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Supplemental Information

Measuring Intracellular Viscosity in Conditions of Hypergravity

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1. Supplementary Figures

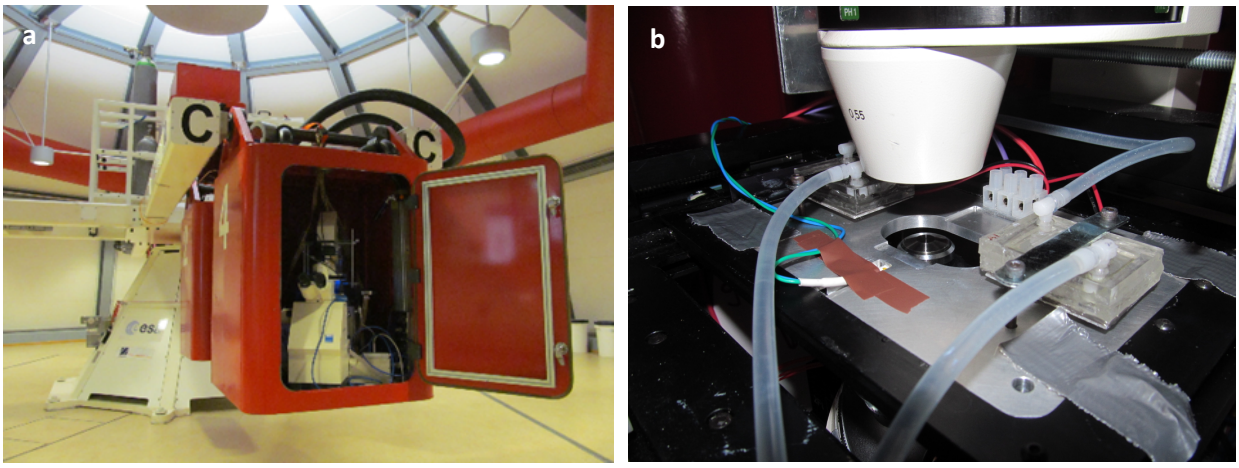


Figure S1. Images of the experimental setup. (a) The inverted microscope mounted inside an LDC gondola, which also contained the excitation source and camera. (b) View of the custom-built microscope stage showing the water-cooled portion of the peltier modules. A recess was machined into the microscope stage to tightly hold the sample in place.

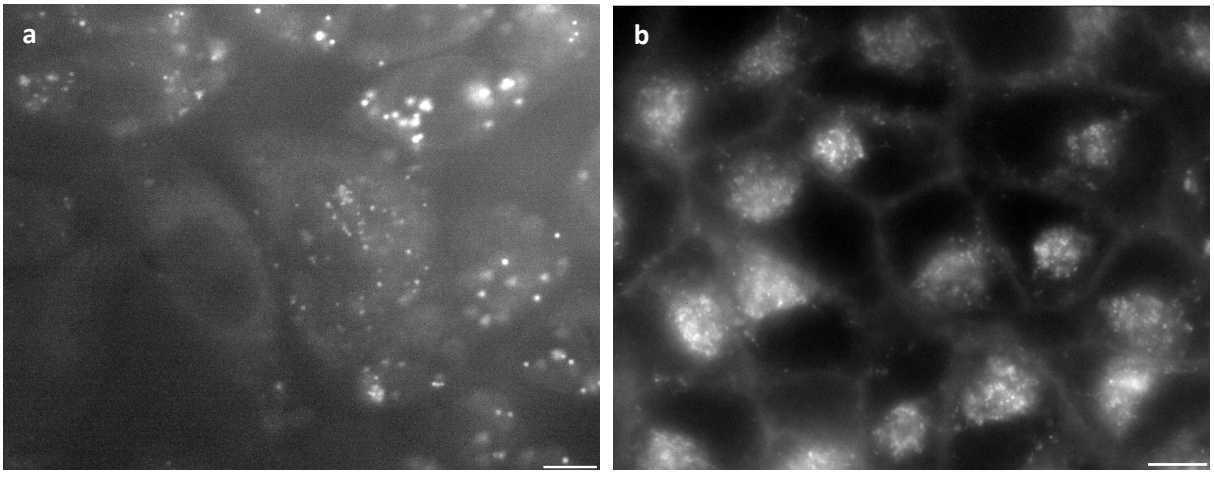


Figure S2. Fluorescence intensity images, acquired on an inverted microscope attached to a cooled system, of HeLa cells stained with (a) rotor **1** at 1g, 200 ms exposure; and (b) rotor **2** at 1 g, 200 ms exposure. Scale bars: 10 µm.

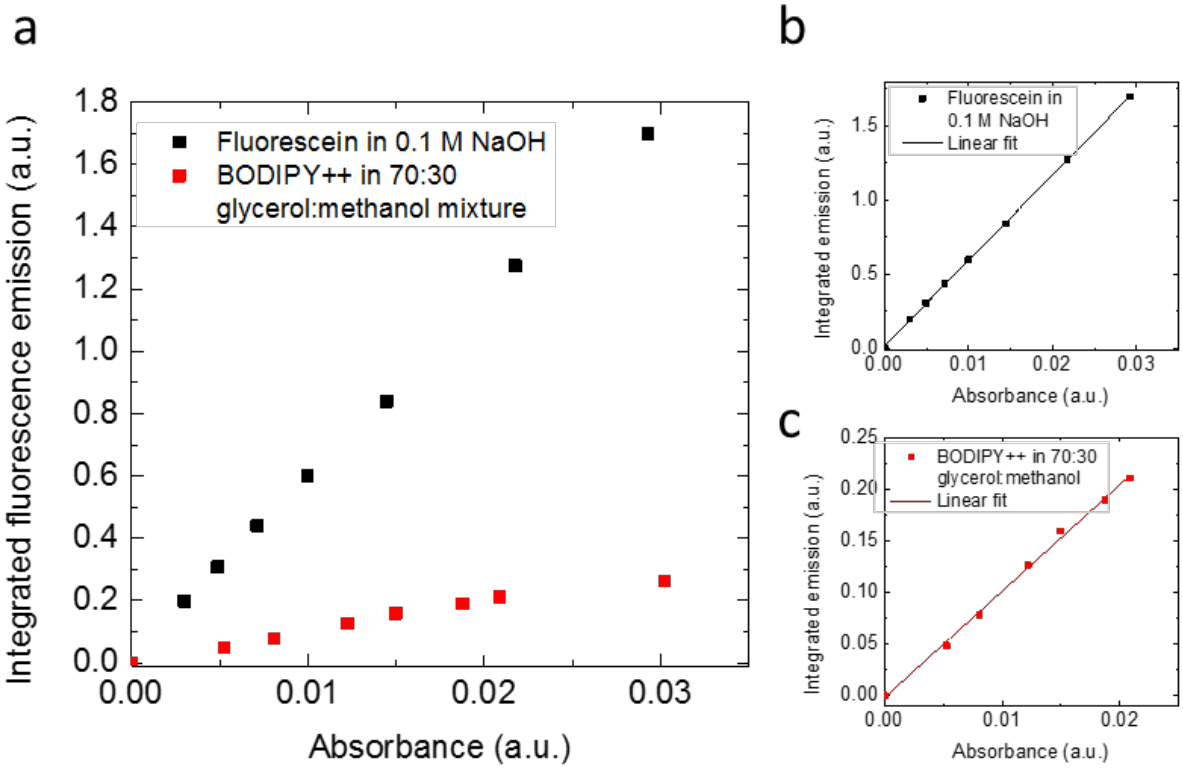


Figure S3. Determination of the quantum yield of rotor **2** in a 70:30 v/v mixture of glycerol/methanol at 20°C. The quantum yield of **2** was estimated by fitting the absorbance vs. integrated fluorescence emission intensity to a linear model and comparing the slope with the linear fit of absorbance vs. integrated fluorescence emission intensity of the reference dye fluorescein. (a) Absorbance vs. integrated fluorescence emission intensity of fluorescein in 0.1 M NaOH and **2** in 70:30 MeOH:glycerol mixture. The quantum yield was obtained via equation $\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X{}^2}{\eta_{ST}{}^2}\right)$ where according to the linear fitting shown in panels (b, c), the numerical values of slopes are (b) $Grad_{ST}$ = 57.56 for fluorescein and (c) $Grad_x$ = 10.30 for **2**. Details of the calculation are found in the Methods section of the main text.

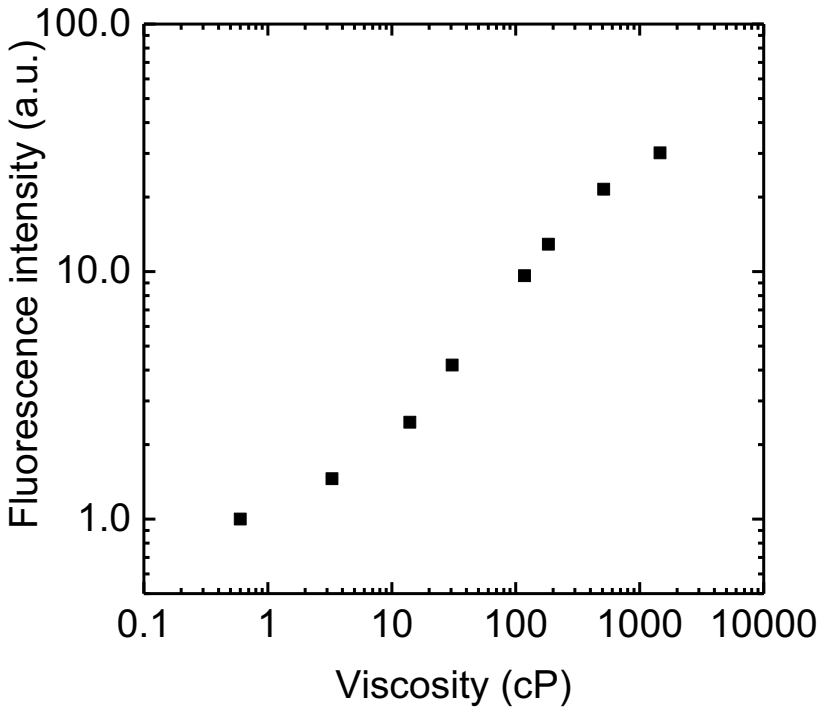


Figure S4. The viscosity sensitivity of molecular rotor **2** fluorescence. Rotor **2** emission intensity at 511 nm as a function of viscosity of methanol/glycerol mixtures, demonstrating over 30 times increase of fluorescence intensity over viscosities of 1 – 1 000 cP.

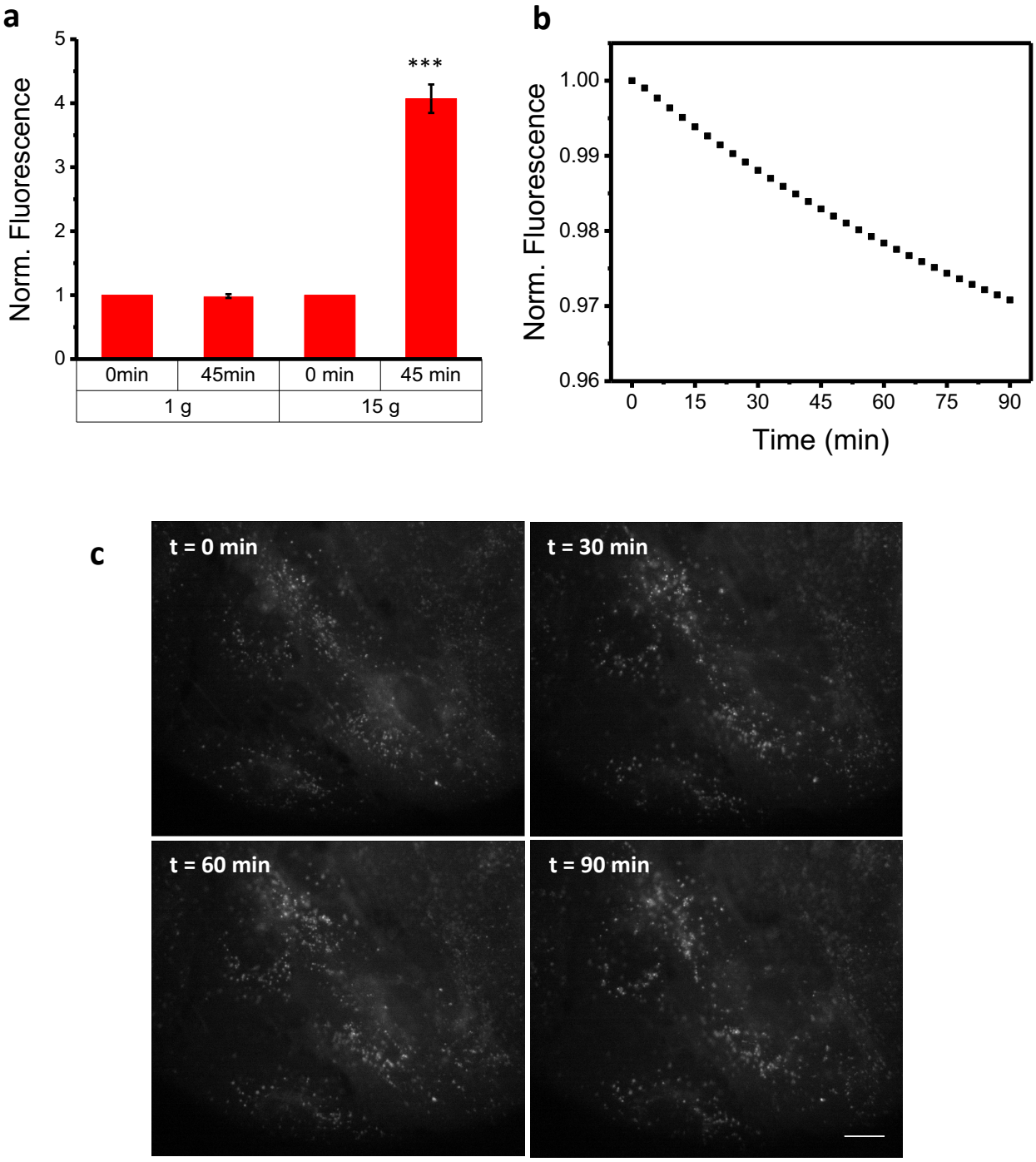


Figure S5. Fluorescence intensity changes of **2** in MC3T3-E1 cells. (a) Average relative fluorescence intensity change in individual cells at 1 g over 45 min and when exposed to hypergravity (15 g) across a 45 min profile, p = 0.001, n = 10, error bars show standard deviation across measured cells. p-value calculated using one way paired Student's t-test. (b) Relative fluorescence intensity change at 1 g over 90 min, showing a minimal total photobleaching effect of 3%. (c) Fluorescence images of cells at 1 g over 90 min. Scale bar 10 µm.

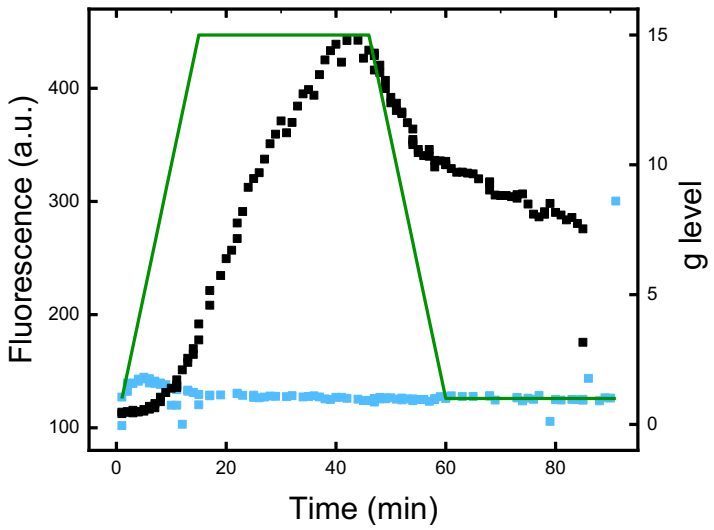


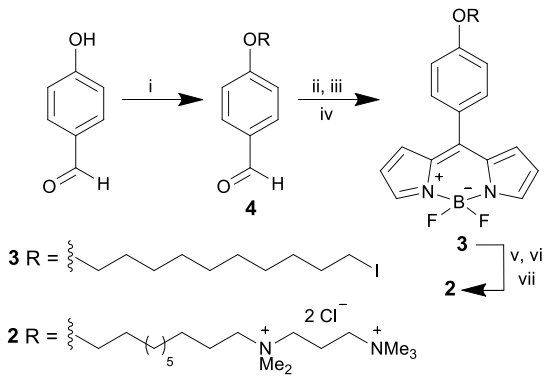
Figure S6. Fluorescence intensity changes of **2** in GPMVs (blue) as g level (green) is changed, with a 15 min ramp. Fluorescence response of **2** in MC3T3-E1 cells (black) is shown for comparison.

2. Synthesis and compound characterization

2.1 General Materials and Methods

The manipulation of all air and/or water sensitive compounds was carried out using standard inert atmosphere techniques. All chemicals were used as received from commercial sources without further purification. Anhydrous solvents were used as received from commercial sources. Analytical thin layer chromatography (TLC) was carried out on Merck® aluminium backed silica gel 60 GF254 plates and visualization when required was achieved using UV light or I_2. Flash column chromatography was performed on silica gel 60 GF254 using a positive pressure of nitrogen with the indicated solvent system. Where mixtures of solvents were used, ratios are reported by volume. Nuclear magnetic resonance spectra were recorded on 400 MHz spectrometers at ambient probe temperature. Chemical shifts for ^{1}H NMR spectra are recorded in parts per million from tetramethylsilane with the solvent resonance as the internal standard (chloroform: δ = 7.26 ppm or methanol: δ = 3.31 ppm). ^{13}C NMR spectra were recorded with complete proton decoupling. Chemical shifts are reported in parts per million from tetramethylsilane with the solvent resonance as the internal standard ($^{13}CDCl_3$: 77.0 ppm or $^{13}CD_3OD$: 49.00 ppm). ^{19}F NMR spectra were recorded with complete proton decoupling. Chemical shifts are reported in parts per million referenced to the standard hexafluorobenzene: −164.9 ppm. Mass spectra were carried out using ElectroSpray Ionization (ESI), and only molecular ions are reported.

2.2 Synthetic Procedures



Scheme 1. Synthesis of **Rotor 2**: (i) 1,10-Diiododecane, K_2CO_3, DMF, Yield: 65%; (ii) neat pyrrole, TFA, Yield: 94%; (iii) DDQ, CH_2Cl_2 and then (iv) $BF_3{\cdot}(OEt_2)_2$, Et_3N, CH_2Cl_2, Yield: 24%;[1] (v) *N,N,N′,N′*-tetramethyl-1,3-propanediamine, THF, RT and then (vi) CH_3I, DMF, and finally (vii) Dowex® 1x8 200 mesh ion-exchange column, H_2O. Yield: 44%.

4-(10-iododecyloxy)benzaldehyde (4). 1,10-Diiododecane (25 g, 63.4 mmol) was added to a mixture of 4-hydroxybenzaldehyde (1 g, 8.2 mmol) and potassium carbonate (2 g, 14.5 mmol) in dry *N,N*-dimethylformamide (25 mL). The reaction mixture was stirred at 80°C for 5 h, then cooled down to room temperature and diluted with CH_2Cl_2 (150 mL). The organic solution was washed with H_2O (3 x 100 mL), dried over anhydrous $MgSO_4$, filtered and the solvents were removed by rotary evaporation. The crude product was purified by flash chromatography on silica gel (2:1 CH_2Cl_2:petroleum ether), R_f 0.40, to give a colorless oil. Yield: 2.1 g (65%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.86 (s, 1H), 7.82 (d, *J* = 8.8 Hz, 2H), 6.99 (d, *J* = 8.8 Hz, 2H), 4.02 (t, *J* = 6.3 Hz, 2H), 3.17 (t, *J* = 6.8 Hz, 2H), 1.80 (m, 4H), 1.36 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ_C 190.88, 164.34, 132.07, 129.84, 114.84, 68.48, 33.60, 30.55, 29.49, 29.40, 29.35, 29.12, 28.58, 26.02, 7.41. **HRMS** (ESI-TOF) m/z 389.0984 ($C_{17}H_{25}O_2I$, $[M]^+$, requires 389.0978).

BODIPY 3. 4-(10-iododecyloxy)benzaldehyde (**4**) (2.1 g, 5.4 mmol) was dissolved in freshly distilled pyrrole (30 mL, 432 mmol) and the resulting solution was degassed by sparging with N_2 for 20 minutes before the addition of TFA (0.1 mL, 1.3 mmol). The mixture was stirred for 3 hours at room temperature, diluted with CH_2Cl_2 (100 mL) and then washed consecutively with H_2O (100 mL), $NaHCO_3$ (100 mL, 0.5M) and H_2O (100 mL). The organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated using a rotary evaporator. The excess pyrrole was removed using high vaccum to give the dipyrromethane as a dark viscous oil. The crude dipyrromethane was purified by flash chromatography on silica gel (2:1 CH_2Cl_2:petroleum ether), R_f 0.35, to give a green viscous oil. Yield: 2.5 g (94%). The dipyrromethane (2.5 g, 4.9 mmol) was dissolved in CH_2Cl_2 (200 mL) and DDQ (1.34 g, 5.9 mmol) was added. The reaction mixture was stirred at room temperature shielded from light for 1 hour. Then, Et_3N (6 mL, 43 mmol) was added, followed immediately by the addition of $BF_3{\cdot}(OEt_2)_2$ (5 mL, 40.5 mmol) and the reaction mixture was stirred at room temperature overnight. The organic solution was washed with H_2O (100 mL), NH_4Cl (100 mL, 0.5M), $NaHCO_3$ (100 mL, 0.5 M) and finally H_2O (100 mL), and then dried over anhydrous $MgSO_4$, filtered and evaporated to give a black viscous oil which was purified by column chromatography on silica gel (7:1 petroleum ether:ethyl acetate), R_f 0.20, to give **BODIPY 3** as a red-orange sticky solid. Yield: 717 mg (24%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.91 (br s, 2H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.04 (d, *J* = 8.8 Hz, 2H), 6.98 (d, *J* = 4.4 Hz, 2H), 6.55 (dd, *J* = 4.4, 1.9 Hz, 2H), 4.05 (t, *J* = 6.4 Hz, 2H), 3.19 (t, *J* = 7.3 Hz, 2H), 1.84 (m, 4H), 1.33 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ_C 161.88, 147.68, 143.44, 134.95, 132.59, 131.48, 126.23, 118.36, 114.66, 68.45, 33.64, 30.60, 29.56, 29.46, 29.44, 29.28, 28.64, 26.14, 7.45; **^{19}F NMR** (377.5 MHz, $CDCl_3$) δ_F –143.19 (q, $J_{F\text{-}B}$ = 29.7 Hz); **HRMS** (ESI-TOF) m/z 531.1481 ($C_{25}H_{30}BN_2OF_2$, $[M\text{-}F^-]^+$, requires 531.1480)

Rotor 2. To a solution of **BODIPY 3** (240 mg, 0.43 mmol) in 16 mL of THF was added *N,N,N′,N′*-tetramethyl-1,3-propanediamine (3 mL, 17.9 mmol). The resulting mixture was stirred at room temperature overnight, during which time a dark-red waxy compound precipitated out from the reaction mixture. The solvent and excess of *N,N,N′,N′*-tetramethyl-1,3-propanediamine were removed by evaporation under reduced pressure and the crude product was washed several times with diethyl ether. This mono-charged intermediate, which

was used without further purification, was dissolved in DMSO (6 mL) and iodomethane (3 mL, 48.2 mmol) was added to the solution. After stirring the reaction mixture at room temperature overnight, the solvent was evaporated under reduced pressure to give a dark red crude product which was purified by column chromatography on silica gel (methanol, and then a mixture of 3:1 methanol:0.5 M NH_4Cl), R_f 0.2. Fractions were evaporated at 30°C to give a mixture of **rotor 2** and NH_4Cl which was further dissolved in methanol and successively filtered to remove most of NH_4Cl. The red-orange crude, which was still contaminated with NH_4Cl according to ^{1}H-NMR, was dissolved in methanol and a saturated solution of NH_4PF_6 in H_2O was added in order to exchange counter-ions. The precipitate was isolated by filtration, washed thoroughly with H_2O, methanol and diethyl ether. Finally, the red-orange solid was dissolved in acetone and passed through a Dowex® 1x8 200 mesh ion-exchange column (H_2O). Fractions were evaporated to dryness (at 30°C) to give **rotor 2** as a red-orange wax. Yield: 121 mg (44%).

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.92 (br s, 2H), 7.60 (d, *J* = 8.3 Hz, 2H), 7.13 (d, *J* = 8.3 Hz, 2H), 7.05 (d, *J* = 3.4 Hz, 2H), 6.63 (dd, *J* = 3.4, 1.4 Hz, 2H), 4.10 (t, *J* = 6.3 Hz, 2H), 3.42 (m, 6H), 3.23 (s, 9H), 3.17 (s, 6H), 2.35 (m, 2H), 1.83 (m, 4H), 1.42 (m, 12H); **^{13}C NMR** (100 MHz, CD_3OD) δ_C 163.44, 149.01, 144.52, 136.01, 133.79, 132.58, 127.20, 119.50, 115.77, 69.40, 66.47, 63.92, 61.61, 54.07, 51.41, 30.58, 30.52, 30.44, 30.27, 27.42, 27.13, 23.67, 18.68; **^{19}F NMR** (377.5 MHz, CD_3OD) δ_F –145.68 (q, $J_{F\text{-}B}$=28.6 Hz); **HRMS** (ESI-TOF) m/2z 284.2023 ($C_{33}H_{51}BN_4OF_2$, $[M\text{-}2Cl^-]^+$, requires 284.2057)

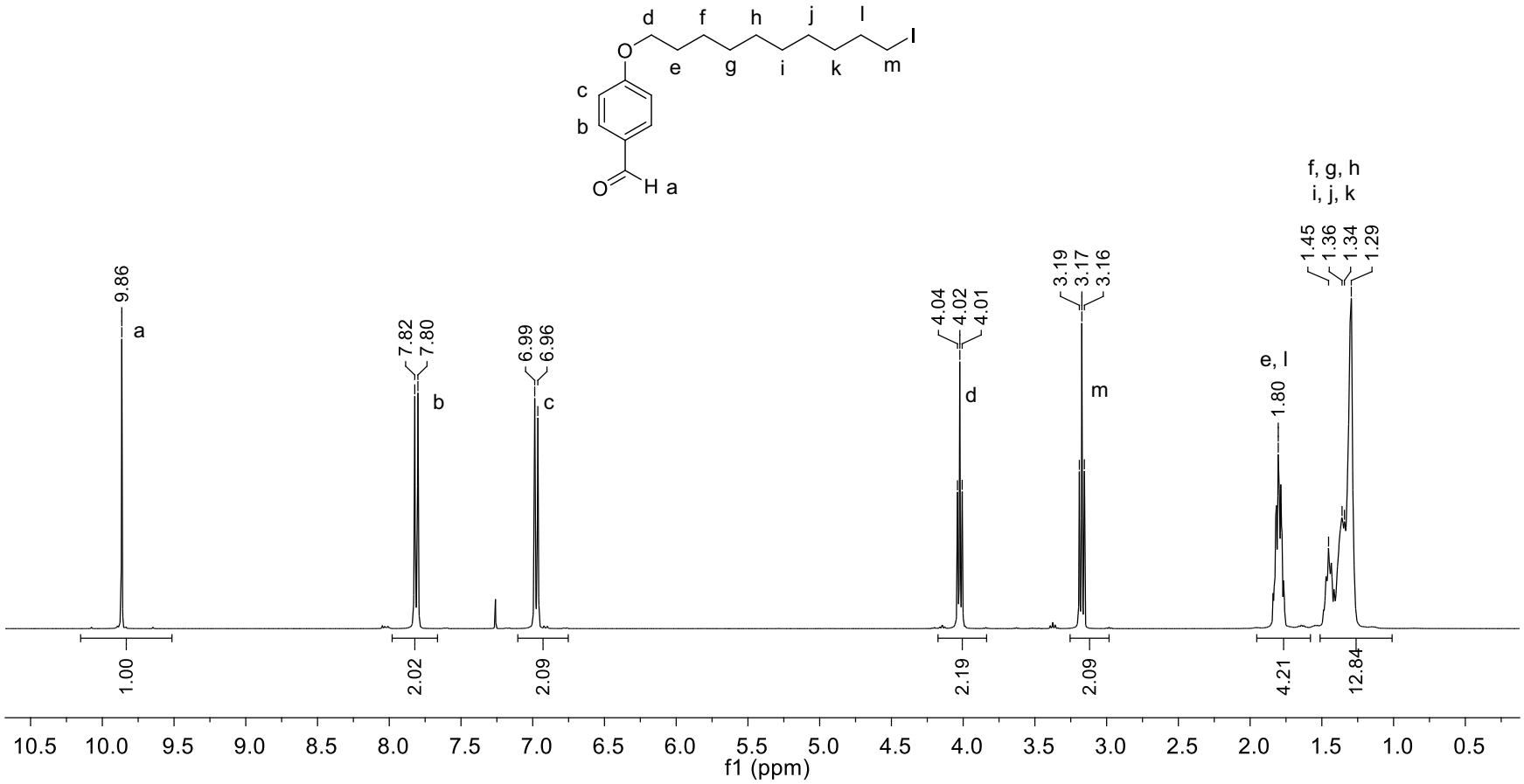


Figure S7. ^{1}H NMR spectrum of **4** (400 MHz, $CDCl_3$).

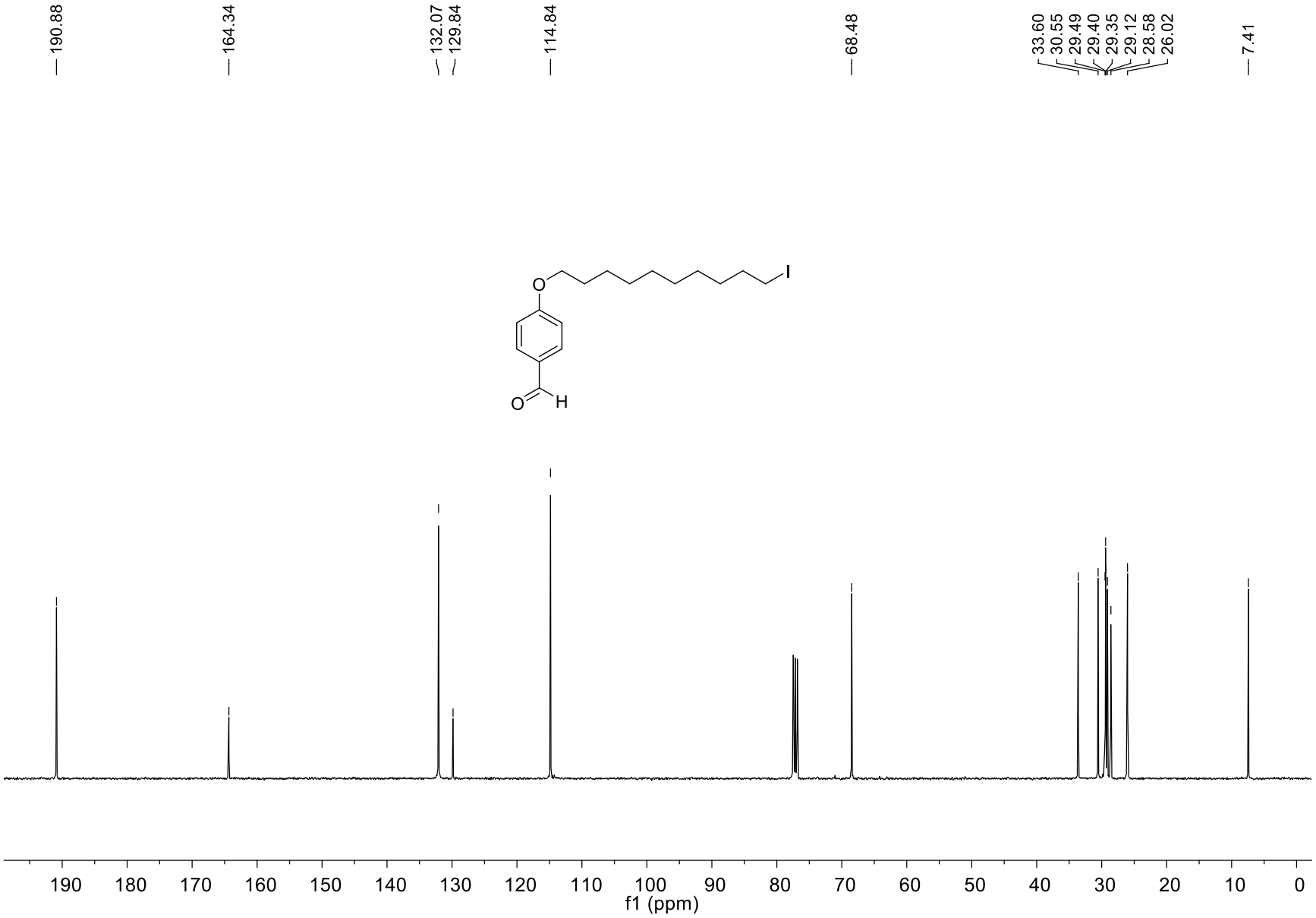


Figure S8. ^{13}C NMR spectrum of **4** (100 MHz, $CDCl_3$).

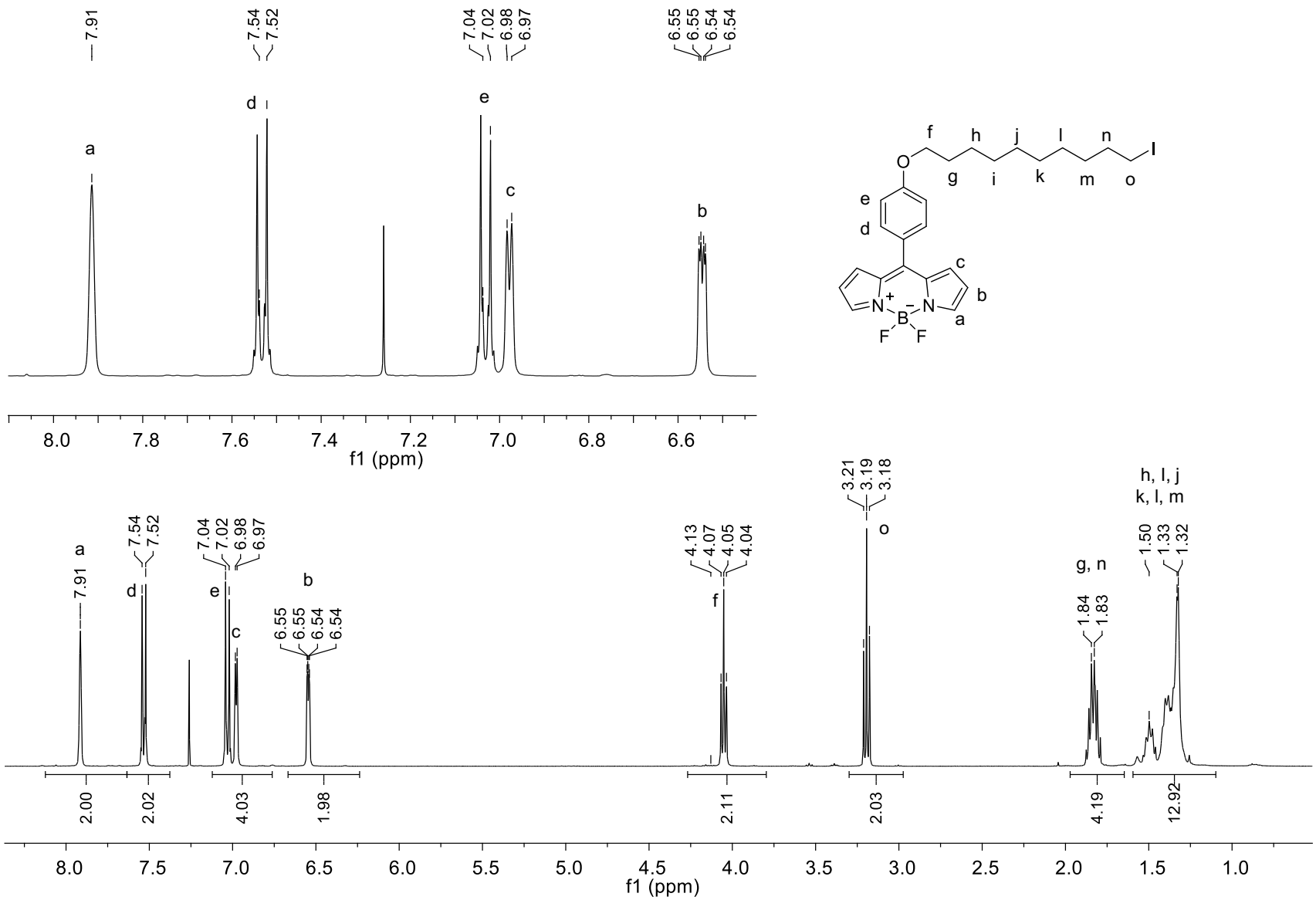


Figure S9. ^{1}H NMR spectrum of **BODIPY 3** (400 MHz, $CDCl_3$).

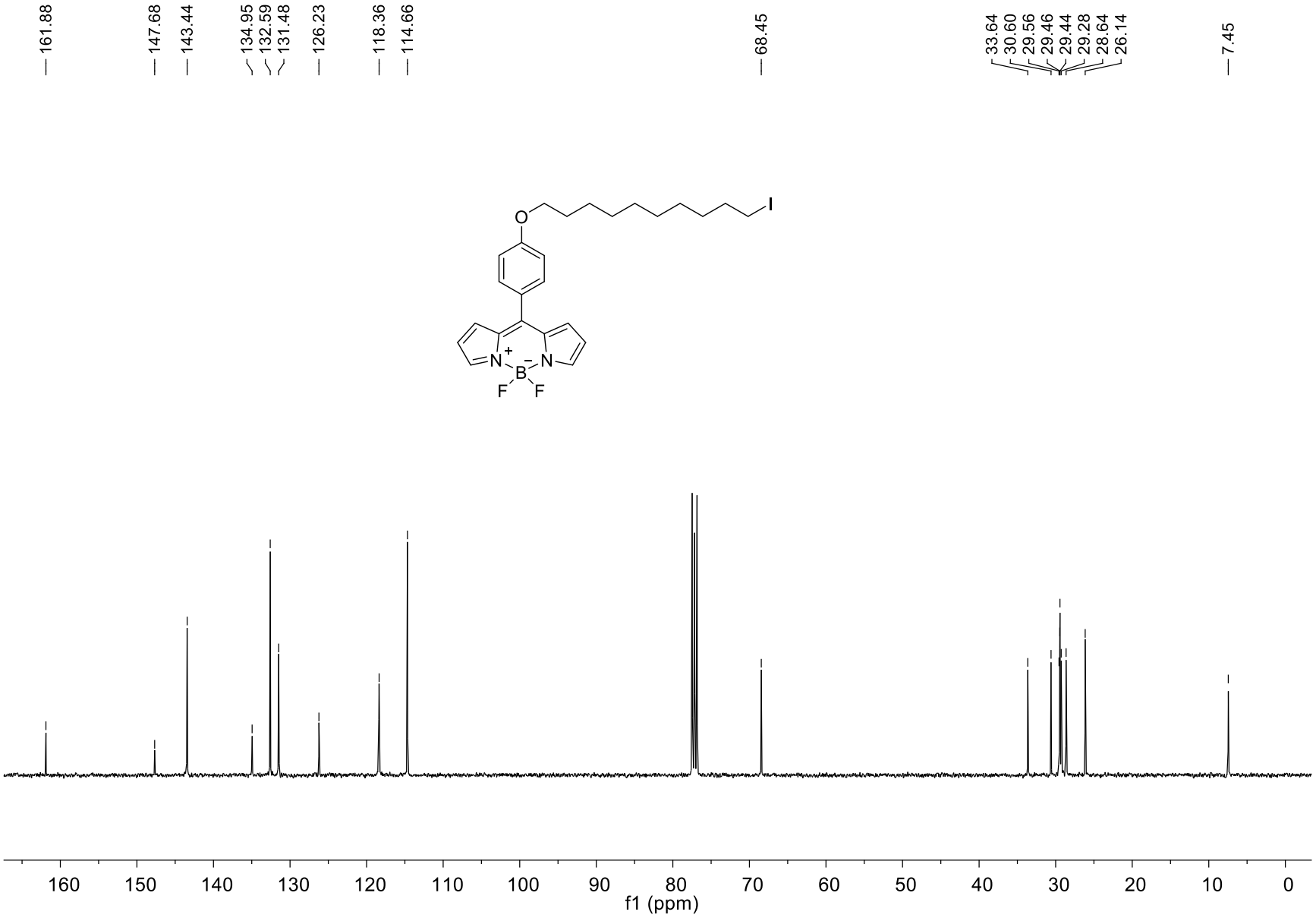


Figure S10. ^{13}C NMR spectrum of **BODIPY 3** (100 MHz, $CDCl_3$).

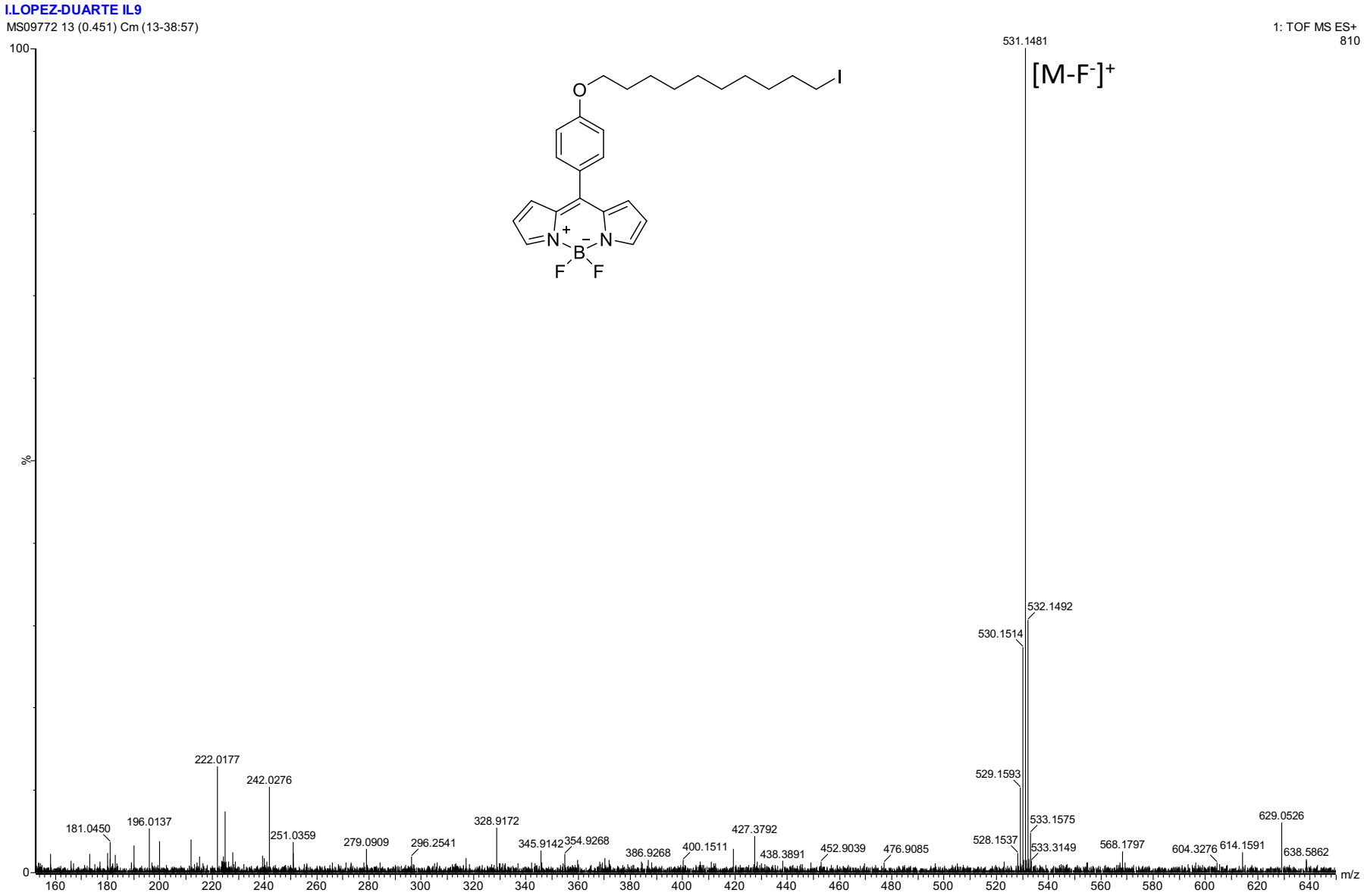


Figure S11. HRMS (ESI-TOF) mass spectrum of **BODIPY 3**.

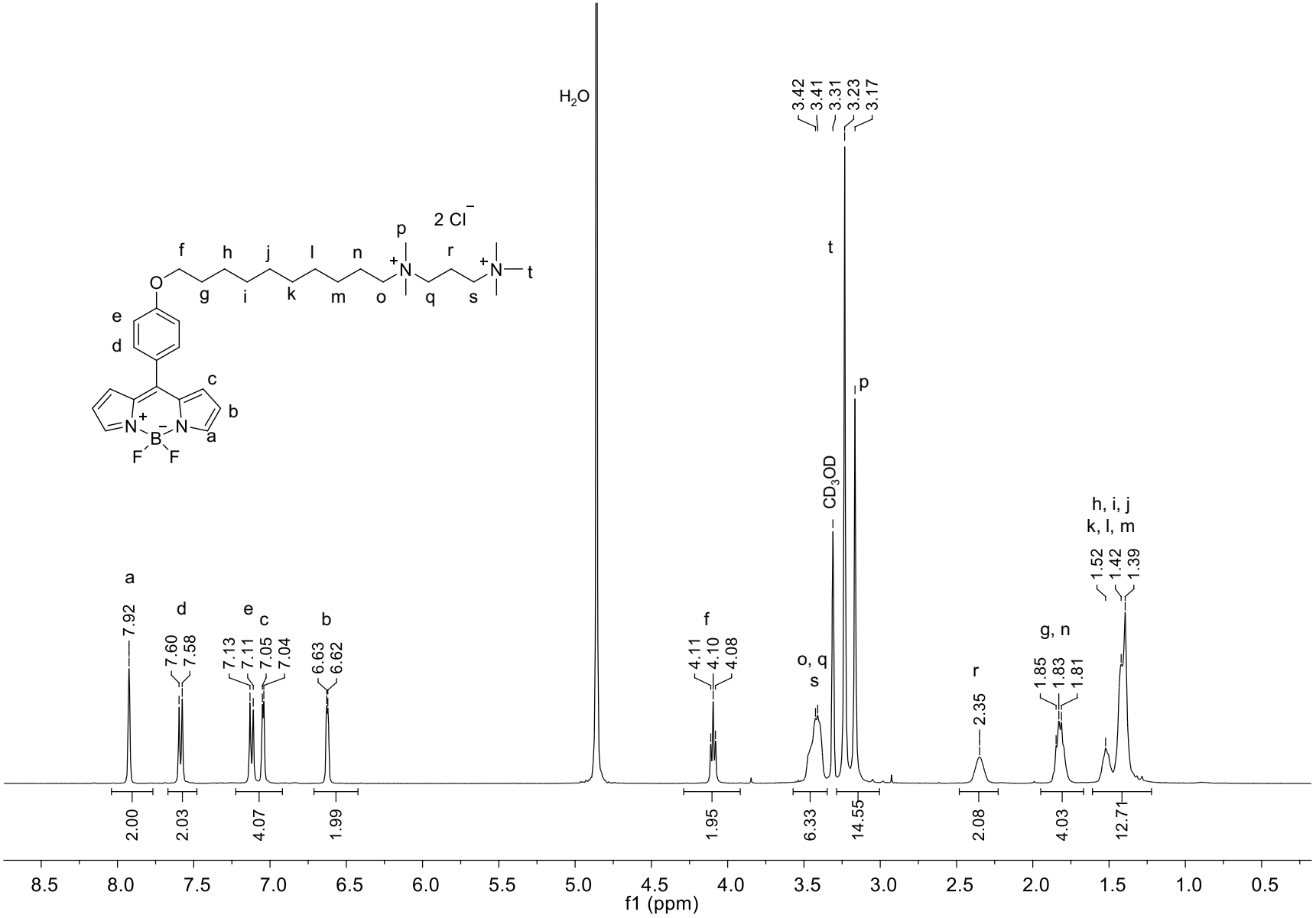


Figure S12. ^{1}H NMR spectrum of **rotor 2** (400 MHz, CD_3OD).

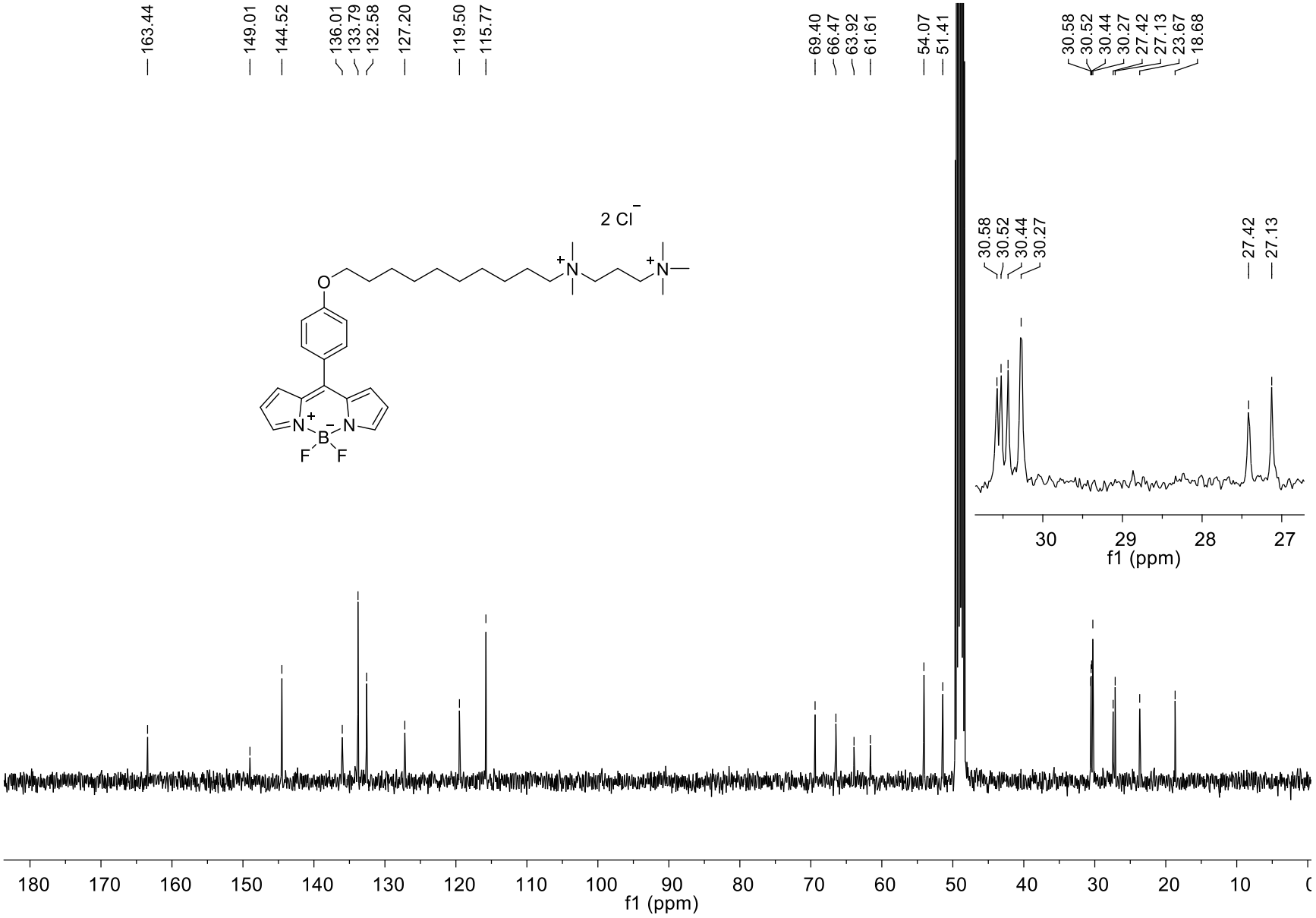


Figure S13. ^{13}C NMR spectrum of **rotor 2** (100 MHz, CD_3OD).

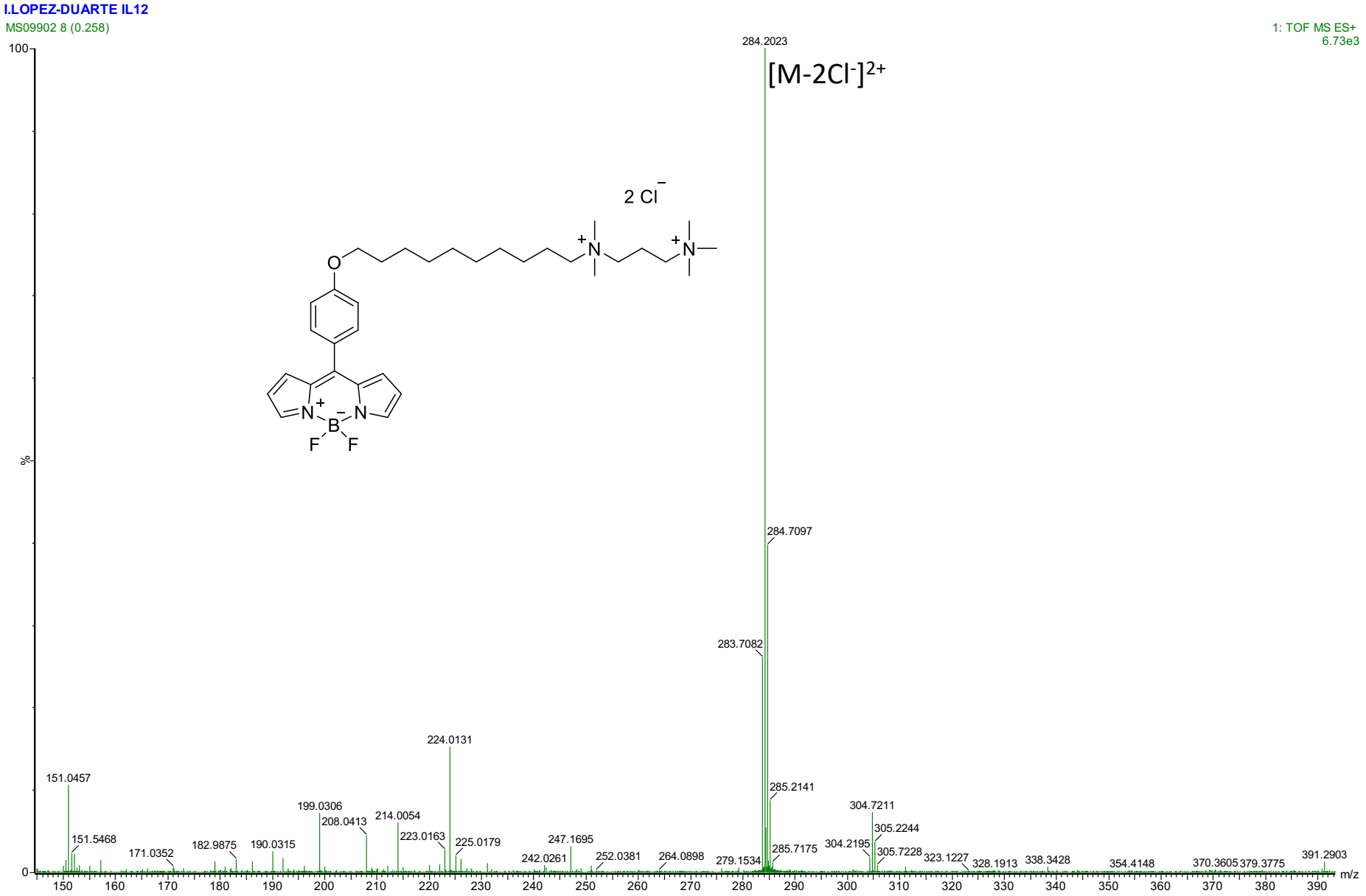


Figure S14. HRMS (ESI-TOF) mass spectrum of **rotor 2**.

3. Supplementary References

1. (a) Wagner, R. W.; Lindsey J. S. *Pure & Appl. Chem*. **1996**, *68 (7)*, 1373-1380. (b) Loudet, A.; Burgess, K. *Chem. Rev*., **2007**, *107*, 4891-4932.