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Sydnone-Coumarins as Clickable Turn-On Fluorescent Sensors for Molecular Imaging

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Supplementary Information

Table of content

I. General Information

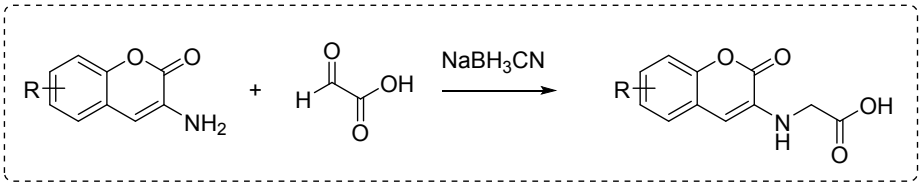
Organic solvents (Aldrich) were used without further purification. Purifications of reactions products were carried out by flash chromatography using Merck silica gel (40-63 µm). FT-ATR-IR spectra were recorded on a Perkin-Elmer UAR Two Spectrum spectrometer and are reported as wavelength numbers (cm^{-1}). ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) were measured on a Brucker Avance 400 MHz spectrometer. 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), doublet (d), triplet (t). 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 (pompe 2545, mass: ZQ2000) mass Spectrometer. Unless otherwise noted, all other commercially available reagents and solvents were used without further purification. HPLC were performed on a Shimadzu System (SPD-M20A detector, CTO-10AC oven, SIL-20A injector, LC-20AB pump) with a Phenyl column (3.5µm, 4.6x100mm). Melting points were measured on a Büchi B-545 and are reported in °C. Absorbances and fluorescences for the screening were measured on a Molecular Device SpectraMax® M5[e]. Absorbances were measured on a Varian Cary® 50 UV-Vis spectrophotometer. Fluorescence spectra were obtained on a HORIBA FluoroMax®-4 fluorimeter.

For the protein labeling:

The Mini-Protean III electrophoresis system, 4-20% gradient mini-gels, Migrating buffer and Precision Plus Molecular marker were from Bio-Rad Laboratories (Germany). Fluorescence measurement were performed with VersaDoc MP 4000 Molecular Digital Imaging System (Bio-Rad) or a LAS4000 biomolecular imaginer (GE healthcare) allowing visualization of bands relative to fluorescent proteins. Blue LED was used as light source and the emission collection was at 530nm for MP 4000 imager and at 515nm for LAS4000 imager. Esquire HCT Electrospray ion trap mass spectrometer (Bruker Daltonik GmbH, Germany) in the positive ion mode coupled on-line to an Agilent 1100 HPLC and a 4800 spectrometer MALDI-TOF/TOF Proteomics Analyzer (Applied Biosystems, Foster City, CA) were used for mass spectrometry analysis. Experiments with MYO-ALK use as starting material MYO-ALK prepared as described in previous work[i]. Experiments with MYO-**I** use as starting material MYO-**I** mixture prepared as described later.

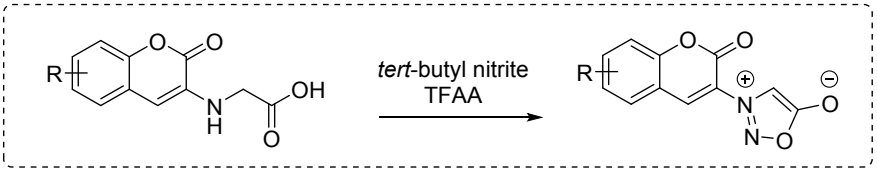
II. Synthetic Procedure and Analytical Data

General Procedure A:



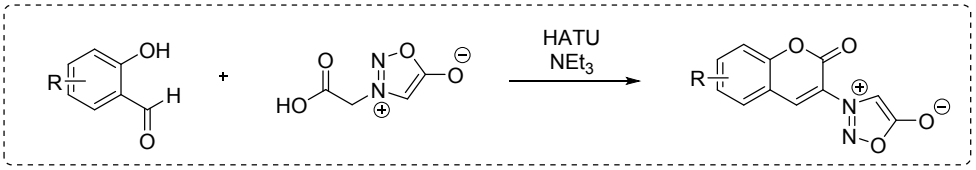
Into a solution of aminocoumarin (1 eq.) in methanol, at 0 °C, sodium acetate (2 eq.), AcOH (4 eq.), glyoxylic acid (1.5 eq.) and sodium cyanoborohydride (1 eq.) were added in this order. The solution was stirred at room temperature for 18 hours. After condensation, a celite® filtration was performed and washed thoroughly with EtOAc/AcOH 99/1. The filtrate was extracted with EtOAc. Organics layers were collected, dried over $MgSO_4$ and evaporated under *vacuum*. A column chromatography was performed in DCM/MeOH/AcOH: (9/0.9/0.1).

General Procedure B:



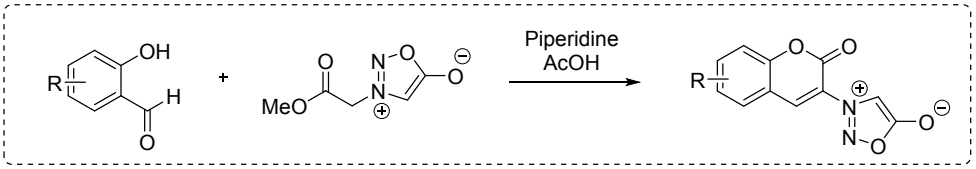
Tert-butylnitrite (1.1 eq.) was poured on glycine (1 eq.). The reaction mixture was stirred for 3 hours. Trifluoroacetic anhydride (2.5 eq.) was added into the reactional mixture after evaporation. The reaction mixture was stirred for 4 hours. A column chromatography was performed in heptane/EtOAc: (7/3).

General Procedure C:



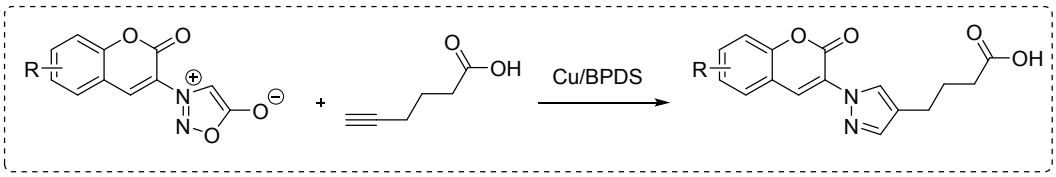
In a round bottom flask, HATU (3 eq.), triethylamine (3 eq.) and the acidsydnone **13** (3 eq.) were stirred in DMF at room temperature overnight. Then salicylaldehyde (1 eq.) and triethylamine(2 eq.) were added to the reaction mixture and stirred at room temperature for 8h. The solvent was evaporated under *vacuum*, the residue was then extracted with DCM and water. The organic layers were collected, dried over $MgSO_4$ and evaporated under *vacuum*. A column chromatography was performed on the crude product.

General Procedure D:



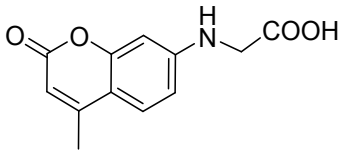
A mixture containing n-butanol, salicylaldehyde (1 eq.), methylestersydnone **S3** (1.2 eq.), piperidine (0.15 eq.) and AcOH (0.5 eq.) was refluxed overnight. Upon cooling to room temperature, the reaction mixture was evaporated under *vacuum* and a column chromatography was performed on the solid residue.

General Procedure E:



A solution of copper sulfate pentahydrate (0.2 eq.), bathophenanthrolinedisulfonic acid (0.2 eq.) and triethanolamine (1 eq.) was prepared in a minimum of water and was added into coumarin sydnone (1 eq.) and hexynoïc acid (1.2 eq.) in DMF. If a precipitate appeared, DMF was added until it faded away. Then sodium ascorbate (2 eq.) was added to the reaction mixture and stirred at 60 °C overnight. After cooling to room temperature, the reaction mixture was evaporated under *vacuum*. The liquid residue was quenched by a solution of HEDTA 0.05M and extracted with EtOAc and HCl (1M).

(4-methyl-2-oxo-2H-chromen-7-yl)glycine (S1)



$C_{12}H_{11}NO_4$

MW: 233 g.mol^{-1}

Compound **S1** was obtained in 30% yield, 320 mg (red solid) from 7-amino-4-methyl-2H-chromen-2-one (800 mg, 4.57 mmol), glyoxylic acid (631 mg, 6.85 mmol), sodium acetate (740 mg, 9.13 mmol), AcOH (1.04 ml, 18.27 mmol) and sodium cyanoborohydride (283 mg, 4.57 mmol) using general procedure **A**.

MP: 192–194 °C;

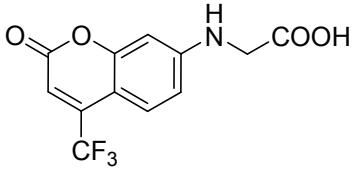
^{1}H NMR (400MHz, DMSO-d_6, δ ppm): 7.45 (d, *J* = 8.8 Hz, 1H), 6.78 (s, 1H), 6.64 (dd, *J* = 1.9, 8.8 Hz, 1H), 6.40 (d, *J* = 1.9 Hz, 1H), 5.93 (s, 1H), 3.83 (s, 2H), 2.31 (s, 3H);

^{13}C NMR (100MHz, DMSO-d_6, δ ppm): 172.3, 160.8, 155.5, 153.8, 152.1, 125.9, 110.4, 109.3, 107.9, 97.0, 44.8, 18.1;

IR (cm^{-1}): 3364, 2918, 2854, 1654, 1592, 1403, 1368, 1216, 1072, 921, 814, 650, 538;

LCMS (ESI) *m/z*: $C_{12}H_{12}NO_4$ $[M+H]^+$ 234.

2-oxo-4-(trifluoromethyl)-2H-chromen-7-yl)glycine (S2)



$C_{12}H_8F_3NO_4$

MW: 287 g.mol^{-1}

Compound **S2** was obtained in 78% yield, 782 mg (yellow solid) from 7-amino-4-(trifluoromethyl)-2H-chromen-2-one (800 mg, 3.49 mmol), glyoxylic acid (481 mg, 5.23 mmol), sodium acetate (572 mg, 6.98 mmol), AcOH (700 µl, 14.0 mmol) and sodium cyanoborohydride (216 mg, 3.49 mmol) using general procedure **A**.

MP: 213–215 °C;

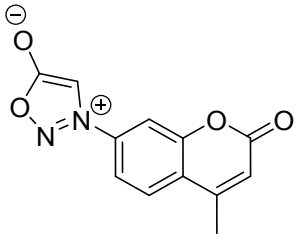
^{1}H NMR (400MHz, DMSO-d$_6$, δ ppm): 7.39 (dd, *J* = 9.1, 2.0 Hz, 1H), 7.25 (t, *J* = 6.0 Hz, 1H), 6.75 (dd, *J* = 2.0, 9.1 Hz, 1H), 6.54 (d, *J* = 2.0 Hz, 1H), 6.00 (s, 1H), 3.96 (d, *J* = 6.0 Hz, 2H);

^{13}C NMR (100MHz, DMSO-d$_6$, δ ppm): 171.8, 159.4, 156.6, 152.9, 140.2 (q, *J* = 30.6Hz), 125.2, 122.1 (q, *J* = 273.8 Hz) 111.6, 107.7, 102.0, 97.4, 45.5;

IR (cm^{-1}): 3358, 2904, 1742, 1675, 1593, 1543, 1416, 1350, 1287, 1207, 1133, 895, 833, 724, 645, 521, 470;

LCMS (ESI) *m/z*: $C_{12}H_9F_3NO_4$ $[M+H]^+$: 288.

3-(4-methyl-2-oxo-2H-chromen-7-yl)-1,2,3-oxadiazol-3-ium-5-olate (1)



$C_{12}H_8N_2O_4$

MW: 244 g.mol^{-1}

Compound **1** was obtained in 97% yield, 96 mg (red solid) from compound **S1** (92.8 mg, 0.40 mmol), terbutylnitrite (53 µL, 0.44 mmol) and trifluoroacetic anhydride (139 µL, 1 mmol) using general procedure **B**.

MP: 209–211 °C;

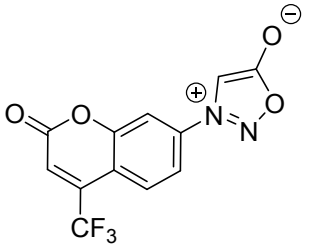
^{1}H NMR (400MHz, DMSO-d$_6$, δ ppm): 8.07–7.92 (m, 4H), 6.60 (s, 1H), 2.52 (s, 3H, under DMSO signal);

^{13}C NMR (100MHz, DMSO-d$_6$, δ ppm): 168.3, 158.9, 153.1, 152.3, 136.0, 127.4, 122.6, 116.8, 116.3, 110.0, 95.3, 18.1;

IR (cm^{-1}): 3128, 3055, 2922, 2853, 1767, 1736, 1613, 1576, 1457, 1373, 1163, 1087, 969, 937, 873, 797, 725, 703, 613, 573, 455;

LCMS (ESI) *m/z*: $C_{12}H_9N_2O_4$ $[M+H]^+$: 245.

3-(2-oxo-4-(trifluoromethyl)-2H-chromen-7-yl)-1,2,3-oxadiazol-3-ium-5-olate (2)



$C_{12}H_5F_3N_2O_4$

MW: 298 g.mol^{-1}

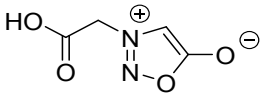
Compound **2** was obtained in 51% yield, 56 mg (red solid) from compound **S2** (105 mg, 0.37 mmol), terbutylnitrite (42 µL, 0.41 mmol) and trifluoroacetic anhydride (129 µL, 0.92 mmol) using general procedure **B**.

MP: 222–224 °C;

^{1}H NMR (400MHz, DMSO-d$_6$, δ ppm): 8.23 (s, 1H), 8.00 (s, 2H), 7.96 (s, 1H), 7.29 (s, 1H);

^{13}C NMR (100MHz, DMSO-d_6, δ ppm): 168.3, 157.6, 154.1, 137.9 (q, *J* = 33.2 Hz), 136.6, 126.6, 122.8, 119.9, 117.5, 116.2, 111.0, 95.4;
IR (cm^{-1}): 3450, 3154, 3093, 2929, 1732, 1617, 1581, 1453, 1437, 1405, 1355, 1326, 1268, 1199, 1175, 1143, 1032, 987, 963, 943, 904, 890, 869, 827, 772;
LCMS (ESI) *m/z*: $C_{12}H_6F_3N_2O_4$ $[M+H]^+$: 299.

3-(carboxymethyl)-1,2,3-oxadiazol-3-ium-5-olate (13)

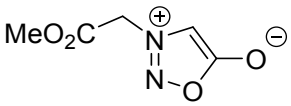


$C_4H_4N_2O_4$
MW: 144 $g.mol^{-1}$
Yield: 28%
Brown solid

To a stirred solution of iminodiacetic acid (6.70 g, 50.0 mmol) in 8.4 mL of concentrated hydrochloric acid, and 12.5 mL of water at 0 °C was added dropwise a solution of sodium nitrite (6.90 g, 100 mmol) in water (25.0 mL) over a period of 40 min. The solution was warmed gradually to ambient temperature. The aqueous solution was then extracted continuously with ether (40 mL) for 48 h and the ethereal extract was dried over $MgSO_4$ before evaporation of the ether to obtain 3.80 g (23.5 mmol, 47%) of the intermediate nitroso compound. A solution of the later in acetic anhydride (21.0 mL) was heated at reflux for 20 min. The reaction mixture was cooled and the excess of acetic anhydride was removed under *vacuum*. 30 mL of water were added to the residue and the solution was washed twice with DCM. The aqueous layer was then evaporated. 2.00 g (13.8 mmol, 28%) of compound **13** were isolated.

^{1}H NMR (400 MHz, MeOD-d_4, δ ppm): 6.88 (s, 1H), 5.32 (s, 2H);
^{13}C NMR (100 MHz, MeOD-d_4, δ ppm): 172.1, 167.1, 98.8, 54.5;
IR (cm^{-1}): 1732, 1393, 1195, 1179, 1068, 950, 747, 722, 699, 642, 514;
HRMS (ESI): *m/z* calcd for $C_4H_3N_2O_4$ $[M-H]^-$: 143.0093; found: 143.0092.

***3-(2-methoxy-2-oxoethyl)-1,2,3-oxadiazol-3-ium-5-olate (S3*)**

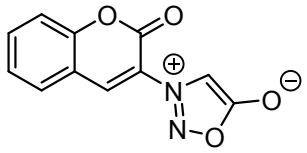


$C_5H_6N_2O_4$
MW: 158 $g.mol^{-1}$
Yield: 91%
White solid

To a solution of (trimethylsilyl)diazomethane (2M in hexane, 2.2 mL, 4.40 mmol) was added dropwise a solution of sydnone **13** (320 mg, 2.20 mmol) in dry MeOH (5.0 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight. The reaction was quenched with AcOH and the aqueous layer was extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$ and evaporated under reduced pressure. 316 mg (2.00 mmol, 91%) of compound **S3** were isolated.

MP: 112–114 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.11 (s, 1H), 5.52 (s, 2H), 3.77 (s, 3H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.5, 165.3, 97.2, 53.3, 53.1;
IR (cm^{-1}): 3150, 3035, 2958, 1763, 1754, 1718, 1483, 1435, 1356, 1221, 1194, 1179, 1070, 998, 978, 944, 880, 760, 730, 714, 669;
LCMS (ESI): *m/z*: $C_5H_7N_2O_4$ $[M+H]^+$: 159.

3-(2-oxo-2H-chromen-3-yl)-1,2,3-oxadiazol-3-ium-5-olate (3).



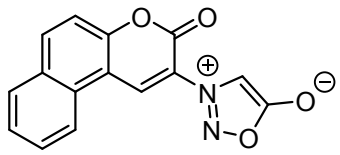
$C_{11}H_6N_2O_4$

MW: 230 g.mol^{-1}

Compound **3** was obtained in 70% yield, 16 mg (yellow solid) from compound **13** (43.2 mg, 0.3 mmol) using general procedure **C** and purified by column chromatography (Heptane/EtOAc: 7/3).

MP: 184–186 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.92 (s, 1H), 7.95 (dd, *J* = 1.7, 7.8 Hz, 1H), 7.84 (ddd, *J* = 1.7, 7.4, 8.5 Hz, 1H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.52 (td, *J* = 0.9, 7.6, Hz, 1H), 7.49 (s, 1H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm) : 168.0, 154.5, 153.4, 140.5, 134.9, 130.4, 125.7, 121.6, 117.2, 116.6, 97.9;
IR (cm^{-1}): 3661, 3131, 1719, 1626, 1608, 1568, 1436, 1401, 1334, 1287, 1258, 1220, 1131, 1065, 945, 836, 756, 692;
HRMS (ESI) *m/z* calcd for $C_{11}H_7N_2O_4$ $[M+H]^+$: 231.0400; found: 231.0402.

3-(3-oxo-3H-benzo[f]chromen-2-yl)-1,2,3-oxadiazol-3-ium-5-olate (4)



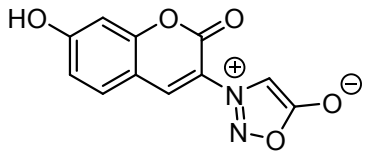
$C_{15}H_8N_2O_4$

MW: 280 g.mol^{-1}

Compound **4** was obtained in 78% yield, 45 mg (yellow solid) from compound **S3** (42 mg, 0.26 mmol) using general procedure **D** and purified by column chromatography (Heptane/EtOAc: 8/2).

MP: 214–216 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.68 (s, 1H), 8.61 (d, *J* = 8.5 Hz, 1H), 8.41 (d, *J* = 9.1 Hz, 1H), 8.14 (d, *J* = 8.2 Hz, 1H), 7.82 (m, 1H), 7.75–7.69 (m, 2H), 7.57 (s, 1H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.1, 154.6, 154.2, 137.6, 136.5, 130.1, 129.3, 129.2 (2C), 126.9, 122.6, 120.9, 116.5, 111.8, 98.0;
IR (cm^{-1})**:** 3111, 2929, 2850, 1863, 1724, 1628, 1566, 1516, 1463, 1434, 1395, 1365, 1342, 1287, 1213, 1174, 1111, 1060, 994, 937, 842, 819, 780, 757, 707, 674;
HRMS (ESI) *m/z* calcd for $C_{15}H_9N_2O_4$ $[M+H]^+$: 281.0557; found: 281.0558.

3-(7-hydroxy-2-oxo-2H-chromen-3-yl)-1,2,3-oxadiazol-3-ium-5-olate (5)



$C_{11}H_6N_2O_5$

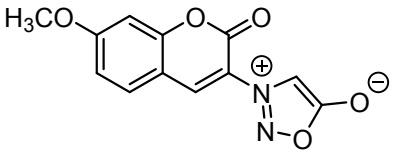
MW: 246 g.mol^{-1}

Compound **5** was obtained in 68% yield, 150 mg (orange solid) from compound **S3** (142 mg, 0.9 mmol) using general procedure **D** and purified by column chromatography (Heptane/EtOAc: 2/8).

MP: 247–249°C ;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.72 (s, 1H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.44 (s, 1H), 6.88 (m, 1H), 6.79 (s, 1H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.4, 166.9, 165.8, 156.2, 155.0, 140.6, 132.1, 115.6, 108.9, 102.6, 97.7;

IR (cm^{-1}) **:** 3182, 3110, 3051, 2944, 1734, 1699, 1598, 1460, 1431, 1404, 1383, 1342, 1326, 1271, 1252, 1236, 1199, 1181, 1128, 1114, 1070, 1005, 974, 942, 879, 854, 801, 776, 753, 732, 721, 689 .
LCMS (ESI) ***m/z***: $C_{11}H_7N_2O_4$ $[M+H]^+$ 247**.**

3-(7-((l1-oxidanyl)-l5-methyl)-2-oxo-2H-chromen-3-yl)-1,2,3-oxadiazol-3-ium-5-olate (6)



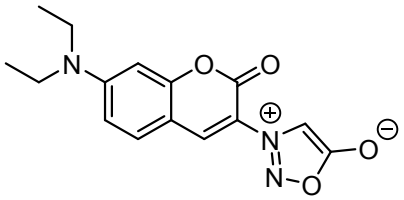
$C_{12}H_8N_2O_5$

MW: 260 $g.mol^{-1}$

Compound **6** was obtained in 68% yield, 97 mg (yellow solid) from compound **S3** (142.2 mg, 0.9 mmol) using general procedure **D** and purified by recrystallization in MeOH.

MP: 205–207 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.84 (s, 1H), 7.86 (d, *J* = 8.8 Hz, 1H), 7.48 (s, 1H), 7.22 (d, *J* = 2.5 Hz, 1H), 7.12 (dd, *J* = 2.5 Hz, 8.8 Hz, 1H), 3.93 (s, 3H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.1, 165.0, 155.8, 154.8, 140.6, 131.7, 118.2, 114.1, 110.6, 101.0, 97.8, 56.5;
IR (cm^{-1})**:** 3159, 3092, 2918, 1784, 1723, 1603, 1516, 1458, 1396, 1361, 1264, 1250, 1156, 1125, 1055, 1014, 989, 918, 857, 822, 759, 714, 643;
HRMS (ESI) *m/z* calcd for $C_{12}H_9N_2O_5$ $[M+H]^+$: 261.0506; found: 261.0508.

3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-1,2,3-oxadiazol-3-ium-5-olate (7)



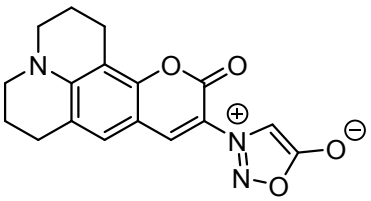
$C_{15}H_{15}N_3O_4$

MW: 301 $g.mol^{-1}$

Compound **7** was obtained in 85% yield, 51 mg (red solid) from compound **S3** (38 mg, 0.24 mmol) using general procedure **D** and purified by column chromatography (Toluene/EtOAc: 8/2).

MP: 221–223 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.61 (s, 1H), 7.63 (d, *J* = 9.1 Hz, 1H), 7.43 (s, 1H), 6.87 (dd, *J* = 2.5, 9.1 Hz, 1H), 6.70 (d, *J* = 2.5 Hz, 1H), 3.50 (q, *J* = 7.1 Hz, 4H), 1.15 (t, *J* = 7.1 Hz, 6H) ;
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.1, 158.7, 155.2, 152.8, 140.1, 131.6, 113.2, 110.6, 105.7, 97.3, 96.4, 44.4 (2C), 12.3 (2C);
IR (cm^{-1}): 3197, 3045, 2962, 2933, 1749, 1716, 1612, 1581, 1511, 1439, 1382, 1340, 1254, 1183, 1130, 1073, 956, 923, 824, 747, 703, 671;
HRMS (ESI) *m/z* calcd for $C_{15}H_{16}N_3O_4$ $[M+H]^+$: 302.1135; found: 302.1136.

3-(11-oxo-2,3,6,7-tetrahydro-1H,5H,11H-pyrano[2,3-f]pyrido[3,2,1-ij]quinolin-10-yl)-1,2,3-oxadiazol-3-ium-5-olate (8)



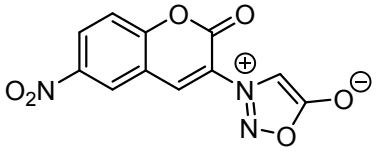
$C_{17}H_{15}N_3O_4$

MW: 325 $g.mol^{-1}$

Compound **8** was obtained in 84% yield, 33 mg (red solid) from compound **S3** (19 mg, 0.12 mmol) using general procedure **D** and purified by column chromatography (Heptane/EtOAc: 8/2).

MP: 243–245 °C;
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.17 (s, 1H), 7.34 (s, 1H), 6.98 (s, 1H), 3.36 (m, 4H), 2.88 (t br., *J* = 6.5 Hz, 2H), 2.77 (t br., *J* = 6.5 Hz, 2H), 2.04–1.97 (m, 4 H);
The product was not soluble enough to perform ^{13}C NMR
IR (cm^{-1}): 3197, 30444, 2950, 2925, 2851, 1735, 1696, 1618, 1579, 1481, 1459, 1442, 1432, 1373, 1323, 1279, 1185, 1152, 1042, 956, 819, 744, 721, 688;
HRMS (ESI) *m/z* calcd for $C_{17}H_{16}N_3O_4$ $[M+H]^+$: 326.1136; found: 326.1140.

3-(6-nitro-2-oxo-2H-chromen-3-yl)-1,2,3-oxadiazol-3-ium-5-olate (9)



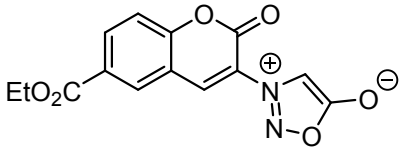
$C_{11}H_5N_3O_6$

MW: 275 g.mol^{-1}

Compound **9** was obtained in 21% yield, 17 mg (yellow solid) from compound **13** (130 mg, 0.9 mmol) using general procedure **C** and purified by column chromatography (Toluene/EtOAc: 8/2).

MP: 219–221 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.03 (s, 1H), 8.93 (d, *J* = 2.8 Hz, 1H), 8.60 (dd, *J* = 2.8, 9.2 Hz, 1H), 7.84 (d, *J* = 9.2 Hz, 1H), 7.52 (s, 1H) ;
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.8, 156.8, 153.8, 144.2, 138.9, 129.0, 126.1, 123.4, 118.2, 117.7, 97.9;
IR (cm^{-1})**:** 3146, 3099, 3047, 1873, 1764 1734, 1611, 1522, 1474, 1385, 1342, 1313, 1263, 1249, 1169, 1130, 1113, 1059, 998, 956, 935, 855, 838, 756, 732, 665;
HRMS (ESI) *m/z* calcd for $C_{11}H_6N_3O_6$ $[M+H]^+$: 276.0251; found: 276.0251.

3-(6-(ethoxycarbonyl)-2-oxo-2H-chromen-3-yl)-1,2,3-oxadiazol-3-ium-5-olate (10)



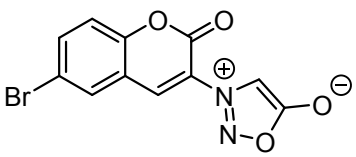
$C_{14}H_{10}N_2O_6$

MW: 302 g.mol^{-1}

Compound **10** was obtained in 49% yield, 44 mg (beige solid) from compound **13** (130 mg, 0.9 mmol) using general procedure **C** and purified by column chromatography (Toluene/EtOAc: 6/4).

MP: 184–186 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.03 (s, 1H), 8.59 (d, *J* = 2.1 Hz, 1H) 8.32 (dd, *J* = 2.1, 8.7 Hz, 1H), 7.71 (d, *J* = 8.7 Hz, 1H), 7.50 (s, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 1.37 (t, *J* = 7.1 Hz, 3H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.9, 164.3, 156.1, 154.2, 139.9, 134.8, 131.8, 127.1, 122.5, 117.4, 117.3, 97.9, 61.4, 14.2;
IR (cm^{-1})**:** 3646, 3135, 3060, 2930, 1858, 1779, 1753, 1707, 1639, 1614, 1524, 1475, 1367, 1289, 1273, 1244, 1196, 1159, 1101, 1060, 984, 953, 833, 763, 729, 668;
HRMS (ESI) *m/z* calcd for $C_{14}H_{11}N_2O_6$ $[M+H]^+$: 303.0612; found: 303.0613.

3-(6-bromo-2-oxo-2H-chromen-3-yl)-1,2,3-oxadiazol-3-ium-5-olate (11)



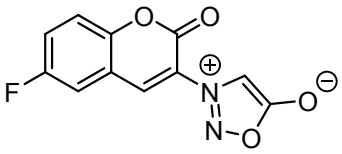
$C_{11}H_5BrN_2O_4$

MW: 309 g.mol^{-1}

Compound **11** was obtained in 66% yield, 61 mg (yellow solid) from compound **13** (130 mg, 0.9 mmol) using general procedure **C** and purified by column chromatography (Heptane/EtOAc: 8/2).

MP: 164–165 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) : 8.91 (s, 1H), 7.98 (d, *J* = 1.8 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 1 H), 7.73 (dd, J = 1.8, 8.4 Hz, 1H), 7.50 (s, 1H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.9, 154.1, 153.7, 139.8, 131.7, 128.9, 128.0, 121.8, 119.8, 116.5, 97.9;
IR (cm^{-1})**:** 3188, 3105, 3063, 1869, 1732, 1627, 1597, 1556, 1486, 1398, 1270, 1207, 1167, 1139, 1114, 1063, 991, 939, 923, 862, 792, 755, 727, 677;
HRMS (ESI) *m/z* calcd for $C_{11}H_6BrN_2O_4$ $[M+H]^+$ (^{79}Br): 308.9505; found: 308.9508.

3-(6-fluoro-2-oxo-2H-chromen-3-yl)-1,2,3-oxadiazol-3-ium-5-olate (12)



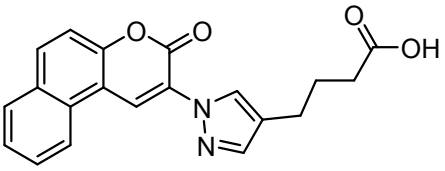
$C_{11}H_5FN_2O_4$

MW: 248 $g.mol^{-1}$

Compound **12** was obtained in 78% yield, 58 mg (yellow solid) from compound **S3** (63 mg, 0.9 mmol) using general procedure **D** and purified by column chromatography (Heptane/EtOAc: 8/2).

MP: 159–161 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.86 (s, 1H), 7.84 (dd, *J* = 3.0, 8.3 Hz, 1H), 7.76–7.67 (m, 2H), 7.51 (s, 1H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.9, 158.4 (d, *J* = 243 Hz), 154.3, 149.9, 139.3 (d, *J* = 2.3 Hz), 122.7, 122.2 (d, *J* = 25 Hz), 118.9 (d, *J* = 8.8 Hz), 118.2 (d, *J* = 11 Hz), 115.4 (d, *J* = 25 Hz), 98.0;
IR (cm^{-1})**:** 3182, 3068, 2916, 1876, 1718, 1617, 1573, 1486, 1460, 1421, 1384, 1318, 1262, 1201, 1167, 1126, 1112, 1064, 998, 955, 937, 889, 821, 756, 716, 692;
HRMS (ESI) *m/z* calcd for $C_{11}H_6FN_2O_4$ $[M+H]^+$: 249.0306; found: 249.0310.

4-(1-(2-oxo-2H-benzo[h]chromen-3-yl)-1H-pyrazole-4-yl)butanoic acid (14)



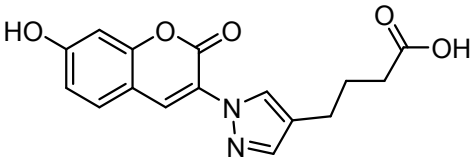
$C_{20}H_{16}N_2O_4$

MW: 348 $g.mol^{-1}$

Compound **14** was obtained in 65% yield, 34 mg (brown powder) from compound **4** (42 mg, 0.15 mmol) and 5-hexynoic acid (20 µL, 0.18 mmol) using general procedure **C** and triturated in Et_2O.

MP: 225–227 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) : 9.09 (s, 1H), 8.55 (d, *J* = 8.5 Hz, 1H), 8.41 (s, 1H), 8.19 (d, *J* = 9.1 Hz, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 7.76–7.72 (m, 2H), 7.66–7.62 (m, 2H), 2.54 (t, *J* = 7.5 Hz, 2H), 2.28 (t, *J* = 7.5 Hz, 2H), 1.82 (quint, *J* = 7.5 Hz, 2H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 174.3, 156.0, 150.8, 141.5, 132.5, 130.1, 128.9, 128.9, 128.7, 128.4, 126.3, 125.6, 125.4, 122.5, 122.4, 116.2, 112.9, 33.2, 25.6, 22.9;
IR (cm^{-1}): 2922, 1714, 16.29, 1609, 1566, 1518, 1444, 1405, 1389, 1337, 1322, 1252, 1189, 1114, 1015, 997, 942, 899, 815, 742;
HRMS (ESI) *m/z* calcd for $C_{20}H_{17}N_2O_4$ $[M+H]^+$: 349.1183; found: 349.1185.

4-(1-(7-hydroxy-2-oxo-2H-chromen-3-yl)-1H-pyrazole-4-yl)butanoic acid (15)



$C_{16}H_{14}N_2O_5$

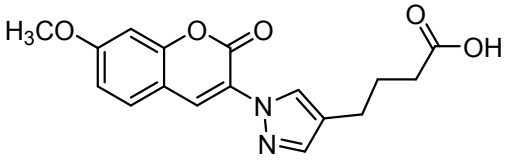
MW: 314 g.mol^{-1}

Yield: 66%

Compound **15** was obtained in 66% yield, 31 mg (orange solid) from compound **5** (37 mg, 0.15 mmol) and 5-hexynoic acid (20 µL, 0.18 mmol) using general procedure **E** and triturated in Et_2O.

MP: 207–209 °C;
^{1}H NMR (400 MHz, MeOD-d_4, δ ppm): 8.24 (s, 1H), 8.22 (s, 1H), 7.60 (s, 1H), 7.56 (d, *J* = 8.6 Hz, 1H), 6.86 (dd, *J* = 2.3, 8.6 Hz, 1H), 6.79 (d, *J* = 2.3 Hz, 1H), 2.60 (t, *J* = 7.5 Hz, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 1.91 (quint , *J* = 7.5 Hz, 2H);
^{13}C NMR (100 MHz, MeOD-d_4, δ ppm): 177.4, 163.2, 158.9, 155.6, 133.6, 131.1 (2C), 123.9, 115.4 (2C), 112.7, 103.4 (2C), 34.4, 27.4, 24.5;
IR (cm^{-1}): 3126, 2922, 1720, 1697, 1665, 1608, 1580, 1509, 1472, 1400, 1319, 1274, 1245, 1174, 1125, 1183, 1007, 979, 917, 858, 807, 758;
HRMS (ESI) *m/z* calcd for $C_{16}H_{15}N_2O_5$ $[M+H]^+$: 315.0975; found: 315.0980.

4-(1-(7-methoxy-2-oxo-2H-chromen-3-yl)-1H-pyrazole-4-yl)butanoic acid (16)



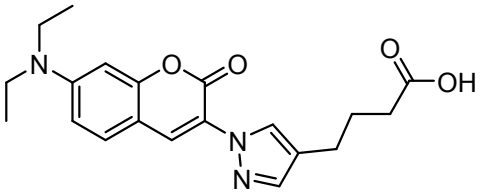
$C_{17}H_{16}N_2O_5$

MW: 328 g.mol^{-1}

Compound **16** was obtained in 65% yield, 32 mg (beige powder) from compound **6** (39 mg, 0.15 mmol) and 5-hexynoic acid (20 µL, 0.18 mmol) using general procedure **E** and purified by column chromatography (Heptane/EtOAc: 8/2) then triturated in Et_2O.

MP: 159–161 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.41 (s, 1H), 8.27 (s, 1H), 7.78 (d, *J* = 8.7 Hz, 1H), 7.65 (s, 1H), 7.1 (d, *J* = 2.4 Hz, 1H), 7.02 (dd, *J* = 2.4, 8.7 Hz, 1H), 3.87 (s, 3H), 2.52–2.49 (m, 2H, under DMSO signal), 2.26 (t, *J* = 7.5 Hz, 2H), 1.78 (quint, *J* = 7.5 Hz, 2H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 174.3, 162.2, 156.4, 153.3, 140.9, 130.7, 129.8, 128.7, 123.0, 122.1, 113.3, 112.1, 100.4, 56.0, 33.2, 25.7, 22.9;
IR (cm^{-1}): 3076, 2919, 1730, 1702, 1618, 1518, 1443, 1401, 1357, 1315, 1284, 1236, 1176, 1163, 1141, 1031, 1023, 982, 865, 824, 800, 763;
HRMS (ESI) *m/z* calcd for $C_{11}H_{17}N_2O_4$ $[M+H]^+$: 329.1132; found: 329.1136.

4-(1-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-1H-pyrazole-4-yl)butanoic acid (17)



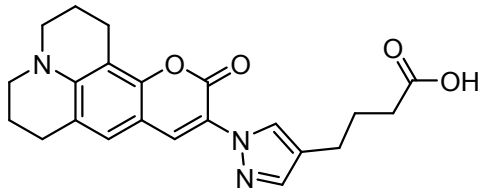
$C_{20}H_{23}N_3O_4$

MW: 369 g.mol^{-1}

Compound **17** was obtained in 31% yield, 17 mg (orange powder) from compound **7** (45 mg, 0.15 mmol) and 5-hexynoic acid (20 µL, 0.18 mmol) using general procedure **E** and purified by column chromatography (Heptane/EtOAc: 8/2) then triturated in Et_2O.

MP: 159–161 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.26 (s, 1H), 8.19 (s, 1H), 7.59–7.57 (m, 2H), 6.77 (dd, *J* = 2.2, 8.9 Hz, 1H), 6.62 (d, *J* = 2.2 Hz, 1H), 3.44 (q, *J* = 7.0 Hz, 4H), 2.50–2.47 (m, 2H, under DMSO peak), 2.25 (t, *J* = 7.4 Hz, 2H), 1.78 (quint., *J* = 7.4 Hz, 2H), 1.13 (t, *J* = 7.0 Hz, 6H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 174.3, 157.0, 154.5, 150.4, 140.1, 132.4, 129.7, 128.5, 121.5, 119.7, 109.7, 107.0, 96.3, 44.1 (2C), 33.2, 25.7, 22.9, 12.3 (2C);
IR (cm^{-1}): 2970, 2927, 1688, 1619, 1602, 1522, 1421, 1400, 1385, 1352, 1261, 1234, 1205, 1130, 1073, 969, 925, 849, 821, 796, 755;
HRMS (ESI) *m/z* calcd for $C_{20}H_{24}N_3O_4$ $[M+H]^+$: 370.1761; found: 370.1762.

4-(1-(11-oxo-2,3,6,7-tetrahydro-1H,5H,11H-pyrano[2,3-f]pyrido[3,2,1-ij]quinolin-10-yl)-1H-pyrazole-4-yl)butanoic acid (Pyr1)



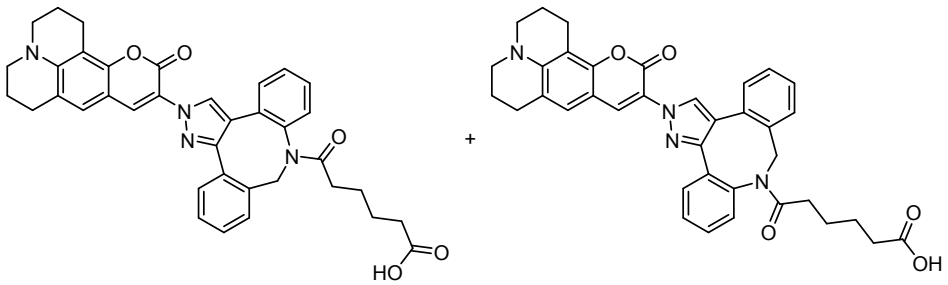
$C_{22}H_{23}N_3O_4$

MW: 394 $g.mol^{-1}$

Compound **18** was obtained in 52% yield, 31 mg (yellow powder) from compound **8** (49 mg, 0.15 mmol) and 5-hexynoic acid (20 µL, 0.18 mmol) using general procedure **E** and purified by column chromatography (Heptane/EtOAc : 8/2).

MP: 165–167 °C;
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.17 (s, 1H), 8.12 (s, 1H), 7.56 (s, 1H), 7.13 (s, 1H), 3.26 (m, 4H), 2.76 (br.t, *J* = 6.4 Hz, 2H), 2.70 (br.t, *J* = 6.4 Hz, 2H), 2.48–2.46 (m, 2H, under DMSO peak), 2.24 (t, *J* = 7.3 Hz, 2H), 1.93–1.83 (m, 4H), 1.77 (quint, *J* = 7.3 Hz, 2H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 174.4, 157.0, 149.4, 145.7, 140.0, 132.6, 128.4, 125.5, 121.5, 119.1, 119.0, 106.9, 105.2, 49.3, 48.8, 33.3, 26.9, 25.8, 23.0, 20.8, 19.9, 19.8;
IR (cm^{-1}): 2941, 2847, 1696, 1620, 1605, 1565, 1516, 1435, 1392, 1322, 1309, 1286, 1244, 1205, 1178, 1153, 1045, 962, 927, 756;
HRMS (ESI) *m/z* calcd for $C_{22}H_{24}N_3O_4$ $[M+H]^+$: 394.1761; found: 394.1759.

6-oxo-6-(2-(11-oxo-2,3,6,7-tetrahydro-1H,5H,11H-pyrano[2,3-f]pyrido[3,2,1-ij]quinolin-10-yl)-2,9-dihydro-8H-dibenzo[b,f]pyrazoleo[4,3-d]azocin-8-yl)hexanoic acid (Pyr2)



$C_{37}H_{34}N_4O_5$

MW: 615 $g.mol^{-1}$

To a solution of **8** (6.24 mg, 0.019 mmol) in DMF (0.1 M) was added DIBAC (6.40 mg, 0.019 mmol). The mixture was stirred at room temperature overnight. The solution was evaporated under reduced pressure. Compounds **19a** and **19b** were obtained as a mixture in quantitative yield (11.6 mg, 0.019 mmol).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.66 (s, 1H), 8.33 (s, 1H), 8.18 (s, 1H), 7.62–7.59 (m, 1H), 7.55–7.42 (m, 5H), 7.40–7.29 (m, 8H), 7.25–7.22 (m, 2H), 7.21–7.16 (m, 4H), 6.15 (d, *J* = 16.6 Hz, 1H), 6.05 (d, *J* = 16.6 Hz, 1H), 4.45 (d, *J* = 16.6 Hz, 1H), 4.44 (d, *J* = 16.6 Hz, 1H), 3.32–3.28 (m, 5H), 2.94 (t, *J* = 6.2 Hz, 3H), 2.81–2.75 (m, 3H), 2.17–2.08 (m, 8H), 2.04–1.96 (m, 8H), 1.86–1.83 (m, 4H), 1.48–1.25 (m, 10H);

*The ^{1}H NMR signals of compounds **19a** and **19b** are undistinguishable.*

IR (cm^{-1}): 2930, 2858, 1702, 1654, 1619, 1602, 1518, 1433, 1390, 1307, 1287, 1195, 1167, 1018, 975; 757, 730;

LCMS (ESI) *m/z*: $C_{37}H_{35}N_4O_5$ $[M+H]^+$ 616.

III. Screening procedure

Reactions were carried out in 96-well plates according to **Figure S1**.

- 1 eq. of coumarin sydnone **1–12** (40 µL of a 40 mM DMF solution),
- 1 eq. of alkyne **A–I** (40 µL of 40 mM DMF solution),
- 40 µL of solution Cu/BPDS/Base in water (a mixture of 0.2 eq. of $CuSO_4.5H_2O$, 0.2 eq. of BPDS and 1 eq. of triethanolamine respectively 8 mM/8 mM/40 mM) and 2 eq. of sodium ascorbate (40 µL of a 80 mM water solution) for lines **A–G** + 1 line
- 80 µL of water for lines **H–I** + 1 line

Each well corresponds to one single combination of coumarin sydnone and alkyne. The reaction plate was sealed with an aluminum foil and incubated at 60 °C under orbital stirring overnight.

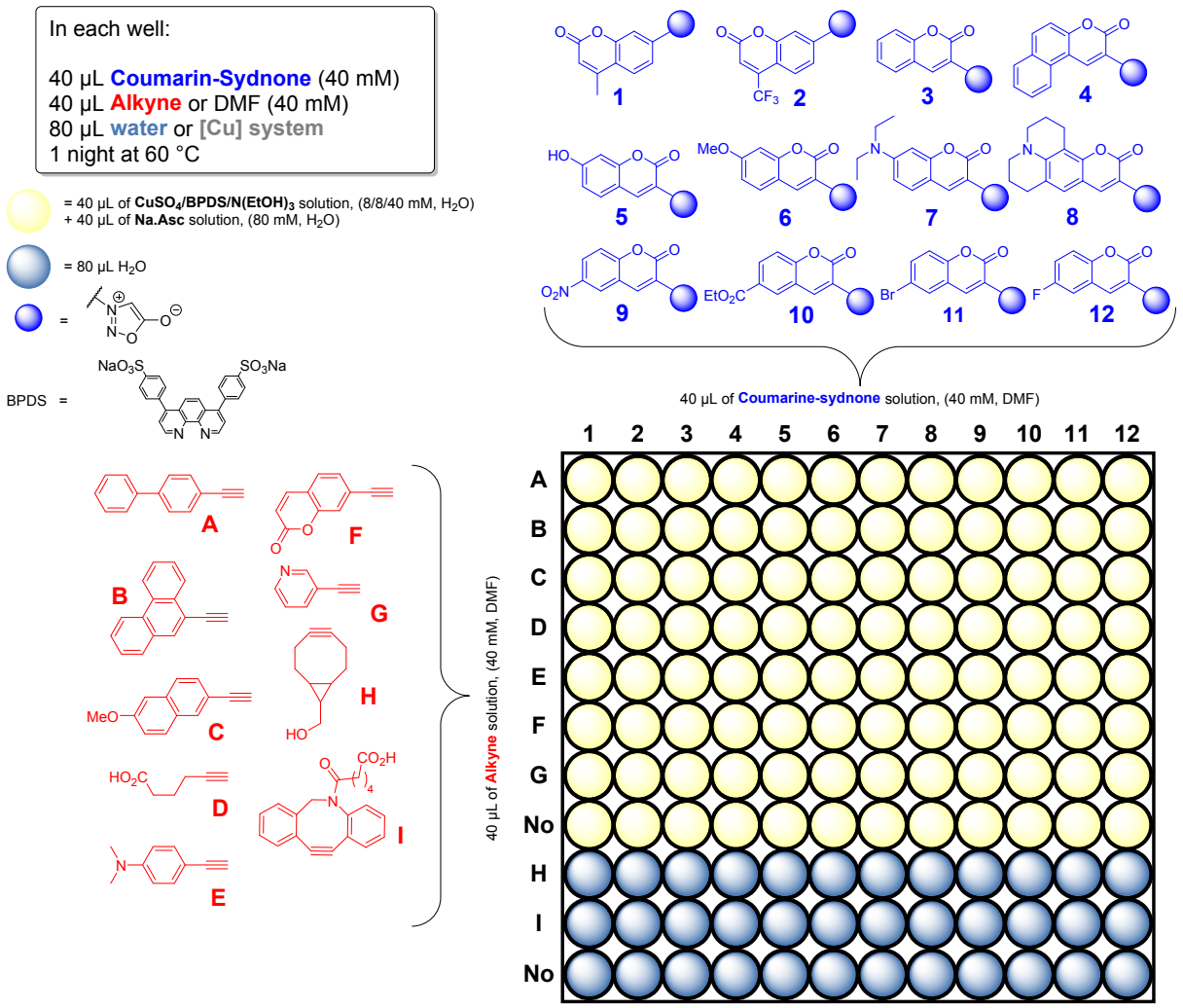


Figure S1 : Screening plate

Then 20 µL of solution of each well were transferred into plate containing 180 µL of a mixture of DMF/water (15/85) in each well. The plate was stirred for 10 minutes and the second dilution of a 20 µL aliquot was carried out in 180 µL of a mixture of DMF/water (15/85) in each well in a black plate with clear bottom. Absorbance was measured for each well. Then 100 µL solution of each well were transferred into black plate with black bottom and fluorescence was measured (**Figure S2**).

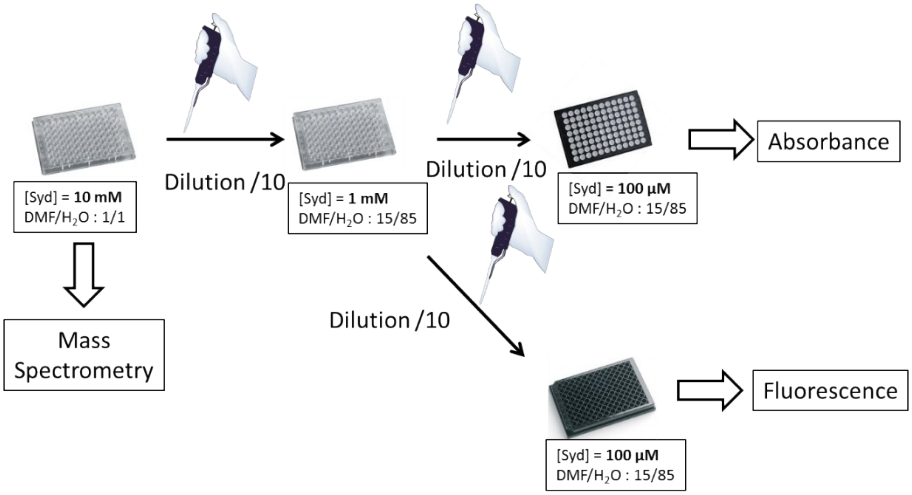


Figure S2 : Procedure for the analysis of the screening plates

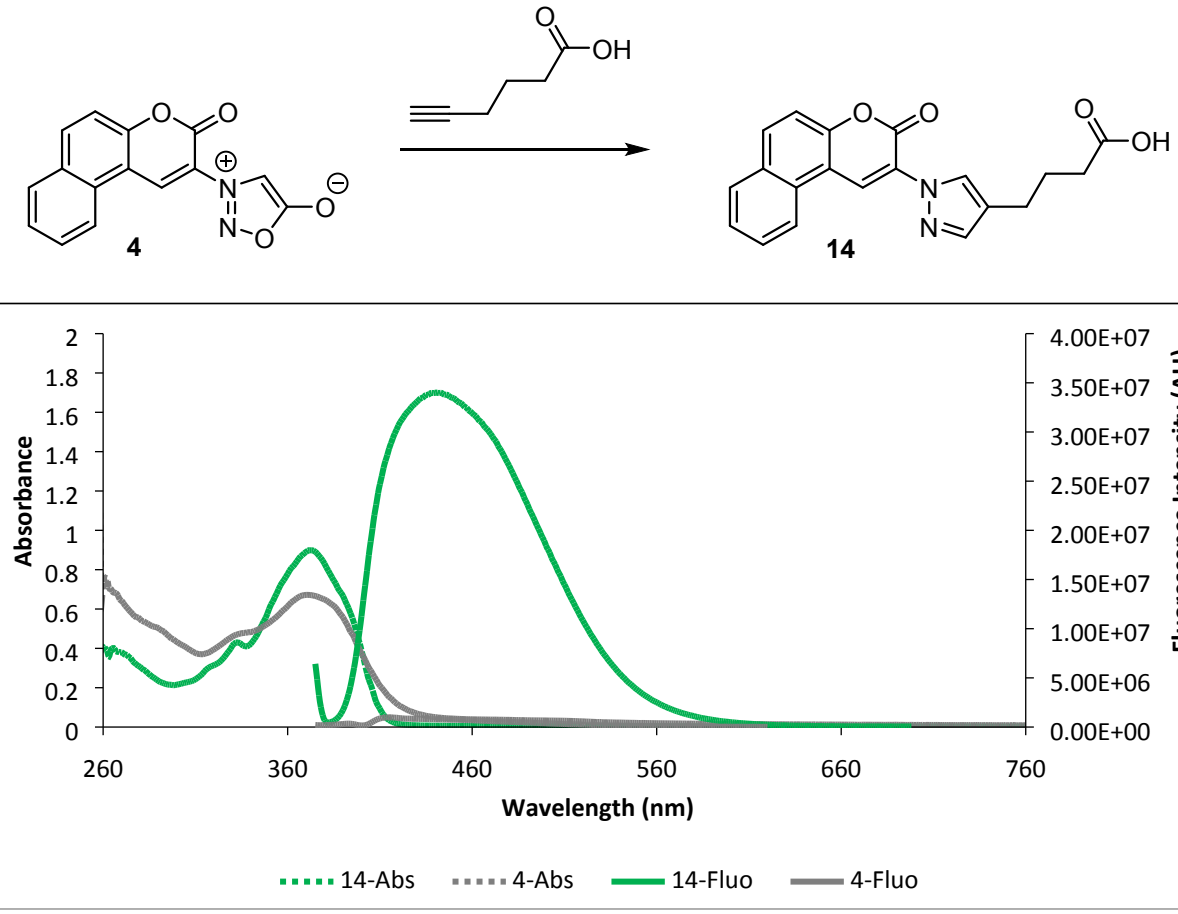
IV. Optical properties

Absorbance an Fluorescence studies :

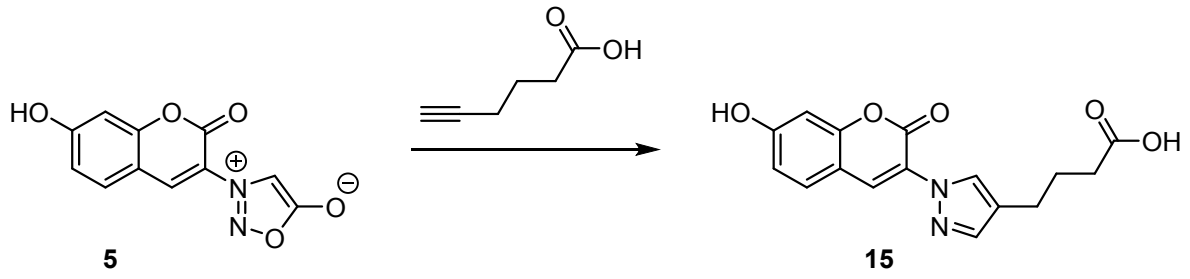
Absorbances were measured on three solutions (50µM, 25µM and 5µM) of compounds in PBS pH 7.4. The molar extinction coefficients were determined by plotting absorbance values versus the solution concentrations and analyzing by linear regression. The molar extinction corresponds to the determined slope.

Fluorescence spectra were measured with excitation at the maximum of absorbance of the pyrazoles.

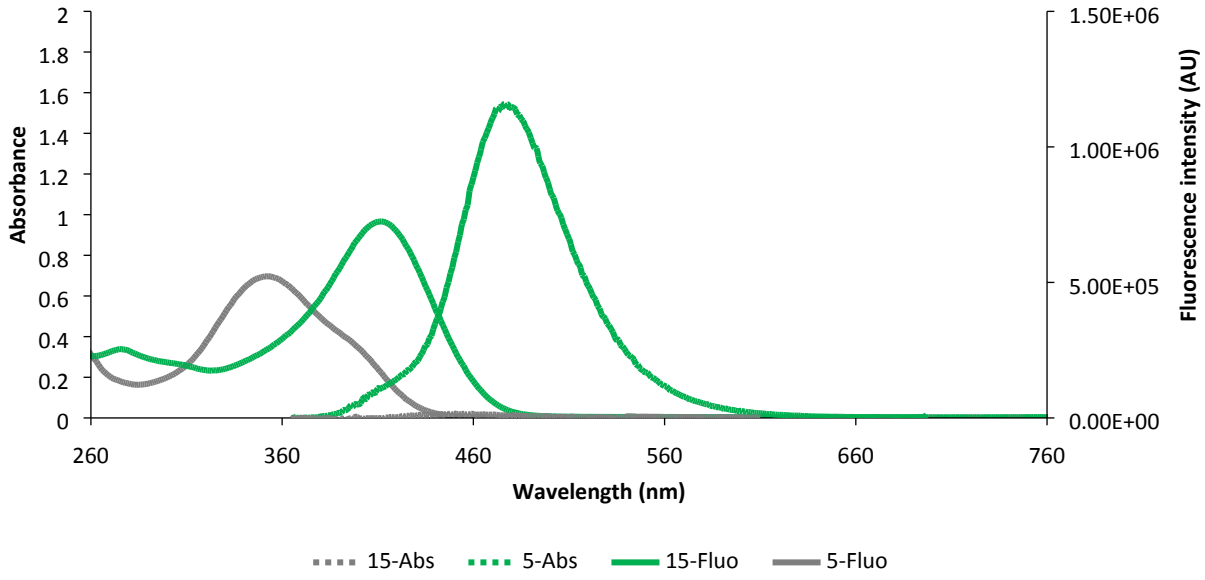
Table S1 : Absorbance and fluorescence curves of sydnones **4-8** and pyrazoles **14–17, Pyr1–2**



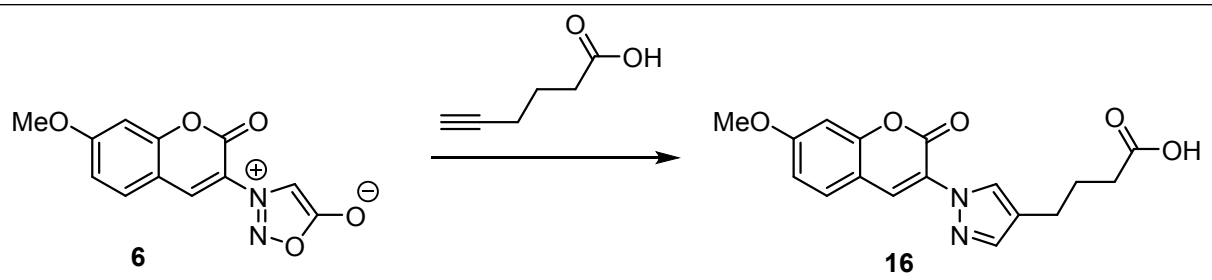
Superposition of absorbance curves of 4 and 14 and fluorescence curves of 4 and 14 excited at the maximum of absorbance of the 14 (λ_{exc} = 372 nm). Spectra of fluorescence were registered at 0.5 µM in PBS pH 7.4, absorbance spectra represented compounds at 50 µM.

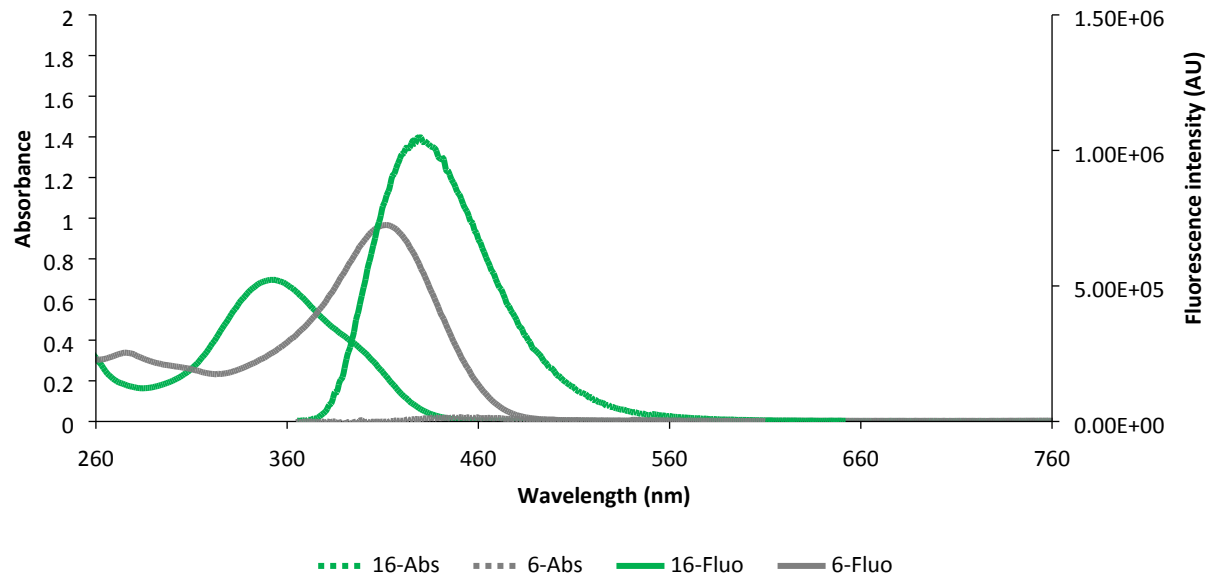


5 **15**

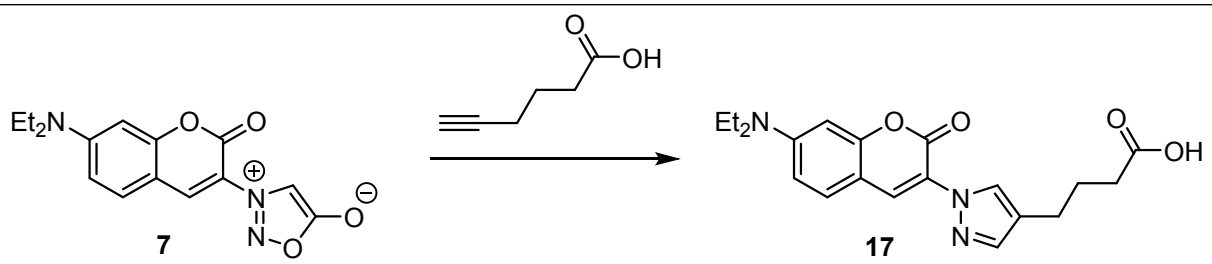


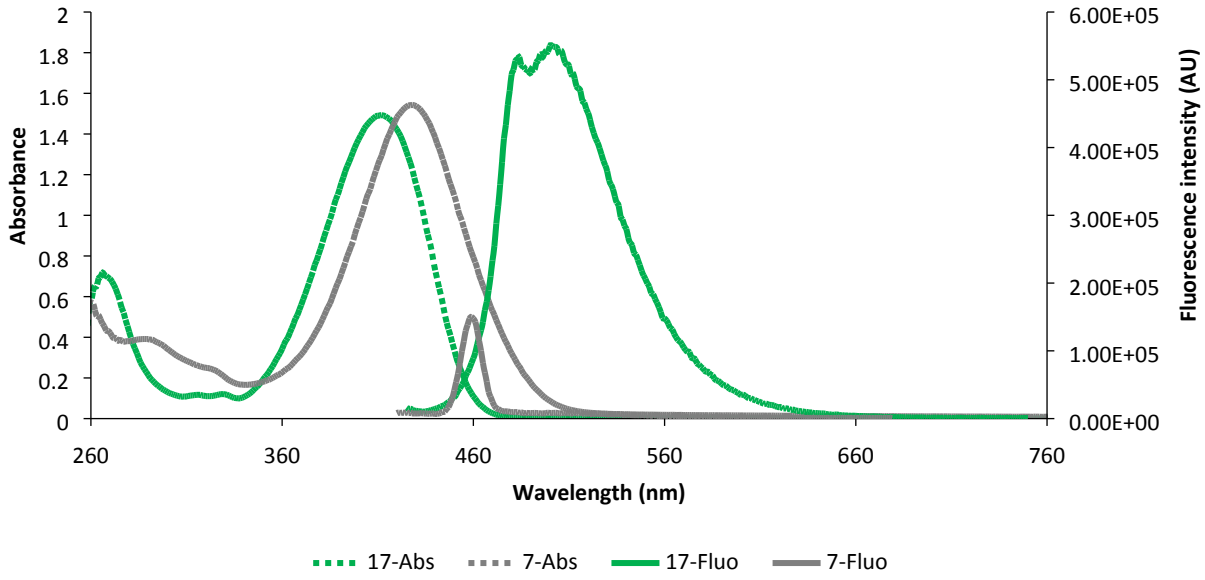
Superposition of absorbance curves of 5 and 15 and fluorescence curves of 5 and 15 excited at the maximum of absorbance of the 15 (λ_{exc} = 355 nm). Spectra of fluorescence were registered at 10 nM in PBS pH 7.4, absorbance spectra represented compounds at 50 µM.



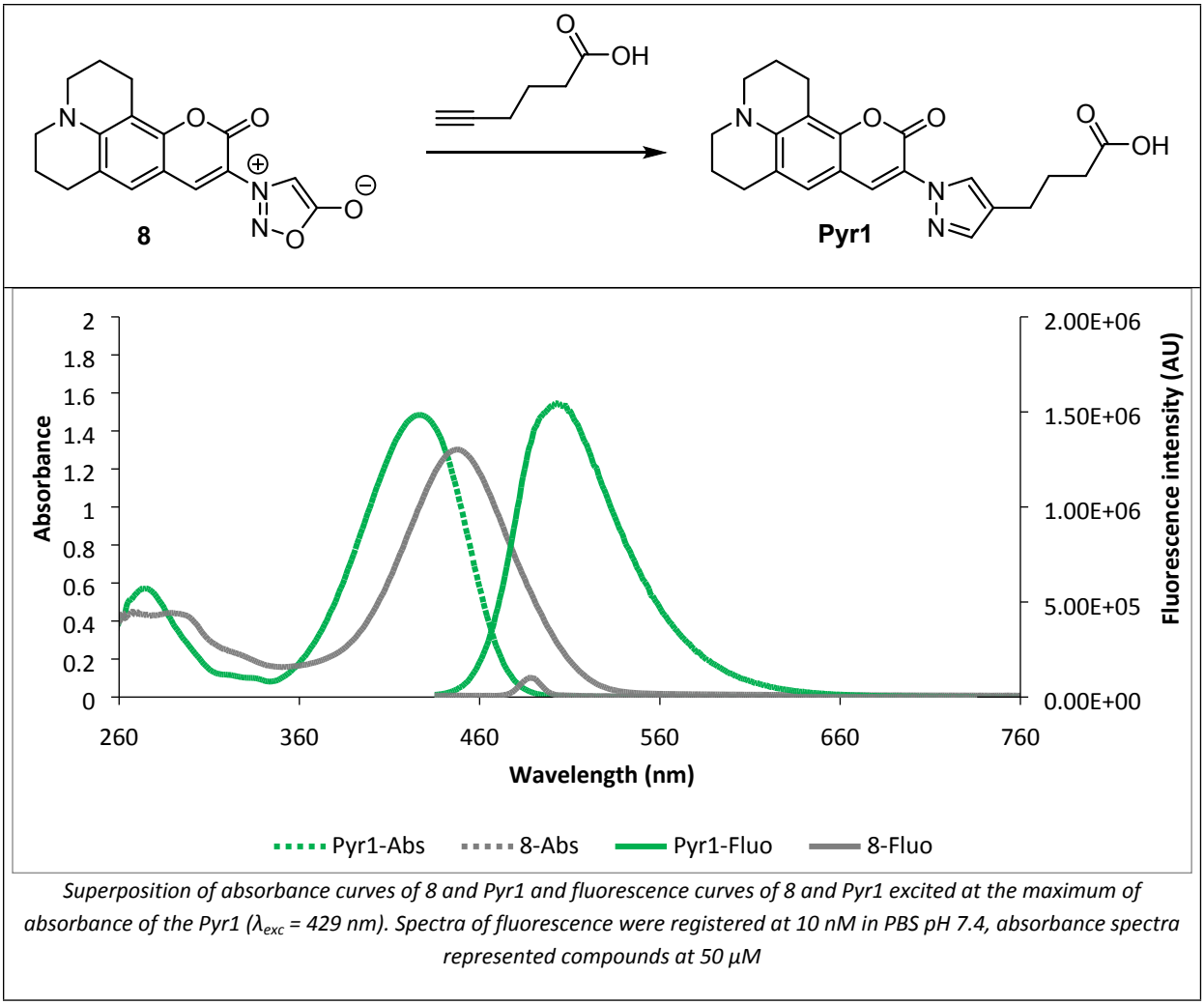


Superposition of absorbance curves of 6 and 16 and fluorescence curves of 6 and 16 excited at the maximum of absorbance of the 16 (λ_{exc} = 353 nm). Spectra of fluorescence were registered at 10 nM in PBS pH 7.4, absorbance spectra represented compounds at 50 µM.

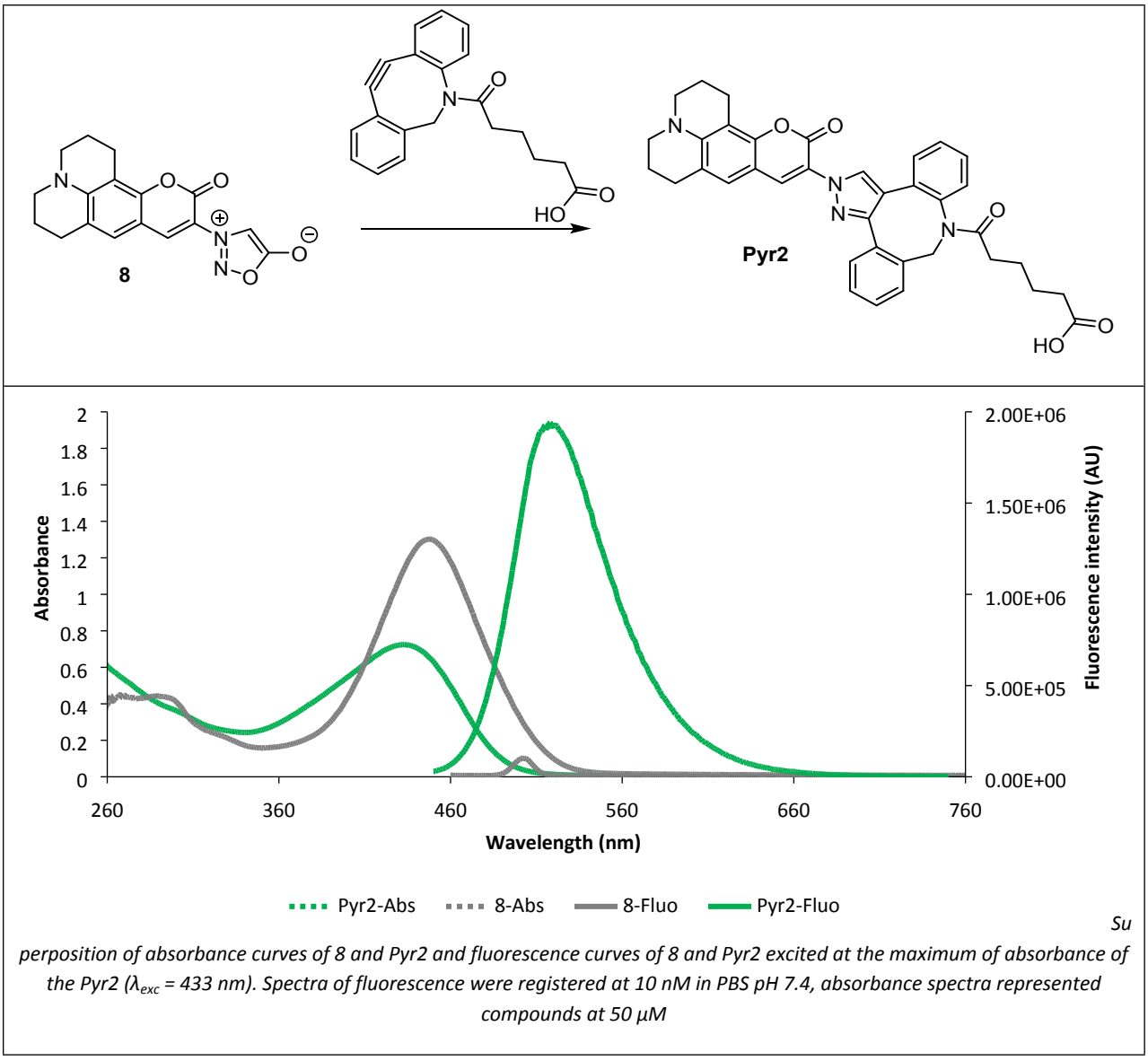




Superposition of absorbance curves of 7 and 17 and fluorescence curves of 7 and 17 excited at the maximum of absorbance of the 17 (λ_{exc} = 412 nm). Spectra of fluorescence were registered at 10 nM in PBS pH 7.4, absorbance spectra represented compounds at 50 µM .



Superposition of absorbance curves of 8 and Pyr1 and fluorescence curves of 8 and Pyr1 excited at the maximum of absorbance of the Pyr1 (λ_{exc} = 429 nm). Spectra of fluorescence were registered at 10 nM in PBS pH 7.4, absorbance spectra represented compounds at 50 µM



Superposition of absorbance curves of 8 and Pyr2 and fluorescence curves of 8 and Pyr2 excited at the maximum of absorbance of the Pyr2 (λ_{exc} = 433 nm). Spectra of fluorescence were registered at 10 nM in PBS pH 7.4, absorbance spectra represented compounds at 50 µM

Quantum yield calculations :

The quantum yield of each probes was calculated in a relative way[ii] with quinine sulfate in a solution of H_2SO_4 0,1 M as a standard. The quantum yield is defined by the following equation:

$$\Phi_x = \Phi_{ref} \cdot \frac{Aref}{Ax} \cdot \frac{Fx}{Fref} \cdot \left(\frac{nx}{nref}\right)^2 \cdot \frac{Dx}{Dref}$$

Entry	Item	Sample	Symbol
1	Quantum yield	Quinine sulfate	Φ_{ref}
2	Absorbance for λ_{max}	Quinine sulfate Coumarin-pyrazole	*Aref* *Ax*
3	Emmision spectra surface	Quinine sulfate Coumarin-pyrazole	*Fref* *Fx*
4	Reflexion index of solvant	Quinine sulfate Coumarin-pyrazole	*nref* *nx*
5	Dilution ratio	Quinine sulfate Coumarin-pyrazole	*Dref* *Dx*

Table S2 : Method for quantum yield calculation

Turn-on

The turn-on values were determined as a ratio between the intensities of pyrazole and sydnone at the maximum of pyrazole emission, using excitation at the maximum of pyrazole absorbance.

Summary of optical properties:

		Sydnone				Pyrazole					
Entry	**Sydnone/Pyrazole**	λ_{ex}	λ_{em}	Φ_f	**ε**	λ_{ex}	λ_{em}	Φ_f	**ε**	**Brill.**	**Turn-on**
1	**4/14**	370	435	0,005	13800	372	441	0,33	18100	5918	38
2	**5/15**	433	516	0,006	26100	355	480	0,53	18100	9539	97
3	**6/16**	349	422	0,006	19100	353	430	0,38	22300	8429	25
4	**7/17**	428	525	0,029	31000	412	499	0,28	29900	8372	51
5	**8/Pyr1**	448	545	0,045	26200	429	520	0,32	29600	9531	164
6	**8/Pyr2**					433	515	0,57	14100	8037	131

Table S3 : Summary of optical properties for sydnones **4–8** and pyrazoles **14–17, Pyr1–2**

V. Kinetics

Measurment of the kinetic constant by HPLC

Reactions of sydnone **8** with DBCO was carried out in PBS/DMSO (90:10) mixtures at 100 µM concentration of **8** and 150 µM concentration of DBCO using the following procedure.
To 989 µL of phosphate buffered saline (PBS 1X) was added, 1 µL of the solution of internal standard (3-fluoro-4-nitrophenol, 100 mM in DMSO), 10 µL of the solution of **8** (10 mM in DMSO) and 1.5 µL of the solution of DBCO (100 mM in DMSO). The reaction mixture was injected in HPLC every 15 min and the conversion was followed by measuring the normalized sydnone peak area.

Second order reaction rate was determined by plotting $-\ln([A]/[B])/([A]_0 - [B]_0)$ versus time and analyzing by linear regression (**Equation S1**). Second order rate constant corresponds to the determined slope.

$$\frac{-\ln\left(\frac{[A]}{[B]}\right)}{[A]_0 - [B]_0} = kt + const$$

Equation S1. [A]—concentration of sydnone (M); [B]—concentration of DBCO (M); t—reaction time (s); k—reaction rate ($M^{-1} \cdot s^{-1}$)
Linear regression curves for the reactive sydnone **8** is illustrated in **Table S4**.

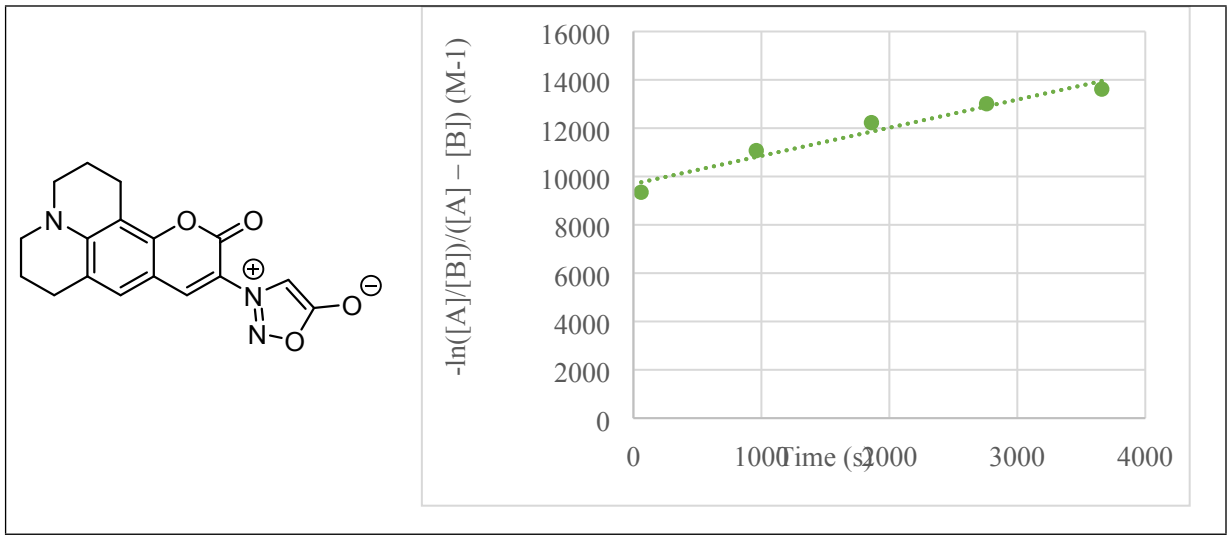


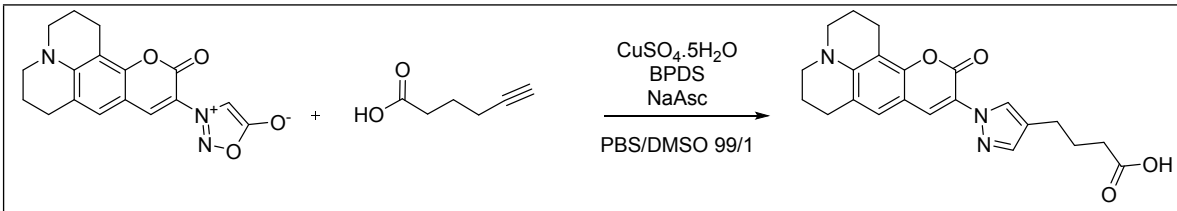
Table S4 : Linear regression curves showing $-\ln([A]/[B])/([A]_0 - [B]_0)$ plotted versus time

Reaction kinetics followed by fluorescence

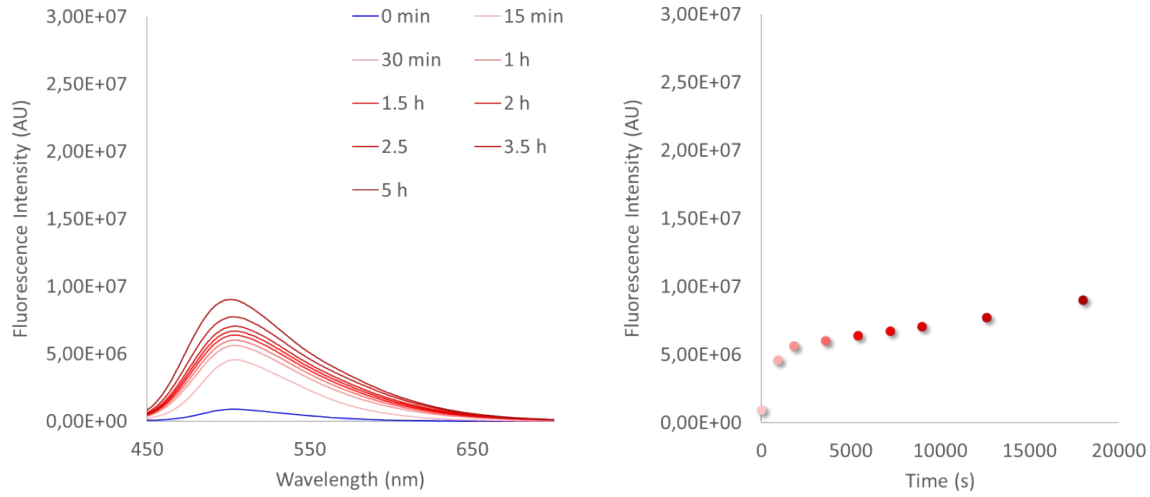
Reactions were carried out in a quartz cuvette. Fluorescence spectrum were registered with excitation at 433 nm, in PBS pH 7,4, containing 1% of DMSO.

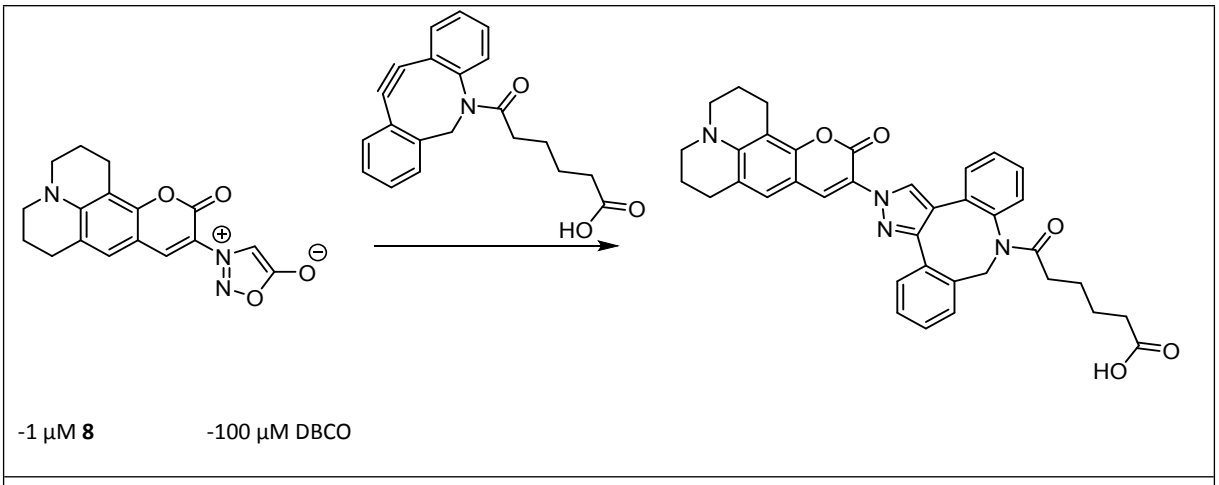
The reaction conditions and the measured spectra are summed up in **Table S5**

Table S5 : Monitoring of the reactions using fluorescence spectroscopy

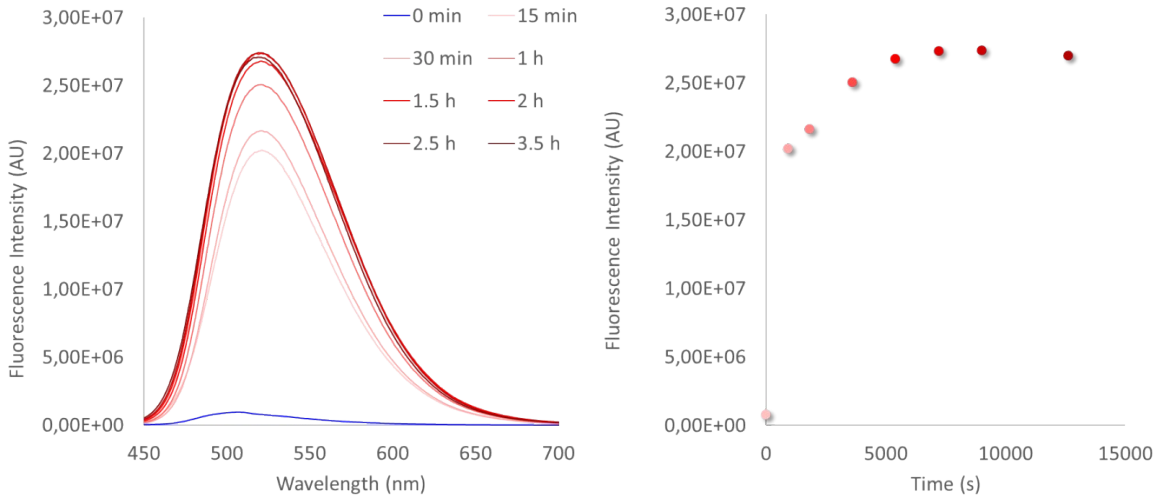


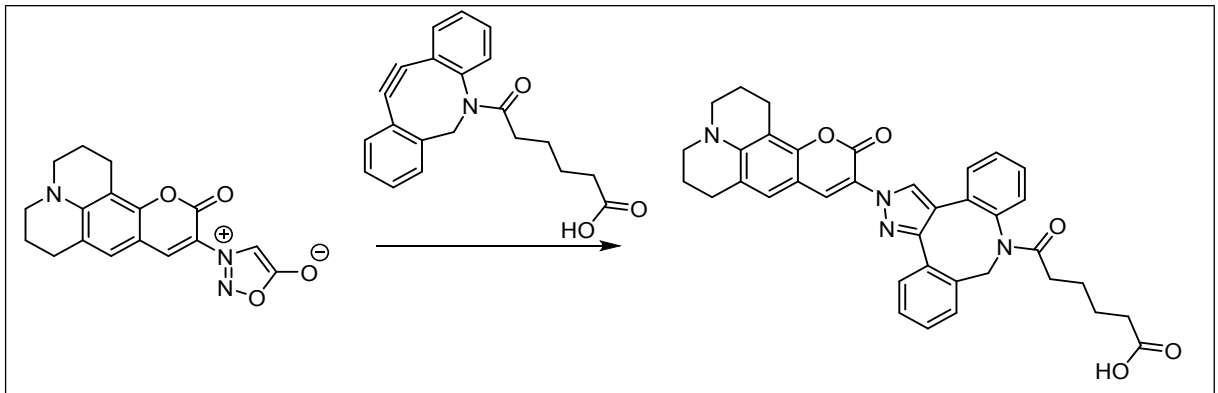
-1 µM **8** -100 µM Alkyne **D** -50 µM $CuSO_4.5H_2O$ -300 µM BPDS -2.5 mM Na. Asc



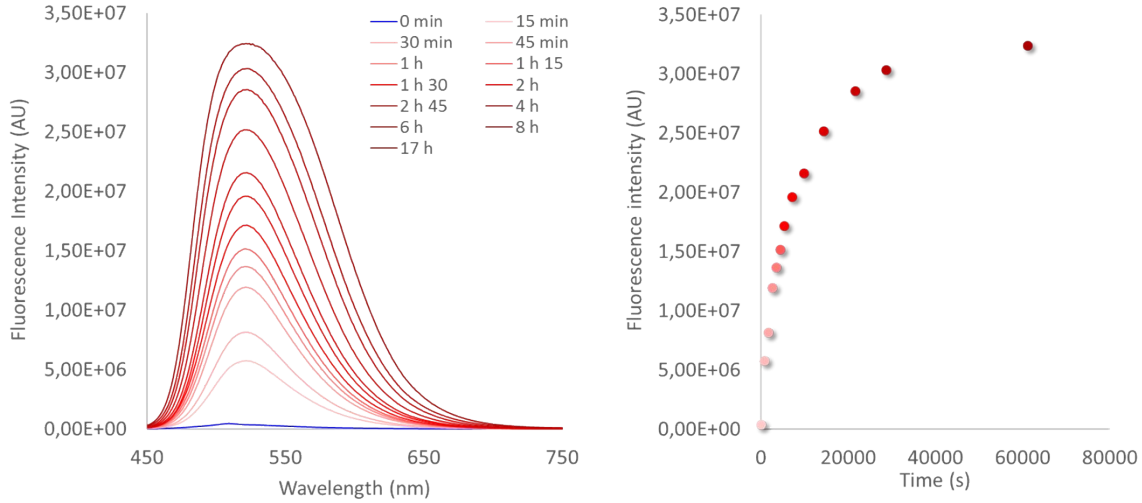


-1 µM **8** -100 µM DBCO





-1 µM **8** -10 µM DBCO



VI. Protein labeling

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) or a LAS4000 biomolecular imaginer 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 and at 515 nm for LAS4000 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. Proteins samples diluted with sinapinic acid matrix solution prepared at 10 mg/mL in H_2O/CH_3CN/TFA (70/30/0.1) were manually spotted on MALDI plate. MS spectra were recorded from crystallized samples using positive linear mode

B- Preparation and characterisation of MYO-I conjugate

Protocol

A solution of 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 (***DBCO-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-**I** sample contains modified Myoglobins bearing respectively one dibenzocyclooctyne (MYO-$\mathbf{I}_1$) or two dibenzocyclooctyne (MYO-$\mathbf{I}_2$) together with unmodified Myoglobin.

Characterisation

- *ESI-MS*

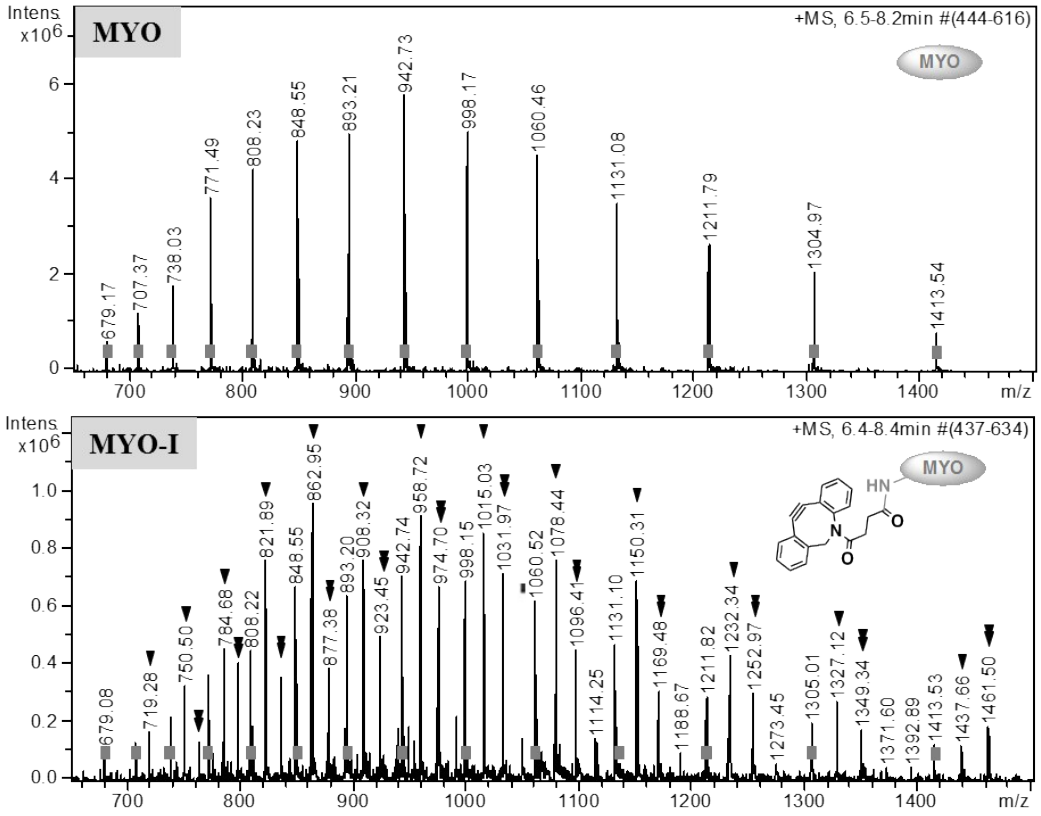


***Figure S3**. ESI-MS spectra of Myoglobin (up) and MYO-**I** samples (down). Grey squares refer to the set of multicharged states of unmodified Myoglobin MYO, black triangles to Myoglobin bearing one DBCO (MYO-**I_1**, panel down) and black double triangles to Myoglobin bearing two DBCO (MYO-**I_2**, panel down).*

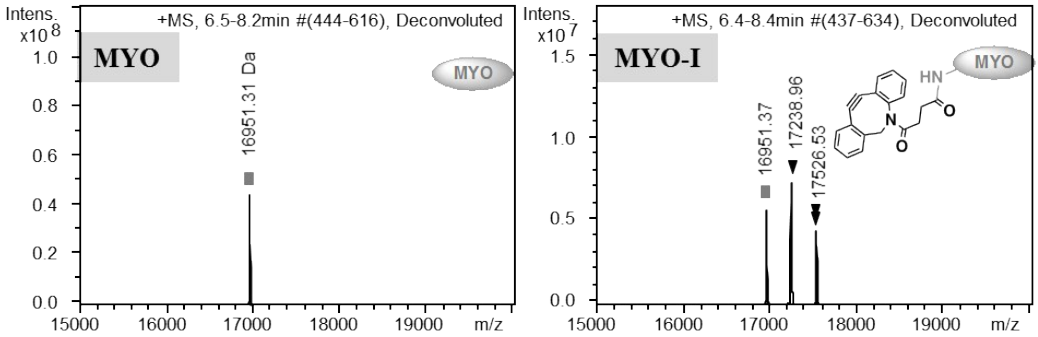
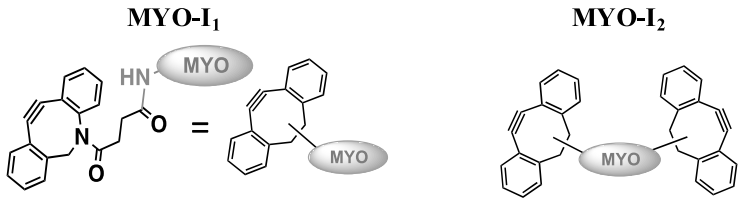


Figure S4. *ESI-MS deconvoluted spectra obtained from MYO (left panel) and MYO-**I** samples (right panel). Grey squares refer to MYO (both panels), black triangles to MYO-**I_1**, (right panel) and black double triangles to MYO-**I_2** (right panel).*

- MYO : 16951.37 Da +/-0.58 Da (expected for GLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASEDLKKHGTVVLTALGGILKKKGHHEAELKPLAQ SHATKHKIPIKYLEFISDAIIHVLHSKHPGDFGADAQGAMTKALELFRNDIAAKYKELGFQG: $C_{769}H_{1212}N_{210}O_{218}S_2$: 16951,30 Da)
- MYO-I_1 : 17238.96 Da +/- 0.40 Da (expected for $C_{788}H_{1225}N_{211}O_{220}S_2$:17238.61 Da)
- MYO-I_2 : 17526.53 Da +/- 0.58 Da (expected for $C_{807}H_{1238}N_{212}O_{222}S_2$:17525.91 Da)



- *MALDI-TOF-MS*

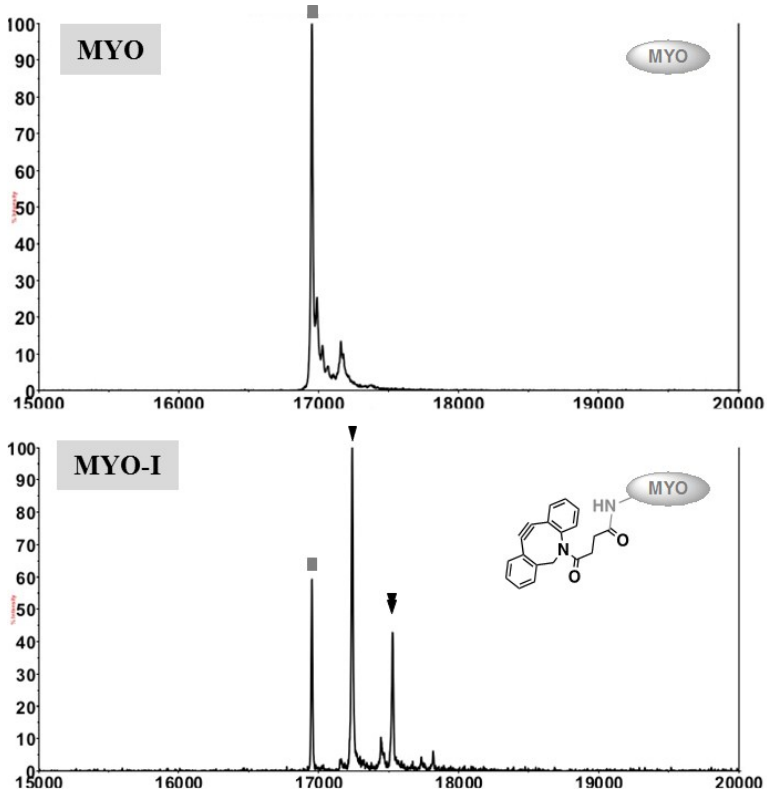


Figure S5. *MALDI-MS spectra of Myoglobin starting material (top panel) and MYO-**I** samples (lower panel). Grey squares refer to MYO (both panels), black triangles to MYO-**I_1**, (lower panel) and black double triangles to MYO-**I_2** (lower panel).*

- *SDS-PAGE*

A part of MYO-**I** 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. The present gel shows a tiny shift between MYO and MYO-**I** migration position. MYO-**I** is detected at a higher position compared to MYO.

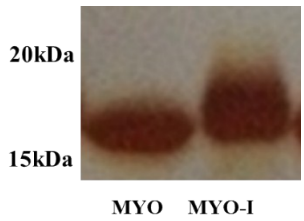
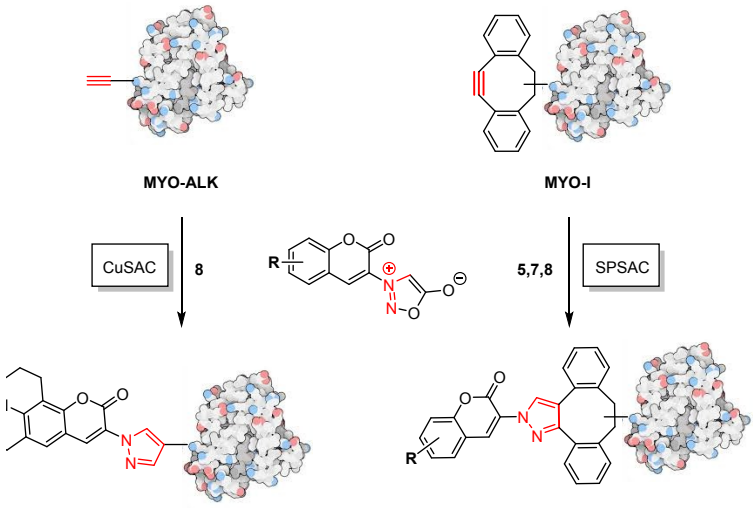


***Figure S6**. Silver stained SDS-PAGE of Myoglobin and **MYO-I**.*

C- Labeling experiments using CuSAC and SPSAC



1. Labeling of Myoglobin-alkyne (MYO-ALK) in buffer via CuSAC

15 µL of **8** in DMSO at 5 mM were mixed and incubated at room temperature for 30 minutes with 7.5 µL of a $CuSO_4$ solution at 24 mM and 7.5 µL of a THPTA prior addition of 7.5 µL of Sodium Ascorbate. The resulting mixture was further incubated for 30 minutes at room temperature and the whole resulting volume of **8**/$CuSO_4$/THPTA or NaAsc was added to 37.5 µL of buffered MYO-ALK protein solution at 4mg/mL in PBK 50 mM pH 8. Solutions mixture of **8**/$CuSO_4$/THPTA/AscNa/MYO-ALK at respective concentrations 1 mM/2.4 mM/3.6 mM/4 mM/184 µM were incubated at room temperature and analyzed by SDS-PAGE and fluorescence imaging after 1h, 5h and overnight incubation. Analysis was performed on protein aliquot of ~5 µg diluted in Laemmli buffer. For this purpose, 15 µL of each experiment were collected at time 1h, 5h and after night incubation and added to Laemmli buffer 2X (15 µL) to lead 1mg/mL sample.

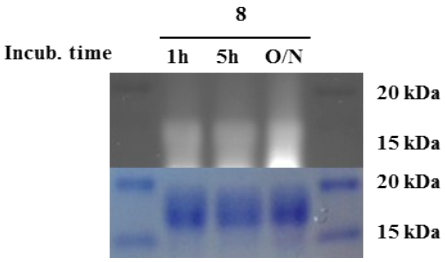


Figure S7 - *SDS-PAGE analysis of labeling experiment of MYO-ALK by **8** in presence of THPTA after different times of incubation at room temperature (1h, 5h and O/N). The 4-20% polyacrylamide gel was scanned for fluorescence with Versadoc MP4000 (top panel) and Coomassie blue stained (lower panel).*

The experiments using THPTA as copper ligand led to the recording of tiny fluorescent signals at the expected molecular weight when **8** is involved in the reaction. These results indicate that the MYO-ALK did react with the sydnone but probably with a very low yield of conversion that may be explained by its low reactivity at room temperature. None fluorescent signals were observed when the other ligand (for example, bathophenantroline) were used as copper ligand (data not shown). As illustrated by the above panels, none evolution of the conversion could be observed overtime despite the myoglobin structure integrity over time during reaction process as checked with the corresponding coomassie blue stained gel (lower panel).

2. Labeling of MYO-I in buffer via SPSAC

Protocol

80 µL of a solution containing 9.44 nmoles of MYO-**I** in PBK buffer 50 mM pH 6.6 were mixed with 20 µL of sydnone **5**, **7** or **8** to lead to a solution containing 94 µM of MYO-**I** and 1 mM of sydnone **5**, **7** or **8**. The solutions were incubated for 1h30 at room temperature before mass spectrometry and SDS-PAGE analysis of reaction mixtures. For this purpose, 10 µL of the reaction mixtures were respectively added to 40 µL of H_2O/CH_3CN :TFA (95/5 :0.1%) or to 30 µL of Laemmli buffer. 2 µg of each proteins adduct were loaded in wells of a 4-20% acrylamide 10 wells gel.

Analysis of labeling experiments

- *LC-ESI-MS*

MYO-conjugates sample were subjected to LC-ESI-MS as well as starting material MYO-**I**. In all 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 (triangles) and myoglobin bearing two pyrazole derivatives (double triangles).

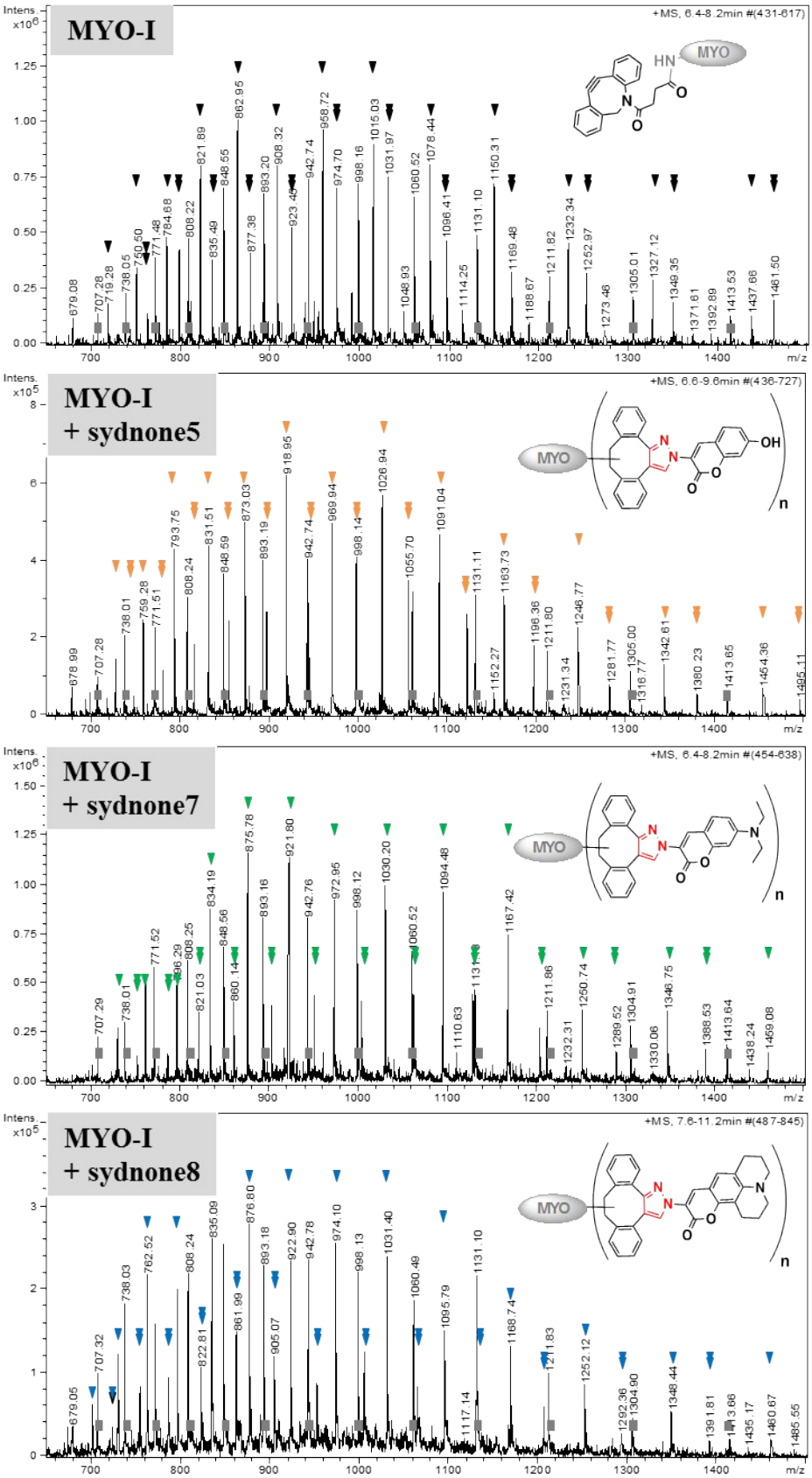


Figure S8 - *ESI-MS spectra of MYO-**I** and of Myoglobin labelled by probe **5**, **7** or **8**. Grey squares refer to unmodified MYO, black triangles to MYO-**I** (n = 1) and black double triangles to MYO-**I** (n = 2), orange triangles (n = 1) and orange double triangles (n = 2) to **5**-derived Myoglobin; green triangles (n = 1) and green double triangles (n = 2) to **7**-derived Myoglobin; blue triangles (n = 1) and blue double triangles (n = 2) to **8**-derived Myoglobin*

Summary of m/z for each charge state of MYO and MYO-conjugates

	measured m/z											
					MYO-I + Sydnone							
						5 R = OH		7 R = NEt_2		8 R = Ncyclo		
	charge state z	MYO	$MYO-I_1$	$MYO-I_2$		n=1	n=2	n=1	n=2	n=1	n=2
	25+	679.08	690.53	702.21		698.57	718.23	700.91	722.63	701.65	724.27
	24+	707.28	719.28	731.13		727.74	748.07	729.97	752.66	730.88	754.31
	23+	738.05	750.50	763.05		759.28	780.52	761.74	785.41	762.52	787.16
	22+	771.48	784.68	797.68		793.75	816.06	796.29	821.03	797.15	822.81
	21+	808.22	821.89	835.49		831.51	854.85	834.19	860.14	835.09	861.99
	20+	848.55	862.95	877.38		873.03	897.51	875.78	903.04	876.80	905.07
	19+	893.20	908.32	923.45		918.95	944.71	921.80	950.47	922.90	952.66
	18+	942.74	958.72	974.70		969.94	997.14	972.95	1003.24	974.10	1005.44
	17+	998.16	1015.03	1031.97		1026.94	1055.70	1030.20	1062.23	1031.40	1064.65
	16+	1060.52	1078.44	1096.41		1091.04	1121.63	1094.48	1128.53	1095.79	1131.10
	15+	1131.10	1150.31	1169.48		1163.73	1196.36	1167.42	1203.67	1168.74	1206.46
	14+	1211.82	1232.34	1252.97		1246.77	1281.77	1250.74	1289.52	1252.12	1292.36
	13+	1305.01	1327.12	1349.35		1342.61	1380.23	1346.75	1388.53	1348.44	1391.81
	12+	1413.53	1437.66	1461.50		1454.36	1495.11	1459.08	1504.23	1460.67	1507.69

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

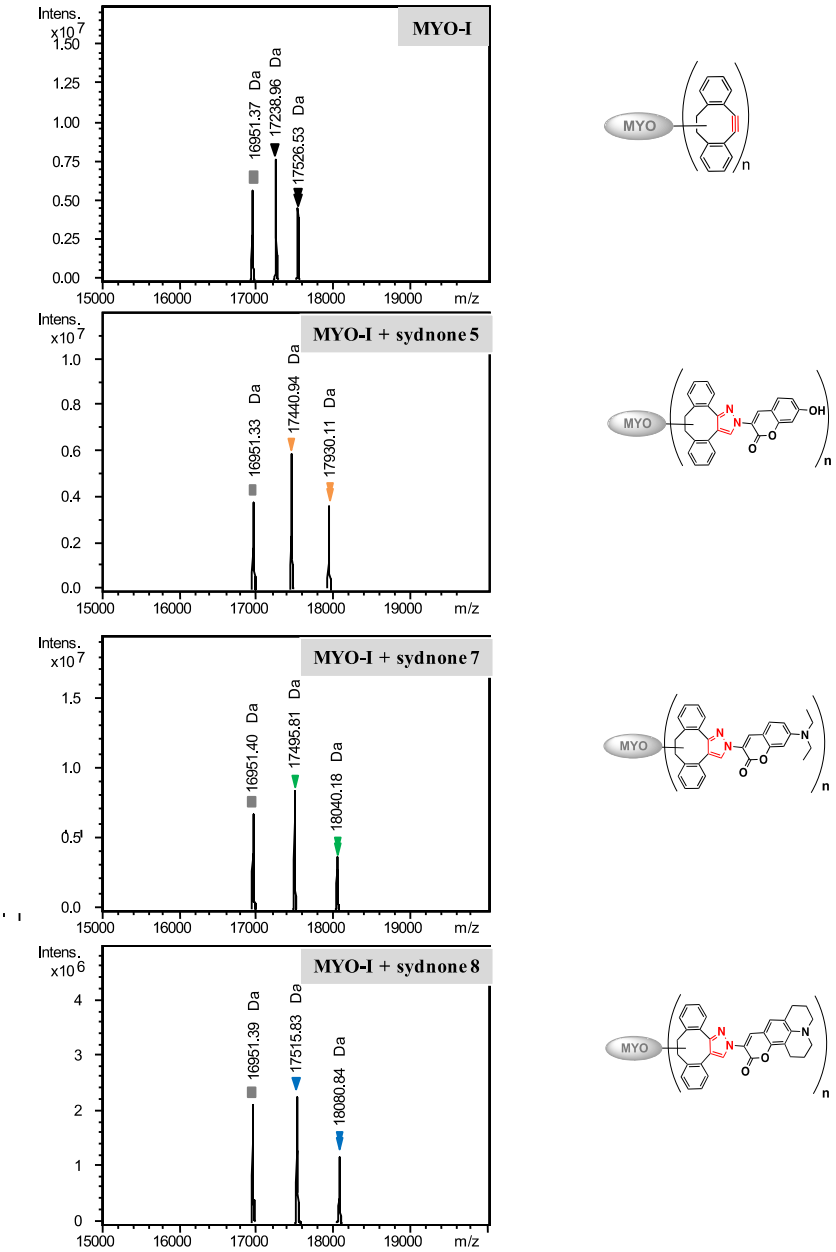
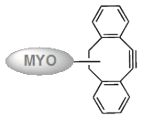


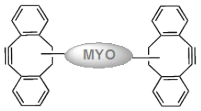
Figure S9 - *Comparison of ESI-MS deconvoluted spectra of MYO-**I** and of Myoglobin labelled by probe **5**, **7** or **8**. Grey squares refer to unmodified MYO, black triangles to MYO-**I** (n = 1) and black double triangles to MYO-**I** (n = 2), orange triangles (n = 1) and orange double triangles (n = 2) to **5**-derived Myoglobin; green triangles (n = 1) and green double triangles (n = 2) to **7**-derived Myoglobin; blue triangles (n = 1) and blue double triangles (n = 2) to **8**-derived Myoglobin*

1st panel	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-I_1 expected at 17238.61 Da($C_{788}H_{1225}N_{211}O_{220}S_2$)
	17526.53 Da +/- 0.58 Da for MYO-I_2 expected at 17525.91 Da($C_{807}H_{1238}N_{212}O_{222}S_2$)
2nd panel	16951.33 Da +/-0.52 Da for MYO expected at 16951.30 Da($C_{769}H_{1212}N_{210}O_{218}S_2$)
	17440.94 Da +/- 0.34 Da for MYO-5_1 expected at 17440.78 Da ($C_{798}H_{1231}N_{213}O_{223}S_2$)
	17930.11 Da +/- 0.46 Da for MYO-5_2 expected at 17930.26 Da ($C_{827}H_{1250}N_{216}O_{228}S_2$)
3rd panel	16951.40 Da +/-0.37 Da for MYO expected at 16951.30 Da ($C_{769}H_{1212}N_{210}O_{218}S_2$)
	17495.81 Da +/- 0.51 Da for MYO-7_1 expected at 17495.90 Da($C_{802}H_{1240}N_{214}O_{222}S_2$)
	18040.18 Da +/- 0.55 Da for MYO-7_2 expected at 18040.50 Da ($C_{835}H_{1268}N_{218}O_{226}S_2$)
4th panel	16951.39 Da +/-0.45 Da for MYO expected at 16951.30 Da ($C_{769}H_{1212}N_{210}O_{218}S_2$)
	17515.83 Da +/- 0.4 Da for MYO-$Pyr2_1$ expected at 17519.92 Da ($C_{804}H_{1240}N_{214}O_{222}S_2$)
	18080.84 Da +/- 0.64 Da for MYO-$Pyr2_2$ expected at 18088.54 Da ($C_{839}H_{1268}N_{218}O_{226}S_2$)

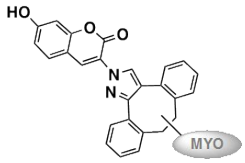
MYO-I_1



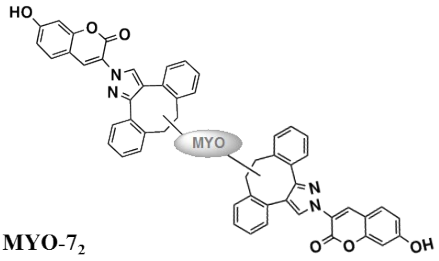
MYO-I_2



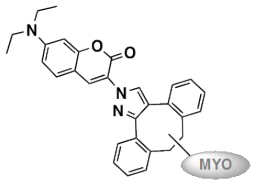
MYO-5_1



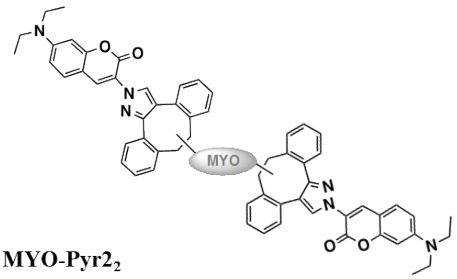
MYO-5_2



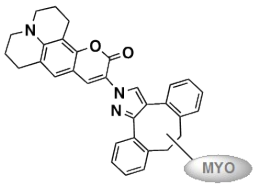
MYO-7_1



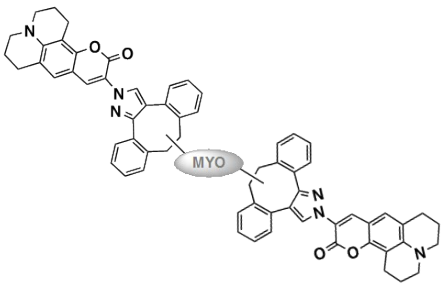
MYO-7_2



MYO-$Pyr2_1$



MYO-$Pyr2_2$



- *MALDI-MS*

MYO-conjugates sample were subjected to MALDI-MS as well as starting material MYO-**I**. The MALDI-MS spectra obtained from the same sydnone derived myoglobin samples confirm the presence in each of these samples of three protein species: unmodified myoglobin (grey squares), myoglobin bearing one pyrazole derivative (triangles) and myoglobin bearing two pyrazole derivatives (double triangles).

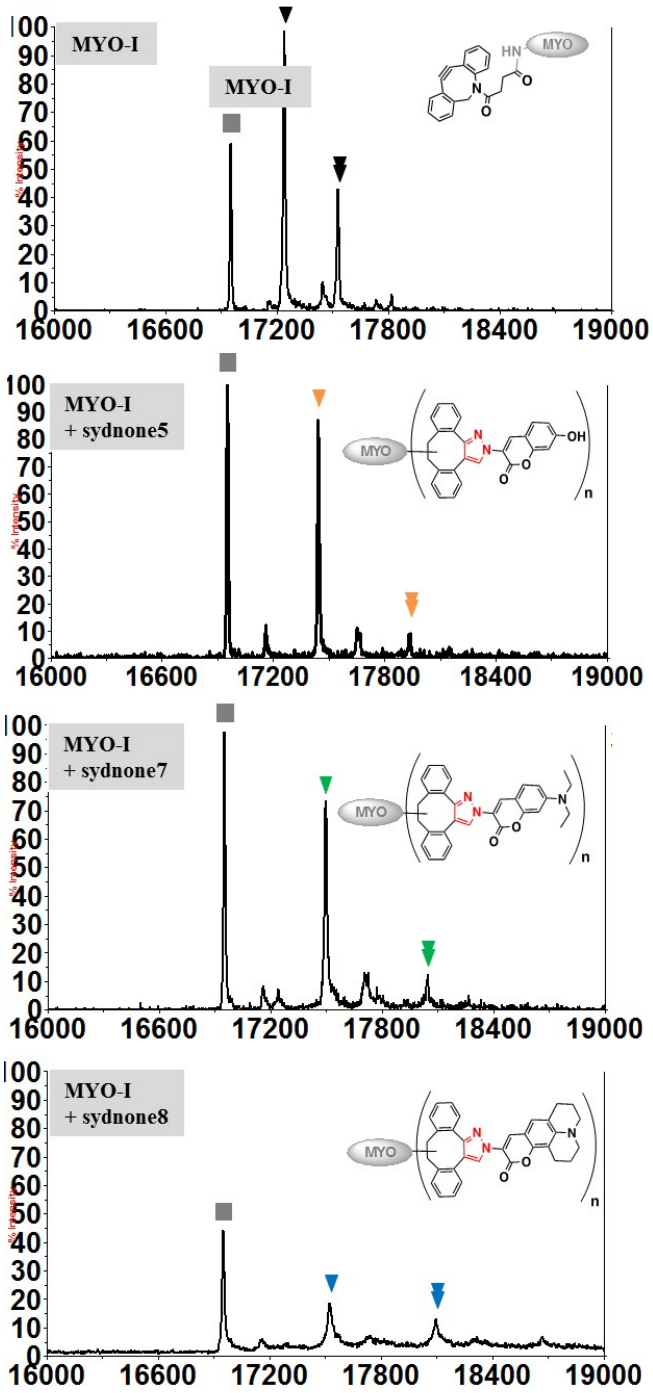


Figure S10 - *Comparison of MALDI-MS spectra of MYO-**I** and of Myoglobin labelled by probe **5**, **7** or **8**. Grey squares refer to unmodified MYO, black triangles to MYO-**I** (n = 1) and black double triangles to MYO-**I** (n = 2), orange triangles (n = 1) and orange double triangles (n = 2) to **5**-derived Myoglobin; green triangles (n = 1) and green double triangles (n = 2) to **7**-derived Myoglobin; blue triangles (n = 1) and blue double triangles (n = 2) to **8**-derived Myoglobin.*

- *SDS-PAGE and fluorescence imaging*

Solutions of *mass-characterized* sydnone myoglobin adducts were analysed by SDS-PAGE using standard condition, Laemmli buffer and 4-20% acrylamide gel. After electrophoretic migration of 0.12 nmoles of proteins species (~ 2 μg), the gel was subjected to fluorescence analysis before being silver-stained.

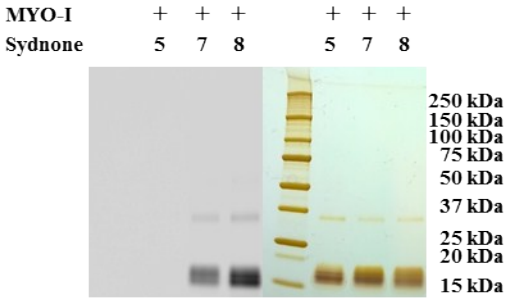


Figure S11 - *SDS-PAGE analysis of labeling experiment of MYO-**I** by Sydnone **5**, **7** or **8**. The 4-20% polyacrylamide gel was scanned for fluorescence with LAS4000 (left panel) or silver stained (right panel).*

Positive fluorescent signal was observed when MYO-**I** was treated with **7** and **8**. None fluorescence signal was observed for **5-**derived Myoglobin as expected at 515 nm. The resulting adducts **7** and **8**-derived myoglobin remains stable under SDS-PAGE conditions and with a valuable signal/noise ratio. 8-derived Myoglobin show the highest fluorescence detection signal that can be identified as the best turn-on probe for protein labeling.

3. Labeling of MYO-I in HeLa Lysate via SPSAC

These experiments were performed to evaluate the potency of sydnone coumarin derivatives to label efficiently and selectively a protein bearing DBCO function in presence of many other proteins. According to the previous results, the turn-on sydnone **8** was chosen to label MYO-**I** in complex media. For this purpose, MYO-**I** was mixed and incubated with a HeLa Lysate before being subjected to the addition of the sydnone **8**.

Protocol

20 μL of a PBK 50 mM pH 8 buffered solution of MYO-**I** (0.18 nmoles, ~ 3 μg), 20 μL of a buffered solution containing MYO-**I** (0.18 nmoles, ~ 3 μg) mixed with 20 μg of HeLa lysate and 20 μL of HeLa lysate buffered solution were respectively subjected to the addition of 5 μL of **8** DMSO solution at 5 mM to lead to 3 mixtures containing respectively MYO-**I** at 7.1 μM in buffer; MYO-**I** at 7.1 μM together with 20 μg of HeLa lysate and 20 μg of HeLa only. Concentration of **8** was set at 1 mM in all experiments. After 1h30 incubation at room temperature, Laemmli buffer 4X was added to each samples and the whole volumes obtained were analysed by SDS-PAGE using 10 wells 4-20% polyacrylamide gel. SDS-PAGE was performed under denaturating conditions.

Analysis of labeling experiments

- *SDS-PAGE and fluorescence imaging*

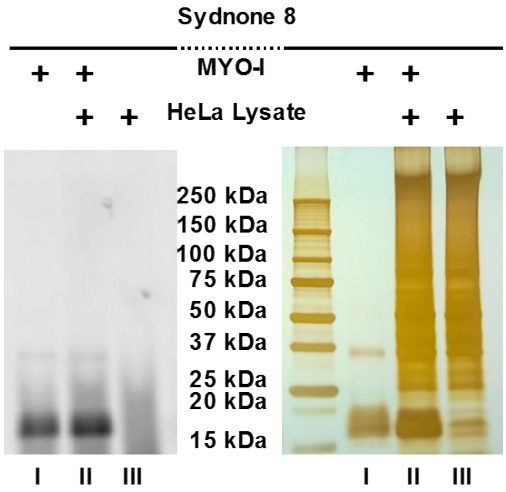


Figure S12 - *SDS-PAGE analysis of labeling experiment of MYO added to HeLa lysate by **8**. Lanes I to III refer to experiments involving **8** respectively added to buffered MYO-**I** (lane I), MYO-**I** mixed to HeLa lysate (lane II) or HeLa lysate only (lane III). The 4-20% polyacrylamide gel was scanned for fluorescence with LAS4000 (left panel) or silver stained (right panel).*

The measured fluorescence signal relative to Myoglobin labeling reach the same intensity range whether the MYO-**I** was preincubated (lane II, left panel) or not (lane I, left panel) with lysate proteins. These results indicate that neither the labeling of MYO-**I** by **8** nor the detection of the fluorescent protein adduct are prevented by the presence of other proteins from the Lysate during the labeling reaction. The latter results together with the absence of any proteins labeling when HeLa lysate was used alone (lane III, left panel) attest the high efficiency of **8** to react in complex media specifically with stained alkyne functionalizing protein leading to fluorescent labeling.

VII. Calculations

Geometry optimizations of the ground states were carried out at the B3LYP/6-31+g(d) level of theory. Geometry optimizations of the first excited states were carried out at the M06-2X/6-31+g(d) level of theory. Frequency calculation was done in all cases to confirm the true minimum nature (no imaginary frequency) of the converged geometry. TDDFT calculations were done at M06-2X/6-311+g(d,p) level of theory using IEFPCM model to include solvent (water) effect. All calculations were done with Gaussian 09 (Revision D.01) software[iii] from Gaussian Inc. and orbitals were plotted with Gaussview 5.0.

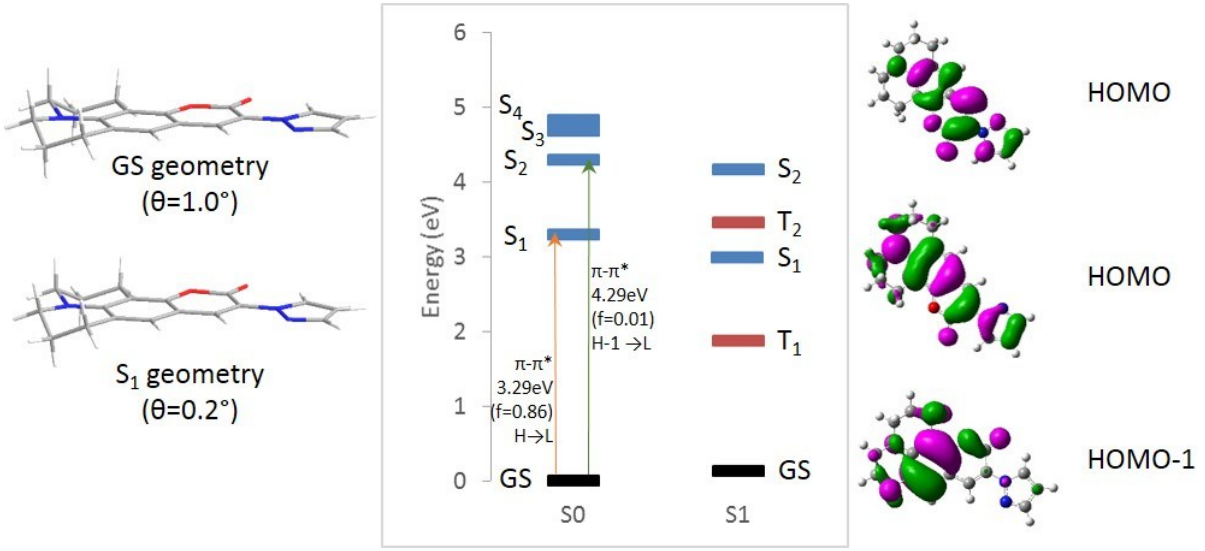
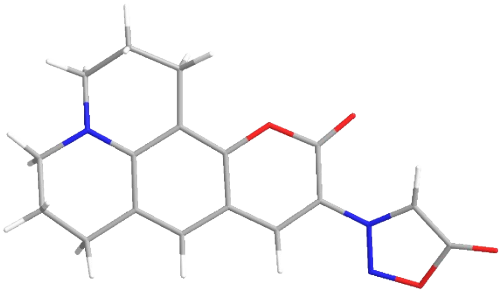


Figure S13 - Left: ground state (top) and first excited state (bottom) optimized geometries for pyrazole-coumarine (θ refers to the dihedral angle between coumarin and pyrazole). Middle: energy diagram of the calculated transitions with their oscillator strength values (f). S0 column refers to calculations carried out using the ground state optimized geometry and S1 to calculations carried out using the first excited state optimized geometry. Right: main orbitals involved in the S_1/T_1 and S_2/T_2 transitions.

Table Sx: Cartesian coordinates for the ground state optimized geometry of sydnone **8**

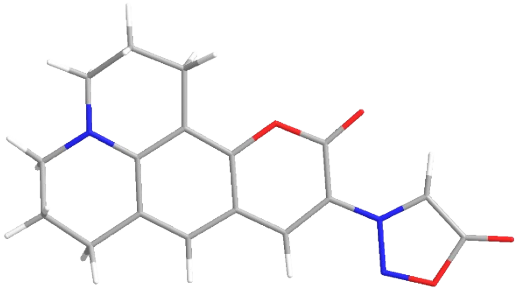


Atom	X (Å)	Y (Å)	Z (Å)
C	-5.833748	-0.202809	-0.195242
C	-4.632645	0.533928	-0.37869
H	-4.481142	1.533568	-0.742732
O	-5.357154	-1.437204	0.370026
N	-4.003455	-1.428159	0.522324
N	-3.623679	-0.250102	0.057288
C	-2.21912	0.013182	0.029635
C	-1.771967	1.3987	0.070525
C	-1.330521	-1.026981	-0.012214
C	0.066853	-0.790122	0.003685
H	-1.705961	-2.045054	-0.054378
C	0.515974	0.545745	0.053986
O	-7.012286	-0.01579	-0.404047
O	-2.461929	2.395598	0.086857
O	-0.394188	1.56991	0.08547
C	1.860924	0.89929	0.066653
C	1.049142	-1.803715	-0.030041

H	0.725855	-2.842124	-0.066781
C	2.8262	-0.145654	0.039313
C	2.397935	-1.517047	-0.001431
N	4.171297	0.159794	0.029029
C	5.186911	-0.886209	0.153929
H	6.114064	-0.497039	-0.281117
H	5.392596	-1.096277	1.218324
C	4.617496	1.525613	0.305677
H	4.650801	1.702824	1.39482
H	5.642425	1.619714	-0.068427
C	4.759536	-2.165862	-0.557907
H	5.530969	-2.932581	-0.423787
H	4.671017	-1.970422	-1.634288
C	3.419167	-2.637017	0.006481
H	3.036531	-3.491933	-0.563067
H	3.569198	-2.993707	1.036741
C	3.705742	2.546536	-0.367104
H	4.056607	3.559206	-0.138895
H	3.765304	2.415939	-1.45519
C	2.264495	2.358338	0.109105
H	1.576135	2.951351	-0.501405
H	2.156672	2.740177	1.135069

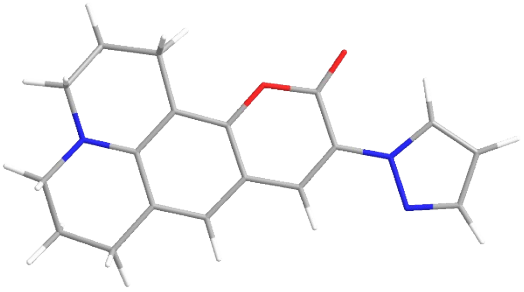
Table Sx: Cartesian coordinates for the first excited state optimized geometry of sydnone **8**

Atom	X (Å)	Y (Å)	Z (Å)
C	5.796311	-0.166846	0.011845
C	4.603522	0.628538	0.001362
H	4.47193	1.697781	-0.003055
O	5.31055	-1.502798	0.012723
N	3.989267	-1.516753	0.003693
N	3.577482	-0.227618	-0.002764
C	2.200922	-0.011869	-0.012214
C	1.7686	1.344049	-0.021225
C	1.3193	-1.12412	-0.015063
C	-0.063356	-0.841658	-0.017293
H	1.681208	-2.142688	-0.013857
C	-0.495929	0.50505	-0.017368
O	6.971689	0.062257	0.01906
O	2.48249	2.337882	-0.022543
O	0.409999	1.542704	-0.031321
C	-1.829645	0.877658	-0.006919
C	-1.07949	-1.822132	-0.018782
H	-0.784102	-2.869942	-0.021352
C	-2.818322	-0.137672	-0.015864
C	-2.422519	-1.504363	-0.028717
N	-4.163598	0.206844	0.021581
C	-5.15988	-0.810135	-0.269179
H	-6.126774	-0.43183	0.078413
H	-5.242218	-0.97868	-1.358926
C	-4.534256	1.56887	-0.323639
H	-4.457587	1.728933	-1.414902
H	-5.5833	1.706953	-0.042644
C	-4.81025	-2.114879	0.430976
H	-5.590487	-2.859025	0.241827
H	-4.770262	-1.936028	1.511996
C	-3.458236	-2.606363	-0.073169
H	-3.111272	-3.461026	0.517792
H	-3.567444	-2.963542	-1.10672
C	-3.643235	2.557033	0.413025
H	-3.957909	3.581892	0.191887
H	-3.762532	2.395544	1.490778



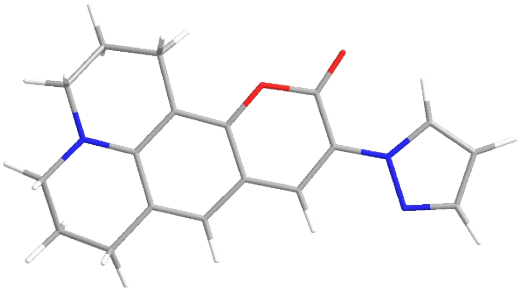
C	-2.188767	2.345972	0.005589
H	-1.514513	2.881824	0.680408
H	-2.01326	2.769379	-0.992756

Table Sx: Cartesian coordinates for the ground state optimized geometry of pyrazole



Atom	X (Å)	Y (Å)	Z (Å)
C	-6.164511	-0.156741	0.01166
C	-5.016826	0.608563	-0.007212
H	-4.853174	1.673027	-0.026245
N	-4.377737	-1.563533	0.029743
N	-3.958063	-0.265192	0.004375
C	-2.565062	-0.006609	-0.009052
C	-2.101367	1.379491	-0.018304
C	-1.664661	-1.035622	-0.010992
C	-0.260278	-0.794599	-0.020147
H	-2.038441	-2.053956	-0.004084
C	0.187608	0.536942	-0.025091
O	-2.786352	2.385236	-0.018974
O	-0.728436	1.55717	-0.028164
C	1.534381	0.896905	-0.026982
C	0.724426	-1.802747	-0.027187
H	0.405579	-2.843429	-0.026791
C	2.500429	-0.142117	-0.035919
C	2.076308	-1.510227	-0.045621
C	-5.705086	-1.494118	0.034202
H	-6.281683	-2.410221	0.054009
H	-7.183945	0.202044	0.009998
N	3.854647	0.169817	-0.002481
C	4.280563	1.538001	-0.284508
H	5.31601	1.639439	0.059025
H	4.278398	1.729484	-1.373455
C	4.853279	-0.868473	-0.240675
H	5.807551	-0.505591	0.15792
H	4.997214	-1.032817	-1.324862
C	4.456176	-2.17809	0.434491
H	5.222636	-2.937973	0.24238
H	4.408347	-2.023121	1.520095
C	3.095991	-2.63231	-0.096853
H	2.72739	-3.494769	0.471358
H	3.214322	-2.975891	-1.13582
C	3.37987	2.543727	0.426003
H	3.721574	3.562788	0.210883
H	3.463935	2.390645	1.509583
C	1.928393	2.359562	-0.022052
H	1.250639	2.924281	0.62665
H	1.792222	2.779943	-1.029564

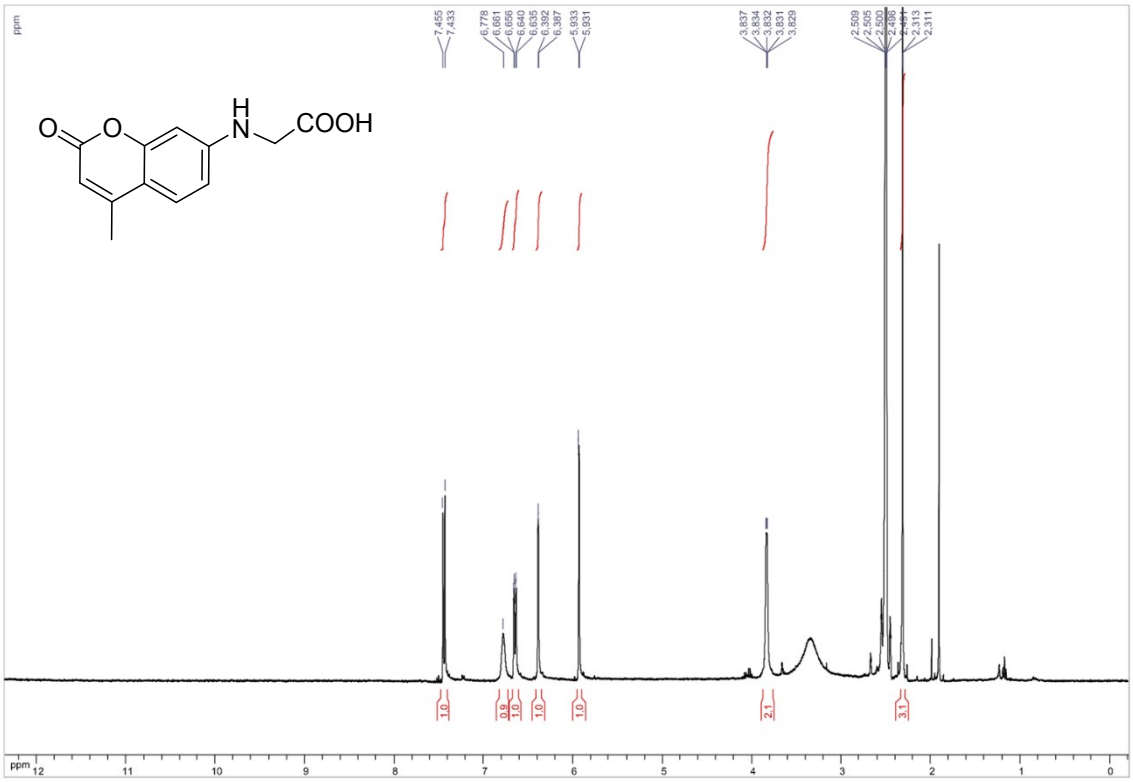
Table Sx: Cartesian coordinates for the first excited state optimized geometry of pyrazole

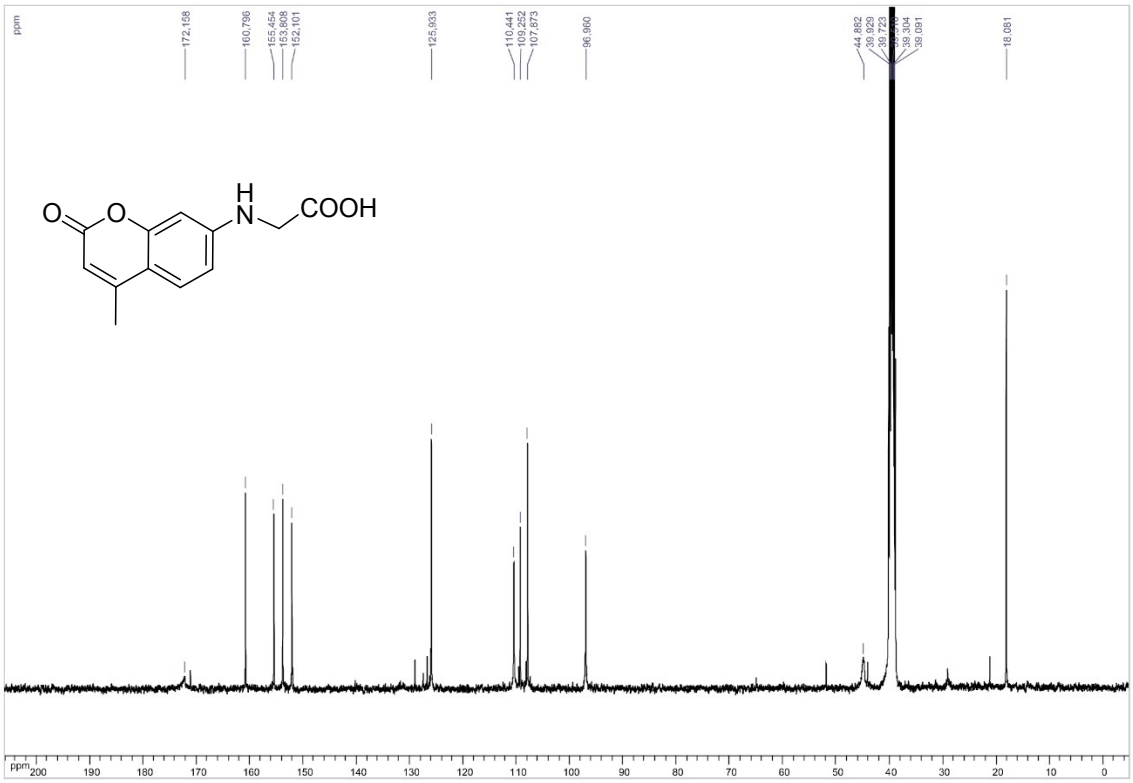


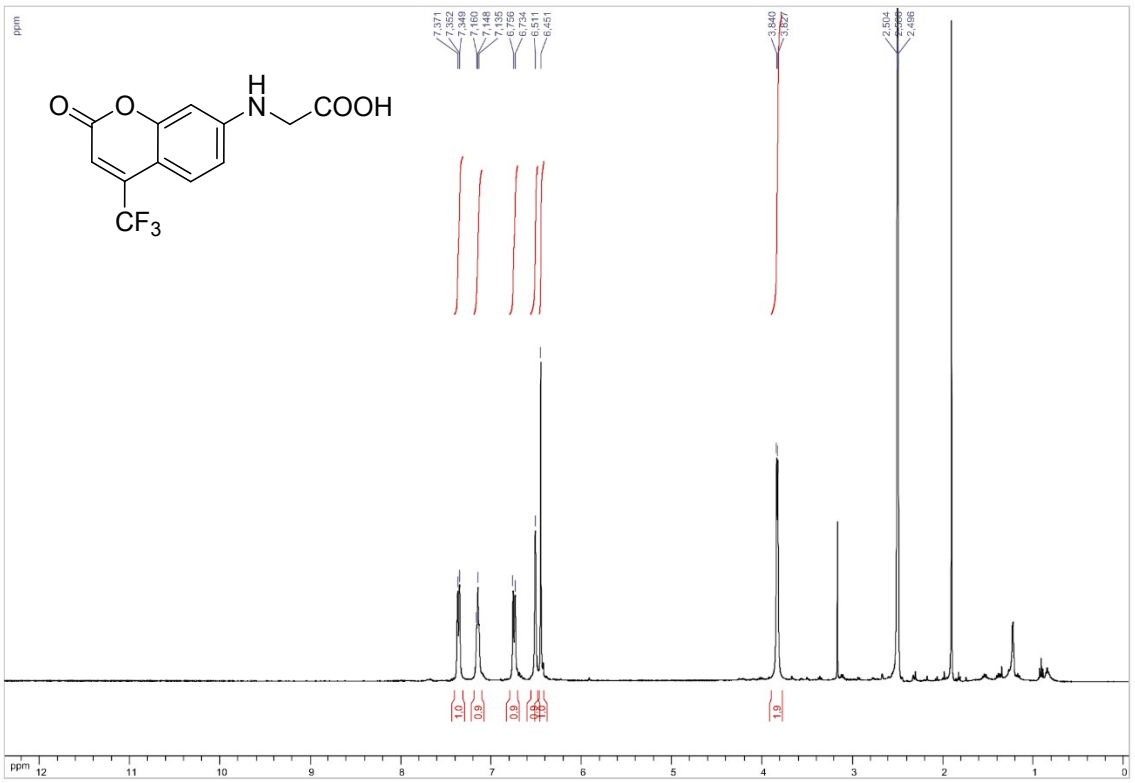
Symbol	X	Y	Z
C	-6.117987	-0.154869	0.101482
C	-4.980642	0.614202	0.060325
H	-4.816442	1.678598	0.057878
N	-4.333604	-1.555826	0.027876

N	-3.91856	-0.258209	0.016799
C	-2.551932	-0.004406	-0.035449
C	-2.106055	1.360395	-0.054238
C	-1.650241	-1.096189	-0.074141
C	-0.273335	-0.824642	-0.082251
H	-2.032869	-2.107599	-0.071271
C	0.171203	0.533098	-0.08178
O	-2.78067	2.374538	-0.03106
O	-0.721697	1.561542	-0.12371
C	1.502695	0.889675	-0.053936
C	0.742563	-1.823504	-0.07494
H	0.432789	-2.866382	-0.078711
C	2.48984	-0.143947	-0.059415
C	2.082823	-1.518813	-0.078675
C	-5.651757	-1.494592	0.078697
H	-6.225361	-2.411722	0.096766
H	-7.138347	0.196309	0.141395
N	3.821783	0.185977	-0.015748
C	4.239312	1.56259	-0.235251
H	5.265775	1.65757	0.131035
H	4.249555	1.781083	-1.318316
C	4.847561	-0.834934	-0.168312
H	5.76886	-0.444311	0.274723
H	5.044626	-1.008757	-1.241563
C	4.430427	-2.13675	0.498649
H	5.221092	-2.881807	0.367067
H	4.30379	-1.965671	1.574372
C	3.120563	-2.613609	-0.116872
H	2.740302	-3.497949	0.404823
H	3.300214	-2.913751	-1.159261
C	3.309114	2.528628	0.482301
H	3.65283	3.554982	0.320641
H	3.352307	2.326513	1.559148
C	1.88613	2.348416	-0.032523
H	1.171329	2.902964	0.581775
H	1.799461	2.763202	-1.046849

VIII. Annexes



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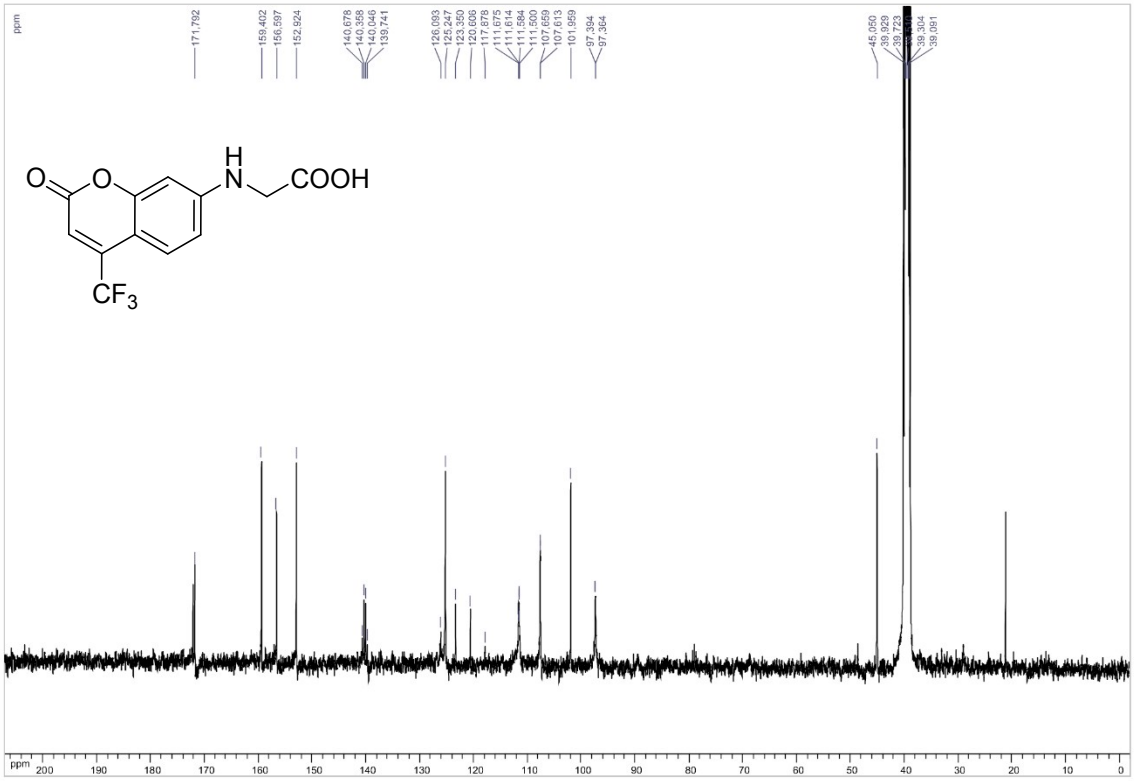
7.349
7.160
7.148
7.135
6.758
6.524
6.511
6.451
3.840
3.827
2.504
2.496
O
O
H
N
COOH
CF3
1.0
0.9
0.9
1.8
1.9
ppm 12 11 10 9 8 7 6 5 4 3 2 1 0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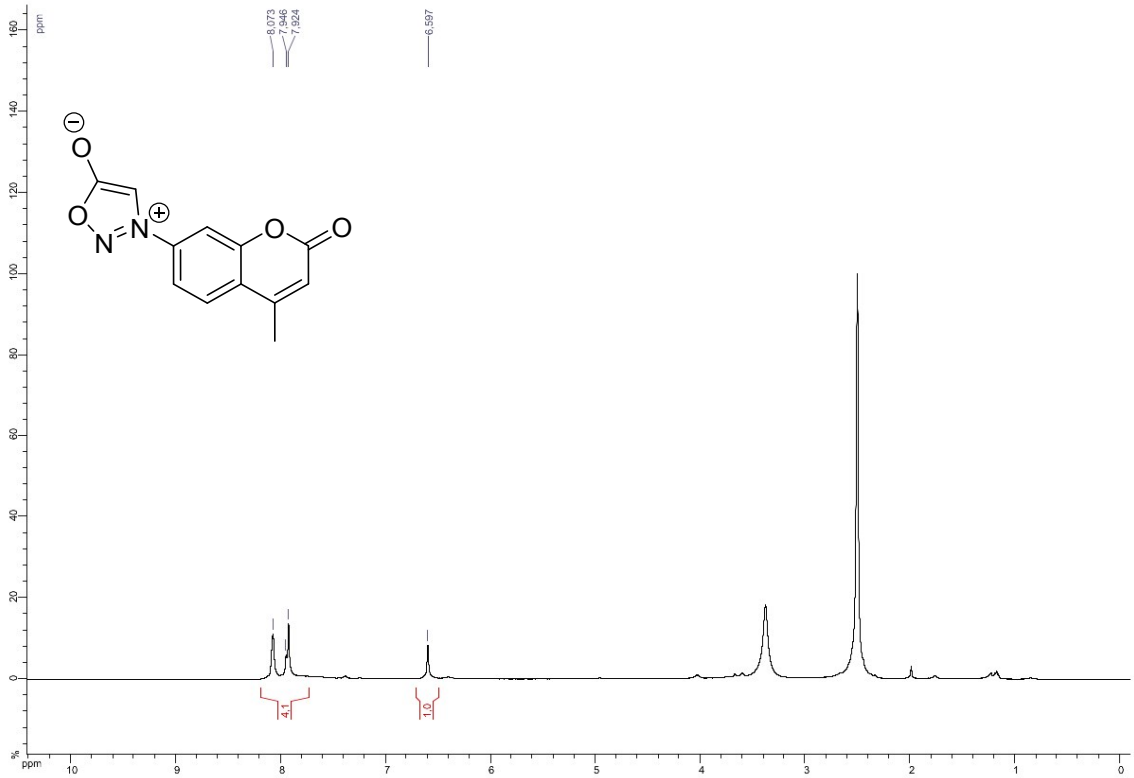
156.597
152.924
140.678
140.358
140.046
139.741
126.093
123.247
123.060
120.606
117.878
111.675
111.614
111.584
111.560
107.659
107.613
101.959
97.394
97.364
45.050
39.929
39.723
39.516
39.304
39.091
O
O
H
N
COOH
CF3
ppm 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0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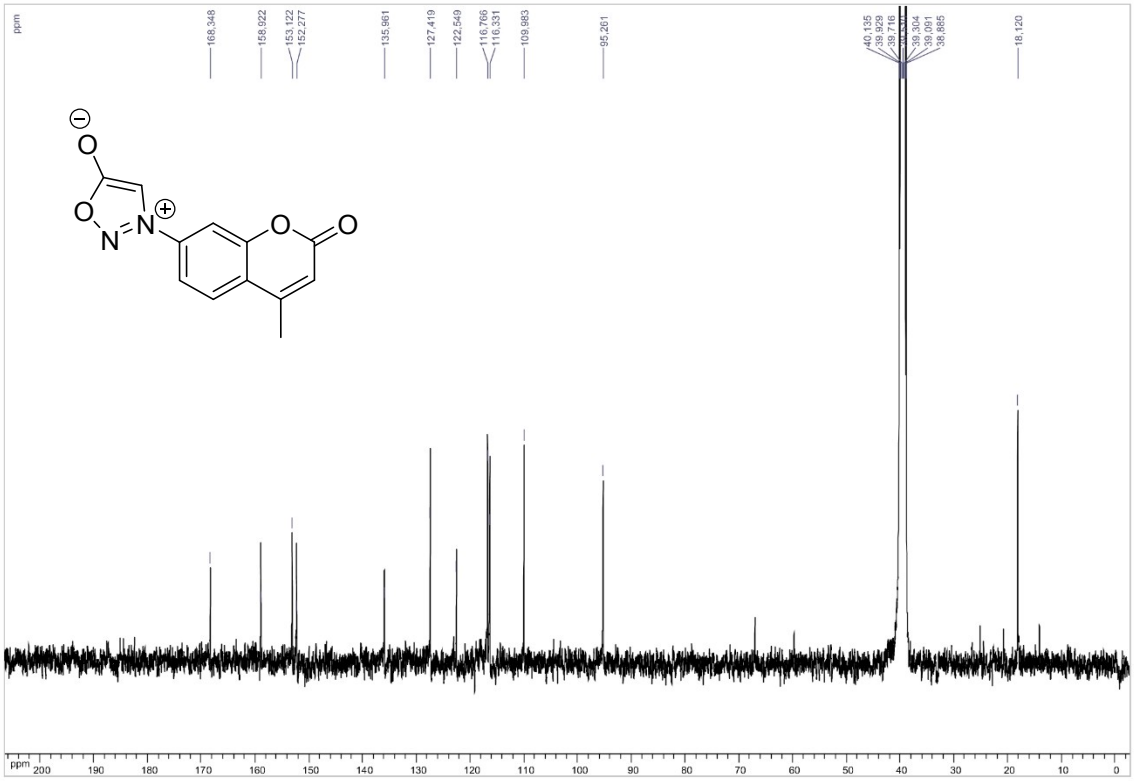
7.946
7.924
6.597
4.1
1.0
ppm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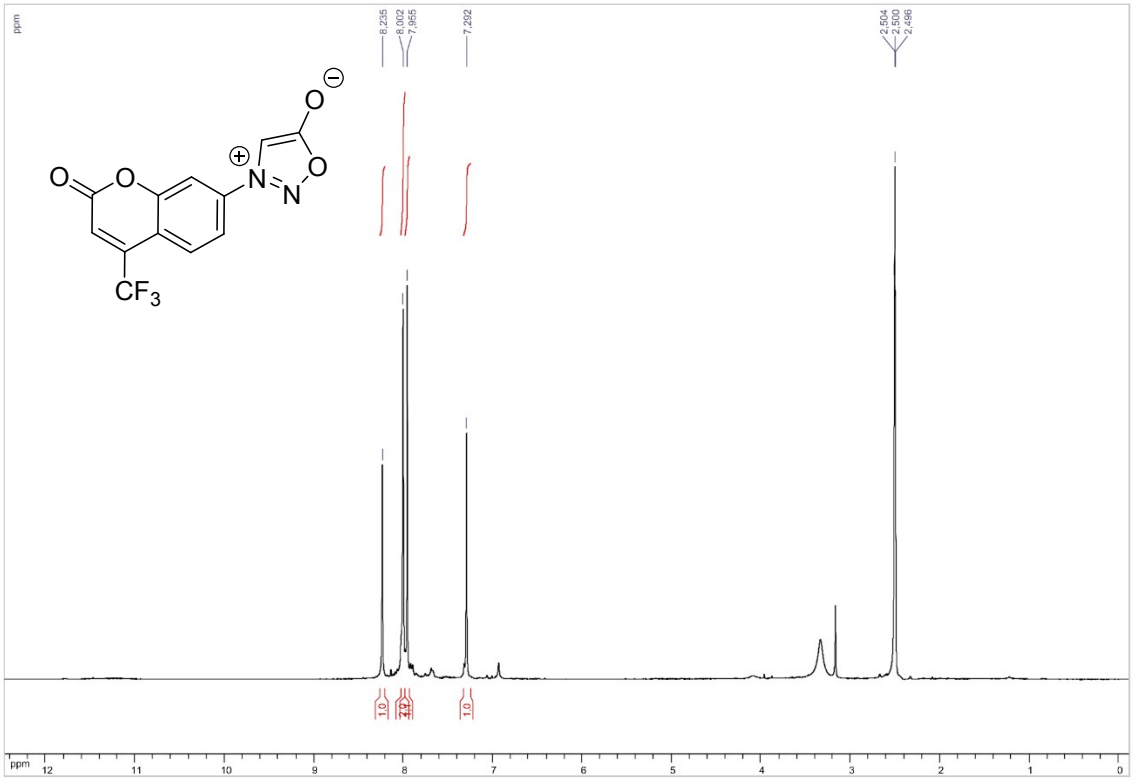
158.922
153.122
152.277
135.961
127.419
122.549
116.766
116.331
109.983
95.261
40.135
39.929
39.716
39.510
39.304
39.091
38.885
18.120
ppm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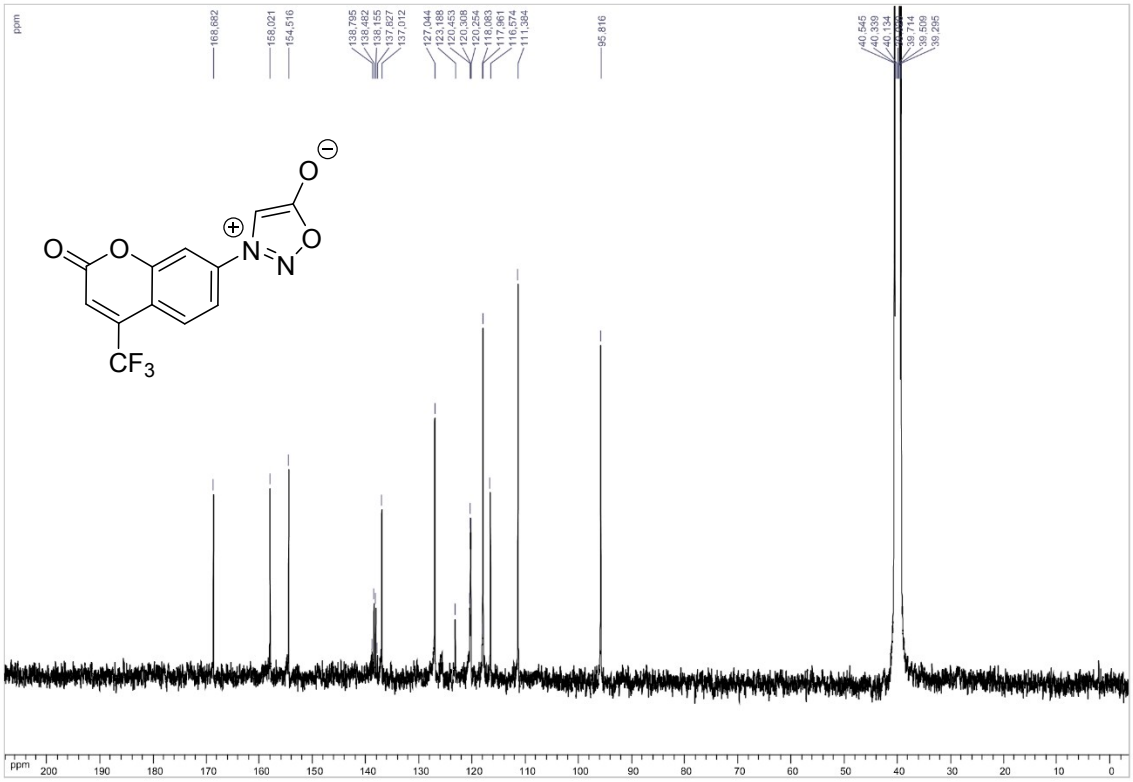
8,002
7,955
7,292
2,504
2,500
2,496
1,0
2,2
1,0
ppm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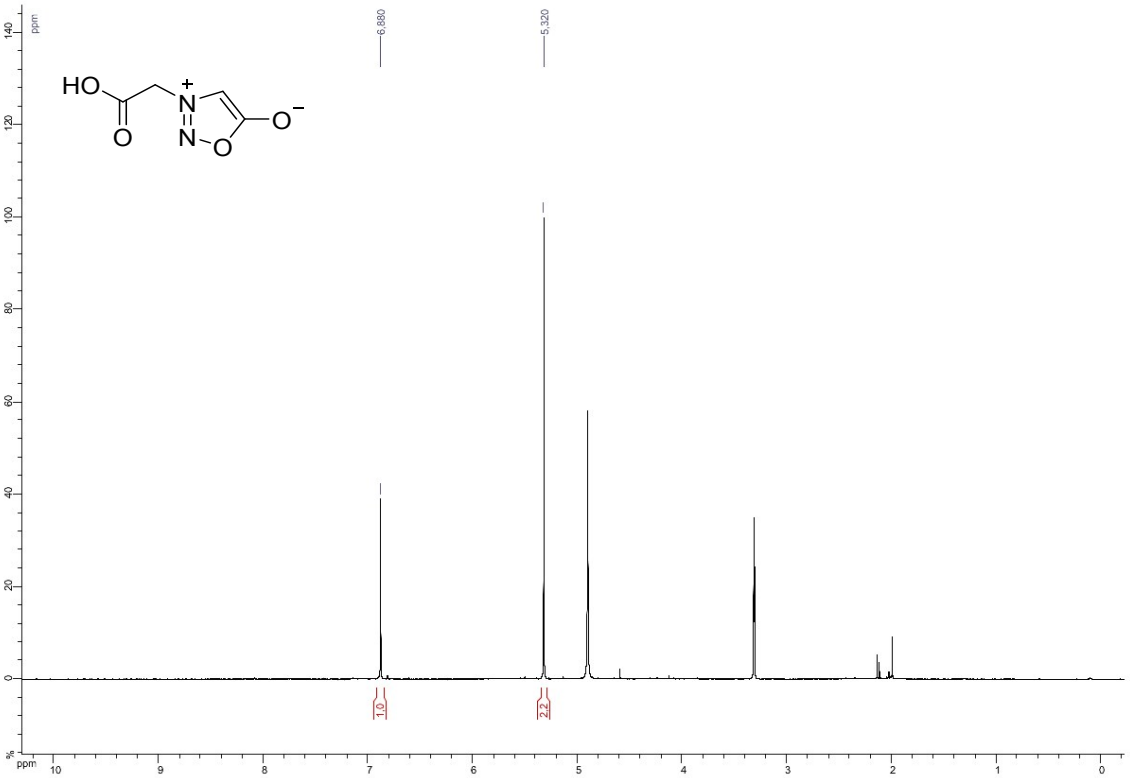
154,516
138,795
138,482
138,155
137,827
137,012
127,044
123,188
120,453
120,308
120,254
118,083
117,961
116,574
111,384
95,816
40,545
40,339
40,134
39,714
39,509
39,295
ppm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O
N
N
O
O
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2,2
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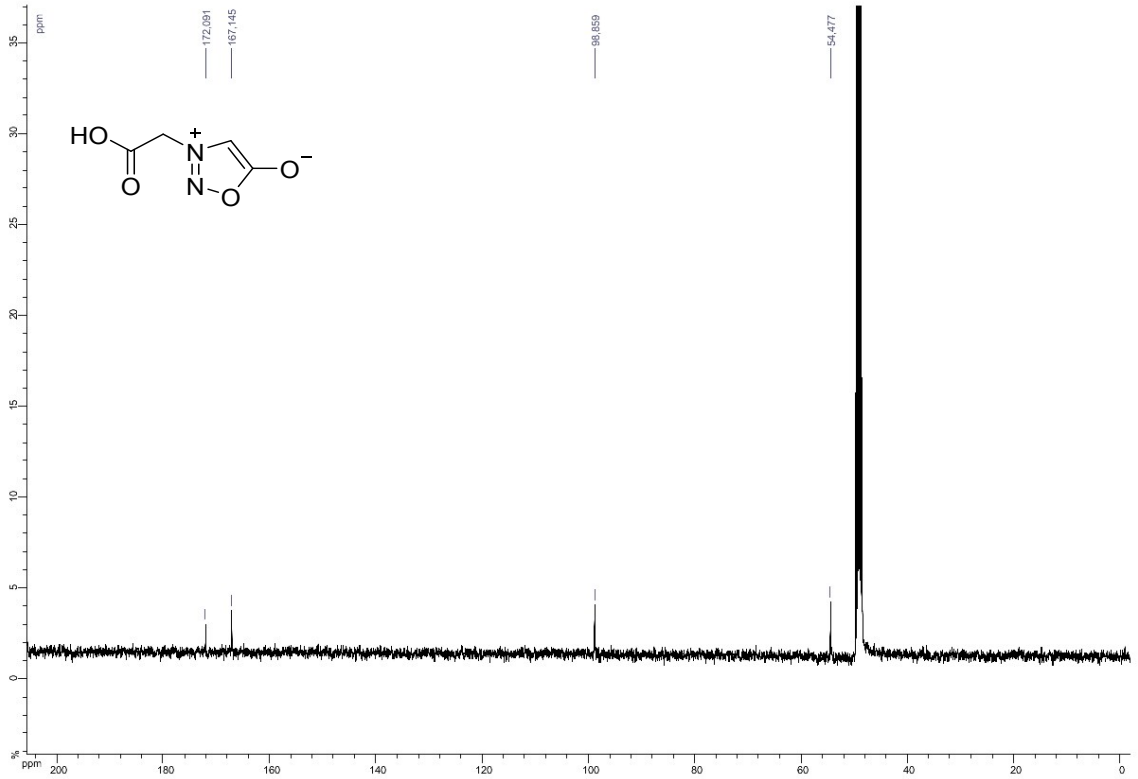
9
8
7
6
5
4
3
2
1
0


HO
O
N
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O
O
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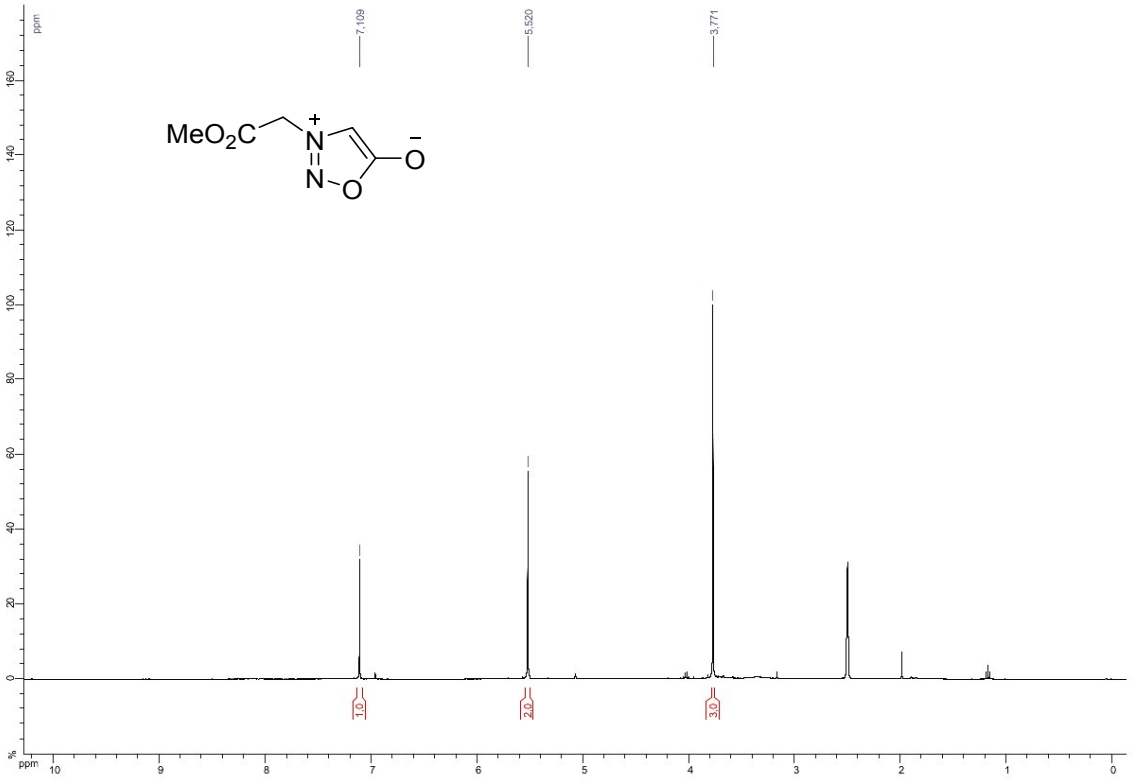
140
120
100
80
60
40
20
0


7.109
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3.771
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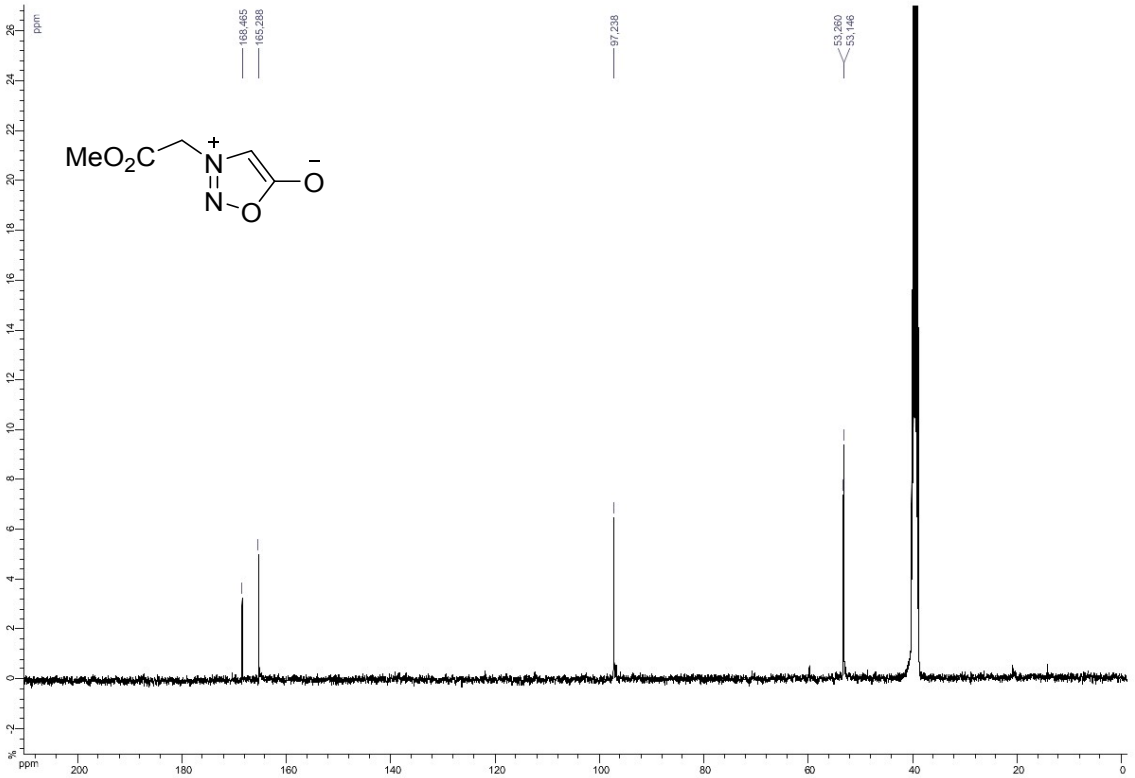
2.0
3.0
ppm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97.238
53.260
53.146
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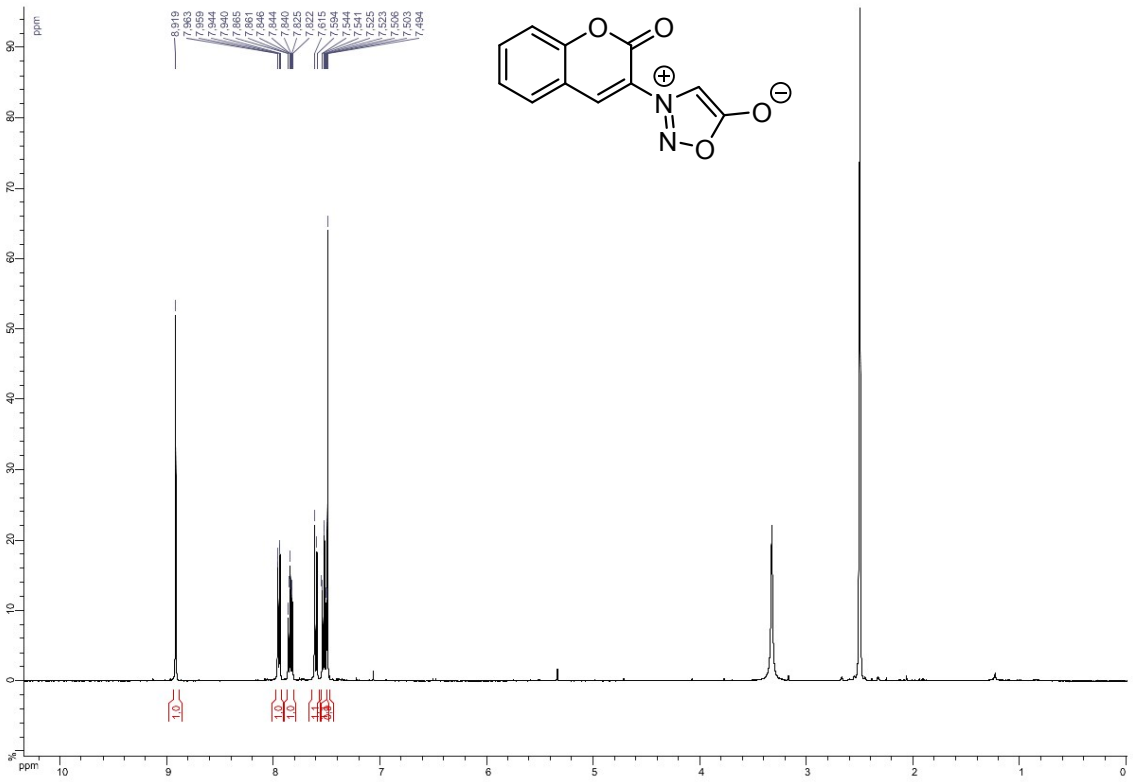
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7.959
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7.940
7.885
7.881
7.848
7.844
7.840
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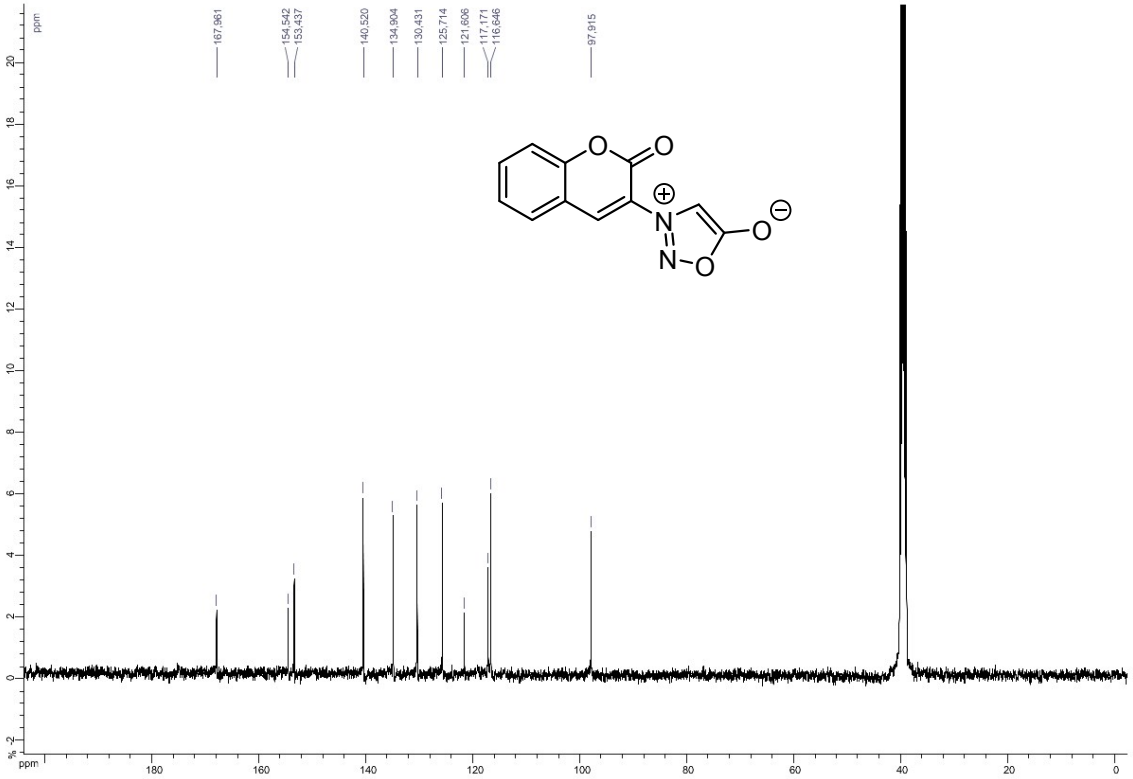
7.822
7.615
7.594
7.544
7.541
7.525
7.523
7.506
7.503
7.494
ppm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140.520
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121.606
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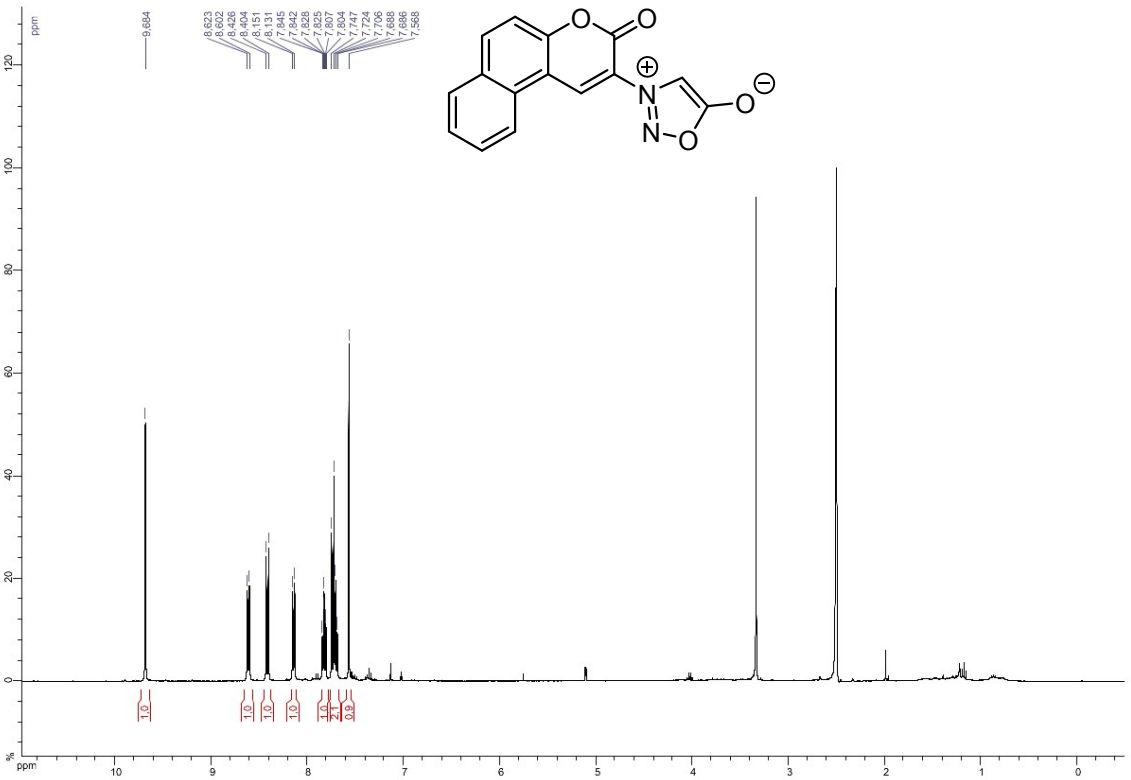
116.646
97.915
ppm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8.426
8.404
8.151
8.131
7.845
7.842
7.828
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7.807
7.804
7.747
7.724
7.706
7.688
7.686
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1.0
1.0
1.0
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0.8
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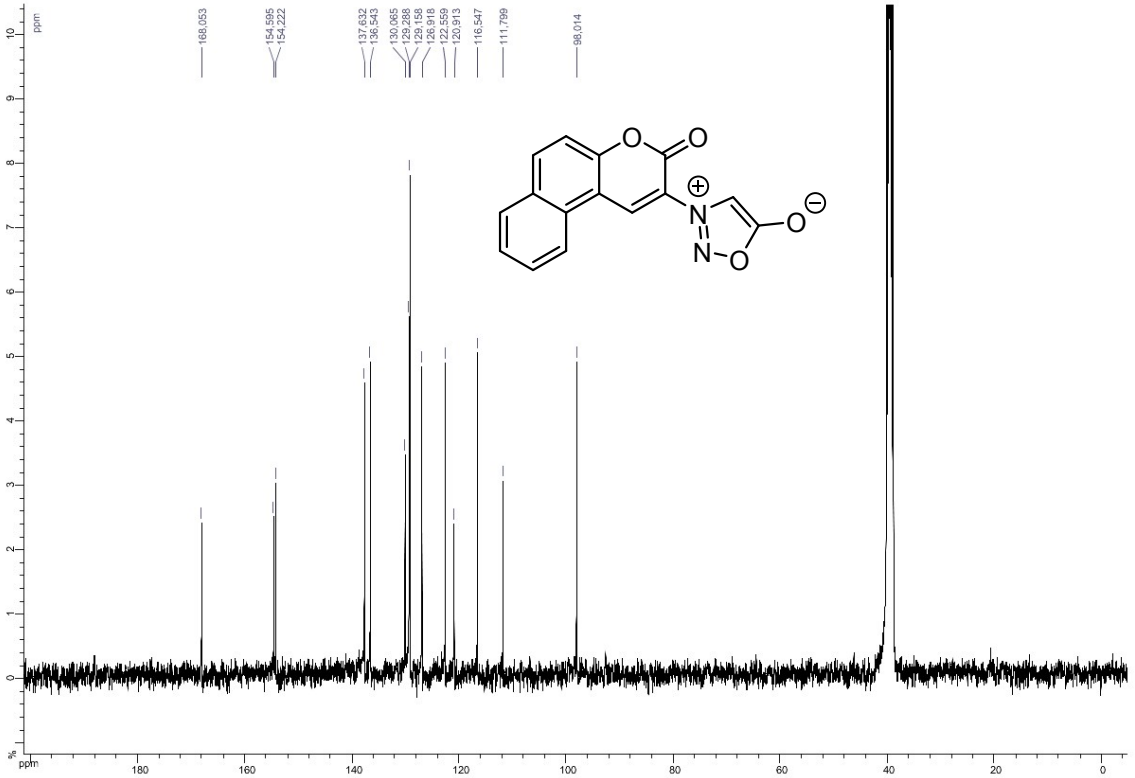
O
N
N
O
O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154.222
137.632
136.543
130.065
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129.158
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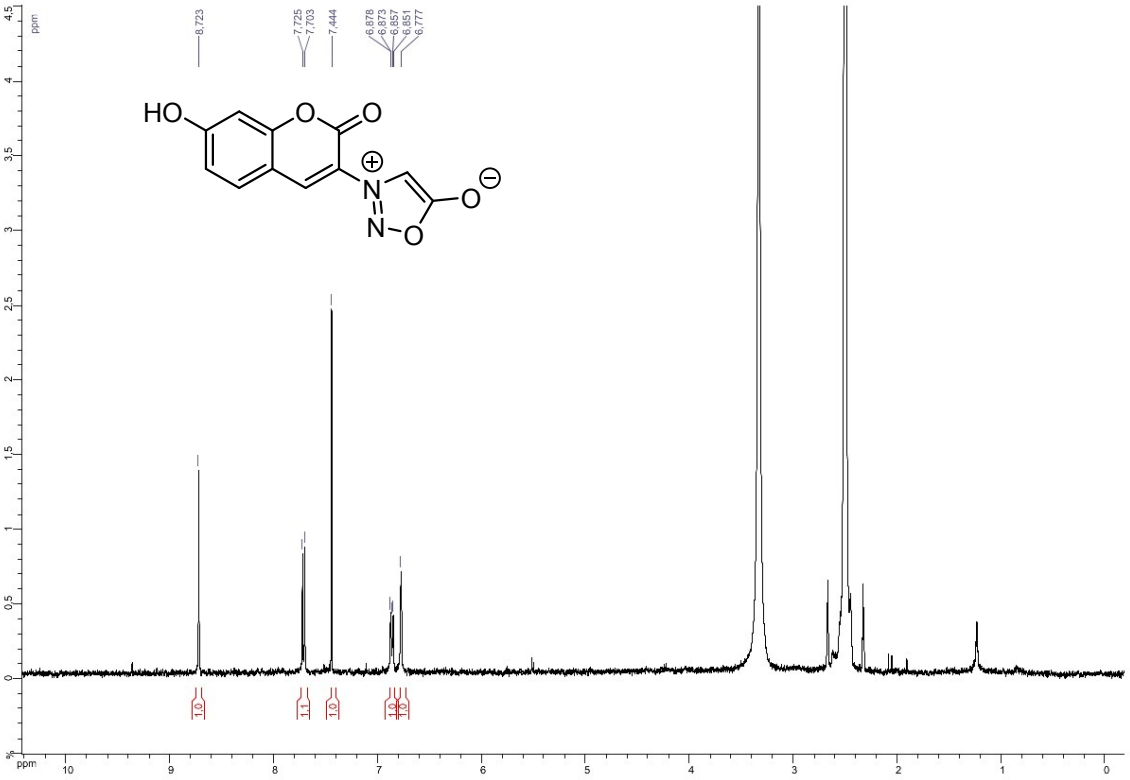
125.559
120.913
116.547
111.799
98.014
O
O
N
N
O
O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6.873
6.857
6.851
6.777
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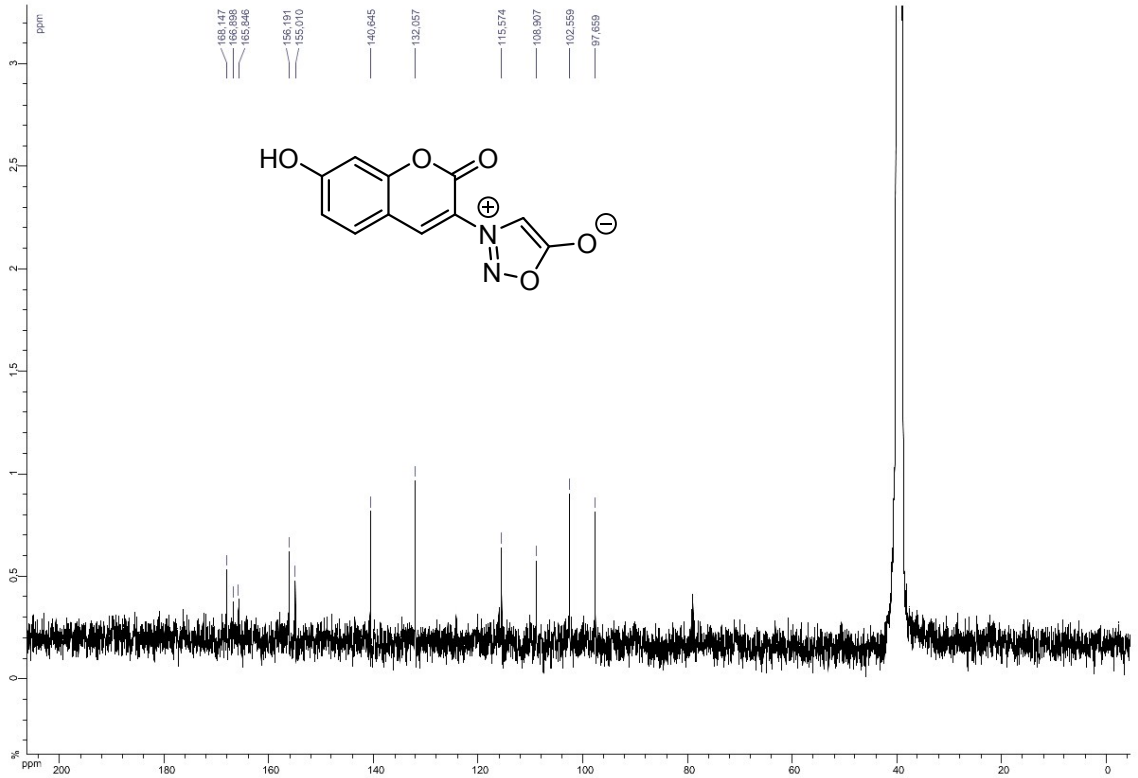
O
ppm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165.846
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140.645
132.057
115.574
108.907
102.559
97.659
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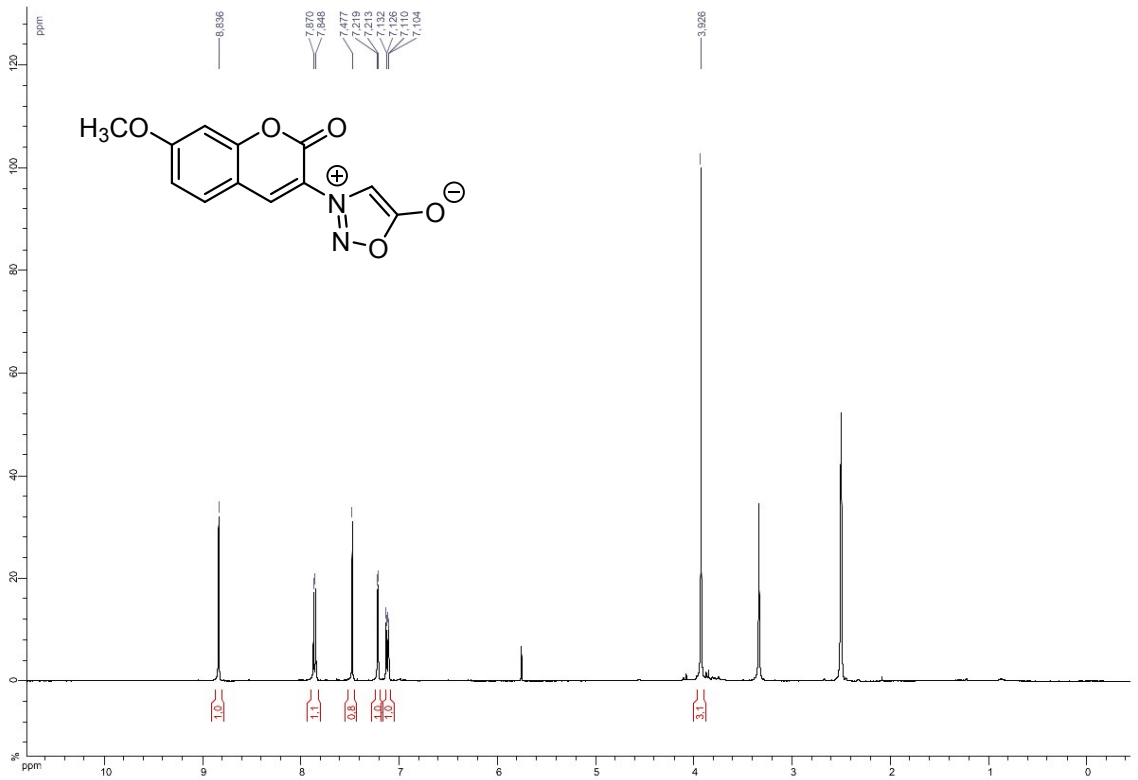
O
N
O
O
ppm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7,219
7,213
7,132
7,126
7,110
7,104
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H3CO
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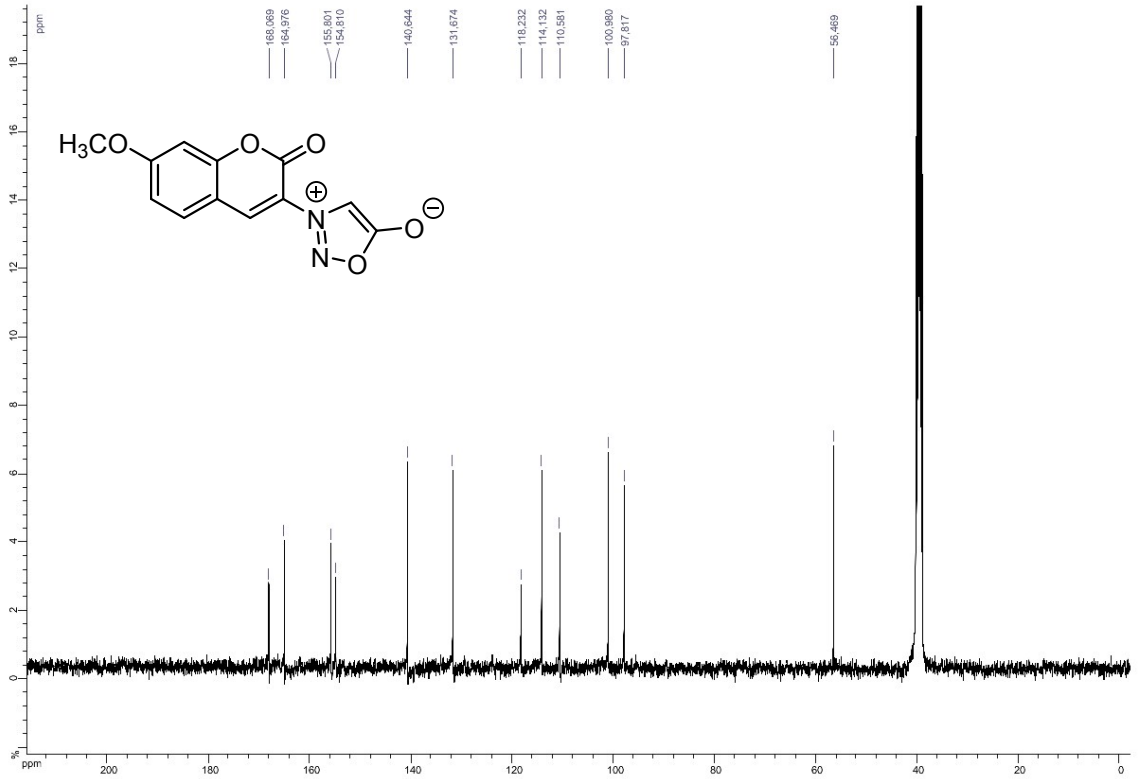
O
N
O
N
O
1,0
1,1
0,8
1,0
1,0
3,1
ppm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154,810
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H3CO
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O
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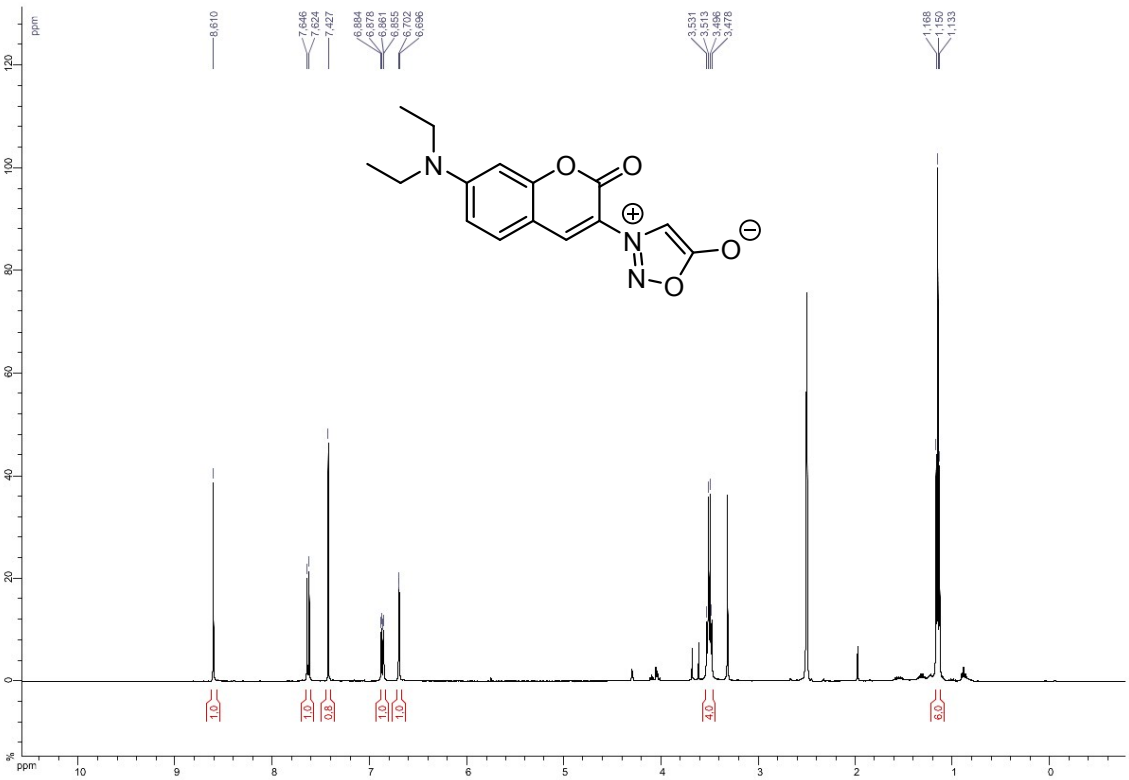
O
N
O
ppm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7.624
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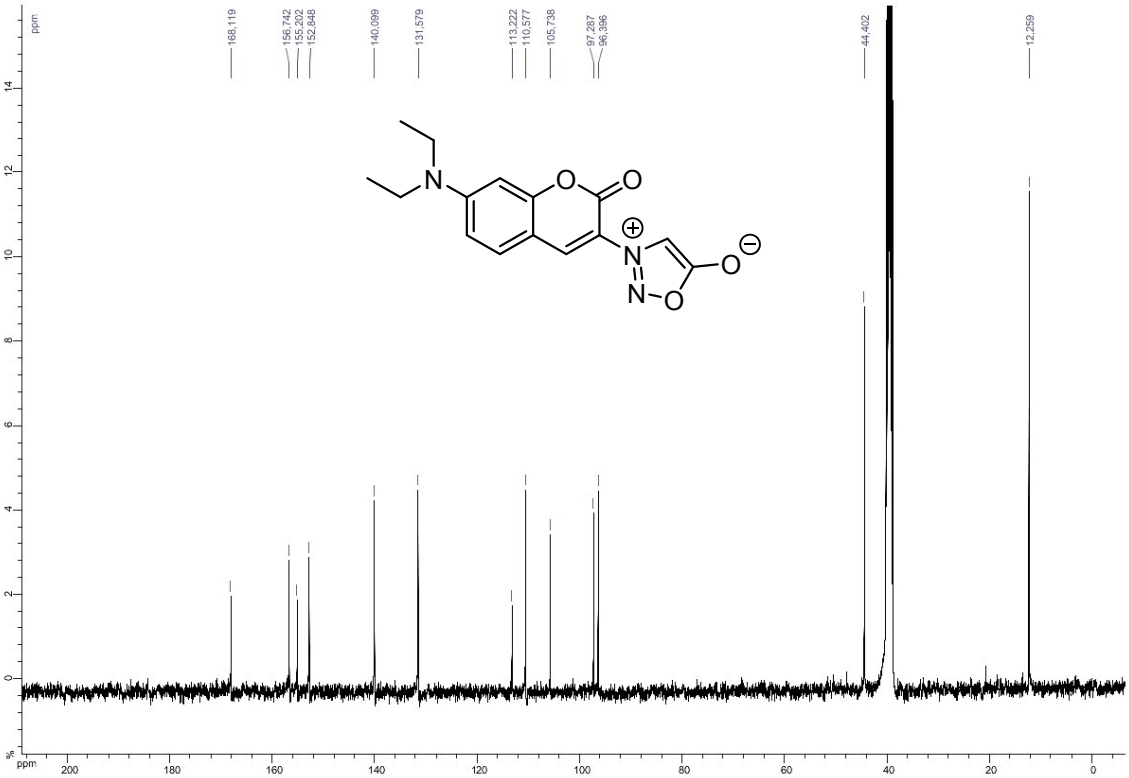
6.884
6.878
6.861
6.855
6.702
6.696
3.531
3.513
3.496
3.478
1.168
1.150
1.133
1.0
1.0
0.8
1.0
1.0
4.0
6.0
ppm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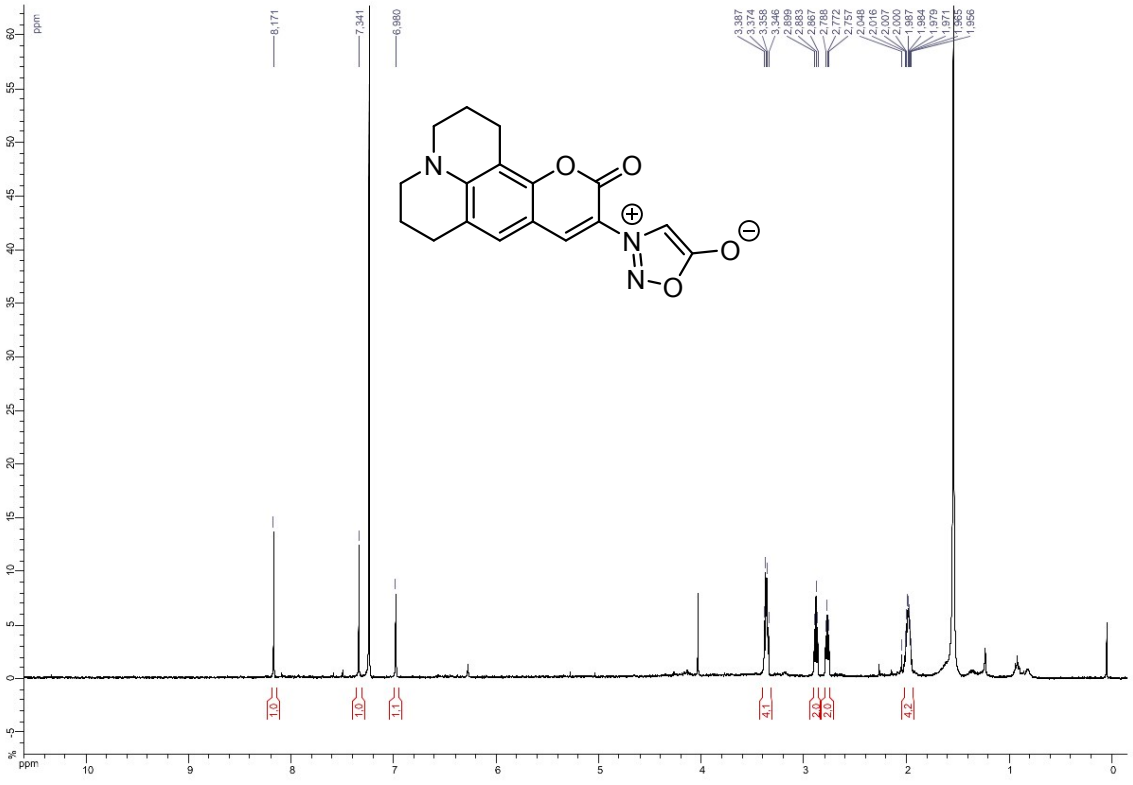
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3,358
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2,867
2,788
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2,757
2,048
2,016
2,007
2,000
1,987
1,964
1,979
1,971
1,965
1,956
1,0
1,0
1,1
4,1
2,0
2,0
4,2
%
ppm
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9
8
7
6
5
4
3
2
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0
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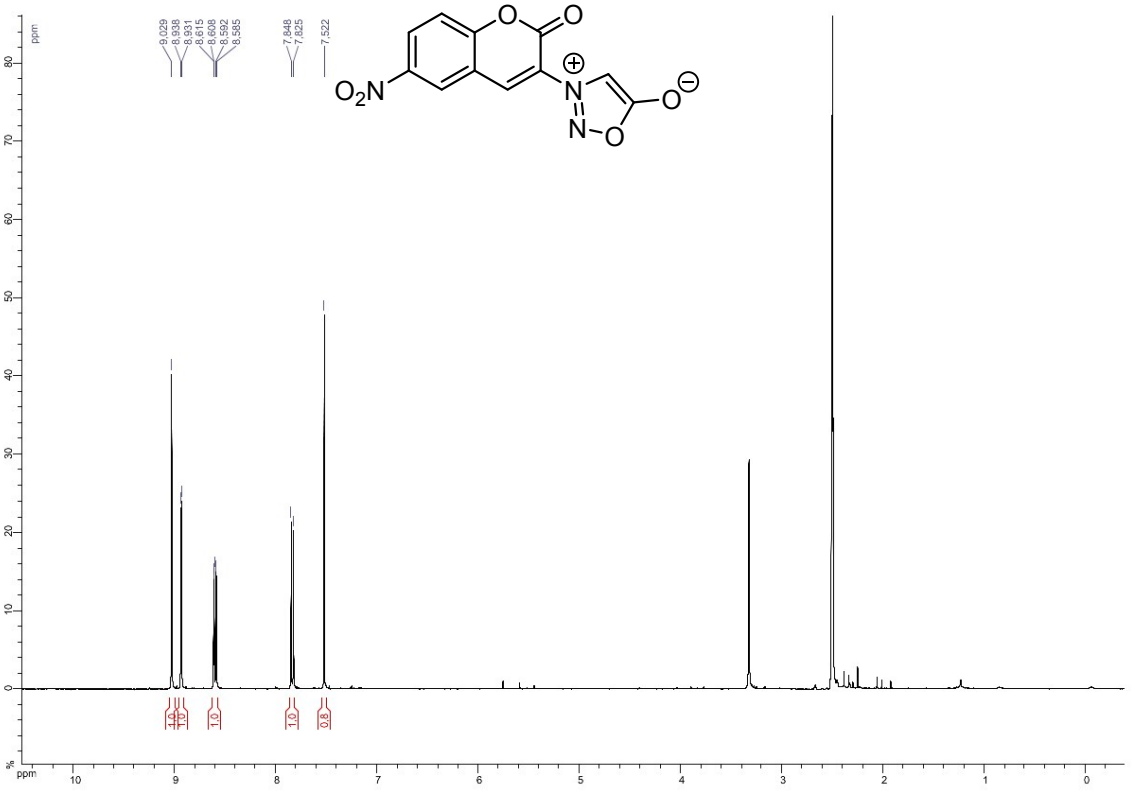
O
N
N
O
O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8.608
8.592
8.585
7.848
7.825
7.522
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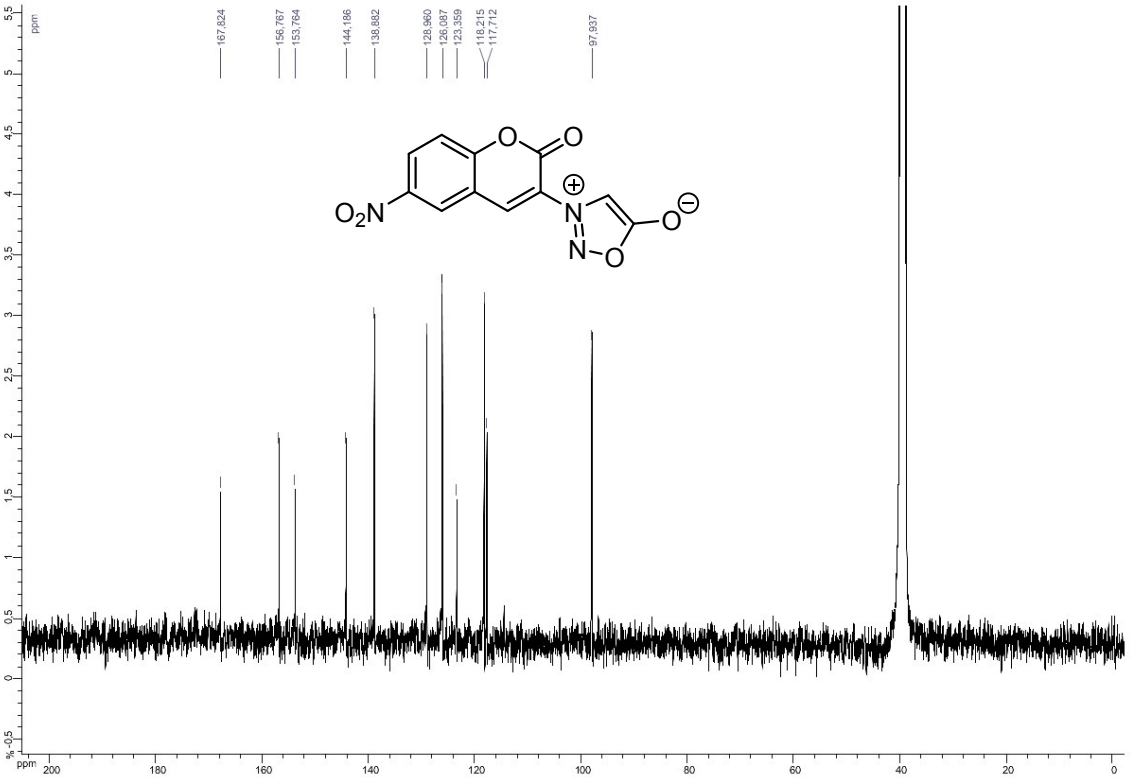
O
N
O
ppm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O
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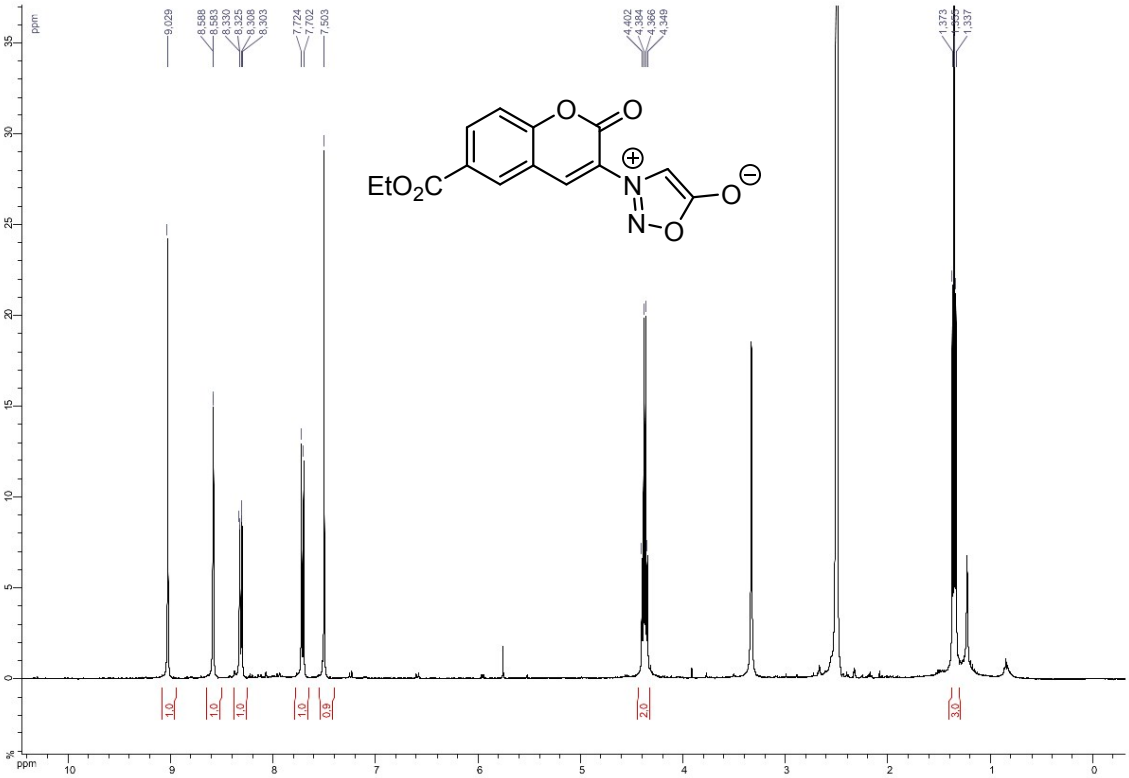
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8.325
8.308
8.303
7.724
7.702
7.503
4.402
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4.366
4.349
1.373
1.355
1.337
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1.0
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2.0
3.0
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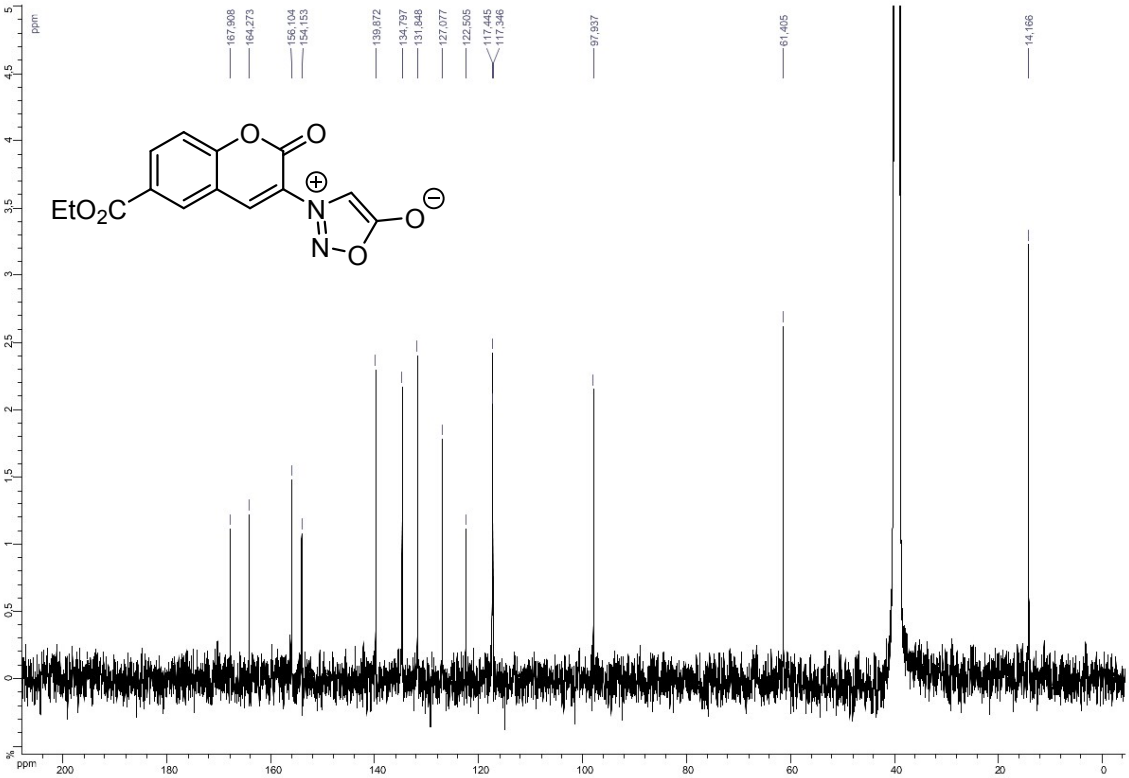
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14.166
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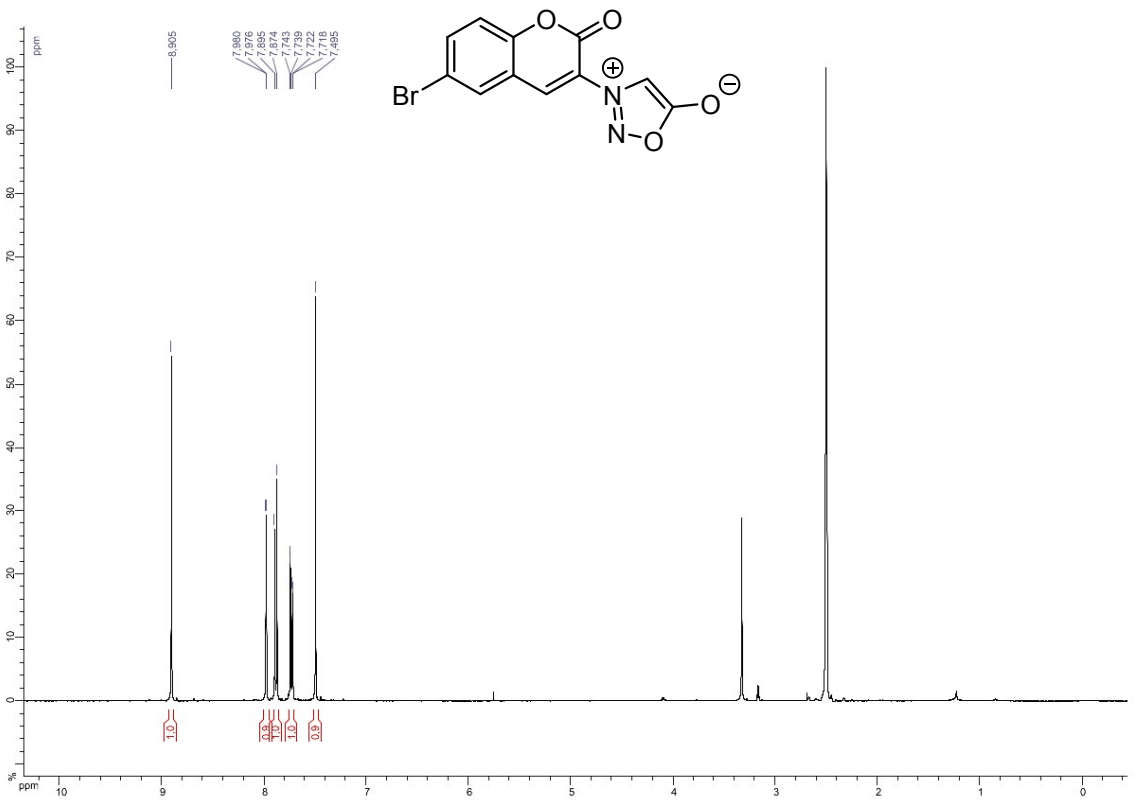
O
ppm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7.874
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7.722
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Br
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N
O
O
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0.8
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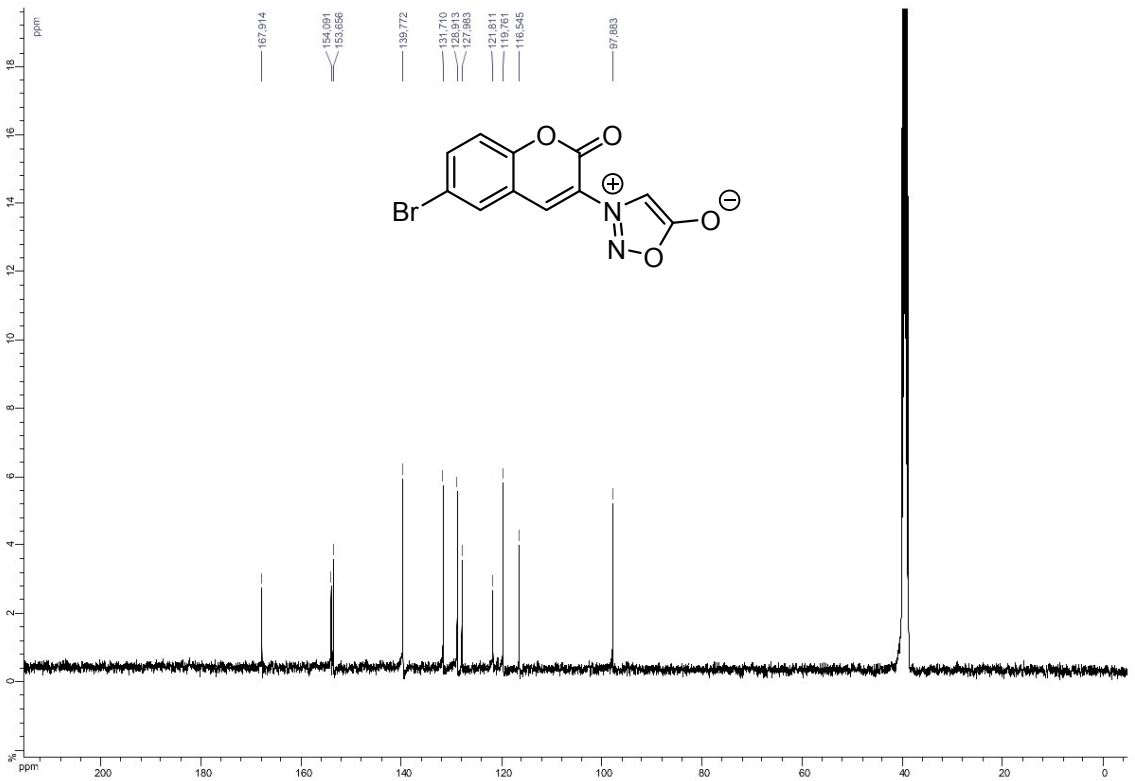
1.0
0.9
ppm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128.913
127.983
121.811
119.761
116.545
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Br
O
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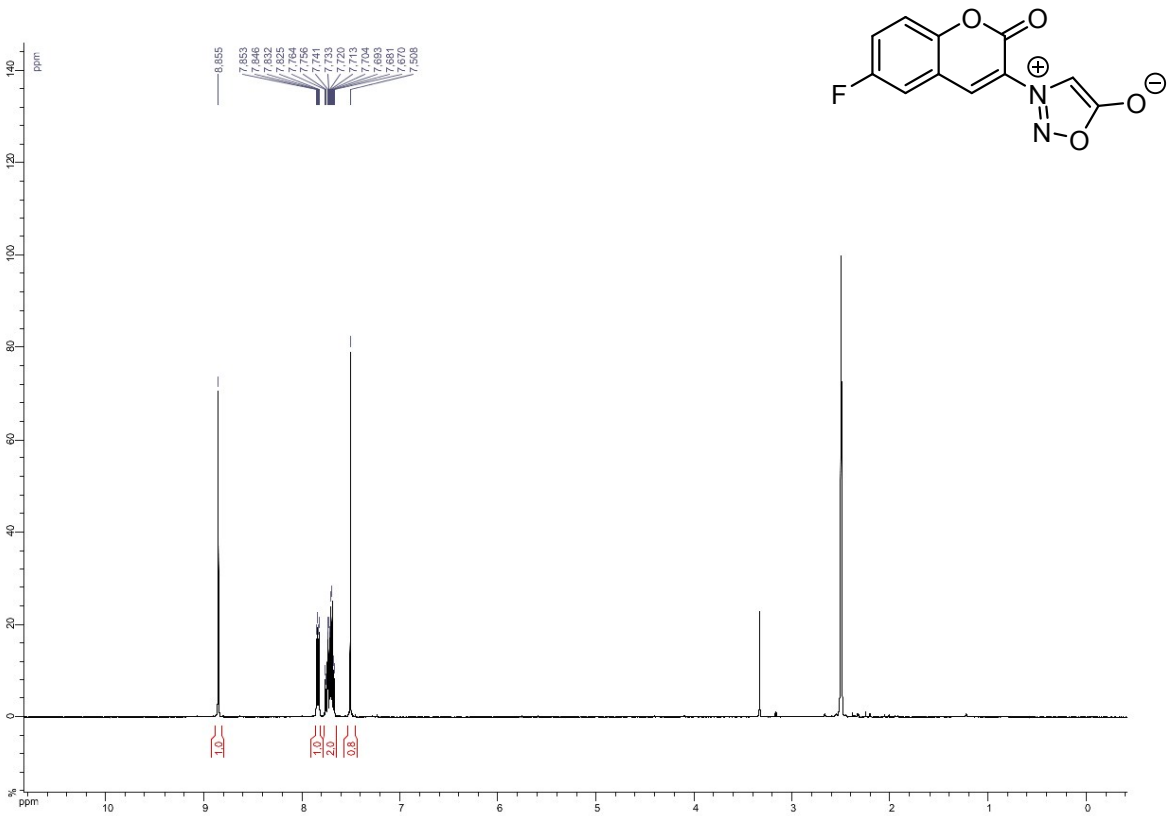
N
O
O
ppm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7,741
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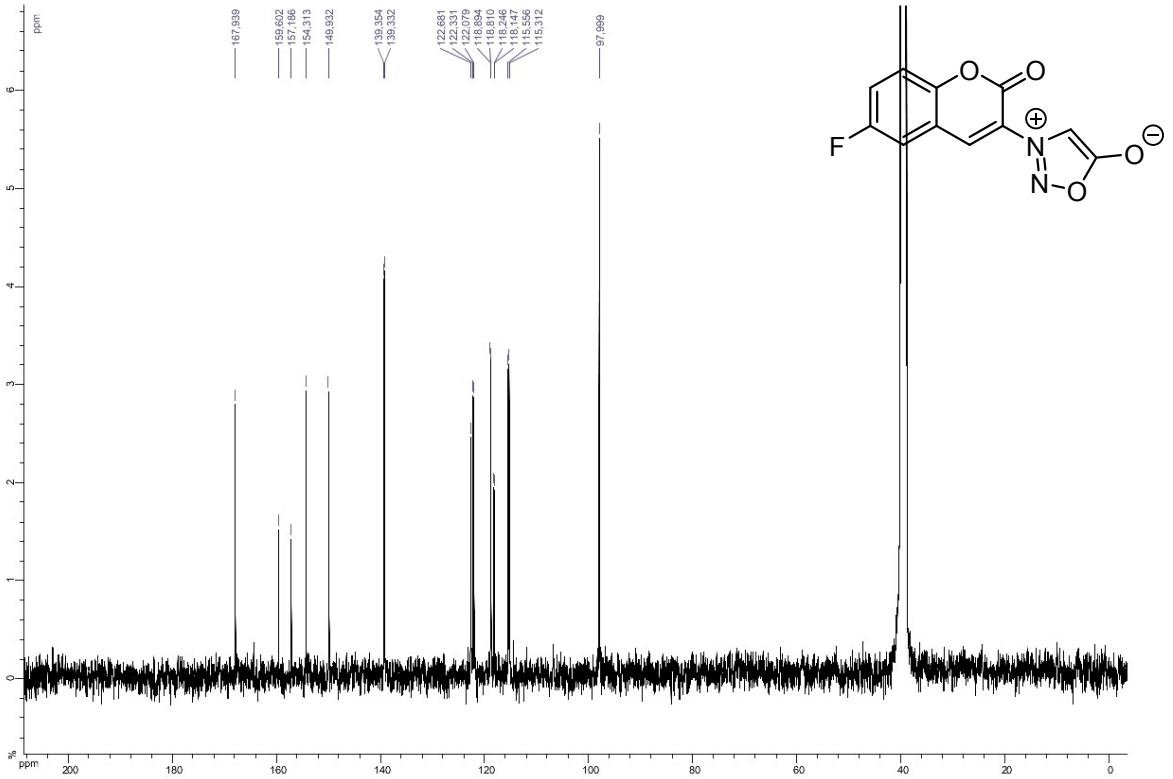
7,720
7,713
7,704
7,693
7,681
7,670
7,508
1,0
1,0
2,0
0,8
F
O
O
N
N
O
O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159,602
157,186
154,313
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139,354
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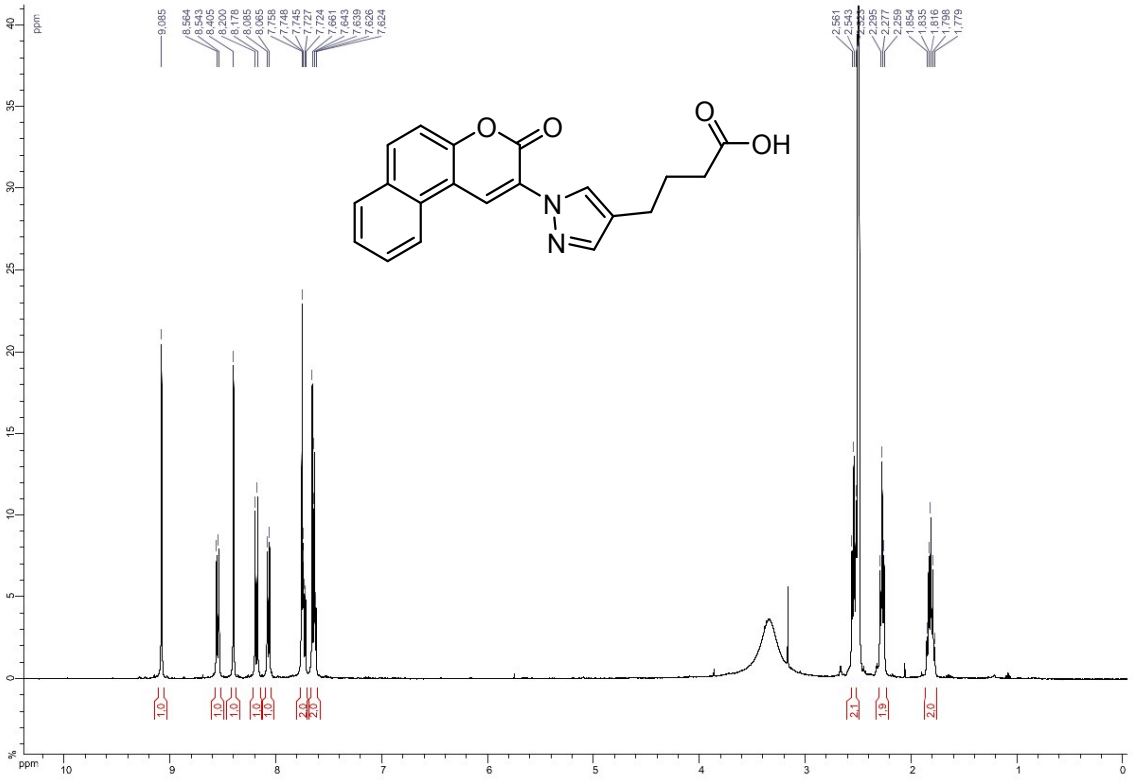
122,331
122,079
118,894
118,810
118,246
115,477
115,556
115,312
97,999
F
O
O
N
N
O
O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8.200
8.178
8.086
8.065
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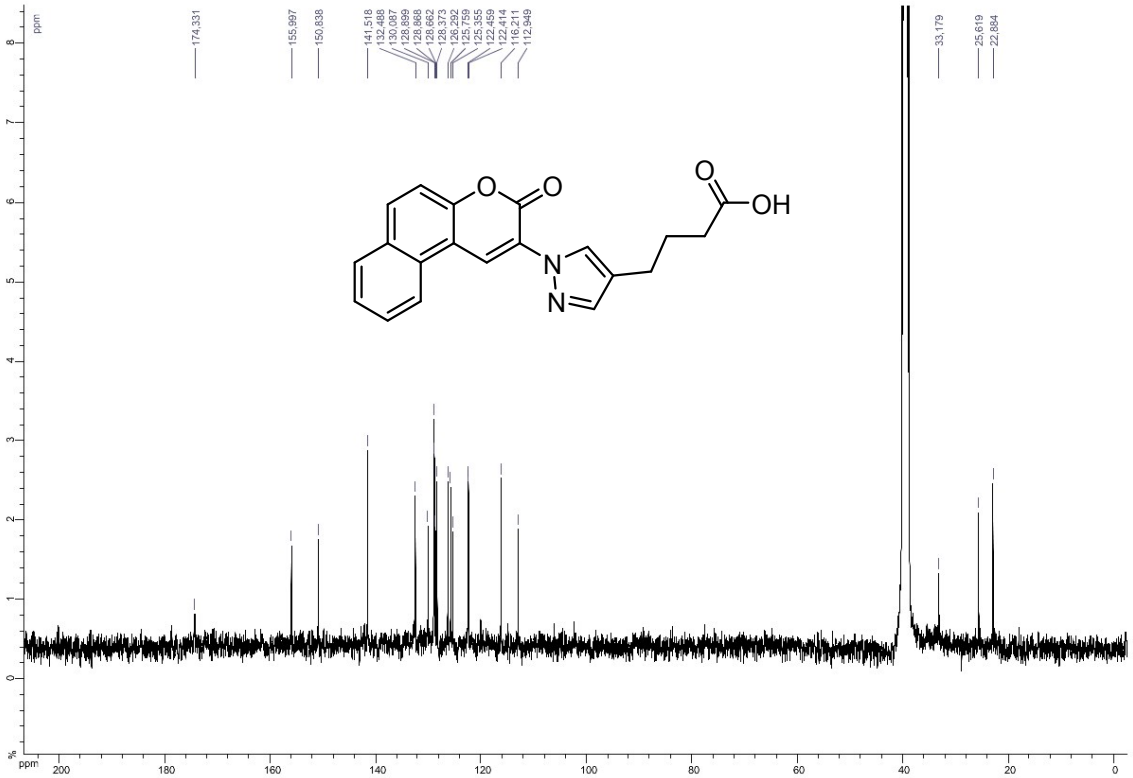
7.748
7.745
7.727
7.724
7.661
7.643
7.639
7.626
7.624
2.561
2.543
2.295
2.277
2.259
1.854
1.835
1.816
1.798
1.779
O
O
OH
N
N
ppm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130.087
128.899
128.868
128.652
128.373
126.292
125.759
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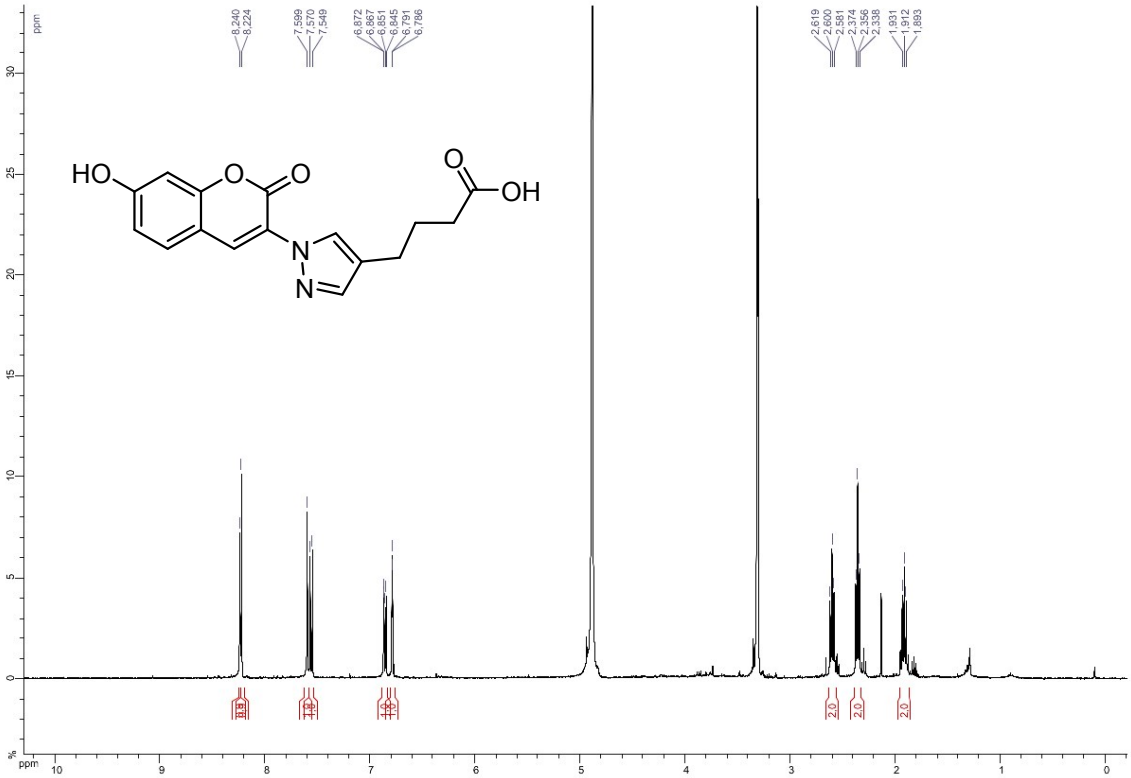
116.211
112.949
33.179
25.619
22.884
O
O
OH
N
N
ppm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6.791
6.786
2.619
2.600
2.581
2.374
2.355
2.336
1.931
1.912
1.893
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O
O
OH
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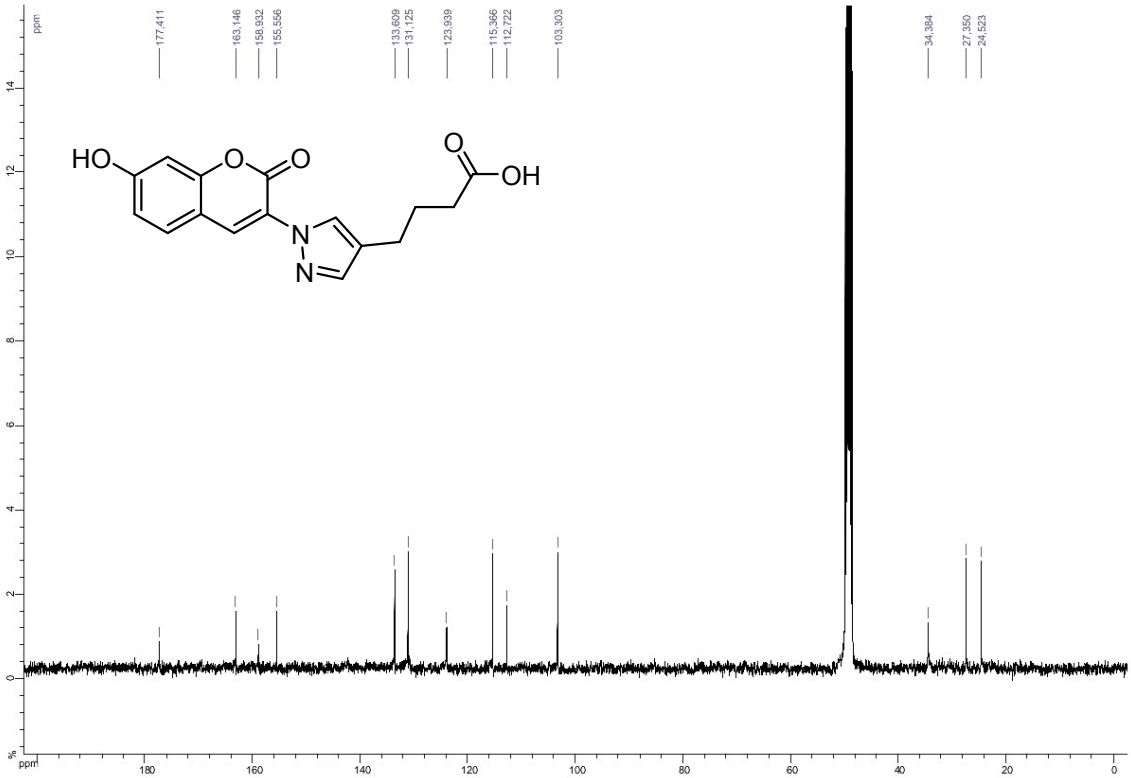
2.0
2.0
2.0


ppm
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158.932
155.556
133.609
131.125
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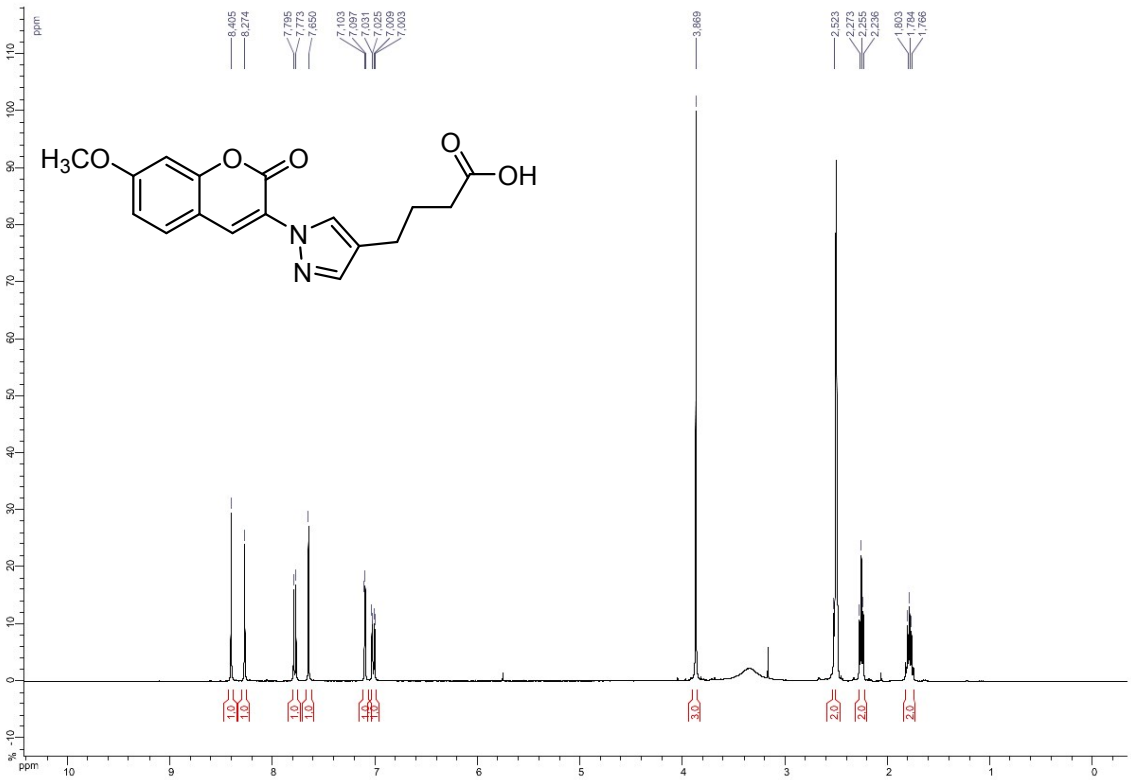
115.366
112.722
103.303
34.384
27.350
24.523
HO
O
O
O
OH
N
N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7.795
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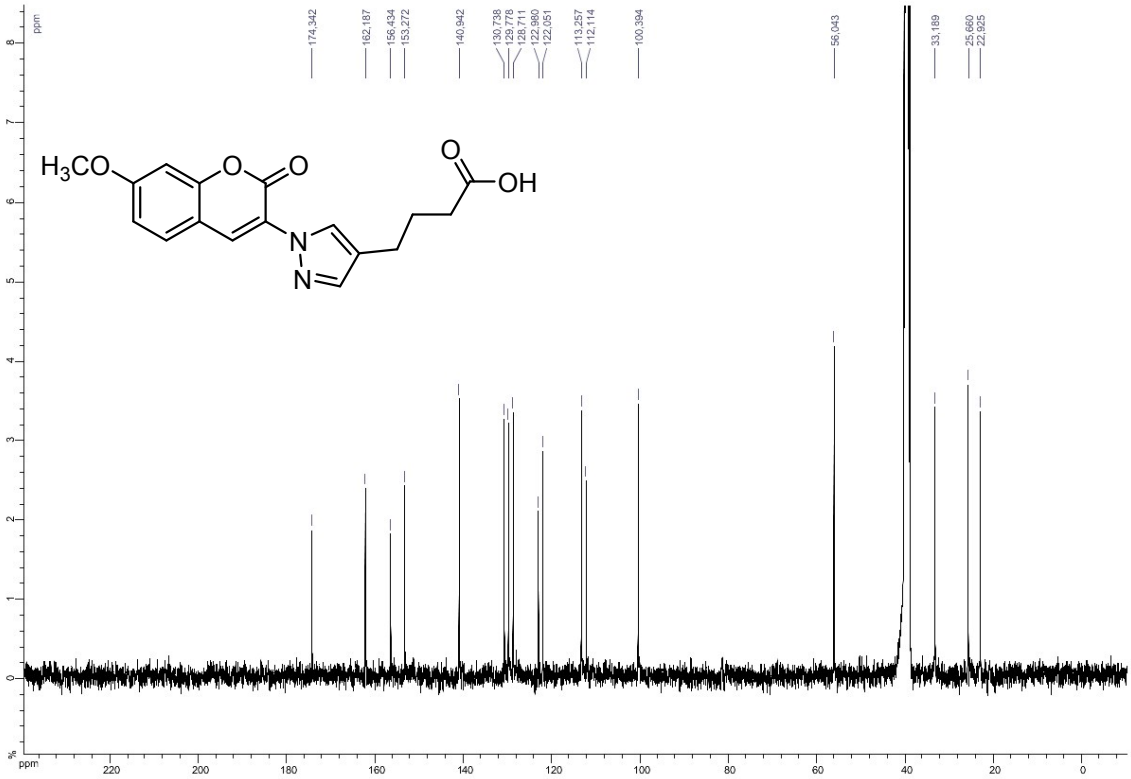
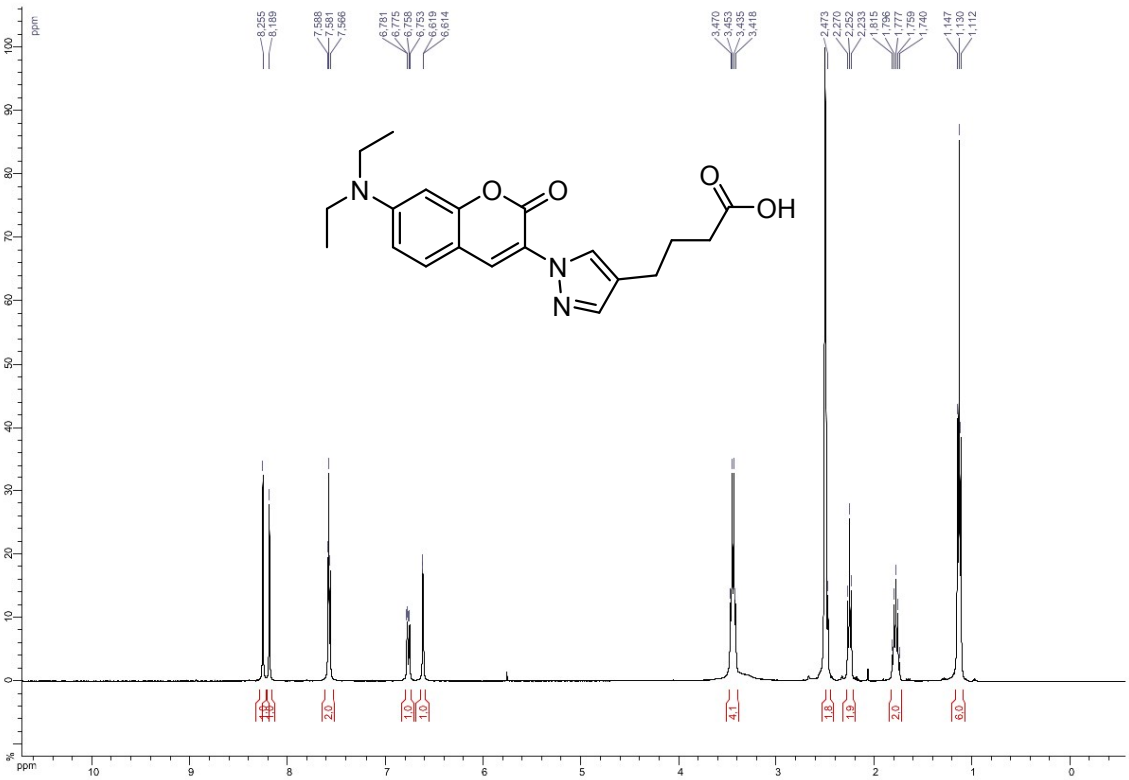
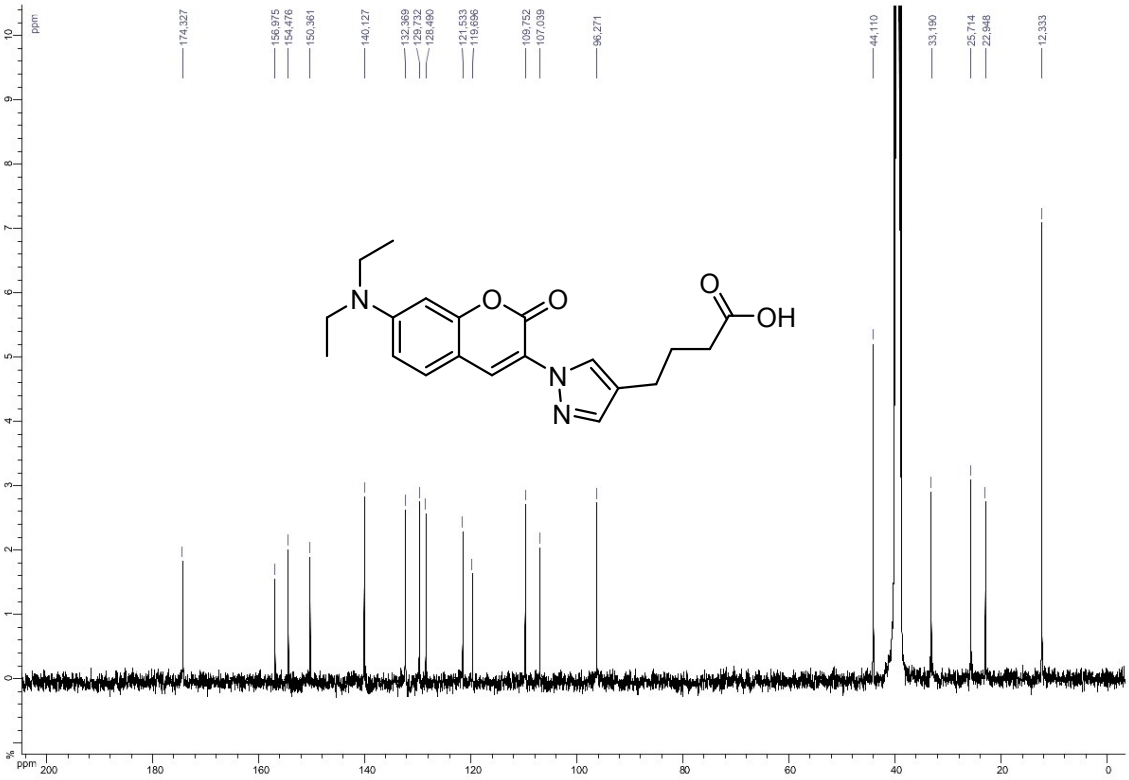
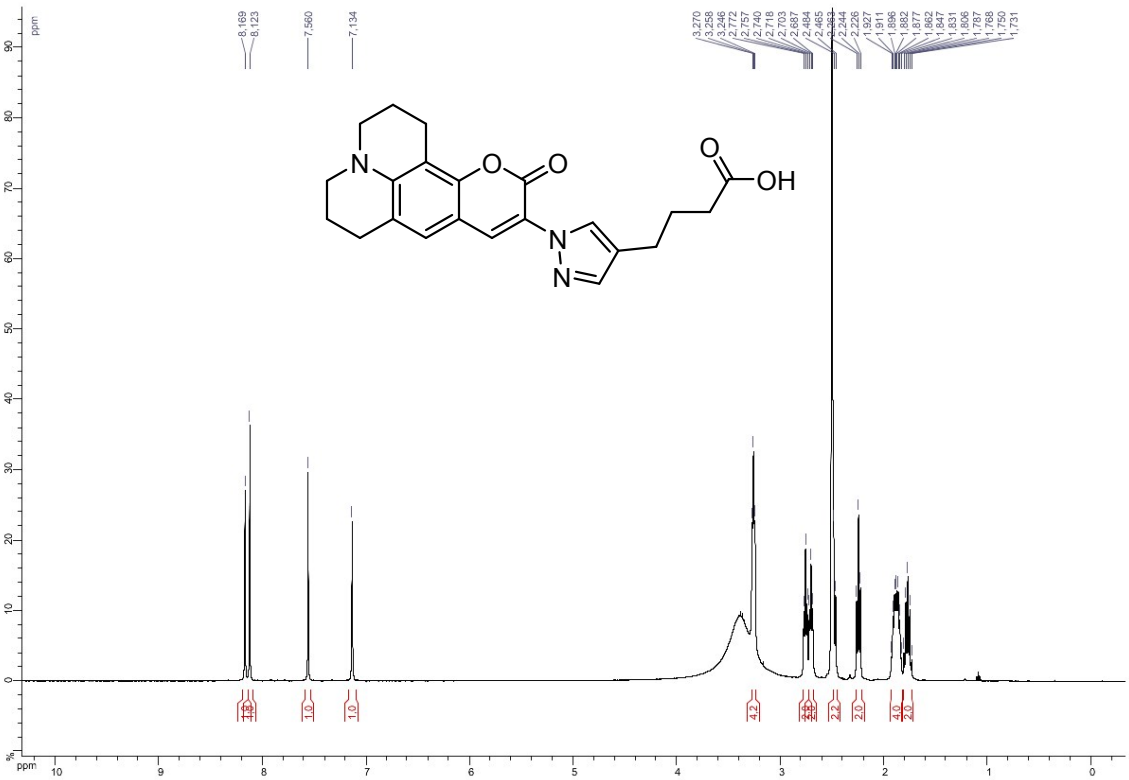
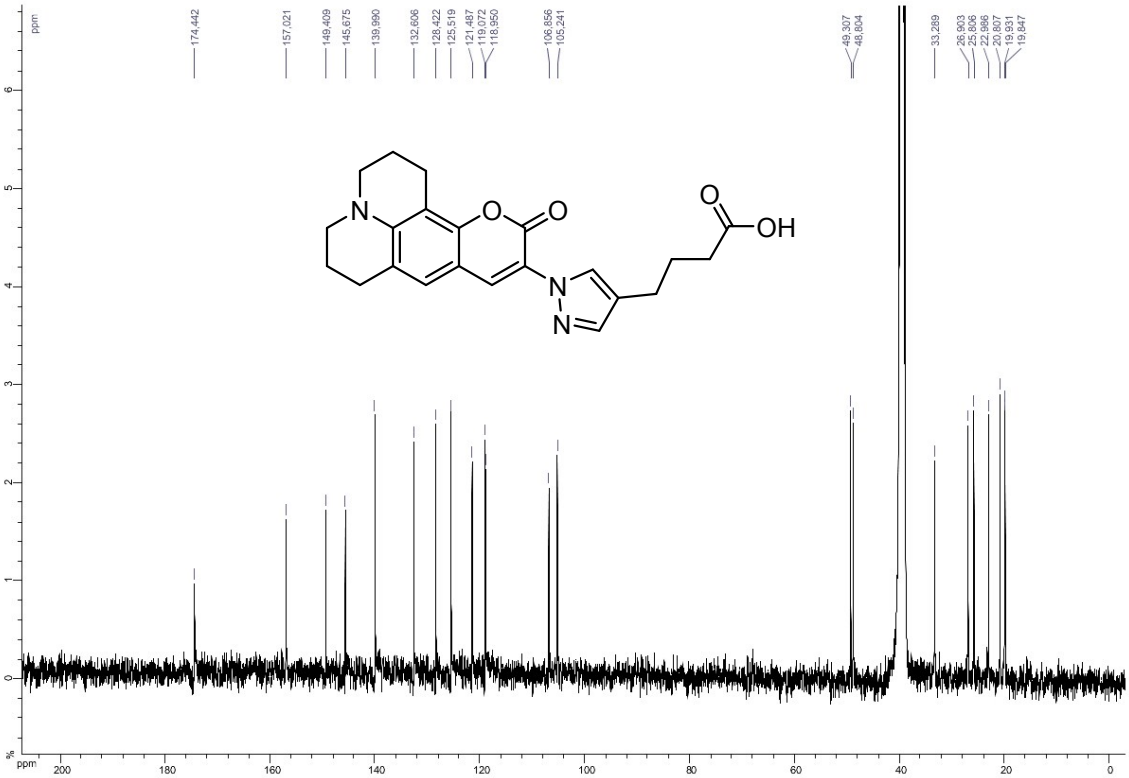
7.650
7.103
7.097
7.031
7.025
7.009
7.003
3.869
2.523
2.273
2.255
2.236
1.803
1.784
1.766
H3CO
O
O
O
OH
N
N
ppm


174.342
162.187
156.434
153.272
140.942
130.738
129.778
128.711
122.980
122.051
113.257
112.114
100.394
56.043
33.189
25.660
22.925
H3CO
O
O
O
OH
N
N
ppm


8.255
8.189
7.588
7.581
7.566
6.781
6.775
6.758
6.753
6.619
6.614
3.470
3.453
3.435
3.418
2.473
2.270
2.252
2.233
1.815
1.796
1.777
1.759
1.740
1.147
1.130
1.112
N
O
O
O
OH
N
N
ppm


174.327
156.975
154.476
150.361
140.127
132.369
129.732
128.490
121.533
119.696
109.752
107.039
96.271
44.110
33.190
25.714
22.948
12.333
N
O
O
O
OH
N
N
ppm


8.169
8.123
7.560
7.134
O
OH
N
O
O
N
N


174.442
157.021
149.409
145.675
139.990
132.606
128.422
125.519
121.487
119.072
118.950
106.856
105.241
49.307
48.804
33.289
26.903
25.805
22.996
20.807
19.931
19.847
O
OH
N
O
O
N
N

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[ii] Williams, A. T. R.; Winfield, S. A.; Miller, J. N. *Analyst* **1983**, *108*, 1067.
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