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# A novel molecular probe sensing polynuclear hydrolyzed aluminum by chelation-enhanced fluorescence

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## ABSTRACT

A novel molecular probe (P2) sensing polynuclear hydrolyzed aluminum by chelation-enhanced fluorescence was described, which was prepared from 1,1'-(1,4-phenylene)bis(7-methyl-1,3,5-octanetrione) and 2-aminomethylpyridine. P2 showed a strong fluorescence response to Al(III) when excited by the optimized wavelength of 400 nm and the  $\lambda_{\text{max,em}}$  at 505 nm indicated a hypsochromic shift of 11 nm to that of free P2, but Cu(II) was observed to quench the fluorescence intensity to nearly zero. The P2–Al(III) complex indicated the best fluorescence response at a pH near 7 and there might be a relevance to the amphoteric property of the aluminum hydroxide. The emission intensity at 505 nm continued to increase until the mole ratio of [Al(III)]/[P2] reached a value of 13 and no stoichiometry was observed during the process, which implied that the sensing target of the P2 probe was not the free aluminum ion but the polynuclear hydrolyzed aluminum. The binuclear hydrolyzed aluminum compounds were selected as model target molecules to simulate the energy-minimized structure of the P2–Al(III) complex to confirm the CHEF mechanism. The enhanced fluorescent images of HeLa cells incubated with 1  $\mu\text{M}$  P2 and 5  $\mu\text{M}$  Al(III) were obtained.

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## 1. Introduction

Aluminum is the most abundant metal in earth's crust. It is frequently exposed throughout nature and extensively used in modern life [1]. Toxicological effects on human health of aluminum are well known, such as Alzheimer's disease [2] and Parkinson's disease [3]. The link between the diseases and special circumstances involved in the food chain have been studied in depth [4]. Therefore, the development of analytical methods for the facile detection of aluminum is of great importance in environmental monitoring and biochemical assays. For this purpose, several methods have been developed for the determination of aluminum, such as atomic absorption spectrometry [5] and atomic emission spectrometry (AAS and AES, respectively) [6], voltammetry [7], etc. Fluorimetry is a well known ultra-trace analytical technique, which is based on fluorescent nanoparticles [8] or fluorescent molecular probes [9], however, compared to the detection of transition metals, only limited examples of fluorimetric determination of aluminum ions were reported, including methods using naphthalene-based molecules [10], fluorescent triphenylene nanoparticles (colloids) [11] and the hybrid ligand

from a binaphthyl scaffold and an amino acid [12], by means of weak coordination interaction and strong solvation effect of aluminum ions. The macrocyclic molecule bearing the 1,2,3-triazole ring and anthraquinone spacers was recently reported to detect Al(III) by the fluorescence method [13], as well.

To avoid sophisticated and laborious synthesis of the probe, we were inspired to design a simple and conveniently prepared molecular probe which could selectively detect Al(III) by the fluorescence method. The chelation-enhanced fluorescence (CHEF) mechanism had been proposed as a design strategy to develop novel fluorescent probes for metal ions [14]. Tang et al. reported a semirigid structural di-hydroxyquinoline-based oxadiazole molecule which selectively senses Cd(II) ions through the CHEF mechanism, wherein separated conjugated units in the semirigid structure would be integrated into one rigid coplanar conjugation system via metal coordination [15]. Based on previous research on the phenylene conjugated multi-carbonyl compounds [16], they were found to be possible candidates for molecular probes with potential fluorescence property as well as selective recognition via coordination [17]. Herein, we now report P2 (Scheme 1) as a novel but simple molecular probe which could give a highly selective fluorescent response to Al(III). The probe was prepared from 1,1'-(1,4-phenylene)bis(7-methyl-1,3,5-octanetrione) and 2-aminomethylpyridine through a condensation reaction, which was deliberately to function as the semirigid structure in the CHEF process.

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## 2. Experimental

### 2.1. General

Chemicals were commercially available and used directly without further purification.  $^1\text{H}$  NMR spectra were recorded on an AVANCE200 NMR Spectrometer (Bruker) in  $\text{CDCl}_3$  using  $(\text{CH}_3)_4\text{Si}$  as an internal standard, and  $J$  values were given in Hz. LC–MS was performed on an HP 1100 HPLC (Agilent), interfaced with a triple–quadrupole mass spectrometer equipped with an ESI ion source and an in-line diode-array ultraviolet–visible detector. HR–ESIMS were recorded on an Acquity UPLC–QToF–MS Premier instrument (Waters) operating in the positive electrospray ionization mode.

### 2.2. Synthesis procedure

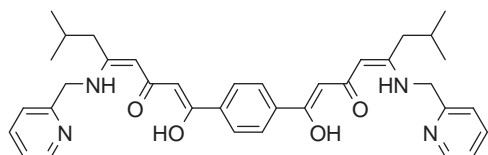
1,1'-(1,4-Phenylene)bis(7-methyl-1,3,5-octanetrione) was prepared via the literature reported method [17]. Synthesis of P2 involved condensations. 0.108 g (1 mmol) of 2-(aminomethyl)pyridine and 0.5 mL pyridine were added to 0.166 g (0.4 mmol) of 1,1'-(1,4-phenylene)bis(7-methyl-1,3,5-octanetrione) dissolved in 10 mL ethanol. The yellow solution was refluxed for 6 h and allowed to cool to room temperature. A yellow solid appeared when cooling slowly after the reaction. This product was purified by crystallization from dichloromethane–ethanol solution to give 0.22 g (93%) yellow crystals.  $^1\text{H}$  NMR ( $\delta$ , ppm/ $\text{CDCl}_3$ ): 15.90(2H, br), 10.98(2H, t), 8.59(2H, m), 7.81(4H, m), 7.72(2H, m), 7.32(2H, m), 7.23(2H, m), 5.88(2H, s), 4.95(2H, s), 4.67(4H, d,  $J=6.36$  Hz), 2.16(4H, d,  $J=7.16$  Hz), 1.90(2H, m), 0.99(12H, d,  $J=6.46$  Hz). ESIMS (ESI+): 298.2((M+2H)/2), 595.4(M+H). HR–ESIMS(ESI+): 595.3276 (calculated for  $\text{C}_{36}\text{H}_{43}\text{N}_4\text{O}_4$ , [M+H]: 595.3284).

### 2.3. Fluorescence measurement

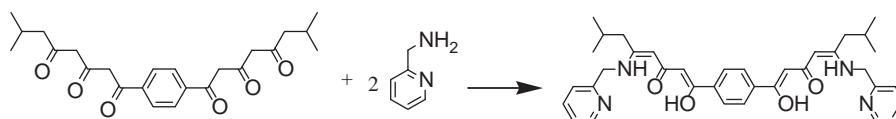
The fluorescence spectra were measured in standard quartz cuvettes on an LS 50B spectrofluorimeter (Perkin Elmer). The relative fluorescence quantum yield was calculated from the integrated area under the corrected emission curve and the Rhodamine B served as a reference [18]. The salts were  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ,  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ ,  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ,  $\text{CsCl}$ ,  $\text{Cu}(\text{NO}_3)_2 \cdot 2.5\text{H}_2\text{O}$ ,  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ,  $\text{KNO}_3$ ,  $\text{NaCl}$ ,  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Sb}(\text{NO}_3)_3$ ,  $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{HgCl}_2$  and  $\text{Pb}(\text{NO}_3)_2$ .

### 2.4. Geometry optimization

The geometry optimization was carried out in the commercially available computer program package (HyperChem Pro. Release 8.0 from Hypercube Inc., USA). The semi-empirical AM1



**Scheme 1.** Novel molecular probe (P2) designed to sense metal ions.



**Scheme 2.** Synthesis of molecular probe (P2) by condensation reaction between 1,1'-(1,4-phenylene)bis(7-methyl-1,3,5-octanetrione) and 2-(aminomethyl)pyridine.

method was used for the geometry optimization of the free probe (P2), UHF was used and in SCF controls, the accelerate convergence was selected and the convergence limit was set as 0.01; in optimization calculation, the algorithm of Polak–Ribiere (conjugated gradient) was adopted and as termination condition, RMS gradient was set as 0.01 kcal/mol. The molecular mechanics MM+ method was used for the geometry optimization of the complex of P1–Al(III), the algorithm of Polak–Ribiere (conjugated gradient) was adopted and as termination condition, RMS gradient was set as 0.001 kcal/mol.

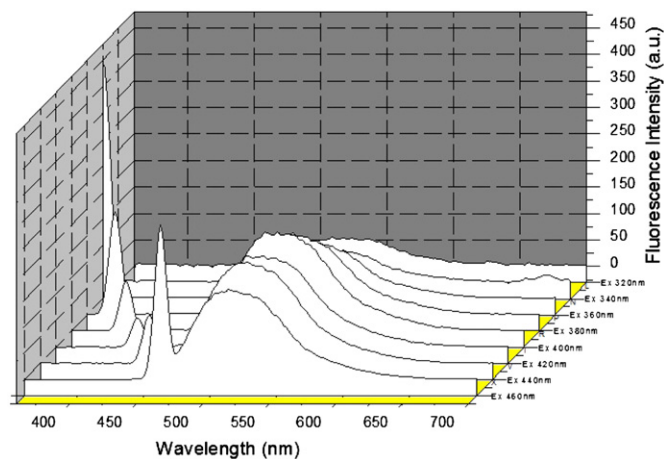
### 2.5. Imaging of HeLa cells incubated with P2 and Al(III)

The HeLa cells were seeded in 24-well flat-bottomed plates, 1 day before imaging. The next day, cells were incubated with P2 (1  $\mu\text{M}$ ) for 30 min at 37  $^\circ\text{C}$ , washed with PBS buffer (pH 7.4) to remove the remaining probe, then the treated cells were incubated with 5  $\mu\text{M}$  Al(III) in culture medium for 20 min at 37  $^\circ\text{C}$ . The fluorescent images of cells were obtained on an Olympus IX71 inverted fluorescence microscope (Olympus Optical Co., Tokyo, Japan) equipped with a 100 W mercury lamp for epi-illumination. The emission from the sample was collected and separated from Rayleigh- and Raman-shifted light by a combination of filters; an excitation filter 425DF45, a dichroic mirror 475DCLP, and an emission filter 530DF30 (Omega Optical, Inc., USA) and then focused into a cooled 16-bit CCD camera (Cascade 650, Photometrics, Tucson, AZ). Image acquisition and processing were performed with the MetaMorph imaging software (Universal Imaging, Downingtown, PA). All measurements were performed at room temperature.

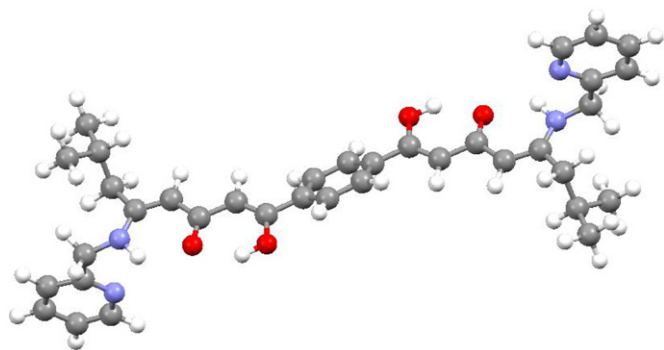
## 3. Results and discussion

### 3.1. Synthesis and characterization of P2 probe

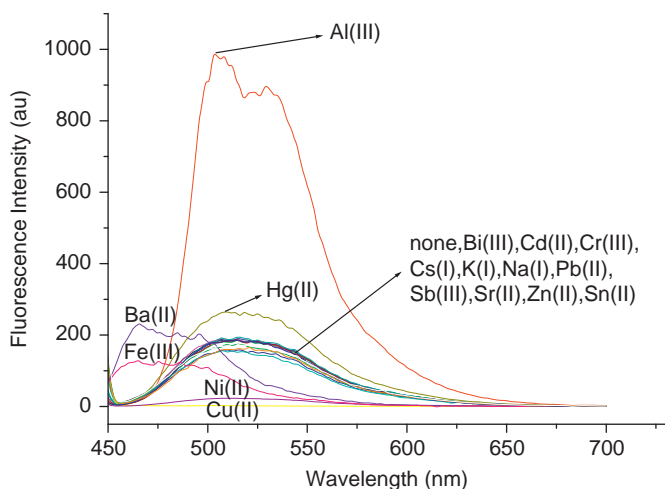
Synthesis involved a typical condensation between one equivalent of 1,1'-(1,4-phenylene)bis(7-methyl-1,3,5-octanetrione) and



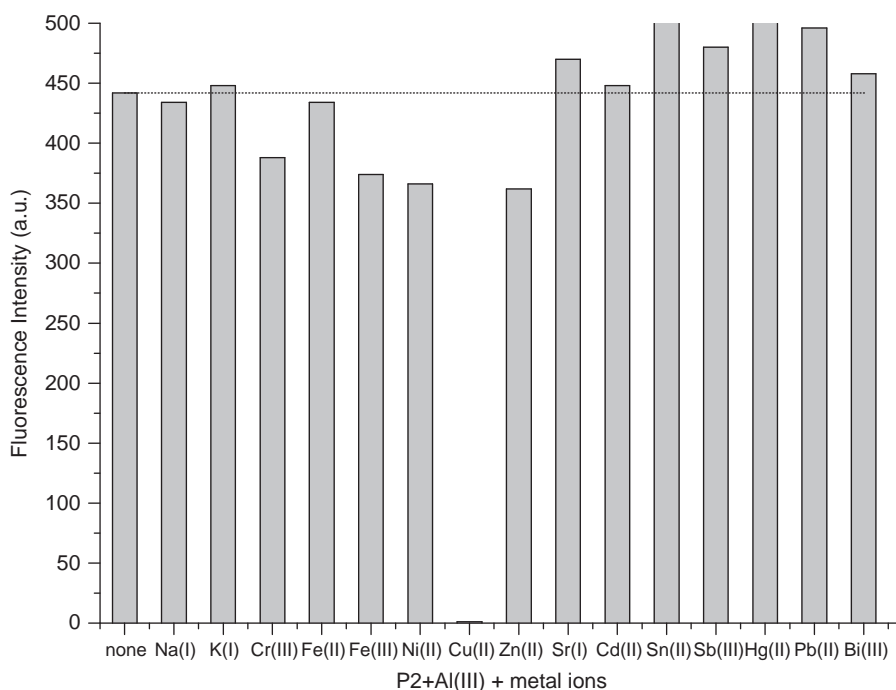
**Fig. 1.** 3D fluorescence spectrum of the free P2 probe (20  $\mu\text{M}$ ) in THF–methanol (1/4, v/v, pH 7.0) at an excitation wavelengths from 240 to 460 nm.



**Fig. 2.** Configuration for the energy-minimized structure of the free P2 (key: gray—C, red—O, white—H, and blue—N). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 3.** Fluorescence spectra of P2 (20  $\mu\text{M}$ ) in the presence of different metal ions (80  $\mu\text{M}$ ) in THF-methanol (1/4, v/v, pH 7.0) at an excitation wavelength of 400 nm.



**Fig. 4.** Fluorescence responses of P2 (10  $\mu\text{M}$ ) at 505 nm containing Al(III) (40  $\mu\text{M}$ ) to different metal ions (40  $\mu\text{M}$ ) in THF-methanol (1/4, v/v, pH 7.0) at an excitation wavelength of 400 nm.

two equivalents of 2-(aminomethyl)pyridine (Scheme 2). Generally, the addition of primary amines to ketones gives the hemiaminals and immediate loss of water gives the enamines, then the target probe (P2) was obtained as a dominant product. The reaction proceeded completely with a yield of nearly 100% and no intermediate (including mono condensation product) was detected by The LC-MS method. The ESI mass spectrum of P2 indicated a weak parent ion peak of 595.4(m/z) as  $M+H$ , but a strong doubly charged ion peak of 298.2(m/z) as  $(M+2H)/2$ . The product was further confirmed using the method of HR-ESIMS (found: 595.3276; calculated for  $C_{36}H_{43}N_4O_4$ ,  $[M+H]$ : 595.3284), too. In the  $^1\text{H}$  NMR spectrum in deuteriochloroform, P2 existed almost entirely in the enol form which was confirmed by the presence of broad resonances at 15.90(enamine proton) and 10.98 ppm (enol proton), as well as the presence of sharp singlets at 5.88 and 4.95 ppm, each integrating to two protons and corresponding to the  $-\text{CH}=\text{}$  groups adjacent to the enamine and enol groups.

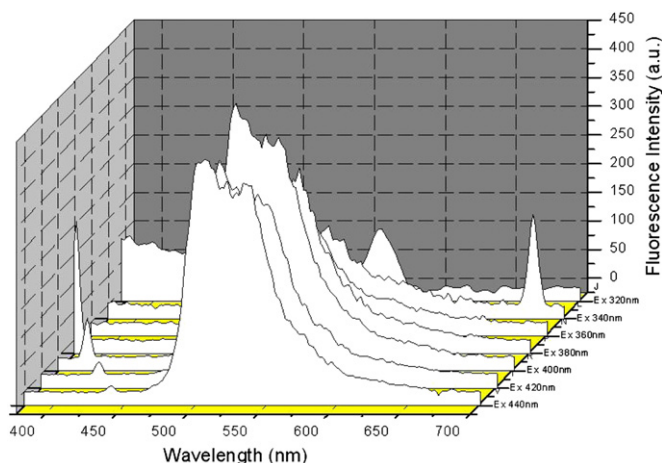
Total fluorescence characterization of the free P2 probe was shown in Fig. 1 in 3D spectrum at excitation wavelengths from 240 to 460 nm. In the fluorescence emission, P2 exhibited a broad peak at the  $\lambda_{\text{max,em}}$  of 516 nm and the fluorescence intensity reached 187 a.u. when excited by light of 400 nm after optimization. However, the fluorescence peaks were relatively weak and inundated by the noise of the resonance fluorescence and Rayleigh scattering of the excitation light to some extent. The geometric simulation of free P2 molecules was carried out by the semi-empirical AM1 method. The total molecular configuration indicated as the centrosymmetric 'trans' conformation and the enol tautomer were optimized as the energy-minimized structure (Fig. 2). The carbonyl group acted as the bridge connecting the imine and enol groups nearby via intramolecular hydrogen bonds into two 6-member rings, which fused with each other, constructing a conjugated plane. However, the coplanarity between the fused rings and the phenylene was not perfect but indicated a dihedral angle of  $30.1^\circ$ , which was similar to that in the crystal structure of its precursor molecule [19]. The torsion

between these conjugated units further prevented from constructing a larger conjugation system and thus led to the low fluorescence intensity of the molecule. Therefore, P2 appeared to be a promising semirigid candidate for CHEF if coplanarity between separated conjugated units was improved upon binding suitable metal ions.

### 3.2. Selective fluorescent sensing of Al(III) by P2

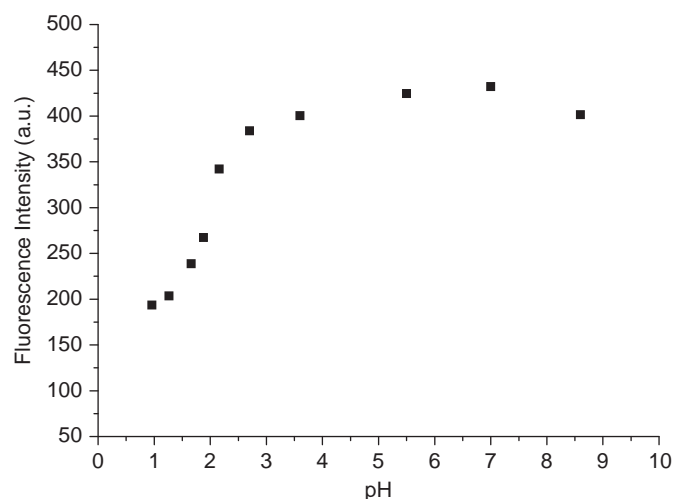
To assess the specificity of the fluorescent sensing, various metal ions were examined in parallel under the same experimental condition. As shown in Fig. 3, P2 showed the strongest fluorescent response to Al(III) and the intensity was distinctly increased to five times when excited by light of 400 nm. Upon addition of Al(III), the  $\lambda_{\max,em}$  indicated a small hypsochromic shift from 11 nm to 505 nm with a relative quantum yield of 0.12. The combination of P2 with Hg(II) had a small fluorescence increase, and there was nearly no effect on the fluorescent curve of P2 by addition of the other metal ions including Bi(III), Cd(II), Cr(III), Cs(I), K(I), Na(I), Pb(II), Sb(III), Sr(II), Zn(II) and Sn(II). Combination of P2 with Ba(II) and Fe(III) did not indicate significant changes of the fluorescence intensities, but large hypsochromic shifts of about 50 nm. However, Ni(II) and Cu(II) obviously reduced the fluorescent response, which indicated quenching effects rather than the CHEF mechanism on this fluorescent probe. Therefore, it could be concluded that P2 probe displayed selective sensitivity to Al(III) instead of the other ions examined. The Cu(II) quenched the fluorescence intensity so thoroughly as to nearly reach zero. Probably, the  $d^9$  electronic configuration of the Cu(II) ions played an important role in the quenching effect in which the electron transition process inducing the fluorescence emission was blocked by orbital overlap between the fluorophore and Cu(II) according to the Dexter energy transfer mechanism [20]. The competition experiments of the P2–Al(III) complex mixing with other metal ions showed that no significant variation is observed in fluorescence intensity except in the case of Cu(II), which quenched the fluorescence intensity to nearly zero (Fig. 4). It was inferred that Cu(II) showed much stronger competition than Al(III) and other metal ions during combination with P2, which was due to the more sufficient orbital overlap by Cu(II)'s elongated-octahedral electronic configuration as a Jahn–Teller effect [21].

The 3D spectrum of the P2–Al(III) complex is shown in Fig. 5 and P2–Al(III) exhibited a broad emission peak centered at 505 nm at the optimized excitation wavelength of 400 nm.

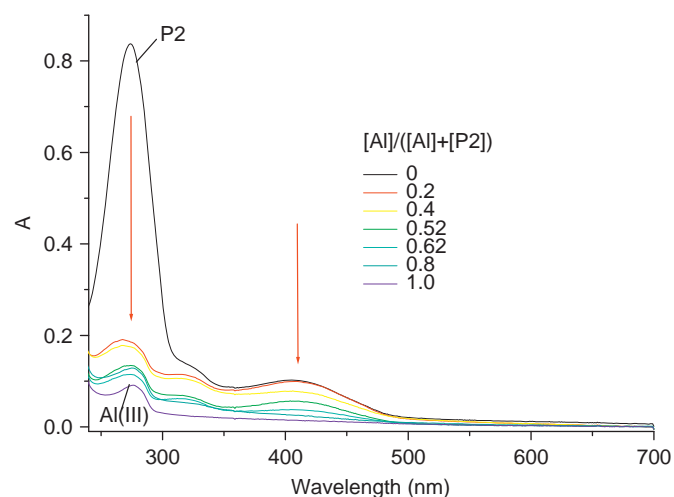


**Fig. 5.** 3D fluorescence spectrum of the P2 (10  $\mu$ M) in the presence of Al(III) ion (40  $\mu$ M) in THF-methanol (1/4, v/v, pH 7.0) using excitation wavelengths from 320 to 440 nm.

The fluorescence emission at the optimized excited condition was strong enough to ignore the noise from the resonance fluorescence or Rayleigh scattering. Fluorescent probes based on coordination are usually disturbed by protons during the detection of metal ions, so it is necessary to study the pH effect on the fluorescence emission of the P2–Al(III) complex. The fluorescent response of the P2–Al(III) complex toward pH values was investigated in THF-methanol (1/4, v/v) mixed solvent in search of this optimal sensing condition. As a experimental result, the P2–Al(III) complex indicated the best fluorescent response at a pH near 7 (Fig. 6), and the emission intensity at 505 nm gradually decreased by increasing either the acid or base concentration. Thus, this optimization experiment enabled us to select neutral pH for the P2 probe to detect Al(III). Furthermore, what merits our special attention is that there might be an interesting relevance to the amphoteric property of the aluminum hydroxide in which the polynuclear polymer had the best forming condition at the strict neutral pH value. Thus the real sensing target of P2 might be the polynuclear aluminum hydroxide, not the free aluminum ion itself, and this could be an example to distinguish polynuclear



**Fig. 6.** Plot of fluorescence emission at 505 nm of the P2 (10  $\mu$ M) in the presence of Al(III) ion (40  $\mu$ M) versus pH in THF-methanol (1/4, v/v) at an excitation wavelength of 400 nm.

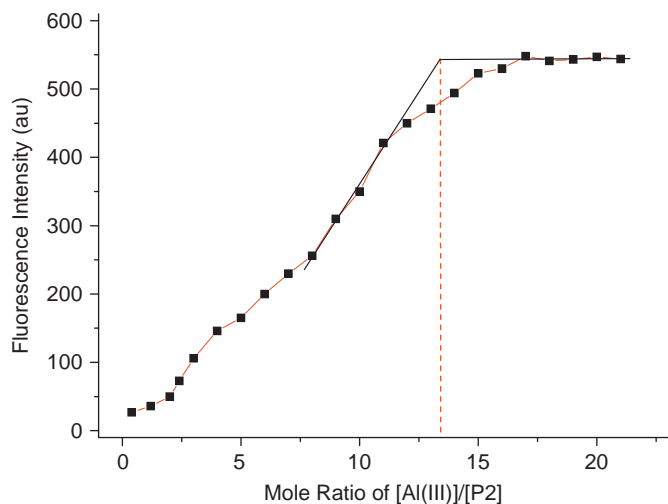


**Fig. 7.** UV-vis spectra of P2–Al(III) in THF-methanol (1/4, v/v, pH 7.0) in the presence of increasing mole ratios of  $[Al]/([Al]+[P2])$  as 0, 0.2, 0.4, 0.52, 0.62, 0.8 and 1.0, successively.  $[Al]+[P2]=10 \mu$ M.

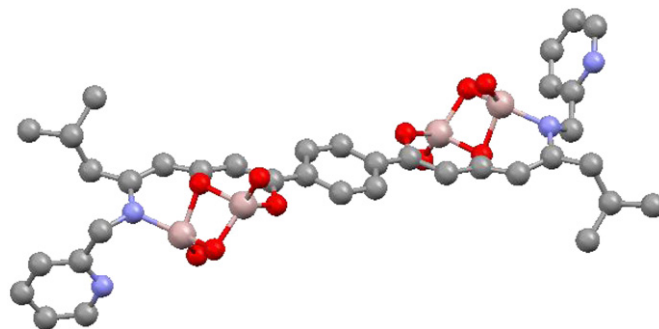
aluminum hydroxide from free aluminum ion by the fluorescence method.

To gain more chemical insight into the sensing mechanism of P2 towards Al(III), a Job plot by the UV–vis experiment was carried out to determine the stoichiometry of the binding event; however, no characteristic stoichiometry was observed in the UV–vis spectra of P2–Al(III) in THF–methanol (Fig. 7). The absorption peak at 275 nm decreased rapidly in the presence of

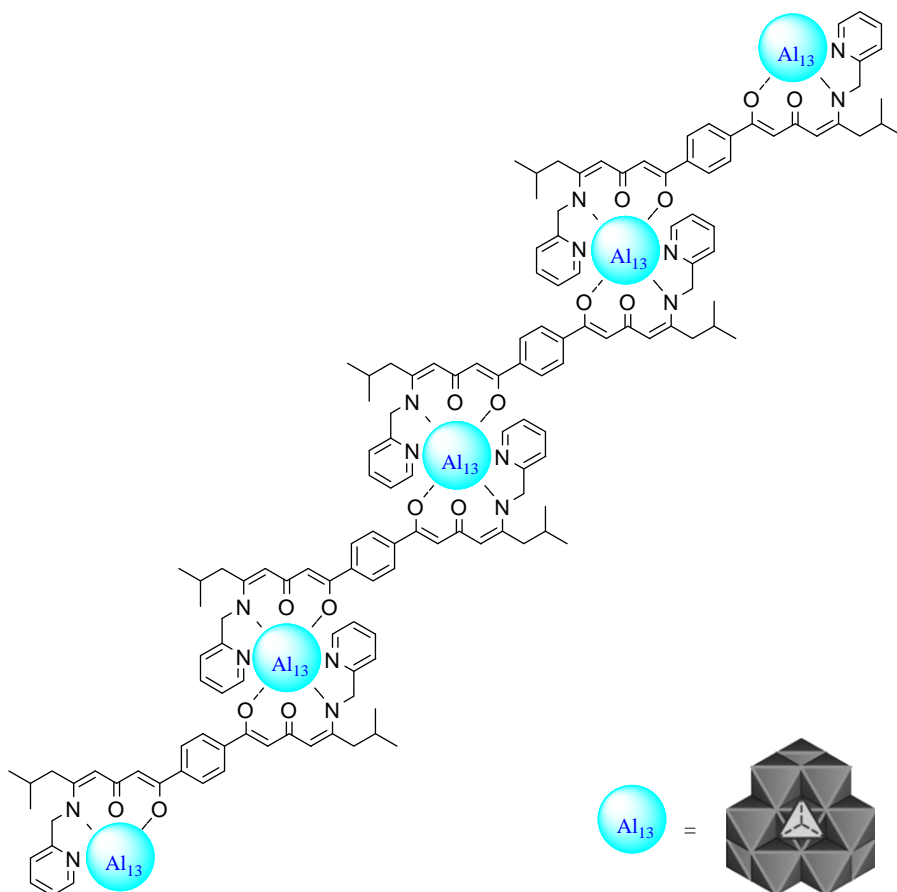
increasing mole ratio of  $[Al]/([Al]+[P2])$ , while the absorption peak at 400 nm changed slightly until diluted to near zero. Upon gradual addition of Al(III) to P2 solution in a fluorescence titration experiment, the emission intensity at 505 nm increased, and the ratiometric study also indicated quite different from other metal detecting probes through the CHEF mechanism reported in recent years which typically had the stoichiometry of metal/probe mole ratio of 1:1 [22] or 1:2 [15] during formation of the probe–metal complex. The emission intensity at 505 nm continued to increase until the mole ratio of  $[Al(III)]/[P2]$  reached a remarkable value near 13 (Fig. 8) and no obvious turning point seemed to appear before it. The continual growth of high metal/probe mole ratio of stoichiometry during spectroscopic titration confirmed that the



**Fig. 8.** Plot of fluorescence emission at 505 nm of the P2(5  $\mu$ M)–Al(III) complex versus the mole ratio of  $[Al(III)]/[P2]$  in THF–methanol (1/4, v/v) at an excitation wavelength of 400 nm.

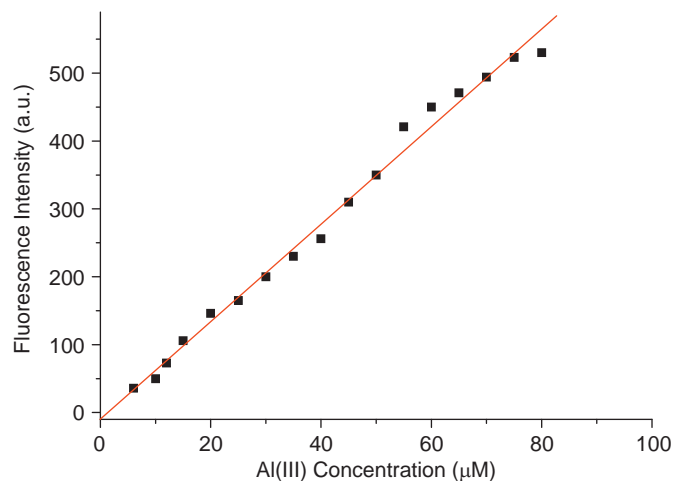


**Fig. 10.** Configuration for the energy-minimized structure of P2 complex with the binuclear hydrolyzed aluminum (key: gray—C, red—O, pink—Al, and blue—N; the hydrogen atoms were omitted for clarity). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 9.** Proposed terrace-like supramolecule assembled by P2 and  $\epsilon$ -Al<sub>13</sub>.

sensing target of the P2 probe was a series of hydrolyzed polynuclear aluminum compounds ( $\text{Al}_p(\text{OH})_q^{(3p-q)+}$ ) with different degrees of polymerization. To go a step further, the ratiometric value of 13 obtained from the fluorescence titration experiment (Fig. 8) inspired us to propose the tridecanuclear aluminum hydrolyzed compound (Baker–Figgis–Keggin structure) as the target molecule sensitized by P2, which was one of the most prevailing polynuclear aluminum hydrolyzed forms in solution [23]. The  $\epsilon$  isomer of the Baker–Figgis–Keggin structure ( $\epsilon\text{-Al}_{13}$ ) was then selected as a sensing example to illustrate the assembling configuration between P2 and  $\epsilon\text{-Al}_{13}$  (Fig. 9), which displayed as an infinite terrace-like supramolecule.

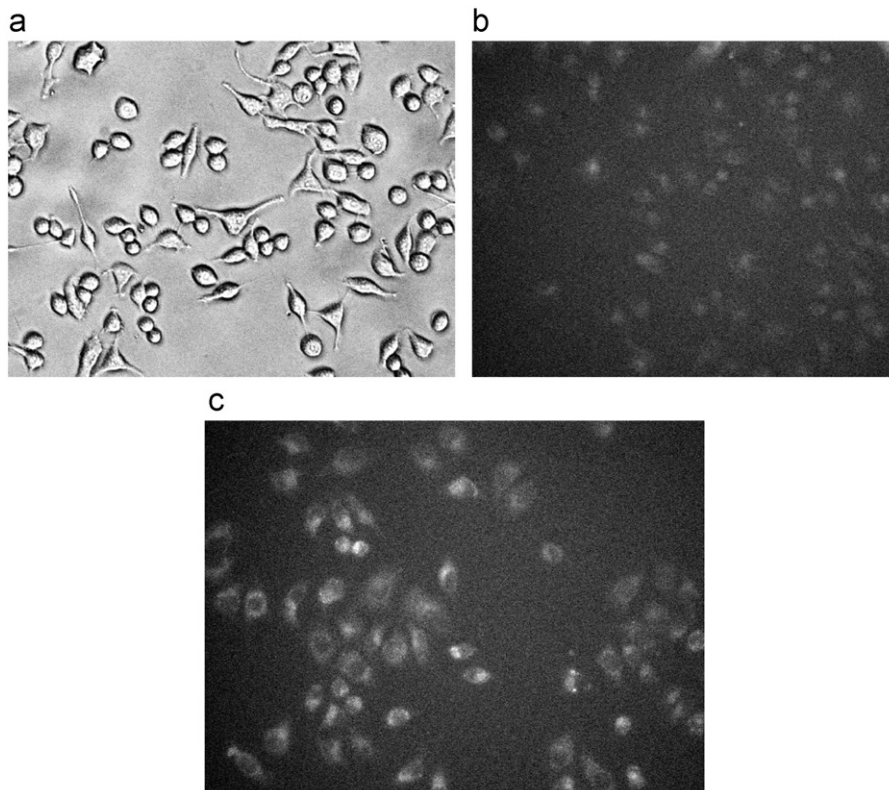


**Fig. 11.** Fluorescence emission changes at 505 nm upon addition of Al(III) (10–80  $\mu\text{M}$ ) to THF–methanol (1/4, v/v, pH 7.0) solution of P2 at a concentration of 5  $\mu\text{M}$ . Excitation wavelength is 400 nm.

The attempt to prepare a single crystal of the P2–Al(III) complex for three-dimensional molecular structure study was carried out but did not succeed. Solid state isolation of the hydrolyzed polynuclear aluminum complexes from solutions usually presented difficulties because of their kinetic lability and high rates of ligand exchange [24], and then the MM+ calculation method in the HyperChem 8.0 package was used to simulate the molecular geometry. Firstly, the binuclear hydrolyzed aluminum compound was selected as a model target molecule and the configuration for the energy-minimized structure of the P2–Al(III) complex is shown in Fig. 10. The carbonyl group acted as the bridge, too, connecting the two aluminum atoms. The imine and enol groups coordinated with the aluminum atoms as well, but the pyridine moiety which was intentionally introduced could not act as an additional dentate coordinating to the Al(III) atom possibly due to the high tension in its structure. The two fusing 6-member rings constructed by the binuclear hydrolyzed aluminum did not keep coplanarity to each other anymore, but one of them became coplanar to the middle phenylene moiety with a dihedral angle of only  $0.3^\circ$ . Obviously, the fluorescence intensity could be increased by the enlargement of conjugation system compared to that of the free P2 molecule. Meanwhile in the electronic structure of P2, the radiationless process via  $n\pi^*$  state induced by  $N$  lone electron pairs was blocked by Al(III) coordination, and this further contributed to the fluorescence enhancement.

### 3.3. The detection limit and cell imaging

Meanwhile, under the optimized conditions, the fluorescence intensity of the solution of P2 was nearly proportional to the amount of Al(III) added in the concentration range of 10–80  $\mu\text{M}$  (Fig. 11), and the equation of linear regression was expressed as  $F = -9.746 + 7.181C$ , with a correlation coefficient of 0.9953.



**Fig. 12.** Fluorescence images of Al(III) in HeLa cells incubated with P2; (a) bright-field transmission image of cells incubated with 1  $\mu\text{M}$  P2, (b) fluorescence image of cells incubated with 1  $\mu\text{M}$  P2, excited by 400 nm light, and (c) fluorescence image of cells incubated with 1  $\mu\text{M}$  P2 and 5  $\mu\text{M}$  Al(III), excited by 400 nm light.

The detection limit was estimated to be 0.3  $\mu\text{M}$  which was quantified using 3 standard deviations above the mean background.

We then evaluated bio-imaging applications of P2 for detection of Al(III) in biological systems. The enhanced fluorescent images of HeLa cells incubated with 1  $\mu\text{M}$  P2 and 5  $\mu\text{M}$  Al(III) were obtained on an Olympus IX71 inverted fluorescence microscope excited by ultraviolet light at 400 nm (Fig. 12). Interestingly, the distribution of different brightnesses in the fluorescence images indicated that the permeability of Al(III) could only reach the superficial region of the cell. The result indicated that P2 probe could be potentially useful for the study of Al(III) bioactivity in living cells.

#### 4. Conclusions

In conclusion, we have described the design, synthesis, and characterization of a novel molecular probe sensing polynuclear hydrolyzed aluminum by chelation-enhanced fluorescence. P2 showed a strong fluorescence response to Al(III) when excited at the optimized wavelength of 400 nm and the  $\lambda_{\text{max,em}}$  at 505 nm indicated a hypsochromic shift of 11 nm to that of free P2, but Cu(II) was observed to quench the fluorescence intensity to nearly zero. The P2–Al(III) complex indicated the best fluorescence response at a pH near 7, and there might be a relevance to the amphoteric property of the aluminum hydroxide. The stoichiometry implied that the sensing target of the P2 probe was not the free aluminum ion but polynuclear hydrolyzed aluminum. The energy-minimized structure of P2 complex with the polynuclear hydrolyzed aluminum was simulated to confirm the CHEF mechanism. The enhanced fluorescent images of HeLa cells incubated with 1  $\mu\text{M}$  P2 and 5  $\mu\text{M}$  Al(III) were obtained.

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