Electronic Supplementary Information (ESI) for:

Thermally-Activated Chemiluminescent Squaraine Rotaxane Endoperoxide With Green Emission

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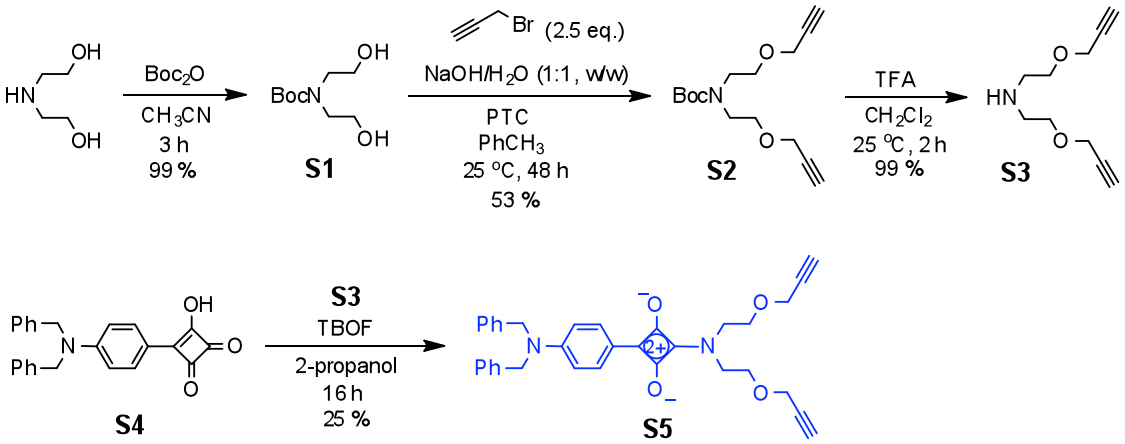
A. Synthesis

Synthesis of Squaraine Dye S5:



Scheme S1: Synthesis of squaraine dye **S5**.

Synthesis of boc-protected diethanolamine (S1)[S1]

Commercially available diethanolamine (1.0 g, 9.9 mmol) was dissolved in acetonitrile (30 mL) in a dried flask under an atmosphere of nitrogen. Di-*t*-butyl-dicarbonate (2.3 g, 11 mmol) was separately dissolved in acetonitrile (20 mL) and added dropwise to the reaction flask over 30 mins. After 3 hours, the solution was evaporated under reduced pressure to yield **S1**, a clear, viscous oil that was used without further purification (2.0 g, 99 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.24 (br. s, 1H), 4.09 (br. s, 1H), 3.76 (br. s, 4H), 3.40 (br. s, 4H), 1.45 (s, 9H).

Synthesis of boc-protected bis(2-(prop-2-yn-1-yloxy)ethyl)amine (S2)

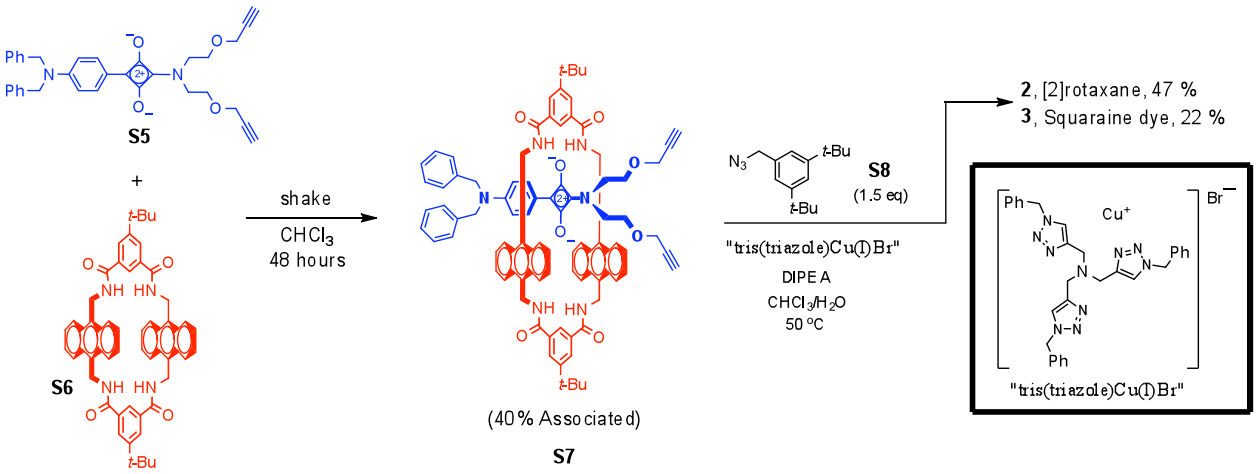
A 50 % $NaOH_{(aq)}$ solution was made by dissolving NaOH (15 g) in H_2O (15 mL). **S1** (11 g, 52 mmol) was dissolved in toluene (30 mL) and slowly poured over the aqueous solution. Phase-transfer catalyst (PTC), tetrabutylammonium bisulfate (40 mg, 0.12 mmol) and propargyl bromide (18 mL, 200 mmol) were added and the layers were gently stirred for 48 h. The organic layer was isolated and evaporated under reduced pressure giving a crude, yellow oil. Purification using column chromatography with silica gel and EtOAc/hexanes (1:1) as the eluent gave pure **S2** (7.8 g, 53 %) as a yellow, viscous oil that slowly decomposes at room temperature. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13 (d, J = 2.5 Hz, 4H), 3.67-3.57 (m, 4H), 3.52-3.36 (m, 4H), 2.41 (t, J = 2.5 Hz, 2H), 1.44 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$, 40°C) δ 155.6, 85.2, 79.9, 74.5, 68.7, 58.3, 48.0, 28.5; HRMS (ESI-TOF) calculated for $C_{15}H_{23}NNaO_4$ $[M+Na]^+$ 304.1516; found 304.1519.

Synthesis of bis(2-(prop-2-yn-1-yloxy)ethyl)amine (S3)

Removal of the protecting group in **S2** (7.8 g, 43 mmol) was achieved using TFA (5 mL) in dichloromethane (15 mL). After 2 h at room temperature, the solvent and excess TFA were removed by reduced pressure. Pure **S3** (99 % yield) was obtained and the product was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (br. s, 1H), 4.22 (d, J = 2.5 Hz, 4H), 3.84 (t, J = 16.0 Hz, 4H), 3.38 (br. s, 4H), 2.51 (t, J = 2.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 76.0, 63.9, 58.6, 47.3, 43.8; HRMS (ESI-TOF) calculated for $C_{10}H_{16}NO_2$ $[M+H]^+$ 182.1176; found 182.1175.

Synthesis of squaraine dye (S5)

Dibenzyl-semi-squaraine **S4**[S2] (490 mg, 1.3 mmol) was dissolved in anhydrous 2-propanol (30 mL) under an atmosphere of nitrogen. **S3** (230 mg, 1.2 mmol) was separately dissolved in anhydrous 2-propanol (15 mL) and added dropwise to the reaction flask. Drying agent, tri-n-butyl orthoformate (1.5 mL) was added to the solution and the reaction was refluxed for 16 h. Concentration under reduced pressure provided crude material that was further purified by column chromatography with silica gel and EtOAc:CH_2Cl_2 (1:5) as eluent to give **S5** (25 %, 170 mg) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, J = 9.0 Hz, 2H), 7.35 (d, J = 10.0 Hz, 2H), 7.29 (d, J = 10.0 Hz, 2H), 7.22 (d, J = 10.0 Hz, 4H), 6.79 (d, J = 9.0 Hz, 2H), 4.73 (s, 4H), 4.26 (t, J = 8.5 Hz, 4H), 4.19 (d, J = 4.0 Hz, 4H), 3.87 (t, J = 8.0 Hz, 4H), 2.45 (t, J = 4.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.6, 152.1, 137.3, 130.2, 129.0, 127.6, 126.6, 119.8, 112.2, 79.1, 75.4, 68.3, 58.5, 54.0, 50.6; HRMS (ESI-TOF) calculated for $C_{34}H_{33}N_2O_4$ $[M+H]^+$ 533.2435; found 533.2425.



Scheme S2: Synthesis of capped squaraine dye **3** and [2]rotaxane **2**.

Synthesis of squaraine rotaxane (2)[S3]

Dye **S5** (45 mg, 0.084 mmol) and macrocycle **S6** (75 mg, 0.089 mmol) were stirred in chloroform (3 mL) for 48 h. Azide **S8** (57 mg, 0.23 mmol), *N*,*N*-diisopropyethylamine (59 mg, 0.46 mmol), and organic soluble tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine copper(I)bromide (5.5 mg, 0.0084 mmol) were added and a layer of water (1 mL) was added to the top of the solution. The reaction was heated to 50 °C for 48 h. The organic layer was separated and copper salts were removed by washing the reaction mixture with Si-EDTA (Silicycle) copper scavenger (80 mg) for 2 hours. Concentration under reduced pressure provided crude material that was further purified by column chromatography with silica gel and EtOAc:$CHCl_3$ (1:1) as eluent to give **2** (47 %, 73 mg) as a yellow-orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (s, 6H), 8.03 (dd, J = 6.5 Hz, J = 3.0 Hz, 4H), 7.77 (dd, J = 7.0 Hz, J = 3.0 Hz, 4H), 7.63 (s, 2H), 7.57-7.54 (m, 4H), 7.30-7.42 (m, 10H), 7.24 (dd, J = 7.0 Hz, J = 3.0 Hz, 4H), 7.18 (d, J = 2.0 Hz, 2H), 6.95 (dd, J =7.0 Hz, J = 3.0 Hz, 4H), 6.43 (d, J = 9.0 Hz, 2H), 5.56 (d, J = 9.0 Hz, 2H), 5.54 (s, 4H), 5.48 (dd, J = 15.0 Hz, J = 6.0 Hz, 4H), 5.17 (dd, J = 15.0 Hz, J = 3.0 Hz, 4H), 4.60 (s, 4H), 4.46 (s, 4H), 3.49 (t, J = 6.0 Hz, 4H), 3.33 (t, J = 5.5 Hz, 4H), 1.48 (s, 18H), 1.29 (s, 36H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.3, 167.2, 152.9, 152.0, 144.4, 136.8, 134.0, 133.5, 130.7, 130.3, 129.7, 129.4, 129.1, 128.2, 128.1, 127.0, 126.3, 125.9, 124.9, 124.8, 123.2, 123.1, 122.8, 121.5, 114.5, 111.0, 68.2, 64.4, 55.1, 53.9, 51.0, 37.3, 35.5, 35.1, 31.6, 31.5; HRMS (ESI-TOF) calculated for $C_{120}H_{131}N_{12}O_8$ $[M+H]^+$ 1868.0207; found 1868.0288.

Synthesis of squaraine dye (3)

Squaraine dye **S5** (110 mg, 0.21 mmol) and azide **S8** (180 mg, 0.73 mmol) were dissolved in chloroform (5 mL). *N*,*N*-diisopropylethylamine (100 µL, 0.57 mmol) and tris(triphenylphosphine) copper(I)bromide (20.0 mg, 0.022 mmol) were added to the solution and the reaction was heated to 50 °C for 72 h. To remove the copper, saturated EDTA (3 mL) was stirred vigorously for 1 h. The crude material from the organic layer was further purified by column chromatography with silica gel and EtOAc:$CHCl_3$ (50:50) solvent as eluent to give **3** (22 %, 47 mg) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, J = 9.0 Hz, 2H), 7.47 (s, 2H), 7.40 (t, J = 2.0 Hz, 2H), 7.29-7.36 (m, 6H), 7.20-7.22 (m, 4H), 7.10 (d, J = 2.0 Hz, 4H), 5.45 (s, 4H), 4.73 (s, 4H), 4.60 (s, 4H), 4.11 (t, J = 5.0 Hz, 4H), 3.78 (t, J = 5.0 Hz, 4H), 1.29 (s, 36H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.6, 152.1, 152.0, 145.6, 144.8, 137.3, 134.0, 130.7, 130.2, 129.1, 127.6, 126.7, 123.0, 122.9, 122.7, 112.3, 68.3, 64.6, 55.0, 54.1, 50.5, 35.1, 31.6; HRMS (ESI-TOF) calculated for $C_{64}H_{78}N_8NaO_4$ $[M+Na]^+$ 1045.6038; found 1045.6042.

B. Photophysical Properties

Photophysical properties were determined using a Lambda 25 (Perkin Elmer) UV-Vis and Fluoromax-3 (Yvon Horiba) fluorescence spectrometers. Spectrophotometric grade solvents were purchased from commercial sources and used without further purification. All measurements were made at room temperature (22 °C) in the prescence of air. Coumarin 6 (Φ_f = 0.93 in chloroform) was used as a standard for quantum yield measurements.[S4]

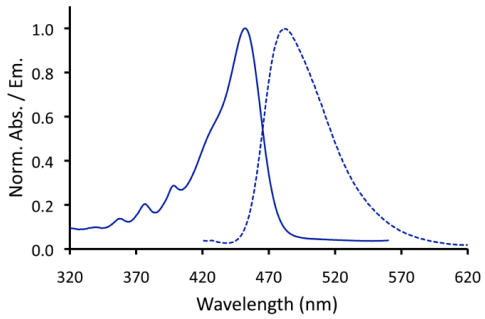


Figure S1: Normalized absorption and emission spectra of squaraine dye **S5** in $CHCl_3$ (5 µM).

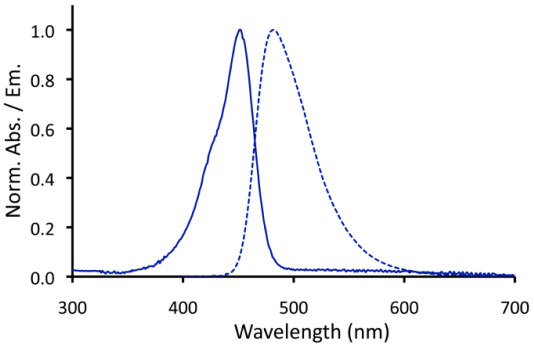


Figure S2: Normalized absorption and emission spectra of squaraine dye **3** in $CHCl_3$ (5 µM).

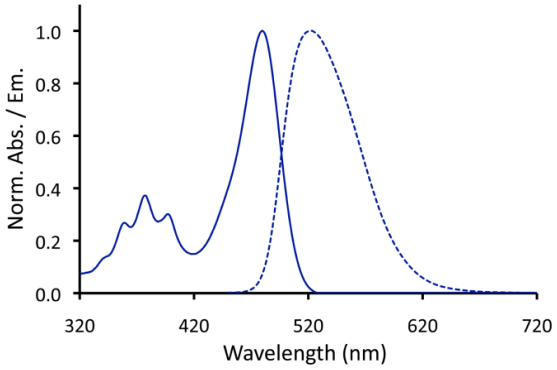
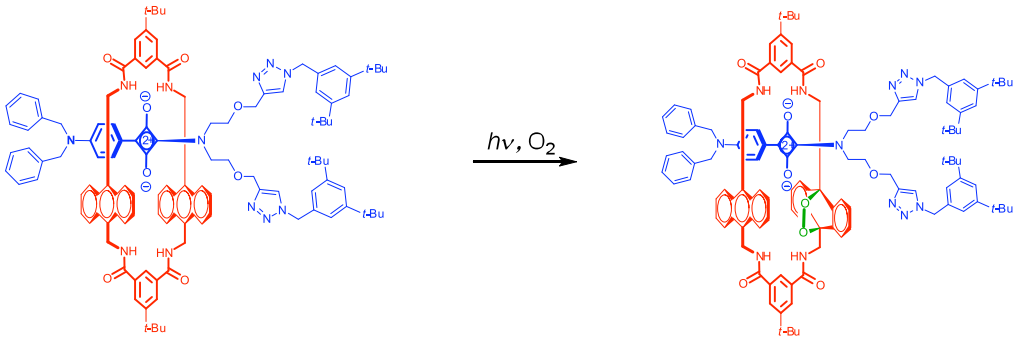


Figure S3: Normalized absorption and emission spectra of squaraine rotaxane **2** in $CHCl_3$ (5 μM).

Table S1: Photophysical properties in $CHCl_3$ (5 μM).

Compound	λ_{abs} (nm)	log ε	λ_{em} (nm)	Φ_f
S5	452	4.41	481	0.06
3	451	4.46	481	0.09
2	479	4.67	522	0.74

C. Synthesis of 2EP



2 **2EP**

Scheme S3: Photooxidation of **2** to **2EP**.

Synthesis of Squaraine Rotaxane Endoperoxide (2EP).
Catalytic Rose Bengal (1.0 mg, 20 mol %) was added to a solution of **2** (4.0 mg, 2.1 μmol) dissolved in $CDCl_3$ (1.0 mL). Oxygen was bubbled through the solution as it was irradiated with 520 nm filtered light for 2 h in an ice bath. **2EP** was purified by eluting the product mixture through a plug of silica gel using an ice-cold eluent of 5 % MeOH in CH_2Cl_2. Isolated fractions were combined and evaporated under reduced pressure in an ice-cold water bath. Additional impurities were removed by triturating the dried residue with 1:2 ether:hexanes (3 x 5 mL) to yield pure **2EP**.

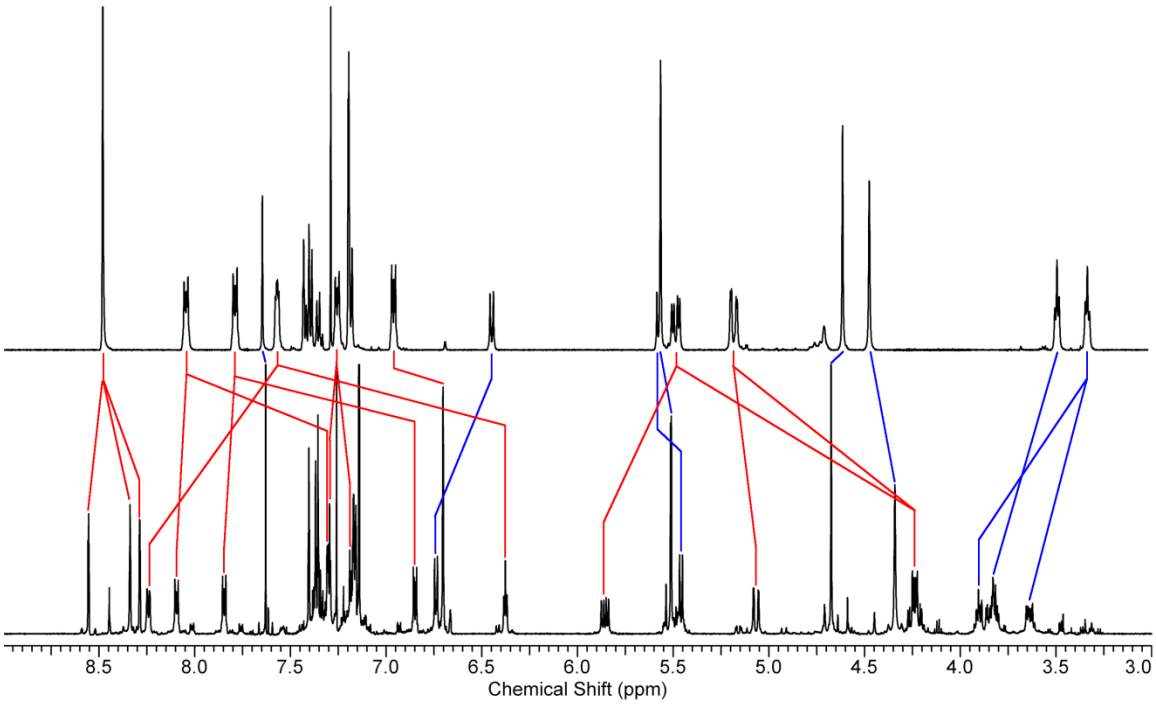


Figure S4: ^{1}H NMR spectra ($CDCl_3$, 600 MHz, 20 °C) of (*top*) pure **2** and (*bottom*) photooxidation to **2EP** after 120 mins of irradiation. For proton assignments of **2** and **2EP**, see Figures S14 and S16, respectively.

Spectral data for **2EP**: Absorption λ_{max} (5 μM, $CHCl_3$) = 473 nm, Fluorescence λ_{max} (1.0 mM, $CHCl_3$) = 533 nm, Fluorescence λ_{max} (5 μM, $CHCl_3$) = 520 nm. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.57 (t, *J* = 1.5 Hz, 2H), 8.35 (t, *J* = 1.5 Hz, 2H), 8.30 (t, *J* = 1.5 Hz, 2H), 8.25 (dd, *J* = 7.5 Hz, *J* = 1.0 Hz, 2H), 8.11 (dd, *J* = 6.5 Hz, *J* = 3.0 Hz, 2H), 7.86 (dd, *J* = 7.0 Hz, *J* = 3.0 Hz, 2H), 7.64 (s, 2H), 7.41 (t, *J* = 2.0 Hz, 2H), 7.39-7.38 (m, 4H), 7.37 (t, *J* = 1.5 Hz, 2H), 7.33-7.30 (m, 4H), 7.19 (dd, *J* = 6.0 Hz, *J* = 3.0 Hz, 2H), 7.17 (m, 4H), 7.15 (d, *J* = 2.0 Hz, 4H), 6.86 (dd, *J* = 7.0 Hz, *J* = 3.0 Hz, 2H), 6.75 (d, *J* = 9.0, 2H), 6.72 (d, *J* = 1.5 Hz, 4H), 6.39 (t, *J* = 5.5 Hz, 2H), 5.87 (dd, *J* = 15.0 Hz, *J* = 7.5 Hz, 2H), 5.52 (d, *J* = 2.5 Hz, 4H), 5.47 (d, *J* = 9.0 Hz, 2H), 5.08 (dd, *J* = 15.0 Hz, *J* = 1.5 Hz, 2H), 4.69 (s, 4H), 4.35 (s, 4H), 4.27 (dd, *J* = 14.0 Hz, *J* = 5.5 Hz, 2H), 4.23 (dd, *J* = 14.0 Hz, *J* = 5.0 Hz, 2H), 3.93-3.81 (m, 6H), 3.67-3.62 (m, 2H), 1.46 (s, 18H), 1.28 (s, 36H); ^{13}C NMR ($CDCl_3$, 150 MHz, -10 °C) δ 176.6, 176.1 172.0, 168.6, 167.2, 166.9, 152.8, 151.9, 150.7, 144.2, 144.1, 136.6, 136.2, 135.8, 133.91, 133.89, 133.8, 133.7, 133.2, 130.9, 130.6, 130.5, 130.4, 130.1, 129.7, 129.6, 129.1, 129.0, 128.4, 127.5, 126.8, 126.2, 125.7, 124.7, 123.4, 123.3, 123.1, 123.0, 122.8, 121.8, 121.0, 113.5, 110.6, 81.1, 67.2, 64.1, 55.0, 52.9, 37.7, 35.4, 35.0, 29.9; HRMS (ESI-TOF) calculated for $C_{120}H_{131}N_{12}O_{10}$ $[M+H]^+$ 1901.0106; found 1901.0141.

^{1}H NMR variable temperature studies of 2EP

The following variable temperature spectra exhibit two-site exchange behavior that matches previous observations of **1EP**.[S5] This data is consistent with an internally directed endoperoxide stereoisomer of **2EP** undergoing conformational rocking. The hypothetical external endoperoxide stereoisomer of **2EP** cannot exhibit this dynamic NMR behavior.

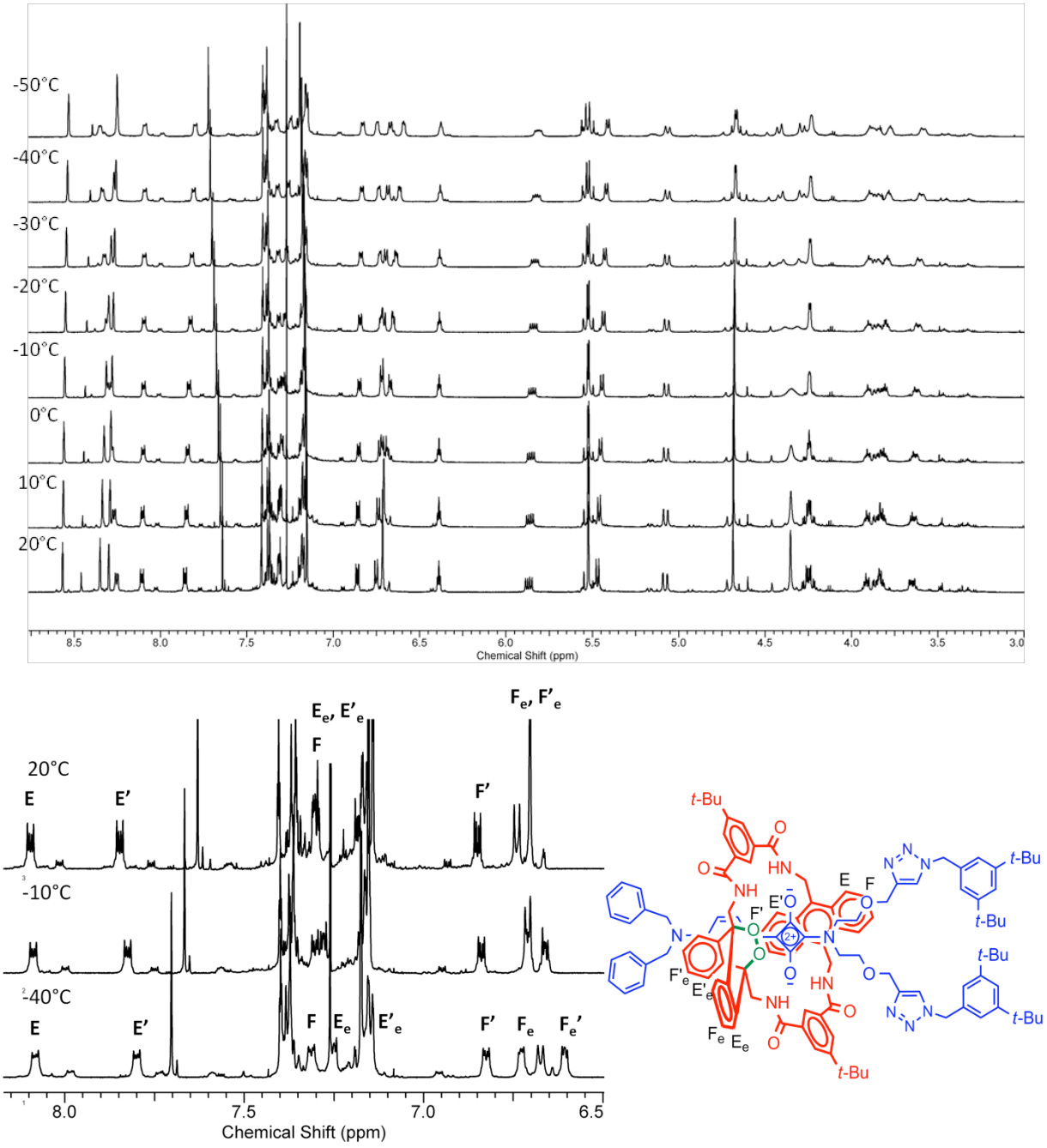


Figure S5: (*top*) Variable temperature ^{1}H NMR (600 MHz, $CDCl_3$) spectra of **2EP** (4 mM) showing two-site exchange of anthracene peaks. (*bottom*) Expansion of spectra highlighting the coalescence of anthracene endoperoxide protons E_e and E_e' and F_e and F_e'.

D. Cycloreversion of 2EP

Kinetic experiments used a 500 MHz ^{1}H NMR spectrometer with a calibrated thermocouple. Samples of **2EP** (4 mM, $CDCl_3$) were prepared in NMR tubes that were not exposed to any light. The ratios of **2EP** to **2** were determined by comparing signal integrations of protons '4' and '9' (Figure S14) for each compound in the mixture. Kinetic measurements at 40 °C and 10 °C were made by scanning the sample every 15 mins and 24 h, respectively, until full cycloreversion was achieved. The 10 °C sample was stored inside an incubator between measurements. First-order rate constants of 0.64 h^{-1} (40 °C) and 0.012 h^{-1} (10 °C) were determined with corresponding half-lives of 1.1 h and 56 h respectively. An activation energy of 98.1 $kJ \cdot mol^{-1}$ was determined by making an Arrhenius plot (rate vs. T^{-1}).

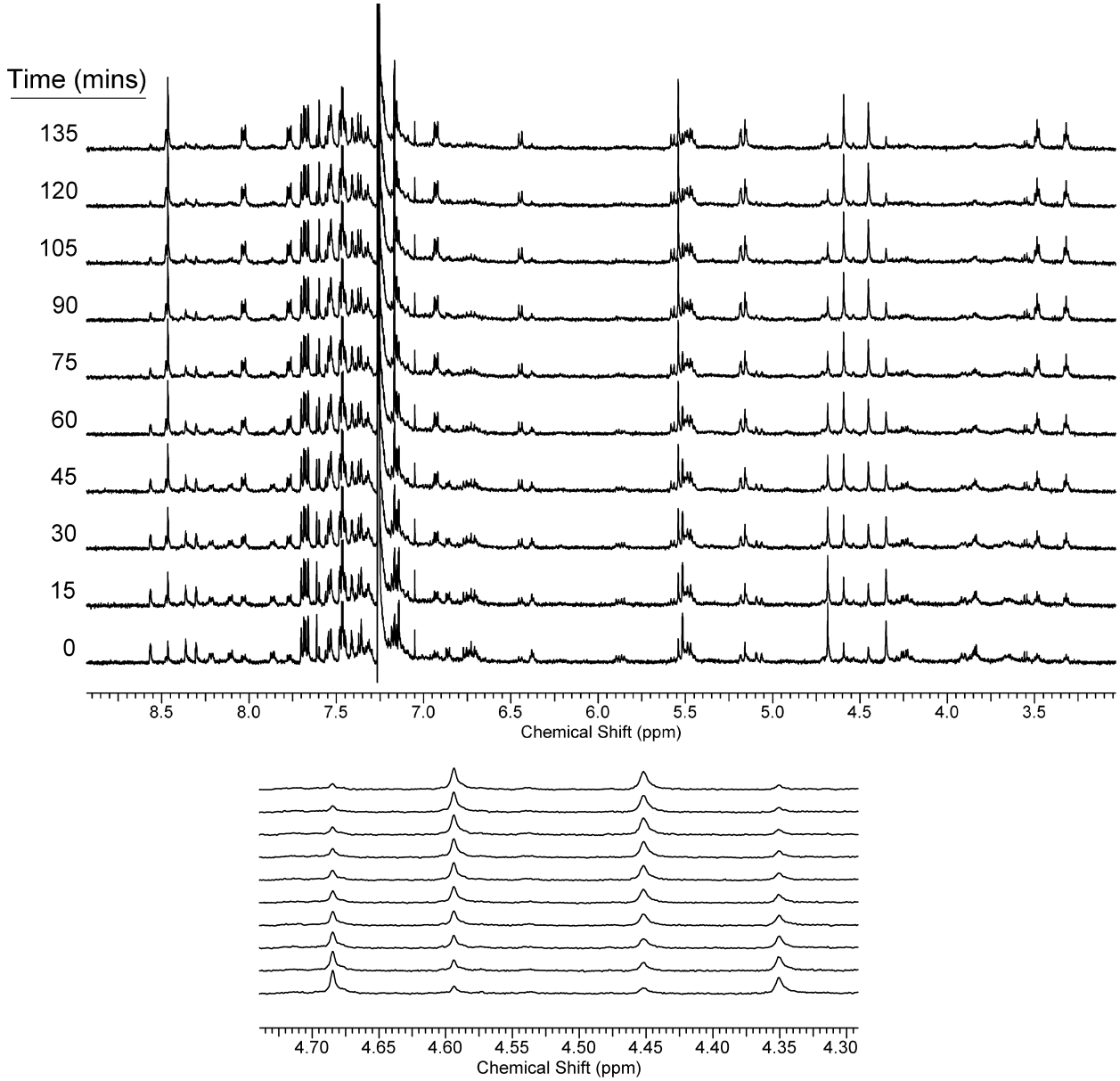


Figure S6 (*top*) ^{1}H NMR (600 MHz, $CDCl_3$) spectra of the cycloreversion of **2EP** (4 mM) to form **2** over 135 mins at 40 °C. (*bottom*) Partial ^{1}H NMR spectra showing the peaks that were monitored for kinetic measurements.

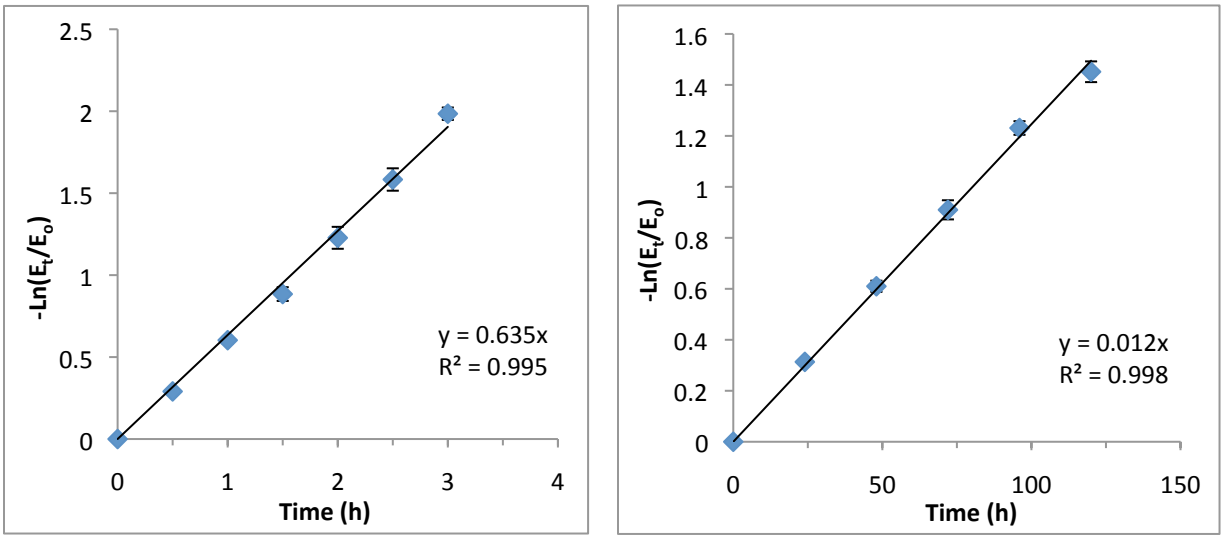
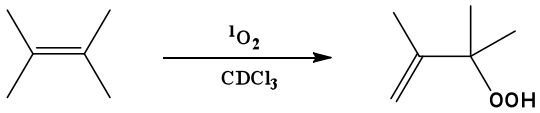


Figure S7: First-order kinetic plots of the cycloreversion of **2EP** at 40 °C (left) and 10 °C (right) in $CDCl_3$ (4 mM).

E. Singlet Oxygen Trapping Experiments



A previously reported trapping method was utilized to measure the amount of singlet oxygen released by **2EP**.[S3] 2,3-dimethyl-2-butene (30 eq, 150 mM) was added to a sample of **2EP** in $CDCl_3$ (5 mM) and the cycloreversion allowed to proceed at 38 °C. ^1H NMR spectra were collected and the trapped hydroperoxide was detected by its characteristic spectral pattern. By comparing intensity of the olefin signals (between 4.9-5.1 ppm) with the amount of regenerated rotaxane **2**, it was calculated that 61±10 % of the released oxygen was singlet oxygen.

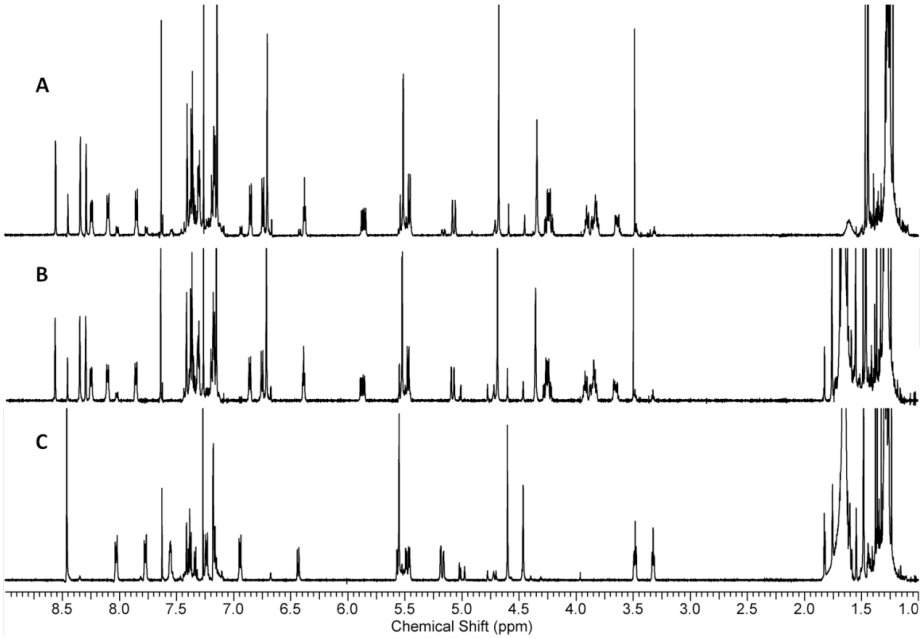


Figure S8: ^{1}H NMR spectra ($CDCl_3$, 600 MHz, 20 °C) of: (A) pure **2EP**, (B) **2EP** immediately following addition of 2,3-dimethyl-2-butene trapping agent, (C) complete cycloreversion to **2** and production of trapped hydroperoxide (spectrum acquired after 48 h at 38 °C).

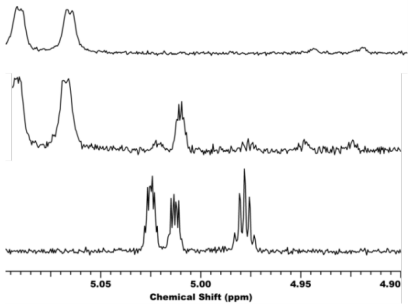


Figure S9: Expansion of Figure S8 showing the two olefin proton signals for the hydroperoxide product gained from reaction of 2,3-dimethyl-2-butene with singlet oxygen.

F. ^{1}H and ^{13}C NMR Spectra

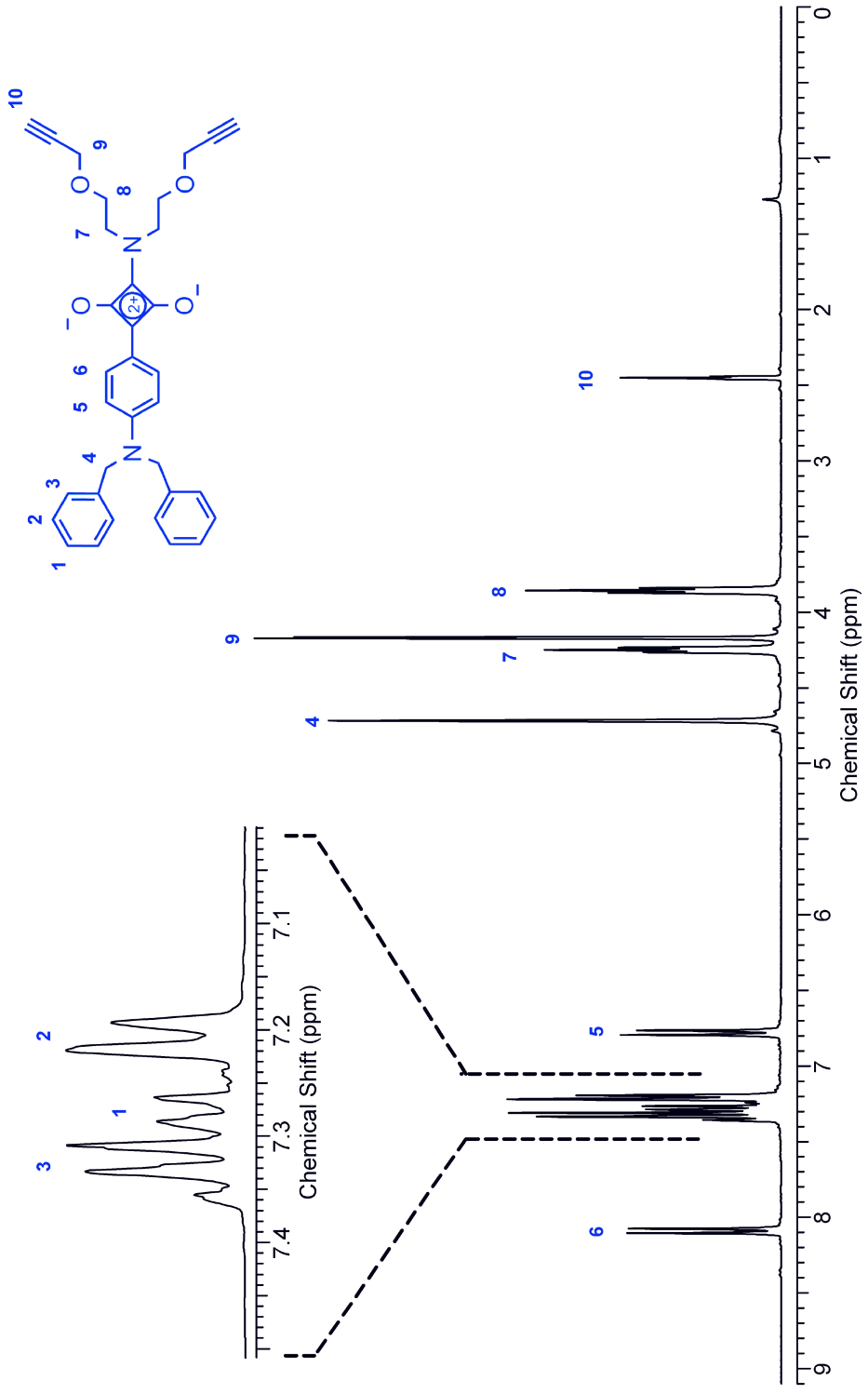


Figure S10: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of squaraine dye **S5**.

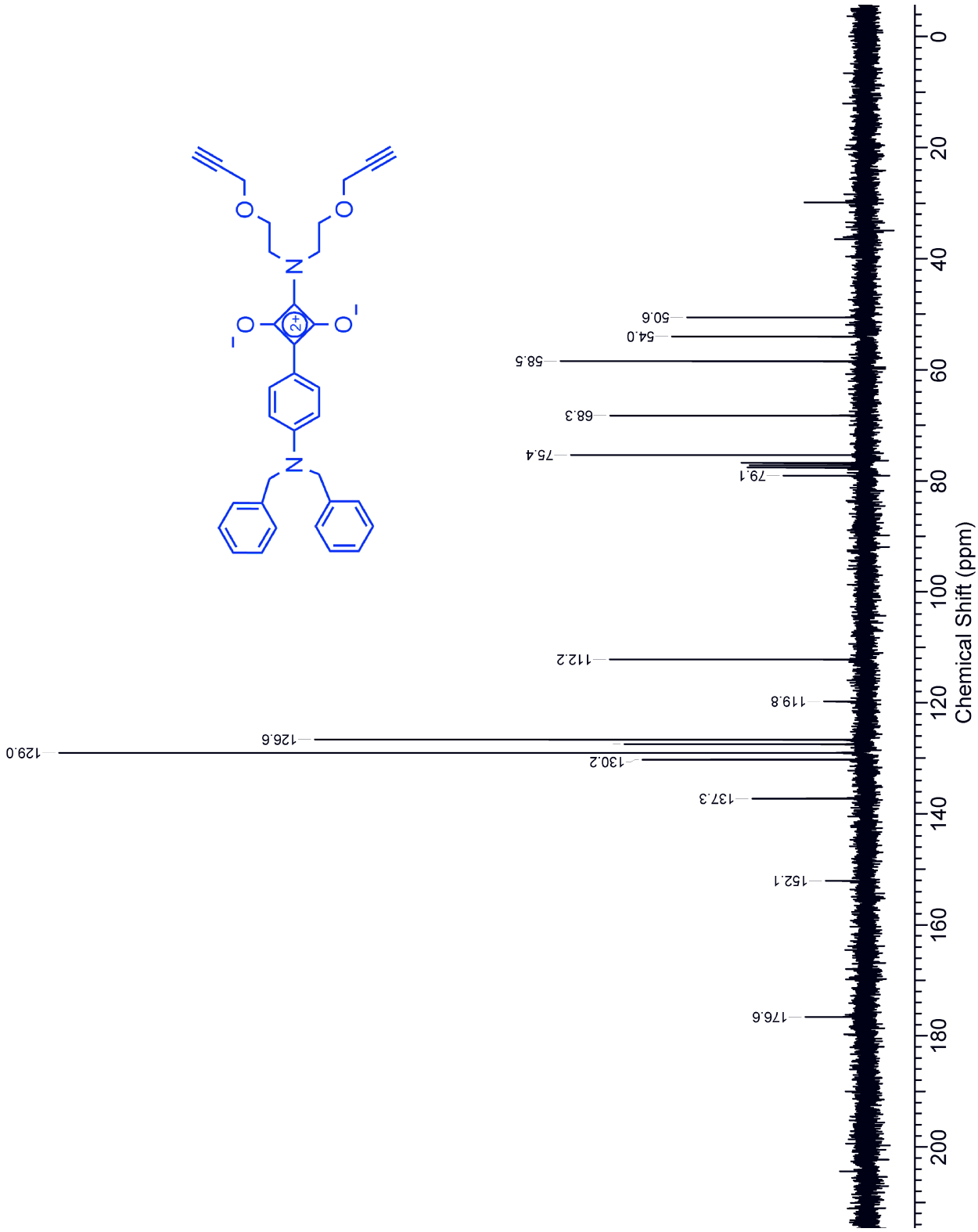


Figure S11: ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of squaraine dye **S5**.

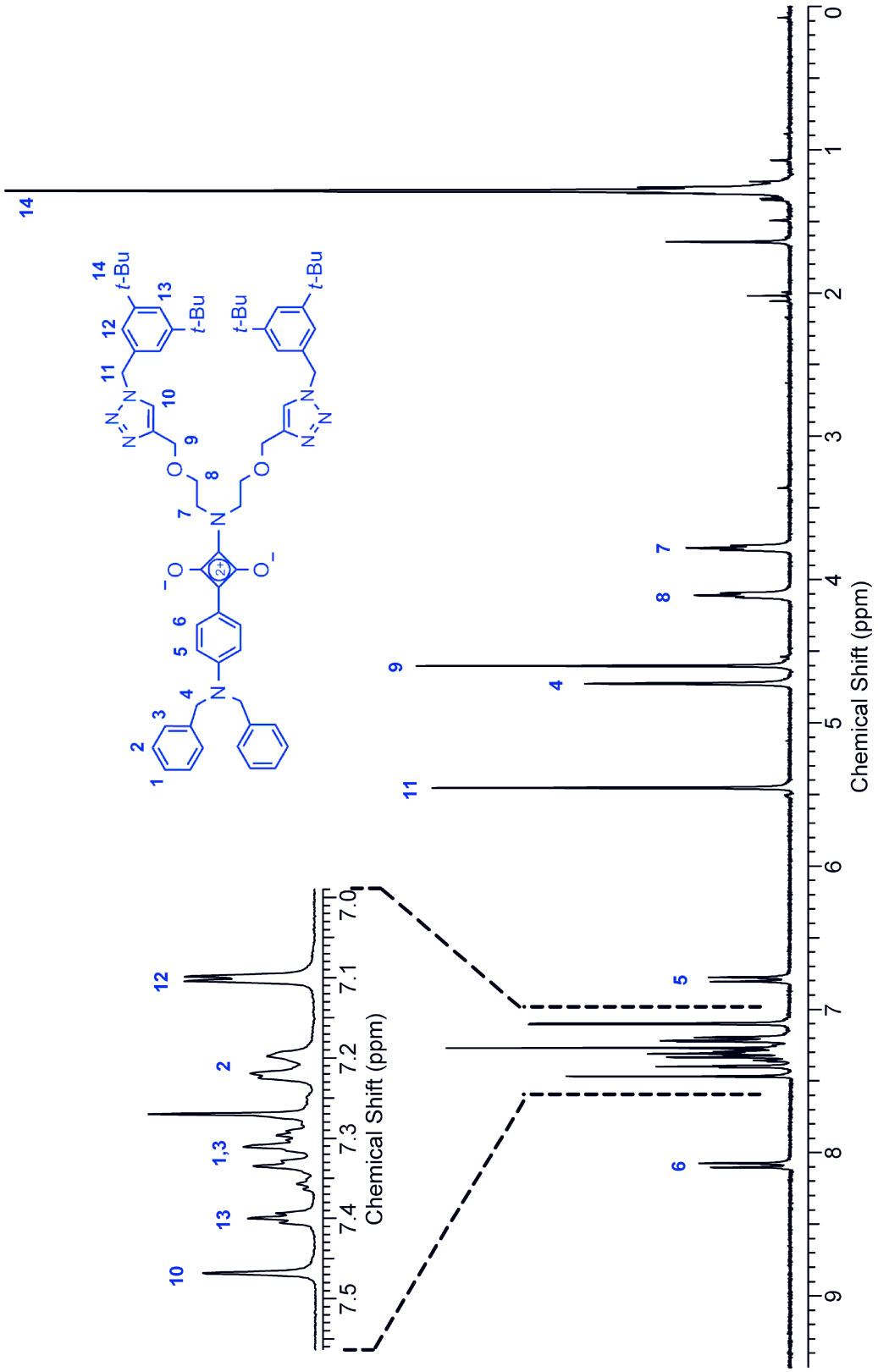


Figure S12: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of squaraine dye **3**.

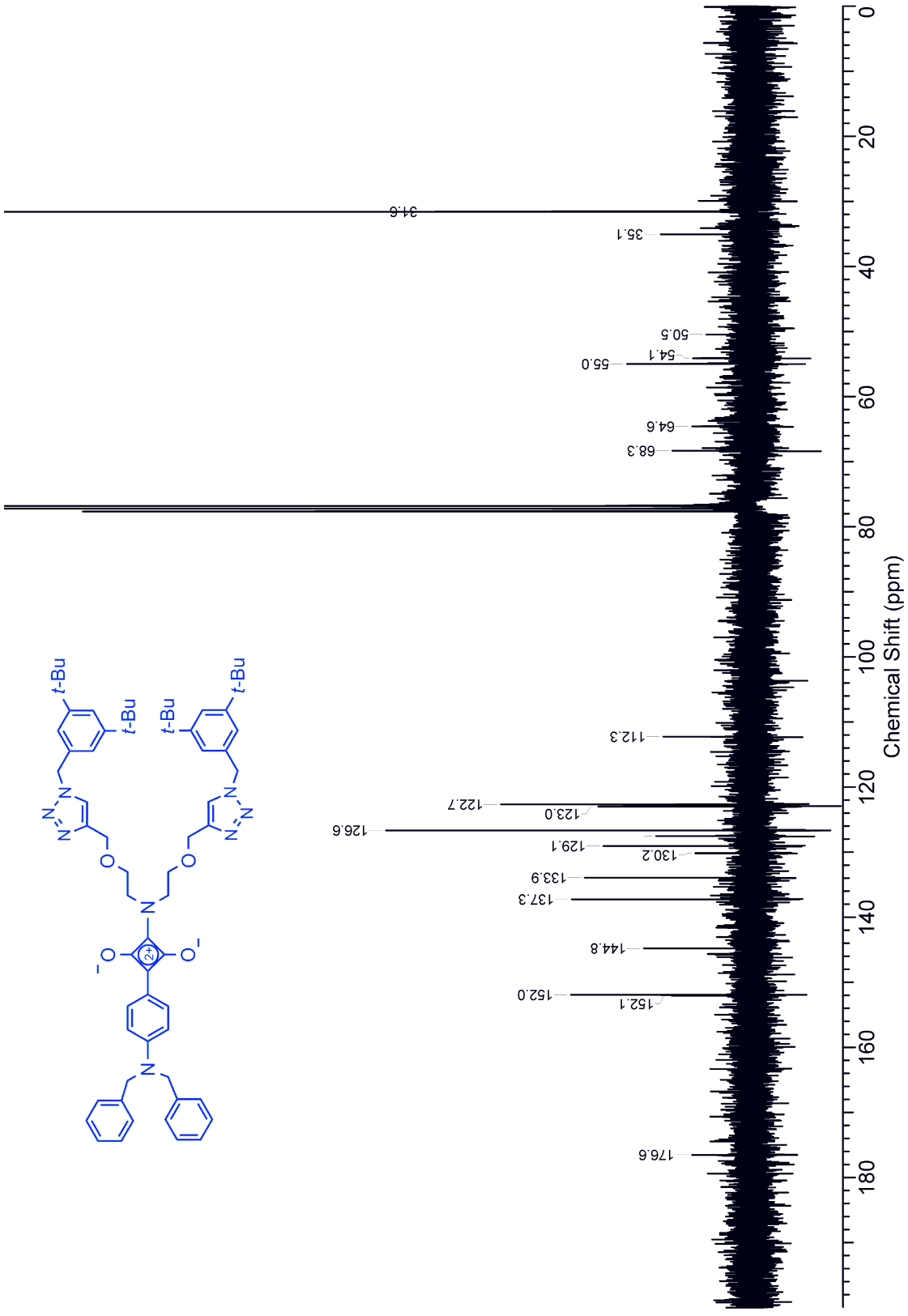


Figure S13: ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of squaraine dye **3**.

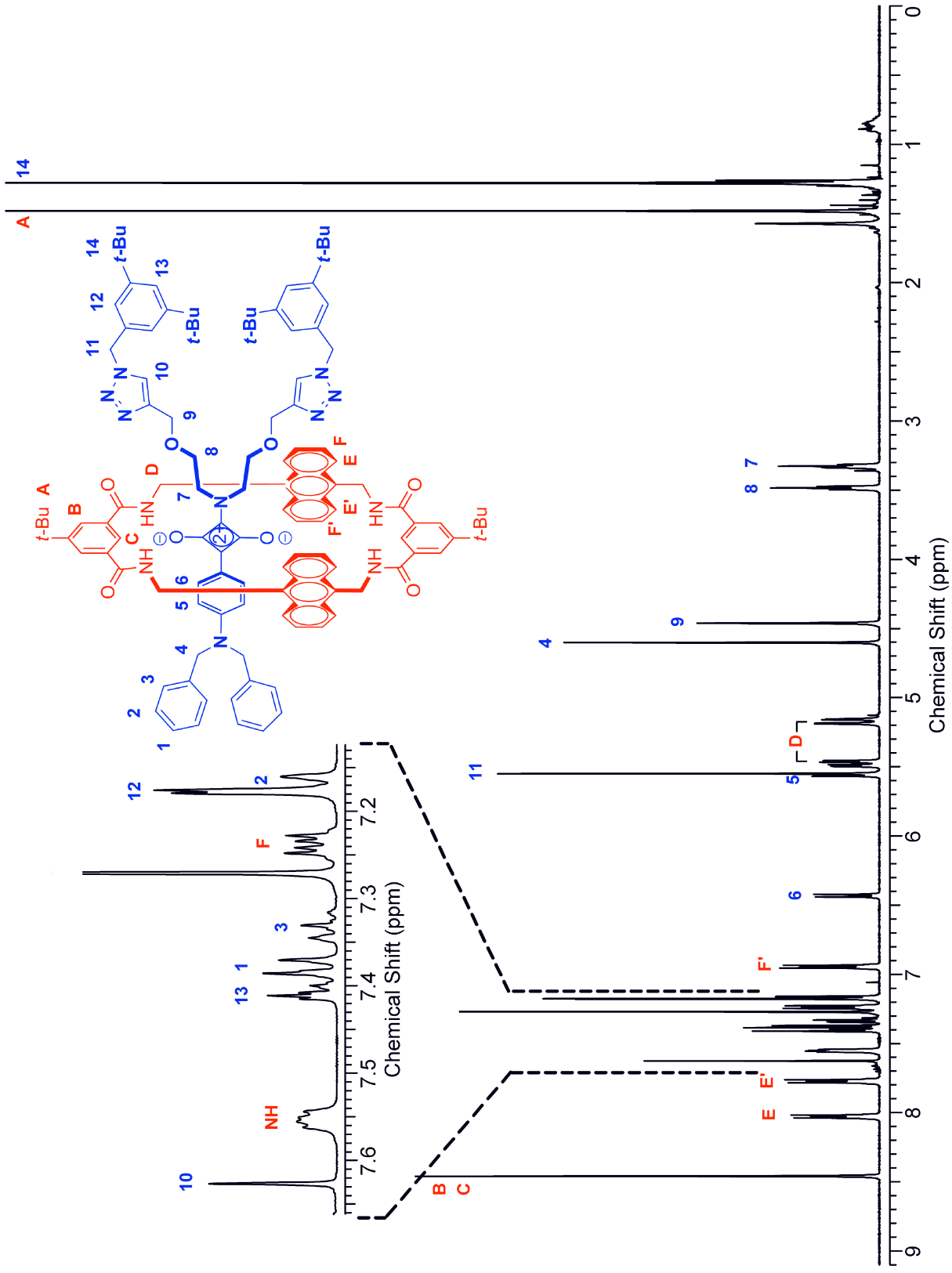


Figure S14: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of squaraine rotaxane **2**.

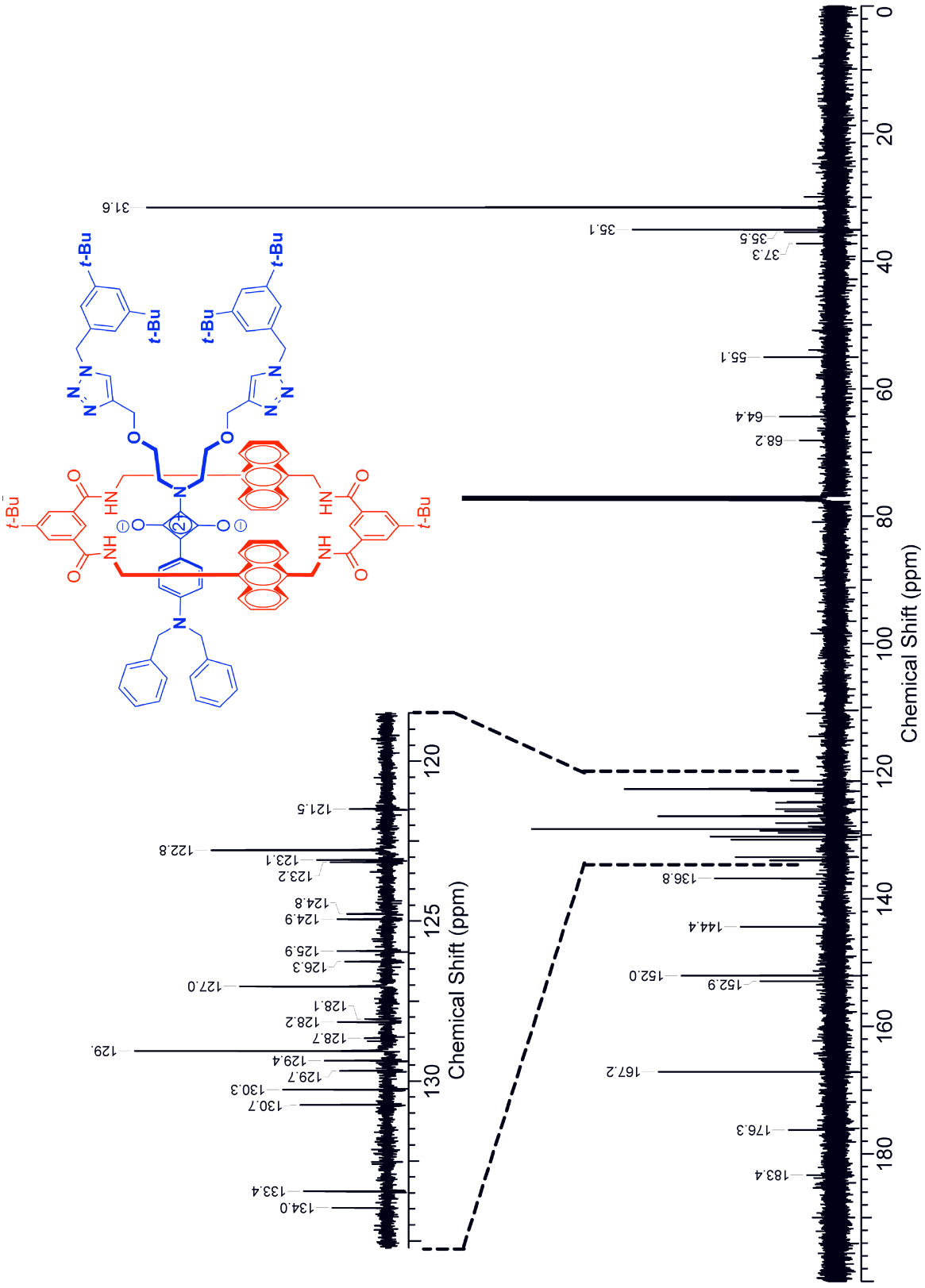


Figure S15: ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of squaraine rotaxane **2**.

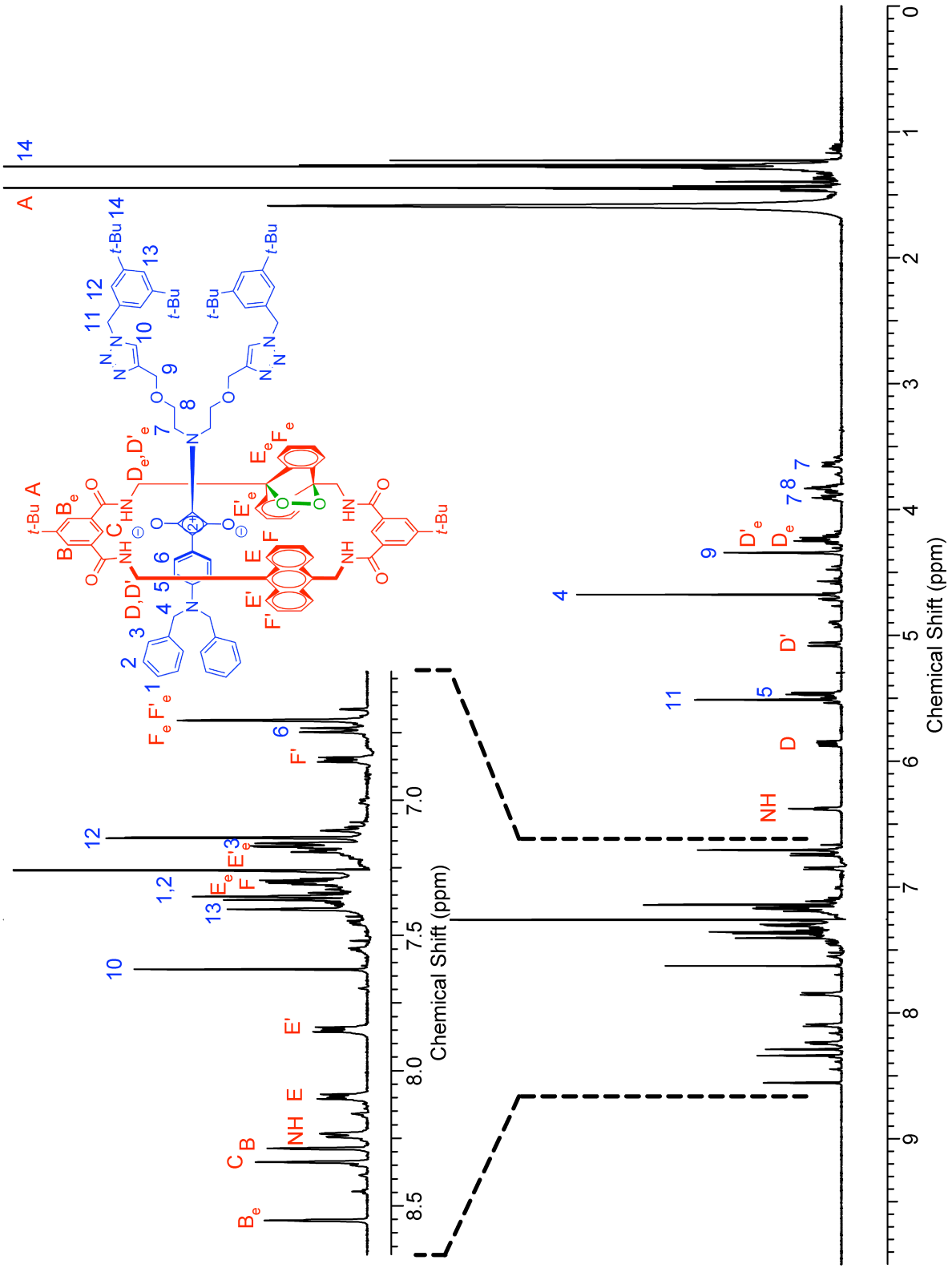


Figure S16: ^{1}H NMR (600 MHz, -10 °C, $CDCl_3$) spectrum of endoperoxide **2EP**.

Multidimensional NMR spectroscopy was used to confirm bond connectivity and establish definitive structural assignments for **2EP**. Chemical shift information obtained from COSY (Figure S17) was consistent with previously published assignments for **1EP**.[S3] COSY cross-peaks identified the well-resolved anthracene coupling partners (blue) and macrocycle amide and benzylic hydrogen coupling partners (red for anthracene coupling, green for endoperoxide coupling). The endoperoxide anthracene partners split at lower temperature due to the system's dynamic behavior (see Figure S5) and are only partially resolved at -10°C.

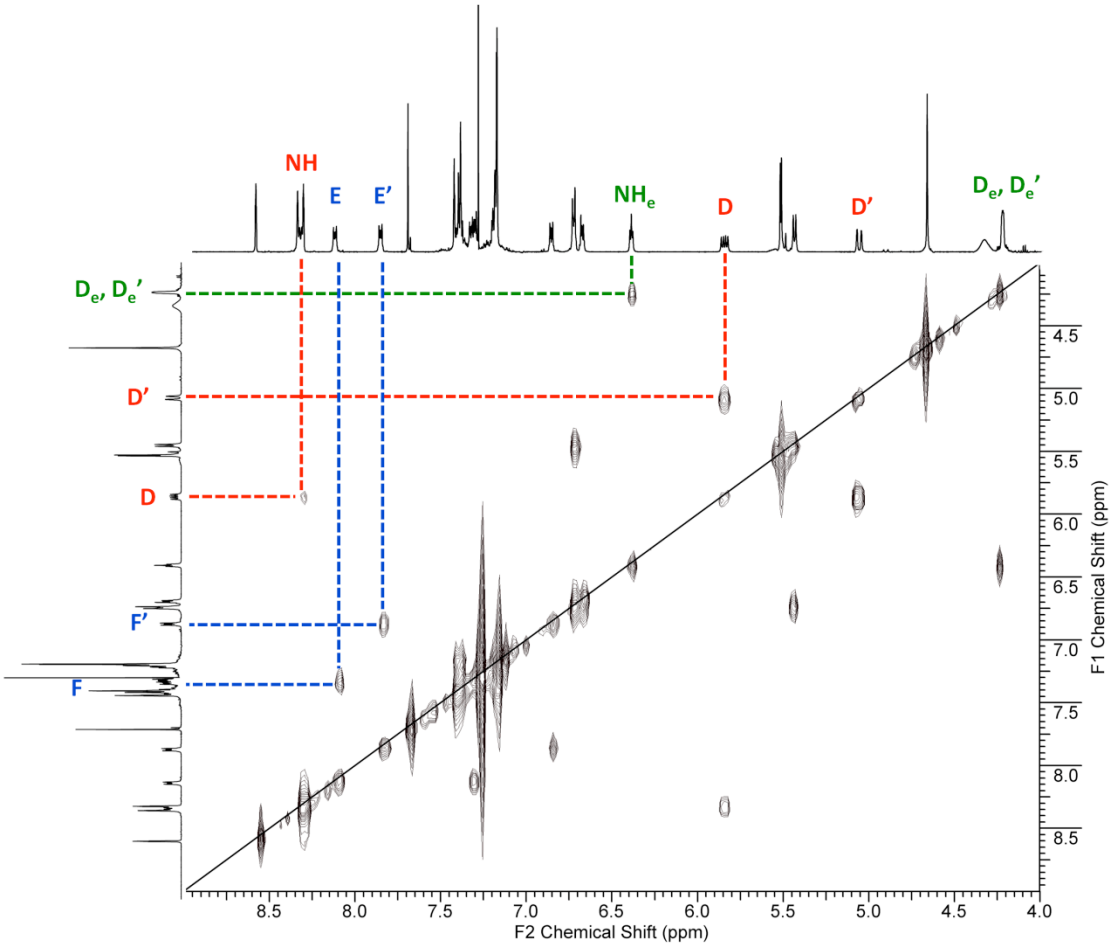


Figure S17: ^{1}H NMR (600 MHz, -10 °C, $CDCl_3$) COSY spectrum of endoperoxide **2EP**.

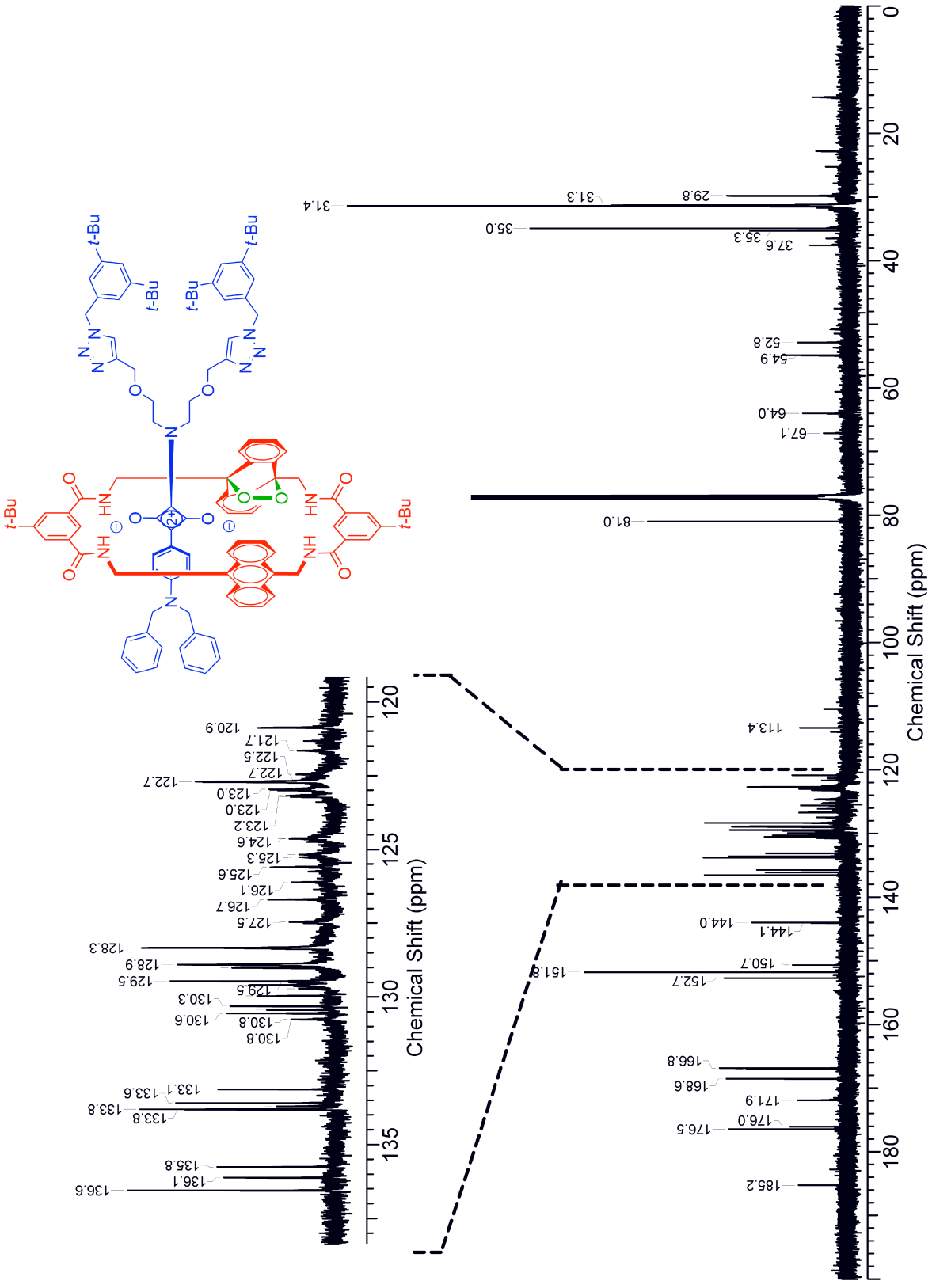


Figure S18: ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of endoperoxide **2EP**.

G. References

[S1] L. S. Berbeci, W. Wang and A. E. Kaifer, *Org. Lett.,* 2008, **10**, 3721.
[S2] N. Fu, J. M. Baumes, E. Arunkumar, B. C. Noll and B. D. Smith, *J. Org. Chem.,* 2009, **74**, 6462.
[S3] J. J. Gassensmith, L. Barr, J. M. Baumes, A. Paek, A. Nguyen and B. D. Smith, *Org. Lett.*, 2008, **10**, 3343.
[S4] M. S. A. Abdel-Mottaleb, M. S. Antonious, M. M. Abo Ali, L. F. M. Ismail, B. A. El-Sayed and A. M. K. Sherief, *J. Chem. Sci*., 1992, **104**, 185.
[S5] J. M. Baumes, J. J. Gassensmith, J. Giblin, J.-J. Lee, A. G. White, W. J. Culligan, W. M. Leevy, M. Kuno and B. D. Smith, *Nature Chem*., 2010, **2**, 1025.