Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Dibenzo[a,c]phenazine-derived near-infrared fluorescence biosensor for detection of lysophosphatidic acid based on the aggregation-induced emission

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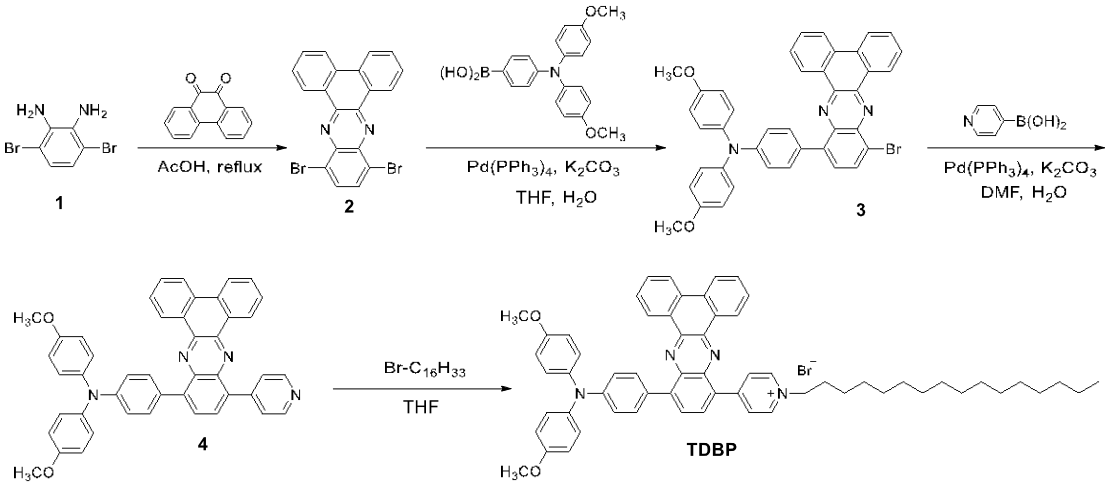
S1. Materials and Measurements

N, *N*-dimethylformamide (DMF) was refluxed with calcium hydride and distilled before use. Tetrahydrofuran (THF) was pre-dried over 4 A° molecular sieves and distilled under an N_2 atmosphere from sodium benzophenone ketyl immediately before use. 3, 6-dibromobenzene-1,2-diamine (compound **1**)[1] and (4-(bis(4-methoxyphenyl)amino)phenyl)boronic acid[2] were prepared according to the previous published literatures. All others chemicals were purchased from commercial sources and used without further purification. Lysophosphatidic acid (LPA) was purchased from the aladdin. The solutions for analytical studies were prepared with deionized water treated with a Milli-Q System (Billerica, MA, USA).

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 400MHz spectrometer with tetramethyl silane (TMS) as the internal reference. Electrospray ionization and time-of-flight analyzer (ESI-TOF) mass spectra were carried out on a Waters Micromass LCT mass spectrometer. Absorption spectra were measured with a Varian Cary 500 UV-Vis spectraphotometer. Fluorescence spectra were measured with a Horiba scientific FluoroMax-4 spectrofluorometer. The SEM micrographs were recorded with a JEOL JSM-6360 scanning electron microscope (SEM). Zeta potentials were recorded on Zeta-size 3000 HS.



Scheme S1. Synthetic route of the compound **TDBP**.

Synthesis of 2. In a 100 mL two-necked round-bottom flask, compound **1** (2.26 g, 8.5 mmol) and phenanthrene-9,10-dione (1.76 g, 8.5 mmol) were dissolved in 25 mL acetic acid under N_2 atmosphere and refluxed for 12 h. Upon cooling, the mixture solution was concentrated using a rotary evaporator and the obtained product was washed by the DCM for three times to afford the pure compound **2** (2.41 g, 65%). ^{1}H NMR ($CDCl_3$, 400 MHz, TMS), δ: 9.48 (d, *J* = 9.0 Hz, 2H), 8.58 (d, *J* = 8.0 Hz, 2H), 8.03 (s, 2H), 7.85 (t, *J* = 8.2 Hz, 2H), 7.79 (t, *J* = 7.2 Hz, 2H).

Synthesis of 3. In a 50 mL two-necked round-bottom flask, compound **2** (435.9 mg, 1 mmol) and $Pd(PPh_3)_4$ (30.0 mg, 0.026 mmol) were dissolved in 15 mL THF under N_2 atmosphere, and then added 5 mL 2 M potassium carbonate aqueous solution to the mixture. After stirring for 30 min at 50 °C, (4-(bis(4-methoxyphenyl)amino)phenyl)boronic acid (433.2 mg, 1 mmol) dissolved in 10 mL THF was dropwise added into the mixture and stirred for another 5 h. Upon cooling, the mixture was poured into saturated brine and extracted with DCM (50 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated using a rotary evaporator. The residue was purified by column chromatography on silica (PE/DCM = 4/1–2/1, v/v) to afford the product as a yellow solid (211.6 mg, 32%). ^{1}H NMR ($CDCl_3$, 400 MHz, TMS), δ: 9.53 (d, *J* = 7.5Hz, 1H), 9.14 (d, *J* = 8.4 Hz, 1H), 8.57 (s, 2H), 8.21 (s, 1H), 7.86 – 7.64 (m, 7H), 7.22 (d, *J* = 8.9 Hz, 4H), 7.15 (d, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 8.9 Hz, 4H), 3.84 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz, TMS), δ: 156.06, 148.57, 142.07, 141.96, 140.82, 140.44, 140.13, 139.67, 132.94, 132.24, 132.20, 131.75, 130.62, 130.38, 130.05, 129.93, 129.59, 128.96, 128.06, 127.98, 126.93, 126.80, 122.85, 122.81, 122.45, 119.57, 114.77, 55.55. HRMS (ESI) (m/z): [$(M+H)^+$] calcd for $C_{40}H_{28}BrN_3O_2$, 662.1443, found: 662.1438.

Synthesis of 4. In a 50 mL two-necked round-bottom flask, compound **3** (99.2 mg, 0.15 mmol) and $Pd(PPh_3)_4$ (30.0 mg, 0.026 mmol) were dissolved in 20 mL DMF under N_2 atmosphere, and then added 5 mL 2 M potassium carbonate aqueous solution to the mixture. After stirring for 30 min at 60 °C, pyridin-4-ylboronic acid (61.5 mg, 0.5 mmol) dissolved in 5 mL H_2O was dropwise added into the mixture and refluxed for another 10 h. Upon cooling, the mixture was poured into saturated brine and extracted with DCM (50 mL × 3). The combined organic layer was washed five times by the water and then dried over anhydrous $MgSO_4$ and concentrated using a rotary evaporator. The residue was purified by column chromatography on silica (DCM/Ethanol = 40/1–20/1, v/v) to afford the product as a red solid (67.4 mg, 68%). ^{1}H NMR ($CDCl_3$, 400 MHz, TMS), δ: 9.17 (d, *J* = 8.0 Hz, 1H), 9.08 (d, *J* = 9.2 Hz, 1H), 8.85 (d, *J* = 6.0 Hz, 2H), 8.56 (d, *J* = 8.0 Hz, 2H), 8.00 (s, 2H), 7.92 (d, *J* = 6.0 Hz, 2H), 7.86 (d, *J* = 8.7 Hz, 2H), 7.79 (t, *J* = 8.2 Hz, 2H), 7.70 (t, *J* = 7.8 Hz, 2H), 7.24 (d, *J* = 9.0 Hz, 4H), 7.18 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 9.0 Hz, 4H), 3.84 (s, 6H). ^{13}C NMR ($CDCl_3$, 100MHz, TMS), δ: 156.10, 149.25, 148.65, 146.80, 141.46, 141.38, 141.10, 140.79, 139.87, 139.64, 135.74, 132.22, 132.15, 132.12, 132.05, 131.87, 130.36, 130.26, 130.19, 130.04, 129.82, 128.59, 128.47, 128.04, 127.99, 127.00, 126.59, 126.39, 125.91, 122.86, 119.44, 114.80, 55.55. HRMS (ESI) (m/z): [$(M+H)^+$] calcd for $C_{45}H_{32}N_4O_2$, 661.2604, found: 661.2600.

Synthesis of TDBP. In a 50 mL two-necked round-bottom flask, compound **7** (330.1 mg, 0.5 mmol) and 1-bromohexadecane (456.3 mg, 1.5 mmol) were dissolved in 20 mL THF under N_2 atmosphere and then stirred at 70 ℃ for 72 hours. Upon cooling, the mixture was poured into saturated brine and extracted with DCM (30 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated using a rotary evaporator. The residue was purified by column chromatography on silica (DCM/Ethanol = 10/1, v/v) to afford the product as a purple solid (106.1 mg, 22%). ^{1}H NMR ($CDCl_3$, 400 MHz, TMS), δ: 9.57 (d, *J* = 5.5 Hz, 2H), 8.60 (d, *J* = 8.0 Hz, 1H), 8.52 (d, *J* = 5.5 Hz, 2H), 8.43 (d, *J* = 8.2 Hz, 1H), 8.09 – 7.91 (m, 3H), 7.64 (dd, *J* = 16.8, 7.5 Hz, 3H), 7.52 – 7.36 (m, 4H), 7.12 (d, *J* = 8.9 Hz, 4H), 6.98 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 4H), 5.14 (*J* = 4.8 Hz, 2H), 3.83 (s, 6H), 2.16 – 2.04 (m, 2H), 1.28 – 1.15 (m, 26H), 0.85 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz, TMS), δ: 155.10, 152.28, 147.57, 143.03, 142.16, 139.55, 139.29, 137.22, 136.92, 130.76, 130.52, 130.16, 128.09, 127.83, 127.62, 127.33, 127.10, 126.02, 121.09, 117.32, 113.74, 54.48, 45.00, 31.30, 30.89, 28.70, 28.64, 28.56, 28.34, 25.30, 21.66, 13.11, 7.68. HRMS (ESI) (m/z): [(M-Br)$^+$] calcd for $C_{61}H_{65}N_4O_2$, 885.5102, found: 885.5103.

S2. Quantification of LPA in the fetal bovine serum (FBS)

For the quantification measurements, a stock solution (1×10^{-3} M) of probe **TDBP** was prepared by dissolving 4.82 mg of **TDBP** into 5 mL of DMSO. A stock solution (1×10^{-3} M) of LPA was dissolved in the pure HEPES buffer solution and fetal bovine serum solution for the spiked and recovered concentration of LPA, respectively. All the measurements were conducted in the DMSO/water (1/1, v/v) solution at the concentration of 100 μM of **TDBP.** Fetal bovine serum was added saturated ammonium sulfate to remove protein by salting out effect and centrifugal operation prior to use.

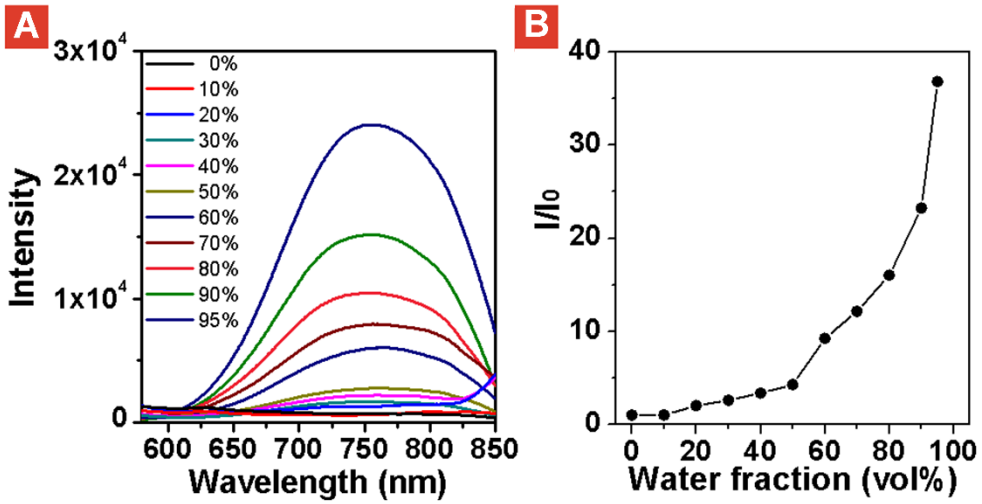


Fig. S1 (A) Fluorescence emission spectra of **TDBP** in DMSO and DMSO/water mixtures with different water fractions (f_w). (B) Plots of relative emission intensity (I/I_0) versus the composition of the DMSO/water mixtures of **TDBP**; I_0 = emission intensity in pure DMSO solution. Solution concentration: 1 ×10^{-4} M; λ_{ex}: 530 nm.

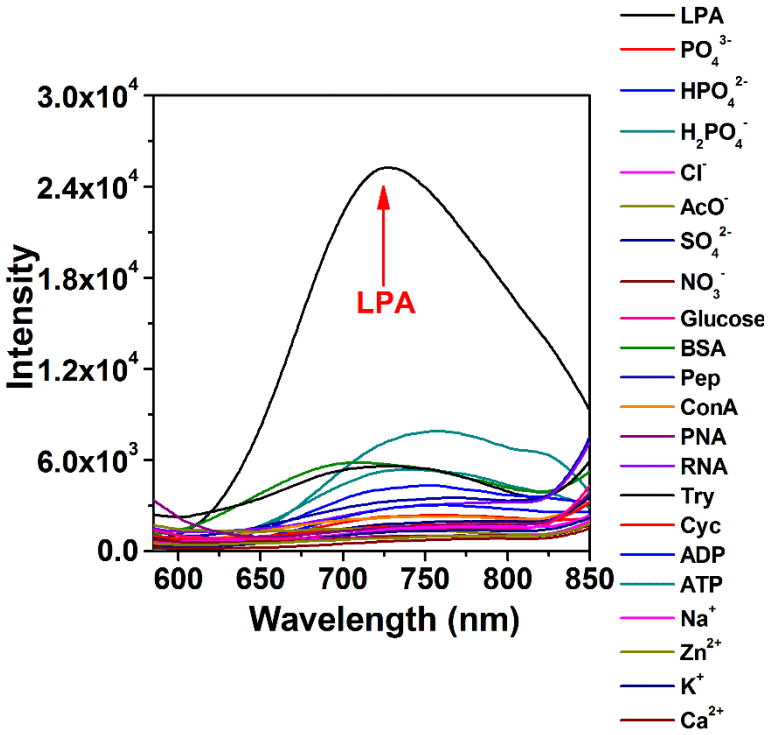


Fig. S2. The fluorescence spectra of **TDBP** (100 μM) to LPA (100 μM) in the presence of various proteins and miscellaneous interference cations and anions (100 μM), λ_{ex}: 530 nm.

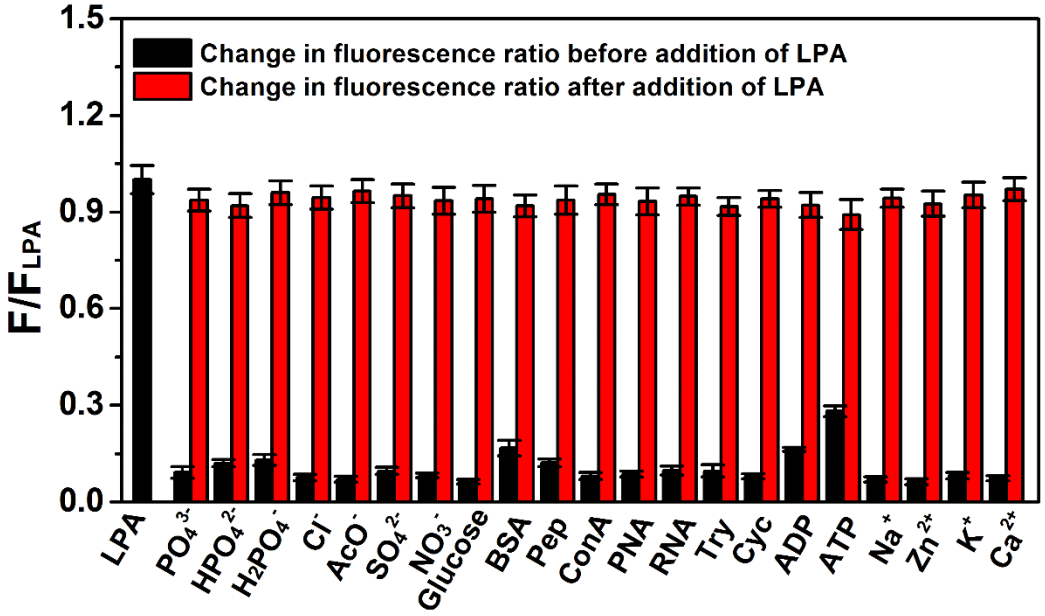
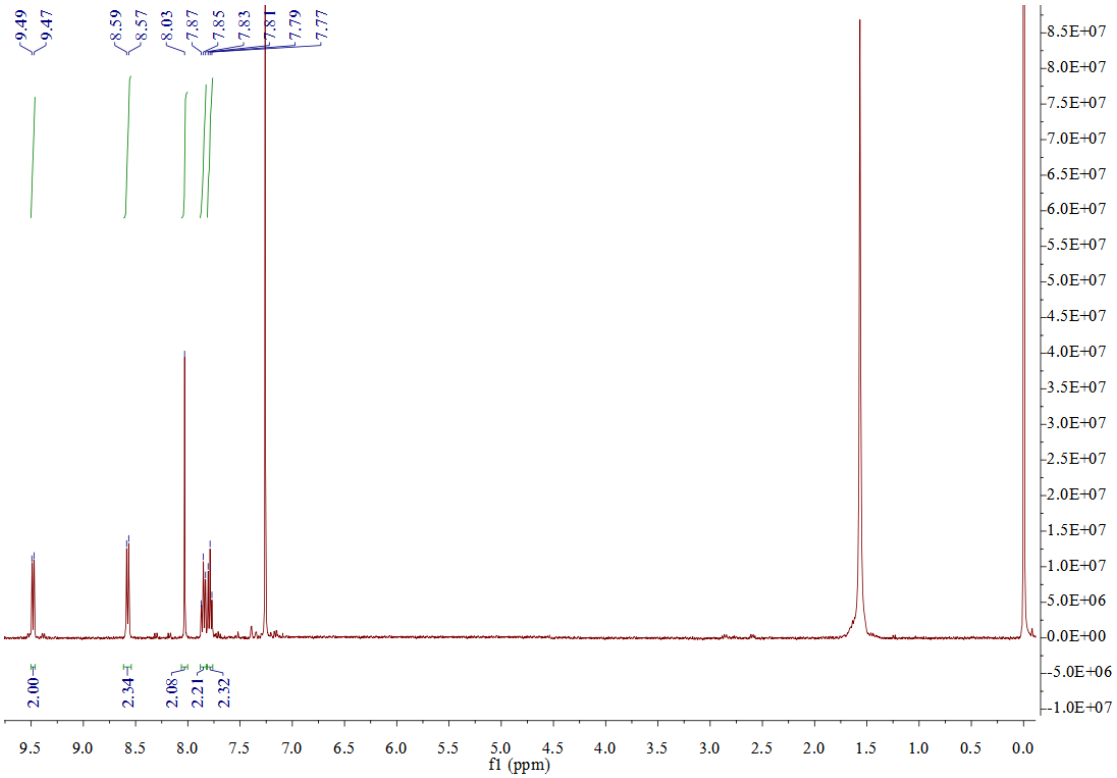


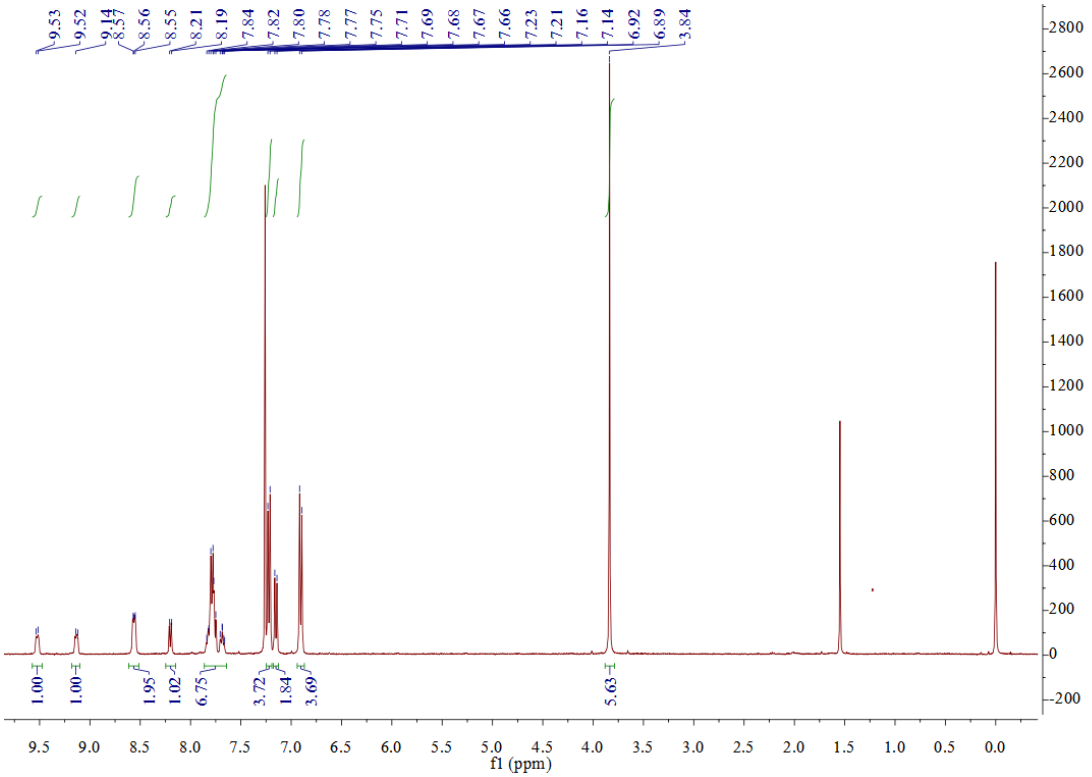
Fig. S3 Change in fluorescence ratio (F/F_{LPA}) of **TDBP** (100 μM) upon addition of 1 equiv. amounts of LPA in the presence of other interfere substrates (1 equiv.) in DMSO/H_2O mixtures (1/1, v/v). F/F_{LPA} represents the fluorescence intensity ratio of other interfere substrates (F) and LPA (F_{LPA}), λ_{ex}: 530 nm.

S6. Characterization

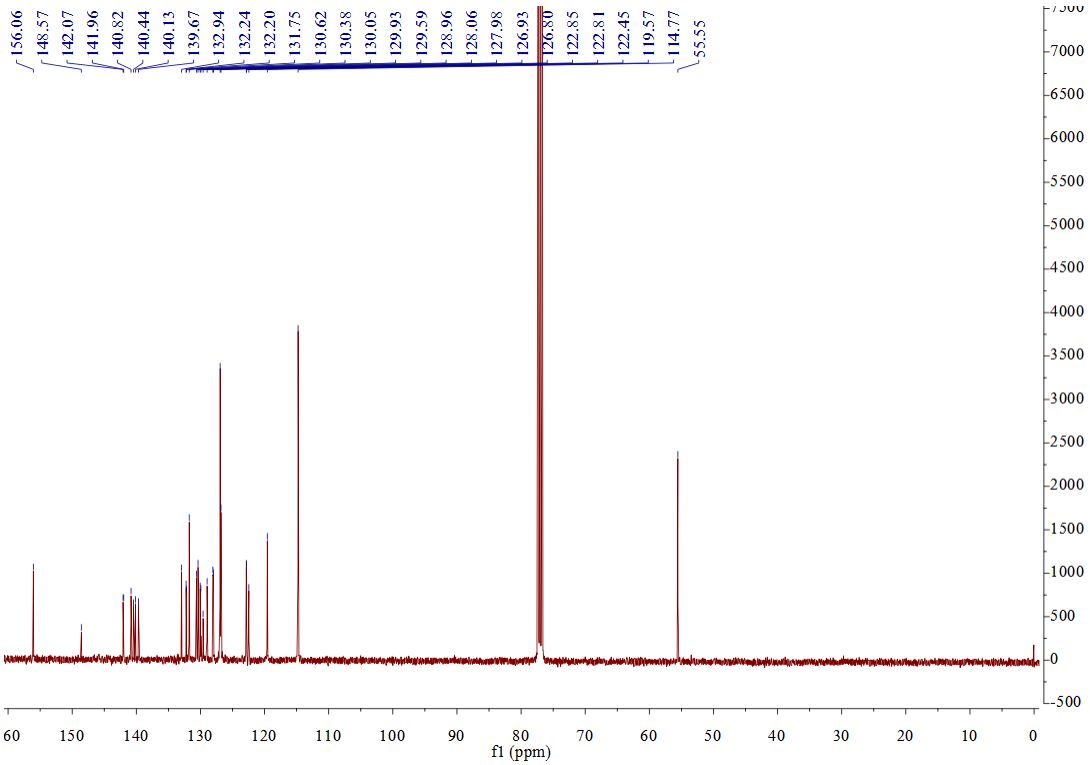
^{1}H NMR of compound 2



^{1}H NMR of compound 3



^{13}C NMR of compound 3



High-Res ESI-TOF mass spectrum of compound 3

Elemental Composition Report **Page 1**

Single Mass Analysis
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Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
43 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-40 H: 0-100 N: 0-3 O: 0-2 Br: 0-1

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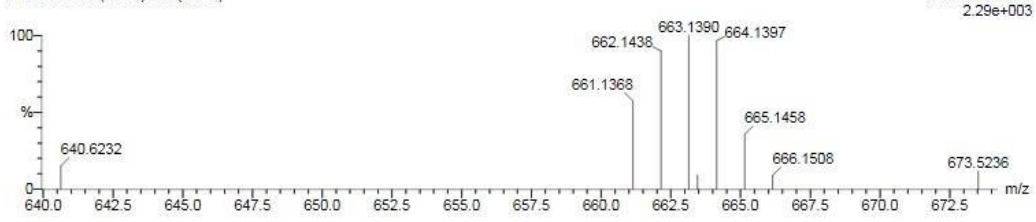
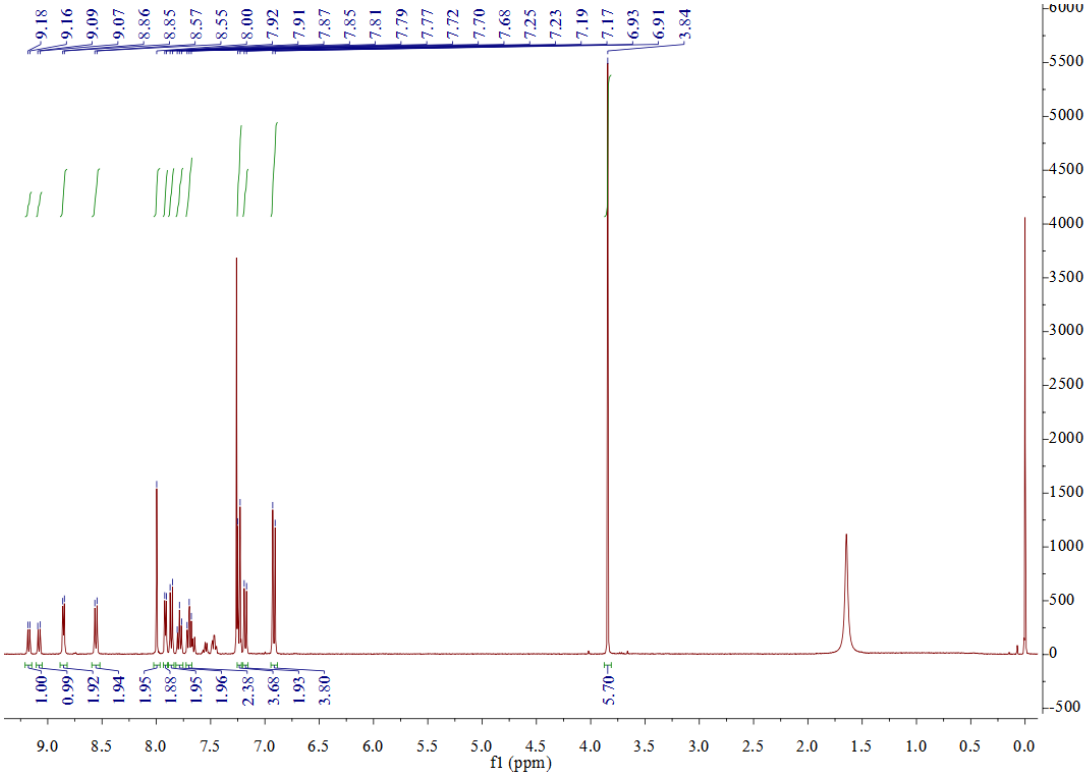
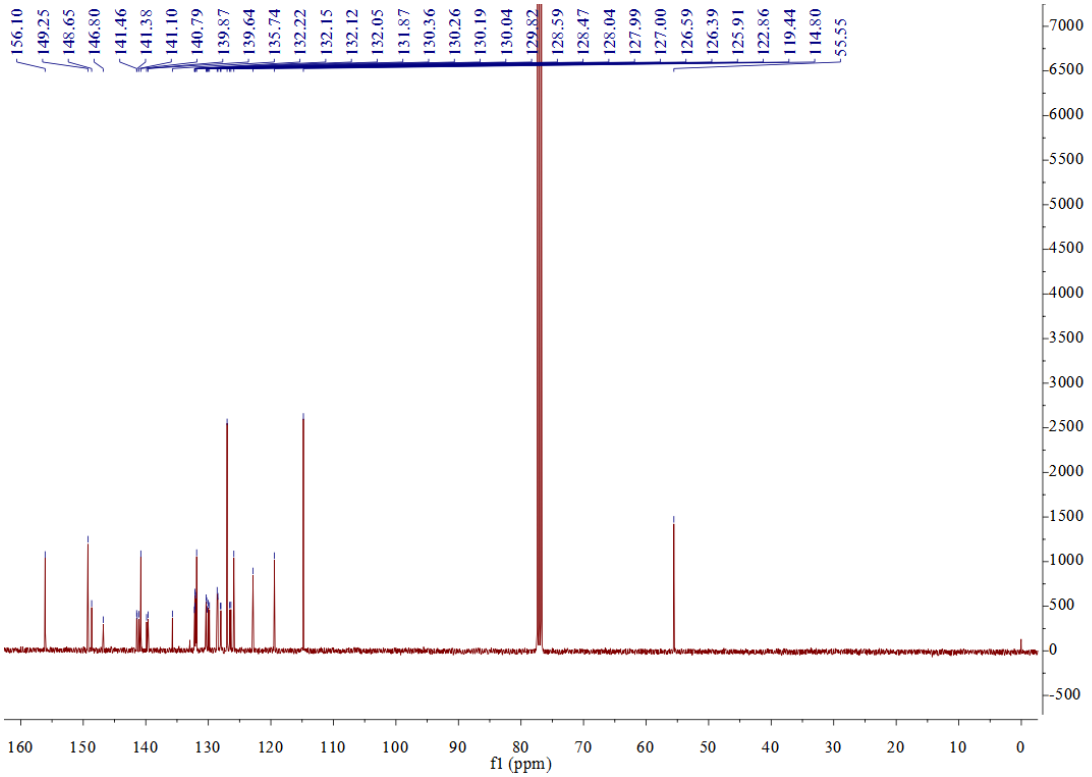


Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
662.1438	662.1443	-0.5	-0.8	27.5	31.5	0.0	C40 H29 N3 O2 Br

^{1}H NMR of compound 4



^{13}C NMR of compound 4



High-Res ESI-TOF mass spectrum of compound 4

Elemental Composition Report **Page 1**

Single Mass Analysis
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Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
85 formula(e) evaluated with 7 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-61 H: 0-66 N: 0-4 O: 0-2

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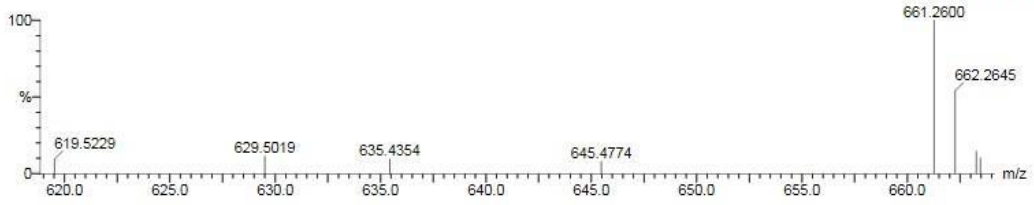
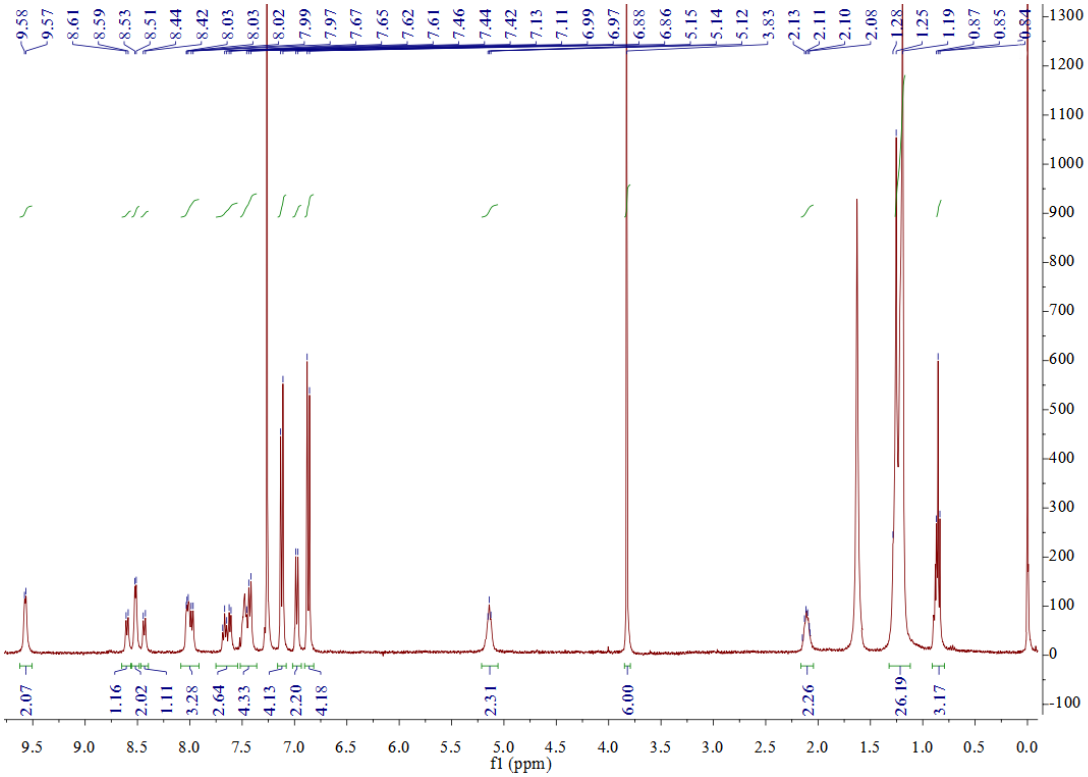
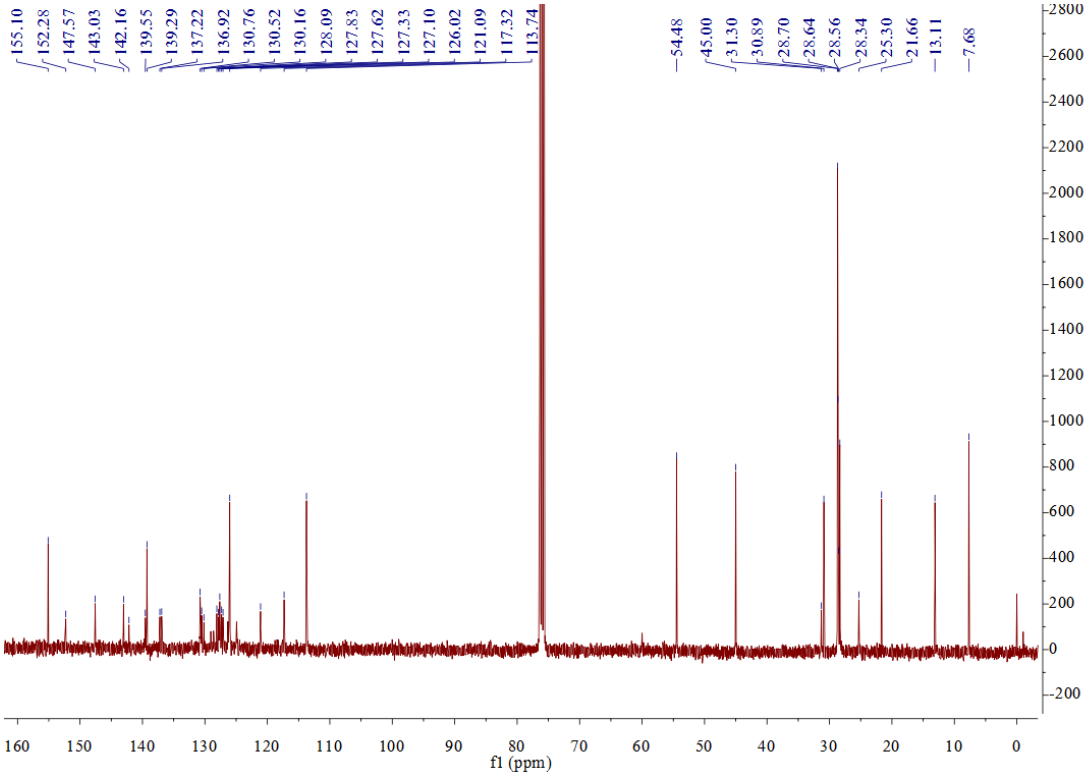


Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
661.2600	661.2604	-0.4	-0.6	31.5	8.4	0.0	C45 H33 N4 O2

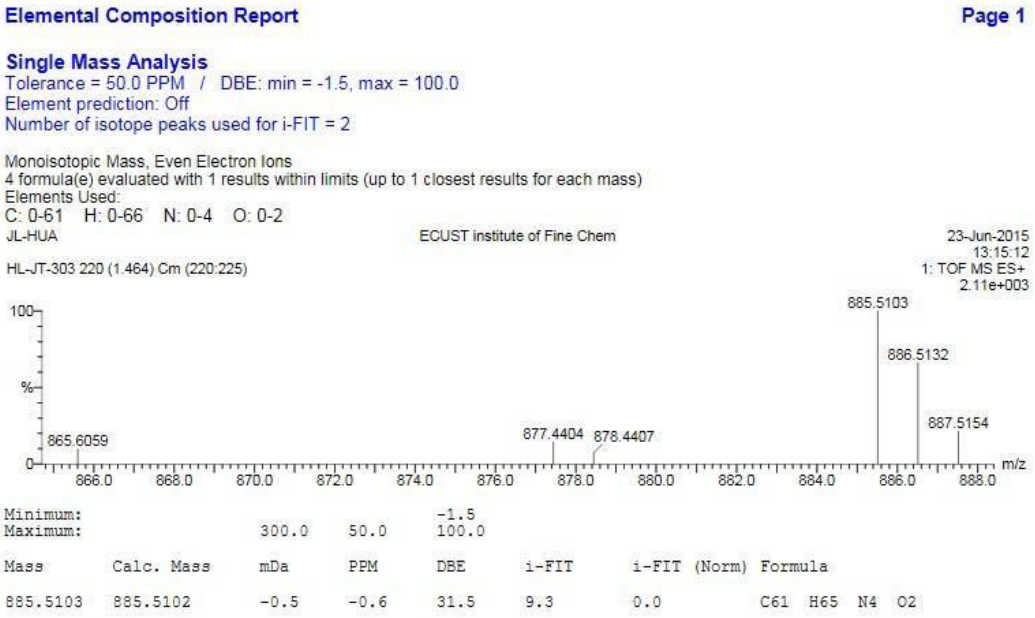
^{1}H NMR of compound TDBP



^{13}C NMR of compound TDBP



High-Res ESI-TOF mass spectrum of compound TDBP



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
4 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-61 H: 0-66 N: 0-4 O: 0-2

JL-HUA ECUST institute of Fine Chem 23-Jun-2015 13:15:12
HL-JT-303 220 (1.464) Cm (220:225) 1: TOF MS ES+ 2.11e+003

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885.5103	885.5102	-0.5	-0.6	31.5	9.3	0.0	C61 H65 N4 O2

S7. References

1. H. N. Tsao, C. Yi, T. Moehl, J. H. Yum, S. M. Zakeeruddin, M. K. Nazeeruddin and M. Grätzel, *ChemSusChem*, 2011, **4**, 591.
2. Y. D. Hang, X. P. He, L. Yang and J. L. Hua, *Biosens. Bioelectron*, 2015, **65**, 420.