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Phosphates Sensing: Two Polyamino-Phenolic Zinc Receptors Able to Discriminate and Signal Phosphates in Water

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Two Zn(II)-dinuclear systems were studied as receptors for phosphates; they were obtained by using the two polyamino-phenolic ligands 3,3′-bis[*N,N*-bis(2-aminoethyl)aminomethyl]-2,2′-dihydroxybiphenyl (**L1**) and 2,6-bis[*N,N*-bis(2-aminoethyl)aminomethyl]phenol (**L2**) in which the difference lies in the spacers between the two dien units, biphenol or phenol in **L1** and **L2**, respectively. The metallo-receptors obtained are able to selectively discriminate phosphate (Pi) from pyrophosphate (PPi) and vice versa in aqueous solution in a wide range of pH ($6 < pH < 10$). The **L1** receptor system shows selectivity toward PPi over Pi, and on the contrary the **L2** system exhibits opposite selectivity. This different selectivity is ascribed to the different Zn(II)−Zn(II) distances between the two metal centers which, showing a similar coordination requirement and binding phosphate in a bridge disposition, fit in a different way with the different guests. Furthermore, NMR studies supported the model of interaction proposed between guests and receptors, highlighting that they are also able to bind biological phosphates such as G6P and ATP at physiological pH. Fluorescence studies showed that the receptor system based on **L1** is able to signal the presence in solution of Pi and PPi at physiological pH; the presence of Pi is detected by a quenching of the emission, that of PPi by an enhancement of it. With the aid of an external colored sensor (PCV), the receptors were then used to produce simple signaling systems for phosphates based on the displacement method; the two chemosensors obtained are able to signal and quantify these anions at physiological pH, preserving the selectivity between phosphate and pyrophosphate and extending it to G6P and ATP.

Introduction

Anions play a fundamental role in both environmental and biological chemistry. Among all anions, phosphate and molecules showing this group are of special interest because of their ubiquitous presence in life. The presence of inorganic phosphate derived from fertilizers, similarly to nitrates, leads to an excessive growth (eutrophication) of aquatic plants and algae that disrupts aquatic life cycles,[1] while sodium and potassium organo-phosphate compounds are among the most used parasiticides in many intensive agricultural activities and are often found in ground waters, leading to severe health problems.[2]

Phosphates are involved in many biological processes spanning a wide range of functions from making up genes together with heterocyclic bases and sugars, to storing energy.[3−6] Among all the biomolecules showing phosphate groups, nucleotides such as adenosine-triphosphate (ATP) and its hydrolytic derivatives play important roles and are thus of great interest for monitoring their presence in solution in real-time; along the same lines, gluocose-6-phosphate (G6P) is linked to ATP in the glycolysis process by the hexokinase enzyme.[7]

Given the importance of these species, it is essential to improve the efficiency of molecular systems able to selectively bind, detect, and in some cases signal them, with the final

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purpose of measuring the levels of the specific anions in environmental and biological matrixes. This is, however, a difficult task because of the presence of the aqueous medium, which strongly competes for the coordination of the anions; the successful development of photochemical systems able to selectively coordinate the target anion and at the same time to signal it is even more difficult.[8,9]

In recent years, two main approaches have been developed to obtain receptors for anions and such for phosphates in water. The most exploited strategies involve (i) metal complexes, in which the coordinated metal center shows an unsaturated coordination environment and thus can bind the anion via classical coordination chemistry;[10] (ii) positively charged hosts, as the polyammonium class, which can interact with the anion mainly via charge−charge interactions. However, H-bonds and π-stacking interactions can aid recognition, mainly in complex phosphates such as nucleotides including ATP, ADP, and others.[11]

It must also be taken into account that the way phosphates interact with a receptor depends not only on the pH of the medium, that is, the degree of protonation, but also on the length of the polyphosphoric chain.

It is well-known that by nature many metal complexes are receptors able to add, catalyze, or transport substrates mainly of anionic character; in addition, the cooperation of two or more metal ions in creating the active center makes it possible to achieve receptors forming stable adducts and showing high selectivity toward specific substrates.[12,13] For this reason, many efforts have recently been made to synthesize ligands with a macrocyclic or non-macrocyclic skeleton able to form dinuclear metal complexes affording this cooperation of the metal ions in forming the active center.[14−16]

Furthermore, the modulation of the distance between the metal centers is crucial, as shown in the natural polynuclear metallo-receptors, which can be used as the key point to obtain selectivity toward specific substrates. In this case, dinuclear zinc complexes are attractive, given the well-known affinity of phosphates and related biomolecules for many natural zinc enzymes with multinuclear zinc sites. Although many receptors for phosphates have been produced in recent years, few of them are able to discriminate phosphate from polyphosphates, highlighting (also by color changes) the presence of the anions in solution.[17] In the field of sensor receptor systems, some of them show the chromophore covalently linked to the molecular framework but, more recently, the chemosensing ensemble method, based on the displacement approach, has been introduced, giving rise to an improvement in research on achieving better sensor-systems for specific guests.[18,19] This competitive method foresees the use of an external secondary guest used as sensor (S) which can be bound by the receptor (R) to form the R-S sensing system. The photoactive species S has different optical properties depending whether it is free or bound in the complex. The affinity between R and S cannot be very high so as to allow it to be released in the medium in the presence of the target guest (G), which should usually show higher affinity toward R to form the R-G adduct; the release of S in the medium indirectly signals the presence of G.

In this study, the binding properties of two dinuclear zinc systems toward the phosphate anions phosphate (Pi), pyrophosphate (PPi), and ATP and G6P (Chart 1) are reported.

The two Zn(II) dimetallic systems used as phosphates receptors were obtained by the two aza-phenolic ligands **L1** and **L2** reported in Chart 1.[20,21] The molecular skeleton of both ligands allows the formation of preorganized dinuclear Zn(II) species in which the two Zn(II) ions can cooperate to bind guests. In particular, some dinuclear species, such as $[Zn_2(H_{-2}\mathbf{L1})]^{2+}$ and $[Zn_2(H_{-1}\mathbf{L2})]^{3+}$, show the zinc ions similarly coordinated (see Scheme 1a), displaced at different fixed distances (longer in **L1** than in **L2**) and able to

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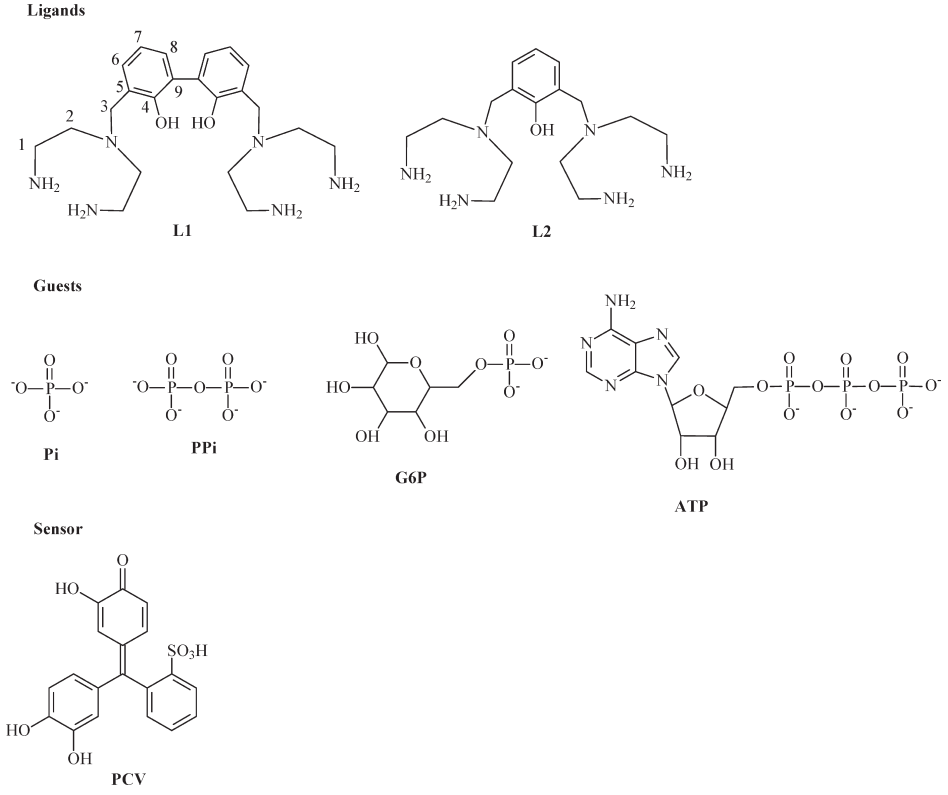
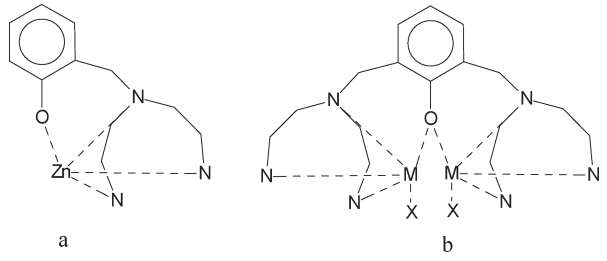
Chart 1. Ligands Together with Labels for the NMR Resonances and the Phosphate Guests Investigated



Scheme 1. Fragment Searched in the CSD[a]



[a] In a, the zinc cation is pentacoordinated; in b, X is a halogen atom.

add guests to saturate their coordination requirement; in addition, both systems provided fluorescent signaling of the presence of Zn(II) in solution.[22]

Complex formation between the dinuclear Zn(II) species and the guests G was investigated by potentiometric, UV−vis, fluorimetric and ^{1}H, ^{31}P NMR measurements, performed in aqueous solution on the system R/G (R1 = 2Zn(II)/**L1** and R2 = 2Zn(II)/**L2**; G = PO_4^{3-}, $P_2O_7^{4-}$, ATP, and G6P).

Two new R-S sensor systems working at physiological pH were prepared and investigated as signaling systems for phosphates in water; the systems are made up of a Zn(II)-dinuclear system R (R1 using **L1**; R2 using **L2**) and pyrocatechol violet (PCV) used as a colorimetric sensor S. The crystal structure of the $[Zn_2(H_{-1}\mathbf{L2})(Br)_2]^+$ cation is also reported.

Experimental Section

Synthesis. Ligand 3,3′-bis[*N*,*N*-bis(2-aminoethyl)aminomethyl]-2,2′-dihydroxybiphenyl (**L1**) and 2,6-bis[*N*,*N*-bis(2-aminoethyl)aminomethyl]phenol (**L2**) were prepared as previously described.[20,21a]

$[Zn_2(H_{-1}L2)(Br)_2](ClO_4)$ (1). A sample of $Zn(ClO_4)_2 \cdot 6H_2O$ (37.2 mg, 0.1 mmol) in water (10 mL) was added to an aqueous solution (10 mL) containing **L2**·6HBr (16.3 mg, 0.05 mmol). The pH of the resulting solution was adjusted to 6 with 0.1 M NaOH; then an excess of NaBr (51.5 mg, 0.1 mmol) was added, and **1** precipitated as a microcrystalline white solid (27 mg, 76%). Crystals suitable for X-ray analysis were obtained by slow evaporation of an aqueous solution containing **1**. Anal. Calcd for $C_{16}H_{31}Br_2ClZn_2N_6O_5$: C 26.94; H 4.38; N 11.78. Found: C 27.1; H 4.5; N 11.8.

Caution!. *Perchlorate salts of organic compounds are potentially explosive; these compounds must be prepared and handled with great care.*

X-ray Crystallography. For compound $[Zn_2(H_{-1}\mathbf{L2})(Br)_2](ClO_4)$(**1**) intensity data were collected by using an Oxford Diffraction XCalibur diffractometer equipped with a CCD area detector. The radiation used was Mo Kα (λ = 0.7107 Å). Data collection was carried on with the CrysAlis CCD[23] program, and data frames were collected with 1° increment in ω. Data reduction was performed with the CrysAlis RED[24] program. Absorption correction was performed with the program SADABS.[25] The structure was then solved by using the SIR97

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program[26] and refined by full-matrix least-squares against F^2 using all data (SHELX97).[27]

The ethylenic chains C(6)−C(7) and C(8)−C(9) show a certain degree of disorder, and as a consequence the carbon atoms C(7) and C(9) were set in double position, the atoms N(2), N(3), C(6), C(7), C(8), and C(9) were refined isotropically, and the hydrogen atoms bonded to these atoms were not introduced. All the other non-hydrogen atoms were refined anisotropically. All the hydrogen atoms (with the exception of the above ones) were set in calculated positions and refined in agreement with the atoms to which they are bound.

Geometrical calculations were performed by PARST97[28] and molecular plots were produced by the Oak Ridge Thermal Ellipsoid Program (ORTEP3).[29]

Crystallographic data and refinement parameters are reported in Table 1.

EMF Measurements. Equilibrium constants for the ternary systems 2Zn(II)/L/G (L = **L1** and **L2**; G = PO_4^{3-} and $P_2O_7^{4-}$) were determined by pH-metric measurements (pH = $-\log[H^+]$) in 0.15 mol dm^{-3} NaCl aqueous solution at 298.1 ± 0.1 K, using the fully automatic equipment already described.[30] Protonation constants of **L1** and **L2**, as well as their stability constants with Zn(II) employed in the calculations, were determined in previous works.[20,22] The EMF data were acquired with the PASAT computer program.[31] A combined glass electrode was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO_2-free NaOH solutions and determining the equivalent point by Gran's method,[32] which gives the standard potential E° and the ionic product of water. At least three potentiometric titrations were performed for each system in the pH range 5−11, using a different molar ratio of the dinuclear complex/substrate ranging from 1:1 to 1:3. All titrations were treated either as single sets or as separate entities for each system; no significant variations were found in the values of the determined constants. The HYPERQUAD computer program was used to process the potentiometric data.[33]

Spectroscopic Experiments. ^{1}H and ^{31}P NMR spectra were recorded on a Bruker Avance 200 instrument, operating at 200.13 and 81.01 MHz, respectively, and equipped with a variable temperature controller. The temperature of the NMR probe was calibrated using 1,2-ethandiol as calibration sample. For the spectra recorded in D_2O, the peak positions are reported with respect to HOD (4.75 ppm) for ^{1}H NMR spectra; ^{31}P NMR chemical shifts are relative to an external reference of 85% H_3PO_4.

For experiments at pH = 7.4 and 8.3, 0.01 M HEPES and TAPS buffer D_2O solutions were used, respectively.

Fluorescence spectra were recorded at 298 K with a Varian Cary Eclipse spectrofluorimeter. UV absorption spectra were recorded at 298 K with a Varian Cary-100 spectrophotometer equipped with a temperature control unit; either HEPES or TAPS was used as buffer for the solution at controlled pH values.

The sensing systems R1-PCV and R2-PCV (R1 = 2Zn(II)/**L1**, R2 = 2Zn(II)/**L2** systems) used to detect the interaction with phosphate anions were prepared by mixing $Zn(ClO_4)_2$, **L1** or **L2** and pyrocathecol violet (PCV) in a 100:50:1 molar ratio in an aqueous solution of 10 mM HEPES buffer pH = 7.4; in this way, the molar ratio calculated on the receptor species R and PCV was 50 to 1 for both systems. The final concentrations of R and PCV were 4.0×10^{-4} and 8.0×10^{-6} M, respectively. Buffered HEPES solutions containing G anions (G = PO_4^{3-}, $P_2O_7^{4-}$, ATP, and G6P) were added to the sensing solution up to 5 equiv with respect to the Zn(II)-complexes R.

The HYPERQUAD computer program was used to process the spectrophotometric data.[33] The processing of the spectrophotometric curve sets for each G/R-PCV systems allowed us to evaluate the association constant of G to the R systems at this pH by referring to the reaction R-PCV + G = R-G + PCV; knowledge of the R + PCV = R-PCV addition constants made it possible to obtain the addition constants as G + R = R-G at this pH value.

Table 1. Crystallographic Data and Refinement Parameters for Compound **1**

empirical formula	$C_{16}H_{31}Br_2ClN_6O_5Zn_2$
formula weight	713.48
temperature (K)	298
wavelength (Å)	0.7107
crystal system, space group	monoclinic, $P2/n$
unit cell dimensions (Å, deg)	a = 10.692(2) b = 10.374(2), β = 98.33(1) c = 11.321(2)
volume (Å^3)	1242.5(4)
Z, D_c (mg/cm^3)	2, 1.907
μ (mm^{-1})	5.293
F(000)	712
crystal size (mm)	0.53 × 0.48 × 0.4
2θ range (deg)	8.2−64.6
reflections collected/unique	4388/3562
data/parameters	3562/123
final R indices [$I > 2\sigma(I)$]	R1 = 0.0676, wR2 = 0.1884
R indices (all data)	R1 = 0.1129, wR2 = 0.2202

Results and Discussion

X-ray Solid State Structures. The asymmetric unit of **1** contains half of the dinuclear metal complex and half of a perchlorate anion. The two halves of the complex and of the perchlorate anion are related by two different 2-fold symmetry axes passing through C(1), C(4), O(1), and Cl(1), respectively (Figure 1).

The zinc cation Zn(1) is pentacoordinated by the donor atoms N(1), N(2), N(3), and O(1), provided by the ligand H_{-1}**L2**$^-$, and by the bromide ion Br(1) (Figure 1). All the zinc-donor atom bond distances are in agreement with those found in the Cambridge Structural Database (CSD,[34] v. 5.28) for analogous compounds (see Table 2).

The resulting coordination polyhedron is a trigonal bipyramid (*tbp*) (τ index[35] = 0.84) with the atoms Br(1) and N(1) occupying the axial positions; the zinc atom is 0.2319(7) Å out of the mean plane defined by N(2), N(3), and O(1) and shifted toward the bromide ion. Lastly the oxygen atom O(1) works as a bridging unit between the two metal ions, keeping them 3.494(1) Å apart.

The two zinc cations are disposed on the opposite sides of the mean plane containing the aromatic ring: the angle defined by the phenolate mean plane (P1) and that defined by Zn(1), Zn(1′) [′ = $-x$ + 3/2, y, $-z$ + 1/2], and O(1) (P2) is 57.5(2)°. Finally, the two bromide ions that complete the coordination sphere of the two metal

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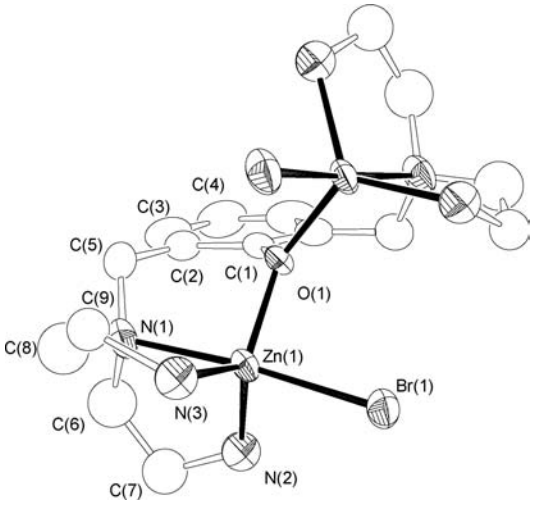


Figure 1. ORTEP3 view of compound **1** (30% probability).

Table 2. Selected Bond Distances (Å) and Angles (deg) for Compound **1**[a]

Zn(1)−O(1)	1.999(3)
Zn(1)−N(1)	2.248(6)
Zn(1)−N(2)	2.089(7)
Zn(1)−N(3)	2.075(7)
Zn(1)−Br(1)	2.535(1)
Zn(1)−Zn(1′)	3.494(1)
O(1)−Zn(1)−N(1)	87.5(2)
O(1)−Zn(1)−N(2)	111.9(2)
O(1)−Zn(1)−N(3)	122.8(2)
O(1)−Zn(1)−Br(1)	98.5(1)
N(1)−Zn(1)−N(2)	82.0(2)
N(1)−Zn(1)−N(3)	81.2(3)
N(1)−Zn(1)−Br(1)	173.4(2)
N(2)−Zn(1)−N(3)	121.5(3)
N(2)−Zn(1)−Br(1)	98.1(2)
N(3)−Zn(1)−Br(1)	93.2(2)
Zn(1)−O(1)−Zn(1′)	121.90(2)

[a] ′: $-x + 1/2 + 1, +y, -z + 1/2$.

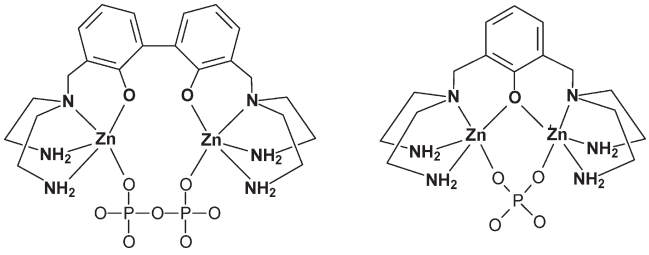
cations are above and below the plane containing the Zn−O−Zn moiety (Figure 1) [Br(1)···Br(1′) distance = 4.334(1) Å]

A search in the Cambridge Structural Database[34] gave five metal complexes[20,21b,36] containing the framework sketched in Scheme 1a: in four of them the zinc cation shows a *tbp* coordination geometry.[20,21,36a]

Two of these five complexes[21b] contain the same metal complex cation $(Zn_2H_{-1}\mathbf{L2})^{3+}$ as **1** but in those cases the metal ions are double-bridged. Besides the oxygen atom of the phenolate moiety, a donor atom, provided by an external ligand (butanolate and azide anion, respectively), completes the Zn(II) coordination sphere.

Although the two complexes show some similarities with **1** (i.e., the disposition of the five donor atoms around the zinc atoms and the position of the metal cations with respect to the phenolate mean plane), the presence of a double-bridged "core" [ZnOXZn] in the $(Zn_2H_{-1}\mathbf{L2})(N_3)^{2+}$ and $(Zn_2H_1\mathbf{L2})(C_4H_9O)^{2+}$ implies some relevant differences between the butanolate and azide complexes

Scheme 2. Proposed Coordination Models of Pi and PPi Anions in the Receptor Species of **L1** and **L2**



and **1**. Indeed, while in the first two complexes the Zn···Zn distance is a little bit longer than 3 Å,[37] in **1** the separation between the two zinc ions is 3.494(1) Å, and the P1−P2 angle is also definitely larger in **1** with respect to the double-bridged complexes: about 57 versus 24°.

It is worth to noting that all the three "mono-bridged" complexes containing the fragment sketched in Scheme 1b retrieved from the CSD show a P1−P2 angle of about 58° (and thus comparable with that observed in **1**).[38] In all cases the halogen ions are chloride.

Binding of Phosphates. The capability of both the R1 and R2 dinuclear systems (R1 = 2Zn(II)/**L1**, R2 = 2Zn(II)/**L2**) to act as host for a series of phosphate guests G (G = PO_4^{3-}, $P_2O_7^{4-}$, ATP and G6P) in aqueous solution were studied by potentiometric, UV−vis, fluorescence, and ^1H, ^{31}P NMR experiments.

Potentiometric Measurements. The R/G systems (R1 = 2Zn(II)/**L1**, R2 = 2Zn(II)/**L2** and G = PO_4^{3-} and $P_2O_7^{4-}$) were potentiometrically studied in 0.15 mol dm^{-3} NaCl aqueous solution at 298.1 K, using different R/G molar ratios; the stability constants for the ternary systems formed are reported in Tables 3 and 4 for **L1** and **L2**, respectively, while the distribution diagrams of the adducts formed are reported in Figure 2.

Both guests interact with the Zn(II)-dinuclear species of both ligands which can be considered receptor systems R for phosphates. Analysis of the data showed that under our experimental conditions, only adducts with 1:1 stoichiometry between the dinuclear complexes (R) and the guests (G) form. This suggests that the guests are able to simultaneously bind both Zn(II) ions, coordinating them in a bridge disposition between the two metal centers which thus cooperatively act in forming the active binding receptor site.

By examining the stability constants and comparing the formed systems, several features were identified. For the R1 system, the more stable adducts for both anions are those formed with the $[Zn_2H_{-2}\mathbf{L1}]^{2+}$ dinuclear species (see Table 3) which behaves, as previously suggested,[20,22] as the best receptor species in binding guests between all the Zn(II)-dinuclear species of **L1**. The addition constants revealed that PPi is better bound than Pi; in fact, while

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(37) A search performed using the CSD evidenced that the Zn···Zn distance in double bridged complexes having the oxygen of a phenolate moiety as one of bridged atoms is composed in the 2.87−3.33 Å range with a mean value of 3.11.

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Table 3. Logarithm of the Formation Constants for the 2Zn(II)/**L1** System with PO_4^{3-} (Pi) and $P_2O_7^{4-}$(PPi) Guests[a]

	LogK	
	L1	
reaction	Pi	PPi
$2Zn^{2+} + H_{-1}L^{-} + G^{n-} + H^{+} = [Zn_2H_{-1}L(HG)]^{4-n}$	37.34(3)	
$2Zn^{2+} + H_{-1}L^{-} + G^{n-} = [Zn_2H_{-1}L(G)]^{3-n}$	31.58(3)	28.55(3)
$2Zn^{2+} + H_{-1}L^{-} + G^{n-} = [Zn_2H_{-2}L(G)]^{2-n} + H^{+}$	21.42(4)	21.98(2)
$2Zn^{2+} + H_{-1}L^{-} + G^{n-} + H_2O = [Zn_2H_{-2}L(OH)(G)]^{1-n} + 2H^{+}$		11.00(2)
$2Zn^{2+} + H_{-1}L^{-} + G^{n-} + 2H_2O = [Zn_2H_{-2}L(OH)_2(G)]^{n-} + 3H^{+}$		−0.23(5)
$[Zn_2H_{-1}L]^{3+} + HG^{1-n} = [Zn_2H_{-1}L(HG)]^{4-n}$	2.22	
$[Zn_2H_{-2}L]^{2+} + HG^{1-n} = [Zn_2H_{-2}L(HG)]^{3-n}$	3.18	3.52
$[Zn_2H_{-2}L]^{2+} + G^{n-} = [Zn_2H_{-2}L(G)]^{2-n}$		5.07
$[Zn_2H_{-2}L(OH)]^{+} + HG^{1-n} = [Zn_2H_{-2}L(OH)(HG)]^{2-n}$	2.87	
$[Zn_2H_{-2}L(OH)]^{+} + G^{n-} = [Zn_2H_{-2}L(OH)(G)]^{1-n}$		3.94
$[Zn_2H_{-2}L(OH)_2] + G^{n-} = [Zn_2H_{-2}L(OH)_2(G)]^{n-}$		3.48

[a] G^{n-} = Pi or PPi, n = 3 or 4 for Pi and PPi, respectively; determined in 0.15 mol dm^{-3} NaCl at 298.1 K. Values in parentheses are the standard deviations on the last significant figure.

Table 4. Logarithm of the Formation Constants for the 2Zn(II)/**L2** System with PO_4^{3-} (Pi) and $P_2O_7^{4-}$(PPi) Guests[a]

	LogK	
	L2	
reaction	Pi	PPi
$2Zn^{2+} + L + G^{n-} + H^{+} = [Zn_2L(HG)]^{5-n}$	37.52(5)	
$2Zn^{2+} + L + G^{n-} = [Zn_2L(G)]^{4-n}$	33.12(3)	28.07(2)
$2Zn^{2+} + L + G^{n-} = [Zn_2H_{-1}L(G)]^{3-n} + H^{+}$	23.02(3)	20.49(3)
$2Zn^{2+} + L + G^{n-} + H_2O = [Zn_2H_{-1}L(OH)(G)]^{2-n} + 2H^{+}$		10.30(4)
$[Zn_2H_{-1}L]^{3+} + H_2G^{2-n} = [Zn_2H_{-1}L(H_2G)]^{5-n}$	2.00	
$[Zn_2H_{-1}L]^{3+} + HG^{1-n} = [Zn_2H_{-1}L(HG)]^{4-n}$	4.46	2.78
$[Zn_2H_{-1}L]^{3+} + G^{n-} = [Zn_2H_{-1}L(G)]^{3-n}$		3.32
$[Zn_2H_{-1}L(OH)]^{2+} + HG^{1-n} = [Zn_2H_{-1}L(OH)(HG)]^{3-n}$	3.19	
$[Zn_2H_{-1}L(OH)]^{2+} + G^{n-} = [Zn_2H_{-1}L(OH)(G)]^{2-n}$		1.96

[a] G^{n-} = Pi or PPi, n = 3 or 4 for Pi and PPi, respectively; determined in 0.15 mol dm^{-3} NaCl at 298.1 K. Values in parentheses are the standard deviations on the last significant figure.

the $[Zn_2H_{-2}\mathbf{L1}]^{2+}$ species forms a more stable adduct with the $P_2O_7^{4-}$ species of PPi with an addition constant of logK = 5.07, Pi shows the highest addition constant of logK = 3.18 for the addition of HPO_4^{2-} to the same dinuclear $[Zn_2H_{-2}\mathbf{L1}]^{2+}$ species. The formation of adducts occurs at pH higher than six (6 < pH < 11) where the dinuclear species that form, as well as the anions, are more charged. As depicted in the distribution diagrams in Figures 2a and 2b, the main species formed are $[Zn_2H_{-2}\mathbf{L1}(HPO_4)]$ and $[Zn_2H_{-2}\mathbf{L1}(P_2O_7)]^{2-}$ for Pi and PPi, respectively. This occurs in the pH range where the $[Zn_2H_{-2}\mathbf{L1}]^{2+}$ species is prevalent in solution[20] (6.5 < pH < 10). An aspect that needs to be taken into account is the different degree of protonation of the two anions, depending on the pH. This is the reason why Pi is mainly bound as HPO_4^{2-} (HG) while PPi as $P_2O_7^{4-}$ (G); indeed, they are the species present at the pH yielding the $[Zn_2H_{-2}\mathbf{L1}]^{2+}$ species and, for the same reason, PPi can also be bound as $HP_2O_7^{3-}$ (HG).

For the R2 system, the most stable adducts with both anions are those formed with the $[Zn_2H_{-1}\mathbf{L2}]^{3+}$ dinuclear species (see Table 4) which is, as previously reported,[21b,22] the best species to add guests among all the Zn(II)-dinuclear species of **L2**. For this system, the stability constants highlight that the $[Zn_2H_{-1}\mathbf{L2}]^{3+}$ species forms more stable adducts with Pi than PPi; the constants (logK) are 4.46 and 3.32 for the addition of HPO_4^{2-} (HG) and $P_2O_7^{4-}$ (G), respectively. Also in this case Pi is better stabilized as the HPO_4^{2-} (HG) and PPi as the $P_2O_7^{4-}$ (G), although the $H_2PO_4^{-}$, as well as the $HP_2O_7^{3-}$ species, are also bound by the Zn(II)-dinuclear species (see Table 4 and Figures 2c and 2d). As depicted in the distribution diagrams of Figure 2c, the main species formed with Pi ($[Zn_2H_{-1}\mathbf{L2}(HPO_4)]^{+}$) is prevalent in solution from pH 6 to pH 10; on the contrary the adducts with PPi are never prevalent in solution with respect to the other species under our experimental conditions.

A further aspect to consider is the real species involved in adduct formation, as well as the exact speciation of the adducts; Tables 3 and 4 report the more probable adducts described above, although other speciations could also be hypothesized; for example, the adduct $[Zn_2H_{-1}\mathbf{L2}(OH)(HPO_4)]$ could also be written as $[Zn_2H_{-1}\mathbf{L2}(PO_4)]$.

Moreover, several adducts made up of R and anions having different speciation may form; for example, the R2 system forms adducts with Pi of $[Zn_2H_{-1}\mathbf{L2}(H_2PO_4)]^{2+}$, $[Zn_2H_{-1}\mathbf{L2}(HPO_4)]^{+}$, and $[Zn_2H_{-1}\mathbf{L2}(OH)(HPO_4)]$ stoichiometries.

One method able to overcome the difficulty in establishing the exact speciation of adducts formed and to determine the binding selectivity of these systems is to calculate the distribution diagrams for the ternary systems (substrate A)-(substrate B)-receptor and to plot the overall percentages of the complexed receptor as a

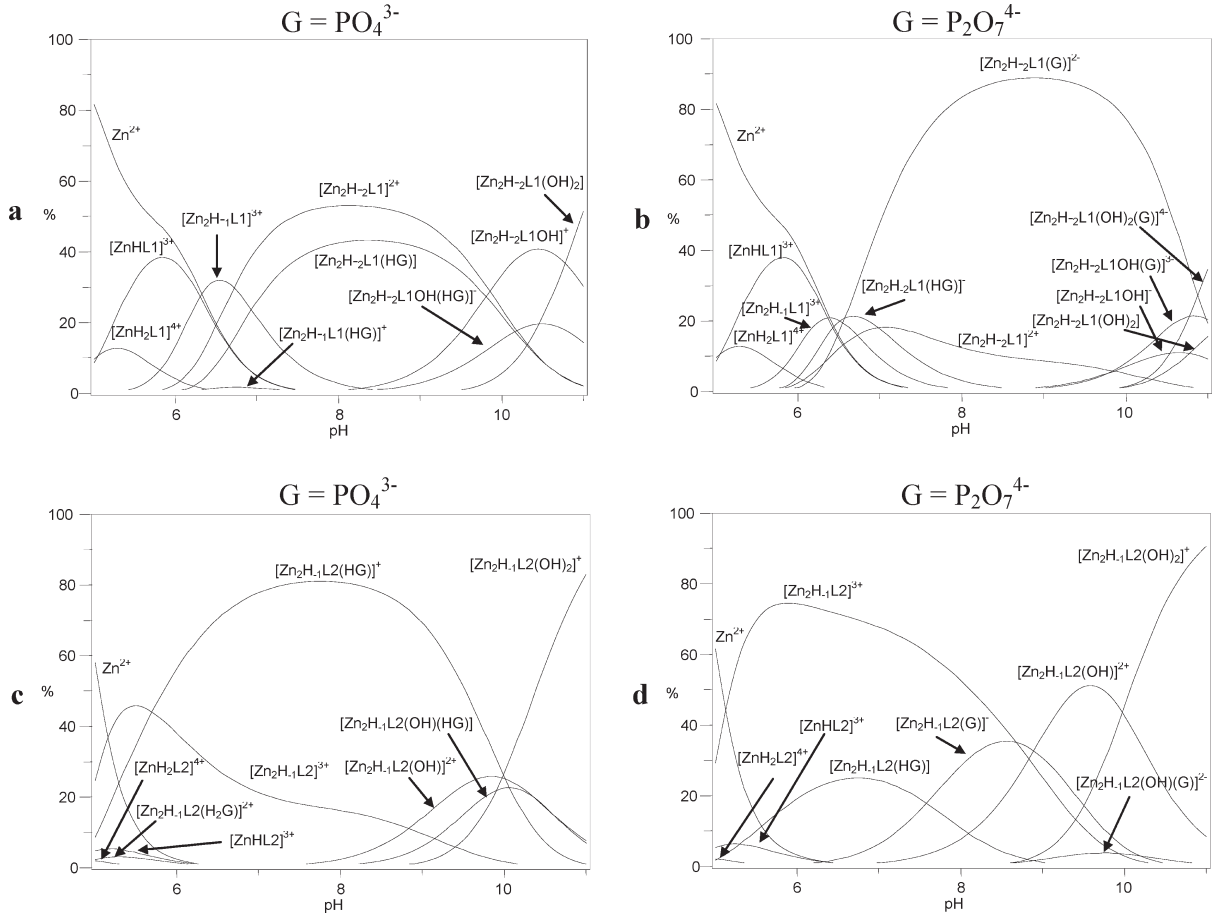

Figure 2. Distribution diagrams of the species for the systems 2Zn(II)/**L1**/G and 2Zn(II)/**L2**/G as a function of pH in aqueous solution, $I = 0.15$ mol dm^{-3} NaCl, at 298.1 K, (G = PO_4^{3-} or $P_2O_7^{4-}$). [**L1**] = [**L2**] = 1×10^{-3} mol dm^{-3}, [Zn^{2+}] = 2×10^{-3} mol dm^{-3}, [G] = 1×10^{-3} mol dm^{-3}. (a) **L1**, G = PO_4^{3-}; (b) **L1**, G = $P_2O_7^{4-}$; (c) **L2**, G = PO_4^{3-}; (d) **L2**, G = $P_2O_7^{4-}$.

function of pH.[11f,39] Such plots can be extended to compare the relative affinities of two receptors for one substrate. This method presents the additional advantage of not requiring any assumption regarding the location of the protons in the host and guest species. Thus, calculating distribution diagrams for the ternary system (receptor 1)/(receptor 2)/substrate and plotting the overall amounts of complexed receptor as a function of pH results in a clear establishment of selectivity patterns in these systems. In this case, the receptors are the Zn(II)-dinuclear systems of **L1** and **L2**, while the substrates are Pi and PPi.

Figure 3 shows the percentage of Pi and PPi bound to the Zn(II)-dinuclear receptor species of **L1** (R1) and **L2** (R2), respectively, as a function of pH. The concentration was calculated for a system containing R, Pi, and PPi in equal concentrations (1×10^{-3} mol dm^{-3}). Figure 3a refers to the R1 dinuclear species and highlights a marked propensity of the R1 system to bind PPi in the presence of the other anion under the same conditions; the binding selectivity was found from neutral to alkaline pH values, reaching a maximum in the pH range 8−10. On the contrary, it can be seen (Figure 3b) that the R2 system has a marked selectivity for phosphate over the pyrophosphate in the pH range 6−10 with a maximum in the pH range 7−8. Such behavior, which is clearly depicted in Figure 3, is in agreement with the greatly more stable complex formed by phosphate with R2 as well as by pyrophosphate with R1 systems.

As regards the comparison between the selectivity of R1 and R2 systems toward each anion, the diagram in Figure 4a shows that Pi is preferentially bound by the R2 species, mainly in the pH range 6−9, while PPi prefers to be bound by the R1 species, mainly in the pH range 7−11 (Figure 4b).

In conclusion, the R1 system shows selectivity toward pyrophosphate over phosphate exhibiting such selectivity also in competition with the R2 system (Figures 3a and 4b), and on the contrary, the latter shows the opposite selectivity and better recognizes phosphate over pyrophosphate also in competition with the R1 system (Figures 3b and 4a).

This important result makes it possible to choose a suitable receptor depending on the target guest to be detected; for example, Figures 3 and 4 show that the best receptor to detect Pi is R2 and the best field of pH at which to recognize it is around the physiological one; on the contrary, PPi is better recognized by the R1 system and mainly at alkaline pH values ($8 < pH < 10$). It is obvious that, when there is a simultaneous presence of both anions in the medium, such selectivity becomes more marked when the concentration of one anion prevails over the other.

Taking into account the topologies of both ligands, as well as the Zn(II)-dinuclear species formed, the main

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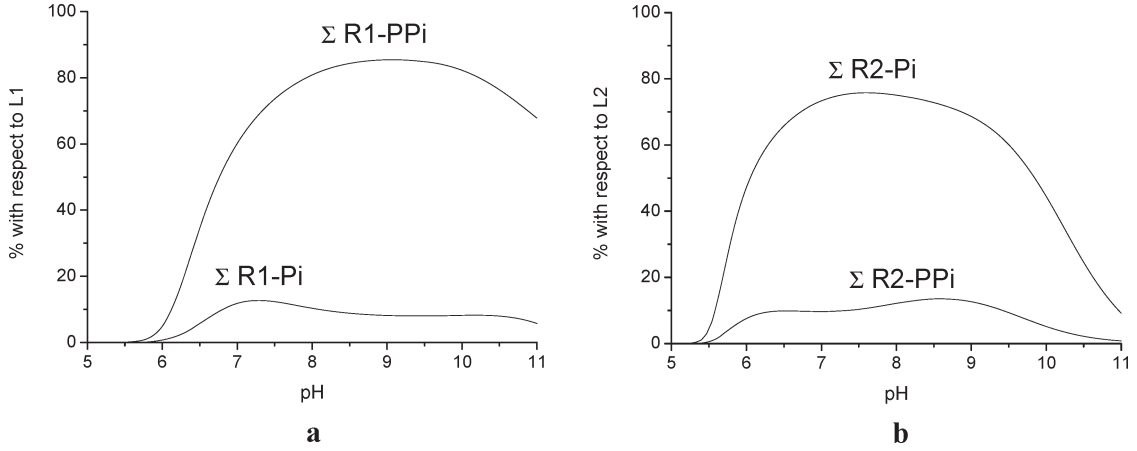

Figure 3. Selectivity diagrams for the systems (a) R1/Pi/PPi and (b) R2/Pi/PPi showing the percentage of the ligand bound to Pi and PPi as a function of pH. All reagents are 1×10^{-3} mol dm^{-3}. Percentages were calculated with respect to L (L = **L1** or **L2**). R1 = ∑Zn(II)-dinuclear species of **L1** bound; R2 = ∑Zn(II)-dinuclear species of **L2** bound.

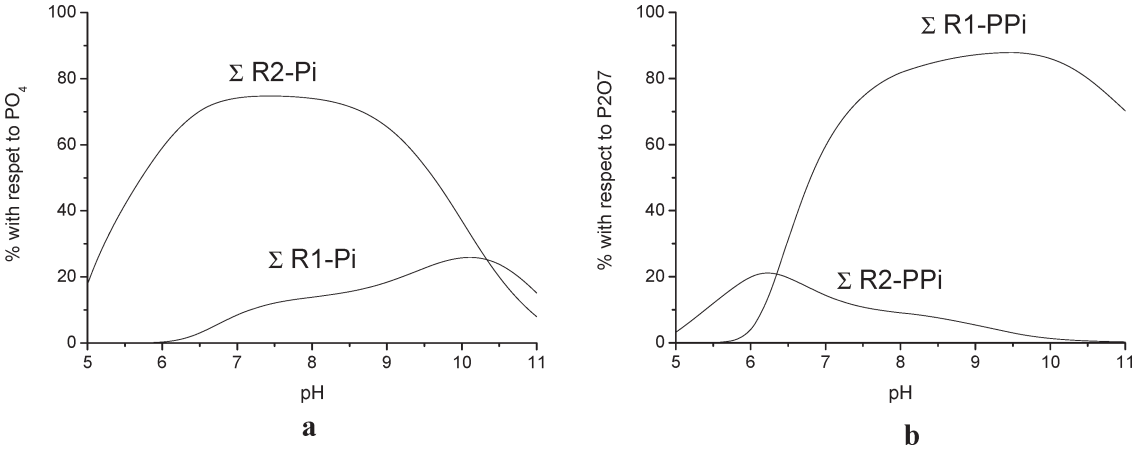

Figure 4. Selectivity diagrams for the systems (a) R1/R2/Pi and (b) R1/R2/PPi showing the percentage of the anion bound to R1 and R2 as a function of pH. All reagents are 1×10^{-3} mol dm^{-3}. Percentages were calculated with respect to L (L = **L1** or **L2**). R1 = ∑Zn(II)-dinuclear species of **L1** bound; R2 = ∑Zn(II)-dinuclear species of **L2** bound.

aspect which stands out upon comparing the dinuclear complexes of **L1** with those of **L2** is the different Zn(II)−Zn(II) distance, which is longer in **L1** than in **L2** systems.[20,21b] This may be the reason why the R1 system shows higher affinity toward PPi with respect to Pi, while the contrary is observed with R2. In other words, a cooperation between both Zn(II) metal centers in stabilizing the guests can be suggested, as reported in similar cases; the guest is displaced in a bridge disposition between the two metals, binding them with two oxygen atoms, one for each Zn(II). In the case of Pi, the two coordinated oxygen atoms better fit the shorter metal−metal distance found in dinuclear species of **L2** with respect to those of **L1** ones; on the contrary, the oxygen atoms of PPi better fit the longer distance in the dinuclear species of **L1**. Comparing Pi and PPi, it is possible to suppose that the latter is involved in the coordination of the two Zn(II) ions with two oxygen atoms not bound to the same phosphorus atom in a Zn−O−P−O−P−O−Zn arrangement, at least in the **L1** system, while Pi is coordinated in a Zn−O−P−O−Zn way as schematically reported in Scheme 2. In fact, if both guests were coordinated in the same way, considering that PPi is more highly charged than Pi in the more stable species ($P_2O_7^{4-}$ vs HPO_4^{2-}), we should expect consistently higher addition constants for pyrophosphate than phosphate in both cases.

In addition, looking at similar **L2** metallo-receptors for phosphates reported in the literature,[18,39−42] some further considerations can be outlined. Indeed, many of these receptors show the two Zn(II) ions stabilized in a way similar to that of **L2** (Scheme 1), and the main difference can be found in the presence of two DPA (bis-(2-pyridilmethyl)amine) units in the place of the diethylentriamine-(dien) ones which are in any case separated by a phenolic function.[18,42] In these systems, the association constants, which were determined spectrophotometrically, were found to be higher for PPi than Pi at physiological pH and, in the case of PPi they are also higher than those with the **L1** system (with differences of at least 3 logarithmic units). The first reason for this can be attributed to the

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different coordination between PPi and Pi in bridging the two Zn(II); in fact, although they are displaced in a bridge disposition between the two metals as suggested in **L1** and **L2**, PPi is coordinated with four oxygen atoms (two for each Zn(II)) while Pi is with only two (one for each Zn(II)) as highlighted by the crystal structures of these systems. This is in agreement to that above suggested for **L1** and **L2** in which PPi is coordinated with two oxygen atoms only thus exhibiting lower addition constants with respect to the other systems. The different binding among the **L2** receptors and those containing the DPA moieties can be traced to the polyamine subunits; the nitrogen atom of the pyridines in the DPA and the primary amines in the dien units exhibit different hard−soft binding properties, conformational hindrance, as well as solvation power. The dien more strongly binds Zn(II) in **L2** and in **L1**, thus mainly favoring a penta-coordinative sphere around the Zn(II), while the softer DPA permits a hexa-coordination environment, thus allowing the coordination of four oxygen atoms of PPi. This aspect is supported by several crystal structures reported for these systems; the Zn(II)-metal complexes of **L1** and **L2** always show each metal penta-coordinated so each Zn(II) ion is able to host one coordinating group only, supporting the proposed Scheme 2.

The stability of the Zn(II)-dinuclear species formed with both **L1** and **L2** does not allow the presence of free ligands, and thus their interaction with the guests in a coordinative competition with the metal complexes should be excluded; however, the formation of adducts between the metal-free **L1** and **L2** and guests Pi and PPi in the pH range 5−11 was considered. The potentiometric method did not reveal the formation of stable adducts in this pH range; taking into account that in this range both ligands show the presence of the negatively charged phenolate group, that the positively charged polyammonium groups are located far from each other, and that the ligands topology does not allow the concentration of the positive charges in a preorganized area as occurs in the macrocyclic polyamines, all these reasons could explain the absence of interactions between L and G, thus making safe the above discussion.

NMR Studies. The two systems R1 and R2 (R1 = 2Zn(II)/**L1**, R2 = 2Zn(II)/**L2**) were examined by ^{1}H and ^{31}P NMR experiments in aqueous buffer D_2O solution. The systems were studied titrating aqueous buffer D_2O solutions at the pH values where the main receptor systems are $[Zn_2H_{-2}\mathbf{L1}]^{2+}$ and $[Zn_2H_{-1}\mathbf{L2}]^{3+}$, respectively,[20,22] as well as at physiological pH, because of the biological importance of the guests investigated. For these reasons, the R1 system was studied at pH = 7.4 and 8.3 while the R2 system was only studied at pH = 7.4. The guests ATP and G6P over Pi and PPi were also added to the systems to investigate the receptor's capability to bind these biological guests.

Table 5 reports, the main ^{1}H and ^{31}P NMR shifts ($\Delta\delta$) exhibited by the adducts with respect to the free receptors measured at pH 7.4, while Figure 5 reports the ^{1}H aromatic resonances obtained by adding Pi, PPi, and ATP to the R1 systems at pH 7.4.

Figure 5 reveals that the addition of all guests perturbs the aromatic part of the **L1** receptor although the influence of each anion was not the same (see also Table 5). The first aspect is the broadening of the adduct resonances with respect to the free receptor, on the NMR time scale, signaling the interaction with the guest. The second one is the downfield shift of the aromatic resonances in the adduct formed; the shift is in the opposite direction to that observed for the phenol hydrogen atoms of the **L2** receptor (see Table 5) indicating that in the presence of metallo-receptors and a conjugated system coordinated with the metal center it is difficult to anticipate, only in terms of electron density, the shift due to the coordination of a guest by the metal center. The third aspect is the different shift exhibited by the resonances H5 and H7 in

Table 5. ^{1}H and ^{31}P NMR Shifts ($\Delta\delta$) of Selected Resonances for the R-G Adducts with Respect to Free Receptors R and Guests G[a] in a HEPES Buffer pH 7.4 Aqueous Solution

	H5	H6	H7	P_α	P_β	P_γ
			R1			
PO_4^{3-}	+0.05	+0.11	+0.05	+1.82		
$P_2O_7^{4-}$	+0.13	+0.19	+0.29	+2.62		
ATP	+0.06	+0.08	+0.15	+0.06	+3.42	+5.83
G6P	+0.01	+0.01	+0.01	+0.78		
			R2			
PO_4^{3-}	−0.07	−0.05		+0.82		
$P_2O_7^{4-}$	−0.13	−0.22		+1.66		
ATP	−0.09	−0.11		−0.16	2.49	4.83
G6P	−0.02	−0.02		+0.38		

[a] R1 = 2Zn(II)/**L1**, R2 = 2Zn(II)/**L2** systems, G = Pi, PPi, ATP and G6P. Charges are omitted for clarity. Values in parentheses are the standard deviations on the last significant figure.

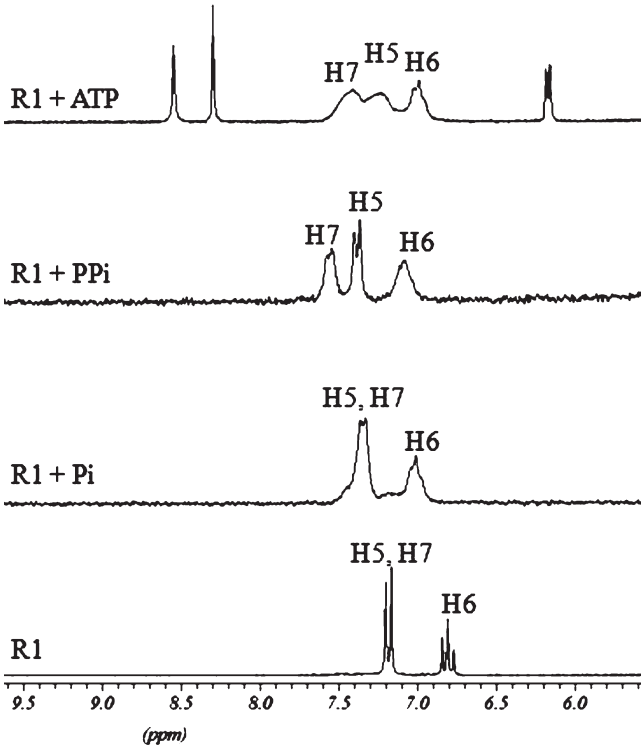

Figure 5. ^{1}H NMR spectra (aromatic protons) in D_2O at pH = 7.4 of R1 (a); R1 + Pi (b); R1 + PPi (c); R1 + ATP (d) in 1: 1 molar ratio; R1 = 2Zn(II)/**L1** system.

the presence of PPi and ATP with respect to those in the Pi and G6P adducts (spectrum not reported but similar to the Pi one). In particular, H5 and H7 become very different in chemical shift, H7 undergoing a greater downfield shift with respect to H5 in the PPi and ATP adducts (in the latter the spectrum is broader although three resonances can be observed) while they exhibit the same shift in the Pi and G6P ones. This can be attributed to a different mutual disposition of the two aromatic rings in several adducts that may be due, as above suggested, to a different interaction of PPi and ATP versus Pi and G6P in the coordination of the two zinc centers affecting the angle between the two rings in a different way; a Zn−O−P−O−P−O−Zn coordination with PPi and ATP versus a necessarily Zn−O−P−O−Zn scheme, with Pi and G6P can be hypothesized.

The last aspect are the shifts exhibited by the phosphorus resonances of several guests; they are always shifted farther downfield in the adducts with the R1 system than in those with R2. In the ^{31}P NMR spectra of ATP, the P_γ and P_β resonances undergo a larger downfield shift in both systems while P_α shows a slight downfield or upfield shift with R1 and R2, respectively (see Table 5). The downfield shift of the P_γ and P_β resonances suggests that in both R1 and R2 systems, this part only of the ATP phosphoric chain is involved in the coordination of the two Zn(II) in a similar coordination to that of PPi in R1; this indirectly suggests that PPi binds the two Zn(II) in a similar way also in the R2 system. The spectrum recorded at pH 8.3 with the R1 system shows analogous spectral behavior.

UV−vis and Fluorescence Measurements. In previous studies it has been shown that the optical properties of free **L1** and **L2** were perturbed by the formation of dinuclear Zn(II)-species, signaling the formation of the complexes, as well as the presence of Zn(II) in aqueous solution.[22] For this reason, the R/G system (R1 = 2Zn(II)/**L1**, R2 = 2Zn(II)/**L2**; G = PO_4^{3-}, $P_2O_7^{4-}$) was studied by UV−vis and fluorescence to investigate if the formation of phosphates adducts (R-G) provides an optical answer for the presence in solution of the guest. The systems were studied by titrating aqueous buffer solutions at pH values where the main receptor systems $[Zn_2H_{-2}\mathbf{L1}]^{2+}$ and $[Zn_2H_{-1}\mathbf{L2}]^{3+}$ are prevalent in solution, as well as at physiological pH. The R1 system was studied at pH = 7.4, 8.3, and 9.3 where the receptor species $[Zn_2H_{-2}\mathbf{L1}]^{2+}$ is prevalent in solution as well as there is the formation of the more stable adduct $[Zn_2H_{-2}\mathbf{L1}(G)]$ (Figure 2a and 2b); the R2 system was studied at pH = 7.4 and 8.3 (Figure 2c and 2d).

The UV−vis spectra with Pi did not show deep changes in the spectral profiles of both systems as well as the addition of PPi to the R2 dinuclear species at the pH values examined; instead, the addition of PPi to the R1 receptor system slightly affects the spectrum in all the pH ranges studied; the λ_{max} shifts from 298 nm at pH = 8.3 to 305 nm with the formation of the $[Zn_2H_{-2}\mathbf{L1}(P_2O_7)]^{2-}$ adduct, preserving the same absorption.

The fluorescence of the R1 receptor system is more greatly affected by the presence in solution of the guests than is the absorption. Figure 6 shows the fluorescence spectral profiles obtained by adding to the R1 system amounts of Pi (Figure 6a) and PPi (Figure 6b) in a buffer pH = 8.3 aqueous solution. The addition of Pi up to the formation of the $[Zn_2H_{-2}\mathbf{L1}(HPO_4)]$ species gives rise to a quenching effect, and a 4-fold decrease in emission was observed although the same λ_{em} was preserved (Figure 6a). On the contrary, the formation of the $[Zn_2H_{-2}\mathbf{L1}(P_2O_7)]^{2-}$ species obtained by adding PPi to the R1 system gave an enhancement effect almost doubling the emission; in this case, a shift of the λ_{em} was also observed, from 379 nm in the free receptor system to 395 nm in the adducts (Figure 6b); a similar behavior was observed at the other pH values.

Table 6. Logarithms of the Addition Constants of G (G = PCV, Pi, PPi, ATP, and G6P) to the R System (R1 = 2Zn(II)/**L1**, R2 = 2Zn(II)/**L2**)[a]

	Log K				
reaction	PCV	Pi	PPi	ATP	G6P
R1 + G = R1-G	3.80(4)[b]	3.1(1) 3.0[c]	4.4(3) 4.2[c]	3.8(1)	2.6(2)
R2 + G = R2-G	3.98(6)	4.4(1) 4.3[c]	2.8(3) 2.9[c]	2.5(1)	3.5(1)

[a] Determined in 0.05 mol dm^{-3} HEPES pH = 7.4 buffer aqueous solution at 298 K. The addition constants for R-G adducts were calculated titrating the R-PCV system. [b] Values in parentheses are the standard diviation on the last significant figure [c] Calculated by potentiometric data at pH = 7.4

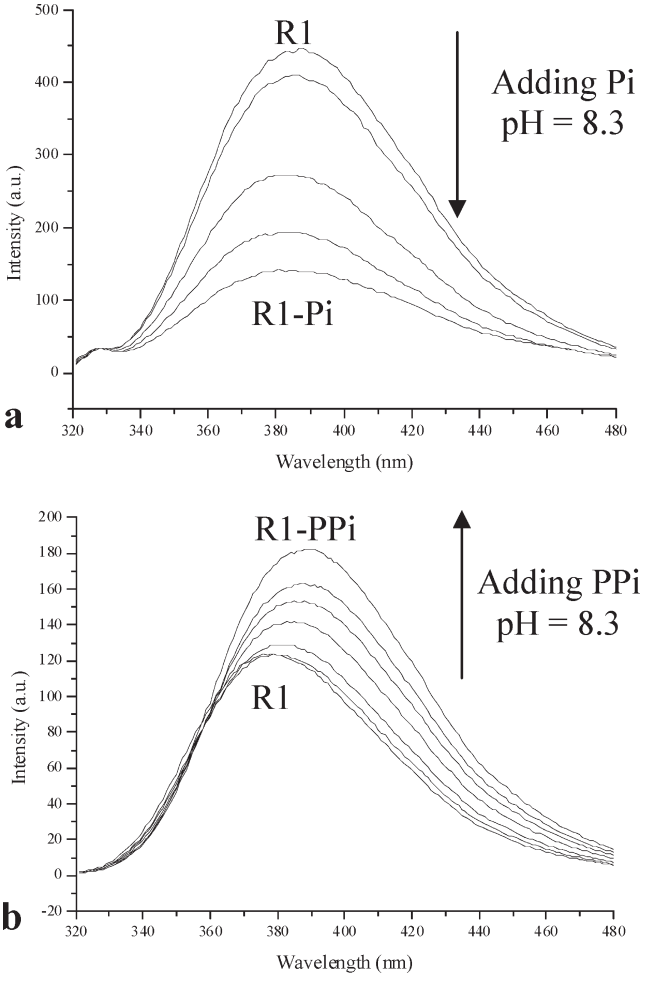

Figure 6. Fluorescence spectra of the 2Zn(II)/**L1** system (R1) in aqueous buffer (TAPS, 5×10^{-2} M) solution at pH = 8.3, obtained by adding several amounts of Pi (a) and PPi (b) up to 2 equiv with respect to [**L1**] = 5.0×10^{-5} M.

Examining the fluorescence experiments carried out on the R2 system, PPi basically does not affect the emission of the system while Pi shifts the λ_{em} slightly along with the formation of the adduct from 308 to 313 nm, while the emission slightly decreases.

The UV−vis data suggest that the guests do not strongly influence the chromophore in either receptor system with the exception of PPi in the R1, where, as previously reported,[22] the binding of the guest probably affects the angle between the aromatic rings of **L1** changing the absorption λ_{max}. The fluorescence of the **L1** receptor changes in the opposite direction by adding the two guests; the drop in fluorescence observed by adding Pi can be ascribed to an increase in electron density of the BPH unit by coordinating the anion which, as reported in similar cases, increases a thermal relaxation negatively affecting emission decay mechanisms;[43] on the contrary, the formation of PPi adducts gives rise, as also suggested by UV−vis experiments, to a conformational change of the BPH unit which increases the emission and shifts the λ_{em}.

The R2 receptor shows very few spectral changes; thus, taking into account that a bridging guest binding the two Zn(II) ions with the same atom such as the hydroxide yields a spectral change in the $[Zn_2H_{-1}\mathbf{L2}]^{3+}$ receptor, it is possible to suppose that Pi as well as PPi are coordinated to the Zn(II) through two oxygen atoms each for one Zn(II) ion as above-reported; in other words, each replacing the bromide in the crystal structure reported herein (Figure 1).

However, considering the changes in the receptor systems because of the binding of the guests, only the R1 system is able to signal the presence in solution of these guests by fluorescence emission.

Sensing of the Phosphates. As highlighted in the above paragraphs, the dinuclear Zn(II) systems can be used as receptors R1 and R2 (R1 = 2Zn(II)/**L1**; R2 = 2Zn(II)/**L2**) for the phosphates G studied (G = PO_4^{3-}, $P_2O_7^{4-}$, ATP, and G6P) in a wide range of pH. Because of the biological importance of these phosphates, a sensing system R-S able to signal and quantify phosphates in aqueous solution at physiological pH was assembled by exploiting the receptor properties of R1 and R2 (see Figures 3 and 4) and the chemosensing ensemble method by using pyrocatechol violet (PCV) as signaling guest (see Experimental Section). PCV is a well-known dye with a strong absorptivity changing in λ_{max} when it is free or engaged in coordination at pH = 7.4.[44] In particular, it shows a main band at λ_{max} = 444 nm (ε = 13000 cm^{-1} mol^{-1} dm^3) when it is free or λ_{max} = 624 nm (sharp, ε = 14000 cm^{-1} mol^{-1} dm^3) when it is bound to the metal ions in the host and the solution appears yellow or blue whether PCV is coordinated or not, respectively (see Figure 7).

The dinuclear receptors R1 and R2 are the systems obtained in solution by mixing Zn(II) and **L1** (R1) or **L2** (R2) in a 2:1 molar ratio; at pH 7.4, as above-reported, only dinuclear species are present in solution for both

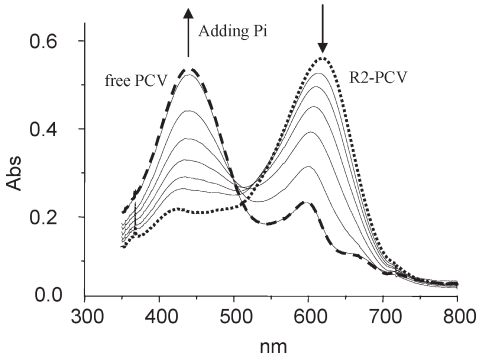

Figure 7. UV−vis spectra of free PCV (dashed line) and for the 2Zn(II)/**L2** system (R2) (dotted line) in aqueous buffer (HEPES, 1×10^{-2} M) solution at pH = 7.4, [PCV] = 4.0×10^{-5} M, [R2] = 2.0×10^{-3} M, and those obtained by adding several amounts of Pi up to 1 equiv with respect to [R2] species (lines).

systems; by potentiometric measurements, R1 consists in the $[Zn_2H_{-1}\mathbf{L1}]^{3+}$ and $[Zn_2H_{-2}\mathbf{L1}]^{2+}$ species, the latter being prevalent over the former, while R2 in the $[Zn_2H_{-1}\mathbf{L2}]^{3+}$ and $[Zn_2H_{-1}\mathbf{L2(OH)}]^{2+}$ species, the former being prevalent over the latter.[22] The R/PCV as well as the R-PCV/G titrations were carried out in a buffered HEPES aqueous solution at pH = 7.4 (see Experimental Section) by UV−vis experiments.

Both dinuclear receptor systems R1 and R2 are able to bind PCV at pH = 7.4 as demonstrated by the disappearance of the band at 444 nm (yellow color) and the appearance of the band at 624 nm (blue color, see Figure 7 for the R2 system). A large amount of R systems (up to 50 equiv) must be added with respect to PCV to obtain its complete coordination, as confirmed by the invariability of the absorption spectra; for this reason, the sensing system is made up of 50 equiv of R (calculated on [L]) with respect to PCV. The spectrophotometric titrations of the R systems with PCV allowed us to evaluate the association constant for the reaction R + PCV = R-PCV at pH = 7.4; the data obtained are reported in Table 6 and confirm the right molar ratio to be used to build the sensing system.

Figure 7 reports a typical titration experiment carried out with the sensing system R2-PCV by adding Pi; when Pi was added to the buffer solution of the R2-PCV system, a turnover of the spectral figure, from complexed to free PCV, was observed (see lines in Figure 7). This can be explained by the replacement of PCV with Pi in the R2 adduct, yielding the release of PCV into the medium. This gives rise to a change in solution color from blue (R2-PCV) to yellow (R2-Pi + free PCV), signaling the presence of Pi. In this case, 1 equiv of Pi with respect to the R2 complex is sufficient to completely release the PCV. In these cases, the spectrophotometric titrations carried out with the R-PCV sensing system reveal the presence in solution of the guest and make it possible to evaluate the addition constants of the several phosphate guests to the R systems as R + G = R-G (see Table 6).

PCV dye is slightly less bound to the R1 system than R2; the values are in agreement with those found in the literature for similar Zn(II) dinuclear systems. The association constants found for R-G adducts are in agreement with those calculated by potentiomeric measurements at pH 7.4, supporting the quality and the reliability of

(43) Mohanty, J.; Pal, H.; Sapre, A. V. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 2193.

(44) Harris, D. C. *Quantitative Chemical Analysis*, 4th ed.; Freeman and Co: New York, 1995; p 352.

the method; for example, the formation of the R2-Pi adduct gives a logK = 4.4 by the chemosensing ensemble and a logK = 4.3 by potentiometric method. This method also allows an estimation of the formation constants of the adduct with ATP and G6P, which could not be obtained by potentiometric measurements because of the complexity and insolubility of the systems formed. The values for the interaction of R1 and R2 with ATP and G6P highlight that ATP is better recognized by the R1 receptor systems while R2 better binds G6P at this pH value (see Table 6). This finding can be explained by a different coordination way of ATP with respect to G6P, similar to that suggested in the case of PPi versus Pi, in binding the two Zn(II) ions. In other words, the phosphoric chain of ATP better fits the Zn−Zn distance in R1 than in R2, and the opposite occurs for G6P. However, the main result reached by using this method is that two simple systems able to sense and signal these guests in aqueous solution at physiological pH are obtained; in addition, it is possible to choose one of them depending on the target guest to detect. No interaction was observed with the common inorganic anions tested (Cl^-, Br^-, ClO_4^-, NO_3^-, and SO_4^{2-}).

Conclusions

In the several studies carried out on the Zn(II) dinuclear systems obtained by using **L1** and **L2** amino phenolic ligands as possible receptors for phosphates in aqueous solution, both Zn(II) dinuclear receptor systems R were able to bind all the phosphates investigated. Potentiometric measurements allowed us to evaluate the formation constants of the adducts with Pi and PPi, highlighting that only the dinuclear systems behave as receptor species; they are able to bind Pi and PPi in a wide range of pH ($6 < pH < 10$). Comparative studies demonstrated different selectivity of the two metallo-receptors toward these guests; the **L1** system (R1) shows higher selectivity toward PPi over Pi on the contrary the **L2** one (R2) exhibits opposite selectivity. The first system shows the best selectivity at pH 8.3 where the best receptor species $[Zn_2H_{-2}\mathbf{L1}]^{2+}$ and the highest charged guest ($P_2O_7^{4-}$) are prevalent in solution; the second one shows the best selectivity at physiological pH where the best receptor species $[Zn_2H_{-1}\mathbf{L2}]^{3+}$ is prevalent, binding Pi preferentially as HPO_4^- species. The different selectivity exhibited by the two systems can be attributed to the different fitting shown by the two guests in binding the Zn(II)−Zn(II) metal centers in a bridging disposition. The longer PPi better fits the longer distance between the two Zn (II) in the **L1** receptor species, and a Zn−O−P−O−P−O−Zn bridge can be suggested; the contrary occurs for **L2** dinuclear species in which the shorter Zn−Zn distance is better fitted by Pi than PPi in a Zn−O−P−O−Zn bridging environment. In all adducts both phosphates bind each Zn (II) ions with one oxygen atom, obtaining a penta-coordination environment around each Zn(II), which, compared with ligand showing the DPA moieties, is due to the presence of the dien units. The favored penta-coordination environment of the two Zn(II) is responsible for the selectivity found, thus making it possible to choose a suitable receptor depending on the guest to detect.

The NMR studies supported the model of interaction proposed between receptors and guests; furthermore, they also highlighted that biologically important phosphates such as G6P and ATP, which show phosphate groups referable to Pi and PPi, respectively, interact with the receptor systems at physiological pH in a way similar to that of their inorganic precursors.

Fluorescence studies showed that the receptor system based on **L1** is able to signal the presence in solution of Pi and PPi at physiological pH by fluorescence emission; the presence of Pi can be detected by a quenching of the emission while PPi is revealed by an enhancement of it.

Taking into account the different selectivity exhibited by these metallo-receptors, two simple chemosensors based on the chemosensing ensemble method and using the dinuclear system coupled with the PCV dye were prepared and tested at physiological pH; these signaling systems were able to detect the presence of the phosphates investigated in solution and to determine their concentration at physiological pH by using a simple colorimetric method.

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Supporting Information Available: Listings of tables of crystallographic data, positional parameters, isotropic and anisotropic thermal factors, bond distances and angles in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.