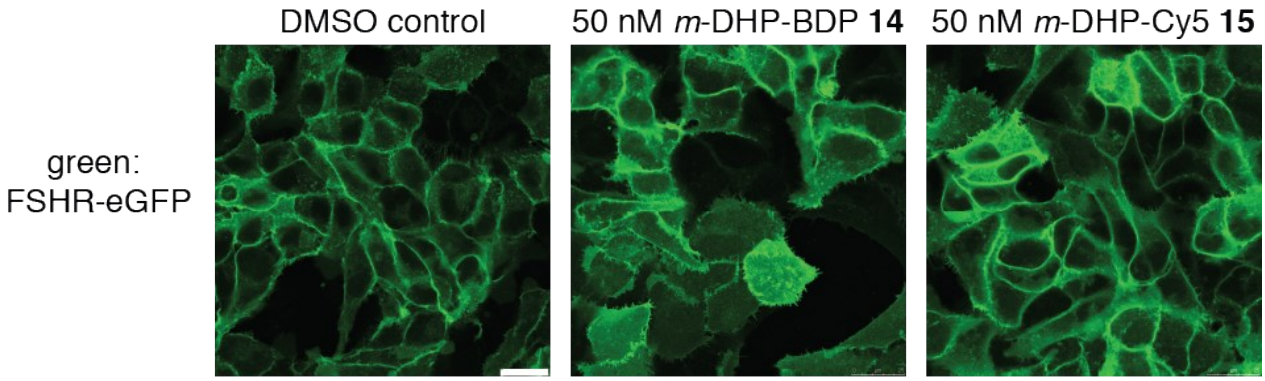
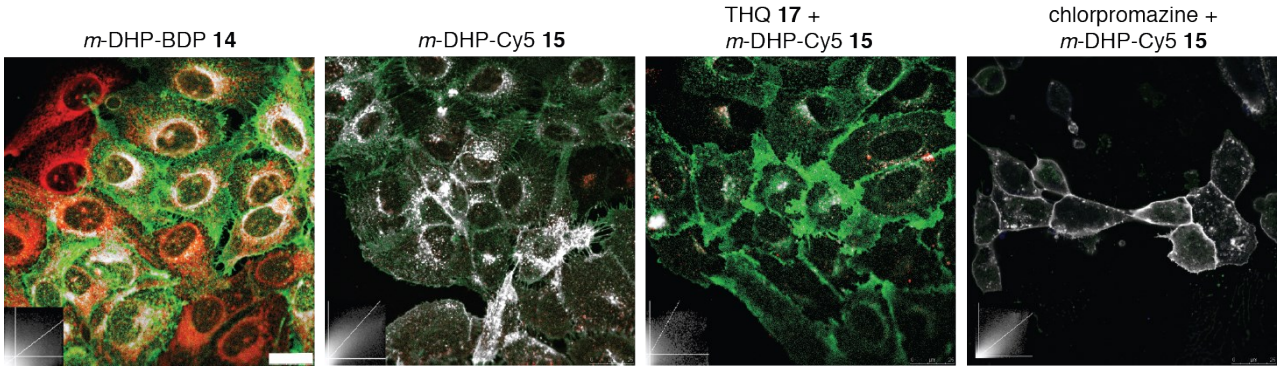
Supplementary information for the manuscript:

Fluorescent Small-Molecule Agonists as Follicle-Stimulating Hormone Receptor Imaging Tools

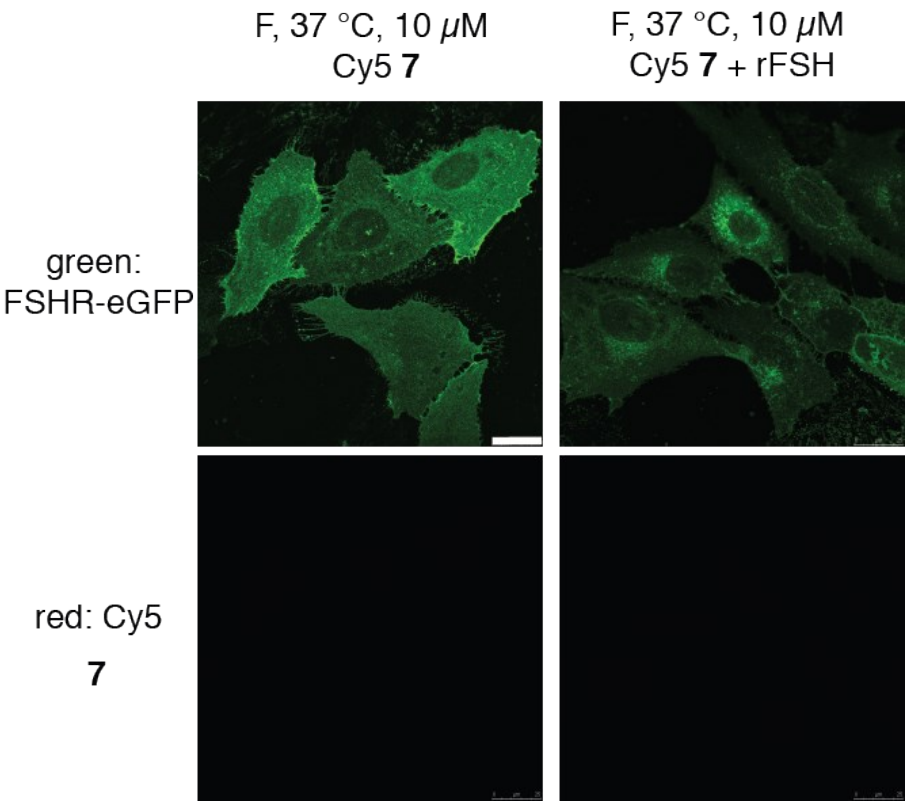
Sascha Hoogendoorn[a,b,*], Gijs H. M. van Puijvelde[c], Gijs A. van der Marel[a], Chris J. van Koppen[d], C. Marco Timmers[e], Herman S. Overkleeft[a,*]



Supplementary Figure 1. Low concentration of agonists (50 nM, 2h) do not induce internalization of the FSHR in U2OS-FSHR cells. Scale bar: 25 um.



Supplementary Figure 2. Colocalization analysis of FSHR-eGFP with **14** or **15** under various conditions. Overlays showing colocalized pixels in white are shown; insert: fluorescence scatter plot. Scalebar: 25 µm. Values for different colocalization parameters are given in Supplementary Table 2.



Supplementary Figure 3. Cy5 dye **7** does not induce FSHR internalization in U2OS-FSHR cells and is not taken up when receptor internalization is induced with recombinant FSH. Scale bar: 25 um.

Supplementary Table 1. Quadrant analysis of flow cytometry data

		Untreated (%)		Treated (%)	
Compound	Quadrant	U2OS	U2OS-FSHR	U2OS	U2OS-FSHR
m-DHP-BDP **14**	UL	0.06±0.01	0.01±0.01	**99.8±0.06**	7.3±1.1
	UR	0.08±0.03	0.09±0.02	0.11±0.02	**92.6±1.1**
	LL	**99.8±0.03**	6.36±0.88	0.06±0.05	0.03±0.02
	LR	0.05±0.03	**93.5±0.87**	0	0.08±0.06
m-DHP-Cy5 **15**	UL	0	0	1.6±0.19	0.59±0.35
	UR	0.03±0.03	0	0.02±0.01	**88.1±2.8**
	LL	**99.9±0.06**	6.2±0.83	**98.4±0.21**	6.1±1.3
	LR	2.43±2.33	**93.9±0.9**	0.06±0.02	5.2±1.9

U2OS or U2OS-FSHR cells were either untreated or treated with 1 μM of compound and analyzed by flow cytometry. Contourplots of GFP vs fluorophore fluorescence were divided in quadrants (UL: upper left; UR: upper right; LL: lower left; LR: lower right) as shown in Figure 3. Percentages (mean ± SEM) calculated from three independent experiments performed in duplo are given. The main population in each group is typeset in boldface.

Supplementary Table 2. Colocalization analysis of *m*-DHP-BDP **14** and *m*-DHP-Cy5 **15** with eGFP-FSHR.

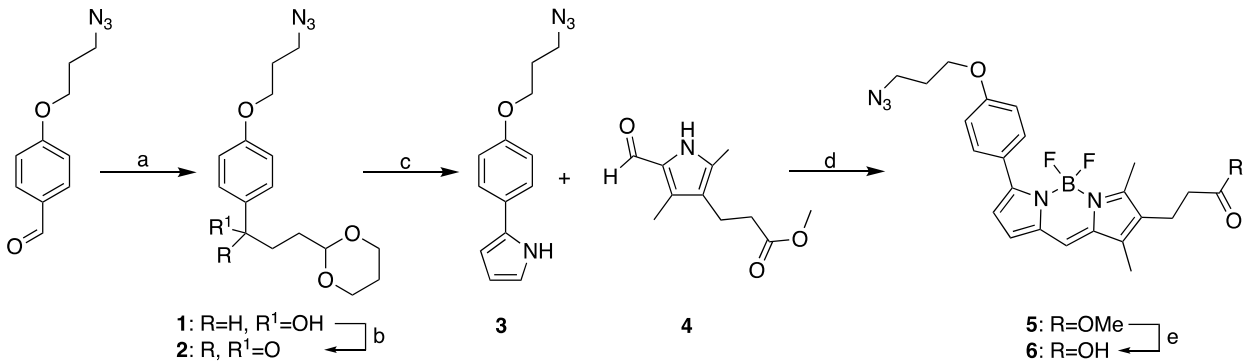
	n (*N*)	**Rr**	**M1**	**M2**	**ICQ**
14	12 (3)	0.4838 ± 0.023	0.9072 ± 0.010	0.9409 ± 0.009	0.1994 ± 0.011
15	22 (5)	0.7271 ± 0.008	0.9460 ± 0.005	0.9164 ± 0.007	0.2852 ± 0.005
10 μM THQ **17 + 15**	6 (2)	0.4637 ± 0.029	0.9027 ± 0.014	0.5942 ± 0.019	0.1658 ± 0.004
10 μM CPZ **+ 15**	10 (2)	0.7314 ± 0.013	0.9476 ± 0.006	0.9225 ± 0.008	0.2906 ± 0.006
20 μM CPZ **+ 15**	9 (2)	0.7581 ± 0.020	0.9437 ± 0.010	0.9254 ± 0.007	0.3352 ± 0.005

n(N): number of pictures n from N independent experiments analyzed. Rr: Pearson's colocalization coefficient. Manders overlap coefficients (M) M1: red; M2: green. ICQ: intensity correlation quotient. Mean +/- SEM is given.

Synthesis

General. All reagents were of commercial grade and used as received unless stated otherwise. Reaction solvents were of analytical grade and when used under anhydrous conditions stored over flame-dried 3 Å molecular sieves. Dichloromethane was distilled over CaH_2 prior to use. Solvents used for column chromatography were of technical grade and distilled before use. All moisture and oxygen sensitive reactions were performed under an argon atmosphere. Flash chromatography was performed on silica gel (Screening Devices BV, 0.04-0.063 mm, 60 Å). Reactions were routinely monitored by TLC analysis on DC-alufolien (Merck, Kieselgel60, F254) with detection by UV-absorption (254/366 nm) where applicable and spraying with a solution of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (25 g/l) and $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$ (10 g/l) in 10% sulfuric acid in water followed by charring at ~150 °C. ^1H and ^{13}C NMR spectra
were recorded on a Bruker AV-400 (400 MHz) or Bruker DMX-600 (600 MHz). Chemical shifts are given in ppm (δ) relative to the residual solvent peak or TMS (0 ppm) as internal standard. Coupling constants are given in Hz. Peak assignments are based on 2D ^1H-COSY and ^{13}C-HSQC NMR experiments. IR measurements (thin film) were conducted on an IRaffinity-1 apparatus and evaluated using IRSolutions software (Shimadzu, Kyoto, Japan). LC-MS measurements were conducted on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI^+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a standard C18 (Gemini, 4.6 mmD x 50 mmL, 5µ particle size, Phenomenex) analytical column and buffers A: H_2O, B: ACN, C: 0.1% aq. TFA. High resolution mass spectra were recorded on a LTQ Orbitrap (Thermo Finnigan) mass spectrometer equipped with an electronspray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL min^{-1}, capillary temperature 250 °C) with resolution R=60000 at m/z 400 (mass range m/z=150-2000) and dioctylphtalate (m/z = 391.28428) as a "lock mass". The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). For reversed-phase HPLC purification of the final compounds a Gilson automated HPLC system equipped with a C18 semiprep column (Gemini C18, 250x10 mm, 5µ particle size, Phenomenex) was used. Cy5 **7** was a kind gift from Marcus Rood (van Leeuwen group, Leiden University Medical Center, The Netherlands)

Synthesis of bifunctional BODIPY dye 6



Supplementary Scheme 1. Reagents and conditions: [**a**] (1,3-dioxan-2-ylethyl)-magnesium bromide (0.5 M in THF), THF, 0 °C → rt, 70%; [**b**] MnO_2, DCM, 79%; [**c**] NH_4OAc, acetic acid; [**d**] i) HBr (aq), MeOH; ii) TEA, $BF_3 \cdot OEt_2$, DCE, 90 °C, **5**: 44% from **2** and hydrolyzed **6**: 10%; [**e**] Me_3SnOH, toluene, reflux, 86%

1-(4-(3-azidopropoxy)phenyl)-3-(1,3-dioxan-2-yl)propan-1-ol (1). Azidobenzaldehyde (1.54 g, 7.5 mmol) was dissolved in dry THF (30 mL) and cooled to 0 °C in an ice-bath. Grignard reagens (1,3-dioxan-2-ylethyl)-magnesium bromide (0.5 M in THF, 15.5 mL, 7.75 mmol, 1.03 eq) was dropwise added over 1 h. The mixture was stirred for an additional hour, while warming up to rt. TLC analysis indicated complete conversion of the reaction, so the reaction was quenched by addition of NH_4OAc (sat aq, 20 mL), followed by extraction with EtOAc (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica column chromatography (20% EtOAc in toluene) resulting in compound **1** as a colorless oil in 70% yield (1.68 g, 5.2 mmol). R_f = 0.3 (2:1 toluene:EtOAc). ^1H NMR (400 MHz, $CDCl_3$): δ 7.28 - 7.21 (m, 2H, 2 x CH_{ar}), 6.90
- 6.73 (m, 2H, 2 x CH_{ar}), 4.61 (dd, *J* = 7.1, 5.8 Hz, 1H, CH), 4.53 (t, *J* = 4.8 Hz, 1H, CH), 4.12 - 4.04 (m, 2H, CH_2), 4.01 (t, *J* = 5.9 Hz, 2H, CH_2), 3.77 - 3.66 (m, 2H, CH_2), 3.49 (t, *J* = 6.7 Hz, 2H, CH_2), 2.80 (s, 1H, OH), 2.12 - 1.96 (m, 3H,

CH_2, CH_2- H_a), 1.91 - 1.78 (m, 2H, CH_2), 1.78 - 1.55 (m, 2H, CH_2), 1.35 - 1.25 (m, 1H, CH_2-H_b). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.96, 137.26, 127.13, 114.35, 102.07, 73.63, 66.93, 64.56, 48.29, 33.40, 31.60, 28.83, 25.72. FT-IR (thin film) v 3447, 2960 (N_3), 2854, 2094, 1611, 1512, 1241, 1136 cm^{-1}. LC/MS analysis (linear gradient 10 → 90% ACN) t_R: 7.46 min, ESI-MS (m/z): $[M + H]^+$: 321.93. ESI-HRMS (m/z): calcd. for $[C_{16}H_{23}N_3O_4 + H]^+$ 322.17163; obsd. 322.17661.

1-(4-(3-azidopropoxy)phenyl)-3-(1,3-dioxan-2-yl)propan-1-one (2). Alcohol **1** (1.28 g, 4 mmol) was dissolved in dry DCM (40 mL) and MnO_2 (3.47 g, 40 mmol, 10 eq) was added. The mixture was stirred for 24 h at room temperature, after which another 10 eq of MnO_2 were added (3.47 g, 40 mmol) and the reaction continued for 20 h. Upon completion, the mixture was filtered over a plug of celite and concentrated. Purification by silica column chromatography (10 → 20% EtOAc in PE) resulted in compound **2** as a colorless oil that solidified over time, in 79%
yield (1.01 g, 3.16 mmol). R_f = 0.5 (1:1 PE:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, *J* = 8.8 Hz, 2H, 2 x CH_{ar}), 6.92 (d, *J* = 8.8 Hz, 2H, 2 x CH_{ar}), 4.66 (t, *J* = 5.0 Hz, 1H, CH), 4.20 - 4.02 (m, 4H, 2 x CH2), 3.88 - 3.68 (m, 2H, CH2), 3.53 (t, *J* = 6.6 Hz, 2H, CH_2), 3.06 (t, *J* = 7.3 Hz, 2H, CH_2), 2.14 - 1.96 (m, 5H, 2 x CH_2, CH_2- H_a), 1.44 - 1.17 (m, 1H, CH_2-H_b).^{13}C
NMR (101 MHz, $CDCl_3$): δ 198.25, 162.52, 130.45, 130.36, 114.18, 101.23, 66.98, 64.79, 48.20, 32.37, 29.59, 28.76, 25.90. FT-IR (thin film) v 2960 (N_3), 2854, 2360, 2098, 1675, 1600, 1508, 1252, 1134 cm^{-1}. LC/MS analysis (linear gradient 10 → 90% ACN) t_R: 8.51 min, ESI-MS (m/z): $[M + H]^+$: 320.07. ESI-HRMS (m/z): calcd. for $[C_{16}H_{21}N_3O_4 + H]^+$ 320.16048; obsd. 320.16035.

2-(4-(3-azidopropoxy)phenyl)-1H-pyrrole (3). Ketone **2** (1.2 g, 3.8 mmol) was dissolved in glacial acetic acid (30 mL) and NH_4OAc (30 g, 380 mmol, 100 eq) was added. The mixture was heated to reflux for 7 h and then allowed to cool to rt, before being poured into ice-water (100 mL). The solution was neutralized with Na_2CO_3 and extracted with DCM (3 x 50 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated. The mixture was purifed by silica column chromatography (0→10% EtOAc in PE) yielding both pyrrole **3** and acetoxypropane as an impurity. Because of the instable nature of the pyrrole it was used as such, without any further purifications. R_f = 0.45 (4:1 toluene:EtOAc). ^{1}H NMR (400 MHz, CDCl3): δ 8.77 (s, 1H, NH), 7.38 (d, *J* = 8.7 Hz, 2H, 2 x CH_{ar}), 6.86 (d, *J* = 8.7 Hz, 2H, 2 x CH_{ar}), 6.76 (d, *J* = 1.2 Hz, 1H, CH_{ar}), 6.45 (s, 1H, CH_{ar}), 6.29 (dd, *J* = 5.5, 2.7 Hz, 1H, CH_{ar}), 3.99 (t, *J* = 6.0 Hz, 2H, CH_2), 3.49 (t, *J* = 6.7 Hz, 2H, CH_2), 2.04 - 2.00 (m, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.98, 131.71, 125.97, 124.94, 118.23, 114.70, 109.52, 104.60, 64.43, 48.04, 28.57.

1,3-dimethyl-2-(2-methoxycarbonylethyl)-5-(4-(3-azido-propoxy)-phenyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (5). Azido-pyrrole **3** (crude, 3.8 mmol) was dissolved in MeOH (30 mL) and carboxyaldehyde pyrrole **4** (794 mg, 3.8 mmol, 1 eq) was added. After cooling of the mixture to 0 °C, HBr (48% in H2O, 430 µL, 1 eq) was added and the reaction continued for 1.5 h at 0 °C. The crude purple HBr salt was filtered off, dissolved and coevaporated with DCE (3 x 50 mL). To a solution of the crude HBr-salt (1.65 g, 3.2 mmol) in DCE (20 mL) were added TEA (1.3 mL, 9.6 mmol, 3 eq) and $BF_3 \cdot OEt_2$ (2 mL, 16 mmol, 5 eq) and the mixture was heated to 90 °C for 15 min, before being concentrated *in vacuo* and purified by silica column chromatography (0 → 2% EtOAc in toluene, followed by 10% EtOAc in toluene + 1% AcOH). After purification both the title BODIPY-methyl ester **5** (812 mg, 1.69 mmol, 44% over 3 steps) and hydrolyzed azido-BODIPY-acid **6** (185 mg, 0.4 mmol, 10%) were obtained as purple solids. R_f = 0.6 (4:1 toluene:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, *J* = 8.8 Hz, 2H, 2 x CH_{ar}), 7.05 (s, 1H, CH_{ar}), 6.94 (d, *J* = 8.8 Hz, 2H, 2 x CH_{ar}), 6.91 (d, *J* = 4.1 Hz, 1H, CH_{ar}), 6.51 (d, *J* = 4.0 Hz, 1H, CH_{ar}), 4.06 (t, *J* = 5.9 Hz, 2H, CH_2), 3.65 (s, 3H, CH_3), 3.49 (t, *J* = 6.7 Hz, 2H, CH_2), 2.69 (t, *J* = 7.7 Hz, 2H, CH_2), 2.52 (s, 3H, CH_3), 2.43 (t, *J* = 7.7 Hz, 2H, CH_2), 2.16 (s, 3H, CH_3), 2.03 (p, *J* = 6.3 Hz, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.89, 159.49, 159.13, 155.52, 139.82, 135.05, 134.31, 130.78, 130.74, 130.70, 129.93, 128.03, 125.71, 123.00, 118.30, 114.22, 64.50, 51.75, 48.24, 33.84, 28.76, 19.46, 13.12, 9.54. ESI-HRMS (m/z): calcd. for $[C_{24}H_{26}BF_2N_5O_3 + H]^+$ 482.21695; obsd. 482.21711.

1,3-dimethyl-2-(2-carboxyethyl)-5-(4-(3-azido-propoxy)-phenyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (6). BODIPY **5** (120 mg, 0.25 mmol) was dissolved in toluene (20 mL). After addition of Me_3SnOH (99 mg, 0.55 mmol, 2.2 eq) the mixture was heated to reflux for 3h and subsequently stirred for 16 h at rt. The organic layer was washed with HCl (aq, 0.1 M, 3 x 30 mL), water (1 x 30 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Silica column chromatography (0 → 0.1% AcOH in 30% EtOAc in toluene) afforded azido-BODIPY-acid **6** (100 mg, 0.21 mmol, 86%) as a purple solid. R_f = 0.3 (4:1 toluene:EtOAc + AcOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (d, *J* = 8.9 Hz, 2H, 2 x CH_{ar}), 7.08 (s, 1H, CH_{ar}), 6.99 - 6.91 (m, 3H, 3 x CH_{ar}), 6.53 (d, *J* = 4.1 Hz,

1H, CH_{ar}), 4.09 (t, *J* = 5.9 Hz, 2H, CH_2), 3.52 (t, *J* = 6.7 Hz, 2H, CH_2), 2.71 (t, *J* = 7.6 Hz, 2H, CH_2), 2.54 - 2.46 (m, 5H, CH_3, CH_2), 2.19 (s, 3H, CH_3), 2.06 (p, *J* = 6.3 Hz, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ 178.49, 159.59, 159.07, 155.85, 139.86, 135.17, 134.34, 130.85, 129.60, 128.24, 125.78, 123.14, 118.53, 114.31, 64.57, 48.34, 33.90, 28.86, 19.30, 13.22, 9.71. ESI-HRMS (m/z): calcd. for $[C_{23}H_{24}BF_2N_5O_3 + H]^+$ 468.20130; obsd. 468.20170.

Synthesis of *m*-DHP

Methyl 3-(hydroxymethyl)benzoate (9). Dimethyl isophtalate (1.94 g, 10 mmol) was dissolved in MeOH (50 mL) and KOH (0.65 g, 12 mmol) was added. The mixture was stirred for 15 h, concentrated *in vacuo* followed by the addition of water, acidification with HCl (aq) and extraction with DCM. After drying ($MgSO_4$), filtration and concentration, the mixture was dissolved in dry THF (20 mL) and cooled to 0 °C. BH_3· THF (20 mL 1 M in THF, 20 mmol) was slowly added at 0 °C, followed by stirring at rt for 45 min, after which the mixture was cooled again and carefully quenched with acetic acid (aq, 50%). The solvent was removed under reduced pressure and the solids dissolved in EtOAc, washed with water, $NaHCO_3$ (sat aq, 4x), dried (Na_2SO_4) and concentrated. Silica column chromatography (0 → 30% EtOAc in PE) yielded pure compound **9** (852 mg, 5.1 mmol, 51% over 2 steps) as a colorless oil. R_f = 0.5 (2:1 PE:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 - 7.97 (m, 1H, CH_{ar}), 7.92 (d, *J* = 7.8 Hz, 1H, CH_{ar}), 7.56 - 7.51 (m, 1H, CH_{ar}), 7.40 (t, *J* = 7.7 Hz, 1H, CH_{ar}), 4.69 (s, 2H, CH_2), 3.89 (s, 3H, CH_3), 3.07 (s, 1H, OH). ^{13}C NMR (101 MHz, CDCl3): δ 167.17, 141.39, 131.41, 130.17, 128.63, 128.54, 127.88, 64.47, 52.15.

Methyl 3-((2-bromo-4-((*4R,7S*)-3-cyano-2-methyl-5-oxo-7-propyl-1,4,5,6,7,8-hexahydroquinolin-4-yl)-6-ethoxyphenoxy)methyl)benzoate (10). To a solution of DHP **8** (0.2 g, 0.45 mmol) in dry THF (10 mL) were added PPh_3 (polymer bound, 0.25 g, 0.75 mmol) and methyl 3-(hydroxymethyl)benzoate **9** (82 mg, 0.5 mmol) and the mixture was cooled to 0 °C. DIAD (0.1 mL, 0.5 mmol) was added dropwise and the reaction continued for 48 h at ambient temperature. LC/MS analysis (linear gradient 10% → 90% ACN/0.1%TFA/H_2O, t_R:10.2 min) showed a conversion of ~90%. The resin was filtered off, the mixture concentrated and the crude product was subjected to silica column chromatography (0 → 30% EtOAc in PE) resulting in an inseparable mixture of product **10** and starting material **8** (239 mg) which was taken to the next step without any further purification. *Rf* = 0.65 (15:1 DCM:MeOH). ESI-MS (m/z): $[M + Na]^+$: 615.07.

3-((2-Bromo-4-((*4R,7S*)-3-cyano-2-methyl-5-oxo-7-propyl-1,4,5,6,7,8-hexa-hydroquinolin-4-yl)-6-ethoxyphenoxy)methyl)benzoic acid (11). Compound **10** (239 mg, containing **8**) was dissolved in MeOH (10 mL) and degassed under argon with sonication. A degassed solution of NaOH (aq, 2 M, 5 mL) was added and the reaction mixture heated to 40 °C for 2 h. The reaction was acidified to pH~5 with HCl (aq, 1 M), extracted with DCM (3x), dried over $MgSO_4$ and concentrated. Silica column chromatography (0 → 10% MeOH in DCM) provided compound **11** (169 mg, 0.29 mmol, 65% over 2 steps) in pure form. R_f = 0.15 (15:1 DCM:MeOH). ^{1}H NMR (400 MHz, MeOD): δ 8.22 (s, 1H, CH_{ar}), 7.98 (d, *J* = 7.7 Hz, 1H, CH_{ar}), 7.72 (d, *J* = 7.5 Hz, 1H, CH_{ar}), 7.45 (t, *J* = 7.6 Hz, 1H, CH_{ar}), 6.92 (s, 1H, CH_{ar}), 6.89 (s, 1H, CH_{ar}), 5.00 (s, 2H, CH_2), 4.47 (s, 1H, CH), 4.14 - 4.03 (m, 2H, CH_2), 2.56 (d, *J* = 16.6 Hz, 1H, CH_2-H_a), 2.47 - 2.34 (m, 2H, CH_2), 2.23 - 2.03 (m, 5H, CH_2-H_b, CH, CH_3), 1.43 (t, *J* = 6.8 Hz, 3H, CH_3), 1.35 (s, 4H, 2 x CH_2), 0.91 (s, 3H, CH_3). ^{13}C NMR (101 MHz, MeOD): δ 1198.36, 169.99, 154.13, 153.56, 147.44, 145.20, 144.40, 139.14, 133.85, 132.46, 130.84, 130.35, 129.37, 124.27, 120.38, 118.74, 113.72, 109.98, 88.33, 75.18, 65.71, 44.05, 39.74, 38.14, 34.80, 33.50, 20.95, 18.04, 15.18, 14.48. $[\alpha]_D^{20}$ = -86° (c = 0.42, $CHCl_3$). ESI-HRMS (m/z): calcd. for $[C_{30}H_{31}BrN_2O_5 + H]^+$ 579.14891; obsd. 579.14882.

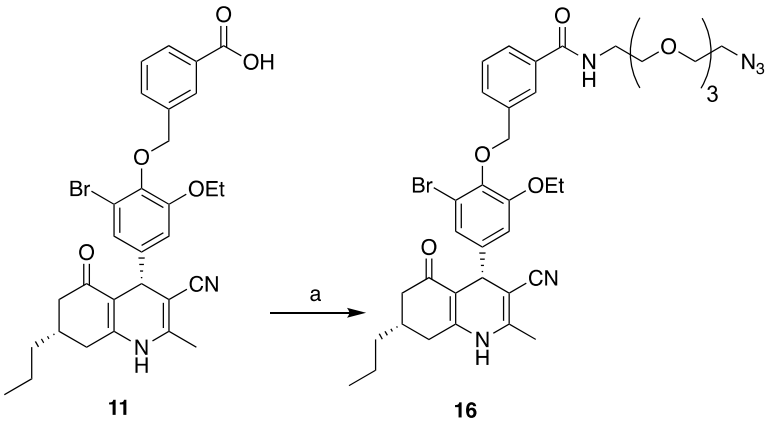
***tert*-Butyl (1-(3-((2-bromo-4-((*4R,7S*)-3-cyano-2-methyl-5-oxo-7-propyl-1,4,5,6,7,8-hexahydroquinolin-4-yl)-6-ethoxyphenoxy)methyl) phenyl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)carbamate (12).** To a solution of **11** (20 mg, 0.034 mmol, 1 eq) in DCM (1 mL) were added 1-Boc-1,11-diamino-tetraethylene glycol spacer (1 mL as a solution in DCM, 0.053 mmol, 1.5 eq) and EEDQ (17 mg, 0.07 mmol, 2 eq). The reaction was monitored by TLC, and upon completion (20 h), the mixture was loaded onto a silica column (0 → 4% MeOH in DCM) to give the Boc-protected DHP **12** as a slightly yellow solid (22 mg, 0.026 mmol, 90%). R_f = 0.35 (15:1 DCM:MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H, CH_{ar}), 7.75 (d, *J* = 7.5 Hz, 1H, CH_{ar}), 7.64 (d, *J* = 7.7 Hz, 1H, CH_{ar}), 7.52 (s, 1H, NH), 7.43 (t, *J* = 7.7 Hz, 1H, CH_{ar}), 7.00 (s, 1H, NH), 6.91 (d, *J* = 1.8 Hz, 1H, CH_{ar}), 6.84 (d, *J* = 1.8 Hz, 1H, CH_{ar}), 5.06 (t, *J* = 5.5 Hz, 1H, NH), 5.03 (s, 2H, CH_2), 4.54 (s, 1H, CH), 4.15 – 4.07 (m, 2H, CH_2), 3.68 – 3.56 (m, 12H, 6 x CH_2), 3.48 (t, *J* = 5.2 Hz, 2H, CH_2), 3.28 – 3.20 (m, 2H, CH_2), 2.45 – 2.27 (m, 3H, CH_2, CH_2-H_a), 2.19 – 1.99 (m, 5H, CH_3, CH, CH_2-H_b), 1.43 (d, *J* = 6.4 Hz, 12H, 4 x CH_3), 1.30 – 1.23 (m, 4H, 2 x CH_2), 0.86 (t, *J* = 6.8 Hz, 3H, CH_3).^{13}C NMR (101 MHz, $CDCl_3$) δ 195.83, 167.94, 156.22, 152.73, 150.12, 145.27, 144.11, 142.50, 138.33, 134.70, 131.30, 128.62, 127.05, 126.63, 123.10, 119.51, 118.09, 113.09, 109.84, 88.00, 81.79, 74.09, 70.61, 70.58, 70.43, 70.28, 69.96, 64.72, 43.55, 40.48, 40.03, 38.37, 37.53, 33.95, 33.58, 28.55. $[\alpha]_D^{20}$ = -84° (c = 0.44, $CHCl_3$).

ESI-HRMS (m/z): calcd. for $[C_{43}H_{57}BrN_4O_9 + H]^+$ 853.33817; obsd. 853.33937.

***m*-DHP-NH$_2$ (13).** Compound **12** (10-20 µmol) was subjected to DCM/TFA (2 mL, 1:1, v/v) for 30 min - 1 h, upon which TLC indicated complete removal of the Boc-group. The mixture was diluted with toluene and concentrated *in vacuo*. Silica column chromatography (2.5% → 5% MeOH in DCM + 1% TEA) afforded amine **76** (R_f = 0.5 (10:1:1 DCM:MeOH:TEA) which was used immediately in the coupling reaction with the different fluorophores. ESI-MS (m/z): $[M + H]^+$: 753.33.

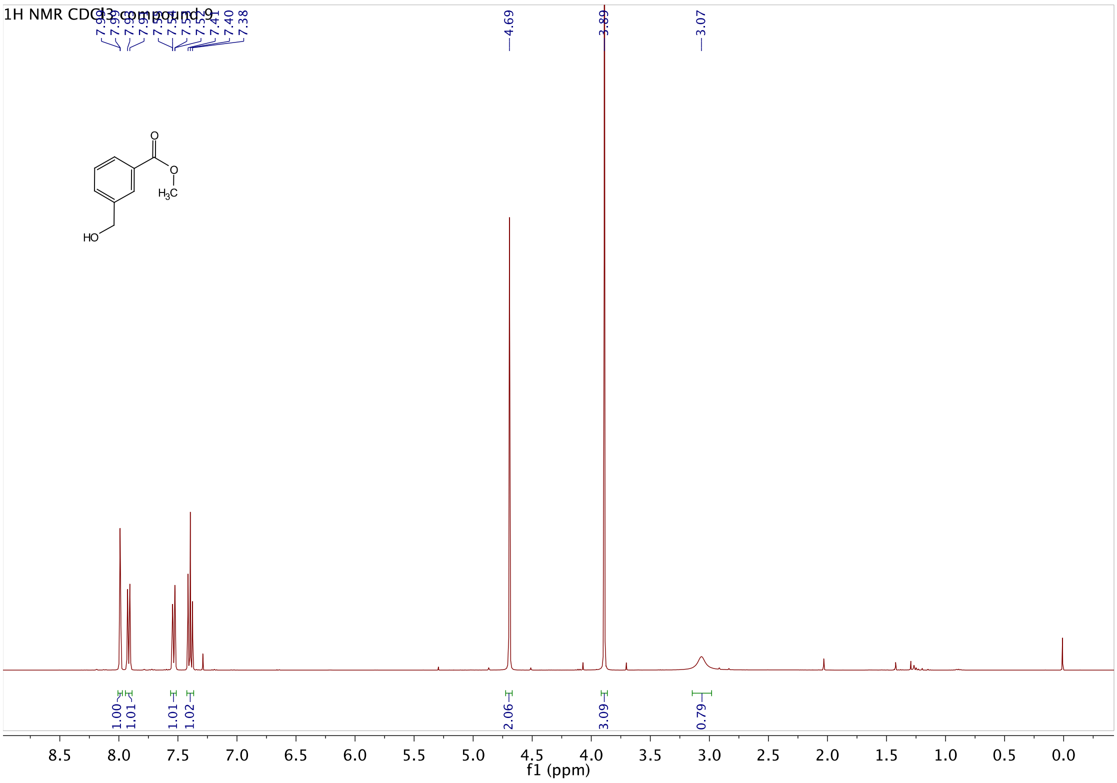
***m*-DHP-BODIPY-N$_3$ (14).** To a solution of amino-DHP **13** (8 mg, 10.6 µmol) and azido-BODIPY acid **6** (6 mg, 10.6 µmol) in DCM (1 mL) were added EDC · HCl (4 mg, 21 µmol, 2 eq), HOBt (2.8 mg, 21 µmol, 2 eq) and TEA (3 µL, 21 µmol, 2 eq). The mixture was stirred for 3 h at room temperature, and subsequently washed with HCl (aq, 1 M, 2 x 5 mL), water (1 x 5 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica column chromatography (0 → 5% MeOH in DCM), gave the product as a purple solid (6.2 mg, 5.1 µmol, 48%). R_f = 0.3 (20:1 DCM:MeOH). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.98 (d, *J* = 1.8 Hz, 1H, CH_{ar}), 7.88 - 7.84 (m, 2H, 2 x CH_{ar}), 7.76 (dt, *J* = 7.8, 1.4 Hz, 1H, CH_{ar}), 7.62 (dt, *J* = 7.8, 1.3 Hz, 1H, CH_{ar}), 7.41 (t, *J* = 7.7 Hz, 1H, CH_{ar}), 7.09 (s, 1H, CH_{ar}), 6.98 - 6.94 (m, 3H, 3 x CH_{ar}), 6.91 (d, *J* = 2.0 Hz, 1H, NH), 6.87 (d, *J* = 5.6 Hz, 1H, CH_{ar}), 6.80 (d, *J* = 2.0 Hz, 1H, CH_{ar}), 6.53 (d, *J* = 4.1 Hz, 1H, CH_{ar}), 6.45 (s, 1H, CH_{ar}), 6.15 (t, *J* = 5.6 Hz, 1H, NH), 5.02 (d, *J* = 2.0 Hz, 2H, CH_2), 4.55 (s, 1H, CH), 4.13 - 4.07 (m, 4H, 2 x CH_2), 3.67 - 3.62 (m, 6H, 3 x CH_2), 3.61 - 3.59 (m, 2H, CH_2), 3.56 - 3.52 (m, 4H, 2 x CH_2), 3.50 - 3.47 (m, 2H, CH_2), 3.41 (t, *J* = 5.1 Hz, 2H, CH_2), 3.34 - 3.29 (m, 2H, CH_2), 2.72 (t, *J* = 7.2 Hz, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.45 (dd, *J* = 16.1, 4.0 Hz, 1H, CH_2-H_a), 2.32 - 2.24 (m, 4H, 2 x CH_2), 2.18 (d, *J* = 7.2 Hz, 4H, CH_3, CH), 2.10 - 2.05 (m, 6H, CH_3, CH_2-H_b, CH_2), 1.42 (t, *J* = 7.0 Hz, 3H, CH_3), 1.35 - 1.28 (m, 4H, 2 x CH_2), 0.91 - 0.87 (m, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 195.63, 171.89, 167.72, 159.80, 159.62, 155.46, 152.79, 149.35, 144.73, 144.14, 142.28, 140.15, 138.10, 135.05, 134.78, 134.58, 131.31, 130.87, 128.71, 128.01, 127.07, 126.71, 125.91, 123.01, 122.96, 119.27, 118.43, 118.07, 114.35, 113.03, 110.15, 88.41, 74.19, 70.56, 70.36, 70.29, 69.96, 69.83, 64.71, 64.65, 48.38, 43.48, 39.92, 39.45, 38.28, 37.54, 36.27, 33.93, 33.76, 28.92, 20.09, 19.93, 18.65, 14.96, 14.19, 13.34, 9.77. ESI-HRMS (m/z): calcd. for $[C_{61}H_{71}BBrF_2N_9O_9 + H]^+$ 1202.46920; obsd. 1202.47131.

***m*-DHP-Cy5 (15).** Amino-DHP **13** (5 mg, 6.6 µmol, 1.1 eq) was added to a pre-activated solution of Cy5-acid **7** (5 mg, 6 µmol), PyBOP (3.4 mg, 6.6 µmol, 1.1 eq) and DiPEA (3 µL, 18 µmol, 3 eq) in DMF (1 mL). The mixture was stirred for 3 h at room temperature, before the solvent was removed *in vacuo* and the residue purified by RP-HPLC (25 → 45% ACN in 12', buffer A: 10 mM NH_4OAc) or silica column chromatography (0 → 2% H_2O in 40% MeOH in $CHCl_3$). Crystallization from DCM/MeOH/hexanes and lyophilization from H_2O yielded the product as a bright blue solid (5 mg, 3.3 µmol, 55%). R_f = 0.7 (1:1:1:1 EtOAc:n-BuOH:AcOH:H_2O). ^{1}H NMR (600 MHz, MeOD): δ 8.27 (td, *J* = 13.0, 8.1 Hz, 2H, 2 x =CH), 8.03 (s, 1H, CH_{ar}), 7.92 - 7.85 (m, 4H, 4 x CH_{ar}), 7.79 (d, *J* = 7.8 Hz, 1H, CH_{ar}), 7.68 (d, *J* = 7.6 Hz, 1H, CH_{ar}), 7.46 (t, *J* = 7.7 Hz, 1H, CH_{ar}), 7.37 (d, *J* = 8.8 Hz, 1H, CH_{ar}), 7.28 (d, *J* = 8.2 Hz, 1H, CH_{ar}), 6.89 (dd, *J* = 15.7, 1.8 Hz, 2H, 2 x CH_{ar}), 6.68 (t, *J* = 12.4 Hz, 1H, =CH), 6.39 (d, *J* = 13.7 Hz, 1H, =CH), 6.31 (d, *J* = 13.7 Hz, 1H, =CH), 5.00 (s, 2H, CH_2), 4.58 (s, 1H, SO_3H), 4.47 (s, 1H, CH), 4.17 - 4.10 (m, 2H, CH_2), 4.10 - 4.04 (m, 4H, 2 x CH_2), 3.69 - 3.56 (m, 10H, 5 x CH_2), 3.56 - 3.51 (m, 2H, CH_2), 3.45 (t, *J* = 5.4 Hz, 2H, CH_2), 3.29 - 3.26 (m, 2H, CH_2), 2.90 (t, *J* = 6.8 Hz, 2H, CH_2), 2.60 (dd, *J* = 16.8, 4.5 Hz, 1H, CH_2-H_a), 2.47 - 2.40 (m, 2H, CH_2-H_b, CH_2-H_a), 2.17 (t, *J* = 7.3 Hz, 3H, CH_2, CH), 2.15 - 2.09 (m, 4H, CH_3, CH_2-H_b), 1.99 - 1.93 (m, 4H, 2 x CH_2), 1.80 - 1.70 (m, 14H, 4 x CH_3, CH_2), 1.67 - 1.62 (m, 2H, CH_2), 1.41 (t, *J* = 7.0 Hz, 3H, CH_3), 1.39 - 1.34 (m, 4H, 2 x CH_2), 0.95 - 0.88 (m, 3H, CH_3). ^{13}C NMR (151 MHz, MeOD): δ 198.37, 175.80, 175.35, 175.15, 170.07, 156.31, 156.10, 154.16, 153.67, 147.56, 145.27, 144.93, 144.81, 144.49, 143.41, 143.34, 142.64, 142.60, 139.34, 135.82, 132.62, 129.58, 128.45, 128.09, 128.04, 128.01, 124.31, 121.33, 120.47, 118.74, 113.78, 111.72, 111.62, 109.98, 105.52, 105.33, 88.30, 75.27, 71.59, 71.57, 71.28, 71.19, 70.57, 70.53, 65.75, 50.59, 50.53, 45.07, 43.80, 40.97, 40.30, 39.79, 38.19, 36.63, 34.88, 33.57, 28.15, 27.89, 27.34, 27.13, 26.50, 23.53, 20.97, 18.06, 15.23, 14.47. LC/MS analysis (linear gradient 10 → 90% ACN) t_R: 6.67 min, ESI-MS (m/z): [M + H]+: 1501.27. ESI-HRMS (m/z): calcd. for $[C_{73}H_{91}BrN_6O_{17}S_3 + H]^+$ 1501.48385; obsd. 1501.48711.



Supplementary Scheme 2. Reagents and conditions: [**a**] 11-azido-3,6,9-trioxaundecan-1-amine hydrochloride, EEDQ, DiPEA, 74%

Synthesis of *m*-DHP-N_3 (16). To a solution of **11** (30 mg, 0.052 mmol, 1 eq) in DCM (1 mL) were added spacer 11-azido-3,6,9-trioxaundecan-1-amine hydrochloride (1 mL as a solution in DCM, 0.052 mmol, 1 eq), EEDQ (15 mg, 0.06 mmol, 1.2 eq) and DiPEA (26 µL, 0.15 mmol, 3 eq). Reaction progress was slow, so after 24 h another 2 eq of EEDQ and 1.5 eq of spacer were added. The reaction was monitored by TLC, and upon completion, the mixture was washed with HCl (aq, 1 M, 2x), water (2x), dried (MgSO4) and concentrated.
Pure product (30 mg, 0.039 mmol, 74%) was obtained by silica column chromatography (0→2% MeOH in DCM). R_f = 0.7 (12:1 DCM:MeOH). FT-IR (thin film) v 3294, 2924, 2866, 2360, 2343, 2201, 2110, 1644, 1498, 1426, 1384, 1275, 1124, 1045 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H, CH_{ar}), 7.73 (d, *J* = 7.8 Hz, 1H, CH_{ar}), 7.66 (d, *J* = 10.1 Hz, 2H, CH_{ar} + N*H*), 7.45 (t, *J* = 7.7 Hz, 1H, CH_{ar}), 7.00 (s, 1H, N*H*), 6.91 (d, *J* = 1.8 Hz, 1H, CH_{ar}), 6.86 (d, *J* = 1.9 Hz, 1H, CH_{ar}), 5.03 (s, 2H, CH_2), 4.55 (s, 1H, C*H*), 4.19 – 4.03 (m, 2H, CH_2), 3.75 – 3.54 (m, 14H, 7 x CH_2), 3.40 – 3.27 (m, 2H, CH_2), 2.48 – 2.23 (m, 3H, CH_2 + CH_2-H1), 2.17 – 2.02 (m, 5H, CH_3 + C*H* + CH_2-H2), 1.43 (t, *J* = 7.0 Hz, 3H, CH_3), 1.33 – 1.18 (m, 4H, 2 x CH_2), 0.85 (t, *J* = 6.9 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.93, 168.01, 152.71, 150.35, 145.39, 144.04, 142.57, 138.42, 134.68, 131.34, 128.63, 127.13, 126.44, 123.14, 119.57, 118.09, 113.03, 109.70, 87.87, 74.01, 70.75, 70.67, 70.41, 70.08, 69.80, 64.68, 50.74, 43.54, 40.05, 38.43, 37.50, 33.92, 33.51, 19.84, 18.44, 14.95, 14.15. ESI-HRMS (m/z): calcd. for $[C_{38}H_{47}BrN_6O_7 + H]^+$ 779.27624; obsd. 779.27652. $[\alpha]_D^{20}$ = -102° (c = 0.5, $CHCl_3$).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7.40
7.38
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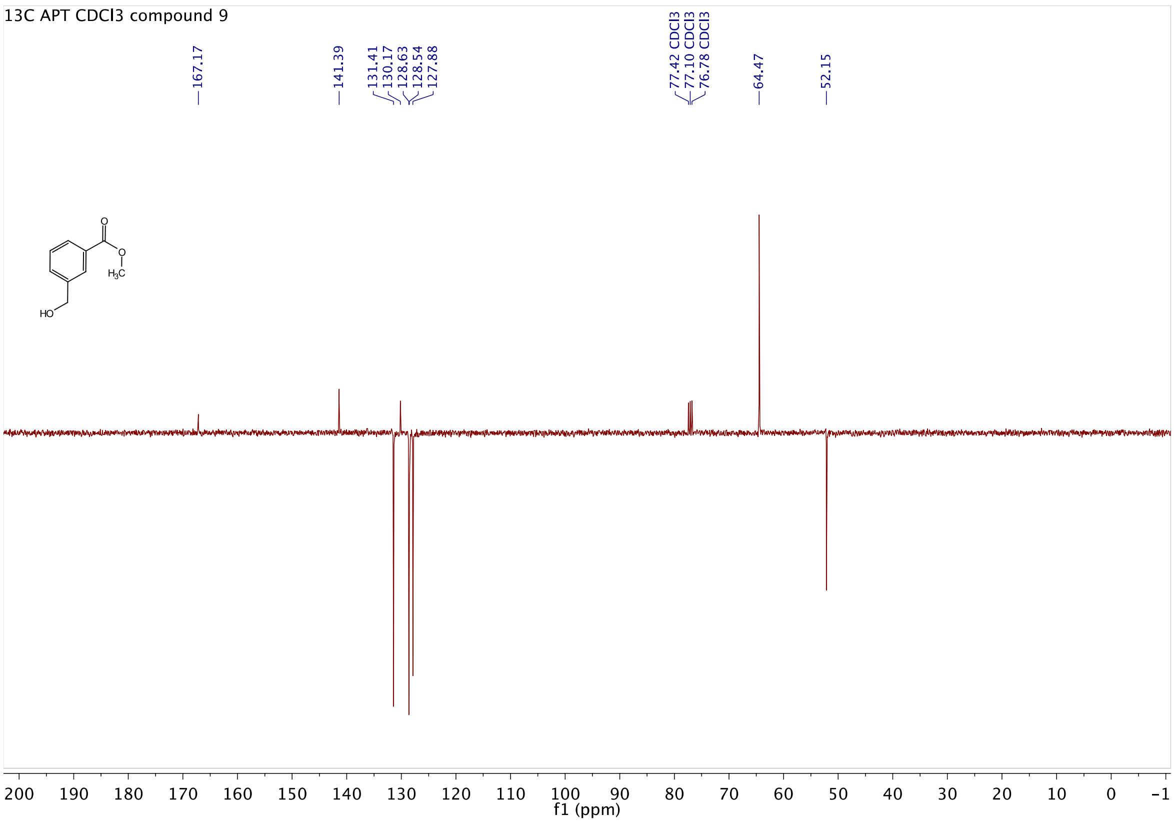
3.07
1.00
1.01
1.01
1.02
2.06
3.09
0.79
f1 (ppm)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141.39
131.41
130.17
128.63
128.54
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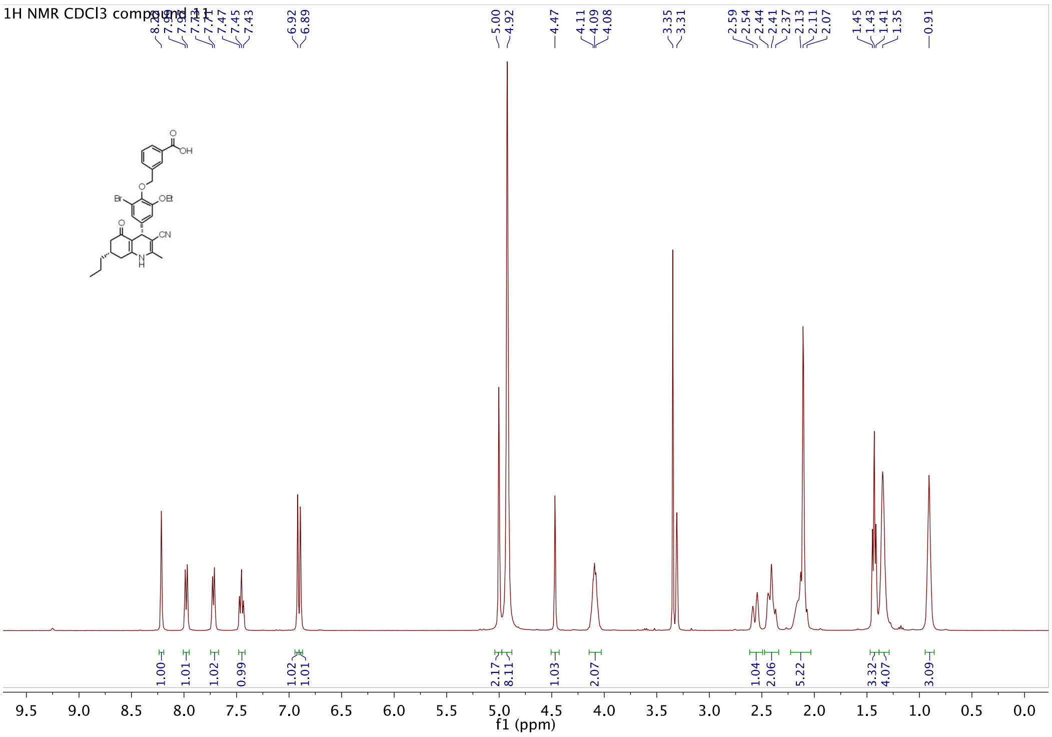
76.78 CDCl3
64.47
52.15
f1 (ppm)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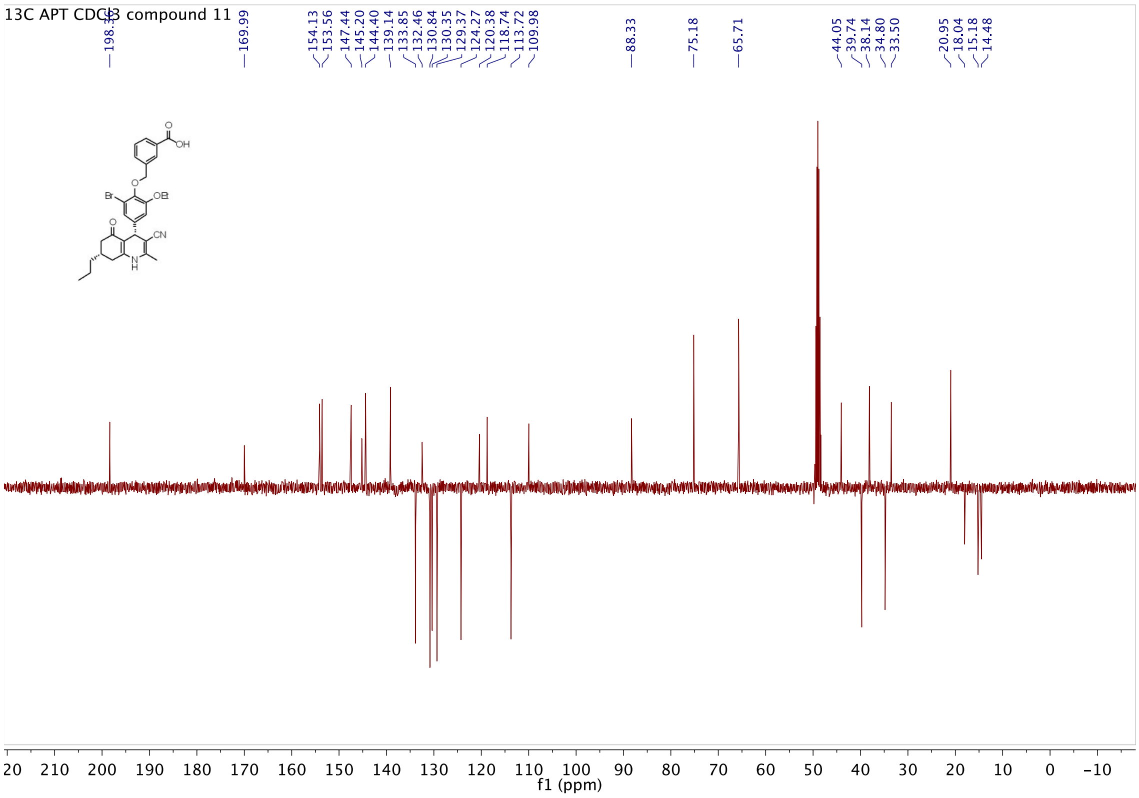
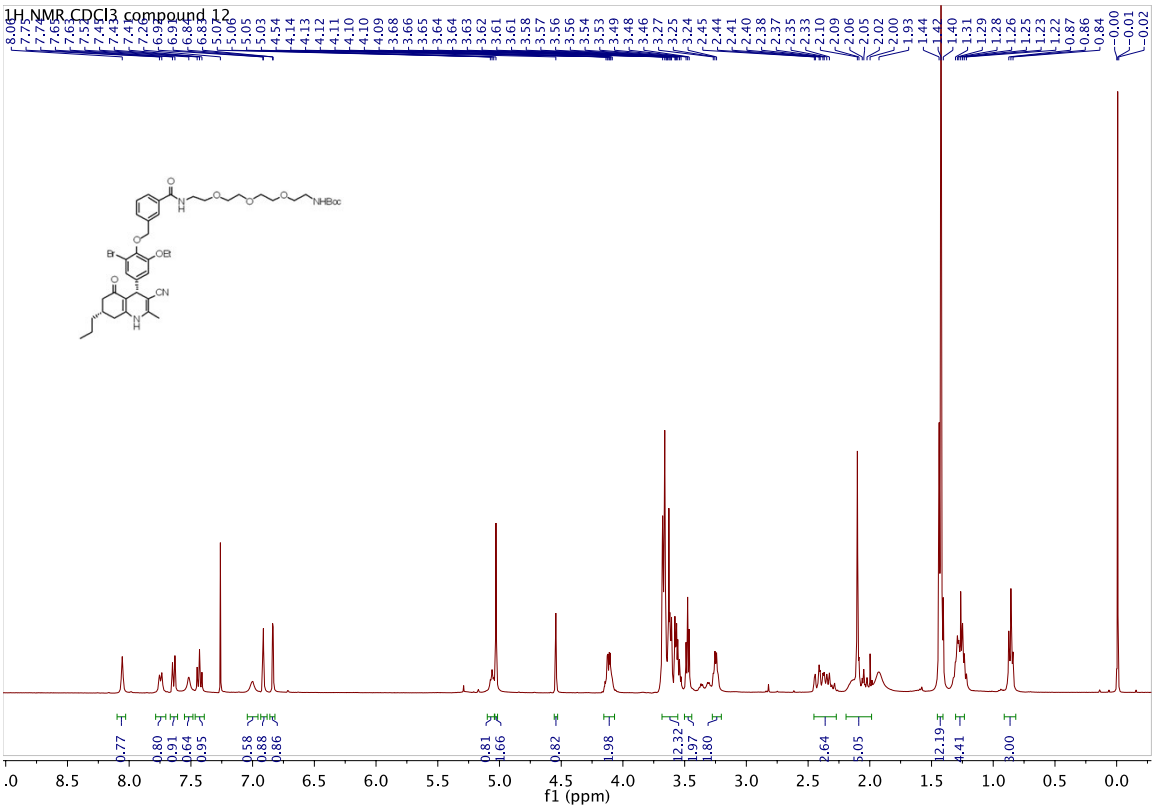
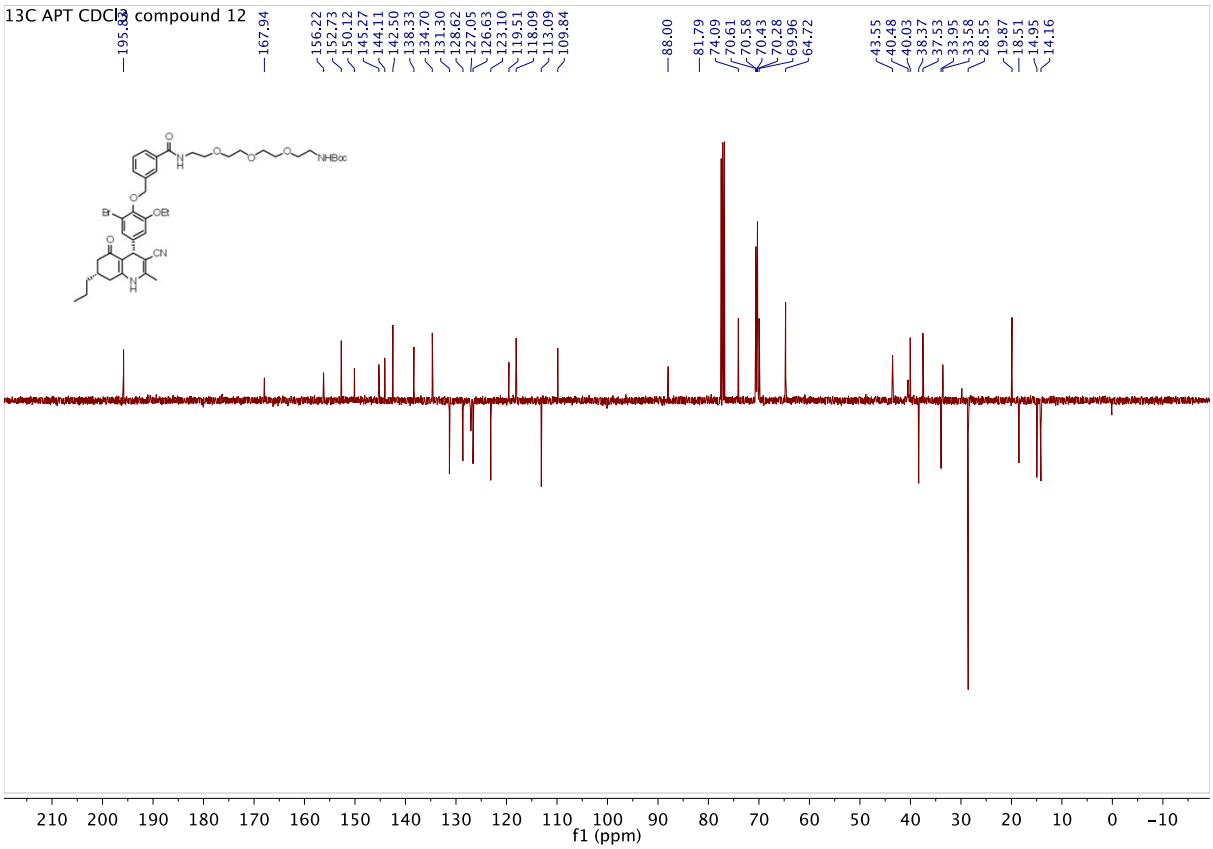
f1 (ppm)

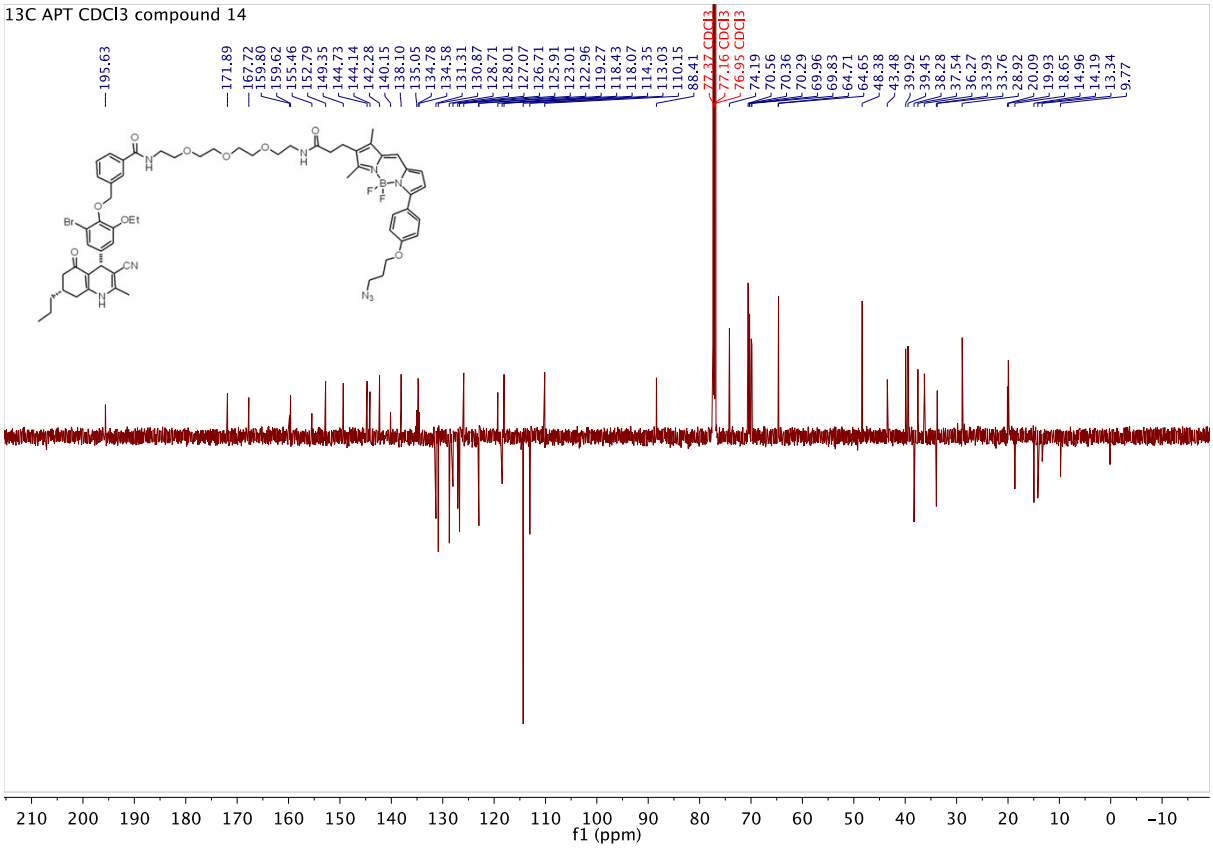

13C APT CDCl3 compound 11
f1 (ppm)

1H NMR CDCl3 compound 12



13C APT CDCl3 compound 12

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155.46
152.79
149.35
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140.15
138.10
135.05
134.78
134.58
131.31
130.87
128.71
128.01
127.07
126.71
125.91
123.01
122.96
119.27
118.43
118.07
114.35
113.03
110.15
88.41
77.37 CDCl3
77.16 CDCl3
76.95 CDCl3
74.19
70.56
70.36
70.29
69.96
69.83
64.71
64.65
48.38
43.48
39.92
39.45
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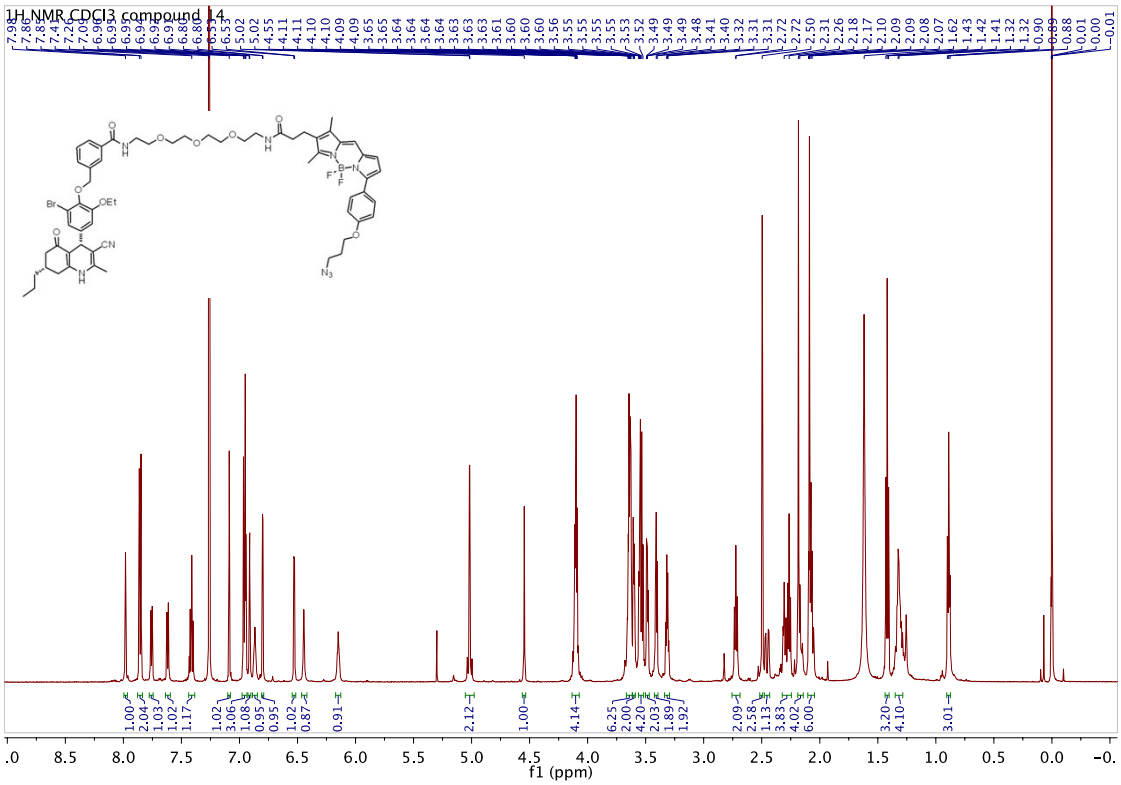
14.96
14.19
13.34
9.77
f1 (ppm)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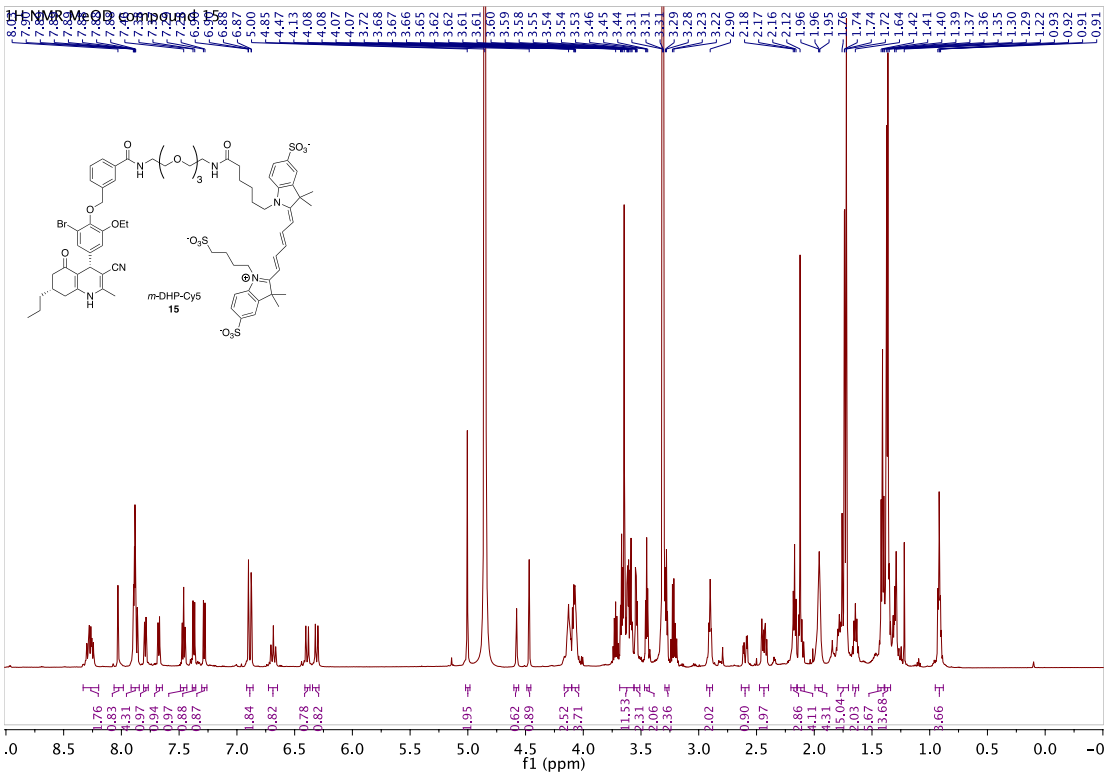
f1 (ppm)


1H NMR MeOD compound 15
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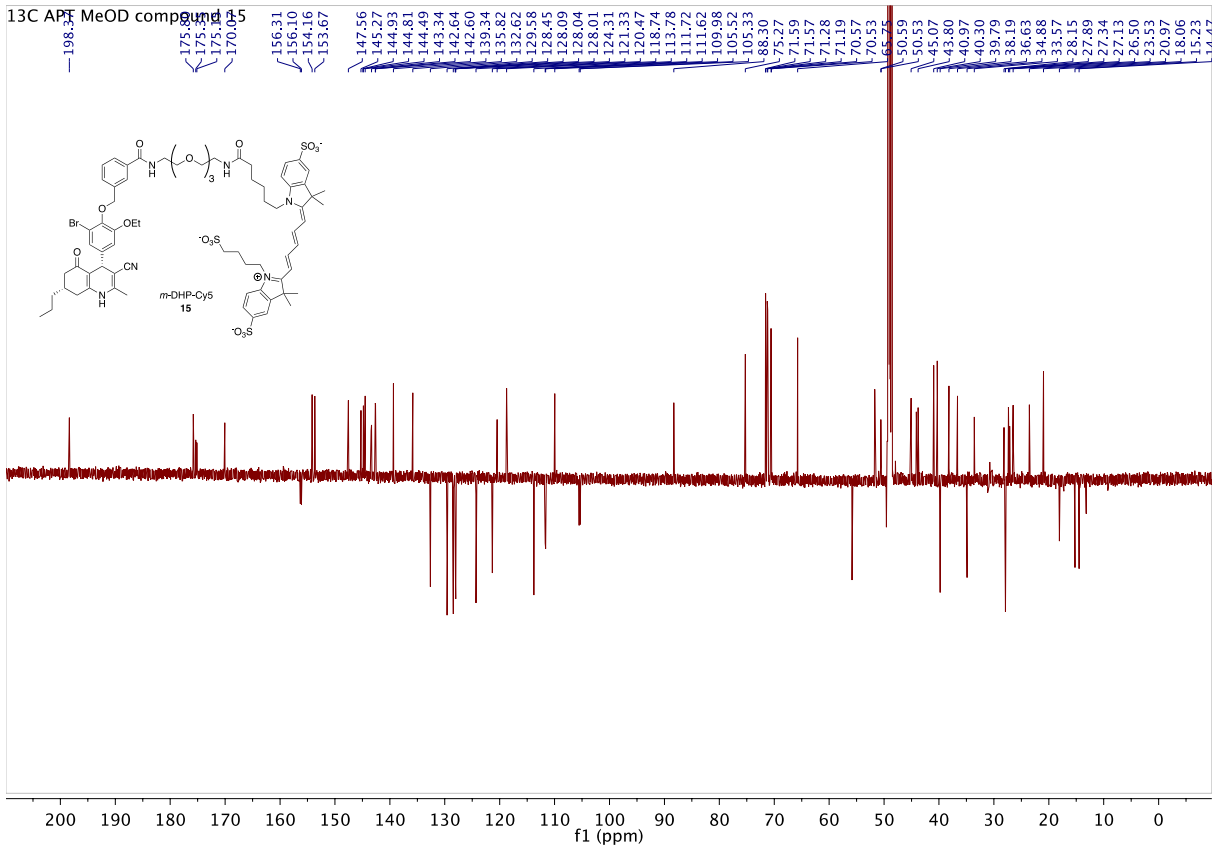
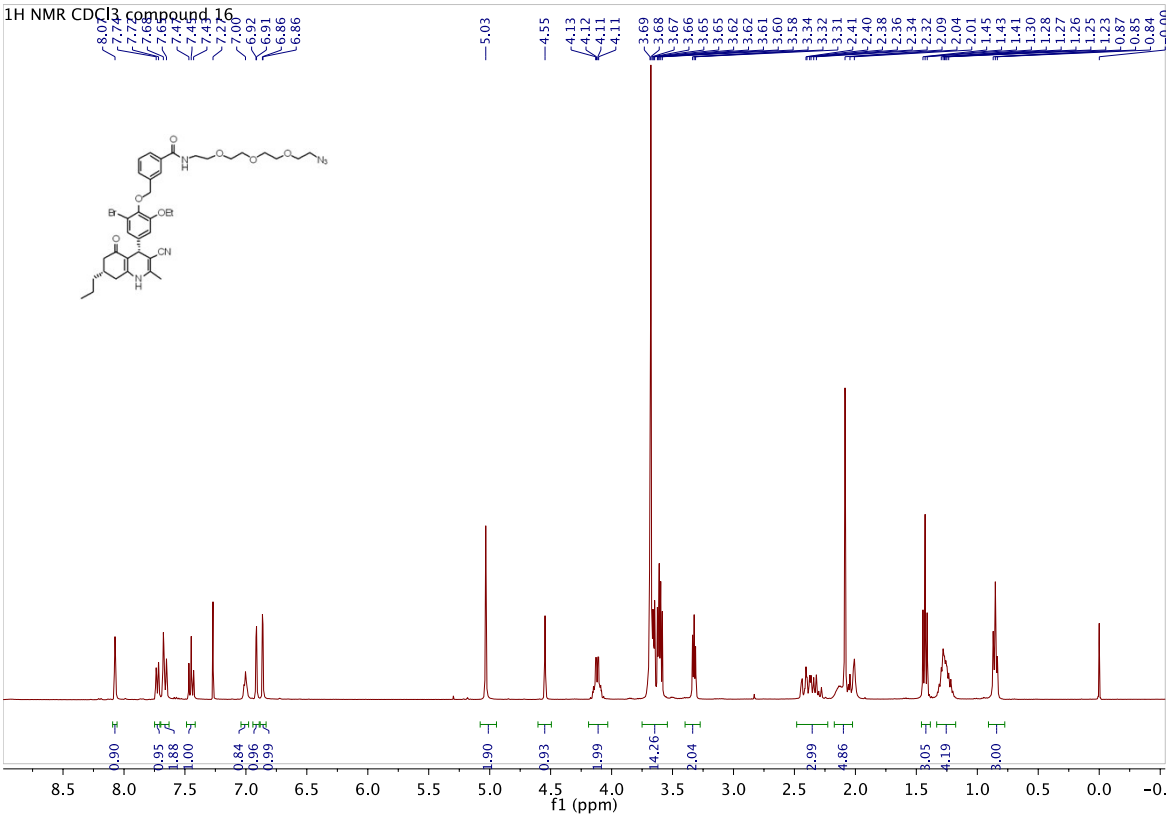
15
f1 (ppm)


13C APT MeOD compound 15
m-DHP-Cy5
15
f1 (ppm)

1H NMR CDCl3 compound 16



13C APT CDCl3 compound 16