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Short communication

Solution and structural binding studies of phosphate with thiophene-based azamacrocycles

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ABSTRACT

Two thiophene-based monocyclic receptors **L1** and **L2** have been studied for phosphate binding in solutions (D_2O and DMSO-d_6) by ^1H NMR and ^{31}P NMR titrations, and in the solid state by single crystal X-ray analysis. Results from ^1H NMR titrations suggest that the ligands bind phosphate anions in a 1:2 binding mode in DMSO-d_6, with the binding constants of 5.25 and 4.20 (in log *K*), respectively. The binding of phosphate to **L1** and **L2** was further supported by ^{31}P NMR in D_2O at pH = 5.2. The crystal structure of the phosphate complex of **L1** reveals unambiguous proof for the formation of a ditopic complex *via* multiple hydrogen bonds from NH···O and CH···O interactions.

Phosphate is a key building block of nucleic acids, playing critical roles in many biochemical processes [1]. The translocation of phosphate between DNA and proteins in living cells is an essential step in the regulation of metabolic processes [2]. Energy production and storage processes within the body are regulated by phosphorylated compounds, such as adenosine triphosphate [3]. It is known that the phosphate level in serum and saliva is linked to several diseases including hyperparathyroidism, vitamin D deficiency and *Fanconi* syndrome [4]. It is also widely used in fertilizer and drug-related industries [5]. Because of its significant roles in environment, healthcare and biochemical applications, molecular recognition of phosphate by synthetic molecules is a growing area of current research in supramolecular chemistry [6]. However, phosphate binding in water is unfavorable due to its high free energy of hydration ($\Delta G^0 = -465$ J/mol) [7]. Polyamine-based receptors that are water soluble, are often used to bind phosphates in water over a wide range of pH [8]. For examples, Martell and coworkers synthesized hexaazamacrocyclic ligands and used them to encapsulate a pyrophosphate *via* four hydrogen bonds [9]. A hexaprotonated 26-membered polyammonium macrocycle reported by Bowman-James and coworkers was shown to form a complex with six di-hydrogen phosphate anions and two neutral phosphoric acid molecules, illustrating dipotic behavior of the receptor [10]. Bianchi, García-España, Paoletti and coworkers reported a tetraprotonated macrocycle [18]aneN$_6$ that binds two pyrophosphate anions *via* NH···O and CH···O bonds [11]. Lu and coworkers showed that an octa-protonated *p*-xylyl-based cryptand encapsulated a phosphate anion *via* multiple hydrogen bonds [12]. Other reported receptors that are neutral molecules including amides [13], thioamides [14], ureas [15], thioureas [16], pyrroles [17] and indoles [18] were shown to bind phosphate in organic solvents. Our recent studies on various macrocycle-based receptors indicated that hydrogen bonding and electrostatic interactions are major binding forces in stabilizing complexes; thus providing insight into the binding modes of anions and conformations of host molecules [19–21]. Herein, we report the binding aspects of two thiophene-based macrocycles **L1** and **L2** *via* ^1H NMR and ^{31}P NMR in solutions. We also report the structural characterization of a phosphate complex with **L1**, forming a ditopic complex with one dihydrogen phosphate and one monohydrogen phosphate.

The hexamine ligands **L1** and **L2** were synthesized through Schiff-base condensation reaction (Scheme 1) of the corresponding dipodal amine with two equivalents of 2,5-thiophendicarboxaldehyde followed by the reduction with $NaBH_4$, as reported earlier [20–22]. The tosylate salts were prepared by reacting the free ligands with *p*-toluenesulfonic acid in methanol. The analysis of ^1H NMR data suggested the formation of an adduct with six tosylate groups providing six positive charges on the macrocyclic moiety. The phosphate complex was synthesized by the addition of a few drops of aqueous phosphoric acid to **L1** dissolved in methanol. Crystals suitable for X-ray analysis were grown from the slow evaporation of the solution at room temperature.

^1H NMR titrations of $[H_6\mathbf{L1}]^{6+}$ and $[H_6\mathbf{L2}]^{6+}$ were performed to study the binding interactions with phosphate using *n*-$Bu_4N^+ H_2PO_4^-$ ($TBAH_2PO_4$) in D_2O as well as in DMSO-d_6. The incremental addition of the anion (20 mM) to $[H_6\mathbf{L1}]^{6+}$ (2 mM) in D_2O (pH = 5.2) resulted in a downfield shift of aliphatic protons. The non-linear regression analysis of the shift change of several independent protons of $[H_6\mathbf{L1}]^{6+}$

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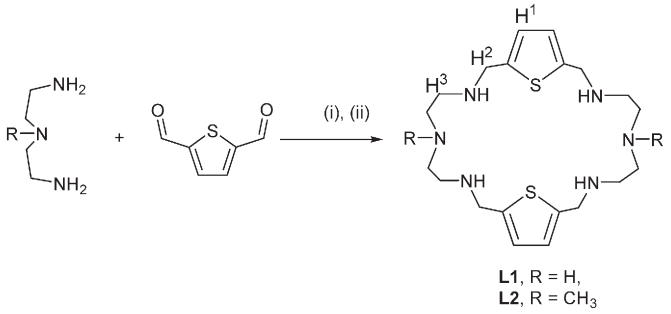

Scheme 1. Synthetic scheme for the macrocycles **L1** and **L2**: Reaction conditions: (i) methanol, 0 °C, 24 h stirring; (ii) $NaBH_4$, methanol, overnight stirring.

showed moderate binding for phosphate ($\log K = 2.0\ M^{-1}$), providing a good fit of a 1:1 binding model [23]. However, under identical conditions, there was no significant change in any protons of $[H_6\mathbf{L2}]^{6+}$ upon the addition of $TBAH_2PO_4$ to the host solution, indicating weak host-guest interactions in D_2O. We, therefore, proceeded to investigate the binding properties of $[H_6\mathbf{L1}]^{6+}$ and $[H_6\mathbf{L2}]^{6+}$ in DMSO-d_6. Fig. 1 displays the stacking of ^{1}H NMR titration spectra of $[H_6\mathbf{L1}]^{6+}$ obtained after the increasing amount of phosphate anion (ranging from 0 to 10 equivalents), showing a gradual upfield shift of both the aromatic and the aliphatic protons of the macrocyclic moiety at room temperature. The changes in the chemical shift of the aromatic proton (Ar*H*) as a function of the anion concentration are displayed in Fig. 1. The non-linear regression analysis of the changes in the chemical shift of the ligand as recorded with an increasing amount of anionic solution provided the best fit for a 1:2 binding model [24]. The ligand $[H_6\mathbf{L2}]^{6+}$ also shows a similar binding trend with phosphate in DMSO-d_6. Association constants presented in Table 1 suggest that the binding process of each ligand involves the formation of both a 1:1 (ligand:anion) complex and a 1:2 (ligand:anion) complex. However, a 1:2 complex is stronger than a 1:1 complex in DMSO-d_6, supporting the X-ray structure (discussed later). A similar binding mode was previously reported for phosphate with [26] $aneN_6C_6$ [10]. As shown in Table 1, the overall binding constant of **L1** for phosphate in DMSO-d_6 is slightly higher than that of **L2** which could be due to the reduced hydrogen bonding ability of the methylated compound.

The interaction of $[H_6\mathbf{L1}]^{6+}$ and $[H_6\mathbf{L2}]^{6+}$ with $TBAH_2PO_4$ in D_2O was also investigated by phosphorus ^{31}P NMR at room temperature.

Table 1
Association constants of the ligands for phosphate as determined by ^{1}H NMR titrations.[a]

Ligand	Log *K*	
	D_2O	DMSO-d_6
L1	2.0 (1:1)	1.54 (1:1) 5.25 (1:2)
L2	[b]	1.92 (1:1) 4.20 (1:2)

[a] Estimated error was less than 15%.
[b] No significant NMR shift was observed.

Because of the lower sensitivity of ^{31}P NMR compared to ^{1}H NMR, a higher concentration of $TBAH_2PO_4$ (10 mM) was loaded to an NMR tube, and a host solution (25 mM) was prepared as a titrant. The ^{31}P resonance was calibrated against an aqueous phosphoric acid used in a sealed capillary tube. Fig. 2 shows the ^{31}P NMR spectra of $TBAH_2PO_4$ (10 mM) after the addition of two equivalents of $[H_6\mathbf{L1}]^{6+}$ and $[H_6\mathbf{L2}]^{6+}$ in D_2O. As clearly shown in Fig. 2a, the signal at $\delta_P = 0.218$ ppm for the free $TBAH_2PO_4$ shifts upfield to $\delta_P = -1.635$ ppm ($\Delta\delta_P = 1.853$ ppm) after the addition of $[H_6\mathbf{L1}]^{6+}$ (2 equivalents). The other ligand $[H_6\mathbf{L2}]^{6+}$ also shows similar shifting to $\delta_P = -1.404$ ppm but to a lesser extent ($\Delta\delta_P = 1.622$ ppm), indicating a lower affinity for the phosphate anion than that with $[H_6\mathbf{L1}]^{6+}$. This upfield shift suggest the formation of the phosphate complex due to the strong electrostatic interactions, where the bound phosphate is shielded by the interacting macrocycle. Similar upfield shifts of phosphorus resonance were reported due to the complexation by polyamine-based hosts [12,25].

The phosphate complex of **L1** was obtained by reacting the free macrocycle with phosphoric acid. Crystals suitable for X-ray analysis were obtained by recrystallization of the salt in methanol/water. The structural analysis of the phosphate complex reveals that the salt crystallizes in the triclinic space group (*P*1) and the asymmetric unit consists of two hexaprotonated macrocycles, three monohydrogen phosphate (HPO_4^{2-}), six di-hydrogen phosphate ($H_2PO_4^-$) and eight water molecules to yield a molecular formula of $2(C_{20}H_{40}N_6S_2)^{6+}\cdot 3(HPO_4)^{2-}\cdot 6(H_2PO_4)^-\cdot 8(H_2O)$. One hydrogen phosphate (P9) is disordered about 90:10 into two orientations. One water molecule (O8W) is also found to be disordered about 80:20. Each macrocycle is fully protonated and the total positive charges (12+) from the two hexaprotonated receptors are balanced by the

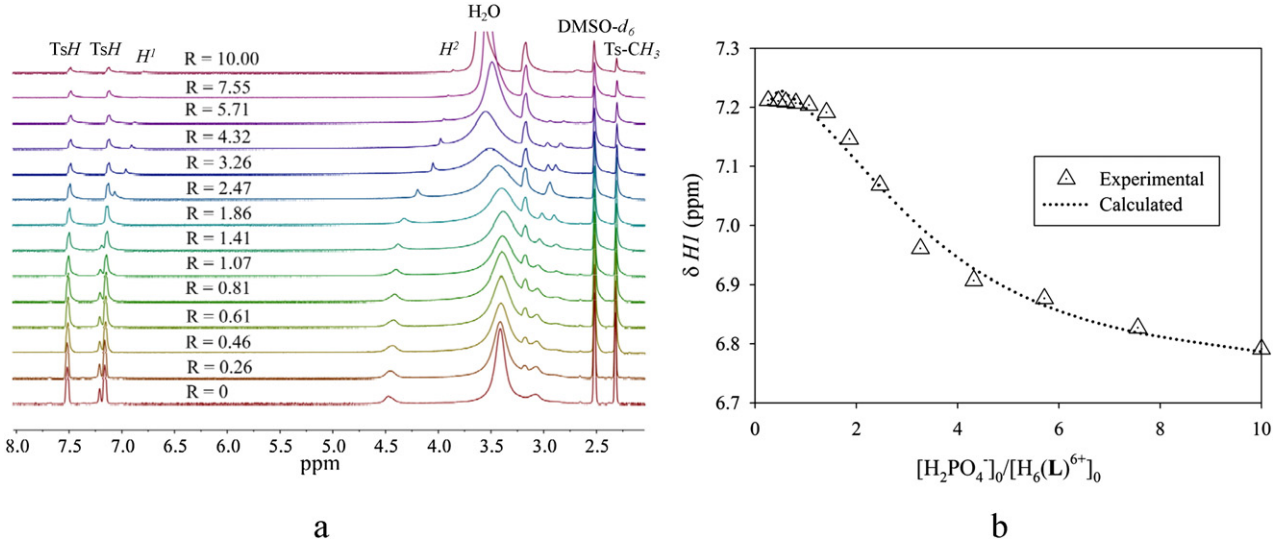

Fig. 1. (a) ^{1}H NMR spectra of $[H_6\mathbf{L1}](Ts)_6$ (2 mM) with an increasing amount of $TBAH_2PO_4$ (20 mM) in DMSO-d_6. (b) Titration curve of $[H_6\mathbf{L1}](Ts)_6$with $TBAH_2PO_4$ showing the change in the chemical shift of Ar-*H* (H1) against an increasing concentration of phosphate in DMSO-d_6.