**A Near-IR Uncaging Strategy Based on Cyanine Photochemistry**

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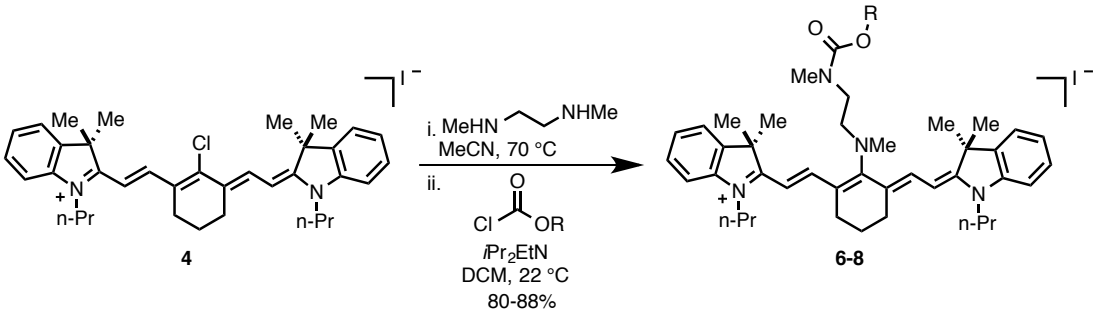
Supporting Information – Table of Contents

**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 commercially obtained reagents were used as received. See *JOC Standard Abbreviations and Acronyms* for abbreviations (available at http://pubs.acs.org/userimages/ContentEditor/1218717864819/joceah_abbreviations.pdf). Flash column chromatography was performed using normal phase silica gel (60 Å, 230-400 mesh, RediSep® Normal-phase Silica Flash Columns) or reversed phase (100 Å, 20-40 micron particle size, RediSep® Rf Gold® Reversed-phase C18) on a CombiFlash® Rf 200i (Teledyne Isco Inc). Preparative HPLC was performed with a Waters® 2545 Binary Gradient Module equipped with a Waters® 2767 Sample Manager fraction collector and a Luna 10 μm C18 110 Å (75 x 30 mm) column obtained from Phenomenex, Inc. 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. 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% MeOH/0.1% aqueous formic acid over 4.5 min at a flow rate of 0.2 mL/min. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on Varian and Bruker spectrometers (at 400 or 500 MHz or at 100 or 125 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. Data for $^{13}$C NMR spectra are reported in terms of chemical shift. IR spectra were recorded on a Varian 640-IR spectrometer and are reported in terms of frequency of absorption ($cm^{-1}$). 96-well plate-based absorbance and fluorescence measurements were performed on a BioTek Synergy 2 multi-mode microplate reader operated by Gen5 2.01 software. Coumarin fluorescence was monitored using a 360/40 nm excitation filter and a 460/40 nm emission filter. Absorbance 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 5 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. Light intensity measurements were performed with a Thorlabs PM200 optical power and energy meter fitted with an S120VC standard Si photodiode power sensor (200-1100 nm, 50 mW). Flow cytometry was performed at the CCR Flow Cytometry Core (NCI-Frederick) using a BD LSRII SORP analyzer operating laser lines at 488 nm (EGFP) and 561 nm (tdTomato). Cellular imaging was performed using a Zeiss LSM UV510 confocal laser-scanning microscope. Cells

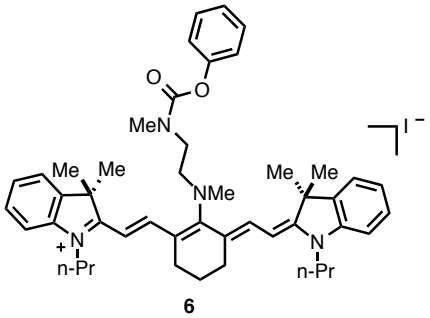
were plated on Nunc Lab-Tek® II chambered #1.5 German borosilicate coverglass (Thermo Fisher Scientific, Inc.). Hoescht 33342 staining (1 μM incubated for 1 h) was imaged using a Diode 405-30 laser (405 nm ex., 420-480 nm bandpass em.), EGFP using an Argon/2 laser (488 nm ex., 505-550 bandpass em.), LysoTracker® Red DND-99 staining (0.5 μM incubated for 3 h) using a DPSS laser (561 nm ex., 585-600 nm spectral em.), and **11** using a HeNe633 laser (633 nm ex., 650 longpass em.). All images result from 63x magnification using a plan-apochromat oil immersion objective. Image processing was conducted with ImageJ 1.47.

## Experimental Procedures

### General Procedure for Synthesis of Carbamates 6-8



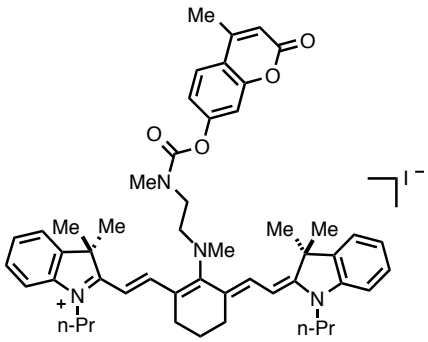

**(6-8):** To a solution of IR-780 iodide **4** (100 mg, 0.15 mmol) in MeCN (5 mL) was added *N*,*N*'-dimethylethylenediamine (80 μL, 0.75 mmol). The green solution was heated to 70 °C in a sealed vial for 5 min as the reaction color transitioned from green to dark blue. LC/MS analysis showed the consumption of **4**. The reaction was cooled to room temperature and diluted with DCM (30 mL). The organic phase was washed twice with saturated aqueous $NaHCO_3$ and dried over $Na_2SO_4$. The intermediate diamine solution in DCM was filtered and used directly in the next step. To a solution of the chloroformate (0.15 mmol) in DCM (5 mL) was added *N*,*N*-diisopropylethylamine (52 μL, 0.30 mmol) and the intermediate diamine solution in DCM. The dark blue solution was stirred for 10 min, at which time LC/MS analysis showed consumption of the diamine intermediate. The solution was concentrated *in vacuo* and the residue was purified by silica gel chromatography (100% EtOAc, then 0→10% MeOH/DCM) to afford carbamates **6-8**.




**6**

**(6):** Synthesized according to the general procedure above to afford 102 mg of **6** (81% yield) as a dark blue solid. $^1$H NMR (DMSO, 400 MHz, 70 °C) $\delta$ 7.58 (d, $J$ = 13.5 Hz, 2H), 7.45 (d, $J$ = 7.4 Hz, 2H), 7.39 – 7.27 (m, 4H), 7.22 (d,

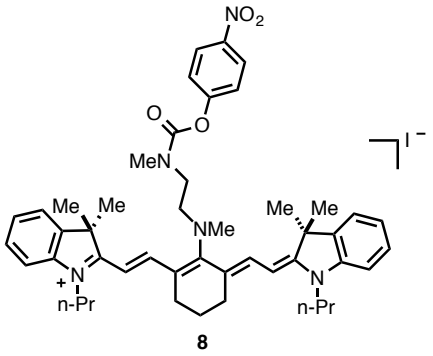
*J* = 7.9 Hz, 2H), 7.20 – 7.08 (m, 3H), 7.07 – 6.99 (m, 2H), 5.93 (d, *J* = 13.5 Hz, 2H), 4.08 – 3.89 (m, 6H), 3.74 – 3.65 (m, 2H), 3.46 (s, 3H), 3.00 (s, 3H), 2.55 – 2.51 (m, 4H), 1.84 – 1.71 (m, 6H), 1.58 (s, 12H), 0.97 (t, *J* = 7.4 Hz, 6H); $^{13}$C NMR (DMSO, 100 MHz, 70 °C) $\delta$ 172.4, 168.5, 153.7, 150.8, 142.5, 140.6, 139.9, 128.8, 127.9, 124.8, 123.2, 122.8, 121.7, 121.2, 109.5, 95.8, 54.9, 47.35, 47.32, 44.8, 44.0, 34.3, 28.2, 24.3, 21.1, 19.6, 10.8; IR (thin film) 1722, 1507, 1446, 1340, 1258 cm$^{-1}$; HRMS (ESI) calculated for $C_{47}H_{59}N_4O_2$ ($M^+$) 711.4638, observed 711.4627.




**7**

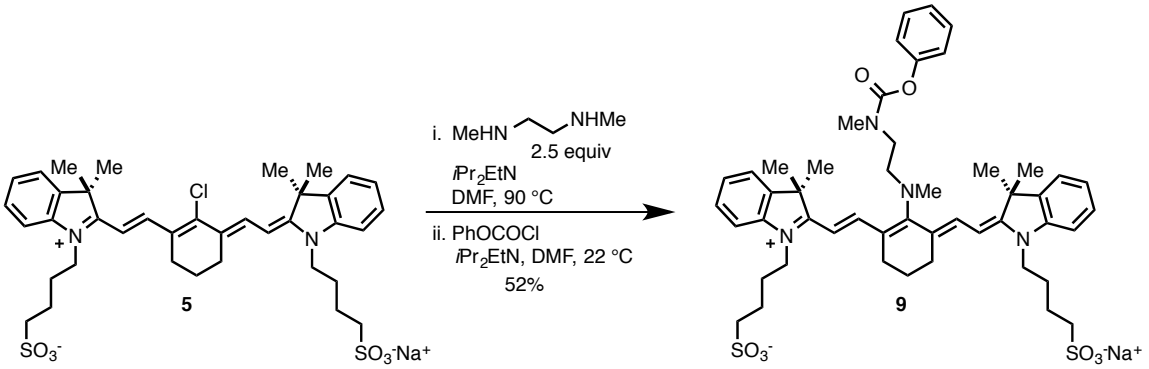
**(7):** Synthesized according to the general procedure above to afford 110 mg of **7** (80% yield) as a dark blue solid. 4-Methylumbelliferone chloroformate was obtained by dropwise addition of a slurry of 1 equivalent of the sodium salt of 4-methylumbelliferone in MeCN to phosgene (5 equivalents, PhMe solution) at 0 °C.[1] The reaction was allowed to warm to room temperature over 30 min, after which time the volatiles were concentrated *in vacuo*. The crude solid was slurried in MeCN and filtered, removing the more insoluble diaryl carbonate. The filtrate was concentrated to afford 4-methylumbelliferone chloroformate as a white solid. The analytical data for **7** are as follows: $^1$H NMR (DMSO, 400 MHz, 70 °C) $\delta$ 7.73 (d, *J* = 8.7 Hz, 1H), 7.58 (d, *J* = 13.3 Hz, 2H), 7.45 (d, *J* = 7.4 Hz, 2H), 7.33 (t, *J* = 7.7 Hz, 2H), 7.22 (d, *J* = 7.9 Hz, 2H), 7.16 – 7.05 (m, 4H), 6.30 (s, 1H), 5.93 (d, *J* = 13.3 Hz, 2H), 4.09 – 3.89 (m, 6H), 3.76 – 3.65 (m, 2H), 3.46 (s, 3H), 3.02 (s, 3H), 2.55 – 2.51 (m, 4H), 2.38 (s, 3H), 1.88 – 1.67 (m, 6H), 1.59 (s, 12H), 0.96 (t, *J* = 7.4 Hz, 6H); $^{13}$C NMR (DMSO, 100 MHz, 70 °C) $\delta$ 172.3, 168.6, 159.2, 153.2, 153.0, 152.4, 142.5, 140.7, 140.0, 128.0, 125.8, 123.2, 122.9, 121.8, 117.9, 117.6, 116.7, 113.2, 109.6, 109.2, 95.9, 54.8, 47.46, 47.41, 44.9, 44.1, 34.3, 28.2, 24.4, 21.1, 19.6, 17.7, 10.9; IR (thin film) 1727, 1614, 1546, 1509, 1453, 1373 cm$^{-1}$; HRMS (ESI) calculated for $C_{51}H_{61}N_4O_4$ ($M^+$) 793.4687, observed 793.4679.

[1] Neurosearch A/S, Den. Novel 1,4-diazabicyclo[3.2.2]nonane derivatives and their medical use. WO2007135122; 2007



**8**

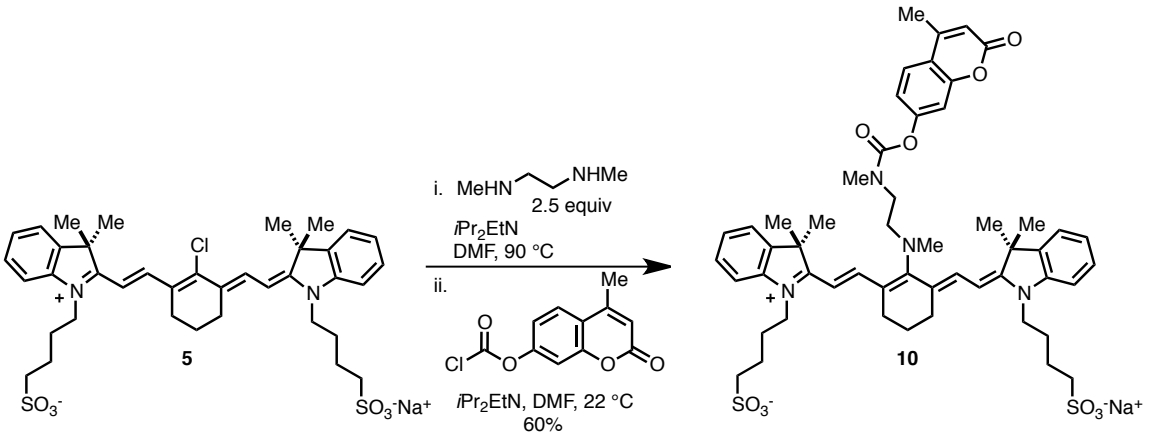
**(8):** Synthesized according to the general procedure above to afford 117 mg of **8** (88% yield) as a dark blue solid. $^1$H NMR (DMSO, 400 MHz, 70 °C) $\delta$ 8.22 (d, $J$ = 8.6 Hz, 2H), 7.57 (d, $J$ = 13.5 Hz, 2H), 7.46 (d, $J$ = 7.4 Hz, 2H), 7.38 – 7.30 (m, 4H), 7.23 (d, $J$ = 7.9 Hz, 2H), 7.13 (t, $J$ = 7.4 Hz, 2H), 5.94 (d, $J$ = 13.5 Hz, 2H), 4.01 (t, $J$ = 7.2 Hz, 4H), 3.96 (t, $J$ = 6.7 Hz, 2H), 3.77 – 3.64 (m, 2H), 3.44 (s, 3H), 3.03 (s, 3H), 2.55 – 2.51 (m, 4H), 1.85 – 1.69 (m, 6H), 1.59 (s, 12H), 0.96 (t, $J$ = 7.4 Hz, 6H); $^{13}$C NMR (DMSO, 100 MHz, 70 °C) $\delta$ 172.1, 168.6, 155.7, 152.7, 144.2, 142.5, 140.7, 140.0, 128.0, 124.7, 123.1, 122.9, 122.2, 121.8, 109.6, 95.9, 54.7, 47.4, 47.4, 44.9, 44.0, 34.3, 28.2, 24.3, 21.1, 19.6, 10.8; IR (thin film) 1715, 1507, 1444, 1363 cm$^{-1}$; HRMS (ESI) calculated for $C_{47}H_{58}N_5O_4$ (M+H) 756.4489, observed 756.4480.



**9**

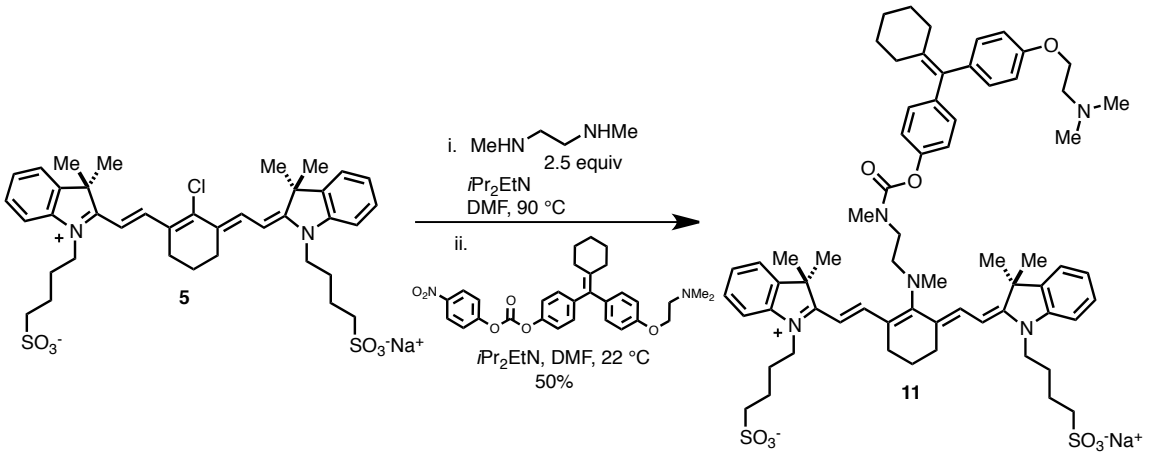
**(9):** To a solution of IR-783 **5** (95 mg, 0.13 mmol) in DMF (3 mL) was added *N,N'*-dimethylethylenediamine (34 μL, 0.32 mmol) and diisopropylethylamine (44 μL, 0.25 mmol). The green solution was heated to 90 °C in a sealed vial for 10 min as the reaction color transitioned from green to dark blue. LC/MS analysis showed the consumption of **5**. The reaction was cooled to room temperature and precipitated into $Et_2O$ (40 mL). The slurry was centrifuged, the supernatant discarded, and the blue pellet was resuspended in $Et_2O$ (20 mL). The procedure was repeated, and the blue pellet was dissolved in DMF (5 mL). Diisopropylethylamine (44 μL, 0.25 mmol) was added, followed by dropwise addition of phenylchloroformate (17 μL, 0.14 mmol) in DMF (0.1 mL). The reaction was stirred for 10 min at room temperature. After this time the solution was precipitated into $Et_2O$ (80 mL), centrifuged, and the crude pellet was purified by reversed-phase preparative HPLC (10→95% (MeCN/0.1% v/v $NH_4OH$/water). After lyophilization, **9** was obtained (60 mg, 52%) as a dark blue solid. $^1$H NMR ($CD_3OD$, 400 MHz, 65 °C) $\delta$ 7.68 (d, $J$ = 13.3 Hz, 2H), 7.38 – 7.23 (m, 6H), 7.19 – 7.06 (m, 5H), 7.06 – 6.98 (m, 2H), 5.94 (d, $J$ = 13.3 Hz, 2H), 4.10 – 3.92 (m, 6H), 3.73 (s, 2H), 3.51 (s, 3H), 3.04 (s, 3H), 2.86 (t, $J$ =

7.0 Hz, 4H), 2.55 (t, $J$ = 6.6 Hz, 4H), 2.03 – 1.81 (m, 10H), 1.60 (s, 12H);[2] IR (thin film) 2930, 1716, 1508, 1453, 1368 $cm^{-1}$; HRMS (ESI) calculated for $C_{49}H_{63}N_4O_8S_2$ ($MH^+$) 899.4082, observed 899.4098.




**(10):** To a solution of IR-783 **5** (37 mg, 0.049 mmol) in DMF (1.5 mL) was added *N,N'*-dimethylethylenediamine (13 μL, 0.12 mmol) and diisopropylethylamine (10 μL, 0.049 mmol). The green solution was heated to 90 °C in a sealed vial for 10 min as the reaction color transitioned from green to dark blue. LC/MS analysis showed the consumption of **5**. The reaction was cooled to room temperature and precipitated into $Et_2O$ (40 mL). The slurry was centrifuged, the supernatant discarded, and the blue pellet was resuspended in $Et_2O$ (20 mL). The procedure was repeated, and the blue pellet was dissolved in DMF (3 mL). Diisopropylethylamine (34 μL, 0.20 mmol) was added, followed by dropwise addition of 4-methylumbelliferone chloroformate (48 mg, 0.20 mmol, see compound **7** for procedure) in MeCN (4 mL). The reaction was stirred for 10 min at room temperature. The solution was precipitated into $Et_2O$ (80 mL), centrifuged, and the crude pellet was purified by reversed-phase preparative HPLC (10→95% MeCN/0.1% v/v $NH_4OH$/water). After lyophilization, **10** was obtained (30 mg, 60%) as a dark blue solid. $^1H$ NMR ($CD_3OD$, 400 MHz, 65 °C) $\delta$ 7.81 – 7.55 (m, 3H), 7.37 – 7.24 (m, 4H), 7.18 – 7.02 (m, 6H), 6.20 (s, 1H), 5.95 (d, $J$ = 13.5 Hz, 2H), 4.10 – 3.96 (m, 6H), 3.79 – 3.69 (m, 2H), 3.59 – 3.49 (m, 3H), 3.06 (s, 3H), 2.86 (t, $J$ = 6.8 Hz, 4H), 2.56 (t, $J$ = 6.7 Hz, 4H), 2.38 (s, 3H), 2.02 – 1.82 (m, 10H), 1.60 (s, 12H); IR (thin film) 2932, 1724, 1615, 1508, 1452, 1366 $cm^{-1}$; HRMS (ESI) calculated for $C_{53}H_{65}N_4O_{10}S_2$ ($MH^+$) 981.4137, observed 981.4151.

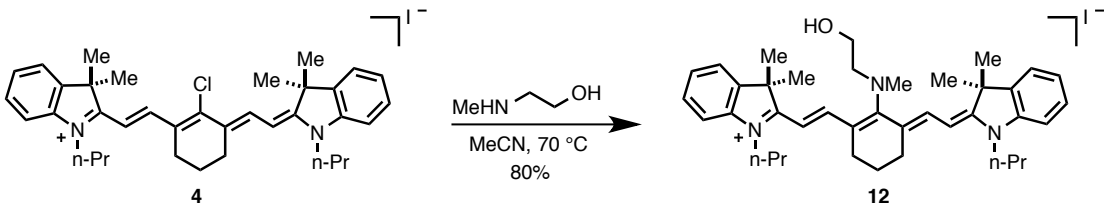
[2] With *N*-methyl carbamates **9**, **10**, and **11**, high temperature NMR (65 °C) was required to resolve the carbamate rotomers. However, these sulfonates proved unstable over many hours at these elevated temperatures in $CD_3OD$ (as well as several other solvents), which precluded obtaining $^{13}C$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



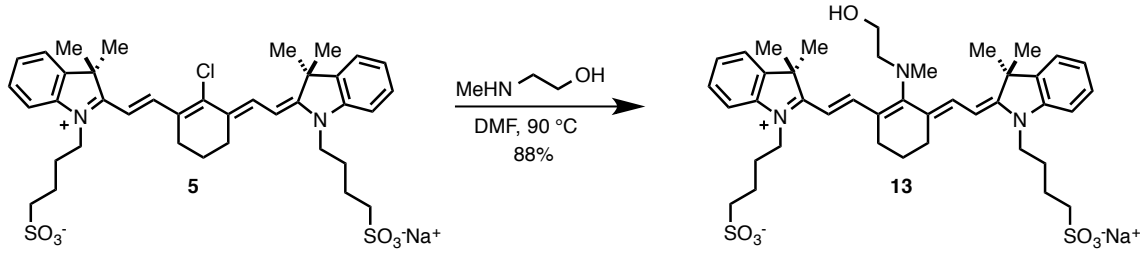

**(11):** To a solution of IR-783 **5** (50 mg, 0.060 mmol) in DMF (2 mL) was added *N,N'*-dimethylethylenediamine (16 μL, 0.15 mmol) and diisopropylethylamine (10 μL, 0.060 mmol). The green solution was heated to 90 °C in a sealed vial for 10 min as the reaction color transitioned from green to dark blue. LC/MS analysis showed the consumption of **5**. The reaction was cooled to room temperature and precipitated into $Et_2O$ (40 mL). The slurry was centrifuged, the supernatant discarded, and the blue pellet was resuspended in $Et_2O$ (20 mL). The procedure was repeated, and the blue pellet was dissolved in DMF (10 mL). In a separate flask 4-hydroxycyclofen[3] (53 mg, 0.15 mmol) was combined with diisopropylethylamine (52 μL, 0.30 mmol) and THF (3 mL). 4-Nitrophenylchloroformate (40 mg, 0.20 mmol) in THF (2 mL) was added via slow dropwise addition to the 4-hydroxycyclofen/THF solution at room temperature. The clear, light yellow solution was stirred for 10 min, after which time the reaction was quenched with aqueous $NaHCO_3$ and pyridine (20 μL). The reaction was diluted with DCM (30 mL) and stirred vigorously for 1 h. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated until ~3 mL of volume remained. This crude solution of cyclofen/4-nitrophenyl mixed carbonate was used immediately in the next step. Diisopropylethylamine (21 μL, 0.12 mmol) was added to the Cy7-diamine/DMF solution prepared above, followed by a solution of the mixed carbonate in THF (~3 mL). The reaction was stirred for 1 h at room temperature. The solution was precipitated into $Et_2O$ (100 mL), centrifuged, and the crude pellet was purified by reversed-phase chromatography (10→60% (MeCN/0.1% v/v $NH_4OH$/water). The solvent was concentrated *in vacuo* to afford **11** (35 mg, 50%) as a dark blue solid. $^1H$ NMR ($CD_3OD$, 400 MHz, 65 °C) $\delta$ 7.76 – 7.48 (m, 2H), 7.37 – 7.24 (m, 4H), 7.17 – 7.03 (m, 4H), 7.00

[3] Sinha, D. K.; Neveu, P.; Gagey, N.; Aujard, I.; Benbrahim-Bouzidi, C.; Le Saux, T.; Rampon, C.; Gauron, C.; Goetz, B.; Dubruille, S.; Baaden, M.; Volovitch, M.; Bensimon, D.; Vriz, S.; Jullien, L. *ChemBioChem* **2010**, *11*, 653.

– 6.77 (m, 8H), 5.99 – 5.77 (m, 2H), 4.27 (t, *J* = 5.1 Hz, 2H), 4.15 – 3.99 (m, 2H), 4.00 – 3.89 (m, 4H), 3.79 – 3.63 (m, 2H), 3.63 – 3.51 (m, 3H), 3.47 (t, *J* = 5.1 Hz, 2H), 3.01 (s, 3H), 2.91 (s, 6H), 2.88 – 2.78 (m, 4H), 2.57 – 2.41 (m, 4H), 2.18 – 2.09 (m, 2H), 2.10 – 2.01 (m, 2H), 1.98 – 1.76 (m, 10H), 1.62 – 1.45 (m, 18H); IR (thin film) 2927, 1715, 1509, 1454, 1375 $cm^{-1}$; HRMS (ESI) calculated for $C_{66}H_{86}N_5O_9S_2$ ($MH^+$) 1156.5861, observed 1156.5879.



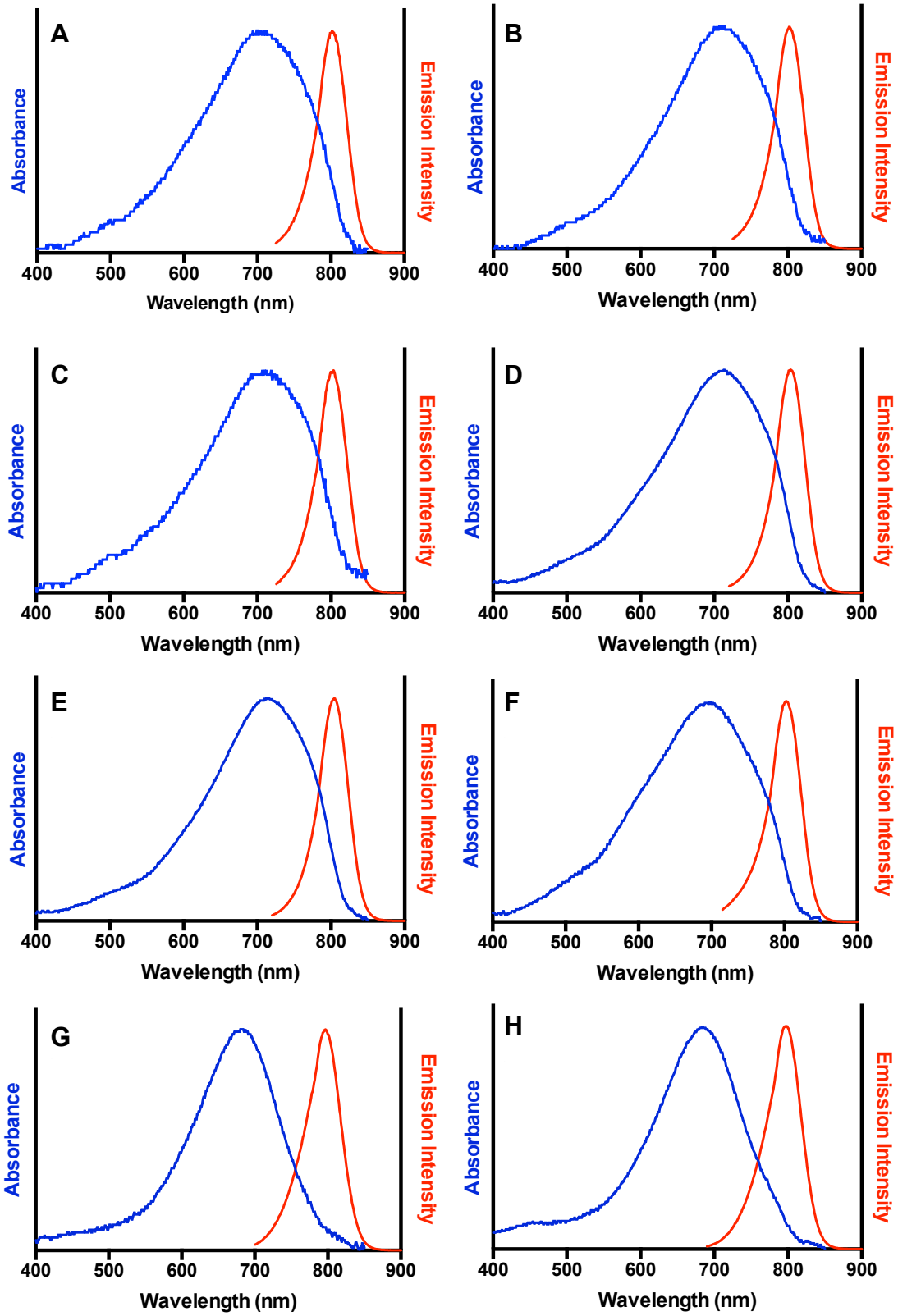

**(12):** To a solution of IR-780 iodide **4** (100 mg, 0.150 mmol) in MeCN (5 mL) was added *N*-methylethanolamine **14** (60 µL, 0.75 mmol). The solution was heated to 70 °C in a sealed vial for 20 min as the reaction color transitioned from green to dark blue. After this time LC/MS analysis showed complete consumption of **4**. The solution was concentrated *in vacuo*, and the residue was purified by silica gel chromatography (100% EtOAc, then 0→10% MeOH/DCM) to afford **12** (85 mg, 80%) as a dark blue solid. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 7.69 (d, *J* = 13.0 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 4H), 7.09 (d, *J* = 7.5 Hz, 2H), 6.89 (d, *J* = 7.9 Hz, 2H), 5.69 (d, *J* = 13.0 Hz, 2H), 4.12 – 3.94 (m, 4H), 3.81 (t, *J* = 7.4 Hz, 4H), 3.55 (s, 3H), 2.47 (t, *J* = 6.6 Hz, 4H), 1.90 – 1.76 (m, 6H), 1.67 (s, 12H), 1.04 (t, *J* = 7.4 Hz, 6H); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 177.1, 168.7, 143.1, 142.3, 140.6, 128.2, 123.7, 123.2, 122.4, 108.9, 95.1, 60.1, 59.5, 48.2, 45.4, 45.1, 29.5, 25.0, 22.0, 20.4, 11.9; IR (thin film) 1544, 1509, 1444, 1345 $cm^{-1}$; HRMS (ESI) calculated for $C_{39}H_{52}N_3O$ ($M^+$) 578.4110, observed 578.4096.




**(13):** To a solution of IR-783 **5** (500 mg, 0.67 mmol) in DMF (7 mL) was added *N*-methylethanolamine **14** (267 µL, 3.34 mmol). The solution was heated to 90 °C in a sealed vial for 15 min as the reaction color transitioned from green to dark blue. After this time LC/MS analysis showed complete consumption of **5**. The reaction was cooled to room temperature and precipitated into $Et_2O$ (100 mL). 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 purified by reversed-phase chromatography (0→50% (MeCN/0.1% v/v $NH_4OH$/water). The solvent was concentrated *in vacuo* to afford **13** (462 mg, 88%) as a dark blue solid. $^1H$ NMR ($CD_3OD$, 400 MHz) $\delta$ 7.76 (d, $J$ = 13.3 Hz, 2H), 7.37 (d, $J$ = 7.5 Hz, 2H), 7.31 (t, $J$ = 8.0 Hz, 2H), 7.15 (d, $J$ = 8.0 Hz, 2H), 7.10 (t, $J$ = 7.5 Hz, 2H), 5.95 (d, $J$ = 13.3 Hz, 2H), 4.08 – 3.96 (m, 4H), 3.93 – 3.82 (m, 4H), 3.47 (s, 3H), 2.87 (t, $J$ = 6.8 Hz, 4H), 2.53 (t, $J$ = 6.5 Hz, 4H), 2.00 – 1.89 (m, 8H), 1.84 (p, $J$ = 6.6 Hz, 2H), 1.65 (s, 12H); $^{13}C$ NMR ($CD_3OD$, 100 MHz) $\delta$ 177.6, 170.3, 144.3, 144.1, 141.8, 129.5, 125.4, 124.3, 123.1, 110.7, 96.9, 61.2, 60.5, 52.0, 45.1, 44.0, 29.5, 28.6, 26.9, 25.6, 23.7, 23.2; IR (thin film) 2926, 1511, 1453, 1346 $cm^{-1}$; HRMS (ESI) calculated for $C_{41}H_{56}N_3O_7S_2$ ($MH^+$) 766.3554, observed 766.3561.

**Absorbance and Emission Spectra of 6-13**



Normalized absorbance and emission curves of 1 μM **6** (A), **7** (B), **8** (C), **9** (D), **10** (E), **11** (F), **12** (G), and **13** (H) in DMSO. Absorbance and emission profiles of **6-13** are similar to those reported for structurally similar amino-Cy7s.[4,5]

**Determination of Quantum Yields and Molar Absorption Coefficients**

Quantum yields ($\Phi_f$) were determined in DMSO or PBS relative to ICG ($\Phi_f$ = 0.13 in DMSO[6], 0.012 in PBS[7]), from plots of integrated fluorescence intensity vs. absorbance, 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, $\Phi$ is the fluorescence quantum yield, *Grad* is the gradient of the integrated fluorescence intensity vs. absorbance plot, and $\eta$ is the refractive index of the solvent.[8,9,10,11] Measurements were performed in 10 mm path length quartz cuvettes (Hellma 111-QS), maintained at 25 °C, with the absorbance of all dye solutions ≤ 0.08 in order to maximize illumination homogeneity and optical transparency and minimize reabsorption effects.[10] ICG standard and test compound solutions were excited at their absorbance maxima.

Molar absorption coefficients (ε) were determined in DMSO or PBS using Beer's law, from plots of absorbance vs. concentration. Measurements were performed in 10 mm path length quartz cuvettes (Hellma 111-QS), maintained at 25 °C, with absorbance at the highest concentration ≤ 0.08 (see above) (Table S1).

Measured $\Phi_f$ and ε for **6-11** are similar to those reported for structurally-similar amino-Cy7s.[4,5]


4. Peng, X.; Song, F.; Lu, E.; Wang, Y.; Zhou, W.; Fan, J.; Gao, Y. *J. Am. Chem. Soc.* **2005**, *127*, 4170.
5. Cao, X.; Lin, W.; He, L. *Org. Lett.* **2011**, *13*, 4716.
6. Benson, R. C.; Kues, H. A. *J. Chem. Eng. Data* **1977**, *22*, 379.
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8. Parker, C. A.; Rees, W. T. *Analyst* **1960**, *85*, 587.
9. Williams, A. T. R.; Winfield, S. A. *Analyst.* **1983**, *108*, 1067.
10. Rurack, K.; Spieles, M. *Anal. Chem.* **2011**, *83*, 1232.
11. Samanta, A.; Vendrell, M.; Das, R.; Chang, Y.-T. *Chem. Commun.* **2010**, *46*, 7406.

**Table S1. Optical Properties of 6-11**

| Compound | $\lambda_{abs}$ (nm) | $\lambda_{em}$ (nm) | $\Phi_f$ | $\varepsilon$ ($M^{-1}$ $cm^{-1}$) |
|---|---|---|---|---|
| **6**[a] | 704 | 804 | 0.38 | 5.44 x $10^4$ |
| **7**[a] | 705 | 802 | 0.42 | 6.38 x $10^4$ |
| **8**[a] | 705 | 802 | 0.38 | 4.49 x $10^4$ |
| **9**[b] | 674 | 789 | 0.32 | 5.24 x $10^4$ |
| **10**[b] | 676 | 791 | 0.29 | 5.15 x $10^4$ |
| **11**[b] | 668 | 774 | 0.30 | 4.00 x $10^4$ |

[a]Measured in DMSO relative to ICG ($\Phi_f$ = 0.13); [b]Measured in 50 mM PBS (pH 7.5) with 1% (v/v) DMSO relative to ICG ($\Phi_f$ = 0.012).

**General Plate Photolysis Procedure**

Samples in 96-well plates (Corning black opaque polypropylene for fluorescence, Corning UV-transparent acrylic copolymer for absorbance) were irradiated using a 690 nm LED (L690-66-60, Marubeni America Co.) at the stated intensity (mW/cm$^2$) as measured using a power meter. Sample wells did not exceed 25 °C over the irradiation time course. Test samples were diluted from a 10 mM stock in DMSO to a final concentration of 50 μM for absorbance studies and 50 nM for fluorescence studies in the relevant test media. 4-Nitrophenol and 4-methylumbelliferone were included as positive controls in each absorbance and fluorescence assay, respectively, with media serving as the negative control. Plates receiving no irradiation and minimal exposure to ambient light were always run in parallel. All experiments were run at least in triplicate, with standard deviation ≤ 5% in all cases. Half-lives were calculated by exponential fitting.

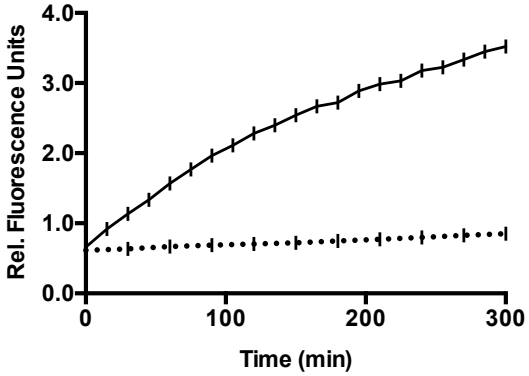



**Figure S1.** Photolysis of 50 nM **10** in 50 mM HEPES (pH 7.5) with 1% (v/v) DMSO with 1 mW/cm$^2$ 690 nm LED light. Solid lines indicate the sample was irradiated whereas broken lines indicate the sample was not irradiated.

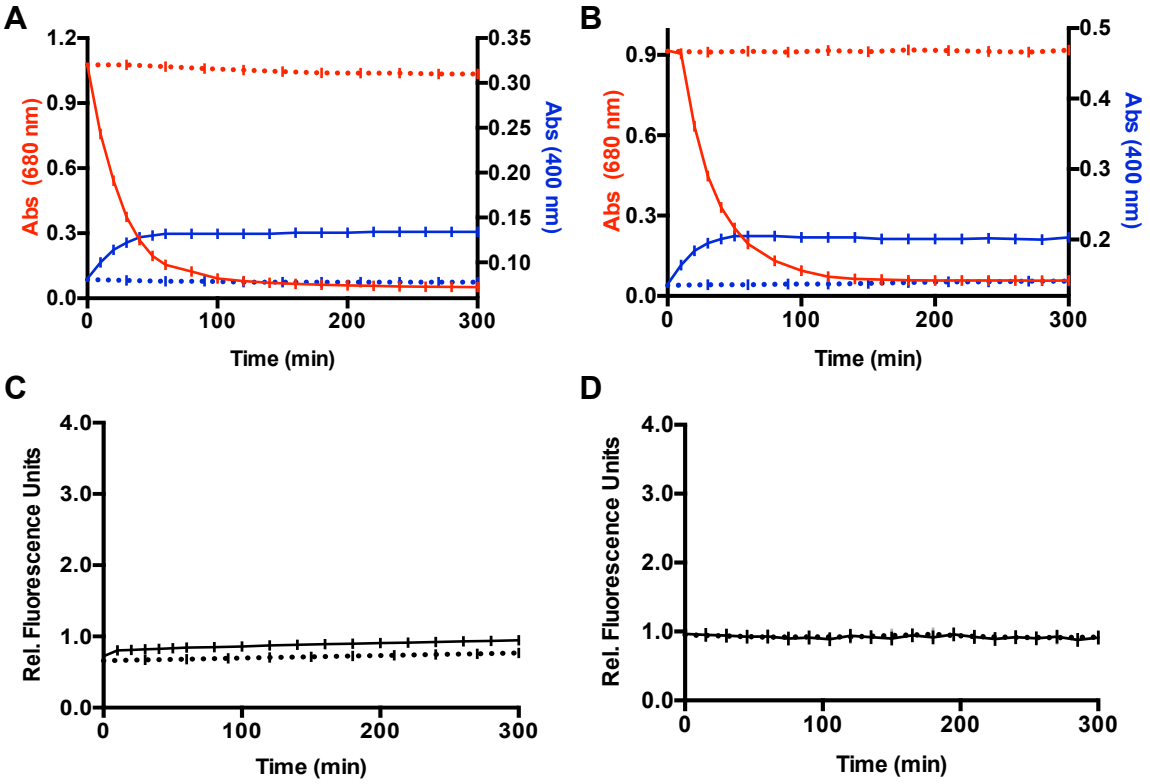



**Figure S2.** Photolysis of **6** and **9**. (A) 50 µM **6** in 50 mM HEPES (pH 7.5) with 5% (v/v) DMSO; (B) 50 µM **6** in DMEM buffered with 50 mM HEPES (pH 7.5) with 10% (v/v) FBS and 0.1% (v/v) DMSO; (C) 50 nM **6** in 50 mM HEPES (pH 7.5) with 5% (v/v) DMSO; (D) 50 nM **9** in 50 mM HEPES (pH 7.5) with 1% (v/v) DMSO. Solid lines indicate the sample was irradiated whereas broken lines indicate the sample was not irradiated. Samples in HEPES buffer were irradiated with 1 mW/cm$^2$ and samples in DMEM with 3 mW/cm$^2$ from a 690 nm LED light. The modest increase in 400 nm absorbance observed for **6** is attributed to other photolysis products.

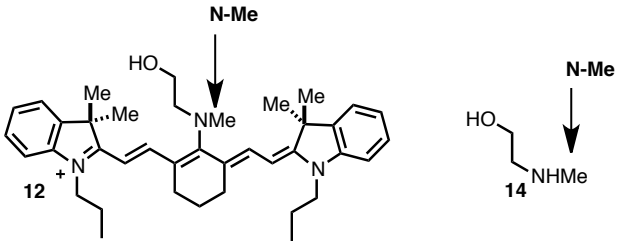
**NMR Photolysis**

A solution containing **12** (0.35 mg, 0.0005 mmol) and dimethyl sulfone (0.05 mg, 0.0005 mmol) in $D_2O$ (250 μL) and $d_4$-methanol (250 μL) was prepared in an NMR tube. An NMR spectrum (32 scans, 30 sec. relaxation) was obtained and the sample was then irradiated for 24 h with 5 mW/cm$^2$ 690 nm light, after which time a second NMR spectrum was obtained. A second sample was maintained in the dark for 14 d, over which time little change was observed by NMR. For this experiment, a 500 MHz Varian system equipped with cryogenic probe was used. The experiment was run in triplicate and representative spectral data is shown on page 15. The $CH_2Cl_2$ (5.47) signal served as a second internal standard.

The identity of *N*-methylethanolamine (**14**) and 3,3-dimethyl-1-propylindolin-2-one (**15**)[12] were determined by doping experiments involving additions of dilute solutions of these compounds in $d_4$-methanol to the NMR tube. The (*E*)-2-(3,3-dimethyl-1-propylindolin-2-ylidene)acetaldehyde (**16**) was verified in a similar fashion but by comparing the signals with the with the commercially available (*E*)-2-(1,3,3-trimethylindolin-2-ylidene)acetaldehyde[13] which exhibits nearly identical signals for the aryl, vinyl, and aldehydic peaks.

The conversion was measured by comparing the integrations of the well-resolved *N*-methyl shift in **12** and **14** as shown below. Precise quantification of the other products was not possible due to overlapping signals.

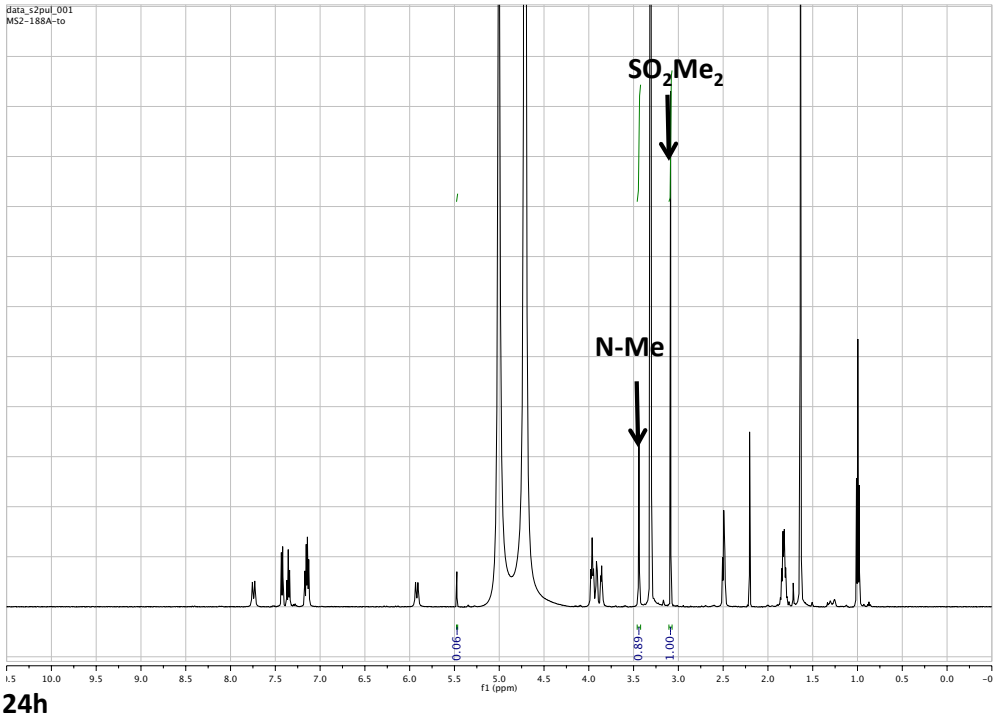
***Key Signals***



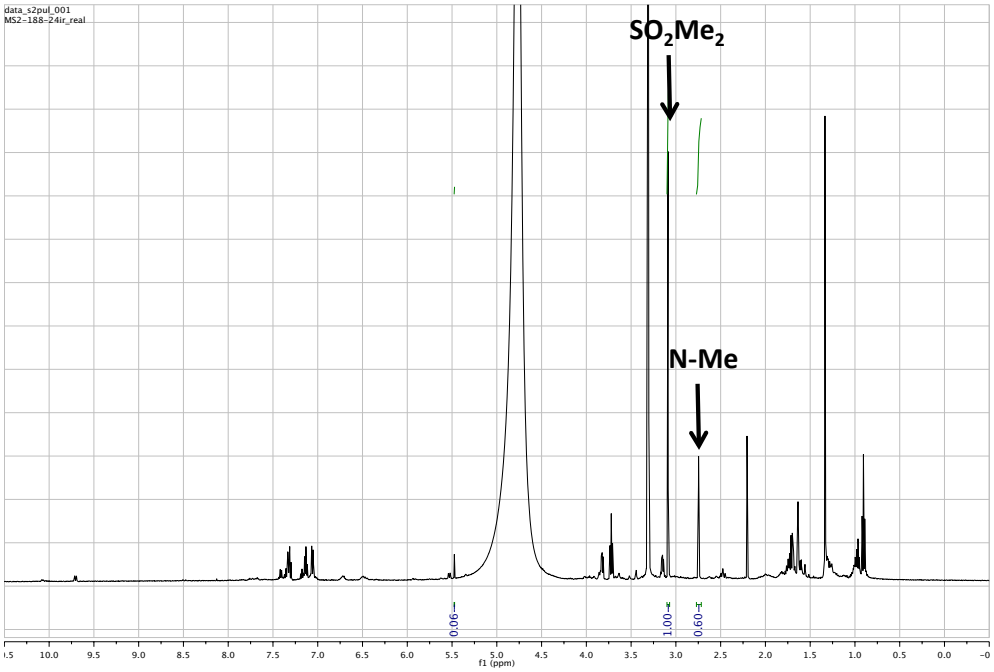

12. Prepared by alkylation of 3,3-dimethylindolin-2-one with *n*-propyl iodide and NaH in DMF, see: Robertson, D. W.; Krushinski, J. H.; Beedle, E. E.; Wyss, V.; Pollock, G. D.; Wilson, H.; Kauffman, R. F.; Hayes, J. S. *J. Med. Chem.* **1986**, *29*, 1832.
13. Reported to exist in the (*E*) configuration. See: Zemlyanoi, V. N.; Mushkalo, I. L.; Kornilov, M. Yu.; Boldeskul, I. E.; Dekhtyar, M. L. *J. Heterocyclic Chem.* **1983**, *19*, 293.

*NMR Data*

**t = 0h**




**t = 24h**



**Procedure for Time-Dependent LC/HRMS Analysis of 10**

A 20 mM stock of **10** in DMSO was diluted into water to yield a 20 μM solution into a HPLC vial. Analyses were conducted on a Thermo-Fisher LTQ-Orbitrap-XL hybrid mass spectrometer system operated at a resolution of 30,0000 (FWHM) in positive ion mode. Separations were carried out on a narrow-bore (50 X 2.1 mm) Phenomenex Kinetex reversed-phase XB-C18 (2.6 $\mu$m) core-shell column coupled with a Security Guard Ultra UPHLC $C_{18}$ guard column (5 X 2.1 mm). The columns were eluted at a flow rate of 250 $\mu$l/min with a linear, 10-min, 2 - 90% gradient of $CH_3CN/H_2O$ containing 0.1% HCOOH followed by a 10-min hold at 90% $CH_3CN$ before gradient reset and equilibration. The column was connected directly (by-passing the diode array UV detector) to the Ion MAX API electrospray ion source of the mass spectrometer to minimize further photolytically-induced decomposition during analysis. After a time zero LC/HRMS run, the sample was irradiated for 30 minutes at 22°C with 10 $mW/cm^2$ 690 nm (± 20 nm) light source. The irradiation was halted, and after a time 30 minute LC/HRMS run the sample was heated to 37°C and then analyzed at 60 and 90 minutes.

**Calculated and Observed HRMS Masses for Photolysis Products**

**Peak 1 (25):** HRMS (ESI) calculated for $C_{15}H_{19}N_2O_4$ $(M+H)^+$ 291.1339, observed 291.1347.

**Peak 2 (24):** HRMS (ESI) calculated for $C_{22}H_{28}NO_5S$ $(M+H)^+$ 418.1683, observed 418.1689.

**Peak 3 (26):** HRMS (ESI) calculated for $C_{10}H_9O_3$ $(M+H)^+$ 177.0546, observed 177.0550.

**Peak 4 (20):** HRMS (ESI) calculated for $C_{16}H_{22}NO_4S$ $(M+H)^+$ 324.1264, observed 324.1267.

**Peak 5 (22):** HRMS (ESI) calculated for $C_{14}H_{20}NO_4S$ $(M+H)^+$ 298.1108, observed 298.1111.

**Peak 6 (19):** HRMS (ESI) calculated for $C_{37}H_{44}N_3O_8S$ $(M+H)^+$ 690.2844, observed 690.2859.

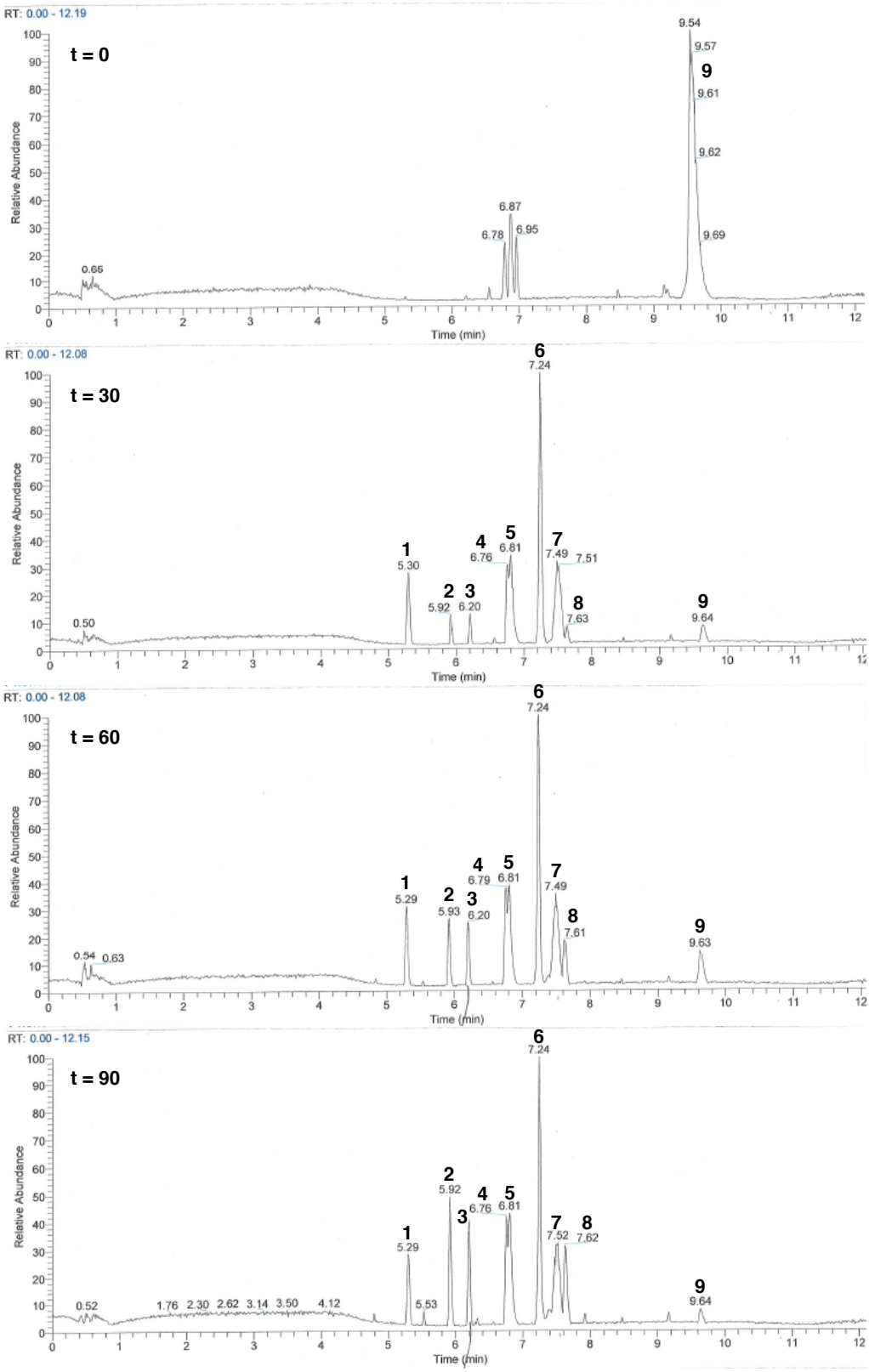
**Peak 6 (21):** HRMS (ESI) calculated for $C_{39}H_{46}N_3O_8S$ $(M+H)^+$ 716.3000, observed 716.3011.

**Peak 7 (17,18):** HRMS (ESI) calculated for $C_{53}H_{65}N_4O_{12}S_2$ $(M+H)^+$ 1013.4035, observed 1013.4057.

**Peak 8 (23):** HRMS (ESI) calculated for $C_{24}H_{30}NO_5S$ $(M+H)^+$ 444.1839, observed 444.1848.

**Peak 9 (10):** HRMS (ESI) calculated for $C_{53}H_{65}N_4O_{10}S_2$ $(M+H)^+$ 981.4137, observed 981.4144.

**LC/HRMS Base Peak TIC of Photolysis of 10 at t = 0, 30, 60, and 90 minutes**



**Procedure for Relative Ion Analysis of Photolysis of 10**

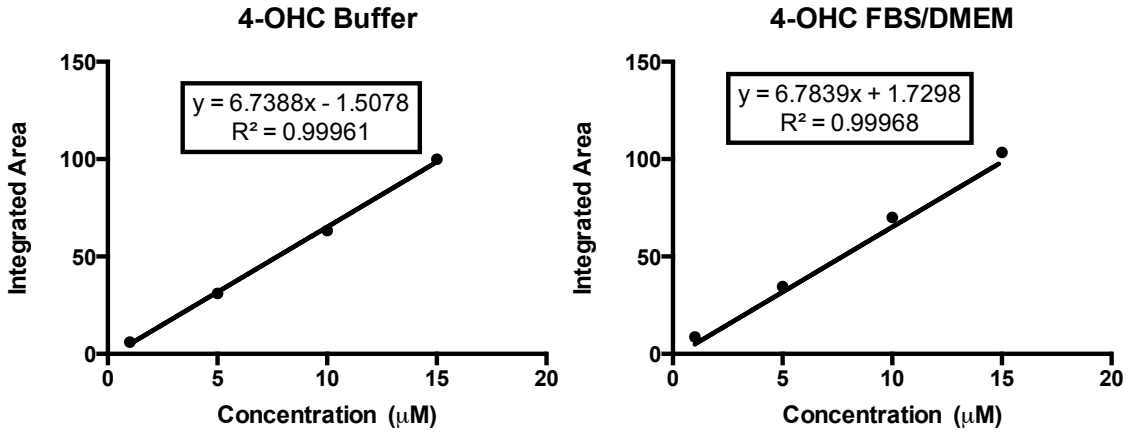
A 20 mM stock of **10** in DMSO was diluted into water to yield a 10 μM solution. Phenylalanine (40 μM) was used as an internal standard. The photolysis was run for 30 min at rt with 10 mW/cm$^2$ 690 nm (± 20 nm) light in a 12-well plate. After 30 min, the reaction was transferred to a vial and heated to 37 °C. Aliquots were taken at 0 (prior to irradiation), 30, 60 and 90 min and immediately analyzed by a direct loop injection method with a Shimadzu LCMS-2020 Single Quadrupole instrument (normal resolution). The relative ion counts in Figure 3 were calculated by integrating the extracted ion chromatogram (EIC) of the *m/z* of **10**, **17/18**, **19**, **21**, and **26** and dividing by the ion count of the phenylalanine internal standard.[14]

**Procedure for Photolysis of 11**

The HPLC yields of 4-hydroxycyclofen (4-OHC) during the photolysis experiment were determined by an external calibration method.[15] A calibration curve was constructed with varying concentrations of 4-OHC plotted against the integrated area of the drug peak (see below). The solutions for calibration were generated from a 1.0 mM stock solution of 4-OHC via dilution in 9:1 (v/v) DMEM/FBS to afford 1, 5, 10, and 15 μM solutions. From each of these standards, 250 μL was taken and diluted into 250 μL of acetonitrile (to precipitate serum proteins from FBS). The samples were briefly vortexed and then centrifuged for 1 min at 12,000 rpm. The supernatant was transferred to a vial and analyzed by HPLC. The calibration samples were analyzed on an Agilent 1260 Infinity HPLC utilizing a Kinetex 5 μm C18 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. This method of sample preparation and analysis was identical to what is used in the photolysis experiment (see below) to ensure consistency between the calibration curves and the experimental runs. A nearly identical calibration curve was obtained from 50 mM HEPES (pH 7.5) buffer demonstrating that the protein precipitation procedure did not alter the observed concentration values.

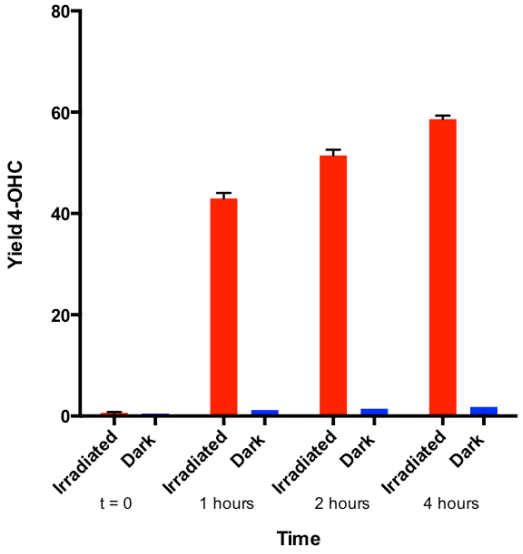
14. Bereman, M. S.; Young, D. D.; Deiters, A.; Muddiman, D. C. *J. Proteome Res.* **2009**, *8*, 3764.
15. Pigini, D.; Cialdella, A. M.; Faranda P.; Tranfo, G. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1013.

**HPLC Calibration Curves for 4-OHC**



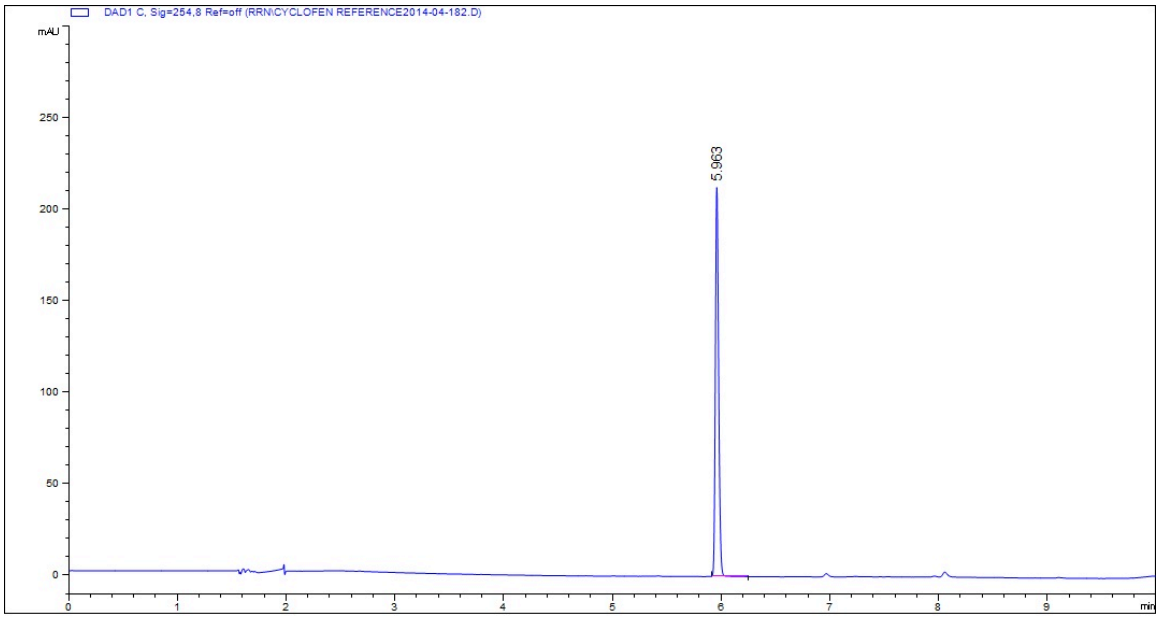

Stock solutions of **11** (2 mM in DMSO) were used to generate the reactions for photolysis. A 7.5 μL aliquot from the 2 mM DMSO stock was taken and diluted up to 1.5 mL with 9:1 (v/v) DMEM/FBS containing 0.5% (v/v) DMSO to afford a 10 μM solution. The photolysis was run for 30 min at 22 °C with 10 mW/cm$^2$ 690 nm (± 20 nm) light in a 12-well plate in experimental triplicate concurrently with a dark control. After 30 min, the reactions were transferred to vials and heated to 37 °C. After 1, 2, and 4 hours of heating, 250 μL of the reaction solution was taken and diluted into 250 μL of acetonitrile. The samples were then prepared as outlined above and immediately analyzed by HPLC.

**Figure S3: Yield of 4-OHC at 0, 1, 2, and 4 h**

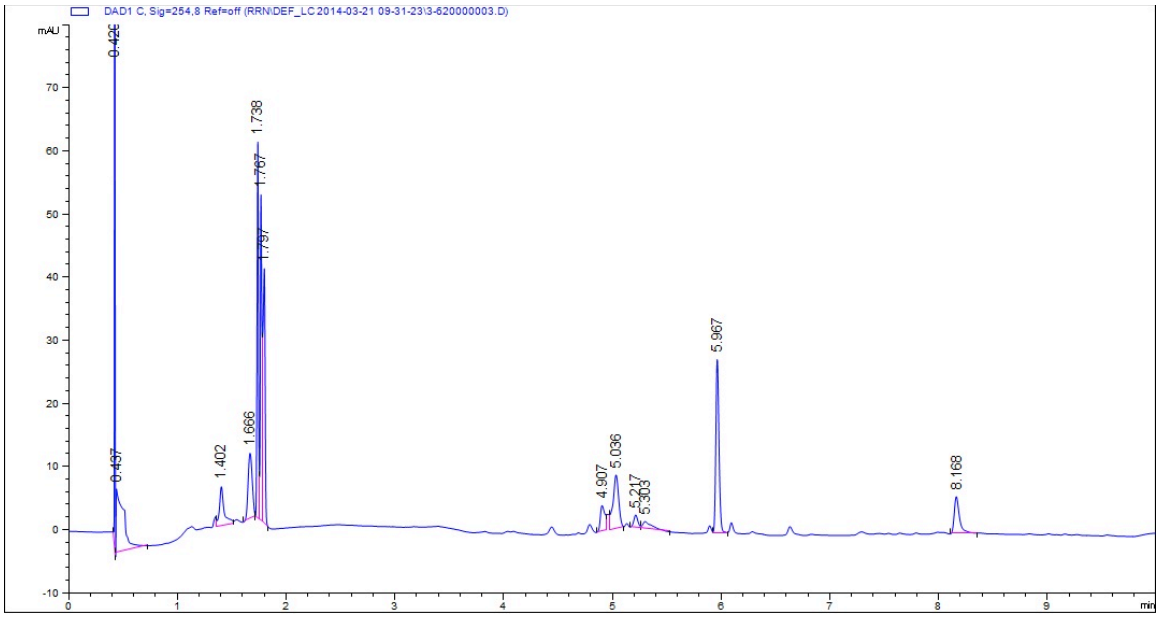


**Chromatogram of authentic 4-OHC at 254 nm (A). Chromatogram of photolysis experiment of 11 at t = 4 h at 254 nm (B).**

**A. Trace of 4-OHC**




**B. Trace of photolysis at t = 4 h**



**Cell Culture**

The estrogen receptor-positive MCF7 human breast cancer cell line and HeLa cell line was obtained from the NCI DTP, DCTD Tumor Repository. MCF7 and HeLa cells were cultured at 37 °C in an atmosphere of 20% $O_2$ and 5% $CO_2$ in DMEM supplemented with 4 mM L-glutamine, 25 mM D-glucose, 44 mM sodium bicarbonate, and 10% heat-inactivated fetal bovine serum. 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.

Mouse embryonic fibroblasts (MEFs) were obtained as described below and maintained at 37 °C in an atmosphere of 20% $O_2$ and 5% $CO_2$ in DMEM supplemented with 4 mM L-glutamine, 25 mM D-glucose, 44 mM sodium bicarbonate, 1% (v/v) penicillin-streptomycin, and 10% heat-inactivated fetal bovine serum. Stock cultures were maintained as above.

**Cell Photolysis and Cytotoxicity**

Cells were seeded into 96-well plates and allowed to adhere overnight. Initial seeding densities were kept sufficiently low to ensure cells were in exponential growth for the duration of the assay. Media was replaced with that containing caged **11**, caged **13**, or 4-hydroxycyclofen. Media alone (no inhibitor) was included in all assays as a negative control. Plates were exposed to 10 mW/cm$^2$ from a 690 nm LED for 30 min or kept dark. Following a 72 h incubation period, 20 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) from a 5 mg/mL stock in PBS was added to each well and incubated for 4 h at 37 °C. Media was removed, 100 μL of DMSO added to each well to solubilize MTT formazan, and absorbance at 550 nm was read using a microplate reader. Drug effects were expressed as % cell viability relative to the no inhibitor control. Half-maximal inhibitory concentrations ($IC_{50}$) were obtained from sigmoidal curve fits of % viability vs. concentration data.

**MEF Line Isolation**

All animal studies were approved by the NCI ACUC. Primary MEFs were isolated from E13.5 Rosa$^{CreER/mTmG}$ embryos generated by crosses between homozygous *Rosa26CreER$^T$* and Rosa-*mT/mG* mice. Time-mated females were euthanized and embryos collected in sterile PBS. The head and viscera were removed and body was minced in 1 mL of 0.25% trypsin without EDTA and incubated at 37 °C for 5 min in a 15 mL conical tube. Tissue was triturated by pipetting vigorously and the trypsin reaction was stopped by addition of 5 mL DMEM with 15%

FBS. Cells were plated 1 embryo per 25 $cm^2$ flask and then passaged the next day and subsequently as described above.

**MEF Photoactivation**

Cells were seeded into 6-well plates and allowed to reach near confluency overnight. Media was replaced with DMEM containing **11**, **13**, or 4-hydroxycyclofen at the indicated concentration and incubated for 2 h. The cells were washed with warm PBS and covered with fresh media. Alternatively, cells were continuously exposed to the test compound (no media exchange). The dish was irradiated with 10 $mW/cm^2$ from a 690 nm LED for 30 min or kept dark. Cells were incubated for 48-72 h prior to flow cytometric analysis for EGFP expression (1 or 5 μM) or for confocal microscopy (1 μM).

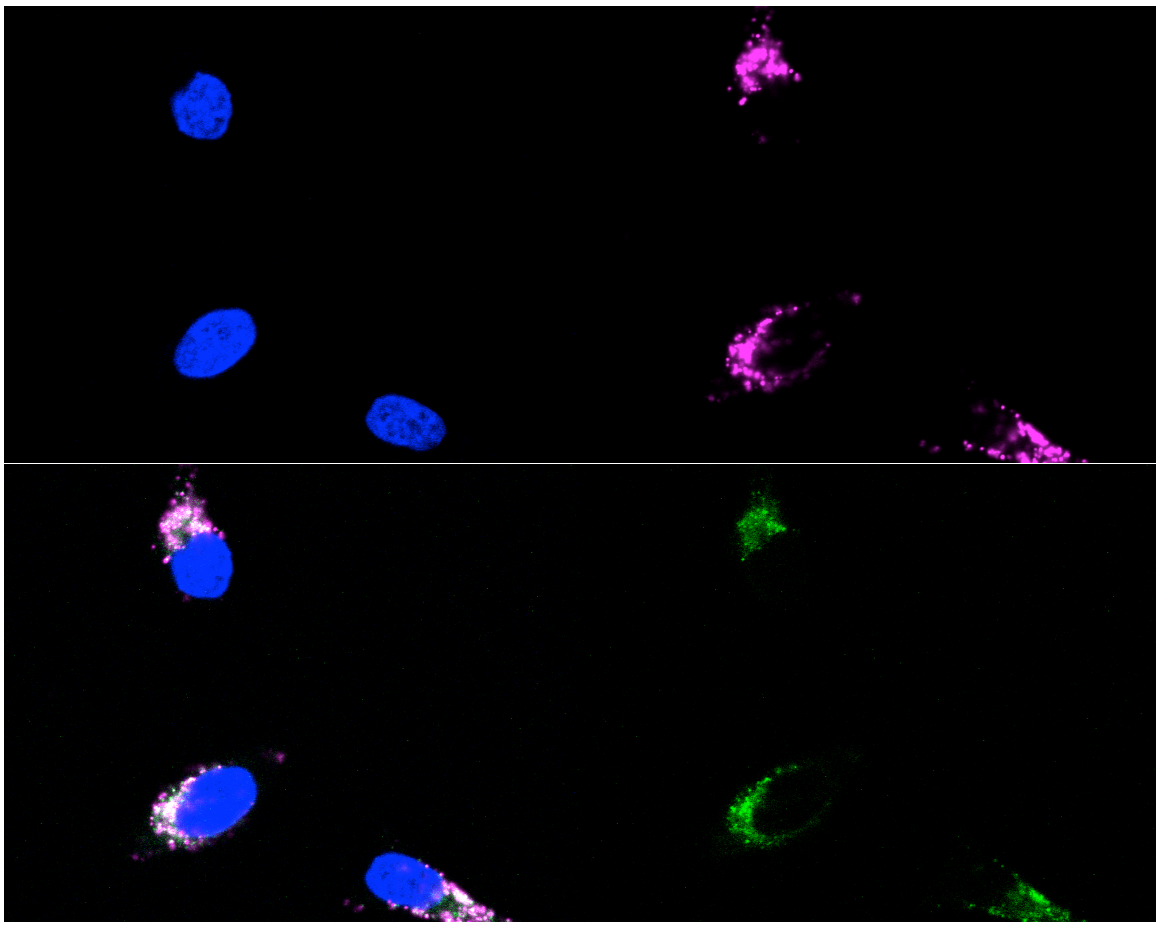
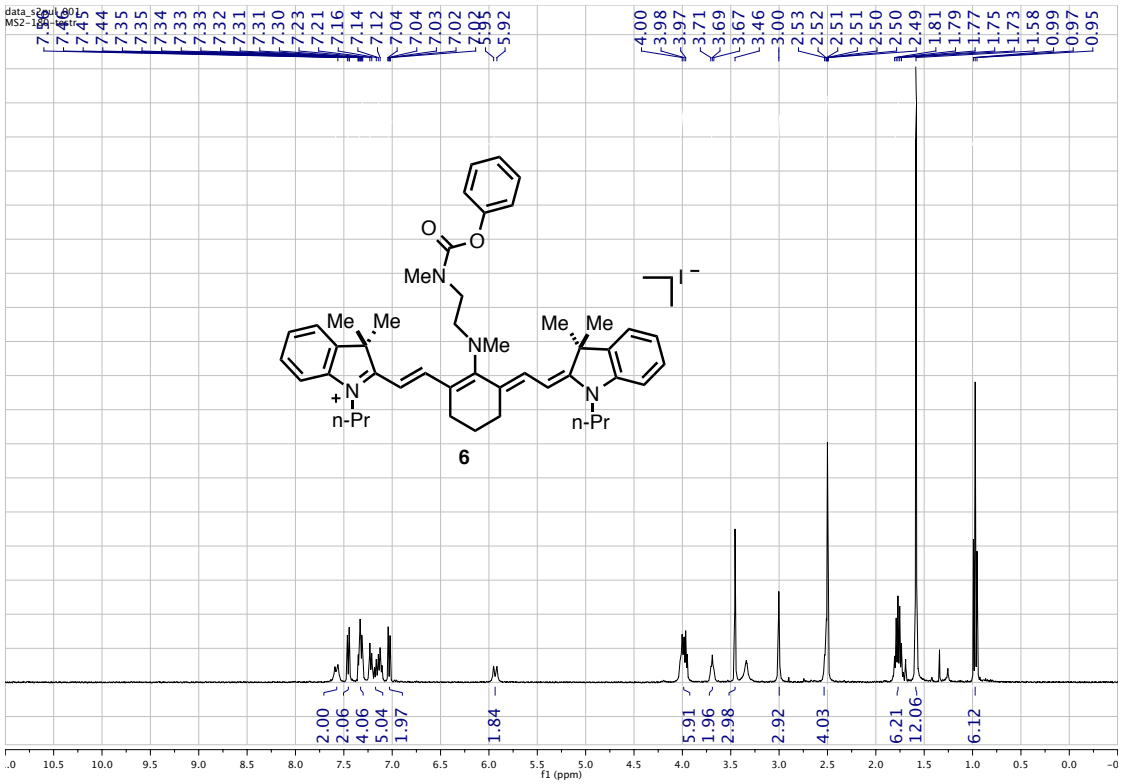
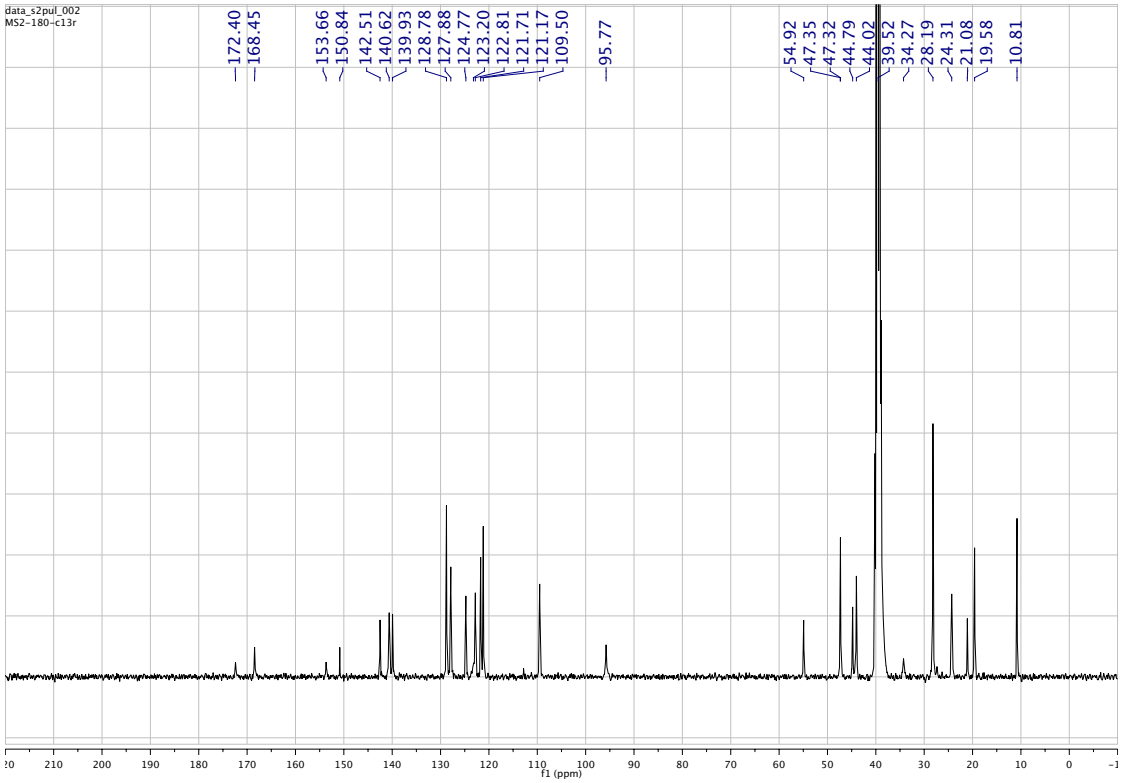
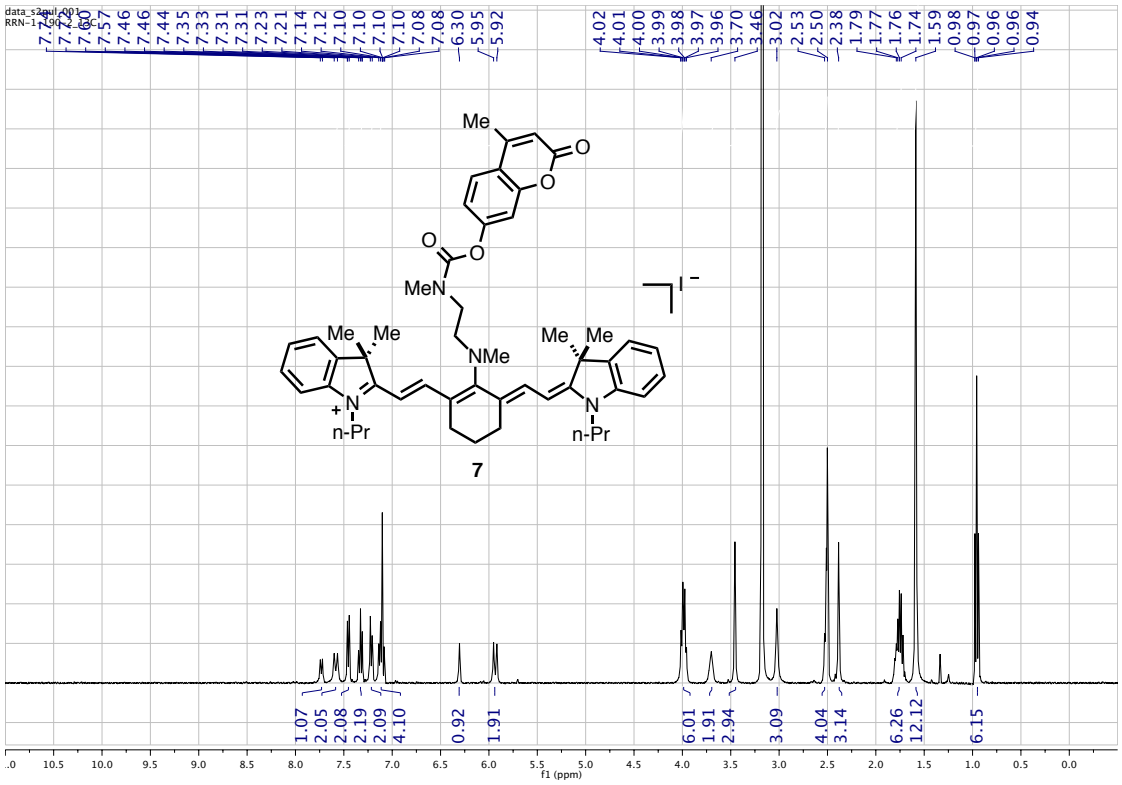


**Figure S4.** Confocal microscopy images of live HeLa cells treated with **11** (1 μM), LysoTracker® Red DND-99 (0.5 μM), and Hoechst 33342 (1 μM). Clockwise from top left, emission from Hoescht (nuclear staining), emission from **11**, emission from LysoTracker (lyosomal staining), and overlay are shown.

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

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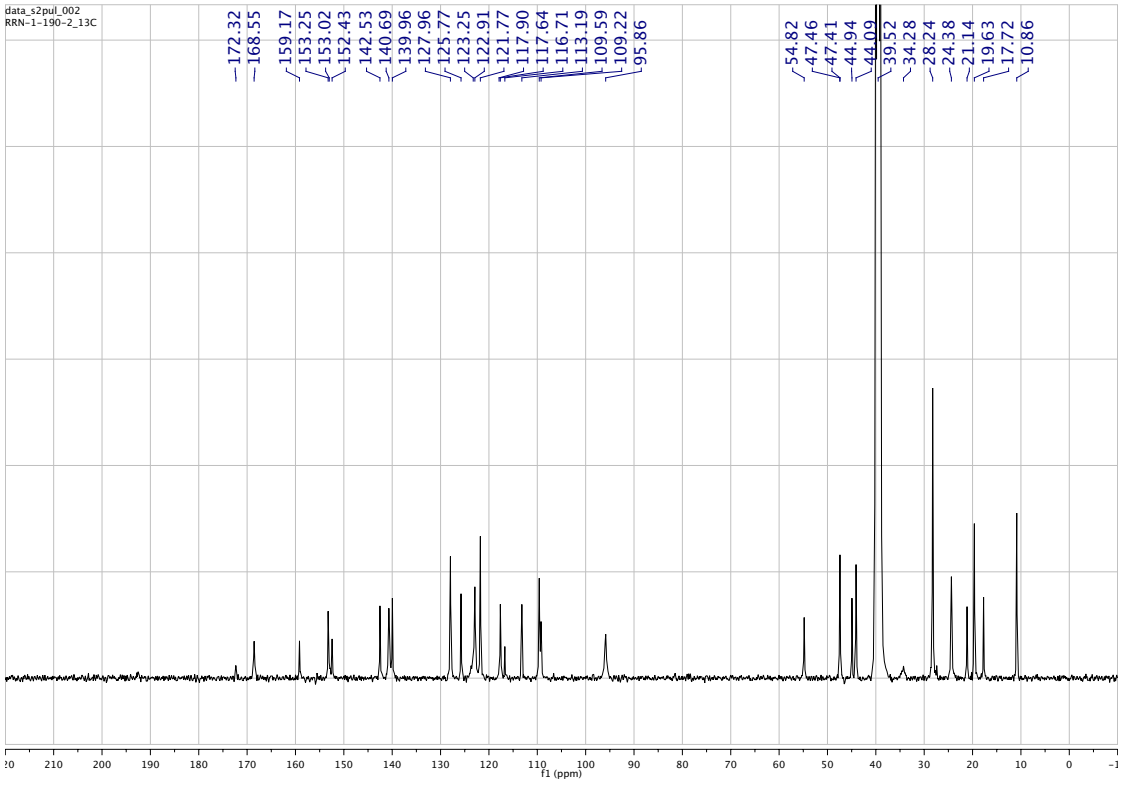
N
+
n-Pr
N
n-Pr
7
f1 (ppm)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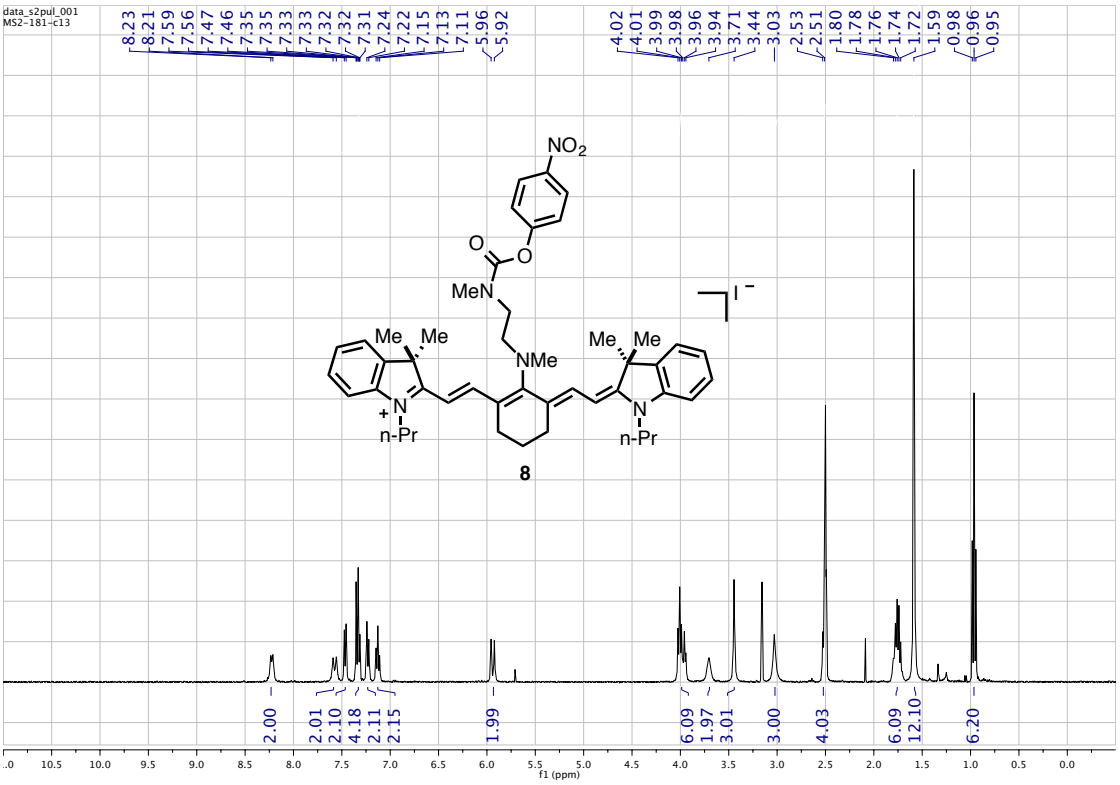
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N
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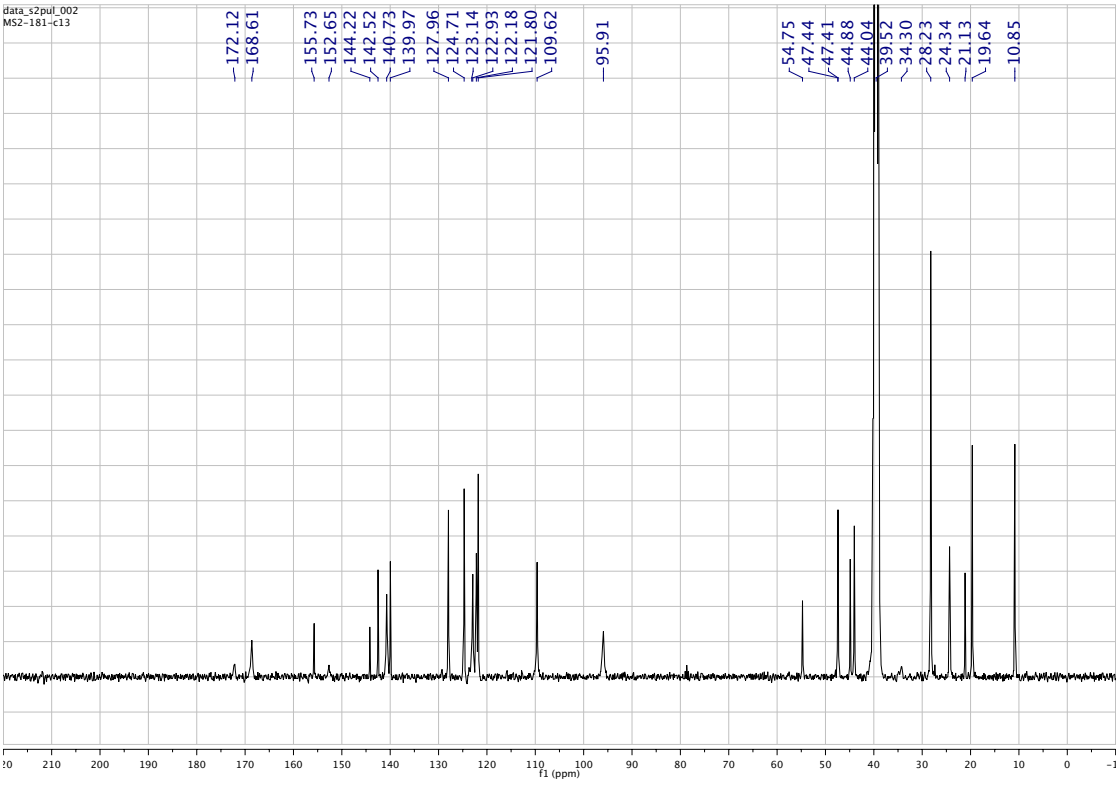
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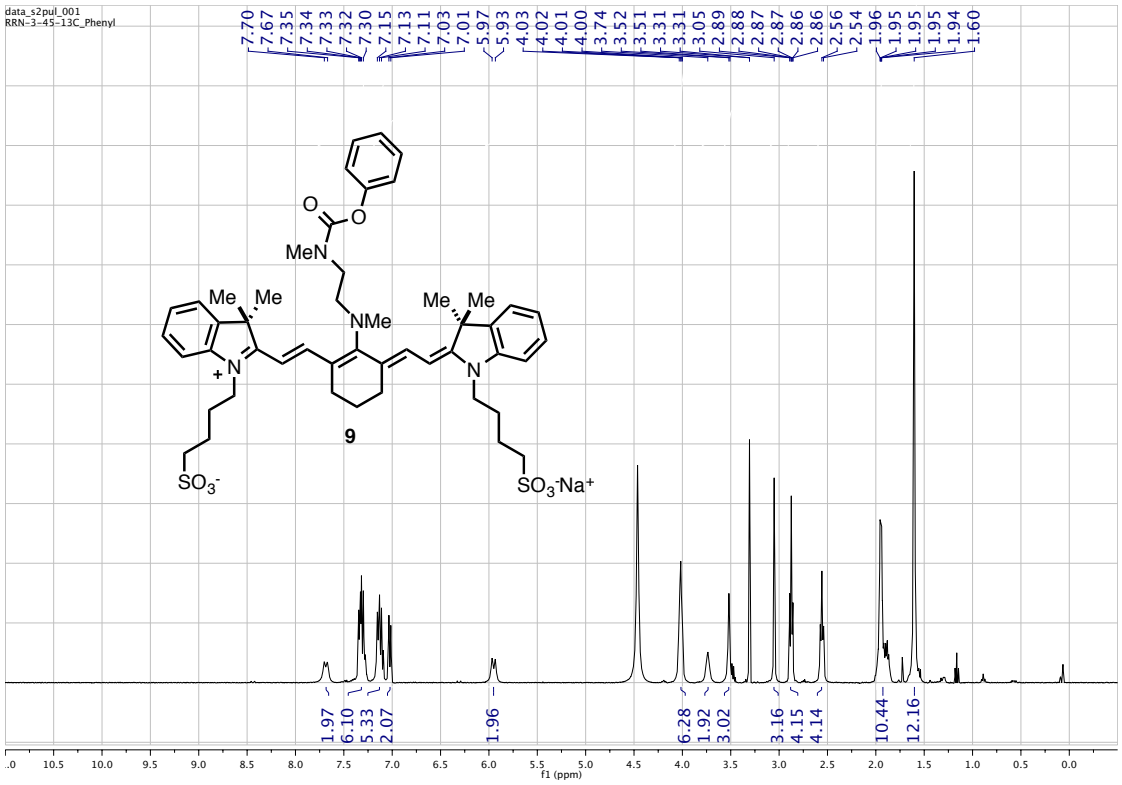
10.85
f1 (ppm)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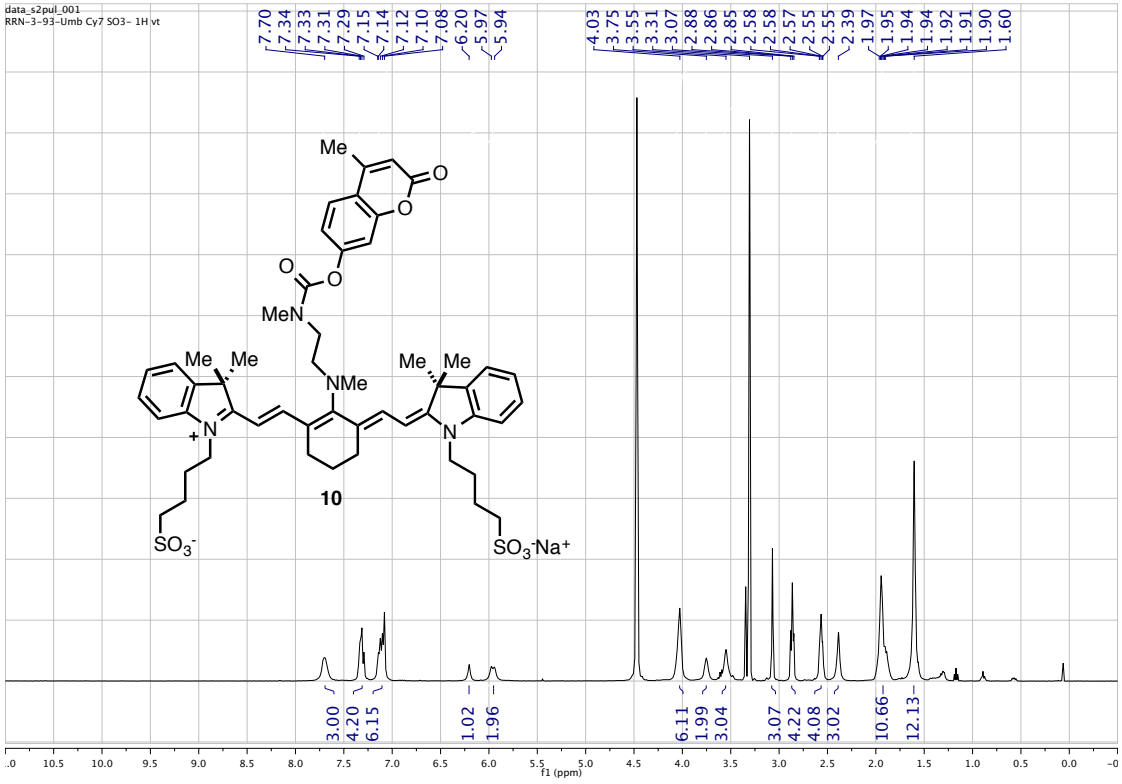
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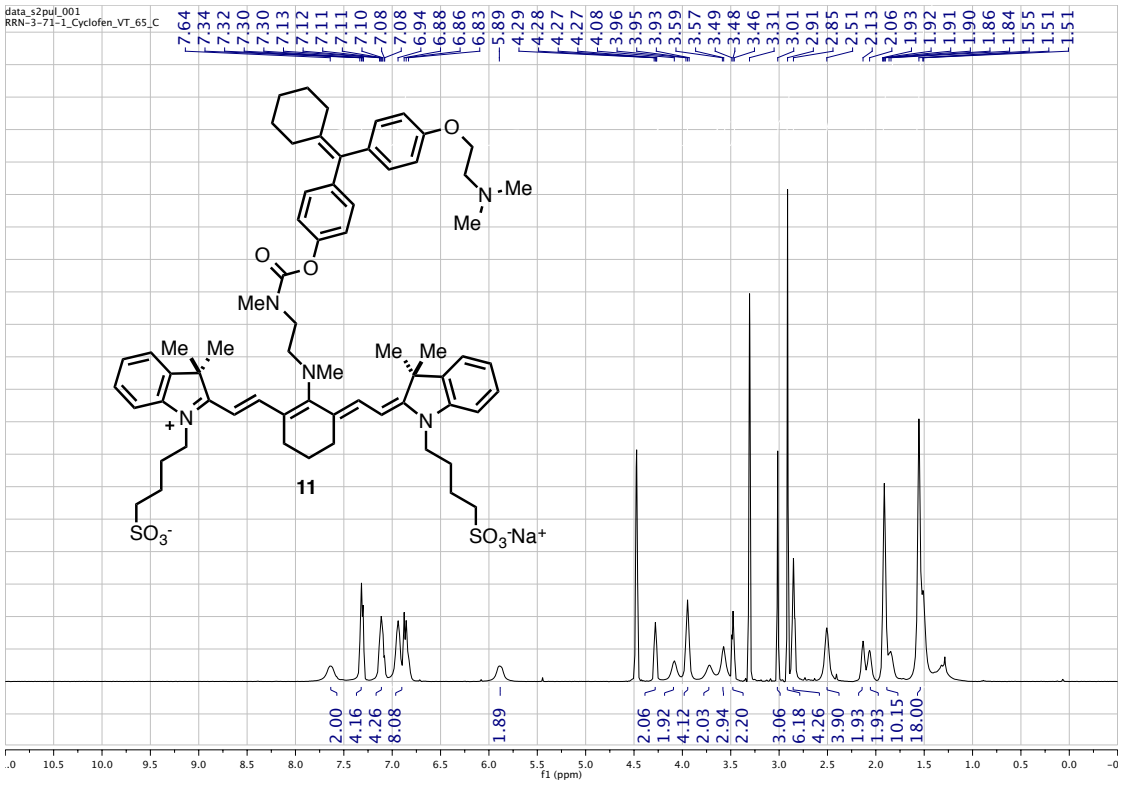
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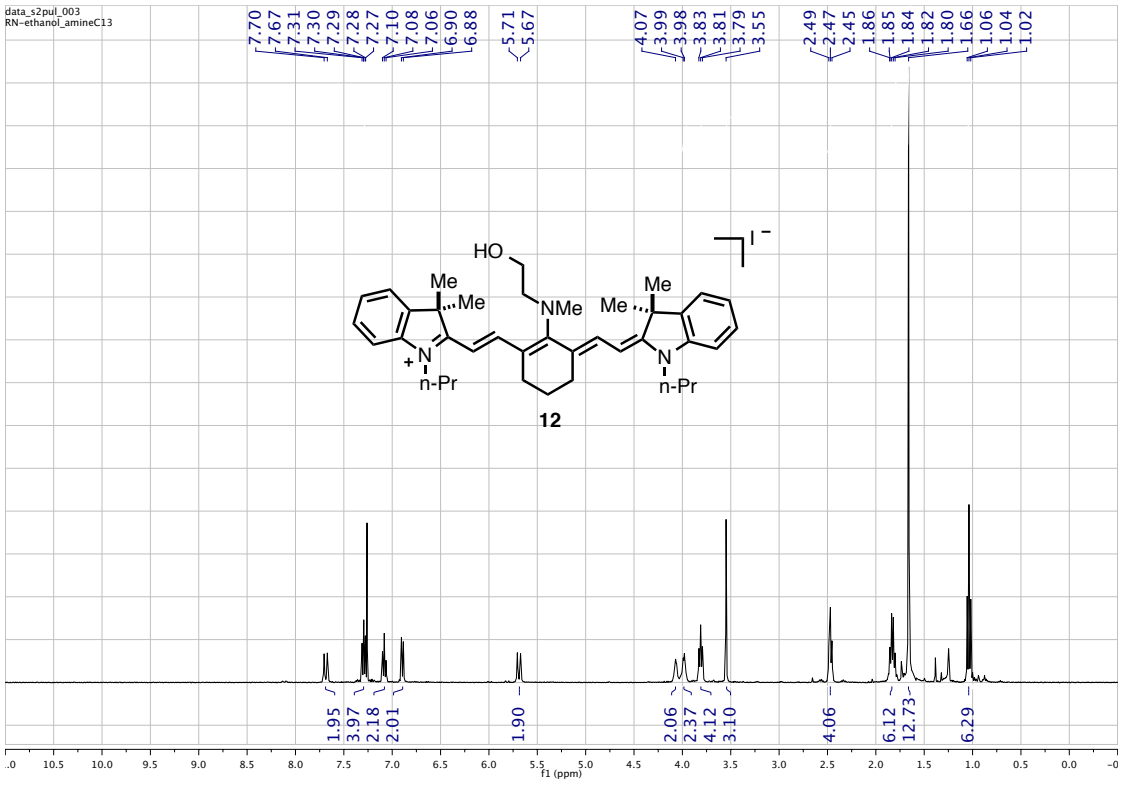
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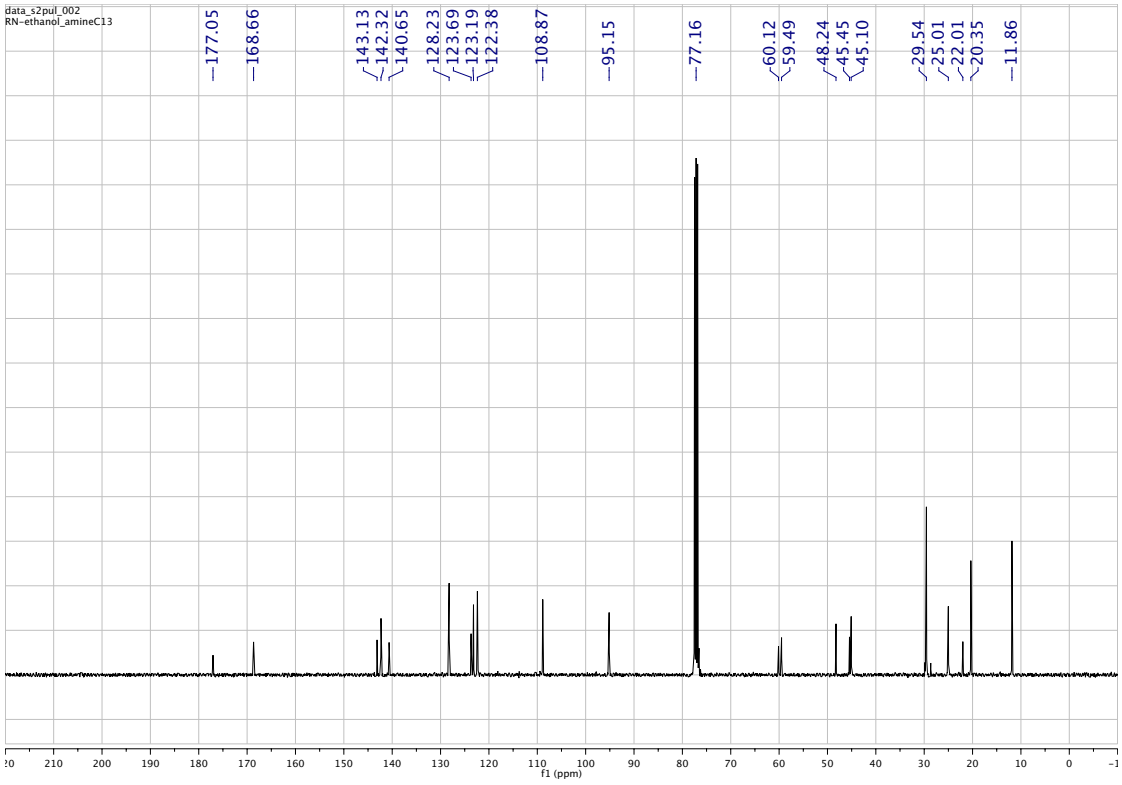
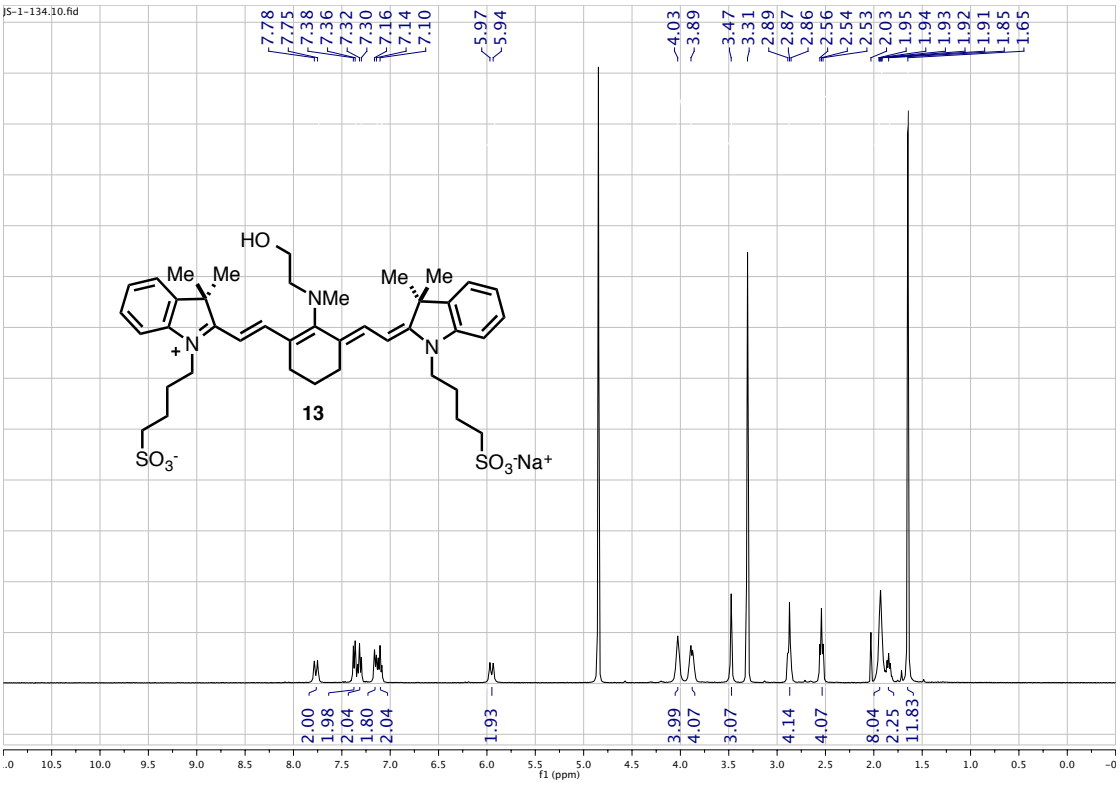
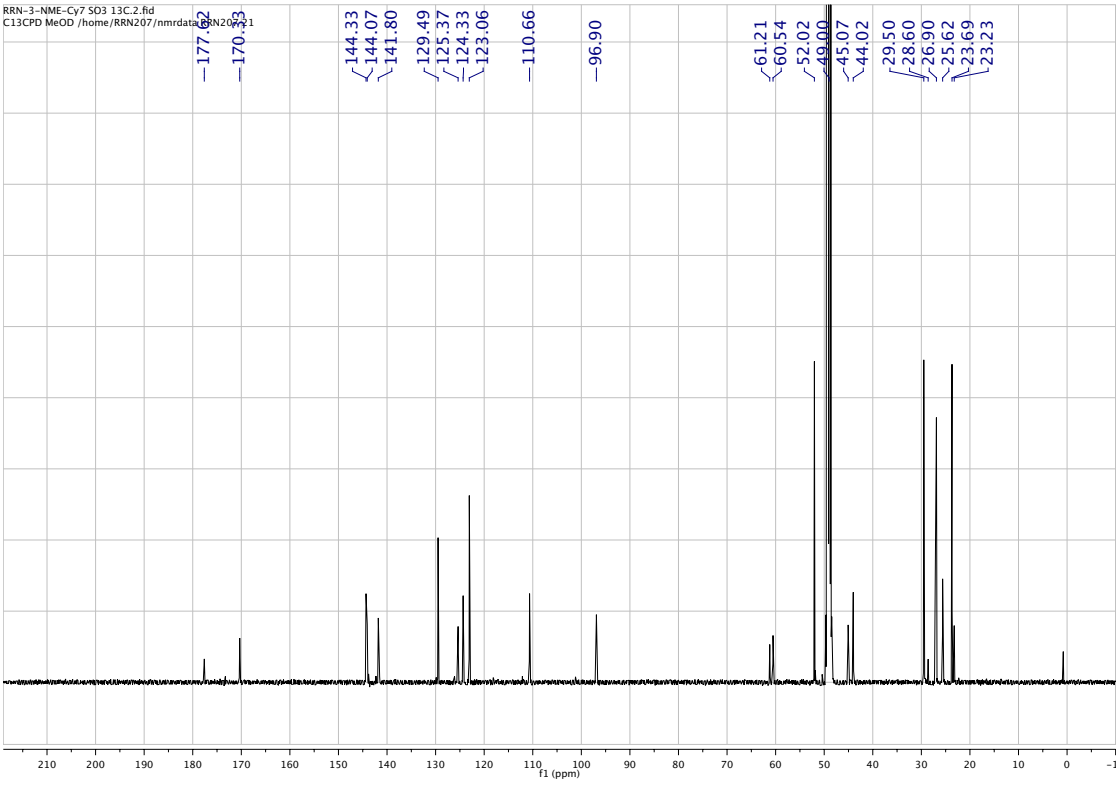
5.67
4.07
3.99
3.98
3.83
3.81
3.79
3.55
2.49
2.47
2.45
1.86
1.85
1.84
1.82
1.80
1.66
1.06
1.04
1.02
HO
NMe
Me
Me
Me
Me
N
n-Pr
N
n-Pr
I −
12
1.95
3.97
2.18
2.01
1.90
2.06
2.37
4.12
3.10
4.06
6.12
12.73
6.29
f1 (ppm)


data_s2pul_002
RN-ethanol_amineC13
177.05
168.66
143.13
142.32
140.65
128.23
123.69
123.19
122.38
108.87
95.15
77.16
60.12
59.49
48.24
45.45
45.10
29.54
25.01
22.01
20.35
11.86
f1 (ppm)


JS-1-134.10.fid
HO
NMe
Me Me
Me Me
N
+N
13
SO3-
SO3-Na+
7.78
7.75
7.38
7.36
7.32
7.30
7.16
7.14
7.10
5.97
5.94
4.03
3.89
3.47
3.31
2.89
2.87
2.86
2.56
2.54
2.53
2.03
1.95
1.94
1.93
1.92
1.91
1.85
1.65
2.00
1.98
2.04
1.80
2.04
1.93
3.99
4.07
3.07
4.14
4.07
8.04
2.25
11.83
f1 (ppm)


RRN-3-NME-Cy7 SO3 13C.2.fid
C13CPD MeOD /home/RRN207/nmrdata/RRN207/1
177.62
170.33
144.33
144.07
141.80
129.49
125.37
124.33
123.06
110.66
96.90
61.21
60.54
52.02
49.00
45.07
44.02
29.50
28.60
26.90
25.62
23.69
23.23
f1 (ppm)