Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Cyclen-functionalized perylenebisimides as sensitive and selective fluorescent sensors for Pb^{2+} in aqueous solution

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Contents

1. General Remarks

NMR spectra were obtained on a Bruker AMX-400. The ^{1}H NMR (400 MHz) chemical shifts were measured relative to $CDCl_3$ or DMSO-d_6 as the internal reference ($CDCl_3$: δ = 7.26 ppm; DMSO-d_6: δ = 2.50 ppm). The ^{13}C NMR (100 MHz) chemical shifts were given using $CDCl_3$ and DMSO-d_6 as the internal standard ($CDCl_3$: δ = 77.16 ppm; DMSO-d_6: δ = 39.52 ppm). High-resolution mass spectra (HR-MS) were obtained with a Waters-Q-TOF-Premier (ESI). Melting points were determined with XRC-1 and are uncorrected. Absorption spectra were detected on a HITACHI U-2910 spectrometer. Fluorescent emission spectra were collected on a Horiba Jobin Yvon-Edison fluoromax-4 fluorescence spectrometer.

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. n-Octylperylene-3,4:9,10-tetracarboxylic-3,4-anhydride-9,10-imide (**2**),[1] 10-(2-aminoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate tri-*tert*-butyl ester (**3**)[2] were prepared according to the literature procedures. Solvents were dried over CaH_2 or sodium and freshly distilled prior to use. Unless otherwise indicated, all syntheses and manipulations were carried out under N_2 atmosphere.

DMSO was HPLC grade and water was distilled for twice in the optical spectroscopic studies. Chloride (Zn^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, Pb^{2+}, Ba^{2+}, Mn^{2+}, Cr^{3+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}), nitrate (Ag^{+}) were prepared in water as stock solutions for each measurement. Each time a 3 mL of receptor solution was filled in a quartz cell of 1 cm of optical path length and the stock solution of metal ion was dropped into a quartz cell using a microsyringe. The volume of metal ions stock solution added was less than 100 μL to remain the concentration of receptor constant. The excitation and emission slits of fluorescence spectra were set at 2.0 nm if not specified. Fluorescence images was examined under a fluorescence microscopy (OLYMPUS IX71) irradiated by green light source (540-580 nm).

2. Synthesis and characterization of PBIs

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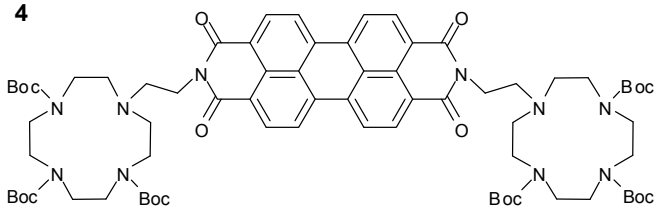

3,4,9,10-perylenetetracarboxylic dianhydride **1** (235 mg, 0.60 mmol) and 10-(2-aminoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate tri-*tert*-butyl ester **3** (680 mg, 1.32 mmol) were added into DMF (10 mL) under N_2 and stirred for 20 h at 120 ºC. After cooling to room temperature, the mixture was poured into 20 mL water and followed by addition of 50 mL CH_2Cl_2. After stirring for 30 min, the organic phase was separated and washed with water (3×10 mL). The organic phases were dried over Na_2SO_4 and a red residue was obtained after removing the solvent under reduced pressure. Then the residue was purified by column chromatography on silica gel eluting with CH_2Cl_2/EtOAc (1:3, v/v) to give the desired product as a dark -red solid (580 mg) at a yield of 70%. Mp = 150-152 ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.59 (d, J = 8.0 Hz, 4H), 8.52 (s, 4H), 4.39 (t, J = 7.4 Hz, 4H), 3.58-3.36 (m, 24H), 3.00-2.90 (m, 12H), 1.45 (s, 54H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.78, 162.74, 156.2, 155.8, 155.5, 133.9, 133.8, 130.8, 128.7, 125.5, 122.8, 79.5, 79.3, 54.9, 53.6, 49.9, 47.9, 28.8, 28.6 ppm; HRMS (ESI): m/z calcd for $C_{74}H_{103}N_{10}O_{16}$ $[M+H]^+$ 1387.7554, found 1387.7535.

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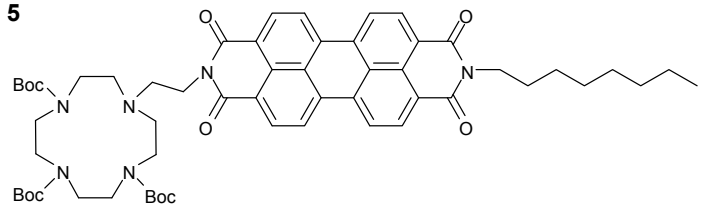
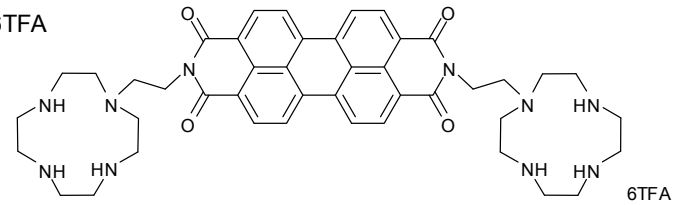


A mixture of **2** (112 mg, 0.22 mmol), **3** (103 mg, 0.20 mmol) and 2 mL DMF were heated under N_2 at 100 ºC for 20 h. After cooling to room temperature, the mixture was dispersed in 20 mL water and followed by addition of 50 mL CH_2Cl_2. After

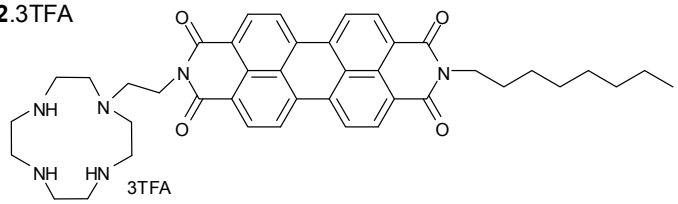
stirring for 30 min, the organic phase was separated and washed with water (3×10 mL). The organic phases were dried over Na_2SO_4 and a red residue was obtained after removing the solvent under reduced pressure. Then the residue was purified by column chromatography on silica gel eluting with CH_2Cl_2/EtOAc (1:1, v/v) to give the desired product as a dark-red solid (101 mg) at a yield of 50%. mp: 123-125 ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.49 (s, 4H), 8.33 (s, 4H), 4.37 (s, 2H), 4.18 (s, 2H), 3.56-3.45 (m, 12H), 3.04-2.93 (m, 6H), 1.77 (s, 2H), 1.46-1.29 (m, 37H), 0.88 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.5, 155.9, 155.5, 133.2, 133.0, 130.3, 128.3, 124.9, 122.9, 122.4, 122.3, 79.6, 79.5, 79.3, 54.8, 53.3, 49.9, 47.8, 47.2, 40.8, 31.9, 29.7, 29.5, 29.4, 28.8, 28.6, 28.1, 27.4, 22.8, 14.2 ppm; HRMS (ESI): m/z calcd for $C_{57}H_{73}N_6O_{10}$ $[M+H]^+$ 1001.5388, found 1001.5376.

PBI-**1**.6TFA



The compound **4** was dissolved in CH_2Cl_2/TFA (1:1) and stirred for 12 h at room temperature. The solvent and excess trifluoroacetic acid were removed under reduced pressure, giving a dark-red solid. The solid was dissolved in water and washed with CH_2Cl_2 (3×10 mL). After phase separation, the solvent was removed in vacuo to give the product as a red solid without further purification. Yield: 100%. Mp > 300 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.72 (t, J = 8.8 Hz, 4H), 8.45 (d, J = 7.2 Hz, 4H), 4.23 (s, 4H), 3.14-2.82 (m, 36H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 162.6, 158.8, 158.5, 133.3, 130.4, 127.8, 124.6, 123.6, 121.8, 118.6, 115.6, 49.7, 47.7, 44.6, 42.5, 42.0 ppm; HRMS (ESI+): m/z calcd for $C_{44}H_{55}N_{10}O_4$ $[M+H]^+$ 787.4408, found 787.4398.

PBI-**2**.3TFA



The compound **5** was dissolved in CH_2Cl_2/TFA (1:1) and stirred for 12 h at room temperature. The solvent and excess trifluoroacetic acid were removed under reduced pressure, giving a dark-red solid as the product without further purification. Yield: 100%. Mp > 300 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.17-8.00 (m, 8H), 7.29 (brs, 2H), 4.19 (s, 2H), 3.94 (s, 2H), 3.17-2.85 (m, 18H), 1.67 (s, 2H), 1.38-1.31 (m, 10H), 0.90 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 162.1, 161.7, 132.7, 132.2, 129.8, 129.4, 127.1, 126.8, 123.8, 123.6, 123.1, 122.9, 121.2, 121.0, 49.1, 47.6, 44.6, 43.3, 42.5, 42.0, 31.2, 28.7, 28.6, 27.2, 26.6, 22.0, 13.9 ppm; HRMS (ESI+): m/z calcd for $C_{42}H_{49}N_6O_4$ $[M+H]^+$ 701.3815, found 701.3799.

3. References

1. Y. Nagao and T. Misono, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 1191.

2. R. Reichenbach-Klinke, M. Kruppa and B. König, *J. Am. Chem. Soc.*, 2002, **124**, 12999.

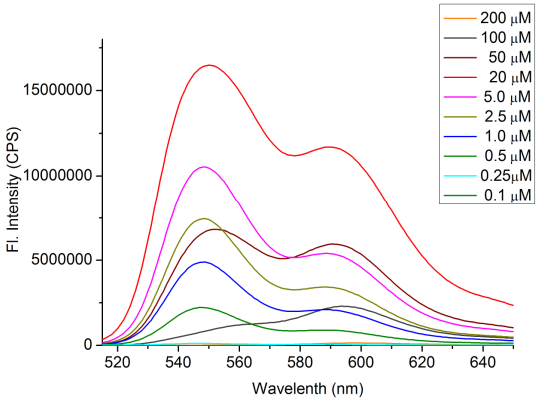


Figure S1. Fluorescent responses of PBI-**1** (0.1 μM - 200 μM) in HEPES (10 mM, pH = 7.2). (λex = 495 nm, λem = 548 nm)

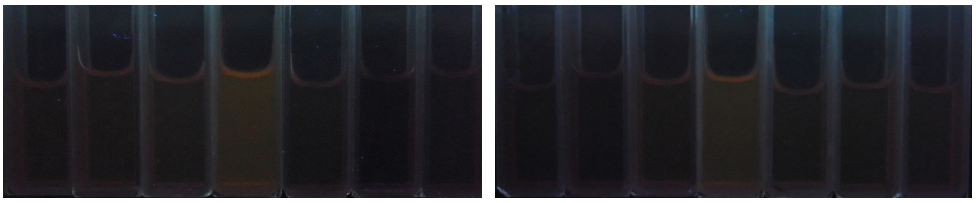


Figure S2. The color change of PBI-**1** (30 μM) in HEPES (10 mM, pH = 7.2) under a UV lamp (365 nm) by addition of 2 equiv. different metal ions (from left to right： no metal ion, Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Ag^{+}) (left) and after 5 days (right).

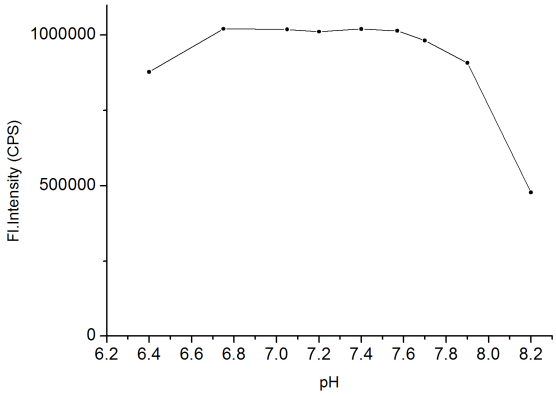


Figure S3. Effect of pH on the fluorescence intensity at 548 nm of PBI-**1** (5 μM) in buffer solution. The pH of solution was adjusted by aqueous solution of NaOH (1 M) and HCl (1 M).

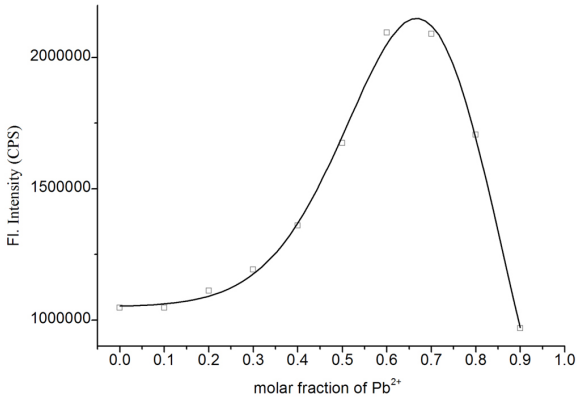


Figure S4. Job's plot of PBI-**1** and Pb^{2+}. The total concentration of PBI-**1** and Pb^{2+} were kept at 10 μM in HEPES (10 mM, pH = 7.2). (λex = 495 nm, λem = 548 nm).

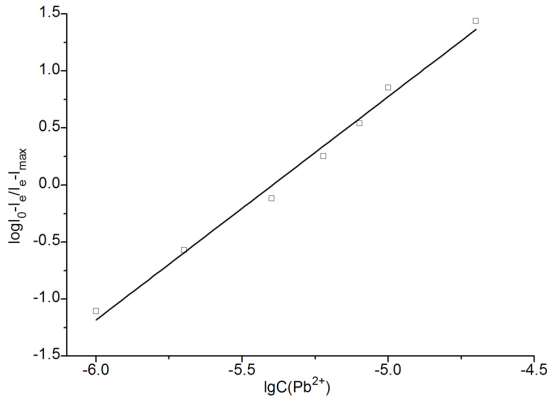


Figure S5. Fluorescence intensity of PBI-**1** at 548 nm as a function of lg[Pb^{2+}] (2.50 - 50 μM) in the condition of the Pb^{2+} titration. (r = 0.990)

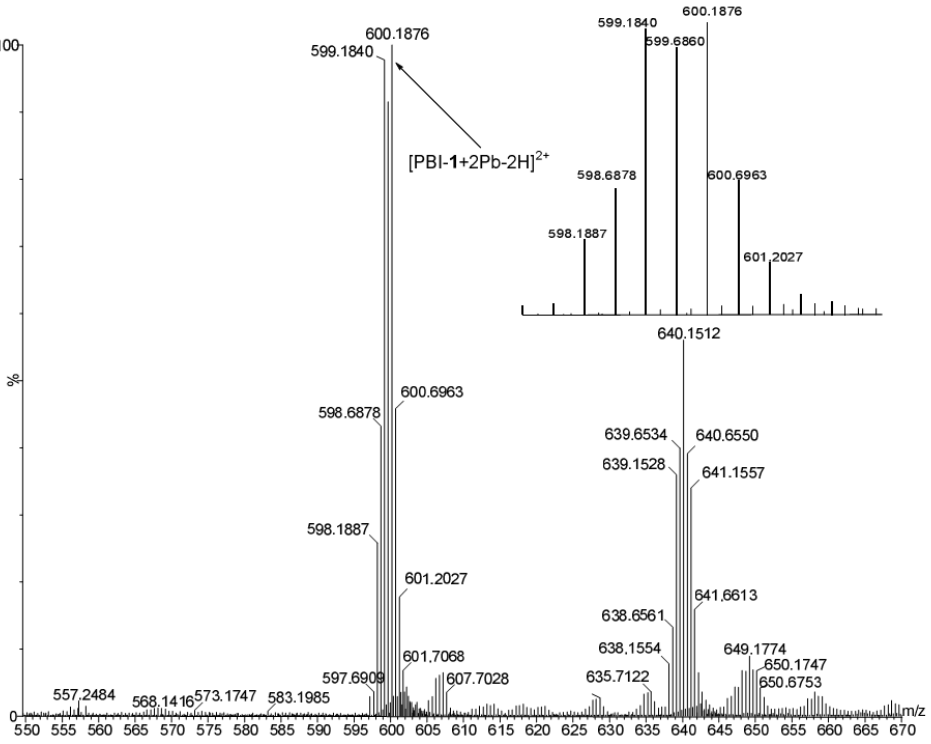


Figure S6. The ESI-TOF mass spectrum of a mixture of PBI-**1** and $Pb(ClO_4)_2$.

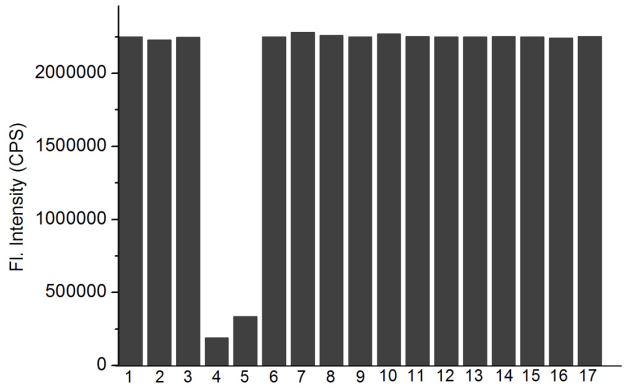


Figure S7. Fluorescent responses of PBI-**1** (5 μM) in 10 mM HEPES (pH = 7.2) to various metal ions (5 equiv.), followed by Pb^{2+} (5 equiv.): 1, none; 2, Zn^{2+}; 3, Cd^{2+}; 4, Cu^{2+}; 5, Hg^{2+}; 6, Ag^{+}; 7, Fe^{2+}; 8, Fe^{3+}; 9, Mn^{2+}; 10, Cr^{3+}; 11, Co^{2+}; 12, Ni^{2+}; 13, Na^{+}; 14, K^{+}; 15, Ca^{2+}; 16, Ba^{2+}; 17, Mg^{2+}. (λex = 495 nm, λem = 548 nm)

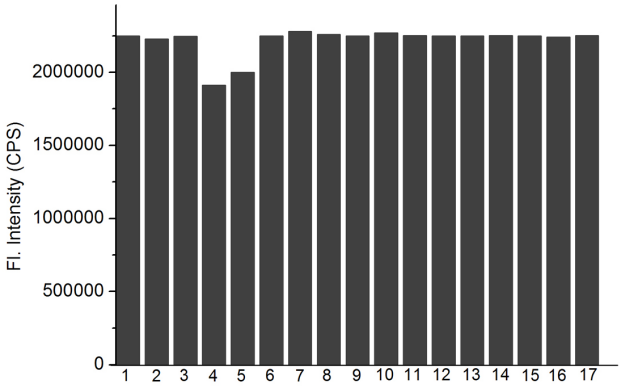


Figure S8. Fluorescent responses of PBI-**1** (5 μM) in 10 mM HEPES (pH=7.2) to various metal ions (5 equiv.) in the presence of Pb^{2+} (5 equiv.): 1, none; 2, Zn^{2+}; 3, Cd^{2+}; 4, Cu^{2+}; 5, Hg^{2+}; 6, Ag^{+}; 7, Fe^{2+}; 8, Fe^{3+}; 9, Mn^{2+}; 10, Cr^{3+}; 11, Co^{2+}; 12, Ni^{2+}; 13, Na^{+}; 14, K^{+}; 15, Ca^{2+}; 16, Ba^{2+}; 17, Mg^{2+}. (λex = 495 nm, λem = 548 nm)

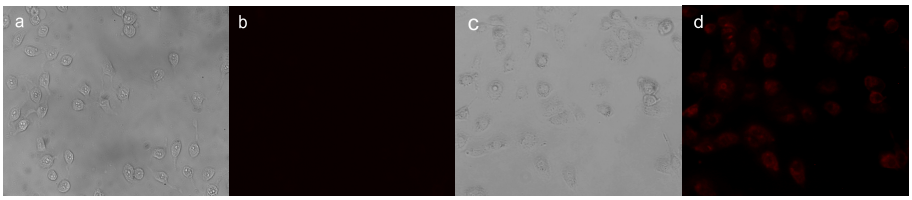


Figure S9. Fluorescence microscopy images of HepG2 cells incubated with (b) PBI-**1**, (d) PBI-**2** in D-HBSS; (a)/(c) were the brightfield images corresponding to (b)/(d), respectively.

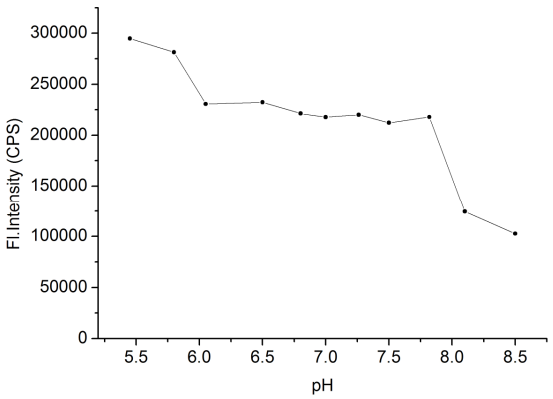


Figure S10. Effect of pH on the fluorescence intensity at 545 nm of PBI-**2** (5 μM) in buffer solution. The pH of solution was adjusted by aqueous solution of NaOH (1 M) and HCl (1 M). (λex = 490 nm, λem = 545 nm, slit = 8 nm/8 nm)

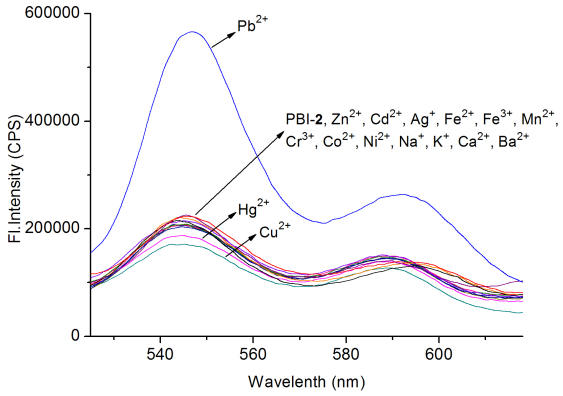


Figure S11. Fluorescent responses of PBI-**2** (5 μM) in 10 mM HEPES/DMSO (v/v = 90/10, pH = 7.2) to various metal ions (25 μM) respectively. (λex = 490 nm, λem = 545 nm, slit = 8 nm/8 nm)

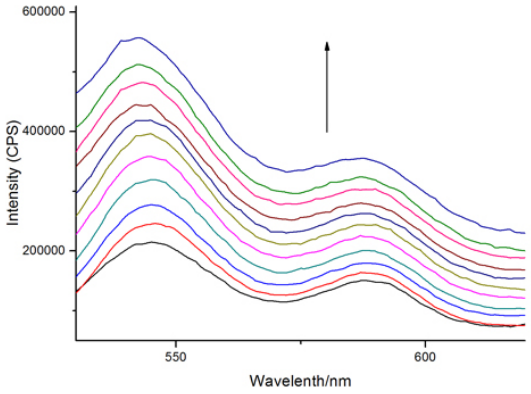


Figure S12. Fluorescence responses of PBI-**2** (5 μM) in 10 mM HEPES/DMSO (v/v = 90/10, pH = 7.2) to various concentrations of Pb^{2+}. (λex = 490 nm, λem = 545 nm, slit = 8 nm/8 nm)

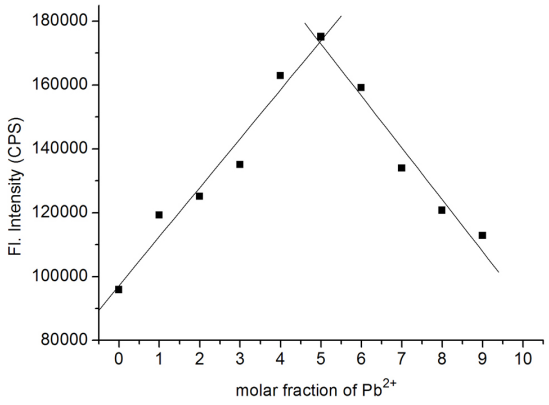


Figure S13. Job's plot of PBI-**2** and Pb^{2+}. The total concentration of PBI-**2** and Pb^{2+} were kept at 10 μM in 10 mM HEPES/DMSO (v/v = 90/10, pH = 7.2). (λex = 490 nm, λem = 545 nm, slit = 8 nm/5 nm).

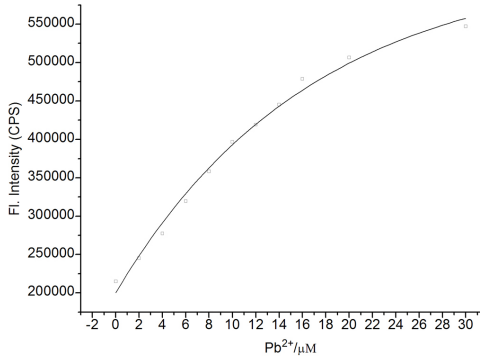


Figure S14. The nonlinear curve fitting for PBI-**2** of the fluorescence intensity at 545 nm against the added amount of Pb^{2+} (2 - 30 μM).

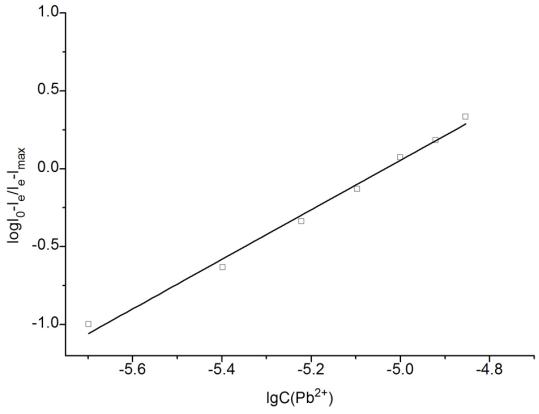


Figure S15. Fluorescence intensity of PBI-**2** at 545 nm as a function of lg[Pb^{2+}] (2 – 15 μM) in the condition of the Pb^{2+} titration. (r = 0.991).

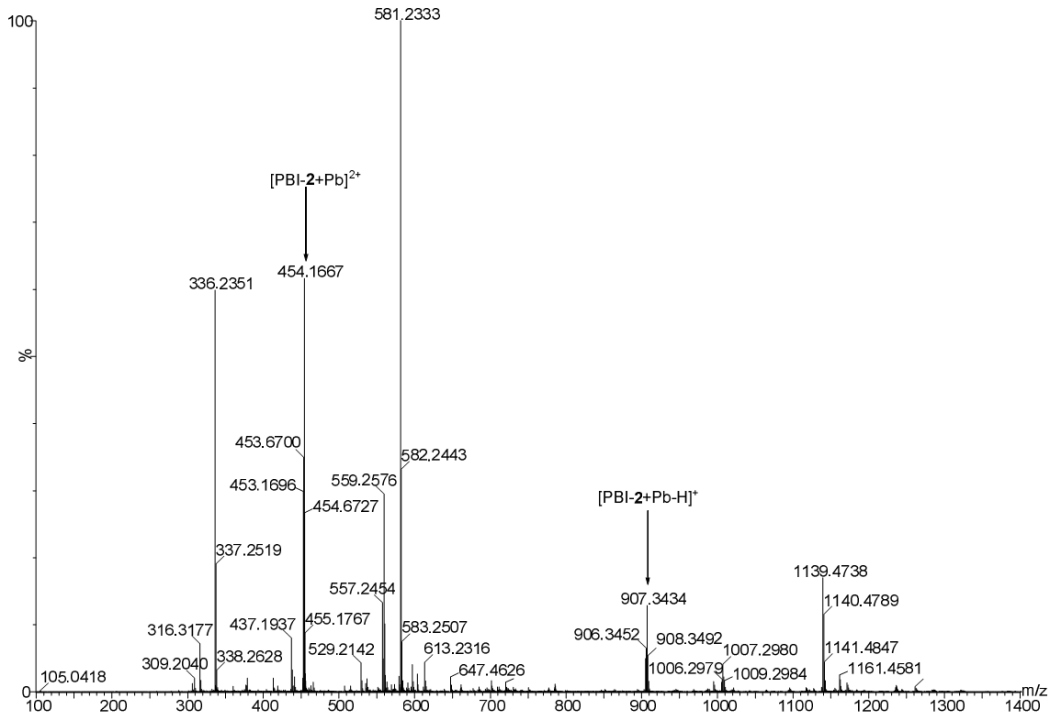
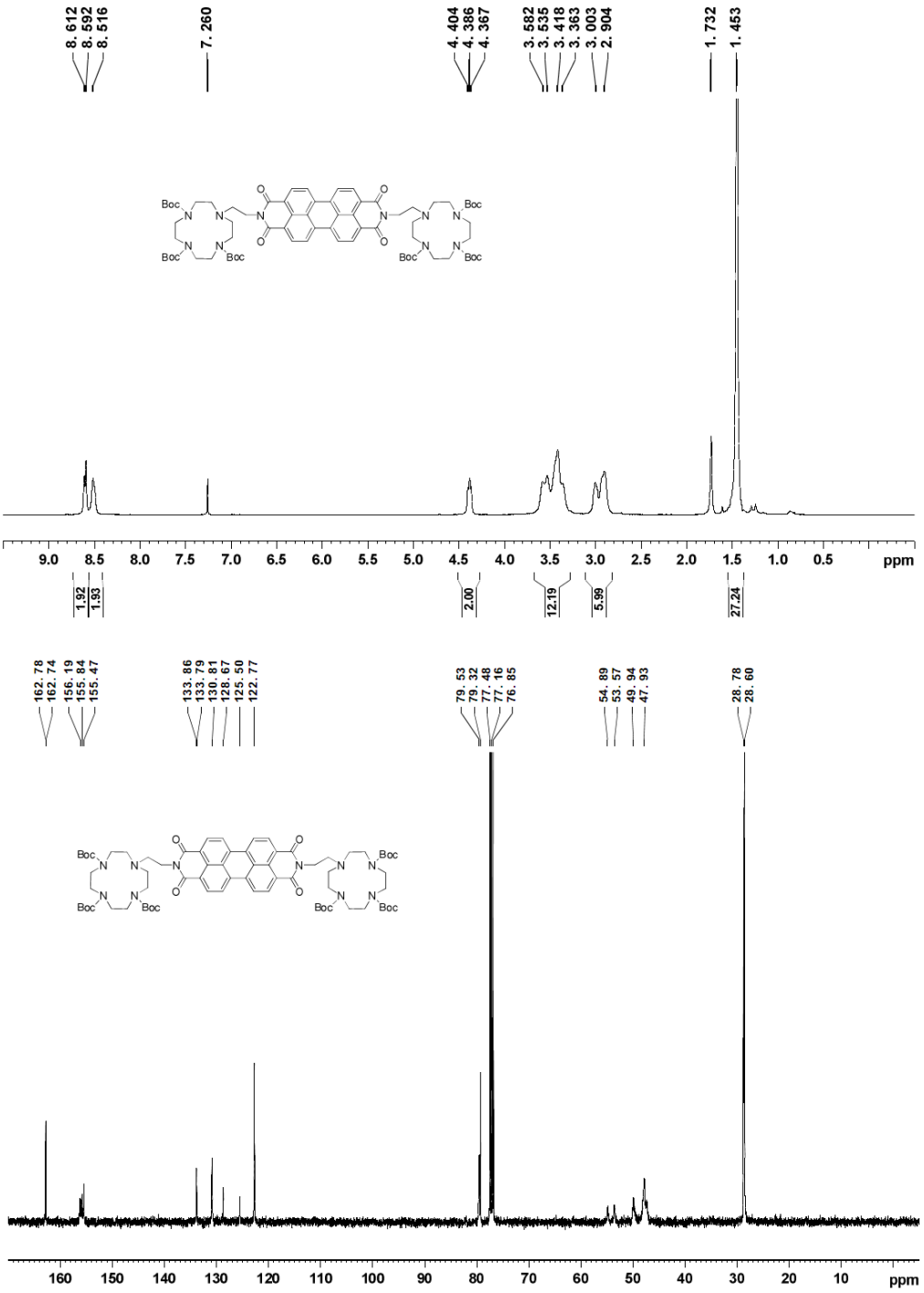
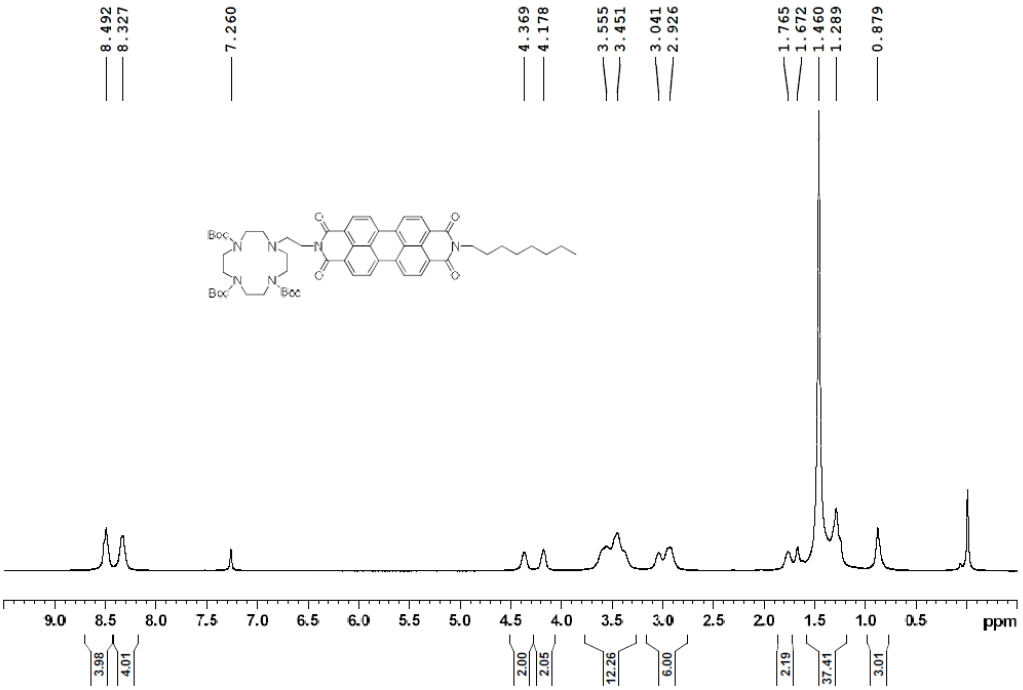


Figure S16. The ESI-TOF mass spectrum of a mixture of PBI-**2** and $Pb(ClO_4)_2$.

VI. Copies of ^{1}H and ^{13}C NMR spectra

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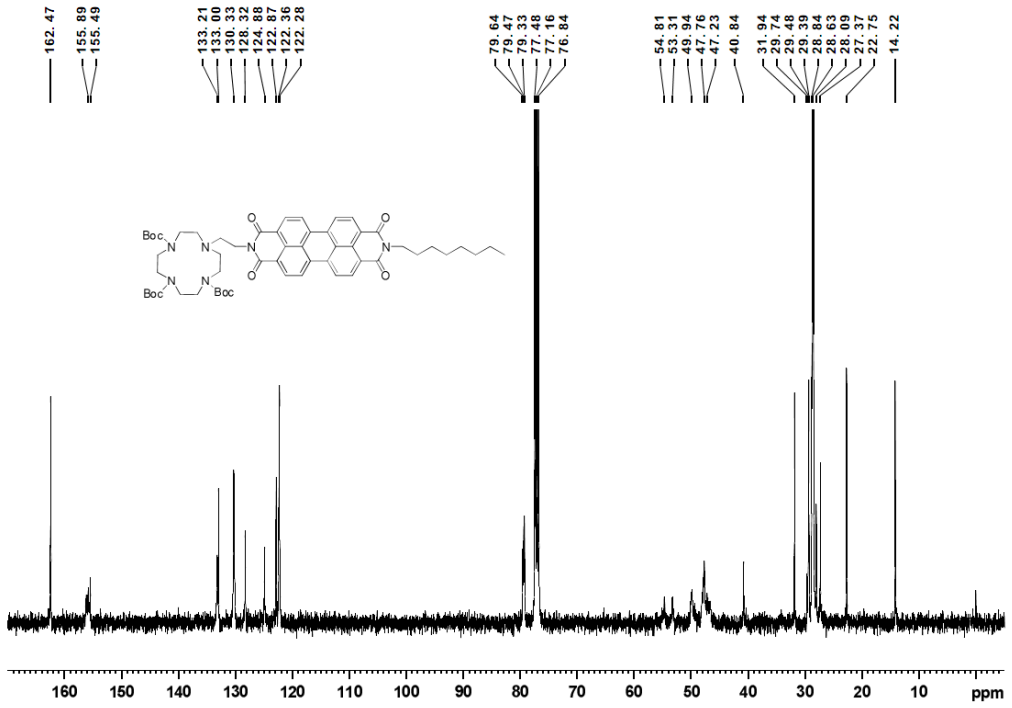
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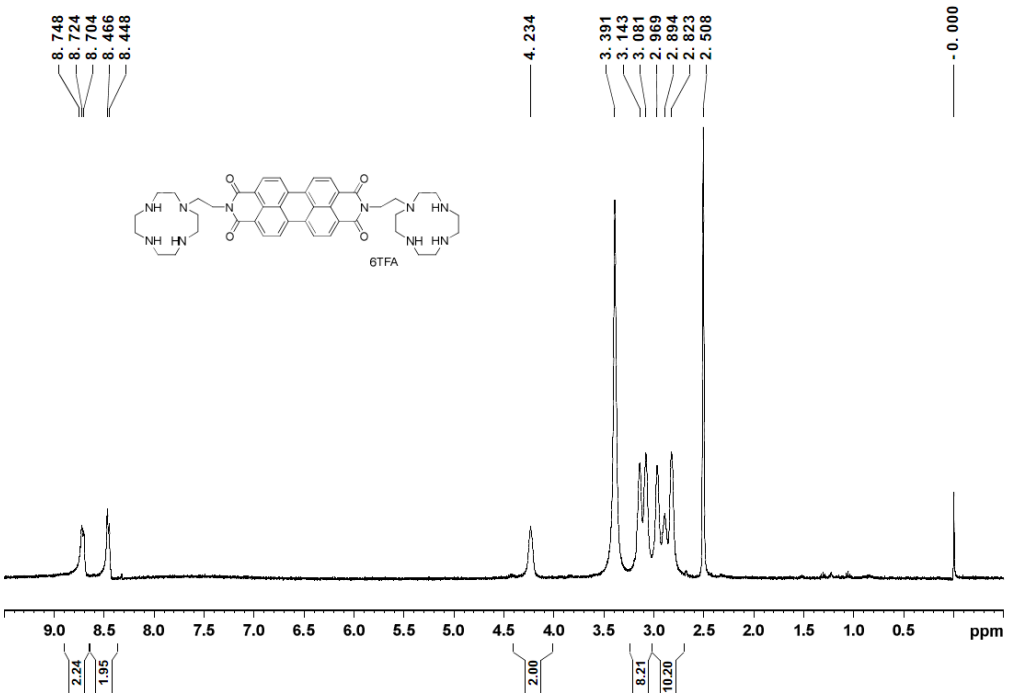
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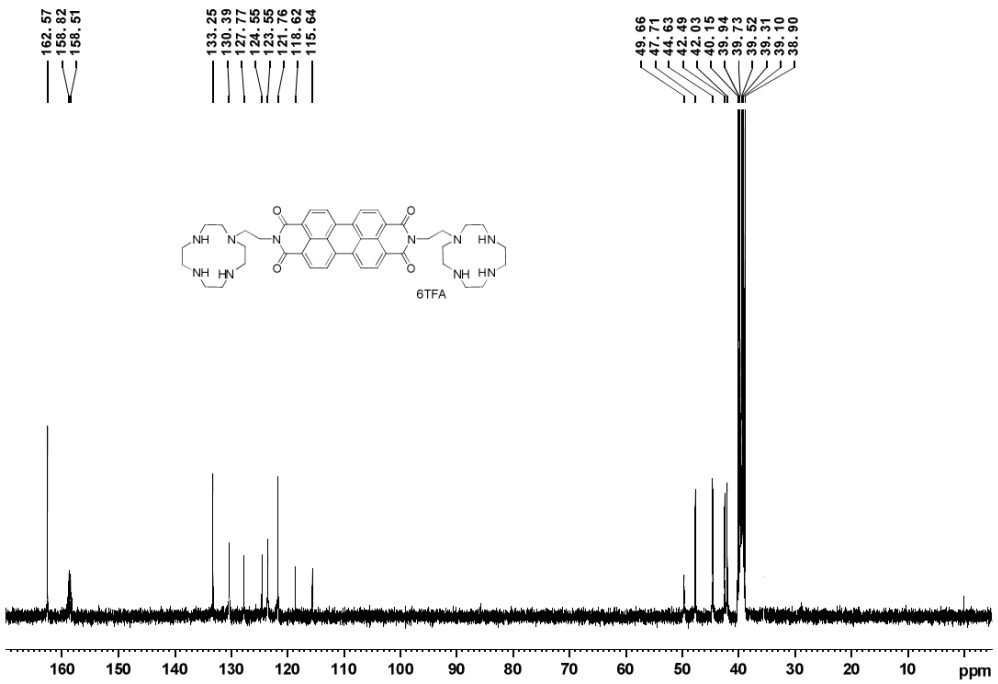
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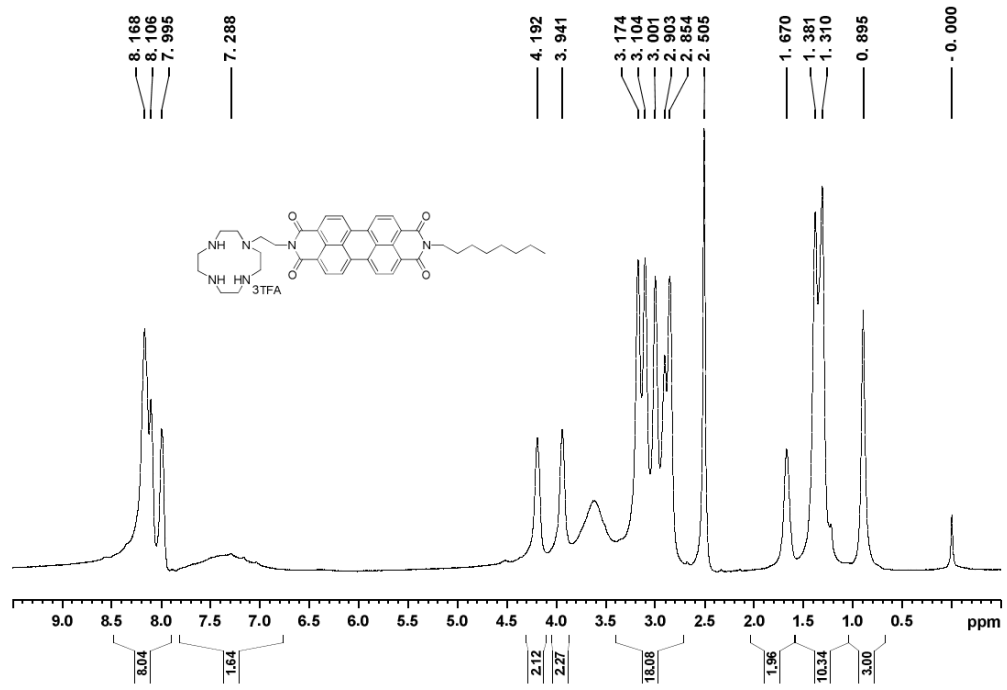
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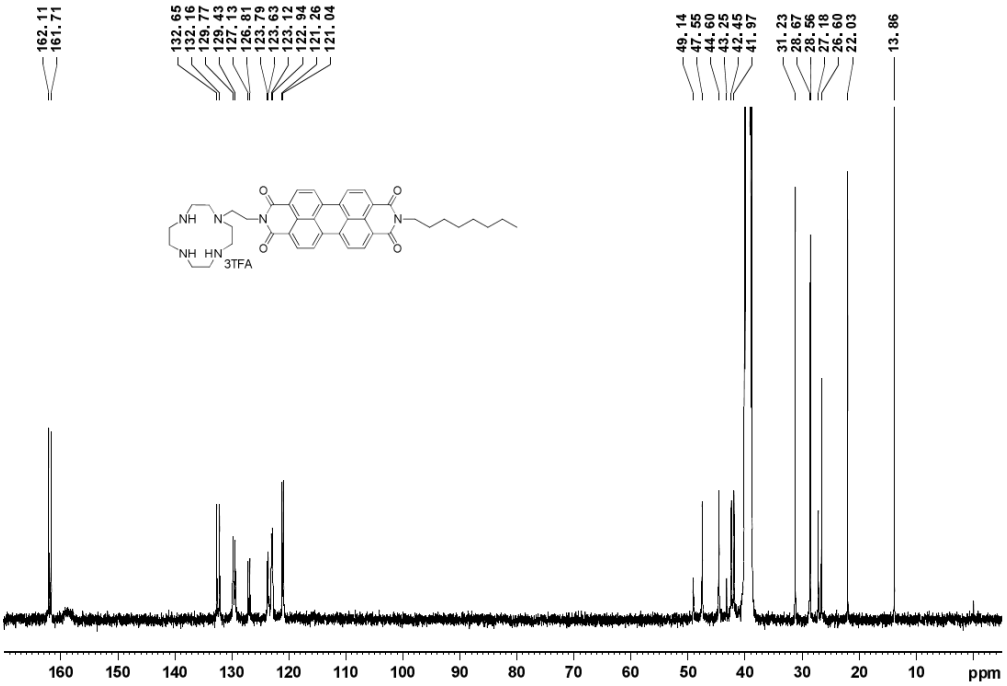
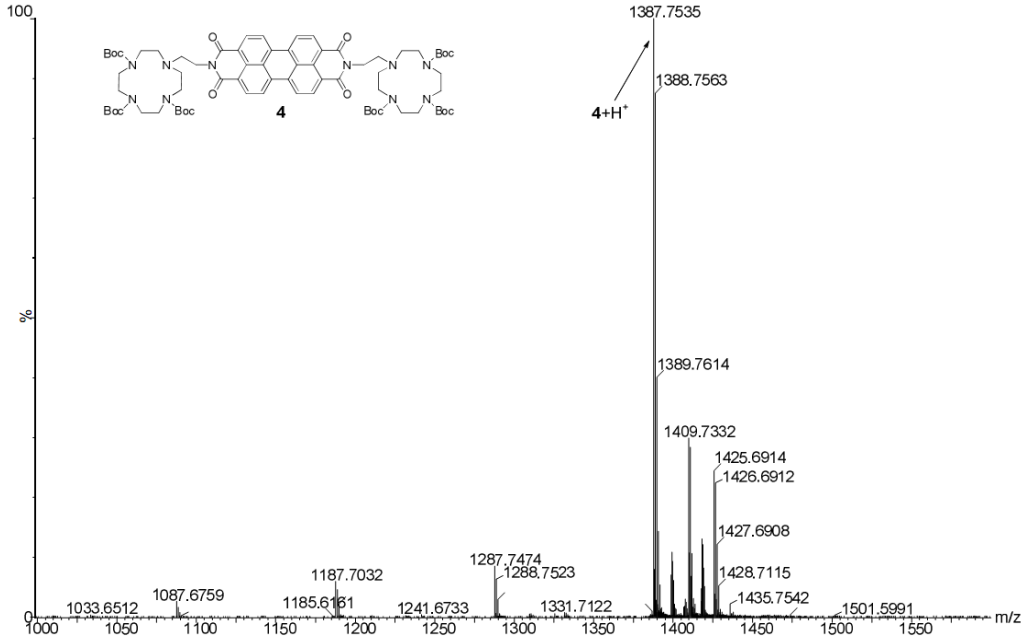
1.64
2.12
2.27
18.08
1.96
10.34
3.00


162.11
161.71
132.65
132.16
129.77
129.43
127.13
126.81
123.79
123.63
123.12
122.94
121.26
121.04
49.14
47.55
44.60
43.25
42.45
41.97
31.23
28.67
28.56
27.18
26.60
22.03
13.86
NH
N
N
N
NH HN
3TFA
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


4
4+H+
1387.7535
1388.7563
1389.7614
1409.7332
1425.6914
1426.6912
1427.6908
1428.7115
1435.7542
1501.5991
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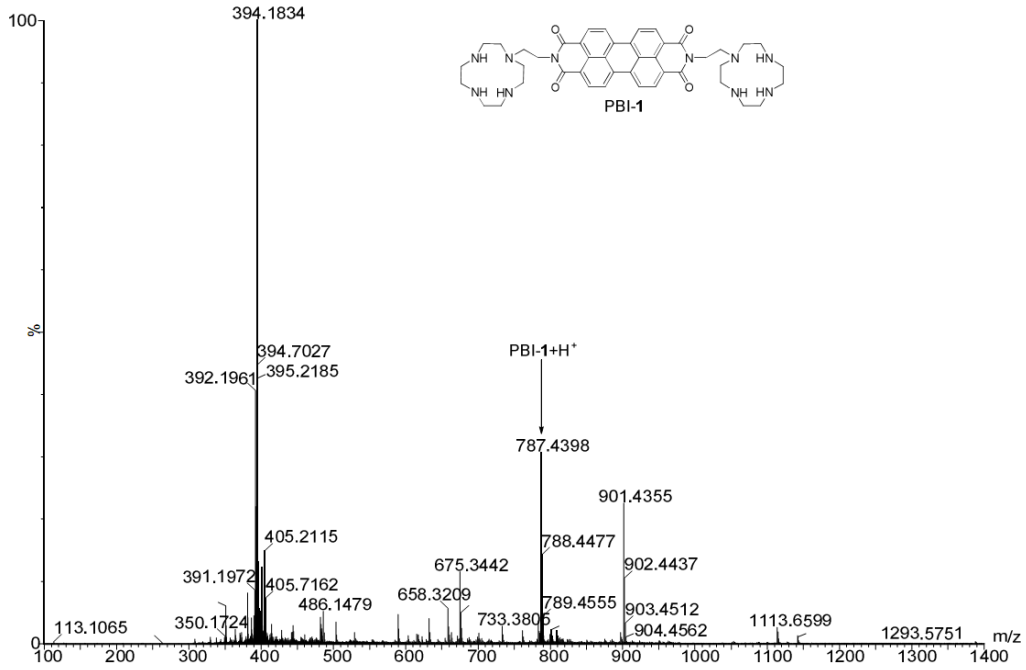
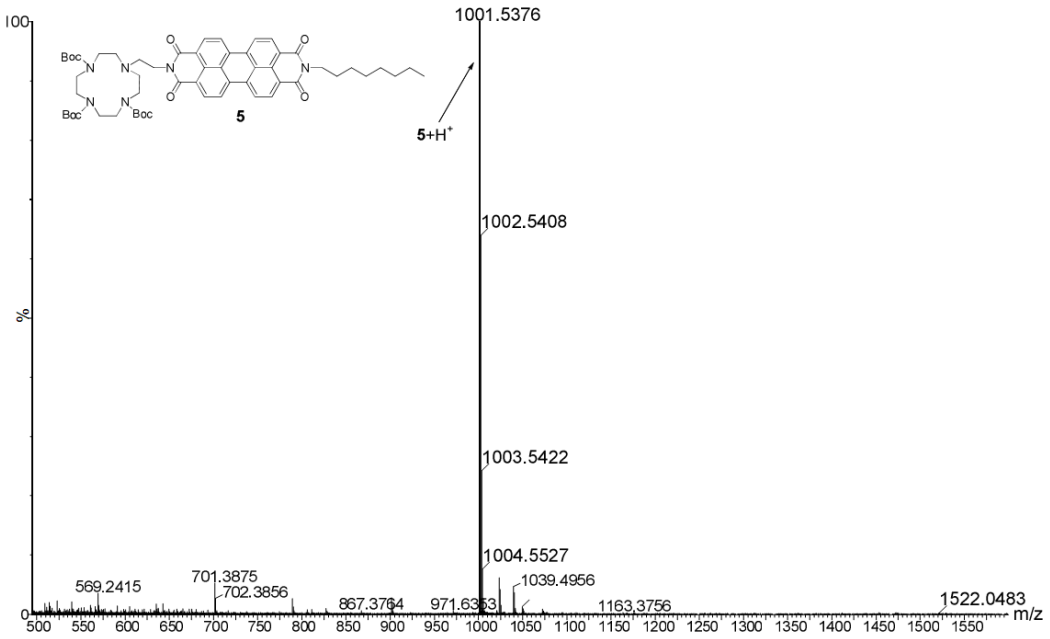
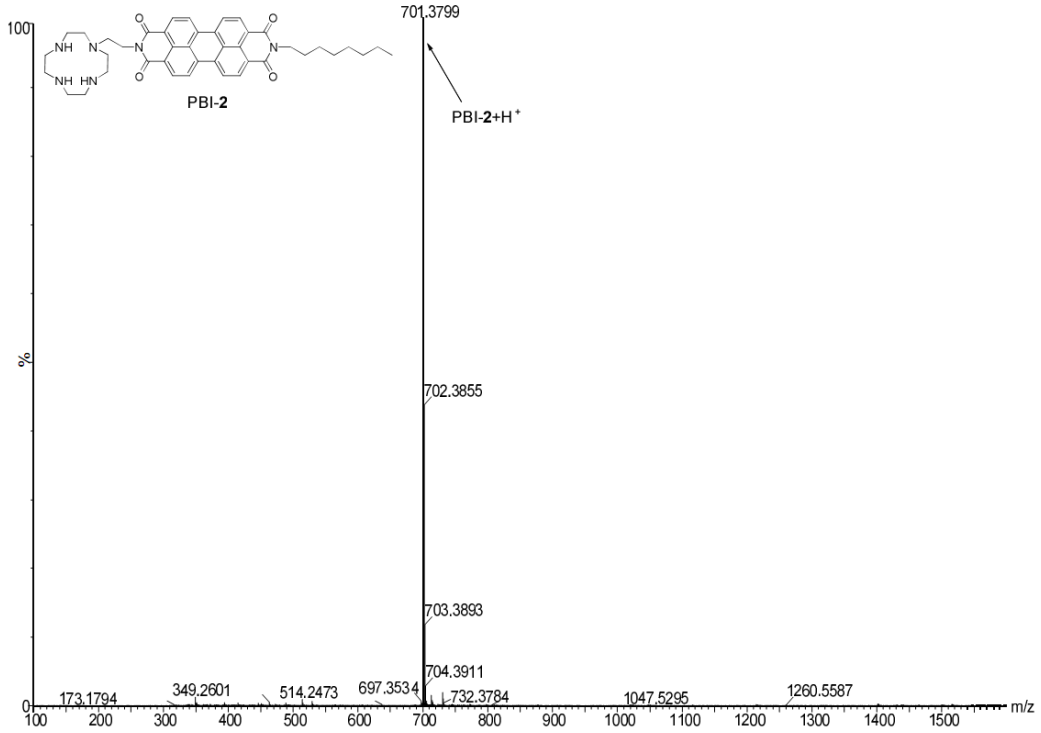
1187.7032
1087.6759
1033.6512
1185.6161
1241.6733
1331.7122
m/z


PBI-1
PBI-1+H+
394.1834
394.7027
395.2185
392.1961
787.4398
901.4355
405.2115
788.4477
675.3442
902.4437
391.1972
405.7162
658.3209
486.1479
789.4555
903.4512
733.3805
904.4562
113.1065
350.1724
1113.6599
1293.5751
m/z


100
%
0
Boc
Boc
Boc
Boc
5
5+H+
1001.5376
1002.5408
1003.5422
1004.5527
1039.4956
569.2415
701.3875
702.3856
867.3764
971.6353
1163.3756
1522.0483
500 550 600 650 700 750 800 850 900 950 1000 1050 1100 1150 1200 1250 1300 1350 1400 1450 1500 1550
m/z


100
%
0
NH
NH HN
PBI-2
PBI-2+H+
701.3799
702.3855
703.3893
704.3911
173.1794
349.2601
514.2473
697.3534
732.3784
1047.5295
1260.5587
100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500
m/z