Electronic Supplementary Material (ESI) for ChemComm.

Sydnones-Based Turn-On Fluorogenic Probes for No-Wash Protein Labeling and in-Cell Imaging

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Supplementary Information

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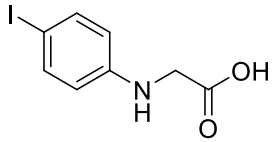
I. General Information

Organic solvents (Aldrich) were used without further purification. Unless otherwise noted, all other commercially available reagents and solvents were used without further purification. Purifications of reactions products were carried out by flash chromatography using Merck silica gel (40-63 µm). FT-ATR-IR spectra were recorded on a Perkin-Elmer UAR Two Spectrum spectrometer and are reported as wavelength numbers (cm^{-1}). ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) were measured on a Brucker Avance 400 MHz spectrometer. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) were measured on a Brucker Avance 600 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ) downfield from residual solvents peaks and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), broad singlet (br. s), doublet (d), triplet (t), quartet (q) and quintet (quin). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). Electrospray mass spectra were obtained using an ESI-Quadripole autopurify, Waters (pump: 2545, mass: ZQ2000) mass Spectrometer. Melting points were measured on a Büchi B-545 and are reported in °C. Absorbances were measured on a Varian Cary® 50 UV-Vis spectrophotometer. Fluorescence spectra were obtained on a HORIBA FluoroMax®-4 fluorimeter. Fluorescence analyses for kinetic studies were recorded on a molecular Device SpectraMax® M5e.

II. Synthesis and Analytical Data for Sydnones Syd 1-9

2-[(4-iodophenyl)amino]acetic acid **(Int1)**

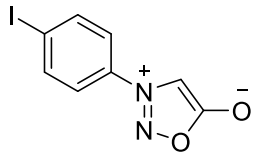


$C_8H_8INO_2$
MW: 277.06 $g.mol^{-1}$
Light purple solid
Yield: 97%

To a solution of *p*-iodoaniline (11.0 g, 50.0 mmol) in MeOH (350 mL) at 0 °C was added NaOAc (8.20 g, 100 mmol), glacial acetic acid (11.5 mL, 200 mmol), glyoxylic acid monohydrate (6.90 g, 75.0 mmol) and $NaBH_3CN$ (3.14 g, 50.0 mmol). The solution was warmed slowly to room temperature and stirred overnight. The reaction mixture was then filtered through a plug of silica gel and washed with a solution of acetic acid 1% in EtOAc. Brine was added and the aqueous layer was extracted twice with EtOAc. The organic layers were combined, dried over $MgSO_4$ and evaporated under reduced pressure. 12.6 g (45.0 mmol, 97%) of compound **Int4** were isolated.

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.55 (br. s, 1H), 7.32 (d, *J* = 8.6 Hz, 2H), 6.12 (br. s, 1H), 6.39 (d, *J* = 8.6 Hz, 2H), 3.77 (s, 2H).
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 172.3, 148.0, 137.0 (2C), 114.8 (2C), 76.7, 44.4.
IR (cm^{-1}): 3417, 2883, 1873, 1715, 1589, 1505, 1435, 1392, 1349, 1316, 1292, 1270, 1234, 1181, 1145, 1113, 1059, 993, 914, 810.
LCMS (ESI): *m/z*: 278 $[M+H]^+$.
Mp.: 119–121 °C.

***3-(4-iodophenyl)-1,2,3λ^5-oxadiazol-3-ylium-5-olate* (1)**

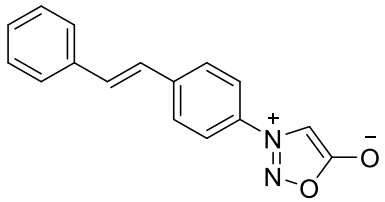


$C_8H_5IN_2O_2$
MW: 288.04 g.mol^{-1}
Beige solid
Yield: 75%

To a solution of compound **Int1** (12.6 g, 45.0 mmol) in THF (80 mL) was added *t*-butyl nitrite (5.88 mL, 49.5 mmol). The mixture was stirred at room temperature for 1 h and trifluoroacetic anhydride (6.88 mL, 49.5 mmol) was added. After 3 h stirring at room temperature, EtOAc was added and the reaction was quenched with a saturated solution of $NaHCO_3$. The aqueous layer was extracted three times with EtOAc. The organic layers were combined, dried over $MgSO_4$ and evaporated under reduced pressure. 9.77 g (33.9 mmol, 75%) of compound **1** were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.08 (d, *J* = 8.7 Hz, 2H), 7.80 (s, 1H), 7.73 (d, *J* = 8.7 Hz, 2H).
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 168.4, 138.9 (2C), 134.2, 123.3 (2C), 99.6, 94.9.
IR (cm^{-1}): 3115, 1725, 1578, 1491, 1439, 1412, 1115, 1020, 1003, 947, 857, 819.
LCMS (ESI): *m/z*: 289 $[M+H]^+$.
Mp.: 150–152 °C.
HRMS (ESI): *m/z* calcd for $C_8H_6N_2O_2I$ $(M+H^+)$: 288.9474; found: 288.9464.

***3-{4-[(E)-2-phenylethenyl]phenyl}-1,2,3λ^5-oxadiazol-3-ylium-5-olate* (Syd1)**

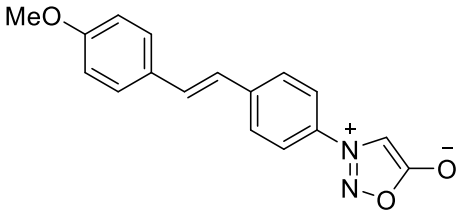


$C_{16}H_{12}N_2O_2$
MW: 264.28 g.mol^{-1}
Yellow solid
Yield: 45%

To a solution of styrene (521 mg, 5.00 mmol) in DMF (20 mL) was added $Pd(OAc)_2$ (22.5 mg, 1.00 mmol), dppe (39.8 mg, 1.00 mmol) and Et_3N (279 µL, 2.00 mmol). The mixture was warmed to 80 °C and compound **1** (288 mg, 1.00 mmol) was added over a period of 12 h. The solution was stirred 12 more hours at 80 °C before being filtered through a plug a Celite and evaporated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, heptane/EtOAc 7/3). 119 mg (0.45 mmol, 45%) of compound **Syd1** were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.70 (s, 4H), 7.54 (m, 2H), 7.39 (m, 2H), 7.32 (m, 1H), 7.24 (d, *J* = 16.2 Hz, 1H), 7.13 (d, *J* = 16.2 Hz, 1H), 6.74 (s, 1H).
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 169.1, 141.9, 136.3, 133.4, 132.6, 129.0 (2C), 128.9, 128.0 (2C), 127.0 (2C), 126.2, 121.6 (2C), 93.4.
IR (cm^{-1}): 3111, 1758, 1453, 1174, 1006, 964, 945, 848, 826.
LCMS (ESI): *m/z*: 265 $[M+H]^+$.
Mp.: 172–174 °C.
HRMS (ESI): *m/z* calcd for $C_{16}H_{12}N_2O_2$ $(M+H^+)$: 265.0977; found: 265.0972.

***3-{4-[(E)-2-(4-methoxyphenyl)ethenyl]phenyl}-1,2,3λ^5-oxadiazol-3-ylium-5-olate* (Syd2)**

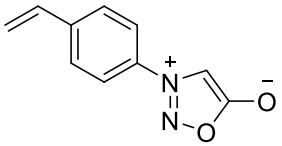


$C_{17}H_{14}N_2O_3$
MW: 294.31 g.mol^{-1}
Dark yellow solid
Yield: 45%

To a solution of 4-vinylanisole (671 mg, 5.00 mmol) in DMF (20 mL) was added $Pd(OAc)_2$ (22.5 mg, 1.00 mmol), dppe (39.8 mg, 1.00 mmol) and Et_3N (279 µL, 2.00 mmol). The mixture was warmed to 80 °C and compound **1** (288 mg, 1.00 mmol) was added over a period of 12 h. The solution was stirred 12 more hours at 80 °C before being filtered through a plug a Celite and evaporated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, heptane/EtOAc 7/3). 118 mg (0.45 mmol, 45%) of compound **Syd2** were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.68 (s, 4H), 7.49 (m, 2H), 7.19 (d, *J* = 16.2 Hz, 1H), 7.00 (d, *J* = 16.2 Hz, 1H), 6.93 (m, 2H), 6.72 (s, 1H), 3.85 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 168.9, 160.1, 142.1, 132.8, 132.0, 128.9, 128.2 (2C), 127.4 (2C), 123.8, 121.3 (2C), 114.3 (2C), 93.2, 55.3.
IR (cm^{-1}): 3128, 1763, 1596, 1571, 1508, 1456, 1360, 1298, 1250, 1173, 1025, 1005, 969, 944, 835.
LCMS (ESI): *m/z*: 295 $[M+H]^+$.
Mp.: 174–176 °C.
HRMS (ESI): *m/z* calcd for $C_{17}H_{15}N_2O_3$ $(M+H^+)$: 295.1083; found: 295.1079.

***3-(4-ethenylphenyl)-1,2,3λ^5-oxadiazol-3-ylium-5-olate* (2)**



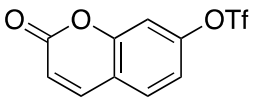
$C_{10}H_8N_2O_2$
MW: 188.19 g.mol^{-1}
Brown solid
Yield: 83%

To a solution of compound **1** (864 mg, 3.00 mmol) in DMF (60 mL) was added successively tetravinyltin (0.60 mL, 3.30 mmol), lithium chloride (382 mg, 9.00 mmol) and $PdCl_2(PPh_3)_2$ (105 mg, 0.15 mmol). The mixture was stirred at room temperature overnight. The solution was evaporated under reduced pressure and the residue was dissolved in dichloromethane and a solution of NaOH 1 M. This mixture was stirred during 1 h and the aqueous layer was extracted with dichloromethane. The organic layers were combined, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, heptane/EtOAc 7/3). 466 mg (2.48 mmol, 83%) of compound **2** were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.68 (d, *J* = 8.8 Hz, 2H), 7.62 (d, *J* = 8.8 Hz, 2H), 6.77 (dd, *J* = 11.1 Hz, *J* = 17.5 Hz, 1H), 6.72 (s, 1H), 5.90 (d, *J* = 17.5 Hz, 1H), 5.47 (d, *J* = 11.1 Hz, 1H).
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 169.1, 142.0, 134.9, 127.9 (2C), 121.5 (2C), 117.9, 93.5, 31.1.
IR (cm^{-1}): 3114, 1760, 1451, 946, 926, 855, 844.
LCMS (ESI): *m/z*: 189 $[M+H]^+$.
Mp.: 86–88 °C.

HRMS (ESI): *m/z* calcd for $C_{10}H_9N_2O_2$ ($M+H^+$) : 189.0664; found: 189.0655.

***2-oxo-2H-chromen-7-yl trifluoromethanesulfonate* (Int2)**

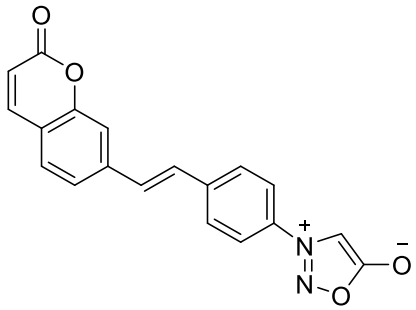


$C_{10}H_5F_3O_5S$
MW: 294.20 g.mol^{-1}
Pale yellow oil
Yield: 91%

Compound **Int2** was synthesized according to a reported procedure.[1]

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.72 (d, *J* = 9.6 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 1H), 7.29 (d, *J* = 2.3 Hz, 1H), 7.23 (dd, *J* = 8.6 Hz, *J* = 2.3 Hz, 1H), 6.50 (d, *J* = 9.6 Hz, 1H).
LCMS (ESI): *m/z*: 295 $[M+H]^+$.

***3-{4-[(E)-2-(2-oxo-2H-chromen-7-yl)ethenyl]phenyl}-1,2,3λ^5-oxadiazol-3-ylium-5-olate* (Syd3)**



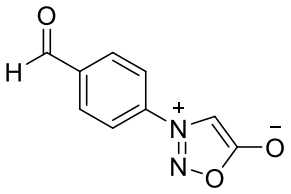
$C_{19}H_{12}N_2O_4$
MW: 332.08 g.mol^{-1}
Dark yellow solid
Yield: 40%

To a solution of compound **2** (94.1 mg, 0.50 mmol) in DMF (5.0 mL) was added compound **Int2** (221 mg, 0.75 mmol), $Pd(OAc)_2$ (11.2 mg, 0.05 mmol), dppe (19.2 mg, 0.05 mmol) and Et_3N (139 µL, 1.00 mmol). The mixture was stirred at 80 °C overnight. The reactional mixture was filtered through a plug of Celite, washed with EtOAc and evaporated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, dichloromethane/EtOAc 9/1). 65.9 mg (0.20 mmol, 40%) of compound **Syd3** were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.64 (m, 3H), 7.54–7.43 (m, 4H), 7.07 (d, *J* = 1.4 Hz, 1H), 6.80 (dd, *J* = 17.4 Hz, *J* = 10.9 Hz, 1H), 6.40 (d, *J* = 9.6 Hz, 1H), 5.93 (d, *J* = 17.4 Hz, 1H), 5.51 (d, *J* = 10.9 Hz, 1H).
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 166.6, 160.3, 153.9, 143.3, 142.7, 142.2, 134.8, 133.2, 128.4, 128.2 (2C), 125.0 (2C), 122.8, 118.7, 118.4, 117.4, 114.5, 106.2.
IR (cm^{-1}): 1725, 1612, 1267, 1224, 1101, 948, 844.
LCMS (ESI): *m/z*: 333 $[M+H]^+$.
Mp.: 176–178 °C
HRMS (ESI): *m/z* calcd for $C_{19}H_{13}N_2O_4$ ($M+H^+$) : 333.0875; found: 333.0872.

[1] Thompson, A. L. S.; Kabalka, G. W.; Akula, M. R.; Huffman J. W. Synthesis **2004**, *4*, 547–550.

3-(4-formylphenyl)-1,2,3λ^5-oxadiazol-3-ylium-5-olate **(3)**

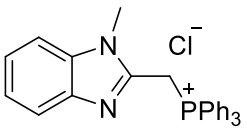


$C_9H_6N_2O_3$
MW: 190.16 g.mol^{-1}
Orange solid
Yield: 52%

To a solution of compound **2** (282 mg, 1.50 mmol) in a mixture acetone/water 3/1 (6 mL) was added NMO (264 mg, 2.25 mmol) and OsO_4 (2.5 wt% in *t*BuOH, 0.94 mL, 0.075 mL). The mixture was stirred at room temperature during 4 h. A 25% solution of $Na_2S_2O_3$ was added and the aqueous layer was extracted six times with ethyl acetate. The organic layers were combined, dried over $MgSO_4$ and evaporated under reduced pressure. The residue was solubilized in a mixture acetone/water 3/1 and $NaIO_4$ (642 mg, 3.00 mmol) was added. The mixture was stirred at room temperature during 6 h. The suspension was filtered through a plug of Celite and washed with EtOAc. The aqueous layer was extracted three times with EtOAc. The organic layers were combined, dried over $mgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, heptane/EtOAc 7/3 → 5/5). 148 mg (0.78 mmol, 52%) of compound **3** were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.48 (s, 1H), 8.50 (d, *J* = 8.6 Hz, 2H), 8.29 (d, *J* = 8.6 Hz, 2H), 7.21 (s, 1H).
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 190.1, 168.7, 139.0, 138.6, 131.5 (2C), 122.2 (2C), 93.9.
IR (cm^{-1}): 3148, 1751, 1702, 1598, 1455, 1295, 1206, 1226, 949, 825.
LCMS (ESI): *m/z*: 191 $[M+H]^+$.
Mp.: 145–147 °C.
HRMS (ESI): *m/z* calcd for $C_9H_7N_2O_3$ $(M+H^+)$: 191.0457; found: 191.0451.

[(1-methyl-1H-1,3-benzodiazol-2-yl)methyl]triphenylphosphanium chloride **(Int3)**



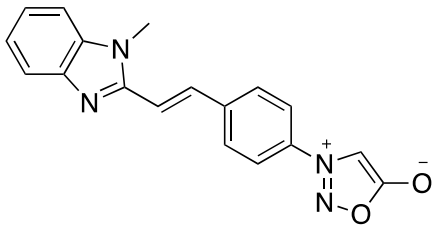
$C_{27}H_{24}ClN_2P$
MW: 442.93 g.mol^{-1}
White solid
Yield: 89%

Compound **Int3** was synthesized according to reported procedure.[2]

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.92 (m, 6H), 7.71 (m, 3H), 7.59 (td, *J* = 8.0 Hz, *J* = 3.5 Hz, 6H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 3.5 Hz, 2H), 7.17–7.12 (m, 1H), 5.98 (d, *J* = 14.5 Hz, 2H), 3.86 (s, 3H).
LCMS (ESI): *m/z*: 408 $[M-Cl]^+$.

[2] Dumat, B.; Bordeau, G.; Faurel-Paul, E.; Mahuteau-Betzer, F.; Saettel, N.; Metge, G.; Fiorini-Debuisschert, C.; Charra, F.; Teulade-Fichou, M.-P. *J. Am. Chem. Soc.* **2013**, *135*, 12697–12706.

***3-{4-[(E)-2-(1-methyl-1H-1,3-benzodiazol-2-yl)ethenyl]phenyl}-1,2,3λ^5-oxadiazol-3-ylium-5-olate* (Syd4)**

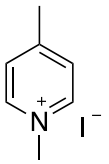


$C_{18}H_{14}N_4O_2$
MW: 318.34 g.mol^{-1}
Yellow solid
Yield: 89%

To a solution of compound **Int3** (97.4 mg, 0.22 mmol) in THF (1.0 mL) at -78 °C was added dropwise *n*-buthyl lithium (2.5 M in hexanes, 0.01 mL, 0.24 mmol). The solution was warmed to 0 °C. After 1 h at 0 °C, a solution of compound **3** (38.0 mg, 0.20 mmol) in THF (1.0 mL) was added at -78 °C. After which, the reactional mixture was warmed slowly to room temperature and stirred overnight. Water and dichloromethane was added and the aqueous layer was extracted with dichloromethane. The organic layers were combined, dried over $mgSO_4$ and evaporated under reduced pressure. The crude product was purified by preparative TLC (SiO_2, dichloromethane/MeOH 98/2, Et_3N 1%). 57.0 mg (0.18 mmol, 89%) of compound **Syd4** were isolated.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 8.14 (d, *J* = 8.8 Hz, 2H), 8.01 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 15.9 Hz, 1H), 7.86 (s, 1H), 7.74 (d, *J* = 15.9 Hz, 1H), 7.63 (d, *J* = 7.7 Hz, 1H), 7.58 (d, *J* = 7.7 Hz, 1H), 7.28–7.21 (m, 2H), 3.98 (s, 3H).
^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 168.5, 150.4, 142.8, 140.0, 136.1, 134.0, 133.6, 128.8 (2C), 122.4, 122.3, 121.8 (2C), 118.7, 117.5, 110.4, 94.7, 29.7.
IR (cm^{-1}): 1729, 1454, 1390, 1332, 1236, 1008, 948, 869, 825, 742, 721, 688, 514.
LCMS (ESI): *m/z*: 319 $[M+H]^+$.
Mp.: 242–244 °C.
HRMS (ESI): *m/z* calcd for $C_{18}H_{15}N_4O_2$ $(M+H^+)$: 319.1195; found: 319.1196.

***1,4-dimethylpyridin-1-ium iodide* (Int4)**



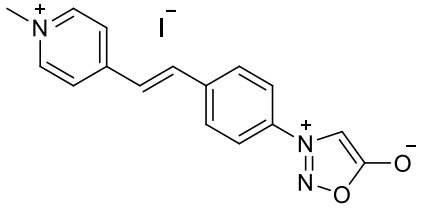
$C_7H_{10}IN$
MW: 235.07 g.mol^{-1}
Light pink solid
Yield: 95%

Compound **Int4** was synthesized according to a reported procedure.[3]

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 8.81 (d, *J* = 6.5 Hz, 2H), 7.95 (d, *J* = 6.5 Hz, 2H), 4.26 (s, 3H), 2.59 (s, 3H).

[3] Busi, M.; Cantadori, B.; Boccini, F.; De Zorzi, R.; Geremia, S.; Dalcanale, E. *Eur. J. Org. Chem.* **2011**, *2011*, 2629–2642.

***3-{4-[(E)-2-(1-methylpyridin-1-ium-4-yl)ethenyl]phenyl}-1,2,3λ⁵-oxadiazol-3-ylium-5-olate iodide* (Syd5)**

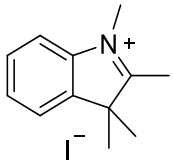


$C_{16}H_{14}IN_3O_2$
MW: 407.21 g.mol^{-1}
Dark brown solid
Yield: 73%

To a solution of compounds **3** (83.7 mg, 0.44 mmol) and **Int4** (94.0 mg, 0.40 mmol) in MeOH (8.0 mL) was added three drops of piperidine. The mixture was stirred at 75 °C during 24 h. Et_2O was added and the mixture was cooled to 0 °C and stirred during 1 h, after which the precipitate was filtered. 119 mg (0.29 mmol, 73%) of compound **Syd5** were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.93 (d, *J* = 6.6 Hz, 2H), 8.27 (d, *J* = 6.6 Hz, 2H), 8.14–8.02 (m, 5H), 7.87 (s, 1H), 7.73 (d, *J* = 16.4 Hz, 1H), 4.29 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 168.5, 151.7, 145.4 (2C), 138.9, 138.2, 135.0, 129.4 (2C), 126.3, 124.0 (2C), 122.2 (2C), 94.8, 47.2.
IR (cm^{-1}): 345, 3035, 1732, 1622, 1518, 1447, 1341, 1183, 985, 950, 845.
LCMS (ESI): *m/z*: 280 $[M\text{-}I]^+$.
Mp.: 100 °C (decomp.)
HRMS (ESI): *m/z* calcd for $C_{16}H_{14}N_3O_2$ (M-I$^-$) : 280.1086; found: 280.1081.

***1,2,3,3-tetramethyl-3H-indol-1-ium iodide* (Int5)**



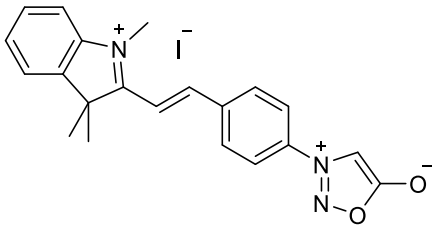
$C_{12}H_{16}IN$
MW: 301.17 g.mol^{-1}
Red solid
Yield: 84%

Compound **Int5** was synthesized according to a reported procedure.[4]

^{1}H NMR (400 MHz, MeOD-d_4, δ ppm): 7.87–7.81 (m, 1H), 7.79–7.73 (m, 1H), 7.68–7.62 (m, 2H), 4.86 (s, 3H) 4.05 (s, 3H), 1.60 (s, 6H).
^{13}C NMR (100 MHz, MeOD-d_4, δ ppm): 197.7, 143.7, 143.2, 131.1, 130.4, 124.5, 116.2, 55.8, 36.6, 30.8, 22.7 (2C).
LCMS (ESI): *m/z*: 174 $[M\text{-}I]^+$.

[4] Wu, I.-C.; Yu, J.; Ye, F.; Rong, Y.; Gallina, M. E.; Fujimoto, B. S.; Zhang, Y.; Chan, Y.-H.; Sun, W.; Zhou, X.-H.; Wu, C.; Chiu, D. T. *J. Am. Chem. Soc.* **2015**, *137*, 173–178.

***3-{4-[(E)-2-(1,3,3-trimethyl-3H-indol-1-ium-2-yl)ethenyl]phenyl}-1,2,3λ^5-oxadiazol-3-ylium-5-olate iodide* (Syd6)**

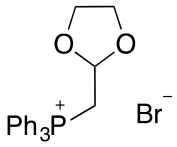


$C_{21}H_{20}IN_3O_2$
MW: 473.31 g.mol^{-1}
Dark brown solid
Yield: 44%

To a solution of compounds **3** (523 mg, 2.75 mmol) and **Int5** (753 mg, 2.50 mmol) in MeOH (8.0 mL) was added six drops of piperidine. The mixture was stirred at 40 °C during 16 h. EtOAc was added and the mixture was cooled to 0 °C and stirred during 1 h, after which the precipitate was filtered. 518 mg (1.09 mmol, 44%) of compound **Syd6** were isolated.

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.53 (d, *J* = 8.6 Hz, 2H), 8.48 (d, *J* = 16.6 Hz, 1H), 8.17 (d, *J* = 8.6 Hz, 2H), 7.99–7.96 (m, 1H), 7.95 (s, 1H), 7.94–7.90 (m, 1H), 7.87 (d, *J* = 16.6 Hz, 1H), 7.70–7.64 (m, 2H), 4.23 (s, 3H), 1.82 (s, 6H).
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 181.8, 168.5, 149.7, 144.0, 141.8, 138.0, 136.6, 131.7 (2C), 130.0, 121.1, 123.0, 122.0 (2C), 116.0, 115.7, 95.1, 52.6, 35.1, 25.0 (2C).
IR (cm^{-1}): 1750, 1614, 1586, 1555, 1451, 1169, 1008, 947, 831.4, 811.5, 762.8, 723, 514.
LCMS (ESI): *m/z*: 346 [M-I]$^+$.
Mp.: 228–230 °C.

***(1,3-dioxolan-2-ylmethyl)triphenylphosphanium bromide* (Int6)**



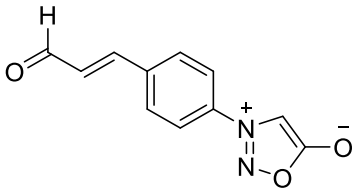
$C_{22}H_{22}BrO_2P$
MW: 426.29 g.mol^{-1}
White solid
Yield: 44%

Compound **Int6** was synthesized according to a reported procedure.[5]

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.91–7.71 (m, 15H), 5.19 (m, 1H), 4.22 (dd, *J* = 14.3 Hz, *J* = 4.7 Hz, 2H), 3.72 (m, 4H).
LCMS (ESI): *m/z*: 349 [M-Br]$^+$.

[5] Cresp, T. M.; Sargent, M. V.; Vogel, P. *J. Chem. Soc., Perkin Trans. 1* **1974**, 37–41.

***3-{4-[(1E)-3-oxoprop-1-en-1-yl]phenyl}-1,2,3λ^5-oxadiazol-3-ylium-5-olate* (4)**

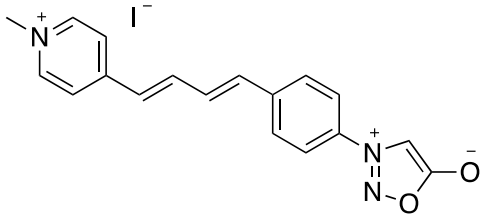


$C_{11}H_8N_2O_3$
MW: 216.20 g.mol^{-1}
Yellow solid
Yield: 67%

To a solution of compound **3** (114 mg, 0.60 mmol) in dichloromethane (20 mL) was added TDA-1 (0.192 mL, 0.60 mmol), a saturated solution of K_2CO_3 (20 mL) and compound **Int6** (256 mg, 0.60 mmol). The solution was refluxed overnight. The aqueous layer was extracted three times with dichloromethane. The organic layers were combined, washed with H_2O, dried over $MgSO_4$ and evaporated under reduced pressure to obtain compound the corresponding sydnone-dioxolane with triphenylphosphine oxide. The residue was dissolved in a mixture THF/ water 1/1 (12 mL) and acetic acid (3.0 mL) was added. The aqueous layer was extracted three times with dichloromethane. The organic layers were combined, washed with a saturated solution of $NaHCO_3$, dried over $mgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, heptane/EtOAc 8/2 → 2/8). 87.0 mg (0.40 mmol, 67%) of compound **4** were isolated.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.78 (d, *J* = 7.5 Hz, 1H), 7.82 (s, 4H), 7.54 (d, *J* = 16.0 Hz, 1H), 6.81 (dd, *J* = 16.0 Hz, *J* = 7.5 Hz, 1H), 6.79 (s, 1H).
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.0, 168.9, 148.9, 138.3, 136.0, 131.4 (2C), 130.1, 122.1 (2C), 93.6.
IR (cm^{-1}): 1740, 1673, 1624, 1451, 1121, 980, 947, 815, 726.
LCMS (ESI): *m/z*: 217 $[M+H]^+$.
Mp.: 181-183 °C.

***3-{4-[(1E,3E)-4-(1-methylpyridin-1-ium-4-yl)buta-1,3-dien-1-yl]phenyl}-1,2,3λ^5-oxadiazol-3- ylium-5-olate iodide* (Syd7)**



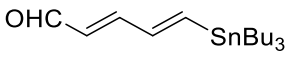
$C_{18}H_{16}IN_3O_2$
MW: 433.25 g.mol^{-1}
Brown solid
Yield: 20%

To a solution of compounds **4** (541 mg, 2.50 mmol) and **Int4** (534 mg, 2.27 mmol) in MeOH (50 mL) was added six drops of piperidine. The mixture was stirred at 60 °C during 6 h. Et_2O was added and the mixture was cooled to 0 °C and stirred during 1 h, after which the precipitate was filtered. 194 mg (0.45 mmol, 20%) of compound **Syd7** were isolated.

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.84 (d, *J* = 6.7 Hz, 2H), 8.19 (d, *J* = 6.7 Hz, 2H), 7.99 (d, *J* = 8.8 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H), 7.84 (dd, *J* = 15.2 Hz, *J* = 10.8 Hz, 1H), 7.84 (s, 1H), 7.47 (dd, *J* = 15.5 Hz, *J* = 10.8 Hz, 1H), 7.17 (d, *J* = 15.2 Hz, 1H), 7.00 (d, *J* = 15.5 Hz, 1H), 4.25 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.5, 151.9, 145.1 (2C), 140.8, 139.9, 137.6, 134.1, 131.1, 128.7 (2C), 128.5, 123.6 (2C), 121.9 (2C), 94.7, 47.0.
IR (cm^{-1}): 3457, 3033, 1735, 1643, 1602, 1517, 1448, 1006, 949, 869, 814, 725, 509.
LCMS (ESI): *m/z*: 306 $[M\text{-}I]^+$.
Mp.: 218-220 °C.
HRMS (ESI): *m/z* calcd for $C_{18}H_{16}N_3O_2$ (M-I$^-$) : 306.1237; found: 306.1240.

***(2E,4E)-5-(tributylstannyl)penta-2,4-dienal* (Int7)**

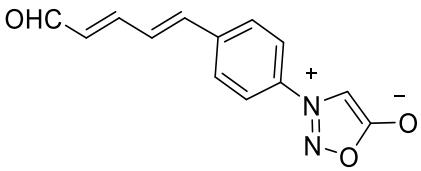


$C_{17}H_{32}OSn$
MW: 372.15 g.mol^{-1}
Yellow oil
Yield: 51%

Compound **Int7** was synthesized according to a reported procedure from pyridine in 3 steps.[6]

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 9.56 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 18.8 Hz, 1H), 7.00 (dd, *J* = 15.3 Hz, 10.2 Hz, 1H), 6.79 (dd, *J* = 18.8 Hz, 10.2 Hz, 1H), 6.06 (dd, *J* = 15.0 Hz, 8.0 Hz, 1H), 1.56–1.48 (m, 6H), 1.36–1.28 (m, 6H), 1.04–0.88 (m, 15H).

***3-(4-((1E,3E)-5-oxopenta-1,3-dien-1-yl)phenyl)-1,2,3-oxadiazol-3-ium-5-olate* (5)**



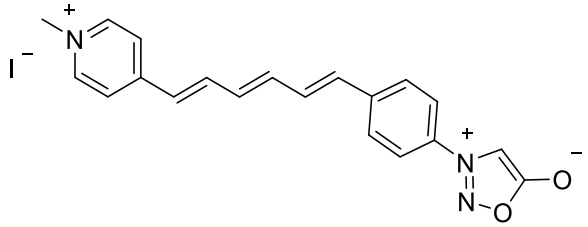
$C_{13}H_{10}N_2O_3$
MW: 242.07 g.mol^{-1}
light yellow solid
Yield: 56%

To a solution of compound **1** (150 mg, 0.521 mmol) in dioxane (8 mL) was added **Int7** (252 mg, 0.677 mmol), NEt_3 (53 mg, 0.073 mL, 0.521 mmol), and $PdCl_2(PPh_3)_2$ (19 mg, 0.026 mmol) successively. The reaction mixture was stirred at 60 °C for overnight. Then the solvent was evaporated under reduced pressure and the residue was dissolved in 10 mL of CH_2Cl_2 followed by a 0.1 M solution of NaOH (5mL). Then the reaction mixture was stirred for 1 h and the aqueous layer was extracted with CH_2Cl_2 (3x15 mL). The organic layers were combined, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by chromatography (heptane/EtOAc=3:1) to obtain a light yellow solid as product **5** (71 mg, 0.293 mmol) in 56 % yield.

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.63 (d, *J* = 8.0 Hz, 1H), 8.0–7.91 (m, 4H), 7.82 (s, 1H), 7.57–7.41 (m, 2H), 7.29 (d, *J* = 15.2 Hz, 1H), 6.34 (dd, *J* = 14.8, 8.0 Hz, 1H).
^{13}C NMR (100 MHz, DMF-d_7, δ ppm): 194.2, 169.0, 152.0, 140.3, 139.9, 135.2, 133.5, 130.3, 129.3 (2C), 122.4 (2C), 94.8.
IR (cm^{-1}): 3476, 3138, 2919, 2849, 1740, 1674, 1620, 1599, 1452, 1394, 1234, 1157, 1116, 1013, 988, 946, 842.
LC MS (ESI) *m/z* : 243 $[M+H]^+$.
Mp.: 178–180 °C.

[6] Michels, T. D.; Rhee, J. U.; Vanderwal, C. D.; *Org. Lett*., **2008**, *10*(*21*), 4787-4790.

3-(4-((1E,3E,5E)-6-(1-methylpyridin-1-ium-4-yl)hexa-1,3,5-trien-1-yl)phenyl)-1,2,3-oxadiazol-3-ium-5-olate iodide (Syd8)



$C_{20}H_{18}IN_3O_2$
MW: 459.04 g.mol^{-1}
yellow solid
Yield: 37%

To a solution of compound **5** (20 mg, 0.0826 mmol) and **Int4** (19 mg, 0.0826 mmol) in MeOH (3.5 mL) was added one drops of piperidine. The mixture was stirred at 60 °C for 10 hrs. Then the reaction mixture was allowed to cool at the room temperature for 1 h followed by addition Et_2O (2.5 mL) and subsequently, the mixture was cooled at 0 °C for 1 h to recrystallize the compound. Then the precipitate was filtered to isolate the sydnone **Syd8** (14.1 mg, 0.0307 mmol) in 37 % as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 8.80 (d, *J* = 6.5 Hz, 2H), 8.13 (d, *J* = 6.4 Hz, 2H), 7.96 (d, *J* = 8.8 Hz, 2H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.82 (s, 1H), 7.79–7.75 (m, 1H), 7.38 (dd, J = 15.6 Hz, 10.8 Hz, 1H), 7.01–6.79 (m, 4H), 4.23 (s, 3H).

^{13}C NMR (101 MHz, DMSO-d$_6$, δ ppm): 194.40 , 168.83 , 152.35 , 142.52 , 140.67 , 135.75 , 134.08 , 132.75, 132.14, 131.66 , 128.65 , 122.17 , 94.95.

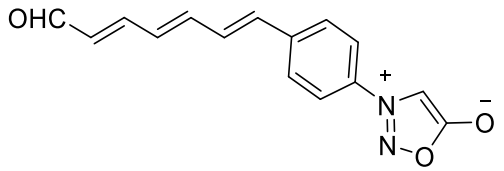
IR (cm^{-1}): 3441, 3063, 2925, 1742, 1641, 1619, 1598, 1516, 1449, 1366, 1189, 1150, 1006, 948, 878, 826.

LC MS (ESI) *m/z* : 332 $[M-I]^+$.

Mp.: 249-252 °C.

HRMS (ESI) : *m/z* calcd for $C_{20}H_{18}N_3O_2$ $[M-I]^+$: 332.1395; found: 332.1394.

3-(4-((1E,3E,5E)-7-oxohepta-1,3,5-trien-1-yl)phenyl)-1,2,3-oxadiazol-3-ium-5-olate (6)

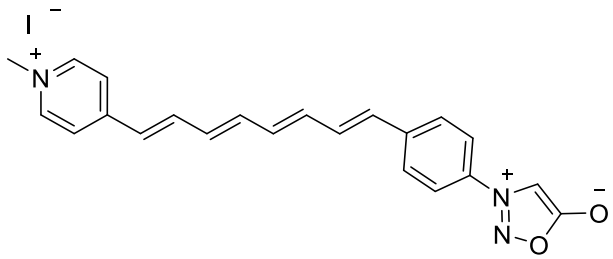


$C_{15}H_{12}N_2O_3$
MW: 268.08 g.mol^{-1}
yellow solid
Yield: 37%

To a stirred solution of unsaturated aldehyde **5** (40 mg, 0.165 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added TDA-1 (69 mg, 0.07 mL, 0.215 mmol) followed by a saturated solution of K_2CO_3 (10 mL) and compound **Int6** (91 mg, 0.215 mmol). The reaction mixture was stirred vigorously at reflux temperature for overnight. The aqueous layer was extracted three times with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$ and evaporated under reduced pressure to obtain protected aldehyde with triphenylphosphine oxide. The residue was dissolved in mixture of THF:H_2O (1:1) 10 mL followed by addition of excess acetic acid (3mL). The aqueous layer was extracted three times with CH_2Cl_2. The organic layers were combined and washed with saturated solution of $NaHCO_3$, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by column chromatography (DCM/EtOAc/MeOH=30:19:1) to obtain the desired aldehyde **6** (28 mg, 0.104 mmol, 63 %) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.58 (d, *J* = 8.0 Hz, 1H), 7.96–7.85 (m, 4H), 7.81 (s, 1H), 7.46 (dd, *J* = 15.1 Hz, 11.2 Hz, 1H), 7.31 (dd, *J* = 15.6 Hz, 10.9 Hz, 1H), 7.04 (dd, *J* = 15.1 Hz, 10.4 Hz, 2H), 6.79 (dd, *J* = 14.8 Hz, 11.2 Hz, 1H), 6.28 (dd, *J* = 15.1 Hz, 8.0 Hz, 1H).
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 194.0, 168.4, 152.0, 142.1, 140.3, 135.4, 133.7, 132.4, 131.8, 131.3, 128.3 (2C), 121.8 (2C), 94.6.
IR (cm^{-1}): 3444, 3075, 3024, 2853, 1750, 1672, 1589, 1603, 1512, 1483, 1435, 1332, 1179, 1116, 1027, 996, 748, 718.
LC MS (ESI) *m/z* : 269 $[M+H]^+$.
Mp.: 166-168 °C.

***3-(4-((1E,3E,5E,7E)-8-(1-methylpyridin-1-ium-4-yl)octa-1,3,5,7-tetraen-1-yl)phenyl)-1,2,3-oxadiazol-3-ium-5-olate iodide* (Syd9)**



$C_{22}H_{20}IN_3O_2$
MW: 485.06 g.mol^{-1}
brown solid
Yield: 32%

To a solution of compound **6** (17 mg, 0.0634 mmol) and **Int4** (15 mg, 0.0634 mmol) in MeOH (5 ml) was added one drop of piperidine. The mixture was stirred at 60 °C for 10 hrs. Then the reaction mixture was allowed to cool at the room temperature for 1 h followed by addition Et_2O (4 mL) and subsequently, the mixture was cooled at 0 °C for 1 h to recrystallize the compound. Then the precipitate was filtered to isolate the sydnone **Syd9** (10 mg, 0.021 mmol) in 32% as a brown solid.

^{1}H NMR (400 MHz, DMF-d_7, δ ppm): 8.99 (d, *J* = 6.4 Hz, 2H), 8.26 (d, *J* = 6.4 Hz, 2H), 8.03 (d, *J* = 3.1 Hz, 2H), 7.94–7.85 (m, 3H), 7.79 (s, 1H), 7.38 (dd, *J* = 15.5 Hz, 10.0 Hz, 1H), 6.97 (d, *J* = 15.5 Hz, 3H), 6.91–6.77 (m, 3H), 4.47 (s, 3H).
^{13}C NMR (101 MHz, DMF-d_7, δ ppm): 168.8, 152.9, 145.2 (2C), 141.8, 141.3, 141.1, 137.6, 134.9, 133.2, 133.0, 132.4, 128.1 (2C), 128.0, 127.0, 123.5 (2C), 121.9 (2C), 94.3, 46.9.
IR (cm^{-1}): 3436, 2931, 2869, 2811, 1650, 1495, 1438, 1387, 1254, 1095, 1062, 864.
LC MS (ESI) *m/z*: 358 $[M-I]^+$.
Mp.: 136 °C (decomp.)
HRMS (ESI): *m/z* calcd for $C_{22}H_{20}N_3O_2$ $[M-I]^+$: 358.1548; found: 358.1550.

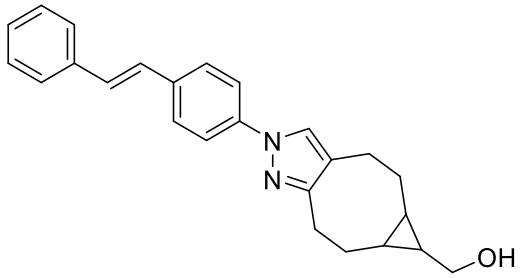
III. Synthesis and Analytical Data of pyrazoles Pyr1-9, Pyr5'-Pyr9'

General procedures for the preparation of cycloadducts

Procedure A : To a solution of sydnone (1.0 eq) in acetonitrile (0.1 M) was added the cyclooctyne (1.5 eq). The mixture was stirred at room temperature overnight. The solution was evaporated under reduced pressure and purified by flash chromatography (SiO_2, heptane/EtOAc).

Procedure B : To a solution of sydnone (1.0 eq) in acetonitrile (0.1 M) was added the cyclooctyne (1.05 eq). The mixture was stirred at room temperature overnight. The solution was evaporated under reduced pressure.

(11-{4-[(E)-2-phenylethenyl]phenyl}-10,11-diazatricyclo[7.3.0.0^{4,6}]dodeca-1(12),9-dien-5-yl)methanol **(Pyr1)**

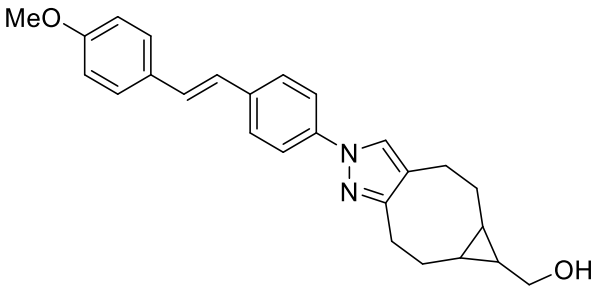


$C_{25}H_{26}N_2O$
MW: 370.50 g.mol^{-1}
Beige solid
Yield: 82%

The title compound **Pyr1** was obtained in 82% yield (30.2 mg, 0.08 mmol) from compound **Syd1** (26.4 mg, 0.10 mmol) and BCN (22.5 mg, 0.15 mmol) according to procedure A. The crude product was purified by flash chromatography (SiO_2, heptane/EtOAc 8/2 → 5/5).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.65–7.49 (m, 8H), 7.36 (m, 2H), 7.09 (s, 2H), 3.73 (m, 2H), 3.13 (ddd, *J* = 14.9 Hz, *J* = 8.2 Hz, *J* = 2.3 Hz, 1H), 2.86 (ddd, *J* = 14.9 Hz, *J* = 8.4 Hz, *J* = 2.4 Hz, 1H), 2.75 (ddd, *J* = 14.9 Hz, *J* = 8.9 Hz, *J* = 2.5 Hz, 1H), 2.56 (ddd, *J* = 14.9 Hz, *J* = 8.5 Hz, *J* = 2.5 Hz, 1H), 2.36–2.21 (m, 2H), 1.61 (br. s, 1H), 1.49–1.32 (m, 2H), 1.22–1.10 (m, 3H).
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 154.7, 139.3, 137.4, 134.7, 128.8 (2C), 128.5, 127.9, 127.8, 127.6 (2C), 126.6 (2C), 126.3, 122.6, 118.4 (2C), 60.2, 27.7, 25.0, 23.6, 23.7, 21.9, 21.3, 21.1.
IR (cm^{-1}): 3302, 2918, 1596, 1519, 1406, 1383, 1053, 1021, 964, 952, 816.
LCMS (ESI): *m/z*: 371 $[M+H]^+$.
Mp.: 192–194 °C.
HRMS (ESI): *m/z* calcd for $C_{25}H_{27}N_2O$ $(M+H^+)$: 371.2123; found: 371.2108.

***(11-{4-[(E)-2-(4-methoxyphenyl)ethenyl]phenyl}-10,11-diazatricyclo[7.3.0.0^{4,6}]dodeca-1(12),9-dien-5-yl)methanol* (Pyr2)**

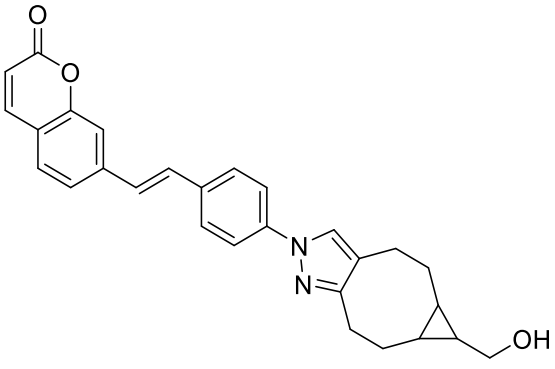


$C_{26}H_{28}N_2O_2$
MW: 400.52 g.mol^{-1}
Beige solid
Yield: 88%

The title compound **Pyr2** was obtained in 88% yield (35.0 mg, 0.09 mmol) from compound **Syd2** (29.4 mg, 0.10 mmol) and BCN (22.5 mg, 0.15 mmol) according to procedure A. The crude product was purified by flash chromatography (SiO_2, heptane/EtOAc 9/1).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.62 (s, 1H), 7.58 (m, 2H), 7.52 (m, 2H), 7.45 (m, 2H), 7.05 (d, *J* = 16.3 Hz, 1H), 6.96 (d, *J* = 16.3 Hz, 1H), 6.90 (m, 2H), 3.83 (s, 3H), 3.77–3.67 (m, 2H), 3.12 (ddd, *J* = 14.9 Hz, *J* = 8.3 Hz, *J* = 2.7 Hz, 1H), 2.86 (ddd, *J* = 14.9 Hz, *J* = 8.3 Hz, *J* = 2.7 Hz, 1H), 2.75 (ddd, *J* = 14.9 Hz, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 2.55 (ddd, *J* = 14.9 Hz, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 2.36–2.21 (m, 2H), 1.68 (br. s, 1H), 1.49–1.38 (m, 2H), 1.23–1.12 (m, 3H).
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 159.4, 154.6, 139.0, 135.0, 130.2, 128.0, 127.8 (2C), 127.3 (2C), 126.3, 125.8, 122.5, 118.4 (2C), 114.3 (2C), 60.3, 55.5, 27.7, 25.0, 23.8, 23.7, 21.9, 21.3, 21.1.
IR (cm^{-1}): 2923, 1604, 1564, 1519, 1386, 1248, 1025, 972, 951, 837.
LCMS (ESI): *m/z*: 401 $[M+H]^+$.
Mp.: 215–217 °C.
HRMS (ESI): *m/z* calcd for $C_{26}H_{29}N_2O_2$ $(M+H^+)$: 401.2229; found: 401.2219.

***7-[(E)-2-{4-[5-(hydroxymethyl)-10,11-diazatricyclo[7.3.0.0^{4,6}]dodeca-1(12),9-dien-11-yl]phenyl}ethenyl]-2H-chromen-2-on* (Pyr3)**



$C_{28}H_{26}N_2O_3$
MW: 438.53 g.mol^{-1}
Yellow solid
Yield: 79%

The title compound **Pyr3** was obtained in 79% yield (34.6 mg, 0.08 mmol) from compound **Syd3** (33.2 mg, 0.10 mmol) and BCN (22.5 mg, 0.15 mmol) according to procedure A. The crude product was purified by flash chromatography (SiO_2, heptane/EtOAc 7/3 → 5/5).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.69 (d, *J* = 9.6 Hz, 1H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.16 (s, 1H), 7.12 (d, *J* = 8.5 Hz, 2H), 7.01 (dd, *J* = 7.9 Hz, *J* = 1.0 Hz, 1H), 6.64 (dd, *J* = 17.5 Hz,

J = 10.8 Hz, 1H), 6.45 (d, *J* = 9.6 Hz, 1H), 5.69 (d, *J* = 17.5 Hz, 1H), 5.23 (d, *J* = 10.8 Hz, 1H), 3.75 (m, 2H), 3.15 (ddd, *J* = 15.0 Hz, *J* = 8.2 Hz, *J* = 2.8 Hz, 1H), 2.81 (ddd, *J* = 14.9 Hz, *J* = 8.6 Hz, *J* = 2.9 Hz, 1H), 2.77 (ddd, *J* = 12.3 Hz, *J* = 8.5 Hz, *J* = 2.8 Hz, 1H), 2.48 (ddd, *J* = 15.1 Hz, *J* = 7.9 Hz, *J* = 3.0 Hz, 1H), 2.39–2.29 (m, 1H), 2.29–2.20 (m, 1H), 1.62 (br. s, 1H) 1.57–1.39 (m, 2H), 1.31–1.16 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 160.5, 154.2, 154.0, 143.0, 139.1, 138.8, 136.3, 135.9, 134.8, 128.0, 126.8 (2C), 126.4, 124.7 (2C), 121.2, 118.5, 118.3, 117.3, 114.6, 60.2, 27.5, 24.9, 23.7, 22.8, 22.2, 21.1, 21.1.

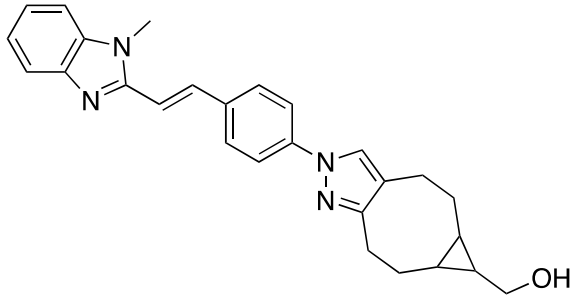
IR (cm^{-1}): 2925, 1732, 1617, 1516, 1372, 1103, 938, 909, 845.

LCMS (ESI): *m/z*: 439 $[M+H]^+$.

Mp.: 123–125 °C.

HRMS (ESI): *m/z* calcd for $C_{28}H_{27}N_2O_3$ $(M+H^+)$: 439.2022; found: 439.2023.

(11-{4-[(E)-2-(1-methyl-1H-1,3-benzodiazol-2-yl)ethenyl]phenyl}-10,11-diazatricyclo[7.3.0.0^{4,6}] dodeca-1(12),9-dien-5-yl)methanol **(Pyr4)**



$C_{27}H_{28}N_4O$
MW: 424.55 g.mol^{-1}
Yellow solid
Yield: 99%

The title compound **Pyr4** was obtained in 99% yield (42.5 mg, 0.10 mmol) from compound **Syd4** (31.8 mg, 0.10 mmol) and BCN (15.8 mg, 0.11 mmol) according to procedure B.

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.23 (s, 1H), 7.87 (d, *J* = 9.0 Hz, 2H), 7.84 (d, *J* = 16.0 Hz, 1H), 7.78 (d, *J* = 9.0 Hz, 2H), 7.63—7.58 (m, 1H), 7.56—7.51 (m, 1H), 7.48 (d, *J* = 16.0 Hz, 1H), 7.25—7.18 (m, 2H), 4.29 (t, *J* = 5.1 Hz, 1H), 3.94 (s, 3H), 3.48 (quin, *J* = 5.2 Hz, 2H), 2.99 (ddd, *J* = 14.9 Hz, *J* = 8.1 Hz, *J* = 2.5 Hz, 1H), 2.82 (ddd, *J* = 14.9 Hz, *J* = 8.1 Hz, *J* = 2.5 Hz, 1H), 2.64 (ddd, *J* = 14.7 Hz, *J* = 8.9 Hz, *J* = 2.6 Hz, 1H), 2.48 (ddd, *J* = 14.7 Hz, *J* = 8.9 Hz, *J* = 2.6 Hz, 1H), 2.21—2.09 (m, 2H), 1.43—1.26 (m, 2H), 1.05—0.93 (m, 3H).

^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 154.0, 151.1, 142.8, 139.6, 136.1, 135.0, 132.7, 128.6 (2C), 126.9, 122.3, 122.0, 121.9, 118.4, 117.2 (2C), 113.7, 110.1, 57.4, 29.6, 27.1, 24.1, 23.1, 22.7, 21.4, 20.1, 19.9.

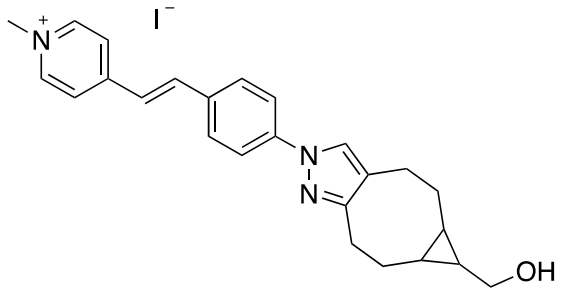
IR (cm^{-1}): 3339, 2918, 1603, 1565, 1519, 1462, 1437, 1383, 1329, 1122, 1030, 953, 816, 740.

LCMS (ESI): *m/z*: 426 $[M+H]^+$.

Mp.: 193–195 °C.

HRMS (ESI): *m/z* calcd for $C_{27}H_{29}N_4O$ $(M+H^+)$: 425.2341; found: 425.2331.

4-[(E)-2-{4-[5-(hydroxymethyl)-10,11-diazatricyclo[7.3.0.0^{4,6}]dodeca-1(12),9-dien-11-yl]phenyl} ethenyl]-1-methylpyridin-1-ium iodide **(Pyr5)**

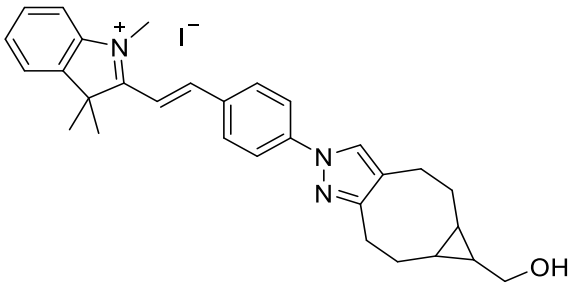


$C_{25}H_{28}IN_3O$
MW: 513.42 g.mol^{-1}
Brown solid
Yield: 99%

The title compound **Pyr5** was obtained in 99% yield (25.5 mg, 0.05 mmol) from compound **Syd5** (20.4 mg, 0.05 mmol) and BCN (7.89 mg, 0.05 mmol) according to procedure B.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 8.35 (d, *J* = 6.8 Hz, 2H), 7.77 (s, 1H), 7.70 (d, *J* = 6.8 Hz, 2H), 7.52 (d, *J* = 16.3 Hz, 1H), 7.34 (m, 4H), 7.00 (d, *J* = 16.3 Hz, 1H), 3.80 (t, *J* = 5.2 Hz, 1H), 3.75 (s, 3H), 2.97 (t, *J* = 5.6 Hz, 2H), 2.48 (ddd, *J* = 14.8 Hz, *J* = 7.9 Hz, *J* = 2.6 Hz, 1H), 2.32 (ddd, *J* = 15.0 Hz, *J* = 8.2 Hz, *J* = 2.6 Hz, 1H), 2.14 (ddd, *J* = 14.8 Hz, *J* = 8.9 Hz, *J* = 3.0 Hz, 1H), 1.97 (ddd, *J* = 15.0 Hz, *J* = 10.9 Hz, *J* = 3.0 Hz, 1H), 1.71—1.56 (m, 2H), 0.93—0.75 (m, 2H), 0.54—0.41 (m, 3H).
^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 154.5, 152.5, 145.1 (2C), 140.7, 139.9, 131.7, 129.5 (2C), 127.1, 123.3 (2C), 122.8, 122.4, 117.4 (2C), 57.4, 46.9, 27.1, 24.0, 23.1, 22.6, 21.4, 20.1, 19.9.
IR (cm^{-1}): 3382, 2921, 1599, 1520, 1380, 1179, 1020, 949, 839, 748.
LCMS (ESI): *m/z*: 386 $[M-I]^+$.
HRMS (ESI): *m/z* calcd for $C_{25}H_{28}N_3O$ $(M-I^-)$: 386.2232; found: 386.2226.

2-[(E)-2-{4-[5-(hydroxymethyl)-10,11-diazatricyclo[7.3.0.0^{4,6}]dodeca-1(12),9-dien-11-yl]phenyl}ethenyl]-1,3,3-trimethyl-3H-indol-1-ium iodide **(Pyr6)**



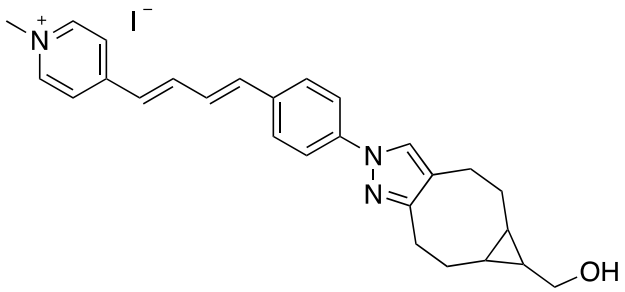
$C_{30}H_{34}IN_3O$
MW: 579.53 g.mol^{-1}
Red solid
Yield: 82%

The title compound **Pyr6** was obtained in 82% yield (47.5 mg, 0.08 mmol) from compound **Syd6** (47.3 mg, 0.10 mmol) and BCN (16.5 mg, 0.11 mmol) according to procedure B.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 8.42 (d, *J* = 16.4 Hz, 1H), 8.39 (s, 1H), 8.31 (d, *J* = 8.8 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H), 7.91–7.85 (m, 2H), 7.71–7.58 (m, 3H), 4.15 (s, 3H), 3.47 (m, 2H), 3.00 (ddd, *J* = 14.9 Hz, *J* = 7.9 Hz, *J* = 2.7 Hz, 1H), 2.84 (ddd, *J* = 14.9Hz, *J* = 7.9 Hz, *J* = 2.6 Hz, 1H), 2.71–7.62 (m, 1H), 2.54–2.45 (m, 1H), 2.22–2.09 (m, 2H), 1.80 (s, 6H), 1.44–1.26 (m, 2H), 1.06–0.90 (m, 3H).
^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 181.6, 155.4, 152.6, 143.5, 142.7, 141.8, 132.3 (2C), 130.9, 129.3, 128.9, 127.5, 123.5, 122.8, 117.2 (2C), 115.1, 112.0, 57.4, 52.0, 34.4, 27.0, 25.4 (2C), 23.8, 23.00, 22.4, 21.3, 20.0, 19.8.

IR (cm^{-1}): 3396, 2930, 1591, 1575, 1536, 1373, 1298, 1184, 1175, 945, 757.
LCMS (ESI): *m/z*: 453 $[M\text{-}I]^+$.
Mp.: 125–127 °C.

***4-[(1E,3E)-4-{4-[5-(hydroxymethyl)-10,11- diazatricyclo[7.3.0.0^{4,6}]dodeca-1(12),9-dien-11-yl]phenyl} buta-1,3-dien-1-yl]-1-methylpyridin-1-ium iodide* (Pyr7)**

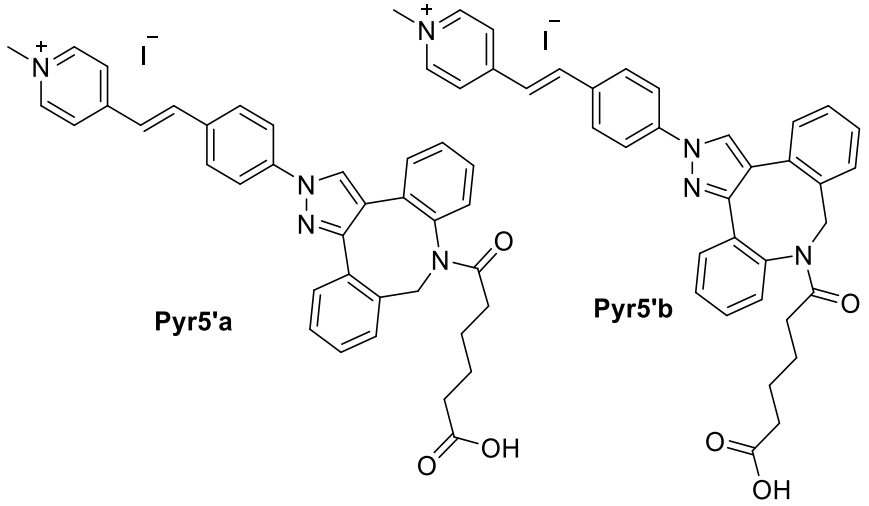


$C_{27}H_{30}IN_3O$
MW: 539.46 g.mol^{-1}
Brown solid
Yield: 86%

The title compound **Pyr7** was obtained in 86% yield (46.6 mg, 0.09 mmol) from compound **Syd7** (43.3 mg, 0.10 mmol) according to procedure B.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 8.77 (d, *J* = 6.7 Hz, 2H), 8.21 (s, 1H), 8.12 (d, *J* = 6.7 Hz, 2H), 7.81 (dd, *J* = 15.6 Hz, *J* = 10.6 Hz, 1H), 7.76 (d, *J* = 9.0 Hz, 2H), 7.68 (d, *J* = 9.0 Hz, 2H), 7.26 (dd, *J* = 15.4 Hz, *J* = 10.6 Hz, 1H), 7.07 (d, *J* = 15.6 Hz, 1H), 6.87 (d, *J* = 15.4 Hz, 1H), 4.28 (t, *J* = 5.0 Hz, 1H), 4.21 (s, 3H), 3.44 (t, *J* = 5.6 Hz, 2H), 2.95 (ddd, *J* = 14.7 Hz, *J* = 7.8 Hz, *J* = 2.5 Hz, 1H), 2.80 (ddd, *J* = 14.9 Hz, *J* = 8.1 Hz, *J* = 2.4 Hz, 1H), 2.63 (ddd, *J* = 14.9 Hz, *J* = 9.2 Hz, *J* = 2.8 Hz, 1H), 2.45 (ddd, *J* = 14.7 Hz, *J* = 8.3 Hz, *J* = 2.6 Hz, 1H), 2.18–2.04 (m, 2H), 1.40–1.23 (m, 2H), 1.01–0.91 (m, 3H).
^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 154.2, 152.3, 144.9 (2C), 141.9, 139.9, 139.6, 132.6, 128.7 (2C), 127.5, 127.0, 126.3, 123.2 (2C), 122.5, 117.4 (2C), 57.4, 46.8, 27.1, 24.0, 23.1, 22.7, 21.4, 20.1, 20.0.
IR (cm^{-1}): 3389, 2935, 1640, 1589, 1513, 1379, 1189, 1015, 952, 747.
LCMS (ESI): *m/z*: 413 $[M\text{-}I]^+$.
HRMS (ESI): *m/z* calcd for $C_{27}H_{30}N_3O$ (M-I) : 412.2383 ; found: 412.2384.

***4-[(E)-2-{4-[13-(5-carboxypentanoyl)-3,4,13/14-triazatetracyclo[13.4.0.0^{2,6}.0^{7,12}] nonadeca-1(19),2,5,7,9,11,15,17-octaen-4-yl]phenyl}ethenyl]-1-methylpyridin-1-ium iodide* (Pyr5'a) */ 4-[(E)-2-{4-[14-(5-carboxypentanoyl)-3,4,13/14-triazatetracyclo[13.4.0.0^{2,6}.0^{7,12}] nonadeca-1(19),2,5,7,9,11,15,17-octaen-4-yl]phenyl}ethenyl]-1-methylpyridin-1-ium iodide* (Pyr5'b)**

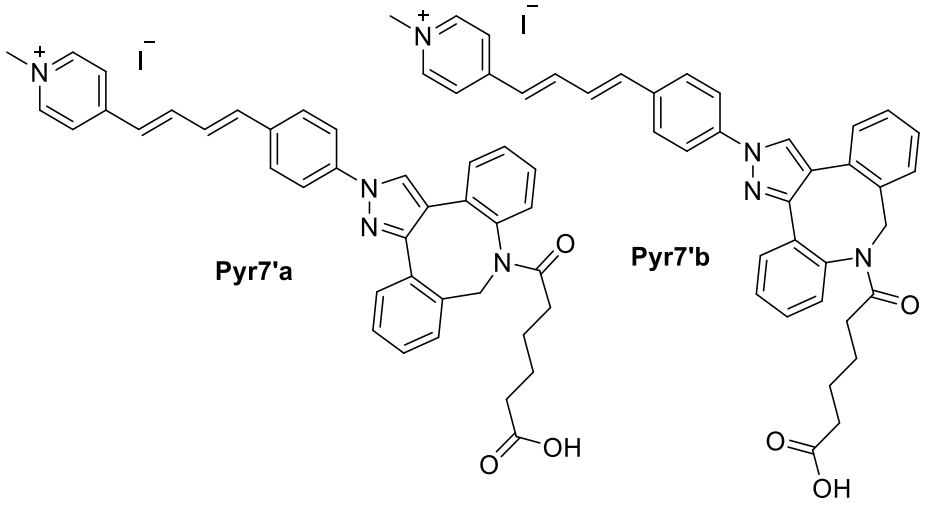


$C_{36}H_{33}IN_4O_3$
MW: 696.59 g.mol^{-1}
Brown solid
Yield: 97%

Compounds **Pyr5'a** and **Pyr5'b** were obtained as a mixture in 97% yield (16.9 mg, 0.025 mmol) from compound **Syd5** (10.4 mg, 0.024 mmol) and DIBAC (8.40 mg, 0.025 mmol) according to procedure B.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 8.88 (m, 6H), 8.21 (d, *J* = 6.7 Hz, 4H), 8.09—8.01 (m, 6H), 7.93–7.86 (m, 4H), 7.63—7.21 (m, 18H), 5.97 (d, *J* = 17.2 Hz, 1H), 5.93 (d, *J* = 17.2 Hz, 1H), 4.49 (d, *J* = 17.2 Hz, 1H), 4.48 (d, *J* = 17.2 Hz, 1H), 4.24 (s, 6H), 1.93 (t, *J* = 6.9 Hz, 2H), 1.86 (t, *J* = 6.9 Hz, 2H), 1.80—1.67 (m, 2H), 1.62—1.67 (m, 2H), 1.30—1.04 (m, 10H).
The ^{1}H NMR signals of compounds **Pyr5'a** *and* **Pyr5'b** *are undistinguishable.*
IR (cm^{-1}): 3429, 2956, 1732, 1234, 1160, 1048, 1025, 1005, 762.
LCMS (ESI): *m/z*: 570 $[M-I]^+$.
HRMS (ESI): *m/z* calcd for $C_{36}H_{33}N_4O_3$ $(M-I^-)$: 569.2547; found: 569.2548.

4-[(1E,3E)-4-{4-[13-(5-carboxypentanoyl)-3,4,13-triazatetracyclo[13.4.0.0^{2,6}.0^{7,12}]nonadeca-1(19),2,5,7,9,11,15,17-octaen-4-yl]phenyl}buta-1,3- dien-1-yl]-1-methylpyridin-1-ium iodide **(Pyr7'a)** ***/ 4-[(1E,3E)-4-{4-[14-(5-carboxypentanoyl)-3,4-diazatetracyclo[13.4.0.0^{2,6}.0^{7,12}]nonadeca-1(19),2,5,7,9,11,15,17-octaen-4-yl]phenyl}buta-1,3- dien-1-yl]-1-methylpyridin-1-ium iodide*** **(Pyr7'b)**

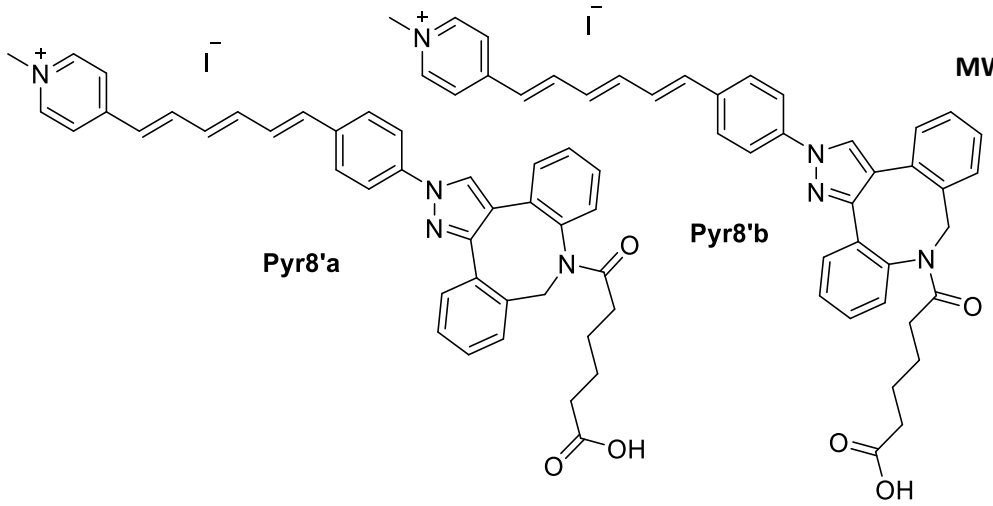


$C_{38}H_{35}IN_4O_3$
MW: 722.61 g.mol^{-1}
Brown solid
Yield: 97%

Compounds **Pyr7'a** and **Pyr7'b** were obtained as a mixture in 97% yield (16.8 mg, 0.023 mmol) from compound **Syd7** (10.4 mg, 0.024 mmol) and DIBAC (8.40 mg, 0.025 mmol) according to procedure B.

^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 8.81–8.77 (m, 5H), 8.14 (d, *J* = 6.7 Hz, 4H), 8.00–7.93 (m, 4H), 7.88–7.75 (m, 6H), 7.73–7.39 (m, 10H), 7.37–7.19 (m, 9H), 7.12 (d, *J* = 15.3 Hz, 2H), 6.91 (d, *J* = 15.4 Hz, 2H), 5.97 (d, *J* = 17.2 Hz, 1H), 5.92 (d, *J* = 17.2 Hz, 1H), 4.49 (d, *J* = 17.2 Hz, 1H), 4.47 (d, *J* = 17.2 Hz, 1H), 4.21 (s, 6H), 1.98–1.84 (m, 4H), 1.80–1.68 (m, 2H), 1.63–1.48 (m, 2H), 1.31–1.06 (m, 10H).
The ^{1}H NMR signals of compounds **Pyr7'a** *and* **Pyr7'b** *are undistinguishable.*
IR (cm^{-1}): 3346, 2927, 1643, 1591, 1514, 1402, 955, 761.
LCMS (ESI): *m/z*: 596 $[M-I]^+$.
HRMS (ESI): *m/z* calcd for $C_{38}H_{35}N_4O_3$ $(M-I^-)$: 595.2704 ; found: 595.2709.

***4-((1E,3E,5E)-6-(4-(8-(5-carboxypentanoyl)-8,9-dihydro-2H-dibenzo[b,f]pyrazolo[4,3-d]azocin-2-yl)phenyl)hexa-1,3,5-trien-1-yl)-1-methylpyridin-1-ium iodide* (Pyr8'a) */ 4-((1E,3E,5E)-6-(4-(9-(4-carboxybutyl)-8,9-dihydro-2H-dibenzo[b,f]pyrazolo[3,4-d]azocin-2-yl)phenyl)hexa-1,3,5-trien-1-yl)-1-methylpyridin-1-ium* (Pyr8'b)**

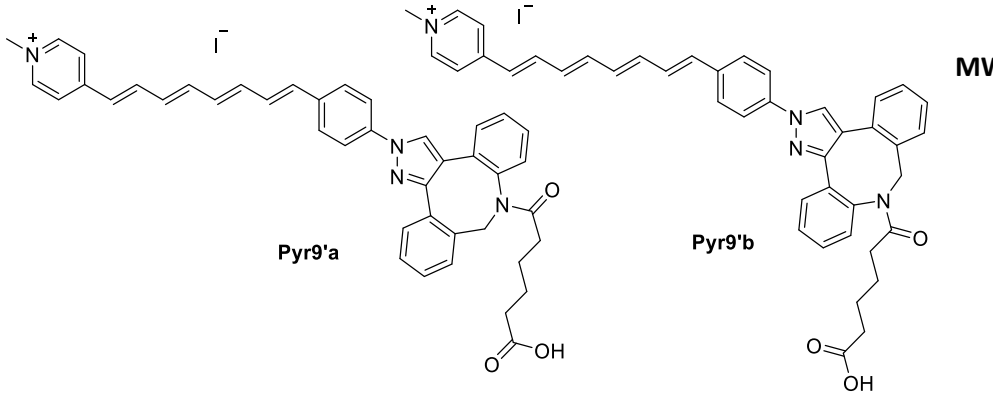


$C_{40}H_{37}IN_4O_3$
MW: 748.67 g.mol^{-1}
Brown solid
Yield: 97%

Compounds **Pyr8'a** and **Pyr8'b** were obtained as a mixture from compound **Syd8** (4.2 mg, 9.1 µmol) and DIBAC (8.40 mg, 0.025 mmol) according to procedure B.

HRMS (ESI): *m/z* calcd for $C_{40}H_{37}N_4O_3$ (M-I$^-$) : 621.2859 ; found: 621.2860.

***4-((1E,3E,5E,7E)-8-(4-(8-(5-carboxypentanoyl)-8,9-dihydro-2H-dibenzo[b,f]pyrazolo[4,3-d]azocin-2-yl)phenyl)octa-1,3,5,7-tetraen-1-yl)-1-methylpyridin-1-ium iodide* (Pyr9'a)*/ 4-((1E,3E,5E,7E)-8-(4-(9-(5-carboxypentanoyl)-8,9-dihydro-2H-dibenzo[b,f]pyrazolo[3,4-d]azocin-2-yl)phenyl)octa-1,3,5,7-tetraen-1-yl)-1-methylpyridin-1-ium* (Pyr9'b)**



$C_{42}H_{39}IN_4O_3$
MW: 774.70 g.mol^{-1}
Brown solid
Yield: 96%

Compounds **Pyr9'a** and **Pyr9'b** were obtained as a mixture from compound **Syd9** (5.9 mg, 12.2 µmol) and DIBAC (4.1 mg, 13.1 µmol) according to procedure B.

HRMS (ESI): *m/z* calcd for $C_{42}H_{39}N_4O_3$ (M-I$^-$) : 647.3017 ; found: 647.3015.

IV. Absorbance and fluorescence properties of sydnones and pyrazoles

Absorbances were measured on three solutions (50 µM, 25 µM and 5 µM) of compounds in water/DMSO (8:2) mixtures for all sydnones except **Syd2** (DMSO 100%). The molar extinction coefficients were determined by plotting absorbance values versus the solution concentrations and analyzing by linear regression **(Equation 2)**. The molar extinction coefficient corresponds to the determined slope.

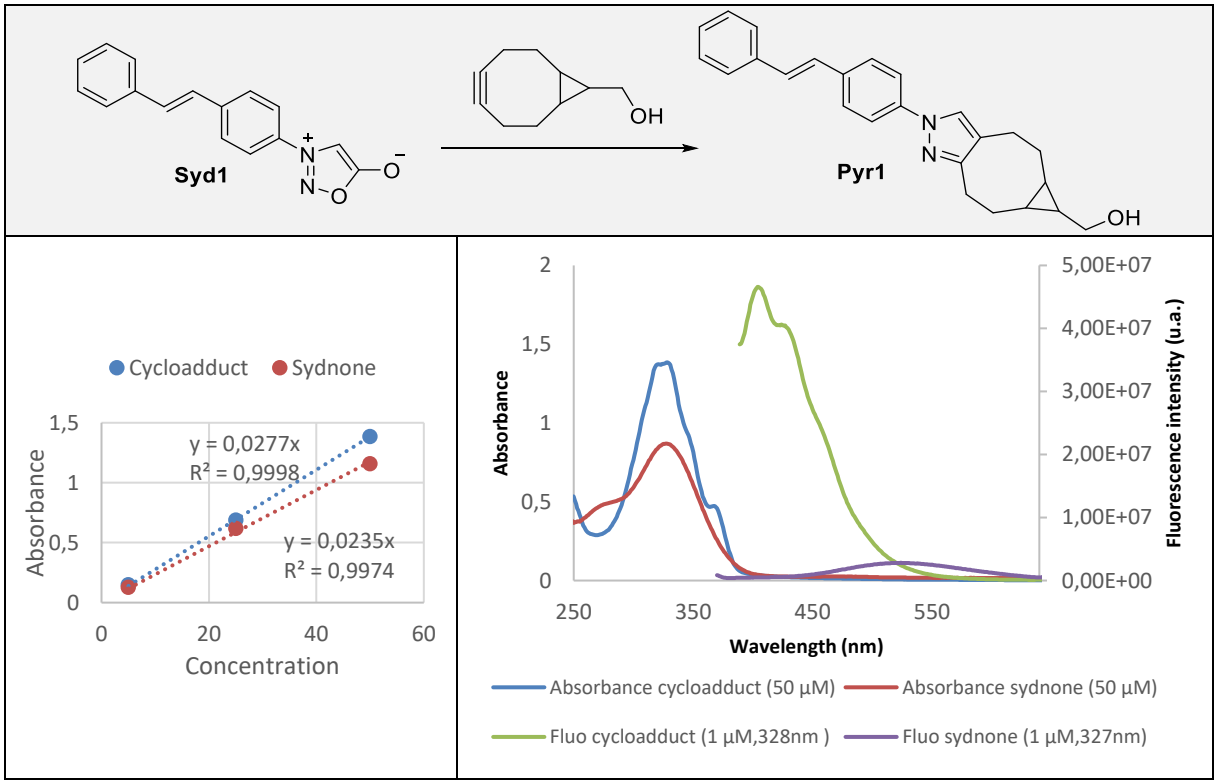
$$A = \varepsilon l C$$

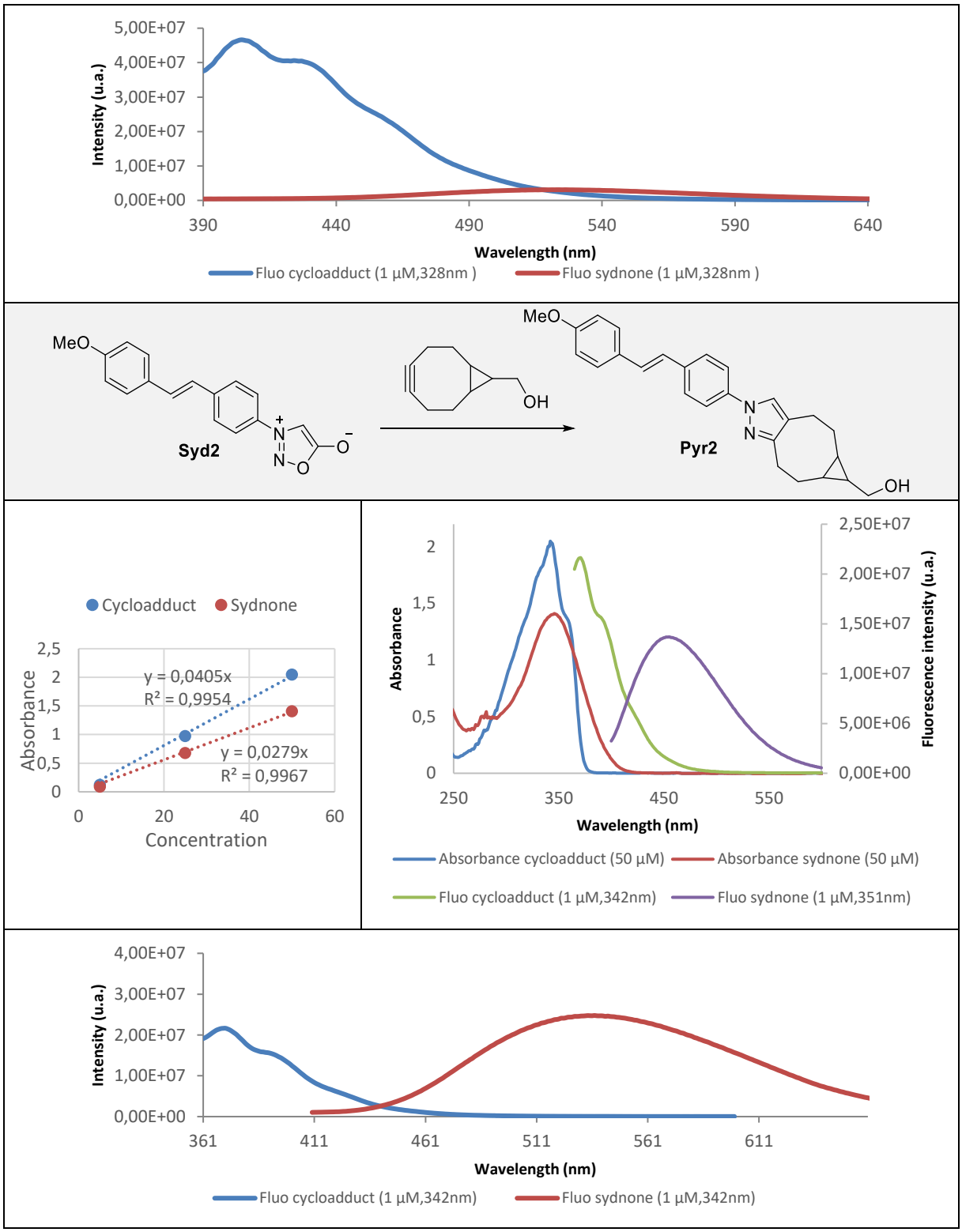
Equation 1. *A – absorbance of the sample; ε – molar extinction coefficient (L. mol^{-1}. cm^{-1}); l – pathlengh (cm); C – solution concentration (mol. L^{-1})*

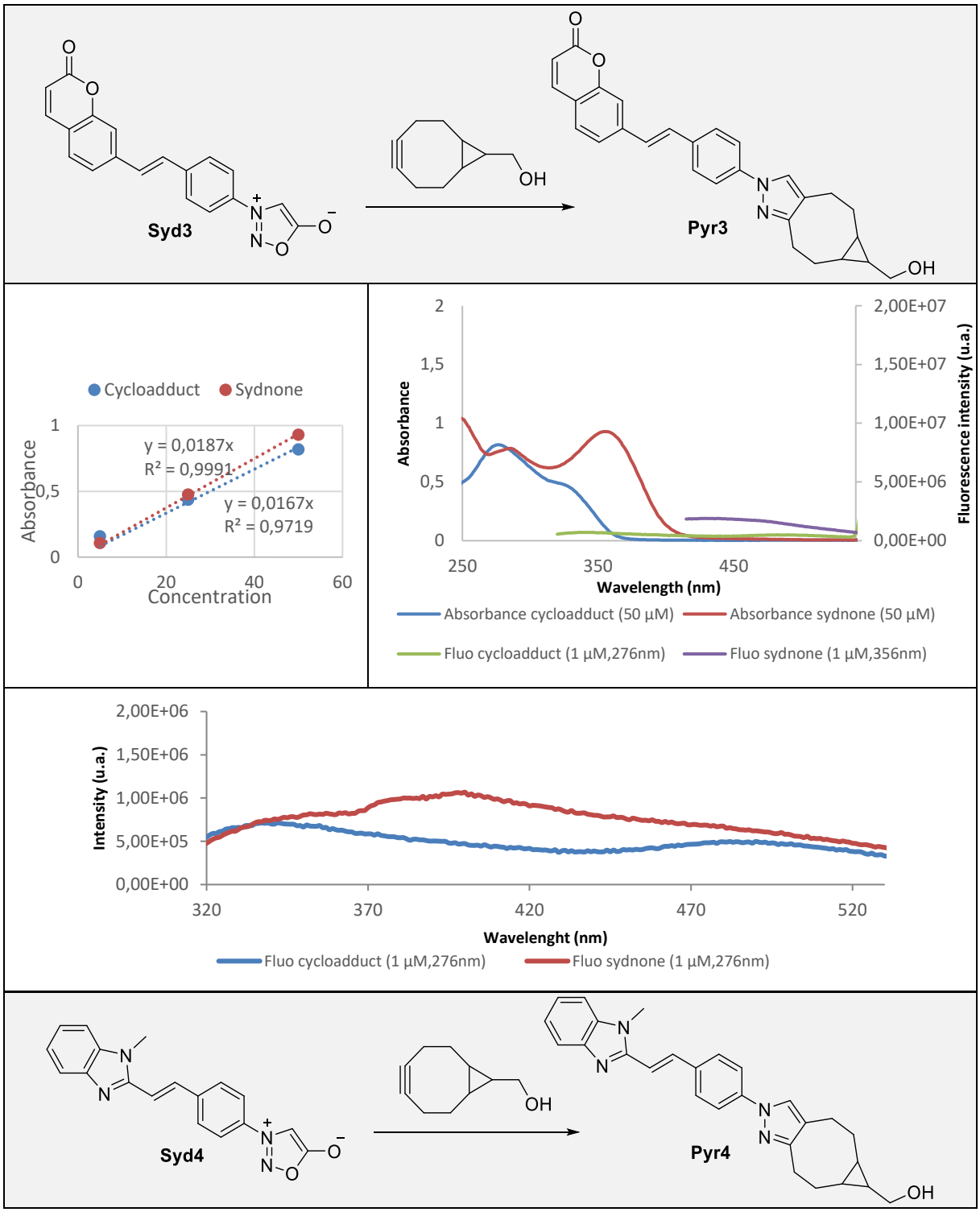
Fluorescences were measured on 1 µM solutions of compounds in water/DMSO (8:2) mixtures (or DMSO 100% for **Syd2**) excited at a wavelength corresponding to the maximum of absorbance of the sydnone or the cycloadduct.

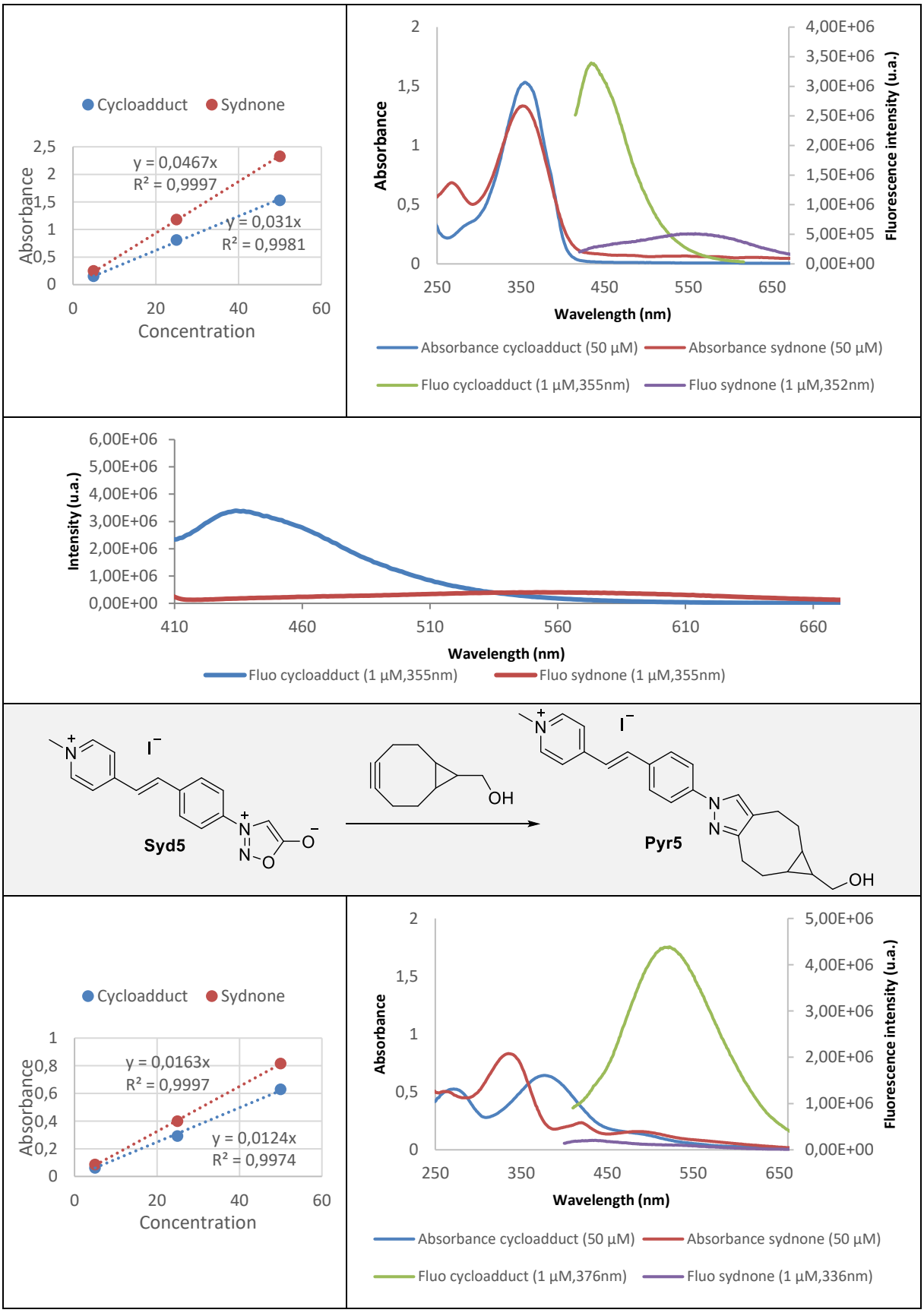
Absorbance and fluorescence spectra for each sydnone/cycloadduct couple are reported in **Table S1**.

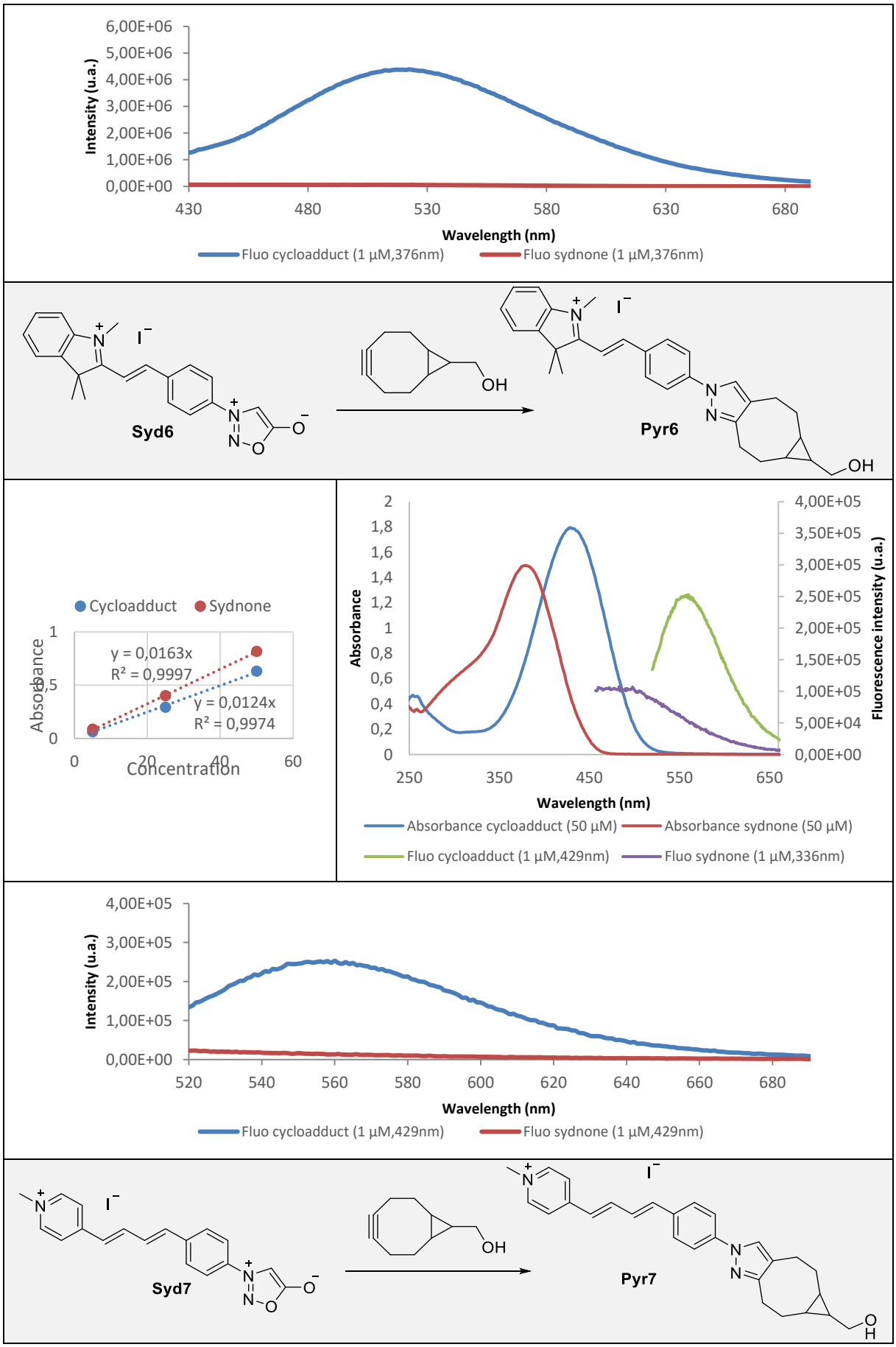
Table S1. *Superposition of absorbance spectra of sydnones and corresponding cycloadducts and fluorescence spectra of sydnones and cycloadducts excited at their respective maximum of absorbance. Superposition of fluorescence spectra for sydnones and cyloadducts excited at the maximum of absorbance of the cycloadduct.*

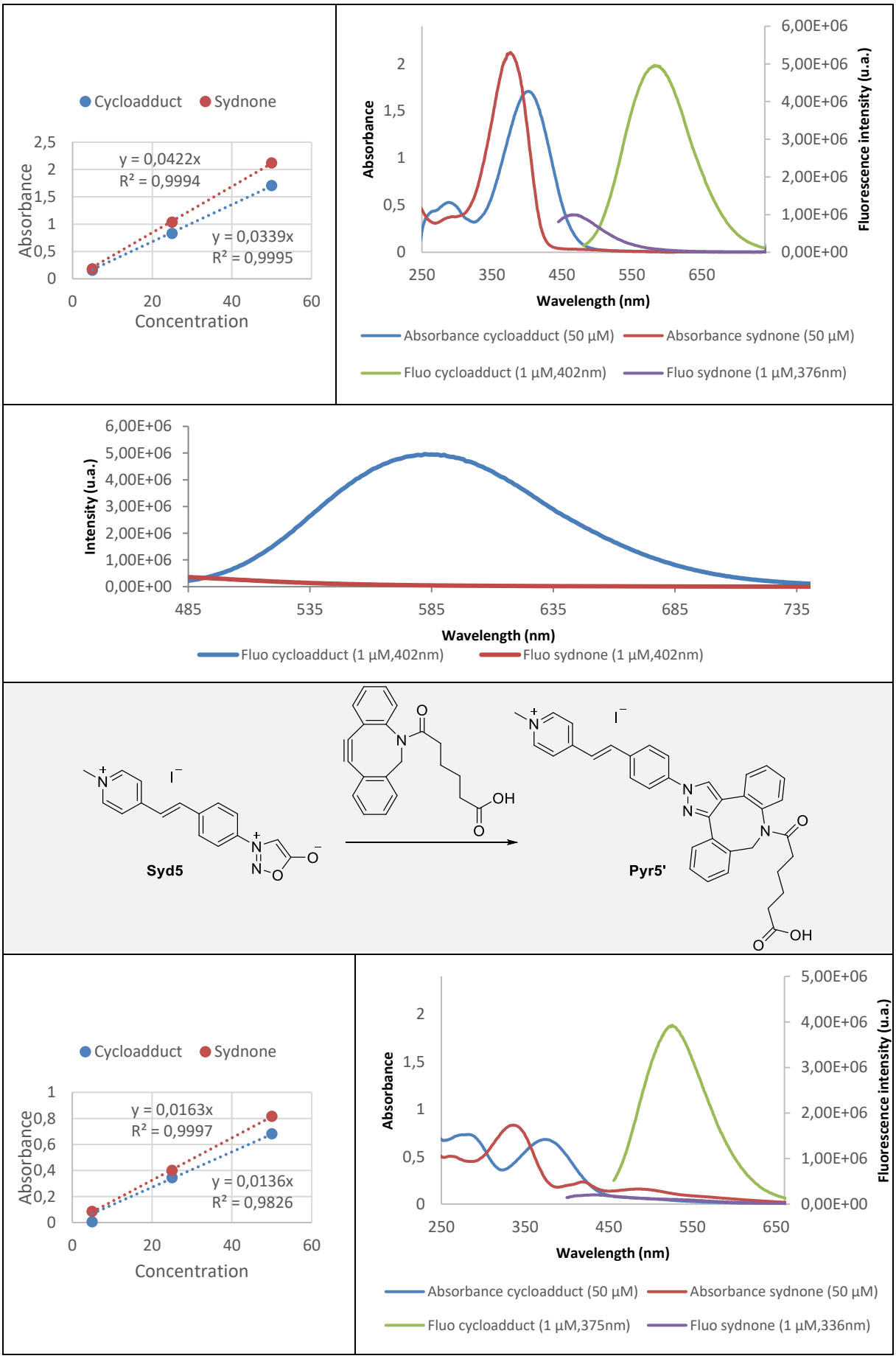


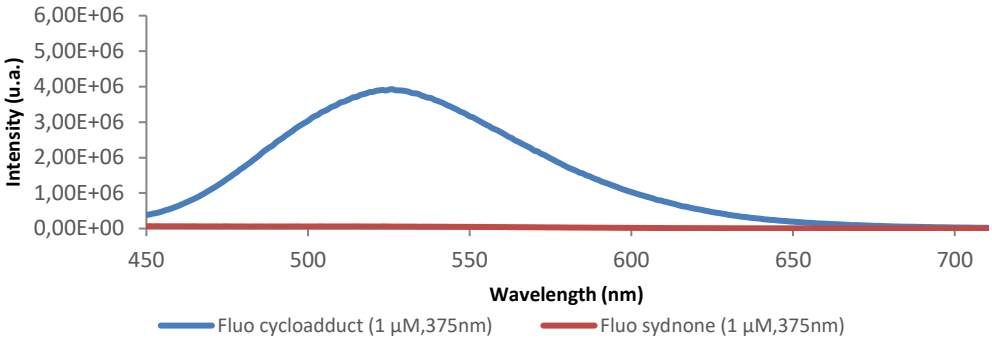

5,00E+07
4,00E+07
3,00E+07
2,00E+07
1,00E+07
0,00E+00
Intensity (u.a.)
390
440
490
540
590
640
Wavelength (nm)
Fluo cycloadduct (1 µM,328nm)
Fluo sydnone (1 µM,328nm)
MeO
Syd2
OH
Pyr2
Cycloadduct
Sydnone
y = 0,0405x
R² = 0,9954
y = 0,0279x
R² = 0,9967
Absorbance
Concentration
2,50E+07
2,00E+07
1,50E+07
1,00E+07
5,00E+06
0,00E+00
Fluorescence intensity (u.a.)
250
350
450
550
Absorbance cycloadduct (50 µM)
Absorbance sydnone (50 µM)
Fluo cycloadduct (1 µM,342nm)
Fluo sydnone (1 µM,351nm)
4,00E+07
3,00E+07
2,00E+07
1,00E+07
0,00E+00
361
411
461
511
561
611
Fluo cycloadduct (1 µM,342nm)
Fluo sydnone (1 µM,342nm)

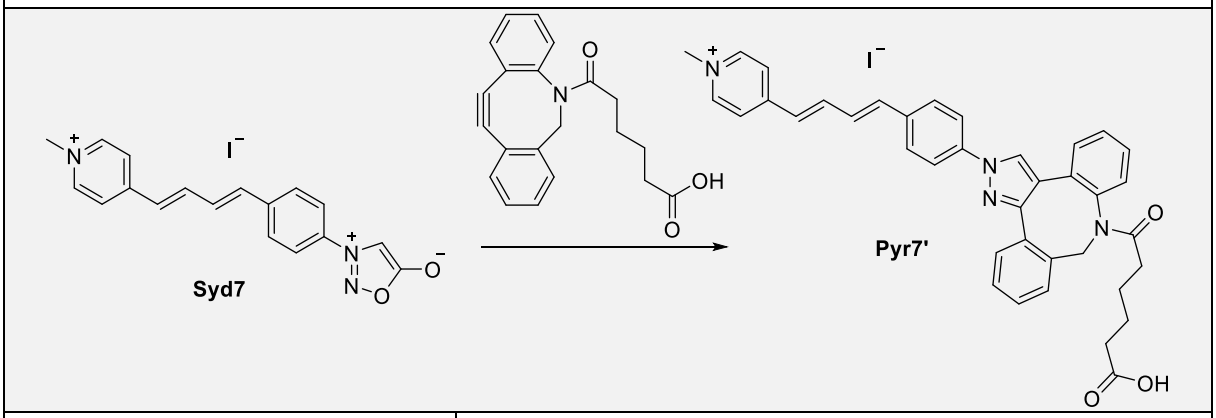

Syd3
OH
Pyr3
OH
Cycloadduct
Sydnone
y = 0,0187x
R² = 0,9991
y = 0,0167x
R² = 0,9719
Absorbance
Concentration
Absorbance
Fluorescence intensity (u.a.)
Wavelength (nm)
Absorbance cycloadduct (50 µM)
Absorbance sydnone (50 µM)
Fluo cycloadduct (1 µM,276nm)
Fluo sydnone (1 µM,356nm)
Intensity (u.a.)
Wavelenght (nm)
Fluo cycloadduct (1 µM,276nm)
Fluo sydnone (1 µM,276nm)
Syd4
OH
Pyr4
OH

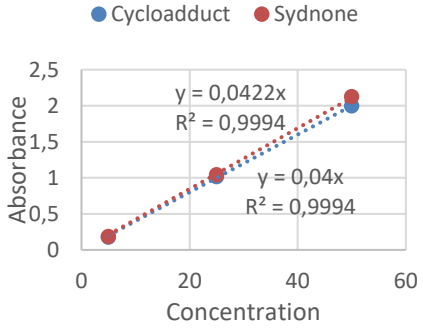

Cycloadduct
Sydnone
y = 0,0467x
R² = 0,9997
y = 0,031x
R² = 0,9981
Absorbance
Concentration
Absorbance
Fluorescence intensity (u.a.)
Wavelength (nm)
Absorbance cycloadduct (50 µM)
Absorbance sydnone (50 µM)
Fluo cycloadduct (1 µM,355nm)
Fluo sydnone (1 µM,352nm)
Intensity (u.a.)
Wavelength (nm)
Fluo cycloadduct (1 µM,355nm)
Fluo sydnone (1 µM,355nm)
Syd5
OH
Pyr5
OH
Cycloadduct
Sydnone
y = 0,0163x
R² = 0,9997
y = 0,0124x
R² = 0,9974
Absorbance
Concentration
Absorbance
Fluorescence intensity (u.a.)
Wavelength (nm)
Absorbance cycloadduct (50 µM)
Absorbance sydnone (50 µM)
Fluo cycloadduct (1 µM,376nm)
Fluo sydnone (1 µM,336nm)

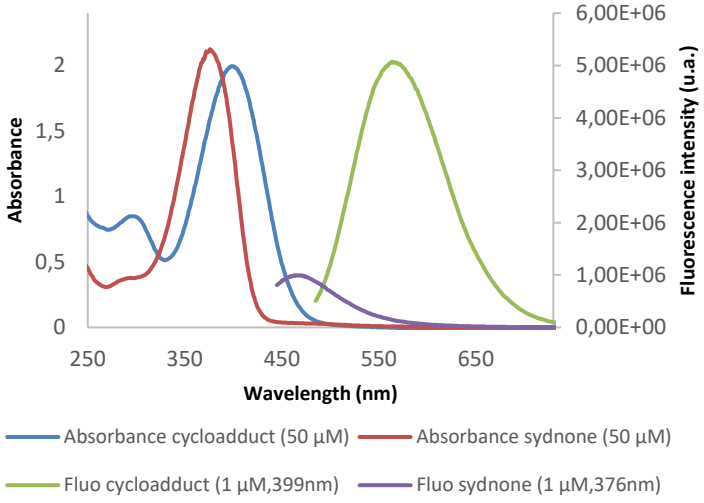

6,00E+06
5,00E+06
4,00E+06
3,00E+06
2,00E+06
1,00E+06
0,00E+00
Intensity (u.a.)
430
480
530
580
630
680
Wavelength (nm)
Fluo cycloadduct (1 µM,376nm)
Fluo sydnone (1 µM,376nm)
Syd6
OH
Pyr6
Cycloadduct
Sydnone
y = 0,0163x
R² = 0,9997
y = 0,0124x
R² = 0,9974
Absorbance
Concentration
Absorbance
Fluorescence intensity (u.a.)
Wavelength (nm)
Absorbance cycloadduct (50 µM)
Absorbance sydnone (50 µM)
Fluo cycloadduct (1 µM,429nm)
Fluo sydnone (1 µM,336nm)
4,00E+05
3,00E+05
2,00E+05
1,00E+05
0,00E+00
Intensity (u.a.)
520
540
560
580
600
620
640
660
680
Wavelength (nm)
Fluo cycloadduct (1 µM,429nm)
Fluo sydnone (1 µM,429nm)
Syd7
OH
Pyr7

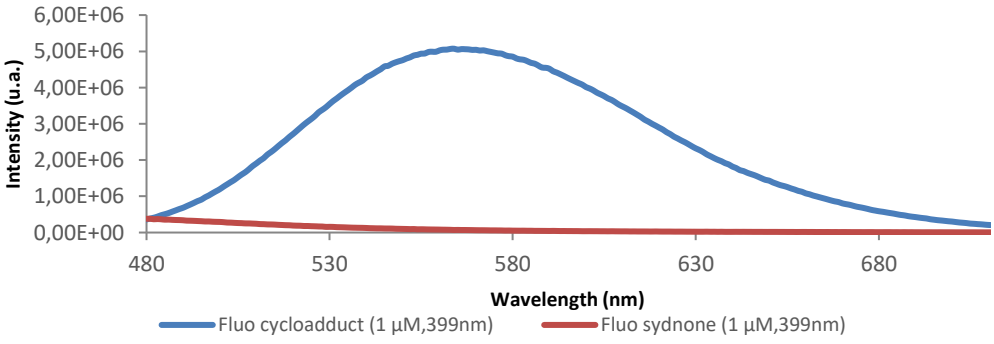

Cycloadduct
Sydnone
y = 0,0422x
R² = 0,9994
y = 0,0339x
R² = 0,9995
Absorbance
Concentration
Absorbance
Fluorescence intensity (u.a.)
Wavelength (nm)
Absorbance cycloadduct (50 µM)
Absorbance sydnone (50 µM)
Fluo cycloadduct (1 µM,402nm)
Fluo sydnone (1 µM,376nm)
Intensity (u.a.)
Fluo cycloadduct (1 µM,402nm)
Fluo sydnone (1 µM,402nm)
Syd5
Pyr5'
Cycloadduct
Sydnone
y = 0,0163x
R² = 0,9997
y = 0,0136x
R² = 0,9826
Absorbance
Concentration
Absorbance
Fluorescence intensity (u.a.)
Wavelength (nm)
Absorbance cycloadduct (50 µM)
Absorbance sydnone (50 µM)
Fluo cycloadduct (1 µM,375nm)
Fluo sydnone (1 µM,336nm)

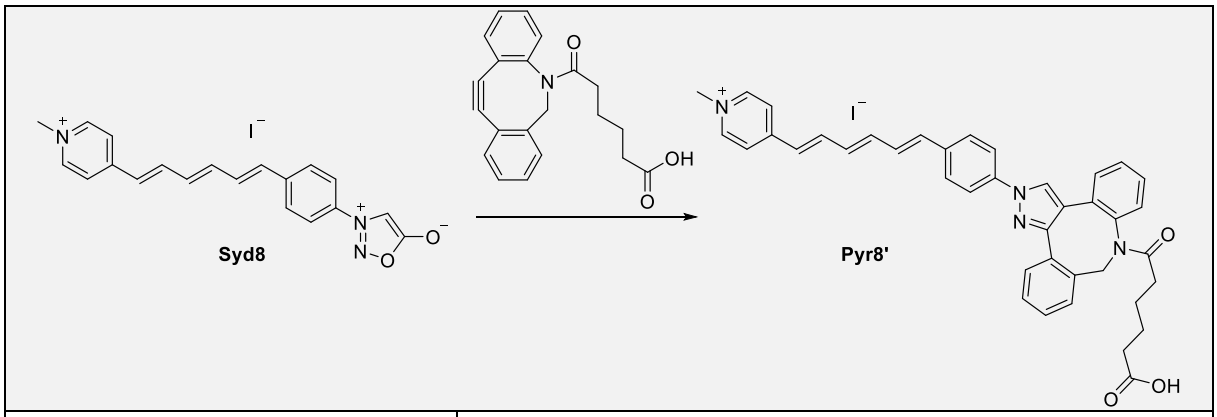

6,00E+06
5,00E+06
4,00E+06
3,00E+06
2,00E+06
1,00E+06
0,00E+00
Intensity (u.a.)
450
500
550
600
650
700
Wavelength (nm)
Fluo cycloadduct (1 µM,375nm)
Fluo sydnone (1 µM,375nm)

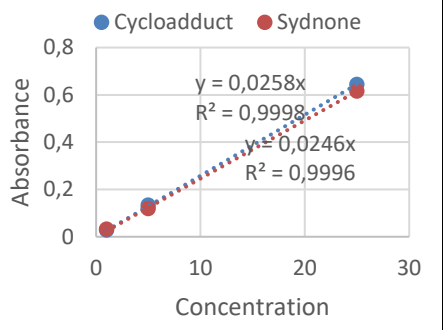

Syd7
Pyr7'
OH

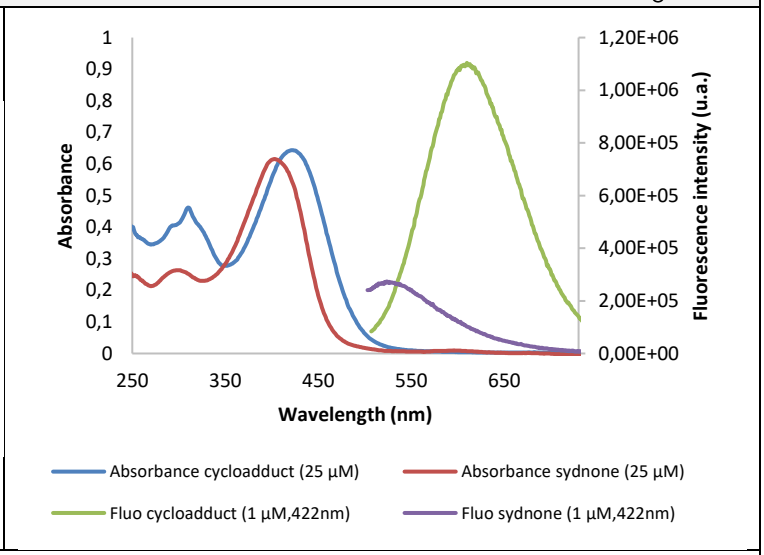

Cycloadduct
Sydnone
y = 0,0422x
R² = 0,9994
y = 0,04x
R² = 0,9994
Absorbance
Concentration

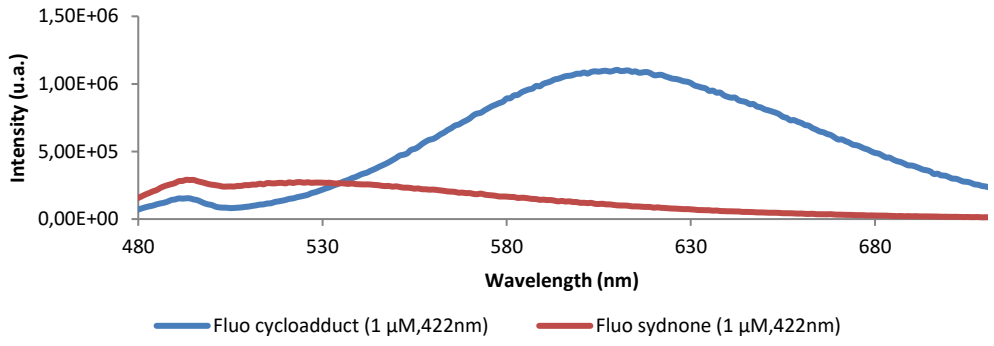

Absorbance
Fluorescence intensity (u.a.)
Wavelength (nm)
Absorbance cycloadduct (50 µM)
Absorbance sydnone (50 µM)
Fluo cycloadduct (1 µM,399nm)
Fluo sydnone (1 µM,376nm)

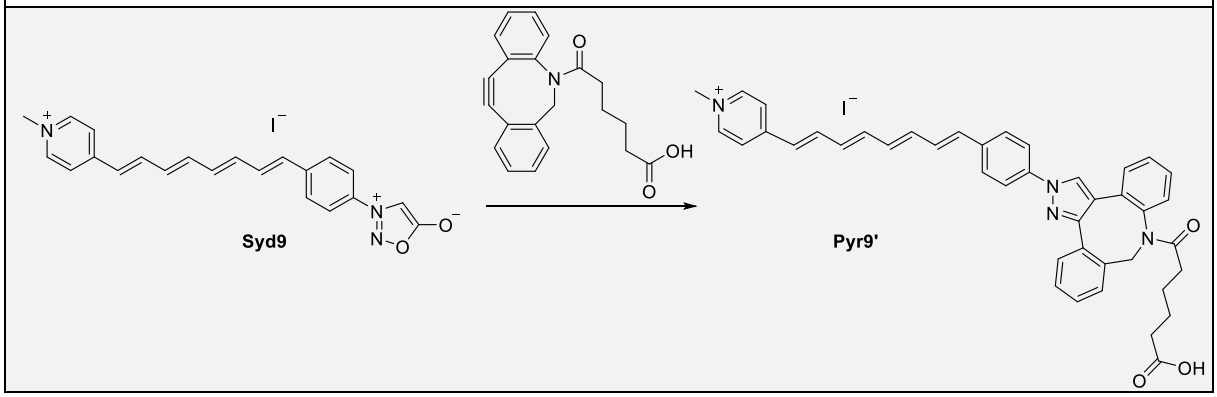

6,00E+06
5,00E+06
4,00E+06
3,00E+06
2,00E+06
1,00E+06
0,00E+00
Intensity (u.a.)
480
530
580
630
680
Wavelength (nm)
Fluo cycloadduct (1 µM,399nm)
Fluo sydnone (1 µM,399nm)

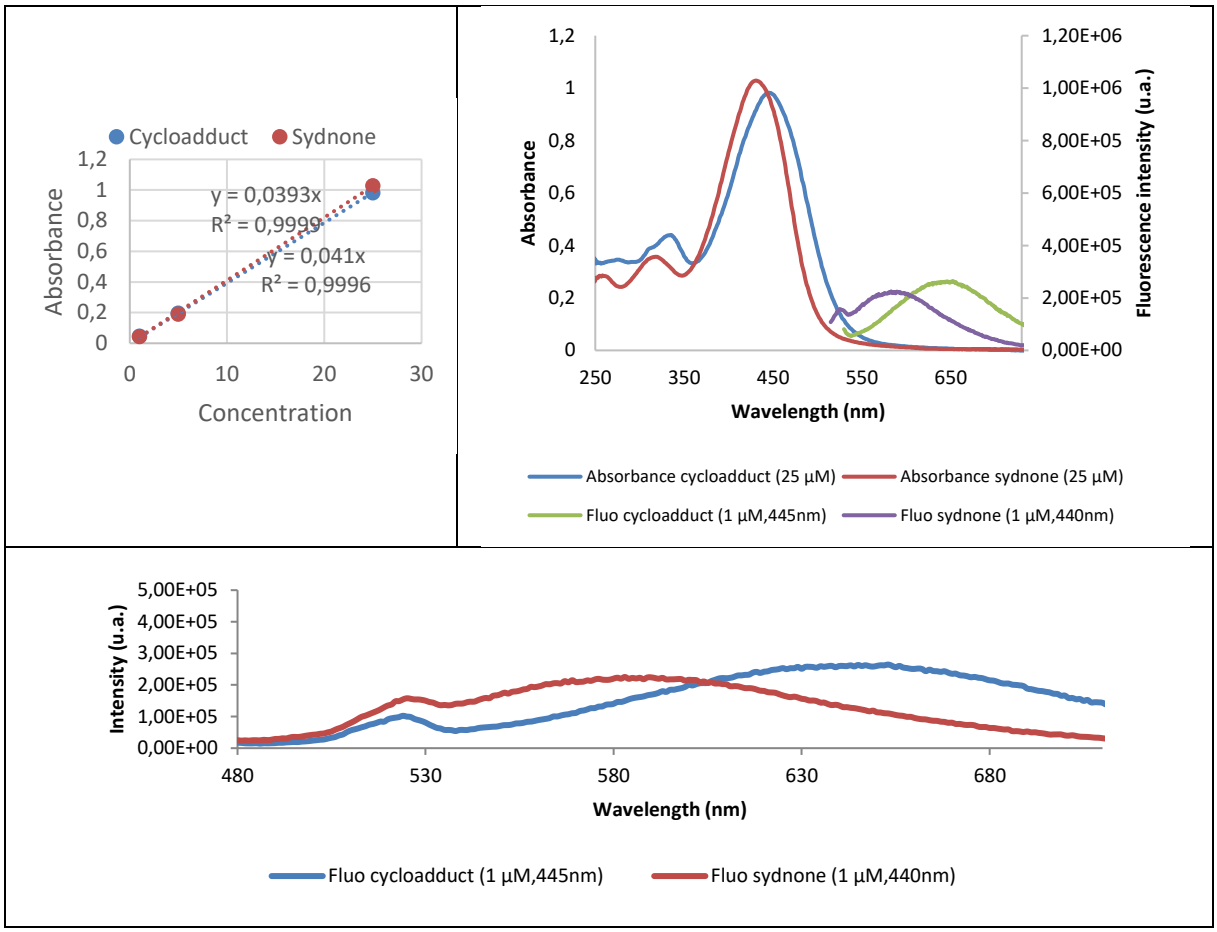

Syd8
Pyr8'


Cycloadduct
Sydnone
y = 0,0258x
R² = 0,9998
y = 0,0246x
R² = 0,9996
Absorbance
Concentration


Absorbance
Fluorescence intensity (u.a.)
Wavelength (nm)
Absorbance cycloadduct (25 µM)
Absorbance sydnone (25 µM)
Fluo cycloadduct (1 µM,422nm)
Fluo sydnone (1 µM,422nm)


Intensity (u.a.)
Wavelength (nm)
Fluo cycloadduct (1 µM,422nm)
Fluo sydnone (1 µM,422nm)


Syd9
Pyr9'



Summary of the fluorescence properties of pyridinium-based sydnones and pyrazoles:

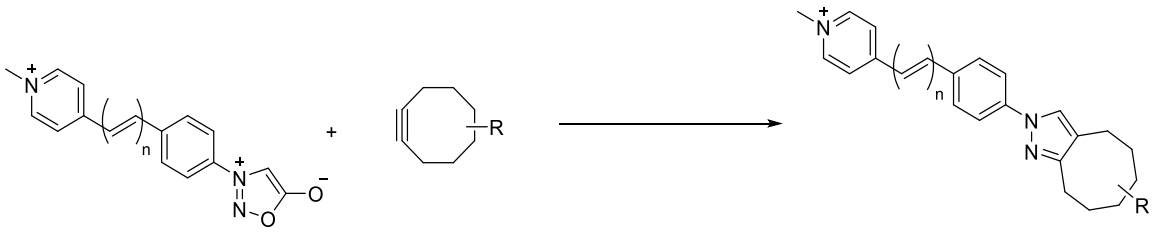


Table S2. *Characterization of the fluorescence properties of Syd10, 12-14 and Pyr10', 12'-14'*

			Sydnone				Pyrazole					
Entrée	n	alcyne	λ_{ex} (nm)	λ_{em} (nm)	Φ_f	ε	λ_{ex} (nm)	λ_{em} (nm)	Φ_f	ε	Brill.	Turn-on
1	1	BCN	337	434	0,003	12400	376	521	0,066	16300	1076	78
2	1	DIBAC					375	526	0,040	13600	544	71
3	2	BCN	376	466	0,008	42200	401	582	0,047	33900	1593	97
4	2	DIBAC					399	567	0,047	40000	1880	73
5	3	DIBAC	403	530	0.002	24600	422	612	0.007	25600	191	11
6	4	DIBAC	431	588	0.002	41045	445	652	0.002	39300	95	2.4

V. Kinetic studies

Reactions of sydnones **Syd5** and **Syd7** with **BCN** were carried out in PBK 5 mM/DMSO (8:2), culture medium/DMSO (8:2), cellular lysate/DMSO (8:2), plasma/DMSO (8:2) and glutathione (1 mM in PBK 5 mM)/DMSO (8:2) at 100 µM concentration of sydnones and 150 µM concentration of **BCN** using the following procedure:

To 800 µL of the chosen medium was added 85 µL of DMSO, 100 µL of the solution of sydnone (1 mM in DMSO) and 15 µL of the solution of **BCN** (10 mM in DMSO). 3 x 200 µL of the reaction mixture were dropped off a 96-well opaque plate and the emission was measured every minute after excitation at the appropriate wavelength until the reaction reaches 10% conversion.

Reactions of sydnones **Syd5, Syd7** and **Syd8** with **DIBAC** were carried out in PBK 5 mM/DMSO (8:2), diluted culture medium*/DMSO (8:2), diluted cellular lysate*/DMSO (8:2), diluted plasma*/DMSO (8:2) and glutathione (1 mM in PBK 5 mM)/DMSO (8:2) at 10 µM concentration of sydnones and 15 µM concentration of **DIBAC** using the following procedure:

To 160 µL of the chosen medium was added 14 µL of DMSO, 20 µL of the solution of sydnone (100 µM in DMSO) and 6 µL of the solution of **DIBAC** (500 µM in DMSO) in a 96-well opaque plate and the emission was measured every second after excitation at the appropriate wavelength until the reaction reaches 10% conversion. This operation was repeated 3 times.

**Culture medium, plasma and cellular lysate were used as diluted solutions in PBK 1M to avoid the low signal/noise ratio due to the internal fluorescence of these media and the low fluorescence intensity of the sample at the concentration of the experiment.*

Correspondences between fluorescence and concentrations were established by plotting the calibration curve obtained by measuring the emission after the excitation at the appropriate wavelength of solutions for 0.5%, 2.5%, 5%, 7.5% and 10% conversion of the reaction.

Table S3. *Concentrations of sydnone and cycloadduct in the medium for 10%, 7.5%, 5%, 2.5% and 0.5% conversion of the reaction at 100 µM.*

Conversion	10%	7.5%	5%	2.5%	0.5%
$C_{sydnone}$	90 µM	92.5 µM	95 µM	97.5 µM	99.5 µM
$C_{cycloadduct}$	10 µM	7.5 µM	5 µM	2.5 µM	0.5 µM

Table S4. *Concentrations of sydnone and cycloadduct in the medium for 10%, 7.5%, 5%, 2.5% and 0.5% conversion of the reaction at 10 µM.*

Conversion	10%	7.5%	5%	2.5%	0.5%
$C_{sydnone}$	9 µM	9.25 µM	9.5 µM	9.75 µM	9.95 µM
$C_{cycloadduct}$	1 µM	0.75 µM	0.5 µM	0.25 µM	0.05 µM

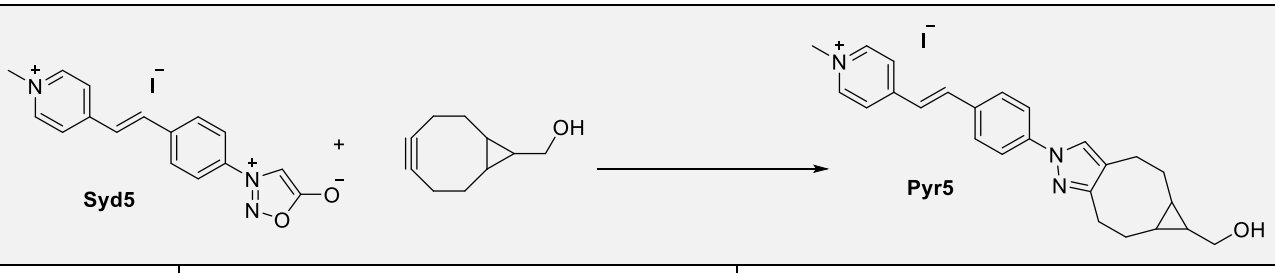
Second order reaction rate was determined by plotting $\ln([A]/[B])/([A] - [B])$ versus time and analyzing by linear regression **(Equation S2)**. Second order rate constant corresponds to the determined slope.

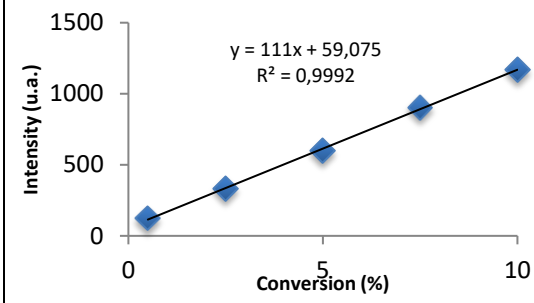
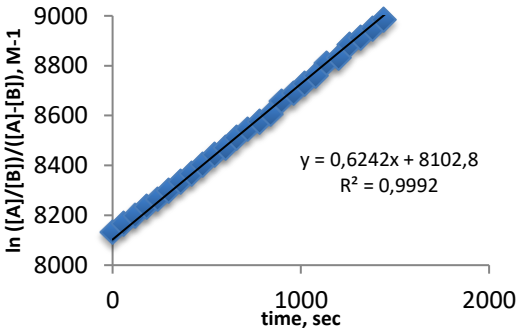
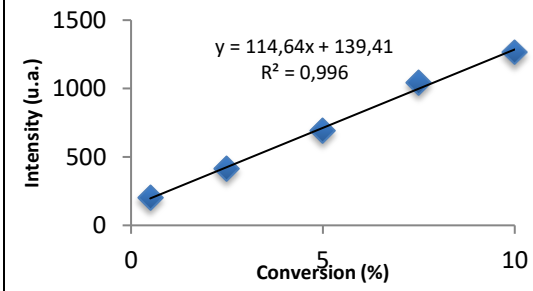
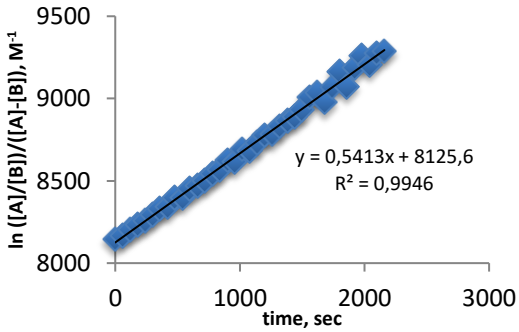
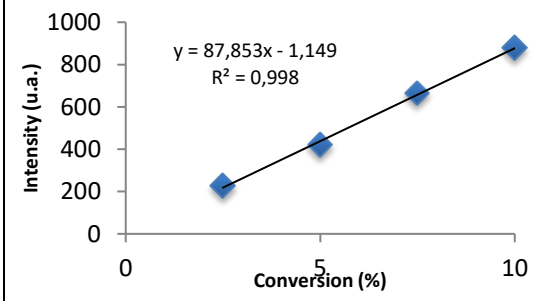
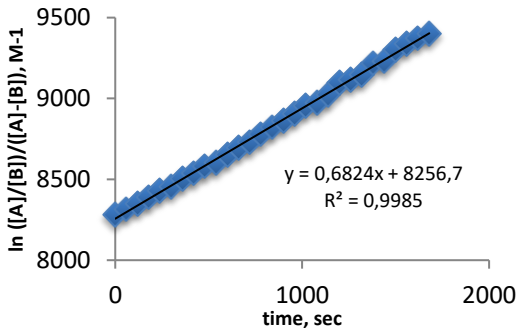
$$\frac{\ln\left(\frac{[A]}{[B]}\right)}{[A]-[B]} = kt + const$$

Equation S2. *[A] – concentration of sydnones (M); [B] – concentration of BCN (M); t – reaction time (sec); k – reaction rate ($M^{-1}\cdot sec^{-1}$).*

Calibration curves and linear regression curves are illustrated in **Tables S5, S6, S7, S8** and **S9**.

Table S5. *Calibration curves showing fluorescence intensity plotted versus conversion and linear regression curves showing ln([A]/[B])/([A] – [B]) plotted versus time for the reaction between sydnone* **Syd5** *and* **BCN** *in various media.*



PBK 5mM/ DMSO (8:2)		
Culture medium/ DMSO (8:2)		
Cellular lysate/ DMSO (8:2)		

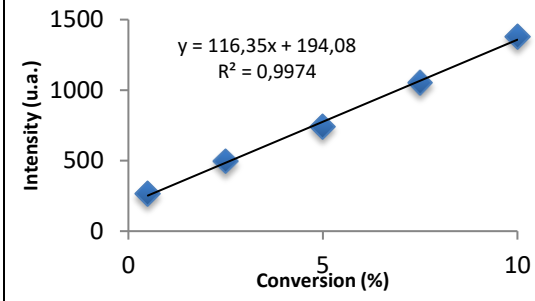
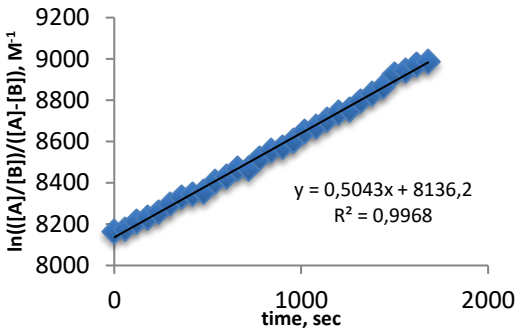
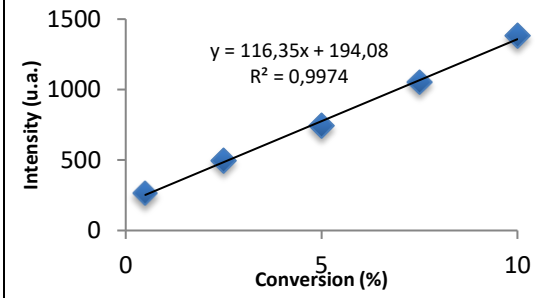
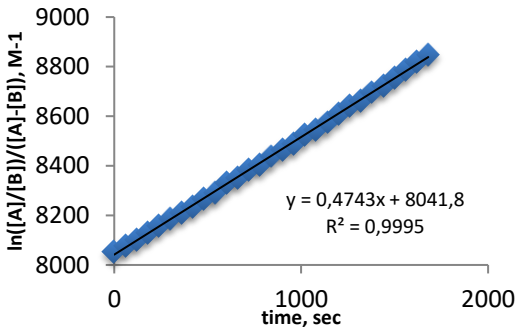
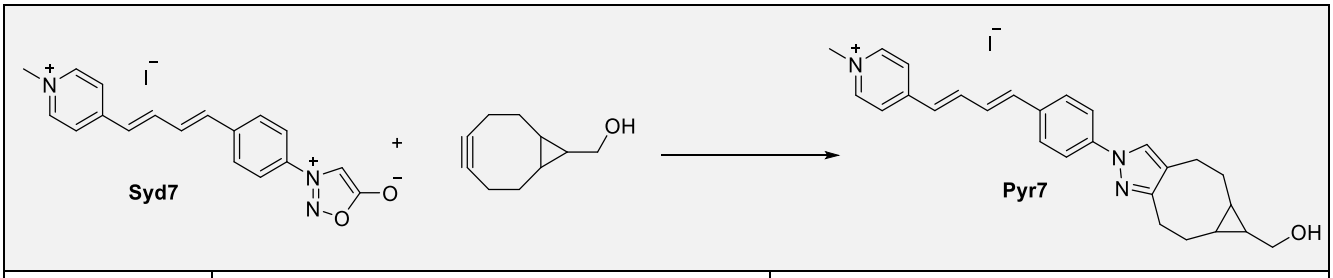
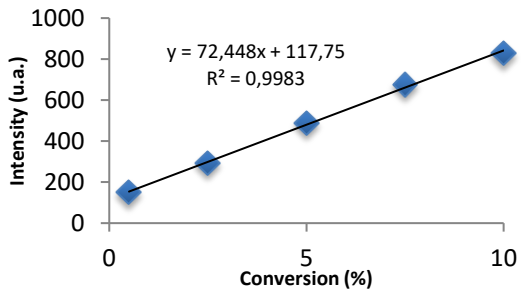
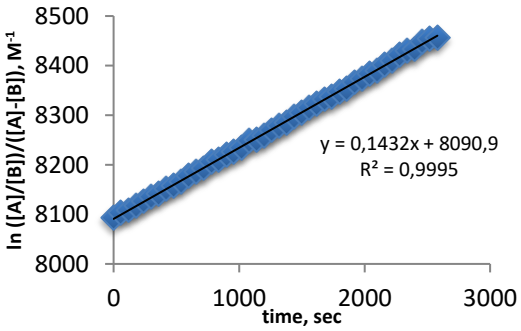
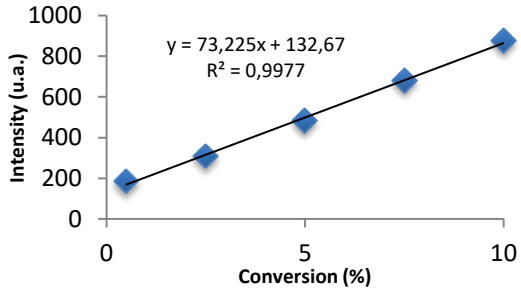
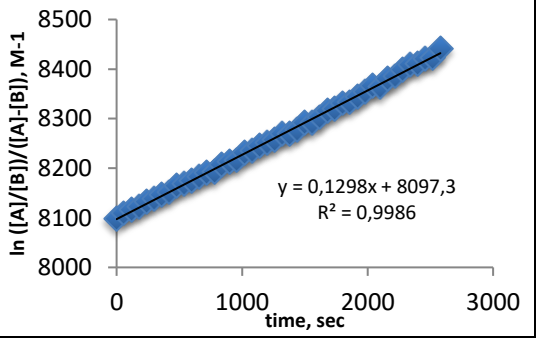
Plasma/ DMSO (8:2)		
Glutathion 1mM (in PBK 5mM)/ DMSO (8:2)		

Table S6. *Calibration curves showing fluorescence intensity plotted versus conversion and linear regression curves showing ln([A]/[B])/([A] – [B]) plotted versus time for the reaction between sydnone* **Syd7** *and* **BCN** *in various media.*



PBK 5mM/ DMSO (8:2)		
Culture medium/ DMSO (8:2)		

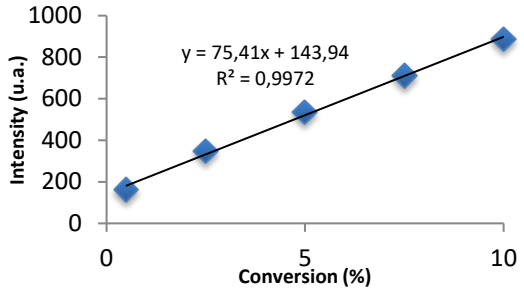
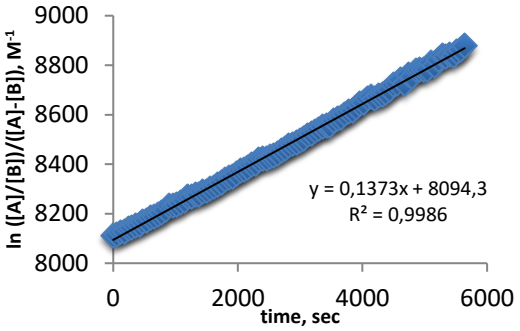
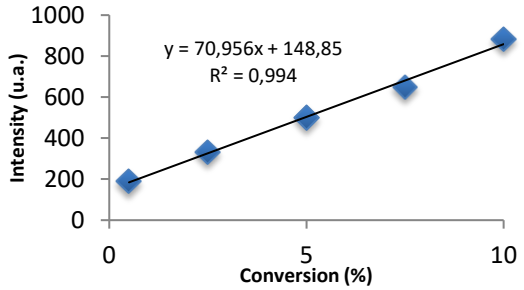
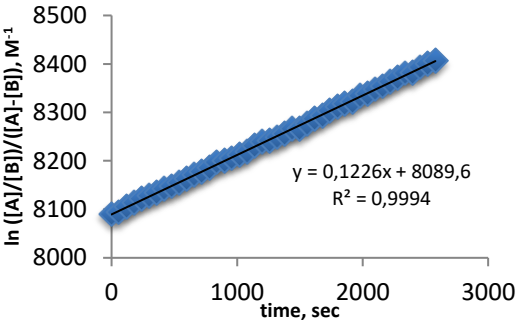
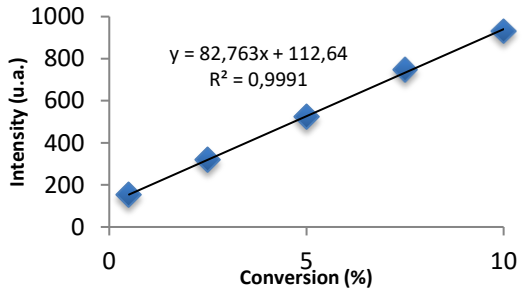
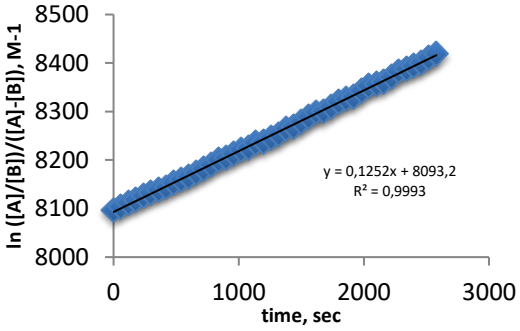
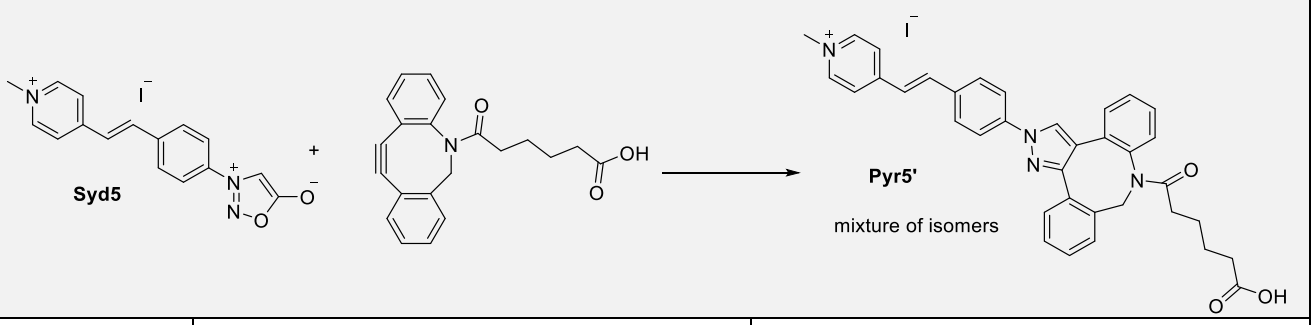
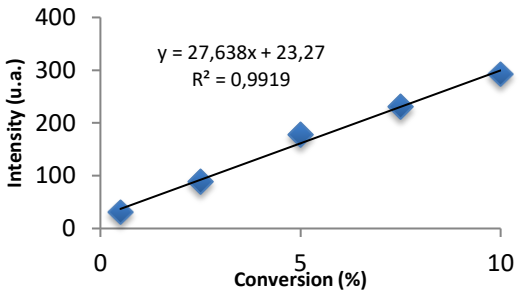
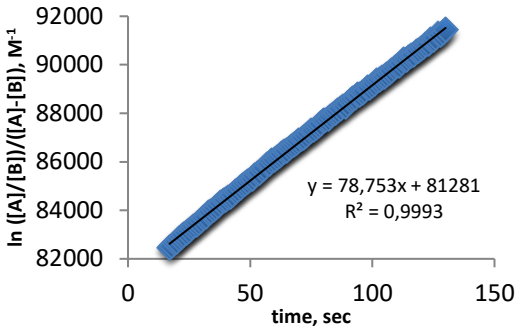
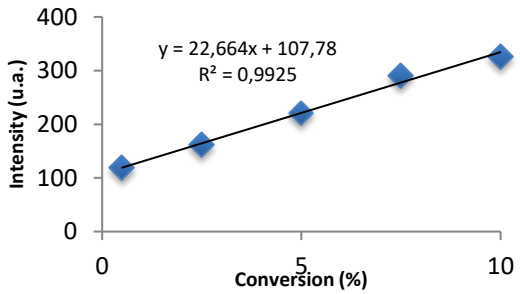
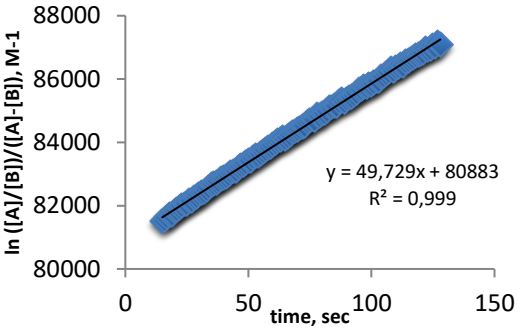
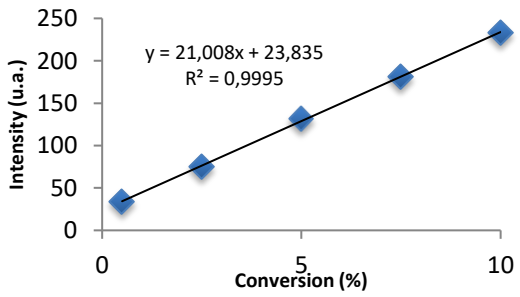
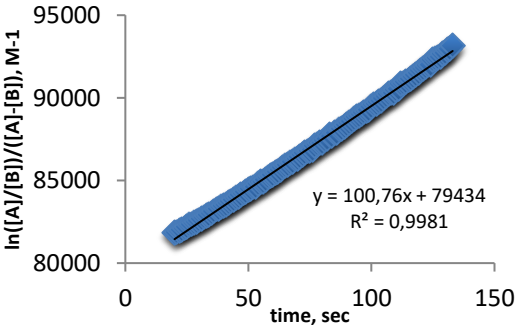
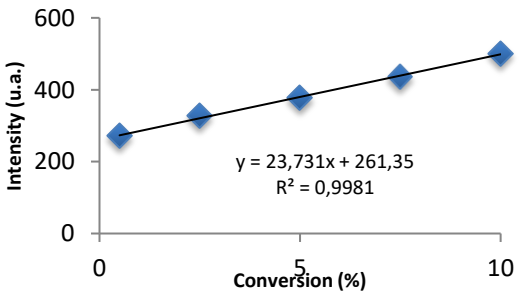
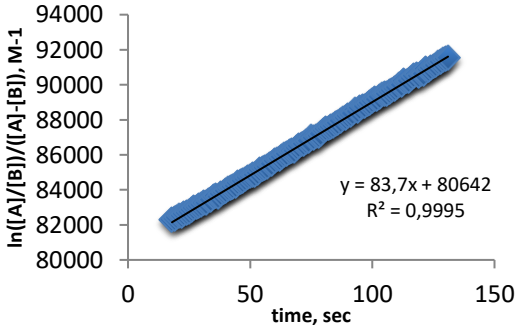
Cellular lysate/ DMSO (8:2)		
Plasma/ DMSO (8:2)		
Glutathion 1mM (in PBK 5mM)/ DMSO (8:2)		

Table S7. *Calibration curves showing fluorescence intensity plotted versus conversion and linear regression curves showing ln([A]/[B])/([A] – [B]) plotted versus time for the reaction between sydnone* **Syd5** *and* **DIBAC** *in various media.*



PBK 5mM/ DMSO (8:2)		
Culture medium/ DMSO (8:2)		
Cellular lysate/ DMSO (8:2)		
Plasma/ DMSO (8:2)		

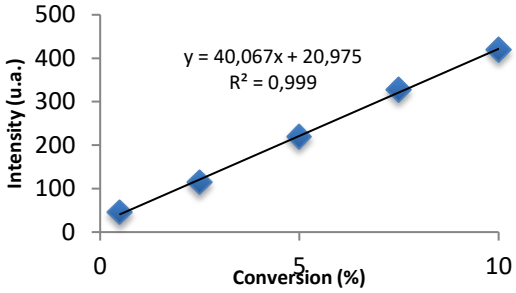
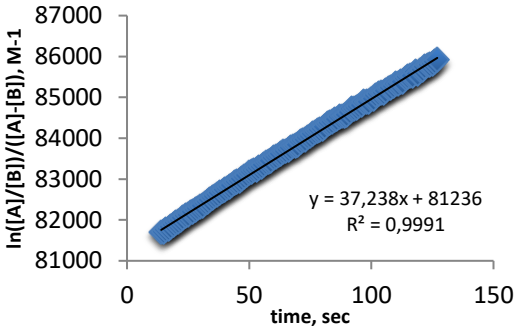
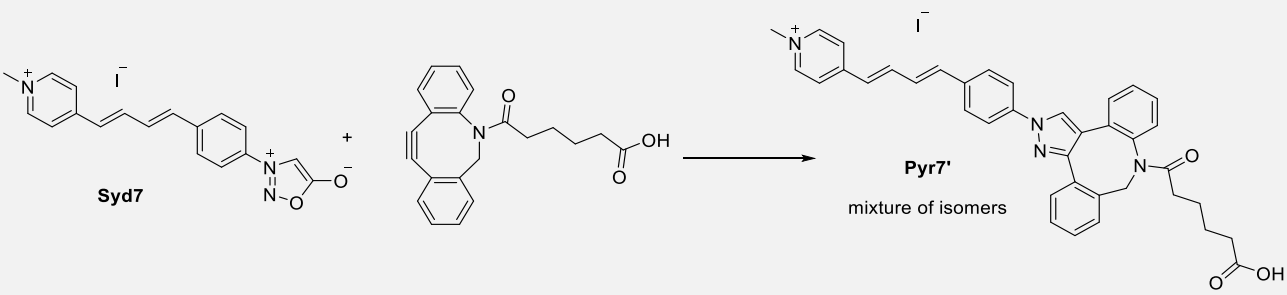
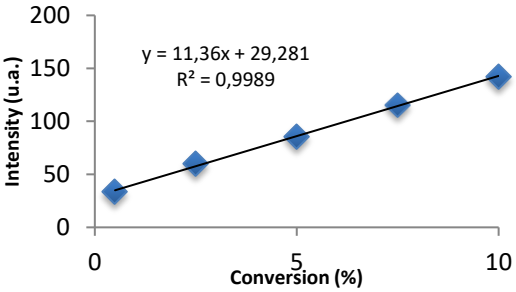
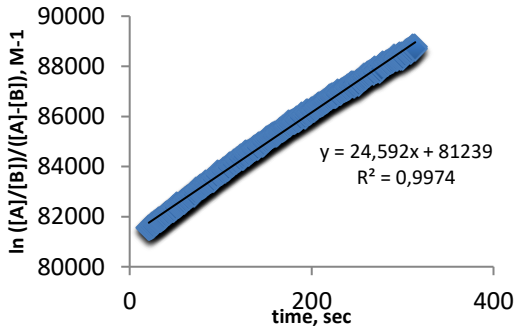
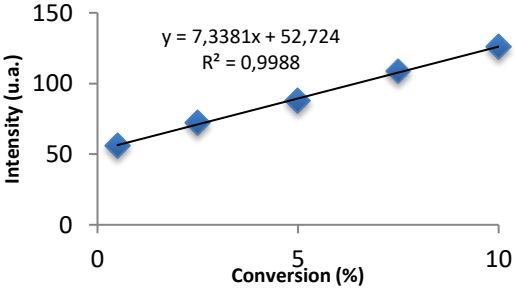
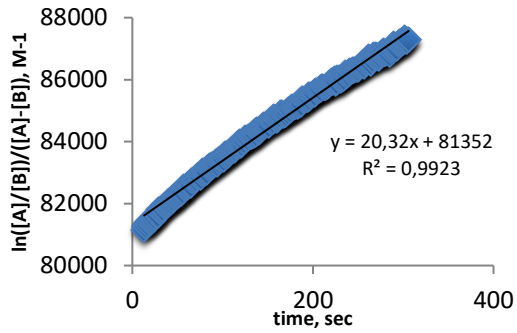
Glutathion 1mM (in PBK 5mM)/ DMSO (8:2)		

Table S8. *Calibration curves showing fluorescence intensity plotted versus conversion and linear regression curves showing ln([A]/[B])/([A] – [B]) plotted versus time for the reaction between sydnone* **Syd7** *and* **DIBAC** *in various media.*



PBK 5mM/ DMSO (8:2)		
Culture medium/ DMSO (8:2)		

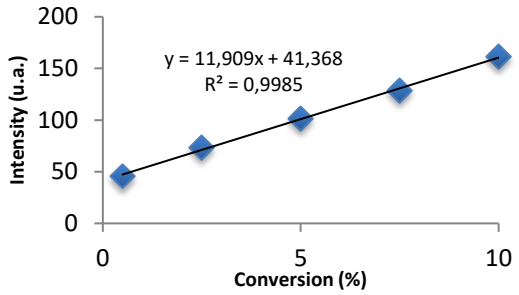
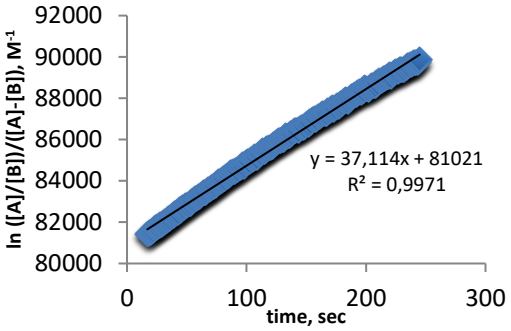
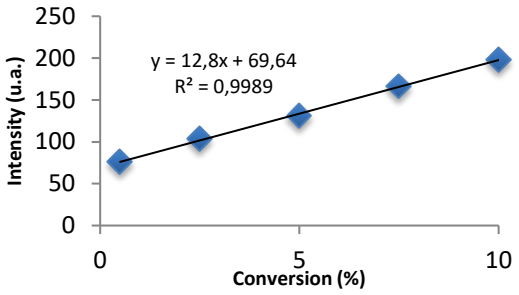
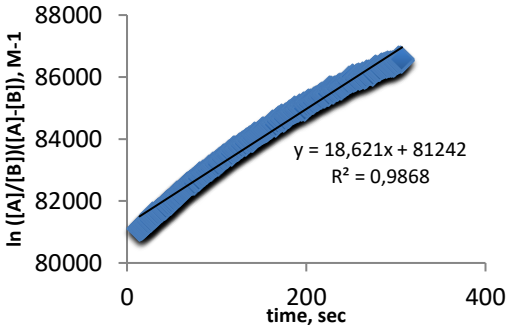
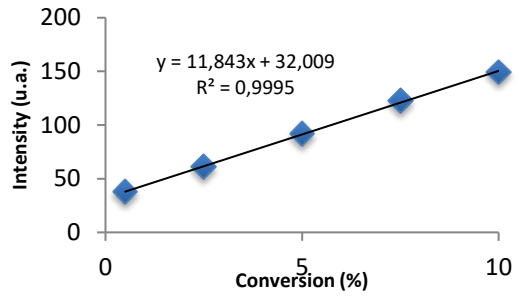
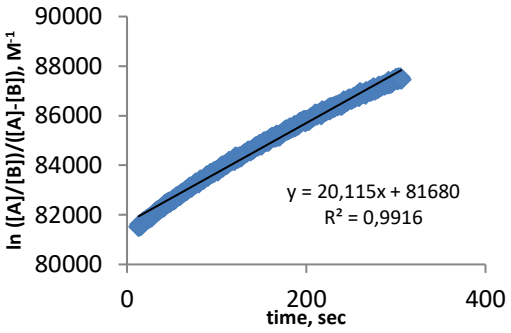
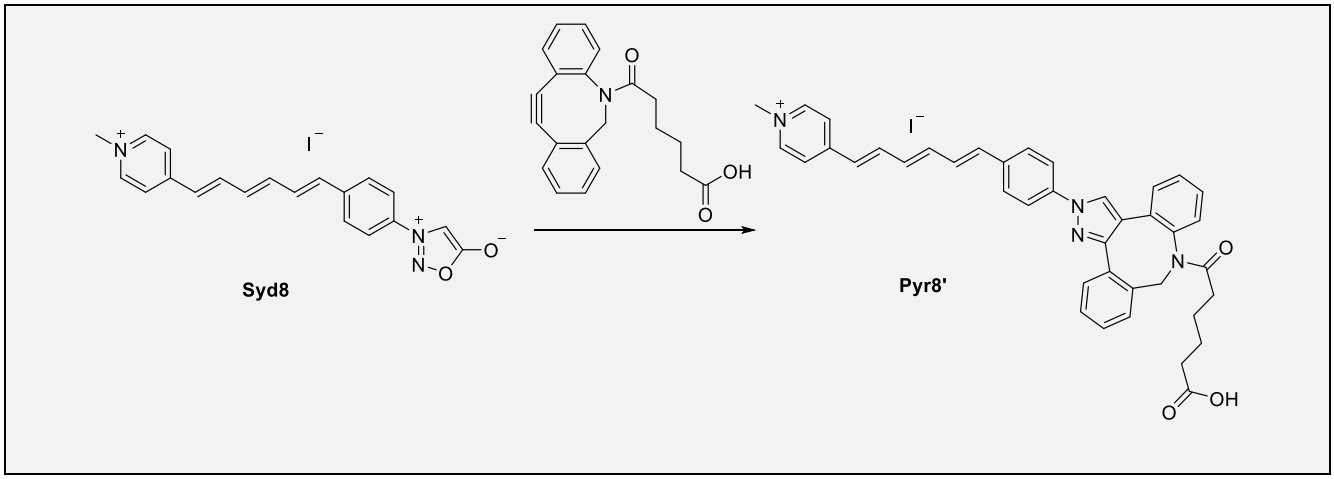
Cellular lysate/ DMSO (8:2)		
Plasma/ DMSO (8:2)		
Glutathion 1mM (in PBK 5mM)/ DMSO (8:2)		

Table S9. *Calibration curves showing fluorescence intensity plotted versus conversion and linear regression curves showing ln([A]/[B])/([A] – [B]) plotted versus time for the reaction between sydnone* **Syd8** *and* **DIBAC** *in various media.*



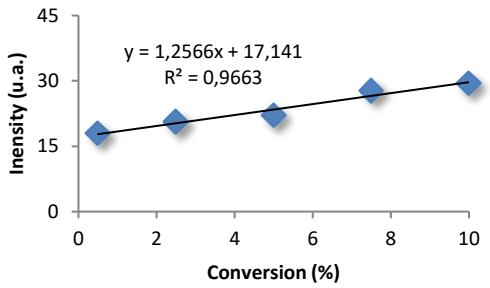
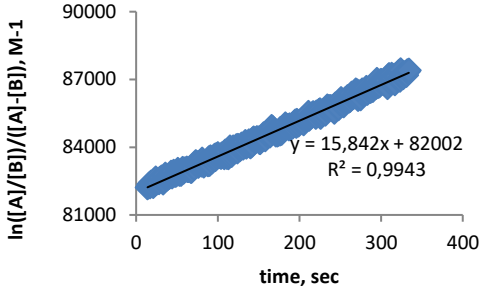
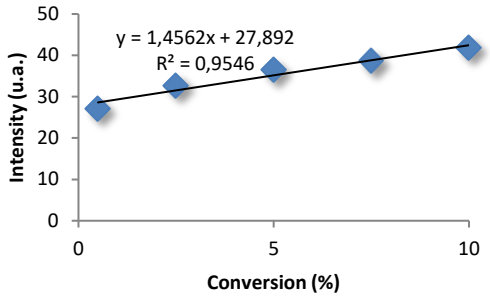
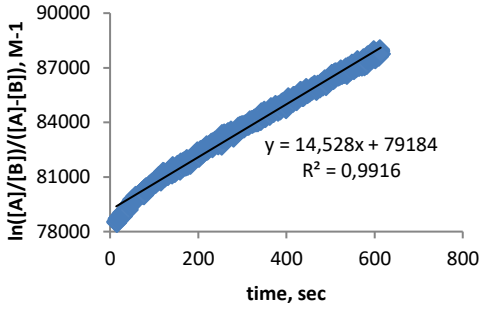
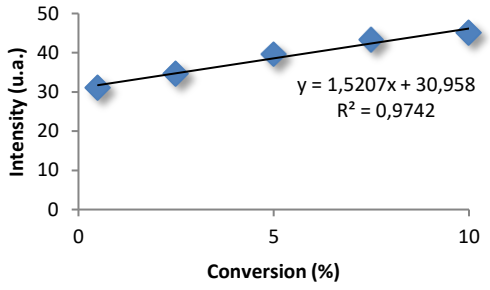
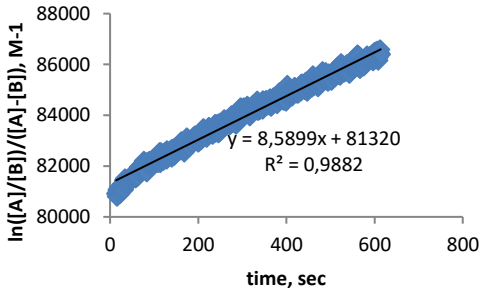
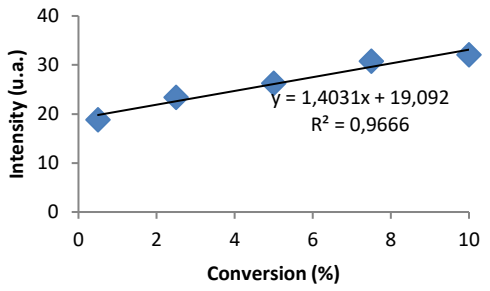
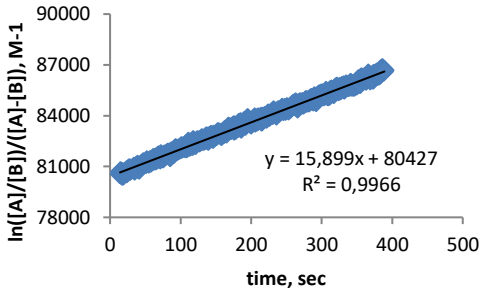
PBK 5mM/ DMSO (8:2)		
Cellular lysate/ DMSO (8:2)		
Plasma/ DMSO (8:2)		
Glutathion 1mM (in PBK 5mM)/ DMSO (8:2)		

Table S10. *Summary of kinetic data obtained with BCN and DIBAC*

Entrée	n	Alcyne	PBK	Lysat cellulaire	Plasma dilué	Glutathion 1 mM
1	**1**	**BCN**	0,624 ± 0,014	0,683 ± 0,009	0,504 ± 0,022	0,474 ± 0,003
2	**2**	**BCN**	0,152 ± 0,002	0,137 ± 0,006	0,128 ± 0,007	0,130 ± 0,001
3	**1**	**DIBAC**	78,81 ± 9,51	102,11 ± 14,9	82,73 ± 25,96	35,29 ± 1,51
4	**2**	**DIBAC**	24,67 ± 6,32	36,28 ± 7,71	20,37 ± 6,77	20,00 ± 2,19
5	**3**	**DIBAC**	12.63 ± 2.89	14.52 ± 1.33	8.59 ± 2.6	15.74 ± 1.98

VI. Protein Labelling

A- METHODS

SDS-PAGE

Proteins samples in loading buffer were boiled at 95 °C for 5 min and subsequently loaded into wells of a 4 - 20% mini-SDS gel. The Mini-Protean III electrophoresis system was used with tris/glycine migrating buffer.

FLUORESCENCE IMAGING

Fluorescence measurements were performed with VersaDoc MP 4000 Molecular Digital Imaging System (Bio-Rad) allowing visualization of bands relative to fluorescent proteins. Blue LED was used as light source and the emission collection was at 530 nm for MP 4000 imager.

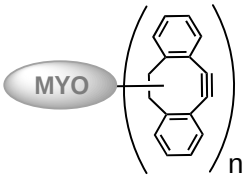
LC-ESI-MS

In this study, the LC step was performed before the ESI-MS with the only aim to desalt efficiently the sample as well as to avoid any signal saturation by the chemical sydnone. LC-ESI-MS experiments were carried out using an Esquire HCT Electrospray ion trap mass spectrometer in the positive ion mode coupled on-line to an Agilent 1100 HPLC. Nebulization and desolvation ESI conditions were optimized to obtain maximum sensitivity. Acidified samples were injected onto a monolith reverse-phase micro-column (ProSwift RP-4H, 1.0 x 50 mm, Thermo-Scientific) equilibrated in 0.1 % TFA/water and elution was carried out at 200 µL/min with a fast linear gradient of acetonitrile in 0.1 % TFA. During elution, the flow was split with 10 % directed to the electrospray mass spectrometer and 90 % to the diode array UV-Vis detector. HyStar/EsquireControl softwares were used for full scan MS acquisitions. DataAnalysis software was used for data processing and obtention of deconvoluted spectra.

MALDI-MS

Proteins sample were analysed using a 4800 spectrometer MALDI-TOF/TOF Proteomics Analyzer (Applied Biosystems, Foster City, CA). Proteins samples diluted with sinapinic acid matrix solution prepared at 10 mg/mL in H2O/CH3CN/TFA (70/30/0.1) were manually spotted on MALDI plate. MS spectra were recorded from crystallized samples using positive linear mode.

B- Preparation of Myo-DIBAC conjugate



A solution of Myoglobin from equine heart (22.5 mg, 1.32 µmol) purchased from Sigma-Aldrich in PBNa 100 mM pH 7,4 (9 mL) was treated at room temperature with dibenzocyclooctyne-*N*-hydroxysuccinimidyl ester (***DIBAC-NHS***) (1.86 mg, 4.62 µmol), previously suspended in 600 µL of DMF. After 3 h of gentle shaking, 2.25 mL of PBNa 100 mM pH 7.4 were added to the reaction mixture that was then dialysed overnight against PBK 25 mM pH 8 and aliquoted as 11.75 nmoles of Myoglobin. Aliquotes were freeze-dried and kept at -20 °C. Mass spectrometry analysis (ESI and MALDI) indicated that the obtained **Myo-DIBAC** sample contains modified Myoglobins bearing respectively one dibenzocyclooctyne (**Myo-DIBAC$_1$**) or two dibenzocyclooctyne (**Myo-DIBAC$_2$**) together with unmodified Myoglobin.

Myo-DIBAC$_1$ **Myo-DIBAC$_2$**

ESI-MS analysis

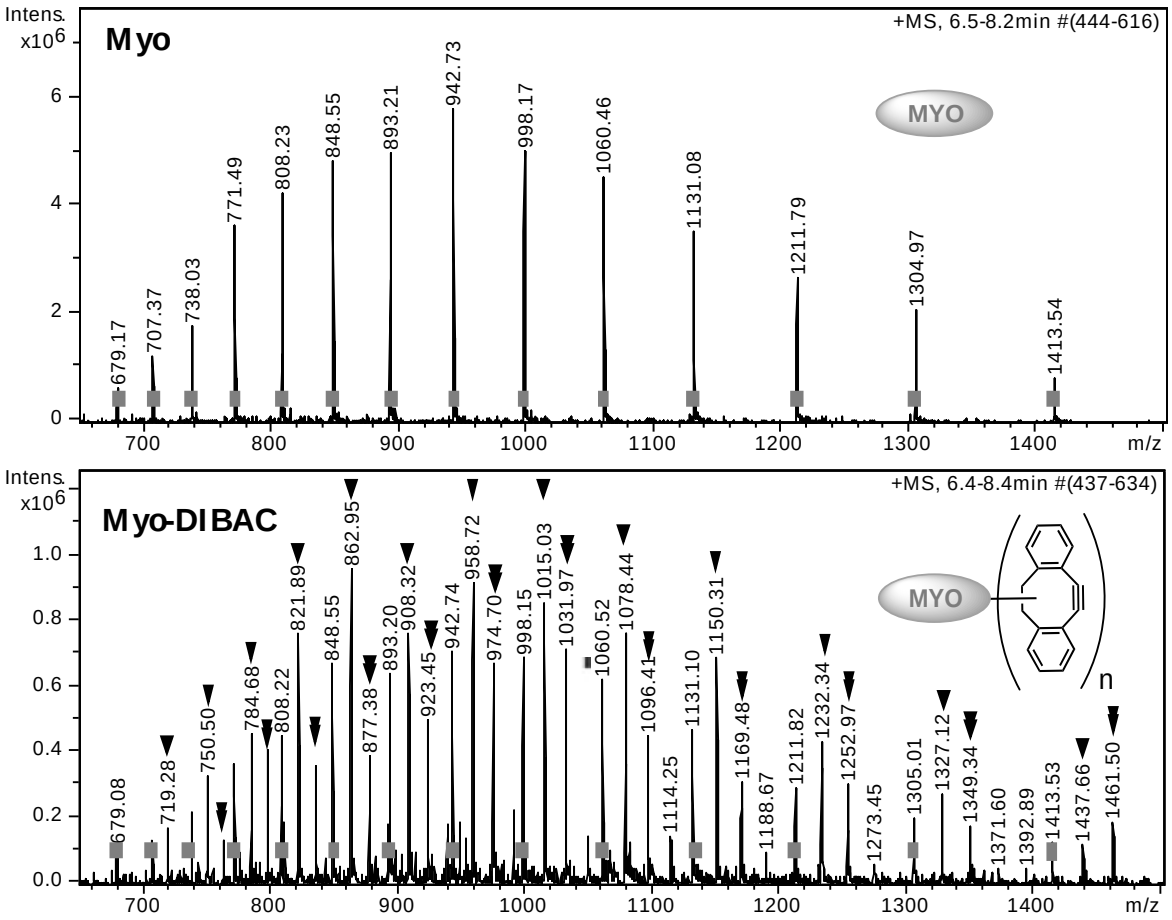


Figure S1. *ESI-MS spectra of Myoglobin (up) and Myo-DIBAC samples (down). Grey square refers to the set of multicharged states of unmodified Myoglobin, black triangles to the set relative to Myoglobin bearing one* ***DIBAC*** *(**Myo-DIBAC$_1$**, down) and black double triangles to the set relative to Myoglobin bearing two* ***DIBAC*** *(**Myo-DIBAC$_2$**, down).*

ESI-MS spectra deconvolution using Data analysis software gave access to corresponding proteic specie molecular weight:

Myo : 16951,37 Da +/-0,6 Da (expected for $C_{769}H_{1212}N_{210}O_{218}S_2$: 16951,30 Da)

Myo-DIBAC$_1$: 17238,96 Da +/- 0,4 Da (expected for $C_{788}H_{1225}N_{211}O_{220}S_2$:17238,61 Da)

Myo-DIBAC$_2$: 17526,53 Da +/- 0,57 Da (expected for $C_{807}H_{1238}N_{212}O_{222}S_2$:17525,91 Da).

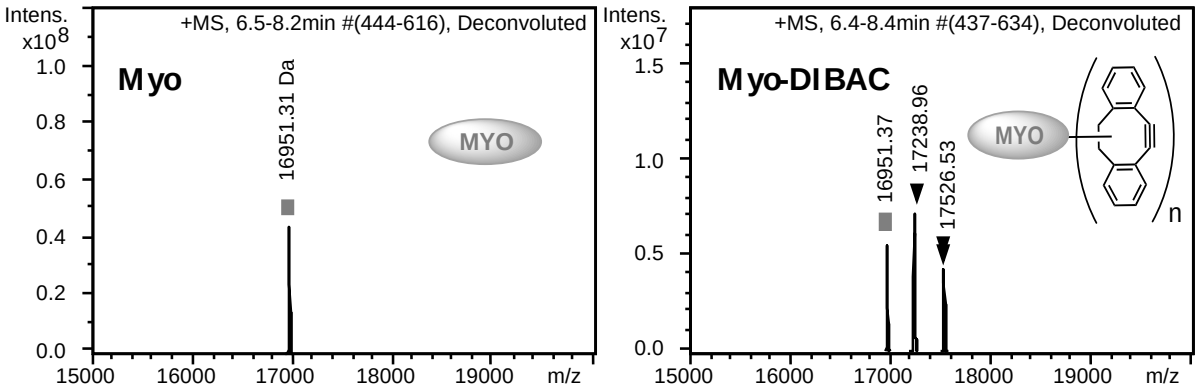


Figure S2. *ESI-MS deconvoluted of Myoglobin (left) and **Myo-DIBAC** (right). Grey square refers to the set of multicharged states of unmodified Myoglobin, black triangles to the set relative to Myoglobin bearing one DIBAC (Myo-DIBAC$_1$, left) and black double triangles to the set relative to Myoglobin bearing two DIBAC (Myo-DIBAC$_2$, right).*

MALDI-TOF analysis

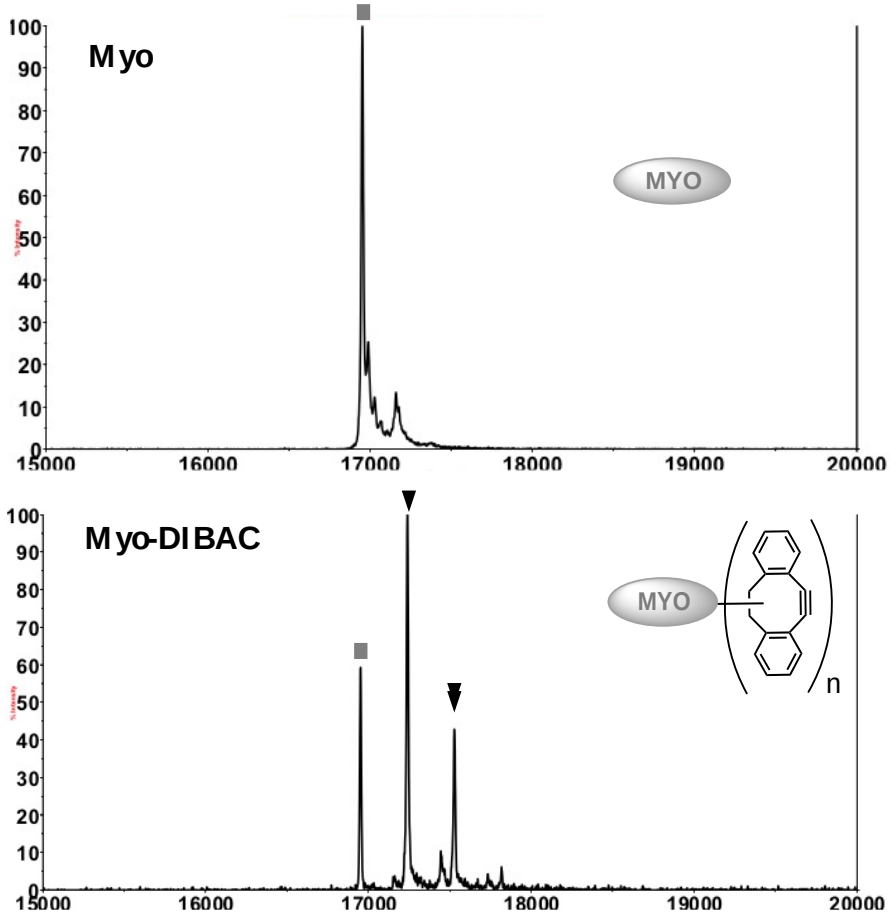


Figure S3. *MALDI-MS spectra of Myoglobin (up) and **Myo-DIBAC** (down). Grey square refers to the set of multicharged states of unmodified Myoglobin, black triangles to the set relative to Myoglobin bearing one DIBAC (Myo-DIBAC$_1$, up) and black double triangles to the set relative to Myoglobin bearing two DIBAC (Myo-DIBAC$_2$, down).*

SDS-PAGE analysis

A part of **Myo-DIBAC** sample as well as an equimolar reference of native unmodified Myoglobin (0.06 nmoles) were analysed by SDS-PAGE using standard condition, Laemmli buffer, 4–20% acrylamide gel and silver staining.

Protein migration on SDS-PAGE

Equal amount of **Myo-DIBAC** (10 µg, 1 µg/µl) and Myoglobin (10 µg, 1 µg/µl) as negative control in Laemmli buffer were loaded in wells of a 4%–20% gradient gel after being heated up to 95 °C for 5 min. SDS-PAGE (Bio-Rad® Tris/glycine migrating buffer system, mini-Protean III electrophoresis system) was performed under denaturating conditions

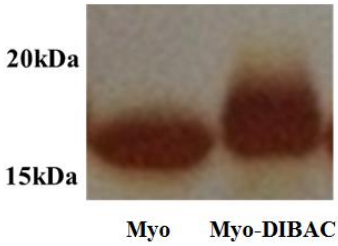
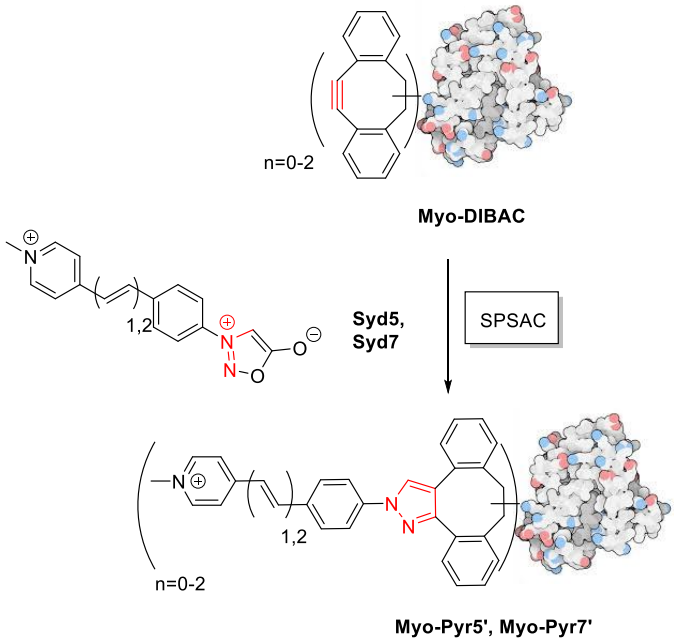


Figure S4. *Silver stained SDS-PAGE of Myoglobin and **Myo-DIBAC**.*

C- SPSAC between Syd10/Syd12 and Myo-DIBAC in solution



Protocol

A **Myo-DIBAC** (11.75 nmoles) solution in PBK 50 mM pH 8 (45 µL) was mixed with 5 µL of DMSO solubilized **Syd5**/**Syd7** at 10 mM (50 nmoles). The final 50 µL solution mixture containing 235 µM of **Myo-DIBAC** and 1 mM of **Syd5**/**Syd7** was incubated for two hours at room temperature to give **Myo-Pyr5'**/**Pyr7'** sample.

ESI-MS

Each **Myo-Pyr5'/Pyr7'** samples were analysed by LC-ESI-MS. In both cases, the ESI-MS spectra shows the presence of three sets of multicharged states corresponding respectively to three type of Myoglobin entities: unmodified myoglobin (grey squares), myoglobin bearing one pyrazole derivative ($PyrX_1$ = colored triangles) and myoglobin bearing two pyrazole derivatives ($PyrX_2$ = colored double triangles).

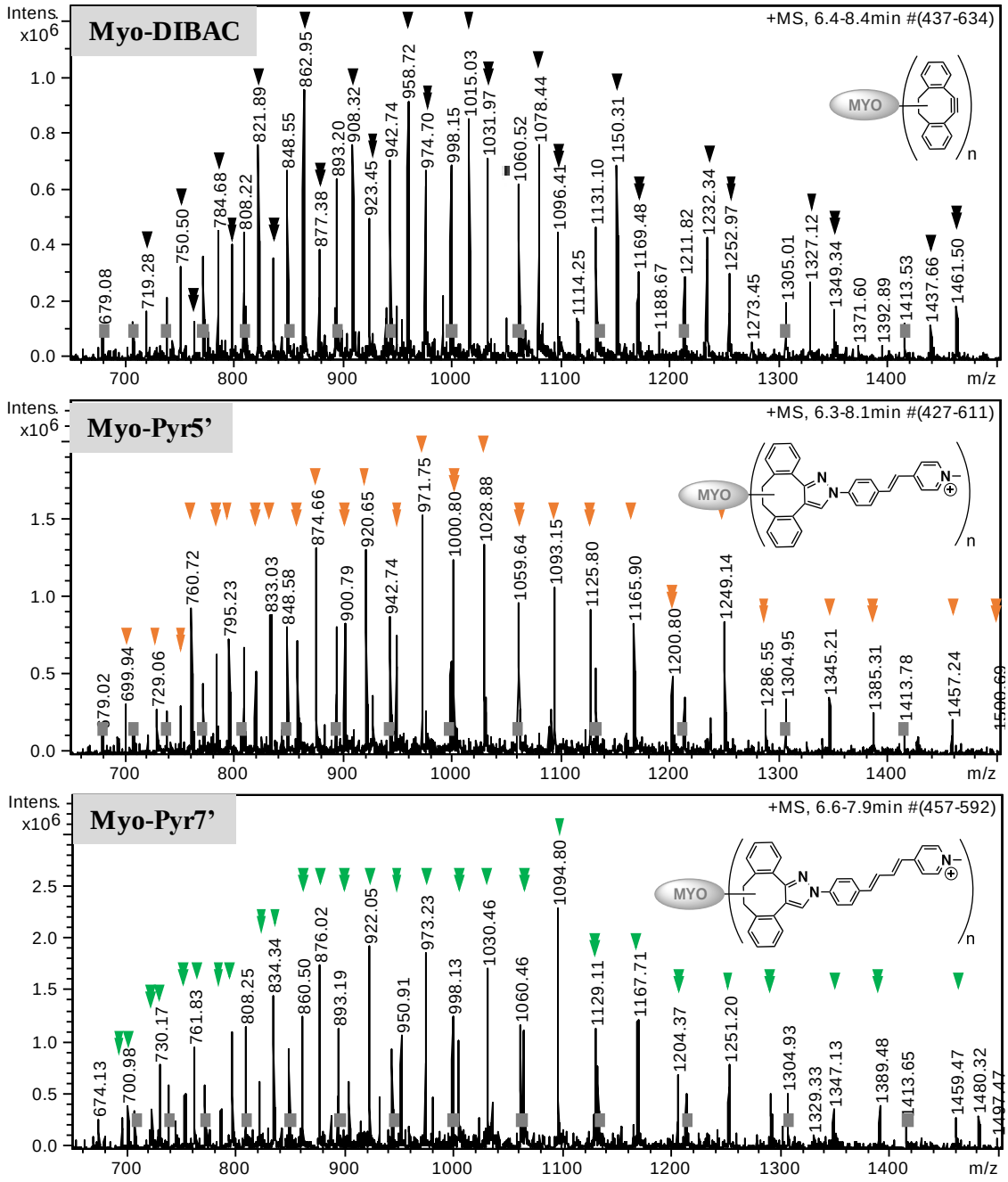


Figure S5. *ESI-MS spectra of **Myo-DIBAC**, **Myo-Pyr5'** and **Myo-Pyr7',** resulting from the labelling of **Myo-DIBAC** by **Syd5** or **Syd7** probes. Grey squares refer to unmodified MYO, black triangles to **Myo-DIBAC** (n = 1) and black double triangles to **Myo-DIBAC** (n = 2), orange triangles (n = 1) and orange double triangles (n = 2) to **Syd5**-derived Myoglobin; green triangles (n = 1) and green double triangles (n = 2) to **Syd7**-derived Myoglobin.*

Table S11. *Summary of m/z for each charge state of MYO and MYO-conjugates*

	measured m/z						
charge state z	**Myo**	**Myo-DIBAC$_1$**	**Myo-DIBAC$_2$**	**Myo-Pyr5'$_1$**	**Myo-Pyr5'$_2$**	**Myo-Pyr7'$_1$**	**Myo-Pyr7'$_2$**
25+	679.17	690.54	702.21	699.94	720.75	700.98	722.82
24+	707.37	719.28	731.14	729.06	750.76	730.17	753.04
23+	738.03	750.50	763.05	760.72	783.52	761.83	785.82
22+	771.49	784.68	797.68	795.23	818.96	796.44	821.39
21+	808.23	821.89	835.49	833.03	857.96	834.34	860.50
20+	848.55	862.95	877.38	874.66	900.79	876.02	903.41
19+	893.21	908.32	923.45	920.65	948.19	922.05	950.91
18+	942.73	958.72	974.70	971.75	1000.80	973.23	1003.73
17+	998.17	1015.03	1031.97	1028.88	1059.64	1030.46	1062.69
16+	1060.46	1078.44	1096.41	1093.15	1125.80	1094.80	1129.11
15+	1131.08	1150.31	1169.48	1165.90	1200.80	1167.71	1204.37
14+	1211.79	1232.34	1252.97	1249.14	1286.55	1251.20	1290.06
13+	1304.97	1327.12	1349.34	1345.21	1385.31	1347.13	1389.48
12+	1413.54	1437.66	1461.50	1457.24	1500.69	1459.47	1505.01

ESI-MS spectra deconvolution using *Data Analysis Software* gave access to corresponding proteic specie molecular weights.

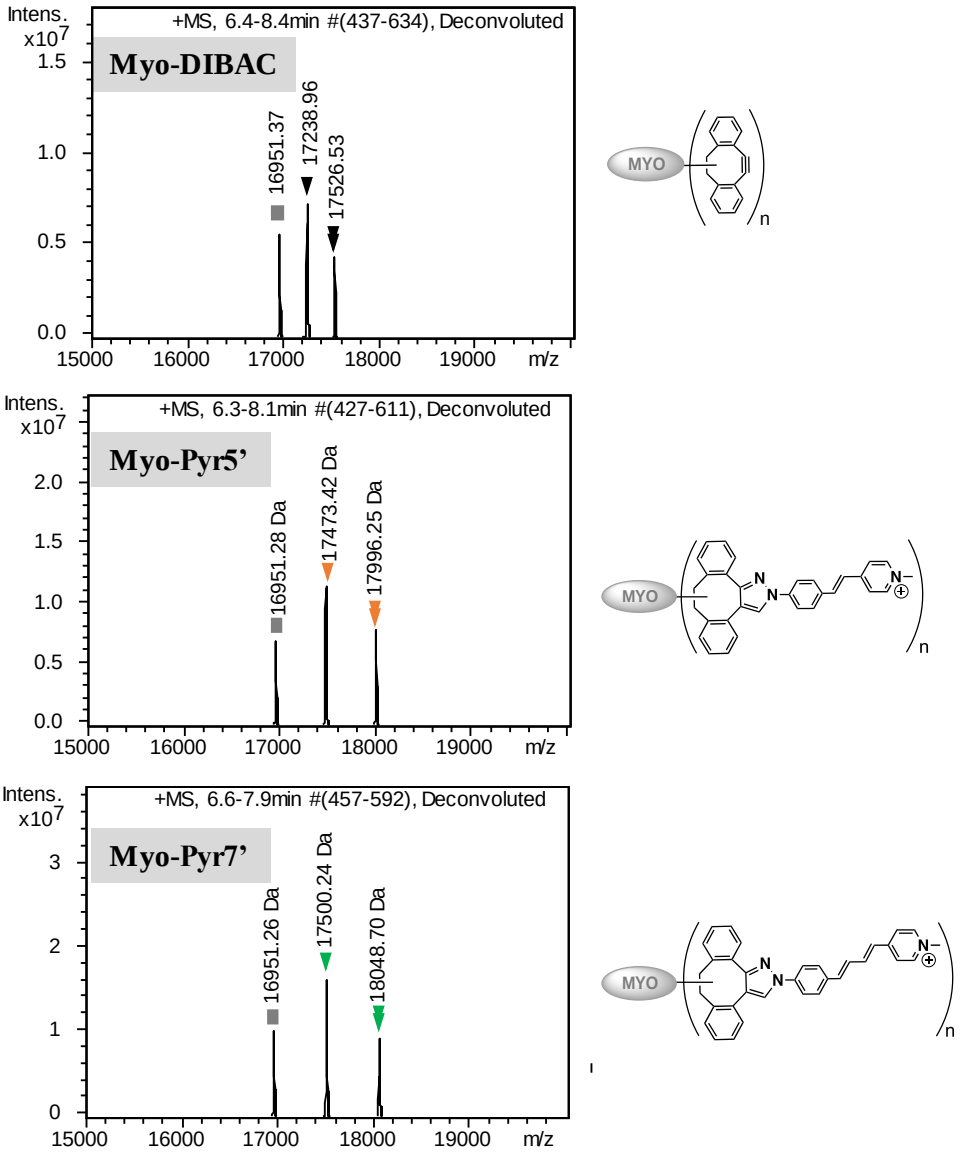
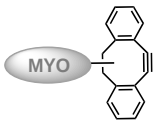
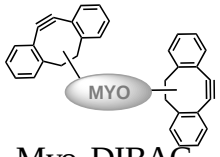


Figure S6. *Comparison of ESI-MS deconvoluted spectra of **Myo-DIBAC**, **Myo-Pyr5'** and **Myo-Pyr7'** resulting from the labelling of **Myo-DIBAC** by **Syd5** or **Syd7** probes. Grey squares refer to unmodified MYO, black triangles to **Myo-DIBAC** (n = 1) and black double triangles to **Myo-DIBAC** (n = 2), orange triangles (n = 1) and orange double triangles (n = 2) to **Syd5**-derived Myoglobin; green triangles (n = 1) and green double triangles (n = 2) to **Syd7**-derived Myoglobin.*

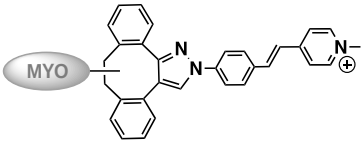
1st panel Myo-DIBAC	16951.37 Da +/-0.58 Da for Myo expected at 16951.30 Da ($C_{769}H_{1212}N_{210}O_{218}S_2$)
	17238.96 Da +/- 0.40 Da for Myo-DIBAC$_1$ expected at 17238.61 Da($C_{788}H_{1225}N_{211}O_{220}S_2$)
	17526.53 Da +/- 0.58 Da for Myo-DIBAC$_2$ expected at 17525.91 Da($C_{807}H_{1238}N_{212}O_{222}S_2$)
2nd panel Myo-Pyr5'	16951.28 Da +/-0.45 Da for Myo expected at 16951.30 Da($C_{769}H_{1212}N_{210}O_{218}S_2$)
	17473.42 Da +/- 0.37 Da for Myo-Pyr5'$_1$ expected at 17474.90 Da ($C_{803}H_{1239}N_{214}O_{220}S_2$)
	17996.25 Da +/- 0.73 Da for Myo-Pyr5'$_2$ expected at 17998.51 Da ($C_{837}H_{1266}N_{218}O_{222}S_2$)
3rd panel Myo-Pyr7'	16951.26 Da +/-0.59 Da for Myo expected at 16951.30 Da ($C_{769}H_{1212}N_{210}O_{218}S_2$)
	17500.24 Da +/- 0.58 Da for Myo-Pyr7'$_1$ expected at 17500.94 Da ($C_{805}H_{1241}N_{214}O_{220}S_2$)
	18048.70 Da +/- 0.73 Da for Myo-Pyr7'$_2$ expected at 18050.58 Da ($C_{841}H_{1270}N_{218}O_{222}S_2$)



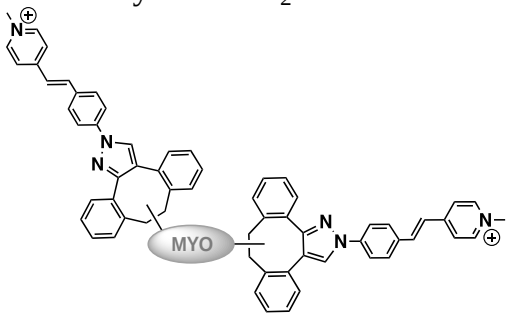
Myo-DIBAC$_1$



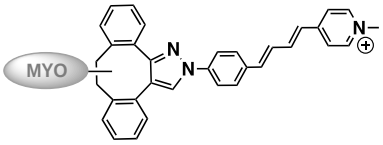
Myo-DIBAC$_2$



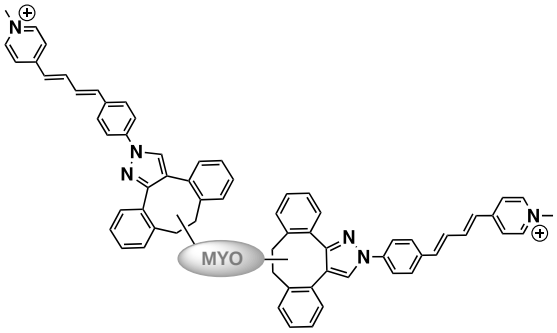
Myo-Pyr5'$_1$



Myo-Pyr5'$_2$



Myo-Pyr7'$_1$



Myo-Pyr7'$_2$

MALDI-MS

MALDI-MS spectra obtained from the same MYO-pyrX samples confirm the presence of three protein species in each of these samples: unmodified MYO, MYO-pyrX$_1$ and MYO-pyrX$_2$.

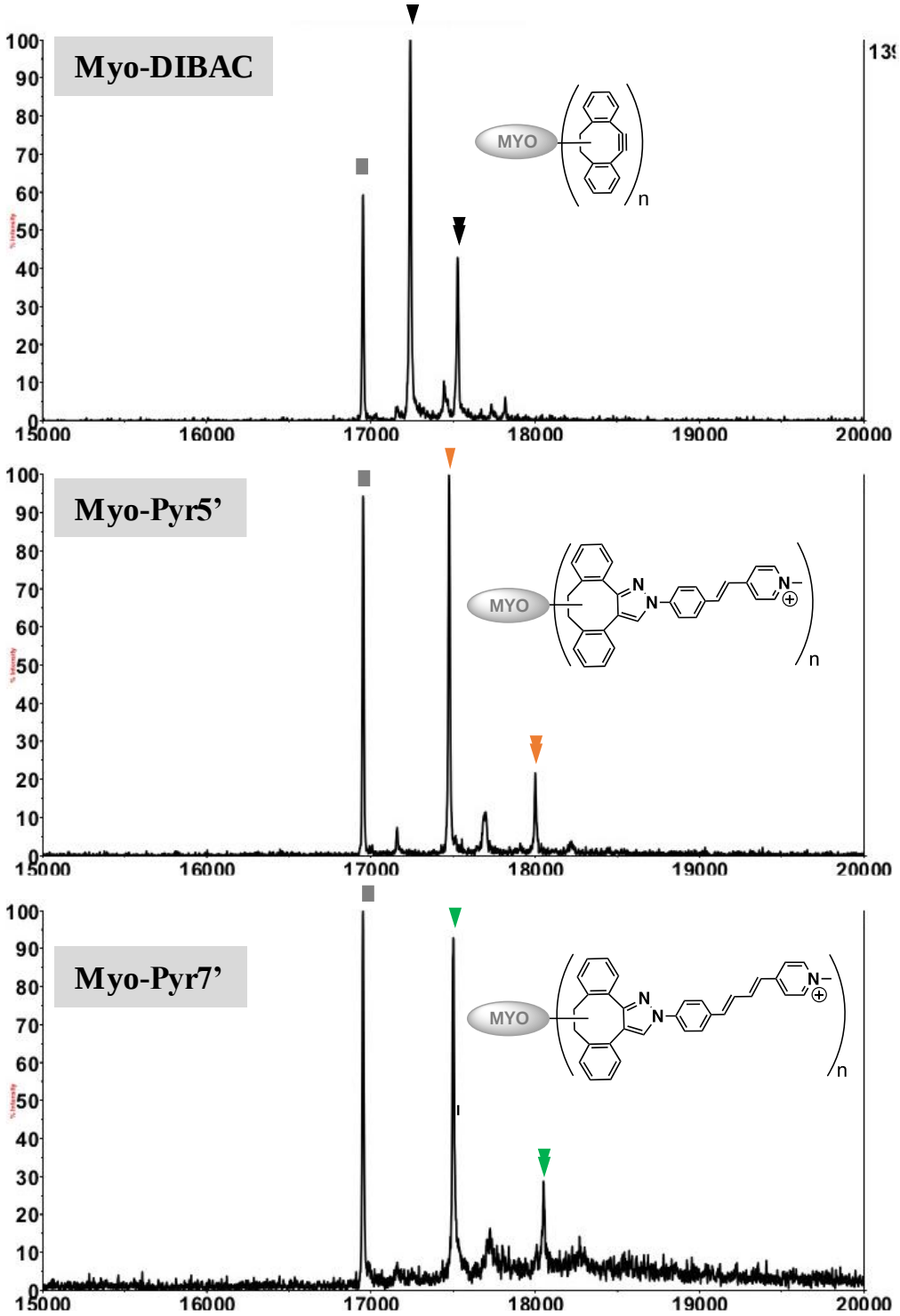


Figure S7- *Comparison of MALDI-MS spectra of **Myo-DIBAC**, **Myo-Pyr5'** and **Myo-Pyr7'** resulting from the labelling of **Myo-DIBAC** by **Sy5** or **Syd7** probes. Grey squares refer to unmodified MYO, black triangles to **Myo-DIBAC** (n = 1) and black double triangles to **Myo-DIBAC** (n = 2), orange triangles (n = 1) and orange double triangles (n = 2) to **Syd5**-derived Myoglobin; green triangles (n = 1) and green double triangles (n = 2) to **Syd7**-derived Myoglobin.*

According to these spectra, Myo-PyrX_2 shows poorest desorption properties compared to Myo-PyrX_1. This could be related to its hydrophobic structure and the ability of MALDI-MS technology to be less prone to detect hydrophobic species.

SDS-PAGE and Fluorescence imaging

Both solutions of *mass-charaterized* **Syd5** and **Syd7** adducted myoglobins were analysed by SDS-PAGE using standard condition, Laemmli buffer and 4 – 20 % acrylamide gel. The gel was first subjected to fluorescence analysis (upper panels) and then to silver staining (lower panel). The detection threshold using Versadoc imager was shown to be better for **Myo-Pyr7'** than for **Myo-Pyr5'**. The positive detection of Myo-PyrX could be achieved after 5 minutes 95 °C heating and electrophoretic migration. Quantity down to 3 µg was detected in the case of labeled **Myo-Pyr7'**.

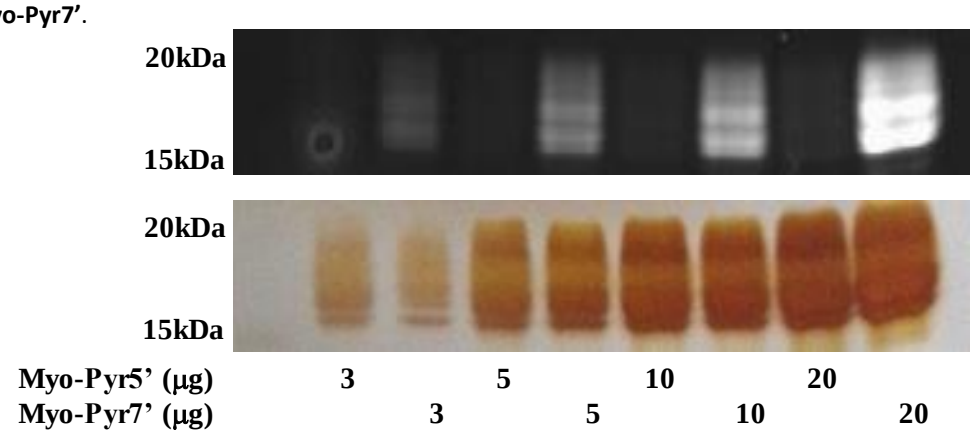


Figure S8- ***Myo-Pyr5'*** *and* ***Myo-Pyr7'*** *(3, 5, 10 and 20 µg) analysed by SDS-PAGE on a 4 - 20 % polyacrylamide gel : scanned for fluorescence with Versadoc MP4000 (upper panel) or silver stained (lower panel)*

D- *"On-gel SPSAC"* method

The *on-gel SPSAC* method refers to a new labelling strategy where a turn-on sydnone probe solution is dispensed on a SDS-PAGE gel to specifically enlighten octyne proteins already present in the gel. The gel is overlaid by a buffered solution of sydnone and fluorescence is visualised using an imager. The overlay step is illustrated on Figure S9.

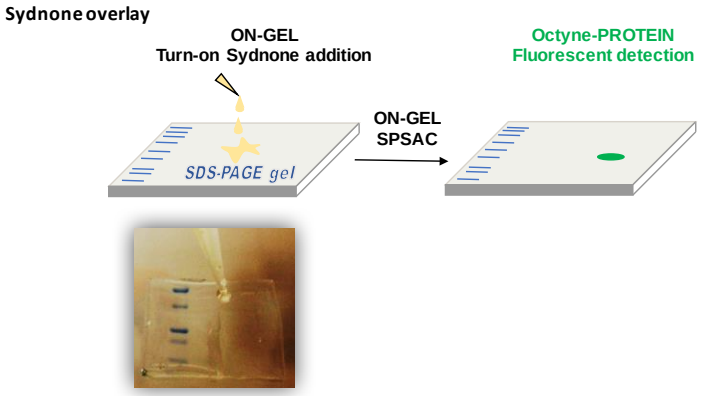


Figure S9*. Overlay step: Gel is overlaid with a solution of sydnone*

On-gel SPSAC Protocol

After protein migration, the gel was first incubated in a fixing solution (30 % EtOH, 5 % Acetic acid in deionized water, 2 x 30 min), rinsed with water (4 x 10 min) and carefully settled on a glass plate. The gel was then overlaid with a solution of sydnone at 100 µM in PBK 5 mM pH 8. Interaction between the gel and the sydnone solution was maintained for 5 min or more when specified. The solution was removed from the gel using a blotting paper. The gel was then scanned on Molecular Imager® VersaDoc™ MP 4000 to detect the fluorescent protein adduct.

E- Syd5/Syd7 labelling with Myo-DIBAC using *On-gel SPSAC* method

Protocol

Equal amount of **Myo-DIBAC** (10 µg, 1 µg/µl in Laemmli buffer) and Myoglobin (10 µg, 1 µg/µl in Laemmli buffer) were loaded in wells of a 4 % - 20 % gradient gel after being heated up to 95 °C for 5 min. SDS-PAGE (Tris/glycine migrating buffer system) was performed under denaturating conditions. After migration, *On-gel SPSAC* protocol was then performed with a solution of sydnone (**Syd5/Syd7**) for 5 min**.** Following the fluorescence revelation, the SDS-PAGE was revealed following the standard coomassie blue procedure.

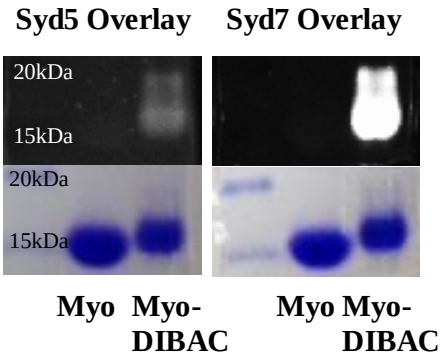


Figure S10. *On-gel SPSAC method using turn-on probe **Syd5** and **Syd7** after migration of **Myo-DIBAC** and Myo (10 µg) set as control. 4 - 20 % SDS gel were overlaid with a solution of **Syd5** or **Syd7** (100 µM) for 5 min, scanned for fluorescence (upper panels) and coomassie stained (lower panels).*

F- Study of detection threshold of Myo-DIBAC with Syd7 using *On-gel SPSAC* method

Protocol

A range of quantities of Myoglobin-DIBAC (100 ng, 250 ng, 500 ng, 1 µg, 2 µg, 5 µg, 10 µg) were loaded and migrated on a (4 % - 20 %) SDS-PAGE from a mother solution at 1 µg/µl. After migration, *On-gel SPSAC* protocol was then performed with a solution of sydnone (**Syd7**) during 5 min for the 1 µg, 2 µg, 5 µg, 10 µg samples and during 20 min in the case of 100 ng, 250 ng, 500 ng samples. Following the fluorescence revelation (upper panels), the 100 ng - 500 ng part of the SDS-PAGE was then revealed following the standard silver-Nitrate procedure and the 1 µg - 10 µg part was revealed using the coomassie blue procedure.

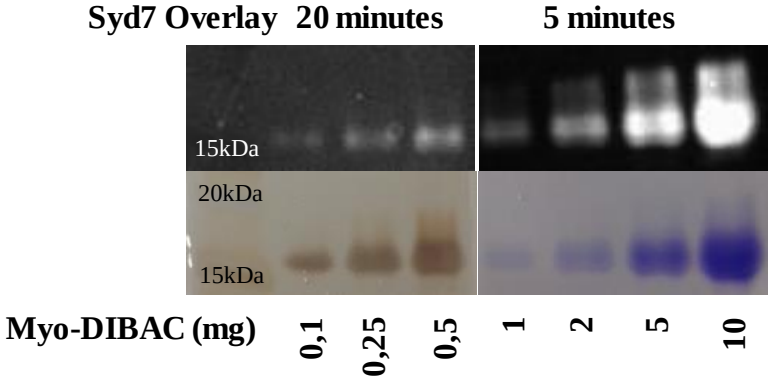


Figure S11. *Detection threshold of **Myo-DIBAC** using on-gel SPSAC method. After migration of **Myo-DIBAC** quantities from 10 µg down to 100 ng, 4 – 20 % SDS gel was overlaid with a solution of **Syd7** (100 µM) for 5 min to 20 min, scanned for fluorescence (upper panels), coomassie stained (right lower panel) or silver-stained (left lower panel).*

G- Study of Syd7 probe specificity to stain Myo-DIBAC in complex media (HSA or HeLa Lysate):

Protocol

Three solutions of **Myo-DIBAC**, HeLa Lysate and HSA were prepared in Leammli buffer at respectively 1 µg/µL, 1.65 µg/µL and 1.65 µg/µL. To produce **Myo-DIBAC** spiked-in lysate or HSA solutions, a solution of **Myo-DIBAC** (2 µg/µL) in PBK 50 mM pH 8 was diluted ten times with a HeLa Lysate solution (2.2 µg/µL) or with buffered solution of HSA (2.2 µg/µL) before the addition of Laemmli buffer. To do so, a **Myo-DIBAC** solution (10 µL) was added to HeLa lysate solution or HSA solution (91 µL) before the addition of Laemmli blue 4X (33.6 µL). Both resulting solutions contained **Myo-DIBAC** at 0.15 µg/µL together with HeLa lysate or HSA at 1.48 µg/µL. From these samples in loading buffer, 2 µg of **Myo-DIBAC** (2 µL), 20 µg of HSA (12.1 µL), 20 µg of HeLa lysate (12.1 µL) and mixtures of **Myo-DIBAC** /HSA and **Myo-DIBAC** /HeLa Lysate (13.4 µL, 2 µg / 20 µg,) were loaded in wells of 4 % - 20 % gradient SDS-gel. SDS-PAGE (Tris/glycine migrating buffer system) was performed under denaturating conditions. After migration, *On-gel SPSAC* protocol was performed with a solution of sydnone (**Syd7**) for 5 min. Following the fluorescence revelation, the SDS-PAGE was revealed following the standard coomassie blue procedure.

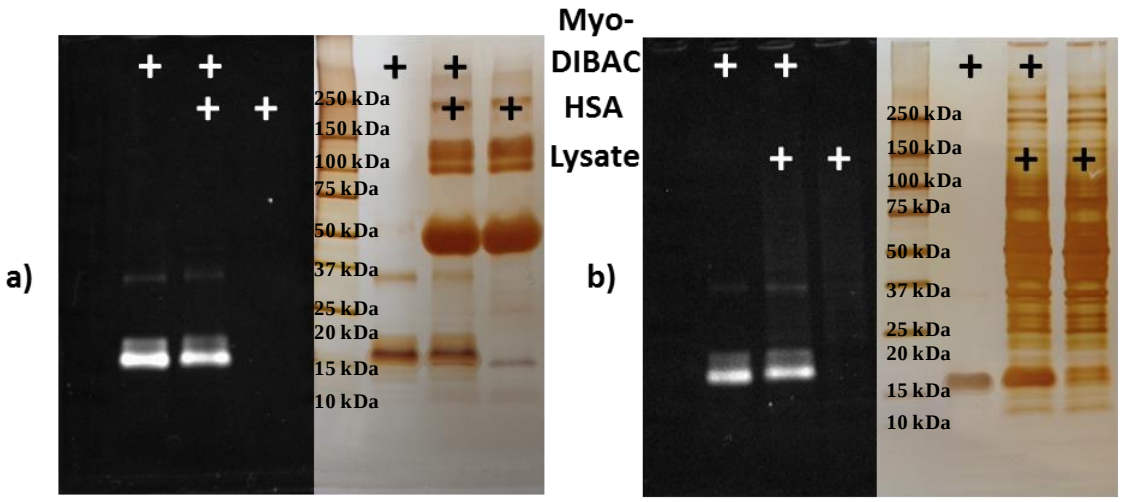


Figure S12. *On-gel SPSAC specificity after migration of **Myo-DIBAC** mixed or not to HSA (a) or to HeLa Lysate (b) and HSA and Lysate controls (lanes III and VI). 4 – 20 % SDS gel were overlaid with a solution of **Syd7** (100 µM) for 5 min, scanned for fluorescence (left panels) and silver stained (right panels).*

VII. Cell culture and confocal microscopy general procedures

The hepatocyte carcinoma cells Huh-7 were grown in low-glucose Dulbecco's modified Eagle's medium (DMEM) (SIGMA-Aldrich, St-Louis, MO) supplemented with 10% fetal bovine serum (FBS) (SIGMA), 100 U/mL penicillin, 100 µg/mL streptomycin (Eurobio) at 37°C in 5% CO_2 humidified atmosphere.

The day before experiment, 2.5 x 104 Huh-7 cells per well were seeded in eight-chambered Lab-Tek plates (Ref 155409, Nunc, Rochester, NY) in 500 µL complete medium. Cells were incubated with 50 µM of **Syd7** for 90 min. Medium was removed and cells were then incubated with BCN at different concentrations for 30 min. In other assays, cells were incubated first with BCN and then with **Syd7**. Nuclei labeling was obtained with Hoechst 33258 (SIGMA) at 5 µg/mL after 30 min incubation. Pictures were taken in red-phenol free DMEM with a Leica TSC SPE confocal microscope (Leica Microsystems GmbH, Mannheim, Germany) using 405 nm and 488 nm laser.

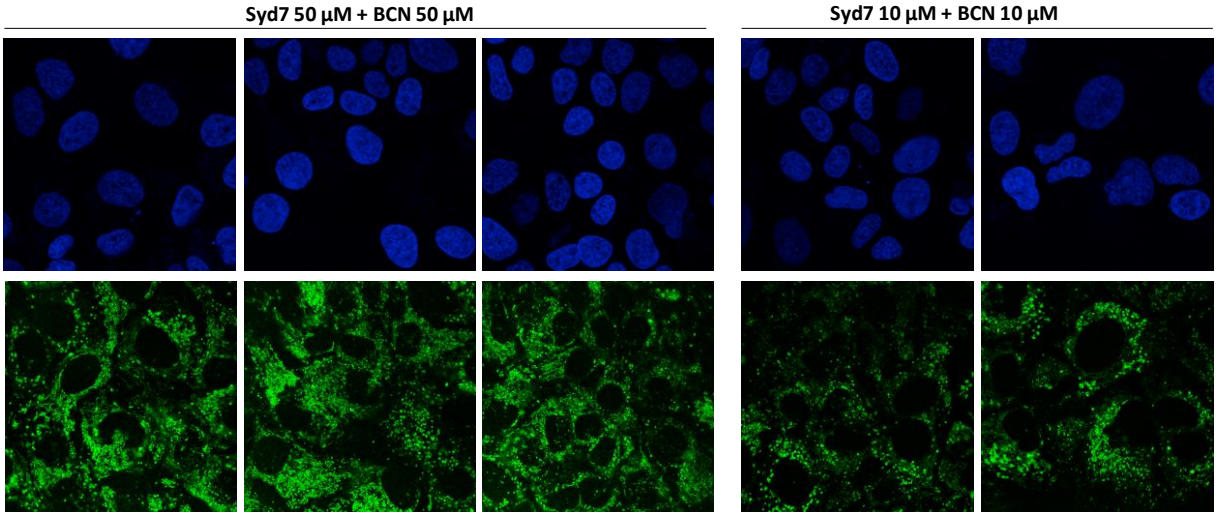


Figure S13. *In cell SPSAC reactions. Cells were incubated with 50 µM or 10 µM of **Syd7** for 90 min, then medium was removed and cells were then incubated with 50 µM or 10 µM of BCN for 90 min. Nuclei labelling was obtained with Hoechst 33258. Pictures were taken with a confocal microscope using 488 nm laser.*

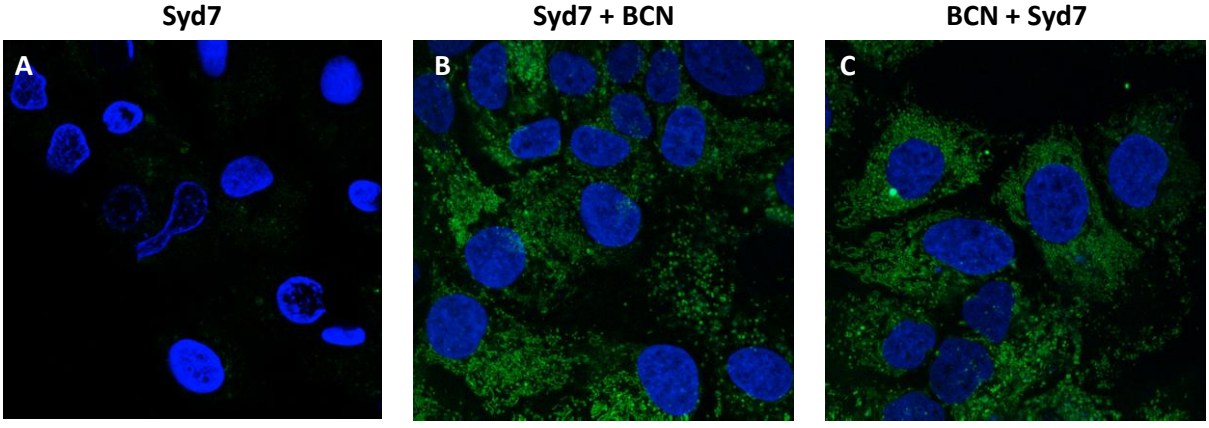
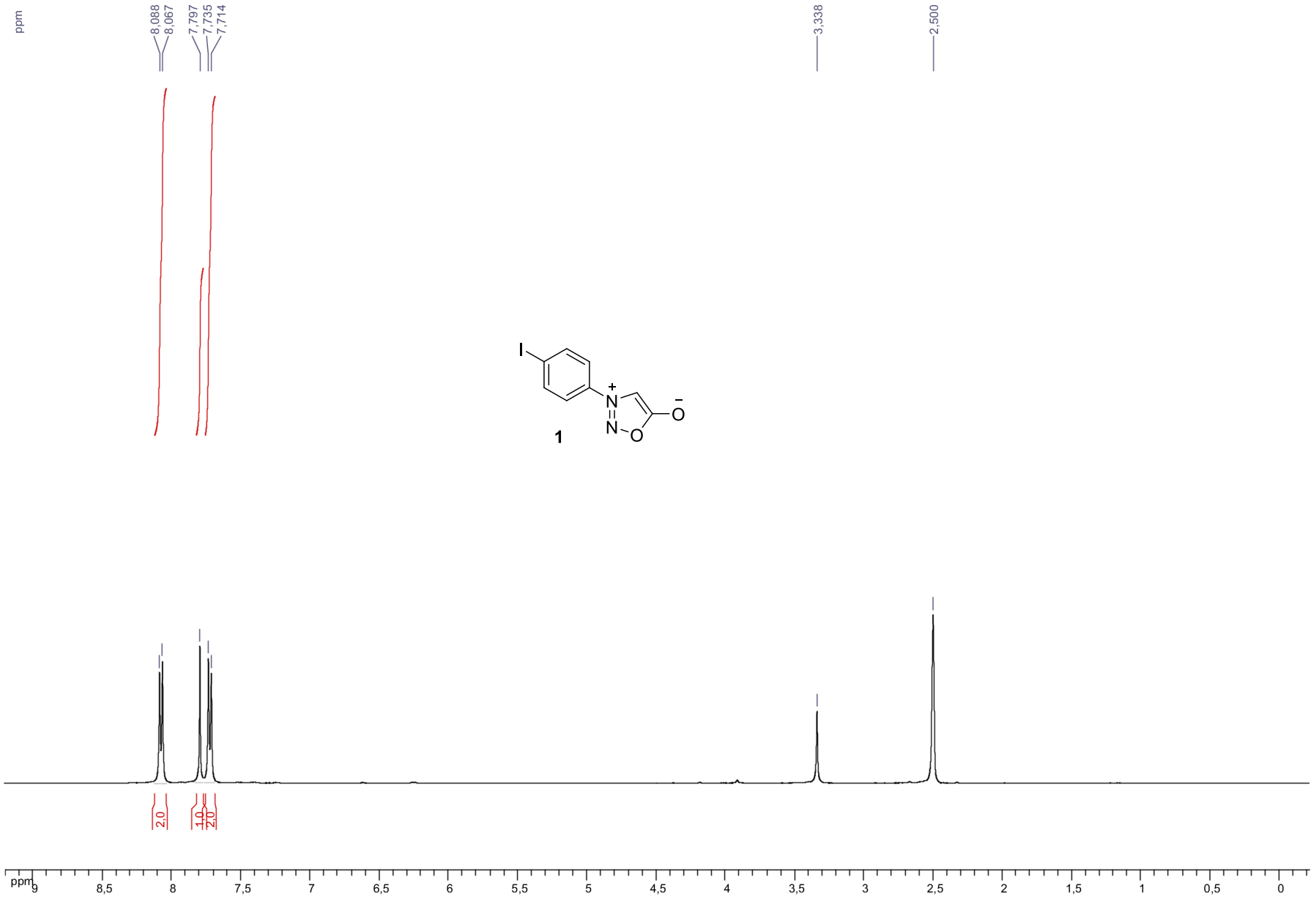


Figure S14. *In cell SPSAC reactions. A) Control experiment in absence of BCN; B) Cells were incubated with 50 µM of **Syd7** for 90 min, then medium was removed and cells were then incubated with 50 µM of BCN for 90 min; C) Inverse addition: cells were incubated with 50 µM of BCN for 90 min, then medium was removed and cells were then incubated with 50 µM of **Syd7** for 90 min. Nuclei labelling was obtained with Hoechst 33258. Pictures were taken with a confocal microscope using 488 nm laser.*

VIII. ^{1}H NMR and ^{13}C NMR spectra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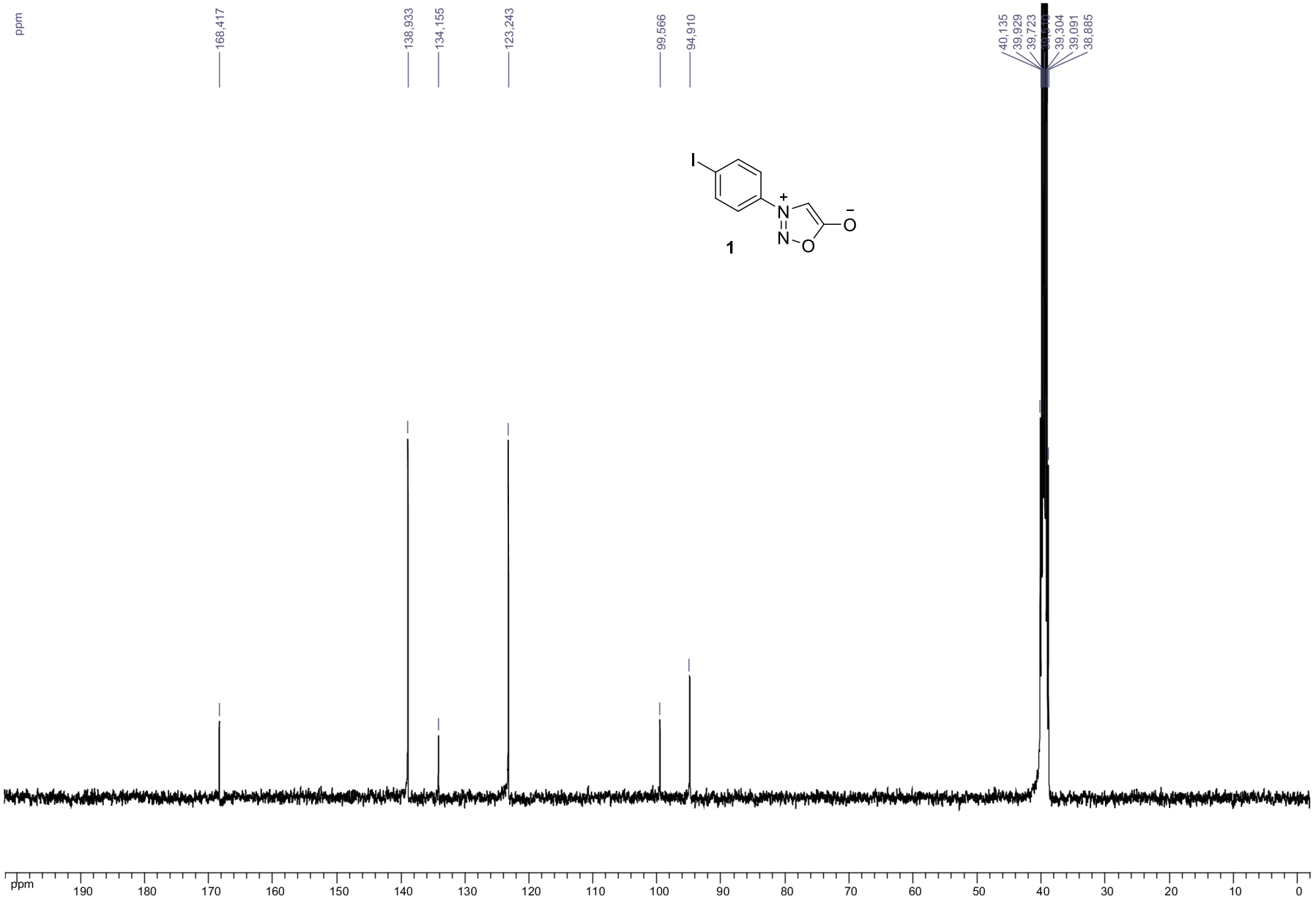
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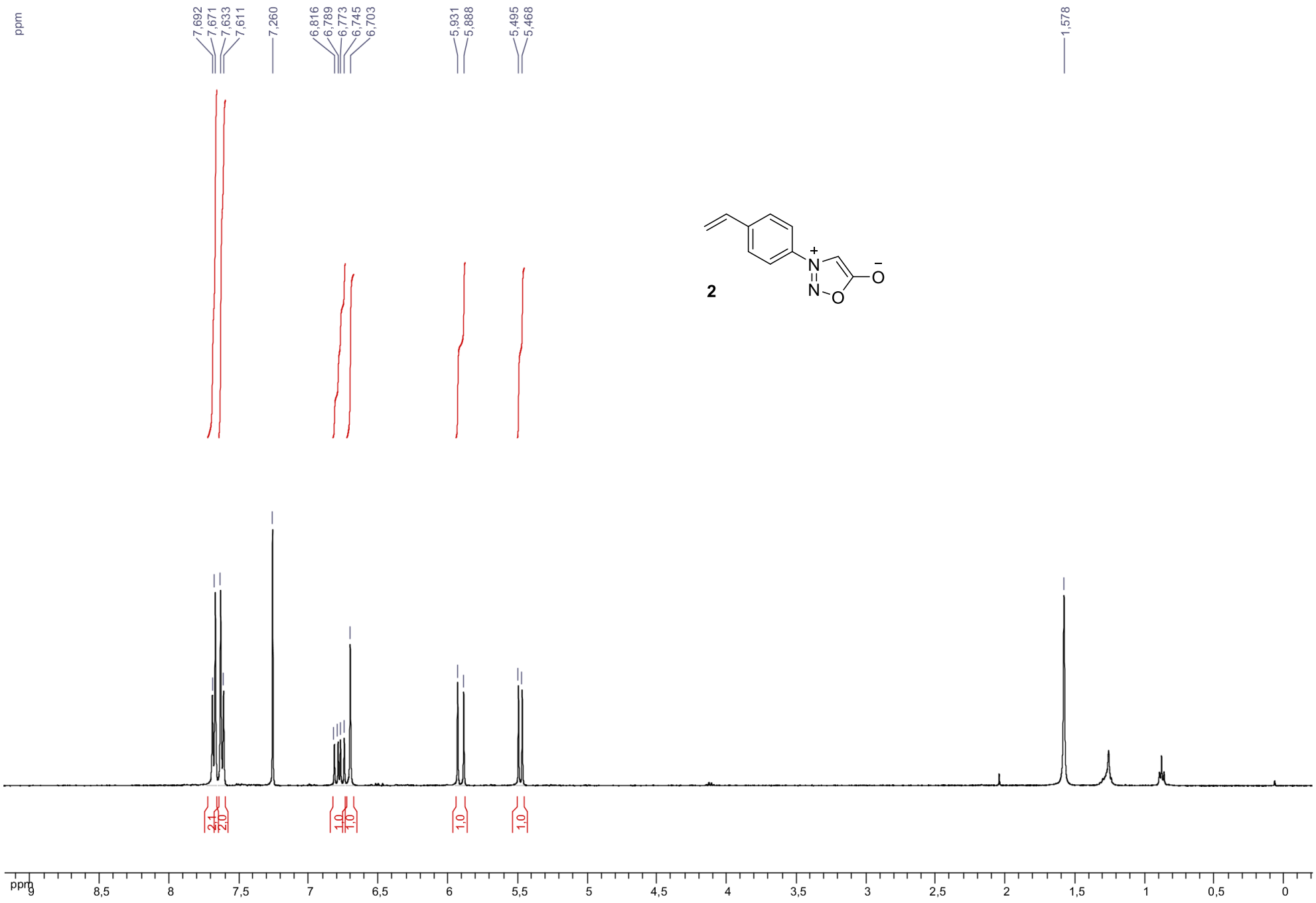
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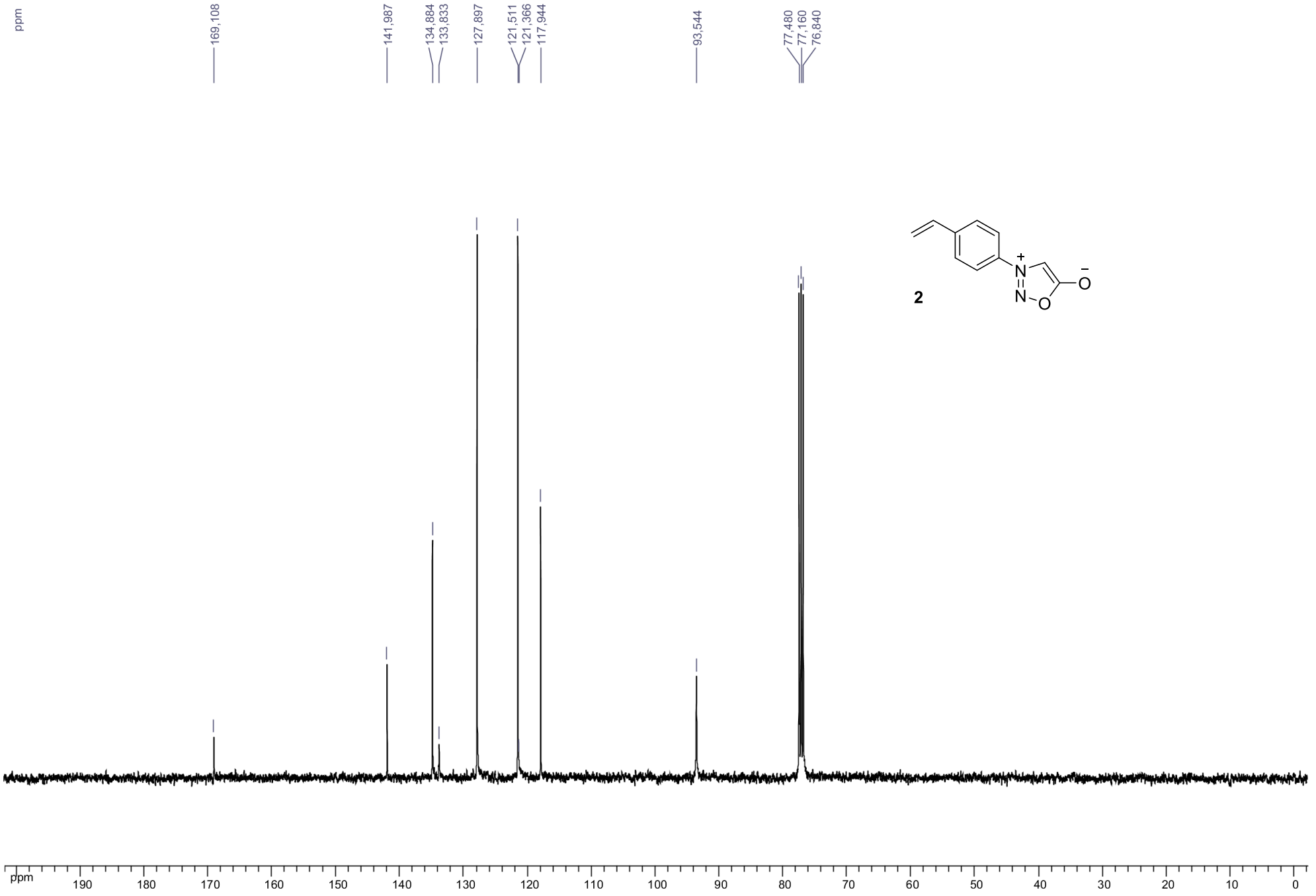
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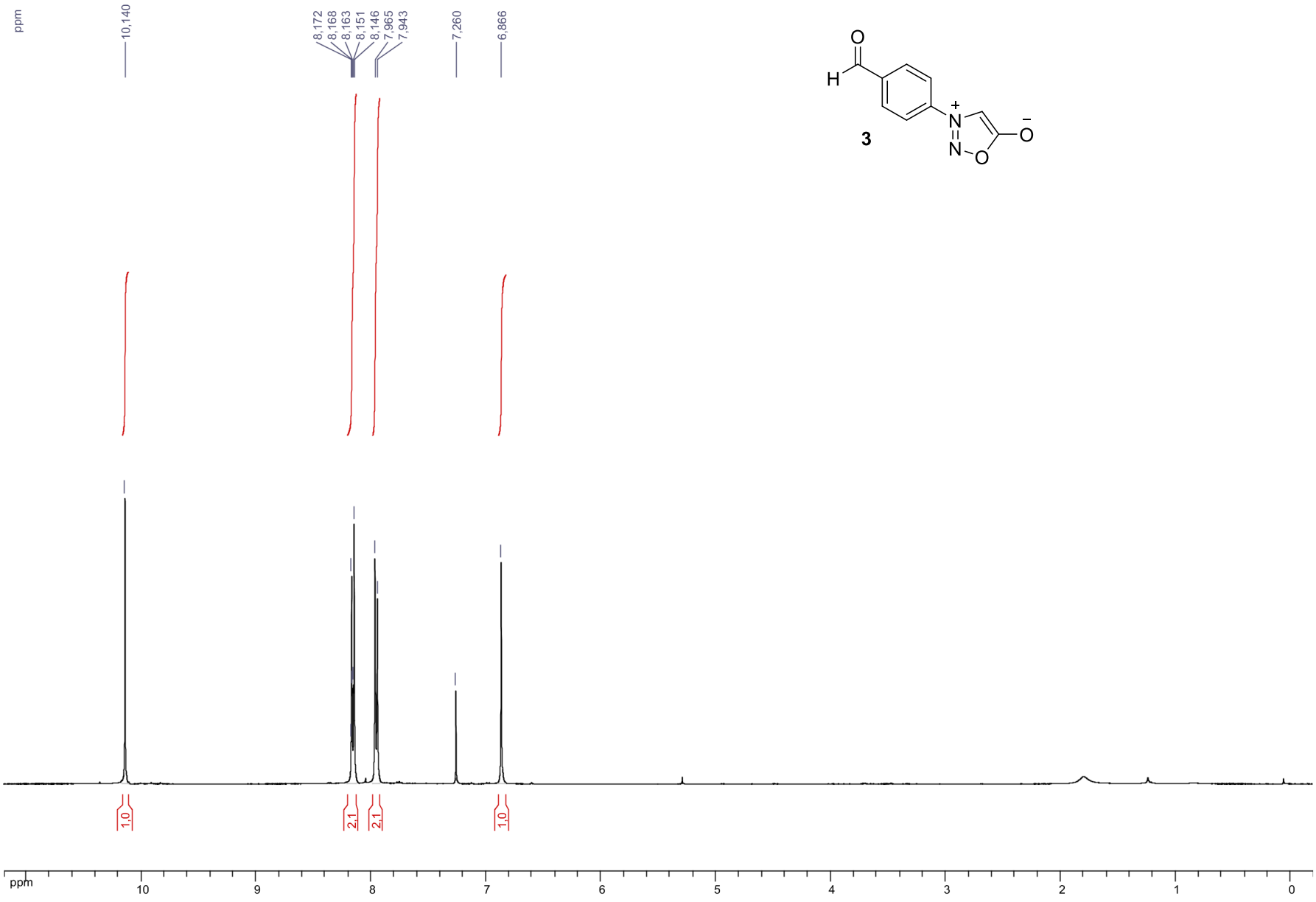
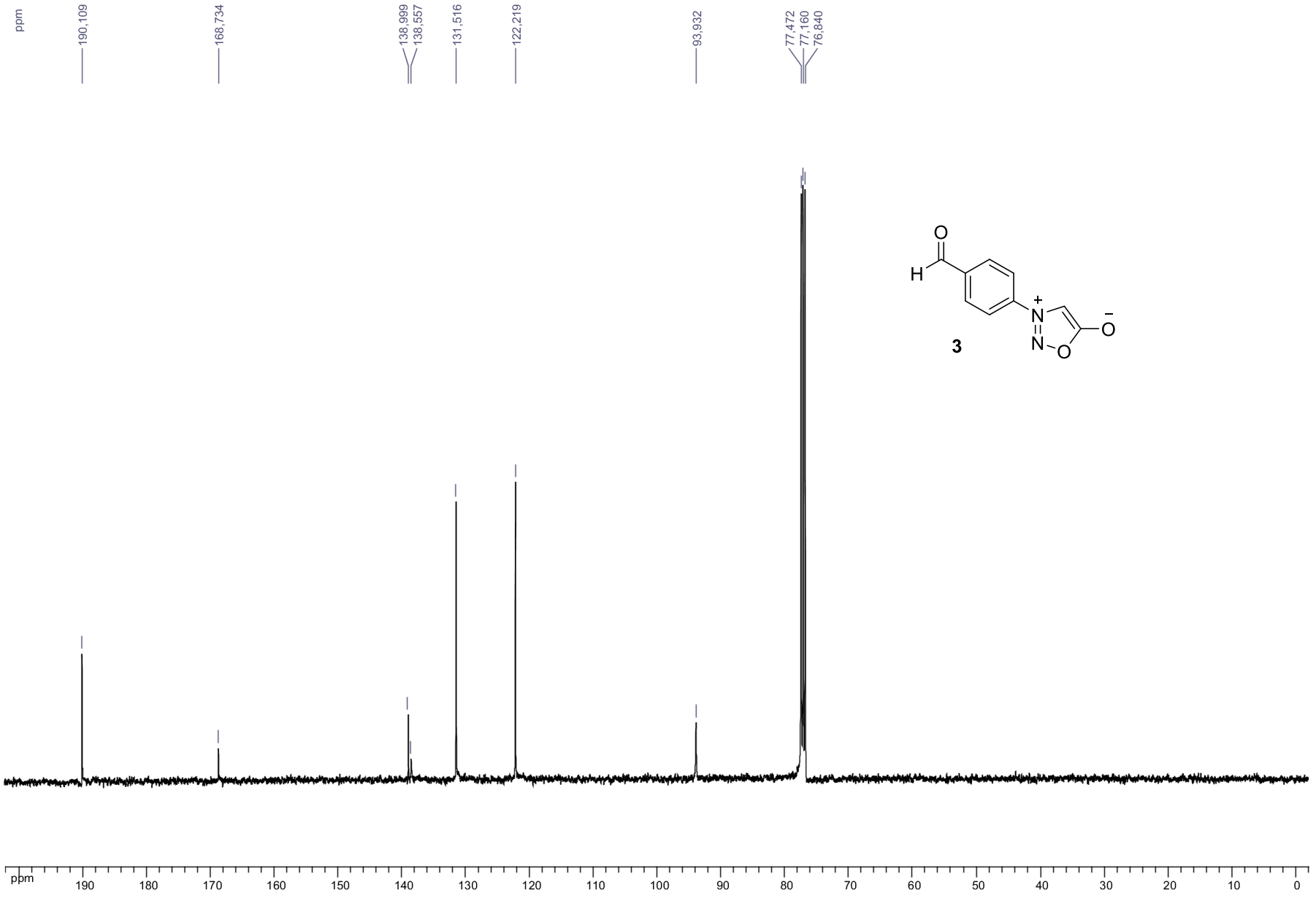
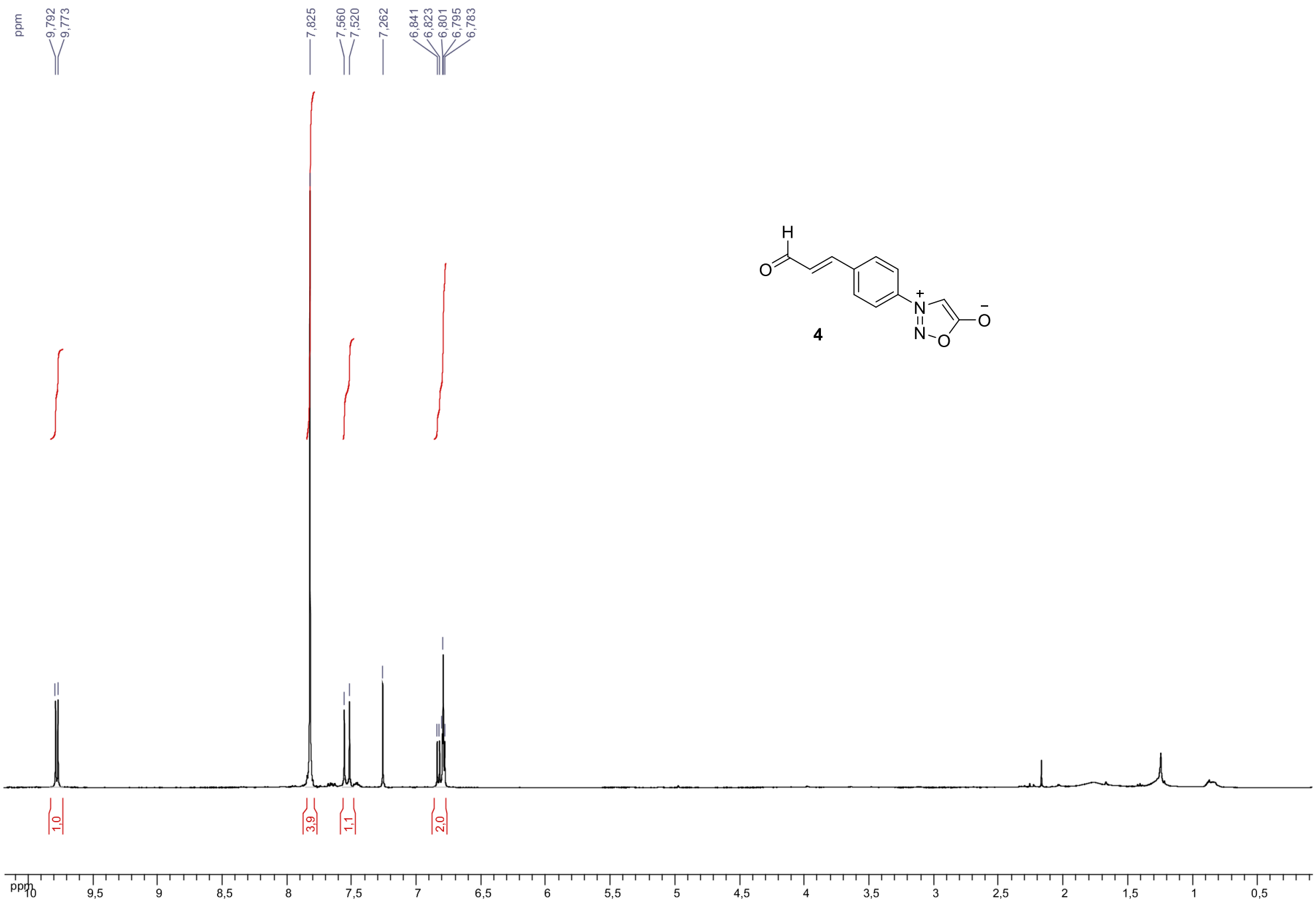
76,840
2
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

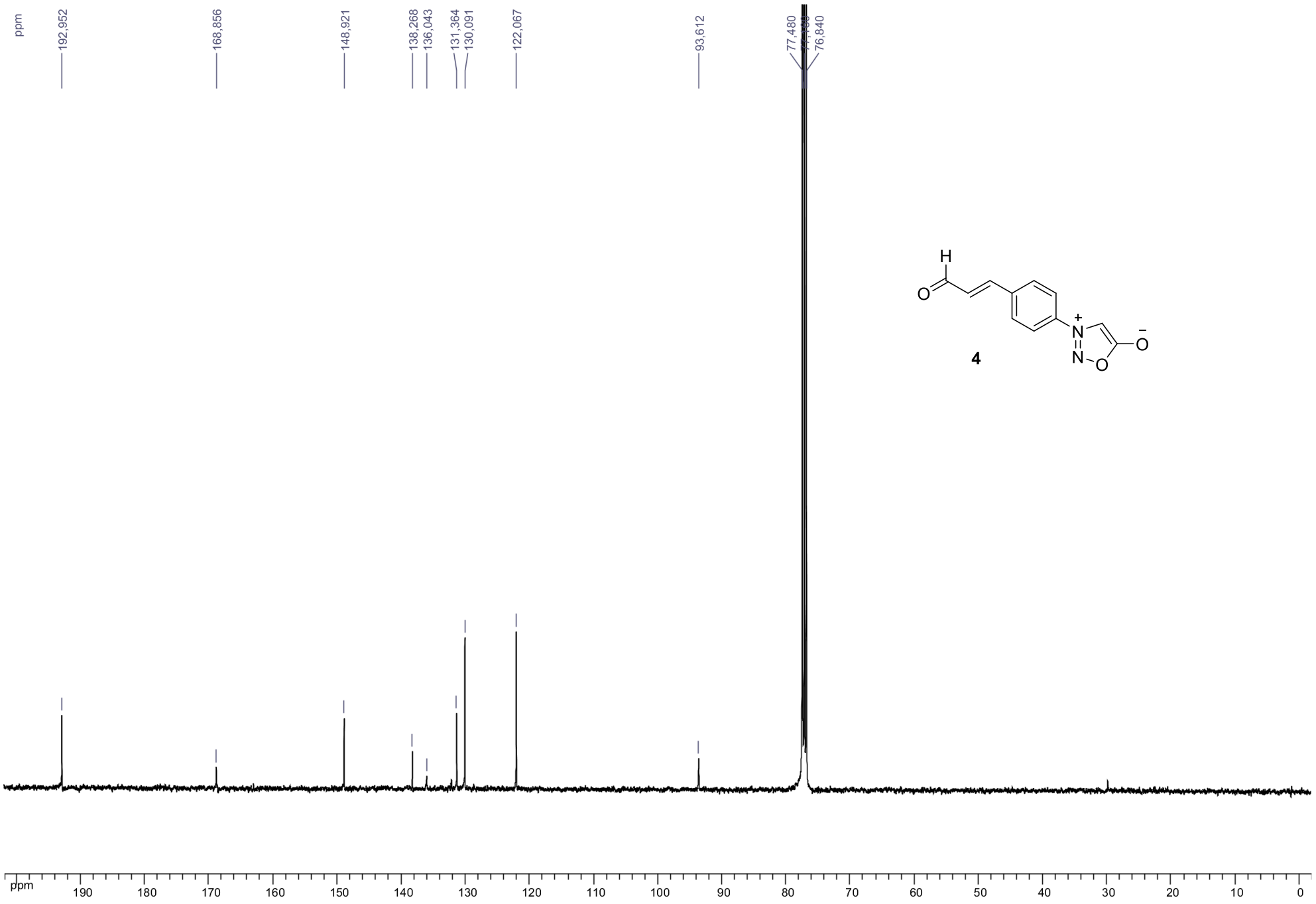

ppm
10,140
8,172
8,168
8,163
8,151
8,146
7,965
7,943
7,260
6,866
1,0
2,1
2,1
1,0
ppm
10
9
8
7
6
5
4
3
2
1
0

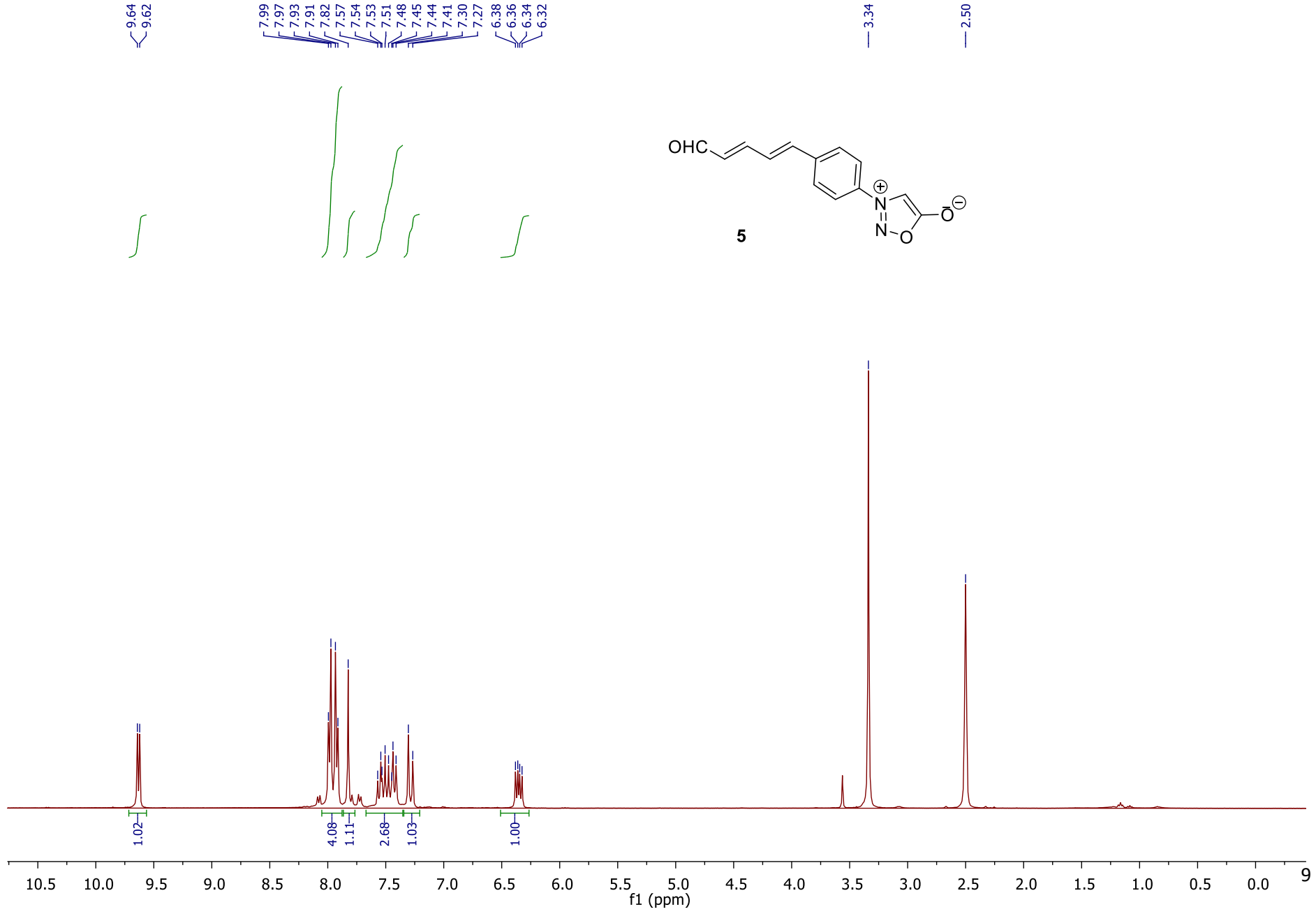
O
H
N
N
O
O

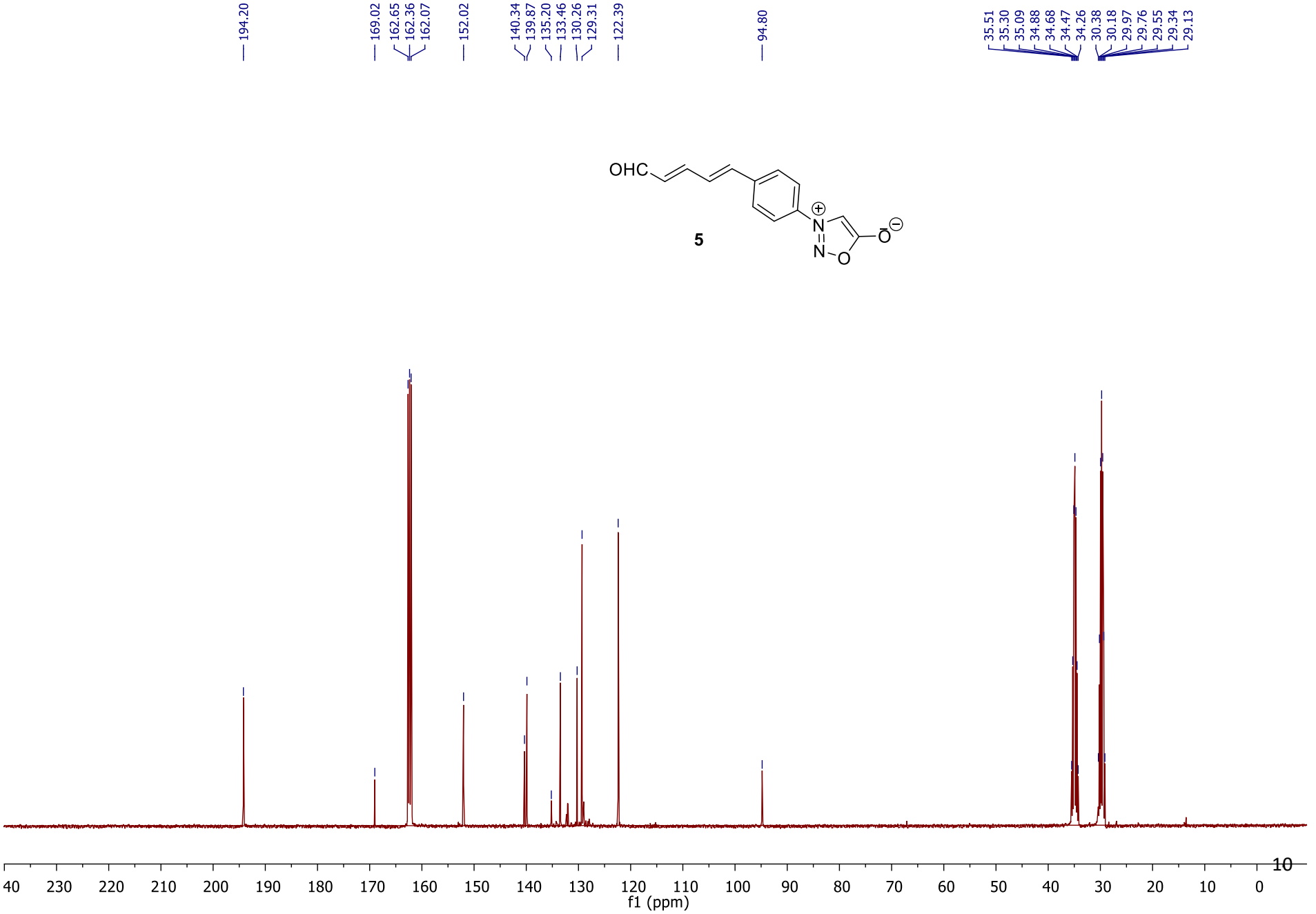
3

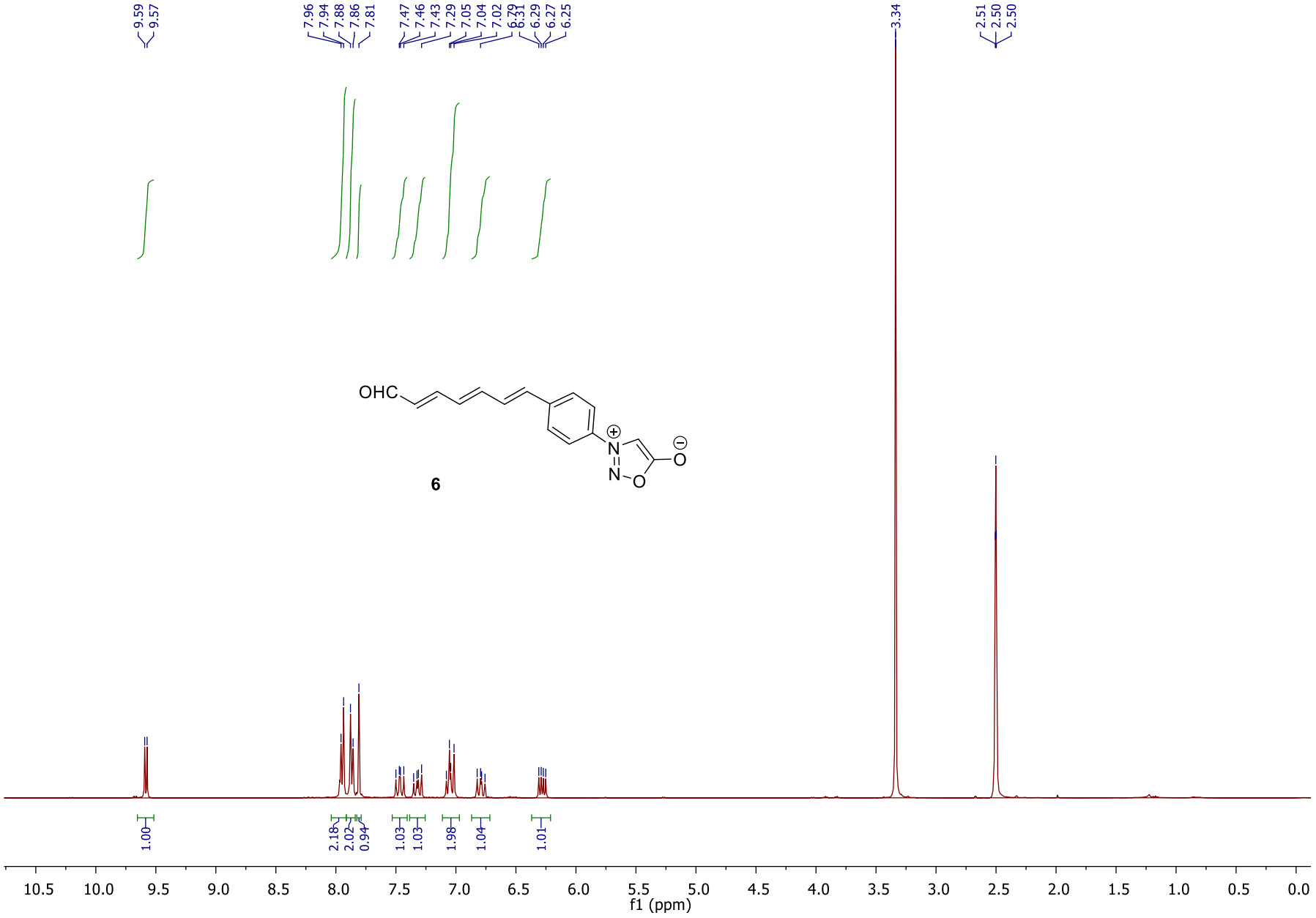

ppm
190,109
168,734
138,999
138,557
131,516
122,219
93,932
77,472
77,160
76,840
O
H
N
N
O
O
3
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

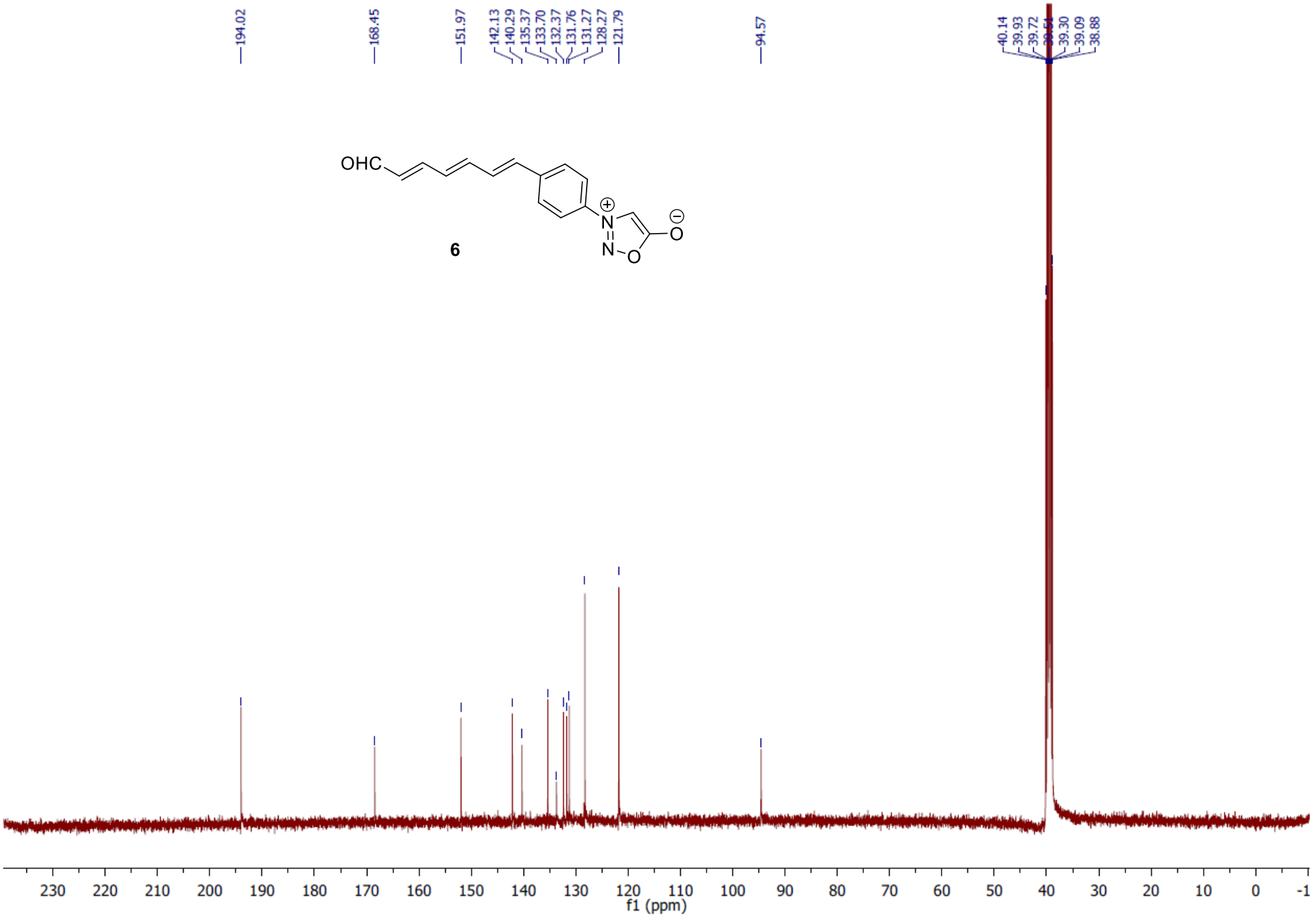

ppm
9,792
9,773
7,825
7,560
7,520
7,262
6,841
6,823
6,801
6,795
6,783
4
1,0
3,9
1,1
2,0
ppm
10
9,5
9
8,5
8
7,5
7
6,5
6
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5

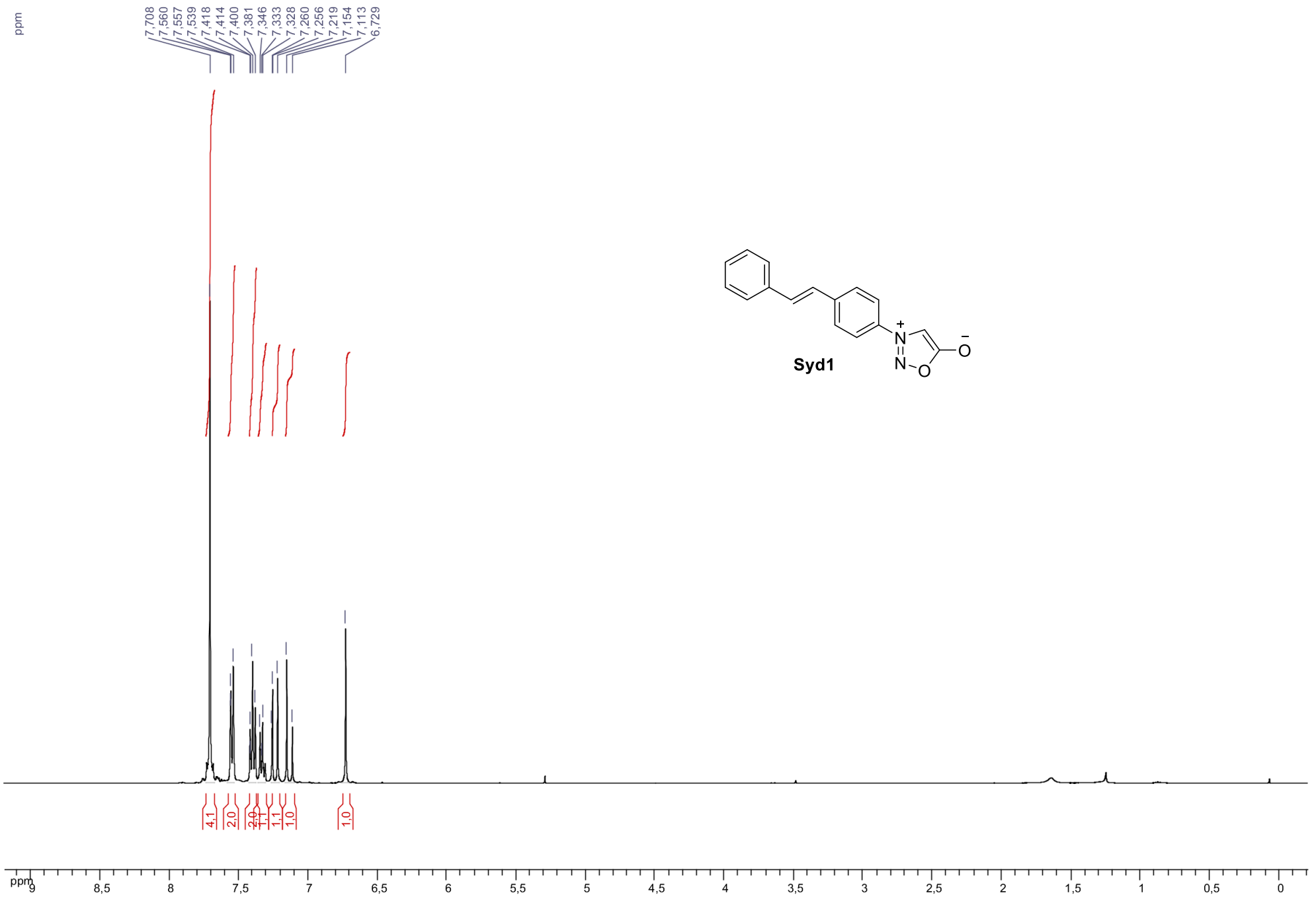

ppm
192,952
168,856
148,921
138,268
136,043
131,364
130,091
122,067
93,612
77,480
76,840
H
O
N
N
O
O
4
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


9.64
9.62
7.99
7.97
7.93
7.91
7.82
7.57
7.54
7.53
7.51
7.48
7.45
7.44
7.41
7.30
7.27
6.38
6.36
6.34
6.32
3.34
2.50
OHC
N
N
O
O
5
1.02
4.08
1.11
2.68
1.03
1.00
10.5
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
0.0
f1 (ppm)

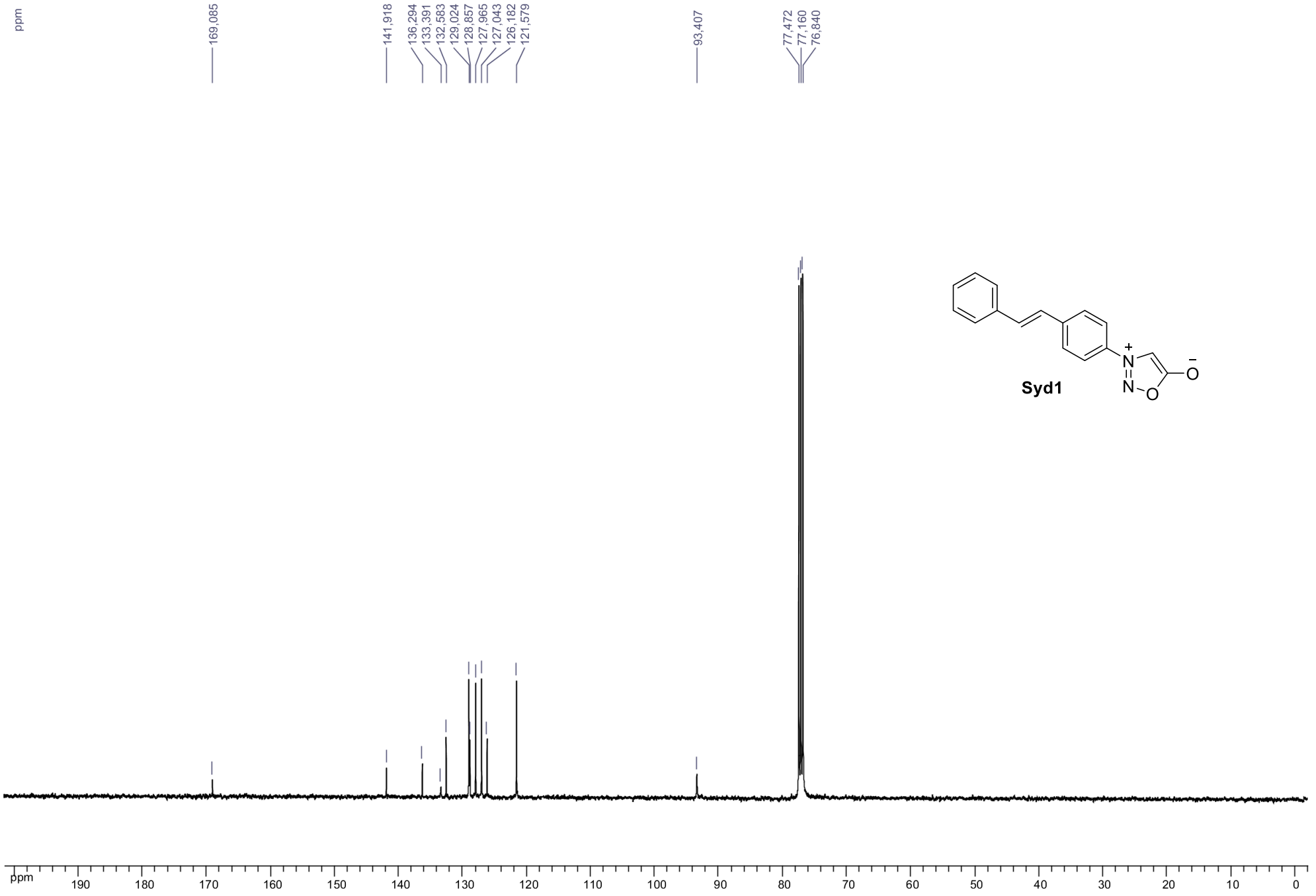

194.20
169.02
162.65
162.36
162.07
152.02
140.34
139.87
135.20
133.46
130.26
129.31
122.39
94.80
35.51
35.30
35.09
34.88
34.68
34.47
34.26
30.38
30.18
29.97
29.76
29.55
29.34
29.13
OHC
N
N
O
O
5
40
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

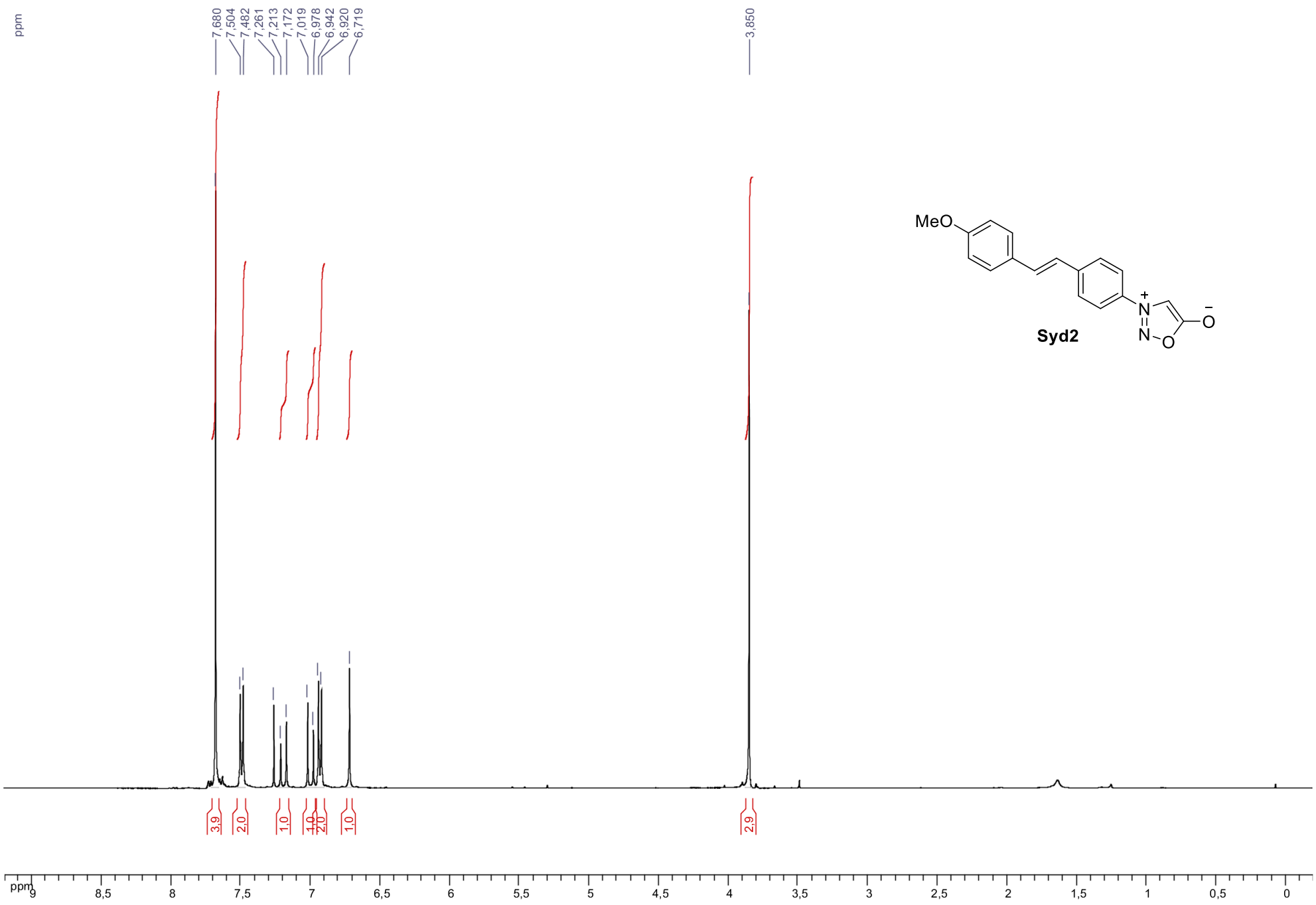

9.59
9.57
7.96
7.94
7.88
7.86
7.81
7.47
7.46
7.43
7.29
7.05
7.04
7.02
6.79
6.31
6.29
6.27
6.25
3.34
2.51
2.50
2.50
OHC
N
N
O
O
6
1.00
2.18
2.02
0.94
1.03
1.03
1.98
1.04
1.01
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

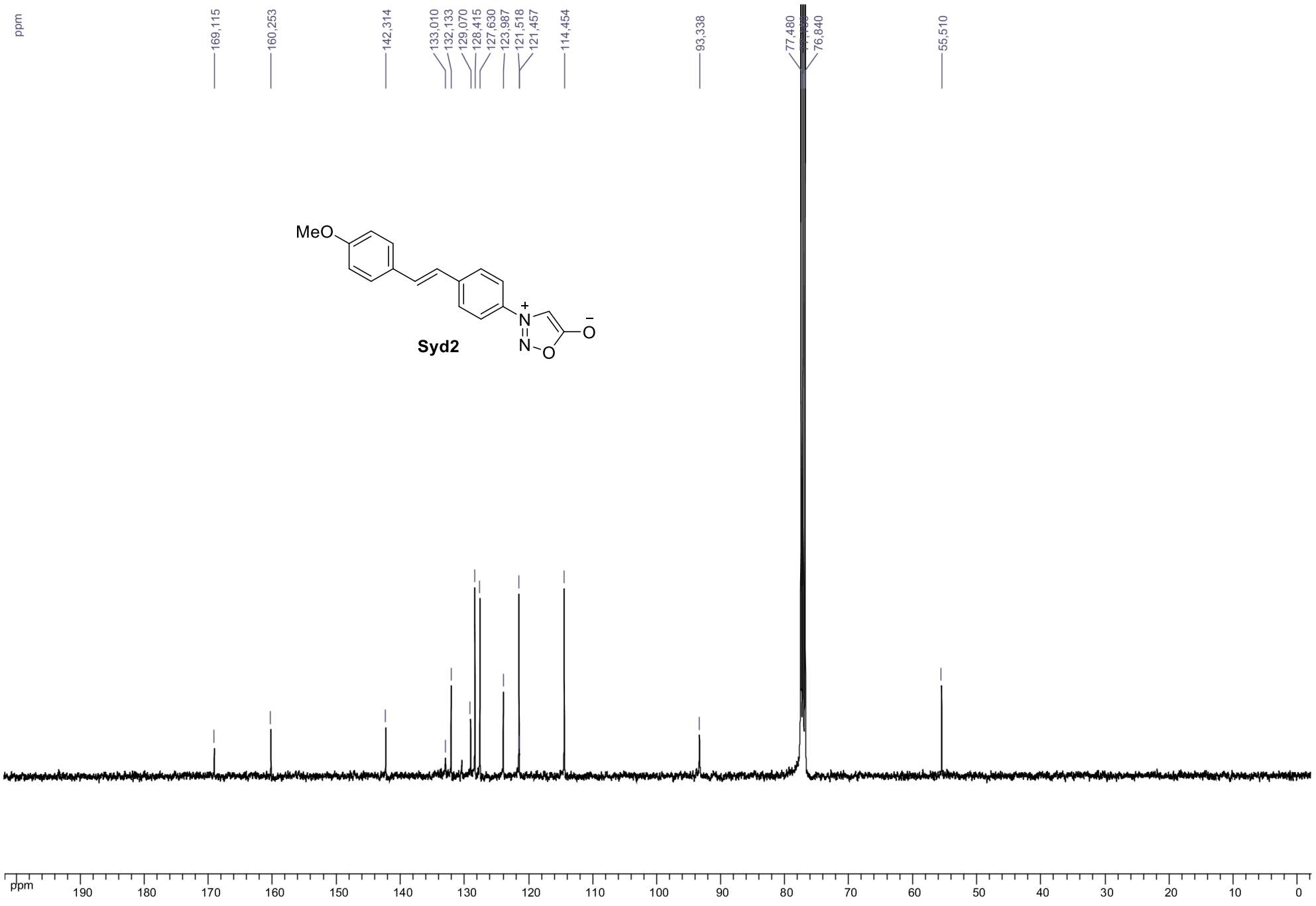

194.02
168.45
151.97
142.13
140.29
135.37
133.70
132.37
131.76
131.27
128.27
121.79
94.57
40.14
39.93
39.72
39.51
39.30
39.09
38.88
OHC
N
O
N
O
6
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

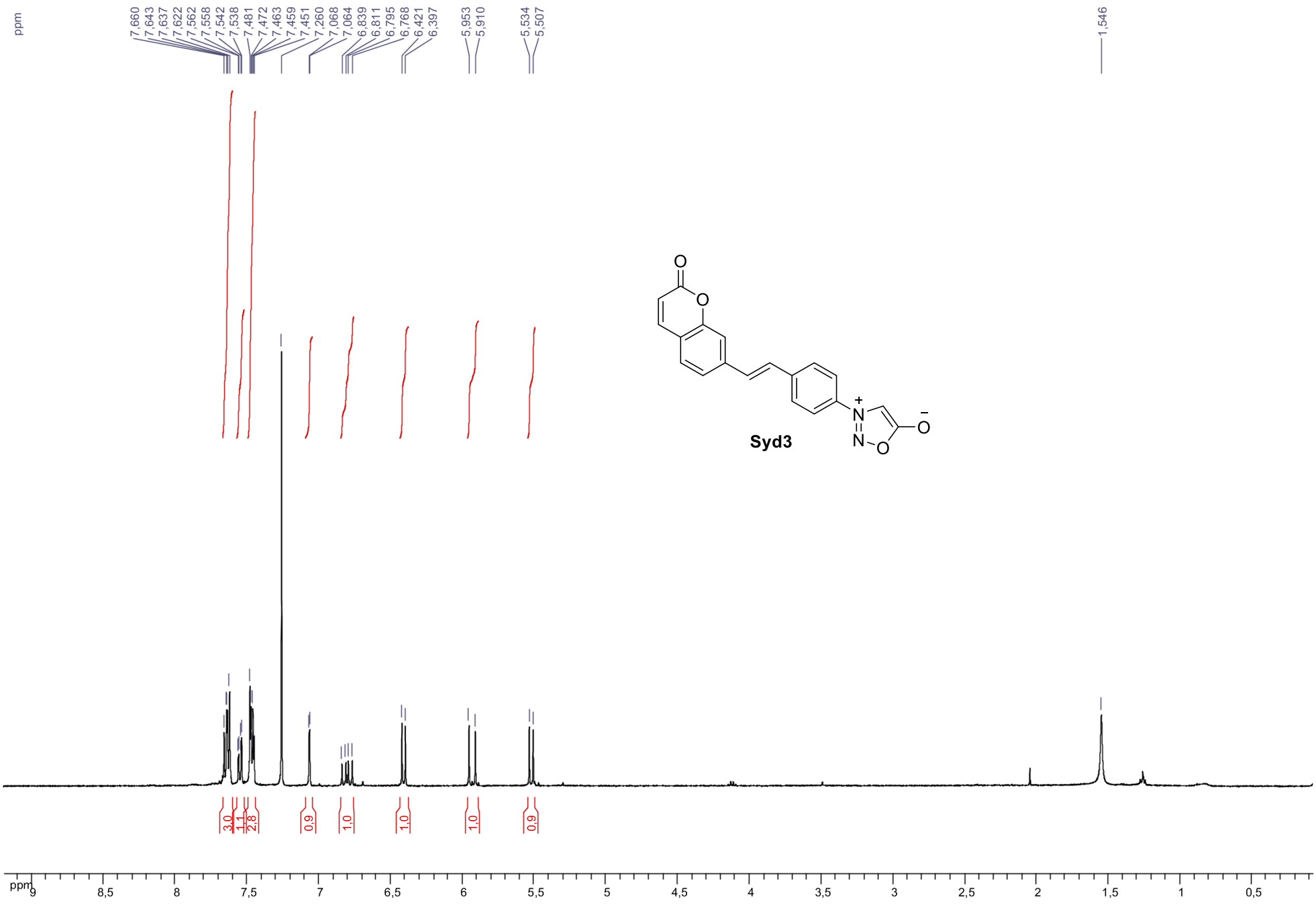

ppm
7,708
7,560
7,557
7,539
7,418
7,414
7,400
7,381
7,346
7,333
7,328
7,260
7,256
7,219
7,154
7,113
6,729
4,1
2,0
2,0
1,1
1,1
1,0
1,0
ppm
9
8,5
8
7,5
7
6,5
6
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0

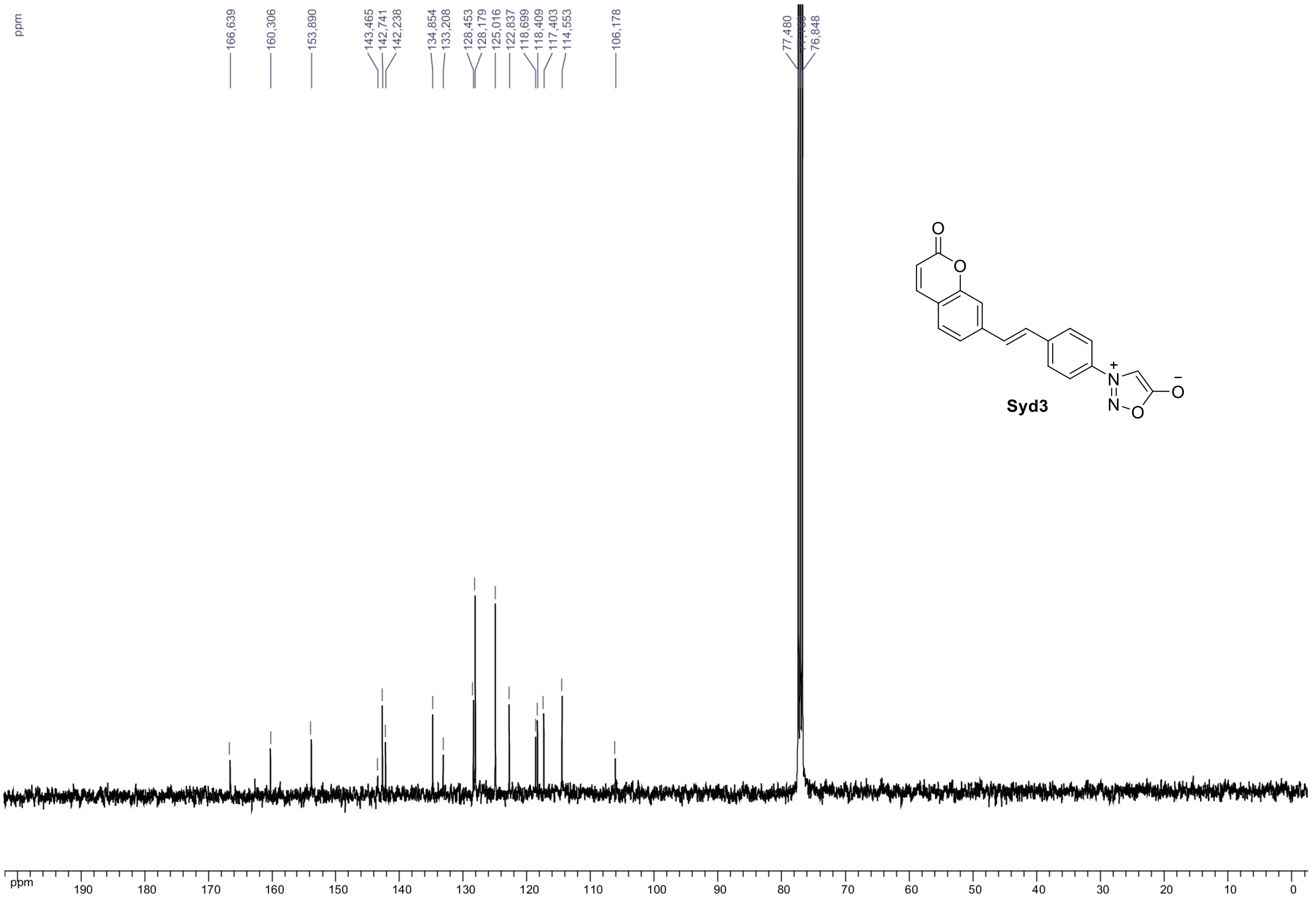
Syd1

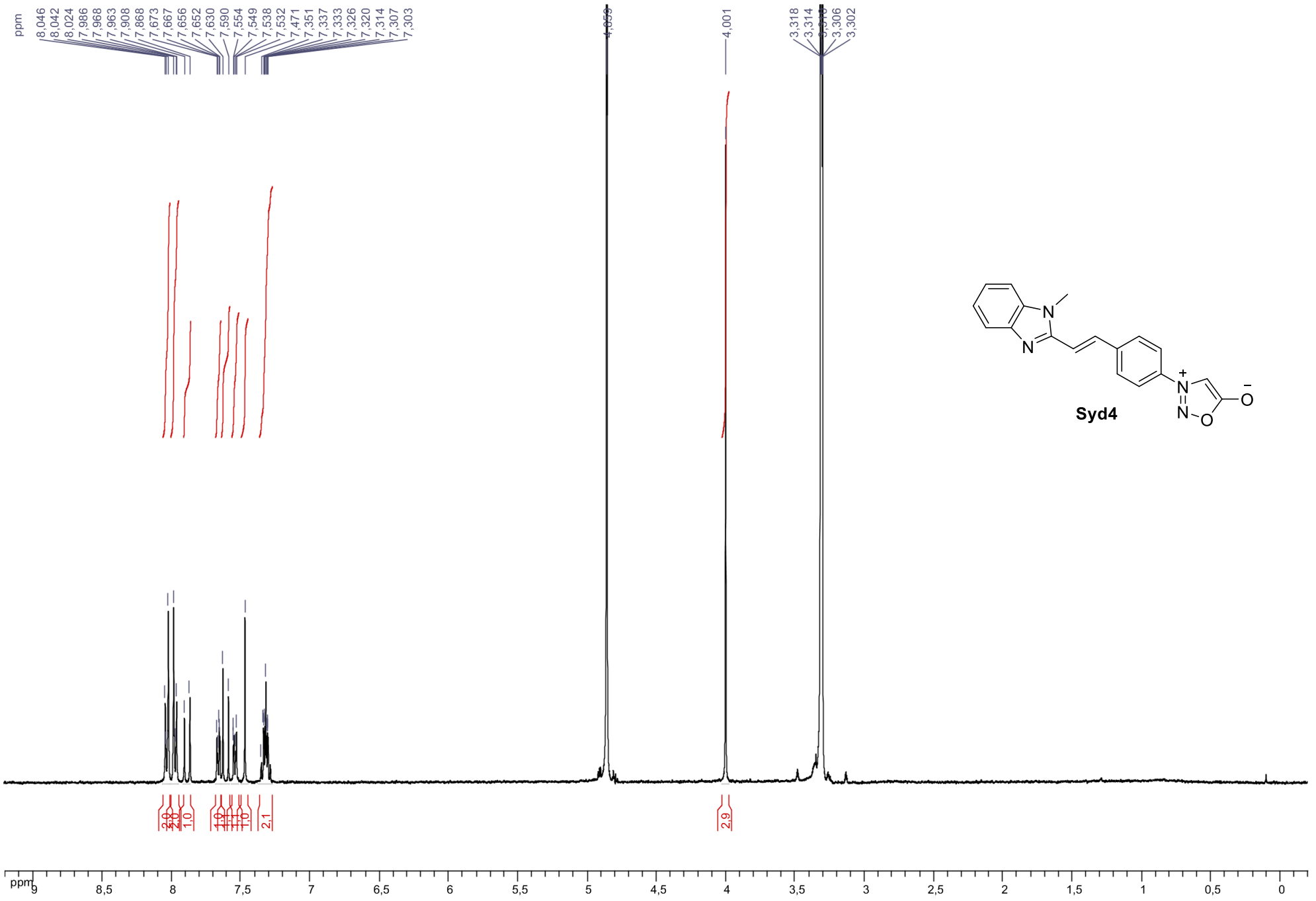

ppm
169,085
141,918
136,294
133,391
132,583
129,024
128,857
127,965
127,043
126,182
121,579
93,407
77,472
77,160
76,840
Syd1
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

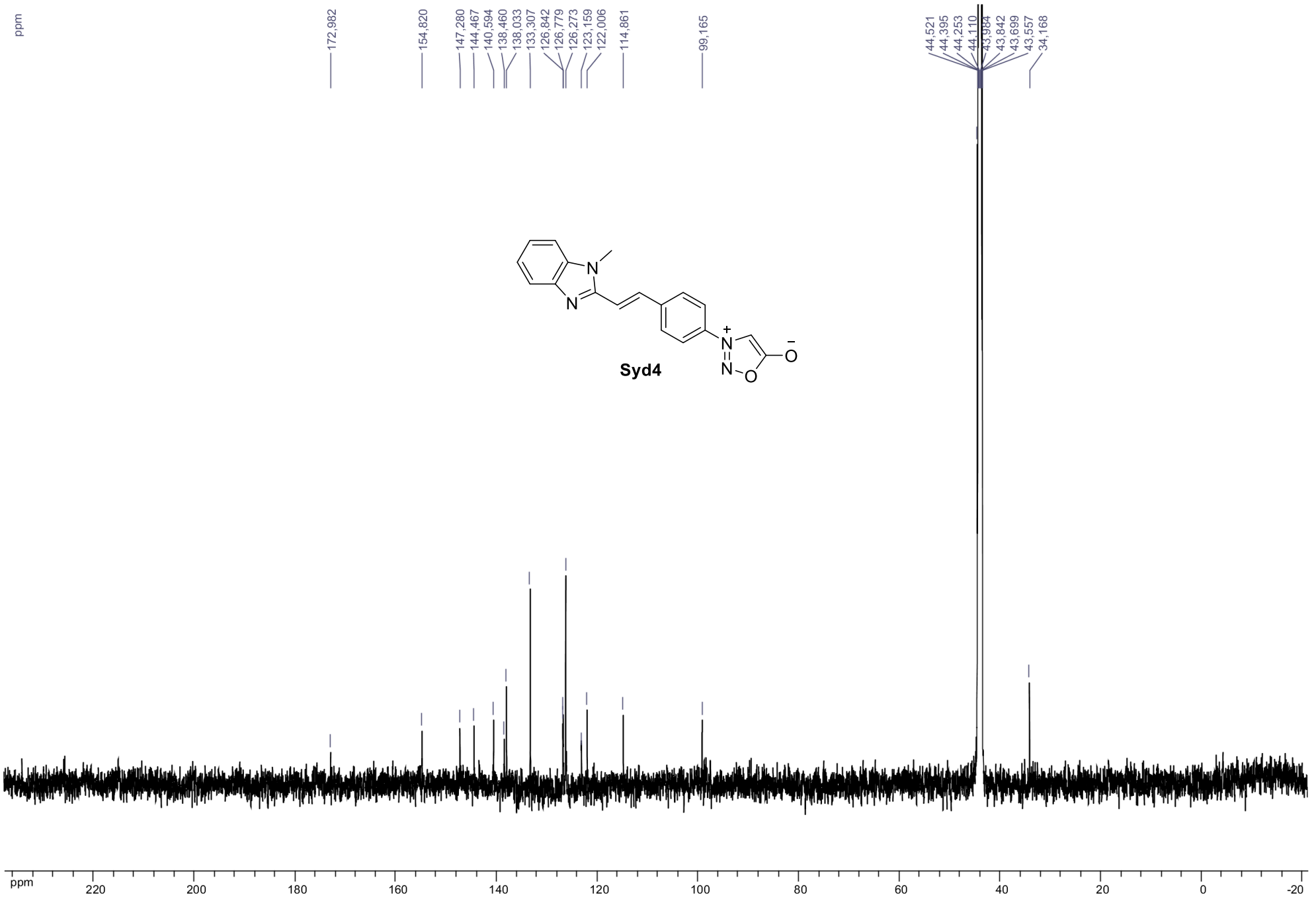

ppm
7,680
7,504
7,482
7,261
7,213
7,172
7,019
6,978
6,942
6,920
6,719
3,850
MeO
Syd2
3,9
2,0
1,0
1,0
2,0
1,0
2,9
ppm
9
8,5
8
7,5
7
6,5
6
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0

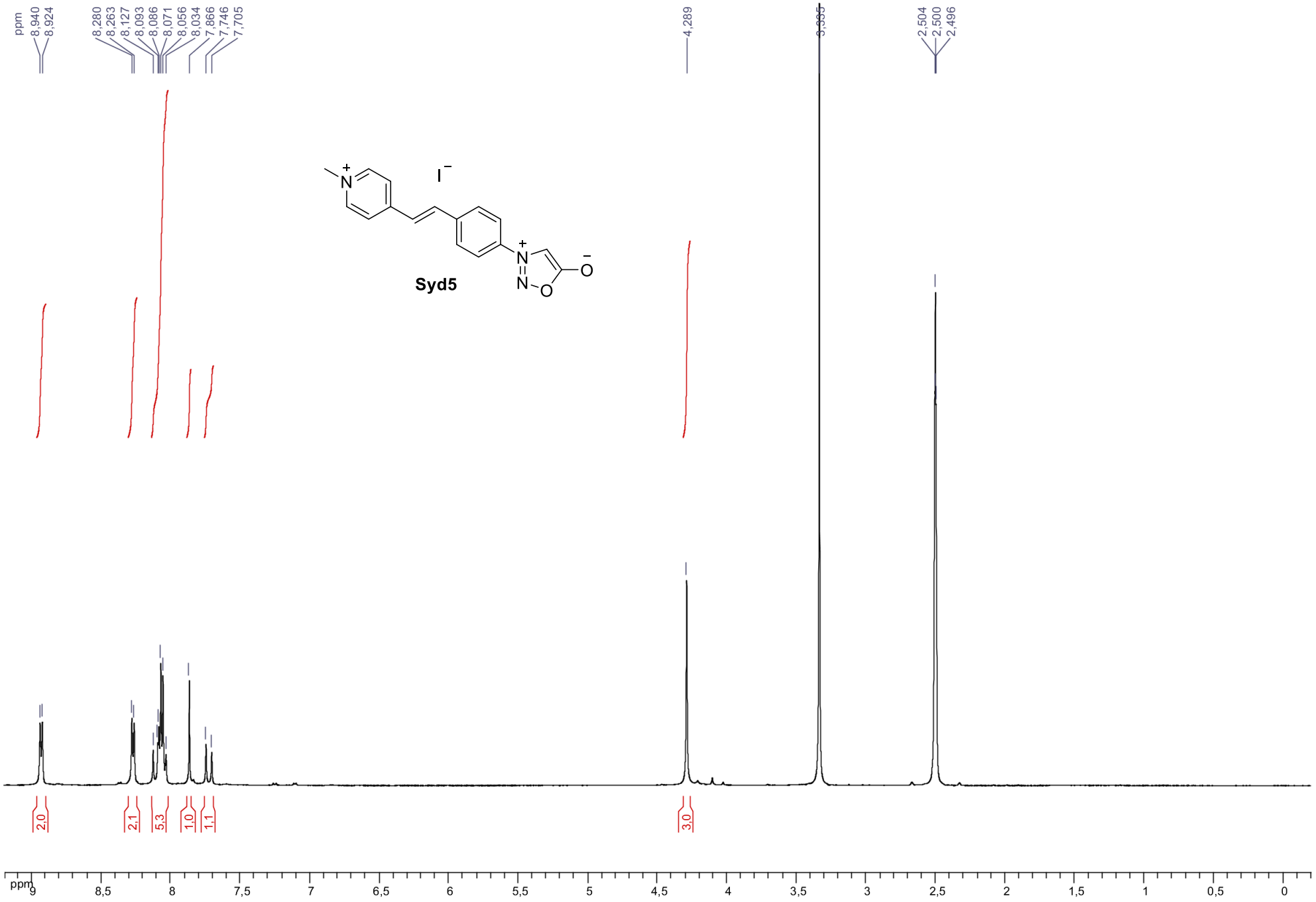

ppm
169,115
160,253
142,314
133,010
132,133
129,070
128,415
127,630
123,987
121,518
121,457
114,454
93,338
77,480
76,840
55,510
MeO
Syd2
N
N
O
O
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

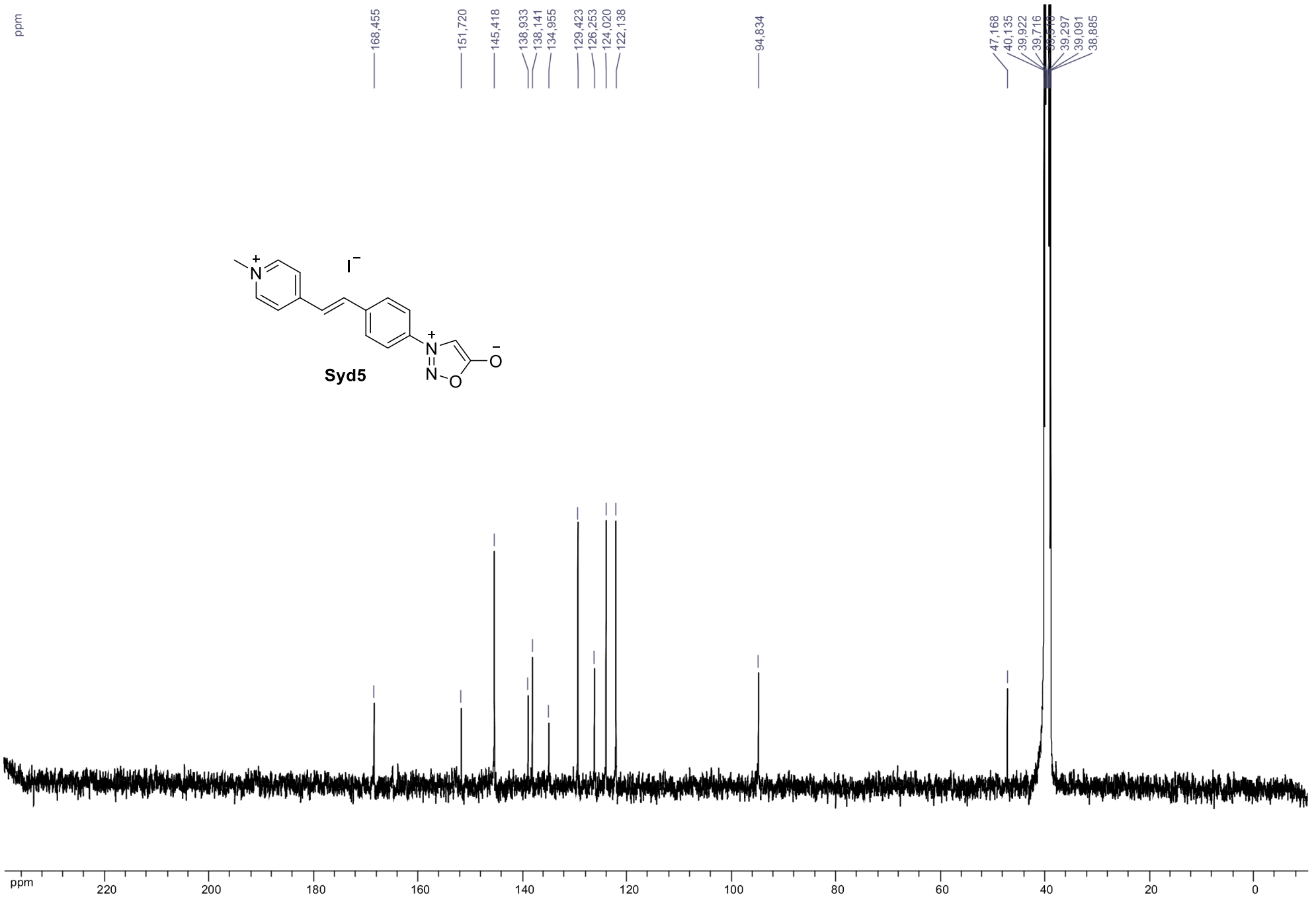

ppm
7,660
7,643
7,637
7,622
7,562
7,558
7,542
7,538
7,481
7,472
7,463
7,459
7,451
7,260
7,068
7,064
6,839
6,811
6,795
6,768
6,421
6,397
5,953
5,910
5,534
5,507
1,546
Syd3
3,0
1,1
2,8
0,9
1,0
1,0
1,0
0,9
ppm
9
8,5
8
7,5
7
6,5
6
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5

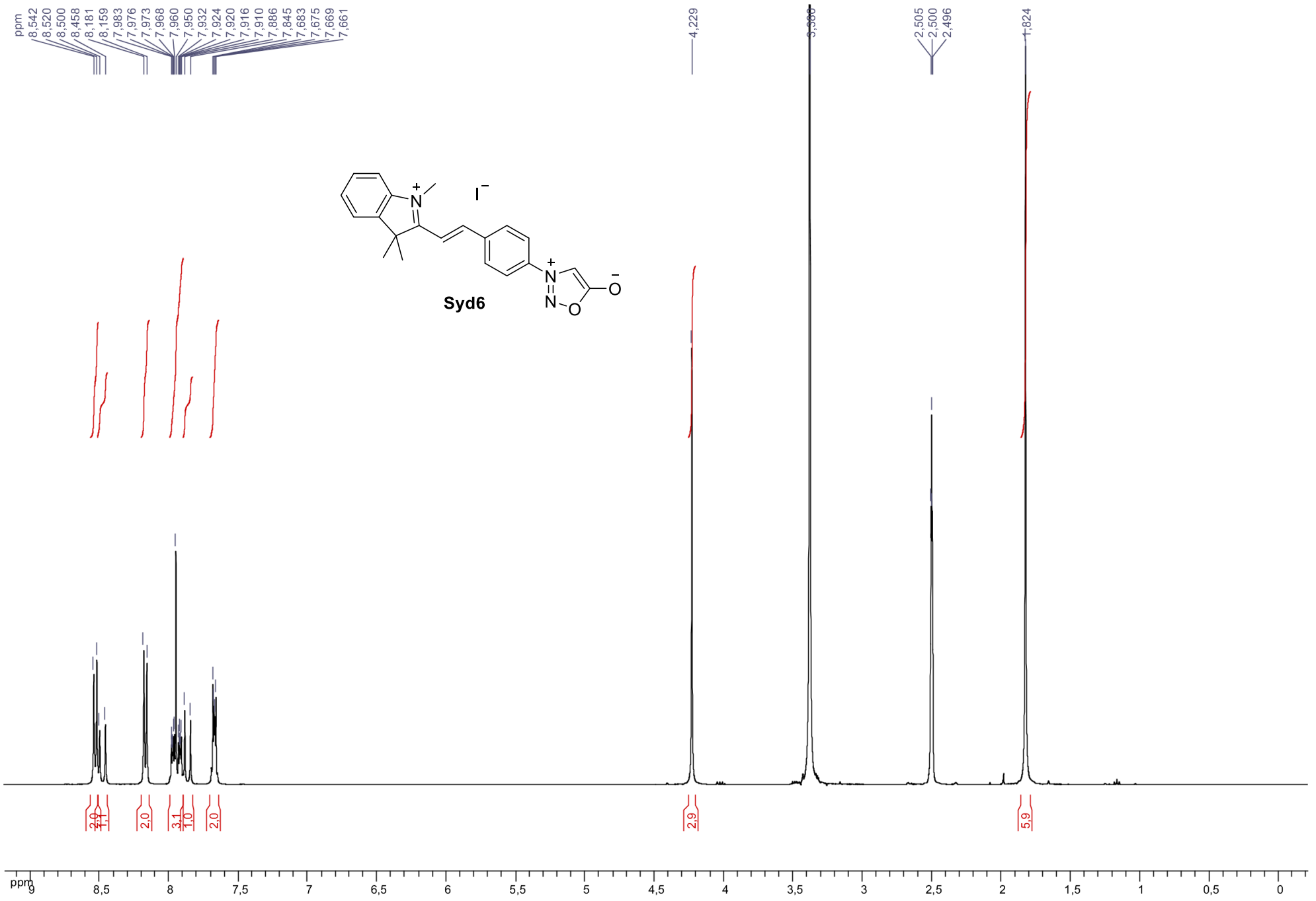

ppm
166,639
160,306
153,890
143,465
142,741
142,238
134,854
133,208
128,453
128,179
125,016
122,837
118,699
118,409
117,403
114,553
106,178
77,480
76,848
Syd3
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

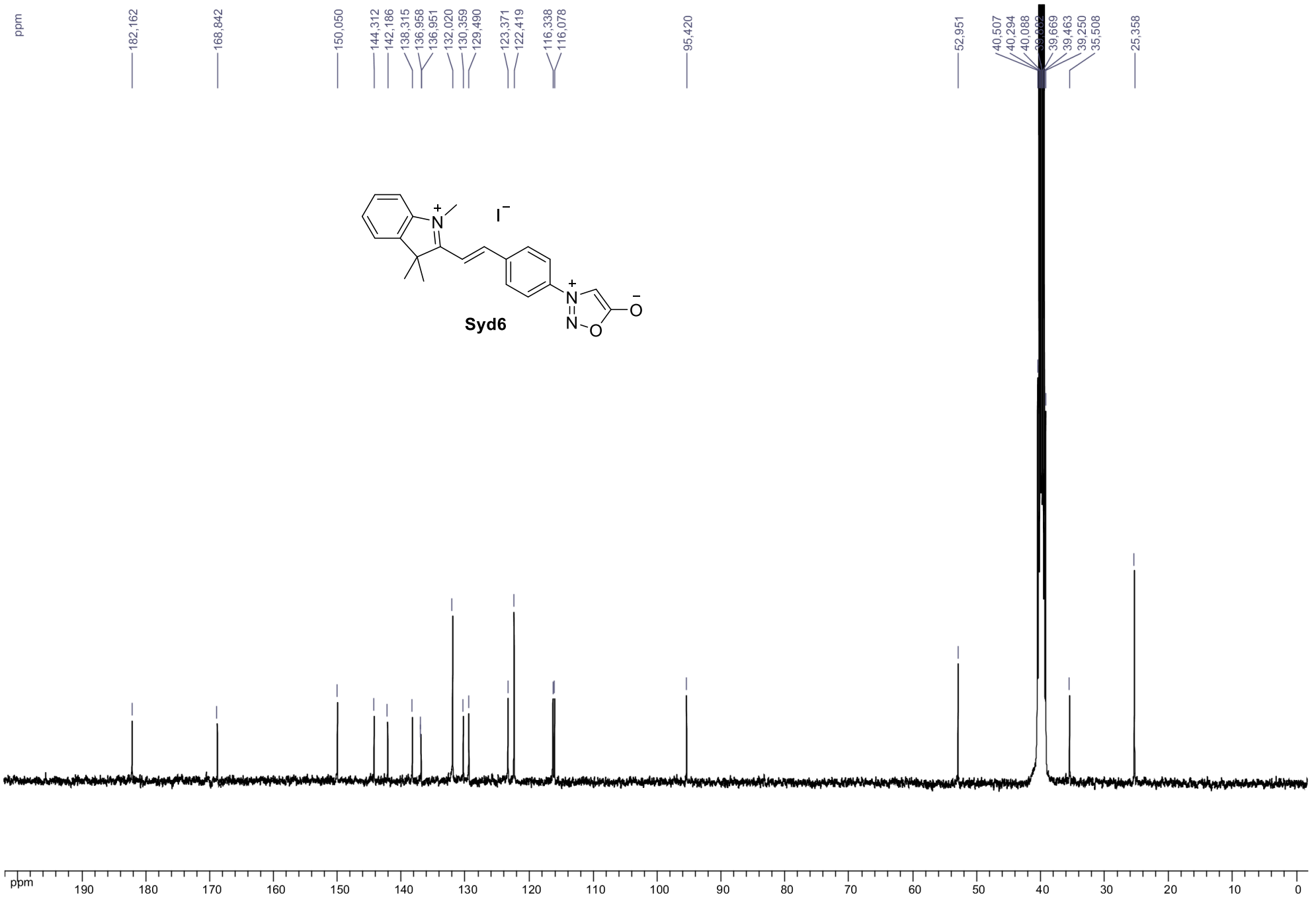

ppm
8,046
8,042
8,024
7,986
7,968
7,963
7,908
7,868
7,673
7,667
7,656
7,652
7,630
7,590
7,554
7,549
7,538
7,532
7,471
7,351
7,337
7,333
7,326
7,320
7,314
7,307
7,303
4,058
4,001
3,318
3,314
3,306
3,302
2,0
2,0
2,0
1,0
1,0
1,1
1,1
1,0
2,1
2,9
ppm
9
8,5
8
7,5
7
6,5
6
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
Syd4

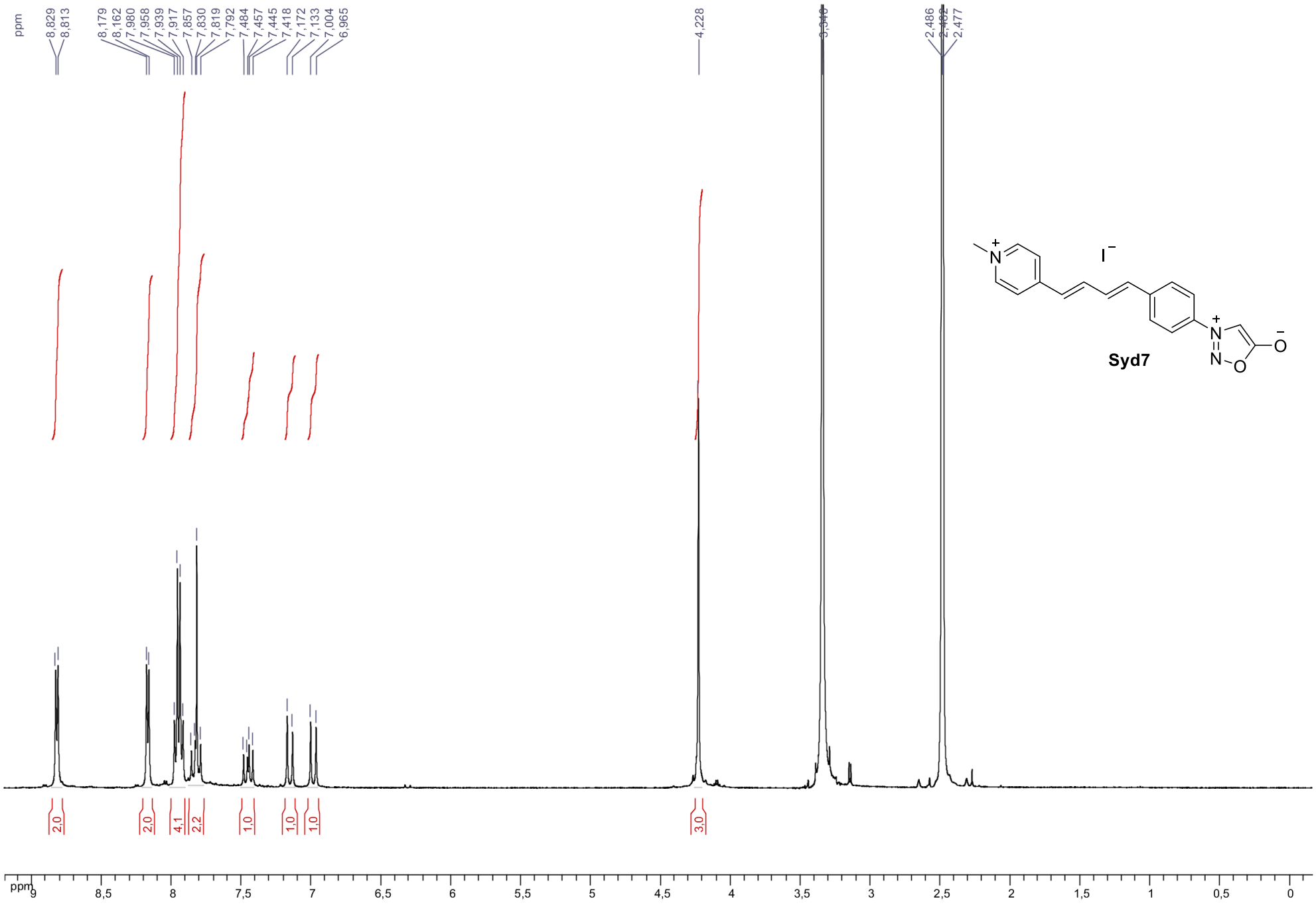

ppm
172,982
154,820
147,280
144,467
140,594
138,460
138,033
133,307
126,842
126,779
126,273
123,159
122,006
114,861
99,165
44,521
44,395
44,253
44,110
43,984
43,842
43,699
43,557
34,168
Syd4
ppm
220
200
180
160
140
120
100
80
60
40
20
0
-20

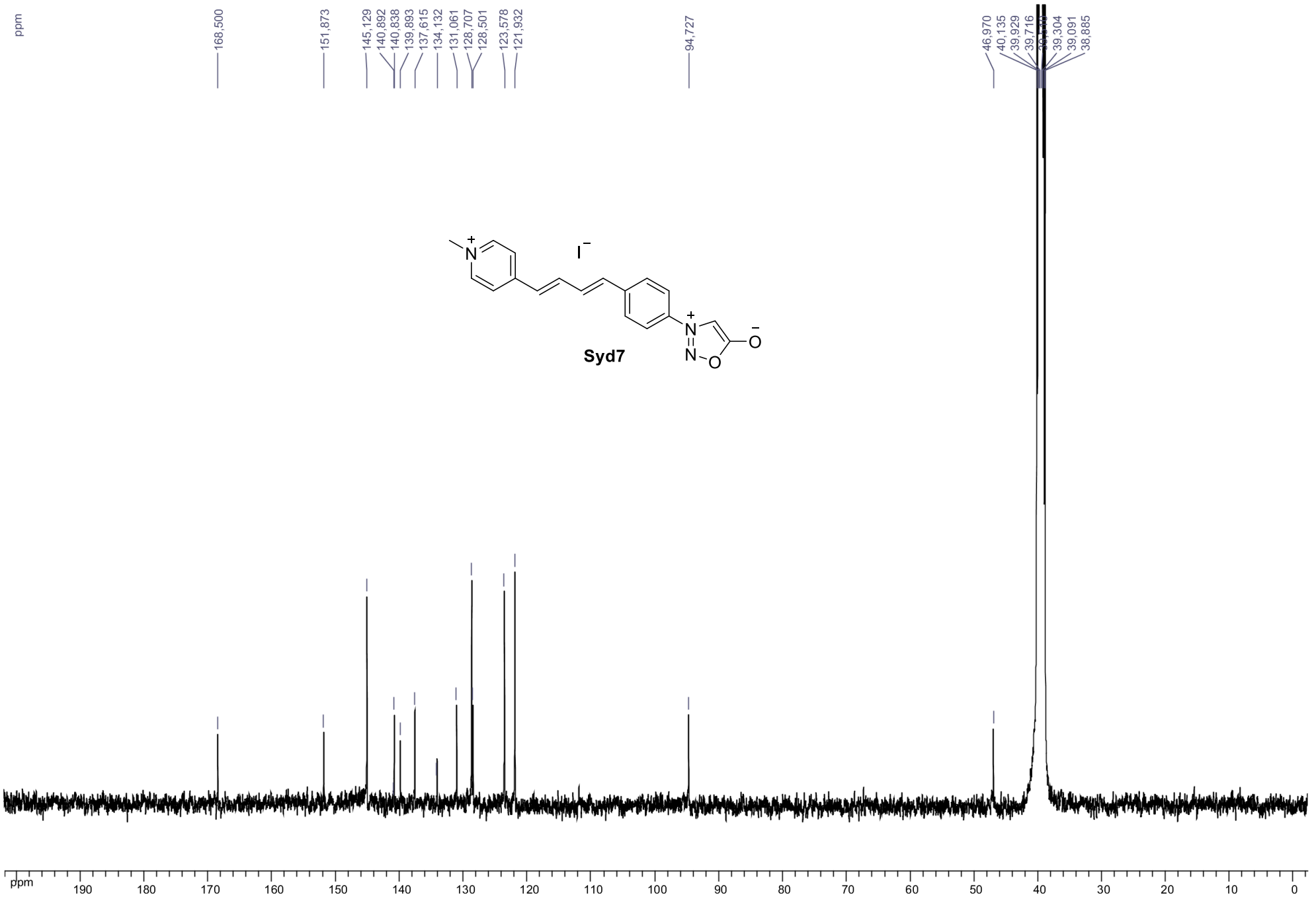

ppm
8,940
8,924
8,280
8,263
8,127
8,093
8,086
8,071
8,056
8,034
7,866
7,746
7,705
4,289
3,335
2,504
2,500
2,496
Syd5
2,0
2,1
5,3
1,0
1,1
3,0
ppm
9
8,5
8
7,5
7
6,5
6
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0

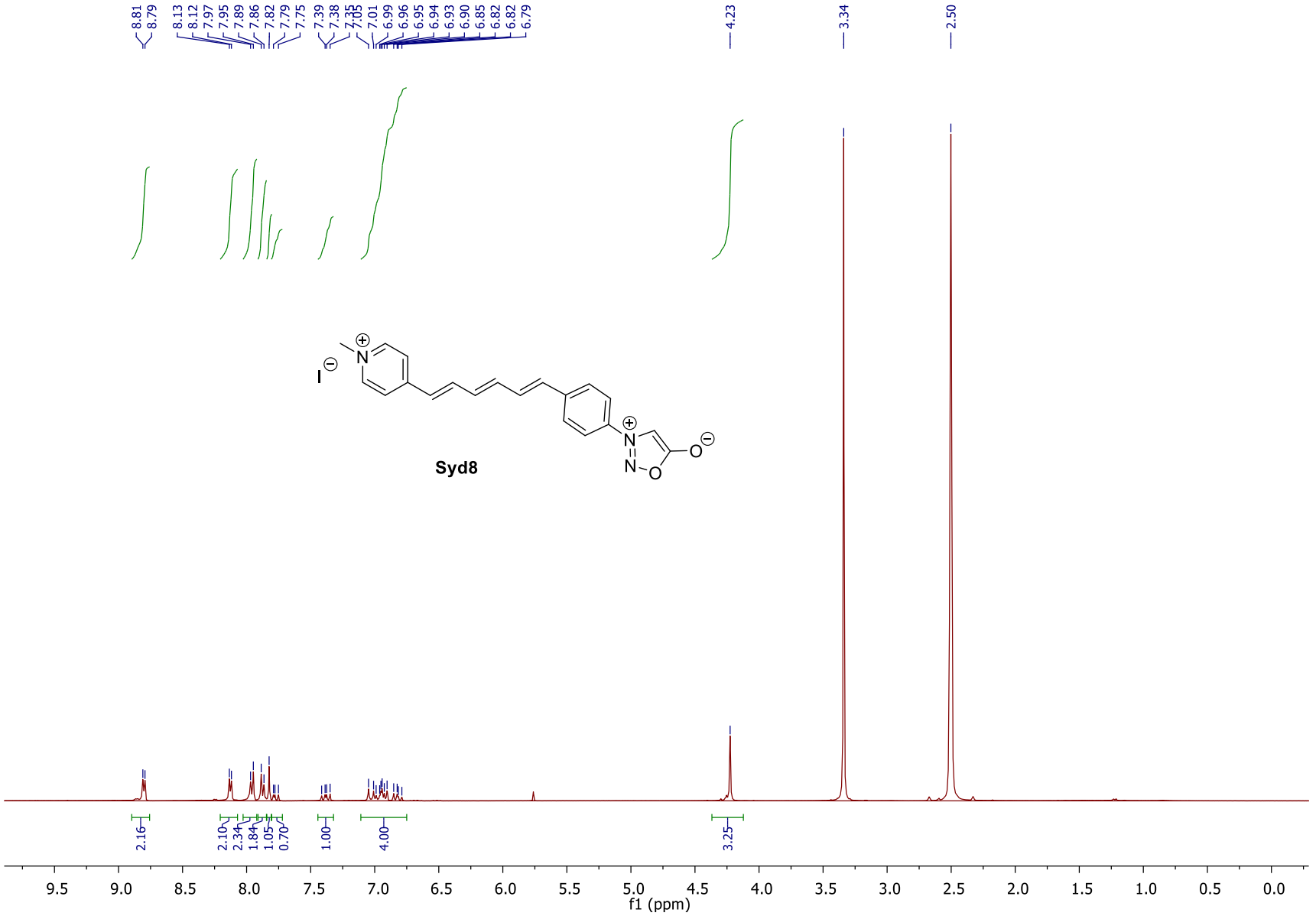

ppm
168,455
151,720
145,418
138,933
138,141
134,955
129,423
126,253
124,020
122,138
94,834
47,168
40,135
39,922
39,716
39,510
39,297
39,091
38,885
Syd5
ppm
220
200
180
160
140
120
100
80
60
40
20
0

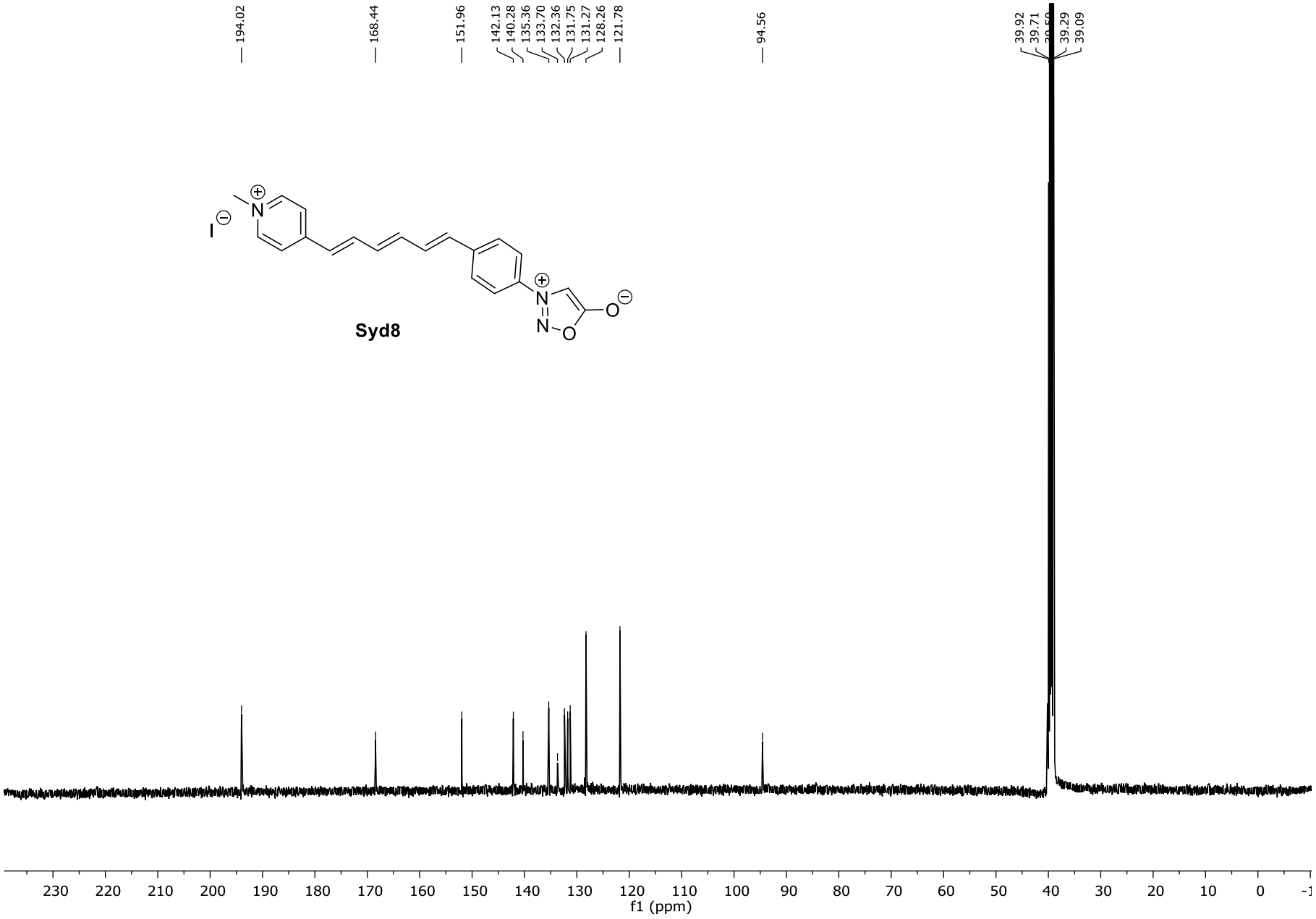

ppm
8,542
8,520
8,500
8,458
8,181
8,159
7,983
7,976
7,973
7,968
7,960
7,950
7,932
7,924
7,920
7,916
7,910
7,886
7,845
7,683
7,675
7,669
7,661
4,229
3,386
2,505
2,500
2,496
1,824
I⁻
Syd6
2,0
1,1
2,0
3,1
1,0
2,0
2,9
5,9
ppm
9
8,5
8
7,5
7
6,5
6
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0

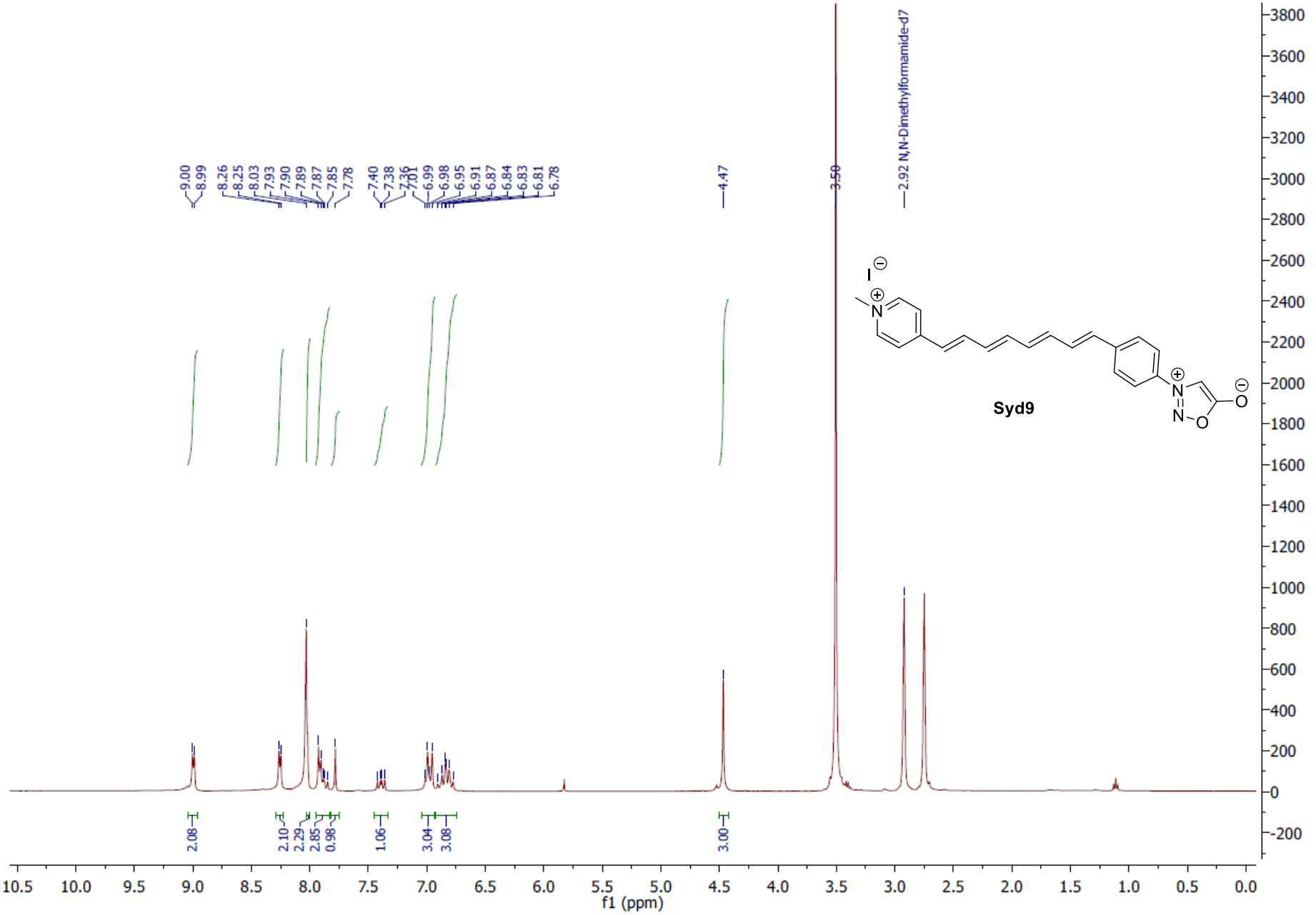

ppm
182,162
168,842
150,050
144,312
142,186
138,315
136,958
136,951
132,020
130,359
129,490
123,371
122,419
116,338
116,078
95,420
52,951
40,507
40,294
40,088
39,669
39,463
39,250
35,508
25,358
I⁻
Syd6
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

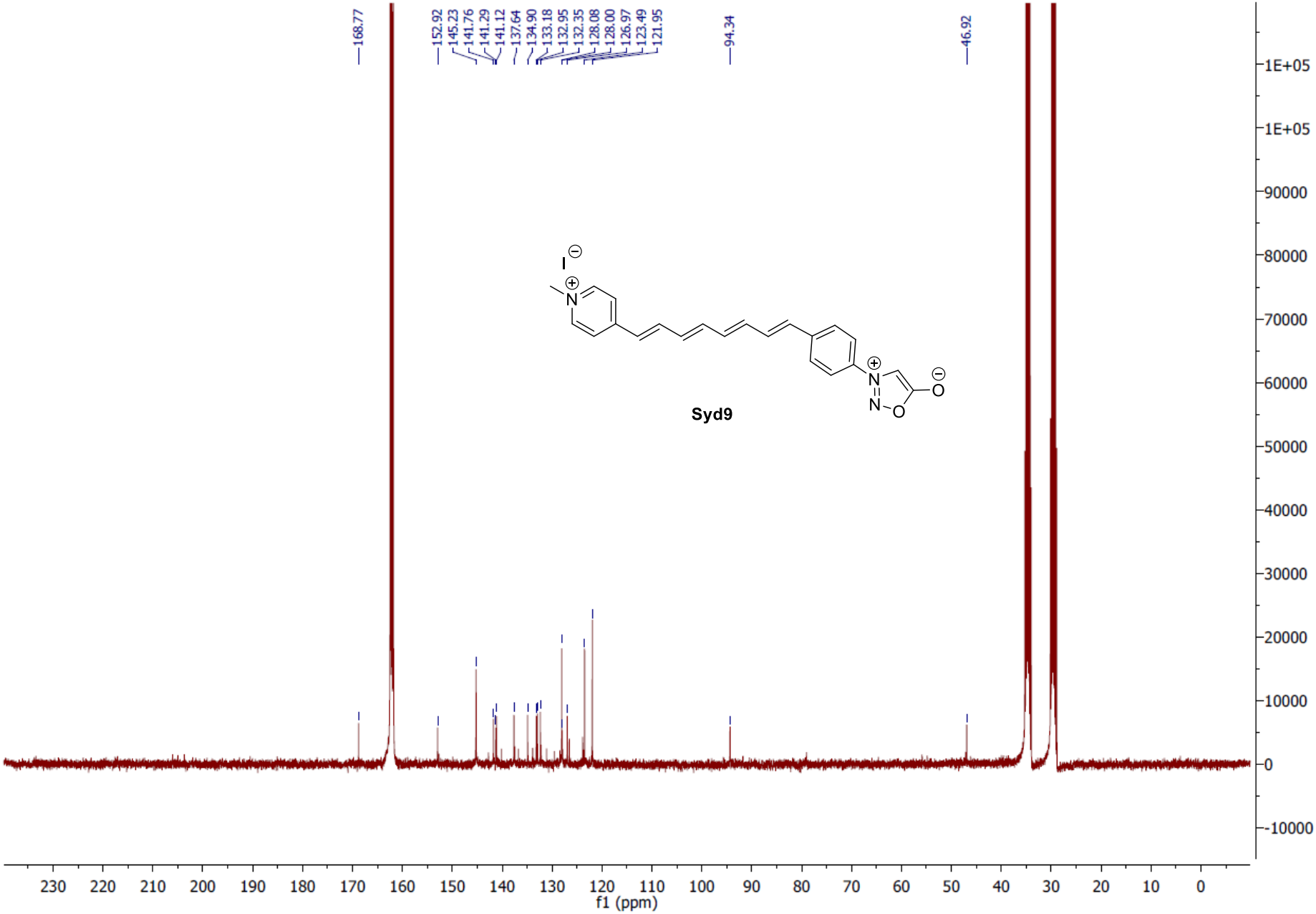

ppm
8,829
8,813
8,179
8,162
7,980
7,958
7,939
7,917
7,857
7,830
7,819
7,792
7,484
7,457
7,445
7,418
7,172
7,133
7,004
6,965
4,228
3,310
2,486
2,477
2,0
2,0
4,1
2,2
1,0
1,0
1,0
3,0
Syd7

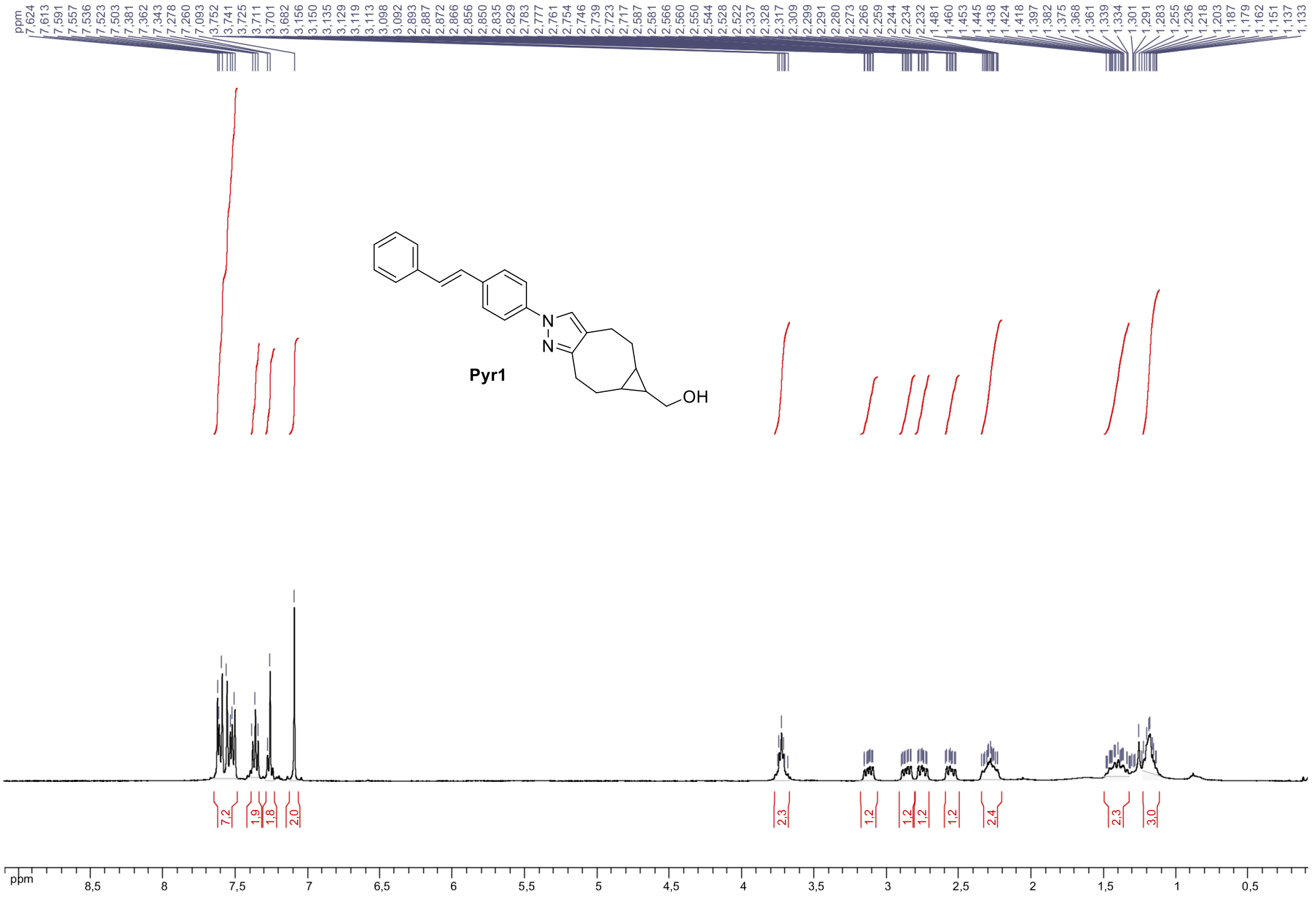

ppm
168,500
151,873
145,129
140,892
140,838
139,893
137,615
134,132
131,061
128,707
128,501
123,578
121,932
94,727
46,970
40,135
39,929
39,716
39,304
39,091
38,885
I⁻
Syd7
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


8.81
8.79
8.13
8.12
7.97
7.95
7.89
7.86
7.82
7.79
7.75
7.39
7.38
7.35
7.05
7.01
6.99
6.96
6.95
6.94
6.93
6.90
6.85
6.82
6.82
6.79
4.23
3.34
2.50
Syd8
2.16
2.10
2.34
1.84
1.05
0.70
1.00
4.00
3.25
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
0.0
f1 (ppm)


194.02
168.44
151.96
142.13
140.28
135.36
133.70
132.36
131.75
131.27
128.26
121.78
94.56
39.92
39.71
39.29
39.09
Syd8
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


9.00
8.99
8.26
8.25
8.03
7.93
7.90
7.89
7.87
7.85
7.78
7.40
7.38
7.36
7.01
6.99
6.98
6.95
6.91
6.87
6.84
6.83
6.81
6.78
4.47
3.50
2.92 N,N-Dimethylformamide-d7
Syd9
2.08
2.10
2.29
2.85
0.98
1.06
3.04
3.08
3.00
f1 (ppm)


168.77
152.92
145.23
141.76
141.29
141.12
137.64
134.90
133.18
132.95
132.35
128.08
128.00
126.97
123.49
121.95
94.34
46.92
Syd9
1E+05
1E+05
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7,503
7,381
7,362
7,343
7,278
7,260
7,093
3,752
3,741
3,725
3,711
3,701
3,682
3,156
3,150
3,135
3,129
3,119
3,113
3,098
3,092
2,893
2,887
2,872
2,866
2,856
2,850
2,835
2,829
2,783
2,777
2,761
2,754
2,746
2,739
2,723
2,717
2,587
2,581
2,566
2,560
2,550
2,544
2,528
2,522
2,337
2,328
2,317
2,309
2,299
2,291
2,280
2,273
2,266
2,259
2,244
2,234
2,232
1,481
1,460
1,453
1,445
1,438
1,424
1,418
1,397
1,382
1,375
1,368
1,361
1,339
1,334
1,301
1,291
1,283
1,255
1,236
1,218
1,203
1,187
1,179
1,162
1,151
1,137
1,133
N
N
OH
Pyr1
7,2
1,9
1,8
2,0
2,3
1,2
1,2
1,2
1,2
2,4
2,3
3,0
ppm
8,5
8
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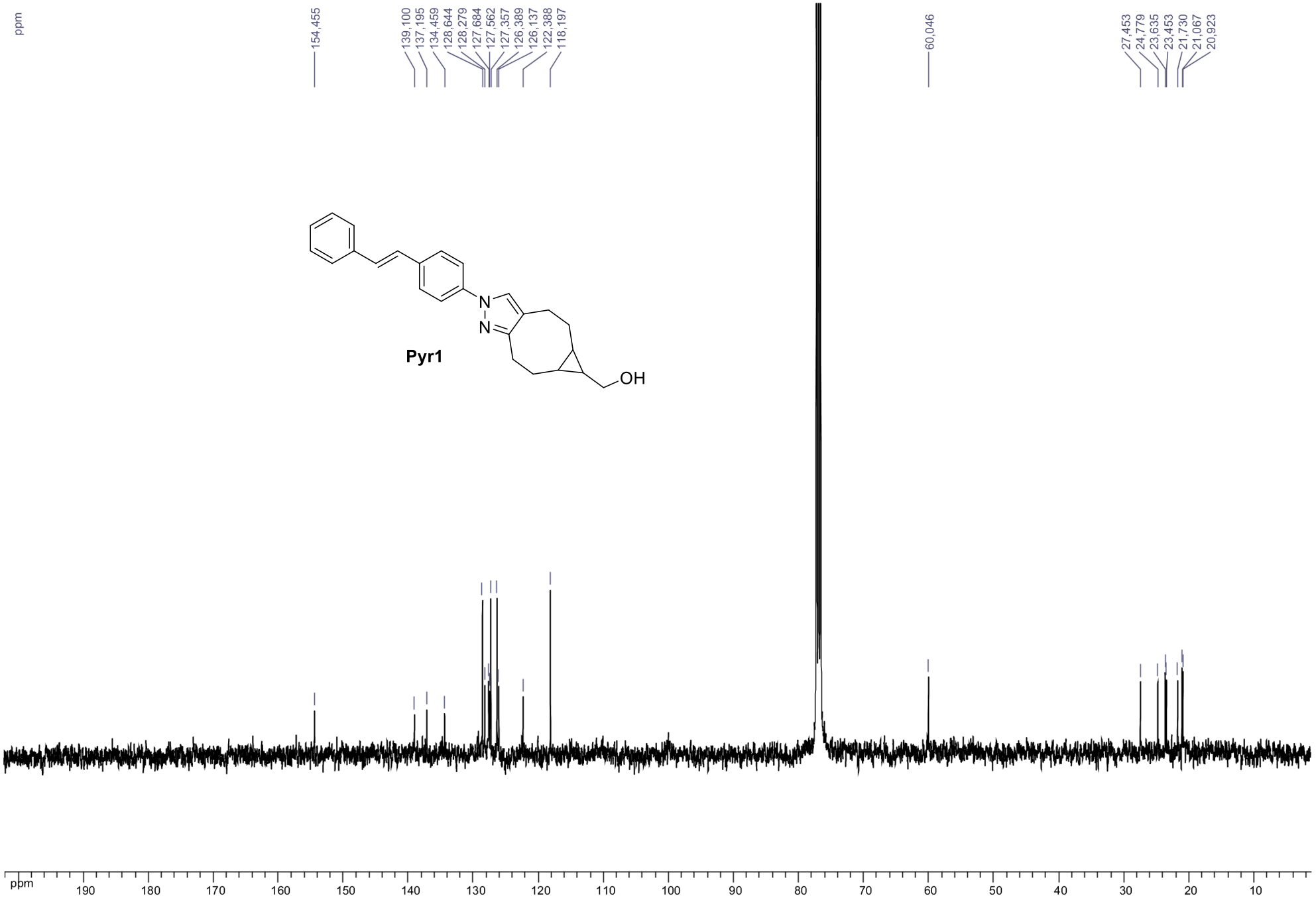
6,5
6
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5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154,455
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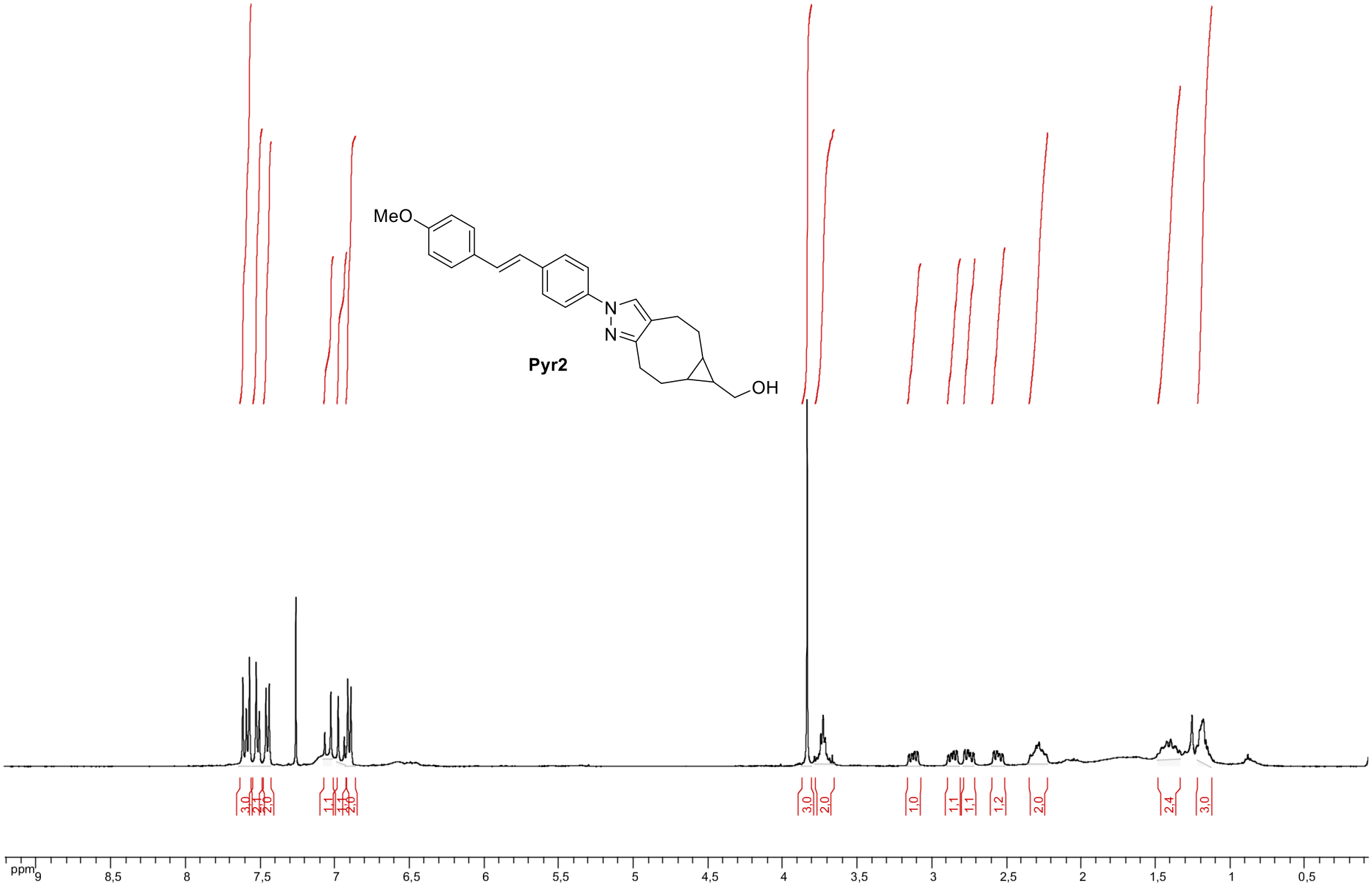
137,195
134,459
128,644
128,279
127,684
127,562
127,357
126,389
126,137
122,388
118,197
60,046
27,453
24,779
23,635
23,453
21,730
21,067
20,923
N
N
OH
Pyr1
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


MeO
N
N
OH
Pyr2
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2,1
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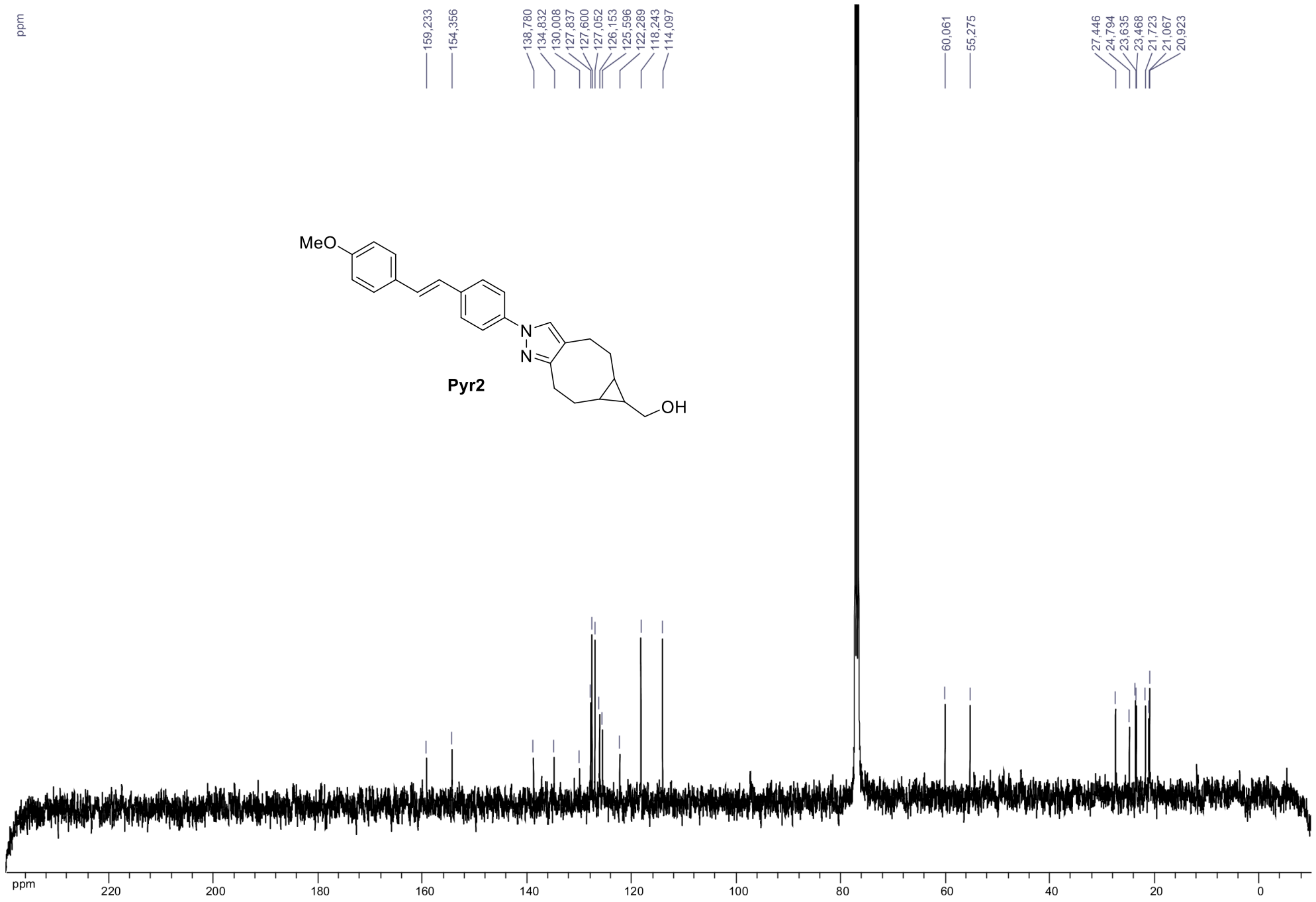
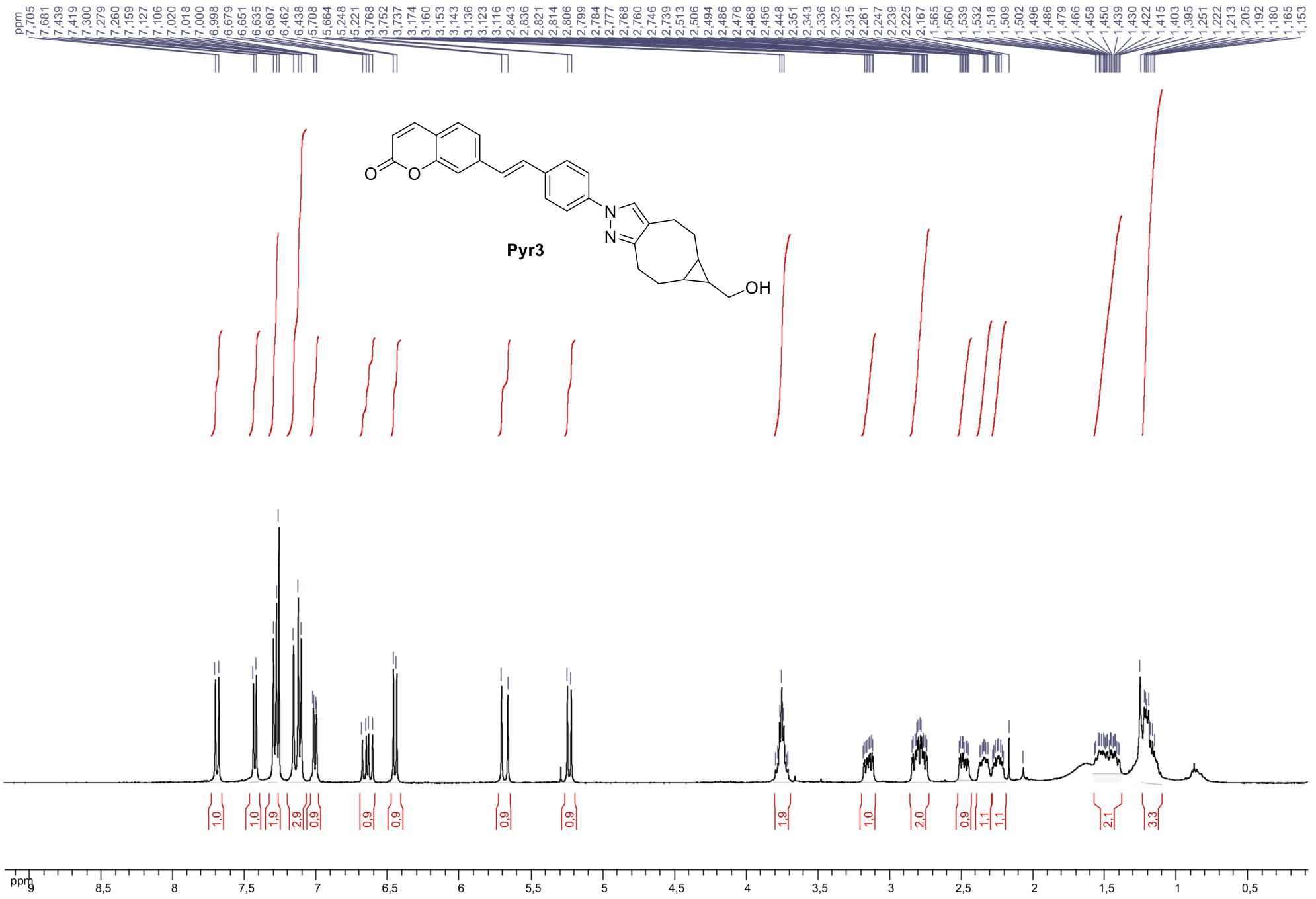
1,1
1,1
2,0
3,0
2,0
1,0
1,1
1,1
1,2
2,0
2,4
3,0
ppm
9
8,5
8
7,5
7
6,5
6
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5


ppm
159,233
154,356
138,780
134,832
130,008
127,837
127,600
127,052
126,153
125,596
122,289
118,243
114,097
60,061
55,275
27,446
24,794
23,635
23,468
21,723
21,067
20,923
MeO
N
N
OH
Pyr2
ppm
220
200
180
160
140
120
100
80
60
40
20
0


ppm
7,705
7,681
7,439
7,419
7,300
7,279
7,260
7,159
7,127
7,106
7,020
7,018
7,000
6,998
6,679
6,651
6,635
6,607
6,462
6,438
5,708
5,664
5,248
5,221
3,768
3,752
3,737
3,174
3,160
3,153
3,143
3,136
3,123
3,116
2,843
2,836
2,821
2,814
2,806
2,799
2,784
2,777
2,768
2,760
2,746
2,739
2,513
2,506
2,494
2,486
2,476
2,468
2,456
2,448
2,351
2,343
2,336
2,325
2,315
2,261
2,247
2,239
2,225
2,167
1,565
1,560
1,539
1,532
1,518
1,509
1,502
1,496
1,486
1,479
1,466
1,458
1,450
1,439
1,430
1,422
1,415
1,403
1,395
1,251
1,222
1,213
1,205
1,192
1,180
1,165
1,153
O
O
N
N
OH
Pyr3
1,0
1,0
1,9
2,9
0,9
0,9
0,9
0,9
0,9
1,9
1,0
2,0
0,9
1,1
1,1
2,1
3,3
ppm
9
8,5
8
7,5
7
6,5
6
5,5
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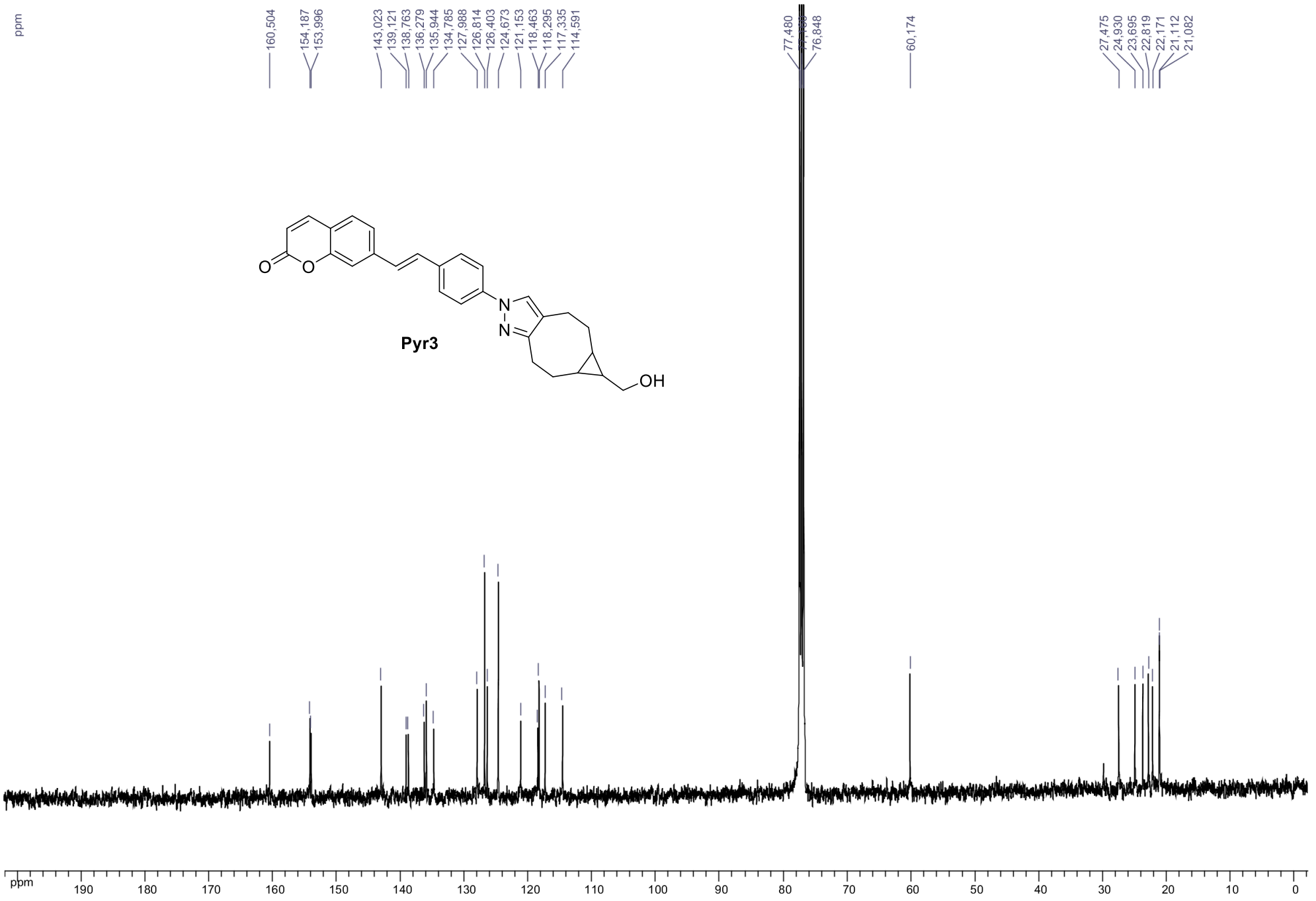
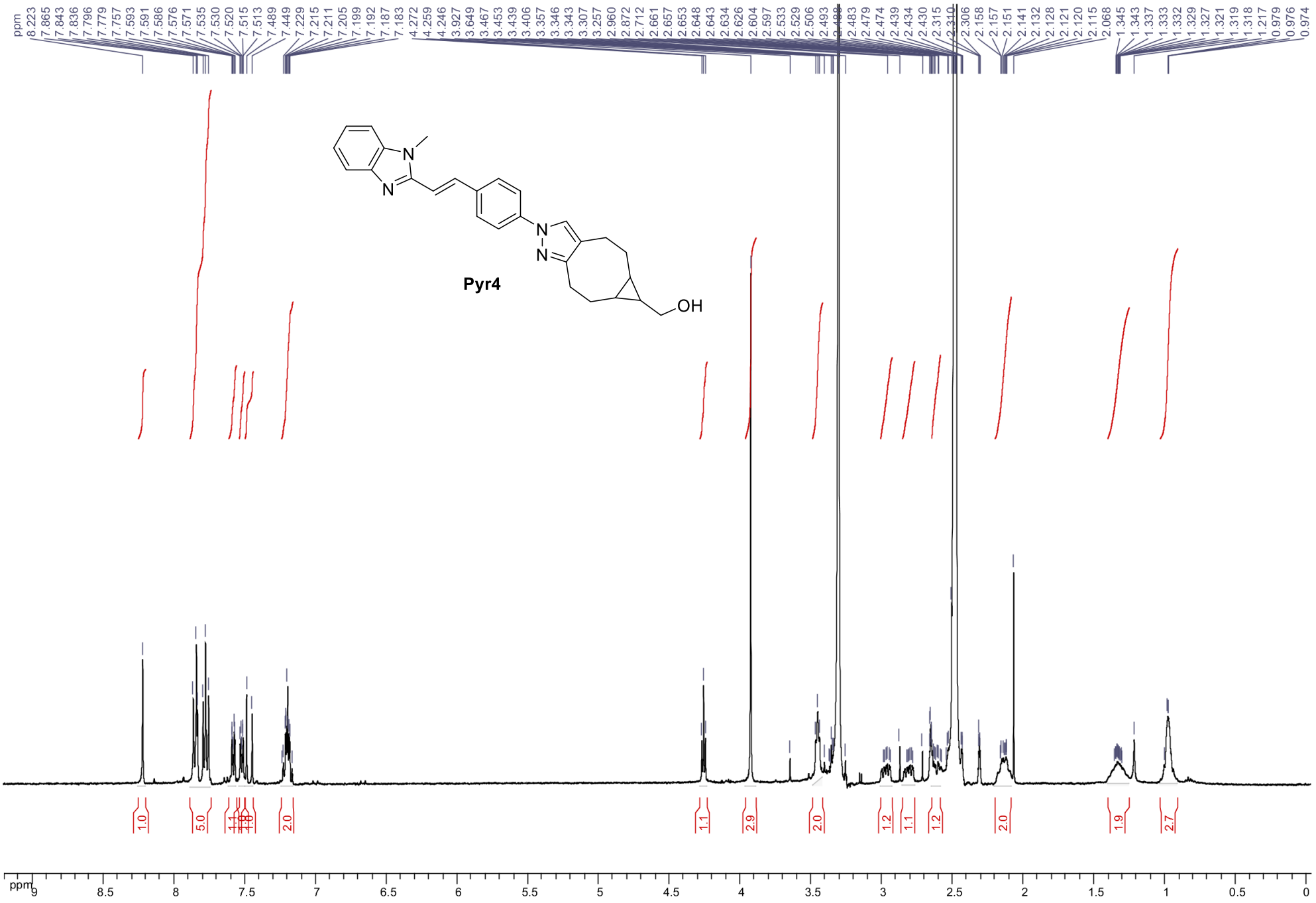
4,5
4
3,5
3
2,5
2
1,5
1
0,5


ppm
160,504
154,187
153,996
143,023
139,121
138,763
136,279
135,944
134,785
127,988
126,814
126,403
124,673
121,153
118,463
118,295
117,335
114,591
77,480
76,848
60,174
27,475
24,930
23,695
22,819
22,171
21,112
21,082
O
O
N
N
OH
Pyr3
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


ppm
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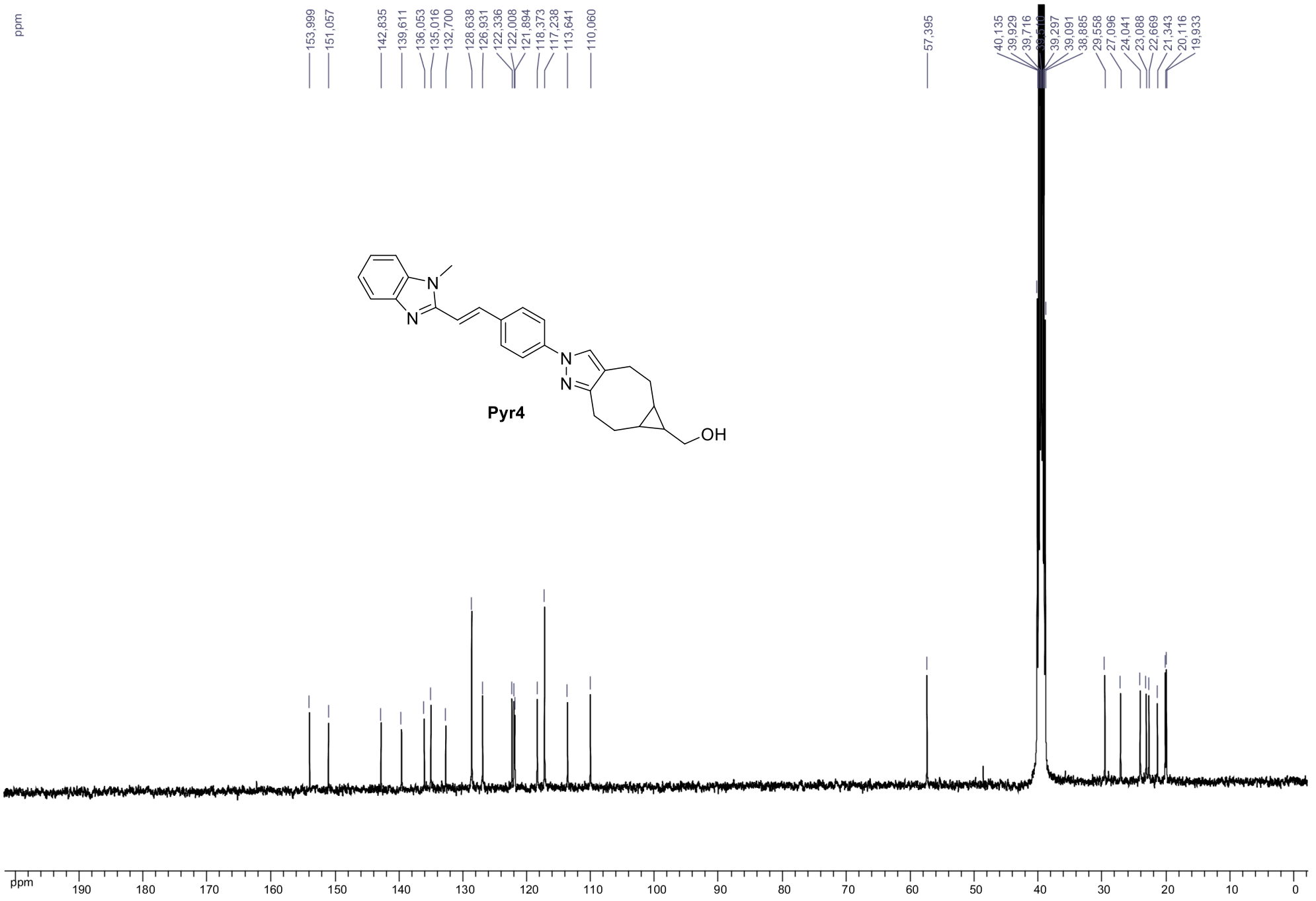
7.843
7.836
7.796
7.779
7.757
7.593
7.591
7.586
7.576
7.571
7.535
7.530
7.520
7.515
7.513
7.489
7.449
7.229
7.215
7.211
7.205
7.199
7.192
7.187
7.183
4.272
4.259
4.246
3.927
3.649
3.467
3.453
3.439
3.406
3.357
3.346
3.343
3.307
3.257
2.960
2.872
2.712
2.661
2.657
2.653
2.648
2.643
2.634
2.626
2.604
2.597
2.533
2.529
2.506
2.493
2.483
2.479
2.474
2.439
2.434
2.430
2.315
2.310
2.306
2.158
2.157
2.151
2.141
2.132
2.128
2.121
2.120
2.115
2.068
1.345
1.343
1.337
1.333
1.332
1.329
1.327
1.321
1.319
1.318
1.217
0.979
0.976
0.974
N
N
N
N
OH
Pyr4
1.0
5.0
2.0
1.1
2.9
2.0
1.2
1.1
1.2
2.0
1.9
2.7
ppm
9
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0


ppm
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139,611
136,053
135,016
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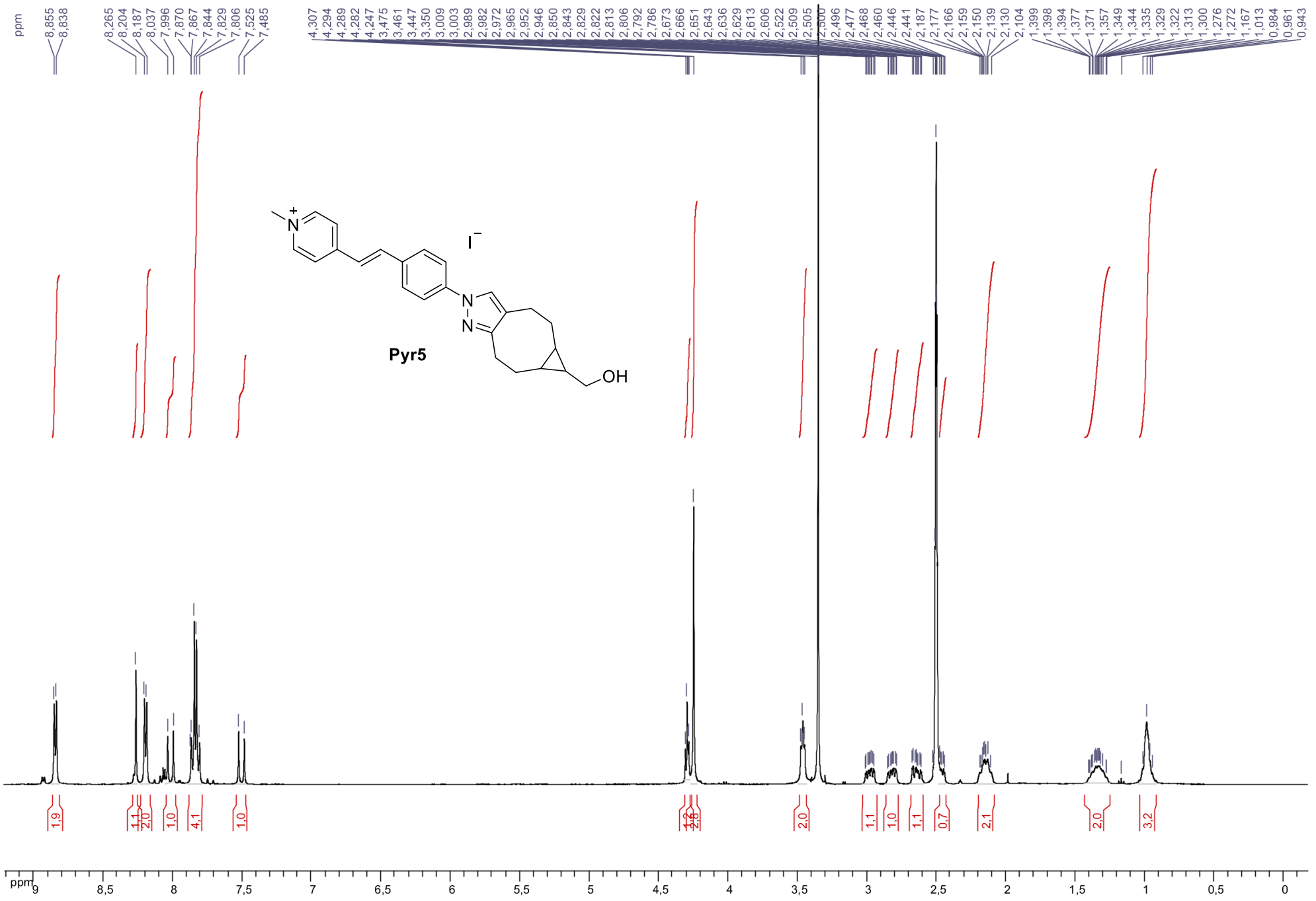
126,931
122,336
122,008
121,894
118,373
117,238
113,641
110,060
57,395
40,135
39,929
39,716
39,297
39,091
38,885
29,558
27,096
24,041
23,088
22,669
21,343
20,116
19,933
N
N
N
N
OH
Pyr4
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


ppm
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8,204
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8,037
7,996
7,870
7,867
7,844
7,829
7,806
7,525
7,485
4,307
4,294
4,289
4,282
4,247
3,475
3,461
3,447
3,350
3,009
3,003
2,989
2,982
2,972
2,965
2,952
2,946
2,850
2,843
2,829
2,822
2,813
2,806
2,792
2,786
2,673
2,666
2,651
2,643
2,636
2,629
2,613
2,606
2,522
2,509
2,505
2,500
2,496
2,477
2,468
2,460
2,446
2,441
2,187
2,177
2,166
2,159
2,150
2,139
2,130
2,104
1,399
1,398
1,394
1,377
1,371
1,357
1,349
1,344
1,335
1,329
1,322
1,313
1,300
1,276
1,272
1,167
1,013
0,984
0,961
0,943
I⁻
N
N
N
OH
Pyr5
1,9
1,1
2,0
1,0
4,1
1,0
1,2
2,8
2,0
1,1
1,0
1,1
0,7
2,1
2,0
3,2
ppm
9
8,5
8
7,5
7
6,5
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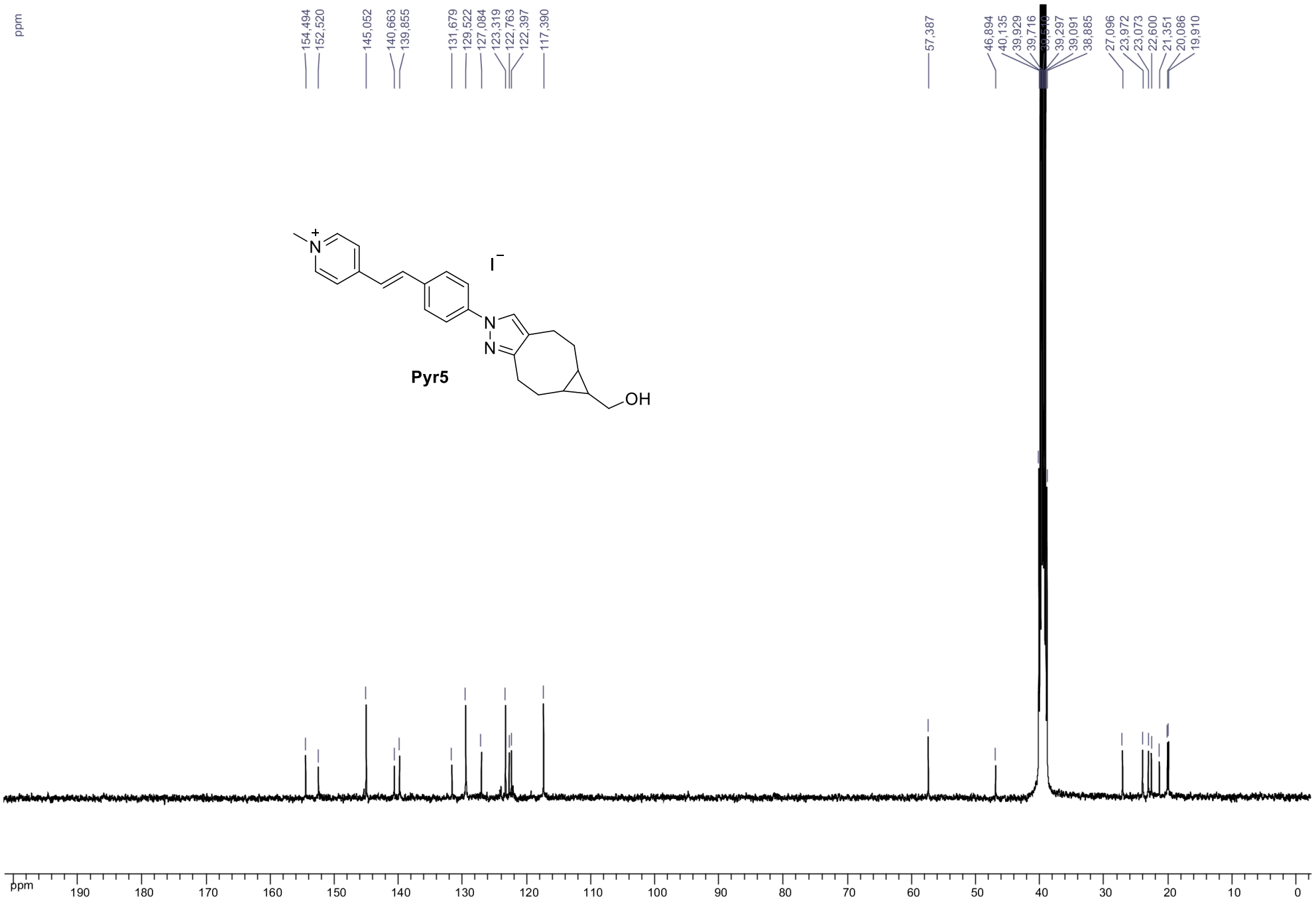
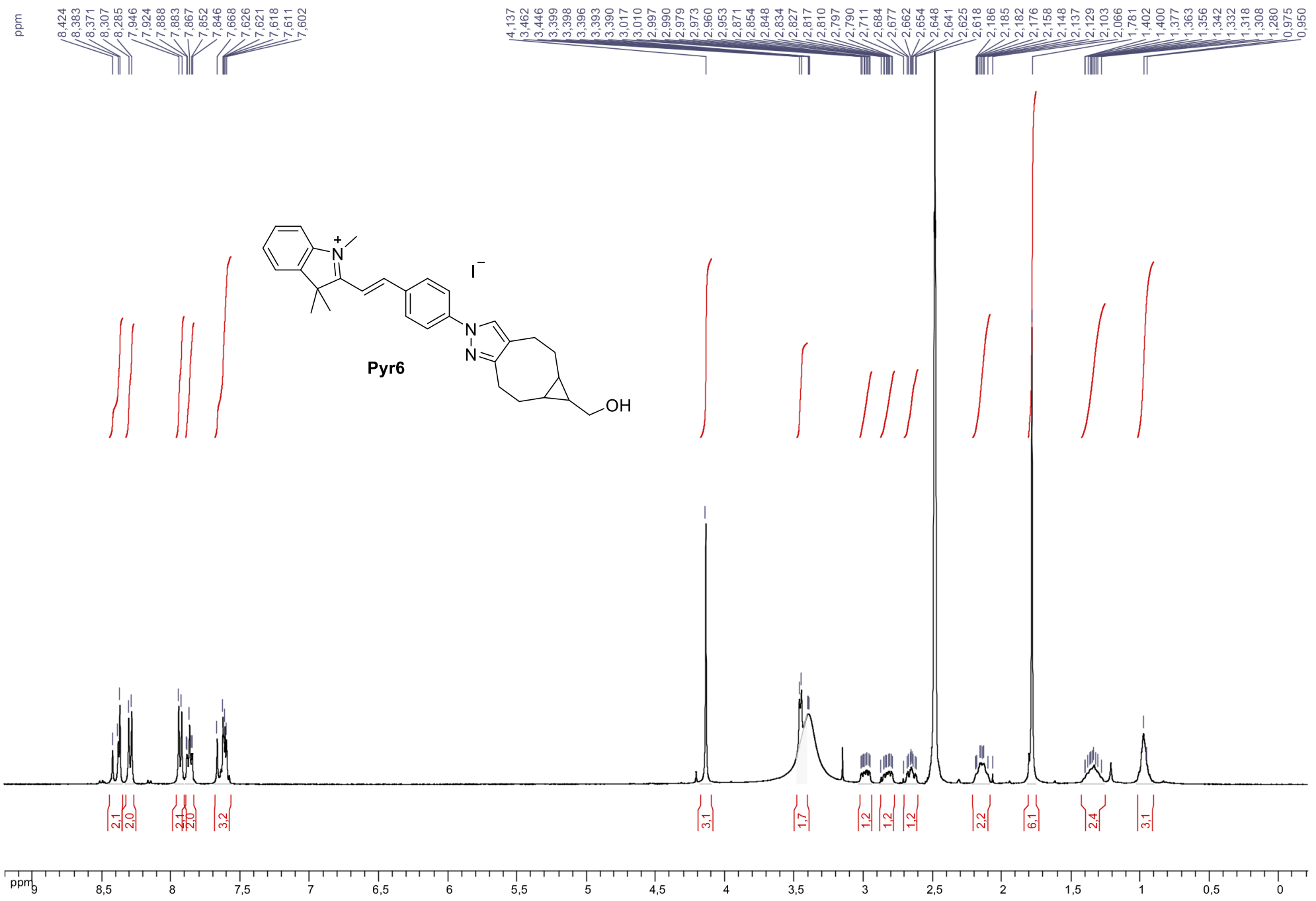
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0


ppm
154,494
152,520
145,052
140,663
139,855
131,679
129,522
127,084
123,319
122,763
122,397
117,390
57,387
46,894
40,135
39,929
39,716
39,540
39,297
39,091
38,885
27,096
23,972
23,073
22,600
21,351
20,086
19,910
I⁻
N
N
OH
Pyr5
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


ppm
8,424
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8,371
8,307
8,285
7,946
7,924
7,888
7,883
7,867
7,852
7,846
7,668
7,626
7,621
7,618
7,611
7,602
4,137
3,462
3,446
3,399
3,398
3,396
3,393
3,390
3,017
3,010
2,997
2,990
2,979
2,973
2,960
2,953
2,871
2,854
2,848
2,834
2,827
2,817
2,810
2,797
2,790
2,711
2,684
2,677
2,662
2,654
2,648
2,641
2,625
2,618
2,186
2,185
2,182
2,176
2,158
2,148
2,137
2,129
2,103
2,066
1,781
1,402
1,400
1,377
1,363
1,356
1,342
1,332
1,318
1,308
1,280
0,975
0,950
I⁻
N
N
OH
Pyr6
2,1
2,0
2,1
2,0
3,2
3,1
1,7
1,2
1,2
1,2
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8
7,5
7
6,5
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5,5
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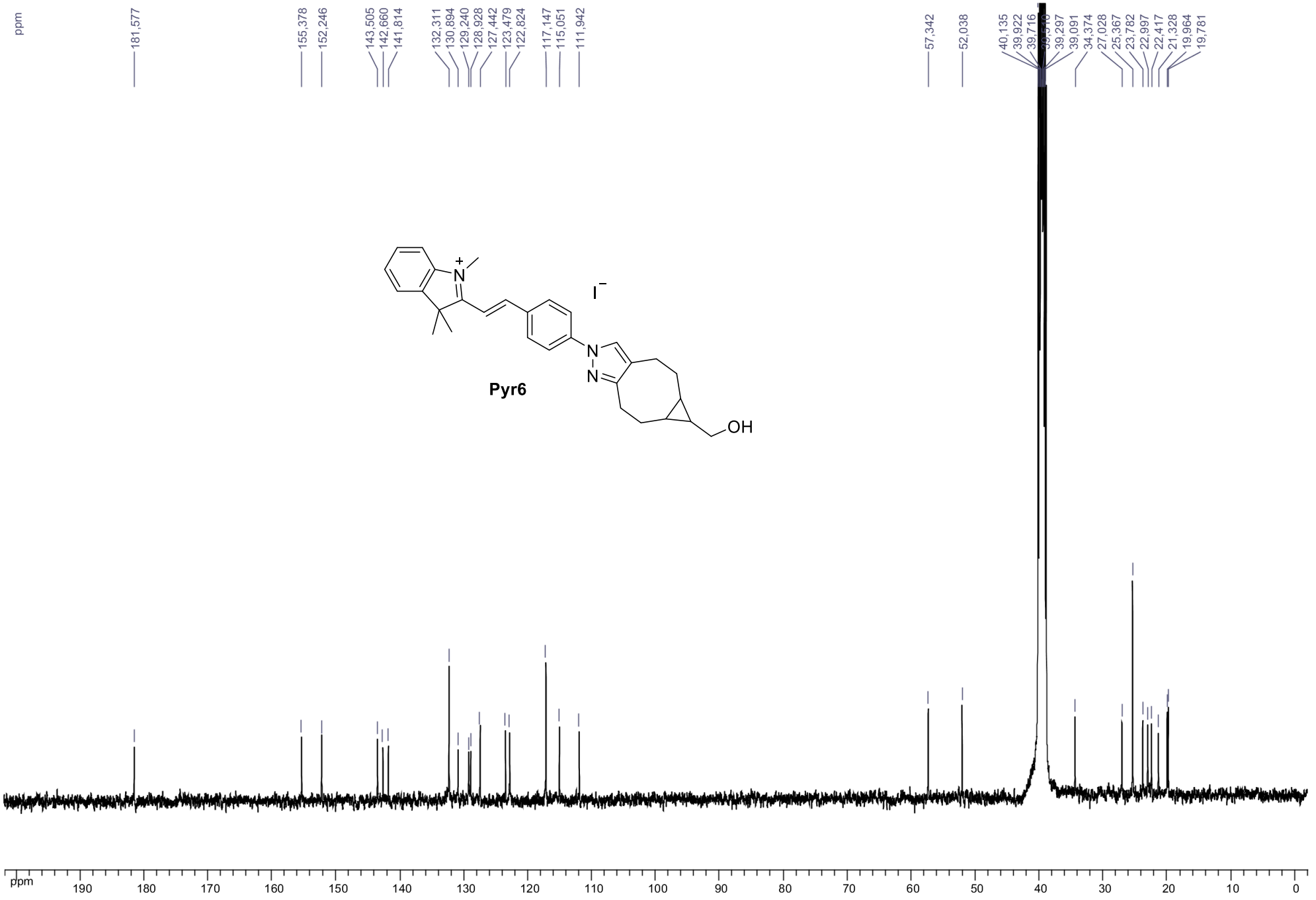
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0


ppm
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142,660
141,814
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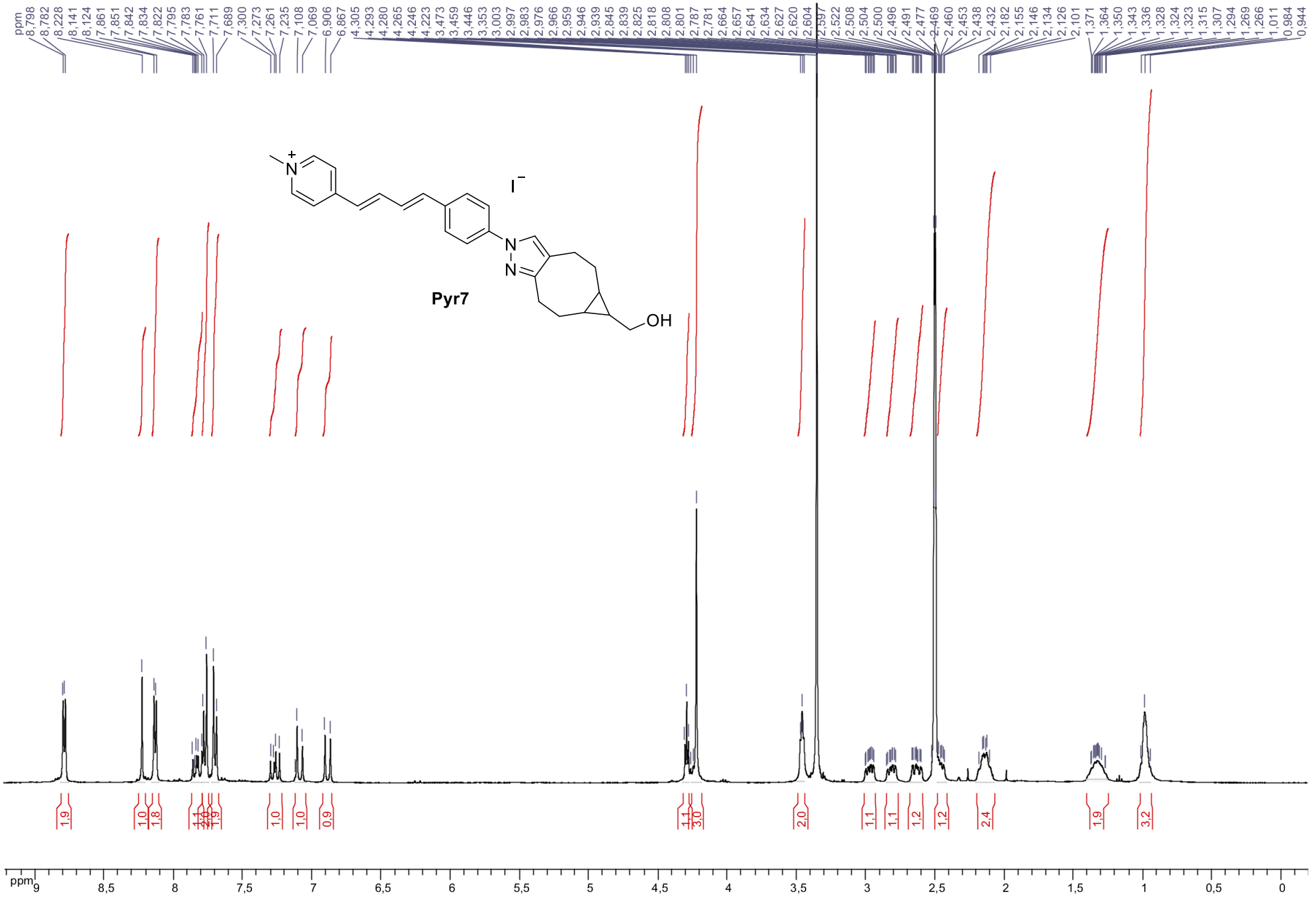
130,894
129,240
128,928
127,442
123,479
122,824
117,147
115,051
111,942
57,342
52,038
40,135
39,922
39,716
39,510
39,297
39,091
34,374
27,028
25,367
23,782
22,997
22,417
21,328
19,964
19,781
I⁻
N
N
OH
Pyr6
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


ppm
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7.842
7.834
7.822
7.795
7.783
7.761
7.711
7.689
7.300
7.273
7.261
7.235
7.108
7.069
6.906
6.867
4.305
4.293
4.280
4.265
4.246
4.223
3.473
3.459
3.446
3.353
3.003
2.997
2.983
2.976
2.966
2.959
2.946
2.939
2.845
2.839
2.825
2.818
2.808
2.801
2.787
2.781
2.664
2.657
2.641
2.634
2.627
2.620
2.604
2.597
2.522
2.508
2.504
2.500
2.496
2.491
2.477
2.469
2.460
2.453
2.438
2.432
2.182
2.155
2.146
2.134
2.126
2.101
1.371
1.364
1.350
1.343
1.336
1.328
1.324
1.323
1.315
1.307
1.294
1.269
1.266
1.011
0.984
0.944
N
I
N
N
OH
Pyr7
1,9
1,0
1,8
1,0
1,0
1,9
1,0
1,0
0,9
1,1
3,0
2,0
1,1
1,1
1,2
1,2
2,4
1,9
3,2
ppm
9
8,5
8
7,5
7
6,5
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5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
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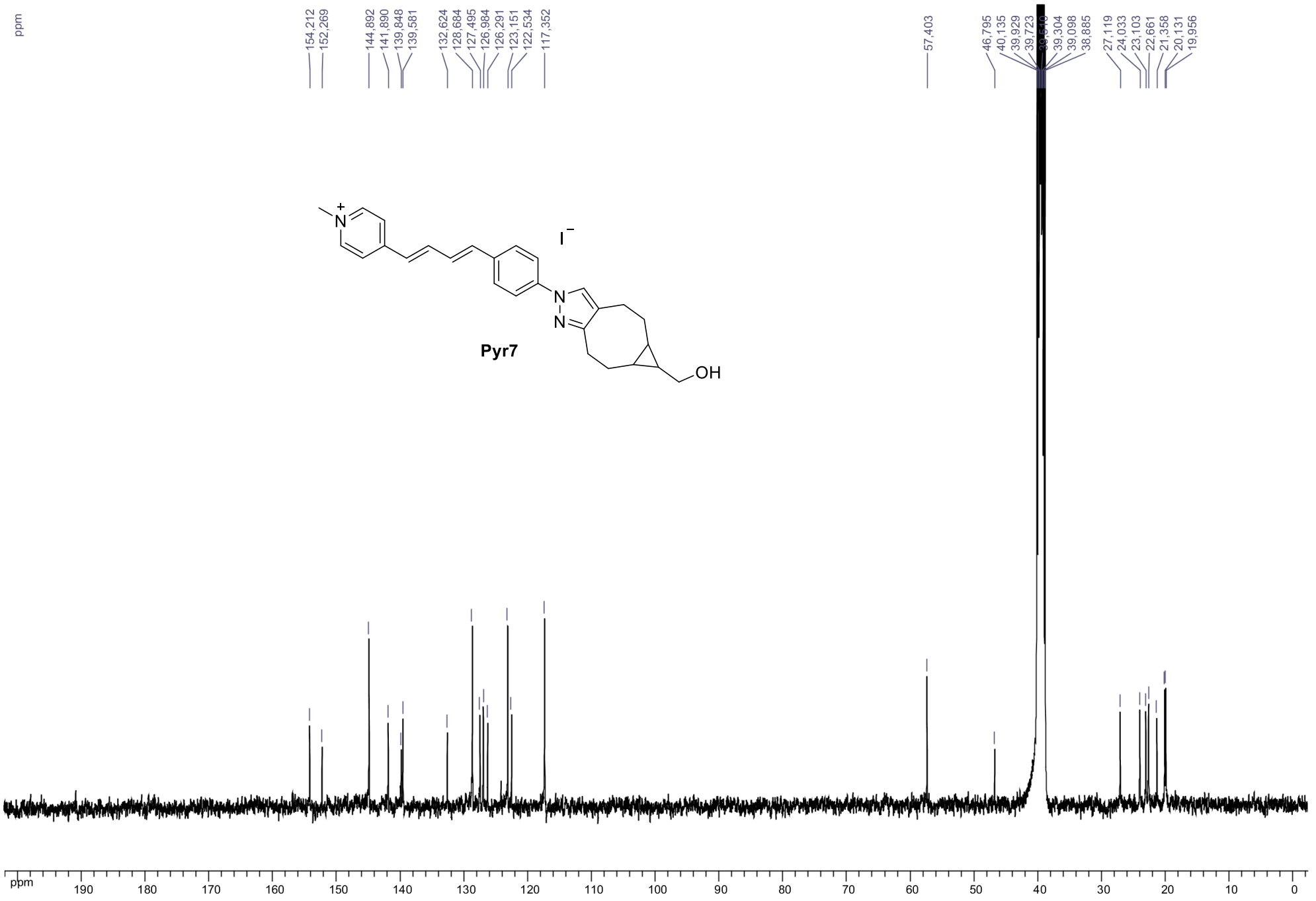
0


ppm
154,212
152,269
144,892
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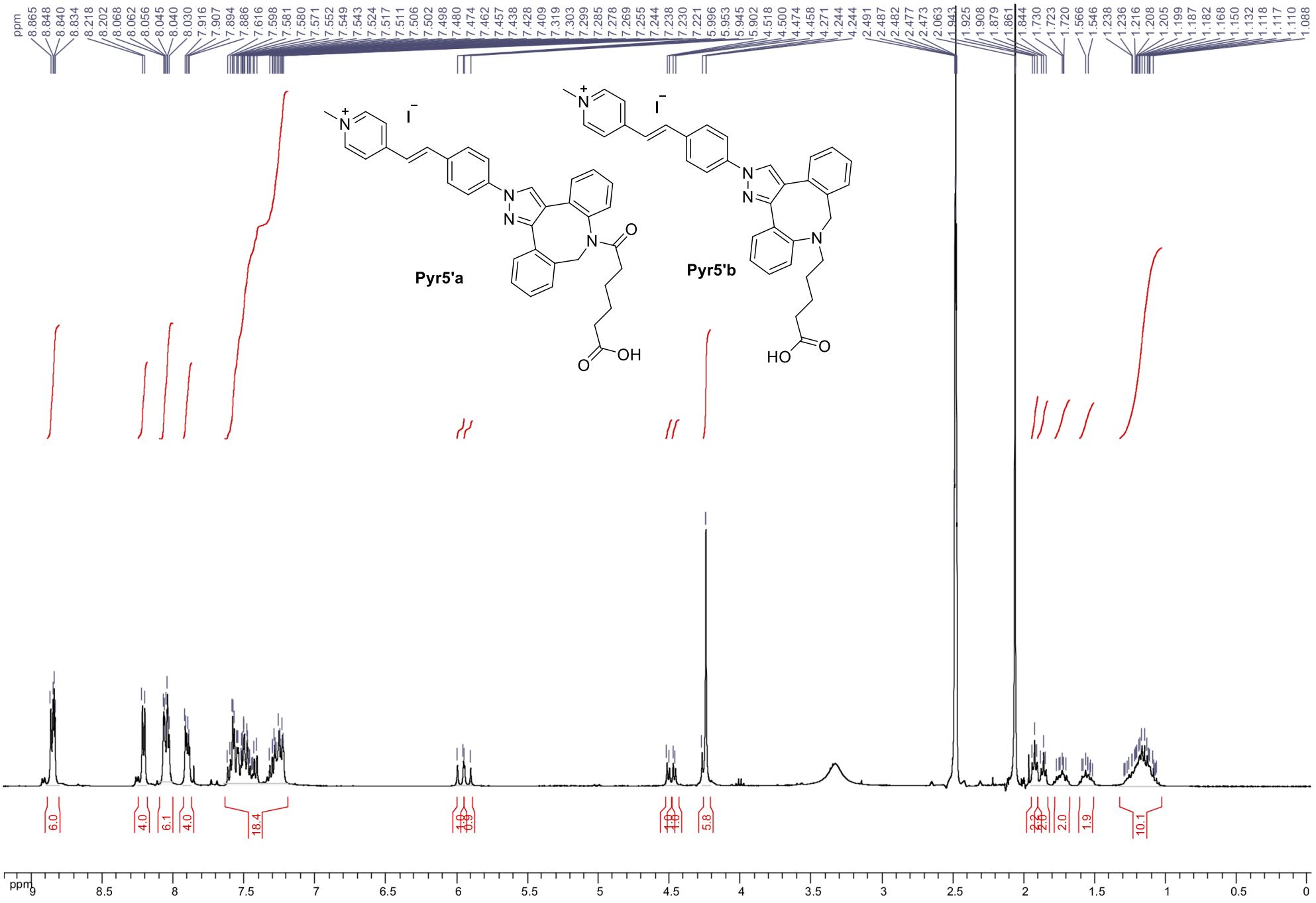
139,848
139,581
132,624
128,684
127,495
126,984
126,291
123,151
122,534
117,352
57,403
46,795
40,135
39,929
39,723
39,540
39,304
39,098
38,885
27,119
24,033
23,103
22,661
21,358
20,131
19,956
I⁻
N
N
OH
Pyr7
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


ppm
8.865
8.848
8.840
8.834
8.218
8.202
8.068
8.062
8.056
8.045
8.040
8.030
7.916
7.907
7.894
7.886
7.616
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Pyr5'b
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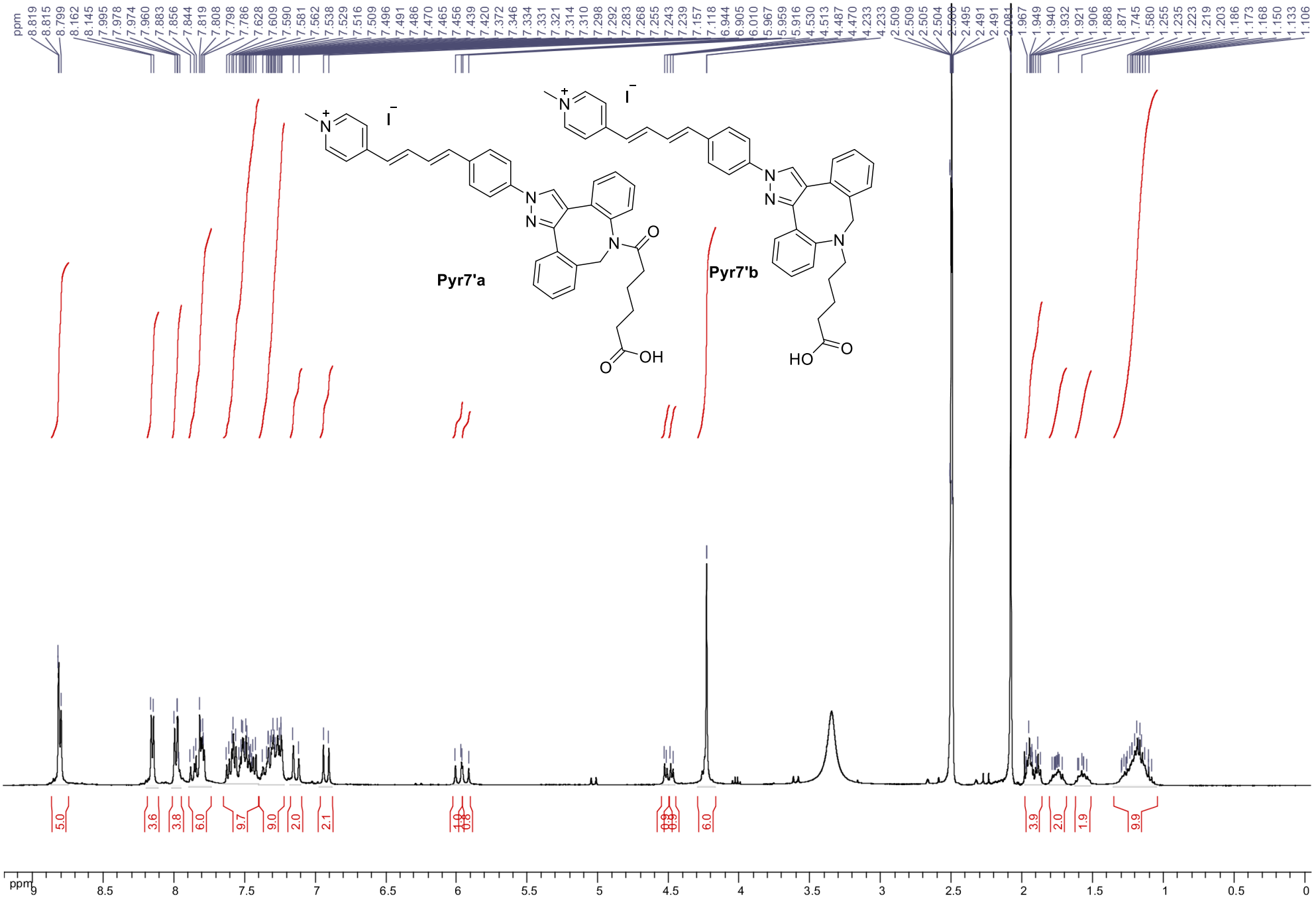
Pyr7'a
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