Selective recognition of H2PO4 by a cholestane-imidazole-zinc ensemble

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Selective recognition of $\text{H}_2\text{PO}_4^-$ by a cholestane-imidazole-zinc ensemble

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ABSTRACT

A new facile amphiphile cholestane-based zinc complex 4 containing a 3-aminopropylimidazole moiety at the 3α and 7α positions of cholestane was designed and synthesized. Recognition selectivity of the new receptor 4 with various anions was assessed by $^1$H NMR titration. Dihydrogen phosphate showed the highest binding affinity among all the tested anions ($K_a = 4.4 \times 10^{5} \text{M}^{-1}$).

Organized assembly of molecules through non-covalent interactions is a powerful tool for generating defined molecular architectures. Such types of assemblies have been effectively utilized in host–guest chemistry. Research in the chemistry of anion recognition is expanding and interest in the field of supramolecular chemistry continues due to its significant role in a wide variety of environmental, clinical, chemical, and biological applications. Receptors that can selectively bind and detect the phosphate derivatives have actively been investigated in recent years. Of all the anions, recognition of the phosphate anion is vital because of its significant role in signal transduction, energy storage, and gene expression and functions of proteins, including enzymes, transcription factors, hormonal receptor sites, and biological membranes, have been recognized. Zn(II) is highly regulated under normal physiological conditions, as this metal ion plays a key role in a wide variety of processes such as DNA and RNA syntheses, transmission of genetic messages, growth and development, signal transduction, apoptosis, brain and immune function, and lipid metabolism. In addition, the zinc ion is also closely involved in intercellular signaling and neuromodulation functions. More specifically, several groups reported various zinc complexes for the recognition of anions containing di-(2-picolyl)amine as metal binding site.

On the other hand, steroid motifs are an important class of macrocyclic compounds and offer several attractive features that allow them to be used as building blocks in the construction of molecular receptors. One distinct advantage is their high degree of preorganization when they are inserted into a receptor, because the rigid steroid frame enforces a preorganized binding site and provides a high degree of homogeneity. Cholesterol-based receptors are the least synthesized and used in supramolecular chemistry due to the limited usefulness by their low degree of functionalization. Our main aim was to design and synthesize receptors functionalized at the C-3 and C-7 positions of 5α-cholestane with amino groups for molecular recognition. For that, we have also described several methods for the preparation of amines through reductive amination. The 5α-skeleton of cholestane provides more space between the functionalized positions at C-3 and C-7, and the introduction of the axial 3- and 7-amino groups provides the two necessary inwardly-directed hydrogen bonding sites. We have also synthesized novel cholestane-based receptors containing amide, and imidazole as recognizing pendants at the 3 and 7-positions of 5α-cholestane for anions and dicarboxylic acid recognition. Recently, cholestane-based fluorescent imidazolium receptors 2 and 3 bridged with 9,10-dimethyleanthracene and 4,5-dimethyleneacridine, respectively, have been synthesized. Receptor 2...
showed the highest selectivity toward dihydrogen phosphate whereas 3 was found to be selective toward hydrogen pyrophosphate.

Herein, we report the synthesis and binding affinity of a new cholestane-imidazole-zinc ensemble 4 bearing two binding imidazole units at the C3 and C7 positions of 5α-cholestane which are connected with zinc through coordination bond.

The cholestane-imidazole-zinc ensemble 4 was prepared as shown in Scheme 1. Compound 1 was obtained through a one-step reductive amination of 5α-cholestane-3,7-dione with 3-aminopropylimidazole as described in our previous literature. The subsequent reaction of 1 with zinc perchlorate under reflux in CH3CN for 12 h gave the corresponding zinc-–imidazole complex 4. The 1H and 13C NMR, elemental analysis and HR-FAB-mass confirmed the structure of 4.

The metalloccycle phane formation study was initiated by the reaction of 1 with various metal cations. The formation was investigated by monitoring its interaction with various metal cations such as Hg2+, Cu2+, and Zn2+ through 1H NMR spectroscopic technique in DMSO-d6. These studies indicate that 1 showed the highest selectivity toward Zn(ClO4)2. When the Zn2+ was added successively to the solution of 1 in DMSO-d6, the significant downfield shifts of both imidazole H-2 protons were observed. A similar result was observed with Hg(ClO4)2 and Cu2+ but the extent of H-2 chemical shifting was less than that of Zn(ClO4)2, whereas in the case of Cu2+ after the addition of 1 equiv, the H-2 protons of both imidazoles disappeared completely. So, considering these results, we are encouraged to make the zinc complex with 1 which can be further utilized for anions recognition.

To investigate the nature and strength of complexation of 1 with Zn(ClO4)2, 1H NMR titration was carried out in DMSO-d6. The H-2 protons of both imidazoles significantly shifted toward the imidazole nitrogen of 1. Furthermore, the titration of 1 with Zn(ClO4)2 was confirmed by its HR-FAB mass and elemental analysis data, which indicated a 1:1 stoichiometry for this complex. The peak at 781.4122 in HR-FAB mass spectrum of 1H NMR data gave the association constant of 105 M-1, 4.4 which also confirmed the 1H NMR peaks of H2PO4 after the successive addition of H2PO4 and HPO42- ions (Figs. S-3 and S-5).

The association constants (K, M-1) were estimated to be comparable to those of other anions, as depicted in Table 1. The association constants were calculated from the chemical shift changes of the H-2 protons of imidazoles by using the WinE-NMR software, which suggested a 1:1 complex formation. The 1:1 stoichiometry of anions with 4 is also confirmed by 1H NMR Job's plot binding with H2PO4- ion (Fig. S-4). From the 1H NMR titration, the association constants of 4 in DMSO-d6 with F-, Cl-, Br-, CH3COO-, SO42-, and H2PO4- were calculated to be 1.2 × 10^5 M-1, 4.4 × 10^4 M-1, 8.9 × 10^4 M-1, 8.8 × 10^4 M-1, 9.9 × 10^3 M-1, and 4.4 × 10^3 M-1, respectively. Due to its negligible chemical shift values the association constant of I- could not be calculated.

The present study revealed that 4 containing hydrogen bond donors as H-2 protons of imidazole which are adjacent to the Zn(II) center increase the affinity of anions to the metal center as well as the extra two 3,7-NH hydrogen bond donors which also contributed to the firm binding of the anions. The binding constant observed for ensemble 4 toward dihydrogen phosphate is found to be three times higher than that of the receptor 2 and ten times greater than that of 3.

In order to understand the nature, as well as the binding mode of 4 with anions, a molecular mechanic calculation was conducted, that was optimized at the Hartree–Fock 3-21G (s) level using Spartan 04 software. Energy minimization of 4 (Fig. S-5) clearly indicates that zinc metal coordination with two imidazoles of 1, well organize the all binding sites of 4, making it appropriate for anion recognition. H2PO4- was found to be more strongly bonded to both zinc metal ion and both the imidazole H-2 protons through C—H—O and Zn2+-O2- bonds. Whereas two axially situated –NH hydrogen donors and two C2-H imidazole hydrogen donors were all found to have contributed to bind spherical shaped Br– anion (Fig. S-5). The distances calculated for both imidazole N-3 with Zn(II) are found to be a = 1.85 Å and b = 1.84 Å which also match with the literature values. Furthermore distances calculated for the interaction of 4 with H2PO4- are indicated by the dotted lines in Figure 4. The distances observed for host guest interactions are c = 2.30 Å, d = 2.10 Å, e = 2.22 Å, f = 2.23 Å, g = 1.96 Å, and h = 3.48 Å for H2PO4- ion. However, it was not possible to determine the

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**Scheme 1.** Synthesis of cholestane-imidazole-zinc ensemble.
chemical shift values by NMR titration because of the assimilation of N–H protons in the up field area.

The interaction of 4 with dihydrogen phosphate is also investigated through partial $^{31}$P NMR spectroscopy study in DMSO-$d_6$ (Fig. 5). In the $^{31}$P NMR spectrum, phosphorus peak of dihydrogen phosphate shifted considerably upfield from its original position $d_{0.15}$ to $d_{0.98}$ after the 1 equiv addition of 4 ($\Delta d = -1.13$). Significant upfield shift of the phosphorous peak of dihydrogen phosphate clearly revealed that dihydrogen phosphate is strongly bound with the zinc complex 4. The complexation of 4 with dihydrogen phosphate is also confirmed by FAB mass (Fig. S-6). The FAB mass spectrum revealed the formation of complex 4–$\text{H}_2\text{PO}_4^-$ as a 1:1 stoichiometry with the molecular ion peak at $m/z$ 895.5, which can be assigned as the signal for [M+$\text{H}_2\text{PO}_4^+$+$\text{H}_2\text{O}$+$\text{HClO}_4^-$]. Due to the amphoteric properties of Zn$^{2+}$, a water molecule participates in the coordination sphere as a fourth or fifth ligand.8a The attempts to grow single crystals of complex 4–$\text{H}_2\text{PO}_4^-$ that are suitable for X-ray structure determination were not successful.

The necessity of metal ion coordination for anions selectivity were also judged by treating 1 with F$^-$, Cl$^-$, Br$^-$, I$^-$, CH$_3$COO$^-$, $\text{H}_2\text{PO}_4^-$, $\text{HSO}_4^-$ and HP$_2\text{O}_7^3-$ (2 equiv) in the form of tetrabutylammonium salts, but none of the anions induced any changes in the chemical shift of the imidazole H-2 proton (Fig. S-7). However, the addition of zinc ion to ligand 1 followed by dihydrogen phosphate, a significant downfield chemical shift was observed (Fig. S-8). These observations reveal that the importance of the metal complex formation is a pre-requisite for selective the recognition of anions.

In conclusion, we have synthesized a new cholestane-imidazole-zinc ensemble 4 as an anion receptor, which showed the highest selectivity toward the $\text{H}_2\text{PO}_4^-$ through hydrogen bonding and
...coordinative interaction. Both imidazole C2-H hydrogen bond donors adjacent to a Zn(II) center were found to increase the affinity of phosphate to the Zn(II) center. The highest selectivity of 4 toward H2PO4− might be caused due to the better-defined substrate binding sites, their reduced structural flexibility, and increased uniformity due to the hydrogen binding and metal ion coordination.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.03.047.

References and notes


12. (a) Brotherhood, P. R.; Davis, A. P.


16. Compound 4: 1H NMR (DMSO-d6) δ 0.61 (s, 3H, 18-CH3), 0.75 (s, 3H, 19-CH3), 0.83 (d, J = 6.6 Hz, 26-CH3); 0.84 (d, J = 7.4 Hz, 21-CH3); 1.79–1.88 (m, 4H, –CH2); 2.55–2.82 (m, 4H, –CH2); 4.07–4.25 (m, 4H, –OCH2–); 6.05 (s, 2H, im-H5), 7.34 (s, 2H, im-H4), 7.85 (s, 2H, im-H2); 13C NMR (DMSO-d6) δ 12.0, 18.9, 19.5, 20.6, 21.5, 22.7, 22.9, 23.5, 23.6, 24.9, 27.7, 28.0, 31.0, 31.2, 31.4, 32.1, 35.5, 36.1, 36.4, 38.9, 39.3, 40.7, 42.6, 43.1, 44.1, 45.1, 45.8, 50.4, 52.7, 54.0, 56.2; Elemental analysis: Anal. Calcd for C45H56Cl2N6O8Zn: C, 54.1, 45.8, 50.4, 52.7, 54.0, 56.2; Found: C, 54.97; H, 7.49; N, 8.97; Elemental analysis: Anal. Calcd for C39H66Cl2N6O8Zn-ClO4: 781.4126, Found: 781.4122.


18. Energy minimization was carried out using Spartan'04 for Windows (Wavefunction, Inc.: Irvine, CA).