Supporting information

Carbazole Substituted 2-Aminobenzamide Compounds: Synthesis, Fluorescence ON-OFF-ON Sensing of Zn(II) and PPi Ions, Assay for Alkaline Phosphatase, and Computational Study

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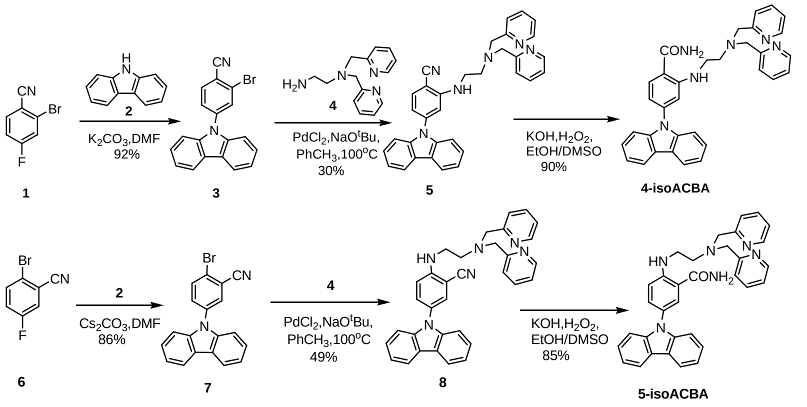
Experimental Section

General. All chemicals were purchased as reagent grade and used without further purification. Buchwald–Hartwig cross-coupling reactions were performed in flame-dried glassware under argon. Toluene was distilled from calcium hydride. The reactions were monitored by analytical thin-layer chromatography (TLC) on silica gel F_{254} glass plates and visualized under UV light (254 and 365 nm) and/or by staining with ninhydrin. Flash column chromatography was performed on silica gel (200–300 mesh). ^{1}H NMR spectra were recorded with a Bruker Avance III 400 MHz NMR spectrometer at 20 °C. Chemical shifts (in ppm) were determined relative to tetramethylsilane ($\delta = 0$ ppm) in deuteriated solvents. Coupling constants in Hz were measured from the one-dimensional spectra. ^{13}C NMR or ^{13}C attached-protontest (^{13}C-Apt) spectra were recorded with the 400 MHz NMR spectrometer (100 MHz) and calibrated with $CDCl_3$ ($\delta = 77.23$ ppm). High-resolution mass spectra were recorded with Waters LCT Premier XE or Bruker micrOTOF-QII mass spectrometer. Low-resolution mass spectra were determined by Agilent 7890A GC-5975CMS. UV-vis absorption and emission spectra were recorded with a GBC Cintra 10e UV/Vis spectrophotometer and a Varian Cary Eclipse spectro-fluorimeter, respectively, in a quartz cell with a 1 cm path length.



2-bromo-4-carbazolylbenzonitrile (3):

Carbazole (167 mg, 1.00 mmol, 1.00 equiv.) was dissolved in DMF (4 ml), and 2-bromo-4-fluorobenzonitrile (200 mg, 1.00 mmol, 1.00 equiv.) and K_2CO_3 (276 mg, 2.00 mmol, 2.00 equiv.) were added. The reaction mixture was stirred in a 60 °C oil bath until the starting material had been completely consumed as detected by TLC. The solution was then allowed to cool to room temperature, and the DMF was evaporated under vacuum to leave a yellowish oil. The crude oil was then diluted with DCM (150 mL), washed with saturated NaCl solution, and dried with $MgSO_4$. After removal of the solvent, the mixture was purified by column chromatography (Hexanes/EtOAc = 12/1) to give **3**

(319 mg, 0.92 mmol, 92%) as a colorless solid. Mp. 193–194 °C. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.14 (d, J = 7.8 Hz, 2 H), 7.99 (d, J = 1.9 Hz, 2 H), 7.90 (d, J = 8.3 Hz, 1 H), 7.71 (dd, J = 8.3, 2.0 Hz, 1 H), 7.44-7.49 (m, 4 H), 7.34–7.38 (m, 2 H). ^{13}C-Apt (100 MHz, CDCl$_3$): δ 142.9, 139.6, 135.5, 130.7, 126.6, 125.4, 124.2, 121.4, 120.7, 116.9, 113.7, 109.5. MS (ESI): *m/z* [M]$^+$ 346.1. Anal. Calcd for $C_{19}H_{11}BrN_2 \cdot 1/3H_2O$: C, 64.60; H, 3.33; N, 7.93. Found: C, 64.94; H, 3.31; N, 7.59.

2-({2-[bis(pyridin-2-ylmethyl)amino]ethyl}amino)-4-carbazolylbenzamide (4-isoACBA):

A Schlenk flask was charged with compound 3 (108 mg, 0.31 mmol, 1.00 equiv.), BPEA **4** (76 mg, 0.31 mmol, 1.00 equiv.), sodium *tert*-butoxide (75 mg, 0.78 mmol, 2.50 equiv.), palladium chloride (11 mg, 0.06 mmol, 0.20 equiv.), 1,1'-bis(diphenylphosphanyl)ferrocene (DPPF; 33 mg, 0.06 mmol, 0.20 equiv.), and toluene (10 mL) under argon. The flask was immersed in an oil bath at 100 °C with stirring until the starting material had completely disappeared as judged by TLC analysis. The solution was then allowed to cool to room temperature, diluted with DCM (100 mL), filtered through Celite, and concentrated. The crude product was purified by column chromatography (CH_2Cl_2 /MeOH = 40/1) on silica gel to give **5** (48 mg, 0.094 mmol, 30%) as a dark-red viscous oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ 2.92 (t, J = 5.84 Hz, 2 H), 3.21 (d, J = 4.60, 2 H), 3.92 (s, 4 H), 6.04 (bs, 1 H), 6.71 (d, J = 1.48 Hz, 1 H), 6.86 (dd, J = 8.24, 1.68 Hz, 1 H), 7.38-7.43 (m, 4 H), 7.28-7.31 (m, 2 H), 7.61 (d, J = 7.64 Hz, 3 H), 7.69 (td, J = 7.72, 1.60 Hz, 2 H), 8.12 (d, J = 7.76 Hz, 2 H). KOH (48 mg, 0.86 mmol, 9.15 equiv.) and compound **5** (48 mg, 0.094 mmol, 1.00 equiv.) were added to a solution of EtOH and DMSO (4/1, 1.5 mL). The reaction mixture was stirred in a 40 °C oil bath, and H_2O_2 (30%, 0.3 mL) was slowly added dropwise through a syringe over 0.5 h. Then the resulting solution was stirred for another 2.0 h until the disappearance of benzonitrile as shown by TLC. After removal of the solvent by rotary evaporation, the mixture was diluted with DCM (150 mL), washed with saturated NaCl solution, dried with $MgSO_4$, filtered, and concentrated. The mixture was purified by column chromatography (CH_2Cl_2/MeOH = 20/1) to give 4-isoACBA (45 mg, 0.085 mmol, 90%) as a pale yellow solid. Mp. 75–77 °C. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.43 (d, J = 4.4 Hz, 2 H), 8.14 (d, J = 7.8 Hz, 2 H), 7.74 (dd, J = 7.6, 12.4 Hz, 3 H), 7.41 (d, J = 4.1 Hz, 4 H), 7.27–7.29 (m, 4 H), 7.06 (t, J = 5.4 Hz, 2 H), 6.77 (dd, J = 8.3, 1.8 Hz, 1 H), 6.72 (s, 1 H), 3.93 (s, 4 H), 3.28 (s, 2 H), 2.95 (t, J = 5.9 Hz, 2 H), 2.90 (s, 2 H). ^{13}C-Apt (100 MHz, CDCl$_3$): δ 171.3, 159.0, 151.2, 148.8, 142.3, 140.4, 136.6, 129.9, 126.0, 123.6, 123.2, 122.1, 120.3, 120.1, 112.6, 112.4, 109.6, 60.6, 52.6, 40.6, 29.7. HRMS (ESI-TOF): *m/z* Calcd for $C_{33}H_{31}NO_6$ $[M+H]^+$ 527.2599, found: 527.2561.

2-bromo-5-carbazolylbenzonitrile (7):

Carbazole (167 mg, 1.00 mmol, 1.00 equiv.) was dissolved in DMF (4 ml), and 2-bromo-5-fluorobenzonitrile (300 mg, 1.50 mmol, 1.50 equiv.) and Cs_2CO_3 (648 mg, 2.00 mmol, 2.00 equiv.) were added. The reaction mixture was stirred at room temperature for 30 min and then heated in an 80 °C oil bath until the starting material had been completely consumed as detected by TLC. The solution was then allowed to cool to room temperature, and the DMF was evaporated under vacuum to leave a yellowish oil. The crude oil was then diluted with DCM (150 mL), washed with saturated NaCl solution, and dried with $MgSO_4$. After removal of the solvent, the mixture was purified by column chromatography (hexanes/EtOAc = 12/1) to give **7** (300 mg, 0.86 mmol, 86%) as a colorless solid. Mp. 125–127 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 8.14 (d, *J* = 7.7 Hz, 2 H), 7.90-7.93 (m, 2 H), 7.69-7.72 (m, 1 H), 7.42-7.46 (m, 2 H), 7.32-7.37 (m, 4 H). ^{13}C-Apt (100 MHz, $CDCl_3$): δ 140.1, 137.8, 134.9, 132.4, 132.2, 126.5, 123.9, 123.3, 121.1, 120.7, 117.6, 116.4, 109.2. MS (ESI): *m/z* $[M]^+$ 346.0. Anal. Calcd for $C_{19}H_{11}BrN_2$: C, 65.73; H, 3.19; N, 8.07. Found: C, 65.90; H, 3.09; N, 8.11.

2-({2-[bis(pyridin-2-ylmethyl)amino]ethyl}amino)-4-carbazolylbenzamide (5-isoACBA):

A Schlenk flask was charged with compound 7 (149 mg, 0.43 mmol, 1.05 equiv.), BPEA (99 mg, 0.41 mmol, 1.00 equiv.), sodium *tert*-butoxide (99 mg, 1.03 mmol, 2.50 equiv.), palladium chloride (15 mg, 0.08 mmol, 0.20 equiv.), 1,1′-bis(diphenylphosphanyl)ferrocene (DPPF; 46 mg, 0.08 mmol, 0.20 equiv.), and toluene (10 mL) under argon. The flask was immersed in an oil bath at 100 °C with stirring until the starting material had completely disappeared as judged by TLC analysis. The solution was then allowed to cool to room temperature, diluted with DCM (100 mL), filtered through Celite, and concentrated. The crude product was purified by column chromatography (CH_2Cl_2/MeOH = 40/1) on silica gel to give **8** (100 mg, 0.20 mmol, 49%) as a colorless solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.59 (d, *J* = 4.7 Hz, 2 H), 8.13 (d, *J* = 7.7 Hz, 2 H), 7.74 (td, *J* = 7.7, 1.5 Hz, 2 H), 7.65 (d, *J* = 7.8 Hz, 2 H), 7.56 (d, *J* = 2.4 Hz, 1 H), 7.47 (dd, *J* = 8.8, 2.3 Hz, 1 H), 7.40 (t, *J* = 7.5 Hz, 2 H), 7.26 (dd, *J* = 17.1, 7.5 Hz, 4 H), 7.19 (t, *J* = 5.7, 2 H), 6.73 (d, *J* = 8.9 Hz, 1 H), 6.06 (bs, 1 H), 3.95 (s, 4 H), 3.34 (s, 2 H), 2.98 (t, *J* = 5.7 Hz, 2 H).

KOH (100 mg, 1.79 mmol, 8.95 equiv.) and compound **8** (99 mg, 0.20 mmol, 1.00 equiv.) were added to a solution of EtOH and DMSO (4/1, 2.0 mL). The reaction mixture was stirred in a 40 °C oil bath, and H_2O_2 (30%, 0.5 mL) was slowly added dropwise through a syringe over 0.5 h. Then the resulting solution was stirred for another 2.0 h until the disappearance of benzonitrile as shown by TLC. After removal of the solvent by rotary evaporation, the mixture was diluted with DCM (150mL), washed with saturated NaCl solution, dried with $MgSO_4$, filtered, and concentrated. The mixture was purified by column chromatography (CH_2Cl_2/MeOH = 15/1) to give 5-isoACBA (88 mg, 0.17 mmol, 85%) as a pale green solid. Mp. 210–212 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 8.52 (d, J = 4.3 Hz, 2 H), 8.25 (bs, 1 H), 8.13 (d, J = 7.6 Hz, 2 H), 7.79 (d, J = 7.7 Hz, 2 H), 7.69 (t, J = 7.4 Hz, 2 H), 7.51 (s, 1 H), 7.39 (t, J = 7.4 Hz, 3 H), 7.26 (t, J = 10.5 Hz, 4 H), 7.15 (t, J = 5.3 Hz, 2H), 6.76 (d, J = 8.8 Hz, 1H), 5.75 (bs, 2 H), 3.93 (s, 4 H), 3.38 (t, J = 5.7 Hz, 2 H), 2.93 (t, J = 5.7 Hz, 2 H). ^{13}C-Apt (100 MHz, $CDCl_3$): δ 171.1, 149.2, 148.6, 141.6, 137.5, 132.9, 127.7, 125.9, 124.3, 124.1, 123.0, 122.8, 120.3 (2C), 119.7, 114.2, 113.1, 109.6, 59.8, 52.5, 40.1, 29.7. HRMS (ESI-TOF): m/z Calcd for $C_{33}H_{31}NO_6$ $[M+H]^+$ 527.2599, found: 527.2562.

Spectroscopic Materials and Methods: Stock solutions (0.5 mM) of zinc perchlorate were prepared in HEPES buffer (10 mM HEPES, pH = 7.4). Stock solutions (1.0 mM) of 4-isoACBA and 5-isoACBA were prepared in DMSO. The 4-isoACBA-Zn(II) and 5-isoACBA-Zn(II) complex solutions were prepared by *in situ* mixing of Stock solutions (0.5 mM) of zinc perchlorate (2.0 mL) and Stock solutions (1.0 mM) of 4-isoACBA and 5-isoACBA (1.0 mL), and then diluted to 100 ml, respectively. All the fluorescence spectra of probes were also measured in HEPES buffer (10 mM HEPES, pH = 7.4, 1% (v/v) DMSO) and the excitation wavelength was 350 nm with excitation and emission slit widths of 5 nm at room temperature. The association constants of the complexes between PPi or metal ions and the probes were determined according to the literature procedures.[1-2]

Assay for alkaline phosphatase: The fluorescence turn-off assay for ALP was carried out in 10 mM HEPES buffer of pH 7.4 at 37 ℃. Generally, the solutions containing (i) 10 μM 4-isoACBA-Zn(II) + 10 μM $Na_4P_2O_7$ and (ii) 10 μM 5-isoACBA-Zn(II) + 10 μM $Na_4P_2O_7$ were prepared respectively, and the resulted mixture was incubated for 10 mins. Upon the addition of an aliquot of ALP (Sigma product number P7640, from bovine intestinal mucosa), fluorescence intensity for 4-isoACBA-Zn(II) (λ_{ex} = 350 nm, λ_{em} = 426 nm) and for 5-isoACBA-Zn(II) (λ_{ex} = 350 nm, λ_{em} = 464 nm) was recorded as a function of time with different ALP concentrations.

DFT Calculation: The ground state structures of sensors were optimized using density functional theory (DFT) with B3LYP functional and 6-31G (d) basis set. No imaginary frequencies were found in frequency analysis of all calculated structures. All calculations were performed using the Gaussian 09.[3]

Reference:

(1) N. Shao, H. Wang, X. Gao, R. Yang, W. Chan, *Anal. Chem.*, **2010**, *82*, 4628-4636.

(2) K. A. Conners, *Binding constants*, Wiley: New York, 1987.

(3) M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian 09; Revisions A.02 ed., Gaussian, Inc.: Wallingford CT, 2009.

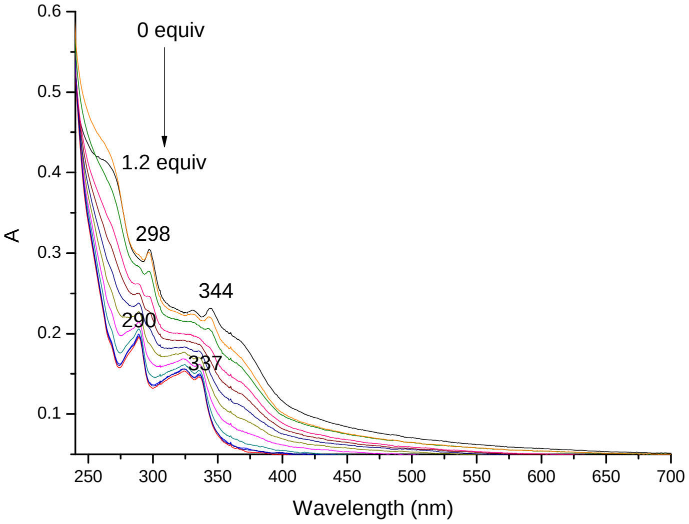


Figure S1a UV absorption spectra of 4-isoACBA (10 μM) upon addition of Zn^{2+} (0-1.2 equiv) in 10 mM HEPES buffer (pH = 7.4).

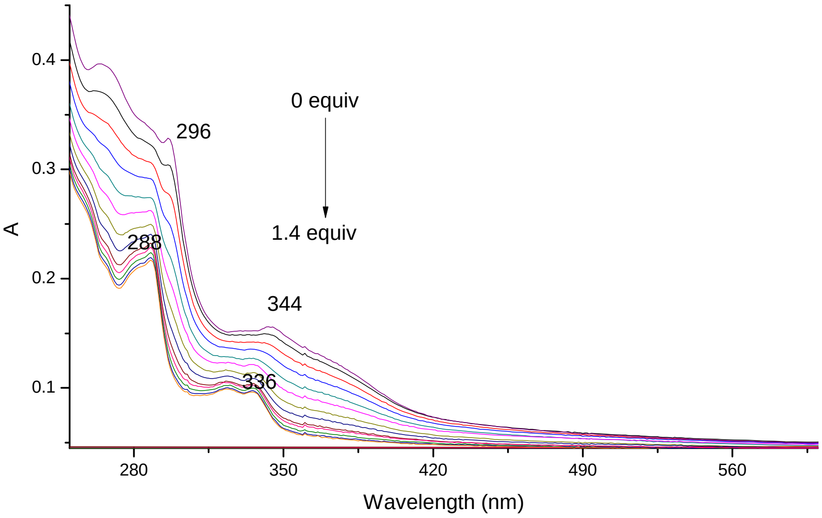
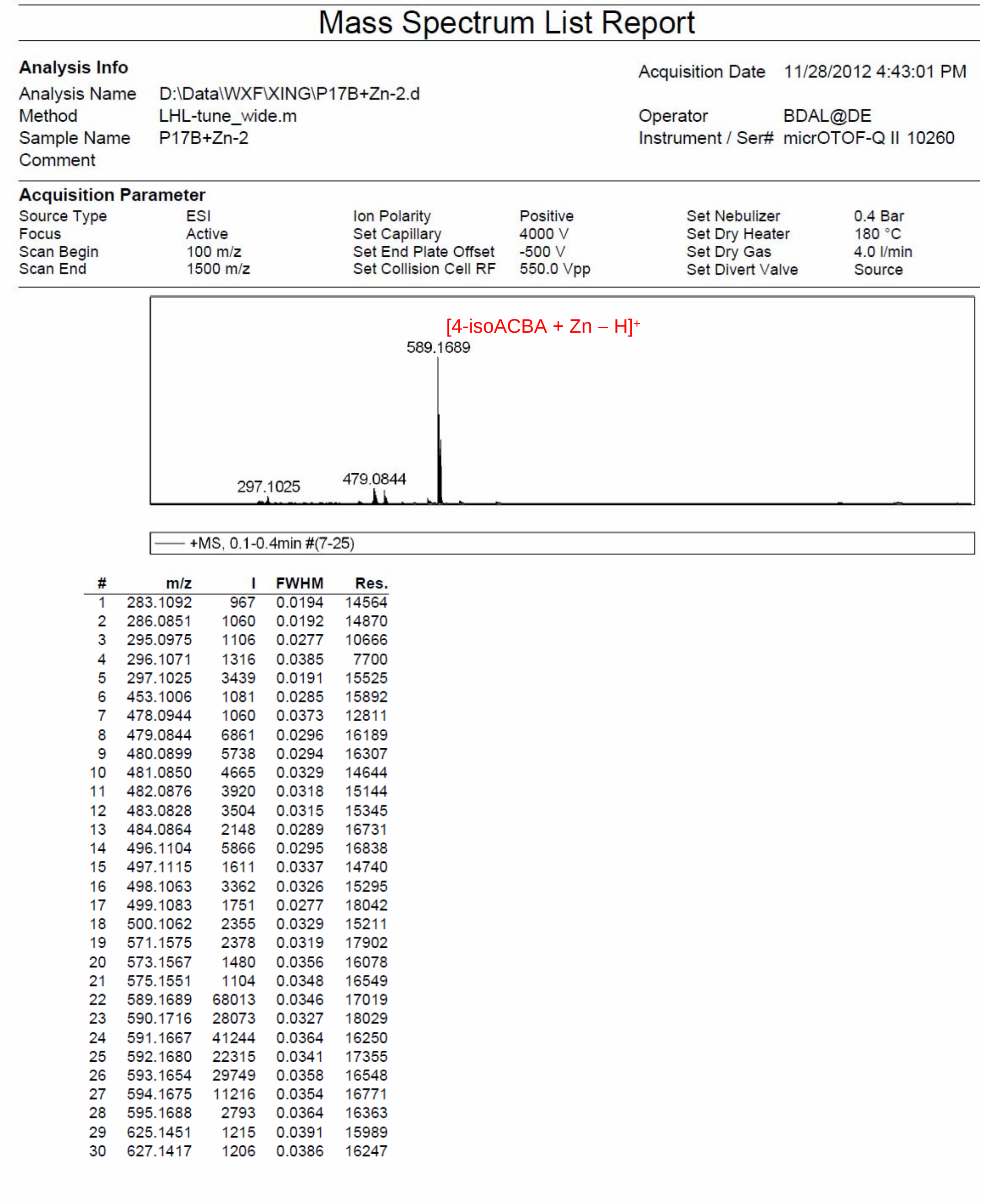


Figure S1b UV absorption spectra of 5-isoACBA (10 μM) upon addition of Zn^{2+} (0-1.4 equiv) in 10 mM HEPES buffer (pH = 7.4).



Mass Spectrum List Report

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Comment			

Acquisition Parameter

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Scan End	1500 m/z	Set Collision Cell RF	550.0 Vpp	Set Divert Valve	Source

#	m/z	I	FWHM	Res.
1	283.1092	967	0.0194	14564
2	286.0851	1060	0.0192	14870
3	295.0975	1106	0.0277	10666
4	296.1071	1316	0.0385	7700
5	297.1025	3439	0.0191	15525
6	453.1006	1081	0.0285	15892
7	478.0944	1060	0.0373	12811
8	479.0844	6861	0.0296	16189
9	480.0899	5738	0.0294	16307
10	481.0850	4665	0.0329	14644
11	482.0876	3920	0.0318	15144
12	483.0828	3504	0.0315	15345
13	484.0864	2148	0.0289	16731
14	496.1104	5866	0.0295	16838
15	497.1115	1611	0.0337	14740
16	498.1063	3362	0.0326	15295
17	499.1083	1751	0.0277	18042
18	500.1062	2355	0.0329	15211
19	571.1575	2378	0.0319	17902
20	573.1567	1480	0.0356	16078
21	575.1551	1104	0.0348	16549
22	589.1689	68013	0.0346	17019
23	590.1716	28073	0.0327	18029
24	591.1667	41244	0.0364	16250
25	592.1680	22315	0.0341	17355
26	593.1654	29749	0.0358	16548
27	594.1675	11216	0.0354	16771
28	595.1688	2793	0.0364	16363
29	625.1451	1215	0.0391	15989
30	627.1417	1206	0.0386	16247

Figure S2a HRMS spectrum of 4-isoACBA-Zn(II) complex.

Mass Spectrum List Report

Analysis Info

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Comment			

Acquisition Parameter

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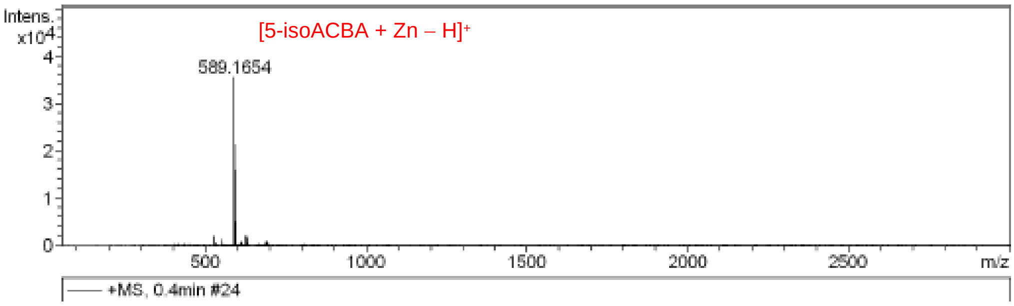


#	m/z	I	FWHM	Res.
1	406.3533	245	0.0271	15009
2	436.2090	260	0.0274	15921
3	527.2522	1968	0.0315	16751
4	528.2539	1128	0.0241	21941
5	533.1757	422	0.0265	20147
6	549.2335	1178	0.0315	17437
7	550.2349	473	0.0254	21621
8	589.1654	35398	0.0286	20572
9	590.1677	14913	0.0274	21577
10	591.1643	21250	0.0323	18307
11	592.1647	10305	0.0321	18475
12	593.1625	15740	0.0321	18502
13	594.1636	4832	0.0360	16500
14	595.1677	1244	0.0346	17209
15	596.1646	247	0.0353	16866
16	610.8134	489	0.0346	17653
17	612.8071	730	0.0295	20752
18	625.1411	1981	0.0359	17436
19	626.1416	908	0.0314	19966
20	627.1380	2077	0.0332	18918
21	628.1416	910	0.0350	17933
22	629.1368	1527	0.0284	22174
23	630.1358	501	0.0367	17189
24	631.1326	380	0.0419	15052
25	685.0920	259	0.0246	27850
26	689.1186	666	0.0365	18881
27	691.1179	844	0.0293	23554
28	693.1153	472	0.0344	20171
29	694.1202	235	0.0368	18842
30	807.7187	234	0.0240	33641

Figure S2b HRMS spectrum of 5-isoACBA-Zn(II) complex.

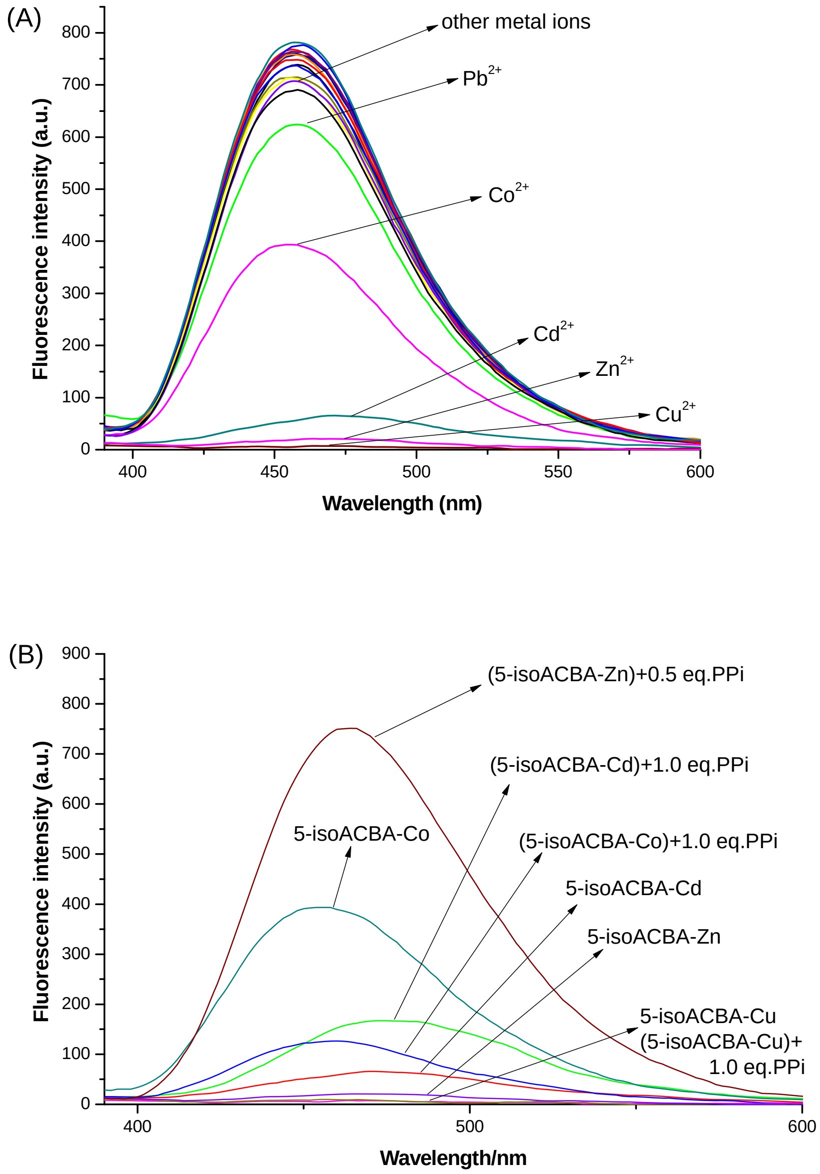


Figure S3 Fluorescence spectra of (a) 5-isoACBA on the addition of various metal ions and (b) 5-isoACBA**-**M and 5-isoACBA-M on the addition of PPi. *Experimental conditions*: 5-isoACBA and 4-isoACBA-M (10 μM, 10 mM HEPES buffer, pH 7.4), 10 μM Li^+, K^+, Rb^+, Cs^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, Sm^{2+}, Co^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+}, 5-10 μM PPi, λ_{ex} = 350 nm.

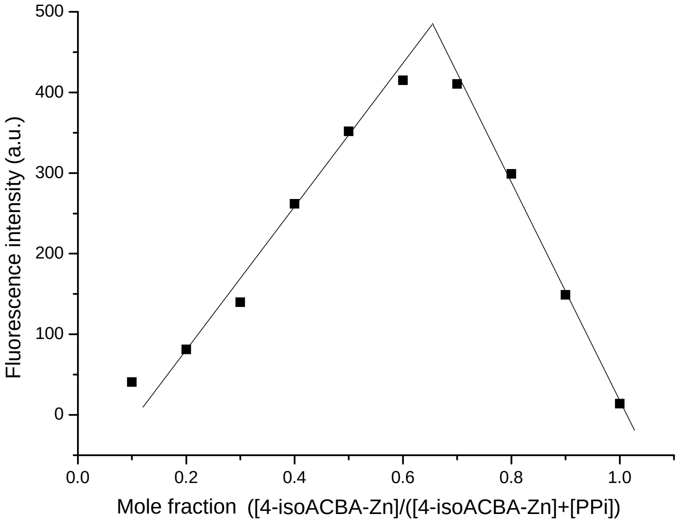


Figure S4a. Job's plot for the binding between 4-isoACBA-Zn(II) and PPi. [4-isoACBA-Zn(II)] + [PPi] = 0.01 mM.

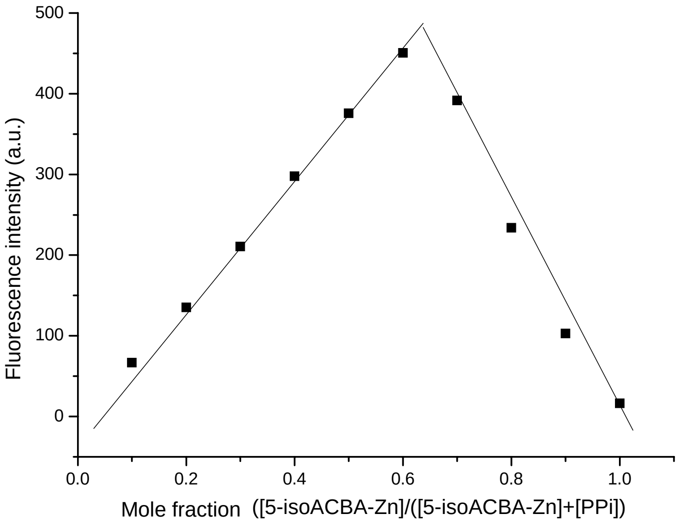


Figure S4b. Job's plot for the binding between 5-isoACBA-Zn(II) and PPi. [5-isoACBA-Zn(II)] + [PPi] = 0.01 mM.

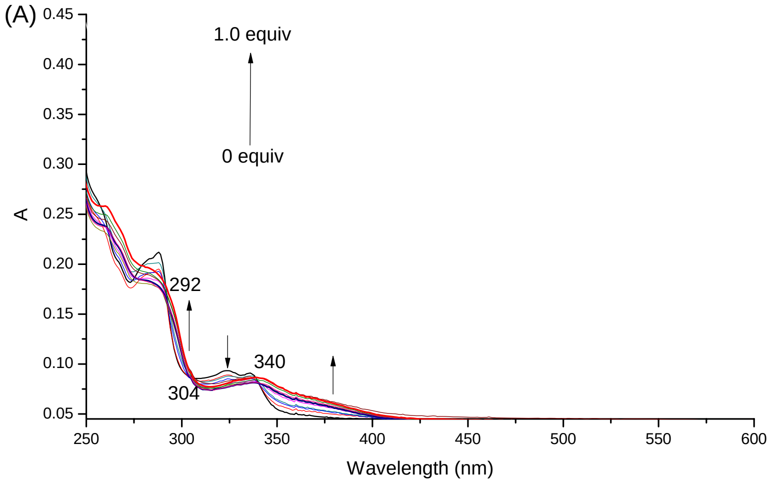
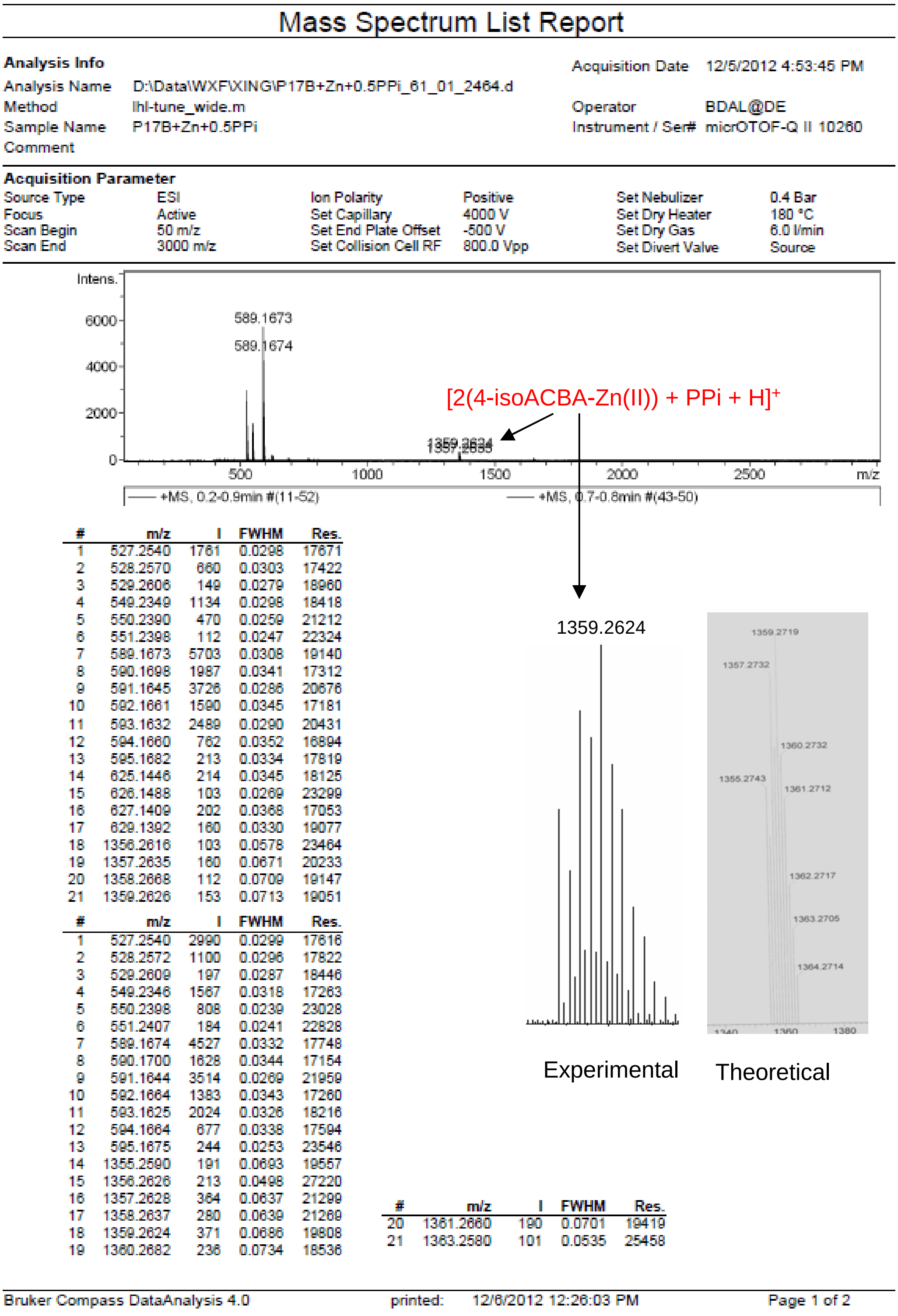


Figure S5. UV-vis absorption spectra of 5-isoACBA-Zn(II) (10 μM) upon addition of PPi in 10 mM HEPES buffer (pH = 7.4).



Mass Spectrum List Report

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Comment			

Acquisition Parameter

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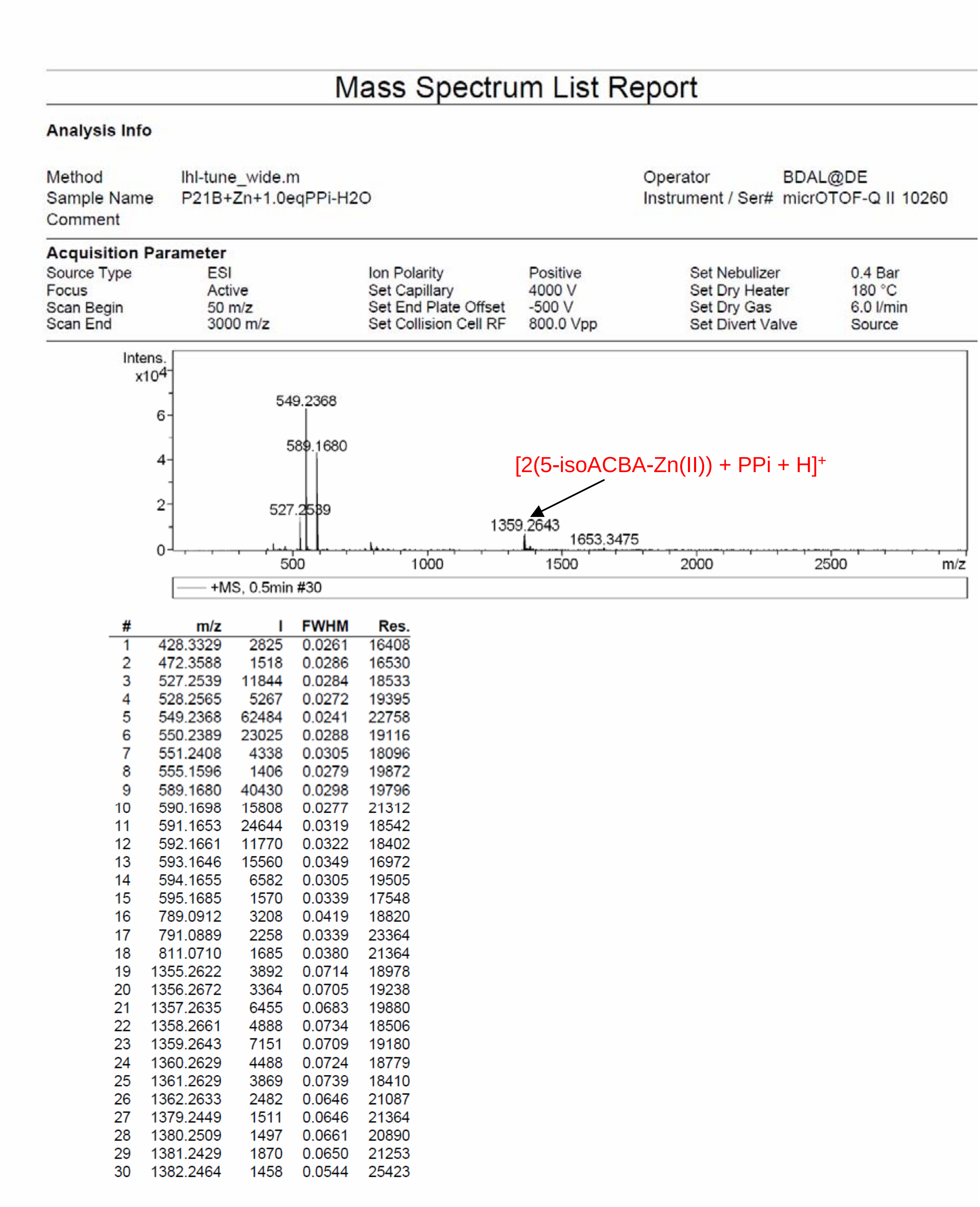
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	800.0 Vpp	Set Divert Valve	Source

#	m/z	I	FWHM	Res.
1	527.2540	1761	0.0298	17671
2	528.2570	660	0.0303	17422
3	529.2606	149	0.0279	18960
4	549.2349	1134	0.0298	18418
5	550.2390	470	0.0259	21212
6	551.2398	112	0.0247	22324
7	589.1673	5703	0.0308	19140
8	590.1698	1987	0.0341	17312
9	591.1645	3726	0.0286	20676
10	592.1661	1590	0.0345	17181
11	593.1632	2489	0.0290	20431
12	594.1660	762	0.0352	16894
13	595.1682	213	0.0334	17819
14	625.1446	214	0.0345	18125
15	626.1488	103	0.0269	23299
16	627.1409	202	0.0368	17053
17	629.1392	160	0.0330	19077
18	1356.2616	103	0.0578	23464
19	1357.2635	160	0.0671	20233
20	1358.2668	112	0.0709	19147
21	1359.2626	153	0.0713	19051

#	m/z	I	FWHM	Res.
1	527.2540	2990	0.0299	17616
2	528.2572	1100	0.0296	17822
3	529.2609	197	0.0287	18446
4	549.2346	1567	0.0318	17263
5	550.2398	808	0.0239	23028
6	551.2407	184	0.0241	22828
7	589.1674	4527	0.0332	17748
8	590.1700	1628	0.0344	17154
9	591.1644	3514	0.0269	21959
10	592.1664	1383	0.0343	17260
11	593.1625	2024	0.0326	18216
12	594.1664	677	0.0338	17594
13	595.1675	244	0.0253	23546
14	1355.2590	191	0.0693	19557
15	1356.2626	213	0.0498	27220
16	1357.2628	364	0.0637	21299
17	1358.2637	280	0.0639	21269
18	1359.2624	371	0.0686	19808
19	1360.2682	236	0.0734	18536
20	1361.2660	190	0.0701	19419
21	1363.2580	101	0.0535	25458

Bruker Compass DataAnalysis 4.0 printed: 12/6/2012 12:26:03 PM Page 1 of 2

Figure S6a HRMS spectrum of 4-isoACBA-Zn(II)-PPi obatined in positive mode on Bruker micrOTOF-QII mass spectrometer.



Mass Spectrum List Report

Analysis Info

Method	lhl-tune_wide.m	Operator	BDAL@DE
Sample Name	P21B+Zn+1.0eqPPi-H2O	Instrument / Ser#	micrOTOF-Q II 10260
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	800.0 Vpp	Set Divert Valve	Source

#	m/z	I	FWHM	Res.
1	428.3329	2825	0.0261	16408
2	472.3588	1518	0.0286	16530
3	527.2539	11844	0.0284	18533
4	528.2565	5267	0.0272	19395
5	549.2368	62484	0.0241	22758
6	550.2389	23025	0.0288	19116
7	551.2408	4338	0.0305	18096
8	555.1596	1406	0.0279	19872
9	589.1680	40430	0.0298	19796
10	590.1698	15808	0.0277	21312
11	591.1653	24644	0.0319	18542
12	592.1661	11770	0.0322	18402
13	593.1646	15560	0.0349	16972
14	594.1655	6582	0.0305	19505
15	595.1685	1570	0.0339	17548
16	789.0912	3208	0.0419	18820
17	791.0889	2258	0.0339	23364
18	811.0710	1685	0.0380	21364
19	1355.2622	3892	0.0714	18978
20	1356.2672	3364	0.0705	19238
21	1357.2635	6455	0.0683	19880
22	1358.2661	4888	0.0734	18506
23	1359.2643	7151	0.0709	19180
24	1360.2629	4488	0.0724	18779
25	1361.2629	3869	0.0739	18410
26	1362.2633	2482	0.0646	21087
27	1379.2449	1511	0.0646	21364
28	1380.2509	1497	0.0661	20890
29	1381.2429	1870	0.0650	21253
30	1382.2464	1458	0.0544	25423

Figure S6b HRMS spectrum of 5-isoACBA-Zn(II)-PPi.

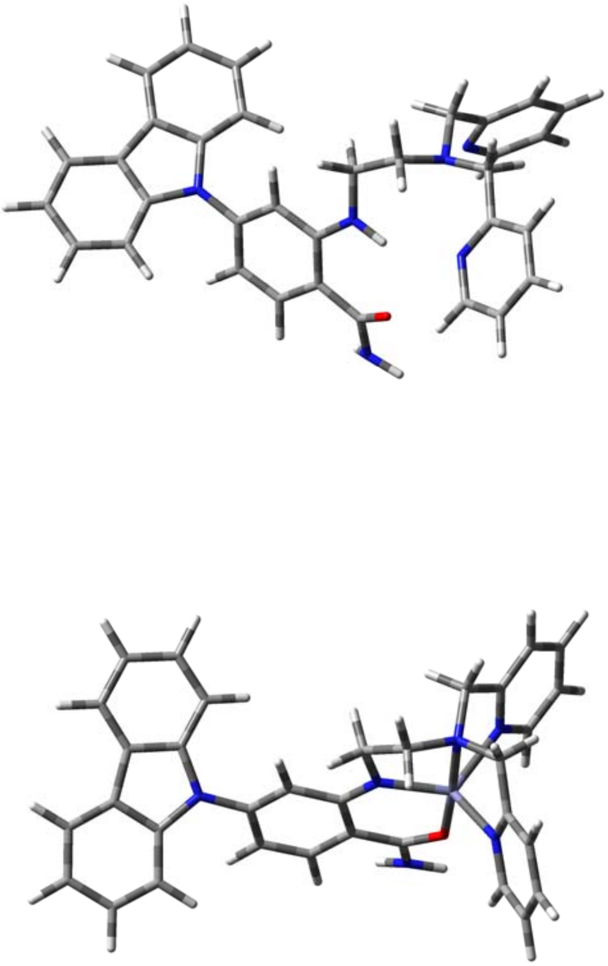
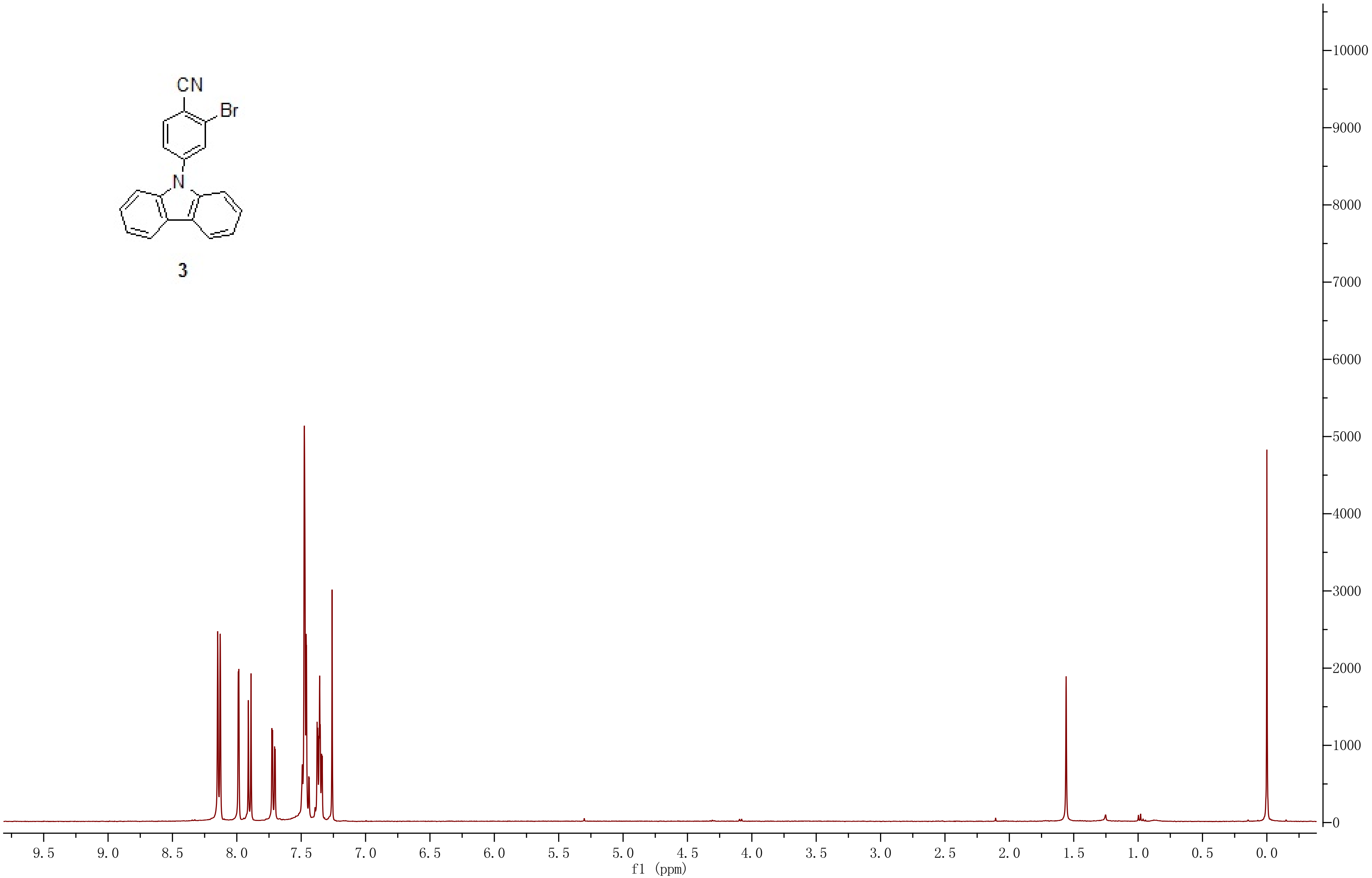
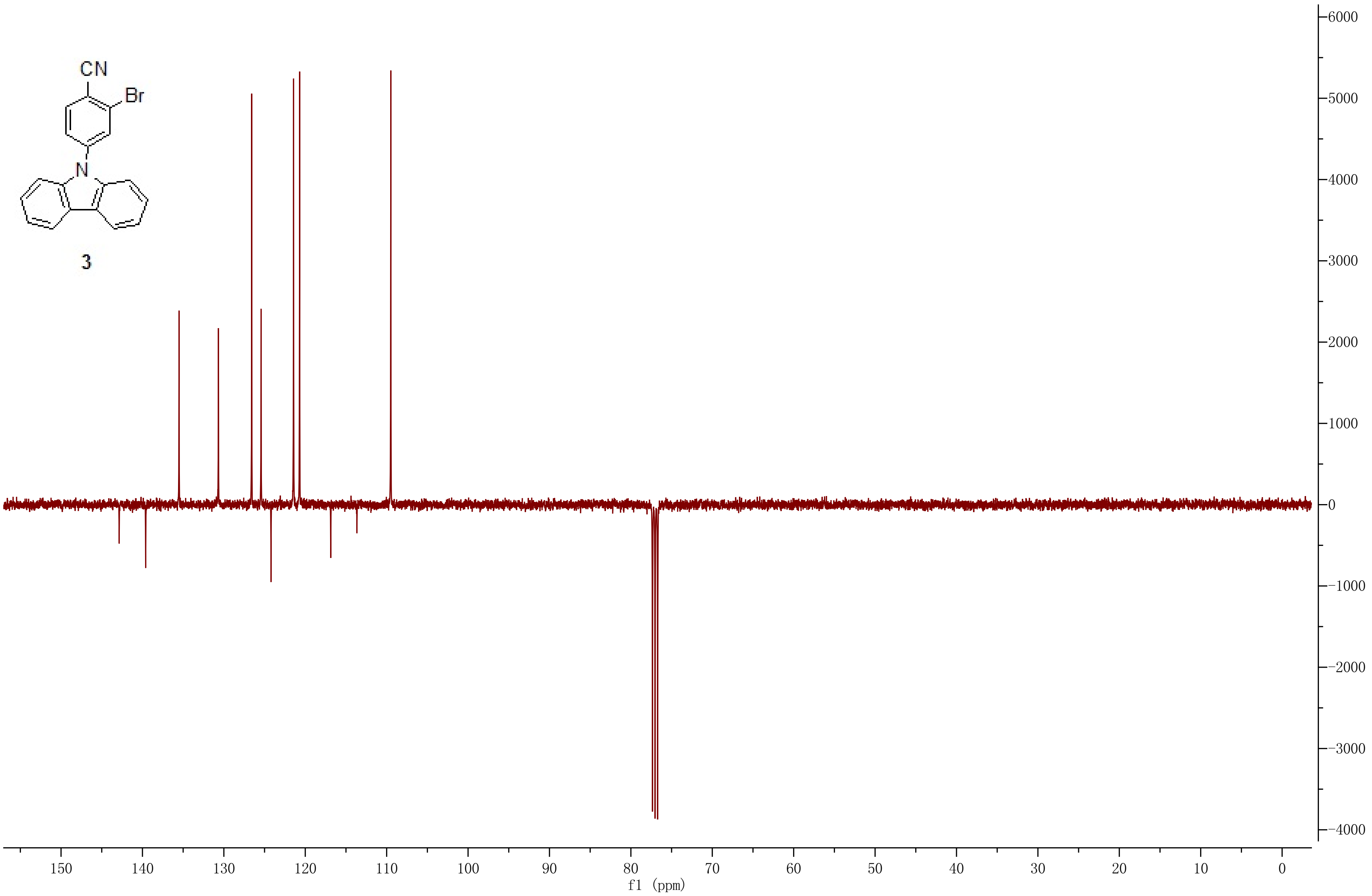
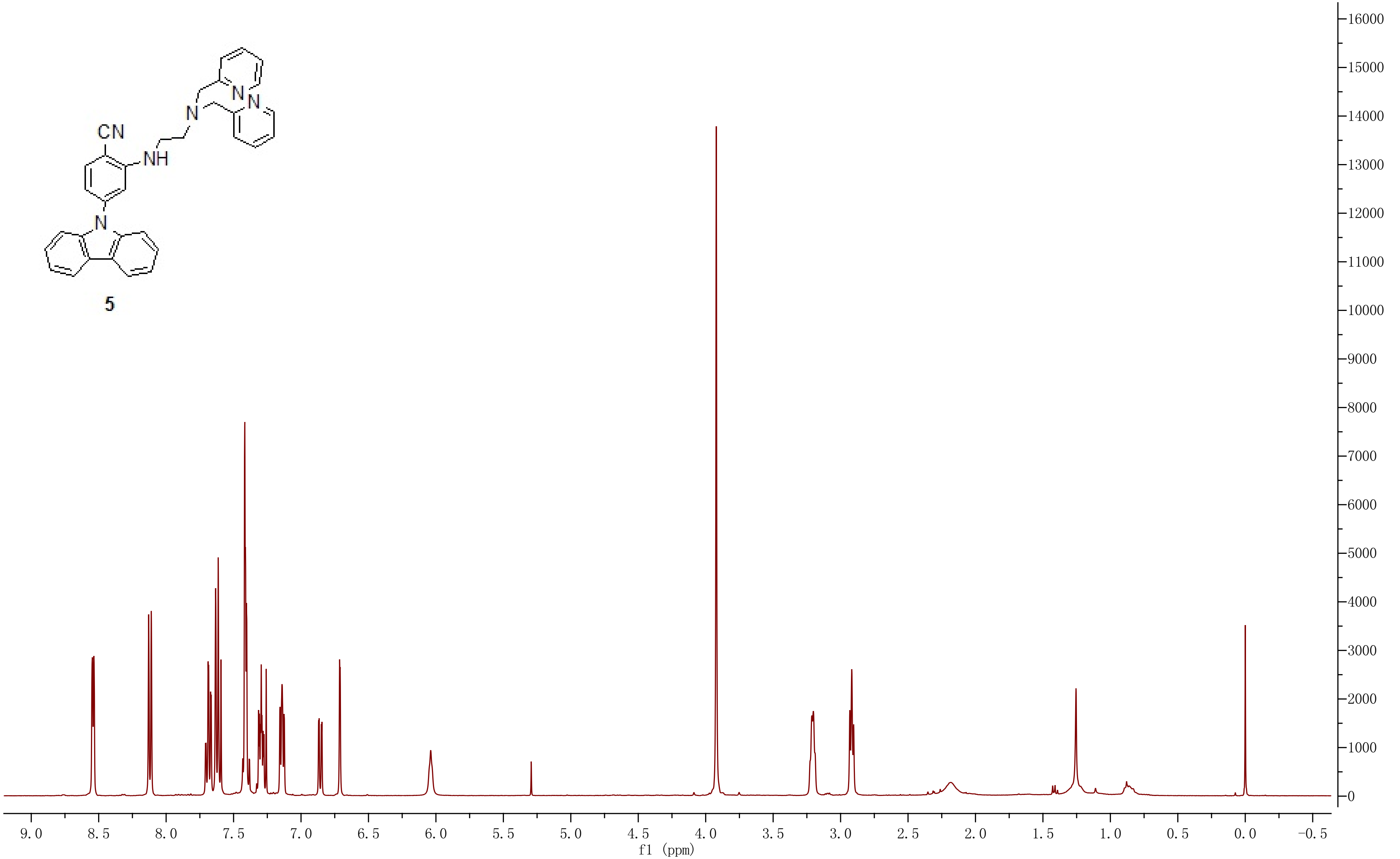


Figure S7. Calculated structure for 4-isoACBA and 4-isoACBA-Zn(II).


CN
Br
N
3
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
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
0.0
f1 (ppm)


CN
Br
N
3
6000
5000
4000
3000
2000
1000
0
−1000
−2000
−3000
−4000
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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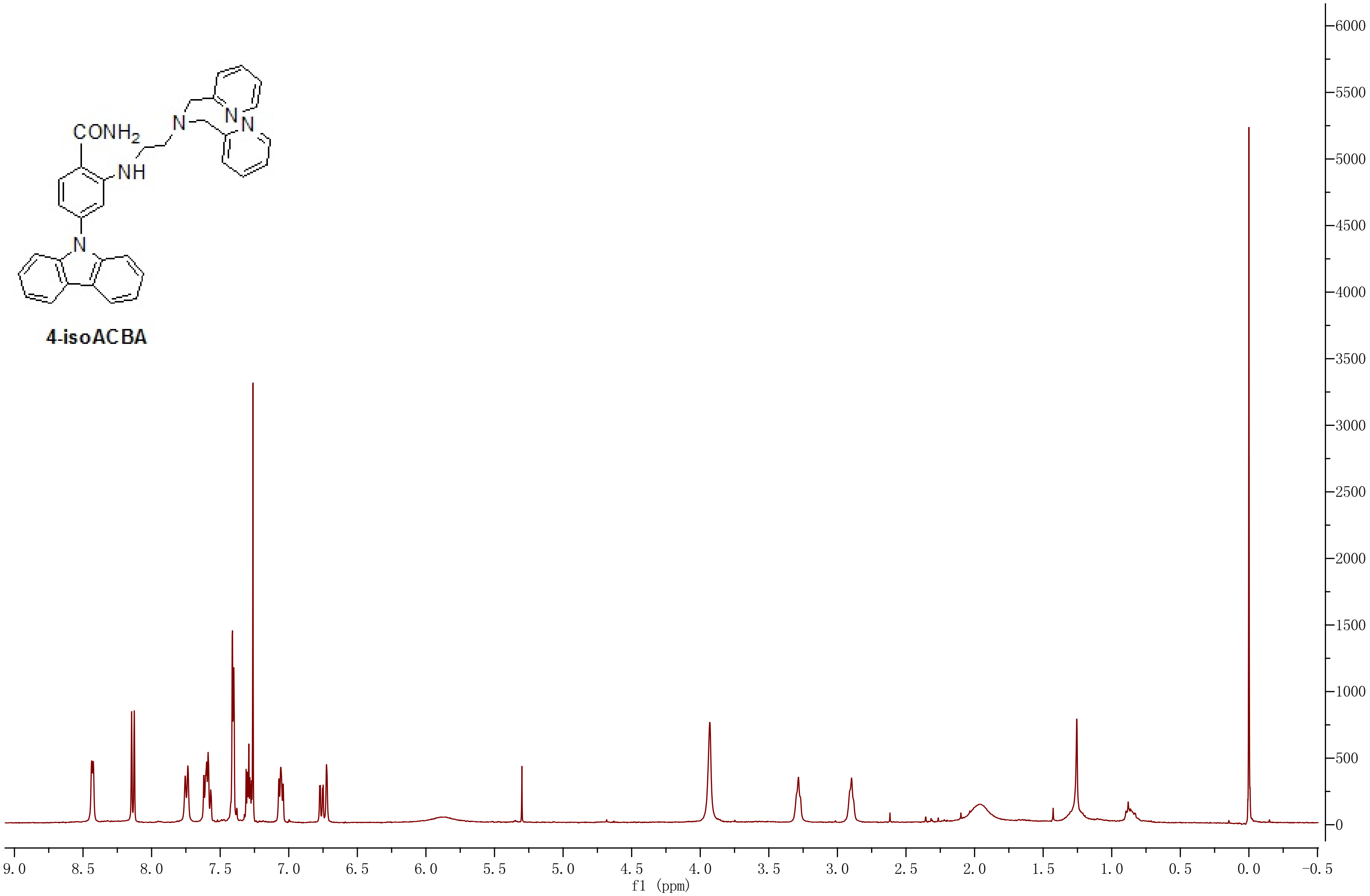
NH
N
N
N
N
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

5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N
N
N
4-isoACBA
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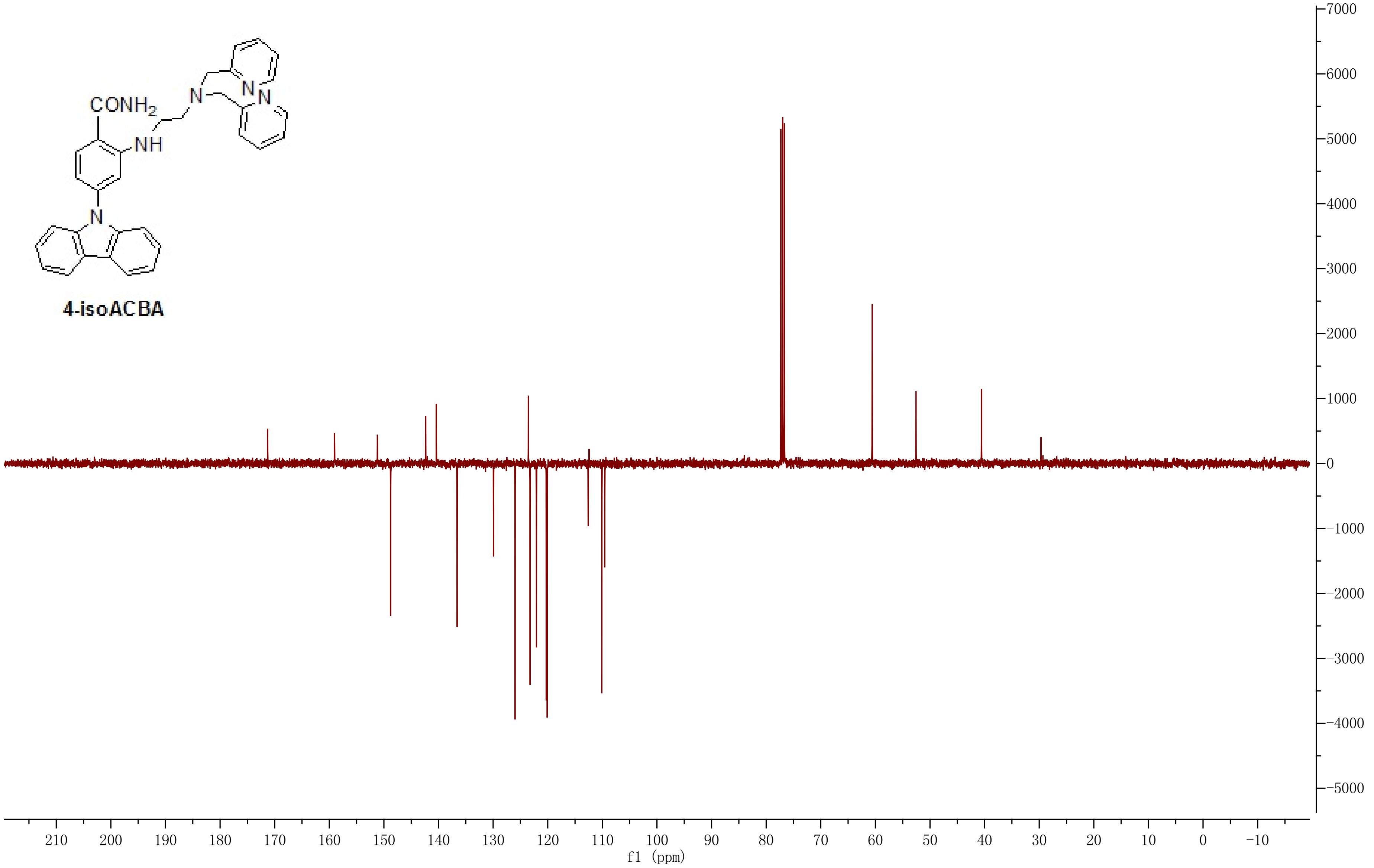
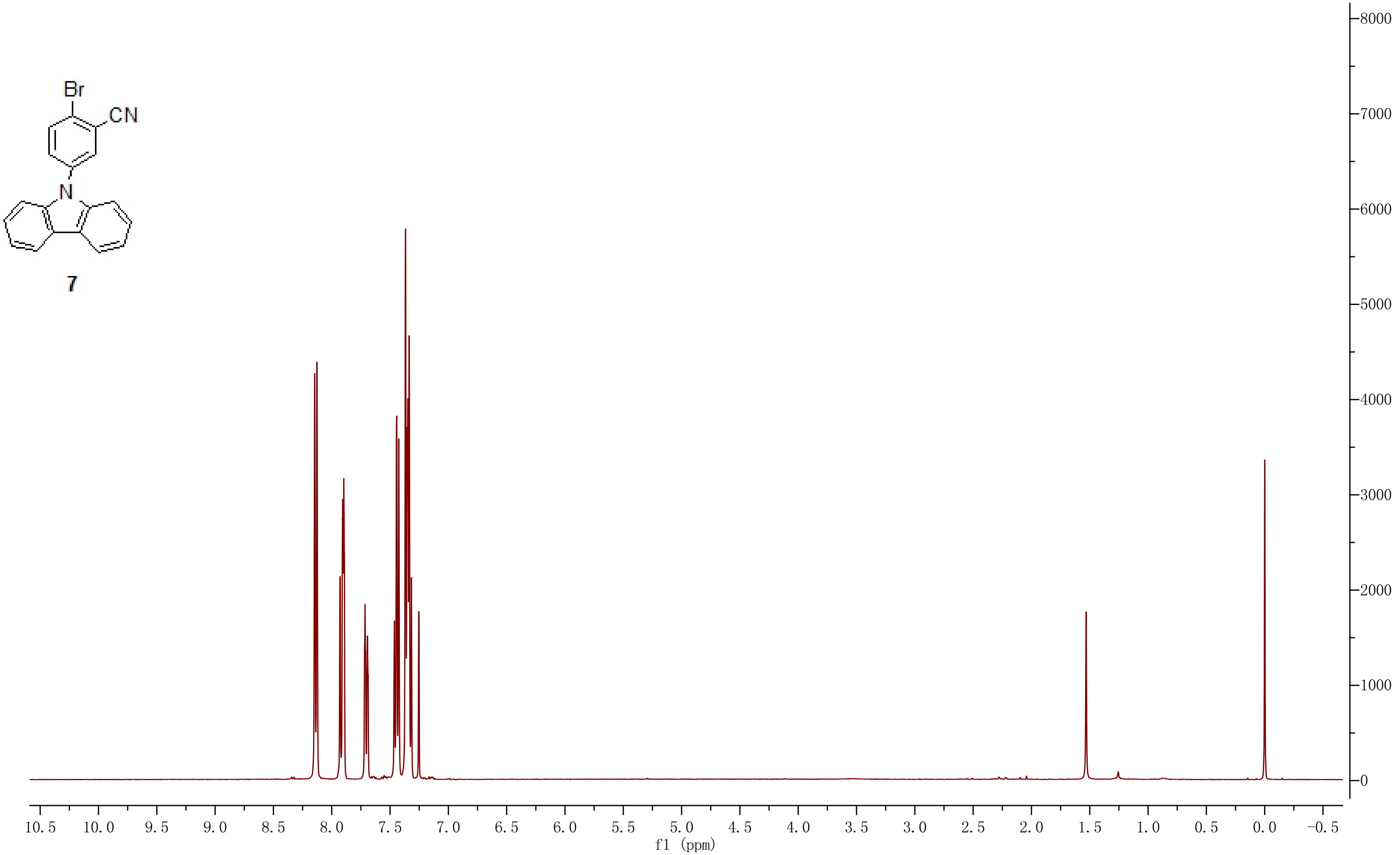
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


CONH2
NH
N
N
N
N
4-isoACBA
7000
6000
5000
4000
3000
2000
1000
0
−1000
−2000
−3000
−4000
−5000
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

Br
CN
N

7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5000
4000
3000
2000
1000
0
10.5
10.0
9.5
9.0
8.5
8.0
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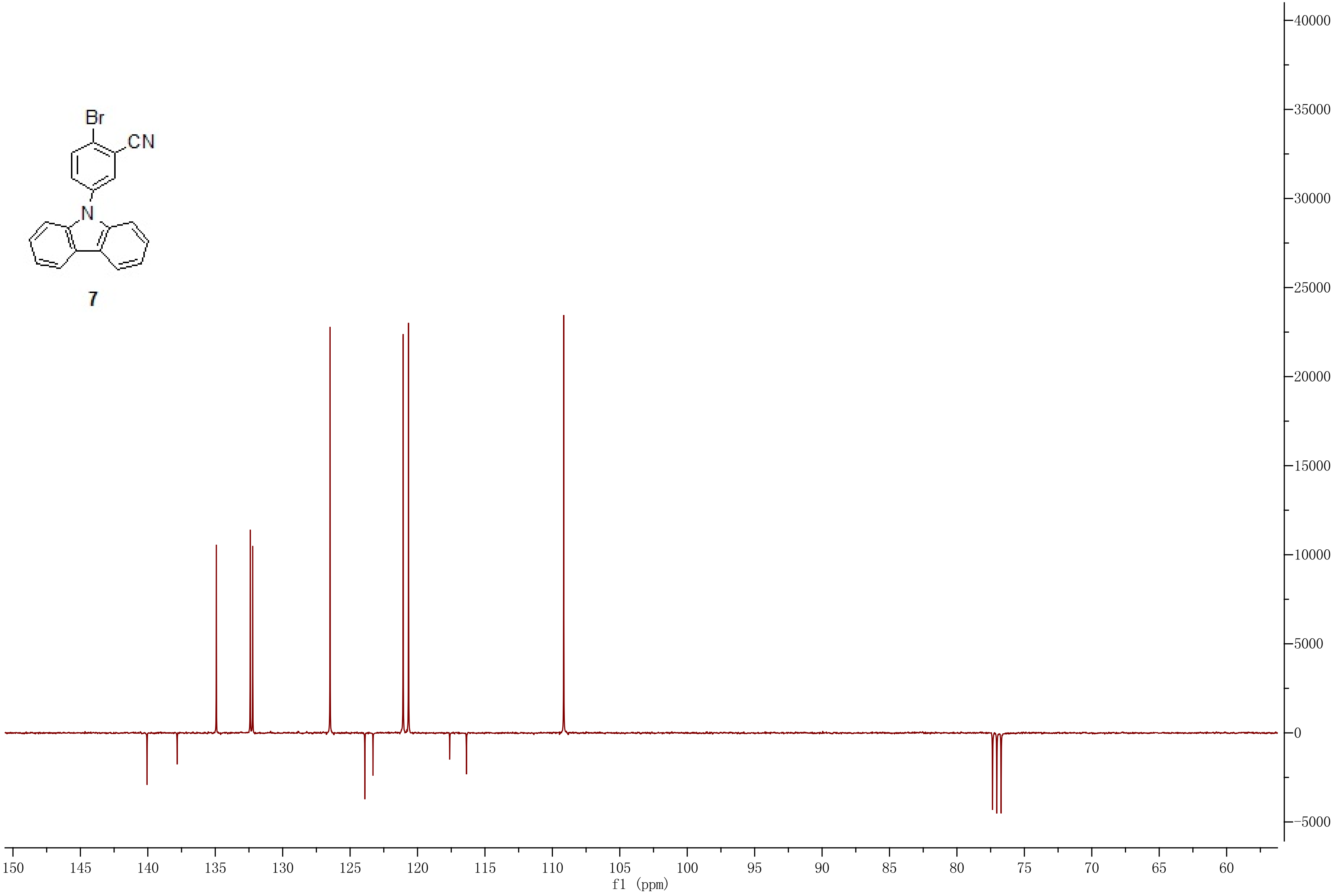
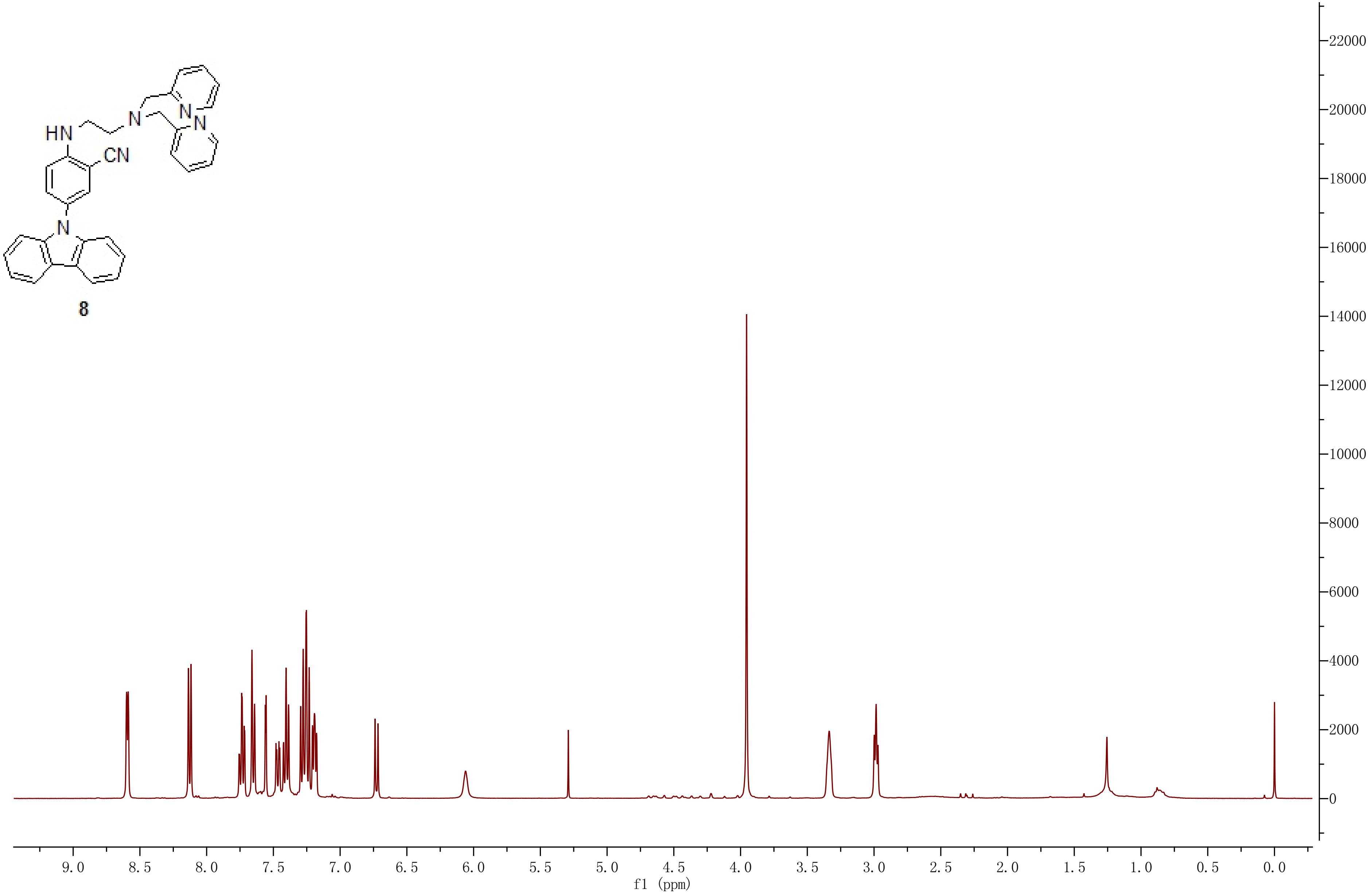
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

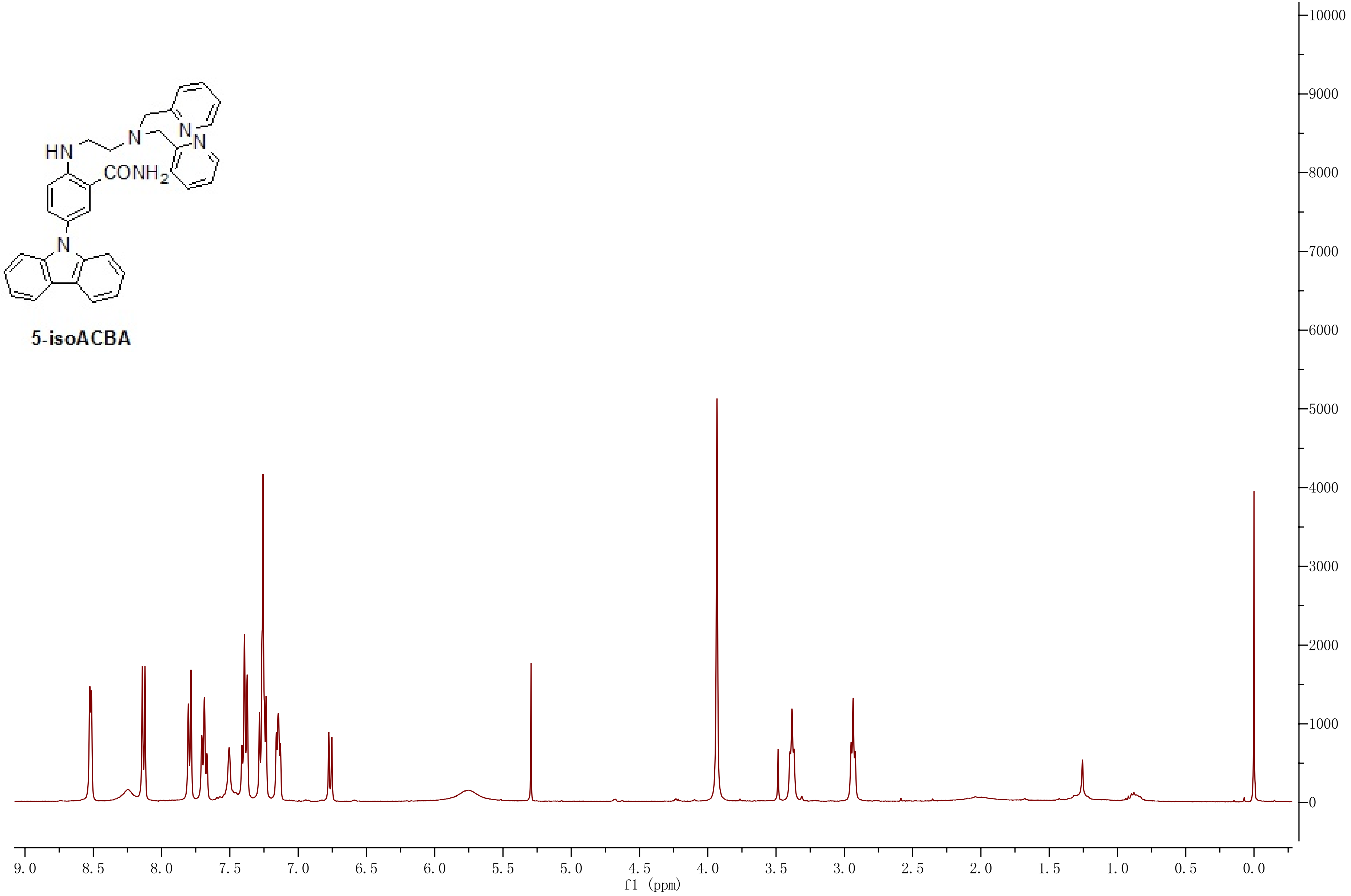
Br
CN
N

7


40000
35000
30000
25000
20000
15000
10000
5000
0
−5000
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
f1 (ppm)

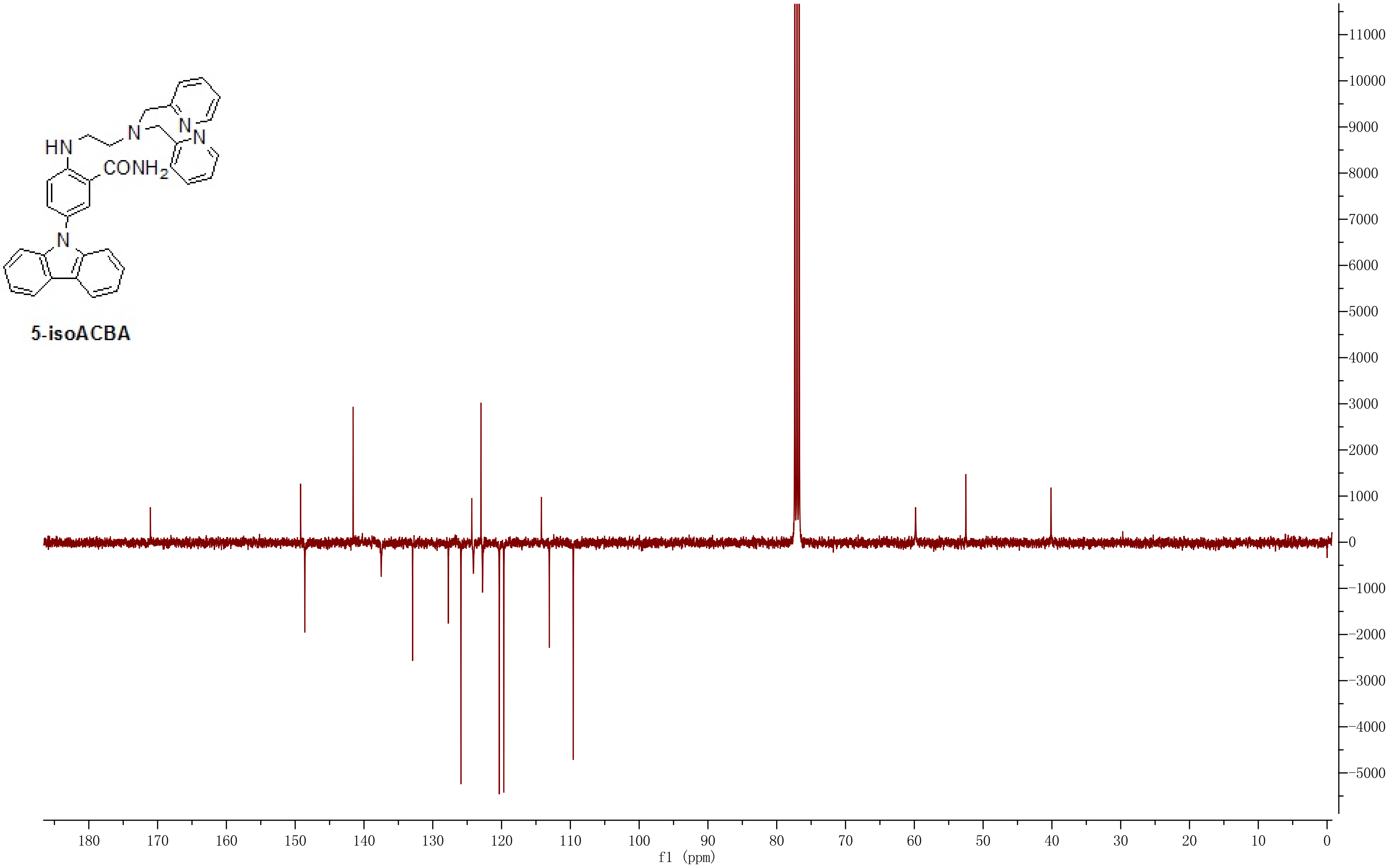

HN
CN
N
N
N
N
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

8



5-isoACBA

HN
N
N
N
CONH$_2$
N
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
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)


HN
N
N
N
CONH$_2$
N
5-isoACBA
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
−1000
−2000
−3000
−4000
−5000
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)