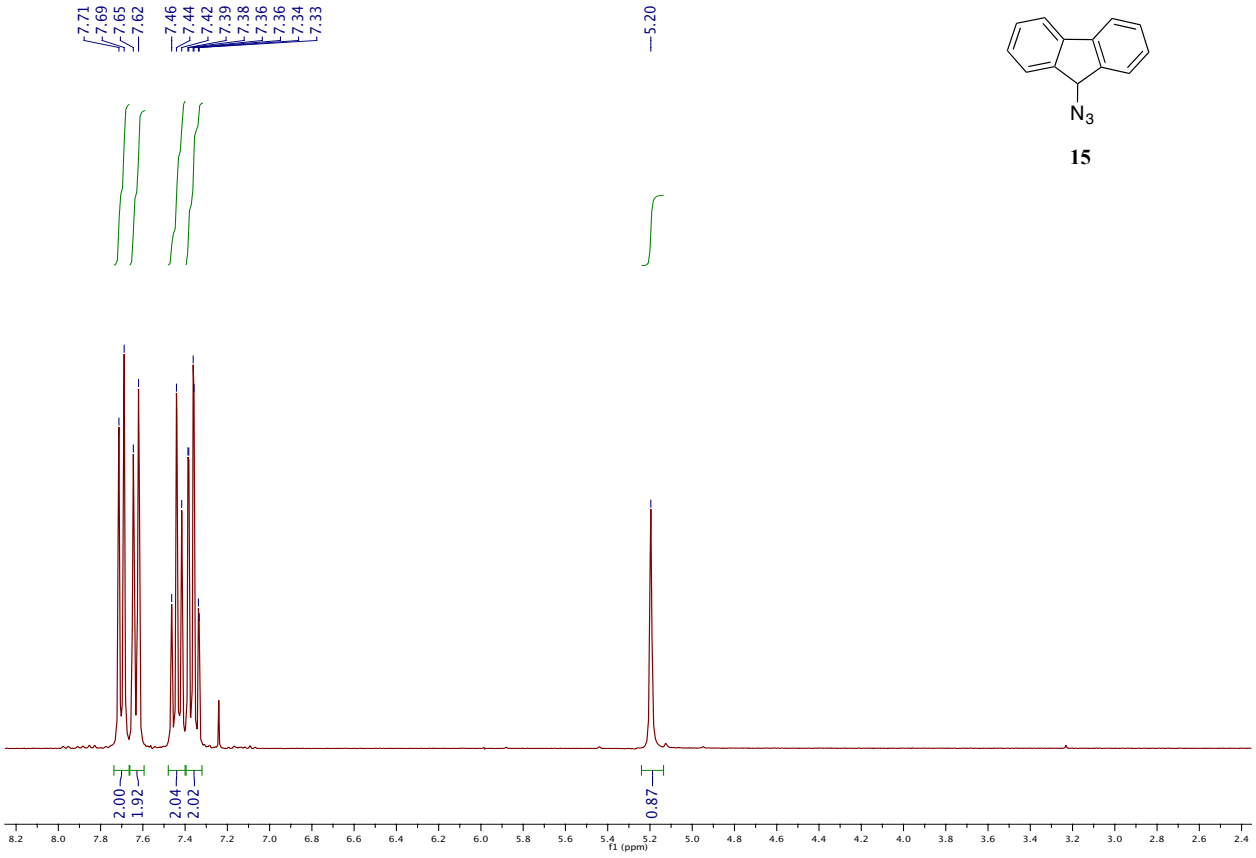
4.31
4.28
4.26
4.23
1.31
1.29
1.26
4.00
6.06
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


161.11
61.66
14.39
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

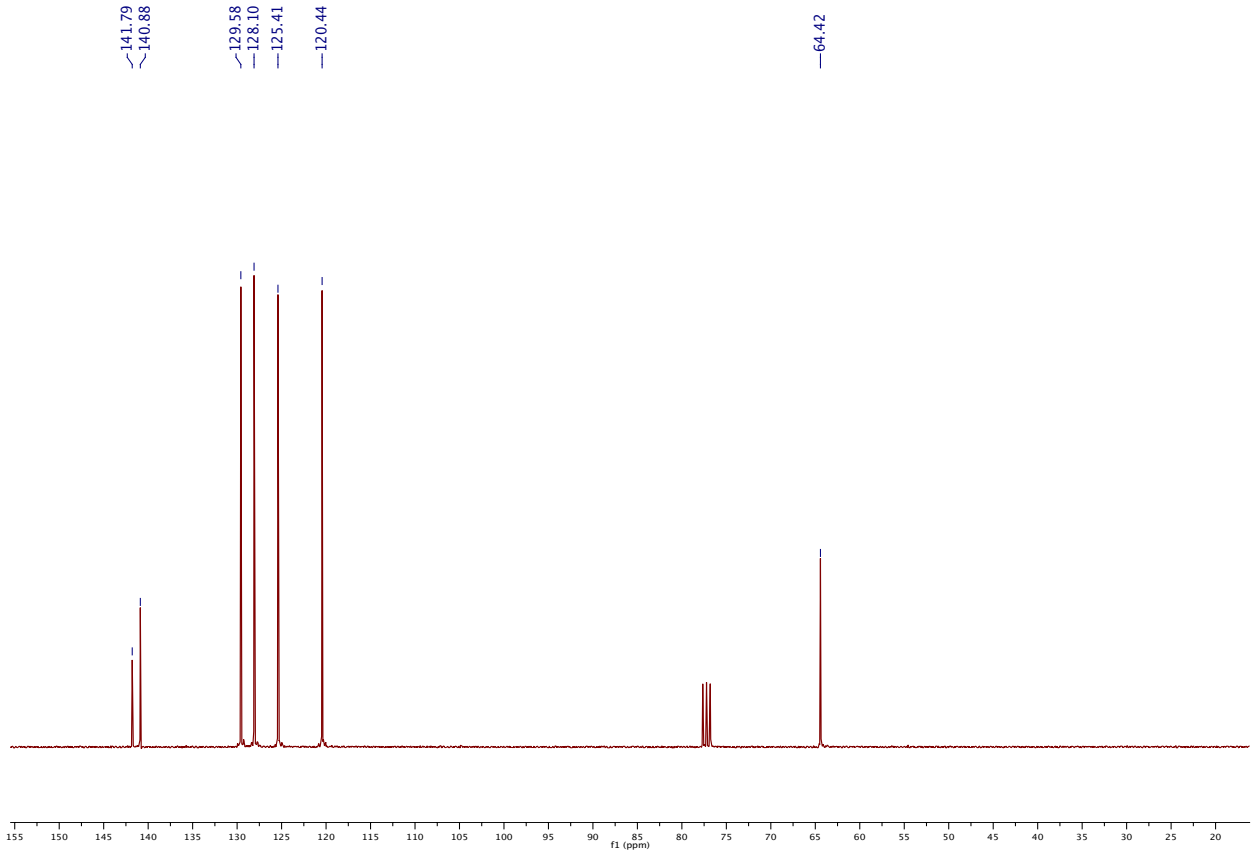

7.06
7.04
7.01
6.99
6.96
6.93
6.86
6.84
7.80
2.00
f1 (ppm)

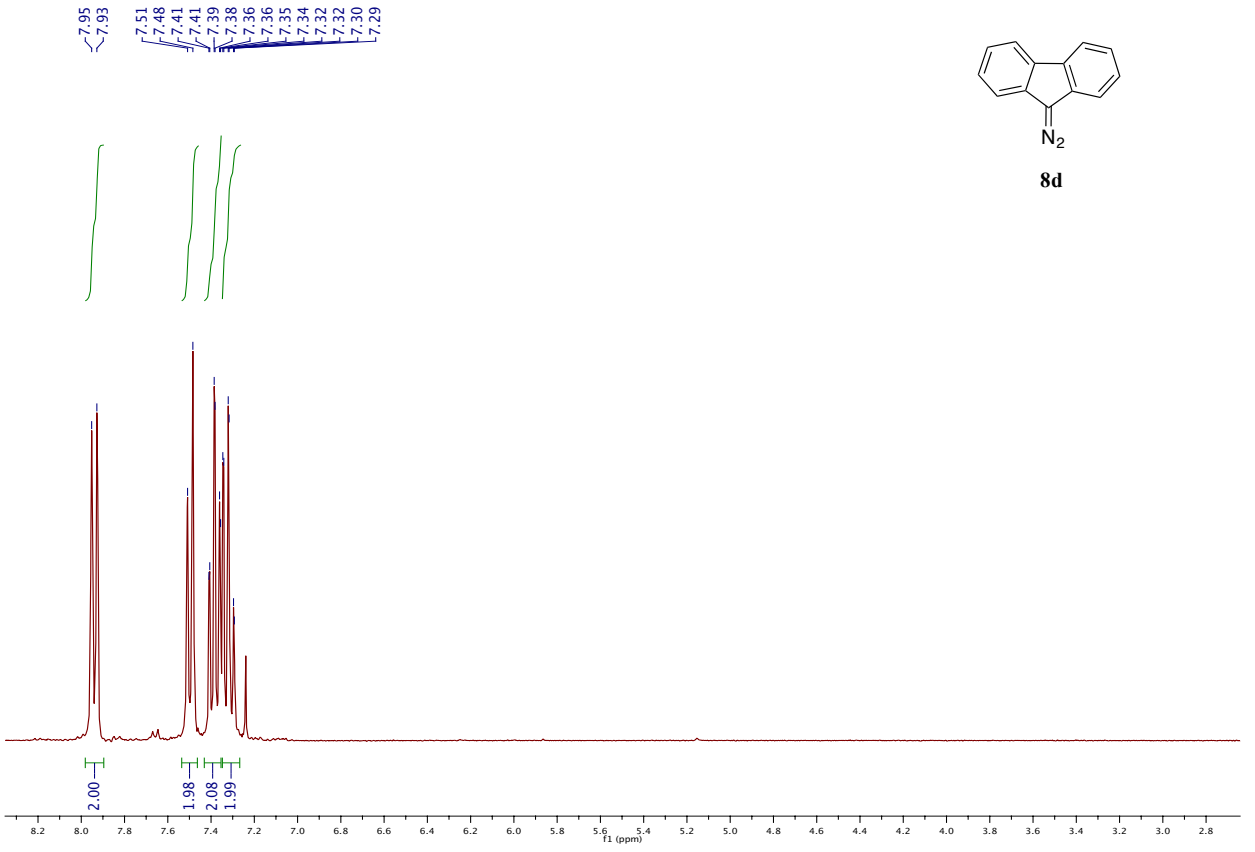
8c


129.76
129.33
125.82
125.39
f1 (ppm)

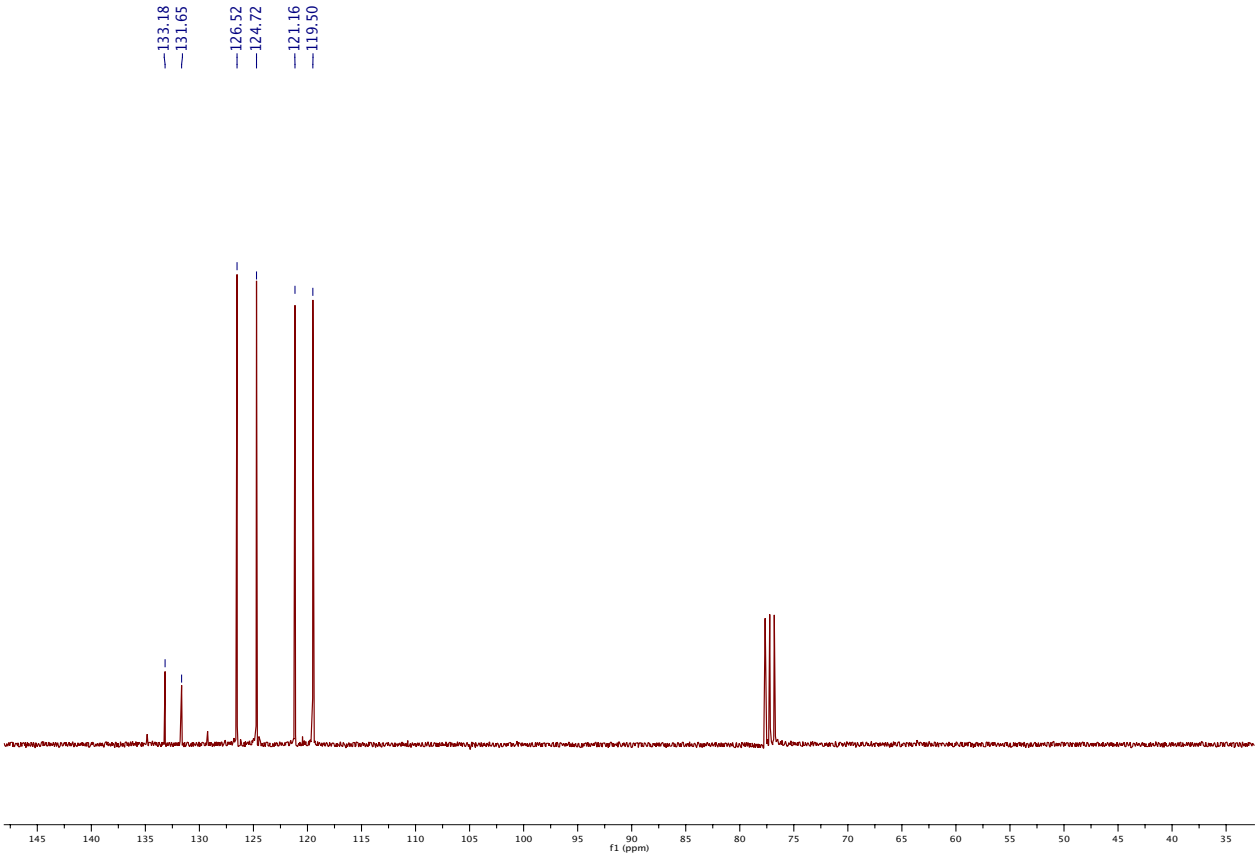

7.71
7.69
7.65
7.62
7.46
7.44
7.42
7.39
7.38
7.36
7.36
7.34
7.33
5.20
2.00
1.92
2.04
2.02
0.87
f1 (ppm)

15


141.79
140.88
129.58
128.10
125.41
120.44
64.42
f1 (ppm)


7.95
7.93
7.51
7.48
7.41
7.41
7.39
7.38
7.36
7.36
7.35
7.34
7.32
7.32
7.30
7.29
2.00
1.98
2.08
1.99
f1 (ppm)

8d


133.18
131.65
126.52
124.72
121.16
119.50
f1 (ppm)

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