A NIR fluorescent probe for the detection of fluoride ions and its application for *in vivo* bioimaging

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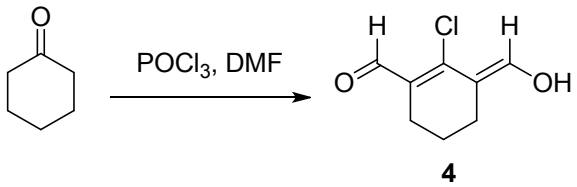
Supporting Information

Table of contents

1. General procedure for the synthesis of compounds 4

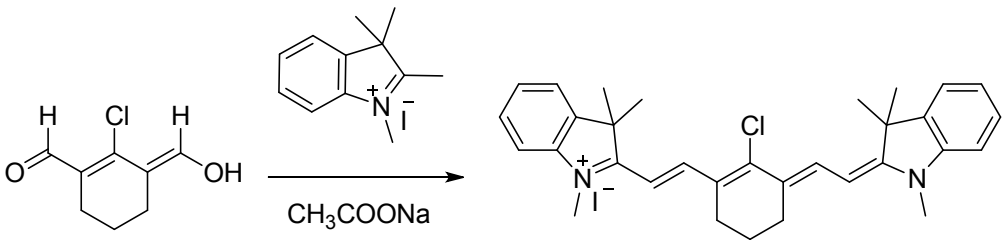


4

Scheme S1 The synthesis of compound 4

Cyclohexanecarboxaldehyde was synthesized according to previous report[1]. A mixture of 40 mL dimethylformamide (0.52 mol) and 40 mL of methylene chloride was chilled in an ice bath for 30 min. 37 mL phosphorus oxychloride (0.41 mol) and cyclohexanone (10 g, 0.10 mol) was added dropwise to above mixture with stirring. The solution was refluxed for 4 h, cooled, poured onto 200 g of ice, and allowed to stand overnight. The yellow solid was collected with a yield of 9.56 g (54.38%).

2. General procedure for the synthesis of compound 5

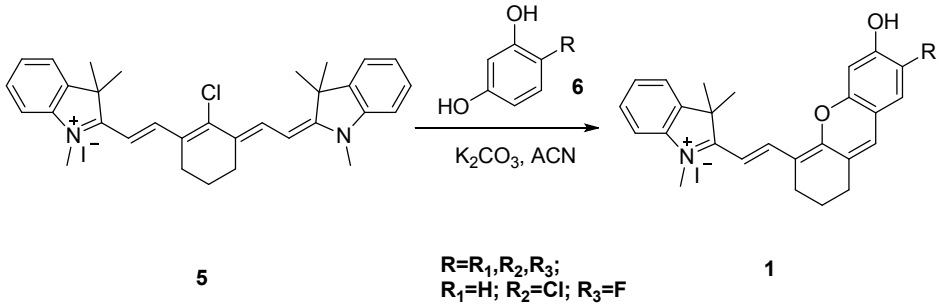


4 **5**

Scheme S2 The synthesis of compound 5

Compound 5 was synthesized according to previous report[1]. 1, 2, 3, 3 – tetrametheyl - 3H -indolium iodide (903 mg, 3.0 mmol), sodium acetate trihydrate (408.2 mg, 3.0 mmol) and 129.4 mg (0.75 mmol) of 3bohexanecarboxaldehyde were dissolved in 12 mL acetic anhydride in a flask. The mixture was heated at 70 ºC refluxing with constant stirring. After 10 min, another 129.6 mg (0.75 mmol) of 3bohexanecarboxal-dehyde was added into reacting solution. 30 min later, the reaction solution was cooled to room temperature and the mixture was dropped into the methyl ether in an ice bath and rude green powder was filtrated after precipitation. The crude was purified by column chromatography on silica gel with a mixture of CH_2Cl_2 and CH_3OH as eluent. Removal of solvent under vacuum afforded pure deep green powder 547.5 mg (59.73%).

3. General procedure for the synthesis of compounds 1



Scheme S3 The synthesis of compound 1

4. References

1) L. Wang, J. Jin, X. Chen, H.-H. Fan, B. K. F. Li, K.-W. Cheah, N. Ding, S. Ju, W-T. Wong and C. Li, *Organic & Biomolecular Chemistry*, 2012, 10, 5366.

5. Fluorescence spectra, UV absorption spectrum and color change

5.1 Color change

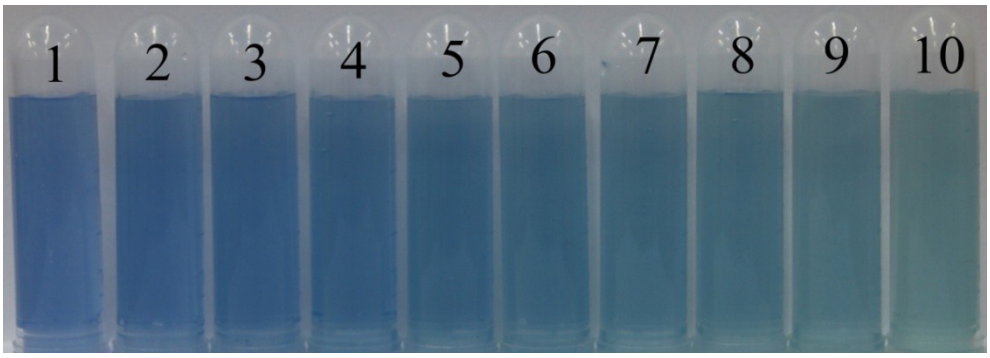


Fig. S1 The color change of 3c (10 μM) upon addition of NaF in a PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4) solution. Each picture was recorded at 20 min after the addition of NaF (1-10 : 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 3.0 equiv.). The color changes of 3a and 3b are same as 3c.

5.2 Photostability

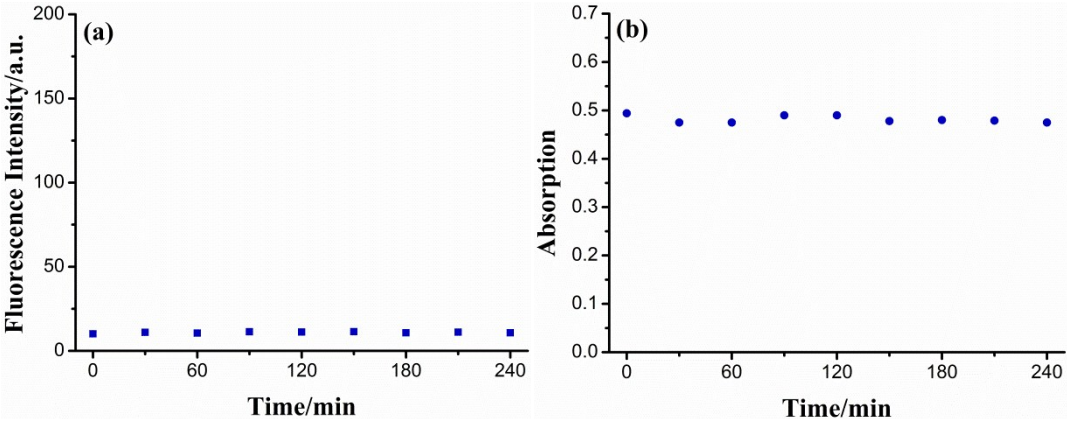


Fig. S2 Time-dependent of 3a (10 μM, PBS/DMSO = 3:7 (v/v, 0.01 M, pH =7.4) solution) under sustained illumination. (a) Fluorescence spectra. (b) UV absorption spectra.

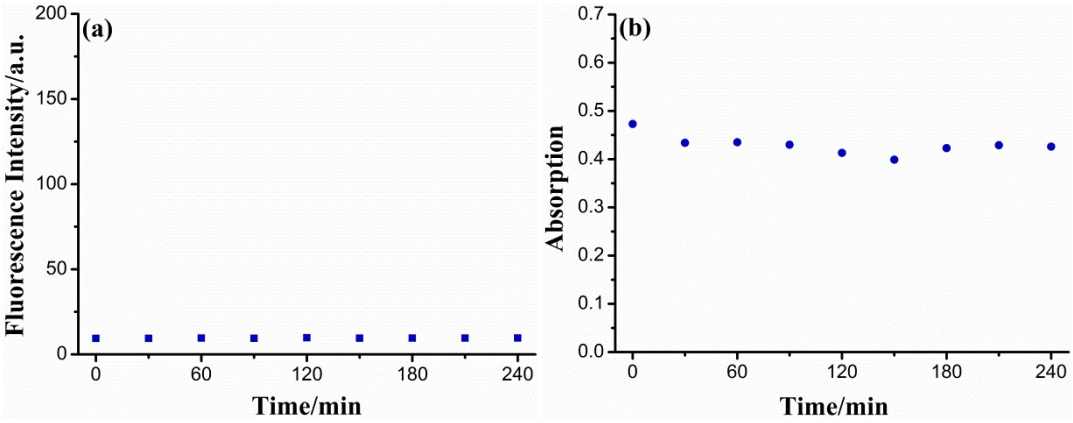


Fig. S3 Time-dependent of 3b (10 μM) under sustained illumination. (a) Fluorescence spectra. (b) UV absorption spectra.

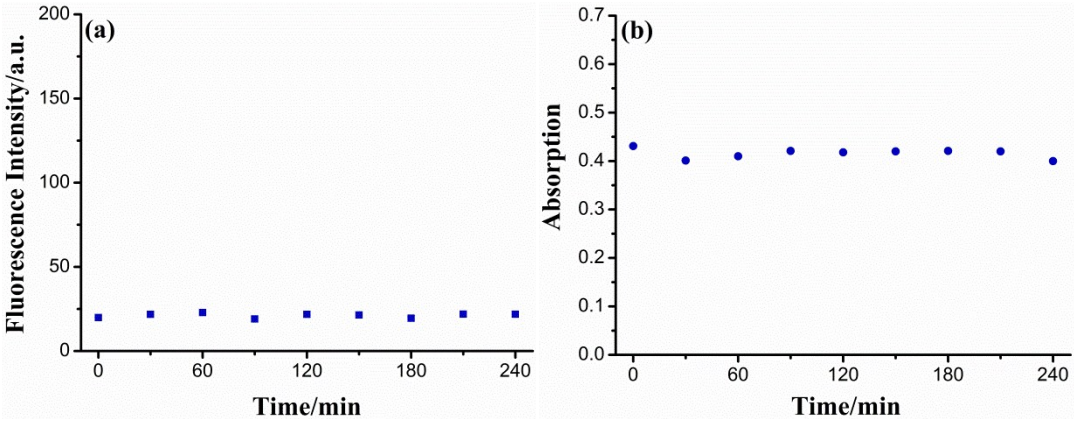


Fig. S4 Time-dependent of 3c (10 μM) under sustained illumination. (a) Fluorescence spectra. (b) UV absorption spectra.

5.3 Fluorescence spectra and UV absorption spectrum of 1a and 3a

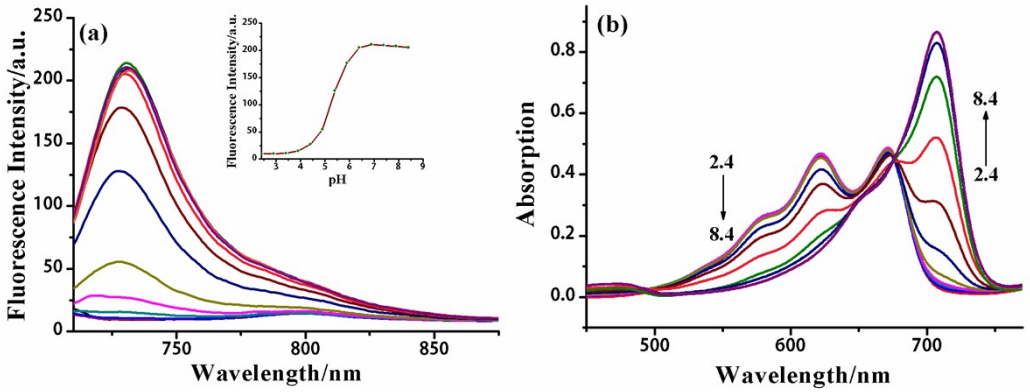


Fig. S5 (a) The fluorescence intensity changes depending the pH (2.4 - 8.4) of 1a (10 μM), λ_{ex} = 690 nm. Inset: the linear relationship of fluorescence intensity with pH (2.4 - 8.4) of 1a (10 μM). (b) The UV-Vis absorption of 1a (10 μM) at pH from 2.4 to 8.4. All data were measured in PBS/DMSO=3 : 7 (v/v, 0.01 M, pH = 2.4 - 8.4).

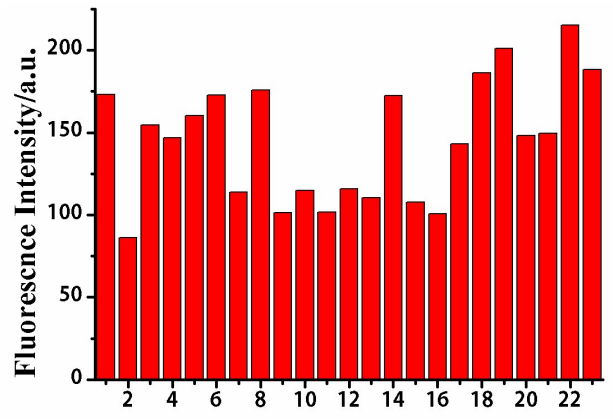


Fig. S6 Fluorescence response of 3a (10 μM) in the presence of NaF (3.0 equiv.) with other analytes (10.0 equiv.) in a PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4) solution. The analytes: (1) F^-; (2) F^- + SCN^-; (3) F^- + HS^-; (4) F^- + $S_2O_3^{2-}$; (5) F^- + SO_4^{2-}; (6) F^- + Ac^-;(7) F^- + $H_2PO_4^{2-}$; (8) F^- + HCO_3^-; (9) F^- + NO_3^-; (10) F^- + I^-; (11) F^- + Br^-; (12) F^- + Cl^-; (13) F^- + CO_3^{2-}; (14) F^- + Cu^{2+}; (15) F^- + Zn^{2+}; (16) F^- + Mn^{2+}; (17) F^- + Ni^{2+}; (18) F^- + Mg^{2+}; (19) F^- + Fe^{2+}; (20) F^- + Ca^{2+}; (21) F^- + Al^{3+}; (22) F^- + K^+; (23) F^- + Na^+.

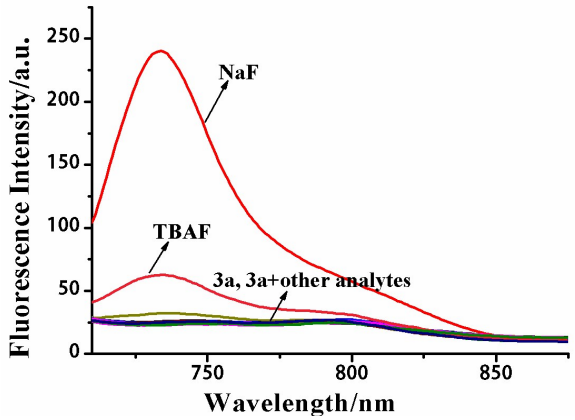


Fig. S7 Fluorescence response of 3a (10 μM) to various analytes (3.0 equiv.) in a PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH =7.4) solution. Other analytes ($H_2PO_4^{2-}$; Ac^-; SO_4^-; $S_2O_3^{2-}$; HS^-; SCN^-; Cl^-; Br^-; I^-; CO_3^{2-}; NO_3^-; HCO_3^-; Cu^{2+}; Zn^{2+}; Mn^{2+}; Ni^{2+}; Mg^{2+}; Fe^{2+}; Ca^{2+}; Al^{3+}; K^+; Na^+). λ_{ex} = 690 nm

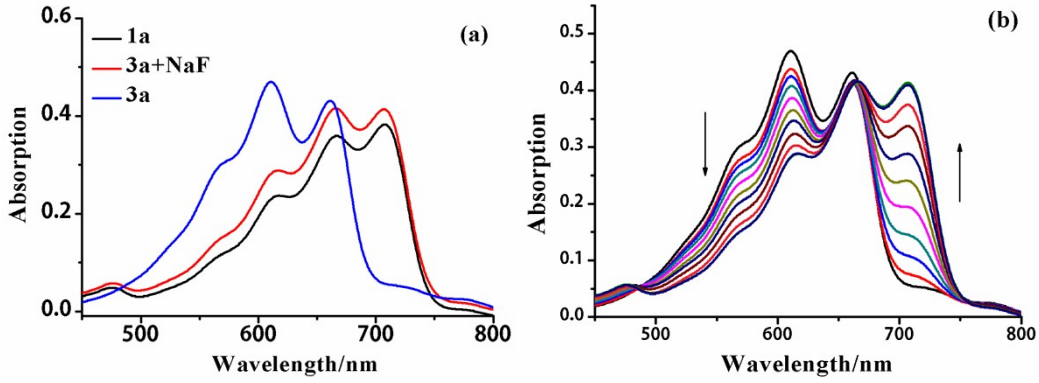


Fig. S8 (a) The UV-vis absorption of 1a (10 μM), 3a (10 μM) and 3a (10 μM)+NaF (3.0 equiv.). Measured in PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4). (b) UV titration of 3a (10 uM) upon addition of NaF in a PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4) solution. Each spectrum was recorded at 10 min after the addition of NaF (0.0 - 10.0 equiv.).

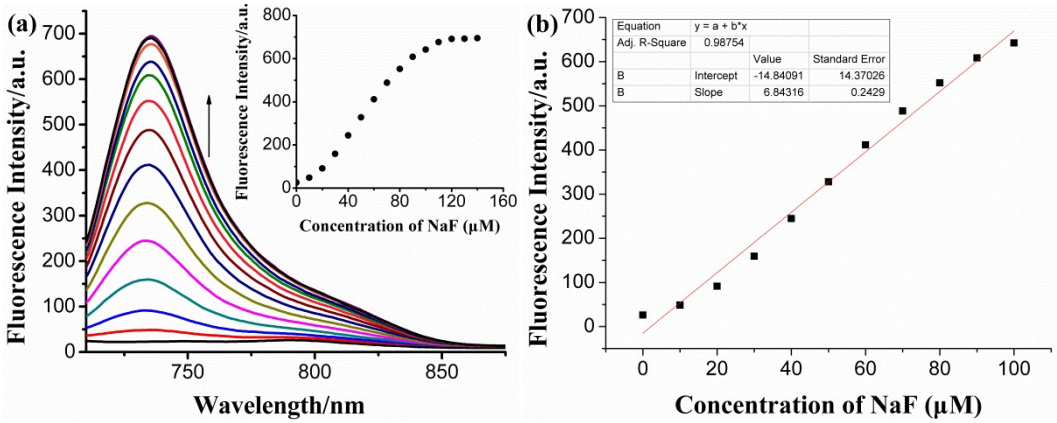


Fig. S9 (a) Fluorescence titration studies of 3a (10 μM) upon addition of NaF (0.0 - 13.0 equiv.) in a PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4) solution at room temperature. Inset: The relationship between the fluorescent intensity and NaF concentration. (b) The linear correlation of probe 3a between fluorescence intensity and concentration of F^- in the range of 0 to 100 μM.

5.4 Fluorescence spectra and UV absorption spectrum of 1b and 3b

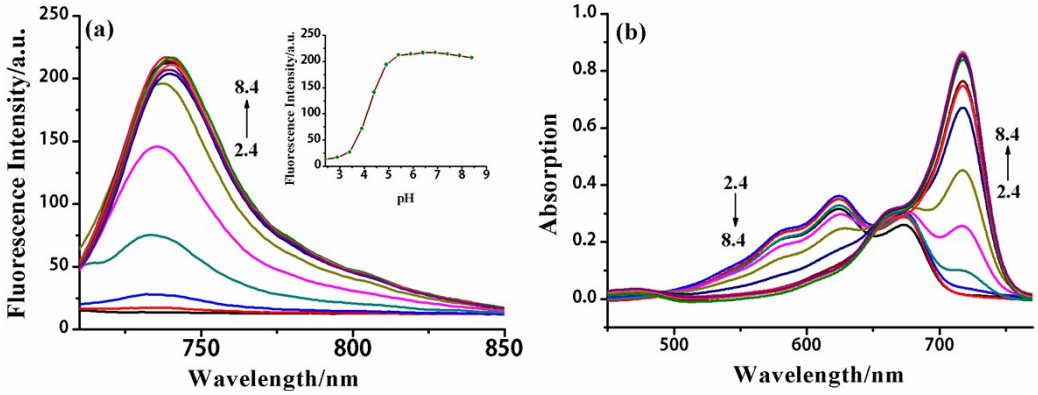


Fig. S10 (a) The fluorescence intensity changes depending the pH (2.4 - 8.4) of 1b (10 μM), λ_{ex} = 690 nm. Inset: the linear relationship of fluorescence intensity with pH (2.4 - 8.4) of 1b (10 μM). (b) The UV-Vis absorption of 1b (10 μM) at pH from 2.4 to 8.4. All data were measured in PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 2.4 - 8.4).

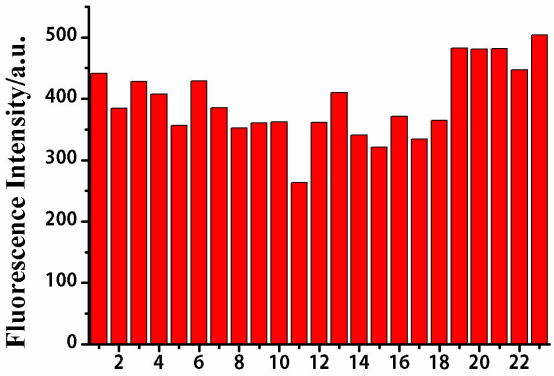


Fig. S11 Fluorescence response of 3b (10 μM) in the presence of NaF (3.0 equiv.) with other analytes (10.0 equiv.) in a PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4) solution. The analytes: (1) F^-; (2) $F^- + SCN^-$; (3) $F^- + HS^-$; (4) $F^- + S_2O_3^{2-}$; (5) $F^- + SO_4^{2-}$;(6) $F^- + Ac^-$; (7) $F^- + H_2PO_4^{2-}$; (8) $F^- + HCO_3^-$; (9) $F^- + NO_3^-$; (10) $F^- + I^-$; (11) F^-+ Br^-; (12) $F^- + Cl^-$; (13) $F^- + CO_3^{2-}$;(14) $F^- + Cu^{2+}$; (15) $F^- + Zn^{2+}$; (16) $F^- + Mn^{2+}$; (17) $F^- + Ni^{2+}$; (18) $F^- + Mg^{2+}$; (19) $F^- + Fe^{2+}$; (20) $F^- + Ca^{2+}$; (21) $F^- + Al^{3+}$; (22) $F^- + K^+$; (23) $F^- + Na^+$.

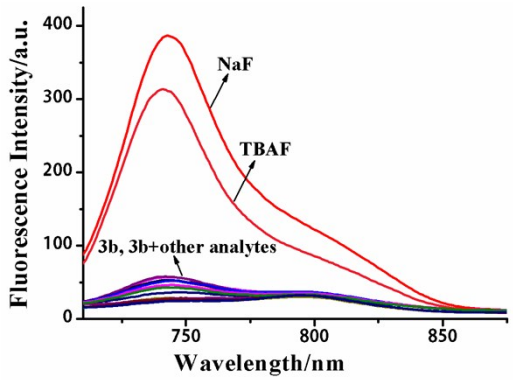


Fig. S12 Fluorescence response of 3b (10 μM) to various anions (3.0 equiv.) in a PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4) solution. Other analytes $H_2PO_4^{2-}$; Ac^-; SO_4^-; $S_2O_3^{2-}$; HS^-; SCN^-; Cl^-; Br^-; I^-; CO_3^{2-}; NO_3^-; HCO_3^-; Cu^{2+}; Zn^{2+}; Mn^{2+}; Ni^{2+}; Mg^{2+}; Fe^{2+}; Ca^{2+}; Al^{3+}; K^+; Na^+).

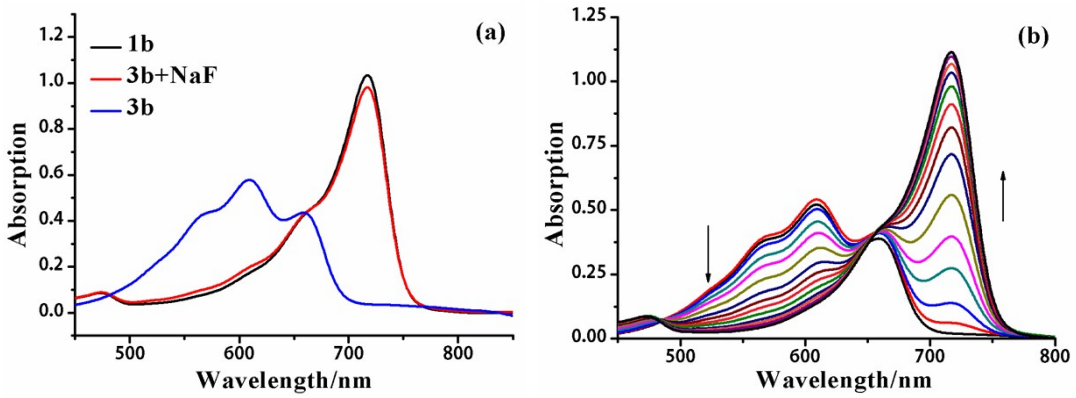


Fig. S13 (a) The UV-vis absorption of 1b (10 μM), 3b (10 μM) and 3b (10 μM)+NaF (3.0 equiv.). Measured in PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4). (b) UV titration of 3b (10 uM) upon addition of NaF in a PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4) solution. Each spectrum was recorded at 10 min after the addition of NaF (0.0 - 12.0 equiv.).

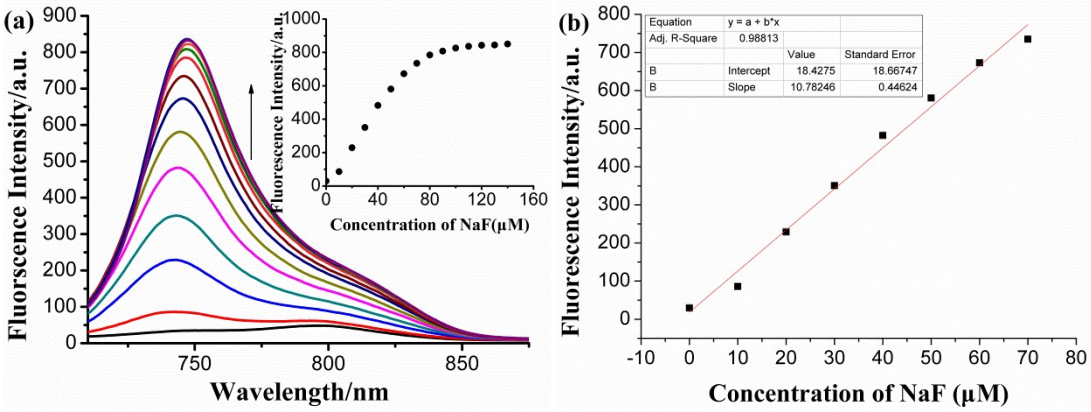


Fig. S14 (a) Fluorescence titration studies of 3b (10 μM) upon addition of NaF (0.0 - 12.0 equiv.) in a PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4) solution at room temperature. Inset: The linear relationship between the fluorescent intensity and NaF concentration. (b) The linear correlation of probe 3b between fluorescence intensity and concentration of F^- in the range of 0 to 70 μM.

5.5 Fluorescence spectra and UV absorption spectrum of 1c and 3c

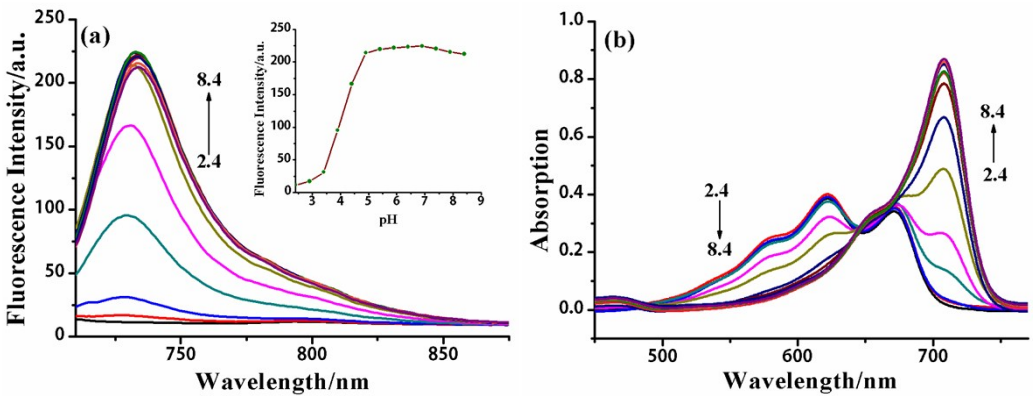


Fig. S15 (a) The fluorescence intensity changes depending the pH (2.4 - 8.4) of 1c (10 μM). λ_{ex} = 690 nm. Inset: the linear relationship of fluorescence intensity with pH (2.4 - 8.4) of 1c (10 μM) (b) The UV-vis absorption of 1c (10 μM) at pH from 2.4 to 8.4. All data were measured in PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 2.4 - 8.4).

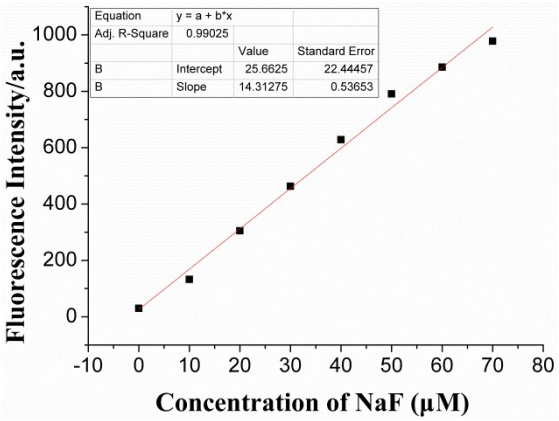


Fig S16 The linear correlation of probe 3c between fluorescence intensity and concentration of F^- in the range of 0 to 70 μM.

6. HPLC experiments

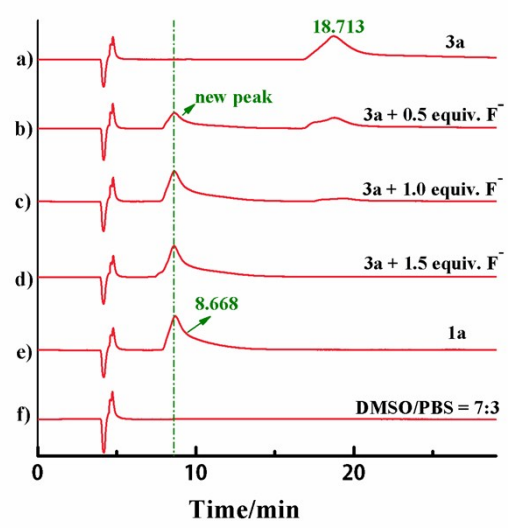


Fig. S17 HPLC chromatograms analysis, a) **3a** (0.2 mM). b) **3a** (0.2 mM)+NaF (0.5 equiv.). c) **3a** (0.2 mM)+NaF (1.0 equiv.). d) **3a** (0.2 mM)+NaF (1.5 equiv.). e) **1a** (0.2 mM). f) DMSO/PBS= 7:

3. Conditions: incubation for 10 min in a PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4) solution at room temperature.

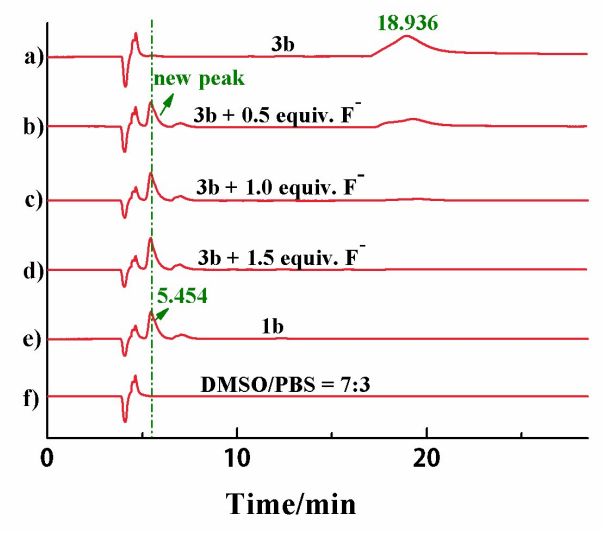


Fig. S18 HPLC chromatograms analysis, a) **3b** (0.2 mM). b) **3b** (0.2 mM)+NaF (0.5 equiv.). c) **3b** (0.2 mM)+NaF (1.0 equiv.). d) **3b** (0.2 mM)+NaF (1.5 equiv.). e) **1b** (0.2 mM). f) DMSO/PBS= 7: 3. Conditions: incubation for 10 min in a PBS/DMSO = 3 : 7 (v/v, 0.01 M, pH = 7.4) solution at room temperature.

7. MTT assay

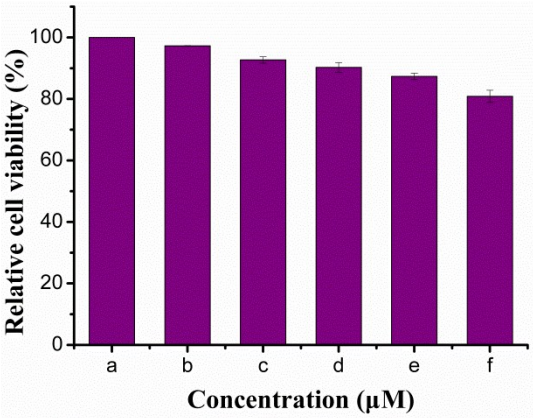


Fig. S19 Cytotoxicity of **3a** on HepG2 cells determined by MTT. a) 0 μM. b) 5 μM. c) 10 μM. d) 20 μM. e) 40 μM. f) 80 μM.

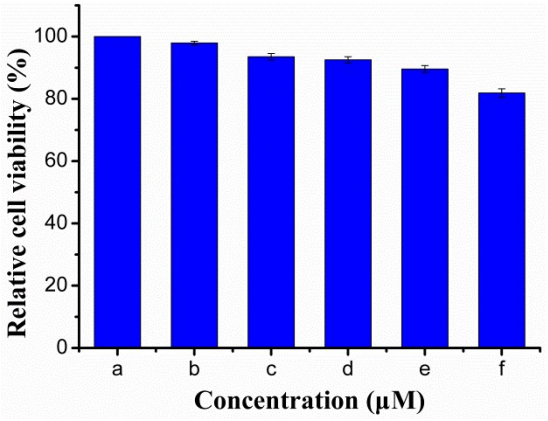


Fig. S20 Cytotoxicity of **3b** on HepG2 cells determined by MTT. a) 0 μM. b) 5 μM. c) 10 μM. d) 20 μM. e) 40 μM. f) 80 μM.

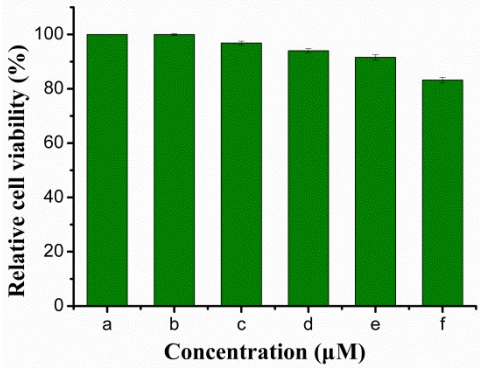


Fig. S21 Cytotoxicity of **3c** on HepG2 cells determined by MTT. a) 0 μM. b) 5 μM. c) 10 μM. d) 20 μM. e) 40 μM. f) 80 μM.

8. Cell and animal imaging

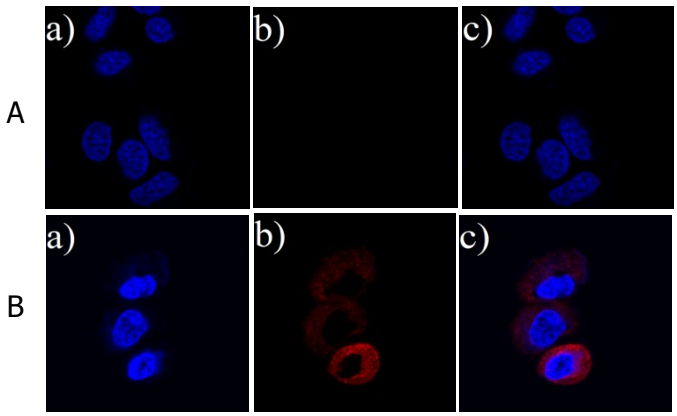
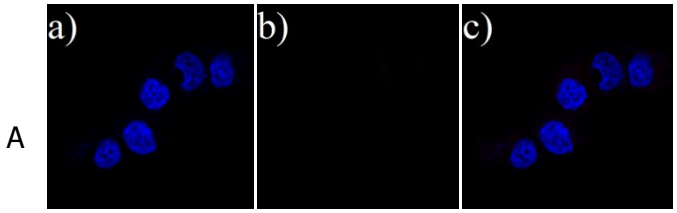


Fig. S22 Confocal microscope images of probe 3a in *HepG2* cells. A: control. B: Image of the cells incubated with **3a** (20 μM) for 1 h, and further incubated with NaF (100 μM) for 10 min. (a) emissions from the blue channel. (b) emissions from the red channel, and (c) Merge.



B

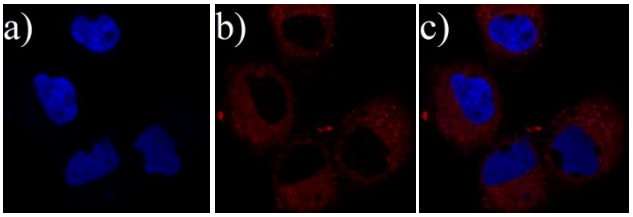


Fig. S23 Confocal microscope images of probe 3b in *HepG2* cells. A: control. B: Image of the cells incubated with **3b** (20 μM) for 1 h, and further incubated with NaF (100 μM) for 10 min. (a) emissions from the blue channel, (b) emissions from the red channel, and (c) Merge.

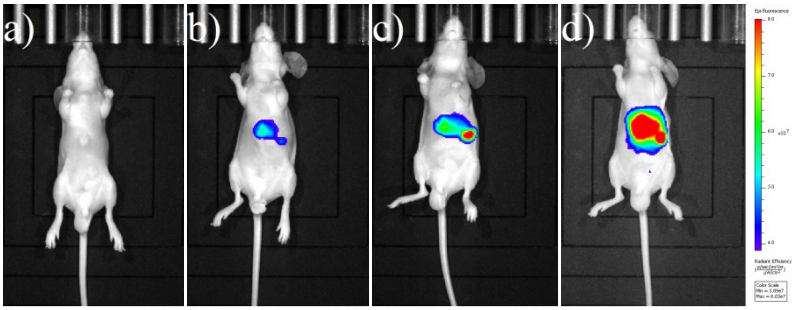


Fig. S24 Fluorescence images of **3c** in mouse. a) Mice injected with probe **3c** (20 μM). b) Mice injected with **3c** (20 μM) and 28 mgF/kg sodium fluoride recorded at 15 min. c) Mice injected **3c** (20 μM) with 28 mgF/kg sodium fluoride recorded at 2 h. d) Mice injected **3c** (20 μM) with 28 mgF/kg sodium fluoride recorded at 4 h.

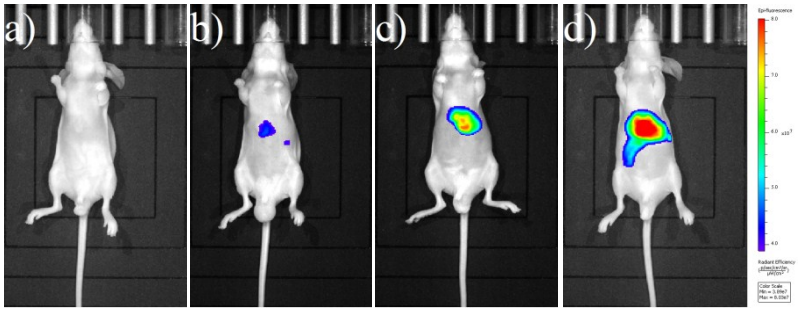


Fig. S25 Fluorescence images of **3c** in mouse. a) Mice injected with probe **3c** (20 μM). b) Mice injected with **3c** (20 μM) and 12 mgF/kg sodium fluoride recorded at 15 min. c) Mice injected **3c** (20 μM) with 12 mgF/kg sodium fluoride recorded at 2 h. d) Mice injected **3c** (20 μM) with 12 mgF/kg sodium fluoride recorded at 4 h.

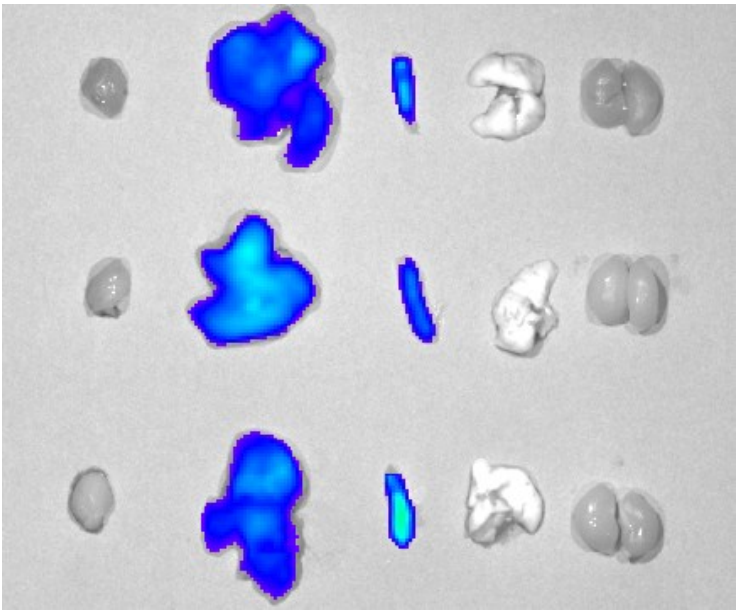


Fig. S26. Organic imaging, from left to right: heart, liver, spleen, lungs and kidney.

9. HRMS data

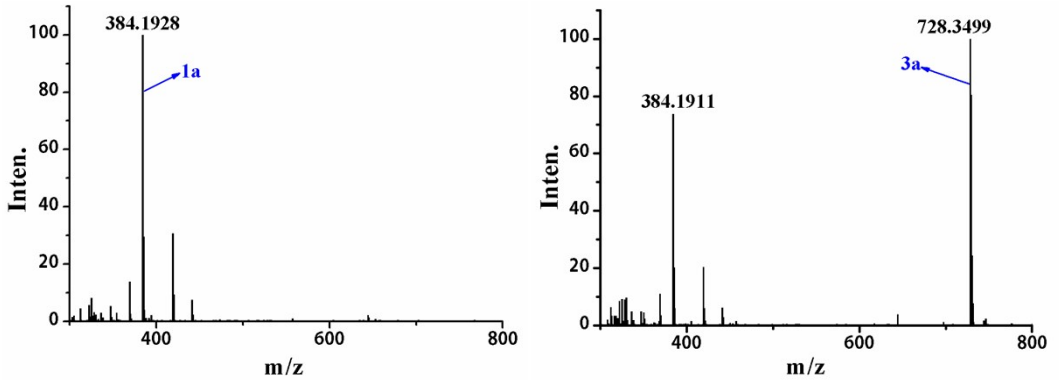


Fig. S27 ESI mass spectrum of 1a and 3a

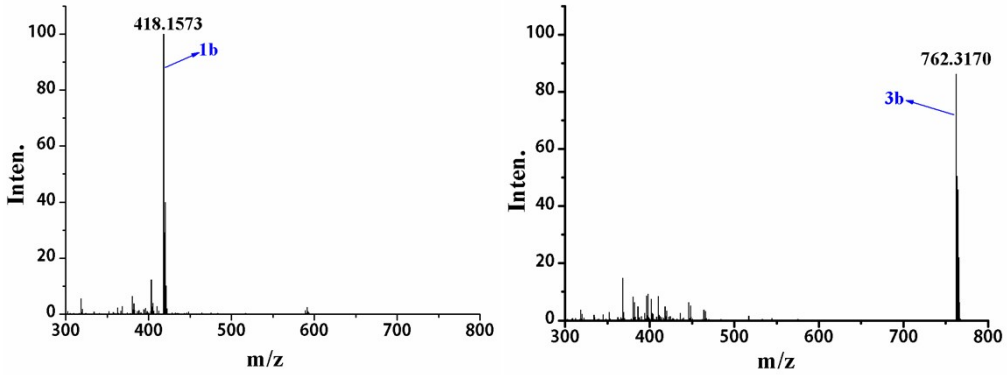


Fig. S28 ESI mass spectrum of 1b and 3b

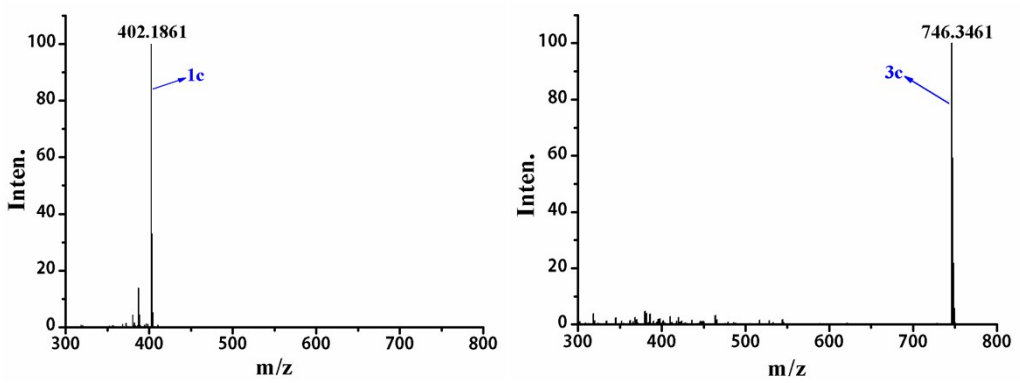


Fig. S29 ESI mass spectrum of 1c and 3c

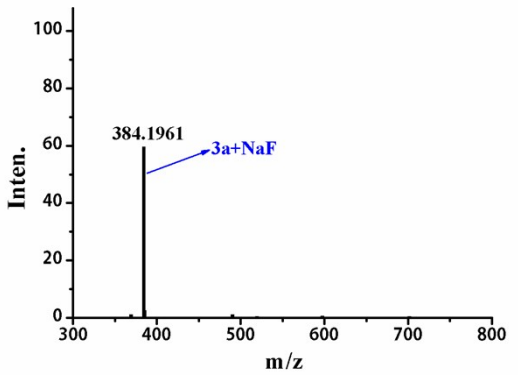


Fig. S30 ESI mass spectrum of 3a+NaF

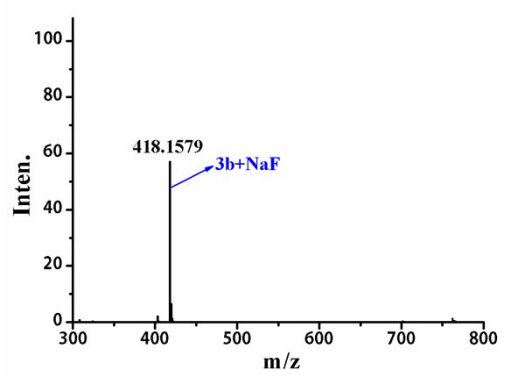


Fig. S31 ESI mass spectrum of 3b+NaF

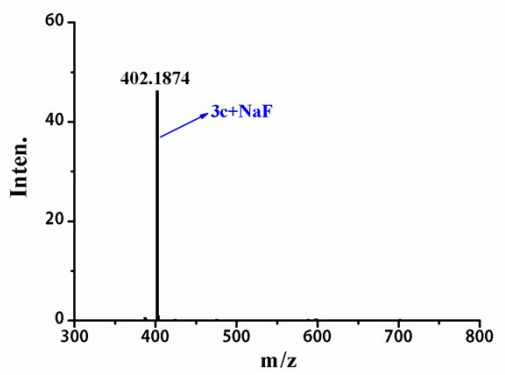
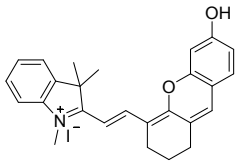
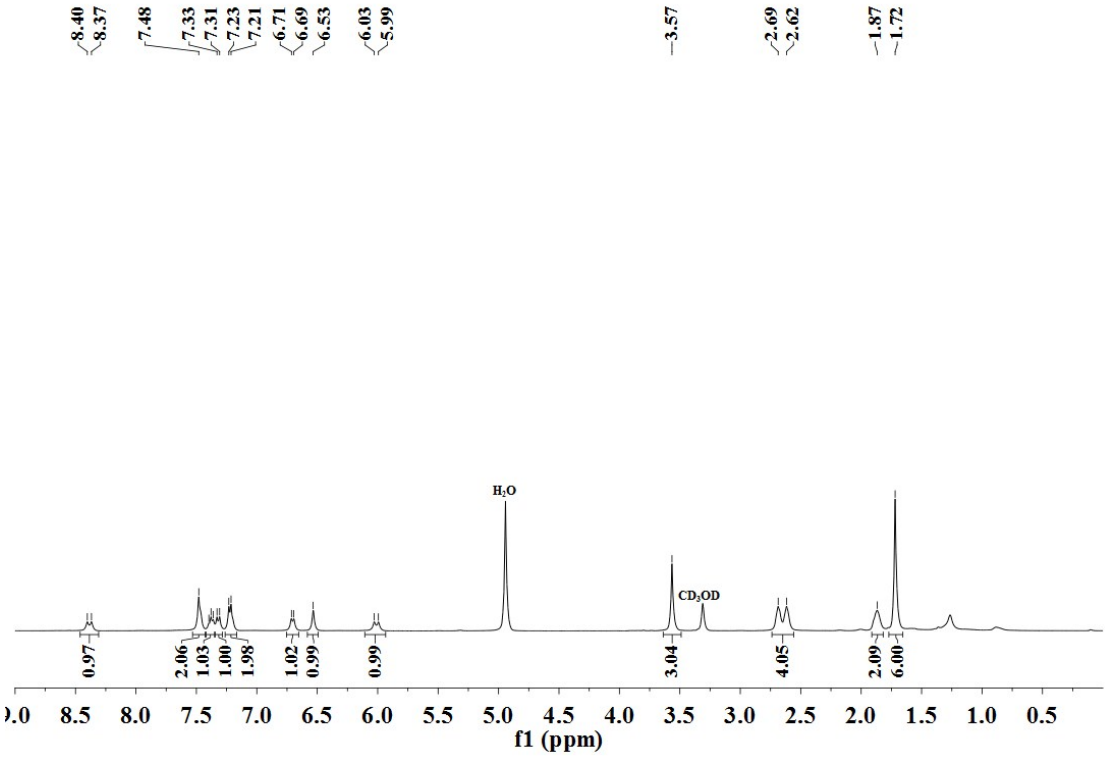


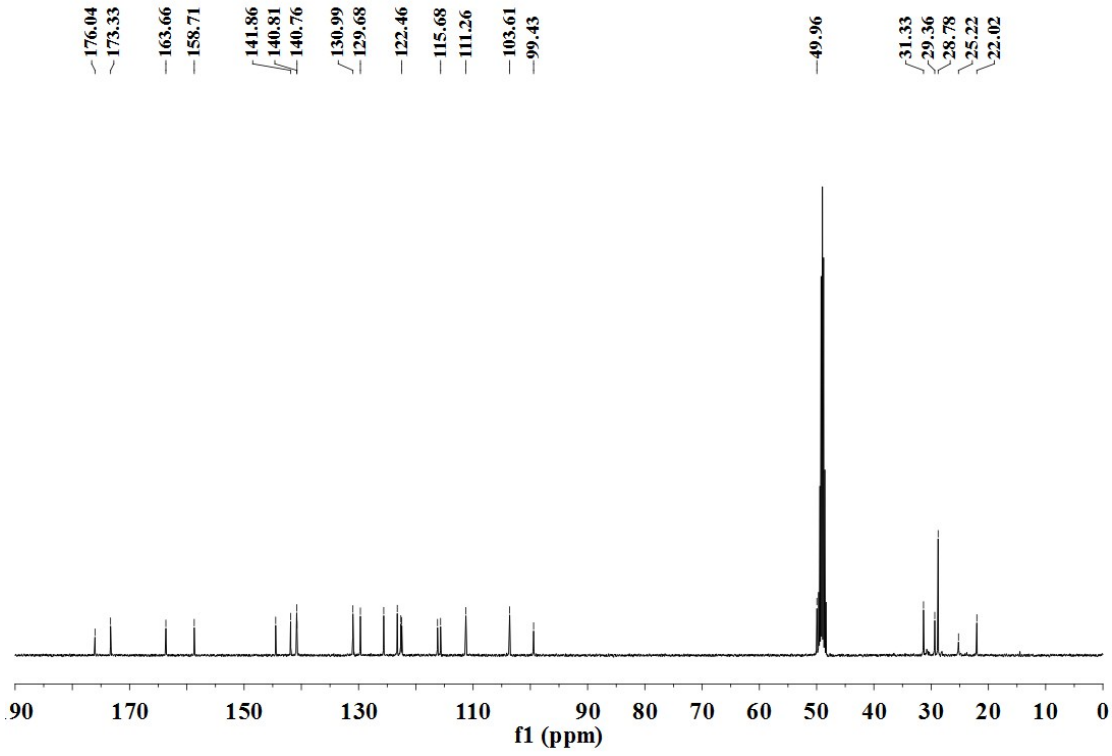
Fig. S32 ESI mass spectrum of 3c+NaF

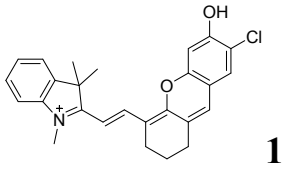
10. ^{1}H NMR, ^{13}C NMR data



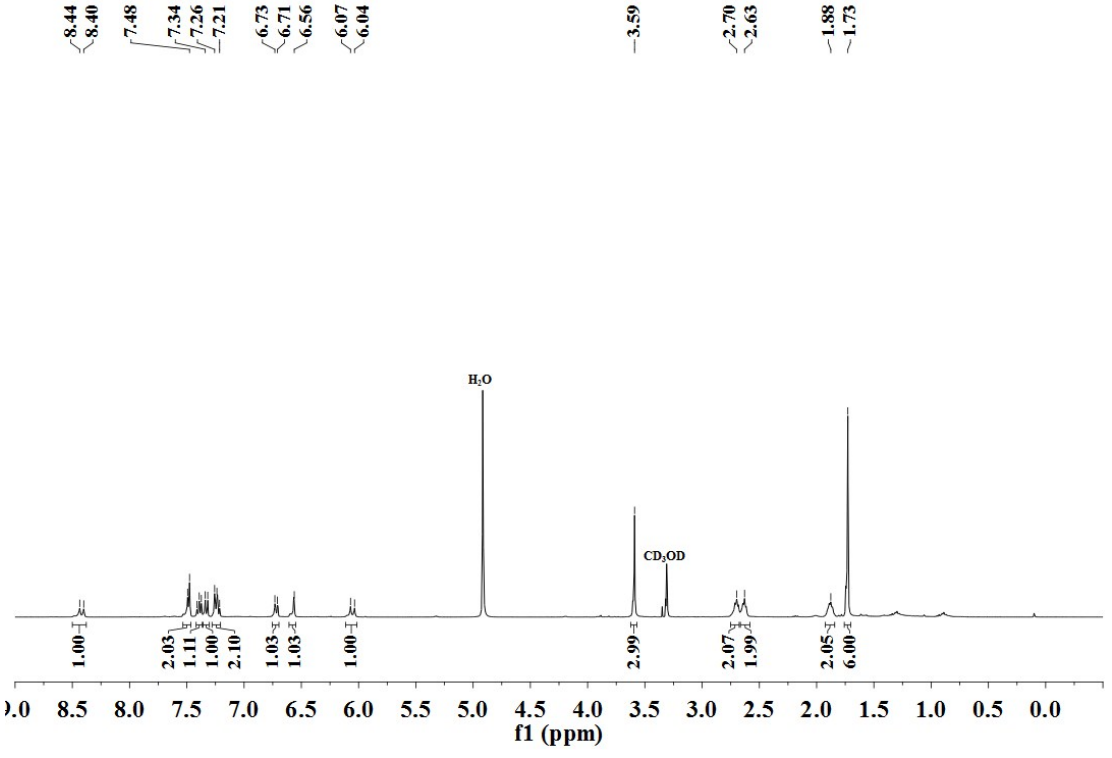
1a

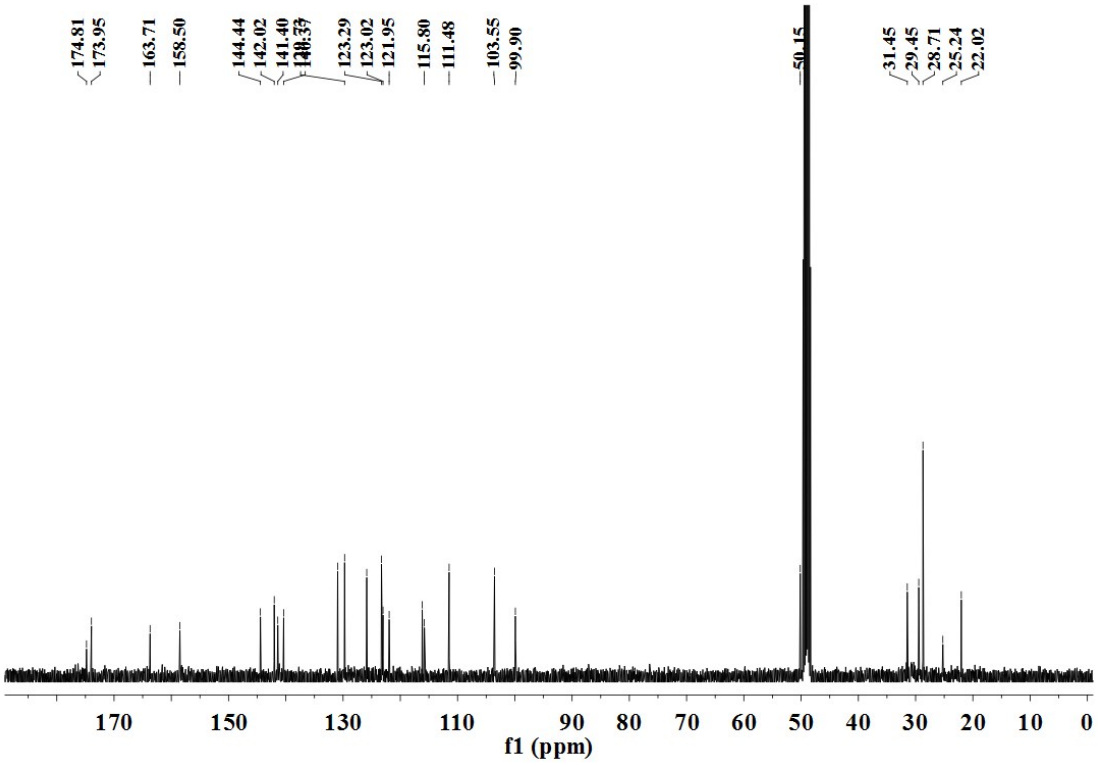


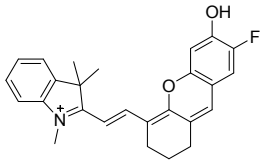

176.04
173.33
163.66
158.71
141.86
140.81
140.76
130.99
129.68
122.46
115.68
111.26
103.61
99.43
49.96
31.33
29.36
28.78
25.22
22.02
f1 (ppm)


OH
Cl
O
N+

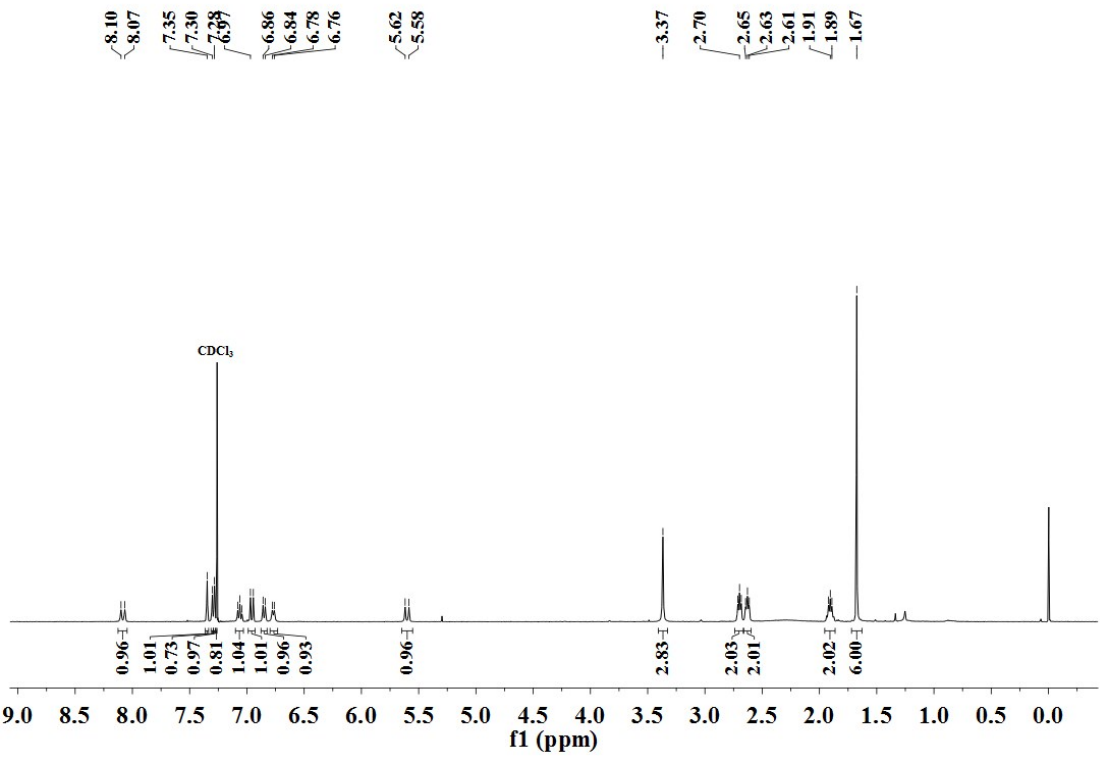
1b

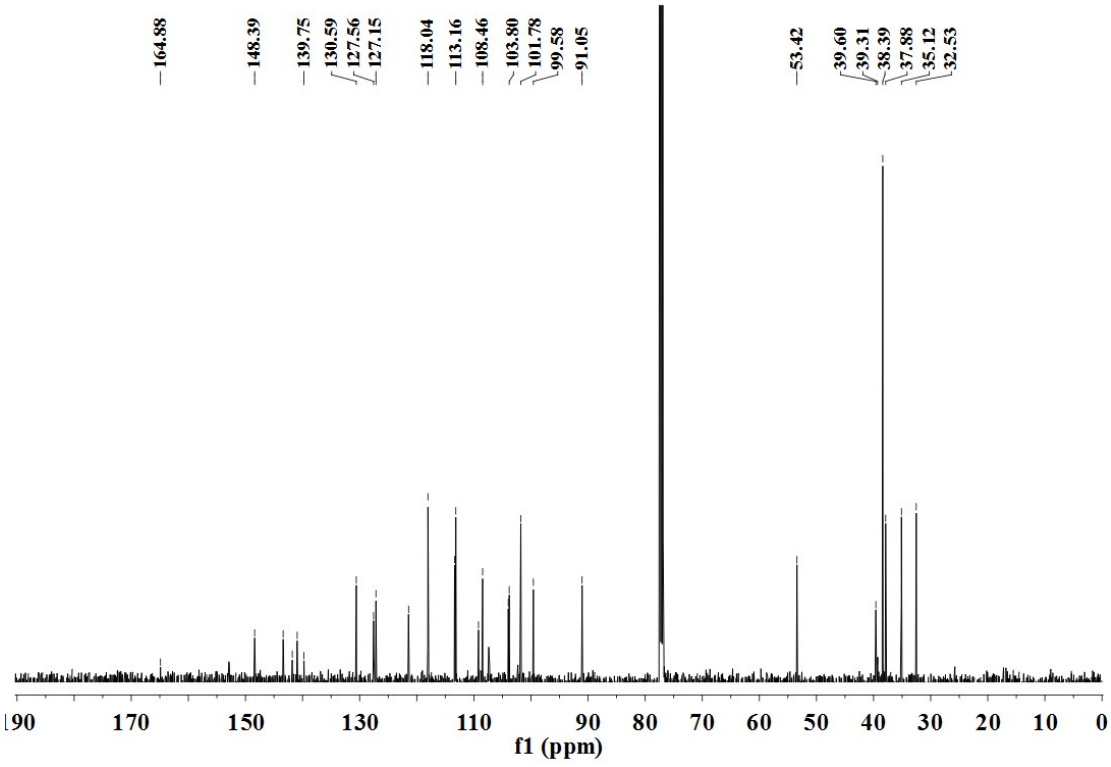

8.44
8.40
7.48
7.34
7.26
7.21
6.73
6.71
6.56
6.07
6.04
3.59
2.70
2.63
1.88
1.73
H2O
CD3OD
1.00
2.03
1.11
1.00
2.10
1.03
1.03
1.00
2.99
2.07
1.99
2.05
6.00
f1 (ppm)

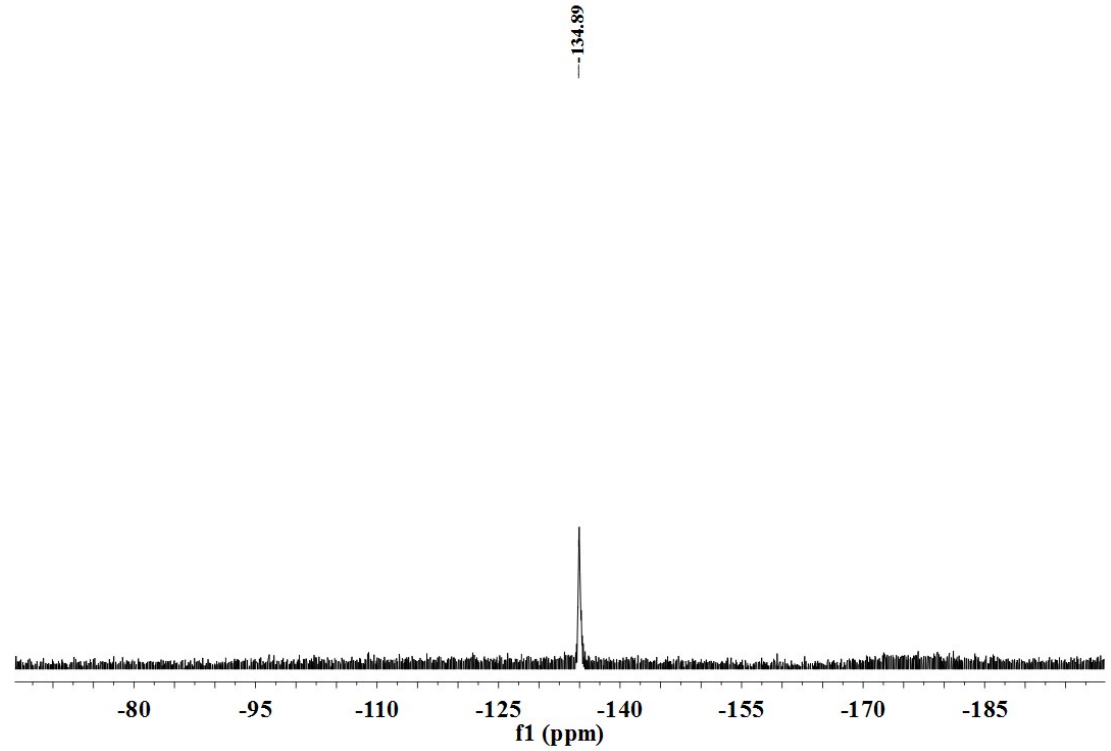

174.81
173.95
163.71
158.50
144.44
142.02
141.40
123.29
123.02
121.95
115.80
111.48
103.55
99.90
50.15
31.45
29.45
28.71
25.24
22.02
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

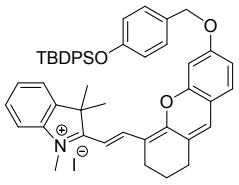

OH
F
O
N+

1c

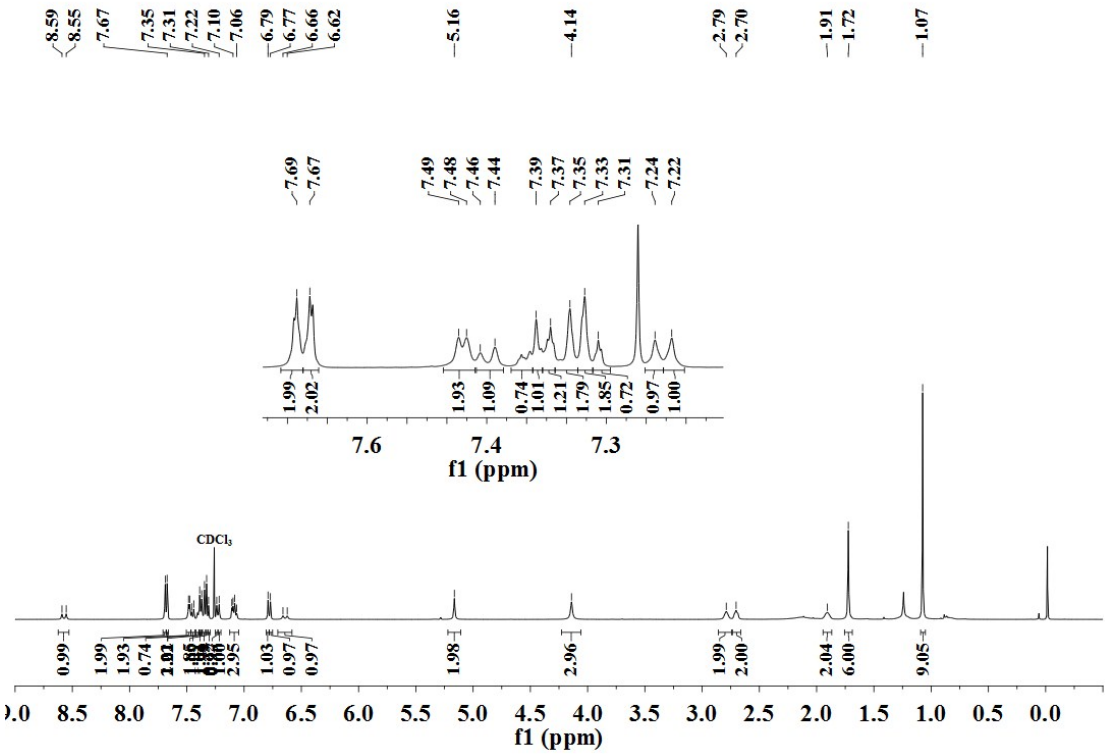

8.10
8.07
7.35
7.30
7.28
6.97
6.86
6.84
6.78
6.76
5.62
5.58
3.37
2.70
2.65
2.63
2.61
1.91
1.89
1.67
CDCl3
0.96
1.01
0.73
0.97
0.81
1.04
1.01
0.96
0.93
0.96
2.83
2.03
2.01
2.02
6.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

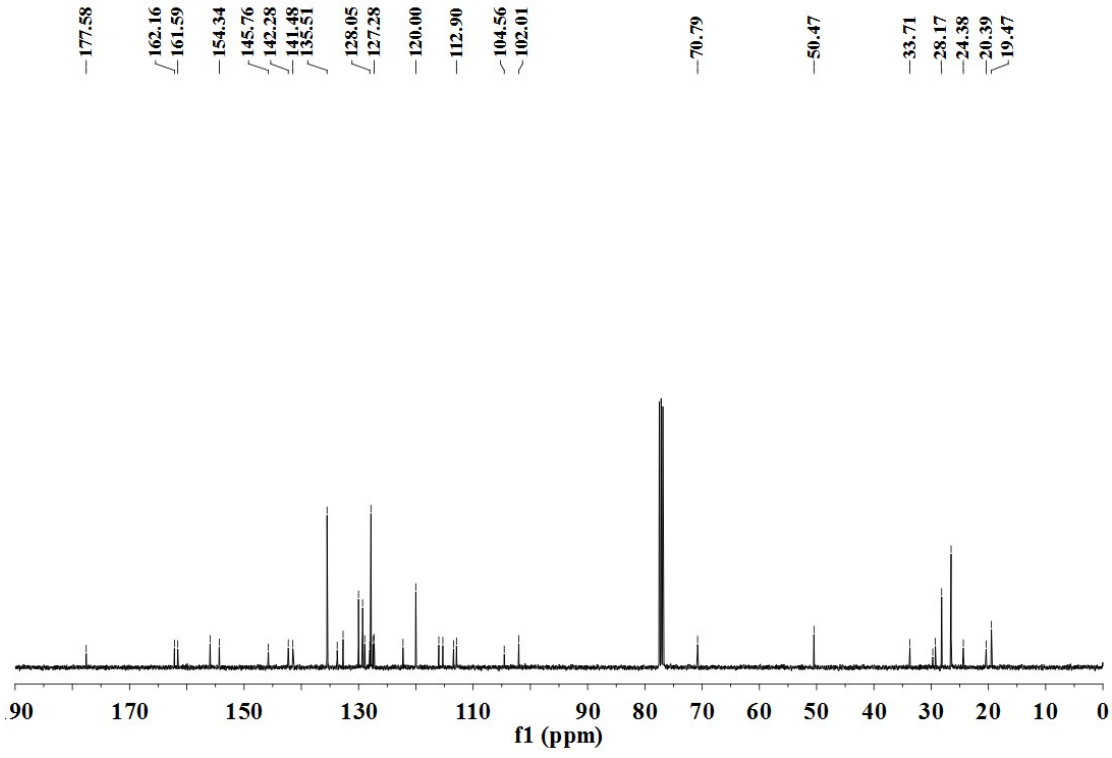

164.88
148.39
139.75
130.59
127.56
127.15
118.04
113.16
108.46
103.80
101.78
99.58
91.05
53.42
39.60
39.31
38.39
37.88
35.12
32.53
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

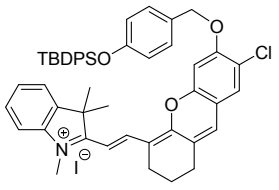

134.89
-80
-95
-110
-125
-140
-155
-170
-185
f1 (ppm)


TBDPSO
O
O
N

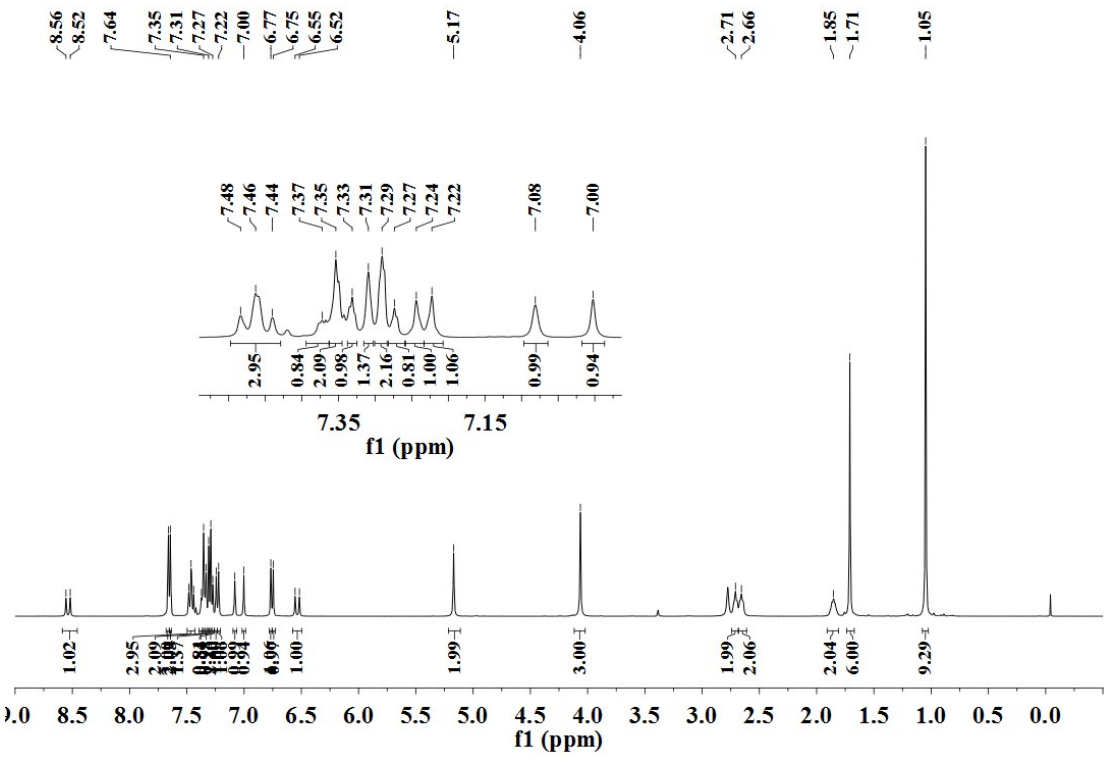
3a


8.59
8.55
7.67
7.35
7.31
7.22
7.10
7.06
6.79
6.77
6.66
6.62
5.16
4.14
2.79
2.70
1.91
1.72
1.07
7.69
7.67
7.49
7.48
7.46
7.44
7.39
7.37
7.35
7.33
7.31
7.24
7.22
1.99
2.02
1.93
1.09
0.74
1.01
1.21
1.79
1.85
0.72
0.97
1.00
7.6
7.4
7.3
f1 (ppm)
CDCl3
0.99
1.99
1.93
0.74
1.03
0.97
0.97
1.98
2.96
1.99
2.00
2.04
6.00
9.05
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


177.58
162.16
161.59
154.34
145.76
142.28
141.48
135.51
128.05
127.28
120.00
112.90
104.56
102.01
70.79
50.47
33.71
28.17
24.38
20.39
19.47
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


TBDPSO
O
Cl
O
N
I

3b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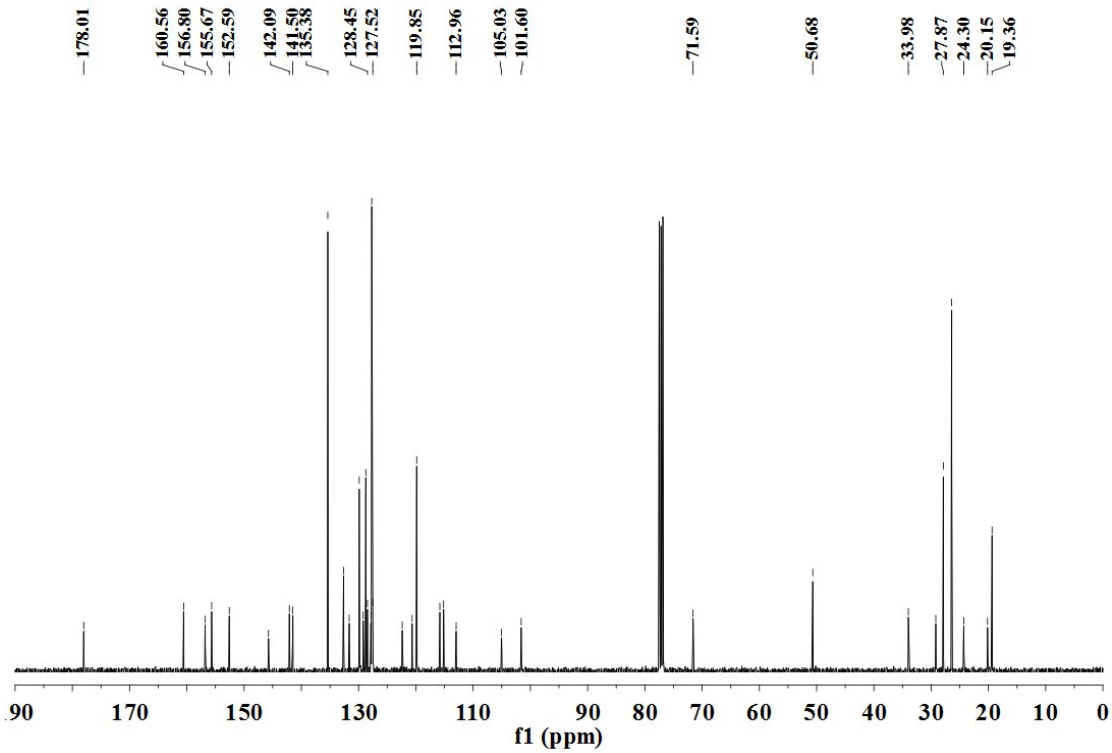
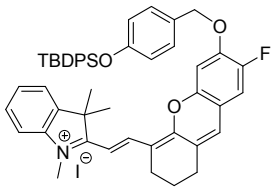
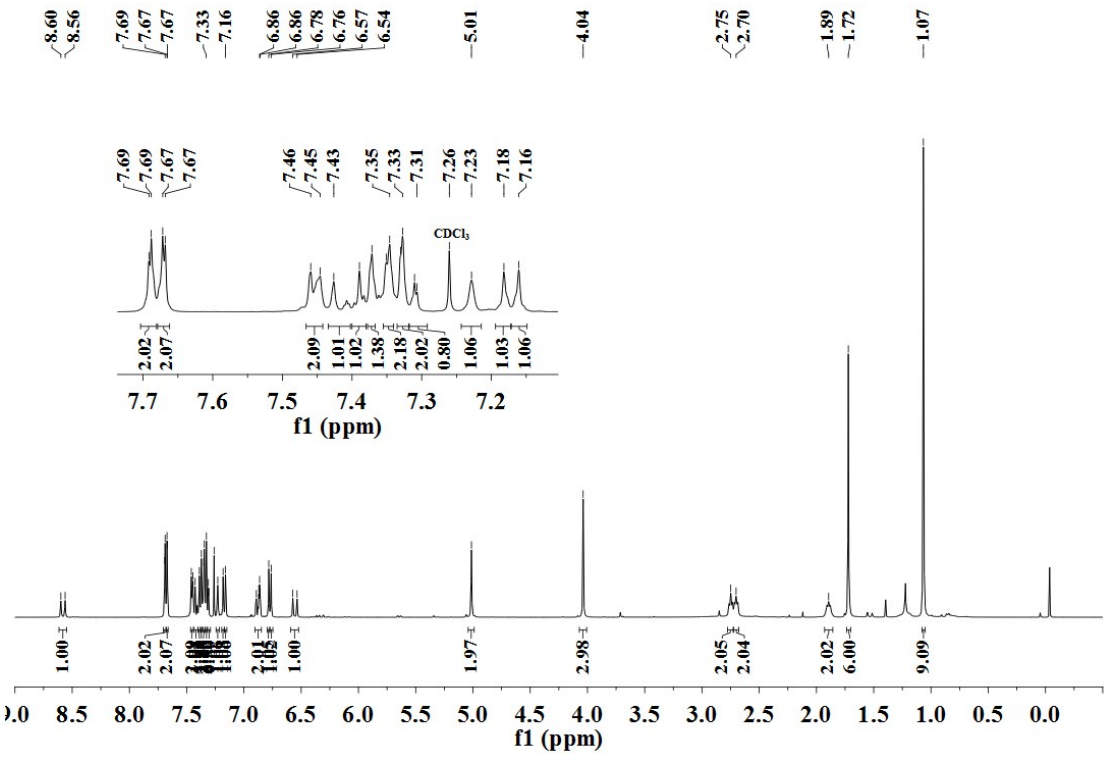
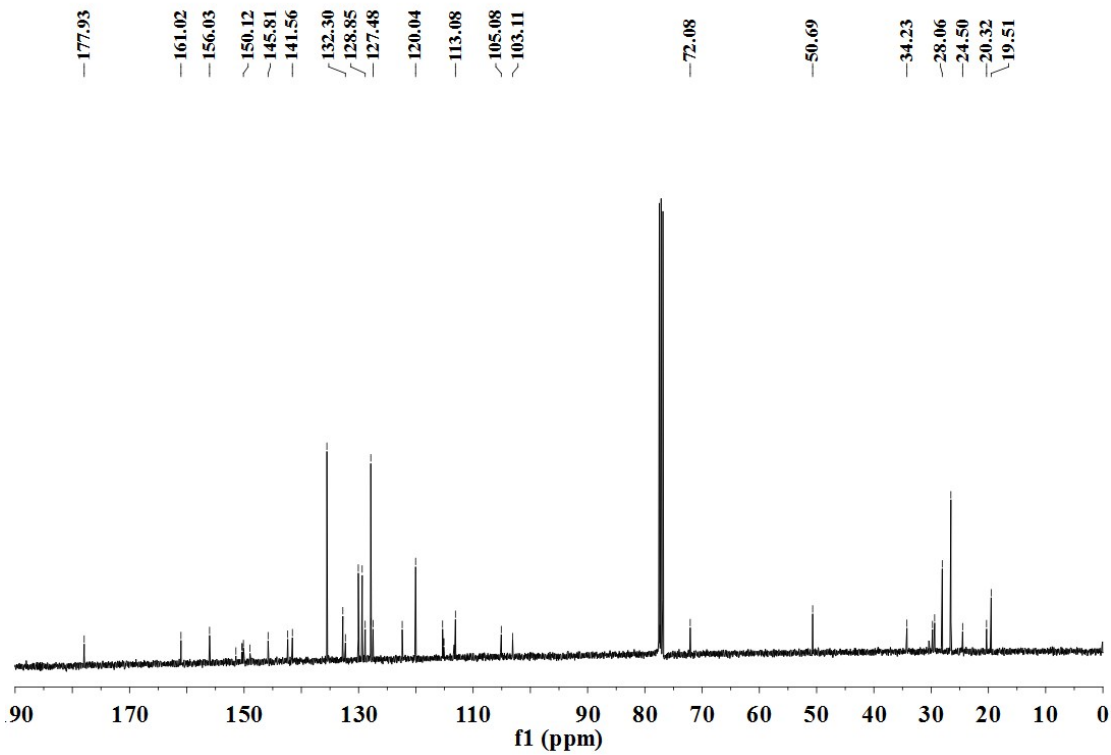
7.64
7.35
7.31
7.27
7.22
7.00
6.77
6.75
6.55
6.52
5.17
4.06
2.71
2.66
1.85
1.71
1.05
7.48
7.46
7.44
7.37
7.35
7.33
7.31
7.29
7.27
7.24
7.22
7.08
7.00
2.95
0.84
2.09
0.98
1.37
2.16
0.81
1.00
1.06
0.99
0.94
7.35
7.15
f1 (ppm)
1.02
2.95
1.00
1.99
3.00
1.99
2.06
2.04
6.00
9.29
f1 (ppm)

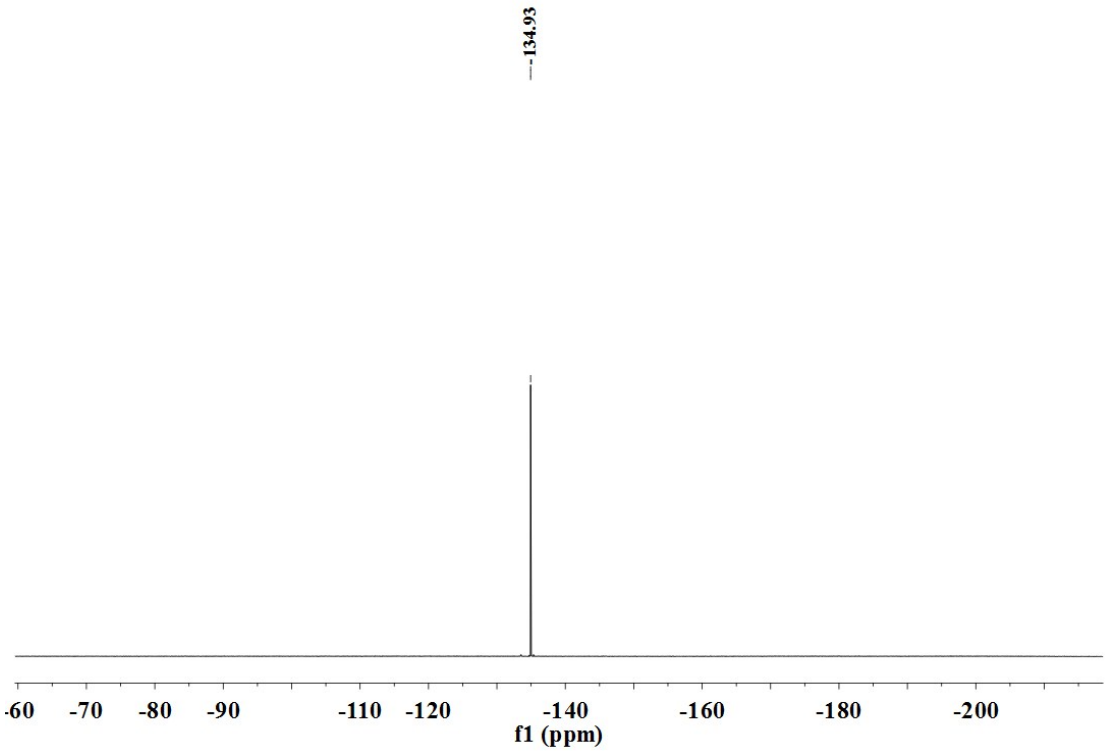

178.01
160.56
156.80
155.67
152.59
142.09
141.50
135.38
128.45
127.52
119.85
112.96
105.03
101.60
71.59
50.68
33.98
27.87
24.30
20.15
19.36
f1 (ppm)


TBDPSO
F
O
O
N
I

3c


8.60
8.56
7.69
7.67
7.67
7.33
7.16
6.86
6.86
6.78
6.76
6.57
6.54
5.01
4.04
2.75
2.70
1.89
1.72
1.07
CDCl3
f1 (ppm)


177.93
161.02
156.03
150.12
145.81
141.56
132.30
128.85
127.48
120.04
113.08
105.08
103.11
72.08
50.69
34.23
28.06
24.50
20.32
19.51
f1 (ppm)


-134.93
-60
-70
-80
-90
-110
-120
-140
-160
-180
-200
f1 (ppm)