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A new tetranaphthoimidazolium receptor was synthesized and reported as a selective fluorescent chemosensor for phytate, myo-inositol hexakisphosphate (IP₆). In a 100% aqueous solution at pH 7.4, chemosensor 1 showed a selective fluorescence enhancement for IPs over IP₃, phosphate, pyrophosphate, AMP, ADP, and ATP. An excimer emission at 465 nm linearly increases in the range of 300 nM to 1 μM with a detection limit of 2.28 × 10⁻⁷ M. In addition, first live cell imaging of IP₆ has been demonstrated by using a synthetic receptor.

Inositol 1,4,5-trisphosphate (IP₃) is known as an important second messenger in intracellular signal transduction processes, which can also control the cellular Ca²⁺ concentration.¹ myo-Inositol hexakisphosphate (phytate, IP₆) is a fully phosphorylated form of inositol (Fig. 1), which is found in blood, urine, and intracellular fluids.² IP₆ has been regarded as an antinutrient due to its ability to chelate essential trace minerals, such as Fe, Zn, and Ca.³ recent studies report beneficial properties of IP₆, such as blood-glucose-lowering and lipid-lowering effects, antioxidative properties and anticancer activities.⁴ In addition, a previous study suggested that phytic acid is a cofactor in DNA repair by nonhomologous end-joining.⁵ Another report using yeast mutants also suggested that intracellular phytic acid may be involved in mRNA export from the nucleus to the cytosol.⁶ However, the exact physiological roles of intracellular phytic acid are still unclear.⁷

Accordingly, various detection methods for the determination of IP₆ have been reported, such as refractive index HPLC analysis for phytate itself or of its hydrolysis products (inositol and phosphate),⁸ flow injection-capillary zone electrophoresis,⁹ and gas chromatography mass spectrometry.⁹ However, these methods suffer from a time consuming sample pretreatment process or advanced instruments. A fluorescent chemosensing approach certainly has advantages over these methods, in particular, the opportunity for in vivo imaging.⁹ There have been some efforts to sense IP₃ and IP₆ via fluorescent changes. These methods were based on a ligand exchange in which metal ions were removed from metal complexes by IPs. Notably, the Ahn group reported a fluorogenic chemosensing ensemble for IP₆ using an eosine and Cu²⁺ complex.¹² Han group, on the other hand, reported a colorimetric sensing system for IP₆ using a combination of tris-Zn benzene derivative as the receptor unit and 11-mercaptoundecylphosphoric acid functionalized gold nanoparticles as the reporter unit.¹³ Recently, Kubo and coworker reported fluorescence sensing of IP₆ without using metal ions as the binding sites, in which an isothiouronium-attached polythiophene showes a selective fluorescence quenching effect with IP₆ at pH 5.5.¹⁴

**Scheme 1.** Synthesis of tetranaphthoimidazolium receptor 1.

Imidazolium-based receptors have been actively studied due to the unique ionic hydrogen bonding interactions between imidazolium (C-H)¹⁰ groups and anions.¹⁵ Specifically, fluorescent receptors bearing imidazoliums, naphthoimidazolium or bisbenzoimidazoliums have been recently utilized as selective fluorescent chemosensors for various anionic targets.¹⁶ As described above, previously reported examples utilized metal ion-complex systems. In most of these systems, metal ions were removed by IP₆ and thus cannot be easily applied to...
image IP$_6$ in the cell.

In the current study, we synthesized a new tetranaphthoimidazolium receptor 1, which shows a selective fluorescence enhancement with IP$_6$ in a 100% aqueous system at pH 7.4.

Naphthoimidazoliums maintain the unique properties of imidazolium, such as ionic hydrogen bonding interactions, and they are inherently fluorescent, so there is no need to introduce additional fluorophores.

For the synthesis of fluorescent chemosensor 1, 1-methyl-1H-naphtho[2,3-d]imidazole 2 was first synthesized according to the reported procedure. A mixture of 2 and 1,2,4,5-tetrakis(bromomethyl)benzene in acetonitrile afforded 1 at an 80% yield (Scheme 1). Compound 1 was fully characterized by high-resolution FAB mass spectroscopy, $^1$H NMR, and $^{13}$C NMR spectroscopy (Supporting Information).

**Fig. 2** (a) Fluorescent spectra of 1 (5 μM) in HEPES buffer (0.02 M, pH 7.4) upon addition of sodium salts of of F$^-$, Cl$^-$, CH$_3$CO$_2^-$, HPO$_4^{2-}$, NO$_3^-$, HSO$_4^-$, PPI, AMP, ADP, ATP, IP$_3$, and IP$_8$ (1 equiv.) in HEPES buffer (0.02 M, pH 7.4). (b) Job’s plot of 1 with IP$_7$ in HEPES buffer (0.02 M, pH 7.4).

The selectivity of 1 was tested with sodium salts of F$^-$, Cl$^-$, CH$_3$CO$_2^-$, HPO$_4^{2-}$, ClO$_4^-$, NO$_3^-$, HSO$_4^-$, pyrophosphate (PPI), AMP, ADP, ATP, IP$_3$, and IP$_8$ (1 equiv.) in HEPES buffer (0.02 M, pH 7.4). A relatively small monomeric emission at 370 nm and large excimer emission at 465 nm were observed in the fluorescence spectrum, as shown in Fig. 2. Among these various anionic analysers, only IP$_6$ showed a selective fluorescence enhancement at 465 nm. On the other hand, simple anions such as, PPI, AMP, ADP and ATP did not induce any significant fluorescence change. Fig. S6 demonstrates the fluorescence titrations of 1 (5 μM) in HEPES buffer (0.02 M, pH 7.4) upon adding of 0-1 equiv. of IP$_6$. A Job plot showed 1:1 stoichiometry between 1 and IP$_6$, as shown in Fig. 2b. An excimer emission at 465 nm linearly increases in the range of 300 nM to 1 μM with a detection limit of 2.28 × 10$^{-7}$ M (Fig. S7).

**Fig. 3** Fluorescence changes of 1 (5 μM) with sodium salts of HPO$_4^{2-}$, PPI, AMP, ADP, ATP, IP$_3$, and IP$_8$ (1 equiv.) in HEPES buffer (0.02 M, pH 7.4).

The partial $^1$H NMR spectra of 1 with IP$_6$ in DMSO-d$_6$-D$_2$O (9:1, v/v) is presented in Fig. 4. Imidazolium C-2 proton (H$_a$) appears as a small signal a result of exchange with D$_2$O because of the acidic nature of this proton. However, a downfield shift of this proton could be clearly observed upon the addition of IP$_6$. Benzylic hydrogens also displayed slight downfield shift. These changes can be attributed to the possible hydrogen bonding interactions between these protons and phosphate groups of IP$_6$. On the other hand, there were upfield shifts for aromatic protons, which are probably due to the excimer formations between two naphthoimidazolium groups.

**Fig. 5** Calculated structures and schematics for binding modes for the complexes of 1 with IP$_3$ and IP$_6$. Hydrogen atoms were omitted for clarity and dashed red lines indicate interactions of the phosphate with hydrogen atoms (>2.5 Å).

To obtain an insight into the binding modes and fluorescence behaviors of 1 with IP$_3$ and IP$_6$, we carried out density functional theory (DFT) and time-dependent DFT (TDDFT) calculations with the M06-2x functional using a suite of Gaussian 09 programs. The optimized structures of 1, 1+IP$_3$ and 1+IP$_6$ are shown in Fig. 5. Compound 1 is well stacked with the naphthoimidazolium moieties at distances of about 4.8 Å, which can lead to intramolecular excimer formation. The phosphate groups of IP$_3$/IP$_6$ and the (C─H)$^+$ of the imidazolium moieties as well as hydrogen atoms in the alkyl side-chain of the imidazolium moieties are involved in interacting with IP$_3$ and IP$_6$. The π-stacking distance between naphthoimidazolium moieties was shortened to be 4.1 Å and 3.6 Å for 1+IP$_3$ and 1+IP$_6$, respectively. In binding with IP$_6$, the naphthoimidazolium moieties are closer each other than in...
binding with IP$_3$. In addition, the interplanar dihedral angle (C$_1$-N$_1$-N$_2$-C$_2$) of 1+IP$_3$ is calculated to be 34° due to the space for motions, while it keeps almost stacking with the dihedral angle of 4° in 1+IP$_6$ due to the limited space through the strong interactions with six phosphates. This structural feature is consistent with NMR experimental data, and responsible for the stronger fluorescence of 1 in binding with IP$_6$ than IP$_3$.

To investigate the fluorescence property of receptor upon addition of IP$_6$, TDDFT calculations were performed. The important orbital transitions to the excitation and the corresponding orbital shapes were shown in Fig. S8. The major transition of 1 comes from HOMO→LUMO+3 and HOMO-1→LUMO+2 transitions. Though these orbitals are localized in four naphthoimidazolium groups, the on-site transition is likely to be dominated considering the weak fluorescence observed in experiment. Whereas in 1+IP$_6$, HOMO-3→LUMO+8 and HOMO-4→LUMO+6 transitions where the electrons in one naphthoimidazolium group might interact with holes in another one resulting excimer emission.

Finally, probe 1 was further applied for live cell imaging. Fluorescence images of HeLa cells (adenocarcinoma) and WI38 VA-13 subclone 2RA cells (normal) labeled are explained in Fig. 6 and Fig. S9. It can be seen faint fluorescence is observed in the labeled cells, however incubation with phytic acid (5 and 50 µM) induced strong fluorescence (Fig. 6). Probe 1 successfully passed through the cytoplasm and nuclei of cells. To identify the cytotoxic effect of 1, HeLa cells were seeded in a 24-well plate. The cells were incubated with 0, 1, 5, and 50 µM 1 for 24 h at 37 °C, and cell viability was determined by counting live cells. When the cells were treated with 50 µM of 1, cell viability was more than 99% compared to those without 1 treatment (Fig. S10).

These results indicated that 1 is nontoxic and may play a role as a bio-probe for intracellular phytic acid, which has very useful applications in bioimaging assays.

In conclusion, we report a new tetrannaphthoimidazo[d]imidazolium receptor 1 as the fluorescent chemosensor for phytate, myo-inositol hexakisphosphate (IP$_6$), in 100% aqueous solution at pH 7.4. The fluorescent receptor 1 displayed a selective fluorescence enhancement with IP$_6$. The other simple anions, phosphate, pyrophosphate, AMP, ADP, ATP and IP$_3$ did not induce any significant fluorescence change. The possible binding modes and fluorescence changes are also explained by theoretical calculations. We further showed the first successful in vivo imaging of IP$_6$ in cells by using a relatively simple naphthoimidazolium-based fluorescent probe. By using this relatively simple receptor, we could obtain reasonable selectivity for IP$_6$ in 100% aqueous solution at pH 7.4.

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### Notes and references

2. V. Raboy, Phytochemistry, 2003, 64, 1033.
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