

ELECTRONIC SUPPLEMENTARY INFORMATION

Geminal cross-coupling synthesis, ion-induced emission and lysosome imaging of cationic tetraarylethene oligoelectrolytes

Qi-Yuan Zhou,[#] Bo Xin,[#] Ya-Long Wang,[#] Chong Li,^{*} Ze-Qiang Chen, Qi Yu, Zhen-Li Huang, and Ming-Qiang Zhu^{*}

CONTENTS

- 1. EXPERIMENTAL:**
 - (a) MATERIALS AND INSTRUMENTATION**
 - (b) SYNTHESIS PROCEDURES**
 - (c) CELL CULTURE AND IMAGING**
- 2. SUPPLEMENTARY NOTES**
- 3. ¹H NMR SPECTRA of OF_n AND OF_n⁺**
- 4. UV AND FL SPECTRA OF OF_n AND OF_n⁺ IN THF OR WATER**
- 5. DENSITY FUNCTIONAL THEORY CALCULATIONS**
- 6. FLUORESCENCE SPECTRA**
- 7. FLUORESCENCE COLOCALIZATION IMAGING OF HELA CELLS**
- 8. FLUORESCENCE QUANTUM YIELDS**
- 9. NMR SPECTRA**
- 10. MASS SPECTRA**
- 11. REFERENCES**

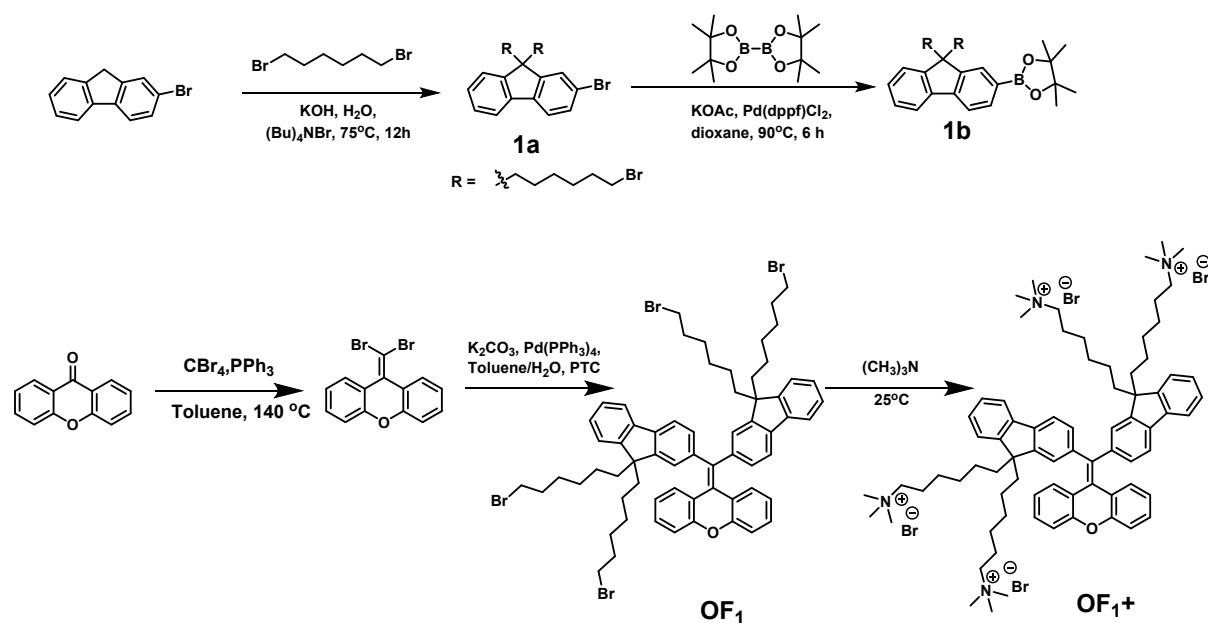
1. EXPERIMENTAL

(a) MATERIALS AND INSTRUMENTATION

All commercially available starting materials, reagents and solvents were used as supplied, unless otherwise stated, and were purchased from Energy, Aladdin, Alfa Aesar, J&K and Sinopharm Chemical Reagent Co. Ltd. All reactions were carried out under a dry nitrogen atmosphere and the temperatures were measured externally. Toluene, dioxane and tetrahydrofuran (THF) were dried using sodium wire and benzophenone as the indicator. Other solvents were dried over CaH₂ with stirring overnight followed by distillation under reduced pressure. Reported yields are isolated yields. Purification of all final products was accomplished by gravity column chromatography, using silica gel. For qualitative purity tests of all intermediates and final products, a single spot (visualized using UV-light at 254 nm and 365 nm) was obtained on thin film chromatography plate. The UV-Vis absorption and photoluminescence emission of the compounds were recorded on Shimadzu UV-VIS-NIR Spectrophotometer (UV-3600) and Edinburgh instruments (FLS 920 spectrometers), respectively. The NMR spectra were recorded using a 600M Bruker Ascend™ 600 MHz in CDCl₃ or CD₂Cl₂ and an internal standard of tetramethylsilane was used. The relative fluorescence quantum yields were estimated using N,N'-di(2,6-diisopropylphenyl)terrylene-3,4:11,12-tetracarboxdiimides in chloroform as standard material. The Mass spectra were recorded using an Agilent 1100 LC/MSD Trap. MALDI TOF mass spectra were recorded with a MALDI-TOF-TOF (Bruker ultrafleXtreme).

(b) SYNTHESIS PROCEDURES

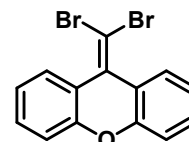
The 9-(dibromomethylene)-9H-xanthene was synthesized via the Corey-Fuchs reaction from 9H-xanthene-9-one with a high yield according to previous works.^{1,2}



Scheme S1. The synthesis route to OF₁ and OF₁⁺

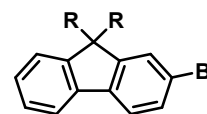
The synthesis of 9-(dibromomethylene)-9H-xanthene ^{1,2}

9H-xanthen-9-one (3.92 g, 20.0 mmol), carbon tetrabromide (13.265 g, 40.0 mmol), and triphenyl phosphine (17.530 g, 80.0 mmol) were combined in 100 mL of anhydrous toluene. The mixture was refluxed at 140°C for 72 h. Column chromatography of the crude product on silica gel with hexanes as the eluent gave 9-(dibromomethylene)-9H-xanthene as a white solid, followed by recrystallization from hexane/diethyl ether to obtain a white crystal in 74% yield. ¹H NMR (600 MHz, CDCl₃) δ 7.98 (dd, *J* = 7.9, 1.4 Hz, 2H), 7.34 (m, 2H), 7.23 (dd, *J* = 8.2, 0.9 Hz, 2H), 7.16 (m, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 152.80, 131.61, 129.36, 128.13, 123.64, 122.56, 116.58, 87.47.



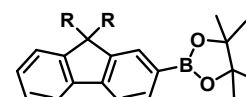
The synthesis of compound 1a ³

Under a nitrogen atmosphere, 50 wt% sodium hydroxide aqueous solution (50 mL) was added to the mixture of 2-bromofluorene (9.8 g, 40 mmol), tetrabutylammonium bromide (1.28 g, 4 mmol) and 1, 6-Dibromohexane (150 mL). The reaction mixture was stirred at 70°C for 12 h. After cooling down to room temperature, the organic layer was separated and the water layer was extracted with DCM. The combined organic solution was washed with water several times. After evaporation of the dichloromethane, the residual liquid reagent was removed under vacuum. The crude product was purified by column chromatography over silica gel with hexane as the eluent to give a colorless liquid (19.01 g, yield 83.2%). ¹H NMR (600 MHz, CDCl₃) δ 7.69 – 7.65 (m, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.48 – 7.43 (m, 2H), 7.37 – 7.29 (m, 3H), 3.28 (t, *J* = 6.8 Hz, 4H), 2.00 – 1.87 (m, 4H), 1.65 (p, *J* = 6.9 Hz, 4H), 1.19 (p, *J* = 7.6 Hz, 4H), 1.12 – 1.03 (m, 4H), 0.65 – 0.53 (m, 4H). ¹³C NMR (151 MHz, CDCl₃) δ 152.56, 149.90, 140.14, 140.01, 130.03, 127.57, 127.08, 126.03, 122.78, 121.10, 121.03, 119.82, 55.24, 40.12, 33.91, 32.61, 28.99, 27.75, 23.46.



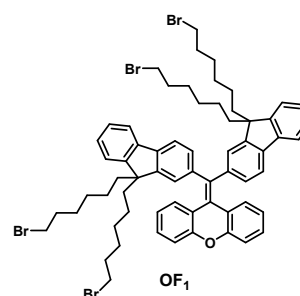
The synthesis of compound 1b ⁴

Under a nitrogen atmosphere, bis(pinacolato)diboron (11.43 g, 45 mmol), **1a** (17.14g, 30 mmol), KOAc (11.76 g, 120 mmol), and Pd(dppf)Cl₂ (1.10 g, 1.5 mmol) were dissolved in dioxane (300 mL) and heated to 90°C and stirred for 6 h. After cooling down to room temperature and evaporation of the 1, 4-dioxane. The residue was dissolved with 100ml dichloromethane, and washed with water three times, the organic layer was dried over magnesium sulfate. The solvent was removed under vacuum. The crude product was purified by column chromatography over silica gel with hexane/dichloromethane as the eluent to give a light white solid (13.73g, yield 74%). ¹H NMR (600 MHz, CDCl₃) δ 7.81 (d, *J* = 7.5 Hz, 1H), 7.75 – 7.66 (m, 3H), 7.33 (d, *J* = 5.8 Hz, 3H), 3.26 (t, *J* = 6.9 Hz, 4H), 2.06 – 1.92 (m, 2H), 1.63 (p, *J* = 7.0 Hz, 4H), 1.39 (s, 12H), 1.16 (p, *J* = 7.5 Hz, 4H), 1.09 – 1.01 (m, 4H), 0.63 – 0.51 (m, 4H). ¹³C NMR (151 MHz, CDCl₃) δ 150.90, 149.46, 144.10, 140.91, 133.83, 128.69, 127.59, 126.82, 122.82, 120.16, 119.03, 83.74, 54.95, 40.06, 33.95, 32.64, 29.00, 27.74, 24.96, 23.43.



The synthesis of compound **OF₁**

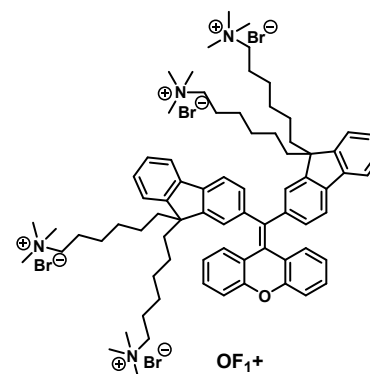
A mixture of 1,1-dibromo-2,2-diphenylene (0.352 g, 1 mmol), **1b** (1.48 g, 2.4 mmol), Pd(PPh₃)₄ (0.120 g, 0.1 mmol), tetrabutylammonium hydrogen sulfate (0.034 g, 0.1 mmol), K₂CO₃ (0.414 g, 3 mmol) were stirred in toluene (30 mL) and water (15 mL) under nitrogen atmosphere overnight at 90°C. After cooling down to room temperature, the organic layer was separated and 50 mL dichloromethane was added. The organic solution was washed with water three times. The organic layer was dried over magnesium sulfate, and the solvent was removed under vacuum.

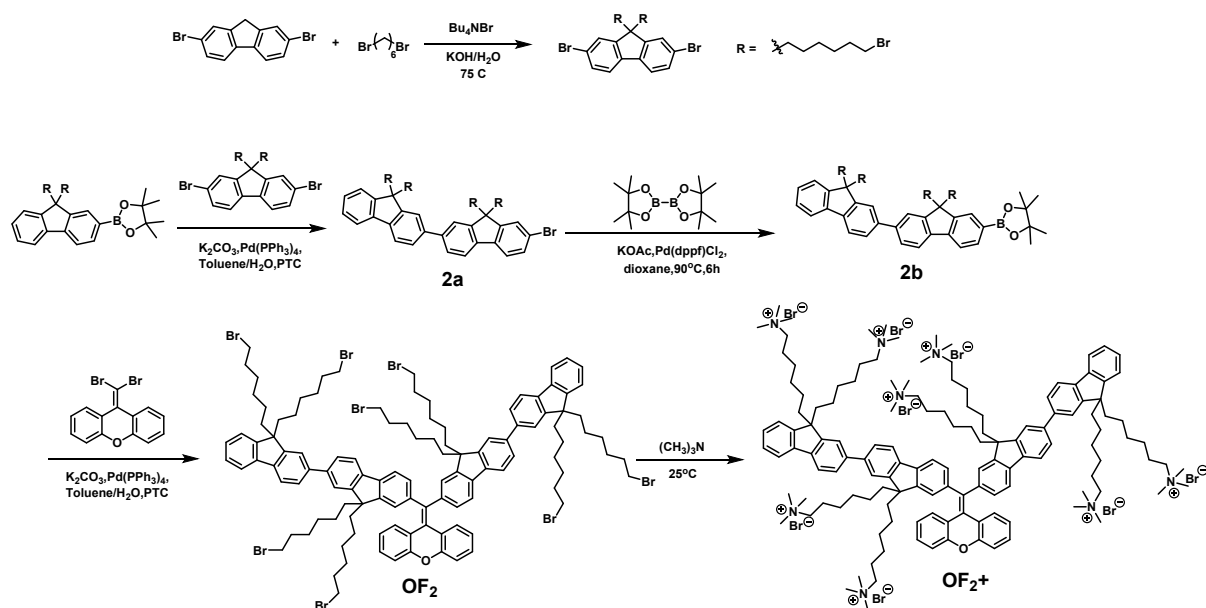


After filtration, the residue was purified by column chromatography over silica gel with hexane/dichloromethane as the eluent to give a red solid (0.987 g, 84%). ¹H NMR (600 MHz, CDCl₃) δ 7.68 – 7.61 (m, 4H), 7.33 – 7.27 (m, 6H), 7.25 – 7.21 (m, 4H), 7.18 (d, *J* = 7.5 Hz, 2H), 7.13 – 7.09 (m, 4H), 6.65 – 6.60 (m, 2H), 3.25 (t, *J* = 6.8 Hz, 8H), 1.91 – 1.78 (m, 8H), 1.62 (p, *J* = 7.1 Hz, 8H), 1.21 – 1.08 (m, 8H), 0.99 (p, *J* = 7.4 Hz, 8H), 0.62 – 0.48 (m, 8H). ¹³C NMR (151 MHz, CDCl₃) δ 153.51, 150.48, 142.56, 140.75, 139.77, 139.57, 129.03, 128.55, 127.95, 127.10, 126.93, 125.01, 124.82, 124.12, 122.67, 122.29, 120.01, 119.73, 116.39, 54.85, 40.17, 33.93, 32.66, 29.07, 27.79, 26.92, 23.50. MALDI-TOF-MS calcd. for C₆₄H₇₀Br₄O: 1174.88; found: [M⁺] 1174.76.

The synthesis of **OF₁⁺**

In a 25 ml flask, **OF₁** (100 mg) was dissolved into 10 mL THF. 5 mL trimethylamine (TMA) solution in ethanol (33%) was then added at -78°C. After stirred at room temperature for 24 h, some solid precipitated. Then trimethylamine (TMA) solution in ethanol (33%) was added to the mixture until the solid was completely dissolved. After stirring for another 24 h, the solvent was evaporated at vacuum. The resulting solid mixture was washed with THF twice, collected by centrifugation and dried overnight in vacuum at 45°C to afford **OF₁⁺** (110 mg, 92%) as a light yellow solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.83 – 7.74 (m, 4H), 7.48 – 7.40 (m, 4H), 7.35 – 7.30 (m, 4H), 7.29 – 7.21 (m, 6H), 7.01 (d, *J* = 8.0 Hz, 2H), 6.66 (t, *J* = 7.4 Hz, 2H), 3.25 – 3.15 (m, 8H), 3.00 (s, 36H), 2.00 – 1.84 (m, 8H), 1.46 (t, *J* = 7.6 Hz, 8H), 1.08 – 0.94 (m, 16H), 0.60 – 0.39 (m, 8H). ¹³C NMR (151 MHz, DMSO) δ 153.41, 150.67, 142.47, 140.62, 140.50, 139.73, 129.02, 128.93, 128.23, 127.80, 127.48, 124.62, 124.35, 123.30, 122.84, 120.88, 120.50, 116.85, 65.49, 54.98, 52.51, 29.25, 25.92, 23.79, 22.52.

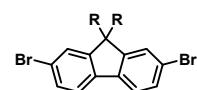




Scheme S2. The synthesis of OF₂ and OF₂⁺

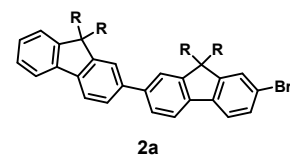
The synthesis of 2, 7-dibromo-9, 9-bis(6-bromo-hexyl)fluorene

Under a nitrogen atmosphere, 50 wt% sodium hydroxide aqueous solution (50 mL) was added to the mixture of 2, 7-dibromofluorene (12.96 g, 40 mmol), tetrabutylammonium bromide (1.28 g, 4 mmol) and 1, 6-Dibromohexane (150 mL). The reaction mixture was stirred at 70°C for 12 h. After cooling down to room temperature, the organic layer was separated and the water layer was extracted with DCM. The combined organic solution was washed with water several times. After evaporation of the dichloromethane, the residual liquid reagent was removed under vacuum. The crude product was purified by column chromatography over silica gel with hexane as the eluent to give a white solid (22.6 g, yield 87%). ¹H NMR (600 MHz, CDCl₃) δ 7.52 (d, *J* = 8.0 Hz, 2H), 7.46 (dd, *J* = 8.1, 1.6 Hz, 2H), 7.43 (d, *J* = 1.3 Hz, 2H), 3.29 (t, *J* = 6.8 Hz, 4H), 1.96 – 1.88 (m, 4H), 1.67 (p, *J* = 6.9 Hz, 4H), 1.20 (p, *J* = 7.5 Hz, 4H), 1.08 (p, *J* = 7.6 Hz, 4H), 0.63 – 0.54 (m, 4H). ¹³C NMR (151 MHz, CDCl₃) δ 152.14, 139.05, 130.31, 126.06, 121.55, 121.22, 55.54, 40.04, 33.88, 32.60, 28.94, 27.75, 23.45.



The synthesis of compound 2a

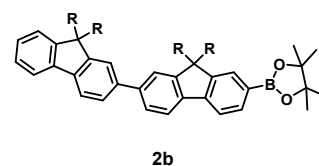
A mixture of 2,7-dibromo-9,9-bis(6-bromo-hexyl)fluorene (19.5 g, 30 mmol), 1b (6.18 g, 10 mmol), K₂CO₃ (6.9 g, 50 mmol), tetrabutylammonium hydrogen sulfate (0.335 g, 1 mmol) Pd(PPh₃)₄ (0.1155 g, 0.1 mmol) were stirred in toluene (120 mL) and H₂O (50 mL) under a nitrogen atmosphere over night at 90°C. After cooling down to room temperature, the organic layer was separated and 100 mL dichloromethane was added. The organic solution was washed with water three times. the organic layer was dried over magnesium sulfate, the residue was purified by column chromatography over silica gel with hexane/dichloromethane as the eluent to give a white solid (6.9 g, 65%). ¹H NMR (600 MHz, CDCl₃) δ 7.78 (d, *J* = 7.8 Hz, 1H), 7.76 – 7.73 (m, 2H), 7.65 (t, *J* = 8.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 3H), 7.49 (d, *J* = 7.5 Hz, 2H), 7.38 – 7.30 (m, 3H), 3.28 (q, *J* = 6.9 Hz, 8H), 2.11 – 1.96 (m, 8H), 1.66 (h, *J* = 7.1 Hz, 8H), 1.22 (h, *J* = 7.6 Hz, 8H), 1.12 (h, *J* = 7.7 Hz, 8H), 0.77 – 0.63 (m, 8H). ¹³C NMR (151 MHz, CDCl₃) δ 152.82, 151.16, 150.75, 150.56, 140.98, 140.68, 140.55, 140.19, 139.81, 139.25, 130.16, 127.20, 126.98,



126.44, 126.20, 126.11, 122.84, 121.18, 121.08, 120.12, 120.03, 119.84, 55.43, 55.09, 40.21, 40.15, 33.96, 33.93, 32.59, 29.03, 28.99, 27.74, 23.57.

The synthesis of compound 2b

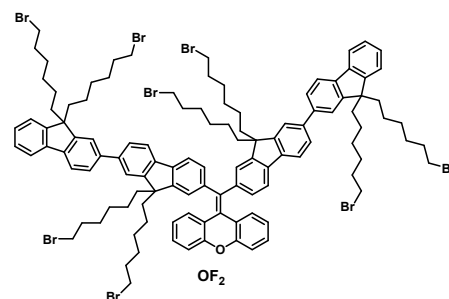
Under a nitrogen atmosphere, bis(pinacolato)diboron (2.476 g, 9.75 mmol), 2a (6.9 g, 6.5 mmol), KOAc (2.548 g, 26 mmol), and Pd(dppf)Cl₂ (0.238 g, 0.325 mmol) were dissolved in 1, 4-dioxane (100 mL) and heated to 90°C and stirred for 6 h. After cooling to room temperature and evaporation of the 1, 4-dioxane. The residue was dissolved with 100 ml



dichloromethane, and washed with water three times, the organic layers were dried over magnesium sulfate. The solvent was removed under vacuum. The crude product was purified by column chromatography over silica gel with hexane/dichloromethane as the eluent to give a light white solid (6.265g, yield 87%). ¹H NMR (600 MHz, CDCl₃) δ 7.84 (d, *J* = 7.5 Hz, 1H), 7.83 – 7.77 (m, 2H), 7.77 – 7.73 (m, 3H), 7.66 (d, *J* = 7.8 Hz, 2H), 7.61 (s, 2H), 7.38 – 7.34 (m, 2H), 7.34 – 7.30 (m, 1H), 3.27 (td, *J* = 6.8, 4.2 Hz, 8H), 2.12 – 2.01 (m, 8H), 1.65 (h, 8H), 1.40 (s, 12H), 1.21 (h, *J* = 7.5 Hz, 8H), 1.10 (h, *J* = 7.2 Hz, 8H), 0.77 – 0.64 (m, 8H). ¹³C NMR (151 MHz, CDCl₃) δ 151.75, 151.13, 150.58, 149.77, 145.22, 143.77, 140.96, 140.74, 140.45, 140.40, 140.19, 133.95, 128.77, 127.15, 126.96, 126.22, 122.84, 121.27, 121.23, 120.46, 120.01, 119.83, 119.13, 83.78, 55.16, 55.08, 40.23, 40.09, 33.97, 33.96, 32.62, 32.61, 29.05, 29.00, 27.74, 27.73, 24.97, 23.58, 23.52.

The synthesis of compound OF₂

A mixture of 1,1-dibromo-2,2-diphenylene (0.106 g, 0.3 mmol), 2b (7.97 g, 0.72 mmol), Pd(PPh₃)₄ (0.034 g, 0.03 mmol), tetrabutylammonium hydrogen sulfate (0.01 g, 0.03 mmol), K₂CO₃ (0.207 g, 1.5 mmol) were stirred in toluene (10 mL) and water (5 mL) under nitrogen atmosphere overnight at 90°C. After cooling down to room temperature, the organic layer was separated and 30ml dichloromethane was added. The organic solution was washed with water three times. The organic layer was dried over magnesium sulfate, and then the solvent was removed under vacuum. After filtration, the residue was purified by column

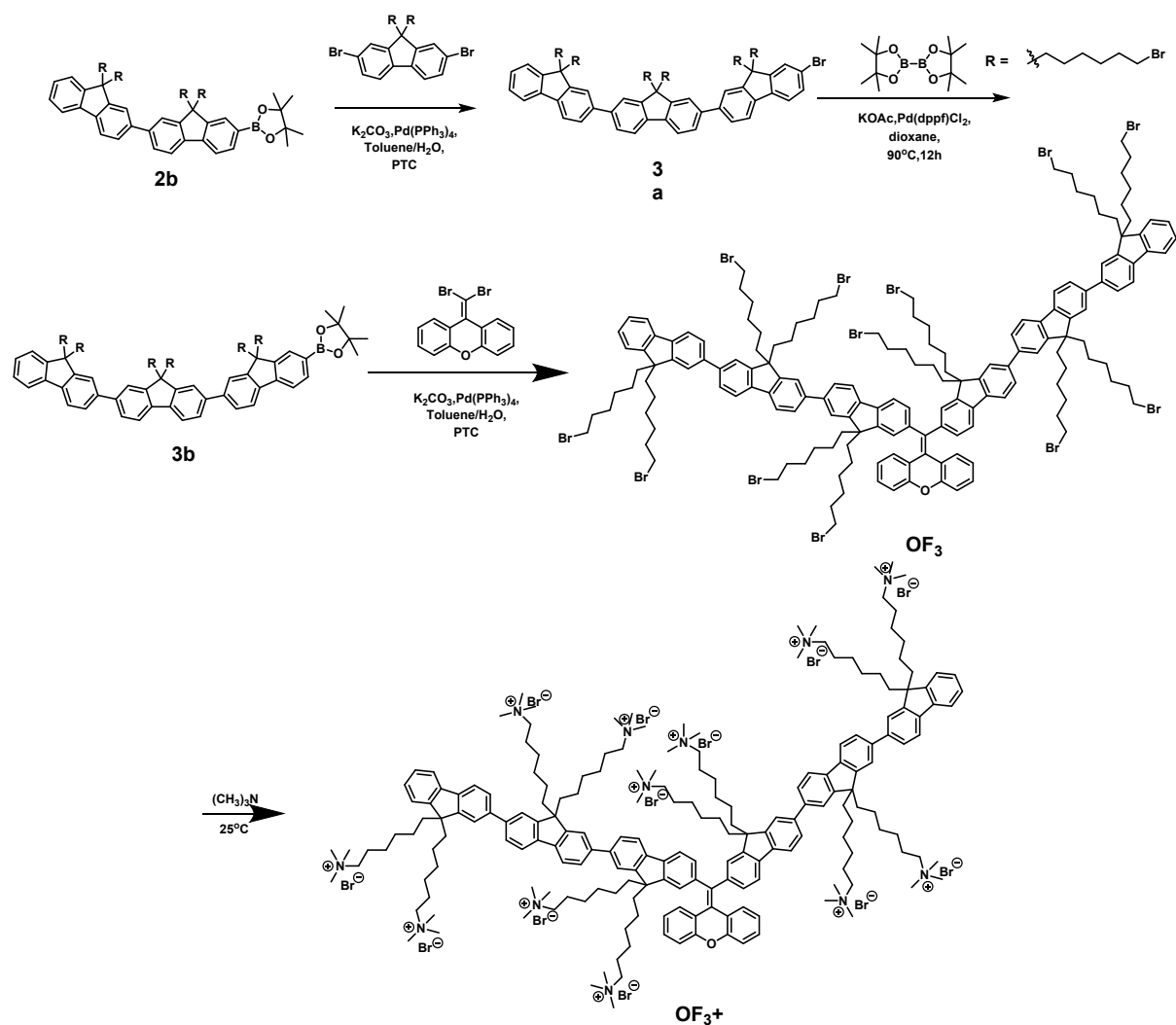
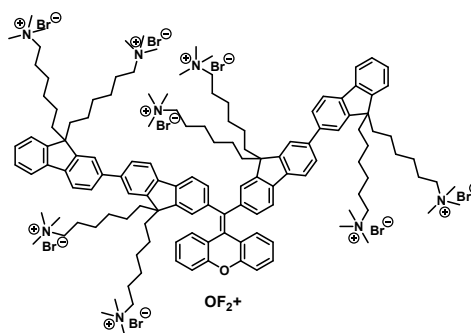


chromatography on silica gel using DCM/PE as the eluent to give a yellow solid (0.482g, 74.6%). ¹H NMR (600 MHz, Methylene Chloride-*d*₂) δ 7.90 – 7.65 (m, 16H), 7.48 – 7.32 (m, 10H), 7.23 (d, *J* = 9.9 Hz, 6H), 6.74 (s, 2H), 3.33 (s, 16H), 2.21 – 1.93 (m, 16H), 1.80 – 1.63 (m, 16H), 1.35 – 1.19 (m, 16H), 1.19 – 1.03 (m, 16H), 0.83 – 0.64 (m, 16H). ¹³C NMR (151 MHz, CDCl₃) δ 153.52, 151.30, 151.12, 150.79, 150.56, 142.63, 140.74, 140.43, 140.40, 140.35, 140.01, 139.45, 129.05, 128.69, 128.01, 127.13, 126.95, 126.29, 126.16, 124.79, 124.23, 122.83, 122.32, 121.15, 121.08, 120.15, 120.03, 119.99, 119.81, 116.45, 55.08, 55.05, 40.22, 33.99, 32.66, 32.60, 29.08, 29.04, 27.81, 27.73, 23.60, 23.57. MALDI-TOF-MS calcd. for C₁₁₄H₁₃₀Br₈O: 2155.53; found: [M⁺] 2155.26.

The synthesis of compound OF₂⁺

Into a 50 ml flask, OF₂ (100 mg) was dissolved into 10 mL THF. 5 mL trimethylamine (TMA) solution in ethanol (33%) was then added at -78°C. After stirred at room temperature for 24 h, some solid precipitated. Then trimethylamine (TMA) solution in ethanol (33%) was added to the mixture till the solid was dissolved. After stirring for another 48 h, the solvent was evaporated at vacuum. The resulting solid mixture was washed with THF twice, collected by centrifugation and dried overnight in vacuum at 45°C to afford OF₂⁺ (105 mg, 86%) as

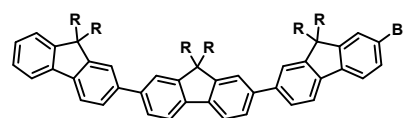
a light yellow solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.96 – 7.84 (m, 12H), 7.79 (d, *J* = 8.1 Hz, 4H), 7.50 (d, *J* = 7.6 Hz, 4H), 7.39 – 7.24 (m, 10H), 7.06 (d, *J* = 7.7 Hz, 2H), 6.69 (t, *J* = 7.6 Hz, 2H), 3.29 – 3.14 (m, 16H), 3.07 – 2.93 (m, 68H), 2.16 – 1.95 (m, 16H), 1.58 – 1.37 (m, 16H), 1.12 – 0.97 (m, 32H), 0.66 – 0.50 (m, 16H).



Scheme S3. Synthesis of compounds OF₃ and OF₃⁺

The synthesis of compound 3a

A mixture of 2,7-diBromo-9,9-bis(6-bromohexyl)fluorine (9.75 g, 15mmol), **2b** (5.54 g, 5 mmol), K₂CO₃ (3.45 g, 25 mmol), tetrabutyl-ammonium hydrogen sulfate (0.168 g, 0.5 mmol) Pd(PPh₃)₄ (0.058 g, 0.05 mmol) were stirred in toluene (60 mL) and H₂O (25 mL) under a nitrogen atmosphere over night at 90 °C.

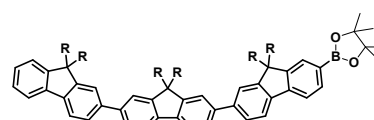


3a

After cooling down to room temperature, the organic layer was separated and 100 mL dichloromethane was added. The organic solution was washed with water for three times and then the organic layers were dried over magnesium sulfate. The residue was purified by column chromatography over silica gel with hexane/dichloromethane as the eluent to give a white solid (5.509 g, 71%). ¹H NMR (600 MHz, CDCl₃) δ 7.86 – 7.73 (m, 5H), 7.73 – 7.56 (m, 9H), 7.53 – 7.46 (m, 2H), 7.41 – 7.31 (m, 3H), 3.28 (s, 12H), 2.19 – 1.92 (m, 12H), 1.68 (s, 12H), 1.24 (s, 12H), 1.13 (s, 12H), 0.87 – 0.64 (m, 12H). ¹³C NMR (151 MHz, CDCl₃) δ 152.83, 151.47, 151.42, 151.15, 150.79, 150.58, 140.95, 140.75, 140.58, 140.45, 140.38, 140.24, 139.96, 139.81, 139.30, 130.19, 127.17, 126.98, 126.45, 126.34, 126.20, 126.12, 122.85, 121.28, 121.20, 121.11, 120.14, 120.03, 119.83, 55.45, 55.29, 55.09, 40.24, 40.16, 33.98, 33.94, 32.61, 32.57, 29.05, 29.02, 29.00, 27.75, 27.72, 23.66, 23.58.

The synthesis of compound 3b

Under a nitrogen atmosphere, bis(pinacolato)diboron (0.762 g, 3 mmol), 3a (3.1g, 2mmol), KOAc (0.784 g, 8 mmol), and Pd(dppf)Cl₂ (0.073 g, 0.1 mmol) were dissolved in dioxane (50 mL) and heated to 90°C and stirred for 6 h. After cooling to room temperature and evaporation of the 1, 4-dioxane. The residue was dissolved in 50 mL

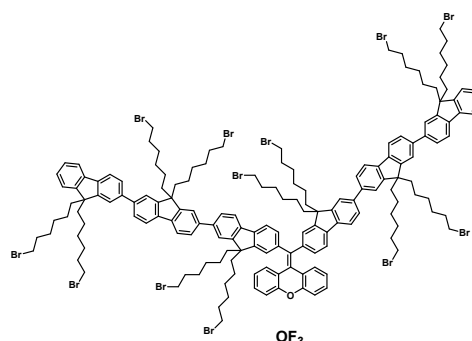


3b

dichloromethane, and washed with water three times, the organic layers were dried over magnesium sulfate. The solvent was removed under vacuum. The crude product was purified by column chromatography over silica gel with hexane/dichloromethane as the eluent to give a light white solid (2.4g, yield 75%). ¹H NMR (600 MHz, CDCl₃) δ 7.86 – 7.74 (m, 8H), 7.72 – 7.66 (m, 4H), 7.64 (d, *J* = 8.7 Hz, 4H), 7.39 – 7.32 (m, 3H), 3.32 – 3.24 (m, 12H), 2.16 – 2.02 (m, 12H), 1.72 – 1.62 (m, 12H), 1.41 (s, 12H), 1.26 – 1.17 (m, 12H), 1.17 – 1.06 (m, 12H), 0.82 – 0.66 (m, 12H). ¹³C NMR (151 MHz, CDCl₃) δ 151.77, 151.43, 151.14, 150.57, 149.77, 143.75, 140.93, 140.75, 140.51, 140.43, 140.40, 140.24, 140.10, 140.01, 133.96, 128.77, 127.15, 126.96, 126.32, 126.20, 122.84, 121.32, 121.27, 121.19, 120.49, 120.11, 120.02, 119.82, 119.14, 83.78, 83.49, 55.27, 55.17, 55.09, 40.24, 40.10, 33.98, 32.63, 32.61, 32.59, 29.05, 29.00, 27.74, 25.02, 24.98, 23.67, 23.59, 23.53.

The synthesis of compound OF₃

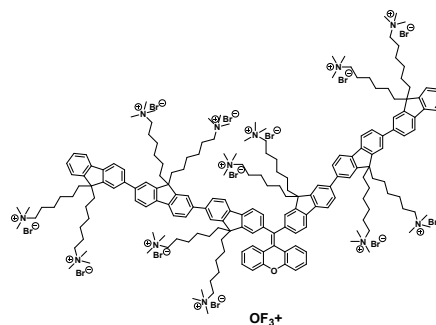
A mixture of 1,1-dibromo-2,2-diphenylene (0.106 g, 0.3 mmol), 3b (1.15 g, 0.72 mmol), Pd(PPh₃)₄ (0.034 g, 0.03 mmol), tetrabutyl-ammonium hydrogen sulfate (0.01 g, 0.03 mmol), K₂CO₃ (0.207 g, 1.5 mmol) were stirred in toluene (10 mL) and water (5 mL) were stirred under nitrogen atmosphere overnight at 90°C. After cooling down to room temperature, the organic layer was separated and 30 mL dichloromethane was added. The organic solution was washed with water three times. The organic layers were dried over magnesium sulfate,



and the solvent was removed under vacuum. After filtration, the residue was purified by column chromatography on silica gel using DCM/PE as the eluent to give a red solid (0.592 g, 63%). ^1H NMR (600 MHz, Methylene Chloride- d_2) δ 7.93 – 7.63 (m, 28H), 7.45 – 7.28 (m, 10H), 7.28 – 7.12 (m, 6H), 6.71 (s, 2H), 3.29 (s, 24H), 2.20 – 1.91 (m, 24H), 1.78 – 1.59 (m, 24H), 1.30 – 1.04 (m, 48H), 0.85 – 0.60 (m, 24H). ^{13}C NMR (151 MHz, CDCl_3) δ 153.51, 151.42, 151.33, 151.13, 150.80, 150.56, 142.65, 140.74, 140.48, 140.42, 140.39, 140.05, 140.01, 139.45, 129.05, 128.70, 128.02, 127.15, 126.96, 126.29, 126.18, 125.09, 124.79, 124.24, 122.83, 122.32, 121.26, 121.18, 121.09, 120.16, 120.09, 120.02, 119.82, 116.45, 55.27, 55.08, 55.06, 40.24, 34.01, 33.99, 32.67, 32.60, 32.58, 29.09, 29.04, 29.02, 27.81, 27.74, 27.72, 23.65, 23.61, 23.58. MALDI-TOF-MS calcd. for $\text{C}_{164}\text{H}_{190}\text{Br}_{12}\text{O}$: 3136.17; found: $[\text{M}+1]$ 3137.32.

The synthesis of compound OF_3^+

Into a 25 mL flask, OF_3 (100 mg) was dissolved into 10 mL THF. A 5 mL solution of trimethylamine (TMA) in ethanol (33%) was then added at -78°C . After stirred at room temperature for 24 h, some solid precipitated formed. Then trimethylamine (TMA) solution in ethanol (33%) was added to the mixture till the solid was dissolved. After stirring for another 72 h, the solvent was evaporated at vacuum. The resulting solid mixture was washed with THF twice, collected by centrifugation and dried overnight in vacuum at 45°C to afford OF_3^+ (99 mg, 81%) as a light yellow solid.



^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 8.00 – 7.80 (m, 28H), 7.55 – 7.46 (m, 4H), 7.40 – 7.24 (m, 10H), 7.07 (d, $J = 7.7$ Hz, 2H), 6.75 – 6.65 (m, 2H), 3.33 – 3.17 (m, 24H), 3.02 (d, $J = 32.0$ Hz, 107H), 2.32 – 1.95 (m, 24H), 1.61 – 1.39 (m, 24H), 1.17 – 1.00 (m, 48H), 0.70 – 0.50 (m, 24H).

(c) CELL CULTURE AND IMAGING

HeLa cells were seeded in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) containing 10% of fetal bovine serum (FBS) at 37°C and 5% CO_2 in a humidified incubator. For confocal microscopy imaging experiments, HeLa cells were transferred into glass bottom Petri dishes. After attachment, the cells were exposed to DMEM containing OF_n^+ (1-3) for 2 h. Specially, the external OF_n^+ (1-3) dyes do not need to be washed because OF_n^+ (1-3) are naturally non-fluorescent in aqueous solution and the cells were imaged directly by a Confocal Laser Scanning Microscopy. For the colocalization imaging, we incubated the cells with OF_n^+ (1-3) at $5 \mu\text{M}$ for 2 h to label lysosomes, followed by treatment with LysoTracker Red or MitoTracker Red at 50 nM for another 45 mins. LysoTracker Red and MitoTracker Red in cells was observed by excitation at 640 nm, and fluorescence emission was collected between 660 nm and 750 nm using a confocal laser-scanning microscope (Zeiss LSM710, Germany). While, OF_n^+ ($n = 1-3$) in cells were visualized by excitation at 405 nm and fluorescence emission was collected between 415 nm and 475 nm.

2. SUPPLEMENTARY NOTES

Note S1 The fluorescence enhancement mechanism of salt or ionic effect of OF_n^+

When the salt concentration is increased, some of the water molecules are attracted by the salt ions, which decreases the number of water molecules available to interact with OF_1^+ and destroys the solvation effect. This results in reducing the solubility of OF_1^+ in water and causing the aggregation of OF_1^+ molecules. Hence the addition of inorganic salts into OF_1^+ aqueous solution activates the restriction of intramolecular motion (RIM) process and switch on fluorescence emission. It is believable that the fluorescence enhancement is mainly attributed to the salt or ionic effect rather than the pH value. (Fig. S5)

3. 1H NMR SPECTRA of OF_n and OF_n^+

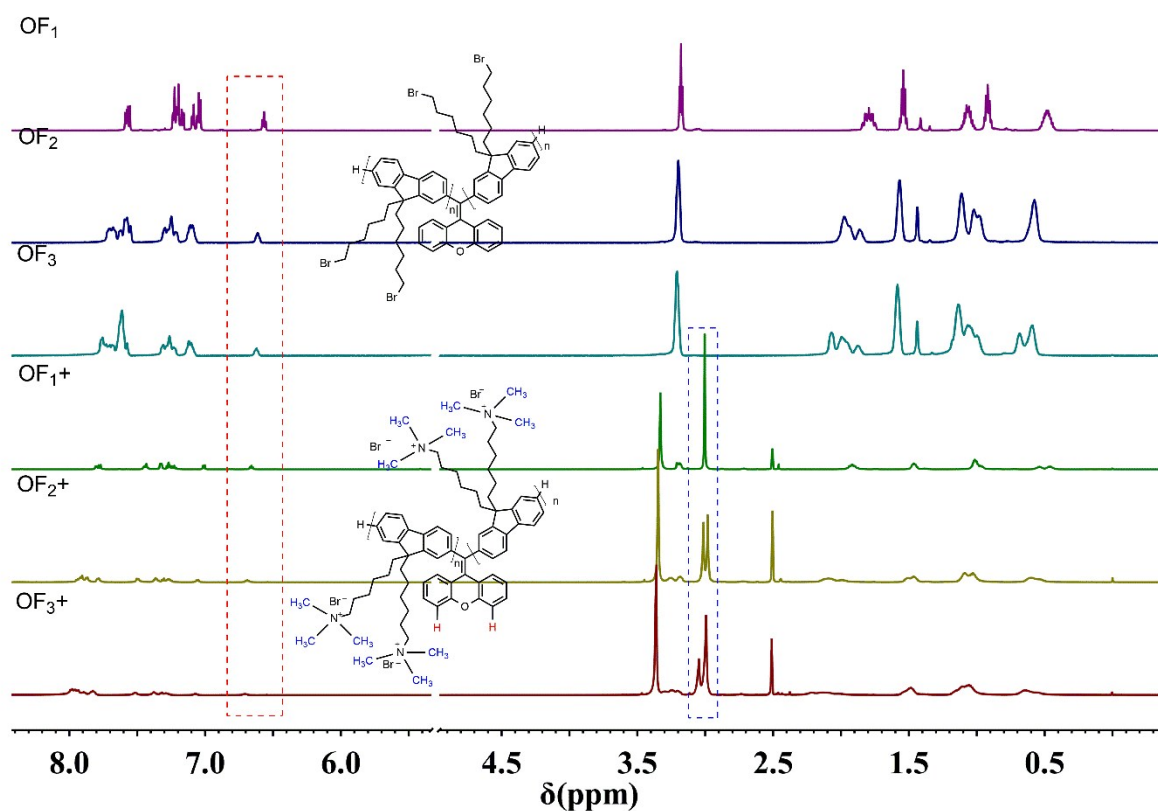


Fig. S1 1H NMR of OF_n in CD_2Cl_2 and OF_n^+ in DMSO.

4. UV AND FL SPECTRA OF OF_n AND OF_n+ IN THF OR WATER

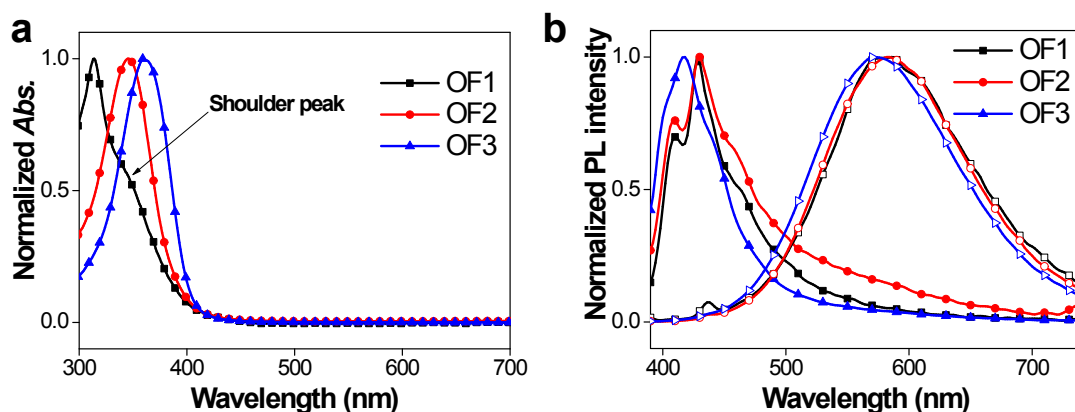


Fig. S2 Normalized UV-vis absorption spectra and fluorescence spectra of **OF_n**. (a) Normalized UV-vis absorption in THF. (b) Normalized fluorescence spectra in THF (line + solid symbol graphics) and in water (line + hollow symbol graphics). The concentration of each **OF_n** is 2 μ M, the excitation wavelength is 380 nm.

5. DENSITY FUNCTIONAL THEORY CALCULATIONS

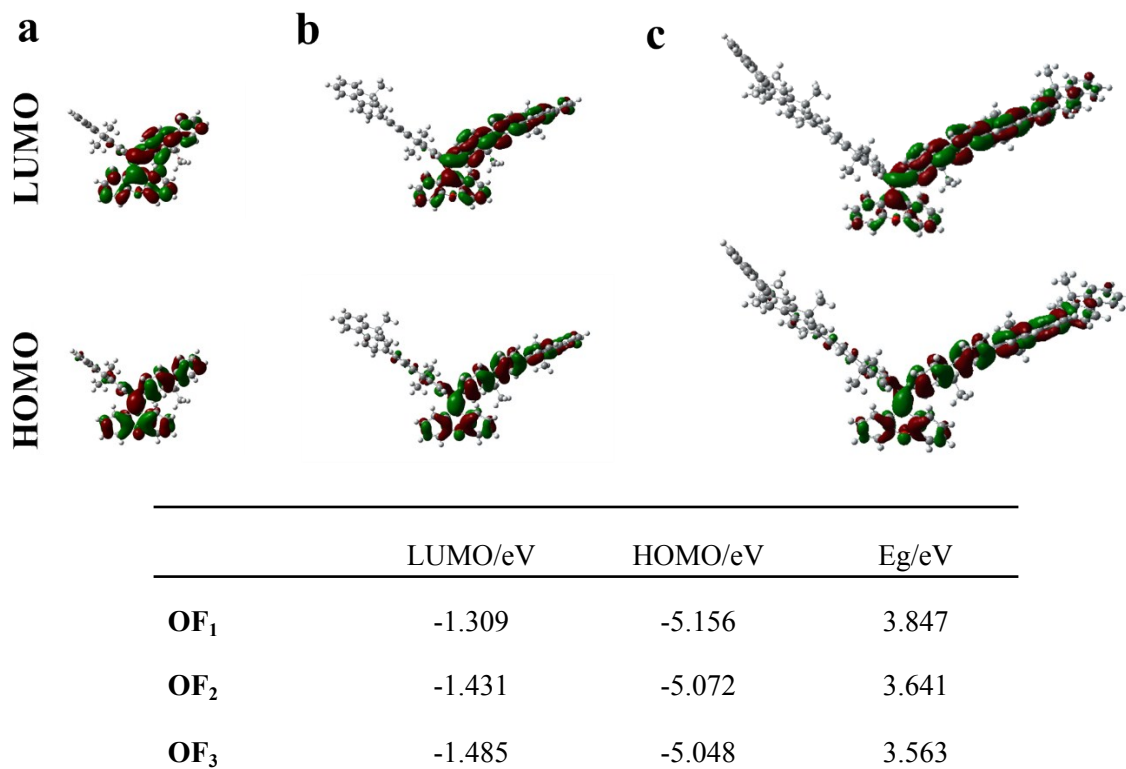


Fig. S3 Molecular orbital amplitude plots of HOMO and LUMO energy levels of **OF_n** calculated using B3LYP/6-31G(d) basis set in Gaussian 09 program. Eg (energy gap) = LUMO - HOMO. (a) **OF₁**; (b) **OF₂**; (c) **OF₃**.

6. FLUORESCENCE SPECTRA

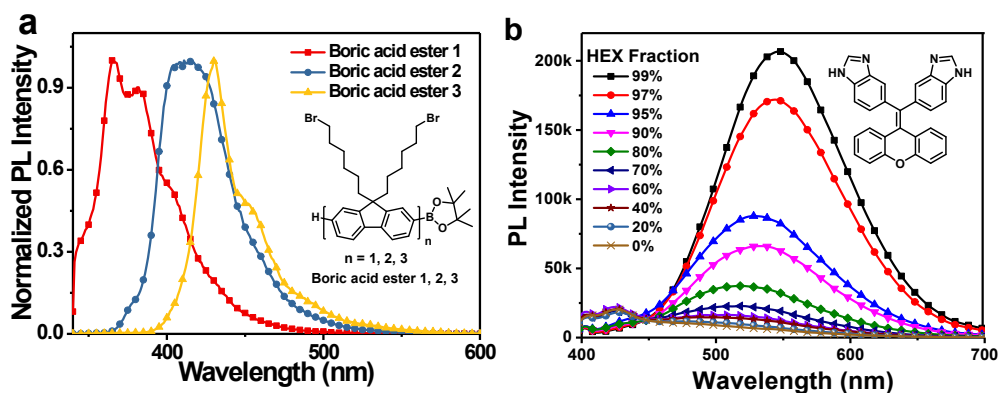


Fig. S4 (a) Normalized fluorescence spectra of solid fluorene boric acid esters. Inset: the structure of fluorene boric acid esters. The excitation wavelength is 330 nm. (b) Emission spectra of 9-methylene-9H-xanthene derivative in DCM-Hexane with different hexane content. The concentration of dyes is kept at 1.0×10^{-5} M. The excitation wavelength is 370 nm. Inset: the structure of the compounds.

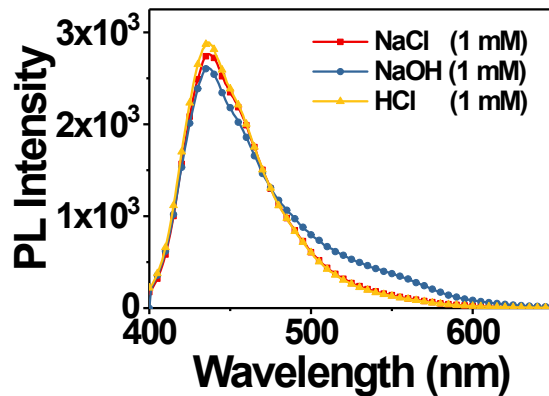


Fig. S5 Fluorescence spectra of OF_1^+ in NaCl (1 mM), aqueous solution of NaOH (1 mM, pH = 11) and HCl (1 mM, pH = 3). The concentration of each OF_1^+ is 2 μ M, the excitation wavelength is 380 nm.

7. Fluorescence colocalization imaging of HeLa Cells

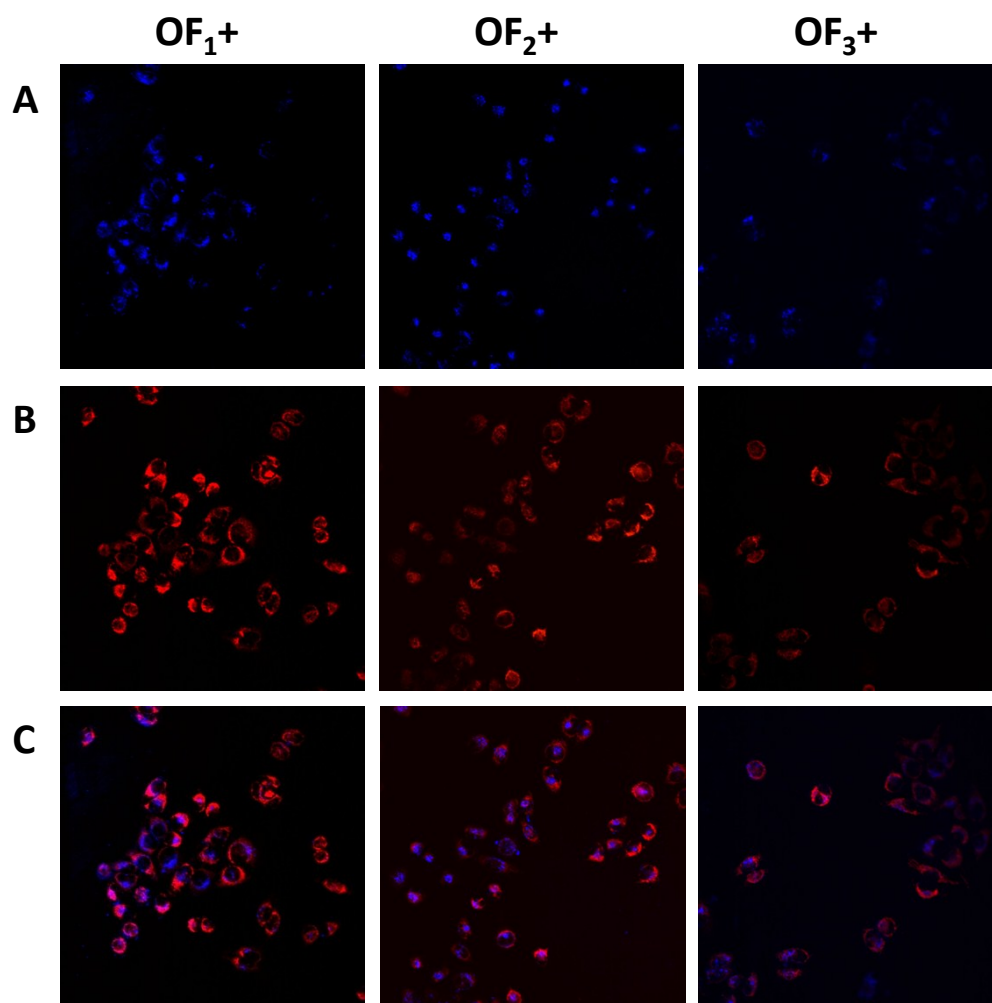


Fig. S6 Fluorescent colocalization images of HeLa cells stained with (A) OF_n+ (5 μ M) for 2 h and (B) MitoTracker red FM (MT, 50 nM) for 30 min. (C) Panels A and B merged. The Pearson's correlation coefficients between the green-yellow emissions from OF_n+ ($n = 1-3$) and the red emissions from MitoTracker Red were determined to be 12.4% (OF_1+), 32.7% (OF_2+) and 44.5% (OF_3+), respectively, demonstrating OF_n+ ($n = 1-3$) target poor to mitochondria. Excitation wavelength: 330–385 nm (for TPE-TPP) and 540–580 nm (for MT).

8. FLUORESCENCE QUANTUM YIELDS

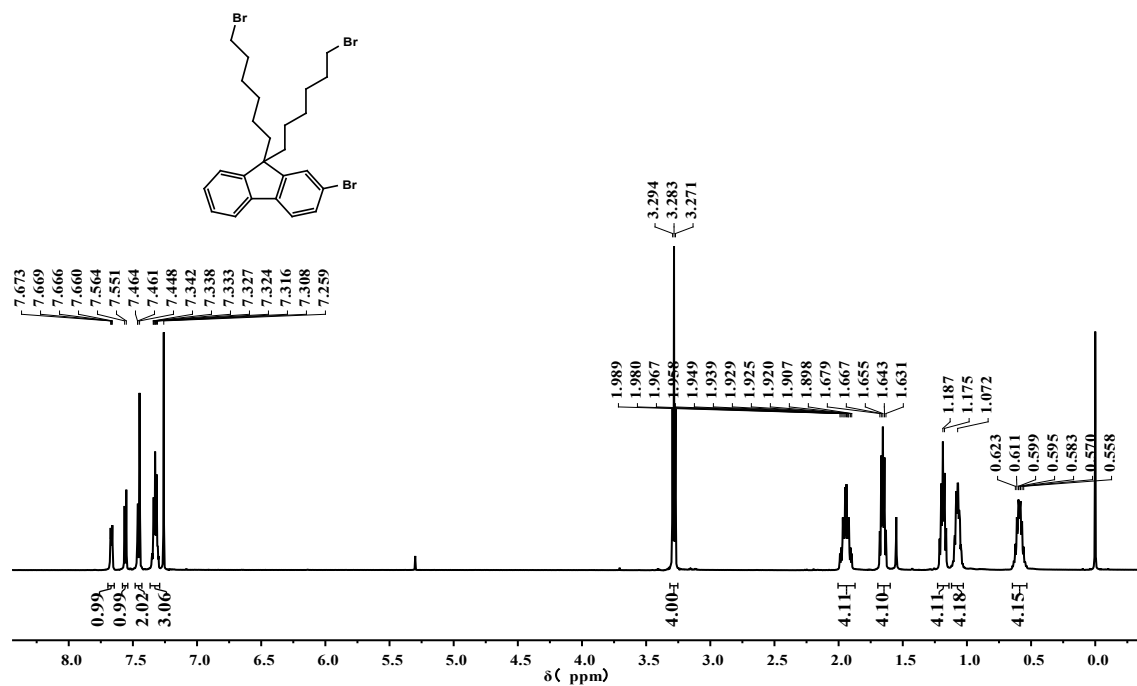
Table S1 The relative fluorescence quantum yields in different solvents.

	$\lambda_{\text{abs,sol}}(\text{nm})^{\text{a}}$	$\lambda_{\text{PL,sol}}(\text{nm})^{\text{b}}$	$\lambda_{\text{PL,agg}}(\text{nm})^{\text{c}}$	$\Phi_{\text{F,sol}}^{\text{d}}$	$\Phi_{\text{F,agg}}^{\text{e}}$
OF₁	314	428	586	0.90%	12.12%
OF₂	345	430	582	0.59%	32.22%
OF₃	360	418	572	1.69%	35.85%
OF₁+	315	428	572	2.89%	29.95%
OF₂+	349	442	556	2.09%	32.85%
OF₃+	368	428	550	4.20%	38.62%

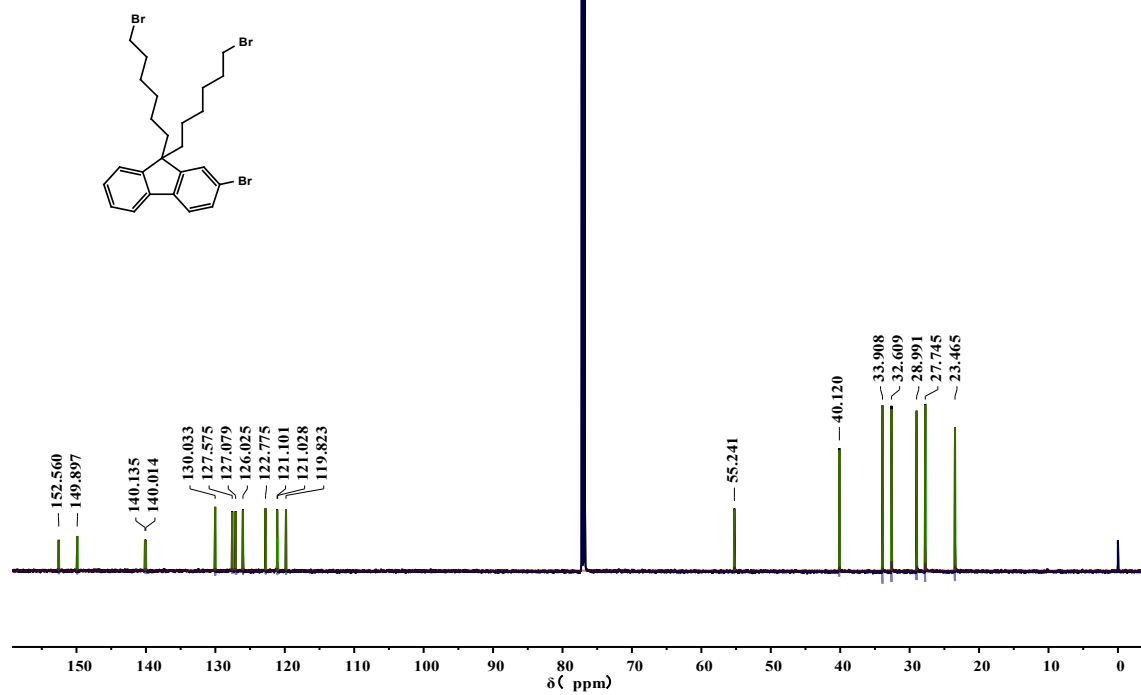
^a The maximum absorption wavelength of **OF_n** in THF and **OF_n+** in DMSO; ^b The maximum emission wavelength of **OF_n** in THF and **OF_n+** in DMSO; ^c The maximum emission wavelength of **OF_n** in water and **OF_n+** in THF; ^d Fluorescence quantum yields of **OF_n** in THF and **OF_n+** in DMSO. ^e Fluorescence quantum yields of **OF_n** in water and **OF_n+** in THF. All the fluorescence quantum yields were measured using quinine sulfate ($\Phi_{\text{F}} = 55\%$ in 0.1 M H₂SO₄) as standard reference.

9. NMR SPECTRA.

zhumingqing-20170215-2#

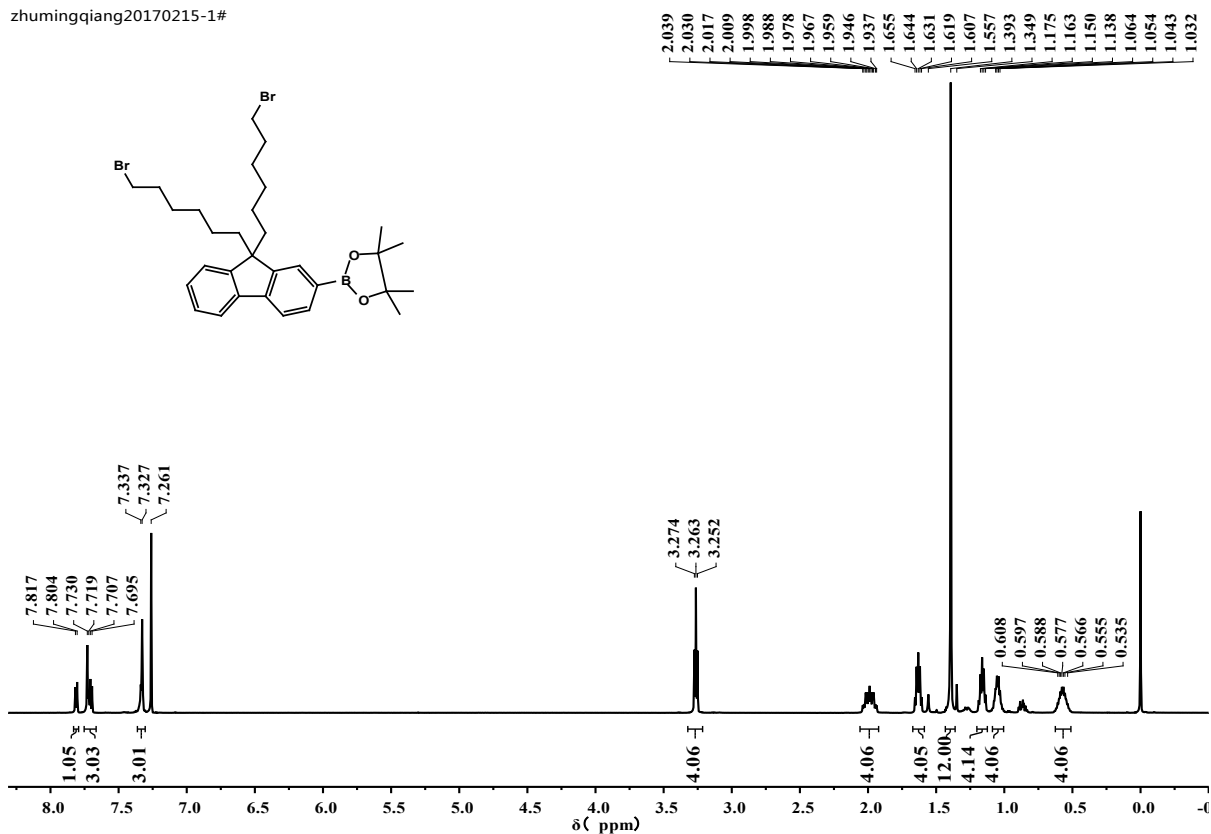


zhumingqiang-170224-4#

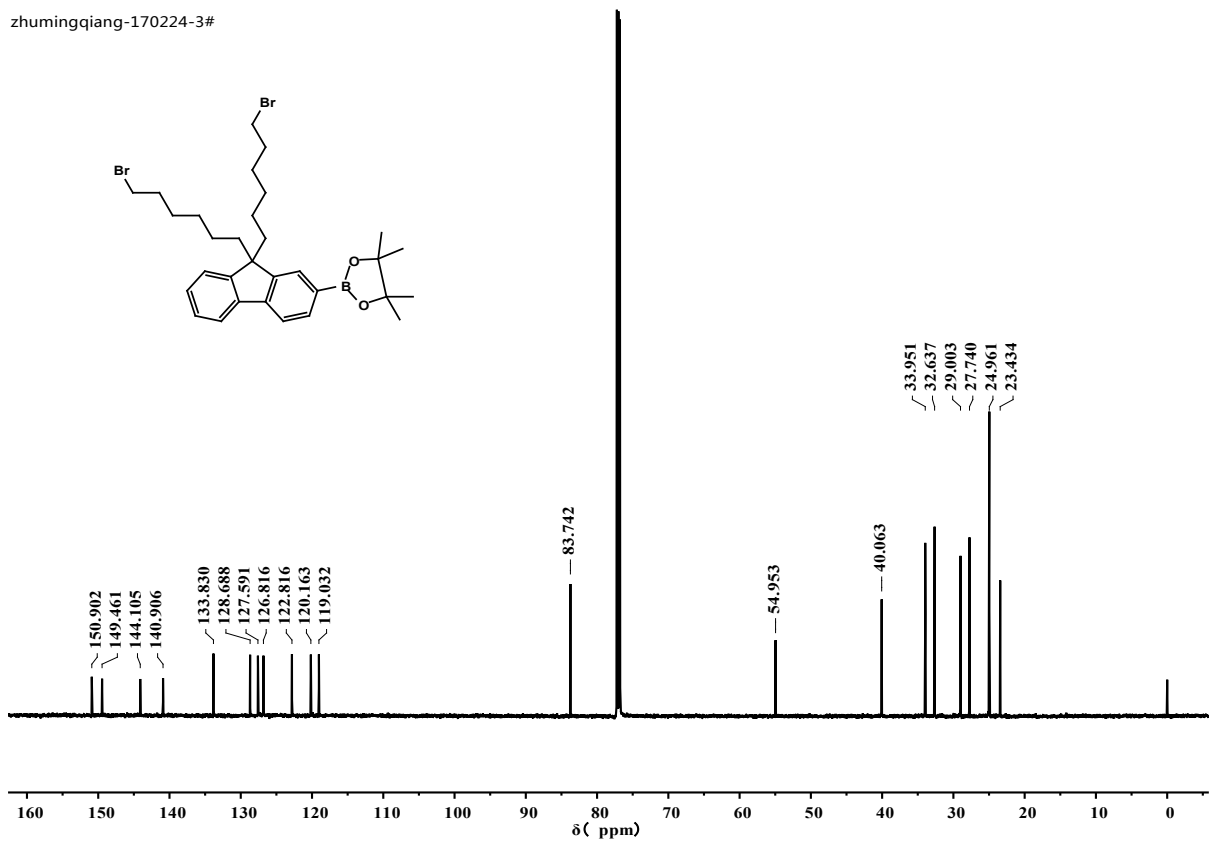


The NMR spectra of **1a**

zhumingqiang20170215-1#

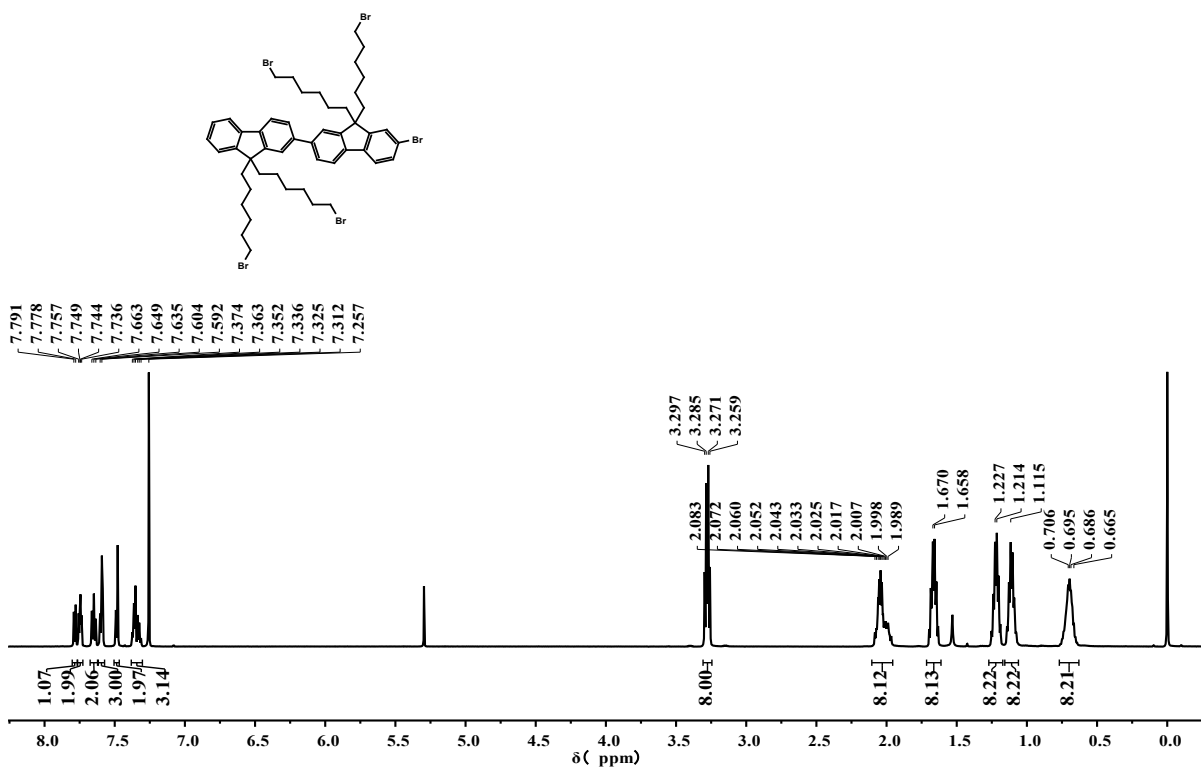


zhumingqiang-170224-3#

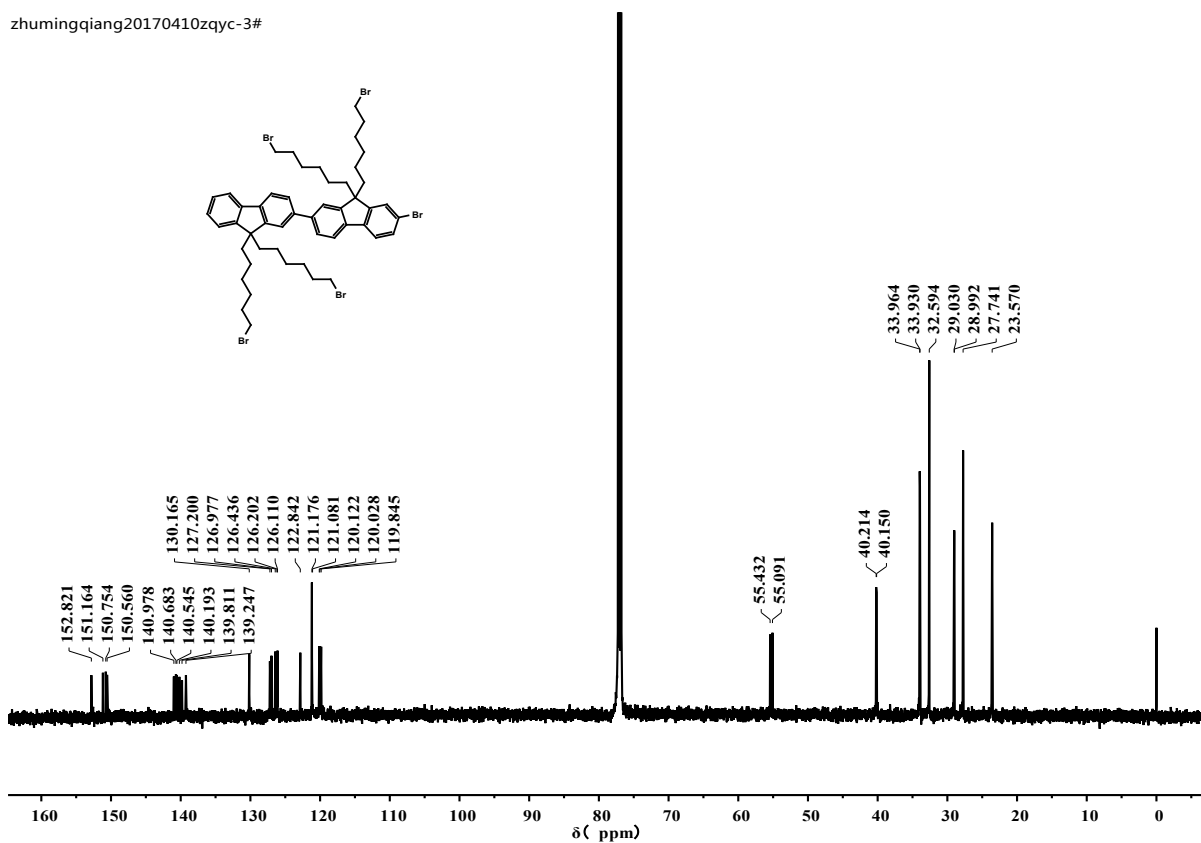


The NMR spectra of **1b**

zhumingqiang20170317-1#

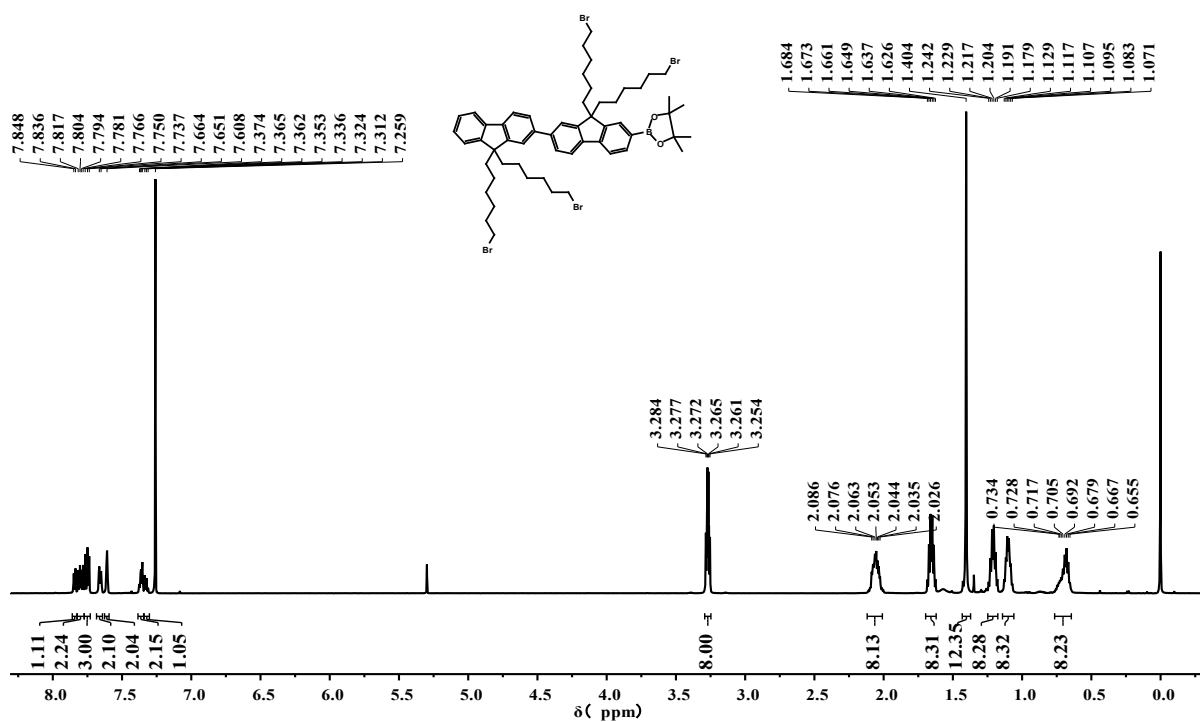


zhumingqiang20170410zqyc-3#

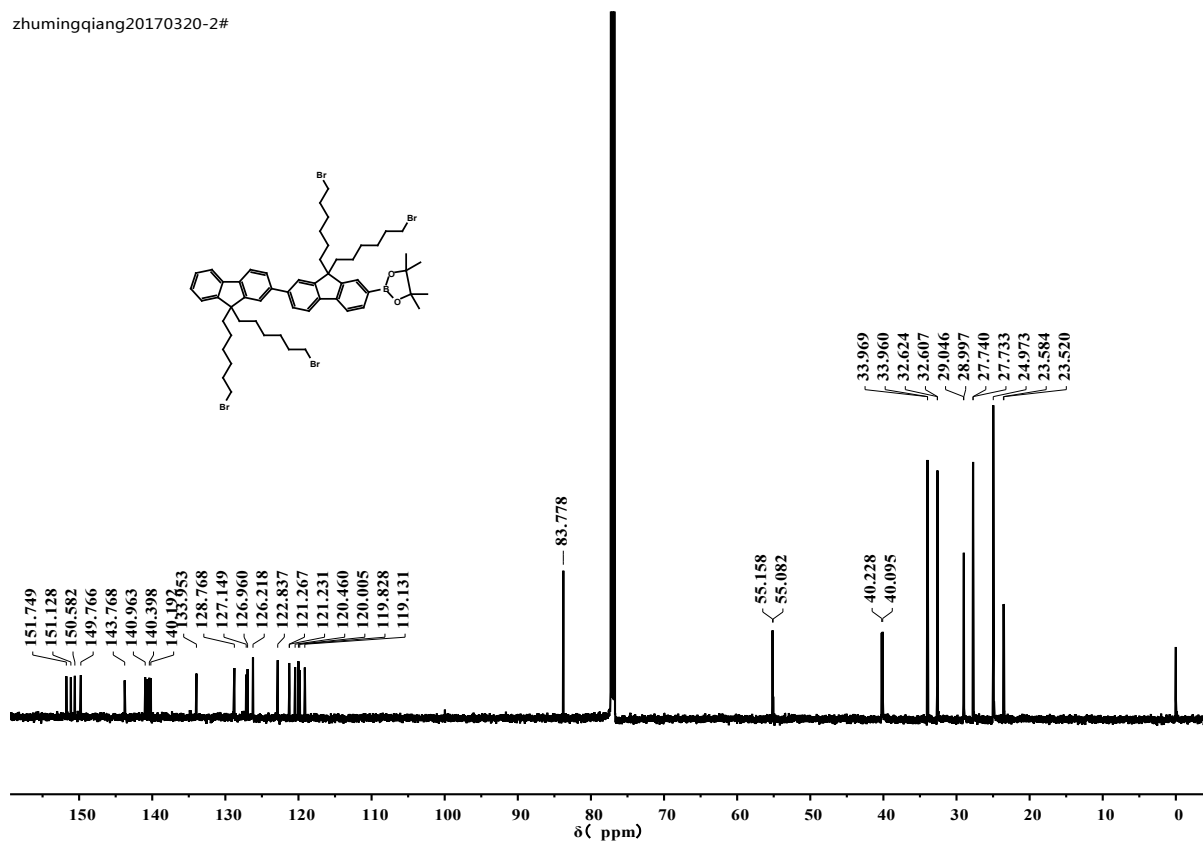


The NMR spectra of 2a

zhumingqiang20170317-2#

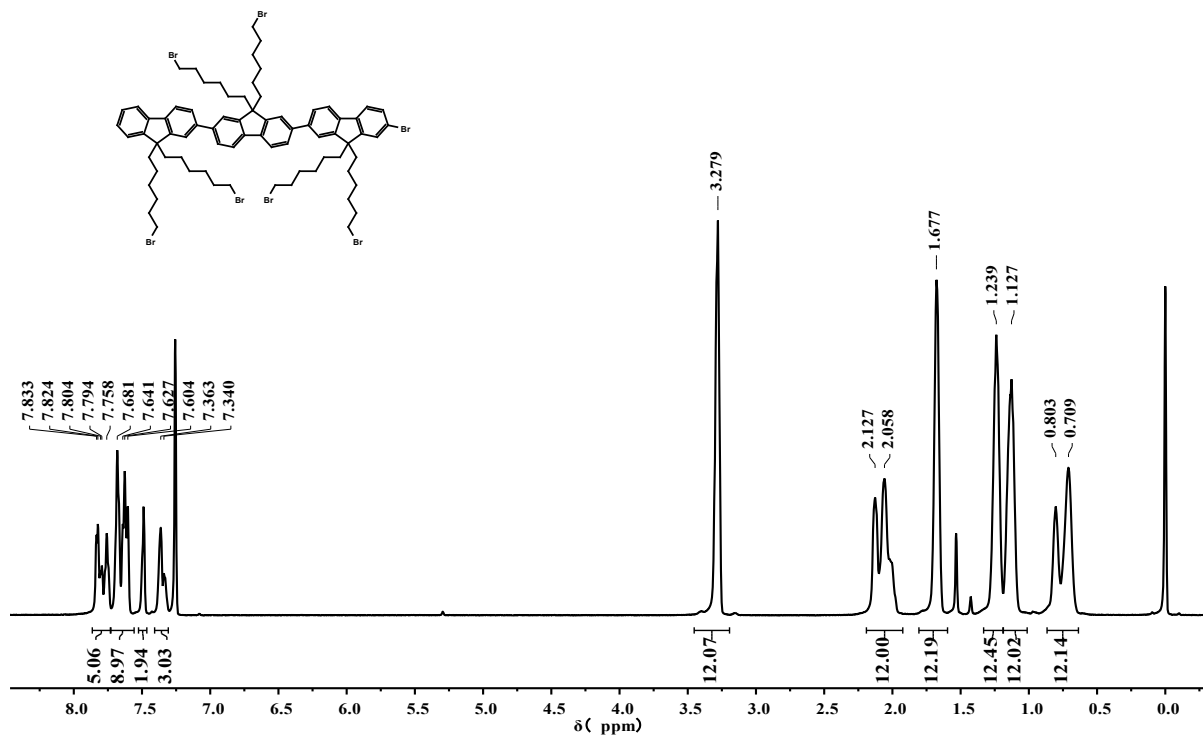


zhumingqiang20170320-2#

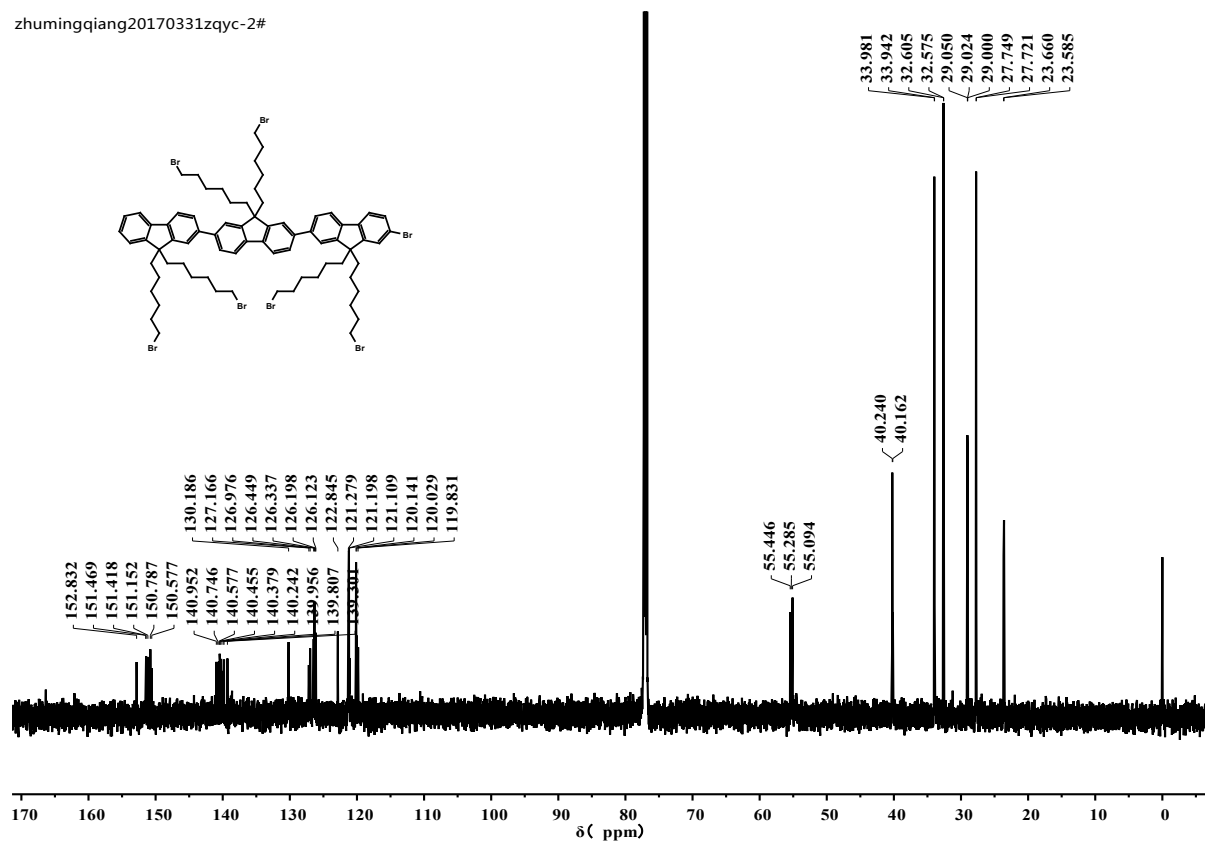


The NMR spectra of **2b**

zhumingqiang20170331zqy-2#

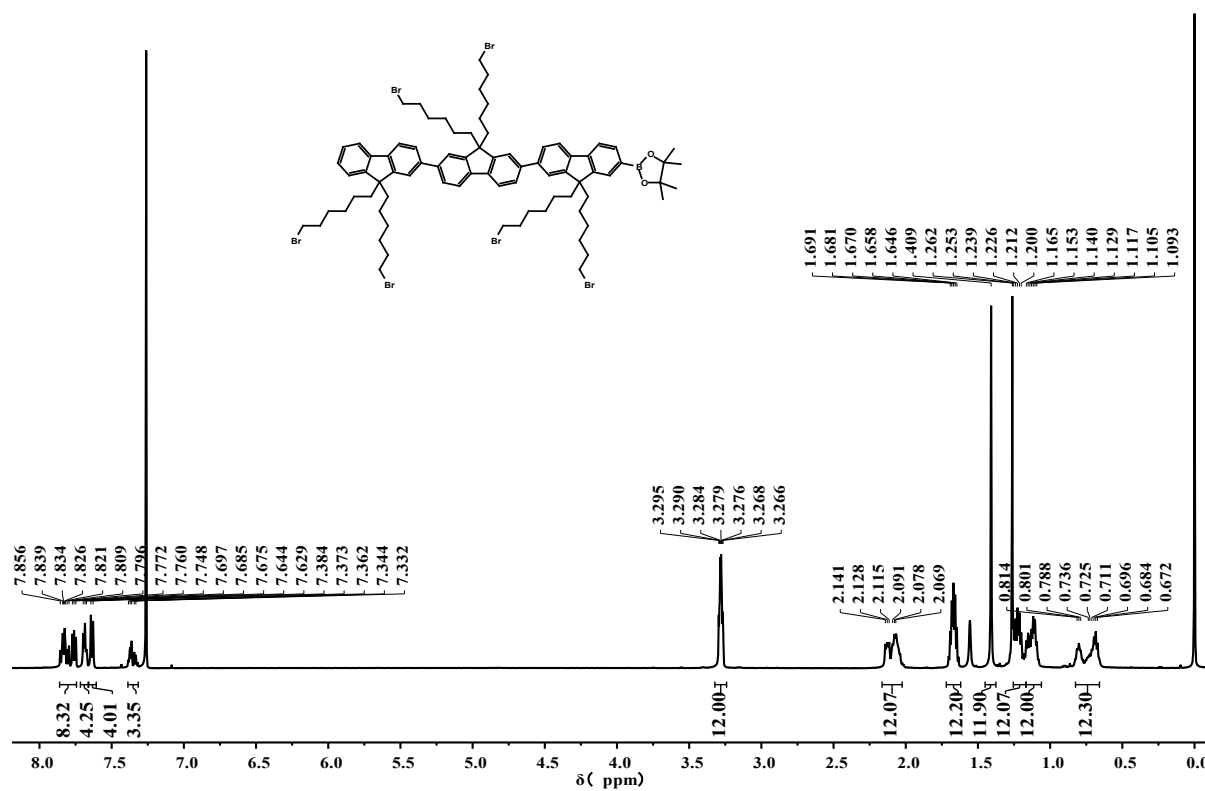


zhumingqiang20170331zqyc-2#

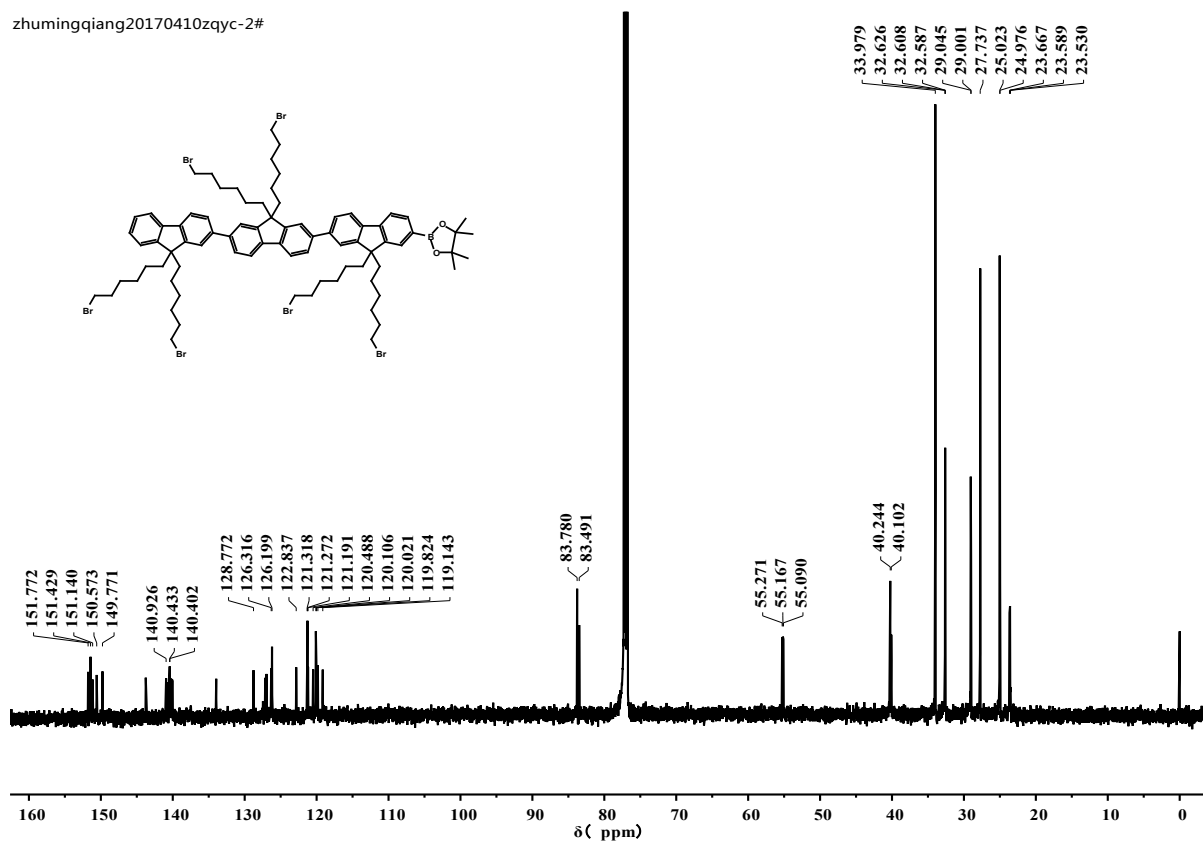


The NMR spectra of 3a

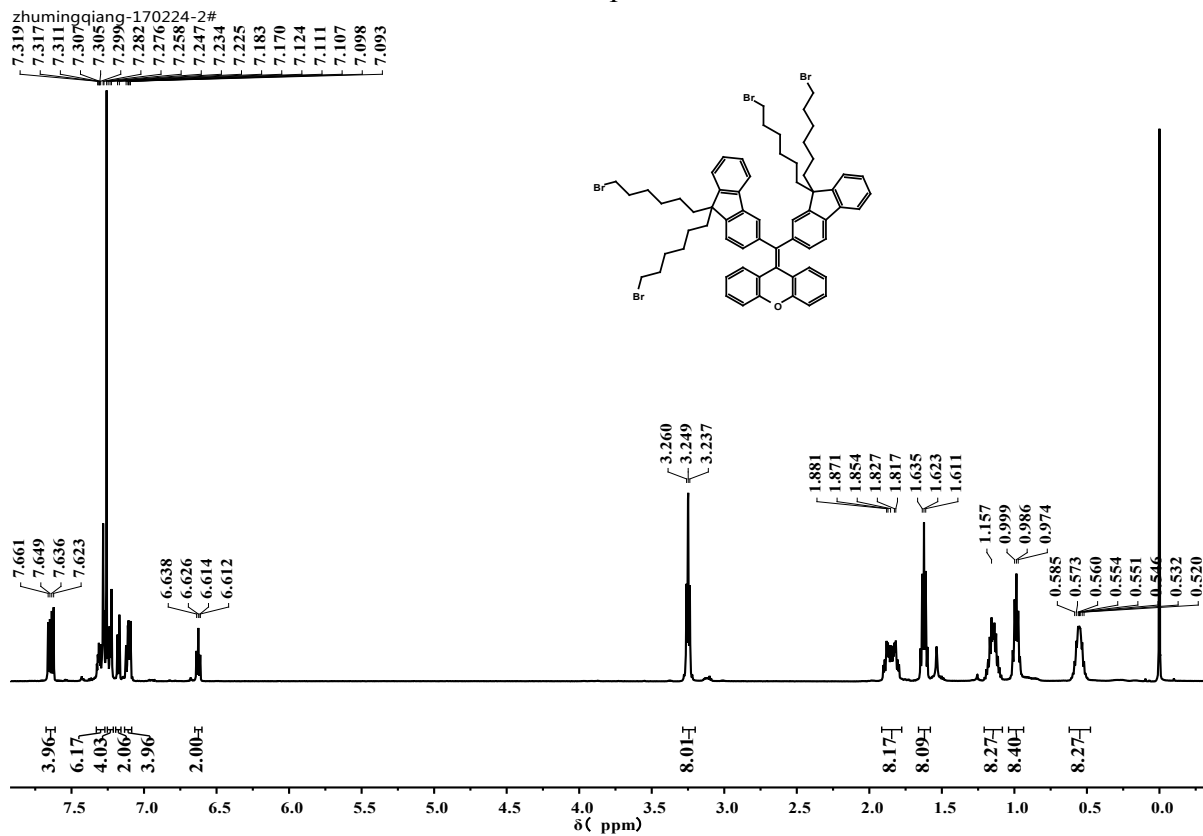
zhumingqiang20170407zqy-1#



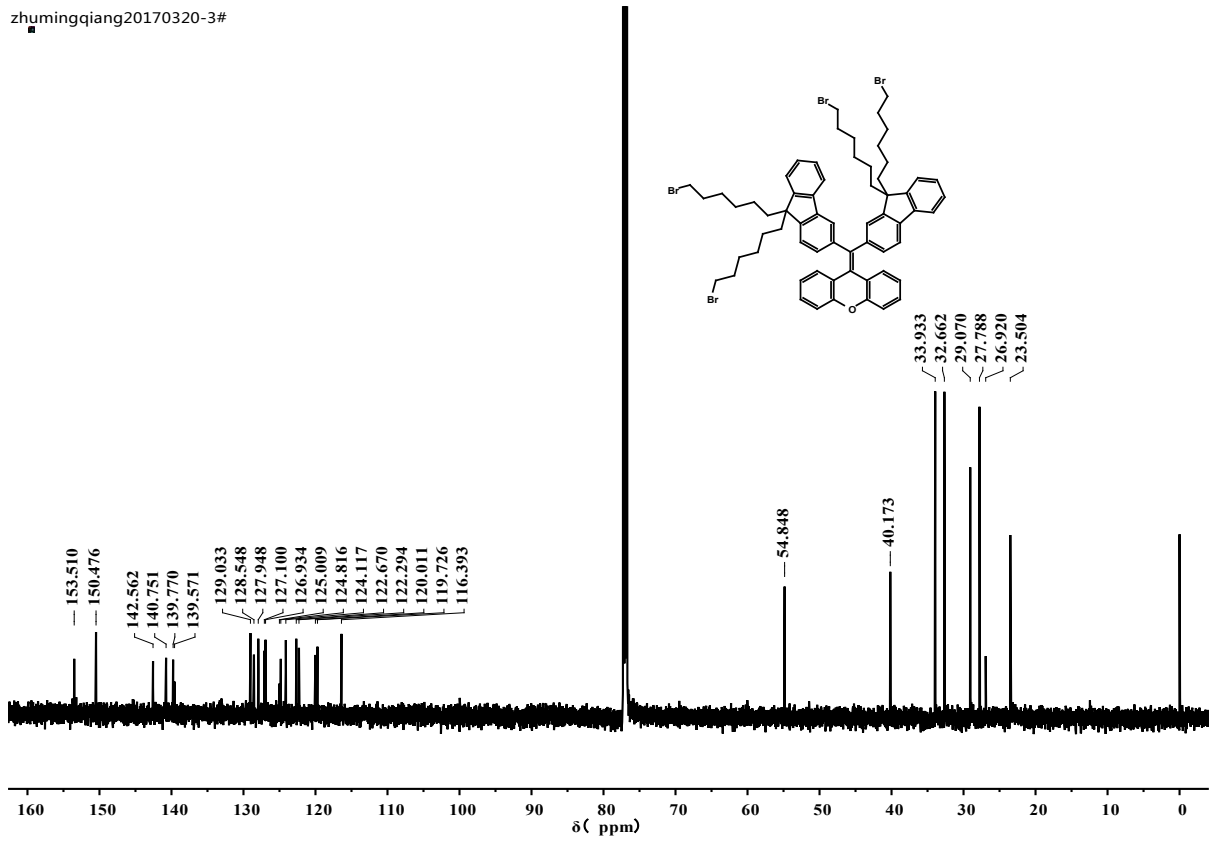
zhumingqiang20170410zqyc-2#



The NMR spectra of **3b**

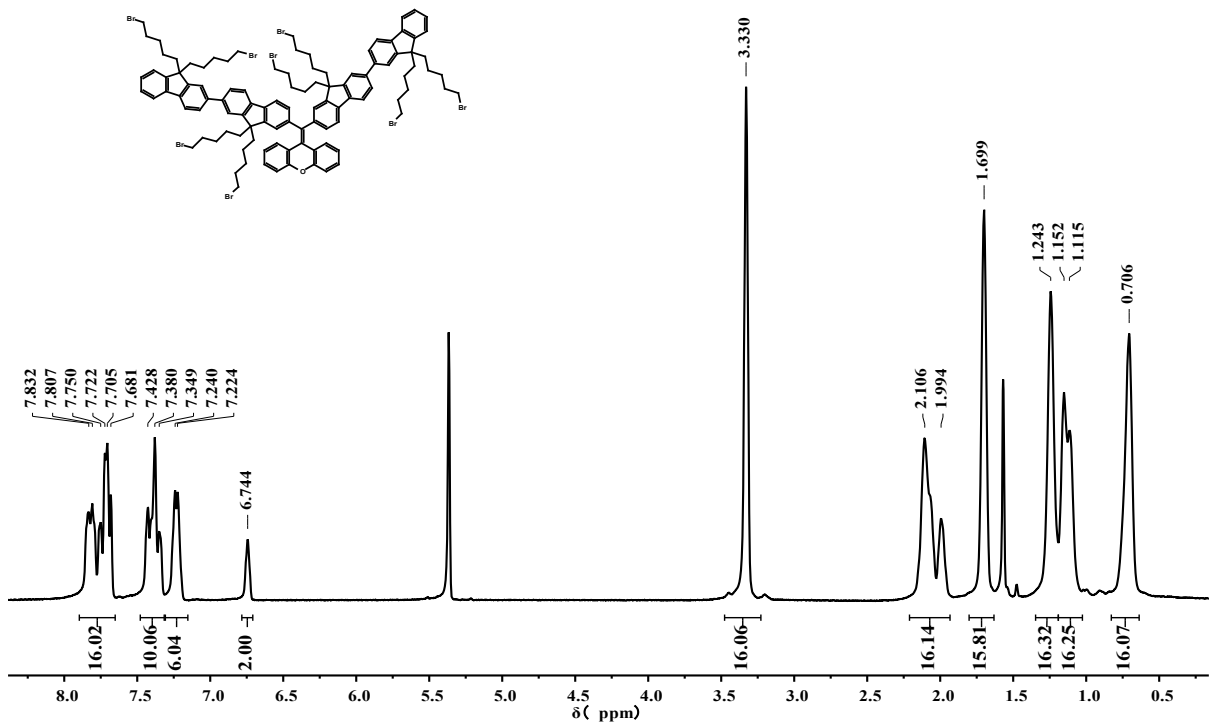


zhumingqiang20170320-3#

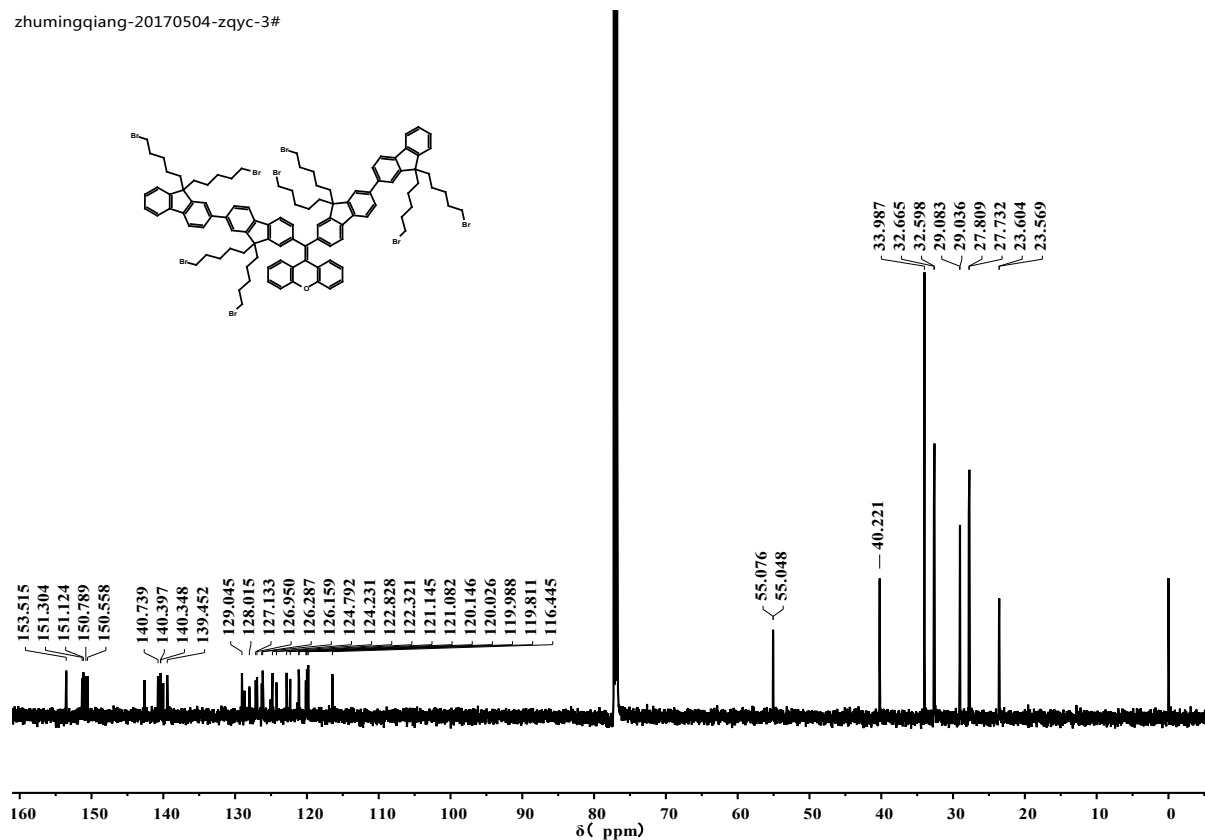


The NMR spectra of OF₁

zhumingqiang20170331zqy-1#

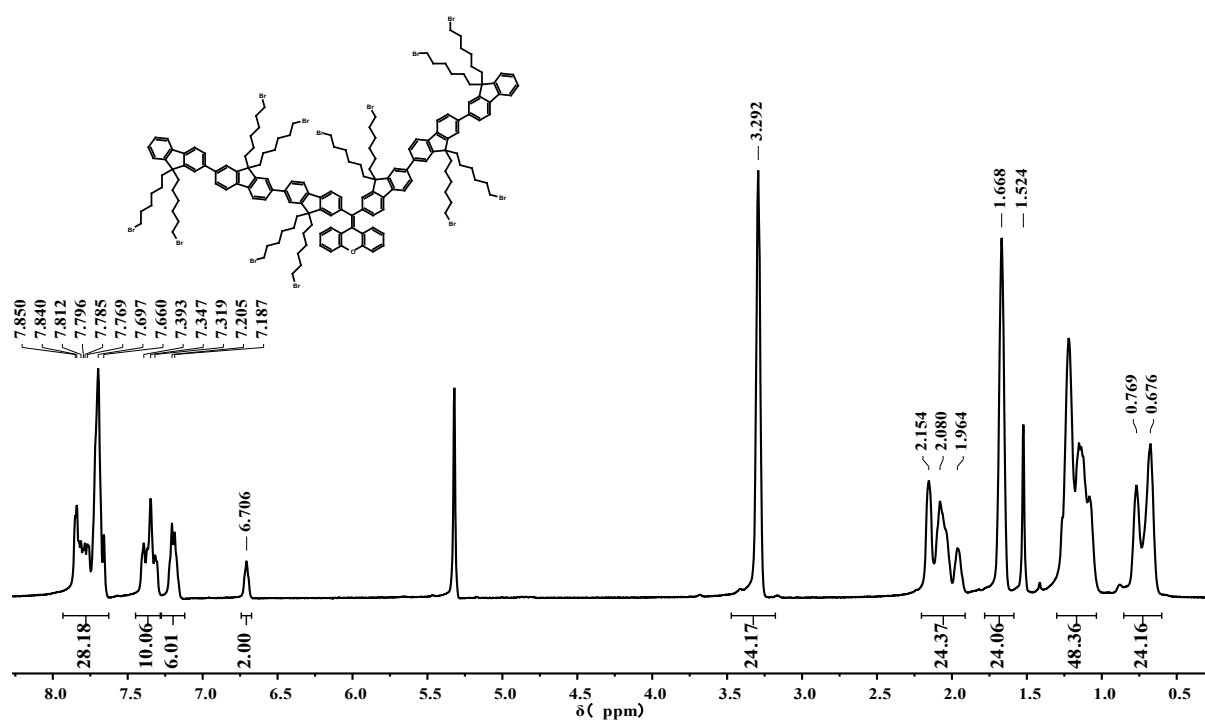


zhumingqiang-20170504-zqyc-3#

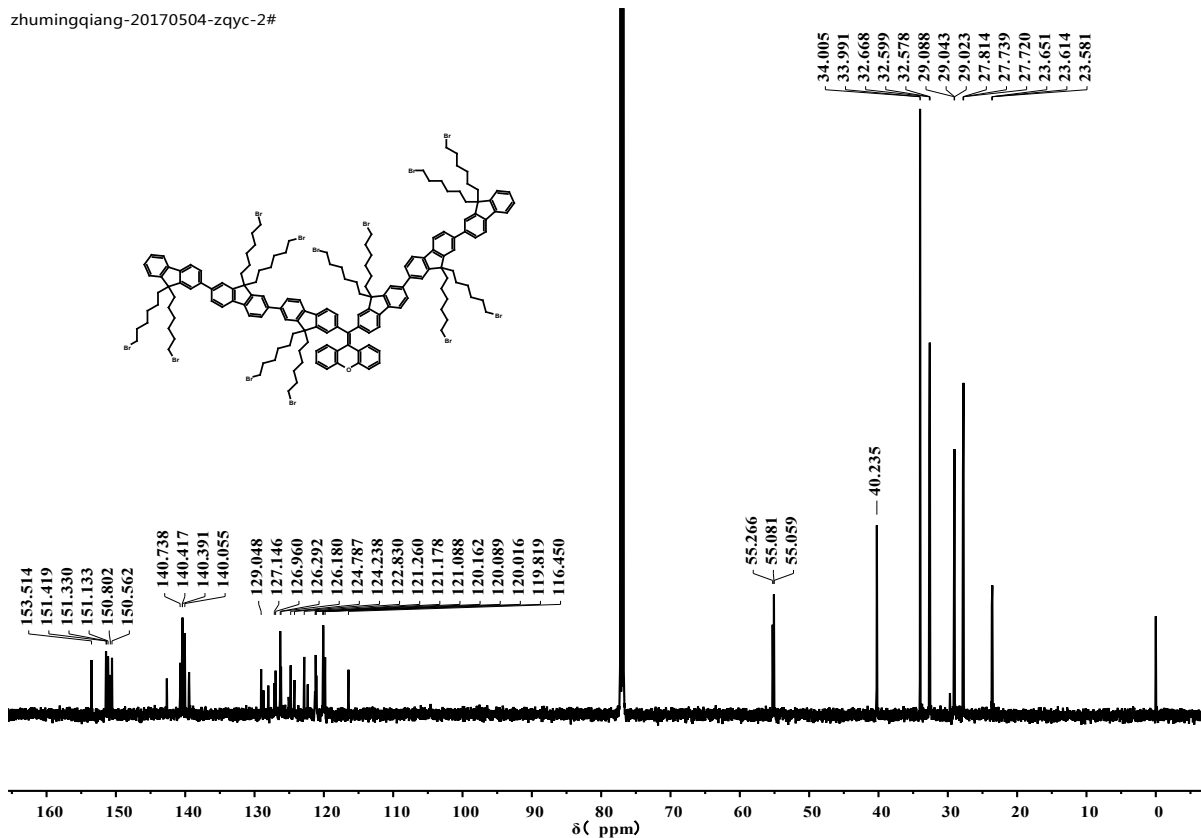


The NMR spectra of OF_2

zhumingqiang20170414zqy-3#

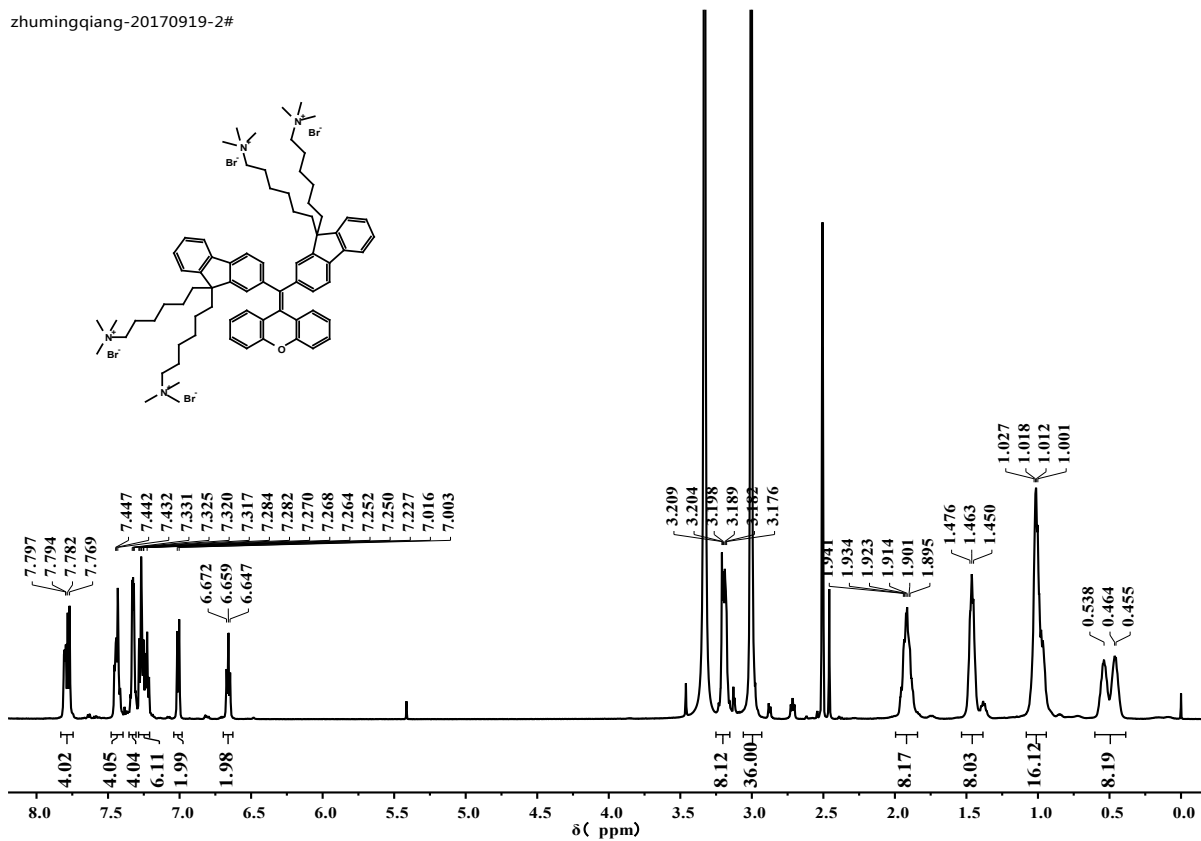


zhumingqiang-20170504-zqyc-2#

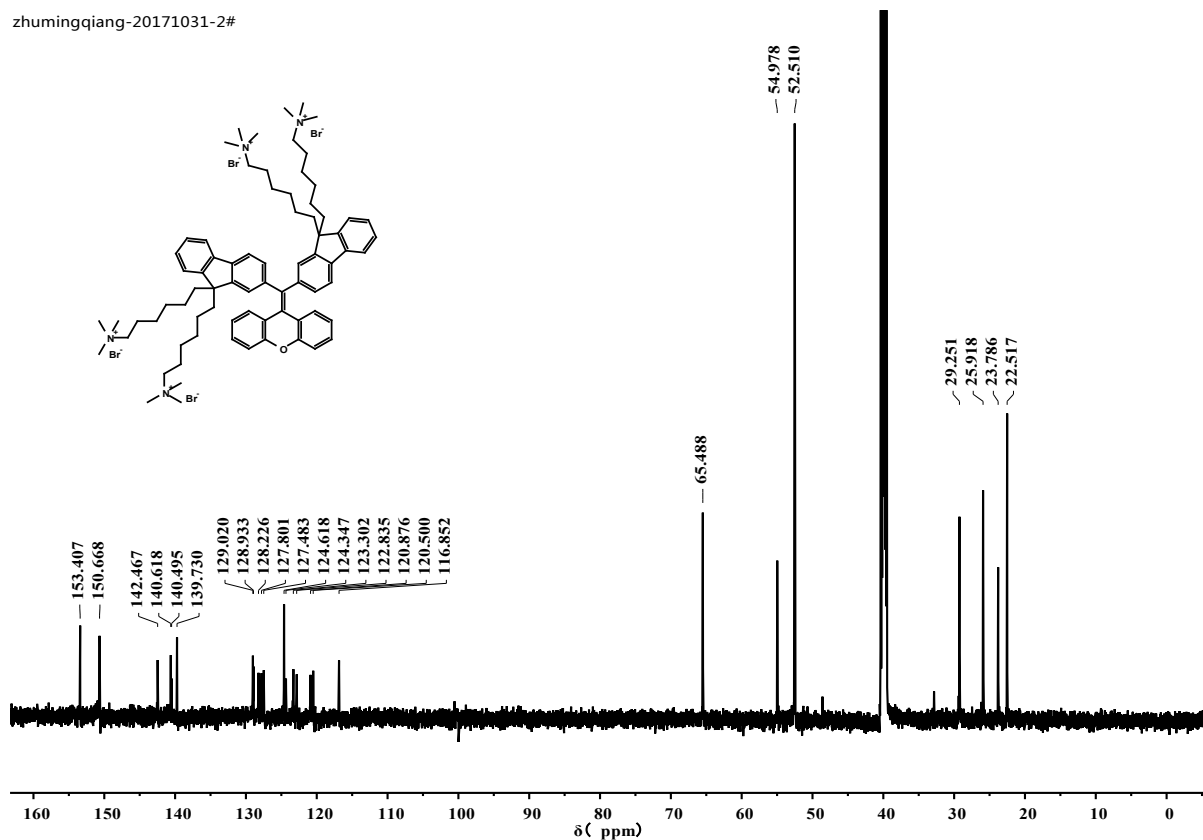


The NMR spectra of OF₃

zhumingqiang-20170919-2#

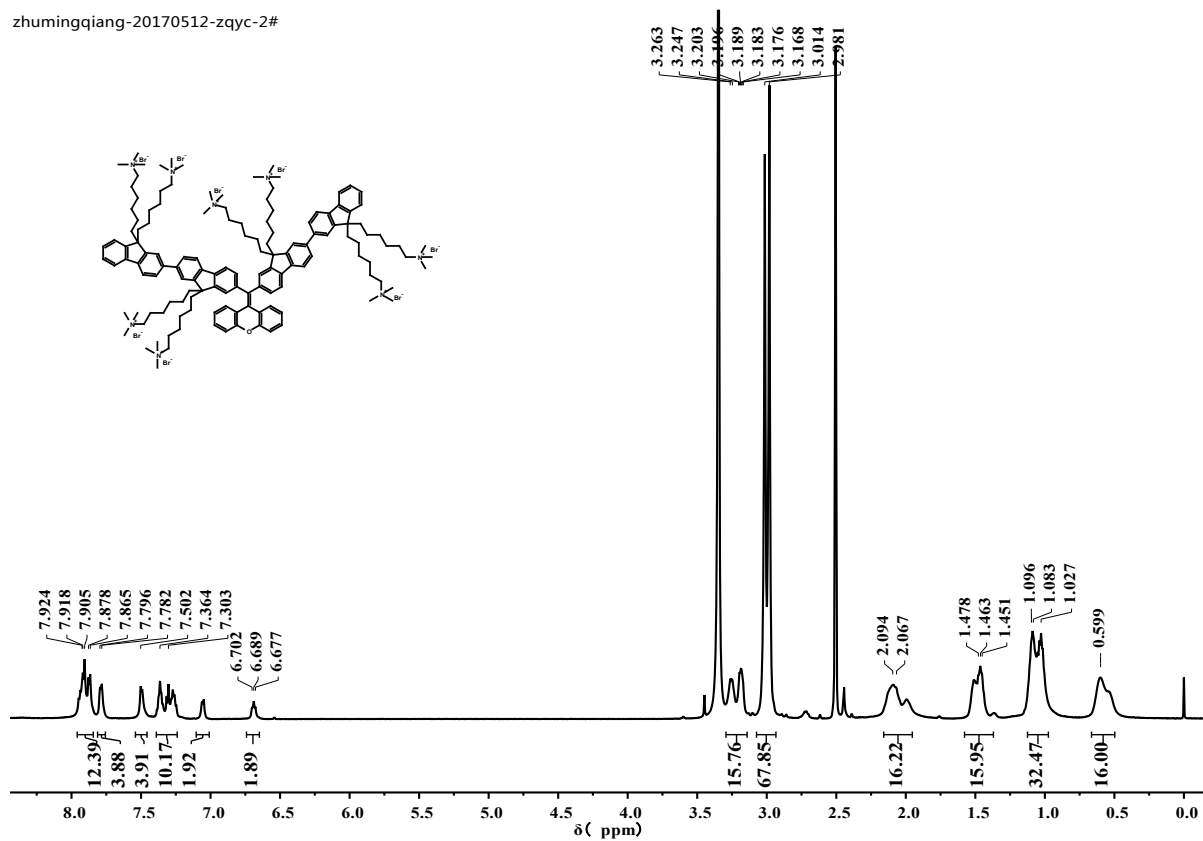


zhumingqiang-20171031-2#

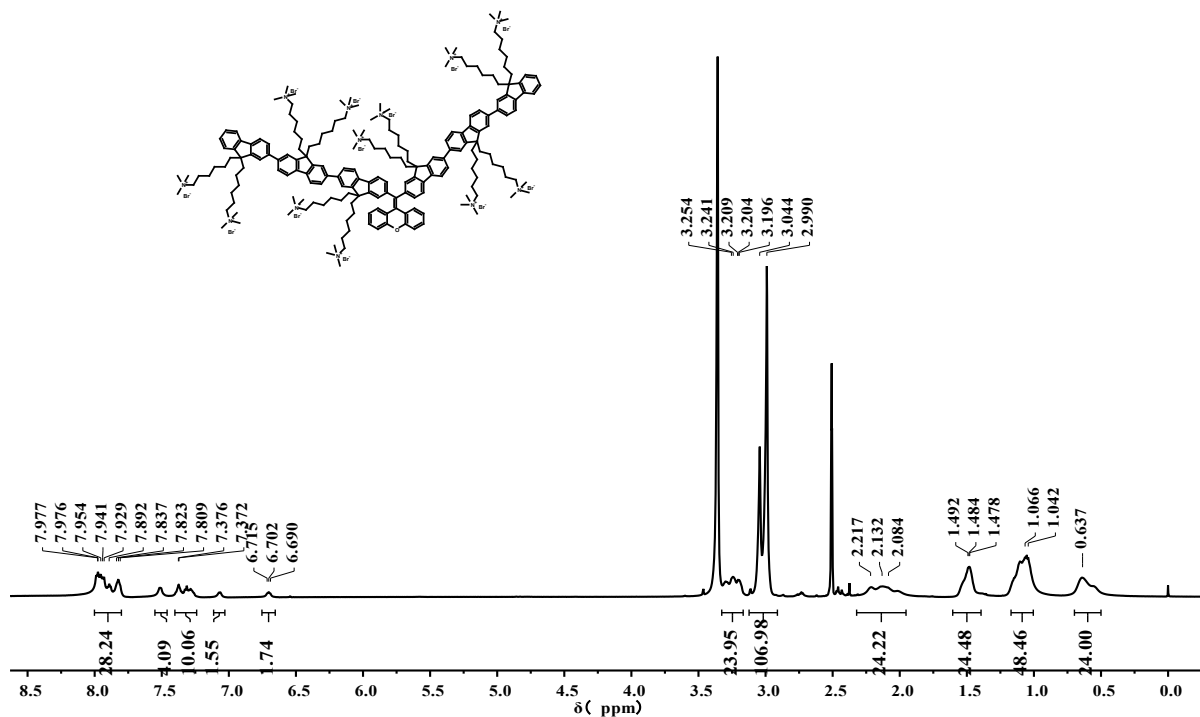


The NMR spectra of **OF₁⁺**

zhumingqiang-20170512-zqyc-2#



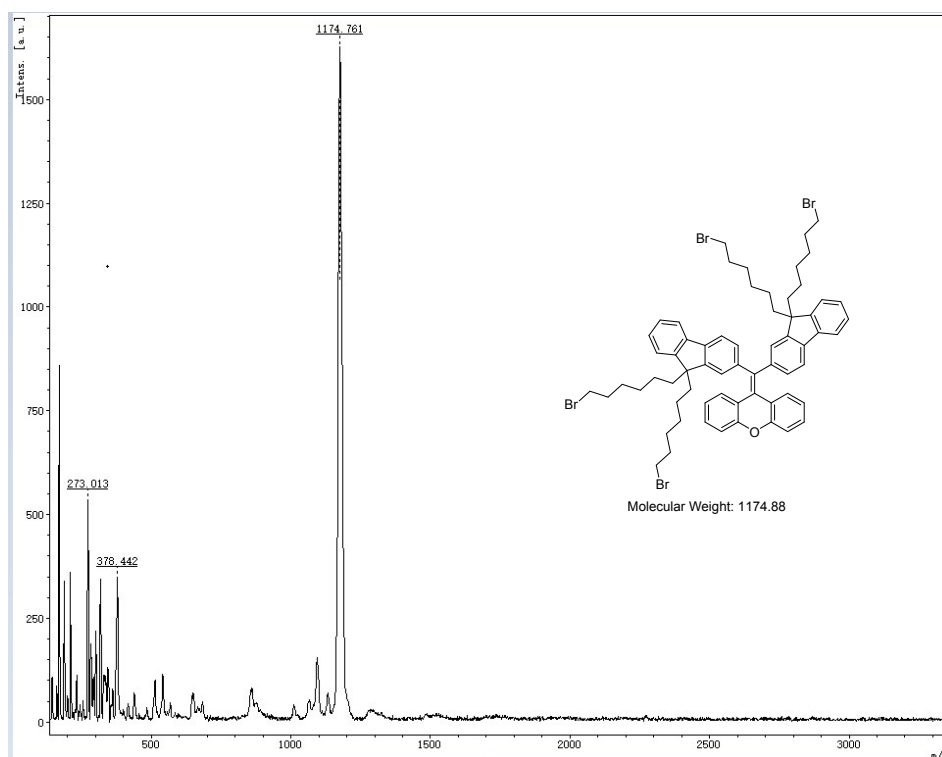
The NMR spectra of **OF₂⁺**



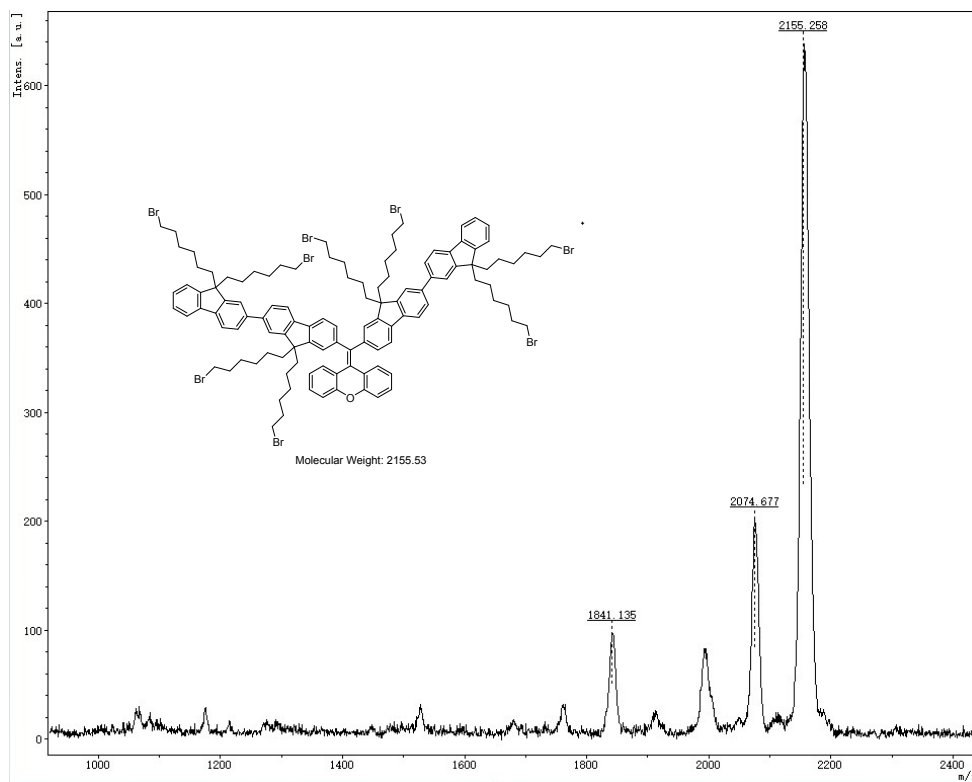
The NMR spectra of OF_3^+

10. MASS SPECTRA.

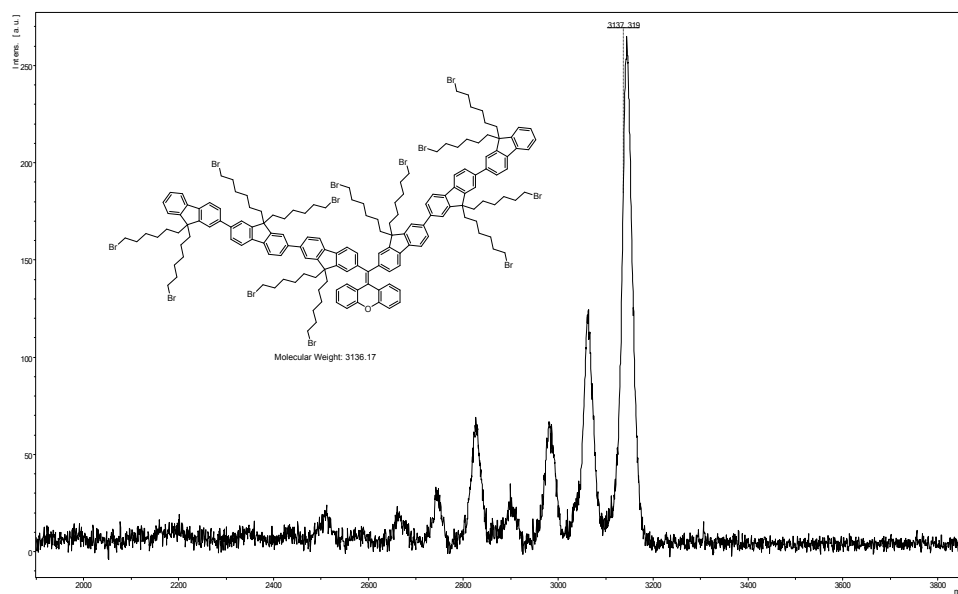
OF_1



OF₂



OF₃



11. REFERENCES.

- (1) P. M. Donovan, L. T. Scott, *J. Am. Chem. Soc.*, 2004, **126**, 3108-3112
- (2) G.-F. Zhang, H. Wang, M. P. Aldred, T. Chen, Z.-Q. Chen, X. Meng and M.-Q. Zhu, *Chem. Mater.*, 2014, **26**, 4433-4446.
- (3) C. C. Hung, H. C. Wu, Y. C. Chiu, S. H. Tung, W. C. Chen, *J. Polym. Sci. Pol. Chem.*, 2016, **54**, 3224-3236.
- (4) L. Zhu, J. Pan, C. M. Christensen, B. C. Lin, M. A. Hickner, *Macromolecules*, 2016, **49**, 3300-3309.