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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.

Keywords

Azamacrocycle; Ditopic complex; Anion binding; Phosphate complex

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 (ΔG^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

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Appendix A. Supplementary material CCDC 1035333 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_ request/cif. Supplementary data associated with this article can be found, in the online version, at doi: ???.

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* ^{1}H 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 ^{1}H 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.

^{1}H NMR titrations of [H$_6$**L1**]$^{6+}$ and [H$_6$**L2**]$^{6+}$ were performed to study the binding interactions with phosphate using *n*-Bu$_4$N$^+$H$_2$PO$_4^-$ (TBAH$_2$PO$_4$) in D_2O as well as in DMSO-d_6. The incremental addition of the anion (20 mM) to [H$_6$**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$_6$**L1**]$^{6+}$ 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$_6$**L2**]$^{6+}$ upon the addition of TBAH$_2$PO$_4$ to the host solution, indicating weak host-guest interactions in D_2O. We, therefore, proceeded to investigate the binding properties of [H$_6$**L1**]$^{6+}$ and [H$_6$**L2**]$^{6+}$ in DMSO-d_6. Figure 1 displays the stacking of ^{1}H NMR titration spectra of [H$_6$**L1**]$^{6+}$ obtained after the increasing amount of phosphate anion (ranging 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 Figure 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$_6$**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. 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. Figure 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 Figure 2a, the signal at δ_P = 0.218 ppm for the free $TBAH_2PO_4$ shifts upfield to δ_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 δ_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. Crystallographic data are presented in Table 2. 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 negative charges provided by three doubly-charged monohydrogen phosphates and six singly-charged dihydrogen phosphate anions.

As shown in Figure 3, two identical macrocycles in the asymmetric unit possess a non-crystallographic inversion center, forming a sandwich type complex with four bound phosphate groups and two water molecules. Two water molecules serve as linkers to macrocycles as well as to bound dihydrogen phosphates locating between the macrocycles (Figure 3a). Each macrocycle adopts the shape of a flat ellipsoid and binds two phosphate groups; one is monohydrogen phosphate and the other is dihydrogen phosphate, located above and below the elliptical plane. The complex is stabilized through electrostatic interactions and multiple H-bonds. A list of H-bond distances with bond angles are shown in Table 3. Figure 3c shows one macrocycle of the asymmetric unit, with two bound phosphates held by hydrogen bonding interactions with the macrocyclic cation. The monohydrogen phosphate (P76) is held by two NH···O (2.684 and 2.747 Å) and two CH···O (3.266 and 3.388 Å) bonds, while the dihydrogen phosphate interacts through two strong NH···O bonds (N15H···O70 = 2.932 and N15H···O73 = 2.677 Å) and one weak NH···O

bonds (N39H···O80 = 3.266 Å). As a result, the monohydrogen phosphate is closer to the cavity than the dihydrogen phosphate. The involvement of CH···O interactions is well documented in the literature [21]. Extensive hydrogen bonds between the macrocycle and water molecules are also present.

In conclusion, we report the phosphate binding properties of two simple thiophene-based monocyclic receptors **L1** and **L2** by ^{1}H NMR and ^{31}P NMR studies in two different polar solvents (D_2O and DMSO-d_6). Both ligands show lessened interactions with the anion in competitive D_2O due to their weak hydrogen bonding ability in the highly polar solvent. However, after switching the solvent from D_2O to DMSO-d_6, the binding affinity is significantly increased, exhibiting the formation of 1:2 complexes. The ^{31}P NMR has been successfully used as a probe to identify the chemical environment of bound and free phosphates in D_2O. Structural characterization of the phosphate complex with **L1** suggests that both NH···O and CH···O interactions play roles in stabilizing the host-guest complex.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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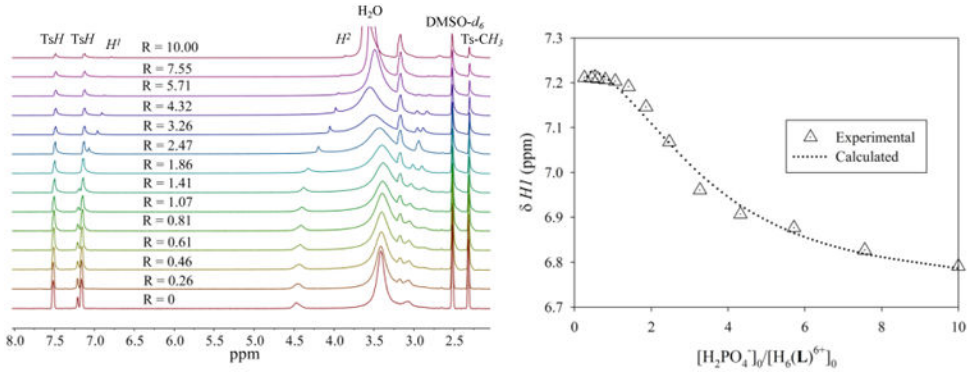


Figure 1.

(a) ^{1}H NMR spectra of [H_6**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**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.

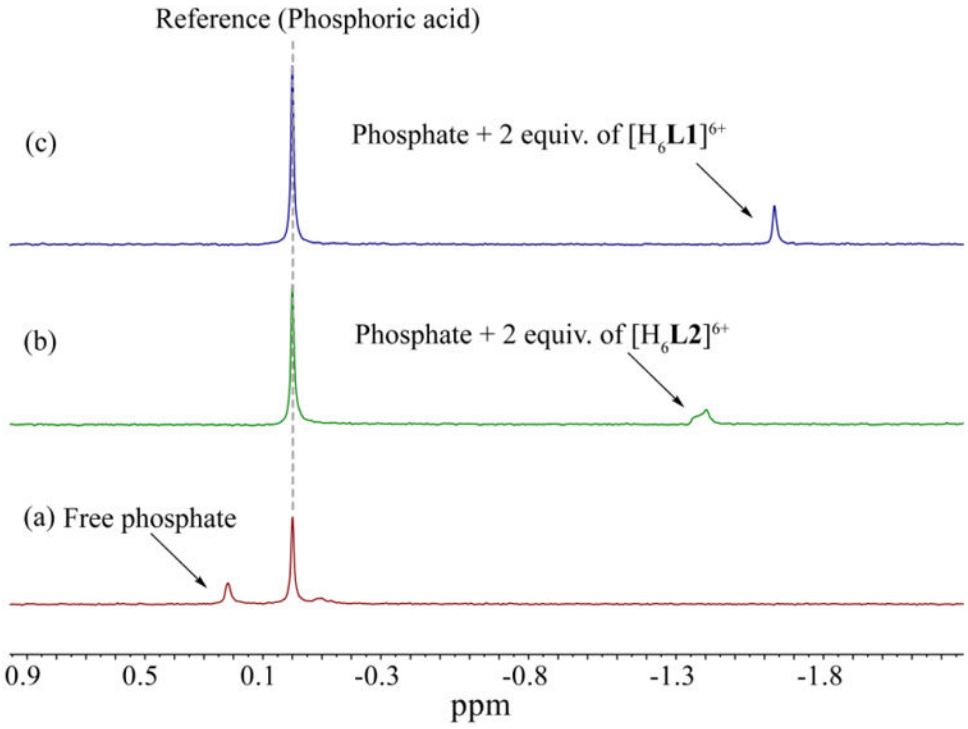


Figure 2.

^{31}P NMR spectra of n-$Bu_4N^+H_2PO_4^-$ in D_2O recorded at room temperature (a) free $TBAH_2PO_4$ (δ_P = 0.218 ppm) (10 mM) (b) $TBAH_2PO_4$ + 2 equivalents of [H_6**L2**] $(Ts)_6$ (δ_P = − 1.404 ppm) and (c) $TBAH_2PO_4$ + 2 equivalents of [H_6**L1**] $(Ts)_6$ (δ_P = − 1.6357 ppm)

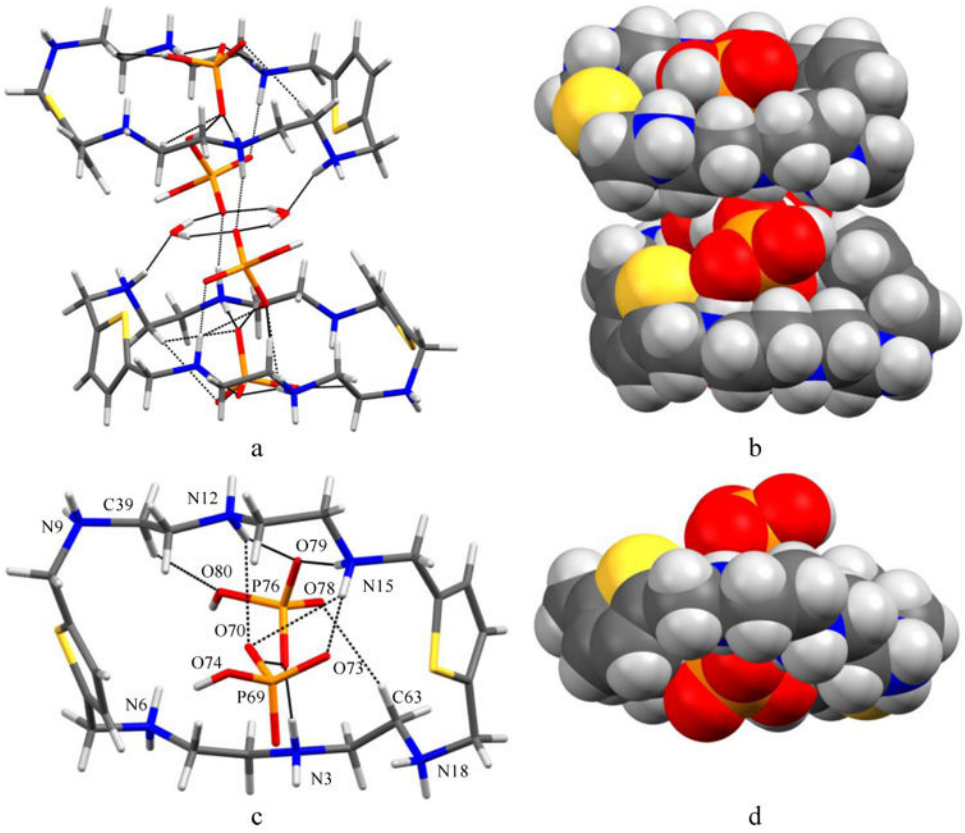
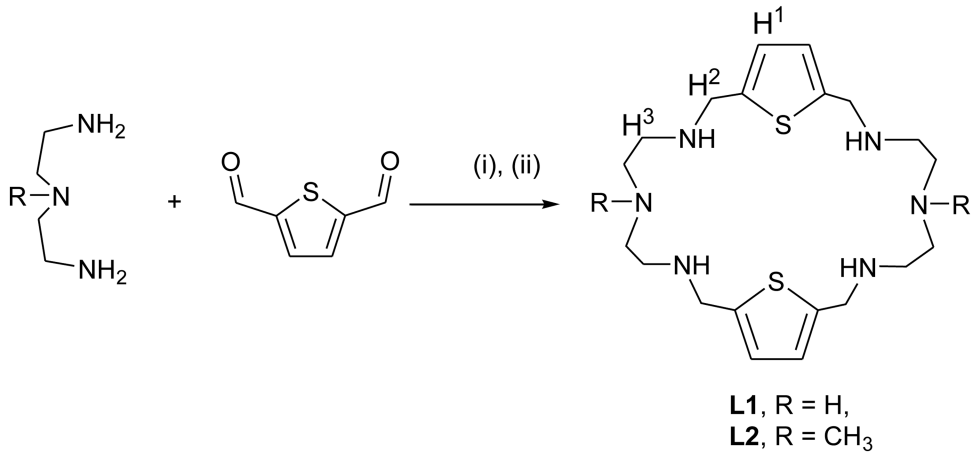


Figure 3.

Top: crystal structure of the phosphate complex showing $[(H_6\mathbf{L1})_2{\cdot}(HPO_4)_2{\cdot}(H_2PO_4)_2{\cdot}(H_2O)_2]^{6+}$ moiety in capped-stick view (a), and space filling view (b). Bottom: crystal structure of the phosphate complex showing $[(H_6\mathbf{L1}){\cdot}(HPO_4){\cdot}(H_2PO_4)]^{3+}$ moiety in capped-stick view (c), and space filling view (d).



Scheme 1.

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

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.