Supporting Information for:

Exploring viscosity, polarity and temperature sensitivity of BODIPY-based molecular rotors

Aurimas Vyšniauskas,[a]‡ Ismael López-Duarte,[a] Nicolas Duchemin,[a] Thanh-Truc Vu,[a] Yilei Wu,[a] Ekaterina M. Budynina,[b] Yulia A. Volkova,[b] Eduardo Peña Cabrera,[c] Diana E. Ramírez-Ornelas,[c] Marina K Kuimova[a]*

a. Chemistry Department, Imperial College London, Exhibition Road, SW7 2AZ, UK.

E-mail: m.kuimova@imperial.ac.uk

b. Department of Chemistry, Lomonosov Moscow State University, 119991, Russia.

c. Departamento de Química DCNE, Universidad de Guanajuato, Col. Noria Alta S/N

Guanajuato, Gto. 36050, Mexico.

‡ Present address: Center of Physical Sciences and Technology, Sauletekio av. 3, Vilnius, LT-10257, Lithuania.

Table of Contents

1. Additional data **p. 2-6**

2. Synthesis and compound characterization **p. 7-25**

3. References **p. 26**

1. Additional data

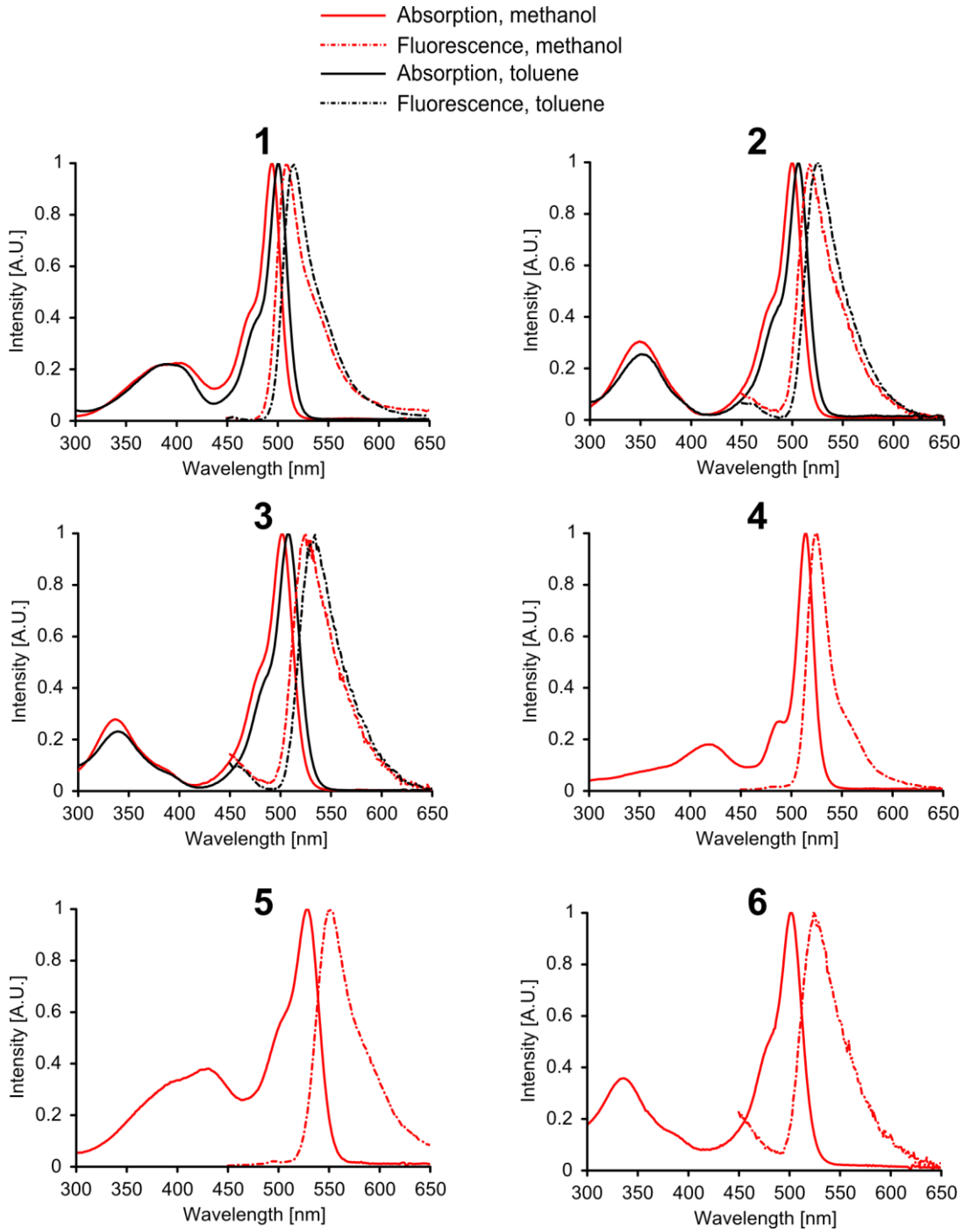


Figure S1. Absorption (solid lines) and fluorescence (dotted lines) spectra of BODIPY-based molecular rotors in methanol (red) and toluene (black). The excitation wavelengths for fluorescence spectra were 400 nm (**1**), 340 nm (**2, 3, 6**), 420 nm (**4**) and 430 nm (**5**).

Table S1. Positions of peaks in spectra in Figure S1 for all dyes.

	Solvent	Absorption /nm	Fluorescence /nm
1	Methanol	495	509
	Toluene	501	516
2	Methanol	500	518
	Toluene	506	526
3	Methanol	502	525
	Toluene	508	534
4	Methanol	515	525
5	Methanol	529	551
6	Methanol	502	525

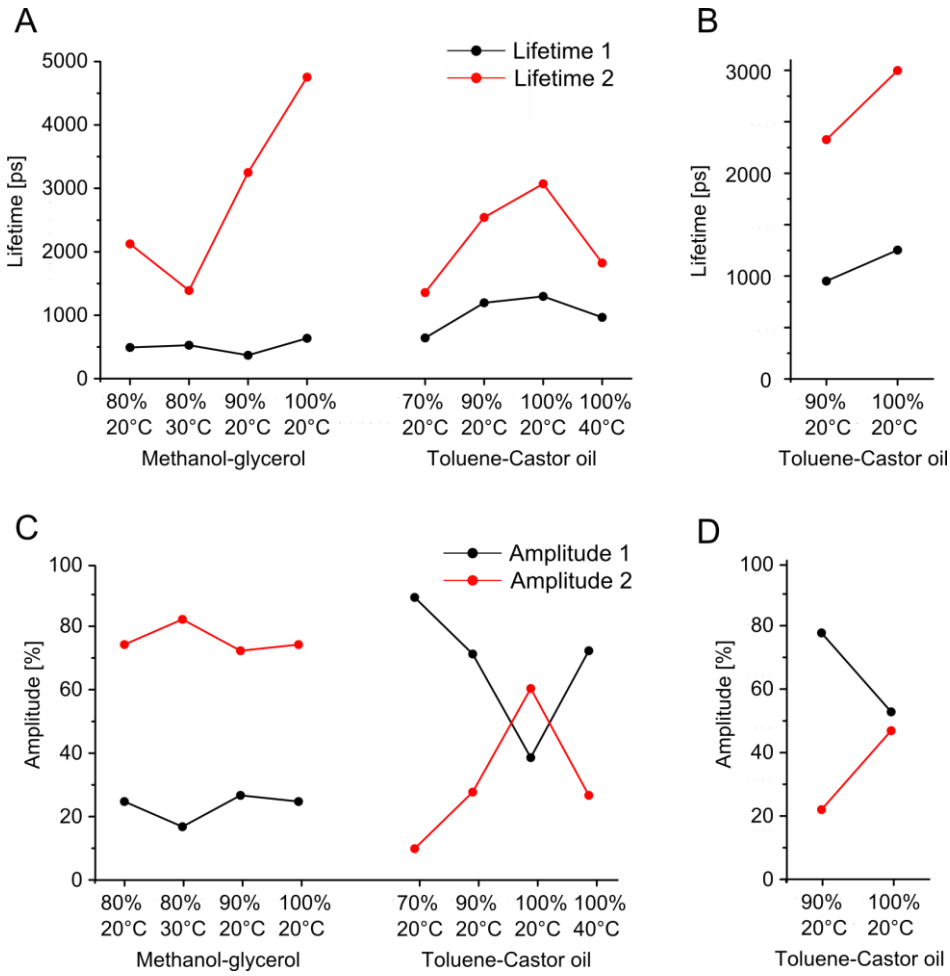


Figure S2. Lifetimes and amplitudes of biexponential fluorescence decays of dyes **2** and **3**. The top row shows the individual lifetime components of **2** (A) and **3** (B); the bottom row contains the amplitudes of **2** (C) and **3** (D). The solvent mixtures and temperatures at which biexponential decays were obtained are shown on *x* axes.

Table S2. Parameters of the global fits of the calibration data of **1**, **2** and **3** in Figure 5, main text.

	Solvent mixture	a_1	a_2	a_3	a_4	a_5
1	Methanol-glycerol	6.7	-0.80	0.025	-0.58	$1.6 \cdot 10^8$
	Toluene-Castor oil	0.61	-0.70	0.12	-1.32	$1.4 \cdot 10^8$
2	Methanol-glycerol	46.8	-0.76	0.067	0	$1.1 \cdot 10^8$
	Toluene-Castor oil	0.48	-0.57	0.57	-1.43	$5.6 \cdot 10^7$
3	Methanol-glycerol	16.8	-0.88	0.060	0	$1.3 \cdot 10^8$
	Toluene-Castor oil	0.69	-0.55	1.4	-1.20	$-5.7 \cdot 10^7$

The global fitting of the data, taking into account the environmental polarity

In an attempt to create a single equation to globally fit the datasets measured in both methanol-glycerol and toluene-Castor oil mixtures for each rotor we have expanded Equation 6 (main text) with additional empirical polarity-dependent terms. In order to fit the data we had to use polarity-dependent terms instead of constants a_1, a_3 and a_4, resulting in the Equation S1 below.

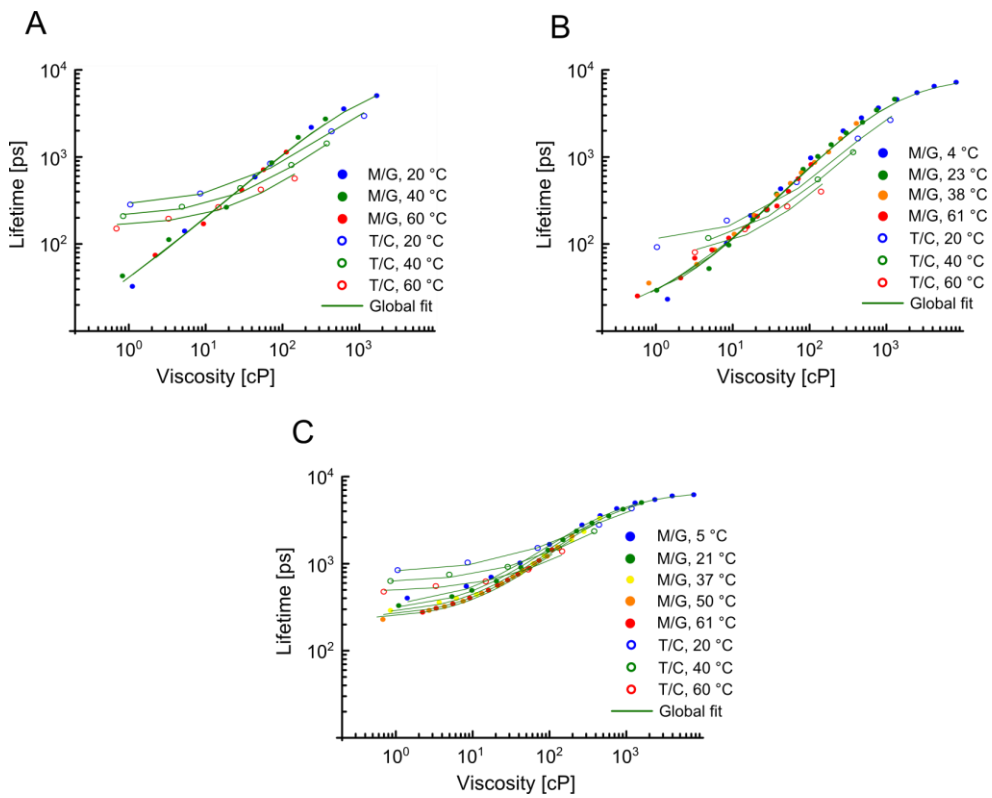


Figure S3. The global fits of lifetime data in Figure 2, main text. The data for dyes **2**, **3** and **1** are shown in A), B) and C), respectively. The data obtained in methanol-glycerol and toluene-Castor oil mixtures were fitted globally together using Equation S1:

$$\tau = \frac{1}{\frac{1}{\left(a_1 + a_2\varepsilon\right)\eta^{a_3} + \left(a_4 + a_5\varepsilon\right)} e^{\left(a_6 + a_7\varepsilon\right)/T} + a_8} \qquad \text{(S1)}$$

$a_{1...8}$ are free parameters, η is viscosity, T is temperature and ε is the average dielectric constant for the solvent mixtures used. The value was 3.5 for toluene-Castor oil and 38 for methanol-glycerol mixtures.

In order to fit the data for one fluorophore in two types of solvent mixtures using a single global fit we had to include polarity-dependent empirical terms instead of constants a_1, a_3 and a_4 in Equation 6, main text. Constant a_4 needed to be replaced because the activation energy barrier for rotation is polarity-dependent, as explained in main text. Interestingly, this was not enough in order to have a good fit and we also needed to replace a_1 and a_3 with the polarity-dependent terms. The fitting parameters are shown in Table S3 below.

Table S3. Parameters of the full global fits of the calibration data of **1**, **2** and **3** in Figure S3.

	a_1	a_2	a_3	a_4	a_5	a_6	a_7	a_8
1	-0.25	0.19	-0.79	6.0	0.86	$-1.4\cdot10^3$	22	$1.5\cdot10^{-4}$
2	-1.34	0.49	-0.85	2.3	0.32	$-1.1\cdot10^3$	28	$1.3\cdot10^{-4}$
3	-4.34	1.32	-0.72	2.15	0.12	$-1.5\cdot10^3$	39	$1.1\cdot10^{-4}$

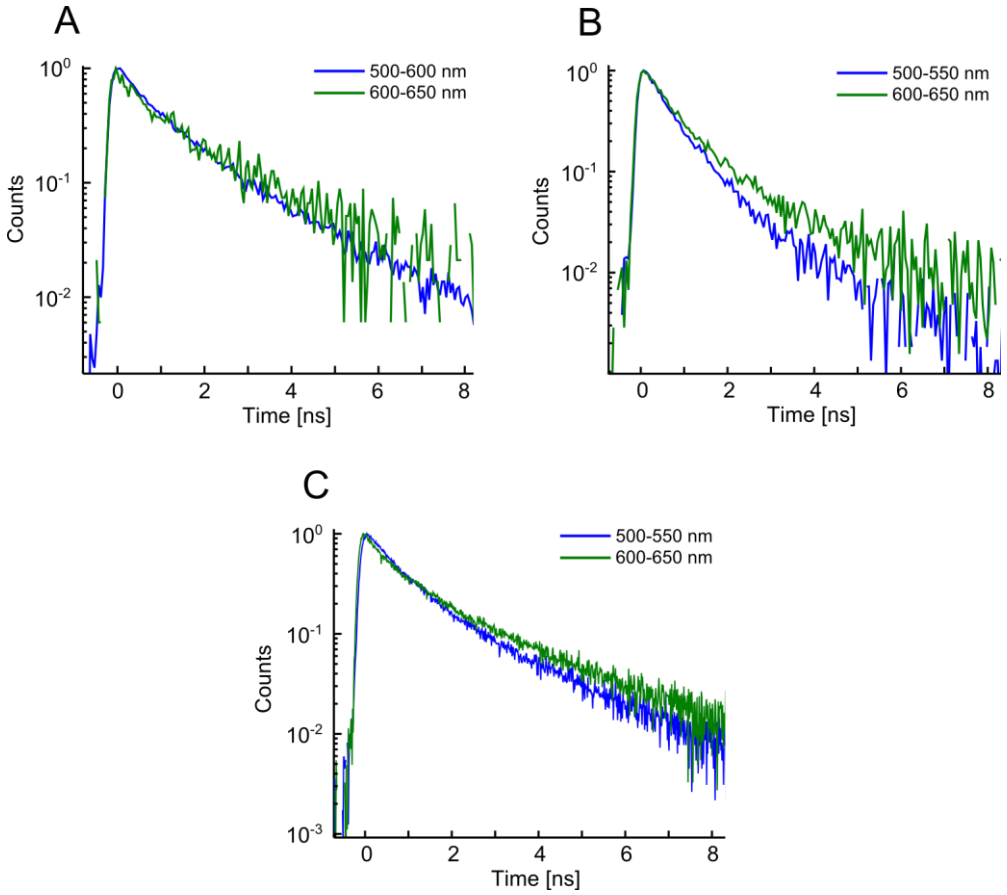


Figure S4. The checks for aggregation of dyes **2** (A), **3** (B) and **6** (C) in SK-OV-3 cells. BODIPY aggregates are known to emit at a higher wavelength and have a longer fluorescence lifetime than BODIPY monomers. In order to ascertain their presence, the fluorescence decays were measured at two ranges of wavelengths. The decays measured in A, B and C are similar, which indicates a small to negligible degree of aggregation of **2**, **3** and **6**.

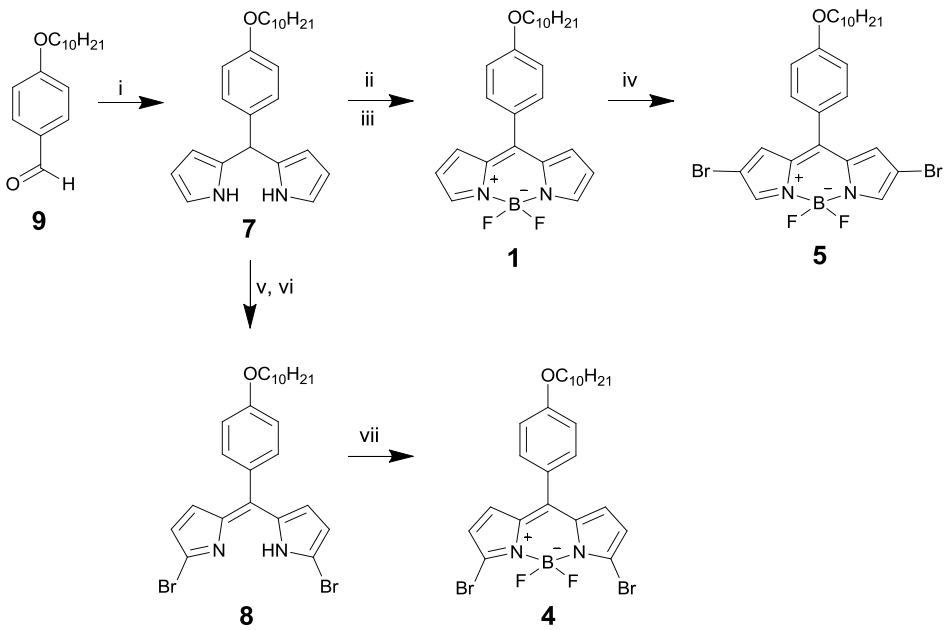
2. Synthesis and compound characterization

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 (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 ($^{13}CD_3OD$: 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

BODIPY 4 and BODIPY 5



Scheme S1. Synthesis of **BODIPY 4** and **BODIPY 5:** (i) neat pyrrole, TFA; (ii) DDQ, CH_2Cl_2 and then (iii) $BF_3{\cdot}(OEt_2)_2$, Et_3N, CH_2Cl_2; (iv) NBS, DMF/CH_2Cl_2, Yield: 53%; (v) NBS, THF, and then (vi) DDQ, THF, Yield: 35%; (vii) $BF_3{\cdot}(OEt_2)_2$, Et_3N, toluene, Yield: 89%;

Compound **9**,[1] dypyrromethane **7**[1] and **BODIPY 1**[1] were prepared according to previously published procedures.

Dibromodipyrromethene 8. A solution of dipyrromethane **7** (600 mg, 1.6 mmol) in dry THF (60 mL) was cooled to -78 °C under argon. NBS (590 mg, 3.34 mmol) was added in two portions over 1h. Once the NBS was dissolved, a solution of DDQ (390 mg, 1.6 mmol) in THF (5 mL) was added dropwise. The reaction mixture was allowed to warm up to room temperature overnight and the solvent was removed under vacuum. The crude product was purified by flash chromatography on silica gel (1:10 CH_2Cl_2:petroleum ether) to give **8** as an orange solid. Yield: 300 mg (35%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 12.50 (br s, 1H), 7.38 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 6.55 (d, *J* = 4.4 Hz, 2H), 6.36 (d, *J* = 4.4 Hz, 2H), 4.03 (t, *J* = 6.5 Hz, 2H), 1.83 (m, 2H), 1.51 (m, 2H), 1.29 (m, 12H), 0.90 (m, 3H); **HRMS** (ESI-TOF) m/z 533.0808 ($C_{25}H_{31}Br_2N_2O$ $[M+H]^+$, requires 533.0803).

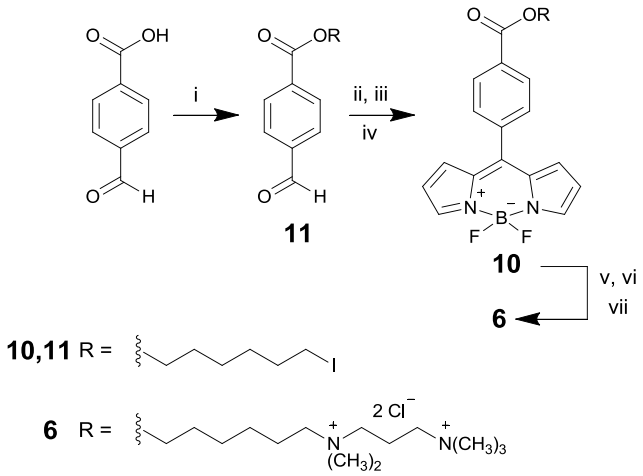
BODIPY 4. A mixture of dipyrromethene **8** (300 mg, 0.56 mmol), Et_3N (0.78 mL; 5.6 mmol) and $BF_3.Et_2O$ (0.69 mL, 0.56 mmol) in toluene (15 mL) was stirred at room temperature for 1h. The reaction mixture was treated with water (10 mL), and the organic solution was dried over anhydrous $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography on silica gel (10:1 petroleum ether:ethyl acetate) to give **BODIPY 4** as an orange solid. Yield: 290 mg (89%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.45 (d, *J* = 8.4 Hz, 2H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 4.3 Hz, 2H), 6.55 (d, *J* = 4.3 Hz, 2H), 4.05 (t, *J* = 6.5 Hz, 2H), 1.84 (m, 2H), 1.50 (m, 2H), 1.29 (m, 12H), 0.90 (t, *J* = 6.3 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ_C 161.78, 143.46, 135.40, 132.28, 131.69, 131.52, 124.56, 122.40, 114.62, 68.37, 31.87, 29.55, 29.34, 29.30, 29.10, 26.00, 22.67, 21.05, 14.11; **HRMS** (ESI-TOF) m/z 580.0692 ($C_{25}H_{29}BBr_2F_2N_2O$ $[M]^+$, requires 580.0708).

BODIPY 5. To a solution of **BODIPY 1** (620 mg, 1.46 mmol) in DMF/CH_2Cl_2 (50 mL/50 mL) was added dropwise a solution of NBS (413 mg, 3.50 mmol) in CH_2Cl_2 (30 mL) at room temperature.[2] The mixture was stirred at room temperature for 2 h. After removal of solvents under vacuum, the crude product was purified by column chromatography on silica gel (2:1 CH_2Cl_2:hexane) to give **BODIPY 5** as an orange solid. Yield: 433 mg (53%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.82 (s, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.06 (d, *J* = 8.5 Hz, 2H), 7.01 (s, 2H), 4.07 (t, *J* = 6.4 Hz, 2H), 1.86 (m, 2H), 1.50 (m, 2H), 1.29 (m, 12H), 0.90 (t, *J* = 6.3 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ_C 162.51, 147.22, 143.19, 134.41, 132.56, 131.51, 125.11, 114.94, 106.80, 68.48, 31.89, 29.54, 29.32, 29.06, 25.98, 22.66, 14.11; **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ_F -144.90 (q, J_{F-B} = 27.9 Hz); **HRMS** (ESI-TOF) m/z 580.0705 ($C_{25}H_{29}BN_2OF_2Br_2$ $[M]^+$, requires 580.0708).

BODIPY 6



Scheme S2. Synthesis of **BODIPY 6**: (i) 1,6-diiodohexane, K_2CO_3, DMF, Yield: 67%; (ii) neat pyrrole, TFA, Yield: 96%; (iii) DDQ, CH_2Cl_2 and then (iv) $BF_3{\cdot}(OEt_2)_2$, Et_3N, CH_2Cl_2, Yield: 20%; (v) *N,N,N',N'*-tetramethyl-1,3-propanediamine, THF, and then (vi) CH_3I, DMF, and finally (vii) Dowex® 1x8 200 mesh ion-exchange column, H_2O. Yield: 41%.

4-(6-iodohexyloxycarbonyl)benzaldehyde (11). 1,6-Diiodohexane (25 g, 74 mmol) was added to a mixture of 4-formylbenzoic acid (1 g, 6.6 mmol) and potassium carbonate (1.8 g, 13 mmol) in dry *N,N*-dimethylformamide (25 mL). The reaction mixture was stirred at 75°C for 3 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 0.35, to give a colorless oil. Yield: 1.65 g (67%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 10.10 (s, 1H), 8.19 (d, *J* = 8.8 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H), 4.36 (t, *J* = 6.3 Hz, 2H), 3.20 (t, *J* = 6.7 Hz, 2H), 1.86 (m, 4H), 1.48 (m, 4H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ_C 191.53, 165.52, 139.08, 135.31, 130.09, 129.45, 65.40, 33.22, 30.06, 28.43, 24.96, 6.70.

BODIPY 10. 4-(6-iodohexyloxycarbonyl)benzaldehyde (**11**) (1.6 g, 4.4 mmol) was dissolved in freshly distilled pyrrole (20 mL, 288 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 45 minutes 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 0.38, to give a green viscous oil. Yield: 2 g (96%). The dipyrromethane (2 g, 4.2 mmol) was dissolved in CH_2Cl_2 (50 mL) and DDQ (0.96 g, 4.2 mmol) was added. The reaction mixture was stirred at room temperature shielded from light for 45 min. Then, Et_3N (2 mL, 14.3 mmol) was added, followed immediately by the addition of $BF_3{\cdot}(OEt_2)_2$ (1.5 mL, 11.9 mmol) and the reaction mixture was stirred at room temperature overnight.[1] 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 (6:1 petroleum ether:ethyl acetate), R_f 0.30, to give **BODIPY 10** as a red-orange sticky solid. Yield: 457 mg (20%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.21 (d, *J* = 8.8 Hz, 2H), 7.98 (br s, 2H), 7.66 (d, *J* = 8.8 Hz, 2H), 6.89 (d, *J* = 3.9 Hz, 2H), 6.58 (dd, *J* = 3.9, 1.1 Hz, 2H), 4.40 (t, *J* = 6.4 Hz, 2H), 3.22 (t, *J* = 6.8 Hz, 2H), 1.88 (m, 4H), 1.52 (m, 4H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ_C 165.72, 145.77, 144.80, 137.91, 134.72, 132.41, 131.40, 130.40, 129.54, 118.92, 65.35, 33.29, 30.15, 28.55, 25.07, 6.78; **^{19}F NMR** (377.5 MHz, $CDCl_3$) δ_F –143.98 (q, J_{F-B} = 27.5 Hz); **HRMS** (ESI-TOF) m/z 521.0732 ($C_{22}H_{21}BN_2O_2F_2I$, $[M]^+$, requires 521.0709, 100%).

BODIPY 6. To a solution of **BODIPY 10** (150 mg, 0.28 mmol) in 2 mL of THF was added *N,N,N′,N′*-tetramethyl-1,3-propanediamine (3 mL, 18 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 DMF (2 mL) and iodomethane (1 mL, 16 mmol) was added to the solution.[3] 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 **BODIPY 6** 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 **BODIPY 6** as a red-orange wax. Yield: 75 mg (41%).

^{1}H NMR (400 MHz, CD_3OD) δ_H 8.22 (d, *J* = 8.3 Hz, 2H), 8.01 (br s, 2H), 7.75 (d, *J* = 8.3 Hz, 2H), 6.98 (d, *J* = 4.4 Hz, 2H), 6.64 (dd, *J* = 4.4, 1.9 Hz, 2H), 4.42 (t, *J* = 5.6 Hz, 2H), 3.45 (m, 6H), 3.23 (s, 9H), 3.18 (s, 6H), 2.39 (m, 2H), 1.87 (m, 4H), 1.62 (m, 2H), 1.52 (m, 2H); **^{13}C NMR** (100 MHz, CD_3OD) δ_C 167.34, 147.30, 146.30, 139.44, 136.13, 133.81, 132.81, 132.01, 130.76, 120.35, 66.47, 64.02, 61.71, 54.16, 51.58, 29.77, 27.16, 26.85, 23.73, 18.79; **^{19}F NMR** (377.5 MHz, CD_3OD) δ_F –145.57 (q, J_{F-B}=27.8 Hz); **HRMS** (ESI-TOF) m/2z 270.1746 ($C_{30}H_{43}BF_2N_4O_2$, $[M-2Cl^-]^{2+}$, requires 270.1723).

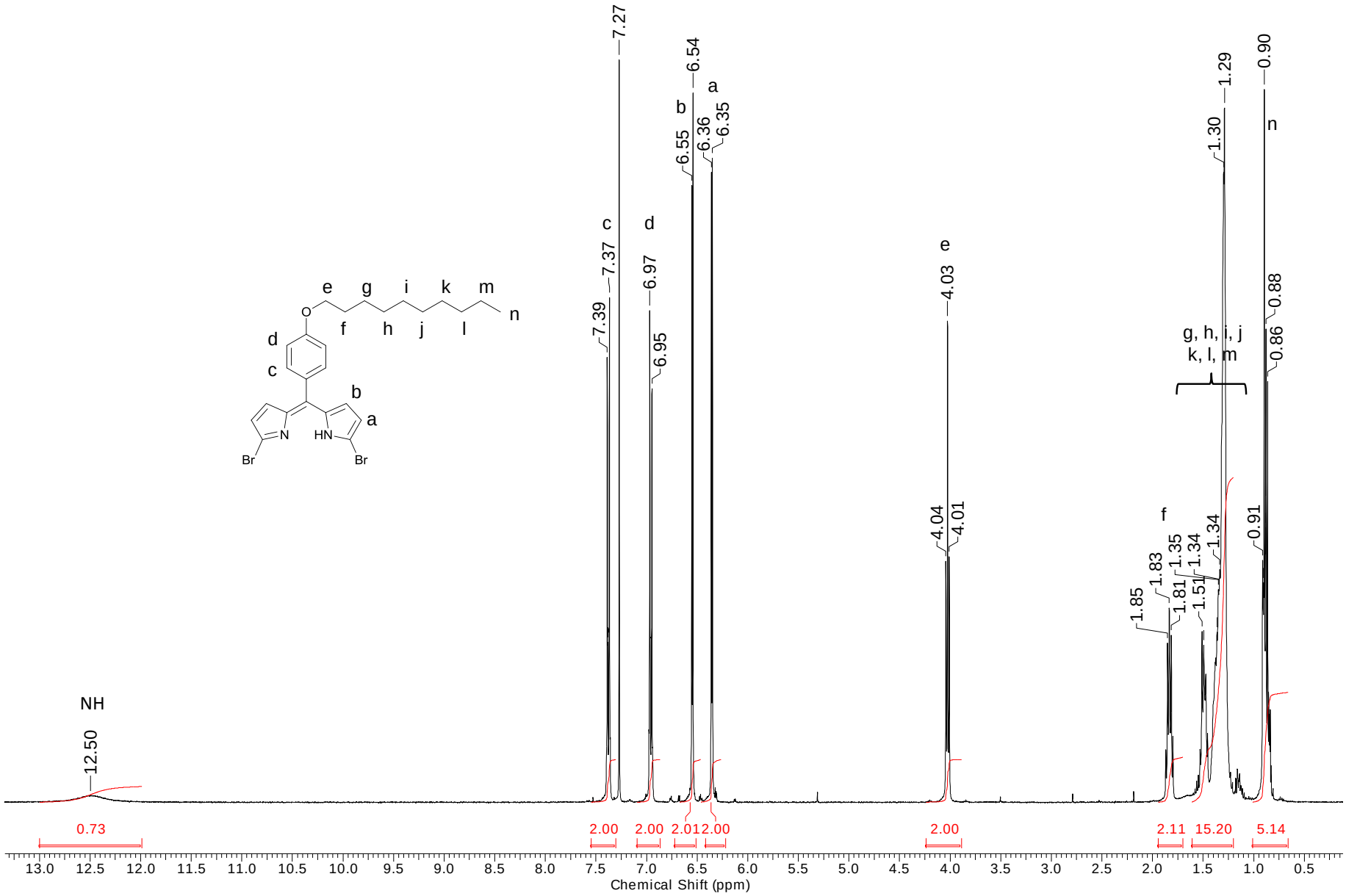


Figure S5. ^{1}H NMR spectrum of **8** (400 MHz, $CDCl_3$).

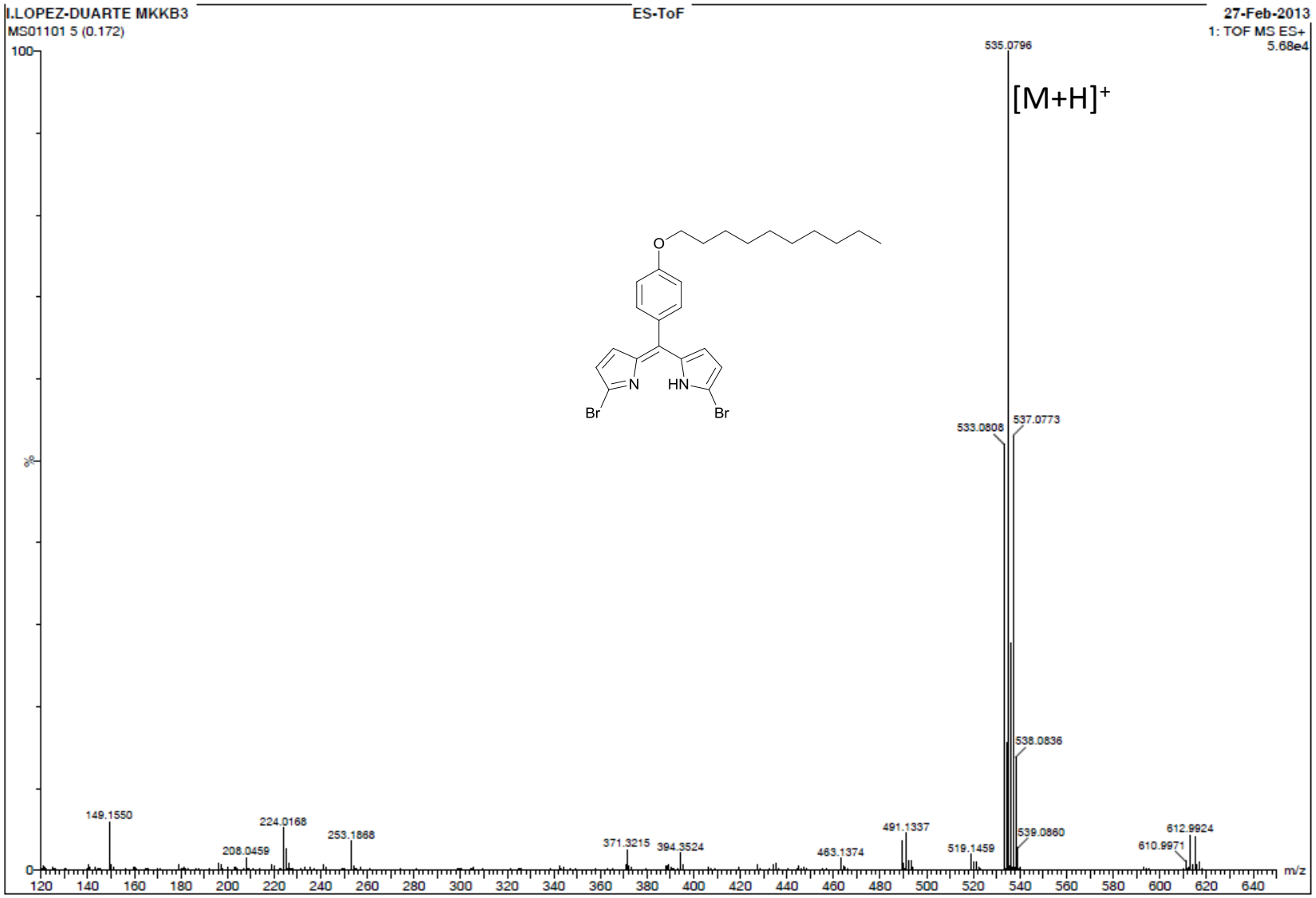


Figure S6. HRMS (ESI-TOF) mass spectrum of **8**.

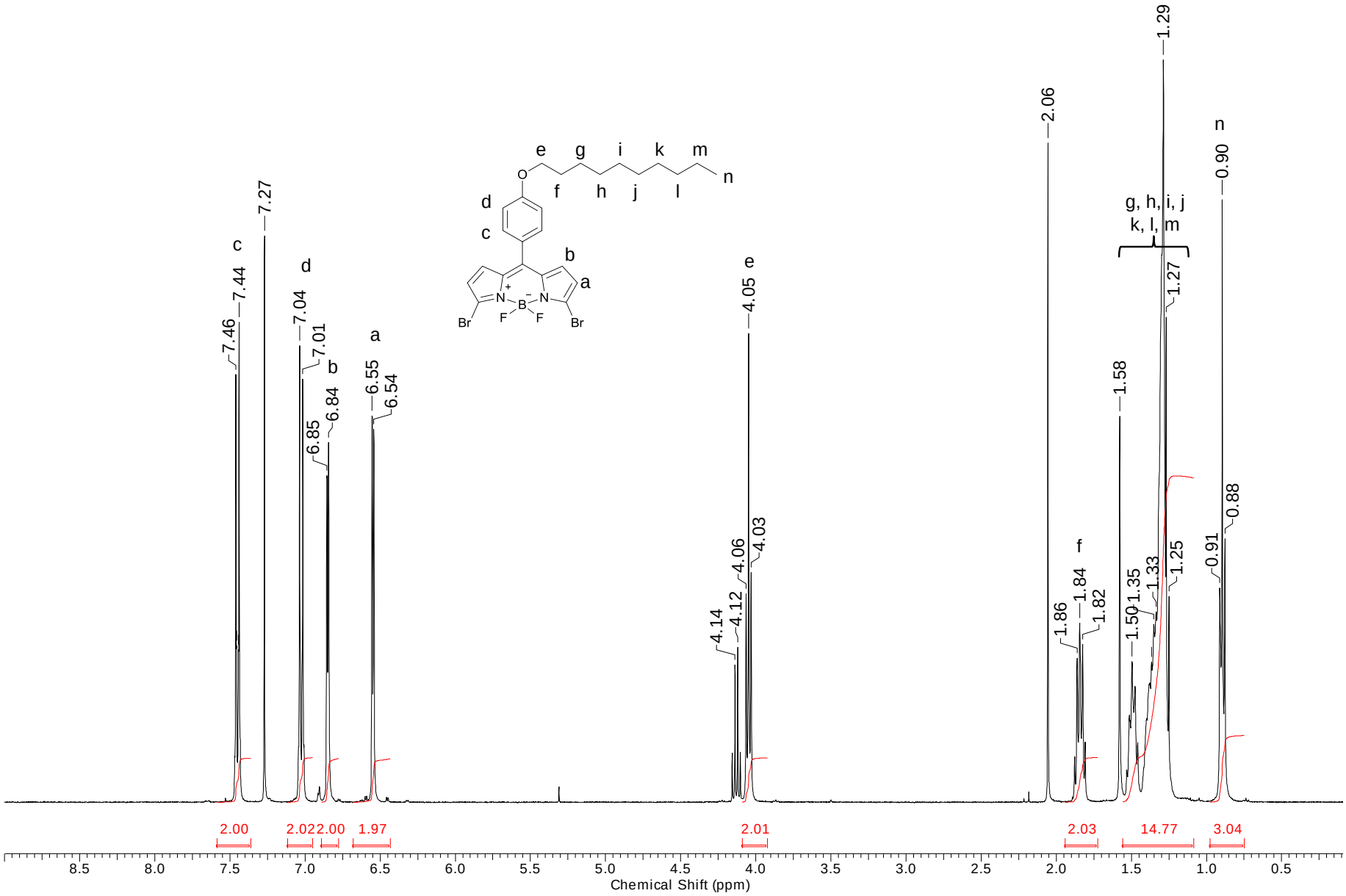


Figure S7. ^{1}H NMR spectrum of **BODIPY 4** (400 MHz, $CDCl_3$).

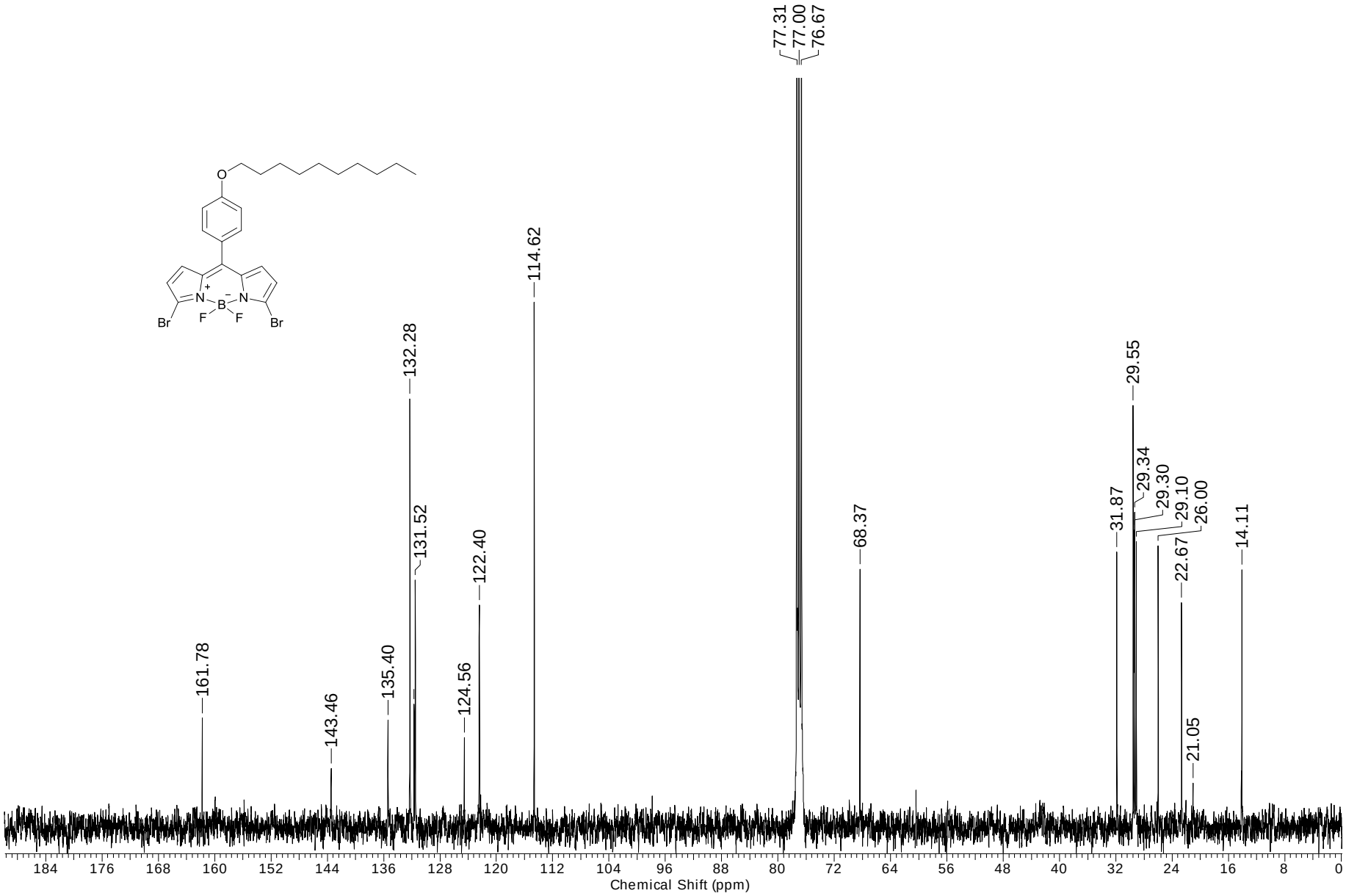


Figure S8. ^{13}C NMR spectrum of **BODIPY 4** (100 MHz, $CDCl_3$).

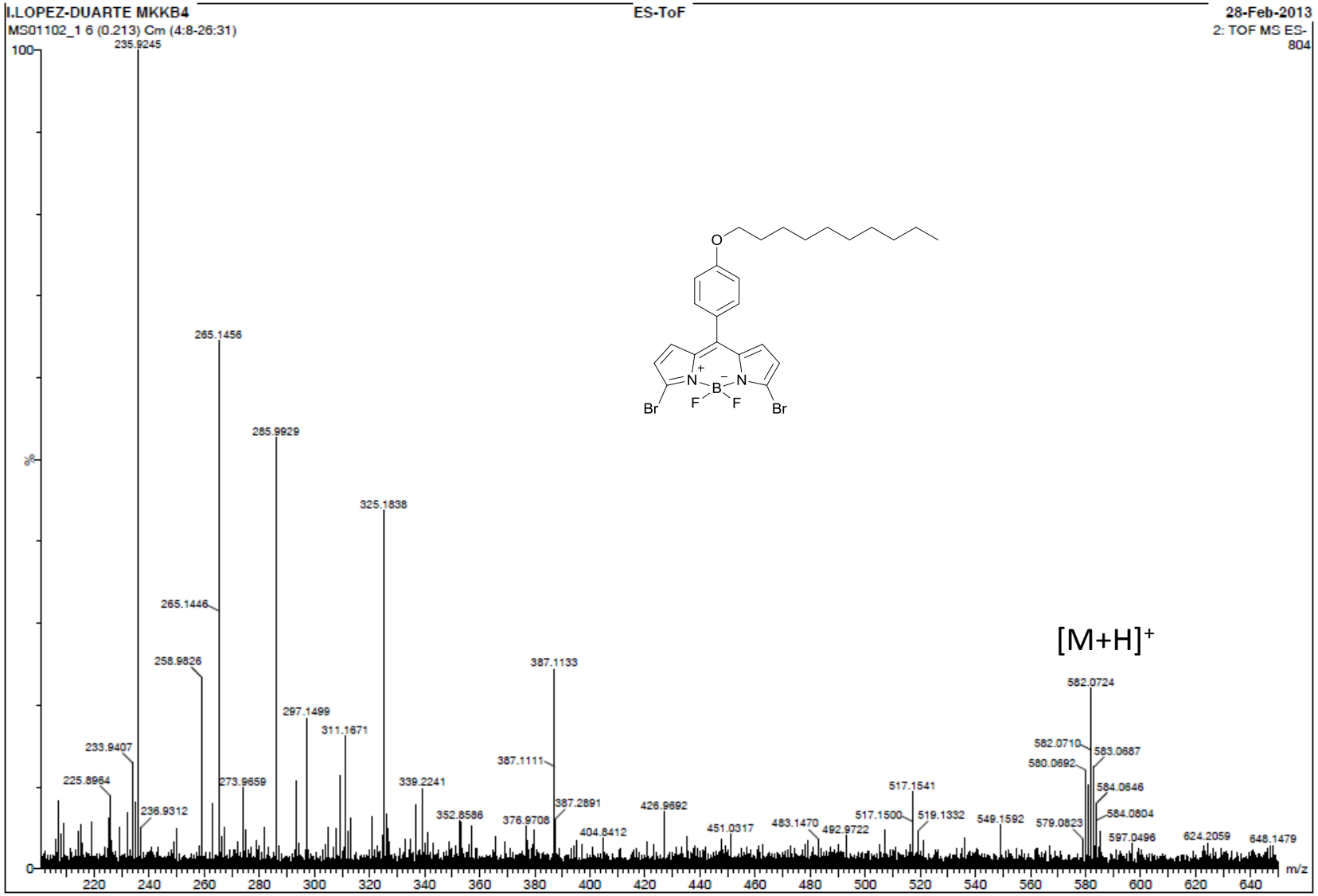


Figure S9. HRMS (ESI-TOF) mass spectrum of **BODIPY 4**.

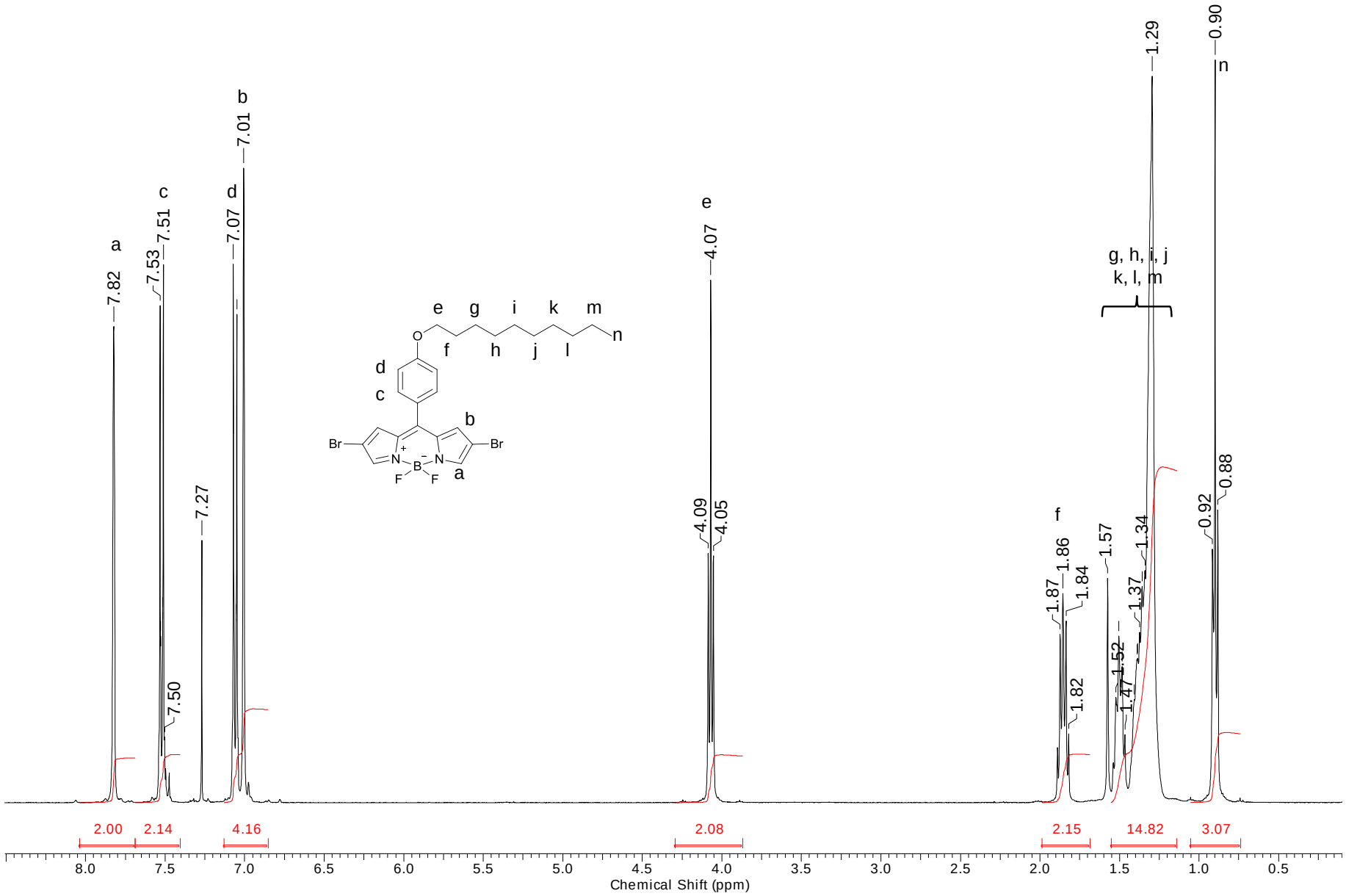


Figure S10. ^{1}H NMR spectrum of **BODIPY 5** (400 MHz, $CDCl_3$).

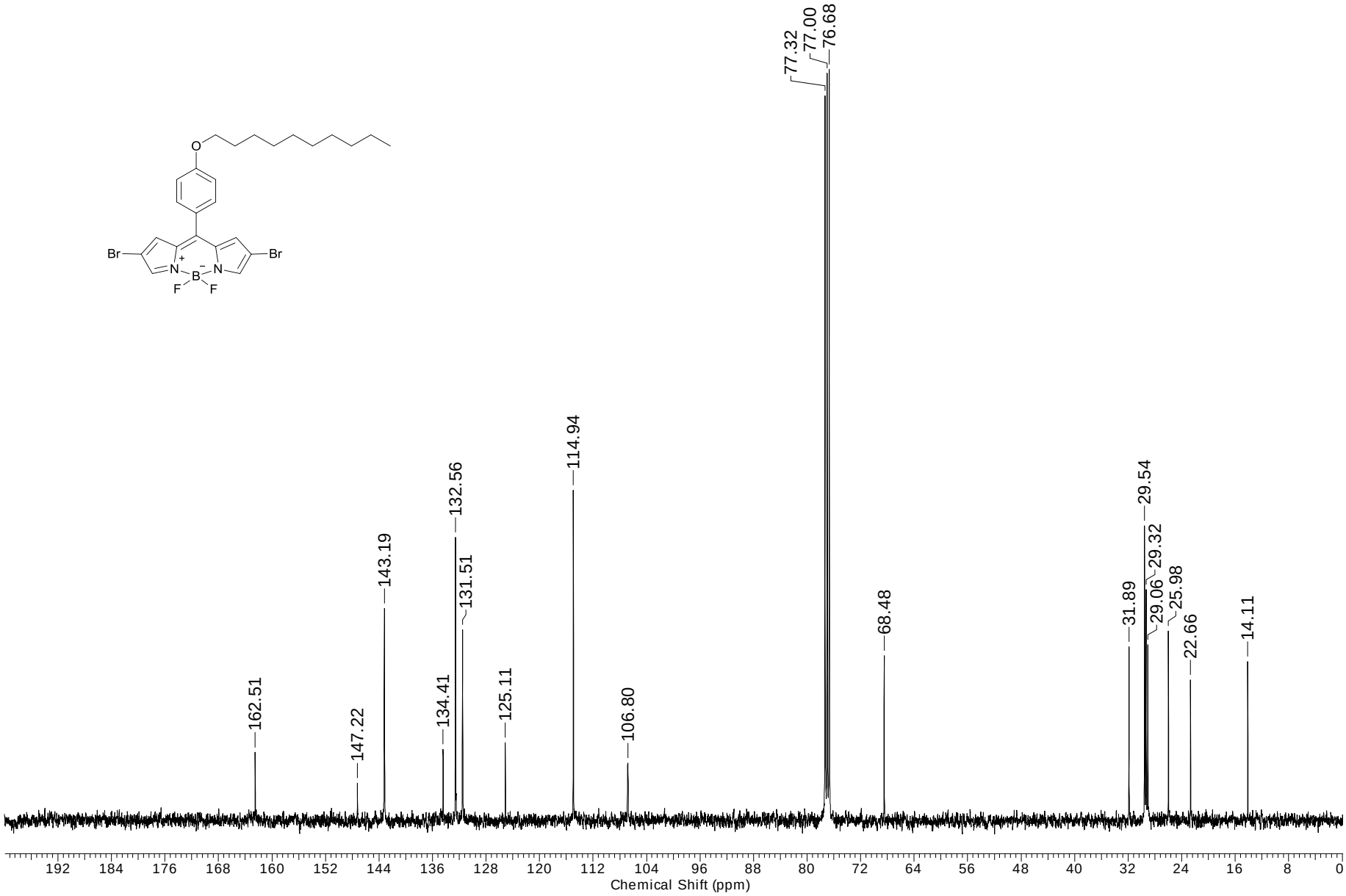


Figure S11. ^{13}C NMR spectrum of **BODIPY 5** (100 MHz, $CDCl_3$).

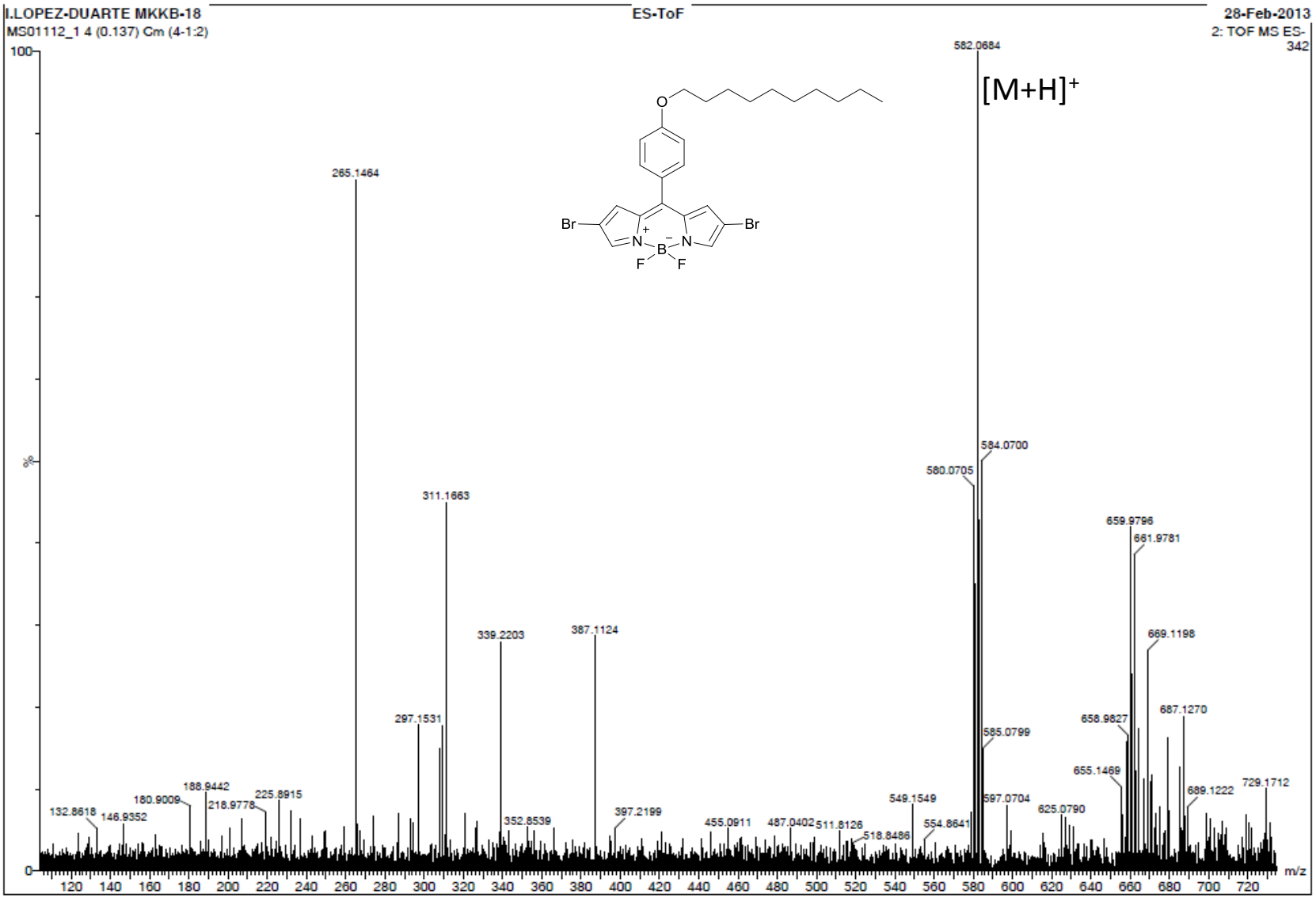


Figure S12. HRMS (ESI-TOF) mass spectrum of **BODIPY 5**.

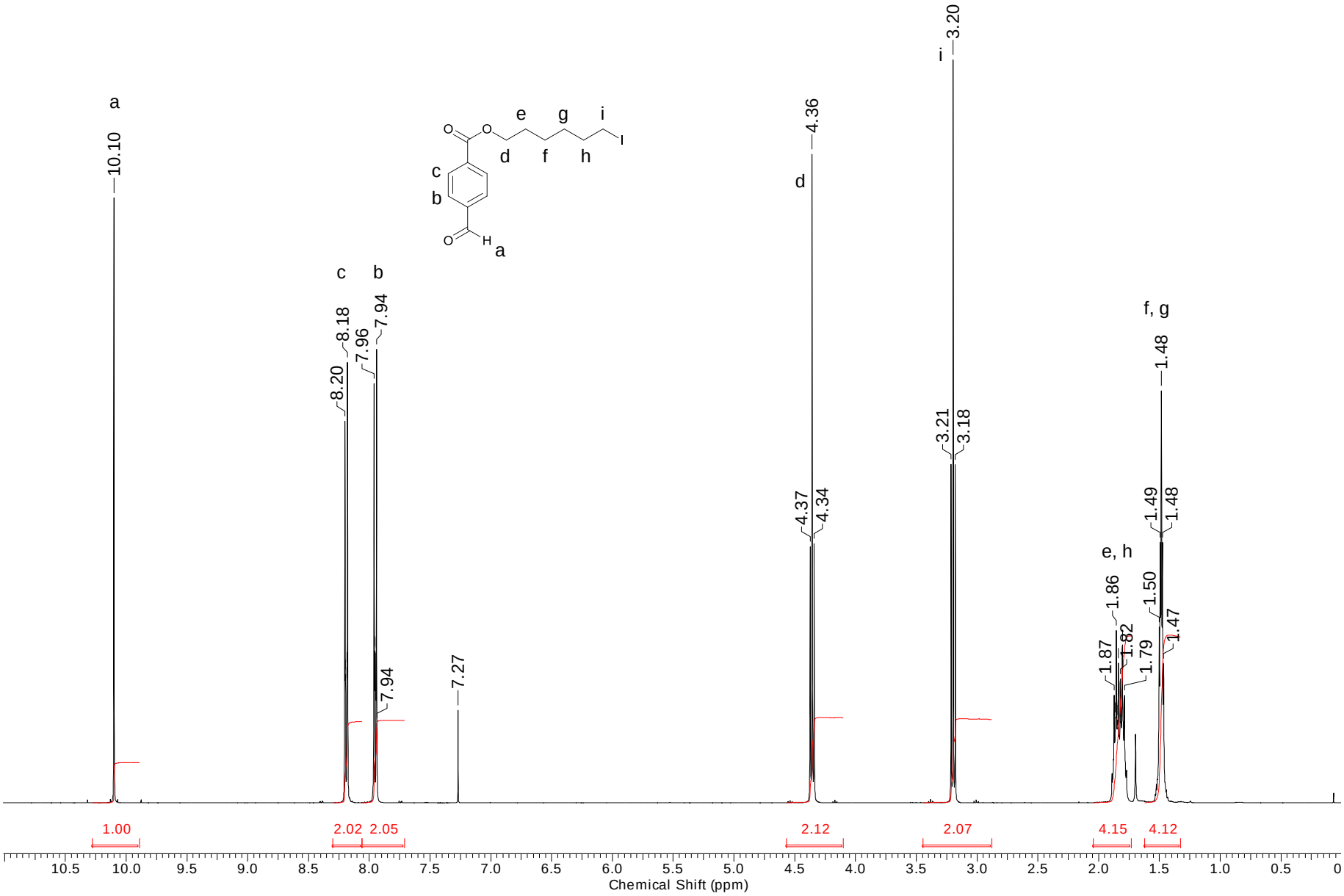


Figure S13. ^{1}H NMR spectrum of **11** (400 MHz, $CDCl_3$).

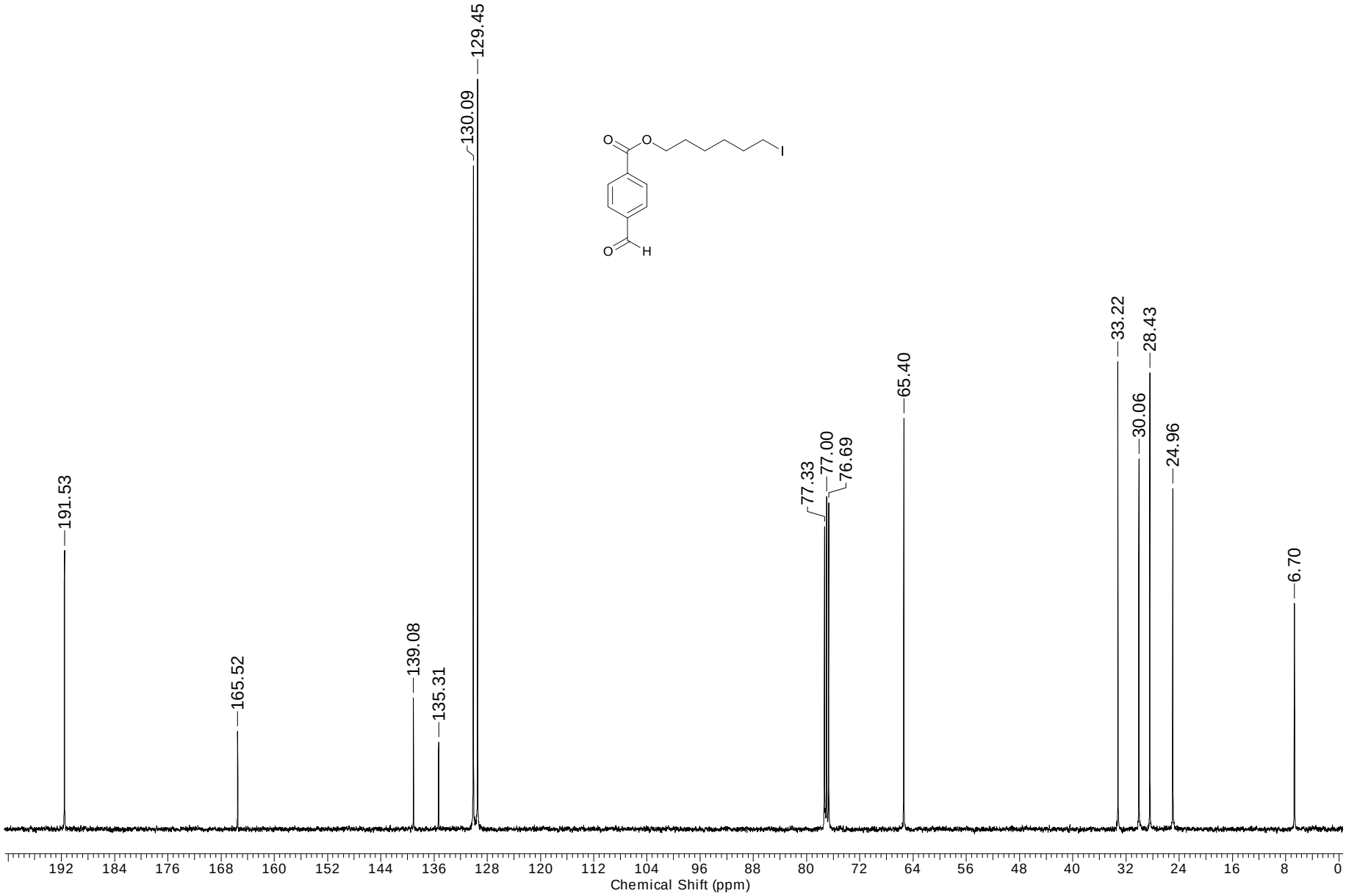


Figure S14. ^{13}C NMR spectrum of **11** (100 MHz, $CDCl_3$).

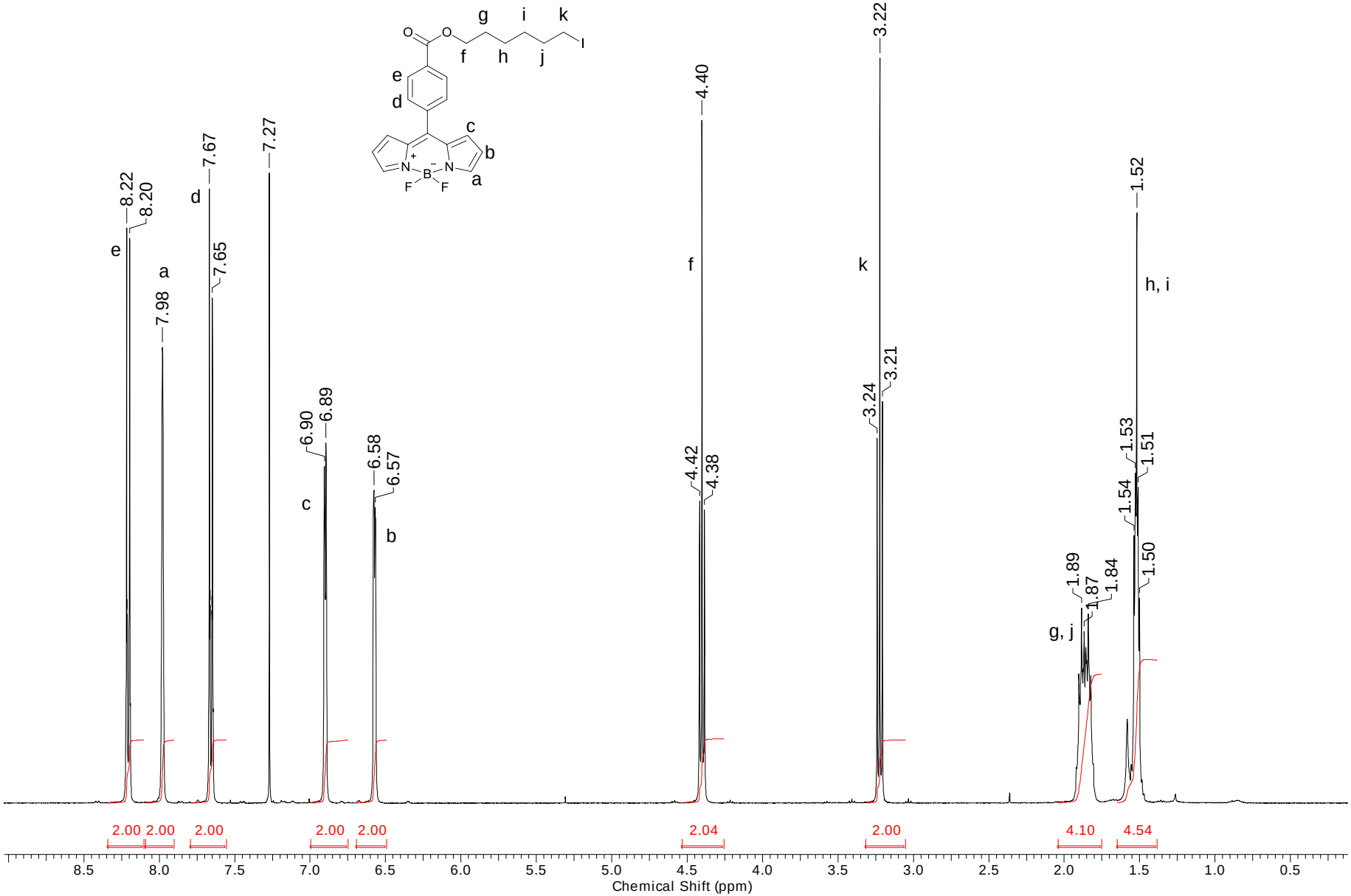


Figure S15. ^{1}H NMR spectrum of **BODIPY 10** (400 MHz, $CDCl_3$).

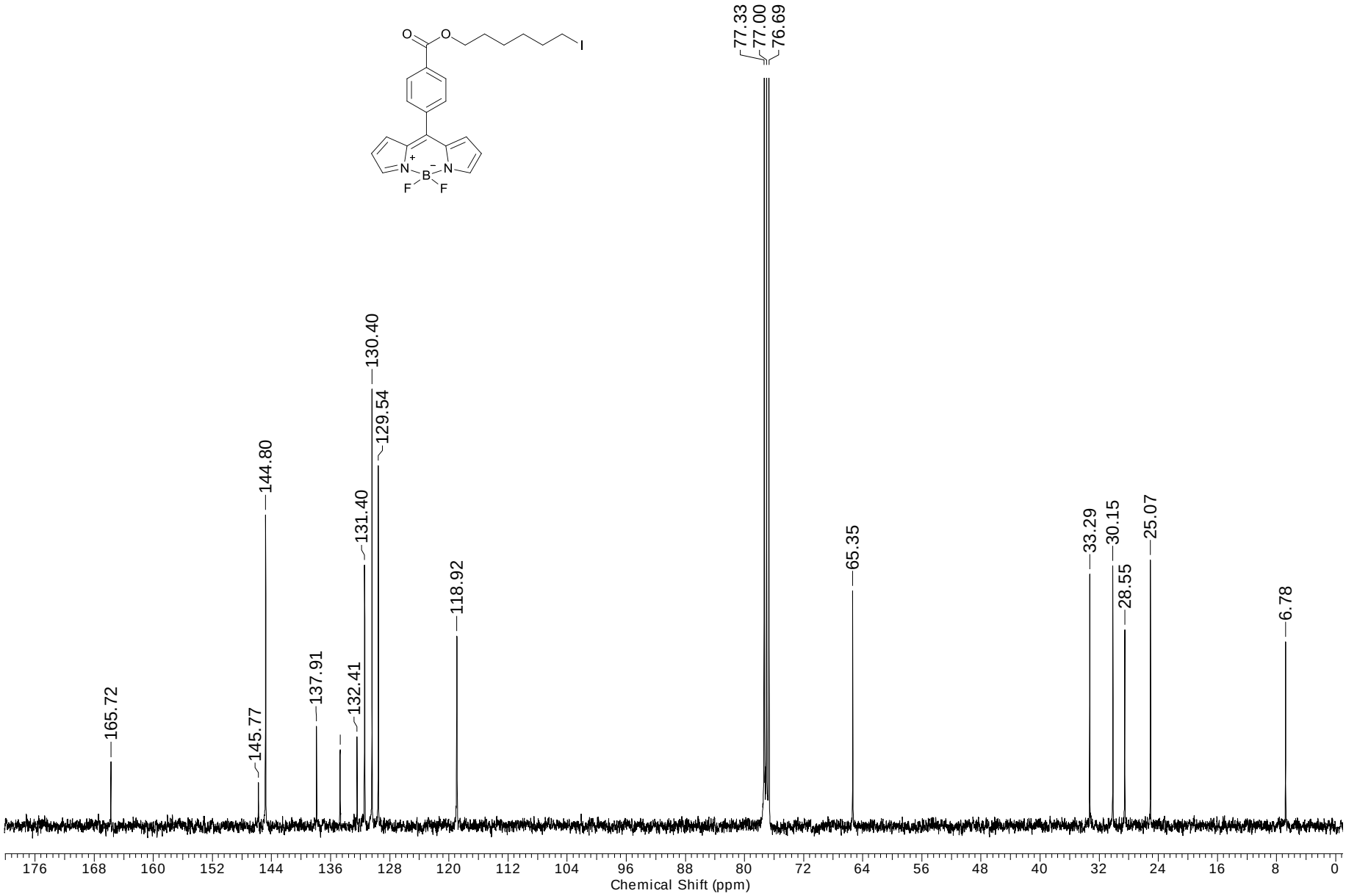


Figure S16. ^{13}C NMR spectrum of **BODIPY 10** (100 MHz, $CDCl_3$).

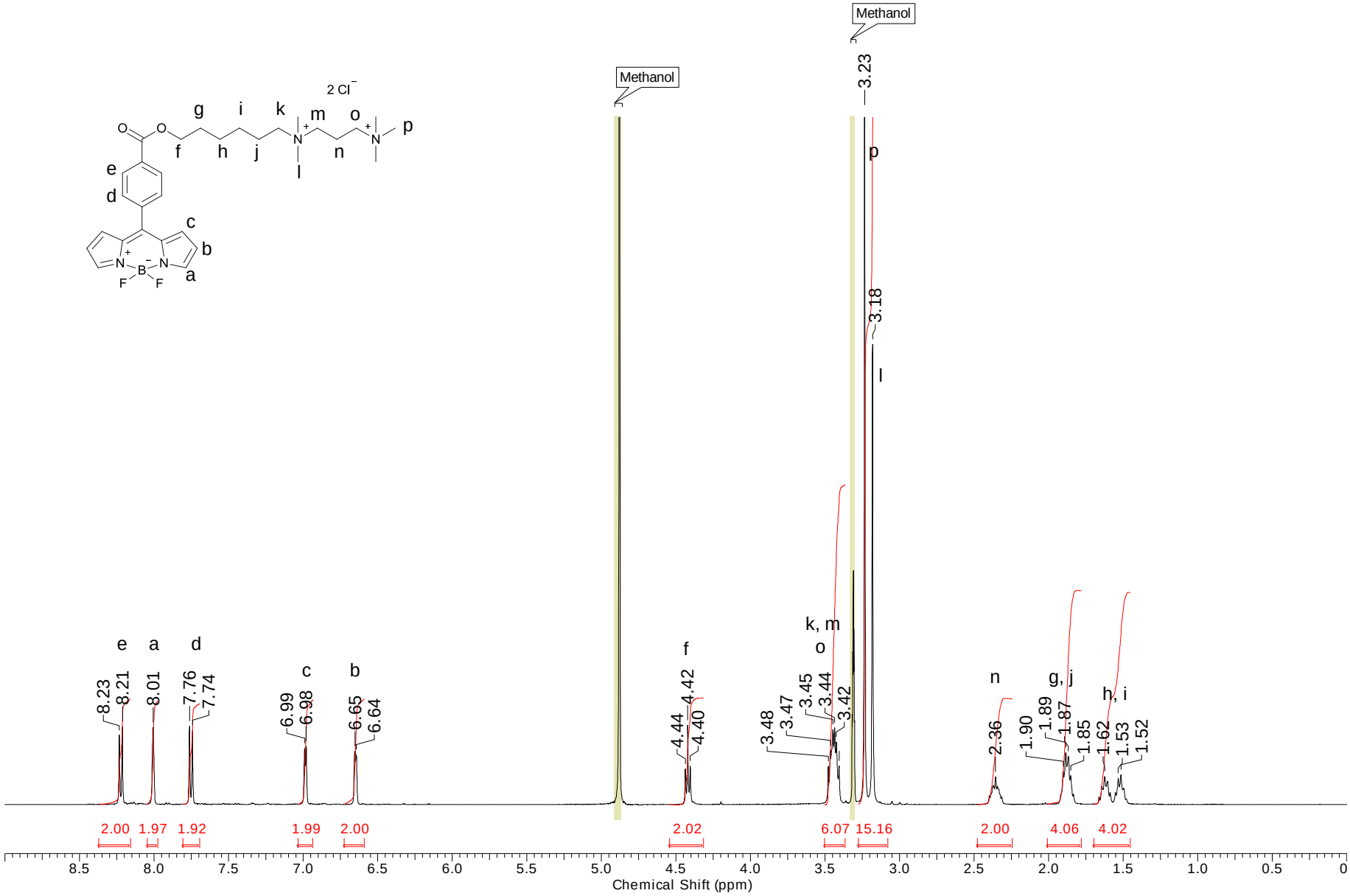


Figure S17. ^{1}H NMR spectrum of **BODIPY 6** (400 MHz, CD_3OD).

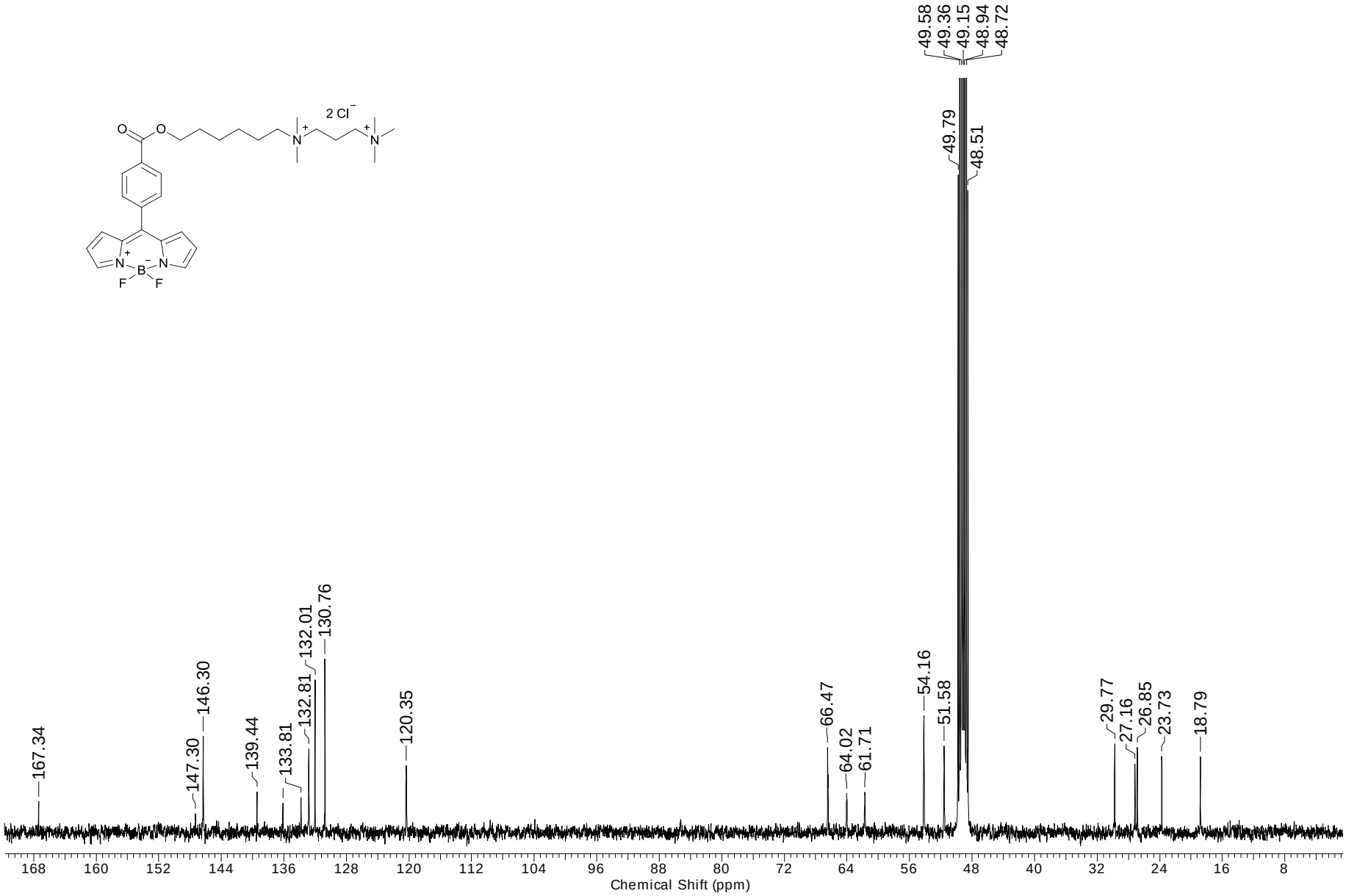


Figure S18. ^{13}C NMR spectrum of **BODIPY 6** (100 MHz, CD_3OD).

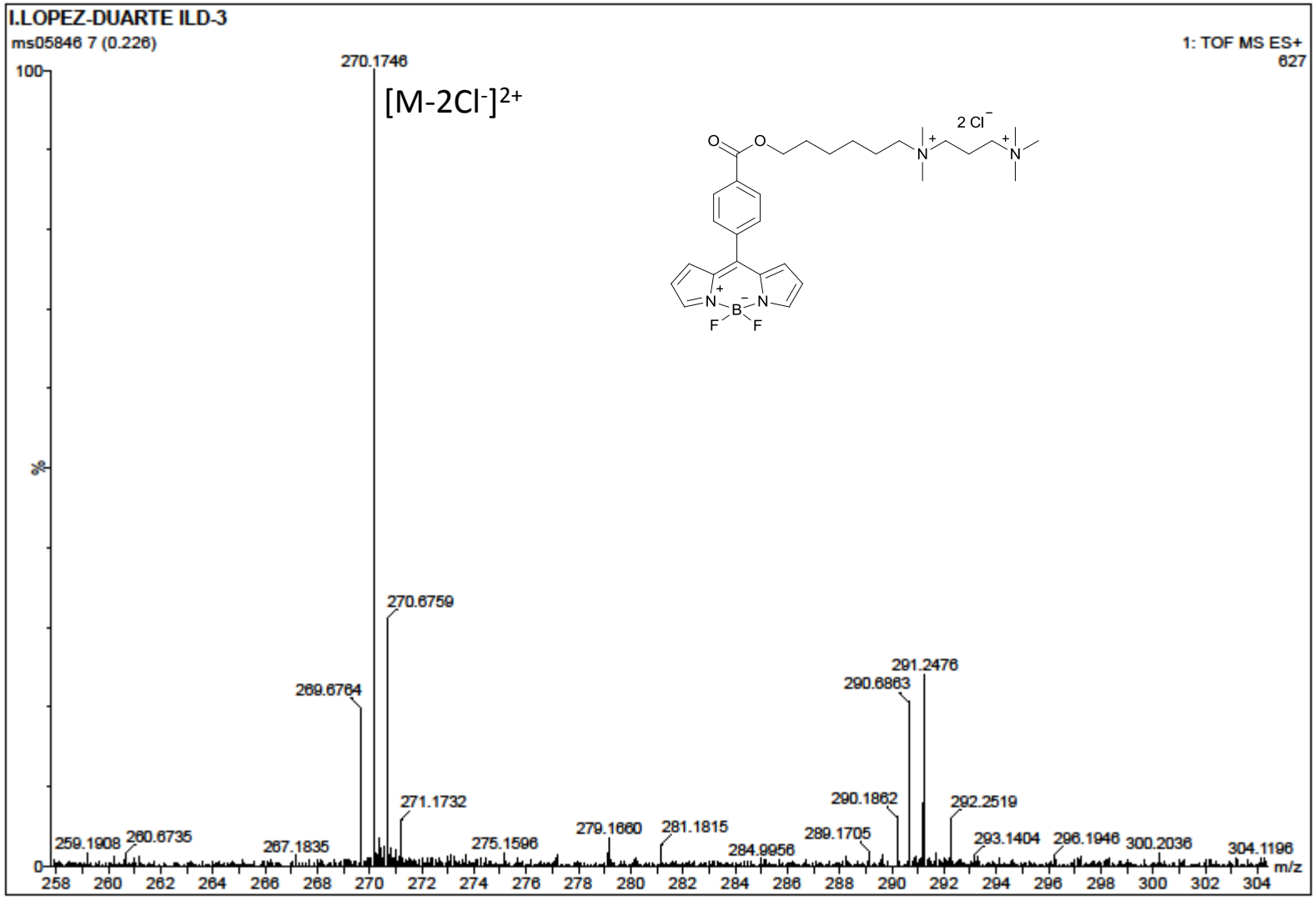


Figure S19. HRMS (ESI-TOF) mass spectrum of **BODIPY 6**.

3. References

1. (a) Wagner, R. W.; Lindsey J. S. *Pure & Appl. Chem*. **1996**, *68 (7)*, 1373-1380. (b) Kuimova, M. K.; Yahioglu, G.; Levitt J. A.; Suhling, K. *J. Am. Chem. Soc*., **2008**, *130*, 6672–6673. (c) Levitt, J. A.; Kuimova, M. K.; Yahioglu, G.; Chung, P.H; Suhling, K.; Phillips, D. *J. Phys. Chem. C*, **2009**, *113*, 11634–11642.

2. Hayashi, I; Yamaguchi, S.; Cha, W. Y.; Kim, D.; Shinokubo, H. *Org Lett.,* **2011***, 13 (12),* 2992-2995.

3. López-Duarte, I.; Vu, T. T.; Izquierdo, M. A.; Bull, J.A.; Kuimova, M. K. *Chem. Comm.* **2014**, *50*, 5282-5284.