

Synthesis and Characterization of a Dansyl-Based Fluorescent Probe for Analytical Purposes †

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Abstract: With the aim of investigating the development of an easily synthesized dansyl-based fluorescent probe for detecting heavy metal-based nanomaterials in aqueous solution, we have synthesized and characterized a Schiff base ligand, which we have named H₃L. This derives from the reaction of 4-formyl-3-hydroxybenzoic acid with N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide. H₃L incorporates a suitable O,N-binding domain that can bind heavy metal ions at the surface of the nanoparticles.

Keywords: schiff base; fluorescent probes; NMR spectroscopy; UV-Vis spectroscopy

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1. Introduction

Human and environmental health risks of heavy metal-based nanomaterials are demanding affordable and reliable techniques for their detection [1–4]. This would increase safety related to the handling and release into the environment of metal-based nanomaterials. Due to their selectivity, sensitivity and simplicity, chemical sensors such as fluorescent probes are very promising methods to be used not only for detection, but for quantification as well. Some optical sensors have been developed for detection of Ag NPs [5–7], but investigation of fluorescent sensors to detect and quantify CuO NPs remains virtually unexplored. Recently, we have explored the chelating potential of two easily synthesized Schiff base for CuO NPs sensing [8,9]. Now, we have chosen for our investigation the Schiff base H₃L, which derives from the reaction of 2-(aminomethyl)aniline with tosyl chloride and 4-formyl-3-hydroxybenzoic acid (Figure 1).

2. Results and Discussion

H₃L can be obtained in a quick two-step synthesis. In the first step, 2-(aminomethyl)aniline reacts selectively through the aminomethyl group with dansyl chloride to form N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide. Subsequent nucleophilic addition of N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide to 4-formyl-3-hydroxybenzoic acid yields the desired Schiff base ligand H₃L (Figure 1). The obtaining of H₃L has been demonstrated using FT-IR, UV-Vis and NMR spectroscopies.

The most characteristic infrared bands of H₃L, such as ν N-H, ν C=O, ν C=N, ν_{as} OSO and ν_s OSO have been highlighted in Figure 2, top. The infrared spectrum of H₃L exhibits

unequivocally two new strong sharp bands at about 1613 and 1686 cm^{-1} , which are attributable to the formation of the imino group, and to the presence of the carboxylic acid group, respectively. Infrared spectroscopy evidenced also the absence of three bands at about 1632, 3383 and 3472 cm^{-1} , which were attributed to δ HNH, ν_s HNH and ν_{as} HNH modes, respectively in the spectrum of N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide (Figure 2, bottom). Consequently, infrared spectroscopy provides strong evidence of the condensation of 4-formyl-3-hydroxybenzoic acid with N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide through its non-dansylated amino group.

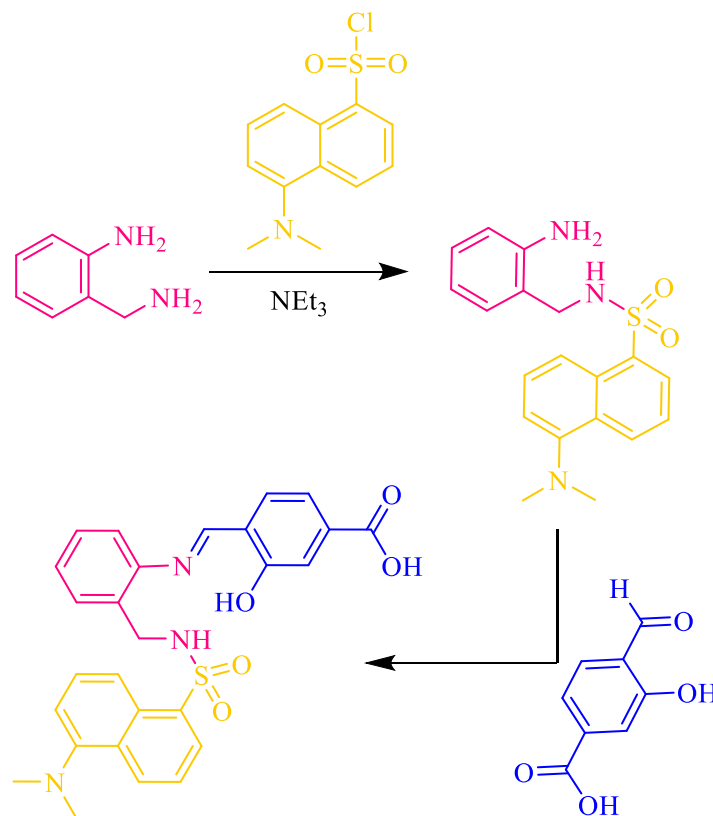


Figure 1. Schematic representation of the synthesis of H₃L in two steps: (i) selective dansylation of a primary diamine and (ii) aldiminic condensation of N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide with 4-formyl-3-hydroxybenzoic acid.

Figure 3 shows the ^1H NMR spectra of H₃L (top) and N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide (bottom), with signal assignment on a 3D model of each molecule. The most remarkable observation in the ^1H NMR spectrum of H₃L is the presence of the imine proton at about 8.0 ppm (singlet) and the disappearance at 3.9 ppm of the amino group of N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide. This is a clear evidence of the condensation of N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide with 4-formyl-3-hydroxybenzoic acid yielding the desired Schiff base ligand H₃L. Thus, NMR spectroscopy supported the obtaining of H₃L. The most remarkable signals in the ^1H NMR spectrum of N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide are the corresponding to methylene (doublet), amine (singlet) and sulfonamide (triplet) protons. The observation of these three signals is clear evidence of the selective dansylation of the aminomethyl group during the first step of the synthesis.

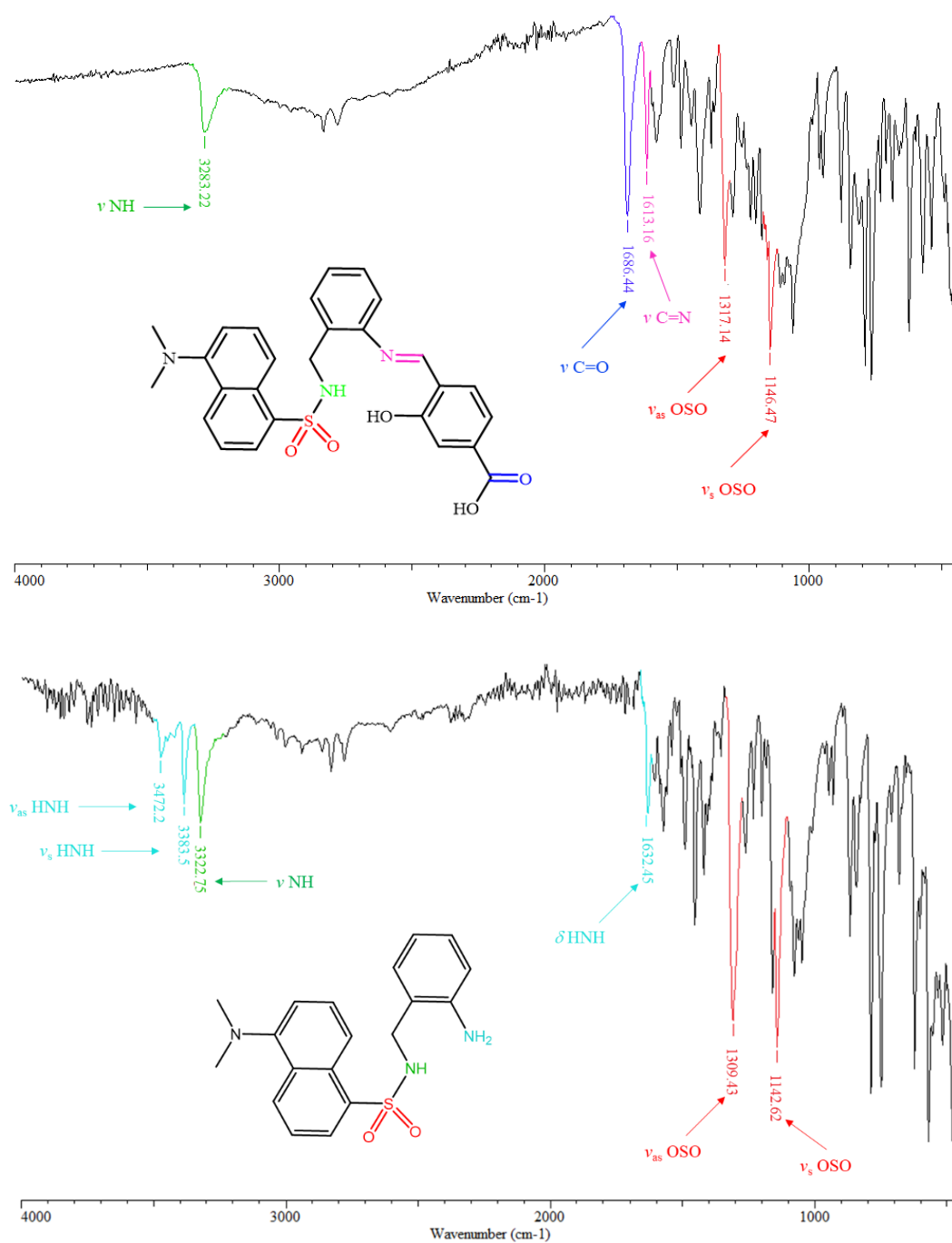


Figure 2. View of the ATR-IR spectra of H₃L (**top**) and N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**bottom**), with signal assignment of remarkable functional groups of the molecules on the corresponding schemes.

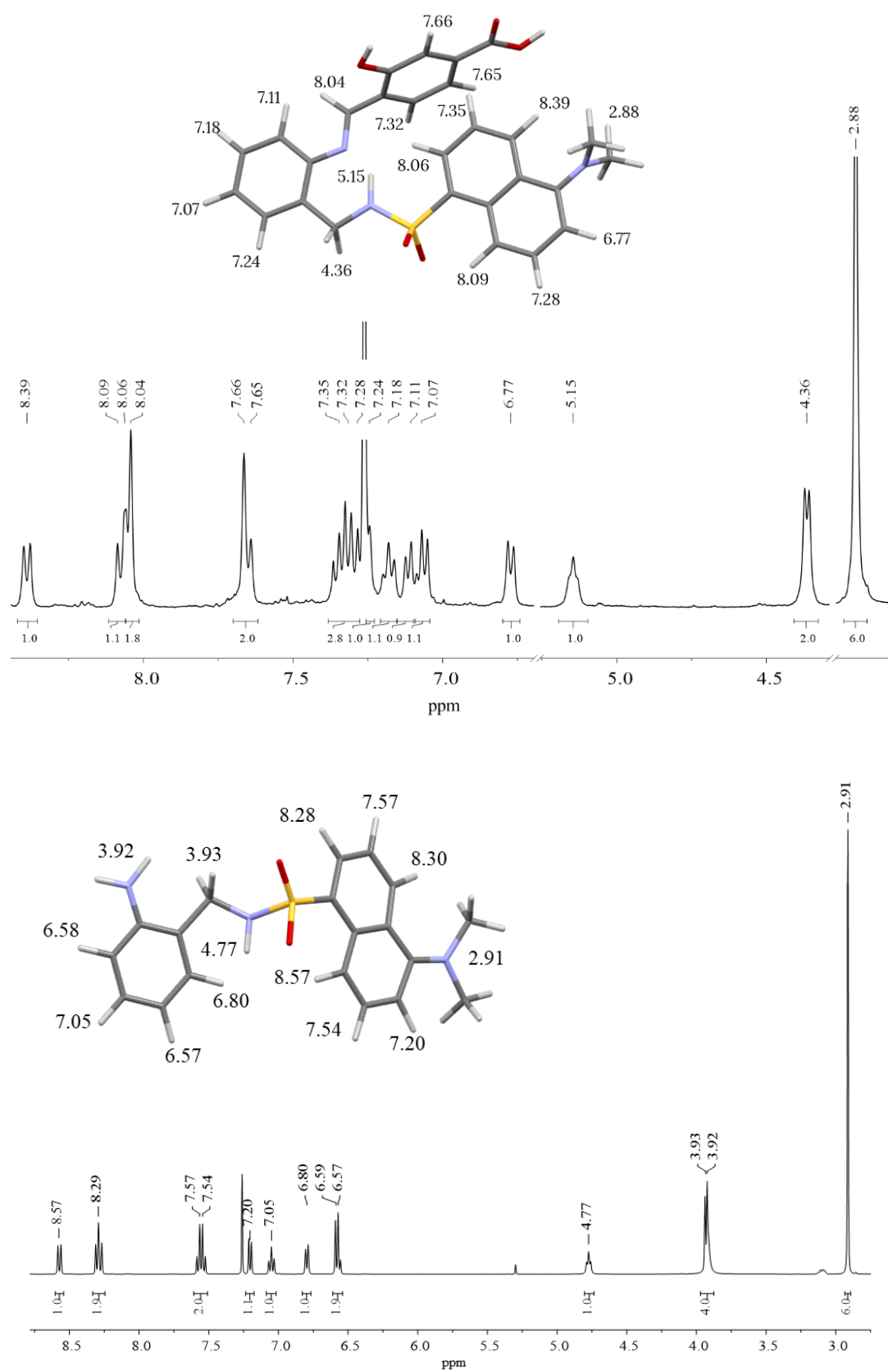


Figure 3. ^1H NMR spectra (in chloroform- d) of H_3L (**top**) and N -(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**bottom**), with signal assignment on a 3D model of each molecule.

H₃L displayed three intense intraligand absorption bands centered at about 225, 250 and 345 nm (Figure 4), corresponding to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the conjugated system. The absorption band at about 225 nm is in accordance with the presence of the carboxyl group. The observation of two bands with maxima at about 250 and 345 nm evidenced the absorption behavior of the dansyl moiety [10]. The shoulder at about 265 nm can be attributed to the imino group [11] of H₃L.

An ethanol solution of H₃L emitted a maximal green fluorescence at 520 nm when it was exposed to visible light with a wavelength of 400 nm. The fluorescence behavior could be assigned to the dansyl moiety, known for the typical charged-transfer band between the donor dimethylamino group and the sulfonyl unit, centered around $\lambda_{em} = 520$ nm, as previously described [10]. The study of the influence of pH on the fluorescence spectrum of H₃L (Figure 5) showed that pH values of about 6 (and lower ones) result in protonation of the dimethylamino group, pH values of about 8 lead to deprotonation of the carboxylic acid group, but when pH was about 9, deprotonation of the phenol group was achieved, and pH values of 11 lead to deprotonation of the sulfonamide group. Therefore, varying the pH of the medium, the Schiff base ligand can achieve different forms, such as H₄L⁺, H₃L, H₂L⁻, HL²⁻ and L³⁻, being the most emissive the anionic forms.

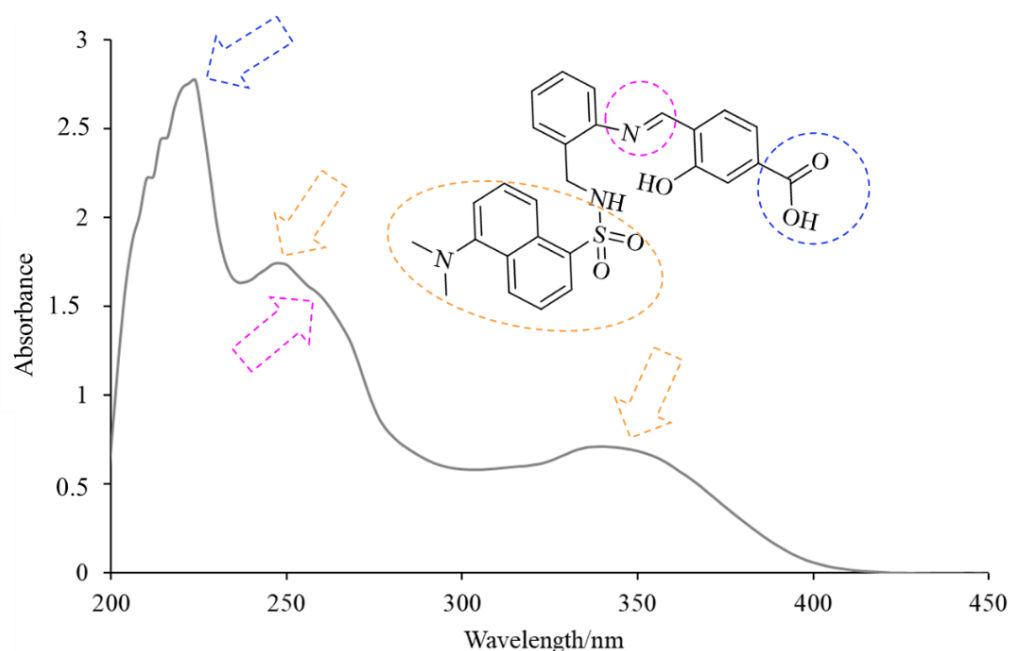


Figure 4. View of the UV-Vis spectrum of H₃L in ethanol solution.

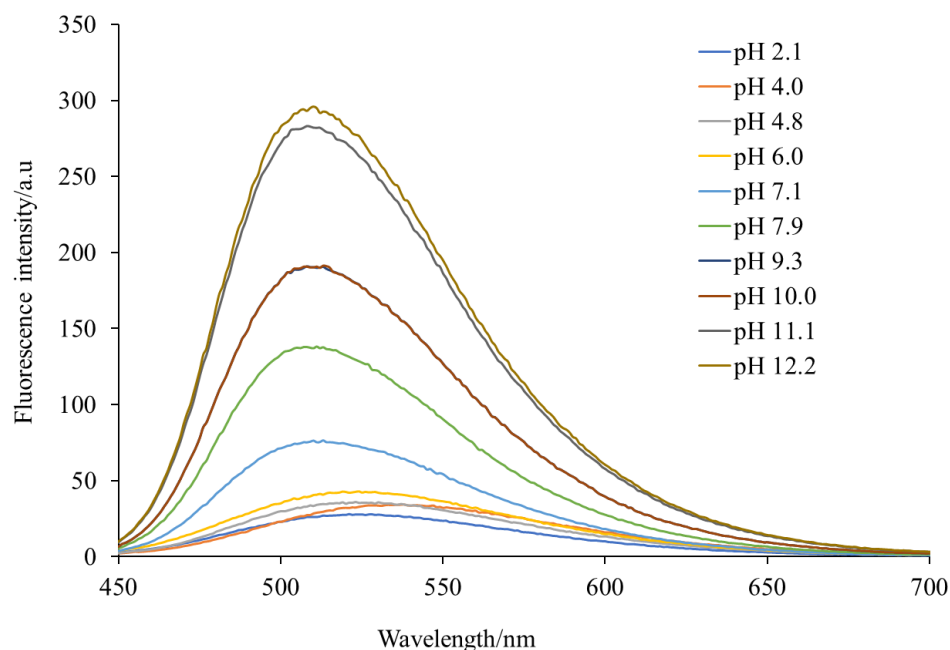


Figure 5. Influence of pH on the fluorescence spectrum of H₃L measured in ethanol at room temperature.

Preliminary studies showed that at pH value of about 12 favors the interaction between H₃L and CuO nanomaterials. This was evidenced by a pronounced decrease in the fluorescence intensity of an ethanol solution of H₃L when CuO is added in increasing amounts. Figure 6 shows the gradual quenching of the fluorescence intensity of an ethanol solution of H₃L after the addition of CuO nanopowder.

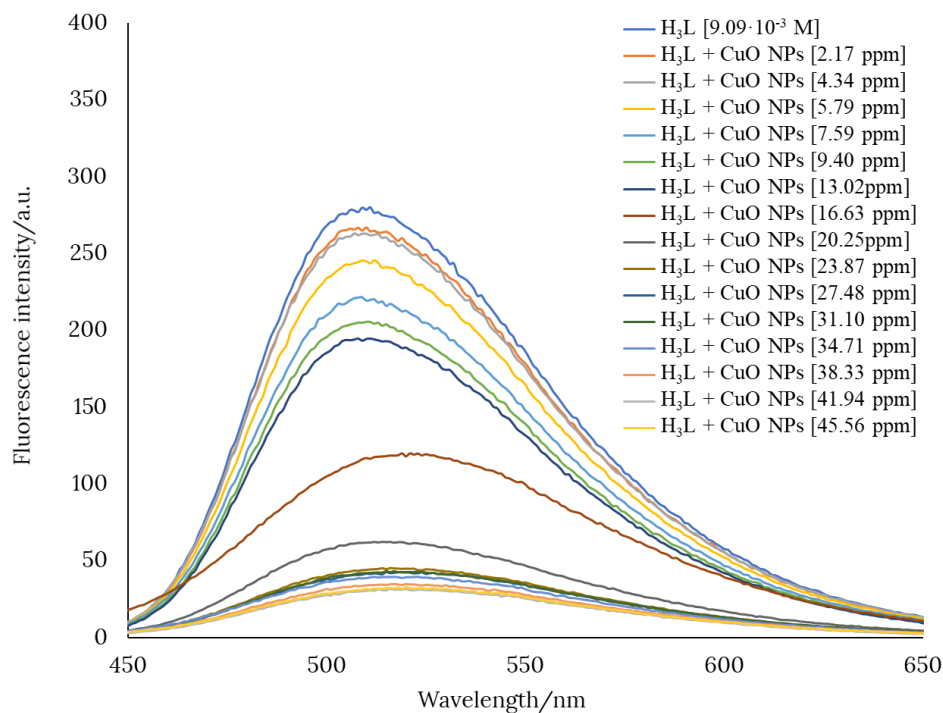


Figure 6. Variation of the fluorescence intensity of an ethanol solution of H₃L after the addition of CuO nanopowder.

3. Conclusions

As conclusion, we can say that we have synthesized and characterized a dansyl-based fluorescent ligand that deserves be explored as a probe for detecting heavy metal-based nanomaterials in aqueous solution.

4. Experimental Section

Materials and Methods

All starting materials and reagents were commercially available and were used without further purification. ^1H NMR spectra (400 MHz) were measured in deuterated chloroform. J values are given in Hertz. The spectra of the samples were recorded using ATR-FTIR spectrometer (Spectrum Two, Spectrometer FT-IR UATR, PerkinElmer, Waltham, MA, USA).

N-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide

The compound was prepared in a 250 mL round flask dissolving 2.35 mmol (0.6339 g) of dansyl chloride in 60 mL of CH_2Cl_2 . In a second flask 2.59 mmol (0.3162 g) of 2-aminobenzylamine was dissolved in 40 mL of CH_2Cl_2 , at which 2.59 mmol (0.2621 g) of triethylamine were added. This second solution was added in the first-round flask and stirred 18 h at reflux temperature. Evaporation to 1/3 of its volume under vacuum and a small portion of diethyl ether was added until the solution became cloudy. After this, it was necessary to filtrate under vacuum and washed with deionized water to separate the triethylamine hydrochloride present. The yellow solid was air-dried and weighted to calculate the final yield.

Yield: 0.604 g (75%). ^1H NMR (400 MHz, chloroform-*d*, δ in ppm): δ 8.57 (d, J = 8.5 Hz, 1H), 8.29 (t, J = 8.8 Hz, 2H), 7.57 (t, J = 8.3 Hz, 1H), 7.54 (t, J = 8.6 Hz, 1H), 7.20 (d, J = 7.5 Hz, 1H), 7.05 (t, J = 7.7 Hz, 1H), 6.80 (d, J = 7.4 Hz, 1H), 6.58 (d, J = 7.6 Hz, 1H), 6.57 (t, J = 7.0 Hz, 1H), 4.77 (t, J = 6.1 Hz, 1H), 3.93 (d, J = 7.1 Hz, 2H), 2.91 (s, 6H). UV-Vis (ethanol, $4.02 \cdot 10^{-5}\text{M}$, λ in nm) 254, 294, 346. Fluorescence λ/nm : λ_{em} 520 (λ_{ex} 340). ATR-FTIR (ν in cm^{-1}): 3472 w (ν_{as} HNH), 3384 m, sh (ν_{s} HNH), 3323 m (ν NH), 1632 m (δ HNH), 1309 s (ν_{as} OSO), 1143 s (ν_{s} OSO)

*H*₃*L*

The compound could be obtained in a 250 mL round flask dissolving the compound *N*-(2-aminobenzyl)-5-(dimethylamino)naphthalene-1-sulfonamide (0.09 g, 0.25 mmol) in CHCl_3 (20 mL). Then, while the magnetic stirring is on, a solution of 4-formyl-3-hydroxybenzoic acid (0.0294 g, 0.25 mmol) is added in a molar ratio of 1:1 along with 20 mL CHCl_3 . A yellow-orange mixture was obtained and then the reflux was switched on for 8 h, using a modified Dean-Stark. During this time, the state of the reaction was monitored by TLC (using a mixture of hexane and ethyl acetate with a 60:40 ratio as eluent). After the 8 h, the yellow-orange solution was concentrated in the rotary evaporator to dryness and a light orange precipitate was obtained. Then 10 mL of diethyl ether were added to the precipitate and the resulting suspension is left to stir for 6 h. After this time, it is filtered under vacuum and an orange-coloured precipitate is obtained, which is left to dry in the oven for a couple of hours. The ether fraction corresponds to impurities that were present.

Yield: 0.099 g (78%); ^1H NMR (400 MHz, chloroform-*d*) δ 8.39 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 9.6 Hz, 2H), 8.05 (d, J = 6.9 Hz, 1H), 8.04 (s, 1H), 7.66 (s, 1H), 7.65 (d, J = 8.6 Hz, 1H), 7.39–7.26 (m, 3H), 7.24 (d, 1H), 7.18 (t, J = 7.3 Hz, 1H), 7.11 (d, J = 7.4 Hz, 1H), 7.07 (t, J = 7.6 Hz, 1H), 6.77 (d, J = 7.6 Hz, 1H), 5.15 (t, J = 5.6 Hz, 1H), 4.36 (d, J = 5.8 Hz, 2H), 2.88 (s, 6H). UV-Vis (ethanol, 10^{-5}M , λ in nm) 224, 248, 340; Fluorescence λ/nm : λ_{em} 546 (λ_{ex} 400). ATR-FTIR (ν in cm^{-1}): $\nu(\text{NH})_{\text{sulfonamide}}$ 3283, $\nu(\text{C}=\text{O})$ 1686, $\nu(\text{C}=\text{N}_{\text{imine}})$ 1613, $\nu_{\text{as}}(\text{SO}_2)$ 1317, $\nu_{\text{s}}(\text{SO}_2)$ 1146.

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J.S.-M.; visualization, A.M.G.-D. and M.F.; supervision, J.S.-M. and P.B.-B.; project administration, P.B.-B.; funding acquisition, P.B.-B. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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