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Design of ICT-PET fluorescent probes for zinc(II) based on 5-aryl-2,2′-bipyridines

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ABSTRACT

Rational design of selective and sensitive ‘off-on’ fluorescent probes for Zn(II) cations exploiting both PET and ICT mechanisms of sensing is illustrated by the synthesis and application of 5-aryl-2,2′-bipyridines modified with a dipicolylaminomethyl fragment. The aryl substituent provides tuning of the properties.

Zinc(II) is the second most abundant *d*-block metal ion in the human brain.[1] Sensitive methods for detection of zinc ions attract attention since zinc plays a crucial role in many biological processes.[2] Fluorescence spectroscopy is a powerful method for sensing and imaging metal cations at submicromolar concentrations.[3,4] Photoinduced electron transfer (PET) and intermolecular charge transfer (ICT) are the most widely used mechanisms of fluorescence signal modification used in the design of Zn(II) chemosensors.[5,6] PET provides significant enhancement of a fluorescence signal with increased concentration of the metal ions and gives ‘off-on’ sensors.[7] However, in PET probes isolation of the signaling and sensing parts through aliphatic spacers cannot give a shift of the fluorescence maxima. On the contrary, in ICT sensors the signaling and sensing parts are combined in one conjugated system, and binding of an analyte leads to blue or red shift of the fluorescence usually without significant changes of intensity, thus a ratiometric analysis can be applied.[8] A combination of these two techniques promises better results in ‘turn on’ ratiometric sensor design since the effect of color alteration due to ICT would be amplified by intensity increase due to PET.[6] This idea has been illustrated by V. Kozhevnikov when 6,6″-bis(aminomethyl)-5,5″-bis-(4-bromophenyl)-2,2′:6′,2″-terpyridine showed both a red-shift of the maximum and an enhancement of intensity of luminescence upon increasing the Zn(II) concentration in buffered aqueous solution.[9] In this case, the terpyridine moiety played the role of the metal binding site and the chromophore simultaneously. A similar effect was reported for 6-dipicolylaminomethyl-2-pyridylbenzimidazole.[10] Both PET and ICT mechanisms can be assumed for sensors, where an amino or oxy group conjugated with a chromophore takes part in binding the Zn(II) cation.[11]

Zinc(II) complexes of bipyridine, phenanthroline, or terpyridine exhibit bright luminescence. Extended conjugated systems provide significant red-shift of luminescence of the ligands and especially Zn(II) complexes, thus a number of ICT Zn(II) sensors based on oligopyridines have been described.[12] In particular, 5-aryl-2,2′-bipyridines can be considered as an appropriate platform for the design of new Zn(II) sensors.[13] In order to increase the selectivity of a sensor, the bipyridine chelating site should be extended with specific Zn(II) ion receptors, that is, dipicolylamine (DPA).[6] To exploit the PET mechanisms the amine should be attached at the α-position of the bipyridine through an appropriate spacer, for example a methylene group.

The targeted ligands **1a,b** were synthesized according to the strategy shown on Scheme 1. Key intermediates, 5-aryl-6′-bromomethyl-2,2′-bipyridines **2**, were obtained by the ‘1,2,4-triazine′ methodology starting from synthetically available triazines **3**.[14] Reaction of **2** with dipicolylamine resulted in the desired ligands **1a,b**.[15] The aryl substituent in **1** plays a dual role. Firstly, it increases the amphiphilic properties of the ligand, which is important for biological imaging applications.[2] Secondly, the aryl modulates absorption and emission properties of the ligand and the Zn-complex that shifts the emission maxima to the visible area, and increases the Stokes shift.

Zinc(II) complexes of the new ligands were isolated from the reaction of **1** with $Zn(ClO_4)_2$. Single crystals of $[Zn(\mathbf{1a})](ClO_4)_2$ were obtained by recrystallization from methanol. X-ray crystallography[16] revealed the 1:1 composition of the complex ion $[Zn(\mathbf{1a})]^{2+}$ (Fig. 1). Pentacoordinated zinc(II) atoms have distorted trigonal

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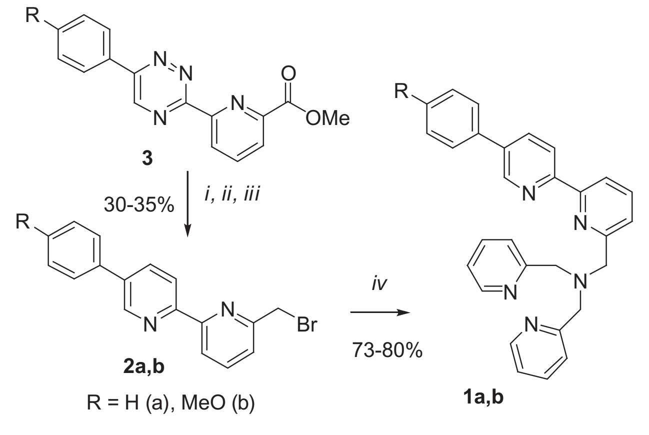

Scheme 1. Synthesis of ligands. Reagents and conditions: (i) 2,5-norbornadiene, *o*-xylene, reflux, 48 h; (ii) $NaBH_4$, EtOH, reflux, 2 h; (iii) PBr_3, CH_2Cl_2, 8 h; (iv) 2,2′-dipicolylamine, K_2CO_3, MeCN, reflux, 8 h.

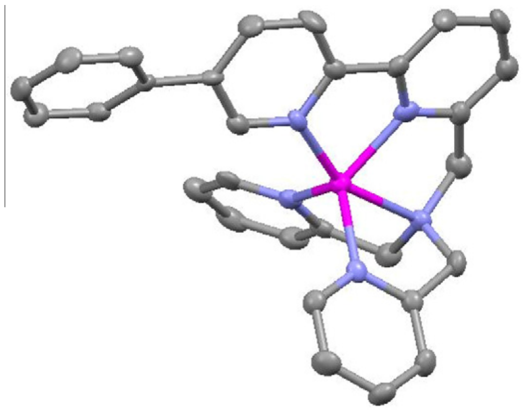

Figure 1. Crystal structure of [Zn(**1a**)]$^{2+}$. Hydrogen atoms are omitted for clarity.

bipyramidal geometry. Distances between zinc and the nitrogens of the pyridine rings at the base of bipyramid are practically equal (2.01–2.03 Å), while the Zn-N distances with the fourth pyridine ring and the amino group at the vertexes of the bipyramid are significantly longer, 2.09 and 2.25 Å correspondingly.

Photophysical properties of the new ligands **1** and their complexes measured in acetonitrile are collected in Table 1. The dipicolylaminomethyl group does not affect the energy of electronic transitions, so absorption and emission spectra of both ligands and complexes are close to those of the parent arylbipyridines.[13a] At the same time, the intensities of fluorescence of the ligands are decreased significantly due to PET. In the Zn-complexes the lone pair of the amino group is involved in the coordination of the metal atom that excludes PET and turns on bright fluorescence. The enormous (35 fold) increase of quantum efficiency of luminescence but with a very small red-shift upon Zn(II) coordination allows the suggestion that **1a** is a typical 'off-on' PET but not ICT sensor. An electron-donating methoxy group in the aryl amplifies intraligand charge-transfer in **1b** resulting in a significant red-shift (60 nm) of fluorescence upon complex formation. At the same time, the coordination results in an eightfold increase of the fluorescence intensity, thus **1b** can be discussed as PET-ICT sensor.

The absorption and emission spectra of **1a** on titrating $Zn(ClO_4)_2$ into the aqueous solution (HEPES buffer) are presented in Figure 2. The absorption and emission maxima are slightly (5–10 nm) shifted toward longer wavelengths with the addition of Zn(II). The fluorescence intensity in an aqueous medium was enhanced but not as dramatically (by the factor of 5.5) as in acetonitrile because of the relatively intense ambient emission of the ligand **1a** in water. However **1a** can be considered as a PET fluorescent probe for Zn(II) in aqueous solutions. The lowest-energy absorption band of the Zn(II) complex of **1b** in the HEPES buffer was 10 nm blue-

Table 1
Photophysical data of **1** and [Zn(**1**)]$(ClO_4)_2$ in acetonitrile

Compound	Absorption, λ_{max}, nm (ε, 10^{-3} $M^{-1}cm^{-1}$)	λ_{em}, nm	Φ[a]
1a	262 (20.5), 303 (31.5)	367	0.006
[Zn(1a)]$(ClO_4)_2$	262 (20.0), 327 (27.1)	377	0.21
1b	263 (11.3), 268 (11.2), 313 (25.6)	395	0.12
[Zn(1b)]$(ClO_4)_2$	262 (15.2), 345 (17.8)	455	0.94

[a] Quantum yields of fluorescence were measured in acetonitrile relatively to the anthracene (Φ = 0.27 in ethanol[17]).

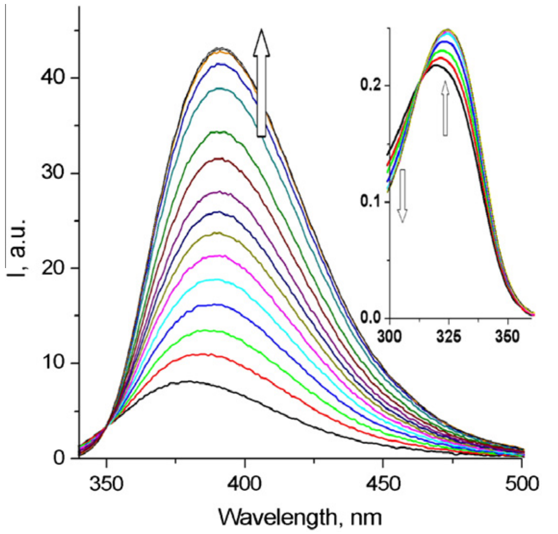

Figure 2. Fluorescence enhancement upon titrating $Zn(ClO_4)_2$ (0 – 0.8 equiv, step 0.05 equiv) to the solution of **1a** (10^{-5} M in HEPES buffer), excitation at isosbestic point 313 nm. Inset: changes of the absorption.

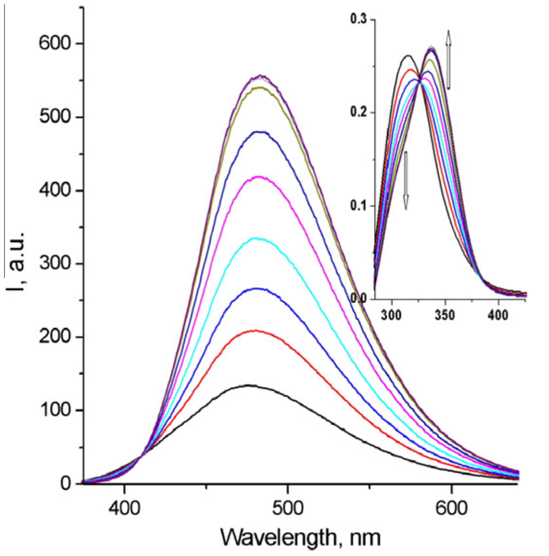

Figure 3. Fluorescence enhancement upon titrating $Zn(ClO_4)_2$ (0 – 0.8 equiv, step 0.1 equiv) to the solution of **1b** (10^{-5} M in HEPES buffer), excitation at isosbestic point 327 nm. Inset: changes of the absorption.

shifted in comparison with the data measured in acetonitrile, while the absorption maximum of the ligand **1b** was not sensitive to the solvent (Fig. 3). On the contrary, the emission maximum of **1b** shifted dramatically (83 nm) toward longer wavelengths in aqueous solution in comparison with acetonitrile. Titrating Zn(II) into the solution of **1b** in the HEPES buffer resulted in a very small (7 nm) red-shift of the emission maxima with a 4.5-fold enhancement of the fluorescence intensity (Fig. 3). Thus in an aqueous medium **1b** worked like a PET but not like a ICT fluorescent probe for Zn(II).

Metal-sensing selectivity was studied for **1a**. The fluorescence ratio values of **1a** in response to various divalent metal ions in the HEPES buffer are depicted in Figure 4. The fluorescence response was not affected by physiologically abundant metal ions such as Na^+, Ca^{2+}, and Mg^{2+}, although Cd^{2+} changed the fluorescence spectra similarly to Zn(II). Transition metals, such as Cu^{2+}, Fe^{2+}, and Mn^{2+} quenched the fluorescence. However Co^{2+}, Hg^{2+}, and particularly Ni^{2+} caused no significant decrease of the fluorescence signal. The ligand **1b** showed similar selectivity.

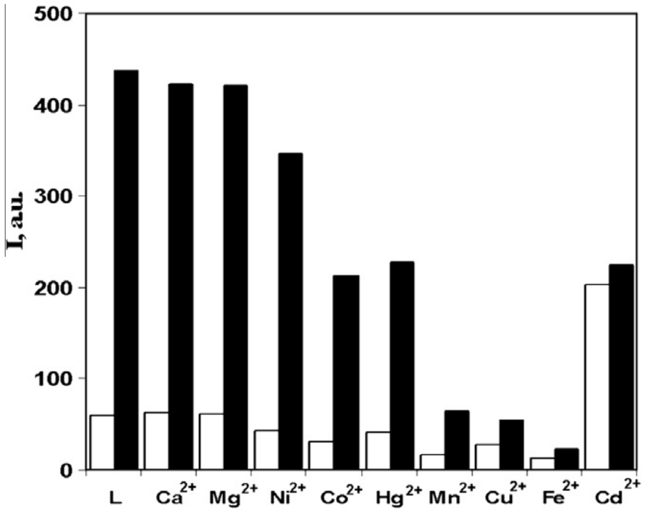

Figure 4. Fluorescence response of **1a** (10 M) to 10 M of various metal ions (blank bars) and followed by addition of 10 M of Zn(II) (black bars) monitored at 391 nm in aqueous solution (25 mM HEPES, 0.1 M NaCl), excitation at 313 nm (isosbestic point of the absorption spectra).

We used DFT calculations (B3LYP/TZVP, COSMO solvation model) to understand the large differences between the photophysical properties of the ligands and the complexes in acetonitrile and water solutions. Geometries of the ligands **1a,b** and their Zn(II) complex cations were calculated, and geometry parameters of $[Zn(\mathbf{1a})]^{2+}$ were close to the crystal data described above. Additionally H-bond complexes of the ligands **1a,b** with a molecule of water linked to the amino group were calculated in order to consider the solvent effect. The calculated energies and localizations of frontier molecular orbitals of the ligands **1a,b** and their complexes with water and Zn(II) are shown in Figure 5.

The HOMO of **1a** is mainly the lone pair of the amino group, while the LUMO is π^* located on the bipyridine fragment. Since the n,π^*-excited state decays through nonradiative pathways, it explains the low intensity of the fluorescence of **1a** in acetonitrile. In the Zn complex of **1a**, the HOMO has π character, so bright emission from π,π^*-excited state was observed. The interaction of **1a** with a molecule of water decreases the energy of the lone pair, thus in the **1a+HOH** complex, the HOMO is π but not n, that is in water solution **1a+HOH** should emit from the π,π^*-excited state, which explains the fluorescence enhancement of **1a** in aqueous media.

An electron-donating methoxy group raises energy of the π orbital, which becomes the HOMO of **1b** in acetonitrile solution, while the lone pair of the amino group is slightly lower in energy. It increases the π,π^* character of the lowest excited state of **1b**, that enhances the intensity of the fluorescence if compared with **1a**. As a result, the PET effect is significantly reduced for **1b** both in acetonitrile and particularly in water. The lowest excited state of **1b** is an ICT state, since the HOMO is mainly localized on the methoxyphenyl fragment, while the LUMO is on the bipyridine moiety, thus excitation increases the polarity of **1b** (Fig. 6). In contrast, the high

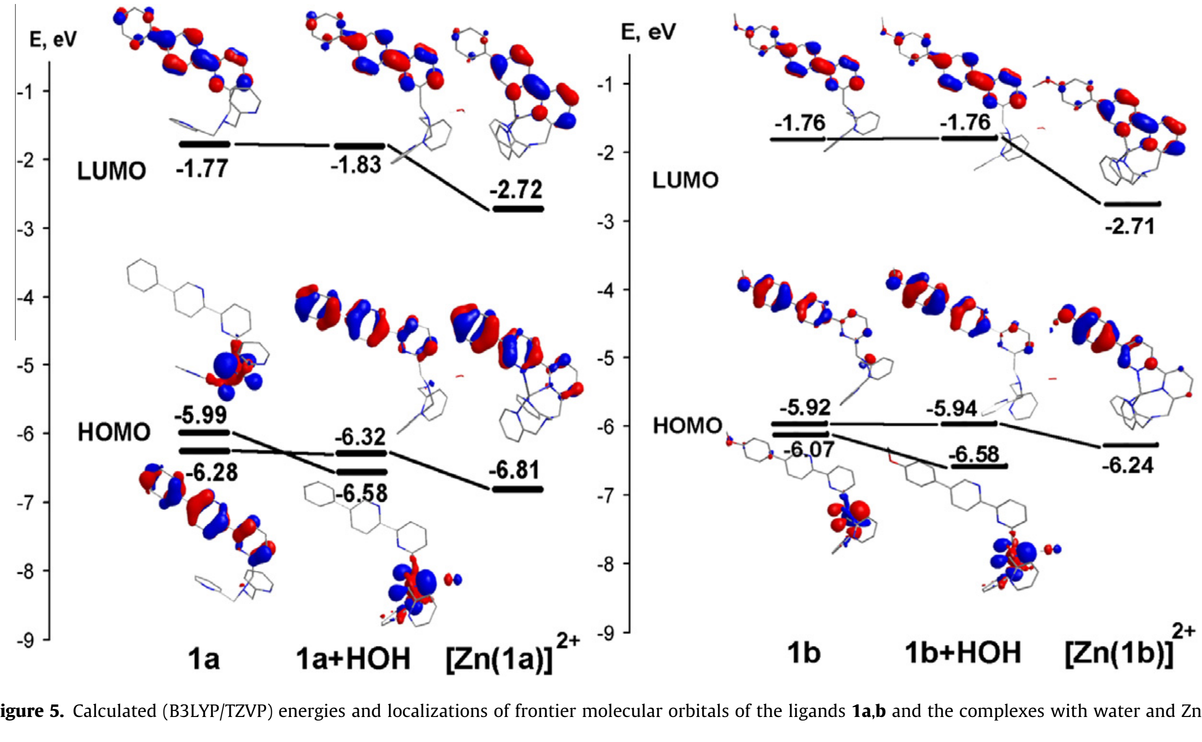

Figure 5. Calculated (B3LYP/TZVP) energies and localizations of frontier molecular orbitals of the ligands **1a**,**b** and the complexes with water and Zn(II).

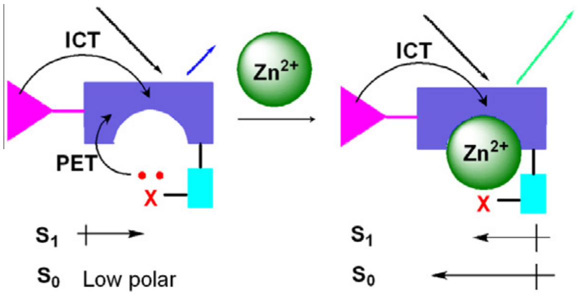

Figure 6. Schematic representation of photoinduced processes, PET and ICT, and changes of the dipole moment upon excitation from the ground state (S_0) to the excited state (S_1) for the ligand before and after Zn(II) coordination.

polarity of the Zn(II) complex of **1b** in the ground state is reduced at the excited state due to charge transfer from the methoxyphenyl fragment to the bipyridine upon excitation. As a result, increasing the solvent polarity (from acetonitrile to water) leads to a decrease in the red shift upon Zn(II) coordination.

In conclusion, arylbipyridines modified with a dipicolylaminomethyl fragment are selective and quite sensitive 'off-on' fluorescent probes for Zn(II) cations. Depending on the substituents in the aryl both the PET and ICT mechanisms of sensing can be exploited. However, the latter is weak in highly polar aqueous media. Generally, in the design of hybrid PET-ICT fluorescent 'turn on' ratiometric probes for Zn(II), two points have to be considered:

(A) The occupied molecular orbitals of the chromophore should be sufficiently lower in energy than the lone pair of the metal binding part to provide PET in aqueous media; DFT calculations are an effective tool for this purpose.

(B) The substituents should not decrease the polarity of the excited state of the Zn-complex providing a large red-shift due to ICT in polar aqueous media, that is the intraligand charge transfer should be directed from the chelating part toward the conjugated electron-withdrawing substituent.

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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.2012.09.027.

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15. *General procedure for the preparation of ligands* ***1*** Mixture of corresponding 6′-bromomethylbipyridine **2** (0.6 mmol), dipicolylamine (0.12 mL, 0.66 mmol), anhydrous potassium carbonate (830 mg, 6 mmol), and dry acetonitrile (90 mL) was stirred under reflux for 12 h. The solvent was removed under reduced pressure. Water (50 mL) was added to the residue, and the resulting mixture was stirred at room temperature for 5 min. The resulting precipitate was filtered off, washed with water and recrystallized from acetonitrile.
16. CCDC 894258 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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