An Acylhydrazone-Based Fluorescent Sensor for Sequential Recognition of Al$^{3+}$ and H$_2$PO$_4^-$

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Abstract: A novel acylhydrazone-based fluorescent sensor NATB was designed and synthesized for consecutive sensing of Al$^{3+}$ and H$_2$PO$_4^-$ . NATB displayed fluorometric sensing to Al$^{3+}$ and could sequentially detect H$_2$PO$_4^-$ by fluorescence quenching. The limits of detection for Al$^{3+}$ and H$_2$PO$_4^-$ were determined to be 0.83 and 1.7 µM, respectively. The binding ratios of NATB to Al$^{3+}$ and NATB-Al$^{3+}$ to H$_2$PO$_4^-$ were found to be 1:1. The sequential recognition of Al$^{3+}$ and H$_2$PO$_4^-$ by NATB could be repeated consecutively. In addition, the practicality of NATB was confirmed with the application of test strips. The sensing mechanisms of Al$^{3+}$ and H$_2$PO$_4^-$ by NATB were investigated through fluorescence and UV–Visible spectroscopy, Job plot, ESI-MS, $^1$H NMR titration, and DFT calculations.

Keywords: aluminum ion; dihydrogen phosphate; acylhydrazone; fluorescent chemosensor; sequential detection; calculations

1. Introduction

Al$^{3+}$, the third most abundant metallic element in nature [1,2], is broadly employed in daily life in packaging materials, pharmaceuticals, food additives, machinery, clinical medicines, and water purification [3,4]. Owing to its widespread usage, Al$^{3+}$ could be readily accumulated in the body, which leads to the development of diverse diseases such as Parkinson’s and Alzheimer’s disease [5,6]. Dihydrogen phosphate (H$_2$PO$_4^-$) is an important component related to many intercellular activities, such as signaling mediation, protein phosphorylation, enzymatic reactions, ion-channel regulation, and so on [7–9]. However, excessive agricultural use of phosphate causes eutrophication or massive algal growth, leading to a deficiency in oxygen levels [10–12]. For these reasons, there has been a strong demand for the development of sensing and detection methods for Al$^{3+}$ and H$_2$PO$_4^-$.

The traditional analytical methods reported for the analysis of cations and anions, such as ICP-AES, AAS, and electrochemical methods, have been largely restricted due to their expensive instruments, complicated procedures, and the need for highly trained operators [13–15]. In contrast, fluorescence methods have shown the advantages of cost-effectiveness, simplicity, easy operation, and high sensitivity [16–18]. While numerous fluorescent chemosensors for a single analyte have been reported, fluorescent chemosensors that allow the sequential sensing of multiple analytes with great selectivity and sensitivity are still needed [19–21] because they are more cost-effective, recyclable and practical [22–24]. Several fluorescent sensors have been addressed for consecutive sensing of Al$^{3+}$ and several anions [25–28] or several cations and H$_2$PO$_4^-$ [29–31]. In addition, Kumar et al. reported a fluorescent sensor for sequential sensing of Al$^{3+}$ and H$_2$PO$_4^-$/HSO$_4^-$ [32]. The practical importance of sequential sensing may have potential applications such as logic gates and molecular switches. Nevertheless, a sequential fluorescent sensor that can exclusively detect Al$^{3+}$ and H$_2$PO$_4^-$ has not been reported to date.
As Al$^{3+}$ is a hard cation, chemosensors containing hard base units, such as nitrogen or oxygen atoms, prefer to coordinate with Al$^{3+}$ [33–35]. In this regard, acylhydrazone derivatives, having oxygen and nitrogen atoms, are expected to be a suitable functional group to design an Al$^{3+}$ chemosensor [36–38]. Naphthalene moieties have been widely applied for the design of fluorescent sensors because of their excellent photophysical properties as a fluorophore [39–41]. Hence, we expected that a compound including both acylhydrazone and naphthalene may operate as a fluorescence chemosensor for Al$^{3+}$.

In the current study, we designed an acylhydrazone-based fluorescent sensor, NATB, which showed green fluorescence emissions with Al$^{3+}$ and could sequentially detect H$_2$PO$_4^-$ through fluorescence quenching with high sensitivity and selectivity. A sensing mechanism of NATB to Al$^{3+}$ and H$_2$PO$_4^-$ was illustrated by fluorescence and UV–Vis spectroscopy, Job plot, ESI-MS, $^1$H NMR titration, and calculations.

2. Experimental Section

2.1. Materials and Equipment

All solvents and reagents were commercially obtained from TCI (TCI, Nihonbashi-Honcho, Tokyo, Japan) and Sigma-Aldrich (MilliporeSigma, Burlington, MA, USA). NMR experiments were conducted using DMSO-$d_6$ as the solvent, and the data were recorded on a Varian spectrometer (Varian, Palo Alto, CA, USA). Fluorescence and UV–Visible spectra were measured with Perkin Elmer machines (Perkin Elmer, Waltham, MA, USA). The quantum yields of NATB and NATB-Al$^{3+}$ were relatively determined with quinine ($\Phi = 0.54$ in $1 \times 10^{-3}$ M H$_2$SO$_4$) as a reference. ESI-MS data were recorded on a Thermo Finnigan machine (Thermo Finnigan LLC, San Jose, CA, USA).

2.2. Synthesis of N$^\prime$-[(E)-(3-tert-butyl-2-hydroxyphenyl)methylidene]-3-hydroxynaphthalene-2-carbohydrazide (NATB)

The intermediate compound, 3-hydroxy-2-naphthohydrazide (1), 1.8 mmol) and 3-hydroxy-2-naphthohydrazide (2, 0.3 mmol) were mixed in absolute EtOH (10 mL) with a catalytic amount of HCl and stirred at room temperature for 1 day. A yellow precipitate was filtered, rinsed with cold absolute EtOH, and dried (77.2 mg, 70.1%); $^{13}$C NMR in DMSO-$d_6$: $\delta$ 163.23 (1C), 156.90 (1C), 153.75 (1C), 151.45 (1C), 135.84 (1C), 130.31 (1C), 129.53 (1C), 128.20 (1C), 125.75 (1C), 123.75 (1C), 119.86 (1C), 118.70 (1C), 115.74 (1C), 110.54 (1C), 77.32 (1H), 7.30 (d, 1H), 7.77 (1H), 1.43 (s, 9H), 8.63 (s, 1H), 8.45 (s, 1H), 7.93 (d, 1H), 7.77 (d, 1H), 7.53 (t, 1H), 7.38 (t, 1H), 7.35 (s, 1H), 7.32 (d, 1H), 7.20 (d, 1H), 6.91 (t, 1H), 1.43 (s, 9H). $^1$H NMR titration, and calculations.

2.3. Preparation of Spectroscopic Experiments

An NATB stock (10 mM) was prepared in DMSO. The stock solutions (20 mM) of varied cations were prepared using their nitrate salts (Al$^{3+}$, Na$^+$, Cr$^{3+}$, Fe$^{2+}$, Ca$^{2+}$, Cd$^{2+}$, Zn$^{2+}$, Pb$^{2+}$, Co$^{2+}$, Cu$^{2+}$, In$^{3+}$, Mn$^{2+}$, Ga$^{3+}$, Ni$^{2+}$, Mg$^{2+}$, Ag$^+$, Hg$^{2+}$ and K$^+$) or perchlorate salt (Fe$^{3+}$). The concentrated solutions (20 mM) of varied anions were prepared using their tetrabutylammonium salts (H$_2$PO$_4^-$, SCN$^-$, BzO$^-$, N$^3_3$, OAc$^-$, and NO$_2^-$), tetrathyrammonium salts (F$^-$, Cl$^-$, Br$^-$, I$^-$ and CN$^-$), sodium salts (S$^{2-}$ and ClO$^-$), or potassium salts (HPO$_4^{2-}$, PO$_4^{3-}$, HSO$_4^-$ and P$_2$O$_5^{4-}$ (PPi)). All spectroscopic experiments were conducted in MeOH.

2.4. Competitive Experiments

For Al$^{3+}$, 6 µL (10 mM) of an NATB stock in DMSO was mixed into MeOH (2 mL) to make 30 µM. A total of 4.5 µL of various cations (20 mM) in DMF was diluted in NATB to make 45 µM. Finally, 4.5 µL (20 mM) of an Al$^{3+}$ stock in DMF was mixed into each solution to produce 45 µM, and their fluorescent spectra were measured.
For $\text{H}_2\text{PO}_4^-$, 6 µL (10 mM) of an NATB stock in DMSO and 4.5 µL (20 mM) of an $\text{Al}^{3+}$ stock in DMF were diluted into MeOH (2 mL) to produce 30 µM of NATB-$\text{Al}^{3+}$. We added 4.5 µL of various anions (20 mM) in $\text{H}_2\text{O}$ to NATB-$\text{Al}^{3+}$ to produce 45 µM. A total of 4.5 µL (20 mM) of an $\text{H}_2\text{PO}_4^-$ stock was diluted into each solution to produce 45 µM. Their fluorescent spectra were measured.

2.5. Determination of Association Constant ($K$)

The association constant ($K$) was calculated using Li’s method [43]. If the ligand ($L$) and the analyte ($M$) form an $m$-$n$ complex, $M_mL_n$, the equilibrium constant of the corresponding complex, $K$, can be expressed by the following equation:

$$[M]^m = \frac{1}{nK} \frac{1}{[L]^n} \frac{1 - \alpha}{\alpha^n}$$

where,

$[M] = \text{the concentration of analyte}$

$[L]_T = \text{the total concentration of ligand}$

and $\alpha$ could be described as:

$$\alpha = \frac{I - I_{\text{max}}}{I_{\text{min}} - I_{\text{max}}}$$

where,

$I = \text{the fluorescence intensity of complex}$

2.6. Calculations

Calculations were achieved with the Gaussian 16 program [44]. Optimal geometries of NATB and NATB-$\text{Al}^{3+}$ were provided with the DFT method [45,46]. B3LYP was selected as the hybrid functional basis set. The 6–31G(d,p) basis set was implemented to all atoms except $\text{Al}^{3+}$ [47,48], and the LANL2DZ basis set was employed for applying ECP to $\text{Al}^{3+}$ [49–51]. No imaginary frequency was found in the optimized states of NATB or NATB-$\text{Al}^{3+}$, indicating their local minima. The solvent effect of MeOH was considered with IEFPCM [52]. Based on the energy-optimized structures of NATB and NATB-$\text{Al}^{3+}$, the plausible UV–Vis transition states were calculated by the TD-DFT method with 20 lowest singlet states.

3. Results and Discussion

The synthesis of NATB was conducted as depicted in Scheme 1. The condensation reaction of 3-(tert-buty1)-2-hydroxybenzaldehyde (1) and 3-hydroxy-2-naphthohydrazide (2) produced the desired product, $N'$-[E-(3-tert-butyl-2-hydroxyphenyl)methylidene]-3-hydroxynaphthalene-2-carbohydrazide (NATB), which was verified with $^1\text{H}$ NMR, $^{13}\text{C}$ NMR (Figures S1 and S2), and ESI-MS.

![Scheme 1. Synthesis of NATB.](image-url)
3.1. Spectroscopic Examination of NATB to Al\(^{3+}\)

To confirm the fluorescence selectivity of NATB, the fluorescence emission was studied with a variety of cations in MeOH (Figure 1). As a result, NATB exhibited notable fluorescence emission at 526 nm with Al\(^{3+}\), while NATB and NATB with other cations showed negligible or no fluorescence emission (\(\lambda_{\text{ex}} = 358\) nm). These outcomes demonstrated that NATB could be utilized as a fluorescent probe for the selective sensing of Al\(^{3+}\). On the other hand, NATB was soluble in aqueous media, but it did not show any selectivity to Al\(^{3+}\). In addition, the fluorescence emission of NATB was examined with various anions including dihydrogen phosphate. NATB had no selectivity for the anions.

![Figure 1](image-url)

**Figure 1.** Fluorescence changes of NATB (30 \(\mu\)M) with a variety of cations (1.5 equiv) in MeOH. Photograph: the fluorescent images of NATB and NATB-Al\(^{3+}\) (\(\lambda_{\text{ex}}: 358\) nm).

To check the concentration-dependent properties of NATB to Al\(^{3+}\), fluorescence titration was carried out (Figure 2). NATB exhibited little fluorescence with a tiny quantum yield (\(\Phi = 0.008\)). However, the continuous increase in Al\(^{3+}\) up to 1.5 equiv significantly enhanced the green fluorescence emission at 526 nm (\(\Phi = 0.162\)). UV–Vis spectrometry was also conducted with Al\(^{3+}\) to examine its photophysical characteristics (Figure 3). Upon the addition of Al\(^{3+}\), the absorption of 310 nm clearly decreased, while a new absorption of 325 nm constantly increased up to 1.5 equiv. An explicit isosbestic point was observed at 315 nm, verifying that the coordination of NATB with Al\(^{3+}\) produced a stable complex.

A 1:1 stoichiometric coordination between NATB and Al\(^{3+}\) was suggested by the Job plot experiment (Figure S3), which was explicitly supported by ESI-MS analysis (Figure S4). The positive ion mass displayed a large peak of 596.16 (m/z), which was correlated to [\(\text{NATB}(\text{H}^+) + \text{Al}^{3+} + 2\text{DMF} + \text{NO}_3^-\)\(^+\)] (calcd. 596.23). The association constant (K) of NATB-Al\(^{3+}\) was confirmed to be \(3.6 \times 10^4\) M\(^{-1}\) (Figure S5) based on Li’s method \[43\]. The detection limit of NATB toward Al\(^{3+}\) was 0.83 \(\mu\)M, based on 3\(\sigma\)/slope (Figure S6).

The \(^{1}H\) NMR titrations were achieved to investigate the binding mechanism of NATB toward Al\(^{3+}\) (Figure 4). Upon the addition of Al\(^{3+}\) to NATB, the proton H\(_{14}\) continually disappeared and the protons H\(_{5}\) and H\(_{6}\) were deshielded. These results indicate that the deprotonated oxygen on the tert-butylphenol group and the oxygen and nitrogen on the acylhydrazone group may be coordinated to Al\(^{3+}\) (Scheme 2).
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**Figure 2.** Fluorescence titration of NATB (30 $\mu$M) with varied amounts of Al$^{3+}$ (0–1.5 equiv) in MeOH.

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**Figure 4.** \(^1\)H NMR titration of NATB with Al\(^{3+}\) in DMSO-\(d_6\).

**Scheme 2.** Sequential recognition mechanism of Al\(^{3+}\) and H\(_2\)PO\(_4^-\) by NATB.

To verify the practicability of NATB as a probe for Al\(^{3+}\), an interference experiment was conducted (Figure S7). NATB could detect Al\(^{3+}\) with other cations without significant interferences, except for In\(^{3+}\), Fe\(^{3+}\) and Cu\(^{2+}\). These three cations bound more tightly to NATB than Al\(^{3+}\). For the practical application of NATB, test kits were prepared by immersing filter paper strips in the NATB solution. When NATB-coated test kits were immersed in a range of concentrations of Al\(^{3+}\) solutions, the obvious green fluorescence emission showed up above 2 mM of Al\(^{3+}\) under UV light (Figure 5a). However, the fluorescence was not displayed when those strips were applied to the same concentration of other cations (Figure 5b). These results indicate the potential applications of NATB in easily recognizing Al\(^{3+}\) without any complicated tools.
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**Figure 5.** Detection of Al\textsuperscript{3+} by NATB-coated test kits (10 mM). (a) NATB-coated test kits immersed in the solution of different Al\textsuperscript{3+} concentrations; (b) NATB-coated test kits immersed in 2 mM of various cation solutions.

### 3.2. Calculations

To comprehend the Al\textsuperscript{3+}-sensing property of NATB, DFT calculations were performed with the Gaussian 16 program (Figure 6). As the Job plot, ESI-MS, and \textsuperscript{1}H NMR titration implied a 1:1 stoichiometric coordination of NATB with Al\textsuperscript{3+}, all calculations were conducted with 1:1 stoichiometry. NATB showed a dihedral angle of 0.013° (1O, 2C, 3N, and 4C) with a planar structure (Figure 6a). The coordination of NATB with Al\textsuperscript{3+} distorted its structure, showing a dihedral angle of 98.875° (Figure 6b).

Based on the energy-minimized structures of NATB and NATB-Al\textsuperscript{3+}, TD-DFT calculations were conducted to inspect the transition energies and molecular orbitals. NATB featured the main absorption induced from the HOMO \rightarrow LUMO (347.28 nm), showing intra-charge transfer (ICT) transition from the tert-butylphenol to the naphthol (Figure S8). The major absorption of NATB-Al\textsuperscript{3+} derived from the HOMO-1 \rightarrow LUMO transition (412.27 nm) also showed a similar ICT transition (Figures S9 and S10). The reduction in the energy gap was consistent with the red-shift of the experimental absorption. These outcomes led us to conclude that the fluorescence turn-on mechanism of NATB to Al\textsuperscript{3+} may be a chelation-enhanced fluorescence (CHEF) effect [53]. Based on experimental data and theoretical calculations, an appropriate binding structure of NATB-Al\textsuperscript{3+} is proposed in Scheme 2.
3.3. Spectroscopic Examination of NATB-Al$^{3+}$ to H$_2$PO$_4^-$

We studied the fluorescence selectivity of NATB-Al$^{3+}$ to a range of anions such as H$_2$PO$_4^-$, Cl$^-$, CN$^-$, OAc$^-$, F$^-$, ClO$_4^-$, I$^-$, N$_3^-$, BzO$^-$, SCN$^-$, Br$^-$, NO$_2^-$, S$^2^-$, HPO$_4^{2-}$, PO$_4^{3-}$, HSO$_4^-$, and PPI in MeOH (Figure 7). Most of the anions did not affect the fluorescence emission of NATB-Al$^{3+}$, while the addition of H$_2$PO$_4^-$ toward NATB-Al$^{3+}$ resulted in significant fluorescence quenching ($\lambda_{\text{ex}} = 358$ nm). The result demonstrated that NATB-Al$^{3+}$ could be used as a chemosensor for H$_2$PO$_4^-$ with fluorescence quenching.

Figure 6. Energy-optimized forms of (a) NATB and (b) NATB-Al$^{3+}$.

Figure 7. Fluorescence changes of NATB-Al$^{3+}$ (30 μM) with various anions (45 μM) in MeOH ($\lambda_{\text{ex}}$: 358 nm). Photograph: the fluorescent images of NATB-Al$^{3+}$ and NATB-Al$^{3+}$-H$_2$PO$_4^-$ ($\lambda_{\text{ex}}$: 358 nm).
The fluorescence titration experiments were conducted to verify the fluorescence quenching ability of H$_2$PO$_4^-$ toward NATB-Al$_3^+$ (Figure 8). The fluorescence of NATB-Al$_3^+$ consistently diminished with the addition of H$_2$PO$_4^-$ up to 1.5 equiv ($\Phi = 0.005$). UV–Vis spectroscopy showed that the continuous addition of H$_2$PO$_4^-$ increased the absorbance at 310 nm, while those at 270 and 325 nm decreased with the explicit isosbestic points at 253 and 315 nm (Figure 9). The UV–Vis spectrum of H$_2$PO$_4^-$ with NATB-Al$_3^+$ is analogous to that of free NATB, implying that the addition of H$_2$PO$_4^-$ released Al$_3^+$ from the NATB-Al$_3^+$ complex (Figure S11).

**Figure 8.** Fluorescence titration of NATB-Al$_3^+$ (30 μM) with various amounts of H$_2$PO$_4^-$ (0–1.5 equiv) in MeOH.

**Figure 9.** UV–Vis changes of NATB-Al$_3^+$ (30 μM) with various amounts of H$_2$PO$_4^-$ (0–1.5 equiv) in MeOH.
The stoichiometry of $\text{H}_2\text{PO}_4^-$ toward $\text{NATB}-\text{Al}^{3+}$ was determined by the Job plot experiment (Figure S12), which exhibited a 1:1 stoichiometry. The mass spectral analysis displayed a peak of 395.06 ($m/z$), which demonstrated the regeneration of $\text{NATB}$ ($[\text{NATB} + \text{H}^+ + \text{MeOH}]^+$; calcd. 395.20) (Figure S13). These outcomes supported the mechanism that the addition of $\text{H}_2\text{PO}_4^-$ released $\text{Al}^{3+}$ from $\text{NATB}-\text{Al}^{3+}$, which resulted in the loss of fluorescence. Based on Li’s method [43], the association constant ($K$) for $\text{H}_2\text{PO}_4^-$ with $\text{NATB}-\text{Al}^{3+}$ was calculated as $1.2 \times 10^4$ M$^{-1}$ (Figure S14). The detection limit of $\text{NATB}-\text{Al}^{3+}$ toward $\text{H}_2\text{PO}_4^-$ was determined as 1.7 µM, based on 3σ/slope (Figure S15).

Importantly, $\text{NATB}$ is the first fluorescent sensor for the consecutive sensing of $\text{Al}^{3+}$ and $\text{H}_2\text{PO}_4^-$ (Table S1). On the other hand, $\text{NATB}$ showed higher detection limits for $\text{Al}^{3+}$ and $\text{H}_2\text{PO}_4^-$ compared to Kumar’s work [32], but it could solely detect $\text{H}_2\text{PO}_4^-$ without the interference of $\text{HSO}_4^-$.

The reversibility in the response of $\text{NATB}$ was verified through the alternative additions of $\text{Al}^{3+}$ and $\text{H}_2\text{PO}_4^-$ (Figure 10). The fluorescence emission of $\text{NATB}$ repeated its enhancing and quenching processes several times without fluorescence efficiency loss. To verify that $\text{NATB}-\text{Al}^{3+}$ is an effective fluorescence probe for $\text{H}_2\text{PO}_4^-$, the interference of other anions was tested (Figure S16). The results indicated that the presence of other anions (1.5 equiv) did not interfere with the fluorescence quenching of $\text{NATB}-\text{Al}^{3+}$ toward $\text{H}_2\text{PO}_4^-$.  

**Figure 10.** Change in fluorescence of $\text{NATB}$ (30 µM) upon alternate addition of $\text{Al}^{3+}$ and $\text{H}_2\text{PO}_4^-$ in MeOH ($\lambda_{\text{ex}}$: 358 nm).
4. Conclusions

An acyhydrazone-based chemosensor NATB was developed and its sequential recognition of Al\(^{3+}\) and H\(_2\)PO\(_4^-\) was studied. NATB showed a strong fluorescence increase with Al\(^{3+}\), and its complex NATB-Al\(^{3+}\) sequentially detected H\(_2\)PO\(_4^-\) by releasing Al\(^{3+}\) with turn-off fluorescence. Importantly, NATB is the first sequential fluorescent probe for selective sensing of Al\(^{3+}\) and H\(_2\)PO\(_4^-\). Detection limits of NATB for Al\(^{3+}\) and H\(_2\)PO\(_4^-\) were calculated as 0.83 and 1.7 \(\mu\)M, respectively, based on 3\(\sigma\)/slope. NATB could repeat sequential recognition of Al\(^{3+}\) and H\(_2\)PO\(_4^-\) several times and could be applied to detect Al\(^{3+}\) in test strips. The sensing mechanism of NATB toward Al\(^{3+}\) and H\(_2\)PO\(_4^-\) was demonstrated with a Job plot, ESI-MS, \(^1\)H NMR spectroscopy, and theoretical calculations. The detection mechanism of NATB toward Al\(^{3+}\) is suggested to be a CHEF effect through DFT calculations.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ma14216392/s1, Table S1: Examples of chemosensors for successive detection related to Al\(^{3+}\) or H\(_2\)PO\(_4^-\) or both; Figure S1: \(^1\)H NMR spectrum of NATB in DMSO-\(d_6\); Figure S2: \(^{13}\)C NMR spectrum of NATB in DMSO-\(d_6\); Figure S3: Job plot for the binding of NATB with Al\(^{3+}\) (50 \(\mu\)M) in MeOH. Fluorescence intensity at 526 nm is plotted as a function of the molar ratio of [Al\(^{3+}\)]/([Al\(^{3+}\)] + [NATB]); Figure S4: Positive-ion ESI mass spectrum of NATB (100 \(\mu\)M) in MeOH upon the addition of 1 equiv of Al\(^{3+}\) in DMF; Figure S5: Li’s equation plot (at 526 nm) of NATB (30 \(\mu\)M) in MeOH, based on fluorescence titration, assuming 1:1 stoichiometry for association between NATB and Al\(^{3+}\); Figure S6: Calibration curve of NATB as a function of Al\(^{3+}\) concentration in MeOH. [NATB] = 30 \(\mu\)M and [Al\(^{3+}\)] = 0–18 \(\mu\)M (\(\lambda_{ex} = 358\) nm); Figure S7: Competitive experiments of NATB (30 \(\mu\)M) toward Al\(^{3+}\) (45 \(\mu\)M) in the presence of other metal ions (45 \(\mu\)M, \(\lambda_{ex} = 358\) nm) in MeOH; Figure S8: (a) The theoretical excitation energies and the experimental UV–Vis spectrum of NATB; (b) The major electronic transition energies and molecular orbital contributions of NATB; Figure S9: (a) The theoretical excitation energies and the experimental UV–Vis spectrum of NATB-Al\(^{3+}\); (b) The major electronic transition energies and molecular orbital contributions of NATB-Al\(^{3+}\); Figure S10: The major molecular orbital transitions and excitation energies of NATB and NATB-Al\(^{3+}\); Figure S11: UV–Vis spectra of NATB and NATB-Al\(^{3+}\) with H\(_2\)PO\(_4^-\) in MeOH, respectively; Figure S12: Job plot for the stoichiometry of NATB-Al\(^{3+}\) with H\(_2\)PO\(_4^-\) (30 \(\mu\)M) in MeOH. Fluorescence intensity at 526 nm is plotted as a function of the molar ratio of [NATB-Al\(^{3+}\)]/[NATB-Al\(^{3+}\)] + [H\(_2\)PO\(_4^-\)]; Figure S13: Positive-ion ESI mass spectrum of NATB-Al\(^{3+}\) (100 \(\mu\)M) in MeOH upon the addition of 1 equiv of H\(_2\)PO\(_4^-\) in H\(_2\)O; Figure S14: Li’s equation plot (at 526 nm) of NATB-Al\(^{3+}\) (30 \(\mu\)M) based on fluorescence titration in MeOH, assuming 1:1 stoichiometry for association between NATB-Al\(^{3+}\) and H\(_2\)PO\(_4^-\); Figure S15: Calibration curve of NATB-Al\(^{3+}\) in MeOH as a function of H\(_2\)PO\(_4^-\) concentration. [NATB-Al\(^{3+}\)] = 30 \(\mu\)M and [H\(_2\)PO\(_4^-\)] = 0.0–18.0 \(\mu\)M (\(\lambda_{ex} = 358\) nm); Figure S16: Interference studies of NATB-Al\(^{3+}\) (30 \(\mu\)M) toward H\(_2\)PO\(_4^-\) (45 \(\mu\)M) in the presence of other anions (45 \(\mu\)M, \(\lambda_{ex} = 358\) nm) in MeOH.

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