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Electronic Supplementary Information

8-Styryl-substituted coralyne derivatives as DNA binding fluorescent probes

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Table of contents

1 Experimental Section

1.1 Equipment

NMR spectra were recorded with a Bruker Avance 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) at room temperature (approximately 22 °C) or with a Varian VNMR-S 600 (^{1}H: 600 MHz, ^{13}C: 150 MHz) at 35 °C (DMSO-d_6). Spectra were processed with the software ACD/NMR Processor Academic Edition 12.02 and referenced to the solvent DMSO-d_6 (^{1}H: δ = 2.50, ^{13}C: δ = 39.5). The chemical shifts are given in ppm. Absorption spectra were recorded with a Cary 100 Bio spectrophotometer in quartz cells (10 mm x 10 mm) with baseline correction. Emission spectra were collected with a Cary Eclipse spectrophotometer in quartz cells (10 mm x 10 mm) at 20 °C. Elemental analyses data were determined with a HEKAtech *EURO*EA combustion analyser by Mr. Rochus Breuer (Universität Siegen, Organische Chemie I). Mass spectra (ESI) were recorded on a Finnigan LCQ Deca (U = 6 kV; working gas: Argon; auxiliary gas: Nitrogen; temperature of the capillary: 200 °C). The melting points were measured with a BÜCHI 545 (BÜCHI, Flawil, CH) and are uncorrected.

1.2 DNA and buffer solutions

All buffer solutions were prepared from purified water (resistivity 18 MΩ cm^{-1}) and biochemistry-grade chemicals. The buffer solutions were filtered through a PVDF membrane filter (pore size 0.45 µm) prior to use. Oligodeoxyribonucleotides (purification: HPLC; quality control: MALDI-TOF; synthesis scale: 1.0 µmol) **F21T** [fluorescein-GGG(TTAGGG)$_3$-tetramethylrhodamine], **22AG** d[A(GGGTTA)$_3$GGG], and **ds26** [d(CA$_2$TCG$_2$ATCGA$_2$T$_2$CGATC$_2$GAT$_2$G)] were purchased from Metabion Int. AG (Planegg/Martinsried). Calf thymus DNA (type I; highly polymerized sodium salt; ε = 12824 cm^{-1} M^{-1}) was purchased from Sigma-Aldrich (St. Louis, MO, USA). BPE buffer: 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, pH 7.0; Na cacodylate buffer: 10 mM $Na(CH_3)_2AsO_2$ x 3 H_2O, 10 mM KCl, 90 mM LiCl, pH 7.2–7.3.

1.3 Methods

1.3.1 Photometric and fluorimetric titrations

The spectrometric titrations were performed according to published protocols.[1] Solutions were prepared for each measurement from stock solutions of the derivatives **4b–d** in MeCN (c = 1 mM). Aliquots of the stock solution were evaporated under a stream of nitrogen, redissolved in DMSO (10% v/v) and BPE buffer to obtain a ligand concentration of c_L = 20 µM. The respective DNA solutions also contained the ligand at the same concentration in order to avoid dilution effects. Aliquots of the ligand solutions were placed into quartz cells and titrated with the DNA solutions in intervals of 0.05–2 equivalents, and absorption or emission spectra were recorded. The titrations were stopped after no changes were

observed in absorption or emission spectra upon addition of at least three two-equivalent portions of the titrant. All spectrometric titrations were performed at least two times to ensure reproducibility. In general, absorption spectra were determined with a detection speed of 120 nm min^{-1} in a range from 300 to 600 nm and subsequently smoothed in the Origin software with the function “adjacent-averaging“ (factor of 10). For the detection of emission spectra the excitation and emission slits were adjusted to 5 nm. The detection speed was 120 nm min^{-1} and the detector voltage was adjusted between 600 and 800 V depending on the fluorescence intensity. The spectra were smoothed with the implemented moving-average function by a factor of 5. Emission spectra in the range between 600 and 800 nm were corrected using an instrument specific correction curve. The binding constants were determined by fitting the binding isotherms from the fluorimetric titrations to the established theoretical model according to the independent-site model (eq. 1), where y is the normalized intensity; $A = 1/K_b$; $B = c_L$, and n is the number of the binding sites per quadruplex DNA.[2] The normalized intensity y was corrected with regard to the change in absorption at the excitation wavelength.

$$y = \frac{A + B + nx - \sqrt{(A + B + nx)^2 - 4Bnx}}{2B} \qquad \text{(Eq. 1)}$$

1.3.2 Thermal denaturation experiments

FRET melting experiments were performed according to published procedures.[3] The stock solutions of the oligonucleotide **F21T** (c = 50 µM), the duplex DNA **ds26** (c = 200 µM) and the ligand (c = 10 µM) were prepared in cacodylate buffer, whereas the ligand solution contained 1% v/v DMSO. The samples were mixed according to Table S1 and transferred into quartz cells (10 mm pathlength) for fluorescence measurements.

Table S1. Composition of the Samples for Fluorimetric DNA Denaturation Experiments with **F21T** in the Absence and in the Presence of **ds26**.

Sample	c_L / µM	V_L / µL [b]	V_{DMSO}	V_{buffer} / µL
1[a]	0	0	10.0	986
2[a]	0.25	25.0	9.75	961
3[a]	0.50	50.0	9.50	937
4[a]	1.00	100	9.00	887
5[a,c]	0	0	10.0	971
6[a,c]	0.25	25.0	9.75	946
7[a,c]	0.50	50.0	9.50	922
8[a,c]	1.00	100	9.00	872

[a] $V_{\mathbf{F21T}}$ = 4 µL, $c_{\mathbf{F21T}}$ = 0.2 µM. [b] c_{DMSO} = 1% (v/v). [c] $V_{\mathbf{ds26}}$ = 15 µL, $c_{\mathbf{ds26}}$ = 3.0 µM.

The excitation wavelength was λ_{ex} = 470 nm and the emission intensity at λ_{fl} = 515 nm was recorded. The excitation and emission slits were adjusted to 5 nm and the detector voltage was adjusted to 600 V. The samples were first heated from 20 °C to 90 °C at a rate of 2.5 °C min^{-1}. After holding the temperature for 5 min, samples were cooled to 10 °C at 1.0 °C min^{-1}. After holding the temperature for 5 min, samples were heated from 10 °C to 95 °C at 0.2 °C min^{-1} and the fluorescence was detected during the latter ramp. The raw spectra were smoothed in the Origin software with the function “adjacent-averaging“ (factor of 20), normalized and plotted as a function of the temperature. The melting temperature was calculated by taking the maximum of the first derivative of the melting curve approximated by the Gaussian function. The shift of the melting temperature was calculated according to eq. 2.

$$\Delta T_m = T_m \text{ (DNA-Ligand)} - T_m \text{ (DNA)} \qquad \text{(Eq. 2)}$$

1.3.3 Determination of fluorescence quantum yields

Solutions were prepared for each measurement from stock solutions of the derivatives **2** and **4a–f** in MeCN (c = 1 mM). Aliquots of the stock solution were evaporated under a stream of nitrogen and redissolved in MeCN. In general, absorption spectra were determined with a scan rate of 120 nm min^{-1} in a range from 200 to 600 nm and subsequently smoothed in the Origin software with the function “adjacent-averaging“ (factor of 10).

For the detection of emission spectra the excitation and emission slits were adjusted to 5 nm. The scan rate was 120 nm min^{-1} and the detector voltage was adjusted to 500 V. The spectra were smoothed with the implemented moving-average function by a factor of 5. The relative fluorescence quantum yields of the coralyne derivatives **4a–f** were determined under identical conditions, *i.e.* the same cuvettes were used and the measurements were performed at a constant temperature with the same settings on the spectrometer (detection wavelength, excitation wavelength, detector voltage, slit bandwidths, collection rate). Coumarin 307 (Φ_{fl} = 0.58 in MeCN)[4] was used as standard. The emission spectra were collected from solutions with Abs. = 0.10 at the excitation wavelength λ_{ex} = 400 nm. After integration of the fluorescence band, the relative fluorescence quantum yields were calculated according to eq. 3.[5]

$$\phi_F = \frac{J_x(1-T_s)}{J_S(1-T_x)} \cdot \frac{n_x^2}{n_S^2} \cdot \phi_{F,S} \qquad \text{(Eq. 3)}$$

The subscripts “x” and “s” refer to the substance under investigation and a reference compound, respectively; $J = \int I_F(\lambda)d\lambda$ is the emission integral over the area of interest; T is the

optical transmittance of the sample solution at the excitation wavelength, λ_{ex}; n is the refractive index of the sample or standard solution; however the quotient of the refractive indexes was neglected because it does not differ significantly from 1.

1.3.4 Cell culture and fluorescence microscopy

NIH 3T3 mouse fibroblasts were cultured at standard conditions (37 °C, 5% CO_2) in Dulbecco's modified Eagle medium (DMEM high glucose; Gibco, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific), 2 mM L-Glutamine (Gibco, Thermo Fisher Scientific), 100 U mL^{-1} penicillin (Gibco, Thermo Fisher Scientific) and 100 µg mL^{-1} streptomycin (Gibco, Thermo Fisher Scientific). Cells were detached with 0.25% Trypsin/EDTA after washing with PBS and collected by centrifugation (270 g for 4 min) in a conical tube and counted with a Neubauer improved cell counting chamber (Brand, Wertheim, Germany). Cells were seeded at a density of 10.000 cells cm^{-2} 48 h prior incubation with 2.5 µL of a 1 mM solution of **4b** in DMSO (final concentration: 2.5 µM) for 1 h in the cell culture medium at 37 °C and 5% CO_2. As a control, cells were also incubated with the same amount of DMSO (2.5 µL mL^{-1}).

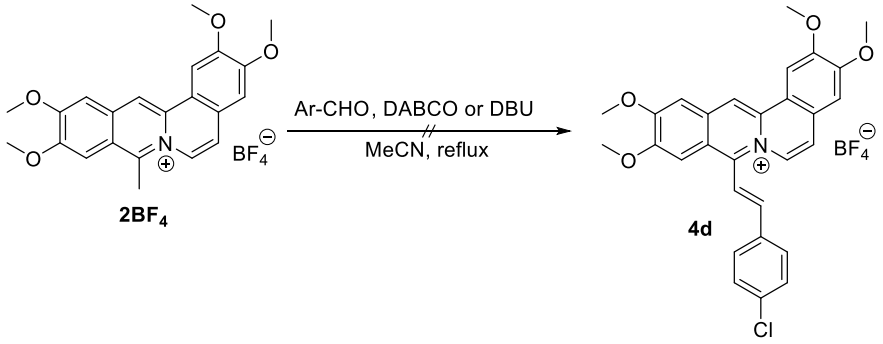
For fluorescence microscopy, cells were washed with PBS (Lonza, Belgium) once, fixed with 4% paraformaldehyde for 20 min at 21 °C and embedded with Mowiol® 4-88 (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) after an additional washing step with PBS. For analysis, images were taken with an Axiovert 135 microscope equipped with an AxioCam MRm and Zen software (Carl Zeiss MicroImaging GmbH, Jena, Germany).

The confocal fluorescence microscopy images were collected employing a Microtime 200 time-resolved confocal fluorescence microscope setup (PicoQuant, Berlin, Germany) comprising an inverted laser scanning microscope (IX-71, Olympus, Hamburg, Germany) and a 40x objective LD Achroplan, NA 0.60 (Zeiss, Oberkochen, Germany). The ligand was excited by a continuous wave laser (LSR-532-U-50) at 532 nm. The fluorescence passed through the cut-off (>545 nm) filter and was split into two beams. One fluorescence beam was detected by a single-photon avalanche diode (PD5CTC, Micro Photon Devices, Bolzano, Italy) and the other beam with a similar diode (PD1CTC) after passing through the band-pass (652–732 nm) filter. The objective was held on a XYZ piezo controller and was scanned in XY-plane in a range of 80 x 80 μm^2 with a pixel resolution of 512 x 512. The dwell time was 4.8 ms/pixel. The data were analyzed using the SymPhoTime (version 5.2.4.0, PicoQuant, Berlin, Germany), and ImageJ and Matlab (MathWorks) software packages.

1.4 Synthesis

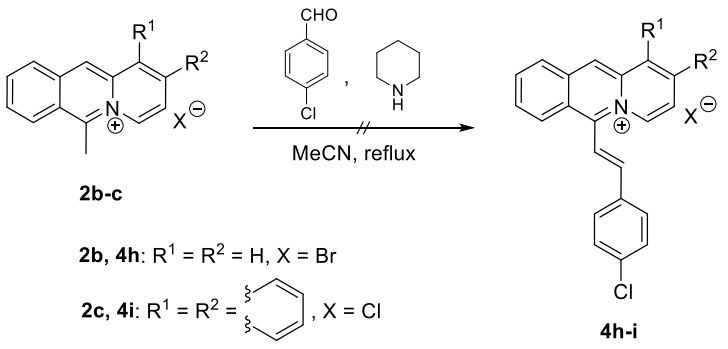
1.4.1 General remarks

We also examined whether the stryryl derivatives **4a–f** could be obtained when other bases are used instead of piperidine. Therefore, the synthesis of the chloro-substituted derivative **4d** was investigated exemplarily. It turned out, however, that the Knoevenagel reaction is not successful when sterically demanding bases such as 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) or 1,4-Diazabicyclo[2.2.2]octane (DABCO) are used because coralyne (**2**) either decomposed or did not react, respectively.



Scheme S1. Attempted synthesis of the coralyne derivative **4d** with DABCO or DBU as base.

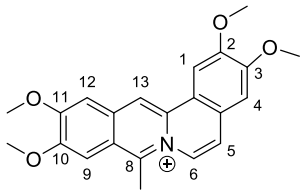
It was shown that the 4-methylbenzo[*a*]quinolizinium readily reacts in a Knoevenagel type reaction.[6] Hence we also investigated, if other quinolizinium derivatives, except coralyne (**2**), that contain a methyl group in α-position to the positively charged nitrogen atom are prone to react in a Knoevenagel type reaction. However, both of the tested substrates, specifically the 6-methylbenzo[*b*]quinolizinium bromide (**2b**)[7] and the 8-methyldibenzo[*a*,*g*]quinolizinium chloride (**2c**),[8] rapidly decomposed upon addition of only *catalytic* amounts of piperidine. Therefore, the four methoxy groups of coralyne are apparently crucial to stabilize the positively charged nitrogen atom and protect the substrate from nucleophilic attacks by the base which subsequently lead to decomposition.[9]



Scheme S2. Attempted synthesis of the styryl-substituted quinolizinium derivatives **4h–i**.

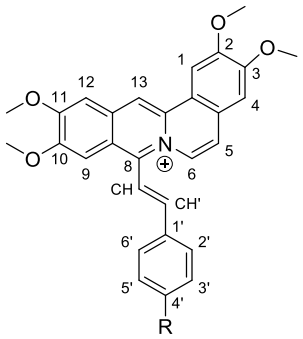
*1.4.2 Synthesis of coralyne (**2**) and its 8-styryl-substituted derivatives **4a–f***

*Coralyne tetrafluoroborate (**2BF$_4$**)*



To a solution of coralyne sulfoacetate (**2SA**)[10] (2.50 g, 4.97 mmol) in H_2O (300 mL) was added $NaBF_4$ (10.9 g, 99.3 mmol) in portions with stirring, after which a viscous yellow mass was formed. The mixture was poured into THF (600 mL) under vigorous stirring and filtered. The yellow solid was washed with Et_2O (2 x 50 mL), recrystallized from MeCN/$MeNO_2$ (1/1), filtered, washed with MeCN (20 mL) and Et_2O (3 x 20 mL) and dried *in vacuo*. The product **2BF$_4$** was obtained as yellow, amorphous solid (1.71 g, 3.79 mmol, 76%). – mp > 300 °C (dec.). – ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 3.31 (s, 3 H, 8-Me), 3.97 (s, 3 H, 10-OMe), 4.07 (s, 3 H, 3-OMe), 4.11 (s, 6 H, 2-OMe, 11-OMe), 7.54 (s, 1 H, 9-H), 7.55 (s, 1 H, 4-H), 7.68 (s, 1 H, 12-H), 7.89 (d, ^3J = 8 Hz, 1 H, 5-H), 8.11 (s, 1 H, 1-H), 8.77 (d, ^3J = 8 Hz, 1 H, 6-H), 9.42 (s, 1 H, 13-H). – El. Anal. for $C_{22}H_{22}BF_4NO_4$ (451.22), calcd (%): C 58.56, H 4.91, N 3.10, found (%): C 58.34, H 4.80, N 3.24. – MS (ESI$^+$): *m*/*z* = 364 (100) $[M–BF_4]^+$.

General procedure for the Knoevenagel reaction of coralyne (2BF$_4$) with aromatic aldehydes in the presence of piperidine (GP 1)[11]



To a suspension of **2BF$_4$** (1.00 mmol) and the respective aldehyde (4.00 mmol) in MeCN was added piperidine (2.00–4.00 mmol) at 80 °C, and the reaction mixture was stirred under reflux for 8–18 h. After cooling to r.t., the mixture was added dropwise to Et_2O (20 mL per 1 mL of MeCN) under vigorous stirring. The precipitate was filtered, washed with water (2 x 20 mL), cold MeOH (10 mL) and Et_2O (3 x 20 mL), which gave the essentially pure product. An analytically pure sample was obtained by recrystallization.

General procedure for the Knoevenagel reaction of coralyne ($2BF_4$) with bis(piperidino)phenylmethane derivatives (GP 2)[11]

A solution of the respective aldehyde, piperidine and *p*-TsOH in toluene was stirred at a Dean-Stark apparatus for 2–3 d under reflux. The solvent was removed *in vacuo* and the residue was redissolved in MeCN (6 mL per 1 mmol of aldehyde). To a part of that solution (3 mL per 100 µmol of **2**) **$2BF_4$** was added and the reaction mixture was stirred under reflux for 3–40 h. After cooling to r.t., the mixture was added dropwise to Et_2O (20 mL per 1 mL of MeCN) under vigorous stirring. The precipitate was filtered, washed with water (2 x 20 mL), cold MeOH (10 mL) and Et_2O (3 x 20 mL), which gave the essentially pure product. An analytically pure sample was obtained by recrystallization.

*(E)-8-Styrylcoralyne tetrafluoroborate (**4a**)*

According to GP 2 freshly distilled benzaldehyde (**3a**) (265 mg, 2.50 mmol, 253 µL) was made to react with piperidine (426 mg, 5.00 mmol, 495 µL) and *p*-TsOH (10 mg) in toluene (25 mL) for 40 h. The intermediate was treated with **$2BF_4$** (102 mg, 226 µmol) for 3 h. The product **4a** was obtained as orange coloured amorphous solid (80.0 mg, 148 µmol, 66%). An analytically pure sample was obtained by recrystallization from MeCN at –25 °C. – mp > 300 °C. – ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 3.96 (s, 3 H, 10-OMe), 3.99 (s, 3 H, 3-OMe), 4.14 (s, 6 H, 2-OMe, 11-OMe), 7.47 (d, ^3J = 17 Hz, 1 H, CH'), 7.52–7.54 (m, 1 H, 4'-H), 7.55 (s, 1 H, 9-H), 7.56–7.59 (m, 2 H, 3'-H, 5'-H), 7.66 (s, 1 H, 4-H), 7.68 (s, 1 H, 12-H), 7.79 (d, ^3J = 17 Hz, 1 H, CH), 7.91 (d, ^3J = 8 Hz, 1 H, 5-H), 7.99 (d, ^3J = 7 Hz, 2 H, 2'-H, 6'-H), 8.27 (s, 1 H, 1-H), 8.86 (d, ^3J = 8 Hz, 1 H, 6-H), 9.62 (s, 1 H, 13-H). – ^{13}C-NMR (150 MHz, DMSO-d_6): δ = 55.9 (10-OMe), 56.1 (3-OMe), 56.4 (2-OMe), 56.6 (11-OMe), 104.5 (C9), 104.7 (C12), 105.0 (C1), 108.2 (C4), 116.5 (C13), 117.6 (CH), 119.7 (C13b), 120.5 (C5), 121.6 (C8a), 124.1 (C4a), 125.5 (C6), 127.9 (C2', C6'), 128.9 (C3', C5'), 130.0 (C4'), 134.0 (C1'), 134.5 (C12a), 135.0 (C13a), 143.4 (CH'), 143.9 (C8), 151.5 (C2), 152.6 (C10), 152.8 (C3), 156.3 (C11). – El. Anal. for $C_{29}H_{26}BF_4NO_4$ x ¼ HBF_4 (548.34), calcd (%): C 62.06, H 4.71, N 2.50, found (%): C 61.87, H 4.62, N 2.37. – MS (ESI$^+$): *m*/*z* = 452 (100) $[M–BF_4]^+$.

*(E)-8-(4'-Dimethylaminostyryl)coralyne tetrafluoroborate (**4b**)*

According to GP 1 **2BF$_4$** (135 mg, 300 µmol) was made to react with 4-dimethylaminobenzaldehyde (**3b**) (179 mg, 1.20 mmol) and piperidine (102 mg, 1.20 mmol, 119 µL) in MeCN (10 mL) for 16 h. The product **4b** was obtained as dark brown, amorphous solid (63.0 mg, 108 µmol, 36%). An analytically pure sample was obtained by recrystallization from MeCN/MeOH 1/1 at –25 °C. – mp > 300 °C. – ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 3.05 (s, 6 H, 4'-NMe$_2$), 3.90 (s, 3 H, 10-OMe), 3.95 (s, 3 H, 3-OMe), 4.08 (s, 3 H, 11-OMe), 4.09 (s, 3 H, 2-OMe), 6.84 (d, ^3J = 8 Hz, 2 H, 3'-H, 5'-H), 7.29 (d, ^3J = 16 Hz, 1 H, CH'), 7.38 (d, ^3J = 16 Hz, 1 H, CH), 7.49 (s, 1 H, 4-H), 7.44 (s, 1 H, 9-H), 7.51 (s, 1 H, 12-H), 7.76–7.78 (m, 3 H, 2'-H, 6'-H, 5-H), 8.04 (s, 1 H, 1-H), 8.87 (d, ^3J = 8 Hz, 1 H, 6-H), 9.29 (s, 1 H, 13-H). – ^{13}C-NMR (150 MHz, DMSO-d_6): δ = 39.7 (overlapped with DMSO, 4'-NMe$_2$), 55.7 (10-OMe), 56.0 (3-OMe), 56.3 (2-OMe), 56.5 (11-OMe), 104.6 (C12), 104.7 (C9), 104.8 (C1), 108.0 (C4), 111.0 (CH), 111.8 (C3', C5'), 115.4 (C13), 119.4 (C13b), 120.1 (C5), 121.2 (C8a), 122.4 (C1'), 123.8 (C4a), 125.1 (C6), 129.5 (C2', C6'), 133.6 (C12a), 134.2 (C13a), 144.3 (CH'), 144.8 (C8), 151.3 (C2), 151.7 (C4'), 152.3 (C10), 152.5 (C3), 156.0 (C11). – El. Anal. for $C_{31}H_{31}BF_4N_2O_4$ x ⅓ HBF_4 (611.67), calcd (%): C 60.87, H 5.16, N 4.58, found: C 60.93, H 4.95, N 4.53. – MS (ESI$^+$): *m*/*z* = 495 (100) [M–BF$_4$]$^+$.

*(E)-8-(4'-Methylstyryl)coralyne tetrafluoroborate (**4c**)*

According to GP 1 **2BF$_4$** (226 mg, 500 µmol) was made to react with 4-methylbenzaldehyde (**3c**) (240 mg, 2.00 mmol, 236 µL) and piperidine (85.2 mg, 1.00 mmol, 100 µL) in MeCN (20 mL) for 8.5 h. The product **4c** was obtained as dark yellow, amorphous solid (180 mg, 325 µmol, 65%). An analytically pure sample was obtained by recrystallization from MeCN/MeOH 1/1 at –25 °C. – mp > 300 °C. – ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 2.43 (s, 3 H, 4'-Me), 3.92 (s, 3 H, 10-OMe), 3.96 (s, 3 H, 3-OMe), 4.11 (s, 6 H, 2-OMe, 11-OMe), 7.38 (d, ^3J = 17 Hz, 1 H, CH'), 7.39 (d, ^3J = 8 Hz, 2 H, 3'-H, 5'-H), 7.44 (s, 1 H, 9-H), 7.55 (s, 1 H, 4-H), 7.57 (s, 1 H, 12-H), 7.66 (d, ^3J = 17 Hz, 1 H, CH), 7.83–7.86 (m, 3 H, 2'-H, 6'-H, 5-H), 8.12 (s, 1 H, 1-H), 8.77 (d, ^3J = 8 Hz, 1 H, 6-H), 9.44 (s, 1 H, 13-H). – ^{13}C-NMR (150 MHz, DMSO-d_6): δ = 21.0 (4'-Me), 55.8 (10-OMe), 56.0 (3-OMe), 56.3 (2-OMe), 56.6 (11-OMe), 104.3 (C9), 104.6 (C12), 104.8 (C1), 108.0 (C4), 116.2 (C13), 116.3 (CH), 119.4 (C13b), 120.4 (C5), 121.4 (C8a), 124.0 (C4a), 125.2 (C6), 127.9 (C2', C6'), 129.5 (C3', C5'), 132.2 (C1'), 133.8 (C12a), 134.3 (C13a), 140.0 (C4'), 143.5 (CH'), 143.8 (C8), 151.4 (C2), 152.5 (C10), 152.6 (C3), 156.2 (C11). – El. Anal. for $C_{30}H_{28}BF_4NO_4$ (553.36), calcd (%): C 65.12, H 5.10, N 2.53, found (%): C 65.13, H 5.15, N 2.65. – MS (ESI$^+$): *m*/*z* = 466 (100) [M–BF$_4$]$^+$.

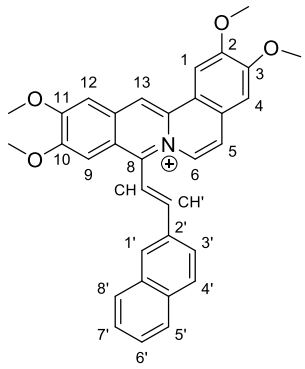
*(E)-8-(4'-Chlorostyryl)coralyne tetrafluoroborate (**4d**)*

According to GP 2 4-chlorobenzaldehyde (**3d**) (703 mg, 5.00 mmol) was made to react with piperidine (511 mg, 6.00 mmol, 594 µL) and *p*-TsOH (10 mg) in toluene (50 mL) for 3 d. The intermediate was treated with **2BF$_4$** (226 mg, 500 µmol) for 40 h. The product **4d** was obtained as ochre coloured amorphous solid (257 mg, 448 µmol, 90%). An analytically pure sample was obtained by recrystallization from MeCN/MeOH 1/1 at –25 °C. – mp > 300 °C. – ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 3.95 (s, 3 H, 10-OMe), 3.98 (s, 3 H, 3-OMe), 4.13 (s, 6 H, 2-OMe, 11-OMe), 7.45 (d, ^3J = 17 Hz, 1 H, CH'), 7.50 (s, 1 H, 9-H), 7.62 (s, 1 H, 4-H), 7.64 (d, ^3J = 8 Hz, 2 H, 3'-H, 5'-H), 7.65 (s, 1 H, 12-H), 7.79 (d, ^3J = 17 Hz, 1 H, CH), 7.89 (d, ^3J = 8 Hz, 1 H, 5-H), 7.99 (d, ^3J = 8 Hz, 2 H, 2'-H, 6'-H), 8.22 (s, 1 H, 1-H), 8.83 (d, ^3J = 8 Hz, 1 H, 6-H), 9.57 (s, 1 H, 13-H). – ^{13}C-NMR (150 MHz, DMSO-d_6): δ = 55.9 (10-OMe), 56.1 (3-OMe), 56.4 (2-OMe), 56.6 (11-OMe), 104.4 (C9), 104.6 (C12), 105.0 (C1), 108.1 (C4), 116.6 (C13), 118.4 (CH), 119.6 (C13b), 120.5 (C5), 121.6 (C8a), 124.1 (C4a), 125.4 (C6), 128.9 (C3', C5'), 129.7 (C2', C6'), 133.9 (C1'), 133.9 (C12a), 134.5 (C13a), 134.6 (C4'), 142.1 (CH'), 143.5 (C8), 151.5 (C2), 152.7 (C10), 152.8 (C3), 156.3 (C11). – El. Anal. for $C_{29}H_{25}BClF_4NO_4$ (573.78), calcd (%): C 60.71, H 4.39, N 2.44, found (%): C 60.62, H 4.48, N 2.63. – MS (ESI$^+$): *m*/*z* = 486 (100) $[M–BF_4]^+$.

*(E)-8-(4'-Methoxystyryl)coralyne tetrafluoroborate (**4e**)*

According to GP 2 4-methoxybenzaldehyde (**3e**) (681 mg, 5.00 mmol, 608 µL) was made to react with piperidine (852 mg, 10.0 mmol, 990 µL) and *p*-TsOH (10 mg) in toluene (50 mL) for 54 h. The intermediate was treated with **2BF$_4$** (180 mg, 400 µmol) for 3 h. The product **4e** was obtained as orange coloured amorphous solid (182 mg, 320 µmol, 80%). An analytically pure sample was obtained by recrystallization from MeCN at –25 °C. – mp > 300 °C. – ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 3.88 (s, 3 H, 4'-OMe), 3.92 (s, 3 H, 10-OMe), 3.94 (s, 3 H, 3-OMe), 4.09 (s, 6 H, 2-OMe, 11-OMe), 7.13 (d, ^3J = 9 Hz, 2 H, 3'-H, 5'-H), 7.36 (d, ^3J = 17 Hz, 1 H, CH'), 7.42 (s, 1 H, 9-H), 7.51–7.55 (m, 3 H, CH, 4-H, 12-H), 7.79 (d, ^3J = 8 Hz, 1 H, 5-H), 7.91 (d, ^3J = 9 Hz, 2 H, 2'-H, 6'-H), 8.06 (s, 1 H, 1-H), 8.75 (d, ^3J = 8 Hz, 1 H, 6-H), 9.35 (s, 1 H, 13-H). – ^{13}C-NMR (150 MHz, DMSO-d_6): δ = 55.4 (4'-OMe), 55.8 (10-OMe), 56.0 (3-OMe), 56.3 (2-OMe), 56.5 (11-OMe), 104.4 (C9), 104.6 (C12), 104.7 (C1), 107.9 (C4), 114.4 (C3', C5'), 114.6 (CH), 115.9 (C13), 119.5 (C13b), 120.3 (C5), 121.3 (C8a), 123.9 (C4a), 125.1 (C6), 127.5 (C1'), 129.6 (C2', C6'), 133.7 (C12a), 134.1 (C13a), 143.2 (CH'), 143.9 (C8), 151.3 (C2), 152.5 (C10), 152.6 (C3), 156.1 (C11), 161.0 (C4'). – El. Anal. for $C_{30}H_{28}BF_4NO_5$ (569.36), calcd (%): C 63.29, H 4.96, N 2.46, found (%): C 63.27, H 4.85, N 2.64. – MS (ESI$^+$): *m*/*z* = 482 (100) $[M–BF_4]^+$.

*(E)-8-[2-(Naphth-2-yl)vinyl]coralyne tetrafluoroborate (**4f**)*



According to GP 1 **2BF$_4$** (135 mg, 300 µmol) was made to react with 2-naphthaldehyde (**3f**) (187 mg, 1.20 mmol) and piperidine (102 mg, 1.20 mmol, 119 µL) in MeCN (10 mL) for 16 h. The product **4f** was obtained as ochre coloured, amorphous solid (127 mg, 215 µmol, 72%). An analytically pure sample was obtained by recrystallization from MeCN/MeOH (1/1; and a few drops of $MeNO_2$) at –25 °C. – mp > 300 °C. – ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 3.96 (s, 3 H, 10-OMe), 4.01 (s, 3 H, 3-OMe), 4.15 (s, 6 H, 2-OMe, 11-OMe), 7.61–7.65 (m, 4 H, 9-H, CH, 6'-H, 7'-H), 7.69 (s, 1 H, 4-H), 7.73 (s, 1 H, 12-H), 7.95 (d, ^3J = 8 Hz, 1 H, 5-H), 7.96 (d, ^3J = 17 Hz, 1 H, CH), 8.01–8.03 (m, 2 H, 5'-H, 8'-H), 8.11 (d, ^3J = 8 Hz, 1 H, 4'-H), 8.24 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1 H, 3'-H), 8.33 (s, 1 H, 1-H), 8.35 (s, 1 H, 1'-H), 8.95 (d, ^3J = 8 Hz, 1 H, 6-H), 9.69 (s, 1 H, 13-H). – ^{13}C-NMR (150 MHz, DMSO-d_6): δ = 56.0 (10-OMe), 56.1 (3-OMe), 56.4 (2-OMe), 56.7 (11-OMe), 104.6 (C9), 104.8 (C12), 105.2 (C1), 108.3 (C4), 116.6 (C13), 118.0 (CH), 119.8 (C13b), 120.6 (C5), 121.7 (C8a), 124.0 (C3'), 124.2 (C4a), 125.6 (C6), 126.8 (C7'), 127.2 (C6'), 127.7 (C5'), 128.4 (C8'), 128.5 (C4'), 129.0 (C1'), 132.6 (C2'), 132.9 (C8a'), 133.6 (C4a'), 134.1 (C12a), 134.7 (C13a), 143.5 (CH'), 144.1 (C8), 151.5 (C2), 152.7 (C10), 152.8 (C3), 156.3 (C11). – El. Anal. for $C_{33}H_{28}BF_4NO_4$ (589.39), calcd (%): C 67.25, H 4.79, N 2.38, found: C 66.77, H 4.52, N 2.83. – MS (ESI$^+$): *m*/*z* = 502 (100) $[M–BF_4]^+$.

*(E)-8-[2-(Anthracene-9-yl)vinyl]coralyne tetrafluoroborate (**4g**)*

According to GP 1 **2BF$_4$** (135 mg, 300 µmol) was made to react with anthracene-9-carbaldehyde (**3g**) (247 mg, 1.20 mmol) and piperidine (102 mg, 1.20 mmol, 119 µL) in MeCN (10 mL) for 16 h. The ^{1}H-NMR spectroscopic analysis of the crude product revealed that coralyne was decomposed during the reaction.

2 Spectroscopic data

2.1 Spectrofluorimetric titrations

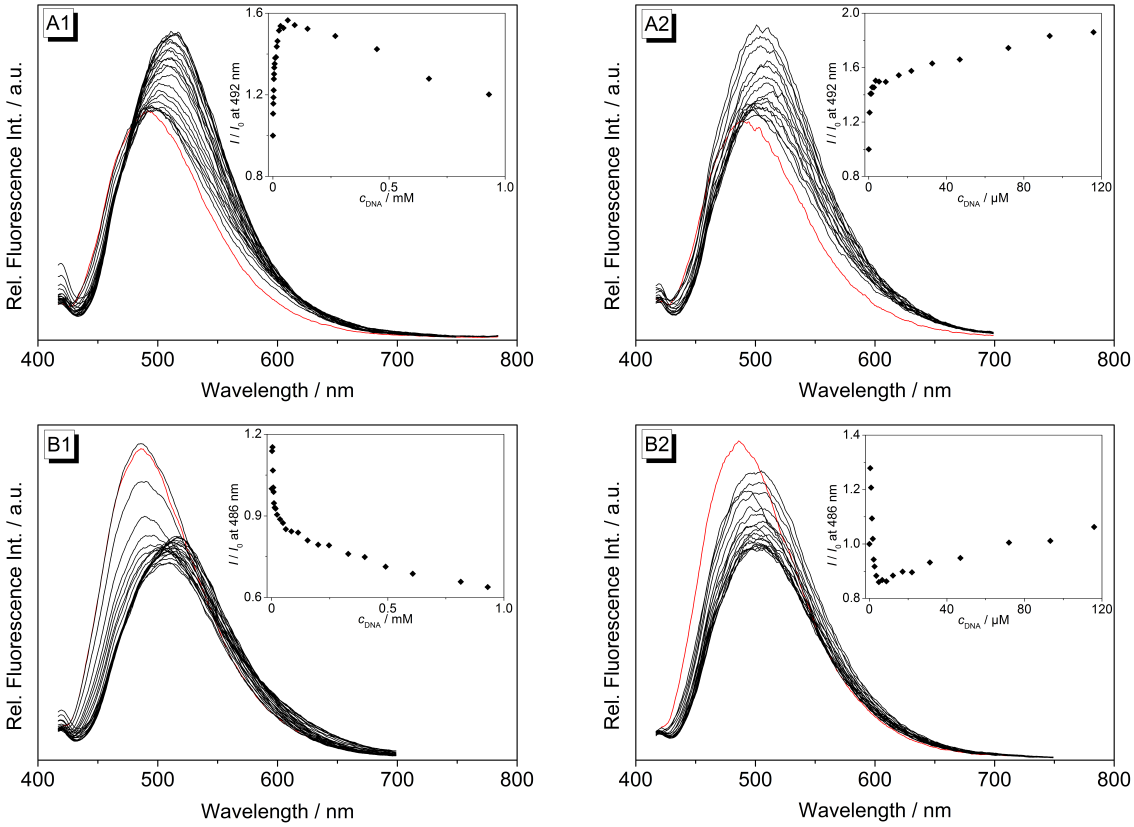


Figure S1. Spectrofluorimetric titration of ct DNA (A1, B1) and **22AG** (A2, B2) to the coralyne derivatives **4c** (A) and **4d** (B) in phosphate buffer (c_{Na^+} = 16 mM, pH 7.0; with 10% v/v DMSO) [c_L = 20 µM, $c_{ct\text{-}DNA,\ bp}$ = 1.34 mM, $c_{\mathbf{22AG}}$ = 360 µM; λ_{ex} = 400 nm]. Insets: Plot of the relative fluorescence intensity I / I_0 (corrected with regard to the change in absorption at the excitation wavelength) *versus* DNA concentration. The emission spectra of the pure ligand solutions are depicted in red.

2.2 Emission spectra of 4b

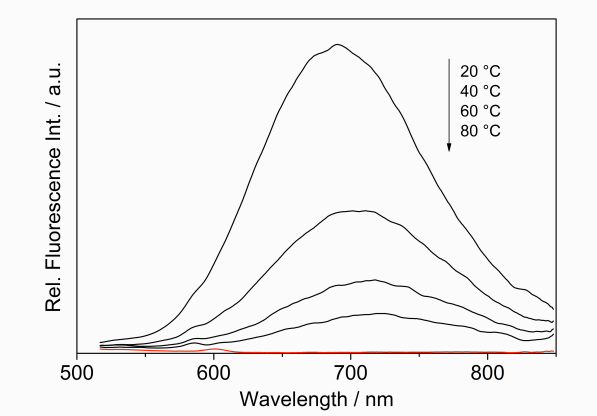


Figure S2. Emission spectra of **4b** (c = 2.5 µM, with 0.25% v/v DMSO) in glycerol (black lines) and in phosphate buffer (c_{Na^+} = 16 mM, pH 7.0) at 20 °C (red line); λ_{ex} = 500 nm. The arrow indicates the change in emission intensity with increasing temperature.

2.3 Thermal Denaturation Experiments

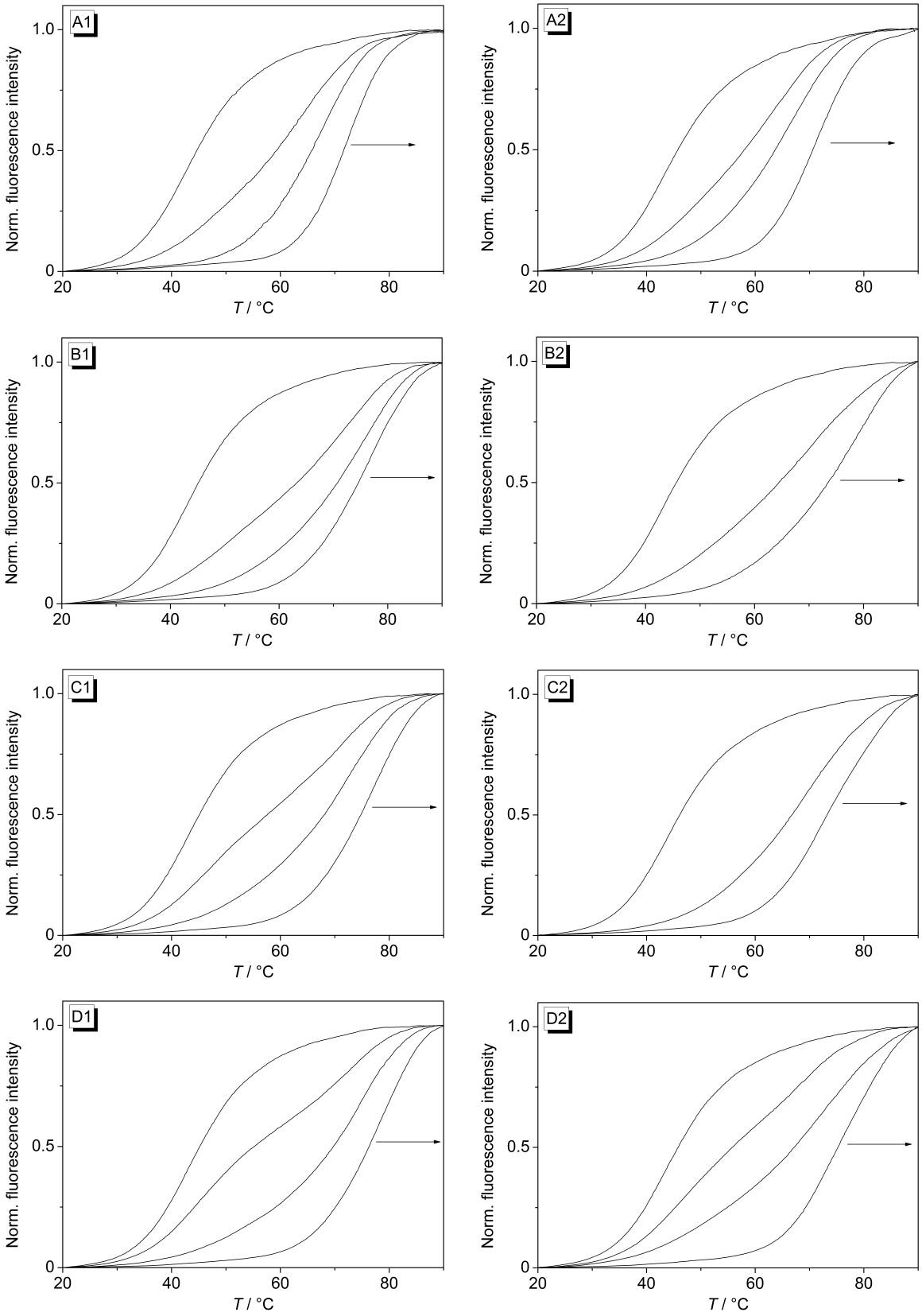


Figure S3. Normalized changes in emission intensity of **F21T** (0.2 μM) at 515 nm between 20 °C and 90 °C in presence of the ligands **2** (A), **4b** (B), **4c** (C) and **4d** (D) without (1) and with (2) the duplex DNA **ds26** (3 μM) in aqueous KCl-LiCl-Na-cacodylate buffer (10 mM K^+, 10 mM Na^+, 90 mM Li^+, pH 7.2) at different ligand-DNA ratios (0, 1.3, 2.5, 5.0); λ_{ex} = 470 nm. In B2 and C2 the melting profile for *LDR* = 1.3 has been omitted.

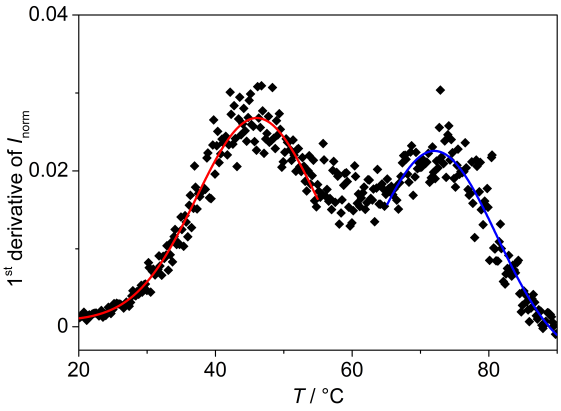


Figure S4. First derivative of the normalized change in emission intensity (Figure S3 D1) at *LDR* = 1.3. The red and the blue line represent the best fit according to the Gaussian function of the first maximum at $T_m \approx 46$ °C and of second maximum at $T_m \approx 72$ °C, respectively.

Table S2. Selectivity of DNA Stabilization of the Ligands **2** and **4b–d**.

	$\Delta\Delta T_m$ / ° C at different *LDR*[a]	
Ligand	2.5	5.0
2	1.7	0.5
4b	6.9	0.9
4c	2.6	2.7
4d	2.6	1.5

[a] $\Delta\Delta T_m = \Delta T_m$ (**F21T** + **ds26**) – ΔT_m (**F21T**); conditions cf. Table 3.

3 ^{1}H-NMR spectra

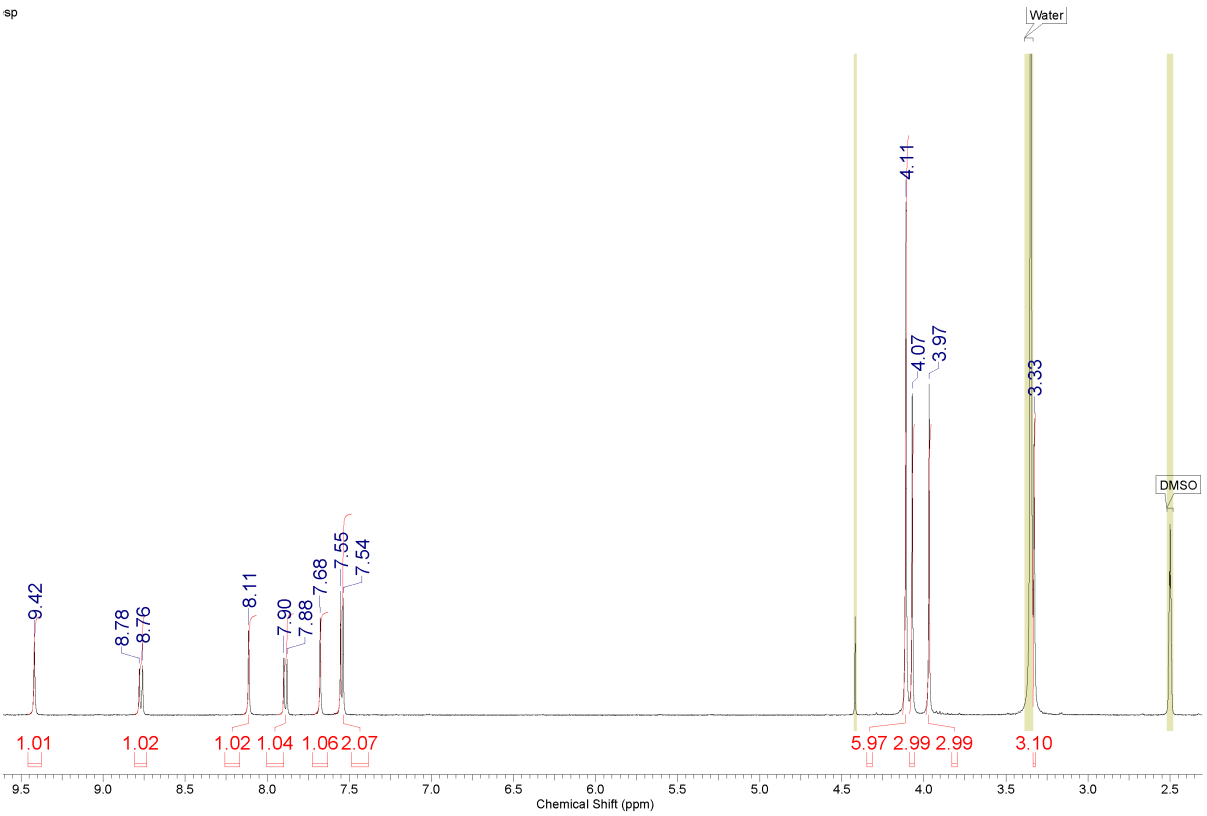


Figure S5. ^{1}H-NMR spectrum (400 MHz) of coralyne tetrafluoroborate (**2BF$_4$**) in DMSO-d_6.

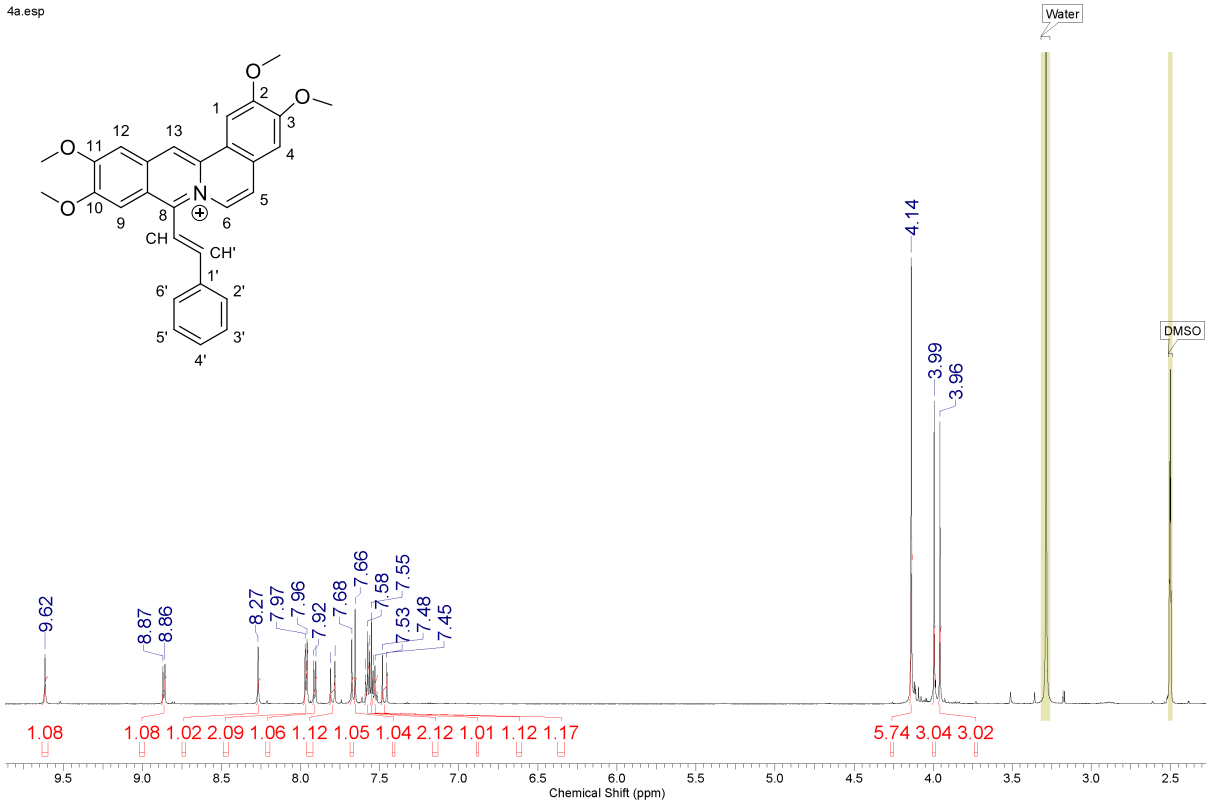


Figure S6. ^{1}H-NMR spectrum (600 MHz) of (*E*)-8-styrylcoralyne tetrafluoroborate (**4a**) in DMSO-d_6.

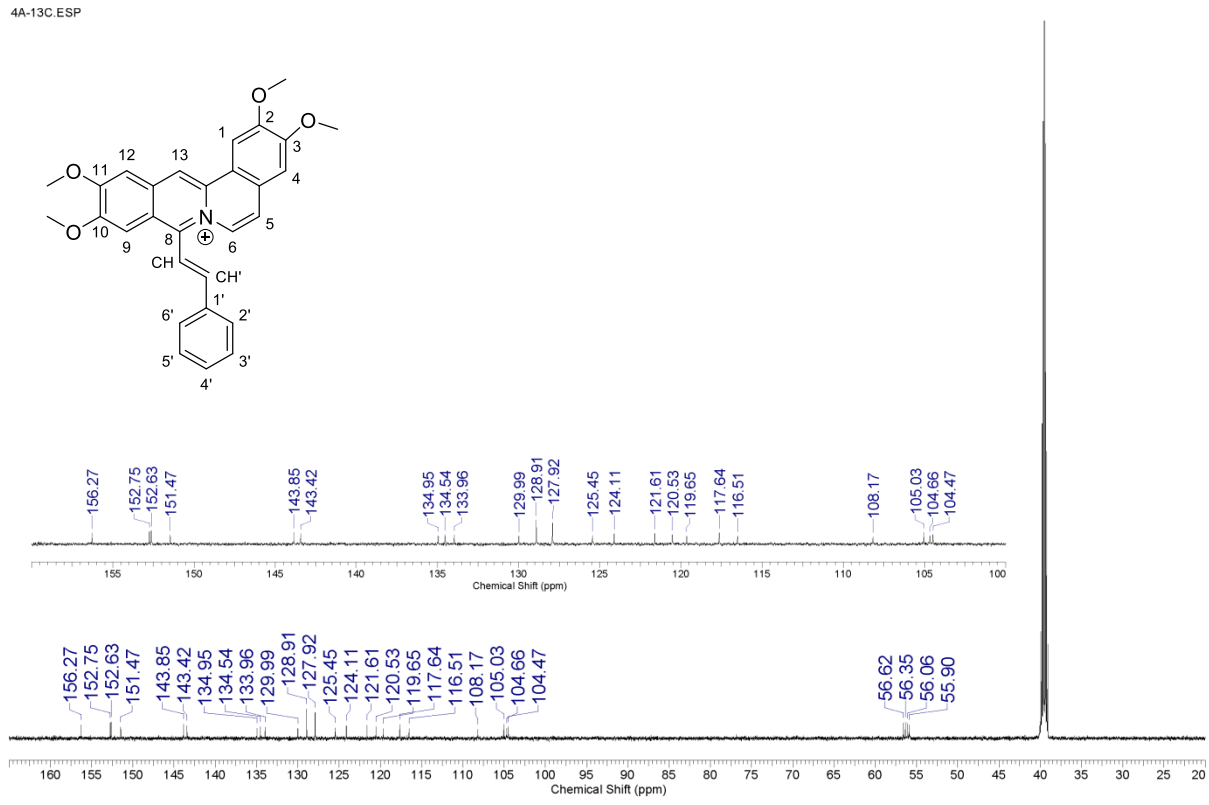


Figure S7. ^{13}C-NMR spectrum (150 MHz) of (*E*)-8-styrylcoralyne tetrafluoroborate (**4a**) in DMSO-d_6.

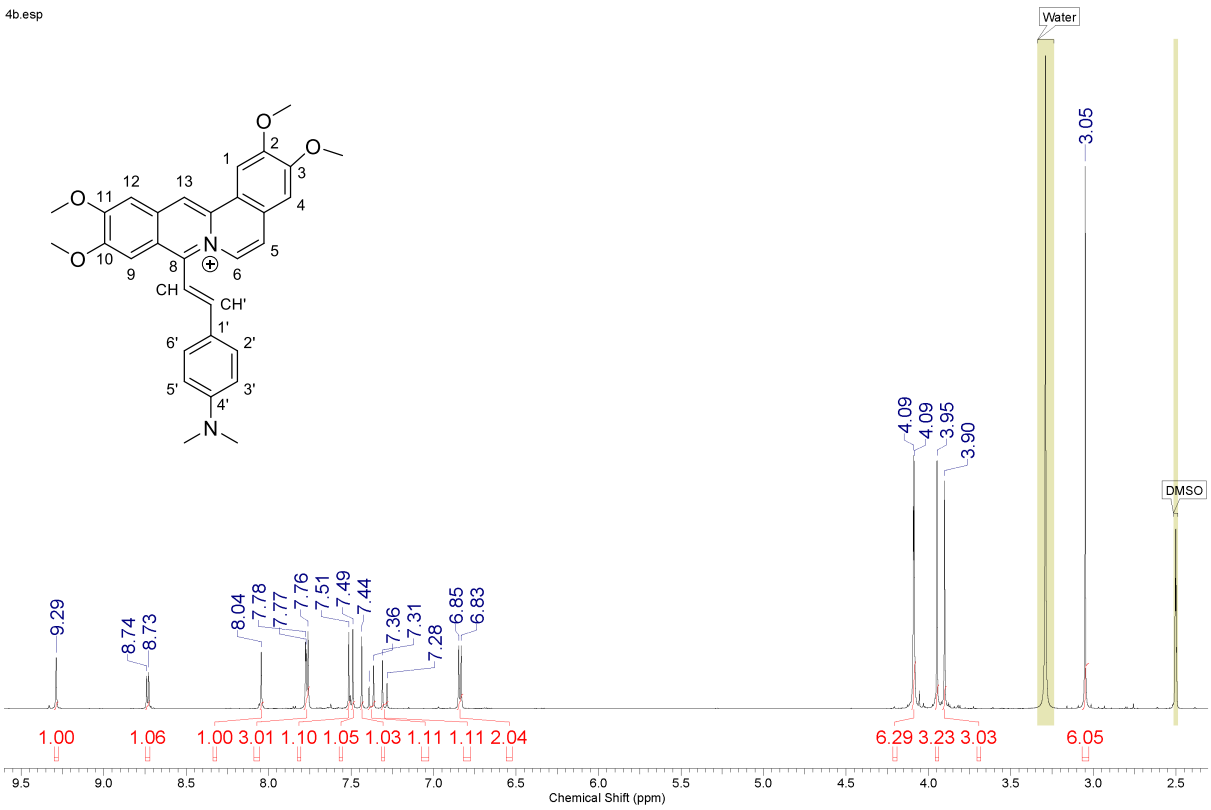


Figure S8. ^{1}H-NMR spectrum (600 MHz) of (*E*)-8-(4'-dimethylaminostyryl)coralyne tetrafluoroborate (**4b**) in DMSO-d_6.

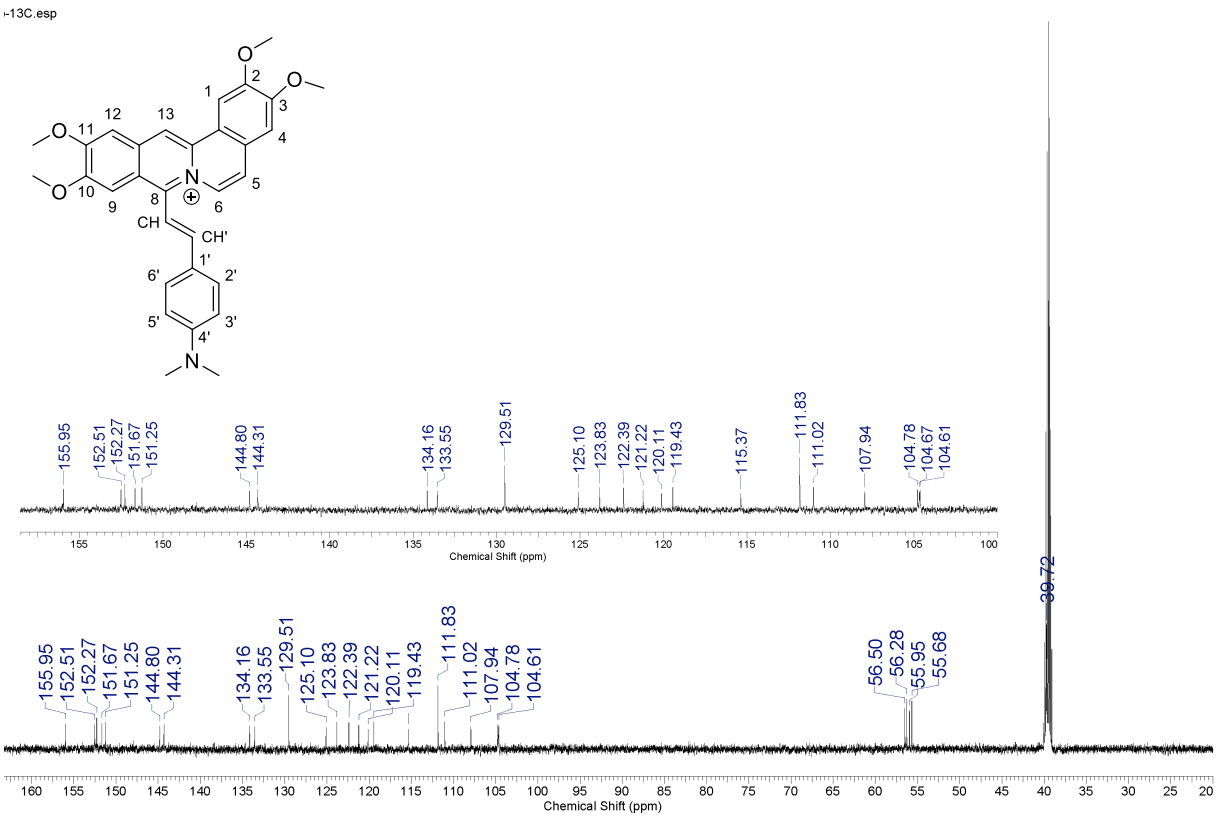


Figure S9. ^{13}C-NMR spectrum (150 MHz) of (*E*)-8-(4'-dimethylaminostyryl)coralyne tetrafluoroborate (**4b**) in DMSO-d_6.

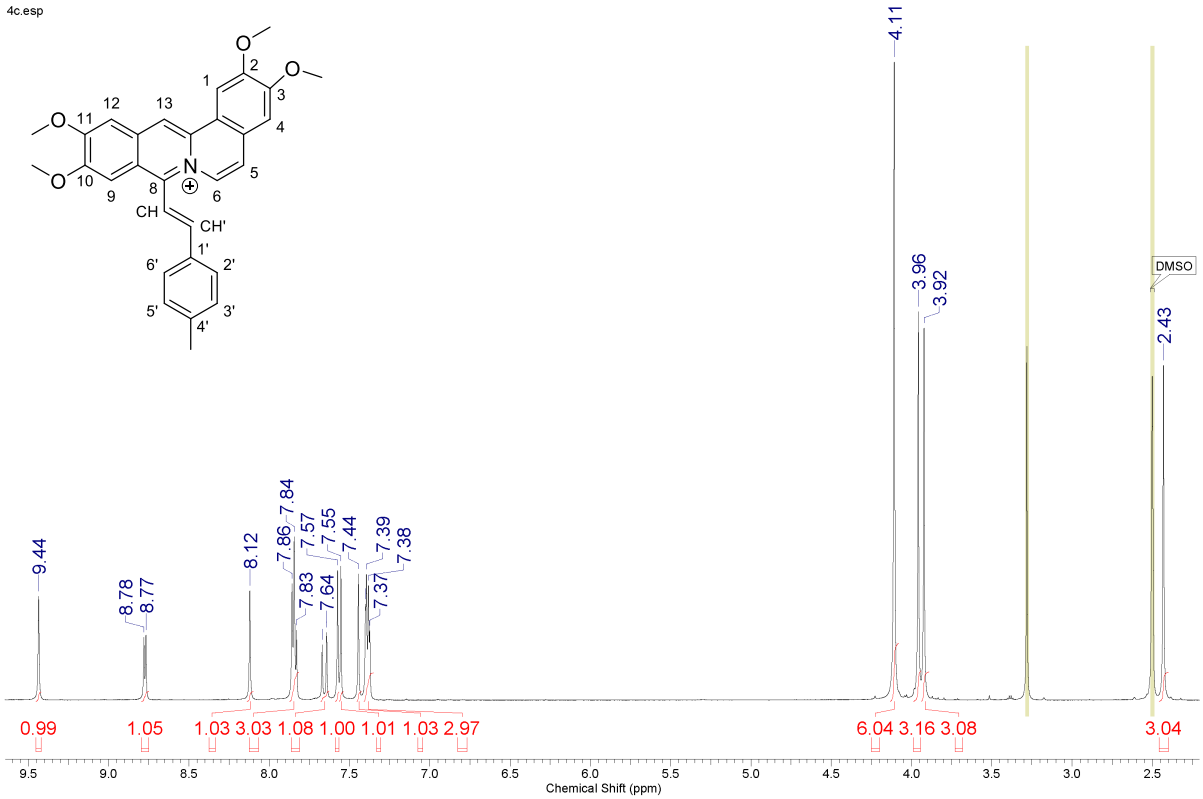


Figure S10. [1]H-NMR spectrum (600 MHz) of (*E*)-8-(4'-methylstyryl)coralyne tetrafluoroborate (**4c**) in DMSO-d_6.

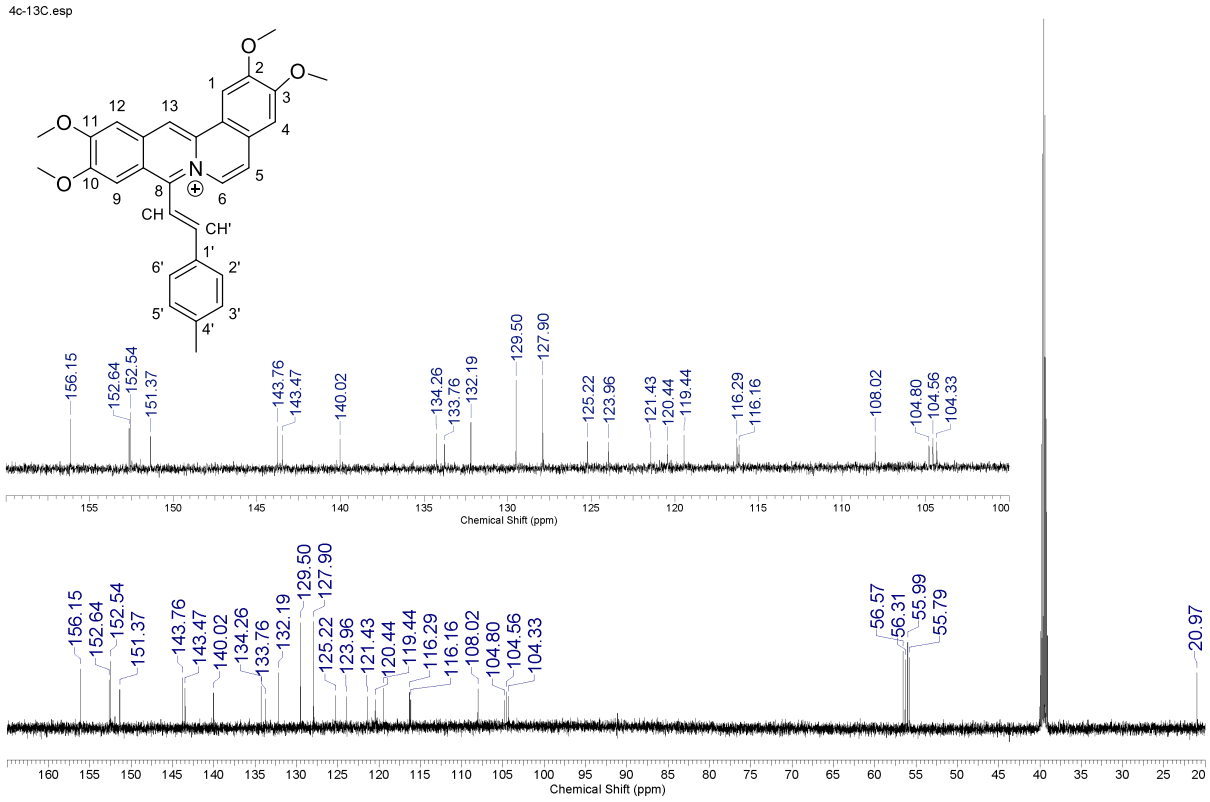


Figure S11. [13]C-NMR spectrum (150 MHz) of (*E*)-8-(4'-methylstyryl)coralyne tetrafluoroborate (**4c**) in DMSO-d_6.

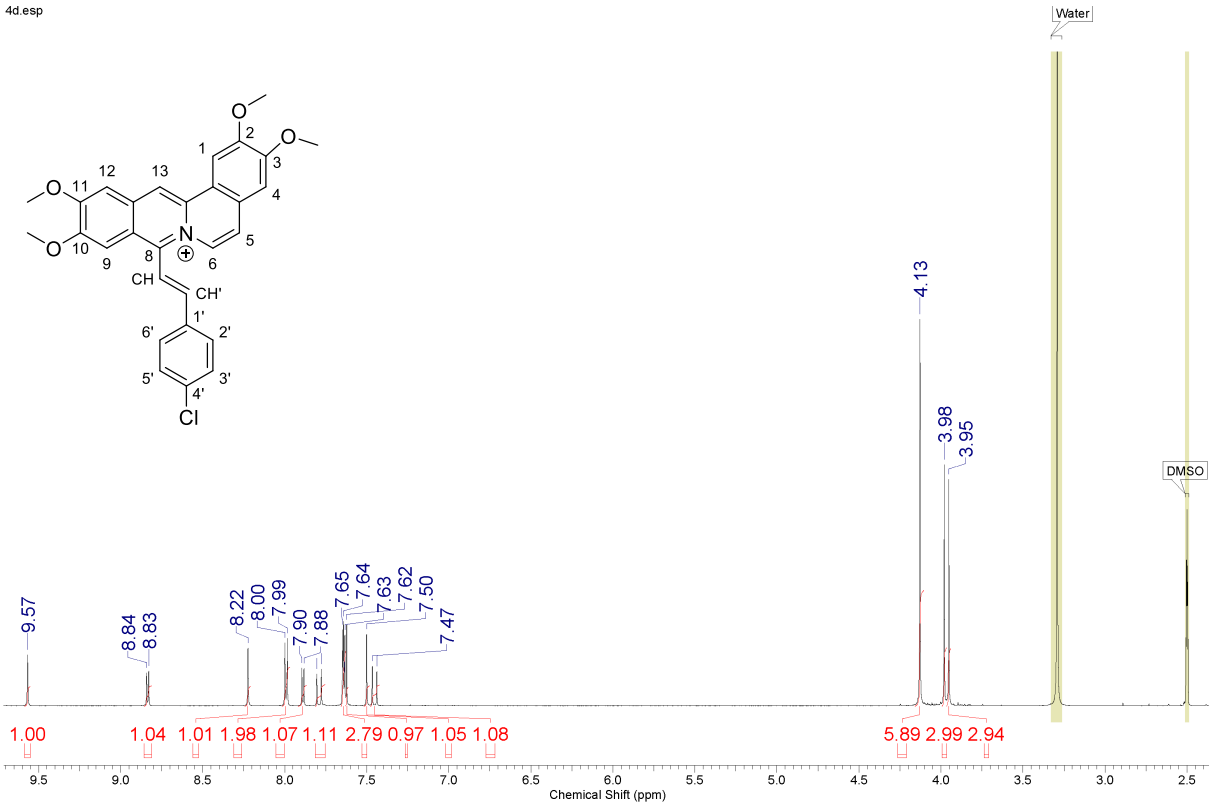


Figure S12. [1]H-NMR spectrum (600 MHz) of (*E*)-8-(4'-chlorostyryl)coralyne tetrafluoroborate (**4d**) in DMSO-d_6.

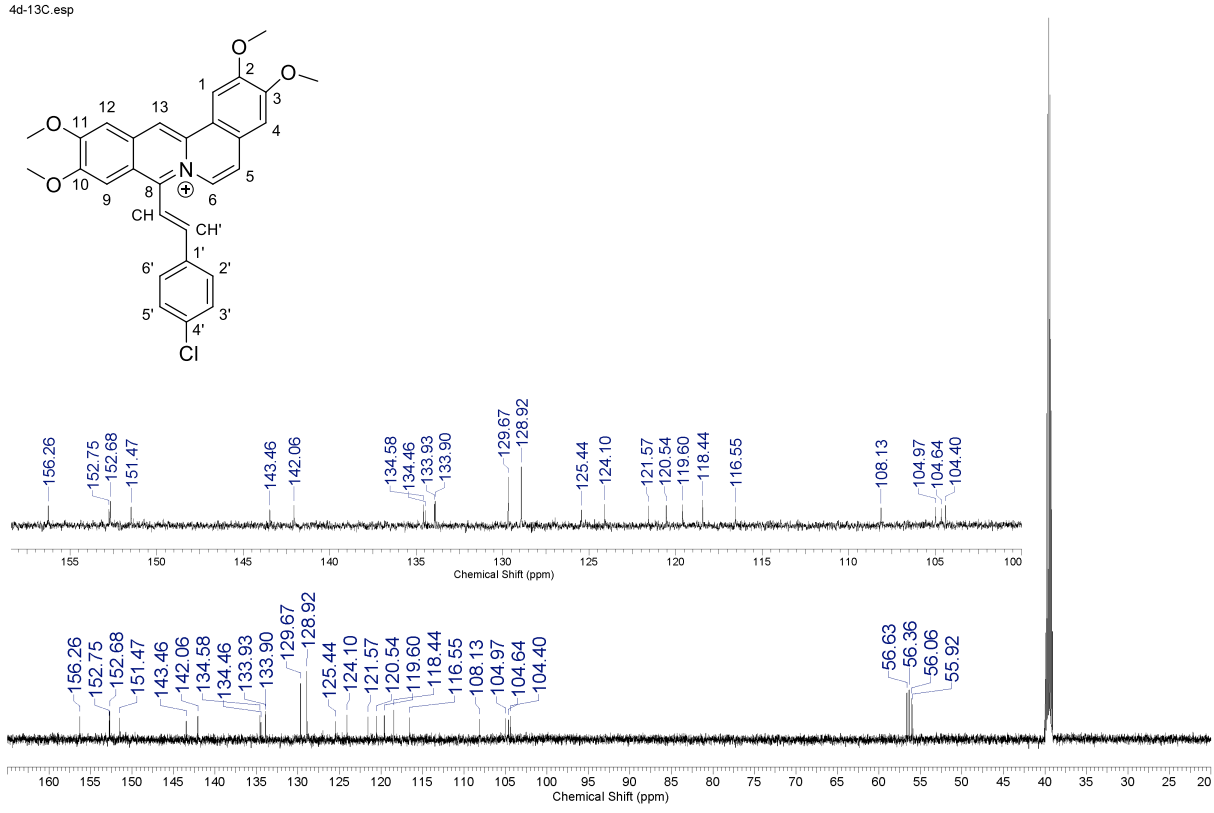


Figure S13. [13]C-NMR spectrum (150 MHz) of (*E*)-8-(4'-chlorostyryl)coralyne tetrafluoroborate (**4d**) in DMSO-d_6.

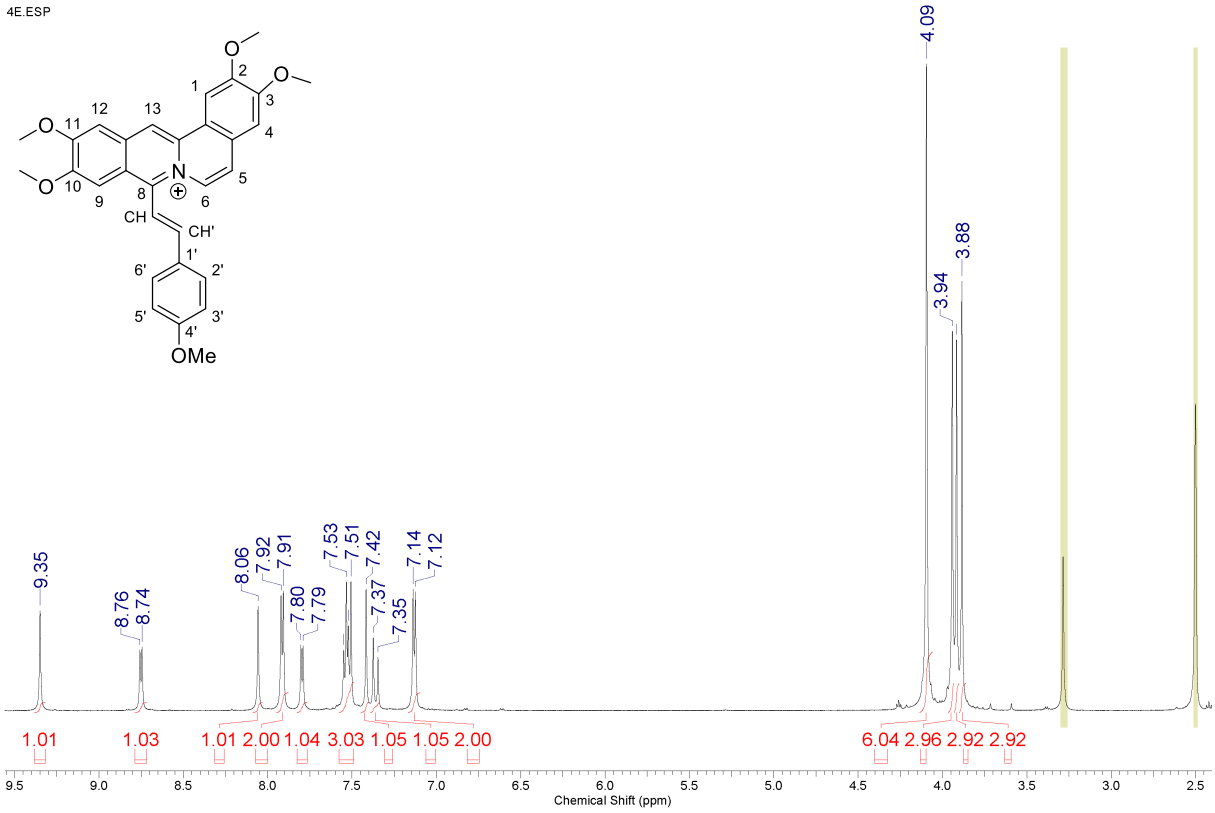


Figure S14. ^{1}H-NMR spectrum (600 MHz) of (*E*)-8-(4'-methoxystyryl)coralyne tetrafluoroborate (**4e**) in DMSO-d_6.

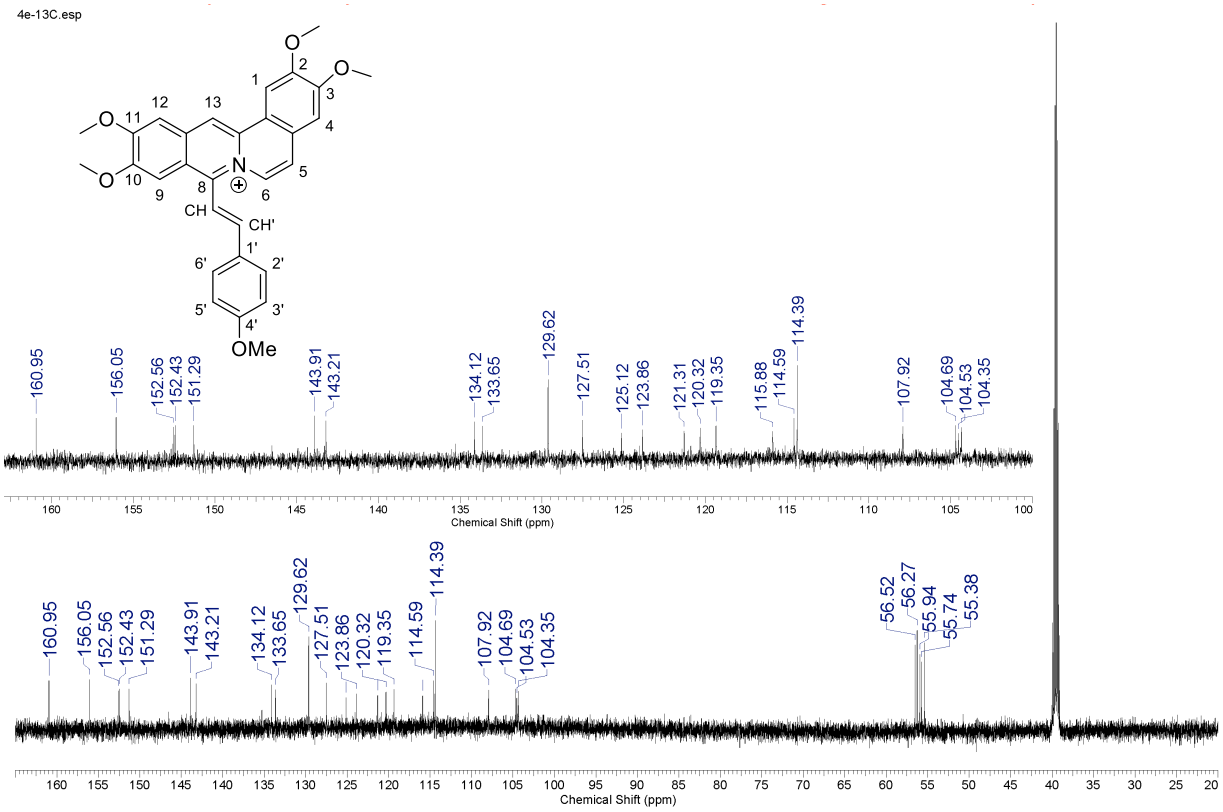


Figure S15. ^{13}C-NMR spectrum (150 MHz) of (*E*)-8-(4'-methoxystyryl)coralyne tetrafluoroborate (**4e**) in DMSO-d_6.

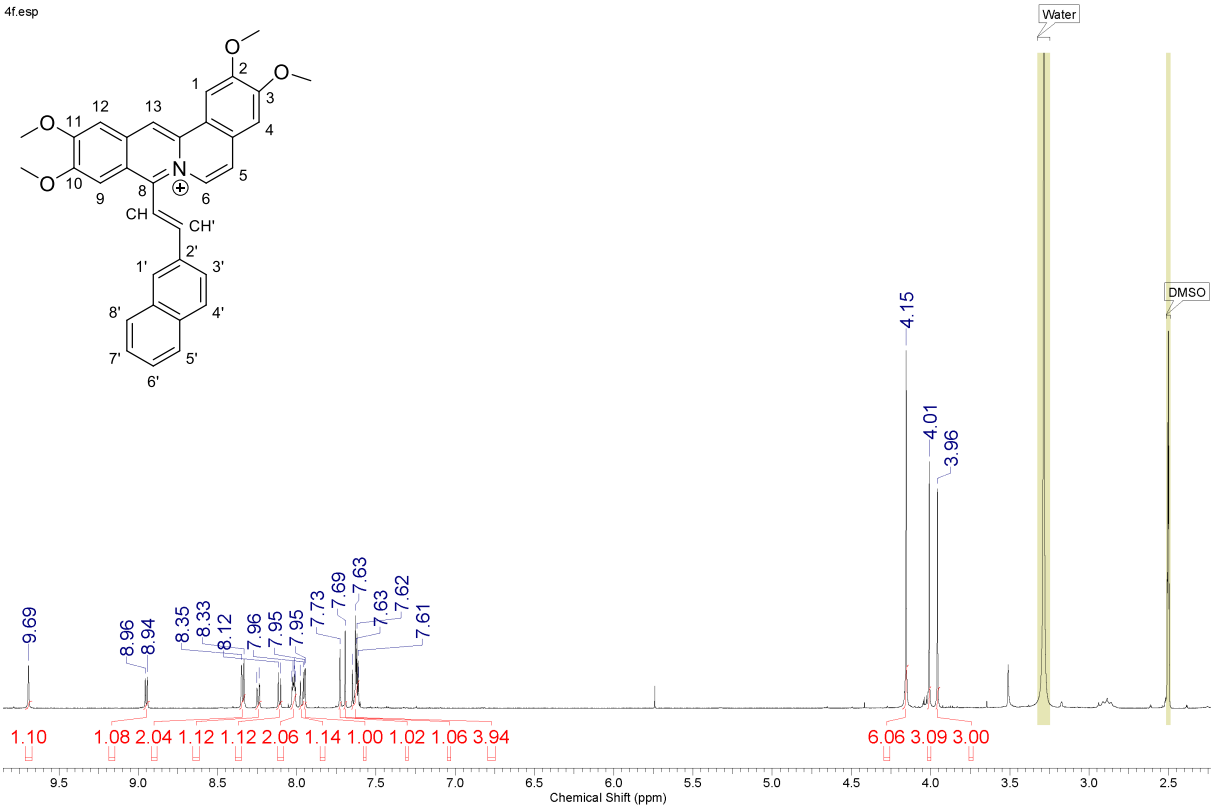


Figure S16. ^{1}H-NMR spectrum (600 MHz) of (*E*)-8-[2-(naphth-2-yl)vinyl]coralyne tetrafluoroborate (**4f**).

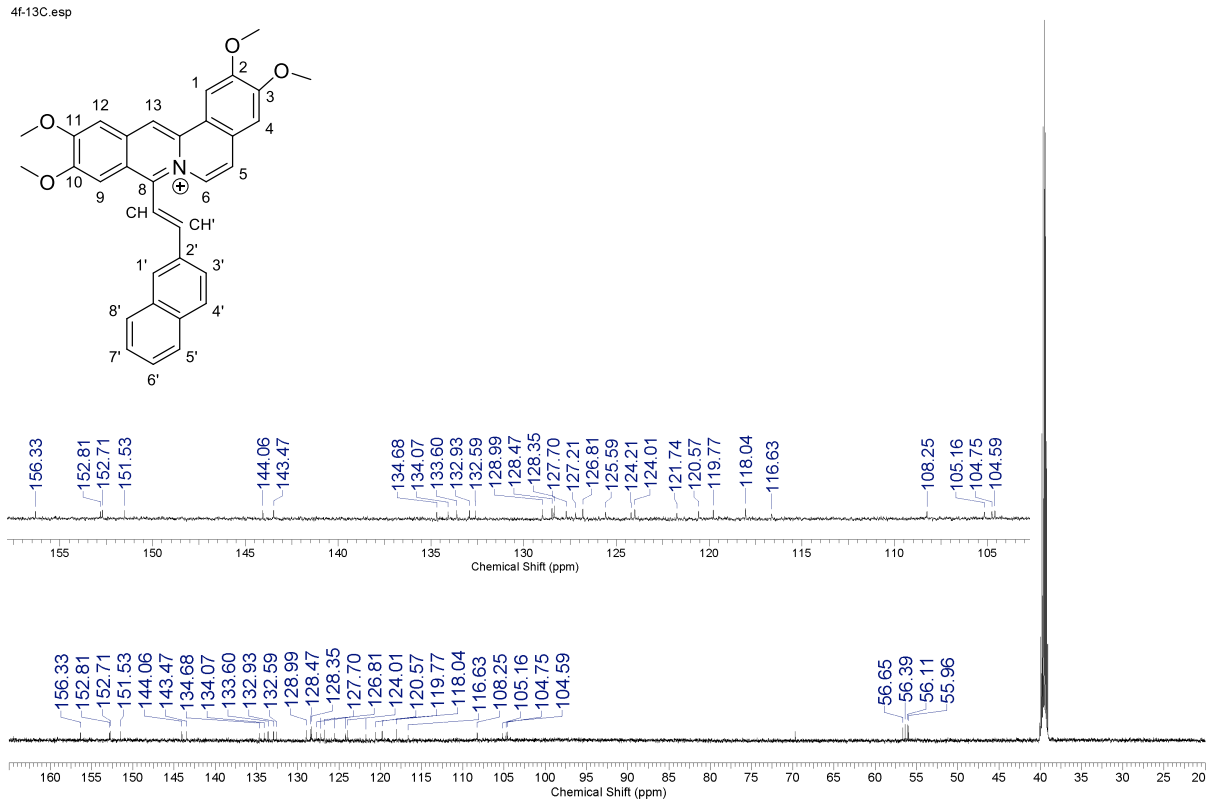


Figure S17. ^{13}C-NMR spectrum (150 MHz) of (*E*)-8-[2-(naphth-2-yl)vinyl]coralyne tetrafluoroborate (**4f**).

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