Supporting Information

Al(III)-Responsive "Off-On" Chemosensor Based on Rhodamine Derivative and Its Application in Cell Imaging

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Contents

Fig. S1

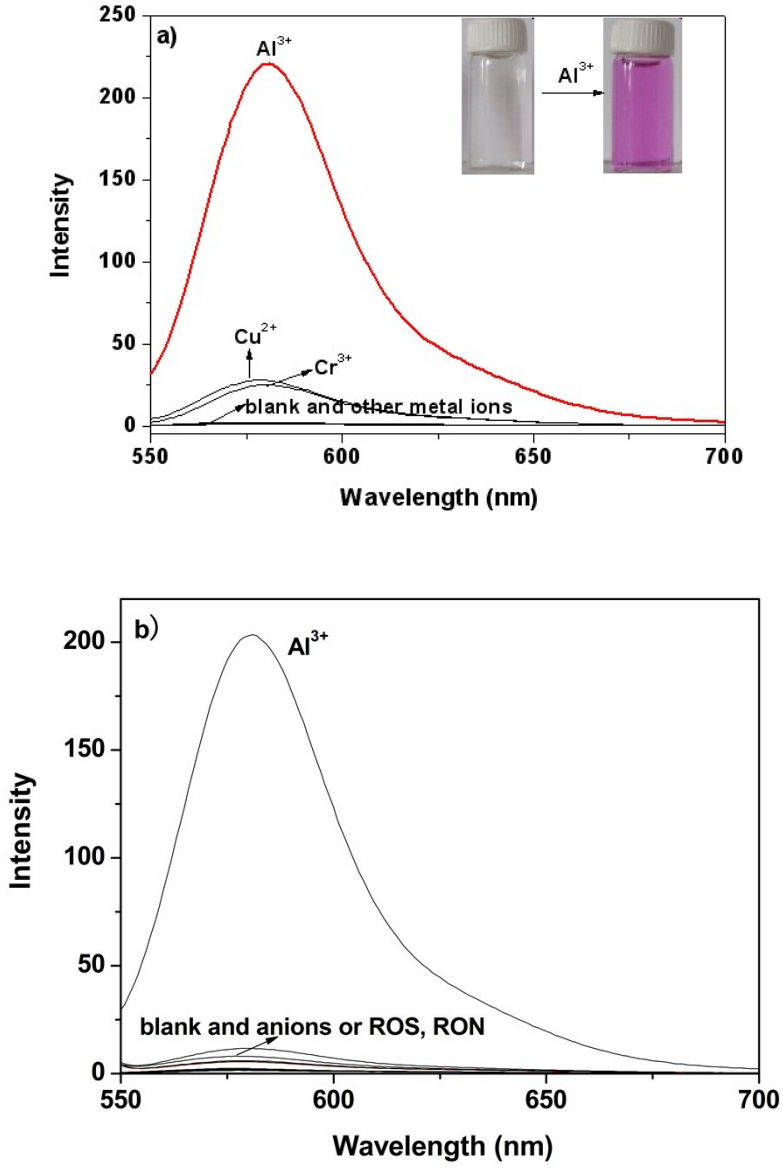


Fig. S1 a) Fluorescence emission spectra of **P** (10 μM) to different metal ions (10 μM) in ethanol-water solution (9:1, v:v, pH5.8, 20 mM HEPES); b) Fluorescence emission spectra of **P** (10 μM) to different anion ions and ROS or RNS (10 μM) in ethanol-water solution (9:1, v:v, pH5.8, 20 mM HEPES).

Fig. S2

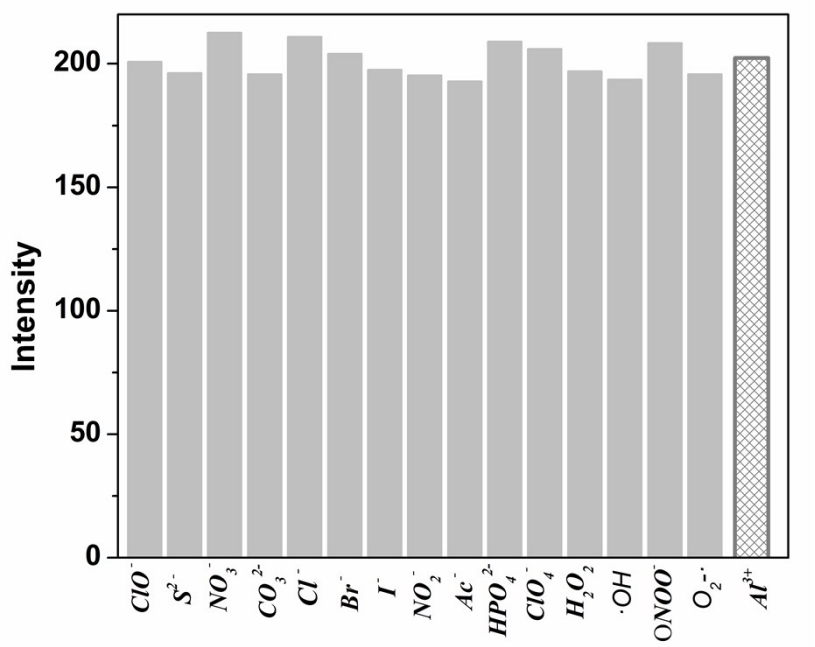


Fig. S2 Fluorescence response of **P** (10 μM) to Al^{3+} ions (10μM) or to a mixture of the specified anion ions and ROS or RNS (50 μM) with Al^{3+} ions (10 μM) in ethanol-water solution (9:1, v:v, pH5.8, 20 mM HEPES).

Fig. S3

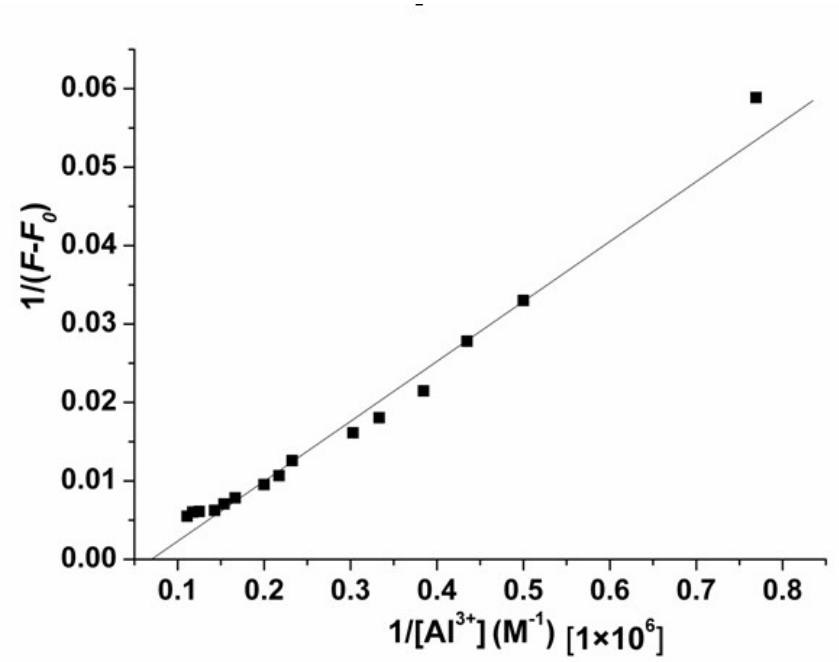


Fig. S3 Benesi-Hildebrand plot of **P**, assuming 1:1 stoichiometry for association between **P** and Al^{3+}.

Fig. S4

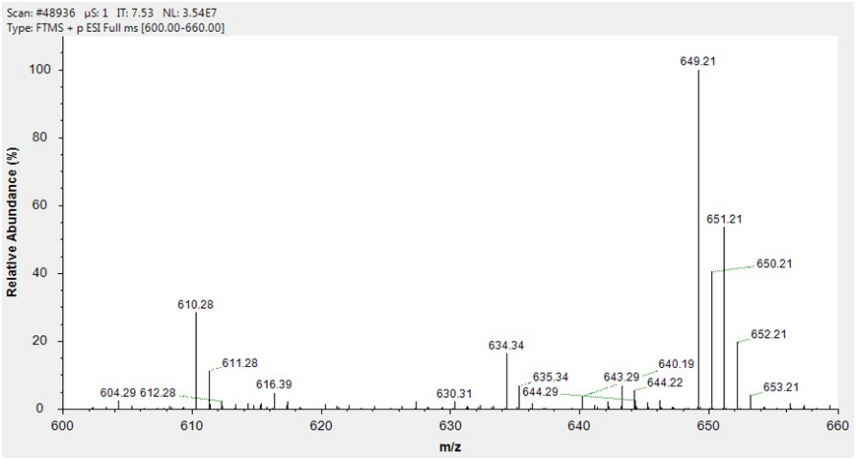


Fig. S4 ESI-MS of **P**-Al^{3+} complex.

Fig. S5

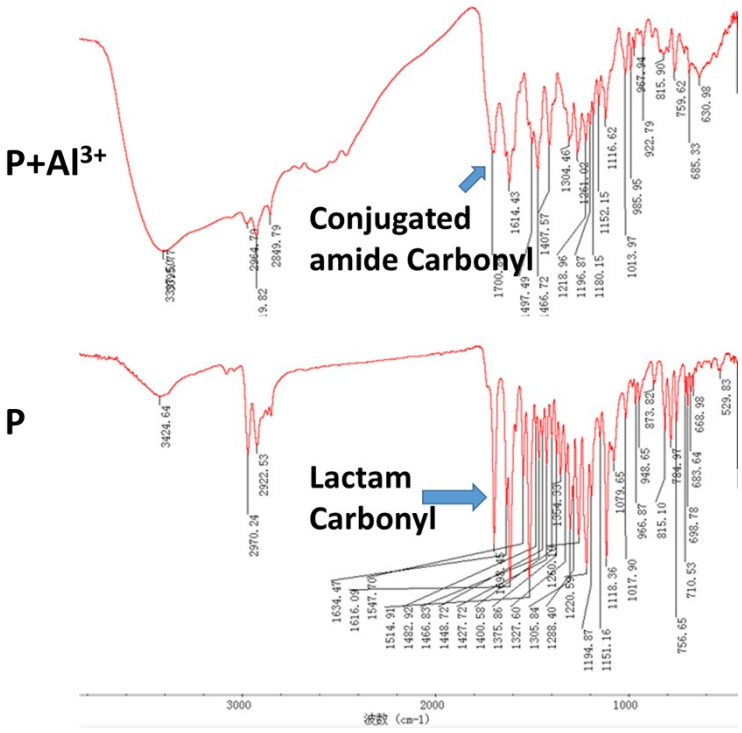
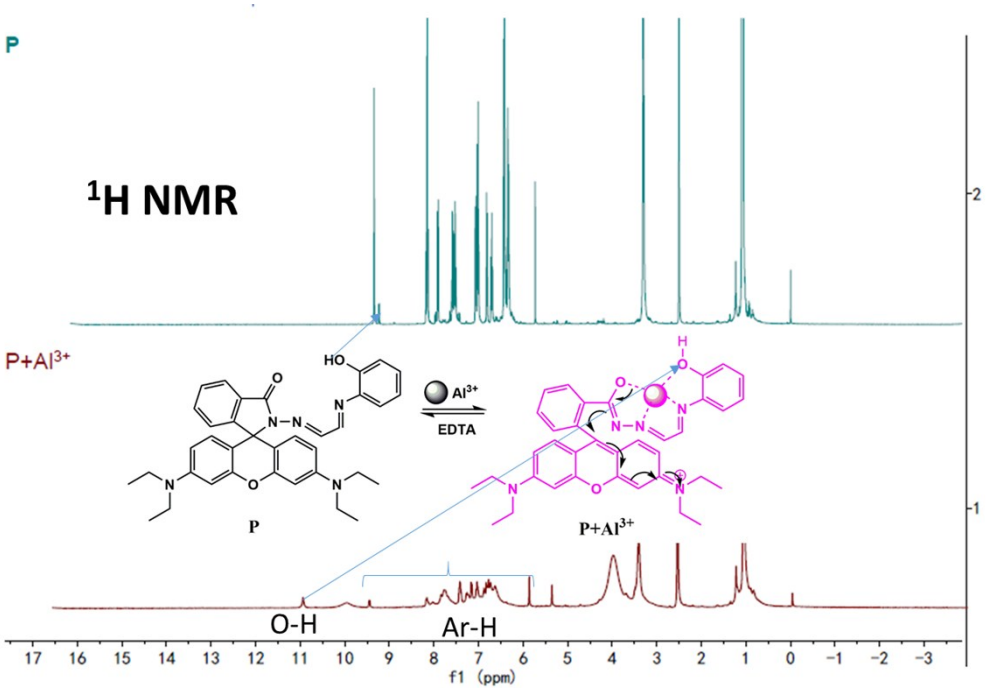


Fig. S5 Contrast of FT-IR spectrum between **P** and **P**-Al^{3+} complex

Fig. S6

a)



b)

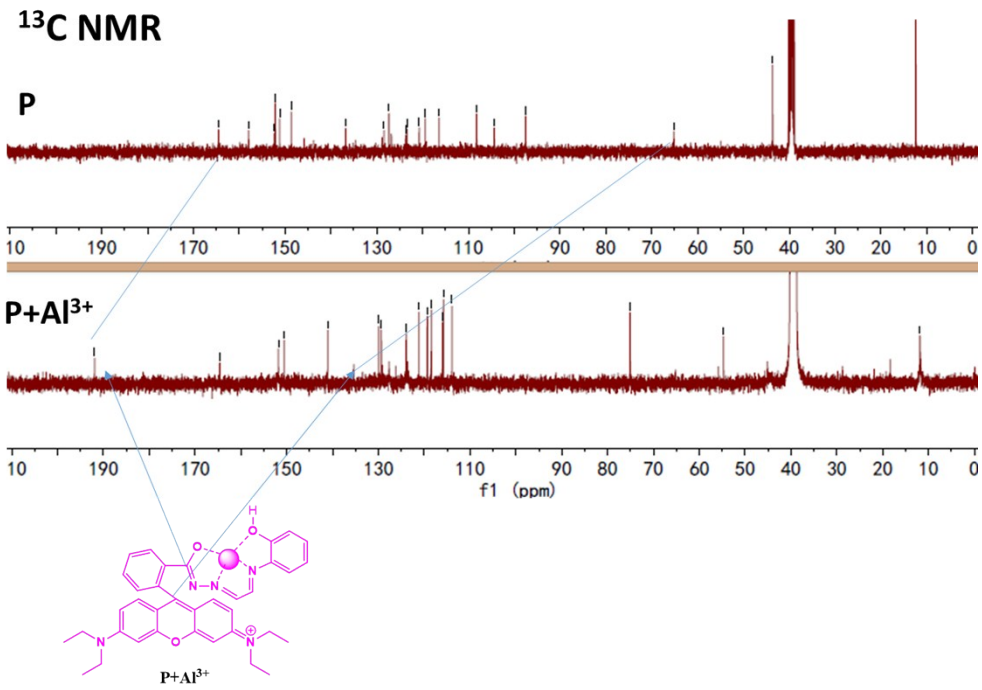


Fig. S6 a) Contrast of ^{1}H NMR spectrum between **P** and **P**-Al^{3+} complex; b) Contrast of ^{13}C NMR spectrum between **P** and **P**-Al^{3+} complex

Fig. S7

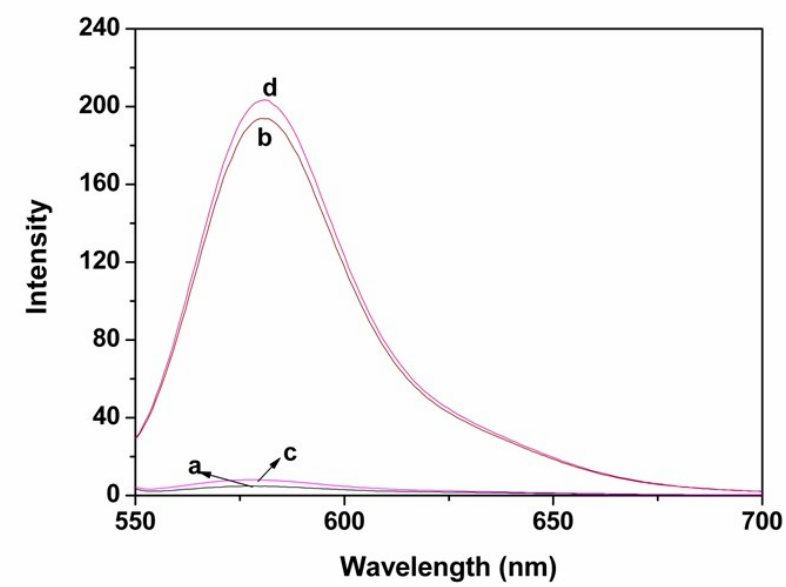


Fig. S7 Reversible titration response of **P** to Al^{3+} in ethanol-water solution (9:1, v:v, pH5.8, 20 mM HEPES): (a) **P** (10 μM); (b) **P** (10 μM) with Al^{3+} (10 μM); (c) **P** (10 μM) with Al^{3+} (10 μM) and then addition of EDTA (20 μM); (d) **P** (10 μM) with Al^{3+} (10 μM) and EDTA (20 μM) and then addition of Al^{3+} (30 μM).

Fig. S8

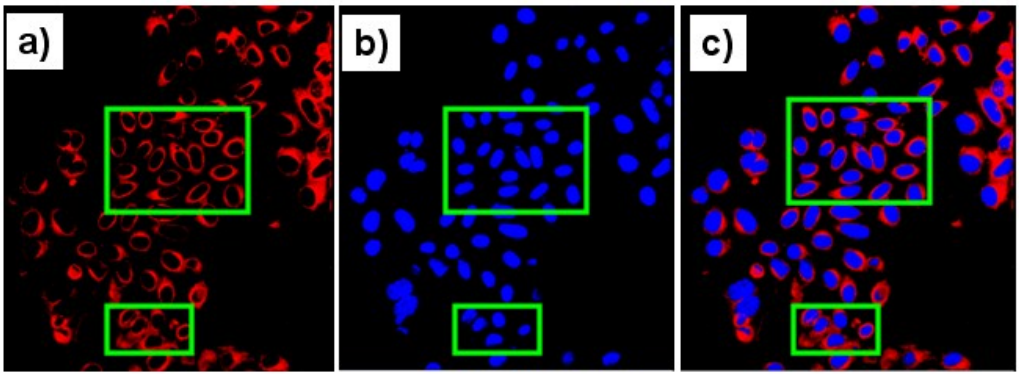


Fig. S8 Confocal fluorescence images of HepG2 cells incubated with **P** (10 μM) and Hoechst 33342 (1 μg/mL) for 30 min. Cells loaded with Al^{3+} (10 μM), then treated with **P** (10 μM) and Hoechst 33342 (1 μg/mL) for 30 min. (a) Red channel with **P**; (b) Blue channel with Hoechst 33342; (c) Overlay of images showing fluorescence from Hoechst 33342 (b) and **P** (a).

Fig. S9

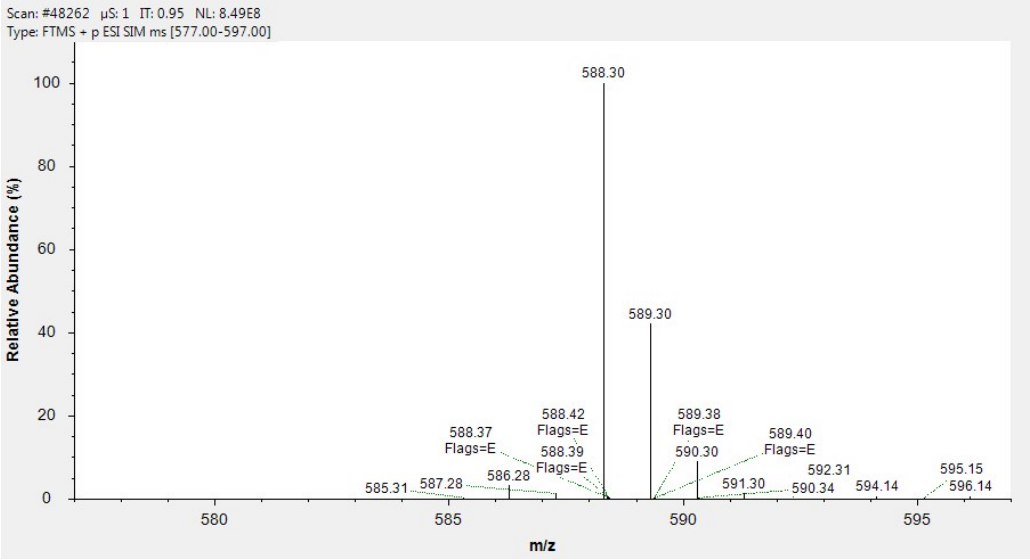


Fig. S9 ESI-MS of **P**

Fig. S10

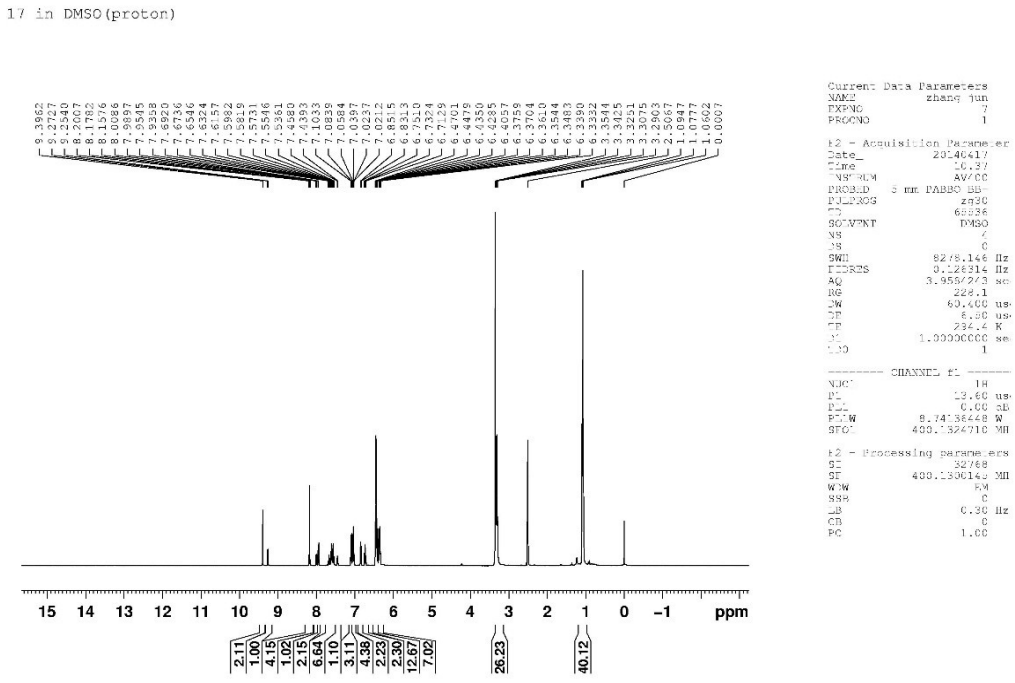


Fig. S10 ^{1}H NMR spectrum of **P**

Fig. S11

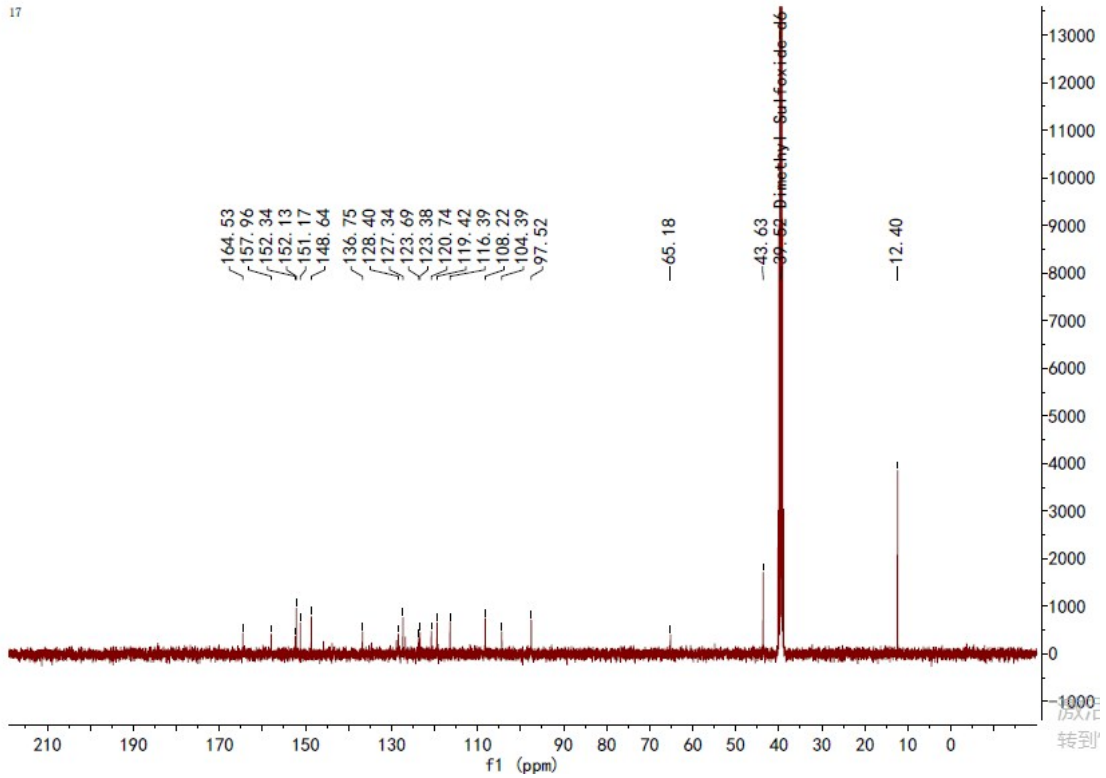


Fig. S11 ^{13}C NMR spectrum of **P**

Table 1 Performances comparison of turn on fluorescent chemosensors for Al^{3+} ion.

Fluorescence parameter	Fluorescence reagents	Reproducibility	LOD (μM)	Fluorescence quantum yield	Fluorescence lifetime	Detection media	Cell applications	Binding constant (M^{-1})	Ref.
$\lambda_{ex}/_{em}$=418/518 nm	Pyrazoline derivative	Reversible	NA	0.574	NA	H_2O-CH_3CN (1:1, v/v, pH 7.2, 20 mM HEPES)	3T3-L1, qualitative analysis	2.13×10^3	[17]
$\lambda_{ex}/_{em}$=445/525 nm	Naphthaldehyde derivative	Reversible	0.001	NA	NA	Ethanol	NA	2.5×10^3	[18]
$\lambda_{ex}/_{em}$=350/532 nm	Pyrrolidine derivative	Reversible	NA	NA	NA	CH_3CN	NA	0.87×10^4	[19]
$\lambda_{ex}/_{em}$=520/587 nm	Rhodamine derivative	Reversible	0.57	0.303	NA	H_2O-CH_3CN (3:7, v/v, pH 7.4, HEPES)	SiHa cells, qualitative analysis	1.4×10^4	[20]
$\lambda_{ex}/_{em}$ =350/526 nm	Naphthalimide derivative	Reversible	0.34	0.48	NA	H_2O-ethanol (1:1, v/v, pH 7.2, Tris-HCl)	NA	2.6×10^4	[21]
$\lambda_{ex}/_{em}$=500/582 nm	Rhodamine derivative	Reversible	0.11	0.51	NA	H_2O-ethanol (1:4, v/v, pH 7.2, 20 mM HEPES)	NA	7.03×10^3	[22]
$\lambda_{ex}/_{em}$=345/490 nm	Benzophenone azine derivative	Irreversible	0.27	NA	NA	Methanol	NA	NA	[23]
λex/em=560/584 nm	Rhodamine derivative	Reversible	0.059	NA	NA	H_2O-CH_3CN (3:7, v/v, pH 7.4, 1 mM HEPES)	SiHa cells, qualitative analysis	6.42×10^4	[24]
$\lambda_{ex}/_{em}$=510/580 nm	Rhodamine derivative	Reversible	0.16	0.45	NA	Ethanol-H_2O (9:1, v:/v, pH 5.8, 20 mM HEPES)	HepG2 cells, qualitative and quantitative analysis	6.9×10^4	This work

Cal. 1: Quantum yield of chemosensor **P**

The quantum yield (Φ) of the Al^{3+} rhodamine complex denotes the fluorescence quantum yield. It is obtained by comparison of the integrated area of the corrected emission spectrum of the sample with that of a solution of rhodamine in ethanol, which has a quantum yield of 0.89. The quantum yield (Φ) can be calculated from multiple measurements (n = 3) with the following equation, where absorbance can be obtained from the absorption spectra and ∫F can be calculated by summation of fluorescence intensity. Consequently, the quantum yield of the Al^{3+}-rhodamine complex can be calculated as 0.45.

$$\phi_{sample} = \frac{Abs_{standard}\phi_{standard}\int F_{sample}}{Abs_{sample}\int F_{standard}}$$

Cal. 2 Binding constant of Al^{3+} and chemosensor **P**

The binding constant was determined according to Benesi-Hildebrand method as follows: When assuming a 1:1 stoichiometry for interaction between chemosensor **P** and Al^{3+}, the equilibrium is given by following equation:

$$\mathbf{P} + \mathbf{Al}^{3+} \xrightarrow{k} \mathbf{P} \bullet \mathbf{Al}^{3+} \quad (1)$$

The association constant, k, is therefore expressed as:

$$k = \frac{[\mathrm{P} \bullet \mathrm{Al}^{3+}]}{[\mathrm{P}][\mathrm{Al}^{3+}]} = \frac{[\mathrm{P} \bullet \mathrm{Al}^{3+}]}{([\mathrm{P}]_0 - [\mathrm{P} \bullet \mathrm{Al}^{3+}])([\mathrm{Al}^{3+}]_0 - [\mathrm{P} \bullet \mathrm{Al}^{3+}])} \quad (2)$$

[**P**·Al^{3+}], [**P**], and [Al^{3+}] represent the equilibrium concentrations of the complex, free **P**, and free Al^{3+}, respectively. [**P**]$_0$ and [Al^{3+}]$_0$ are the initial concentrations of **P** and Al^{3+}, respectively. If [Al^{3+}]$_0$>> [**P**·Al^{3+}], the Eq. 2 can be simplified as follows:

$$k = \frac{[\mathrm{P} \bullet \mathrm{Al}^{3+}]}{([\mathrm{P}]_0 - [\mathrm{P} \bullet \mathrm{Al}^{3+}])[\mathrm{Al}^{3+}]_0} \quad (3)$$

Eq. 3 is transformed to:

$$\frac{1}{[\mathrm{P}\bullet \mathrm{Al}^{3+}]} = \frac{1}{k[\mathrm{P}]_0[\mathrm{Al}^{3+}]_0} + \frac{1}{[\mathrm{P}]_0} \quad (4)$$

Fluorescence intensity is given as follows:

$$F_0 = k_0[\mathrm{P}]_0 (5)$$

$$F = k_0[\mathrm{P}]_0 + k_\infty[\mathrm{P}\bullet \mathrm{Al}^{3+}] (6)$$

$$F_{\max} = k_0[\mathrm{P}]_{\max} + k_\infty[\mathrm{P}\bullet \mathrm{Al}^{3+}]_{\max} (7)$$

where, F_0 is the absorbance of **P** without Al^{3+}, F is the fluorescence intensity of **P** obtained with Al^{3+}, F_{max} is the fluorescence intensity of **P** in the presence of excess amount of Al^{3+}. By means of Eqs. 5, 6 and 7, the following equation is obtained:

$$\frac{F_{\max} - F_0}{F - F_0} = \frac{[\mathrm{P}\bullet \mathrm{Al}^{3+}]_{\max}}{[\mathrm{P}\bullet \mathrm{Al}^{3+}]} \quad (8)$$

In the presence of excess amount of Al^{3+}, $[\mathbf{P}\cdot Al^{3+}]_{max}$ is almost equal to $[\mathbf{P}]_0$. The Eq. 8 can therefore be replaced as follows:

$$\frac{F_{\max} - F_0}{F - F_0} = \frac{[\mathrm{P}]_0}{[\mathrm{P}\bullet \mathrm{Al}^{3+}]} (9)$$

Using Eq. 4 and 9, the Benesi-Hildebrand equation is obtained as:

$$\frac{1}{F - F_0} = \frac{1}{K(F_{\max} - F_0)[\mathrm{Al}^{3+}]_0} + \frac{1}{F_{\max} - F_0} \quad (10)$$

F_0 is the fluorescence intensity of **P** without Al^{3+}, F is the fluorescence intensity of **P** obtained with Al^{3+}, F_{max} is the fluorescence intensity of **P** in the presence of excess amount of Al^{3+}, K is the binding constant (M^{-1}) and was determined from the slope of the linear plot. Therefore, the binding constant is obtained of 6.9×10^4 M^{-1}.