Please note: This version of the ESI published 28/04/2022 replaces the previous version published 20/09/2019. Detailed DNA sequences have been added to page S2 and Scheme S1 has been replaced to correct abbreviated compounds HDM and BMN.

Supporting Information

Ultra-sensitive recognizing of AP-site in DNA at the Single-cell Level: One molecular rotor sequentially self-regulated to form multiple stable conformations

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1. Procedures section

A.R. grade of solvents and reagents were used in this work. Column chromatographic was used to purify compounds and silica gel (200-300 mesh) was used as fillers. DNA, RNA, Triacylglycerol Acylhydrolase, Lysozyme, Proteinase, Histone, Collagen, Hemoglobin, BSA, β-amylase, Trypsin and Chymotrypsin were obtained from Sigma Chemical Co. (USA). AP-sites in DNA were induced by nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) as reference method.[S1, S2] AP-sites Quantification Kit (DNA Damage counting kit) was purchased from Dojindo (Japan). Doubly purified water was used in all experiments, which was prepared using by a Milli-Q system. **BMN-Fluors** and intermediate products as stock were used in spectrographic determination and cell experiments. NMR spectra were obtained from Avance 400 or 600 MHz spectrometer (Bruker Co., Switzerland). Lambd 950 spectrophotometer from PerkinElmer (USA) and a LS-55 spectrophotometer from PerkinElmer were used to detect the absorption spectra and fluorescence spectra in vitro. Ultra-high-resolution electro-spray time-of-flight mass spectrometry (Compact) was used to detect the molecular mass. BD FACSCanto II (USA) was used to sort cells in flow cytometer analysis.

The different sequences of DNA were used in the all spectrum measurements, which were synthesized by the Thermo Fisher Scientific. The sequences of DNA were listed as follow:

DNA sequence: TTCTAGGCTCCTAGGACCCC TTCTAGGCTCCTAGGACCCC TTCTAGGCTCCTAGGACCCC;

2 AP sites in DNA sequence: TTCTAGG(RDG)(RDG)CCTAGGACCCC TTCTAGGCTCCTAGGACCCC TTCTAGGCTCCTAGGACCCC;

4 AP sites in DNA sequence: TTCTAG(RDG)(RDG)(RDG)(RDG)CTAGGACCCC TTCTAGGCTCCTAGGACCCC TTCTAGGCTCCTAGGACCCC;

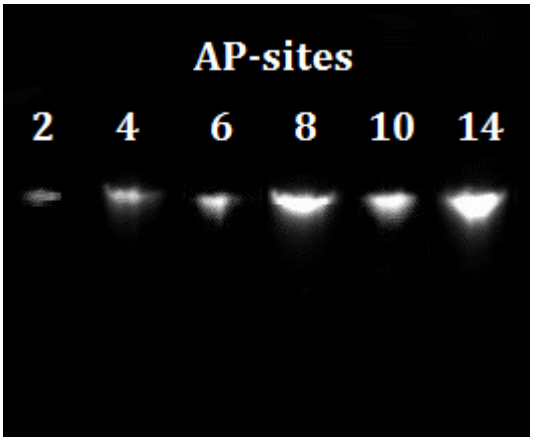
6 AP sites in DNA sequence: TTCTA(RDG)(RDG)(RDG)(RDG)(RDG)(RDG)TAGGACCCC TTCTAGGCTCCTAGGACCCC TTCTAGGCTCCTAGGACCCC;

8 AP sites in DNA sequence: TTCT(RDG)(RDG)(RDG)(RDG)(RDG)(RDG)(RDG)(RDG)AGGACCCC TTCTAGGCTCCTAGGACCCC TTCTAGGCTCCTAGGACCCC;

10 AP sites in DNA sequence: TTC(RDG)(RDG)(RDG)(RDG)(RDG)(RDG)(RDG)(RDG)(RDG) (RDG)GGACCCC TTCTAGGCTCCTAGGACCCC TTCTAGGCTCCTAGGACCCC;

14 AP sites in DNA sequence: TTC(RDG)(RDG)(RDG)(RDG)(RDG)(RDG)(RDG)(RDG)(RDG)(RDG) (RDG)(RDG)(RDG)(RDG)CCC TTCTAGGCTCCTAGGACCCC TTCTAGGCTCCTAGGACCCC

Gel electrophosis of the different AP sites in DNA sequencing



5-formyl uracil in DNA sequencing: 5'-D(*TP*TP*CP*TP*AP*GP*XP*GP*TP*CP*CP*TP*AP*GP*GP* AP* CP*CP*CP*C)-3',

1.1 Spectrographic determination in vitro

A Lambd 950 spectrophotometer from PerkinElmer (USA) and a LS-55 spectrophotometer from PerkinElmer (USA) were used to measure absorption spectra and fluorescence spectra, respectively. In all spectral experiments, the final solutions contained < 5.0‰ DMSO. Each experiment was carried out in five replicates (n = 5). The relative fluorescence quantum yields were determined using Rhodamine B (Φ_F = 0.97 in methanol) by the following equation:

$$\Phi_x = \Phi_s(F_x/F_s)(A_s/A_x)(\lambda_{exs}/\lambda_{exx})(n_x/n_s)^2 \quad (1)$$

where Φ represents quantum yield; F is the integrated area under the corrected emission spectrum; A is absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; n is the refractive index of the solution (because of the low concentrations of the solutions, 10^{-7}-10^{-8} mol/L, the refractive indices of the solutions were replaced with those of the solvents); and the subscripts x and s refer to the unknown and the standard, respectively. The detection limit was calculated by three times the standard deviation divided by the slope of the blank. The data were obtained from replicate experiments (n = 5).

1.2 ^{1}H NMR Titration in D_2O

All ^{1}H NMR titration spectra were obtained at 25 ℃ using a Bruker 600-MHz spectrometer equipped. Data were acquired and processed using Bruker TopSpin 2.1 software and the software package Sparky. **d1-BMN** (3.0 mM) and HDM (3.0 mM) in D_2O was used in ^{1}H NMR titration.

1.3 Photostability in solution

d1-BMN (3.0 μM) in PBS buffer (pH 7.4) at 25 °C. The solutions were irradiated by a 500W iodine-tungsten lamp situated 250 mm away for 7 h. An aqueous solution of sodium nitrite (50 g/L) was placed between the samples and the lamp as a light filter (to cut off the light shorter than 400 nm) and as a heat filter. The photostabilities were expressed in terms of remaining absorption (%) calculated from the changes of absorbance at the absorption maximum before and after irradiation by iodine-tungsten lamp. The absorbance was determined. The data were obtained from replicate experiments (n = 5).

1.4 Quantum Calculations

All the quantum chemical calculations were done with the Gaussian 16 suite. The geometry optimizations of the dyes were performed using density functional theory (DFT) with Becke's three- parameter hybrid exchange function with Lee-Yang-Parrgradient-corrected correlation functional (B3-LYP functional).

Gaussian 16 was used in quantum chemical, and the work of Han was as reference for setting up calculations parameter[S3]. The density functional theory (DFT)[S4] with B3-LYP and B3LYP-D3 functional were used in the geometry optimizations of the dyes. And 6–31G* basis set was utilized. No constraints to bonds/angles/dihedral angles were applied in the calculations and all atoms were free to optimize. The time-dependent density functional theory (TD-DFT)[S5,S6] at the B3LYP/6-31G** level was used to calculate electronic transition energies and corresponding oscillator strengths.

1.5 Molecular docking

All works of molecular docking were conducted in Yinfo Cloud Platform (http://cloud.yinfotek.com/). The UCSF DOCK 6.9 software was used for the molecular docking. The chemical structure of the small molecule **d1-BMN** was drawn by JSME and converted to 3D structure with energy minimization in MMFF94 force field. The crystal structure of DNA Polymerase- damaged DNA complex was taken from the RCSB Protein Data Bank. PDB encoding is 4Q45. NDB encoding is NA2985. DOI: 10.2210/pdb4Q45/pdb. The DMS tool[S7] was employed to build molecular surface of the receptor using a probe atom with a 1.4 Å radius. The binding pocket was defined by the crystal ligand and spheres were generated filling the site by employing the Sphgen module in UCSF Chimera.[S8] A box enclosing the spheres was set with center of (45.397, 58.906, 38.962) and sizes of (18.842, -23.879, 113.892), within which grids necessary for rapid score evaluation were created by the Grid module. Finally, DOCK 6.9[S9] program was utilized to conduct semi-flexible docking where 10000 different orientations were produced. Clustering analysis were performed (RMSD threshold was set 2.0 Å) for candidate poses and the best scored ones were output.

DNA sequencing:5'-D(*TP*CP*TP*AP*GP*GP*GP*TP*CP*CP*TP*AP*GP*GP*AP*CP*CP*C)-3',

Damaged DNA sequencing:5'-D(*TP*CP*TP*AP*GP*GP*(RDG)P*TP*CP*CP*TP*AP*GP*GP*AP*CP*CP*C)-3'.

1.6 Molecular dynamics simulation

Molecular dynamics simulation (MD) was performed using the sander module implemented in the Amber 16 suite, with the BSC1 force field used for the DNA. Hydrogen atoms and sodium ions (to neutralize the negative charges) were added to the DNA with the tleap utility. The simulation system was immersed in a truncated octahedral box of TIP3P explicit water, extended 10 Å outside the DNA on all sides. To start the MD simulation, the simulation system was energy-minimized during 10,000 conjugate-gradient steps. After energy minimization, the system was heated in the NVT ensemble from 0 to 300 K over 50 ps. This procedure was followed by 50 ps of NPT simulation at 300 K and 1 atm pressure using the Langevin dynamics algorithm. After equilibration, 20ns production MD simulation was performed. In all MD simulations, bonds involving hydrogen atoms were constrained using the SHAKE algorithm. Long range interactions were treated using the particle-mesh Ewald (PME) method and a non-bonded interaction cutoff of 10 Å was used. A time step of 2.0 fs was used and coordinates of the system were saved every 2ps.

1.7 Cell Culture

Hepg 2 cell lines were obtained from the Chinese Academy of Medical Sciences. The red-free Dulbecco's Modified Eagle's Medium (DMEM, WelGene) and eagle's minimum essential medium (MEM, WelGene) supplemented with penicillin/streptomycin and 10 % fetal bovine serum (FBS; Gibco) were used for culture cells in a CO_2 incubator at 37 ℃. One day before imaging, the cells mentioned above were seeded into confocal dishes with well glass bottom (MatTek, 1# glass, 0.13-0.16 mm). They were incubated at 37 ℃ in 5.0 wt %/vol CO_2 for 24 h. And then, the cells were incubated with OPM at a certain concentration.

1.8 Single Cell Gel Electrophoresis Assay

Briefly, cells are mixed with 0.5% low-melting agarose at 37 ^{o}C and then placed on a microscope slide coated with 0.5% normal agarose. When the agarose has solidified, an additional layer of agarose is added. After the preparation of the three layers of this material, the cells are lysed in a detergent solution for at least 1.0 h and then the slides are put into a neutral buffer in a electrophoresis chamber, allowing the DNA unwinding, the electrophoresis is carried out, resulting in the migration of small pieces from the core of DNA, toward the electric field. After electro phoresis the slides are rinsed with PBS and cells are stained with a dye (EB). The method is also described in detail in the reference S10 and S11.

Sample Preparation: Cell samples (HepG 2 cell) should be prepared immediately before starting the assay. Cell samples should be handled under yellow light to prevent DNA damage from ultraviolet light. Buffers should be cooled to 4 ℃ to inhibit endogenous damage occurring during sample preparation and to inhibit repair in cells. PBS must be calcium and magnesium free to inhibit endonuclease activities. The appropriate controls should also be included. Optimal results in the CometAssay® are usually obtained with 500-1000 cells per CometSlideTM sample area. Using 50 μL of a cell suspension at 1×10^5 cells per ml combined with 500 μL of LMAgarose will provide the correct agarose concentration and cell density for optimal results when spreading 50 μL per well.

Controls: A sample of untreated cells should always be processed to control for endogenous levels of damage within cells, and for damage that may occur during sample preparation. Control cells and treated cells should be

handled in an identical manner. Treatment will generate significant oxidative damage in the majority of cells, thereby providing a positive control for each step in the CometAssay.

Neutral CometAssay:

1. Prepare Lysis Solution and cool at 4 ℃ for at least 20 minutes before use.

2. Melt LMAgarose in a beaker of boiling water for 5 minutes, with the cap loosened, and then cool in a 37 ℃ water bath for at least 20 minutes.

3. Combine cells at 1×10^5/mL with molten LMAgarose (at 37 ℃) at a ratio of 1:10 (v/v) and immediately pipette 50 µL onto CometSlide™. Use side of pipette tip to spread agarose/cells over sample area. Comet LMAgarose (molten and at 37 ℃ from step 2) 500 µL Cells in 1X PBS (Ca^{2+} and Mg^{2+} free) at 1×10^5/ml 50 µL.

4. Place slides flat at 4 ℃ in the dark (*e.g.* place in refrigerator) for 10 minutes. A 0.5 mm clear ring appears at edge of CometSlide™ area. Increasing gelling time to 30 minutes improves adherence of samples in high humidity environments.

5. Immerse slides in 4 ℃ Lysis Solution for 1 hour or overnight for added sensitivity.

6. Remove slides from Lysis Buffer, drain excess buffer from slide and gently immerse in 50 ml of 4 ℃ 1× Neutral Electrophoresis Buffer for 30 minutes.

7. For the CometAssay® ES unit, add ~850 mL 4 ℃ 1× Neutral Electrophoresis Buffer, place slides in electrophoresis slide tray and cover with Slide Tray Overlay. Set power supply to 21 volts and apply voltage for 45 min at 4 ℃. For other electrophoresis units, align slides equidistant from electrodes, add 1× Neutral Electrophoresis Buffer not to exceed 0.5 cm above slides, and apply voltage at 1 volt per cm (measured electrode to electrode).

8. Drain excess Neutral Electrophoresis Buffer and gently immerse slides in DNA Precipitation Solution for 30 minutes at room temperature.

9. Immerse slides in 70% ethanol for 30 minutes at room temperature.

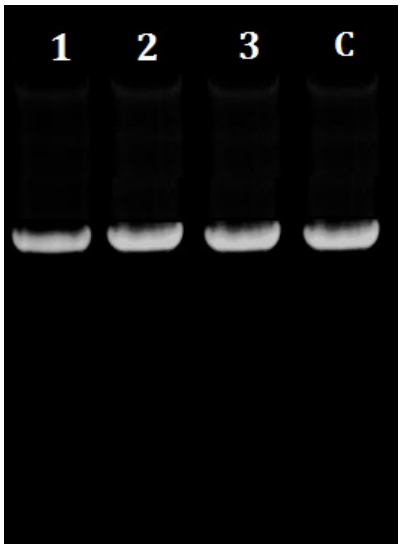
10. Dry samples at 37 ℃ for 10-15 minutes. Drying brings all the cells in a single plane to facilitate observation. Samples may be stored at room temperature, with desiccant prior to scoring at this stage.

11. Place 100 μL of diluted EB or **d1-BMN** onto each circle of dried agarose and stain 30 minutes (room temperature) in the dark. Gently tap slide to remove excess EB or **d1-BMN** solution and rinse briefly in water. Allow slides to dry completely at 37 ℃.

Data Analysis: In healthy cells the fluorescence is confined to the nucleoid (comprised of high molecular weight DNA): undamaged DNA is supercoiled and thus, does not migrate very far out of the nucleoid under the influence of an electric current. Whereas in cells that have accrued DNA damage, migrating fragments (comet tail) from the nucleoid (comet head) are observed. The negatively charged DNA migrates toward the anode and the extrusion length reflects increasing relaxation of supercoiling, which is indicative of damage. In neutral comet assays, Tail Moment is primarily used.

Quantitative Analysis: The DNA damage was assessed with percentage of DNA in the tail (TI %) and for this purpose one hundred cells were assessed per slide by using Comet assay IV image analysis system (Perceptive Instruments, UK). EB maximum excitation is 300 nm and 360 nm, EB maximum emission is 590 nm. **d1-BMN** maximum excitation is 354 and 400 nm, **d1-BMN** maximum emission is 414, 440, 468, 495, 524 or 562 nm. Fluorescein filter is adequate.

Gel electrophoresis was used to verify that DNA containing AP-sites not converted to single strand break during the assay in this work. Gel electrophoresis results indicated that DNA not converted to single strand break before (1), during (2), and after the assay (3) compare with the control group (C).



The DNA gel electrophoresis before (1), during (2), and after the assay (3) compare with the control group (C).

1.9 Cytotoxicity

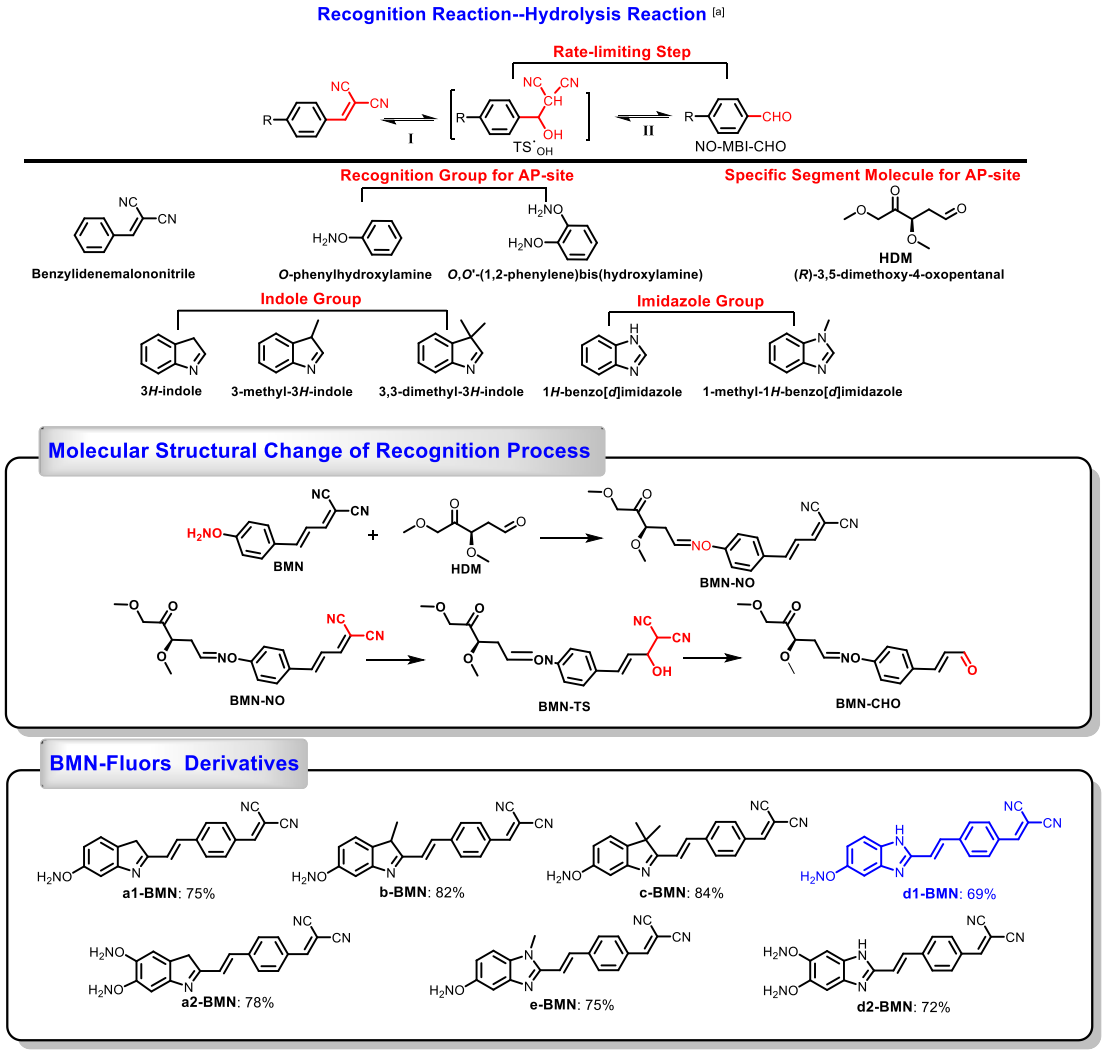
Hepg 2 cell lines and NIH 3T3 cell lines were prepared for cell viability studies in 96-well plates (1×10^5 cells per well that were incubated in 100 μL). The cells were incubated for an additional 24 h with dyes **d1-BMN** in different concentrations. Subsequently, 100 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co. U.S.A.) was added into each well, followed by further incubation for 4 h at 37 ℃. The DMEM was remove and DMSO (200 μL/well) added to dissolve the reddish-blue crystals. Optical density (OD) was determined by a microplate reader (Spectra Max M5, Molecular Devices) at 570 nm with subtraction of the absorbance of the cell-free blank volume at 630 nm. The results from the six individual experiments were averaged. The relative cell viability (100%) was calculated using the following equation:

$$\text{Cell viability (\%)} = (OD_{dye}\text{-}OD_{k\text{-}dye})/(OD_{ctrl}\text{-}OD_{k\text{-}ctrl}) \times 100 \quad (2)$$

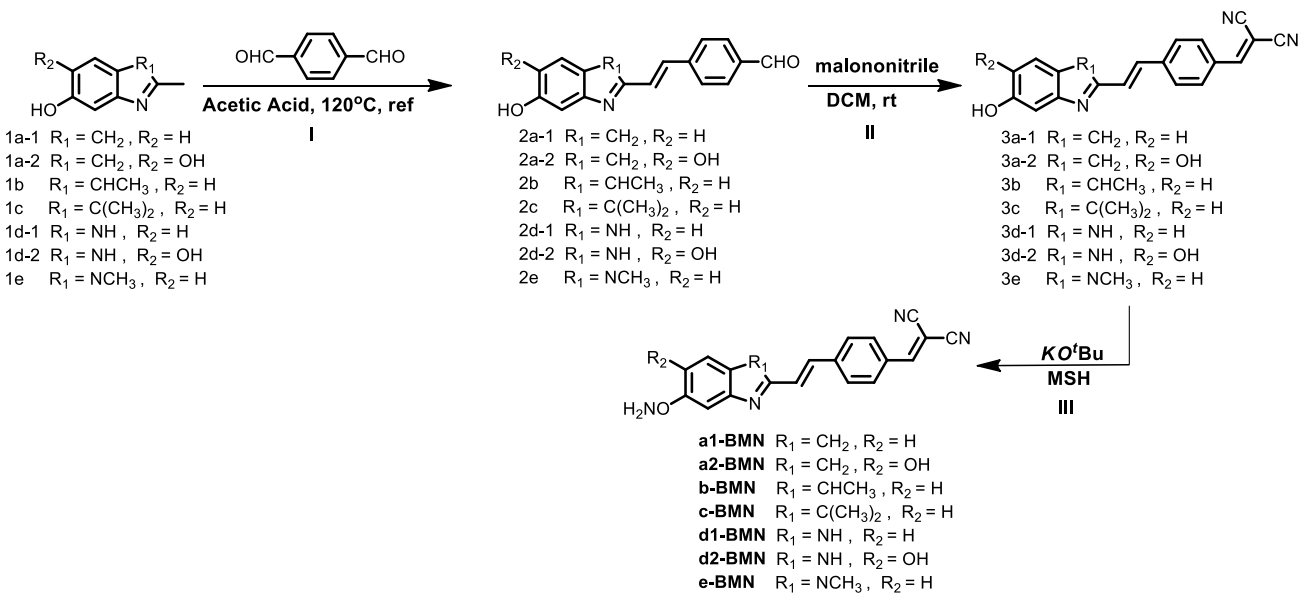
1.10 Flow cytometry

Hepg 2 cell lines with incubated by **d1-BMN** (3.0 μM) for 10 min, then the cells were washed. And they were dispersed into PBS solution at level of 10,000 cells/500 μL. Samples were analyzed with the laser (405 nm) on a flow cytometer (BD FACSCanto II, USA). The average fluorescence intensity in 10,000 cells was obtained and analyzed with BD FACSDiva software. Each individual cell should contain a mixture of AP-site with different sizes, thus, the same cell can give a mixture of fluorescence signals, for example red, orange, yellow, green and blue fluorescence. So, to sort the cell into different channels, we used the sorting function of flow cytometry to in succession and repeatedly sort the cell. Furthermore, in every control experiment for different fluorescence channels, they were added fluorescence compensation to obtain absolutely strong fluorescence signal for the target collection channel.

2. Derivative molecule and synthetic route.



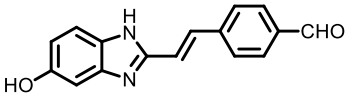
Scheme S1. Derivative molecules. The hydrolysis transformation of 2-(4-vinylbenzylidene)malononitrile derivatives.



Scheme S2. The synthetic route of **BMN-Fluors** derivatives.

3. Synthetic procedures of BMN-Fluors derivatives.

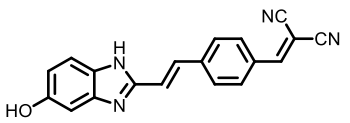
The synthesis of 2d-1 ((*E*)-4-(2-(5-hydroxy-1*H*-benzo[*d*imidazol-2-yl)vinyl)benzaldehyde)



(*E*)-4-(2-(5-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzaldehyde

1d-1 (2-methyl-1*H*-benzo[*d*]imidazol-5-ol (2.6 mmol, 386 mg)), p-Phthalaldehyde (3.0 mmol, 400 mg) was added into acetic acid solution. Under stirring, they was heated to refluxed temperature (120 ^{o}C). After complete consumption of **1d-1** monitored by TLC, concentrated hydrochloric acid (1.0 mL) was added into the mixture solution, and was standed for 1.0 h. Then, there appeared brown solid in the mixture solution, and it were filtrated. The filter liquor was regulated by sodium hydroxide to neutral. Than, the crude product appeared, which was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (50:1 to 5:1, v/v) to obtain a yellow solid **2d-1** ((*E*)-4-(2-(5-hydroxy-1*H*-benzo[*d*]I midazol-2-yl)vinyl)benzaldehyde). Yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$), *δ:* 12.32 (s, 1H), 10.06 (s, 1H), 7.98 (d, 2H, *J* = 5.26 Hz), 7.89 (d, 2H, *J* = 5.27 Hz), 7.80 (d, 1H, *J* = 10.95 Hz), 7.60 (d, 1H, *J* = 10.95 Hz), 7.47 (d, 1H, *J* = 10.95 Hz), 7.36 (d, 1H, *J* = 10.95 Hz), 7.23 (d, 1H, *J* = 5.24 Hz), 5.35 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ:* 190.7, 151.7, 143.3, 141.5, 140.9, 136.1, 133.4, 131.8, 129.7, 129.0, 124.0, 116.6, 114.6, 111.4, 102.6. HRMS: m/z calcd for $C_{16}H_{12}N_2O_2$: 264.0899, found: 264.0905.

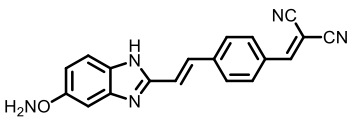
The synthesis of 3d-1 ((*E*)-2-(4-(2-(5-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(5-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

2d-1 (2.0 mmol, 530 mg) and malononitrile (4.0 mmol, 265 mg) were suspended in DCM, and then they was at room temperature under stirring for 4.0 h. Than, the crude product appeared, which was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 1:1, v/v) to obtain a yellow solid **3d-1**. Yield 73%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 12.05 (s, 1H), 7.95 (s, 1H), 7.88 (d, 2H, *J* = 5.26 Hz), 7.77 (d, 1H, *J* = 10.95 Hz), 7.65 (d, 2H, *J* = 5.27 Hz), 7.57 (d, 1H, *J* = 10.95 Hz), 7.44 (d, 1H, *J* = 10.80 Hz), 7.33 (d, 1H, *J* = 10.80 Hz), 7.18 (d, 1H, *J* = 10.80 Hz), 5.36 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 161.8, 151.6, 141.6, 140.1, 135.9, 133.2, 131.4, 130.6, 128.9, 124.0, 116.7, 113.5, 111.4, 102.5, 81.5. HRMS: m/z calcd for $C_{19}H_{12}N_4O$: 312.1011, found: 312.1019.

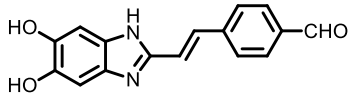
The synthesis of d1-BMN ((*E*)-2-(4-(2-(5-(aminooxy)-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(5-(aminooxy)-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

3d-1 (2.76 mmol, 860 mg) was dissolved in 4 mL of methanol, and then potassium *tert*-butoxide (309 mg, 2.76 mmol) was added. The mixture was allowed to stir for 0.5 h under N_2 atmosphere. Methanol was removed, and the residue was taken up in 2 mL of dichloromethane. The freshly prepared *O*-mesitylsulfonylhydroxylamine (378 mg, 1.76 mmol) in 2 mL of dichloromethane was then added under ice cooling. The mixture was allowed to stir for 1 h, and dichloromethane was then removed under reduce pressure to afford the corresponding product. It was purified by column chromatography on silica gel eluted with DCM/Methanol (100:1 to 10:1, v/v) to obtain a yellow solid **d1-BMN**. Yield 69%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 12.07 (s, 1H), 7.98 (s, 1H), 7.90 (d, 2H, *J* = 5.26 Hz), 7.79 (d, 1H, *J* = 10.95 Hz), 7.67 (d, 2H, *J* = 5.27 Hz), 7.59 (d, 1H, *J* = 10.95 Hz), 7.46 (d, 1H, *J* = 10.80 Hz), 7.35 (d, 1H, *J* = 10.80 Hz), 7.20 (d, 1H, *J* = 10.80 Hz), 6.85 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 161.8, 151.6, 141.6, 140.1, 136.9, 133.2, 131.4, 130.6, 128.9, 124.4, 116.7, 113.5, 111.4, 102.5, 81.5. HRMS: m/z calcd for $C_{19}H_{13}N_5O$: 327.1120, found: 327.1129.

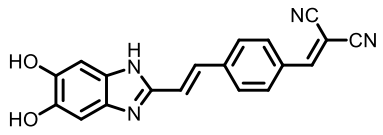
The synthesis of 2d-2 ((*E*)-4-(2-(5,6-dihydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzaldehyde)



(*E*)-4-(2-(5,6-dihydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzaldehyde

The synthesis of **2d-2** was followed by the procedure described in previous method for **2d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (50:1 to 2:1, v/v) to obtain a yellow solid **2d-2** ((*E*)-4-(2-(5,6-dihydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzaldehyde). Yield 80%. ^{1}H NMR (400 MHz, $CDCl_3$), *δ:* 12.30 (s, 1H), 10.06 (s, 1H), 7.98 (d, 2H, *J* = 5.26 Hz), 7.89 (d, 2H, *J* = 5.27 Hz), 7.47 (d, 2H, *J* = 10.95 Hz), 7.34 (d, 1H, *J* = 10.59 Hz), 7.21 (d, 1H, *J* = 10.59 Hz), 5.35 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ:* 191.7, 143.0, 141.5, 138.9, 136.1, 133.4, 132.8, 129.7, 129.0, 124.0, 103.5. HRMS: m/z calcd for $C_{16}H_{12}N_2O_3$: 280.0848, found: 280.0844.

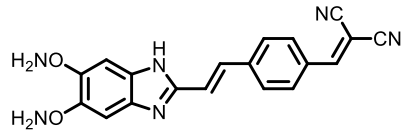
The synthesis of 3d-2 ((*E*)-2-(4-(2-(5,6-dihydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(5,6-dihydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **3d-2** was followed by the procedure described in previous method for **3d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 1:1, v/v) to obtain a yellow solid **3d-2**. Yield 70%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 12.30 (s, 1H), 7.98 (d, 2H, *J* = 5.26 Hz), 7.89 (d, 2H, *J* = 5.27 Hz), 7.64 (s, 1H), 7.47 (d, 2H, *J* = 10.95 Hz), 7.34 (d, 1H, *J* = 10.55 Hz), 7.21 (d, 1H, *J* = 10.55 Hz), 5.36 (s, 2H). ^{13}C NMR (100 MHz, CDCl3): δ: 161.7, 141.5, 138.9, 136.7, 133.4, 132.8, 130.6, 129.0, 124.0, 113.6, 103.5, 81.4. HRMS: m/z calcd for $C_{19}H_{12}N_4O_2$: 328.0960, found: 328.0962.

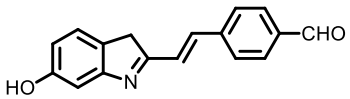
The synthesis of d2-BMN ((*E*)-2-(4-(2-(5,6-bis(aminooxy)-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(5,6-bis(aminooxy)-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **d2-BMN** was followed by the procedure described in previous method for **d1-BMN**. It was purified by column chromatography on silica gel eluted with DCM/Methanol (100:1 to 10:1, v/v) to obtain a yellow solid **d2-BMN**. Yield 54%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 12.32 (s, 1H), 7.98 (d, 2H, *J* = 5.26 Hz), 7.89 (d, 2H, *J* = 5.26 Hz), 7.64 (s, 1H), 7.47 (d, 2H, *J* = 10.95 Hz), 7.34 (d, 1H, *J* = 10.95 Hz), 7.21 (d, 1H, *J* = 10.95 Hz), 6.78 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 161.5, 141.5, 138.9, 136.5, 133.4, 132.8, 130.3, 129.0, 124.5, 113.6, 103.8, 81.8. HRMS: m/z calcd for $C_{19}H_{14}N_6O_2$: 358.1178, found: 358.1182.

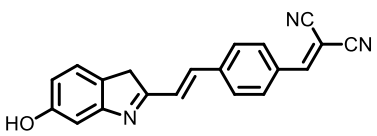
The synthesis of 2a-1 ((*E*)-4-(2-(6-hydroxy-3*H*-indol-2-yl)vinyl)benzaldehyde)



(*E*)-4-(2-(6-hydroxy-3*H*-indol-2-yl)vinyl)benzaldehyde

The synthesis of **2a-1** was followed by the procedure described in previous method for **2d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 10:1, v/v) to obtain a yellow solid **2a-1** ((*E*)-4-(2-(6-hydroxy-3*H*-indol-2-yl)vinyl)benzaldehyde). Yield 80%. ^{1}H NMR (400 MHz, $CDCl_3$), *δ:* 10.06 (s, 1H), 7.98 (d, 2H, *J* = 5.26 Hz), 7.89 (d, 2H, *J* = 5.27 Hz), 7.80 (d, 1H, *J* = 10.95 Hz), 7.60 (d, 1H, *J* = 10.95 Hz), 7.47 (d, 1H, *J* = 10.95 Hz), 7.36 (d, 1H, *J* = 10.59 Hz), 7.21 (d, 1H, *J* = 10.59 Hz), 5.35 (s, 1H), 3.04 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ:* 190.7, 164.6, 156.4, 140.9, 136.1, 131.8, 129.7, 129.8, 129.6, 129.0, 124.0, 120.1, 114.6, 109.4, 35.3. HRMS: m/z calcd for $C_{17}H_{13}NO_2$: 263.0946, found: 263.0948.

The synthesis of 3a-1 ((*E*)-2-(4-(2-(5,6-dihydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile)

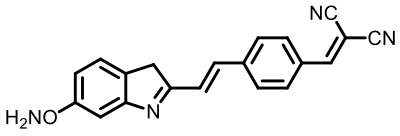


(*E*)-2-(4-(2-(6-hydroxy-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **3a-1** was followed by the procedure described in previous method for **3d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 1:1, v/v) to obtain a yellow solid **3a-1**. Yield 73%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.94 (s, 1H), 7.86 (d, 2H, *J* = 5.26 Hz), 7.75 (d, 1H, *J* = 10.95 Hz), 7.63 (d, 2H, *J* = 5.27 Hz), 7.55 (d, 1H, *J* = 10.95 Hz), 7.42 (d, 1H, *J* = 10.95 Hz), 7.31 (d, 1H, *J* = 10.85 Hz), 7.17 (d, 1H, *J*

= 10.95 Hz), 5.35 (s, 1H), 3.04 (s, 2H). ^{13}C NMR (100 MHz, CDCl3): δ: 164.6, 161.8, 157.6, 156.8, 134.9, 131.2, 130.4, 129.6, 128.9, 123.4, 119.8, 116.7, 114.5, 113.4, 109.5, 81.6, 35.4. HRMS: m/z calcd for $C_{20}H_{13}N_3O$: 311.1059, found: 311.1063.

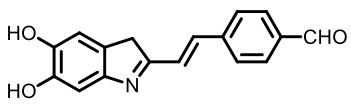
The synthesis of a1-BMN ((*E*)-2-(4-(2-(6-(aminooxy)-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(6-(aminooxy)-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **a1-BMN** was followed by the procedure described in previous method for **d1-BMN**. It was purified by column chromatography on silica gel eluted with DCM/Methanol (100:1 to 10:1, v/v) to obtain a yellow solid **a1-BMN**. Yield 63%. ^{1}H NMR (400 MHz, $CDCl_3$), δ7.99 (s, 1H), 7.91 (d, 2H, *J* = 5.26 Hz), 7.80 (d, 1H, *J* = 10.95 Hz), 7.68 (d, 2H, *J* = 5.27 Hz), 7.60 (d, 1H, *J* = 10.95 Hz), 7.47 (d, 1H, *J* = 10.95 Hz), 7.36 (d, 1H, *J* = 10.90 Hz), 7.22 (d, 1H, *J* = 10.95 Hz), 6.87(s, 2H), 3.04 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 164.6, 161.8, 157.6, 156.8, 134.9, 131.2, 130.4, 129.6, 128.9, 123.4, 119.8, 116.7, 114.5, 113.4, 109.5, 81.6, 35.4. HRMS: m/z calcd for $C_{20}H_{14}N_4O$: 326.1168, found: 326.1165.

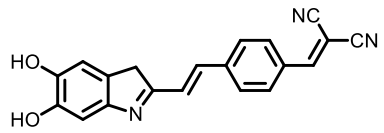
The synthesis of 2a-2 ((*E*)-4-(2-(5,6-dihydroxy-3*H*-indol-2-yl)vinyl)benzaldehyde)



(*E*)-4-(2-(5,6-dihydroxy-3*H*-indol-2-yl)vinyl)benzaldehyde

The synthesis of **2a-2** was followed by the procedure described in previous method for **2d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 10:1, v/v) to obtain a yellow solid **2a-2**. Yield 75%. ^{1}H NMR (400 MHz, $CDCl_3$), *δ:* 10.06 (s, 1H), 7.98 (d, 2H, *J* = 5.26 Hz), 7.89 (d, 2H, *J* = 5.27 Hz), 7.47 (d, 2H, *J* = 5.26 Hz), 7.34 (d, 1H, *J* = 10.59 Hz), 7.21 (d, 1H, *J* = 10.59 Hz), 5.34 (s, 2H), 3.04 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ:* 191.7, 164.4, 148.6, 145.3, 144.0, 140.9, 136.1, 129.7, 129.0, 125.0, 120.0, 117.6, 111.4, 35.3. HRMS: m/z calcd for $C_{17}H_{13}NO_3$: 279.0895, found: 279.0898.

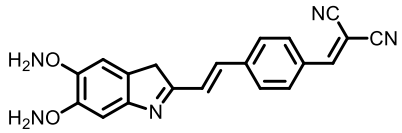
The synthesis of 3a-2 ((*E*)-2-(4-(2-(5,6-dihydroxy-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(5,6-dihydroxy-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **3a-2** was followed by the procedure described in previous method for **3d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 1:1, v/v) to obtain a yellow solid **3a-2**. Yield 56%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.98 (d, 2H, *J* = 5.26 Hz), 7.89 (d, 2H, *J* = 5.27 Hz), 7.64 (s, 1H), 7.47 (d, 2H, *J* = 10.95 Hz), 7.34 (d, 1H, *J* = 10.55 Hz), 7.21 (d, 1H, *J* = 10.55 Hz), 5.34 (s, 2H), 3.04 (s, 2H). ^{13}C NMR (100 MHz, CDCl3): δ: 164.8, 161.6, 148.3, 145.6, 144.1, 134.2, 130.4, 129.6, 128.9, 125.4, 120.4, 117.7, 113.5, 111.4, 102.5, 81.4, 35.2. HRMS: m/z calcd for $C_{20}H_{13}N_3O_2$: 327.1008, found: 327.1002.

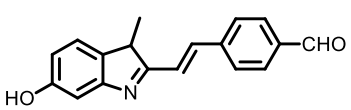
The synthesis of a2-BMN ((*E*)-2-(4-(2-(5,6-bis(aminooxy)-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(5,6-bis(aminooxy)-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **a2-BMN** was followed by the procedure described in previous method for **d1-BMN**. It was purified by column chromatography on silica gel eluted with DCM/Methanol (100:1 to 2:1, v/v) to obtain a yellow solid **d2-BMN**. Yield 54%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.98 (d, 2H, *J* = 5.26 Hz), 7.89 (d, 2H, *J* = 5.27 Hz), 7.65 (s, 1H), 7.47 (d, 2H, *J* = 10.95 Hz), 7.34 (d, 1H, *J* = 10.95 Hz), 7.21 (d, 1H, *J* = 10.95 Hz), 6.78 (s, 4H), 3.04 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 164.7, 161.6, 148.3, 145.5, 144.1, 134.2, 130.4, 129.6, 128.8, 125.4, 120.4, 117.7, 113.5, 111.4, 102.5, 81.4, 35.2. HRMS: m/z calcd for $C_{20}H_{15}N_5O_2$: 357.1226, found: 357.1222.

The synthesis of 2b ((*E*)-4-(2-(6-hydroxy-3-methyl-3*H*-indol-2-yl)vinyl)benzaldehyde)

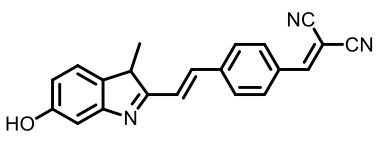


(*E*)-4-(2-(6-hydroxy-3-methyl-3*H*-indol-2-yl)vinyl)benzaldehyde

The synthesis of **2b** was followed by the procedure described in previous method for **2d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 10:1, v/v) to obtain a yellow solid **2b**. Yield 83%. ^{1}H NMR (400 MHz, $CDCl_3$), *δ:* 10.06 (s, 1H), 7.98 (d, 2H, *J* =

5.26 Hz), 7.89 (d, 2H, *J* = 5.27 Hz), 7.80 (d, 1H, *J* = 10.95 Hz), 7.60 (d, 1H, *J* = 10.95 Hz), 7.47 (d, 1H, *J* = 10.95 Hz), 7.36 (d, 1H, *J* = 10.59 Hz), 7.21 (d, 1H, *J* = 10.59 Hz), 5.34 (s, 1H), 3.23 (s, 1H), 1.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ:* 191.7, 164.6, 157.4, 154.9, 140.9, 136.1, 130.8, 129.7, 129.0, 126.0, 114.6, 109.4, 35.2, 18.1. HRMS: m/z calcd for $C_{18}H_{15}NO_2$: 277.1103, found: 277.1108.

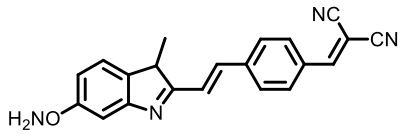
The synthesis of 3b ((*E*)-2-(4-(2-(6-hydroxy-3-methyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(6-hydroxy-3-methyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **3b** was followed by the procedure described in previous method for **3d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 1:1, v/v) to obtain a yellow solid **3b**. Yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.94 (s, 1H), 7.86 (d, 2H, *J* = 5.26 Hz), 7.75 (d, 1H, *J* = 10.95 Hz), 7.63 (d, 2H, *J* = 5.27 Hz), 7.55 (d, 1H, *J* = 10.95 Hz), 7.42 (d, 1H, *J* = 10.95 Hz), 7.31 (d, 1H, *J* = 10.85 Hz), 7.17 (d, 1H, *J* = 10.95 Hz), 5.35 (s, 1H), 3.23 (s, 1H), 1.31 (s,3H). ^{13}C NMR (100 MHz, CDCl3): δ: 164.7, 160.9, 157.1, 154.9, 134.9, 130.4, 129.6, 128.9, 126.4, 120.0, 114.7, 113.5, 109.4, 81.5, 35.8, 18,6. HRMS: m/z calcd for $C_{21}H_{15}N_3O$: 325.1215, found: 325.1209.

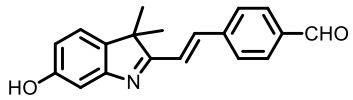
The synthesis of b-BMN ((*E*)-2-(4-(2-(6-(aminooxy)-3-methyl-3H-indol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(6-(aminooxy)-3-methyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **b-BMN** was followed by the procedure described in previous method for **d1-BMN**. It was purified by column chromatography on silica gel eluted with DCM/Methanol (100:1 to 10:1, v/v) to obtain a yellow solid **b-BMN**. Yield 61%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.99 (s, 1H), 7.91 (d, 2H, *J* = 5.26 Hz), 7.80 (d, 1H, *J* = 10.95 Hz), 7.68 (d, 2H, *J* = 5.27 Hz), 7.60 (d, 1H, *J* = 10.95 Hz), 7.47 (d, 1H, *J* = 10.95 Hz), 7.36 (d, 1H, *J* = 10.90 Hz), 7.22 (d, 1H, *J* = 10.95 Hz), 6.87(s, 2H), 3.23 (s, 1H) , 1.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 164.5, 161.2, 157.1, 154.9, 134.9, 130.4, 129.6, 128.9, 126.4, 120.3, 114.7, 113.5, 109.4, 81.5, 35.8, 18,6. HRMS: m/z calcd for $C_{21}H_{16}N_4O$: 340.1324, found: 340.1315.

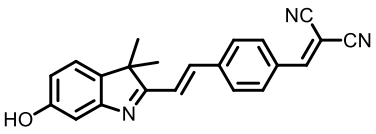
The synthesis of 2c ((*E*)-4-(2-(6-hydroxy-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzaldehyde)



(*E*)-4-(2-(6-hydroxy-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzaldehyde

The synthesis of **2c** was followed by the procedure described in previous method for **2d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 10:1, v/v) to obtain a yellow solid **2c**. Yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$), *δ:* 10.06 (s, 1H), 7.98 (d, 2H, *J* = 5.26 Hz), 7.89 (d, 2H, *J* = 5.27 Hz), 7.80 (d, 1H, *J* = 10.95 Hz), 7.60 (d, 1H, *J* = 10.95 Hz), 7.47 (d, 1H, *J* = 10.95 Hz), 7.36 (d, 1H, *J* = 10.59 Hz), 7.21 (d, 1H, *J* = 10.59 Hz), 5.35 (s, 1H), 1.17 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ:* 191.3, 157.1, 153.4, 145.4, 140.9, 136.1, 129.7, 129.0, 128.1, 120.0, 114.6, 109.4, 39.1, 30.3. HRMS: m/z calcd for $C_{19}H_{17}NO_2$: 291.1259, found: 291.1251.

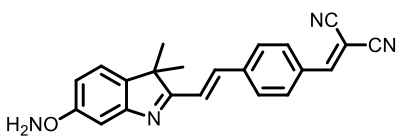
The synthesis of 3c ((*E*)-2-(4-(2-(6-hydroxy-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(6-hydroxy-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **3c** was followed by the procedure described in previous method for **3d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 1:1, v/v) to obtain a yellow solid **3c**. Yield 70%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.94 (s, 1H), 7.86 (d, 2H, *J* = 5.26 Hz), 7.75 (d, 1H, *J* = 10.95 Hz), 7.63 (d, 2H, *J* = 5.27 Hz), 7.55 (d, 1H, *J* = 10.95 Hz), 7.42 (d, 1H, *J* = 10.95 Hz), 7.31 (d, 1H, *J* = 10.85 Hz), 7.17 (d, 1H, *J* = 10.95 Hz), 5.35 (s, 1H), 1.20 (s, 6H). ^{13}C NMR (100 MHz, CDCl3): δ: 164.5, 161.3, 157.1, 153.4, 145.4, 134.1, 130.7, 129.7, 129.0, 128.1, 114.6, 113.6, 109.4, 81.4, 39.1, 30.3. HRMS: m/z calcd for $C_{22}H_{17}N_3O$: 339.1372, found: 339.1379.

The synthesis of c-BMN ((*E*)-2-(4-(2-(6-(aminooxy)-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile)

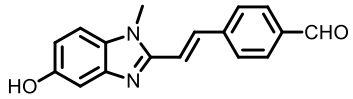


(*E*)-2-(4-(2-(6-(aminooxy)-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **c-BMN** was followed by the procedure described in previous method for **d1-BMN**. It was purified by column chromatography on silica gel eluted with

DCM/Methanol (100:1 to 10:1, v/v) to obtain a yellow solid **c-BMN**. Yield 67%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.99 (s, 1H), 7.91 (d, 2H, *J* = 5.26 Hz), 7.80 (d, 1H, *J* = 10.95 Hz), 7.68 (d, 2H, *J* = 5.27 Hz), 7.60 (d, 1H, *J* = 10.95 Hz), 7.47 (d, 1H, *J* = 10.95 Hz), 7.36 (d, 1H, *J* = 10.90 Hz), 7.22 (d, 1H, *J* = 10.95 Hz), 6.87(s, 2H), 1.19 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: δ: 164.5, 161.3, 157.1, 153.4, 145.4, 134.1, 130.7, 129.7, 129.0, 128.1, 114.6, 113.6, 109.4, 81.4, 39.1, 30.3. HRMS: m/z calcd for $C_{22}H_{18}N_4O$: 354.1481, found: 354.1485.

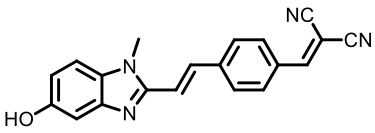
The synthesis of 2e ((*E*)-4-(2-(5-hydroxy-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzaldehyde)



(*E*)-4-(2-(5-hydroxy-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzaldehyde

The synthesis of **2e** was followed by the procedure described in previous method for **2d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 10:1, v/v) to obtain a yellow solid **2e**. Yield 87%. ^{1}H NMR (400 MHz, $CDCl_3$), *δ:* 10.06 (s, 1H), 7.98 (d, 2H, *J* = 5.26 Hz), 7.89 (d, 2H, *J* = 5.27 Hz), 7.80 (d, 1H, *J* = 10.95 Hz), 7.60 (d, 1H, *J* = 10.95 Hz), 7.47 (d, 1H, *J* = 10.95 Hz), 7.36 (d, 1H, *J* = 10.95 Hz), 7.21 (d, 1H, *J* = 5.24 Hz), 5.35 (s, 1H), 3.38 (s, 3H);^{13}C NMR (100 MHz, $CDCl_3$): *δ:* 190.7, 151.7, 143.3, 141.5, 140.9, 136.1, 133.4, 131.8, 129.7, 129.0, 124.7, 116.6, 114.6, 111.4, 103.6, 34.1. HRMS: m/z calcd for $C_{17}H_{14}N_2O_2$: 278.1055, found: 278.1058.

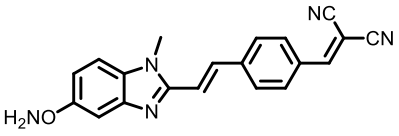
The synthesis of 3e ((*E*)-2-(4-(2-(5-hydroxy-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(5-hydroxy-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **3e** was followed by the procedure described in previous method for **3d-1**. It was purified by column chromatography on silica gel eluted with DCM/Ethyl Acetate (100:1 to 1:1, v/v) to obtain a yellow solid **3e**. Yield 75%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.95 (s, 1H), 7.88 (d, 2H, *J* = 5.26 Hz), 7.77 (d, 1H, *J* = 10.95 Hz), 7.65 (d, 2H, *J* = 5.27 Hz), 7.57 (d, 1H, *J* = 10.95 Hz), 7.44 (d, 1H, *J* = 10.95 Hz), 7.33 (d, 1H, *J* = 10.85 Hz), 7.18 (d, 1H, *J* = 10.95 Hz), 5.36 (s, 1H), 3.38 (s, 3H). ^{13}C NMR (100 MHz, CDCl3): δ: 161.8, 151.6, 141.6, 140.1, 135.9, 133.2, 131.4, 130.6, 128.9, 124.0, 116.7, 113.5, 111.4, 102.5, 81.5, 34.2. HRMS: m/z calcd for $C_{20}H_{14}N_4O$: 326.1168, found: 325.1159.

The synthesis of e-BMN ((*E*)-2-(4-(2-(5-(aminooxy)-1-methyl-1H-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile)



(*E*)-2-(4-(2-(5-(aminooxy)-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

The synthesis of **e-BMN** was followed by the procedure described in previous method for **d1-BMN**. It was purified by column chromatography on silica gel eluted with DCM/Methanol (100:1 to 10:1, v/v) to obtain a yellow solid **e-BMN**. Yield 57 %. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.99 (s, 1H), 7.91 (d, 2H, *J* = 5.26 Hz), 7.80 (d, 1H, *J* = 10.95 Hz), 7.68 (d, 2H, *J* = 5.27 Hz), 7.60 (d, 1H, *J* = 10.95 Hz), 7.47 (d, 1H, *J* = 10.95 Hz), 7.36 (d, 1H, *J* = 10.90 Hz), 7.23 (d, 1H, *J* = 10.95 Hz), 6.87(s, 2H), 3.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 161.8, 151.6, 141.6, 140.1, 136.9, 133.2, 131.4, 130.6, 128.9, 124.4, 116.7, 113.5, 111.4, 102.5, 81.5, 34.1. HRMS: m/z calcd for $C_{20}H_{15}N_5O$: 341.1277, found: 341.1271.

4. The basic optical data of BMN-Fluors

Table S1. The basic optical data of BMN-Fluors

		a1-BMN		a2-BMN	b-BMN		c-BMN	d1-BMN		d2-BMN	e-BMN	
No AP-site	λ_{ex}/nm	375		403	377		379	400		415	393	
	ε / M^{-1} cm^{-1}	143670		148976	125432		114576	154307		167321	165427	
	λ_{em}/nm	562		564	567		562	605		612	589	
	Φ	0.019		0.017	0.013		0.010	0.021		0.025	0.032	
Encounter with AP-site in the first few seconds	λ_{ex}/nm	373		399	371		372	397		412	390	
	ε / M^{-1} cm^{-1}	157300		163110	137332		125446	168947		183195	181122	
	λ_{em}/nm	562		513	567		562	605		533	589	
	Φ	0.17		0.022	0.19		0.21	0.66		0.031	0.54	
2 of AP-site in DNA (20 bp) after 300 s	λ_{ex}/nm	325		351	325		326	354		367	342	
	ε / M^{-1} cm^{-1}	134177		139133	117144		107005	144111		156265	154497	
	λ_{em}/nm	402	562	513	402	567	401	414	605	533	425	589
	Φ	0.02	0.14	0.11	0.03	0.16	0.03	0.93	0.08	0.23	0.13	0.46
4 of AP-site in DNA (20 bp) after 300 s	λ_{ex}/nm	325		351	325		326	354		367	342	
	ε / M^{-1} cm^{-1}	134145		139154	117117		107034	144155		156243	154407	
	λ_{em}/nm	402	562	513	402	567	401	441	605	533	425	589
	Φ	0.06	0.11	0.13	0.07	0.13	0.06	0.88	0.08	0.27	0.27	0.33
6 of AP-site in DNA (20 bp) after 300 s	λ_{ex}/nm	326		353	324		326	354		365	345	
	ε / M^{-1} cm^{-1}	134156		139167	117109		107056	144152		156223	154415	
	λ_{em}/nm	402	562	513	402	567	401	468	605	533	425	589
	Φ	0.11	0.08	0.14	0.10	0.10	0.10	0.84	0.06	0.31	0.35	0.27
8 of AP-site in DNA (20 bp) after 300 s	λ_{ex}/nm	323		353	324		325	355		365	345	
	ε / M^{-1} cm^{-1}	134136		139107	117135		107196	144182		156129	154376	
	λ_{em}/nm	402	562	513	402	567	401	498	605	533	425	589
	Φ	0.15	0.05	0.16	0.14	0.07	0.13	0.80	0.05	0.35	0.43	0.15
10 of AP-site in DNA (20 bp) after 300 s	λ_{ex}/nm	323		353	324		325	355		365	345	
	ε / M^{-1} cm^{-1}	134001		138967	117017		107088	144037		156066	154260	
	λ_{em}/nm	402	562	513	402	567	401	524	605	533	425	589
	Φ	0.19	0.03	0.18	0.17	0.04	0.16	0.78	0.07	0.39	0.51	0.07
14 of AP-site in DNA (20 bp) after 300 s	λ_{ex}/nm	323		353	324		325	355		365	345	
	ε / M^{-1} cm^{-1}	134106		139076	117109		107172	144150		156188	154381	
	λ_{em}/nm	402	562	513	402	567	401	552	605	533	425	589
	Φ	0.23	0.01	0.23	0.20	0.02	0.19	0.71	0.05	0.45	0.59	0.02

[a] Fluorescence enhancement fold; Φ fluorescence quantum yield; ε Molar extinction coefficient.

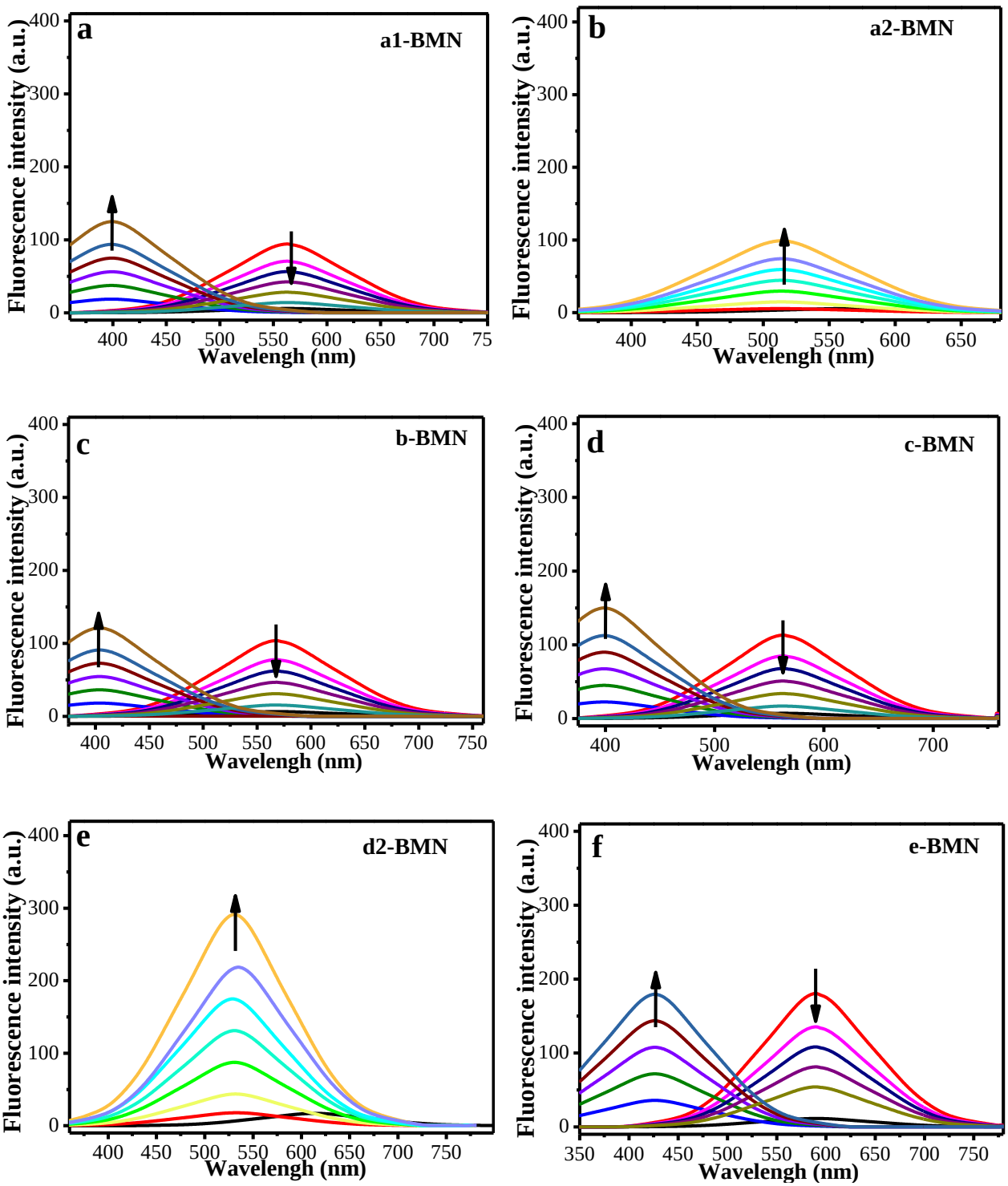


Figure S1. Spectral data of **BMN-Fluors** (3.0 μM) for AP-sites (2, 4, 6, 8, 10 and 14 AP-sites/20 bp DNA) in PBS buffer (pH =7.4) at the different reaction time (0, few seconds and 300 s). a. a1-BMN, b. a2-BMN, c. b-BMN, d. c-BMN, e. d2-BMN, f. e-BMN. The amount of AP-sites in DNA sequence was quantitatively detected by AP-sites Counting Kit (Dojindo, Japan, see Supporting Information).

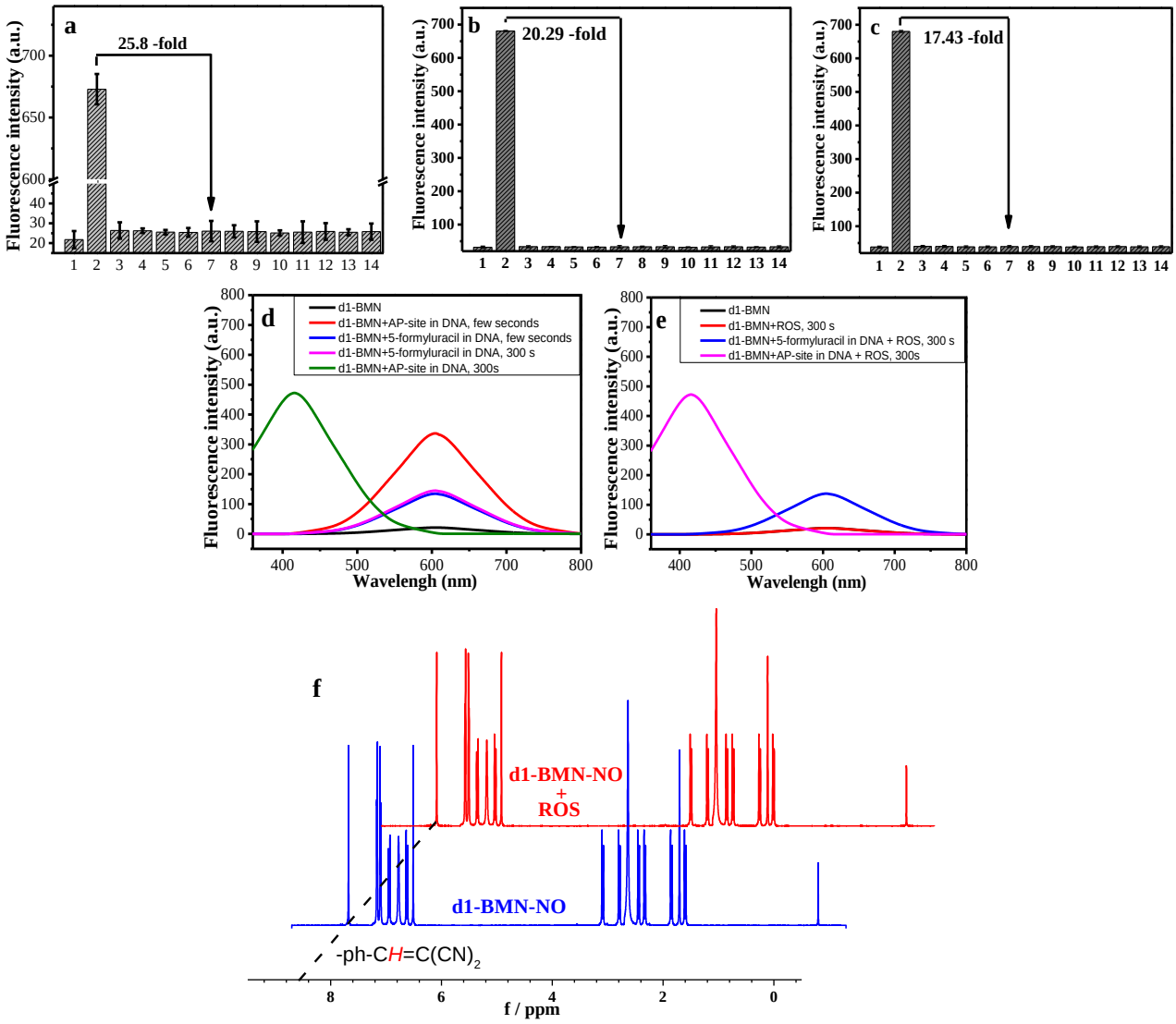


Figure S2. (a) Selective experiment; 1, control; 2, AP-Site in DNA; 3, HSA; 4, RNA; 5, triacylglycerol acylhydrolase; 6, lysozyme; 7, proteinase k; 8, histone; 9, collagen; 10, hemoglobin; 11, BSA; 12, β-amylase; 13, trypsin; and 14, chymotrypsin; (b) The influence of protein. 1. Control, 2. AP-Site in DNA, 3. P-Beta Aminopropionaldehyde, 4. amino transferase, 5. NAT, 6. Cyclodextrin glucanotransferase, 7. Alpha-Cyclodextringlucosyltransferase, 8. Dnmt3a(cytosine-5)-methyltransferase 3A), 9. α-Glucosyltransferasetreated stevia, 10. Kinase (phosphorylating), 11. Glutamic oxalacetic transaminase, 12. Acyltransferase, 13. lactose synthetase, 14. Phosphotransacetylase; (c) The influence of ions. 1. Control, 2. AP-Site in DNA 3. Al^{3+} (0.10 mM), 4. NO_3^- (0.30 mM), 5. Ni^{2+} (0.10 mM), 6. Cl^- (0.20 mM), 7. Mg^{2+} (0.10 mM), 8. SO_4^{2-} (0.10 mM), 9. CO_3^{2-} (0.050 mM), 10. Na^+ (0.10 mM), 11. K^+ (0.10 mM), 12. Ca^{2+} (0.10 mM), 14. $H_2PO_4^-$ (0.10 mM). Excitation wavelength = 400 nm. emission wavelength = 552 nm. **d1-BMN**: 3.0 μM. Data were obtained from replicate experiments (n = 5). (d) Spectral data of **d1-BMN** (3.0 μM) for AP-sites (2 AP-sites/20 bp DNA) and 5-formyluracil in DNA in PBS buffer (pH =7.4) at the different reaction time (0, few seconds and 300 s). Excitation wavelength = 400 nm or 354 nm. (e) Spectral data of **d1-BMN** (3.0 μM) for AP-sites (2 AP-sites/20 bp DNA) and 5-formyluracil in DNA in PBS buffer (pH =7.4) at 300 s. Excitation wavelength = 400 nm or 354 nm. (f) NMR spectra between d1-BMN-NO (3.0 mM) and ROS in D_2O.

5. Molecular docking and molecular dynamics simulation.

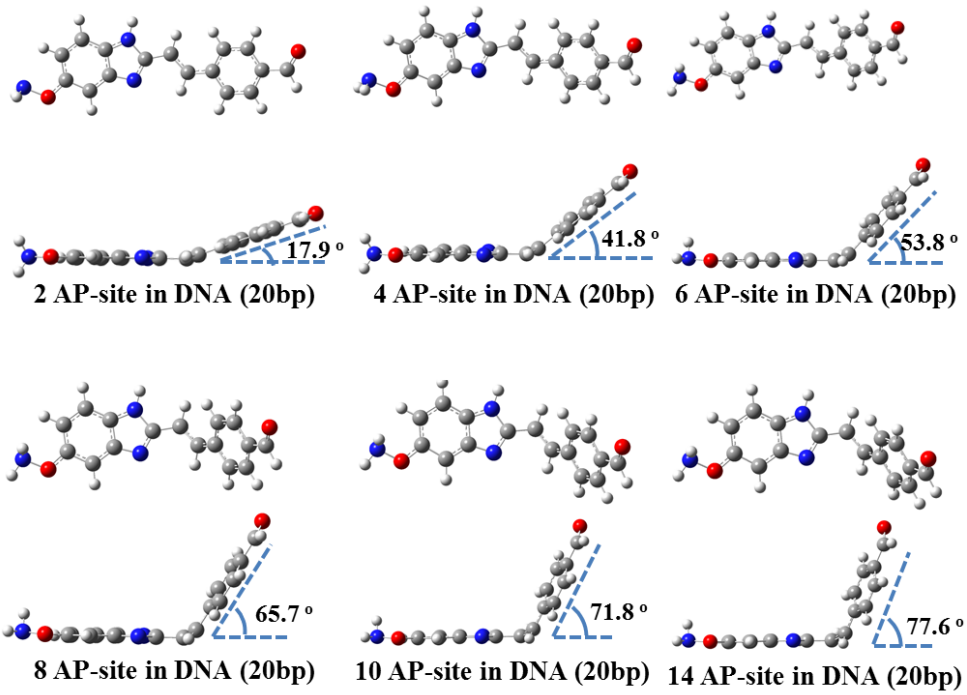


Figure S3. The molecular configuration of **d1-BMN-CHO** in different amount of AP-site in DNA (20 bp). The molecular twist angles are calculated and listed in the figure.

Molecular docking and simulated molecular conformation parameters

(1) Molecular docking parameter

d1-BMN gets the different best configuration parameters by molecular docking as follow:

Configuration 1. (The angle between two planes is 17.9°)

@<TRIPOS>ATOM

1	C	44.4801	-0.3341	142.3689	C.ar	1	UNL111	0.2272
2	C	43.2020	-0.3518	142.9111	C.ar	1	UNL111	-0.1516
3	C	42.5840	0.7731	143.4566	C.ar	1	UNL111	-0.1500
4	C	43.3260	1.9611	143.4340	C.ar	1	UNL111	-0.1500
5	C	44.6194	2.0147	142.8955	C.ar	1	UNL111	0.0825
6	C	45.2115	0.8627	142.3600	C.ar	1	UNL111	-0.1500
7	N	44.8228	-1.5762	141.8929	N.ar	1	UNL111	-0.5653
8	C	43.7973	-2.3635	142.1476	C.ar	1	UNL111	0.1415
9	N	42.7882	-1.6502	142.7521	N.ar	1	UNL111	0.0332
10	C	43.6439	-3.7352	141.8266	C.2	1	UNL111	-0.1050
11	C	43.4700	-4.2066	140.5811	C.2	1	UNL111	-0.1784

```
     12 C          43.3128   -5.6373  140.2728 C.ar     1  UNL111       0.0284
     13 C          43.8924   -6.1450  139.1006 C.ar     1  UNL111      -0.1500
     14 C          43.7375   -7.4907  138.7513 C.ar     1  UNL111      -0.1500
     15 C          42.9885   -8.3356  139.5723 C.ar     1  UNL111       0.0862
     16 C          42.3933   -7.8418  140.7342 C.ar     1  UNL111      -0.1500
     17 C          42.5507   -6.4961  141.0780 C.ar     1  UNL111      -0.1500
     18 C          42.8239   -9.7541  139.1971 C.2      1  UNL111       0.4238
     19 O          41.7218  -10.2606  139.0265 O.2      1  UNL111      -0.5700
     20 O          45.2070    3.2635  142.9243 O.3      1  UNL111       0.0175
     21 N          46.4617    3.3011  142.1891 N.4      1  UNL111      -0.8200
     22 H          41.6328    0.7326  143.8549 H        1  UNL111       0.1500
     23 H          42.9090    2.8205  143.8246 H        1  UNL111       0.1500
     24 H          46.1654    0.8944  141.9675 H        1  UNL111       0.1500
     25 H          41.9142   -2.0116  143.0254 H        1  UNL111       0.2700
     26 H          43.6675   -4.4169  142.6011 H        1  UNL111       0.1500
     27 H          43.4452   -3.5317  139.8008 H        1  UNL111       0.1500
     28 H          44.4391   -5.5188  138.4891 H        1  UNL111       0.1500
     29 H          44.1748   -7.8569  137.8911 H        1  UNL111       0.1500
     30 H          41.8383   -8.4685  141.3378 H        1  UNL111       0.1500
     31 H          42.1020   -6.1310  141.9327 H        1  UNL111       0.1500
     32 H          43.6656  -10.3385  139.0744 H        1  UNL111       0.0600
     33 H          47.1152    2.6329  142.5975 H        1  UNL111       0.3600
     34 H          46.8582    4.2396  142.2412 H        1  UNL111       0.3600
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     3     2     3    ar
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     5     3     4    ar
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     7     5     6    ar
     8     5    20     1
     9     6     1    ar
    10     7     8    ar
    11     8     9    ar
    12     8    10     1
    13    10    11     2
    14    11    12     1
    15    12    17    ar
    16    12    13    ar
    17    13    14    ar
    18    14    15    ar
    19    15    16    ar
    20    15    18     1
    21    16    17    ar
    22    18    19     2
```

23	20	21	1
24	3	22	1
25	4	23	1
26	6	24	1
27	9	25	1
28	10	26	1
29	11	27	1
30	13	28	1
31	14	29	1
32	16	30	1
33	17	31	1
34	18	32	1
35	21	33	1
36	21	34	1

Configuration 2. (The angle between two planes is 41.8^{o})

@<TRIPOS>ATOM

1	C	33.6422	16.0308	128.7400	C.ar	1	UNL111	0.2272
2	C	34.9319	16.3684	129.1281	C.ar	1	UNL111	-0.1516
3	C	35.3234	16.4980	130.4604	C.ar	1	UNL111	-0.1500
4	C	34.3334	16.2650	131.4236	C.ar	1	UNL111	-0.1500
5	C	33.0211	15.9235	131.0673	C.ar	1	UNL111	0.0825
6	C	32.6617	15.8070	129.7175	C.ar	1	UNL111	-0.1500
7	N	33.5502	15.9592	127.3713	N.ar	1	UNL111	-0.5653
8	C	34.7443	16.2653	126.9058	C.ar	1	UNL111	0.1415
9	N	35.6132	16.5052	127.9450	N.ar	1	UNL111	0.0332
10	C	35.1760	16.3047	125.5567	C.2	1	UNL111	-0.1050
11	C	34.3647	16.5185	124.5079	C.2	1	UNL111	-0.1784
12	C	34.8373	16.5549	123.1144	C.ar	1	UNL111	0.0284
13	C	34.0864	17.2611	122.1630	C.ar	1	UNL111	-0.1500
14	C	34.4800	17.2920	120.8210	C.ar	1	UNL111	-0.1500
15	C	35.6268	16.6032	120.4216	C.ar	1	UNL111	0.0862
16	C	36.3762	15.8838	121.3538	C.ar	1	UNL111	-0.1500
17	C	35.9778	15.8558	122.6934	C.ar	1	UNL111	-0.1500
18	C	36.0359	16.6348	119.0033	C.2	1	UNL111	0.4238
19	O	37.0735	16.1153	118.6114	O.2	1	UNL111	-0.5700
20	O	32.1714	15.7011	132.1322	O.3	1	UNL111	0.0175
21	N	31.3054	14.5521	131.9178	N.4	1	UNL111	-0.8200
22	H	36.2889	16.7516	130.7221	H	1	UNL111	0.1500
23	H	34.5767	16.3470	132.4232	H	1	UNL111	0.1500
24	H	31.6960	15.5617	129.4487	H	1	UNL111	0.1500
25	H	36.5658	16.7366	127.8549	H	1	UNL111	0.2700
26	H	36.1801	16.1597	125.3675	H	1	UNL111	0.1500
27	H	33.3591	16.6644	124.6882	H	1	UNL111	0.1500
28	H	33.2339	17.7634	122.4564	H	1	UNL111	0.1500
29	H	33.9257	17.8213	120.1299	H	1	UNL111	0.1500

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      30 H           37.2221   15.3737  121.0549 H       1  UNL111       0.1500
      31 H           36.5282   15.3156  123.3792 H       1  UNL111       0.1500
      32 H           35.4271   17.1106  118.3192 H       1  UNL111       0.0600
      33 H           30.6420   14.4777  132.6892 H       1  UNL111       0.3600
      34 H           31.8705   13.7041  131.8726 H       1  UNL111       0.3600
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      4     2     9    ar
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      6     4     5    ar
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      8     5    20     1
      9     6     1    ar
     10     7     8    ar
     11     8     9    ar
     12     8    10     1
     13    10    11     2
     14    11    12     1
     15    12    17    ar
     16    12    13    ar
     17    13    14    ar
     18    14    15    ar
     19    15    16    ar
     20    15    18     1
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     22    18    19     2
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     24     3    22     1
     25     4    23     1
     26     6    24     1
     27     9    25     1
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     32    16    30     1
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     36    21    34     1
```

Configuration 3. (The angle between two planes is 53.8°)

@<TRIPOS>ATOM

1	C	35.1940	16.5311	123.1848	C.ar	1	UNL111	0.2272
2	C	36.4177	15.8929	123.3369	C.ar	1	UNL111	-0.1516
3	C	37.2697	15.6027	122.2716	C.ar	1	UNL111	-0.1500
4	C	36.8298	15.9946	121.0007	C.ar	1	UNL111	-0.1500
5	C	35.5997	16.6398	120.8105	C.ar	1	UNL111	0.0825
6	C	34.7662	16.9111	121.9043	C.ar	1	UNL111	-0.1500
7	N	34.5800	16.7001	124.4019	N.ar	1	UNL111	-0.5653
8	C	35.3840	16.1630	125.2972	C.ar	1	UNL111	0.1415
9	N	36.5172	15.6761	124.6880	N.ar	1	UNL111	0.0332
10	C	35.2174	16.1062	126.7033	C.2	1	UNL111	-0.1050
11	C	34.0368	16.2284	127.3316	C.2	1	UNL111	-0.1784
12	C	33.8893	16.1659	128.7948	C.ar	1	UNL111	0.0284
13	C	32.6536	15.7800	129.3349	C.ar	1	UNL111	-0.1500
14	C	32.4593	15.7413	130.7197	C.ar	1	UNL111	-0.1500
15	C	33.5032	16.1016	131.5739	C.ar	1	UNL111	0.0862
16	C	34.7339	16.5021	131.0514	C.ar	1	UNL111	-0.1500
17	C	34.9222	16.5395	129.6668	C.ar	1	UNL111	-0.1500
18	C	33.2920	16.0638	133.0348	C.2	1	UNL111	0.4238
19	O	32.2793	15.5909	133.5359	O.2	1	UNL111	-0.5700
20	O	35.3227	16.9842	119.5028	O.3	1	UNL111	0.0175
21	N	34.0318	17.6405	119.3654	N.4	1	UNL111	-0.8200
22	H	38.1740	15.1258	122.4117	H	1	UNL111	0.1500
23	H	37.4278	15.8030	120.1816	H	1	UNL111	0.1500
24	H	33.8569	17.3804	121.7702	H	1	UNL111	0.1500
25	H	37.2741	15.2438	125.1457	H	1	UNL111	0.2700
26	H	36.0591	15.9613	127.2828	H	1	UNL111	0.1500
27	H	33.1901	16.3737	126.7597	H	1	UNL111	0.1500
28	H	31.8791	15.5217	128.7036	H	1	UNL111	0.1500
29	H	31.5491	15.4480	131.1078	H	1	UNL111	0.1500
30	H	35.5028	16.7700	131.6855	H	1	UNL111	0.1500
31	H	35.8300	16.8448	129.2825	H	1	UNL111	0.1500
32	H	34.0220	16.4486	133.6543	H	1	UNL111	0.0600
33	H	34.0805	18.3338	118.6188	H	1	UNL111	0.3600
34	H	33.7899	18.1019	120.2424	H	1	UNL111	0.3600

@<TRIPOS>BOND

1	1	2	ar
2	1	7	ar
3	2	3	ar
4	2	9	ar
5	3	4	ar
6	4	5	ar
7	5	6	ar
8	5	20	1

9	6	1	ar
10	7	8	ar
11	8	9	ar
12	8	10	1
13	10	11	2
14	11	12	1
15	12	17	ar
16	12	13	ar
17	13	14	ar
18	14	15	ar
19	15	16	ar
20	15	18	1
21	16	17	ar
22	18	19	2
23	20	21	1
24	3	22	1
25	4	23	1
26	6	24	1
27	9	25	1
28	10	26	1
29	11	27	1
30	13	28	1
31	14	29	1
32	16	30	1
33	17	31	1
34	18	32	1
35	21	33	1
36	21	34	1

Configuration 4. (The angle between two planes is 65.7°)

@<TRIPOS>ATOM

1	C	35.58408	1.67050	121.0135	C.ar	1	UNL111	0.2272
2	C	34.56160	1.75900	121.4744	C.ar	1	UNL111	-0.1516
3	C	34.06720	3.86550	121.9381	C.ar	1	UNL111	-0.1500
4	C	34.66080	9.80550	121.9189	C.ar	1	UNL111	-0.1500
5	C	35.69552	10.0735	121.4611	C.ar	1	UNL111	0.0825
6	C	36.16920	4.31350	121.0060	C.ar	1	UNL111	-0.1500
7	N	35.85824	7.88100	120.6089	N.ar	1	UNL111	-0.5653
8	C	35.03784	11.8175	120.8254	C.ar	1	UNL111	0.1415
9	N	34.23056	8.25100	121.3392	N.ar	1	UNL111	0.0332
10	C	34.91512	18.6760	120.5526	C.2	1	UNL111	-0.1050
11	C	34.77600	21.0330	119.4939	C.2	1	UNL111	-0.1784
12	C	34.65024	28.1865	119.2318	C.ar	1	UNL111	0.0284
13	C	35.11392	30.7250	118.2351	C.ar	1	UNL111	-0.1500
14	C	34.99000	37.4535	117.9386	C.ar	1	UNL111	-0.1500
15	C	34.39080	41.6780	118.6364	C.ar	1	UNL111	0.0862

```
      16 C          33.91464   39.2090   119.6240 C.ar     1     UNL111       -0.1500
      17 C          34.04056   32.4805   119.9163C.ar      1     UNL111       -0.1500
      18 C          34.25912   48.7705   118.3175 C.2      1     UNL111        0.4238
      19 O          33.37744   51.3030   118.1725 O.2      1     UNL111       -0.5700
      20 O          36.16560   16.3175   121.48560.3       1     UNL111        0.0175
      21 N          37.16936   16.5055   120.8607N.4       1     UNL111       -0.8200
      22 H          33.30624   3.66300   122.2766 H        1     UNL111        0.1500
      23 H          34.32720   14.1025   122.2509 H        1     UNL111        0.1500
      24 H          36.93232   4.47200   120.6723 H        1     UNL111        0.1500
      25 H          33.53136   10.0580   121.5710 H        1     UNL111        0.2700
      26 H          34.93400   22.0845   121.2109 H        1     UNL111        0.1500
      27 H          34.75616   17.6585   118.8306 H        1     UNL111        0.1500
      28 H          35.55128   27.5940   117.7157 H        1     UNL111        0.1500
      29 H          35.33984   39.2845   117.2074 H        1     UNL111        0.1500
      30 H          33.47064   42.3425   120.1371 H        1     UNL111        0.1500
      31 H          33.68160   30.6550   120.6427 H        1     UNL111        0.1500
      32 H          34.93248   51.6925   118.2132 H        1     UNL111        0.0600
      33 H          37.69216   13.1645   121.2078 H        1     UNL111        0.3600
      34 H          37.48656   21.1980   120.9050 H        1     UNL111        0.3600
@<TRIPOS>BOND
      1     1     2    ar
      2     1     7    ar
      3     2     3    ar
      4     2     9    ar
      5     3     4    ar
      6     4     5    ar
      7     5     6    ar
      8     5    20     1
      9     6     1    ar
     10     7     8    ar
     11     8     9    ar
     12     8    10     1
     13    10    11     2
     14    11    12     1
     15    12    17    ar
     16    12    13    ar
     17    13    14    ar
     18    14    15    ar
     19    15    16    ar
     20    15    18     1
     21    16    17    ar
     22    18    19     2
     23    20    21     1
     24     3    22     1
     25     4    23     1
     26     6    24     1
```

27	9	25	1
28	10	26	1
29	11	27	1
30	13	28	1
31	14	29	1
32	16	30	1
33	17	31	1
34	18	32	1
35	21	33	1
36	21	34	1

Configuration 5. (The angle between two planes is 71.8°)

@<TRIPOS>ATOM

1	C	34.6689	16.3720	126.6468	C.ar	1	UNL111	0.2272
2	C	33.4222	15.8164	126.3916	C.ar	1	UNL111	-0.1516
3	C	32.5663	15.3586	127.3930	C.ar	1	UNL111	-0.1500
4	C	33.0271	15.4868	128.7096	C.ar	1	UNL111	-0.1500
5	C	34.2806	16.0418	129.0035	C.ar	1	UNL111	0.0825
6	C	35.1172	16.4871	127.9708	C.ar	1	UNL111	-0.1500
7	N	35.2817	16.7480	125.4764	N.ar	1	UNL111	-0.5653
8	C	34.4540	16.4183	124.5055	C.ar	1	UNL111	0.1415
9	N	33.3074	15.8637	125.0252	N.ar	1	UNL111	0.0332
10	C	34.6105	16.6238	123.1122	C.2	1	UNL111	-0.1050
11	C	35.6831	16.2329	122.4048	C.2	1	UNL111	-0.1784
12	C	36.8351	15.5371	123.0010	C.ar	1	UNL111	0.0284
13	C	36.9386	14.1464	122.8496	C.ar	1	UNL111	-0.1500
14	C	37.9980	13.4409	123.4299	C.ar	1	UNL111	-0.1500
15	C	38.9570	14.1266	124.1778	C.ar	1	UNL111	0.0862
16	C	38.8594	15.5082	124.3499	C.ar	1	UNL111	-0.1500
17	C	37.7983	16.2080	123.7683	C.ar	1	UNL111	-0.1500
18	C	40.0708	13.3771	124.7924	C.2	1	UNL111	0.4238
19	O	40.9908	12.9145	124.1292	O.2	1	UNL111	-0.5700
20	O	34.5770	16.1220	130.3493	O.3	1	UNL111	0.0175
21	N	35.6382	17.0779	130.6256	N.4	1	UNL111	-0.8200
22	H	31.6447	14.9481	127.1761	H	1	UNL111	0.1500
23	H	32.4272	15.1637	129.4848	H	1	UNL111	0.1500
24	H	36.0434	16.8912	128.1802	H	1	UNL111	0.1500
25	H	32.5330	15.5522	124.5033	H	1	UNL111	0.2700
26	H	33.8500	17.1057	122.6077	H	1	UNL111	0.1500
27	H	35.7006	16.4302	121.3920	H	1	UNL111	0.1500
28	H	36.2253	13.6380	122.3039	H	1	UNL111	0.1500
29	H	38.0703	12.4189	123.3059	H	1	UNL111	0.1500
30	H	39.5687	16.0119	124.9053	H	1	UNL111	0.1500
31	H	37.7231	17.2283	123.9046	H	1	UNL111	0.1500
32	H	40.0742	13.2383	125.8150	H	1	UNL111	0.0600

```
      33 H           35.2613   18.0250  130.5867 H       1  UNL111       0.3600
      34 H           36.3766   16.9769  129.9292 H       1  UNL111       0.3600
@<TRIPOS>BOND
      1     1     2    ar
      2     1     7    ar
      3     2     3    ar
      4     2     9    ar
      5     3     4    ar
      6     4     5    ar
      7     5     6    ar
      8     5    20     1
      9     6     1    ar
     10     7     8    ar
     11     8     9    ar
     12     8    10     1
     13    10    11     2
     14    11    12     1
     15    12    17    ar
     16    12    13    ar
     17    13    14    ar
     18    14    15    ar
     19    15    16    ar
     20    15    18     1
     21    16    17    ar
     22    18    19     2
     23    20    21     1
     24     3    22     1
     25     4    23     1
     26     6    24     1
     27     9    25     1
     28    10    26     1
     29    11    27     1
     30    13    28     1
     31    14    29     1
     32    16    30     1
     33    17    31     1
     34    18    32     1
     35    21    33     1
     36    21    34     1
```

Configuration 6. (The angle between two planes is 77.6^{o})

```
@<TRIPOS>ATOM
      1 C           33.5603   16.1115  126.1212 C.ar    1  UNL111       0.2272
      2 C           34.9072   16.2515  126.4283 C.ar    1  UNL111      -0.1516
      3 C           35.4176   16.1210  127.7195 C.ar    1  UNL111      -0.1500
      4 C           34.4889   15.8316  128.7276 C.ar    1  UNL111      -0.1500
```

```
      5 C          33.1219   15.6847  128.4534 C.ar     1  UNL111       0.0825
      6 C          32.6433   15.8279  127.1438 C.ar     1  UNL111      -0.1500
      7 N          33.3500   16.2754  124.7737 N.ar     1  UNL111      -0.5653
      8 C          34.5294   16.5344  124.2462 C.ar     1  UNL111       0.1415
      9 N          35.5013   16.5131  125.2195 N.ar     1  UNL111       0.0332
     10 C          34.8518   16.7524  122.8836 C.2      1  UNL111      -0.1050
     11 C          35.9858   16.3349  122.2975 C.2      1  UNL111      -0.1784
     12 C          37.0388   15.5935  123.0104 C.ar     1  UNL111       0.0284
     13 C          38.1901   16.2806  123.4227 C.ar     1  UNL111      -0.1500
     14 C          39.2306   15.6075  124.0717 C.ar     1  UNL111      -0.1500
     15 C          39.1274   14.2344  124.3016 C.ar     1  UNL111       0.0862
     16 C          37.9946   13.5340  123.8841 C.ar     1  UNL111      -0.1500
     17 C          36.9582   14.2116  123.2355 C.ar     1  UNL111      -0.1500
     18 C          40.2302   13.5273  124.9828 C.2      1  UNL111       0.4238
     19 O          41.2367   14.1075  125.3705 O.2      1  UNL111      -0.5700
     20 O          32.3417   15.3752  129.5493 O.3      1  UNL111       0.0175
     21 N          31.0328   14.8661  129.1701 N.4      1  UNL111      -0.8200
     22 H          36.4236   16.2314  127.9213 H        1  UNL111       0.1500
     23 H          34.8203   15.7236  129.6991 H        1  UNL111       0.1500
     24 H          31.6374   15.7267  126.9364 H        1  UNL111       0.1500
     25 H          36.4635   16.6610  125.0731 H        1  UNL111       0.2700
     26 H          34.1701   17.2658  122.3032 H        1  UNL111       0.1500
     27 H          36.1261   16.5445  121.2968 H        1  UNL111       0.1500
     28 H          38.2704   17.2941  123.2454 H        1  UNL111       0.1500
     29 H          40.0701   16.1233  124.3788 H        1  UNL111       0.1500
     30 H          37.9224   12.5185  124.0535 H        1  UNL111       0.1500
     31 H          36.1257   13.6900  122.9193 H        1  UNL111       0.1500
     32 H          40.1502   12.5101  125.1373 H        1  UNL111       0.0600
     33 H          31.0048   13.8571  129.3173 H        1  UNL111       0.3600
     34 H          30.8604   15.0709  128.1858 H        1  UNL111       0.3600
@<TRIPOS>BOND
     1    1    2   ar
     2    1    7   ar
     3    2    3   ar
     4    2    9   ar
     5    3    4   ar
     6    4    5   ar
     7    5    6   ar
     8    5   20    1
     9    6    1   ar
    10    7    8   ar
    11    8    9   ar
    12    8   10    1
    13   10   11    2
    14   11   12    1
    15   12   17   ar
```

16	12	13	ar
17	13	14	ar
18	14	15	ar
19	15	16	ar
20	15	18	1
21	16	17	ar
22	18	19	2
23	20	21	1
24	3	22	1
25	4	23	1
26	6	24	1
27	9	25	1
28	10	26	1
29	11	27	1
30	13	28	1
31	14	29	1
32	16	30	1
33	17	31	1
34	18	32	1
35	21	33	1
36	21	34	1

(2) Simulated molecular conformation parameters

The molecular configuration parameters of conformation 6:

HETATM	1	P	RDG	7	31.771	53.794	46.797	1.00	0.00	P
HETATM	2	OP2	RDG	7	32.589	52.643	46.378	1.00	0.00	O
HETATM	3	OP1	RDG	7	30.546	54.211	46.020	1.00	0.00	O1-
HETATM	4	O5’	RDG	7	32.745	55.119	46.995	1.00	0.00	O
HETATM	5	C5’	RDG	7	34.062	55.069	47.560	1.00	0.00	C
HETATM	6	1H5’	RDG	7	33.946	55.322	48.614	1.00	0.00	H
HETATM	7	2H5’	RDG	7	34.407	54.054	47.366	1.00	0.00	H
HETATM	8	C4’	RDG	7	35.045	56.064	46.928	1.00	0.00	C
HETATM	9	O4’	RDG	7	34.835	57.298	47.397	1.00	0.00	O
HETATM	10	4HHO	RDG	7	35.732	56.965	47.181	1.00	0.00	H
HETATM	11	C3’	RDG	7	36.476	55.733	47.252	1.00	0.00	C
HETATM	12	C2’	RDG	7	36.874	55.842	48.765	1.00	0.00	C
HETATM	13	C1’	RDG	7	37.507	54.522	49.141	1.00	0.00	C
HETATM	14	H1’	RDG	7	38.575	54.512	49.303	1.00	0.00	H
HETATM	15	N3	RDG	7	36.637	53.548	49.401	1.00	0.00	N
HETATM	16	O1	RDG	7	37.436	52.421	49.801	1.00	0.00	O
HETATM	17	C2	RDG	7	36.753	51.320	50.230	1.00	0.00	C
HETATM	18	C1	RDG	7	37.243	50.664	51.388	1.00	0.00	C
HETATM	19	C6	RDG	7	36.555	49.647	52.027	1.00	0.00	C

```
HETATM   20  C5  RDG     7       35.456  49.146  51.336  1.00  0.00           C
HETATM   21  C4  RDG     7       34.905  49.747  50.169  1.00  0.00           C
HETATM   22  C3  RDG     7       35.522  50.861  49.616  1.00  0.00           C
HETATM   23  H2  RDG     7       35.067  51.311  48.746  1.00  0.00           H
HETATM   24  N1  RDG     7       33.664  49.163  49.825  1.00  0.00           N
HETATM   25  C7  RDG     7       33.544  48.295  50.833  1.00  0.00           C
HETATM   26  N2  RDG     7       34.576  48.199  51.646  1.00  0.00           N
HETATM   27  H5  RDG     7       34.731  47.565  52.417  1.00  0.00           H
HETATM   28  C8  RDG     7       32.456  47.538  50.967  1.00  0.00           C
HETATM   29  C9  RDG     7       31.341  47.727  50.123  1.00  0.00           C
HETATM   30  C10 RDG     7       30.016  47.144  50.325  1.00  0.00           C
HETATM   31  C11 RDG     7       29.096  47.299  49.294  1.00  0.00           C
HETATM   32  C12 RDG     7       27.796  46.812  49.469  1.00  0.00           C
HETATM   33  C13 RDG     7       27.390  46.236  50.655  1.00  0.00           C
HETATM   34  C14 RDG     7       28.384  46.131  51.708  1.00  0.00           C
HETATM   35  C15 RDG     7       29.650  46.616  51.553  1.00  0.00           C
HETATM   36  H13 RDG     7       30.353  46.434  52.352  1.00  0.00           H
HETATM   37  H12 RDG     7       28.087  45.648  52.627  1.00  0.00           H
HETATM   38  C16 RDG     7       26.026  45.926  50.914  1.00  0.00           C
HETATM   39  O2  RDG     7       25.545  45.429  51.921  1.00  0.00           O
HETATM   40  H14 RDG     7       25.330  46.091  50.104  1.00  0.00           H
HETATM   41  H11 RDG     7       27.109  46.950  48.648  1.00  0.00           H
HETATM   42  H10 RDG     7       29.401  47.710  48.343  1.00  0.00           H
HETATM   43  H9  RDG     7       31.532  48.234  49.188  1.00  0.00           H
HETATM   44  H8  RDG     7       32.349  46.891  51.825  1.00  0.00           H
HETATM   45  H3  RDG     7       36.928  49.320  52.986  1.00  0.00           H
HETATM   46  H1  RDG     7       38.095  51.074  51.910  1.00  0.00           H
HETATM   47 1H2' RDG     7       37.594  56.621  49.016  1.00  0.00           H
HETATM   48 2H2' RDG     7       36.003  56.026  49.394  1.00  0.00           H
HETATM   49  H3' RDG     7       36.705  54.751  46.837  1.00  0.00           H
HETATM   50  O3' RDG     7       37.241  56.774  46.730  1.00  0.00           O
TER      51      RDG     7
CONECT    1    4    2    3
CONECT    2    1
CONECT    3    1
CONECT    4    1    5
CONECT    5    4    6    7    8
CONECT    6    5
CONECT    7    5
CONECT    8    5   11    9   10
CONECT    9    8
CONECT   10    8
CONECT   11    8   49   50   12
CONECT   12   11   13   48   47
CONECT   13   12   14   15
CONECT   14   13
```

CONECT 15 13 16
CONECT 16 15 17
CONECT 17 16 22 18
CONECT 18 17 46 19
CONECT 19 18 20 45
CONECT 20 19 26 21
CONECT 21 20 22 24
CONECT 22 17 21 23
CONECT 23 22
CONECT 24 21 25
CONECT 25 24 28 26
CONECT 26 20 25 27
CONECT 27 26
CONECT 28 25 29 44
CONECT 29 28 30 43
CONECT 30 29 31 35
CONECT 31 30 32 42
CONECT 32 31 33 41
CONECT 33 32 38 34
CONECT 34 33 37 35
CONECT 35 30 34 36
CONECT 36 35
CONECT 37 34
CONECT 38 33 40 39
CONECT 39 38
CONECT 40 38
CONECT 41 32
CONECT 42 31
CONECT 43 29
CONECT 44 28
CONECT 45 19
CONECT 46 18
CONECT 47 12
CONECT 48 12
CONECT 49 11
CONECT 50 11

The molecular configuration parameters of conformation 5:

HETATM 1 P RDG 7 31.457 52.655 47.700 1.00 0.00 P
HETATM 2 OP2 RDG 7 32.288 51.539 47.211 1.00 0.00 O1-
HETATM 3 OP1 RDG 7 30.339 53.150 46.876 1.00 0.00 O
HETATM 4 O5’ RDG 7 32.476 53.860 47.919 1.00 0.00 O
HETATM 5 C5’ RDG 7 33.746 53.755 48.505 1.00 0.00 C
HETATM 6 1H5’ RDG 7 33.649 53.930 49.576 1.00 0.00 H
HETATM 7 2H5’ RDG 7 34.215 52.810 48.231 1.00 0.00 H
HETATM 8 C4’ RDG 7 34.507 54.854 47.727 1.00 0.00 C

```
HETATM    9  O4' RDG     7      34.182  56.072  48.142  1.00  0.00           O
HETATM   10 4HHO RDG     7      34.859  55.703  47.535  1.00  0.00           H
HETATM   11  H4' RDG     7      34.360  54.682  46.660  1.00  0.00           H
HETATM   12  C3' RDG     7      36.029  54.754  47.825  1.00  0.00           C
HETATM   13  C2' RDG     7      36.640  55.053  49.262  1.00  0.00           C
HETATM   14  C1' RDG     7      37.253  53.866  49.909  1.00  0.00           C
HETATM   15  H1' RDG     7      38.257  53.911  50.305  1.00  0.00           H
HETATM   16  N3  RDG     7      36.472  52.747  50.074  1.00  0.00           N
HETATM   17  O1  RDG     7      37.278  51.829  50.769  1.00  0.00           O
HETATM   18  C2  RDG     7      36.737  50.613  51.094  1.00  0.00           C
HETATM   19  C1  RDG     7      37.119  49.895  52.275  1.00  0.00           C
HETATM   20  C6  RDG     7      36.482  48.713  52.646  1.00  0.00           C
HETATM   21  C5  RDG     7      35.375  48.265  51.922  1.00  0.00           C
HETATM   22  C4  RDG     7      34.948  48.978  50.736  1.00  0.00           C
HETATM   23  C3  RDG     7      35.507  50.170  50.378  1.00  0.00           C
HETATM   24  H2  RDG     7      35.056  50.666  49.532  1.00  0.00           H
HETATM   25  N1  RDG     7      33.911  48.254  50.156  1.00  0.00           N
HETATM   26  C7  RDG     7      33.707  47.256  51.010  1.00  0.00           C
HETATM   27  N2  RDG     7      34.558  47.181  52.059  1.00  0.00           N
HETATM   28  H5  RDG     7      34.468  46.591  52.874  1.00  0.00           H
HETATM   29  C8  RDG     7      32.705  46.411  50.886  1.00  0.00           C
HETATM   30  C9  RDG     7      31.743  46.510  49.876  1.00  0.00           C
HETATM   31  C10 RDG     7      30.333  46.283  50.037  1.00  0.00           C
HETATM   32  C11 RDG     7      29.615  45.804  48.944  1.00  0.00           C
HETATM   33  C12 RDG     7      28.394  45.265  49.164  1.00  0.00           C
HETATM   34  C13 RDG     7      27.736  45.305  50.436  1.00  0.00           C
HETATM   35  C14 RDG     7      28.577  45.690  51.577  1.00  0.00           C
HETATM   36  C15 RDG     7      29.917  46.196  51.365  1.00  0.00           C
HETATM   37  H13 RDG     7      30.596  46.536  52.133  1.00  0.00           H
HETATM   38  H12 RDG     7      28.230  45.618  52.598  1.00  0.00           H
HETATM   39  C16 RDG     7      26.406  44.892  50.618  1.00  0.00           C
HETATM   40  O2  RDG     7      25.847  44.991  51.747  1.00  0.00           O
HETATM   41  H14 RDG     7      25.874  44.402  49.816  1.00  0.00           H
HETATM   42  H11 RDG     7      27.937  44.820  48.292  1.00  0.00           H
HETATM   43  H10 RDG     7      30.107  45.807  47.982  1.00  0.00           H
HETATM   44  H9  RDG     7      32.039  46.446  48.839  1.00  0.00           H
HETATM   45  H8  RDG     7      32.705  45.520  51.495  1.00  0.00           H
HETATM   46  H3  RDG     7      36.794  48.204  53.546  1.00  0.00           H
HETATM   47  H1  RDG     7      37.953  50.289  52.838  1.00  0.00           H
HETATM   48 1H2' RDG     7      37.433  55.790  49.131  1.00  0.00           H
HETATM   49 2H2' RDG     7      35.899  55.509  49.918  1.00  0.00           H
HETATM   50  H3' RDG     7      36.413  53.845  47.360  1.00  0.00           H
HETATM   51  O3' RDG     7      36.456  55.971  47.141  1.00  0.00           O
TER      52      RDG     7
CONECT    1    3    2    4
CONECT    2    1
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CONECT    3    1
CONECT    4    1    5
CONECT    5    4    8    7    6
CONECT    6    5
CONECT    7    5
CONECT    8    5    9   10   11
CONECT    9    8
CONECT   10    8
CONECT   11    8
CONECT   12   50   51   13
CONECT   13   12   14   48   49
CONECT   14   13   15   16
CONECT   15   14
CONECT   16   14   17
CONECT   17   16   18
CONECT   18   17   23   19
CONECT   19   18   47   20
CONECT   20   19   21   46
CONECT   21   20   27   22
CONECT   22   21   23   25
CONECT   23   18   22   24
CONECT   24   23
CONECT   25   22   26
CONECT   26   25   29   27
CONECT   27   21   26   28
CONECT   28   27
CONECT   29   26   30   45
CONECT   30   29   44   31
CONECT   31   30   32   36
CONECT   32   31   33   43
CONECT   33   32   34   42
CONECT   34   33   39   35
CONECT   35   34   38   36
CONECT   36   31   35   37
CONECT   37   36
CONECT   38   35
CONECT   39   34   41   40
CONECT   40   39
CONECT   41   39
CONECT   42   33
CONECT   43   32
CONECT   44   30
CONECT   45   29
CONECT   46   20
CONECT   47   19
CONECT   48   13
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CONECT 49 13
CONECT 50 12
CONECT 51 12

The molecular configuration parameters of conformation 4:

HETATM 1 P RDG 7 25.467 53.239 47.334 1.00 0.00 P
HETATM 2 OP2 RDG 7 24.186 52.953 46.660 1.00 0.00 O1-
HETATM 3 OP1 RDG 7 25.549 54.448 48.139 1.00 0.00 O
HETATM 4 O5’ RDG 7 26.511 53.302 46.118 1.00 0.00 O
HETATM 5 C5’ RDG 7 27.967 53.452 46.275 1.00 0.00 C
HETATM 6 1H5’ RDG 7 28.289 54.478 46.453 1.00 0.00 H
HETATM 7 2H5’ RDG 7 28.244 52.941 47.197 1.00 0.00 H
HETATM 8 C4’ RDG 7 28.573 52.777 45.095 1.00 0.00 C
HETATM 9 O4’ RDG 7 28.569 53.520 44.025 1.00 0.00 O
HETATM 10 4HHO RDG 7 29.415 53.360 44.495 1.00 0.00 H
HETATM 11 H4’ RDG 7 28.035 51.841 44.946 1.00 0.00 H
HETATM 12 C3’ RDG 7 30.033 52.456 45.345 1.00 0.00 C
HETATM 13 C2’ RDG 7 30.325 51.738 46.686 1.00 0.00 C
HETATM 14 C1’ RDG 7 29.705 50.359 46.778 1.00 0.00 C
HETATM 15 H1’ RDG 7 28.888 50.047 46.145 1.00 0.00 H
HETATM 16 N3 RDG 7 30.248 49.472 47.572 1.00 0.00 N
HETATM 17 O1 RDG 7 29.460 48.330 47.715 1.00 0.00 O
HETATM 18 C2 RDG 7 30.087 47.250 48.314 1.00 0.00 C
HETATM 19 C1 RDG 7 31.104 47.408 49.245 1.00 0.00 C
HETATM 20 C6 RDG 7 31.720 46.246 49.780 1.00 0.00 C
HETATM 21 C5 RDG 7 31.159 45.034 49.464 1.00 0.00 C
HETATM 22 C4 RDG 7 30.196 44.830 48.463 1.00 0.00 C
HETATM 23 C3 RDG 7 29.578 45.978 47.992 1.00 0.00 C
HETATM 24 H2 RDG 7 28.832 45.877 47.218 1.00 0.00 H
HETATM 25 N1 RDG 7 29.867 43.492 48.308 1.00 0.00 N
HETATM 26 C7 RDG 7 30.758 42.858 49.115 1.00 0.00 C
HETATM 27 N2 RDG 7 31.460 43.766 49.820 1.00 0.00 N
HETATM 28 H5 RDG 7 32.168 43.493 50.487 1.00 0.00 H
HETATM 29 C8 RDG 7 30.869 41.515 49.380 1.00 0.00 C
HETATM 30 C9 RDG 7 30.242 40.580 48.545 1.00 0.00 C
HETATM 31 C10 RDG 7 30.362 39.162 48.756 1.00 0.00 C
HETATM 32 C11 RDG 7 30.099 38.290 47.704 1.00 0.00 C
HETATM 33 C12 RDG 7 30.084 36.904 48.017 1.00 0.00 C
HETATM 34 C13 RDG 7 30.381 36.443 49.322 1.00 0.00 C
HETATM 35 C14 RDG 7 30.772 37.333 50.327 1.00 0.00 C
HETATM 36 C15 RDG 7 30.746 38.702 50.011 1.00 0.00 C
HETATM 37 H13 RDG 7 30.782 39.423 50.815 1.00 0.00 H
HETATM 38 H12 RDG 7 30.981 36.998 51.332 1.00 0.00 H
HETATM 39 C16 RDG 7 30.231 35.014 49.584 1.00 0.00 C
HETATM 40 O2 RDG 7 30.352 34.533 50.750 1.00 0.00 O
HETATM 41 H14 RDG 7 30.057 34.375 48.732 1.00 0.00 H

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HETATM   42  H11 RDG     7       29.649  36.199  47.324  1.00  0.00           H
HETATM   43  H10 RDG     7       29.658  38.614  46.773  1.00  0.00           H
HETATM   44  H9  RDG     7       29.944  40.850  47.543  1.00  0.00           H
HETATM   45  H8  RDG     7       31.476  41.118  50.181  1.00  0.00           H
HETATM   46  H3  RDG     7       32.415  46.363  50.599  1.00  0.00           H
HETATM   47  H1  RDG     7       31.467  48.368  49.581  1.00  0.00           H
HETATM   48 1H2' RDG     7       31.392  51.520  46.639  1.00  0.00           H
HETATM   49 2H2' RDG     7       30.146  52.365  47.559  1.00  0.00           H
HETATM   50  H3' RDG     7       30.308  51.808  44.513  1.00  0.00           H
HETATM   51  O3' RDG     7       30.703  53.632  45.318  1.00  0.00           O
TER      52      RDG     7
CONECT    1    2    3    4
CONECT    2    1
CONECT    3    1
CONECT    4    1    5
CONECT    5    4    8    6    7
CONECT    6    5
CONECT    7    5
CONECT    8    5    9   11   10
CONECT    9    8
CONECT   10    8
CONECT   11    8
CONECT   12   50   51   13
CONECT   13   12   48   49   14
CONECT   14   13   16   15
CONECT   15   14
CONECT   16   14   17
CONECT   17   16   18
CONECT   18   17   23   19
CONECT   19   18   47   20
CONECT   20   19   21   46
CONECT   21   20   27   22
CONECT   22   21   23   25
CONECT   23   18   22   24
CONECT   24   23
CONECT   25   22   26
CONECT   26   25   27   29
CONECT   27   21   26   28
CONECT   28   27
CONECT   29   26   45   30
CONECT   30   29   31   44
CONECT   31   30   32   36
CONECT   32   31   33   43
CONECT   33   32   42   34
CONECT   34   33   39   35
CONECT   35   34   36   38
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CONECT   36   31   35   37
CONECT   37   36
CONECT   38   35
CONECT   39   34   40   41
CONECT   40   39
CONECT   41   39
CONECT   42   33
CONECT   43   32
CONECT   44   30
CONECT   45   29
CONECT   46   20
CONECT   47   19
CONECT   48   13
CONECT   49   13
CONECT   50   12
CONECT   51   12
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The molecular configuration parameters of conformation 3:

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HETATM    1  P    RDG     7      31.288  55.764  47.049  1.00  0.00           P
HETATM    2  OP2  RDG     7      30.324  55.505  45.965  1.00  0.00           O
HETATM    3  OP1  RDG     7      31.132  57.105  47.688  1.00  0.00           O1-
HETATM    4  O5'  RDG     7      32.823  55.651  46.566  1.00  0.00           O
HETATM    5  C5'  RDG     7      33.214  54.428  45.985  1.00  0.00           C
HETATM    6  1H5' RDG     7      33.035  53.608  46.680  1.00  0.00           H
HETATM    7  2H5' RDG     7      32.523  54.218  45.168  1.00  0.00           H
HETATM    8  C4'  RDG     7      34.655  54.495  45.497  1.00  0.00           C
HETATM    9  O4'  RDG     7      34.900  55.713  45.036  1.00  0.00           O
HETATM   10  4HHO RDG     7      35.435  55.096  45.580  1.00  0.00           H
HETATM   11  H4'  RDG     7      34.725  53.674  44.783  1.00  0.00           H
HETATM   12  C3'  RDG     7      35.703  54.189  46.616  1.00  0.00           C
HETATM   13  C2'  RDG     7      35.369  54.784  48.055  1.00  0.00           C
HETATM   14  C1'  RDG     7      35.987  54.044  49.166  1.00  0.00           C
HETATM   15  H1'  RDG     7      36.776  54.486  49.757  1.00  0.00           H
HETATM   16  N3   RDG     7      35.509  52.816  49.360  1.00  0.00           N
HETATM   17  O1   RDG     7      36.169  52.285  50.479  1.00  0.00           O
HETATM   18  C2   RDG     7      35.871  50.968  50.797  1.00  0.00           C
HETATM   19  C1   RDG     7      36.664  50.378  51.663  1.00  0.00           C
HETATM   20  C6   RDG     7      36.514  49.094  52.049  1.00  0.00           C
HETATM   21  C5   RDG     7      35.531  48.390  51.367  1.00  0.00           C
HETATM   22  C4   RDG     7      34.579  48.961  50.442  1.00  0.00           C
HETATM   23  C3   RDG     7      34.798  50.296  50.168  1.00  0.00           C
HETATM   24  H2   RDG     7      34.099  50.826  49.537  1.00  0.00           H
HETATM   25  N1   RDG     7      33.706  47.986  49.947  1.00  0.00           N
HETATM   26  C7   RDG     7      34.111  46.915  50.606  1.00  0.00           C
HETATM   27  N2   RDG     7      35.084  47.098  51.472  1.00  0.00           N
HETATM   28  H5   RDG     7      35.425  46.342  52.049  1.00  0.00           H
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HETATM   29  C8  RDG     7       33.468  45.703  50.589  1.00  0.00           C
HETATM   30  C9  RDG     7       32.251  45.601  49.926  1.00  0.00           C
HETATM   31  C10 RDG     7       31.464  44.428  49.813  1.00  0.00           C
HETATM   32  C11 RDG     7       30.321  44.565  49.008  1.00  0.00           C
HETATM   33  C12 RDG     7       29.396  43.495  48.981  1.00  0.00           C
HETATM   34  C13 RDG     7       29.607  42.318  49.686  1.00  0.00           C
HETATM   35  C14 RDG     7       30.830  42.144  50.331  1.00  0.00           C
HETATM   36  C15 RDG     7       31.780  43.190  50.428  1.00  0.00           C
HETATM   37  H13 RDG     7       32.568  43.144  51.166  1.00  0.00           H
HETATM   38  H12 RDG     7       30.981  41.267  50.945  1.00  0.00           H
HETATM   39  C16 RDG     7       28.688  41.221  49.714  1.00  0.00           C
HETATM   40  O2  RDG     7       28.847  40.078  50.233  1.00  0.00           O
HETATM   41  H14 RDG     7       27.707  41.500  49.360  1.00  0.00           H
HETATM   42  H11 RDG     7       28.424  43.677  48.547  1.00  0.00           H
HETATM   43  H10 RDG     7       30.205  45.445  48.392  1.00  0.00           H
HETATM   44  H9  RDG     7       31.870  46.399  49.305  1.00  0.00           H
HETATM   45  H8  RDG     7       33.854  44.882  51.175  1.00  0.00           H
HETATM   46  H3  RDG     7       37.259  48.599  52.655  1.00  0.00           H
HETATM   47  H1  RDG     7       37.419  51.070  52.005  1.00  0.00           H
HETATM   48 1H2' RDG     7       35.769  55.798  48.084  1.00  0.00           H
HETATM   49 2H2' RDG     7       34.297  54.841  48.243  1.00  0.00           H
HETATM   50  H3' RDG     7       35.787  53.109  46.729  1.00  0.00           H
HETATM   51  O3' RDG     7       36.903  54.707  46.064  1.00  0.00           O
TER      52      RDG     7
CONECT    1    4    3    2
CONECT    2    1
CONECT    3    1
CONECT    4    1    5
CONECT    5    4    7    8    6
CONECT    6    5
CONECT    7    5
CONECT    8    5    9   10   11
CONECT    9    8
CONECT   10    8
CONECT   11    8
CONECT   12   13   50   51
CONECT   13   12   48   49   14
CONECT   14   13   15   16
CONECT   15   14
CONECT   16   14   17
CONECT   17   16   18
CONECT   18   17   23   19
CONECT   19   18   47   20
CONECT   20   19   21   46
CONECT   21   20   27   22
CONECT   22   21   23   25
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CONECT 23 18 22 24
CONECT 24 23
CONECT 25 22 26
CONECT 26 25 29 27
CONECT 27 21 26 28
CONECT 28 27
CONECT 29 26 30 45
CONECT 30 29 31 44
CONECT 31 30 32 36
CONECT 32 31 33 43
CONECT 33 32 34 42
CONECT 34 33 35 39
CONECT 35 34 36 38
CONECT 36 31 35 37
CONECT 37 36
CONECT 38 35
CONECT 39 34 40 41
CONECT 40 39
CONECT 41 39
CONECT 42 33
CONECT 43 32
CONECT 44 30
CONECT 45 29
CONECT 46 20
CONECT 47 19
CONECT 48 13
CONECT 49 13
CONECT 50 12
CONECT 51 12

The molecular configuration parameters of conformation 2:

HETATM 1 P RDG 7 32.169 51.996 49.141 1.00 0.00 P
HETATM 2 OP2 RDG 7 32.506 51.267 47.944 1.00 0.00 O
HETATM 3 OP1 RDG 7 30.958 52.780 49.254 1.00 0.00 O1-
HETATM 4 O5’ RDG 7 33.402 52.894 49.537 1.00 0.00 O
HETATM 5 C5’ RDG 7 34.661 52.379 49.896 1.00 0.00 C
HETATM 6 1H5’ RDG 7 34.579 52.158 50.960 1.00 0.00 H
HETATM 7 2H5’ RDG 7 34.871 51.540 49.232 1.00 0.00 H
HETATM 8 C4’ RDG 7 35.782 53.412 49.911 1.00 0.00 C
HETATM 9 O4’ RDG 7 35.432 54.517 50.579 1.00 0.00 O
HETATM 10 4HHO RDG 7 36.361 54.204 50.542 1.00 0.00 H
HETATM 11 H4’ RDG 7 36.033 53.580 48.864 1.00 0.00 H
HETATM 12 C3’ RDG 7 37.144 52.976 50.510 1.00 0.00 C
HETATM 13 C2’ RDG 7 37.180 52.532 51.959 1.00 0.00 C
HETATM 14 C1’ RDG 7 37.583 51.118 52.294 1.00 0.00 C
HETATM 15 H1’ RDG 7 38.555 50.955 52.734 1.00 0.00 H

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HETATM   16  N3  RDG     7      36.722  50.161  51.966  1.00  0.00           N
HETATM   17  O1  RDG     7      37.377  48.984  52.423  1.00  0.00           O
HETATM   18  C2  RDG     7      36.571  47.894  52.398  1.00  0.00           C
HETATM   19  C1  RDG     7      36.606  47.057  53.510  1.00  0.00           C
HETATM   20  C6  RDG     7      35.780  45.889  53.603  1.00  0.00           C
HETATM   21  C5  RDG     7      34.904  45.717  52.515  1.00  0.00           C
HETATM   22  C4  RDG     7      34.803  46.558  51.392  1.00  0.00           C
HETATM   23  C3  RDG     7      35.628  47.680  51.370  1.00  0.00           C
HETATM   24  H2  RDG     7      35.476  48.377  50.560  1.00  0.00           H
HETATM   25  N1  RDG     7      33.722  46.149  50.557  1.00  0.00           N
HETATM   26  C7  RDG     7      33.234  45.088  51.199  1.00  0.00           C
HETATM   27  N2  RDG     7      33.929  44.813  52.280  1.00  0.00           N
HETATM   28  H5  RDG     7      33.607  43.964  52.722  1.00  0.00           H
HETATM   29  C8  RDG     7      32.133  44.445  50.727  1.00  0.00           C
HETATM   30  C9  RDG     7      31.464  44.982  49.605  1.00  0.00           C
HETATM   31  C10 RDG     7      30.027  45.045  49.567  1.00  0.00           C
HETATM   32  C11 RDG     7      29.460  45.652  48.443  1.00  0.00           C
HETATM   33  C12 RDG     7      28.033  45.760  48.356  1.00  0.00           C
HETATM   34  C13 RDG     7      27.240  45.436  49.481  1.00  0.00           C
HETATM   35  C14 RDG     7      27.854  44.938  50.654  1.00  0.00           C
HETATM   36  C15 RDG     7      29.222  44.749  50.675  1.00  0.00           C
HETATM   37  H13 RDG     7      29.657  44.324  51.567  1.00  0.00           H
HETATM   38  H12 RDG     7      27.225  44.674  51.491  1.00  0.00           H
HETATM   39  C16 RDG     7      25.829  45.766  49.605  1.00  0.00           C
HETATM   40  O2  RDG     7      25.115  45.512  50.617  1.00  0.00           O
HETATM   41  H14 RDG     7      25.415  46.300  48.762  1.00  0.00           H
HETATM   42  H11 RDG     7      27.632  46.124  47.422  1.00  0.00           H
HETATM   43  H10 RDG     7      30.191  45.876  47.680  1.00  0.00           H
HETATM   44  H9  RDG     7      32.086  45.154  48.739  1.00  0.00           H
HETATM   45  H8  RDG     7      31.771  43.571  51.248  1.00  0.00           H
HETATM   46  H3  RDG     7      35.849  45.277  54.491  1.00  0.00           H
HETATM   47  H1  RDG     7      37.272  47.325  54.316  1.00  0.00           H
HETATM   48 1H2' RDG     7      37.862  53.181  52.509  1.00  0.00           H
HETATM   49 2H2' RDG     7      36.238  52.856  52.401  1.00  0.00           H
HETATM   50  H3' RDG     7      37.498  52.141  49.905  1.00  0.00           H
HETATM   51  O3' RDG     7      37.827  54.205  50.259  1.00  0.00           O
TER      52      RDG     7
CONECT    1    4    3    2
CONECT    2    1
CONECT    3    1
CONECT    4    1    5
CONECT    5    4    6    7    8
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CONECT    7    5
CONECT    8    5   11   12    9
CONECT    9    8   10
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CONECT   10    9
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CONECT   17   16   18
CONECT   18   17   19   23
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CONECT   22   21   23   25
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CONECT   24   23
CONECT   25   22   26
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CONECT   31   30   36   32
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CONECT   33   32   42   34
CONECT   34   33   35   39
CONECT   35   34   36   38
CONECT   36   31   35   37
CONECT   37   36
CONECT   38   35
CONECT   39   34   40   41
CONECT   40   39
CONECT   41   39
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CONECT   48   13
CONECT   49   13
CONECT   50   12
CONECT   51   12
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The molecular configuration parameters of conformation 1:

HETATM	1	P	RDG	7	31.493	53.146	47.761	1.00	0.00	P
HETATM	2	OP2	RDG	7	32.341	52.699	46.679	1.00	0.00	O
HETATM	3	OP1	RDG	7	31.049	54.525	47.752	1.00	0.00	O1-
HETATM	4	O5’	RDG	7	32.196	52.927	49.178	1.00	0.00	O
HETATM	5	C5’	RDG	7	33.501	52.589	49.323	1.00	0.00	C
HETATM	6	1H5’	RDG	7	33.707	52.212	50.325	1.00	0.00	H
HETATM	7	2H5’	RDG	7	33.765	51.800	48.620	1.00	0.00	H
HETATM	8	C4’	RDG	7	34.454	53.758	49.074	1.00	0.00	C
HETATM	9	O4’	RDG	7	34.122	54.817	49.701	1.00	0.00	O
HETATM	10	4HHO	RDG	7	35.044	54.539	49.514	1.00	0.00	H
HETATM	11	H4’	RDG	7	34.276	53.896	48.007	1.00	0.00	H
HETATM	12	C3’	RDG	7	35.877	53.324	49.365	1.00	0.00	C
HETATM	13	C2’	RDG	7	36.255	53.049	50.860	1.00	0.00	C
HETATM	14	C1’	RDG	7	36.777	51.710	51.250	1.00	0.00	C
HETATM	15	H1’	RDG	7	37.800	51.548	51.554	1.00	0.00	H
HETATM	16	N3	RDG	7	35.971	50.678	51.077	1.00	0.00	N
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HETATM	19	C1	RDG	7	36.604	47.301	52.218	1.00	0.00	C
HETATM	20	C6	RDG	7	35.842	46.123	52.145	1.00	0.00	C
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HETATM	22	C4	RDG	7	34.252	47.069	50.486	1.00	0.00	C
HETATM	23	C3	RDG	7	34.907	48.266	50.627	1.00	0.00	C
HETATM	24	H2	RDG	7	34.585	49.069	49.981	1.00	0.00	H
HETATM	25	N1	RDG	7	33.048	46.663	49.832	1.00	0.00	N
HETATM	26	C7	RDG	7	32.779	45.490	50.398	1.00	0.00	C
HETATM	27	N2	RDG	7	33.760	45.033	51.204	1.00	0.00	N
HETATM	28	H5	RDG	7	33.727	44.173	51.732	1.00	0.00	H
HETATM	29	C8	RDG	7	31.763	44.676	50.126	1.00	0.00	C
HETATM	30	C9	RDG	7	30.814	44.968	49.110	1.00	0.00	C
HETATM	31	C10	RDG	7	29.583	44.170	48.844	1.00	0.00	C
HETATM	32	C11	RDG	7	28.861	44.446	47.626	1.00	0.00	C
HETATM	33	C12	RDG	7	27.683	43.765	47.367	1.00	0.00	C
HETATM	34	C13	RDG	7	27.265	42.754	48.247	1.00	0.00	C
HETATM	35	C14	RDG	7	27.965	42.449	49.481	1.00	0.00	C
HETATM	36	C15	RDG	7	29.100	43.175	49.739	1.00	0.00	C
HETATM	37	H13	RDG	7	29.619	43.060	50.679	1.00	0.00	H
HETATM	38	H12	RDG	7	27.533	41.802	50.230	1.00	0.00	H
HETATM	39	C16	RDG	7	25.967	42.192	47.954	1.00	0.00	C
HETATM	40	O2	RDG	7	25.322	41.355	48.649	1.00	0.00	O
HETATM	41	H14	RDG	7	25.564	42.475	46.993	1.00	0.00	H
HETATM	42	H11	RDG	7	27.144	43.929	46.446	1.00	0.00	H
HETATM	43	H10	RDG	7	29.256	45.245	47.017	1.00	0.00	H
HETATM	44	H9	RDG	7	31.036	45.775	48.427	1.00	0.00	H

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HETATM   45  H8  RDG     7       31.655  43.746  50.665  1.00  0.00           H
HETATM   46  H3  RDG     7       36.221  45.277  52.699  1.00  0.00           H
HETATM   47  H1  RDG     7       37.448  47.345  52.891  1.00  0.00           H
HETATM   48 1H2' RDG     7       37.136  53.643  51.103  1.00  0.00           H
HETATM   49 2H2' RDG     7       35.368  53.262  51.457  1.00  0.00           H
HETATM   50  H3' RDG     7       36.131  52.442  48.777  1.00  0.00           H
HETATM   51  O3' RDG     7       36.651  54.436  48.996  1.00  0.00           O
TER      52      RDG     7
CONECT    1    2    3    4
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CONECT    3    1
CONECT    4    1    5
CONECT    5    4    6    8    7
CONECT    6    5
CONECT    7    5
CONECT    8    5   10    9   12
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CONECT   12    8   13   50   51
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CONECT   38   35
CONECT   39   34   41   40
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CONECT 40 39
CONECT 41 39
CONECT 42 33
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CONECT 50 12
CONECT 51 12

6. Gaussian result

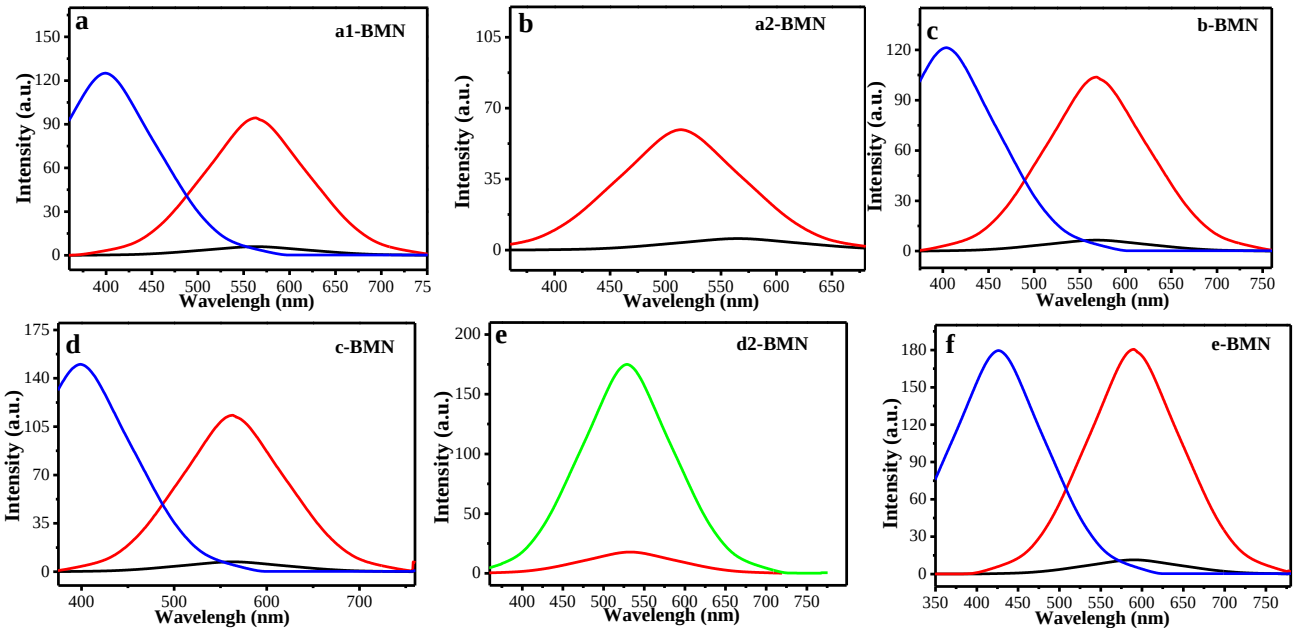

Figure S4. The simulated fluorescence emission spectra by Gaussian 16.

7. Biocompatibility of d1-BMN

Prior to precede single cell gel electrophoresis assay, the biocompatibility of **d1-BMN** such as photostability, biological pH stability, cell toxicity, water solubility and so on, were evaluated by fluorescence intensity as the evaluation parameter, respectively.

7.1 The photostability of d1-BMN under irradiated by iodine-tungsten lamp in PBS buffer

d1-BMN (3.0 μM) retained more than 98% of fluorescence intensity in PBS buffer after they were continuously irradiated by an iodine-tungsten lamp for 6 h. Such kind of photostability of **d1-BMN** ensures that it can provide a stable response signal during single cell assay.

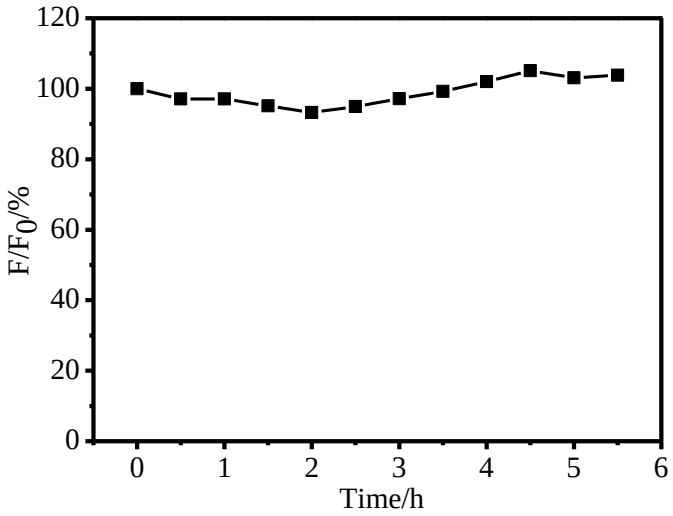

Figure S5. The photostability of d1-BMN (3.0 μM) in PBS buffer (pH 7.4) at 25 °C. Iodine-tungsten lamp: 500W. Excitation wavelength = 400 nm. Emission wavelength = 605 nm.

7.2 The biological stability of d1-BMN in cells

d1-BMN (3.0 μM) retained more than 98.5 % of fluorescence intensity in living cell after they were continuously irradiated by an laser at 405 nm (initial power = 2.6 mM) for 6 h. Such kind of biological stability of **d1-BMN** ensures that it can provide a stable response signal during single cell assay.

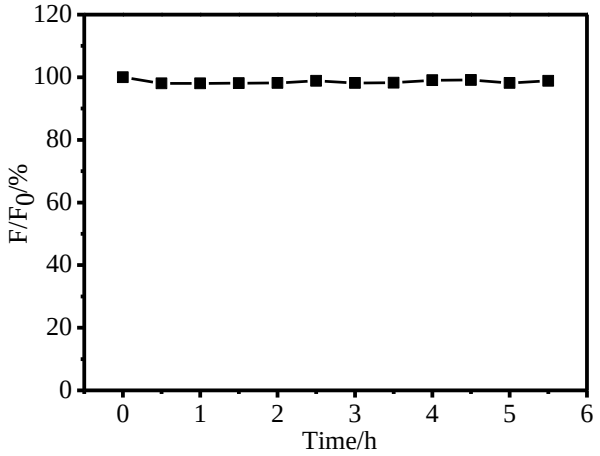

Figure S6. The biological stability of d1-BMN (3.0 μM) in cell. Excitation wavelength = 405 nm. Emission wavelength = 605 nm. The endogenic AP-site was inhibited by O-phenylhydroxylamine. F_0 is the fluorescence intensity at the reaction time of 0, F is the fluorescence intensity at the reaction time of 1 to 6 h.

7.3 Cell toxicity of d1-BMN

The MTT assay results revealed that greater than 95% of cells survived after incubation with **d1-BMN** (5.0 μM) for 24 h. Even the incubation concentration of **d1-BMN** increased to 10 μM, greater than 95 % of the cells still survived. Such high survival rate is of great significance for the single cell assay.

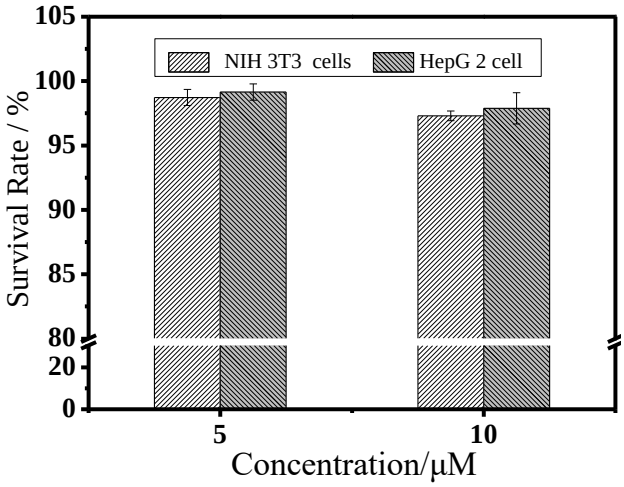

Figure S7. Cell toxicity of d1-BMN (5.0 and 10.0 μM) for HepG2 cells and NIH 3T3 cells. The cells were incubated for an additional 24 h with dyes **d1-BMN** in different concentrations Optical density (OD) was determined by a microplate reader (Spectra Max M5, Molecular Devices) at 570 nm with subtraction of the absorbance of the cell-free blank volume at 630 nm. The results from the six individual experiments were averaged.

7.4 The amount change of AP-site

Before the experiment, the endogenic AP-site was was inhibited by O-phenylhydroxylamine, and then, the cells were incubated by **d1-BMN** for 6.0 h. During this process, whether any new sites are generated is detected in real time. The result indicated that there is no any new AP-site being created. This is of great significance for the single cell assay of damage site-AP-site.

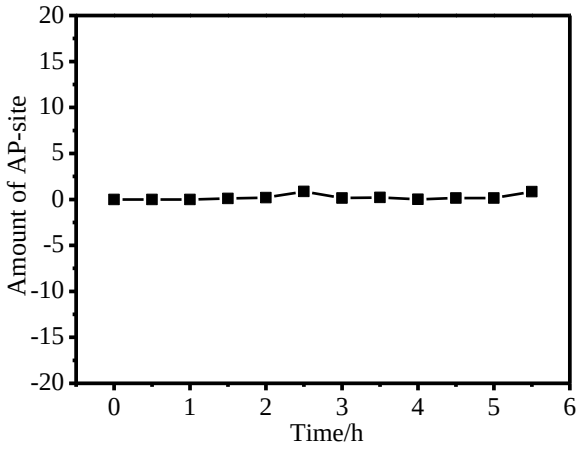

Figure S8. The amount change of damage site. HepG2 cells were incubated by **d1-BMN** (5.0 μM) for different time. The endogenic AP-site was inhibited by O-phenylhydroxylamine.

7.5 The pH-stability of d1-BMN

There was no any influence on the fluorescent signals when the pH of phosphate buffer was at 3.7–10.7. Such kind of pH-stability of **d1-BMN** ensures that it can provide a stable response signal during single cell assay in cellular environment.

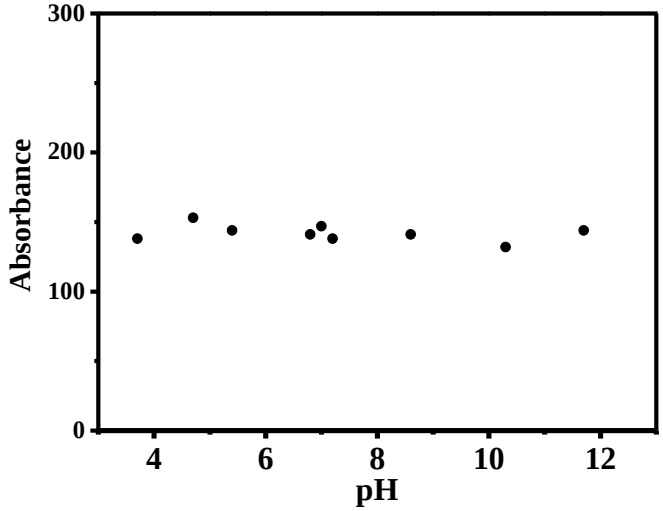

Figure S9. The pH-stability of d1-BMN (3.0 μM) in pH range from 3.7 to 11.7.

7.6 The water solubility of d1-BMN

The solubility of **d1-BMN** was 10 μM. Such kind of water solubility of **d1-BMN** ensures that it is suitable for the signal during single cell assay.

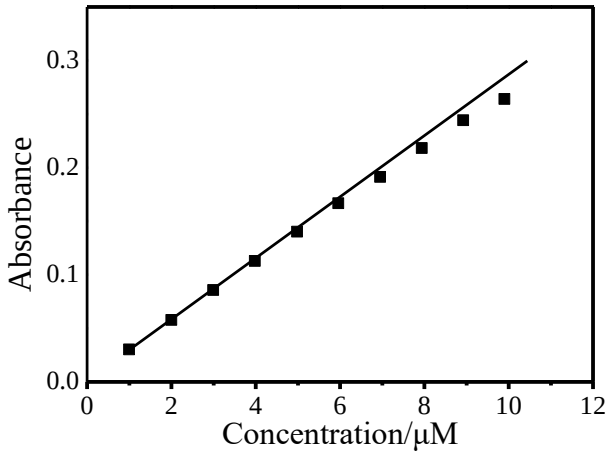

Figure S10. The water solubility of d1-BMN (3.0 μM) in PBS buffer (pH 7.4) at 25 ℃.

8. Single cell gel electrophoresis assay

Table S2. Single cell gel electrophoresis assay results in Hepg 2 cells (n = 200).

		Comet Length**	Tail Length**	Tail Moment**	Olive tail Moment**
Blank group	0	50.01 ±4.16	2.06 ±2.01	0.05 ±0.07	0.07 ±0.11
	5.0	50.19 ±2.34	2.14 ±1.67	0.05 ±0.02	0.07 ±0.14
	15	51.41 ±1.54	2.23 ±1.97	0.05 ±0.04	0.07 ±0.07
	45	52.57 ±3.62	2.32 ±1.41	0.05 ±0.03	0.07 ±0.04
Control groups☆	0	53.21 ±5.97	2.12 ±2.31	0.05 ±0.12	0.08 ±0.10
	5.0	80.73 ±6.54	32.06 ±3.12	20.13 ±5.45	19.34 ±6.21
	15	108.43 ±7.38	52.56 ±12.79	36.74 ±6.89	30.83 ±8.21
	45	123.24 ±7.21	67.35 ±12.34	43.05 ±9.23	38.35 ±9.65
Experimental groups★	0	52.57 ±2.13	2.29 ±0.27	0.05 ±0.17	0.07 ±0.12
	5.0	86.79 ±8.48	39.13 ±10.10	25.21 ±5.30	17.64 ±6.22
	15	105.37 ±8.43	50.57 ±7.78	31.17 ±6.44	31.38 ±5.21
	45	129.12 ±6.59	68.67 ±9.14	43.46 ±8.98	40.67 ±3.76

** P < 0.01; ★ Staining with **d1-BMN**; ☆ Staining with Ethidium Bromide (EB).

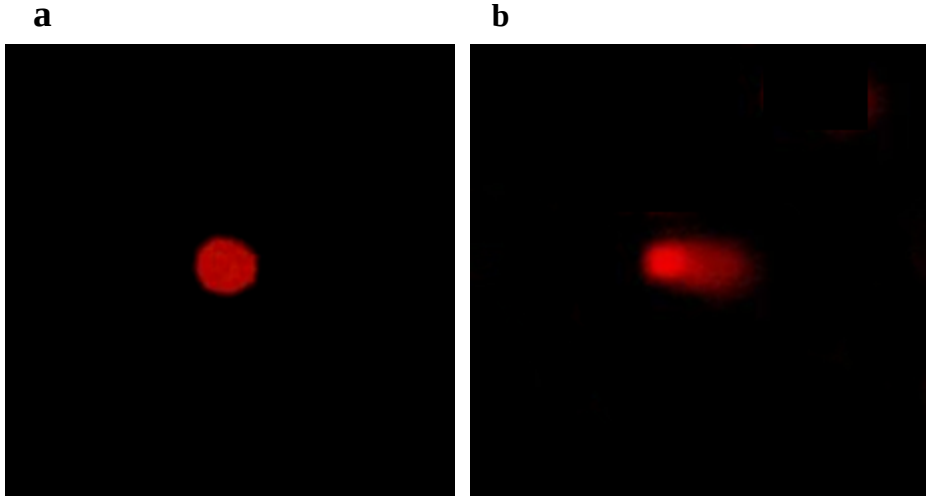

Figure SS-8. a and b. The single cell gel electrophoresis assay of **d1-BMN** (5.0 μM) in Hepg 2 cell by incubating with ROS (a no ROS and b with ROS).

9. Sort different degree of damaged cell by flow cytometry

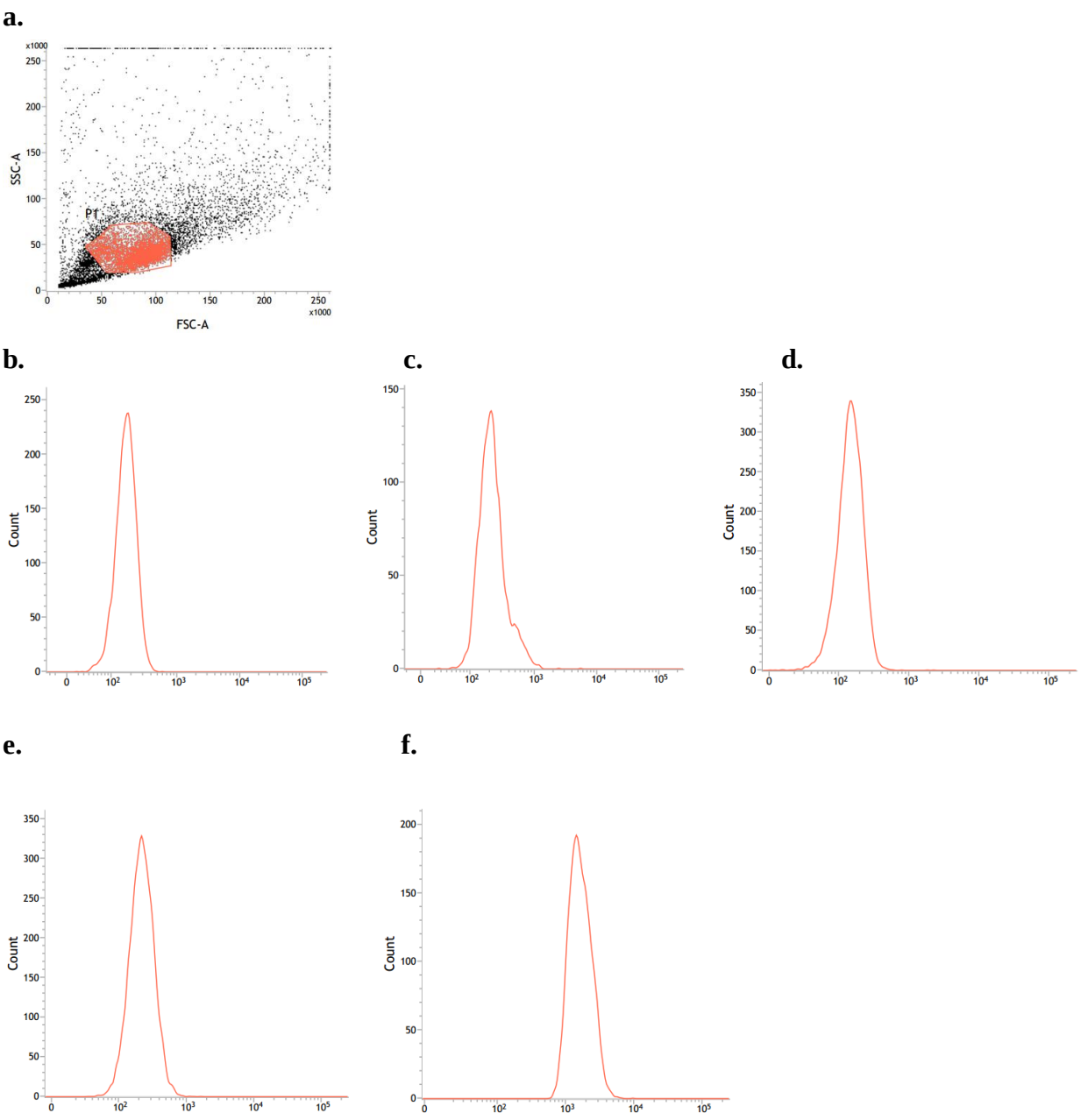

Figure S11. Sort different degree of damaged cell by flow cytometry. d1-BMN (10.0 μM), Hepg 2 cell. a. Cell count scatter plot, 10000 cells. b-d. Cell count histogram at different fluorescence (red, orange, yellow, green and blue).

References.

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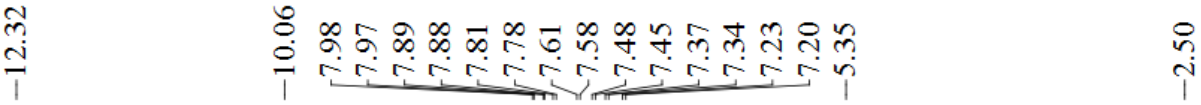
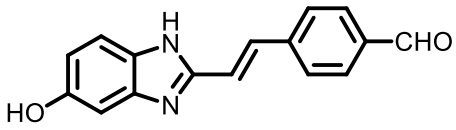
Attached Spectra.

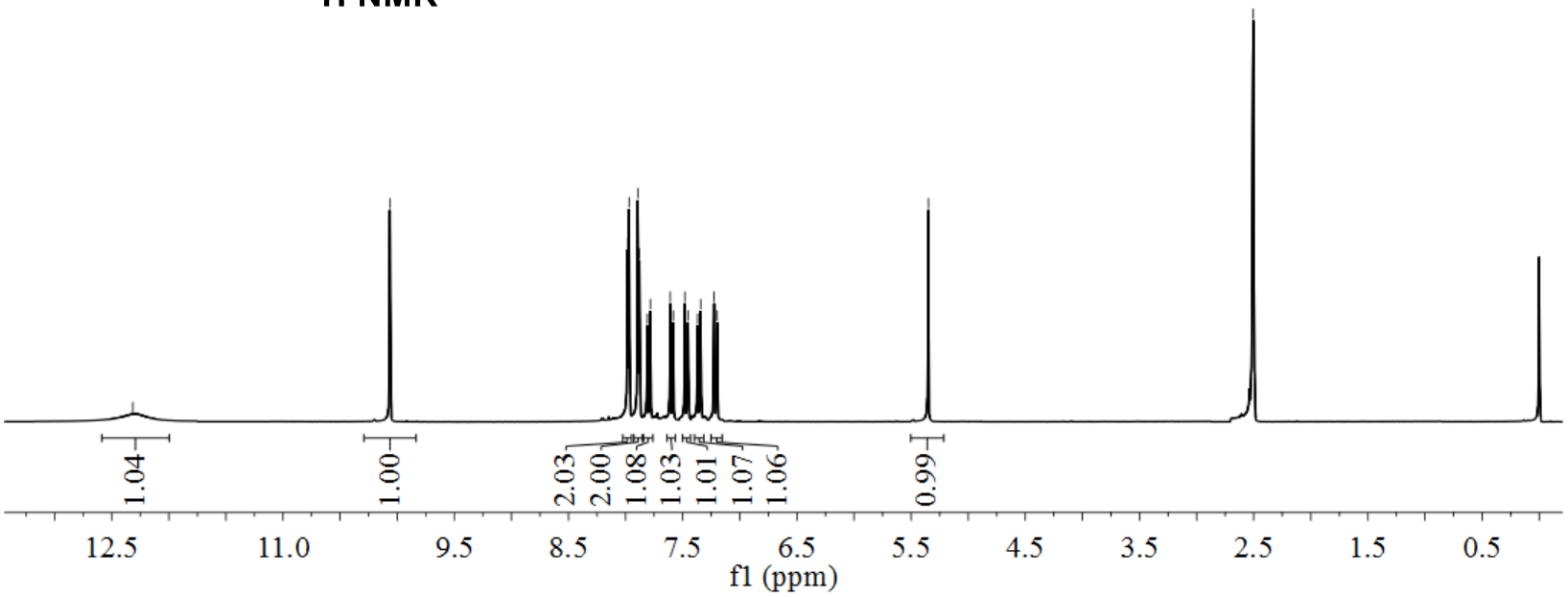


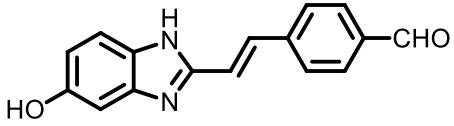


2d-1

(*E*)-4-(2-(5-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzaldehyde

^{1}H NMR

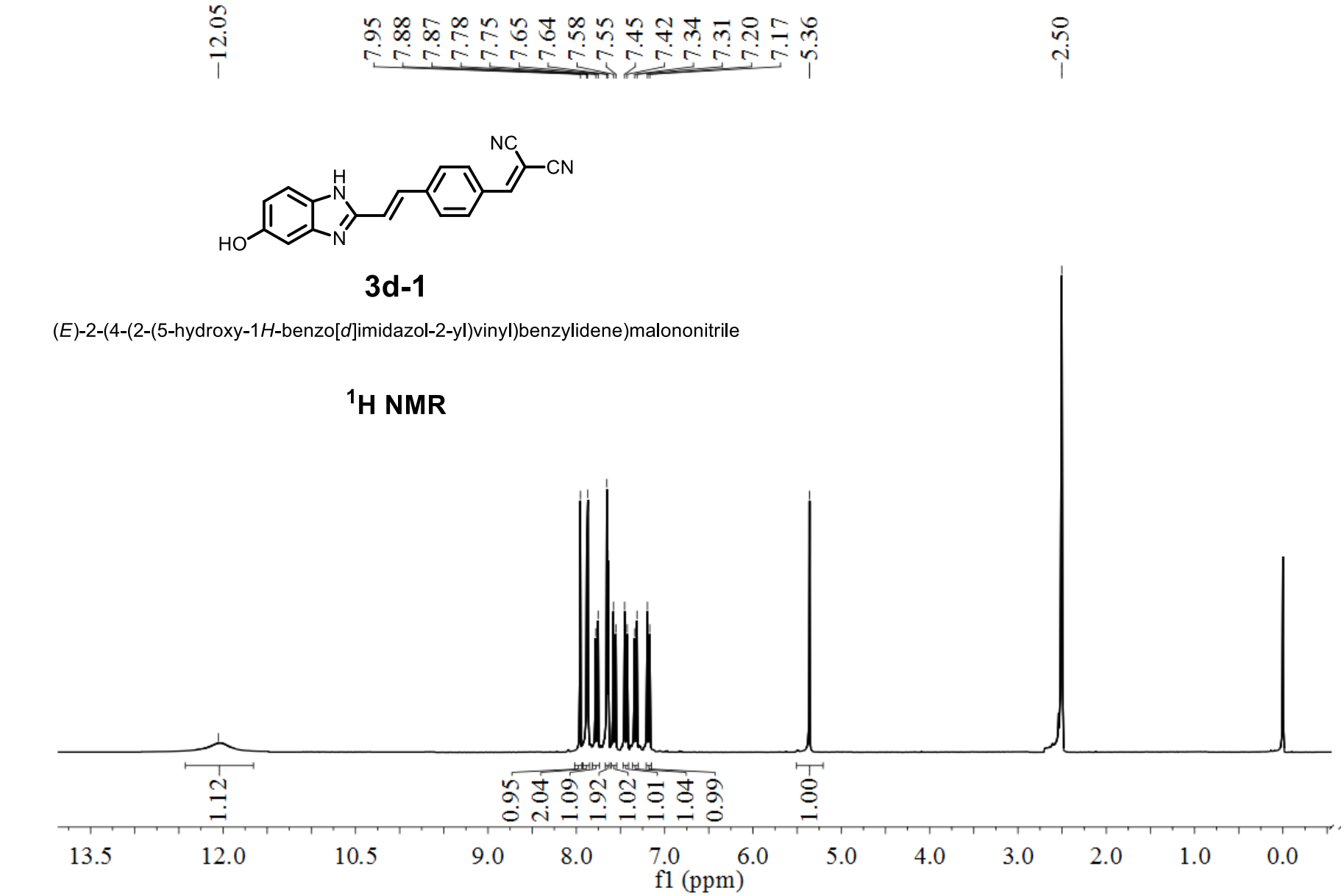




2d-1

(*E*)-4-(2-(5-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzaldehyde

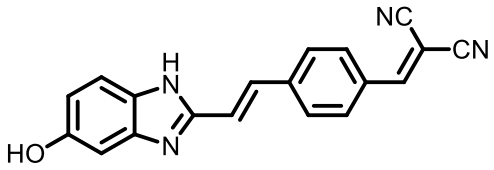
^{13}C NMR



3d-1

(*E*)-2-(4-(2-(5-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

^{1}H NMR



3d-1

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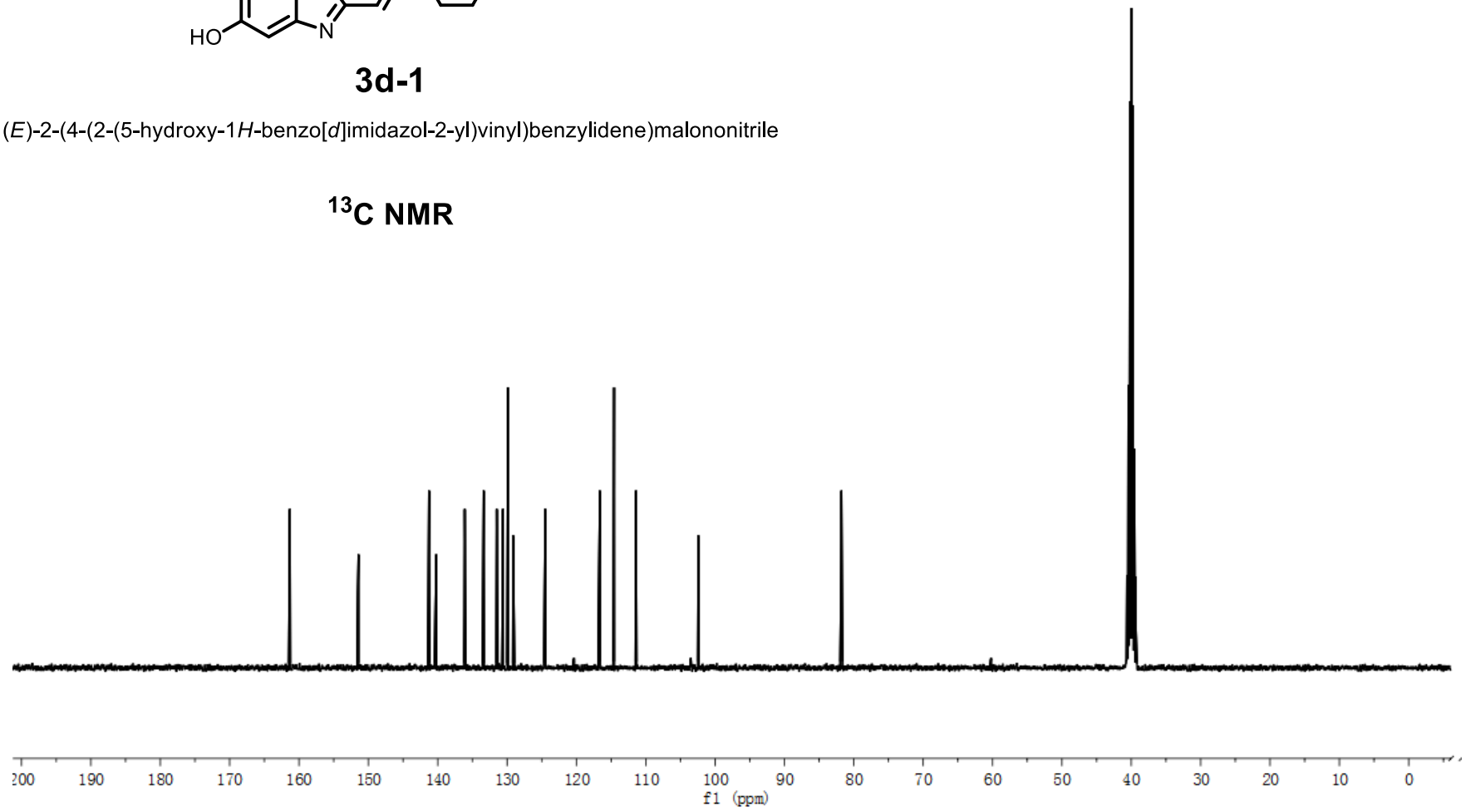
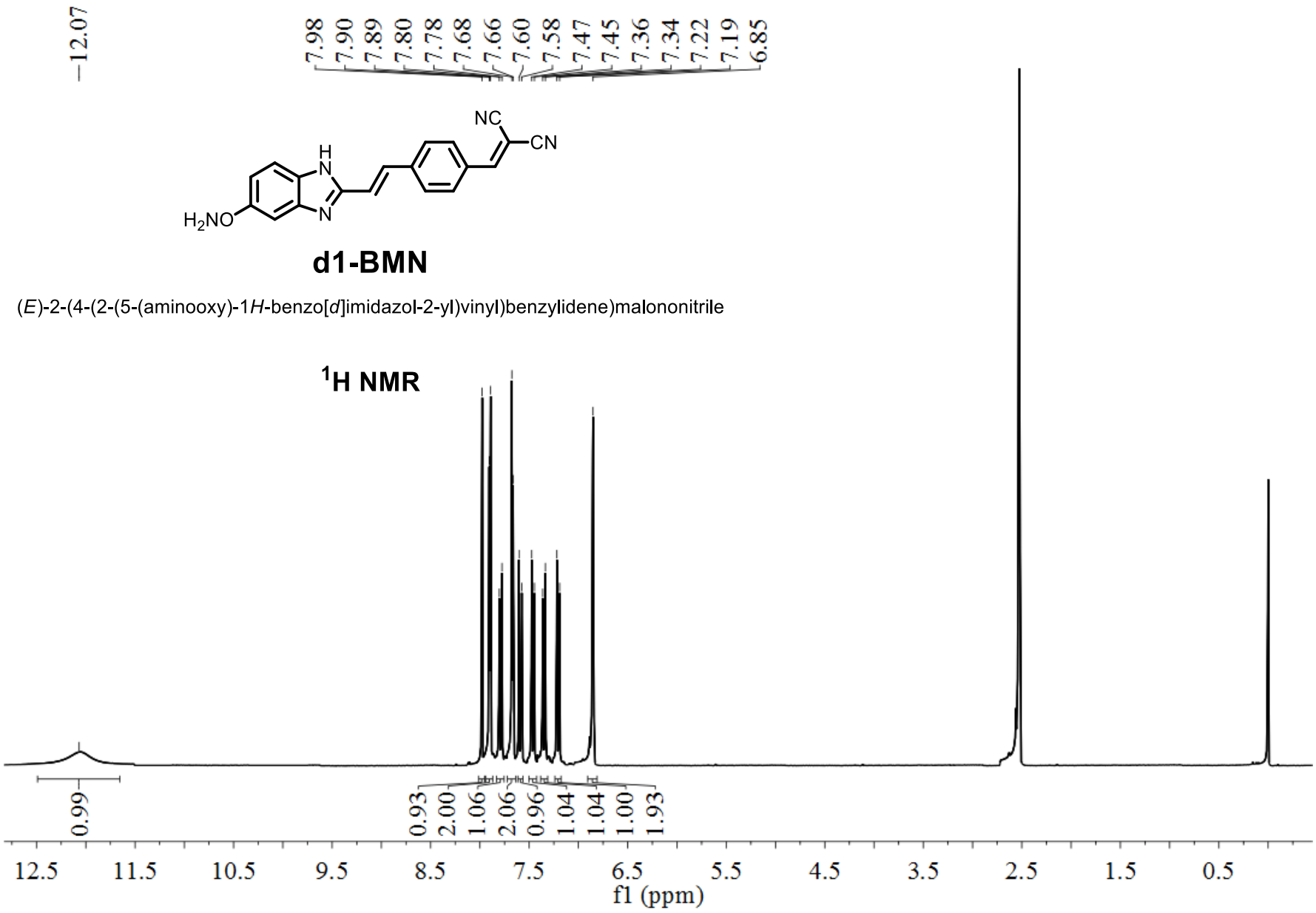
^{13}C NMR

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7.19
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NC
CN
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N
H2NO
d1-BMN
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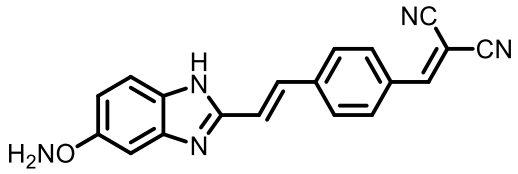
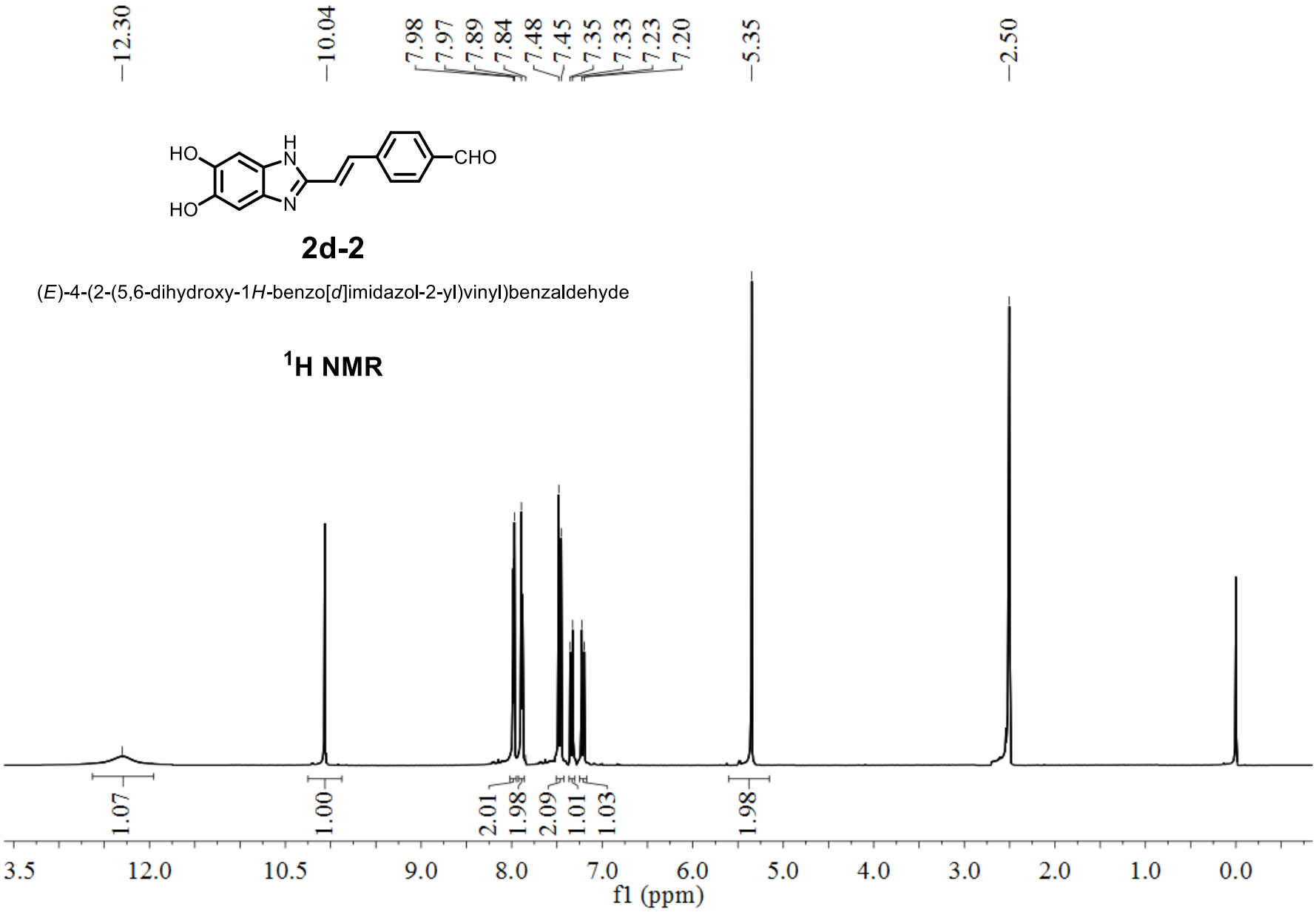
2.00
1.06
2.06
0.96
1.04
1.04
1.00
1.93
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
f1 (ppm)

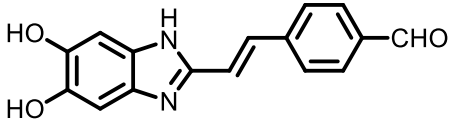


d1-BMN

(*E*)-2-(4-(2-(5-(aminooxy)-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

^{13}C NMR

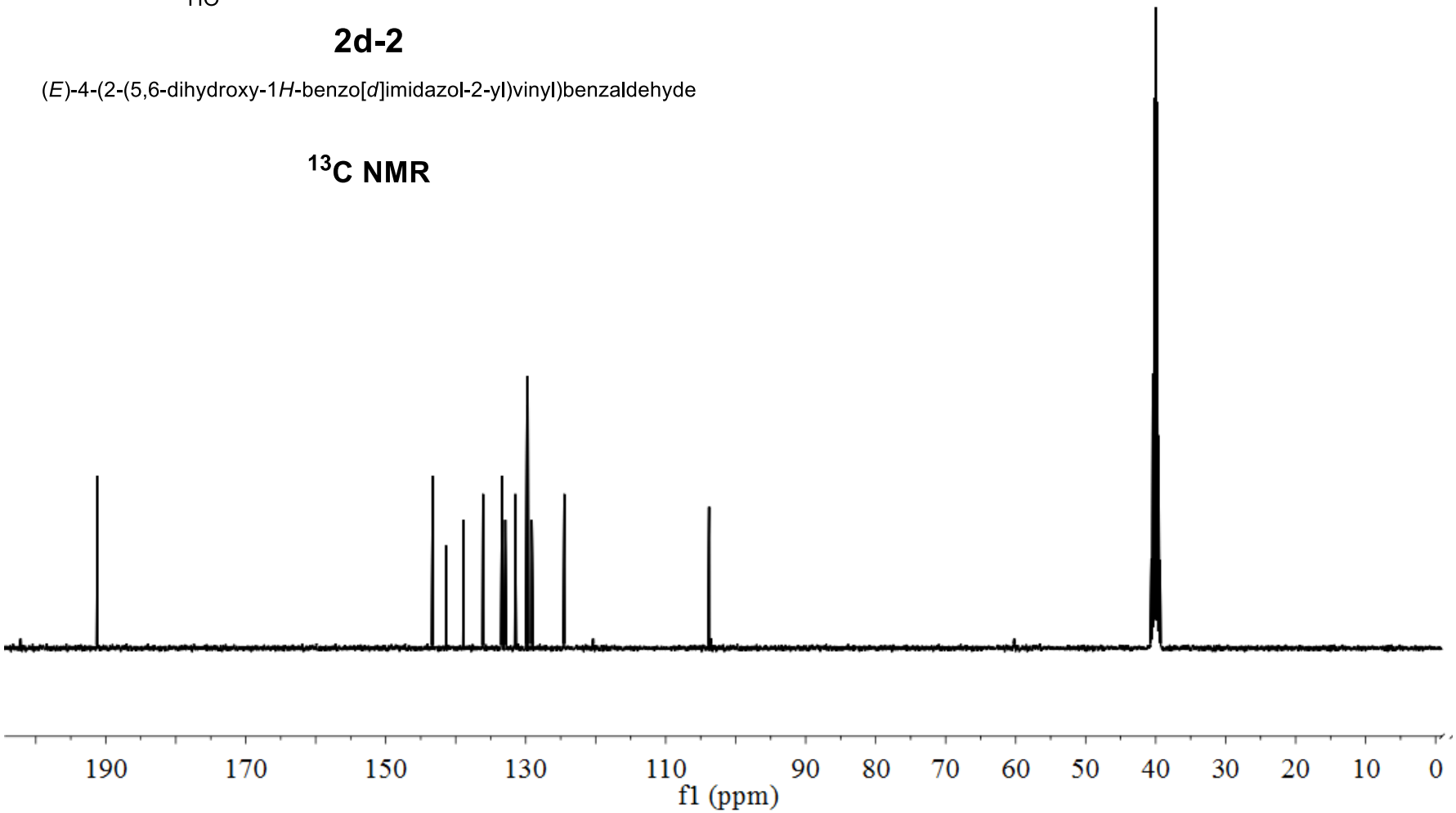

12.30
10.04
7.98
7.97
7.89
7.84
7.48
7.45
7.35
7.33
7.23
7.20
5.35
2.50
HO
HO
H
N
N
CHO
2d-2
(E)-4-(2-(5,6-dihydroxy-1H-benzo[d]imidazol-2-yl)vinyl)benzaldehyde
1H NMR
1.07
1.00
2.01
1.98
2.09
1.01
1.03
1.98
3.5
12.0
10.5
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

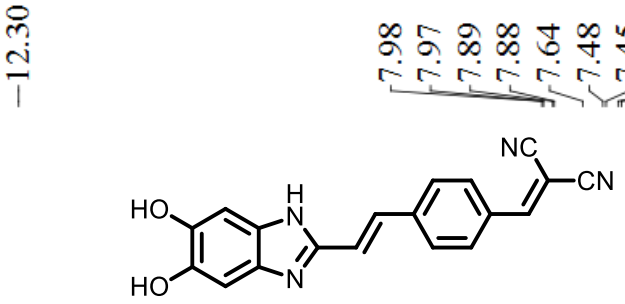


2d-2

(*E*)-4-(2-(5,6-dihydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzaldehyde

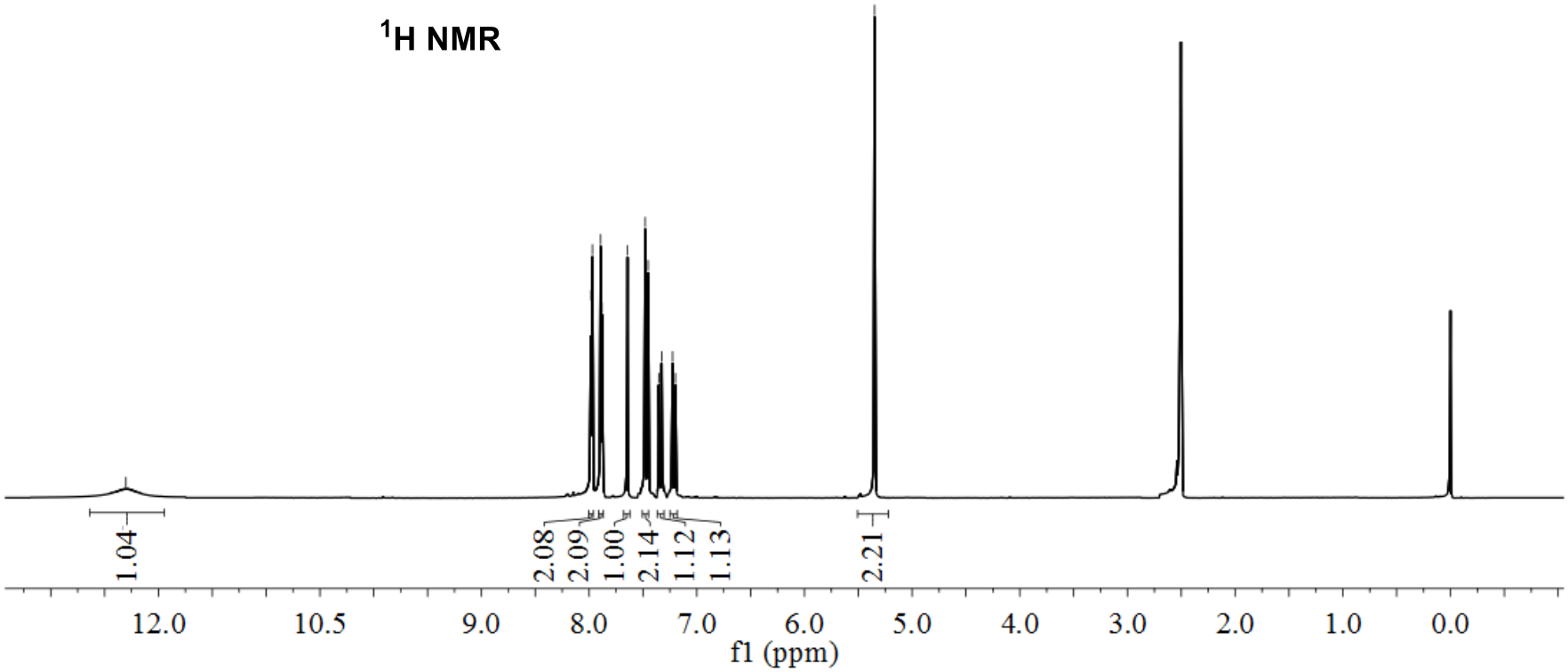
^{13}C NMR

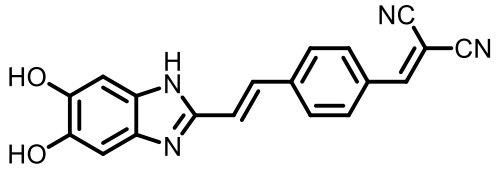




3d-2

(*E*)-2-(4-(2-(5,6-dihydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

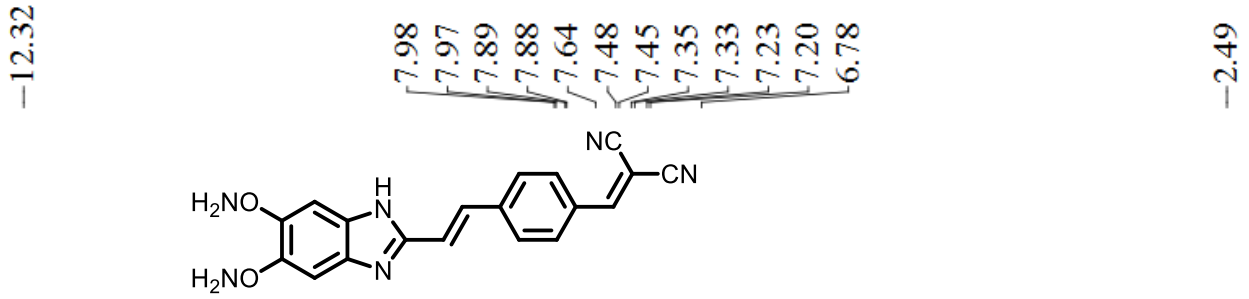




3d-2

(*E*)-2-(4-(2-(5,6-dihydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

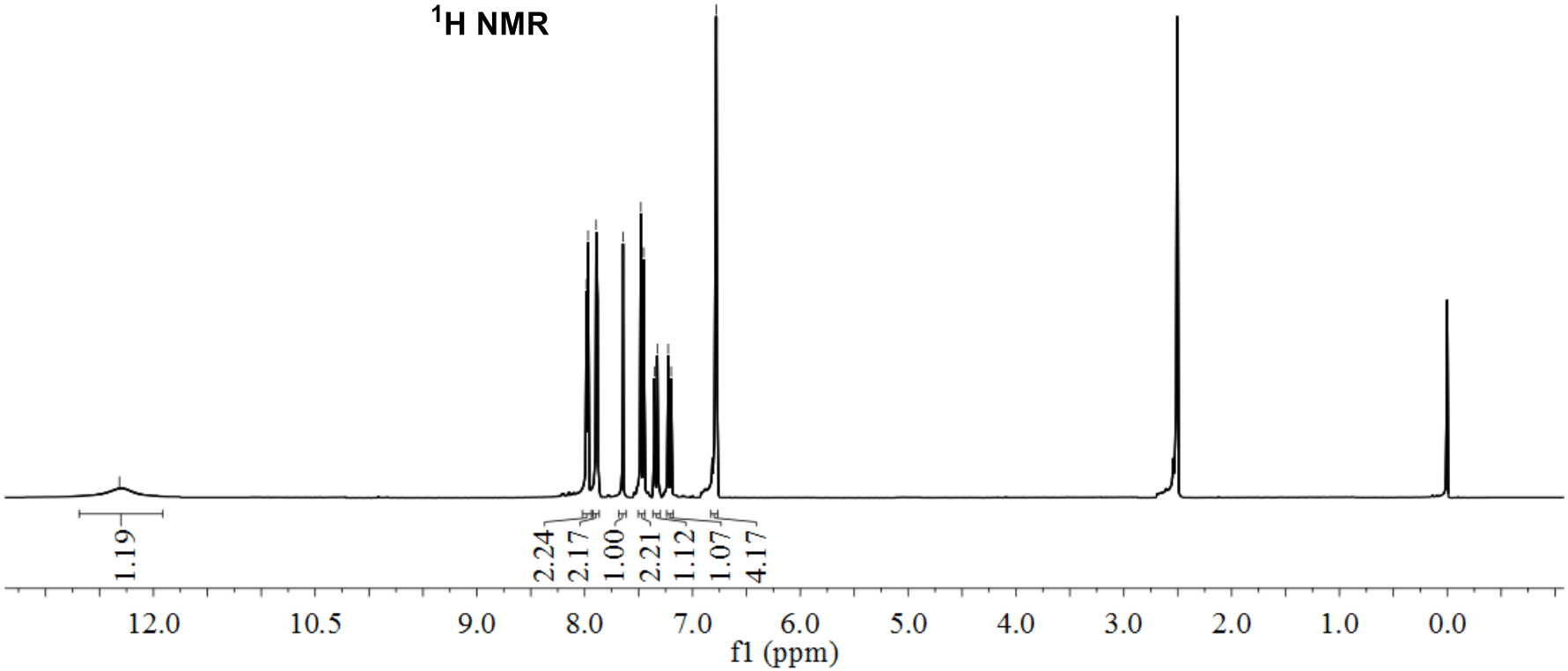
^{13}C NMR

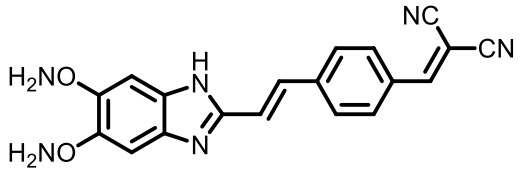


d2-BMN

(*E*)-2-(4-(2-(5,6-bis(aminooxy)-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

^{1}H NMR

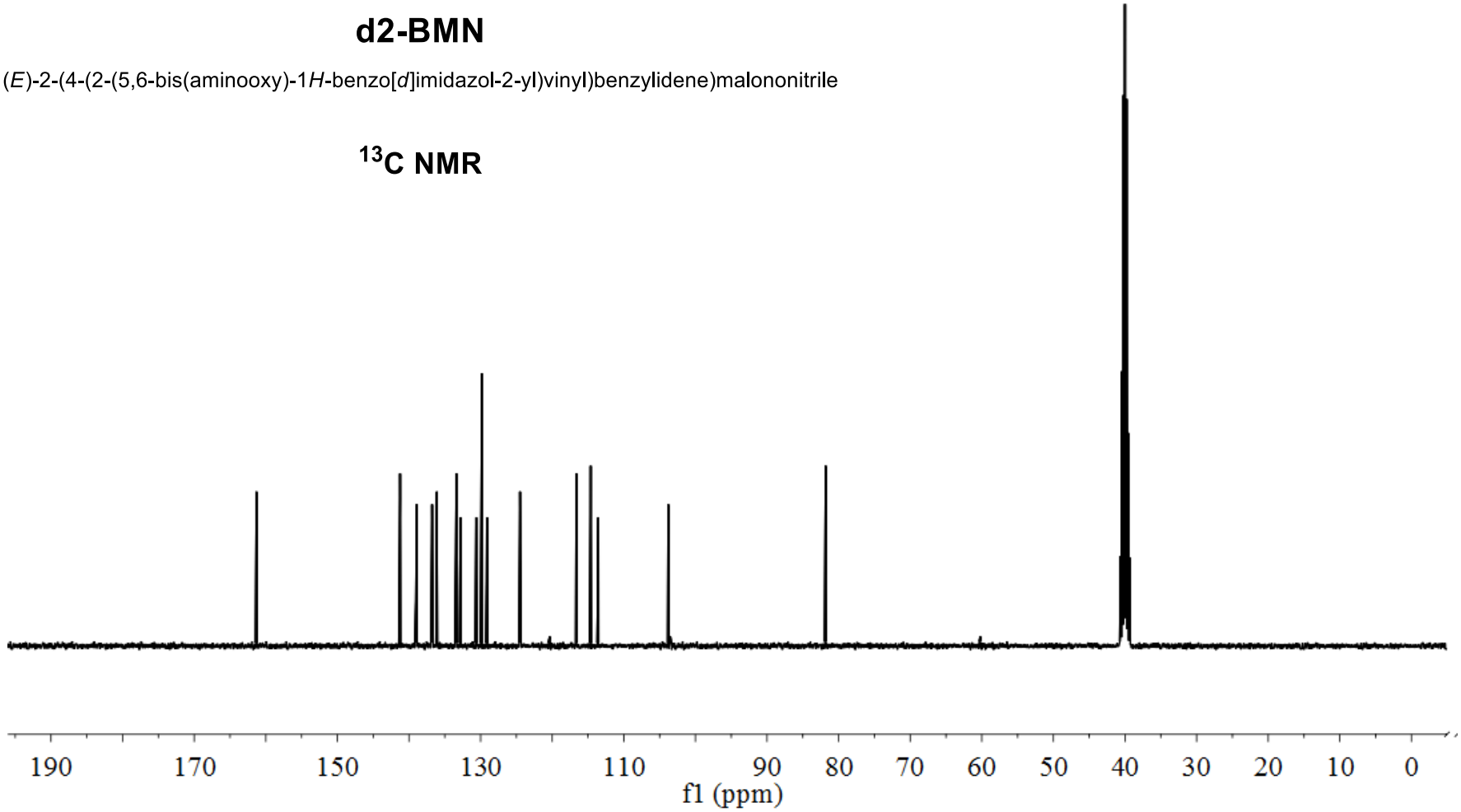


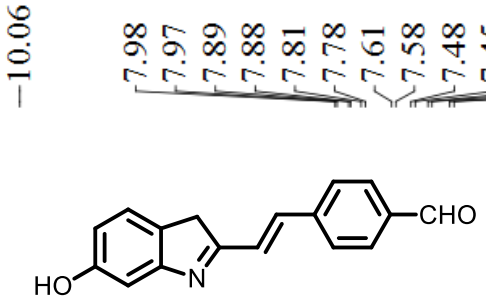


d2-BMN

(*E*)-2-(4-(2-(5,6-bis(aminooxy)-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

^{13}C NMR

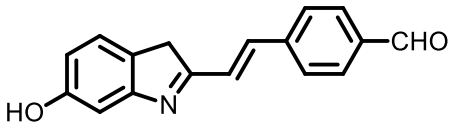




2a-1

(*E*)-4-(2-(6-hydroxy-3*H*-indol-2-yl)vinyl)benzaldehyde

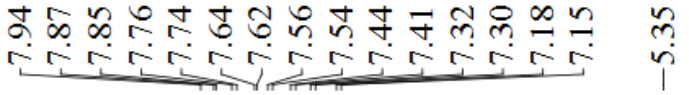
^{1}H NMR

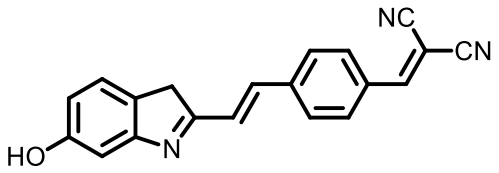


2a-1

(*E*)-4-(2-(6-hydroxy-3*H*-indol-2-yl)vinyl)benzaldehyde

^{13}C NMR

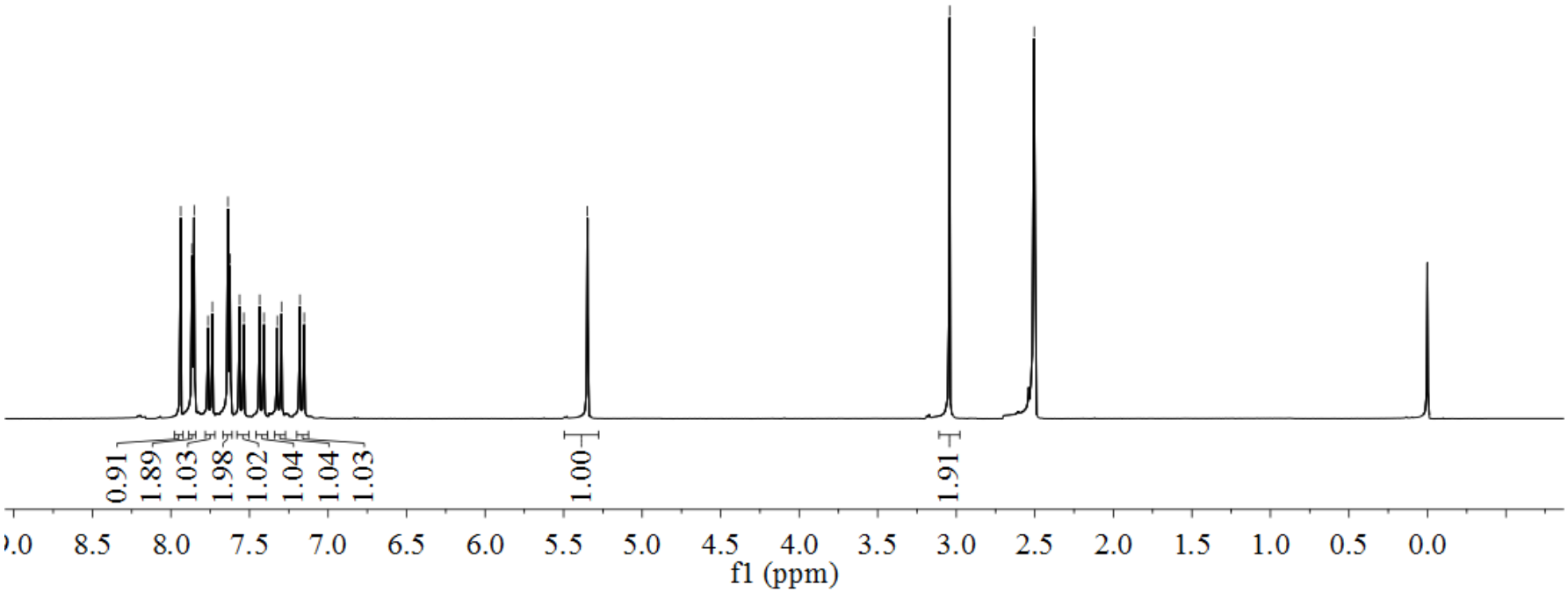


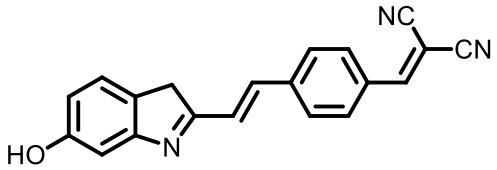


3a-1

(*E*)-2-(4-(2-(6-hydroxy-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{1}H NMR

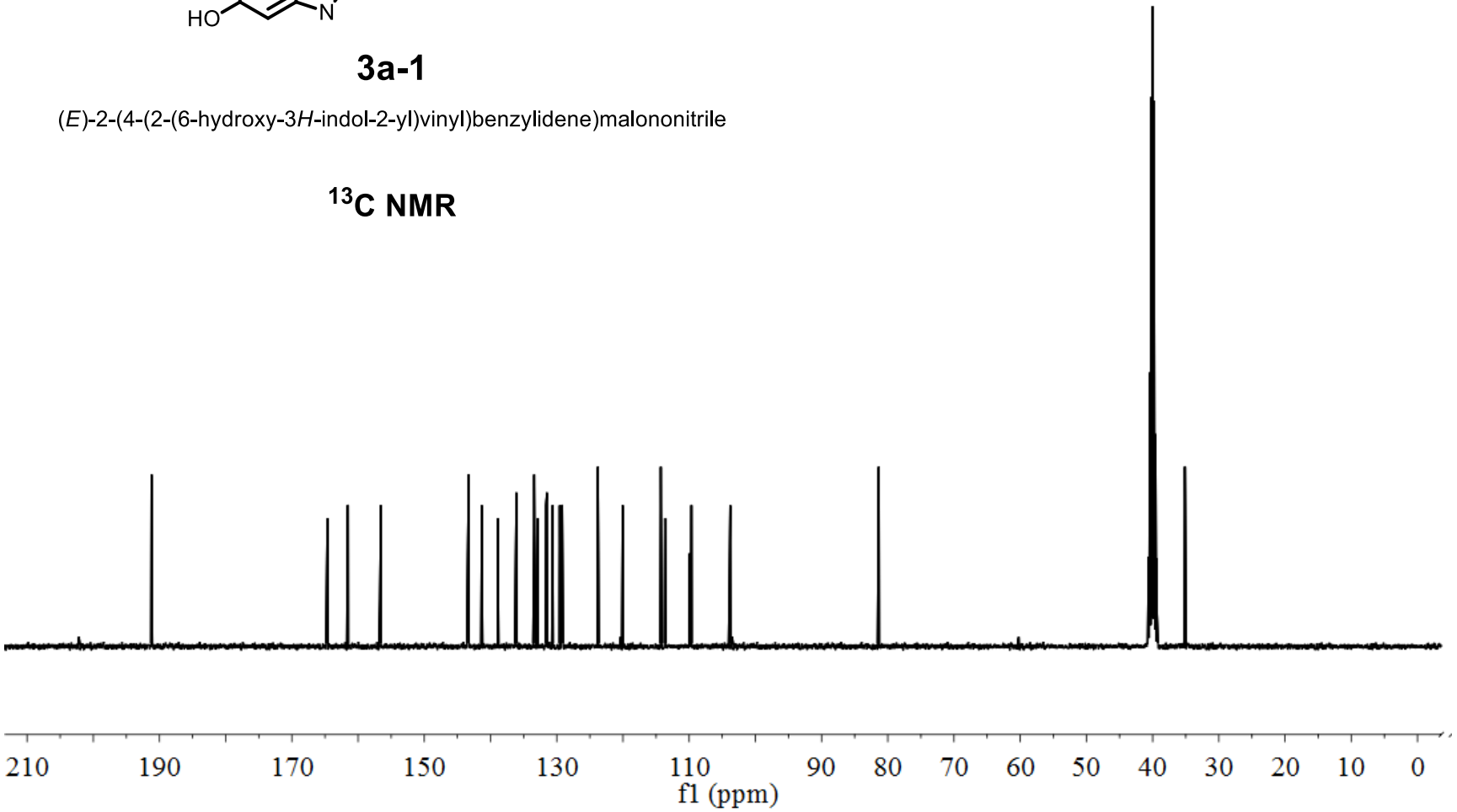


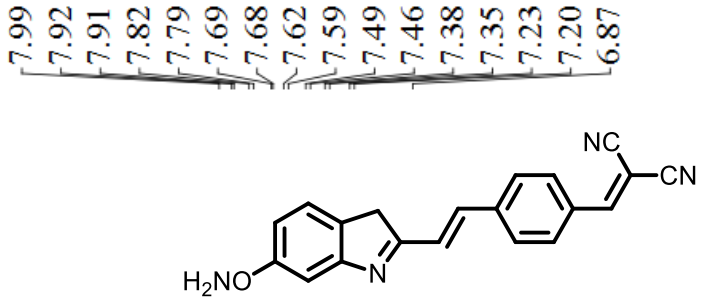


3a-1

(*E*)-2-(4-(2-(6-hydroxy-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{13}C NMR

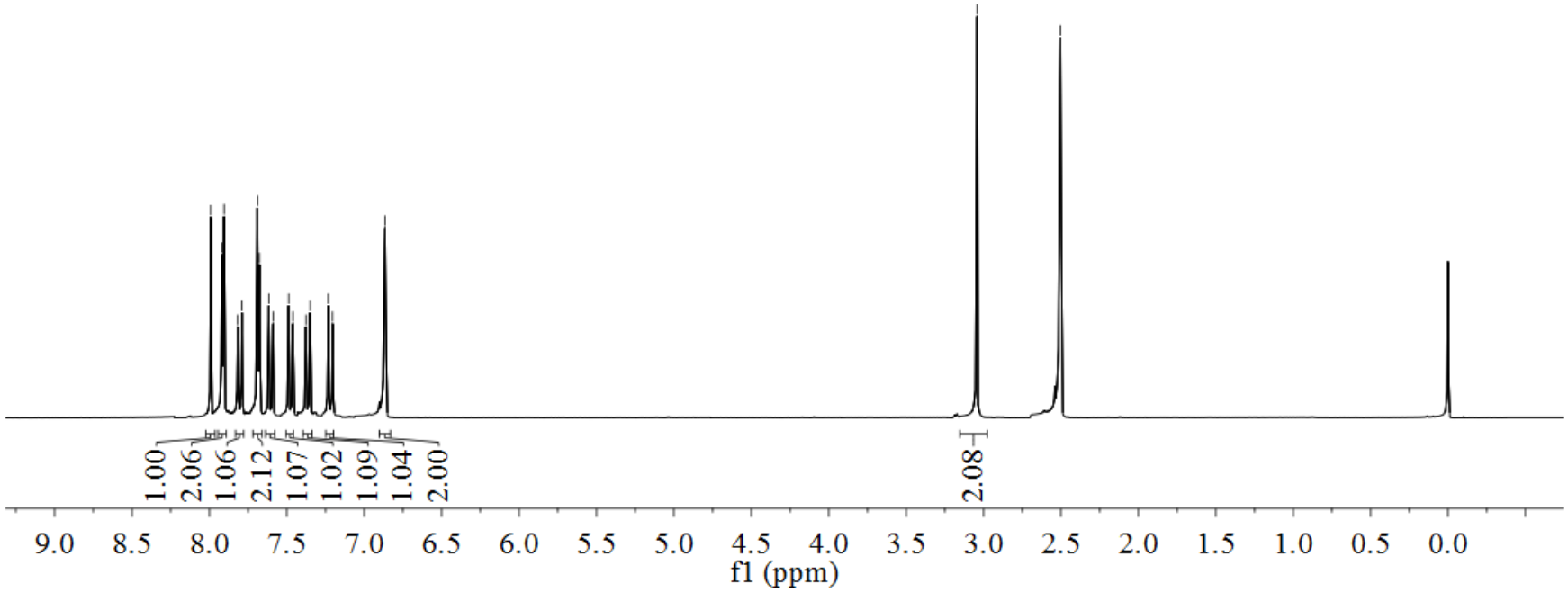


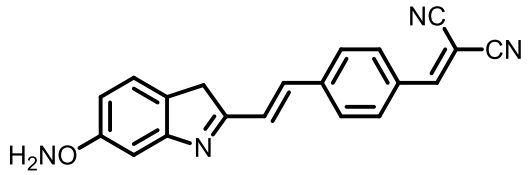


a1-BMN

(*E*)-2-(4-(2-(6-(aminooxy)-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{1}H NMR

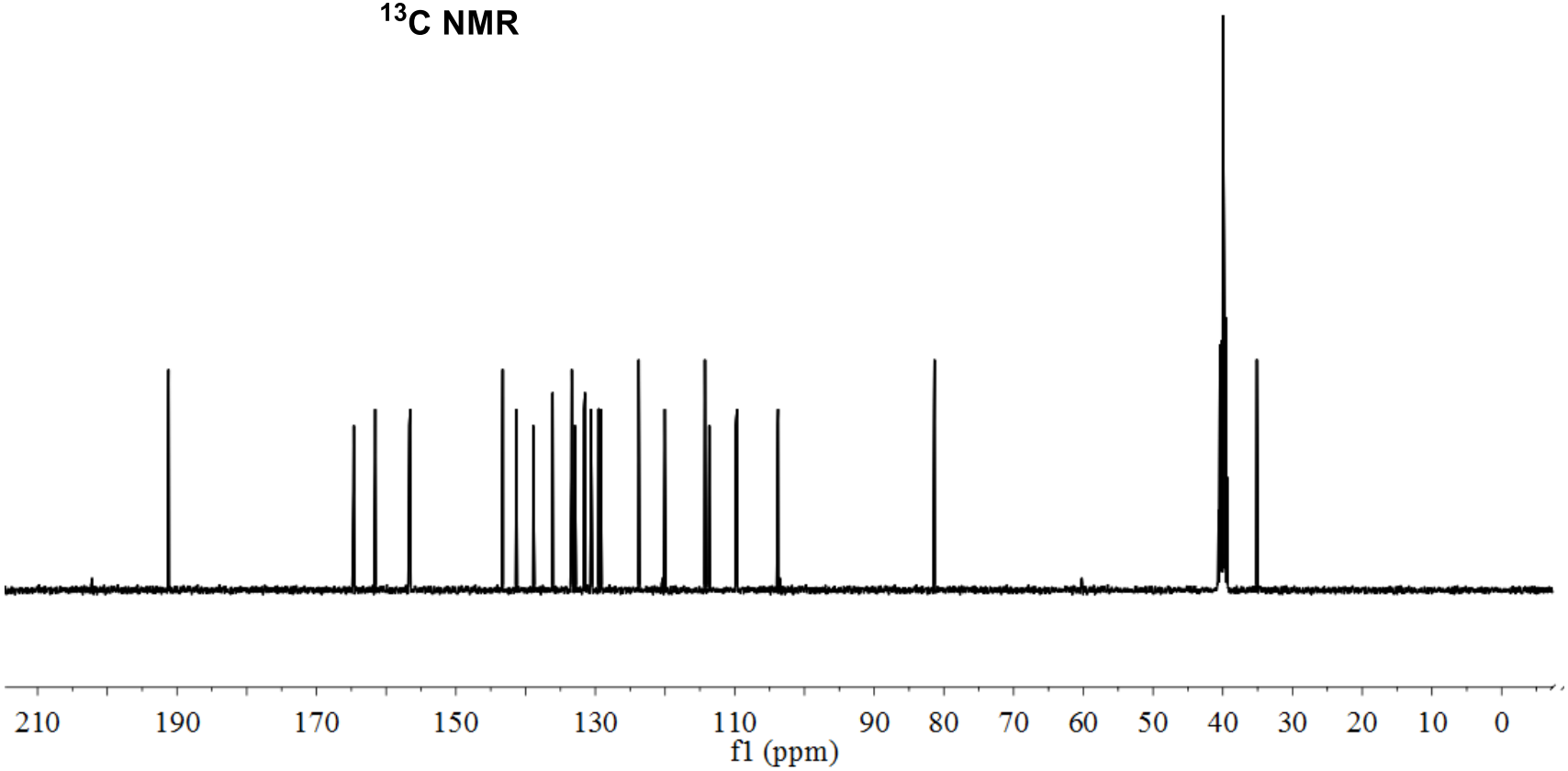


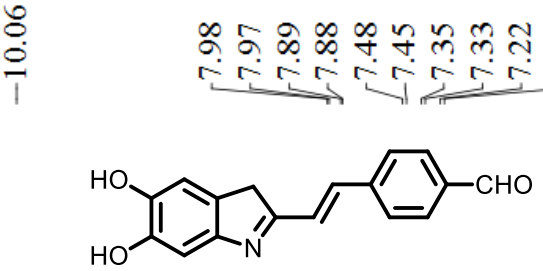


a1-BMN

(*E*)-2-(4-(2-(6-(aminooxy)-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{13}C NMR

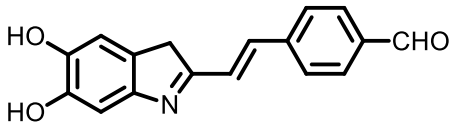




2a-2

(*E*)-4-(2-(5,6-dihydroxy-3*H*-indol-2-yl)vinyl)benzaldehyde

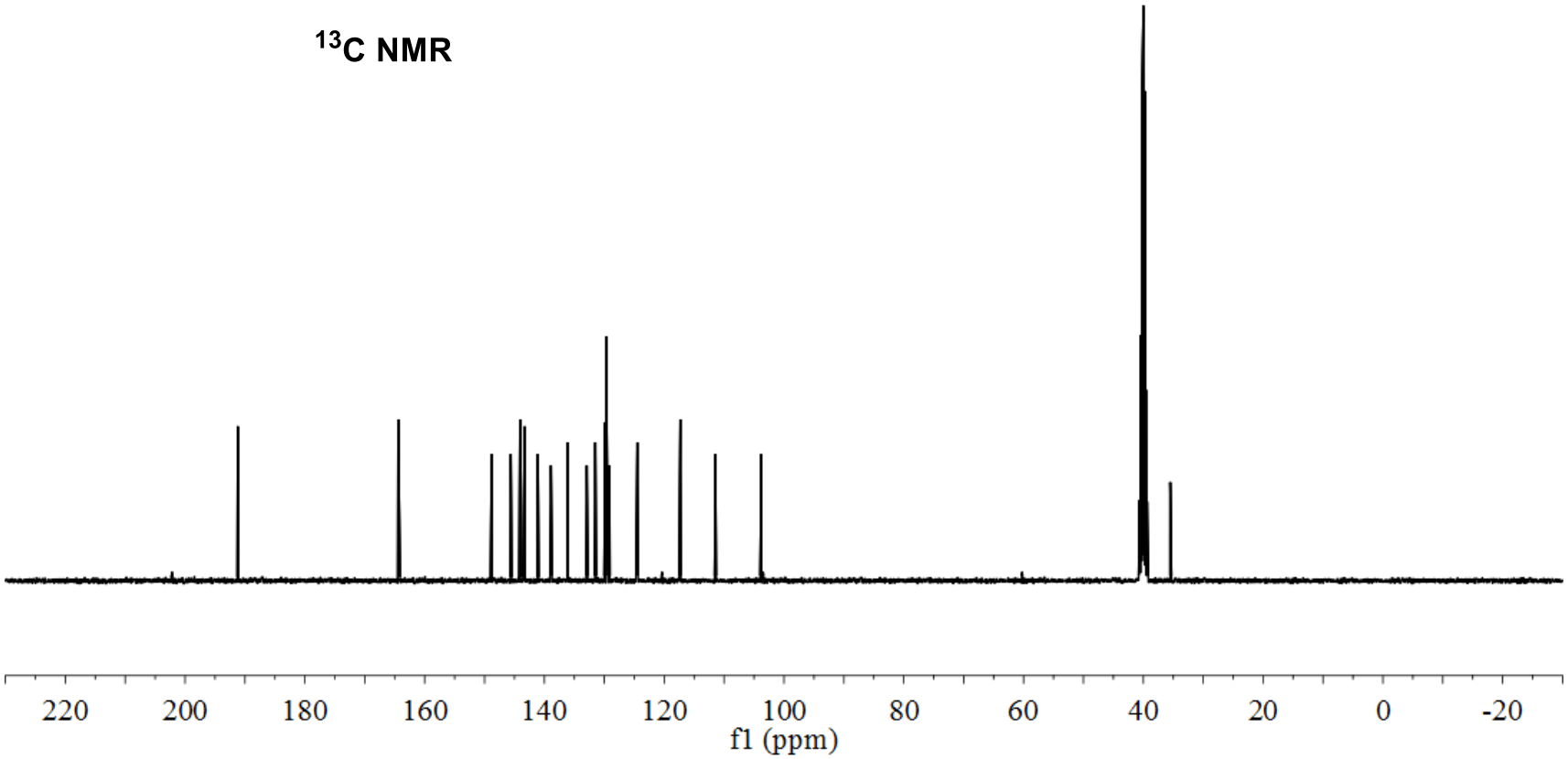
^{1}H NMR

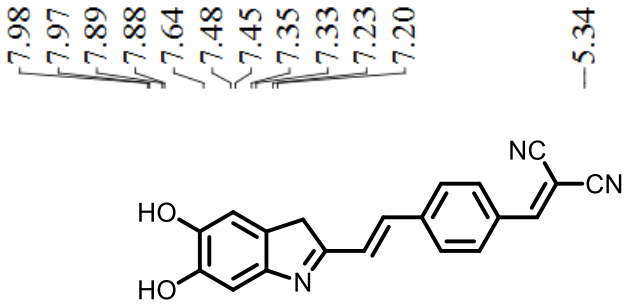


2a-2

(*E*)-4-(2-(5,6-dihydroxy-3*H*-indol-2-yl)vinyl)benzaldehyde

^{13}C NMR

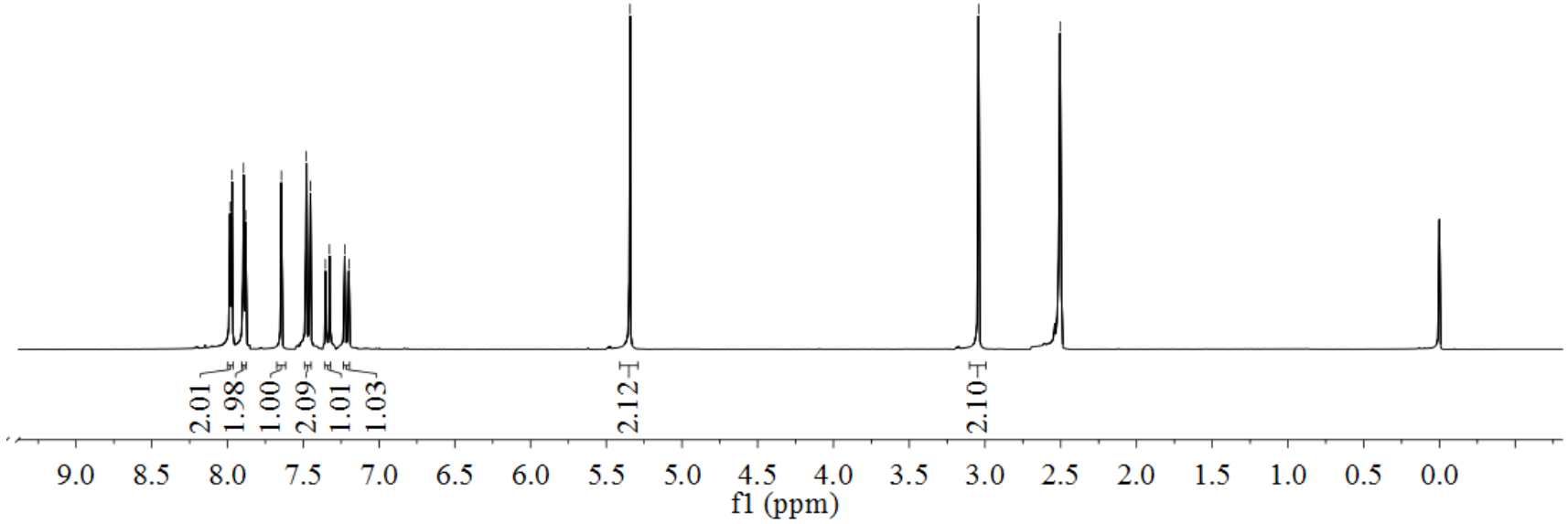


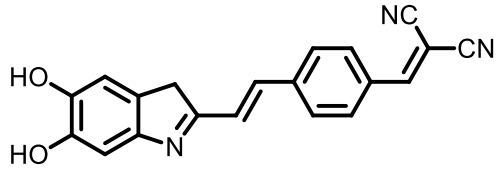


3a-2

(*E*)-2-(4-(2-(5,6-dihydroxy-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{1}H NMR

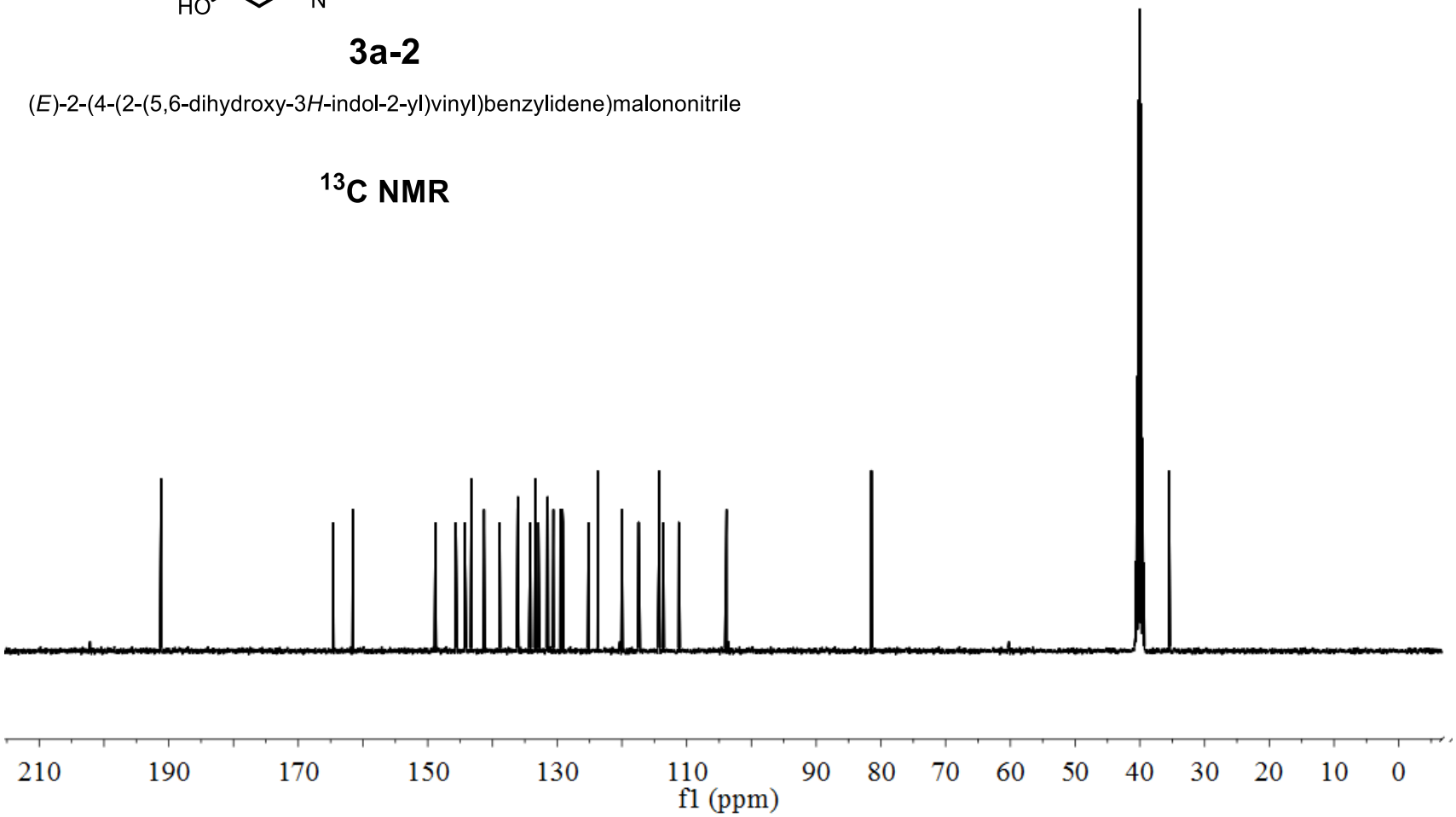


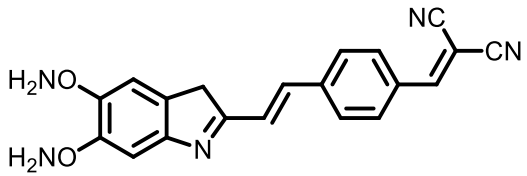


3a-2

(*E*)-2-(4-(2-(5,6-dihydroxy-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{13}C NMR

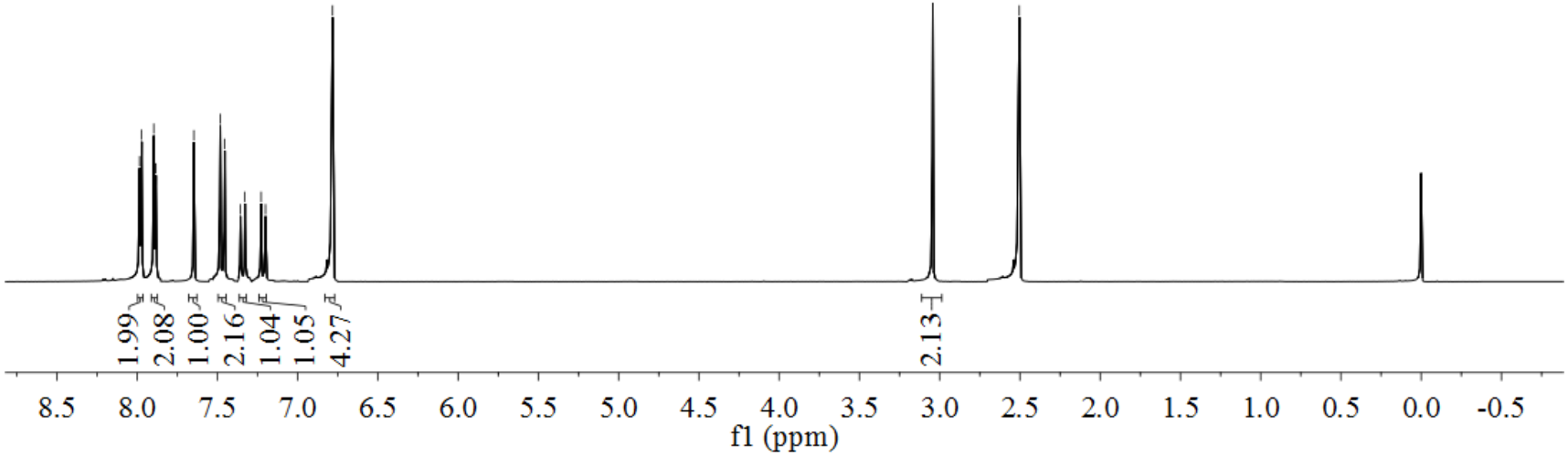


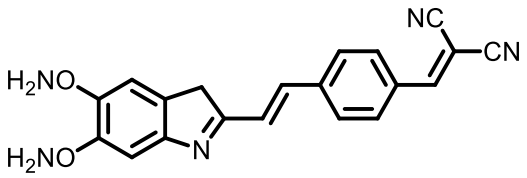


a2-BMN

(*E*)-2-(4-(2-(5,6-bis(aminooxy)-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{1}H NMR

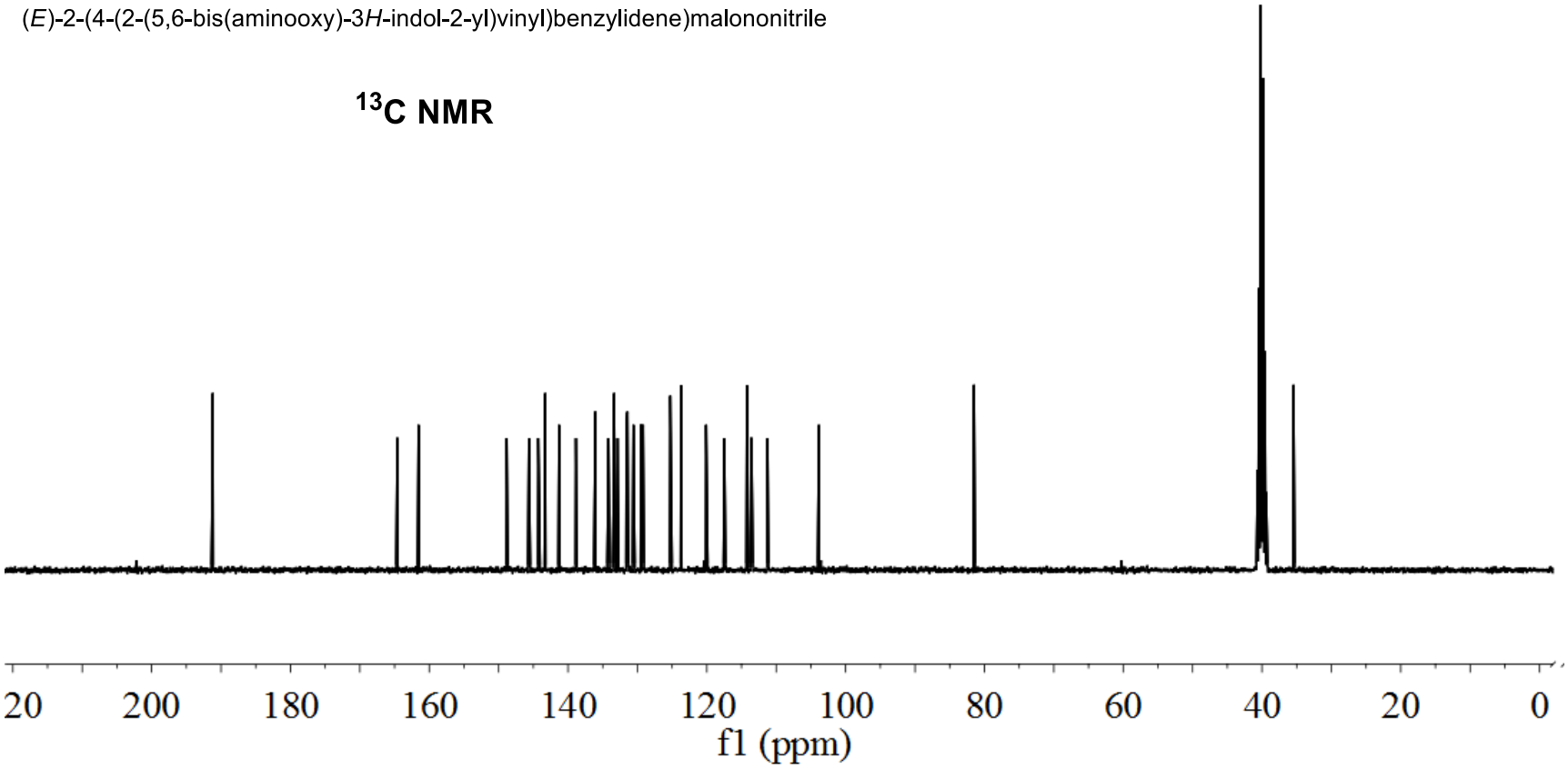


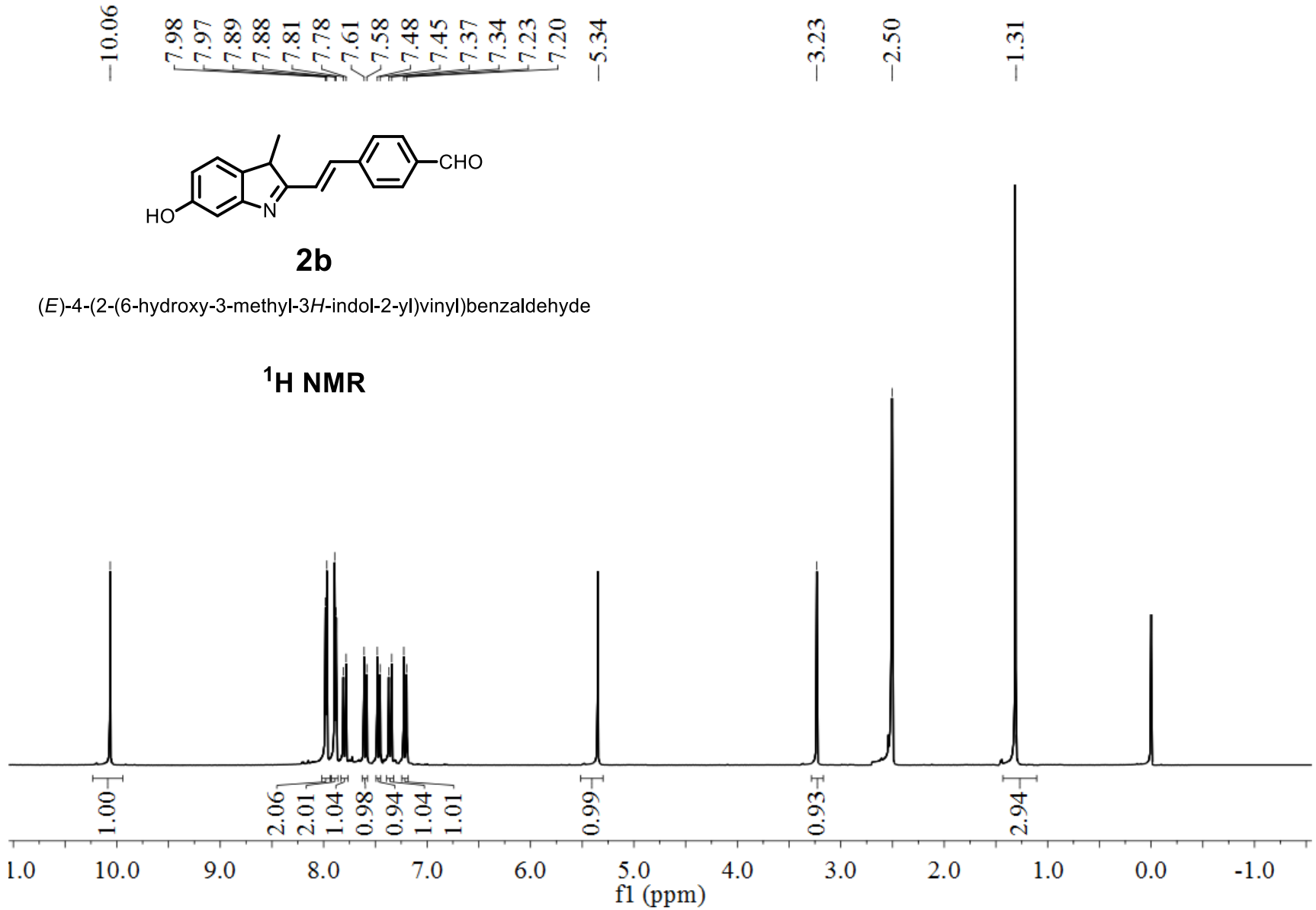


a2-BMN

(*E*)-2-(4-(2-(5,6-bis(aminooxy)-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{13}C NMR

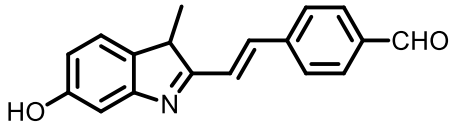




2b

(*E*)-4-(2-(6-hydroxy-3-methyl-3*H*-indol-2-yl)vinyl)benzaldehyde

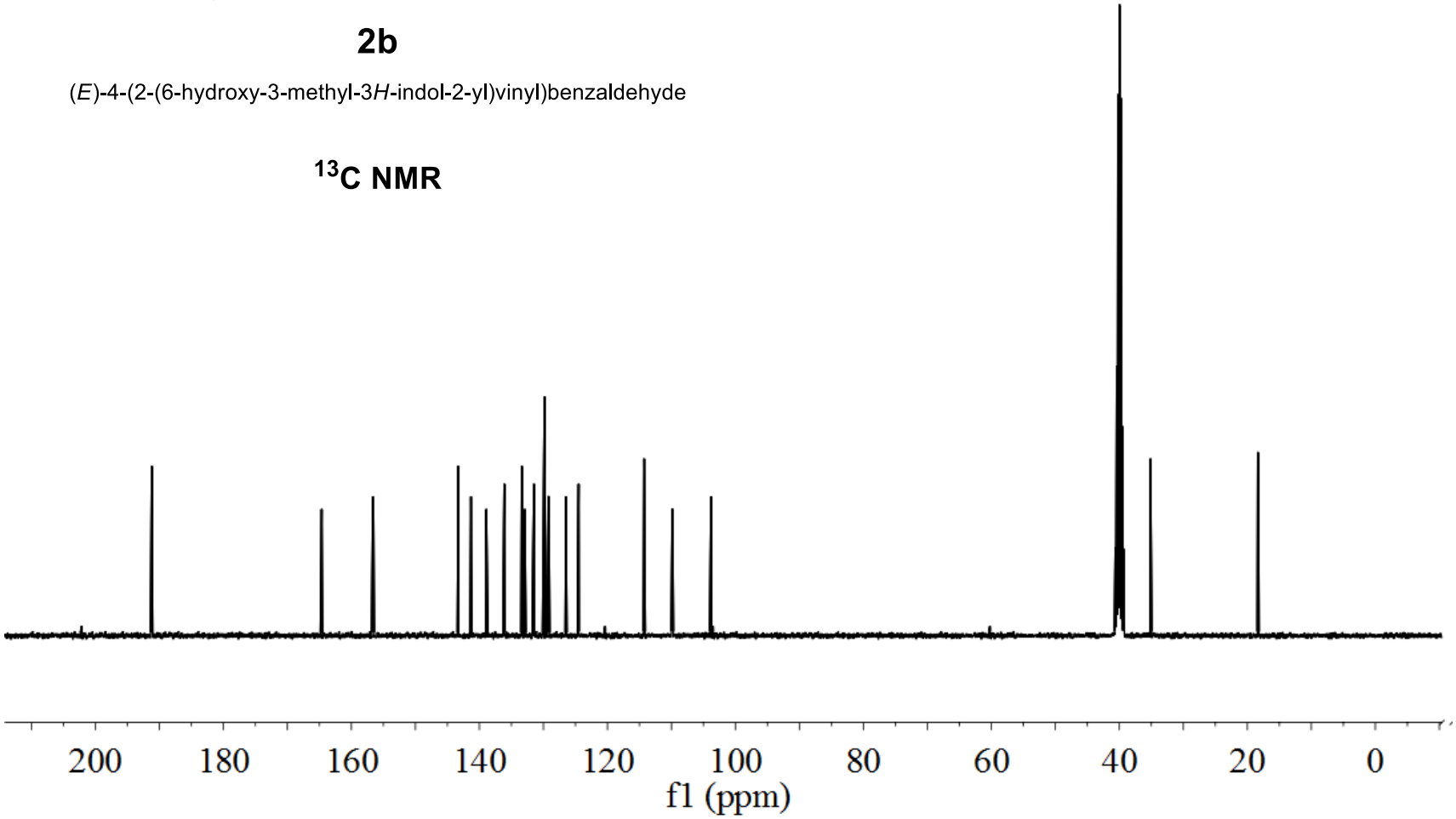
^{1}H NMR

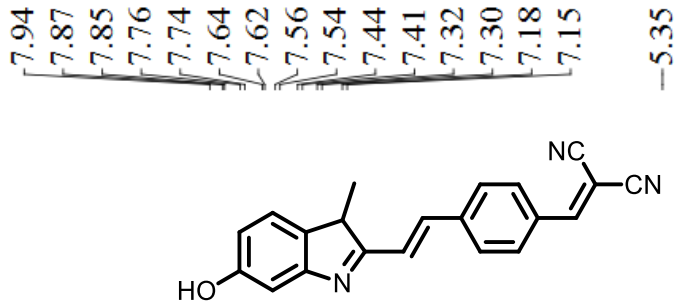


2b

(*E*)-4-(2-(6-hydroxy-3-methyl-3*H*-indol-2-yl)vinyl)benzaldehyde

^{13}C NMR

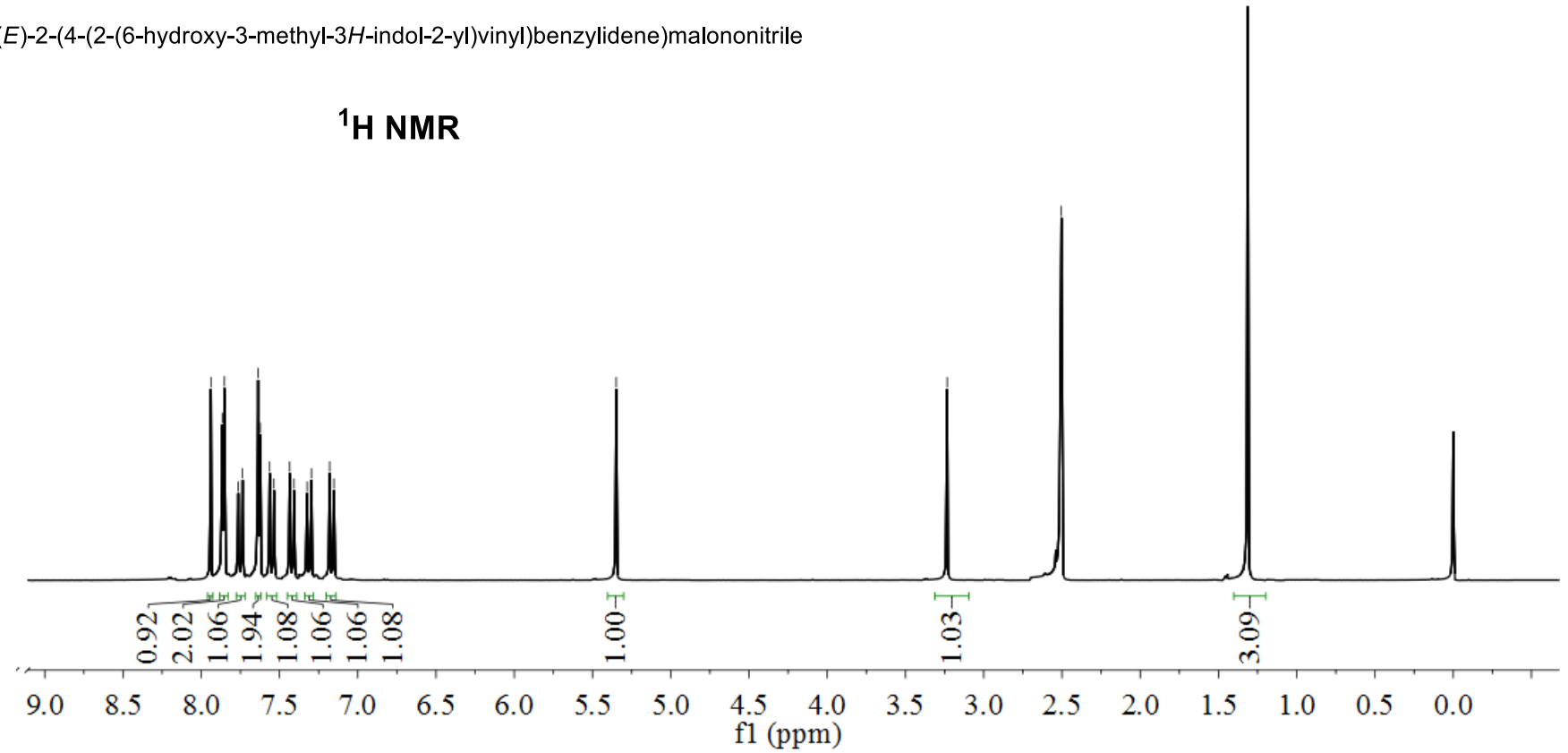


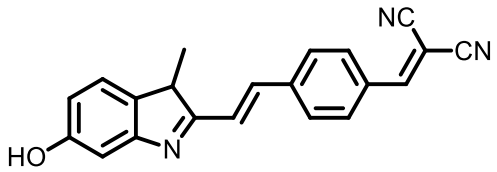


3b

(*E*)-2-(4-(2-(6-hydroxy-3-methyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{1}H NMR

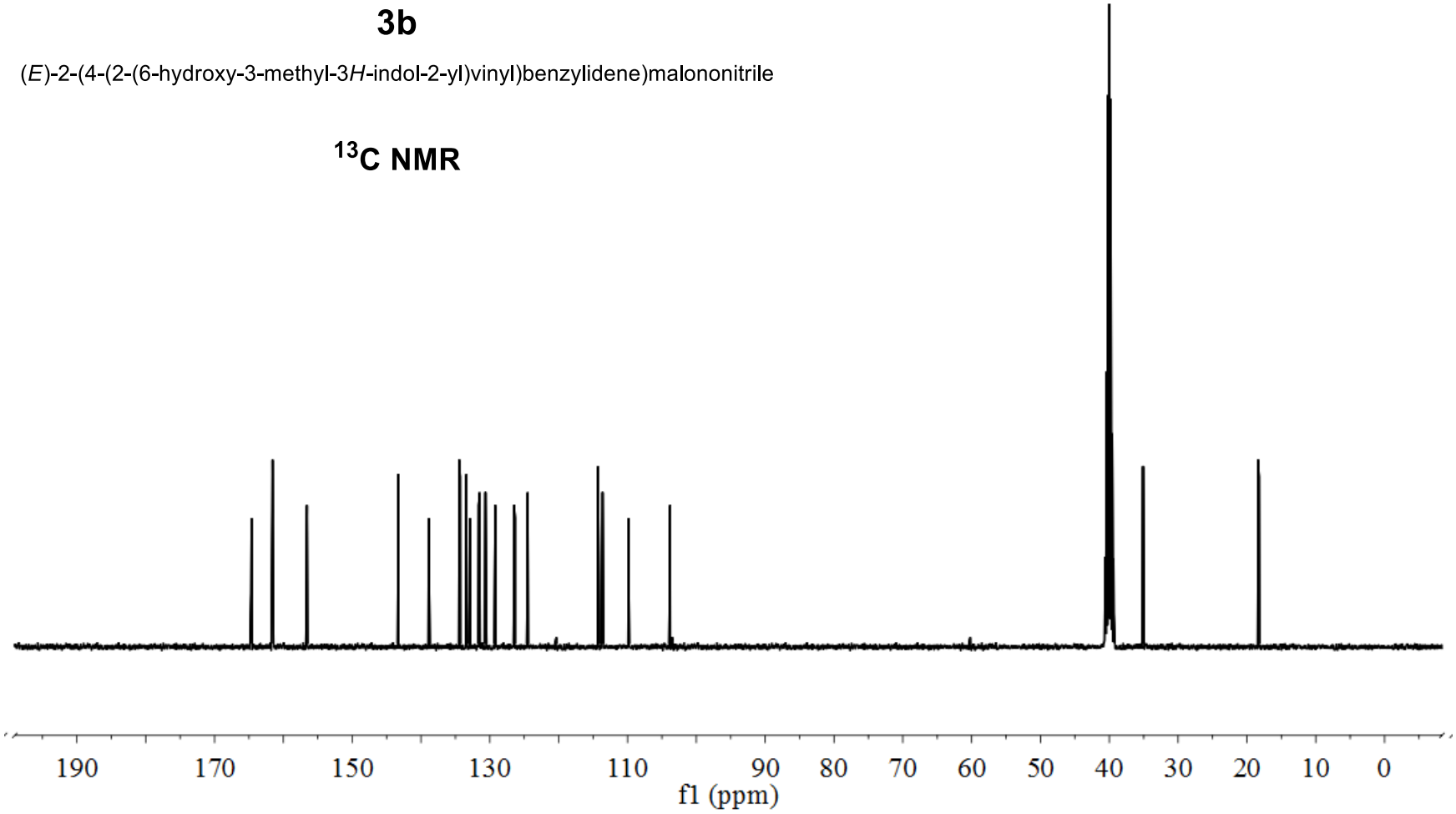


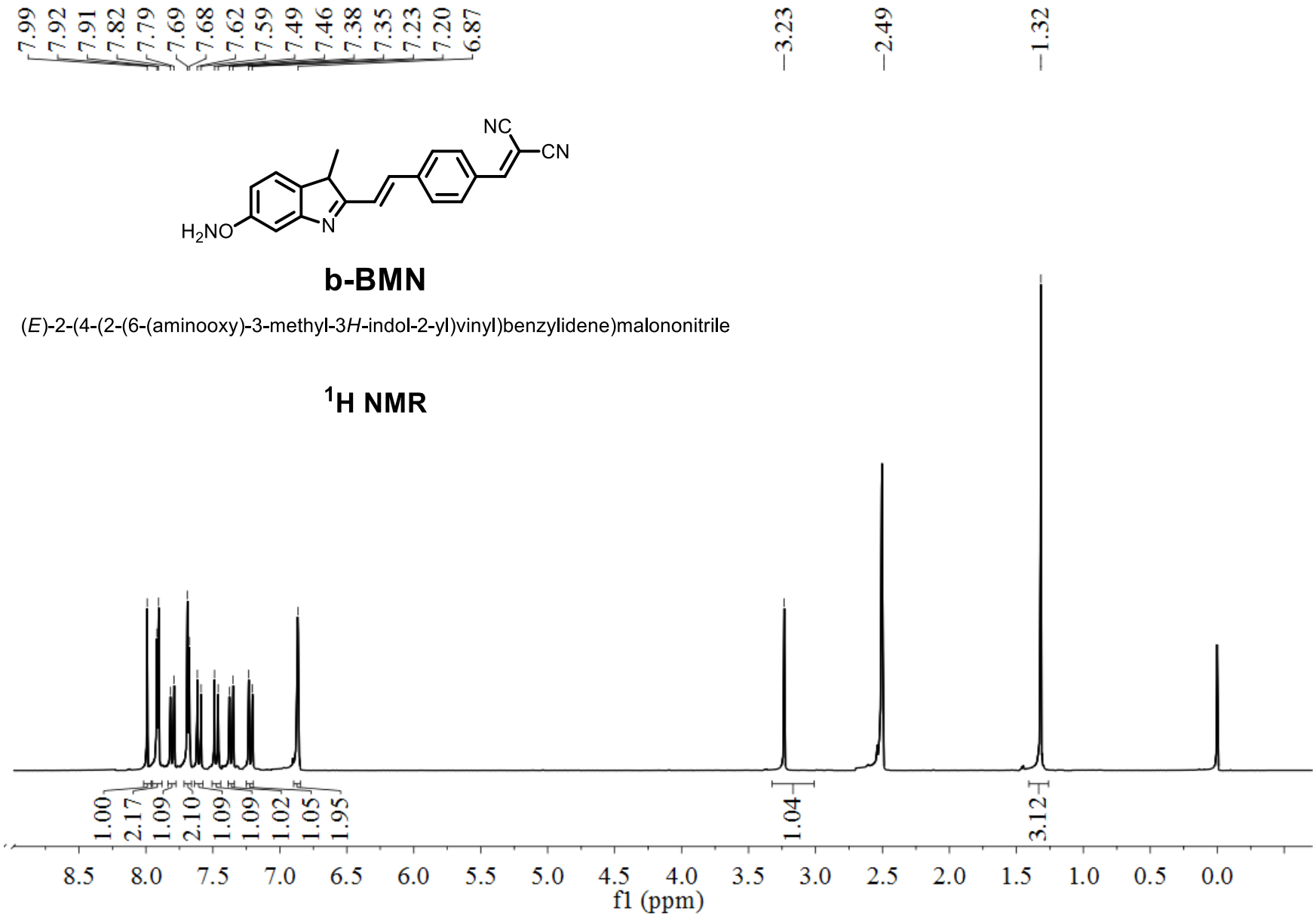


3b

(*E*)-2-(4-(2-(6-hydroxy-3-methyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{13}C NMR

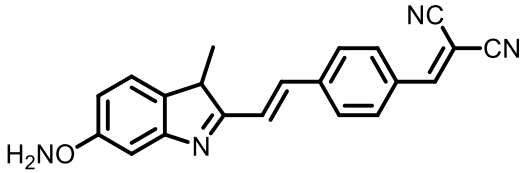




b-BMN

(*E*)-2-(4-(2-(6-(aminooxy)-3-methyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

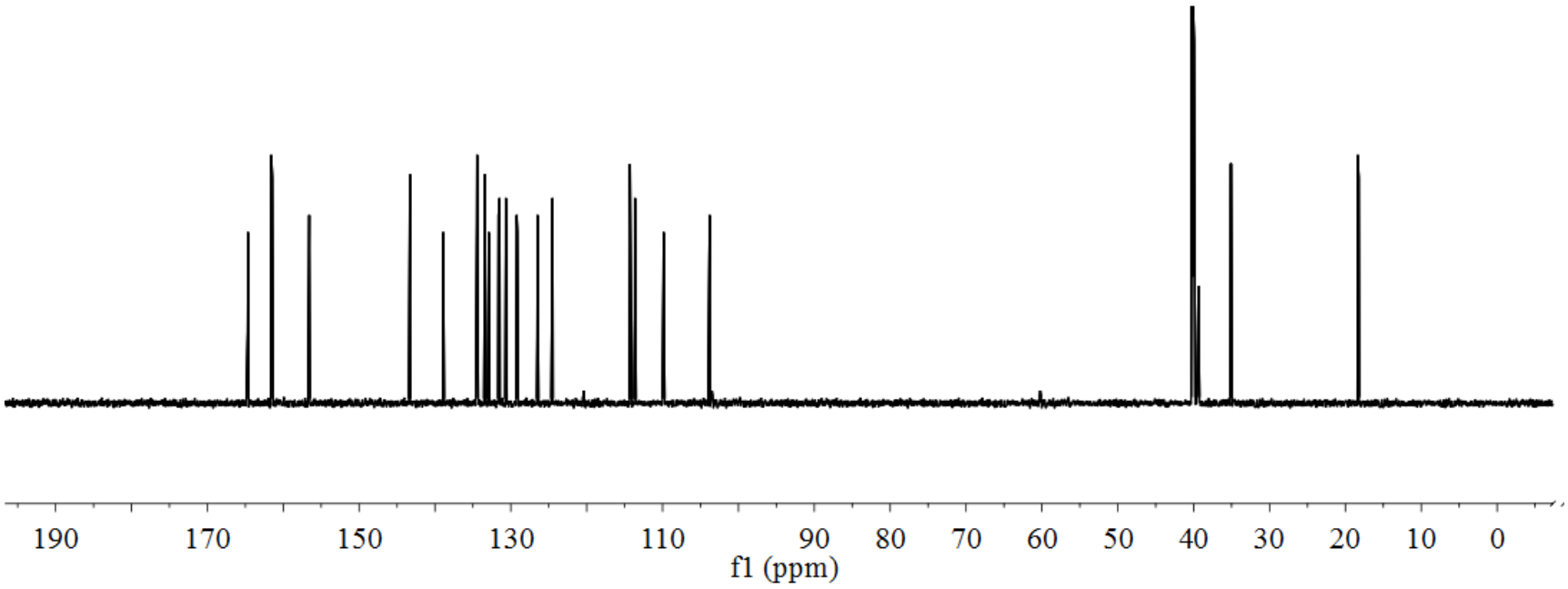
^{1}H NMR

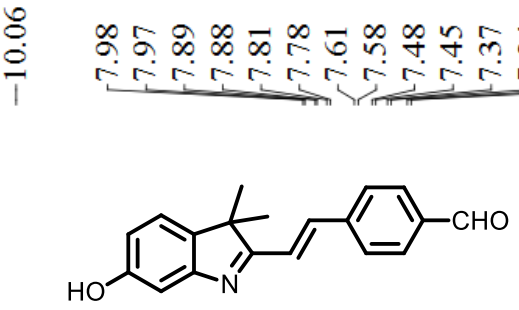


b-BMN

(*E*)-2-(4-(2-(6-(aminooxy)-3-methyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{13}C NMR

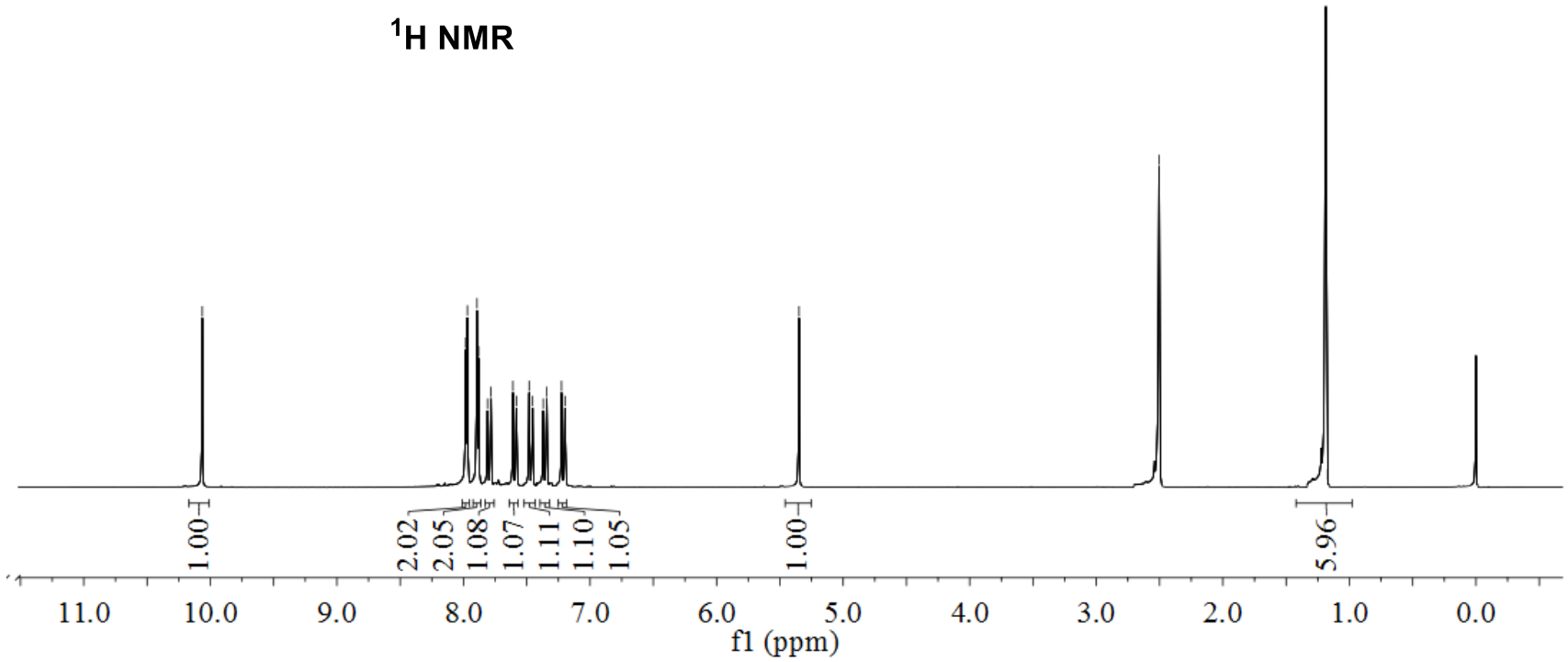


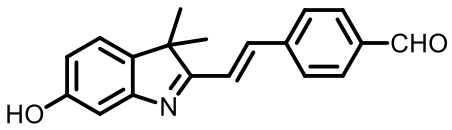


2c

(*E*)-4-(2-(6-hydroxy-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzaldehyde

^{1}H NMR

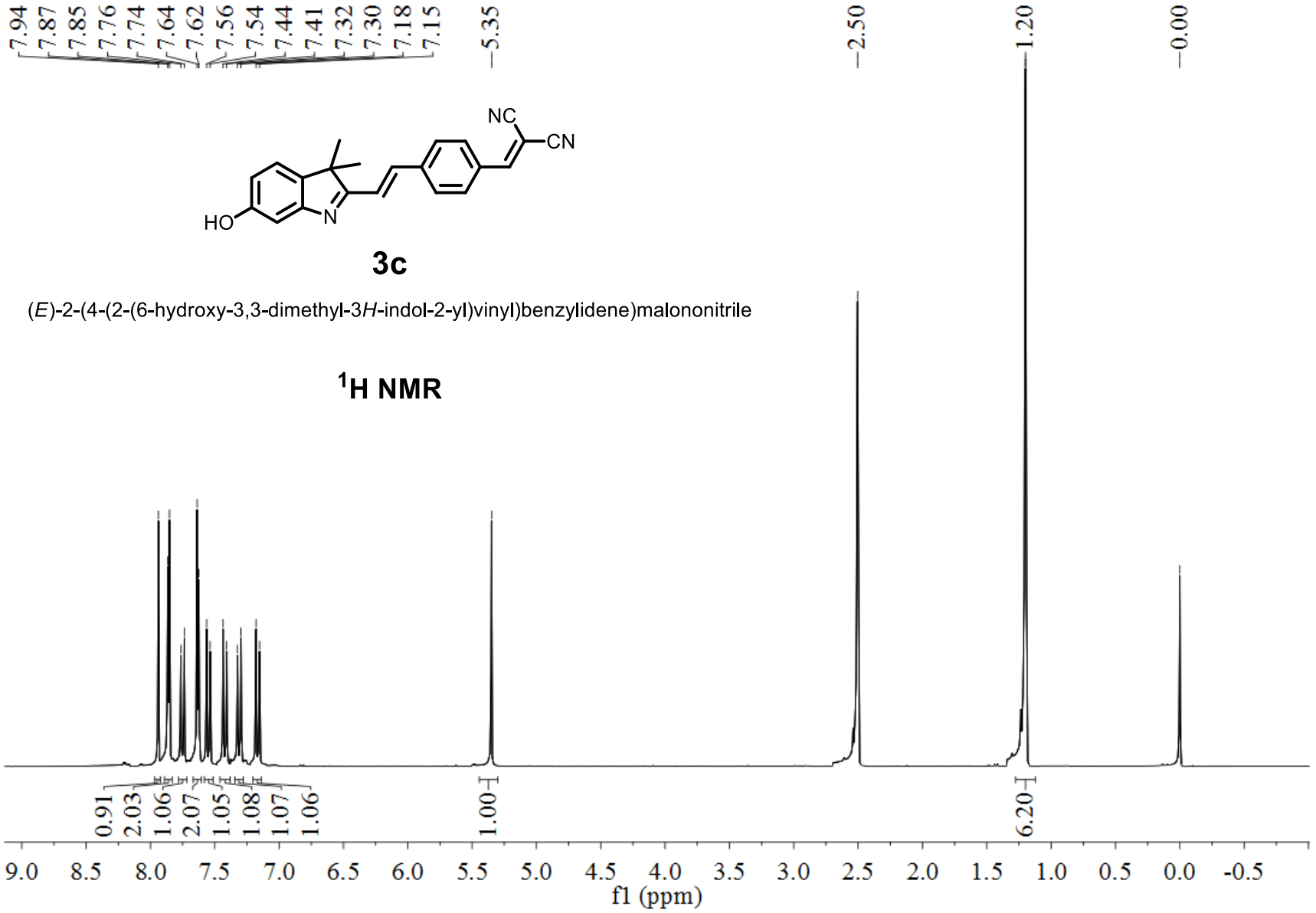




2c

(*E*)-4-(2-(6-hydroxy-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzaldehyde

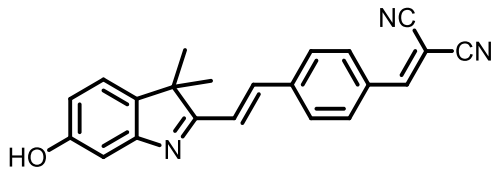
^{13}C NMR



3c

(*E*)-2-(4-(2-(6-hydroxy-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

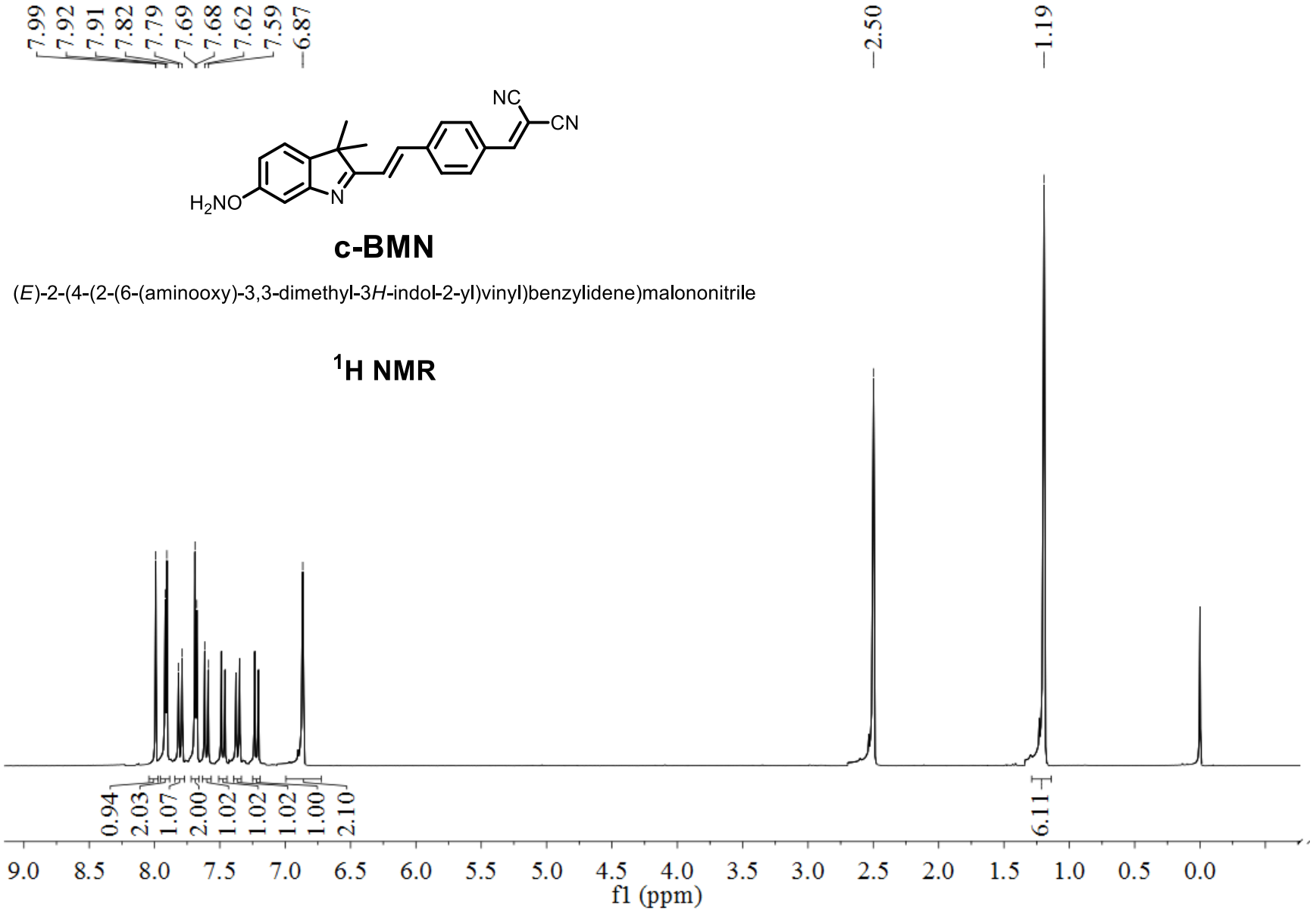
^{1}H NMR



3c

(*E*)-2-(4-(2-(6-hydroxy-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

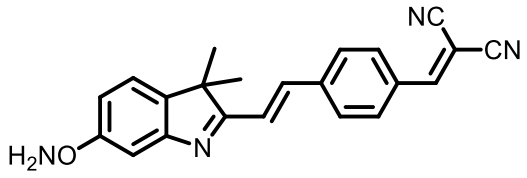
^{13}C NMR



c-BMN

(*E*)-2-(4-(2-(6-(aminooxy)-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

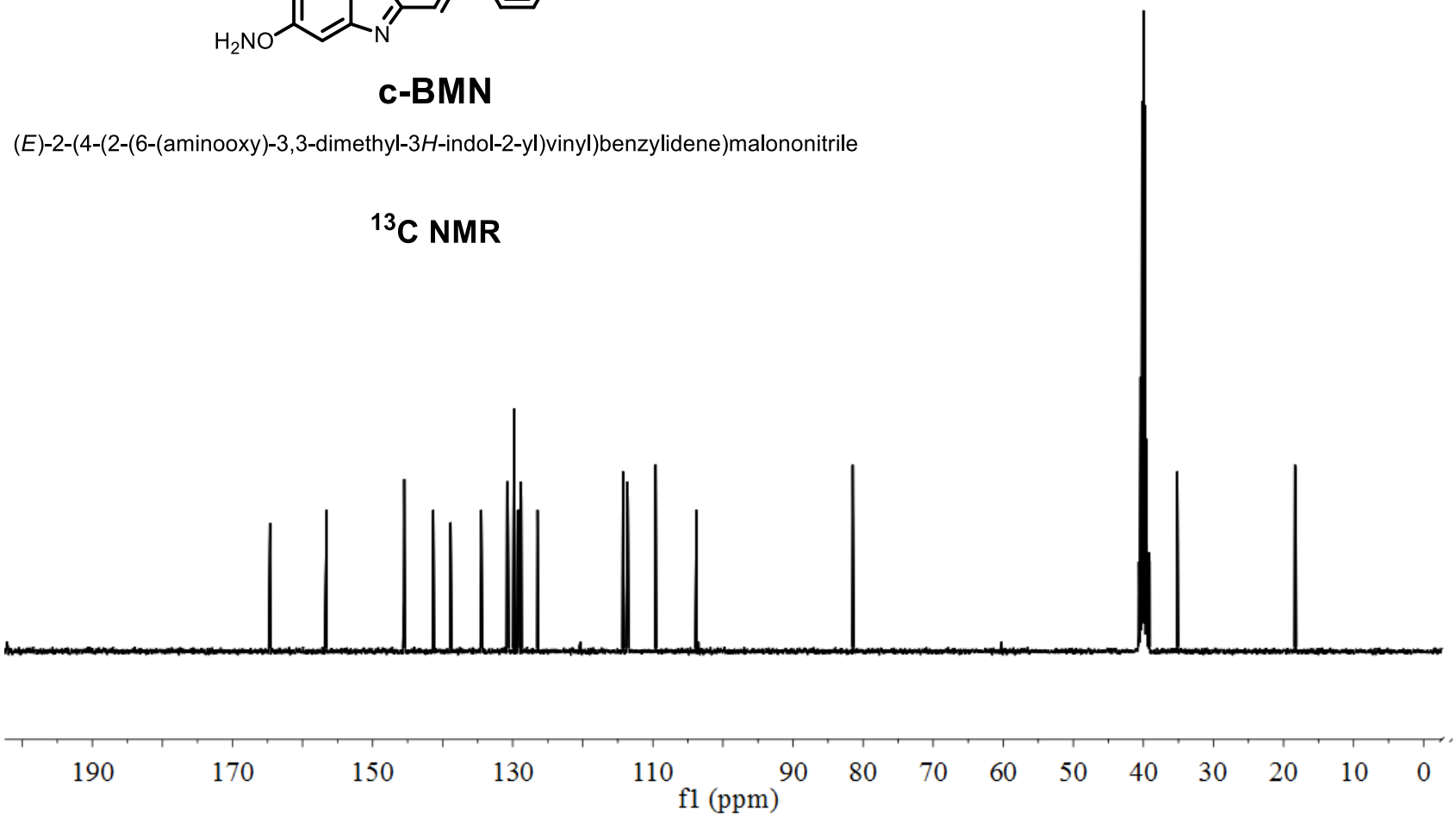
^{1}H NMR

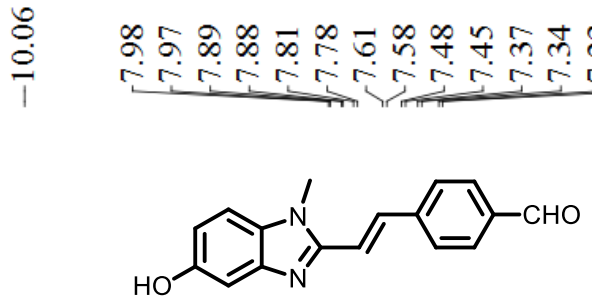


c-BMN

(*E*)-2-(4-(2-(6-(aminooxy)-3,3-dimethyl-3*H*-indol-2-yl)vinyl)benzylidene)malononitrile

^{13}C NMR

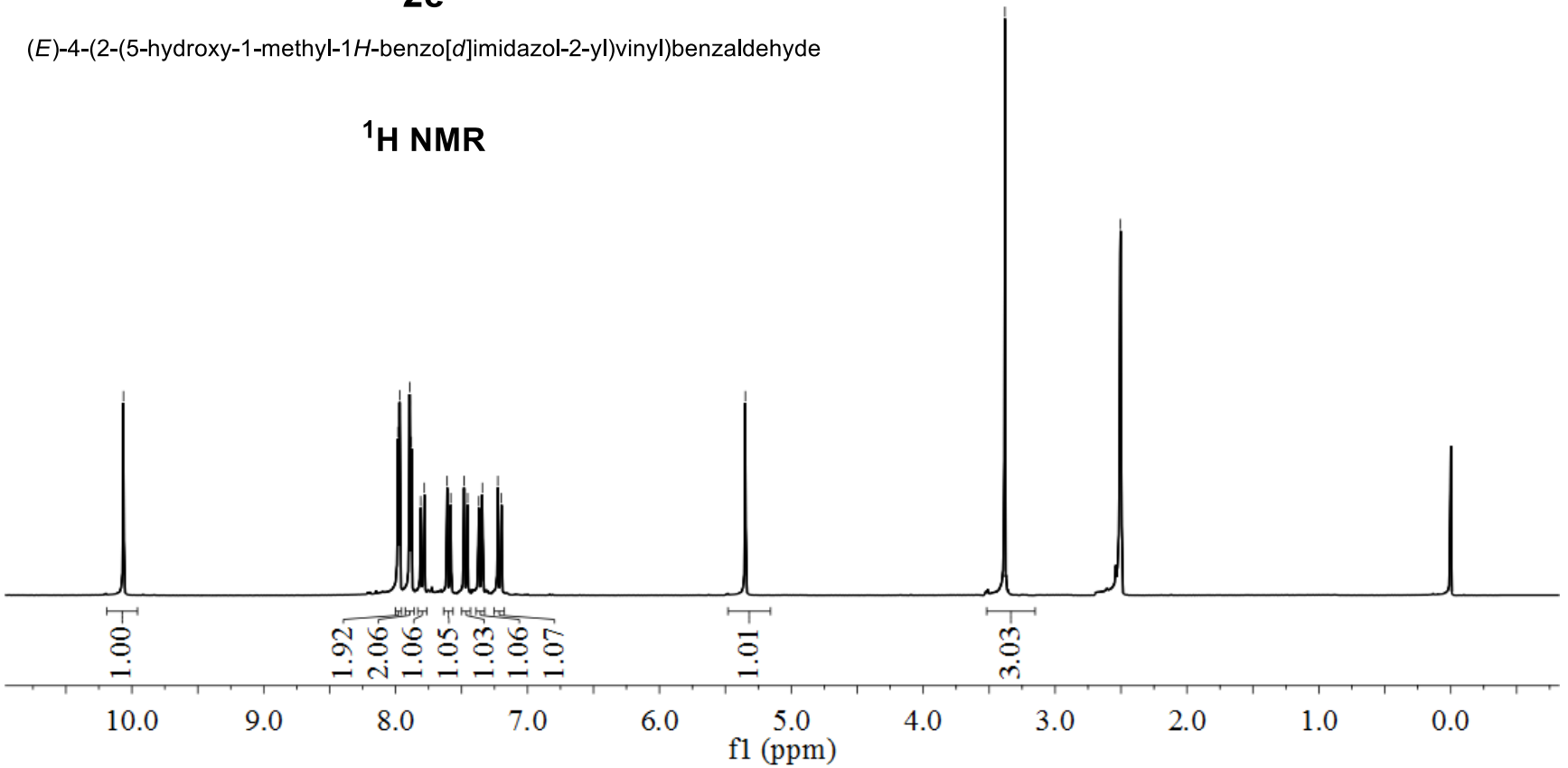


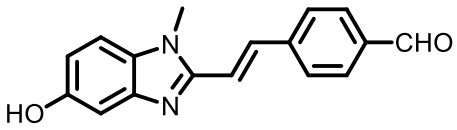


2e

(*E*)-4-(2-(5-hydroxy-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzaldehyde

^{1}H NMR

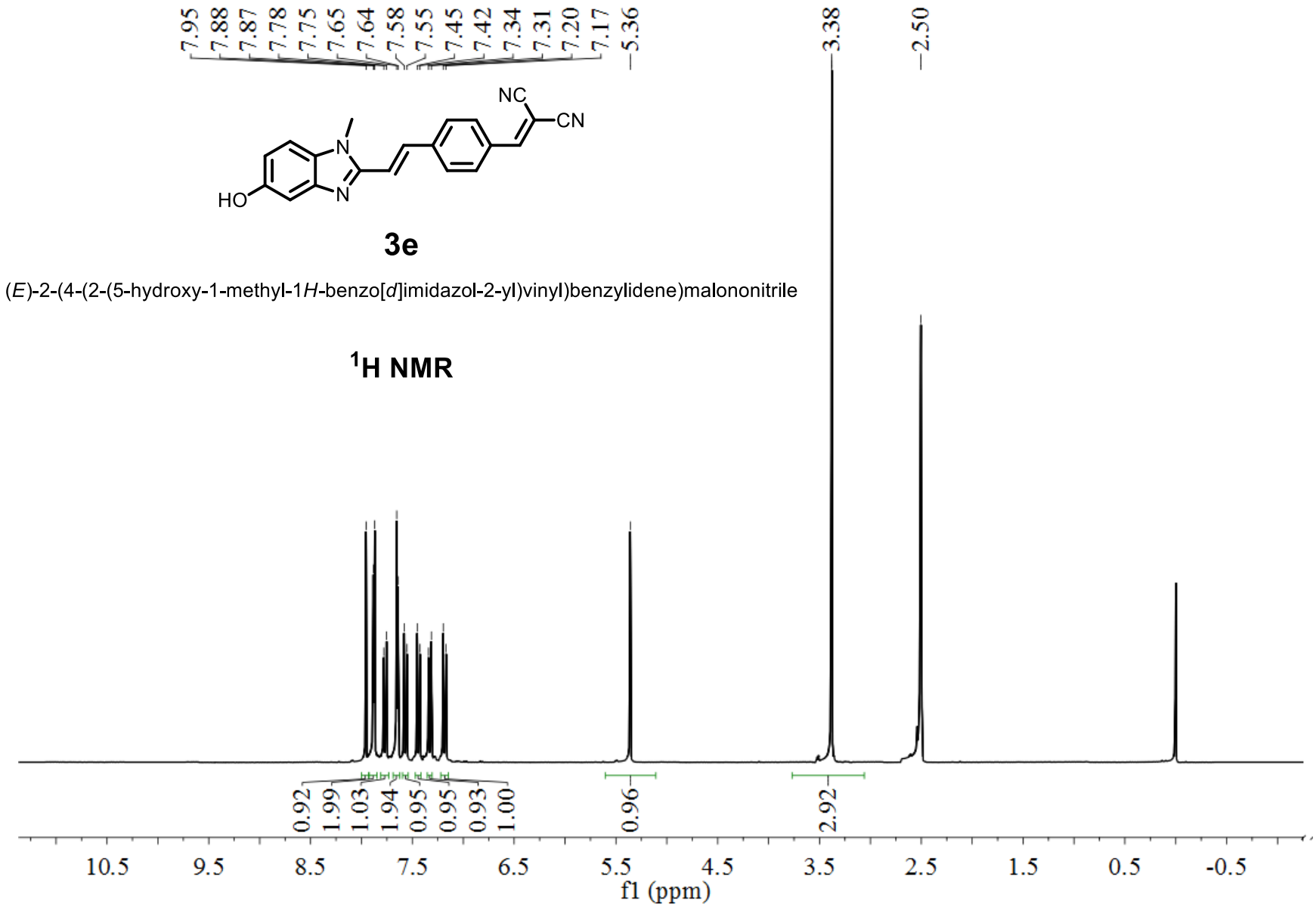




2e

(*E*)-4-(2-(5-hydroxy-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzaldehyde

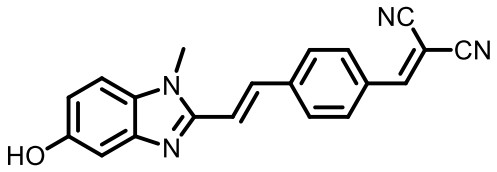
^{13}C NMR



3e

(*E*)-2-(4-(2-(5-hydroxy-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

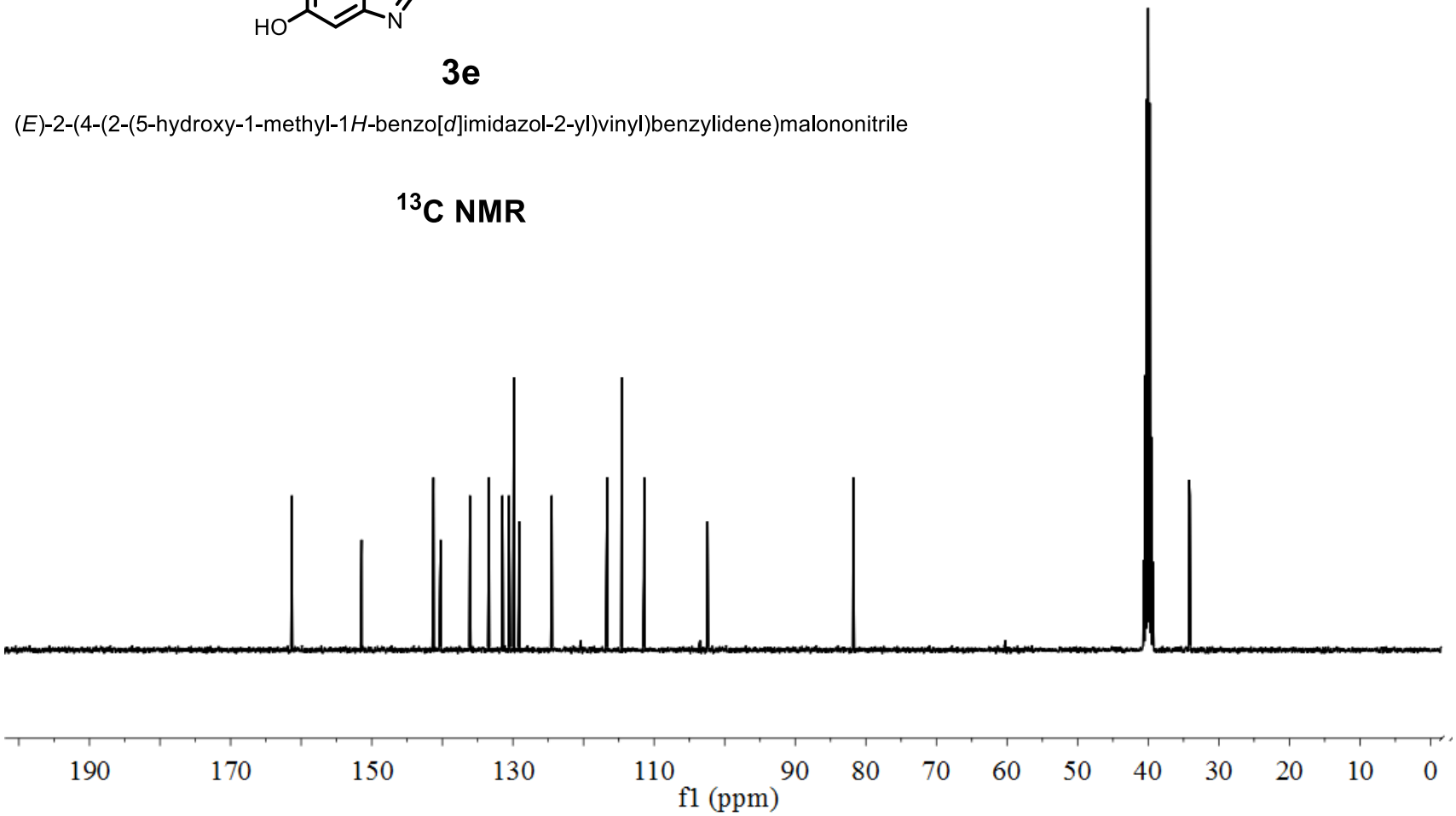
^{1}H NMR

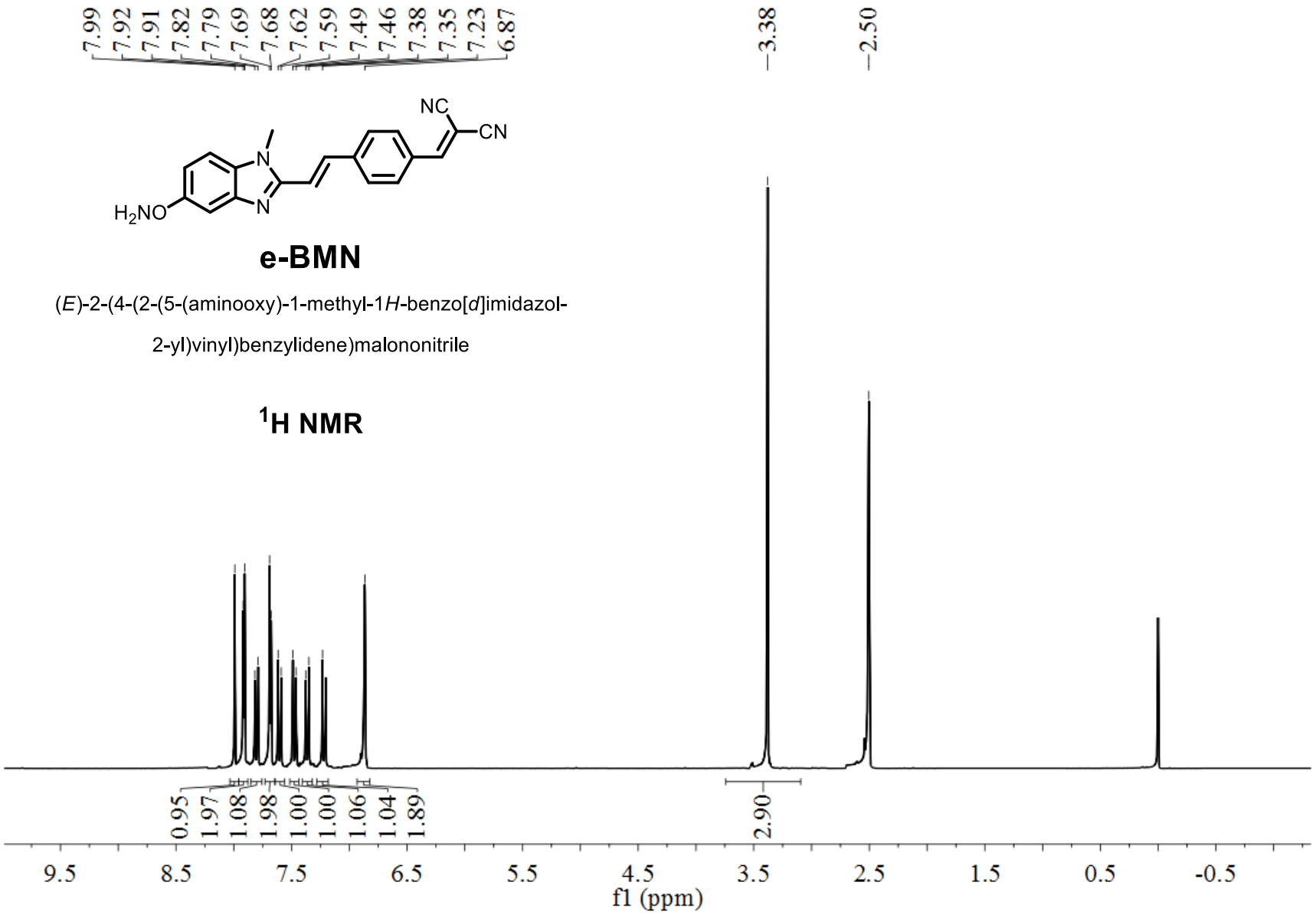


3e

(*E*)-2-(4-(2-(5-hydroxy-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

^{13}C NMR

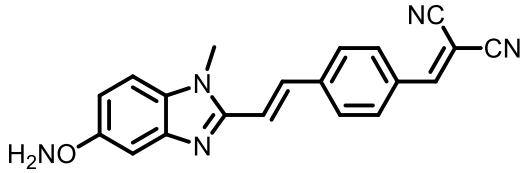




e-BMN

(*E*)-2-(4-(2-(5-(aminooxy)-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

^{1}H NMR

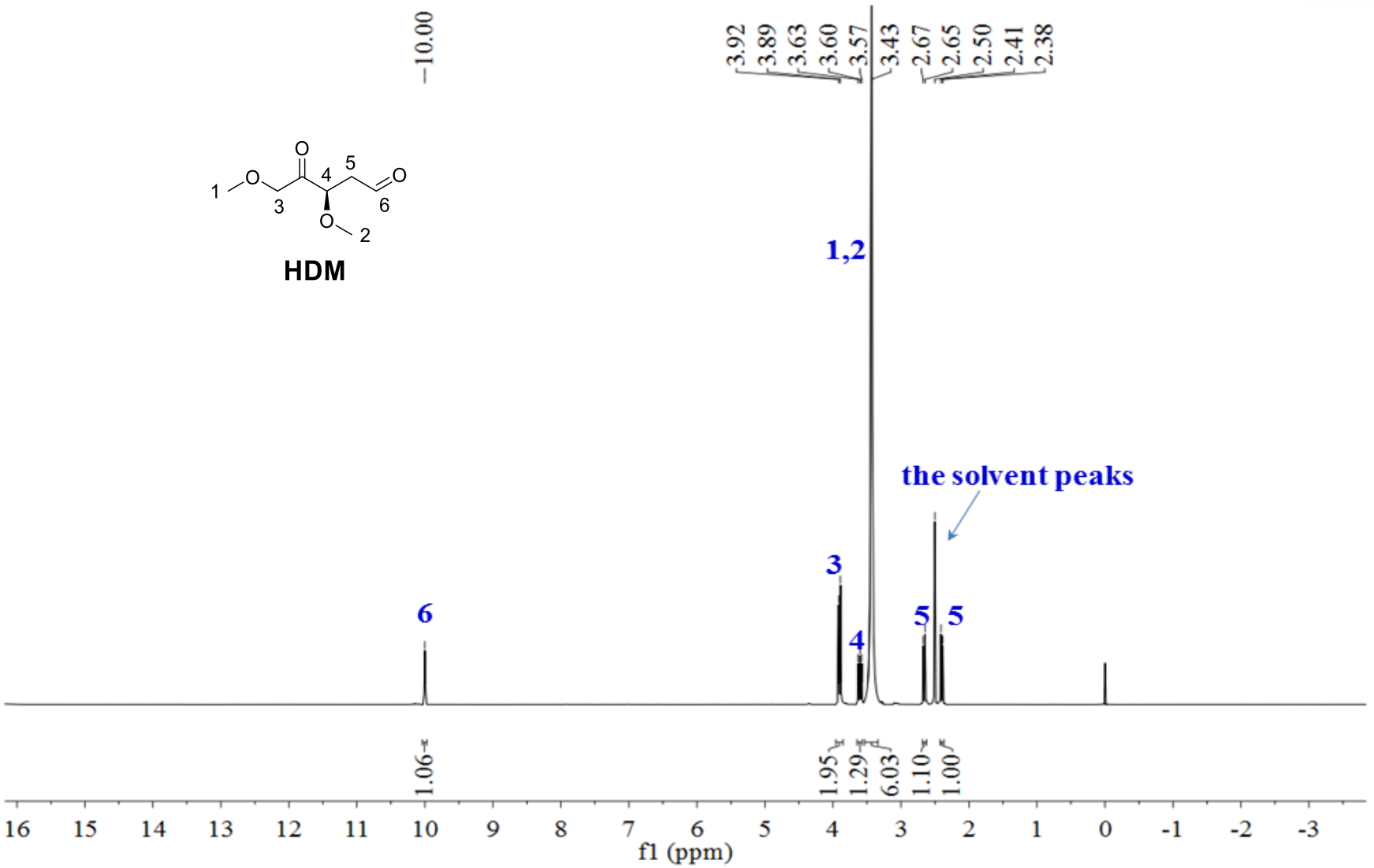


e-BMN

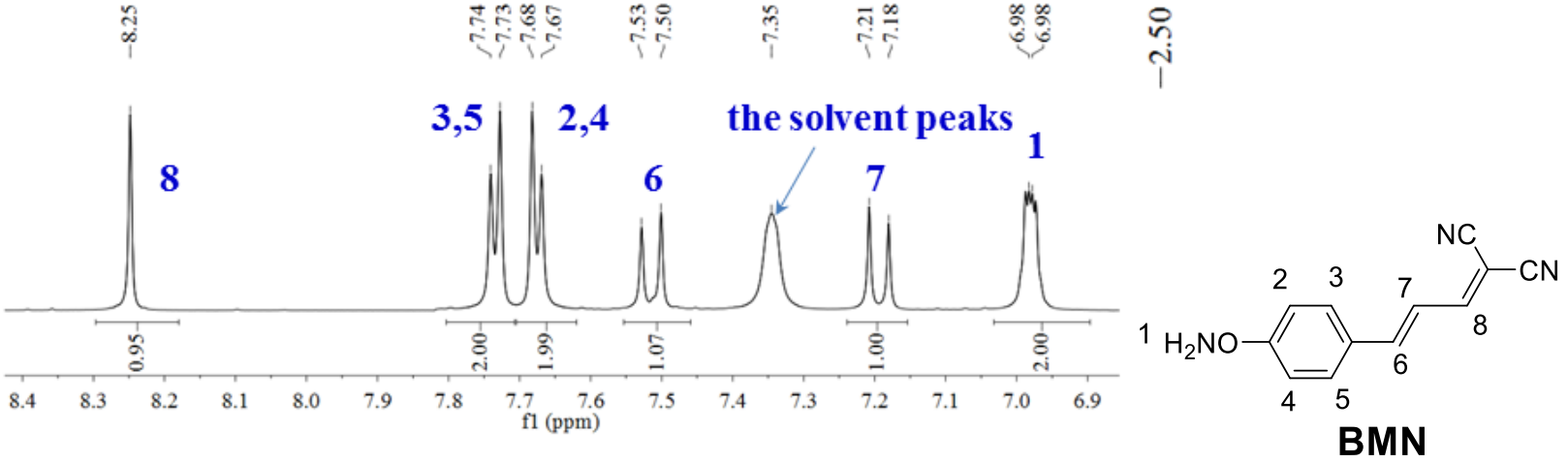
(*E*)-2-(4-(2-(5-(aminooxy)-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)benzylidene)malononitrile

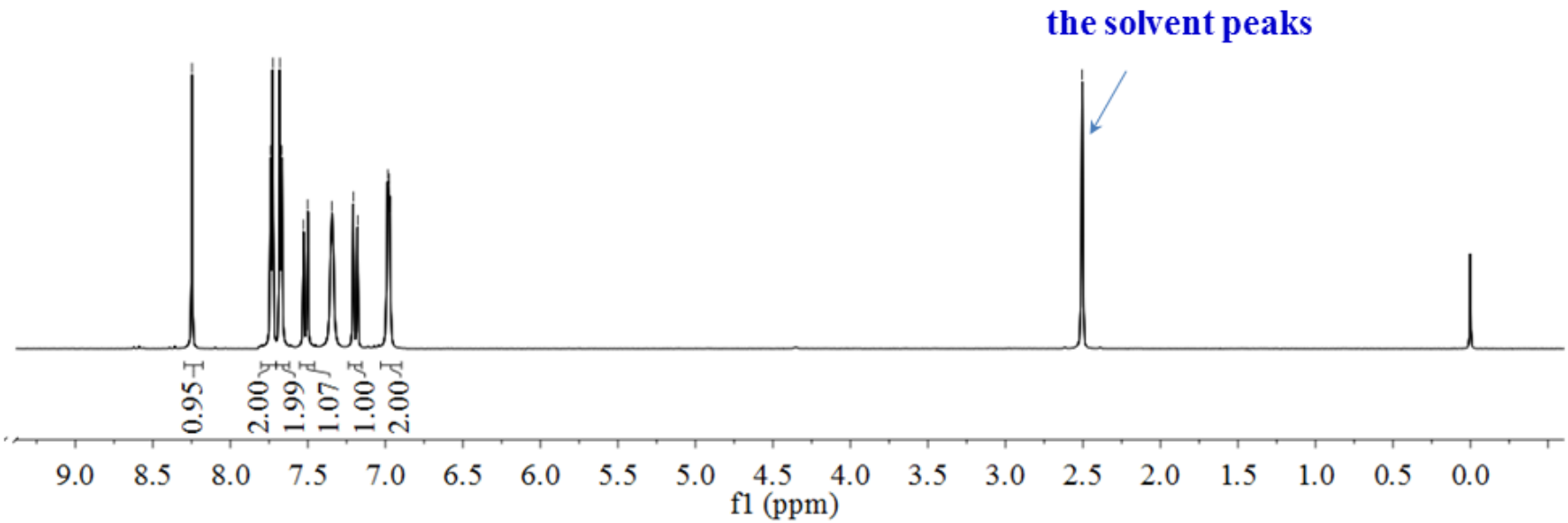
^{13}C NMR

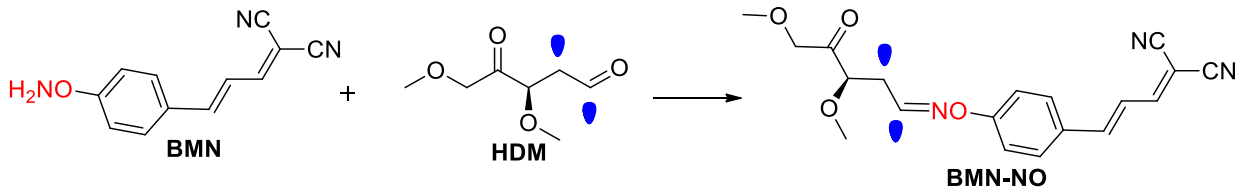
The raw data in ^{1}H NMR titration

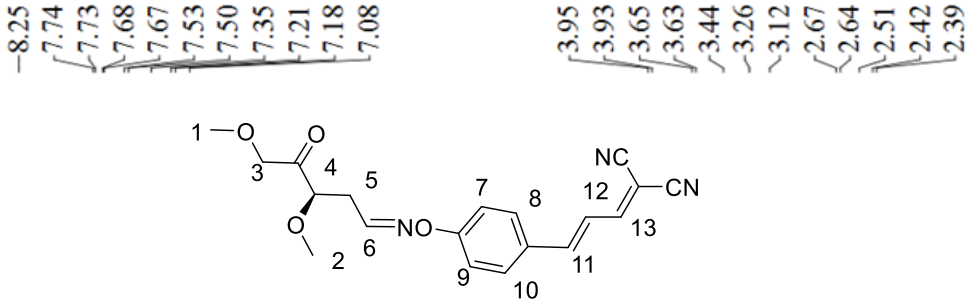


HDM


8.25
7.74
7.73
7.68
7.67
7.53
7.50
7.35
7.21
7.18
6.98
6.98
2.50
3,5
2,4
the solvent peaks
1
8
6
7
0.95
2.00
1.99
1.07
1.00
2.00
8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9
f1 (ppm)
NC
CN
2 3
7
8
1 H2NO
6
4 5
BMN

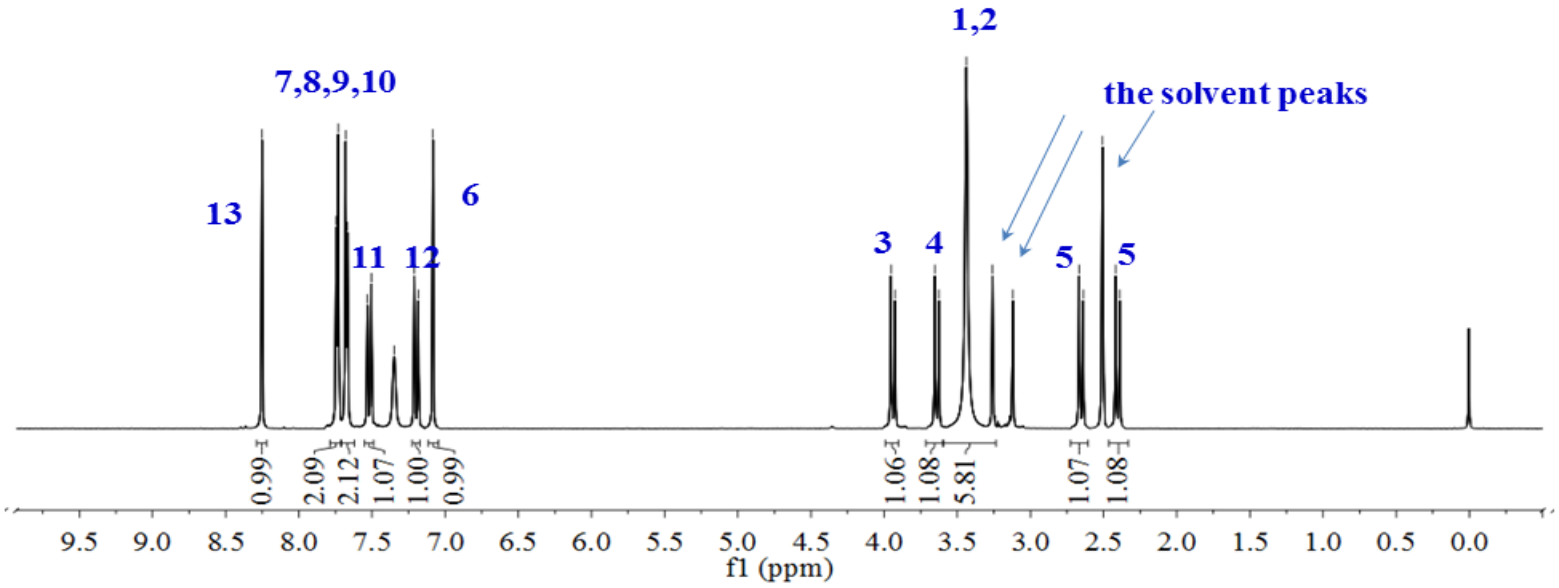

the solvent peaks
0.95
2.00
1.99
1.07
1.00
2.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

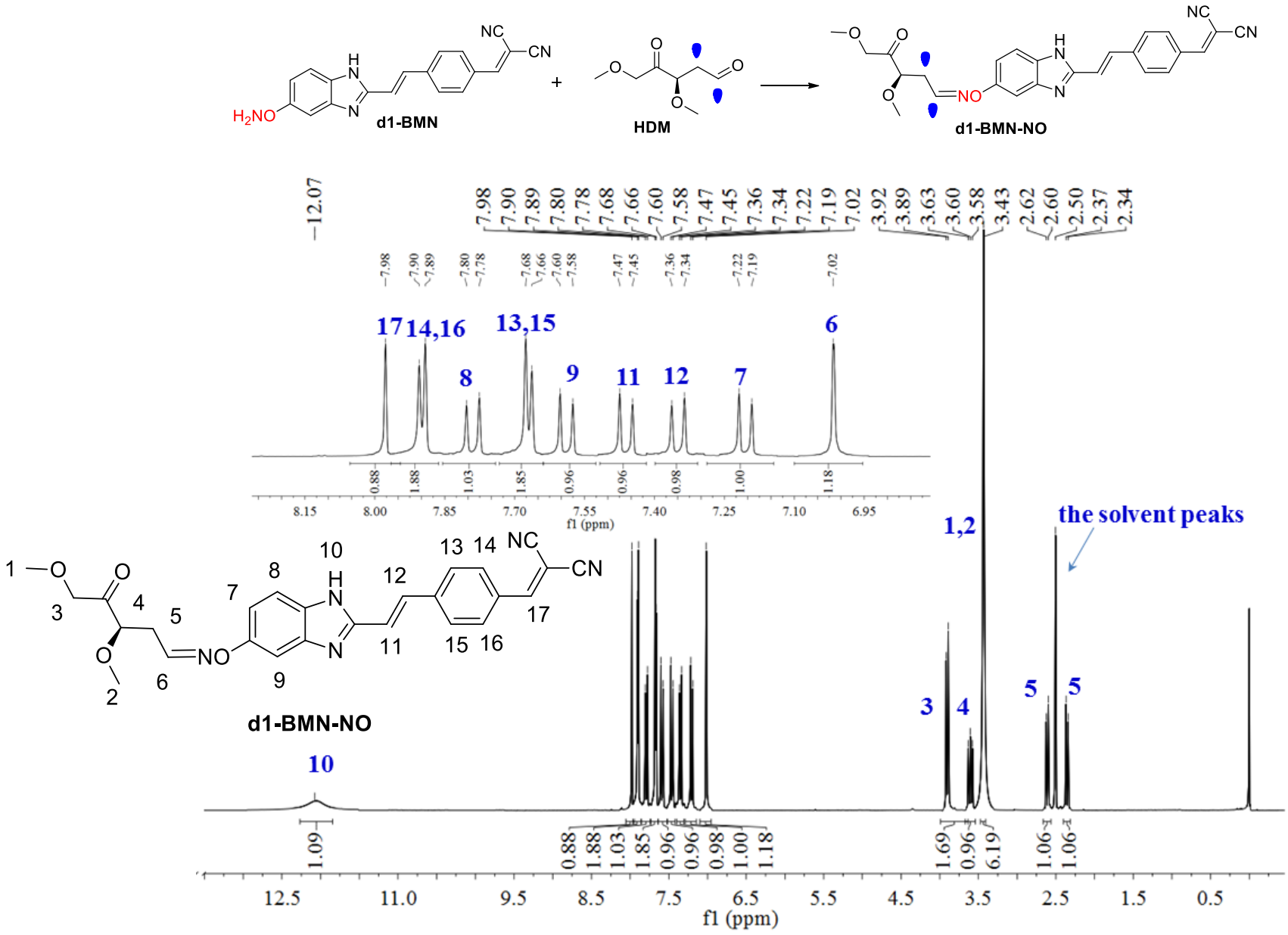

NC
CN
H_2NO
BMN
+
O
O
O
O
HDM
NO
BMN-NO

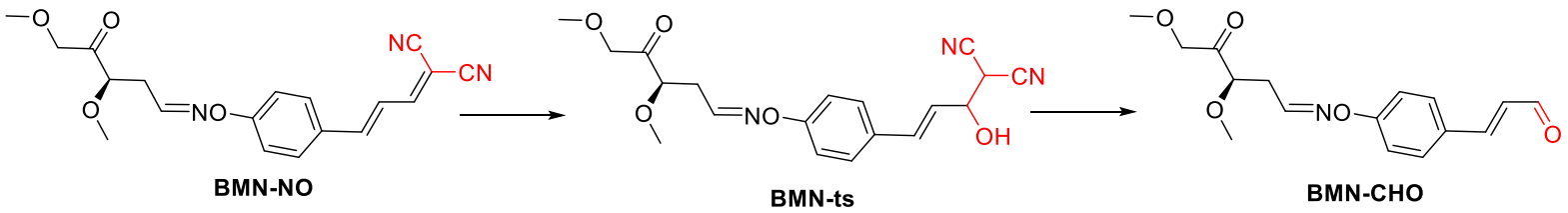

8.25
7.74
7.73
7.68
7.67
7.53
7.50
7.35
7.21
7.18
7.08
3.95
3.93
3.65
3.63
3.44
3.26
3.12
2.67
2.64
2.51
2.42
2.39

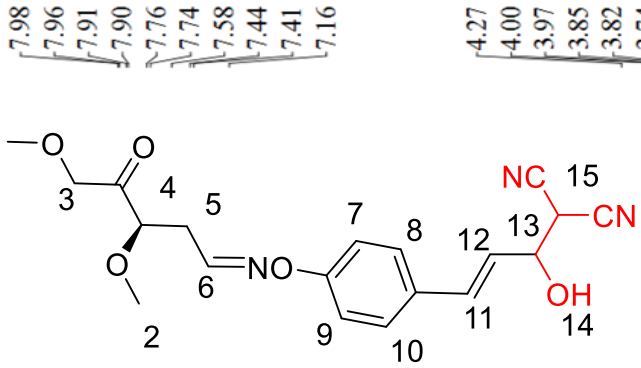
1
O
3
O
4
5
O
2
6
NO
7
8
9
10
NC
CN
12
13
11

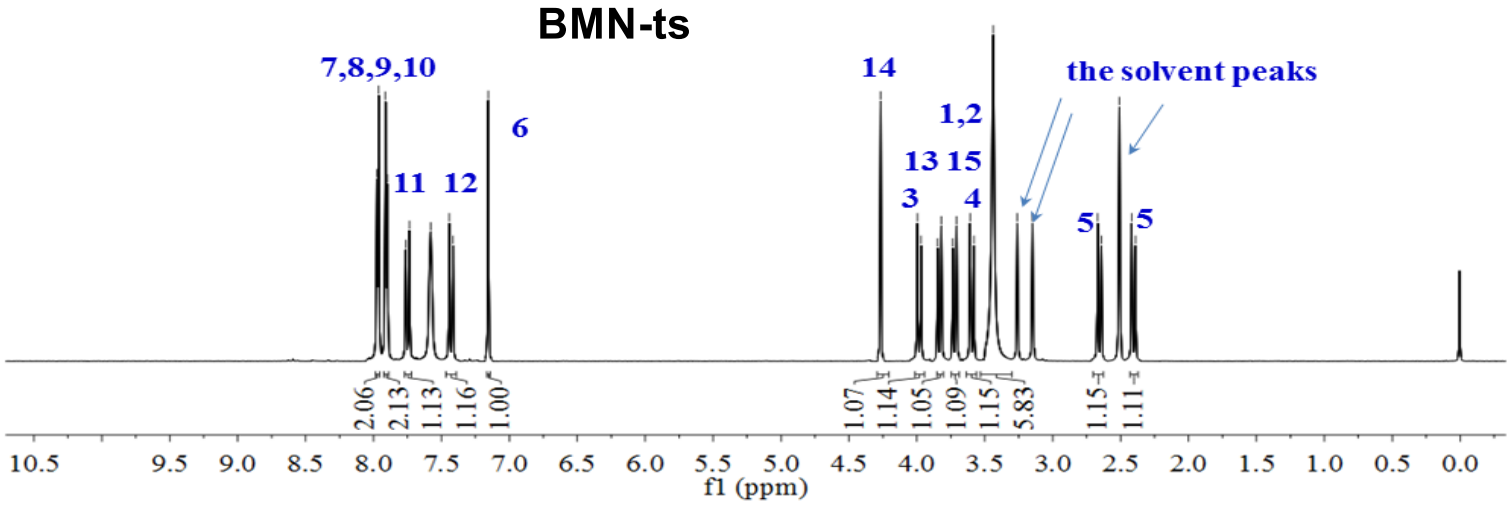
BMN-NO

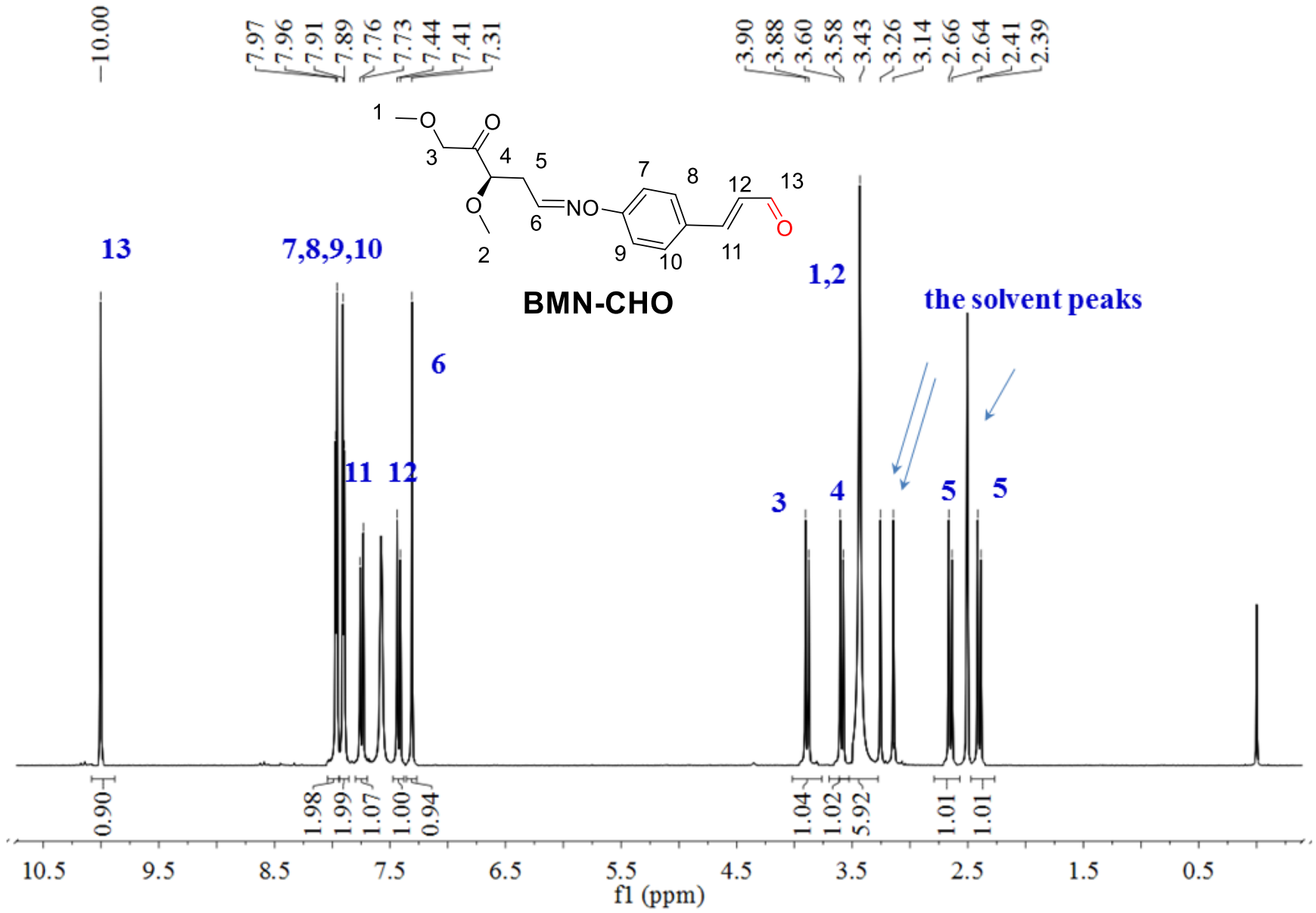

1,2
7,8,9,10
the solvent peaks
13
6
11
12
3
4
5
5
0.99
2.09
2.12
1.07
1.00
0.99
1.06
1.08
5.81
1.07
1.08
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

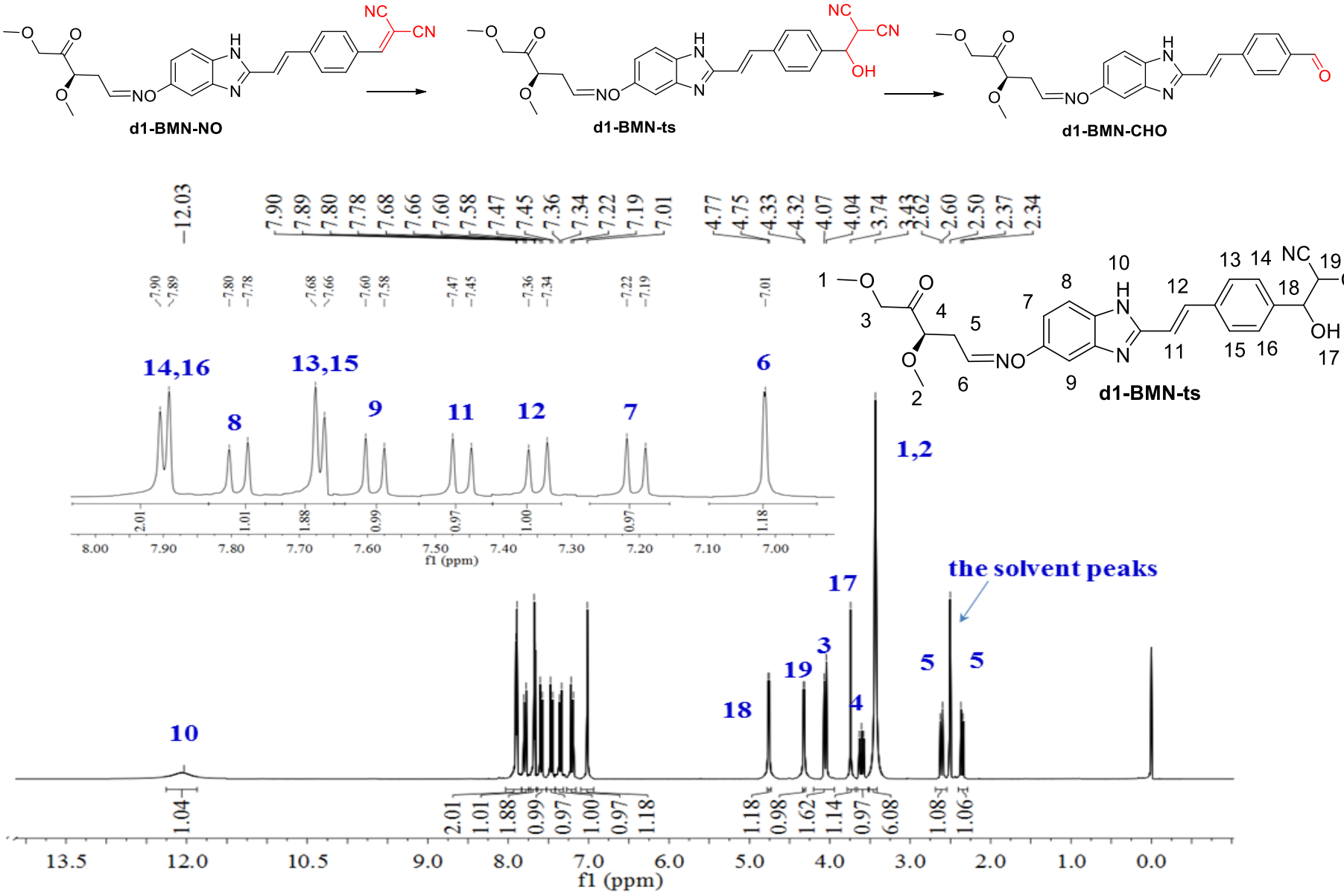

d1-BMN
HDM
d1-BMN-NO
d1-BMN-NO
the solvent peaks
f1 (ppm)

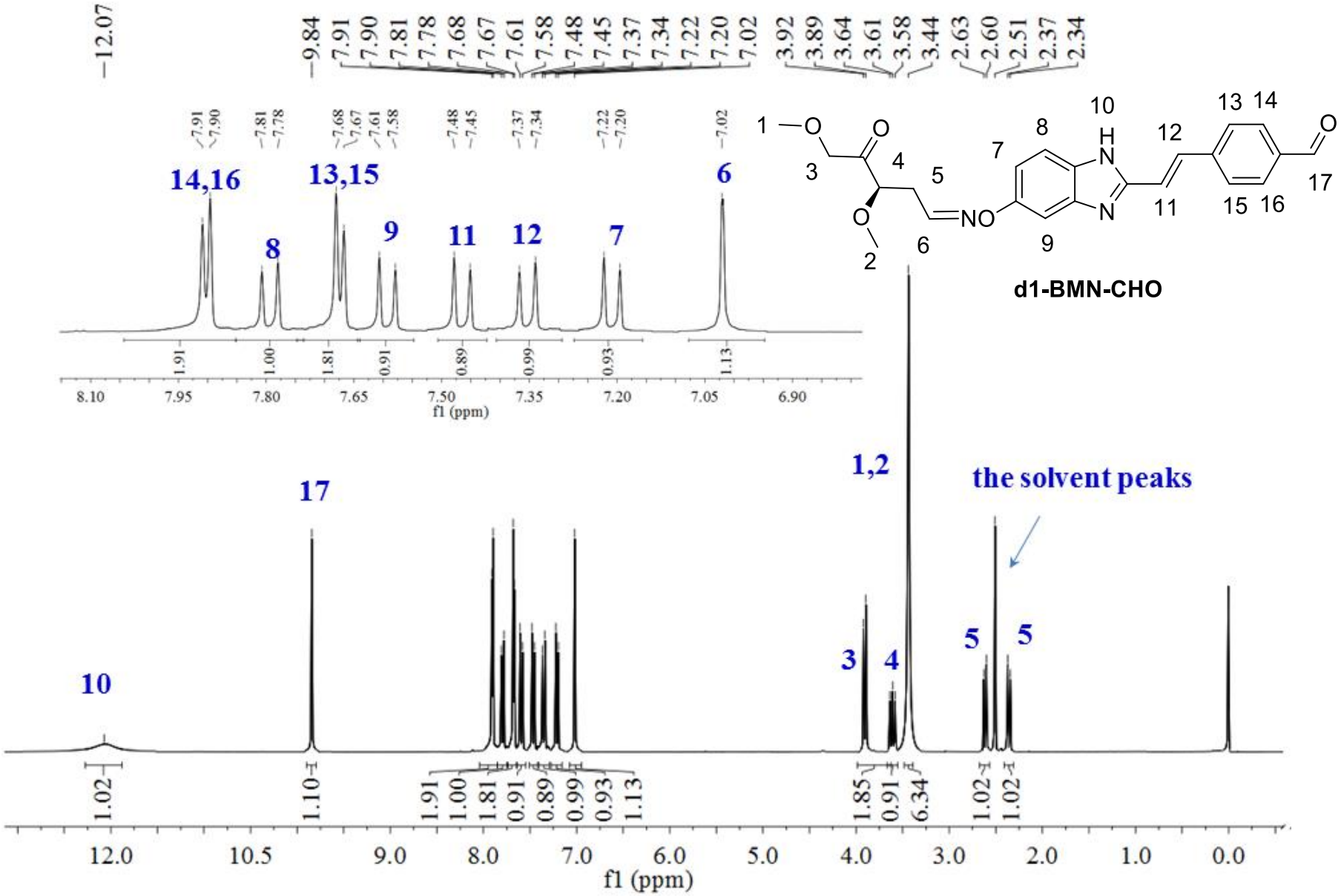

BMN-NO
BMN-ts
BMN-CHO


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
NC
CN
OH
NO


BMN-ts
7,8,9,10
11
12
6
14
1,2
13
15
3
4
5
5
the solvent peaks
f1 (ppm)


10.00
7.97
7.96
7.91
7.89
7.76
7.73
7.44
7.41
7.31
3.90
3.88
3.60
3.58
3.43
3.26
3.14
2.66
2.64
2.41
2.39
BMN-CHO
13
7,8,9,10
6
11
12
1,2
3
4
5
5
the solvent peaks
0.90
1.98
1.99
1.07
1.00
0.94
1.04
1.02
5.92
1.01
1.01
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
f1 (ppm)


d1-BMN-NO
d1-BMN-ts
d1-BMN-CHO
d1-BMN-ts
14,16
13,15
8
9
11
12
7
6
1,2
17
3
19
18
4
5
5
10
the solvent peaks
f1 (ppm)


d1-BMN-CHO
f1 (ppm)
the solvent peaks