Supporting Information

Near-infrared electrochemiluminescence in water through regioselective sulfonation of diaza [4] and [6]helicene dyes

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S-I. Synthesis and characterizations

Reagents

Phosphate-buffered saline (PBS) solution was prepared by dissolving NaCl (8.00 g, 137.0 mmol), KCl (0.20 g, 2.7 mmol), Na_2HPO_4 (1.44 g, 10.0 mmol) and K_2HPO_4 (0.24 g, 1.8 mmol) in 1 L of deionized water. The pH value was 7.4. TPA was dissolved in PBS and the pH was adjusted to 7.4 with phosphoric acid.

Standard cationic [4]helicene and [6]helicene were prepared according to reported procedures.[1] Reagents were used as purchased, unless otherwise stated. Reactions were conducted under N_2 atmosphere using standard Schlenk technics, unless otherwise stated. Column chromatography were performed using Siliaflash P60 silicagel (40-63 μm, 60 Å).

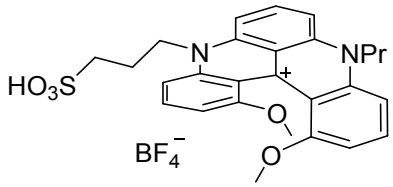
Analytical methods and apparatus

NMR spectra were recorded on Brucker Advance II+ AMX-500 and AMX-400 spectrometers at room temperature (otherwise noted). NMR chemical shifts are given in ppm (δ) relative to Me_4Si with solvent resonances used as internal standards (CD_2Cl_2: 5.32 ppm for ^1H and 53.84 for ^{13}C; CD_3OD: 3.31 ppm for ^1H and 49.0 for ^{13}C. **Melting points** (M.P.) were measured in open capillary tubes with a Buchi B-550 melting points apparatus and are uncorrected. **IR spectra** were recorded with a Perkin-Elmer 1650. FT-IR spectrometer using a diamond ATR Golden Gate sampling. **Electrospray mass spectra** were obtained on a Finnigan SSQ 7000 spectrometer QSTAR pulsar *i* (AB / MDS Sciex), ESI (TIS)/nanoESI/APCI-QqTof or on a Xevo G2 Tof (TOF), ESI (positive polarity) by the Department of Mass Spectroscopy of the University of Geneva. **UV-Vis-NIR absorption spectra** were recorded on a JASCO V-650 spectrophotometer at 20°C. **Steady-state fluorescence spectra** were measured using a Varian Cary 50 Eclipse spectrofluorimeter and were corrected for the wavelength-dependent sensitivity of the detection. Fluorescence quantum yields Φ were measured in diluted solution with an optical density lower than 0.1 using the following equation:

$$\frac{\Phi_x}{\Phi_r} = \left(\frac{A_r(\lambda)}{A_x(\lambda)}\right)\left(\frac{n_x^2}{n_r^2}\right)\left(\frac{D_x}{D_r}\right)$$

where A is the absorbance at the excitation wavelength (λ), n the refractive index and D the integrated intensity. “r” and “x” stand for reference and sample. The fluorescence quantum yields were measured relative to oxazine 725 in ethanol (Φ = 0.11). Excitations of reference and sample compounds were performed at the same wavelength.

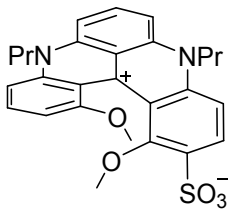
Compound [4]-C3-Sulfo



1,13-dimethoxy-5-propyl-5,9-dihydroquinolino[2,3,4-kl]acridin-13b-ylium tetrafluoroborate[2] (50 mg, 0.11 mmol, 1 equiv) was dissolved in 2 mL of acetonitrile. 1,3-propanesultone (133 mg, 1.09 mmol, 10 equiv) and DIPEA (0.27 mL, 1.64 mmol, 15 equiv) were added and the mixture was refluxed overnight. The solvent was then evaporated, the crude residue was dissolved in DCM and washed with an aqueous diluted solution of HBF_4. The organic layer was dried over Na_2SO_4, filtered and concentrated. Purification by silicagel column chromatography using DCM/acetone (80/20), then DCM/methanol (90/10, R_f = 0.40) afforded the pure product as a green solid (59 mg, 94% yield).

M.P.: 265°C (decomposition). **^{1}H NMR (CD_2Cl_2, 500 MHz):** δ = 8.24 (t, ^3J = 9 Hz, 1H, CH_{Ar}), 7.96 – 7.90 (m, 2H, CH_{Ar}), 7.89 – 7.83 (m, 1H, CH_{Ar}), 7.74 (d, ^3J = 9 Hz, 1H, CH_{Ar}), 7.40 (d, ^3J = 9 Hz, 1H, CH_{Ar}), 7.32 (d, ^3J = 9 Hz, 1H, CH_{Ar}), 6.85 – 6.79 (m, 2H, CH_{Ar}), 5.01 (m, 2H, CH_2), 4.58 (m, 1H), 4.36 (m, 1H, CH_2), 3.74 (s, 6H, OCH_3), 3.05 (t, ^3J = 6 Hz, 2H, CH_2), 2.52 (m, 2H, CH_2), 2.15 (m, 2H, CH_2), 1.24 (t, ^3J = 7 Hz, 3H, CH_3). **^{13}C NMR (CD_2Cl_2, 126 MHz):** δ = 160.1 (C_{quat}), 159.8 (C_{quat}), 143.0 (C_{quat}), 142.7 (C_{quat}), 142.4 (C_{quat}), 139.4 (C_{quat}), 139.0 (C_{quat}), 137.7 (CH), 137.2 (CH), 137.0 (CH), 119.7 (C_{quat}), 113.6 (C_{quat}), 108.5 (CH), 107.4 (CH), 106.2 (CH), 104.6 (CH), 103.0 (CH), 102.9 (CH), 55.9 (OCH_3), 52.0 (CH_2), 50.0 (CH_2), 48.2 (CH_2), 22.9 (CH_2), 20.0 (CH_2), 11.3 (CH_3). **^{19}F NMR (CD_2Cl_2, 282 MHz):** δ = 152.4. **UV-vis:** λ_{max} (CH_3OH) = 617 nm (ε = 14,600 $L.mol^{-1}.cm^{-1}$). **IR (neat, cm^{-1}):** ν = 3444, 3099, 2938, 1602, 1578, 1522, 1494, 1470, 1343, 1251, 1166, 1136, 1093, 1032, 946, 868, 813, 760, 716, 644, 619. **HRMS (ESI)** calculated for [M+]: 493.1792 ($C_{27}H_{29}N_2O_5S^+$), Found 493.1769.

Compound [4]-monoSulfo

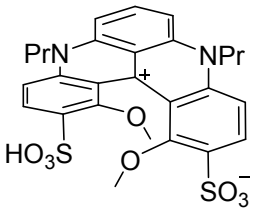


1,13-dimethoxy-5,9-dipropyl-5,9-dihydro-13bH-quinolino[2,3,4-kl]acridin-13b-ylium tetrafluoroborate (50 mg, 0.1 mmol, 1 equiv) was dissolved in 4 mL of polyphosphoric acid. Concentrated H_2SO_4 (96%, 0.1 mL) was then added and the reaction mixture warmed to 60 °C for 1

hour under mechanical stirring. The reaction mixture was then cooled to room temperature and hydrolyzed by addition of 5 mL of water. The crude mixture was then extracted with DCM and the organic phase washed with water (3x10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification over silica gel using DCM/MeOH (95/5, Rf= 0.24) as eluent afforded the pure compound as a green solid (28 mg, 57% yield).

M. P.: 98 °C (decomposition) **^{1}H NMR (CD_3OD, 500 MHz):** δ = 8.43 (d, *J* = 9.3 Hz, 1H), 8.33 (t, *J* = 8.5 Hz, 1H), 8.01 (dd, *J* = 8.9, 8.1 Hz, 1H), 7.80 (d, *J* = 9.3 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.71 (d, *J* = 8.5 Hz, 1H), 7.61 (d, *J* = 8.9 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 1H), 4.85 – 4.73 (m, 2H), 4.68 – 4.59 (m, 1H), 4.59 – 4.49 (m, 1H), 3.84 (s, 3H), 3.03 (s, 3H), 2.18 – 2.07 (m, 4H), 1.26 – 1.20 (m, 6H). **^{13}C NMR (CD_3OD, 126 MHz):** δ = 161.9 (C_{quat}), 159.7 (C_{quat}), 144.4 (C_{quat}), 143.9 (C_{quat}), 143.6 (C_{quat}), 140.3 (C_{quat}), 140.2 (C_{quat}), 139.3 (CH_{Ar}), 138.3 (CH_{Ar}), 136.8 (CH_{Ar}), 133.5 (C_{quat}), 120.7 (C_{quat}), 116.3 (C_{quat}), 113.3 (C_{quat}), 111.9 (CH_{Ar}), 108.0 (CH_{Ar}), 106.8 (CH_{Ar}), 106.5 (CH_{Ar}), 104.8 (CH_{Ar}), 62.9 (OCH_3), 56.9 (OCH_3), 52.2 (NCH_2), 21.0 (CH_2), 20.9 (CH_2), 11.1 (2 CH_3). **UV-vis:** λ_{max} (CH_3CN) = 628 nm (ε = 12,550 $L.mol^{-1}.cm^{-1}$). **IR (neat, cm^{-1}):** ν = 3459, 2967, 1607, 1579, 1497, 1345, 1250, 1171, 1040. **HRMS (ESI+)** calculated for $[M+H]^+$: 493.1792 ($C_{27}H_{29}N_2O_5S$), Found 493.1785.

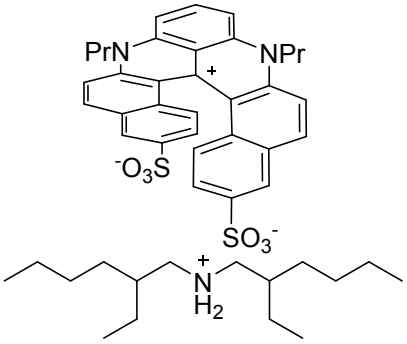
Compound [4]-diSulfo



1,13-dimethoxy-5,9-dipropyl-5,9-dihydro-13bH-quinolino[2,3,4-kl]acridin-13b-ylium tetrafluoroborate (100 mg, 0.2 mmol) was dissolved in 6 mL of polyphosphoric acid. Concentrated H_2SO_4 (96%, 0.5 mL) was then added and the reaction mixture warmed to 80 °C for 2 hours under mechanical stirring. The reaction mixture was then cooled to room temperature and hydrolyzed by addition of 8 mL of water. The crude mixture was then extracted with DCM until the organic phase became colorless. NaOH pellets were then carefully added to the aqueous phase at 0 °C until pH = 14. The aqueous phase was then concentrated under reduced pressure and the resulting salts were washed with acetone. The mother liquor was dried over Na_2SO_4, filtrated and concentrated. Purification over silica gel using DCM/MeOH (90/10) as eluent afforded the pure compound as a blue solid (59 mg, 61% yield).

M. P.: 100 °C (decomposition) **^{1}H NMR (CD_3OD, 500 MHz):** δ = 8.52 (d, *J* = 9.4 Hz, 2H), 8.44 (t, *J* = 8.6 Hz, 1H), 7.98 (d, *J* = 9.4 Hz, 2H), 7.86 (d, *J* = 8.6 Hz, 2H), 4.94 – 4.89 (m, 2H), 4.76 – 4.62 (m, 2H), 3.11 (s, 6H), 2.26 – 2.06 (m, 4H), 1.24 (t, *J* = 7.3 Hz, 6H). **^{13}C NMR (CD_3OD, 126 MHz):** δ = 163.0 (C_{quat}), 158.9 (C_{quat}), 145.0 (C_{quat}), 143.5 (C_{quat}), 140.3 (C_{quat}), 139.1 (C_{quat}), 137.2 (CH_{Ar}), 133.7 (CH_{Ar}), 121.7 (C_{quat}), 119.4 (C_{quat}), 117.1 (C_{quat}), 115.7 (C_{quat}), 112.8 (CH_{Ar}), 107.3 (CH_{Ar}), 63.4 (2 OCH_3), 52.6 (2 NCH_2), 21.0 (2 CH_2), 11.10 (2 CH_3). **UV-vis:** λ_{max} (CH_3CN) = 648 nm (ε = 9,880 $L.mol^{-1}.cm^{-1}$). **IR (neat, cm^{-1}):** ν = 3437, 1679, 1619, 1573, 1494, 1439, 1367, 1341, 1251, 1182, 1131, 1054, 975, 843, 802. **HRMS (ESI+)** calculated for $[M+H]^+$: 573.1360 ($C_{27}H_{29}N_2O_8S_2$), Found 573.1370.

Compound [6]-diSulfo



To a flask containing 7,11-dipropyl-7,11-dihydro-17cH-benzo[a]benzo[5,6]quinolino[2,3,4-kl]acridin-17c-ylium tetrafluoroborate (54 mg, 0.1 mmol) was added conc. H_2SO_4 (1 mL). The resulting solution was stirred at 20 °C for 48 hours until completion of the reaction as indicated by mass spectrometry. After this time, the reaction mixture was poured onto ice. Once the ice melted, the aqueous solution was washed with CH_2Cl_2 three times in order to remove traces of unreacted starting material and mono sulfonated material. Then, the aqueous solution was extracted once using a 1 M bis(2-ethylhexyl)amine solution in CH_2Cl_2 (the aqueous solution became colorless, the organic layer blue). The resulting organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was dissolved in CH_2Cl_2 and Et_2O (*ca.* 45 mL) was added, leading to the precipitation of the excess of ammonium salt as a white solid. The mother liquor was separated from the precipitate, evaporated and the precipitation was repeated five times in total. The residue was further purified by flash chromatography (SiO_2, 10 x 2 cm) with first a gradient of MeOH (up to 5%) in CH_2Cl_2 to remove impurities and then with a mixture of $CH_3CN/CH_3OH/CH_3COOH$ (89:10:01, R_f = 0.12) to afford the product as a blue solid (54 mg, 63% yield).

R_f ($CH_3CN/CH_3OH/CH_3COOH$ (89:10:01), SiO_2): 0.12. **M. P.:** >150 °C **^{1}H NMR (500 MHz, CD_3OD):** δ = 8.50 (d, *J* = 9.3 Hz, 2H), 8.35-8.31 (m, 3H), 8.25 (d, *J* = 9.5 Hz, 2H), 7.89 (d, *J* = 8.5 Hz, 2H), 7.32 (d, *J* = 8.8 Hz,

2H), 7.26 (dd, *J* = 8.9, 1.8 Hz, 2H), 5.02-4.96 (m, 2H), 4.71-4.66 (m, 2H), 2.92 (dd, *J* = 7.0, 2.6 Hz, 3H), 2.31 – 2.07 (m, 4H), 1.80 – 1.69 (m, 2H), 1.54 – 1.22 (m, 20H), 0.95-0.91 (m, 10H). **^{13}C NMR (126 MHz, CD_3OD):** δ = 145.5 (C), 144.3 (C), 143.2 (C), 140.0 (CH), 139.3 (C), 136.5 (CH), 131.1 (C), 130.1 (C), 127.2 (CH), 126.5 (CH), 123.5 (CH), 123.0 (C), 117.3 (CH), 117.2 (C), 108.2 (CH), 53.3 (CH_2), 53.3 (CH_2), 52.5 (CH_2), 37.6 (CH), 31.3 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 24.5 (CH_2), 24.4 (CH_2), 24.0 (CH_2), 21.5 (CH_2), 14.4 (CH_3), 11.2 (CH_3), 10.5 (CH_3), 10.5 (CH_3). **UV-vis:** λ_{max} (CH_3CN) = 616 nm (ε = 16,700 $L.mol^{-1}.cm^{-1}$). **IR (neat, cm^{-1}):** ν 3455, 2959, 2931, 2873, 1611, 1572, 1547, 1526, 1507, 1487, 1460, 1336, 1260, 1230, 1177, 1156, 1118, 1092, 1032, 997, 903, 853, 830, 755, 732, 697, 647, 629. **HRMS (ESI–)** calculated for $[M]^-$: 611.1305 ($C_{33}H_{27}N_2O_8S_2$), Found 611.1288; **(ESI+)** calculated for $[M+2H]^+$: 613.1462 ($C_{33}H_{29}N_2O_8S_2$), Found 613.1443; **(ESI+)** calculated for $[M]^+$: 242.2843 ($C_{16}H_{36}N$), Found 242.2867.

S-II. ^{1}H, ^{13}C and HRMS characterizations

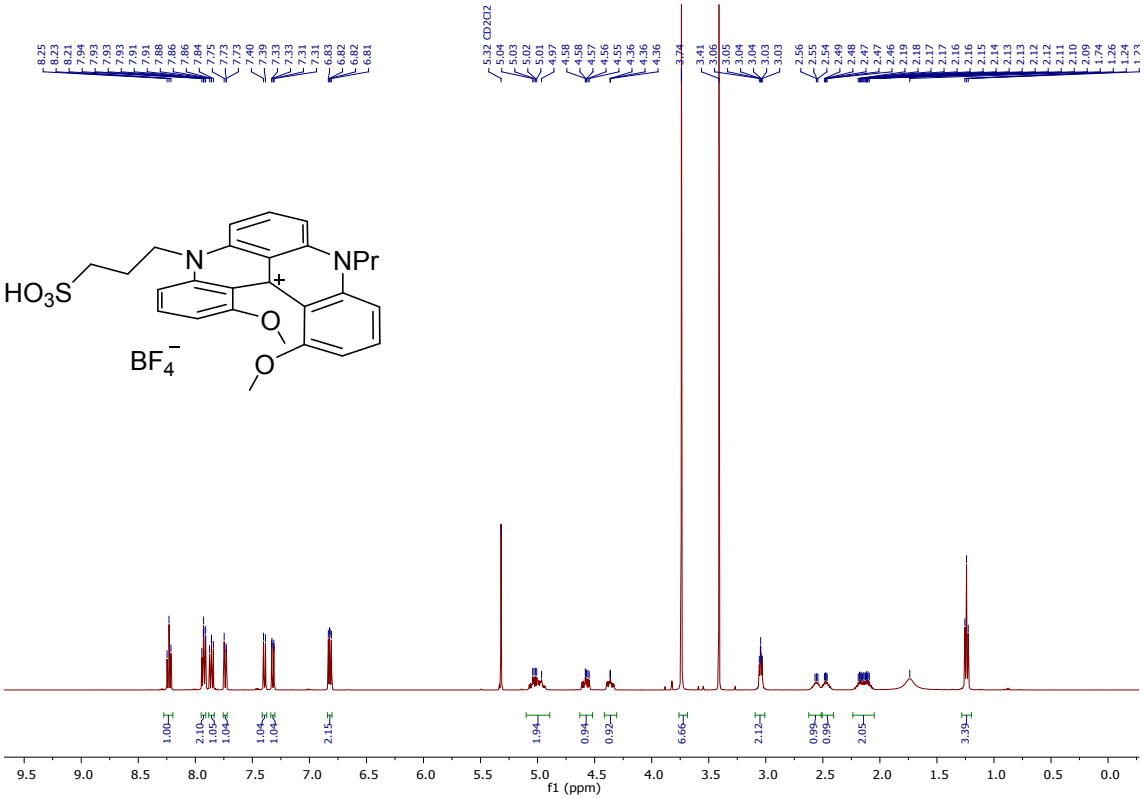


Figure S 1. ^{1}H NMR (CD_2Cl_2, 400 MHz) spectrum of compound **[4]-C3-Sulfo**.

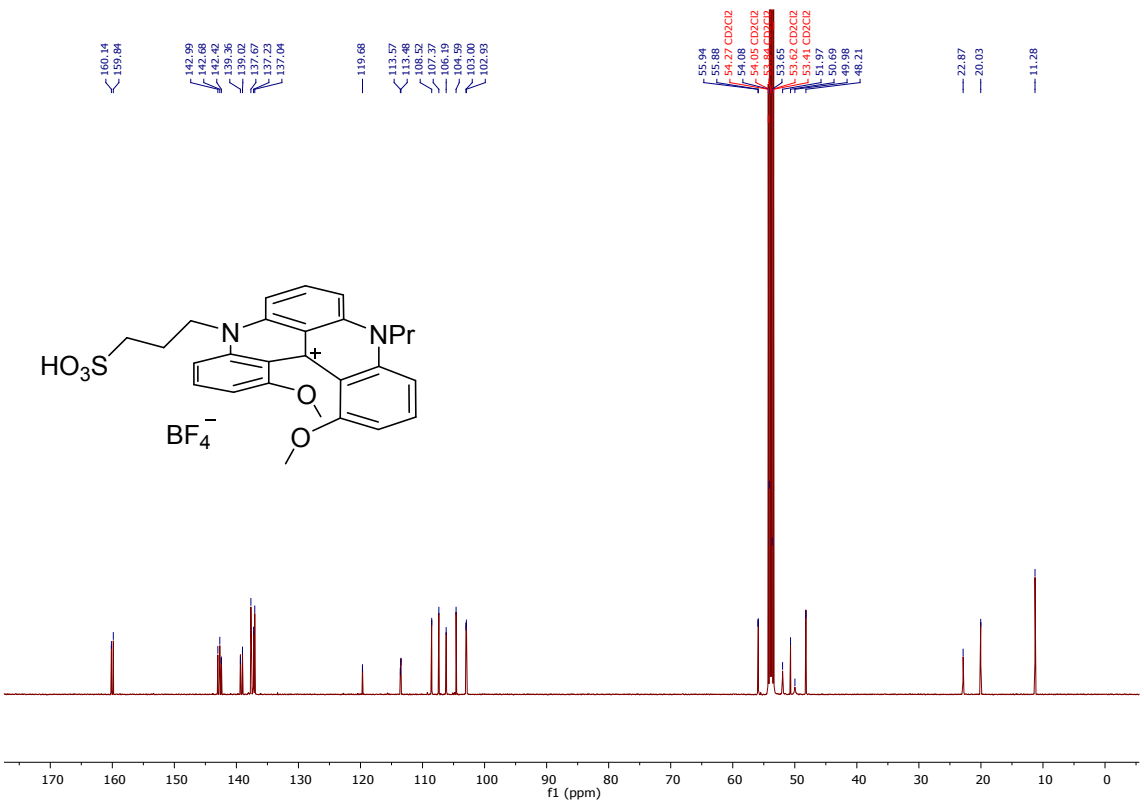


Figure S 2. ^{13}C NMR (CD_2Cl_2, 126 MHz) spectrum of compound **[4]-C3-Sulfo**.

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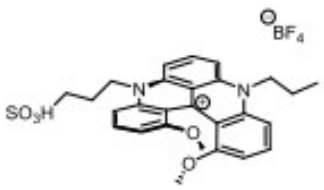
Submitter:	PASCAL	Date of reception:	12/12/14
Sample name:	SPA015	Date of certificate:	22/12/14
Sample number:	7605	Data filename:	MS03GE-141219-HT-A001
Operator:	Eliane Sandmeier	Instrument:	QSTAR XL (AB/MDS Sciex)
Principal investigator:	Dr. Sophie Michalet	Ionisation mode:	ESI (positive mode)

Expected Formula	Observed *m/z* $[M]^+$	Expected *m/z* (amu)	Accuracy (ppm)
$C_{27}H_{29}N_2O_5S$	493.1769	493.1792	-4.7



SPA015

Chemical Formula: $C_{27}H_{29}N_2O_5S^+$

Exact Mass: 493.1792

Molecular Weight: 493.5940

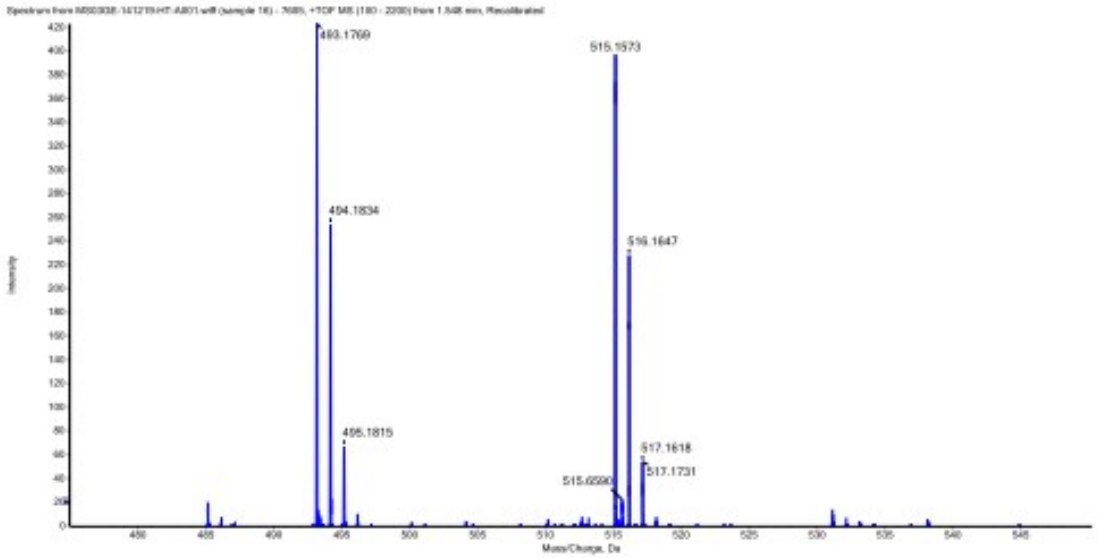


Figure S 3. HRMS spectrum of compound **[4]-C3-Sulfo**.

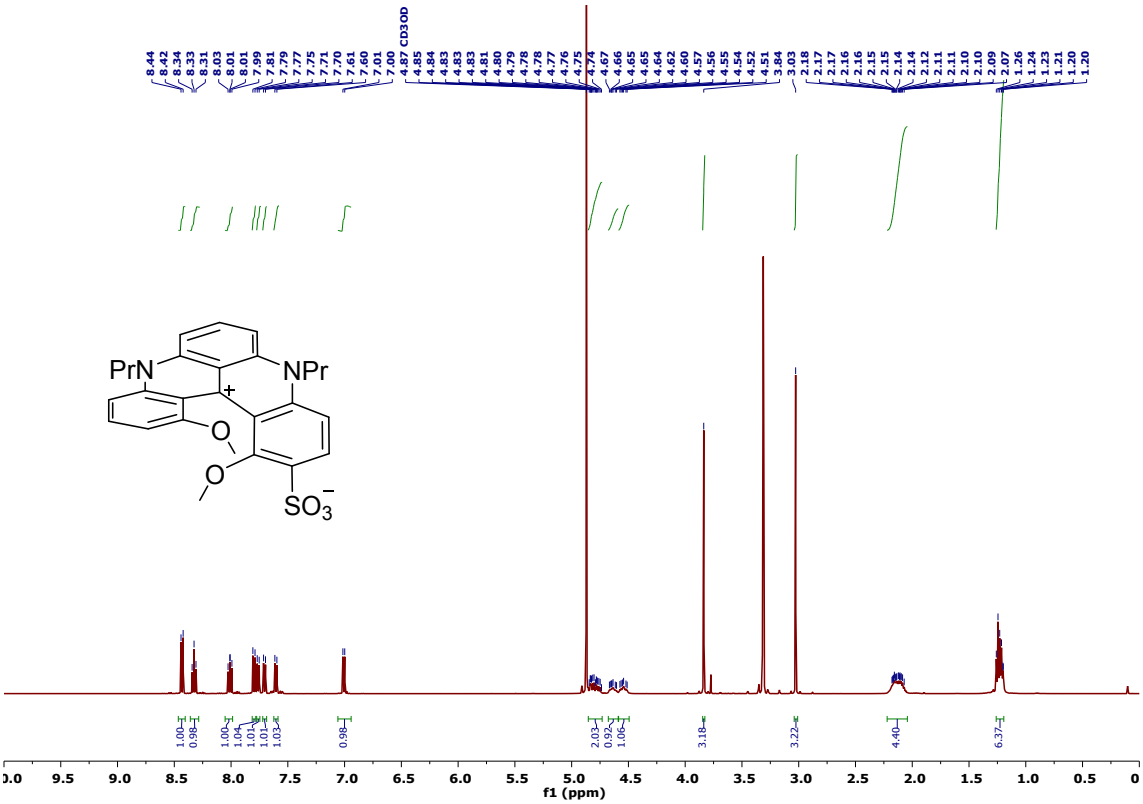


Figure S 4. ^{1}H NMR (CD_3OD, 400 MHz) spectrum of compound **[4]-monoSulfo**.

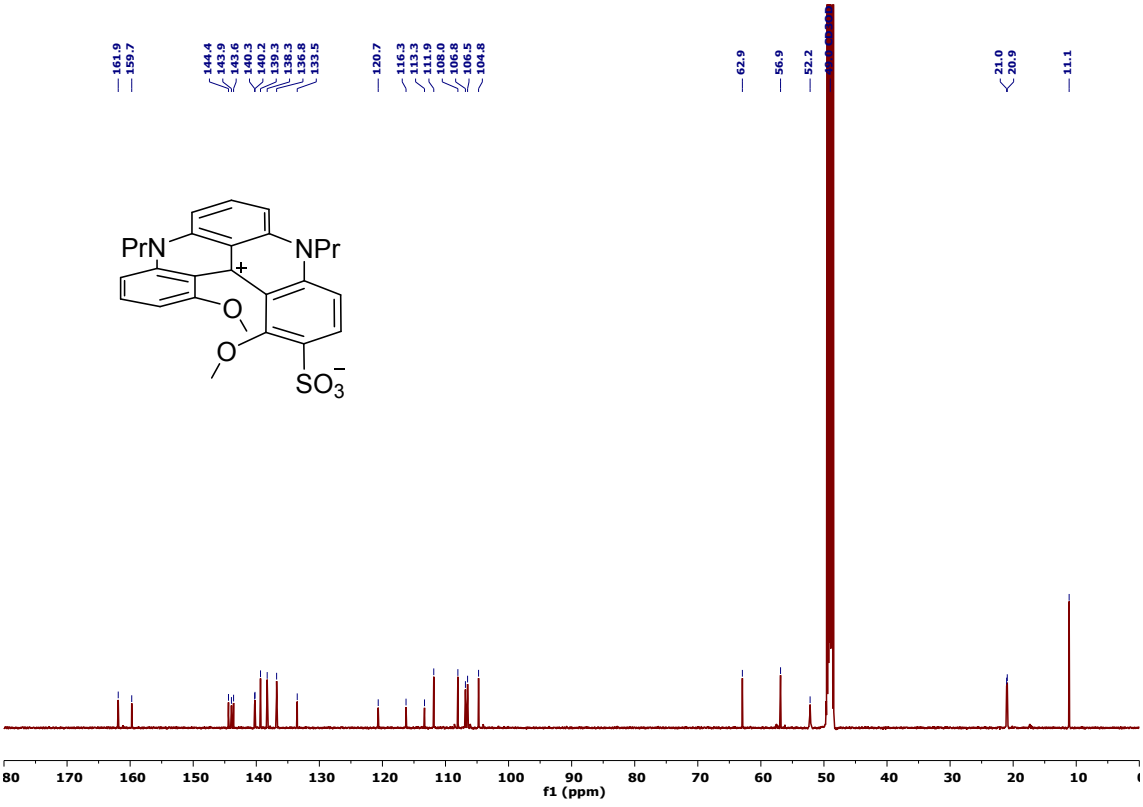


Figure S 5. ^{13}C NMR (CD_3OD, 126 MHz) spectrum of compound **[4]-monoSulfo**.

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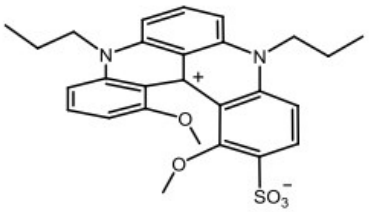
Submitter:	DUWALD	Date of reception:	18/10/2016
Sample name:	DUR307	Date of certificate:	24/10/2016
Sample number:	8503	Data filename:	SMS10GE-161020-ES-A005
Operator:	Eliane Sandmeier	Instrument:	QSTAR Pulsar (AB/MDS Sciex)
Principal investigator:	Dr. Sophie Michalet	Ionisation mode:	ESI (positive mode)

Expected Formula	Observed *m/z* $[M+H]^+$	Expected *m/z* (amu)	Accuracy (ppm)
$C_{27}H_{29}N_2O_5S$	493.1785	493.1792	-1.4



Chemical Formula: $C_{27}H_{28}N_2O_5S$
Exact Mass: 492.17

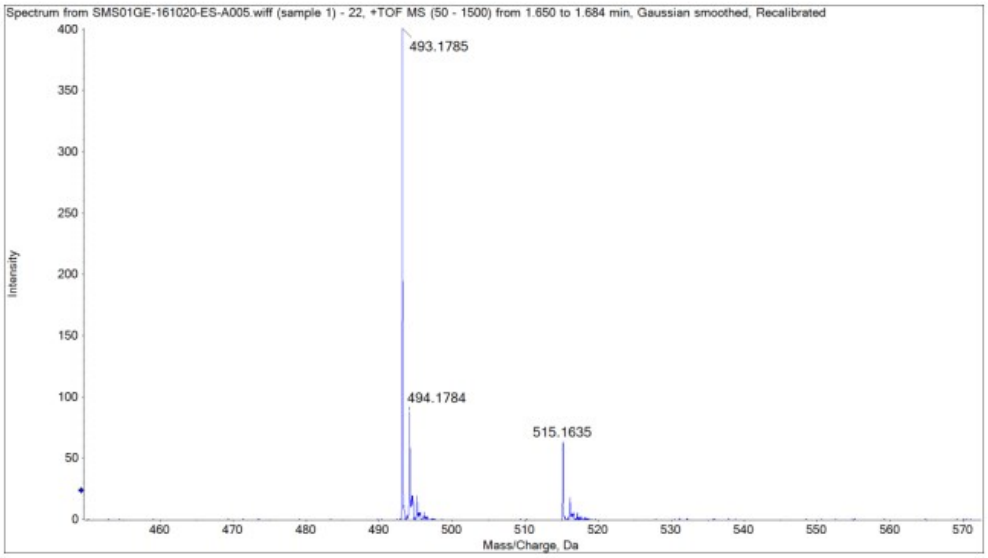


Figure S 6. HRMS spectrum of compound **[4]-monoSulfo**.

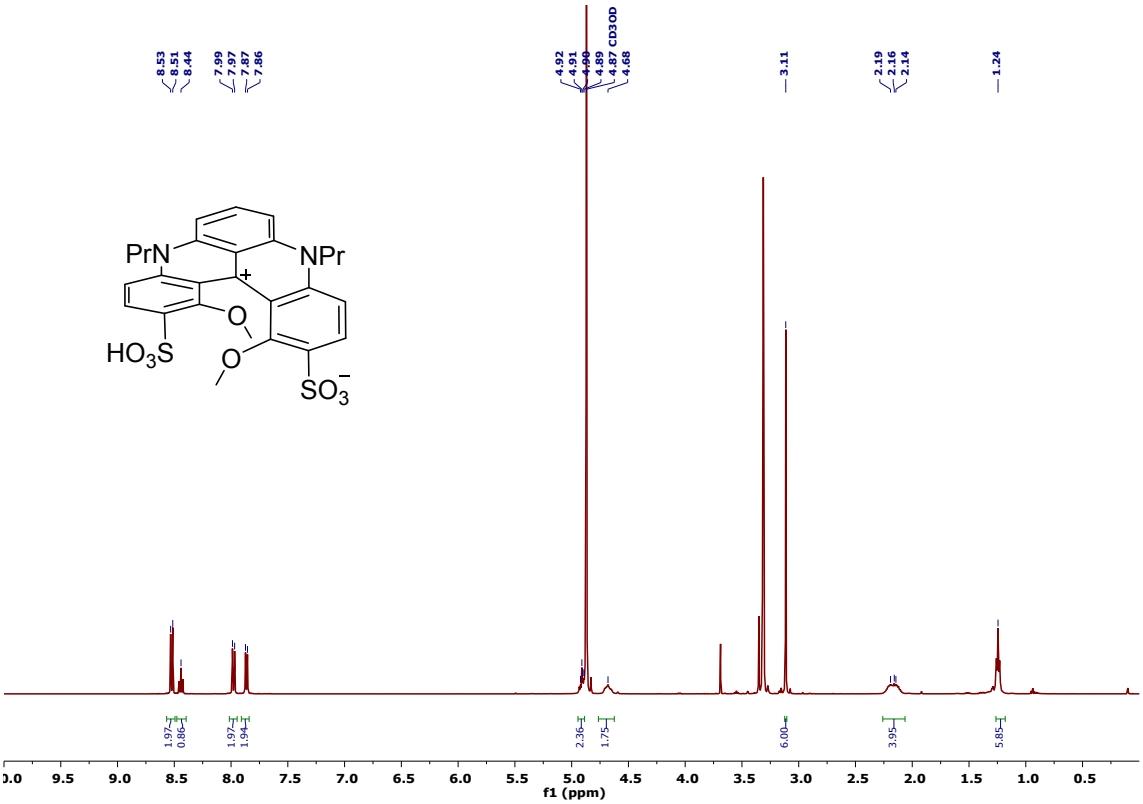


Figure S 7. ^{1}H NMR (CD_3OD, 400 MHz) spectrum of compound **[4]-diSulfo**.

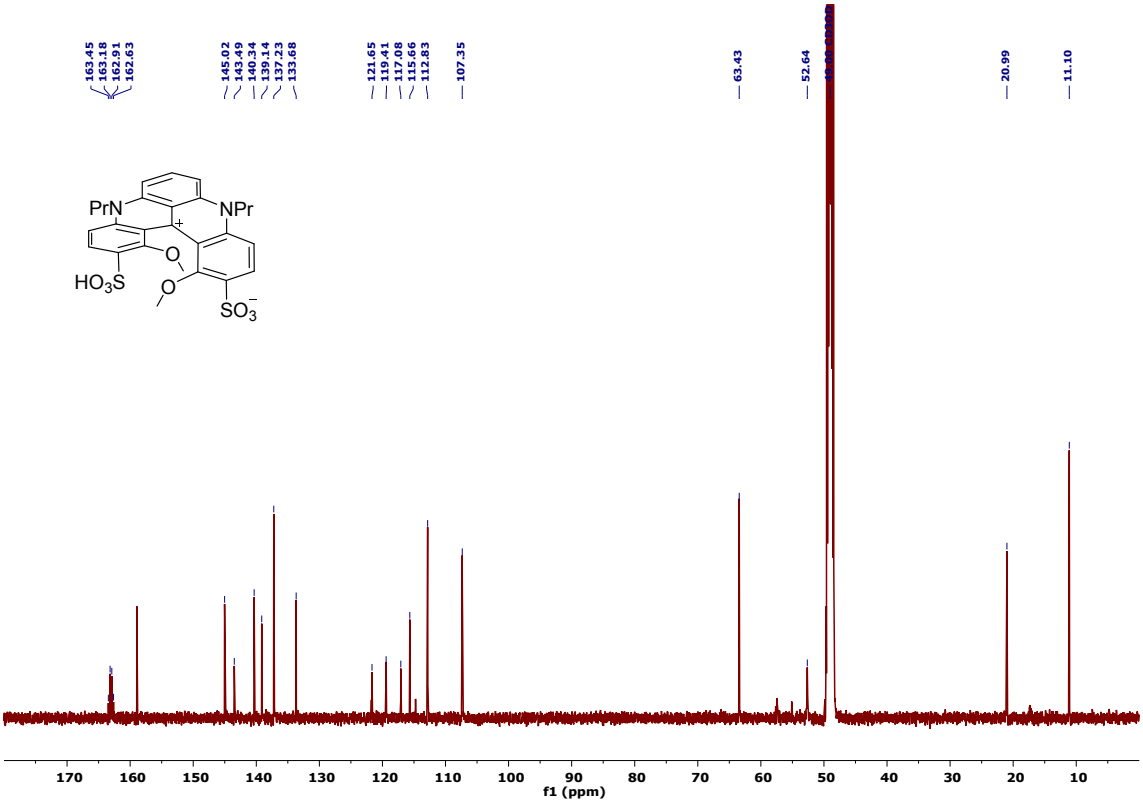


Figure S 8. ^{13}C NMR (CD_3OD, 126 MHz) spectrum of compound **[4]-diSulfo**.

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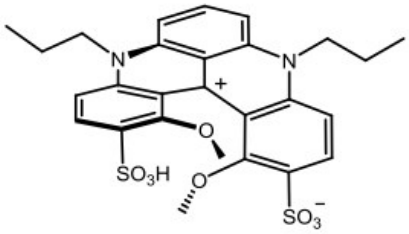
Submitter:	DUWALD	Date of reception:	18/10/2016
Sample name:	DUR310	Date of certificate:	24/10/2016
Sample number:	8504	Data filename:	SMS10GE-161020-ES-A006
Operator:	Eliane Sandmeier	Instrument:	QSTAR Pulsar (AB/MDS Sciex)
Principal investigator:	Dr. Sophie Michalet	Ionisation mode:	ESI (positive mode)

Expected Formula	Observed *m/z* $[M+H]^+$	Expected *m/z* (amu)	Accuracy (ppm)
$C_{27}H_{29}N_2O_8S_2$	573.1370	573.1360	1.8



Chemical Formula: $C_{27}H_{28}N_2O_8S_2$
Exact Mass: 572.13

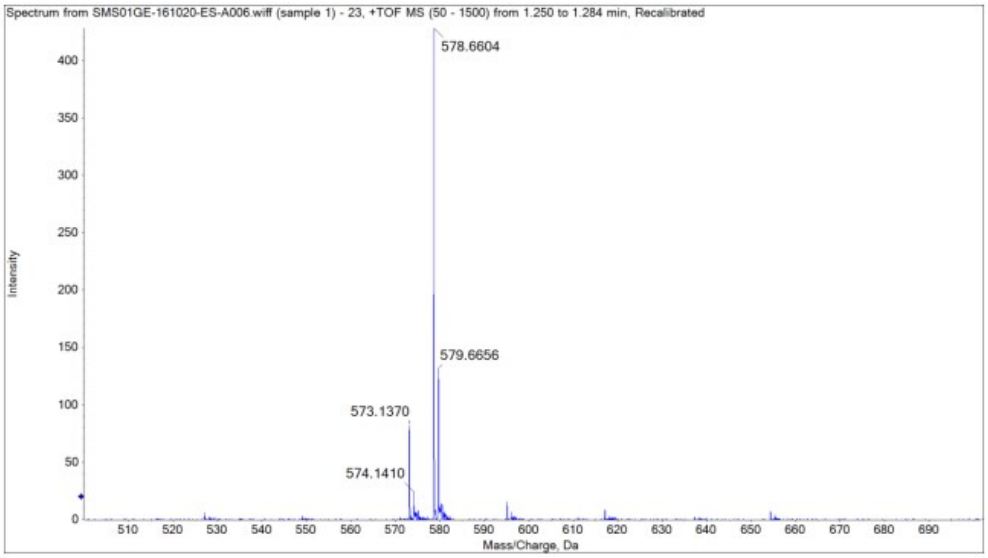


Figure S 9. HRMS spectrum of compound **[4]-diSulfo**.

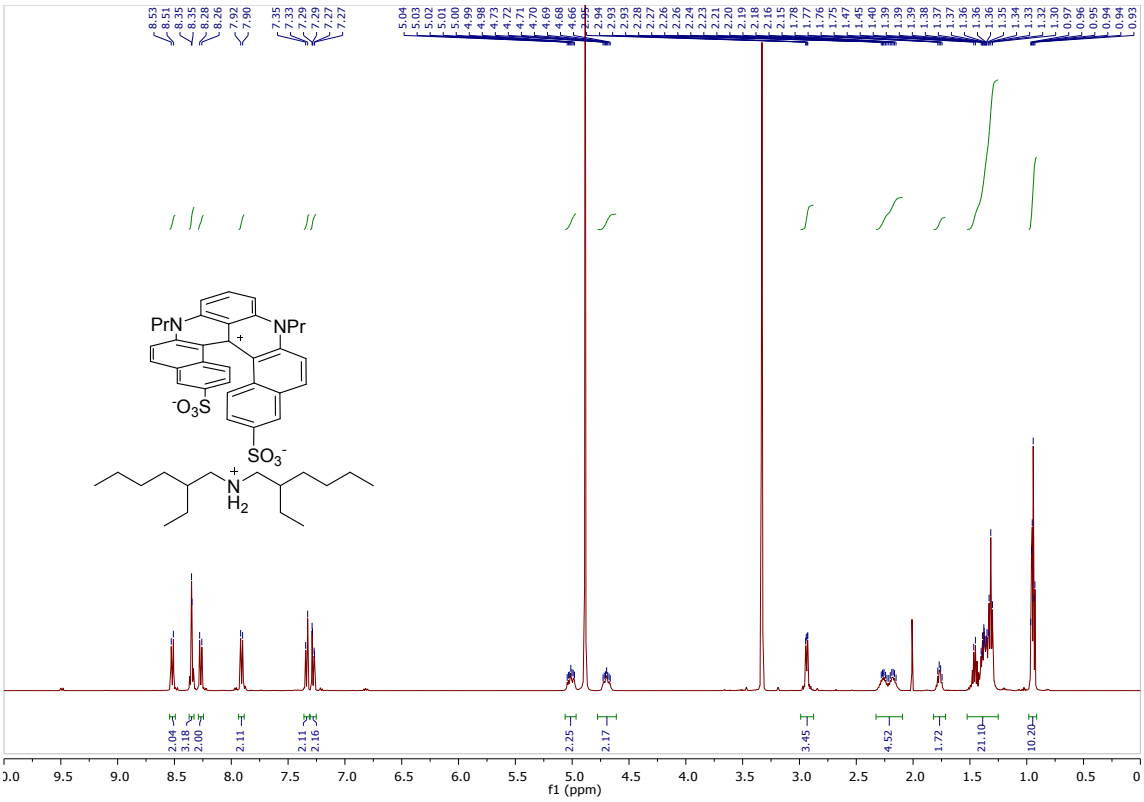


Figure S 10. ^{1}H NMR (CD_3OD, 400 MHz) spectrum of compound **[6]-diSulfo**.

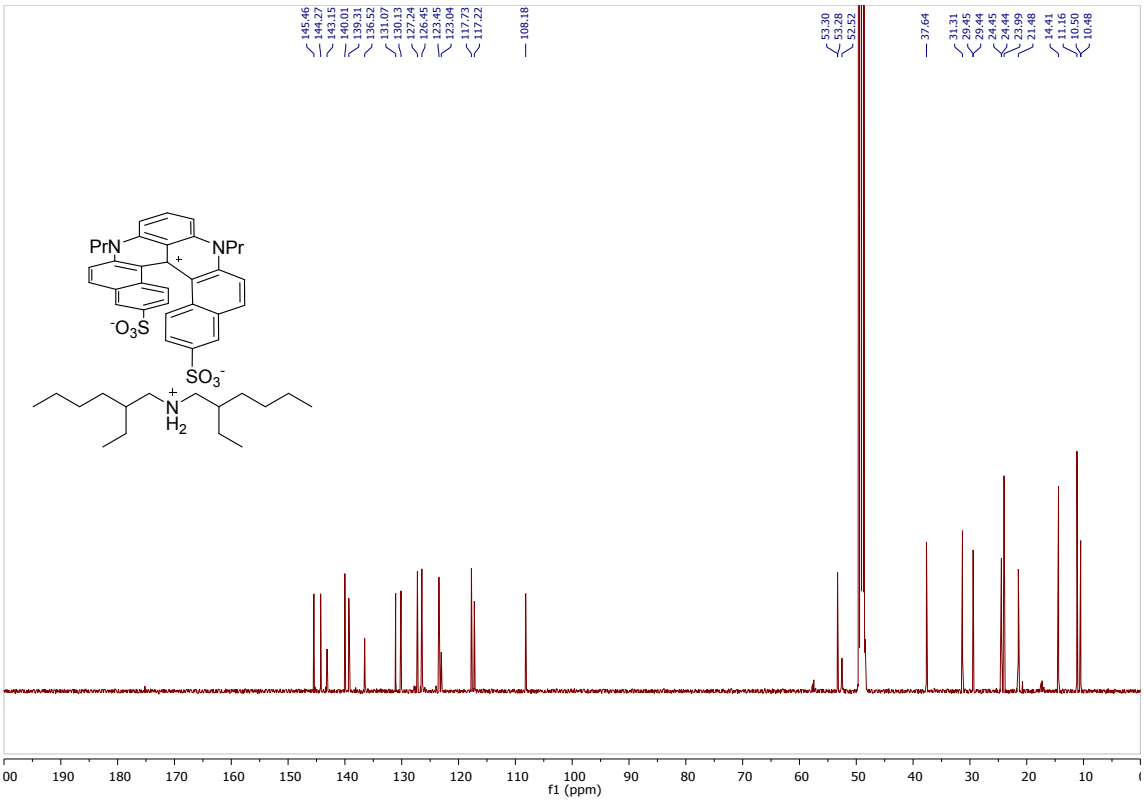


Figure S 11. ^{13}C NMR (CD_3OD, 126 MHz) spectrum of compound **[6]-diSulfo**.

Mass Spectrometry Core Facility

Lacour Group – University of Geneva

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FACULTÉ DES SCIENCES

ESI-HRMS – Certificate of Analysis

Applicant:	Johann Bosson	Date of certificate:	10/12/19
Sample name:	JB527_pos	Instrument:	Xevo G2 Tof (TOF)
Folder:	101219.PRO	Mobile phase:	MeOH (100 µl/min)
Analyst:	Stéphane Grass	Ionisation mode:	ESI (positive polarity)

Elemental Formula	Ion type	*Masslynx values **** *calc. m/z*	*meas. m/z*	Calc. *m/z*	Meas. *m/z*	Accuracy [a] *(ppm)*
$C_{33}H_{29}N_2O_6S_2$	$[M+H]^+$	*613.1467*	*613.1448*	613.1462	613.1443	-3.1

[a] *Mass spectrum is calibrated by the use of the MS lockspray system (LeuEnk calibration solution).*

**** MassLynx software does not take into account the mass of the electron for ionic species, therefore the shift of m/z 0.000459.*

Zoomed mass spectrum – Isotopic distribution.

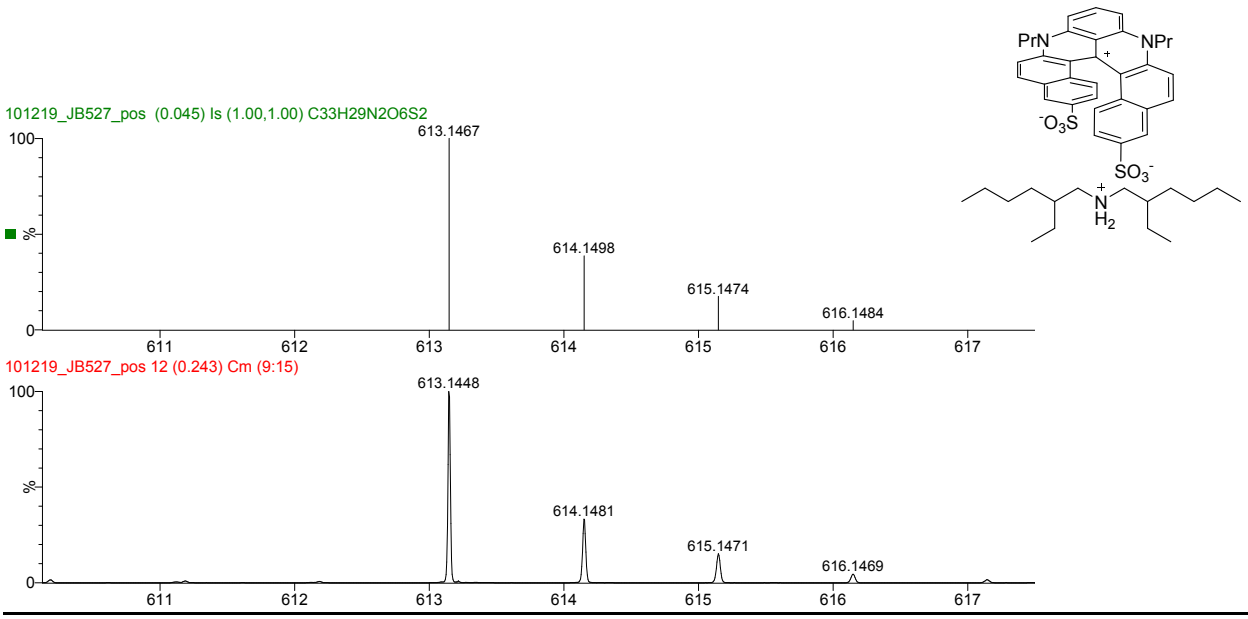

Figure S 12. Zoomed HRMS spectrum of compound **[6]-diSulfo**.

Mass Spectrometry Core Facility

Lacour Group – University of Geneva

ESI-HRMS – Certificate of Analysis

Applicant:	Johann Bosson	Date of certificate:	10/12/19
Sample name:	JB527_pos2	Instrument:	Xevo G2 Tof (TOF)
Folder:	101219.PRO	Mobile phase:	MeOH (100 µl/min)
Analyst:	Stéphane Grass	Ionisation mode:	ESI (positive polarity)

Elemental Formula	Ion type	*Masslynx values* *** *calc. m/z*	*meas. m/z*	Calc. *m/z*	Meas. *m/z*	Accuracy [a] *(ppm)*
$C_{16}H_{36}N$	$[M+H]^+$	*242.2848*	*242.2872*	242.2843	242.2867	9.9

[a] *Mass spectrum is calibrated by the use of the MS lockspray system (LeuEnk calibration solution).*

**** MassLynx software does not take into account the mass of the electron for ionic species, therefore the shift of m/z 0.000459.*

Zoomed mass spectrum – Isotopic distribution.

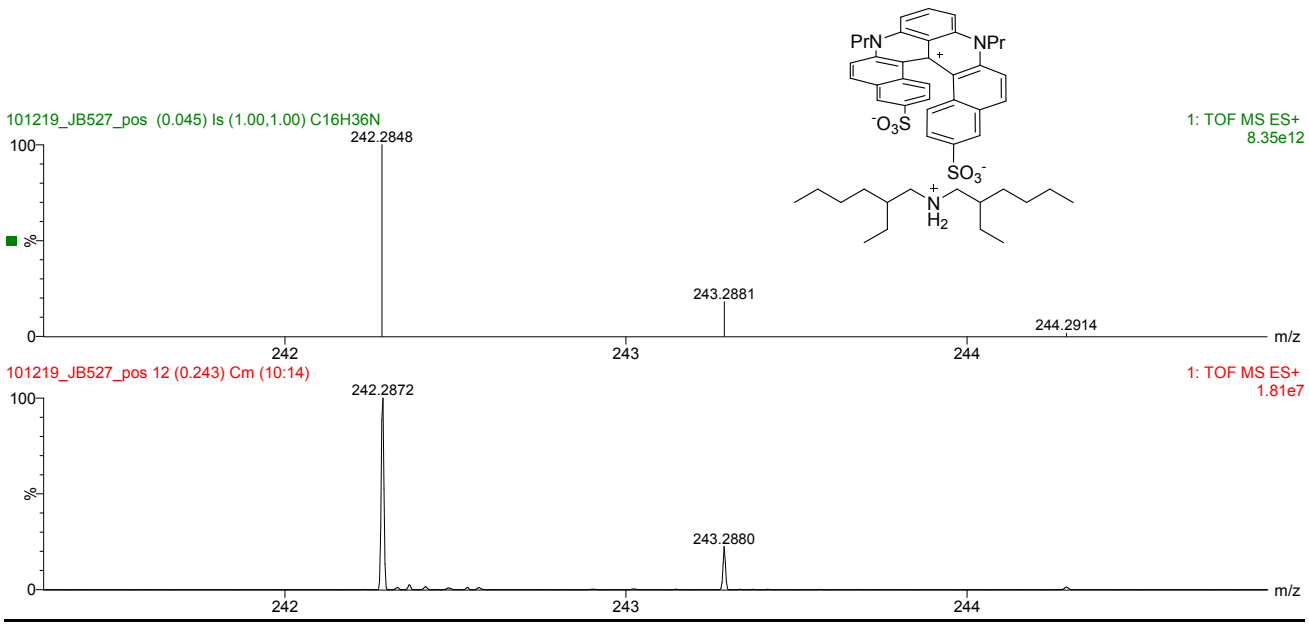

Figure S 13. Zoomed HRMS spectrum of compound **[6]-diSulfo**.

Mass Spectrometry Core Facility

Lacour Group – University of Geneva

ESI-HRMS – Certificate of Analysis

Applicant:	Johann Bosson	Date of certificate:	10/12/19
Sample name:	JB527_neg	Instrument:	Xevo G2 Tof (TOF)
Folder:	101219.PRO	Mobile phase:	MeOH (100 µl/min)
Analyst:	Stéphane Grass	Ionisation mode:	ESI (negative polarity)

Elemental Formula	Ion type	*Masslynx values **** calc. *m/z*	*Masslynx values **** meas. *m/z*	Calc. *m/z*	Meas. *m/z*	Accuracy [a] *(ppm)*
$C_{33}H_{27}N_2O_6S_2$	$[M\text{-}H]^+$	*611.131*	*611.1243*	611.1305	611.1238	-11.0

[a] *Mass spectrum is calibrated by the use of the MS lockspray system (LeuEnk calibration solution).*

**** MassLynx software does not take into account the mass of the electron for ionic species, therefore the shift of m/z 0.000459.*

Zoomed mass spectrum – Isotopic distribution.

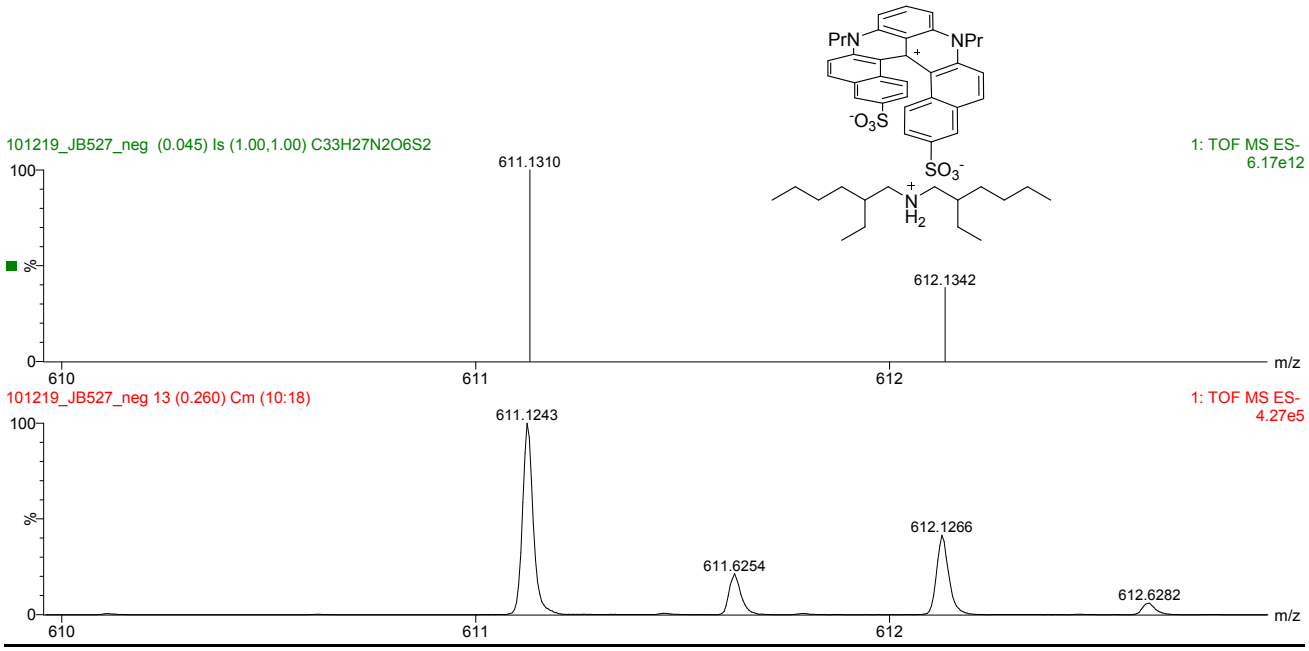

Figure S 14. Zoomed HRMS spectrum of compound **[6]-diSulfo**.

S-III. X-ray crystallography

Data were collected on an Agilent Supernova using Cu Kα1 radiation.

Figure S1 shows a view of the asymmetric unit with displacement ellipsoids at 50 percent probability. Table S1 displays information on the refinement.

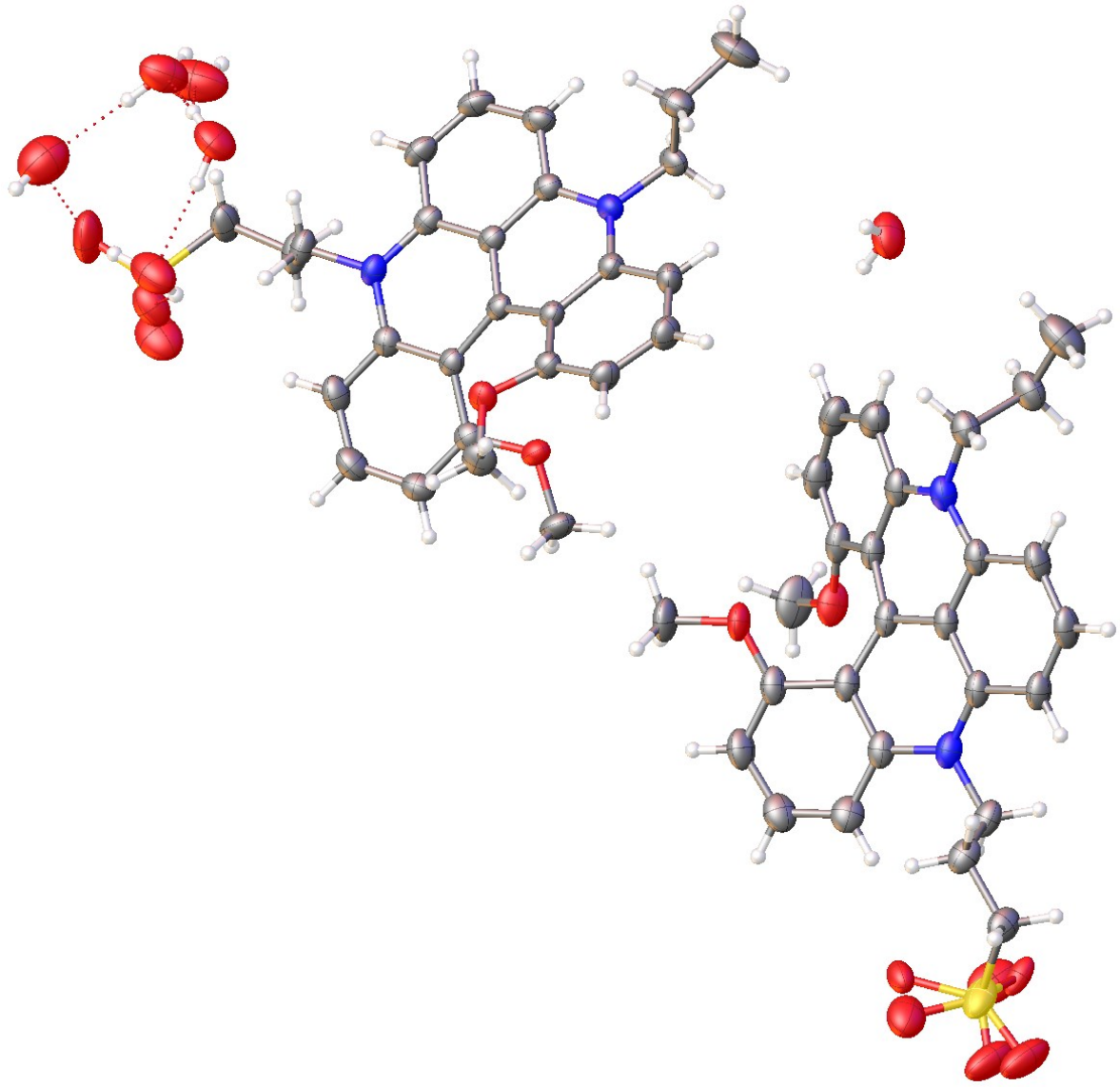

Figure S 15. View of the asymmetric unit (displacement ellipsoids at 50 percent probability) of the crystal structure of **[4]-C3-Sulfo** (only *M* enantiomers shown).

The crystal is twinned (non-merohedral twinning). However only one twin component was integrated. (data using both twin components were not as good since the second component is diffracting in a weaker way).

The data quality does not allow to assert the hydrogen positions unambiguously.

One sulfonate group is disordered and was refined using two components. The following restraints/constraints were applied

SADI S8B O11B S8B O11B S8B O9B S8B O9B S8B O10C S8B O10B

SADI 0.04 O9C O11C O11C O10C O10C O9C

SADI 0.04 O9B O10B O10B O11B O11B O9B

RIGU S8B O11B O10B O9B

RIGU S8B O11B O10C O9B

EADP O11C O11B

Table S 1: crystallographic data for **[4]-C3-Sulfo**

CCDC number	2008862	
Empirical formula	C54 H66 N4 O15 S2	
Formula weight	1075.22	
Temperature	180.15 K	
Wavelength	1.54184 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 10.1378(3) Å	a= 87.576(2)°
	b = 10.9386(3) Å	b= 85.907(2)°
	c = 23.3534(6) Å	g = 86.452(2)°
Volume	2576.32(11) $Å^3$	
Z	2	
Density (calculated)	1.386 Mg/m^3	
Absorption coefficient	1.560 mm^{-1}	
F(000)	1140	
Crystal size	0.15 x 0.128 x 0.063 mm^3	
Theta range for data collection	3.798 to 73.677°.	
Index ranges	-12<=h<=12, -13<=k<=13, -28<=l<=28	

Reflections collected	35732
Independent reflections	10120 [R(int) = 0.0458]
Completeness to theta = 67.684°	98.9 %
Absorption correction	Analytical
Max. and min. transmission	0.910 and 0.815
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	10120 / 29 / 719
Goodness-of-fit on F^2	1.043
Final R indices [I>2sigma(I)]	R1 = 0.0664, wR2 = 0.1860
R indices (all data)	R1 = 0.0793, wR2 = 0.1976
Extinction coefficient	n/a
Largest diff. peak and hole	1.082 and -0.608 e.$Å^{-3}$

S-IV. Solubility study

[4]quinacridine	[4]helicene	[6]helicene
		

Table S2. Pictures of **[4]quinacridine**, **[4]helicene** and **[6]helicene** in PBS (left) and in acetonitrile (right) at concentrations *ca.* 10^{-3} M.

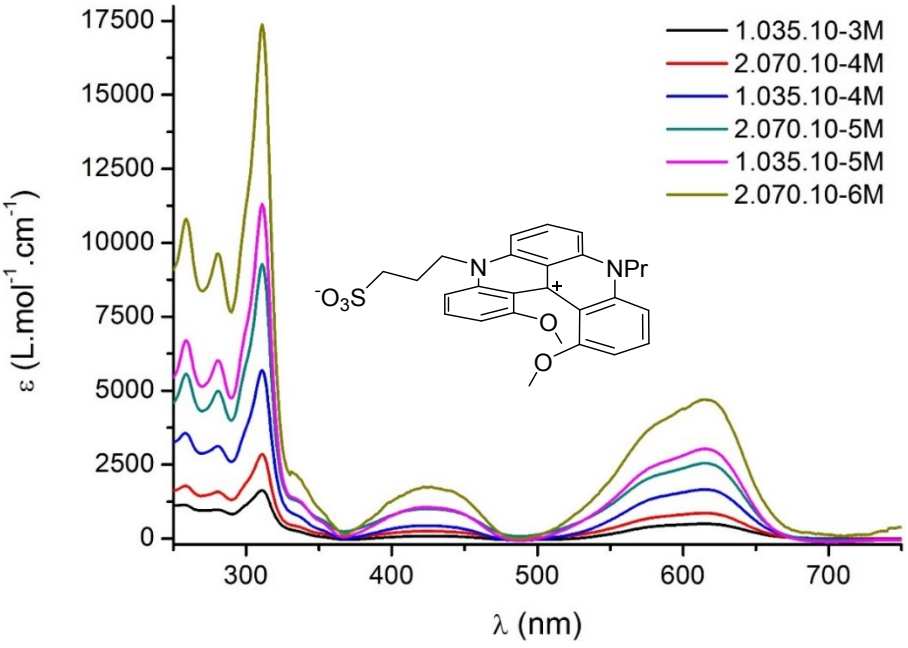

Figure S 16. Electronic absorption spectra of compound **[4]-C3-Sulfo** in PBS recorded at different concentrations.

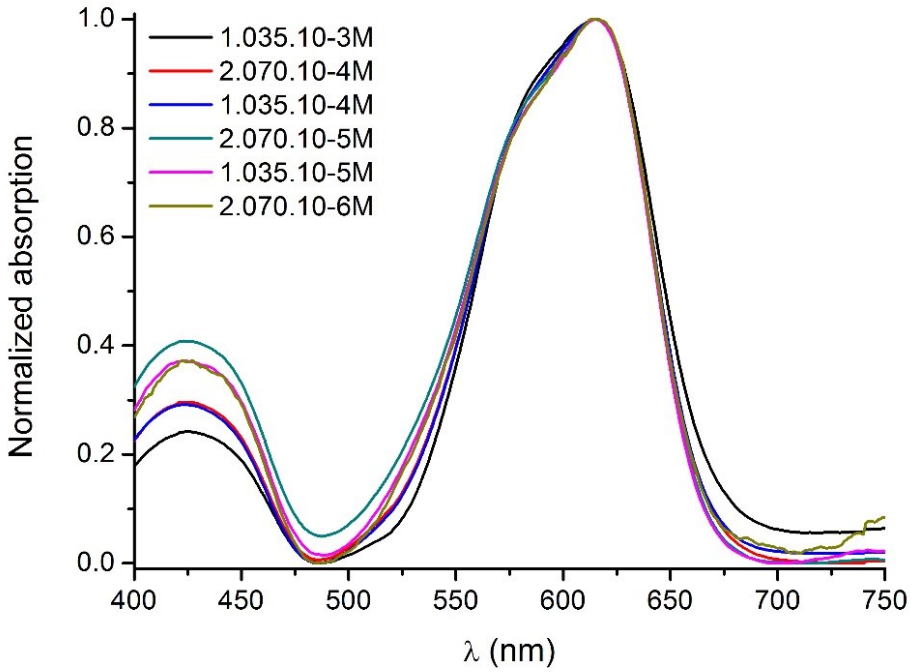

Figure S 17. Normalized electronic absorption spectra of compound **[4]-C3-Sulfo** in PBS recorded at different concentrations.

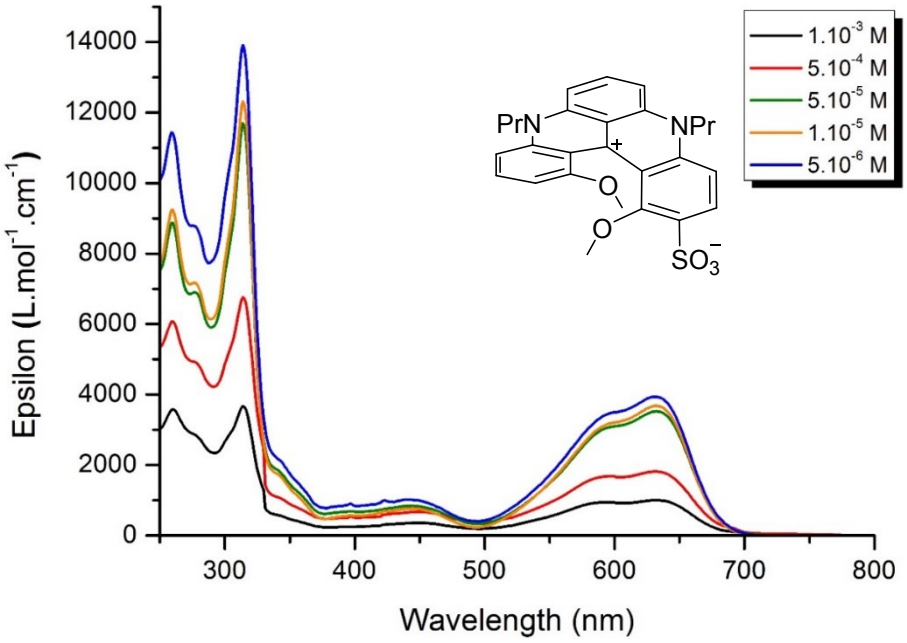

Figure S 18. Electronic absorption spectra of compound **[4]-monoSulfo** in PBS recorded at different concentrations.

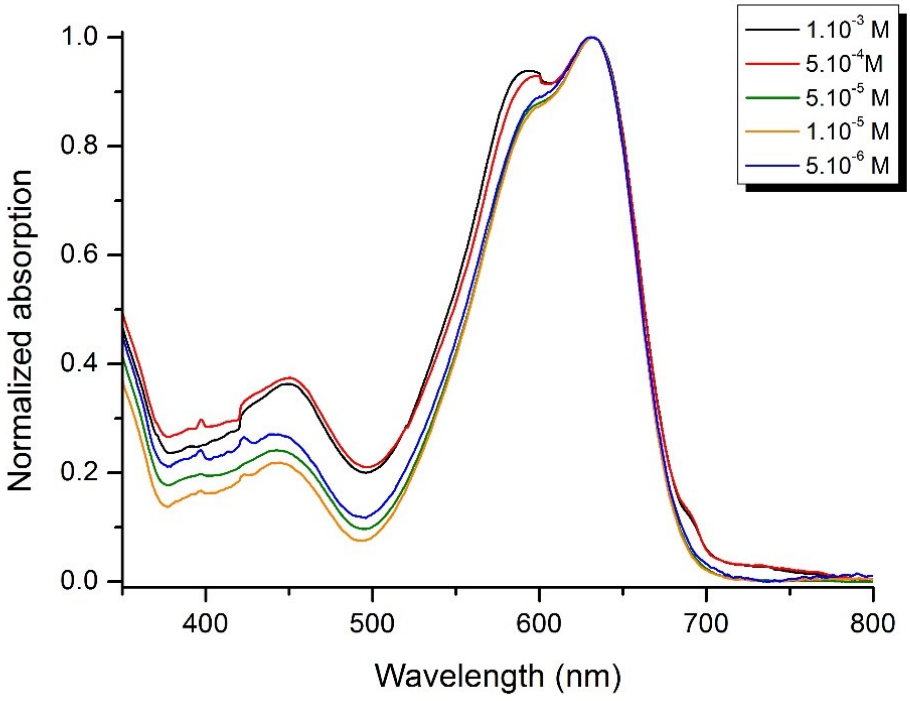

Figure S 19. Normalized electronic absorption spectra of compound **[4]-monoSulfo** in PBS recorded at different concentrations.

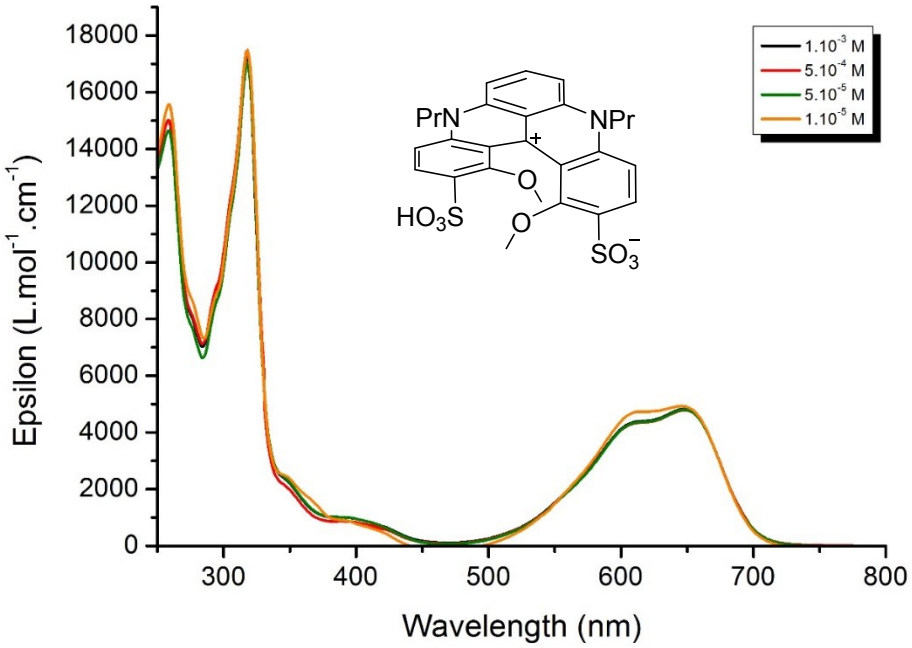

Figure S 20. Electronic absorption spectra of compound **[4]-diSulfo** in PBS recorded at different concentrations.

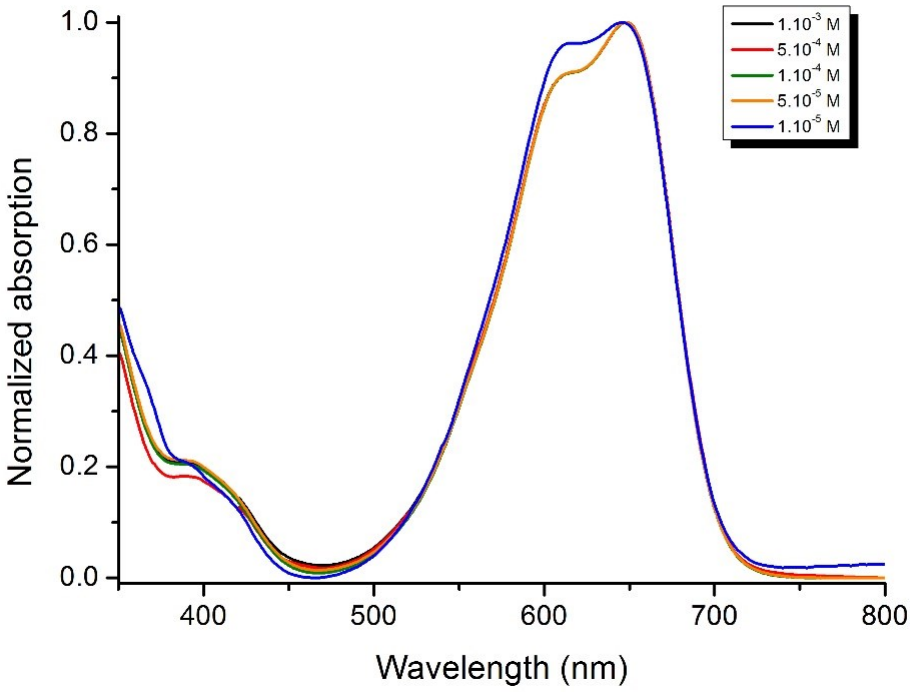

Figure S 21. Normalized electronic absorption spectra of compound **[4]-diSulfo** in PBS recorded at different concentrations.

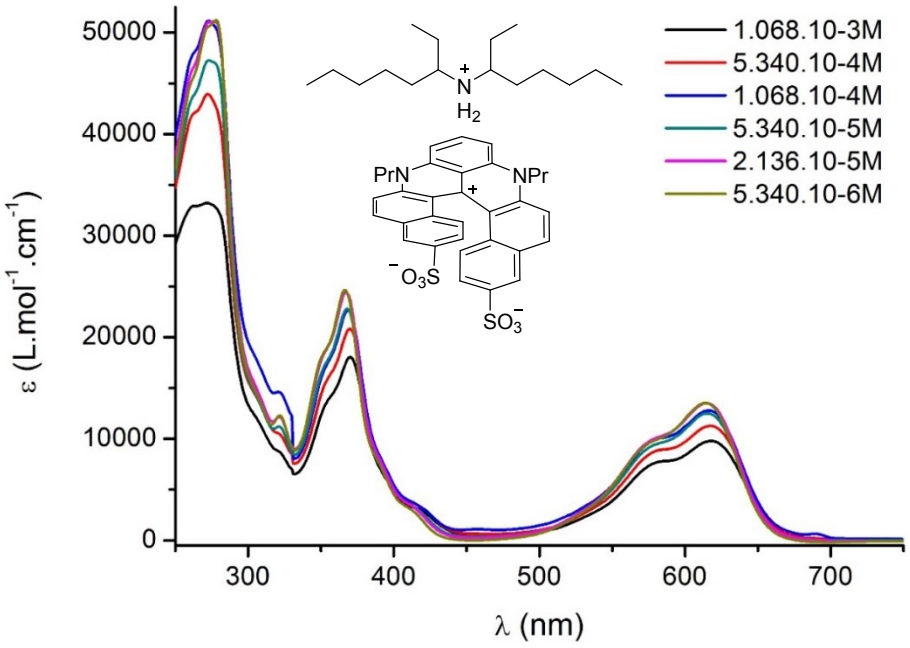

Figure S 22. Electronic absorption spectra of compound **[6]-diSulfo** in PBS recorded at different concentrations.

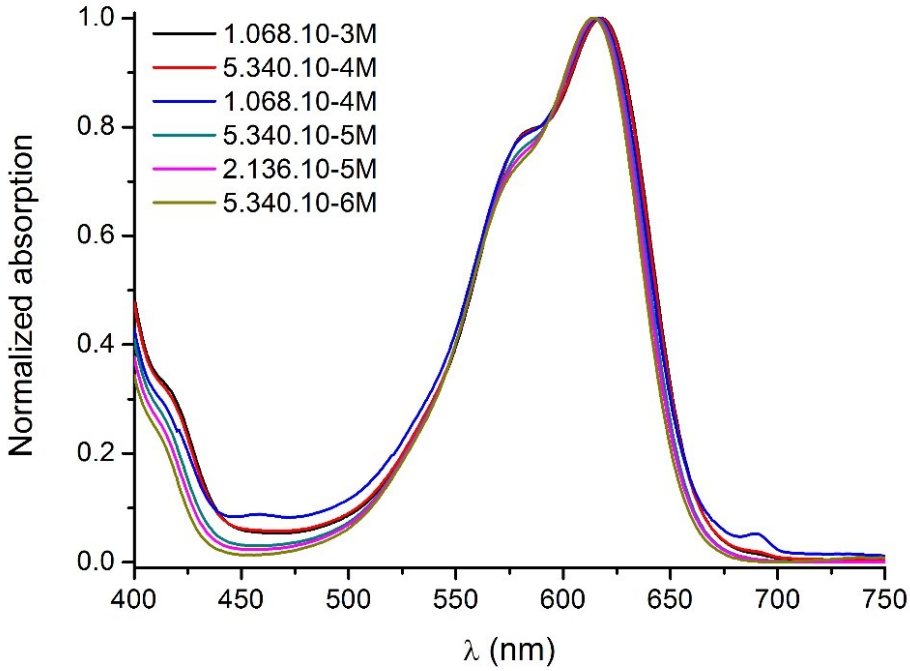

Figure S 23. Normalized electronic absorption spectra of compound **[6]-diSulfo** in PBS recorded at different concentrations.

S-V. Electrochemical characterization

Voltammetric experiments were performed with a PGSTAT30 Autolab potentiostat connected to a conventional three-electrode cell, consisting in a silver-wire pseudo-reference electrode, a platinum-wire auxiliary electrode, and a platinum or glassy carbon working electrode. Prior to measurements, the working electrode was polished with alumina slurry of different sizes, rinsed thoroughly with Milli-Q water between each polishing step, and sonicated in water.

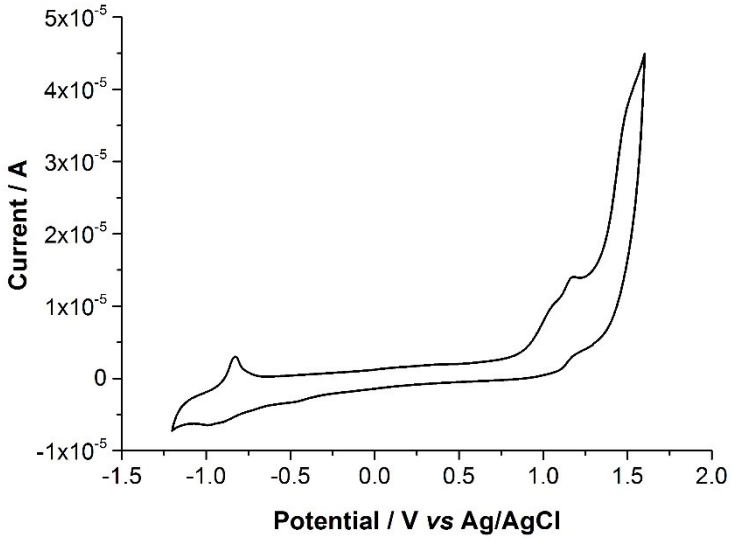

Figure S24. CV recorded with $5x10^{-4}$ M of **[6]-diSulfo** in 0.1 M PBS degassed for 5 minutes. Scan rate 0.1 V s^{-1}.

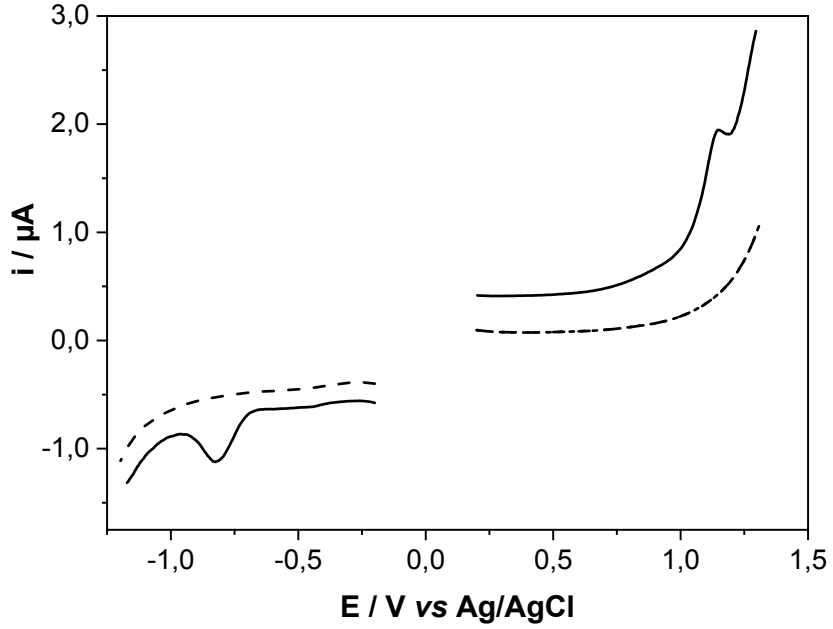

Figure S25. DPV recorded with 2×10^{-5} M of **[4]-monoSulfo** (plain line) in 0.1 M PBS degassed for 5 minutes. DPV of PBS alone (d&shed line).

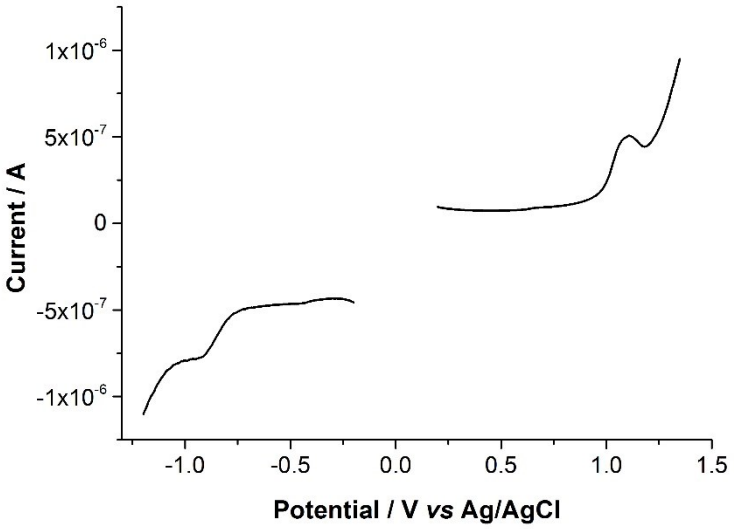

Figure S26. DPV recorded with 2×10^{-5} M of **[4]-C3-Sulfo** in 0.1 M PBS degassed for 5 minutes.

S-VI. ECL efficiency, spectra and mechanisms

ECL emission was collected with a Hamamatsu R5070 photomultiplier tube with a Hamamatsu C9525 high-voltage power supply. The PMT detector was held at -750 V and placed at a defined distance of few millimeters from the working electrode. The output signal was amplified by a Keithley 6485 Picoammeter, then it was acquired via the second input channel of the µAutolab type III potentiostat.

ECL spectra were recorded with a Princeton Instruments Acton SpectraPro 2300i after the CCD camera, cooled to -110 °C with liquid N2. The optical fiber connected to the device was located at a defined distance of few millimeters from the working electrode

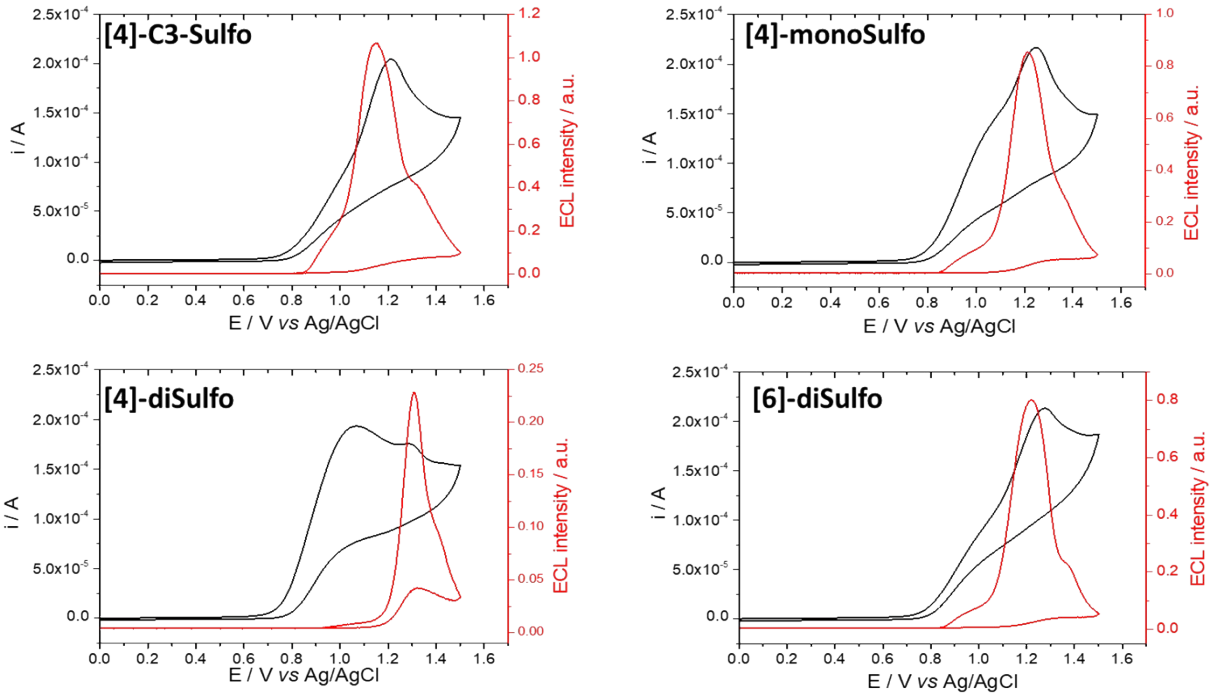

Figure S27. Voltammetric (black curve) and ECL (red curve) responses of the different diaza [4] and [6]helicenes recorded at a concentration of 2×10^{-5} M in 0.1 M degassed PBS containing 0.05 M TPA. Scan rate: 0.1 $V.s^{-1}$.

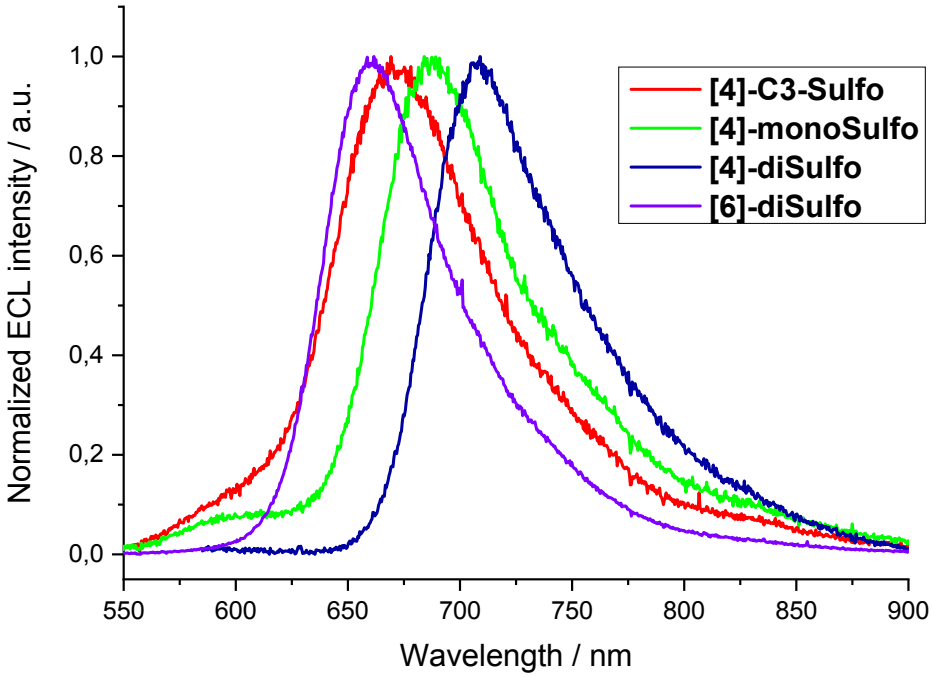

Figure S28. ECL spectra recorded with each dye (2×10^{-5} M) and TPA as coreactant (5×10^{-2} M) in 0.1 M PBS (pH 7.4). E = 1.4 V *vs.* Ag/AgCl.

The pre-peak appearing before the oxidation of the helicene dyes (noted HEL) can be described by the so-called "revisited" route" or "low oxidation potential ECL" route.[3] In this pathway, only the coreactant TPA is oxidized at the electrode surface and the resulting radicals, $TPA^{\bullet+}$ and $TPA^{\bullet}$, react with the helicene dyes to generate their excited states:

$TPA - e \rightleftharpoons TPA^{\bullet+}$ (S1)

$TPA^{\bullet+} \rightleftharpoons TPA^{\bullet} + H^{+}$ (S2)

$TPA^{\bullet} + HEL \rightleftharpoons HEL^{\bullet-} + TPA'$ (side product) (S3)

$TPA^{\bullet+} + HEL^{\bullet-} \rightleftharpoons HEL^{*} + TPA$ (S4)

$HEL^{*} \rightleftharpoons HEL + h\nu$ (S5)

The predominant ECL signal occurs at potentials where the helicene dyes are oxidized. The corresponding main ECL pathway follows the reaction sequence including Eq. S1, S2, S5 and the following Equations:

$HEL - e \rightleftharpoons HEL^{\bullet+}$ (S6)

$HEL^{\bullet+} + TPA \rightleftharpoons HEL + TPA^{\bullet+}$ (S7)

$HEL^{\bullet+} + TPA^{\bullet} \not\rightarrow HEL^{*} + TPA'$ (side product) (S8)

The excited state of the helicene dyes could also be generated by another electron-transfer reaction involving the reduced and oxidized forms, $HEL^{\bullet-}$ and $HEL^{\bullet+}$ (generated following Eq. S3 and S6, respectively), represented as:

$HEL^{\bullet+} + HEL^{\bullet-} \not\rightarrow HEL^{*} + HEL$ (S10)

However, in the present case, considering the low stability of the electrogenerated species, this latter reaction should not contribute significantly to the ECL emission.

The ECL efficiency Φ_{ECL} was calculated using the following equation:

$$\Phi_{ECL} = \Phi_{ECL}^{0} \frac{I}{Q} \frac{Q^{0}}{I^{0}} \quad \text{(S11)}$$

where Φ_{ECL} and Φ_{ECL}^{0} are the ECL efficiencies of the tested helicenes and of the reference $[Ru(bpy)_3]^{2+}$ compound, I and I° are integrated ECL intensities of the tested helicenes and of the $[Ru(bpy)_3]^{2+}$ compound, and Q and Q° are the charges passed for the tested helicenes and for $[Ru(bpy)_3]^{2+}$, respectively. We used $[Ru(bpy)_3]^{2+}$ as a reference (Φ_{ECL}^{0} = 100%).

S-VII. References

[1] a) C. Herse, D. Bas, F. C. Krebs, T. Bürgi, J. Weber, T. Wesolowski, B. W. Laursen and J. Lacour, *Angew. Chem. Int. Ed.* **2003**, *42*, 3162-3166; b) B. W. Laursen and F. C. Krebs, *Chem. Eur. J.* **2001**, *7*, 1773-1783; c) F. Torricelli, J. Bosson, C. Besnard, M. Chekini, T. Buergi and J. Lacour, *Angew. Chem. Int. Ed.* **2013**, *52*, 1796-1800.

[2] A. Wallabregue, P. Sherin, J. Guin, C. Besnard, E. Vauthey and J. Lacour, *Eur. J. Org. Chem.* **2014**, 6431-6438.

[3] a) W. Miao, J.-P. Choi and A. J. Bard, *J. Am. Chem. Soc*., **2002**, 124, 14478-14485. b) J. I. Kim, I.-S. Shin, H. Kim and J.-K. Lee, *J. Am. Chem. Soc*., **2005**, *127*, 1614-1615. c) M. Sentic, M. Milutinovic, F. Kanoufi, D. Manojlovic, S. Arbault and N. Sojic, *Chem. Sci*., **2014**, *5*, 2568-2572. d) Z. Chen and Y. Zu, *J. Phys. Chem. C*, **2009**, *113*, 21877-21882. e) M.-J. Li, Z. Chen, V. W.-W. Yam and Y. Zu, *ACS Nano*, **2008**, *2*, 905-912.