Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Informations for

1,8-Naphthyridine-based molecular clips for off-on fluorescence sensing of Zn^{2+} in living cells

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Contents

1. Experimental Section

Reagents and instruments

All reagents and solvents were of AR grade and used without further purification unless otherwise noted. All solvents were dried using standard procedure prior to use.

^{1}H NMR and ^{13}C NMR spectra were recorded on a VARIAN INOVA-400 spectrometer with chemical shifts reported as ppm (in DMSO-d_6 or $CDCl_3$, TMS as internal standard). Mass spectrometric data were obtained on HP1100LC/MSD and LCQ-Tofmass spectrometers. Fluorescence emission spectra were obtained using EDINBURGH FS920 luminescence spectrometer. For all fluorescent measurements, both excitation and emission slit widths were 1 nm. Optical absorption spectra were measured on a TU-1900 Uv/Vis spectrophotometer at room temperature. Cell imaging were measured on Nikon eclipase TE2000-5 inverted fluorescence microscopy.

General procedures of spectra detection

Stock solutions (2×10^{-2} M) of the CH_3CN perchlorate salts of K^+, Na^+, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Fe^{3+}, Ag^+, Pb^{2+} and Hg^{2+} were prepared and diluted to the appropriate concentration. Stock solution of **DP1** and **DP2** (1 mM) were also prepared in distilled CH_3CN solution. Test solutions were prepared by placing 40 uL of host stock solution into a quartz cell of 1 cm optical path length including 2 mL CH_3CN:H_2O (1:1, v/v, containing 0.01 M HEPES, pH=7.21) solution, and then adding an appropriate aliquot of each metals stock by using a micro-syringe. The fluorescence quantum yield was determined using optically matching solutions of Rhodamine-6G (Φ_f = 0.94 in ethanol) as the standard at an excitation wavelength of 500 nm. Excitation and emission slit widths were modified to adjust the luminescent intensity in a suitable range. All the spectroscopic measurements were performed at least in triplicate and averaged.

NMR titration method

All NMR spectra were measured on a VARIAN INOVA-400 spectrometer at 298 K. A solution (1 mM) of recepter **DP1** in DMSO-d_6 was titrated with sufficit quantum of $Zn(ClO_4)_2$ by using a micro-syringe. The chemical shift changes of the proton of **DP1** were monitored.

Cell incubation and imaging

HeLa cells were cultured in 1640 supplemented with 10% FCS (Invitrogen). Cells were seeded in 24-well flat-bottomed plates for Nikon eclipase TE2000-5 inverted fluorescence microscopy. After 12 h, HeLa cells were incubated with 5 μM compound **DP1** (in the culture medium containing 0.5% DMSO) for 15 min at 37 ^oC under 5% CO_2 and then washed with phosphate-buffered saline (PBS) three times before incubating with 20 eq Zn^{2+} for another 30 min, and cells were rinsed with PBS three times again. The fluorescence imaging of intracellular Zn^{2+} in HeLa cells was observed under Nikon eclipase TE2000-5 inverted fluorescence microscopy with a 20×objective lens (excited with blue light). For all images, the microscope settings, such as brightness, contrast, and exposure time were held constant to compare the relative intensity of intracellular Zn^{2+} fluorescence.

Computational details

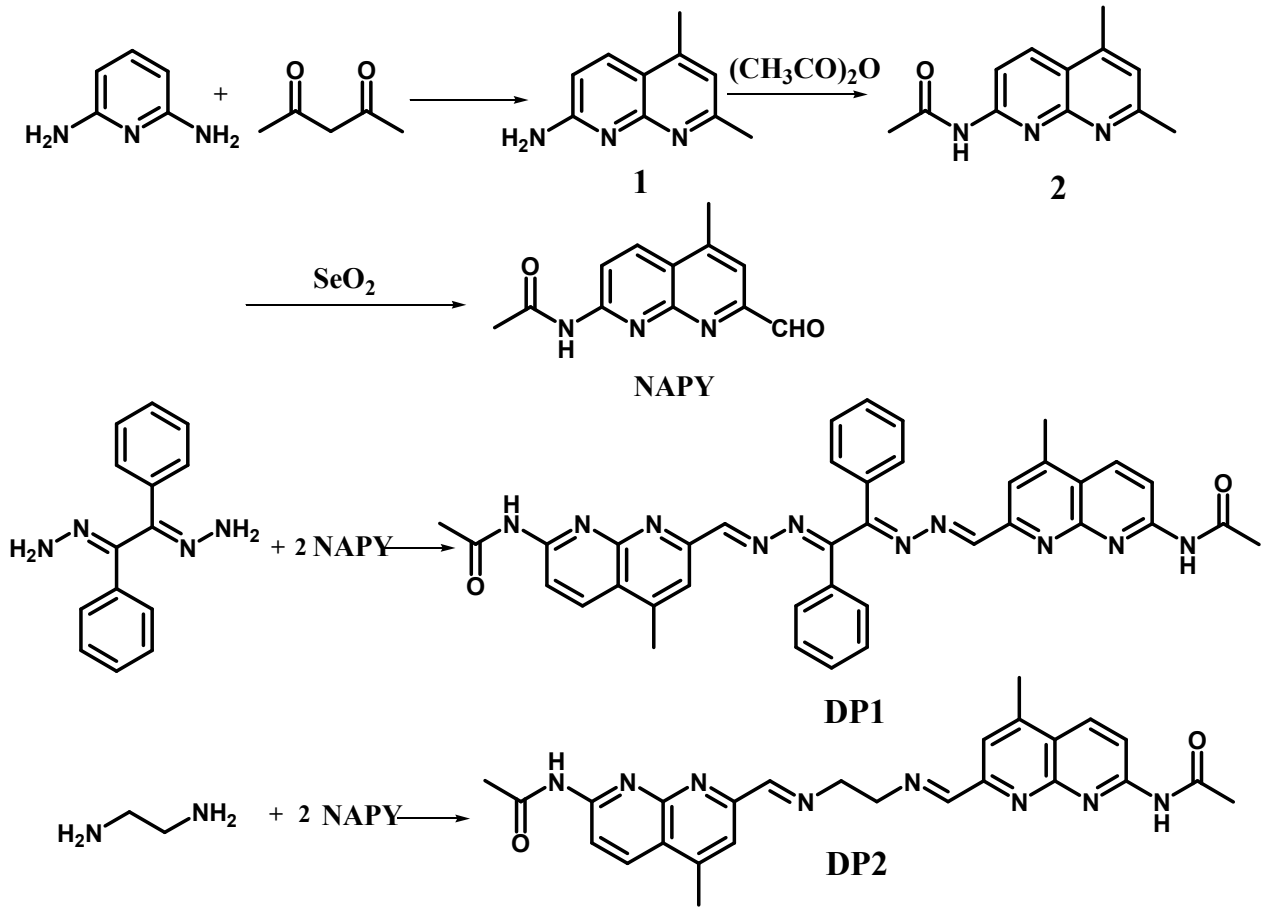
Frontier molecular orbitals have been performed at the Becke3LYP (B3LYP) level of the density functional theory. The SDD basis set is used to describe Zn and 6-31G(d) basis set was used for all the other atoms.

Method of ESI-MS spectra for DP1+Zn^{2+}

The reaction mixtures of Zn(II) perchlorate and **DP1** in a 10:1 molar ratio in 30 mL mixed solvents of dichloromethane and methanol were stirred at room temperature for 5 hours, then filtered and concentrated to 5 mL. Addition of diethyl ether gave yellow solids. The crude products were redissolved in dichloromethane for the ESI-MS spectra.

Synthesis of DP1 and DP2

The synthetic scheme for the preparation of the **DP1** and **DP2** are shown in Scheme S1.



Scheme S1. The synthetic procedure of **DP1** and **DP2**

Synthesis of **7-Amino-2,4-dimethyl-1,8-naphthyridine** (**1**)

1 was synthesized and purified following the literature procedures described previously.[1] ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.99 (d, $1H_{Ar}$, J = 8Hz), 6.92 (s, $1H_{Ar}$), 6.73 (d, $1H_{Ar}$, J = 8Hz), 5.82 (d, $2H_{NH2}$, J = 6.4Hz), 2.83 (s, $3H_{CH3}$), 2.54 (s, $3H_{CH3}$).

Synthesis of **7-acetylamino-2,4-dimethyl-1,8-naphthyridine** (**2**)

2 was synthesized and purified following the literature procedures described previously.[2] ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.26 (s, $1H_{NH}$), 8.47 (d, $1H_{Ar}$, J = 8Hz), 8.32 (d, $1H_{Ar}$, J = 8Hz), 7.12 (s, $1H_{Ar}$), 2.70 (s, $3H_{CH3}$), 2.65 (s, $3H_{CH3}$), 2.28 (s, $3H_{CH3}$).

Synthesis of **7- acetylamino-4-methyl-1,8-naphthyridine-2-aldehyde** (**NAPY)**

NAPY was synthesized and purified following the literature procedures described previously.[3] ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.20 (s, $1H_{CHO}$), 9.07 (s, $1H_{NH}$), 8.68 (d, $1H_{Ar}$, J = 8Hz), 8.45 (d, $1H_{Ar}$, J = 8Hz), 7.87 (s, $1H_{Ar}$), 2.79 (s, $3H_{CH3}$), 2.32 (s, $3H_{CH3}$).

Synthesis of **DP1**

Benzyl dihydrazone (0.24g, 1 mmol) was added slowly with stirring to a solution of **NAPY** (0.458g, 2.0 mmol) in 50 mL of methanol. The resulting solution was refluxed for 12 h under a nitrogen atmosphere (Scheme S1). The pale yellow precipitate that formed was filtered, washed with methanol and dried under vacuum. Yield **DP1**: 86%.. Anal calc. for $C_{38}H_{32}N_{10}O_2$: C 69.08, H 4.88, N 21.20, O 4.84%. Found: C 69.10, H 4.90, N 21.17, O 4.83%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.05 (s, $1H_{NH}$), 8.53 (d, $1H_{Ar}$, J = 8Hz), 8.48 (S, $2H_{Ar}$), 8.38 (d, $1H_{Ar}$, J = 8Hz), 7.55 (m, $5H_{Ar}$), 3.17 (s, $3H_{CH3}$), 2.15 (s, $3H_{CH3}$); ^{13}C NMR (400 MHz, DMSO-d_6) δ: 164.91, 163.55, 163.38, 157.09, 147.52, 144.12, 136.16, 133.01, 131.90, 129.51, 128.59, 126.59, 122.14, 114.33, 114.14, 54.78, 26.12. MS: m/z 598.56 $(M+Na)^+$.

Synthesis of **DP2**

DP2 was synthesized in the same methed as that of **PD1** as a pale yellow powder. Anal calc. for $C_{26}H_{26}N_8O_2$: C 64.72, H 5.43, N 23.22, O 6.63%. Found: C 64.65, H 5.42, N 23.28, O 6.65%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.08 (s, $1H_{NH}$), 8.56 (d, $1H_{Ar}$, J = 8Hz), 8.46 (s, $1H_{Ar}$), 8.38 (d, $1H_{Ar}$, J = 8Hz), 7.90 (s, $1H_{CH}$), 4.11 (s, $2H_{CH2}$), 2.69 (s, $3H_{CH3}$), 2.17 (s, $3H_{CH3}$); ^{13}C NMR (100

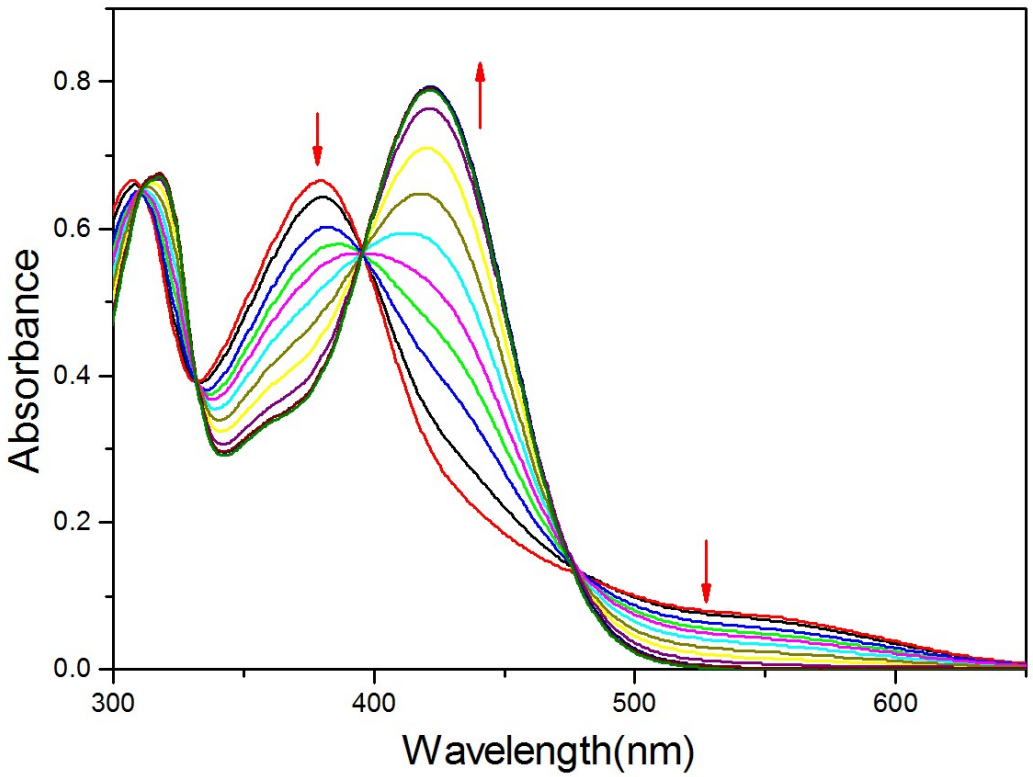
MHz, DMSO-d_6) δ: 162.12, 161.87, 157.89, 152.08, 134.39, 131.63, 130.41, 128.88, 127.54, 109.52, 97.12, 45.17, 12.37. MS: m/z 483.35 $(M+H)^+$; m/z 484.36 $(M+2H)^+$.

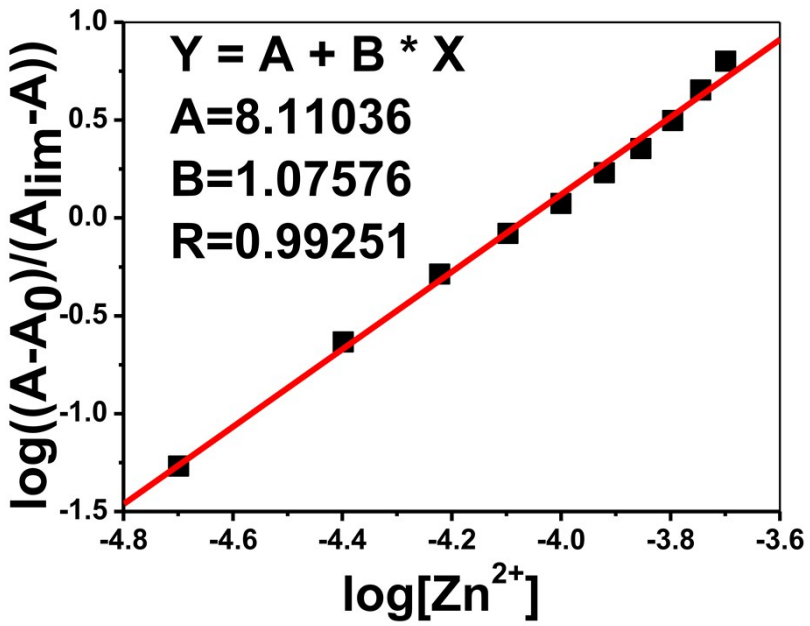
1 Z. X. Li, W.F. Fu, M. M. Yu, X. J. Zhao, Y. Chen, *Dyes and Pigments*, 2007, 75, 516.

2 C. He, S.J. Lippard, *Tetrahedron*, 2000, 56, 8245 .

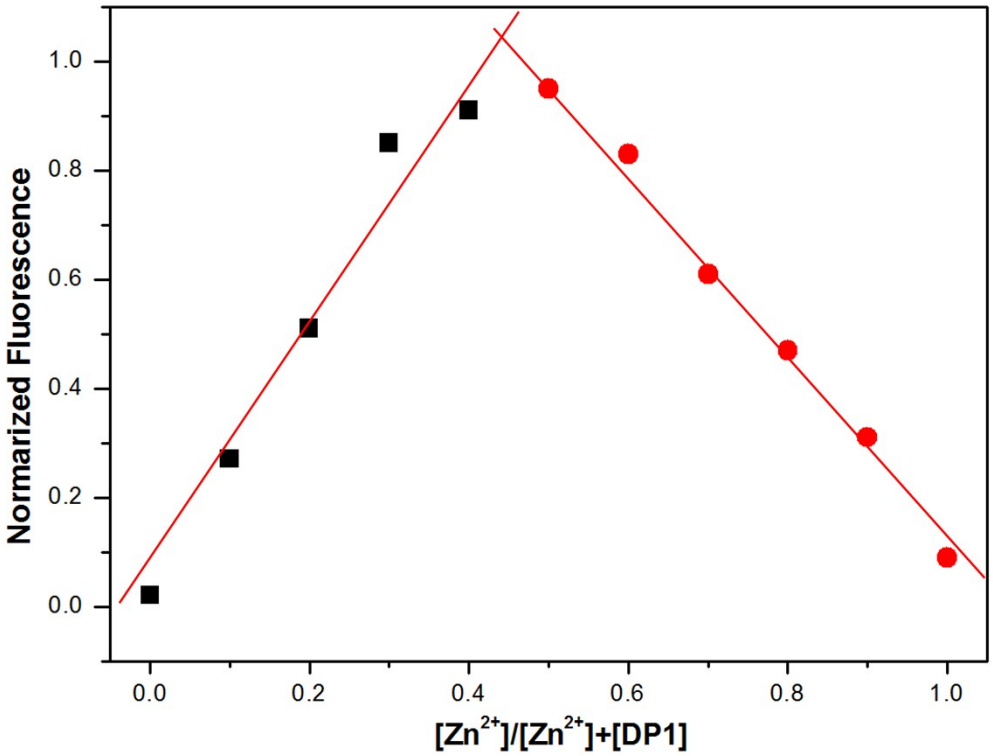
3 R.A. Henry, P.R. Hammond, *J. Heterocyclic Chem.,* 1977, 1109 .

2. Figure S1 Top: Absorption spectra of **DP1** upon addition of increasing amounts of $Zn(ClO_4)_2$ in CH_3CN:H_2O(1:1, v/v, containing 0.01 M HEPES, pH=7.21). Bottom: The plot of Zn^{2+} concentration versus absorbance at 424 nm.

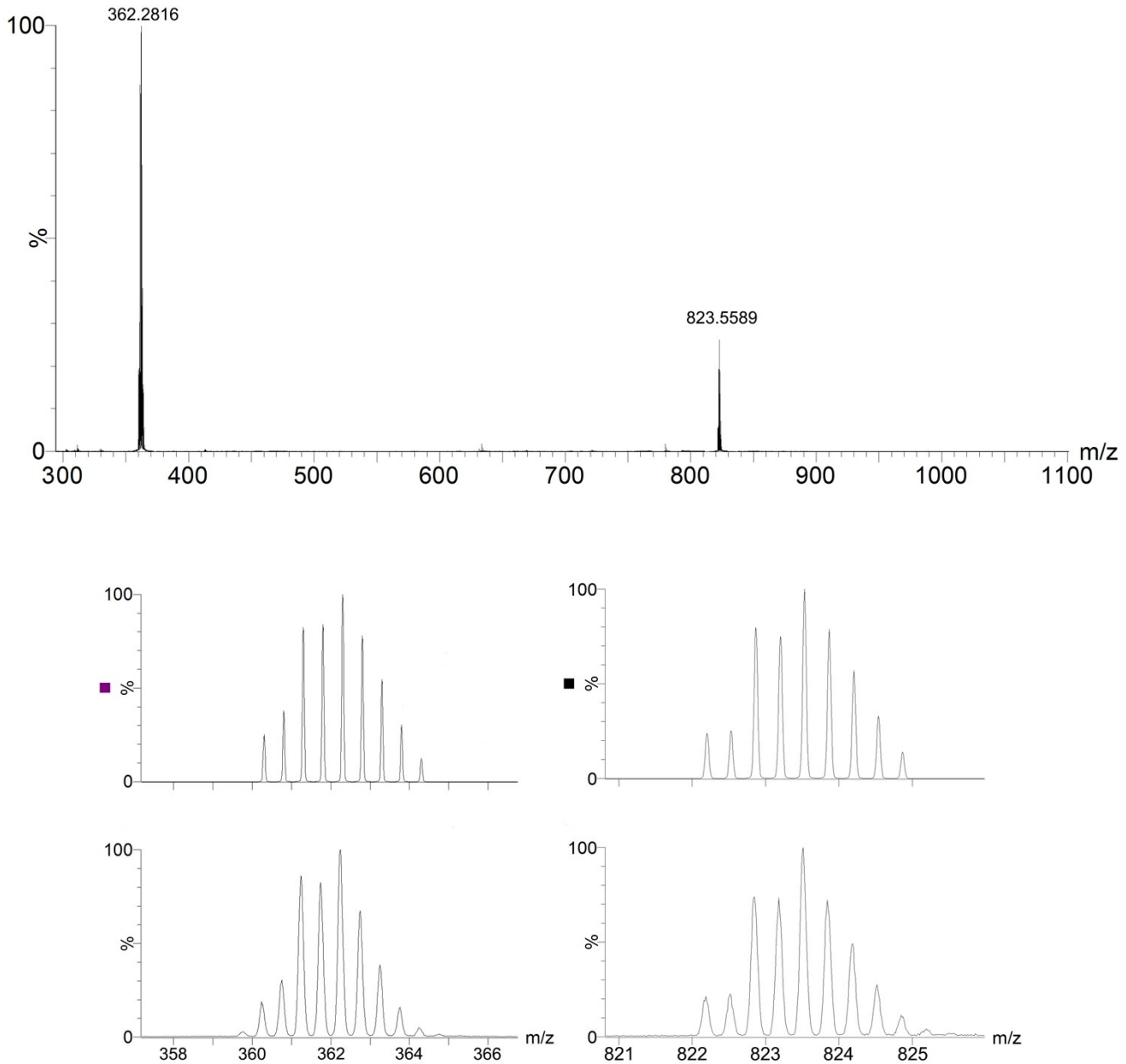




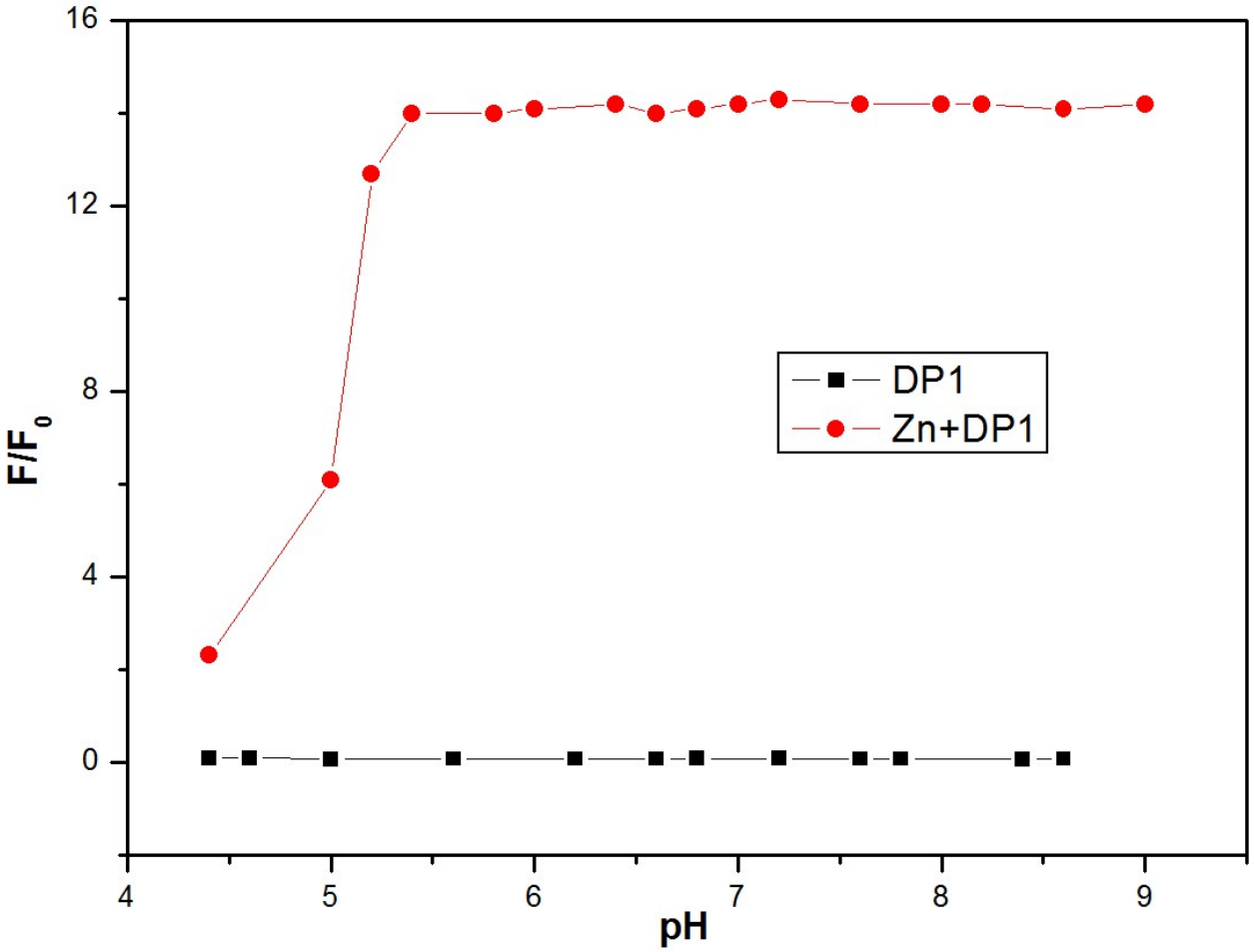
3. Figure S2 Binding analysis using the method of Job's plot. Fluorescence intensity at 518 nm in $CH_3CN:H_2O$ (1:1, v/v, containing 0.01 M HEPES, pH=7.21) of compound **DP1** varied upon addition of $Zn(ClO_4)_2$ with an excitation at 424 nm.



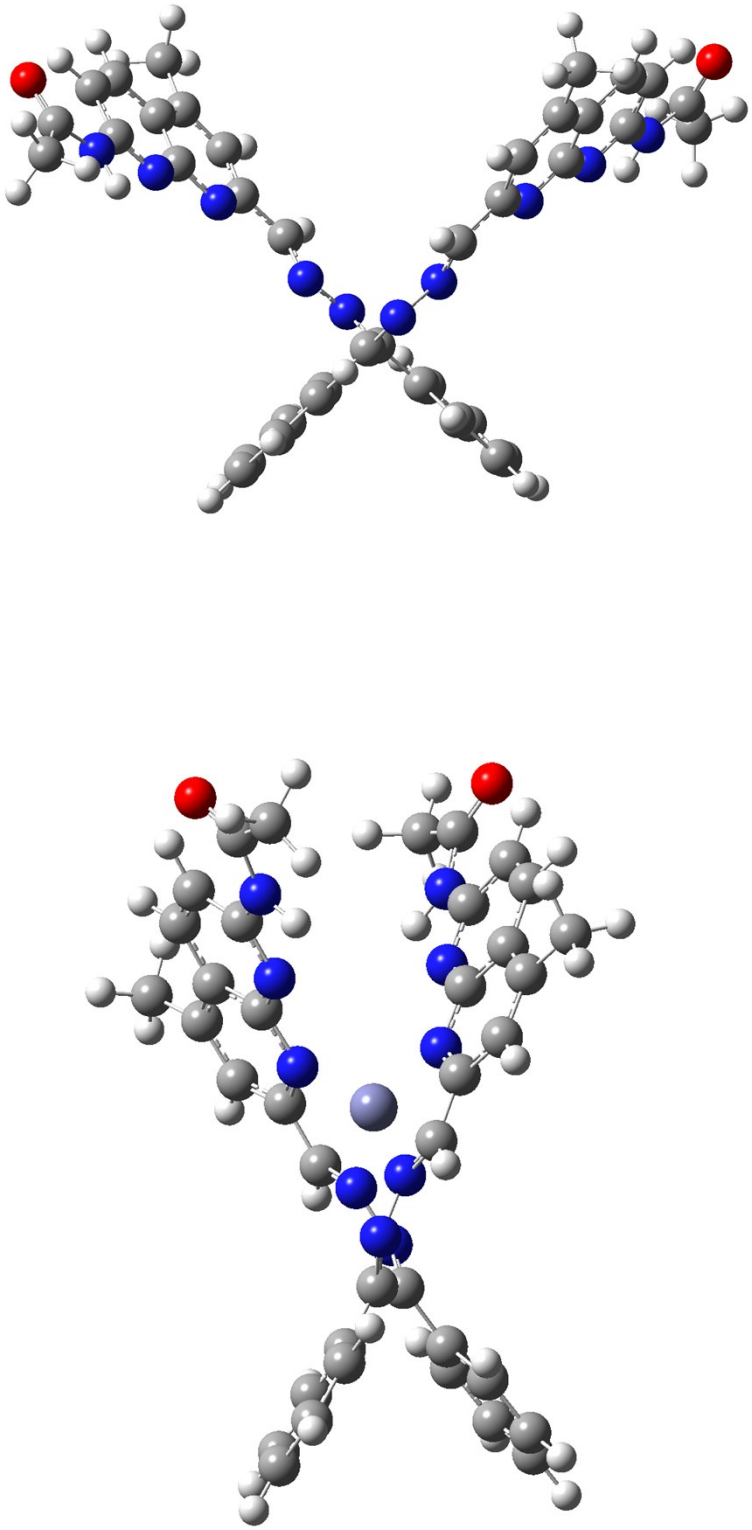
4. Figure S3 ESI-MS spectra of **DP1** in the presence of Zn^{2+} exhibited two peaks at m/z = 362.28 and 823.56, assignable to [**DP1**+Zn]$^{2+}$ and [**DP1**+Zn+ClO_4]$^{+}$ complexation species, respectively. The high resolved spectra exhibit the measured (bottom pictures) and simulated (top ones) isotopic patterns at m/z 362.28 and m/z 823.56, respectively.



5. Figure S4 The pH-dependent fluorescence response of **DP1** (20 μM) and **DP1**+Zn^{2+} in CH_3CN/H_2O (1:1, v/v).

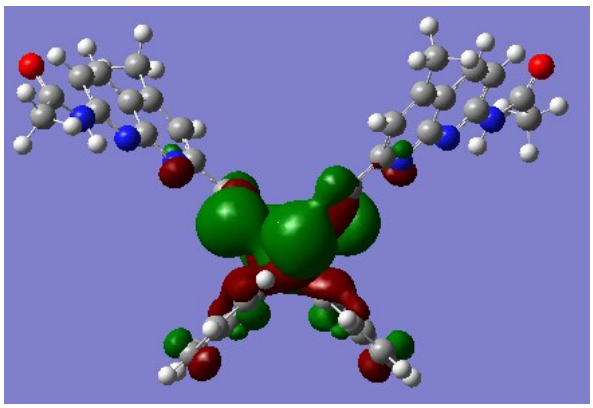


6. Figure S5 Optimized geometry of **DP1** and **DP1**-Zn^{2+}.

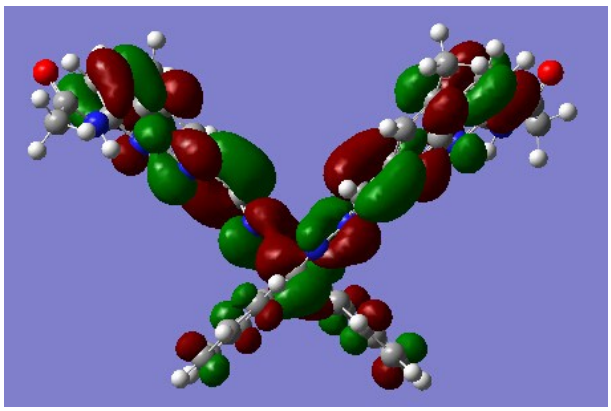


7. Figure S6 Frontier orbitals of **DP1**(a) and **DP1**-Zn^{2+} complexation species (b) based on their optimized geometries.

a

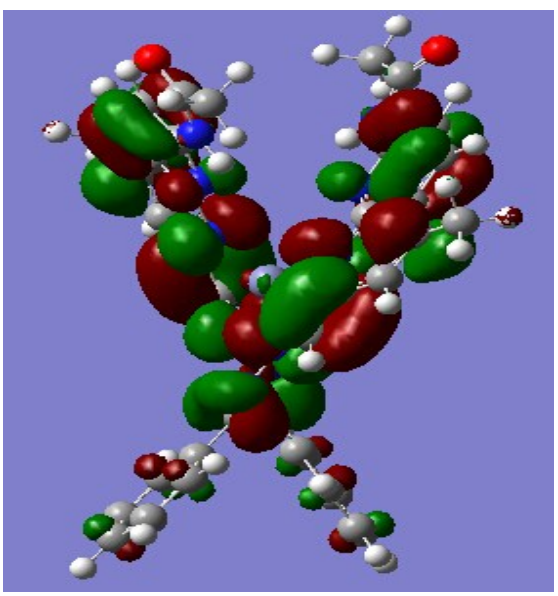


HOMO



LUMO

b



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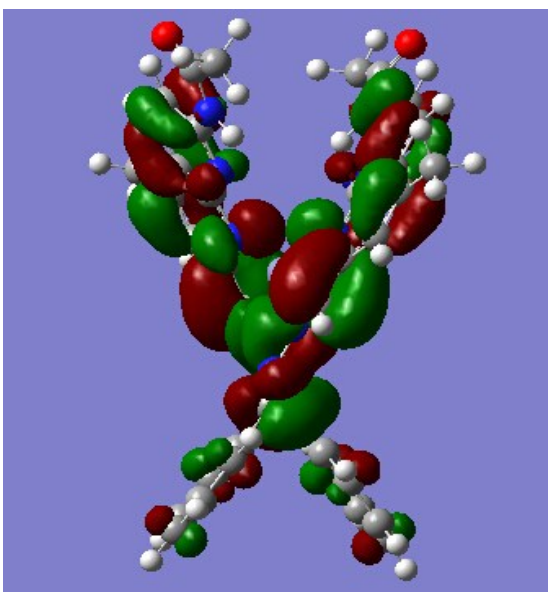
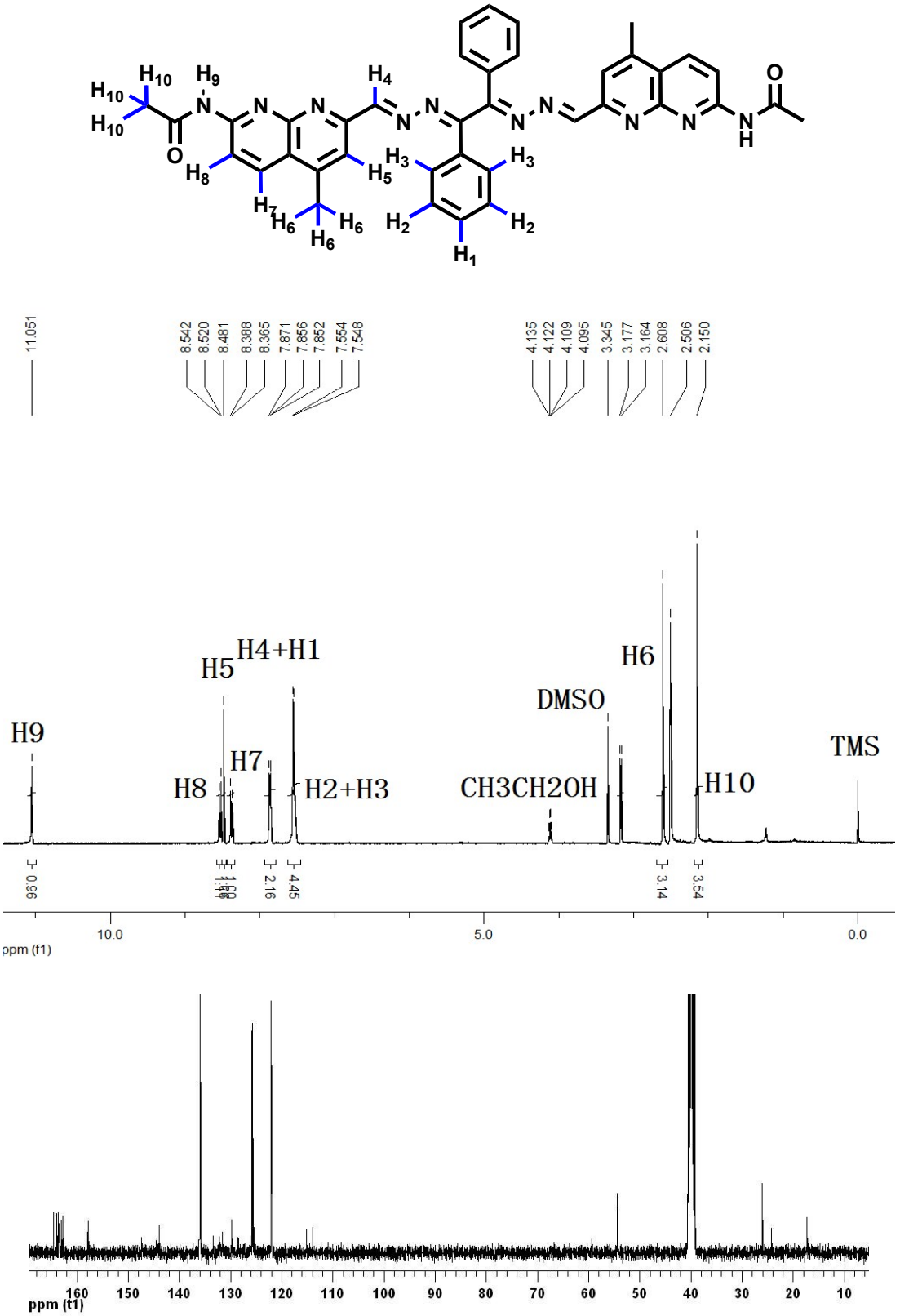
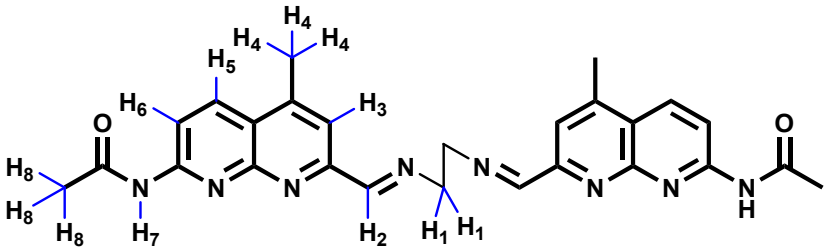


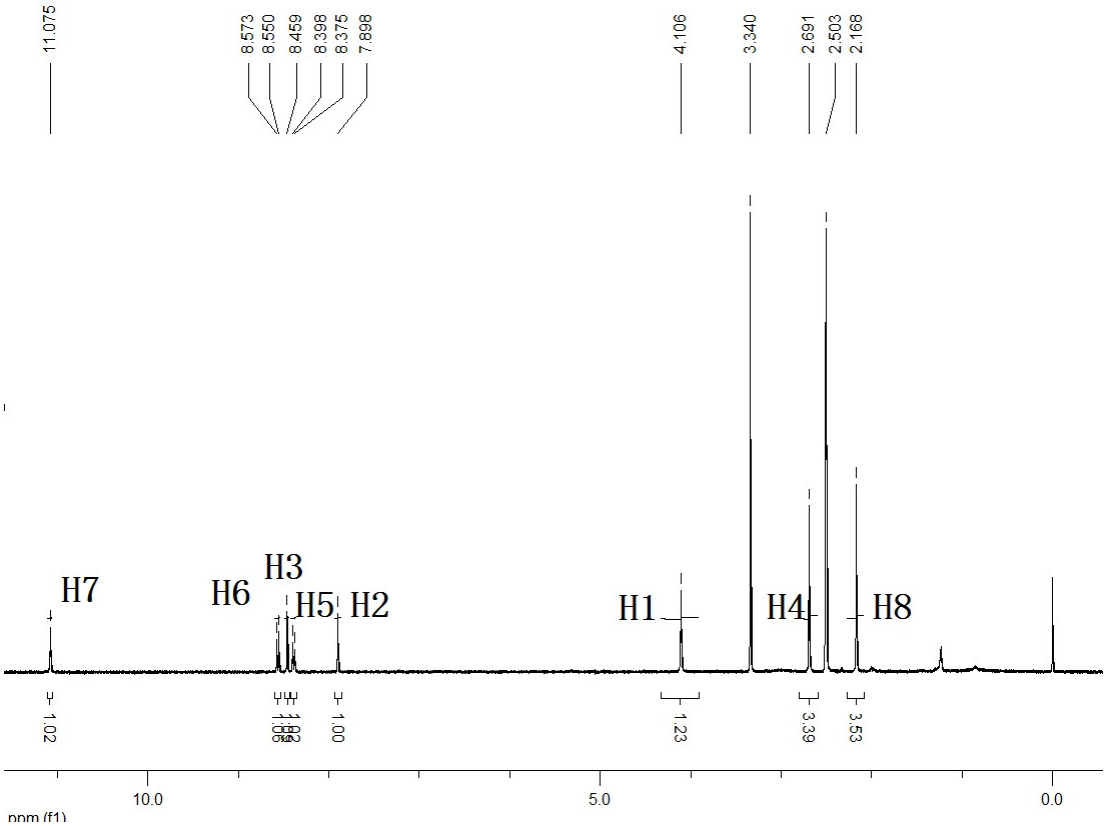
LUMO

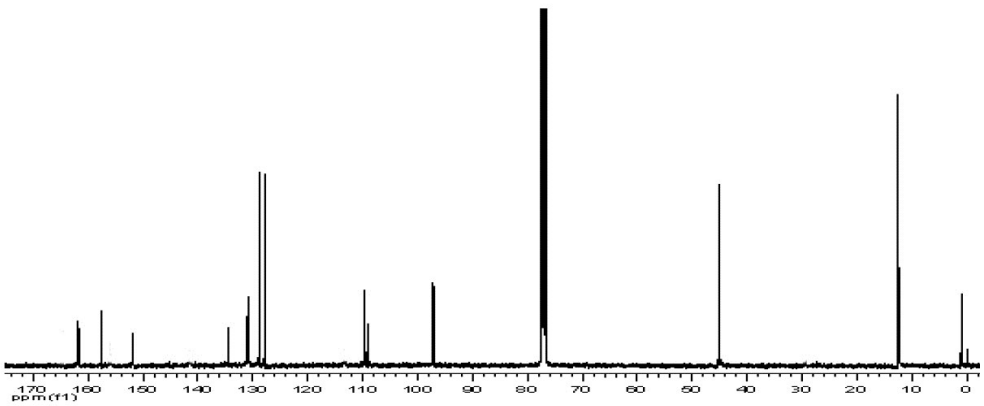
8. Figure S7 ^{1}H-NMR and ^{13}C-NMR spectra of **DP1**.



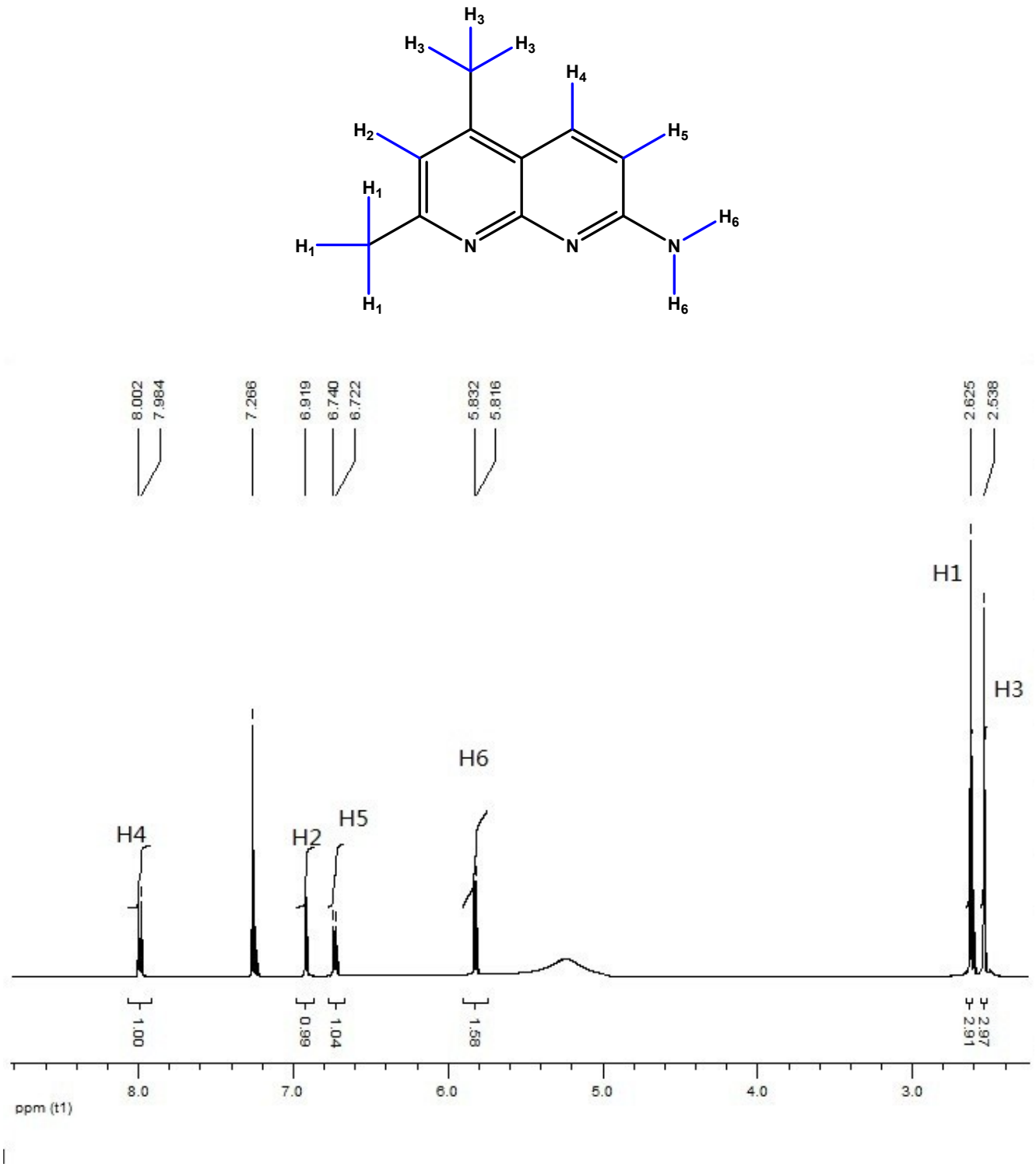
9. Figure S8 ^{1}H-NMR and ^{13}C-NMR spectra of **DP2.**



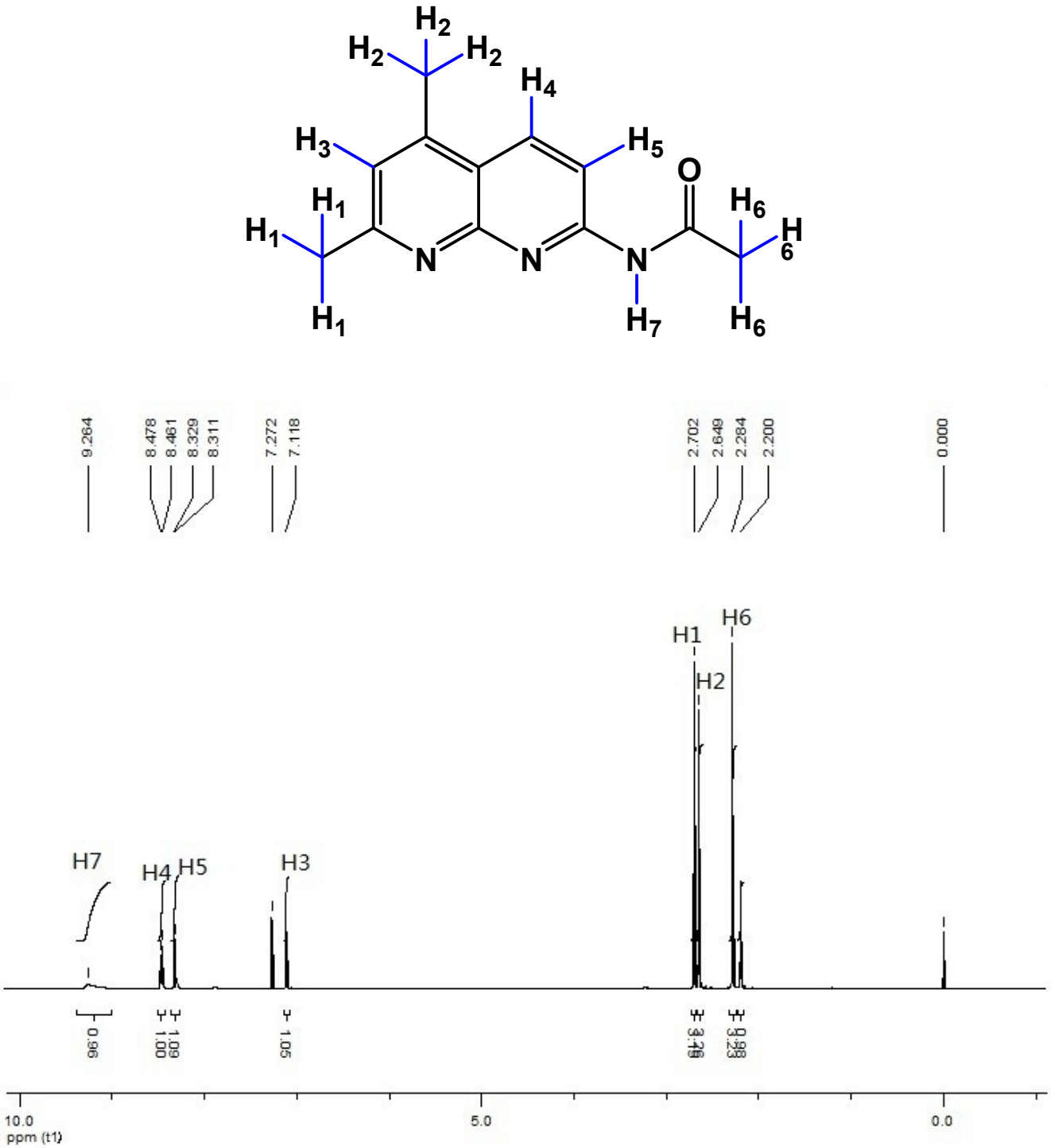




10. Figure S9 ^{1}H-NMR spectra of **1.**



11. Figure S10 ^{1}H-NMR spectra of **2.**



12. Figure S11 ^{1}H-NMR spectra of **NAPY.**

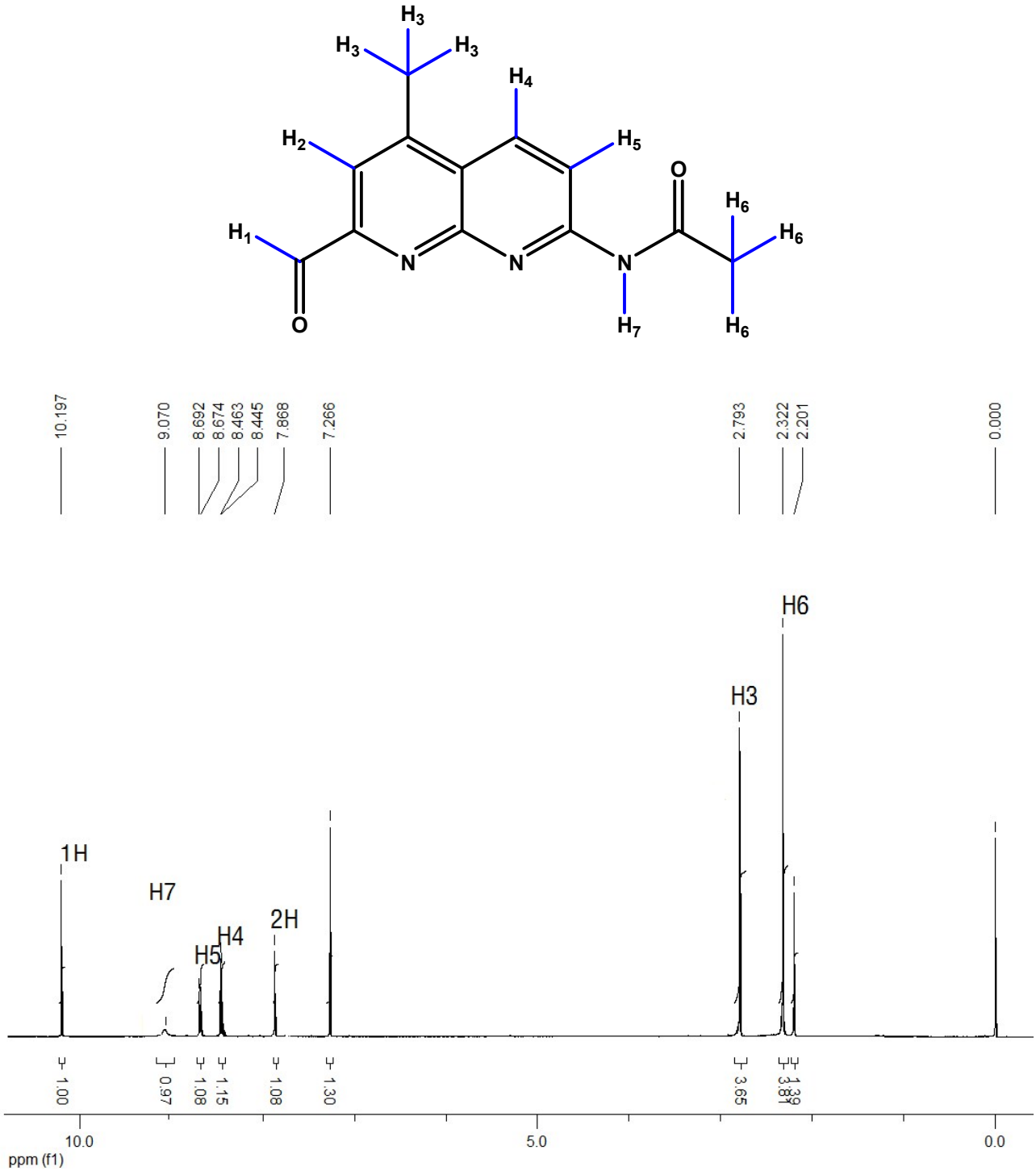


Figure S12 MS spectra of **DP1**.

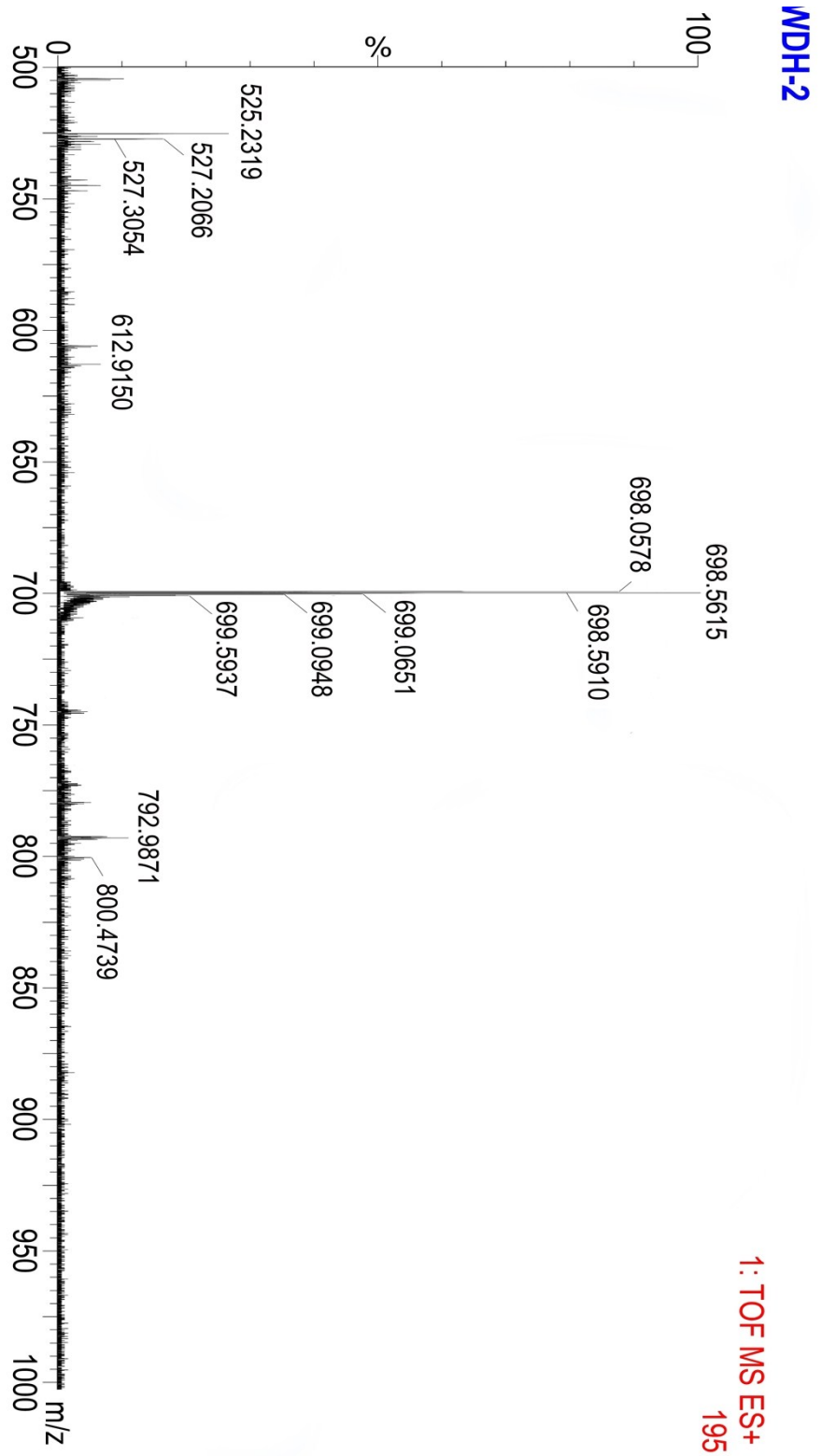


Figure S13 MS spectra of **DP2.**

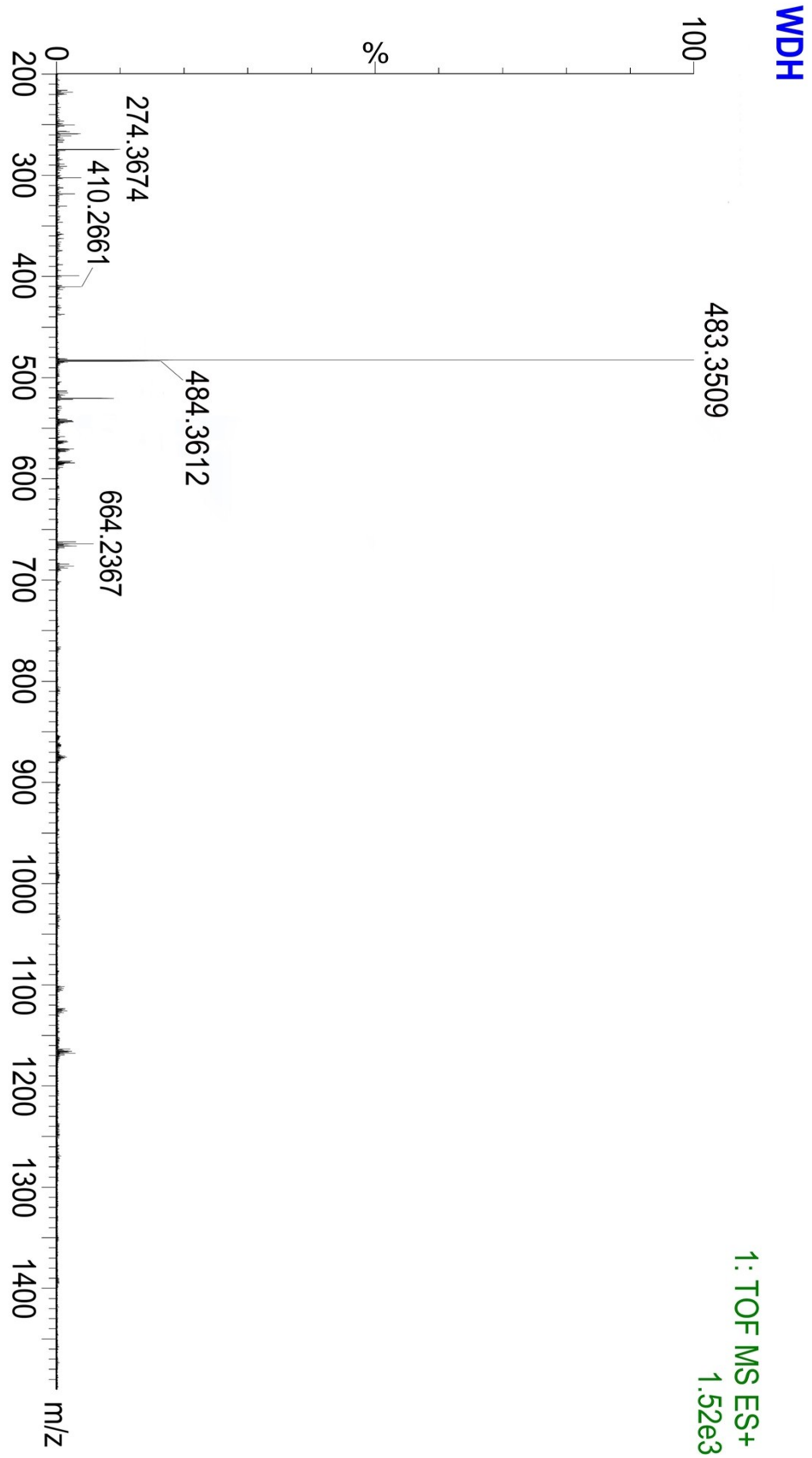


Figure S14 Calculations for detection limit of **DP1** with Zn^{2+}

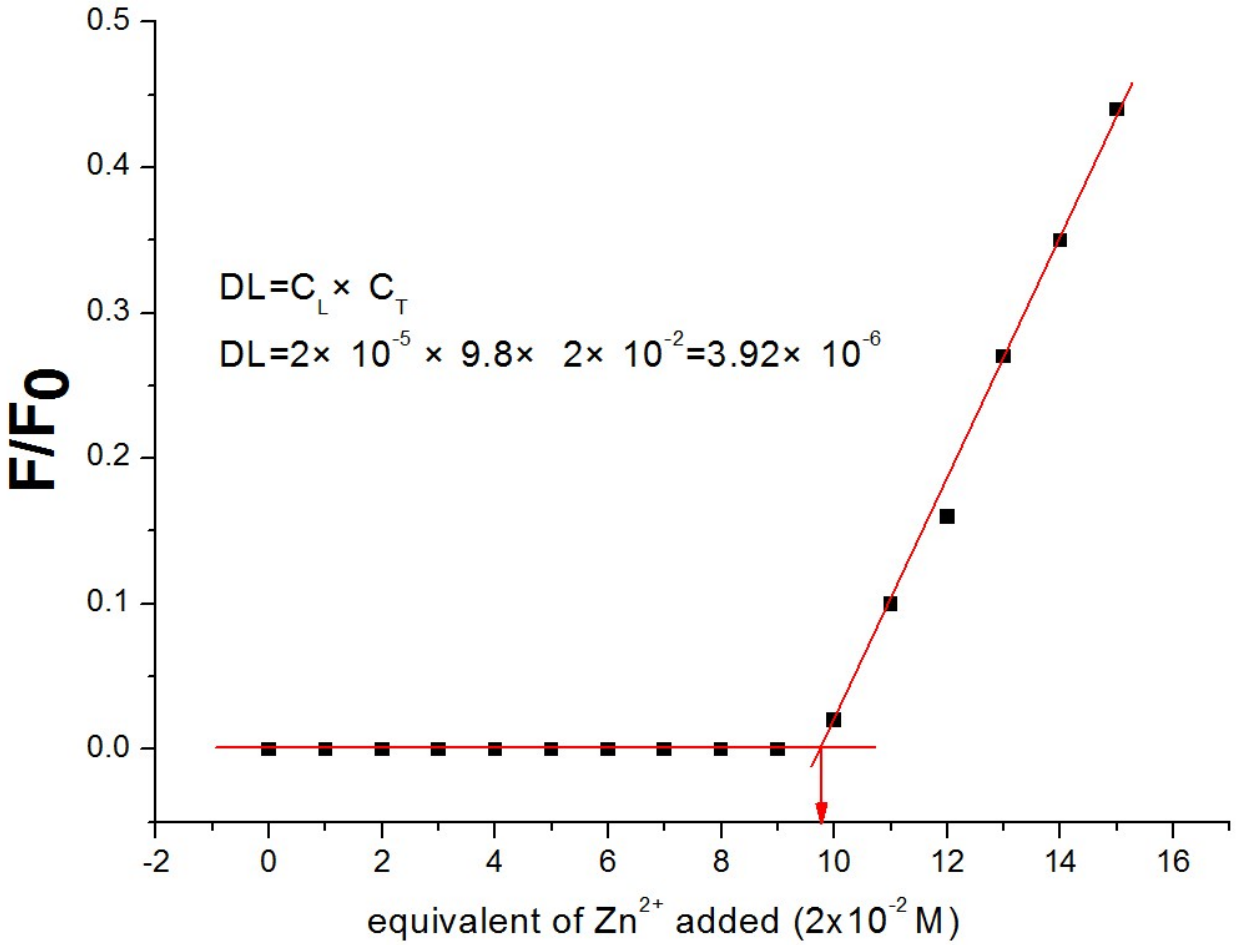


Figure S15 UV-Vis spectral data of **DP2**