

Characterization of an Al³⁺-selective fluorescent probe based on a benzoyl hydrazine derivative and its application in cell imaging

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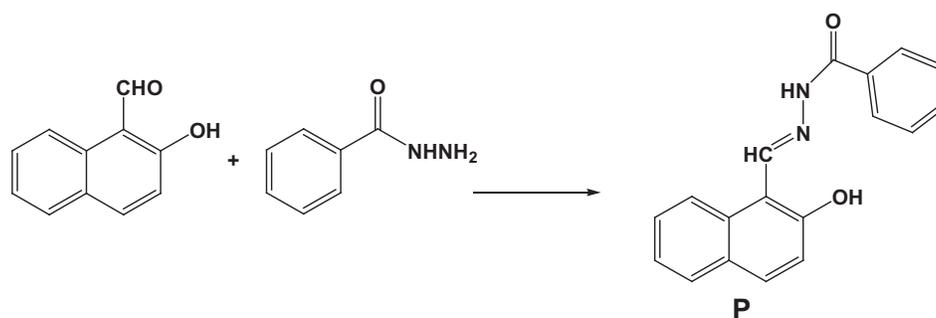
Abstract: A fluorescent probe based on benzoyl hydrazine was synthesized and characterized as an Al³⁺-selective fluorescent probe. This probe showed good selectivity towards Al³⁺ compared to other common ions. Under optimized experimental conditions, the probe exhibited a linear dynamic response for Al³⁺ from 5.0×10^{-7} to 4.5×10^{-6} M with a detection limit of 1.3×10^{-7} M in ethanol–water solution (9:1, v:v, pH 6.8, 20 mM HEPES). Furthermore, it was used for imaging of Al³⁺ in living cells with satisfying results.

Key words: Fluorescent probes, Al³⁺, cell imaging

1. Introduction

Fluorescence techniques offer significant advantages over other methods for species monitoring inside living cells because of the nondestructive character, instantaneous response, and availability of a wide range of indicator dyes, and many biologically important species such as metal ions, anions, and amino acids have been successfully detected by this method in vitro and in vivo.^{1–3} Because of the attractive electronic and photophysical properties of metal complexes of Schiff bases, particular attention has been paid to the synthesis and study of these compounds.^{4,5} In addition, Schiff base derivatives incorporating a fluorescent moiety are appealing tools for optical sensing of metal ions.^{4–8} Among the metal ions, aluminum is a nonessential element for living systems, but the ionic radius and charge of Al³⁺ make it a competitive inhibitor of several essential elements like Mg²⁺, Ca²⁺, and Fe³⁺.⁸ Therefore, the detection of chelatable aluminum (Al³⁺) in biological studies has attracted much attention recently.^{9–11} However, the lack of spectroscopic characteristics and poor coordination ability compared to transition metals mean the detection of Al³⁺ has always been problematic.⁸ For this reason, the development of Al³⁺ probes is more difficult than those of other metal ions. In general, Al³⁺, being a hard acid, prefers hard donor sites like N and O in its coordination sphere. As a result, most of the reported Al³⁺ probes contain mixed N and O donor sites.^{8,12–14} With the above-mentioned in mind, in this work a Schiff base compound containing N and O donor sites was synthesized and successfully characterized as an Al³⁺-selective probe (Scheme 1).

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Scheme 1. Synthesis route of probe **P**.

2. Results and discussion

2.1. Effects of pH on **P** and **P** with Al^{3+}

The influence of pH on fluorescence was determined first. As shown in Figure 1, the emission intensities of the free probe **P** can be negligible in the range pH 4–10, suggesting that probe **P** is stable over a wide pH range. However, a significant fluorescence enhancement was measured upon addition of Al^{3+} in the pH range 4–6.8, which is attributed to coordination of **P** with Al^{3+} . For the natural sample considered, further UV-vis and fluorescent studies were carried out in ethanol–water solution (9:1, v:v, 20 mM HEPES, pH 6.8).

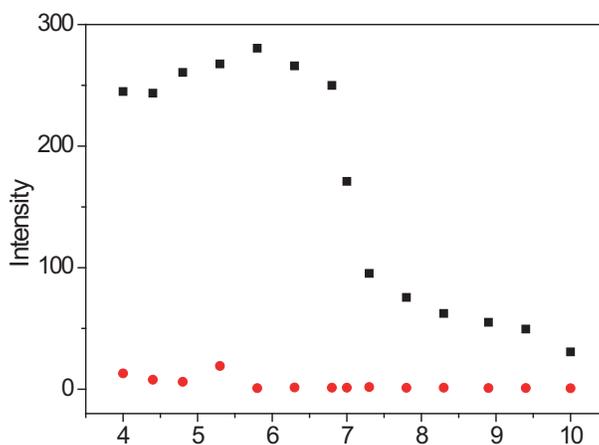


Figure 1. pH-dependent spectrum of **P** (10 μM) (●) and **P** (10 μM) plus Al^{3+} (50 μM) (■) in HEPES buffers as a function of different pH values in ethanol–water solution (9:1, v:v, 20 mM HEPES).

2.2. UV-vis spectral response of **P**

Absorption spectra of **P** were obtained in ethanol–water solution (9:1, v:v, 20 mM HEPES, pH 6.8) as shown in Figure 2. The addition of Al^{3+} to the solution of **P** (10 μM) caused an obvious red-shift in the UV region (Figure 2a), and with the addition of different concentration of Al^{3+} , there was a regular change in the UV spectra (Figure 2b). These results clearly suggested the binding of **P** with Al^{3+} .

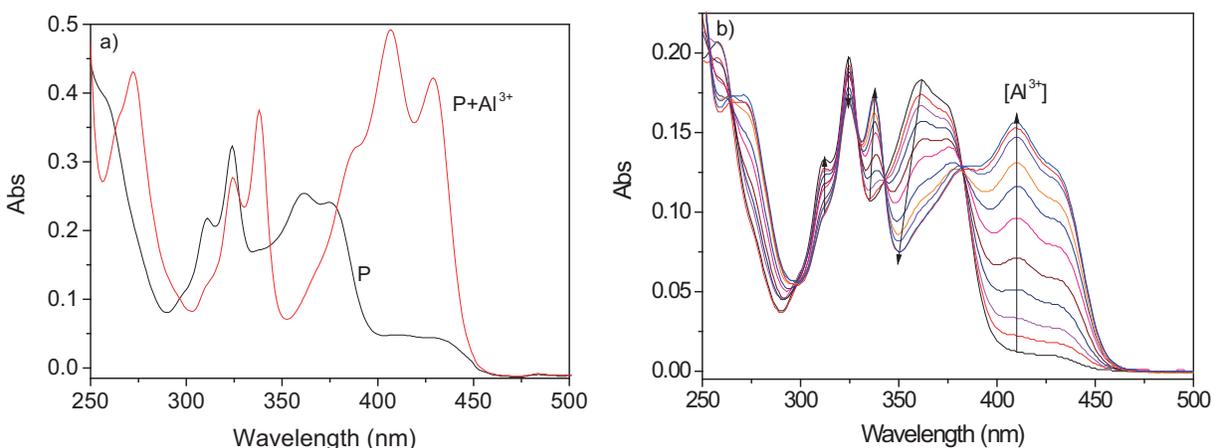


Figure 2. a) The absorption spectra of **P** (10 μM) with Al³⁺ (50 μM) in ethanol–water solution (9:1, v:v, pH6.8, 20 mM HEPES); b) Absorbance of **P** (10 μM) with various concentrations of Al³⁺ (0–10 μM) in ethanol–water solution (9:1, v:v, pH 6.8, 20 mM HEPES).

2.3. Fluorescent signaling of Al³⁺

For an excellent probe, high selectivity is a matter of necessity. Related metal ions, including Na⁺, K⁺, Ca²⁺, Cd²⁺, Mg²⁺, Co²⁺, Zn²⁺, Pb²⁺, Ni²⁺, Hg²⁺, Ag⁺, Cu²⁺, Fe³⁺, Al³⁺, and Cr³⁺, were used to evaluate the metal ions binding properties of **P** by fluorescence spectroscopy (Figure 3). The results showed that the proposed probe **P** has good selectivity to Al³⁺, which was also confirmed by the interference experiment (Figure S1). Upon the addition of increasing concentration of Al³⁺, the intensity increased drastically, and a linear relationship was observed to exist between the relative fluorescent intensity and the concentration of Al³⁺ in the range of 5.0×10^{-7} to 4.5×10^{-6} M with a detection limit of 1.3×10^{-7} M (Figure 4).

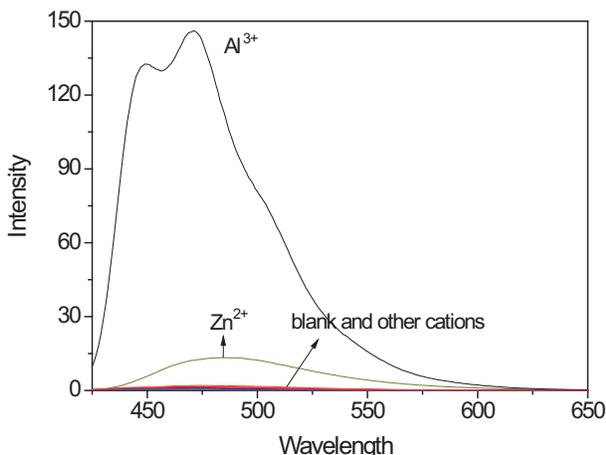


Figure 3. Fluorescent emission spectra of **P** (10 μM) to different metal ions (50 μM) in ethanolwater solution (9:1, v:v, pH 6.8, 20 mM HEPES).

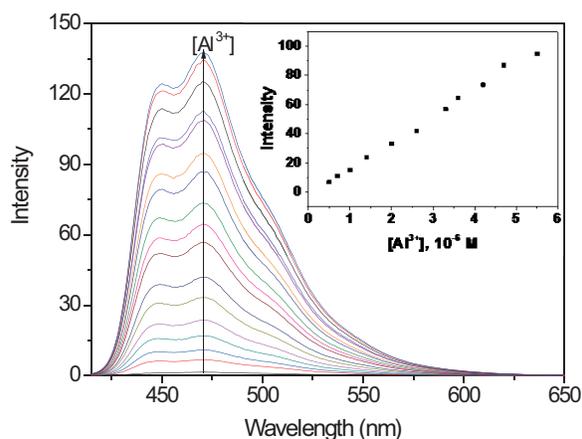


Figure 4. Fluorescence spectra of **P** (10 μM) in the presence of different amounts of Al³⁺ (0–10 μM) in ethanol–water solution (9:1, v:v, pH 6.8, 20 mM HEPES).

2.4. The proposed reaction mechanism

The method of continuous variation (Job's method) was used to determine the stoichiometry of the **P**-Al³⁺ complex (Figure 5). As expected, the result indicated a 1:1 stoichiometry of Al³⁺ to **P** in the complex. In the mass spectra of Al³⁺-**P** complex (Figure S2), 351.1 corresponded to [**P** + Al³⁺ + Cl⁻ - H⁺]⁺ and 387.7 corresponded to [**P** + Al³⁺ + 2Cl⁻]⁺, also supporting the binding mode of **P** with Al³⁺. The association constant *K* was determined from the slope to be $2.2 \times 10^4 \text{ M}^{-1}$, by plotting the fluorescence intensity $1/(F-F_0)$ against $1/[\text{Al}^{3+}]$. According to the results, the plausible binding mechanism of **P** in the present system is schematically depicted in Scheme 2, and the enhancement of fluorescence may be caused by blocking the C=N isomerization rather than another mechanism. A reversibility experiment was carried out and the results showed that the reaction of Al³⁺ with proposed probe **P** was reversible (Figure S3).

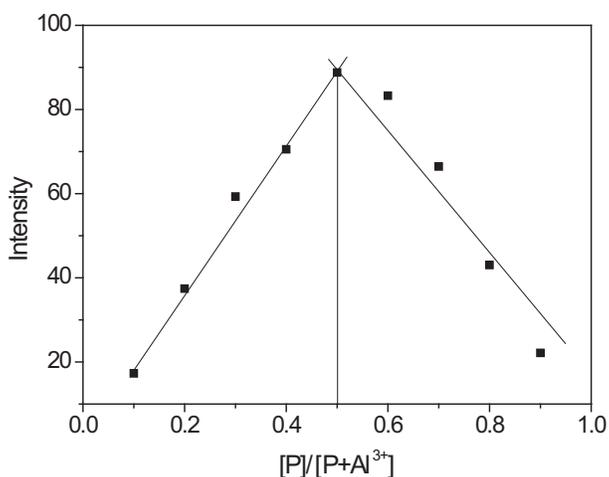
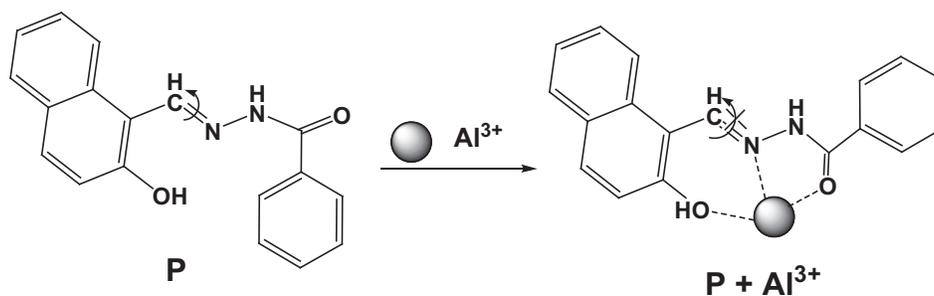


Figure 5. Job's plot of **P** with Al³⁺ in ethanol–water solution (9:1, v:v, pH 6.8, 20 mM HEPES). Total concentrations of **P** and Al³⁺ were kept at a fixed 20 μM .



Scheme 2. Proposed binding mode between **P** and Al³⁺.

2.5. Preliminary analytical application

To further demonstrate the practical applicability of the probe **P** to detect Al³⁺ in living cells, fluorescence images of H1-7701 cells were recorded before and after addition of Al³⁺.

The cells were supplemented with only **P** in the growth medium for 30 min at 37 °C, which led to no fluorescence as determined by laser scanning confocal microscopy (ex = 405 nm) (Figure 6a). In contrast, when

loaded with 1 μM AlCl_3 for 30 min, a bright fluorescence was detected (Figure 6b). These results suggested that probe **P** can penetrate the cell membrane and might be used for detecting Al^{3+} in living cells.

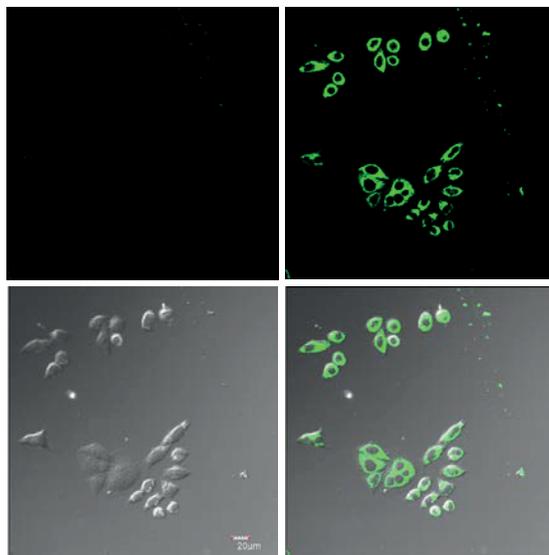


Figure 6. Confocal fluorescence images in HI-7701 cells ($\text{ex} = 405 \text{ nm}$). (a) Cells incubated with 20 μM **P** in PBS buffer for 30 min; (b) Cells incubated with 20 μM **P** in PBS buffer for 30 min, and then further incubated with 1 μM Al^{3+} for 30 min, washed three times; (c) Brightfield image of cells shown in panel a) and b); (d) Overlay of b) and c).

3. Experimental section

3.1. Reagents and instruments

All reagents and solvents were of analytical grade and used without further purification.

UV-Vis spectra were obtained on a Hitachi U-2910 spectrophotometer. Fluorescence emission spectra were obtained on a Hitachi 4600 spectrofluorometer. Mass (MS) spectra were recorded on a Thermo TSQ Quantum Access Agilent 1100 system. Nuclear magnetic resonance (NMR) spectra were measured with a Bruker AV 400 instrument and chemical shifts are given in ppm from tetramethylsilane (TMS).

3.2. Synthesis of compound **P**¹⁵

2-Hydroxy-1-naphthaldehyde (1.0 mmol) and benzoichydrazide (1.0 mmol) were mixed and stirred in ethanol (30 mL) at 80 $^{\circ}\text{C}$ for 4 h and then cooled to room temperature. The white precipitate so obtained was filtered and dried under vacuum and used directly. Yields: 85.3%. MS: m/z 291.30 $[\text{M} + 1]^+$; 313.22 $[\text{M} + \text{Na}]^+$. ^1H NMR (δ ppm, d_6 -DMSO): 12.81 (s, 1H), 12.23 (s, 1H), 9.52 (s, 1H), 8.24 (d, 1H, $J = 8.5$), 8.01 (d, 2H, $J = 7.2$), 7.95 (d, 1H, $J = 9.0$), 7.91 (d, 1H, $J = 8.1$), 7.67 (d, 1H, $J = 7.4$), 7.64 (d, 1H, $J = 5.4$), 7.63 (d, 1H, $J = 7.9$), 7.59 (d, 1H, $J = 7.0$), 7.43 (t, 1H, $J = 7.4$), 7.26 (d, 1H, $J = 9.0$). ^{13}C NMR (δ ppm, d_6 -DMSO): 163.42, 158.92, 147.77, 133.64, 133.58, 132.99, 132.53, 129.89, 129.55, 128.72, 128.67, 128.48, 124.44, 121.49, 119.81, 109.43 (Figures S4–S6).

3.3. General spectroscopic methods

Metal ions and probe **P** were dissolved in deionized water and DMSO to obtain 1.0 mM stock solutions, respectively. Before spectroscopic measurements, the solution was freshly prepared by diluting the high concentration stock solution to the corresponding desired concentration. For all measurements, excitation and emission slit widths were 5 nm and excitation wavelength was 405 nm.

4. Conclusions

In summary, we describe an Al³⁺-selective fluorescent probe. This proposed probe has good selectivity and sensitivity to Al³⁺ compared to other common ions. In addition, we have demonstrated that **P** can be used to detect Al³⁺ in living cells. It is anticipated that the proposed probe will significantly promote studies on the effects of Al³⁺ in biological systems.

Acknowledgments

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Supplementary

Characterization of an Al³⁺-selective fluorescent probe based on a benzoyl hydrazine derivative and its application in cell imaging

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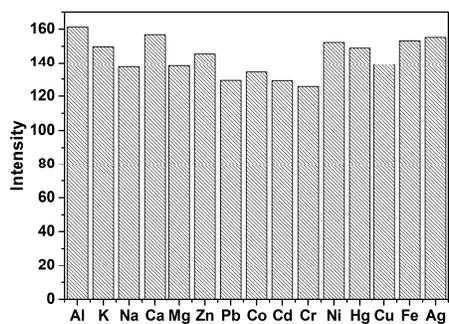


Figure S1. Fluorescence response of **P** (10 μM) to 10 μM Al³⁺ and to the mixture of 50 μM other metal ions with 10 μM Al³⁺.

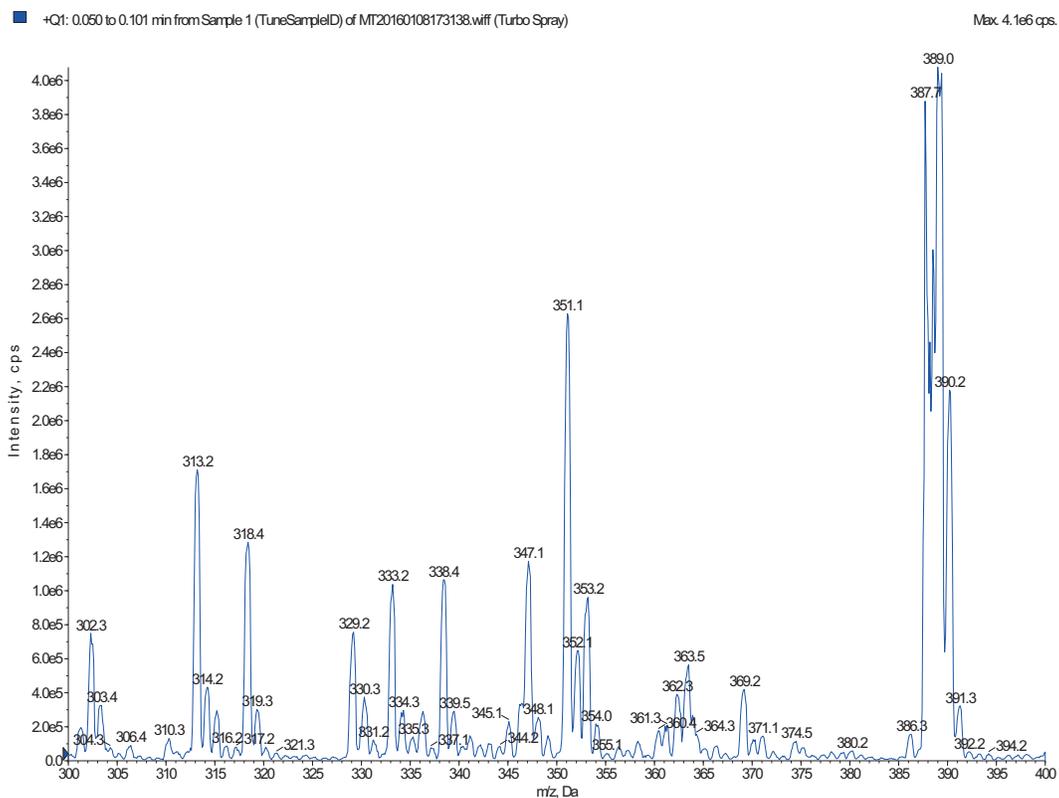


Figure S2. ESI-MS mass spectrum of **P** with Al³⁺.

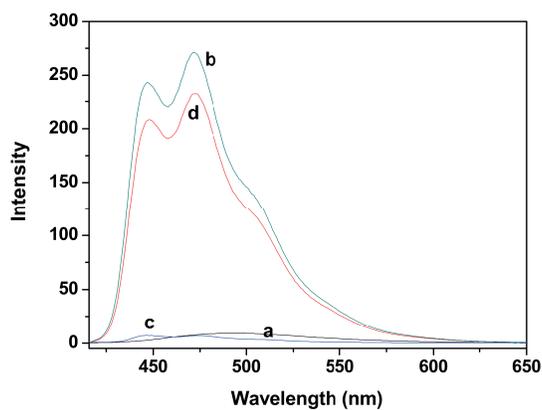


Figure S3. Reversible titration response of **P** to Al^{3+} in ethanol–water solution (9:1, v:v, pH 6.8, 20 mM HEPES) (a) **P** (10 μM); (b) **P** (10 mM) with Al^{3+} (50 μM); (c) **P** (10 μM) with Al^{3+} (50 μM) and then addition of EDTA (100 μM); (d) **P** (10 μM) with Al^{3+} (50 μM) and EDTA (100 μM) and then addition of 200 μM Al^{3+} .

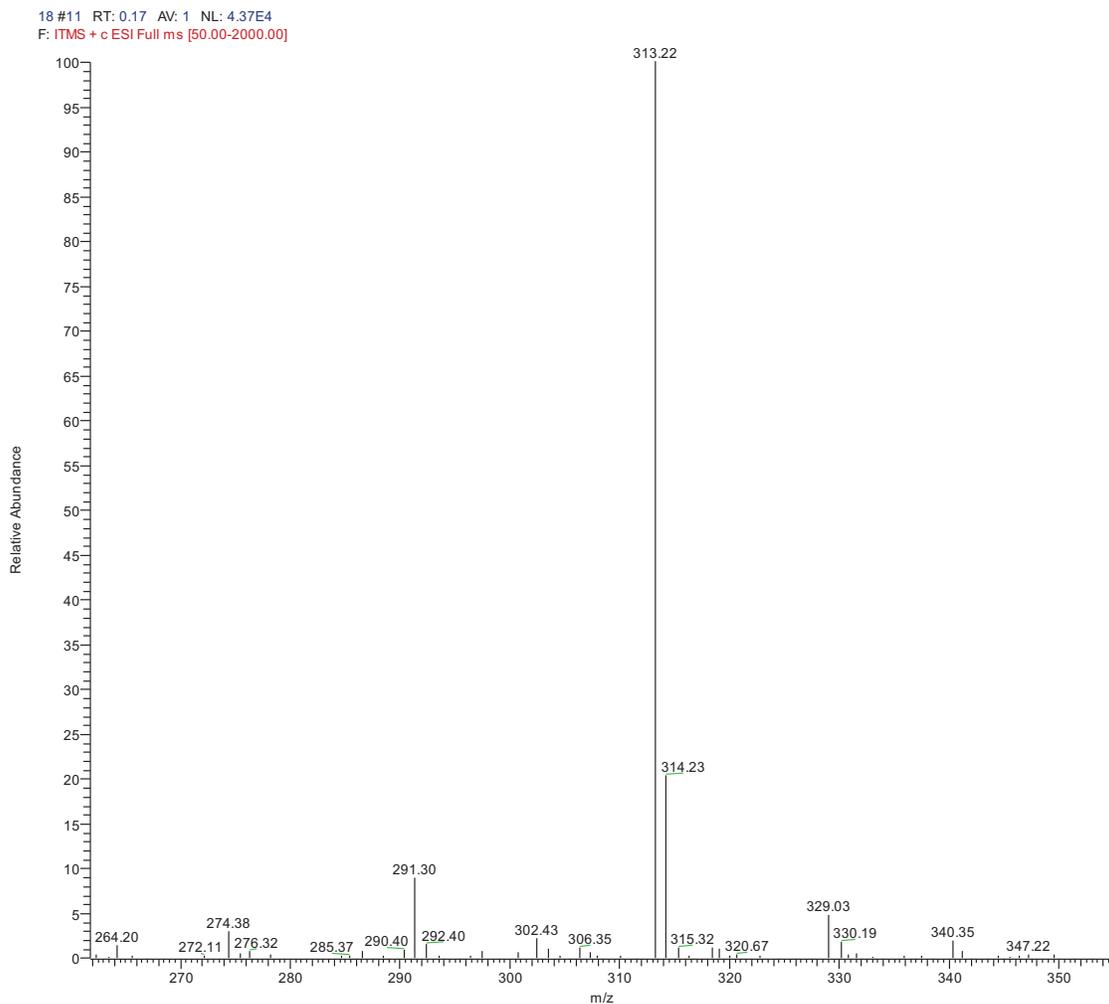
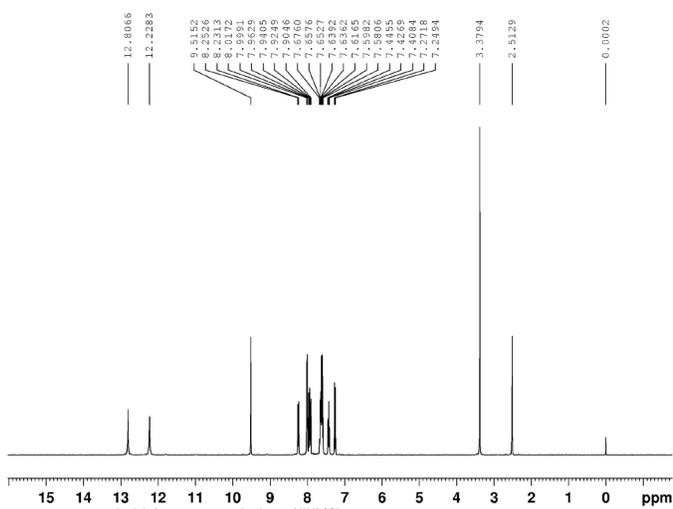


Figure S4. ESI-MS mass spectrum of **P**.

27 in DMSO (proton)



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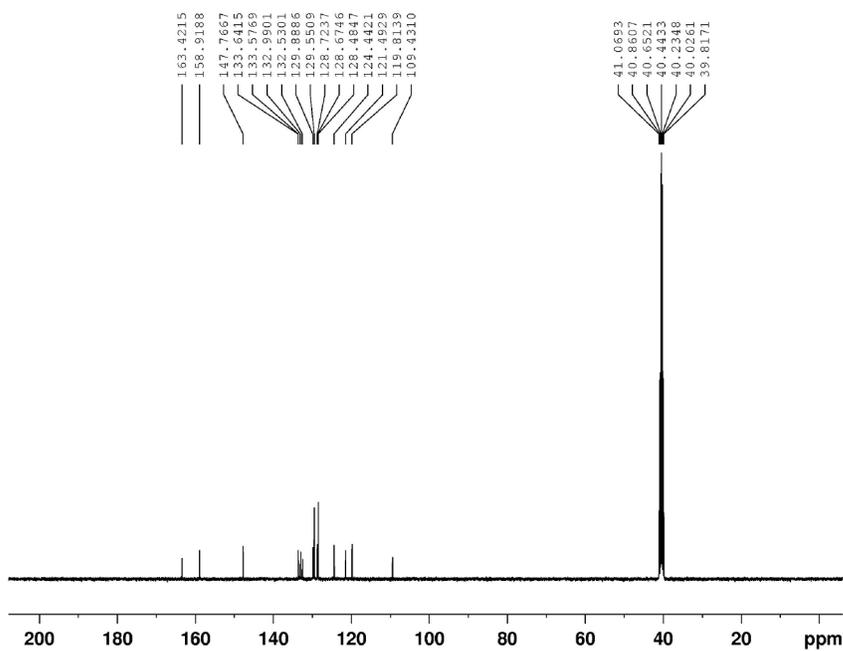
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----- CHANNEL f1 -----
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P1       13.60 usec
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SFO1     400.1324710 MH

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Figure S5. ¹H NMR spectrum of P.

27 in DMSO (c13)



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DE       6.50 usec
TE       297.3 K
D1       2.00000000 sec
D11      0.03000000 sec
TDO      1

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PL12    14.50 dB
PL13    14.50 dB
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Figure S6. ¹³C NMR spectrum of P.