Supporting Information

Fluorogenic Sydnone-Modified Coumarins Switched-On by Copper-Free Click Chemistry

Camille Favre[1,2] and Frédéric Friscourt*[1,2]

[1]Institut Européen de Chimie et Biologie, Université de Bordeaux, 2 rue Robert Escarpit, 33607 Pessac, France.

[2]Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, CNRS UMR5287, Bordeaux, France

Email: f.friscourt@iecb.u-bordeaux.fr

Table of Contents

Materials and Methods	S2
Synthetic Procedures	S4
Protein labeling	S7
Kinetics data (Figs. S1-S2)	S8
Absorption and Fluorescence Spectra (Figs. S3-S7)	S9
Computational data (Tables S1-S4)	S11
References	S15
^{1}H and ^{13}C NMR Spectra	S16

Materials and Methods

All reactions were carried out under a dry argon environment. All solvents were of reagent grade and used as received. Dichloromethane and tetrahydrofuran were dried over alumina columns under nitrogen through a solvent purification system. Chemicals and reagents were used as commercially supplied without any further purification unless otherwise stated. Room temperature refers to ambient temperature (20-22 °C). Reactions were monitored by Thin Layer Chromatography (TLC) using aluminum backed silica gel 60 (F254) plates, visualized using UV254 nm and 365 nm and potassium permanganate dips as appropriate. Column chromatography was carried out using silica gel G60 (Fluka analytical, 230-400 mesh, 40-63 μm particle size, 60 Å) as the stationary phase. Azide-FITC **17** was synthesized according to the literature.[1]

NMR Spectroscopy

^{1}H and ^{13}C-NMR spectra were recorded on a Brüker 300 MHz spectrometer and kinetics measurements were recorded on a Brüker 400 MHz. Chemical shifts are reported in δ units, parts per million (ppm) downfield from TMS. Coupling constants (*J*) are reported in Hertz (Hz) without adjustments; therefore, due to limits in digital resolution, in some cases there are small differences (<1 Hz) in the measured *J* value of the same coupling constant determined from different signals. Splitting patterns are designed as follows: s – singlet, d – doublet, t – triplet, dd – doublet of doublets, dt – doublet of triplets, td – triplet of doublets, ddd – doublet of doublet of doublets, tt – triplet of triplets, sp – septet, hept – heptet, m – multiplet, br – broad. Various 2D techniques experiments were used to establish the structures and to assign the signals.

Mass Analysis

High-resolution mass spectra were obtained with an electrospray ionization Thermo Exactive orbitrap mass spectrometer.

X-ray crystallography

Data collections were performed at the IECB X-ray facility on a high flux microfocus Rigaku FRX rotating anode at the copper kα wavelength equipped with a Dectris Pilatus 200K hybrid detector at 150K. The crystals were mounted on cryo-loops after quick soaking on Paratone—N oil from Hampton research and flash-frozen. The data were processed with the CrysalisPro software version 38.43. Crystal structures were solved using SHELXT.[2] Both structures were refined using

SHELXL 2017 version.[3] Full-matrix least-squares refinement were performed on F^2 for all unique reflections, minimizing $w(Fo^2 - Fc^2)^2$, with anisotropic displacement parameters for non-hydrogen atoms. All H atoms found in difference electron-density maps were refined freely, all the other were treated as riding on their parent C or N atoms. Data statistics are reported in the cif files.
CCDC numbers: Compound **4**: CCDC 1844074 and compound **5**: CCDC 1844087.

Absorption and Fluorescence Measurements

UV−vis and Fluorescence spectra were recorded at 25 ± 0.1 °C using a SpectraMax M2E Multi-Mode Cuvette/Microplate Reader from molecular devices using a cuvette with 1 cm path length.

Quantum Yield Determination

Quantum yields were determined from the slope of the integrated fluorescence emission between 340 and 700 nm (excitation at 320 nm) for **11**, 360 and 700 nm (excitation at 340 nm) for **5** and **14**, 370 and 700 nm (excitation at 350 nm) for **4** and **13** versus absorbance using quinine sulfate in 1.0 N H_2SO_4 (Φ_F = 0.54 ± 0.03) as fluorescence standard. For each compound, four data points were acquired with absorbances ranging between 0.1 and 0.6 (l = 1 cm).

In-Gel Visualization

Following reaction with sydnone probes **4** or **5**, some protein samples were analyzed via SDS-PAGE (10% gradient) and visualized by in-gel fluorescence scanning (BioRad, ChemiDoc MP imager, trans-UV excitation (302 nm)/standard emission filter). The gels were then stained with Coomassie Blue to reveal total protein content.

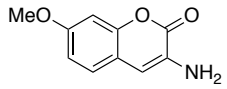
Computational Studies

All quantum chemical studies were performed with the Gaussian 09 (Rev. A02) software package.[4] Molecular structures for compounds **4**, **5**, **15** and **16** were obtained by optimizing their gas-phase geometries by DFT with the B3LYP hybrid functional and Pople's 6-311G(d) split valence basis set. To account for bulk solvent effects, the gas-phase geometries were further geometry-optimized with inclusion of the polarizable continuum model (PCM), which creates a solute cavity based on a set of overlapping spheres.[5] Coordinates for the final geometries are listed in Tables S1-S4. All structures were validated based on vibrational frequency analysis to ensure a stationary point on the ground state potential surface. Vertical excitation energies were computed based on the solvent-equilibrated ground-state geometries using TD-DFT with the 6-311+G(d) basis set with

added diffuse functions. Electron density differences between the ground and respective excited states were determined from the corresponding Gaussian cube output files and visualized with the VMD software package.[6]

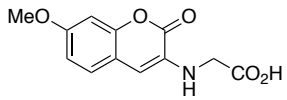
Synthetic Procedures

3-Amino-7-methoxy-coumarin 9.



A solution of dicyclohexylcarbodiimide (2.48 g, 12.0 mmol) and *N*-acetylglycine (2.81 g, 24.0 mmol) was stirred in dichloromethane (40 mL) at room temperature overnight. The resulting solid was filtered off and washed with dichloromethane (30 mL). The remaining filtrate, containing anhydride **7**, was then concentrated under reduced pressure and the remaining residue was dissolved in *N*,*N*′-dimethylformamide (12 mL), to which 2-hydroxy-4-methoxybenzaldehyde **6** (913 mg, 6.0 mmol) and sodium acetate (738 mg, 9.0 mmol) were added. The resulting mixture was then refluxed at 120 °C for 1 h. The solution was cooled down to room temperature and water was added. The precipitate was filtered off, washed with water (30 mL), triturated in cold ethanol (10 mL), re-filtered and washed with cold ethanol (10 mL) to afford 7-methoxy-3-*N*-acetylamino-coumarin **8**. Without any further purification, the coumarin **8** was dissolved in a mixture of concentrated hydrochloric acid (20 mL), ethanol (10 mL) and water (10 mL). The solution was then refluxed for 1 h. After being cooled down to room temperature, the mixture was neutralized by addition of solid sodium bicarbonate. The resulting precipitate was filtered off, washed with water (100 mL) and dried under reduced pressure to afford pure 3-aminocoumarin **9** (859 mg, 75%) as a light yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (s, 3H), 4.05 (br s, 2H), 6.70 (s, 1H), 6.80-6.83 (m, 2H), 7.20 (d, J = 9.3 Hz, 1H) in agreement with the literature data.[7]

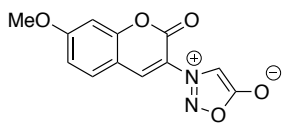
[(7-Methoxy-2-oxo-2H-chromen-3-yl)amino]acetic acid 10.



A solution of 3-aminocoumarin **9** (180 mg, 0.94 mmol) and bromoacetic acid (130 mg, 0.94 mmol) in water (10 mL) was stirred at 95 °C for 3 h. After being cooled to 0 °C, the formed precipitate was filtered off, washed with water (30 mL), triturated in dichloromethane, re-filtered and dried under reduced pressure to afford the pure coumarin acetic acid **10** (95 mg, 41%) as a light brown solid: ^{1}H NMR (300 MHz, methanol-d_4): δ 3.82 (s, 3H), 3.93 (s, 2H), 6.48 (s, 1H), 6.83-6.86 (m, 2H), 7.32 (d, J = 9.3 Hz, 1H); ^{13}C NMR

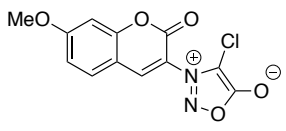
(75 MHz, DMSO-d_6): δ 44.6 (CH_3), 55.6 (CH_2), 100.5 (CH), 106.1 (CH), 112.4 (CH), 114.7 (C), 126.0 (CH), 130.5 (C), 148.6 (C), 157.9 (C), 158.7 (C), 171.2 (C); HRMS (*m/z*): $[M-H]^-$ calcd. for $C_{12}H_{10}NO_5$, 248.0564; found, 248.0564.

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



t-Butyl nitrite (0.03 mL, 0.25 mmol) was added dropwise to a solution of glycine **10** (51 mg, 0.2 mmol) in tetrahydrofuran (3 mL) and was stirred at room temperature for 1 h. Trifluoroacetic anhydride (0.03 mL, 0.25 mmol) was then added to the previous solution and the reaction mixture was stirred for an additional hour. The mixture was then quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL). The organic layer was extracted with dichloromethane (3 × 15 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (10 g) using a mixture of dichloromethane and ethyl acetate (95:5) to afford the coumarin sydnone **4** (35 mg, 68%) as a yellow solid. The compound could be recrystallized from methanol if necessary: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.96 (s, 3H), 6.94 (d, *J* = 2.3 Hz, 1H), 7.03 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.32 (s, 1H), 7.61 (d, *J* = 8.8 Hz, 1H), 8.45 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.4 (CH_3), 97.4 (CH), 101.1 (CH), 110.4 (C), 115.2 (CH), 116.8 (C), 131.2 (CH), 138.2 (CH), 154.5 (C), 156.4 (C), 166.0 (C), 168.8 (C); HRMS (*m/z*): $[M+H^+]$ calcd. for $C_{12}H_9N_2O_5$, 261.0506; found, 261.0507.

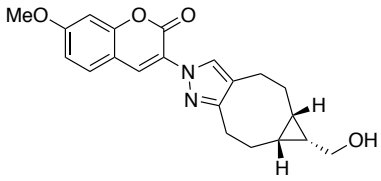
4-Chloro-3-(7-methoxy-2-oxo-2H-chromen-3-yl)-1,2,3-oxadiazol-3-ium-5-olate 5.



N-Chlorosuccinimide (27 mg, 0.2 mmol) was added to a solution of sydnone **4** (44 mg, 0.17 mmol) in *N*,*N*'-dimethylformamide (3 mL) and the mixture was stirred at room temperature overnight. Water was added and the mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with water (3 × 10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (5 g) using a mixture of petroleum ether and ethyl acetate (6:4) to afford the pure chlorosydnone **5** (31 mg, 62%) as a pink-red solid. The compound could be recrystallized from methanol if necessary: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.97 (s, 3H), 6.96 (d, *J* = 2.4 Hz, 1H), 7.03 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.60 (d, *J* = 8.7 Hz, 1H), 8.15 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.5 (CH_3), 101.4 (CH), 110.2 (C), 115.0 (CH), 116.6

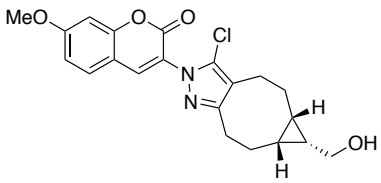
(C), 131.2 (CH), 143.4 (CH), 154.1 (C), 157.2 (C), 163.6 (C), 166.3 (C); HRMS (*m/z*): [M+H$^+$] calcd. for $C_{12}H_8ClN_2O_5$, 295.0116; found, 295.0117.

3-(6-(Hydroxymethyl)-5,5a,6,6a,7,8-hexahydrocyclopropa[5,6]cycloocta[1,2-c]pyrazol-2(4H)-yl)-7-methoxy-2H-chromen-2-one 13.



A solution of sydnone **4** (12 mg, 0.05 mmol) and BCN (**12**) (7.5 mg, 0.05 mmol) in a mixture of methanol (4 mL) and dichloromethane (1 mL) was stirred at room temperature for 4 h. The volatiles were removed under reduced pressure and the residue was purified by flash chromatography on silica gel (5 g) using a mixture of dichloromethane and methanol (99:1) to afford the pyrazole **13** (8 mg, 44%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.11-1.22 (m, 3H), 1.31-1.48 (m, 2H), 1.61 (br s, 1H), 2.21-2.35 (m, 2H), 2.53 (ddd, *J* = 15.0, 8.5, 2.3 Hz, 1H), 2.74 (ddd, *J* = 14.9, 8.9, 2.7 Hz, 1H), 2.86 (ddd, *J* = 15.0, 8.3, 2.6 Hz, 1H), 3.09 (ddd, *J* = 14.9, 8.3, 2.6 Hz, 1H), 3.72 (d, *J* = 7.1 Hz, 2H), 3.88 (s, 3H), 6.86 (d, *J* = 2.3 Hz, 1H), 6.90 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 8.21 (s, 1H), 8.30 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.0 (CH), 21.1 (CH), 21.9 (CH), 23.5 (CH_2), 23.7 (CH_2), 24.8 (CH_2), 27.5 (CH_2), 55.9 (CH_3), 60.2 (CH_2), 100.5 (CH), 112.7 (C), 113.6 (CH), 122.2 (C), 123.3 (C), 128.6 (CH), 128.9 (CH), 130.6 (CH), 153.4 (C), 155.1 (C), 157.1 (C), 162.2 (C); HRMS (*m/z*): [M+H$^+$] calcd. for $C_{21}H_{23}N_2O_4$, 367.1652; found, 367.1655.

3-(3-Chloro-6-(hydroxymethyl)-5,5a,6,6a,7,8-hexahydrocyclopropa[5,6]cycloocta[1,2-c]pyrazol-2(4H)-yl)-7-methoxy-2H-chromen-2-one 14.



A solution of chlorosydnone **5** (15 mg, 0.05 mmol) and BCN (**12**) (7.5 mg, 0.05 mmol) in a mixture of methanol (4 mL) and dichloromethane (1 mL) was stirred at room temperature for 3 h. The volatiles were removed under reduced pressure and the residue was purified by flash chromatography on silica gel (5 g) using a mixture of tert-butyl methyl ether and methanol (99:1) to afford the pure chloropyrazole **14** (15 mg, 75%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13-1.21 (m, 3H), 1.31-1.49 (m, 2H), 1.66 (br s, 1H), 2.23-2.32 (m, 2H), 2.46 (ddd, *J* = 15.3, 8.8, 2.7 Hz, 1H), 2.71 (ddd, *J* = 15.1, 8.8, 2.7 Hz, 1H), 2.84 (ddd, *J* = 15.3, 8.3, 2.6 Hz, 1H), 3.02 (ddd, *J* = 15.0, 8.4, 2.7 Hz, 1H), 3.72 (d, *J* = 7.0 Hz, 2H),

3.90 (s, 3H), 6.88-6.93 (m, 2H), 7.44 (d, J = 8.4 Hz, 1H), 7.86 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.0 (CH), 21.1 (CH), 22.0 (CH), 22.9 (CH_2), 23.3 (CH_2), 24.0 (CH_2), 28.4 (CH_2), 56.1 (CH_3), 60.1 (CH_2), 100.9 (CH), 111.8 (C), 113.9 (CH), 118.2 (C), 122.4 (C), 127.5 (C), 129.7 (CH), 141.0 (CH), 154.9 (C), 155.7 (C), 157.8 (C), 163.9 (C); HRMS (*m/z*): [M+H$^+$] calcd. for $C_{21}H_{22}ClN_2O_4$, 401.1263; found, 401.1264.

Protein labeling

Preparation of BSA-BCN.

A solution of BSA (400 µL, 20 mg/mL) in PBS (pH 7.4) was incubated with a solution of NHS-activated BCN ester[8] (100 µL, 25 mM) in DMSO overnight at room temperature. The excess of low molecular weight NHS-activated BCN ester was removed by spin-filtration (MWCO = 3 kDa). BSA-BCN was then resuspended in PBS (pH 7.4) and stored at 4 °C.

Fluorogenic labeling of BSA-BCN with sydnone-modified coumarins 4 and 5.

BSA-BCN conjugate (32.5 µL, 2 mg/mL) was incubated with sydnone **4** or **5** (12.5 µL, 1 mM in DMF) at 37 °C for 3 h and analyzed by in-gel fluorescence imaging.

Kinetics Measurements

The rate measurements of cycloaddition of BCN (**12**) with sydnone-modified coumarins **4** and **5** were conducted by using ^{1}H-NMR spectroscopy (Brüker 400 MHz) at 25 °C. A 10 mM solution of sydnone **4** or **5** (0.4 mL) in MeOD:$CDCl_3$ (4:1) was added to a thermally equilibrated solution of BCN (**12**) (20 mM, 0.2 mL) in a mixture of MeOD:$CDCl_3$ (4:1), leading to a mixture of both reactants in 1:1 ratio with a respective concentration of 6.66 mM. Reactions were monitored by following the decay of characteristic peaks of sydnones **4** and **5** as well as the formation of characteristic pyrazole peaks. Consumption of starting materials followed a second-order equation and the second-order rate constants were obtained by least squares fitting of the data to a linear equation.

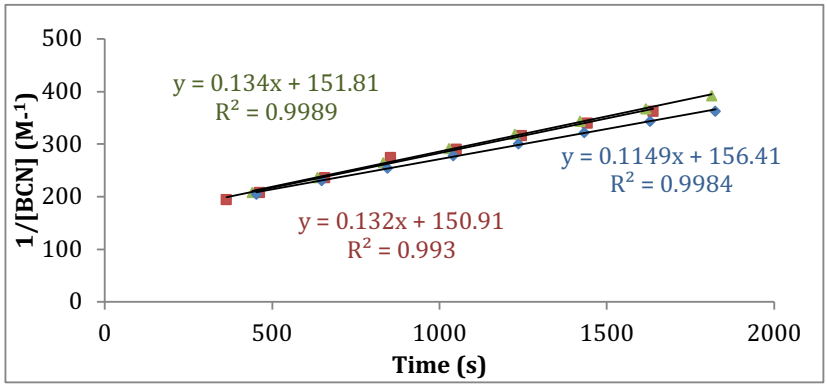


Figure S1: Kinetics measurement plots of BCN (**12**) and sydnone **4**. Decay of **4**: 3.95 (s, OCH_3), 7.35 (s, CHsyd), 8.66 (s, CHar) and formation of the corresponding pyrazole **13**: 3.89 (s, OCH_3), 8.13 (s, CHar), 8.16 (s, CHar).

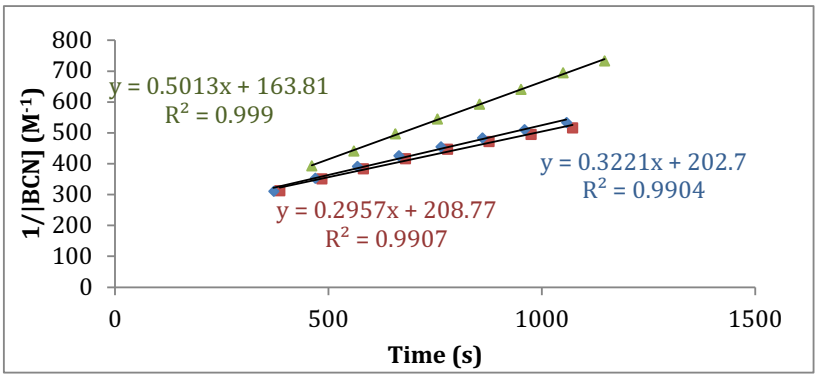


Figure S2: Kinetics measurement plots of BCN (**12**) and sydnone **5**. Decay of **5**: 3.97 (s, OCH_3), 7.75 (d, CHar), 8.61 (s, CHar) and formation of the corresponding pyrazole **14**: 3.92 (s, OCH_3), 7.62 (d, CHar), 8.09 (s, CHar).

Absorption and Fluorescence Spectra

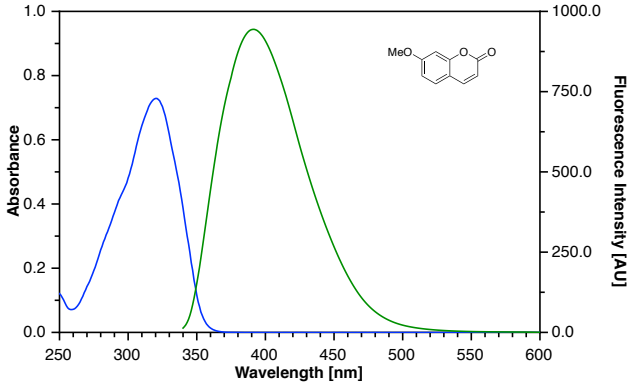


Figure S3. Absorption (blue trace (50 μM in MeOH at 25 °C)) and fluorescence emission spectra (green trace) of 7-methoxycoumarin (**11**).

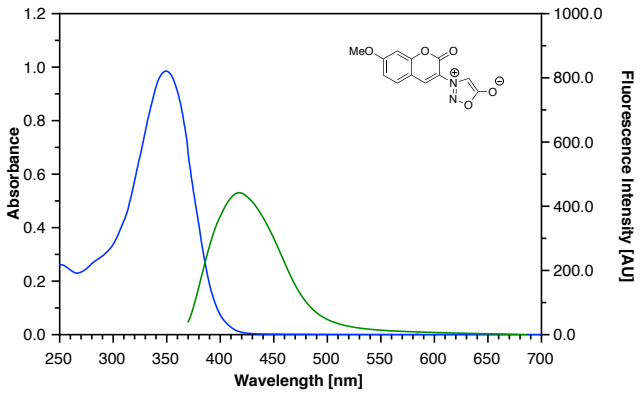


Figure S4. Absorption (blue trace (50 μM in MeOH at 25 °C)) and fluorescence emission spectra (green trace) of sydnone-coumarin **4**.

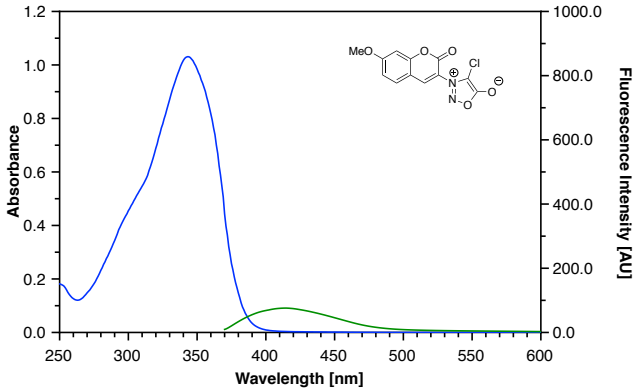


Figure S5. Absorption (blue trace (50 μM in MeOH at 25 °C)) and fluorescence emission spectra (green trace) of chlorosydnone-coumarin **5**.

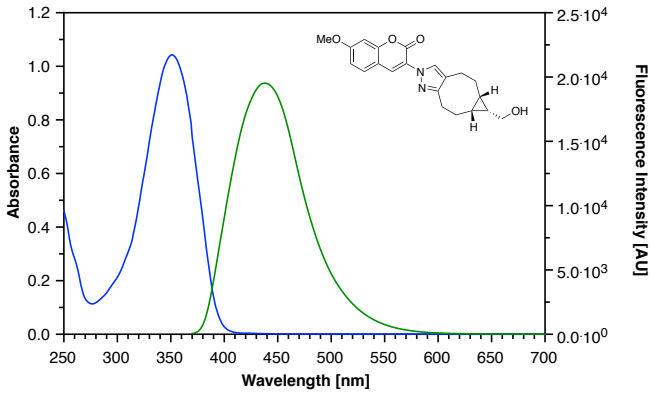


Figure S6. Absorption (blue trace (50 μM in MeOH at 25 °C)) and fluorescence emission spectra (green trace) of pyrazole-coumarin **13**.

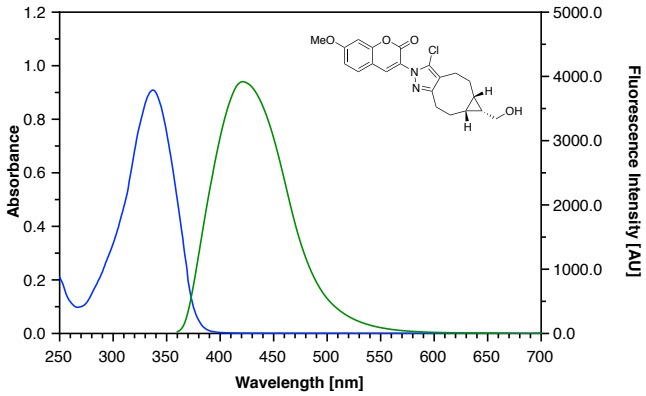


Figure S7. Absorption (blue trace (50 μM in MeOH at 25 °C)) and fluorescence emission spectra (green trace) of chloropyrazole-coumarin **14**.

Computational data

Cartesian Coordinates for Geometry Optimized Structures

Table S1: Cartesian atomic coordinates for the geometry-optimized structure of sydnone **4** (B3LYP/6-311G(d), PCM with methanol as solvent)

Atom	x/Å	y/Å	z/Å
H	-3.14409	2.07604	-0.15856
C	-2.85245	1.03729	-0.07057
C	-2.11308	-1.65600	0.16221
C	-1.52030	0.67535	-0.05234
C	-3.82885	0.03716	0.02780
C	-3.45125	-1.31844	0.14574
C	-1.11267	-0.67046	0.06075
H	-4.19753	-2.09675	0.22362
H	-1.82177	-2.69659	0.25185
O	-5.10204	0.46781	0.00120
C	-6.17103	-0.48366	0.09176
H	-6.14098	-1.18609	-0.74386
H	-7.08376	0.10402	0.04220
H	-6.13395	-1.02469	1.03970
O	-0.59190	1.67681	-0.14381
C	0.77205	1.46588	-0.14017
C	1.17998	0.07173	-0.03427
C	0.28066	-0.94314	0.07178
H	0.63070	-1.96461	0.16380
O	1.49348	2.42942	-0.22056
N	2.58054	-0.21325	-0.06312
C	3.58033	0.49852	0.49428
H	3.42063	1.42809	1.00449
N	2.96216	-1.31639	-0.66706
O	4.30690	-1.35805	-0.48412
C	4.77467	-0.21591	0.24949
O	5.94901	-0.08122	0.50344

Table S2: Cartesian atomic coordinates for the geometry-optimized structure of chlorosydnone **5** (B3LYP/6-31G(d), PCM with methanol as solvent)

Atom	x/Å	y/Å	z/Å
H	-3.44781	1.91285	-0.77894
C	-3.18071	0.93267	-0.40511
C	-2.50685	-1.60722	0.56717
C	-1.86126	0.52960	-0.35848
C	-4.17712	0.05398	0.04138
C	-3.83263	-1.22554	0.52914
C	-1.48737	-0.74299	0.12597
H	-4.59399	-1.91108	0.87422
H	-2.23970	-2.58875	0.94235
O	-5.43476	0.52132	-0.03371
C	-6.52193	-0.30255	0.40938
H	-6.58584	-1.21758	-0.18325
H	-7.41640	0.29518	0.25580
H	-6.42047	-0.54635	1.46905
O	-0.91581	1.41544	-0.80343
C	0.43794	1.14428	-0.82172
C	0.80709	-0.16056	-0.29337
C	-0.10404	-1.06707	0.14400
H	0.22237	-2.03384	0.51153
O	1.18761	1.97805	-1.26254
N	2.20081	-0.48847	-0.31630
C	3.20201	0.09322	0.37352
N	2.58754	-1.47782	-1.09232
O	3.93340	-1.56721	-0.88577
C	4.39922	-0.59209	0.03420
O	5.55984	-0.50871	0.35221
Cl	3.01617	1.34165	1.51524

Table S3: Cartesian atomic coordinates for the geometry-optimized structure of pyrazole **15** (B3LYP/6-31G(d), PCM with methanol as solvent)

Atom	x/Å	y/Å	z/Å
H	-3.60581	2.01496	-0.18644
C	-3.26046	0.99340	-0.08768
C	-2.36791	-1.64476	0.17008
C	-1.90793	0.70344	-0.05924
C	-4.18077	-0.05520	0.01597
C	-3.72698	-1.38265	0.14587
C	-1.42239	-0.60980	0.06689
H	-4.42706	-2.20271	0.22822
H	-2.02064	-2.66742	0.27050
O	-5.48360	0.30653	-0.01795
C	-6.48946	-0.70538	0.08438
H	-6.42213	-1.41438	-0.74450
H	-7.43855	-0.17748	0.03412
H	-6.41818	-1.23785	1.03605
O	-1.03216	1.75110	-0.15888
C	0.34090	1.61225	-0.15844
C	0.84980	0.24837	-0.01409
C	-0.00868	-0.80185	0.09598
H	0.39604	-1.80106	0.19548
O	0.99425	2.62421	-0.27321
N	2.24626	0.04997	-0.02782
N	2.73401	-1.20133	-0.26599
C	4.05047	-1.09950	-0.13280
C	4.44031	0.23026	0.20228
C	3.25940	0.92983	0.26210
H	3.05470	1.96447	0.47191
C	4.93859	-2.28452	-0.34119
H	5.65382	-2.10923	-1.15080
H	4.34715	-3.16592	-0.59208
H	5.52260	-2.50731	0.55718
C	5.82899	0.74418	0.42844
H	6.45880	0.61094	-0.45707
H	6.32457	0.22073	1.25234
H	5.82164	1.80865	0.67077

Table S4: Cartesian atomic coordinates for the geometry-optimized structure of chloropyrazole **16** (B3LYP/6-31G(d), PCM with methanol as solvent)

Atom	x/Å	y/Å	z/Å
H	-3.82119	-1.60574	1.20959
C	-3.51545	-0.75582	0.61252
C	-2.73504	1.44323	-0.93099
C	-2.17599	-0.45556	0.44470
C	-4.47737	0.05490	-0.00187
C	-4.08052	1.16316	-0.77764
C	-1.74973	0.64427	-0.32599
H	-4.81402	1.79798	-1.25499
H	-2.42855	2.29475	-1.52863
O	-5.76033	-0.30569	0.20800
O	-1.26419	-1.26831	1.06225
C	-6.81037	0.46843	-0.38231
H	-6.79487	1.49711	-0.01471
H	-7.73407	-0.01432	-0.07362
H	-6.74003	0.46004	-1.47253
C	0.10549	-1.06576	1.00248
C	0.54469	0.04715	0.16474
C	-0.34561	0.86451	-0.45135
H	0.01597	1.70060	-1.04022
O	0.81052	-1.81234	1.63530
N	1.93612	0.30530	0.10526
N	2.44829	1.46060	0.62221
C	3.75868	1.39381	0.41372
C	4.13409	0.18926	-0.24497
C	2.93583	-0.46561	-0.41634
C	4.66157	2.50197	0.85411
H	5.41168	2.14671	1.56693
H	4.08197	3.29278	1.33217
H	5.20238	2.93634	0.00787
C	5.50519	-0.24410	-0.65501
H	5.47888	-1.20144	-1.17784
H	6.16433	-0.35752	0.21114
H	5.97299	0.48624	-1.32194
Cl	2.61723	-1.96512	-1.21263

References.

(1) Kii, I.; Shiraishi, A.; Hiramatsu, T.; Matsushita, T.; Uekusa, H.; Yoshida, S.; Yamamoto, M.; Kudo, A.; Hagiwara, M.; Hosoya, T., *Org. Biomol. Chem.* **2010**, *8*, 4051.

(2) Sheldric, G. M. *Acta Cryst. A,* **2015**, *A71*, 3.

(3) Sheldrick, G. M. *Acta Cryst. A*, **2008**, *A64*, 112.

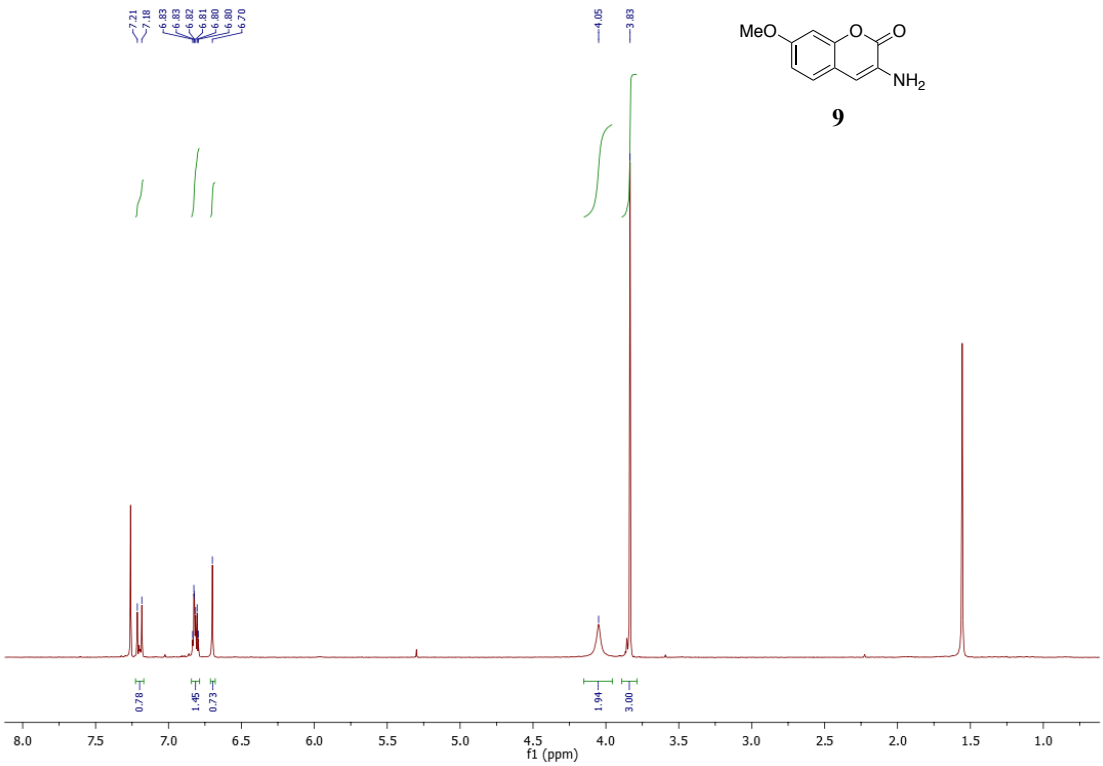
(4) Frisch, M. J. T., G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J.; Gaussian 09; Gaussian, Inc.: Wallingford CT, 2009.

(5) Tomasi, J.; Mennucci, B.; Cammi, R. *Chem. Rev.* **2005**, *105*, 2999.

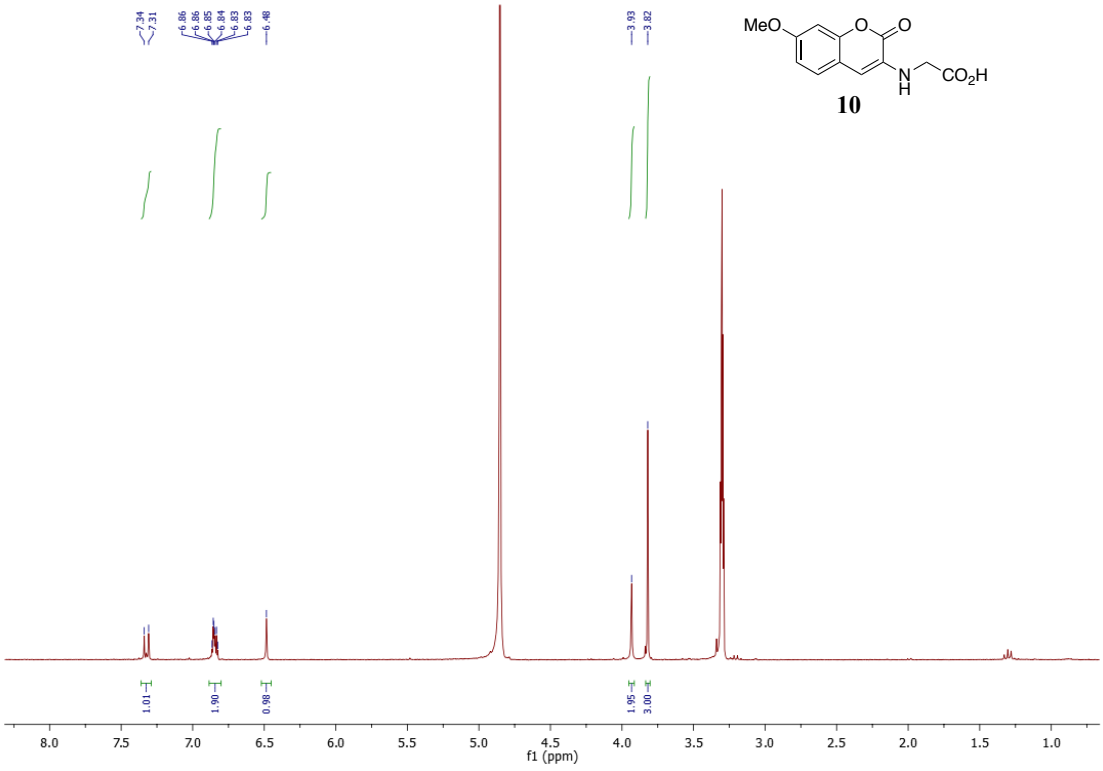
(6) Humphrey, W., Dalke, A. and Schulten, K. *J. Molec. Graphics*, **1996**, *14*, 33.

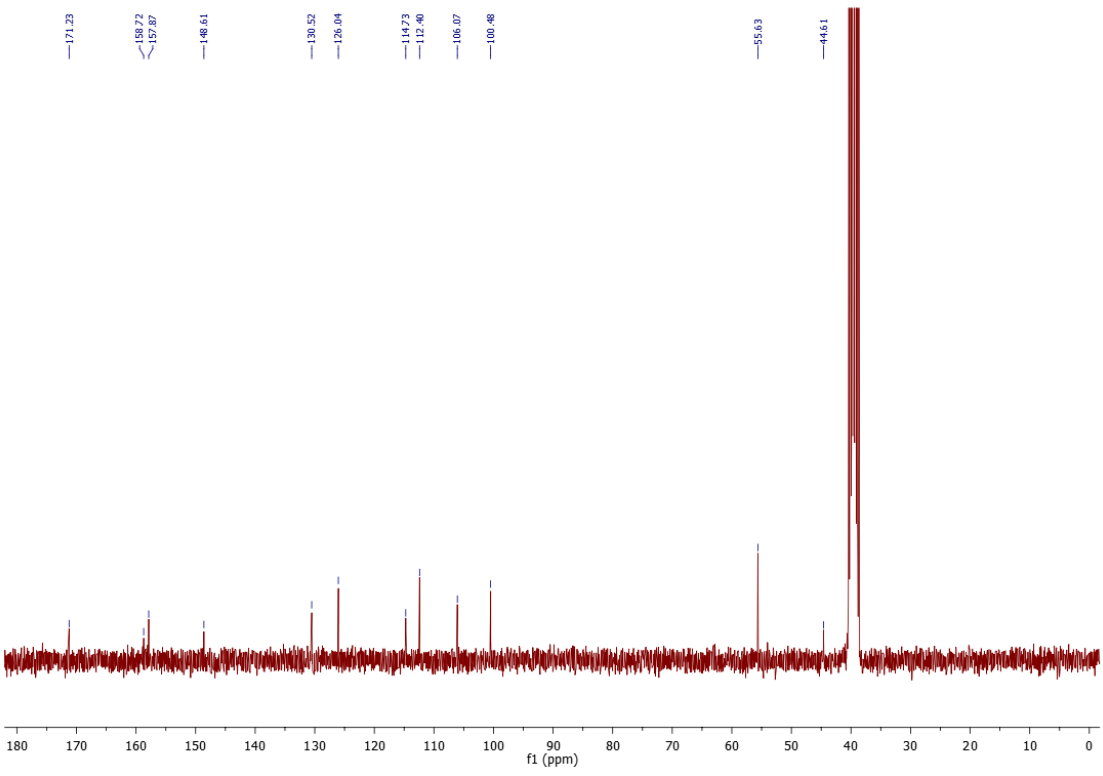
(7) Kudale, A. A.; Kendall, J.; Warford, C.C.; Wilkins, N. D.; and Bodwell, G. J. *Tetrahedron Lett.*, **2007**, *48*, 5077.

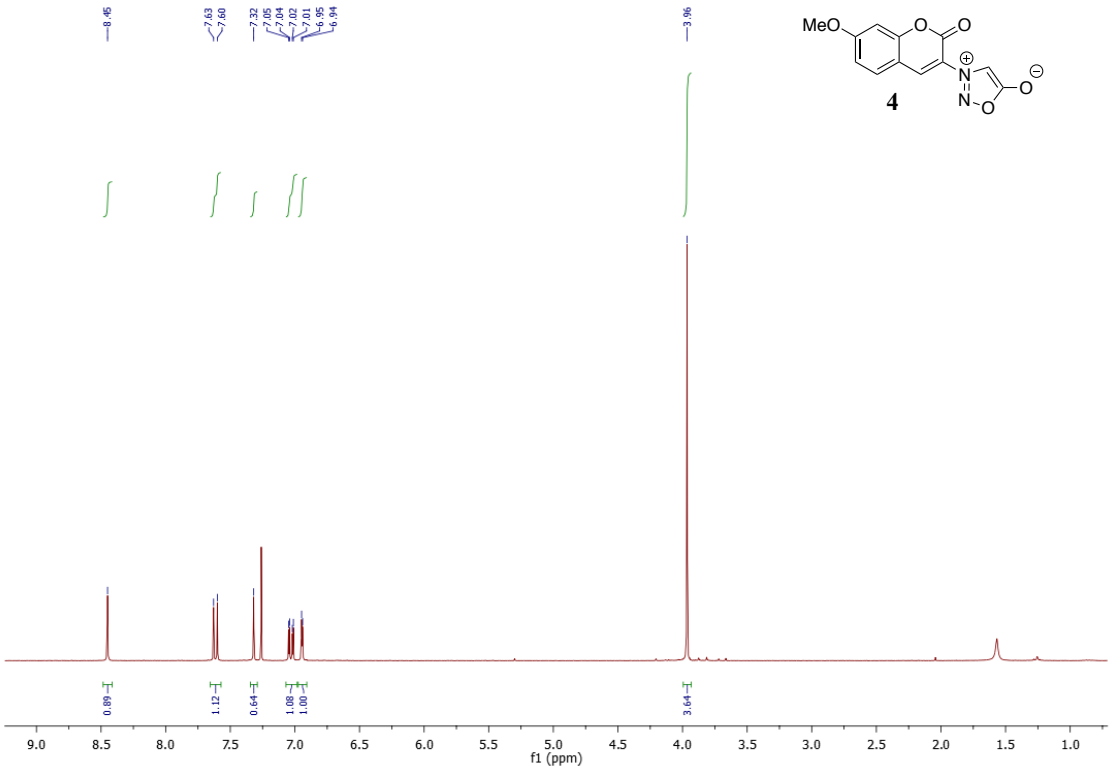
(8) Deforest, C. A.; Tirell, D. A. *Nat. Mat.* **2015***, 14*, 523.

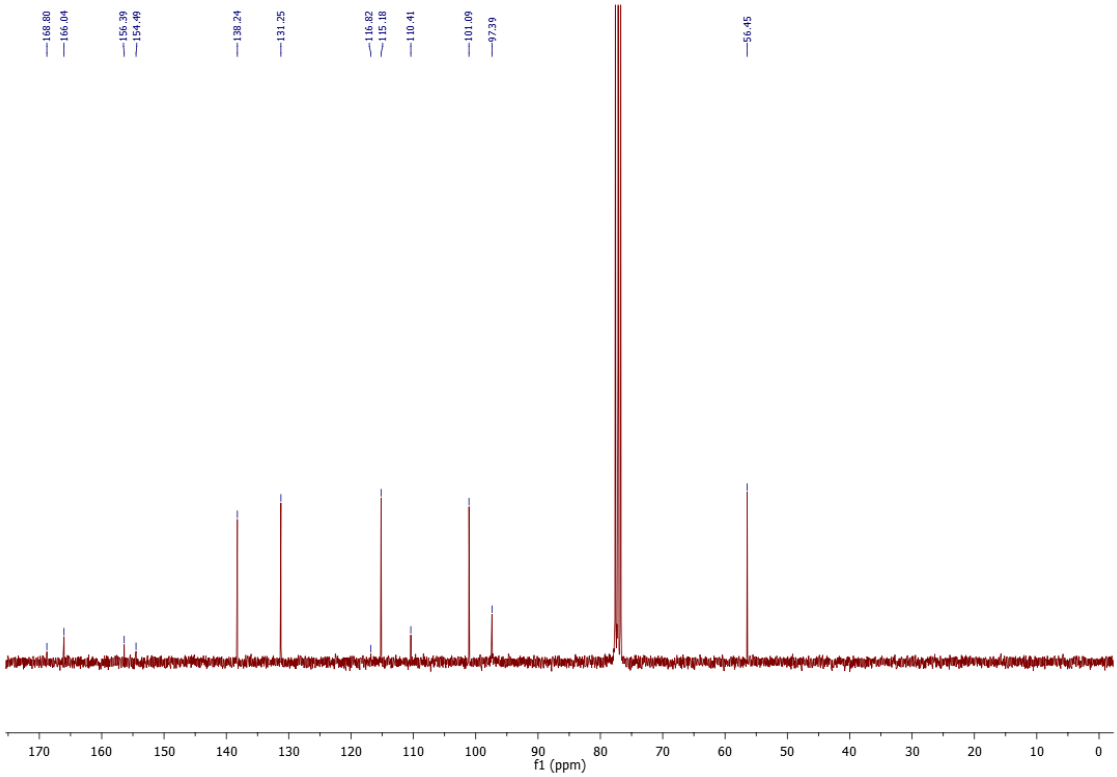

7.21
7.18
6.83
6.83
6.82
6.81
6.81
6.80
6.80
6.70
4.05
3.83
MeO
O
O
NH2
0.78
1.46
0.73
1.94
3.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

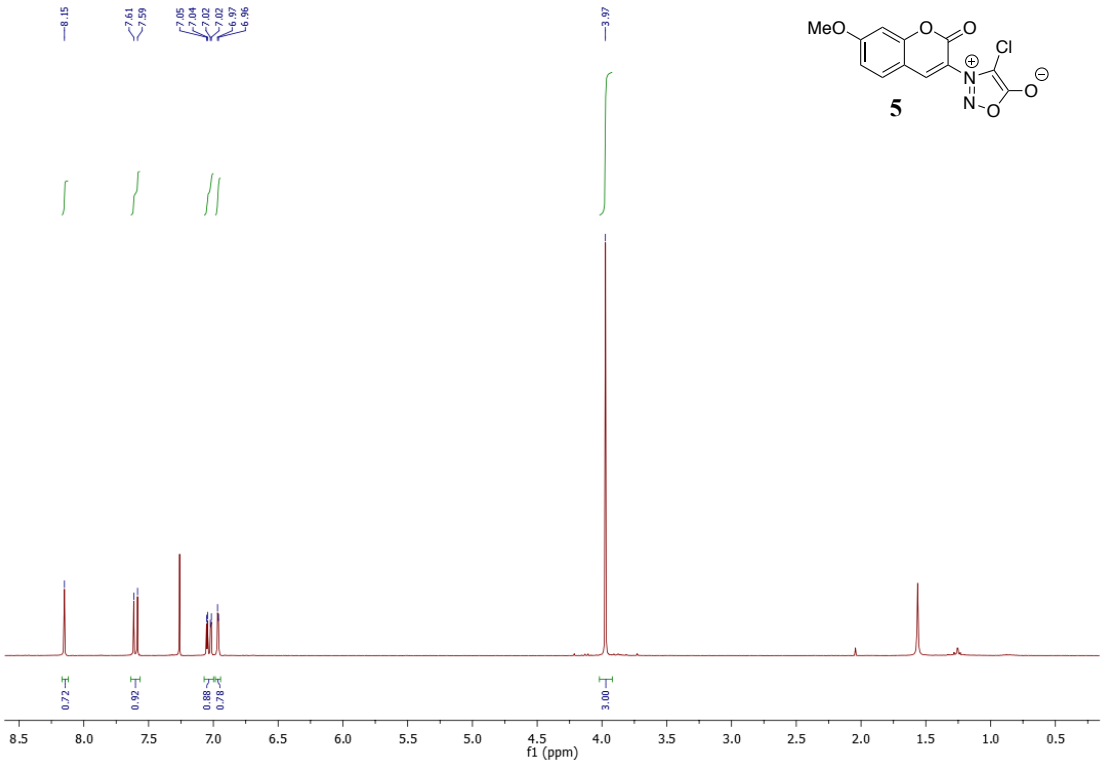
9

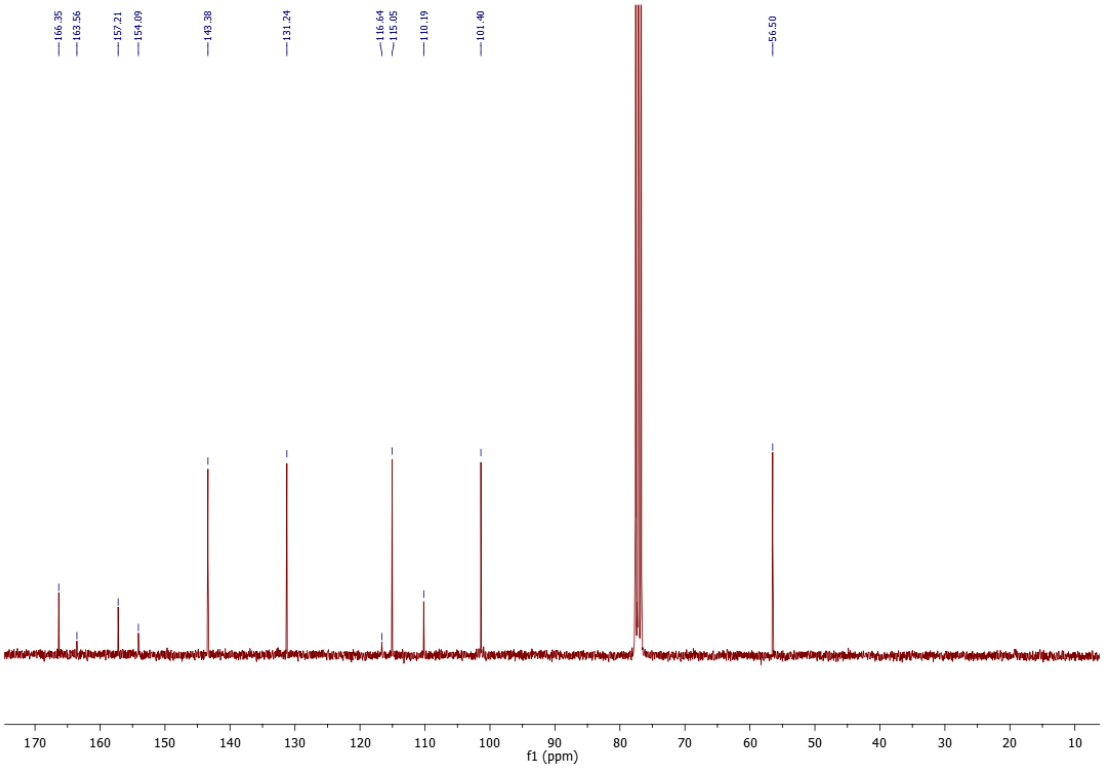

7.34
7.31
6.86
6.86
6.85
6.84
6.83
6.83
6.48
3.93
3.82
MeO
O
O
N
H
CO2H
10
1.01
1.90
0.98
1.95
3.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

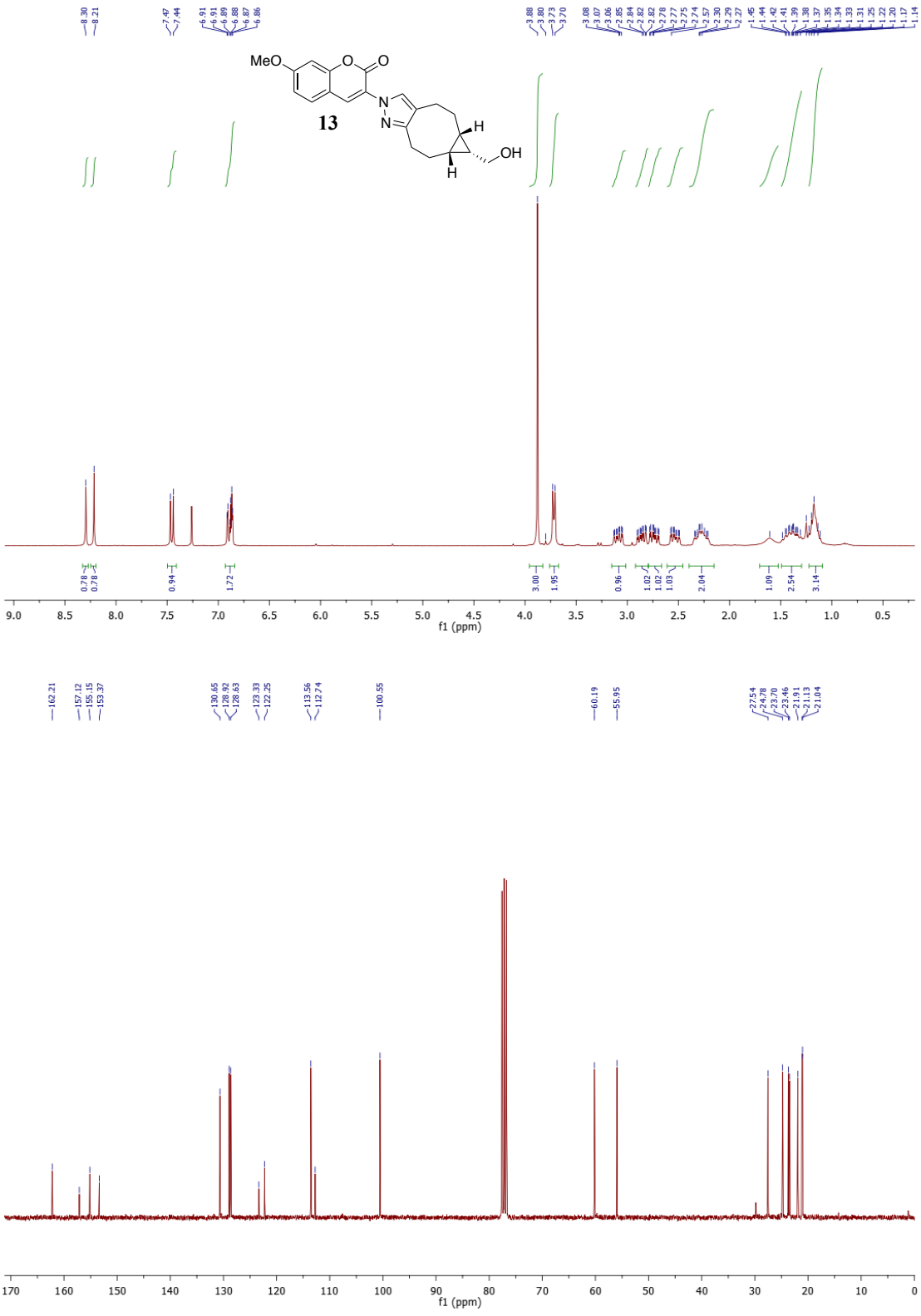

171.23
158.72
157.87
148.61
130.52
126.04
114.73
112.40
106.07
100.48
55.63
44.61
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

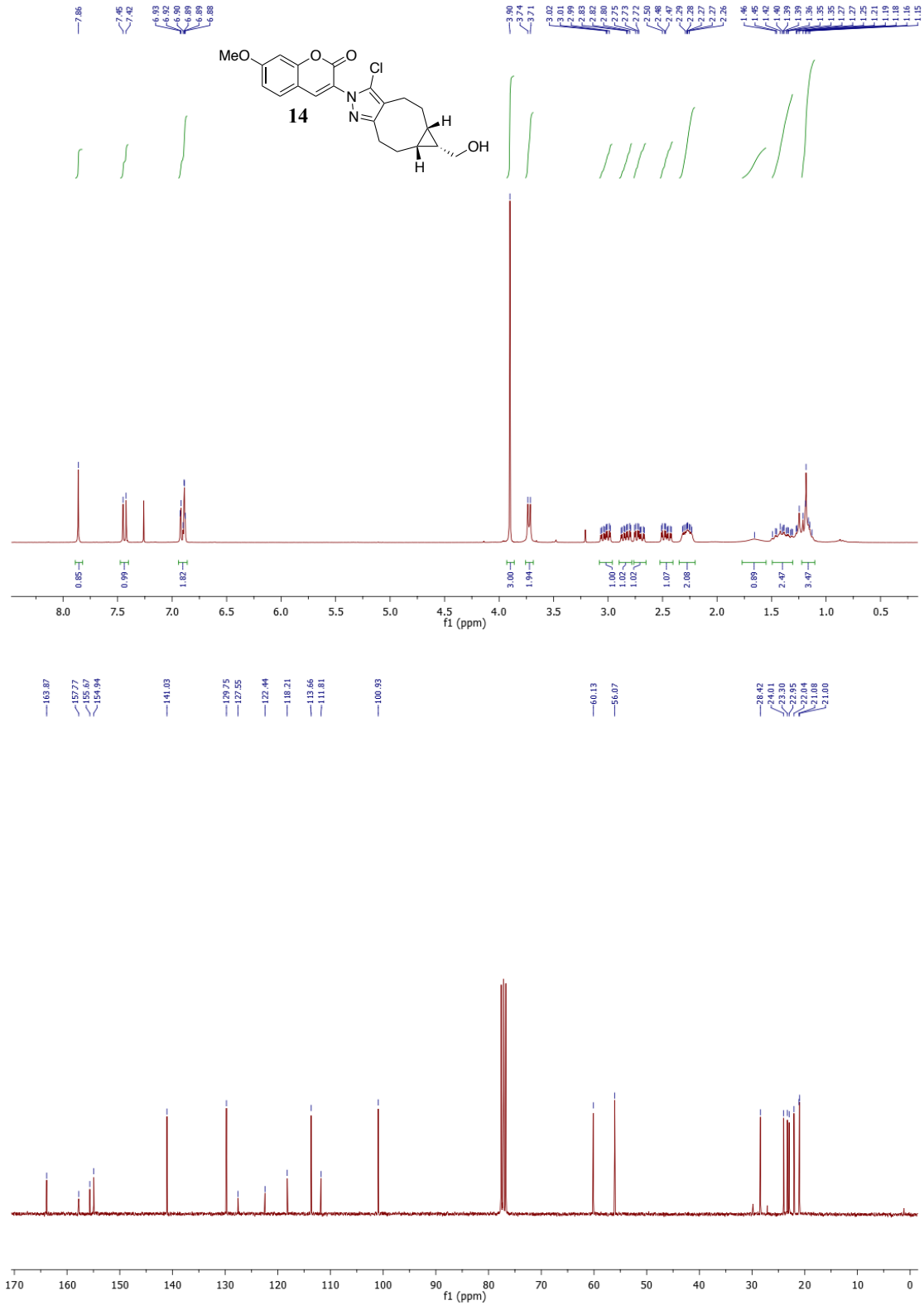

8.45
7.63
7.60
7.32
7.05
7.04
7.02
7.01
6.95
6.94
3.96
MeO
O
O
N
N
O
O
4
0.89
1.12
0.64
1.08
1.00
3.64
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


168.80
166.04
156.39
154.49
138.24
131.25
116.82
115.18
110.41
101.09
97.39
56.45
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


8.15
7.61
7.59
7.05
7.04
7.02
7.02
6.97
6.96
3.97
MeO
O
O
Cl
N
N
O
O
5
0.72
0.92
0.88
0.78
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


166.35
163.56
157.21
154.09
143.38
131.24
116.64
115.05
110.19
101.40
56.50
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


MeO
O
O
N
N
H
OH
H
13
f1 (ppm)
f1 (ppm)


MeO
O
O
Cl
N
N
H
OH
H
14
f1 (ppm)
f1 (ppm)