Research paper

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A novel fluorescent chemosensor for Cu²⁺ ion based on a new hexadentate ligand receptor: X-ray single crystal of the perchlorate salt of the ligand, ion selectivity assays and TD-DFT study

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Abstract

New hexadentate chelates, H_2L^1 and L^1 were synthesized. The Schiff base imine ligand, L^1 , was prepared by reaction of 2,2'-[ethane-1,2-diylbis(oxy)]dibenzaldehyde and propanolamine, and characterized by infrared (IR), ¹H-NMR and ¹³C-NMR spectroscopies. The amine ligand, H_2L^1 , was prepared in the form of perchlorate salt ([H₄L¹](ClO₄)₂,) by reduction of L¹ with NaBH₄, and was characterized by IR (in KBr pellet), ¹H-NMR, ¹³C-NMR and 2D NMR correlation spectroscopy (COSY) (in solution). The X-ray crystal structure of [H₄L¹](ClO₄)₂ was also determined and discussed. Fluorescence studies showed that H_2L^1 is a highly selective novel fluorescence chemosensor for the Cu²⁺ cation. The sensor shows selectivity for Cu²⁺ over 12 other metal cations. In presence of Cu²⁺ (5 equivalents in the μ M range) the fluorescence emission of the sensor, at 309 nm, is quenched by more than 98 %. TD-DFT calculations show that ligand to metal charge transfer transitions in the complex formed between Cu²⁺ and the amine ligand contribute to the quenching of the fluorescence. An explanation based on TD-DFT results is also proposed for the weak fluorescence quenching in the presence of Zn²⁺.

Keywords: Hexadentate Schiff base; X-ray structure; Fluorescence; Cu²⁺ chemosensor; TD-DFT

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1. Introduction

Schiff bases are compounds in which an aldehyde or ketone carbonyl functional group has been substituted by an imine or azomethine group. These compounds are extensively used for industrial applications and also show a vast range of biological activities. Schiff bases were first reported by Hugo Schiff, in 1864 [1]. Since then, a large diversity of methods for their synthesis have been reported. The classical synthesis of these compounds involves the condensation of a primary amine with a carbonyl compound [2] under specific conditions (e.g., azeotropic distillation), and is described by the following chemical equation: $RNH_2 + R-COH \longrightarrow R-N=CH-R + H_2O.$

The chemical and physical properties of Schiff bases and their reduced amine forms have been investigated extensively in different contexts, such as synthesis, identification and qualitative determination of aldehydes or ketones, purification of carbonyl or amino compounds, or protection of these compounds in complex or sensitive reactions [3,4]. In the present work, we explore the use of a hexadentate Schiff base in its reduced salt form as a chemosensor for the Cu^{2+} cation.

Development of new fluorescent chemosensors is a challenging and important field of research in supramolecular chemistry [5-7]. In particular, ion sensing is of great relevance for the understanding of biochemical processes taking place in cellular media [8]. Among the essential transition metals in biological systems, the most abundant ones are Fe, Zn and Cu [9,10].

Other heavy metal ions, such as Hg^{2+} and Cd^{2+} , constitute a serious risk to living organisms due to their high toxicity. Systems based on coumarin have been used in the development of fluorescent chemosensors for the detection of anions (e.g., CN^- , F^-), metal ions (such as Cu^{2+}/Cu^+ , Zn^{2+} , Fe^{3+}/Fe^{2+} , Hg^{2+} , Cd^{2+} , Al^{3+} , alkali metal ions, etc.), reactive sulfur species, biological thiols, reactive oxygen and nitrogen species, etc. These studies have recently been reviewed [11]. Near-infrared fluorescence sensors

are also relevant in the context of applications to *in vivo* bioimaging, due to the low background interference, high image sensitivity and minimal damage to tissues [12].

Fluorescent sensors for Cu^{2+} ion have been developed and used to clarify the physiological role of this metal ion [13-17], which is known to be involved in several cellular processes, such as functional and structural strengthening of proteins and gene expression, and also in processes taking place in human nervous system [18,19]. Both high and low copper concentrations can disrupt normal activity of living systems and lead to diseases, such as Alzheimer's [20], Menkes-Wilson disease [21] and prion-based infections [22]. In spite of the considerable attention that has been given to the design of sensitive and selective fluorescent sensors for the detection of Cu^{2+} ions [23-25], the search for better Cu^{2+} chemosensors is still an active field of research. The Cu^{2+} ion is a strong quencher of fluorescence both *via* energy and electron transfer mechanisms. However, many copper sensors developed hitherto exhibit a fluorescence "turn-off" response that frequently leads to limited resolution [26-30].

Naphthol derived [31] and oligothiophene-phenylamine derived [32] Schiff base ligands have been reported, respectively, as selective fluorescent probes for the detection of Zn^{2+} [31] and Hg^{2+} and Cu^{2+} [32]. The Mn(II)/(III) complexes of a hexadentate bisaldehyde-type Schiff base have also been characterized and the ligand was used for the determination of Fe(III) [33].

As described in details below, in the present investigation a novel hexadentate Schiff base (L¹) and its reduced amine derivative form (in its neutral H₂L¹, and cationic $[H_4L^1]^{2+}$ forms) were synthesized and characterized. The capability of H₂L¹ to act as a highly sensitive fluorescent chemosensor for Cu²⁺ ion is demonstrated.

2. Experimental

2.1. General information

2,2'-[ethane-1,2-diylbis(oxy)]dibenzaldehyde was prepared according to the literature method [34]. Salicylaldehyde and NaBH₄ were obtained from Merck. Infrared (IR) and nuclear magnetic resonance (NMR) spectra were measured on a Perkin Elmer FT-IRGX and a Bruker DPX 300 spectrometer, respectively. The fluorescence spectra were recorded on a Sinco FS2 spectrofluorimeter; excitation and emission band widths were set as 5.0 and 10.0 nm, respectively.

2.2 X-ray crystal structure determination

Single crystal X-ray data for compound $[H_4L^1](ClO_4)$ were collected at 298 K on a Bruker Stoe IPDS-II diffractometer diffractometer using graphite monochromated MoK_{α} radiation ($\lambda = 0.71073$ Å). The linear absorption coefficients, scattering factors for the atoms, and anomalous dispersion corrections were taken from the International Tables for X-ray Crystallography [35]. The data integration and reduction were carried out with the SAINT software [36]. An empirical absorption correction was applied to the collected reflections using SADABS [37], and the space group was determined using XPREP [38]. The structure was solved by the direct method using SHELXTL-2017 [39] and refined on F² by full-matrix least-squares using the SHELXL-2017 program package [40]. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms attached to carbon atoms were positioned geometrically and treated as riding atoms using SIR-97 default parameters.

2.3. Syntheses (see Scheme 1)

2.3.1. Preparation of the precursor aldehyde (2,2'-[ethane-1,2-diylbis(oxy)]dibenzaldehyde)

1,2-dibromoethane (0.1 mol) and NaOH (0.2 mol) were mixed and heated under reflux in 500 mL aqueous ethanol (300 mL H₂O and 200 mL EtOH). Salicylaldehyde (0.2 mol) in 30 mL EtOH was added, and the

solution was heated under reflux for 4 days. After cooling, the resulting yellow crystals were filtered, washed and recrystallized (yield: 87%, purity > 98%) [34,41].

2.3.2. Preparation of ligand L¹

The precursor aldehyde (0.27 g, 1 mmol) and propanolamine (0.150 g, 2 mmol) were mixed and heated at 40 °C for 30 min in EtOH (30 mL). The solution was filtered and the filtrate was reduced to *ca*. 10 mL. The resulting yellow crystalline product was filtered off, washed with cold ethanol, and dried. Yield: 90%. Anal. Calc. for C₂₂H₂₈N₂O₄: C, 68.71; H, 7.29; N, 7.32. Found: C, 68.36; H, 7.17; N, 7.26. IR (KBr, cm⁻¹): 1637 (imine), ¹H-NMR (CDCl₃, 300 MHz): δ 1.80 (m, 4H), 3.60 (t, 4H), 3.67 (t, 4H), 4.32 (m, 4H), 6.90-7.86 (m, 8H), 8.58 (s, 2H). ¹³C-NMR (CDCl₃, 75 MHz): δ 33.5 (C-10), {59.5, 61.4, 67.2 (C-1, C-9, C11)}, {112.6, 121.4, 124.7, 127.4, 132.0 (aromatic rings)}, {157.9, 157.2 (C-2, C-8)}.

2.3.3. Preparation of H₂L¹ and crystalline [H₄L¹](ClO₄)₂

The Schiff base ligand L¹ was reduced with NaBH₄, in ethanol solution at room temperature, to its amine H_2L^1 form. After stirring the solution during 3 h, a white precipitate was formed. The precipitate was then filtered, washed with cold ethanol and dried. Anal. Calc. for C₂₂H₃₂N₂O₄: C, 68.01; H, 8.30; N, 7.21. Found: C, 67.62; H, 8.18; N, 7.19. IR (KBr, cm⁻¹): 3302 (vN–H). The precipitate (H₂L¹) was subsequently dissolved (0.39 g, 1 mmol) in an equimolar acetonitrile/methanol solution. NaClO₄ (0.25 g, 2 mmol) dissolved in methanol (5 mL) was then added and the solution heated at 40 °C for 30 min in presence of HCl. The solution was filtered and the filtrate was reduced to *ca*. 10 mL. The resulting white crystalline product was filtered off, washed with cold ethanol, and dried. The afforded material, [H₄L¹](ClO₄)₂, was recrystallized from an equimolar acetonitrile/methanol solution. Yield: 82%. Anal. Calc. for C₂₂H₃₄Cl₂N₂O₁₂: C, 44.82; H, 5.77 N, 4.75. Found: C, 44.76; H, 5.67; N, 4.68. IR (KBr, cm⁻¹): 1497 (vC=C), 3299 (vN–H). ¹H-NMR (CDCl₃, 300 MHz): δ 1.65 (m, 4H), 2.79 (t, 4H), 3.74 (t, 4H), 3.80 (m, 4H), 4.40 (s, 4H), 6.94-7.26 (m, 8H). ¹³C-NMR (CDCl₃, 75 MHz): δ 30.7 (C-10), {48.6, 49.0, 63.8, 66.7

(C-1, C-8, C9, C11)}, 111.5-156.7 (aromatic rings), DEPT 135°: {30.7, 48.6, 49.0, 63.7, 66.7 (CH₂ aliphatic)}, {111.5, 121.0, 128.5, 130.2 (CH aromatics)}.

Crystals for single crystal X-ray structure determination were obtained by slow diffusion of diethyl ether vapour into a methanol-ethanol solution of the compound.

2.4. Spectroscopic response experiments

Stock solutions (1 mM) of Ni²⁺, Co²⁺, Al³⁺, Mn²⁺, Mg²⁺, Zn²⁺, Hg²⁺, Pb²⁺, Cd²⁺, Ag⁺, Cr³⁺, and Cu²⁺ in H₂O/dimethyl formamide (DMF) (1:1, v/v) were prepared from the corresponding nitrate salts. Stock solutions of L¹ and H₂L¹ (0.2 mM) in DMF were also prepared. The test solutions were then obtained by adding 0.1 mL of the probe's stock solution and an appropriate aliquot of each metal stock solution in a test tube, and subsequently diluting the obtained mixture with H₂O-DMF (1:1, v/v) to give the final concentrations of the probe (10 μ M) and metal ions (in the 0-50 μ M range).

2.5. Computational Details

The structure of the Cu(II)/H₂L¹ complex was investigated at the density functional theory (DFT) level with the CAM-B3LYP [42] exchange correlation functional using the GAUSSIAN09 [43] code. The LANL2DZ [44,45] valence and effective core potential (ECP) functions were used for copper and the double- ζ plus polarization 6–31G(d,p) [46,47] basis set was used for the remaining atoms. Cu(II) ion has an open-shell electronic configuration (d^9), and therefore the calculations were carried out using the unrestricted (U-CAM-B3LYP) formalism. A conformational study was carried out to determine the most stable conformers of the H₂L¹ ligand in water. Starting from the modified X-ray structure of the [H₄L¹]²⁺ fragment, conformers were generated by using the molecular mechanics Dreiding force field [48] as implemented in MarvinSketch [49] and the lower energy 22 conformers were then optimized at the CAM-B3LYP/6–31G(d,p) level of theory. The lowest energy conformer has *anti* conformation and *C*_i symmetry.

This conformer shows OH. N intramolecular hydrogen bridges. The second most stable conformer is only 0.25 kJ/mol above the lowest energy conformer and has syn conformation. It also contains OH...N and NH^{...}O intramolecular hydrogen bridges. The nature of the stationary points found was checked by vibrational analysis, and only those which are true minima (with no imaginary frequencies) were considered. Single point DFT calculations were carried out to obtain the energy difference between the syn conformation adopted by the ligand in the complex and the *anti* conformation of the free ligand. To do this, the metal ion was formally removed from the optimized geometry of the complex and the terminal deprotonated oxygen atoms were saturated so as to have the same chemical formula as the free ligand. Since a very large number of intramolecular interactions (in particular, H-bridges) becomes possible involving the terminal OH and the nitrogen atoms, and the intramolecular network established depends on the orientation given to the added hydrogen atoms, we have used the ReadOpt option of GAUSSIAN09 to optimize the coordinates of these two hydrogen atoms, while maintaining the remaining coordinates of this syn conformation frozen. We have then compared the energy of this syn conformation with the energy of the most stable *anti* conformation of the free ligand H_2L^1 . In these calculations, the tight convergence criterion (scf=tight) was used. The single point calculations were repeated including the effect of the solvent water through the PCM model [50,51]. Respectively, the 40 and 10 lowest-energy vertical electronic excited states were calculated for the optimized geometries of the complex and of the ligand by time-dependent DFT (TD-DFT) [52] using the same functional and basis sets. The values of $\langle S^2 \rangle$ obtained from the U-DFT calculations for the complex were compared with the expected values for the spin state to confirm that spin contamination was negligible in all cases. The same basis sets and functional were used in the DFT and TD-DFT (restricted formalism) calculations for the Zn(II) complex.



Scheme 1. Syntheses of L^1 and H_2L^1 and $[H_4L^1]^{2+}$ (the cation was produced as perchlorate salt).

3. Results and Discussion

3.1. Syntheses and characterization of the compounds

The Schiff base compound L^1 was synthesized as described above, and then reduced to the amine H_2L^1 using NaBH₄. The H_2L^1 compound was subsequently converted to its perchlorate salt, $[H_4L^1](ClO_4)_2$.

The compounds were found to be quite stable in air and they can be stored in a desiccator for long periods of time without decomposing. Both the imine and the amine derivative, in its perchlorate salt form ([H₄L¹](ClO₄)₂), were characterized by, IR, elemental analysis and both ¹H- and ¹³C-NMR. The perchlorate salt of the amine species was further investigated by Distortionless Enhancement by Polarization Transfer (DEPT) 135° and 2D NMR correlation spectroscopy (COSY) experiments, and single crystal X-ray diffraction (IR and NMR data are provided in Figures S1-S8, in the Supporting

Information; X-ray data is available from the Cambrigde Crystalographic Data Centre, CCDC #1424449; http://www.ccdc.cam.ac.uk/conts/retrieving.html).

Condensation of the primary amino group of the propanolamine precursor during the synthesis of L^1 is confirmed by the lack of bands in the characteristic N–H stretching region (3150–3450 cm⁻¹) in the infrared spectrum of the reaction product, and also by the presence of a strong imine C=N stretching band at 1637 cm⁻¹ (Figure S1). On the other hand, in the IR spectrum of $[H_4L^1](ClO_4)_2$ (Figure S2), the band corresponding to the C=N stretching mode of the imine group is absent, while bands due to the stretching vibrations of the amine N–H bonds are easily observed superimposed with the broad feature due to the OH stretching modes (particularly characteristic is the band observed at 3299 cm⁻¹). These observations confirm the successful reduction of L^1 to H_2L^1 , followed by transformation of the later into the corresponding perchlorate salt.

All obtained NMR data (¹H-NMR, ¹³C-NMR, DEPT 135° and COSY), as well as the X-ray crystal structure of $[H_4L^1](ClO_4)_2$, are fully consistent with the proposed structures for the compounds (Figures S3-S8; see also assignments of ¹H-NMR and ¹³C-NMR spectra in the Experimental section). The COSY data obtained for the amine compound (Figure S8) show the characteristic splitting of the diastereotopic protons in the aliphatic region, as expected for this species.

Suitable crystals of $[H_4L^1](ClO_4)_2$ were obtained by slow diffusion of diethyl ether vapour into a methanol-ethanol solution of the compound. A summary of the crystal data, and data collection and refinement details are given in Table 1. An ORTEP diagram of the molecular structure of $[H_4L^1]^{2+}$ unit in the crystal is shown in Figure 1 and the crystal packing is presented in Figure 2a.

| Table 1. Crystal data and structure 1 | refinement parameters for $[H_4L^1](ClO_4)_2$ crystal. |
|---------------------------------------|--|
| Empirical formula | $C_{22}H_{34}Cl_2N_2O_{12}$ |
| Formula weight | 589.41 |
| Temperature (K) | 293 |

| Wavelength | Κα |
|--|------------------------------------|
| Crystal system | Monoclinic |
| Space group | P 21/c |
| Unit cell dimensions | |
| a (Å) | 5.533 (11) |
| $b(\dot{A})$ | 18.318 (4) |
| <i>c</i> (Å) | 13.798 (3) |
| α | 90.000 |
| β | 99.240 (3) |
| γ | 90.000 |
| $V(Å^3)$ | 1380.4 (5) |
| Ζ, Ζ΄ | 2, 1/2 |
| D_{calc} (Mg/m ³) | 1.699 |
| F(000) | 702 |
| θ range for data collection (°) | 2.68–25.99° |
| Index ranges | $-6 \le h \le 6, -22 \le k \le 0,$ |
| | -17 <= 1 <= 17 |
| Reflections collected | 2712 |
| Independent reflections | 1923 |
| R _i | 0.016 |
| Absorption correction | Empirical |
| Max. and min. transmission | 0.854 and 0.774 |
| Refinement method | Full matrix least-squares on |
| | \mathbf{F}^2 |
| restraints | 1 |
| $R[F^2 > 2\sigma(F^2)]$ | 0.0913 |
| $wR(F^2)$ | 0.3046 |

The X-ray crystal structure shows that the two aromatic rings of the $[H_4L^1]^{2+}$ moiety in the $[H_4L^1](ClO_4)_2$ crystal are coplanar, and the aliphatic chains are nearly perpendicular to the plane of the rings, pointing to opposite directions (Figure 2b). The conformation about the central $C_1-C_{1,3}$ bond, as well as that of the terminal hydroxyl group is *anti* (OCCO and HOCC dihedrals = 180°), while those about the C_8-N_1 , N_1-C_9 , C_9-C_{10} and $C_{10}-C_{11}$ bonds are *gauche*, *anti*, *anti* and *gauche'*, respectively (*gauche* and *gauche'* corresponding to conformation-defining dihedral angles of ~60 and – 60°, respectively). The distance between the terminal OH groups (O/O) is 14.24 Å and the longest C/C distance between carbon atoms of the aromatic rings is 14.60 Å. The crystal unit cell contains two $[H_4L^1]^{2+}$ units and four perchlorate anions (Z = 2), and an asymmetric unit with Z' equal to $\frac{1}{2}$, i.e., one perchlorate anion and half of the $[H_4L^1]^{2+}$ unit are enough to totally determine the crystal structure upon application of the symmetry figures of the crystal. The crystal packing results in short contacts between all oxygen

atoms of the perchlorate anion and hydrogen atoms from the $[H_4L^1]^{2+}$ cation, two of these corresponding to weak H-bonds of type O^{...}HN (2.597, 2.713 Å), one to a O^{...}HO H-bond (2.233 Å), and the remaining to non-classic O^{...}HC_(aromatic/aliphatic) interactions (with lengths in the 2.626-2.713 Å range). The H-bond network displays also a H-bond interaction between the nitrogen atoms and hydroxyl oxygen atoms of neighbouring molecules (2.905 Å).



Fig. 1. ORTEP graph of the $[H_4L^1]^{2+}$ moiety in the $[H_4L^1](ClO_4)_2$ crystal. The asymmetric unit is constituted by the left-half of the $[H_4L^1]^{2+}$ cation and one perchlorate anion (shown in the figure); the second half of the $[H_4L^1]^{2+}$ cation is shown for clarity.



Fig. 2. a) Packing diagram for $[H_4L^1](ClO_4)_2$ crystal, showing the unit cell axes; **b**) view of the same fragment of the crystal unit cell shown in Figure 1 but oriented to stress the relative orientations of the aromatic rings and of the aliphatic chain of the $[H_4L^1]^{2+}$ fragment, as well as the O^{...}HN H-bond established between the perchlorate anion and the nitrogen tom of the cation (right).

3.2. Fluorescence response of the amine ligand to Cu²⁺and other cations

The fluorescence response of both the imine and amine compounds in presence of various metal ions $(Ni^{2+}, Co^{2+}, Al^{3+}, Mn^{2+}, Mg^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, Ag^+, Cr^{3+} and Cu^{2+})$ was investigated in H₂O-DMF (1:1 v/v) solution. The results obtained with the imine compound were discourageous. On the other hand, for the amine ligand, its intense fluorescence emission with maximum at 309 nm (upon excitation at 274 nm) was found to be quenched at different levels in presence of the different metal ions. The addition (of 5.0 equivalents) of Ni²⁺, Co²⁺, Al³⁺, Mn²⁺, Mg²⁺ and Zn²⁺ cations to the solution of the amine ligand caused partial reduction of the fluorescence emission (20-30 %), whereas the addition of Hg²⁺, Pb²⁺, Cd²⁺, Ag⁺ and Cr³⁺ cations lead to a stronger quenching of the fluorescence (35-55 %). Noteworthy, the addition of Cu²⁺ caused an almost total quenching of the fluorescence (over 98 %) (Figure 3a).



Fig. 3. a) Fluorescence emission spectra of the H_2L^1 ligand ($\lambda_{ex.} = 274$ nm) obtained in the presence of 5.0 equivalents of different metal ions: Ni²⁺, Co²⁺, Al³⁺, Mn²⁺, Mg²⁺, Zn²⁺, Hg²⁺, Pb²⁺, Cd²⁺, Ag⁺, Cr³⁺ or Cu²⁺, in H₂O-DMF (1:1 v/v). The excitation and emission band widths were 5.0 and 10.0 nm, respectively; **b**) series of fluorescence emission spectra of H_2L^1 ($\lambda_{ex.} = 274$ nm) in H₂O-DMF (1:1, v/v; [H₂L¹] = 10.0 μ M) in presence of different concentrations of Cu²⁺ (0.0-50.0 μ M; step of 5 μ M). The arrow indicates the direction of increasing Cu²⁺ concentration (see also Figure 4).



Fig. 4. Fluorescence intensity (intensity of the fluorescence emission band of H_2L^1 at 309 nm) as a function of the Cu²⁺ concentration, in the range 0.0–50.0 μ M.

To further evaluate the affinity of the ligand towards Cu^{2+} , a fluorescence titration assay was carried out. As Figure 3b shows, the addition of successively increasing amounts of Cu^{2+} (within the 0.0–50.0 µM range) to the ligand solutions causes a gradual quenching of the emission of the ligand at 309 nm. As mentioned previously, the addition of 5.0 equivalents of Cu^{2+} causes the emission to decrease by more than 98%. Moreover, 2.5 equivalents of the cation are already enough to reduce the emission by more than 50 %. In fact, as can be seen in Figure 4, a good linear correlation is observed between the fluorescence emission intensity and the Cu^{2+} concentration.

To help us determine the stoichiometry of the complex formed between Cu^{2+} and the amine ligand, the Job plot method was used. The Job function F_{Job} is calculated from Eq. (1) [53]:

$$F_{Job} = (1-x) F_0 - F$$
 (1)

where F and F₀ are the fluorescence intensities in the presence and in the absence of Cu²⁺, respectively, *x* is the mole fraction of Cu²⁺, and the sum of the concentrations of Cu²⁺ and the ligand is kept constant (10 μ M). The Job plot (Figure 5) shows a maximum at *x* = 0.5, indicating that the stoichiometry of the complex is 1:1.



Fig. 5. Job plot for the H_2L^1/Cu^{2+} system, indicating that the complex formed between the two species has 1:1 stoichiometry. The sum of the concentrations of Cu^{2+} and H_2L^1 was maintained equal to 10 μ M.

To check the practical applicability of the ligand as a selective fluorescence sensor for Cu^{2+} , the effect of addition of other metal ions to a solution of the ligand, in the presence of Cu^{2+} (1.0 equivalent), on the ligand fluorescence was examined. As shown in Figure 6, the fluorescence quenching in the presence of Cu^{2+} is not significantly affected by the competing ions, indicating that the compound can indeed be used as a highly-selective sensitive fluorescence chemosensor for Cu^{2+} detection.



Fig. 6. Fluorescence response of H_2L^1 (L, in the legend) to various metal ions (M) in aqueous DMF (50:50, v/v). The green bar corresponds to the fluorescence intensity in absence of metal ion; blue bars correspond to the fluorescence intensity after the addition of a given metal ion (50 µM) to a 10 µM solution of H_2L^1 ; the red bars show the change of the emission which occurs with the subsequent addition of 50 µM Cu²⁺ to the solution ($\lambda_{ex.} = 274$ nm).

3.3. DFT and TD-DFT study of the Cu²⁺/H₂L¹ complexation and of the fluorescence quenching mechanism

Based on the knowledge of the 1:1 stoichiometry of the Cu^{2+}/H_2L^1 complex formed in solution, DFT calculations were carried out to investigate its geometry. With this ligand, Cu^{2+} can form six, five-, or four-coordinated complexes. Several input structures with different coordination numbers were used in the calculations and both the protonated and deprotonated states of the terminal OH groups in the complex were also considered. Irrespectively of the protonation state of the ligand after complexation, a six-coordinated metal centre, with all the functional groups of the ligand coordinated, was found to be the most stable configuration. These calculations suggest the structure presented in Figure 7 as the most probable structure for the complex formed in solution. Based on the knowledge of the geometries of analogous complexes [54,55], we suggest that the coordinated OH groups most likely will be deprotonated. Table S1 includes selected calculated structural parameters for the metal centre of the complex. The predicted Cu-N (2.163 and 2.168 Å) and Cu – O_(terminal) (1.866 and 1.872 Å) bond lengths are within the expected values for analogous Cu(II) complexes [54,55]. The Cu-O_(ether) bonds are predicted to be very weak, with bonds lengths 2.616 and 2.751 Å, in the range of those reported for the crystal structure of an analogous complex [54].



Fig. 7. Two perspectives of the DFT/U-CAM-B3LYP optimized structure proposed for the complex [CuH₂L¹].

In the Cu^{2+} complex, the ligand adopts a *syn* conformation. It is interesting to compare the energy of this conformation with that of the most stable *anti* conformer of the free ligand. To assess this aspect, single point DFT calculations were carried out for the *syn* conformation adopted by the ligand in the complex, after formal removal of the metal ion and saturation of the deprotonated oxygen atoms (only the coordinates of the H atoms used to saturate the deprotonated oxygen atoms were optimized, as indicated in the Computational Details section). These calculations showed that in the gas phase the *anti* conformation of the free ligand is 17.07 kJ/mol lower in energy compared with the *syn* conformation adopted in the complex (and 44.76 kJ/mol in water solution).

For the complex to form, this energy penalty involving the conformation of the ligand should be overcome by the stabilization occurring with the bonding between the ligand and the metal. In our previous study [56], we have indeed reported the unfavorable conformational rearrangement of an analogous ligand upon complexation. In that study, the X-ray crystal structure of a Cd^{2+} complex showed the ligand in a *syn* conformation, while theoretical DFT calculations revealed that the most stable conformation of the free ligand was the *anti* conformation. That previous study, for which we obtained the X-ray structure of the complex, supports the present conclusion that it is possible for the complex to form, in spite of having un unfavorable conformational rearrangement of the ligand. For the ligand of the present study, we believe that the formation of the complex is further facilitated by the fact that there is a *syn* conformer of the free ligand which is only 0.25 kJ/mol above the lowest energy *anti* conformer, and therefore will be present in solution in large proportion.

To obtain additional information on the fluorescence quenching mechanism occurring due to the formation of the $Cu(II)/H_2L^1$ complex, TD-DFT calculations were carried out for both the free ligand and the $Cu(II)/H_2L^1$ complex. With the exception of some rare cases, fluorescence properties are usually determined by the lowest excited state with the same multiplicity as the ground state. Figure 8 shows the

main contributions to the lowest energy electronic singlet and doublet excitations, respectively for the ligand and for the Cu(II) complex. The ligand's lowest excited singlet state involves transitions from the HOMO-3 and HOMO-2 orbitals, which have a mixture of π and *n* character, respectively to the LUMO+1 and LUMO orbitals, with π^* character. Although these transitions are parity forbidden, this state will be populated by internal conversion from higher energy excited states and the decay from the *S*₁ excited state will give rise to the fluorescence emission observed for the ligand. On the other hand, the first doublet excited state for the Cu(II)/H₂L¹ complex has a major contribution (66%) from a β-spin HOMO-1 to LUMO excitation, which involves essentially charge transfer from the ligand to the metal (d_{z^2} orbital), and therefore has a dominant ligand-to-metal charge transfer (LMCT) character (Figure 8). Additionally, Table 2 and Figure 9 show that the next low-lying excited states for the complex all have LMCT character. This type of transition provides a mechanism for the nonradiative deactivation of the excited state in the complex. This mechanism readily explains the quenching of the fluorescence. However, we cannot rule out that energy transfer from the excited ligand to the Cu(II) centre may also occur, and partially contribute to the radiationless deactivation of the excited state.



Fig. 8. Dominant contributions to the lowest energy singlet (left) and doublet (right) electronic excited sates for the H_2L^1 ligand and the [CuH₂L¹] complex, respectively, calculated at the DFT/U-CAM-B3LYP level.

| Excited | Energy | $\lambda_{calc.}^{a}$ | f^{a} | Main contributions (%) | Dominant character |
|---------|--------|-----------------------|------------------|--|---|
| state | (eV) | (nm) | | | |
| | | | | H ₂ L ¹ Ligand | |
| S_1 | 5.38 | 230 | 0.003 | H-1→L+1 (24%) + H→L (28%) | $\pi \rightarrow \pi^{*+}n \rightarrow \pi^{*}$ |
| | | | | [CuH ₂ L ¹] complex | |
| D_1 | 1.61 | 768 | 0.000 | H-1→Lβ (66%) | LMCT |
| D_2 | 1.94 | 643 | 0.006 | H→Lβ (42%) + H-12→Lβ (14%) | LMCT |
| D_3 | 2.17 | 571 | 0.009 | H-10→Lβ (31%) + H-2→Lβ (21%) | LMCT |
| D_5 | 3.20 | 388 | 0.042 | H→Lβ (53%) + H-8→Lβ (11%) | LMCT |
| D_6 | 3.51 | 353 | 0.072 | H-2→Lβ (66%) | LMCT |

| Table 2. Verti | cal excitation en | ergies, waveler | ngths, oscillato | r strengths ar | nd main co | ontributions to | the excited | states |
|----------------------------|-------------------|-----------------|------------------|----------------|------------|-----------------|-------------|--------|
| of the [CuH ₂ L | 1] complex calcu | lated at the TD | -DFT/U-CAM | -B3LYP leve | el. | | | |

^a For the complex, results are shown for the region up to 250 nm and $f \ge 0.006$. Additionally, the first singlet excited state of the ligand and first doublet excited state of the complex, both with lower *f*, are also listed.



Fig. 9. Diagram showing the main transitions contributing to the excited states of the complex $[CuH_2L^1]$.

3.4. TD-DFT analysis of the fluorescence quenching results for the Zn²⁺/H₂L¹ system

The fluorescence response experiments show that the fluorescence intensity of H_2L^1 suffers little change in the presence of various metals, such as Al(III), Co(II), Ni(II), Zn(II), etc. (Figures 3a and 6). We were

also interested in understanding the reasons for these different behaviours, compared with Cu(II). In this study, we have chosen one particular case, Zn(II), and we have carried out DFT and TD-DFT studies on the Zn(II)/H₂L¹ system to help explain the experimental results. We will assume that Zn(II) will form a complex with H₂L¹ and that this complex is analogous to the Cu(II) complex. Based on this assumption, we have optimized the structure of the Zn(II) complex and carried out TD-DFT calculations to obtain information on its vertical singlet excitation energies and character of excited states. Selected structural parameters and TD-DFT results are presented in Table S2 and Table 3 respectively. The TD-DFT results show that the weak quenching of the fluorescence in the presence of Zn(II) comes from the fact that the S_1 excited state of the Zn(II) complex has the same character and is very similar to the S_1 excited state of the free ligand (its energy is also only slightly lower in the complex) (Figures 8 and 10). This excited state of the Zn(II) complex is localized on the ligand and corresponds to a $\pi \rightarrow \pi^* + n \rightarrow \pi^*$ excitation, exactly as in the free ligand. The molecular orbitals involved in the higher energy excitations listed in Table 3 are shown in Figure S9.

| Excited | Energy | $\lambda_{\text{calc.}^{\mathbf{a}}} f^{\mathbf{a}}$ | | Main contributions (%) | Dominant character |
|---------|--------|--|-------|--|---|
| state | (eV) | (nm) | | | |
| | | | | H ₂ L ¹ Ligand | |
| S_1 | 5.38 | 230 | 0.003 | H-1→L+1 (24%) + H→L (28%) | $\pi \rightarrow \pi^{*+}n \rightarrow \pi^{*}$ |
| | | | | [ZnH ₂ L ¹] complex | |
| S_1 | 5.30 | 233 | 0.043 | H-4→L+1 (34%) + H→L+1 (22%) + | $\pi \rightarrow \pi^{*+}n \rightarrow \pi^{*}$ |
| | | | | H-5→L+1 (18%) | |
| S_2 | 5.34 | 232 | 0.038 | H-5→L (34%) + H-4→L (21%) + | $\pi \rightarrow \pi^{*+}n \rightarrow \pi^{*}$ |
| | | | | H-7→L+2 (13%) | |
| S_7 | 6.00 | 207 | 0.105 | H→L (23%) + H→L+3 (19%) + | $\pi \rightarrow \pi^{*+}n \rightarrow \pi^{*}$ |
| S_8 | 6.04 | 205 | 0.042 | H-1→L+2 (21%) + H-5→L+2 (17%) + | $\pi \rightarrow \pi^{*+}n \rightarrow \pi^{*}$ |
| | | | | H-7→L (14%) | |
| So | 6 14 | 202 | 0.026 | H-1→L+1 (78%) | $n \rightarrow \pi^*$ |

Table 3. Vertical excitation energies, wavelengths, oscillator strengths and main contributions to the excited states of the $[ZnH_2L^1]$ complex calculated at the TD-DFT/CAM-B3LYP level.

^a Results are shown for the region up to 200 nm and f \geq 0.010 for the complex. Additionally, the first singlet excited state of the ligand, with *f*=0.003, is also listed.



Fig. 10. Diagram showing the main transitions contributing to the S_1 excited state of the model considered for the [ZnH₂L¹] complex.

4. Conclusions

In this study, the syntheses and characterization of a new hexadentate Schiff base and its reduced amine derivative were reported. The amine derivative was found to be a promising highly-selective sensitive fluorescence chemosensor for the detection of Cu^{2+} .

The structures of the compounds were characterized from their infrared and NMR (^{13}C , ^{1}H -NMR, DEPT 135°, COSY) spectra, and elemental analysis. The single crystal structure for the amine derivative, in its perchlorate salt form, was also reported and discussed. Fluorescence studies were carried out and these revealed that the amine compound presents high selectivity and appropriate fluorescence response to the Cu²⁺ cation, and therefore can be considered a promising fluorescence chemosensor for the Cu²⁺ cation. This amine chemosensor shows selectivity for Cu²⁺ over twelve other metal cations, and it works at the \Box M range of concentrations. It also exhibits over 98% of fluorescence quenching in presence (of 5 equivalents) of the copper ion. DFT and TD-DFT calculations showed that LMCT transitions in the complex formed between Cu²⁺ and the amine ligand provide a pathway for the radiationless deactivation of the excited state. The theoretical results also provide a possible explanation for the weak fluorescence

quenching of the ligand in the presence of Zn(II). These results open up the possibility of using the new amine chemosensor as a fluorescent probe for applications in a vast range of fields where the analytical determination of Cu^{2+} is relevant. We also hope that this study can motivate future research, including the study of the use of this ligand as a sensor in aqueous medium in the absence of DMF, which could have impact in environmental sciences and biological applications.

Supplementary data

Figures S1, S2 and S3, showing the room temperature infrared spectra of L^1 , H_2L^1 and $[H_4L^1](ClO_4)_2$ in KBr pellet. Figures S4 to S9, presenting the NMR data for the studied imine and amine compounds. Figure S10 showing the selected molecular orbitals of the $[ZnH_2L^1]$ complex. Tables S1 and S2 presenting selected geometrical parameters for the optimized structures. CCDC 1424449 contains the supplementary crystallographic data for $[H_4L^1](ClO_4)_2$. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44)1223-336-033, or e-mail: deposit@ccdc.cam.ac.uk.

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Highlights

- New hexadentate Schiff base and its reduced amine derivative were synthesized
- Amine derivative is a promising highly-selective fluorescence chemosensor for Cu²⁺
- Sensor exhibits fluorescence quenching over 98 % in presence of the copper ion
- Sensor works at the μM range of concentrations
- LMCT transitions in the complex contribute to the quenching of the fluorescence

27

Author Statement:

Reza Golbedaghi: Conceptualization, Methodology, Investigation, Writing- Original draft preparation.

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