Synthesis and evaluation of an imidazo-benzocrown ether functionalised amino acid as a new fluorimetric chemosensor for metal ion detection

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Abstract: A new unnatural phenylalanine derivative bearing benzimidazole and crown ether as coordinating/reporting units was synthesized, and its evaluation as colorimetric/fluorimetric chemosensor was carried out in acetonitrile solution. Compound **3** was tested for metal ion detection (such as Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Na^{+}, Ni^{2+}, Pd^{2+} and Zn^{2+}). The sensory properties and signaling mode, as seen by spectrofluorimetric titrations, showed that amino acid derivative **3** is a fluorimetric chemosensor, being highly sensitive for Cu^{2+}, Hg^{2+} and Pd^{2+}. The metal cation sensing properties displayed by compound **3** showed that it is a promising candidate for sensory applications.

Keywords: Phenylalanine; Imidazo-benzocrown ether; Fluorescence; Metal ion detection.

1. Introduction

The field of fluorescent chemosensors has grown in recent years due to their importance in applications such as in material sciences, biomedical analysis, environmental sciences and analytical chemistry [1].

The design of heteroditopic chemosensors that contain two different binding sites for targeting substances over large concentration ranges and capable of analysing multiple analytes simultaneously is a new and emerging topical field of supramolecular chemistry [2].

Among supramolecular host molecules, crown ethers occupy a special position due to their wide use in the design of new chemosensors based on their unique ability to combine with the cations of alkali metals, with very high selectivity and accessibility. In addition to alkali metals, crown ethers are also effective complexing reagents for the cations of alkali-earth metals, and when nitrogen and sulfur atoms are inserted into their structure they become sensitive to transition metal ions [3].

Additionally, the introduction of an imidazole in the phenylalanine skeleton was expected to increase the overall fluorescence of the resulting amino acid derivative.

Having these facts in mind, and following our research interests that include the synthesis and evaluation of fluorimetric chemosensors for anions and cations based on heterocycles and amino acids [4], a new imidazo-benzocrown ether functionalised amino acid **3** was synthesized and its evaluation as a fluorescent chemosensor is now reported.

2. Experimental

2.1. General procedure for the synthesis of benzimidazolyl phenylalanine 3

A solution of *N*-*t*-butyloxycarbonyl-4-formyl-L-phenylalanine methyl ester **1** (1 equiv) and 4-amino-5-nitrobenzo-18-crown-6 **2** (1 equiv) in absolute ethanol (3 mL) was treated with $Na_2S_2O_4$ (3 equiv), dissolved in a small volume of water, and heated at 80 ºC with stirring for 15 h. The mixture was poured into water (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was dried with magnesium sulphate and evaporated under reduced pressure to give the crude benzimidazolyl phenylalanine **3** which was submitted to silica gel column chromatography using mixtures of dichloromethane and *n*-hexane of increasing polarity as eluent. The fractions containing the purified product were collected and evaporated under vacuum.

*Benzimidazolyl phenylalanine methyl ester (**3**)*

Starting from phenylalanine derivative **1** (0.34 g, 0.11 × 10^{-3} mol) and benzocrown ether derivative **2** (0.035 g, 0.11 × 10^{-3} mol), compound **3** was obtained as a yellow oil (0.044 g, 70%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42 (s, 9H, $C(CH_3)_3$), 2.83-2.88 3.06-3.10 (m, 1H, β-CH_2), 3.68-3.98 (m, 19H, 8 x CH_2 crown and OCH_3), 4.55-4.57 (m, 1H, α-H), 5.30 (d, *J* 7.2 Hz, 1H, NH Boc), 7.04 (s, 1H, H4' and H7'), 7.19 (d, *J* 7.6 Hz, 2H, H2 and H6), 8.15 (d, *J* 7.2 Hz, 2H, H3 and H5); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 28.32 ($C(CH_3)_3$), 38.08 (β-CH_2), 51.74 (CH_2 crown), 52.35 (OCH_3), 54.42 (α-C), 68.03 (CH_2 crown), 68.64 (CH_2 crown), 69.36 (CH_2 crown), 69.94 (CH_2 crown), 70.44 (CH_2 crown), 70.52 (CH_2 crown), 80.09 ($C(CH_3)_3$), 98.69 (C4', C7'), 126.86 (C3, C5), 130.02 (C2, C6), 146.17 (C5', C6'), 155.23 (C2' and C=O Boc), 172.08 (C=O ester); UV/Vis (ethanol, nm): λ_{max} (log ε) = 384 (4.20); MS: *m/z* (ESI) 586 ($[M+H]^+$, 100), 586 (20); HMRS: *m/z* (ESI) calc. for $C_{30}H_{40}N_3O_9$ 586.27553, found 586.27591.

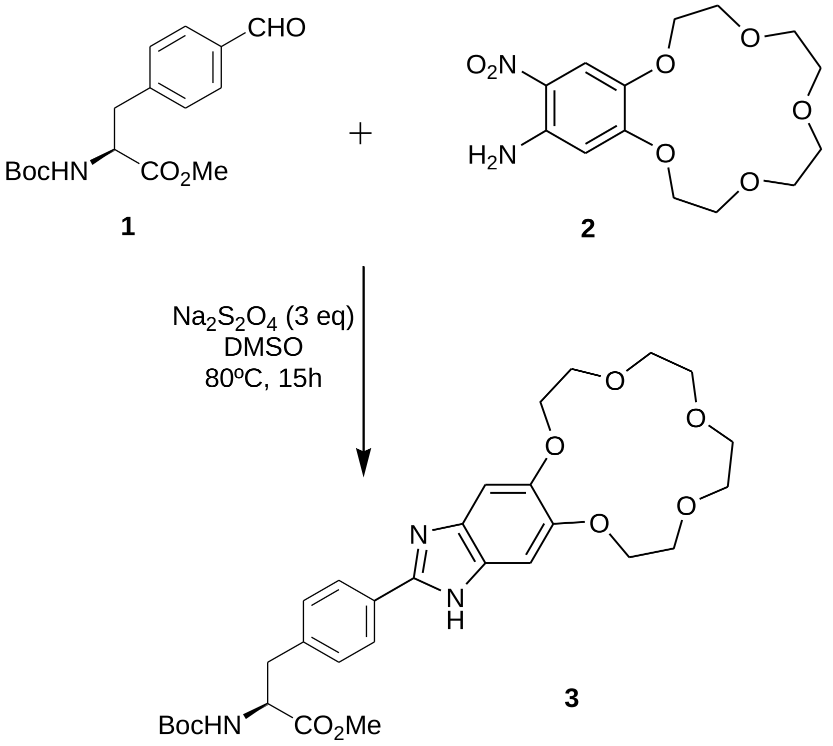
2.2. Spectrofluorimetric titrations and chemosensing studies of benzimidazolyl phenylalanine 3

Solutions of phenylalanine derivative **3** (*ca*. 1.0 × 10^{-5} M) and of the cations under study (ca. 1.0 × 10^{-2} M) were prepared in UV-grade acetonitrile (in the form of hexahidratated tetrafluorborate salts for Cu^{2+}, Co^{2+}, Ni^{2+} and Pd^{2+}, and perchlorate salts for Cd^{2+}, Ca^{2+}, Na^+, Cr^{3+}, Zn^{2+}, Hg^{2+}, $Fe^{2+}$and Fe^{3+}). Titration of the compound with the several metallic cations was performed by the sequential addition of equivalents of metal cation to the phenylalanine 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**. The binding stoichiometry of the phenylalanine derivative with metal cations was determined by Hiperquad Software.

3. Results and discussion

3.1. Synthesis

Compound **3** was synthesized in 70% yield by condensation of *N*-*t*-butyloxycarbonyl-4-formyl-L-phenylalanine methyl ester **1** with 4-amino-5-nitrobenzo-15-crown-5-ether **2** in the presence of $Na_2S_2O_4$ in DMSO at 80 ºC for 15 h (Scheme 1, Table 1). The synthesized compound was fully characterized by the usual spectroscopic techniques.



Scheme. Synthesis of benzimidazolyl phenylalanine **3**.

The photophysical properties of compound **3** in absolute ethanol were evaluated and the UV/Vis absorption and emission spectra of degassed 10^{-5} M solution were measured (Table 1). Relative fluorescence quantum yield were calculated using 9,10-diphenylanthracene in ethanol as standard (Φ_F = 0.95) [5].

Table 1. Yield, UV-visible absorption and fluorescence data for benzimidazolyl phenylalanine **3**, in absolute ethanol.

Cpd.	Yield (%)	UV/Vis		Fluorescence		
		λ_{max}	log ε	λ_{em}	Stokes' shift (nm)	Φ_F
3	70	325	4.20	384	59	0.70

3.2. Spectrofluorimetric titrations of compound 3 with cations in acetonitrile solution

To explore the sensing ability of compound **3** in solution in the presence of Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Na^{+}, Ni^{2+}, Pd^{2+} and Zn^{2+}, a preliminary sensing study was performed with addition of 100 equivalents of metal ion to a 100 μM acetonitrile solution of compound **3** (Figures 1 and 2). For all metals, there were no changes in the absorption spectra (ground state) of compound **3** but in the emission spectra (excited state) noticeable changes occurerd. So, spectrofluorimetric titrations were performed to understand where and how the interaction with the different metal ions was occurring.

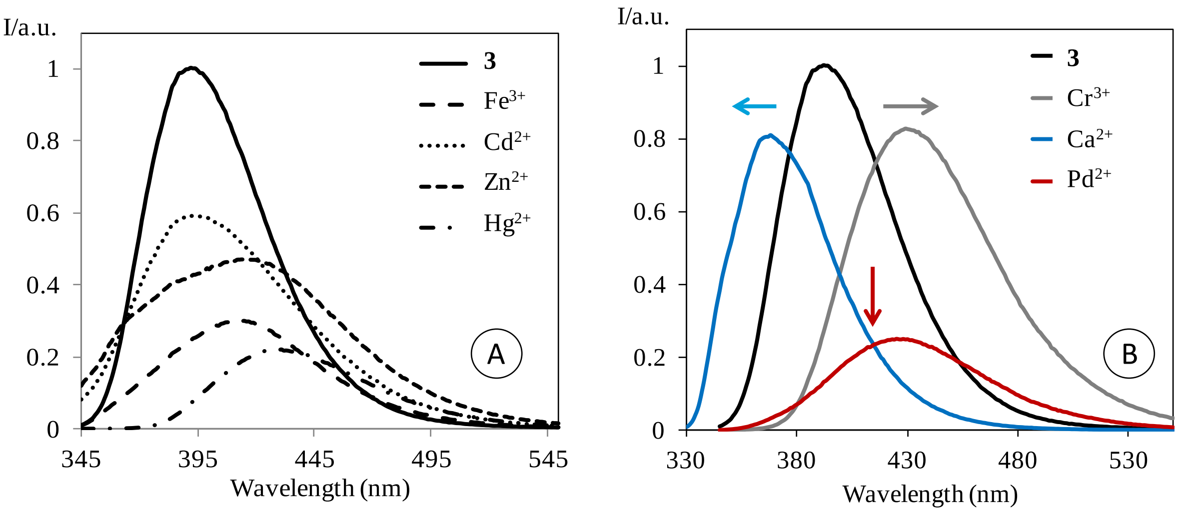


Figure 1. Fluorescence spectra of acetonitrile solutions of compound **3** after addition of 100 equivalents of Fe^{3+}, Cd^{2+}, Zn^{2+} and Hg^{2+} (A), and Cr^{3+}, Ca^{2+} and Pd^{2+} (B).

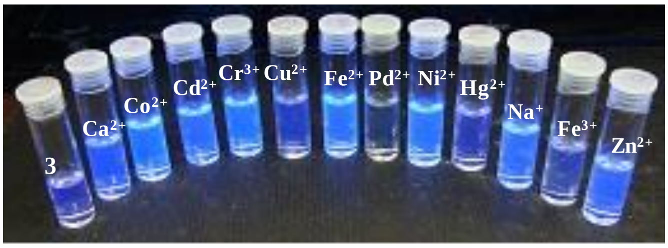


Figure 2. Emission under irradiation at 365 nm of some ethanolinc solutions of compound **3** in the presence of 100 equivalents of several cations.

The stronger interaction was observed in the presence of $Pd^{2+}$and Cu^{2+} where only 1.6 and 3.0 equivalents are enough to quench the emission, respectively (see Figures 3 and 4). A CHEQ (chelation enhancement of the quenching) effect in the fluorescence emission for both metals was observed. In the case of Zn^{2+}, Cr^{3+}, Cd^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+} and Fe^{2+}, that normally produce a CHEF effect (chelation enhancement of fluorescence), a ~40% till ~70% quenching effect was also observed. Moreover, a large red shift (30 to 45 nm) in the emission band was observed for several metals. In some cases, a new red-shifted band appeared, whereas a different behavior (a blue shift) was observed when Ca^{2+} and Na^{+} were added (Figure 1).

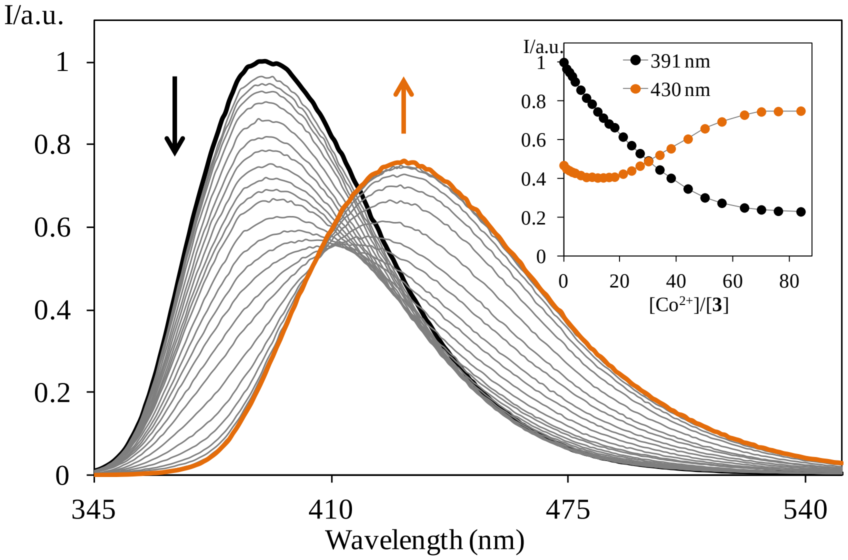


Figure 3. Spectrofluorimetric titrations of compound **3** in the presence of Co^{2+}, in acetonitrile solution. ([**3**]=10µM, T=298K, λ_{exc} **3** = 325 nm). Inset: normalised emission at 391 nm and 430 nm as a function of added metal equivalents.

With the addition of Co^{2+}, compound **3** showed a small red shift on the fluorescence emission spectra and a new band enhanced at ~430 nm (Figure 2). The emission spectra showed the formation of a well-defined isoemissive point at 416 nm. These results suggest the existence of two or more species in solution, and also that the interaction is taking place between the metal ion and the nitrogen of the imidazo moiety. The complexation constant fits to a mononuclear species for compound **3** with value of log β 3.571 ± 0.011.

The more efficient interaction was between compound **3** and Pd^{2+}, which only required 1.6 equivalents to quench almost 80% of the initial fluorescence. However, the best result was obtained for Cu^{2+} with a complete quenching of the fluorescence emission, reaching a plateau at 3.0 equivalents of metal ion. The mononuclear species was formed with complexation constants with values of log β 5.188 ± 0.025 and log β 7.800 ± 0.012 for Pd^{2+} and Cu^{2+}, respectively.

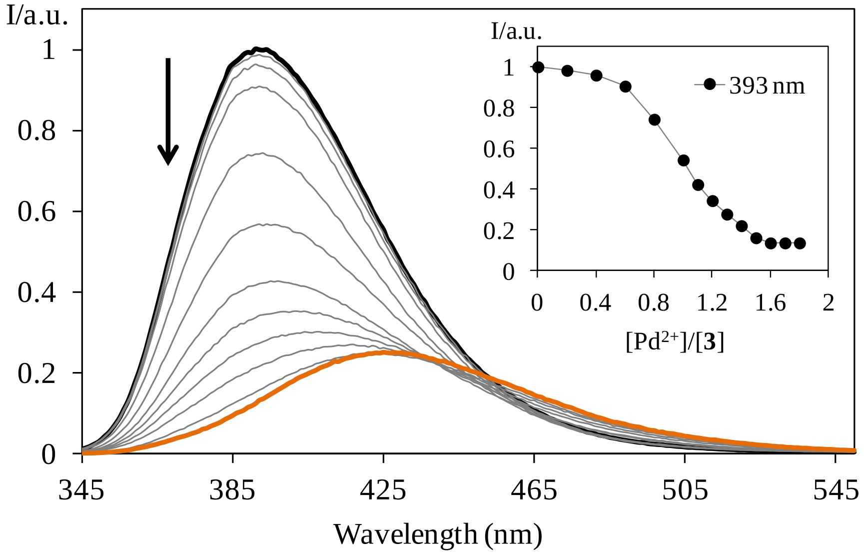


Figure 4. Spectrofluorimetric titrations of compound **3** in the presence of Pd^{2+}, in acetonitrile solution. ([**3**]=10μM, T=298K, λ_{exc} **3** = 325 nm). Inset: normalised emission at 393 nm as a function of added metal equivalents.

4. Conclusions

Compound **3** was synthesized in excellent yield (70%) using a simple experimental procedure. The sensory ability was evaluated for several metal ions by spectrofluorimetric titrations in acetonitrile, and it was found that benzimidazolyl-phenylalanine **3** was a suitable chemosensor for Hg^{2+}, Cu^{2+} and Pd^{2+} showing higher sensitivity for these cations when compared to Co^{2+}, Cr^{3+}, Fe^{3+}, Ni^{2+}, Zn^{2+} and Cd^{2+}, as an almost complete fluorescence quenching was achieved with the addition of 1.6 and 3.0 metal equivalents, respectively.

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