Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION

A Selective and Sensitive Fluorescent Albumin Probe for the Determination of Urinary Albumin

Ying-Yi Wu,[1] Wan-Ting Yu,[1] Tai-Cheng Hou,[1] Tao-Kai Liu,[1] Chi-Ling Huang,[1] I-Chia Chen[1,2] and Kui-Thong Tan[1, 2, *]

1) Department of Chemistry, National Tsing Hua University, 101 Sec. 2, Kuang Fu Rd, Hsinchu 30013, Taiwan (ROC)

2) Frontier Research Center on Fundamental and Applied Sciences of Matters, National Tsing Hua University, 101 Sec. 2, Kuang Fu Rd, Hsinchu 30013, Taiwan (ROC)

Materials and instruments

Chemicals and reagents were purchased from Sigma-Aldrich and TCI. They were used without further purification. All solvents were used after appropriate distillation or purification. Besides the HCAII protein which was purified in the laboratory, all other proteins used in the selectivity test were purchased from Sigma-Aldrich and used without further purification. Sodium phosphate buffered saline (PBS buffer) was diluted from commercially available concentrates supplied by Amersco. Thin layer chromatography (TLC) was performed on TLC-aluminum sheets (Silica gel 60 F254, Merck). Flash column chromatography was performed with silica gel (230-400 mesh, Merck). HPLC analysis was performed with analytical column (EC 150/4.6 Nucleosil 300-5 C18, Macherey-Nagel).

^{1}H, and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC-400 with chemical shifts (δ) reported in ppm relative to the solvent residual signals of $CDCl_3$ (7.24 ppm) and coupling constants reported in Hz. Absorption spectra were recorded using Hitachi U-3310 spectrophotometer and fluorescence spectra were recorded using Hitachi F-4500 fluorescence spectrophotometer and TECAN Infinite M200Pro. High resolution mass spectra (HRMS) were recorded on Varian 901-FTMS.

Determine the dissociation constant K_d of HSA and AL-1

To determine the dissociation constant, we used the following equation：

$$y = B_{max} * x/(k_d + x)$$

where B_{max} is the top asymptote; x is the concentration of HSA and y is the fluorescence intensity obtained from **AL-1** (0.2 μM) incubated with different concentrations of HSA.

Determinination of AL-1 quantum yields

The quantum yields of **AL-1** in the absence or presence of HSA were determined by comparing the integrated area of the corrected emission spectrum of samples with a reference. Fluorescein was used as the reference compound with a quantum yield of 0.79 in 0.1M NaOH. The quantum yield can be calculated using the following equation:

$$\Phi = \Phi_R\left(\frac{m}{m_R}\right)\left(\frac{n^2}{n^2{}_R}\right)$$

Where

m = the slope of the line obtained from the plot of the integrated fluorescence intensity versus absorbance.

m_R= the slope of the line obtained from the plot of the integrated fluorescence intensity versus absorbance of reference.

n = the solvent refractive index.

n_R = the solvent refractive index of reference fluorophore.

ϕ = quantum yield of **AL-1**.

ϕ_R= quantum yield of reference.

Fluorescence lifetime measurements

For time-resolved measurements, the excitation laser wavelength was 400 nm and the power used was less than 1 mW. The laser system is a femtosecond mode-locked Ti:sapphire laser generating a pulse train (82 MHz, 800 nm) of which the second-harmonic pulses are generated with ~~a~~ nonlinear crystals (BBO, type I). Picosecond time-resolved fluorescence was measured by time-correlated single-photon counting (TCSPC). The samples were placed in a cuvette, and the fluorescence was filtered through a bandpass filter ($\pm$10 nm) and detected by a multichannel plate photomultiplier (MCP-PMT, Hamamatsu). The instrument response function was set at 40 ps at fwhm. The polarization of laser beam was kept at the magic angle with respect to the detection position. We obtained the time constants from the experimental curves by deconvoluting a biexponential function against the instrument response function with fwhm ~40 ps (assuming Gaussian function).

Fluorometric analysis of urinary albumin with AL-1

AL-1 was dissolved in DMSO to obtain a 10 mM stock solutions. Urine samples were collected from healthy male donors and used at the same day

without further treatment. 10 μM **AL-1** and various concentration of HSA in 100 μL urine (1% DMSO) were incubated for 5 minutes in 96-well plate and measured with TECAN fluorescent plate reader. For the determination of urinary albumin levels with immunoassay, human albumin ELISA kit (ab108788) from Abcam was used according to the manufacturer's protocol.

Determination of urinary albumin with BCP

The urine samples were concentrated before BCP measurements. The urine samples (8 mL) were centrifuged two times at 8000 rpm for 10 minutes by using 30K Ultracel® column. Acetate buffer (0.1 M sodium acetate, 0.1 M acetic acid, pH 5.2) was added to the Ultracel® column and centrifuged. The process was repeated 3 times. Finally, 0.8 mL of acetate buffer was added to the column and the concentrated urine samples were collected. The concentrated urine samples were incubated with 40μM BCP in acetate buffer (1% DMSO) at room temperature for 30 minutes in a 96-well plate. The concentration of the urinary albumin was measured at 605 nm absorption wavelength by using TECAN Infinite M200Pro microplate reader. The values were interporated to the standard curve obtained by using commercial HSA. All reported albumin levels are the average of triplicate measurements.

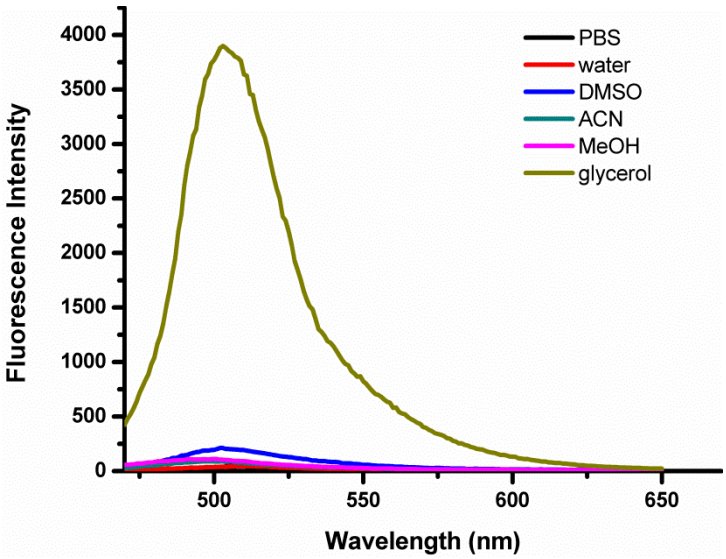


Figure S1. Fluorescent spectra of **AL-1** in different solvents.

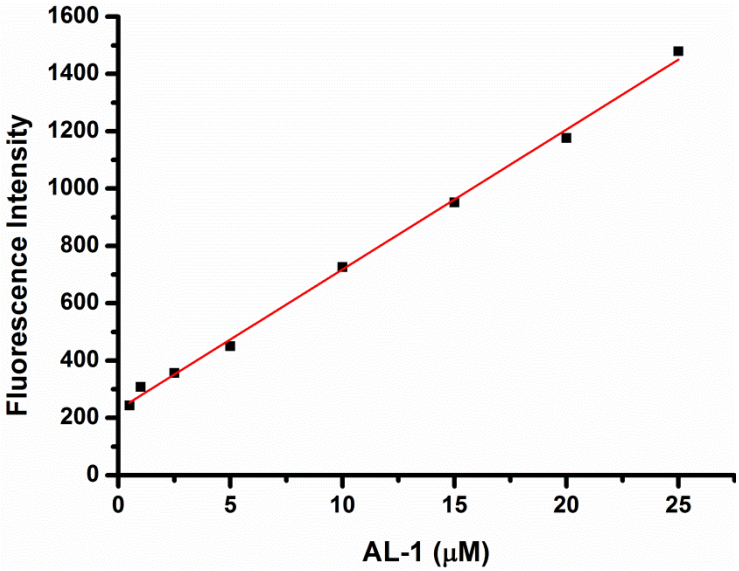


Figure S2. Fluorescence response of **AL-1** at different concentrations in PBS buffer (pH 7.4). The fluorescence intensity increases linearly with increasing concentrations of **AL-1,** which shows that the probe does not undergo any physical transformation such as aggregation, in PBS buffer.

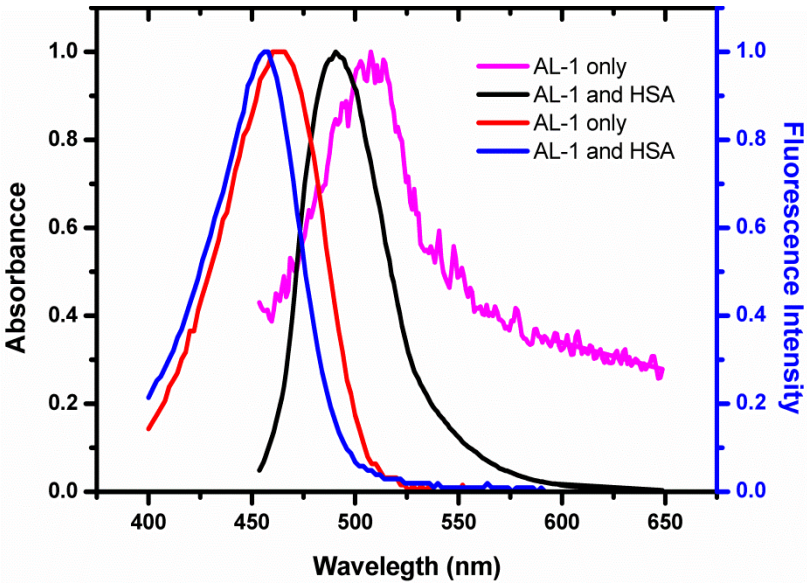


Figure S3. Normalized aborbance and fluorescence spectra of **AL-1** (2 μM) in the absence or presence of HSA (20 μM) in PBS buffer.

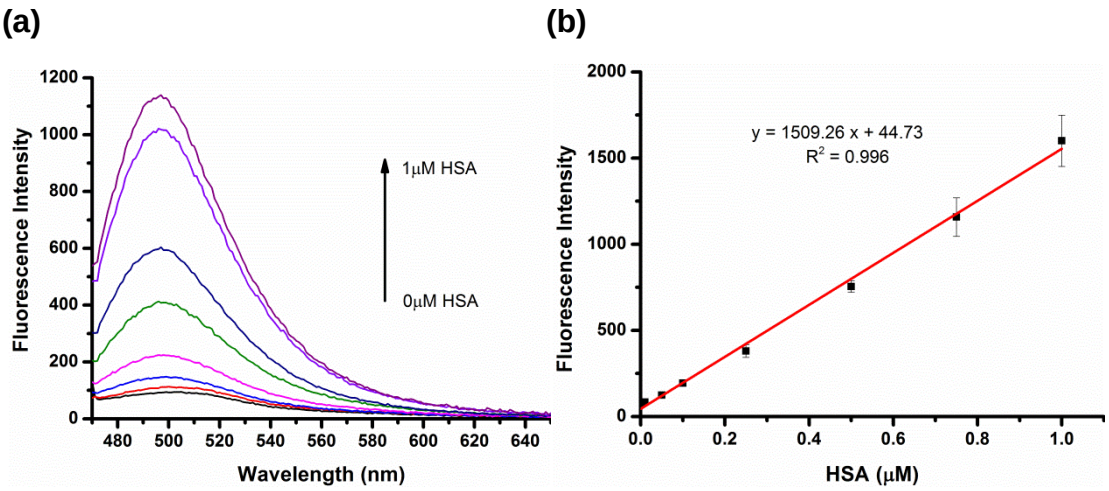
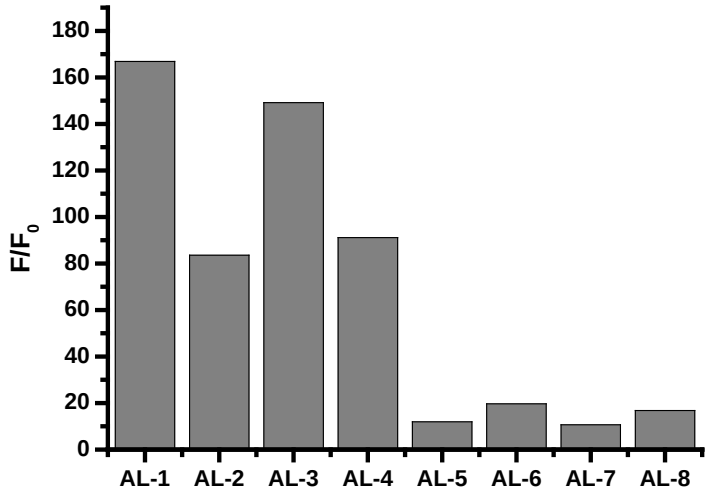


Figure S4. AL-1 limit of detection (LOD) to detect HSA. (a) Fluorescence spectra for the titration of different concentrations of HSA with 10 μM **AL-1**. (b) The fluorescence response is linear in the range of 0 – 1 μM and the LOD is estimated as 6 nM. The LOD was estimated from three times the standard deviation corresponding to the blank sample (AL-1 only).

(a)



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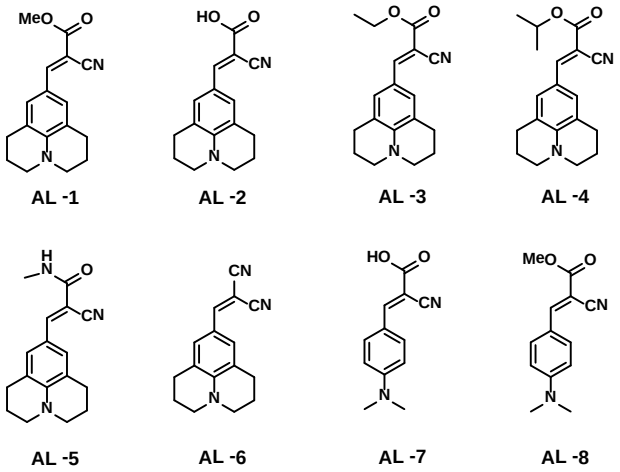


Figure S5. (a) Fluorescence enhancement of different **AL-1** derivatives (2 μM) in the presence of 2 μM HSA. (b) chemical structures of **AL-1** derivatives.

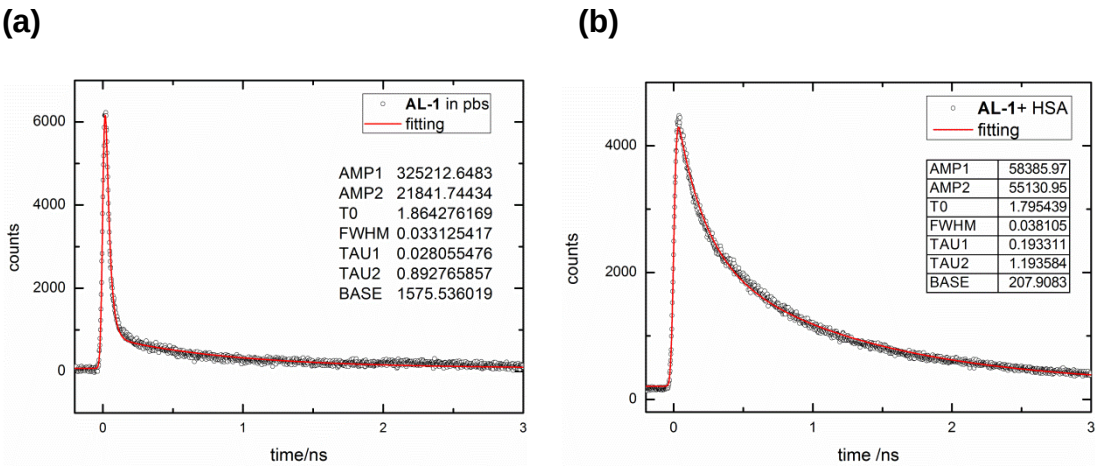


Figure S6. Fluorescence lifetimes of **AL-1**. (A) 10 μM **AL-1** in PBS buffer. (b) 10 μM **AL-1** with 20 μM HSA in PBS buffer.

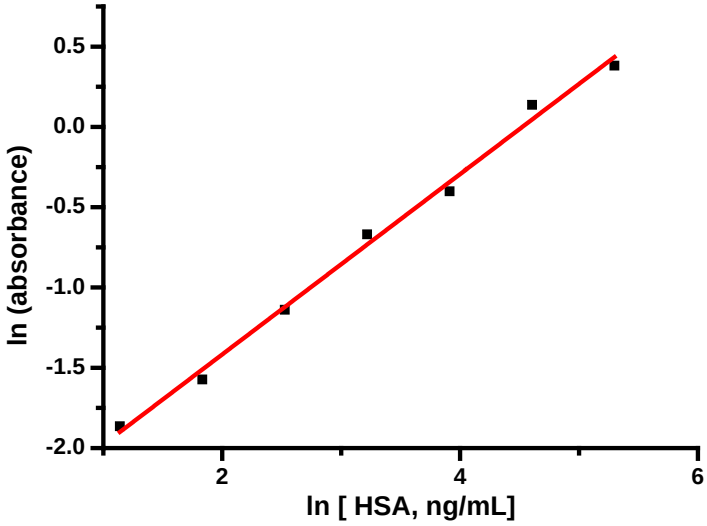


Figure S7. Calibration curve for HSA obtained by immunoassay measurement.

(a) **(b)**

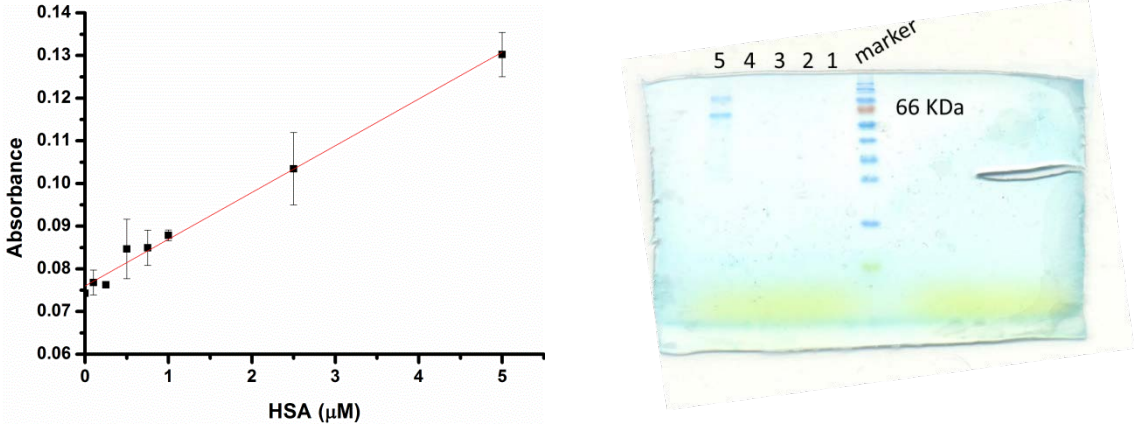


Figure S8. (a) Calibration curve for HSA obtained by Bromocresol purple (BCP) measurement. (b) SDS-PAGE gel of urine samples after centrifugation. 1. Urine filtrate after first centrifugation. 2. Urine filtrate after first centrifugation. 3. Urine filtrate after second centrifugation. 4. Urine filtrate after washing with acetate buffer. 5. 10-fold concentrated urine sample. Urinary albumin is indicated at 66KDa position.

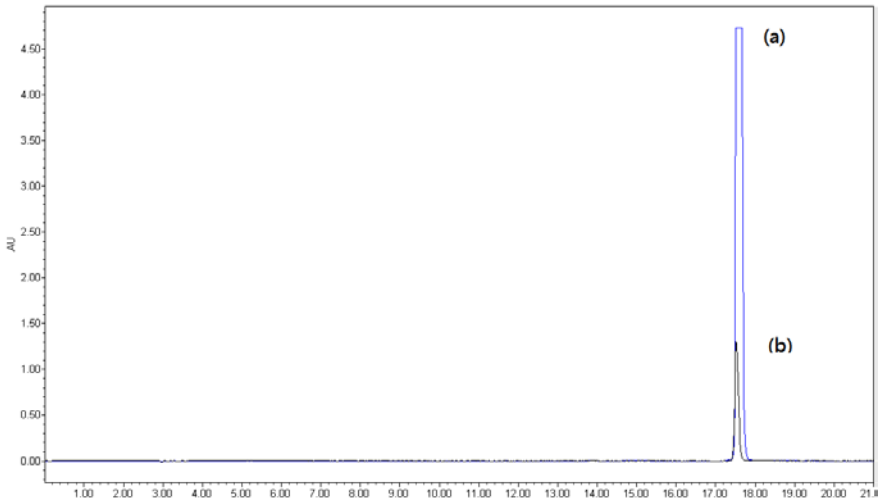
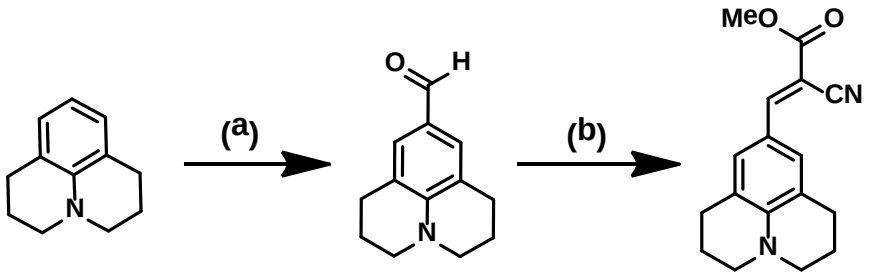


Figure S9. HPLC trace of **AL-1** recorded on (a) 09/12/2013 and (b) 26/05/2014.

Synthesis of AL-1



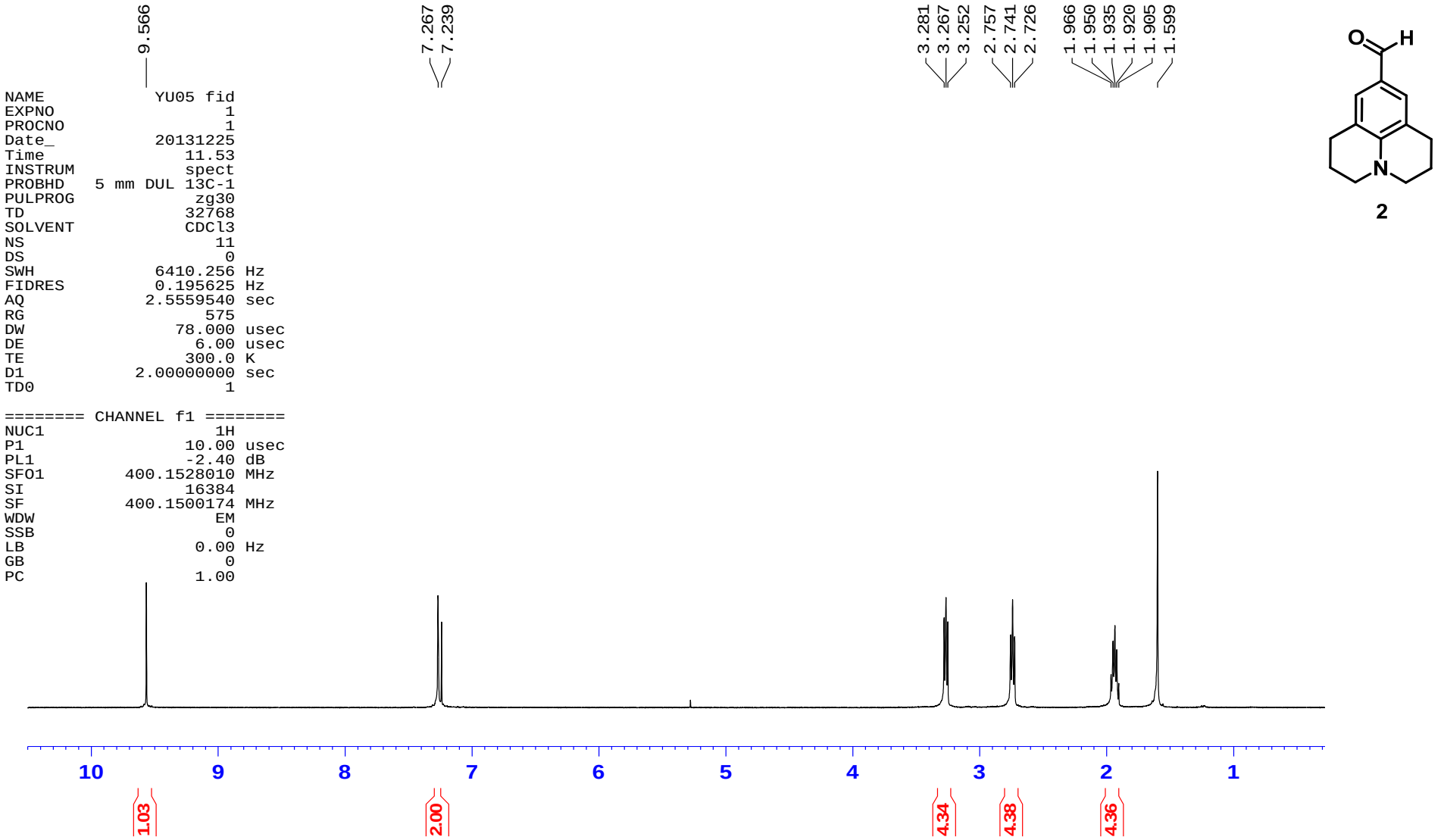
Scheme S1. Synthesis of **AL-1**. (a) $POCl_3$, DMF (b) Methyl cyanoacetate, THF, TEA.

Synthesis of compound 2

Phosphorous oxychloride (1.17 g, 7.62 mmol) was added dropwise to anhydrous DMF (1.67 g, 22.87 mmol) cooled in ice-bath under nitrogen. The mixture was stirred at room temperature for 30 min, and then transferred to a flask containing julolidine (1.2 g, 6.93 mmol) and the resulting mixture was heated at 90 °C for 4 h. After cooling, water (100 mL) was added to the reaction mixture and the mixture was neutralized with sodium bicarbonate. The mixture was then extracted with ethyl acetate and the organic layer was washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to afford compound **2** as a light yellow solid. **Yield**: 90%, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.57 (s, 1H), 7.25 (s, 2H), 3.33 – 3.23 (m, 4H), 2.81 – 2.70 (m, 4H), 2.10 – 1.88 (m, 4H) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 189.86, 147.71, 129.22, 123.72, 120.10, 49.81, 27.45, 21.03 ppm. **HRMS** (ESI): m/z calc. for $C_{13}H_{15}NO$ 201.1154, found 202.1229 $[M+H]^+$.

Synthesis of AL-1

To a solution of Methyl cyanoacetate (120 mg, 1.2 mmol) and compound **2** (161 mg, 0.8 mmol) in THF (5 mL) was added Et_3N (335 μL 2.4 mmol) at room temperature. The reaction mixture was stirred at 50°C for 12 hours. After workup, the residue was purified by column chromatography using ethyl acetate and n-hexane (1:8) as eluent to afford **AL-1** as an orange solid. **Yield**: 89%; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.49 (s, 2H), 3.83 (d, *J* = 11.2 Hz, 3H), 3.37 – 3.26 (m, 4H), 2.80 – 2.67 (m, 4H), 2.07 – 1.85 (m, 4H) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.30, 154.50, 147.71, 131.69, 120.75, 118.31, 91.00, 52.57, 50.12, 29.65, 27.50, 21.04 ppm. **HRMS** (ESI): m/z calc. for $C_{17}H_{18}N_2O_2$ 282.1368, found 283.1439 $[M+H]^+$.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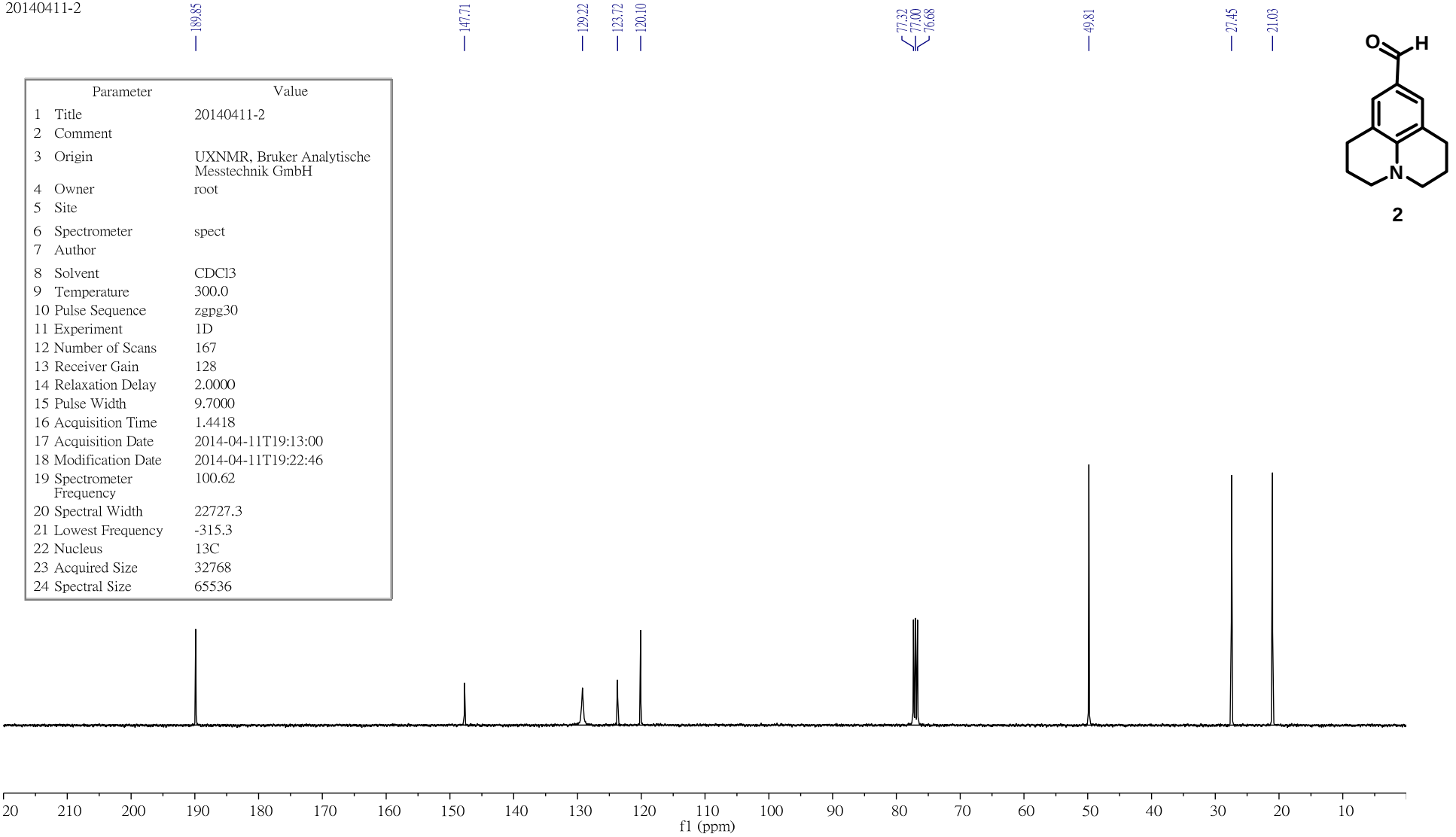

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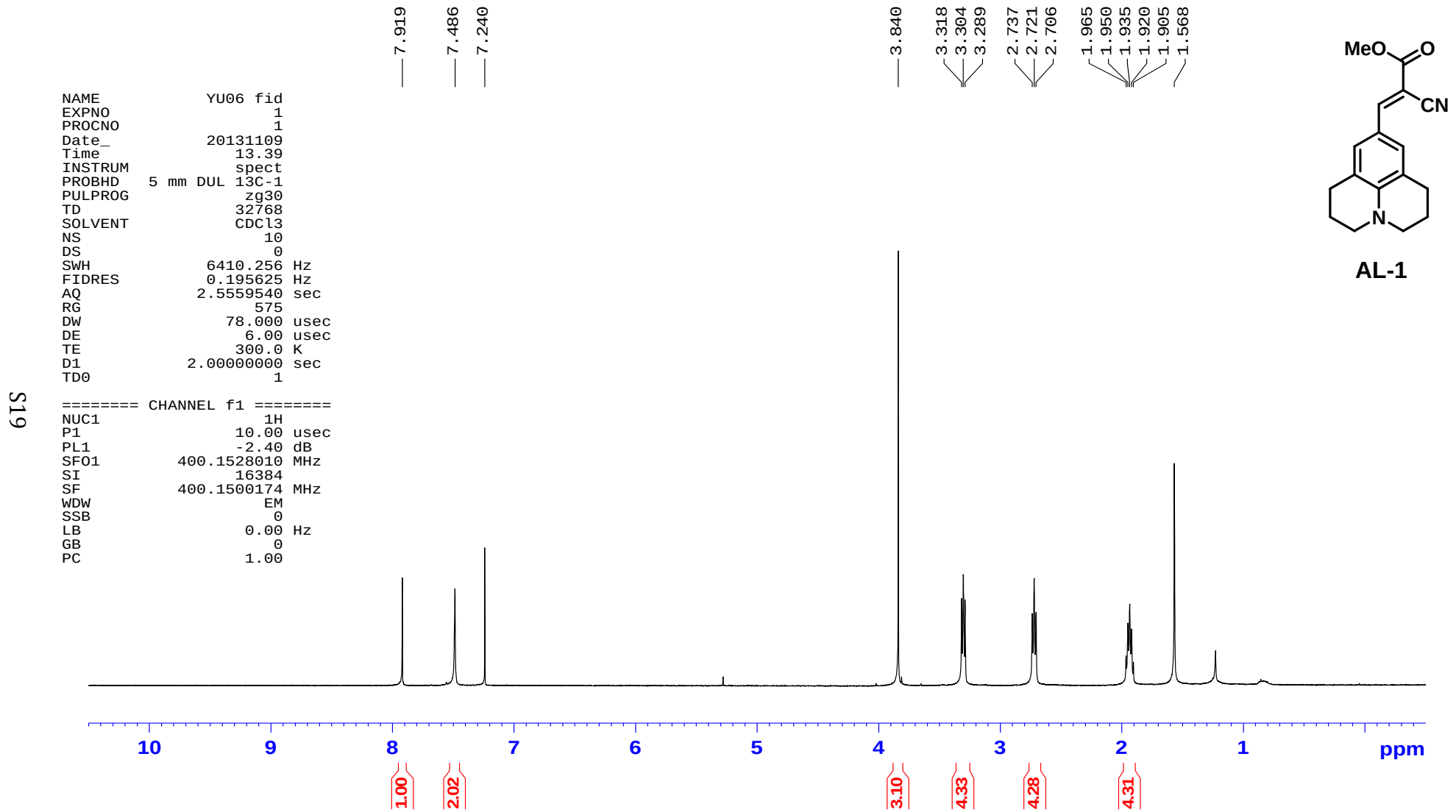
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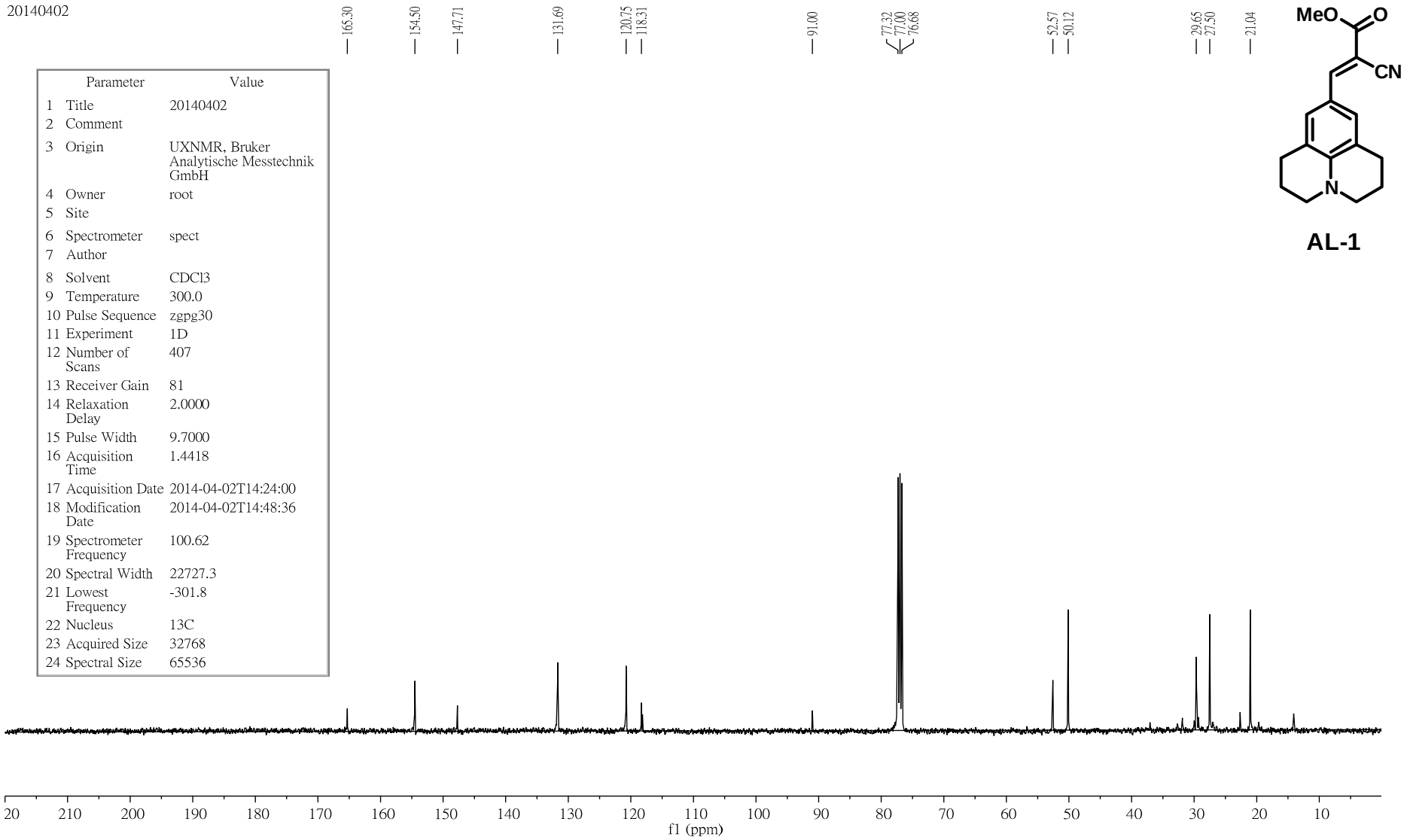

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