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Microwave-assisted synthesis of 3-hydroxy-4-pyridinone/naphthalene conjugates. Structural characterization and selection of a fluorescent ion sensor

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ABSTRACT

Two novel 3-hydroxy-4-pyridinone/naphthalene conjugates (**L1** and **L2**) with different distances between the chelating and the fluorescent moieties were synthesized using conventional heating and microwave irradiation achieving a shorter reaction time. The structure of both compounds was confirmed by X-ray crystallography, revealing that these compounds were isolated as hydrochloride salts in dihydroxypyridinium forms. In solution and in the presence of a base, the tautomeric keto forms may be obtained as it was elucidated by NMR analysis. The dihydroxypyridinium form of **L1** exhibits fluorescence at 450 nm, both in ACN and DMSO, whereas the corresponding keto form exhibits fluorescence at 365 nm. In contrast, the dihydroxypyridinium form of **L2** only fluoresces in DMSO, exhibiting a band at 340 nm, while the keto form is non-fluorescent. These distinct fluorescent behaviors reveal that the tautomeric form in which the ligands are isolated and the distance between the chelating and fluorescent functions strongly influences their fluorescence properties. Ligand **L1** exhibits better fluorescence properties and its fluorescence intensity is quenched in the presence of variable concentration of Cu^{2+}, Zn^{2+}, and Fe^{3+}, thus making it suitable to be used as ion sensor.

1. Introduction

Analytical assays based on functionalized fluorophores are ubiquitous in biotechnology, medicinal chemistry, and environmental sciences.[1,2] In these fields, the design of functionalized molecules, with variable lipophilicity, whose fluorescence properties change in the presence of target metal ions allows to study aspects of metal ion accumulation, trafficking and function or toxicity in living systems in real-time.[3] With this motivation, considerable efforts have been made, in the past few years, to synthesize new simple and easy-to-make sensors able to respond with high sensitivity and efficiency to the presence of different metal ions.

3-Hydroxy-4-pyridinones (3,4-HPO)s constitute a class of ligands with clinical application due to their high affinity towards several biological important metal ions.[4] These compounds have been widely used as chelating agents for the treatment of diseases related with iron overload, such as β-talassemia,[5] and other clinical situations related to the central nervous system injuries caused by Al(III) surplus.[6] (3,4-HPO)s also possess a high affinity for divalent transition metals, like Zn(II), Cu(II), and VO(II). In this context, Zn(II) and VO(IV) 3,4-HPO complexes are currently under study as orally active insulin enhancing drugs.[4,7] The pyridinone structure allows the synthesis of ligands with variable lipophilicity making them useful not only for pharmaceutical applications but also as extractants of metal ions from organic matrices.[4] Fluorescence techniques, particularly those making use of 3,4-HPO fluorescent probes have become increasingly important for the detection of intracellular metal ions in several diseases related with metal ion burdens.[8]

Taking into account the versatile chemistry and the potential clinical application of (3,4-HPO)s, two novel bidentate ligands were prepared both bearing a 3,4-HPO chelator moiety covalently bound to a mono-substituted naphthalene fluorescent platform. The choice of the naphthalene platform is related to its low molecular weight and biological compatibility. Indeed, many naphthalene-based fluorophores have been used to prepare fluorescent sensors[9] and other fluorescent frameworks.[10] Also, the naphthalene skeleton is present in a large number of clinical drugs, such as naphazoline, which is a potent cardiovascular agent, and naproxen, an anti-inflammatory agent.[11] Recently, other important pharmacological properties, such as potential antitumor and antiprotozoal activities were reported for other naphthalene systems.[12]

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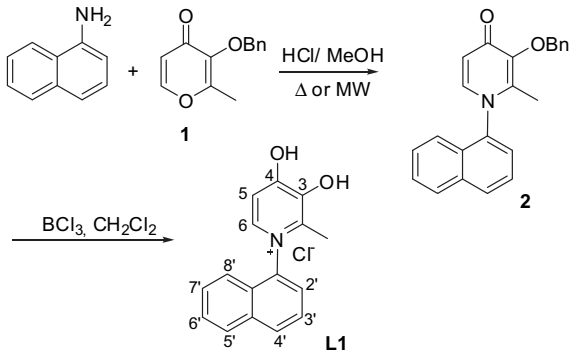
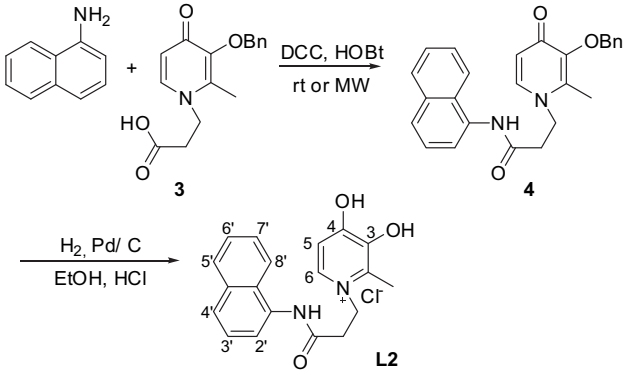
In order to understand the effect of proximity between the chelating and fluorescent functions in the fluorescence properties of naphthalene, we designed ligand **L1** (Scheme 1), which contains the chelating unit and the fluorophore directly attached through the nitrogen atom of the pyridinone ring, and ligand **L2** (Scheme 2) in which the same functions are separated by a $-CH_2CH_2CONH-$ bridge, thus generating a more flexible structure.



Scheme 1. Synthesis of hydrochloride salt of ligand **L1**.



Scheme 2. Synthesis of hydrochloride salt of ligand **L2**.

2. Results and discussion

2.1. Synthesis

Ligand **L1** was efficiently synthesized using a two sequence steps protocol (Scheme 1). First, 1-naphthylamine was allowed to react with 3-benzyloxy-2-methyl-4-pyrone **1**, in a HCl/methanol mixture under reflux (oil-bath) for 24 h, giving rise to derivative **2** in 52% yield. In recent literature reports, it has been demonstrated that many synthetic protocols, in particular protocols regarding the synthesis of organic dyes, can be significantly improved by microwave heating.[13] Taking into account the potential advantages of the direct and rapid microwave heating process, the condensation reaction was conducted using microwave heating under closed vessel conditions. Indeed, increasing the reaction temperature to 140 °C, the reaction leads to derivative **2** in only 60 min, providing a significant reduction of reaction time. Debenzylation was performed with BCl_3 in dichloromethane, under an argon atmosphere, affording the expected hydrochloride salt of ligand **L1** in 82% yield.

In order to prepare ligand **L2** (Scheme 2), the coupling reaction of 1-naphthylamine with 3-benzyloxy-1-(3′-carboxypropyl)-2-methyl-4-pyridinone **3** was carried out through in situ generation of the corresponding activated ester using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt). After stirring at room temperature for 24 h, the reaction provided naphthalene–pyridinone conjugate **4** in 31% yield. When the same reaction was conducted under microwave irradiation (60 min, 55 °C), conjugate **4** was obtained in a significant shorter period of time with similar yield. Subsequent hydrogenolysis, under hydrogen atmosphere in the presence of Pd/C (10%) and HCl, produced the hydrochloride salt of **L2**, with a 65% yield.

In principle, the (3,4-HPO)s are able to exist in two tautomeric forms (enol and keto forms).[14] In the present work, both ligands **L1** and **L2** were isolated as hydrochloride salts in dihydroxypyridinium forms and for both compounds crystal with diffractometric quality were obtained.

2.2. X-ray diffraction

Confirmation of the dihydroxypyridinium forms was obtained from X-ray diffraction data obtained from crystals of both ligands **L1** and **L2** (Figs. 1 and 2). The crystal structure of **L1** is built up from an asymmetric unit composed of one **L1** cation and one chloride anion. Molecules of **L1** are linked in to 1-D polymeric structure through O–H···Cl hydrogen bonding interactions with O···O distances of 2.914(2) and 3.040(2) Å with O–H···Cl angles of 175(4) and 154(3)°, respectively (see Fig. 1, bottom view). Also, the asymmetric unit of **L2** is composed of one molecule of **L2** and one chloride counter ion, which are assembled into a 1-D network of hydrogen bonding interactions as shown in Fig. 2. The chloride anion is bonded to two phenol groups and one N–H amide group of neighboring **L2** cations with Cl···O distances of 2.988(2) and 3.015 (2) Å. The corresponding O–H···O angles are 174(4) and 172(5)°, respectively and N–H···O angle is 177(3) Å.

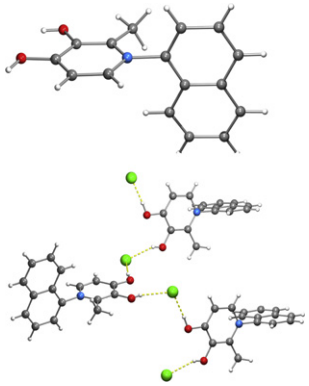


Fig. 1. X-ray single crystal structure of **L1**·**HCl** showing two different features: top, the molecular of **L1**; bottom, the assembly of **L1** molecules by O–H···Cl hydrogen bonding interactions into 1-D polymeric structure.

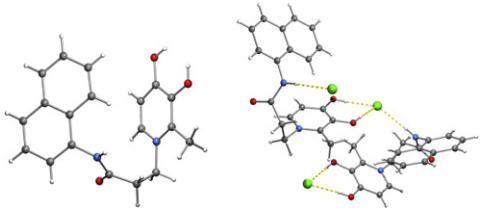


Fig. 2. X-ray single crystal structure of **L2.HCl** showing two different features: left the molecular of **L2**; right, the network of O–H···Cl and O–H···Cl hydrogen bonding interactions.

The spatial disposition of the dihydroxypyridine and naphthalene entities is different in **L1** and **L2** cations. For **L1**, the two aromatic fragments are twisted giving a dihedral angle between their planes of 77.98(5)°, which prevents electronic communication between them in solid state. For **L2**, the dihydroxypyridine intercepts the naphthalene entity at a dihedral angle of 39.43(5)°

and C–H from naphthalene points to dihydroxypyridine at a distance of 2.974(3) Å, which seems to suggest the existence of an edge to face C–H···π interaction. The average C–O quinone bond distance of 1.254(3) Å is typical of a carbonyl group while the C–O distances of 1.333(3) and 1.352(3) Å in **L1** and 1.342(3) and 1.350 (3) Å in **L2** are clearly single bond distances consistent with the presence of phenol groups, as required by the charge balance of the molecular formulas of both organic salts. X-ray single crystal structure of derivative **4** was also obtained and is supplied in Supplementary data.

2.3. Physico-chemical properties in solution

The structure of ligands **L1** and **L2** observed in solution was established by NMR analysis (^{1}H and ^{13}C, 1D, and 2D experiments, including COSY, HSQC, and HMBC spectra for unequivocal assignment of proton and carbon chemical shifts). The ^{1}H NMR spectrum of **L1** in DMSO-d_6 (Fig. 3a) exhibits two doublets at 7.55 ppm and 8.32 ppm ($^3J_{5,6\text{-H}}$=6.8 Hz), corresponding to H-5 and H-6. While H-5 shows strong HMBC correlation with a signal at 143.2 ppm assigned as C-3 (C–OH), H-6 shows HMBC correlation with a signal at 161.0 ppm assigned to C-4 (C–OH) (Fig. 3c). These results provide evidence of the presence of the dihydroxypyridinium form. In order to investigate the presence of other tautomeric forms in solution, solvents having different polarities such as acetonitrile (aprotic solvent) and methanol (protic solvent) were employed.[15] Indeed, both in acetonitrile-d_3 (exhibiting H-5 at 7.38 ppm, H-6 at 7.96 ppm; C-3 at 145.4 ppm and C-4 at 161.8 ppm) and in methanol-d_4 (exhibiting H-5 at δ 7.36 ppm, H-6 at 8.25 ppm; C-3 at 145.1 ppm and C-4 at 161.5 ppm), the dihydroxypyridinium form is the only detectable form in solution. The tautomeric keto form was only achieved when the pH was increased by adding K_2CO_3 directly in the NMR tube. This fact is clearly seen by analysis of the resonance positions of protons H-5 and H-6, which at higher pH appear at lower frequencies, 6.31 and 7.63 ppm (Fig. 3b), and by the resonance positions of carbons C-3 (C–OH) and C-4 (C=O), which appear at 145.6 and 170.4 ppm (Fig. 3d). These chemical shift values are in agreement with the chemical shifts observed for the parent 3-benzyloxy-4-pyridinone derivative **2**, where protons H-5 and H-6 appear at 6.31 and 7.62 ppm and carbons C-3 (C–OBn) and C-4 (C=O) appear at 144.4 and 172.5 ppm.

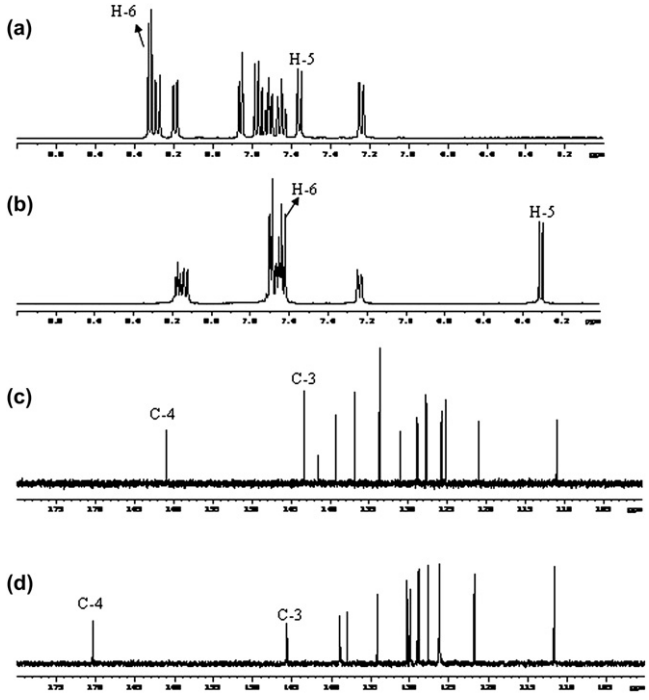
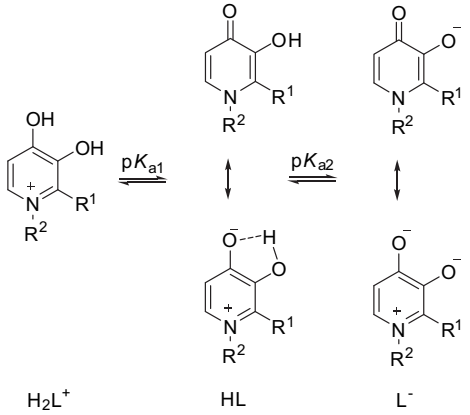


Fig. 3. Aromatic region of NMR spectra of ligand **L1**: (a) ^{1}H NMR in DMSO-d_6, (b) ^{1}H NMR in DMSO-d_6+K_2CO_3, (c) ^{13}C NMR in DMSO-d_6, and (d) ^{13}C NMR in DMSO-d_6+K_2CO_3.

Similar results were observed for ligand **L2** (see Experimental section).

Although these ligands are sparingly soluble in water, once dissolved in DMSO it is possible to prepare aqueous solutions (99% H_2O/1%DMSO) and determine the dissociation constants of **L1** and **L2** by spectrophotometric titration. Isolated as hydrochloride salts, the ligands possess two dissociable protons, which correspond to two hydroxyl groups (Scheme 3).



Scheme 3. Dissociation steps of ligands.

The values obtained for these two acidity constants are pK_{a1}=3.01±0.05 and pK_{a2}=9.61±0.01 for **L1**, and pK_{a1}=3.27±0.03 and pK_{a2}=9.86±0.9 for **L2** and the distribution diagrams as a function of pH are provided in the Supplementary data. The values are within the usual ranges observed for (3,4-HPO)s centered on 3 and 9.4. The slightly lower pK_a values obtained for **L1**, when compared to those obtained for **L2**, are probably due to the close proximity of the aromatic naphthalene skeleton to the pyridinone heterocyclic ring, which provides an electron-withdrawing effect and a concomitant decrease of the protonation constant.

The UV/visible spectra of both ligands (Fig. 4) show an intense band at ca. 290 nm, which is characteristic of the pyridinone π system. A shoulder at ca. 325 nm, assigned to the naphthylamine π system is discernible for **L1** but not for **L2** thus indicating the influence of the two spacers as observed in the X-ray data.

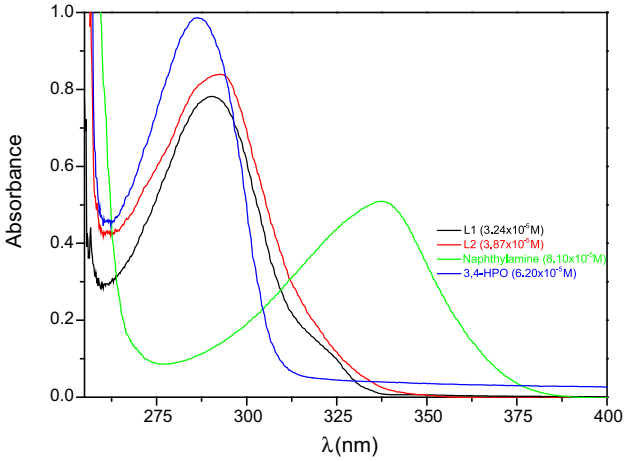


Fig. 4. UV/visible spectra of ligands **L1** and **L2** together with those of naphthylamine and a 3,4-HPO in DMSO.

As observed for most fluorophores the fluorescence properties of naphthylamine are solvent dependent as illustrated in Fig. 5. The naphthylamine fluorescence is observed at 450 nm in water, 425 nm both in methanol and ethanol, 413 nm in ACN and 427 nm in DMSO, showing that this fluorophore is fluorescent in both protic and aprotic solvents.

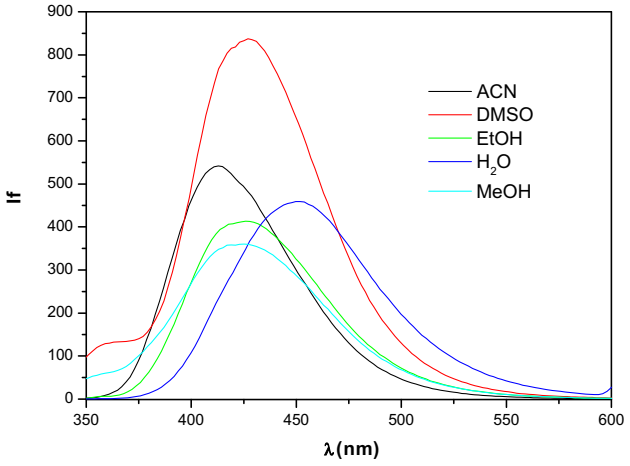


Fig. 5. Fluorescence spectra of naphthylamine in different solvents.

The fluorescence properties of the new ligands based on the naphthylamine fluorophore are also solvent dependent and significantly altered by the bound pyridinone. Ligand **L1** is fluorescent (λ_{em}=450 nm) both in ACN and DMSO but it is non-fluorescent in water, methanol and ethanol, revealing that **L1** is only fluorescent in aprotic solvents. In contrast, ligand **L2** only fluoresces in DMSO exhibiting an emission band at 340 nm. This distinct fluorescence behavior shows that the fluorescence properties are strongly dependent on the distance between the naphthalene and the pyridinone skeletons. Fluorescence spectra obtained for solutions of **L1** prepared by dissolving the dihydroxypyridinium form of the ligand in ACN are depicted in Fig. 6. The corresponding fluorescence spectra in DMSO are provided in the Supplementary data.

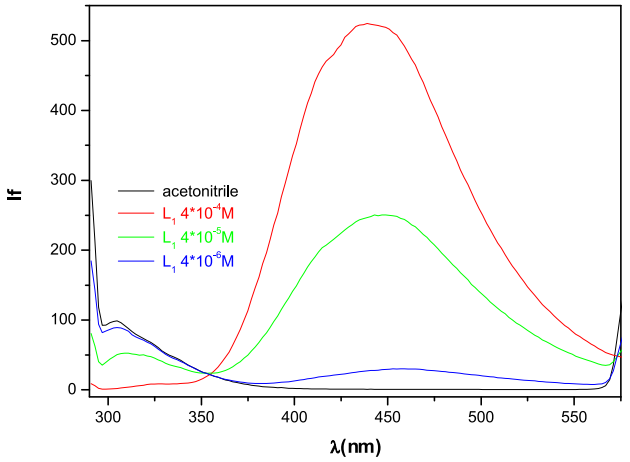


Fig. 6. Fluorescence spectra of dihydroxypyridinium form of **L1** in ACN.

The synthesized ligands show interesting fluorescence properties which depend on the keto and enol tautomeric forms. Considering that the two forms have different fluorescence behavior, the keto forms of both ligands **L1** and **L2** were isolated as described in Experimental section. In fact, the keto form of **L1** is fluorescent at 365 nm while the keto form of **L2** is non-fluorescent. Fluorescence spectra obtained for solutions of enol and keto forms of **L1** in DMSO are depicted in Fig. 7.

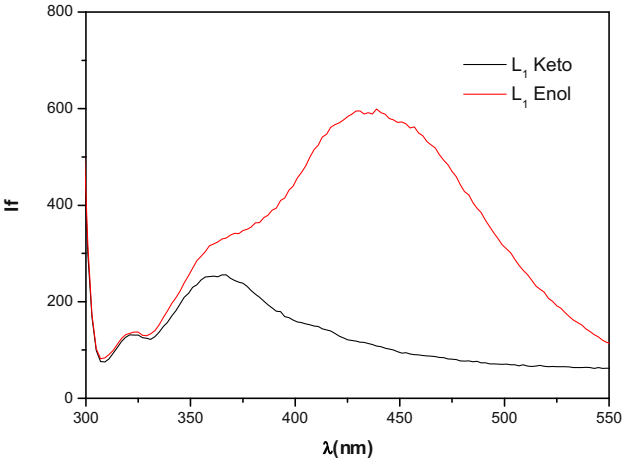


Fig. 7. Fluorescence spectra of enol and keto forms of **L1** in DMSO.

Once recognized the more interesting fluorescence properties of ligand **L1**, the fluorescence behavior of this ligand was studied in the presence of the metal ions Cu^{2+}, Zn^{2+}, and Fe^{3+} in order to evaluate its potential as ion sensor. Fluorescence intensity of **L1** was quenched upon titration of Cu^{2+}, Zn^{2+}, and Fe^{3+} (see Fig. 8 for fluorescence quenching of **L1** in the presence of Cu^{2+} and Fig. 9 for fluorescence quenching of **L1** in the presence of Cu^{2+}, Zn^{2+}, and Fe^{3+}).

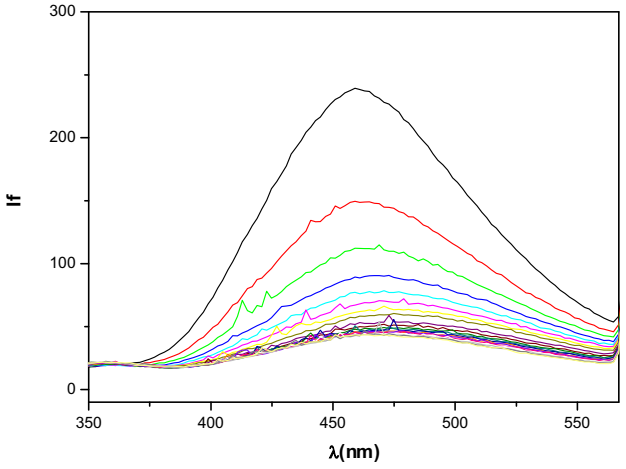


Fig. 8. Fluorescence quenching of **L1** in ACN, in the presence of Cu^{2+}.

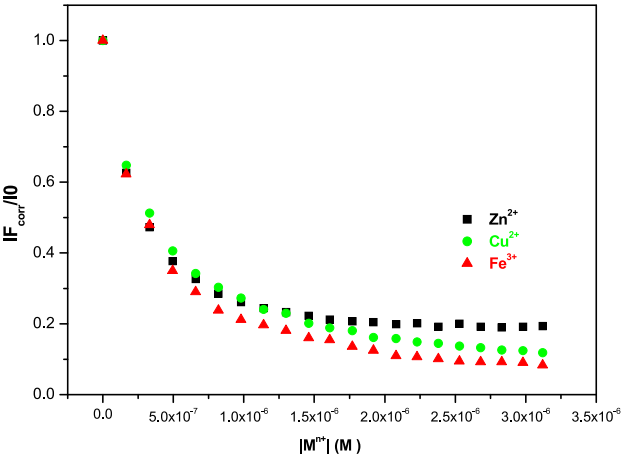


Fig. 9. Fluorescence quenching of **L1** in ACN, in the presence of Cu^{2+}, Zn^{2+}, and Fe^{3+}.

3. Conclusions

Two 3-hydroxy-4-pyridinone/naphthalene conjugates **L1** and **L2** having different distances between the chelating and fluorescent functions were conveniently synthesized using simple and easy-to-make protocols. Both conjugates were isolated in dihydroxypyridinium forms but, in solution and in the presence of a base, the tautomeric keto forms can be also obtained. The fluorescence properties of the ligands are strongly dependent on the tautomeric form in which ligands are isolated and also on the distance between the chelating and fluorescent functions. Therefore ligand **L1** was selected as the compound with more interesting fluorescence properties. In addition, the fluorescence quenching in the presence of variable concentration of Cu^{2+}, Zn^{2+}, and Fe^{3+}, demonstrates that **L1** may be used as a fluorescent ion sensor to detect and quantify those biologically important metal ions. The results obtained in this study are being explored in order to produce other fluorescent (3,4-HPO)s ligands and evaluate their fluorescence behavior.

4. Experimental section

4.1. General information

Reagents and solvents were purchased as reagent-grade, and used without further purification unless otherwise stated. NMR spectra were recorded with Bruker Avance III 400 spectrometer (400.15 MHz for ^1H and 100.63 MHz for ^{13}C). Chemical shifts (δ) are reported in parts per million and coupling constants (J) in hertz; internal standard was TMS. Unequivocal ^1H assignments were made with aid of 2D gCOSY ($^1H/^1H$), while ^{13}C assignments were made on the basis of 2D gHSQC ($^1H/^{13}C$) and gHMBC experiments (delay for long range J C/H couplings were optimized for 7 Hz). Mass spectra were acquired by Unidade De Espectrometria De Masas of Santiago de Compostela and microanalyses were acquired by Unidad De Análisis Elemental of Santiago de Compostela. Flash chromatography was carried out using silica gel Merck (230–400 mesh). Analytical TLC was performed on precoated sheets with silica gel (0.2 mm thick, Merck). Melting points were measured in a glass capillary tube on a Stuart Scientific SMP1 apparatus and are uncorrected.

4.2. Synthesis

3-Benzyloxy-2-methyl-4-pyrone (**1**) and 3-benzyloxy-1-(3′-carboxypropyl)-2-methyl-4-pyridinone (**3**) were prepared as described in literature.[16]

4.3. 3-Benzyloxy-2-methyl-1-naphthyl-4-pyridinone (2)

(*a*) *Using oil-bath reflux*: To a mixture of 3-benzyloxy-2-methyl-4-pyrone **1** (0.51 g, 2.40 mmol), 1-naphthylamine (0.51 g, 3.57 mmol, 1.5 equiv), and methanol (2 mL) was added a 0.2 M solution of HCl (10 mL) and the resulting solution was refluxed for 24 h. After that time, the reaction mixture was neutralized with a solution of NaOH 5% and it was extracted twice with chloroform. The organic layer was washed with water and then dried over anhydrous Na_2SO_4. Upon evaporation of the solvent, the residue was purified by flash chromatography using chloroform as eluent to remove a small amount of unchanged starting material, followed by a mixture of chloroform/methanol (95:5) to give **2**. Compound **2** was further crystallized in chloroform/hexane to give a pale violet solid (0.42 g, 52%). (b) *Using microwave irradiation*: A mixture of 3-benzyloxy-2-methyl-4-pyrone **1** (25.0 mg, 0.12 mmol), 1-naphthylamine (24.8, 0.17 mmol, 1.5 equiv), methanol (0.1 mL), and a 0.2 M solution of HCl (0.5 mL) was placed in a 10 mL reaction vial, which was then sealed and placed in the cavity of a CEM microwave reactor. The reaction was irradiated at 140 °C (1 min ramp to 140 °C and 60 min hold at 140 °C, using 150 W maximum power). Using a similar work-up to that described above, compound **2** was obtained in 19.6 mg (50% of yield). [Found: C, 79.21; N, 4.35; H, 5.58. $C_{23}H_{19}NO_2\cdot 1/2H_2O$ requires C, 78.84; N, 4.00; H, 5.75%]; δ_H (400 MHz; DMSO-d_6) 1.58 (3H, s, CH_3), 5.10 (1H, d, J 11.0 Hz, CH_2C$_6$H$_5$), 5.27 (1H, d, J 11.0 Hz, CH_2C$_6$H$_5$), 6.31 (1H, d, J 7.6 Hz, 5-H), 7.01 (1H, dd, J 8.4 and J 1.0 Hz, 2′-H), 7.32–7.43 (5H, m, CH$_2$C$_6$H$_5$), 7.58–7.68 (4H, m, naph-H), 7.62 (1H, d, J 7.6 Hz, 6-H), 8.10 and 8.13 (2H, 2dd, J 7.4 and J 1.7 Hz, naph-H). δ_C (100 MHz; DMSO-d_6) 13.1 (CH_3), 71.6 (CH_2), 116.1 (C-5), 121.1 (C-2′), 125.5, 125.7, 127.1, 127.9, 128.1, 128.2, 128.5, 128.8, 129.3, 129.8, 133.6, 137.3, 137.4, 140.0 (C-6), 141.3 (C-2), 144.4 (C-3), 172.5 (C-4). m/z (MS, FAB) 342 $(M+H)^+$.

4.4. Synthesis of 3-hydroxy-2-methyl-1-naphthyl-4-pyridinone hydrochloride (L1)

A 1 M solution of boron trichloride in dichloromethane (9.4 mL) was dropped slowly into an ice-bath-cooled suspension of **2** (0.80 g, 2.35 mmol) in dry dichloromethane (47 mL), under an argon atmosphere. The mixture was stirred at room temperature for 5 h. Methanol (15 mL) was added to quench the reaction. After removal of the solvent in vacuum, the residue was precipitated with methanol/acetone to afford the hydrochloride salt of **L1** (0.55 g, 82%) as a pale violet solid. *Dihydroxypyridinium form* mp 267–270 °C. [Found: C, 66.66; N, 4.90; H, 4.77. $C_{16}H_{13}NO_2\cdot HCl$ requires C 66.79, N 4.90, H 4.87%]; δ_H(400 MHz; DMSO-d_6) 2.05 (3H, s, CH_3), 7.24 (1H, dd, J 7.8 and J 1.2 Hz, 2′-H), 7.55 (1H, d, J 6.8 Hz, 5-H), 7.65 (1H, dt, J 7.8 and J 1.2 Hz, naph-H), 7.72 (1H, dt, J 7.8 and J 1.2 Hz, naph-H), 7.77 (1H, t, J 7.8 Hz, naph-H), 7.86 (1H, dd, J 7.8 and J 1.2 Hz, naph-H), 8.19 and 8.28 (2H, d, J 7.8 Hz, naph-H), 8.32 (1H, d, J 6.8 Hz, 6-H), 9.77–11.75 (2H, br, OH). δ_C (100 MHz; DMSO-d_6) 13.8 (CH_3), 111.0 (C-5), 121.0 (C-2′), 125.1, 125.7, 127.6, 127.8, 128.7, 128.9, 131.0, 133.6, 136.8, 139.2 (C-6), 141.5 (C-2), 143.2 (C-3), 161.0 (C-4). m/z (MS, FAB) 252 $(M+H)^+$.

The hydrochloride salt of **L1** (50 mg; 0.174 mmol) was dissolved in a (10:1) mixture of chloroform/methanol and washed twice with a saturated aqueous solution of Na_2CO_3. Then the organic layer was extracted with chloroform and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuum and the resulting residue was crystallized in chloroform/hexane to give quantitatively **L1** in keto form. *Keto form* mp 240–243 °C. [Found: C, 74.76; N, 5.24; H, 5.01. $C_{16}H_{13}NO_2\cdot 1/3H_2O$ requires C, 74.69; N, 5.44; H, 5.35%] calcd δ_H (400 MHz; DMSO-d_6) 1.80 (3H, s, CH_3), 6.31 (1H, d, J 7.2 Hz, 5-H), 7.24 (1H, dd, J 8.0 and J 1.6 Hz, 2′-H), 7.63 (1H, d, J 7.2 Hz, 6-H), 7.63–7.72 (4H, m, naph-H), 8.12–8.19 (2H, m, naph-H). δ_C (100 MHz; DMSO-d_6) 13.0 (CH_3), 111.7 (C-5), 121.8 (C-2′), 126.2, 126.3, 127.7, 128.8, 129.1, 129.97, 129.98, 130.4, 134.2, 138.0 (C-2), 138.9 (C-6), 145.6 (C-3), 170.4 (C-4).

4.5. Synthesis of 1-(*N*-naphthylcarbamoylpropyl)-3-benzyloxy-2-methyl-4-pyridinone (4)

(a) *using standard conditions*: A mixture of 3-benzyloxy-1-(3′-carboxypropyl)-2-methyl-4-pyridinone **3** (1.39 g, 4.84 mmol, 1.2 equiv), DCC (1.08 g, 5.24 mmol, 1.3 equiv), HOBt (0.71 g, 5.24 mmol, 1.3 equiv), and dry DMF (35 mL) was stirred at room temperature, under an argon atmosphere, during 30 min. After that time, 1-naphthylamine (0.58 g, 4.03 mmol) was added and the resulting reaction mixture was allowed to stir at room temperature for 24 h. Upon filtration and removal of the solvent in vacuo, the residue was purified by flash chromatography using as eluent a mixture of chloroform/methanol (95:5) to afford **4** (512 mg, 31%) as a white solid. (b) *Using microwave irradiation*: A mixture of 3-benzyloxy-1-(3′-carboxypropyl)-2-methyl-4-pyridinone **3** (63.1 mg,

0.22 mmol, 1.2 equiv), DCC (49.1 mg, 0.24 mmol, 1.3 equiv), HOBt (32.1 mg, 0.24 mmol, 1.3 equiv), 1-naphthylamine (26.2 mg, 0.18 mmol), and DMA (2 mL) was placed in a 10 mL reaction vial, which was then closed under an argon atmosphere and placed in the cavity of a CEM microwave reactor. The reaction vial was irradiated at 55 °C (1 min ramp to 55 °C and 60 min hold at 55 °C, using 100 W maximum power). The reaction mixture was then purified by flash chromatography using a 95:5 mixture of chloroform/methanol as eluent to give **4** (22.6 mg, 30% yield). [Found: C, 73.29; N, 6.84; H 6.30. $C_{26}H_{24}N_2O_3 \cdot 2/3H_2O$ requires C, 73.57; N, 6.60; H 6.02%]; δ_H (400 MHz; DMSO-d_6) 2.31 (3H, s, CH_3), 2.92 (2H, t, *J* 6.8 Hz, CH_2CH_2CONH), 4.27 (2H, t, *J* 6.8 Hz, CH_2CH_2CONH), 5.03 (2H, s, $CH_2C_6H_5$), 6.17 (1H, d, *J* 7.6 Hz, 5-H), 7.29–7.38 and 7.41–7.44 (5H, 2m, $CH_2C_6H_5$), 7.47–5.56 (3H, m, naph-H), 7.62 (1H, d, *J* 7.2 Hz, naph-H), 7.63 (1H, d, *J* 7.6 Hz, 6-H), 7.77 (1H, d, *J* 8.0 Hz, naph-H), 7.91–7.95 (2H, m, naph-H), 10.08 (1H, s, NH). δ_C (100 MHz; DMSO-d_6) 12.0 (CH_3), 36.6 (CH_2CH_2CONH), 49.3 (CH_2CH_2CONH), 71.9 ($CH_2C_6H_5$), 116.2 (C-5), 121.9, 122.7, 125.5, 125.6, 125.9, 126.1, 127.7, 127.8, 128.2, 128.28, 128.34, 133.2, 133.7, 137.9, 139.7 (C-6), 140.7 (C-2), 145.5 (C-3), 168.8 (CH_2CH_2CONH), 171.0 (C-4). *m*/*z* (MS, FAB) 413 $[M+H]^+$.

4.6. Synthesis of 1-(*N*-naphthylcarbamoylpropyl)-3-hydroxy-2-methyl-4-pyridinone hydrochloride (L2)

A mixture of **4** (0.30 g, 0.73 mmol) and a catalytic amount of 10% Pd/C (w/w) in ethanol (10 mL) and HCl (0.05 mL) was stirred under a hydrogen atmosphere at room temperature for 5 h. The reaction mixture was filtered through Celite and the solvent evaporated in vacuum to give the crude product. The resulting residue was crystallized in methanol/acetone to give the hydrochloride salt of **L2** (0.17 g, 65%) as a white powder. *Dihydroxypyridinium form* mp 224–226 °C. [Found: C, 63.35; N, 7.87; H, 5.14. $C_{19}H_{18}N_2O_3 \cdot HCl$ requires C 63.60, N 7.81, H 5.34%]; δ_H (400 MHz; DMSO-d_6) 2.65 (3H, s, CH_3), 3.16 (2H, t, *J* 6.7 Hz, CH_2CH_2CONH), 4.72 (2H, t, *J* 6.7 Hz, CH_2CH_2CONH), 7.34 (1H, d, *J* 7.0 Hz, 5-H), 7.47–7.55 (3H, m, naph-H), 7.60 (1H, d, *J* 7.2 Hz, naph-H), 7.77 (1H, d, *J* 8.2 Hz, naph-H), 7.86–7.94 (2H, m, naph-H), 8.28 (1H, d, *J* 7.0 Hz, 6-H), 9.57–11.42 (2H, br, OH), 10.25 (1H, s, NH). δ_C (100 MHz; DMSO-d_6) 14.0 (CH_3), 37.2 (CH_2CH_2CONH), 53.9 (CH_2CH_2CONH), 112.0 (C-5), 123.3, 124.0, 126.87, 126.93, 127.2, 127.4, 129.1, 129.5, 134.4, 135.0, 140.0 (C-6), 143.0 (C-2), 144.4 (C-3), 160.3 (C-4), 169.7 (CH_2CH_2CONH). *m*/*z* (MS, FAB) 323 $[M+H]^+$.

The hydrochloride salt of **L2** (100 mg; 0.28 mmol) was dissolved in a (10:1) mixture of chloroform/methanol and washed twice with a saturated aqueous solution of Na_2CO_3. Then the organic layer was extracted with chloroform and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuum and the resulting residue was crystallized in chloroform/hexane to give quantitatively **L2** in keto form. *Keto form* mp 198–200 °C δ_H (400 MHz; DMSO-d_6) 2.41 (3H, s, CH_3), 3.99 (2H, t, *J* 6.8 Hz, CH_2CH_2CONH), 4.35 (2H, t, *J* 6.8 Hz, CH_2CH_2CONH), 6.23 (1H, d, *J* 7.2 Hz, 5-H), 7.47–7.56 (3H, m, naph-H), 7.60 (1H, d, *J* 7.2 Hz, naph-H), 7.65 (1H, d, *J* 7.2 Hz, 6-H), 7.77 (1H, d, *J* 8.4 Hz, naph-H), 7.89–7.94 (2H, m, naph-H), 10.18 (1H, s, NH). δ_C (100 MHz; DMSO-d_6) 11.5 (CH_3), 36.6 (CH_2CH_2CONH), 49.5 (CH_2CH_2CONH), 110.6 (C-5), 121.9, 122.8, 125.5, 125.8, 126.0, 127.7, 128.0, 133.1, 133.6, 137.9 (C-6), 145.3 (C-3), 168.8 (C-4 and CH_2CH_2CONH).

4.7. Spectrophotometric determination of acidity constants

Electronic absorption spectra were recorded with a Varian Cary bio50 spectrophotometer, equipped with a Varian Cary single cell Peltier accessory, using quartz cells with 1 cm path length, thermo stated at 25 °C. Stock solutions of the ligands were prepared by dissolution in DMSO. Acidity constants of both ligands were obtained in aqueous solution by dilution of the right amount of the ligands solution in water (% DMSO less than 1%) and with ionic strength 0.1 M in NaCl. Spectrophotometric pH titrations were performed in stock aqueous solutions of the ligands ($\sim 2.5\times10^{-5}$ M), and aliquots of strong acid or base were added to adjust pH to the desired value. Calculations were performed with the program PHAB using data sets of at least three independent experiments. A typical experiment includes more than 10 solutions in which 10 different pH values have been fixed.

4.8. Fluorescence spectroscopy

Steady-state fluorescence measurements were carried out in a Varian spectrofluorometer, model Cary Eclipse, equipped with a constant-temperature cell holder (Peltier single cell holder). Stock solutions of the compounds were obtained by dissolution in DMSO. All solutions were prepared by dilution of the right amount of the compound solution in the solvent (% DMSO less than 1%). All spectra were recorded with a λ_{exc}=290 nm and emission between 300 and 550 nm for the ligands **L1** and **L2** and λ_{exc}=325 nm and emission between 350 and 550 nm for the naphthylamine. The slit width values used for excitation and emission were 10 nm for **L1** and **L2** in DMSO and 5 nm for **L1** and **L2** in ACN. Fluorescence quenching studies were achieved by successive addition of a constant volume (10 μl) of nitrate metal solutions to the cuvette (final concentration range $0.0-1\times10^{-5}$ M) containing a constant amount of ligand ($\sim 10^{-6}$ M).

4.9. X-ray crystallography

Crystals of **L1** and **L2** with suitable quality for single crystal X-ray determination were grown up from chloroform/methanol solution.

Crystal data of **L1**: $C_{16}H_{14}NO_2Cl$, *M*=287.73, orthorhombic, space group *Pbcn*, *Z*=8, *a*=8.7433(2), *b*=13.1065(4), *c*=24.0925(7) Å, *V*=2760.86(13) Å^3, ρ(calcd)=1.384 Mg m^{-3}, μ=0.277 mm^{-1}. 25,774 reflections were collected and subsequently merged to 3372 unique reflections with a R_{int} of 0.0509. The final refinement of 190 parameters converged to final *R* and R_w indices R_1=0.0516 and wR_2=0.1006 for 2374 reflections with $I>2\sigma(I)$ and R_1=0.0870, and wR_2=0.1116 for all *hkl* data.

Crystal data of **L2**: $C_{19}H_{19}N_2O_3Cl$, *M*=358.81, monoclinic, space group $P2_1$, *Z*=2, *a*=8.2793(5), *b*=7.1661(5), *c*=14.3206(9) Å, *V*=846.69(9) Å^3, β=94.782 (4)°, ρ(calcd)=1.407 Mg m^{-3}, μ=0.247 mm^{-1}. 10,581 reflections were collected, and subsequently merged to 4335 unique reflections with a R_{int} of 0.0264. The final refinement of 237 parameters converged to final *R* and R_w indices R_1=0.0535 and wR_2=0.1331 for 3943 reflections with $I>2\sigma(I)$ and R_1=0.0601, and wR_2=0.1383 for all *hkl* data.

The X-ray data were collected on a CCD Bruker APEX II at 150(2) K using graphite monochromatized Mo Kα radiation (λ=0.71073 Å). The crystals of **L1** and **L2** were positioned at 35 mm from the CCD and the spots were measured using a counting time of 60 s. Data reduction including a multi-scan absorption correction were carried out using the SAINT-NT from Bruker AXS. The structures were solved by direct methods using SHELXS-97.[17] and refined through full-matrix least squares with SHELXL-97.[18] Anisotropic thermal parameters were used for all non-hydrogen atoms. The C–H hydrogen atoms were introduced in the refinement at calculated positions giving thermal parameters equivalent 1.2 times those of the atom to which were attached. The N–H and O–H hydrogen positions in **L1** and **L2** were found from the last Fourier maps and they were included in the structure refinement with individual isotropic temperature factors. Molecular diagrams were drawn with PLATON.[19] CCDC 753940 for **L1**, CCDC 753939 for **L2** and CCDC 753941 for derivative **4** contain 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/datarequest/cif.

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Supplementary data

The supplementary data contains selected NMR spectra (^{1}H, ^{13}C, COSY, HSQC, and HMBC) of derivative **2** and ligand **L1**, X-ray single crystal structure of derivative **4**, distribution diagrams as a function of pH of both ligands **L1** and **L2** and the fluorescence spectra of **L1** in DMSO. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.08.065.

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