Electrophile-Integrating Smiles Rearrangement Provides Previously Inaccessible C4′-*O*-Alkyl Heptamethine Cyanine Fluorophores

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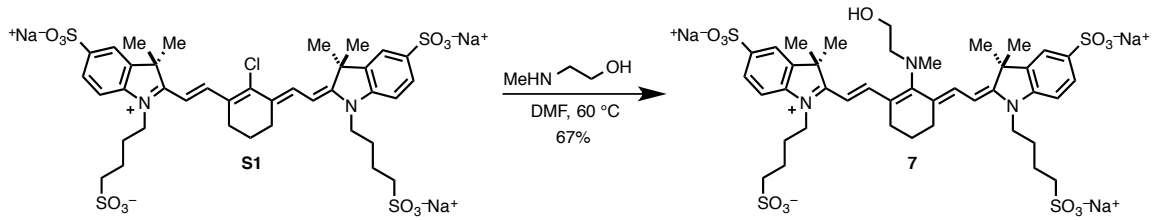
Supporting Information – Table of Contents

General Materials and Methods. Unless stated otherwise, reactions were conducted in oven-dried glassware under an atmosphere of nitrogen or argon using anhydrous solvents (passed through activated alumina columns). All other commercially obtained reagents were used as received. Thin-layer chromatography (TLC) was conducted with E. Merck silica gel 60 F254 pre-coated plates (0.25 mm) and visualized by exposure to UV light (254 nm) or stained with

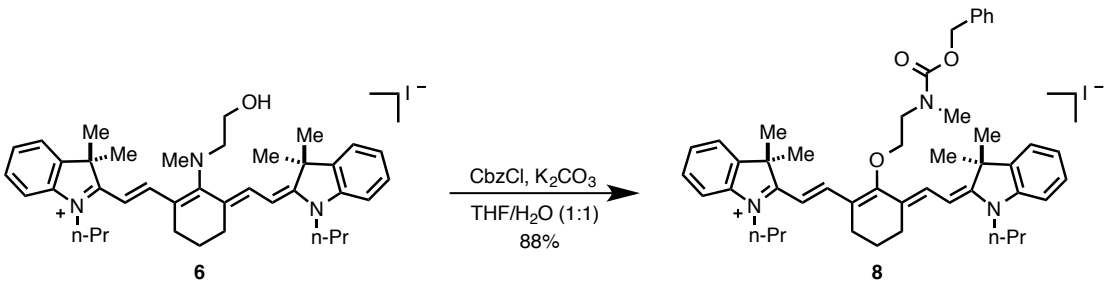
anisaldehyde, ceric ammonium molybdate, potassium permanganate, or iodine. Flash column chromatography was performed using normal phase or reverse phase on a CombiFlash® Rf 200i (Teledyne Isco Inc). Analytical LC/MS was performed using a Shimadzu LCMS-2020 Single Quadrupole utilizing a Kinetex 2.6 μm C18 100 Å (2.1 x 50 mm) column obtained from Phenomenex Inc. Runs employed a gradient of 0→90% MeCN/0.1% aqueous formic acid over 4 minutes at a flow rate of 0.2 mL/min. ^{1}H NMR spectra were recorded on Bruker spectrometers (at 400 or 500 MHz) and are reported relative to deuterated solvent signals. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. ^{13}C NMR spectra were recorded on Varian spectrometers (at 100 or 125 MHz). Data for ^{13}C NMR spectra are reported in terms of chemical shift. IR spectra were recorded on a JASCO FT/IR 4100 spectrometer and are reported in terms of frequency of absorption (cm^{-1}). High-resolution LC/MS analyses were conducted on a Thermo-Fisher LTQ-Orbitrap-XL hybrid mass spectrometer system with an Ion MAX API electrospray ion source in positive ion mode. Separations were carried out on a narrow-bore (50 X 2.1 mm), Zorbax Rapid-Resolution, reversed-phase C18 (3.5 μm) column with a flow rate of 250 μL/min with a 10 min, 2-90% gradient of MeCN/H_2O containing 0.1% HCOOH. Absorption traces for quantum yield measurements were performed on a Shimadzu UV-2550 spectrophotometer operated by UVProbe 2.32 software. Fluorescence traces and quantum yield measurements were recorded on a PTI QuantaMaster steady-state spectrofluorimeter operated by FelixGX 4.0.3 software, with 10 nm excitation and emission slit widths, 0.1 s integration rate, and enabled emission correction. Data analysis and curve fitting were performed using MS Excel 2011 and GraphPad Prism 6. See *JOC Standard Abbreviations and Acronyms* for abbreviations (at http://pubs.acs.org/userimages/ContentEditor/1218717864819/joceah_abbreviations.pdf).

Experimental Procedures

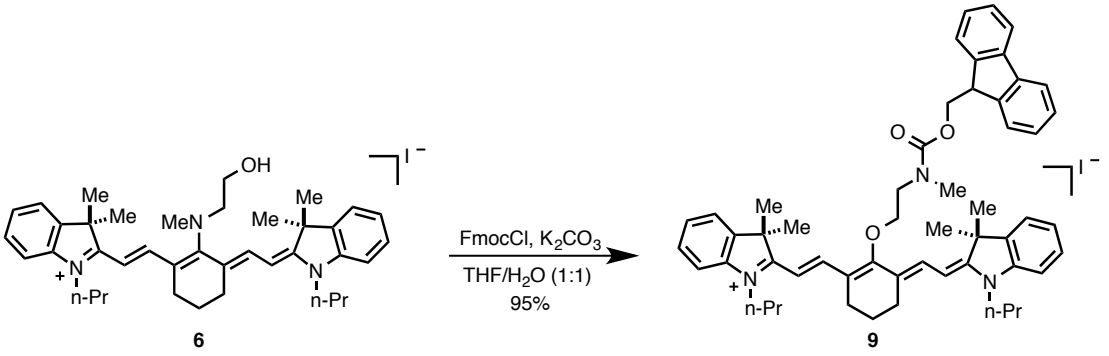


(7): To a solution of cyanine **S1**[1] (115 mg, 0.12 mmol) in DMF (2.5 mL) was added *N*-methylethanolamine (190 μL, 2.41 mmol). The dark green slurry was sonicated for 5 minutes, then heated to 60 °C in a sealed vial for 45 min. After this time, LC/MS analysis showed complete consumption of **S1**, and the reaction color had transitioned from green to dark blue. The reaction was cooled to room temperature and precipitated into Et_2O (100 mL) with a 1 mL DMF vial wash. The slurry was centrifuged, the supernatant discarded, and the blue pellet was resuspended in Et_2O (40 mL). The procedure was repeated, and the crude pellet was dissolved in 5 mL of water for an ion exchange step. A pipet was filled with 1.5 g of Dowex 50W X8 strongly acidic 200-400 mesh resin, washed with 3 mL of water, 5 mL of 1 M H_2SO_4, and finally 3 mL of water. The aqueous solution of the crude **7** was eluted (fast dropwise rate) through the Dowex column into an aqueous $NaHCO_3$ solution (200 mg in 2 mL water). After stirring for 5 minutes, this aqueous solution was purified by reversed-phase chromatography (0→10% MeCN/water) to afford **7** (80 mg, 67%) as a dark blue solid. λ_{max} 644 nm (2 μM PBS); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.85 – 7.77 (m, 4H), 7.73 (d, J = 13.2 Hz, 2H), 7.16 (d, J = 8.3 Hz, 2H), 5.97 (d, J = 13.2 Hz, 2H), 4.10 – 3.89 (m, 7H), 3.54 (s, 3H), 2.97 – 2.81 (m, 4H), 2.57 (t, J = 6.6 Hz, 4H), 2.03 – 1.78 (m, 10H), 1.67 (s, 12H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 175.9, 167.6, 143.2, 142.9, 141.0, 139.4, 125.9, 123.4, 119.4, 108.5, 95.5, 59.8, 58.6, 50.8, 47.3, 44.2, 42.7, 39.5, 28.7, 25.5, 24.3, 22.6, 21.5; IR (thin film) 3412, 1545, 1515, 1478, 1378, 1285 cm^{-1}; HRMS (ESI) calculated for $C_{41}H_{52}N_3O_{13}S_4$; ($M-3H^{-3}$) 307.4122, observed 307.4134.

[1] Hilderbrand, S. A.; Kelly, K. A.; Weissleder, R.; Tung, C. H. *Bioconjugate Chem.* **2005**, *16*, 1275–1281.



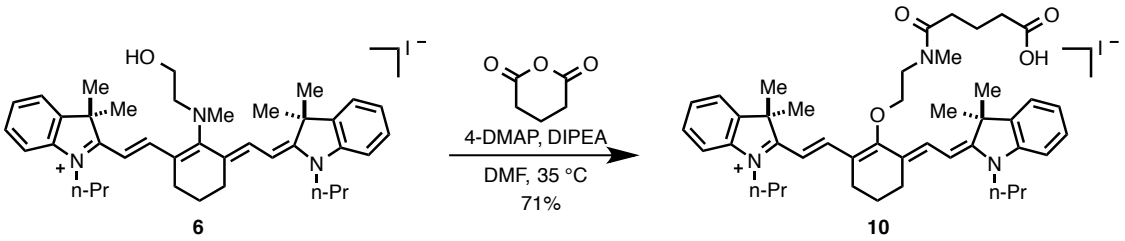
(8): To a solution of **6**[2] (50 mg, 0.072 mmol) in 1:1/THF:H_2O (1 mL) was added benzyl chloroformate (30 μL, 0.217 mmol) and potassium carbonate (50 mg, 0.362 mmol). The biphasic solution was stirred at room temperature for two hours as the reaction color transitioned from dark blue to green. After this time, LC/MS analysis showed complete consumption of **6**. The solution was diluted with saturated aqueous sodium iodide (10 mL), extracted with dichloromethane (2 x 10 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the residue was purified by silica gel chromatography (0→25% MeOH/DCM) affording **8** (54 mg, 88%) as an iridescent green solid. λ_{max} 763 nm (2 μM PBS); ^{1}H NMR (400 MHz, CD_3CN, 70°C) δ 8.10 (d, *J* = 14.2 Hz, 2H), 7.54 – 7.14 (m, 13H), 6.09 (d, *J* = 14.2 Hz, 2H), 5.16 (s, 2H), 4.15 (t, *J* = 5.7 Hz, 2H), 4.02 (t, *J* = 7.4 Hz, 4H), 3.87 (t, *J* = 5.7 Hz, 2H), 3.14 (s, 3H), 2.61 (t, *J* = 6.2 Hz, 4H), 1.92 – 1.80 (m, 6H), 1.66 (s, 12H), 1.03 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (100 MHz, CD_3CN, 70°C) δ 173.6, 171.8, 157.6, 144.1, 142.6, 142.1, 138.7, 129.9, 129.8, 129.2, 129.0, 126.1, 124.3, 123.6, 112.2, 100.9, 76.8, 68.3, 50.8, 50.4, 46.9, 36.9, 29.0, 25.8, 22.4, 21.8, 11.9. IR (thin film) 1698, 1553, 1505, 1361, 1248 cm^{-1}; HRMS (ESI) calculated for $C_{47}H_{58}N_3O_3$ (M^+) 712.4473, observed 712.4446.



(9): To a solution of **6** (38 mg, 0.054 mmol) in 1:1/THF:H_2O (1 mL) was added 9-fluorenylmethyl chloroformate (42 mg, 0.16 mmol) and potassium carbonate (37 mg, 0.27

[2] Gorka, A. P.; Nani, R. R.; Zhu, J.; Mackem, S.; Schnermann, M. J. *J. Am. Chem. Soc.* **2014**, *136*, 14153–14159.

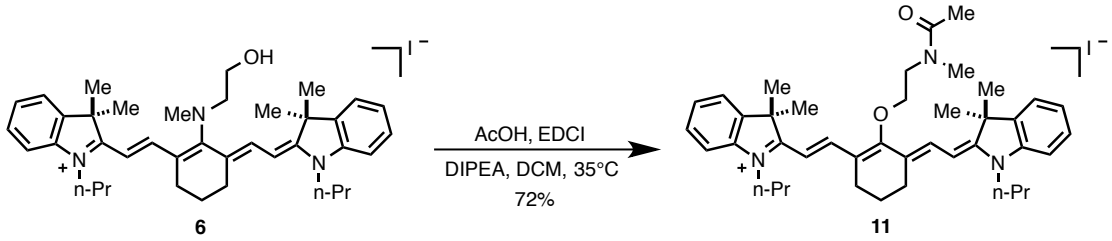
mmol). The biphasic solution was stirred vigorously at room temperature for 15 minutes as the reaction color transitioned from dark blue to green. After this time LC/MS analysis showed complete consumption of **6**. The solution was diluted with saturated aqueous sodium iodide (10 mL), extracted with dichloromethane (2 x 10 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the residue was purified by silica gel chromatography (0→10% MeOH/DCM) affording **9** (48 mg, 95%) as an iridescent green solid. λ_{max} 765 nm (2 μM PBS); ^{1}H NMR (400 MHz, CD_3CN, 70°C) δ 8.02 (d, *J* = 14.2 Hz, 2H), 7.84 – 7.74 (m, 2H), 7.68 – 7.58 (m, 2H), 7.43 – 7.19 (m, 12H), 6.08 (d, *J* = 14.2 Hz, 2H), 4.48 (d, *J* = 6.0 Hz, 2H), 4.27 (t, *J* = 6.0 Hz, 1H), 4.02 (t, *J* = 7.4 Hz, 4H), 3.98 – 3.82 (m, 2H), 3.77 – 3.59 (m, 2H), 3.05 (s, 3H), 2.61 (t, *J* = 6.2 Hz, 4H), 1.91 – 1.79 (m, 6H), 1.61 (s, 12H), 1.02 (t, *J* = 7.4 Hz, 5H);[3] IR (thin film) 1699, 1553, 1505, 1393, 1362, 1246 cm^{-1}; HRMS (ESI) calculated for $C_{54}H_{62}N_3O_3$ (M^+) 800.4786, observed 800.4781.



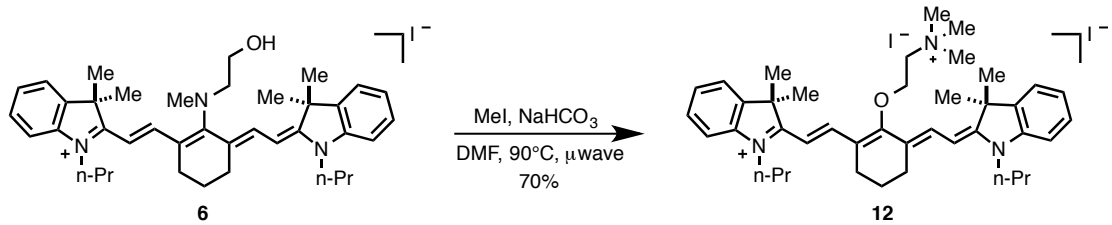
(10): To a solution of **6** (200 mg, 0.28 mmol) in DMF (5 mL) was added 4-dimethylaminopyridine (10 mg, 0.084), diisopropylethylamine (100 μL, 0.56 mmol), and glutaric anhydride (50 mg, 0.43 mmol). The reaction was heated to 35 °C in a sealed vial for 18 hours, during which time the reaction color transitioned from dark blue to green. After this time LC/MS analysis showed complete consumption of **6**. The reaction was diluted with saturated aqueous sodium iodide (10 mL), extracted with dichloromethane (2 x 10 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo* and the green residue was purified by silica gel chromatography (0 → 30% MeOH/DCM) to afford **10** (165 mg, 71%) as an iridescent green solid. λ_{max} 760 nm (2 μM PBS); ^{1}H NMR (CD_3CN, 400 MHz, 70 °C) δ 8.12 (d, *J* = 14.0 Hz, 2H), 7.51 (d, *J* = 7.4 Hz, 2H), 7.41 (m, 2H), 7.29 – 7.21 (m, 4H), 6.11 (d, *J* = 14.0 Hz, 2H), 4.13 (t, *J* = 6.0 Hz, 2H), 4.03 (t, *J* = 7.4 Hz, 4H), 3.92 (t, *J* = 6.0 Hz, 2H), 3.18 (br s, 3H), 2.62 (t, *J* = 6.0 Hz, 4H), 2.44 – 2.36 (m, 2H), 2.34 (t, *J* = 7.3 Hz, 2H), 1.92 – 1.81 (m, 8H), 1.72 (s, 12H), 1.07 – 1.00 (t, *J* = 7.4 Hz,

[3] With carbamate **9** and *N*-methyl amides **10**, **11**, **13-15**, and **19** high temperature NMR (65-75°C) was required to resolve the rotamers. However, these compounds proved unstable over many hours at these elevated temperatures in CD_3OD (as well as in several other solvents), which precluded obtaining ^{13}C NMRs.

6H). IR (thin film) 1723, 1634, 1552, 1506, 1366, 1250 cm^{-1}; HRMS (ESI) calculated for $C_{44}H_{58}N_3O_4$ (M^+) 692.4422, observed 692.4405.

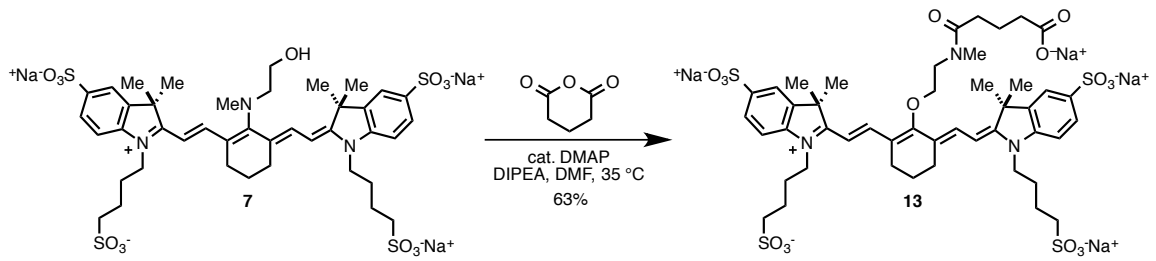


(11): To a solution of alcohol **6** (30 mg, 0.043 mmol), acetic acid (5 μL, 0.09 mmol), and DIPEA (12 μL, 0.090 mmol) in DCM (2 mL) was added EDCI (16 mg, 0.090 mmol) at room temperature. The reaction was heated to 35 °C in a sealed vial for 18 hours, during which time the reaction color transitioned from dark blue to green. After this time, LC/MS analysis showed complete consumption of **6**. The reaction was diluted with saturated aqueous sodium iodide (10 mL), extracted with dichloromethane (2 x 10 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo* and the green residue was purified by silica gel chromatography (0 → 30% MeOH/DCM) affording 23 mg (72%) of **11** as an iridescent green solid. λ_{max} 760 nm (2 μM PBS); ^{1}H NMR (400 MHz, CD_3CN, 75 °C) δ 8.11 (d, *J* = 14.2 Hz, 2H), 7.48 (d, *J* = 7.3 Hz, 2H), 7.40 (td, *J* = 7.8, 1.2 Hz, 2H), 7.30 – 7.19 (m, 4H), 6.10 (d, *J* = 14.2 Hz, 2H), 4.19 – 4.07 (m, 2H), 4.03 (t, *J* = 7.4 Hz, 4H), 3.89 (t, *J* = 5.9 Hz, 2H), 3.24 – 3.04 (m, 3H), 2.61 (t, *J* = 6.0 Hz, 4H), 2.23 – 2.07 (m, 3H), 1.91 – 1.80 (m, 6H), 1.71 (br s, 12H), 1.03 (t, *J* = 7.4 Hz, 6H); IR (thin film) 1634, 1553, 1505, 1394, 1365, 1248 cm^{-1}; HRMS (ESI) calculated for $C_{41}H_{54}N_3O_2$ (M^+) 620.4211, observed 620.4200.



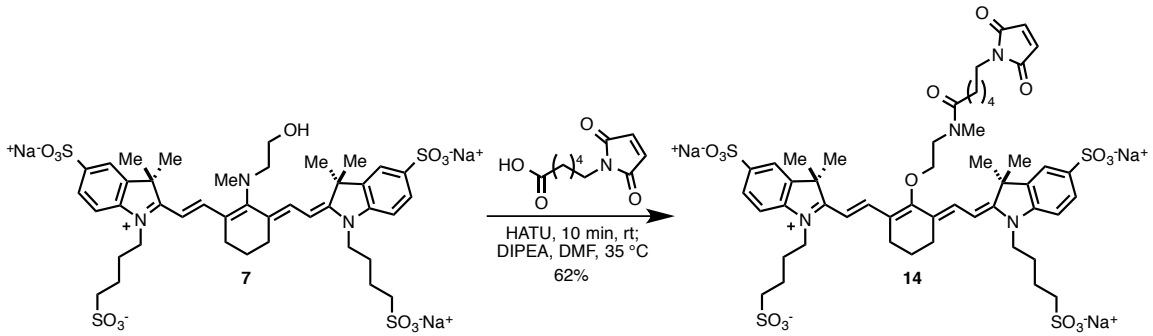
(12): To a microwave vessel containing **6** (13 mg, 0.019 mmol) in dimethylformamide (0.5 mL) was added methyl iodide (17 μL, 0.28 mmol) and $NaHCO_3$ (15 mg, 0.28 mmol). The vessel was sealed, purged with argon, and subjected to 90 °C microwave irradiation for 8 hours, during which time the reaction color transitioned from dark blue to green. After this time LC/MS analysis showed complete consumption of **6**. The reaction was precipitated into diethyl ether (10 mL), centrifuged, and decanted to afford a green residue. The crude material was purified by

silica gel chromatography (0→30% MeOH/DCM) to afford **12** (11 mg, 70%) as a green iridescent solid. λ_{max} 768 nm (2 μM PBS); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.02 (d, J = 14.0 Hz, 2H), 7.54 (d, J = 7.4 Hz, 2H), 7.39 (t, J = 7.7 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.24 (t, J = 7.4 Hz, 2H), 6.19 (d, J = 14.0 Hz, 2H), 4.59 (t, J = 6.2 Hz, 2H), 4.14 (m, 6H), 3.49 (s, 9H), 2.65 (m, 4H), 1.87 (m, 6H), 1.76 (s, 12H), 1.04 (t, J = 7.4 Hz, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.2, 170.2, 143.8, 142.4, 141.0, 129.8, 126.1, 124.0, 123.5, 112.1, 101.3, 70.9, 66.4, 55.3, 50.4, 46.6, 29.1, 25.9, 22.1, 21.8, 11.8; IR (thin film) 1552, 1503, 1393, 1362, 1247 cm^{-1}; HRMS (ESI) calculated for $C_{41}H_{57}N_3O$ (M^{+2}) 303.7245, observed 303.7247.



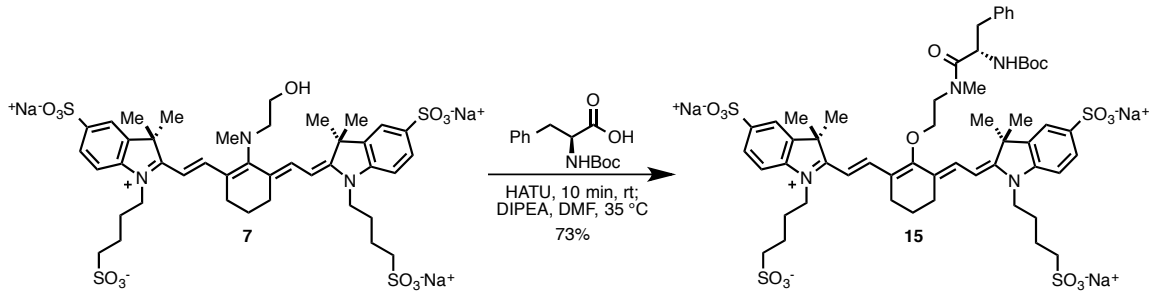
(13): To a solution of **7** (55 mg, 0.055 mmol) in DMF (0.5 mL) was added 4-dimethylaminopyridine (0.4 mg, 0.003), diisopropylethylamine (29 μL, 0.17 mmol), and glutaric anhydride (19 mg, 0.17 mmol). The slurry was heated to 35 °C in a sealed vial for 3 hours, during which time the reaction became homogeneous and dark green. After this time LC/MS analysis showed complete consumption of **7**. The reaction was cooled to room temperature and precipitated into Et_2O (40 mL) with a 1 mL DMF vial wash. The slurry was centrifuged, the supernatant discarded, and the green pellet was resuspended in Et_2O (20 mL). The procedure was repeated, and the crude was purified by reversed-phase chromatography (0→20% MeCN/0.1% v/v aqueous formic acid). The solvent was evaporated, and the crude material was dissolved in 5 mL of water for an ion exchange step. A pipet was filled with 1.5 g of Dowex 50W X8 strongly acidic 200-400 mesh resin, washed with 3 mL of water, 5 mL of 1 M H_2SO_4, and finally 3 mL of water. The aqueous solution of the crude **13** was eluted (fast dropwise rate) through the Dowex column into an aqueous $NaHCO_3$ solution (250 mg in 3 mL water). After stirring for 5 minutes, this aqueous solution was purified by reversed-phase chromatography (0→20% MeCN/water) to afford **13** (39 mg, 63%) as a dark green solid. λ_{max} 764 nm (2 μM PBS); ^{1}H NMR (CD_3OD, 500 MHz, compound exists as a mixture of rotamers, major rotamer is designated by *, minor rotamer denoted by §) δ 8.18 (d, J = 14.2 Hz, 1H*), 8.14 (d, J = 14.2 Hz, 1H§), 7.96 – 7.91 (m, 2H*, 2H§), 7.89 (d, J = 8.4 Hz, 2H*, 2H§), 7.39 – 7.32 (m, 2H*, 2H§), 6.30 – 6.21 (m, 2H*, 2H§), 4.26 – 4.10 (m, 6H*, 6H§), 4.04 (t, J = 4.5 Hz, 1H§), 3.97 (t, J = 4.5 Hz, 1H*), 3.28 (s, 3H*), 3.23 (s, 3H§),

2.90 (t, J = 6.7 Hz, 4H*, 4H§), 2.71 – 2.64 (m, 4H*, 4H§), 2.58 (t, J = 7.7 Hz, 2H§), 2.53 (t, J = 7.7 Hz, 2H*), 2.26 (t, J = 7.2 Hz, 2H*), 2.22 (t, J = 7.2 Hz, 2H§), 2.05 – 1.86 (m, 12H*, 12H§), 1.77 (s, 12H*), 1.74 (s, 12H§); IR (thin film) 1723, 1641, 1555, 1503, 1361, 1233 cm^{-1}; HRMS (ESI) calculated for $C_{46}H_{58}N_3O_{16}S_4$; (M–3H^{-3}) 345.4228, observed 345.4244.

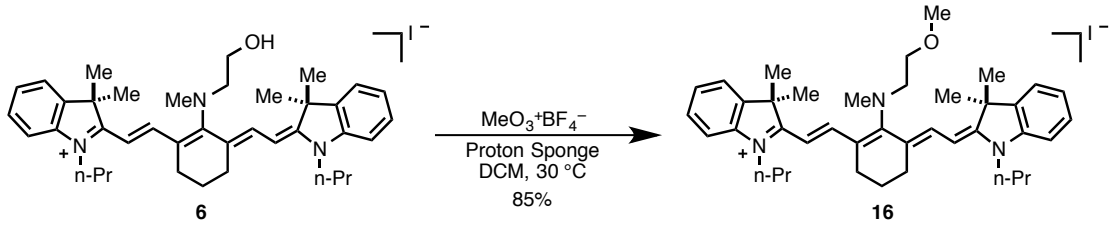


(**14**): 6-Maleimidohexanoic acid (12 mg, 0.058 mmol) and HATU (22 mg, 0.058 mmol) were charged to a 1-dram vial. DMF (0.4 mL) and diisopropylethylamine (15 μL, 0.088 mmol) were added to the vial under argon, and the resulting homogeneous light yellow solution was stirred at room temperature for 10 min. This activated ester/DMF solution was then transferred to a vial containing **7** (29 mg, 0.029 mmol) and DMF (0.4 mL). The deep blue slurry was heated to 35 °C for 1.5 hours as the reaction color transitioned to green, and after which LC/MS analysis showed complete consumption of **7**. The reaction was cooled to room temperature and precipitated into Et_2O (40 mL) with a 1 mL DMF vial wash. The slurry was centrifuged, the supernatant discarded, and the green pellet was resuspended in Et_2O (20 mL). The procedure was repeated, and the crude material was dissolved in 5 mL of water for an ion exchange step. A pipet was filled with 1.5 g of Dowex 50W X8 strongly acidic 200-400 mesh resin, washed with 3 mL of water, 5 mL of 1 M H_2SO_4, and finally 3 mL of water. The aqueous solution of the crude **14** was eluted (fast dropwise rate) through the Dowex column into an aqueous $NaHCO_3$ solution (100 mg in 2 mL water). After stirring for 5 minutes, this aqueous solution was purified by reversed-phase chromatography (0→15% MeCN/water) to afford **14** (21 mg, 62%) as a dark green solid. λ_{max} 766 nm (2 μM PBS); ^{1}H NMR (CD_3OD, 400 MHz, compound exists as a mixture of rotamers, major rotamer is designated by *, minor rotamer denoted by §) δ 8.18 (d, J = 14.1 Hz, 2H*), 8.12 (d, J = 14.1 Hz, 2H§), 7.96 – 7.86 (m, 4H*, 4H§), 7.44 – 7.30 (m, 2H*, 2H§), 6.79 (s, 2H*), 6.76 (s, 2H§), 6.32 – 6.18 (m, 2H*, 2H§), 4.26 – 4.10 (m, 6H*, 6H§), 4.05 – 3.94 (m, 2H*, 2H§), 3.52 (t, J = 6.9 Hz, 2H*), 3.44 (t, J = 6.9 Hz, 2H§), 3.29 (s, 3H*), 3.18 (s, 3H§), 2.90 (t, J = 6.8 Hz, 4H*, 4H§), 2.71 – 2.67 (m, 4H*, 4H§), 2.61 (t, J = 7.5 Hz, 2H§), 2.50 (t, J = 7.4 Hz, 2H*), 2.06 – 1.88 (m, 10H*, 10H§), 1.77 (s, 12H*), 1.74 (s, 12H§), 1.72 – 1.51 (m, 4H*, 4H§), 1.42 – 1.26 (m, 2H*,

2H[§]); IR (thin film) 1701, 1636, 1554, 1507, 1394, 1359, 1254 cm^{-1}; HRMS (ESI) calculated for $C_{51}H_{63}N_4O_{16}S_4$; (M–3H^{-3}) 371.7702, observed 371.7720.

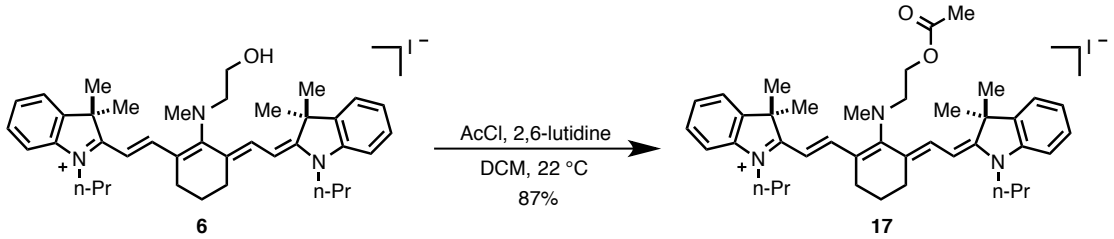


(**15**): Boc-L-phenylalanine (12 mg, 0.046 mmol) and HATU (17 mg, 0.046 mmol) were charged to a 1-dram vial. DMF (0.4 mL) and diisopropylethylamine (12 μL, 0.069 mmol) were added to the vial under argon, and the resulting homogeneous light yellow solution was stirred at room temperature for 10 min. This activated ester/DMF solution was then transferred to a vial containing **7** (23 mg, 0.023 mmol) and DMF (0.4 mL). The deep blue slurry was heated to 35°C for 1.5 hours as the reaction color transitioned to green, at which point LC/MS analysis showed complete consumption of **7**. The reaction was cooled to room temperature and precipitated into Et_2O (40 mL) with a 1 mL DMF vial wash. The slurry was centrifuged, the supernatant discarded, and the blue pellet was resuspended in Et_2O (20 mL). The procedure was repeated, and the crude material was dissolved in 5 mL of water for an ion exchange step. A pipet was filled with 1.5 g of Dowex 50W X8 strongly acidic 200-400 mesh resin, washed with 3 mL of water, 5 mL of 1 M H_2SO_4, and finally 3 mL of water. The aqueous solution of the crude **15** was eluted (fast dropwise rate) through the Dowex column into an aqueous $NaHCO_3$ solution (80 mg in 2 mL water). After stirring for 5 minutes, this aqueous solution was purified by reversed-phase chromatography (0→20% MeCN/water) to afford **15** (21 mg, 73%) as a dark green solid. λ_{max} 768 nm (2 μM PBS); ^{1}H NMR (CD_3OD, 500 MHz, compound exists as a mixture of rotamers, major rotamer is designated by *, minor rotamer denoted by [§]) δ 8.16 (d, J = 14.0 Hz, 2H[§]), 8.09 (d, J = 14.0 Hz, 2H*), 7.94 – 7.83 (m, 4H*, 4H[§]), 7.37 (d, J = 8.3 Hz, 2H*, 2H[§]), 7.32 – 7.09 (m, 4H*, 4H[§]), 6.31 – 6.20 (m, 2H*, 2H[§]), 5.00 – 4.95 (m, 1H*), 4.82 – 4.78 (m, 1H[§]), 4.24 – 4.14 (m, 4H*, 4H[§]), 4.15 – 3.60 (m, 4H*, 4H[§]), 3.15 (s, 3H*), 3.03 (s, 3H[§]), 3.00 – 2.85 (m, 6H*, 6H[§]), 2.76 – 2.62 (m, 4H*, 4H[§]), 2.06 – 1.87 (m, 10H*, 10H[§]), 1.84 – 1.32 (m, 21H*, 21H[§]); IR (thin film) 1701, 1643, 1555, 1507, 1394, 1361, 1255 cm^{-1}; HRMS (ESI) calculated for $C_{55}H_{69}N_3O_{16}S_4$; (M–3H^{-3}) 389.7858, observed 389.7875.



6 16

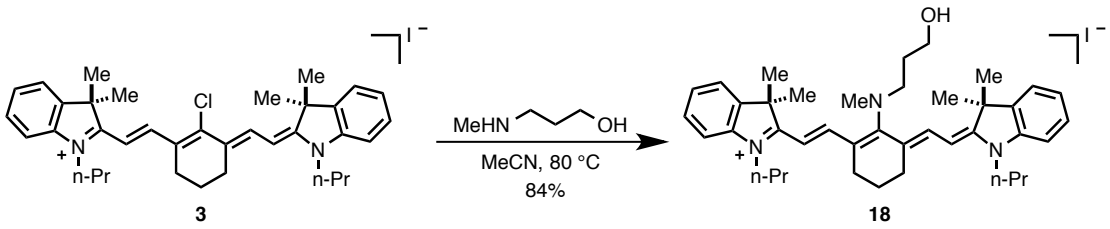
(16): To a mixture of $MeO_3^+BF_4^-$ (38 mg, 0.26 mmol) and Proton Sponge® (16 mg, 0.052 mmol) in DCM (0.5 mL) under argon was added solution of **6** (36 mg, 0.052 mmol) in DCM (2 mL) at room temperature. The blue reaction was heated to 30 °C for 2 hours, at which time LC/MS analysis showed complete consumption of **6**. The reaction was quenched with saturated aqueous $NaHCO_3$ (10 mL) and extracted with dichloromethane (15 mL). The organic layer was washed with saturated aqueous sodium iodide (2 x 5 mL) and dried over Na_2SO_4. The solvent removed *in vacuo* and the blue residue was purified by silica gel chromatography (0→10% MeOH/DCM) affording 31 mg (85%) of **16** as an iridescent blue solid. λ_{max} 668 nm (2 μM PBS); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, *J* = 13.4 Hz, 2H), 7.34 – 7.27 (m, 4H), 7.10 (t, *J* = 7.5 Hz, 2H), 6.95 (d, *J* = 7.9 Hz, 2H), 5.76 (d, *J* = 13.4 Hz, 2H), 3.96 (t, *J* = 4.9 Hz, 2H), 3.86 (t, *J* = 7.3 Hz, 4H), 3.78 (t, *J* = 5.0 Hz, 2H), 3.48 (s, 3H), 3.39 (s, 3H), 2.46 (t, *J* = 6.5 Hz, 4H), 1.91 – 1.78 (m, 6H), 1.64 (s, 12H), 1.04 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.7, 168.7, 142.9, 142.2, 140.3, 128.3, 123.6, 123.3, 122.1, 109.1, 95.5, 70.1, 58.7, 57.2, 48.0, 45.0, 45.0, 29.1, 24.7, 21.8, 20.3, 11.7. IR (thin film) 1547, 1506, 1449, 1343, 1252 cm^{-1}; HRMS (ESI) calculated for $C_{40}H_{54}N_3O$ (M^+) 592.4261, observed 592.4246.



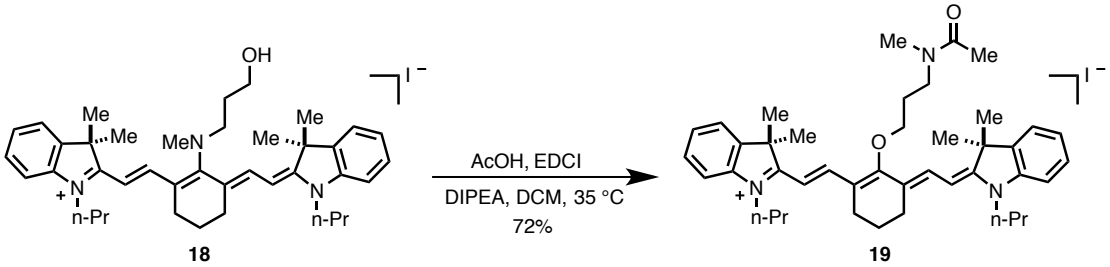
6 17

(17): To a mixture of **6** (30 mg, 0.043 mmol) and 2,6-lutidine (20 μL, 0.17 mmol) in DCM (1 mL) was added acetyl chloride (9.0 μL, 0.13 mmol). The blue solution was stirred for 16 hours at room temperature, at which time LC/MS analysis showed complete consumption of **6**. The solution was diluted with saturated aqueous sodium iodide (5 mL), extracted with dichloromethane (2 x 10 mL), and the organic layer dried over Na_2SO_4. The solvent was removed *in vacuo*, and the blue residue was purified by silica gel chromatography (0→10% MeOH/DCM) affording 28 mg (87%) of **17** as an iridescent blue solid. λ_{max} 689 nm (2 μM PBS); ^{1}H NMR (400

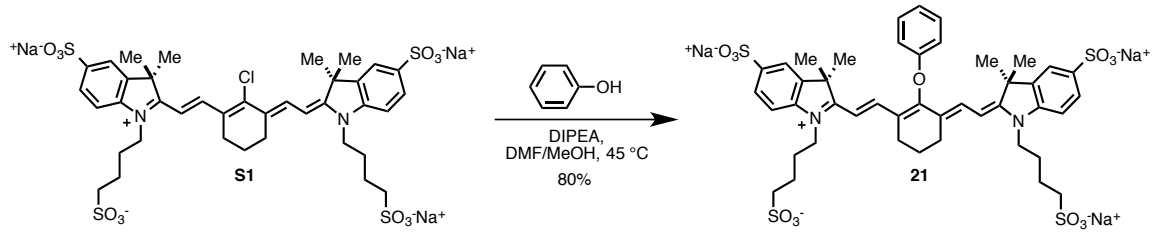
MHz, CD_3CN) δ 7.62 (d, *J* = 13.6 Hz, 2H), 7.46 – 7.38 (d, *J* = 7.3 Hz, 2H), 7.33 (td, *J* = 7.7, 1.2 Hz, 2H), 7.19 – 7.07 (m, 4H), 5.92 (d, *J* = 13.6 Hz, 2H), 4.31 (t, *J* = 5.0 Hz, 2H), 3.99 – 3.82 (m, 6H), 3.40 (s, 3H), 2.48 (t, *J* = 6.3 Hz, 4H), 2.13 (br s, 3H), 1.86 – 1.70 (m, 6H), 1.63 (s, 12H), 1.00 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (100 MHz, CD_3CN) δ 175.3, 171.4, 170.7, 144.1, 143.4, 141.6, 129.3, 125.0, 124.4, 123.1, 110.8, 97.3, 63.5, 57.6, 49.1, 45.9, 45.7, 29.3, 25.5, 22.8, 21.2, 21.0, 11.7. IR (thin film) 1735, 1543, 1503, 1341, 1249 cm^{-1}; HRMS (ESI) calculated for $C_{41}H_{54}N_3O_2$ (M^+) 620.4211, observed 620.4185.



(18): To a solution of IR-780 iodide **3** (315 mg, 0.472 mmol) in MeCN (3 mL) was added 3-methylamino-1-propanol (230 μL, 2.36 mmol). The solution was heated to 80 °C in a sealed vial for 10 minutes as the reaction color transitioned from green to dark blue. After this time LC/MS analysis showed complete consumption of **3**. The reaction mixture was concentrated *in vacuo*, and the residue was purified by silica gel chromatography (0→10% MeOH/DCM) to afford **18** (285 mg, 84%) as a dark blue iridescent solid. ^1H NMR (CD_3CN, 400 MHz) *δ* 7.51 (d, *J* = 13.3 Hz, 2H), 7.38 (d, *J* = 7.3 Hz, 2H), 7.33 – 7.25 (m, 2H), 7.13 – 7.03 (m, 4H), 5.83 (d, *J* = 13.4 Hz, 2H), 3.86 (m, 6H), 3.55 (q, *J* = 5.6 Hz, 2H), 3.40 (s, 3H), 3.02 (t, *J* = 4.9 Hz, 1H), 2.47 (t, *J* = 6.6 Hz, 4H), 2.01 – 1.93 (m, 2H), 1.83 – 1.69 (m, 6H), 1.59 (s, 12H), 0.98 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (CD_3CN, 125 MHz) *δ* 176.8, 169.4, 144.2, 142.2, 141.3, 129.1, 124.3, 123.8, 122.9, 110.3, 95.9, 59.6, 56.8, 48.6, 45.7, 45.4, 32.5, 29.4, 25.4, 22.6, 20.8, 11.6; IR (thin film) 3339, 1540, 1508, 1449, 1344, 1256 cm^{-1}; HRMS (ESI) calculated for $C_{40}H_{54}N_3O$ (M^+) 592.4261, observed 592.4255.



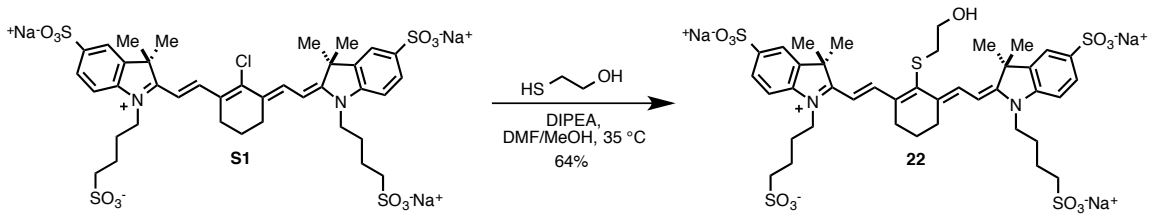
(19): To a solution of alcohol **18** (25 mg, 0.035 mmol), acetic acid (8 μL, 0.14 mmol), and DIPEA (6 μL, 0.035 mmol) in DCM (2 mL) was added EDCI (27 mg, 0.14 mmol) at room temperature. The vial was sealed and the reaction was heated to 35 °C for 48 hours. After this time LC/MS analysis showed complete consumption of **18**. The reaction was diluted with saturated aqueous sodium iodide (10 mL), extracted with dichloromethane (2 x 10 mL), and dried over Na_2SO_4. The solvent removed *in vacuo*, and the green residue was purified by silica gel chromatography (0→10% MeOH/DCM) affording 19 mg (72%) of **19** as an iridescent green solid. ^{1}H NMR (400 MHz, CD_3CN, 72°C; compound exists as a mixture of rotamers, major rotamer is designated by *, minor rotamer denoted by §) δ 8.11 (d, *J* = 14.2 Hz, 2H), 7.49 – 7.45 (m, 2H), 7.42 – 7.37 (m, 2H), 7.27 – 7.20 (m, 4H), 6.10 (d, *J* = 14.2 Hz, 2H), 4.13 – 3.98 (m, 6H), 3.68 – 3.51 (m, 2H), 3.04 (br s, 3H*), 2.94 (br s, 3H§), 2.61 (t, *J* = 6.2 Hz, 4H), 2.31 – 2.17 (m, 2H), 2.07 (br s, 3H§), 2.00 (br s, 3H*), 1.91 – 1.80 (m, 6H), 1.70 (s, 12H), 1.03 (t, *J* = 7.4 Hz, 6H). IR (thin film) 1632, 1551, 1505, 1393, 1360, 1245 cm^{-1}; HRMS (ESI) calculated for $C_{42}H_{56}N_3O_2$ (M^+) 634.4367, observed 634.4355.



(21): 21 was prepared according to procedure of Strekowski et. al.[4] To a solution of **S1** (38 mg, 0.040 mmol) in DMF (0.7 mL) and MeOH (0.7 mL) was added phenol (26 mg, 0.28 mmol) and diisopropylethylamine (48 μL, 0.28 mmol). The dark green solution was heated to 45 °C in a sealed vial for 3 hours, after which time LC/MS analysis showed complete consumption of **S1**. The reaction was cooled to room temperature and precipitated into Et_2O (40 mL). The slurry was centrifuged, the supernatant discarded, and the green pellet was resuspended in Et_2O (20 mL). The procedure was repeated, and the crude material was dissolved in 5 mL of water for an ion exchange step. A pipet was filled with 1.5 g of Dowex 50W X8 strongly acidic 200-400 mesh resin, washed with 3 mL of water, 5 mL of 1 M H_2SO_4, and finally 3 mL of water. The aqueous solution of the crude **21** was eluted (fast dropwise rate) through the Dowex column into an aqueous $NaHCO_3$ solution (70 mg in 2 mL water). After stirring for 5 minutes, this aqueous solution was loaded directly onto a C_{18}Aq column and purified by reversed-phase

[4] Strekowski, L.; Lipowska, M.; Patonay, G. *J. Org. Chem.* **1992**, *57*, 4578–4580.

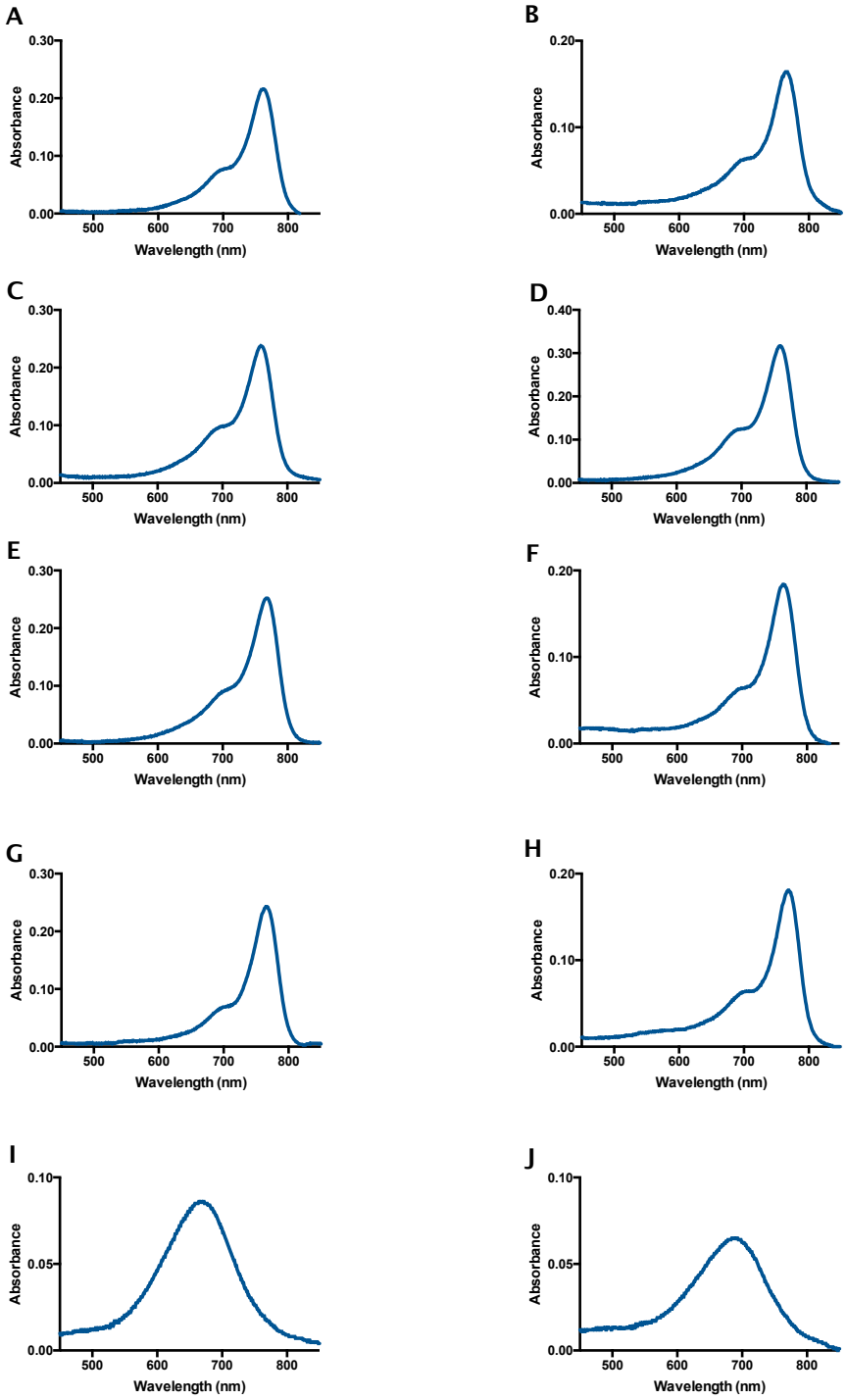
chromatography (0→25% MeCN/water) to afford **21** (32 mg, 80%) as a dark green solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.02 (d, J = 14.2 Hz, 2H), 7.85 (dd, J = 8.3, 1.7 Hz, 2H), 7.78 (d, J = 1.7 Hz, 2H), 7.47 – 7.37 (m, 2H), 7.34 (d, J = 8.3 Hz, 2H), 7.14 (d, J = 8.7 Hz, 2H), 7.06 (t, J = 7.6 Hz, 1H), 6.26 (d, J = 14.2 Hz, 2H), 4.28 – 4.05 (m, 4H), 2.89 (t, J = 6.8 Hz, 4H), 2.82 – 2.75 (m, 4H), 2.11 – 2.01 (m, 2H), 2.01 – 1.87 (m, 8H), 1.37 (s, 12H); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.0, 165.9, 161.3, 144.9, 143.8, 143.1, 142.3, 131.5, 128.1, 124.6, 123.7, 121.2, 115.8, 111.6, 101.9, 51.7, 50.2, 45.0, 28.1, 27.1, 25.2, 23.5, 22.4; IR (thin film) 1558, 1512, 1431, 1400, 1361 cm^{-1}; HRMS (ESI) calculated for $C_{44}H_{49}N_2O_{13}S_4$; (M–3H^{-3}) 313.7367, observed 313.7361.



(22): 22 was prepared according to procedure of Strekowski et. al.[4] To a solution of **S1** (35 mg, 0.037 mmol) in DMF (2 mL) and MeOH (50 μL) was added β-mercaptoethanol (10 μL, 0.15 mmol) and diisopropylethylamine (26 μL, 0.15 mmol). The dark green solution was heated to 35 °C in a sealed vial for 4 hours. The reaction was cooled to room temperature and precipitated into Et_2O (40 mL). The slurry was centrifuged, the supernatant discarded, and the green pellet was resuspended in Et_2O (20 mL). The procedure was repeated, and the crude material was dissolved in 5 mL of water for an ion exchange step. A pipet was filled with 1.5 g of Dowex 50W X8 strongly acidic 200-400 mesh resin, washed with 3 mL of water, 5 mL of 1 M H_2SO_4, and finally 3 mL of water. The aqueous solution of the crude **22** was eluted (fast dropwise rate) through the Dowex column into an aqueous $NaHCO_3$ solution (70 mg in 2 mL water). After stirring for 5 minutes, this aqueous solution was loaded directly onto a C_{18}Aq column and purified by reversed-phase chromatography (0→20% MeCN/water) to afford **22** (23 mg, 64%) as a dark green solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.77 (d, J = 14.1 Hz, 2H), 7.77 (d, J = 1.6 Hz, 2H), 7.65 (dd, J = 8.3, 1.6 Hz, 2H), 7.38 (d, J = 8.3 Hz, 2H), 6.36 (d, J = 14.1 Hz, 2H), 4.96 (t, J = 5.3 Hz, 1H), 4.28 – 4.11 (m, 4H), 3.50 (q, J = 6.3 Hz, 2H), 2.87 (t, J = 6.7 Hz, 2H), 2.71 – 2.61 (m, 4H), 2.56 – 2.51 (m, 4H), 1.89 – 1.72 (m, 10H), 1.70 (s, 12H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 171.9, 155.2, 145.0, 145.0, 142.2, 140.3, 133.4, 126.2, 119.8, 110.4, 101.7, 60.1, 50.7, 48.8, 43.7, 39.1 (observed by HSQC correlation), 27.4, 26.0, 25.7, 22.5, 20.7; IR (thin film) 3410, 1530, 1507, 1432, 1348, 1248 cm^{-1}; HRMS (ESI) calculated for $C_{40}H_{49}N_2O_{13}S_5$; (M–3H^{-3}) 308.3941, observed 308.3937.

Absorption Curves for 8-17

Absorption curves of **8**(A), **9**(B), **10** (C), **11** (D), **12** (E), **13**(F), **14** (G), **15** (H), **16** (I), and **17** (J). **8** and **9** 2 μM in 0.1 M PBS (pH = 7.4) with 20% DMSO (v/v). **10** – **17** 2 μM in 0.1 M PBS (pH = 7.4) with 0.1% DMSO (v/v).

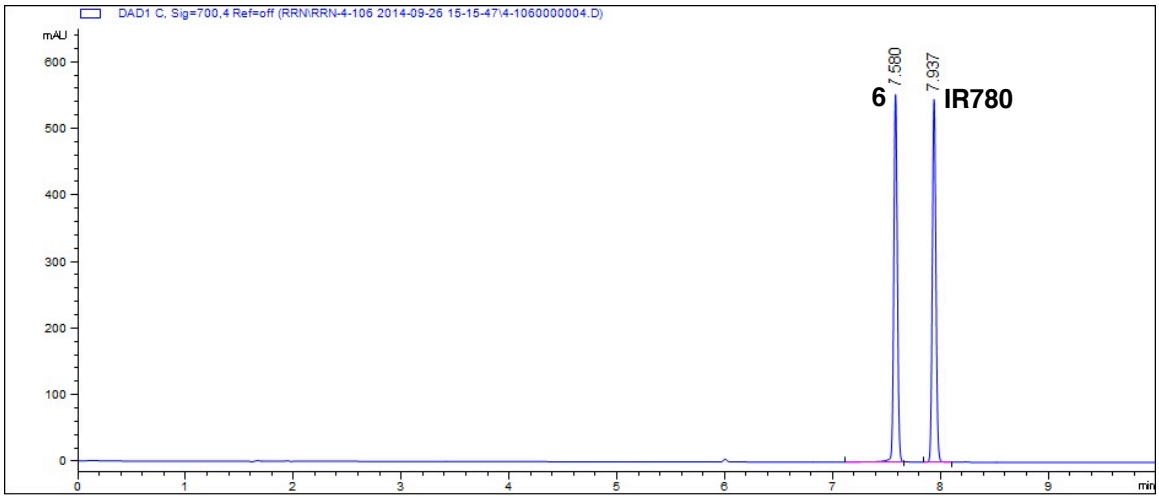


Procedure for Rearrangement Rate Comparison of 6 and 18

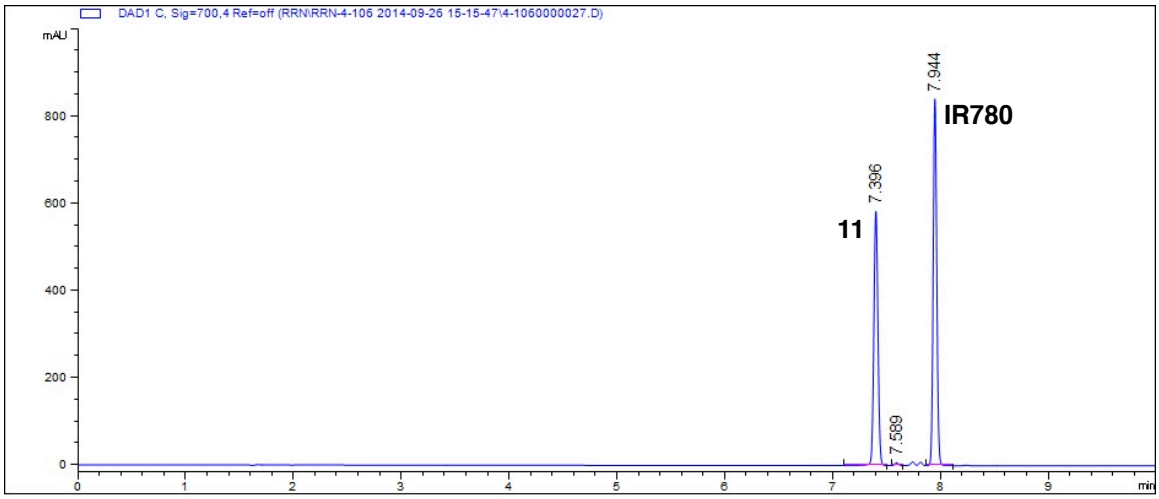
To a solution of alcohol **6** or **18** (0.015 mmol), acetic acid (0.060 mmol), and DIPEA (0.015 mmol) in DCM (0.5 mL) was added an internal standard (IR-780, 0.015 mmol). A t=0 min sample was withdrawn and analyzed by HPLC. EDCI (0.060 mmol) was added and the reaction was heated to 35 °C. At the given time points in Figure 2, a 5 μL aliquot was withdrawn and diluted in 500 μL methanol (to halt the reaction progress). The sample was then analyzed on an Agilent 1260 Infinity HPLC utilizing a Kinetex 5 μm C6-Phenyl 110 Å (4.6 x 250 mm) column (Phenomenex Inc.) with a gradient of 5→98% (10 min) to 98→5% (1 min) MeCN/0.1% aqueous trifluoroacetic acid at a flow rate of 2.0 mL/min. Experiments were run in duplicate and plotted with error bars derived from the standard deviation (<5% in all cases). The peaks were assigned by comparing retention times with purified standards.

Chromatogram of 6 and internal standard at 700 nm (A). Chromatogram of reaction of 6 at t = 300 min at 700 nm (B).

A. Trace at t = 0 min

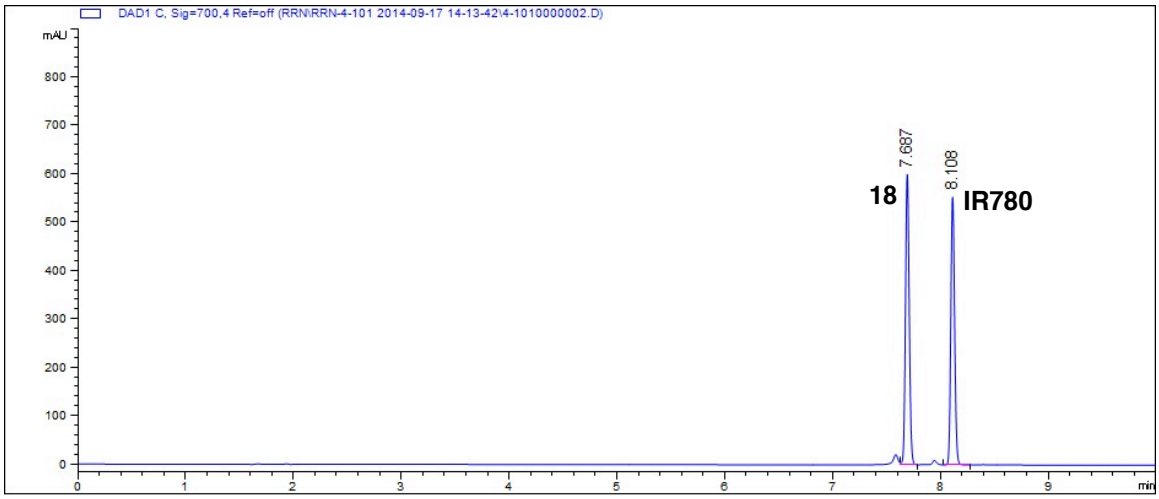


B. Trace at t = 300 min

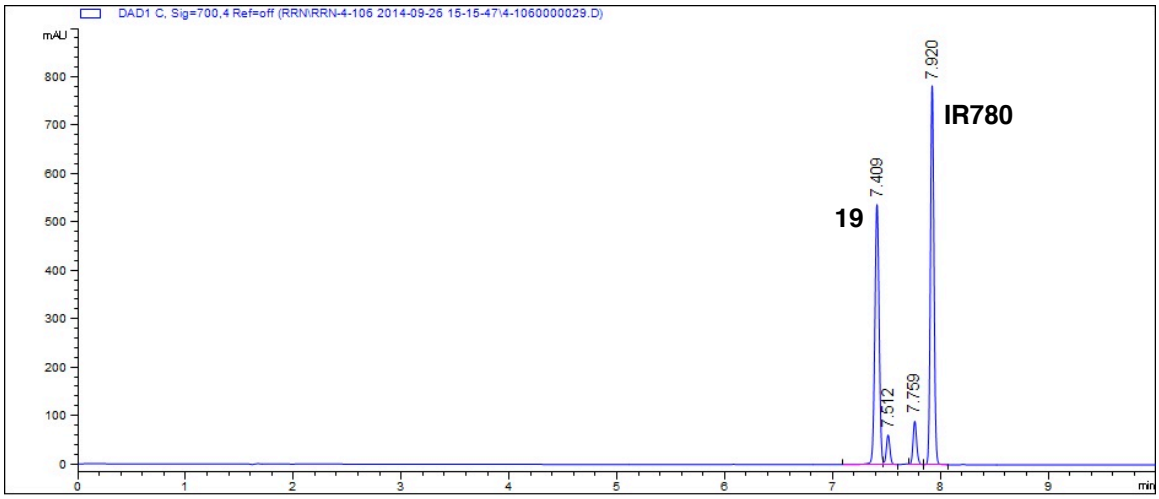


Chromatogram of 18 and internal standard at 700 nm (A). Chromatogram of reaction of 18 at t = 2400 min at 700 nm (B).

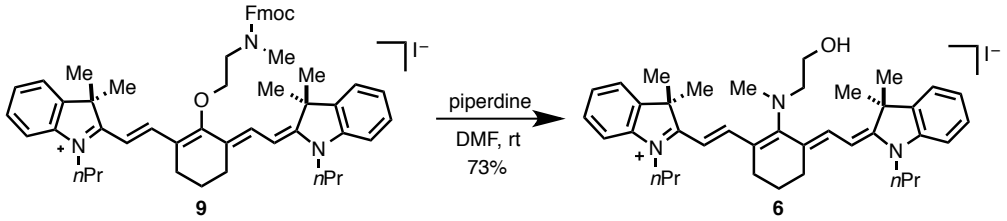
A. Trace at t = 0 min



B. Trace at t = 2400 min



Procedure for Fmoc Deprotection



9 **6**

(6): To a solution of **9** (18 mg, 0.019 mmol) in DMF (300 μL) was added piperidine (10 μL, 0.097 mmol). The green solution was stirred at room temperature for 20 minutes, during which time the reaction color transitioned to dark blue and a precipitate resulted. At this time LC/MS analysis showed a complete conversion of **9** to **6**. The reaction mixture was diluted with DCM (20 mL), washed with water (4 x 5 mL), saturated aqueous sodium iodide (2 x 5 mL), and dried over Na_2SO_4. The volatiles were concentrated *in vacuo*, and the blue residue was purified by silica gel chromatography (50% EtOAc/DCM, 0→25% MeOH/DCM) affording 10 mg (73%) of product. The analytical data were consistent with those for compound **6**.[2]

Determination of Quantum Yields and Molar Extinction Coefficients

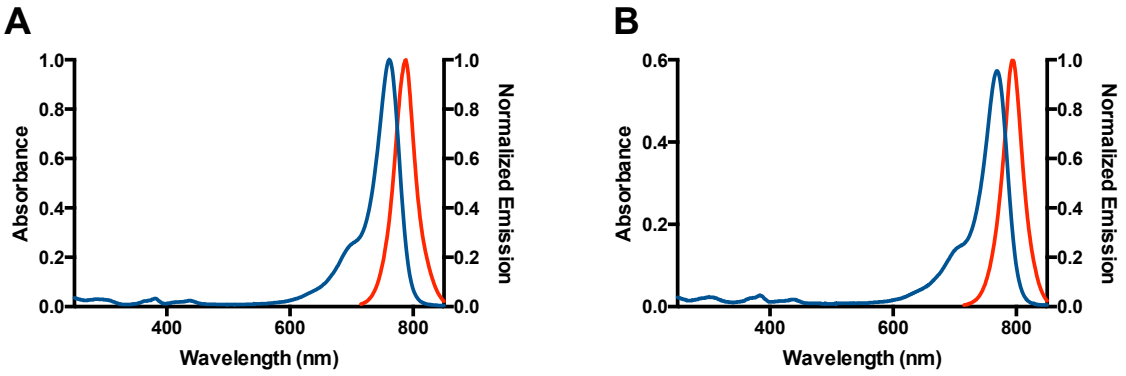
Quantum yields (Φ_f) were determined in methanol relative to ICG and IR783 ($\Phi_f = 0.078$ and 0.084, respectively[5]), from plots of integrated fluorescence intensity versus absorption, according to the following relationship:

$$\Phi_x = \Phi_{st}\left(\frac{Grad_x}{Grad_{st}}\right)\left(\frac{\eta_x}{\eta_{st}}\right)$$

where subscripts *st* and *x* denote standard and test sample, respectively, Φ is the fluorescence quantum yield, *Grad* is the gradient of the integrated fluorescence intensity vs. absorption plot, and η is the refractive index of the solvent.[6,7,8,9] Measurements were performed in 10 mm path length quartz cuvettes (Hellma 111-QS), maintained at 25 °C, with the absorption of all dye solutions ≤ 0.08 in order to maximize illumination homogeneity and optical transparency and minimize reabsorption effects.[7] ICG and IR783 standards and test dye solutions were excited at 70 nm below their absorption maxima.

Molar extinction coefficients (ε) were determined in PBS using Beer's law, from plots of absorption vs. concentration. Measurements were performed in 10 mm path length quartz cuvettes (Hellma 111-QS), maintained at 25 °C, with absorption at the highest concentration ≤ 0.08 (see above).

Absorption and Normalized Emission Curves for 8 and 13



Absorption and normalized emission curves of **8** (A), 7 μM, and **13** (B), 3 μM, for a MeOH sample.

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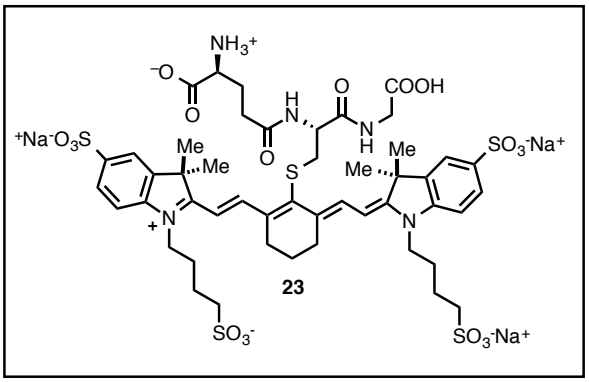
[5] James, N. S.; Chen, Y. H.; Joshi, P.; Ohulchanskyy, T. Y.; Ethirajan, M.; Henary, M.; Strekowski, L.; Pandey, R. K. *Theranostics* **2013**, *3*, 692–702.
[6] Parker, C. A.; Rees, W. T. *Analyst* **1960**, *85*, 587–600.
[7] Williams, A. T. R.; Winfield, S. A.; Miller, J. N. *Analyst* **1983**, *108*, 1067–1071.
[8] Rurack, K.; Spieles, M. *Anal. Chem.* **2011**, *83*, 1232–1242.
[9] Samanta, A.; Vendrell, M.; Das, R.; Chang, Y. T. *Chem. Commun.* **2010**, *46*, 7406–7408.

Procedure for Stability Studies

Stock solutions (5 mM) of **13**, **21**, and **22** were prepared in DMSO. A five hundred-fold dilution in 50 mM PBS buffer (pH = 7.4) was performed to yield 10 μM samples. The samples were analyzed by HPLC (t=0 min) and 5 μL of a 0.2 M glutathione solution in de-ionized water was added to afford a 1 mM final glutathione concentration. The samples were continuously analyzed every 20 minutes by HPLC, and the integrated peak areas of absorption at 780 nm from the starting dyes were plotted versus time. The sample was analyzed on an Agilent 1260 Infinity HPLC utilizing a Kinetex 5 μm Biphenyl 100 Å (4.6 x 250 mm) column (Phenomenex Inc.) with a gradient of 2→98% (4.7 min) to 98→2% (1 min) MeCN/10mM ammonium carbonate at a flow rate of 1.5 mL/min. Experiments were run in duplicate and plotted with error bars derived from the standard deviation (<5% in all cases). After the reaction of **21** and GSH achieved full conversion, the reaction was directly purified by semi-prep HPLC to obtain glutathione-cyanine adduct **23**, which was analyzed by HRMS.



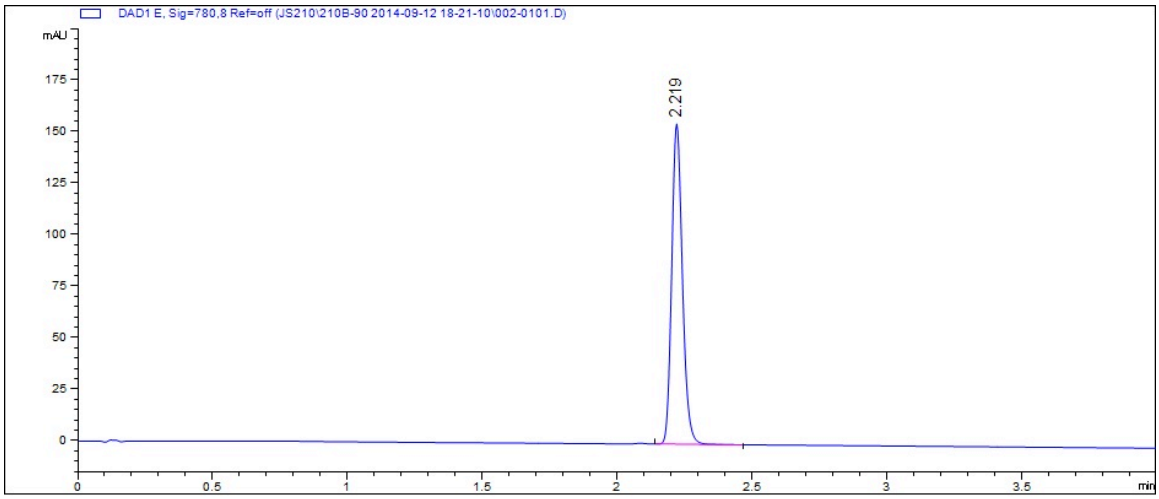
HRMS (ESI) calculated for $C_{48}H_{60}N_5O_{18}S_5$; ($M–3H^{-3}$) 384.7507, observed 384.7500.

Plate Reader Fluorescence Assay

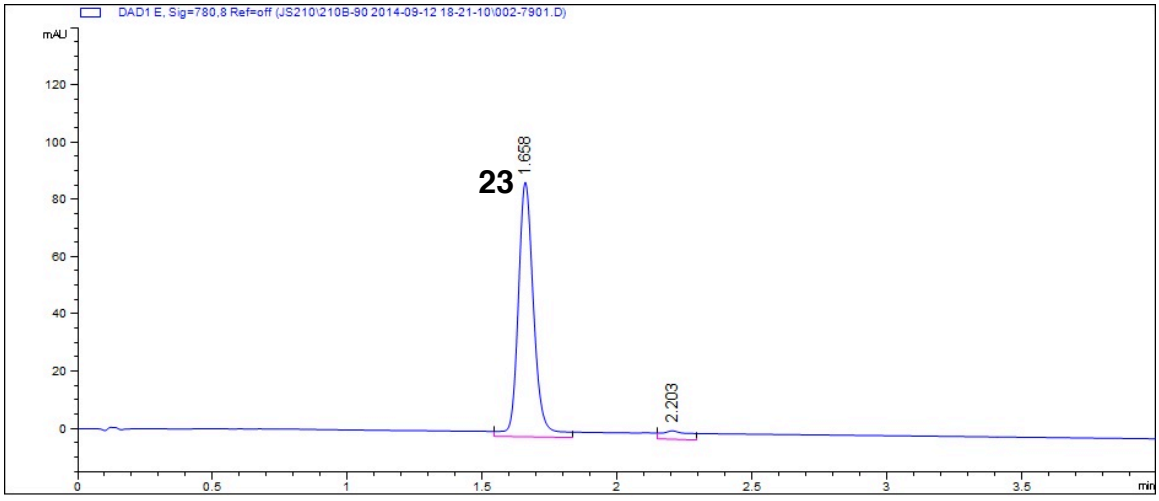
Stock solutions (5 mM) of **13**, **21**, and **22** were prepared in DMSO. Dilutions were performed into 50 mM PBS buffer (pH = 7.4) containing 1 mM reduced glutathione (GSH) to yield 2 μM samples. Fluorescence emission was detected at 790 nm with excitation at 740 nm (9 mm bandwidth) at 20-minute intervals for 500 minutes on a BioTek Synergy Mx plate reader. Conditions were measured in triplicate and plotted with error bars derived from the standard deviation (<5% in all cases).

Chromatogram of 21 at 780 nm (A). Chromatogram of reaction of 21 and glutathione at t = 500 min at 780 nm (B).

A. Trace at t = 0 min

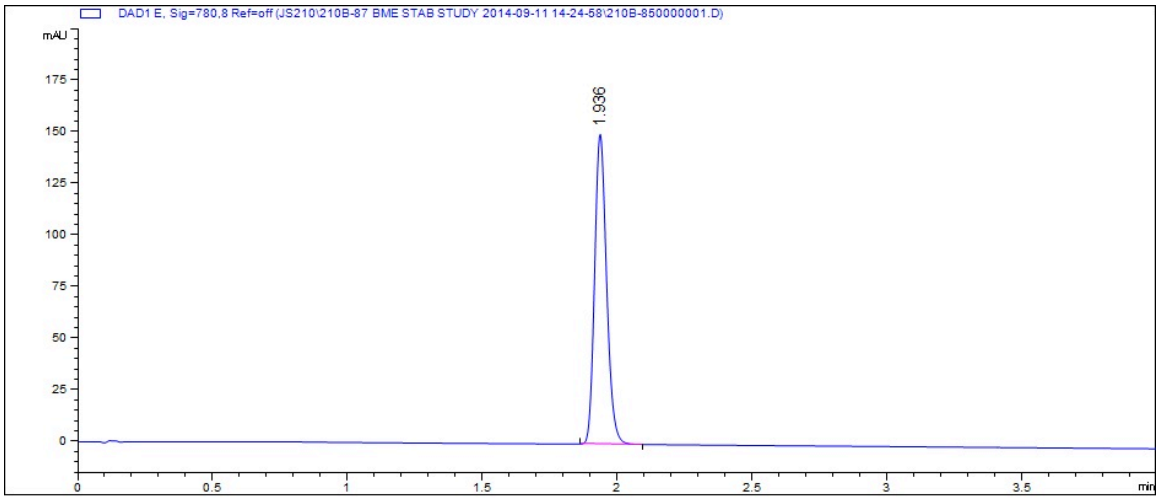


B. Trace at t = 500 min

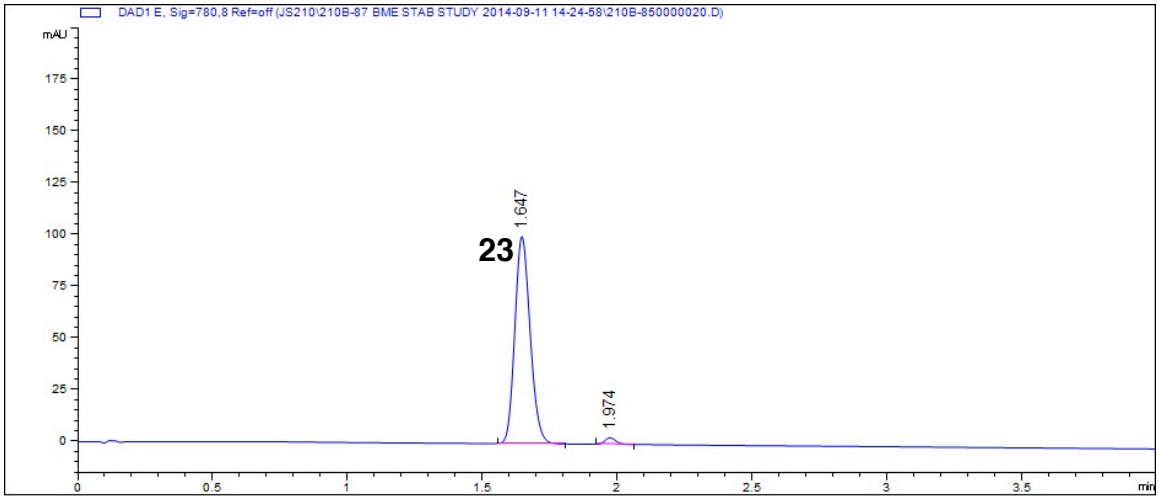


Chromatogram of 22 at 780 nm (A). Chromatogram of reaction of 22 and glutathione at t = 300 min at 780 nm (B).

A. Trace at t = 0 min

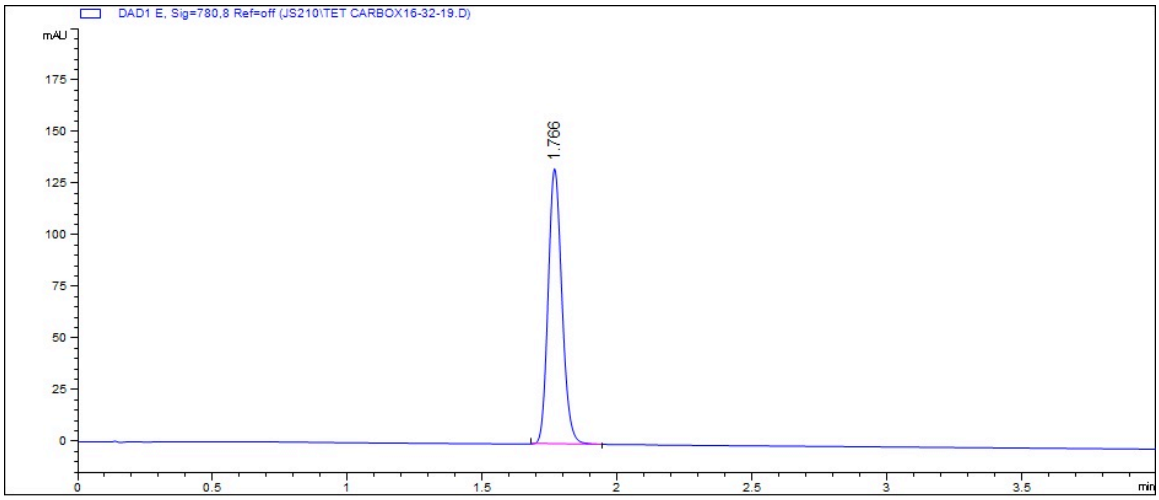


B. Trace at t = 300 min

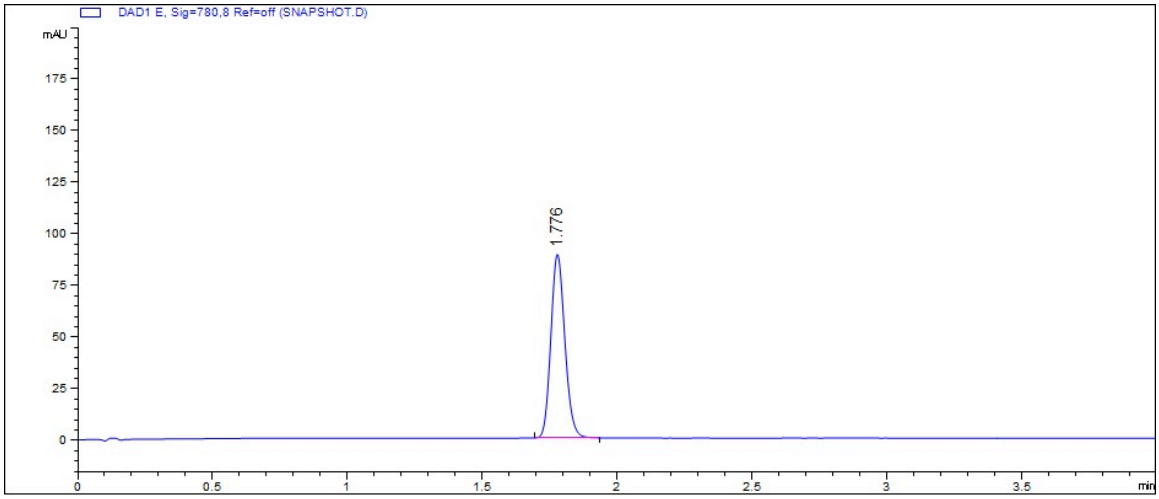


Chromatogram of 13 at 780 nm (A). Chromatogram of reaction of 13 and glutathione at t = 1000 min at 780 nm (B).

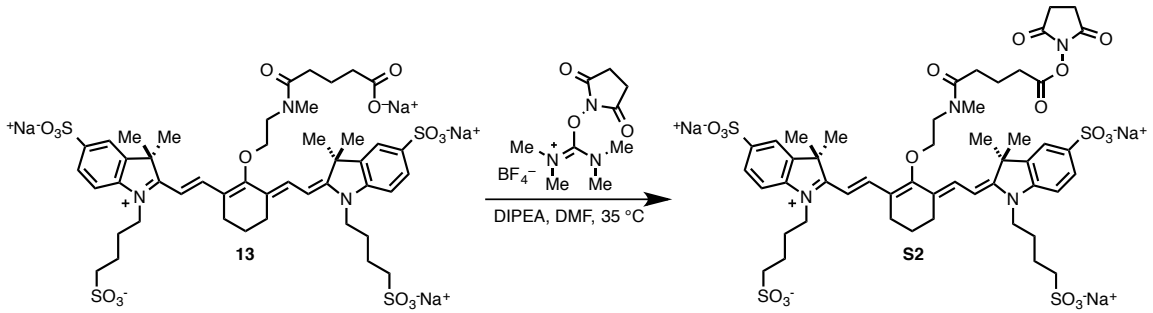
A. Trace at t = 0 min



B. Trace at t = 1000 min



Procedure for NHS Ester Synthesis



A suspension of **13** (14 mg, 0.012 mmol) in toluene (5 mL) was concentrated *in vacuo* to azeotropically remove water. A solution of TSTU (7.5 mg, 0.025 mmol) and diisopropylethylamine (2.2 μL, 0.012 mmol, 1 equivalent) in DMF (500 μL) was added to **13** under an argon atmosphere. This solution was heated to 35°C for 16 hours. After this time LC/MS analysis showed complete consumption of **13**. The reaction was subsequently precipitated into ethyl acetate (45 mL), centrifuged, and the supernatant decanted off. The green pellet was resuspended in Et_2O (20 mL), and the centrifugation procedure was repeated twice. The crude solid (**S2**) was placed under vacuum (< 0.5 Torr) for 1 hour and used directly in the antibody conjugation step.

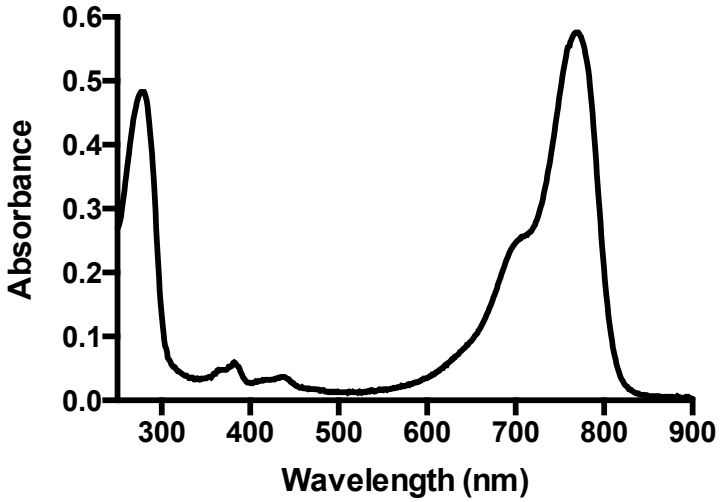
Procedure for Panitumumab Conjugation[10]

All steps were performed under reduced lighting. To 500 μL of 1 M PBS (pH 8.5) in a 1.5 mL microcentrifuge tube was added 500 μL of panitumumab (from a 5 mg/mL commercial stock acquired from Amgen) and 38 μL of a DMSO solution of **S2** (from a 5 mM stock). The resulting mixture was inverted twice to mix and incubated at room temperature for 1 h with gentle mixing. A G10 Sephadex column (GE Healthcare) was primed with 6 column volumes of 0.9% (w/v) saline. The reaction mixture was added to the column and eluted with 0.9% saline. 1 mL fractions were collected. An aliquot of each fraction was diluted tenfold into 1:1 (v/v) MeOH/PBS (0.1 M, pH 7.4) and absorption at 280 and 774 nm recorded. Dye and antibody concentrations were determined from the Beer-Lambert relationship ($C = A/\varepsilon l$), with ε_{dye} = 168,000 M^{-1} cm^{-1} and $\varepsilon_{antibody}$ = 200,000 M^{-1} cm^{-1}, corresponding to the molar extinction coefficients of **13** and panitumumab in 1:1 (v/v) MeOH/PBS, respectively. The degree of labeling (DOL), the average number of dye molecules per antibody, was determined from the

[10] Bhattacharyya, S.; Patel, N. L.; Wei, L.; Riffle, L. A.; Kalen, J. D.; Hill, G. C.; Jacobs, P. M.; Zinn, K. R.; Rosenthal, E. *Med. Chem. Comm.* **2014**, *5*, 1337–1346.

quotient of dye concentration to antibody concentration. The antibody conjugate solution was filtered through a 0.22 μm sterile filter (Acrodisc) and stored at 4 °C.

Absorption Spectrum of panitumumab-13



Cell Culture

The MDA-MB-468 (EGFR/HER1 overexpression[11]) and MCF7 (normal EGFR/HER1 expression[11]) human breast cancer cell lines were obtained from the NCI DTP, DCTD Tumor Repository. MDA-MB-468 was cultured in RPMI supplemented with 2 mM L-glutamine, 11 mM D-glucose, 24 mM sodium bicarbonate, 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B. MCF7 was cultured in DMEM supplemented with 4 mM L-glutamine, 25 mM D-glucose, 44 mM sodium bicarbonate, 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B. Both cell lines were grown at 37 ^{o}C in an atmosphere of 20% O_2 and 5% CO_2. Stock cultures were maintained in continuously exponential growth by weekly passage of the appropriate number of cells following trypsinization with 0.25% Trypsin-EDTA (0.9 mM) in PBS.

[11] (a) Rae, J. M.; Scheys, J. O.; Clark, K. M.; Chadwick, R. B.; Kiefer, M. C.; Lippman, M. E. *Breast Cancer Res. Treat* **2004**, *87*, 87–95. (b) Mamot, C.; Drummond, D. C.; Greiser, U.; Hong, K.; Kirpotin, D. B.; Marks, J. D.; Park, J. W. *Cancer Res.* **2003**, *63*, 3154–3161.

Fluorescence Microscopy

MDA-MB-468 or MCF7 cells (1 x 10^5) were plated on Nunc Lab-Tek® II chambered #1.5 German borosilicate coverglass (Thermo Fisher Scientific, Inc.) and allowed to adhere overnight. Cells were incubated with 100 nM panitumumab-**13** for 2 h, washed twice with PBS, incubated with 1 μM Hoescht 33342 for 0.5 h, washed twice with PBS, and imaged. Fluorescence microscopy was performed using an Evos® FL Auto Imaging System (Life Technologies) at 40x magnification using a coverslip-corrected plan fluorite objective. Near-IR fluorescence was imaged using a Cy7 LED light cube (710/40 nm excitation, 775/46 nm emission) and Hoescht using a DAPI LED light cube (357/44 nm excitation, 447/60 nm emission). Image processing was conducted with ImageJ 1.49f.

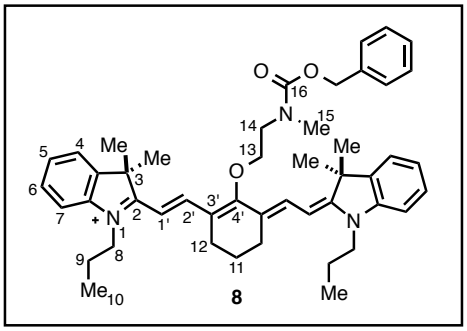
Flow Cytometry

MDA-MB-468 or MCF7 cells (1 x 10^6) were seeded into 6-well plates and allowed to adhere overnight. Cells were incubated with 100 nM panitumumab-**13** for 2 h and washed twice with PBS. Flow cytometric analysis for near-IR fluorescence signal was performed at the CCR Flow Cytometry Core (NCI-Frederick) using a BD LSRII Fortessa analyzer operating a laser line at 647 nm. Data processing was conducted with FlowJo vX.0.7.

Note: *O*-linked vs. *N*-linked assignment

This assignment is made most clearly by the characteristic absorption maxima (*O*-linked ~ 770 nm) and (*N*-linked ~ 680 nm), which has been reported elsewhere.[12] NMR provided additional confirmation. As shown below, full NMR assignment was obtained for two representative rearrangement products. For the remaining compounds, the characteristic absorption maxima and a downfield shift for protons at the C1' and C2' positions provides strong evidence for the assigned structure.

NMR Tables for Selected Compounds



8

Table S1. ^{1}H (400 MHz), ^{13}C (100 MHz), HMBC, and COSY NMR data for **8**, CD_3CN, 45 °C

atom	^{13}C (mult)	^{1}H mult, *J* (Hz)	HMBC[a]	COSY[b]
1′	100.6 (CH)	6.08 (d, *J* = 14.2 Hz, 2H)	2, 2′, 3′	2′
2′	141.7 (CH)	8.07 (d, *J* = 14.2 Hz, 2H)	4′, 12	1′
3′	123.9 (C)			
4′	171.3 (C)			
11	22.2 (CH_2)	1.91–1.78 (m, 2H)	3′, 12	12
12	25.5 (CH_2)	2.59 (t, *J* = 6.1 Hz, 4H)	2′, 3′, 4′, 11	11
13	76.6 (CH_2)	4.11 (t, *J* = 5.5 Hz, 2H)	4′, 14	14
14	50.1 (CH_2)	3.86 (t, *J* = 5.5 Hz, 2H)	13, 15, 16	13

[a]Carbons that correlate to the proton resonance. Optimized for 10 Hz coupling. [b]Protons that correlate to the proton resonance.

[12] a) Peng, X. J.; Song, F. L.; Lu, E.; Wang, Y. N.; Zhou, W.; Fan, J. L.; Gao, Y. L. *J. Am. Chem. Soc.* **2005**, *127*, 4170–4171; b) Pascal, S.; Haefele, A.; Monnereau, C.; Charaf-Eddin, A.; Jacquemin, D.; Le Guennic, B.; Andraud, C.; Maury, O. *J. Phys. Chem. A* **2014**, *118*, 4038–4047.

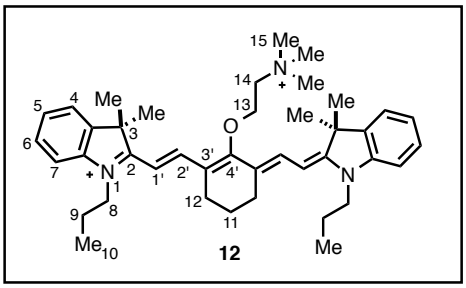
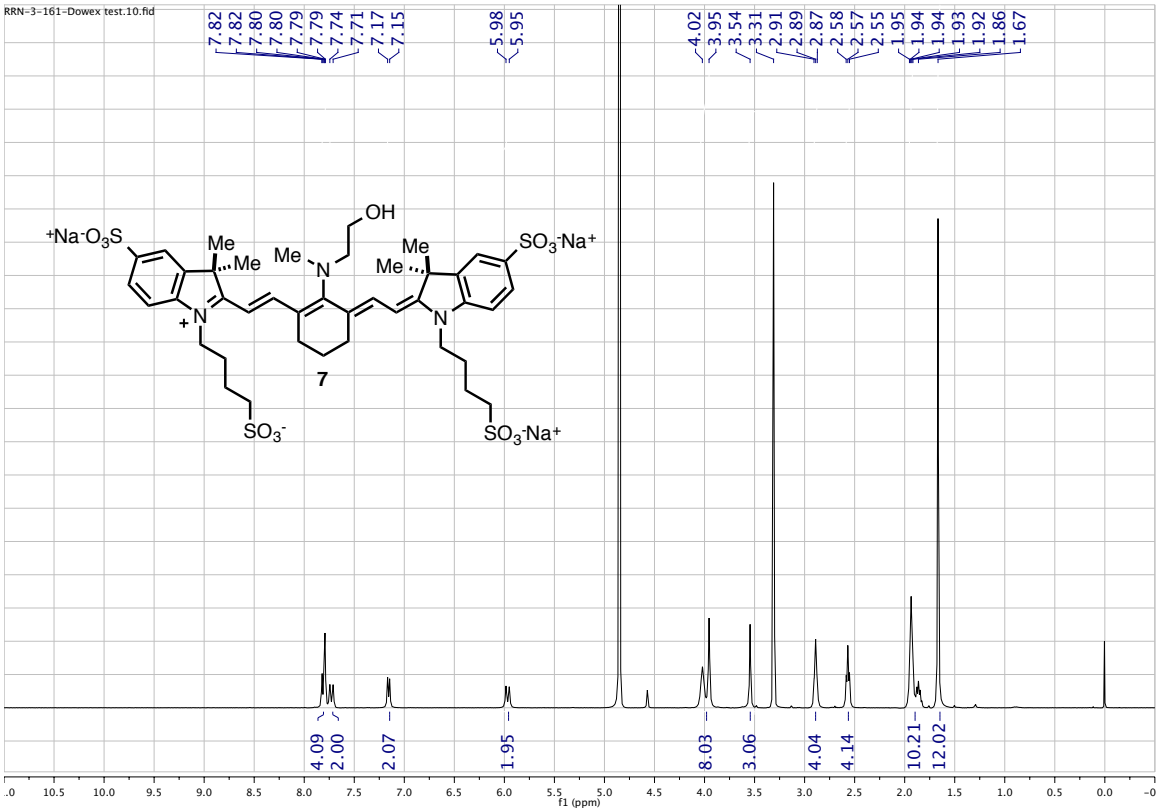


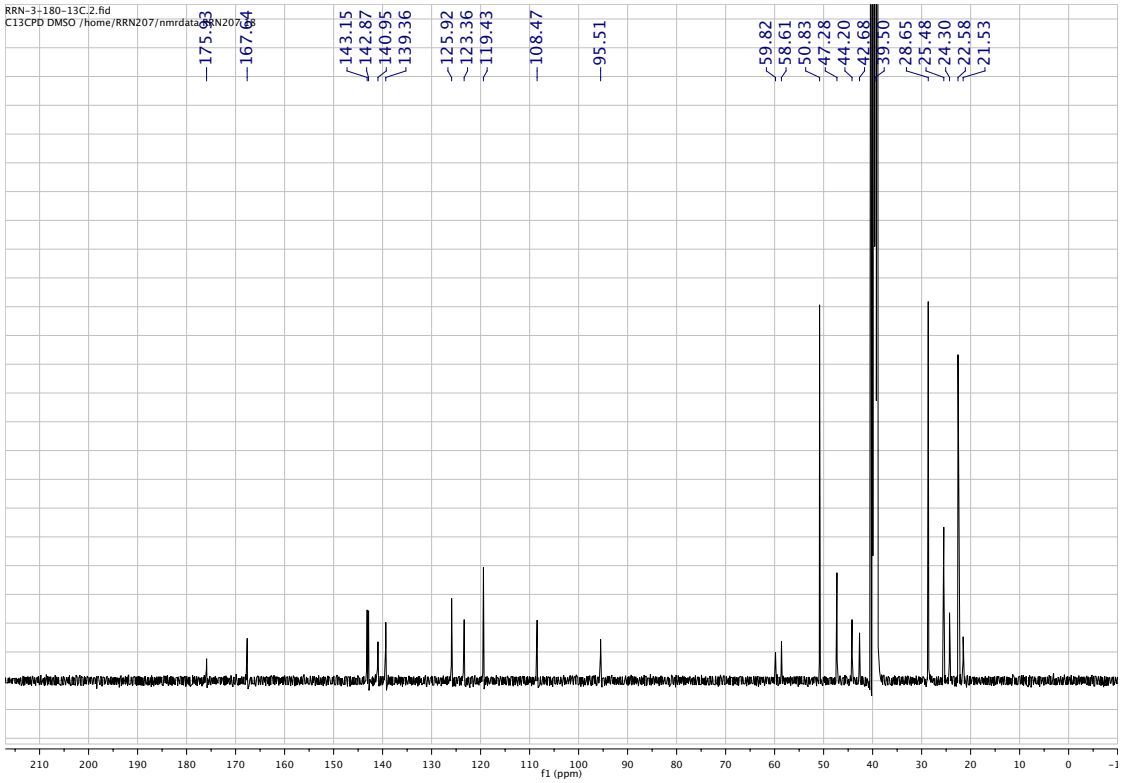
Table S2. ^{1}H (400 MHz), ^{13}C (100 MHz), HMBC, and COSY NMR data for **12**, CD_3OD, 25 °C

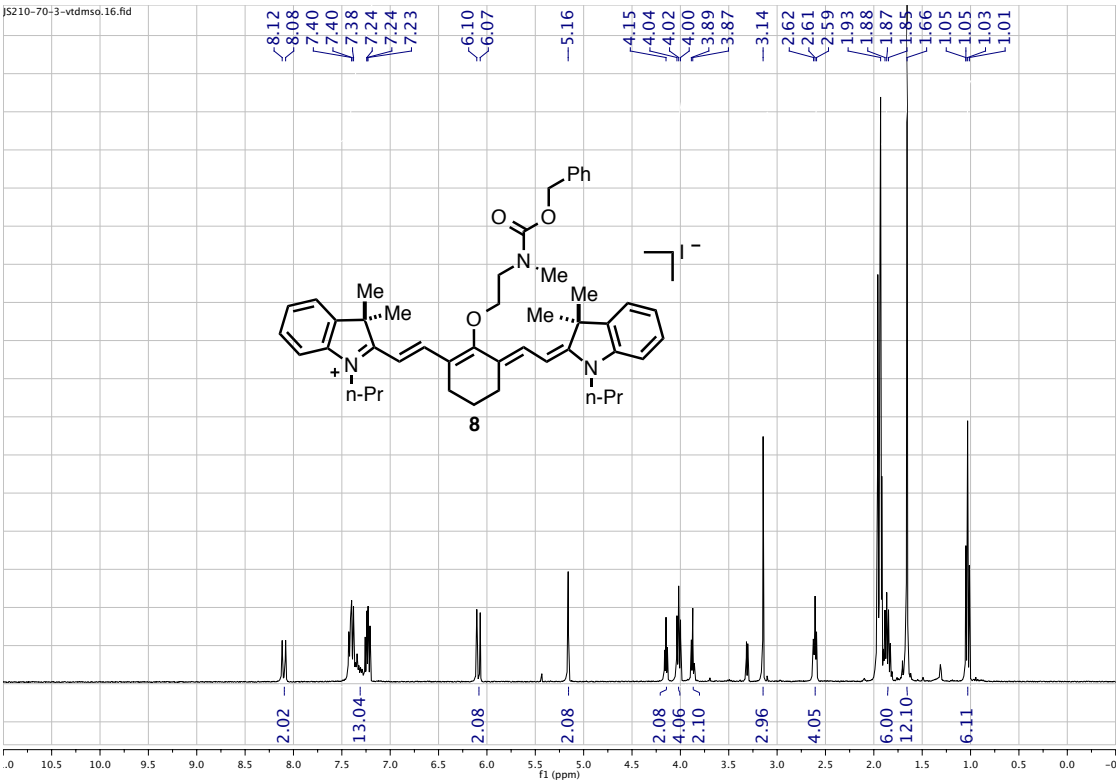
atom	^{13}C (mult)	^{1}H mult, *J* (Hz)	HMBC[a]	COSY[b]
1′	101.3 (CH)	6.20 (d, J = 14.2 Hz, 2H)	2, 2′, 3, 3′	2′
2′	141.0 (CH)	8.03 (d, J = 14.2 Hz, 2H)	2, 4′, 12	1′
3′	124.0 (C)			
4′	170.2 (C)			
11	22.1 (CH_2)	1.97–1.91 (m, 2H)	3′, 12	12
12	25.9 (CH_2)	2.75–2.57 (m, 4H)	2′, 3′, 4′, 11	11
13	70.9 (CH_2)	4.60 (t, J = 6.2 Hz, 2H)	4′, 14	14
14	66.4 (CH_2)	4.23–4.07 (m, 2H)	13, 15	13

[a]Carbons that correlate to the proton resonance. Optimized for 10 Hz coupling. [b]Protons that correlate to the proton resonance.

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



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4.04
4.02
4.00
3.89
3.87
3.14
2.62
2.61
2.59
1.93
1.88
1.87
1.85
1.66
1.05
1.05
1.03
1.01
Ph
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O
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Me
I⁻
Me
Me
Me
Me
O
N
+
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N
n-Pr
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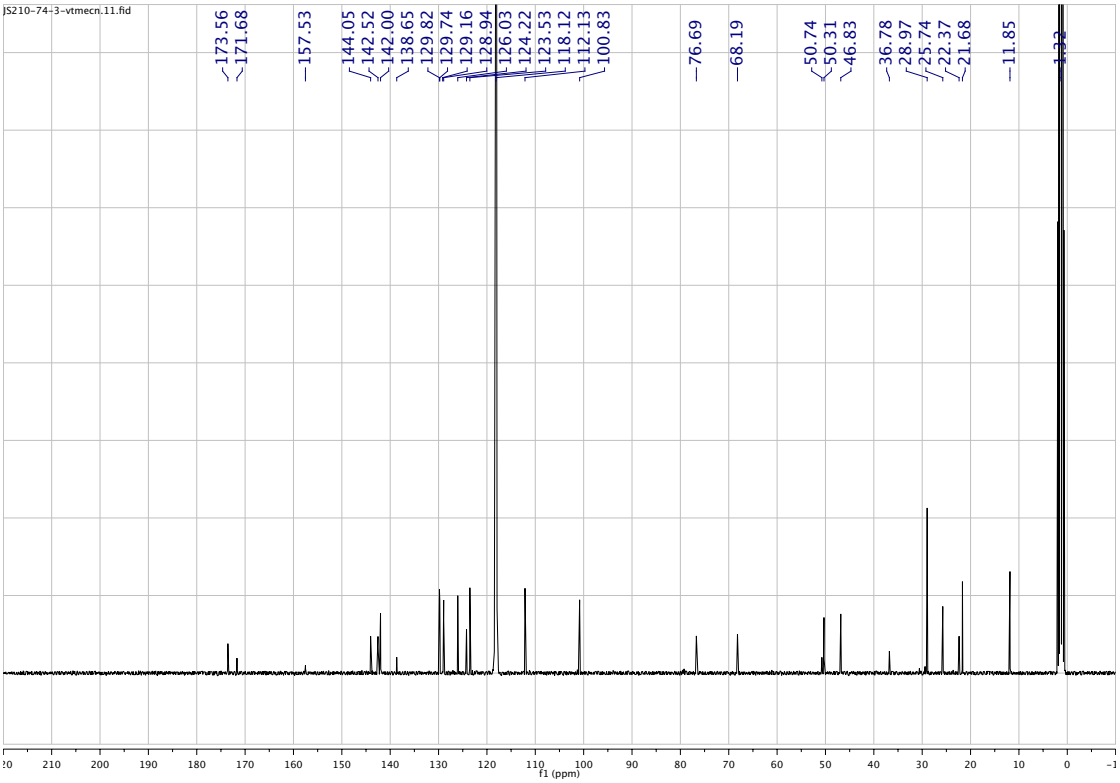
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123.53
118.12
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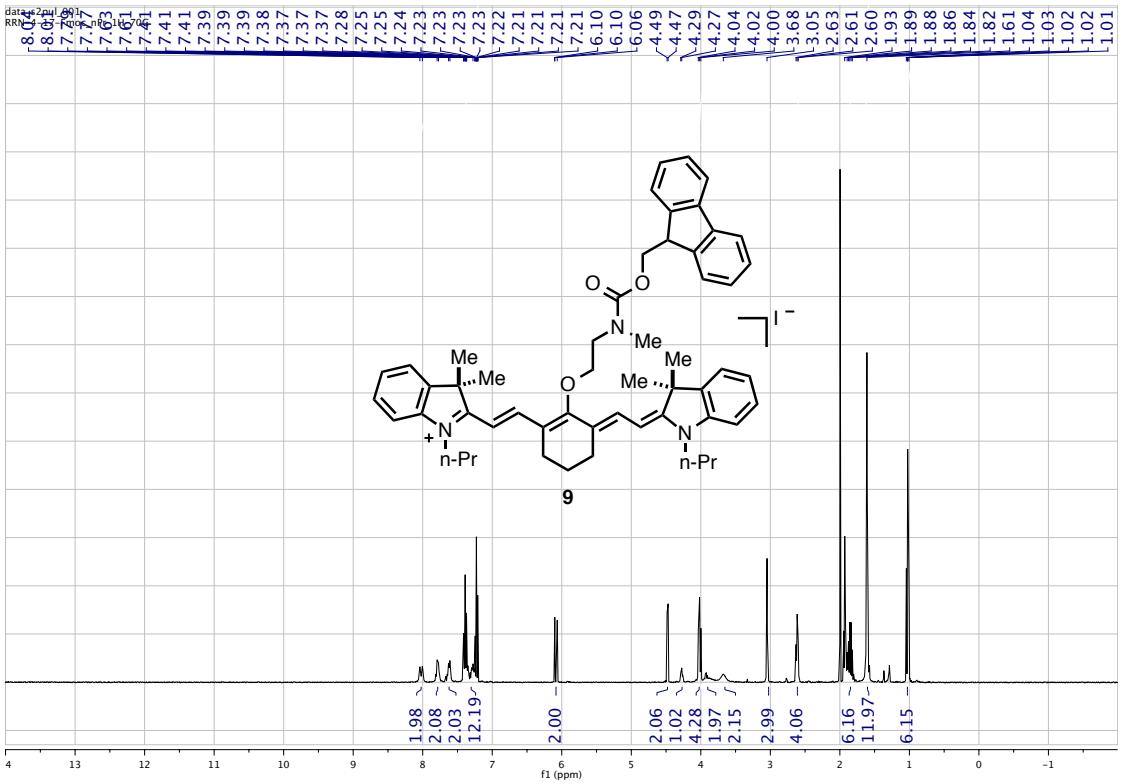
f1 (ppm)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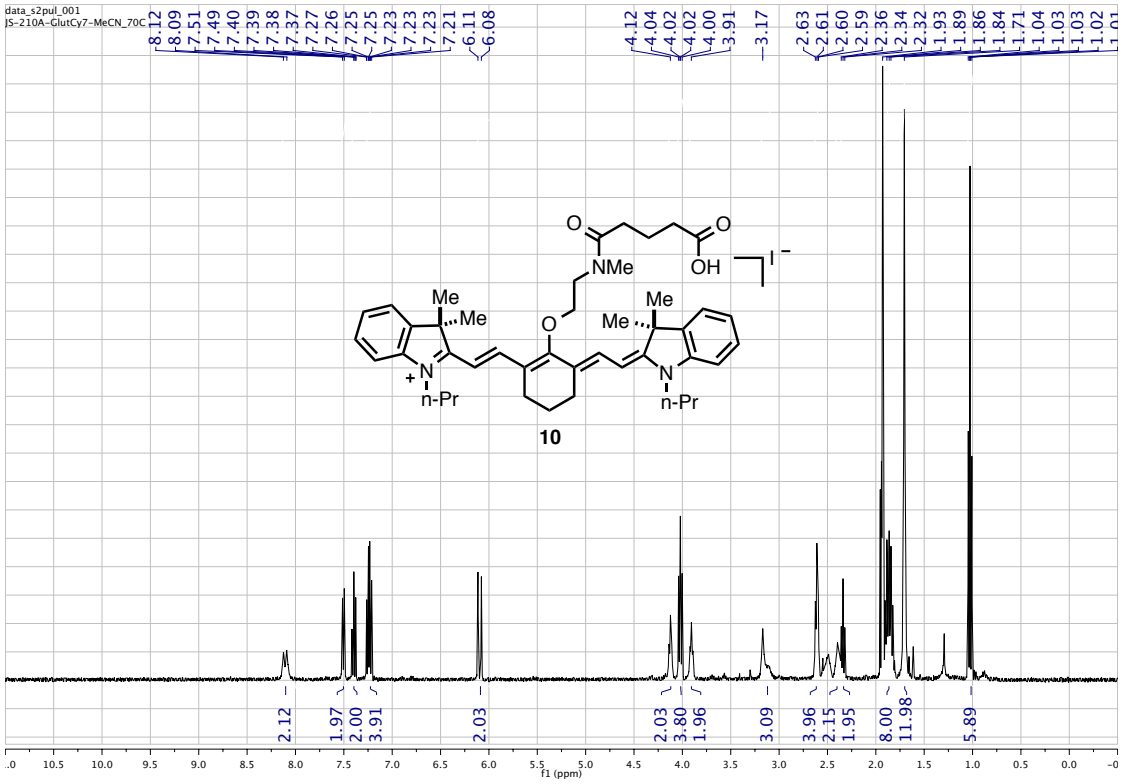
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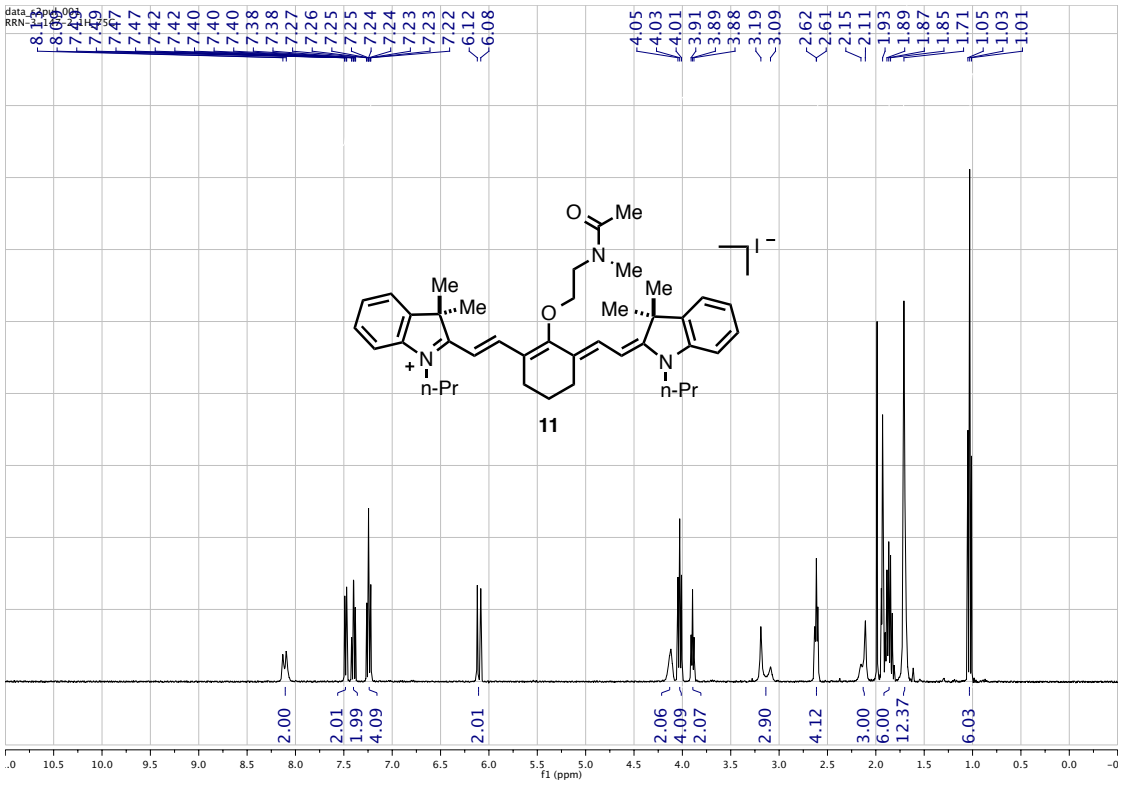
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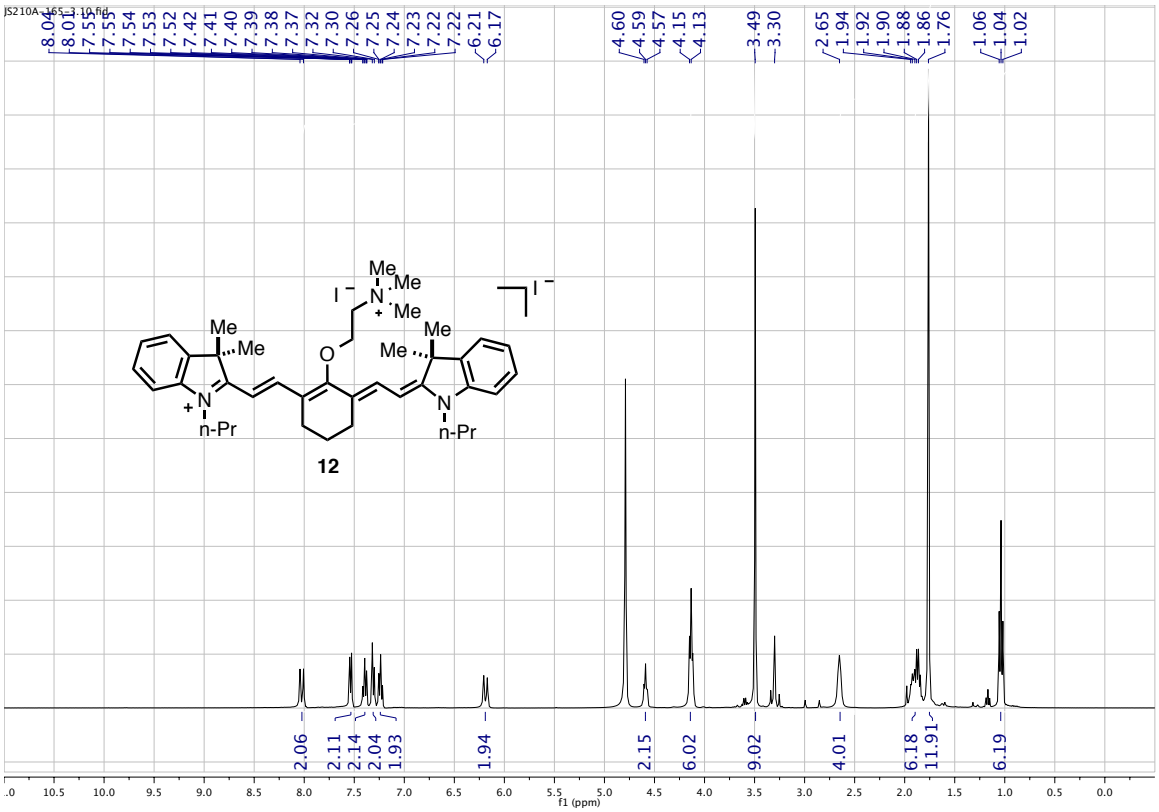
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4.13
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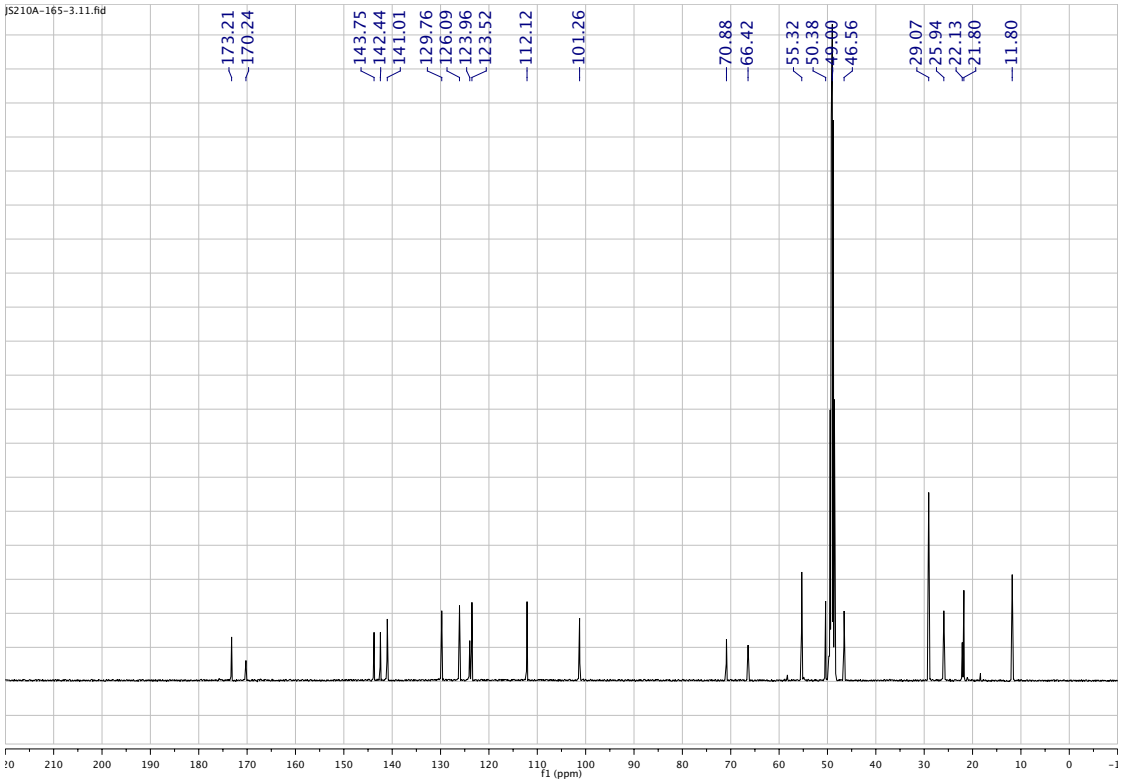
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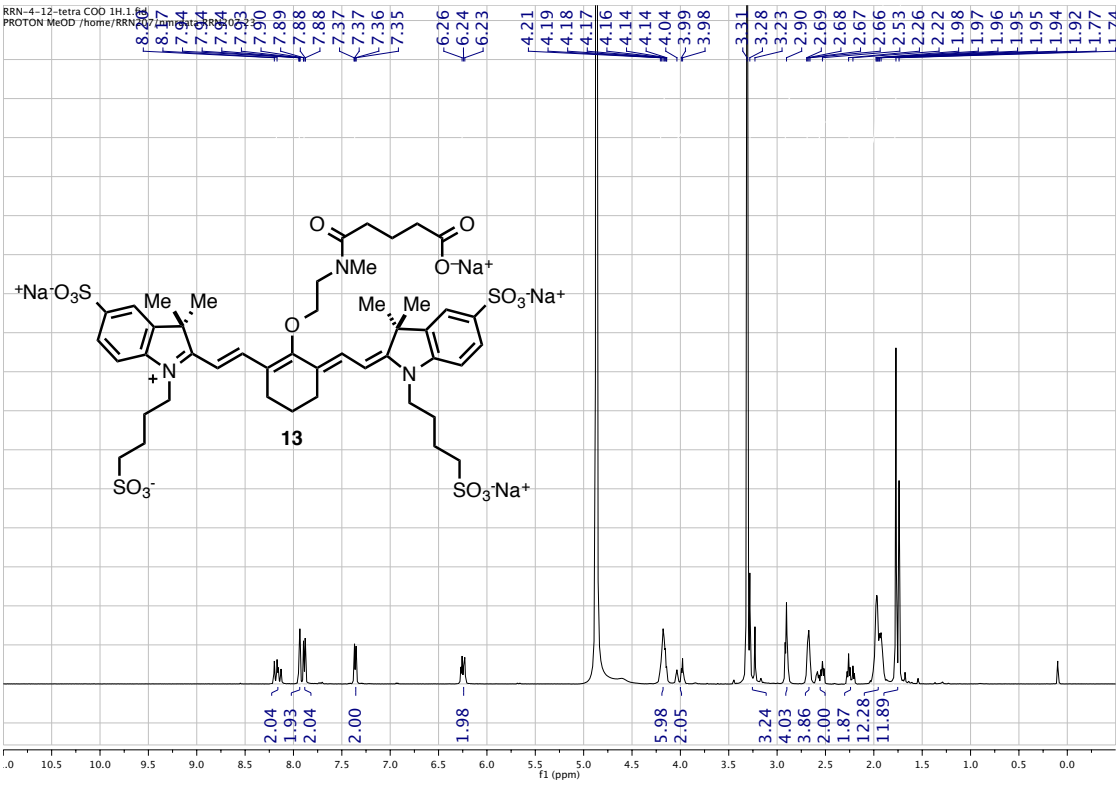
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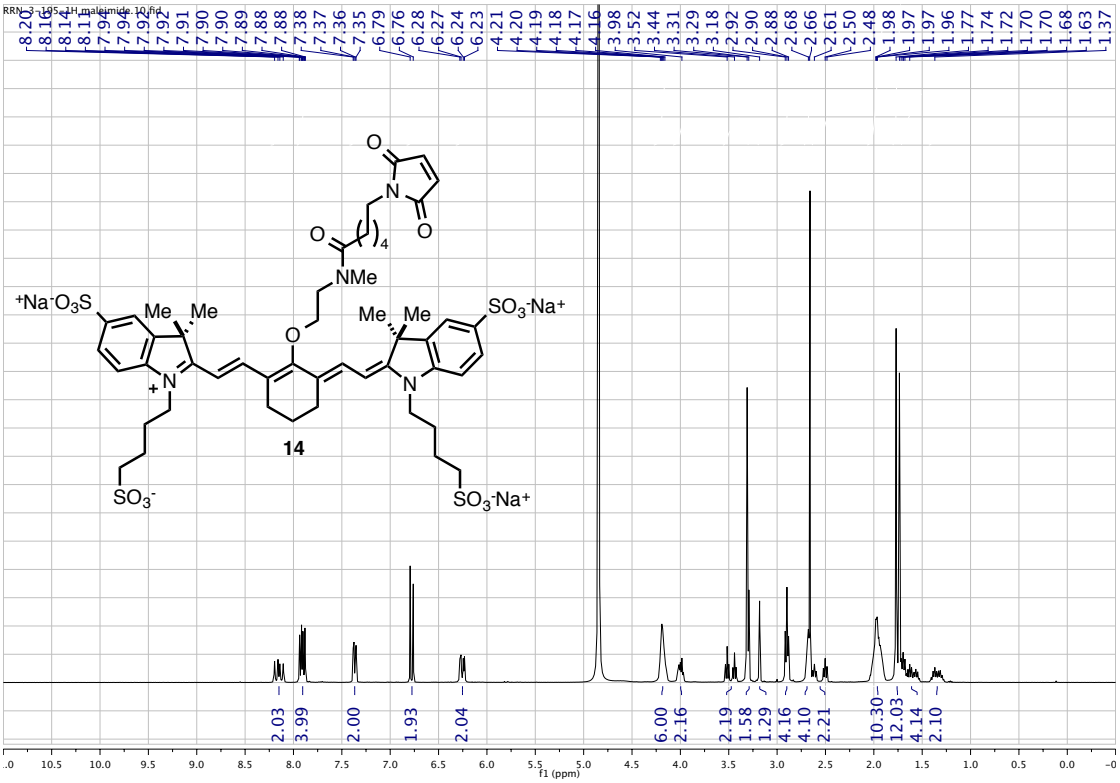
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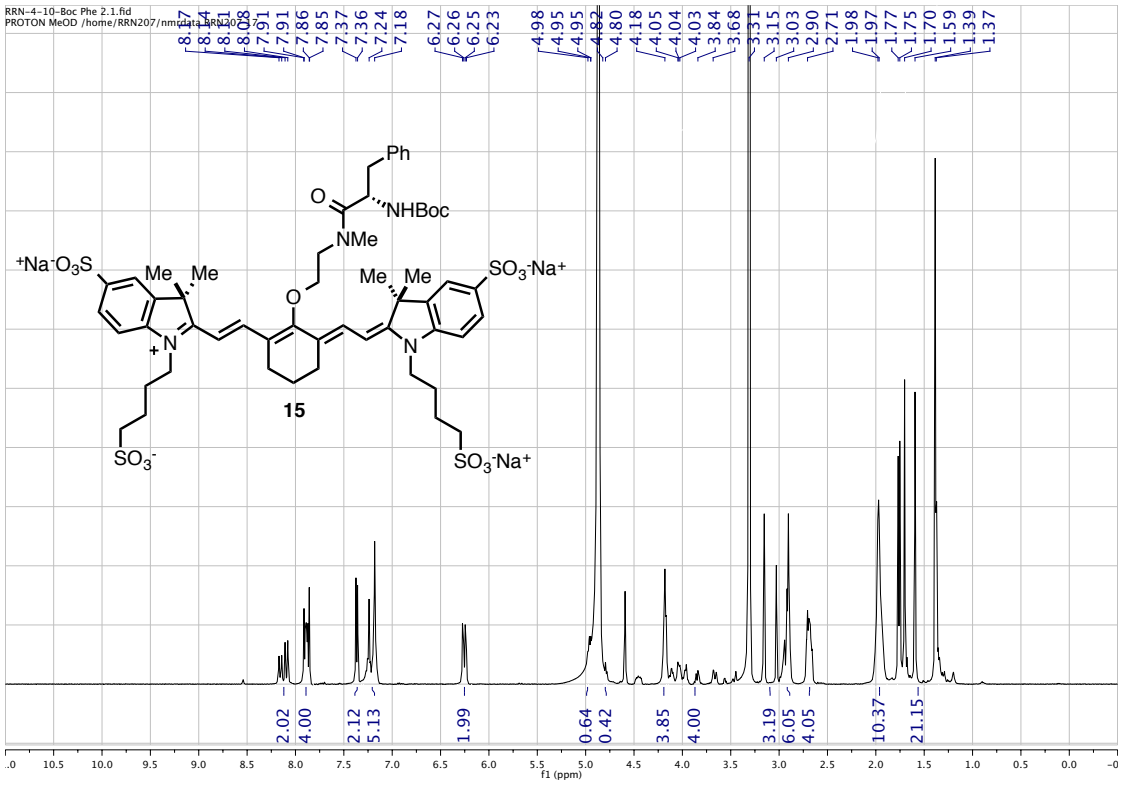
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1.37
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NMe
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Me
O
Me
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SO3-Na+
N
+
N
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SO3-
SO3-Na+
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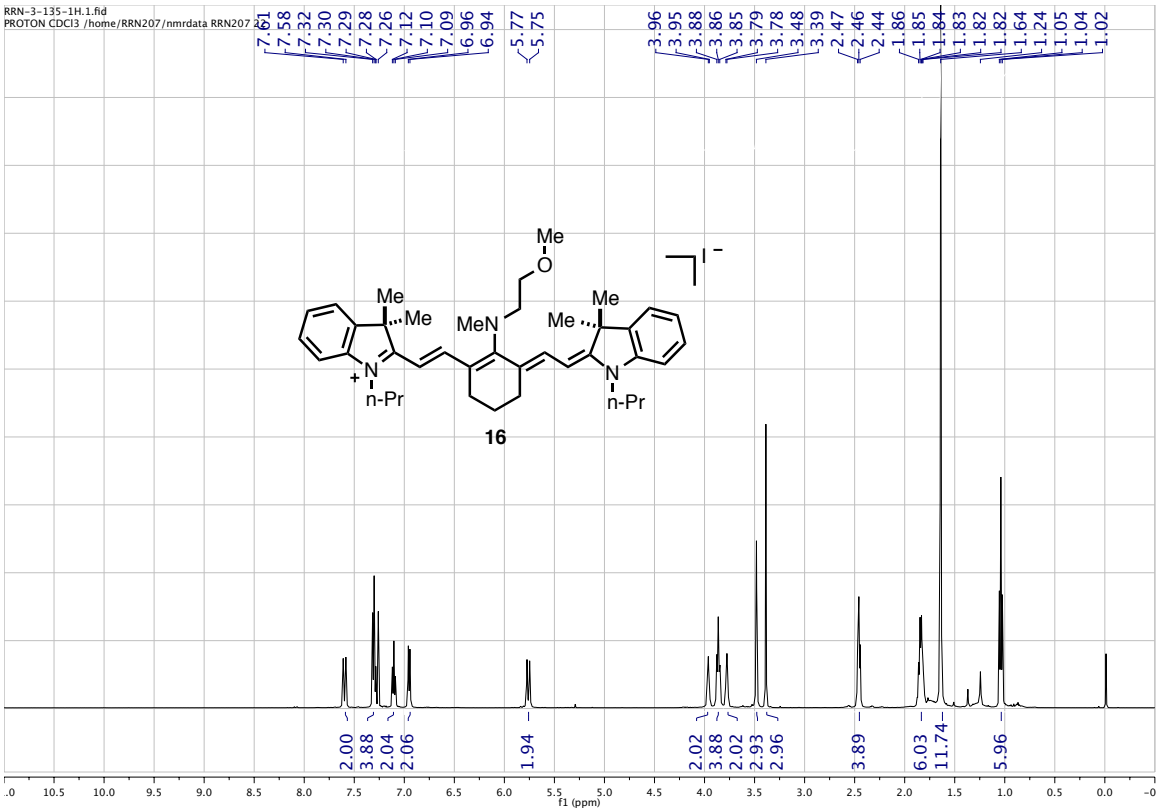
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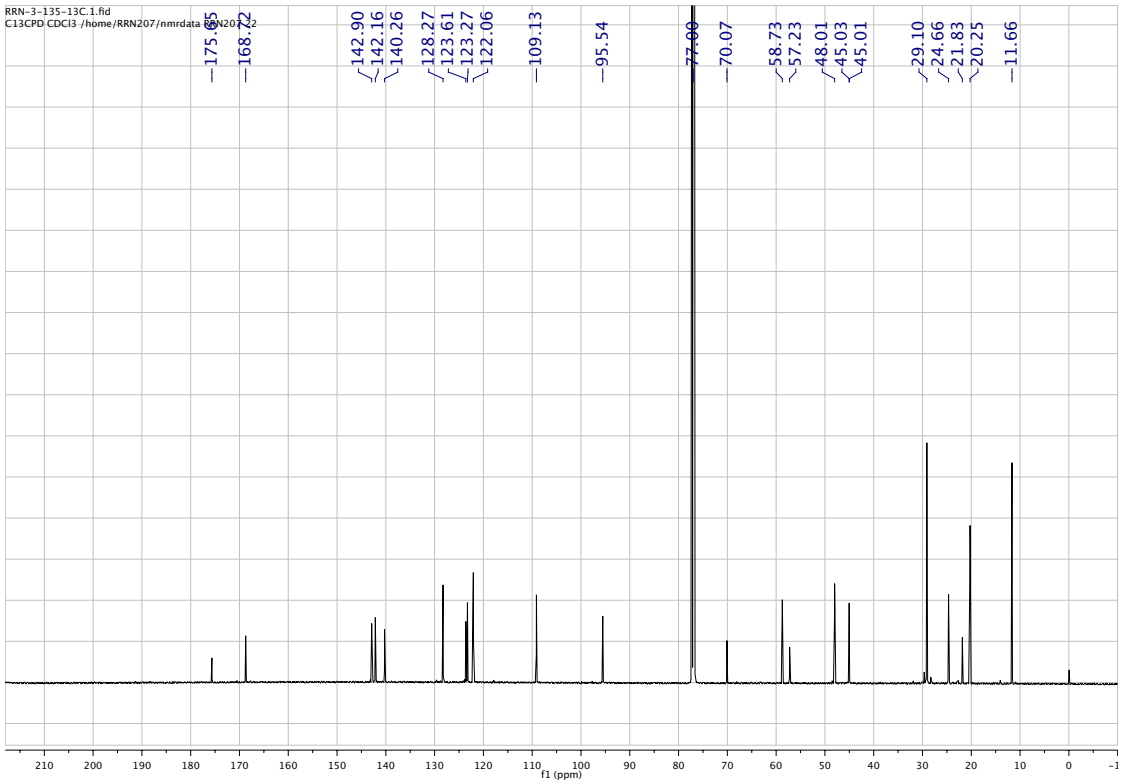
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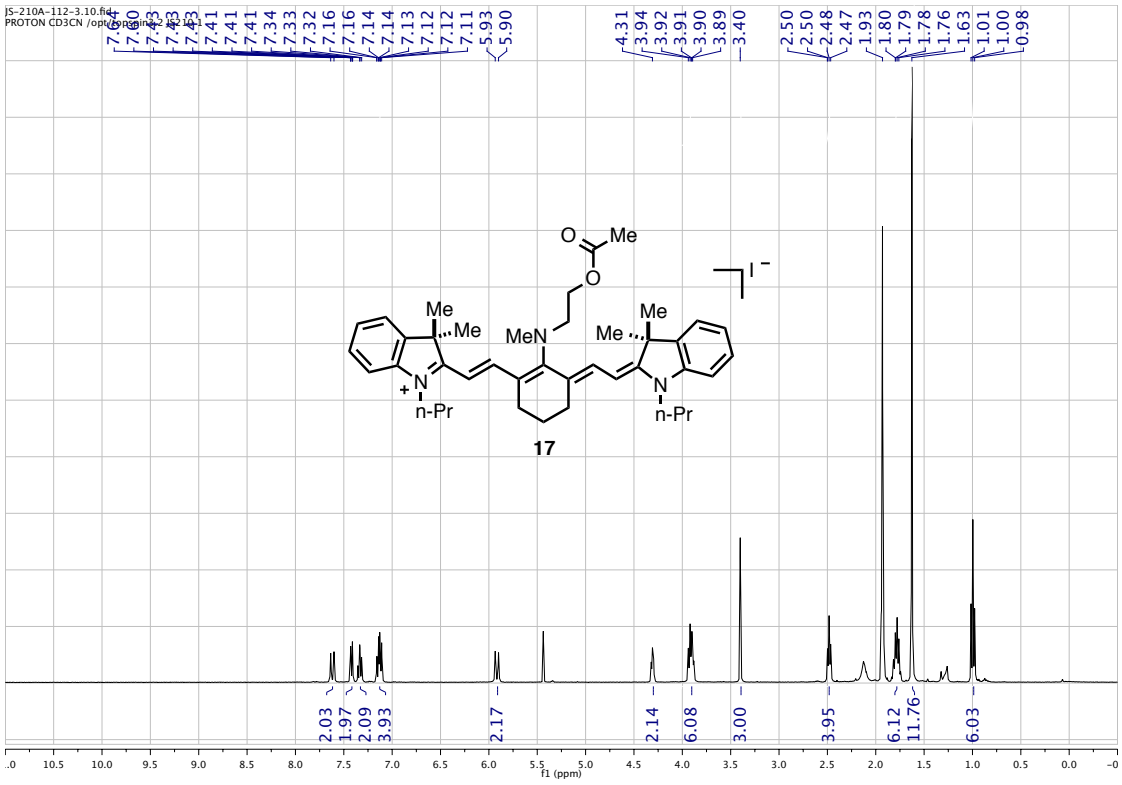
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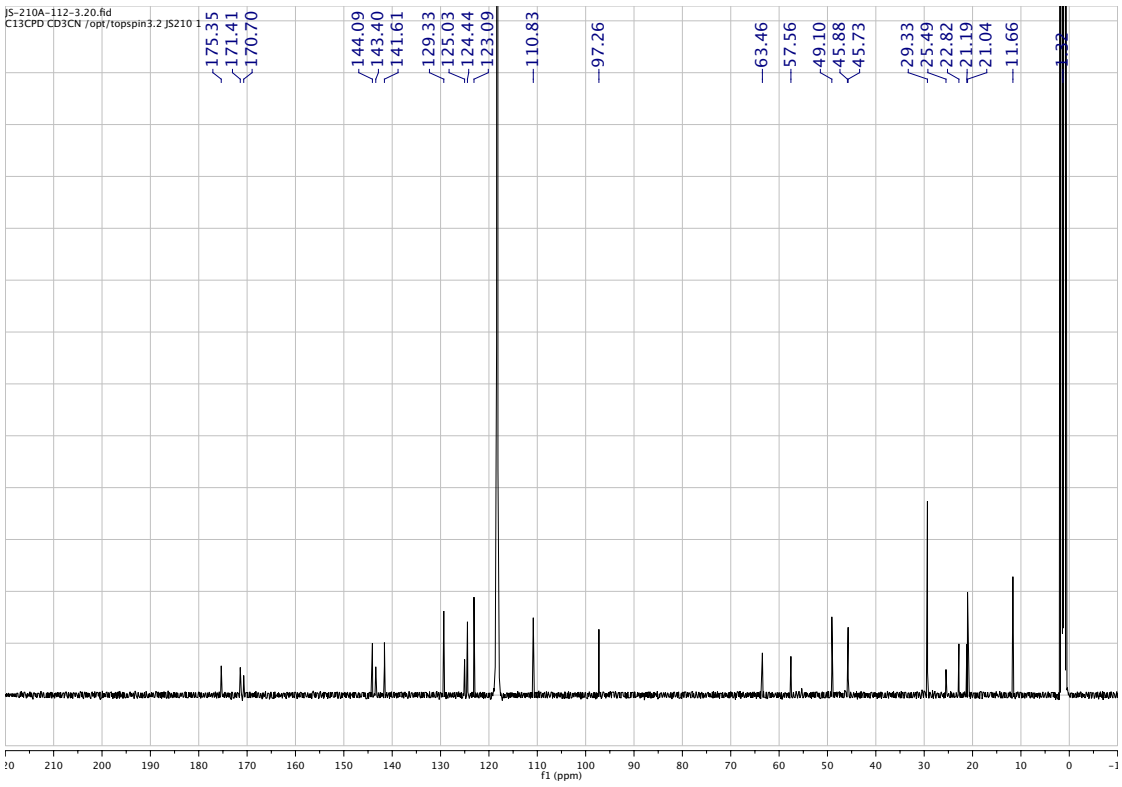
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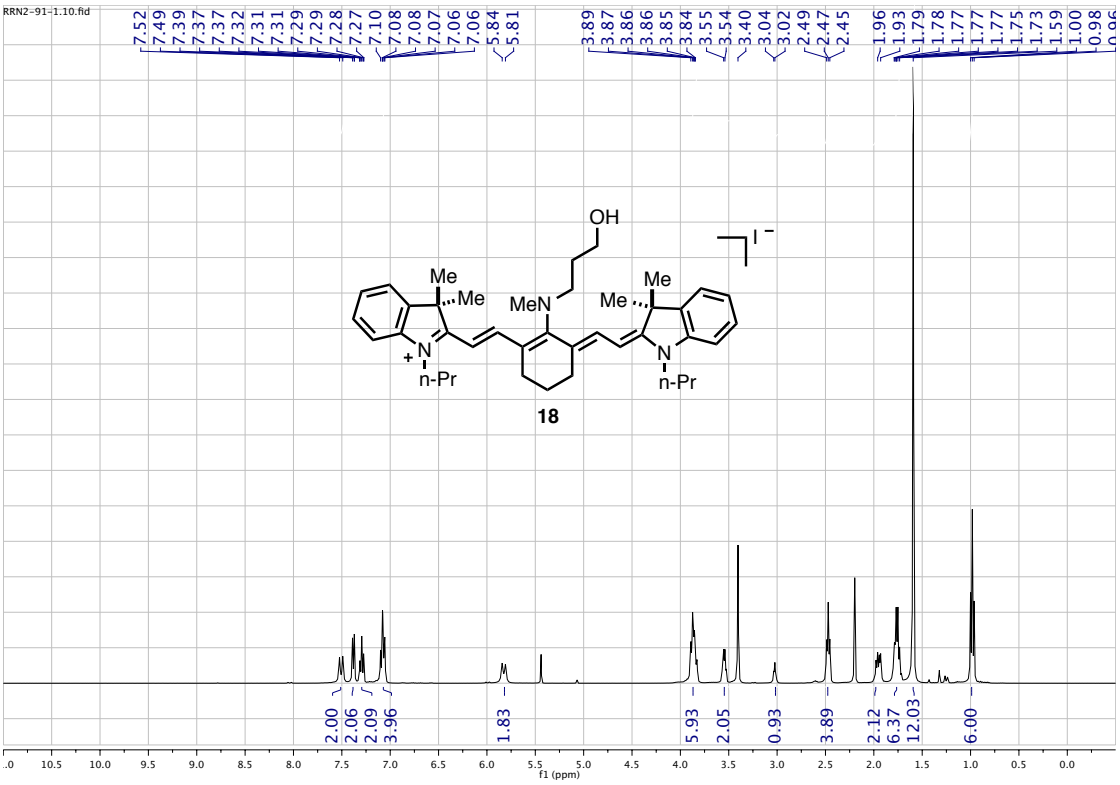
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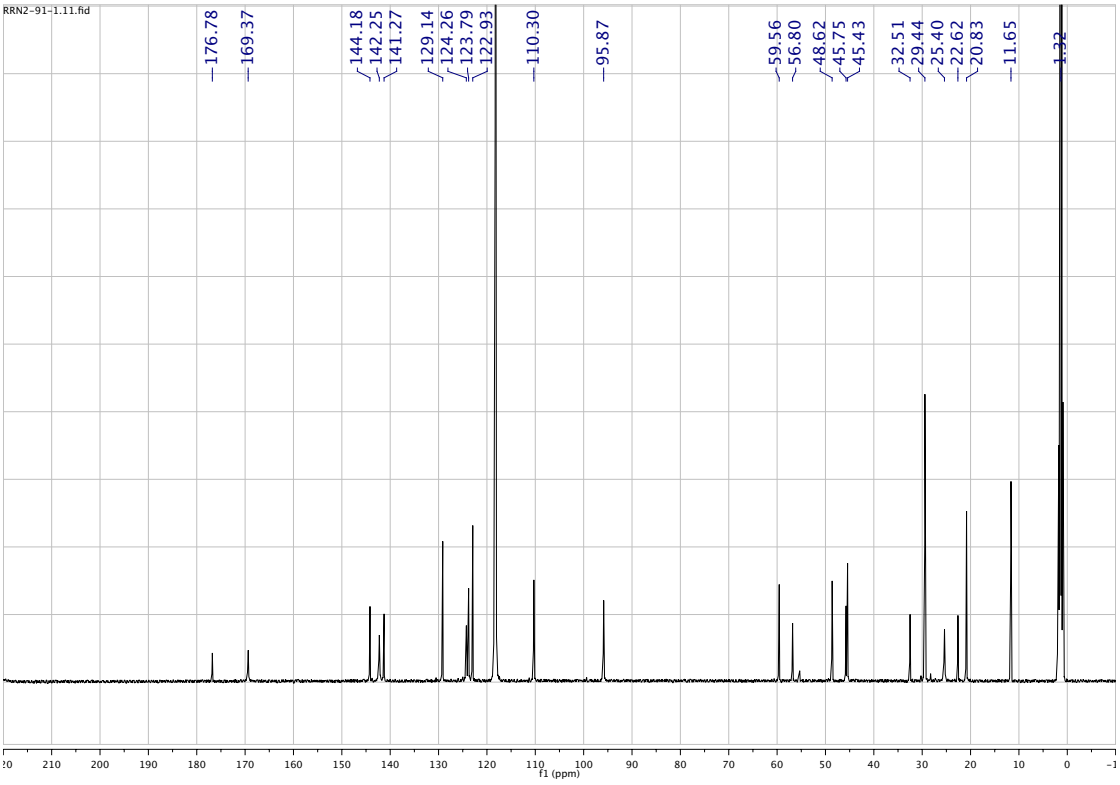
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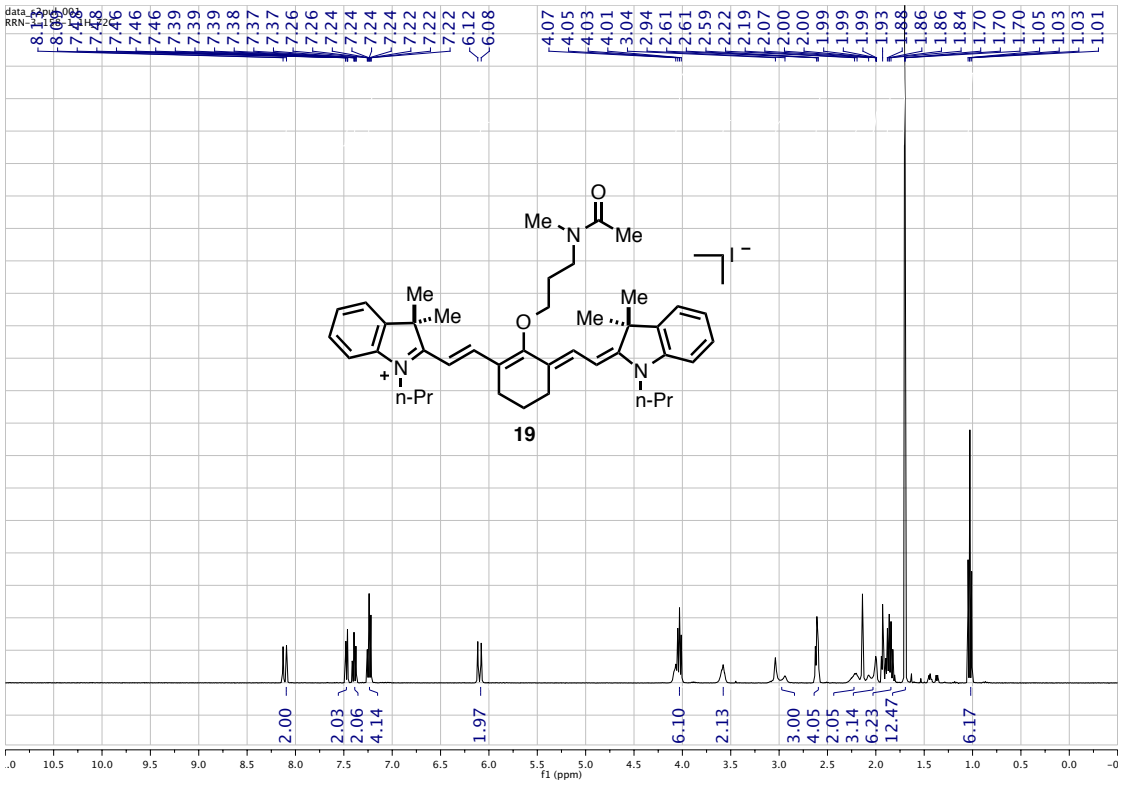
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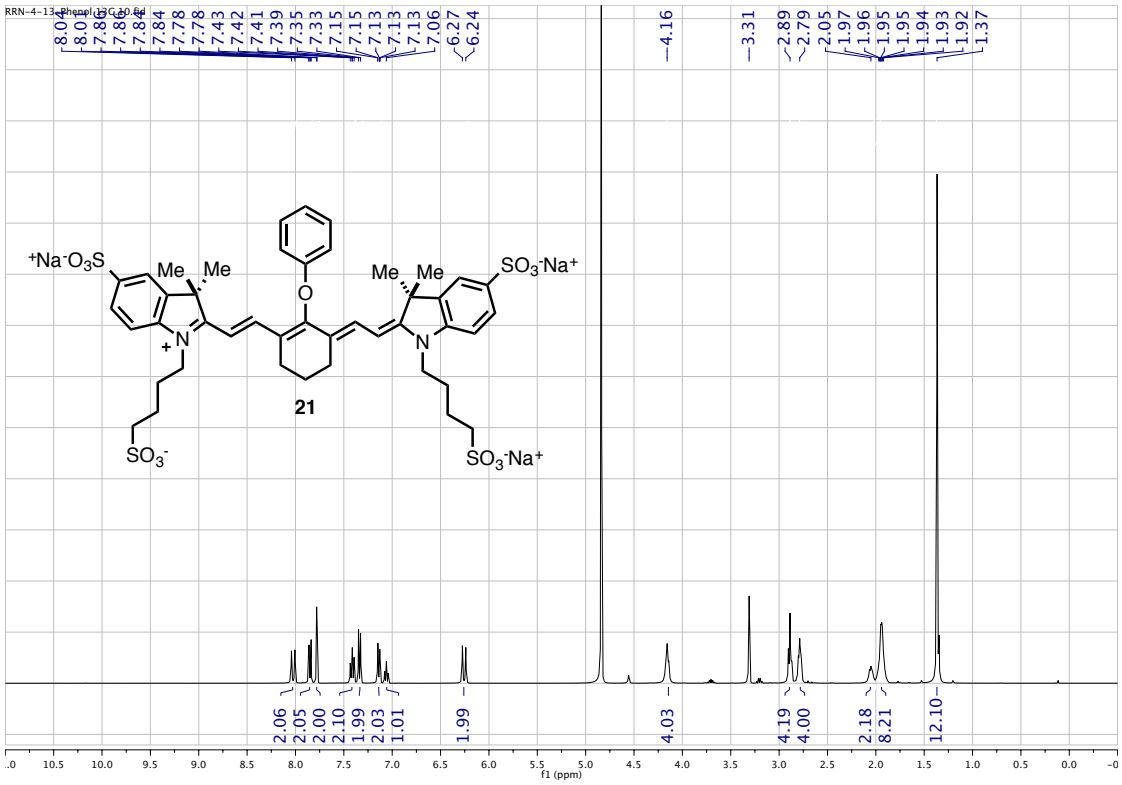
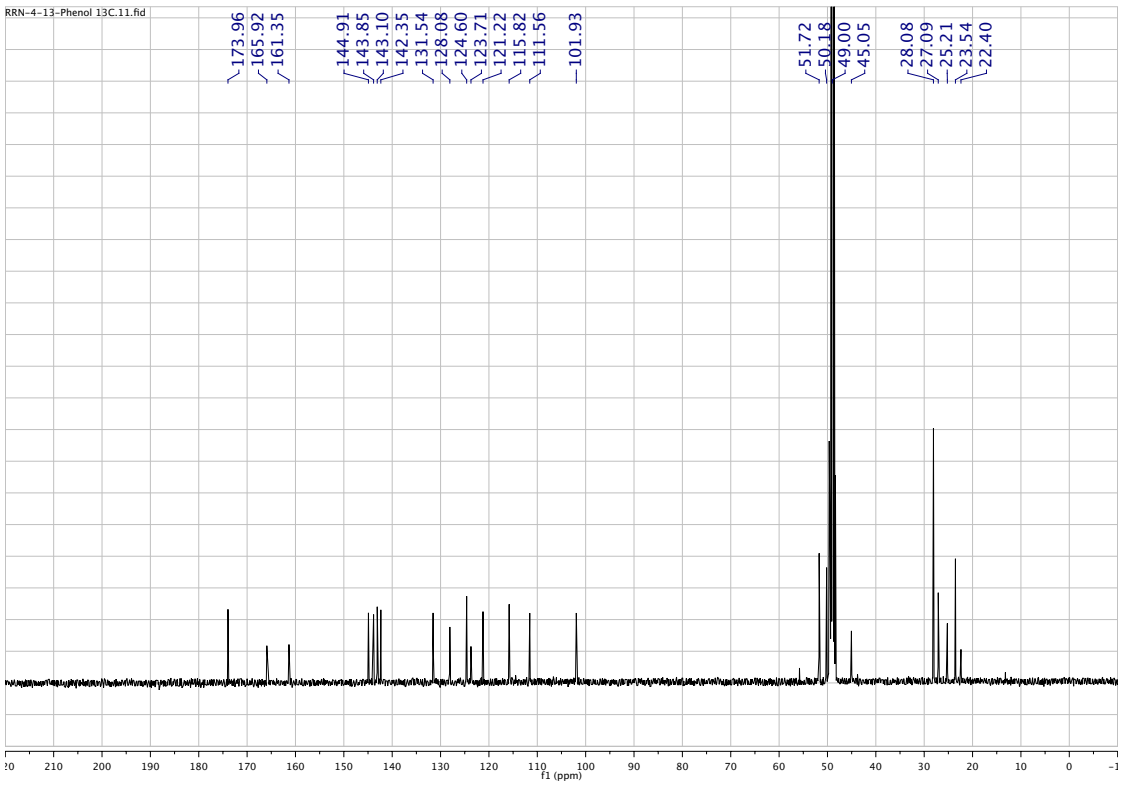
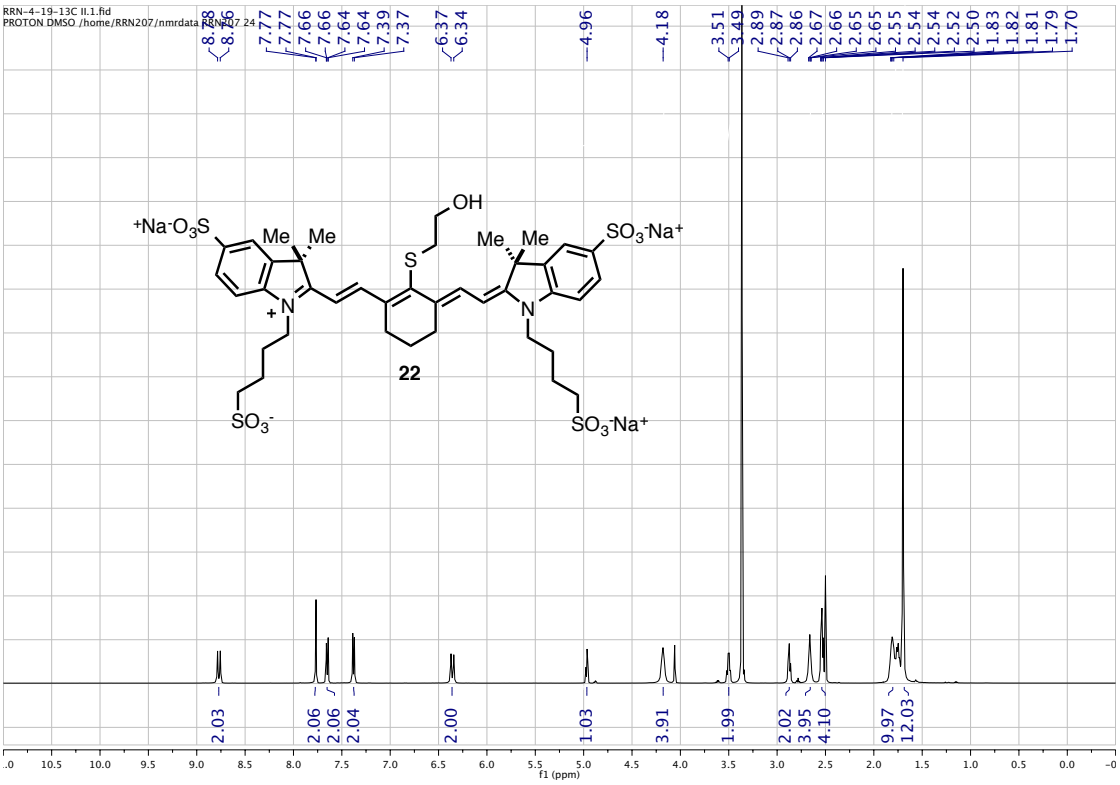
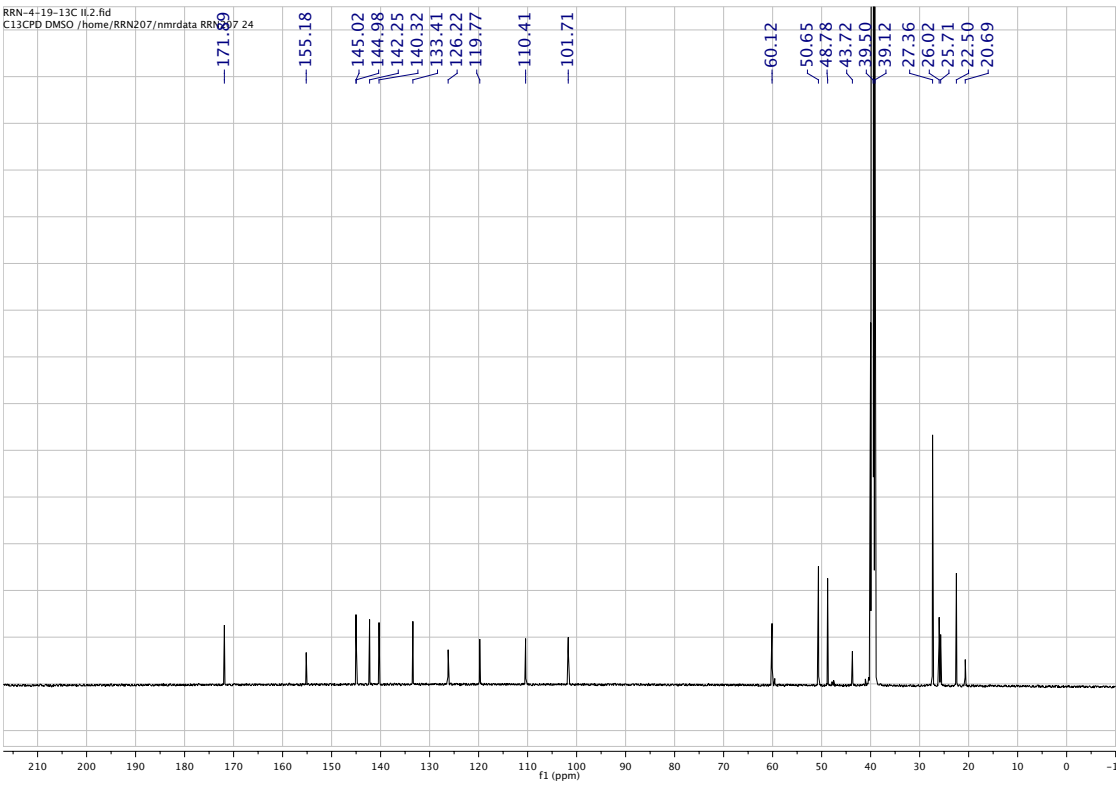
19
f1 (ppm)


RRN-4-13-Phenol 13C.10.fid
8.04
8.01
7.86
7.84
7.84
7.78
7.78
7.43
7.42
7.41
7.39
7.35
7.33
7.15
7.15
7.13
7.13
7.13
7.06
6.27
6.24
4.16
3.31
2.89
2.79
2.05
1.97
1.96
1.95
1.95
1.94
1.93
1.92
1.37
+Na-O3S
Me
Me
O
Me
Me
SO3-Na+
N
+
N
21
SO3-
SO3-Na+
2.06
2.05
2.00
2.10
1.99
2.03
1.01
1.99
4.03
4.19
4.00
2.18
8.21
12.10
f1 (ppm)


RRN-4-13-Phenol 13C.11.fid
173.96
165.92
161.35
144.91
143.85
143.10
142.35
131.54
128.08
124.60
123.71
121.22
115.82
111.56
101.93
51.72
50.18
49.00
45.05
28.08
27.09
25.21
23.54
22.40
f1 (ppm)


RRN-4-19-13C II.1.fid
PROTON DMSO /home/RRN207/nmrdata RRN207 24
8.88
8.86
7.77
7.77
7.66
7.66
7.64
7.64
7.39
7.37
6.37
6.34
4.96
4.18
3.51
3.49
2.89
2.87
2.86
2.67
2.66
2.65
2.65
2.55
2.54
2.54
2.52
2.50
1.83
1.82
1.81
1.79
1.70
+Na-O3S
Me
Me
OH
S
Me
Me
SO3-Na+
N+
N
22
SO3-
SO3-Na+
2.03
2.06
2.06
2.04
2.00
1.03
3.91
1.99
2.02
3.95
4.10
9.97
12.03
f1 (ppm)


RRN-4-19-13C II.2.fid
C13CPD DMSO /home/RRN207/nmrdata RRN207 24
171.89
155.18
145.02
144.98
142.25
140.32
133.41
126.22
119.77
110.41
101.71
60.12
50.65
48.78
43.72
39.50
39.12
27.36
26.02
25.71
22.50
20.69
f1 (ppm)