

Supporting Information for

Shortwave Infrared Fluorofluorophores for Multicolor *In Vivo* Imaging

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Table of Contents

1.	Supplemental figures	S2-S13
2.	Supplemental tables	S14
3.	Synthetic chemistry and counterion exchange procedures	S15-S18
4.	Photophysical measurements	S19-S20
5.	Perfluorocarbon nanoemulsion preparation and applications	S21-S22
6.	General procedure for <i>in vivo</i> experiments and SWIR camera	S23-S24
7.	Spectra	S25-S45
	Absorption and emission spectra	S25
	¹ H-NMR spectra	S26-S31
	¹¹ B-NMR spectra	S32-S35
	¹³ C-NMR spectra	S36-S39
	¹⁹ F-NMR spectra	S40-S45
8.	References	S46

Supplemental figures:

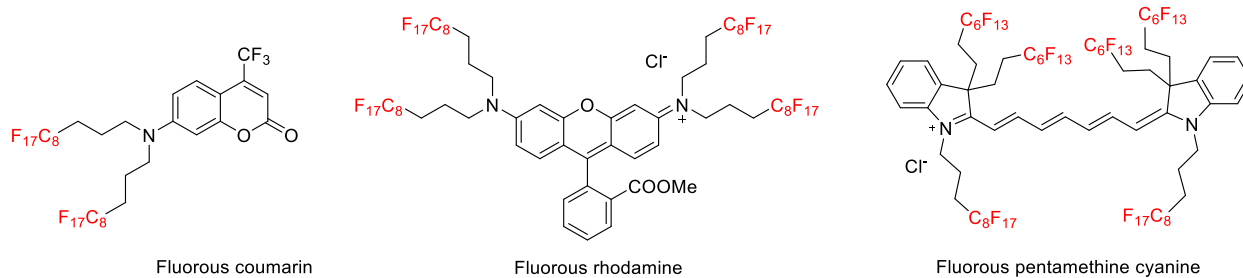


Figure S1. Chemical structures of fluorofluorophores: fluorous coumarin,^[1] fluorous rhodamine,^[1] and fluorous Cy5^[2]

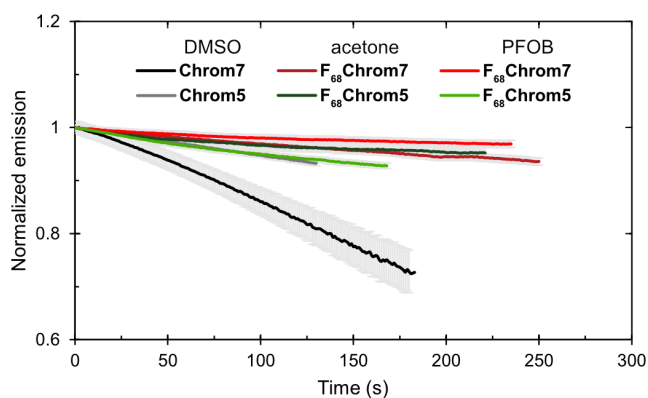


Figure S2. Normalized emission of **Chrom5**, **Chrom7** in DMSO (black, gray) and **F₆₈Chrom5**[B(ArF₂₆)₄], **F₆₈Chrom7**[B(ArF₂₆)₄] in acetone (dark red, dark green) and PFOB (bright red, bright green) under continuous irradiation. Emission detected at 1100 nm LP filtering, with 0.4 ms (974 nm ex. for Chrom7 dyes) or 1 ms (785 nm ex. for Chrom5 dyes) exposure times. Standard deviation (gray error bars) are calculated from three replicates of each solution.

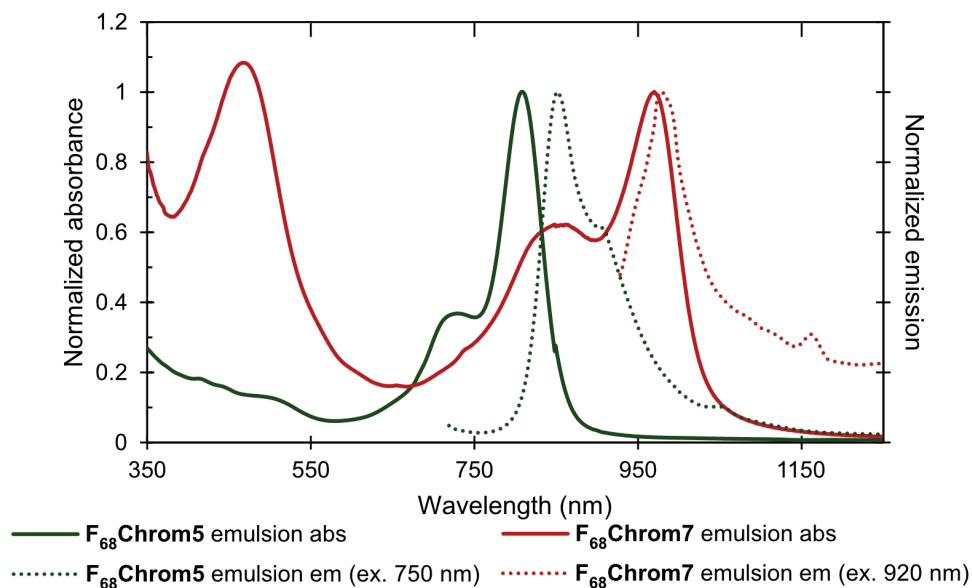


Figure S3. Normalized absorbance and emission spectra of $F_{68}\text{Chrom5}[\text{B}(\text{ArF}_{26})_4]$ or $F_{68}\text{Chrom7}[\text{B}(\text{ArF}_{26})_4]$ -labelled emulsions. Emulsions were prepared according to the “Perfluorocarbon emulsions procedure” with **Pluronic F-68** with 1.2 μM $F_{68}\text{Chrom7}[\text{B}(\text{ArF}_{26})_4]$ and 4.8 μM $F_{68}\text{Chrom5}[\text{B}(\text{ArF}_{26})_4]$. The emulsion solution was placed in a 3 mm cuvette and the absorbance spectrum measured. The empty emulsions were also prepared and subtracted. The peak at 480 nm is assigned to the desymmetrized $F_{68}\text{Chrom7}[\text{B}(\text{ArF}_{26})_4]$. To obtain emission spectra, emulsions containing $F_{68}\text{Chrom5}$ were excited at 750 nm, and emulsions containing $F_{68}\text{Chrom7}$ were excited at 920 nm.

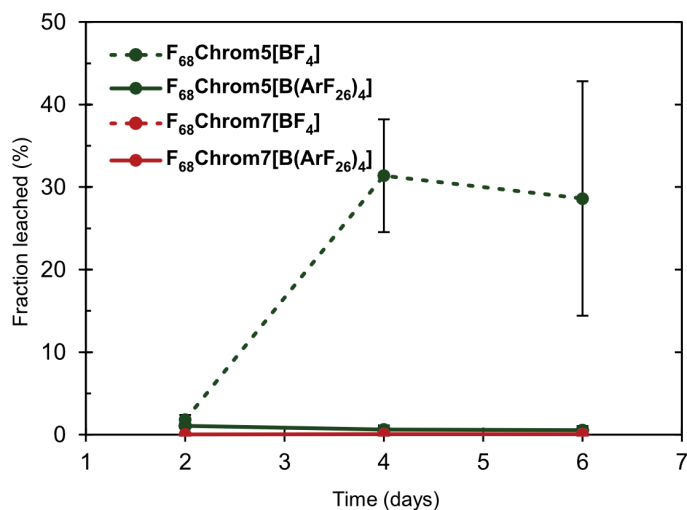


Figure S4. Leaching profiles of **F₆₈Chrom5** and **F₆₈Chrom7** with counterion **BF₄** or **B(ArF₂₆)₄**. Emulsions containing the dyes were prepared according to the “Perfluorocarbon emulsions procedure” with **Pluronic F-68**. The emulsions were then split into 3 equal aliquots, diluted to 1 mL with 1x PBS. 1-octanol was layered on top of the aqueous phase. The entire Eppendorf was wrapped in foil and allowed to rock over a week. Every two days, a 200 μ L aliquot was taken from the octanol layer and the percent leached was quantified by fluorescence. The aliquot was returned to the Eppendorf after measuring fluorescence. The fraction leached is calculated by dividing the integral of the fluorescence of the aliquot by the integral of fluorescence of equimolar dye dissolved in 500 μ L of 1-octanol (equivalent to a 100% leached condition).

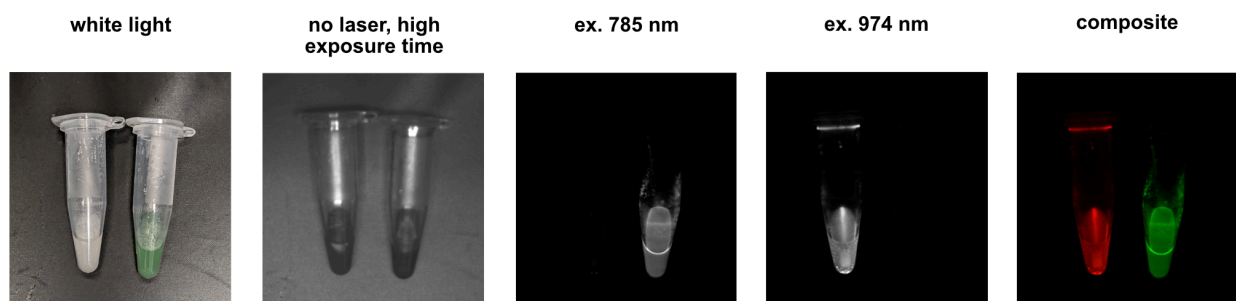


Figure S5. Triggered excitation of emulsions containing **F₆₈Chrom7[B(ArF₂₆)₄]** (0.12 μ M) or **F₆₈Chrom5[B(ArF₂₆)₄]** (0.48 μ M). Emulsions were prepared according to the “Perfluorocarbon emulsions procedure” and imaged directly. The white light image was taken with a phone camera, while the rest are taken on the InGaAs camera. The 785 nm laser is set to flux of 50 mW/cm², and the 974 nm laser is set to flux of 100 mW/cm². Images are collected with a 1100 nm longpass filter. Composite image is created with the “merge colors” tool on ImageJ.

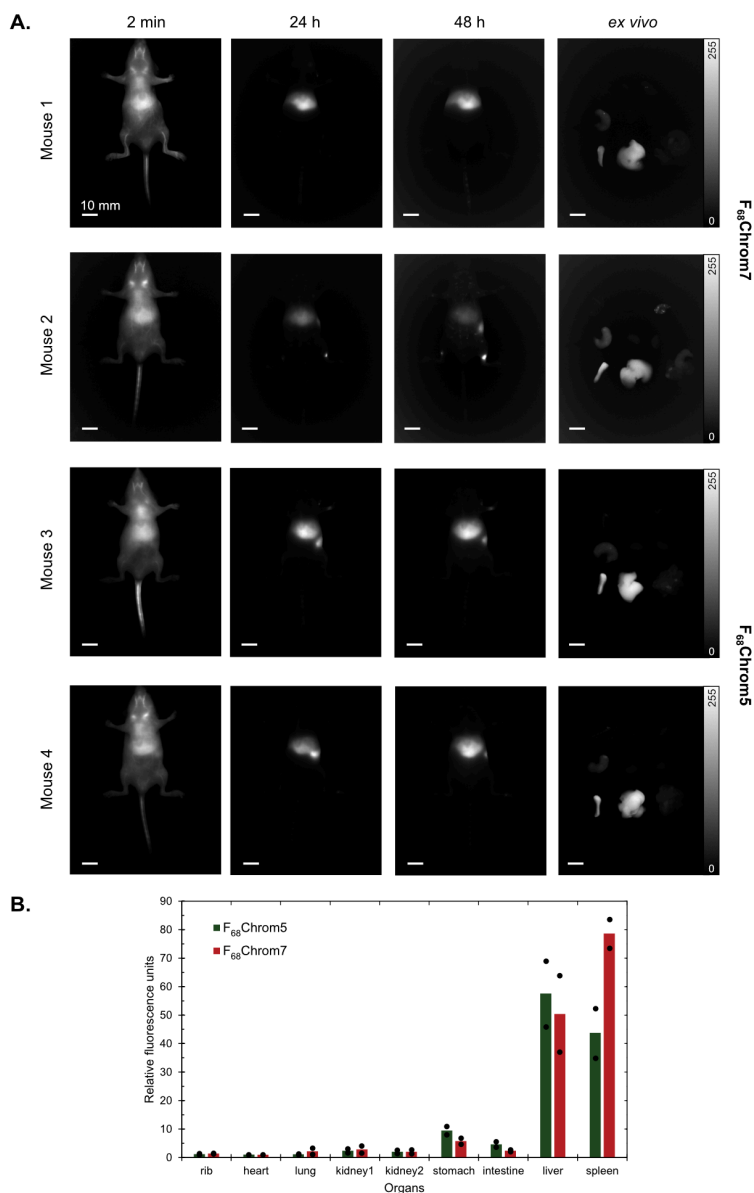


Figure S6. Replicates of Figure 3 (Single color experiments)

A) Dye solutions in PFOB were prepared ($F_{68}\text{Chrom7}[\text{B}(\text{ArF}_{26})_4] = 12 \mu\text{M}$, $F_{68}\text{Chrom5}[\text{B}(\text{ArF}_{26})_4] = 48 \mu\text{M}$). Then emulsions were prepared according to the “general **Pluronic F-68** nanoemulsion preparation procedure.” The emulsions are used as prepared after passing through a $0.45 \mu\text{m}$ syringe filter. Two animals were injected with $100 \mu\text{L}$ of $F_{68}\text{Chrom5}$ - or $F_{68}\text{Chrom7}$ -labelled **Pluronic F-68** emulsions, where mouse 1 and 2 were injected with $F_{68}\text{Chrom7}$ ($1.2 \mu\text{M}$, ex. 974 nm , $100 \text{ mW}/\text{cm}^2$) and mouse 3 and 4 were injected with $F_{68}\text{Chrom5}$ ($4.8 \mu\text{M}$, ex. 786 nm , $50 \text{ mW}/\text{cm}^2$). Images were taken of the animals immediately after tail vein injection, at 24 h, and at 48 h. After sacrificing at 48 h, the organs were excised and imaged to produce the *ex vivo* images. Exposure times ranged from 5 to 30 ms, with longpass filtering at 1100 nm . B) Quantification of organs was performed according to “Image processing for *ex vivo* analysis.” Dots overlaid on bar graph are replicates.

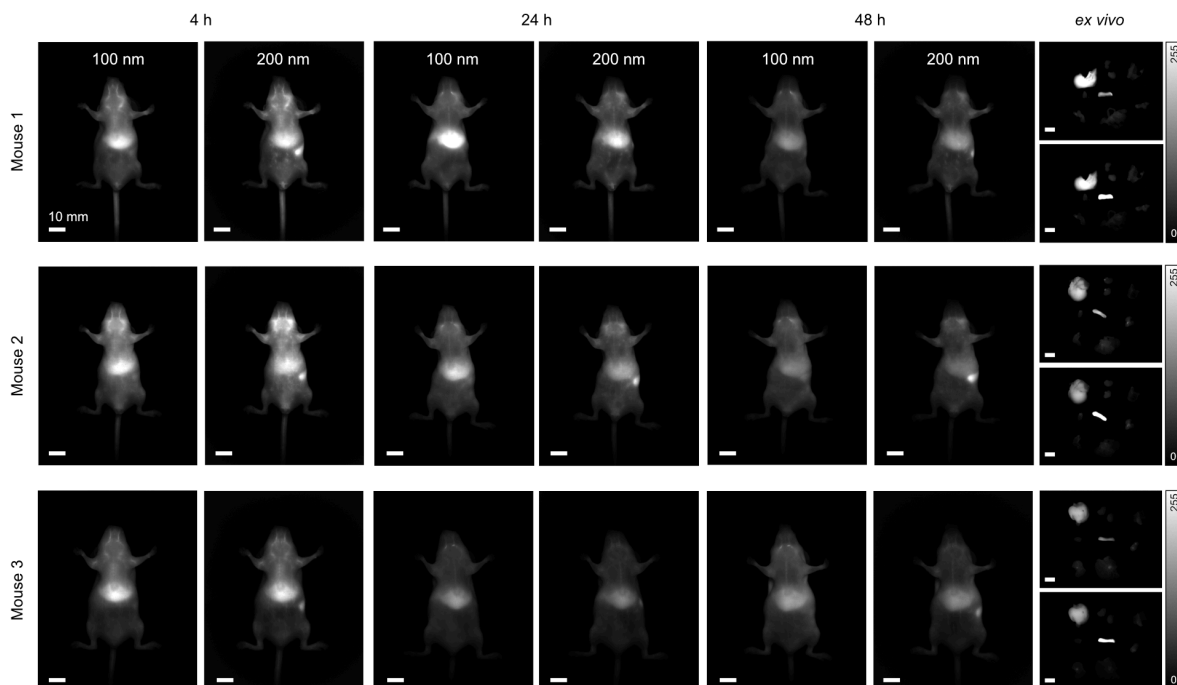


Figure S7. Replicates of Figure 4D (100 and 200 nm)

Dye solutions in PFOB were prepared ($F_{68}\text{Chrom7}[\text{B}(\text{ArF}_{26})_4] = 12 \mu\text{M}$, $F_{68}\text{Chrom5}[\text{B}(\text{ArF}_{26})_4] = 48 \mu\text{M}$). Then emulsions were prepared according to the “general **Pluronic F-68** nanoemulsion preparation procedure.” The emulsions are used as prepared after passing through a $0.45 \mu\text{m}$ syringe filter. $F_{68}\text{Chrom7}$ ($1.2 \mu\text{M}$, ex. 974 nm , 100 mW/cm^2) and $F_{68}\text{Chrom5}$ ($4.8 \mu\text{M}$, ex. 786 nm , 50 mW/cm^2) emulsions were stabilized by **Pluronic F-68**. Three animals were injected with $100 \mu\text{L}$ of $F_{68}\text{Chrom5}$ -labelled 100 nm diameter emulsions and $100 \mu\text{L}$ of $F_{68}\text{Chrom7}$ -labelled 200 nm diameter emulsions. Images were taken of the animals immediately after tail vein injection, at 4 h , 24 h , and 48 h . After sacrificing at 48 h , the organs were excised and imaged to produce the *ex vivo* images. Exposure times ranged from 4 to 7 ms , with longpass filtering at 1100 nm .

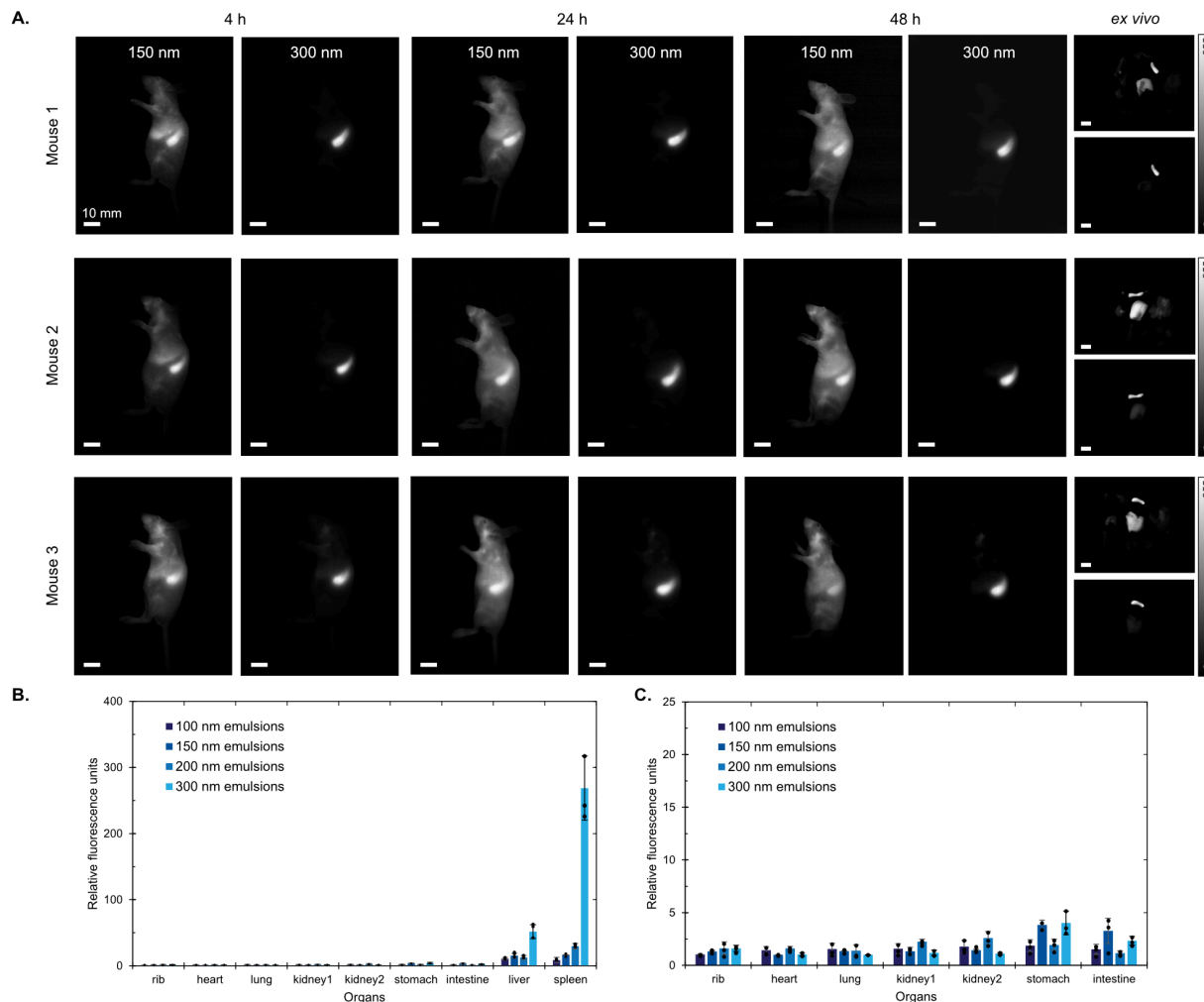


Figure S8. Replicates of Figure 4D (150 and 300 nm)

A) Dye solutions in PFOB were prepared ($F_{68}\text{Chrom7}[\text{B}(\text{ArF}_{26})_4] = 12 \mu\text{M}$, $F_{68}\text{Chrom5}[\text{B}(\text{ArF}_{26})_4] = 48 \mu\text{M}$). Then emulsions were prepared according to the “general **Pluronic F-68** nanoemulsion preparation procedure.” The emulsions are used as prepared after passing through a $0.45 \mu\text{m}$ syringe filter. $F_{68}\text{Chrom7}$ ($1.2 \mu\text{M}$, ex. 974 nm , 100 mW/cm^2) and $F_{68}\text{Chrom5}$ ($4.8 \mu\text{M}$, ex. 786 nm , 50 mW/cm^2) emulsions were stabilized by **Pluronic F-68**. Three animals were injected with $100 \mu\text{L}$ of $F_{68}\text{Chrom5}$ -labelled 150 nm diameter emulsions and $100 \mu\text{L}$ of $F_{68}\text{Chrom7}$ -labelled 300 nm diameter emulsions. Images were taken of the animals immediately after tail vein injection, at 4 h , 24 h , and 48 h . After sacrificing at 48 h , the organs were excised and imaged to produce the *ex vivo* images. Exposure times ranged from 2 to 10 ms , with longpass filtering at 1100 nm . Error bars represent the standard deviation of the three replicates. B) Quantification of organs was performed according to “Image processing for *ex vivo* analysis.” Dots overlaid on bar graph are replicates. Error bars are the standard deviation of the mean, where $n = 3$. C) The same bar graph in B) with the y-axis scaled for better visualization of the smaller intensities.

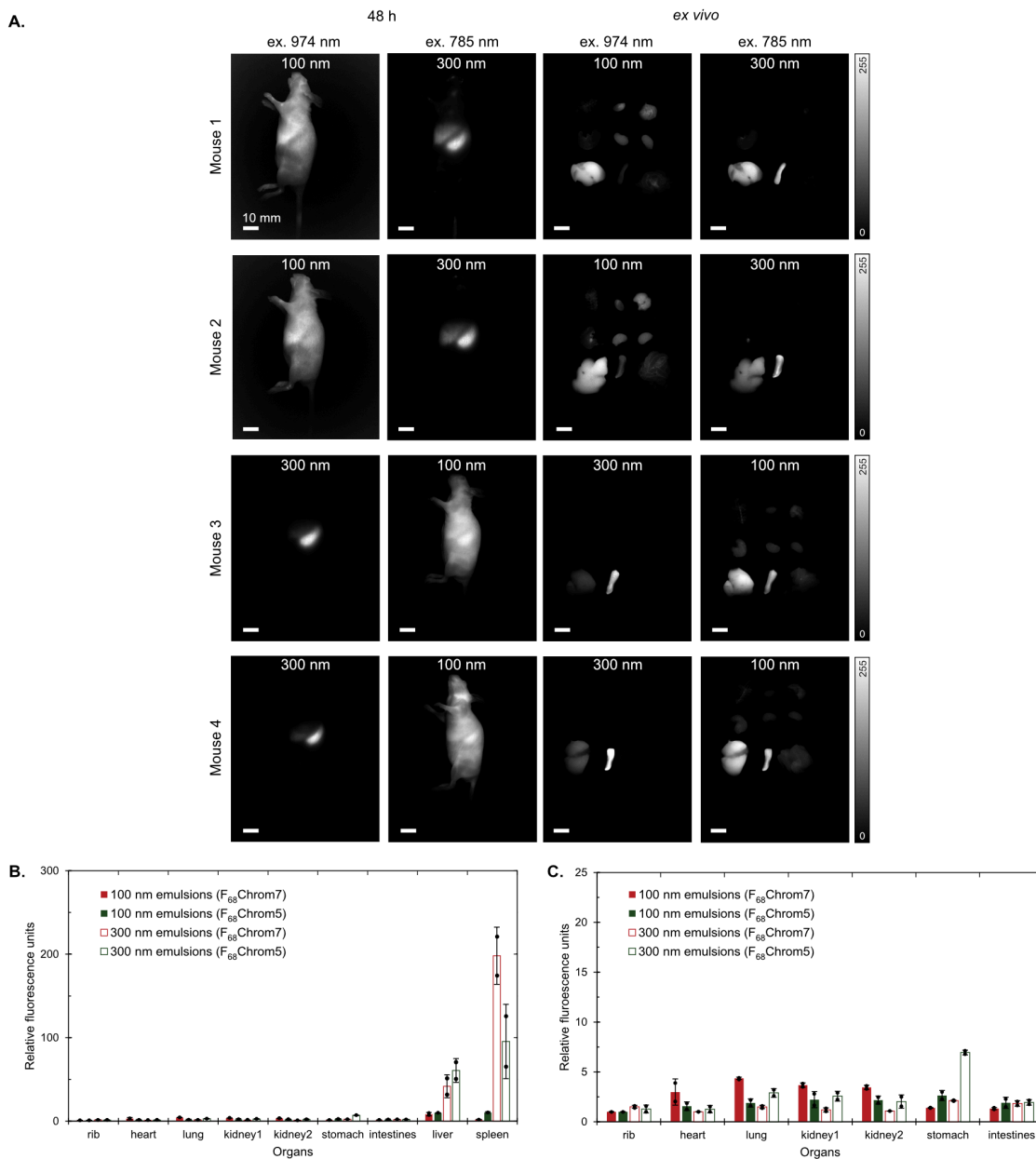


Figure S9. Replicates of Figure 4F,G (Color switch size experiment)

A) Dye solutions in PFOB were prepared ($F_{68}\text{Chrom7}[\text{B}(\text{ArF}_{26})_4] = 12 \mu\text{M}$, $F_{68}\text{Chrom5}[\text{B}(\text{ArF}_{26})_4] = 48 \mu\text{M}$). Then emulsions were prepared according to the “general Pluronic F-68 nanoemulsion preparation procedure.” The emulsions are used as prepared after passing through a $0.45 \mu\text{m}$ syringe filter. $F_{68}\text{Chrom7}$ ($1.2 \mu\text{M}$, ex. 974 nm , 100 mW/cm^2) and $F_{68}\text{Chrom5}$ ($4.8 \mu\text{M}$, ex. 786 nm , 50 mW/cm^2) emulsions were stabilized by Pluronic F-68. Mouse 1 and 2 were injected with $100 \mu\text{L}$ of $F_{68}\text{Chrom7}$ -labelled 100 nm diameter emulsions and $100 \mu\text{L}$ of $F_{68}\text{Chrom5}$ -labelled 300 nm diameter emulsions. Mouse 3 and 4 were injected with $100 \mu\text{L}$ of $F_{68}\text{Chrom7}$ -labelled 300 nm diameter emulsions and $100 \mu\text{L}$ of $F_{68}\text{Chrom5}$ -labelled 100 nm diameter emulsions. Images were taken of the animals immediately after tail vein injection, at 24 h, and 48 h. After sacrificing at 48 h, the organs were excised and imaged to produce the *ex vivo* images. Exposure times ranged from 2 to 10 ms, with longpass filtering at 1100 nm . B) Quantification of organs was performed according to “Image processing for *ex vivo* analysis.” Dots overlaid on bar graph are replicates. Error bars are the standard deviation of the mean, where $n = 2$. C) The same bar graph in B) with the y-axis scaled for better visualization of the smaller intensities.

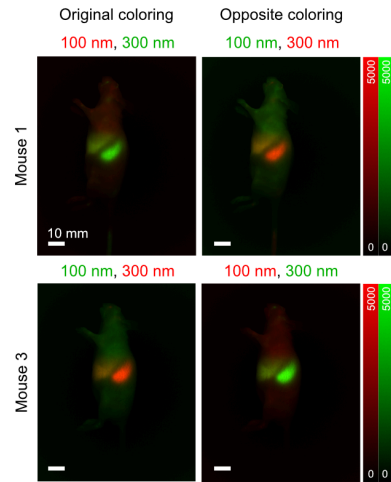


Figure S10. Opposite colors displayed in Figure 4F,G

The same experimental procedures apply from Figure S9. In the “original coloring” column, mouse 1 and 3 are displayed in the original colors in the main text. In the “opposite coloring” column, the single color images were switched from their original corresponding channels, keeping the same brightness settings from the original composite image.

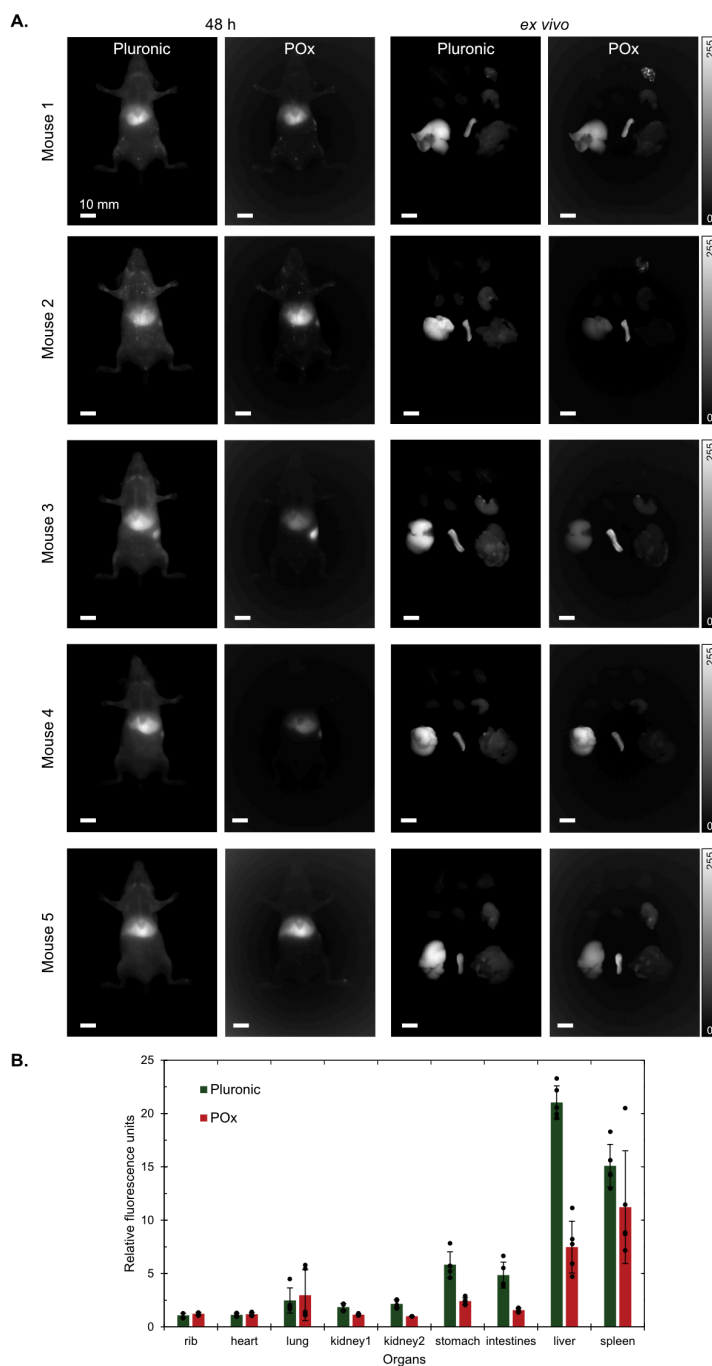


Figure S11. Replicates of Figure 5C (POx vs Pluronic)

A) Dye solutions in PFOB were prepared ($F_{68}\text{Chrom7}[\text{B}(\text{ArF}_{26})_4] = 12 \mu\text{M}$, $F_{68}\text{Chrom5}[\text{B}(\text{ArF}_{26})_4] = 48 \mu\text{M}$). Then emulsions were prepared according to the “Perfluorocarbon emulsions procedures.” The emulsions are used as prepared after passing through a $0.45 \mu\text{m}$ syringe filter. $F_{68}\text{Chrom7}$ ($1.2 \mu\text{M}$, ex. 974 nm , $100 \text{ mW}/\text{cm}^2$) emulsions were stabilized by **POx** (**4**) and $F_{68}\text{Chrom5}$ ($4.8 \mu\text{M}$, ex. 786 nm , $50 \text{ mW}/\text{cm}^2$) emulsions were stabilized by **Pluronic F-68**. Five animals were injected with $100 \mu\text{L}$ of $F_{68}\text{Chrom5}$ -labelled **Pluronic F-68** emulsions and $100 \mu\text{L}$ of $F_{68}\text{Chrom7}$ -labelled **POx** emulsions. Images were taken of the animals immediately after tail vein injection, at 24 h, and 48 h. After sacrificing at 48 h, the organs were excised and imaged to produce the *ex vivo* images. Exposure times ranged from 3 to 10 ms, with

longpass filtering at 1100 nm. B) Quantification of organs was performed according to “Image processing for *ex vivo* analysis.” Dots overlaid on bar graph are replicates. Error bars represent the standard deviation of the three replicates. Significance was calculated using a paired student’s t-test with a 2-tailed distribution assumption.

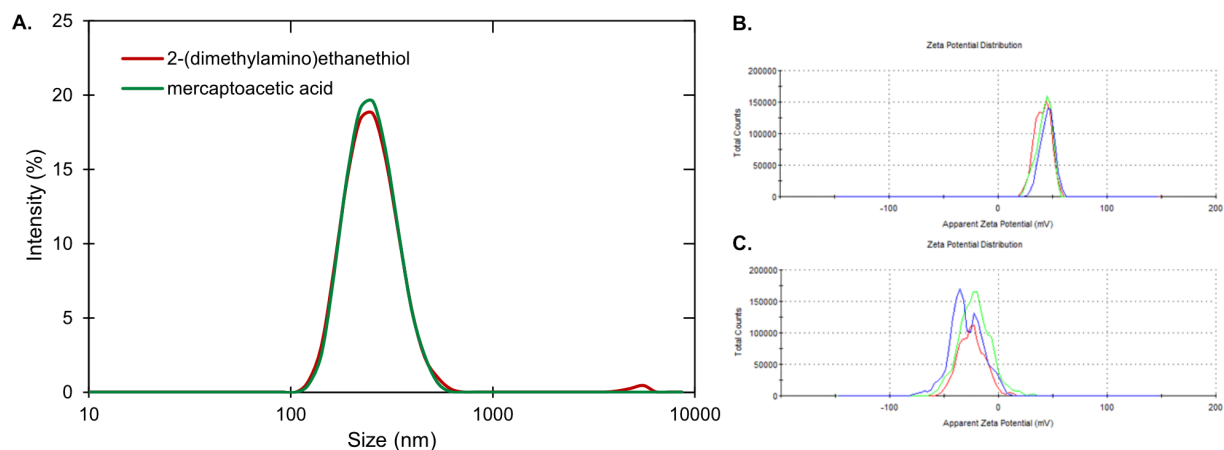


Figure S12. A) Dynamic light scattering intensity graph of emulsions in **Figure 5**. DLS measurements were taken after the thiol-ene reaction. Emulsions were generated according to “Procedure for thiol-ene modified EnePOx (5) emulsions” and diluted 1:100 for the zeta potential measurement. B) Raw zeta potential distributions for positively charged emulsions, with the three replicate measurements shown. C) Raw zeta potential distributions for negatively charged emulsions, with the three replicate measurements shown.

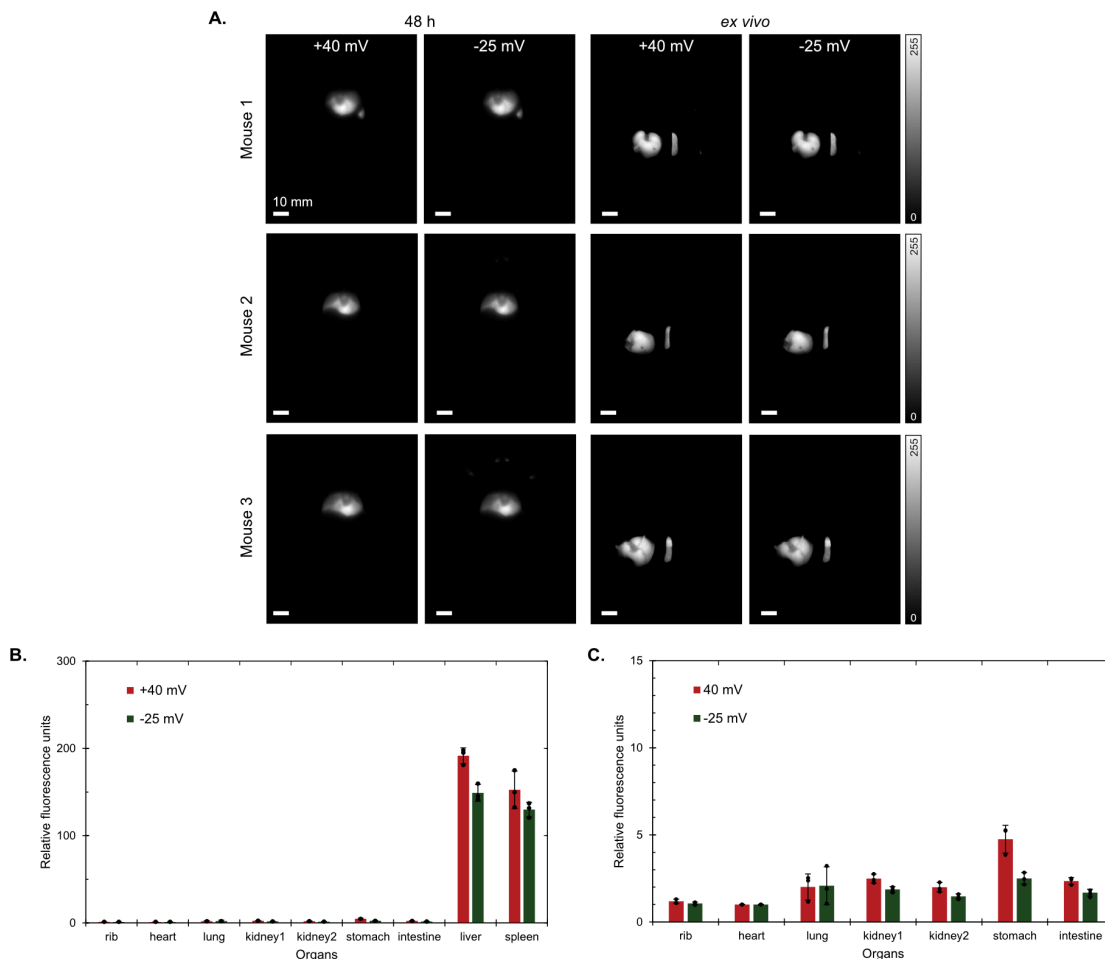


Figure S13. Replicates of Figure 5G (Charge dependence)

A) Dye solutions in PFOB were prepared ($F_{68}\text{Chrom7}[\text{B}(\text{ArF}_{26})_4] = 12 \mu\text{M}$, $F_{68}\text{Chrom5}[\text{B}(\text{ArF}_{26})_4] = 48 \mu\text{M}$). Emulsions were prepared as described in “Procedure for thiol-ene modified EnePOx (**5**) emulsions.” $F_{68}\text{Chrom7}$ ($1.2 \mu\text{M}$, ex. 974 nm, $100 \text{ mW}/\text{cm}^2$) emulsions were negatively charged (-25 mV) and $F_{68}\text{Chrom5}$ ($4.8 \mu\text{M}$, ex. 786 nm, $50 \text{ mW}/\text{cm}^2$) emulsions were positively charged ($+40 \text{ mV}$). Three animals were injected with $100 \mu\text{L}$ of each suspension. Images were taken of the animals immediately after tail vein injection, at 1 h, 24 h, and 48 h. After sacrificing at 48 h, the organs were excised and imaged to produce the *ex vivo* images. Exposure times ranged from 1 to 10 ms, with longpass filtering at 1100 nm. B) Quantification of organs was performed according to “Image processing for *ex vivo* analysis.” Dots overlaid on bar graph are replicates. Error bars represent the standard deviation of the three replicates. Significance was calculated using a paired student’s t-test with a 2-tailed distribution assumption. C) The same bar graph in B) with the y-axis scaled for better visualization of the smaller intensities.

Supplemental tables:**Table S1.** Photophysical measurements of chromenylium polymethine dyes in acetone and PFOB

Dye	F₆₈Chrom5			F₆₈Chrom7		
	BF ₄	B(ArF ₂₆) ₄		BF ₄	B(ArF ₂₆) ₄	
Counterion	acetone	acetone	PFOB	acetone	acetone	PFOB
λ_{abs} (nm)	809	809	807	968	967	957
λ_{em} (nm)	833	832	823	998	997	969
ϵ_{max} (M ⁻¹ cm ⁻¹)	229,000 ± 8000	123,000 ± 2,000	101,000 ± 5,000	167,000 ± 5,000	64,000 ± 1,000	273,000 ± 6,000
Φ_{F} (%)	46 ± 4	41.1 ± 0.7	47.3 ± 0.4	0.98 ± 0.04	1.12 ± 0.02	1.04 ± 0.04

Table S2. Solubility limits of chromenylium polymethine dyes in PFMC and PFOB

Dye	F₆₈Chrom5				F₆₈Chrom7			
	BF ₄		B(ArF ₂₆) ₄		BF ₄		B(ArF ₂₆) ₄	
Counterion	PFMC	PFOB	PFMC	PFOB	PFMC	PFOB	PFMC	PFOB
Solubility limit (μM)	2.37 ± 0.09	1.49 ± 0.09	32.0 ± 0.2	27.5 ± 0.4	4.5 ± 0.1	0.6 ± 0.1	1.17 ± 0.2	1.2 ± 0.2

Synthetic chemistry procedures:

Abbreviations

DCM: dichloromethane, methylene chloride; DIPEA: diisopropylethylamine; DMF: dimethyl formamide; EtOAc: ethyl acetate; EtOH: ethanol; HCl: hydrochloric acid; MeCN: acetonitrile; PFOB: perfluorooctylbromide; PFMC: perfluoromethylcyclohexane; SiO₂: silica gel; THF: tetrahydrofuran.

Materials

3-aminophenol, 1-Iodo-1H,1H,2H,2H,3H,3H-perfluoroundecane, and malonaldehyde bis(phenylimine) monohydrochloride were purchased from Sigma-Aldrich. Ethyl 4,4-dimethyl-3-oxopentanoate was purchased from TCI. 1.4 M methyl magnesium bromide in THF was purchased from Acros Organics. Anhydrous solvents were dispensed directly from a Grubb's-type Phoenix Solvent Drying System constructed by JC Meyer or kept dry under sieves in a Schlenk bomb. All chemicals were used as received unless otherwise stated, with the exception of 2-methyl-4,5-dihydrooxazole, which was distilled prior to use in polymerizations. All reactions were done under dry Schlenk technique unless otherwise noted.

Instrumentation

Microwave reactions were performed using a CEM Discover SP microwave synthesis reactor. All reactions were performed in glass 10 mL microwave reactor vials purchased from CEM with silicone/PTFE caps. Flea micro PTFE-coated stir bars were used in the vials with magnetic stirring set to high and 15 seconds of premixing prior to the temperature ramping. All microwave reactions were carried out with the pressure release limit set to 250 psi (no reactions exceeded this limit to trigger venting) and the maximum wattage set to 300 W (the power applied was dynamically controlled by the microwave instrument and did not exceed this limit for any reactions). Thin layer chromatography was performed with Silica Gel 60 F254 (EMD Millipore) plates and visualized with UV light. Flash chromatography was executed with technical grade silica gel purchased from Sorbtech Technologies with 60 Å pores and 40 – 63 µm mesh particle size. Flash column chromatography was performed on technical grade silica gel with 60 Å pores and 40–63 µm mesh particle size (Sorbtech Technologies). Solvent was removed by rotary evaporation on a Büchi Rotovapor with a Welch self-cleaning dry vacuum pump and further dried by reduced pressure with a Welch DuoSeal pump.

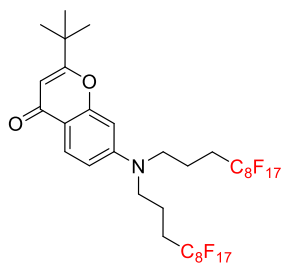
¹H NMR, ¹³C {¹H} NMR, and ¹⁹F {¹H} NMR spectra were recorded on a Bruker AV-400 (¹H, ¹⁹F {¹H}), Bruker Avance-500 (¹H, ¹³C {¹H}), or AV-300 (¹H, ¹⁹F {¹H}) instruments at the UCLA Molecular Instrumentation Center, and chemical shifts are reported in parts per million (ppm). Spectra were recorded in chloroform (CDCl₃) or acetone-*d*₆ and referenced to residual solvent peak. Multiplicities are as indicated: s (singlet), d (doublet), t (triplet), q (quartet), qn (quintet), m (multiplet), bs (broad singlet), and bm (broad multiplet). Coupling constants, *J*, are reported in Hertz (Hz) and integration is provided. Mass spectrometry were performed by electrospray ionization (ESI) on a Waters LCT Premier TOF LC/MS with ACQUITY UPLC. Size Exclusion Chromatography (SEC)/Gel Permeation Chromatography (GPC) was conducted on an Agilent 1260 infinity II high performance liquid chromatography (HPLC) system with a Wyatt Optilab (RI and MALS detection), one Polymer Laboratories PLgel guard column, and two Polymer Laboratories PLgel 5 µm mixed D columns. Eluent was DMF with LiBr (0.1 M) at 40 °C (flow rate: 0.60 mL/min). Masses for analytical measurements were taken on a Sartorius MSE6.6S-000-DM S13 Cubis Micro Balance. Bath sonication was performed using a Branson 3800 ultrasonic cleaner. Probe sonication for nanoemulsion preparation was performed using a QSonica (Q125) probe sonicator. Dynamic light scattering measurements were performed on a Malvern Zetasizer Nano dynamic light scattering instrument. SOP parameters: 10 runs, 10 seconds/run, three measurements, no delay between measurements, 25 °C with 120 second equilibration time. Data are representative of three replicate measurements.

Synthetic procedures:

Synthesis of fluororous aminophenol (**1**)

The synthesis of this material has been reported previously.^[1] To a dry 2-neck flask under positive N₂ pressure, 3-aminophenol (50.0 mg, 0.458 mmol, 1.00 equiv.), 4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl iodide (623 mg, 1.06 mmol, 2.31 equiv.), DIPEA (96 μ L, 0.55 mmol, 1.2 equiv.), and DMF (970 μ L, 0.47 M) are added. The reaction is stirred at 120 °C for 16 hours. The resulting mixture is purified via flash chromatography (SiO₂; hexane:EtOAc 20:1 \rightarrow 15:1) to afford 285 mg (0.277 mmol, 61%) of an off-white solid. R_f = 0.45 in 4:1 hexane:EtOAc. ¹H and ¹⁹F NMR spectra match those of the literature.

Synthesis of fluororous chromone **S1**



To a microwave vial, aminophenol **1** (277.4 mg, 0.3668 mmol, 1.0 equiv.) and neat ethyl 4,4-dimethyl-3-oxopentanoate (1.3 mL, 7.3 mmol, 20. equiv.) are added. The reaction is placed in the microwave and heated at 240 °C for two 5 minute increments (10 minute total reaction time, 150 W), including ramping and cooling cycles. The crude mixture is loaded on silica and purified by flash chromatography from 20:1 \rightarrow 10:1 \rightarrow 8:1 \rightarrow 6:1 \rightarrow 4:1 hexane:EtOAc. The product is isolated as a light yellow solid in 57% yield (0.211 mmol, 240 mg). R_f = 0.07 in 4:1 hexane:EtOAc. ¹H NMR (400 MHz, CDCl₃): δ 8.01 (d, *J* = 9.01 Hz, 1H), 6.69 (dd, *J* = 9.06 Hz, 2.38 Hz, 1H), 6.47 (d, *J* = 2.32 Hz, 1H), 6.16 (s, 1H), 3.49 (t, *J* = 15.17 Hz, 4H), 2.16 (br m, 4H), 1.97 (qn, *J* = 6.92 Hz, 4H), 1.32 (s, 9H). ¹³C NMR (126 MHz, CDCl₃): δ 178.3, 175.3, 159.0, 151.6, 127.3, 114.0, 110.5, 106.3, 97.7, 50.3, 34.4. ¹⁹F NMR (376 MHz, CDCl₃): δ -80.94 (t, *J* = 9.955 Hz, 6F), -113.80 (t, *J* = 13.44 Hz, 4F), 121.79 (br s, 4F), -122.015 (br s, 8F), -122.83 (br s, 4F), -123.39 (br s, 4F), -126.24 (br s, 4F). HRMS (ESI⁺): Calculated for formula C₃₅H₂₅F₃₄NO₂ [M+1]⁺: 1138.1444; found: 1138.1421.

Synthesis of fluororous chromenylium (**2**)

To a dry 2-neck flask under positive N₂ pressure, chromone **S1** (68.5 mg, 0.0602 mmol, 1.00 equiv.) is dissolved in THF (600 μ L, 0.1 M). MeMgBr is added dropwise (1.0 M solution, 0.3011 mmol, 600 μ L, 10 equiv.), and the reaction is stirred at room temperature overnight. The next day, the reaction is quenched with a 5% aqueous solution of HBF₄ to set the counterion. Then the mixture is extracted in DCM (3 x 20 mL). A back extraction was also performed (20 mL). The DCM is removed *in vacuo*, and the dark yellow solid is washed several times with ether (3 x 50 mL portions, with sonication) and filtered. The product is a bright yellow/green solid isolated in 67% yield (0.040 mmol, 45.6 mg). ¹H NMR (400 MHz, acetone-*d*₆): δ 8.32 (d, *J* = 9.69 Hz, 1H), 7.77 (dd, *J* = 9.73 Hz, 2.56 Hz, 1H), 7.59 (s, 1H), 7.51 (d, *J* = 2.53 Hz, 1H), 4.06 (t, *J* = 15.78 Hz, 4H), 2.49 (septet, *J* = 16.2 Hz, 4H), 2.16 (br m, 4H), 1.52 (s, 9 H). ¹³C NMR (126 MHz, acetone-*d*₆): δ 182.0, 167.1, 161.0, 158.1, 130.2, 119.3, 118.6, 113.2, 97.3, 51.1, 38.9, 28.3, 20.1. δ ¹⁹F NMR (376 MHz, acetone-*d*₆): δ -81.63 (t, *J* = 10.03 Hz, 6F), -114.45 (t, *J* = 13.32 Hz, 4F), -122.25 (br s, 4F), -122.43 (br s, 8F), -123.25 (br s, 4F), -123.93 (br s, 4F), -126.71 (br s, 4F), -151.79 (s, 4F). HRMS (ESI⁺): Calculated for formula C₃₆H₂₈F₃₄NO [M]⁺: 1136.1619; found: 1136.1622.

Synthesis of **F₆₈Chrom5[BF₄]**

To a Schlenk flask, chromenylium **2** (21.7 mg, 0.0177 mmol, 2.00 equiv.), malonaldehyde bis(phenylimine) hydrochloride (2.28 mg, 0.00887 mmol, 1.00 equiv.), sodium acetate (3.8 mg, 0.046 mmol, 5.2 equiv.), and acetic anhydride (177 μ L, 0.10 M) are added. The mixture is freeze-pump-thawed thrice before heating to 140 °C for 20 minutes. The reaction is cooled to room temperature and the product is crashed out with toluene (1 mL) and washed several times with toluene (3 x 10 mL portions). The resulting dark green solid is filtered and isolated in 51% yield (0.00452 mmol, 10.9 mg). The crystals are iridescent red. ¹H NMR (500 MHz, acetone-*d*₆): δ 8.17 (t, *J* = 12.7 Hz, 2H), 8.11 (d, *J* = 9.5 Hz, 2H), 7.23 (dd, *J* = 9.4, 2.5 Hz, 2H), 7.08 (s, 1 H), 7.06 (s, 2H), 6.99 (d, *J* = 2.6 Hz, 2H), 6.85 (t, *J* = 12.5 Hz, 1H), 3.86 (t, *J* = 7.7 Hz, 3H), 3.58 (s, 8H), 2.45 (septet, *J* = 7.8 Hz, 12H), 2.14 – 2.08 (br m, 8H), 1.42 (s, 18H). ¹¹B NMR (128 MHz, acetone-*d*₆): δ -1.14 (s). ¹⁹F NMR (376 MHz, acetone-*d*₆): δ -81.64 (t, *J* = 10.0 Hz, 12F), -114.42 (t, *J* = 14.2 Hz, 6F), -122.19 (br s, 6F), -122.41 (br s, 12F), -123.23 (br s, 6F), -123.91 (br s, 6F), -126.70 (br s, 6F), -151.87 (s, 4F). MALDI-TOF MS (ESI⁺): Calculated for formula C₇₅H₅₅F₆₈N₂O₂ [M]⁺: 2307.3172; found: 2307.3190.

Synthesis of **F₆₈Chrom7[BF₄]**

To a Schlenk flask, chromenylium **2** (52.3 mg, 0.0428 mmol, 1.00 equiv.), *N*-((3-(anilino)methylene)-2-chloro-1-cyclohexen-1-yl)methylene)aniline hydrochloride (16.9 mg, 0.0470 mmol, 1.10 equiv.), 2,6-lutidine (9.9 μ L, 0.086 mmol, 2.0 equiv.), and a premade 2.75:1 *n*-butanol:toluene mixture (420 μ L, 0.1 M) are added. The mixture is freeze-pump-thawed thrice before heating to 110 °C to react at reflux for 45 minutes. The reaction is cooled to room temperature and the product is washed with toluene (2 x 20 mL portions), EtOAc (2 x 20 mL portions), and boiling THF (2 x 20 mL portions). The resulting dark purple solid is filtered, washed with toluene again, and isolated in 12% yield (0.00497 mmol, 12.4 mg). ¹H NMR (500 MHz, acetone-*d*₆): δ 8.29 (d, *J* = 14 Hz, 2H), 8.22 (d, *J* = 9.5 Hz, 2H), 7.23 (dd, *J* = 9.4, 2.7 Hz, 2H), 7.15 (d, *J* = 13.9 Hz, 2H), 7.04 (m, 4H), 3.87 (t, *J* = 7.8 Hz, 8H), 2.90 – 2.85 (m, 8H), 2.44 (td, *J* = 19.3, 9.4 Hz, 8H), 2.15 – 2.09 (m, 8H), 1.44 (s, 18H). ¹¹B NMR (128 MHz, acetone-*d*₆): δ -1.14 (s). ¹⁹F NMR (376 MHz, acetone-*d*₆): δ -81.64 (t, *J* = 10.0 Hz, 12F), -114.31 (br t, 8F), -122.19 (br s, 8F), -122.40 (br m, 16F), -123.23 (br s, 8F), -123.91 (br s, 8F), -126.71 (br s, 8F), -151.92 (s, 4F). MALDI-TOF MS (ESI⁺): Calculated for formula C₈₀H₆₀ClF₆₈N₂O₂ [M]⁺: 2407.3252; found: 2407.2887.

Counterion exchange procedures

Either **F₆₈Chrom5** (17.0 mg, 0.00710 mmol) or **F₆₈Chrom7** (4.5 mg, 0.0018 mmol) was aliquoted into a scintillation vial. Two equivalents of **NaB(ArF₂₆)₄** were weighed out and added to the vial. The contents were dissolved in methoxyperfluorobutane (~5 mL/10 mg dye) and sonicated for 1 minute. Exchange was confirmed by TLC in an acetone eluent, where R_f of the exchanged dye is 1, and the R_f of the unexchanged dye is 0. The contents were then purified via silica gel column chromatography with a hexane:DCM eluent. Both exchanged dyes elute at 1:1 hexane:DCM. The fractions deemed pure by UV-Vis analysis were collected and dried *in vacuo*. **F₆₈Chrom5[B(ArF₂₆)₄]**: 21.7 mg, 0.0042 mmol, 60% yield. **F₆₈Chrom7[B(ArF₂₆)₄]**: 3.3 mg, 0.00063 mmol, 35% yield.

F₆₈Chrom5[B(ArF₂₆)₄]

¹H NMR (500 MHz, acetone-*d*₆): δ 8.15 (t, *J* = 13 Hz, 2H), 8.09 (d, *J* = 9.7 Hz, 2H), 7.70 (s, 8H), 7.57 (s, 4H), 7.22 (dd, *J* = 9.5, 2.6 Hz, 2H), 7.07 (s, 2H), 7.04 (s, 2H), 6.99 (d, *J* = 2.6 Hz, 2H), 6.83 (t, *J* = 12.5 Hz, 2H), 3.85 (t, *J* = 7.7 Hz, 8H), 2.43 (septet, *J* = 8.0 Hz, 8H), 2.13 – 2.06 (m, 6H), 2.09 – 1.99 (m, 8H), 1.40 (s, 18H). ¹¹B NMR (128 MHz, acetone-*d*₆): δ -6.56. ¹³C NMR (126 MHz, acetone-*d*₆): δ 170.5, 157.1, 153.1, 149.9, 147.4, 136.7, 127.3, 126.1, 113.3, 111.5, 99.7, 97.7, 49.5, 36.5, 22.4, 18.3. ¹⁹F NMR (376 MHz, acetone-*d*₆): δ -81.66 (t, *J* = 10.2 Hz, 12F), -82.00 (t, *J* = 10.3 Hz, 24F), -111.60 (t, *J* = 14.0 Hz, 12F), -

114.34 (br t, 6F), -122.24 (br s, 16F), -122.43 (br s, 12F), -123.27 (br s, 16F), -123.69 (br s, 12F), -123.93 (br s, 6F), -126.72 (br s, 6F), -127.10 (br s, 6F).

F₆₈Chrom7[B(ArF₂₆)₄]

¹H NMR (500 MHz, acetone-*d*₆): δ 8.27 (d, *J* = 13.7 Hz, 2H), 8.20 (d, *J* = 9.6 Hz, 2H), 7.70 (s, 8H), 7.57 (s, 4H), 7.22 (dd, *J* = 9.6, 2.7 Hz, 2H), 7.13 (d, *J* = 13.8 Hz, 2H), 7.04 (s, 2H), 7.01 (d, *J* = 2.6 Hz, 2H), 3.86 (t, *J* = 7.7 Hz, 8H), 2.43 (septet, *J* = 8.1 Hz, 8H), 1.42 (s, 18H). ¹¹B NMR (128 MHz, acetone-*d*₆): δ -6.57. ¹³C NMR (126 MHz, acetone-*d*₆): δ 170.8, 157.1, 153.1, 146.2, 139.3, 136.7, 130.2, 126.4, 112.4, 99.8, 97.6, 49.6, 36.6, 22.5, 18.2, 13.4. ¹⁹F NMR (376 MHz, acetone-*d*₆): δ -81.66 (t, *J* = 10.1 Hz, 12F), -82.00 (t, *J* = 10.3 Hz, 24F), -111.60 (t, *J* = 14.0 Hz, 12F), -114.33 (t, *J* = 14.0 Hz, 6F), -122.23 (br m, 16F), -122.42 (br m, 12F), -123.29 (br m, 16F), -123.66 (br m, 12F), -123.92 (br m, 6F), -126.54 (br m, 6F), -127.12 (br m, 6F).

Synthesis of POx (4)

This synthesis has been reported previously.^[3] To a flame dried microwave vial, MeCN (0.8 mL, anhydrous) and 2-methyl-4,5-dihydrooxazole (200. μL, 201 mg, 2.36 mmol, 30.0 equiv.) were added. MeOTf (8.9 μL, 13. mg, 0.079 mmol, 1.0 equiv.) was then added and the solution was mixed vigorously. The mixture was heated at 140 °C in the microwave. After 10 minutes, 2-nonyl-4,5-dihydrooxazole (155 μL, 155 mg, 0.786 mmol, 10.0 equiv.) was added under N₂ and heated to 140 °C for 3 minutes, at which point the polymerization was quenched with a saturated KOH in methanol solution (500. μL, 3.90 mmol, 49.0 equiv.) and was stirred at 45 °C overnight. The reaction mixture was evaporated to dryness to yield crude polymer **POx (4)** as a white solid. Polymer was purified by dissolving in DCM and passing through a syringe filter to desalt. The solvent was then removed, and the polymer was redissolved in water and dialyzed in water overnight. The sample was lyophilized to dryness (110.0 mg, 0.02607 mmol, 33% yield). ¹H NMR (500 MHz, CDCl₃): δ 3.44 (m, 139H), 3.04 (m, 3H), 2.32 (m, 20H) 2.11 (m, 79H), 1.58 (m, 19H), 1.25 (m, 115H), 0.86 (t, 29H). SEC (DMF): *M_w* = 8.1 kDa, *M_n* = 7.4 kDa, *D* = 1.1. Measured dn/dc = 0.0591 mL/g.

Synthesis of EnePOx (5)

This synthesis has been reported previously.^[3] To a flame dried microwave vial, MeCN (1.5 mL, anhydrous), 2-methyl-4,5-dihydrooxazole (400. μL, 400 mg, 4.70 mmol, 30.0 equiv.), and 2-(but-3-en-1-yl)-4,5-dihydrooxazole (70. μL, 80 mg, 0.64 mmol, 4.0 equiv.) were added. MeOTf (17.7 μL, 25.7 mg, 0.157 mmol, 1.00 equiv.) was then added and the solution was mixed vigorously. The mixture was heated at 140 °C in the microwave. After 10 minutes, 2-nonyl-4,5-dihydrooxazole (310. μL, 309 mg, 1.57 mmol, 9.81 equiv.) was added under N₂ and heated to 140 °C for 3 minutes, at which point the polymerization was quenched with a saturated KOH in methanol solution (150 μL, 1.2 mmol, 6.0 equiv.) and was stirred at 45 °C overnight. The reaction mixture was evaporated to dryness to yield crude EnePOx (**5**) as a white solid. Polymer was purified by dissolving in DCM and passing through a syringe filter to desalt. The solvent was then removed, and the polymer was redissolved in water and dialyzed in water overnight. The sample was lyophilized to dryness (452.1 mg, 0.09 mmol, 45% yield). ¹H NMR (500 MHz, CDCl₃): δ 5.82 (m, 3H), 5.03 (m, 5H), 3.44 (m, 170H), 3.04 (m, 3H), 2.32 (m, 23H) 2.13 (m, 110H), 1.58 (m, 25H), 1.25 (m, 152H), 0.86 (t, 38H). SEC (DMF): *M_w* = 5.6 kDa, *M_n* = 5.1 kDa, *D* = 1.1. Measured dn/dc 0.0398 mL/g.

Photophysical measurements:

Instrumentation

Absorption spectra are recorded on a Jasco V-770 spectrophotometer. Fluorescence spectra are measured with a Horiba Fluorometer PTI QM-400. Spectra for **F₆₈Chrom5** are taken on the PMT detector, whereas spectra for **F₆₈Chrom7** are taken on the InGaAs detector. Quartz cuvettes (1 cm or 0.3 cm) are used for absorbance and photoluminescence measurements.

Absorption coefficient measurement

The absorption coefficient is calculated according to Beer Lambert's law and is reported as the mean value of three independent determinations, with standard deviation as error:

$$A = \epsilon l c$$

where A is the absorbance in O.D., l is the path length in cm, and c is the concentration in molarity.

All masses are measured on a Sartorius MSE6.6S-000-DM Cubis Micro Balance. The first stock solution is prepared in 1 mL of solvent, measured by Hamilton syringe, and further diluted by Hamilton syringes. At least five concentrations in triplicate are measured before determining the absorption coefficients.

Fluorescence quantum yield measurement

The fluorescence quantum yield Φ_F of a material is defined as follows:

$$\Phi_F = \frac{P_E}{P_A}$$

where P_E , P_A are the number of photons emitted and absorbed respectively. To determine absolute quantum yield, the number of photons absorbed and emitted are measured independently. The quantum yield measurements for **F₆₈Chrom5** are performed with a Horiba petite integrating sphere with the following settings:

Detector: PMT. Excitation: 750 nm. Emission: 770-1200 nm, 720-780 nm. Slit widths: 12 nm. Integration time: 0.1 second.

The quantum yield measurements for **F₆₈Chrom7** are measured relative to IR-26 ($\Phi_F = 0.0005 \pm 0.0003$ in DCM)^[4] according to the formula below:

$$\Phi_{F,x} = \Phi_{F,r}(m_x/m_r)(\eta_x^2/\eta_r^2)$$

where m is the slope of the line obtained from graphing integrated fluorescence intensity over the optical density, η is the refractive index of the solvent (acetone = 1.3586, DCM = 1.4244, PFOB = 1.3048), subscript x represents the **F₆₈Chrom7** and subscript r represents IR-26. The spectra are measured with a Horiba fluorimeter PTI QM-400 on the InGaAs detector. The sample optical density is kept below 0.1 to avoid fluorescence quenching and reabsorption effects. The experimental setup is adjusted and tested on standard dyes. Error for relative measurements is propagated from the error of the reference. Error for absolute measurements is the standard deviation of at least 3 replicate measurements. Emission spectra are baseline corrected.

All relevant photophysical measurements with errors are reported in **Table S1**.

Photobleaching measurements

Absorbance-matched (0.6 O.D.) solutions of **Chrom5**, **Chrom7** in DMSO, **F₆₈Chrom5[B(ArF₂₆)₄]**, **F₆₈Chrom7[B(ArF₂₆)₄]** in acetone, and **F₆₈Chrom5[B(ArF₂₆)₄]**, **F₆₈Chrom7[B(ArF₂₆)₄]** in PFOB were prepared. Note that **Chrom5** and **Chrom7** are not soluble in acetone, and **F₆₈Chrom5[B(ArF₂₆)₄]** and **F₆₈Chrom7[B(ArF₂₆)₄]** are not soluble in DMSO. These solutions were placed in capillary tubes, sealed, and placed on a holder under an InGaAs camera to detect emission intensity. An 1100 nm LP filter was placed over the lens of the camera. See “SWIR Imaging Apparatus” for a description of the setup.

The rate of photobleaching was measured by observing the rate of decrease in emission with continuous irradiation. The video file was taken at 100 fps, but every 100th frame was sampled to obtain the rate of decrease in seconds. The rate of decrease was fit to a mono-exponential decay. Images were background corrected, and a region of interest (ROI) was drawn over each tube. The normalized average intensity and standard deviation of the three trials are plotted in **Figure S2**.

Solubility studies

Solubility limits were obtained by preparing saturated solutions of dyes in the desired solvent, taking aliquots of the supernatant, and using Beer’s law to calculate the concentration. Each measurement was conducted in triplicate. Solubility values are provided in **Table S2**. Errors are standard deviations.

Perfluorocarbon emulsions procedures:

General Pluronic F-68 nanoemulsion preparation procedure

Emulsions are prepared in a 1.5 mL Eppendorf tube using a QSonica (Q125) probe sonicator. A solution of dye in PFOB (20 μ L, either 1.2 μ M **F₆₈Chrom7[B(ArF₂₆)₄]** or 4.8 μ M **F₆₈Chrom5[B(ArF₂₆)₄]**) is added to the bottom of an Eppendorf tube. Then a solution of surfactant in PBS (200 μ L) was layered on top of the PFOB solution. The tube is placed around the point of the probe sonicator, ensuring the probe was not in contact with the sides of the tube but fully submerged into the solution, then clamped and cooled with an ice bath. Immediately the sonicator is activated (35% amplitude, 90 seconds). Upon completion, the emulsions are characterized by DLS (see below) and used for further experiments.

General POx nanoemulsion preparation procedure

Emulsions are prepared in a 1.5 mL Eppendorf tube using a QSonica (Q125) probe sonicator. A 5.6 mg/mL solution of **4** or **5** in DMF is prepared, heating gently with a heat gun until solubilized (~20 seconds). 20 μ L of this solution is added to PBS (200 μ L) and PFOB (20 μ L). The mixture is sonicated (35% amplitude, 90 sec) on ice. The resulting emulsions were then spun in a centrifuge at 6,000 g for 3 mins and the supernatant separated from the emulsion pellet. 200 μ L of PBS were added and the pellet resuspended in PBS. Then 20 μ L of dye in acetone at an equivalent concentration to analogous PF-68 dye stocks is added and rocked for at least 20 mins. The emulsions are centrifuged again and the supernatant separated from the emulsion pellet. The dye-loaded emulsions are resuspended in 200 μ L of PBS and used in further experiments.

Size analysis (Dynamic light scattering)

The bulk emulsion solution is diluted in PBS (50 μ L emulsions in 3 mL 1x PBS) in a plastic 1 cm cuvette. Size is analyzed with a Malvern Zetasizer Nano dynamic light scattering. SOP parameters: 10 runs, 10 seconds/run, three measurements, no delay between measurements, 25 °C with 120 second equilibration time. Collection parameters: Lower limit = 0.6, Upper limit = 1000, Resolution = High, Number of size classes = 70, Lower size limit = 0.4, Upper size limit = 1000, Lower threshold = 0.05, Upper threshold = 0.01. Data are representative of three replicate measurements.

Zeta potential analysis

The bulk emulsion solution is diluted in MilliQ H₂O (20 μ L emulsions in 2 mL MilliQ H₂O) in a plastic 1 cm cuvette. Solution is then transferred to a disposable folded capillary cell for zeta potential measurements. Zeta potential is analyzed with a Malvern Zetasizer Nano. SOP parameters: Minimum: 10 runs, Maximum: 100 runs, 5 measurements, no delay between measurements, Model: Smoluchowski, 25 °C, 120 second equilibration time. Collection parameters: Auto mode. Data are representative of five replicate measurements. Zeta potential error bars represent the standard deviation of the measurements.

Emulsion partition (octanol/water) and leaching studies

An aliquot of dye-containing **Pluronic F-68** nanoemulsion solution (50 μ L) is added to an Eppendorf tube (1.5 mL) containing PBS (950 μ L). The solution is agitated by pipetting to ensure homogeneous suspension of the nanoemulsion. 1-octanol (500 μ L) is then layered on top of the nanoemulsion in PBS solution. Sealed Eppendorf tubes are left on an orbital rocker (40 rotations/min), protected from light, at room temperature, for a set amount of time (2 d, 4 d, 6 d). At each time point, an aliquot of the octanol layer (~200 μ L) is removed from the tube, transferred to a quartz cuvette (3 mm), and the fluorescence spectrum is recorded with the following parameters:

Dye	Excitation wavelength (nm)	Collection wavelengths (nm)	Slit widths (entrance and exit) (nm)
F₆₈Chrom5[BF₄]	750	770-1200	15
F₆₈Chrom5[B(ArF₂₆)₄]	750	770-1200	15
F₆₈Chrom7[BF₄]	920	950-1300	15
F₆₈Chrom7[B(ArF₂₆)₄]	920	950-1300	15

Procedures for perfluorocarbon nanoemulsions to inject in animals

Pluronic F-68 emulsions of different sizes were made with the following conditions:

weight% PF-68 in PBS	Volume of PFOB (μL)	Volume of PBS solution (μL)	Time sonicated (min:sec)	Approximate size (nm)
16	28	200	5:00	100
6	20	200	1:30	150
2.7	20	200	1:30	200
0.6	20	200	1:30	300

Additionally, the 300 nm size emulsions were filtered through a glass fiber 1.2 μm filter (Fisher cat# 03-376-223) to obtain uniform dispersions on the DLS.

Procedure for thiol-ene modified EnePOx (5) emulsions

Empty **EnePOx**-stabilized emulsions are prepared according to the “General POx nanoemulsion preparation procedure.” Note that here the dyes are added after the emulsions are prepared, since the harsh UV light required for the thiol-ene reaction degrades the dyes. To a 600 μL aliquot of empty emulsions, Irgacure 2959 in PBS (575 μL, 0.0150 M, 8.63 mmol) and either mercaptoacetic acid (20.0 μL, 26.4 mg, 0.287 mmol) or 2-(dimethylamino)ethanethiol hydrochloride (22.0 mg, 209 mmol) are added. These emulsions are placed in a 365 nm photoreactor (Lamp: BI365 nm Inspection UV LED lamp, purchased from Risk reactor where output power density >5 mW/cm² at 15” (38 cm), voltage range 90-265V ac, output power: 3 x 325 mW at 365 nm peak; reactor: cardboard box fitted to the lamp and covered with tape and foil for safety) and irradiated for 1 hour at 4 °C. Subsequently, the emulsions are washed with 1x PBS thrice to remove excess thiols, surfactant, and Irgacure. After washing, the emulsions are characterized by DLS and zeta potential (see Figure S12). The dyes were then added in acetone stock solutions (60 μL of acetone in a 600 μL aqueous suspension of emulsions). Once rocked for 20 minutes, the emulsions are washed with 1x PBS again to ensure acetone is removed. These emulsions are then filtered through a 0.45 μm syringe filter and used for *in vivo* experiments.

Procedures for animal work:

General animal procedures

All animal experiments were approved by and conducted in conformity with the UCLA IACUC under protocol number ARC-2018-047. Non-invasive whole mouse imaging was performed on athymic nude female mice (CrI:NU-*Foxn1*tm, catalog #088, 5-8 weeks old), purchased from Charles River Laboratories. Mice were anesthetized with inhaled isoflurane/oxygen set between 2 and 3%. Tail vein injections were performed with a catheter assembled from a 29-gauge needle from an insulin syringe connected through plastic tubing to a syringe prefilled with isotonic saline solution. The bevel of the needle was then inserted into the tail vein and secured using glue. The plastic tubing was then connected to an insulin syringe prefilled with the emulsion suspension. All probes were filtered through a 0.22, 0.45, or 1.2 μm syringe filter prior to *i.v.* injection. 100 μL of each formulation was injected intravenously, never exceeding 200 μL total volume administered. After injection of all formulations, the volume of the catheter was chased with saline to ensure full dosage. Only dyes with the [B(ArF₂₆)₄] counterion were used for *in vivo* experiments.

SWIR imaging apparatus

An InGaAs Camera (Allied Vision Goldeye G-032 Cool TEC2) camera was fitted with a C-mount camera lens (Kowa LM35HC-SW) and emission filters and mounted vertically above an imaging workspace. The camera used a sensor temperature set point of -30 °C. The lasers' (LU1064DLD350-S70AN03 (35 W); LU0980D350-D30AN (35W); LU0890D400-U10AF (40W); LU0785DLU250-S70AN03 (25 W)) output was coupled cube via a 600 nm core fiber-optic bundle (Lumics, LU_LWL0600_0720_220D1A1). The output from the fiber was fixed in an excitation cube (Thorlabs KCB1E), reflected off of a mirror (Thorlabs BBE1-E03), and passed through a positive achromat (Thorlabs AC254-050-AB-ML), 1,100 nm short-pass filters (Edmund Optics #84-768) and an engineered diffuser (Thorlabs ED1-S20-MD) to provide uniform illumination over the working area. The excitation flux was measured over the area of interest with a digital optical power and energy meter (Thorlabs PM100D). Camera and lasers were externally controlled and synchronized by delivering trigger pulses of 5 V Transistor-Transistor Logic to the laser drivers and camera using a programmable trigger controller with pulses generated with an Atmel Atmega328 micro-controller unit and programmed using Arduino Nano Rev 3 MCU (A000005) in the Arduino integrated development environment (IDE). Acquired imaging data is then transferred to the PC via a Gigabit Ethernet interface. For image acquisition, the toolbox of MATLAB programming environment was used in combination with a MATLAB script (CCDA V3, <https://gitlab.com/brunslab/ccda>) to preview and collect the required image data in 14-bit depth.

Image processing for videos

Images were processed using the FIJI distribution of ImageJ. All images were subtracted with a no-laser background (about 200 frames taken at the beginning of every movie). With background subtracted videos, frames of interest were taken, generally the front, left, right, and back sides of the animals. Once the appropriate frames were chosen, the average of the frames (30+ frames) were obtained using the “z-project” feature. These averages were then compressed from the 14-bit (0-14,800 brightness) to the 8-bit depth (0-255 brightness) for display. For 2-color images, the brightness settings were matched before combining channels using the “merge channels” feature. All raw and processed images can be found on BioImage Archive (<https://www.ebi.ac.uk/biostudies/studies/S-BIAD554>).

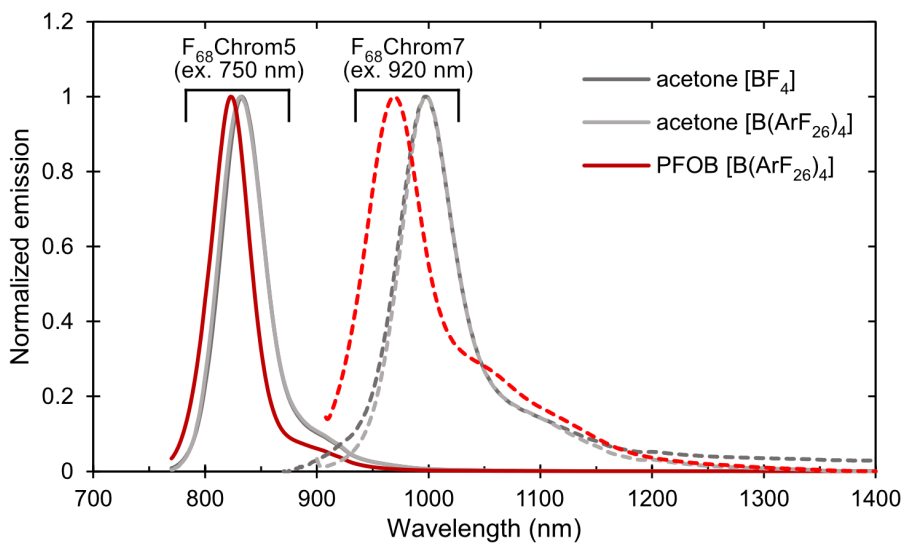
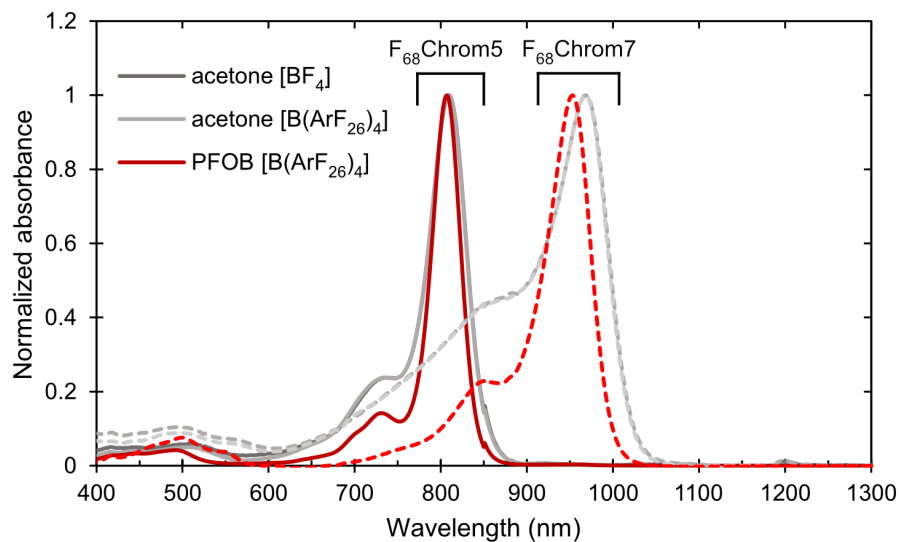
Image processing for *ex vivo* analysis

All images were subtracted with a no-laser background (about 200 frames taken at the beginning of every movie). With background subtracted videos, frames of interest were taken, usually frames 300-600. Regions of interest were highlighted around individual organs using the hand-drawn ROI tool. The mean average intensities were calculated by the “measure” function and reported. For each animal, normalized

fluorescence values are calculated from the lowest signal organ set to 1 as an arbitrary value. The replicate animals were then averaged to generate quantified bar graphs.

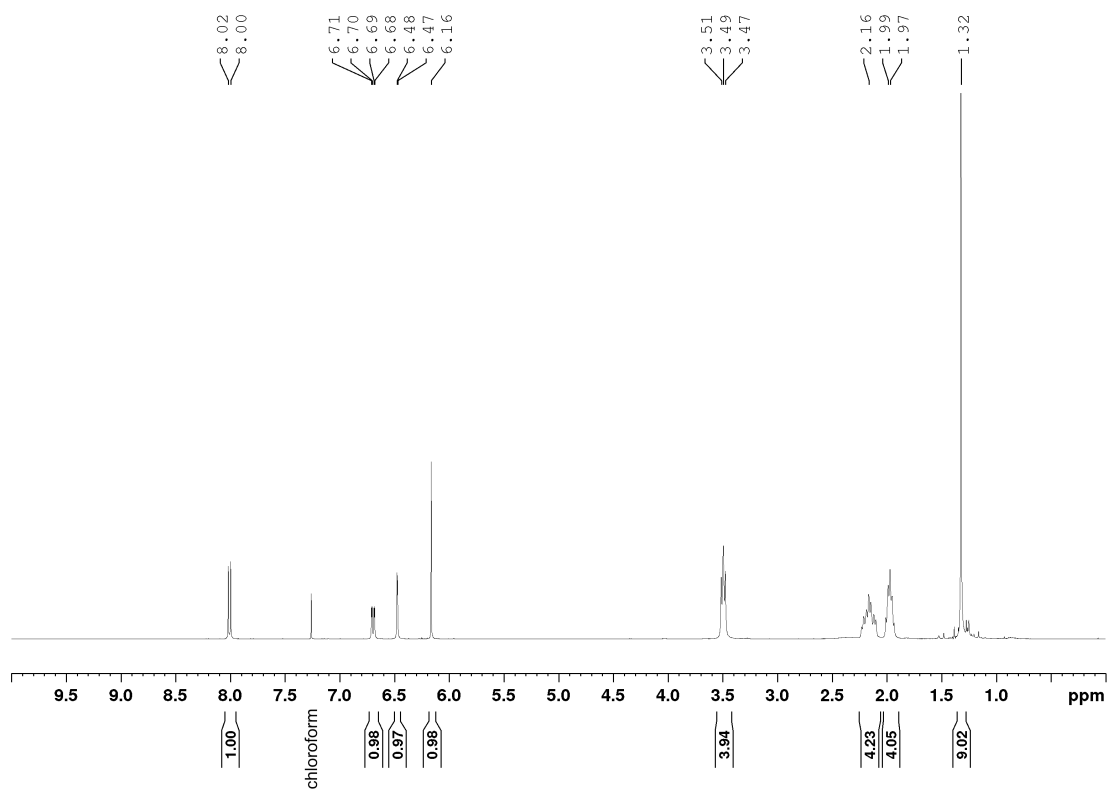
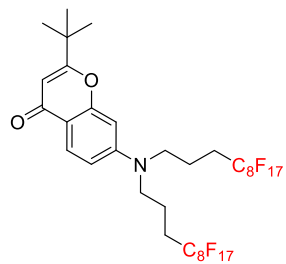
Spectra:

Absorption and emission spectra

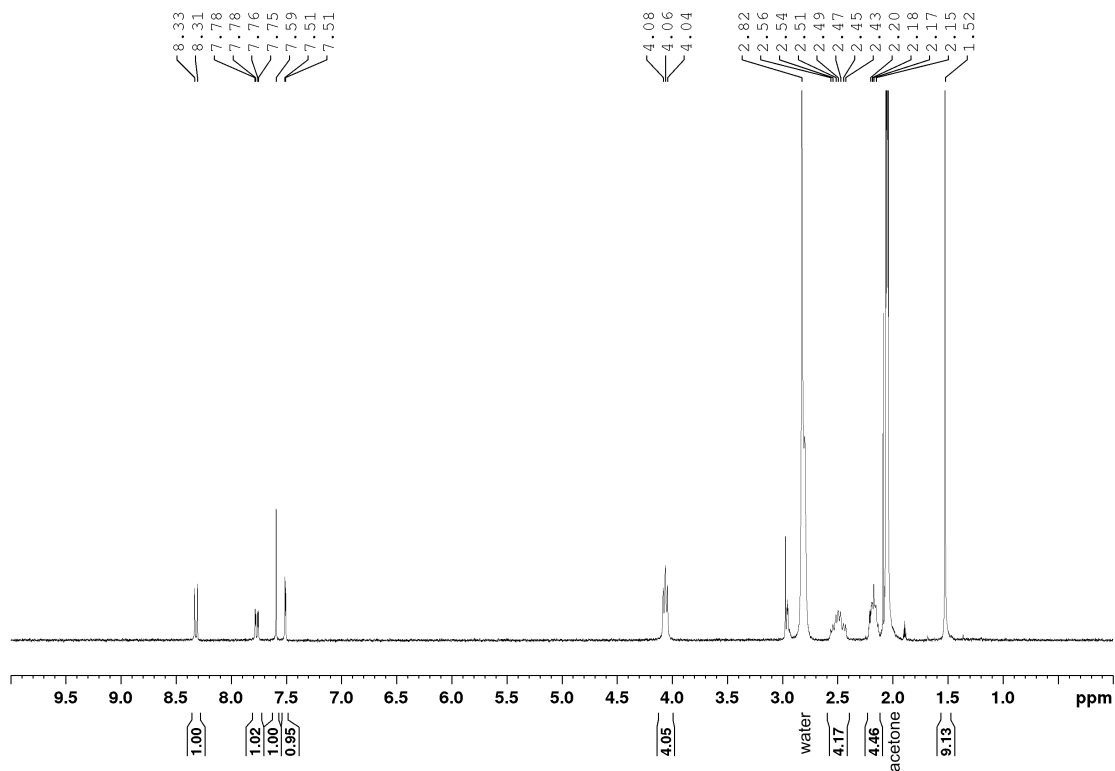
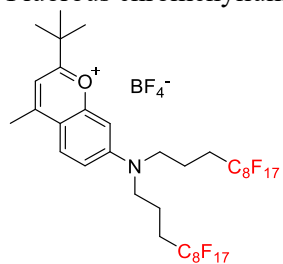


Nuclear magnetic resonance spectra: $^1\text{H-NMR}$

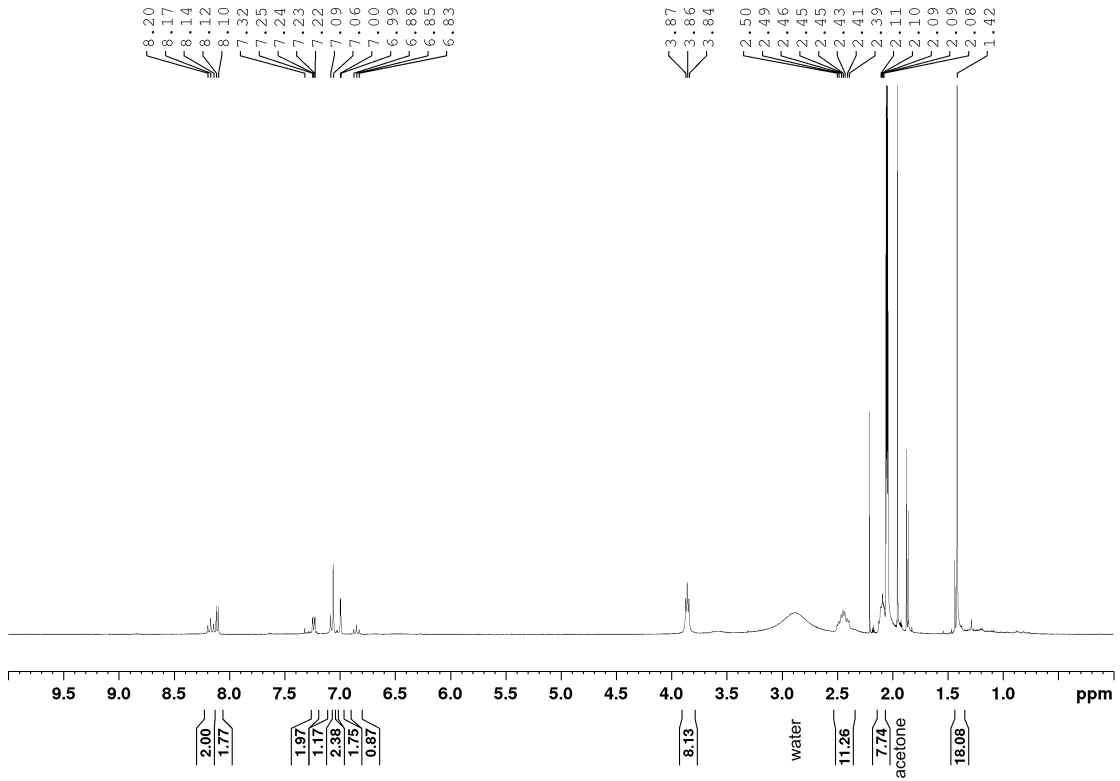
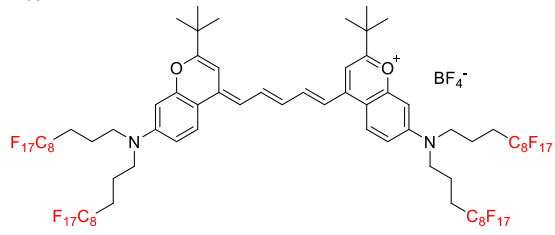
Fluorous chromone S1



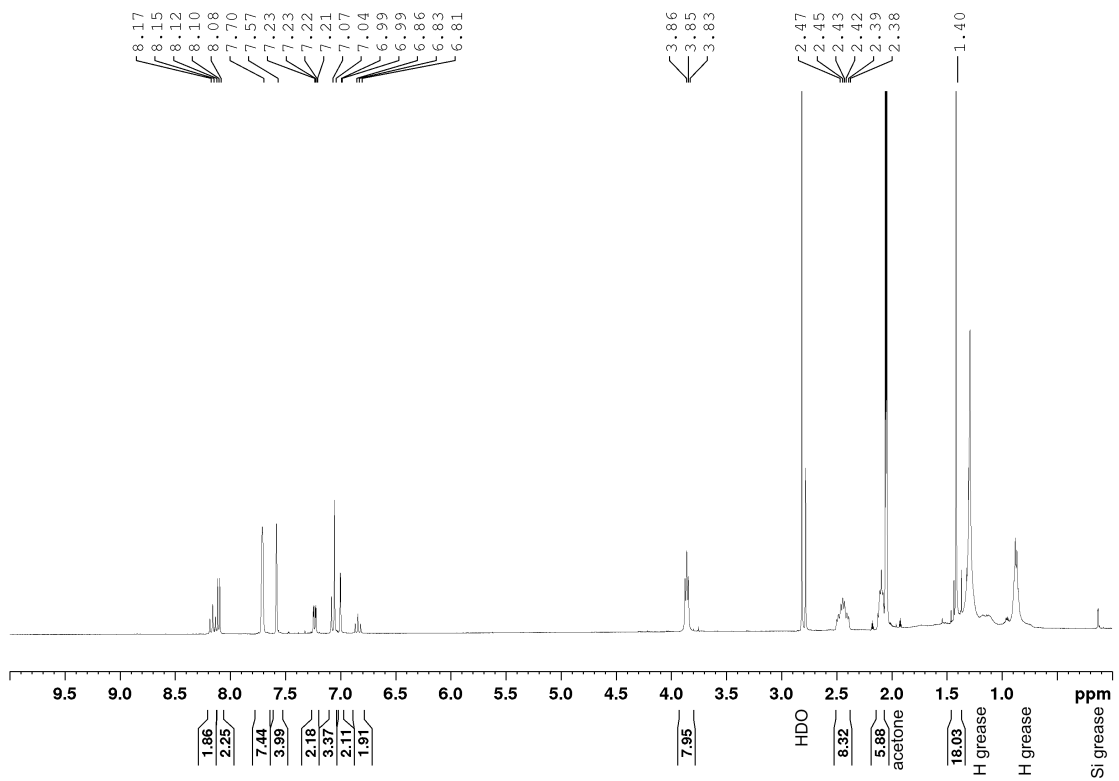
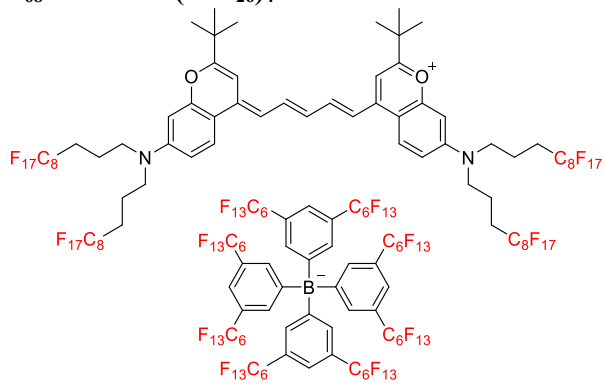
Fluorous chromenylium 2



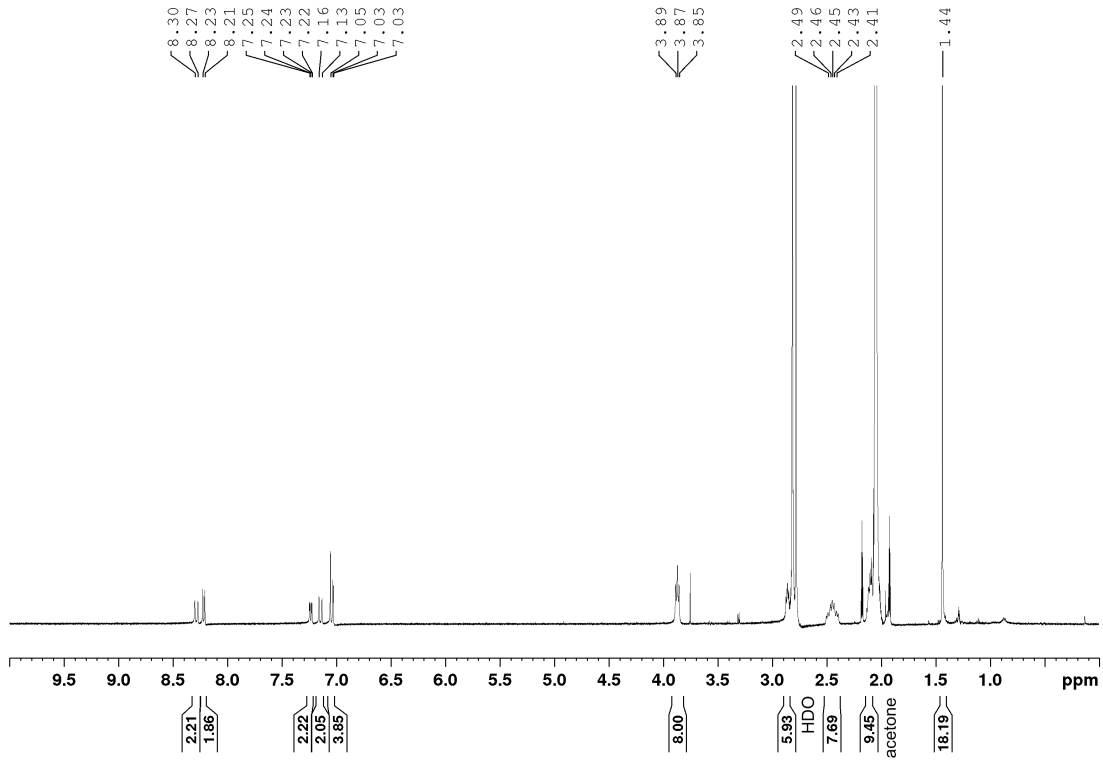
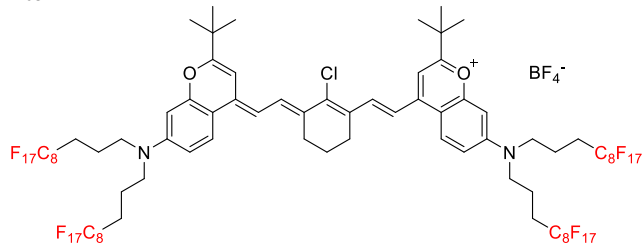
F₆₈Chrom5 BF₄



F₆₈Chrom5 B(ArF₂₆)₄

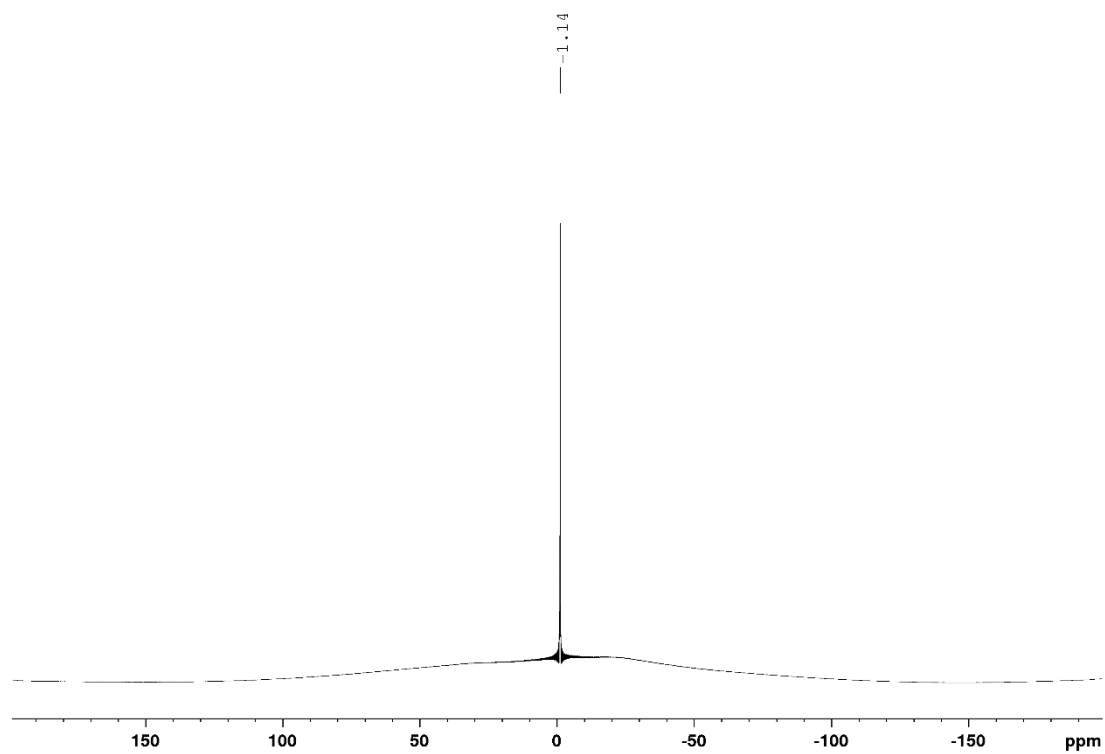
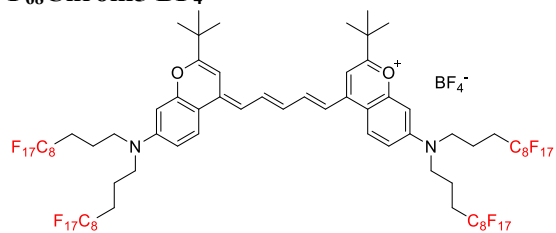


F₆₈Chrom7 BF₄

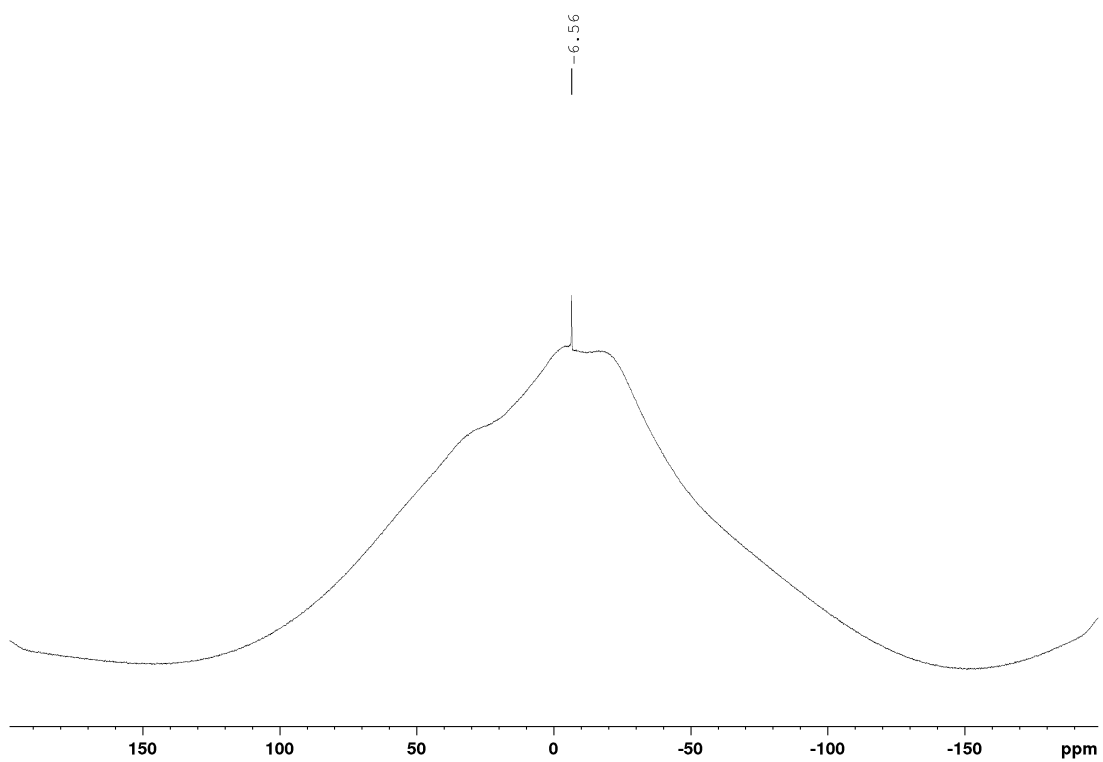
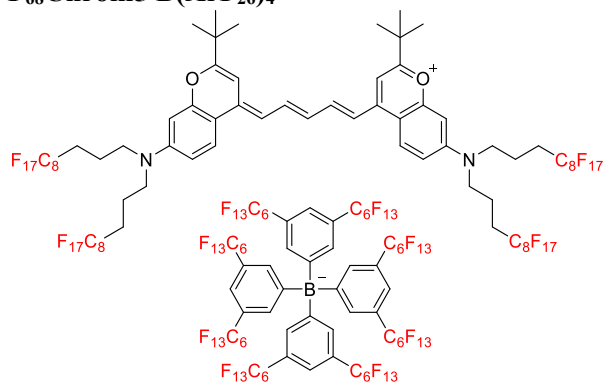


Nuclear magnetic resonance spectra: ^{11}B -NMR

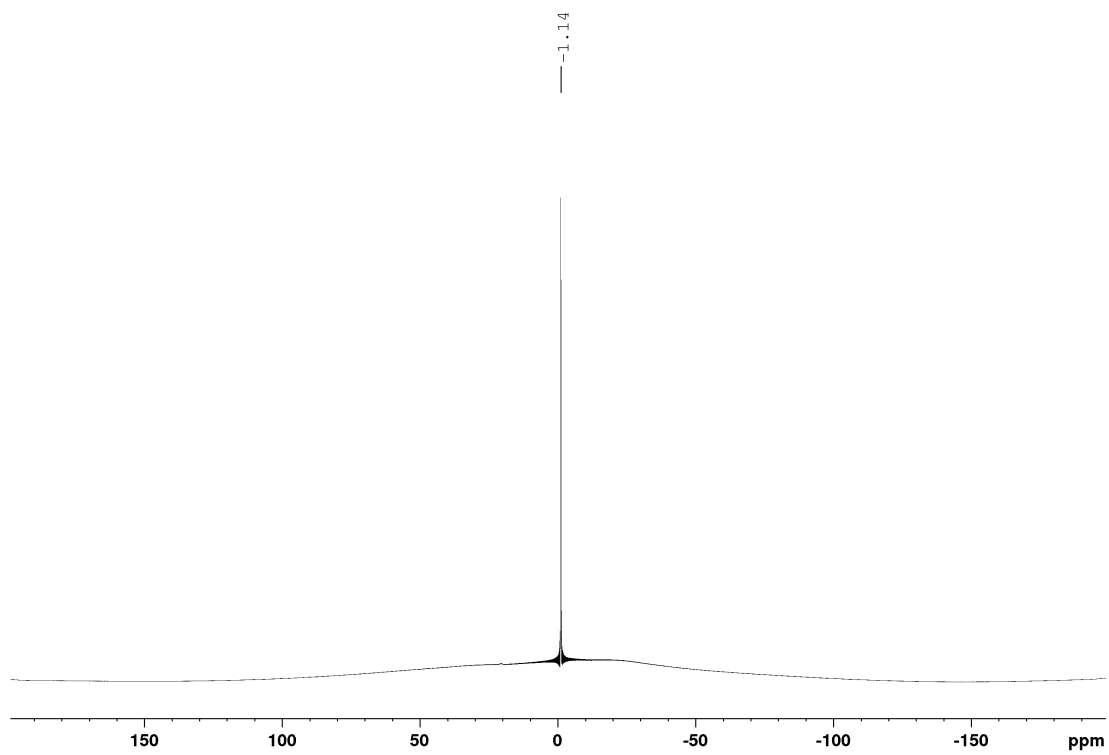
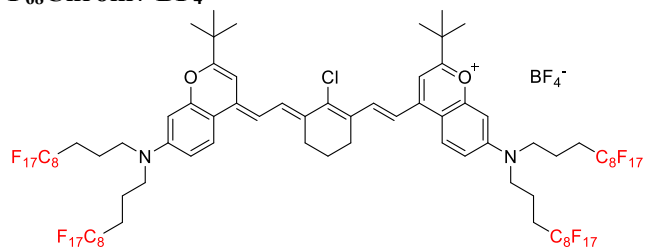
F₆₈Chrom5 BF₄⁻



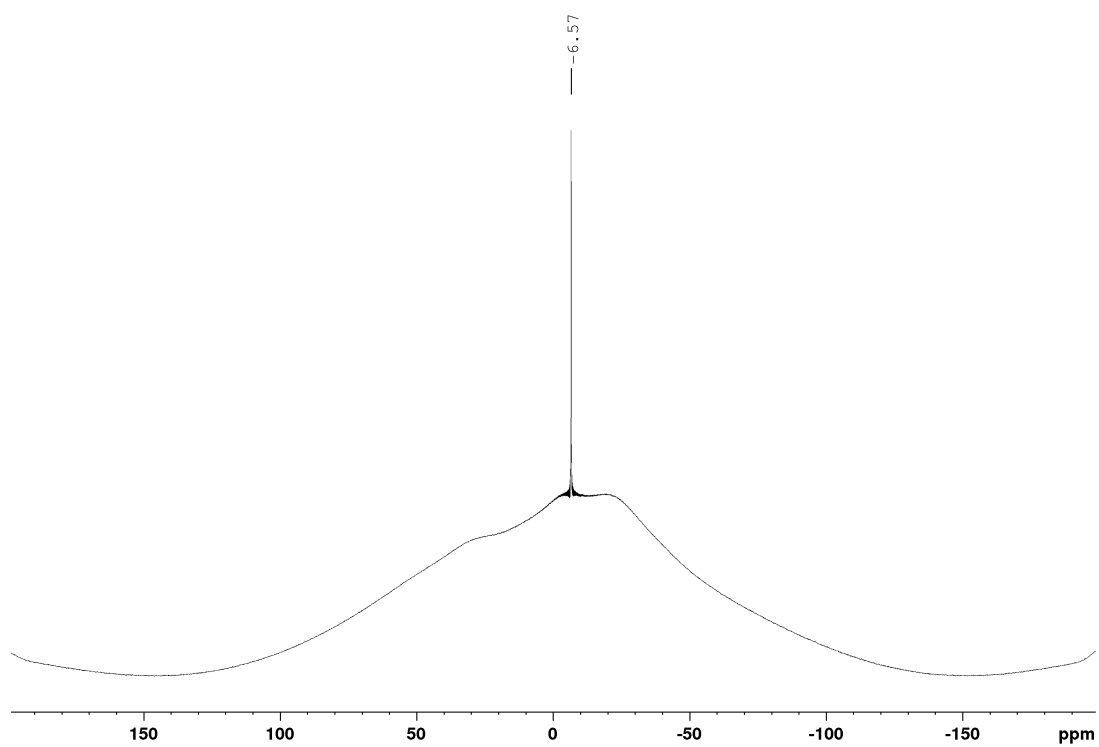
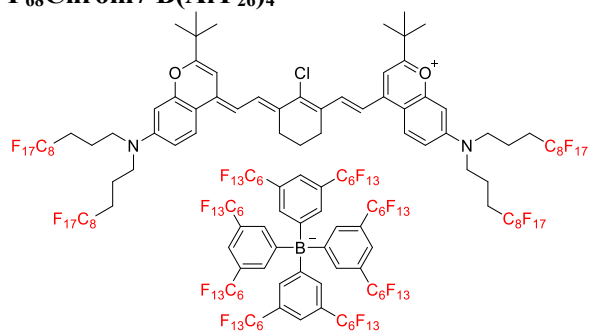
F₆₈Chrom5 B(ArF₂₆)₄



F₆₈Chrom7 BF₄

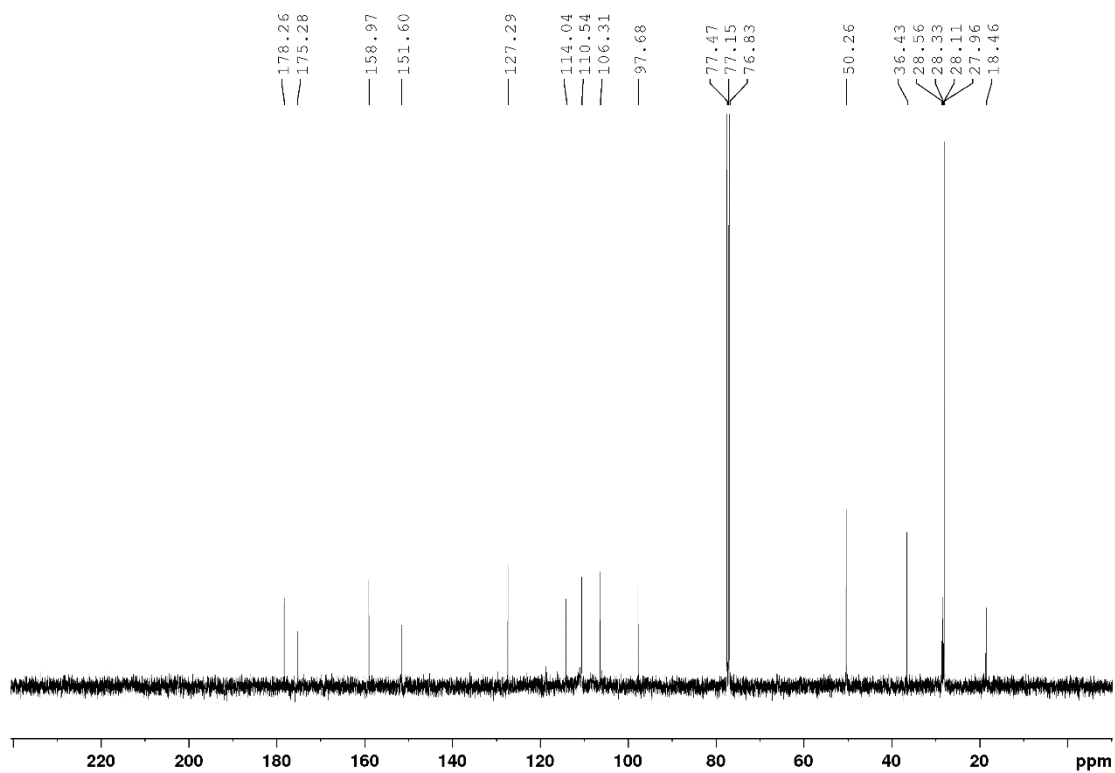
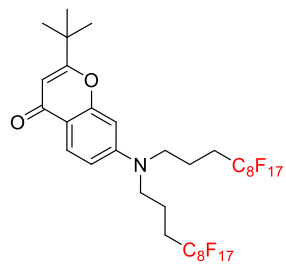


F₆₈Chrom7 B(ArF₂₆)₄

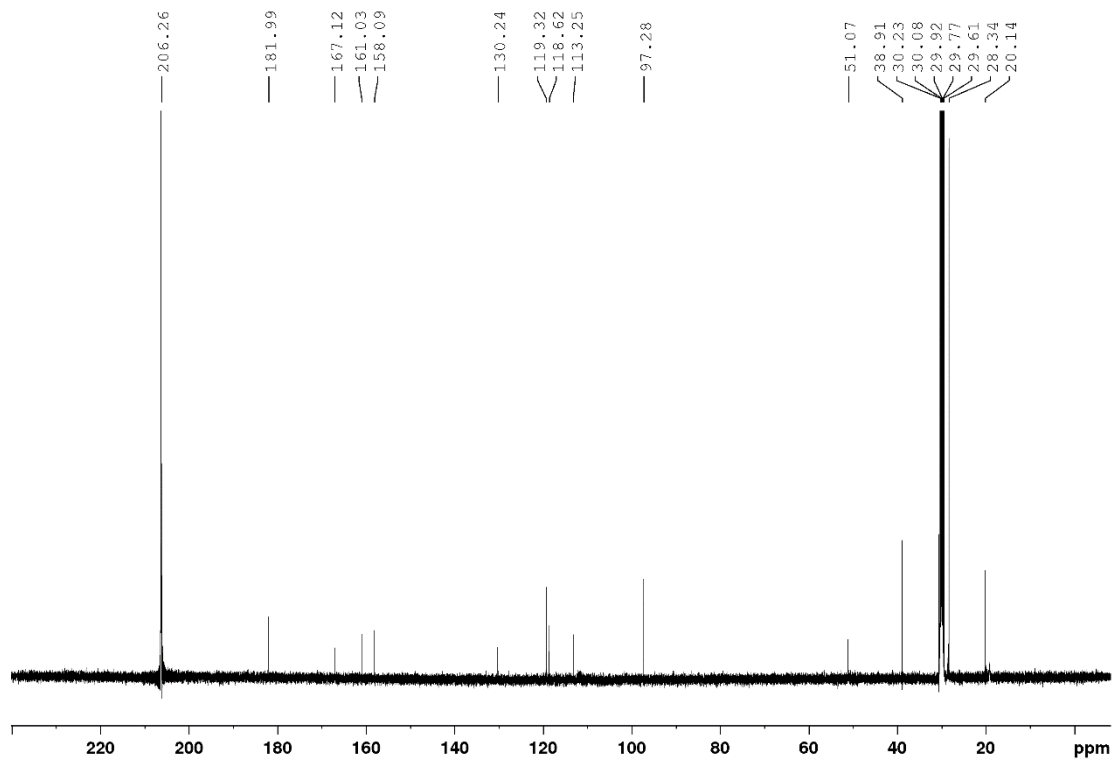
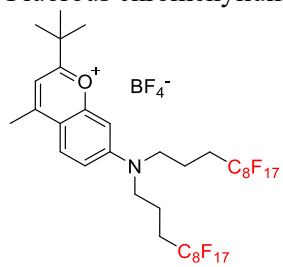


Nuclear magnetic resonance spectra: ^{13}C -NMR

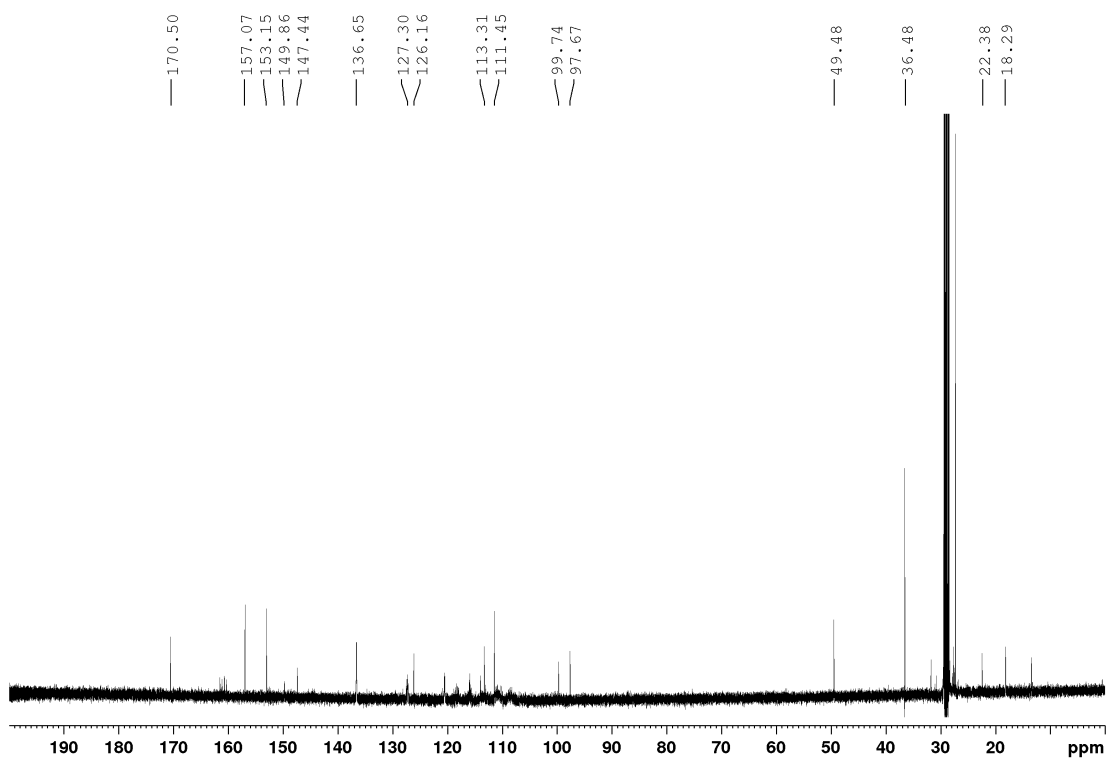
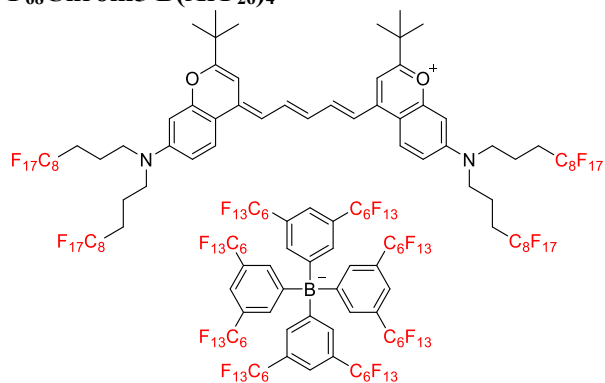
Fluorous chromone S1



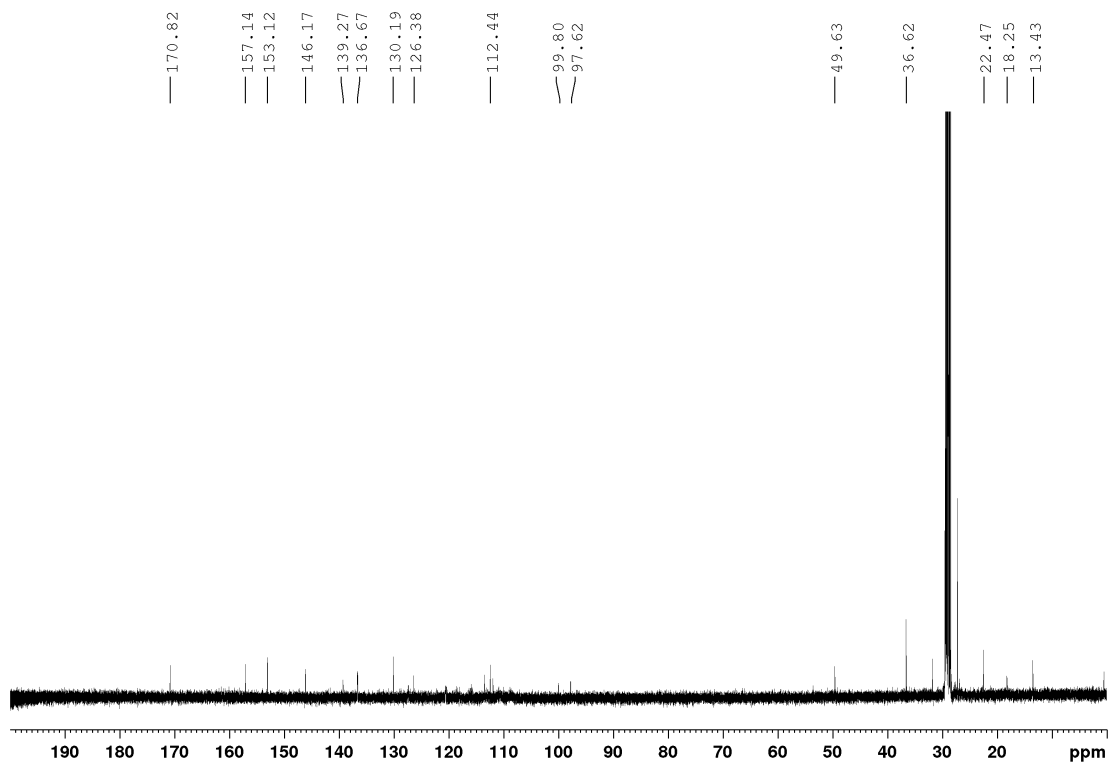
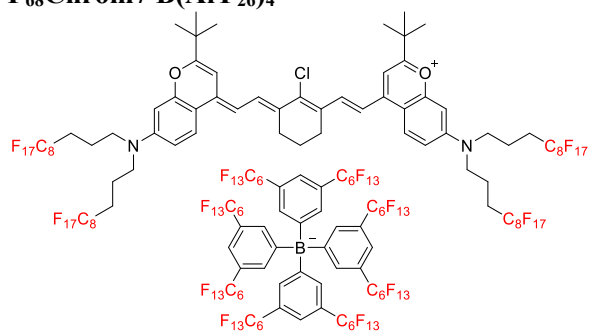
Fluorous chromenylum 2



F₆₈Chrom5 B(ArF₂₆)₄

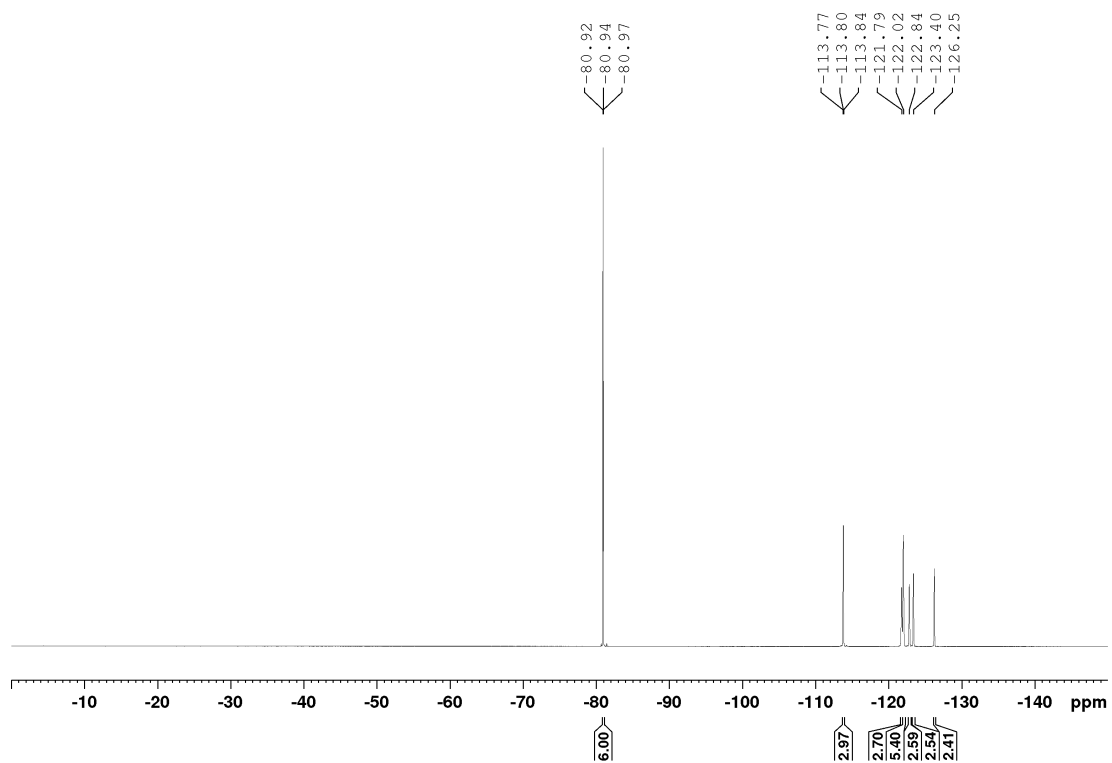
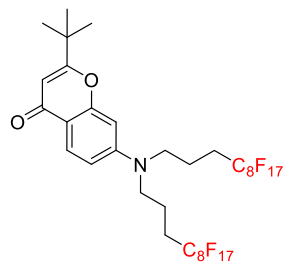


F₆₈Chrom7 B(ArF₂₆)₄

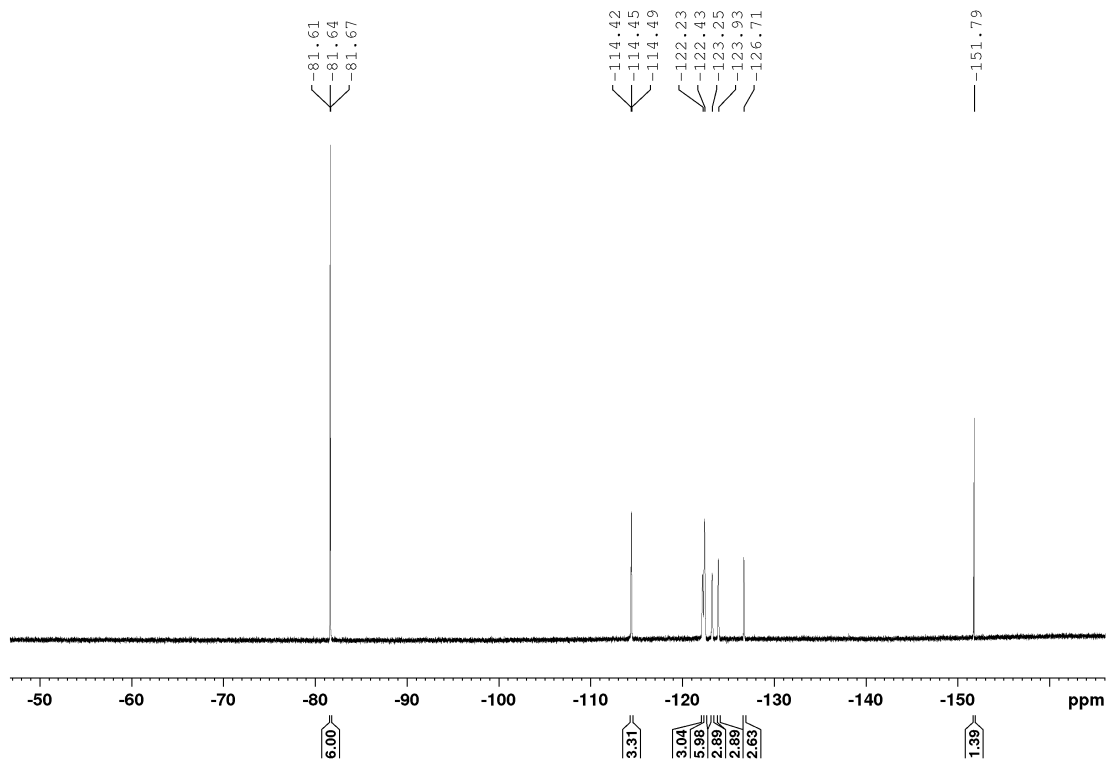
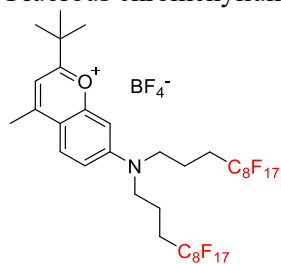


Nuclear magnetic resonance spectra: ^{19}F -NMR

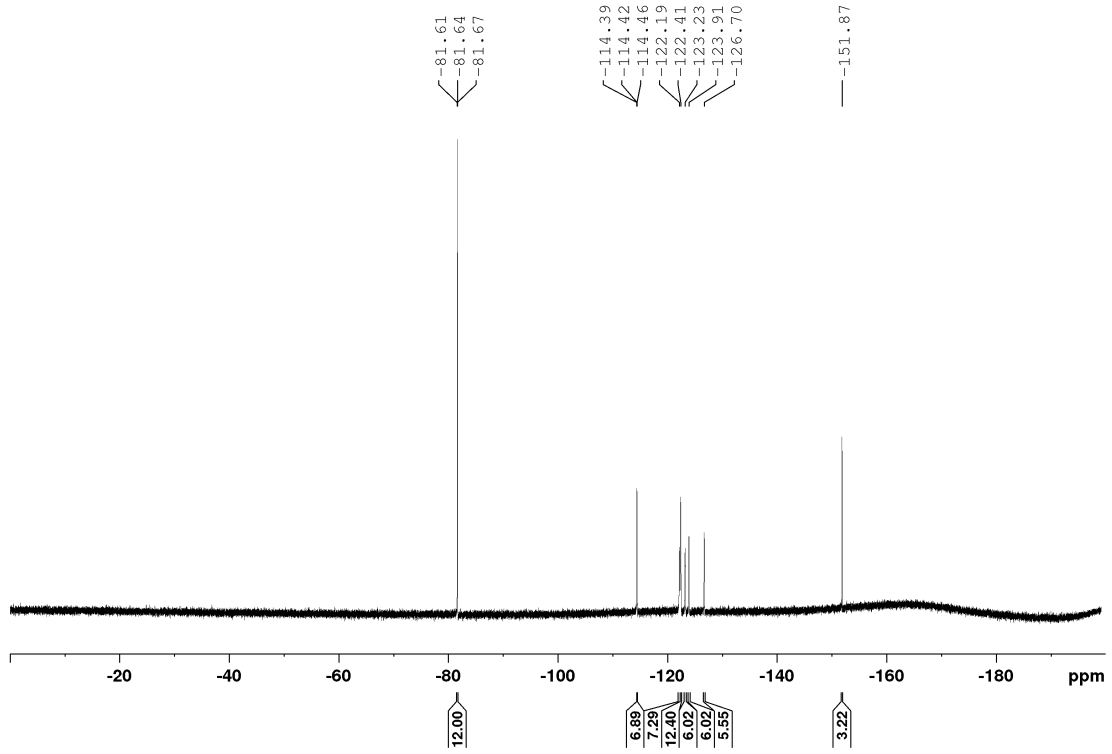
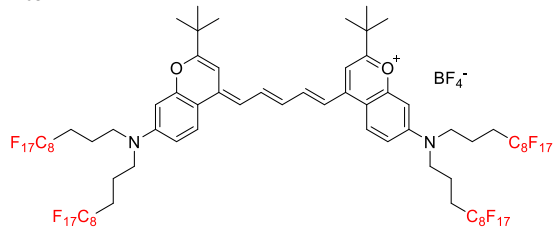
Fluorous chromone S1



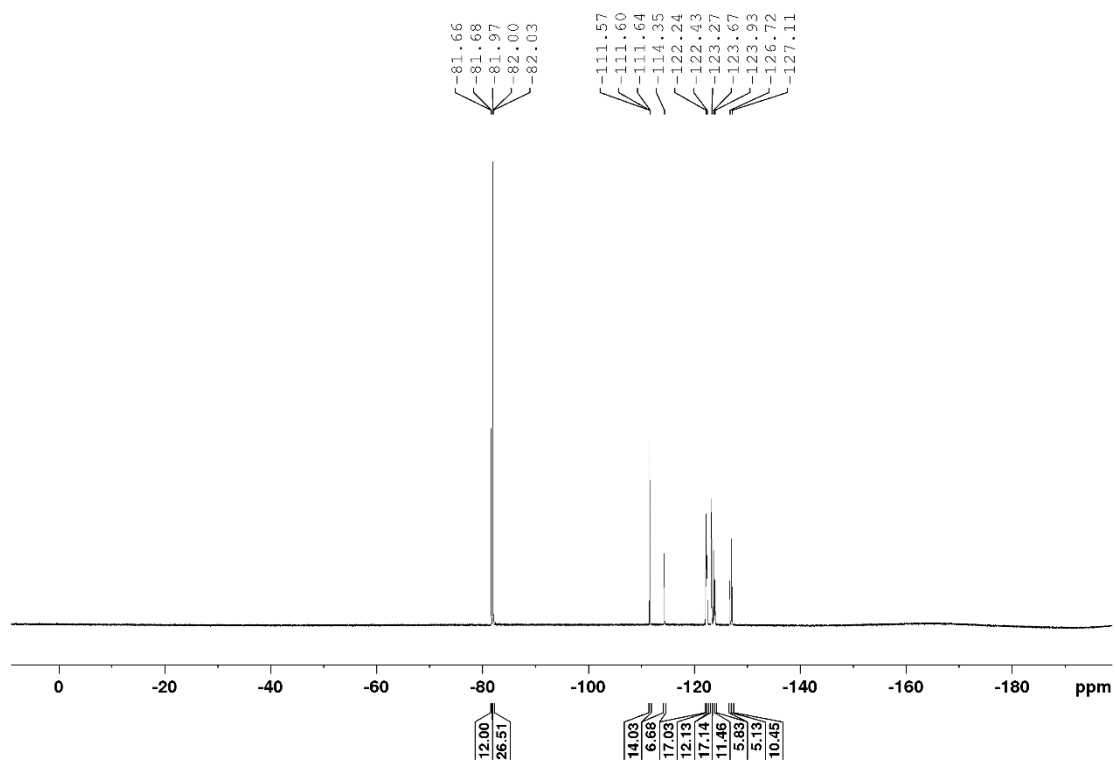
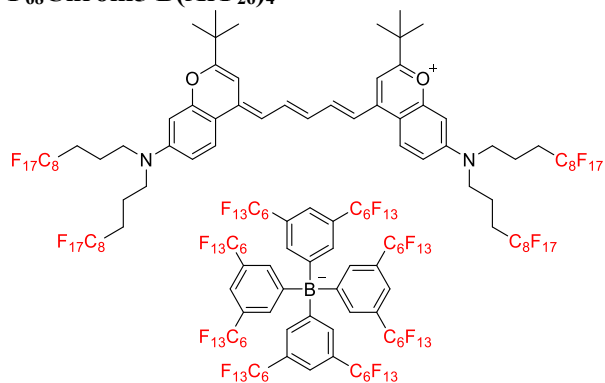
Fluorous chromenylium 2



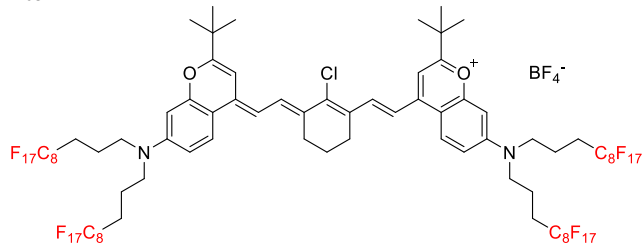
F₆₈Chrom5 BF₄



F₆₈Chrom5 B(ArF₂₆)₄



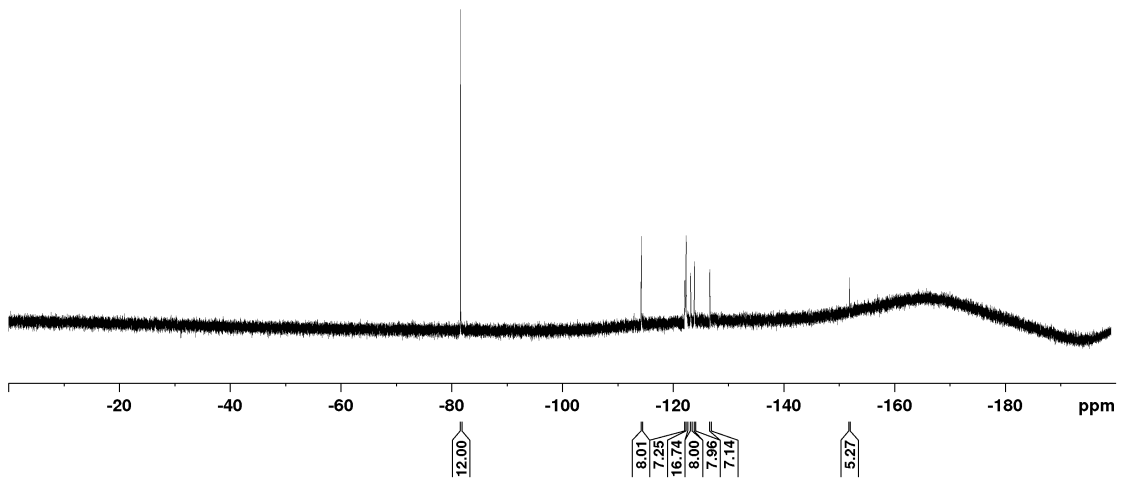
F₆₈Chrom7 BF₄



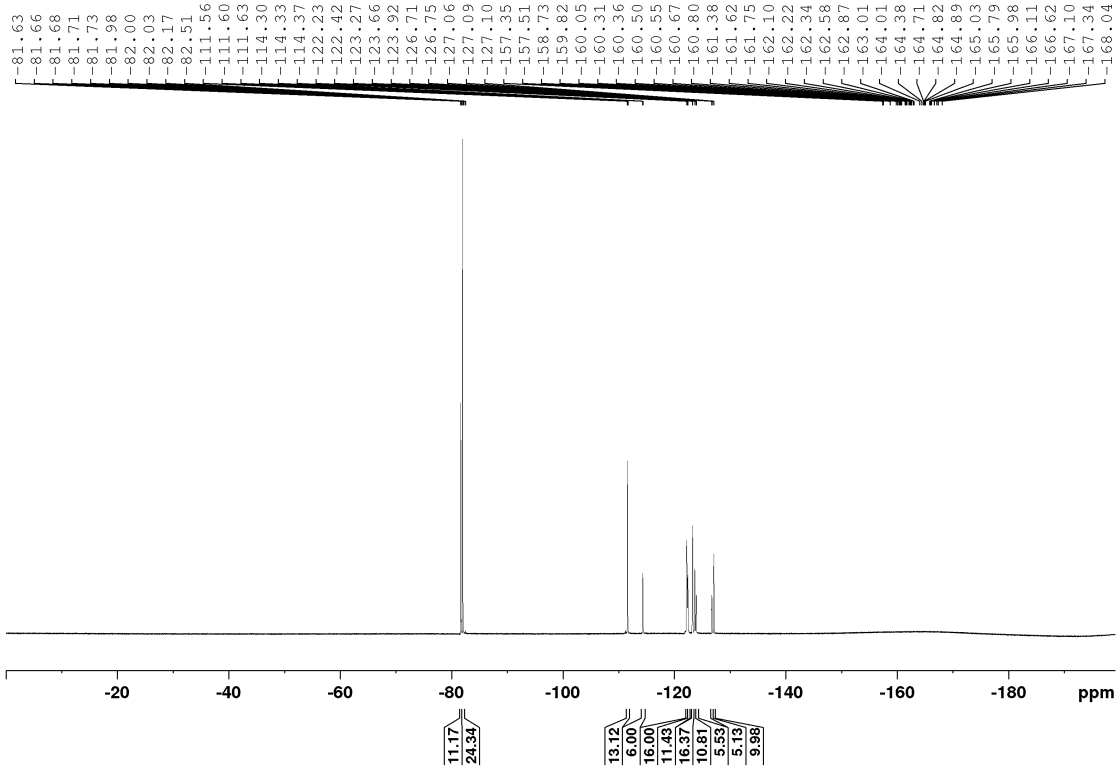
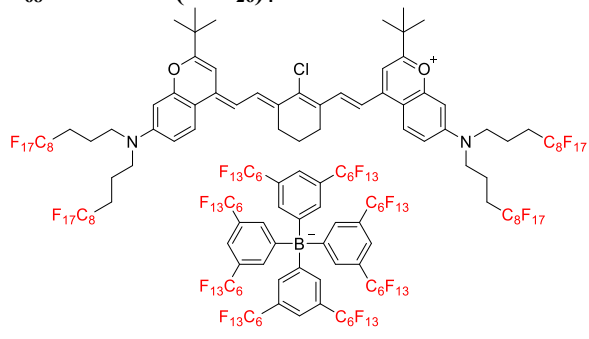
-81.61
-81.64
-81.67

-114.29
-114.31
-122.20
-122.41
-123.24
-123.91
-126.70

-151.93



F₆₈Chrom7 B(ArF₂₆)₄



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- [1] E. M. Sletten, T. M. Swager, *J. Am. Chem. Soc.* **2014**, *136*, 13574–13577.
- [2] K. Yoshinaga, T. Swager, *Synlett* **2018**, *29*, 2509–2514.
- [3] D. A. Estabrook, A. F. Ennis, R. A. Day, E. M. Sletten, *Chem. Sci.* **2019**, *10*, 3994–4003.
- [4] E. D. Cosco, J. R. Caram, O. T. Bruns, D. Franke, R. A. Day, E. P. Farr, M. G. Bawendi, E. M. Sletten, *Angew. Chemie - Int. Ed.* **2017**, *56*, 13126–13129.