Electronic Supplementary Information (ESI) for

Red fluorescent probes based on a BODIPY analogue for selective and sensitive detection of selenols in solutions and in living systems

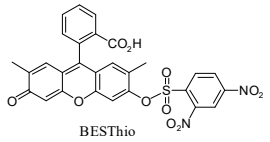
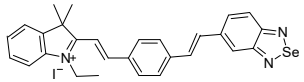
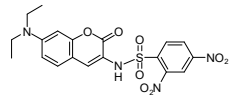
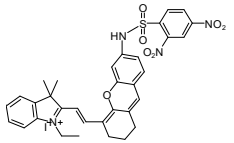
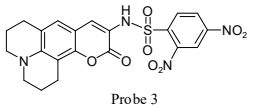
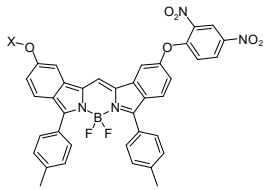
Chun-Guang Dai,[a] *Ji-long Wang,*[b] *and Qin-Hua Song**[a]

[a] Department of Chemistry, University of Science and Technology of China, Hefei 230026, P. R. China. [b] School of Life Sciences, University of Science and Technology of China, Hefei 230027, P. R. China.

E-mail: qhsong@ustc.edu.cn

Contents

I. Table S1. Comparison table of key properties of fluorescent probes for Sec in literatures and in this work

References	Structures of probes	Wavelength maxima $\lambda_{abs}/\lambda_{em}$	LOD	Selectivity I_{Sec}/I_{DTT} for incubation time	Photostability Measured time	Animal imaging
ACIE **2006**, 45, 1810.	 BESThio	450/520 nm	-	>200 for 10 min at pH 5.8	no test	No
CC **2015**, 51, 3102.	 HB	460/580 nm	7.0 nM	2.5-fold for F/F_0 [a] 30 min	Yes 500 s	Mice
JACS **2015**, 137, 757.	 Sel-green	370/502 nm	62 nM	20.2 for 5 min	no test	No
CEJ **2015**, 21, 11696.	 HD-Sec	650/712 nm	-	65-fold for F/F_0	no test	Mice
AC **2016**, 88, 6084.	 Probe 3	380/535 nm	18 nM	120-fold for F/F_0	no test	Zebrafish
This work	 Sel-p1: X = 2,4-dinitrobenzyl Sel-p2: X = CH_3	650/663, 655 nm	16 nM 9 nM	20.9 (Sel-p2) 170-, 40-fold for F/F_0 15 min	Yes 60 min	Mice

[a] F/F_0 expresses the fluorescence increment before and after addition of Sec.

The comparison table (Table S1) displays five reported fluorescent probes for Sec: one sulfonate probe, three sulfonamide probes and one benzoselenadiazole probe. It is well known that both sulfonates and sulfonamides are photolabile. So, the photostability of the four probes hasn't been tested. Among five reported fluorescent probes only one case is a near-IR fluorescent probe (HD-Sec). The selectivity (I_{Sec}/I_{DTT}) of fluorescent probes depends the incubation time, i.e. the longer incubation time, the lower selectivity. The value of F/F_0 expresses the fluorescence increment before and after addition of Sec, and the longer incubation time, the larger value of F/F_0 before finishing the sensing reaction.

II. Photophysical properties of probes and compounds 4 and 5

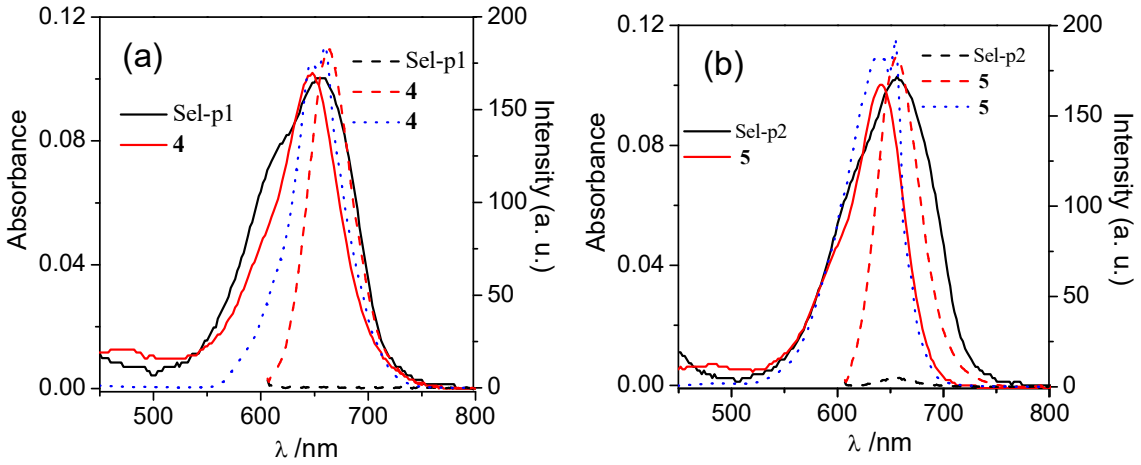


Figure S1. UV/vis absorption spectra (solid) and fluorescence spectra (dash) of Sel-p1, Sel-p2 (black) and compounds **4** and **5** (red), and excitation spectra (dot) of compounds **4** and **5** (blue), in PBS (pH 7.4) buffered water-DMSO (v/v, 1:1).

III. Time-dependent UV/vis absorption spectra of the reaction of Sel-p1/Sel-p2 with Sec

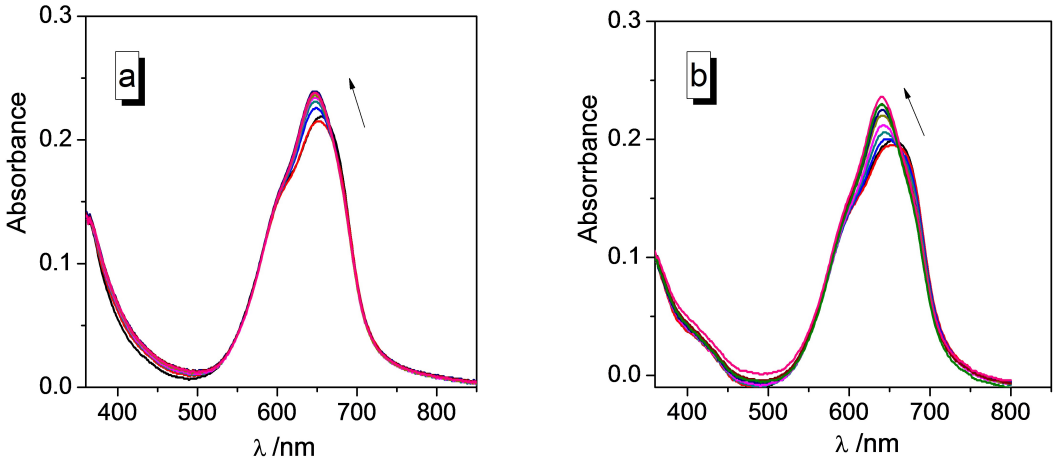


Figure S2. Time-dependent UV/vis absorption spectra of 5 μM Sel-p1 (a) and Sel-p2 (b) by the treatment of equal molar $(Sec)_2$ with DTT (25 μM for Sel-p1 and 12.5 μM for Sel-p2) in the PBS (pH 7.4)–DMSO solution mixture (v/v, 1:1).

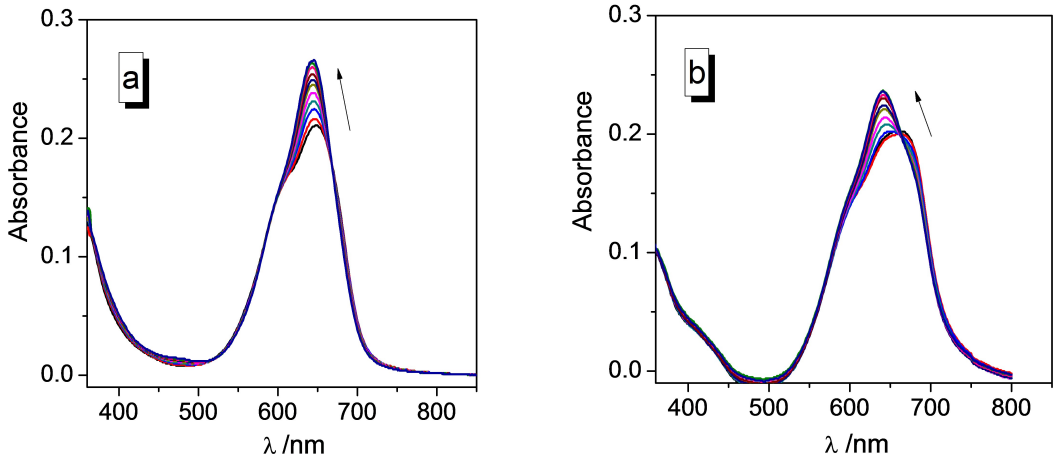


Figure S3. UV/vis absorption spectra of 5 μM Sel-p1 (a) and Sel-p2 (b) in the presence of different concentrations of equal molar $(Sec)_2$ with DTT (0-25 μM for Sel-p1 and 0-12.5 μM for Sel-p2) recorded after 15 min.

IV. HRMS confirmation of the sensing product of Sel-p2 with Sec

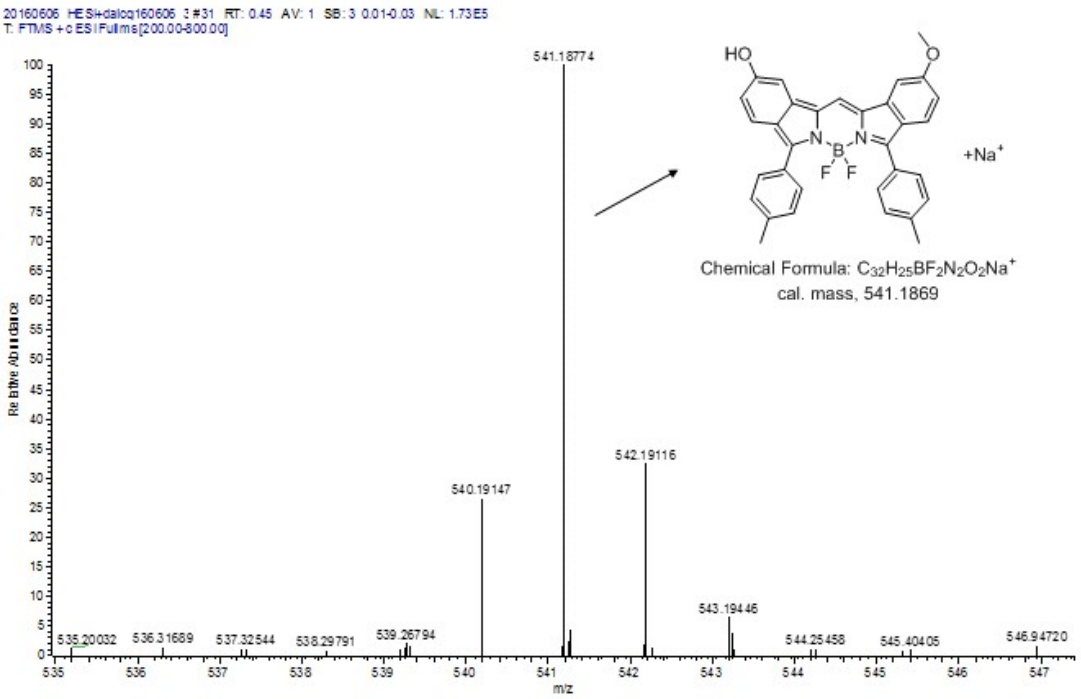


Figure S4. Mass spectrum of the reaction mixture of Sel-p2 incubated with equal molar $(Sec)_2$ and DTT in PBS (pH 7.4) buffered water–DMSO (v/v, 1:1)

V. Measurements of pKa values of compounds 4 and 5

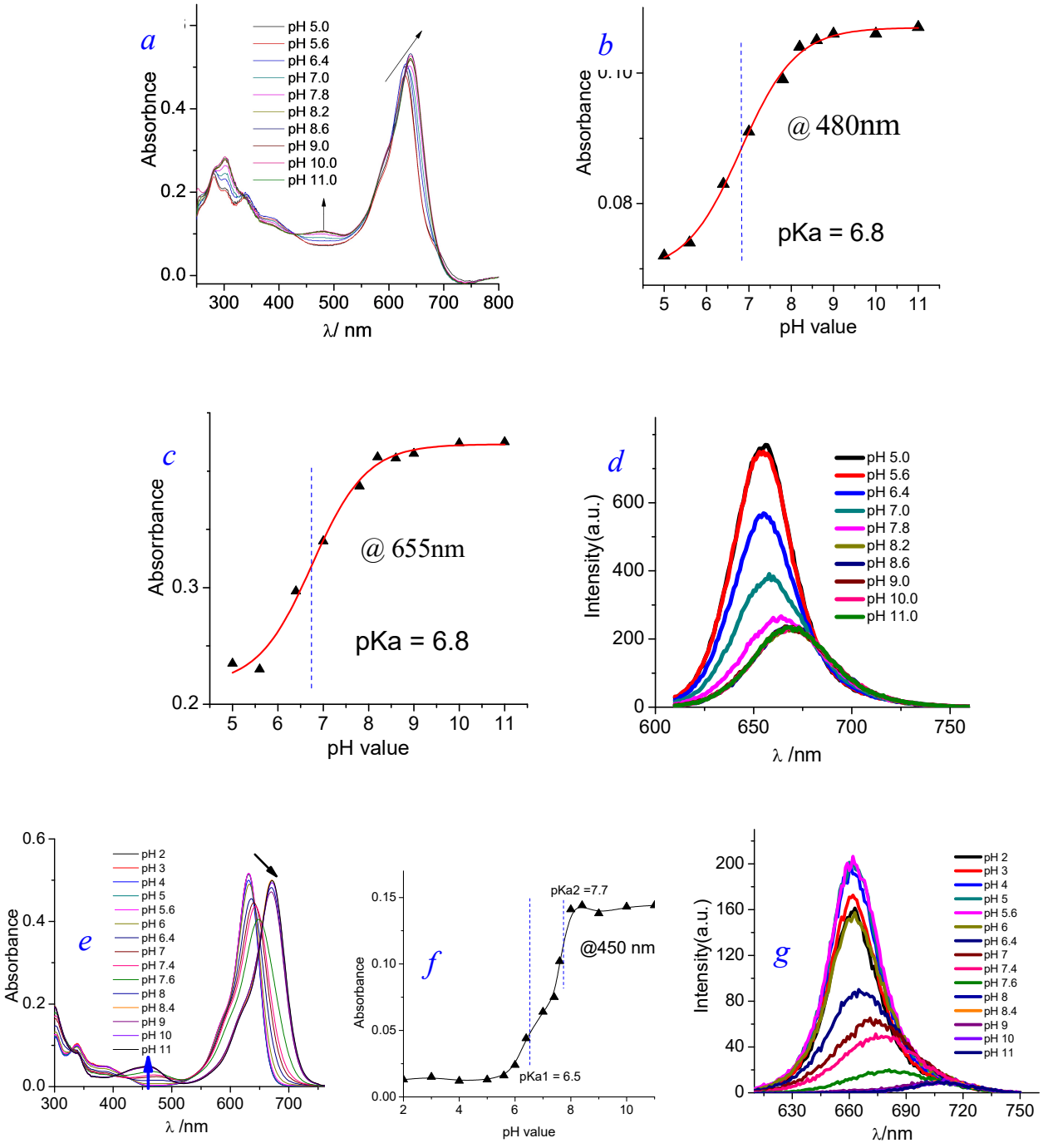


Figure S5. UV/Vis absorption spectra of various solutions of compounds **5** (a) and **4** (e) in the pH range; Plots of absorbance at 480 nm (b) and 655 nm (c) for **5**, and 465 nm for **4** (f) *vs* pH values; Fluorescence spectra of various solutions of compounds **5** (d) and **4** (g) in the pH range.

VI. pH effects on the sensing reaction of Sel-p1/Sel-p2 with Sec

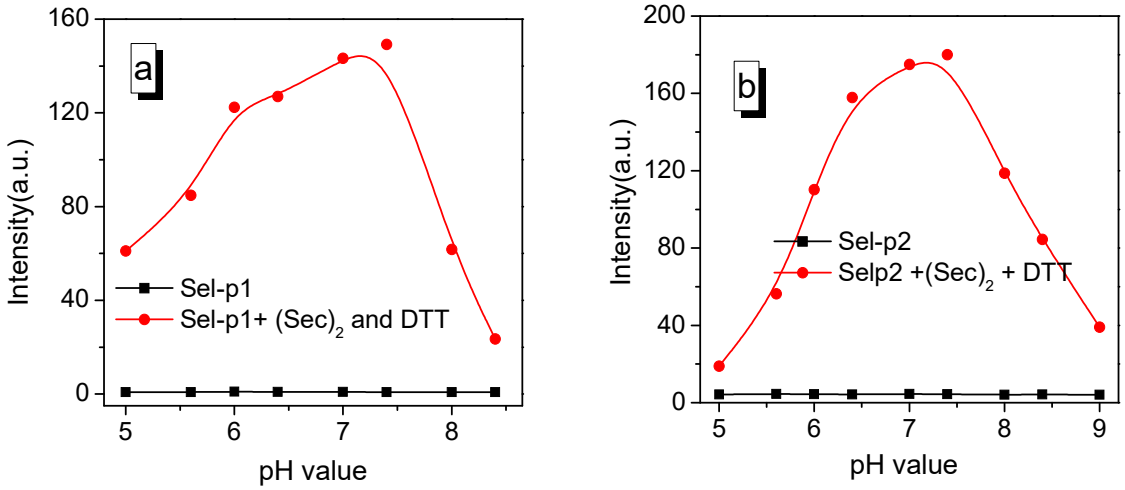


Figure S6. Effects of pH on fluorescence intensity of the probe (5 μM) and the reaction solutions of the probe (5 μM) with equal molar $(Sec)_2$ with DTT (25 μM for Sel-p1 and 12.5 μM for Sel-p2) in PBS (pH 7.4) buffered water–DMSO (v/v, 1:1).

VII. Photostability and thermostability of Sel-p1 and Sel-p2

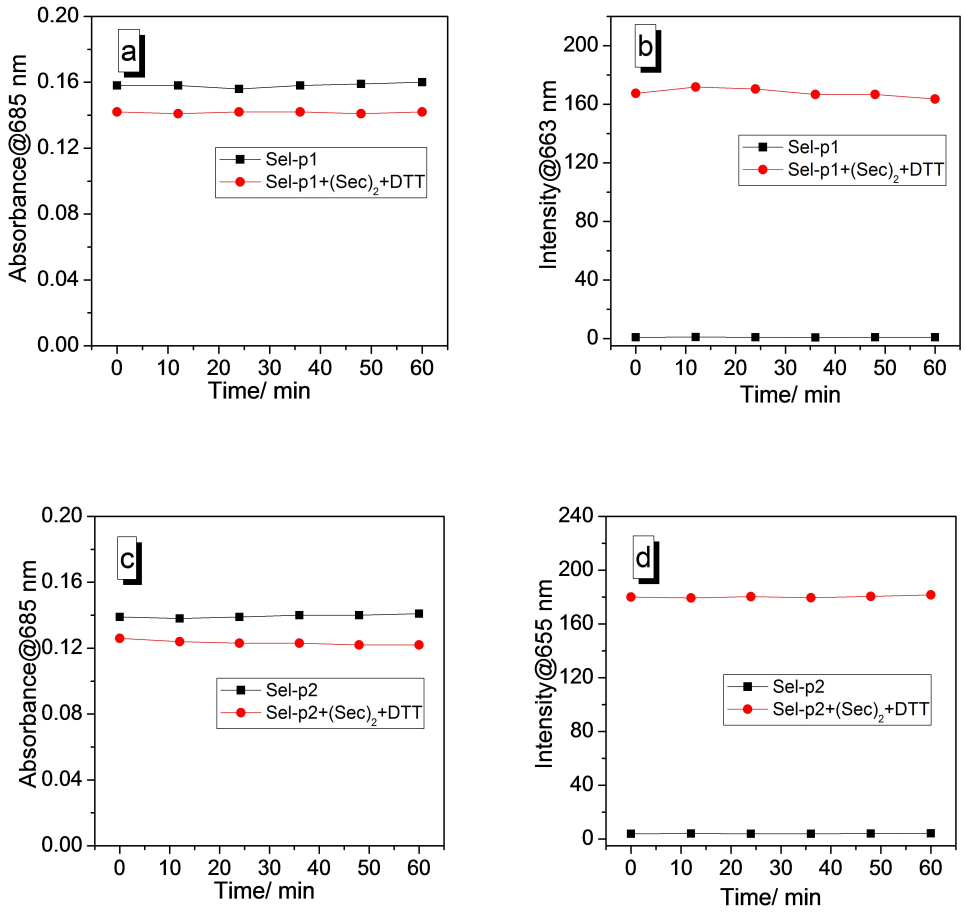


Figure S7. The time courses of UV/vis and fluorescence spectra of probes (5 μM) in the presence of equal molar $(Sec)_2$ with DTT (25 μM) in aqueous solution (DMSO/PBS, v/v 1:1, pH 7.4) recorded for 60 min.

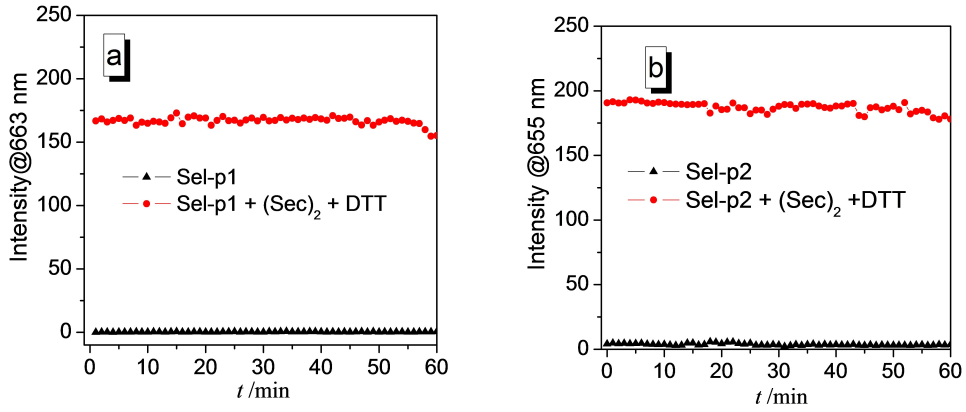
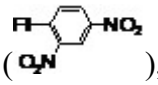
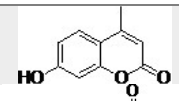
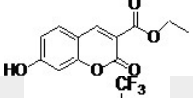
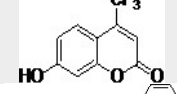
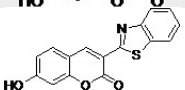
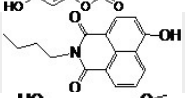
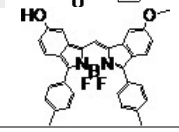


Figure S8. Fluorescence changes of 5 μM probes and the probe (5 μM) in the presence of equal molar $(Sec)_2$ with DTT (25 μM for Sel-p1 and 12.5 μM for Sel-p2) in aqueous solution (DMSO/PBS, v/v 1:1, pH 7.4) respectively under continuous 600 nm irradiation by a xenon lamp (150 W).

VIII. The selectivity of the fluorescent probe for Sec, and the pKa of its related fluorophore

Table S2. The selectivity of the probe (), and the pKa of its related fluorophore (Fl-H)

Fl-H	pKa	Sec[a] (F/F_0)	DTT[a] (F/F_0)	Selectivity[a] (Sec/DTT)
	7.8[1], 7.91[2]	16.5	1.5	11.0
		8.8	1.3	6.8
	7.26[3]	20.1	2.0	10.0
	7.02[4]	72.1	1.5	48.1
	5.8[5]	25.0	1.2	20.8
	6.8[b]	42.9[b]	2.2[b]	19.5[b]

[a] From B. Zhang, C. Ge, J. Yao, Y. Liu, H. Xie and J. Fang, *J. Am. Chem. Soc.*, 2015, **137**, 757-769. [b] From this work.

References

[1] B.N. Mattoo, *Trans. Faraday Soc.*, 1958, **54**, 19-24.

[2] P. M. Nowak, M. Woźniakiewicz, M. Piwowarska and P. K. Jagiellonian, *J. Chromatography A*, 2016, **1446**, 149-157.

[3] M. Savarese, P. A. Netti, C. Adamo, N. Rega and I. Ciofini, *J. Phys. Chem. B*, 2013, **117**, 16165-16173.

[4] V. Ahmed, M. Ispahany, S. Ruttgaizer, G. Guillemette and S. D. Taylor, *Anal. Biochem.*, 2005, **340**, 80-88.

[5] X.-X. Zhang, H. Wu, P. Li, Z.-J. Qu, M.-Q. Tan and K.-L. Han, *Chem. Commun.*, 2016, **52**, 8283-8286.

IX. Cytotoxicity of Sel-p2 and fluorescence imaging of mice

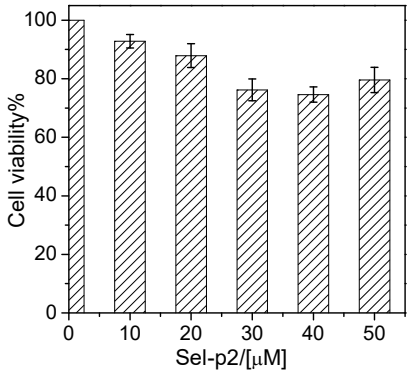


Figure S9. MTT assay of MCF-7 cells in the presence of different concentrations of Sel-p2.

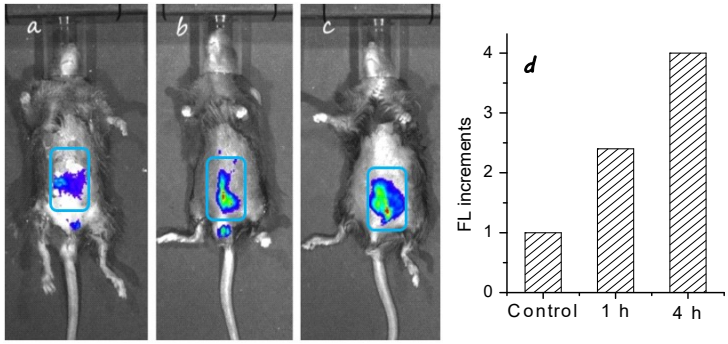
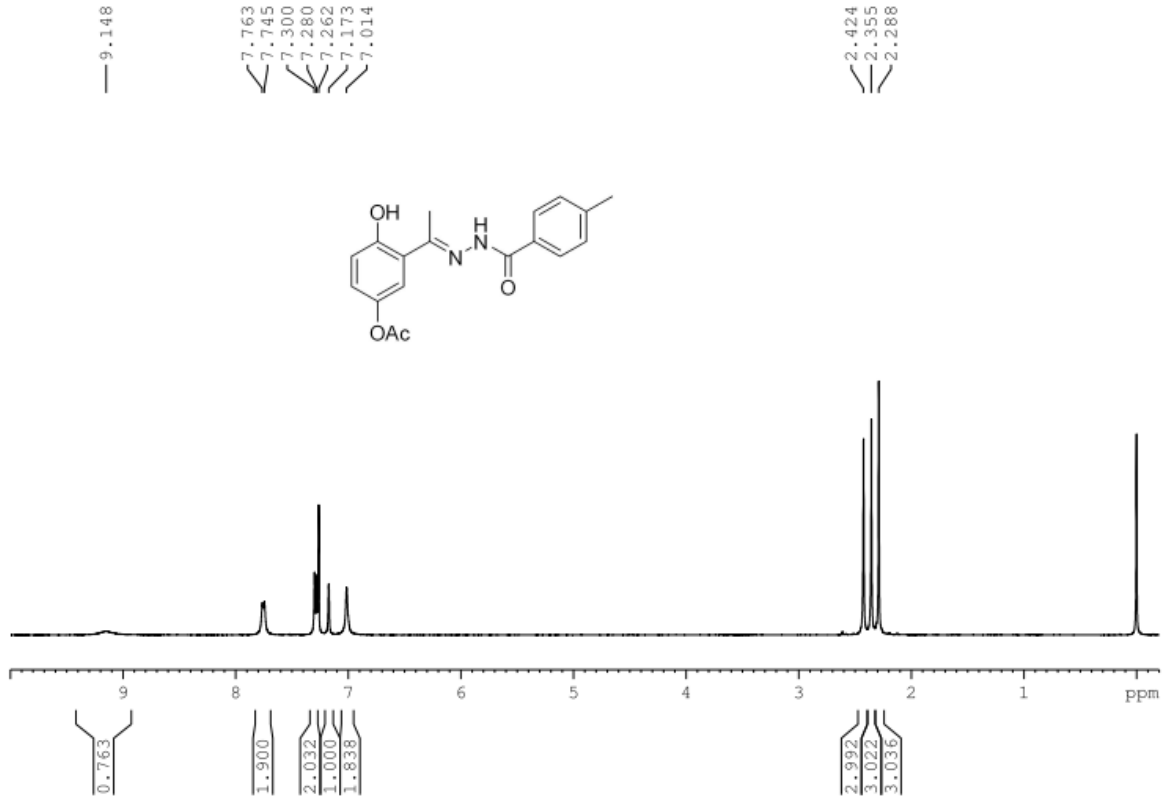
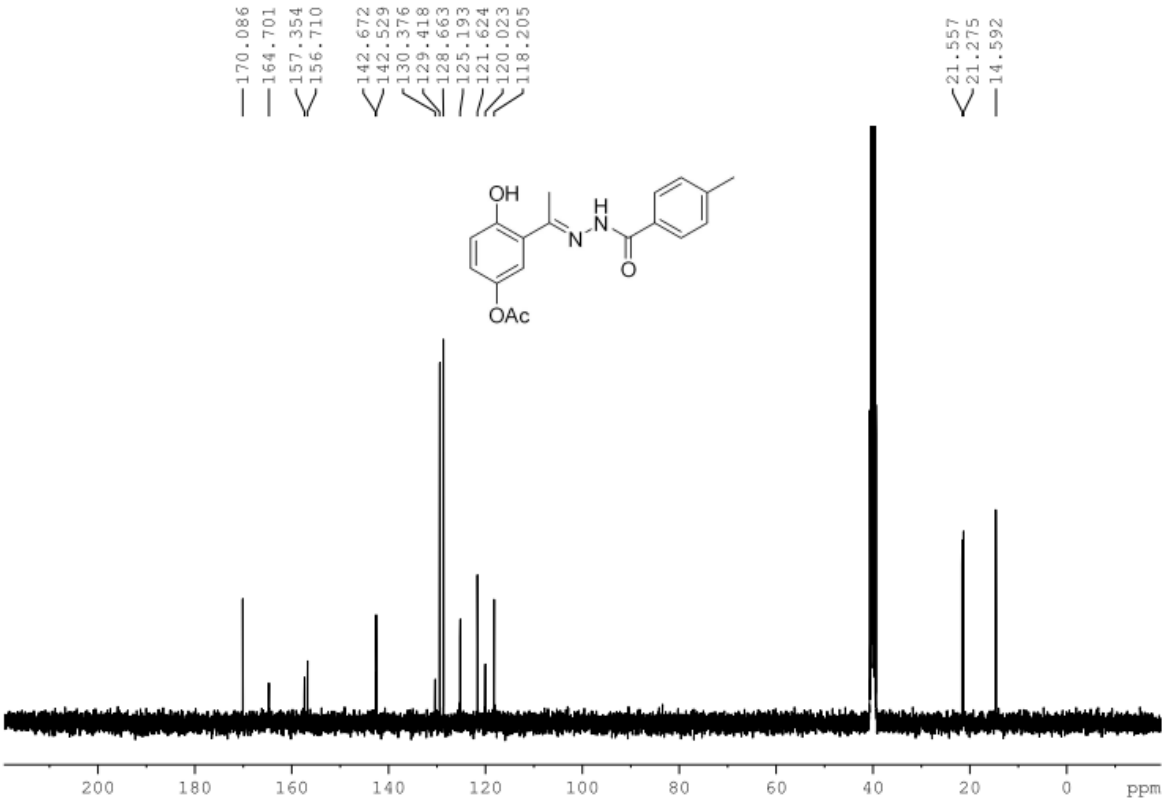
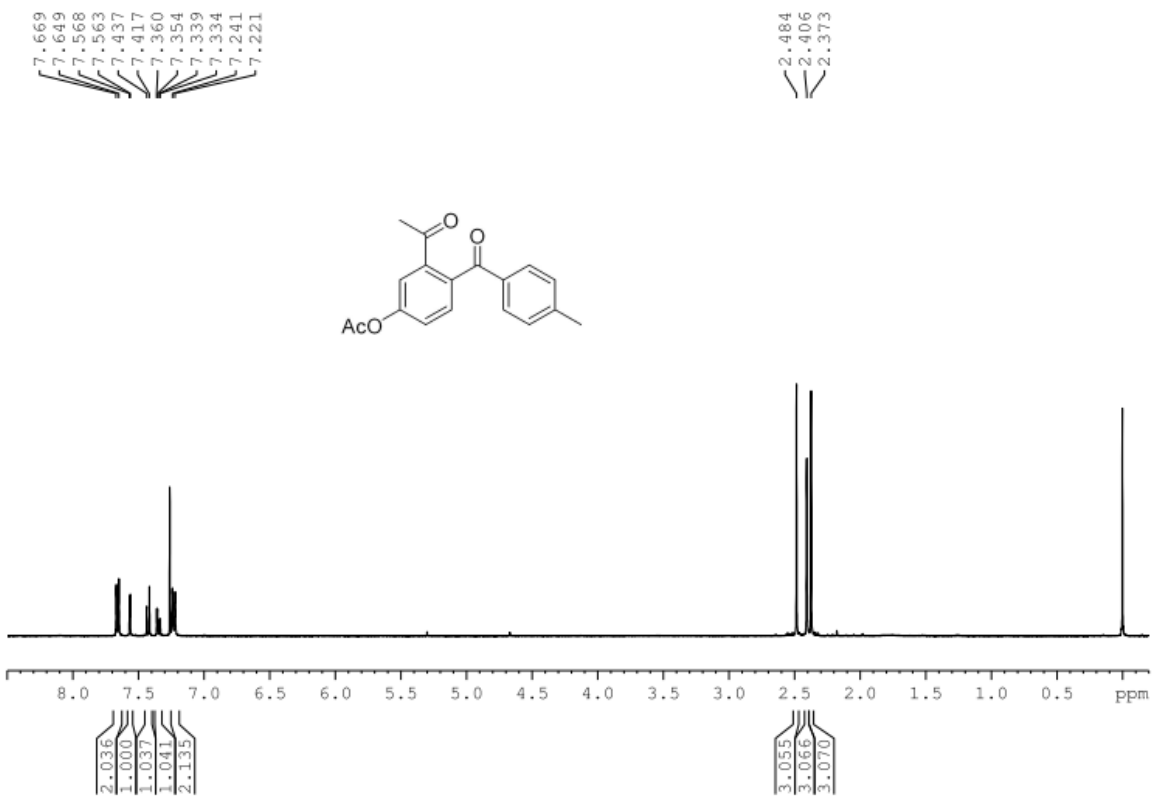


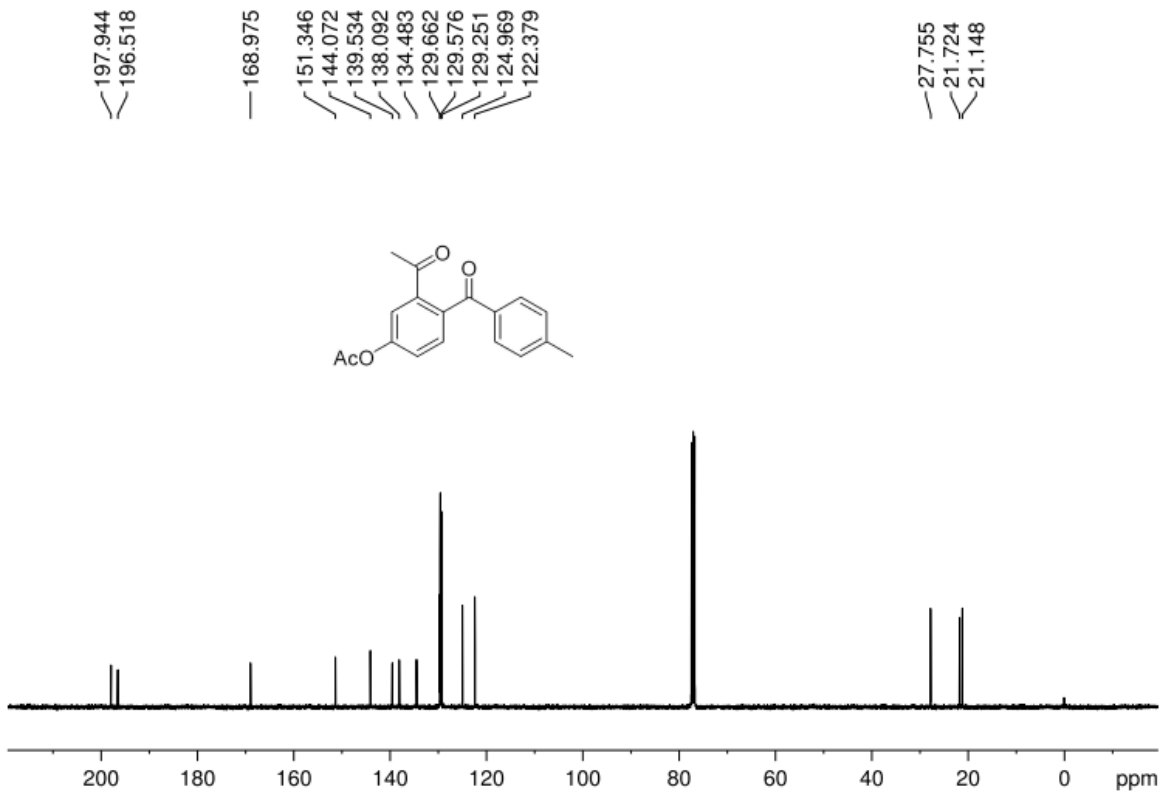
Figure S10. Representative fluorescent images of selenocysteine in mice (a) only the probe Sel-p2 (20 μM), (b) equal molar $(Sec)_2$ with DTT (20 μM in 100 μL saline) and followed Sel-p2 after 1 h, (c) 4 h, (d) fluorescence intensity from the abdominal area of groups a-c.

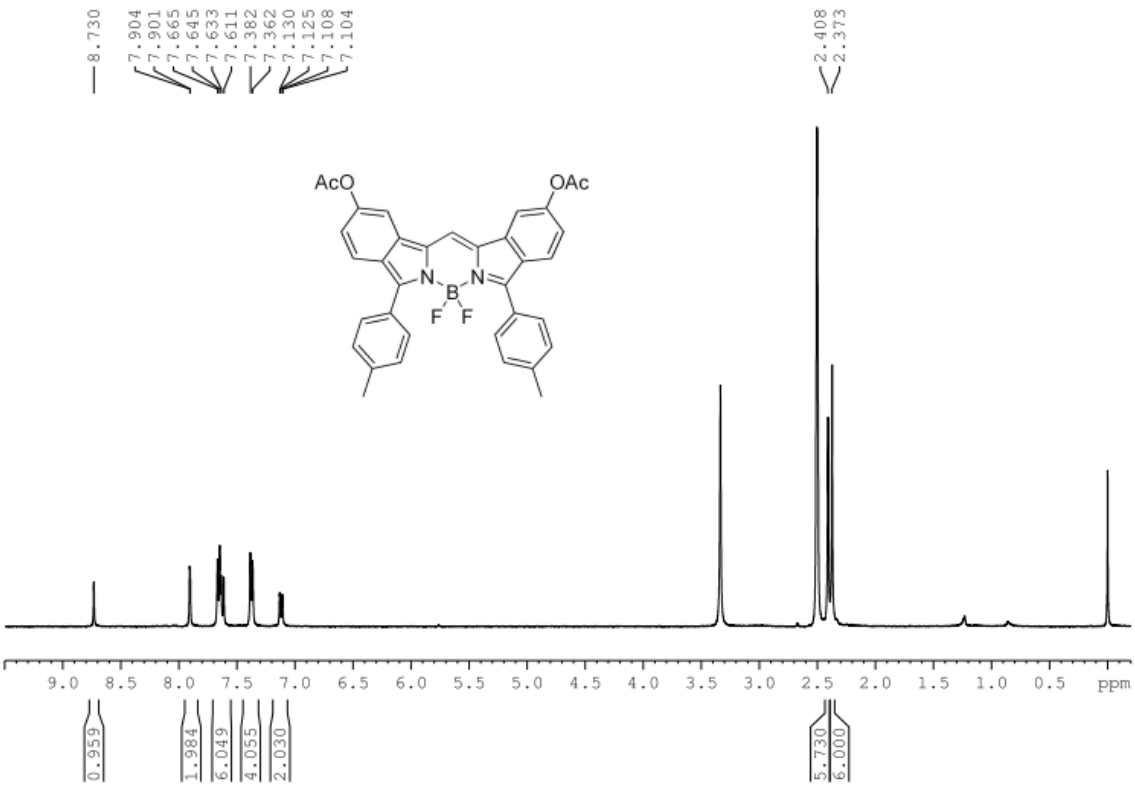
X. Copies of NMR of related compounds.

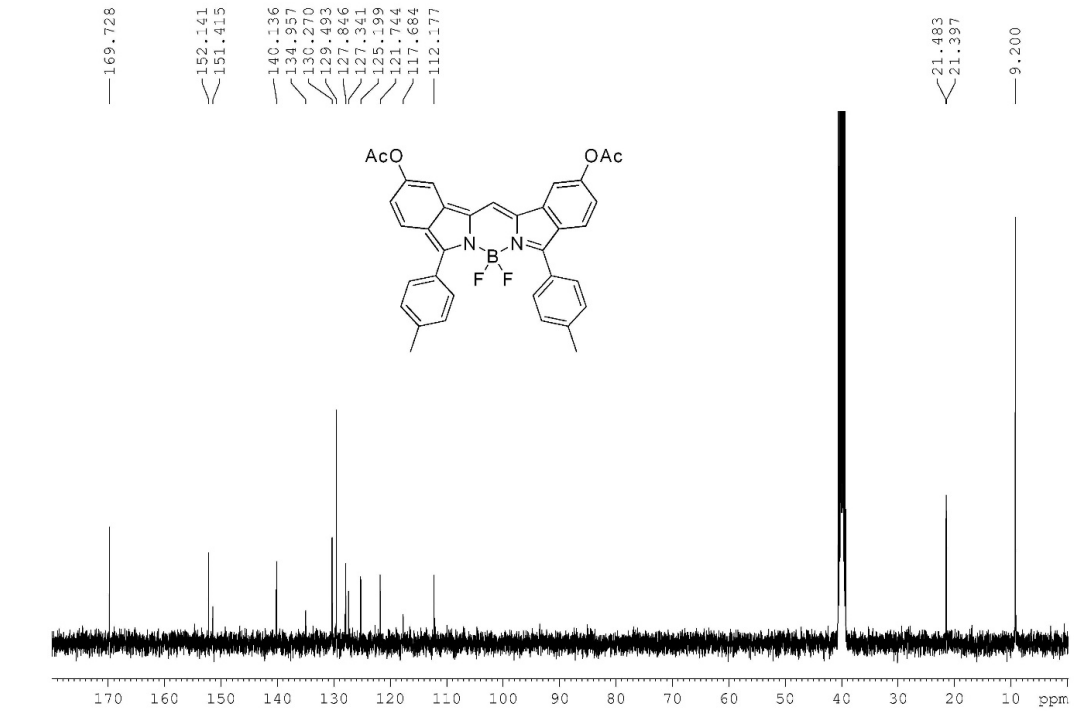


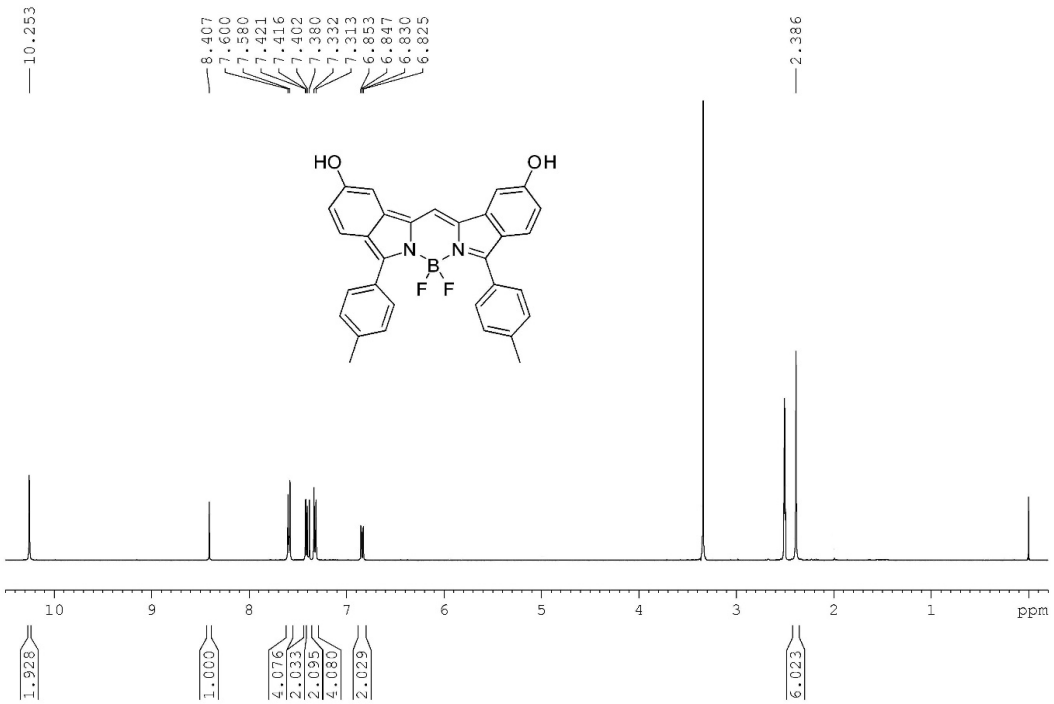


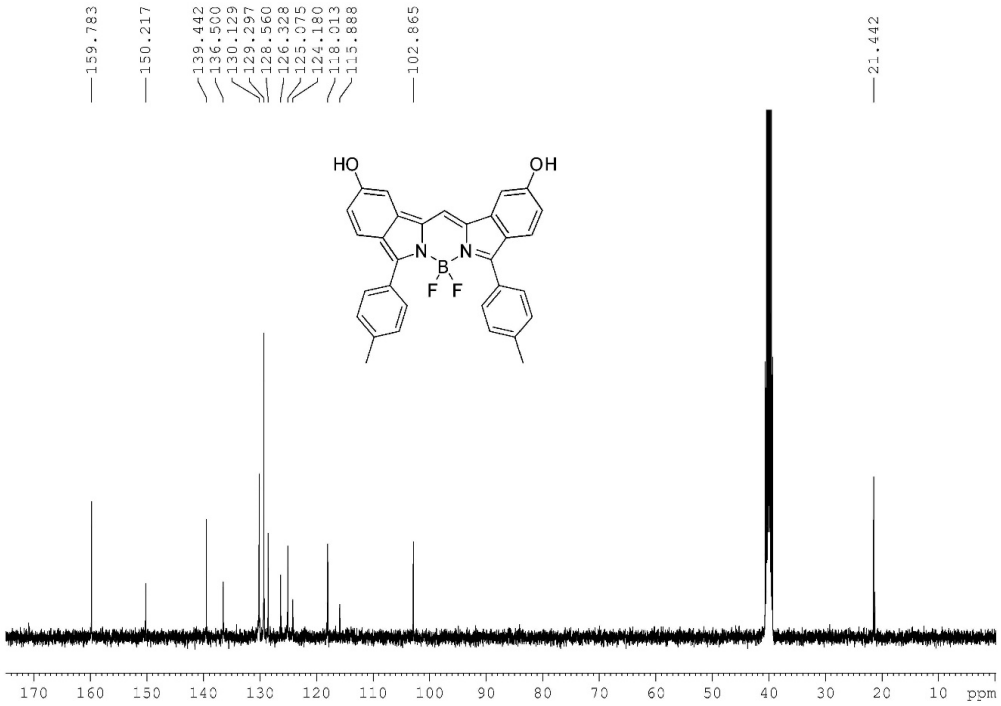

7.669
7.649
7.568
7.563
7.437
7.417
7.360
7.354
7.339
7.334
7.241
7.221
2.484
2.406
2.373
O
O
AcO
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
2.036
1.000
1.037
1.041
2.135
3.055
3.066
3.070

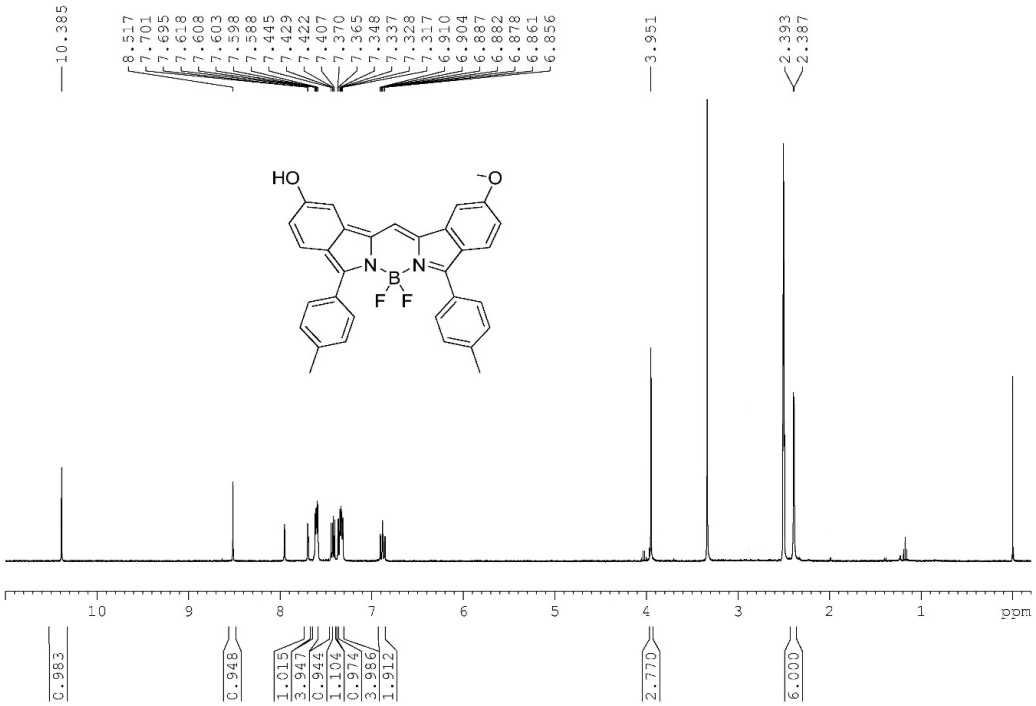

197.944
196.518
168.975
151.346
144.072
139.534
138.092
134.483
129.662
129.576
129.251
124.969
122.379
27.755
21.724
21.148
O
O
AcO
200
180
160
140
120
100
80
60
40
20
0
ppm

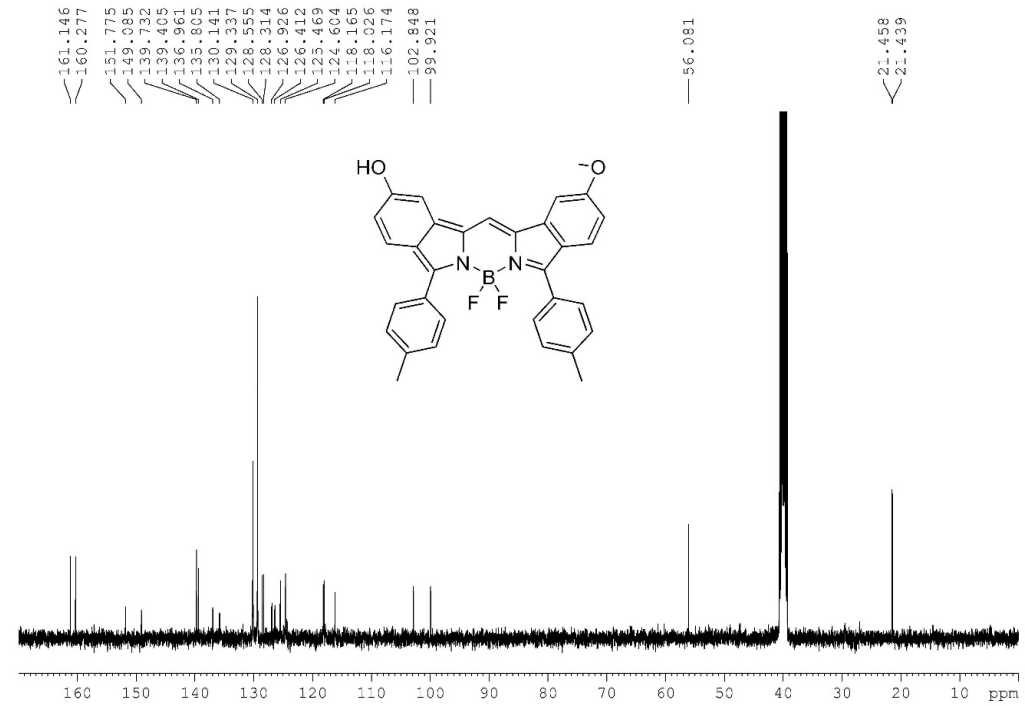

8.730
7.904
7.901
7.665
7.645
7.633
7.611
7.382
7.362
7.130
7.125
7.108
7.104
2.408
2.373
AcO
OAc
N
B
N
F
F
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
0.959
1.984
6.049
4.055
2.030
5.730
6.000

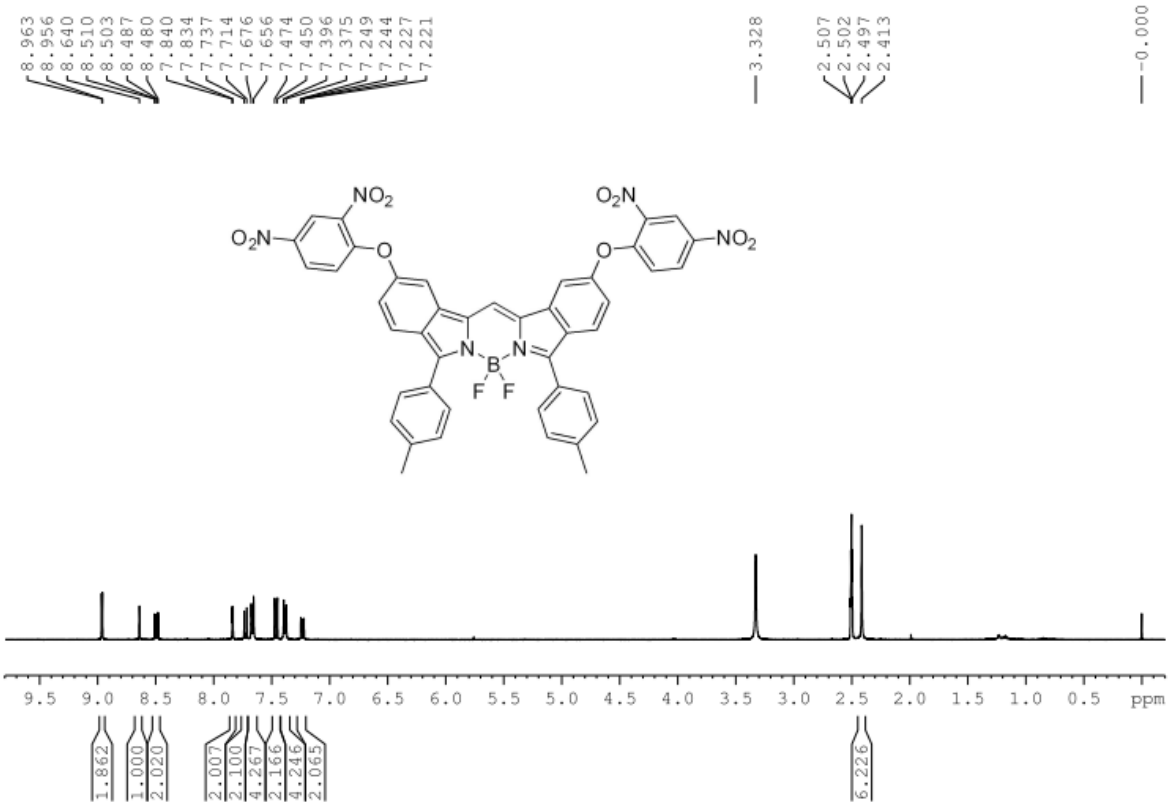

169.728
152.141
151.415
140.136
134.957
130.270
129.493
127.846
127.341
125.199
121.724
117.684
112.177
21.483
21.397
9.200
AcO
OAc
N
B
N
F
F
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

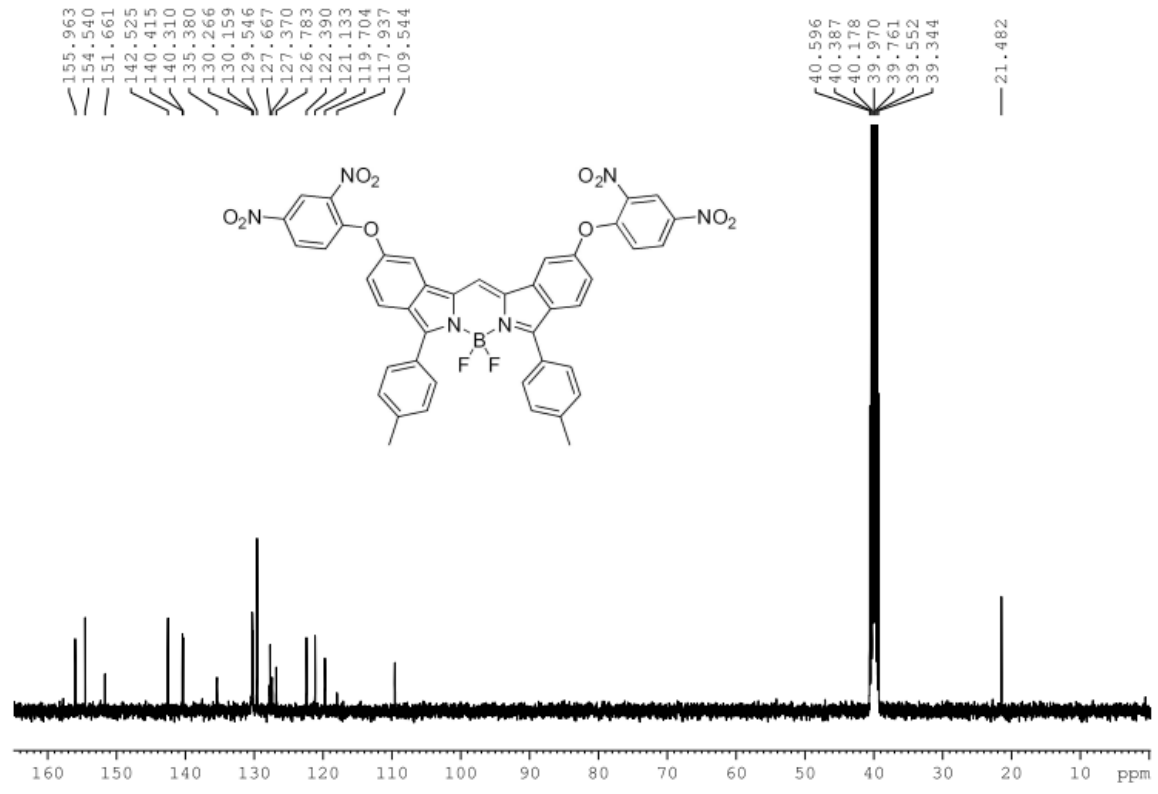

10.253
8.407
7.600
7.580
7.421
7.416
7.402
7.380
7.332
7.313
6.853
6.847
6.830
6.825
2.386
HO
OH
N
B
N
F F
10
9
8
7
6
5
4
3
2
1
ppm
1.928
1.000
4.076
2.033
2.095
4.080
2.029
6.023

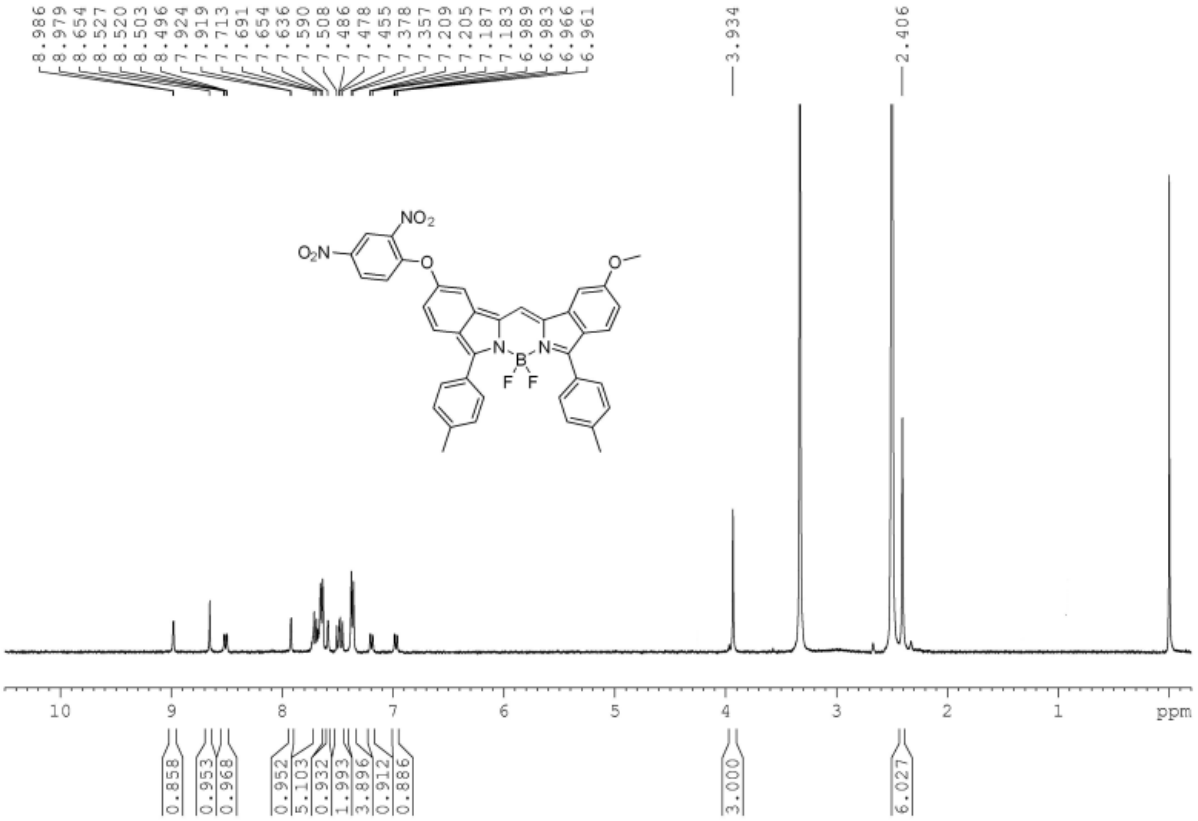

159.783
150.217
139.442
136.500
130.129
129.297
128.560
126.328
125.075
124.180
118.013
115.888
102.865
21.442
HO
OH
N
B
N
F F
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

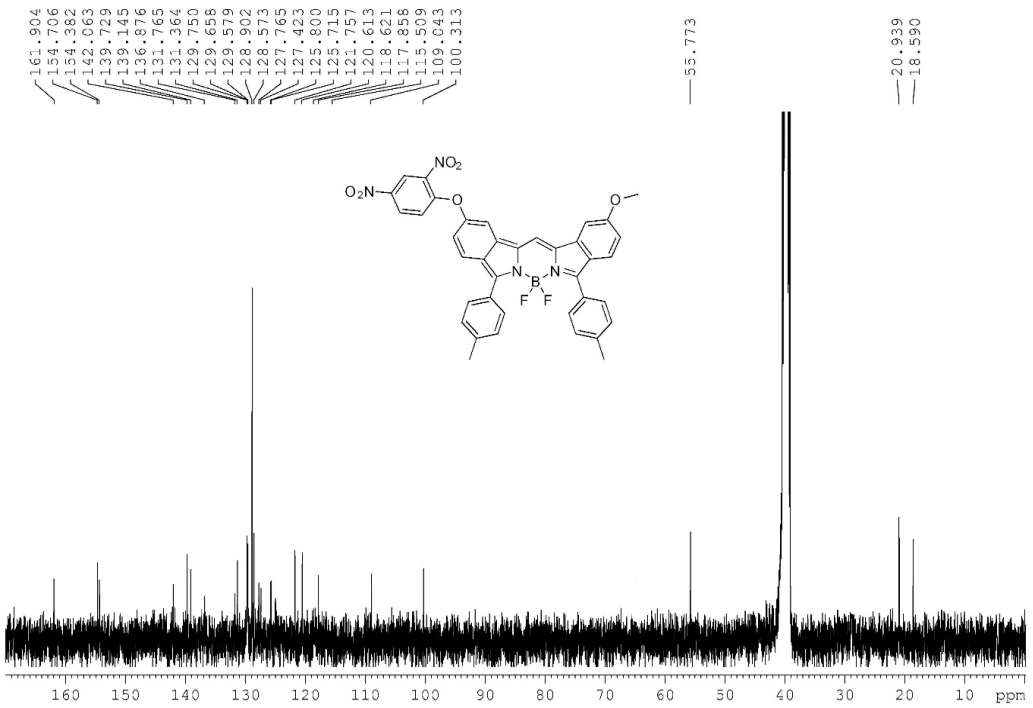

10.385
8.517
7.701
7.695
7.618
7.608
7.603
7.598
7.588
7.445
7.429
7.422
7.407
7.370
7.365
7.348
7.337
7.328
7.317
6.910
6.904
6.887
6.882
6.878
6.861
6.856
3.951
2.393
2.387
HO
O
N
B
N
F
F
10
9
8
7
6
5
4
3
2
1
ppm
0.983
0.948
1.015
3.947
0.944
1.104
0.974
3.986
1.912
2.770
6.000


161.146
160.277
151.775
149.085
139.732
139.405
136.961
135.805
130.141
129.337
128.555
128.314
126.926
126.412
125.469
124.604
118.165
118.026
116.174
102.848
99.921
56.081
21.458
21.439
HO
O
N
B
N
F
F
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


8.963
8.956
8.640
8.510
8.503
8.487
8.480
7.840
7.834
7.737
7.714
7.676
7.656
7.474
7.450
7.396
7.375
7.249
7.244
7.227
7.221
3.328
2.507
2.502
2.497
2.413
0.000
NO2
O2N
O
N
B
F
F
9.5 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 ppm
1.862
1.000
2.020
2.007
2.100
4.267
2.166
4.246
2.065
6.226


155.963
154.540
151.661
142.525
140.415
140.310
135.380
130.266
130.159
129.546
127.667
127.370
126.783
122.390
121.133
119.704
117.937
109.544
40.596
40.387
40.178
39.970
39.761
39.552
39.344
21.482
NO2
O2N
O
N
B
F
F
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


8.986
8.979
8.654
8.527
8.520
8.503
8.496
7.924
7.919
7.713
7.691
7.654
7.636
7.590
7.508
7.486
7.478
7.455
7.378
7.357
7.209
7.205
7.187
7.183
6.989
6.983
6.966
6.961
3.934
2.406
NO2
O2N
O
O
N
B
N
F F
10
9
8
7
6
5
4
3
2
1
ppm
0.858
0.953
0.968
0.952
5.103
0.932
1.993
3.896
0.912
0.886
3.000
6.027


161.904
154.706
154.382
142.063
139.729
139.145
136.876
131.765
131.364
129.750
129.658
129.579
128.902
128.573
127.765
127.423
125.800
125.715
121.757
120.613
118.621
117.858
115.509
109.043
100.313
55.773
20.939
18.590
NO2
O2N
O
O
N
B
N
F F
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm