Supporting Information

Near-Infrared Illumination of Native Tissues for Image-Guided Surgery

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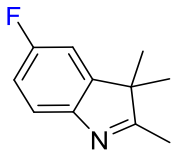
The PDF file includes:

Supplementary Methods

General Synthetic Procedure for the formation of heterocyclic derivatives 1-5.

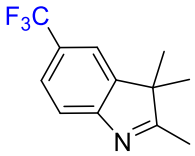
Appropriate 4-substituted phenylhydrazine hydrochloride derivative (20 g) was slowly added to a 250-mL round bottom flask containing 150-mL of vigorously stirring warm glacial acetic acid. After a heterogenous mixture was observed, 3-methyl-2-butanone (3 mol. equiv.) was added. The light brown solution was allowed to heat at reflux for 48-72 h. The reddish brown mixture was concentrated on a rotary evaporator to form a viscous oil after removal of acetic acid. The oil was dissolved in dichloromethane (35 mL) and washed with water (3 x 25 mL) and saturated sodium bicarbonate (3 x 50 mL). The organic layer was extracted, dried over sodium sulfate and obtained, after solvent removal, as a deep-reddish brown oil (Note: fluorine-containing indolenine analog **2** was obtained as a red solid). Compound **1** was a commercially obtained starting material and was not synthesized.



2

5-fluoro-2,3,3-trimethyl-3H-indole (**2**): Yield 84%, MP 146-149 ^{o}C ; ^{1}H NMR (400 MHz, DMSO-d_6) **δ**: 1.24 (s, 6H), 2.19 (s, 3H), 7.09-7.04 (m, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.41 (br s, 1H).

5-chloro-2,3,3-trimethyl-3H-indole (**3**) and *5-bromo-2,3,3-trimethyl-3H-indole* (**4**) have been previously reported by our group.[1]



5

2,3,3-trimethyl-5-(trifluoromethyl)-3H-indole (**5**): Yield 43%, ^{1}H NMR (400 MHz, DMSO-d_6) **δ**: 1.32 (s, 6H), 2.60 (s, 3H), 7.89 (br s, 1H), 8.01 (br s, 1H), 8.24 (br s, 1H).

Synthesis of heterocyclic salts 6-10.

The methylated heterocyclic salts were synthesized using a general method by dissolving the viscous indolenine oil in anhydrous acetonitrile (25 mL) and heated to reflux in the presence of iodomethane (3 mol eq) for 12-18 h. The reaction mixture was allowed to cool to room temperature and the acetonitrile was removed under reduced pressure to afford a red residue. The crude was dissolved in methanol and diethyl ether was added to the solution to afford a light brown solid which was suction filtered and dried to obtain the final product.

1,2,3,3-tetramethyl-3H-indol-1-ium iodide (**6**) was commercially obtained and used in the dye syntheses without purification.

5-fluoro-1,2,3,3-tetramethyl-3H-indol-1-ium iodide (**7**): Yield 81%, ^{1}H NMR (400 MHz, DMSO-d_6) **δ**: 1.29 (s, 6H), 2.31 (s, 3H), 4.11 (s, 3H), 7.21-7.16 (m, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.99 (br s, 1H).

5-chloro-1,2,3,3-tetramethyl-3H-indol-1-ium iodide (**8**) and *5-bromo-1,2,3,3-tetramethyl-3H-indol-1-ium iodide* (**9**) were synthesized as previously described.[1]

1,2,3,3-tetramethyl-5-(trifluoromethyl)-3H-indol-1-ium iodide (**10**): Yield 33%, ^{1}H NMR (400 MHz, DMSO-d_6) **δ**: 1.59 (s, 6H), 2.84 (s, 3H), 4.02 (s, 3H), 8.07 (br s, 1H), 8.15 (br s, 1H), 8.37 (br s, 1H).

Synthesis of Methine Precursor 11-13.

Precursor **11** was obtained commercials from Acros Organics and was used as obtained without modification. Compounds **12** and **13** have been previously described by our laboratory and the compounds were synthesized as described [1].

Synthesis of Methylated Pentamethine Cyanines 14-38.

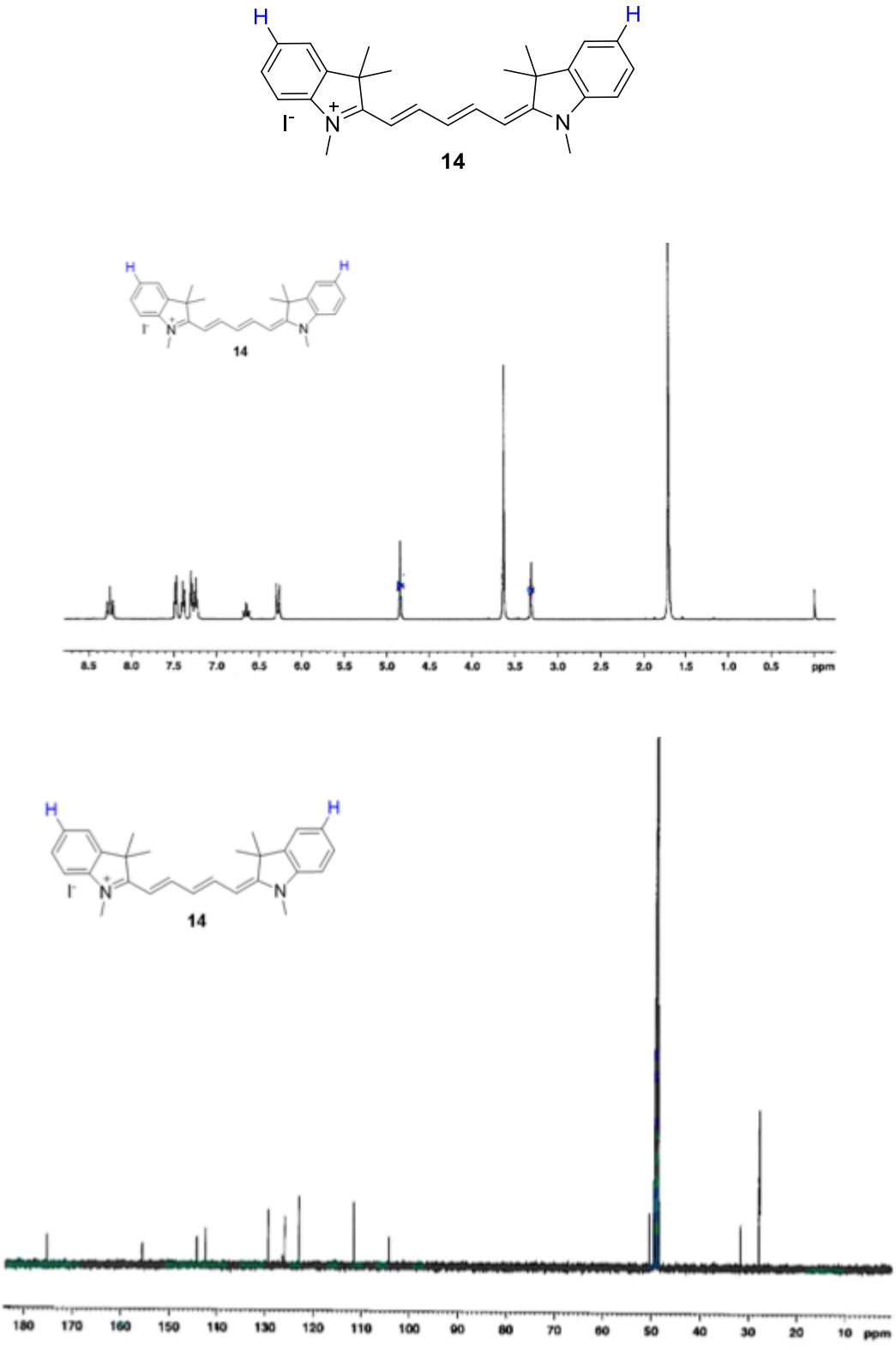


Figure S1. ^{1}H- and ^{13}C-NMR of Compound **14** in MeOD-d_4.

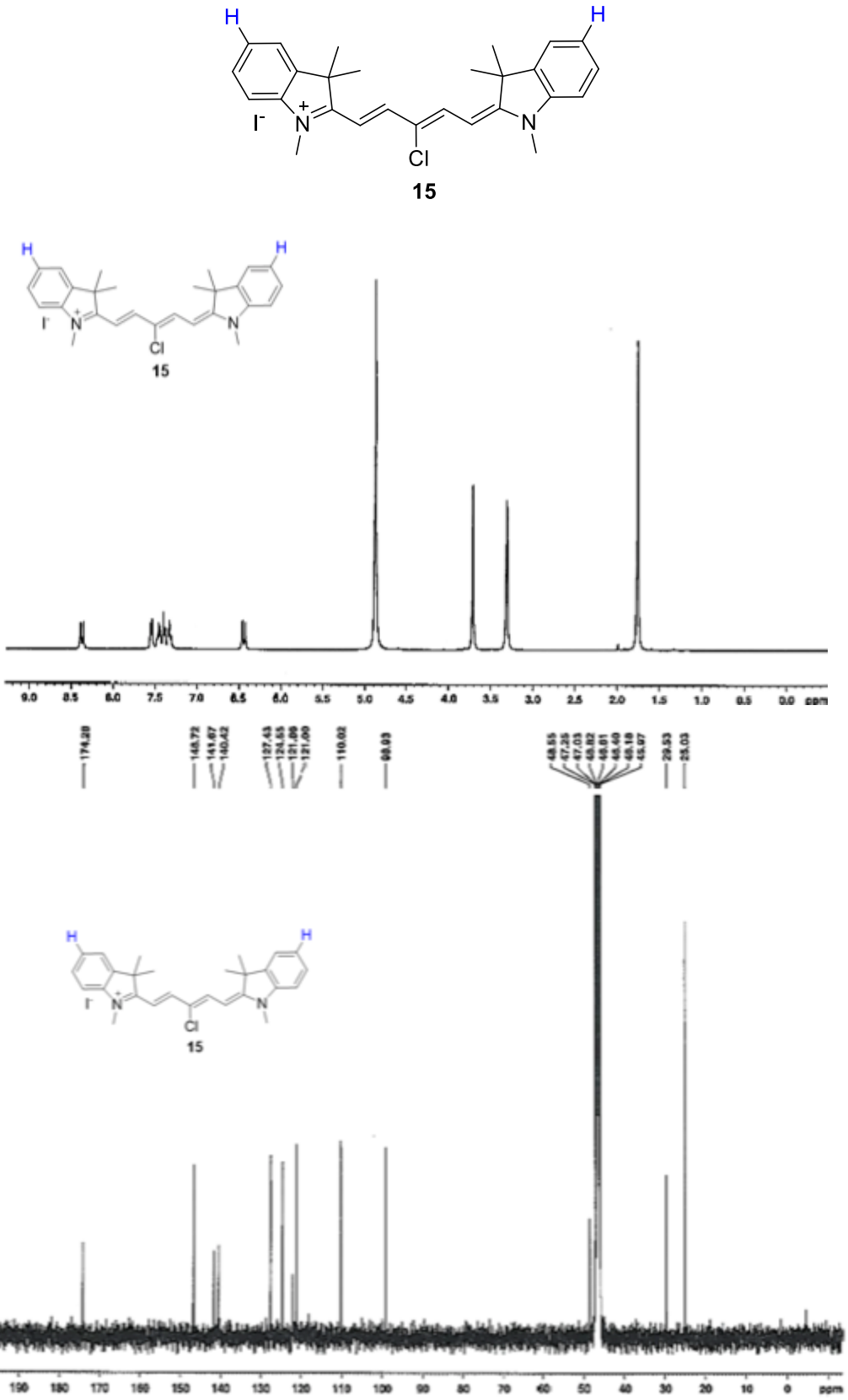


Figure S2. ^{1}H- and ^{13}C-NMR of Compound **15** in MeOD-d_4.

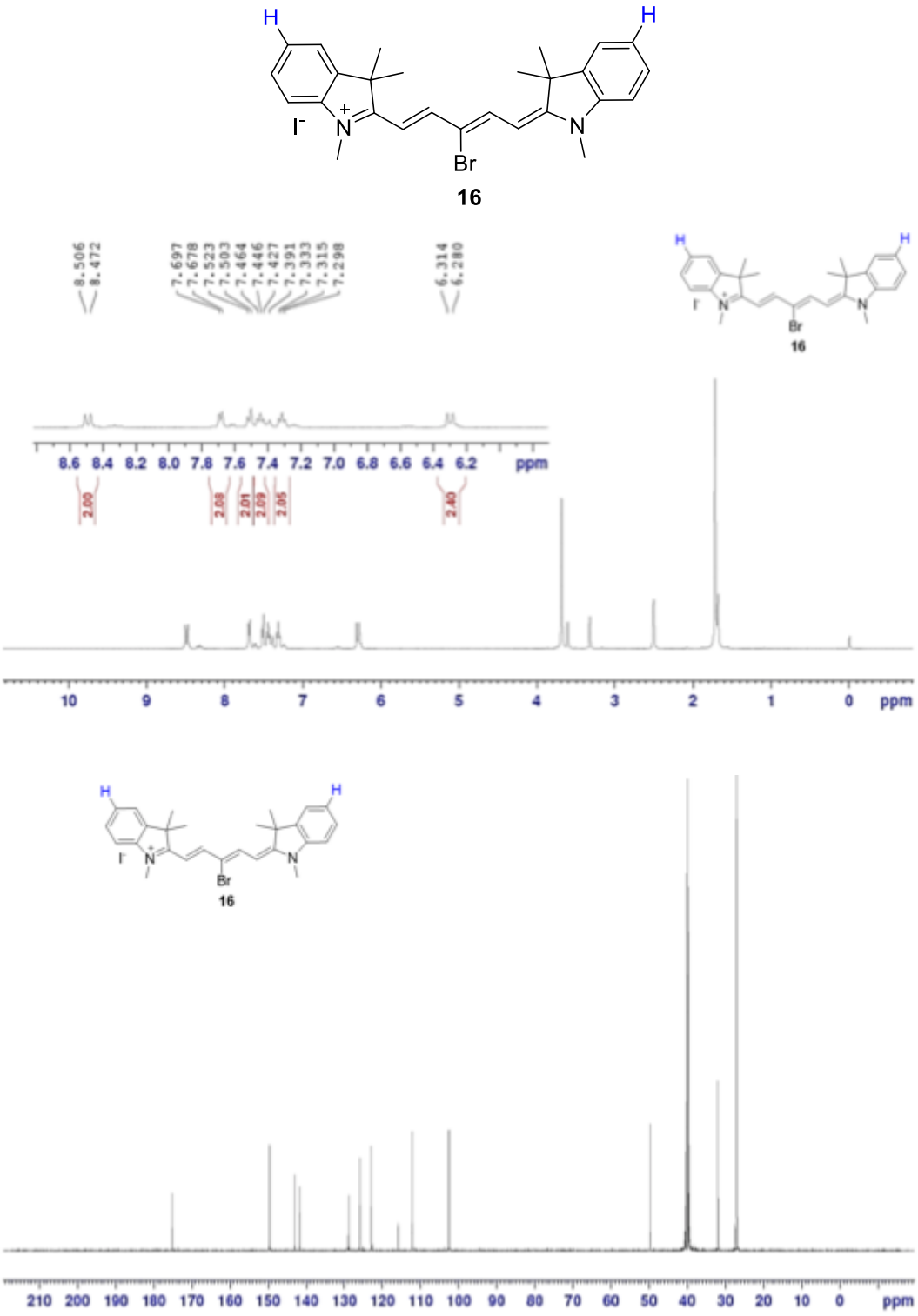
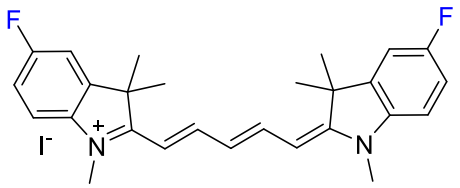


Figure S3. ^{1}H- and ^{13}C-NMR of Compound **16** in MeOD-d_4.



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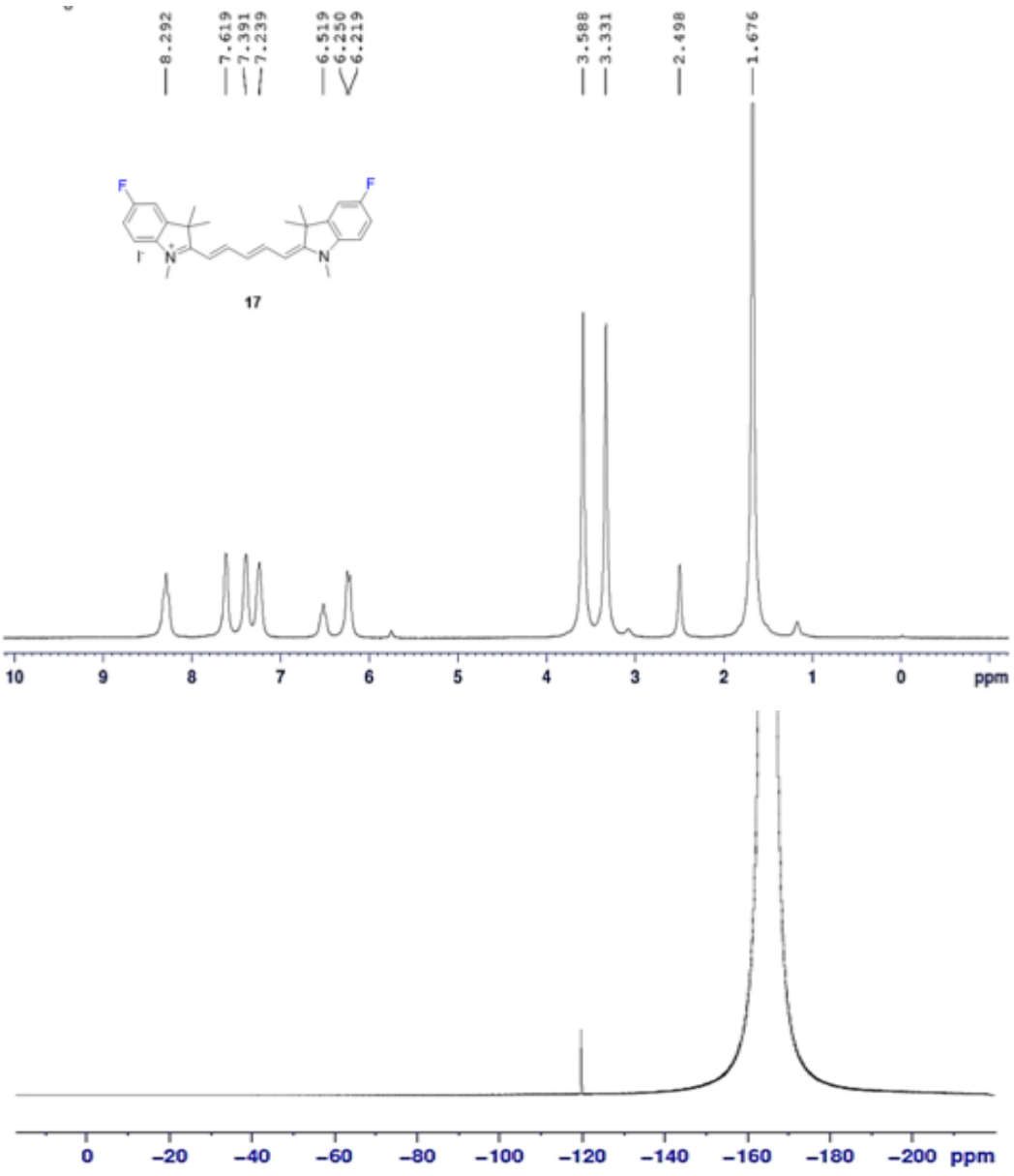
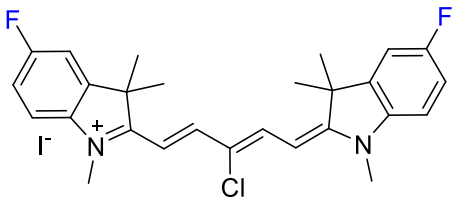


Figure S4. ^{1}H- and ^{19}F-NMR of Compound **17** in DMSO-d_6.



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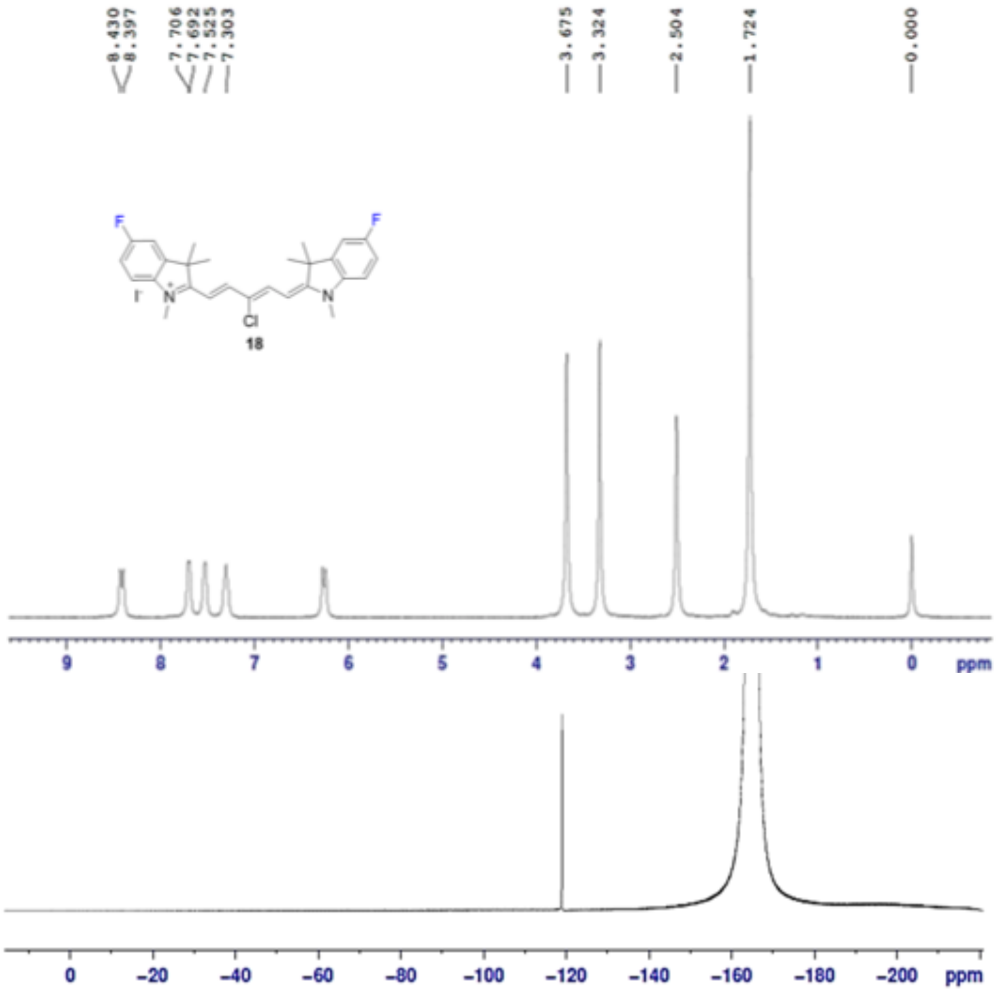
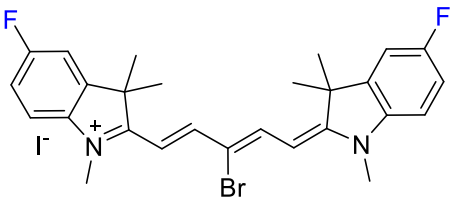


Figure S5. ^{1}H- and ^{19}F-NMR of Compound **18** in DMSO-d_6.



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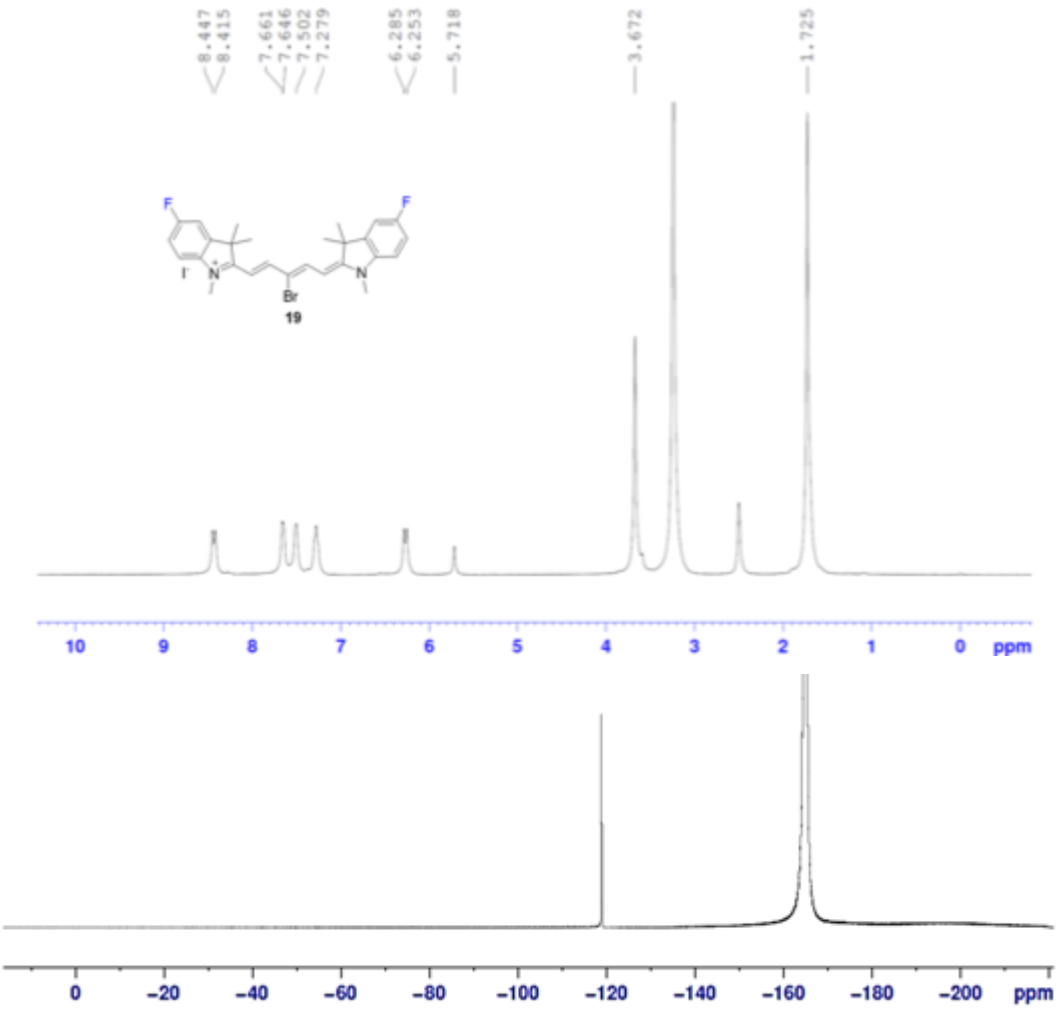


Figure S6. ^{1}H- and ^{19}F-NMR of Compound **19** in DMSO-d_6.

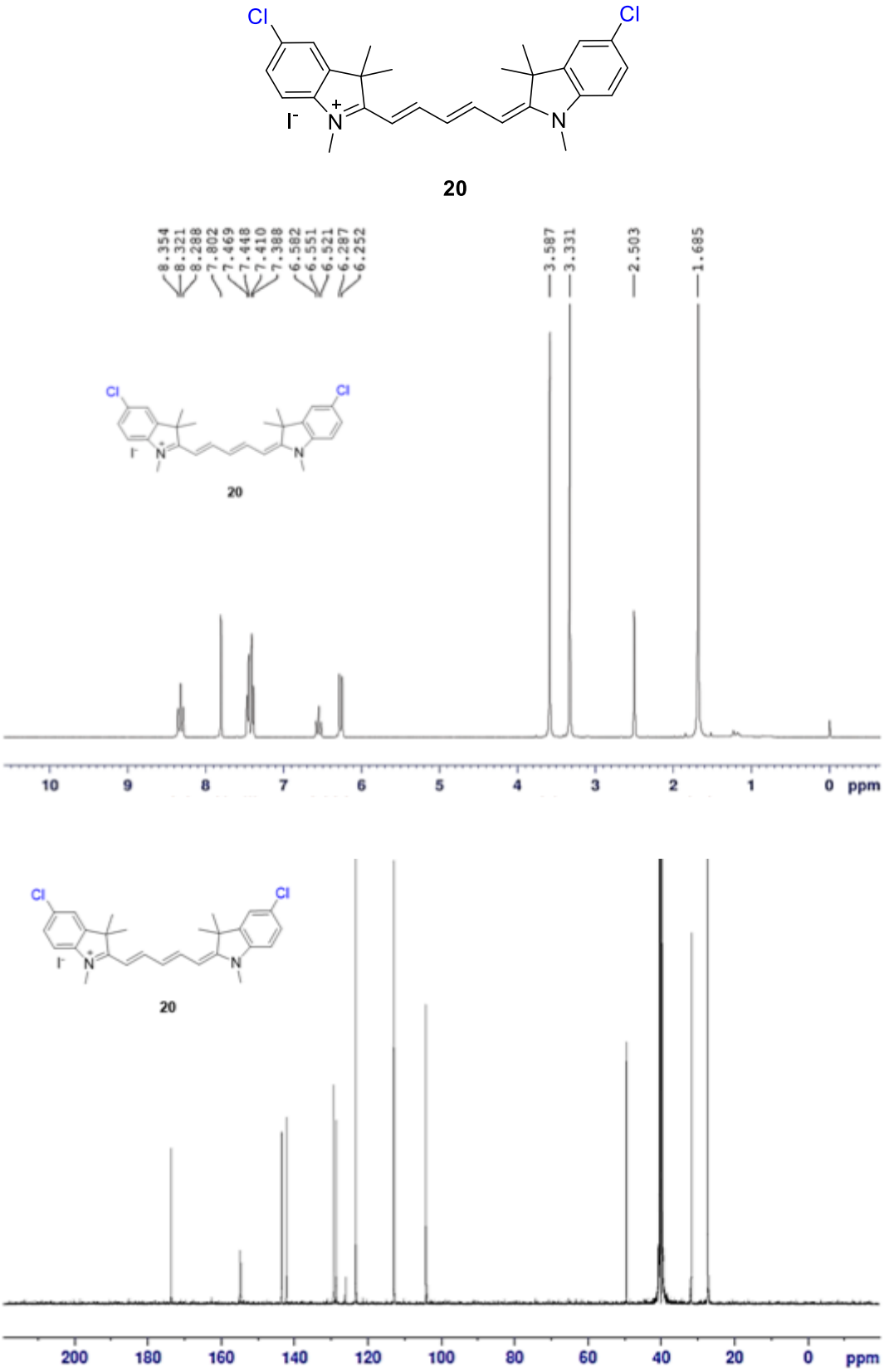


Figure S7. ^{1}H- and ^{13}C-NMR of Compound **20** in DMSO-d_6.

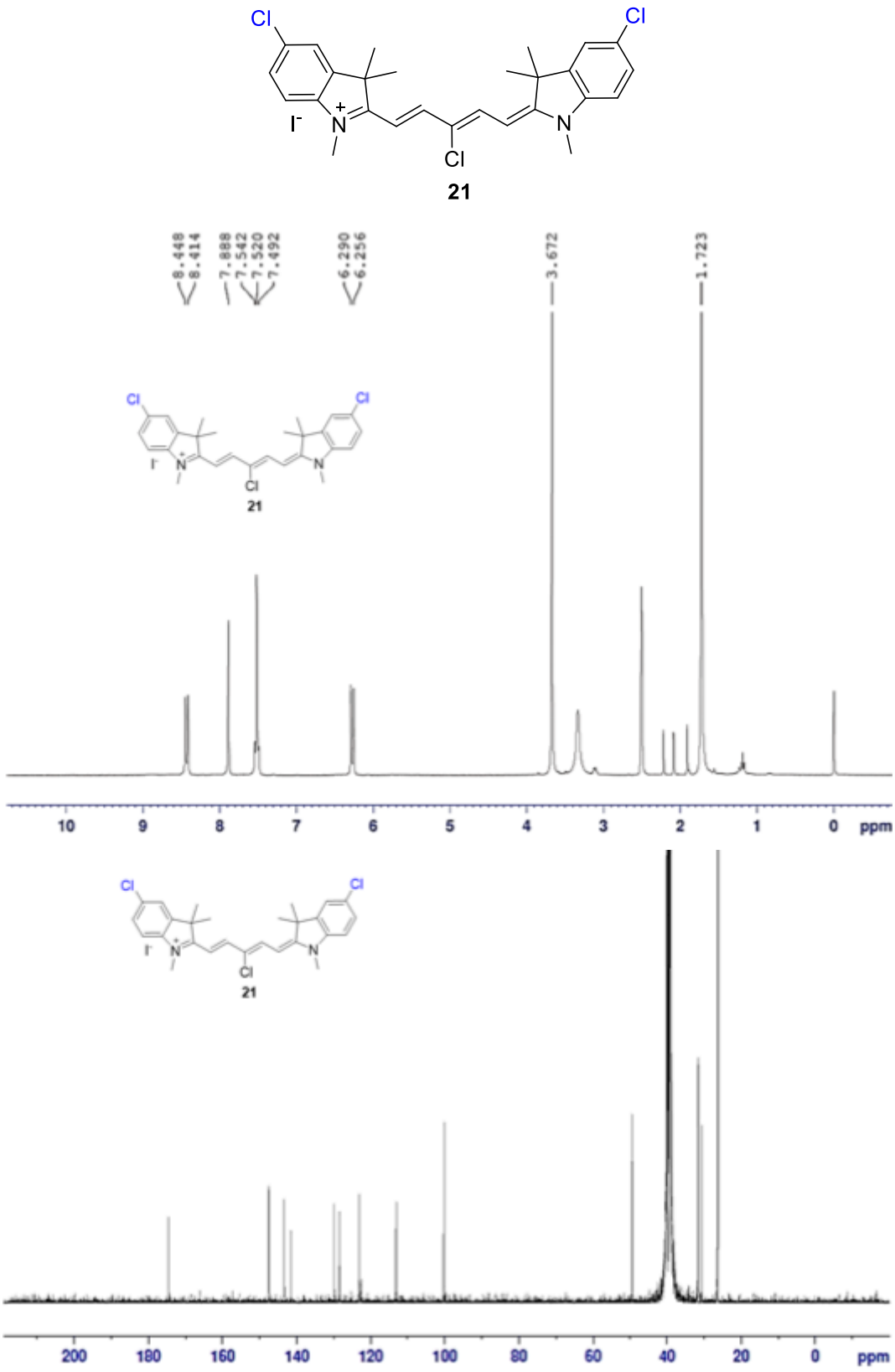


Figure S8. ^{1}H- and ^{13}C-NMR of Compound **21** in DMSO-d_6.

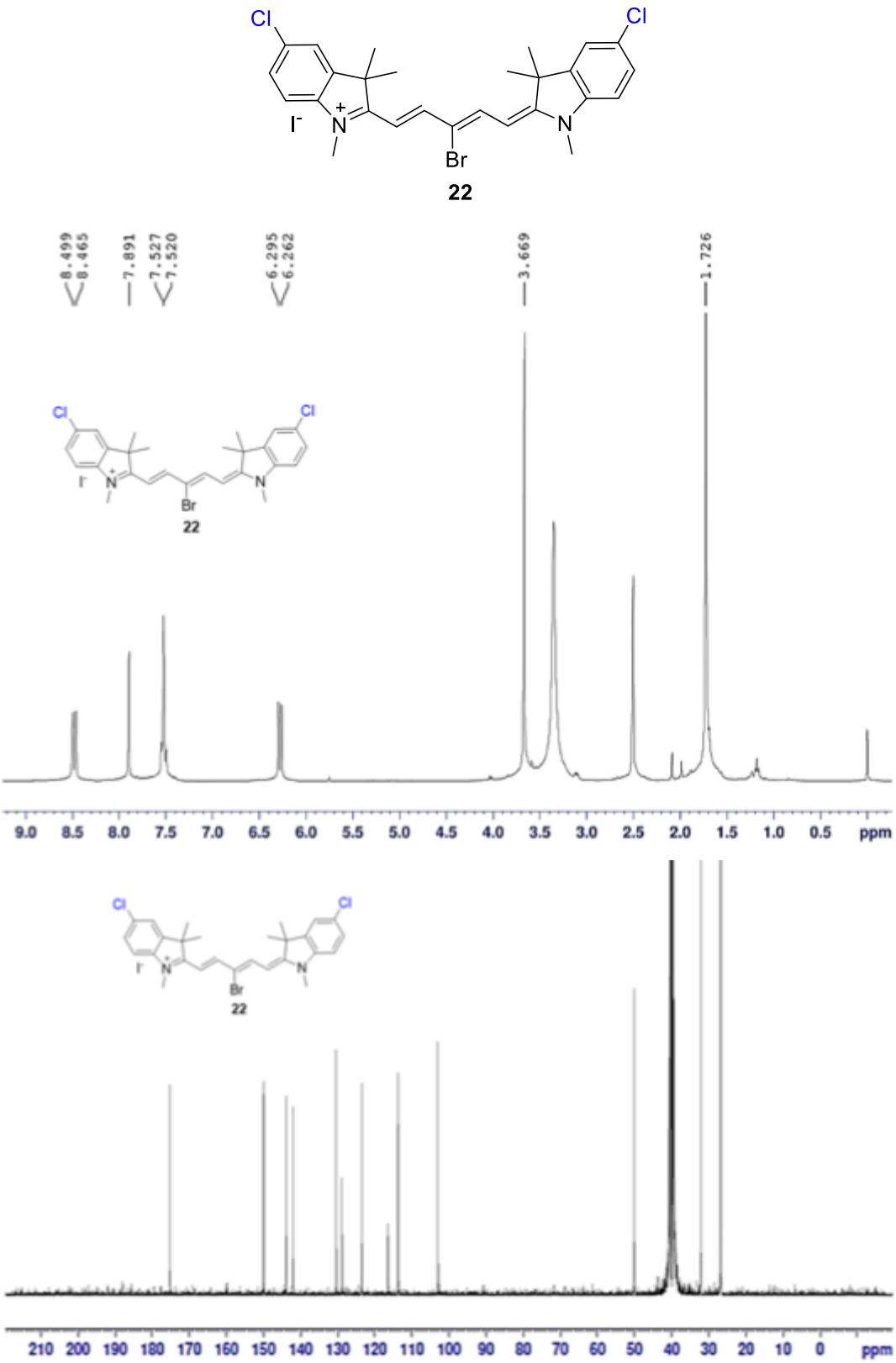


Figure S9. ^{1}H- and ^{13}C-NMR of Compound **22** in DMSO-d_6.

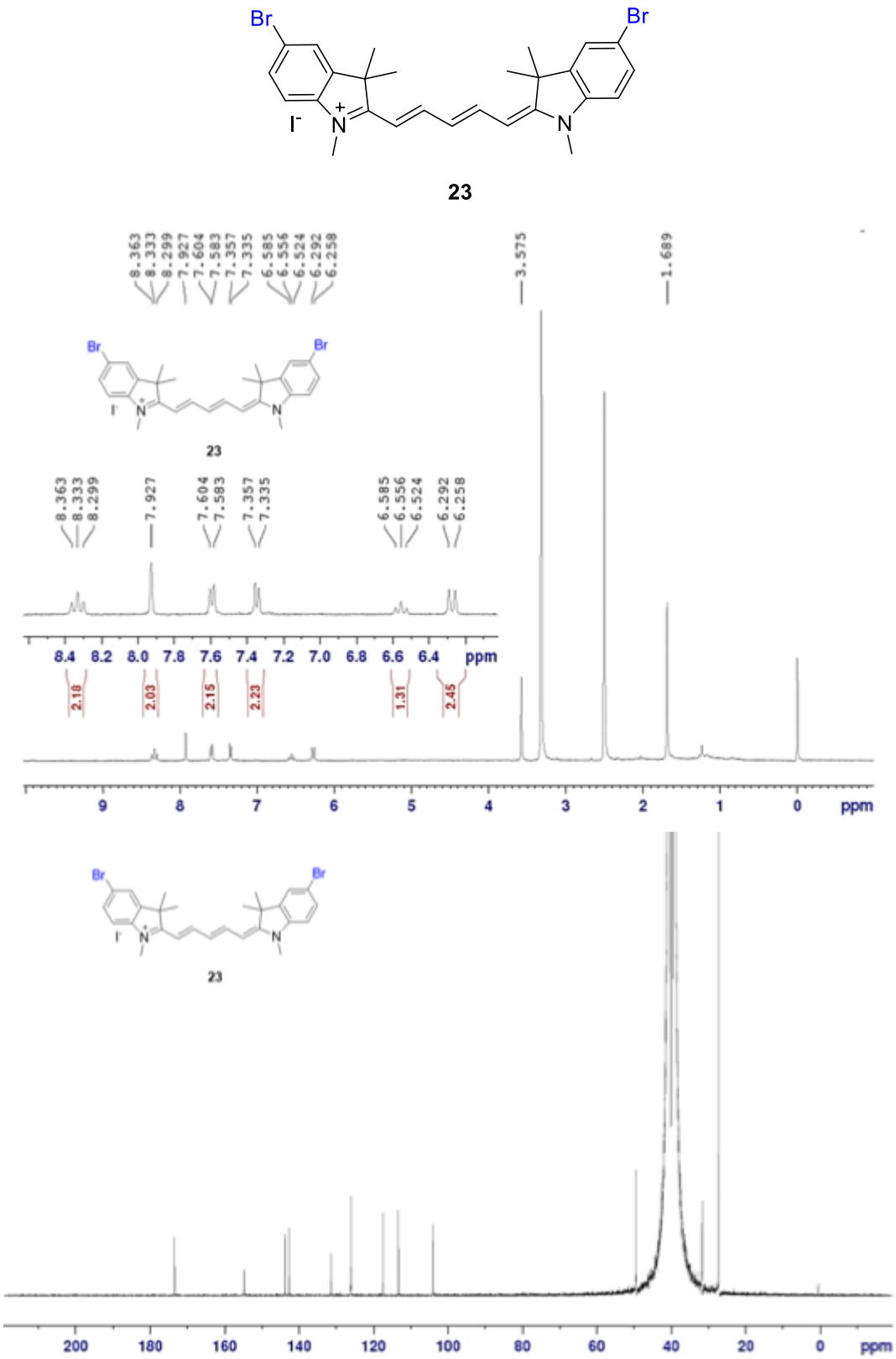
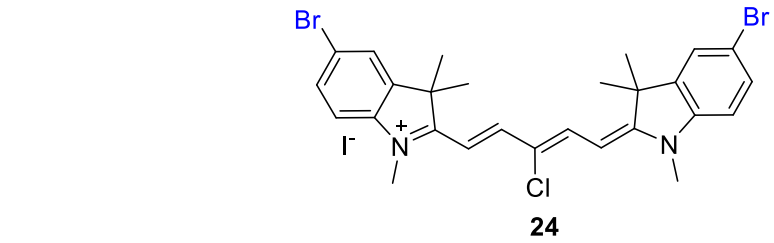


Figure S10. ^{1}H- and ^{13}C-NMR of Compound **23** in DMSO-d_6.



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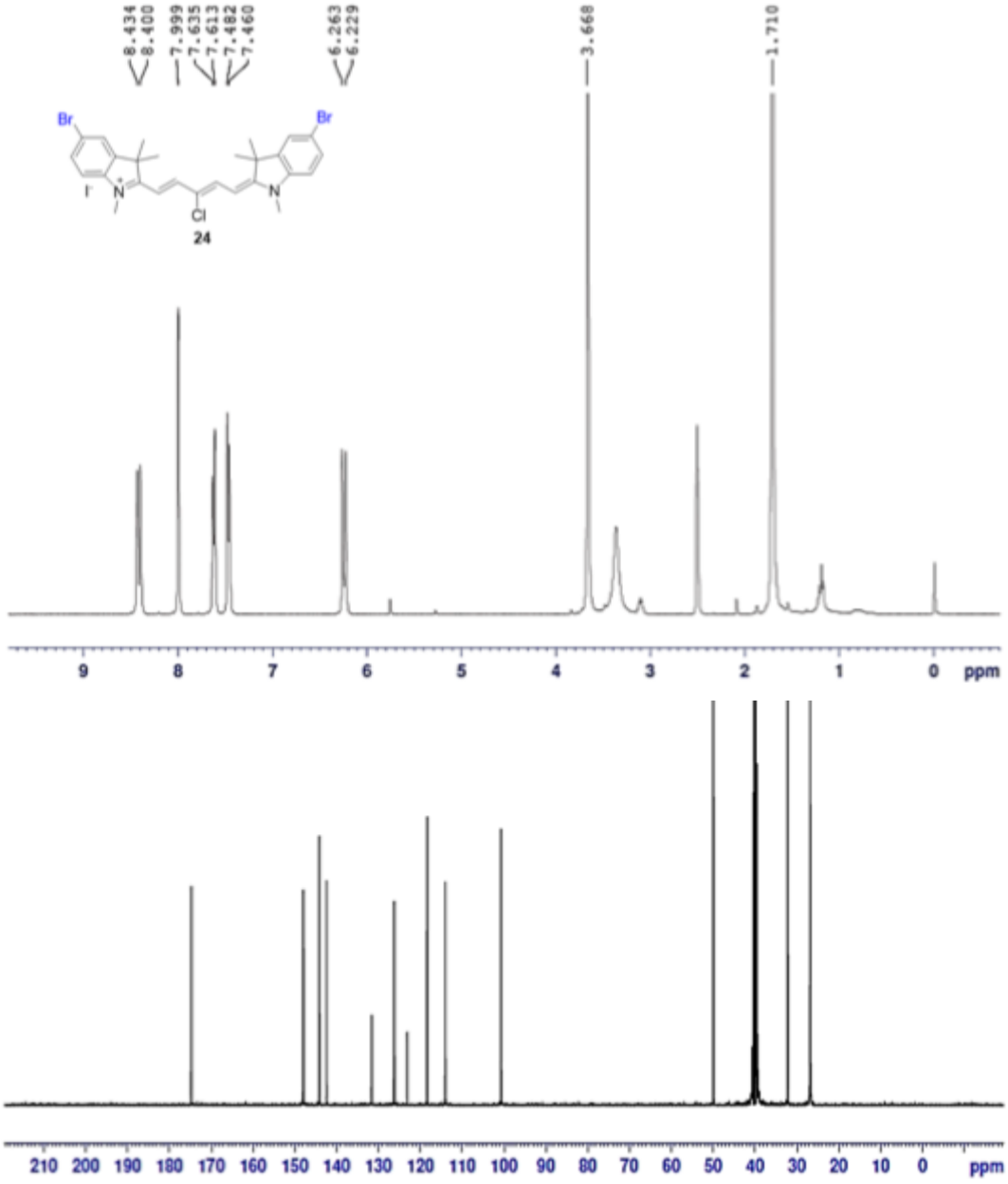


Figure S11. ^{1}H- and ^{13}C-NMR of Compound **24** in DMSO-d_6.

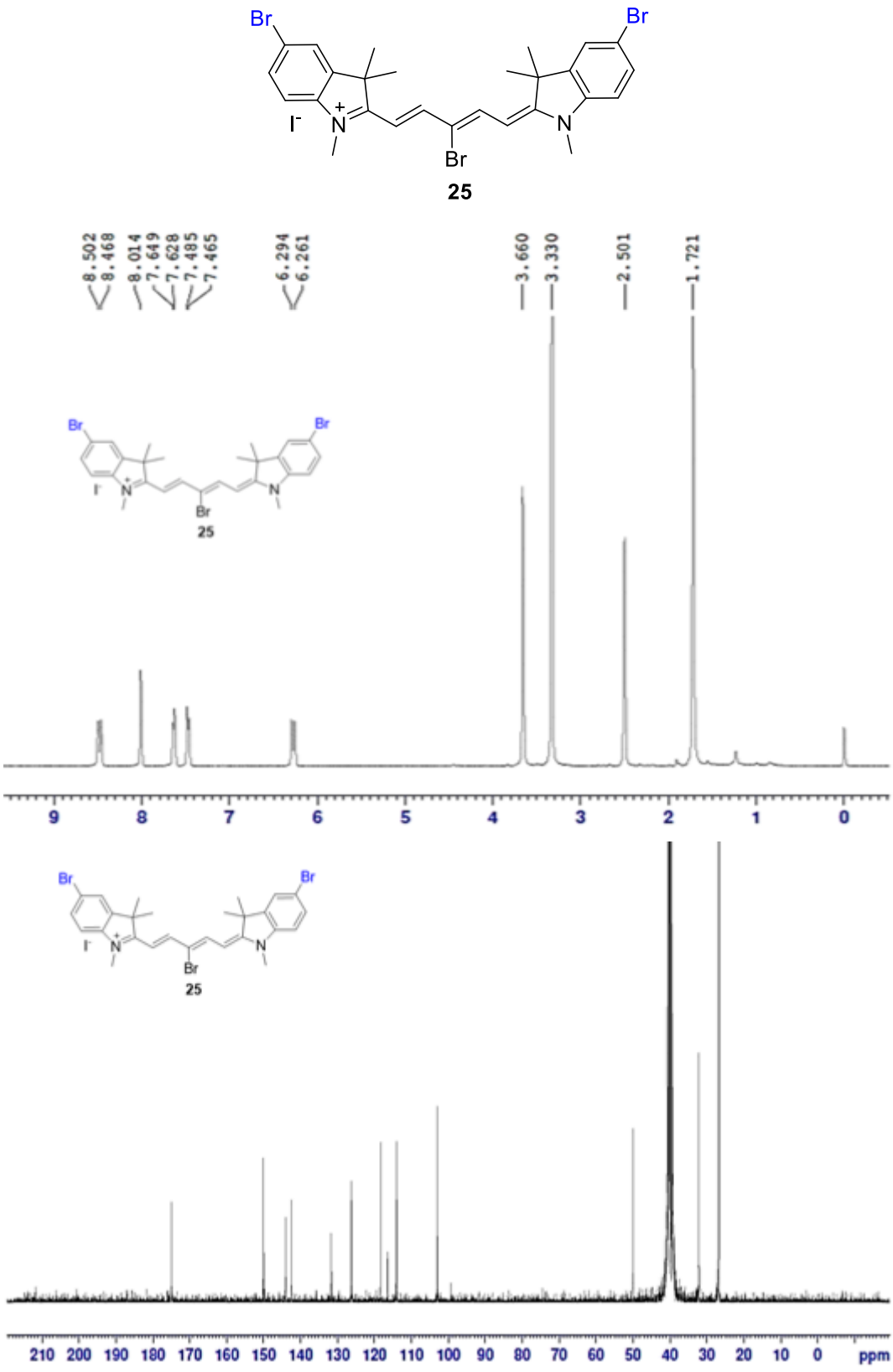
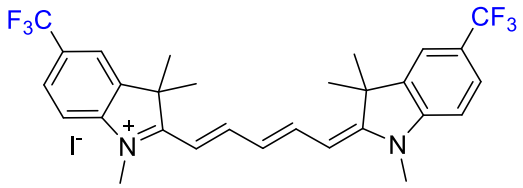


Figure S12. ^{1}H- and ^{13}C-NMR of Compound **25** in DMSO-d_6.



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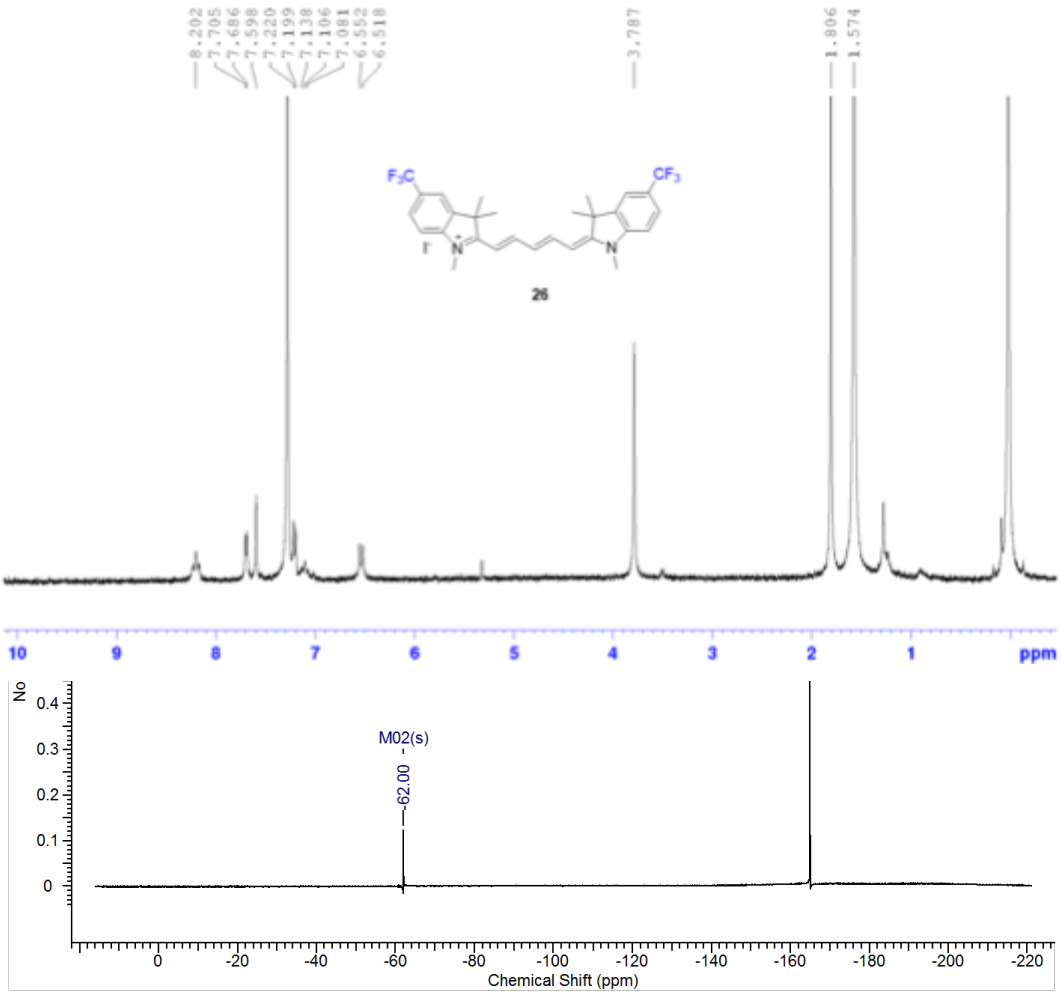


Figure S13. ^{1}H- and ^{19}F-NMR of Compound **26** in $CDCl_3$.

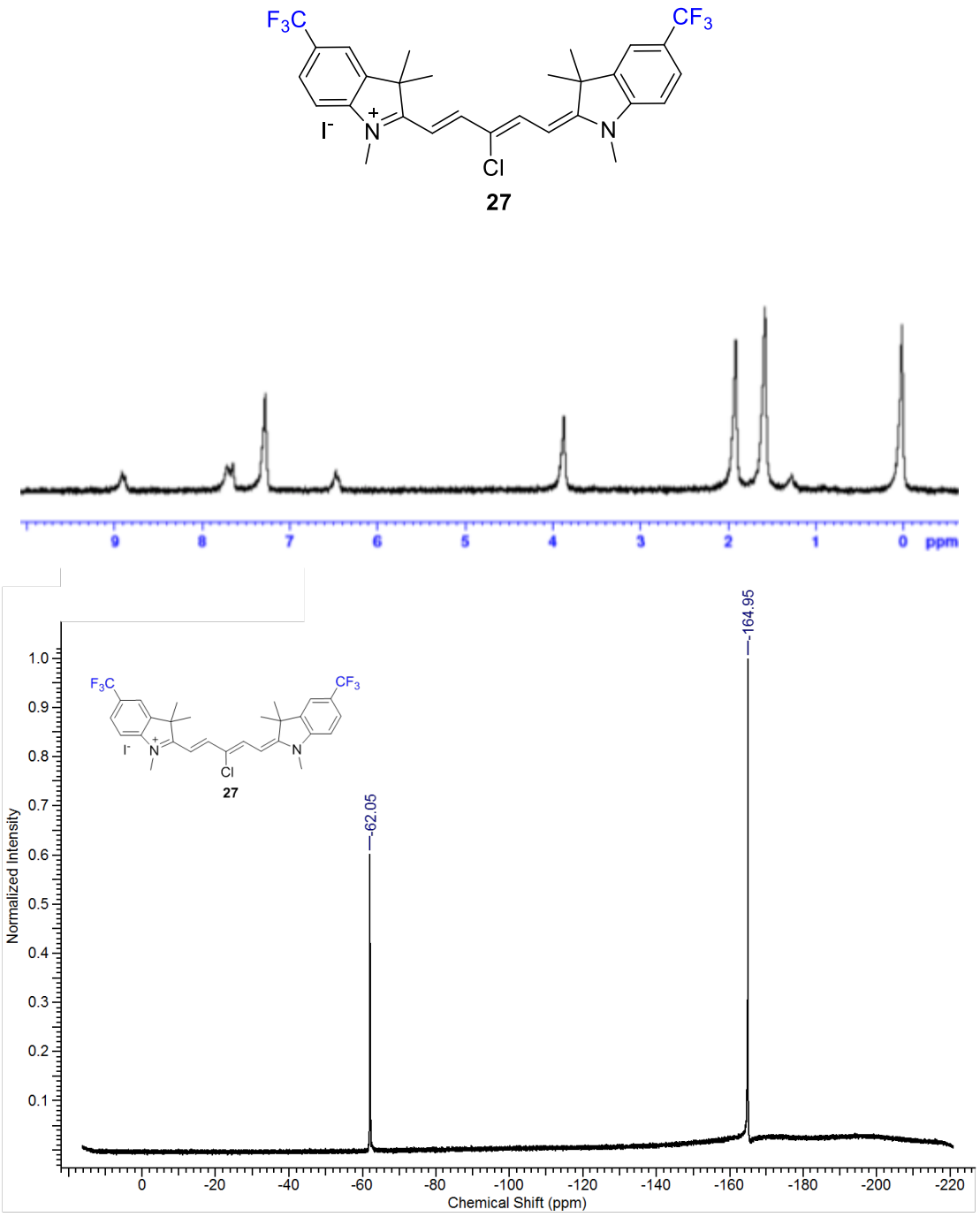


Figure S14. ^{1}H- and ^{19}F-NMR of Compound **27** in $CDCl_3$.

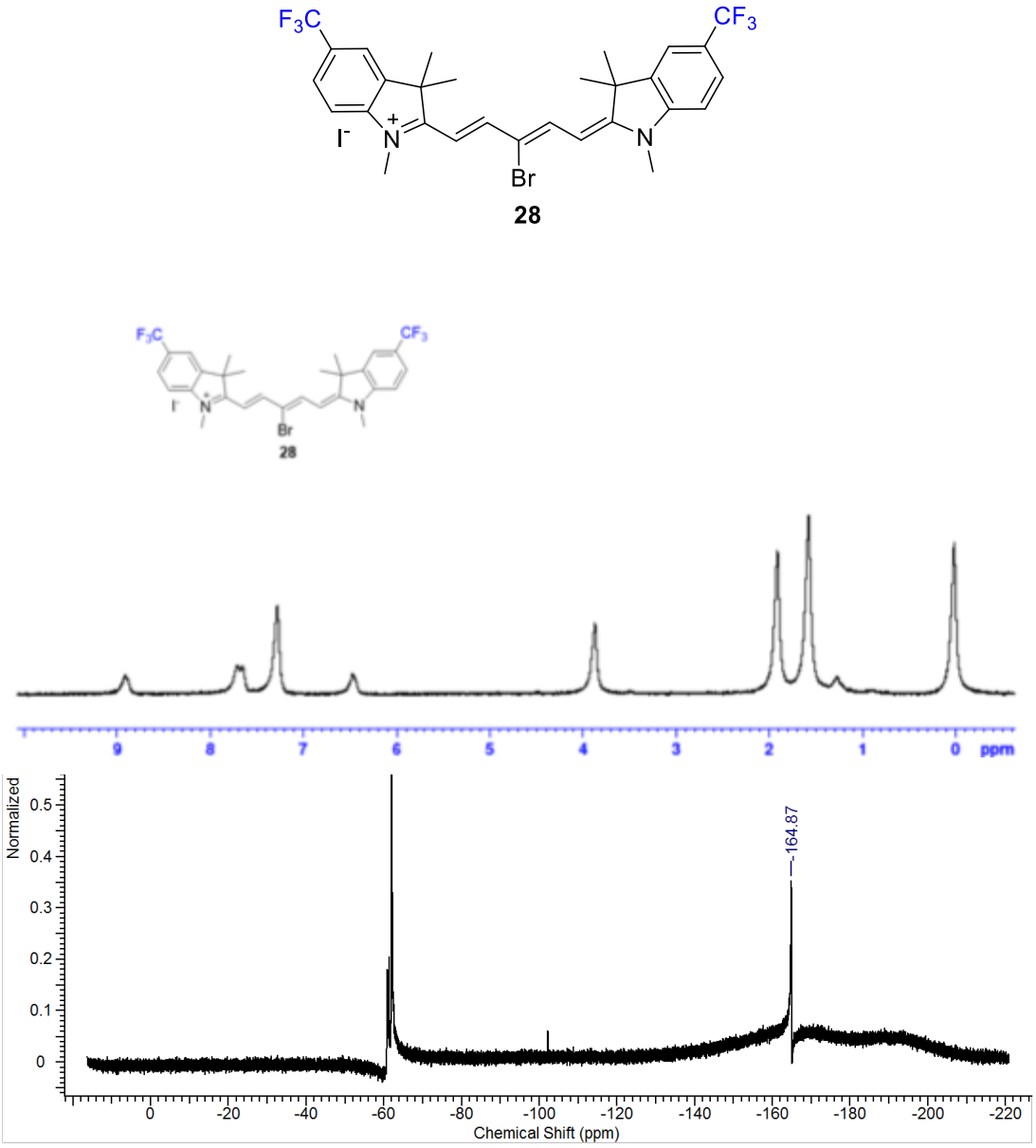


Figure S15. ^{1}H- and ^{19}F-NMR of Compound **28** in $CDCl_3$.

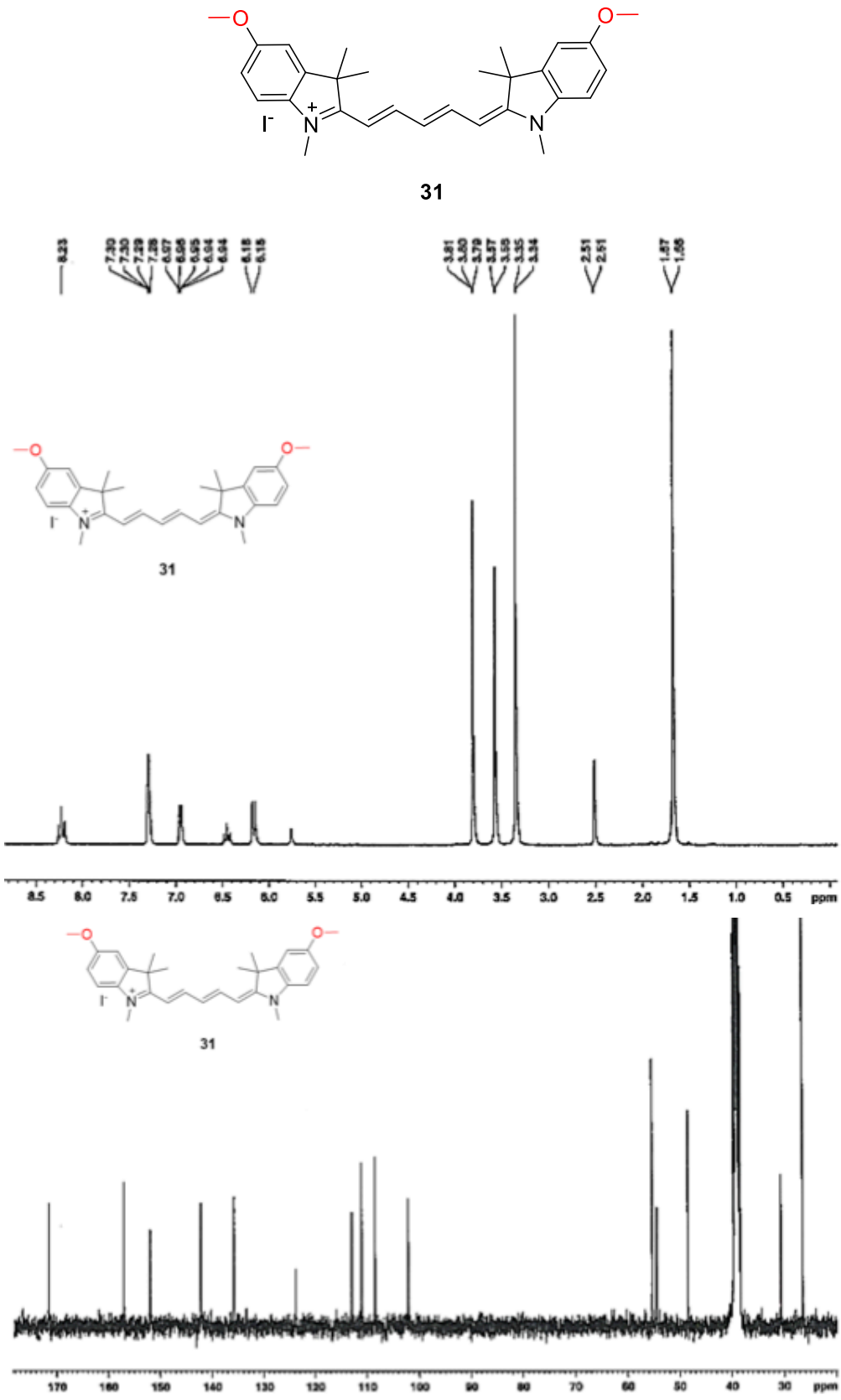


Figure S16. ^{1}H- and ^{13}C-NMR of Compound **31** in DMSO-d_6.

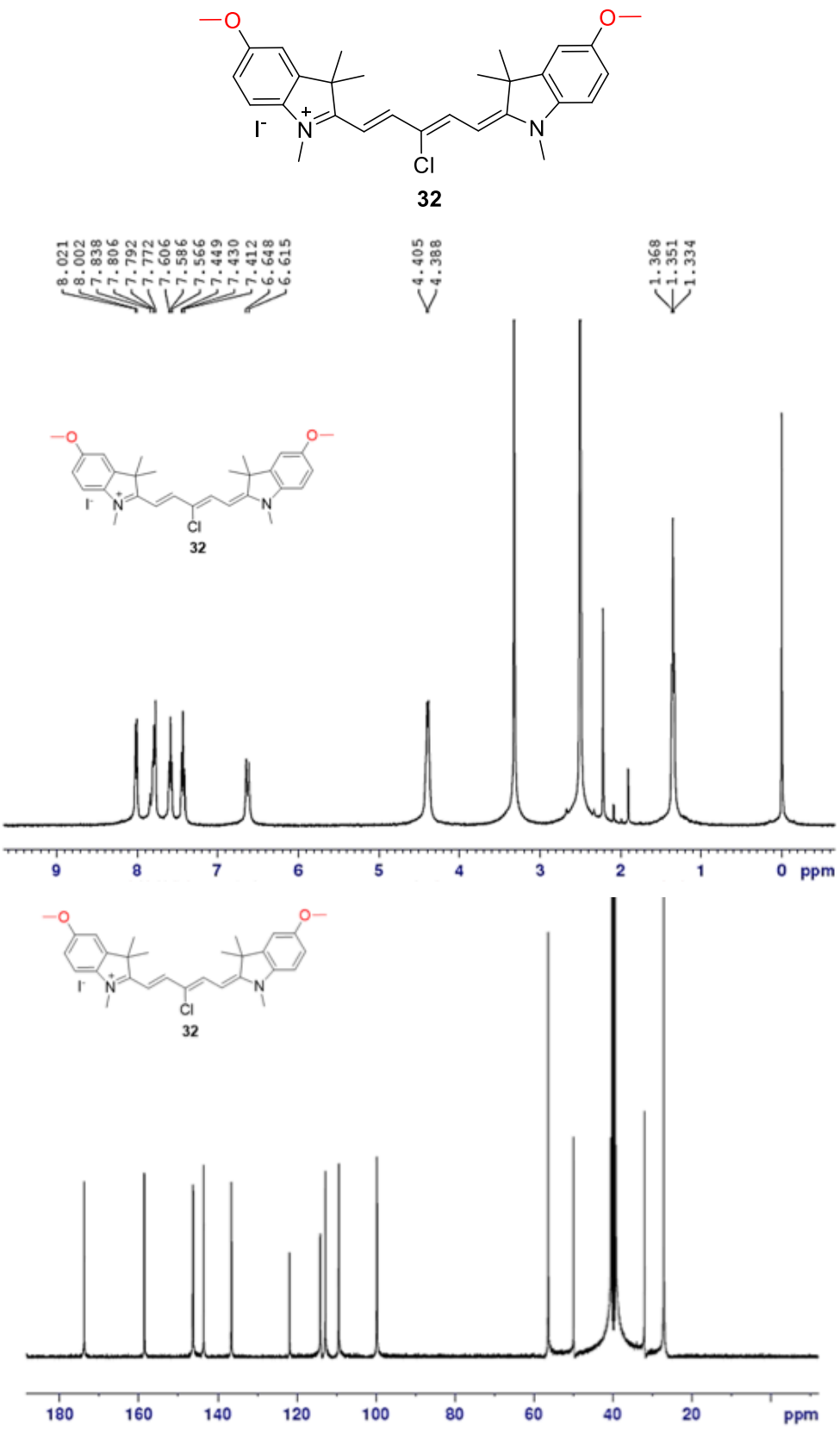


Figure S17. ^{1}H- and ^{13}C-NMR of Compound **32** in DMSO-d_6.

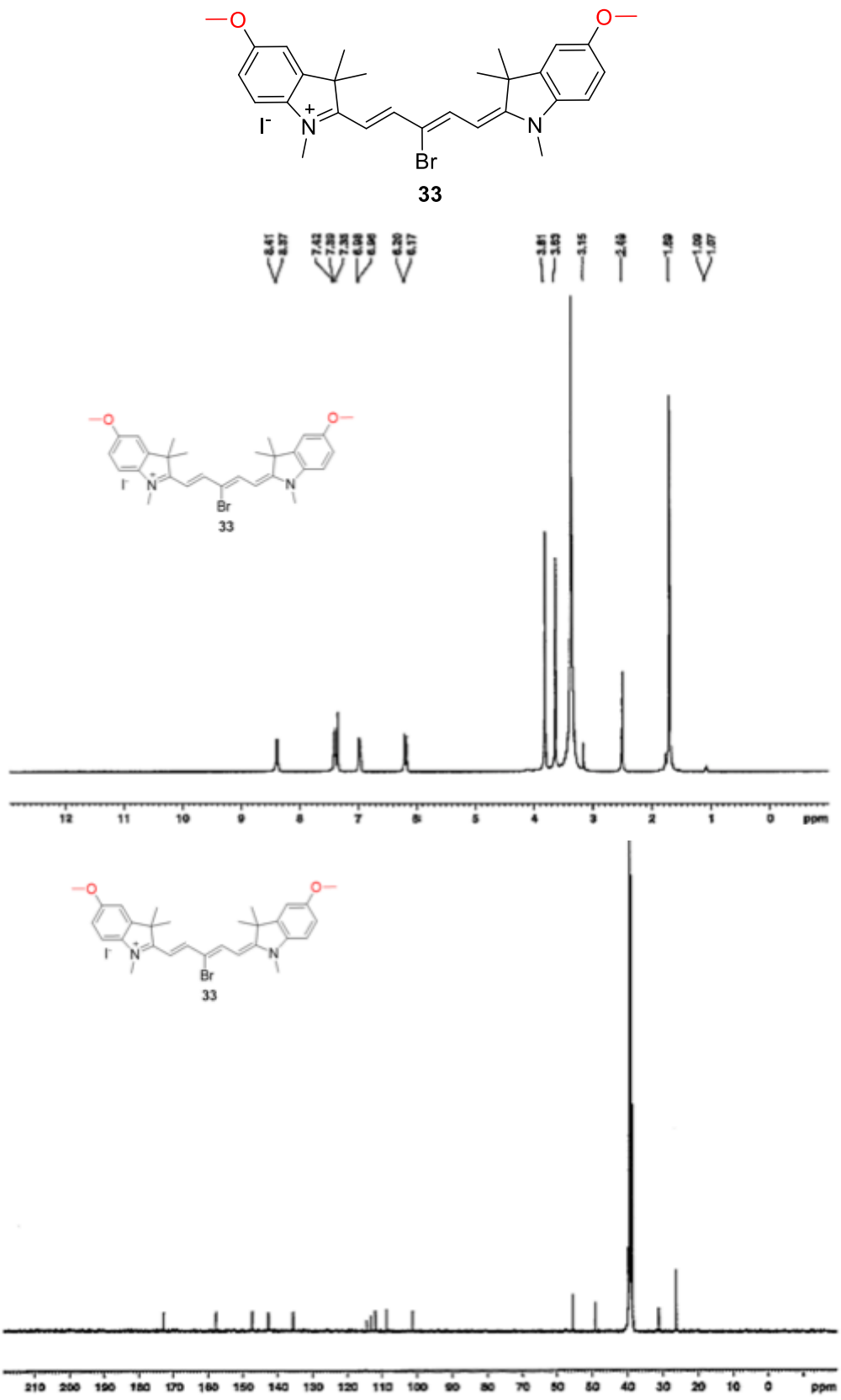
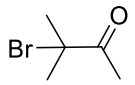
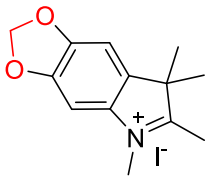


Figure S18. ^{1}H- and ^{13}C-NMR of Compound **33** in DMSO-d_6.



34

3-bromo-3-methyl-2-butanone (**34**): A mixture of 3-methylbutan-2-one (25.00 mL, 233.65 mmol) and acetic acid (38.00 mL) was added to a three-neck oven-dried round bottom flask and maintained at a temperature of 5 ^{o}C. Next, both molecular bromine (12.03 mL, 233.65 mmol) and acetic acid (13.00 mL) was combined in an addition funnel and added dropwise to the round bottom flask. When all of the bromine was added the reaction mixture was allowed to stir overnight at room temperature. To the resulting mixture, water (100 mL) was added, then transferred to a separatory funnel where another 100 mL of water was added and the organic layer was extracted with diethyl ether (3 x 150 mL). The compound was then washed with cold, saturated sodium bicarbonate (4 x 100 mL), dried over anhydrous sodium sulfate, and the reaction mixture was concentrated *in vacuo* to afford yellow oil. The crude product was further purified *via* vacuum distillation (52 ^{o}C , ~ 25 mmHg) to yield a colorless oil. Yield 34%. ^{1}H NMR (400 MHz, CDCl3) **δ:** 1.824 (s, 6H), 2.401 (s, 3H).

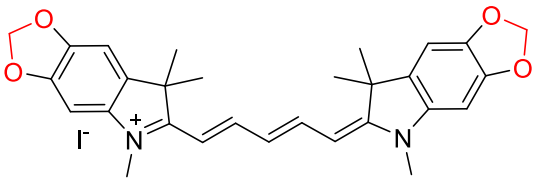


35

6,7,7-trimethyl-7H-[1,3]dioxolo[4,5-f]indole: A solution of DMF, (8 mL), aniline (1.56 g, 11.38 mmol), and potassium carbonate (1.05 g, 6.8 mmol) was brought to 45 ^{o}C in a two-neck round bottom flask with small magnetic stir-bar. 3-Bromo-3-methylbutan-2-one (**34**, 1.86 g, 1 mL) was added to the solution overnight with a syringe pump (Kd Scientific, Model 100). After addition was completed, solution was stirred for 24 h at 45 ^{o}C and monitored by TLC. After TLC showed that the starting material was consumed, dimethylformamide was evaporated *in vacuo*, and concentrate was extracted with toluene (5 x 20 mL) and was washed with deionized water. Toluene was concentrated to an amount of 10-20 mL, and *p*-methyltoluenesulfonic acid (0.110 g, 0.1 mol equiv.) was added to solution. Solution was allowed to reflux for 24h, until TLC showed an absence of starting material. Toluene was evaporated *in vacuo*, and dichloromethane (20 mL) was added to the reaction mixture. Solution was washed with a saturated solution of sodium

carbonate (5 x 50 mL) until the organic layer was a red/brown color. DCM was removed under reduced pressure and the resulting red/brown oil was recovered and the crude cyclic intermediate (obtained in 57% yield) was used without purification. *5,6,7,7-Tetramethyl-7H-[1,3]dioxolo[4,5-f]indol-5-ium iodide* (**35**): This crude product was added to a round bottom flask with acetonitrile (50 mL) and iodomethane (3 mol. eq.). The mixture was heated to 60 ^{o}C for 4h. until the starting material was consumed. The reaction mixture was allowed to cool to rt and diethyl ether (80 mL) was added to the solution resulting in a dark brown oil. Several precipitations from methanol by diluting with diethyl ether afforded a light grey pure solid. Yield 60%, ^{1}H NMR (400 MHz, DMSO-d_6) **δ**: 1.48 (s, 6H) 2.70 (br. s., 3H) 3.90 (s, 3H) 6.19 (s, 2H) 7.48 (s, 1H) 7.64 (s, 1H), ^{13}C NMR (100 MHz, DMSO-d_6) **δ**: 15.43, 21.48, 23.05, 53.61, 55.34, 101.44, 103.34, 125.76, 128.64, 129.34, 139.90, 145.50, 146.91, 147.91, 187.11.

A mixture of indolium salt **35** (1 mmol), bis-iminium salt **11-13** (0.5 mmol), sodium acetate (0.23 mmol) and acetic anhydride (1 mL) was added to an oven dried round bottom flask with magnetic stirring bar. The reaction mixture was heated using a standard oil bath for a particular reaction time and was followed using TLC and UV-Vis-NIR absorption in ethanol. After starting materials were consumed, the reaction was allowed to cool to room temperature. Diethyl ether was added to the round bottom flask resulting in an oily metallic blue residue. The diethyl ether was decanted and the oil was dissolved in a minimal amount of methanol followed by the addition of diethyl ether (50 mL) resulting in the formation of light blue crystals, which were filtered. The crystals were dissolved in dichloromethane leaving unreacted sodium acetate on the funnel. The dichloromethane was removed *in vacuo*. Silica-gel column chromatography eluting with 2-5% methanol in dichloromethane afforded the various pentamethine cyanines **36-38** in their respective yield.



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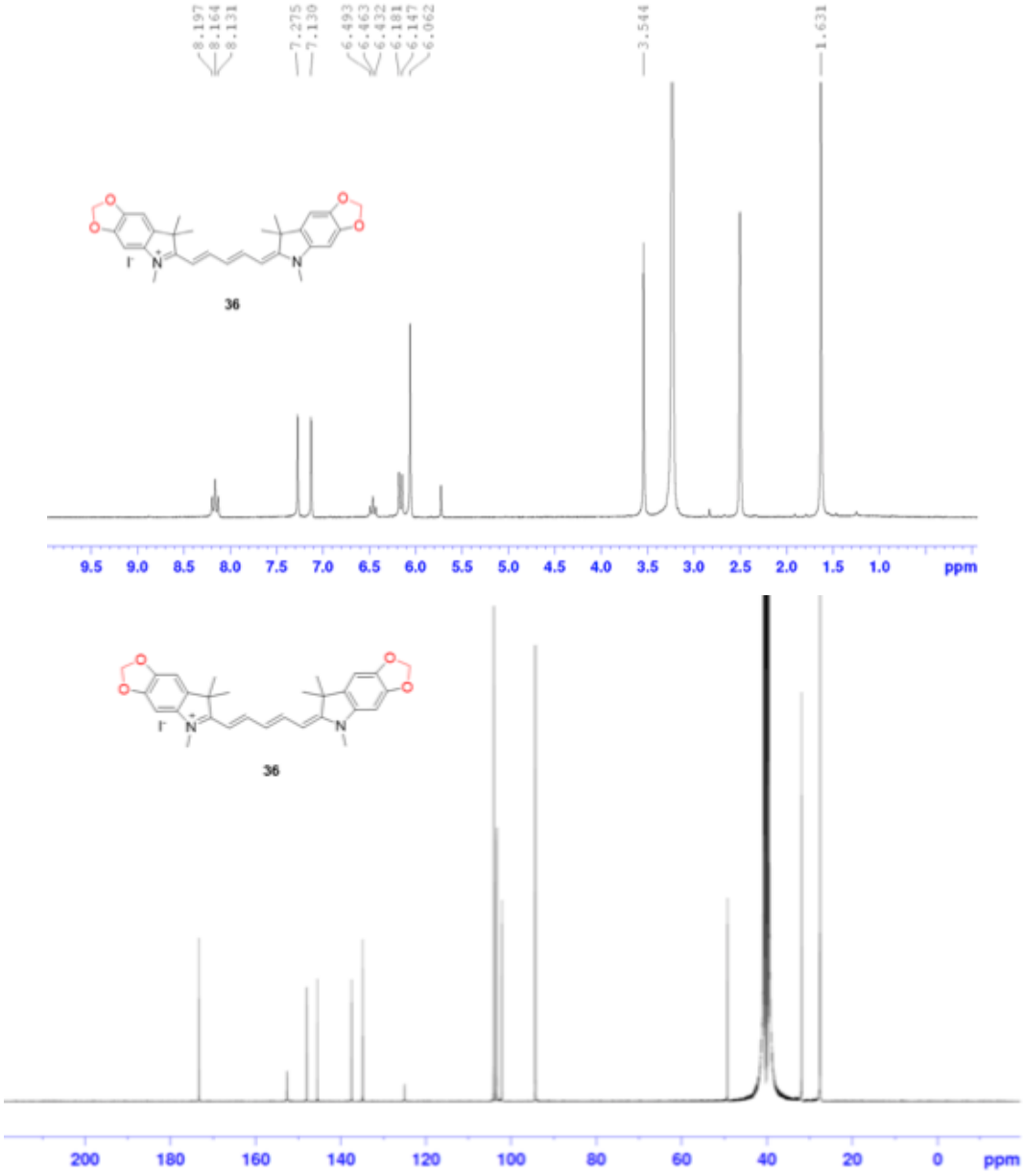
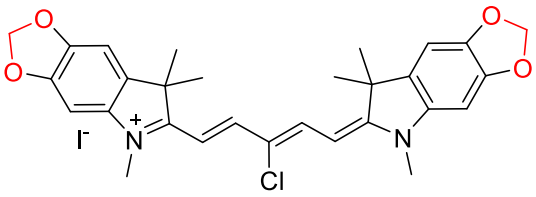


Figure S19. ^{1}H- and ^{13}C-NMR of Compound **36** in DMSO-d_6.



37

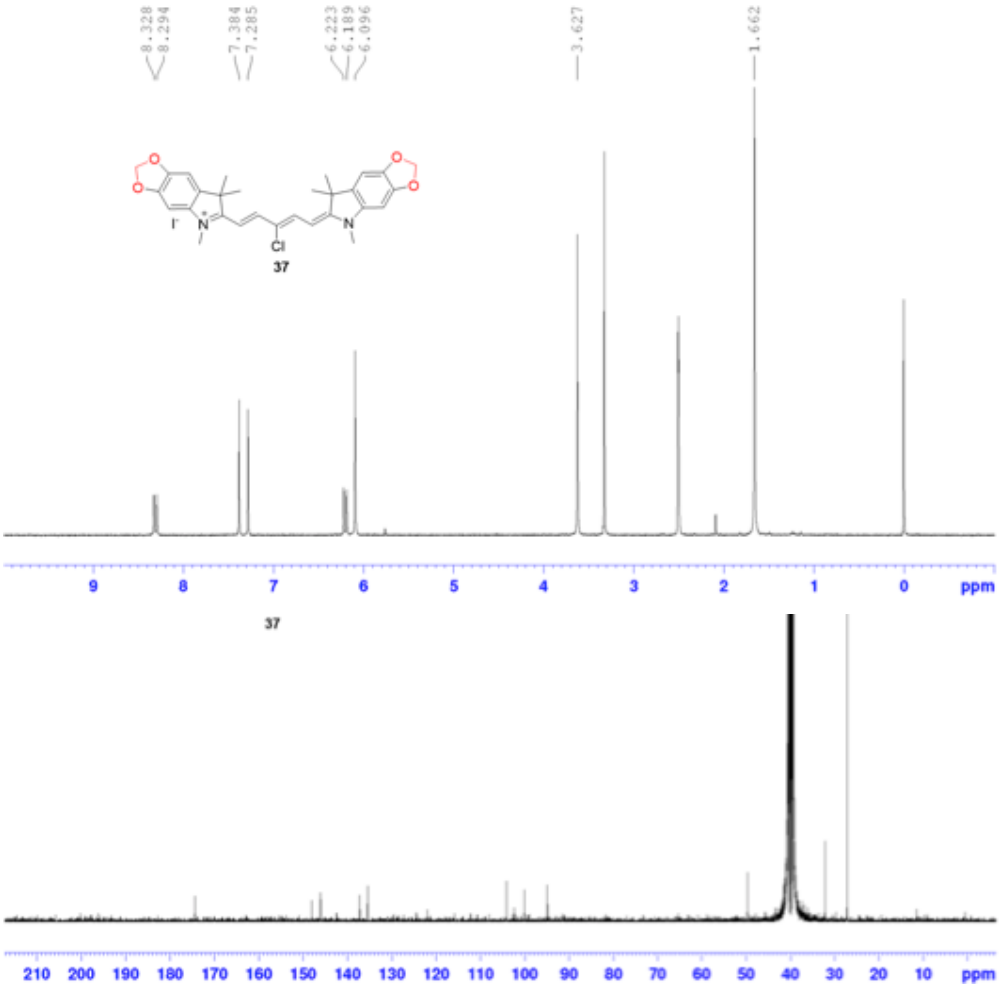
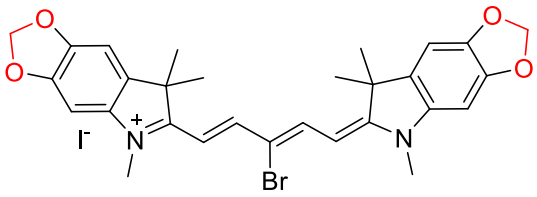


Figure S20. ^{1}H- and ^{13}C-NMR of Compound **37** in DMSO-d_6.



38

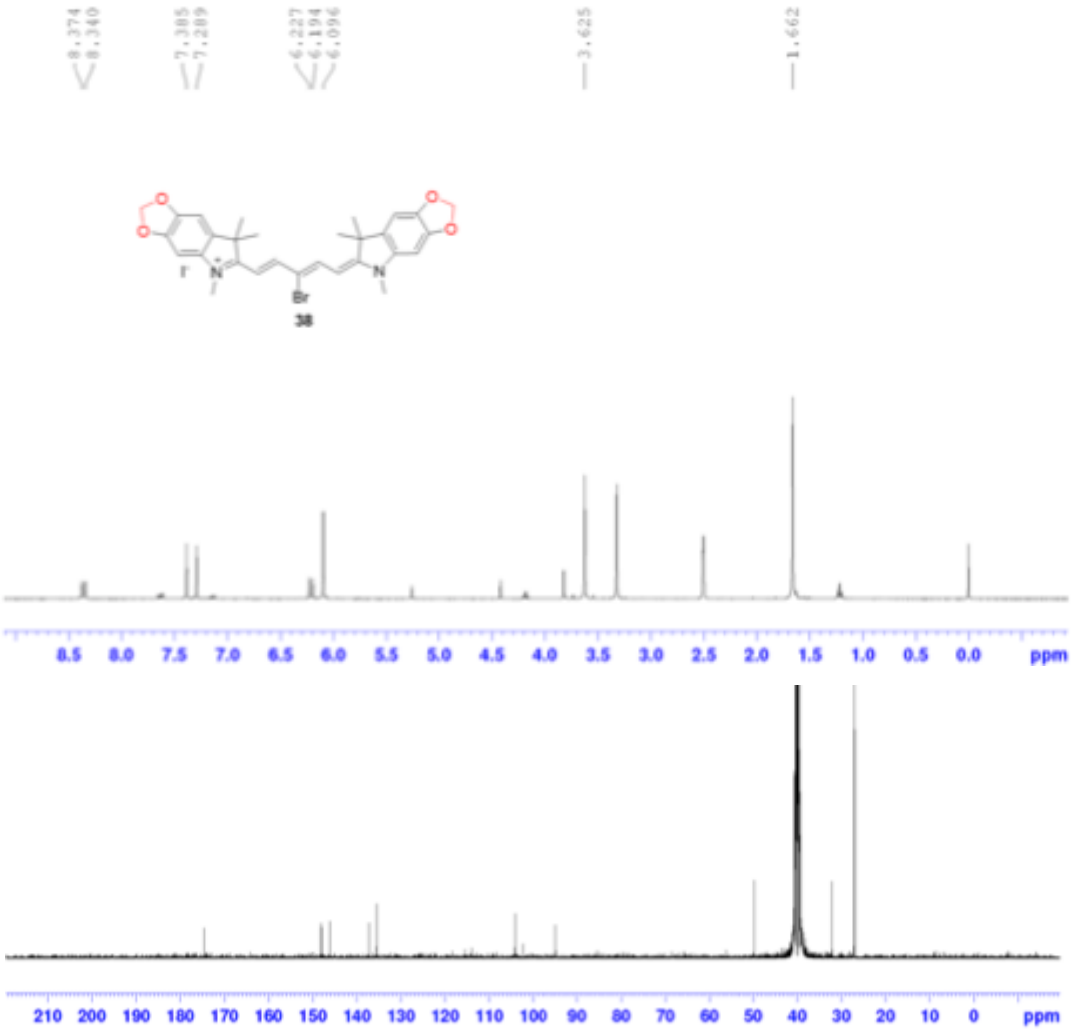


Figure S21. ^{1}H- and ^{13}C-NMR of Compound **38** in DMSO-d_6.

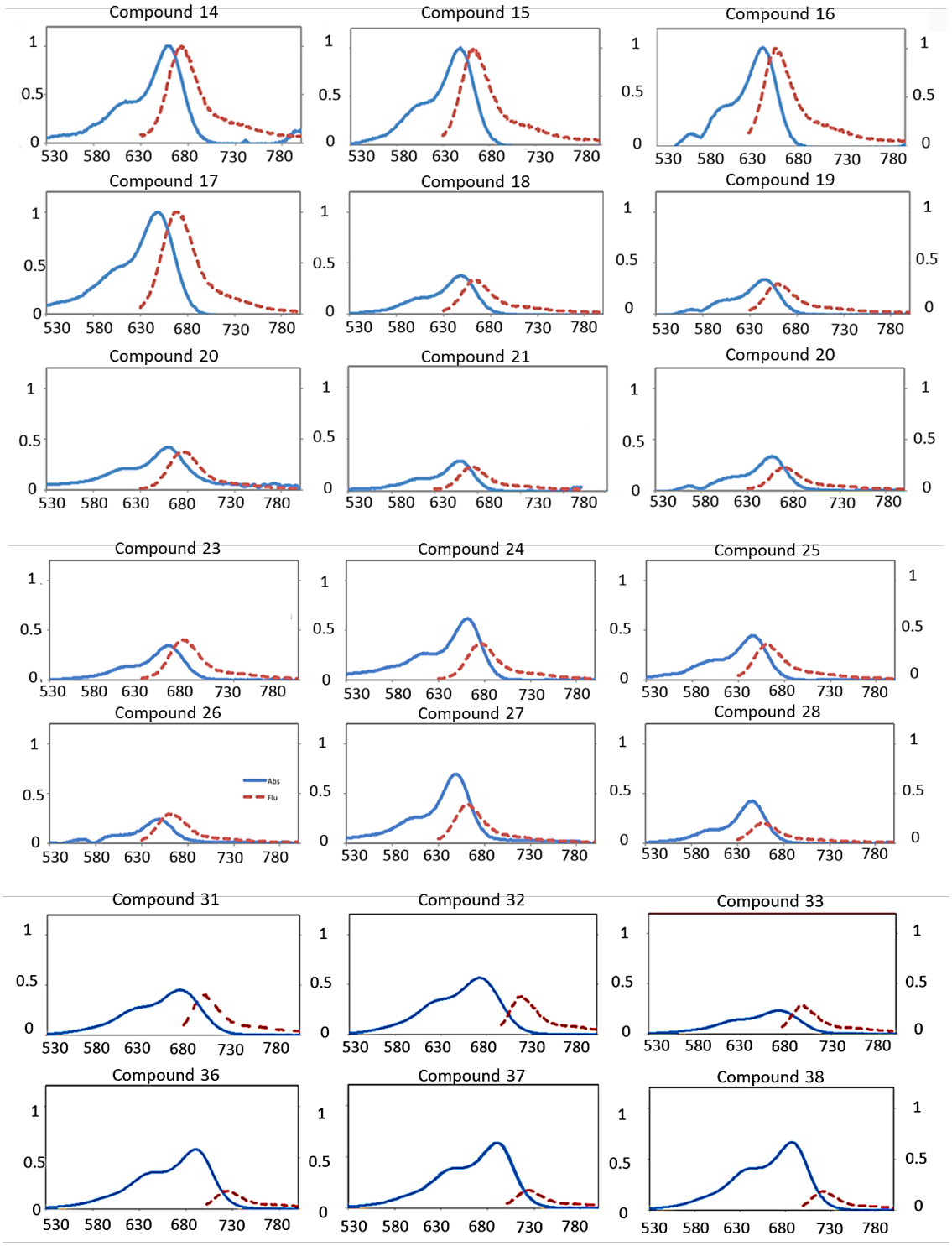


Figure S22. Optical absorption and fluorescence spectra for representative compounds in FBS buffered with HEPES to pH = 7.4 at 37 ºC.

References

1. Owens, E.A., Bruschi, N., Tawney, J.G. & Henary, M. A microwave-assisted and environmentally benign approach to the synthesis of near-infrared fluorescent pentamethine cyanine dyes. *Dyes and Pigments* **113**, 27-37 (2015).