Electronic Supplementary Material (ESI) for ChemComm.

Fluorogenic iminosydnones: bioorthogonal tools for double turn-on click-and-release reactions.

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I. Materials and equipment

All reactions sensitive to air and moisture were carried out under argon in oven-dried glassware.

Reactants and solvents: All chemical products commercially available were purchased from Sigma-Aldrich, Acros and Fluka and used without further purification. Anhydrous solvents: 1,4-dioxane, acetonitrile, DMF, DMSO were purchased in anhydrous form and used without further purification. THF was dried from sodium/benzophenone under nitrogen. Dichloromethane was distilled form calcium hydride under nitrogen. Chloroform was purchased stabilized with amylene.
Purifications: Flash chromatography were performed on silica gel (Merck Kieselgel 60, grading 40-63 µm) or on Alumina (RediSep®Rf, Alumina neutral),

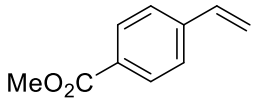
Analysis: Reactions were monitored by TLC carried out on silica 0,25 mm (60 F254, Merck) using UV light as visualizing agent and basic aqueous permanganate as developing agent.
^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) were measured on a Brucker Avance 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from residual solvents peaks and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), broad singlet (br. s), doublet (d), triplet (t), quartet (q), quintet (quint), multiplet (m). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m).
Electrospray mass spectra were obtained using an ESI-Quadripole autopurify, Waters (pump: 2545, mass: ZQ2000) mass Spectrometer.
Infrared spectra (IR) were obtained on a Perkin Elmer system 2000 FT-IR spectrophotometer or a Perkin Elmer UATR TWO FTIR spectrophotometer and are reported as wavelength numbers (cm^{-1}).
Melting points (Mp) were obtained on a BÜCHI Melting Point B-545 and are reported in °C.
Absorbances were measured on a Varian Cary® 50 UV-Vis spectrophotometer.
Fluorescence spectra were obtained on a HORIBA FluoroMax®-4 fluorimeter.
Fluorescence analyses for kinetic studies were recorded on a Molecular Device SpectraMax® $M5_e$.

Cell labeling: Microscopy was performed using a Leica DM 6000 upright microscope using a 63X/1.4 PLAN APO objective. A 50mW diode (405 nm), a 458 nm argon laser and a 633 nm HeNe laser linked to the microscope microscope by an optical fiber assured shuttering and illumination. Image were acquired in different sequences with the following parameters:
-Excitation 633 nm (HeNe laser), Emission 665–700 nm (GaAsP hybrid detector, Hamamatsu), false-colored in red;
- Excitation 458 nm (Argon laser), Emission 540–650 nm (PMT detector, Hamamatsu), false-colored in green;
-Excitation 405 nm (Diode), Emission 415–470 nm (GaAsP hybrid detector, Hamamatsu), false-colored in blue.
Images were acquired and processed using LAS-X and are shown as a single z-plane.
Fluoromount™ Aqueous Mounting Medium was used for preparing the cell samples.

II. Synthetic Procedure and Analytical Data

1) Compound with fluorogenic substituent on the aryl in position N3.

methyl 4-formylbenzoate (SI-1)

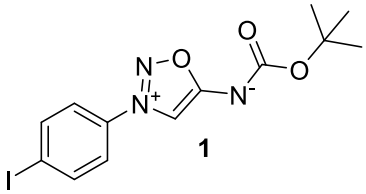


SI-1

$C_{10}H_{10}O_2$
MW: 162 g.mol^{-1}
White solid
Yield: 62%

To an anhydrous solution of Ph_3PMeBr (1.19 g, 3.34 mmol) in THF (10 mL) at -78 °C was added a solution of *n*BuLi in hexanes (2.5 M, 1.4 mL, 3.34 mmol), and the mixture was stirred at 0 °C for 30 min. The mixture was cooled down at -78 °C and a solution of methyl 4-formylbenzoate (498 mg, 3.03 mmol) in anhydrous THF (3 mL) was added dropwise. The mixture was stirred for 1 hour at room temperature. A saturated solution of ammonium carbonate was added and the mixture was extracted with EtOAc. The organic layers were washed with brine, dried iver $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography (SiO_2, Heptane/EtOAc, 95/5) to afford the desired product as a white solid (306 mg, 62%). The spectral data (^{1}H-NMR) was consistent with reported one.[1]
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 8.3 Hz, 2H), 6.73 (dd, *J* = 17.6 Hz, *J* = 10.9 Hz, 1H), 5.85 (d, *J* = 17.6 Hz, 1H), 5.36 (d, *J* = 10.9 Hz, 1H), 3.89 (s, 3H).

(tert-butoxycarbonyl)(3-(4-iodophenyl)-1,2,3-oxadiazol-3-ium-5-yl)amide (1)

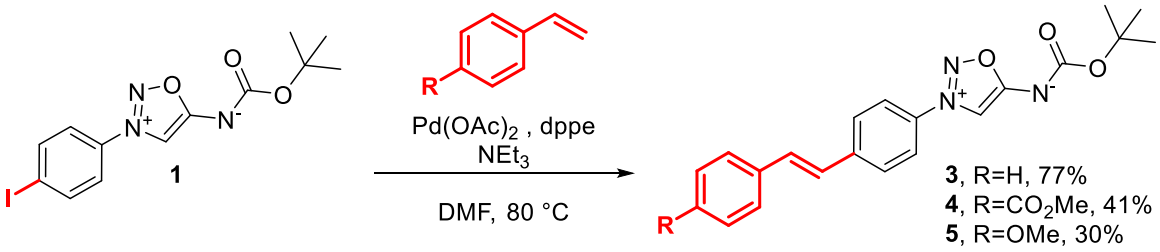


1

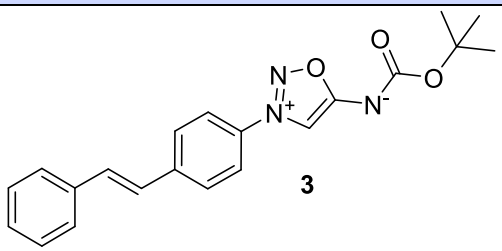
Compound **1** was prepared as described in the literature.[2]

[1] A. Yokoyama, T. Maruyama, K. Tagami, H. Masu, K. Katagiri, I. Azumaya, T. Yokozawa, *Org. Lett.* **2008**, *10*, 3207.
[2] M. Riomet, E. Decuypere, K. Porte, S. Bernard, L. Plougastel, S. Kolodych, D. Audisio, F. Taran, *Chem. Eur. J.* **2018**, *34*, 8535.

Synthetic route to compounds **3**-**5**:



(E)-(tert-butoxycarbonyl)(3-(4-styrylphenyl)-1,2,3-oxadiazol-3-ium-5-yl)amide (3)



$C_{21}H_{21}N_3O_3$
MW: 363 g.mol^{-1}
Yellow solid
Yield: 77%

To a stirred solution of styrene (115 µL, 1.0 mmol), $Pd(OAc)_2$ (4.5 mg, 0.02 mmol), dppe (8.0 mg, 0.02 mmol) and NEt_3 (56 µL, 0.40 mmol) in DMF (2 mL) at 80 °C was added **1** (78 mg, 0.20 mmol) in DMF (2 mL) over a period of 2 hours. The mixture was stirred overnight. After cooling at room temperature, the mixture was filtered over a celite plug and evaporated. The crude product was purified by flash chromatography (SiO_2, heptane/EtOAc, from 80/20 to 70/30) to afford the desired product as a yellow solid (56 mg, 77%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.77 (d, *J* = 8.9 Hz, 2H), 7.72 (d, *J* = 8.9 Hz, 2H), 7.54 (d, *J* = 7.3 Hz, 2H), 7.39 (m, 2H), 7.33 (m, 1H), 7.25 (d, *J* = 16.3 Hz, 1H), 7.13 (d, *J* = 16.3 Hz, 1H), 1.53 (s, 9H).

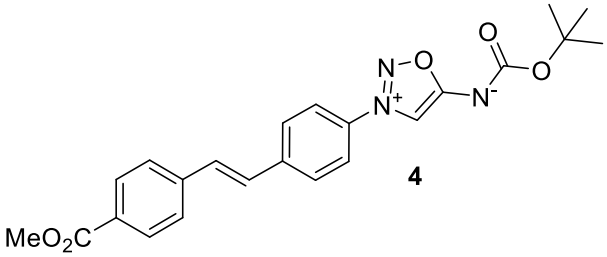
^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 161.1, 142.4, 136.2, 133.0, 132.3, 129.0 (2C), 129.0, 128.0 (2C), 127.1 (2C), 126.0, 121.7 (2C), 102.1, 79.1, 28.4 (3C).

IR (cm^{-1}): 2976, 1655, 1600, 1450, 1366, 1295, 1221, 1163, 1051, 1009, 966, 881, 814.

HRMS (ESI) ***m/z*** calcd for $C_{21}H_{22}N_3O_3$ $[M+H]^+$: 364.1656; found: 364.1656; calcd. for $C_{21}H_{21}N_3NaO_3$. $[M+Na]^+$: 386.1475; found: 386.1473.

Mp.: 179–180 °C.

(E)-(tert-butoxycarbonyl)(3-(4-(4-(methoxycarbonyl)styryl)phenyl)-1,2,3-oxadiazol-3-ium-5-yl)amide (4)



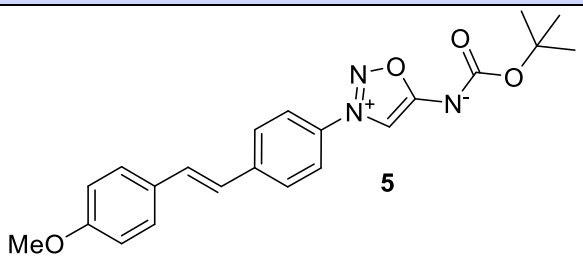
$C_{23}H_{23}N_3O_5$
MW: 421 g.mol^{-1}
Yellow solid
Yield: 41%

To a stirred solution of **SI-1** (264 mg, 1.63 mmol), $Pd(OAc)_2$ (18 mg, 0.08 mmol), dppe (32 mg, 0.08 mmol) and NEt_3 (230 µL, 1.63 mmol) in DMF (8 mL) at 80 °C was added **1** (315 mg, 0.81 mmol) in DMF (8 mL) over a period of 12 hours. The mixture was stirred overnight. After cooling at room temperature, the mixture was filtered over a celite plug and evaporated. The crude product was purified by flash chromatography (SiO_2, DCM/EtOAc, from 100/0 to 90/10) to afford the desired product as a yellow solid (140 mg, 41%).

^{1}H NMR (400 MHz, $CDCl_3$,): δ 8.12 (s, 1H), 8.06 (d, *J* = 8.2 Hz, 2H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 16.9 Hz, 1H), 7.23 (d, *J* = 16.9 Hz, 1H), 3.93 (s, 3H), 1.53 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 166.7, 161.0, 141.8, 140.5, 132.7, 131.8, 130.3 (2C), 130.1, 128.4 (3C), 126.9 (2C), 121.8 (2C), 102.1, 79.1, 52.3, 28.4 (3C).
IR (cm^{-1}): 2977, 1709, 1663, 1606, 1439, 1364, 1306, 1222, 1171, 1110, 972.
HRMS (ESI) *m/z* calcd for $C_{23}H_{24}N_3O_5$ $[M+H]^+$: 422.1710; found: 422.1709; calcd. for $C_{23}H_{24}N_3NaO_5$. $[M+Na]^+$: 444.1530; found: 444.1531.
Mp.: 201–202 °C

(E)-(tert-butoxycarbonyl)(3-(4-(4-methoxystyryl)phenyl)-1,2,3-oxadiazol-3-ium-5-yl)amide (5)

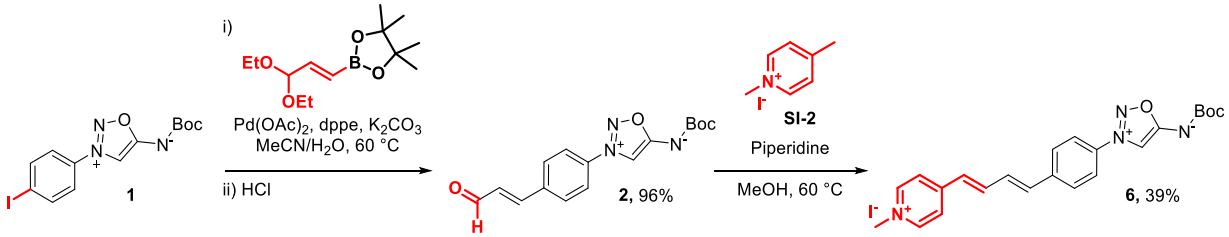


5

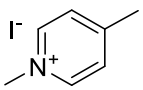
$C_{22}H_{23}N_3O_4$
MW: 393 g.mol^{-1}
Light orange solid
Yield: 30%

To a stirred solution of *p*-vinylanisole (133 µL, 1.0 mmol), $Pd(OAc)_2$ (4.5 mg, 0.02 mmol), dppe (8.0 mg, 0.02 mmol) and NEt_3 (56 µL, 0.40 mmol) in DMF (2 mL) at 80 °C was added **1** (78 mg, 0.20 mmol) in DMF (2 mL) over a period of 2 hours. The mixture was stirred overnight. After cooling at room temperature, the mixture was filtered over a celite plug and evaporated. The crude product was purified by flash chromatography (SiO_2, heptane/EtOAc, from 85/15 to 75/25) to afford the desired product as a yellow solid (24 mg, 30%).
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.74 (d, *J* = 8.7 Hz, 2H), 7.67 (d, *J* = 8.7 Hz, 2H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.19 (d, *J* = 16.3 Hz, 1H), 6.98 (d, *J* = 16.3 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 3.83 (s, 3H), 1.52 (s, 9H).
^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 161.0, 160.3, 142.8, 132.5, 132.0, 129.0, 128.4 (2C), 127.7 (2C), 123.8, 121.6 (2C), 114.4 (2C), 102.0, 79.1, 55.4, 28.4 (3C).
IR (cm^{-1}): 2931, 1658, 1579, 1440, 1364, 1289, 1251, 1156, 1049,1006, 963, 833.
HRMS (ESI) *m/z* calcd for $C_{22}H_{24}N_3O_4$ $[M+H]^+$: 394.1761; found: 394.1762; calcd. for $C_{22}H_{23}N_3NaO_4$. $[M+Na]^+$: 416.1581; found: 416.1581.
Mp.: 153–154 °C.

Synthetic route to compounds **2** and **6**:



1,4-dimethylpyridin-1-ium iodide (SI-2)



SI-2

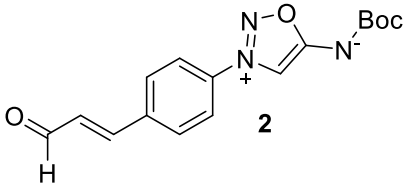
$C_7H_{10}IN$
MW: 235 g.mol^{-1}
Light pink solid
Yield: 95%

Compound **SI-2** was synthesized according to a reported procedure.[3] To a solution of picoline (1.00 g, 10.7 mmol) in dichloromethane (4.0 mL) was added iodomethane (1.0 mL, 16.0 mmol). The mixture

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

was stirred at room temperature during 2 h. Heptane was added and the precipitate formed was filtered. 2.39 g (10.2 mmol, 95%) of compound **SI-2** were isolated.
^{1}H NMR (400 MHz, DMSO-d_6): δ 8.81 (d, *J* = 6.5 Hz, 2H), 7.95 (d, *J* = 6.5 Hz, 2H), 4.26 (s, 3H), 2.59 (s, 3H).

(E)-(tert-butoxycarbonyl)(3-(4-(3-oxoprop-1-en-1-yl)phenyl)-1,2,3-oxadiazol-3-ium-5-yl)amide (2)

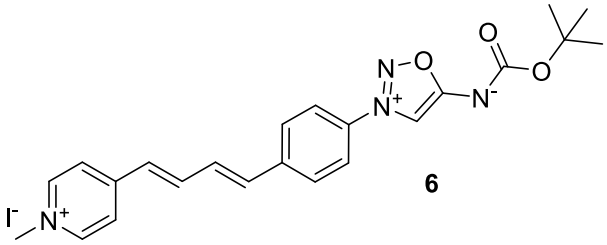


2

$C_{16}H_{17}N_3O_4$
MW: 315 g.mol^{-1}
Orange solid
Yield: 96%

A solution of **1** (387 mg, 1.0 mmol), $Pd(OAc)_2$ (22 mg, 0.10 mmol), Ethylenebis(diphenylphosphine) (40 mg, 0.10 mmol) and K_2CO_3 (415 mg, 3.0 mmol) in MeCN/H_2O (1/1,5 mL) was stirred during 15 minutes at room temperature. Then (E)-2-(3,3-diethoxyprop-1-en-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (282 mg, 1.1 mmol) was added and the reaction was stirred at 60 °C overnight. After cooling to room temperature, HCl 1M was added to reach pH 2 and the mixture was stirred for another 15 minutes. The mixture was extracted with DCM and the organic layers were washed with brine, dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography (SiO_2, DCM/NEt_3 100/0 to 99/1) to afford the desired product as an orange solid (302 mg, 96%).
^{1}H NMR (400 MHz, $CDCl_3$): δ 9.74 (d, *J* = 7.4 Hz, 1H), 8.11 (s, 1H), 7.86 (d, *J* = 8.7 Hz, 2H), 7.80 (d, *J* = 8.7 Hz, 2H), 7.51 (d, *J* = 16.1 Hz, 1H), 6.77 (dd, *J* = 16.1 Hz, 7.4 Hz, 1H), 1.47 (s, 9H).
^{13}C NMR (100 MHz, $CDCl_3$): δ 192.9, 174.8, 160.9, 148.7, 138.8, 135.1, 131.6, 130.3 (2C), 122.3 (2C), 102.4, 79.3, 28.3 (3C).
IR (cm^{-1}) 1662, 1593, 1580, 1441, 1364, 1288, 1216, 1153, 1121, 1048, 1005, 963, 728.
HRMS (ESI) ***m/z*** calcd for $C_{16}H_{18}N_3O_4$ $[M+H]^+$: 316.1292; found: 316.1288.
Mp.74 °C – decomp.

(tert-butoxycarbonyl)(3-(4-((1E,3E)-4-(1-methylpyridin-1-ium-4-yl)buta-1,3-dien-1-yl)phenyl)-1,2,3-oxadiazol-3-ium-5-yl)amide iodide (6)



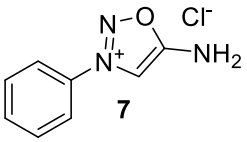
6

$C_{23}H_{25}IN_4O_3$
MW: 532 g.mol^{-1}
Brown solid
Yield: 39%

3 drops of piperidine were added to a solution of **2** (152 mg, 0.39 mmol) and **SI-2** (82 mg, 0.35 mmol) in MeOH (8 mL). The mixture was stirred at 60 °C for 2h. Most of the MeOH was evaporated and cold Et_2O was added. The product was collected by filtration and purified by preparative TLC (Alumina, DCM/MeOH, 90/10), to afford 72 mg (39%) of the desired product as a brown solid.
^{1}H NMR (400 MHz, MeOD-d_4): δ 8.72 (d, *J* = 6.5 Hz, 2H), 8.38 (s, 1H), 8.11 (d, *J* = 6.5 Hz, 2H), 8.02 (d, *J* = 8.8 Hz, 2H), 7.91 (d, *J* = 8.8 Hz, 2H), 7.79 (dd, *J* = 15.6 Hz, *J* = 10.6 Hz, 1H), 7.41 (dd, *J* = 15.6 Hz, *J* = 10.6 Hz, 1H), 7.22 (d, *J* = 15.6 Hz, 1H), 7.03 (d, *J* = 15.6 Hz, 1H), 4.32 (s, 3H), 1.51 (s, 9H).
^{13}C NMR (100 MHz, MeOD-d_4): δ 163.2, 159.9, 153.1, 144.7 (2C), 141.0, 137.8, 133.5, 130.9, 128.6 (2C), 128.1, 123.6 (2C), 122.2 (2C), 78.6, 46.4, 27.1 (3C).
2 quaternary carbons are missing.
IR (cm^{-1}): 1643, 1605, 1293, 1158, 1007.
HRMS (ESI) *m/z* calcd for $C_{23}H_{25}N_4O_3$ $[M]^+$: 405.1921; found: 405.1922;
Mp. 194–196 °C.

2) *Compound with substituent in position N6.*

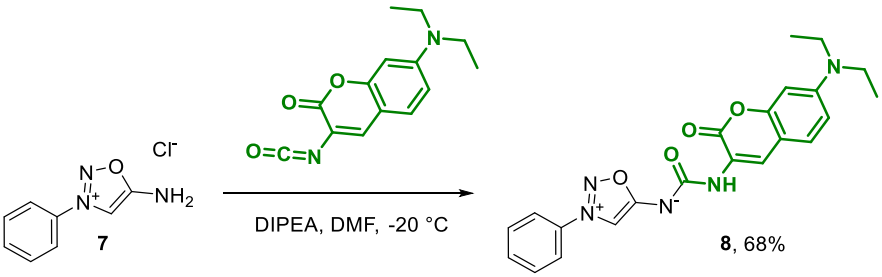
5-amino-3-phenyl-1,2,3-oxadiazol-3-ium chloride 7



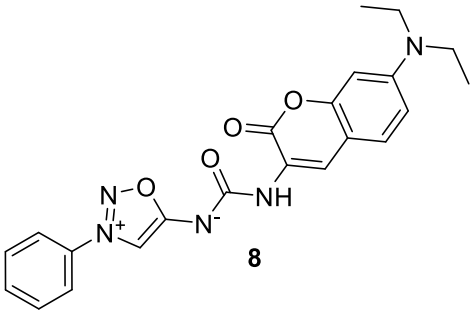
7

Compound **7** was prepared as described in the literature.[2]

Synthetic route to compound **8**:



((7-(diethylamino)-2-oxo-2H-chromen-3-yl)carbamoyl)(3-phenyl-1,2,3-oxadiazol-3-ium-5-yl)amide (8)



8

$C_{22}H_{21}N_5O_4$
MW: 419 g.mol^{-1}
Orange solid
Yield: 68%

A solution of 7-(Diethylamino)coumarin-3-carbonyl azide (8.6 mg, 0.030 mmol) in toluene (2 mL) was stirred at 100 °C for 30 minutes. The solvent was evaporated and the crude isocyanate was used without further purification.

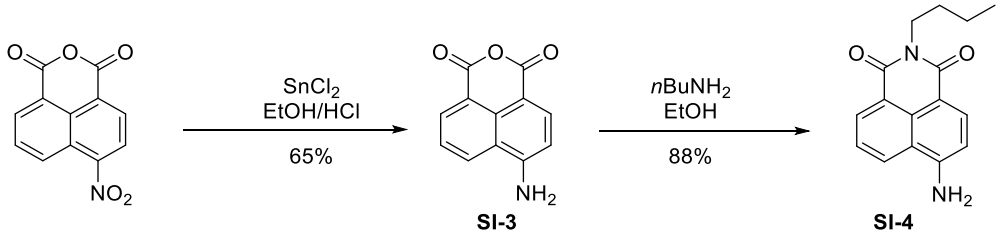
The crude mixture was dissolved in DMF (2.5 mL) and the mixture was stirred at -20 °C under argon atmosphere. **7** (18 mg ,0.090 mmol) was added followed by the slow addition of DIPEA (16 µl, 0.090 mmol) during 30 min. After completion, the mixture was evaporated under *vacuum*. The crude product was purified by column chromatography (Heptane/EtOAc, 60/40) to afford the desired product as an orange solid (8.6 mg, 68%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1H), 8.21 (s, 1H), 7.78–7.81 (m, 3H), 7.62–7.72 (m, 3H), 7.24 (d, *J* = 8.8 Hz, 1H), 6.57 (dd, *J* = 8.8 Hz, *J* = 2.5 Hz, 1H), 6.49 (d, *J* = 2.5 Hz, 1H), 3.37 (q, *J* = 7.1 Hz, 4H) 1.17 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 159.9, 159.7, 152.4, 148.9, 134.2, 133.2, 130.8 (2C), 128.3, 122.4, 121.7 (2C), 121.0, 109.6, 109.2, 102.4, 97.8, 44.9 (2C), 12.7 (2C).

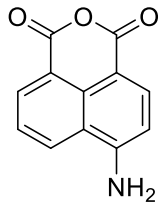
IR (cm^{-1}): 1699, 1649, 1605, 1493, 1354, 1241, 1187, 961, 764.

Synthetic route to compounds **SI-3** and **SI-4**:



SI-3 **SI-4**

6-aminobenzo[de]isochromene-1,3-dione (SI-3)

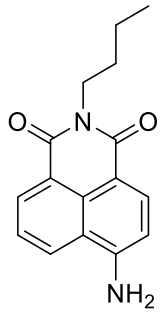


SI-3

$C_{12}H_7NO_3$
MW: 213 g.mol^{-1}
Orange solid
Yield: 65%

The product was synthesized according to a reported procedure.[4]**Erreur ! Signet non défini.** To a stirred cloudy solution of compound 1,8-naphthalic anhydride (1.0 g, 4.1 mmol) in ethanol (2 mL) was added dropwise a solution of $SnCl_2 \cdot 2H_2O$ (4.6 mg, 20.5 mmol) in concentrated hydrochloric acid (3.5 mL) at room temperature. The reaction was heated to reflux for 2 h. After cooling down to room temperature, aqueous solution of Na_2CO_3 (10%) was added to quench the reaction. The precipitate was collected by filtration, washed with water (3 × 10 mL), EtOH and Et_2O and dried *in vacuo* to afford the product **SI-3** as an orange solid (568 mg, 65%), which was directly used without further purification.
^{1}H NMR (400 MHz, DMSO-d_6): δ.8.68 (d, *J* = 8.4 Hz, 1H), 8.42 (d, *J* = 7.3 Hz, 1H), 8.17 (d, *J* = 8.4 Hz, 1H), 7.79 (br.s, 2H), 7.68 (dd, *J* = 7.9 Hz, *J* = 7.3 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H).
LCMS (ESI) m/z: $[M+H]^+$214.

6-amino-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (SI-4)



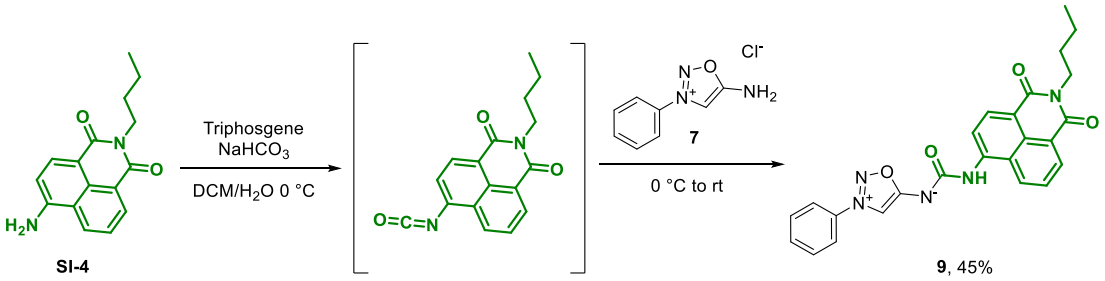
SI-4

$C_{12}H_7NO_3$
MW: 268 g.mol^{-1}
Orange solid
Yield: 88%

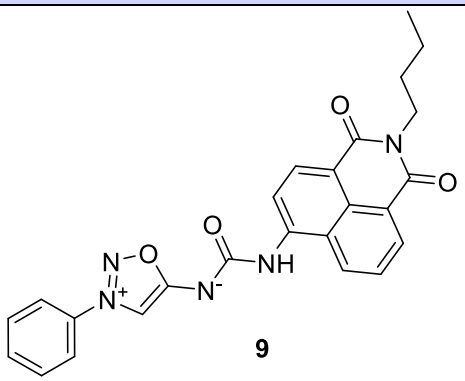
The product was synthesized according to a reported procedure.[4] *n*-butylamine (200 µL, 2.0 mmol) was quickly added to a cloudy solution of **SI-3** (213 mg, 1.0 mmol) in ethanol (20 mL). After refluxing for 16 hours, the reaction was allowed to cool to room temperature. The solvent was removed *in vacuo*, and the crude product was purified by silica gel column chromatography (SiO_2, DCM/MeOH, from 100/0 to 95/5) to give 235 mg of compound **SI-4** (88%).
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (d, *J* = 7.3 Hz, 1H), 8.38 (d, *J* = 8.1 z, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.61 (dd, *J* = 8.4 Hz, *J* = 7.3 Hz, 1H), 6.85 (d, *J* = 8.1 Hz, 1H), 4.99 (br.s, 2H), 4.13 (m, 2H), 1.67 (m, 2H), 1.42 (m, 2H), 0.94 (t, *J* = 7.4 Hz, 3H).
LCMS (ESI) m/z: $[M+H]^+$ 269.

[4] L. Zhang, D. Duan, Y. Liu, C. Ge, X. Cui, J. Sun, J. Fang, *J. Am. Chem. Soc.* **2014**, *136*, 226–233.

Synthetic route to compound **9**:



((2-butyl-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)carbamoyl)(3-phenyl-1,2,3-oxadiazol-3-ium-5-yl)amide (9)



9

$C_{25}H_{21}N_5O_4$
MW: 455 g.mol^{-1}
Yellow solid
Yield: 45%

Triphosgene (20 mg, 0.066 mmol) was suspended in DCM (2 mL) and **SI-4** (52 mg, 0.20 mmol) was added. The suspension was cooled to 0 °C and a solution of $NaHCO_3$ (67 mg, 0.80 mmol) in H_2O (2 mL) was added dropwise. The mixture was stirred for 30 minutes at 0 °C and **7** was added. The mixture was stirred from 0 °C to room temperature overnight. The reaction was quenched with brine and extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered and evaporated under *vacuum*. The product was purified preparative TLC (SiO_2, Heptane/EtOAc) to afford the product as a yellow solid (41 mg, 45%).

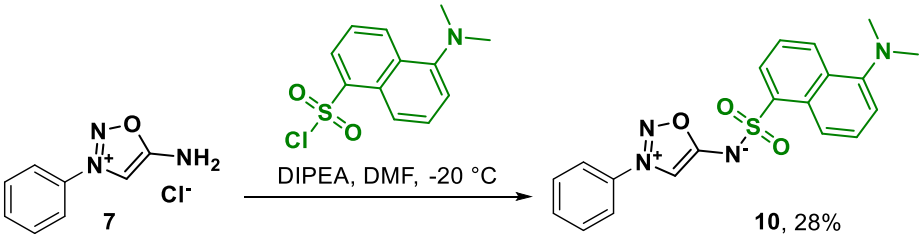
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56–8.64 (m, 3H), 8.32 (s, 1H), 8.28 (d, J = 8.6 Hz, 1H), 8.11 (br.s, 1H), 7.82 (m, 2H), 7.65–7.74 (m, 4H), 4.15 (m, 2H), 1.70 (m, 2H), 1.43 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 164.6, 164.1, 159.3, 141.2, 134.0, 133.5, 133.0, 131.3, 130.8 (2C), 129.3, 126.4, 126.4, 123.5, 122.8, 121.7 (2C), 116.7, 115.8, 103.1, 40.4, 30.5, 20.6, 14.1.

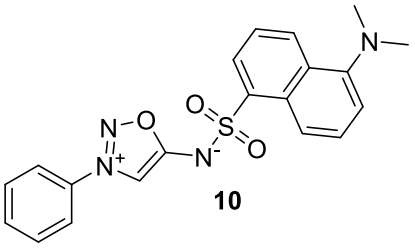
IR (cm^{-1}): 2958, 1650, 1582, 1527, 1494, 1352, 1239, 1191, 1090, 965, 775.

LCMS (ESI) m/z: $[M+H]^+$ 456.

Synthetic route to compounds **10**:



((5-(dimethylamino)naphthalen-1-yl)sulfonyl)(3-phenyl-1,2,3-oxadiazol-3-ium-5-yl)amide (10)



$C_{20}H_{18}N_4O_3S$
MW: 394 g.mol^{-1}
Yield: 28%
White solid

To a solution of **7** (198 mg, 1.0 mmol) in DMF (2 mL) at -20 °C was added dansyl chloride (297 mg, 0.22 mmol). DIPEA (262 µL; 1.5 mmol) was added for 30 minutes at -20 °C. The mixture was stirred while warming up to room temperature for 1 hour. Afterward, NH_4Cl was added and the mixture was extracted with DCM. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The resulting material was purified through flash chromatography (SiO_2, heptane/EtOAc, from 100/0 to 90/10) to afford 110 mg (0.28 mmol, 28% yield) of analytically pure white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (d, J = 8.6 Hz, 1H), 8.47 (d, J = 8.6 Hz, 1H), 8.31 (dd, J = 7.2 Hz, 1.1 Hz, 1H), 7.87 (s, 1H), 7.74 (m, 2H), 7.68 (m, 1H), 7.61 (m, 2H), 7.55 (dd, J = 8.6 Hz, 7.9 Hz, 1H), 7.47 (dd, J = 8.6 Hz, 7.2 Hz, 1H), 7.15 (d, J = 7.9 Hz, 1H), 2.84 (s, 6H).

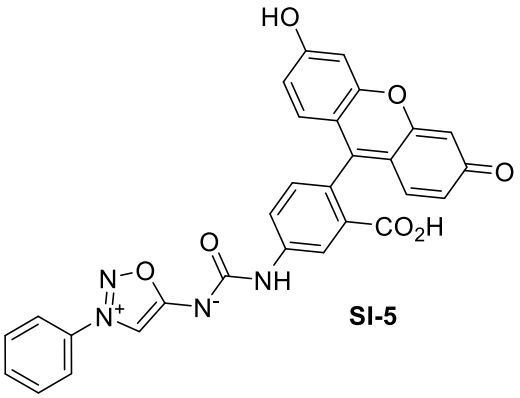
^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 151.5, 137.9, 133.7, 133.5, 130.8 (2C), 130.2, 130.1, 129.8, 128.1, 127.3, 123.2, 121.8 (2C), 121.1, 115.3, 101.0, 45.7 (2C).

IR (cm^{-1}): 3136, 2943, 1603, 1584, 1471, 1363, 1296, 1135, 904, 791, 728, 677, 630, 586, 571.

LCMS (ESI) m/z $[M+H]^+$ 395.

Mp. 202–203 °C.

((3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-5-yl)carbamoyl)(3-phenyl-1,2,3-oxadiazol-3-ium-5-yl)amide (SI-5)



$C_{29}H_{18}N_4O_7$
MW: 534 g.mol^{-1}
Orange solid
Yield: 13%

6-amino-fluorescein (69 mg, 0.20 mmol) and triphosgene (20 mg, 0.066 mmol) were dissolved in freshly distilled acetone (4 mL). $NaHCO_3$ (50 mg, 0.60 mmol) was added and the resulting suspension was stirred for 30 min at 0 °C. **7** (59 mg, 0.30 mmol) was added and the suspension was stirred for 8h

from 0 °C to room temperature. The reaction was degassed through a basic trap (pH=14). The solvent was evaporated under *vacuum* and the crude was purified by preparative TLC (SiO_2, MeOH/DCM, 10/90) to afford the desired product as an orange solid (13 mg, 13%).

^{1}H NMR (400 MHz, MeOD-d_4): δ 8.46 (s, 1H), 8.36 (d, *J* = 2.1 Hz, 1H), 8.02 (m, 2H), 7.85 (dd, *J* = 8.3 Hz, *J* = 2.1 Hz, 1H), 7.72–7.81 (m, 3H), 7.11 (d, *J* = 8.3 Hz, 1H), 6.75 (d, *J* = 8.8 Hz, 2H), 6.67 (d, *J* = 2.4 Hz, 2H), 6.57 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 2H).

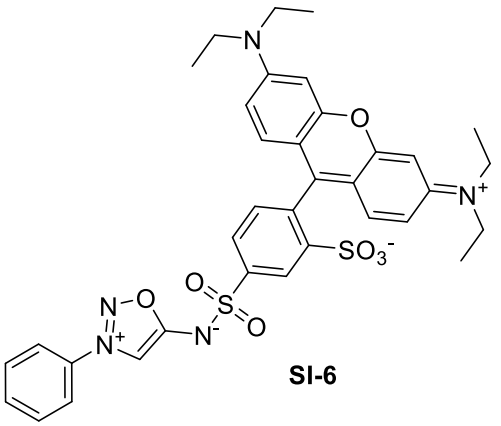
Product was not soluble enough to perform ^{13}C NMR.

IR (cm^{-1}) 3374, 1580, 1468, 1328, 1111.

HRMS (ESI) *m/z* calcd for $C_{29}H_{19}N_4O_7$ $[M+H]^+$: 535.1248; found: 535.1252;
calcd. for $C_{29}H_{20}N_4O_7$. $[M+2H]^{2+}$: 268.0661; found: 268.0664.

Mp. >300 °C.

((4-(6-(diethylamino)-3-(diethyliminio)-3H-xanthen-9-yl)-3-sulfonatophenyl)sulfonyl)(3-phenyl-1,2,3-oxadiazol-3-ium-5-yl)amide (SI-6)



SI-6

$C_{35}H_{35}N_5O_7S_2$
MW: 701 g.mol^{-1}
Dark purple solid
Yield: 28%

To a solution of **7** (39 mg, 0.20 mmol) and sulforhodamine B sulfonyl chloride (58 mg, 0.10 mmol) in DMF (2mL) at -20 °C was added dropwise DIPEA (38 µL, 0.22 mmol) during 30 minutes. The mixture was stirred during 5h from -20 °C to room temperature. The solvent was evaporated under *vacuum* and the crude product was purified by preparative HPLC to afford the desired product as a dark purple solid (20 mg, 28%).

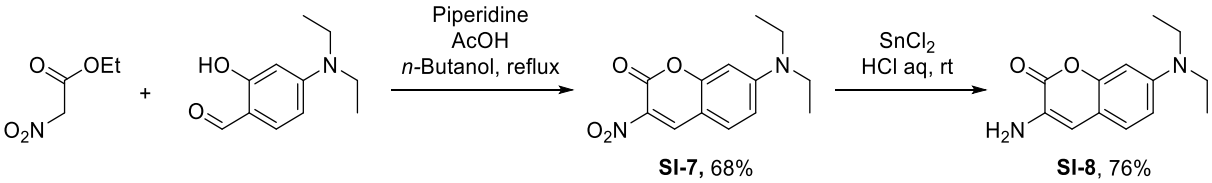
^{1}H NMR (400 MHz, MeOD-d_6): δ 8.75 (d, *J* = 1.8 Hz, 1H), 8.41 (s, 1H), 8.21 (dd, *J* = 8.0 Hz, *J* = 1.8 Hz, 1H), 8.02 (m, 2H), 7.69–7.79 (m, 3H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 9.5 Hz, 2H), 6.96 (dd, *J* = 9.5 Hz, *J* = 2.5 Hz, 2H), 6.90 (d, *J* = 2.5 Hz, 2H), 3.65 (q, *J* = 7.1 Hz, 8H), 1.28 (t, *J* = 7.1 Hz, 12H).

^{13}C NMR (100 MHz, MeOD-d_6): δ 172.6, 159.5 (2C), 158.0, 157.3 (2C), 147.0, 145.8, 135.4, 135.3, 134.6 (2C), 133.8 (2C), 132.6, 131.7 (2C), 129.0, 127.1, 123.6 (2C), 115.4, 115.2 (2C), 104.0, 97.1 (2C), 46.9 (4C), 13.0 (4C).

HRMS (ESI) *m/z* calcd for $C_{35}H_{36}N_5O_7S_2$ $[M+H]^+$: 702.2051; found: 702.2048; calcd. for $C_{35}H_{37}N_5O_7S_2$. $[M+2H]^{2+}$: 351.6062; found: 351.6066; calcd. for $C_{35}H_{35}N_5NaO_7S_2$. $[M+Na]^+$: 724.1870; found: 724.1871

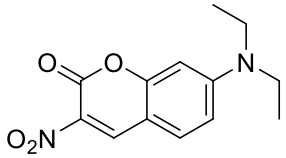
3) *Compound with fluorogenic substituent on the aryl in position N3 and on N6.*

Synthetic route to compounds **SI-7** and **SI-8**:



SI-7, 68% **SI-8**, 76%

7-(diethylamino)-3-nitro-2H-chromen-2-one (SI-7)



SI-7

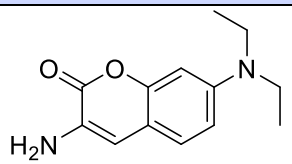
$C_{13}H_{14}N_2O_4$
MW: 262 g.mol^{-1}
Yellow solid
Yield: 68%

The product was synthesized according to a reported procedure.[5] A mixture containing *n*-butanol (2.8 mL), 4-diethylamino salicylaldehyde (193 mg, 1.0 mmol), ethyl nitroacetate (111 µL, 1.0 mmol), piperidine (14 µL) and acetic acid (29 µL) was refluxed for a period of 24 hours. The crude product was evaporated and then purified by column chromatography (SiO_2, Heptane/EtOAc) to afford the desired product as a yellow solid (180 mg, 68%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 (s, 1H), 7.42 (d, *J* = 9.1 Hz, 1H), 6.69 (dd, *J* = 9.1 Hz, *J* = 2.5 Hz, 1H), 6.47 (d, *J* = 2.4 Hz, 1H), 3.48 (q, *J* = 7.2 Hz, 4H), 1.25 (t, *J* = 7.2 Hz, 6H).

LCMS (ESI) m/z: $[M+H]^+$263.

3-amino-7-(diethylamino)-2H-chromen-2-one (SI-8)



SI-8

$C_{13}H_{16}N_2O_2$
MW: 232 g.mol^{-1}
Yellow solid
Yield: 76%

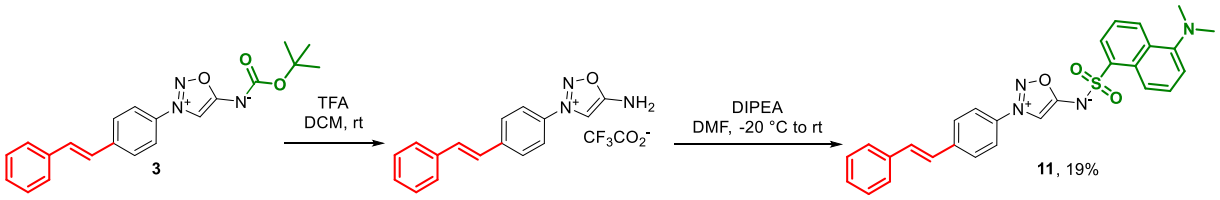
The product was synthesized according to a reported procedure.[5] In a round bottomed flask equipped with a magnetic stirrer, were placed in order, 37.4% HCl (3 mL), stannous chloride dihydrate (1.03 g, 4.58 mmol). To this suspension **SI-7** (160 mg, 0.61 mmol) was added at room temperature in small portions, over a period of thirty minutes. Stirring was continued for 4 h before the solution was poured onto ice and made alkaline using sodium hydroxide solution (5 M). The resulting suspension was then extracted with diethyl ether (2 X 25 mL). The organic layer was washed with water, dried over $MgSO_4$ and concentrated to a pasty residue which upon triturating using heptane gave the desired product as a yellow solid (107 mg, 68%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09 (d, *J* = 8.7 Hz, 1H), 6.68 (s, 1H), 6.55 (dd, *J* = 8.7 Hz, *J* = 2.5 Hz, 1H), 6.50 (d, *J* = 2.5 Hz, 1H), 3.85 (br.s, 2H), 3.35 (q, *J* = 7.1 Hz, 4H), 1.16 (t, *J* = 7.1 Hz, 6H).

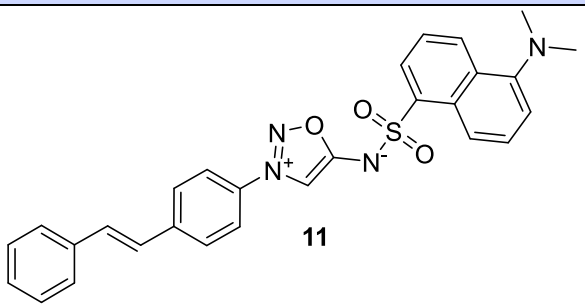
LCMS (ESI) m/z: $[M+H]^+$.233.

[5] K. Sivakumar, F. Xie, B. M. Cash, S. Long, H. N. Barnhill, Q. Wang, *Org. Lett.* **2004**, *6*, 4603.

Synthetic route to compound **11**:



(E)-((5-(dimethylamino)naphthalen-1-yl)sulfonyl)(3-(4-styrylphenyl)-1,2,3-oxadiazol-3-ium-5-yl)amide (11)



$C_{28}H_{24}N_4O_3S$
MW: 496 g.mol^{-1}
Yellow solid
Yield: 19%

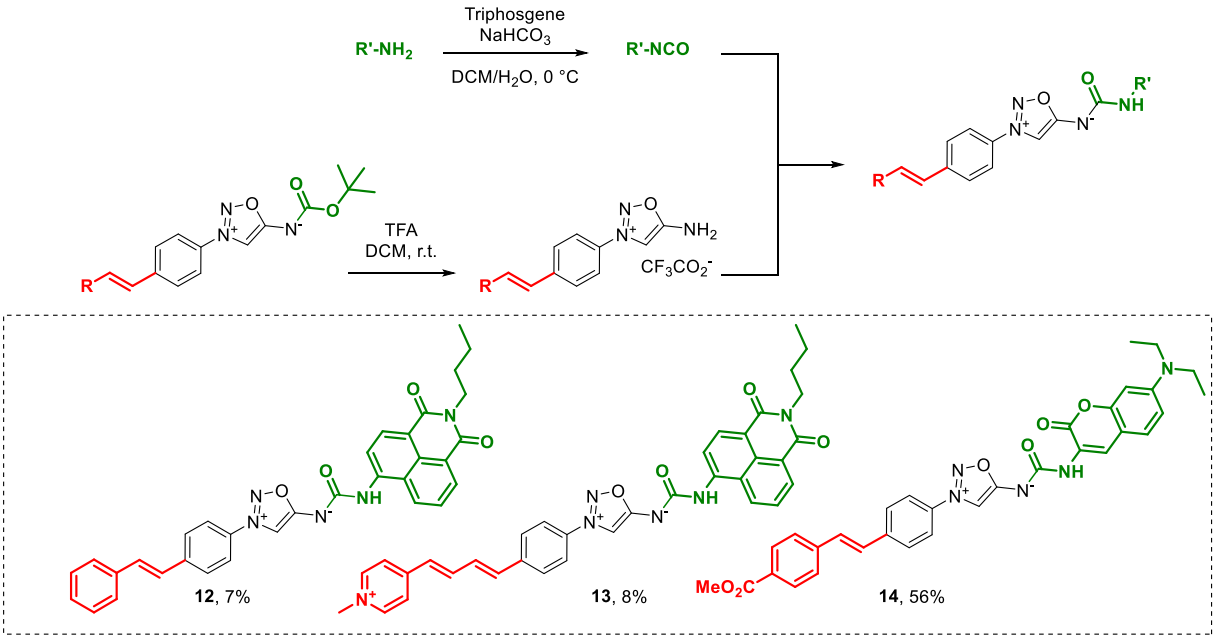
A solution of **3** (27 mg, 0.074 mmol) in HCl/Dioxane (4 mL) was stirred at room temperature for 4 hours. The solvent was evaporated under *vacuum.* The crude product was solubilized in MeOH and evaporated again several times. The residue was dissolved in anhydrous DMF (5 mL) and dansyl chloride (41 mg, 0.15 mmol) was added. The mixture was stirred at -20 °C and DIPEA (28 µL, 0.16 mmol) was added dropwise during 30 minutes at -20 °C. The mixture was stirred from -20 °C to room temperature overnight. The reaction was quenched with a saturated solution of NH_4Cl and extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered and evaporated under *vacuum*. The product was purified by preparative TLC (SiO_2, DCM/MeOH) to afford the product as a yellow solid (7 mg, 19%).

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 8.46 (s, 1H), 8.41 (m, 2H), 8.34 (dd, *J* = 7.4 Hz, *J* = 1.0 Hz, 1H), 8.01(d, *J* = 8.5 Hz, 2H), 7.90 (d, *J* = 8.5 Hz, 2H), 7.66 (m, 2H), 7.60 (ddd, *J* = 8.6 Hz, *J* = 7.4 Hz, *J* = 4.3 Hz, 2H), 7.50 (d, *J* = 16.5 Hz, 1H), 7.31–7.44 (m, 4H), 7.23 (d, *J* = 7.7 Hz, 1H), 2.81 (s, 6H).

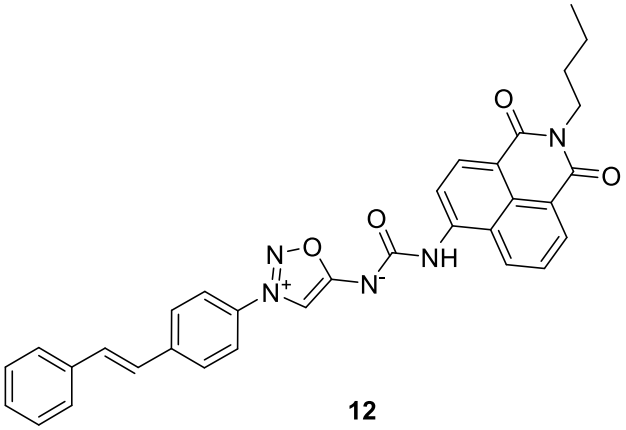
^{13}C NMR (100 MHz, DMSO-d$_6$): δ 169.7, 151.1, 141.8, 138.1, 136.4, 132.1, 131.9, 129.4, 129.0, 128.9 (3C), 128.5, 127.7 (2C), 127.5, 127.1, 127.0 (2C), 126.4, 123.5, 122.8 (2C), 120.4, 115.0, 102.1, 45.1 (2C).

HRMS (ESI) *m/z* calcd for $C_{28}H_{25}N_4O_3S$ $[M+H]^+$: 497.1641; found: 497.1640.

Synthetic route to compounds **12**-**14**:



(E)-((2-butyl-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)carbamoyl)(3-(4-styrylphenyl)-1,2,3-oxadiazol-3-ium-5-yl)amide (12)

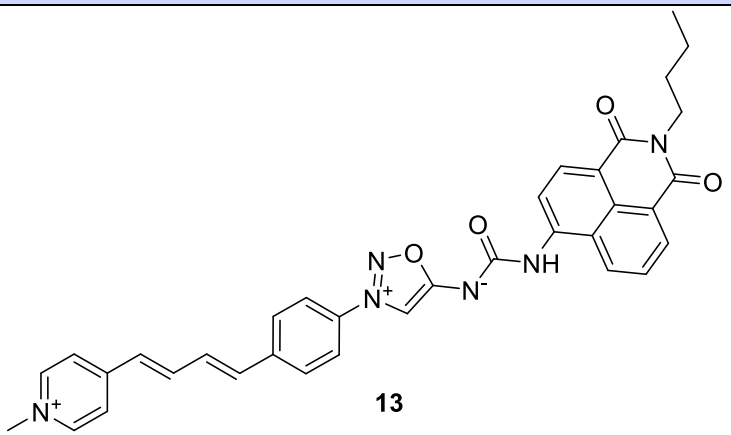


12

$C_{33}H_{27}N_5O_4$
MW: 558 g.mol^{-1}
Yellow solid
Yield: 7%

To a solution of **3** (47 mg, 0.13 mmol) in DCM (1 mL) was added TFA (1 mL). The mixture was stirred at room temperature for 3 hours. The solvent was evaporated under *vacuum.* The crude product was solubilized in MeOH and evaporated again several times in order to get rid of TFA traces.
In another flask triphosgene (13 mg, 0.043 mmol) was suspended in DCM (1.5 mL) and **SI-4** (34 mg, 0.13 mmol) was added. The suspension was cooled to 0 °C and a solution of $NaHCO_3$ (33 mg, 0.39 mmol) in H_2O (1.5 mL) was added dropwise. The mixture was stirred for 30 minutes at 0 °C and the crude deprotected styryl-iminosydnone was added. The mixture was stirred from 0 °C to room temperature overnight. The reaction was quenched with brine and extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered and evaporated under *vacuum*. The product was purified by preparative TLC (SiO_2, MeOH/DCM) to afford the product as a yellow solid (5 mg, 7%).
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57–8.65 (m, 3H), 8.35 (s, 1H), 8.33 (m, 1H), 8.25 (br.s, 1H), 7.82 (d, J = 8.8 Hz, 2H), 7.75 (m, 3H), 7.54 (m, 2H), 7.31–7.42 (m, 3H), 7.27 (d, J = 16.3 Hz, 1H), 7.13 (d, J = 16.3 Hz, 1H), 4.15 (m, 2H), 1.70 (m, 2H), 1.42 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H).
The small quantity of product did not permit to afford a ^{13}C NMR spectra.
HRMS (ESI) *m/z* calcd for $C_{33}H_{28}N_5O_4$ $[M+H]^+$: 558.2136; found: 558.2136;

((2-butyl-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)carbamoyl)(3-(4-((1E,3E)-4-(1-methylpyridin-1-ium-4-yl)buta-1,3-dien-1-yl)phenyl)-1,2,3-oxadiazol-3-ium-5-yl)amide 2,2,2-trifluoroacetate (13)

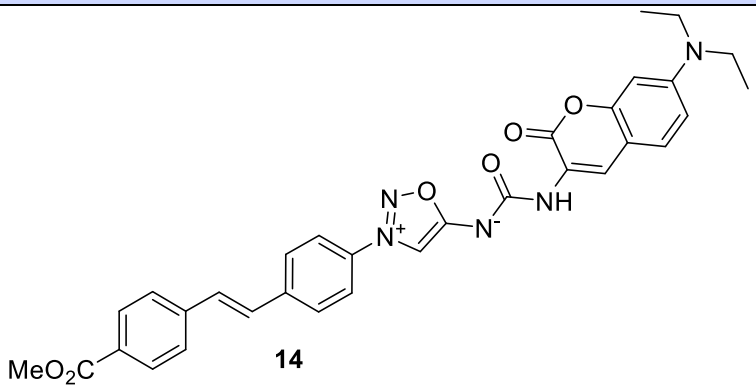


13

$C_{37}H_{31}F_3N_6O_6$
MW: 713 g.mol^{-1}
Orange oil
Yield: 8%

To a solution of **6** (27 mg, 0.05 mmol) in DCM (2 mL) was added TFA (200 µL). The mixture was stirred at room temperature for 3 hours. The solvent was evaporated under *vacuum.* The crude product was solubilized in MeOH and evaporated again several times in order to get rid of TFA traces.
In another flask triphosgene (5 mg, 0.017 mmol) and **SI-4** (13 mg, 0.05 mmol) were dissolved in freshly distilled acetone (1 mL). $NaHCO_3$ (14 mg, 0.17 mmol) was added and the resulting suspension was stirred for 30 min at 0 °C. and the crude deprotected pyridinium-iminosydnone was added. The suspension was stirred overnight from 0 °C to room temperature. The reaction was degassed through a basic trap (pH=14). The solvent was evaporated under *vacuum* and the crude was purified by preparative HPLC to afford the desired product as an orange oil (3 mg, 8%).
^{1}H NMR (400 MHz, MeOD-d_4): δ 8.72 (m, 2H), 8.50–8.60 (m, 6H), 8.09 (d, J = 6.4 Hz, 2H), 8.03 (d, J = 8.4 Hz, 2H), 7.84 (m, 3H), 7.75 (dd, J = 15.6 Hz, J = 10.5 Hz, 1H), 7.35 (dd, J = 15.6 Hz, J = 10.5 Hz, 1H), 7.15 (d, J = 15.6 Hz, 1H), 6.99 (d, J = 15.6 Hz, 1H), 4.32 (s, 3H), 4.13 (m, 2H), 1.69 (m, 2H), 1.44 (m, 2H), 1.00 (t, J = 7.4 Hz, 3H).
The small quantity of product did not permit to afford a ^{13}C NMR spectra.
HRMS (ESI) *m/z* calcd for $C_{35}H_{31}N_6O_4$ $[M]^+$: 599.2401; found: 599.2401;
calcd. for $C_{35}H_{32}N_6O_4$. $[M+H]^{2+}$: 300.1237; found: 300.1240

(E)-((7-(diethylamino)-2-oxo-2H-chromen-3-yl)carbamoyl)(3-(4-(4-(methoxycarbonyl)styryl)phenyl)-1,2,3-oxadiazol-3-ium-5-yl)amide (14)



14

$C_{32}H_{29}N_5O_6$
MW: 580 g.mol^{-1}
Red solid
Yield: 56%

To a solution of **4** (81 mg, 0.19 mmol) in DCM (3 mL) was added TFA (770 µL, 10 mmol). The mixture was stirred at room temperature for 3 hours. The solvent was evaporated under *vacuum.* The crude product was solubilized in MeOH and evaporated again several times in order to get rid of TFA traces.
In another flask triphosgene (19 mg, 0.064 mmol) was suspended in DCM (2 mL) and **SI-8** (44 mg, 0.19 mmol) was added. The suspension was cooled to 0 °C and a solution of $NaHCO_3$ (49 mg, 0.58 mmol) in H_2O (2 mL) was added dropwise. The mixture was stirred for 30 minutes at 0 °C and the

crude deprotected styryl-iminosydnone was added. The mixture was stirred from 0 °C to room temperature overnight. The reaction was quenched with brine and extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered and evaporated under *vacuum*. The product was purified by column chromatography (SiO_2, Heptane/EtOAc) to afford the product as a red solid (62 mg, 56%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1H), 8.17 (s, 1H), 8.00 (d, *J* = 7.8 Hz, 2H), 7.74 (d, *J* = 8.6 Hz, 2H), 7.70 (br.s, 1H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.10–7.19 (m, 3H), 6.53 (dd, *J* = 8.6 Hz, *J* = 2.5 Hz, 1H), 6.37 (d, *J* = 2.5 Hz, 1H), 3.89 (s, 3H), 3.30 (q, *J* = 7.1 Hz, 4H), 1.13 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 173.0, 166.8, 159.5, 152.2, 148.8, 141.7, 140.6, 132.8, 131.5, 130.2 (2C), 130.0, 128.4 (3C), 128.1, 126.9 (2C), 122.1, 121.7 (2C), 120.8, 109.5, 109.1 (2C), 101.8, 97.6, 52.4, 44.8 (2C), 12.6 (2C).

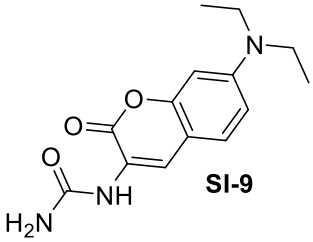
IR (cm^{-1}): 3414, 2970, 1703, 1602, 1352, 1180, 1107, 961, 907, 764, 727.

HRMS (ESI) *m/z* calcd for $C_{32}H_{30}N_5O_6$ $[M+H]^+$: 580.2191; found: 580.2190;
calcd. for $C_{32}H_{31}N_5O_6$. $[M+2H]^{2+}$: 290.6132; found: 290.6135.

Mp.: 140 °C –decomp.

4) Released products

1-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)urea (SI-9)

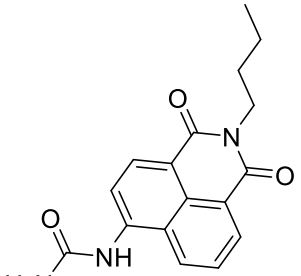


SI-9

$C_{14}H_{17}N_3O_3$
MW: 275 g.mol^{-1}
Yellow solid

8 (8.6 mg, 20.5 µmol) and BCN (3.4 mg, 22.6 µmol) were solubilized in DMSO-d_6 (1 mL). The mixture was stirred at room temperature and the conversion was followed by NMR. Once completed, the reaction was diluted with DCM and HCl 1M was added. The aqueous layer was collected and NaOH 1M was added. The desired product precipitated and was obtained as a yellow solid.
^{1}H NMR (400 MHz, MeOD-d_4): δ 8.21 (s, 1H), 7.30 (d, *J* = 8.8 Hz, 1H), 6.72 (dd, *J* =8.8 Hz, *J* = 2.5 Hz, 1H), 6.53 (d, *J* = 2.5 Hz, 1H), 3.45 (q, *J* = 7.1 Hz, 4H), 1.19 (t, *J* = 7.1 Hz, 6H).
LCMS (ESI) m/z: [M+H]$^+$276**.**

1-(2-butyl-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)urea (SI-10)



SI-10

$C_{17}H_{17}N_3O_3$
MW: 311 g.mol^{-1}
Yellow solid
Yield: 40%

9 (16.7 mg, 37 µmol) and BCN (8.3 mg, 55 µmol) were solubilized in DMSO (1 mL). The mixture was stirred at room temperature overnight. The reaction was diluted with DCM and NH_4Cl_{sat}. The organic layer was collected, dried over $MgSO_4$ and evaporated. The crude mixture was purified by preparative TLC (SiO_2, DCM/MeOH, 95/5) to afford the desired product as a yellow solid (4.6 mg, 40%).
^{1}H NMR (400 MHz, DMSO-d_6): δ 9.30 (s, 1H), 8.63 (d, *J* = 8.8 Hz, 1H), 8.55 (d, *J* = 8.3 Hz, 1H), 8.51 (d, *J* = 8.3 Hz, 1H), 8.41 (d, *J* = 8.5 Hz, 1H), 7.88 (dd, *J* = 8.5 Hz, *J* = 7.3 Hz, 1H), 6.57 (br.s, 2H), 4.03 (t, *J* = 7.8 Hz, 2H), 1.60 (m, 2H), 1.35 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H).
LCMS (ESI) m/z: [M+H]$^+$312**.**

III. Optical properties

Absorbances were measured on three solutions of compounds in DMSO except for compounds **6** and **14** (water/DMSO, 80/20). The molar extinction coefficients were determined by plotting absorbance values versus the concentrations and analyzing by linear regression (Equation 1).The molar extinction coefficient corresponds to the slope.

$$A = \varepsilon \times l \times C$$

Equation 1. Beer-Lambert law (with A= absorbance; ε = molar extinction coefficient ($M^{-1}.cm^{-1}$) ; l = thickness of the cuve (cm) ; C = concentration (M)).

Fluorescences were measured on 1 µm (or 0.1 µM when specified) solutions of compounds in DMSO or water/DMSO (8:2) mixtures excited at a wavelength corresponding to the maximum of absorbance of the cycloadduct or released compound.
For products SI-5 and SI-6 fluorescence was measured on the crude mixture after reaction with BCN
Absorbance and fluorescence spectra for each combination are reported in Table S4.
A summary of optical properties for all compounds is presented in Tables S1-3
Quantum yields were calculated relatively to a standard according to Equation 2**.**

$$\Phi_x = \Phi_{st} \times \left(\frac{A_{st}}{A_x}\right) \times \left(\frac{F_x}{F_{st}}\right) \times \left(\frac{{n_x}^2}{{n_{st}}^2}\right) \times \left(\frac{D_x}{D_{st}}\right)$$

Equation 2. Quantum yield for a compound x comparing to a standard st (with A = absorbance; F = area under the curve of emission; n = refractive index of the solvent; D = factor of dilution between absorbance and fluorescence measurements).

Quinine sulfate and coumarin153 were used as standard for this calculation.

*Table S1 : Fluorescence properties of iminosydnone functionalized with fluorophore in position N3 and their associated pyrazole. Solvent: DMSO. * Water/DMSO, 80/20. Brt: Brightness*

		Iminosydnone				**Pyrazole**					
Entry	Probe	λ_{ex} (nm)	λ_{em} (nm)	Φ_f	ε ($M^{-1}.cm^{-1}$)	λ_{ex} (nm)	λ_{em} (nm)	Φ_f	ε ($M^{-1}.cm^{-1}$)	Brt.	Turn-on
1	**3**	344	446	0.017	25 575	339	374	0.196	33 192	6 505	33
2	**5**	361	591	0.327	24 806	342	370	0.099	27 313	2 703	21
3	**4**	346	419	0.019	22 170	354	455	0.232	28 227	6 548	27
4*	**6**	378	493	0.019	28 128	403	582	0.105	33 948	3 564	19

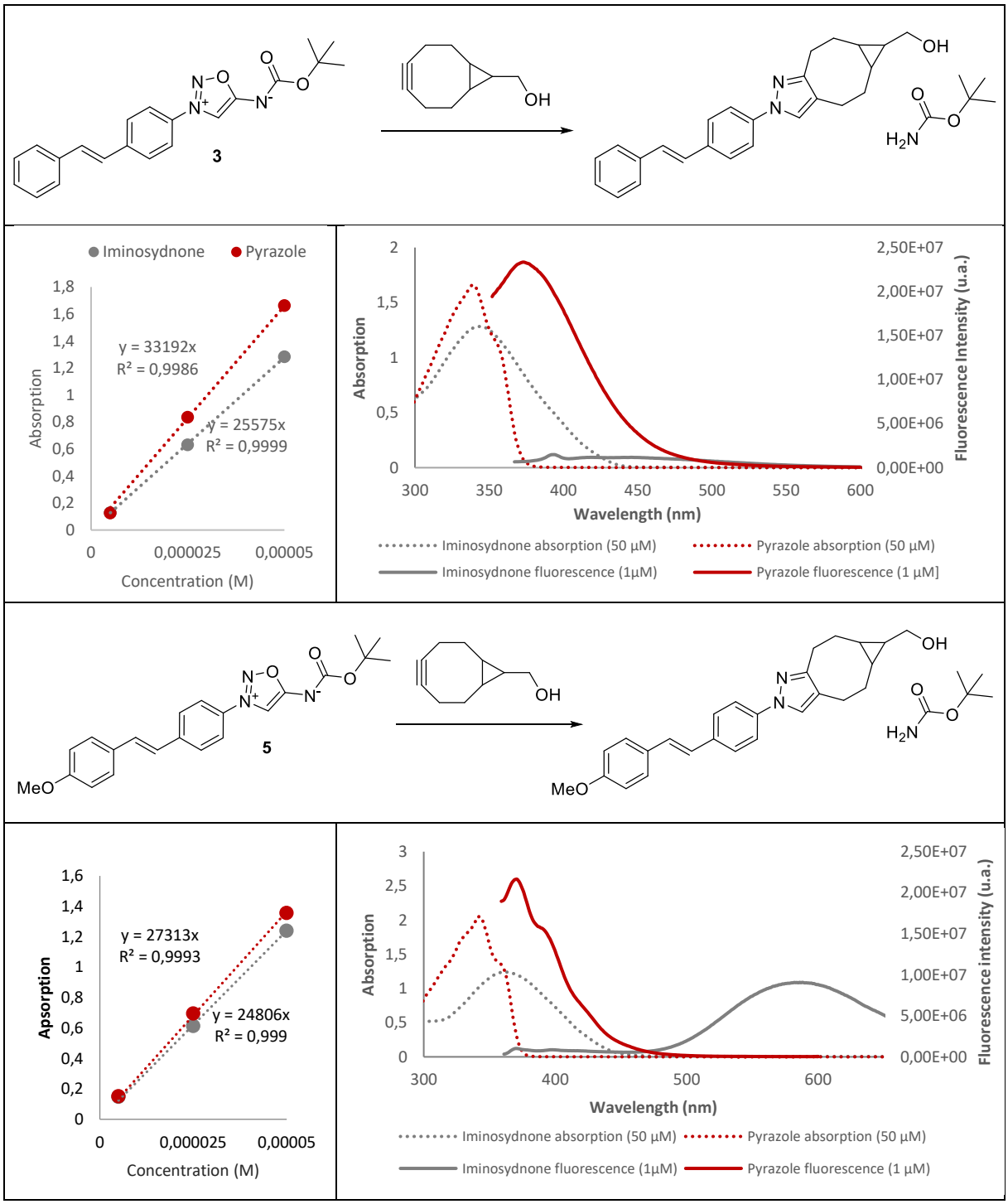
Table S2 : Fluorescence properties of iminosydnone functionalized with fluorophore in position N6 and the associated released products. Solvent: DMSO. Brt: Brightness

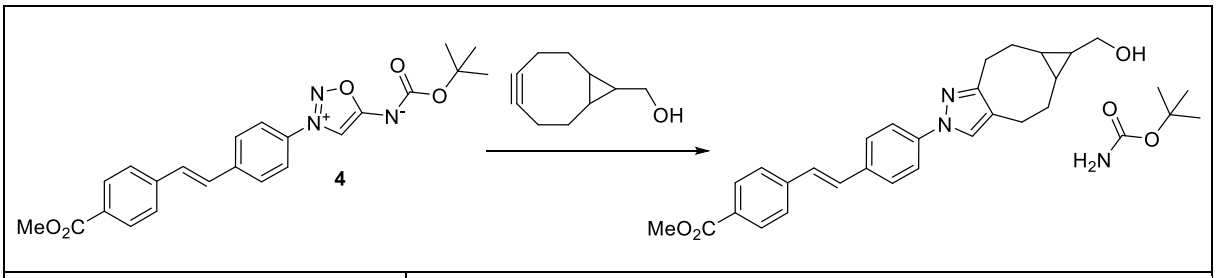
		Iminosydnone				Released fluorophore					
Entry	Probe	λ_{ex} (nm)	λ_{em} (nm)	Φf	ε ($M^{-1}.cm^{-1}$)	λ_{ex} (nm)	λ_{em} (nm)	Φf	ε ($M^{-1}.cm^{-1}$)	Brt.	Turn-on
1	**8**	411	466	0.006	10 294	387	465	0.510	10 516	5 363	86
2	**10**	341	405	0.004	10 294	338	510	0.439	4 884	2 144	282
3	**9**	411	516	0.033	27 929	392	483	0.503	13 001	6 539	69
4*	**SI-5**	495	515	n.d.	35 199	n.d.	515	n.d.	n.d.	n.d.	1,3
5	**SI-6**	566	585	n.d.	92 177	n.d.	585	n.d.	n.d.	n.d.	1

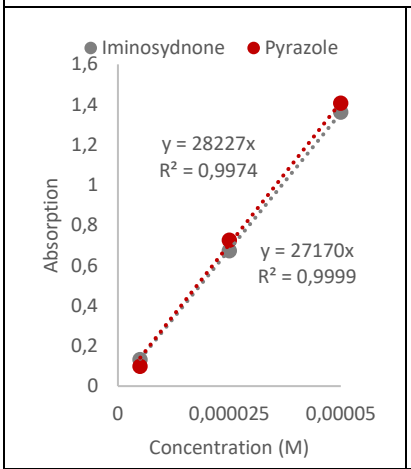
*Table S3 : Fluorescence properties of iminosydnone functionalized with 2 profluorophores and the associated products after reaction with BCN. Solvent: DMSO. * Water/DMSO, 80/20*

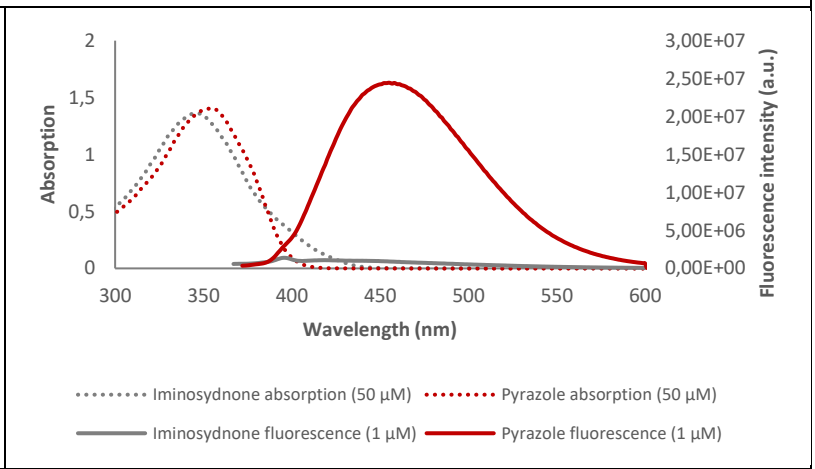
		Iminosydnone				Pyrazole			Released fluorophore		
Entry	Probe	λ_{ex} (nm)	λ_{em} (nm)	Φf	ε ($M^{-1}.cm^{-1}$)	λ_{ex} (nm)	λ_{em} (nm)	Turn-on	λ_{ex} (nm)	λ_{em} (nm)	Turn-on
1	**11**	345	377	0.003	18 654	339	374	29	338	510	97
2	**12**	415	473	0.012	32 349			26	392	483	31
3*	**13**	396	482	0.015	35 561	403	582	18			22
4	**14**	349	467	0.007	29 441	354	455	33	387	465	22

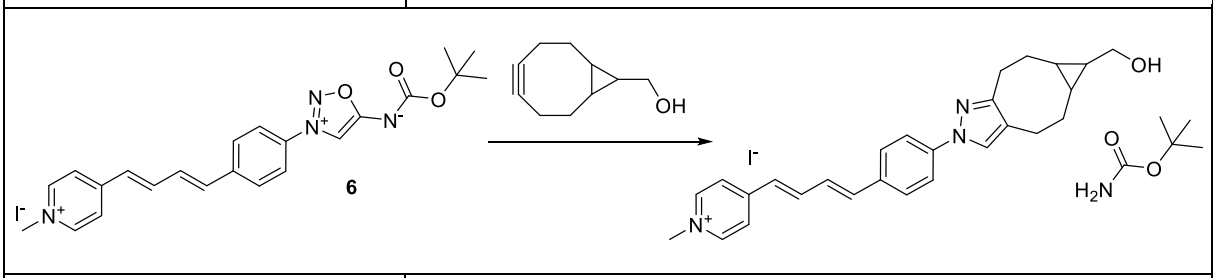
Table S4 : Superposition of absorbance and fluorescence spectra of iminosydnones and corresponding click products

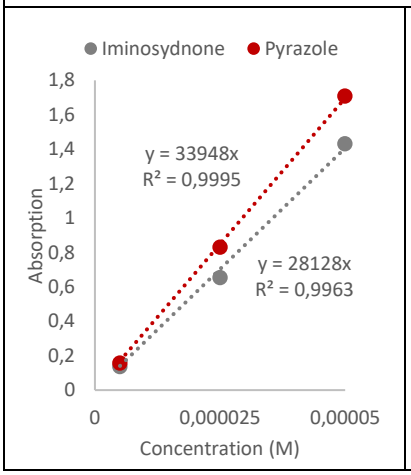


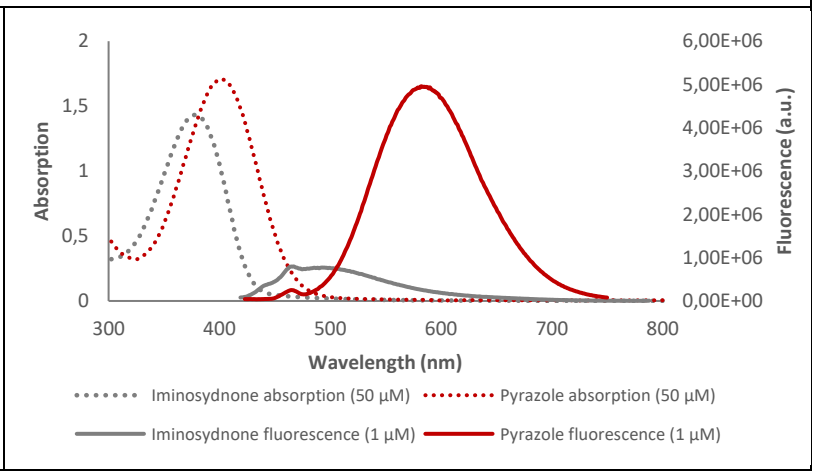

4
OH
OH
MeO2C
H2N

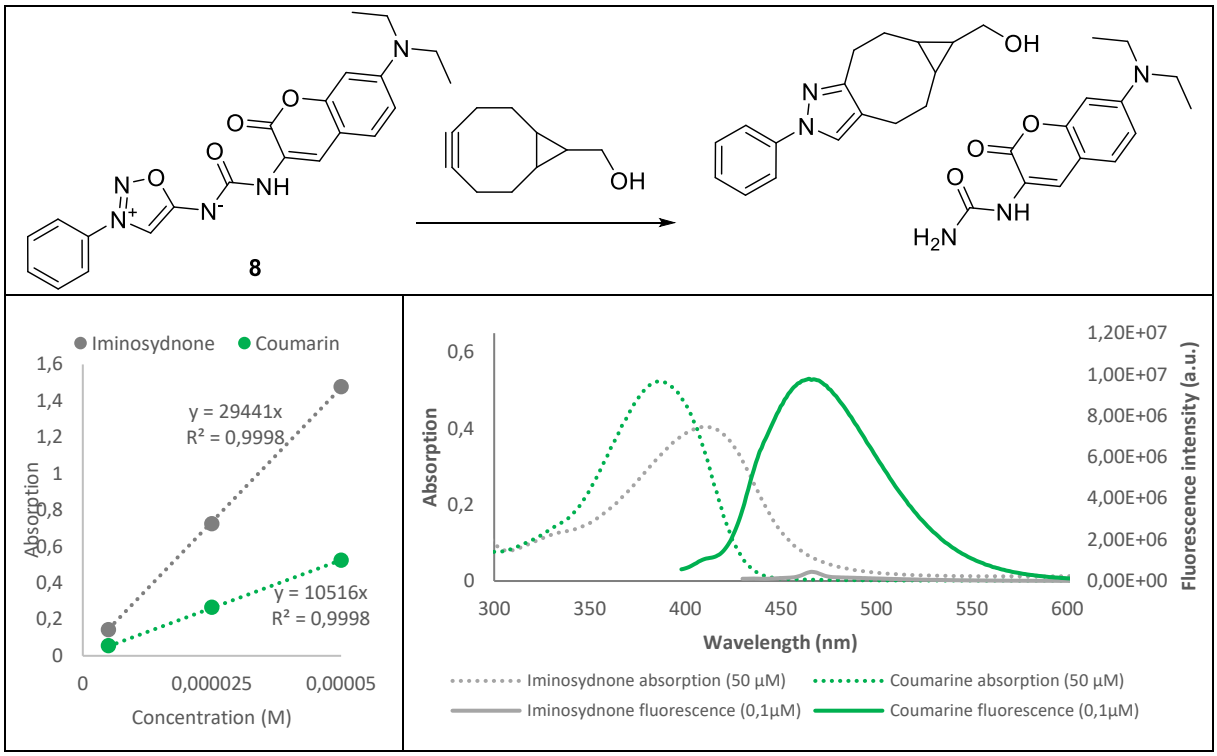

Iminosydnone
Pyrazole
y = 28227x
R² = 0,9974
y = 27170x
R² = 0,9999
Absorption
Concentration (M)
0
0,000025
0,00005

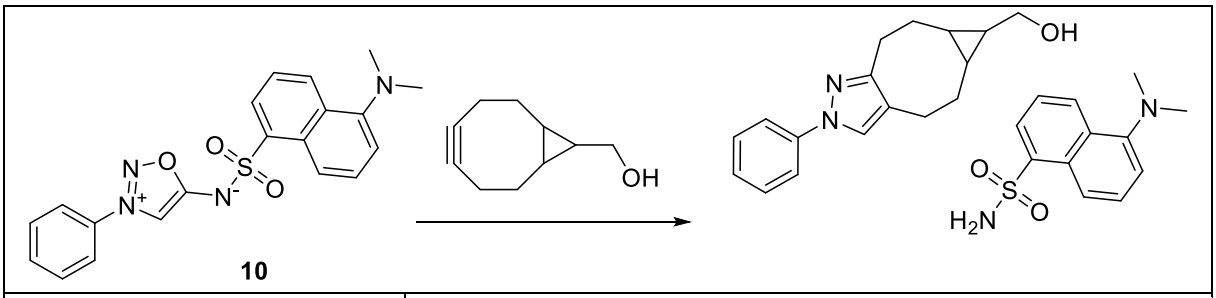

Absorption
Fluorescence intensity (a.u.)
Wavelength (nm)
Iminosydnone absorption (50 µM)
Pyrazole absorption (50 µM)
Iminosydnone fluorescence (1 µM)
Pyrazole fluorescence (1 µM)


6
OH
OH
I-
H2N

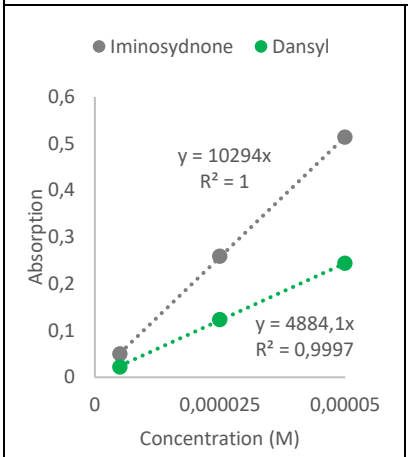

Iminosydnone
Pyrazole
y = 33948x
R² = 0,9995
y = 28128x
R² = 0,9963
Absorption
Concentration (M)
0
0,000025
0,00005

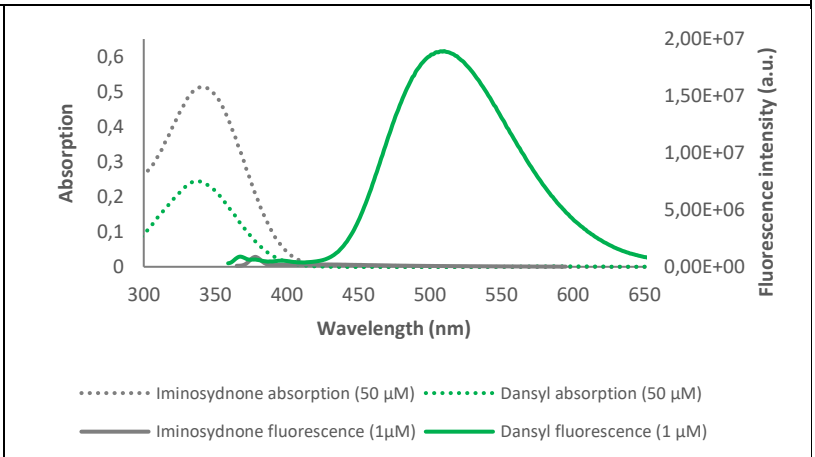

Absorption
Fluorescence (a.u.)
Wavelength (nm)
Iminosydnone absorption (50 µM)
Pyrazole absorption (50 µM)
Iminosydnone fluorescence (1 µM)
Pyrazole fluorescence (1 µM)

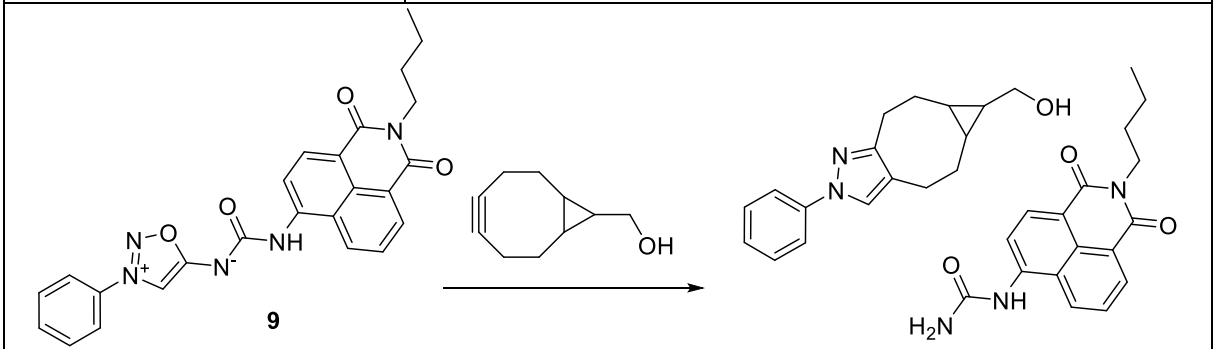

8
OH
OH
NH
NH
H_2N
Iminosydnone
Coumarin
y = 29441x
R² = 0,9998
y = 10516x
R² = 0,9998
Absorption
Concentration (M)
0
0,000025
0,00005
Absorption
Fluorescence intensity (a.u.)
Wavelength (nm)
Iminosydnone absorption (50 µM)
Coumarine absorption (50 µM)
Iminosydnone fluorescence (0,1µM)
Coumarine fluorescence (0,1µM)


OH
OH
H_2N

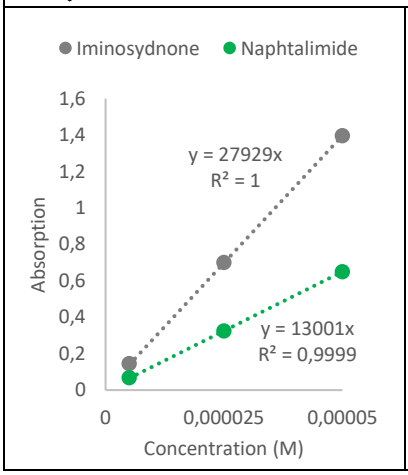
10

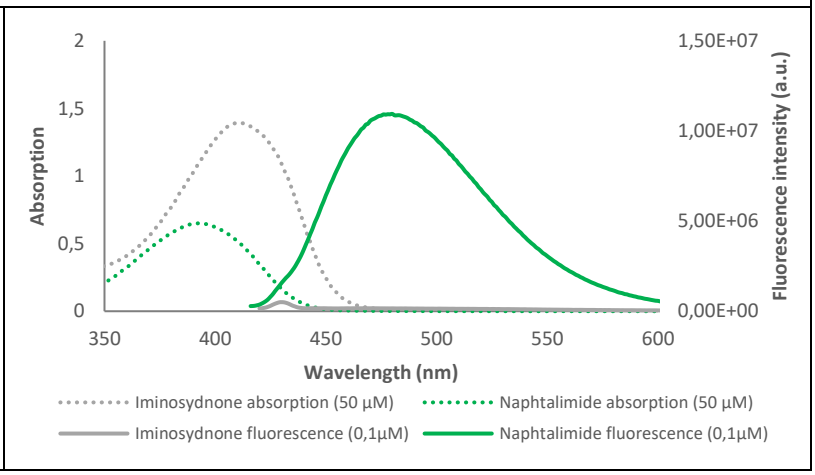

Iminosydnone
Dansyl
y = 10294x
R² = 1
y = 4884,1x
R² = 0,9997
Absorption
Concentration (M)
0
0,000025
0,00005

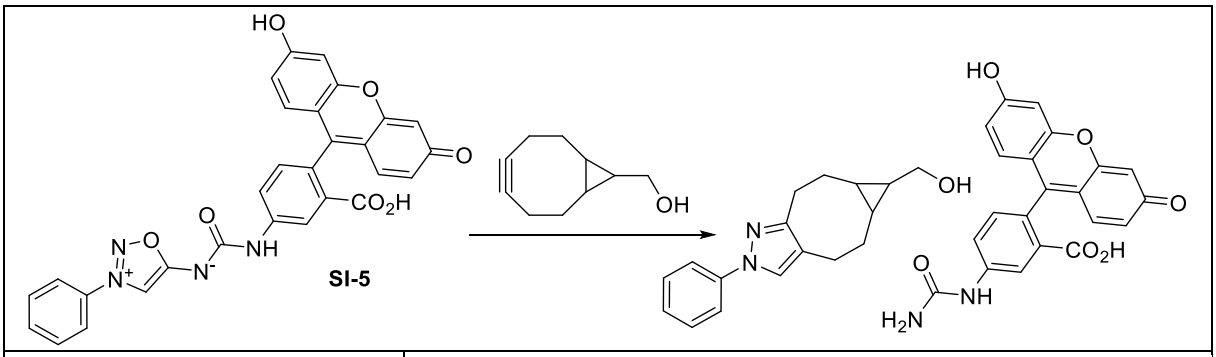

Absorption
Fluorescence intensity (a.u.)
Wavelength (nm)
Iminosydnone absorption (50 µM)
Dansyl absorption (50 µM)
Iminosydnone fluorescence (1µM)
Dansyl fluorescence (1 µM)

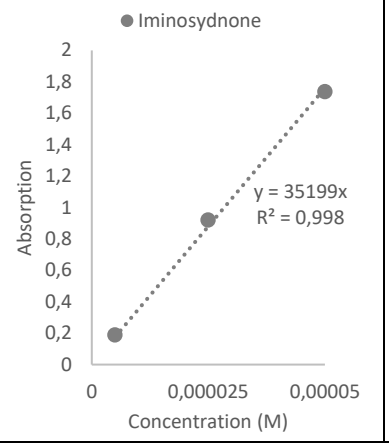

OH
OH
H_2N

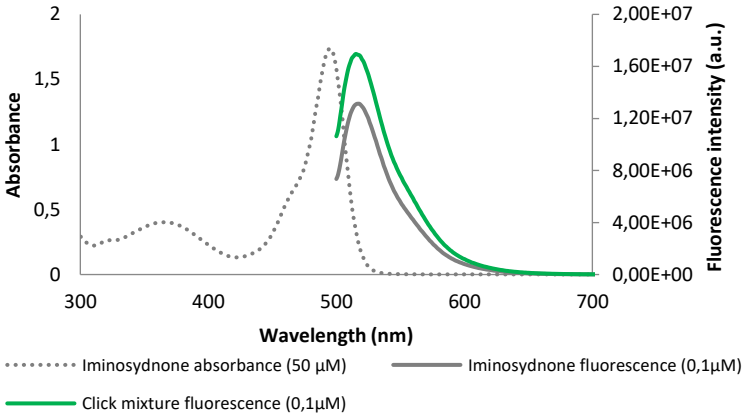
9

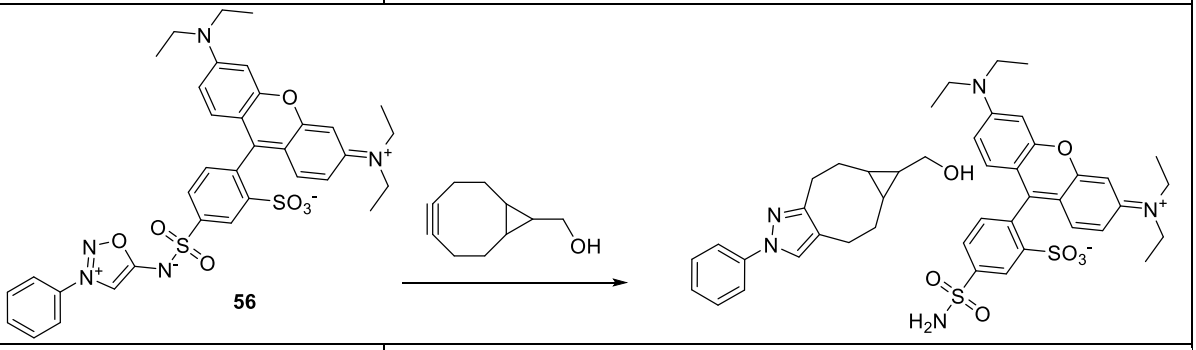

Iminosydnone
Naphtalimide
y = 27929x
R² = 1
y = 13001x
R² = 0,9999
Absorption
Concentration (M)
0
0,000025
0,00005

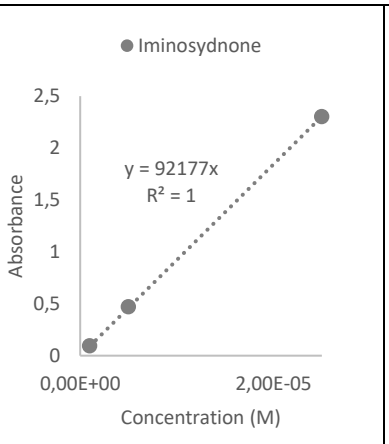

Absorption
Fluorescence intensity (a.u.)
Wavelength (nm)
Iminosydnone absorption (50 µM)
Naphtalimide absorption (50 µM)
Iminosydnone fluorescence (0,1µM)
Naphtalimide fluorescence (0,1µM)

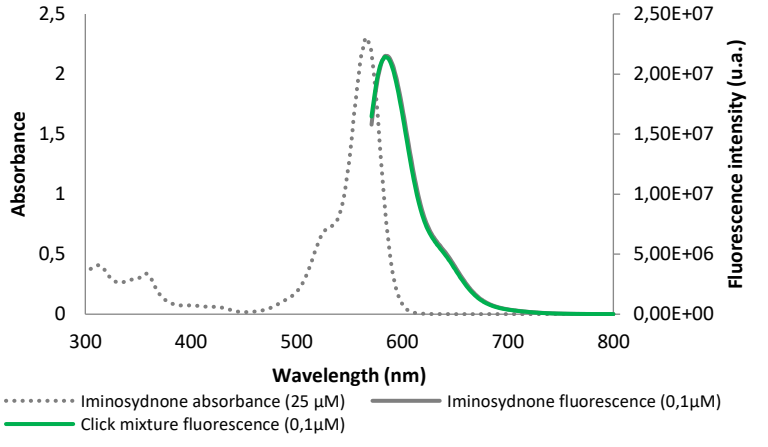

HO
O
O
CO2H
O
NH
N
N
N
SI-5
OH
HO
O
O
CO2H
OH
N
N
O
NH
H2N

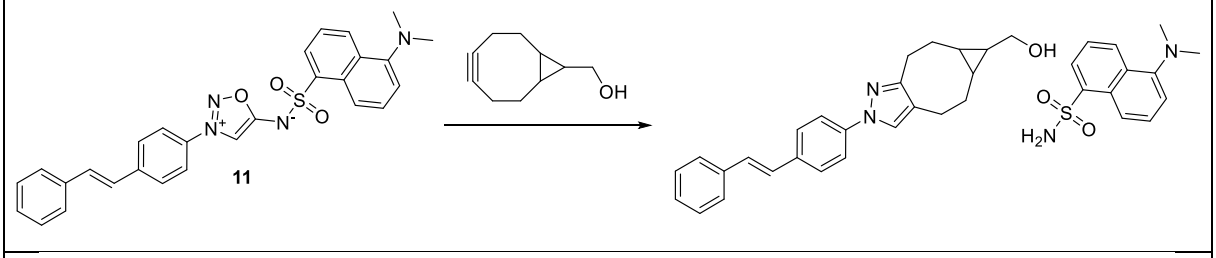

Iminosydnone
Absorption
y = 35199x
R² = 0,998
Concentration (M)
0
0,000025
0,00005
0,2
0,4
0,6
0,8
1
1,2
1,4
1,6
1,8
2

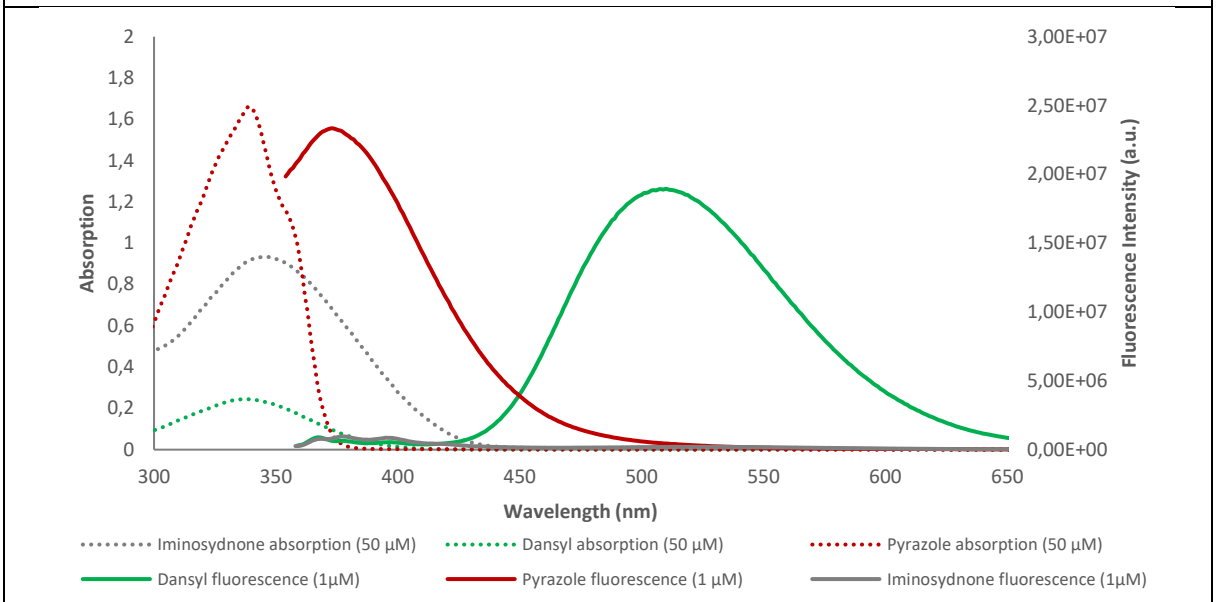

Absorbance
Fluorescence intensity (a.u.)
Wavelength (nm)
300
400
500
600
700
0
0,5
1
1,5
2
0,00E+00
4,00E+06
8,00E+06
1,20E+07
1,60E+07
2,00E+07
Iminosydnone absorbance (50 µM)
Iminosydnone fluorescence (0,1µM)
Click mixture fluorescence (0,1µM)

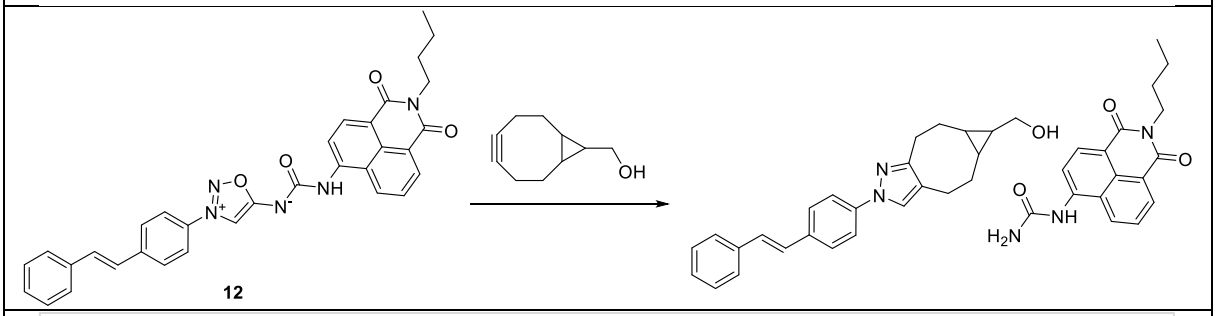

N
O
SO3-
N+
N
O
S
O
N
O
56
OH
N
N
OH
O
SO3-
N+
O
S
O
H2N

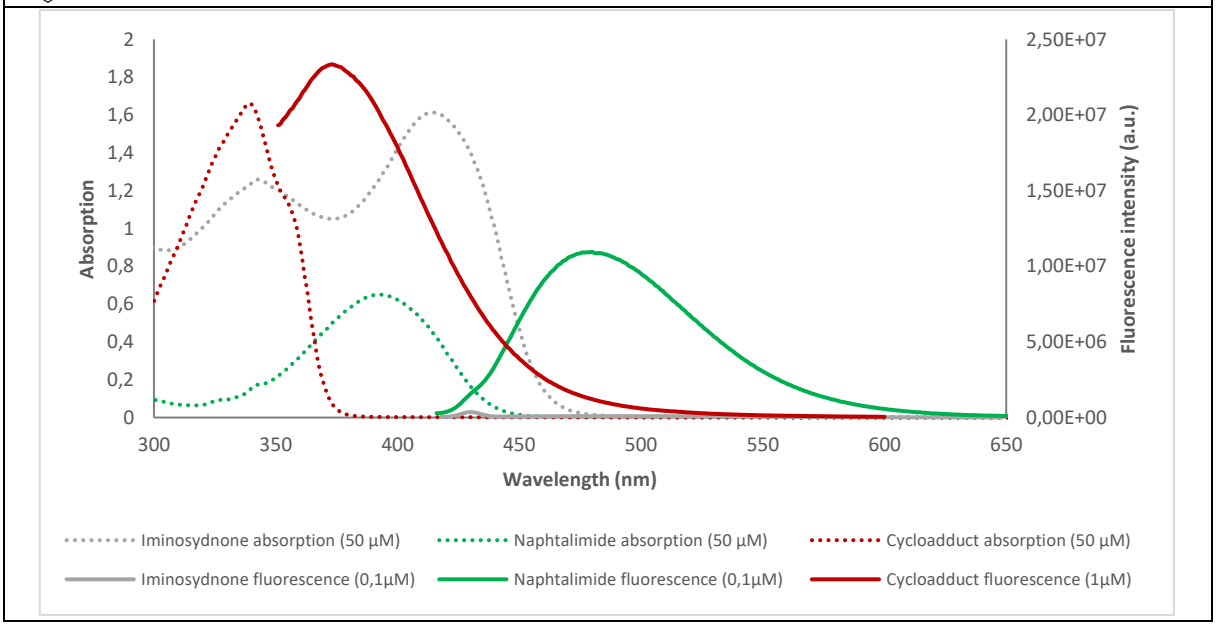

Iminosydnone
Absorbance
y = 92177x
R² = 1
Concentration (M)
0,00E+00
2,00E-05
0
0,5
1
1,5
2
2,5

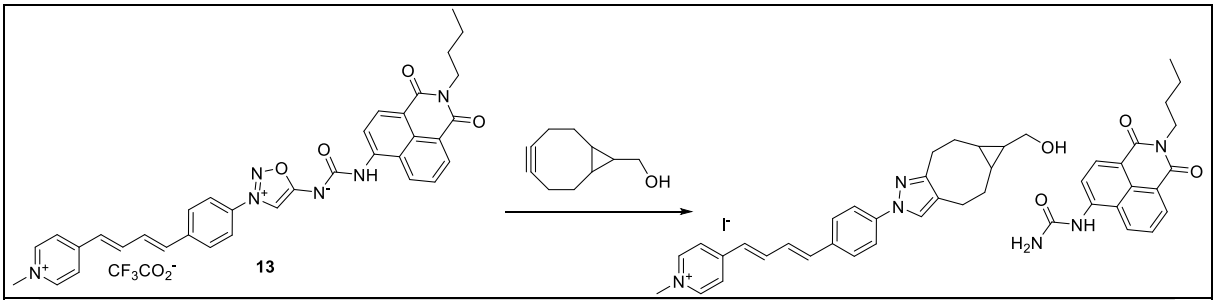

Absorbance
Fluorescence intensity (u.a.)
Wavelength (nm)
300
400
500
600
700
800
0
0,5
1
1,5
2
2,5
0,00E+00
5,00E+06
1,00E+07
1,50E+07
2,00E+07
2,50E+07
Iminosydnone absorbance (25 µM)
Iminosydnone fluorescence (0,1µM)
Click mixture fluorescence (0,1µM)

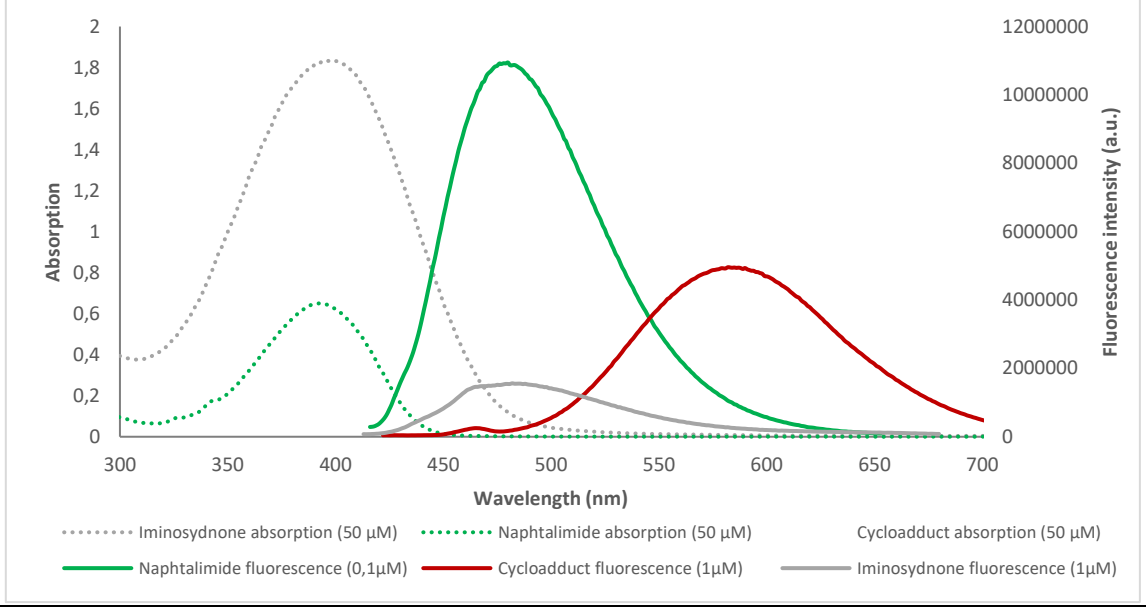

11
OH
OH
H_2N

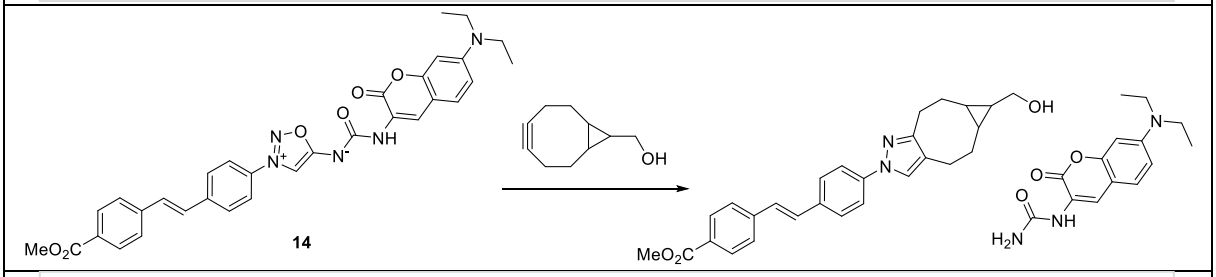

Absorption
Fluorescence Intensity (a.u.)
Wavelength (nm)
Iminosydnone absorption (50 µM)
Dansyl absorption (50 µM)
Pyrazole absorption (50 µM)
Dansyl fluorescence (1µM)
Pyrazole fluorescence (1 µM)
Iminosydnone fluorescence (1µM)

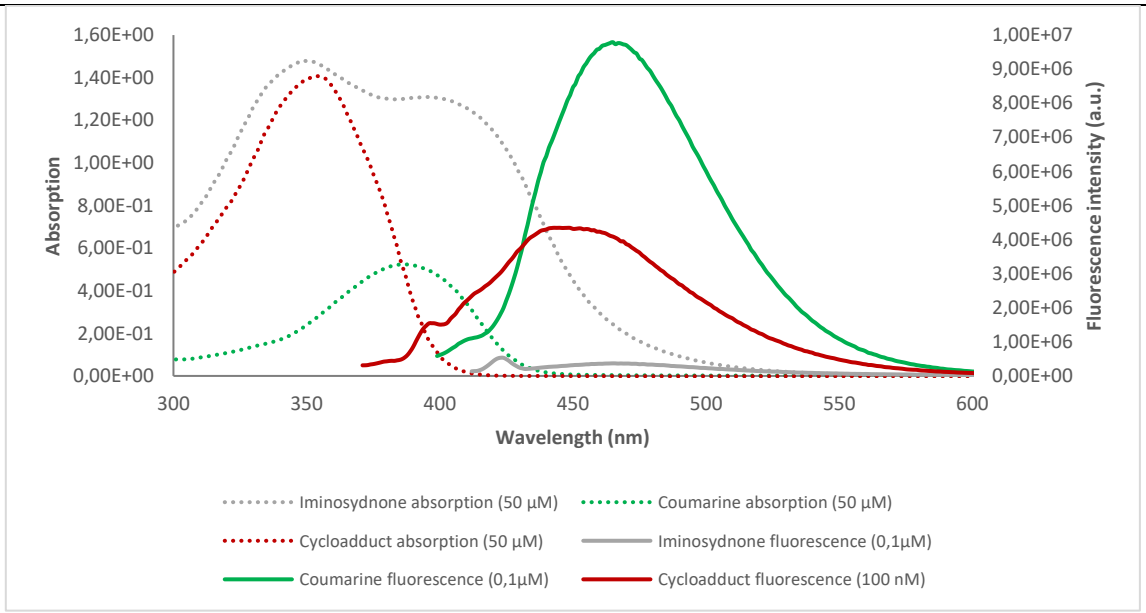

12
OH
OH
NH
H_2N


Absorption
Fluorescence intensity (a.u.)
Wavelength (nm)
Iminosydnone absorption (50 µM)
Naphtalimide absorption (50 µM)
Cycloadduct absorption (50 µM)
Iminosydnone fluorescence (0,1µM)
Naphtalimide fluorescence (0,1µM)
Cycloadduct fluorescence (1µM)

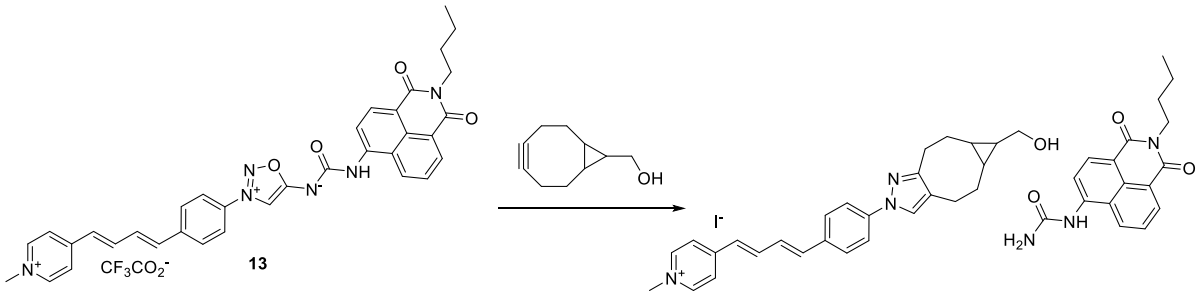

13
OH
I-
OH
NH
NH2


Absorption
Fluorescence intensity (a.u.)
Wavelength (nm)
Iminosydnone absorption (50 µM)
Naphtalimide absorption (50 µM)
Cycloadduct absorption (50 µM)
Naphtalimide fluorescence (0,1µM)
Cycloadduct fluorescence (1µM)
Iminosydnone fluorescence (1µM)


14
MeO2C
OH
NH
NH2


Absorption
Fluorescence intensity (a.u.)
Wavelength (nm)
Iminosydnone absorption (50 µM)
Coumarine absorption (50 µM)
Cycloadduct absorption (50 µM)
Iminosydnone fluorescence (0,1µM)
Coumarine fluorescence (0,1µM)
Cycloadduct fluorescence (100 nM)

IV. Kinetic studies



To **13** (40 µL, 1mM in water, final concentration 10 µM) in PBS 1X (3 920 µL) was added a solution of DBCO (40 µL, 100 mM in DMSO, final concentration 1 mM). The reaction was followed by fluorescence measurement in a single quartz cuvette at two excitations: 405 nm and 458 nm.

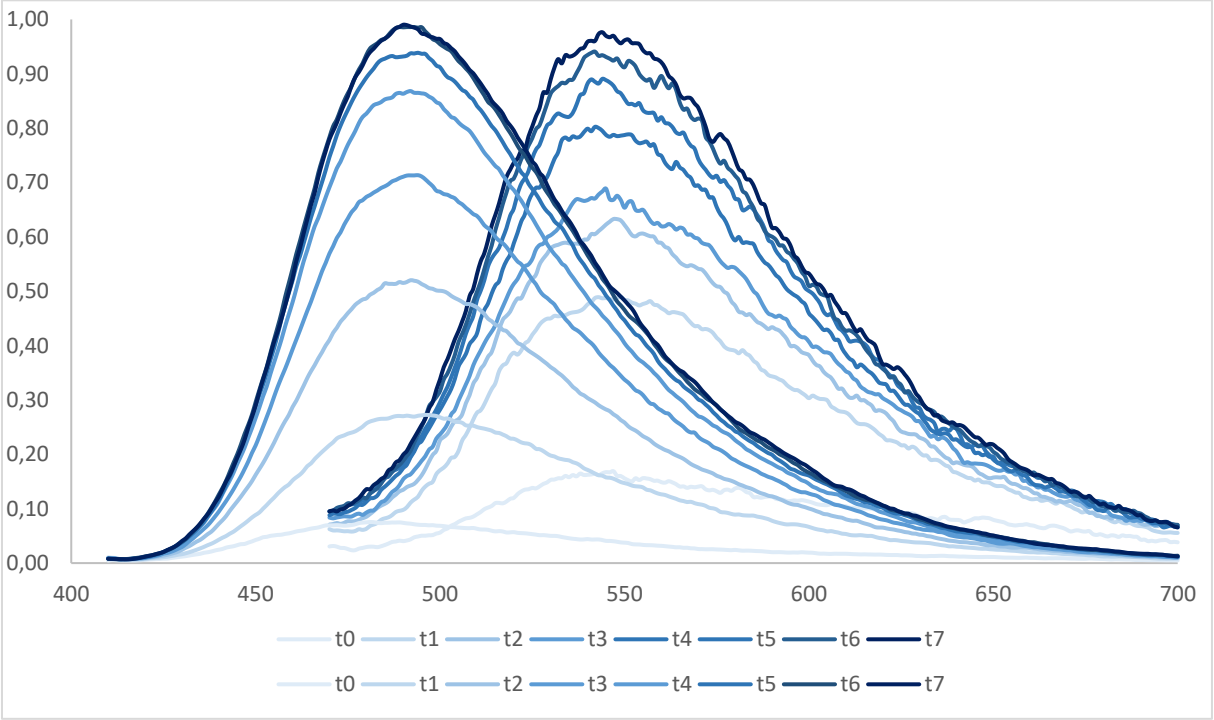


*Figure S1: Fluorescence spectra of reaction between **13** and DBCO over time.*
T1 = 5 min, T2 = 10 min, T3 = 20 min, T4 = 40 min, T5 = 60 min, T6 = 100 min, T7 = 135 min.

Reactions of iminosydnones **13** with **DBCO** was carried out in PBS 1X/DMSO (9:1), plasma/DMSO (9:1) and glutathione (1mM in PBS 1X)/DMSO (9:1) at 10 µM concentration of iminosydnones and 1 mM concentration of **DBCO** using the following procedure:

To 178 µL of the chosen medium in a 96-well opaque plate was added 2 µL of the solution of sydnone (1 mM in water) and 20 µL of the solution of **DBCO** (10 mM in DMSO). This was performed thrice for each medium. The emission was measured every 20 s after excitation at 405 nm.

**Plasma was used as diluted solution (1/1) in PBS 1M to avoid the low signal/noise ratio due to the internal fluorescence of these media and the low fluorescence intensity of the sample at the concentration of the experiment.*

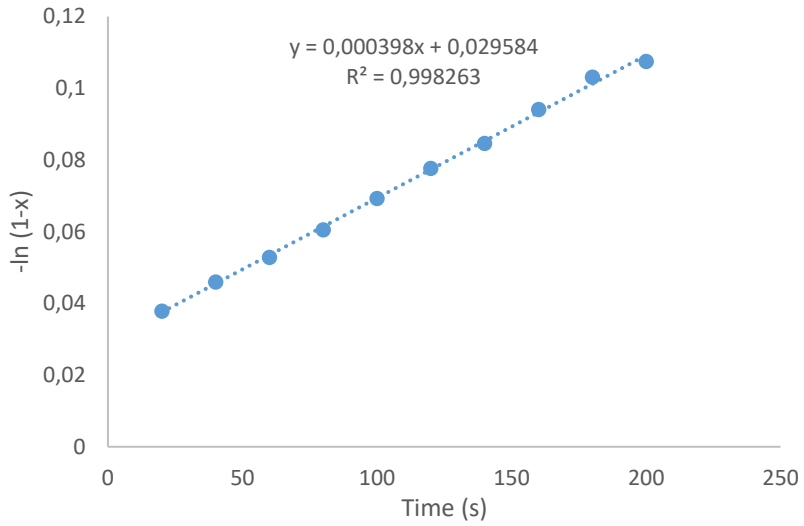
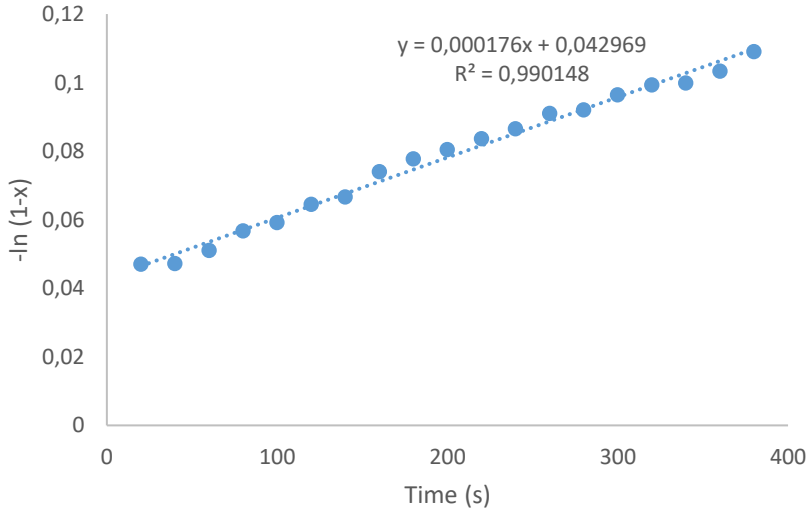
Correspondences between fluorescence and conversions were established by plotting the calibration curve obtained by measuring the emission after the excitation at the appropriate wavelength of solutions at 0% and at full conversion of the reaction.

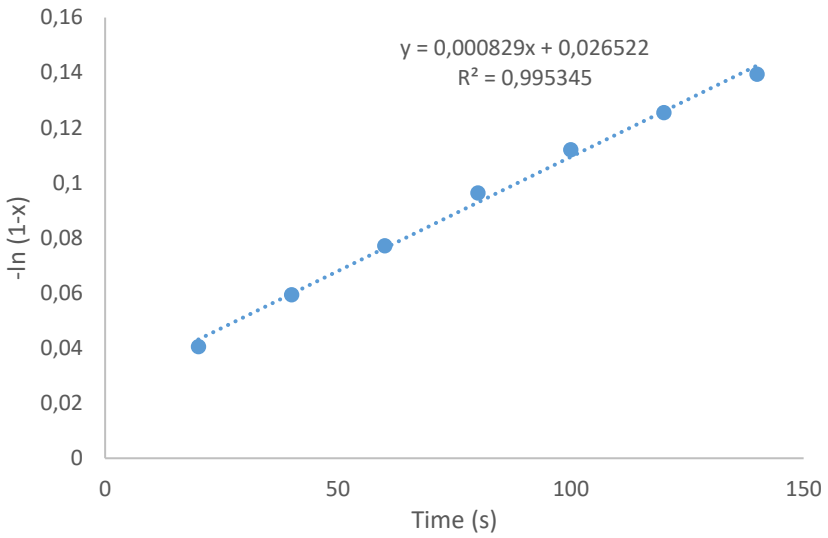
Pseudo-first order reaction rate was obtained by plotting -ln(1-x) versus time and analyzing by linear regression (Equation 3). The second order rate constant was obtained dividing the slope by DBCO concentration *i.e.* 10^{-3} M.

Equation 3: x: reaction conversion; k_{app} : pseudo-first order reaction rate in s^{-1}, k: reaction rate in $M^{-1}.s^{-1}$

$$-\ln(1-x) = k_{app}t + cte = k[DBCO]_0 t + cte$$

Linear regression curves in the 3 media are illustrated in Table S5

PBS 1X	
Plasma	

GSH (1 mM)	

V. Cell culture

Labeling on fixed-cells

CHO cells were grown on Nunc™ Lab-Tek™ II slide in media (HAMF12) containing 10% fetal bovine serum, pyruvate 1mM, 1% non essential aminoacids, glutamine 2mM, penicillin/streptomycin. The cells were then washed with 500 µL PBS, then incubated with 500 µL of PFA 4% during 8 minutes at room temperature and washed three times with 300 µL of PBS. The cells were incubated with 500 µL of Triton 0.2% for 5 minutes at room temperature then washed 3 times with 500 µL of Triton 0.05%-Tween 0.1% and twice with 500 µL of PBS. The cells were then incubated with an solution of **13** (5-10 µM) in HAMF12 for 30 minutes at room temperature and were washed twice with 300 µL of HAMF12. The cells were then incubated with a cycloalkyne solution (200 µM) in HAMF12 during the night at room temperature and were then washed twice with 300 µL of HAMF12. 5 µL of Draq5 solution in 495 µL of HAMF12 were added and the cells were incubated 15 minutes at 37 °C before being washed twice with 500 µL of PBS.

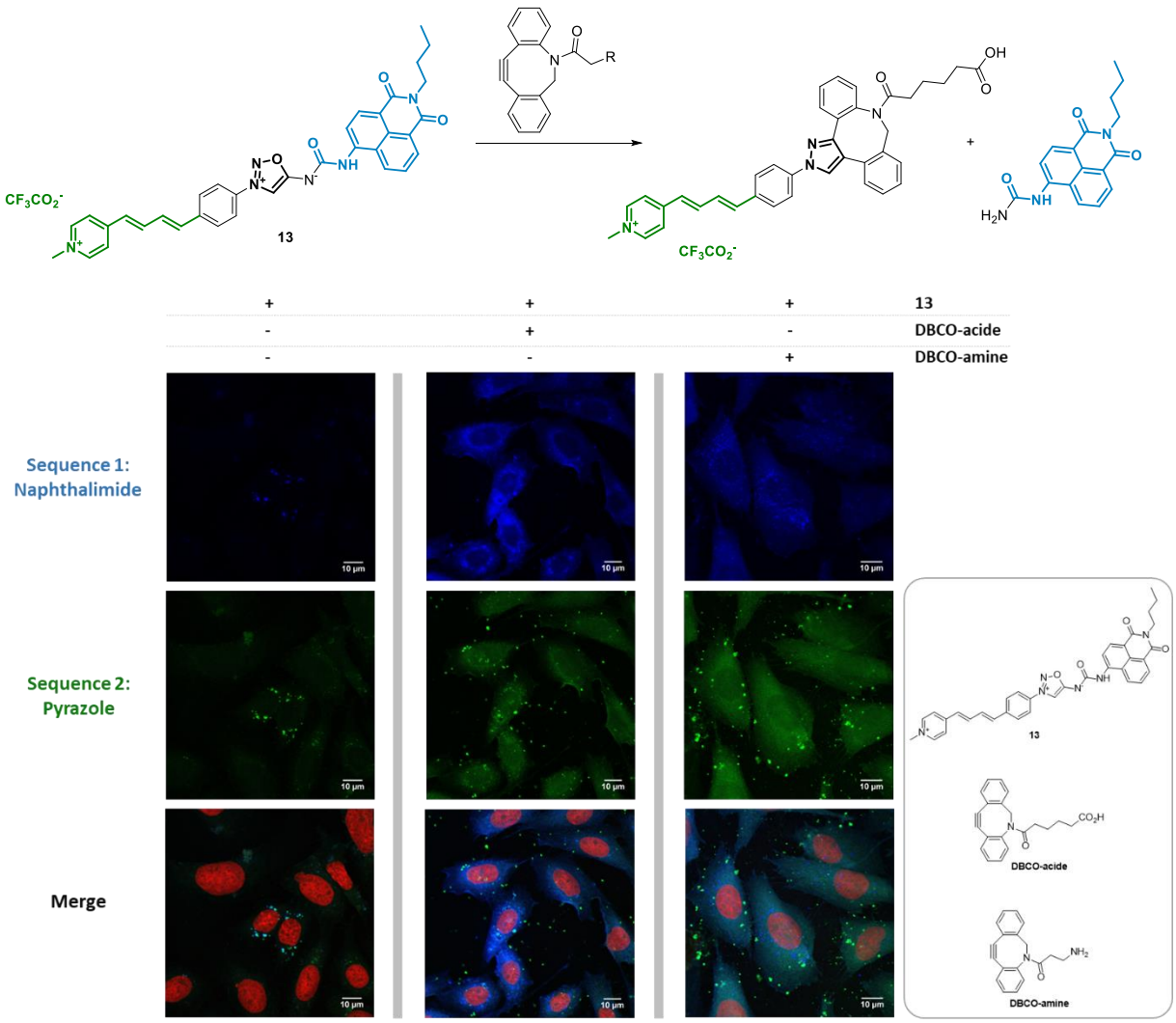


*Figure S2. Cells imaging of the click and release reaction between compound **13** and two DBCO.*

On this first experiment, no difference was observed when we use two different cyclooctyne. The turn-on effect for the click product (green) and the release product (blue) was observed.

When a zoom is applied on a cell, we can observe that after the click and release reaction the fluorescence in the two channel (blue and green) are no more at the same localization meaning that the two fluorogenic part of the molecule are no more connected, thanks to the click and release reaction, and the liberated compounds can move inside the cells (Figure S3).

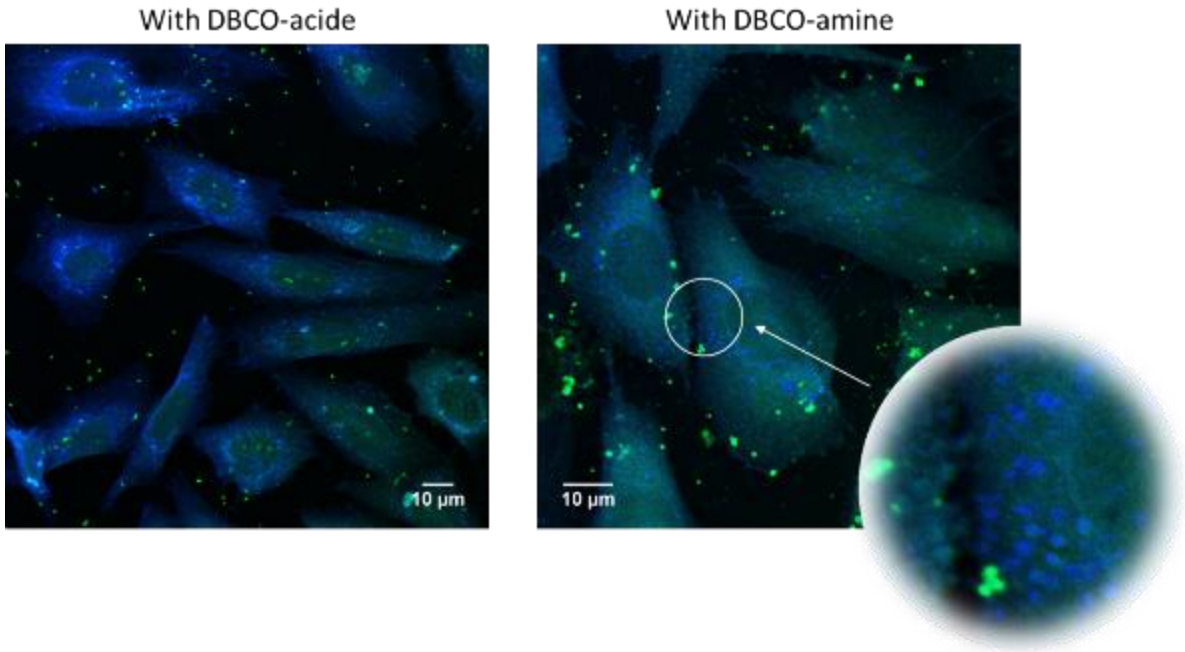


Figure S3. Zoom on some cells from the previous experiment. Different localization of both compound (click product and release prodcut) were observed.

To confirm these results, this experiment was reproduce two times with different batch of CHO cells and the same turn-on effect was observed (Figure S4).

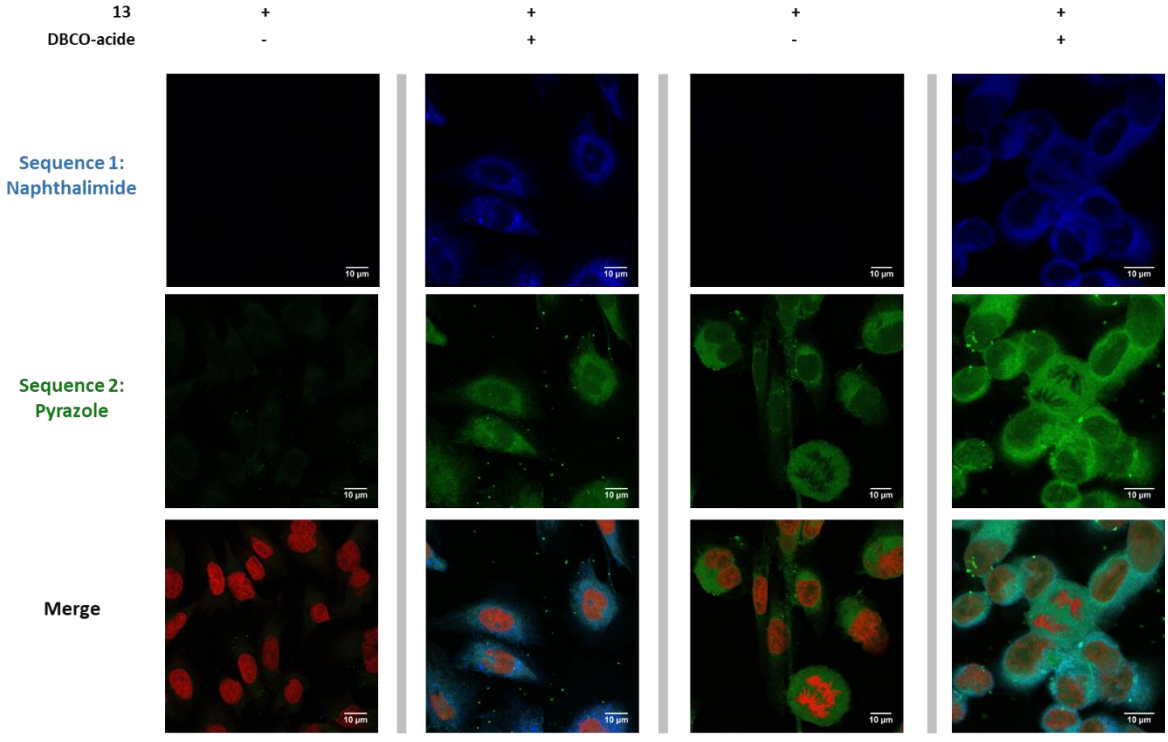


Figure S4. Reproducibility of the experiment.

Labeling on living cells

CHO cells were grown on Nunc™ Lab-Tek™ II slide in media (HAMF12) containing 10% fetal bovine serum, pyruvate 1mM, 1% non essential aminoacids, glutamine 2mM, penicillin/streptomycin. The cells were then washed twice with 500 µL HAMF12. The cells were then incubated with an solution of

13 (5-10 µM) in HAMF12 for 30 minutes at 37 °C and were washed twice with 300 µL of HAMF12. The cells were then incubated with a cycloalkyne solution (200 µM) in HAMF12 for 5 hours at 37 °C and were then washed twice with 300 µL of HAMF12. 5 µL of Draq5 solution in 495 µL of HAMF12 were added and the cells were incubated 15 minutes at 37 °C before being washed twice with 300 µL of HAMF12. The cells were incubated with 500 µL of PFA 4% during 8 minutes at room temperature and washed three times with 500 µL of PBS.

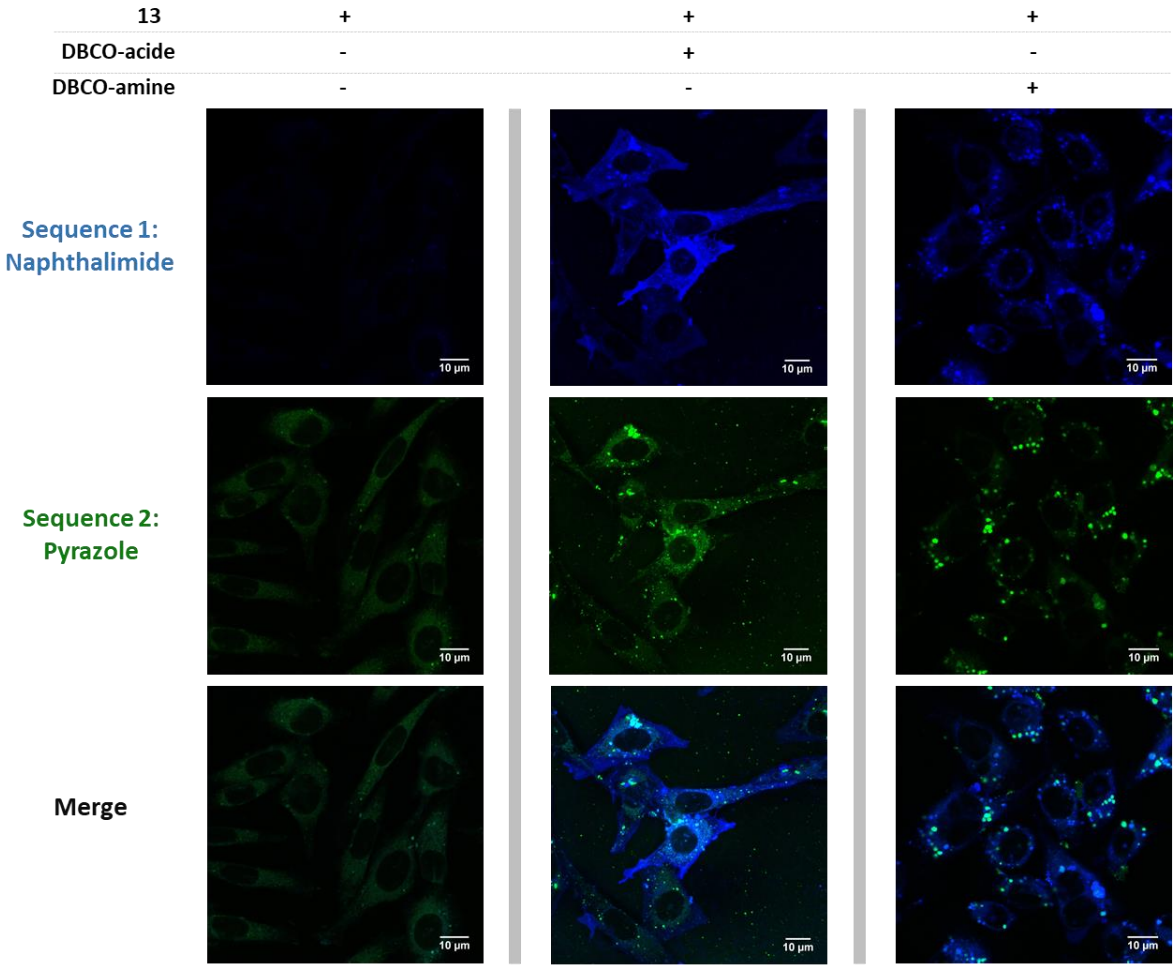
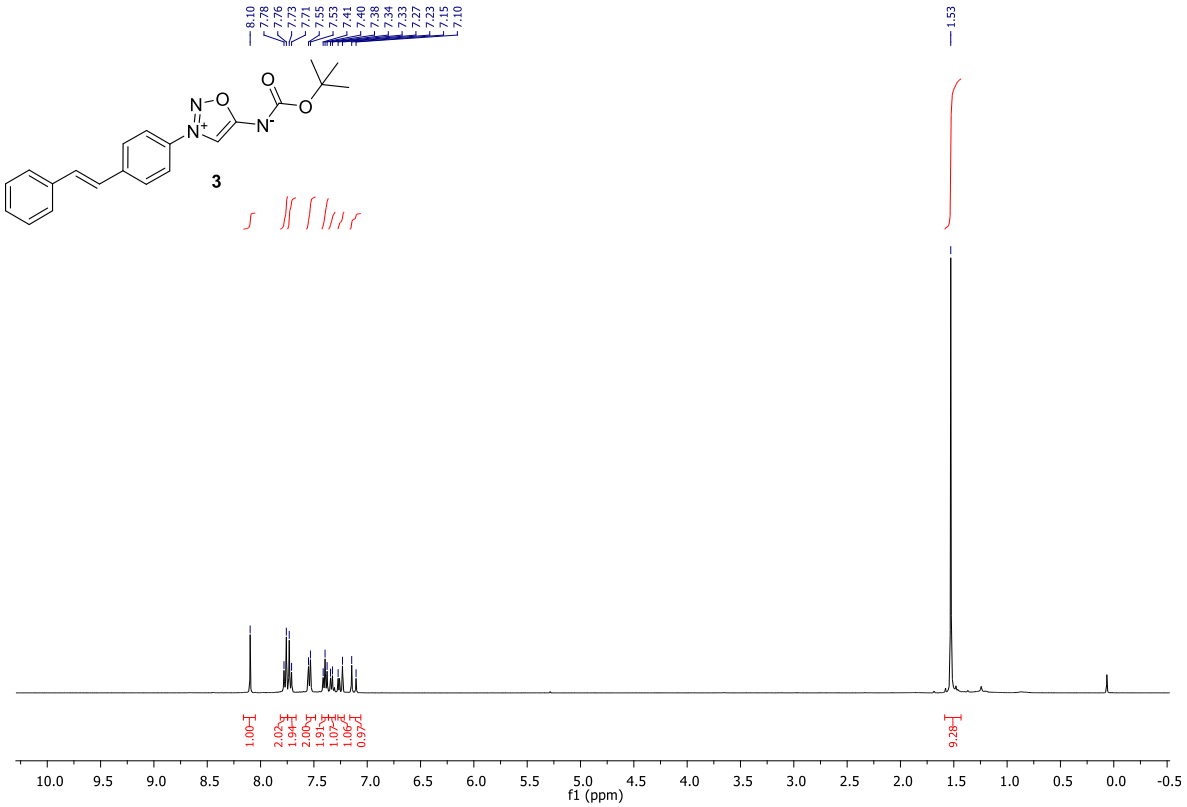


Figure S5. Click and release experiment on living cells.

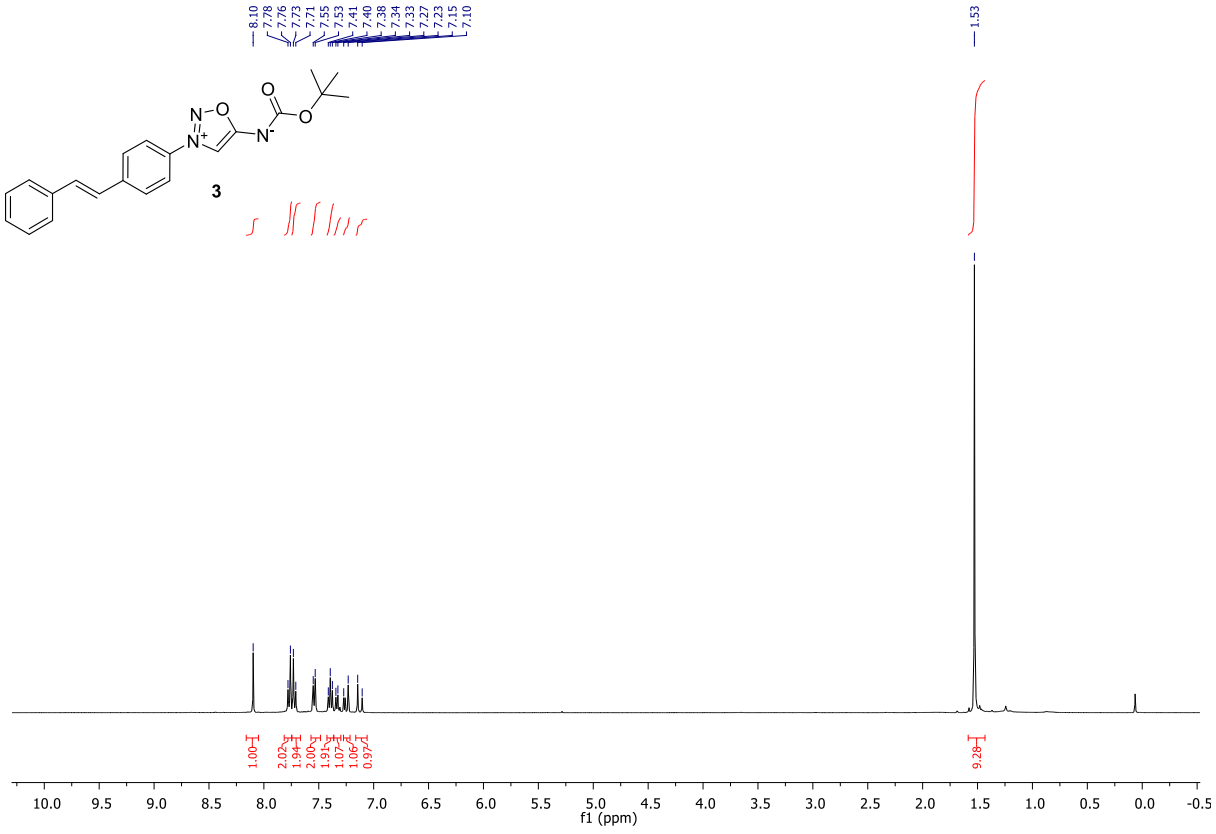
For this experiment, the turn-on effect is still observed for both compound (click product and release product) but the localization of the fluorescence inside cells is different from the experiment with fixed and permeabilized cells.

VI. NMR Spectra

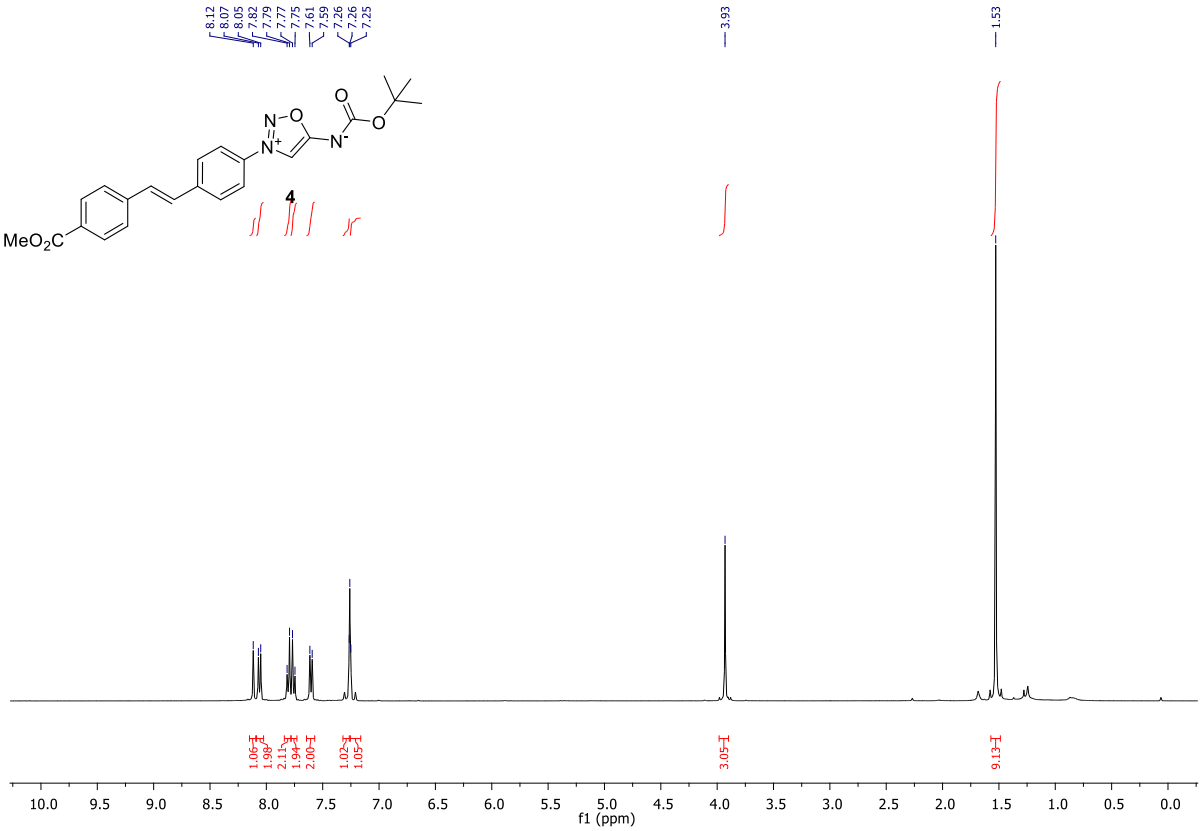
^{1}H NMR (400 MHz), $CDCl_3$, **3**



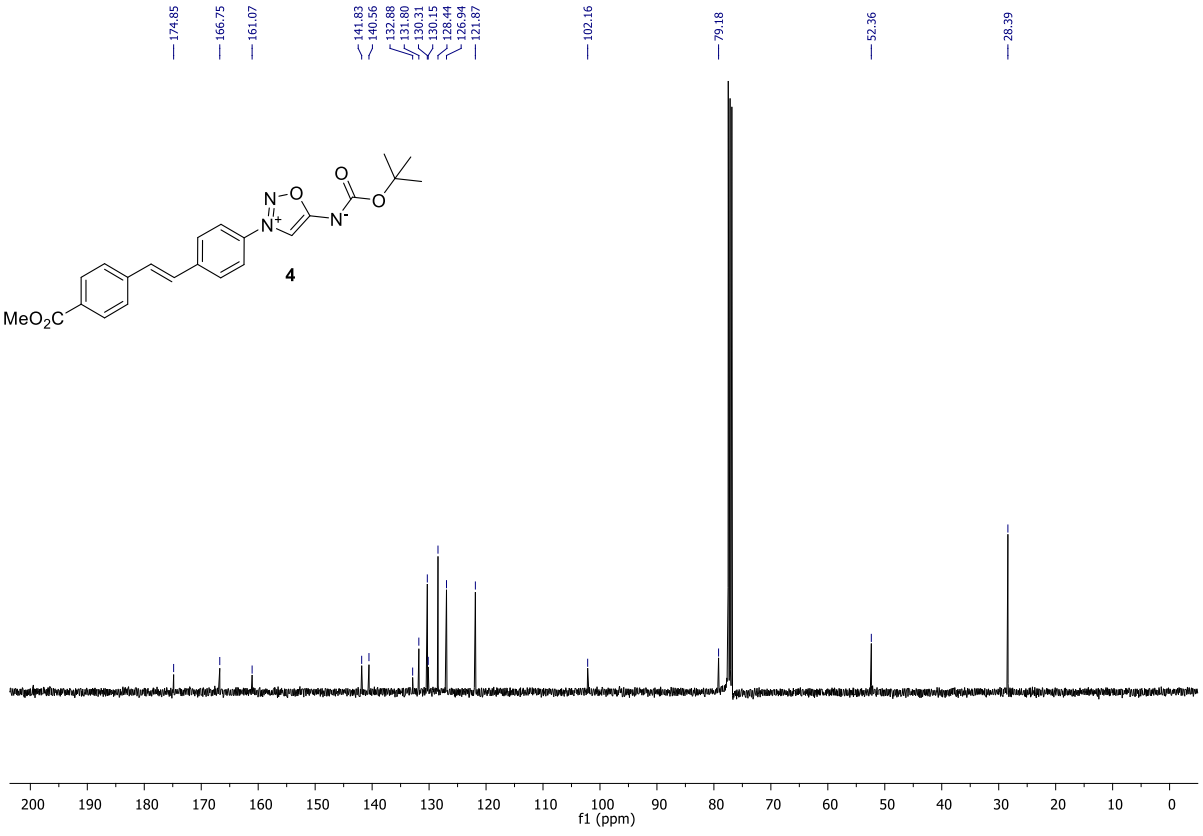
^{13}C NMR (100 MHz), $CDCl_3$, **3**



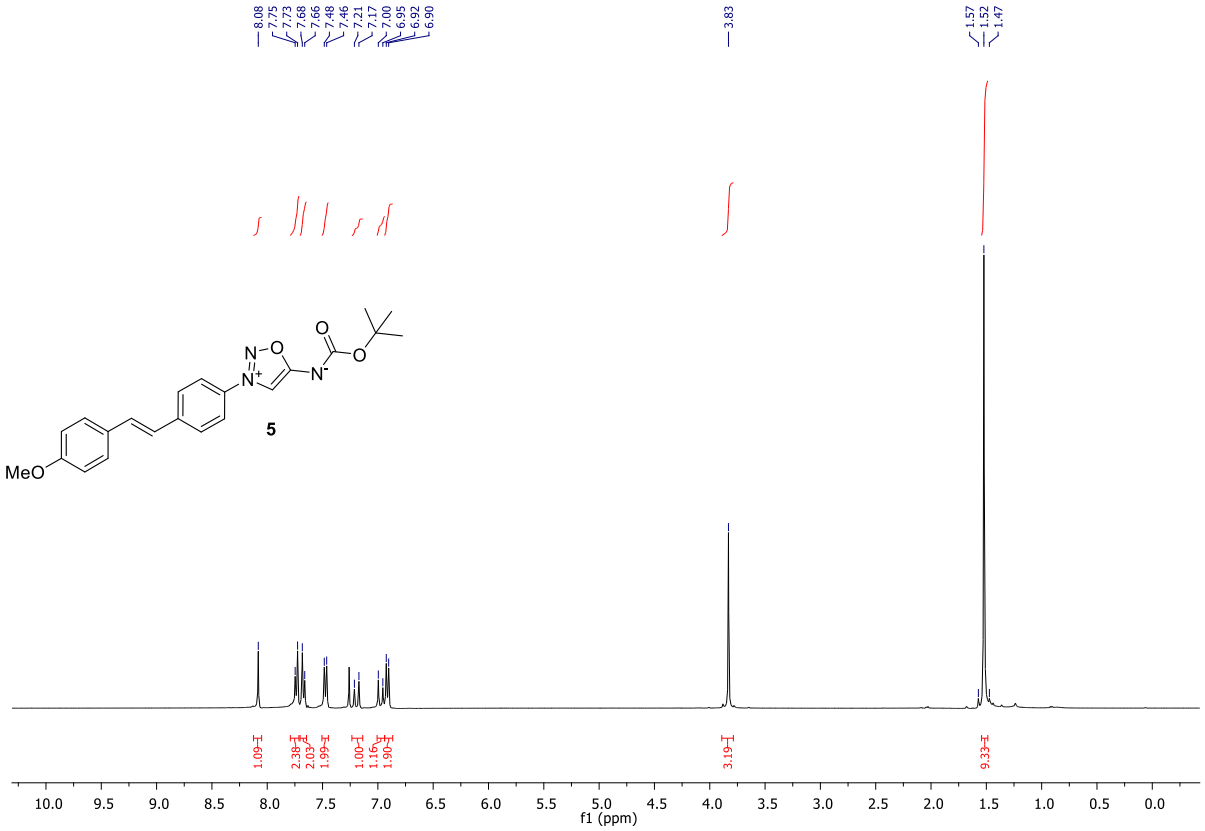
^{1}H NMR (400 MHz), $CDCl_3$, **4**



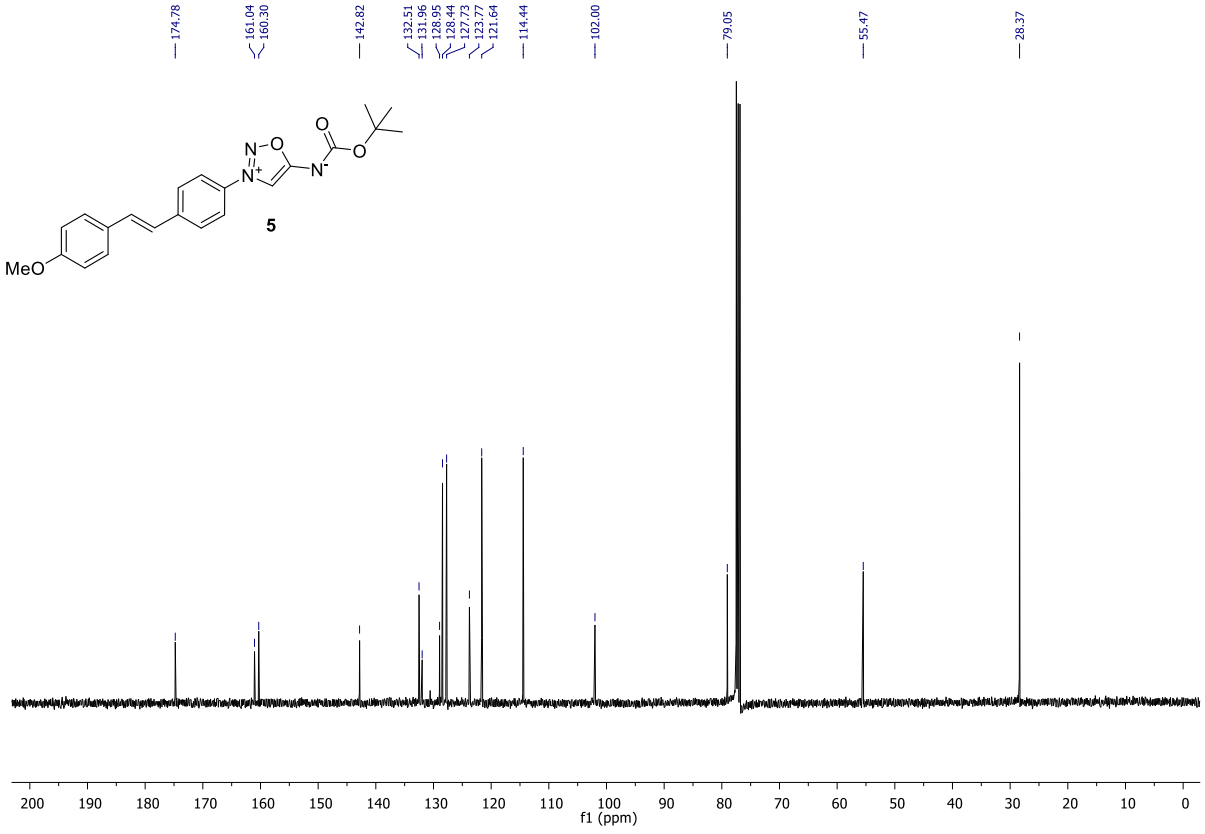
^{13}C NMR (100 MHz), $CDCl_3$, **4**



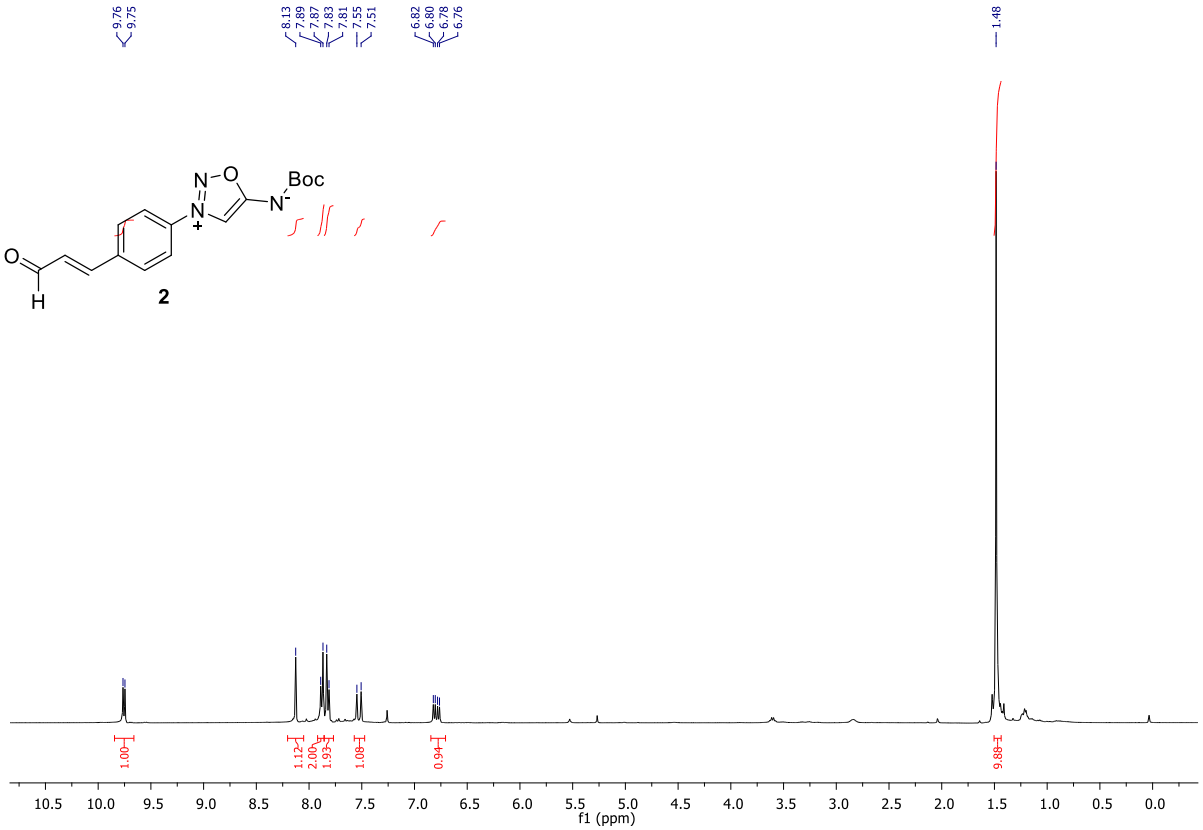
^{1}H NMR (400 MHz), $CDCl_3$, **5**



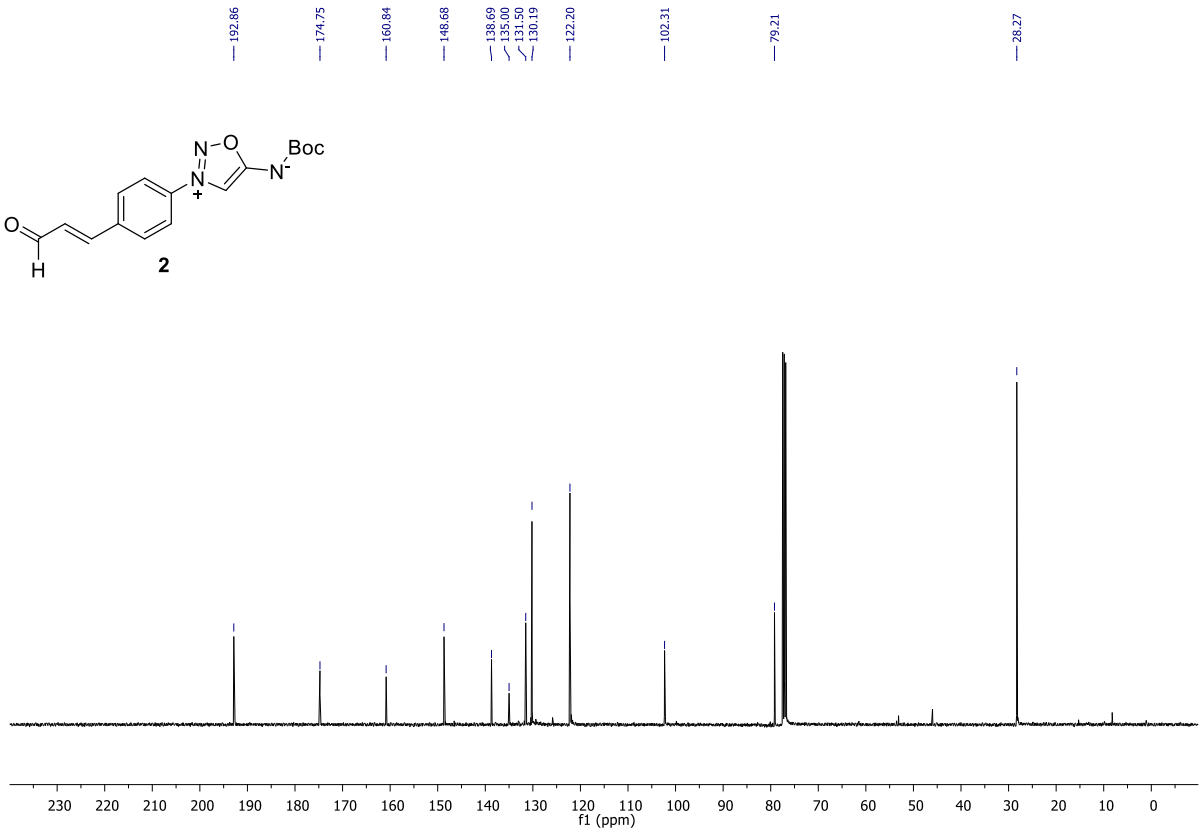
^{13}C NMR (100 MHz), $CDCl_3$, **5**



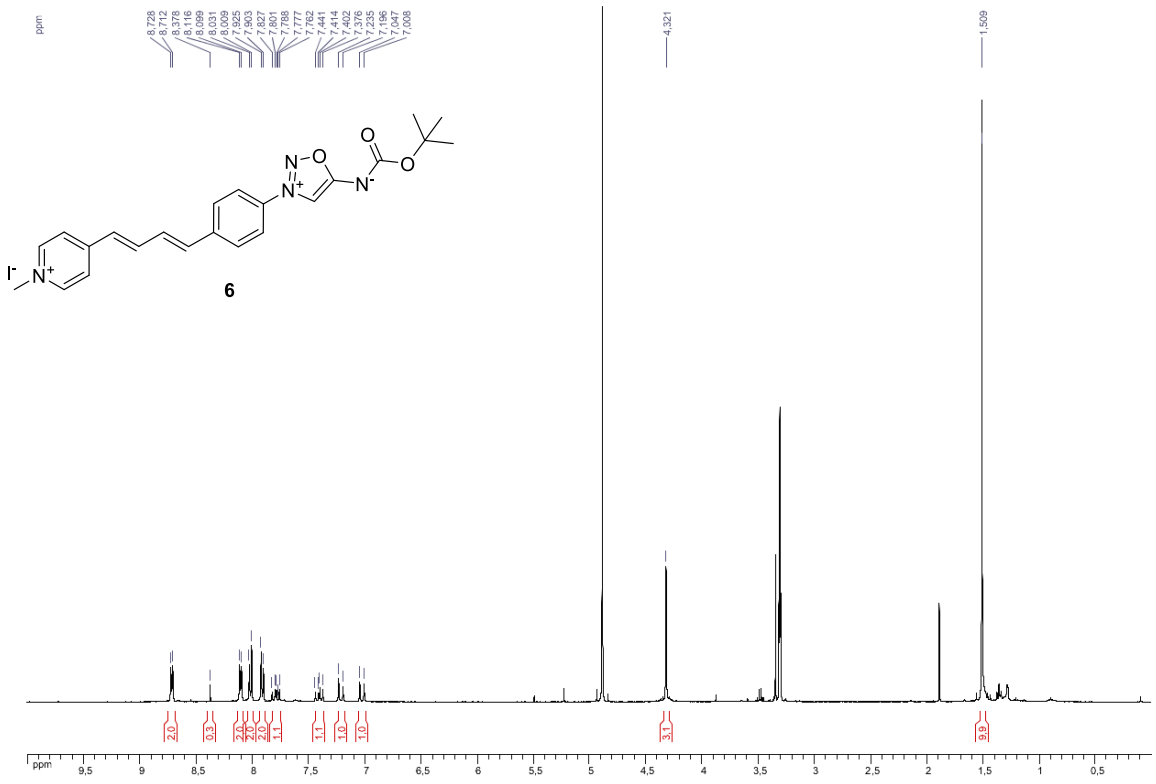
^{1}H NMR (400 MHz), $CDCl_3$, **2**



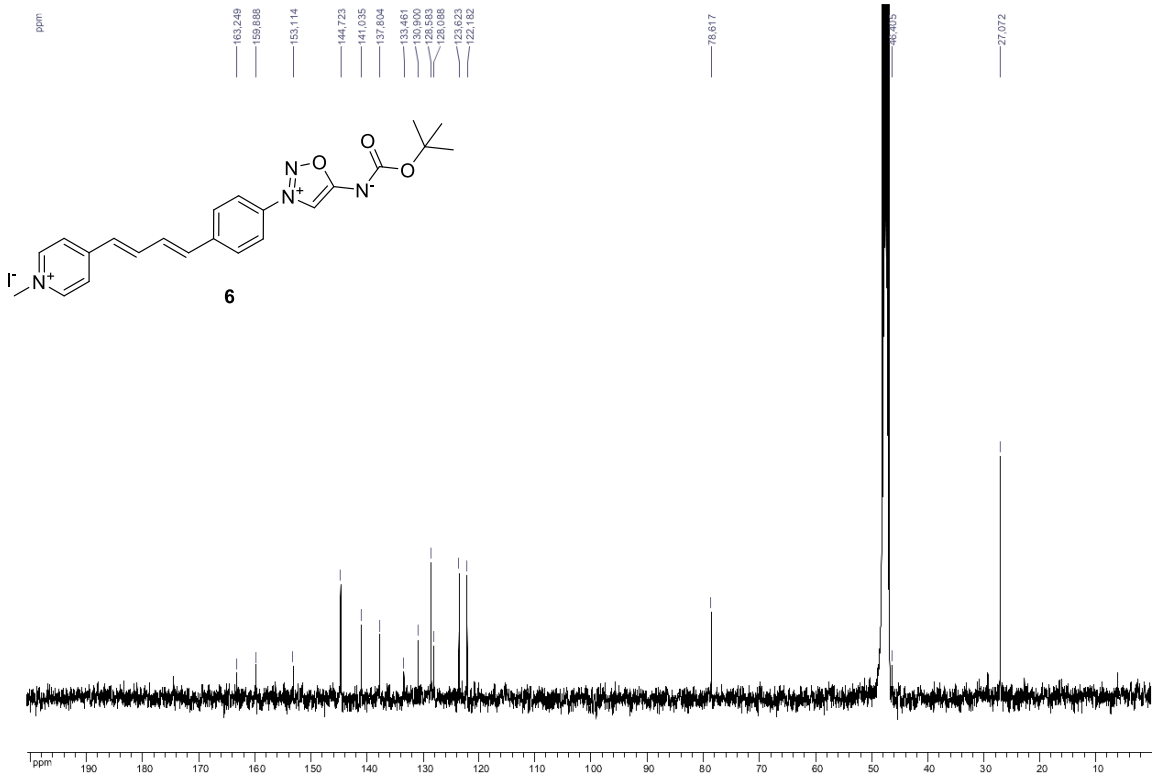
^{13}C NMR (100 MHz), $CDCl_3$, **2**



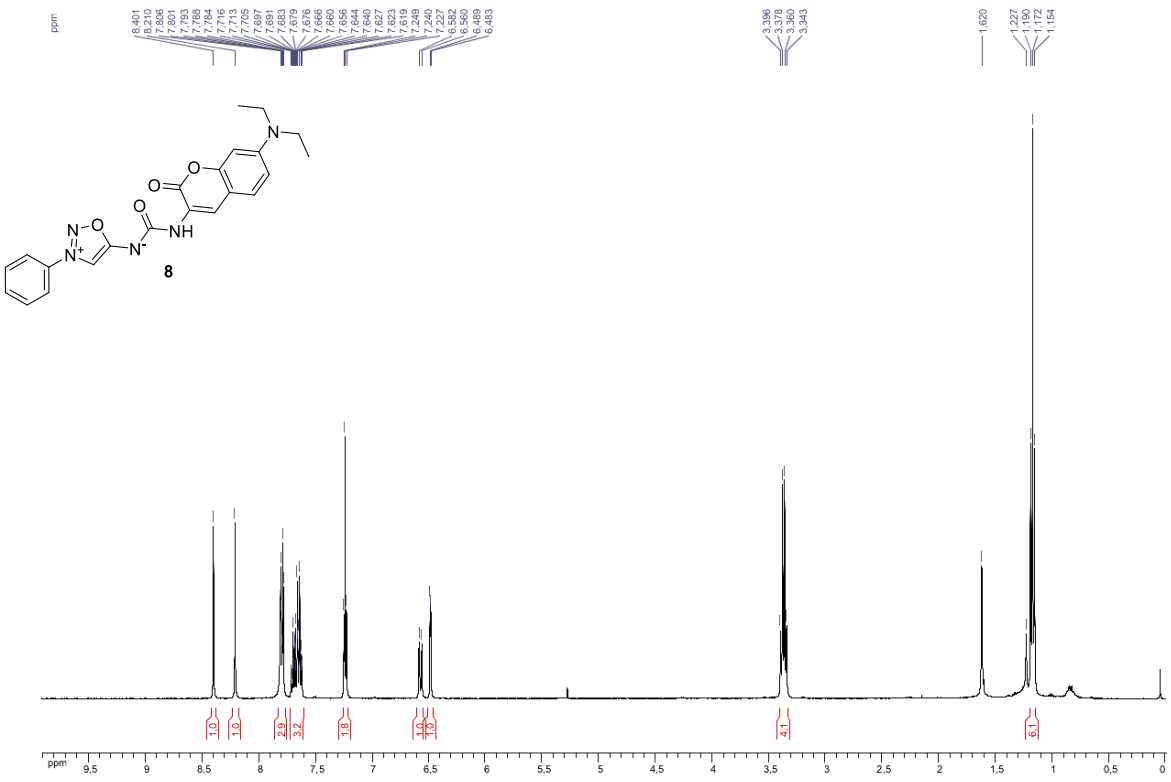
^{1}H NMR (400 MHz), MeOD-d$_4$, **6**



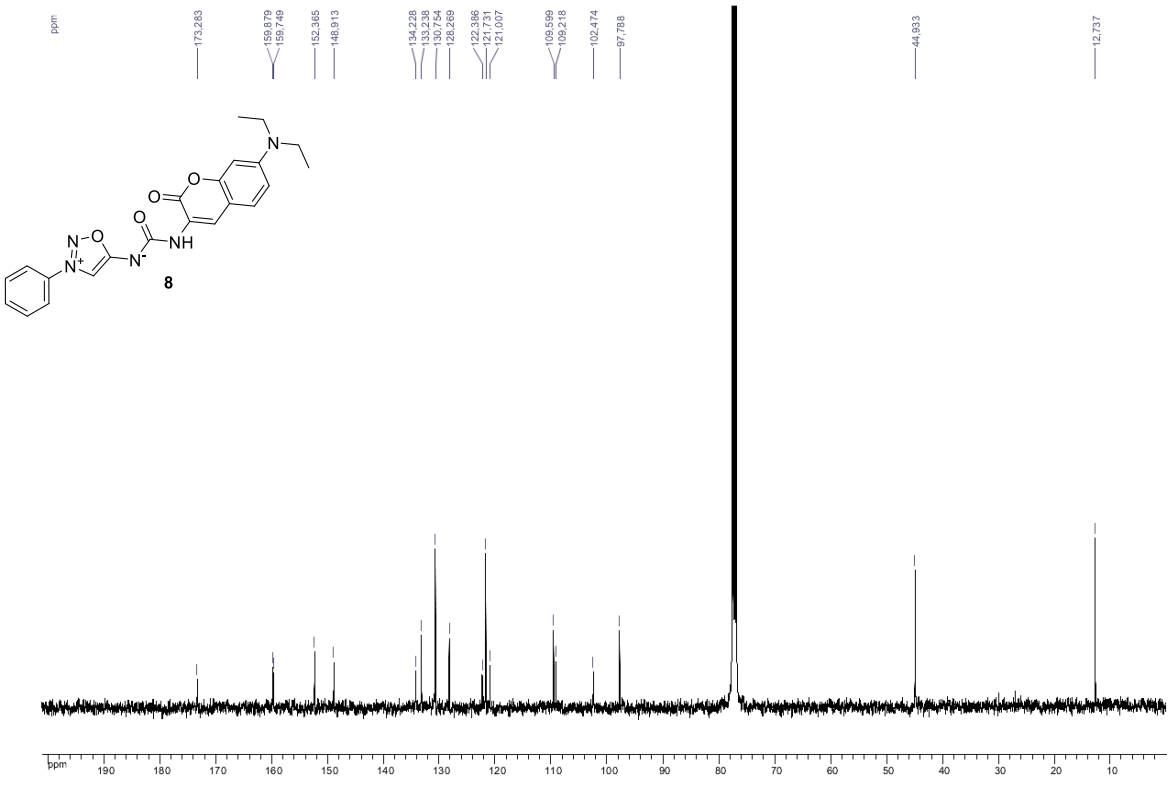
^{13}C NMR (100 MHz), MeOD-d$_4$, **6**



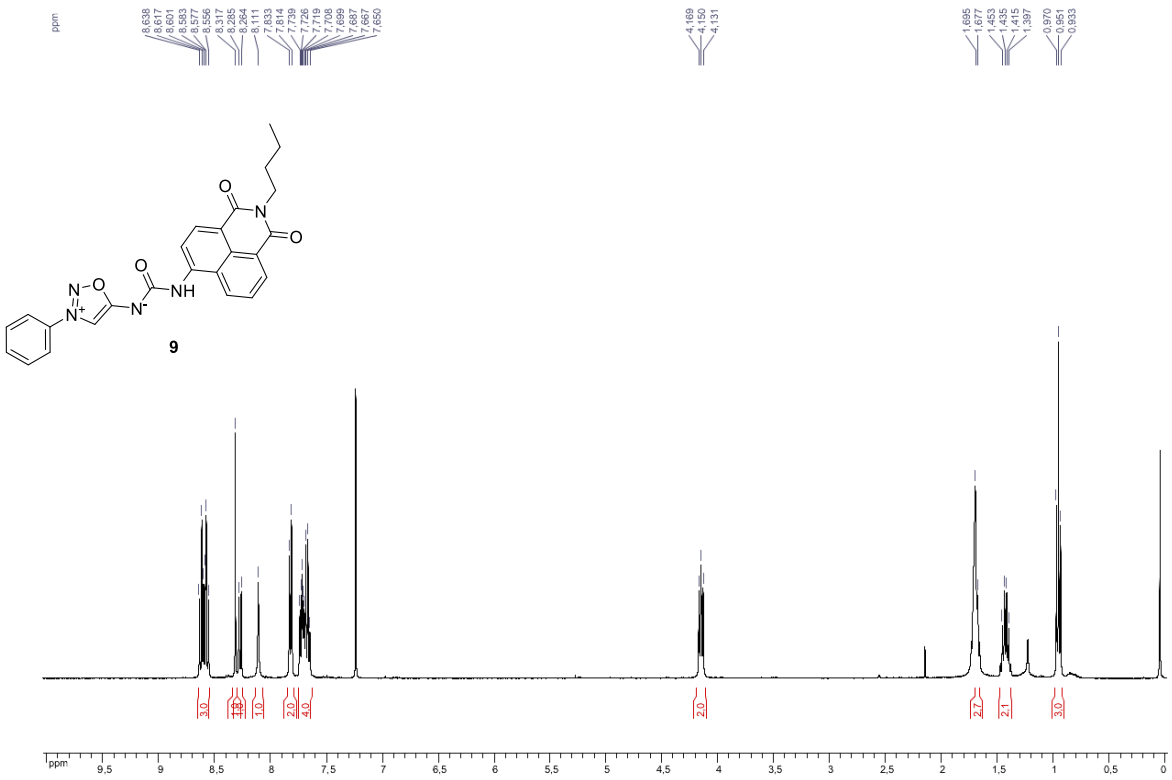
^{1}H NMR (400 MHz), $CDCl_3$, **8**



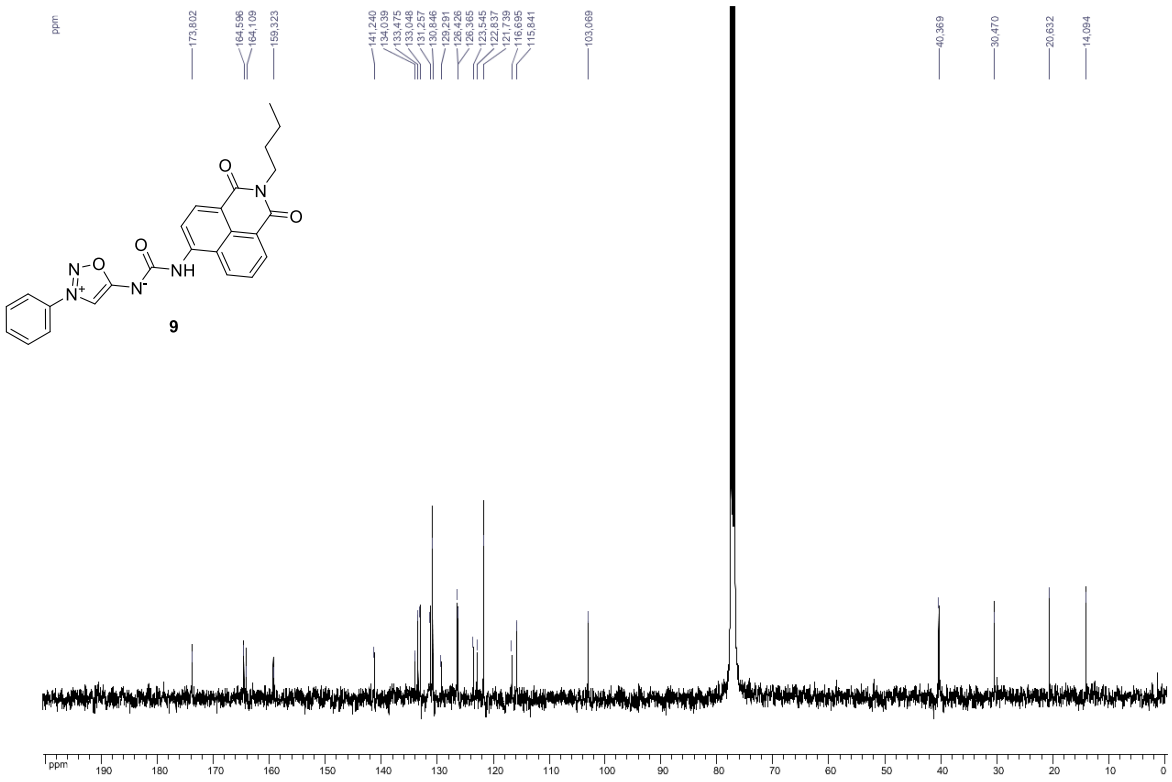
^{13}C NMR (100 MHz), $CDCl_3$, **8**



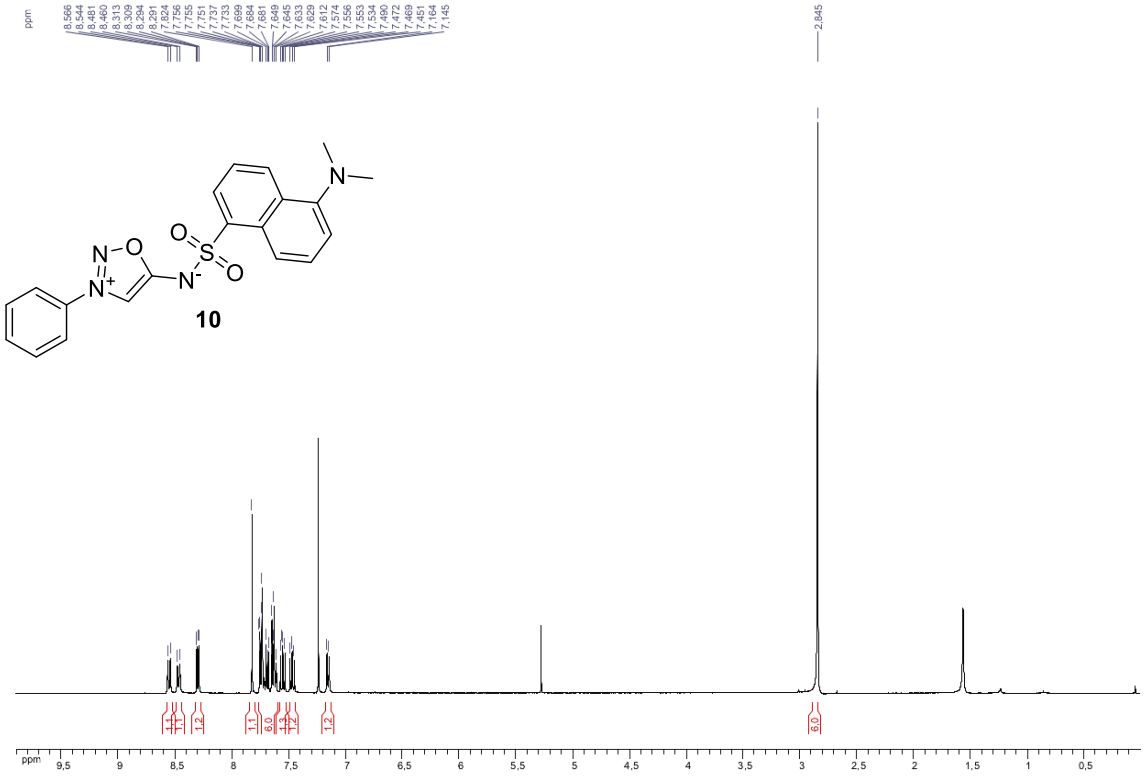
^{1}H NMR (400 MHz), $CDCl_3$, **9**



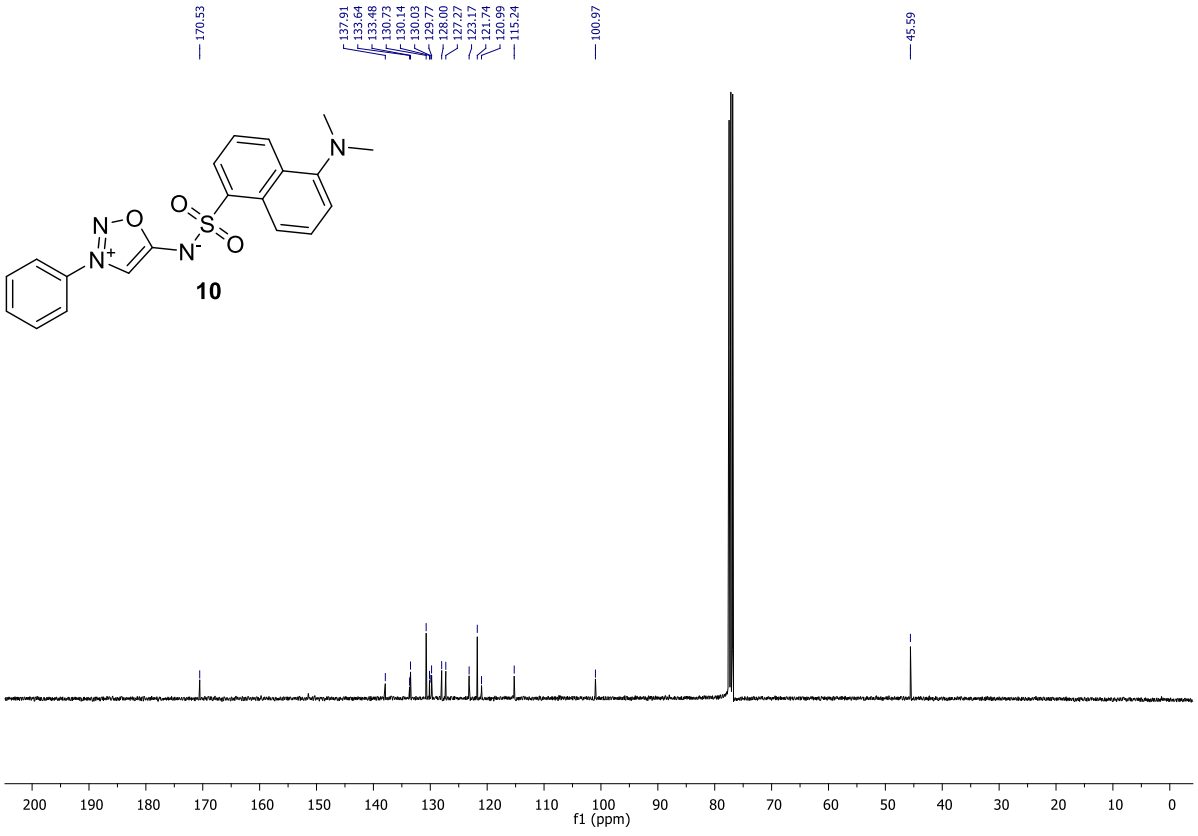
^{13}C NMR (100 MHz), $CDCl_3$, **9**



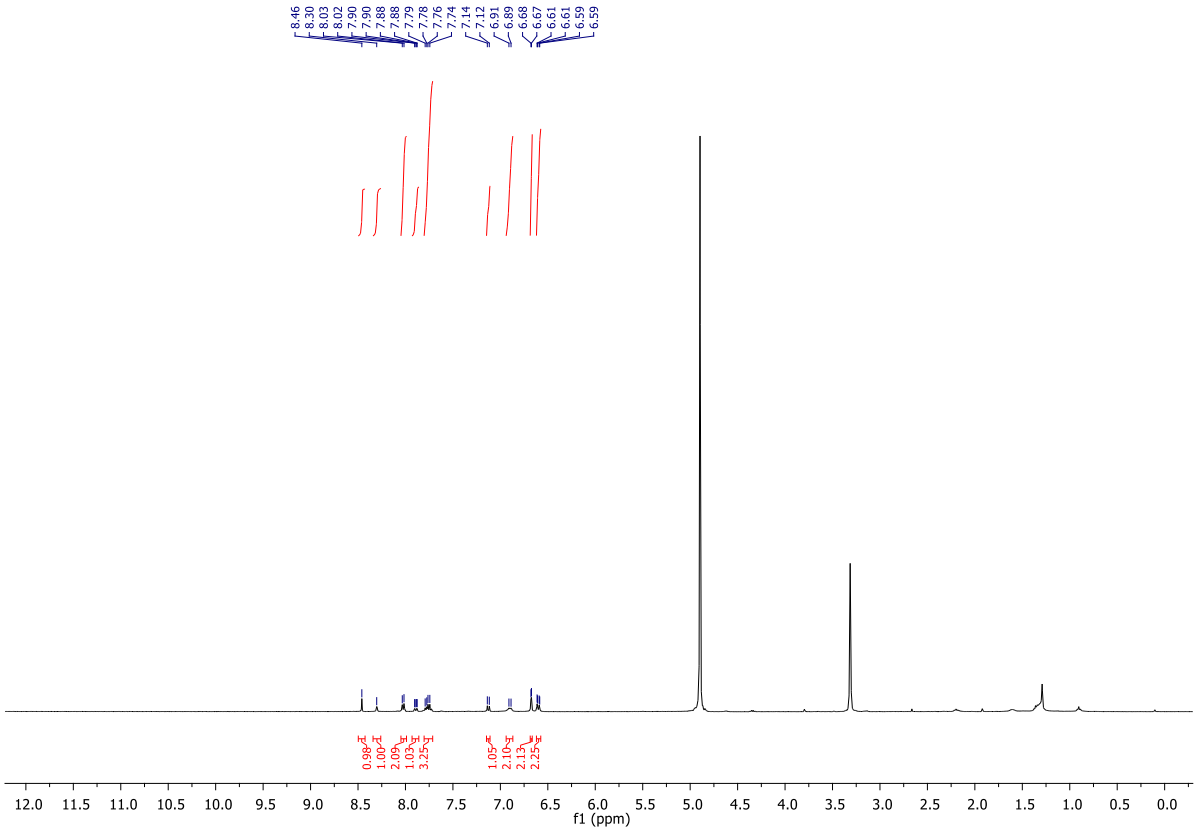
^{1}H NMR (400 MHz), $CDCl_3$, **10**



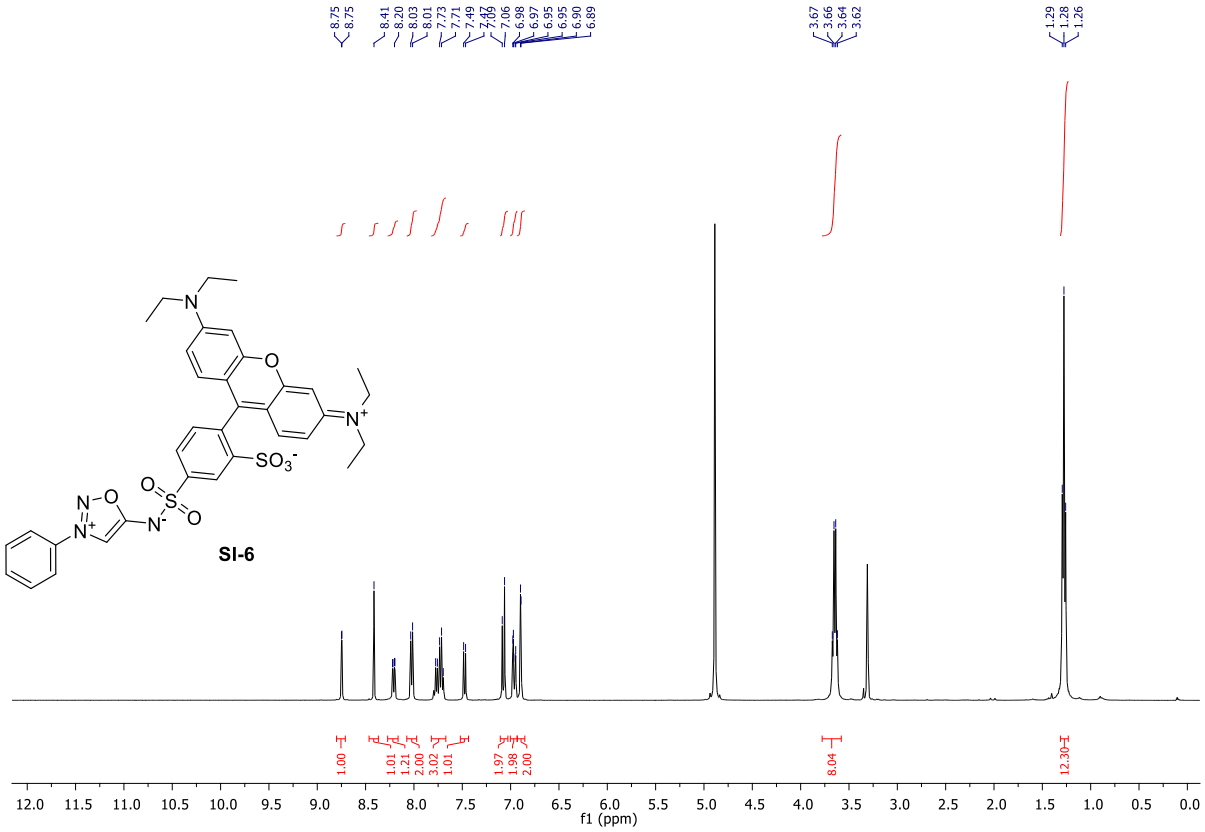
^{13}C NMR (100 MHz), $CDCl_3$, **10**



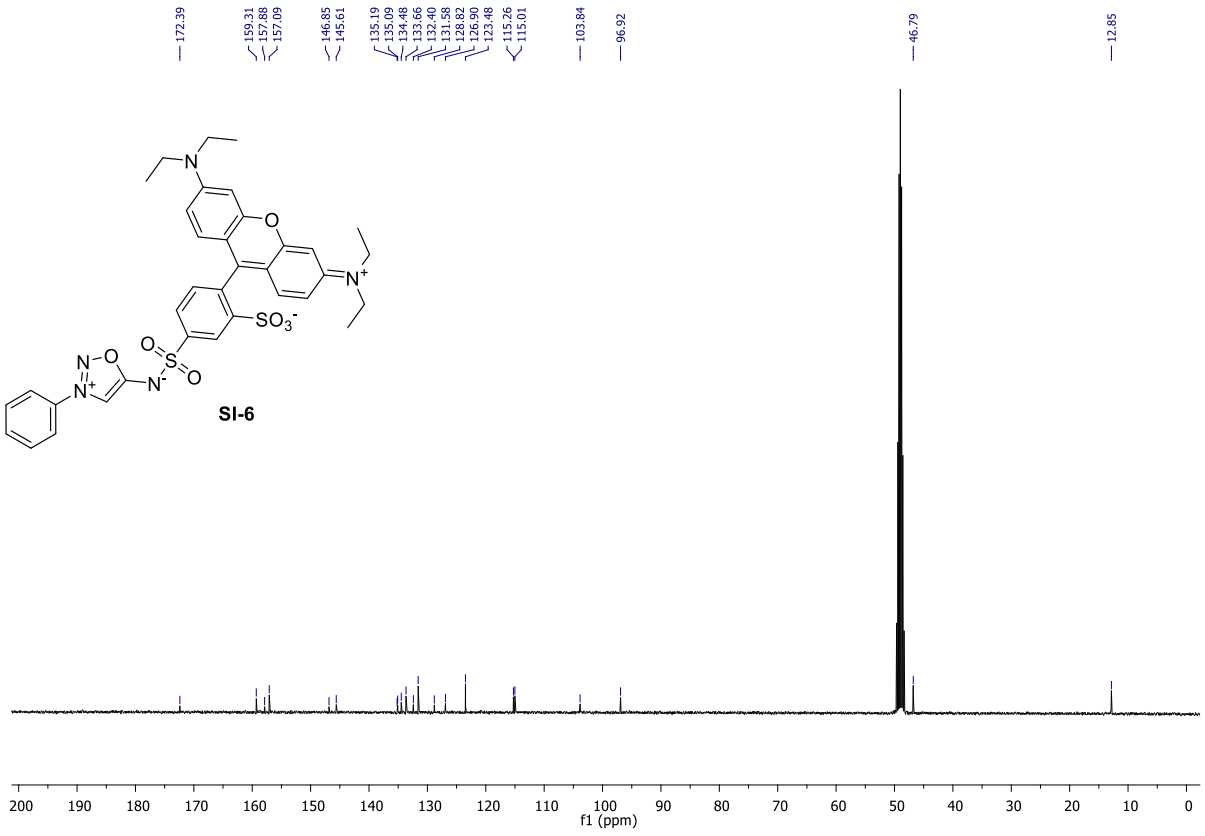
^{1}H NMR (400 MHz), MeOD-d_4, **SI-5**



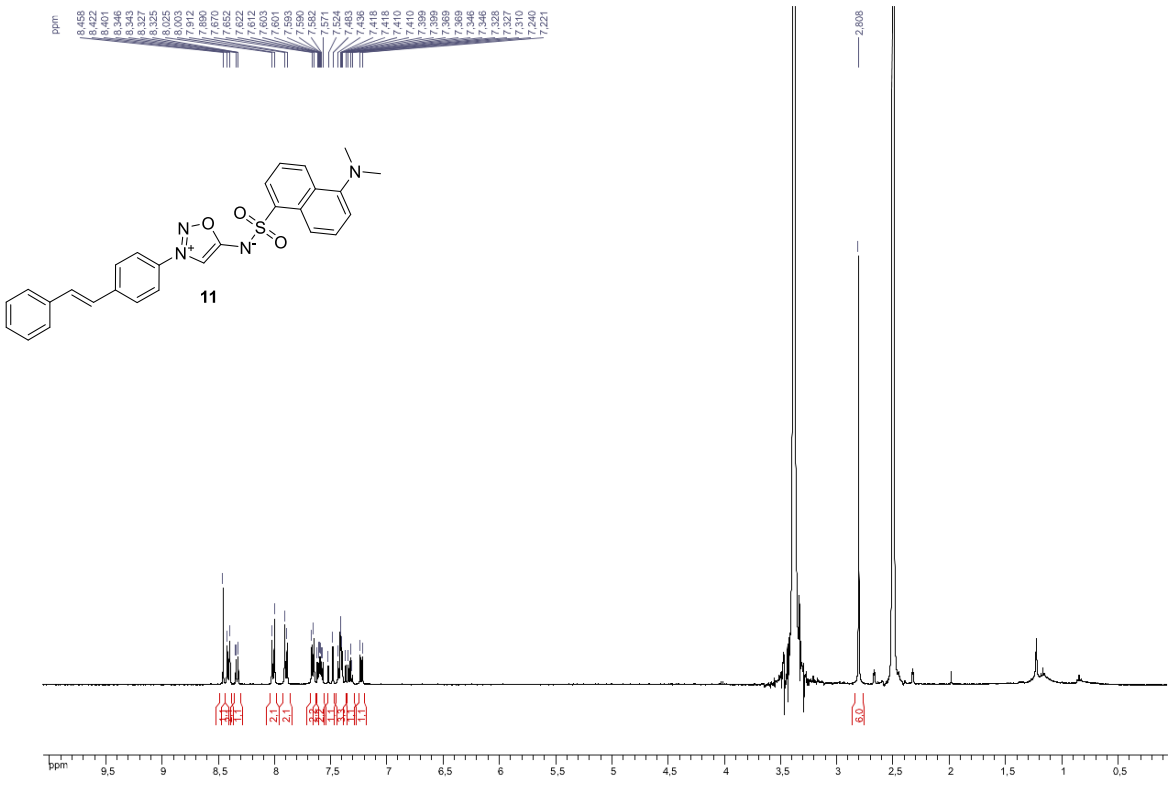
^{1}H NMR (400 MHz), MeOD-d$_4$, **SI-6**



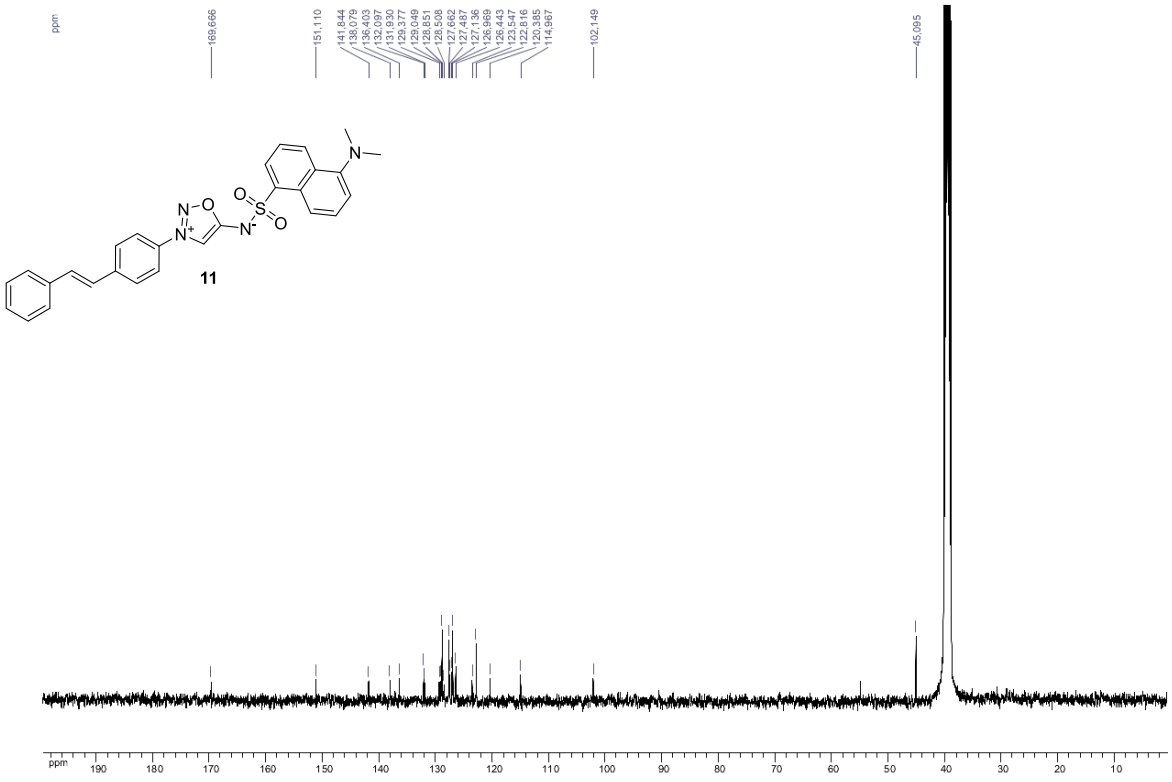
^{13}C NMR (100 MHz), MeOD-d$_4$, **SI-6**



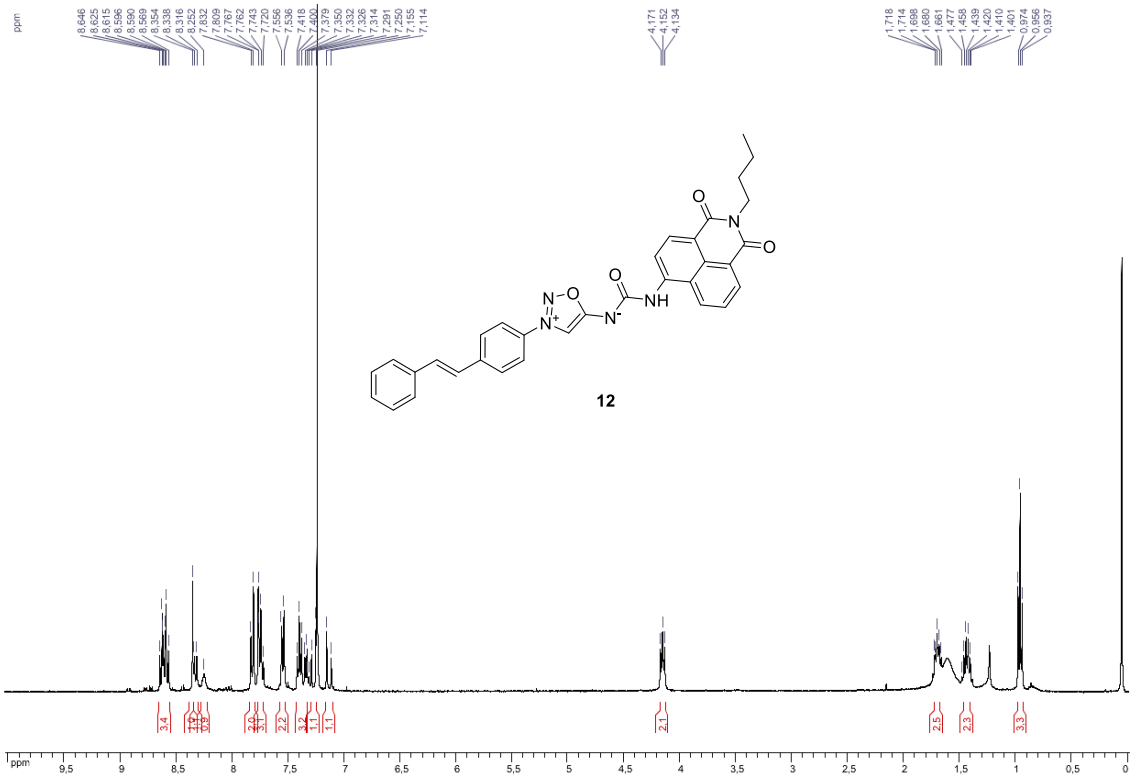
^{1}H NMR (400 MHz), DMSO-d$_6$, **11**



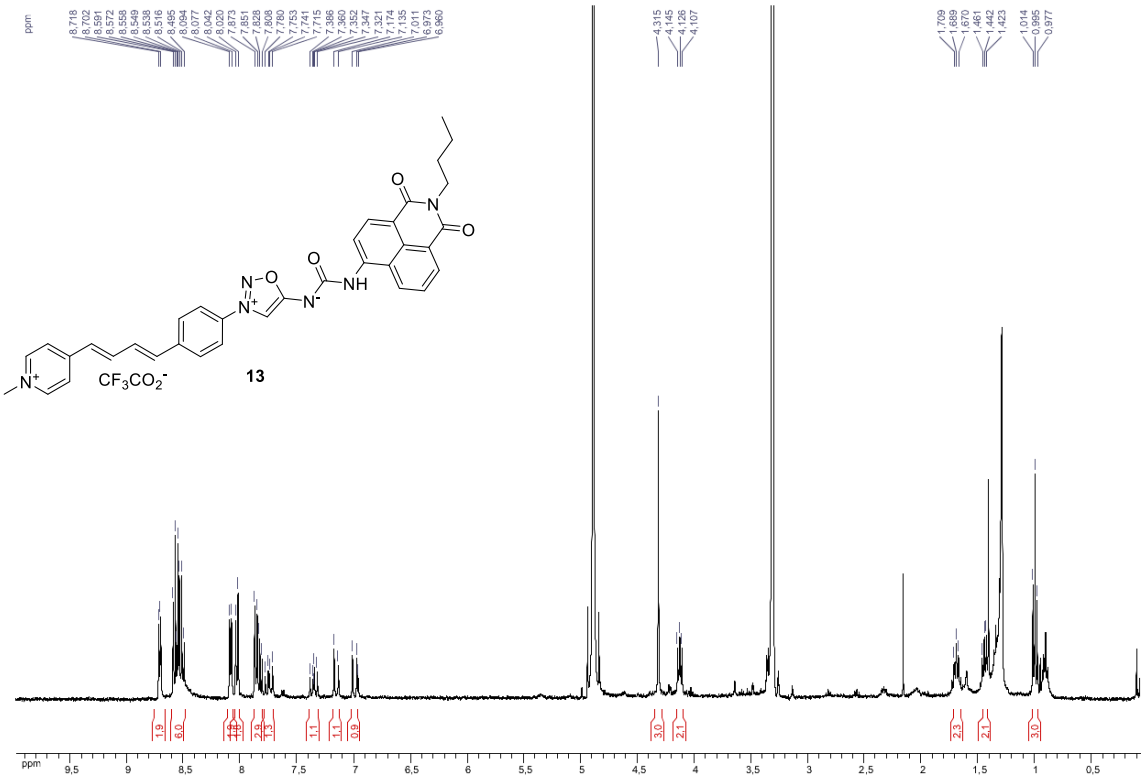
^{13}C NMR (100 MHz), DMSO-d$_6$, **11**



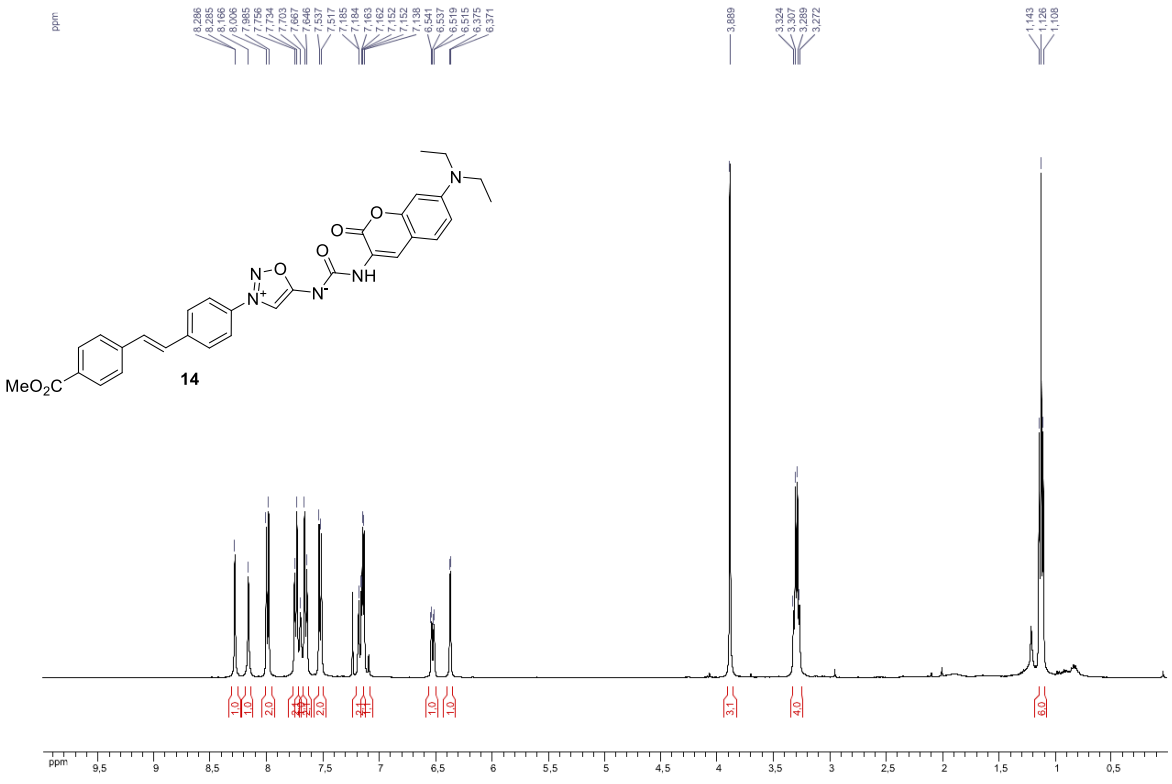
^{1}H NMR (400 MHz), $CDCl_3$, **12**



^{1}H NMR (400 MHz), MeOD-d_4, **13**



^{1}H NMR (400 MHz), $CDCl_3$, **14**



^{13}C NMR (100 MHz), $CDCl_3$, **14**