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Proceeding

Metallic ion sensing with a benzothiazole-based fluorimetric chemosensor†

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Abstract: An unnatural amino acid derivative, *N-tert*-butyloxycarbonyl asparagine benzyl ester bearing a benzothiazole unit at the side chain, was evaluated as fluorimetric chemosensor for several transition metal cations with environmental, biological and analytic relevance. Spectrofluorimetric titrations of the heterocyclic asparagine derivative with the various ions were carried out in acetonitrile and acetonitrile/water (9:1). It was found that the unnatural amino acid had a remarkable fluorimetric response in the presence of Cu^{2+} in acetonitrile and in the presence of Fe^{3+} in acetonitrile/water (9:1).

Keywords: asparagine, benzothiazole, fluorimetric chemosensor, metallic ions

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1. Introduction

The recognition of metallic ions has been given great attention due to their important roles in many biological and environmental processes that can be followed by fluorimetry [1]. The use of fluorimetry in the qualitative and quantitative determination of such species is considered as one of the most effective analytical method for biomedical and environmental monitoring, allowing fast and sensitive detection, usually with low detection limits which are particularly suitable for dilute media [2].

Recent studies have been carried out in the field of design, synthesis and characterization of novel amino acids (alanines, phenylalanines, asparagines) functionalized with heterocyclic rings (benzoxazole, benzimidazole), with different electronic nature having in mind the modulation of their photophysical properties and improvement of chemosensory ability [3-5]. These ensembles can be considered as unnatural amino acids, which can act as building block for intrinsically-labelled fluorescent peptides with enhanced coordination ability [6]. Also, benzothiazoles are known for displaying high relative fluorescence quantum yields and the fine tuning of the optical properties can be achieved by adequate substituent choice at selected positions of the heterocyclic ring [3].

In this communication, we report the chemosensory ability study of a benzothiazole unit coupled to an amino acid core, with potential application as fluorimetric chemosensor for metallic cations, such as Cu^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+}. There is an interest in the development of new sensing systems for these cations since they are examples of the most abundant essential elements in the human body and play vital roles in several physiological processes [7-9], along with being widely used in metallurgical, pharmaceutical and agrochemical industries [10]. As a consequence of their wide variety of applications in life sciences and industry, they have become hazard environmental pollutants [11].

2. Materials and Methods

2.1. General

All reagents and the acetonitrile used in the spectrofluorimetric titration (of spectroscopic grade) were purchased from Sigma-Aldrich, Acros and Fluka and used as received. TLC analysis were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel 60F254) and the spots were visualized under UV light. UV-Visible absorption spectra were obtained using a Shimadzu UV/2501PC spectrophotometer. Fluorescence spectra were collected using a FluoroMax-4 spectrofluorometer.

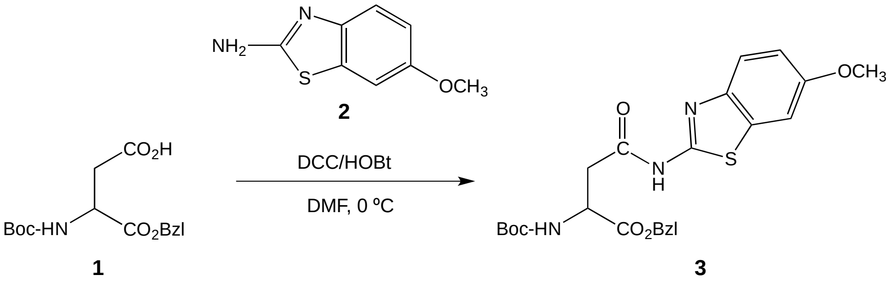
2.2. Spectrofluorimetric titrations

Solutions of benzothiazolyl-asparagine **3** (1×10^{-4} M) and of the metallic cations under study (1×10^{-2} M) were prepared in acetonitrile and acetonitrile/water (9:1). Titration of the asparagine with the several metallic cations (Cu^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+}) was performed by the sequential addition of equivalents of metal cation to the asparagine derivative solution, in a 10 mm path length quartz cuvette and emission spectra were measured by excitation at the wavelength of maximum absorption for compound **3**, with a 10 nm slit.

3. Results and Discussion

3.1. Synthesis of N-tert-butyloxycarbonyl (6-methoxybenzothiazol-2-yl) asparagine **3**

Asparagine derivative **3** was obtained from the corresponding *N-tert*-butyloxycarbonyl asparagine benzyl ester precursor **1** and 2-amino-6-methoxybenzothiazole **2**, by a DCC/HOBt mediated coupling in excellent yield of 91% (Scheme 1). This synthesis was carried out as reported previously by us [3] and the structure of the synthesised compound was confirmed by ^1H and ^{13}C NMR spectroscopy.



Scheme 1. Synthesis of benzothiazolyl-asparagine **3**.

3.2. Spectrofluorimetric titrations with metallic cations

The evaluation of benzothiazolyl-asparagine **3** a fluorimetric chemosensor was carried out by performing spectrofluorimetric titrations in acetonitrile and acetonitrile/water (9:1) in the presence of transition metal cations (Cu^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+}) with biological, environmental and analytical relevance. Acetonitrile was chosen as solvent as it is aprotic and does not interfere by establishing H-bonds with the analyte and/or the sensing system.

The spectral changes after addition of increasing amount of the several ions to a solution of benzothiazolyl-asparagine **3** were evaluated by spectrophotometry and spectrofluorimetry. In the spectrophotometric titrations, no changes were observed in the position or intensity of the absorption band.

However, significant changes could be seen in the fluorescence spectrum of system **3**. In the spectrofluorimetric titrations with Cu^{2+} in acetonitrile, a strong decrease of the fluorescence intensity was observed for compound **3**, with only 1.0 equivalent being necessary to quench 50 % of the initial fluorescence. In Figure 1 is shown the spectrofluorimetric titrations of asparagine **3** with Cu^{2+}, where is revealed that upon addition of increasing amounts of the cation, the band at 319 nm decreased drastically, accompanied by the appearance of a red-shifted band at 420 nm with growing fluorescence intensity that achieved a plateau upon addition of about 20 equivalents of Cu^{2+} (Figure 1A). There was a similar behaviour in the spectrofluorimetric titrations with Fe^{2+} and Fe^{3+}: a decrease of the fluorescence intensity was observed at 320 nm, accompanied by the increase of the fluorescence intensity at 435 and 440 nm, respectively. However, the titration with Fe^{3+} gave an irregular trend of variation (Figure 1B) whereas, by comparison to Cu^{2+}, it was necessary to add a larger amount of Fe^{2+} (120 equivalents) to achieve a fluorescence plateau at the new red-shifted band, with higher fluorescence intensity (Figure 1C). In the spectrofluorimetric titrations with Zn^{2+} in acetonitrile, no significant changes were observed with the addition of increasing amounts of this cation, and so the heterocyclic asparagine does not behave like a chemosensor for Zn^{2+} (Figure 1D). From the obtained results, it can be proposed that the synthesized asparagine is a chemosensor for Cu^{2+} in acetonitrile with high sensitivity.

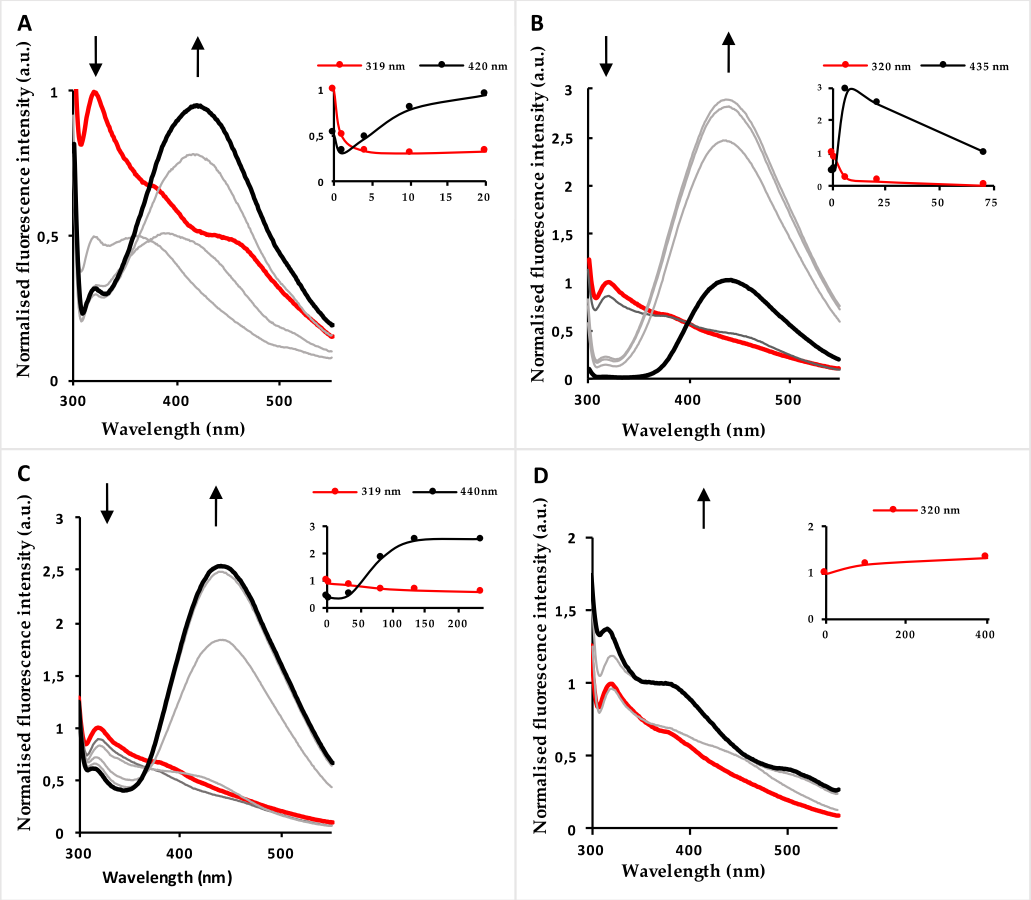


Figure 1. Fluorimetric titrations of benzothiazolyl-asparagine **3** with Cu^{2+}(A), Fe^{3+} (B), Fe^{2+} (C) and Zn^{2+} (D) in acetonitrile [λ_{exc} =290 nm]. Inset: normalised emission as a function of added metal equivalents.

Spectrophotometric titrations in the presence of the same transition metal cations were also carried out in acetonitrile and water (9:1), to evaluate their potential application as chemosensors in aqueous environment. These results, when compared to the chemosensing results obtained in acetonitrile solution, revealed the compound **3** exhibited a strong decrease of the chemosensory ability in mixtures of acetonitrile with water in the presence of Cu^{2+} and Fe^{2+}, with the addition of about 300 equivalents of each cation resulting in a 30% quenching of the initial fluorescence intensity. For Zn^{2+}, the behaviour was similar to that observed previously in acetonitrile, *i.e.* no changes were observed upon sequential addition of this cation (Figure 2).

However, in the spectrofluorimetric titrations with Fe^{3+} in acetonitrile/water (9:1) a steady CHEQ effect (chelation-enhanced quenching) was observed in the fluorescence spectra. In Figure 2B is shown the spectrofluorimetric titration of asparagine **3** with Fe^{3+} where it can be seen that, upon addition of 50 equivalents, there was a decrease in fluorescence of *ca*. 75% of the initial fluorescence intensity at 364 nm. For all cations tested, no new blue- or red-shifted band was formed during titration in acetonitrile/water (9:1).

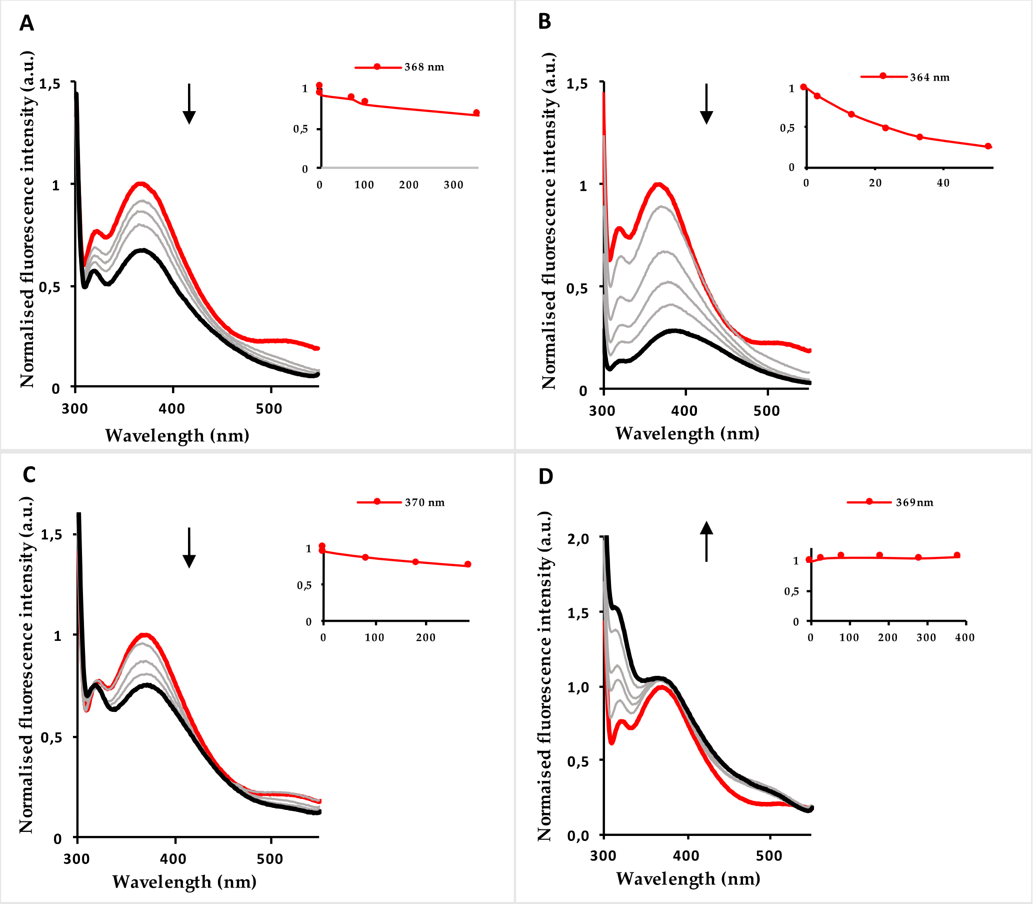


Figure 2. Fluorimetric titrations of benzothiazolyl-asparagine **3** with Cu^{2+} (A), Fe^{3+} (B), Fe^{2+} (C) and Zn^{2+} (D) in acetonitrile/water (9:1) [λ_{exc} =290 nm]. Inset: normalised emission as a function of added metal equivalents.

4. Conclusions

Heterocyclic asparagine **3** containing a benzothiazole unit at its side chain was synthesized and evaluated as fluorescent chemosensor based on amino acid core for a series of relevant transition metal cations (Cu^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+}). From the spectrofluorimetric titrations in acetonitrile, it was found that the tested compound could sense the presence of Cu^{2+}, with a small number of metal equivalents being necessary to achieve a remarkable fluorimetric response. The spectrofluorimetric titrations in acetonitrile/water (9:1) revealed that compound **3** exhibited a decrease of chemosensory ability for Cu^{2+}, $Zn^{2+}$and Fe^{2+}, whereas, in the presence of Fe^{3+} a strong CHEQ effect was observed. The results obtained suggest that this unnatural amino acid can be used for the detection of Cu^{2+} in acetonitrile and of Fe^{3+} in acetonitrile/water (9:1) solution.

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

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