Tetrahedron Letters xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Displacement-based, chromogenic calix[4]pyrrole–indicator complex for selective sensing of pyrophosphate anion

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ARTICLE INFO

Article history:
Received 2 April 2013
Revised 23 April 2013
Accepted 30 April 2013
Available online xxxx

Keywords:
Calix[4]pyrrole
Indicator displacement assay
Pyrophosphate
Azophenolate
Anion recognition

ABSTRACT

A supramolecular complex composed of bis-pyridinium picket calix[4]pyrrole and azophenol indicator is a highly visible colorimetric displacement assay and sensor. The system shows significant selectivity and a higher affinity for pyrophosphate anions over other competing anions.

The design and synthesis of sensitive chemosensors for selective detection of anions[1] have received considerable attention in the chemical, biological, and environmental sciences.[2] The key components in an effective chemosensor are the recognition domain (binding site) and the signaling unit (indicator).[3] In most molecularly constructed sensors, the receptor site and signaling unit are covalently linked to facilitate observable changes associated with the binding interaction. Anslyn and co-workers[4] have developed the indicator-displacement assay (IDA), where sensing of a target analyte is achieved by a binding-induced displacement within a supramolecular receptor–indicator complex. This approach relies upon the competition between the analyte and the indicator for a binding cavity on the receptor; the analyte must have the higher binding affinity. It has been used for the selective sensing of anions such as phosphate,[5] pyrophosphate,[6] nitrate,[7] cyanide,[8] and citrate.[9]

The detection of pyrophosphate anion ($HP_2O_7^{3-}$) is particularly important for the analysis of bioenergetic and metabolic processes.[10] It plays a critical role in energy storage[11] and signal transductions, as well as being a structural component of teeth and bones. Also, it is a product of ATP hydrolysis and participates in many enzymatic reactions[12] such as the adenylate cyclase-catalyzed synthesis of cyclic AMP, aminoacyl tRNA synthetase-catalyzed attachment of amino acids to tRNA in protein synthesis, and DNA sequencing/replication[13] catalyzed by DNA polymerase. High levels of $HP_2O_7^{3-}$ are known to cause several diseases.[14]

Zinc–dipicolylamine (Zn(II)–DPA) and Cu(II)–DPA complexes have been employed as IDAs for the detection of pyrophosphate anions.[15,16] The metal centers become coordination spheres to accommodate the oxoanions. Other receptors for pyrophosphate anion include macrocyclic pyrrole, imidazolium-based macrocycles, and dipyrrolyquinoxalines.[17] We reported a bis-pyridinium calix[4]pyrrole derivative for '*turn on*' fluorescence detection of pyrophosphate in an aqueous organic solvent[18] that utilizes a hydrogen bonding interaction and electrostatic interactions combined with a fluorescent dye-displacement assay. We have also developed a supramolecular receptor–indicator complex[19] composed of bis-pyridinium calix[4]pyrrole and an azo dye for selective recognition of $HP_2O_7^{3-}$ over other competing anions, including F^- and AcO^-. The recognition of F^- in organic media was achieved with a colorimetric IDA using an octamethylcalix[4]pyrrole-(*p*-nitrophenolate) complex by Sessler and co-workers [20] and a merocyanine dye by Machado and co-workers.[21]

Here, we report on an IDA-based colorimetric detection of $HP_2O_7^{3-}$ anion using dicationic calix[4]pyrrole combined with an azo dye indicator. The pyrrole was designed to allow multiple interactions with the guest anion (hydrogen bonding, anion–π interactions, and coulombic interactions). The azo dye, initially bound to the receptor, is replaced by the target analyte, resulting in colorimetric detection (Scheme 1). The *cis*-5,15-(4-pyridyl)-5,10,10,15,20,20-hexamethylcalix[4]pyrrole was prepared in moderate yield by acid-catalyzed condensation of 5-(4-pyridyl)dipyrromethane with acetone. The hexafluorophosphate salt of bis-pyridinium calix[4]pyrrole **1** was obtained via methylation of *cis*-5,15-(4-pyridyl)-5,10,10,15,20,20-hexamethylcalix[4]pyrrole,

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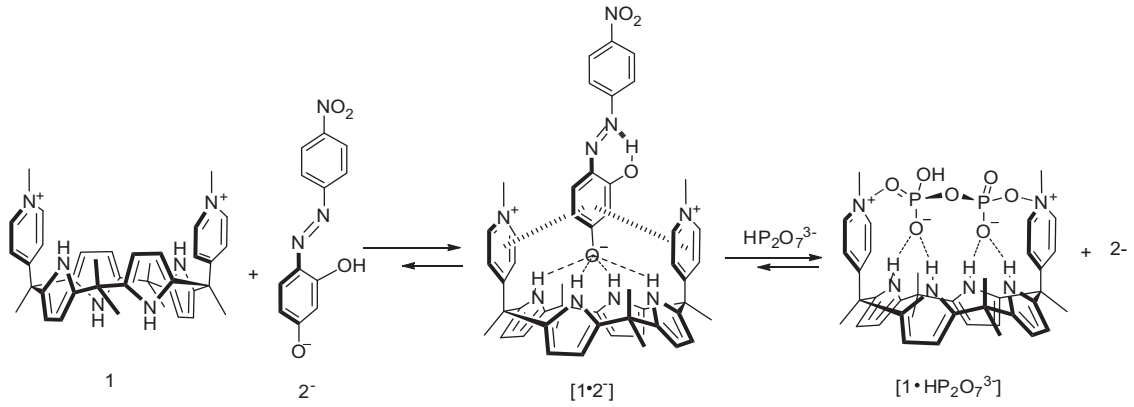
http://dx.doi.org/10.1016/j.tetlet.2013.04.129



Scheme 1. Formation of the complex [**1**·**2**$^-$] and the recognition of $HP_2O_7^{3-}$ via displacement of dye **2**$^-$.

followed by treatment with NH_4PF_6. Tetrabutylammonium salt of azo-dye **2**$^-$ was obtained in good yield by treating 4-(4-nitrophenylazo)resorcinol with tetrabutylammonium hydroxide. The structure of all the compounds was confirmed with spectroscopic means (SI).

The anionic form of indicator **2**$^-$ (10 μM) has a red/pink color with twin absorption bands at 538 nm (ε_{max} = 42,800 l-mol^{-1}cm^{-1}) and 447 nm (ε_{max} = 49,500 l-mol^{-1}cm^{-1}) in acetonitrile. Incremental addition of receptor **1** (0–30 μM) to the solution of **2**$^-$ (10 μM) results in decreased absorbance at 538 nm and increased absorbance at 447 nm, with a 10 nm bathochromic shift, as shown in Figure 1.

The binding of indicator **2**$^-$ to receptor **1** is accompanied by a distinctive color change from pink to yellow. Saturation occurs when 30 μM of receptor is added (Fig. 2). The isosbestic point at 500 nm indicates an equilibrium complexation of receptor **1** and indicator **2**$^-$. A Job plot also supports the 1:1 binding stoichiometry between receptor **1** and indicator **2**$^-$.[22] Analysis of the titration data in Figure 1 with a non-linear least square fit (HypSpec[23]) yields an association constant $K_a = (1.80 \pm 0.04) \times 10^6$ M^{-1}. These observations clearly indicate that formation of the supramolecular receptor–indicator complex [**1**·**2**$^-$] is favorable and that the hydrogen-bonding interactions between the anionic indicator **2**$^-$ and the pyrrole N–H bonds are strong. The binding behavior of the anionic indicator **2**$^-$ and receptor **1** can be monitored by ^{1}H NMR spectroscopy (Fig. 3). When receptor **1** (2.28 mM) is titrated with anionic

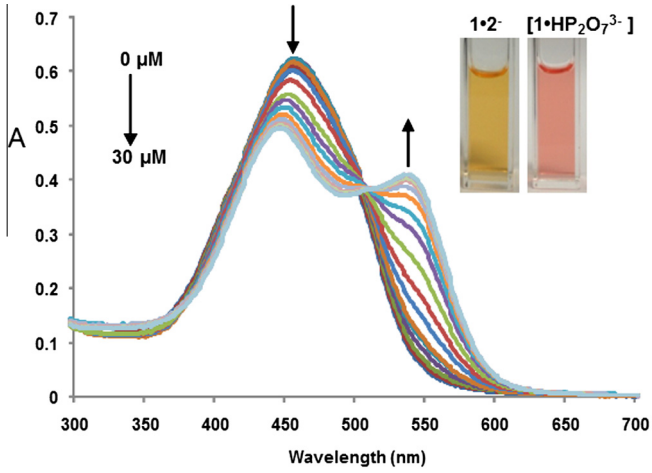


Figure 1. Changes in the absorption spectrum of **1.2**$^-$ (10 μM) upon titration with pyrophosphate ion (0–30 μM) in CH_3CN. The inset displays the color of the [**1.2**$^-$] and the complex [$1H_2P_2O_7^{3-}$].

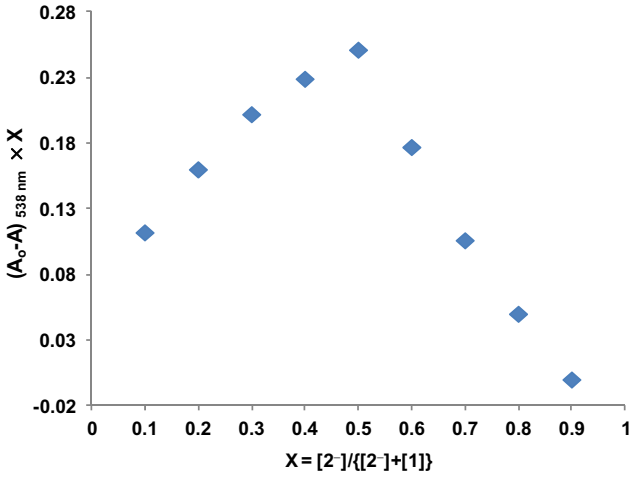


Figure 2. Job plot for the binding between **1** and **2**$^-$ in CH_3CN.

indicator **2**$^-$ in CD_3CN, the signals corresponding to the *ortho*-protons from the anionic center are shifted up-field and the signals from the rest of the aromatic protons become broad relative to the spectrum of pure **2**$^-$ in CD_3CN.

In addition, the signal corresponding to NH protons of receptor **1** shows a considerable down-field shift from δ 7.90 to δ 10.85 ppm (Fig. 3 (iii)), indicating hydrogen bonding between phenolate anion and pyrrole N–H bonds, as well as possible π–π interactions.[24] Only 1.0 equiv of **2**$^-$ is required for complete binding.

The anion recognition properties of the complex [**1**·**2**$^-$] were investigated by UV–vis absorption spectroscopy in CH_3CN. When it is titrated with the anions F^-, CN^-, AcO^-, and Cl^- (as their tetrabutyl ammonium salt, 0–17.5 μM), only small increases in absorbance at 538 nm are observed (Fig. 4). However, a significant change in absorbance, as well as in the visual color, is observed upon titration with pyrophosphate anion ($HP_2O_7^{3-}$). Thus the affinity of pyrophosphate anion toward receptor **1** is strong and is capable of complete replacement of the indicator to form the new complex [**1**·$HP_2O_7^{3-}$]. In contrast, $H_2PO_4^-$, Br^-, and I^- anions do not produce appreciable changes in the absorption spectra.

However, the initial [**1**·**2**$^-$] absorption spectrum is shifted to a broad absorption band at 400–430 nm upon titration with HSO_4^- (17.5 μM). This spectral change is associated with the ionization of monobasic HSO_4^- to dibasic SO_4^- by indicator anion **2**$^-$, which is sufficiently basic to deprotonate HSO_4^-. Formation of the resulting azophenol derivative **2** can be confirmed by comparing the absorption spectra with **2**$^-$. The detection limit[25] for $HP_2O_7^{3-}$ is

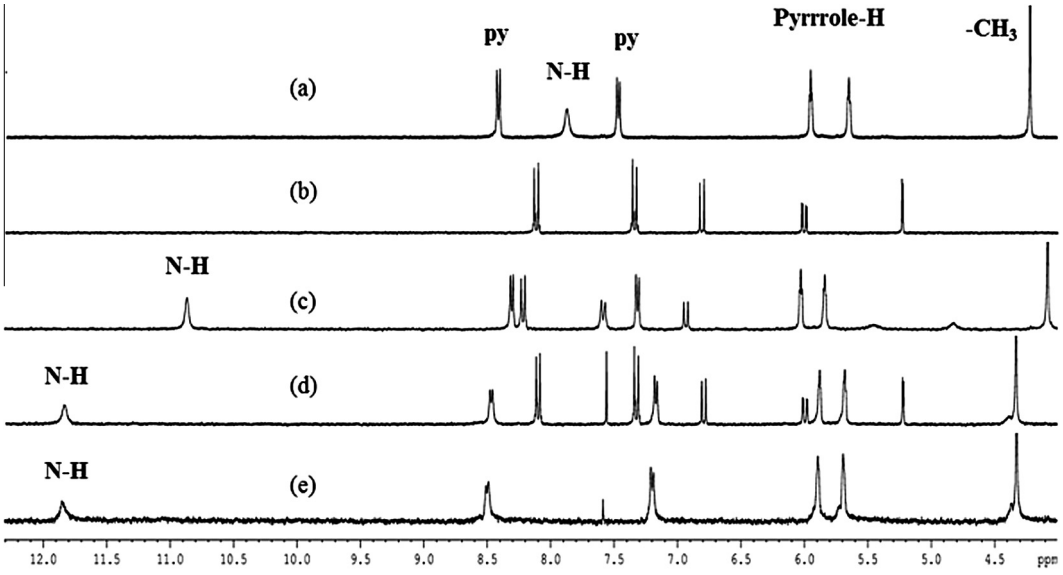


Figure 3. Partial ^{1}H NMR spectra of solutions containing (a) **1** only (2.28 mM), (b) $\mathbf{2}^-$only (2.28 mM) (c) **1**+$\mathbf{2}^-$ (1.0 equiv), (d) [(c) + $HP_3O_7^{3-}$ (1.5 equiv)], and (e) **1** + $HP_2O_7^{3-}$ (1.5 equiv).

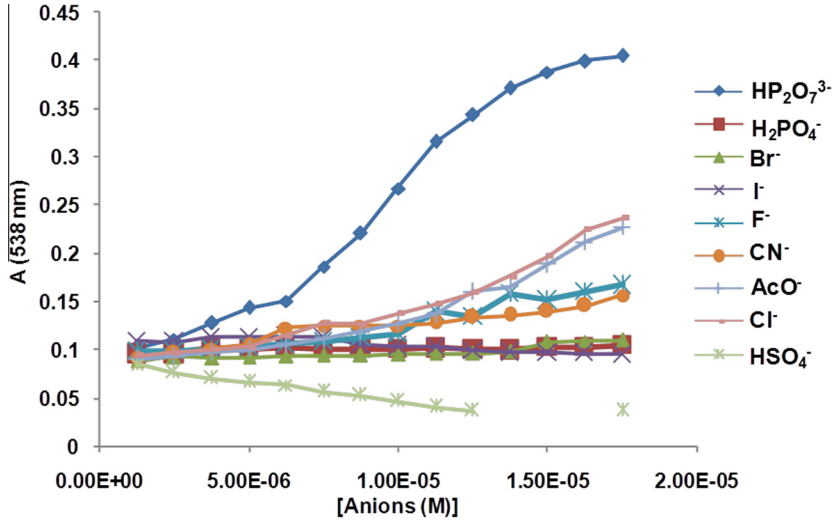


Figure 4. Concentration-dependent absorbance changes at 538 nm with increasing concentrations of various anions at a fixed concentration of [**1**·$\mathbf{2}^-$] in CH_3CN. [**1**]/[$\mathbf{2}^-$] = (30 μM)/(10 μM).

5.6 μM, which is much lower than the intracellular concentration of pyrophosphate (~50 μM).[26] Thus, the sensing complex could be employed in biological systems.

We have previously demonstrated a highly selective fluorescent dye replacement assay sensor for pyrophosphate anion based on a coumarin-calix[4]pyrrole supramolecular complex.[18] Now we show below that the calix[4]pyrrole-based, chromogenic indicator displacement assay for the pyrophosphate anion can selectively recognize anionic analytes as well.

In order to assess the selectivity of the complex [**1**·$\mathbf{2}^-$] for pyrophosphate anion, a solution of complex [**1**·$\mathbf{2}^-$] containing an equimolar concentration of a competing anion such as F^- or AcO^- was titrated with $HP_2O_7^{3-}$ (0–12.5 μM) in CH_3CN. Constant absorbance throughout the titration indicated no significant competition from the other anions. The sensing performance of the complex [**1**·$\mathbf{2}^-$] was also examined with ^{1}H NMR spectroscopy. When the solution of the complex [**1**·$\mathbf{2}^-$] was titrated with 1.5 equiv pyrophosphate anion (as its tetrabutyl ammonium salt) in CD_3CN, exclusive displacement of azophenolate indicator by pyrophosphate anion was observed from the shift of the [**1**·$\mathbf{2}^-$] N–H proton signal from δ 10.85 to δ 11.85 ppm, which correlates with that of the receptor–pyrophosphate complex. These results clearly confirm IDA-based sensing of pyrophosphate anion. The preferable binding of pyrophosphate over fluoride anion was also verified with ^{1}H NMR by mixing equimolar amounts of $HP_2O_7^{3-}$ and F^- to the receptor **1** in CD_3CN; exclusive formation of the receptor–pyrophosphate complex was retained in the presence of F^-. In summary, these results indicate that [**1**·$\mathbf{2}^-$] selectively detects pyrophosphate anion in the presence of common interfering anions such as fluoride or acetate anion.

The higher affinity for pyrophosphate anion over other anions may be attributed to several factors: (1) the presence of appropriately spaced cationic charges; (2) the matching size of the binding domain; and (3) anion–π interactions and the presence of multiple hydrogen-bonding donors. In an ideal IDA system, the binding of the complex formed by the target analyte with the receptor must

be larger than that of the complex of the receptor and indicator. To be more quantitative, the binding constants were calculated from the titration data obtained from UV–vis and ^{1}H NMR spectroscopy using HypSpec.[23] All the titration data were fit with a 1:1 binding stoichiometry. The affinity constant for the direct formation of [**1**·**2**$^{-}$] ($K_a = (1.80 \pm 0.04) \times 10^6$ M^{-1}) is 1/13.6 compared with the formation of [**1**·$HP_2O_7^{3-}$] ($K_a = 2.45 \pm 0.13) \times 10^7$ M^{-1}); thus there is a significant preference for [**1**·$HP_2O_7^{3-}$]. The affinity constant for the formation of [**1**·$HP_2O_7^{3-}$] from [**1**·**2**$^{-}$] by displacement ($K_a = (7.7 \pm 0.11) \times 10^6$ M^{-1}) is less than the value for direct binding. This difference is due to pre-dissociation of [**1**·**2**$^{-}$] to **1** and **2** followed by complexation of **1** with $HP_2O_7^{3-}$. The dissociation constant of the complex [**1**·**2**$^{-}$] is 3.18×10^6 M^{-1}, as calculated by the equilibrium equation.

In conclusion, we have developed a sensitive calix[4]pyrrole-based indicator displacement assay chemosensor displaying significant selectivity for pyrophosphate anion. The dicationic calix[4]pyrrole, armed with bis-pyridinium groups, forms a strong supramolecular receptor–indicator complex with azophenol dye. The system is a highly visible colorimetric IDA sensor for pyrophosphate anion in organic solvent. This work will provide useful insights for the design and development of other IDA-based chemosensors. For example, the interplay between hydrogen bond, anion–π interaction, and electrostatic interaction is crucial for imposing selectivity and is pivotal for selective sensing of anions.

Acknowledgments

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 2009-0087013) to CHL and is partially supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 2012-013930) to JTL.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.04.129.

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