Inorganic Chemistry
Article

Inorg. Chem. **2009**, *48*, 6229–6235 **6229**
DOI: 10.1021/ic900557n

Specific Supramolecular Interactions between Zn^{2+}-Salophen Complexes and Biologically Relevant Anions

Manoli Cano,[†] Laura Rodríguez,*[,†,‡] João Carlos Lima,[†] Fernando Pina,[†] Antonella Dalla Cort,*[,§] Chiara Pasquini,[§] and Luca Schiaffino[§]

[†]*REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Monte de Caparica, Portugal,* [‡]*Departament de Química Inorgànica, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain, and* [§]*Dipartimento di Chimica and IMC−CNR Sezione Meccanismi di Reazione, Università La Sapienza, Box 34 Roma 62, 00185 Roma, Italy*

Received March 21, 2009

Recognition of inorganic phosphates PO_4^{3-}, $P_2O_7^{4-}$, and $P_3O_{10}^{5-}$ and nucleotides AMP^{2-}, ADP^{3-}, and ATP^{4-} by Zn^{2+}-salophen complexes **1** and **2** in ethanol was investigated by different spectroscopic techniques. ^{31}P NMR and mass spectrometry showed that anions of both series are bound by **1** and **2**, while absorption and emission studies revealed that only nucleotides produce relevant changes in the spectral properties of the two hosts. ^{1}H NMR studies proved that the adenine aromatic group is involved in the complexation, thus pointing out the role of supramolecular ditopic receptors played by salophen derivatives toward this class of biologically relevant substrates. The lifetime of the photogenerated triplet state of the Zn^{2+}-salophen compounds was measured by nanosecond laser flash photolysis, and the observed changes upon increasing the concentration of nucleotides allowed the identification of the formation of a 1:0.5 host/guest intermediate complex additionally to the formation of a 1:1 complex.

Introduction

Molecular recognition of anions is gaining increasing relevance in supramolecular and analytical chemistry because of the ubiquitous involvement of anions in biochemical regulation and control.[1−6] A thoroughly tested strategy is complexation through Lewis acid/base or electrostatic interactions that keep the anion close to an adequate signaling unit whose spectral properties are affected by coordination. Fluorescent sensors that display relevant changes in their emission spectra are particularly attractive for such applications because of their higher sensitivity and low detection limits.

Although the vast majority of published works on anion receptors is based on organic frameworks, a number of metal containing receptors have also been described.[7−10] Anion sensing by zinc complexes of polyamine ligands, reported by some of us, is a nice example of this strategy. Since Zn^{2+} has a fully occupied d shell, its polyamine complexes usually exhibit unquenched luminescence and have shown to be highly efficient for such purposes, displaying selective changes of their emissive properties upon anion binding to the metal.[11,12]

Schiff base metal complexes are among the most versatile classes of compounds in coordination chemistry[13] and have found applications in several fields of chemistry, such as

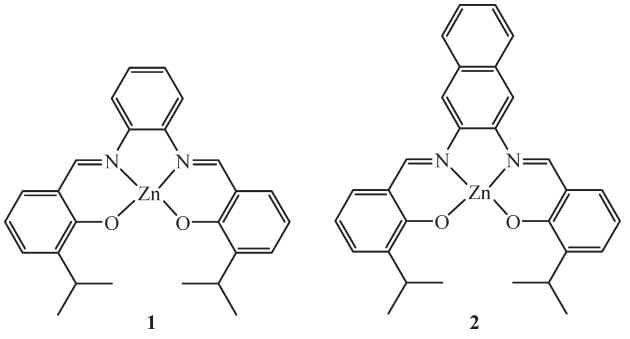
*To whom correspondence should be addressed. E-mail: laura.rodriguez@qi.ub.es (L.R.), antonella.dallacort@uniroma1.it (A.D.C.). Fax: +34 934907725 (L.R.), +39 06490421 (A.D.C.). Phone: +34 934031136 (L.R.), +39 0649913087 (A.D.C.).

(1) Suksai, C.; Tuntulani, T. *Chem. Soc. Rev.* **2003**, *32*, 192.
(2) Sessler, J.-L.; Gale, P. A.; Cho. W.-S. *Anion Receptor Chemistry*; RCS Publishing: Baltimore, MD, 2006.
(3) *Supramolecular Chemistry of Anions*; Bianchi, A., Bowman-James, K., García-España, E., Eds.; Wiley-VCH: New York, 1997.
(4) Kang, S. O.; Begum, R. A.; Bowman-James, K. *Angew. Chem., Int. Ed.* **2006**, *45*, 7882–7894.
(5) Ilioudis, C. A.; Tocher, D. A.; Steed, J. W. *J. Am. Chem. Soc.* **2004**, *126*, 12395–12402.
(6) Katayev, E. A.; Ustynyuk, Y. A.; Sessler, J. L. *Coord. Chem. Rev.* **2006**, *250*, 3004–3037.
(7) Llinares, J. M.; Powell, D.; Bowman-James, K. *Coord. Chem. Rev.* **2003**, *240*, 57–75.
(8) Bowman-James, K. *Acc. Chem. Res.* **2005**, *38*, 671–678.
(9) Albelda, M. T.; Aguilar, J.; Alves, S.; Aucejo, R.; Díaz, P.; Lodeiro, C.; Lima, J. C.; García-España, E.; Pina, F.; Soriano, C. *Helv. Chim. Acta* **2003**, *86*, 3118–3135.
(10) Sancedón, F.; Benito, A.; Lloris, J. M.; Martínez-Manez, R.; Pardo, T.; Soto, J. *Helv. Chim. Acta* **2002**, *85*, 1505–1516.
(11) Anai, T.; Nakata, E.; Koshi, Y.; Ojida, A.; Hamachi, I. *J. Am. Chem. Soc.* **2007**, *129*, 6232–6239.
(12) Rodríguez, L.; Lima, J. C.; Parola, A. J.; Pina, F.; Meitz, R.; Aucejo, R.; García-España, E.; Llinares, J. M.; Soriano, C.; Alarcón, J. *Inorg. Chem.* **2008**, *47*, 6173–6183.
(13) Cozzi, P. G. *Chem. Soc. Rev.* **2004**, *33*, 410–421.

Published on Web 05/28/2009

materials science,[14,15] catalysis,[16,17] structural,[18] and supramolecular chemistry.[19−21] Their Zn^{2+} derivatives are known to be fluorescent and present a vacant coordination position, a consequence of the pentacoordinated tendency of the Zn^{2+} ion. For this reason they appear to be appealing candidates as fluorescent sensors. Some of us, indeed, recently reported on the photophysical properties of the Zn^{2+}-salophen derivatives **1** and **2** and on their ability to selectively discriminate between the members of a series of tertiary amines.[22]

As a continuation of our studies, we carried out an extensive investigation of the binding properties of **1** and **2** toward inorganic phosphates PO_4^{3-}, $P_2O_7^{4-}$, and $P_3O_{10}^{5-}$ and adenosine nucleotides AMP^{2-}, ADP^{3-}, and ATP^{4-}, by means of different, complementary spectroscopic techniques. The results here reported show that Zn^{2+}-salophen complexes **1** and **2**, while binding to both series of phosphates, are efficient fluorescent chemosensors only for adenosine nucleotides, behaving as ditopic supramolecular receptors toward this class of biologically relevant substrates.



Experimental Section

General Procedures. Zinc-salophen complexes **1** and **2** were prepared as previously described.[22] Spectrophotometric grade ethanol was used in all manipulations. $Na_3PO_4 \cdot 12H_2O$ (Aldrich), $Na_4P_2O_7$ (Aldrich), $Na_5P_3O_{10}$ (Aldrich), Na_2AMP (Aldrich), Na_2ADP (Aldrich), and Na_2ATP (Aldrich) were used as received.

Measurements. Absorption spectra were recorded on a Varian Cary 100 Bio UV-spectrophotometer, and fluorescence emission spectra were recorded on a Horiba-Jobin-Yvon SPEX Fluorolog 3.22 spectrofluorimeter at right angle geometry with 2 nm bandwidth excitation and emission slits. The fluorescence and absorption measurements were performed in a 10 mm optical path quartz cuvette (HELMA, Germany) at 25 °C. $^{31}P\{^1H\}$-NMR (δ(85% H_3PO_4) = 0.0 ppm) spectra were recorded on a Bruker DXR Advance 400. MALDI-TOF-MS mass spectra were recorded on a Voyager DE-PRO Biospectrometry Workstation equipped with a nitrogen laser radiating at 337 nm (Applied Biosystems, Foster City, U.S.A.) in the laboratory of mass spectrometry of the REQUIMTE-UNL, and ESI-MS mass spectra were recorded on a LC/MSD-TOF spectrometer at the Universitat de Barcelona. Triplet decay times were measured with a laser flash photolysis LK60 Applied Photophysics system collecting transient absorption decay at 450 nm after laser pulse excitation at 355 nm.

Spectrophotometric and Spectrofluorimetric Titrations. Titrations were carried out at 25 °C in air-equilibrated ethanol by addition of aliquots of 1×10^{-3} M or 1×10^{-2} M solution of the corresponding anion to a 5×10^{-6} M solution of **1** or **2**.

The association constants of the complexes with the anions were obtained from the fit of the spectrophotometric or fluorimetric titrations data with the general equation derived by Lehn,[23] (see eqs S1 and S2 in the Supporting Information).

Molecular Modeling and Semiempirical Calculations. Electronic structure and electronic transitions were calculated with the ZINDO/S semiempirical method with configuration interaction (99 singly excited configurations) in structures previously optimized with the MM+ molecular mechanics method, both methods included in the software package HYPERCHEM 7.0. (Hypercube (2005).[24]

Results and Discussion

^{31}P NMR Spectroscopy and Mass Spectrometry Studies. ^{31}P NMR spectra of freshly prepared 1×10^{-4} M ethanol-d_6 solutions of one anion from each series, namely, ADP^{3-} and PO_4^{3-}, were recorded in the absence and in the presence of 1 equiv of receptor. In both cases the ^{31}P nuclei resonances shift at higher frequencies (lower fields) with respect to the signals of the free anions, when in the presence of the zinc-complexes. Spectra in Figure 1 show what happens in the case of receptor **2**. The two doublet signals at −11.8 ppm corresponding to the non-equivalent phosphorus atoms of the free ADP^{3-} anion are broadened and shifted at about −0.4 ppm upon coordination, while the sharp peak of the free phosphate is shifted from 1.8 to a broadband at about 3.6 ppm. Such behavior is fully consistent with previous data on Zn^{2+}-phosphate association[12] and clearly indicates that Zn^{2+}-salophen derivatives are able to bind phosphate anions. Attempts at increasing the resolution of the broad peak of the complex with ADP^{3-} by lowering the temperature were unsuccessful because of precipitation of complex **2** on cooling. Similar results were obtained for complex **1** (see Supporting Information, Figure S1).

^{31}P NMR spectra of mixtures of compound **2** and ADP^{3-} in 1:0.5, 1:1, and 1:2 ratios, obtained by adding increasing amounts of the phosphate to a solution of **2** were also recorded (Figure 2). The 1:0.5 and 1:1 spectra are quite similar, while the 1:2 spectrum shows the resonances of both free and complexed ADP^{3-}, indicating that in ethanol the complexation equilibrium is slow on the NMR time scale. These results confirm that ADP^{3-} is bound by the Zn^{2+}-salophen complex in ethanol solution, giving a complex of 1:1 stoichiometry.

(14) Di Bella, S. *Chem. Soc. Rev.* **2001**, *30*, 355–366.
(15) Wezenberg, S. J.; Kleij, A. W. *Angew. Chem., Int. Ed.* **2008**, *47*, 2354–2364.
(16) Kleij, A. W.; Lutz, M.; Spek, A. L.; van Leeuwen, P. W. N. M.; Reek, J. N. H. *Chem. Commun.* **2005**, 3661–3663.
(17) Kuil, M.; Goudriaan, P. E.; Kleij, A. W.; Tooke, D. M.; Spek, A. L.; van Leewen, P. W. N. M.; Reek, J. N. H. *Dalton Trans.* **2007**, 2311–2320.
(18) Gao, F.; Ruan, W. J.; Chen, J. M.; Zhang, Y. H.; Zhu, Z. A. *Spectrochim. Acta, Part A* **2005**, *62*, 886–895.
(19) Dalla Cort, A.; Mandolini, L.; Pasquini, C.; Schiaffino, L. *Org. Biomol. Chem.* **2006**, *4*, 4543–4546.
(20) Kitaura, R.; Onoyama, G.; Sakamoto, H.; Matsuda, R.; Noro, S.-I.; Kitagawa, S. *Angew. Chem., Int. Ed.* **2004**, *43*, 2684–2687.
(21) Sun, S.-S.; Stern, C.; Nguyen, S. T.; Hupp, J. T. *J. Am. Chem. Soc.* **2004**, *126*, 6314–6326.
(22) Dalla Cort, A.; Mandolini, L.; Pasquini, C.; Rissanen, K.; Russo, L.; Schiaffino, L. *New J. Chem.* **2007**, *31*, 1633–1638.
(23) Čudić, P.; Žinić, M.; Tomišić, V.; Simeon, V.; Vigneron, J.-P.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1995**, 1073–1075.
(24) *HYPERCHEM*, Version 7.5; Hypercube Inv.: Gainsville, FL, 32601−4256.
(25) Splan, K. E.; Massari, A. M.; Morris, G. A.; Sun, S.-S.; Reina, E.; Nguyen, S. T.; Hupp, J. T. *Eur. J. Inorg. Chem.* **2003**, 2348–2351.

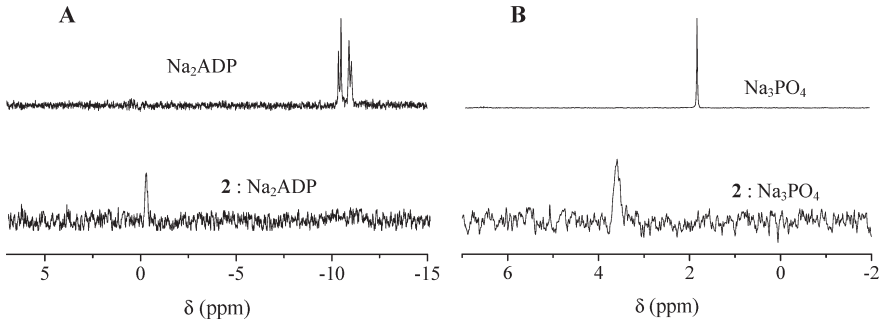


Figure 1. ^{31}P NMR spectra of 1×10^{-4} M ethanol-d_6 solutions of ADP^{3-} (A) and PO_4^{3-} (B) in the absence (upper spectra) and in the presence (lower spectra) of an equimolar amount of compound **2**. All spectra were recorded with $POMe_3$ in acetone-d_6 as external reference.

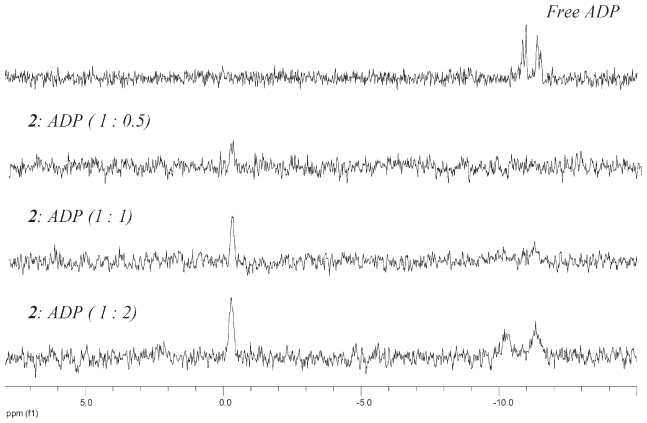


Figure 2. ^{31}P NMR spectra of a 1×10^{-4} M solution of compound **2** in ethanol-d_6 solution in the presence of increasing amounts of ADP^{3-}. The spectrum of ADP^{3-} alone is also shown for comparison. All spectra were recorded with $POMe_3$ in acetone-d_6 as external reference.

Complexes between ADP^{3-} and compounds **1** and **2** were also detected by mass spectrometry. For instance, the mass spectrum of $\mathbf{2}\cdot ADP^{3-}$ in 1:1 ratio (Supporting Information, Figure S2) shows peaks at 544.7 $(M + HADP^{2-} + 3\ EtOH + H_2O)^{2-}$, 511.7 $(M - H^+)^-$, 471.8 $(M + HADP^{2-} + H^+)^{2-}$, 449.8 $(M + HADP^{2-} - 2\ OH)^{2-}$, 319.3 $(M + P_2O_5)^{2-}$, and $(M + HPO_4^{2-})^{2-}$ and the spectrum of $\mathbf{2}\cdot PO_4^{3-}$ in 1:1 ratio (Supporting Information, Figure S3) shows peaks at 591.6 $(M + H_2PO_4^- - CH_3)^-$, 544.5 $(M + H_2PO_4^- - 4CH_3)^-$, 513.6 $(M + H^-)^-$, and 301.6 $(M + HPO_4^{2-})^{2-}$. When a 2:1 mixture of $\mathbf{2}\cdot$and PO_4^{3-} is employed, main peaks at 569.4 $(2\ M + PO_4^{3-} + Na^+ - 2CH_3)^{2-}$, 544.4 $(2\ M + PO_4^{3-} + Na^+ - 4CH_3)^{2-}$, 527.5 $(2\ M + PO_4^{3-} + Na^+ - 6CH_3)^{2-}$, and 515.5 $(2\ M + PO_4^{3-} + Na^+ - 8CH_3)^{2-}$ are observed (Supporting Information, Figure S4), showing that in the gas phase a 2:1 intermediate also exists (attributions are based on simulated isotopic distribution in all cases in which a peak could be simultaneously attributed either to the monometallic or to the bimetallic species). Computer drawings of the $\mathbf{2}\cdot PO_4^{3-}$ complexes in 2:1 and 1:1 ratios for illustration purposes only are shown in Figure 3. ESI-MS mass spectrometry experiments carried out with compound **1** also show the formation of the complexes with both anions (Supporting Information, Figures S5 and S6).

Absorption and Emission Titrations. Absorption spectra of **1** and **2** in ethanol display a high energy band at about 240 nm (ε = 30348 $M^{-1}\cdot cm^{-1}$ (**1**) and 55046 $M^{-1}\cdot cm^{-1}$ (**2**)) and two lower energy bands at 295

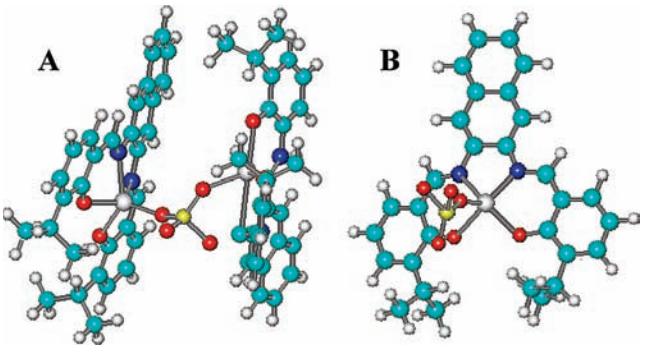


Figure 3. Computer drawn structures of the 2:1 (A) and 1:1 (B) complexes between **2** and PO_4^{3-}.

($\varepsilon = 22816\ M^{-1}\cdot cm^{-1}$) and 405 nm ($\varepsilon = 19287\ M^{-1}\cdot cm^{-1}$) for compound **1** and at 313 ($\varepsilon = 41657\ M^{-1}\cdot cm^{-1}$) and 415 nm ($\varepsilon = 34291\ M^{-1}\cdot cm^{-1}$) for compound **2**. ZINDO/S semiempirical electronic structure calculations indicated that, as already observed for similar derivatives,[25,26] all the observed transitions are predominantly $\pi-\pi^*$ (see Supporting Information, Figure S7). Excitation at the lowest energy bands led to a broad emission centered at 530 and 532 nm for **1** and **2**, respectively.

Addition of increasing amounts of each of the inorganic phosphates to solutions of **1** or **2** did not produce any significant variation on absorption spectra (see Supporting Information, Figure S8) neither at low (5×10^{-6} M) concentrations, nor at concentrations of the same order as those utilized in the ^{31}P NMR experiments (5×10^{-4}–1×10^{-3} M). The near insensitivity of the absorption properties to the presence of an axial ligand is not unprecedented.[27,28]

Remarkable changes were instead observed upon additions of AMP^{2-}, ADP^{3-}, and ATP^{4-} (Figures 4 and 5). Absorption spectra in the 300–500 nm region, where nucleotide absorption is negligible, display a progressive decrease of the intensity of the low energy band at about 405 (**1**)–415 nm (**2**), while the intensity of the band at 295 (**1**)–315 nm (**2**) showed a ~20% decrease. A new band also appears at about 345 nm in both cases. Clear

(26) Silvestri, A.; Barone, G.; Ruisi, G.; Anselmo, D.; Riela, S.; Turco Liveri, V. *J. Inorg. Biochem.* **2007**, *101*, 841–848.
(27) Dalla Cort, A.; De Bernardin, P.; Schiaffino, L. *Chirality* **2009**, *21*, 104–109.
(28) Germain, M. E.; Vargo, T. R.; Khalifah, P. G.; Knapp, M. J. *Inorg. Chem.* **2007**, *46*, 4422–4429.

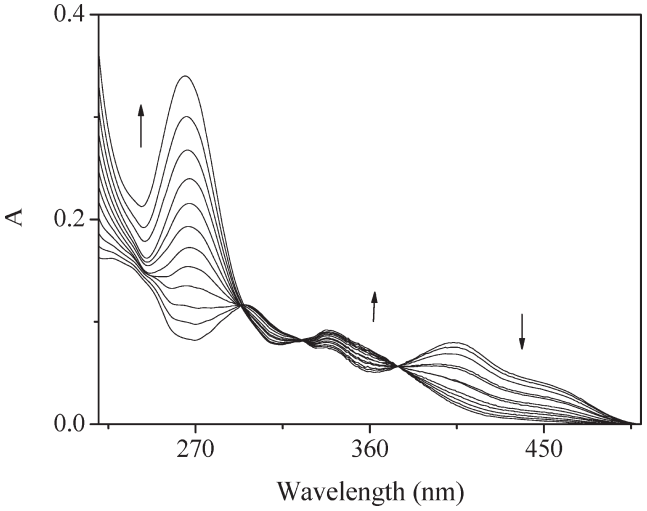


Figure 4. Absorption spectra of a 5×10^{-6} M ethanol solution of compound **1** upon titration with ATP^{4-} at room temperature.

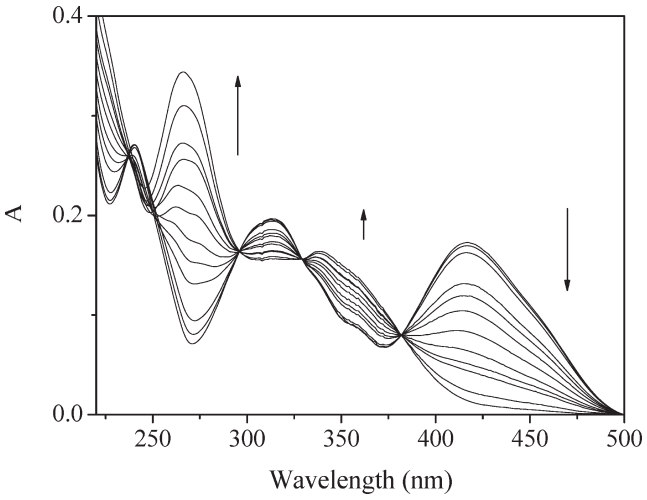


Figure 5. Absorption spectra of a 5×10^{-6} M ethanol solution of compound **2** upon titration with ATP^{4-} at room temperature.

isosbestic points are detected at about 300, 330, and 380 nm.

Similar behavior was already observed in other recently reported chemosensors for ATP^{4-} detection.[29,30] This was attributed to stacking interactions between the aromatic rings of the sensor and the aromatic nucleotide bases. Recently, Kleij and co-workers reported about the demetalation process occurring for similar Zn^{2+}-salophen compounds in the presence of water[31] that also lead to significant changes in the absorption spectra. In our case we can exclude demetalation on the basis of collected evidence. The slow exchange observed for ^{31}P NMR signals of the free and bound phosphate anion in the host/guest 1:2 mixtures is hardly consistent with the

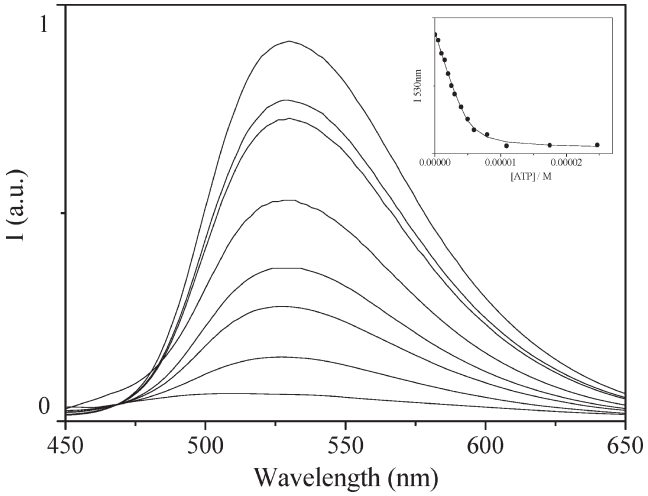


Figure 6. Emission spectra of compound **2** upon titration of a 5×10^{-6} M ethanol solution with ATP^{4-} at room temperature (λ_{exc} = 350 nm). The inset shows the fluorescence intensity at 530 nm vs ATP^{4-} concentration (●); the line represents the fitting of the experimental points assuming a 1:1 stoichiometry.

presence of free ion pairs involved in demetalation. Second, the different behavior observed for inorganic and organic phosphates cannot be accounted by a demetalation hypothesis. Finally, the strongest proof against such a process is the absence of the ^1H NMR signal belonging to the phenolic OH of the free salophen ligand that normally appears shifted in the region 14−12 ppm because of intramolecular hydrogen bonding with the imine nitrogen atoms (see Supporting Information, Figure S9).

Emission titrations were recorded upon excitation of the samples at 350 nm. As observed in the case of absorption spectra, the emission intensity of **1** and **2** remains practically unchanged upon titration with inorganic phosphates, while addition of nucleotides produces a 90−95% quenching of the emission (Figure 6).

Since such a change in the emission spectra was only observed upon addition of nucleotides, this is clearly indicative of the involvement of the nucleotide base in the quenching mechanism. Fluorescence quenching is probably due to a photoinduced electron transfer from the adenosine to the excited state of the fluorophore (i.e., the Zn^{2+}-salophen compound), in analogy to the behavior of other chemosensors containing anthracene fluorophores in the recognition of the same nucleotide.[32,33] This idea is fully confirmed by the negligible variations in emission spectra in inorganic phosphate titrations.

The effect of complexation is well illustrated by the picture in Figure 7 where the green-yellow fluorescence emission of compound **2** is shown in the absence (A) and in the presence of inorganic phosphates (B) or nucleotides (C). The addition of phosphates does not produce any visual change on the emission, while the addition of nucleotides caused the quenching of the green-yellow fluorescence. Zn^{2+}-salophen complexes **1** and **2** are therefore on−off sensors for specific detection of nucleotides.

(29) Bazzicaluppi, C.; Bencini, A.; Bianchi, A.; Danesi, A.; Faggi, E.; Giorgi, C.; Lodeiro, C.; Oliveira, E.; Pina, F.; Valtancoli, B. *Inorg. Chim. Acta* **2008**, *361*, 3410–3419.
(30) Albelda, M. T.; Aguilar, J.; Alves, S.; Aucejo, R.; Díaz, P.; Lodeiro, C.; Lima, J. C.; García-España, E.; Pina, F.; Soriano, C. *Helv. Chim. Acta* **2003**, *86*, 3118–3135.
(31) Wezenberg, S. J.; Escudero-Adán, E. C.; Benet-Buchholz, J.; Kleij, A. W. *Org. Lett.* **2008**, *15*, 3311–3314.
(32) Wang, H.; Chan, W.-H. *Org. Biomol. Chem.* **2008**, *6*, 162–168.
(33) Gunnlaugsson, T.; Davis, A. P.; Hussey, G. M.; Tierney, J.; Glynn, M. *Org. Biomol. Chem.* **2004**, *2*, 1856–1863.

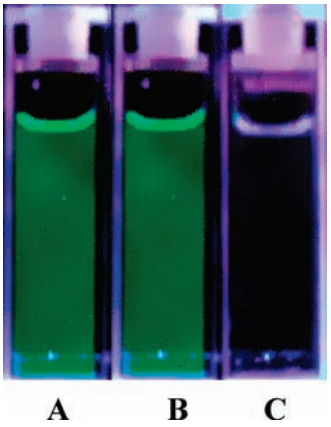


Figure 7. Change on the fluorescent emission of **2** (A) upon addition of 1 equiv of PO_4^{3-} (B) and 1 equiv of ATP^{4-} (C).

Table 1. Association Constant Values (log*K*) for Complexes between Compounds **1** and **2** and Nucleotides

	1		**2**	
	absorption	emission	absorption	emission
AMP^{2-}	5.9	6.0	5.2	5.6
ADP^{3-}	6.7	6.8	7.0	7.1
ATP^{4-}	6.6	6.6	6.4	6.4

In accordance with the results of ^{31}P NMR spectroscopy, absorbance and emission intensity changes upon addition of the nucleotides are well fitted by a binding isotherm for 1:1 complexation (equations S1 and S2, reported as Supporting Information, are used for the fitting of absorption and emission data, respectively) and allowed the determination of the association constants for the formation of the complexes (Table 1). No association constants could be obtained for the inorganic phosphates because of the absence of spectral variations. The formation of 1:1 complexes is supported by the Job plots derived from the UV−visible titration data (see Supporting Information, Figures S10 and S11).

Values retrieved from the fitting of absorption and emission data are in good agreement. The measured values are very high and of the same order of magnitude as those obtained for other similar Zn^{2+}-salophen derivatives recently reported in the literature for complexation with pyridylphosphane[16] and dibenzylammonium[19] compounds.

It was also found that ADP^{3-} is bound with the highest association constants and that the $ADP^{3-} > ATP^{4-} > AMP^{2-}$ trend is followed by both compound **1** and compound **2**. Bathochromic shifts (consistent with those reported for other similar complexes)[34] were observed upon titration with all the members of the nucleotide series: in the case of compound **2**, the emission band is blue-shifted by 18 nm, 10 nm, and 6 nm upon addition of ADP^{3-}, ATP^{4}, and AMP^{2-}, respectively.

The observed trend $ADP^{3-} > ATP^{4-} > AMP^{2-}$ in association constants does not show any correlation with the anion charge or with the number of binding oxygens. Our idea is that such a trend is related to the distance between the donor group and the adenosine moiety that allows the best simultaneous interaction of the metal with the phosphate and the $\pi-\pi$ stacking of adenine with the aromatic region of the receptor. This hypothesis gains support by the finding that the observed bathochromic shifts follow the same trend as the association constants.

(34) Cozzi, P. G.; Dolci, L. S.; Garelli, A.; Montalti, M.; Prodi, L.; Zaccheroni, N. *New J. Chem.* **2003**, *27*, 692–697.

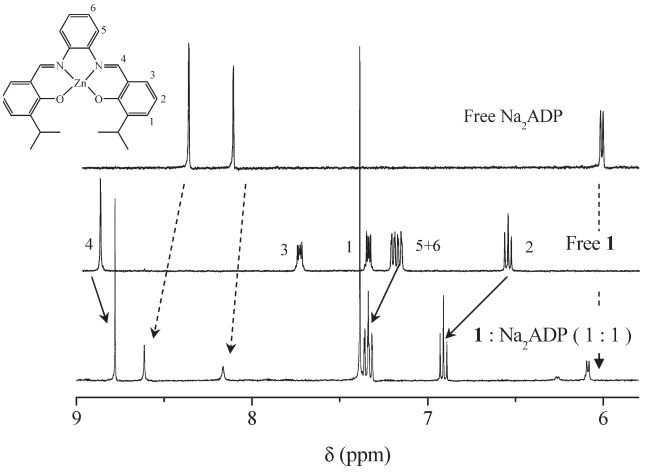


Figure 8. Portions of ^1H NMR spectra in methanol-d_4 of 1×10^{-3} M ADP^{3-}, 1×10^{-3} M **1**, and of their equimolar mixture.

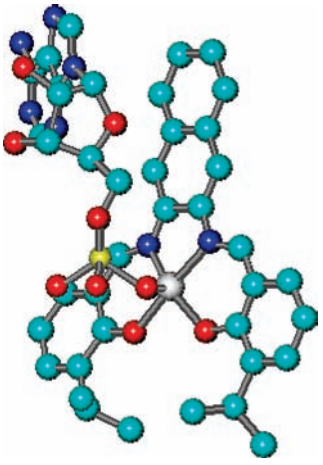


Figure 9. Computer drawing of the **2**:AMP^{2-} complex for illustration purposes only.

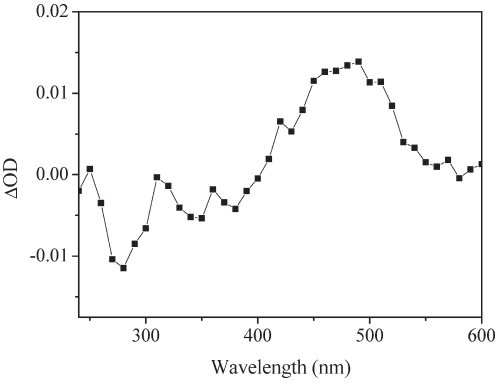


Figure 10. Transient absorption spectrum recorded for compound **2** upon 355 nm laser excitation.

^{1}H NMR Studies. Further evidence for the involvement of the aromatic region of the zinc-salophen derivatives in

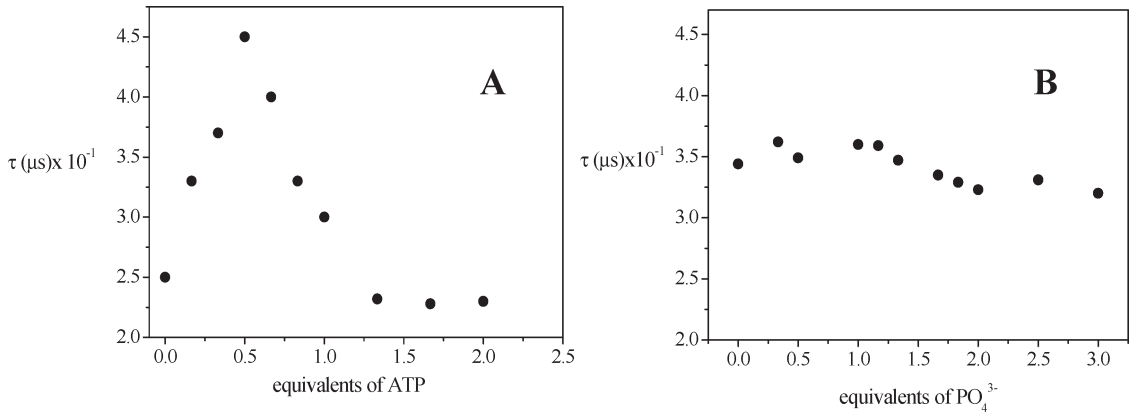


Figure 11. Lifetime values for a 5×10^{-6} M ethanol solution of compound **2** measured upon titration with ATP^{4-} (A) and PO_4^{3-} (B).

the complexation of the organic phosphates comes from ^{1}H NMR studies. Different results were obtained from spectra analysis of the 1:1 mixtures of receptor **1** or **2** with the anions of both series.

Complexation of inorganic phosphates does not produce any relevant change in the ^{1}H NMR spectrum of **1** (see Supporting Information, Figure S12), while, in the presence of AMP^{2-}, ADP^{3-}, or ATP^{4-}, clear global downfield shifts were observed both for the resonances of protons of Zn^{2+}-salophen complexes and of the nucleotide (Figure 8 and Supporting Information, Figure S13).

These data confirm our hypothesis about the existence of stacking interactions that can obviously occur only with nucleotides. A computer drawing of the **2**:AMP^{2-} complex is presented in Figure 9 to illustrate the proposed coordination mode between nucleotides and the salophen compounds. The formation of a Zn−O bond and the parallel disposition between the aromatic rings of the adenosine and those of **2** can be clearly observed in the figure.

Similar attractive interactions between the aromatic part of a water-soluble zinc-salophen derivative and the phenyl ring of phenylalanine have been considered responsible of the marked discrimination between the two enantiomers of the aminoacid.[27]

Laser Flash Photolysis Measurements. Transient absorption spectra of compounds **1** and **2** were recorded using nanosecond laser flash photolysis with 355 nm excitation. As shown in the transient spectrum of **2** (Figure 10), recorded immediately after the laser pulse, the transitions at 250−420 nm (corresponding to the ground state absorption of the complex) are bleached (negative ΔOD) and a new absorption band appears (positive ΔOD) at about 450 nm.

The decay of the 450 nm absorption was fitted with a single exponential law and is quenched by molecular oxygen, which is in agreement with the attribution to triplet−triplet absorption. The recorded value in air-equilibrated solution (0.3 μs) increased by about three times (0.9 μs) after bubbling nitrogen for 45 min. According to previously studies on the photochemistry of salen compounds, the triplet is photogenerated through the recombination of a charge separated singlet excited state that intersystem crosses to the corresponding triplet.[35,36] The lifetime in the microsecond time scale is also in agreement with its spin forbidden deactivation to the ground state.

The emission observed at 530 nm (Figure 6) has a much shorter decay time and is attributed to the decay of the singlet excited state. Since we were not able to record the singlet excited state lifetime with our single photon counting equipment, it is to be concluded that its value is smaller than 100 ps (detection limit of our single photon counting apparatus).

Further laser flash photolysis experiments were carried out in the presence of anions and in conditions similar to those employed for absorption and emission titrations, that is, on 5×10^{-6} M ethanol solutions of compounds **1** and **2** by adding increasing amounts of anions. The decay time of the triplet state was measured at different anion concentrations. Changes in the decay time values were only observed for the addition of nucleotides (Figure 11A) while no significant changes have been observed for titrations with phosphates (Figure 11B).

The observed changes in decay times do not depend linearly on the anion concentration. The initial value of about 0.25 μs increases up to a maximum value of about 0.45 μs upon addition of 0.5 equiv of nucleotide, and decreases afterward until a plateau (ca. 0.22 μs) for a 1:1 stoichiometry is reached, which provides further evidence for 1:1 association. Moreover, the profile indicates the formation of a 1:0.5 intermediate at lower host/guest ratios, a behavior that is in agreement with previous findings on other salen compounds, for which the formation of a similar intermediate with an anion acting as a bridging ligand between two molecules of receptor has already been reported.[34]

The titration data (absorption and fluorescence) previously fitted assuming 1:1 complexation (Table 1) was reanalyzed with the model including 1:1 and 1:0.5 complexes. The data does not demand the presence of 1:0.5 complexes to yield good fits, and the inclusion of the second association process results in unacceptable errors in the determination of the 1:0.5 association constant.

(35) de Castro, B.; Ferreira, R.; Freire, C.; García, H.; Palomares, E. J.; Sabater, M. J. *New J. Chem.* **2002**, *26*, 405–410.

(36) Marquis, S.; Ferrer, B.; Álvaro, M.; García, H. *J. Phys. Chem. A* **2007**, *111*, 6034–6038.

Conclusions

Two different Zn^{2+}-salophen derivatives have been used in molecular recognition of phosphates PO_4^{3-}, $P_2O_7^{4-}$, and $P_3O_{10}^{5-}$ and nucleotides AMP^{2-}, ADP^{3-}, and ATP^{4-}.

^{31}P NMR and mass spectrometry showed that both inorganic phosphate and nucleotide anions are bound by Zn^{2+}-salophen compounds. Nevertheless, ^{1}H NMR, absorption, emission, and laser flash photolysis experiments demonstrate that only association to nucleotides produces relevant changes on the spectral properties of **1** and **2**. This finding, combined with the observation that the trend $ADP^{3-} > ATP^{4-} > AMP^{2-}$ in association constants does not correlate with the anion charge, clearly demonstrates that nucleotides are bound via two main interactions, namely, zinc-phosphate coordination and $\pi-\pi$ stacking between the salophen aromatic rings and the adenosine nucleobase of nucleotides. The simultaneous existence of two clealry distinct interaction sites makes these Zn^{2+}-salophen complexes effective supramolecular receptors for detection of nucleotides.

Acknowledgment. The support and sponsorship provided by COST Action D31/0016 is acknowledged. The authors are also grateful to Fundação para a Ciência e Tecnologia (Portugal) for financial support (PROJECTS) and to the Ministerio de Educación y Ciencia (Spain) for the Project CTQ2006-02362/BQU.

Supporting Information Available: Further details are given in Figures S1−S13 as noted in the text and about the equations used for the fitting of absorption and emission data (equations S1 and S2). This material is available free of charge via the Internet at http://pubs.acs.org.