Supporting information

Red emissive sulfone-rhodols as mitochondrial imaging agents

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Table of Contents

Instrumentation and Materials

All chemicals were used as received unless otherwise noted. All reported ^{1}H NMR spectra were collected using 500 MHz and 600 MHz spectrometers. Chemical shifts (δ ppm) were determined with TMS as the internal reference; *J* values are given in Hz. Chromatography was performed on silicagel (230-400 mesh). Preparative thin layer chromatography (TLC) was carried out using Merck PLC Silica gel 60 F_{254} 1 mm plates. The mass spectra were obtained via electron ionization (EI-MS) or electrospray ionization (ESI-MS). All photophysical studies have been performed with freshly-prepared air-equilibrated solutions at room temperature (298 K).

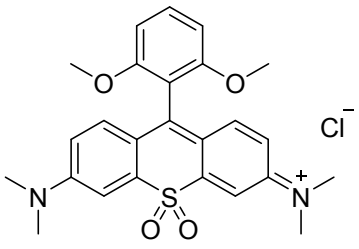
A Shimadzu UV-3600i Plus spectrophotometer and an Edinburgh Instruments Spectrofluorometer FS5 equipped with Hamamatsu R13456 PMT were used to acquire the absorption and emission spectra. Fluorescence lifetimes were measured on Fluorolog TCSPC Horiba. Spectrophotometric grade solvents were used without further purification. Fluorescence quantum yields were determined in toluene, CH_2Cl_2, CH_3CN, EtOH, DMSO and H_2O (with 2% DMSO) using cresyl violet in EtOH (for measurements in CH_2Cl_2, CH_3CN, EtOH, DMSO and H_2O) and sulforhodamine SR101 (for measurements in toluene) as standards. Photostability was determined using an Asahi Spectra Max-350 as a light source and Shimadzu UV-3600i Plus spectrophotometer. FluoroBrite™ DMEM, Foetal Bowine Serum (FBS), 0.25% Trypsin-EDTA, antibiotics (Penicillin/Streptomycin), L-Glutamine were parched from Gibco, and DMEM High Glucose, Dulbeco's Phosphate Buffered Saline from Biowest. The MitoTracker™ Green FM was purchased from Molecular Probes.

Experimental part

General procedure for the preparation of compounds **4**-**7**.

To a solution of bromoarene (2.4 mmol) in 9 mL of anhydrous THF was slowly added sec-BuLi (1.4 M in cyclohexane, 1.7 mL, 2.4 mmol) at −78 °C and the resulting mixture was stirred for 2 h at the same temperature. A suspension of ketone **3** (200 mg, 0.6 mmol) in THF (25 mL) was added dropwise over 20 min. The reaction mixture was allowed to warm up to room temperature overnight. To the solution, 30 mL of 2 M HCl was added and the stirring was continued for 18 h. During this time, the color of the mixture changed from brown to deep green. The mixture was diluted with water and washed five times with Et_2O to remove unreacted xanthone and byproducts. The aqueous layer was then extracted three times with CH_2Cl_2. The combined CH_2Cl_2 layers were dried over Na_2SO_4. The drying agent was filtered off and the filtrate was evaporated. The product was recrystallized from the mixture of Et_2O/DCM/MeOH. However, the pure product was not obtained due to the fast cleavage of methyl group from diethylamino fragment.

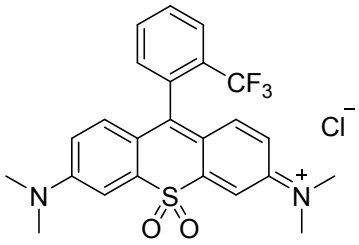
Compound **4**. Yield 70%.



4

It was not possible to get clean NMR spectra, though MS spectrum suggests product **4**. HRMS (ESI) calcd. for $C_{25}H_{27}N_2O_4S$ 451.1692 $[M]^{+\bullet}$, found 451.1696.

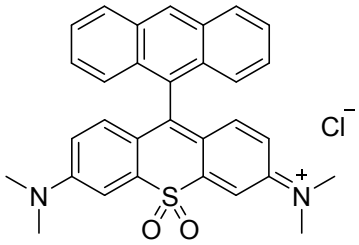
Compound **5**. Yield 57%.



5

It was not possible to get clean NMR spectra, though MS spectrum suggests product **5**. HRMS (ESI) calcd. for $C_{24}H_{22}N_2O_2F_3S$ 459.1354 $[M]^{+\bullet}$, found 459.1318.

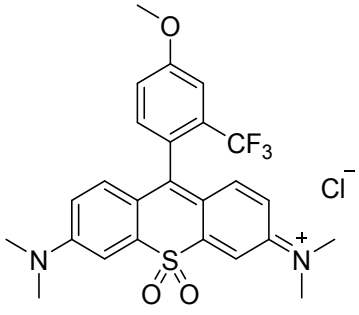
Compound **6**. Yield 42%.



6

It was not possible to get clean NMR spectra, though MS spectrum suggests product **6**. HRMS (ESI) calcd. for $C_{31}H_{27}N_2O_2S$ 491.1793 $[M]^{+\bullet}$, found 491.1793.

Compound **7**. Yield 78%.

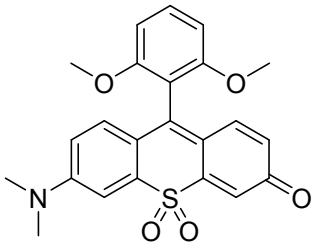


7

It was not possible to get clean NMR spectra, though MS spectrum suggests product 4d. HRMS (ESI) calcd. for $C_{25}H_{24}N_2O_3F_3S$ 489.1460 $[M]^{+\bullet}$, found 489.1460.

General procedure for the preparation of compounds **8-11**. To a solution of SO_2-Rhodamine (0.040 mmol) in 50.0 mL of CH_2Cl_2 and 20.0 mL of 0.5 M NaOH aq. was added. The mixture was stirred for 1 h at room temperature, diluted with DCM, and washed four times with water. The organic layer was dried over Na_2SO_4. The drying agent was filtered off and solvents were evaporated under reduced pressure. The product was purified using column chromatography (silica, CH_2Cl_2 : acetone 95:5). After evaporation of the solvent and drying under vacuum rhodols were obtained as violet-blue solids.

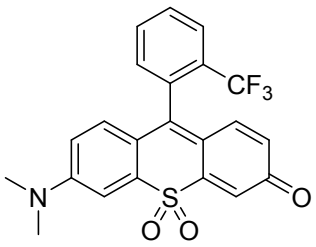
Compound **8**. Yield 25%. M.p. 224-225°C



8

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.45 (t, 1H, J = 8.4 Hz), 7.39 (d, 1H, J = 2.7 Hz), 7.28 (d, 1H, J = 1.9 Hz), 6.94 (d, 1H, J = 9.9 Hz), 6.92 (d, 1H, J = 9.2 Hz), 6.68 (d, 2H, J = 8.4 Hz), 6.60 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.7 Hz), 6.28 (dd, 1H, J_1 = 9.9 Hz, J_2 = 1.9 Hz), 3.69 (s, 6H), 3.14 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 186.9, 160.8, 154.7, 148.1 (2), 143.1, 141.3, 136.6, 134.1, 130.2, 128.5, 124.1, 122.0, 117.2, 115.0, 110.0, 106.7, 58.9, 43.1; HRMS (ESI) calc. for $C_{23}H_{21}NO_5SNa$ 446.1038 $[M + Na]^+$, found 446.1034.

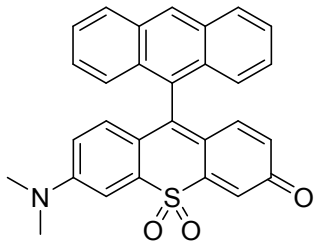
Compound **9**. Yield 25%. M.p. 210-212°C



9

Yield 39%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.89 (d, 1H, J = 7.6 Hz), 7.73 (t, 1H, J = 7.3 Hz), 7.69 (t, 1H, J = 7.5 Hz), 7.43 (d, 1H, J = 2.4 Hz), 7.33 (d, 2H, J = 9.1 Hz), 6.68 (t, 1H, J = 10.1 Hz), 6.65 (s, 1H), 6.58 (dd, 1H, J_1 = 9.1 Hz, J_2 = 2.4 Hz), 6.27 (dm, 1H), 3.17 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 183.6, 151.9, 145.6, 144.9, 140.0, 137.7, 134.6, 133.7, 132.1, 131.2, 129.6, 127.5, 126.9, 126.9, 126.5, 120.8, 118.9, 114.0, 107.5, 40.3; HRMS (ESI) calcd. for $C_{22}H_{17}NO_3SF_3$ 432.0881 $[M + H]^+$, found 432.0874.

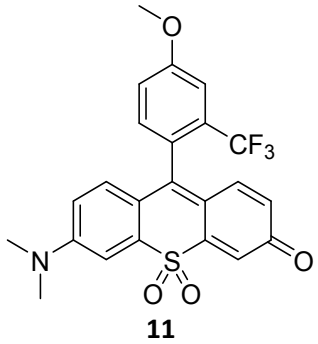
Compound **10**. Yield 11%. M.p. 250°C(dec.)



10

^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.65 (s, 1H), 8.11 (d, 2H, J = 8.52 Hz), 7.69 (d, 2H, J = 8.7 Hz), 7.51 (m, 3H), 7.44 (m, 3H), 6.50 (d, 1H, J = 10 Hz), 6.42 (d, 1H, J = 9.3 Hz), 6.36 (dd, 1H, J_1 = 9.2 Hz, J_2 = 2.8 Hz), 6.10 (dd, 1H, J_1 = 9.9 Hz, J_2 = 1.7 Hz), 3.13 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 183.4, 152.0, 148.1, 144.7, 140.2, 138.1, 135.1, 130.9, 130.2, 128.8, 128.7, 128.3, 127.8, 127.4, 126.0, 125.9, 125.5, 122.3, 119.5, 114.5, 107.5, 40.3; HRMS (ESI) calcd. for $C_{29}H_{22}NO_3S$ 464.1320 $[M + H]^+$, found 464.1337.

Compound **11**. Yield 25%. M.p. 238-240°C



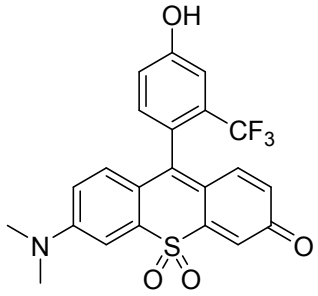
11

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.42 (d, 1H, J = 2.8 Hz), 7.36 (d, 1H, J = 1.9 Hz), 7.31 (d, 1H, J = 2.0 Hz), 7.21 (d, 2H, J = 1.6 Hz), 6.74 (dd, 2H, J_1 = 19.4 Hz, J_2 = 9.6 Hz), 6.59 (dd, 1H, J_1 = 9.2 Hz, J_2 = 2.8 Hz), 6.28 (dd, 1H, J_1 = 10.0 Hz, J_2 = 2.0 Hz), 3.95 (s, 3H), 3.17 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 182.6, 160.2, 151.9, 146.0, 145.0, 140.0, 137.9, 134.7, 132.5, 130.8, 127.5, 126.4, 125.2, 121.3, 119.3, 117.4, 114.0, 107.4, 55.8, 40.3; HRMS (ESI) calcd. for $C_{23}H_{19}NO_4SF_3$ 462.0987 $[M + H]^+$, found 462.0983.

Compound **12**.

A solution of **11** (400 mg, 0.87 mmol) in dry DCM (50 ml) under Ar was cooled to at 0˚C and boron tribromide (225 mg, 0.9 mmol, 0.085 ml) was added dropwise upon stirring. The reaction was allowed to warm to rt and left stirring overnight. The mixture was diluted with 25 ml of saturated sodium bicarbonate and organic layer was separated, dried over sodium sulfate, filtrated and concentrated under the low pressure. The product was purified utilizing column chromatography (CH_2Cl_2 : acetone 95:5).

Yield 50%. M.p. 186-188°C



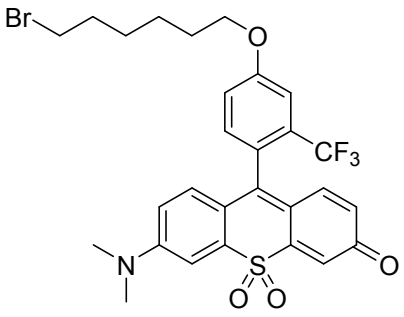
12

^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.59 (s, 1H), 7.35 (1H, d, J = 3 Hz), 7.28 (2H, m), 7.20 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz), 7.01 (1H, d, J = 2 Hz), 6.86 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz), 6.79 (1H, d, J = 10 Hz), 6.72 (1H, d, J = 9.5 Hz), 6.27 (1H, dd, J_1 = 10 Hz, J_2 = 3 Hz), 3.15 (6H, s); ^{13}C NMR (126 MHz, DMSO- d_6) δ: 182.26, 158.40, 151.95, 147.30, 144.88, 139.42, 138.05, 134.86, 133.02, 128.88, 128.64, 128.40, 126.72, 124.52, 124.24, 122.70, 122.34, 119.53, 119.41, 117.91, 114.82, 113.17, 113.13, 107.06; ^{19}F NMR (500 MHz, $CDCl_3$) δ: -58.42; HRMS (ESI) calcd. for $C_{22}H_{17}NO_4SF_3$ 448.0830 $[M + H]^+$, found 448.0820.

Compound **13**.

Compound **12** (194 mg, 0.43 mmol) was dissolved in dry CH_3CN (10 ml) followed by the addition of K_2CO_3 (240 mg, 1.74 mmol). 1,6-dibromohexane (159 mg, 0.65 mmol, 0.1 ml) was added under Ar and the reaction mixture was left refluxing for 18h. After the reaction complete, the solvent was evaporated under reduced pressure and the residue was dissolved in 50 ml of DCM. The organic layer was washes 3 times with water (50 ml), dried over Na_2SO_4 and concentrated in vacuo. The obtained solid was next boiled in 20 ml of hexane, filtered and washed again with hexane. The product was next purified via column chromatography (CH_2Cl_2 : acetone 95:5).

Yield 50%. M.p. 125-127°C(dec.)



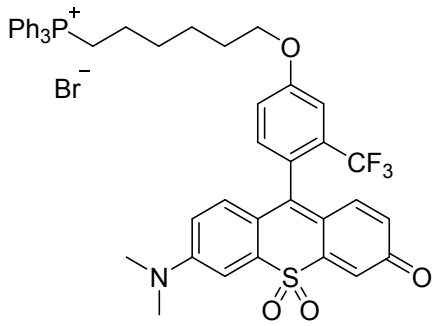
13

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.41 (d, 1H, J = 2 Hz), 7.35 (d, H, J = 2 Hz), 7.31 (d, 1H, J = 2 Hz), 7.19 (m, 2H), 6.77 (d, 1H, J = 10 Hz), 6.73 (d, 1H, J = 9 Hz), 6.58 (dd, 1H, J_1 = 9.5 Hz, J_2 = 3 Hz), 6.28 (dd, 1H, J_1 = 10 Hz, J_2 = 3 Hz), 4.09 (t, 2H, J = 6 Hz), 3.46 (t, 2H, J = 6 Hz), 3.17 (s, 6H), 1.92 (dq, 4H, J_1 = 22 Hz, J_2 = 7 Hz), 1.57 (m, 4H); ^{19}F NMR (500 MHz, $CDCl_3$) δ: -59.53; ^{13}C NMR (126 MHz, $CDCl_3$) δ: 183.63, 159.64, 151.80, 146.11, 144.92, 139.97, 137.94, 134.78, 132.46, 130.67, 127.41, 126.32, 124.94, 121.20, 119.29, 117.64, 113.97, 113.01, 107.38, 77.25, 77.00, 76.75, 68.42, 40.27, 33.70, 32.59, 28.90, 27.83, 25.25. HRMS (ESI) calcd. for $C_{28}H_{27}NO_4SBrF_3Na$ 632.0694 $[M + Na]^+$, found 632.0658.

Compound **14**.

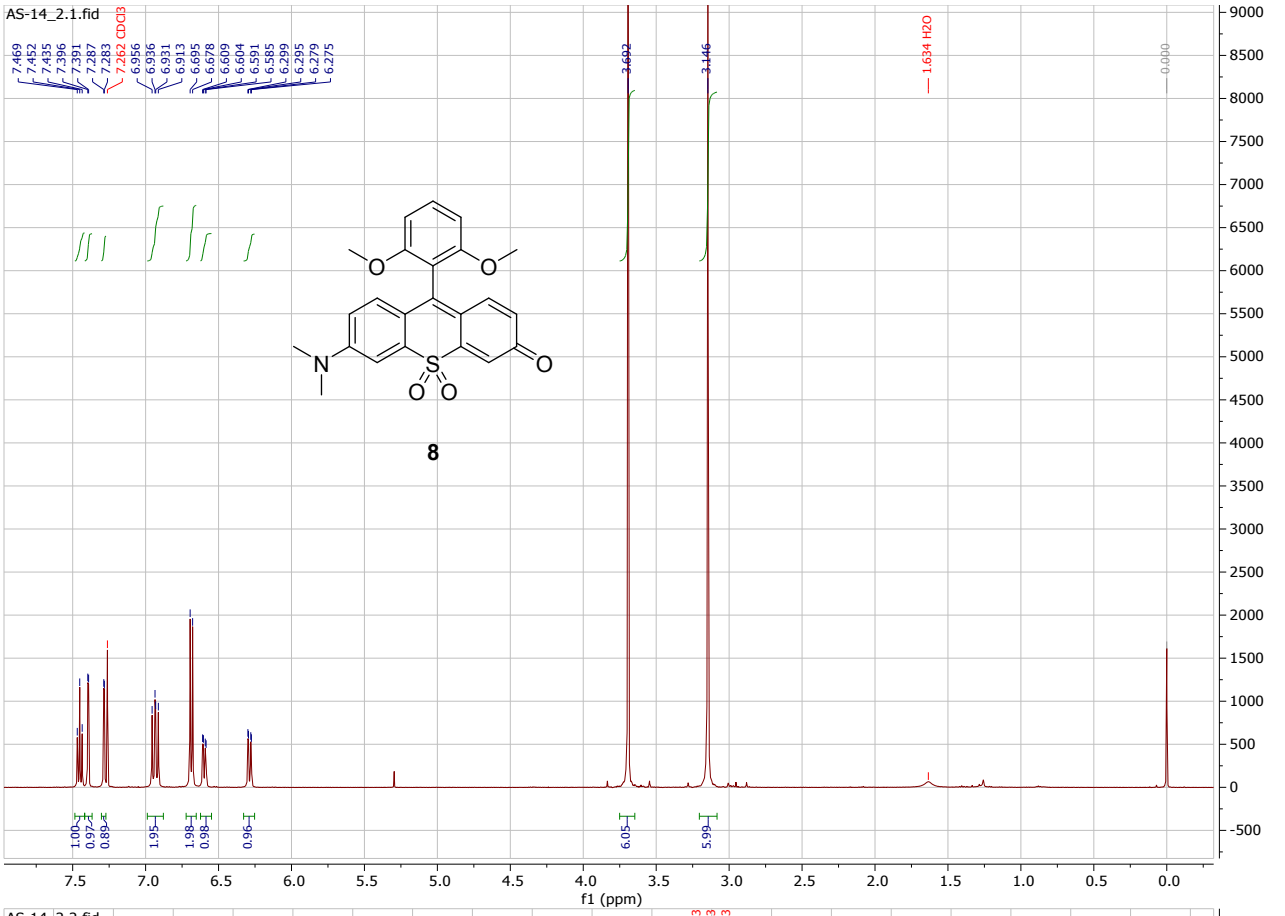
73 mg (0,12 mmol) of **13** with 0,31g (1,2 mmol) of triphenylphosphine in 0,3 ml of DMA was heated at 120° for 35 min under Argon. After cooling the reaction was diluted with a mixture hexane/Et_2O, the crude product was filtered and washed again and next purified via column chromatography on a reversed phase (RP-18) in CH_3CN. After evaporation if the solvent the product was washed with EtOAc for 1h to give 12 mg of the product.

Yield 11%. M.p. 137-139^{o}C(dec.)



14

^{1}H NMR (500 MHz, CD_3CN) δ: 7.74 (bm, 15H), 7.42 (bd, 2H, J_1 = 6.5 Hz,), 7.3 (bs, 2H), 7.13 (bs, 1H), 6.83 (d, 1H, J = 10 Hz), 6.79 (d, 1H, J = 9.5 Hz), 6.73 (dd, 1H, J_1 = 9. Hz, J_2 = 3 Hz), 6.22 (dd, 1H, J_1 = 9 Hz, J_2 = 2 Hz), 4.11 (t, 2H, J = 6.5 Hz), 3.27 (bm, 2H), 3.17 (s, 6H), 1.78 (q, 2H, J = 7.5 Hz), 1.7 (q, 2H, J = 7.5 Hz), 1.63 (q, 2H, J = 7.5 Hz), 1.54 (q, 2H, J = 7.5 Hz); ^{19}F NMR (500 MHz, CD_3CN) δ: -59.95; HRMS (ESI) calcd. for $C_{46}H_{42}NO_4SF_3P$ 792.2524 $[M]^+$, found 792.2505.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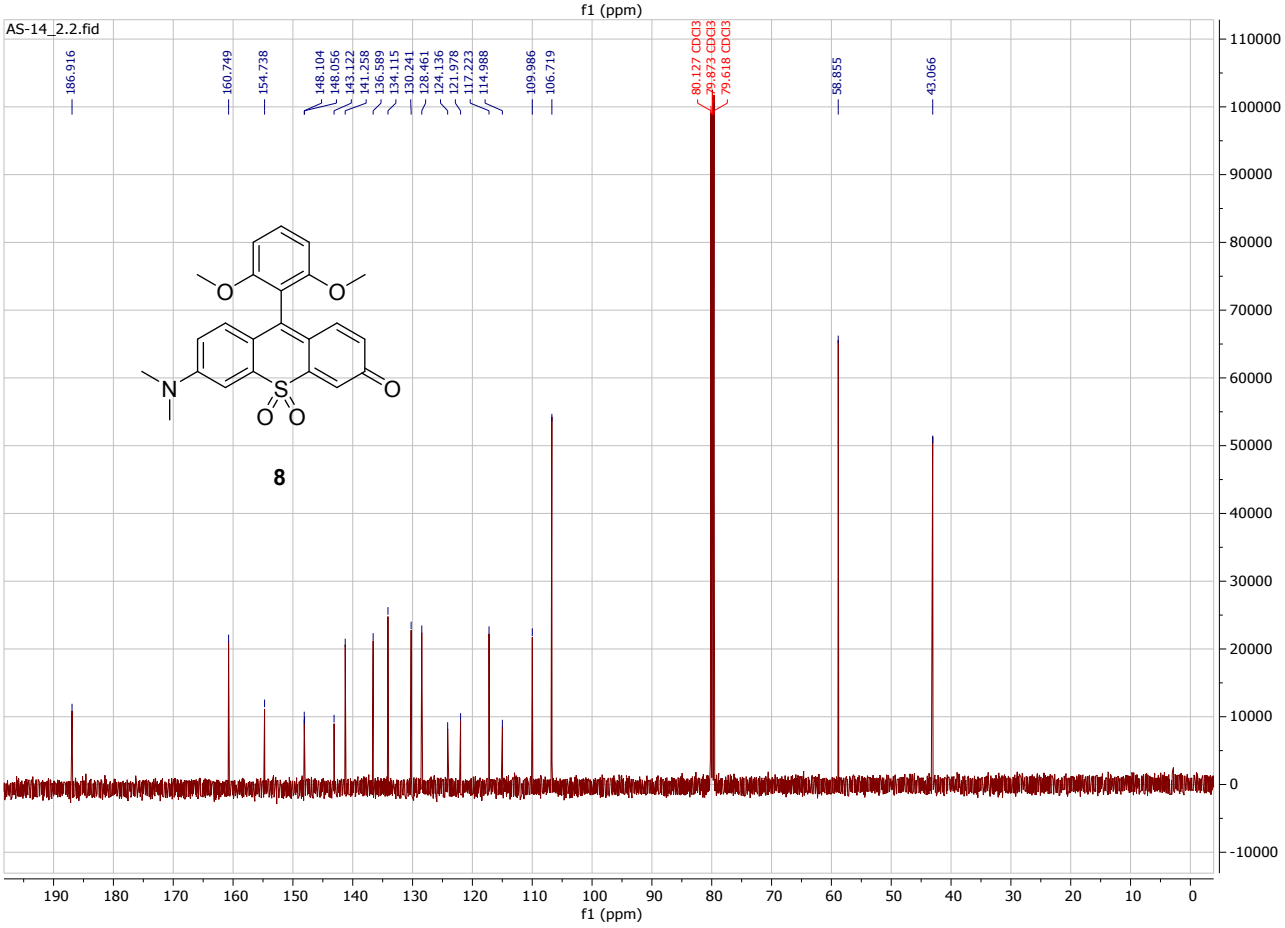
8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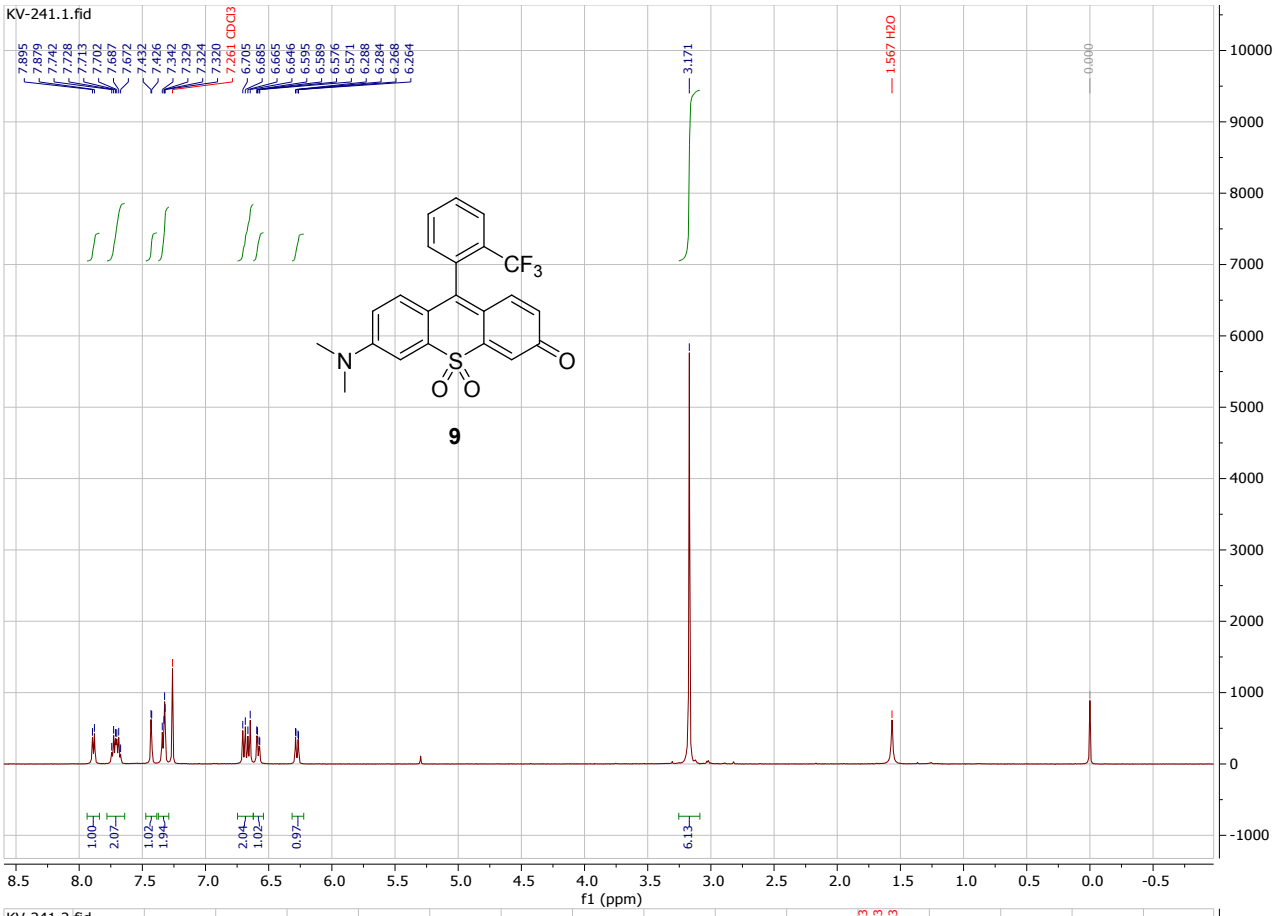
8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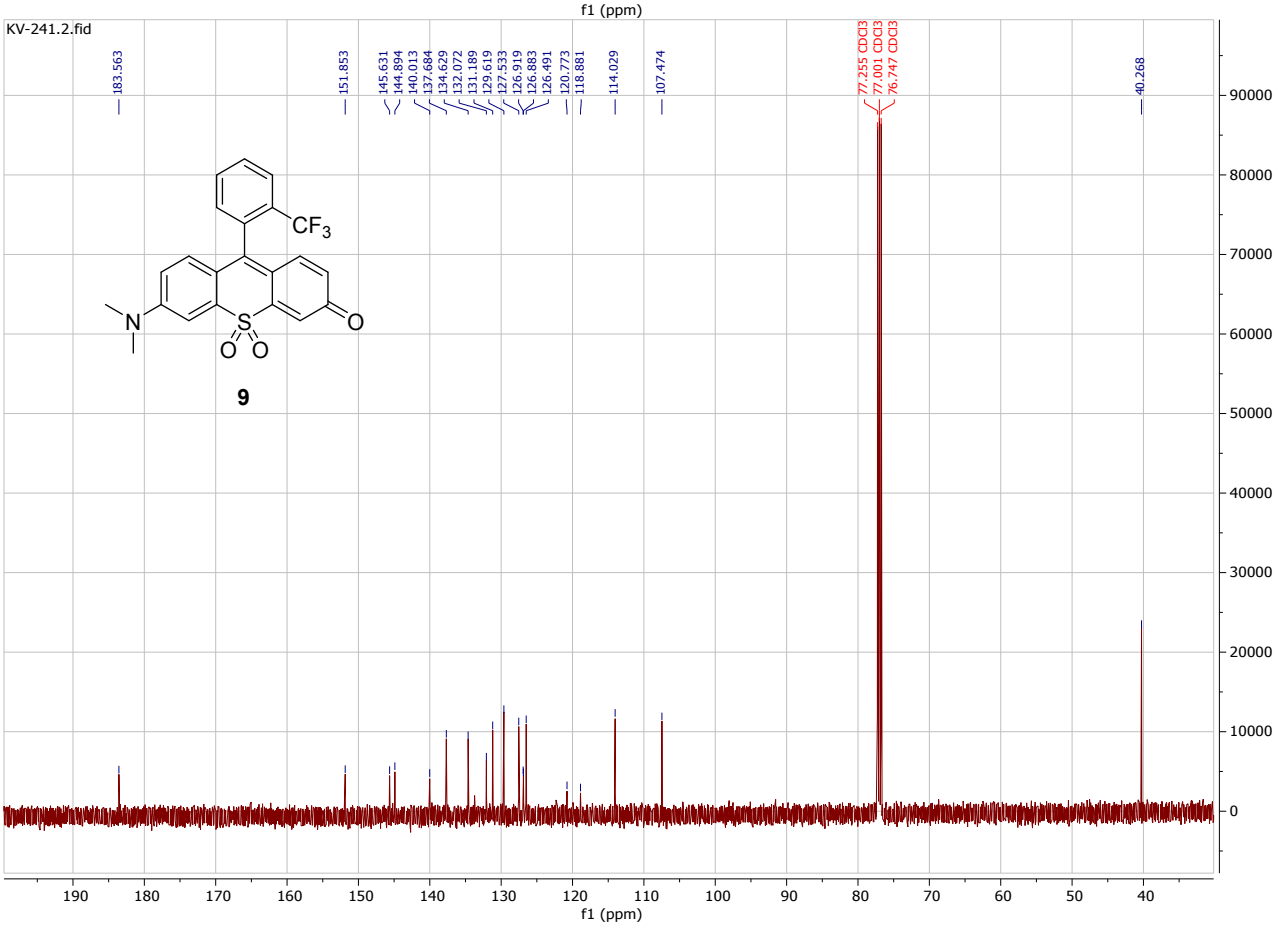
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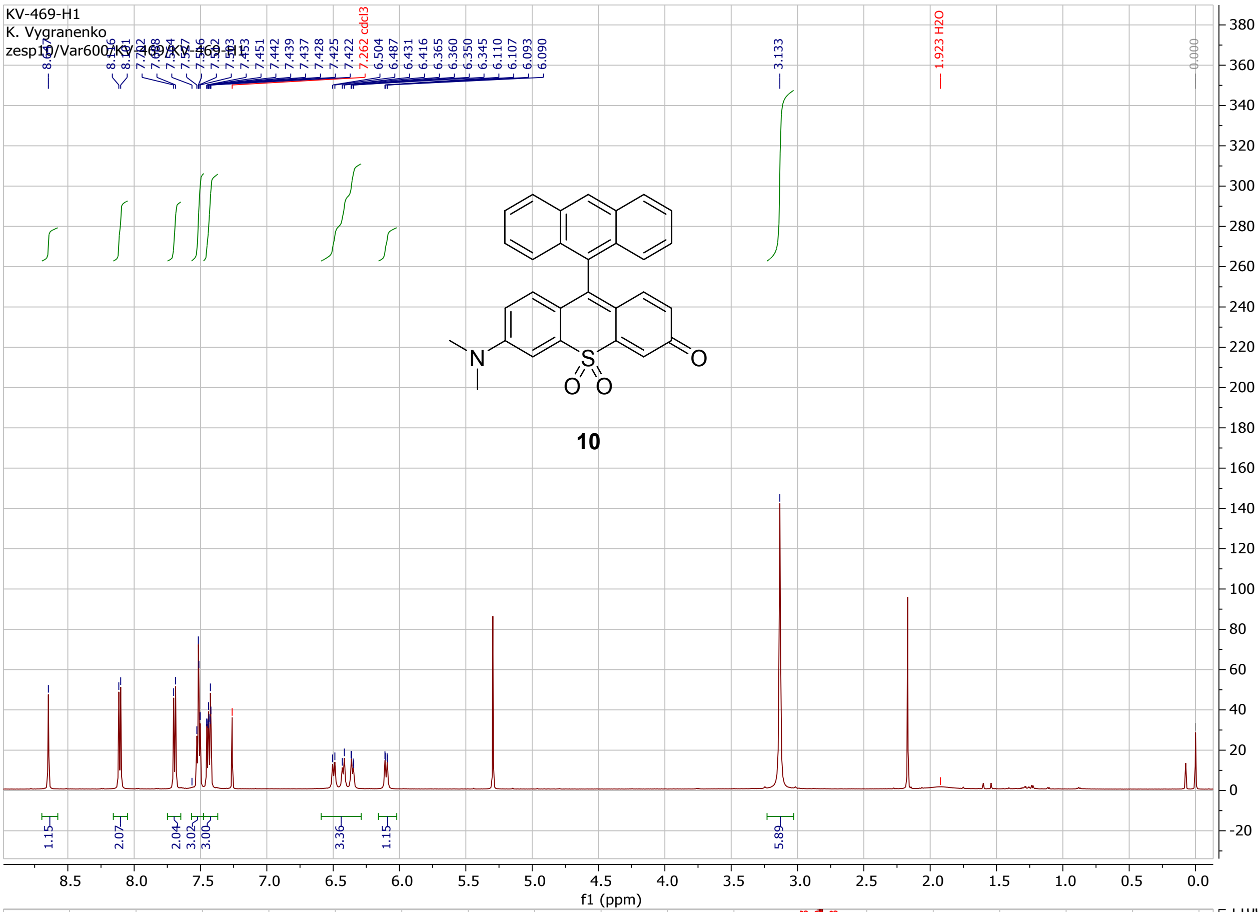
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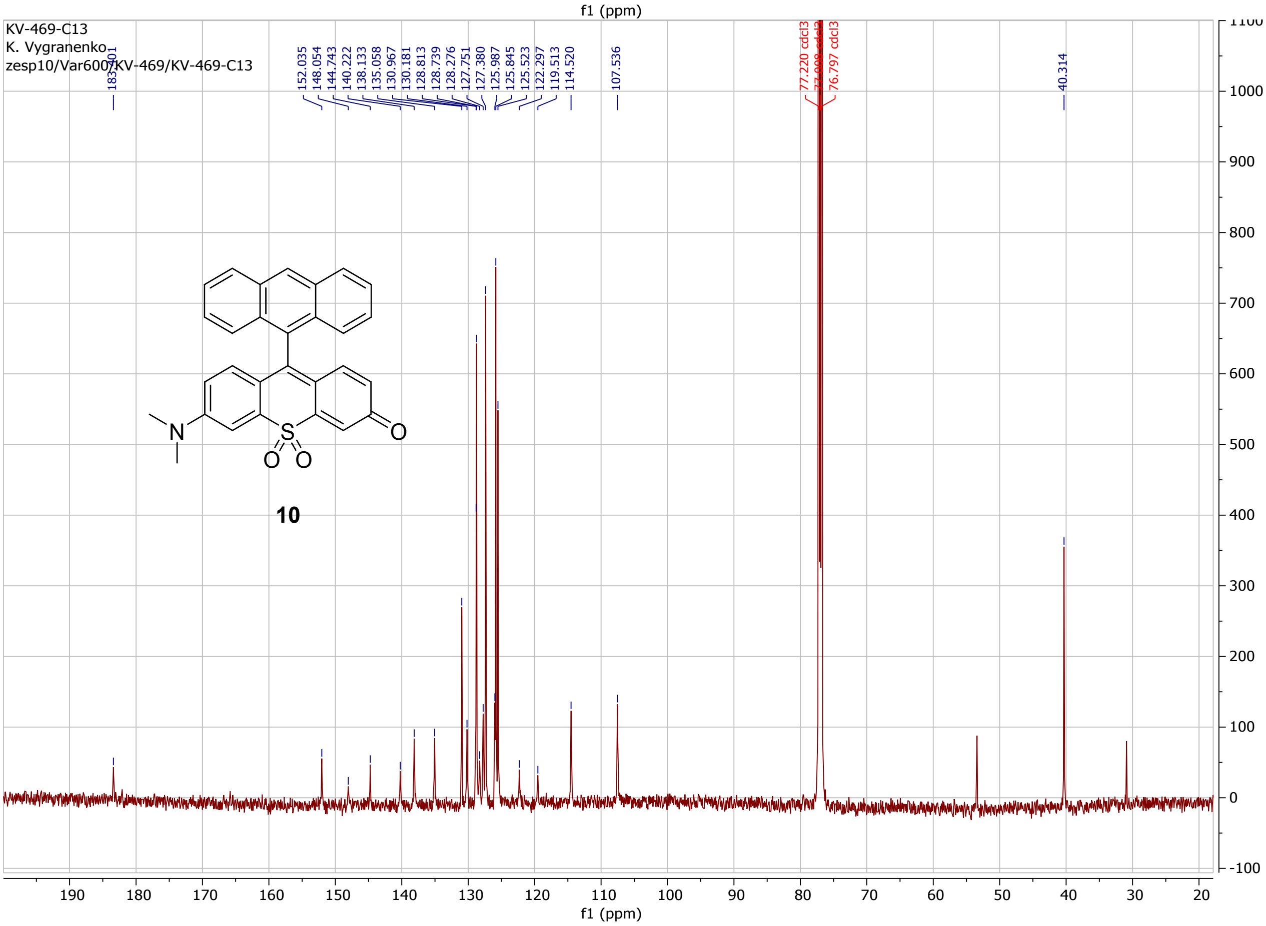
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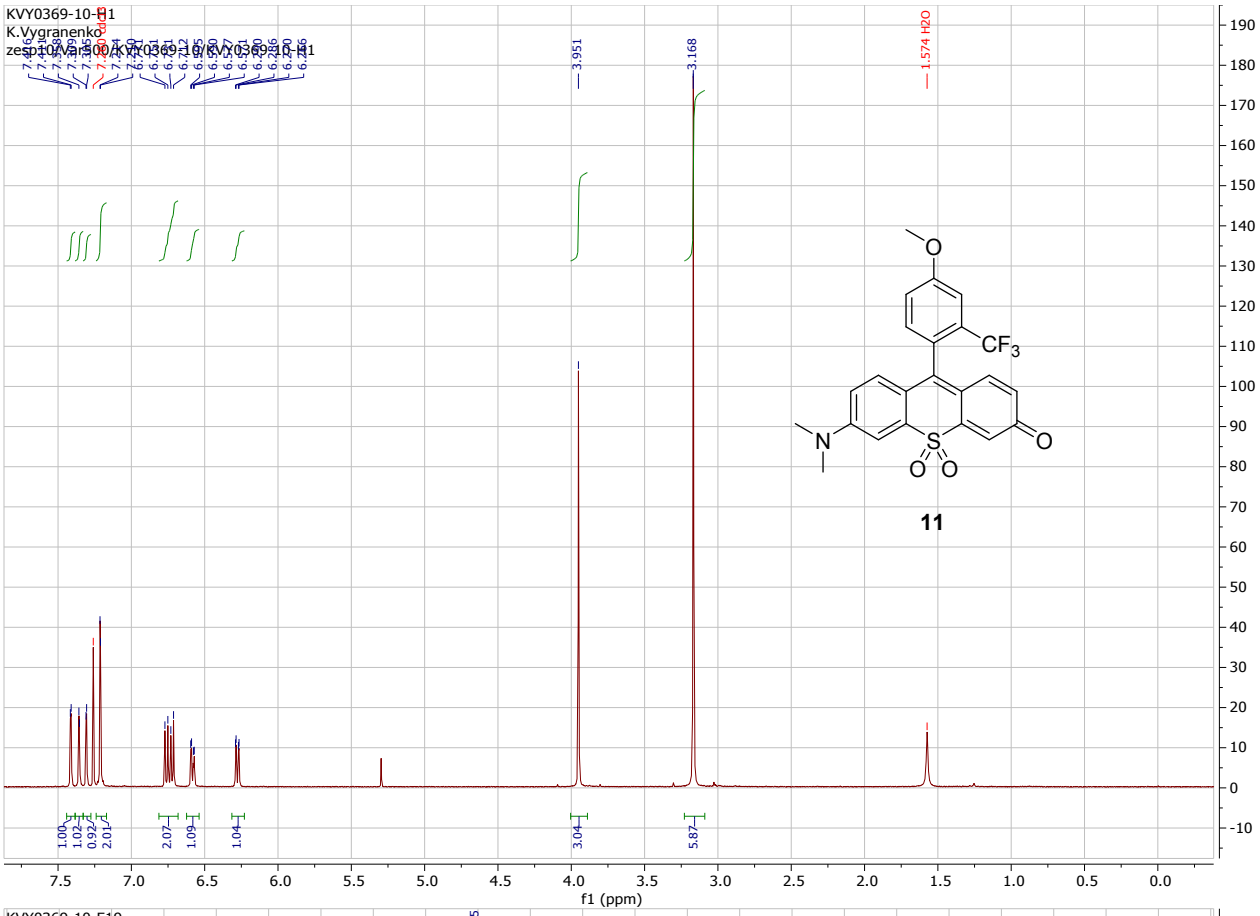
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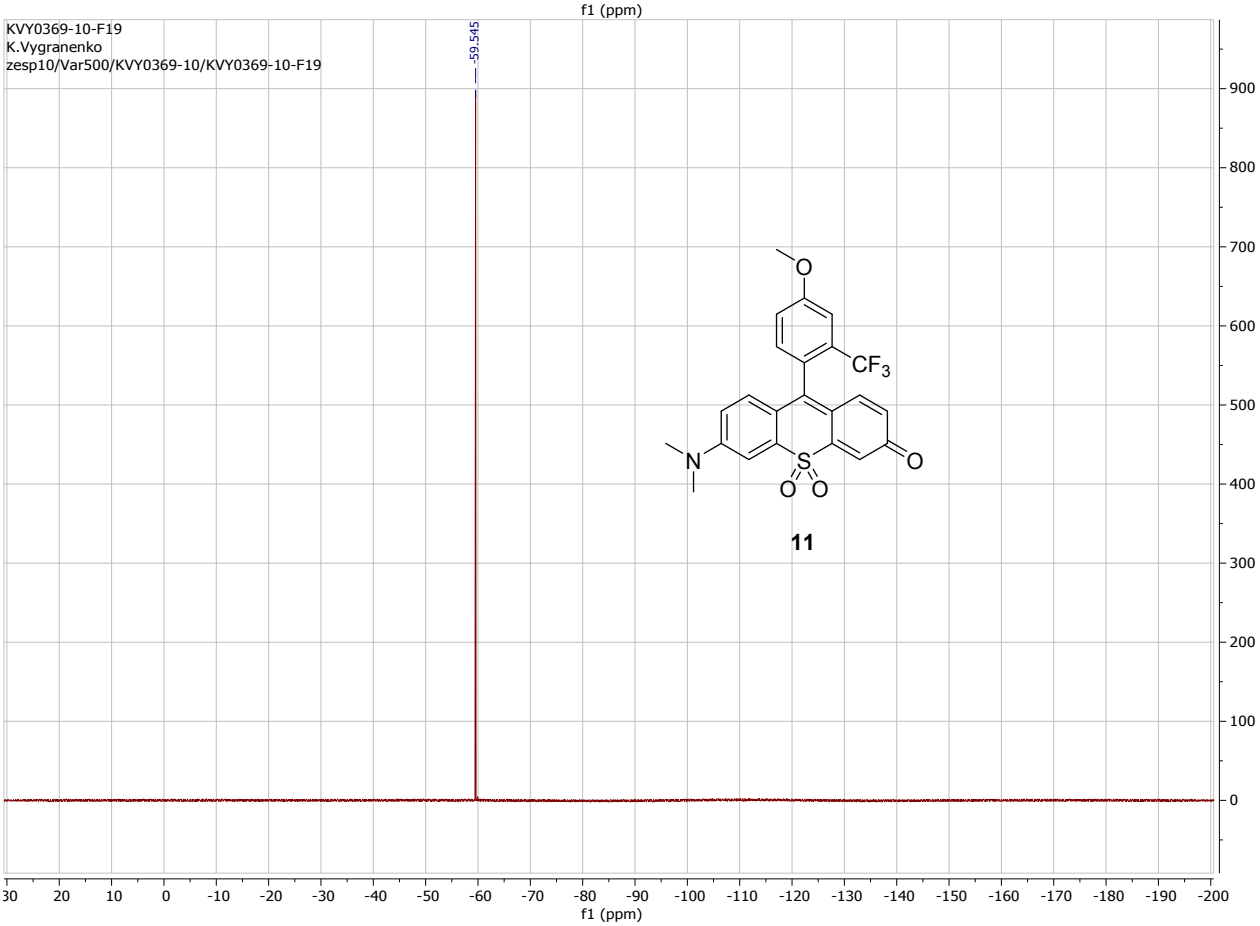
11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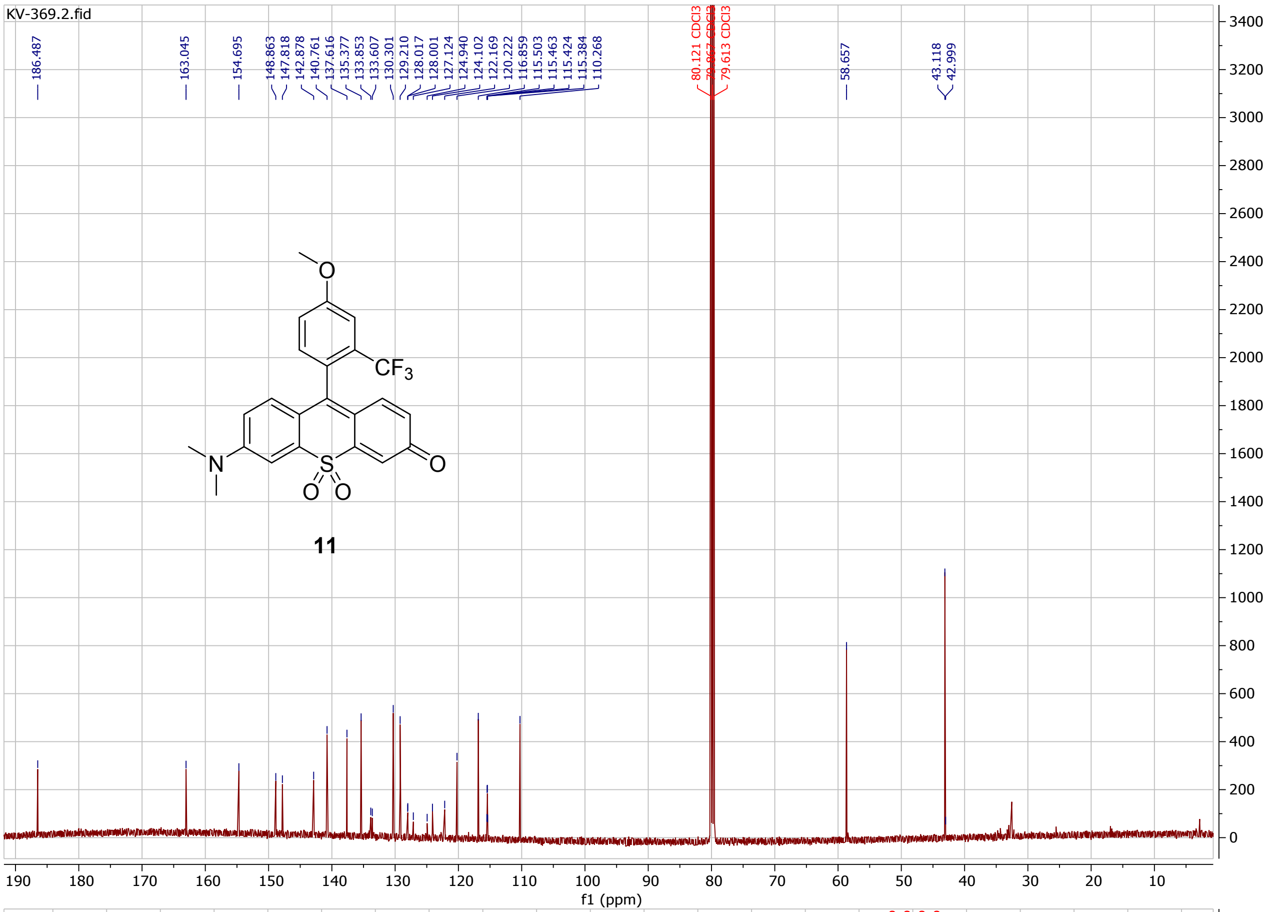
O
11



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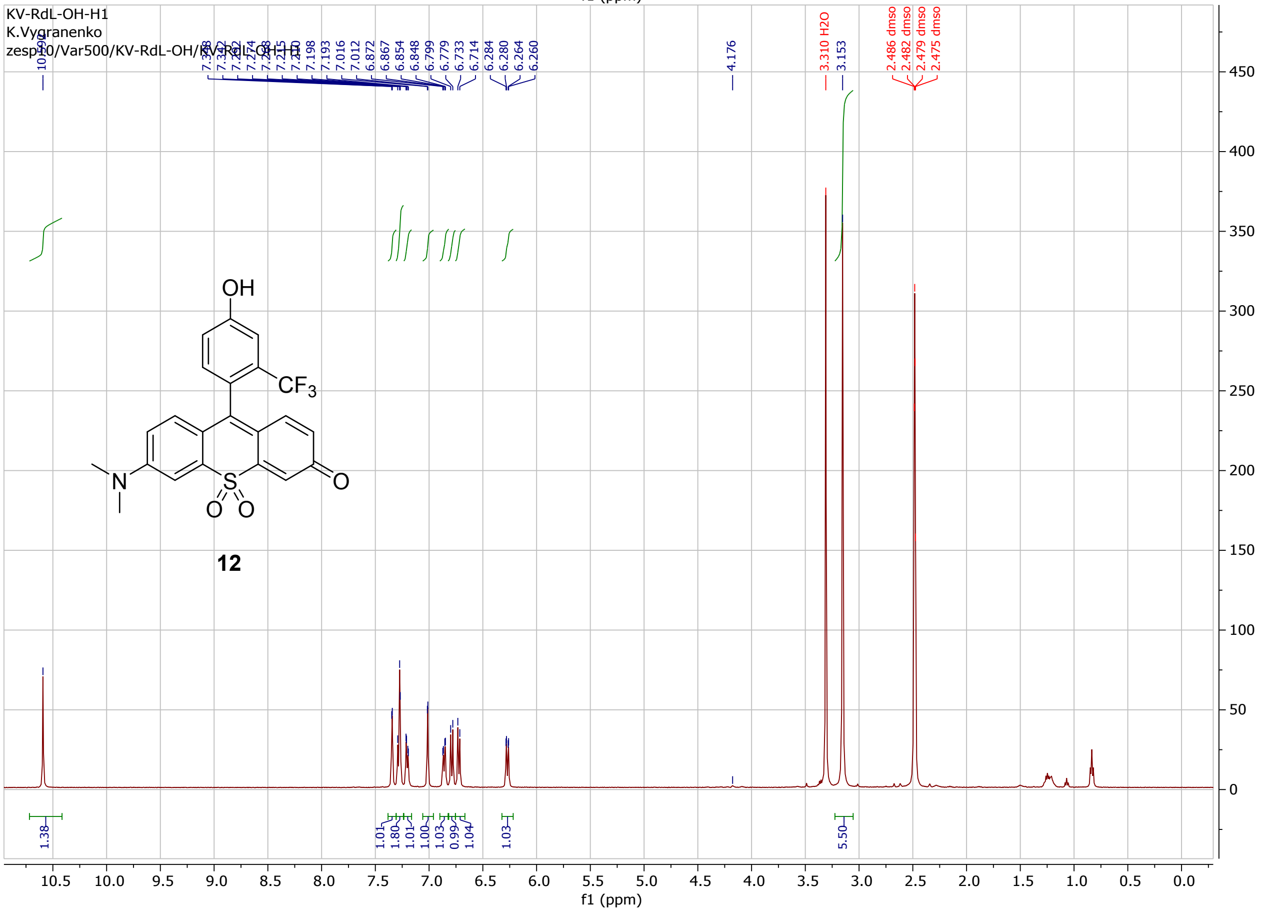

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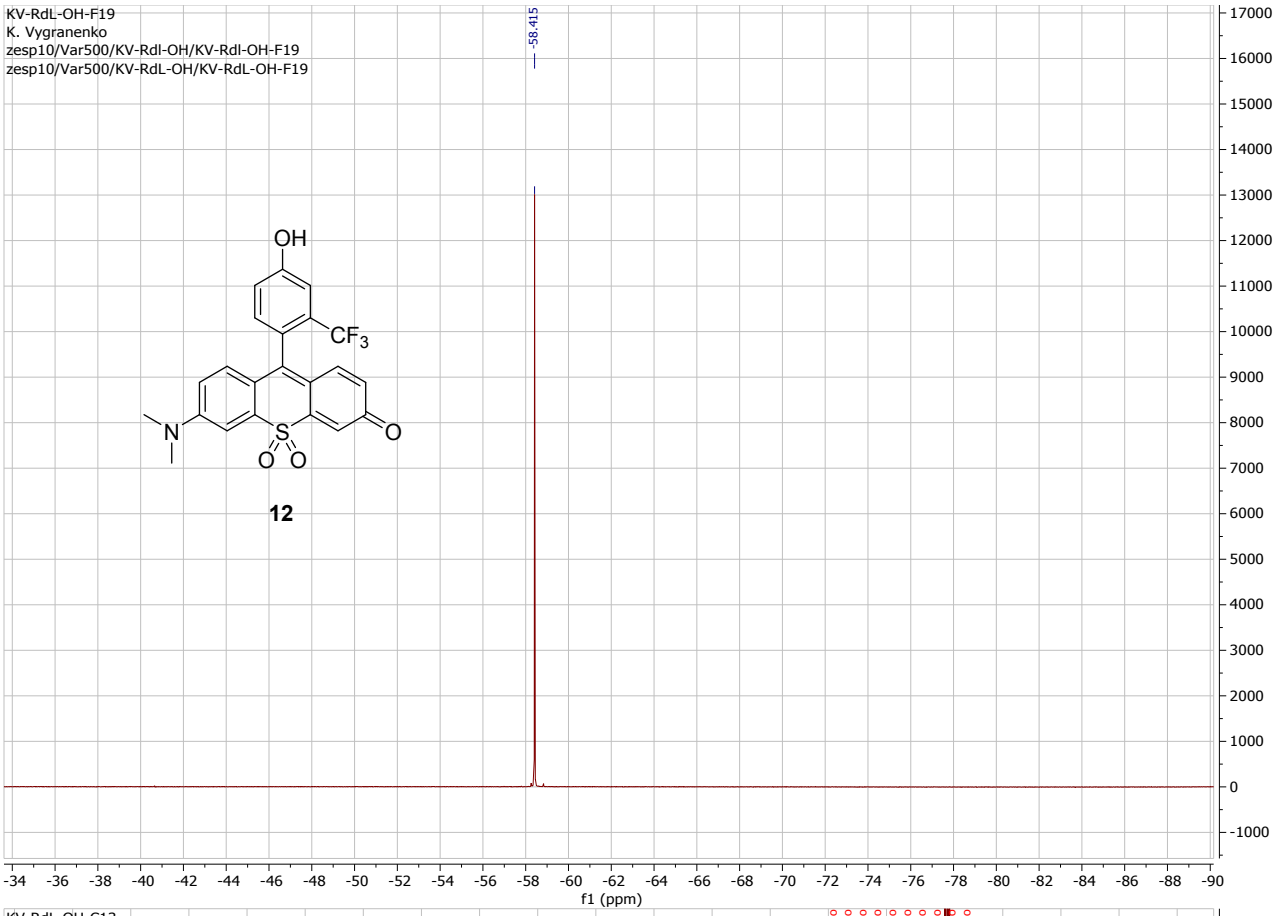
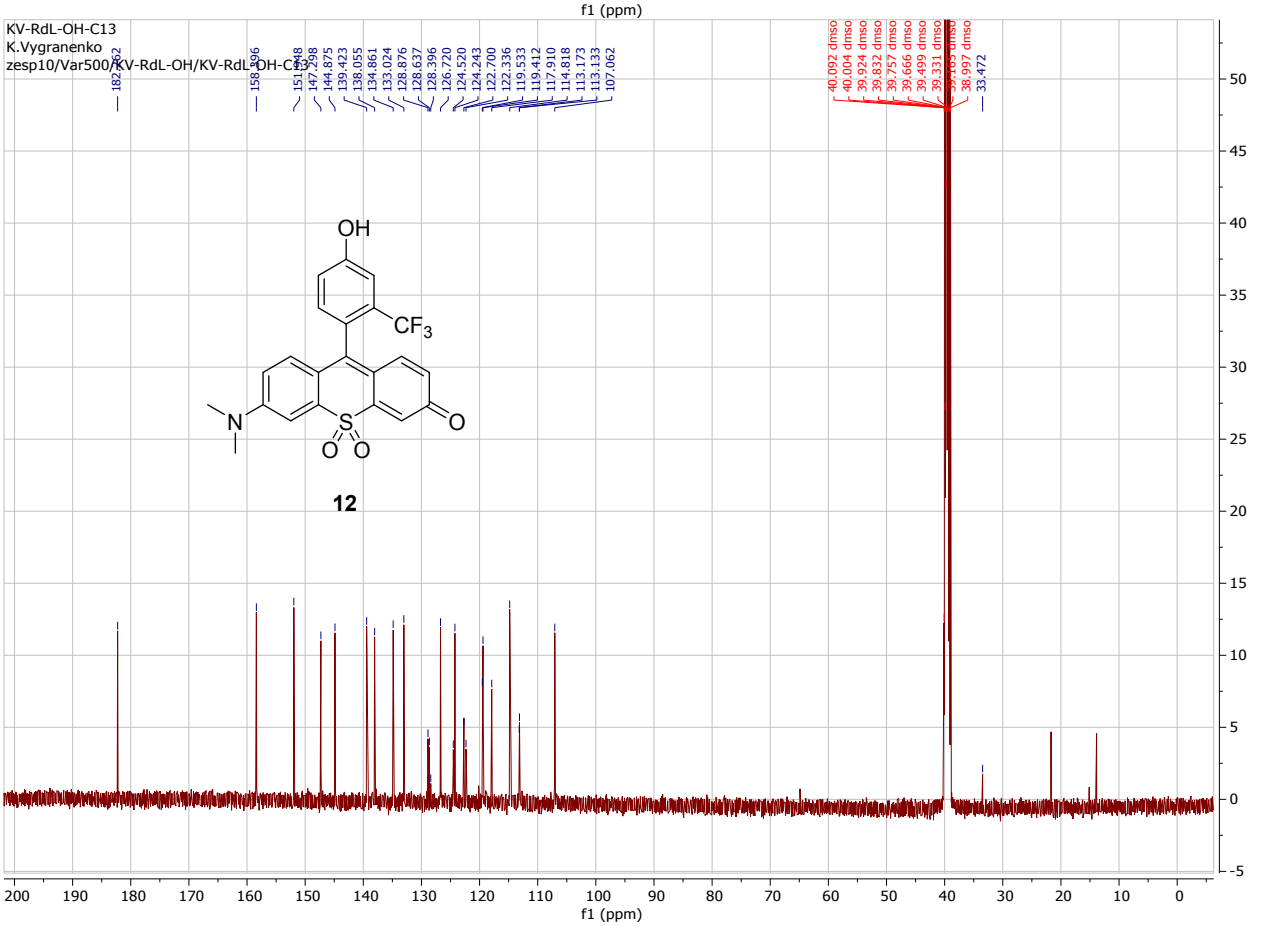

KV-RdL-OH-F19
K. Vygranenko
zesp10/Var500/KV-Rdl-OH/KV-Rdl-OH-F19
zesp10/Var500/KV-RdL-OH/KV-RdL-OH-F19
-58.415
OH
CF3
N
S
O
O
O
12
f1 (ppm)


KV-RdL-OH-C13
K.Vygranenko
zesp10/Var500/KV-RdL-OH/KV-RdL-OH-C13
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158.496
151.248
147.298
144.875
139.423
138.055
134.861
133.024
128.876
128.637
128.396
126.720
124.520
124.243
122.700
122.336
119.533
119.412
117.910
114.818
113.173
113.133
107.062
40.092 dmso
40.004 dmso
39.924 dmso
39.832 dmso
39.757 dmso
39.666 dmso
39.499 dmso
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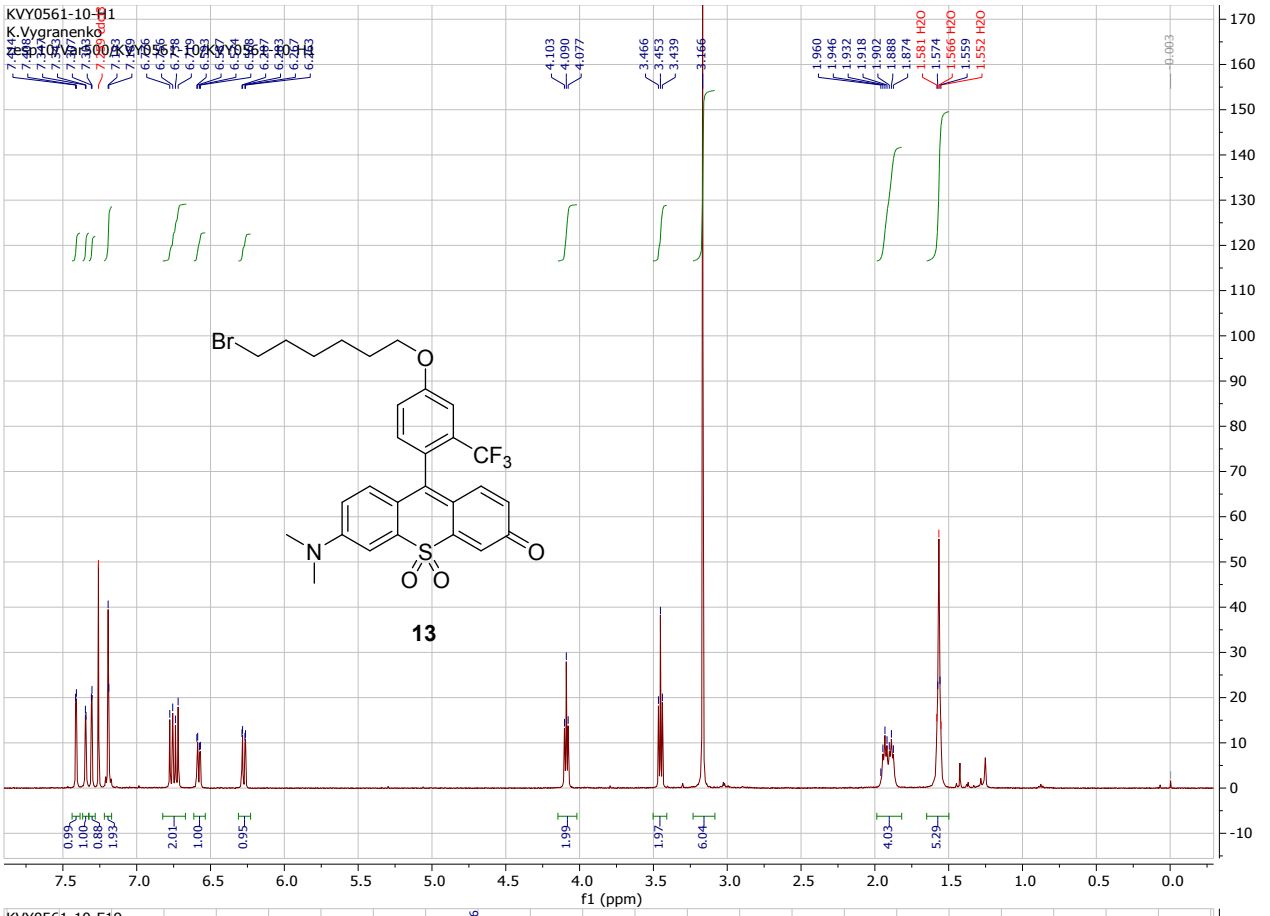
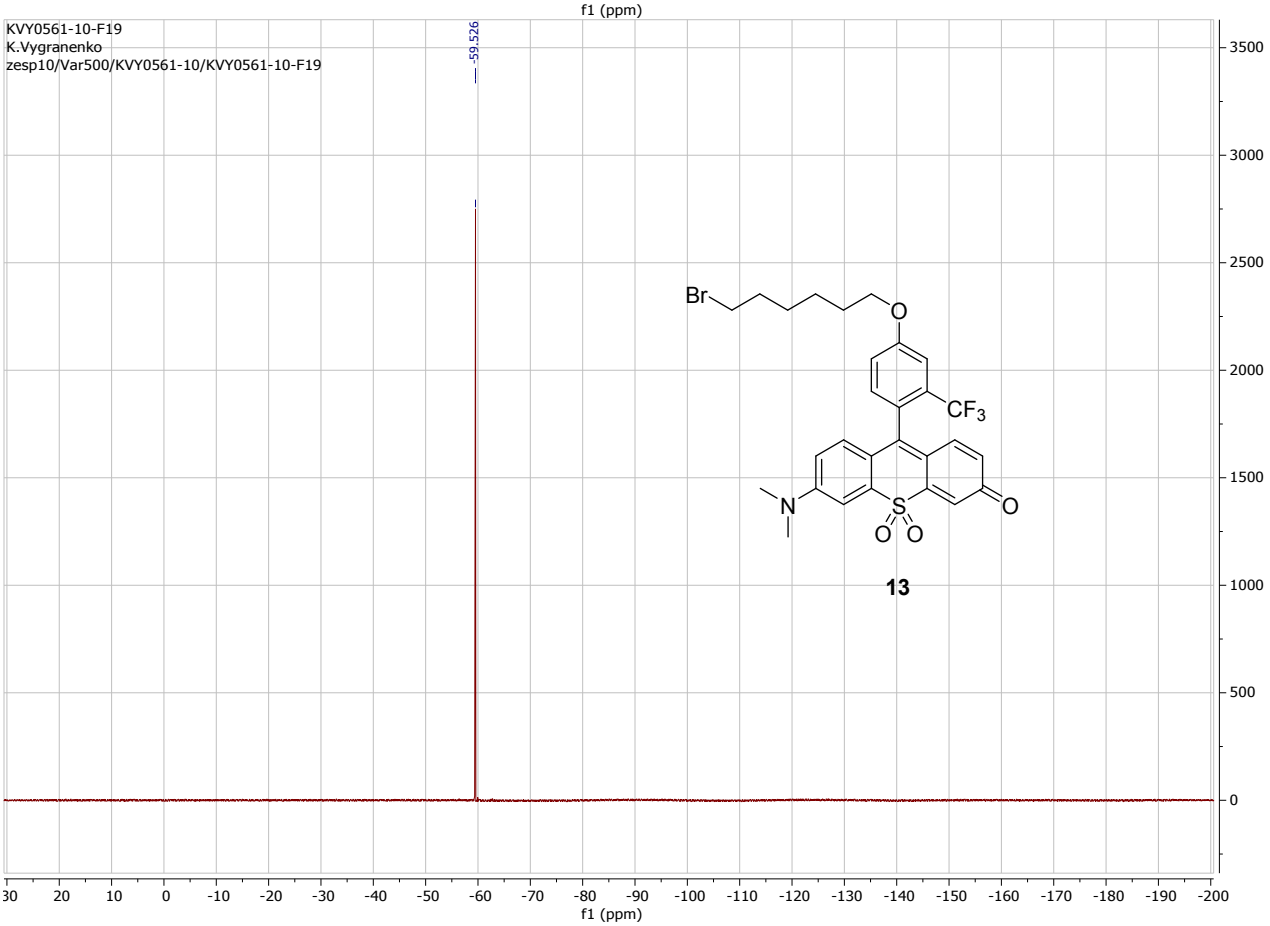
33.472
OH
CF3
N
S
O
O
O
12
f1 (ppm)


KVY0561-10-H1
K.Vygranenko
zesp10/Var500/KVY0561-10/KVY0561-10-H1
Br
O
CF3
N
S
O
O
O
13
f1 (ppm)


KVY0561-10-F19
K.Vygranenko
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O
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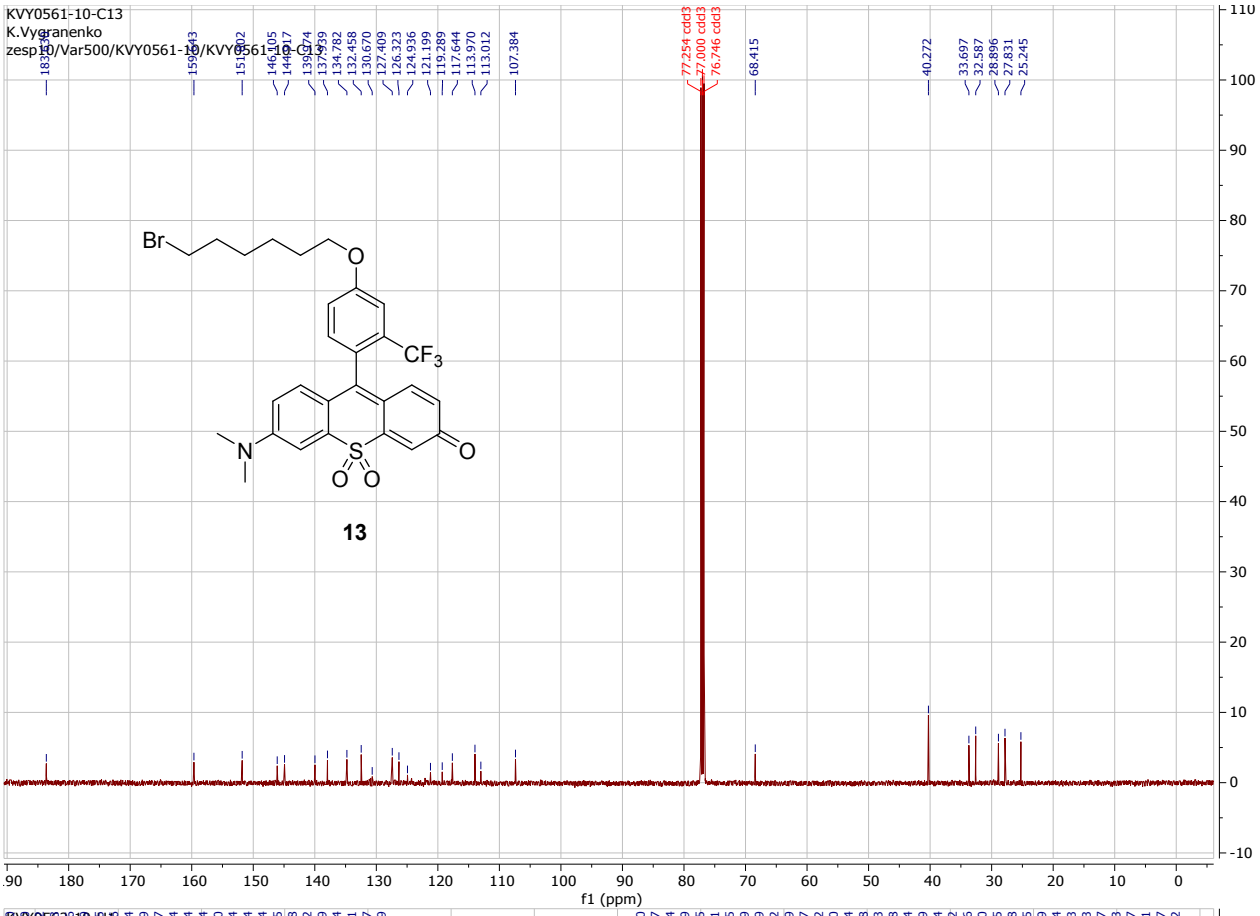
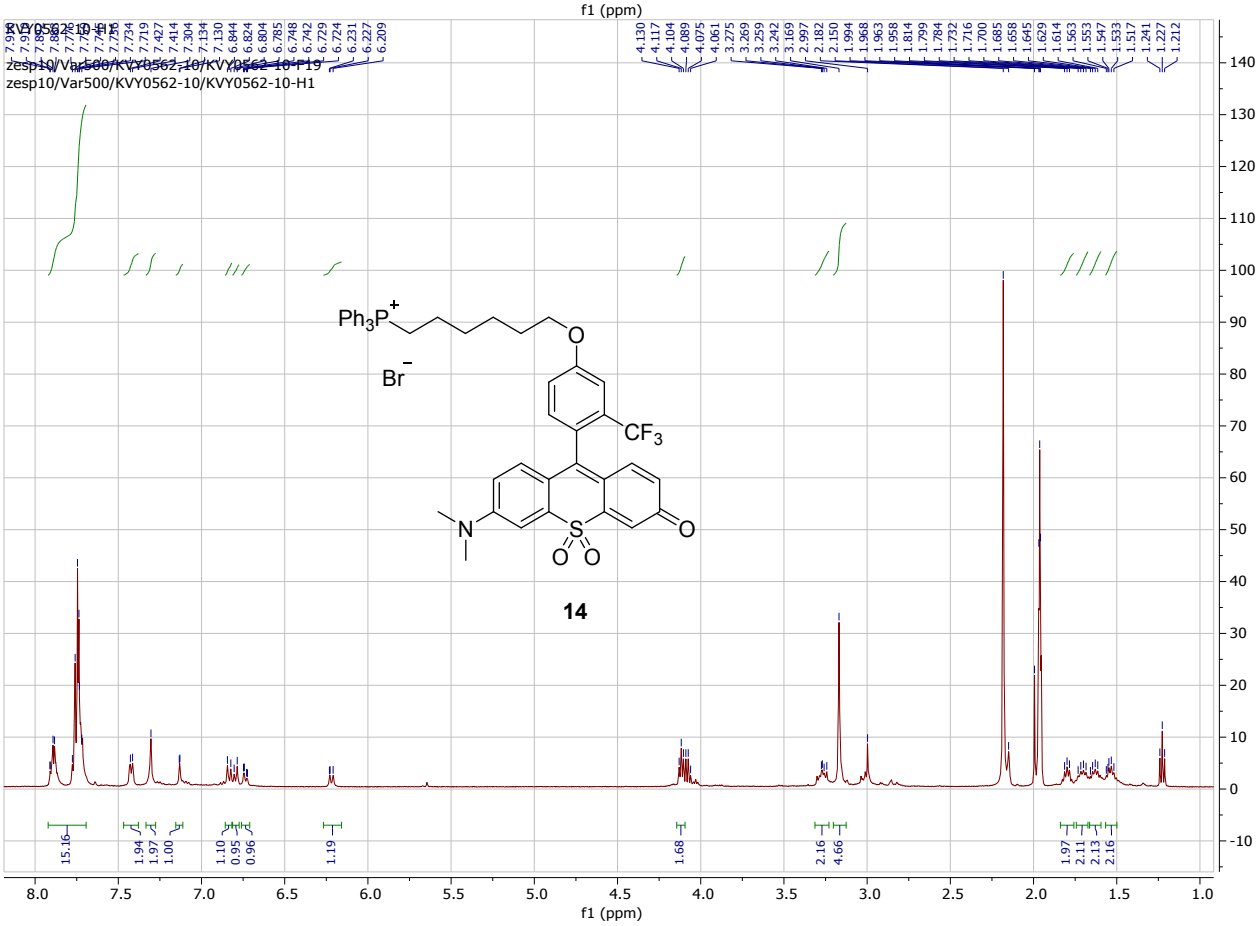
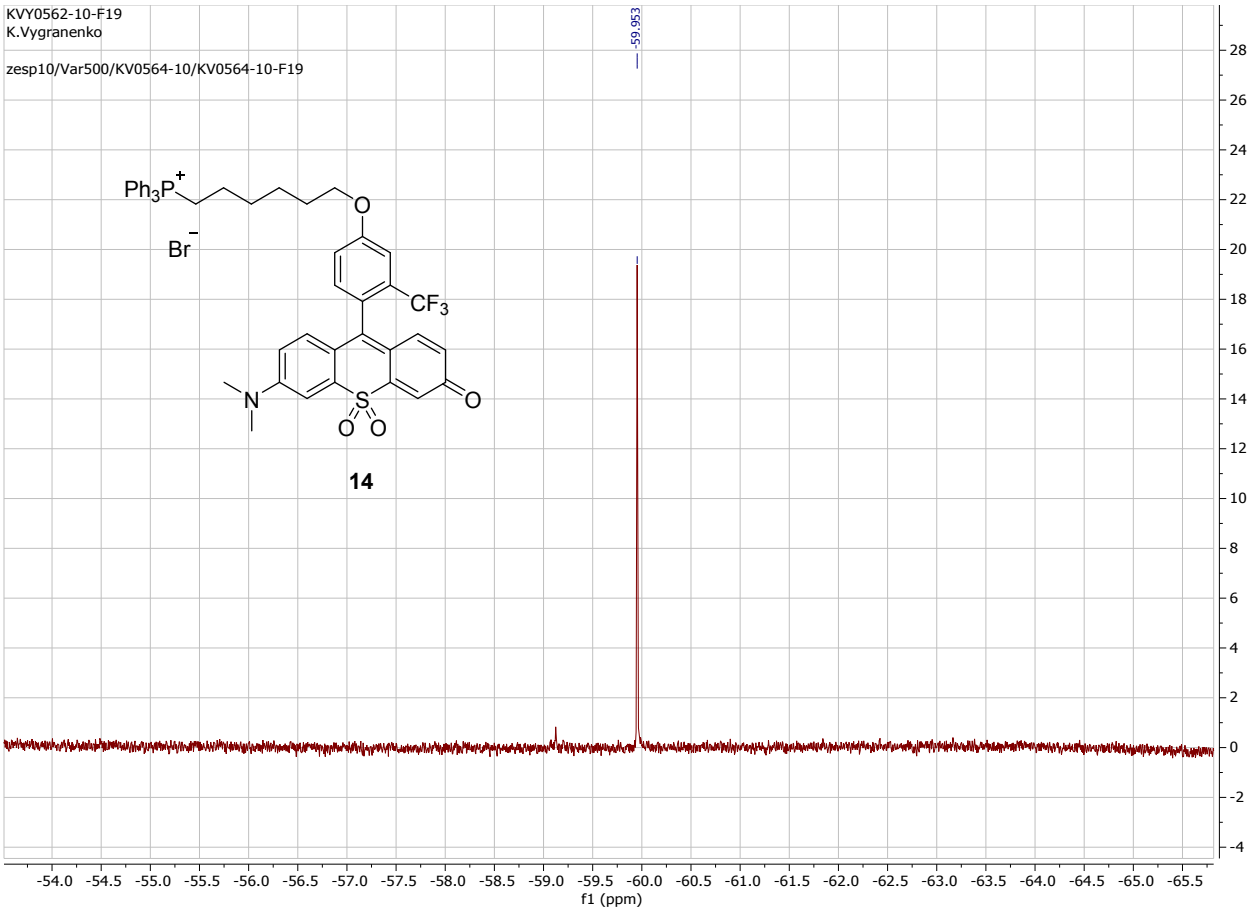
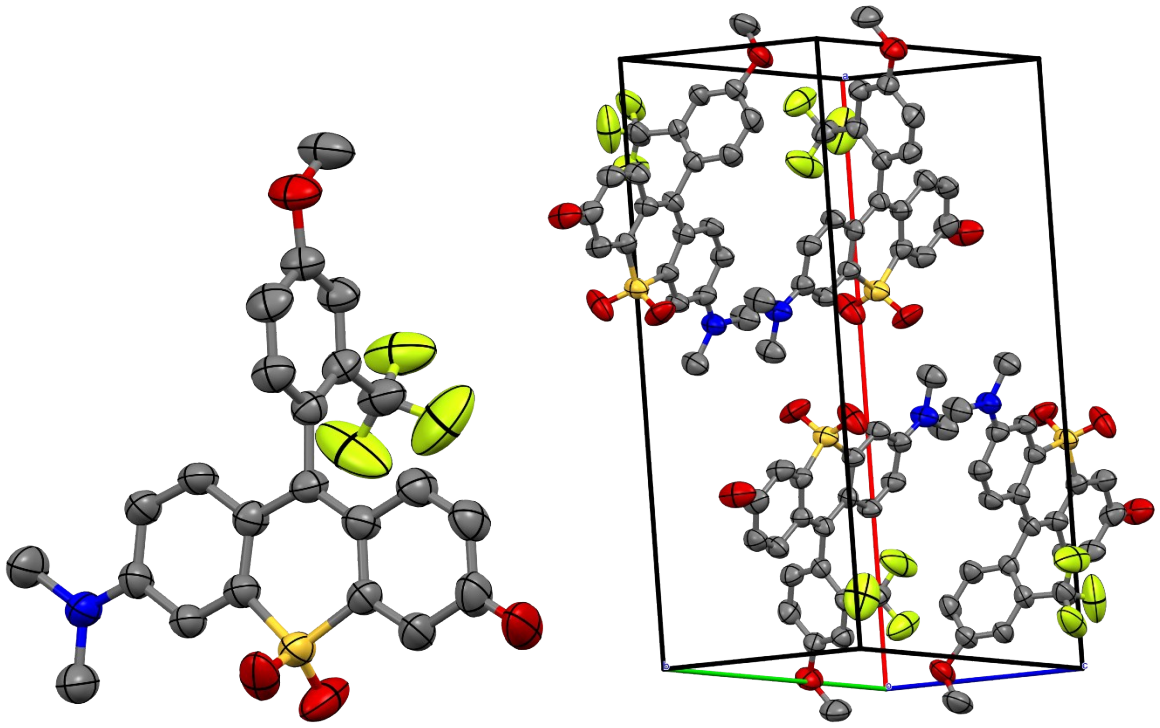
S
O
O
O
13
f1 (ppm)


KVY0561-10-C13
K.Vygranenko
zesp10/Var500/KVY0561-10/KVY0561-10-C13
Br
O
CF3
N
S
O
O
O
13
f1 (ppm)


zesp10/Var500/KVY0562-10/KVY0562-10-H1
Ph3P+
Br−
O
CF3
N
S
O
O
O
14
f1 (ppm)





(a) X-ray structure (b) Crystal packing

Figure S1. X-Ray structure of compound **11** (a) and crystal packing (b).

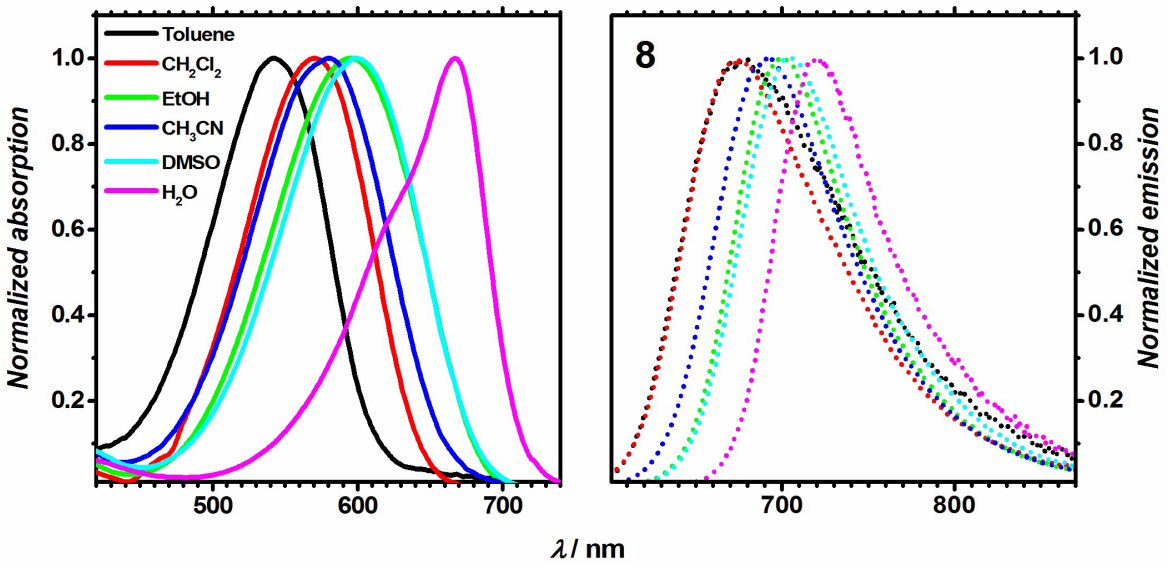


Figure S2. Absorption (solid) and emission (dotted) of compound **8** in toluene, CH_2Cl_2, EtOH, CH_3CN, DMSO, H_2O (containing 2% DMSO).

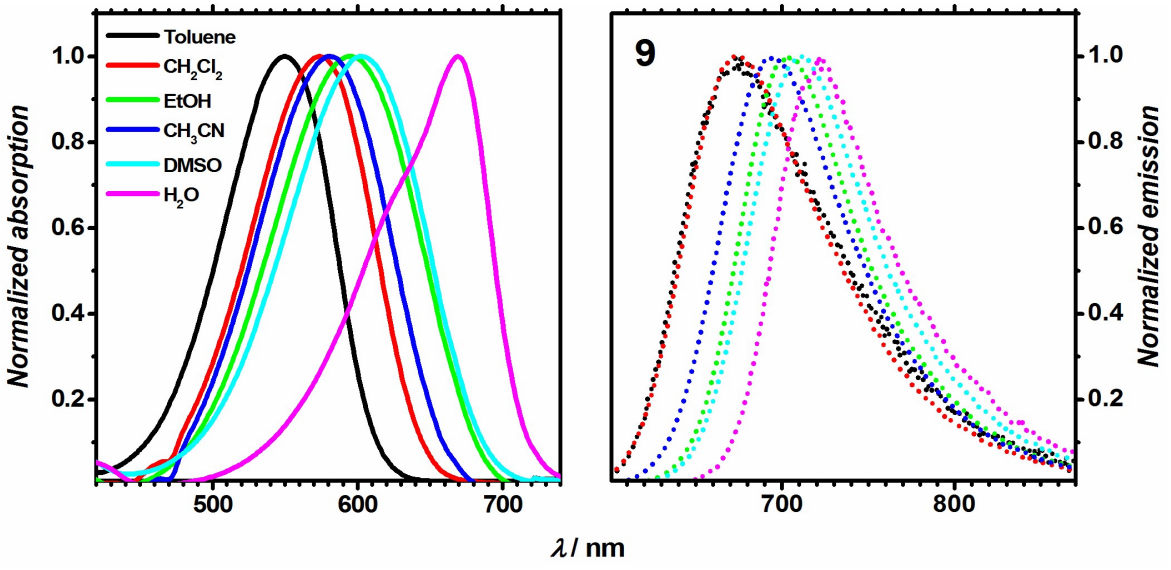


Figure S3. Absorption (solid) and emission (dotted) of compound **9** in toluene, CH_2Cl_2, EtOH, CH_3CN, DMSO, H_2O (containing 2% DMSO).

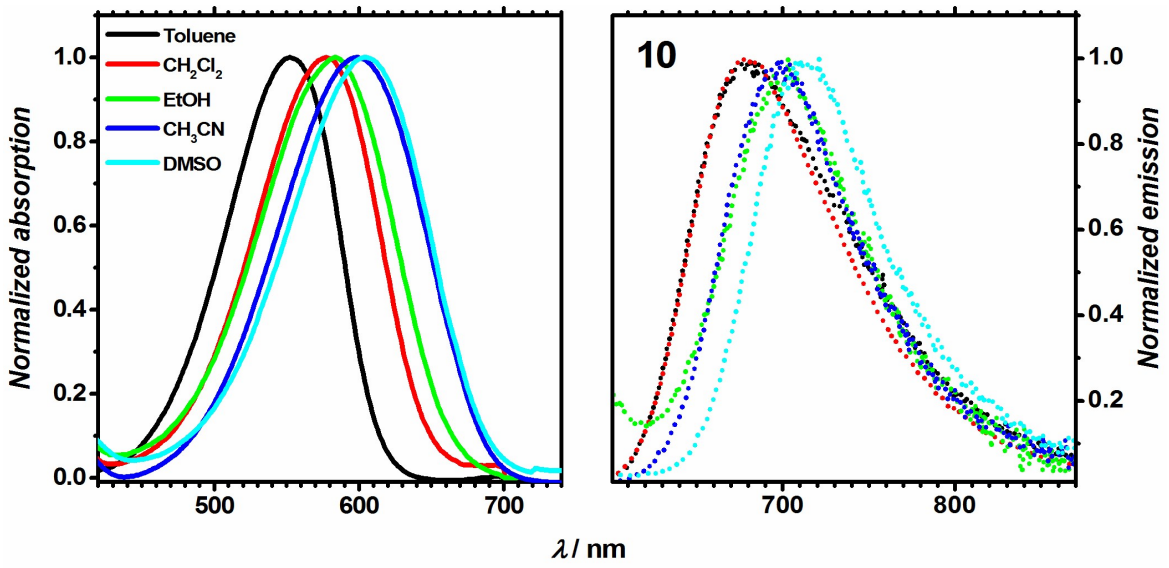


Figure S4. Absorption (solid) and emission (dotted) of compound **10** in toluene, CH_2Cl_2, EtOH, CH_3CN, DMSO, H_2O (containing 2% DMSO).

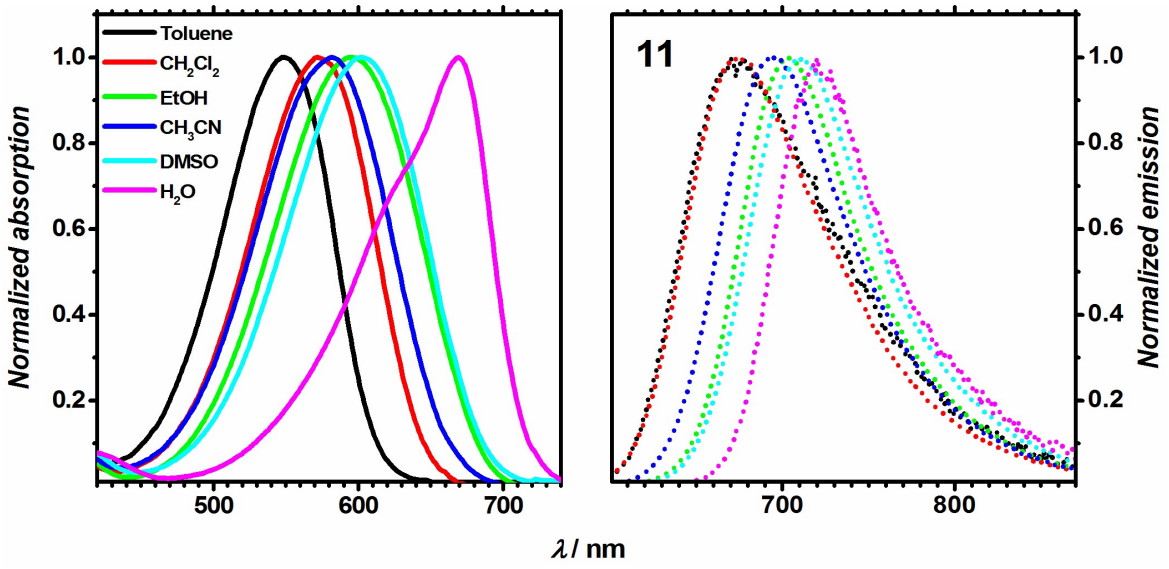


Figure S5. Absorption (solid) and emission (dotted) of compound **11** in toluene, CH_2Cl_2, EtOH, CH_3CN, DMSO, H_2O (containing 2% DMSO).

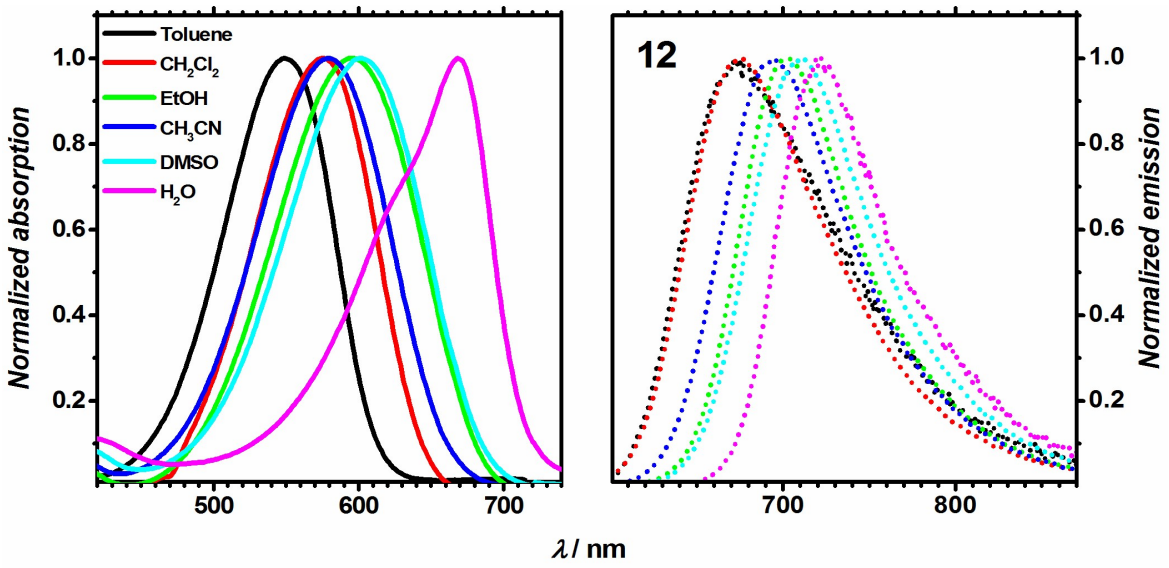


Figure S6. Absorption (solid) and emission (dotted) of compound **12** in toluene, CH_2Cl_2, EtOH, CH_3CN, DMSO, H_2O (containing 2% DMSO).

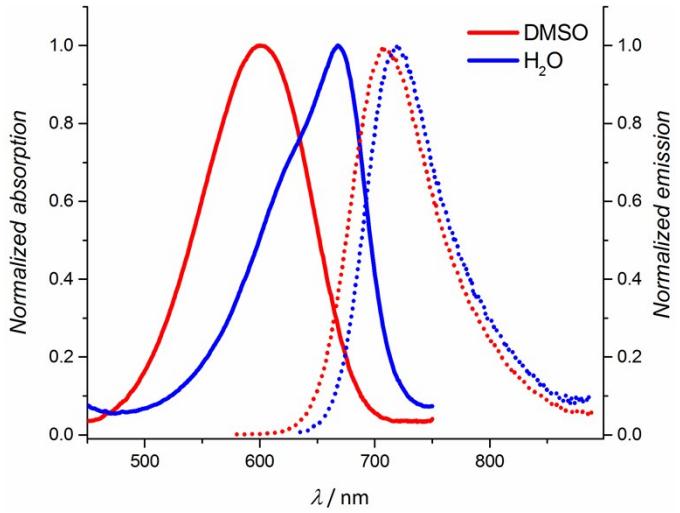


Figure S7. Absorption (solid) and emission (dotted) of compound **14** in DMSO and H_2O (2% of DMSO).

Time-resolved fluorescence data

Table S1. Fluorescence decay data for compounds **8** and **10**.[a]

Comp.	Solvent	λ_{exc} / nm	λ_{obs} / nm	A_1	A_2	τ_1 / ns	τ_2 [ns]	$k_r \cdot 10^{-8}$ / s^{-1}	$k_{nr} \cdot 10^{-8}$ / s^{-1}
8	CH_2Cl_2	336	670			4.20		1.02	1.36
	CH_3CN	336	700			5.24		0.99	0.92
	DMSO	336	700			4.33		1.15	1.16
10	CH_2Cl_2	336	670	38	62	0.46	2.06	2.83	18.9
	CH_3CN	336	700	100		0.32		0.25	31.0
	DMSO	336	700	94	6	0.32	6.13	0.31	31.1

[a]: The values of the radiative k_r and non-radiative k_{nr} rates are approximated on the basis of equations: $k_r = \Phi_f/\tau_1$ and $k_{nr} = 1/\tau_1 - k_r$

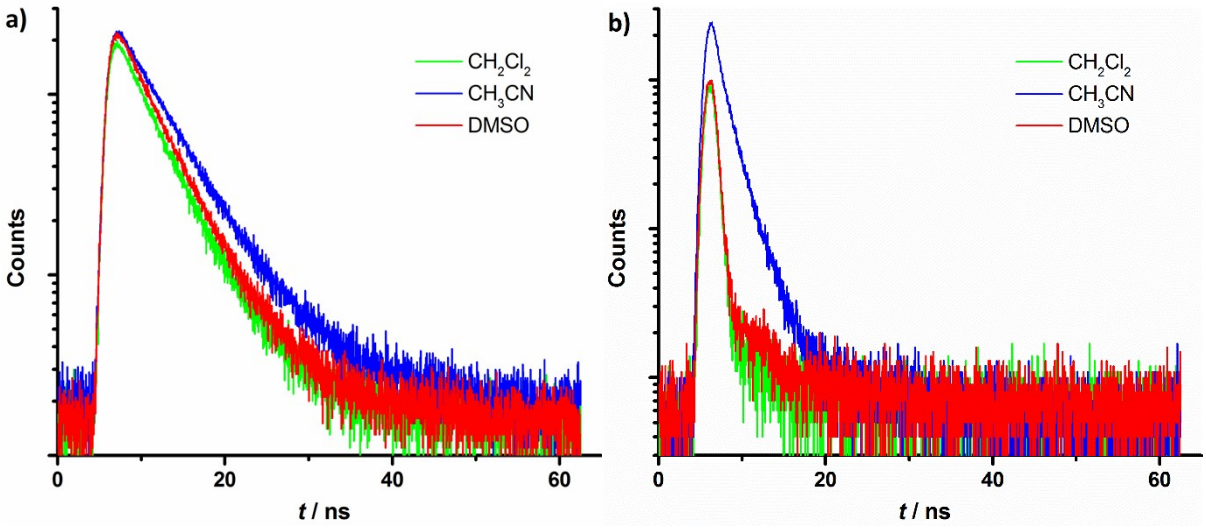
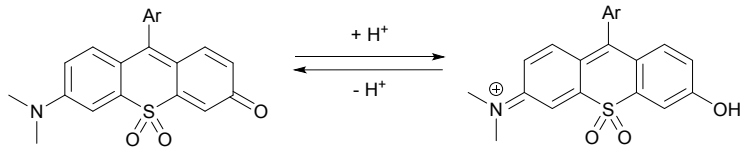


Figure S8. Fluorescence decay for **8** (a) and **10** (b) in CH_2Cl_2, CH_3CN and DMSO.

Absorption dependence on pH



Scheme S1. SO_2-rhodol protonation

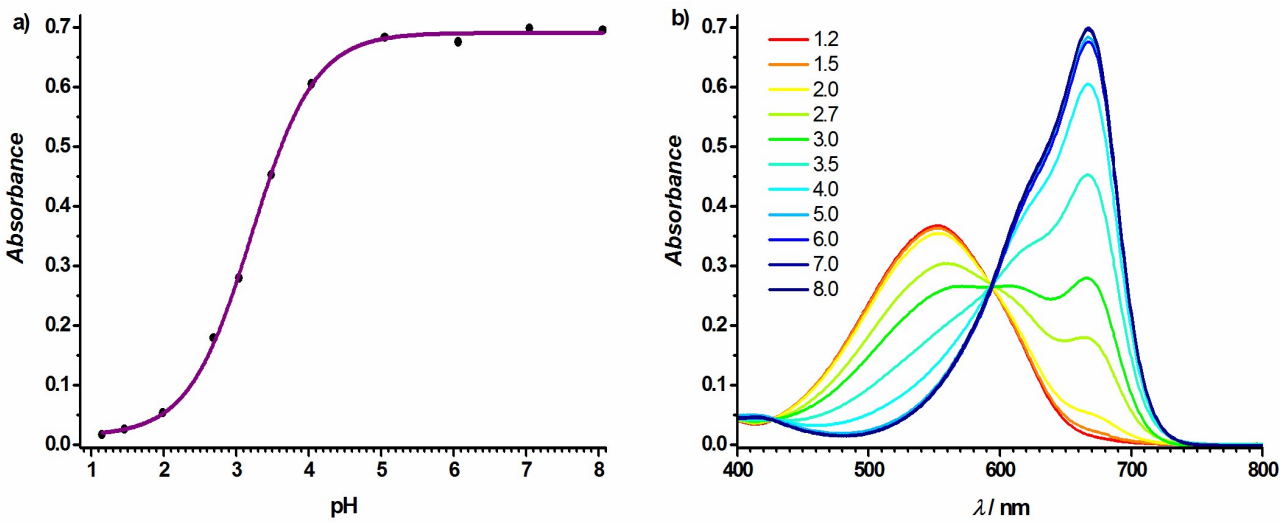


Figure S9. a) Absorption spectra of compound **8** at various pH values. b) The plots of absorbance of compound **8** at 667 nm as function of pH value and their fitting curve (pK_a = 3.21, r = 0.9994).

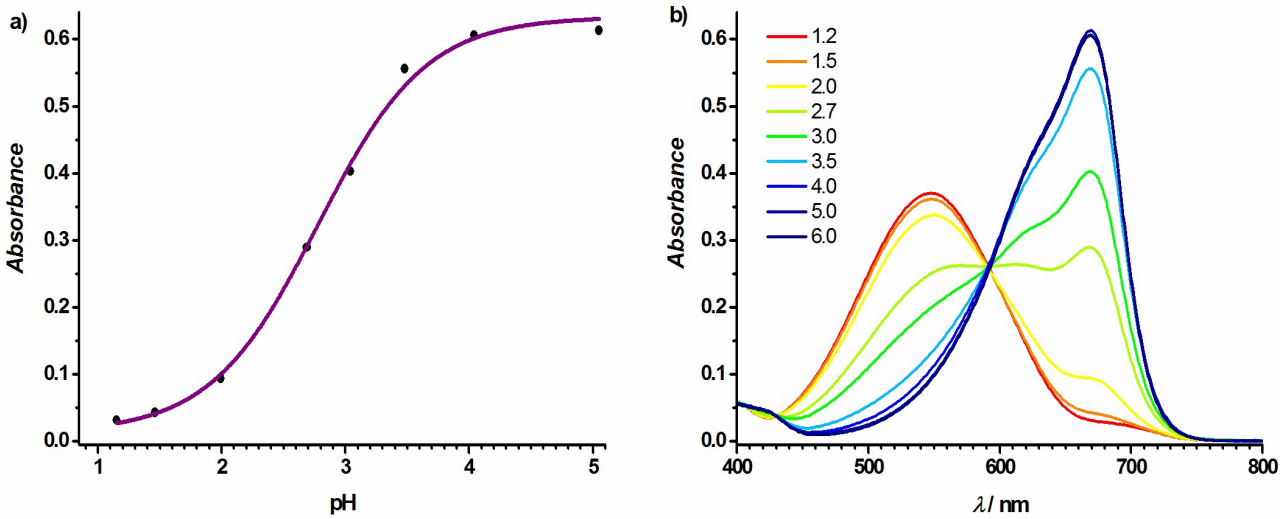


Figure S10. a) Absorption spectra of compound **9** at various pH values. b) The plots of absorbance of compound **9** at 669 nm as function of pH value and their fitting curve (pK_a = 2.78, r = 0.9964).

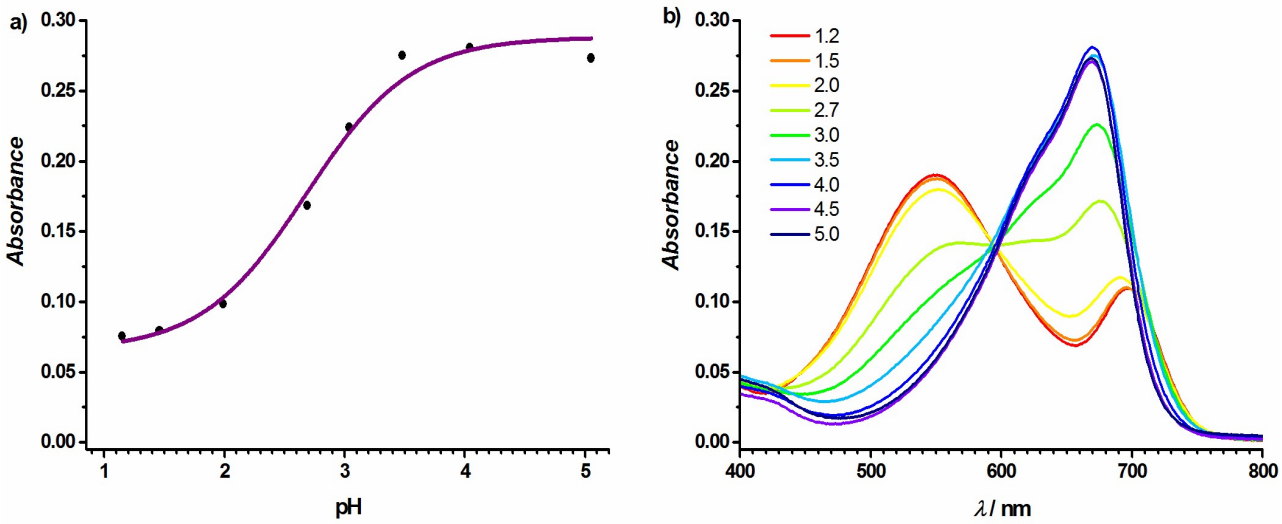


Figure S11. a) Absorption spectra of compound **11** at various pH values. b) The plots of absorbance of compound **11** at 669 nm as function of pH value and their fitting curve (pK_a = 2.68, r = 0.9841).

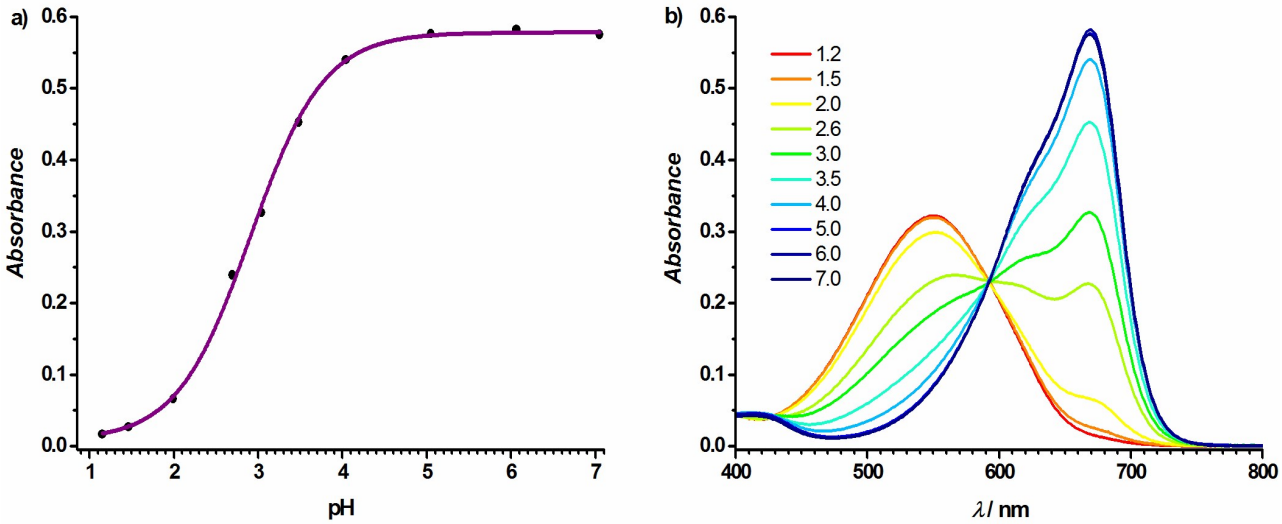


Figure S12. a) Absorption spectra of compound **12** at various pH values. b) The plots of absorbance of compound **12** at 669 nm as function of pH value and their fitting curve (pK_a = 2.91, r = 0.99894).

Compound **10** undergoes the formation of aggregates in DMSO-water media. The latter is not sensitive to a pH change.

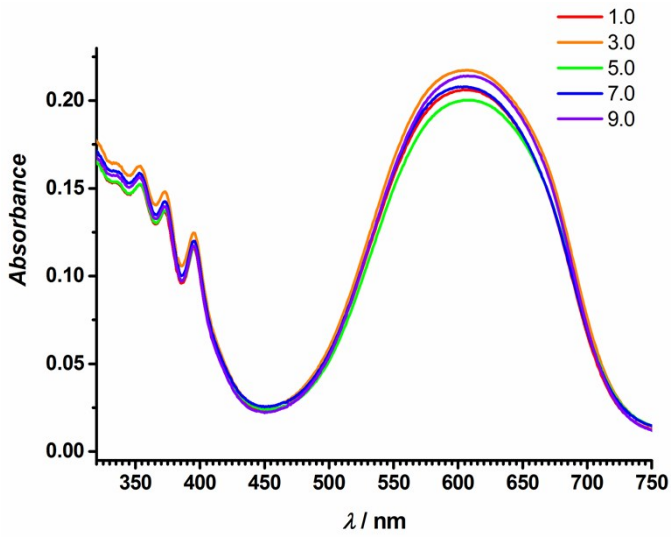


Figure S13. Absorption spectra of compound **10** at various pH values.

Photostability measurements

Photostability was determined through the variation in absorption of each sample at the appropriate absorption maximum wavelength (λ_{abs}) with respect to irradiation time. Ethanol was selected as the solvent. Concentrations giving similar optical densities (A ≈ 1) were used. Quartz cells of samples were irradiated with a 300 W Xe lamp (Asahi spectra MAX-350, light power: 0.16 W/cm2) for 150 min at 25 °C equipped with a UV/vis mirror module through a glass fiber. The absorption spectra were measured at appropriate times during the irradiation. Cresyl violet, Rhodamine 6G and DPP (2,5-dimethyl-3,6-bis(3,4-dimethoxyphenyl)pyrrolo[3,4-c]pyrrole1,4(2H,5H)-dione) were used as references.

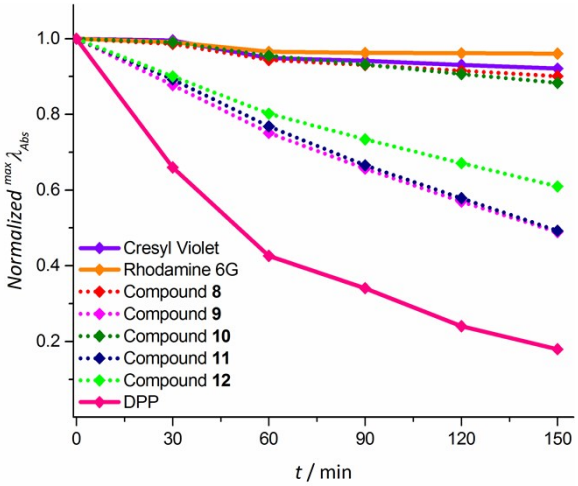


Figure S14. Photostability of sulfone-rhodols compared to the Rhodamine 6G, Cresyl Violet and DPP (2,5-dibutyl-3,6-bis(3,4-dimethoxyphenyl)pyrrolo[3,4-c]pyrrole-1,4(2H,5H)-dione) measured in EtOH using a collimated light source from a 300W Xe lamp.

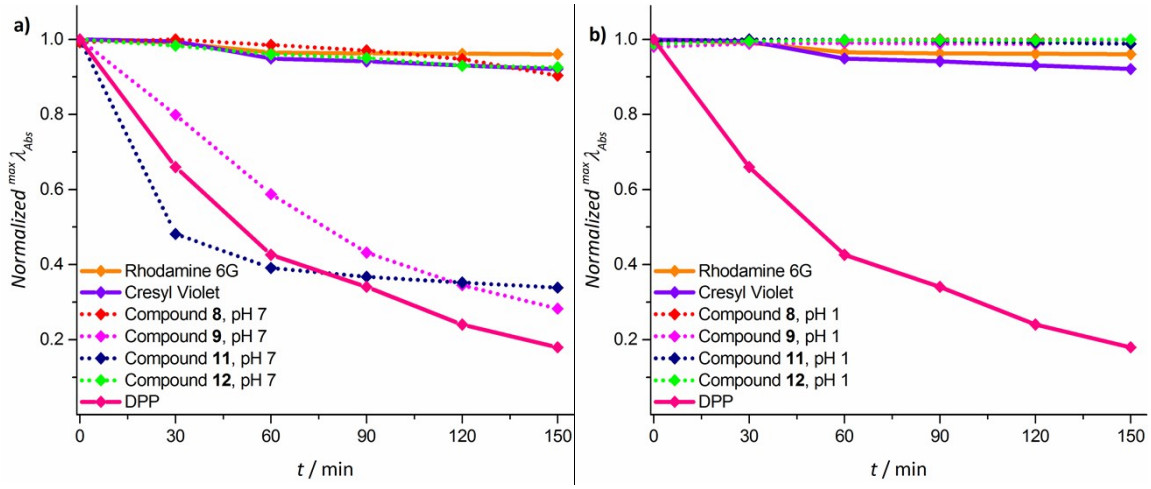


Figure S15. Photostability of sulfone-rhodols at pH 7 (a) and at pH1 (b) (in H_2O containing 2% DMSO) compared to the Rhodamine 6G, Cresyl Violet and DPP (2,5-dibutyl-3,6-bis(3,4-dimethoxyphenyl)pyrrolo[3,4-c]pyrrole-1,4(2H,5H)-dione) in EtOH measured using a collimated light source from a 300W Xe lamp.

Stability experiments were not performed for compound **10** as it precipitated in course of the stability test.

Cell culture conditions

The rat embryonic cardiomyoblast-derived cell line H9C2 were cultured at 37˚C in a humidified atmosphere containing 5% CO_2 in DMEM supplemented with 10% foetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 g/ml streptomycin.

Fluorescence localization of 14 within the cells

The H9C2 cells were loaded with fluorophores in DMEM medium supplemented with 10% foetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 g/ml streptomycin at 37˚C in a humidified atmosphere containing 5% CO_2 for 15-30 minutes with the **14** compound at the final concentration ranging from 200 to 500 nM. The final concentration of the MitoTracker™ Green FM was 150 nM. Both fluorophores were dissolved in DMSO and for the loading were supplemented with 20% Prluronic-127. The final concentration of the Pluronic-127 was kept below 0.05% in the loading buffer. Before measurements, the incubation medium was repleaced with FluoroBriteTM DMEM. The measurements were performed on Olympus IX83 confocal microscope with the water objective 60x UPLSAPO 60XW. The data were transferred to the ImageJ and prepared for presentation.

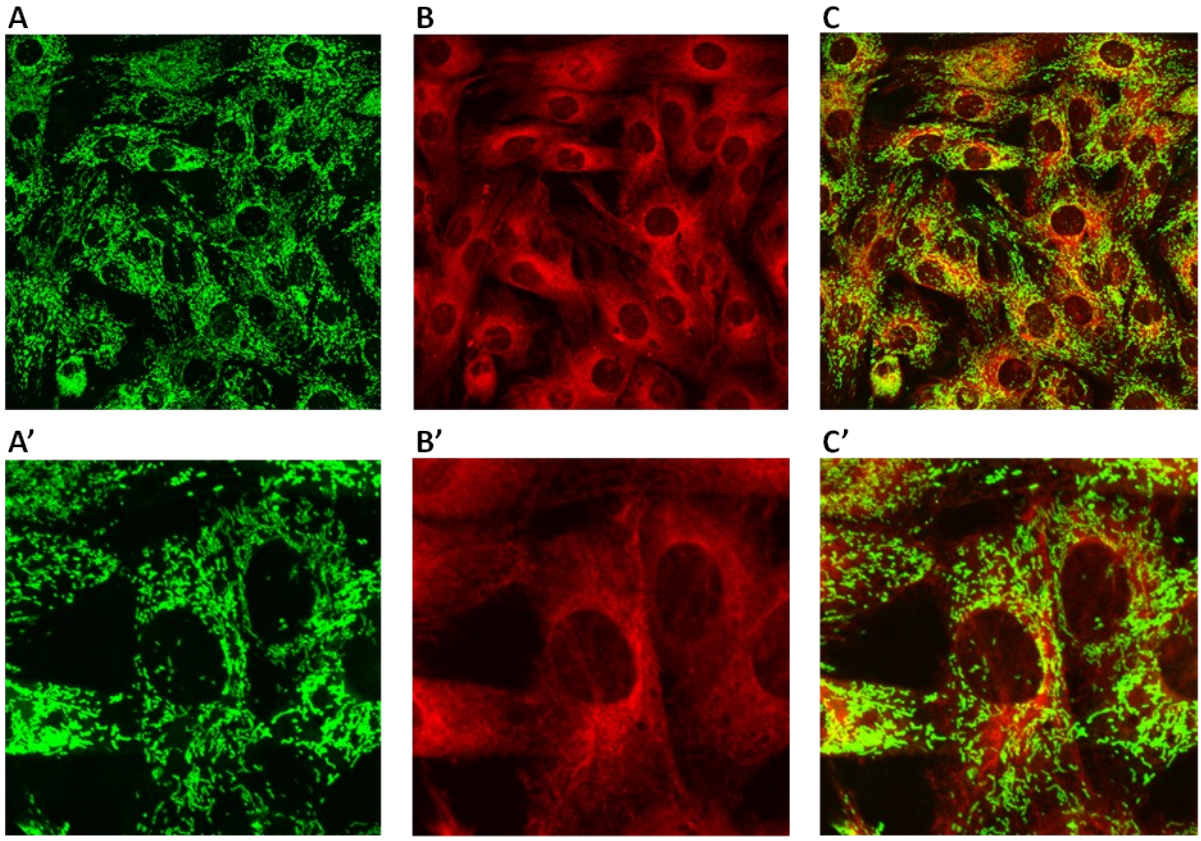


Figure S16. Intracellular localization of **13** compound as detected using confocal fluorescence microscopy. (A; A') The fluorescence of MitoTracker™ Green (green) as a well-established marker for

mitochondria, (B; B') the fluorescence of the **13** (red) recorded with 559 nm excitation wavelength and emission range 610–750 nm, (C; C') overlay picture recorded simultaneously for two fluorophore in living H9C2 cells line. A', B', C' pictures recorded for with higher magnification 3x.

Results

The dye **13** has a unlocalized distribution inside the H9C2 cells.

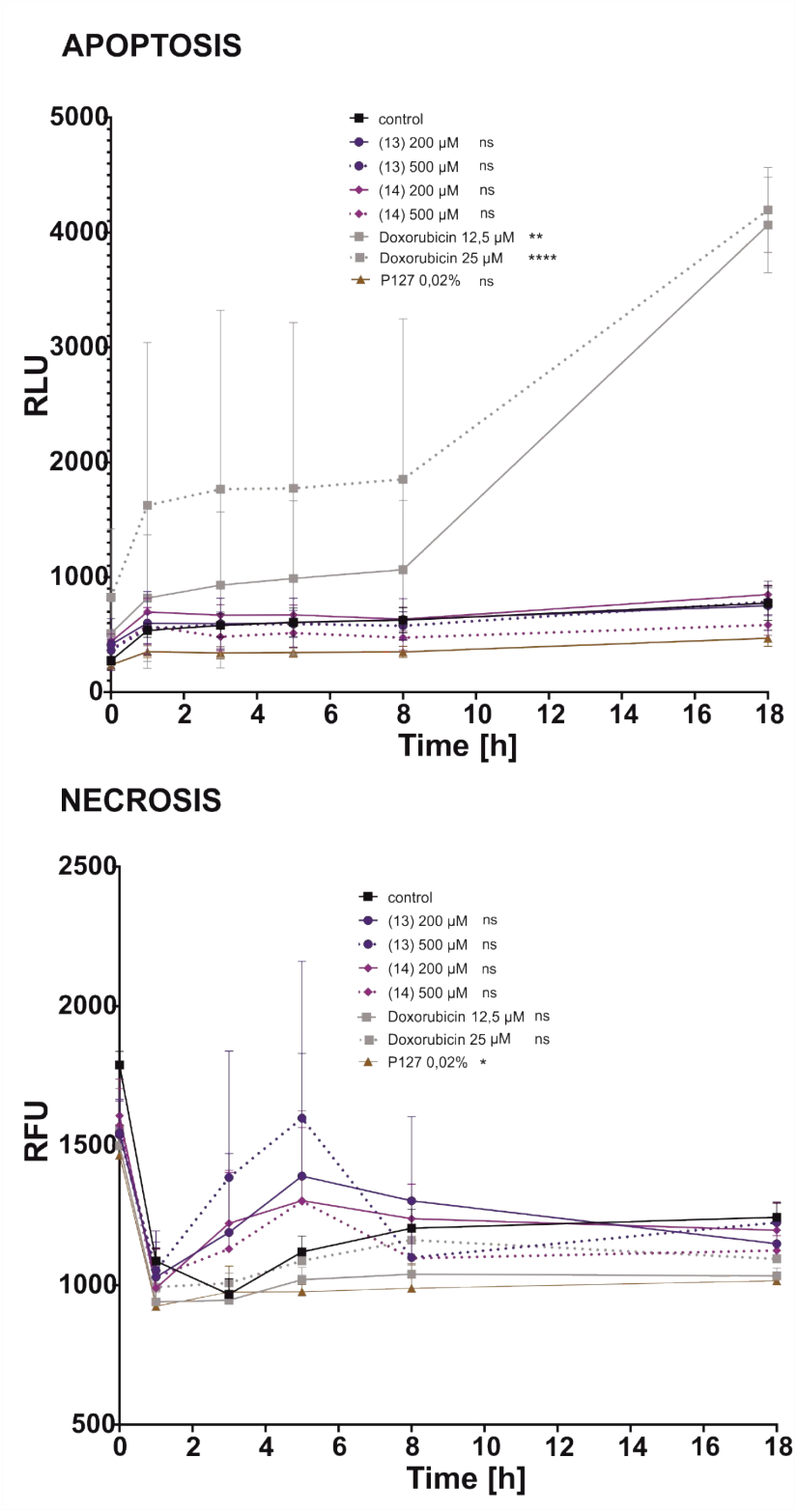


Figure S17. Effect of red emissive sulfonorhodols on apoptosis and necrosis of the H9C2 cells. Change in luminescence (RLU) as a measure of apoptosis and fluorescence (RFU) over the time. Statistical significance relative to the control was determined by two-way ANOVA with Tukey post-hoc test; $p>0.05$ (ns), $p<0.05$(*), $p<0.01$(**), $p<0.0001$(****); n=9

Methods

In order to determine the viability of cells under the influence of the tested red emissive sulfonorhodols **13** and **14**, an annexin V-based apoptosis and necrosis test (RealTime-Glo ™ Annexin V Apoptosis and Necrosis Assay, Promega) was performed, allowing the simultaneous examination of the effect of the substances on the induction of apoptotic and necrotic cell death.

Materials

RealTime-Glo ™ Annexin V Apoptosis and Necrosis Assay, Promega JA1011.