



# Design of a water soluble 1,8-naphthalimide/3-hydroxy-4-pyridinone conjugate: Investigation of its spectroscopic properties at variable pH and in the presence of $\text{Fe}^{3+}$ , $\text{Cu}^{2+}$ and $\text{Zn}^{2+}$



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## ABSTRACT

The synthesis and sensing properties of a new fluorescent probe designed to have a 4-amino-1,8-naphthalimide fluorescent platform functionalized with a 3-hydroxy-4-pyridinone bidentate chelating unit at the 4-position and a terminal aliphatic dimethylamino group at the imide site, are reported. The absorption and fluorescence properties of the ligand were investigated in DMSO and in aqueous solution at variable pH and in the presence of increasing concentration of  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ .

Analysis of the UV–Vis spectra at variable pH allowed the determination of three  $\text{p}K_a$  values ( $\text{p}K_{a1} = 3.19$ ,  $\text{p}K_{a2} = 8.38$ ,  $\text{p}K_{a3} = 9.95$ ) and establishment of the corresponding speciation diagram. Fluorescence spectra obtained in the same conditions show that the fluorescence intensity of the probe decreases with increasing pH and are off above pH 9 as a result of photo-induced electron transfer arising from the aliphatic dimethylamino group. Under physiological pH conditions, the probe shows an absorption band centred at 439 nm and emits in the green at  $\lambda = 536$  nm.

Analysis of UV–Vis and EPR spectra of the ligand in the presence of  $\text{Fe}^{3+}$  and  $\text{Cu}^{2+}$  is consistent with the formation of the corresponding metal ion complexes. The fluorescence intensity of the ligand is quenched in the presence of variable concentrations of  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  and under physiological pH conditions the fluorescence of the probe is ca 92%, 88% and 91% quenched in the presence of  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  respectively.

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## 1. Introduction

Since the early reports by Tsien and co-workers regarding fluorescent probes for calcium [1,2], the design of highly efficient fluorescent probes for sensing and monitoring various chemical species has received an increasing interest from the scientific and industrial communities. Indeed, many fluorescent probes have been developed for different applications including probes sensitive to pH [3], to metal ions [4], to monitor enzymatic activity [5], reactive oxygen and nitrogen species (ROS and RNS) [6] and many other analytes [7].

The most general design concept of fluorescent metal ion probes involves the use of the photoinduced electron transfer (PET) model

based on the covalent linkage of a single or branched chelating architecture and a fluorophore unit. The covalent linkage which separates the two units is typically a short aliphatic spacer that minimizes any ground-state interactions. In these probes, the interaction with the analyte will cause significant changes in the emission spectra accompanied by no, or minor, changes in the absorption spectra of the probes [8].

Originally detailed by de Silva et al. [9a,b] and Czarnik et al. [9c,d], this process has been widely used in the construction of many fluorescent probes, with special emphasis for 4-amino-1,8-naphthalimide based fluorescent probes [10].

4-Amino-1,8-naphthalimides are fluorophores that exhibit bands with an ICT (intramolecular charge transfer) character caused by the electronic conjugation of the electron donating amine and the electron withdrawing imide (Fig. 1) [11]. The ICT character gives rise to a large excited-state dipole and broad absorption and emission bands typically centred at ca 450 and 550 nm, respectively, when recorded

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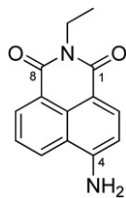


Fig. 1. 4-Amino-1,8-naphthalimide structure.

in water [10]. The spectroscopic properties of 4-amino-1,8-naphthalimides are also highly solvent dependent, noting that polar protic solvents stabilize the ICT character more than apolar solvents. The compounds have fairly simple structures for which facile and straightforward synthetic methods have been established and their structure allows changes on either the amine function in the 4-position of the aromatic naphthalene moiety, or at the imide nitrogen atom [12]. Consequently, the 4-amino-1,8-naphthalimides have been extensively used as strongly absorbing and colourful fluorophores in the design of fluorescent probes, particularly for sensing biologically relevant metal ions, such as  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  [13], and heavy metal ions, like  $\text{Hg}^{2+}$  and  $\text{Pd}^{2+}$  [14].

In most examples, 4-bromo-1,8-naphthalimide, which is a very weak fluorescent compound, has been used as precursor to reach a variety of metal sensing fluorescent probes based on the 4-amino-1,8-naphthalimide platform. These fluorescent probes have been synthesized using mainly two synthetic strategies: (i) direct nucleophilic displacement of the bromine atom of the 4-bromo-1,8-naphthalimide with a functional amine or (ii) initial reaction with a linker and subsequent introduction of the functional moiety. These synthetic approaches have been chosen for the design of many metal sensing fluorescent probes where, in most instances, the nitrogen atom introduced in position 4 of the naphthalimide molecule can be involved in metal ion coordination [15].

Hydroxypyridinones are an important class of *N*-heterocyclic bidentate chelators which show high affinity towards several biological important metal ions, such as Fe(III) [16]. This type of chelator, which include analogues of 1,2-hydroxypyridinone (1,2-HPO), 2,3-hydroxypyridinone (2,3-HPO) and 3,4-hydroxypyridinone (3,4-HPO), have been widely used as decoration agents for the medical treatment of iron overload associated with several diseases [17]. Also some hydroxypyridonate complexes have been applied as Fe(III) chelators for reducing iron toxicity [18].

Among the different types of hydroxypyridinones, the 3,4-HPO class is particularly attractive for pharmaceutical purposes since their structure allows tailoring of their hydrophilic/lipophilic balance (HLB) without significantly changing its chelating properties [19]. Variations in HLB can be achieved by simply introducing appropriate substituents on the endocyclic nitrogen atom of the pyridinone ring, the 3-hydroxy-4-pyridinones are synthesized by the reaction of 3-hydroxy-4-pyrones with primary amines, thus leading to the optimal lipophilicity for delivery or removal of metal ions in the body [19].

In recent years, many 3,4-HPOs modified with fluorescent platforms, like coumarin or fluorescein, have been developed by the Hider's laboratory. The latter probes have shown to be selective fluorescence quenchers for  $\text{Fe}^{3+}$  up to 90% and found application in the determination of cellular iron concentrations [20]. Lately, Callan et al. summarized the development of various molecular and supramolecular fluorescent sensors that have been successfully used for detection of  $\text{Fe}^{3+}$ , many of them based on 3,4-HPO derivatives [21].

Our group has long been interested in the synthesis and solution properties of 3,4-HPO ligands for biomedical applications [19,22]

and more recently on the design of metal ion sensors [23]. Lately, we prepared two bidentate ligands bearing a 3,4-HPO chelator moiety and a naphthalene fluorescent platform that can be used to sense metal ions in organic solvents but due to their very low solubility and lack of fluorescent properties in water are not usable to monitor metal ions in aqueous media [23a].

In the present work we report the design and properties of a new 3,4-HPO probe with high solubility in water and suitable fluorescence properties in aqueous solution. In order to achieve our purpose and in view of the well-known properties of 1,8-naphthalimide derivatives we coupled a 3,4-HPO bidentate chelating unit with the 4-amino-1,8-naphthalimide platform (Fig. 2). The chelator backbone is connected to the fluorophore through the *ortho* position relative to one of the chelating oxygen atoms of the 3,4-HPO moiety, thus providing a more rigid structure and a close proximity between the fluorophore and the binding unit. The 1,8-naphthalimide platform was modified at the imide nitrogen atom in order to accommodate a terminal aliphatic amino group that increases solubility in a pH range determined by the  $\text{pK}_a$  of the amino group. To the best of our knowledge this is the first report of a molecule conjugating the 1,8-naphthalimide platform with a 3-hydroxy-4-pyridinone chelator.

## 2. Experimental

### 2.1. Materials and instrumentation

Chemicals were obtained from Sigma–Aldrich (grade puriss, p.a.) and were used as received unless otherwise specified.

Nuclear magnetic resonance spectra ( $^1\text{H}$  and  $^{13}\text{C}$  NMR) were measured on a Bruker III Avance 400 spectrometer operating at 400.15 MHz and 100.62 MHz for  $^1\text{H}$  and  $^{13}\text{C}$  spectra, respectively. Chemical shifts ( $\delta$ ) are reported in parts per million and coupling constants ( $J$ ) in hertz; internal standard was TMS. Unequivocal  $^1\text{H}$  assignments were made with aid of 2D gCOSY ( $^1\text{H}/^1\text{H}$ ), while  $^{13}\text{C}$  assignments were made on the basis of 2D gHSQC ( $^1\text{H}/^{13}\text{C}$ ) and gHMBC experiments (delay for long range  $J$  C/H couplings were optimized for 7 Hz). Mass spectrometry analysis was performed at the University of Santiago de Compostela (Spain). Melting points were measured in a glass capillary tube on a Stuart Scientific SMP1 apparatus and are uncorrected. FTIR spectra were obtained with a Perkin Elmer FT-IR System (Spectrum BX) with ATR (Attenuated Total Reflectance).

## 3. Synthesis

### 3.1. Synthesis of *N*-(dimethylamino)ethyl-4-bromo-1,8-naphthalimide (3)

Following reported procedures [40], a mixture of 4-bromo-1,8-naphthalic anhydride **1** (0.10 g, 0.36 mmol), *N,N*-dimethylethylenediamine **2** (0.05 mL, 0.43 mmol) and ethanol (1 mL) was heated under reflux for 4 h. After cooling to room temperature (RT), the solid that formed was filtered, washed with water and dried to yield quantitatively naphthalimide **3**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 2.35 (s, 6H,  $\text{N}(\text{CH}_3)_2\text{CH}_2\text{CH}_2-$ ), 2.65 (t, 2H,  $J$  7.2 Hz,  $\text{N}(\text{CH}_3)_2\text{CH}_2\text{CH}_2-$ ), 4.32 (t, 2H,  $J$

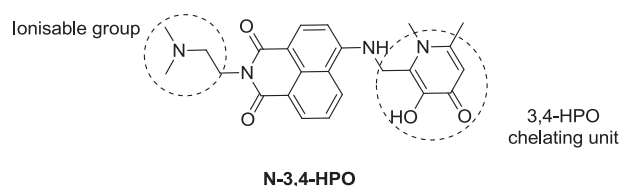


Fig. 2. 1,8-Naphthalimide modified 3,4-HPO (N-3,4-HPO).

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