Supporting information:

Red – light – sensitive BODIPY photoprotecting groups for amines and their biological application in controlling heart rhythm

Kaja Sitkowska[a,b], Martijn F. Hoes[c], Michael Lerch[a], Lucien Lameijer[a,d], Peter van der Meer[c], Wiktor Szymański[a,d],* Ben L. Feringa[a]*

a. Centre for Systems Chemistry, Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands.

b. University of Warsaw, Faculty of Chemistry, Pasteura 1, 02-093 Warsaw, Poland

c. Department of Cardiology, University of Groningen, University Medical Centre Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands

d. Department of Radiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands

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1. General information

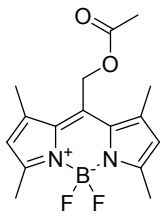
Starting materials, reagents and solvents were purchased from Sigma–Aldrich, Acros and Combi-Blocks and were used without additional purification. Solvents for the reactions were purified by passage through solvent purification columns (MBraun SPS-800). 4-Nitrophenol chloroformate was obtained from Combi-Blocks. Unless stated otherwise, all reactions were carried using standard Schlenk techniques and were run under nitrogen atmosphere in the dark. The reaction progress was monitored by TLC. Thin Layer Chromatography analyses were performed on commercial Kieselgel 60, F254 silica gel plates with fluorescence-indicator UV254 (Merck, TLC silica gel 60 F254). For detection

of components, UV light at λ = 254 nm or λ = 365 nm was used. Column chromatography was performed on commercial Kieselgel 60, 0.04-0.063 mm, Macherey-Nagel.

UPLC traces were measured on Thermo Fisher Scientific LC/MS: UPLC model Vanquish, MS model LTQ with an iontrap and HESI (Heated ESI) ionisation source with positive and negative mode. UV-Vis absorption spectra were recorded on an Agilent 8453 UV/Vis absorption Spectrophotometer. Irradiation with red light was performed using Sahlmann Photochemical Solutions LEDs, LED system (3 x 400 mW, λ_{max} = 652 nm, FWHM 26.4 nm). Obtained UV/vis spectra were baseline corrected. Nuclear Magnetic Resonance spectra were measured with an Agilent Technologies 400-MR (400/54 Premium Shielded) spectrometer (400 MHz). All spectra were measured at room temperature (25°C). Chemical shifts for the specific NMR spectra were reported relative to the residual solvent peak [in ppm; $CDCl_3$: δH = 7.26; $CDCl_3$: δC = 77.16; *d*6-DMSO: δH = 2.50; *d*6-DMSO: δC = 39.52. The multiplicities of the signals are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). All ^{13}C-NMR spectra are ^{1}H-broadband decoupled. High-resolution mass spectrometric measurements were performed using a Thermo scientific LTQ OrbitrapXL spectrometer with ESI ionization. The molecule-ion M^+, $[M + H]^+$ and $[M–X]^+$, respectively, are given in m/z-units. Melting points were recorded using a Stuart analogue capillary melting point SMP11 apparatus.

2. Experimental procedures

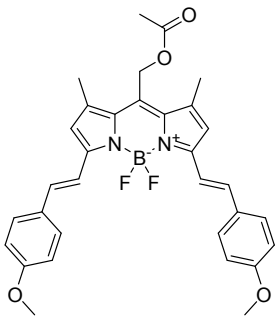
(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazabo-rinin-10-yl)methyl acetate (1)



Compound prepared according to a previously described procedure (47% yield).[1]

Rf. = 0.7 (DCM), M.p. = 184-187°C, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 2.13 (s, 3H, CO**CH$_3$**), 2.36 (s, 6H), 2.53 (s, 6H, 2 x Ar**CH$_3$**), 5.30 (s, 2H, Ar**CH$_2$**CO), 6.08 (s, 2H, 2 x Ar**H**), ^{19}F NMR (376 MHz, Chloroform-*d*) δ -146.43 (dd, *J* = 65.1, 32.5 Hz). HRMS (ESI+) calc. for $[M+H]^+$ ($C_{16}H_{20}BF_2N_2O_2$): 321.1580, found: 321.1585. ^{1}H spectrum in agreement with published data.[1]

(5,5-difluoro-3,7-bis((*E*)-4-methoxystyryl)-1,9-dimethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)methyl acetate (2)



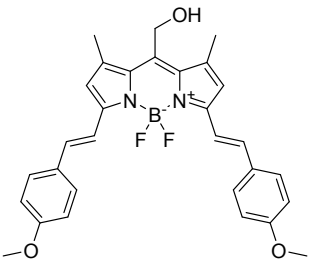
Method a: A solution of compound **1** (0.10 g, 0.31 mmol), *p*-methoxybenzaldehyde (0.20 mL, 1.7 mmol, 5.3 equiv.), piperidine (0.56 mL) and acetic acid (0.56 mL) in dry benzene (50 mL) was heated under reflux with a Dean-Stark apparatus, under nitrogen, till the substrate was consumed (~6 h). The solvent was evaporated; the residue was dissolved in DCM, washed with brine (3x30 mL) and dried with $MgSO_4$. The crude mixture was purified by column chromatography using pentane/EtOAc (4/1->1/1; v/v). Compound **2** was obtained as dark blue solid (75 mg, 43% yield).

Method b: A solution of compound **1** (0.50 g, 1.6 mmol), *p*-methoxybenzaldehyde (2.6 mL, 21 mmol, 14 equiv.), piperidine (1 drop) was heated at 60 °C under vacuum, until the substrate was consumed

(~3 h). The crude mixture was purified by column chromatography using pentane/DCM (4/1->0/1; v/v) as the eluent. Compound **2** was obtained as dark green-purple solid (700 mg, 80% yield).

Rf. = 0.4 (pentane/DCM 1/1; v/v), M.p. = 238-240°C, ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 2.15 (s, 3H), 2.40 (s, 6H), 3.85 (s, 6H), 5.32 (s, 2H), 6.71 (s, 2H), 6.93 (d, *J* = 8.8 Hz, 4H), 7.23 (d, *J* = 16.3 Hz, 2H), 7.53 – 7.62 (m, 6H), ^{19}F NMR (376 MHz, Chloroform-*d*) δ -138.43 (dd, *J* = 66.7, 32.4 Hz). HRMS (ESI+) calc. for $[M+H]^+$ ($C_{32}H_{32}BF_2N_2O_4$) 556.2339, found 556.2344. Spectrum in agreement with published data.[2]

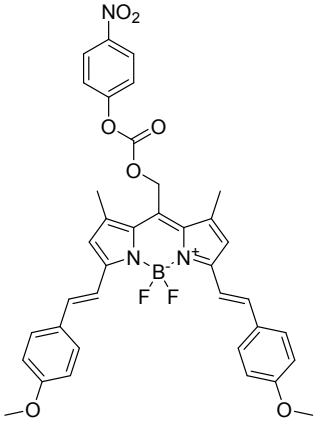
(5,5-difluoro-3,7-bis((*E*)-4-methoxystyryl)-1,9-dimethyl-5H-4*λ*^{4},5*λ*^{4}-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)methanol (3)



To a solution of compound **2** (0.20 g, 036 mmol) in THF (20 mL) and MeOH (20 mL) an aqueous solution of NaOH (0.1 M, 3.6 mL, 1 equiv.) was added and the mixture was stirred at room temperature for 2 h. Subsequently, EtOAc was added, the mixture was washed with brine (3x30 mL) and dried with Na_2SO_4. The crude mixture was purified by column chromatography using DCM and methanol (0.2% MeOH in DCM) as the eluent. The compound was obtained as dark blue solid (100 mg, 54% yield).

RF. = 0.4 (DCM), M.p. = 242-245°C , ^{1}H NMR (400 MHz, Chloroform-*d*) δ 2.56 (s, 6H), 3.86 (s, 6H), 4.93 (d, *J* = 5.5 Hz, 2H), 6.71 (s, 2H), 6.93 (d, *J* = 8.7 Hz, 4H), 7.23 (d, *J* = 16.3 Hz, 2H), 7.53 – 7.61 (m, 6H), ^{19}F NMR (376 MHz, Chloroform-*d*) δ -138.54 (dd, *J* = 67.2, 34.1 Hz). HRMS (ESI+) calc. for $[M+H]^+$ ($C_{30}H_{30}BF_2N_2O_3$) 514.2234, found 514.2232. ^{1}H NMR spectrum in agreement with published data.[2]

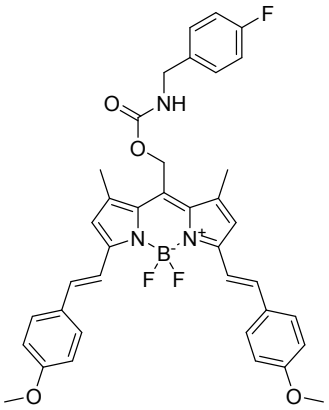
(5,5-difluoro-3,7-bis((*E*)-4-methoxystyryl)-1,9-dimethyl-5H-4*λ*^{4},5*λ*^{4}-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)methyl (4-nitrophenyl) carbonate (4)



To a solution of *p*-nitrophenyl chloroformate (78 mg, 0.39 mmol, 4 equiv.) in dry DCM (10 mL), pyridine (31 *μ*L, 0.39 mmol, 4 equiv.) was added under nitrogen atmosphere. The suspension was then added dropwise to a solution of compound **2** (50 mg, 97 *μ*mol), in dry DCM (10 mL) and DIPEA (57 *μ*L, 0.49 mmol, 5 equiv.) at 0°C, in the dark. The reaction mixture was allowed to warm up and was stirred for 4 h. After this time the crude mixture was purified by column chromatography using DCM as the eluent. Compound **4** was obtained as gold-green solid (60 mg, 91% yield).

Rf. = 0.8 (DCM), M.p. = 242-243°C, ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 2.52 (s, 6H), 3.86 (s, 6H), 5.61 (s, 2H), 6.75 (s, 2H), 6.94 (d, *J* = 7.7 Hz, 4H), 7.26 (d, *J* = 16.1 Hz, 5H), 7.41 (d, *J* = 7.8 Hz, 2H), 7.53 – 7.66 (m, 6H), 8.29 (d, *J* = 7.7 Hz, 2H)., ^{19}F NMR (376 MHz, Chloroform-*d*) δ -138.42 (dd, *J* = 66.7, 32.9 Hz). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 16.0, 55.4, 114.3, 117.0, 119.0, 121.7, 125.4, 126.6, 129.3, 134.5, 137.0, 139.7, 145.6, 152.2, 153.8, 155.3, 160.7. HRMS (ESI+) calc. for $[M+H]^+$ ($C_{37}H_{33}BF_2N_3O_7$): 680.2329, found: 680.2336.

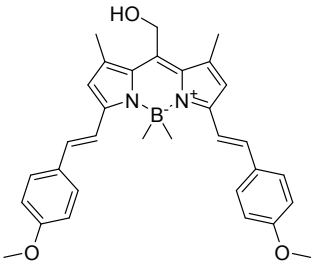
(5,5-difluoro-3,7-bis((*E*)-4-methoxystyryl)-1,9-dimethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)methyl (4-fluorobenzyl)carbamate (5)



To a solution of compound **4** (16 mg, 23 μmol, 1.4 equiv.) in dry THF (0.55 mL), a solution of pyridine in THF (0.5 M, 11 μL, 5.6 μmol, 0.33 equiv.) was added under nitrogen atmosphere. After stirring for 15 minutes at room temperature, a solution of 4-fluorobenzylamine in THF (0.5 M, 34 μL, 17 μmol) in was added. The reaction mixture was then stirred for additional 3 hours. Next, DCM (10 mL) and brine (10 mL) were added and the formed phases were separated. After washing the organic layer with 1 M aq. HCl (3 x 10 mL), 0.1 M aq. NaOH (4 x 10 mL) and brine (2 x 10 mL), it was dried with Na_2SO_4, filtered and the solvent was evaporated. The crude mixture was then purified by flash chromatography using DCM as the eluent. The product was obtained as green solid (11 mg, 97% yield).

Rf. = 0.7 (DCM), M.p. = 215-217^{o}C, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 2.40 (s, 6H), 3.84 (s, 6H), 4.34 (d, *J* = 5.7 Hz, 2H), 5.32 (s, 3H), 6.67 (s, 2H), 6.90 (d, *J* = 8.3 Hz, 4H), 7.01 (t, *J* = 8.3 Hz, 2H), 7.13 – 7.30 (m, 4H), 7.50 - 7.65 (m, 6H), ^{19}F NMR (376 MHz, Chloroform-*d*) δ -138.30 (dd, *J* = 66.5, 30.9 Hz), -114.85 (tt, *J* = 8.8, 5.4 Hz). ^{13}C NMR (101 MHz, Chloroform-*d*) *δ* 15.8, 44.5, 55.4, 58.4, 114.3, 115.6 (d, *J* = 21.6 Hz), 117.0, 118.6, 129.1, 129.2, 129.4, 133.9, 134.5, 136.5, 140.0, 153.3, 155.9, 160.5, 162.2 (d, *J* = 245.9 Hz). HRMS (ESI+) calc. for $[M+H]^+$ ($C_{38}H_{36}BF_3N_3O_4$): 665.2667, found: 665.2674.

(3,7-bis((*E*)-4-methoxystyryl)-1,5,5,9-tetramethyl-5H-*4λ^4*,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)methanol (6)

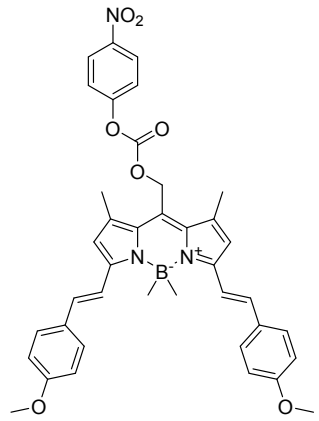


To a solution of compound **3** (0.10 g. 0.19 mmol) in dry THF (10 mL), a solution of CH_3MgBr (1 M, 1.9 mL, 10 equiv.) in THF was added under nitrogen, at room temperature. After stirring the reaction mixture for 30 min, brine (20 mL), NH_4Cl (saturated aq. solution, 5 mL) and DCM (20 mL) were added and the layers were separated. The aqueous layer was extracted with DCM (4 x 10 mL). The combined organic phases were washed with brine and dried with Na_2SO_4. The mixture was purified by column chromatography using DCM as the eluent. The product was obtained as dark blue solid (60 mg, 61%).

Rf. = 0.5 (DCM), M.p. = 213-216^{o}C, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 0.45 (s, 6H), 2.60 (s, 6H), 3.85 (s, 6H), 5.00 (s, 2H), 6.73 (s, 2H), 6.94 (d, *J* = 8.7 Hz, 4H), 7.07 (d, *J* = 16.2 Hz, 2H), 7.42 – 7.56 (m, 6H), ^{13}C NMR (101 MHz, Chloroform-*d*) δ 13.5, 15.8, 29.7, 55.4, 114.4, 117.0, 118.3, 129.4, 137.3, 139.8, 154.8, 160.8, 193.0. HRMS (ESI+) calc. for $[M+H]^+$ ($C_{32}H_{36}BN_2O_3$): 507.2813, found: 507.2805.

(3,7-bis((*E*)-4-methoxystyryl)-1,5,5,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)methyl (4-nitrophenyl) carbonate (7)

To a solution of 4-nitrophenyl chloroformate (40 mg, 0.20 mmol, 4 equiv.) in dry DCM (10 mL), pyridine (18 μL, 0.20 mmol, 4 equiv.) was added under nitrogen atmosphere. The suspension was then added dropwise to a solution of compound **6** (25 mg, 49 μmol), in dry DCM (10 mL) and DIPEA

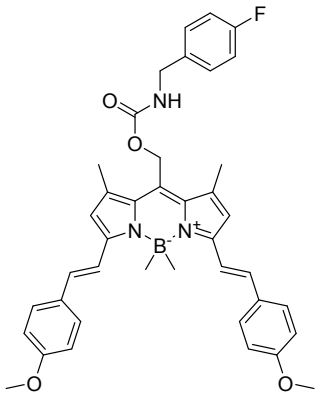


(26 μL, 0.25 mmol, 5 equiv.) at 0°C, in the dark. The reaction mixture was allowed to warm up and was stirred for 4 h. Subsequently, the crude mixture was purified by column chromatography using DCM as the eluent. Compound **7** was obtained as blue solid (9 mg, 27% yield).

Rf. = 0.9 (DCM), M.p. = 152-155°C, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 0.48 (s, 6H), 2.53 (s, 6H), 3.86 (s, 6H), 5.65 (s, 2H), 6.76 (s, 2H), 6.95 (d, *J* = 7.7 Hz, 4H), 7.10 (d, *J* = 16.2 Hz, 2H), 7.42 (d, *J* = 7.8 Hz, 2H), 7.45 – 7.55 (m, 6H), 8.30 (d, *J* = 8.9 Hz, 2H), ^{13}C NMR (101 MHz, Chloroform-*d*) δ 13.9, 16.3, 55.4, 62.8, 114.4, 115.6, 119.0, 119.2, 121.6, 125.4, 126.2, 127.9, 128.5, 129.9, 133.3, 133.3, 136.3, 145.5, 151.1, 152.3, 155.4, 160.2. HRMS (ESI+) calc. for $[M+H]^+$ ($C_{39}H_{39}BN_3O_7$): 672.2831, found: 672.2853.

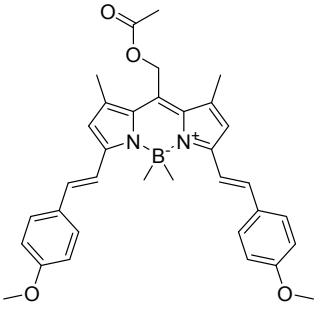
(3,7-bis((*E*)-4-methoxystyryl)-1,5,5,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)methyl (4-fluorobenzyl)carbamate (8)

To a solution of compound **7** (9 mg, 13 μmol, 1.2 equiv.) in dry THF (0.5 mL), a solution of pyridine in THF (0.5 M, 7.3 µL, 3.6 µmol, 0.33 equiv.) was added under nitrogen atmosphere. After stirring for 15 minutes at room temperature, a solution of 4-fluorobenzylamine in THF (0.5 M, 22 μL, 11 μmol) was added. The reaction mixture was then stirred for additional 3 hours. Next, DCM (10 mL) and brine (10 mL) were added and the formed phases were separated. After washing the organic layer with 1 M aq. HCl (3 x 10 mL), 0.1 M aq. NaOH (4 x 10 mL) and brine (2 x 10 mL), it was dried with Na_2SO_4, filtered and the solvent was evaporated. The crude mixture was then purified by flash chromatography using DCM as the eluent. The product was obtained as green solid (4 mg, 55% yield).



Rf. = 0.8 (DCM), M.p. = 112-116°C ^{1}H NMR (400 MHz, Chloroform-*d*) δ 0.45 (s, 6H), 2.47 (s, 6H), 3.85 (s, 6H), 4.39 (d, *J* = 5.1 Hz, 2H), 5.14 (s, 1H), 5.43 (s, 2H), 6.72 (s, 2H), 6.94 (d, *J* = 8.3 Hz, 4H), 7.00 – 7.12 (m, 4H), 7.24 – 7.28 (m, 2H), 7.40 – 7.56 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) *δ* 13.8, 15.8, 44.5, 55.4, 58.4, 104.8, 114.3, 115.6 (d, *J* = 21.4 Hz), 117.0, 118.6, 129.1 (d, *J* = 7.2 Hz), 129.4, 134.5, 134.5, 136.5, 134.0, 153.3, 155.6, 161.2 (d, *J* = 244.9 Hz). HRMS (ESI+) calc. for $[M]^+$ ($C_{40}H_{41}BFN_3O_4$): 642.2934, found: 642.2936.

(3,7-bis((*E*)-4-methoxystyryl)-1,5,5,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)methyl acetate (9)

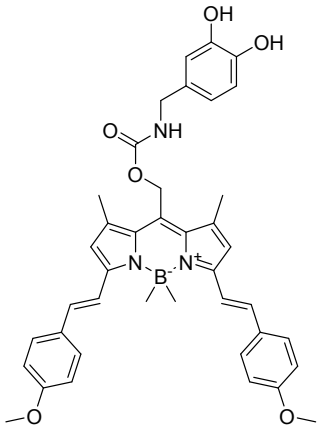


To a solution of acetic acid (2 μL, 33 μmol, 1.7 equiv.) in dry THF (2.5 mL), EDCI (excess) and DMAP (excess) were added under nitrogen. After 5 min of stirring, a solution of compound **6** (10 mg, 20 μmol) in dry THF (2.5 mL) was added. The resulting reaction mixture was stirred at room temperature until no starting material was visible on TLC (about 3 h). Next, brine (5 mL) was added and the formed layers were separated. The organic layer was then washed with aq. $NaHCO_3$ (sat.) (2 x 10 mL) and brine (10 mL) and dried with Na_2SO_4. After the evaporation of the

solvent, the crude mixture was purified by column chromatography using DCM as the eluent. The product was obtained as deep blue crystals (7.6 mg, 59% yield).

Rf. = 0.9 (DCM), M.p. = 161-164°C ^{1}H NMR (400 MHz, Chloroform-*d*) δ 0.47 (s, 6H), 2.17 (s, 3H), 2.43 (s, 6H), 3.86 (s, 6H), 5.39 (s, 2H), 6.73 (s, 2H), 6.94 (d, *J* = 7.6 Hz, 4H), 7.08 (d, *J* = 16.2 Hz, 2H), 7.40 – 7.60 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 16.1, 20.8, 55.4, 58.9, 114.4, 118.8, 119.1, 128.4, 130.0, 130.4, 132.8, 133.3, 136.5, 150.7, 160.1, 170.8. HRMS (ESI+) calc. for $[M+H]^+$ ($C_{34}H_{38}BN_2O_4$): 534.2684, found: 543.2664.

(3,7-bis((*E*)-4-methoxystyryl)-1,5,5,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)methyl (3,4-dihydroxybenzyl)carbamate (10)



To a solution of compound **7** (9.7 mg, 22 μmol) in dry THF (1 mL), a solution of dopamine hydrochloride (8.1 mg, 43 μmol, 2 equiv.) and DIPEA (7.7 μL, 44 μmol, 2 equiv.) in dry DMF (0.5 mL) was added under nitrogen atmosphere. The reaction mixture was stirred at rt till full conversion, as determined by TLC. Afterwards, EtOAc (5 mL) was added and the mixture was washed with brine (10 mL), 1M HCl (aq.) (2 x 10 mL), $NaHCO_{3sat.}$ (2 x 10 mL) and again brine (2 x 10 mL). Then, after it was dried over $MgSO_4$ and the solvents were evaporated, the crude mixture was purified by flash column chromatography using a mixture of DCM and methanol (100% DCM -> 5% methanol in DCM) as the eluent. The product was obtained as blue solid (4.4 mg, 31% yield).

Rf. = 0.3 (5% MeOH in DCM), ^{1}H NMR (400 MHz, acetone-6*d*) *δ* 0.46 (s, 6H), 2.46 (s, 6H), 2.70 (t, *J* = 7.2 Hz, 2H), 3.33 – 3.39 (m, 2H), 3.86 (s, 6H), 5.34 (s, 2H), 6.56 (dd, *J* = 7.9, 1.8 Hz, 1H), 6.73 (dd, *J* = 4.7, 3.3 Hz, 2H), 6.91 (s, 2H), 7.03 (d, *J* = 8.7 Hz, 4H), 7.32 (d, *J* = 16.3 Hz, 2H), 7.50 (d, *J* = 16.3 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 4H). ^{13}C NMR (101 MHz, acetone-6*d*) *δ* 13.4, 15.2, 35.3, 42.5, 42.6, 54.8, 58.0, 114.5, 114.6, 115.1, 115.7, 118.5, 118.8, 120.0, 128.4, 128.6, 129.9, 130.9, 132.2, 133.2, 133.2, 137.3, 143.4, 144.9, 150.5, 160.5. HRMS (ESI-) calc. for $[M+H]^-$ ($C_{41}H_{43}BN_3O_6$) 684.3239, found: 684.3260.

3. Studies on the photochemical properties of compounds **2, 5, 8, 9** and **10**

3.1. Photodeprotection studies

Samples of compounds **2**, **5**, **8**, **9** and **10,** were irradiated with light of λ = 650 nm for indicated amount of time. All the measurements were conducted in a dark room at 23°C on the same day and repeated in the next days for comparisons sake. The samples were irradiated with light of λ = 650 nm for the indicated amounts of time, in 0.2 cm distance from the light source and moved to the spectrometer immediately after each irradiation interval. To ensure the reproducibility of the results, for the irradiation experiments we build a custom system, where the sample was immobilized in a 3d-printed holder and all of the components of the system were fixated with tape.

Half – lives of the compounds were calculated by monoexponential fitting.

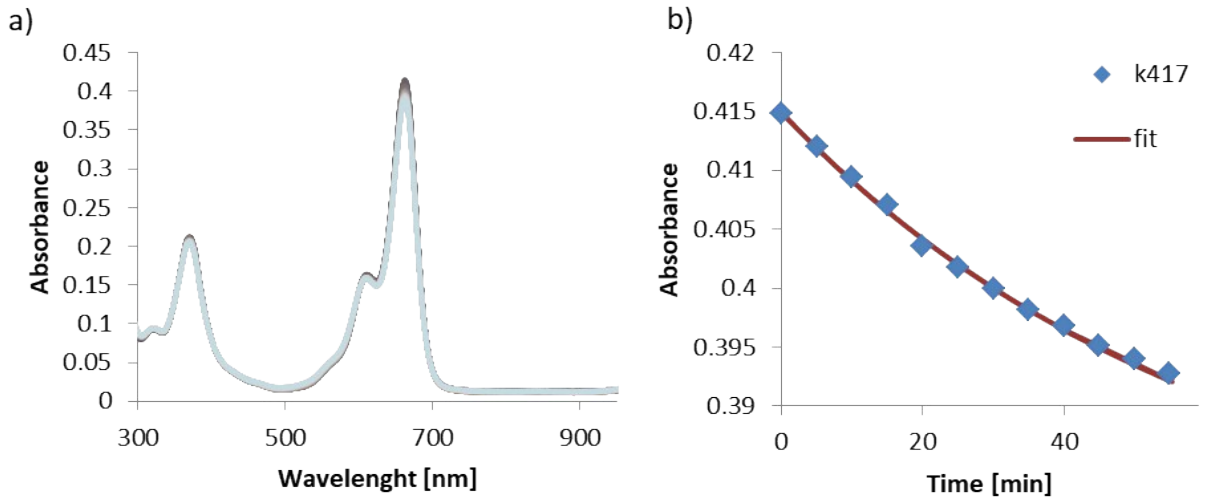


Figure 1. UV-VIS spectra of photodeprotection of compound **2** (10 μmol in 50% Acetonitrile / 5 mmol phosphate buffer pH = 7.5) using λ = 650 nm irradiation: a) spectra (measured every 1 min), b) absorbance at 660 nm with monoexponential fitting (for every 5 spectra for clarity).

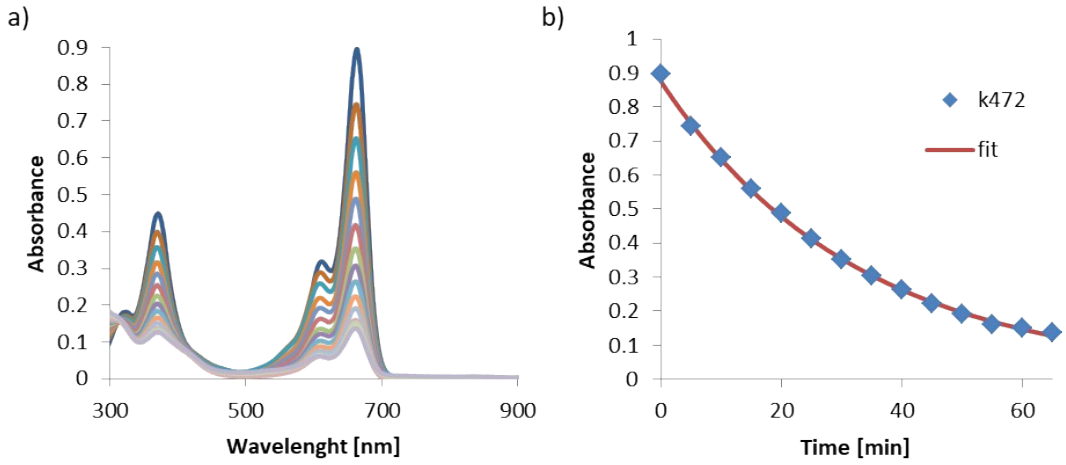


Figure 2. UV-VIS spectra of photodeprotection of compound **5** (10 μmol in 50% Acetonitrile / 5 mmol phosphate buffer pH = 7.5) using λ = 650 nm irradiation: a) spectra (measured every 5 min), b) absorbance at 660 nm with monoexponential fitting.

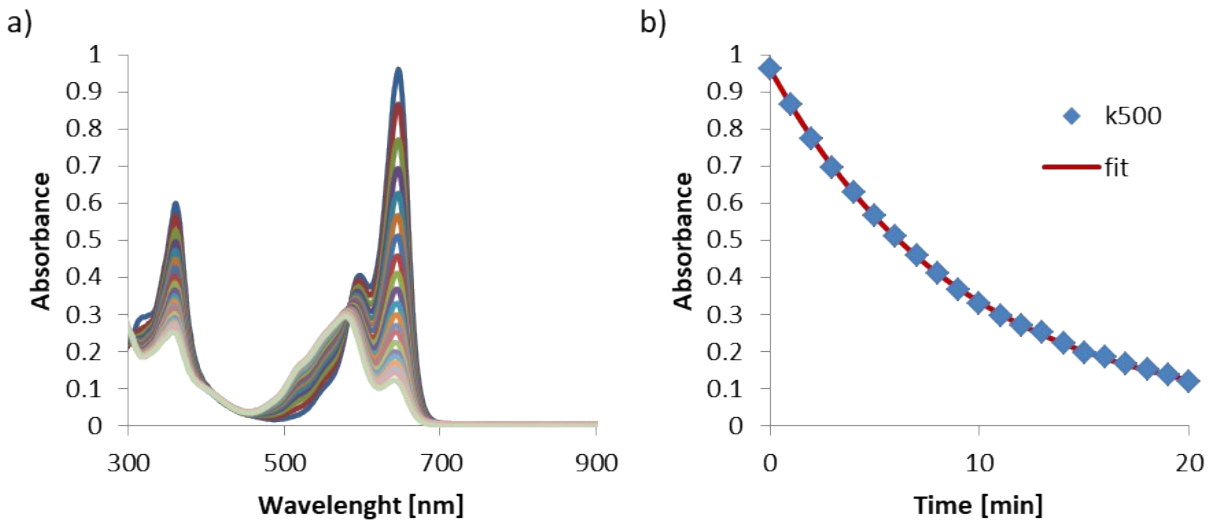


Figure 3. UV-VIS spectra of photodeprotection of compound **8** (10 μmol in 50% Acetonitrile / 5 mmol phosphate buffer pH = 7.5) using λ = 650 nm irradiation: a) spectra (measured every min), b) absorbance at 646 nm with monoexponential fitting.

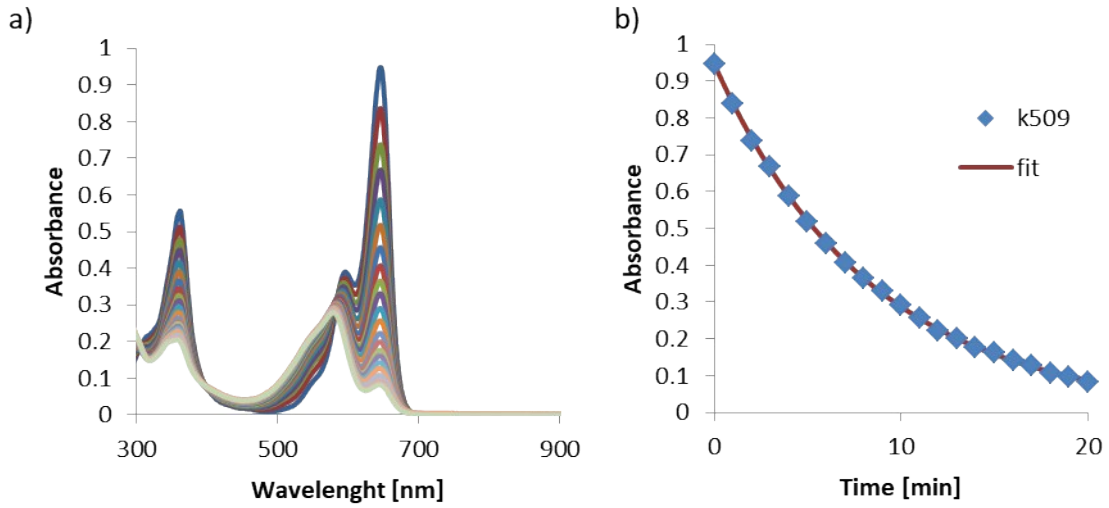


Figure 4. UV-VIS spectra of photodeprotection of compound **9** (10 μmol in 50% Acetonitrile / 5 mmol phosphate buffer pH = 7.5) using λ = 650 nm irradiation: a) spectra (measured every min), b) absorbance at 646 nm with monoexponential fitting.

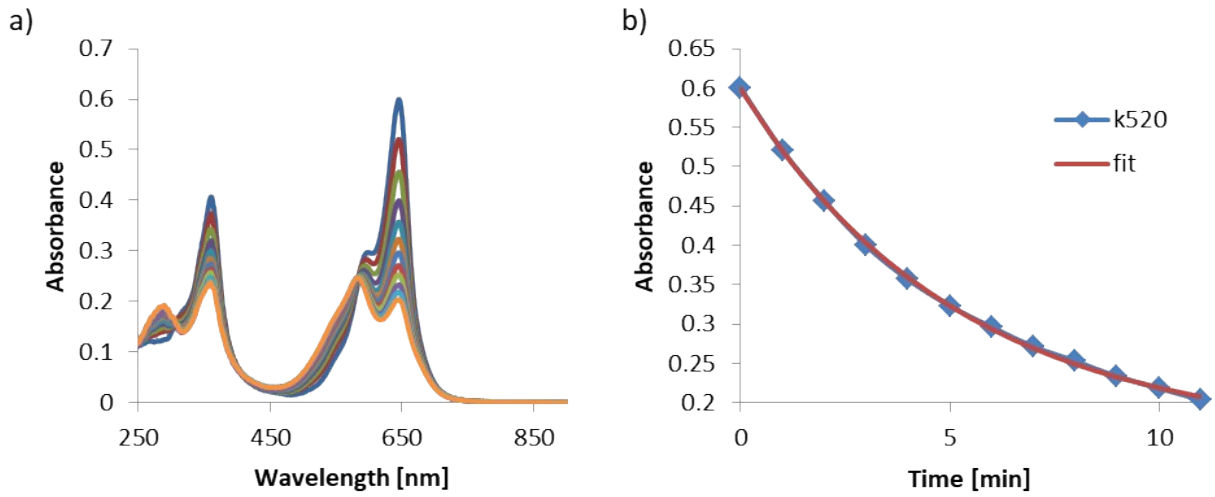


Figure 5. UV-VIS spectra of photodeprotection of compound **10** (10 μmol in 50% Acetonitrile / 5 mmol phosphate buffer pH = 7.5) using λ = 650 nm irradiation: a) spectra (measured every min), b) absorbance at 646 nm with monoexponential fitting.

3.2. Extinction coefficient measurements

For determining the extinction coefficient, 10, 5, 2.5, 1.25 and 0.625 μM 50% Acetonitrile in 5 mM phosphate buffer pH = 7.5 samples of compounds **2**, **5**, **8, 9** and **10** in were prepared and UV-VIS spectra were taken. All the measurements were conducted in a dark room at 23°C on the same day and repeated in the next days for comparisons sake. The samples were irradiated with light of λ = 650 nm for the indicated amounts of time, in 0.2 cm distance from the light source and moved to the spectrometer immediately after each irradiation interval. To ensure the reproducibility of the results, for the irradiation experiments we build a custom system, where the sample was immobilized in a 3d-printed holder and all of the components of the system were fixated with tape.

The coefficients were obtained using Lambert-Beer law.

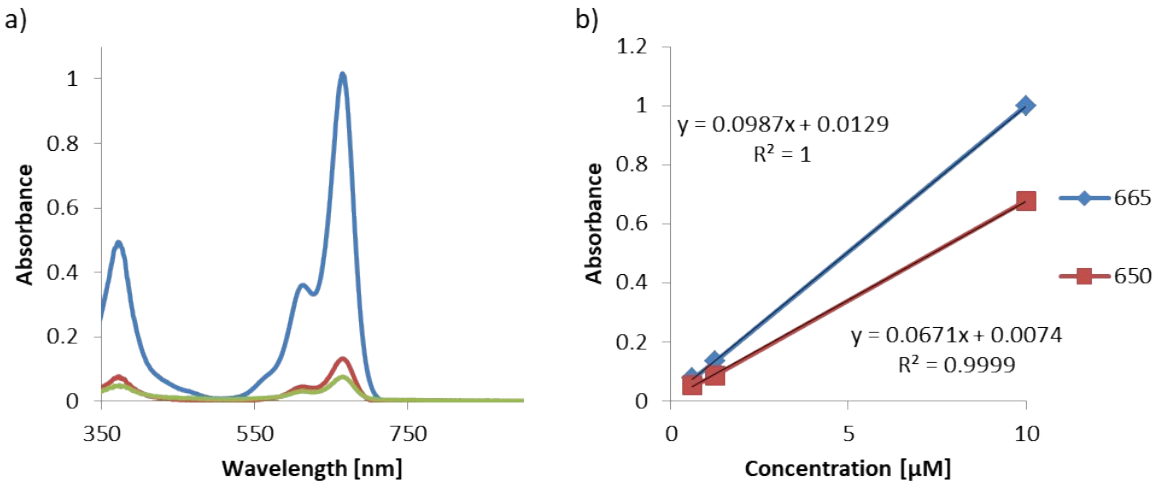


Figure 6. a) UV-VIS spectra of compound **2** at different concentrations (10, 5, 2.5, 1.23, 0.7 μM) in 50% Acetonitrile / 5 mmol phosphate buffer pH = 7.5); b) linear dependence of Absorbance of the compound of its concentration at λ_{max} and λ = 660 nm.

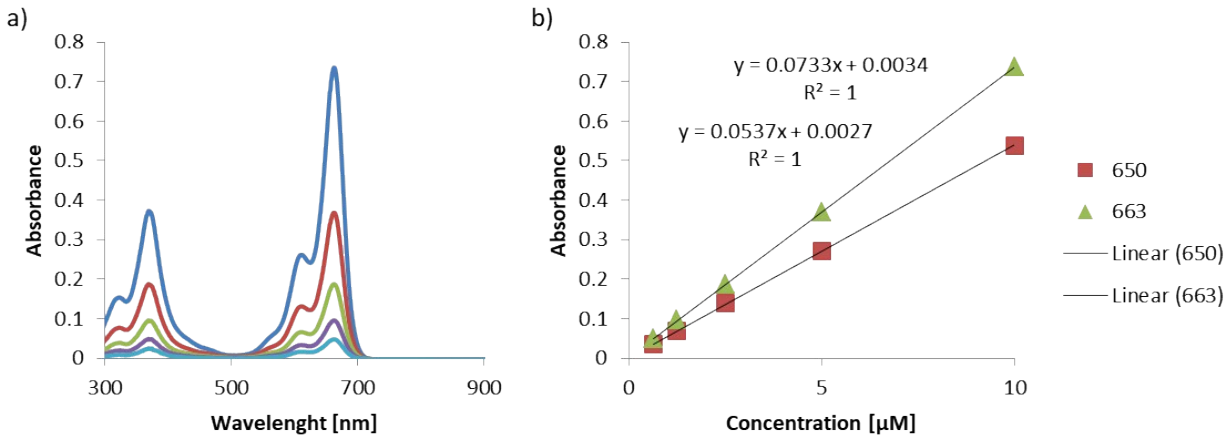


Figure 7. a) UV-VIS spectra of compound **5** at different concentrations (10, 5, 2.5, 1.23, 0.7 μM) in 50% Acetonitrile / 5 mmol phosphate buffer pH = 7.5 ; b) linear dependence of Absorbance of the compound of its concentration at λ_{max} and λ = 660 nm.

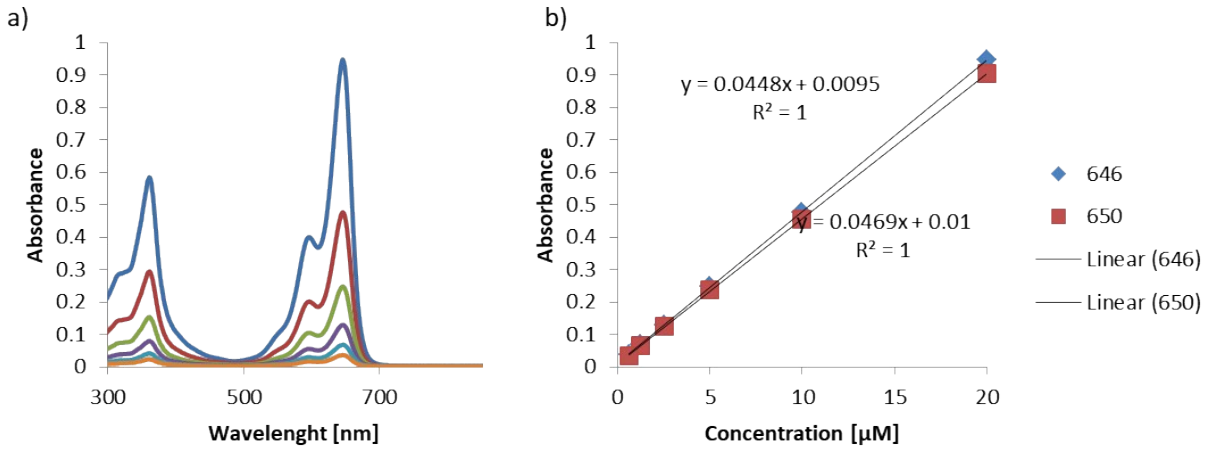


Figure 8. A) UV-VIS spectra of compound **8** at different concentrations (10, 5, 2.5, 1.23, 0.7 μM) in 50% Acetonitrile / 5 mmol phosphate buffer pH = 7.5; B) linear dependence of Absorbance of the compound of its concentration at λ_{max} and λ = 646 nm.

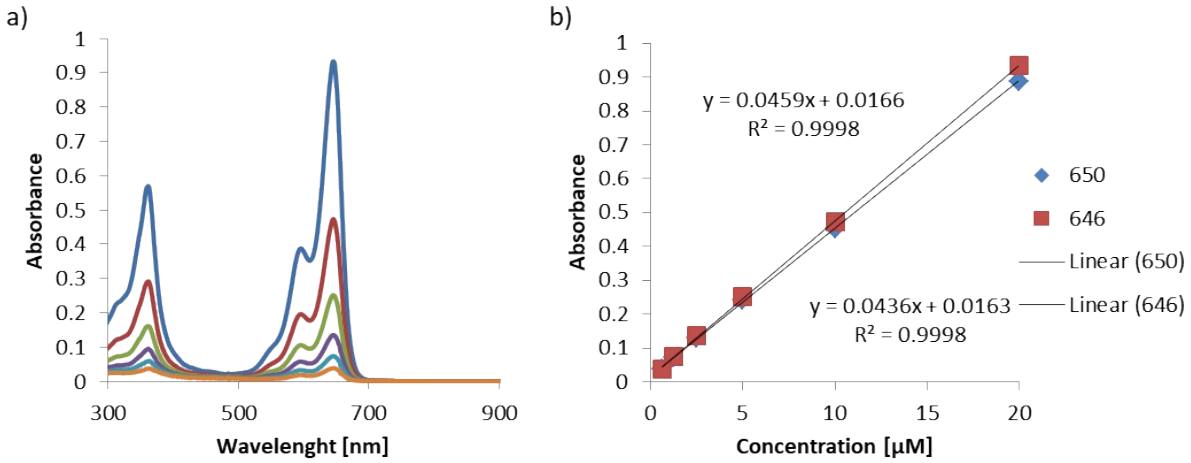


Figure 9. A) UV-VIS spectra of compound **9** at different concentrations (10, 5, 2.5, 1.23, 0.7 μM) in 50% Acetonitrile / 5 mmol phosphate buffer pH = 7.5; B) linear dependence of Absorbance of the compound of its concentration at λ_{max} and λ = 646 nm.

4. Quantum yields measurements

Determination of quantum yield for compound **8**

4.1. Determination of the photon flux

A modification of a standard protocol was applied for the determination of the photon flux. (*Kuhn H. J.; Braslavsky S. E.; Schmidt R. Chemical actinometry (IUPAC Technical Report). Pure Appl. Chem.*, **2004**, *76* (12), 2105) An aqueous H_2SO_4 solution (0.05 M) containing $K_3[Fe(C_2O_4)_3]$ (41 mM, 2 mL, 1 cm quartz cuvette) was irradiated at 20°C for a given period of time in the dark with a 365 nm LED. The solution was then diluted with 1.0 mL of an aqueous H_2SO_4 solution (0.5 M) containing phenanthroline (1 g/L) and NaOAc (122.5 g/L). The absorption at λ= 510 nm was measured and compared to an identically prepared non-irradiated sample. The concentration of $[Fe(phenanthroline)_3]^{2+}$ complex was calculated using its molar absorptivity (ε = 11100 M^{-1} cm^{-1}) and taking into account the dilution. The quantity of Fe^{2+} ions expressed in mol was plotted versus time (expressed in seconds) and the slope, obtained by linear fitting the data points to the equation y = ax +b using Origin software, equals the rate of formation of the Fe^{2+} ion at the given wavelength in standardized conditions. This rate can be converted into the photon flux (I) by dividing it by the quantum yield of $[Fe(phenanthroline)_3]^{2+}$ complex (Φ_{365nm} = 1.29) at 365 nm and by the probability of photon absorption at 365 nm of the Fe^{3+} complex (approximated to 1, because the absorbance of $K_3[Fe(C_2O_4)_3]$ at 365 nm is greater than 2). The obtained photon flux is: I = 4.06x10^{-8} einstein s^{-1}.

4.2. Calibration curve for compound **8** using UPLC.

A series of concentrations of compound **8** in DMSO has been analyzed by UPLC, using UV-Vis detector at λ = 365 nm. UPLC measurements were performed using the following setup: Column: ACQUITY UPLC® HSS T3 1.8µm, 2.1 x 150 mm; Flow: 0.3 mL/min; Eluent A: 0.1% formic acid in water; Eluent B: 0.1% formic acid in acetonitrile; Program: (0-1 min) 5% B; (1-8 min) linear gradient to 90% B; (8-11 min) 90% B; (11-12 min) linear gradient to 5% B; (12-17 min) 5% B.

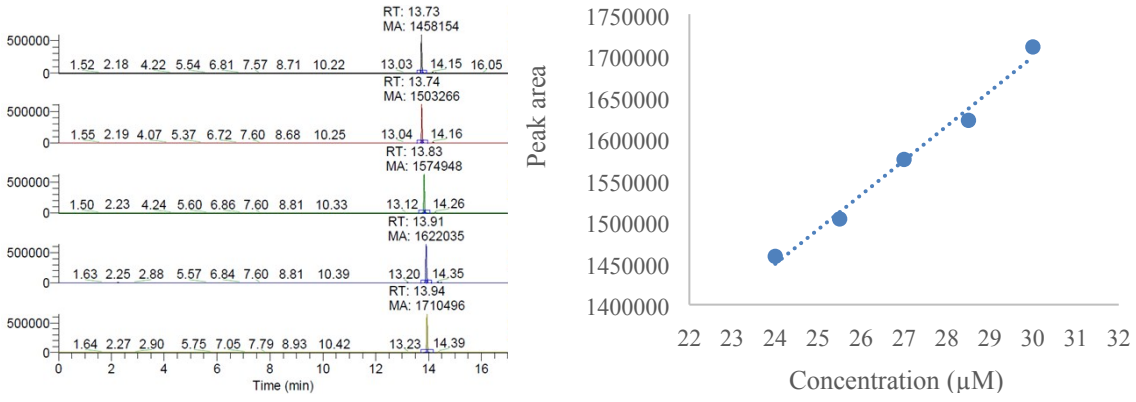


Figure 10. Chromatograms and calibration curve for the determination of the concentration of compound **8**.

4.3. Determination of uncaging kinetics and quantum yield.

3.00 mL of 30 μM solution in MeCN/MilliQ (3:1) was irradiated at 365 nm for 40 min at 25 °C. Every 10 minutes, 50 μL of solution was removed for UPLC analysis.

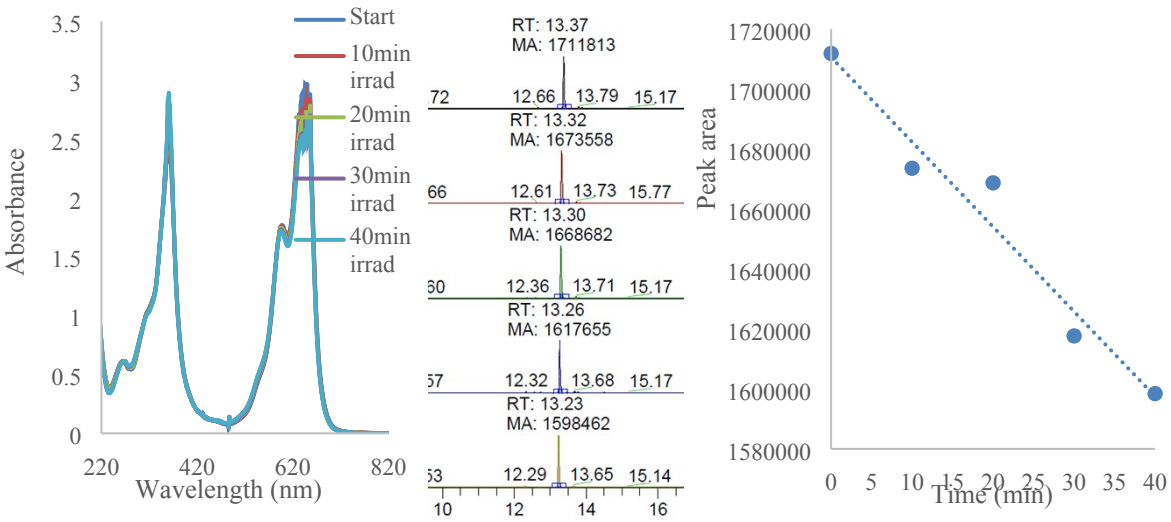


Figure 11. UV-Vis spectra, chromatograms and concentration change for the determination of the quantum yield for the uncaging of compound **8** under 365 nm irradiation.

The measured drop in concentration (-1.133 x 10^{-9} M/s) corresponds to the conversion of 3.4 x 10^{-12} molecules per second. With a photon flux determined for the system (actinometry with $K_3[Fe(C_2O_4)_3]$) to be 4.06x10^{-8} einstein s^{-1}, this gives the **quantum yield of 0.0084%**.

5. Cell culture

HUES9 human embryonic stem cells (hESC; Harvard Stem Cell Institute) were a kind gift from Chad Cowen and were differentiated to generate cardiomyocytes as previously described.[3] Briefly, HUES9 hESC were maintained in Essential 8 medium (A1517001; Thermo Fisher Scientific) on a Geltrex-coated surface (A1413301; Thermo Fisher Scientific), medium was refreshed daily. Cells were incubated under controlled conditions with 37 °C, 5% CO_2 and 100% atmospheric humidity. Differentiation to cardiomyocytes was achieved as described previously.[4] Briefly, hESC were seeded as single cells in Essential 8 medium containing 5 μM Y26732 (S1049, Selleck Chemicals). Once cultures reached 80% confluency, differentiation was initiated (day 0) by switching to RPMI1640 medium (21875-034, Thermo Fisher Scientific) supplemented with 1x B27 minus insulin (A1895601, Thermo Fisher Scientific) and 6 μM CHIR99021 (13122, Cayman Chemical). At day 2, medium was refreshed with RPMI1640 supplemented with 1x B27 minus insulin and 2 μM Wnt-C59 (5148, Tocris Bioscience). From day 4, medium was changed to CDM3 medium and was refreshed every other day (as described by Burridge et al.[5]). To enrich cardiomyocyte populations, differentiated cultures were maintained in glucose-free RPMI1640-based (11879, Thermo Fisher Scientific) CDM3 medium supplemented with 5 mM sodium DL-lactate (CDM3L; L4263, Sigma-Aldrich) for 4 days after day 12.[6] This resulted in >99% pure spontaneously beating cardiomyocytes. Experiments were typically started at day 30.

6. Electrophysiological characterization

hESC-derived cardiomyocytes were seeded at a density of 30.000 cells/well on a fibronectin-coated (F1411, Sigma Aldrich) 48-well CytoView MEA plate (M768-tMEA-48B-5, Axion Biosystems) in CDM3 medium supplemented with 5% KnockOut Serum Replacement (10828028, Thermo Fisher Scientific). Plated cardiomyocytes were maintained for an additional 10 days to assure syncytium formation. AxIS software (Axion Biosystems) Cardiac Beat Detector settings were set as shown in table 1. Other settings were set to default cardiomyocyte settings as recommended in the protocol provided by the manufacturer. Data was collected for at least 30 seconds and compiled statistical data was analyzed using the CiPA analysis tool (Axion Biosystems).

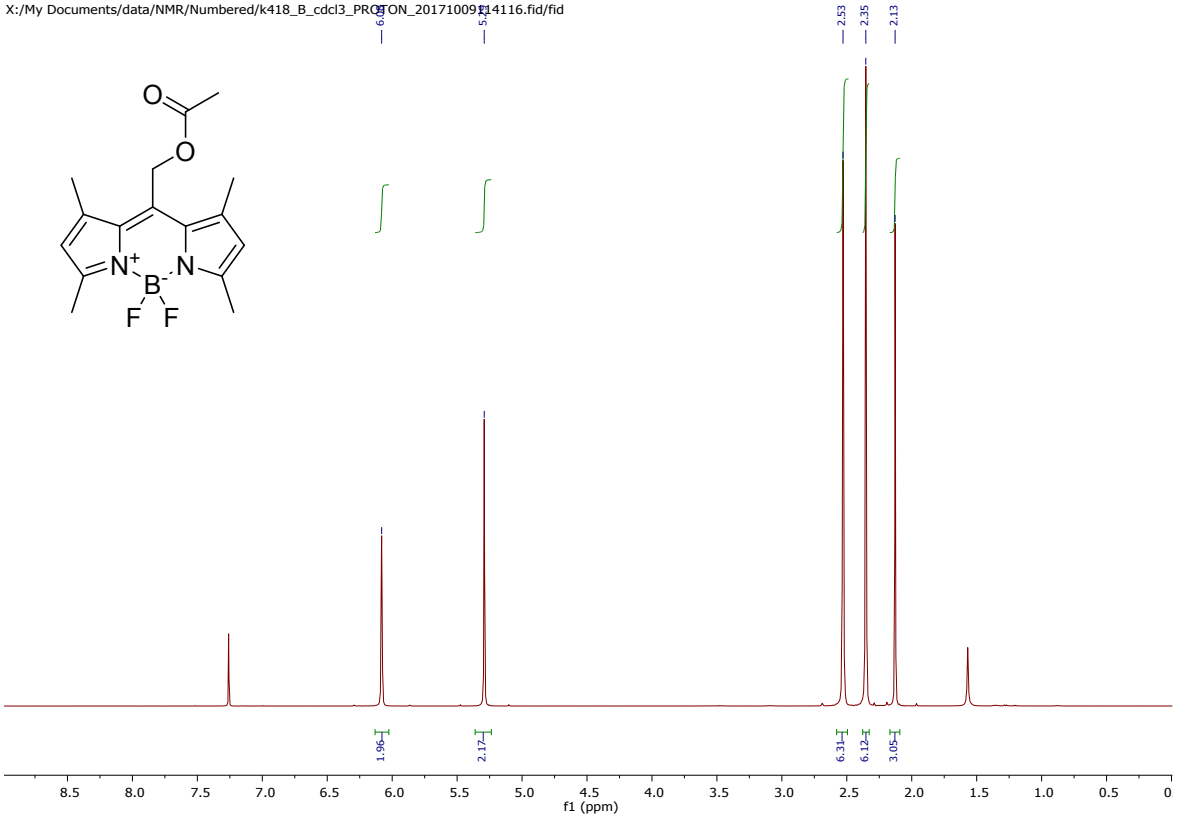
Table 1. Cardiac Beat Detector Settings.

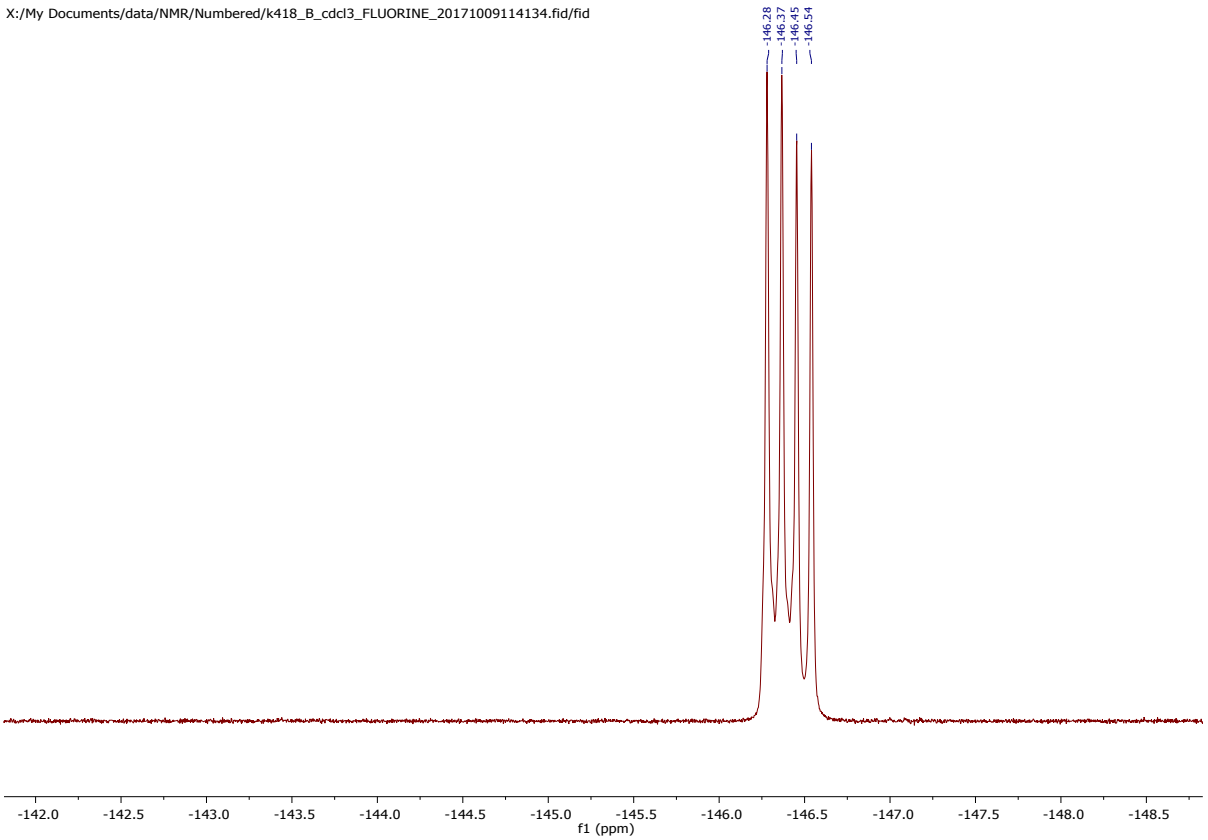
Detection Threshold	200 μV
Min Beat period	250 ms
Field Potential Duration Method	Polynomial regression
Running Average Beat Count	Checked (Use for FPD detection)

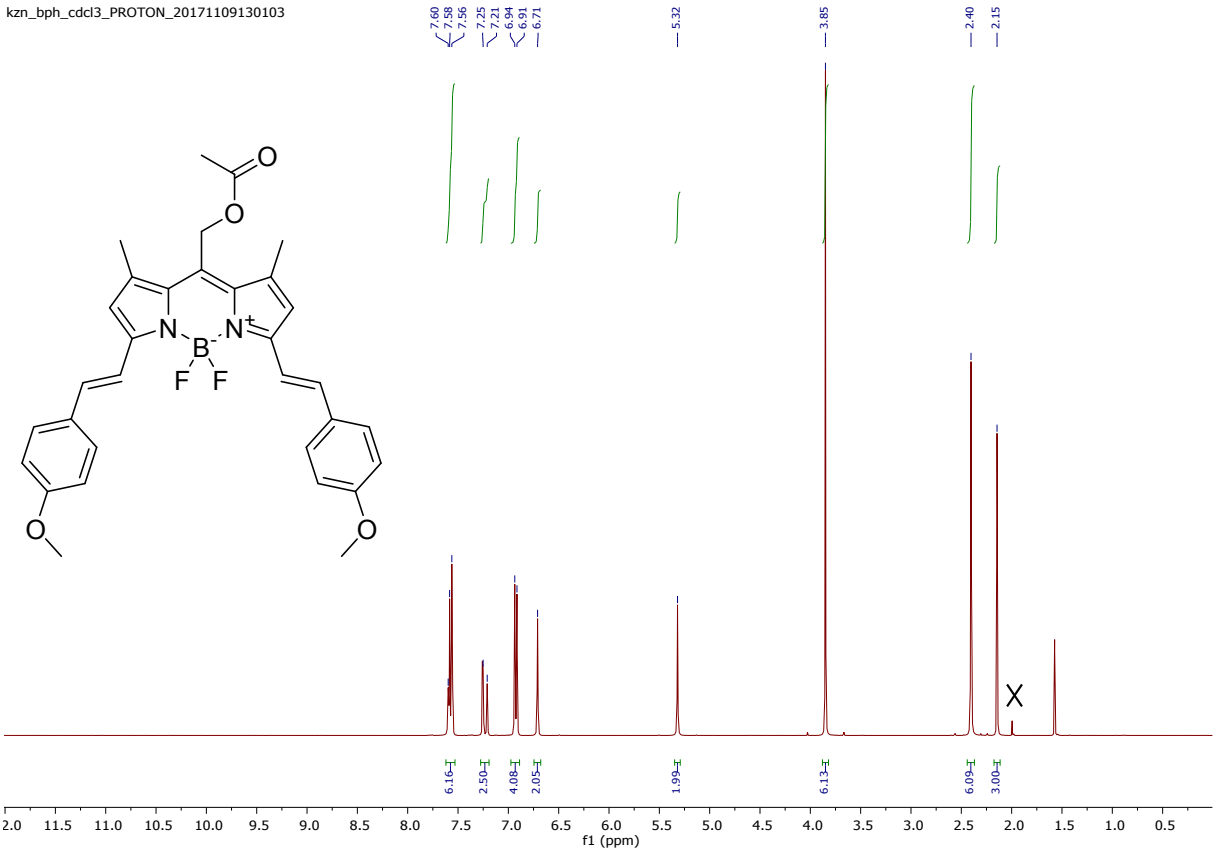
Table 2. Tukey's multiple comparisons test results

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Control vs. Caged dopamine	-2.226	-8,481 to 4,029	No	ns	0.7509
Control vs. Uncaged dopamine	-9.08	-15,89 to -2,273	Yes	**	0.0068
Control vs. Dopamine	-9.436	-16,24 to -2,628	Yes	**	0.0049
Caged dopamine vs. Uncaged dopamine	-6.854	-13,44 to -0,2715	Yes	*	0.0395
Caged dopamine vs. Dopamine	-7.21	-13,79 to -0,6273	Yes	*	0.0288
Uncaged dopamine vs. Dopamine	-0.3559	-7,466 to 6,754	No	ns	0.999

7. NMR Characterization of compounds **1 – 10**



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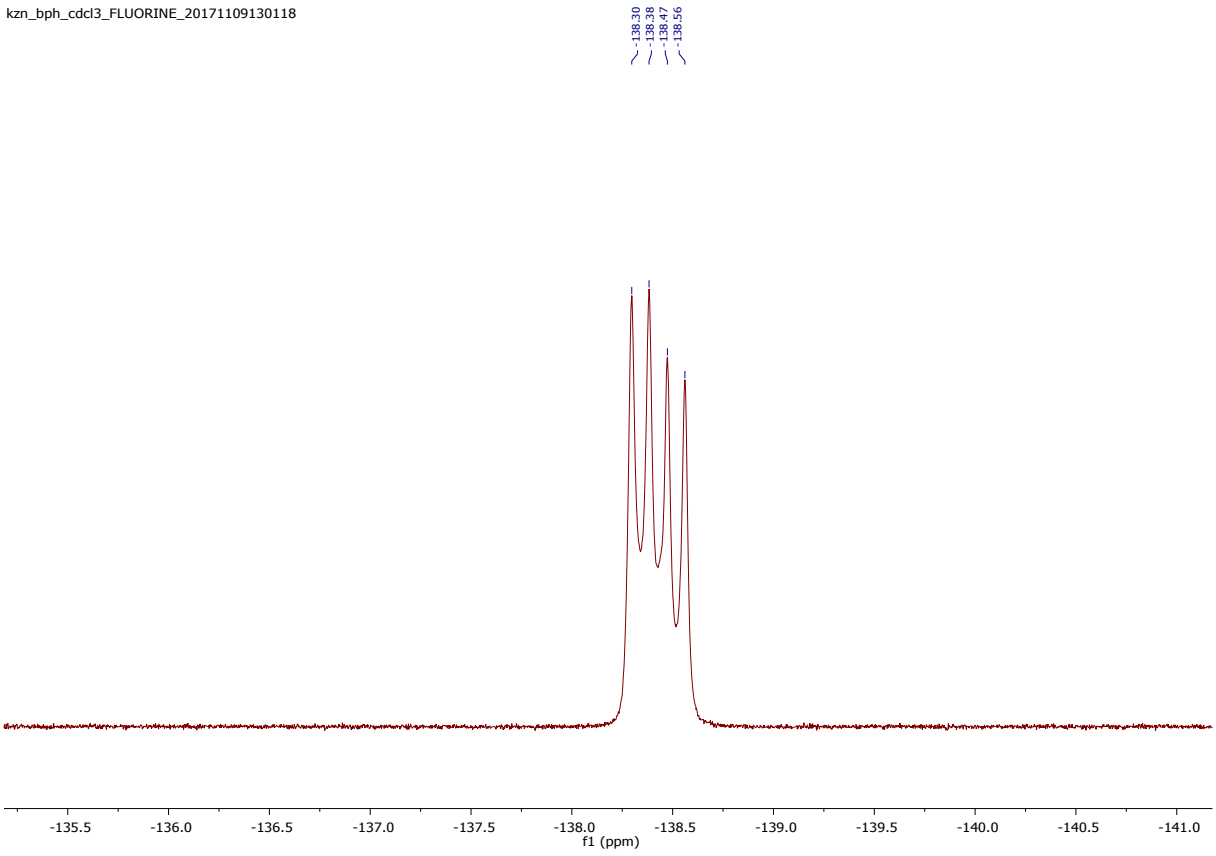
f1 (ppm)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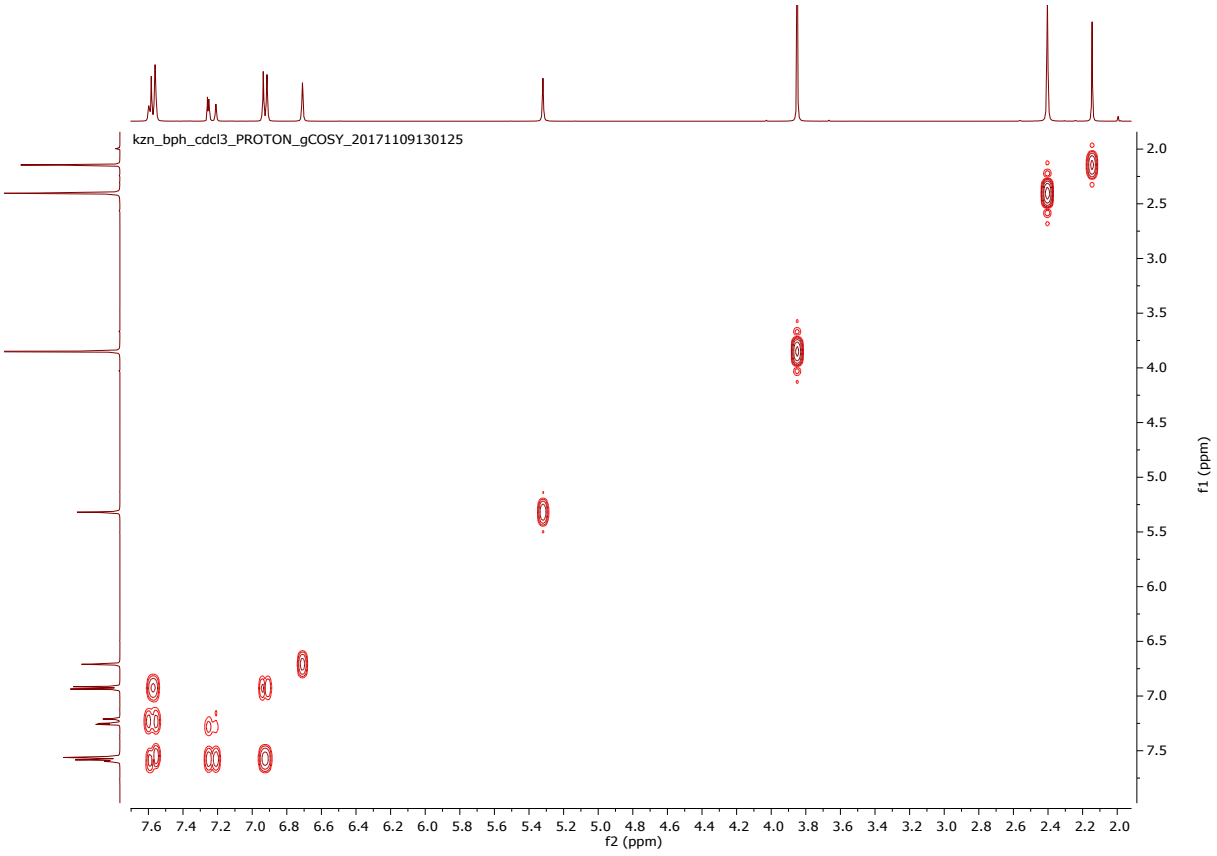
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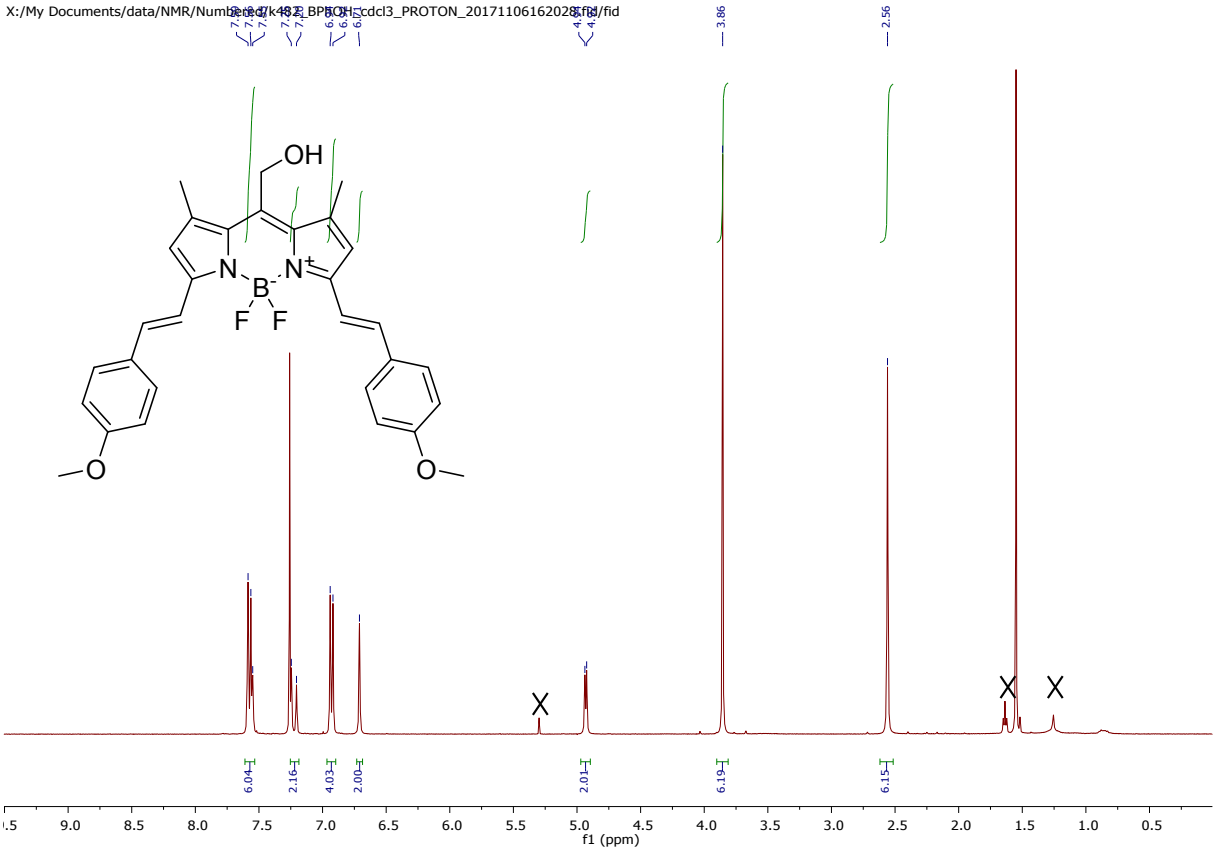
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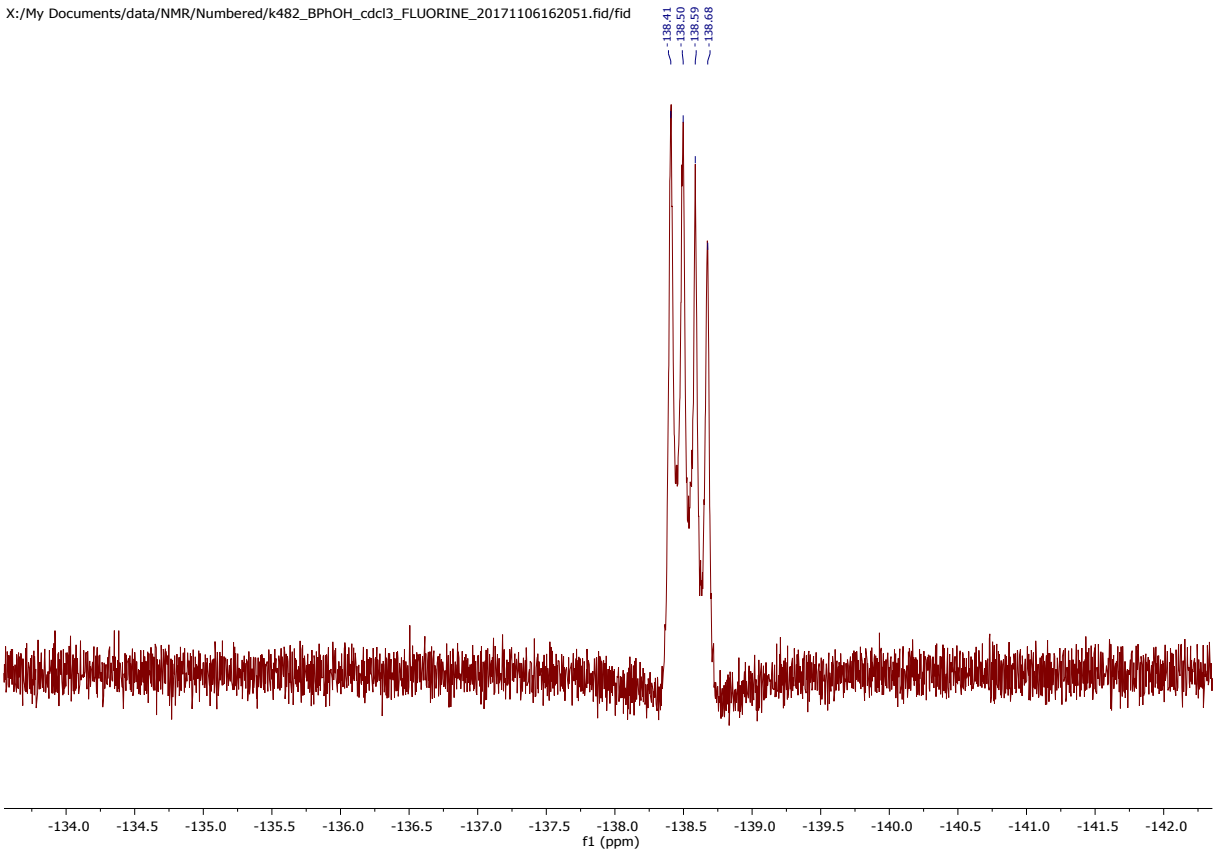
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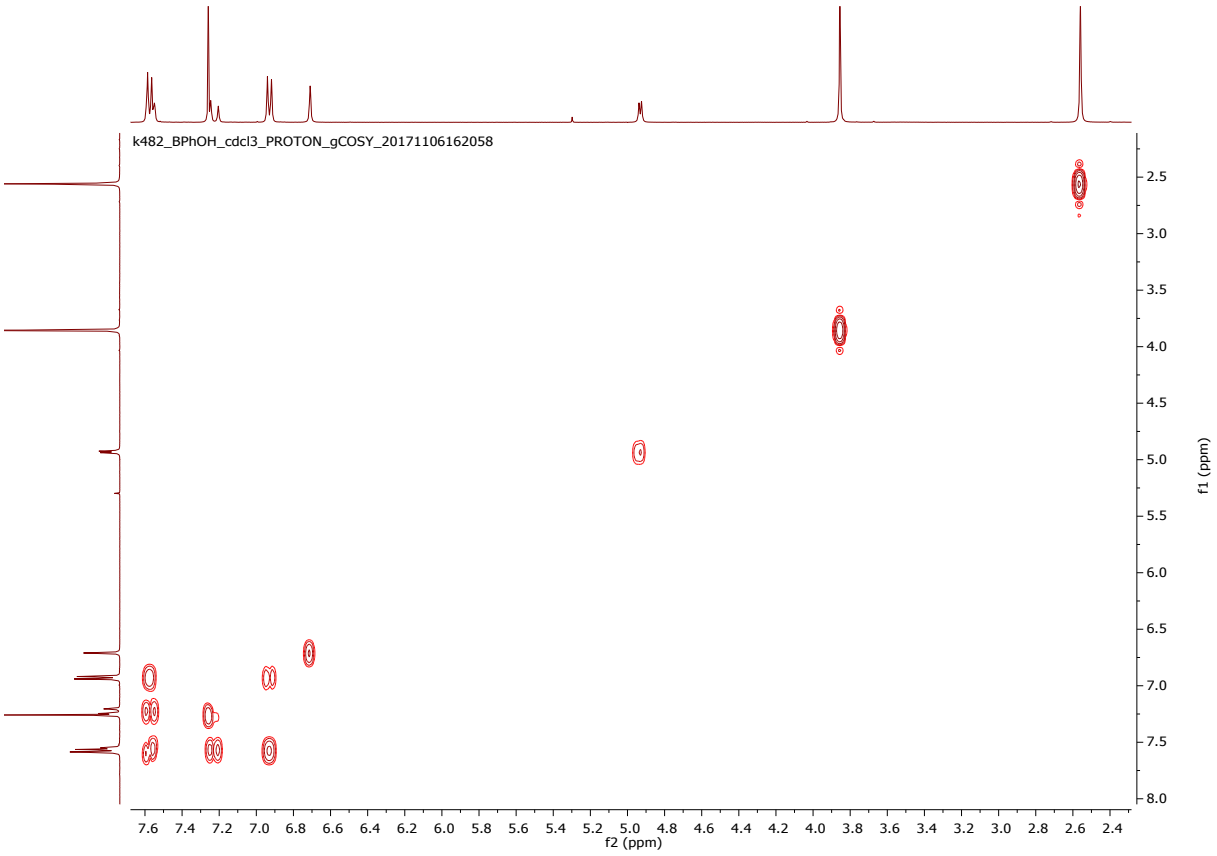
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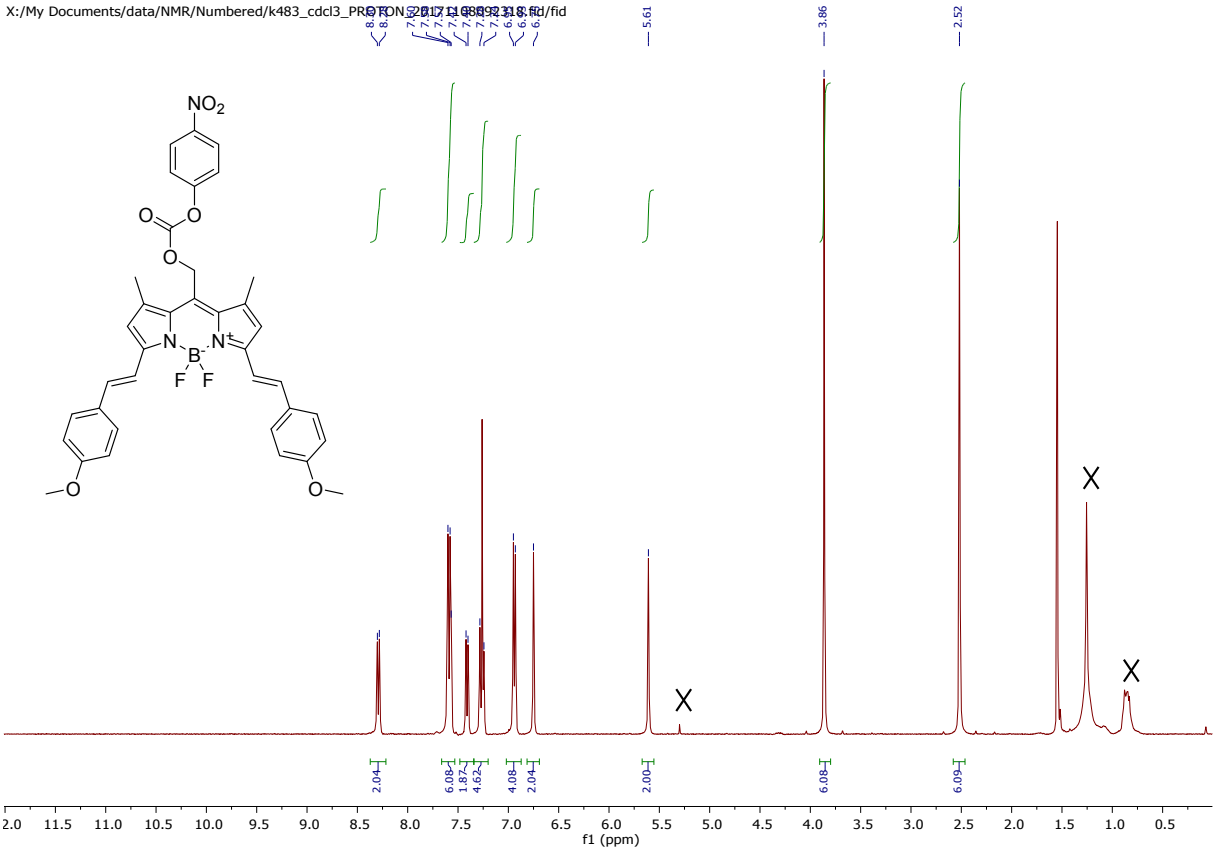
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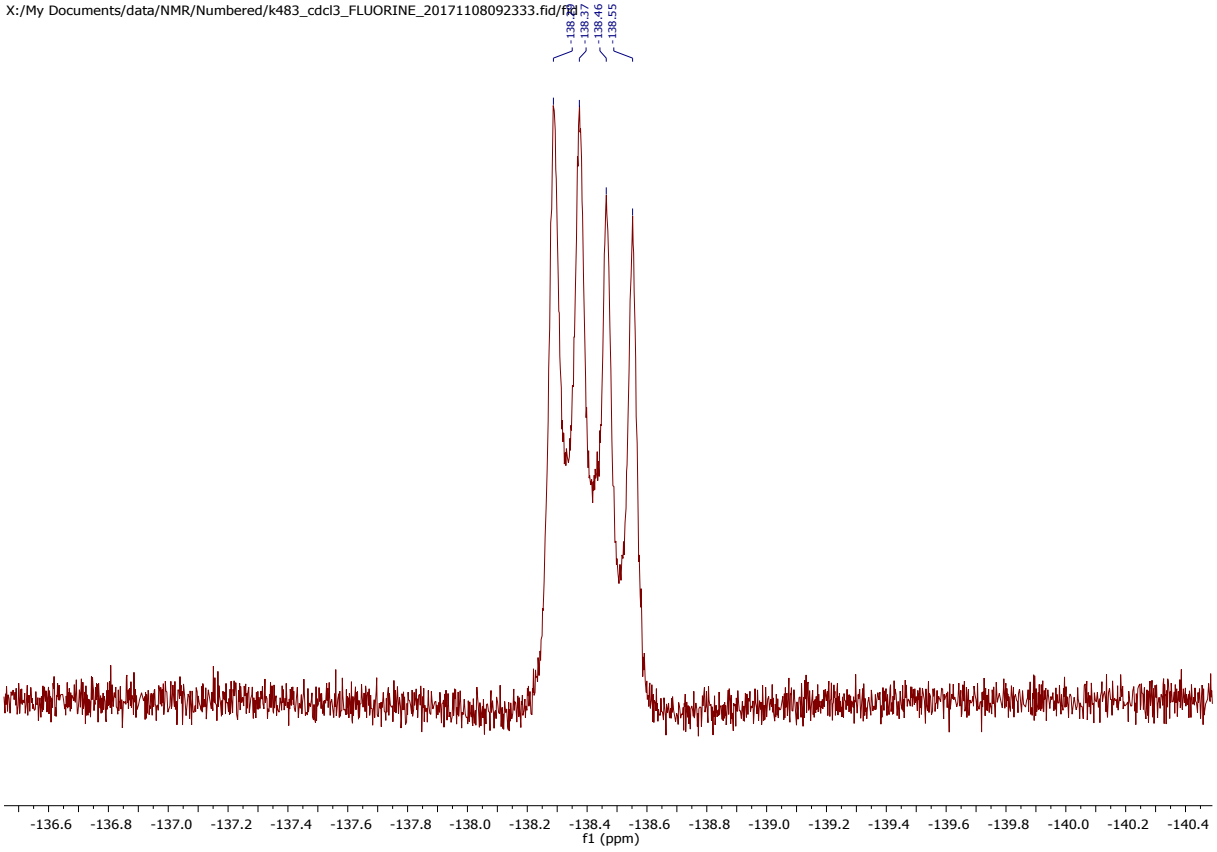
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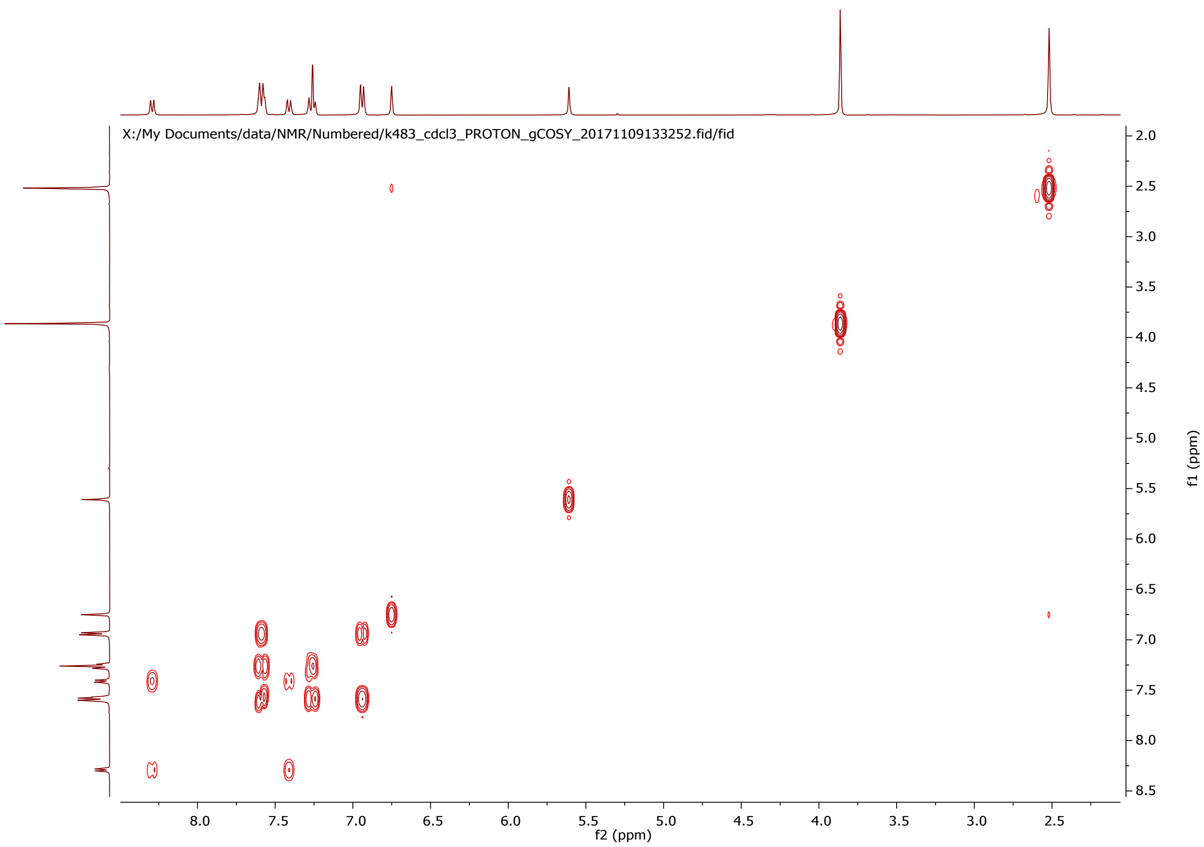
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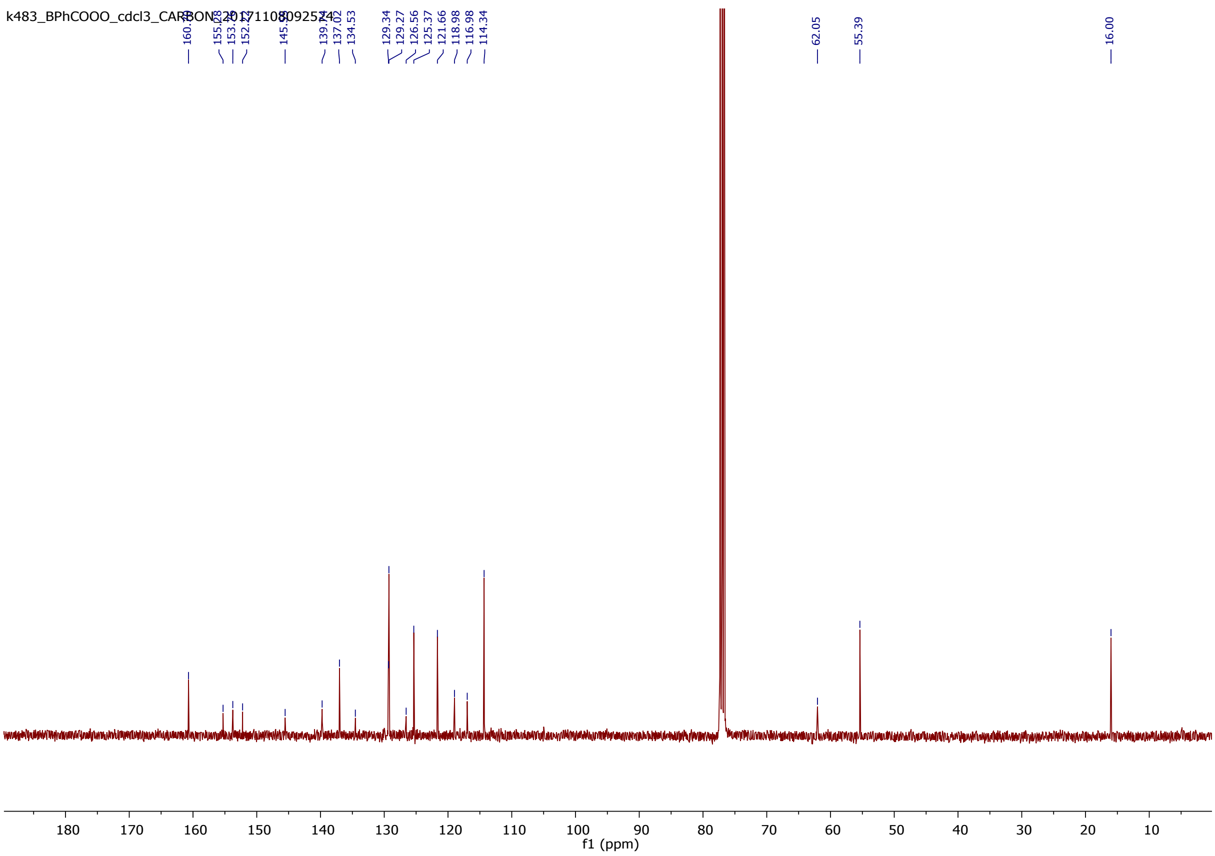
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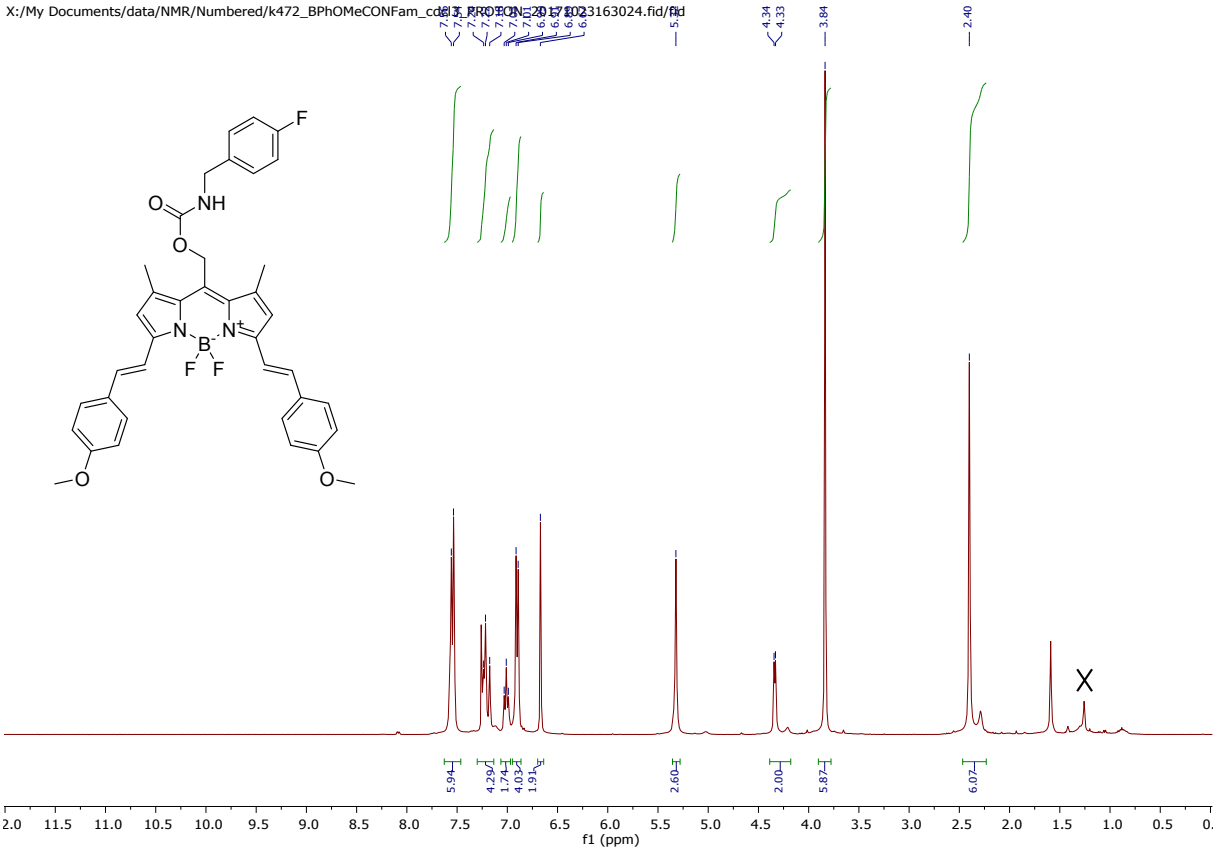
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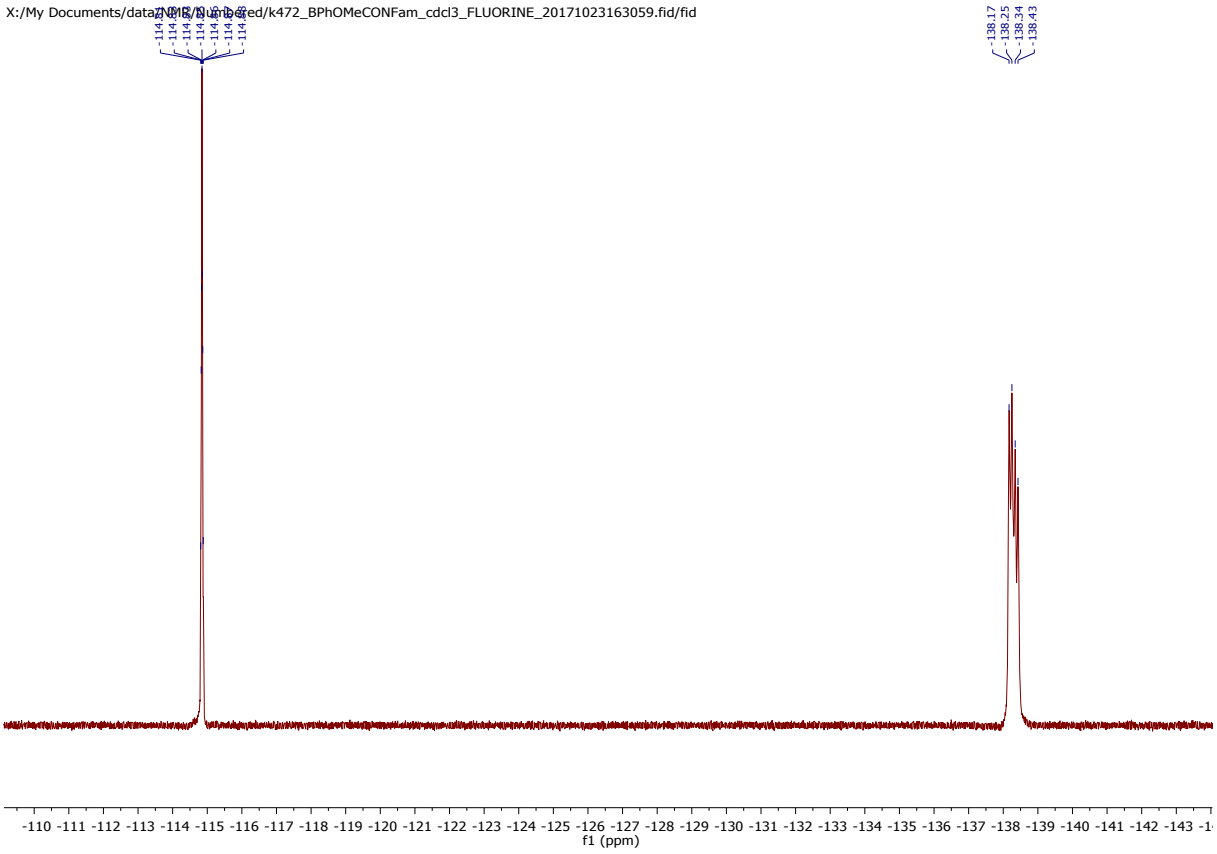
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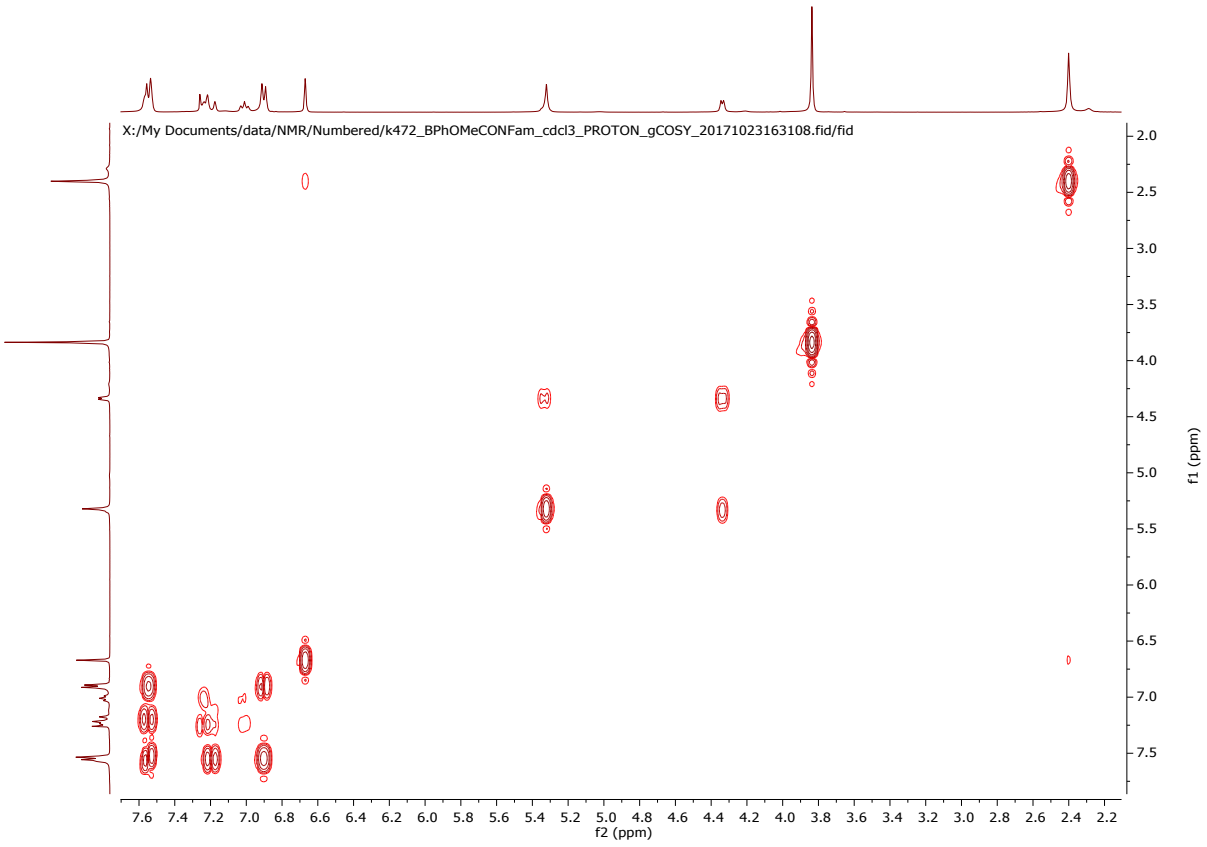
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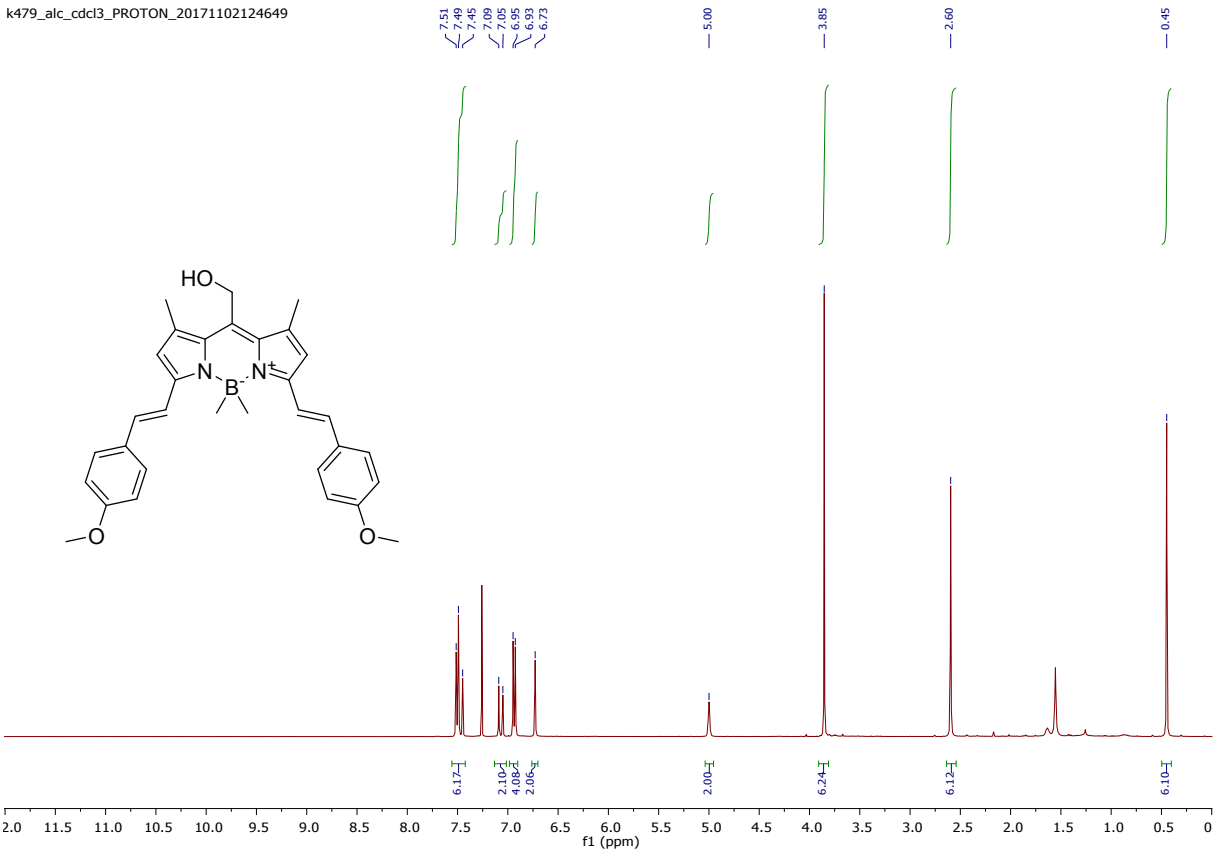
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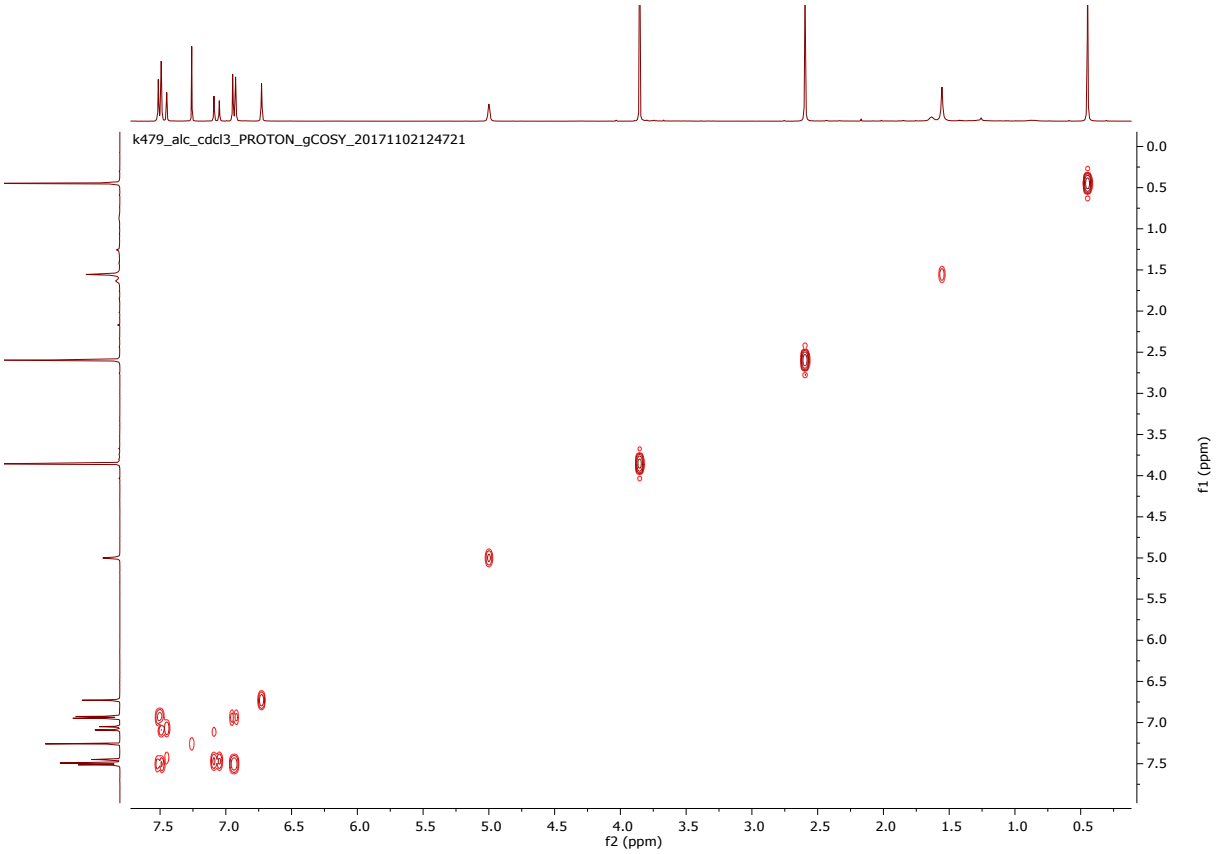
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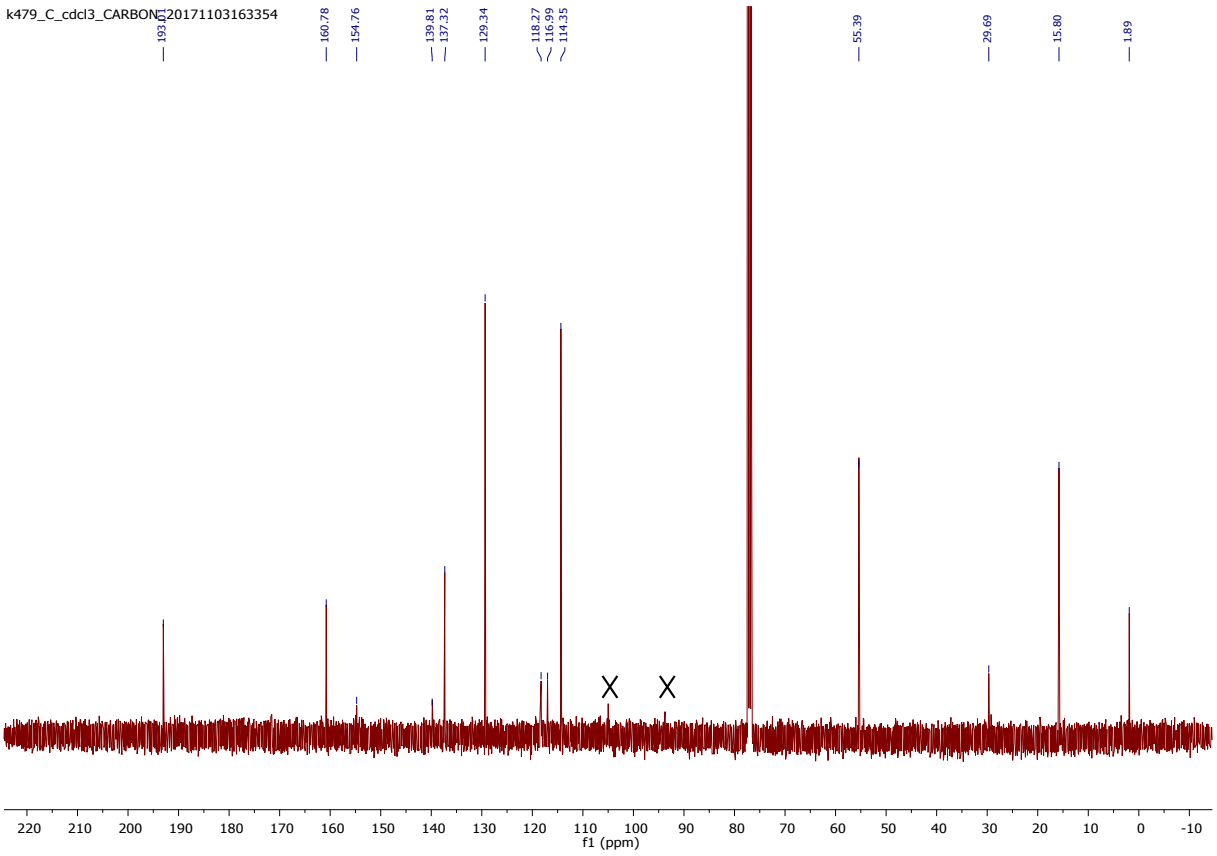
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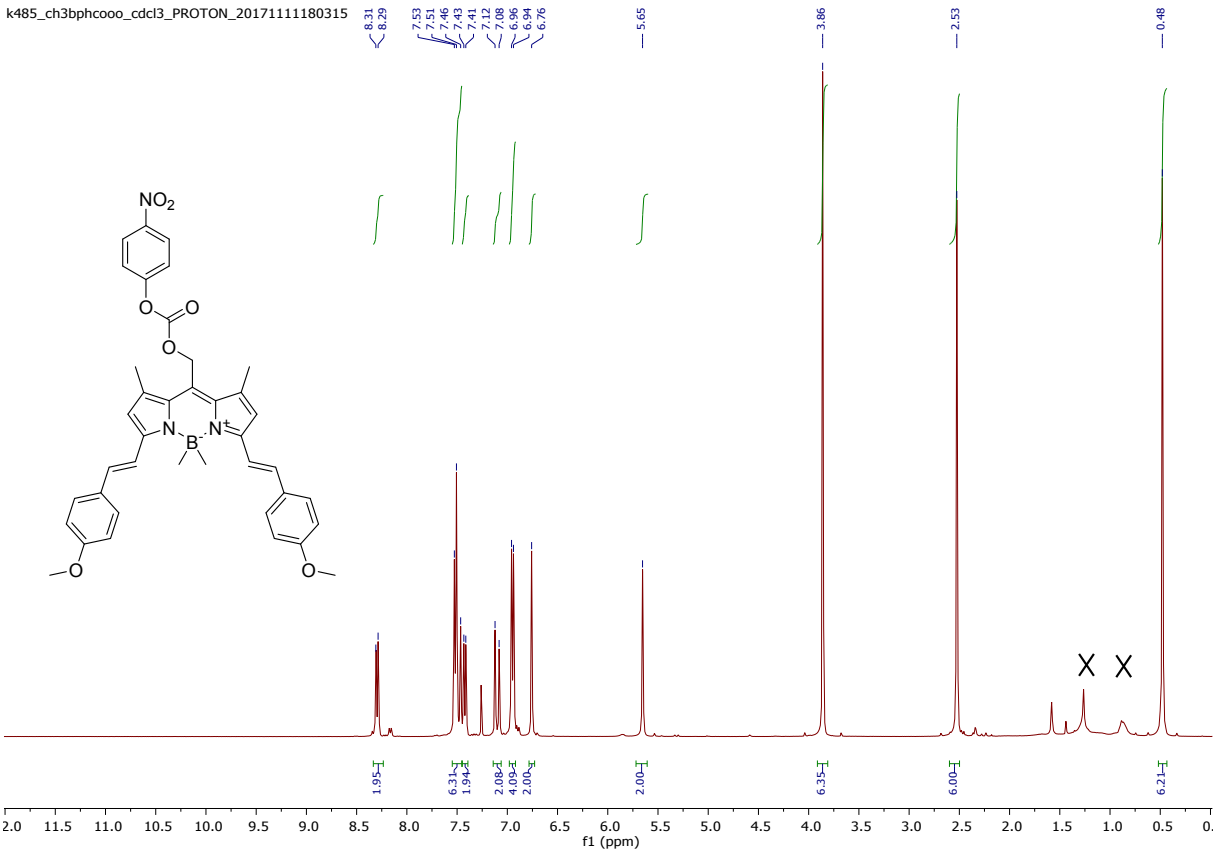
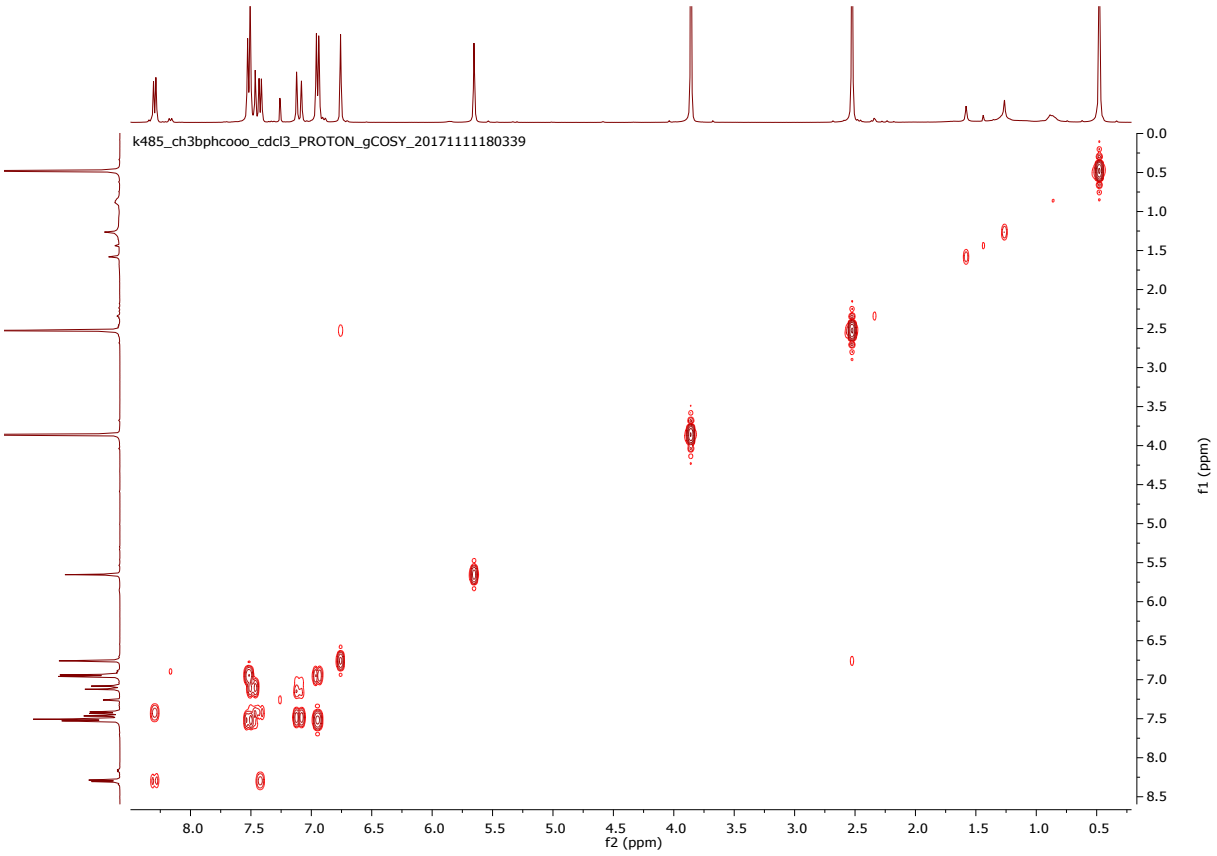
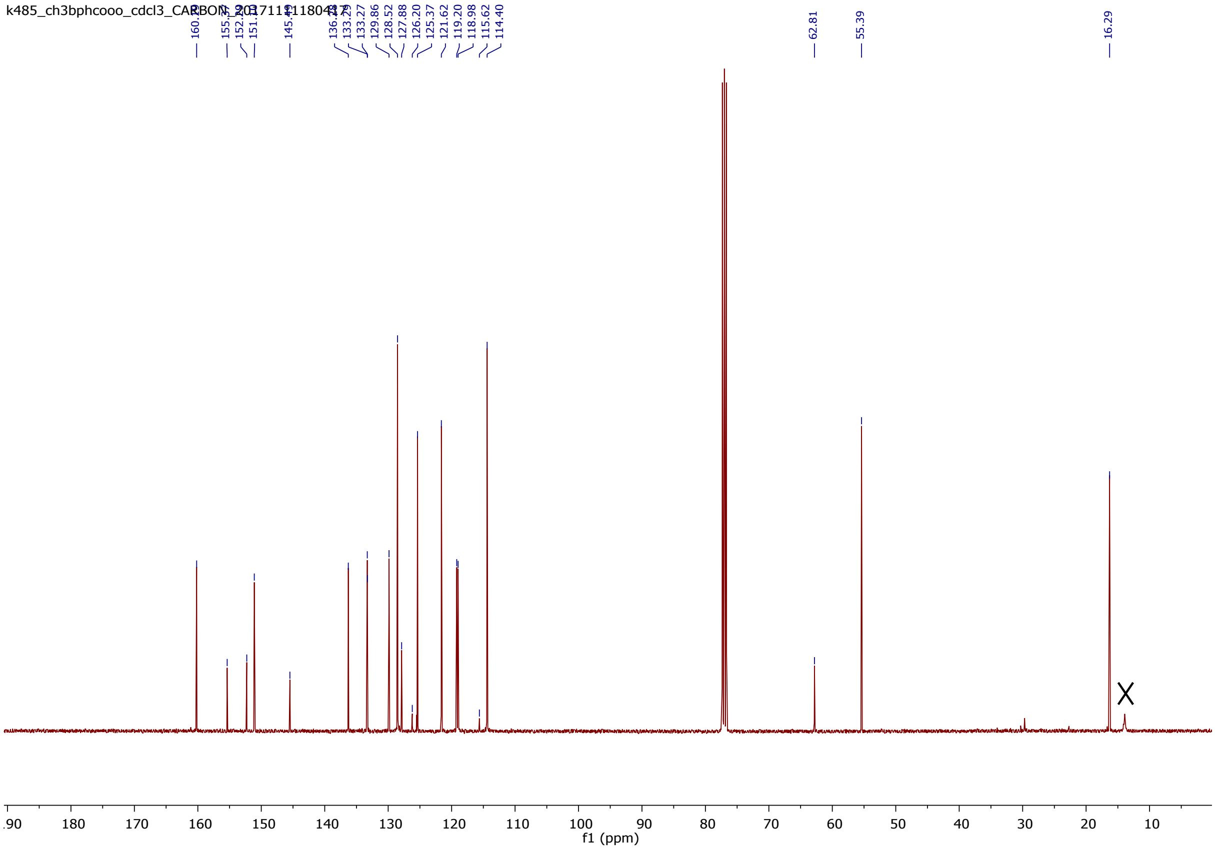
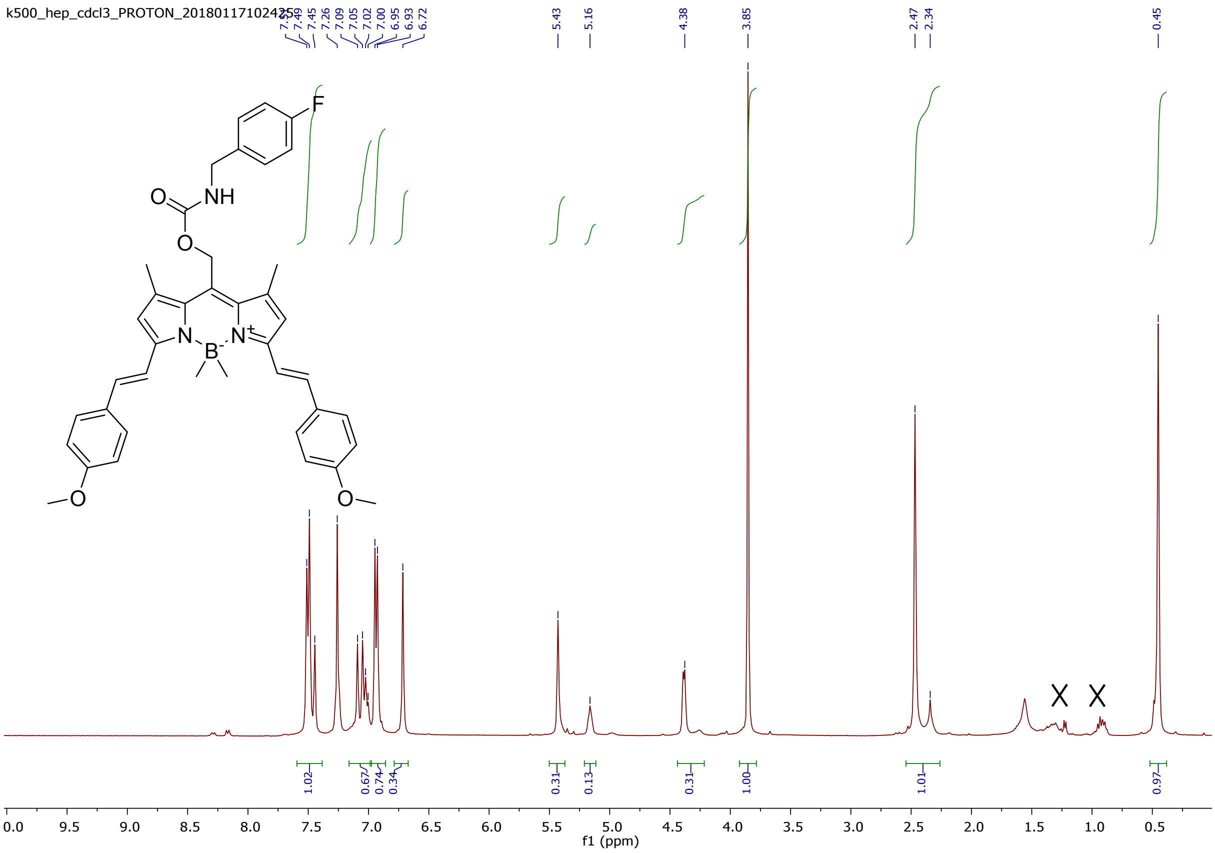
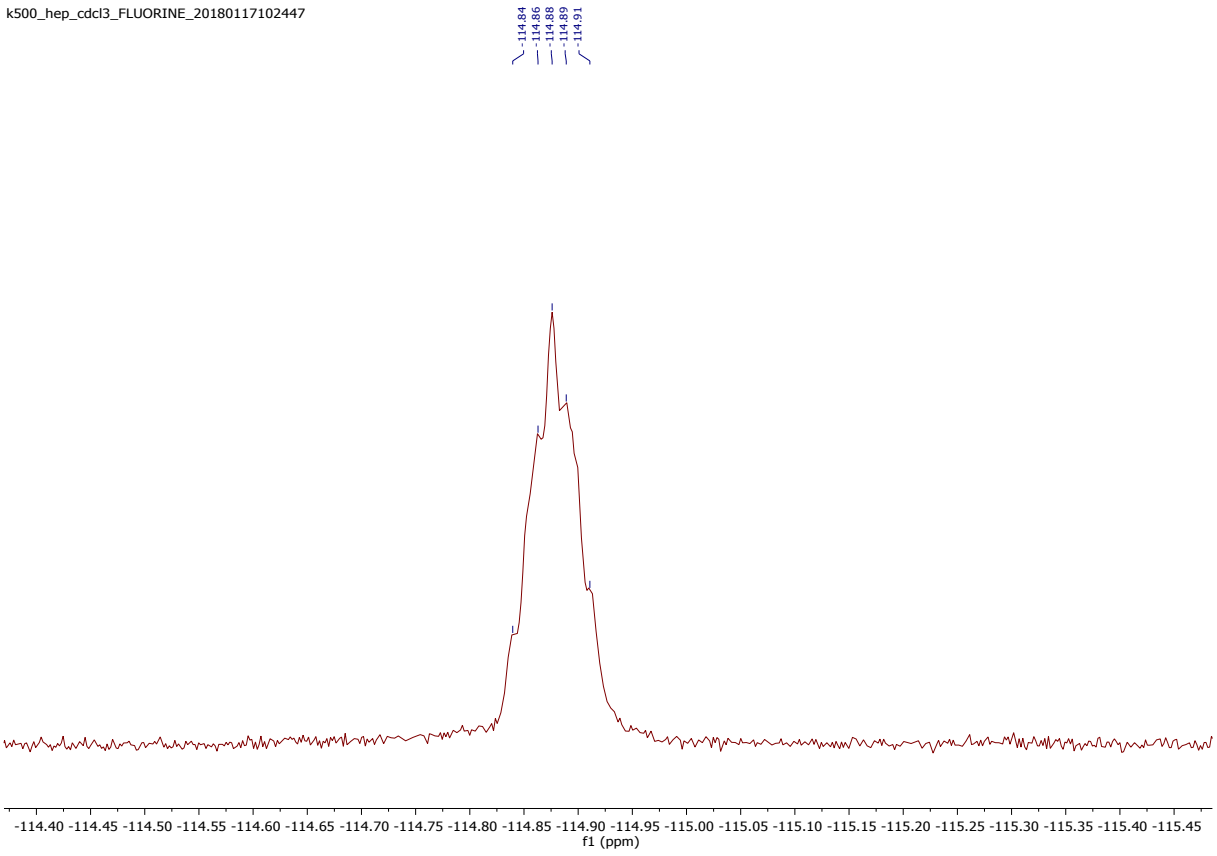
1.89
f1 (ppm)

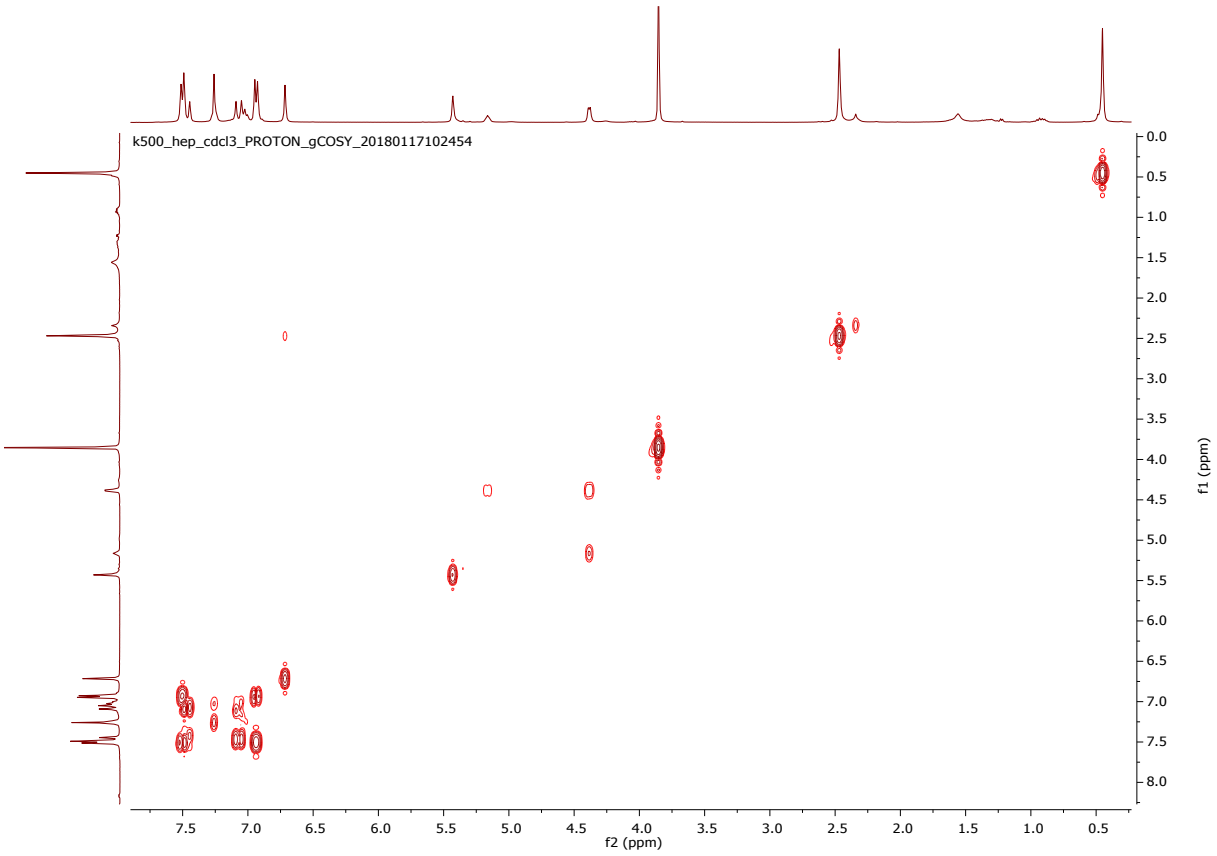

k485_ch3bphcooo_cdcl3_PROTON_20171111180315
8.31
8.29
7.53
7.51
7.46
7.43
7.41
7.12
7.08
6.96
6.94
6.76
5.65
3.86
2.53
0.48
NO2
O
O
O
N
B
N+
O
O
X X
1.95
6.31
1.94
2.08
4.09
2.00
2.00
6.35
6.00
6.21
f1 (ppm)

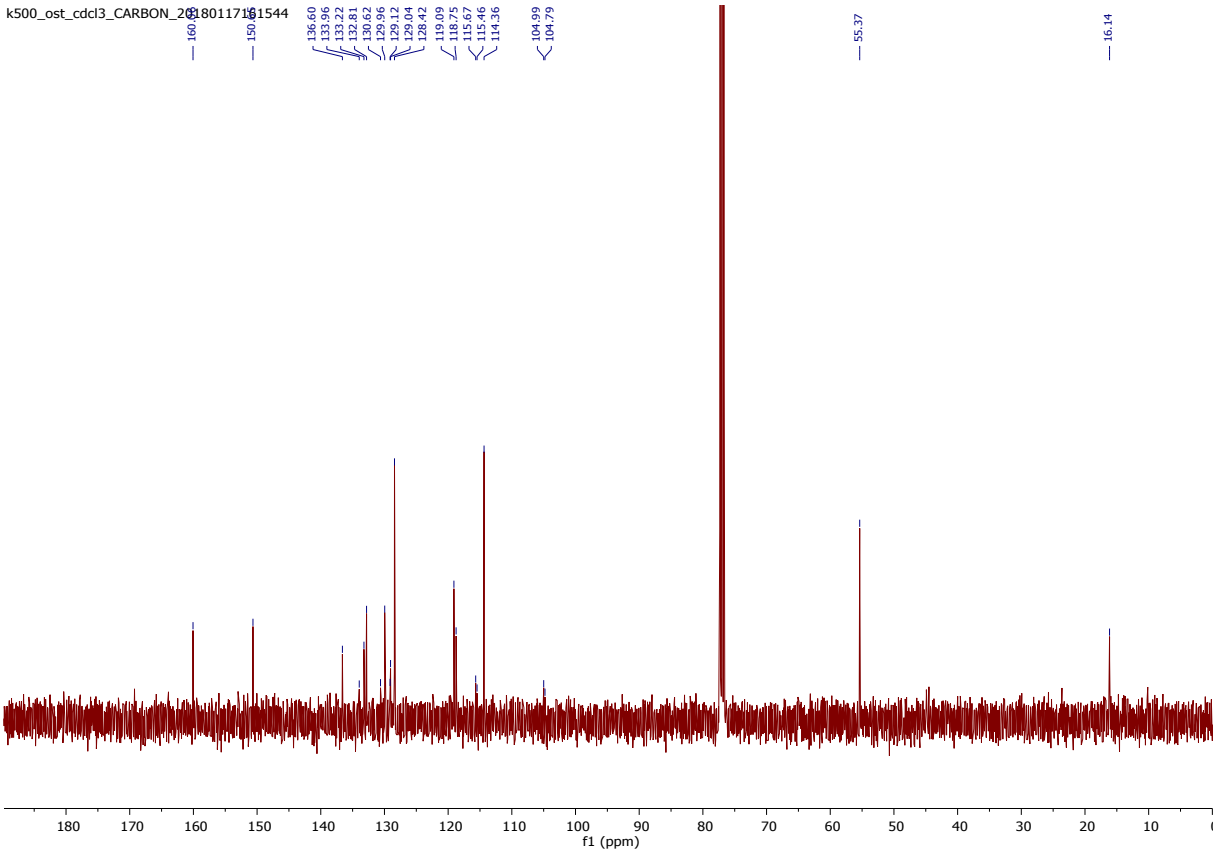

k485_ch3bphcooo_cdcl3_PROTON_gCOSY_20171111180339
f2 (ppm)
f1 (ppm)

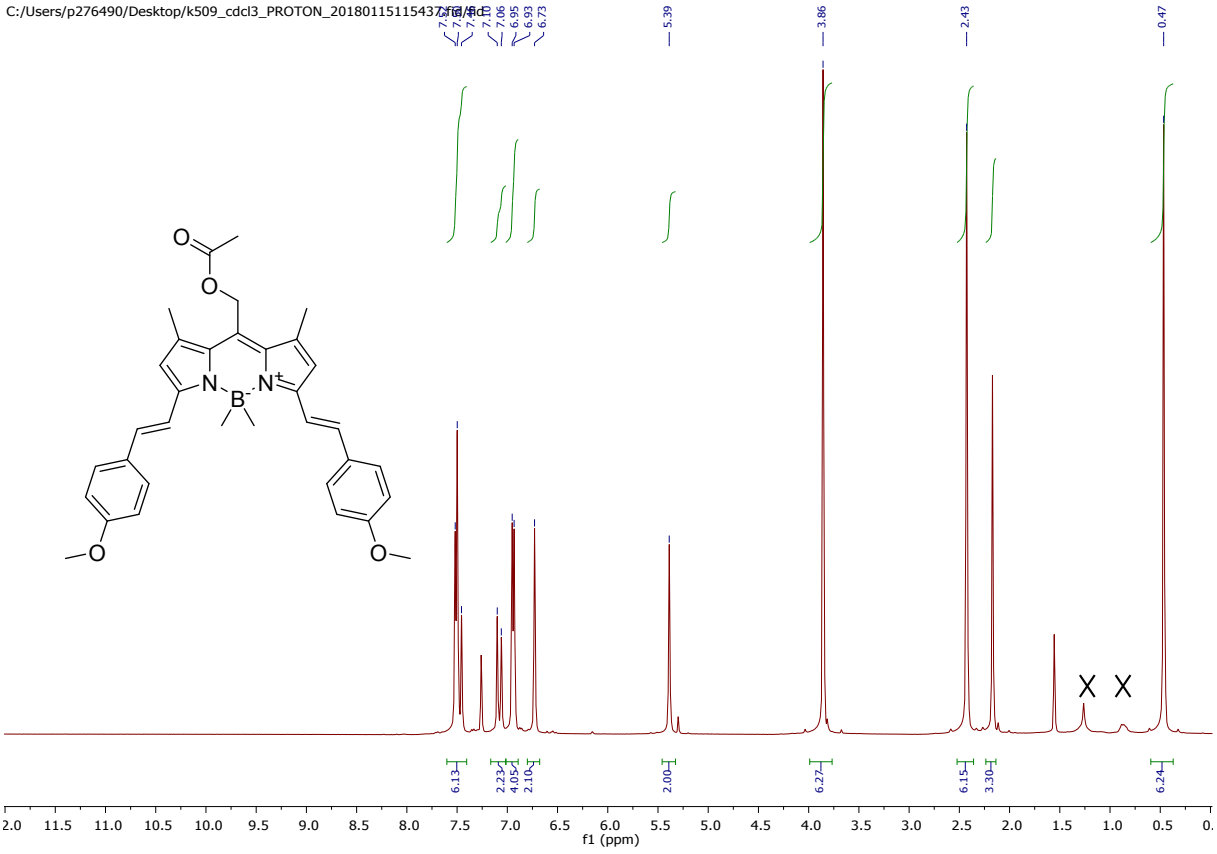

k485_ch3bphcooo_cdcl3_CARBON
129.86
128.52
127.88
126.20
125.37
121.62
119.20
118.98
115.62
114.40
62.81
55.39
16.29
X
f1 (ppm)

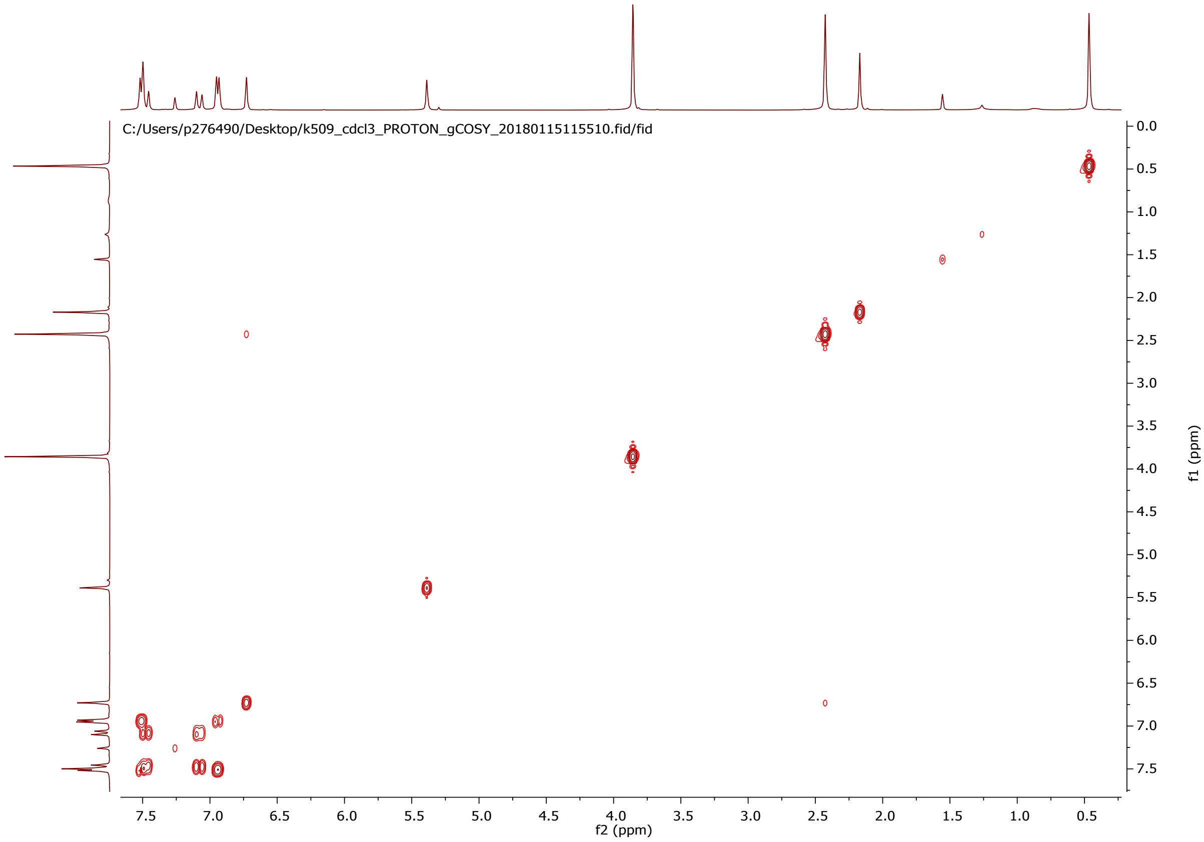

k500_hep_cdcl3_PROTON_20180117102425
7.45
7.26
7.09
7.05
7.02
7.00
6.95
6.93
6.72
5.43
5.16
4.38
3.85
2.47
2.34
0.45
F
O
NH
O
N
B
N+
O
O
X
X
1.02
0.67
0.74
0.34
0.31
0.13
0.31
1.00
1.01
0.97
f1 (ppm)


k500_hep_cdcl3_FLUORINE_20180117102447
-114.84
-114.86
-114.88
-114.89
-114.91
-114.40 -114.45 -114.50 -114.55 -114.60 -114.65 -114.70 -114.75 -114.80 -114.85 -114.90 -114.95 -115.00 -115.05 -115.10 -115.15 -115.20 -115.25 -115.30 -115.35 -115.40 -115.45
f1 (ppm)


k500_hep_cdcl3_PROTON_gCOSY_20180117102454
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
f2 (ppm)
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0
f1 (ppm)


k500_ost_cdcl3_CARBON_20180117161544
160.61
150.16
136.60
133.96
133.22
132.81
130.62
129.96
129.12
129.04
128.42
119.09
118.75
115.67
115.46
114.36
104.99
104.79
55.37
16.14
f1 (ppm)


C:/Users/p276490/Desktop/k509_cdcl3_PROTON_20180115115437.fid/fid
7.46
7.43
7.10
7.06
6.95
6.93
6.73
5.39
3.86
2.43
0.47
6.13
2.23
4.05
2.10
2.00
6.27
6.15
3.30
6.24
f1 (ppm)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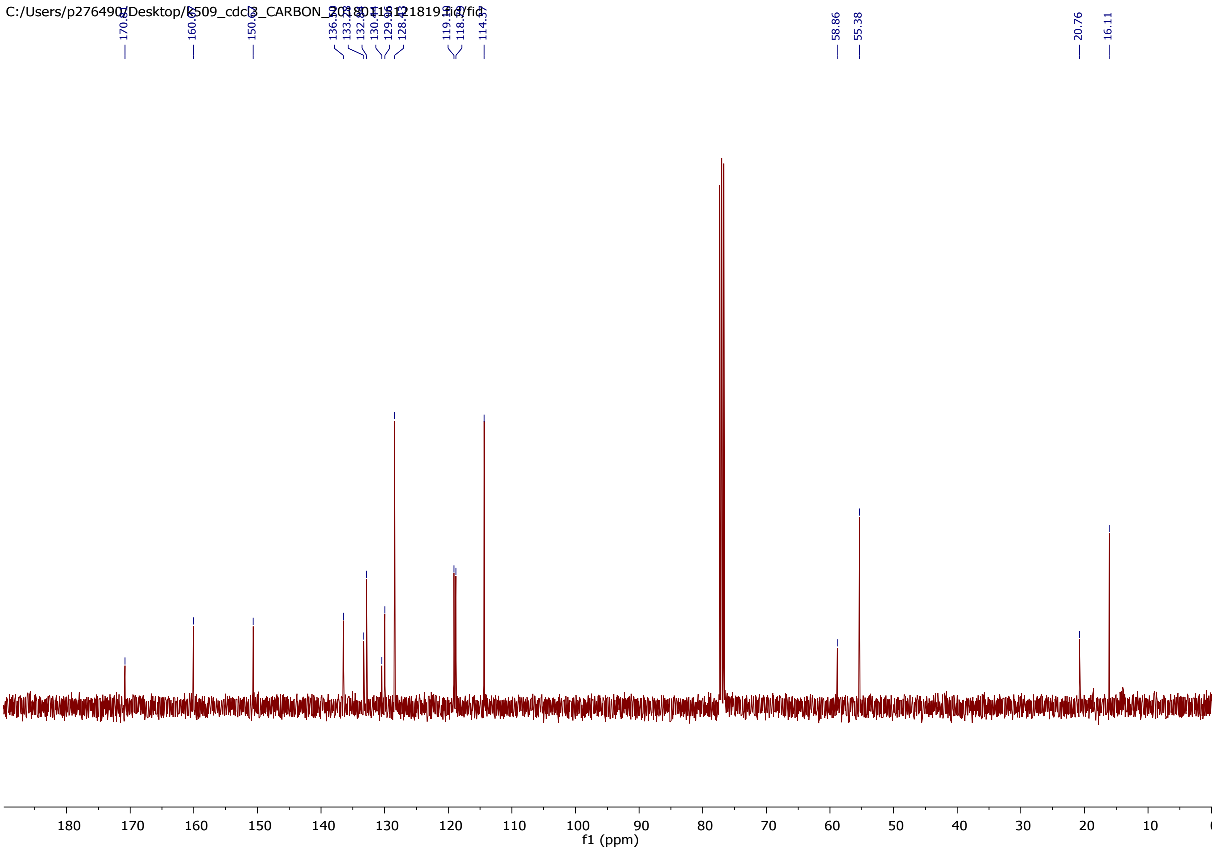
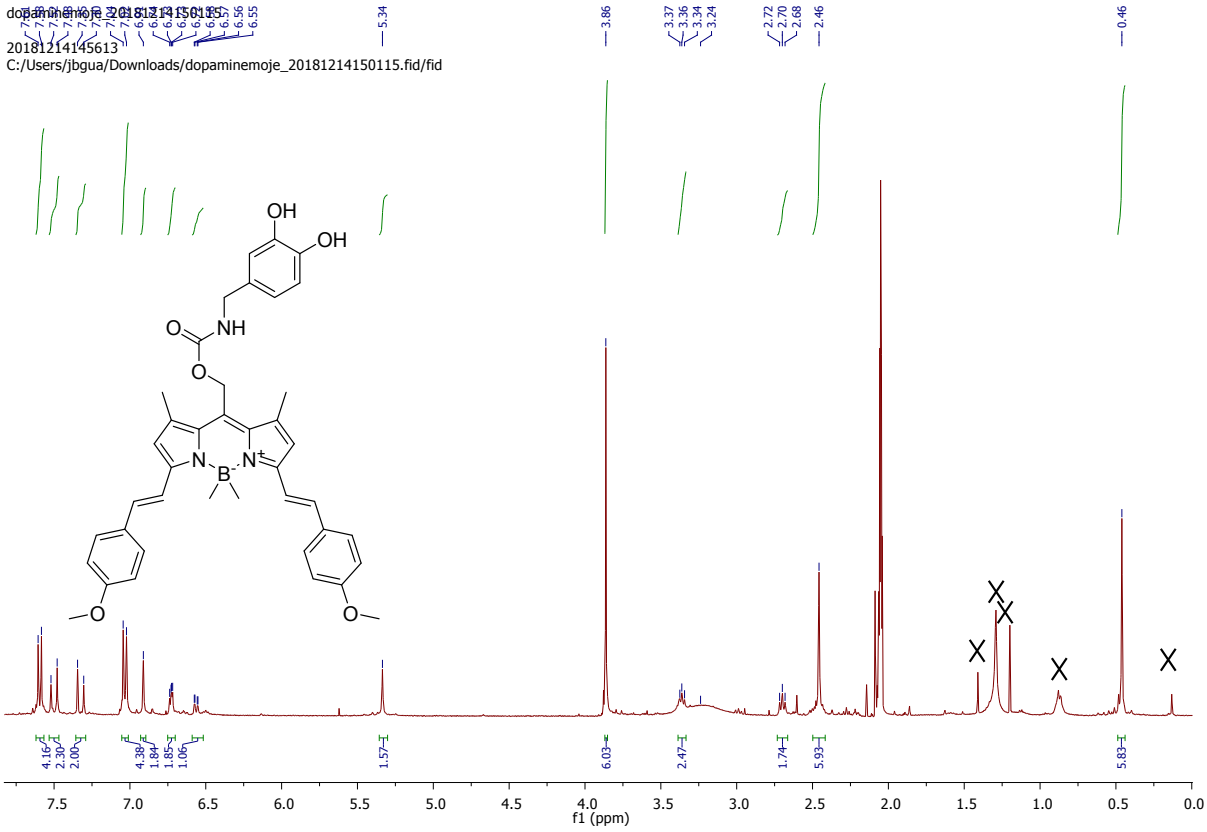
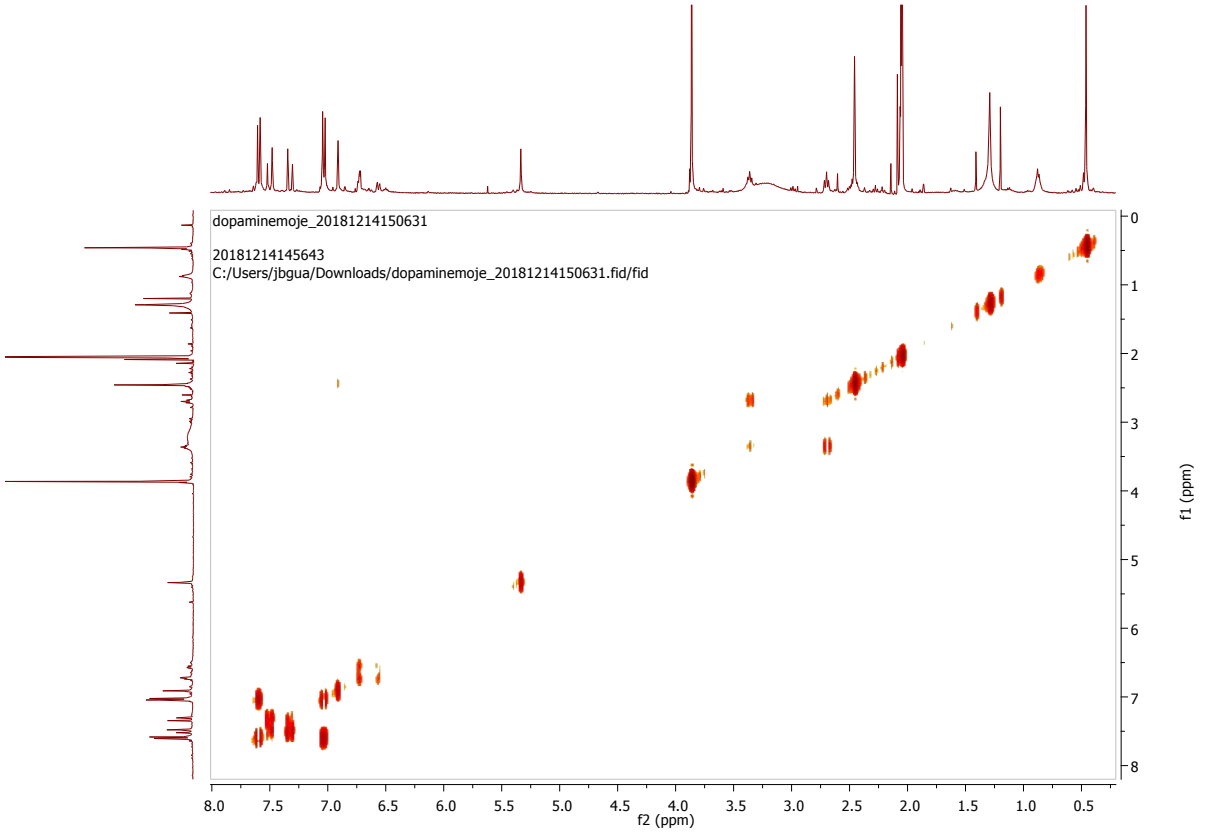
f2 (ppm)
f1 (ppm)


C:/Users/p276490/Desktop/k509_cdcl3_CARBON_20180115121819.fid/fid
58.86
55.38
20.76
16.11
f1 (ppm)


dopaminemoje_20181214150115
20181214145613
C:/Users/jbgua/Downloads/dopaminemoje_20181214150115.fid/fid
OH
OH
NH
O
O
N
B
N+
O
O
f1 (ppm)


dopaminemoje_20181214150631
20181214145643
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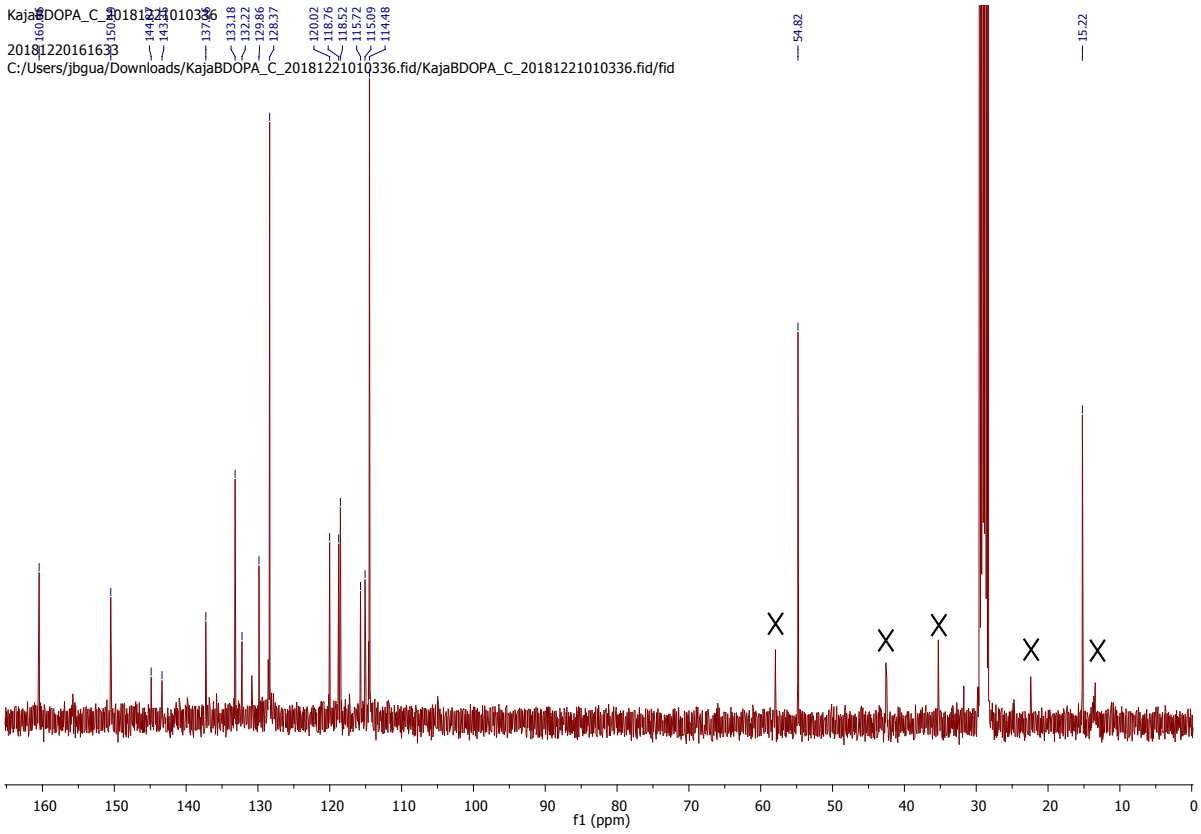
f2 (ppm)
f1 (ppm)



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