Novel functionalised imidazo-benzocrown ethers bearing a thiophene spacer as fluorimetric chemosensors for metal ion detection

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Abstract: Novel phenylalanine derivatives bearing benzimidazole and crown ethers as coordinating/reporting units and thiophene as spacer unit were synthesized, and their evaluation as fluorimetric chemosensors was carried out in acetonitrile and acetonitrile/water solutions. 15-Crown-5 benzimidazolyl phenylalanine methyl ester, 15-crown-5 thienylbenzimidazolyl phenylalanine methyl ester and 18-crown-6 thienylbenzimidazolyl phenylalanine methyl ester were tested for alkaline, alkaline-earth and transition metal ions (such as Na^{+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Ni^{2+}, Pd^{2+} and Zn^{2+}). The different crown ether binding moieties as well as the electronic nature and the length of the π-bridge linked to the benzimidazole heterocycle allowed the fine tuning of the sensory properties as seen by spectrofluorimetric titrations. Therefore, 15-crown-5 benzimidazolyl phenylalanine methyl ester is a fluorimetric chemosensor, being selective and sensitive for Cu^{2+} and Pd^{2+} in aqueous solutions (ACN/H_2O; 80:20). On the other hand, the metal cation sensing properties displayed by 15-crown-5 thienylbenzimidazolyl phenylalanine methyl ester bearing an arylthienyl spacer showed that this is a promising candidate as fluorimetric chemosensor for Fe^{3+}, Pb^{2+} and Pd^{2+} in acetonitrile solution.

1. Introduction

The development of artificial receptors for the recognition of ionic species is currently of great interest as highly selective anion or cation sensing is imperative for many areas, including environmental, biological, clinical, and waste management applications [1]. Fluorescent sensors have been recognized as indispensable tools for monitoring ions and biomolecules with high sensitivity in cells and tissues and their potential for progress can be explained by the distinct advantages offered by fluorescence detection in terms of sensitivity, selectivity, response time and local observation, etc.

Suitable fluorescent reporters must efficiently transduce a binding event into a measurable fluorescence signal, and imidazo-based fluorophores have received increasing attention due to their interesting optical properties. Benzimidazole and its derivatives have been studied in anion and cation

recognition systems that display color changes or fluorescence quenching or enhancement upon binding [2].

Crown ethers occupy a special position among receptors and are widely used in the design of new chemosensors based on their unique ability to coordinate the cations of alkaline metals, along with their fairly high selectivity and accessibility. In addition to alkaline metals, crown ethers are also effective complexing reagents for alkaline-earth and transition metal ions [3].

It has been reported that the combination of thiophene units and crown ethers can modulate the sensory behavior in order to obtain selective Hg^{2+}, Pd^{2+} or Cu^{2+} fluorescent chemosensors taking advantage of cooperative effects in solution for the simultaneous exploration of alkaline/alkaline earth ions in the presence of transition metal ions. The selective and sensitive detection of these heavy or transition metal cations is also an interesting goal due to their importance in environmental and medicinal areas [4].

The design of ditopic chemosensors that contain two different binding sites capable of analysing multiple analytes simultaneously is a new and emerging topical field of supramolecular chemistry [5]. For the construction of fluorophore-labelled peptides and proteins, the insertion of suitable heterocyclic systems at the side chain of natural amino acids, as in the present case the introduction of a fused imidazole in a phenylalanine skeleton, can add extra functionality to the amino acid resulting in the development of functional unnatural amino acids possessing additional properties, such as increased UV absorption and fluorescence. UV-active amino acids are, therefore, valuable tools for biochemistry, cellular biology and cellular imaging applications.

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 [6], new imidazo-benzocrown ether functionalised amino acids **3** and **4** were synthesized and their evaluation as fluorescent chemosensors is now reported. The different π-bridges linked to the benzimidazole coordinating/reporting unit are intended to improve the intramolecular electron delocalization, which will tune the photophysical properties of new sensors and optimize the recognition of target analytes through a greater sensitivity of fluorescence. Additionally, the introduction of different crown ether moieties is intended to improve the selectivity for the recognition of the targets.

2. Experimental

All melting points were measured on a Stuart SMP3 melting point apparatus. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel $60F_{254}$) and spots were visualised under UV light. Chromatography on silica gel was carried out on Merck Kieselgel

(230-240 mesh). NMR spectra were obtained on a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H and 100.6 MHz for ^{13}C using the solvent peak as internal reference at 25 ºC. All chemical shifts are given in ppm using δ_H Me_4Si = 0 ppm as reference and *J* values are given in Hz. Assignments were made by chemical shifts, peak multiplicities and *J* values and were supported by spin decoupling-double resonance and bidimensional heteronuclear correlation techniques. Low and high resolution mass spectrometry analyses were performed at the "C.A.C.T.I. - Unidad de Espectrometria de Masas", at University of Vigo, Spain. Fluorescence spectra were collected using a FluoroMax-4 spectrofluorometer. UV-visible absorption spectra (200–700 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. Luminescence quantum yields were measured using 9,10-diphenylanthracene in ethanol as standard (Φ_F = 0.95) [7]. All commercially available reagents were purchased from Sigma-Aldrich, ACROS, or TCI and used as received. Organic solvents used in the spectroscopic studies were of spectroscopic grade. Compound **1a** was synthesised as reported elsewhere [8] and *N*-(*tert*-butyloxycarbonyl)-4-bromo-L-phenylalanine methyl ester (the precursor for **1b**) was prepared from commercially available 4-bromo-L-phenylalanine by standard protecting group chemistry.

2.1. Synthesis of *N*-(*tert*-butyloxycarbonyl)-4-(5´-formylthiophen-2´-yl)-L-phenylalanine methyl ester, 1b

N-(*tert*-Butyloxycarbonyl)-4-bromo-L-phenylalanine methyl ester (0.170 g, 0.47×10^{-3} mol, 1 equiv) and $Pd(PPH_3)_4$ (0.016 g, 0.014×10^{-3} mol, 0.03 equiv) were stirred in DME (10 mL) during 10 min under inert atmosphere at 80ºC. 5-Formylthiophene boronic acid (0.088 g, 0.57×10^{-3} mol, 1.2 equiv), dissolved in absolute ethanol (1 mL), and Na_2CO_3 2M (0.5 mL, 2 equiv) were added to the previous reaction mixture under inert atmosphere and the progress of the reaction was followed by TLC. Ethyl acetate (10 mL) and saturated NaCl solution (10 mL) were added, the mixture was transferred to an extraction funnel and the layers were separated. The organic layer was washed with water (3 × 15 mL) and NaOH 10% aqueous solution (1 × 15 mL). After drying the organic layer over anhydrous $MgSO_4$, the solvent was removed under vacuum. The crude solid was purified by column chromatography, eluting with dichloromethane-methanol (100:1). *N*-(*tert*-Butyloxycarbonyl)-4-(5´-formylthiophen-2´-yl)-L-phenylalanine methyl ester **1b** was obtained as a white solid (0.101 g, 55%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.41 (s, 9H, $C(CH_3)_3$), 3.08-3.19 (m, 1H, β-CH_2), 3.73 (s, 3H, OCH_3), 4.56-4.61(m, 1H, α-H), 5.07 (d, *J* 7.2 Hz, 1H, NH Boc), 7.19 (d, *J* 8.2 Hz, 2H, H2 and H6), 7.37 (d, *J* 4.0 Hz, 1H, H-3´), 7.59 (d, *J* 8.2 Hz, 2H, H3 and H5), 7.72 (d, *J* 4.0 Hz, 1H, H4´), 9.87 (br s, 1H, CHO) ppm; ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 28.21 ($C(CH_3)_3$), 38.12 (β-CH_2), 52.28 (OCH_3), 54.25 (α-C), 79.92

(*C*(CH_3)$_3$), 123.92 (C3´), 126.41 (C3 and C5), 130.17 (C2 and C6), 131.70 (C4), 137.38 (C1), 141.25 (C4´), 142.27 (C5´), 153.90 (C2´), 154.97 (C=O Boc), 171.54 (C=O ester), 182.70 (CHO) ppm.

2.2. Synthesis of 4´-amino-5´-nitrobenzo-18-crown-6, 2b

4´-Nitrobenzo-18-crown-6 (0.107 g, 0.3 × 10^{-3} mol) was dissolved in methanol/acetic acid (10:1) (5 mL), Pd/C (10 mg) was added and the mixture was stirred in H_2 atmosphere at room temperature for 20 hours. The solvent was removed in a rotary evaporator and 4´-aminobenzo-18-crown-6 was obtained as a yellow oil (0.096 g, 98%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.60-3.66 (m, 12H, 6 × CH_2), 3.81-3.82 (m, 4H, 2 × CH_2), 4.02-4.03(m, 4H, 2 × CH_2), 6.33-6.37 (m, 2H, H3´ and H6´), 6.65-6.67 (m, 3H, H5´ and NH_2) ppm.

4´-Aminobenzo-18-crown-6 (0.078 g, 0.24 × 10^{-3} mol, 1 equiv) was suspended in acetic anhydride (5 mL) and $Cu(NO_3)_2.3H_2O$ (0.058 g, 0.24 × 10^{-3} mol, 1 equiv) was added. The mixture was stirred at room temperature during 1 hour. The mixture was diluted with $CHCl_3$ (10 mL) and saturated Na_2CO_3 solution (10 mL) was added. After complete acetic anhydride hydrolysis, the organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed under vacuum. The crude was submitted to silica gel column chromatography using mixtures of dichloromethane and methanol of increasing polarity as eluent. The fractions containing the purified product were collected and evaporated under vacuum, to yield *N*-(4´-(5´-nitrobenzo-18-crown-6))nitrous amide as a yellow oil (0.081 g, 91%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.67-3.98 (m, 16H, 8 × CH_2), 4.22-4.31(m, 4H, 2 × CH_2), 7.70 (s, 1H, H3´), 8.47 (s, 1H, H6´) ppm.

The previous compound (0.050 g, 0.13 × 10^{-3} mol) was treated with concentrated HCl (2 mL) in 1,2-dicloroethane (10 mL) and diethyl ketone (0.4 mL, 3.9 × 10^{-3} mol, 30 equiv) by stirring under pressure at 80 ºC for 4 hours. The mixture was neutralized with triethylamine. The solvent was removed under vacuum and the residue purified by silica gel column chromatography using mixtures of dichloromethane and methanol of increasing polarity as eluent. 4´-Amino-5´-nitrobenzo-18-crown-6 **2b** was obtained as a yellow oil (0.041 g, 48%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.60-3.75 (m, 12H, 6 × CH_2), 3.85-3.87 (m, 4H, 2 × CH_2), 4.06-4.22 (m, 4H, 2 × CH_2), 6.24 (s, 1H, H3´), 7.42 (s, 1H, H6´) ppm.

2.3. Synthesis of 15-crown-5-benzimidazolyl phenylalanine, 3

A solution of *N*-(*tert*-butyloxycarbonyl)-4-formyl-L-phenylalanine methyl ester **1a** [8] (0.34 g, 0.11 × 10^{-3} mol, 1 equiv) and 4´-amino-5´-nitrobenzo-15-crown-5 **2a** (0.035 g, 0.11 × 10^{-3} mol, 1 equiv) in absolute ethanol (3 mL) was treated with $Na_2S_2O_4$ (0.057 g, 0.33 × 10^{-3} mol, 3 equiv), dissolved in

water (1 mL), and heated at 80 ºC with stirring for 15 h. The mixture was poured into water (20 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layer was dried with anhydrous $MgSO_4$ and evaporated under reduced pressure to give the crude product that 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, giving crown ether benzimidazolyl phenylalanine methyl ester **3** 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 (m, 1H, β-CH_2), 3.06-3.10 (m, 1H, β-CH_2), 3.68-3.98 (m, 19H, 8 × CH_2 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), 52.35 (OCH_3), 54.42 (α-C), 68.03 (CH_2), 68.64 (CH_2), 69.36 (CH_2), 69.94 (CH_2), 70.44 (CH_2), 70.52 (CH_2), 80.09 ($C(CH_3)_3$), 98.69 (C4´, C7´), 126.86 (C3, C5), 130.02 (C2, C6), 131.01 (C4), 137.80 (C1), 138.51 (C3a´, C7a´), 146.17 (C5´, C6´), 155.23 (C2´, C=O Boc), 172.08 (C=O ester); UV/Vis (acetonitrile, nm): λ_{max} (log ε) = 325 (4.20); MS: *m/z* (ESI) 586 ($[M+H]^+$, 100), 586 (20); HMRS: *m/z* (ESI) calc. for $C_{30}H_{40}N_3O_9$ 586.2755, found 586.2759.

2.4. General method for the synthesis of crown ether benzimidazolyl 4-(5´-formylthiophen-2´-yl)-L-phenylalanines 4a-b

A solution of *N*-(*tert*-butyloxycarbonyl)-4-(5´-formylthiophen-2´-yl)-L-phenylalanine methyl ester **1b** (0.050 g, 0.13 × 10^{-3} mol, 1 equiv) and 4´-amino-5´-nitrobenzo-15-crown-5 **2a** (0.042 g, 0.13 × 10^{-3} mol, 1 equiv) or 4´-amino-5´-nitrobenzo-18-crown-6 **2b** (0.048 g, 0.13 × 10^{-3} mol, 1 equiv) in absolute ethanol (3 mL) was treated with $Na_2S_2O_4$ (0.067 g, 0.39 × 10^{-3} mol, 3 equiv), dissolved in water (1 mL), and heated at 80 ºC with stirring for 48 h. The mixture was evaporated under reduced pressure to give the crude benzimidazolyl phenylalanines **4a** and **4b**, respectively. The crudes were submitted to silica gel column chromatography using mixtures of dichloromethane and methanol of increasing polarity as eluent. The fractions containing the purified products were collected and evaporated under vacuum.

2.4.1. 15-Crown-5 benzimidazolyl phenylalanine methyl ester 4a was obtained as a yellow oil (0.034 g, 40%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.45 (s, 9H, $C(CH_3)_3$), 3.04-3.17 (m, 1H, β-CH_2), 3.73-4.05 (m, 16H, 8 × CH_2), 3.74 (s, 3H, OCH_3), 4.55-4.60 (m, 1H, α-H), 5.12 (d, *J* 7.6 Hz, 1H, NH Boc), 7.17-7.22 (m, 2H, H4´ and H7´), 7.19 (d, *J* 7.8 Hz, 2H, H2 and H6), 7.36 (br s, 1H, H3´´), 7.60 (d, *J* 7.8 Hz, 2H, H3 and H5), 8.01 (br s, 1H, H4´´); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 28.31 ($C(CH_3)_3$), 38.03 (β-CH_2), 52.39 (OCH_3), 54.37 (α-C), 67.69 (CH_2), 68.75 (CH_2), 69.22 (CH_2), 69.39

(CH_2), 69.42 (CH_2), 80.03 ($C(CH_3)_3$), 97.31 (C4´, C7´), 124.89 (C3´´), 126.28 (C3, C5), 130.23 (C2, C6), 131.05 (C4), 133.61 (C4´´), 137.73 (C1), 138.59 (C3a´, C7a´), 143.72 (C5´´), 146.70 (C5´, C6´), 147.52 (C5), 151.29 (C2´´), 155.10 (C2´, C=O Boc), 172.06 (C=O ester); UV/Vis (acetonitrile, nm): λ_{max} (log ε) = 370 (4.15); MS: *m/z* (ESI) 668 ($[M+H]^+$, 34), 682 (100), 690 (9), 704 (26); HMRS: *m/z* (ESI) calc. for $C_{34}H_{42}N_3O_9S$ 668.2640, found 668.2636.

2.4.2. 18-Crown-6 benzimidazolyl phenylalanine methyl ester 4b was obtained as a yellow oil (0.036 g, 39%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.44 (s, 9H, $C(CH_3)_3$), 3.01-3.16 (m, 1H, β-CH_2), 3.59-3.86 (m, 20H, 10 × CH_2), 3.74 (s, 3H, OCH_3), 4.56-4.60 (m, 1H, α-H), 5.09 (d, *J* 8.0 Hz, 1H, NH Boc), 7.08 (s, 2H, H4´ and H7´), 7.12-7.16 (m, 2H, H2 and H6), 7.24 (br s, 1H, H3´´), 7.53 (d, *J* 7.2 Hz, 2H, H3 and H5), 7.95 (br s, 1H, H4´´); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 28.29 ($C(CH_3)_3$), 38.03 (β-CH_2), 52.31 (OCH_3), 54.39 (α-C), 67.13 (CH_2), 68.51 (CH_2), 69.24 (CH_2), 69.30 (CH_2), 69,45 (CH_2), 80.01 ($C(CH_3)_3$), 97.88 (C4´, C7´), 124.18 (C3´´), 125.90 (C3 or C5), 125.93 (C3 or C5), 129.26 (C4´´), 129.92 (C2 or C6), 129.99 (C2 or C6), 132.37 (C4), 136.19 (C1), 138.49 (C3a´, C7a´), 143.92 (C5´´), 145.33 (C5´ and C6´), 151.28 (C2´´), 155.09 (C2´, C=O Boc), 172.18 (C=O ester); UV/Vis (acetonitrile, nm): λ_{max} (log ε) = 368 (4.16); MS: *m/z* (ESI) 712 ($[M+H]^+$, 82), 726 (100), 734 (58), 748 (63); HMRS: *m/z* (ESI) calc. for $C_{30}H_{40}N_3O_9$ 712.2901, found 712.2898.

2.5. Spectrofluorimetric titrations and chemosensing studies of benzimidazolyl phenylalanines 3 and 4

Solutions of phenylalanine **3** and **4** (*ca*. 1.0 × 10^{-5} M) and of the cations under study (ca. 1.0 × 10^{-2} M) were prepared in UV-grade acetonitrile and mixtures of acetonitrile and water, in different ratios (in the form of hydrated 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 compounds 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 compounds **3** and **4**. The binding stoichiometry of the phenylalanine derivatives **3** and **4** with the metal cations was determined by Hyperquad software.

3. Results and discussion

3.1. Synthesis

The precursors **1** and **2** (Figure 1) were used in the synthesis of the crown ether-amino acid derivatives **3** and **4a-b**.

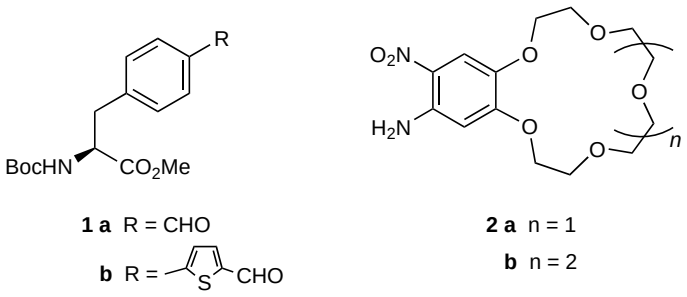
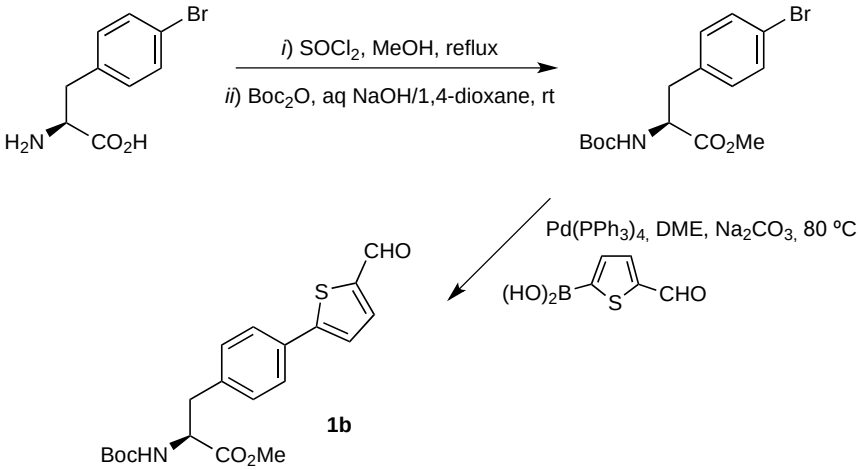
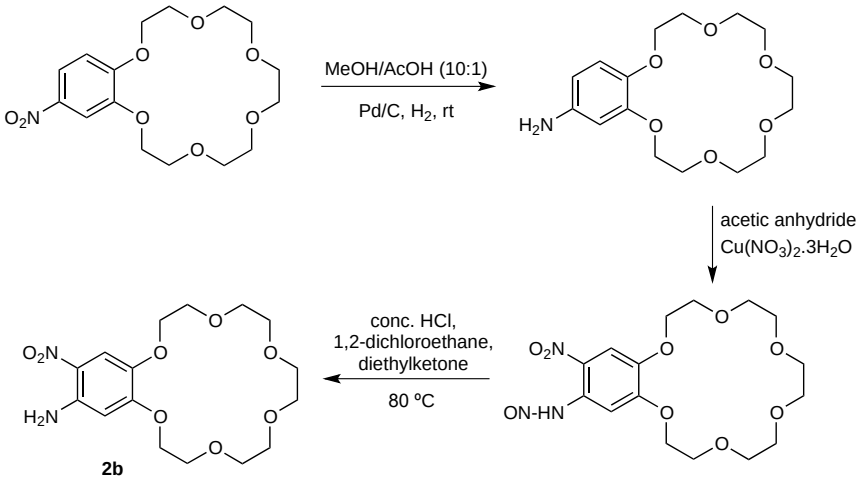


Figure 1. Structure of used precursors **1** and **2**.

N-(*tert*-Butyloxycarbonyl)-4-formyl-L-phenylalanine methyl ester **1a** was synthesised according to a previously published procedure [8] and 4′-amino-5′-nitrobenzo-15-crown-5 **2a** was commercially available. *N*-*tert*-Butyloxycarbonyl-4-(5′-formylthiophen-2′-yl)-L-phenylalanine methyl ester **1b** was obtained by a Suzuki coupling from *N*-(*tert*-butyloxycarbonyl)-4-bromo-L-phenylalanine methyl ester and 2-formyl-5-thiophene boronic acid in moderate yield (57%) (Scheme 1). The precursor 4-bromophenylalanine was commercially available and was protected at its N- and C- terminals by the usual protection methods. The new 4′-amino-5′-nitrobenzo-18-crown-6 **2b** was synthesised from 4′-nitrobenzo-18-crown-6 by reduction of the nitro group (98% yield) and subsequent nitration and denitrosation (91 and 48% yield, respectively) (Scheme 2).



Scheme 1. Synthesis of phenylalanine derivative **1b**.

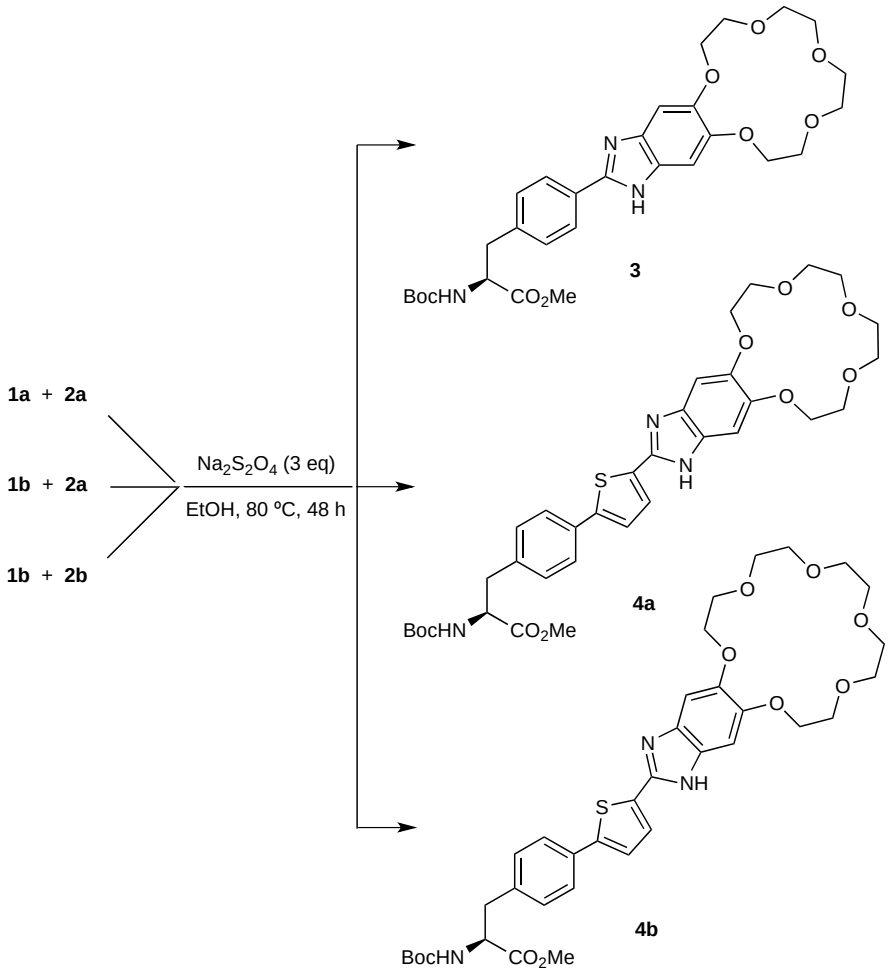


Scheme 2. Synthesis of crown ether derivative **2b**.

Compound **3** was synthesized in 70% yield by condensation of *N*-(*tert*-butyloxycarbonyl)-4-formyl-L-phenylalanine methyl ester **1a** with 4´-amino-5´-nitrobenzo-15-crown-5-ether **2a** in the presence of $Na_2S_2O_4$ in ethanol at 80 ºC, (Scheme 3). Compounds **4a** and **4b** were synthesized from *N*-(*tert*-butyloxycarbonyl)-4-(5´-formylthiophen-2´-yl)-L-phenylalanine methyl ester **1b** and crown ethers **2a** and **2b**, using the same procedure described above, in 40 and 39% yield, respectively. To the best of our knowledge, this is the first time that this synthetic methodology is applied in order to prepare imidazo-benzocrown ether derivatives using as precursors an amino acid, 4´-amino-5´-nitrobenzo-15-crown-5´´ and 4´-amino-5´-nitrobenzo-18-crown-6. The synthesized compounds were fully characterized by the usual spectroscopic techniques.

3.2. Photophysical studies of compounds 3 and 4

The photophysical properties of compounds **3** and **4** in acetonitrile were evaluated and the UV/Vis absorption and emission spectra of degassed 10^{-5} M solutions were measured. Relative fluorescence quantum yields were calculated using 9,10-diphenylanthracene in ethanol as standard (Φ_F = 0.95) [7]. Compounds **3** and **4** exhibited maximum absorption between 325 and 370 nm and emission between 392 and 456 nm. Considering the structure of the compounds, it could be seen that the introduction of the thiophene spacer in **4a** and **4b** induced a bathochromic shift in the absorption band of 45 and 43 nm, respectively, compared to **3** (Table 1, Figure 2 for **3** and **4a** as representative example). This trend was also observed for the emission spectra. The relative fluorescence quantum yields and Stokes' shifts for **4a** and **4b** were also higher than those of **3**, confirming the modulation of the photophysical properties caused by the presence of the thiophene [6d,9].



Scheme 3. Synthesis of crown ether-amino acid derivatives **3** and **4**.

Table 1. Yields, UV-visible absorption and fluorescence data for crown ether benzimidazolyl phenylalanines **3-4** in acetonitrile.

Cpd.	Yield (%)	UV/Vis		Fluorescence			
					Stokes'shift		
		λ_{max}	log ε	λ_{em}	(nm)	(cm^{-1})	Φ_F
3	70	325	4.20	392	67	5259	0.59
4a	40	370	4.15	456	86	5097	0.95
4b	39	368	4.16	456	88	5244	0.94

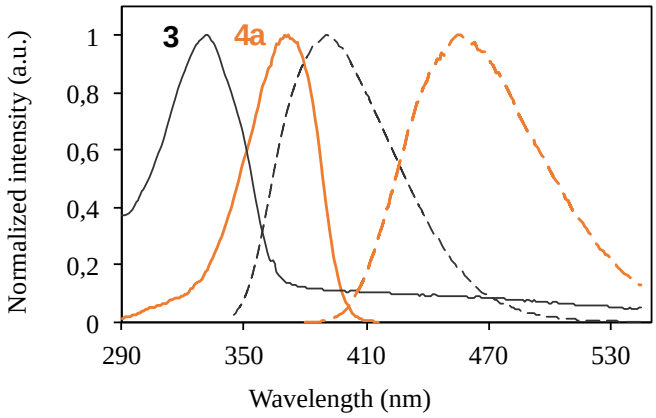


Figure 2. Normalized UV-visible absorption (solid line) and emission spectra (broken line) for compounds **3** and **4a** in acetonitrile (**3**, λ_{exc} = 325 nm; **4a**, λ_{exc} = 370 nm, T = 298 K).

Due to the donor-acceptor character of the synthesised imidazo-benzimidazole crown ethers, it was decided to study the absorption and emission solvatochromic behaviour of derivatives **4** in the presence of protic and aprotic solvents with different polarities such as diethyl ether, 1,4-dioxane, ethanol, acetonitrile, dichloromethane and dimethylsulfoxide (Table 2). Both compounds exhibited positive solvatochromism with respect to their charge transfer (CT) absorption band ($\Delta\upsilon$ = 4444-5161 cm^{-1}). Therefore, compounds **4** appear to be have potential application as solvent polarity probes.

Table 2. Solvatochromic data (λ_{max} and λ_{em}, in nm, and relative fluorescent quantum yields, Φ_F) for benzimidazolyl phenylalanines derivatives **4a-b** with π* values by Kamlet and Taft [10].

		Diethyl ether	Dioxane	EtOH	ACN	DCM	DMSO
	π*	(0.27)	(0.55)	(0.54)	(0.75)	(0.82)	(1.00)
4a	λ_{max} (nm)	363	368	375	370	371	379
	λ_{em} (nm)	435	450	450	456	449	470
	$\Delta\upsilon$ (cm^{-1})	4560	4952	4444	5097	4682	5109
	Φ_F	0.93	0.90	0.91	0.95	0.91	0.90
4b	λ_{max} (nm)	363	369	372	368	368	375
	λ_{em}(nm)	439	447	451	456	449	465
	$\Delta\upsilon$ (cm^{-1})	4769	4729	4709	5244	4902	5161
	Φ_F	0.93	0.90	0.94	0.94	0.92	0.91

3.3. Spectrofluorimetric titrations of compounds 3 and 4 with metallic cations

The modification of phenylalanine through the introduction of UV-active and highly fluorescent heterocycles (benzimidazole, thiophene) and crown ether moieties was expected to provide additional binding sites for a variety of metal ions through the heterocycle donor atoms, as well as improved photophysical properties for the chemosensing studies of compounds **3** and **4a-b**. With these heterocyclic phenylalanine derivatives it was intended to assess the influence of the different π-bridges and of the size of the crown ether at the benzimidazole system in the chemosensing ability of metallic cations.

Considering the biological, environmental and analytical relevance of transition metals 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 interaction of compounds **3-4** with these cations was evaluated through UV-vis and fluorescence spectroscopy in spectrophotometric and spectrofluorimetric titrations in acetonitrile. A preliminary evaluation of the chemosensing ability was performed by addition of 100 equiv of each cation to 1.0×10^{-5} M acetonitrile solutions of **3** and **4a-b** and the changes in the UV-vis absorption and fluorescence spectra were recorded. In the UV-vis absorption spectra, no changes were seen in the bands corresponding to the maximum wavelength of absorption of phenylalanine derivatives **3-4** after addition of up to 100 equiv of each metal cation, but noticeable changes occurred in the emission spectra and in the relative fluorescence quantum yield (Table 3, Figure 3 and Figures S2-S11 in supporting information, for **3** as representative example).

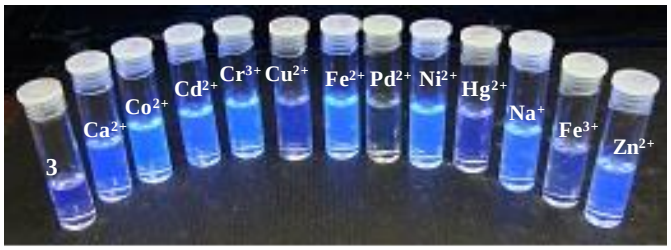


Figure 3. Fluorescence of solutions of phenylalanine **3** (1.0×10^{-5} M) in acetonitrile in the presence of 100 equivalents of several cations, visualised under a 365 nm lamp.

Table 3. Wavelengths of maximum fluorescence (λ_{em}) and relative fluorescent quantum yields (Φ_F) for benzimidazolyl phenylalanines **3**-**4**, in acetonitrile, in the presence of 100 equivalents of several cations ([**3**] = [**4**] = 1.0×10^{-5} M).

Cation	**3**		**4a**		**4b**	
	λ_{em} (nm)	Φ_F	**λ_{em} (nm)**	Φ_F	**λ_{em} (nm)**	Φ_F
Ca^{2+}	366	0.44	433	0.98	438	0.98
Co^{2+}	430	0.55	472	0.61	472	0.15
Cd^{2+}	422	0.38	473	0.64	460	0.45
Cr^{3+}	428	0.55	460	0.59	461	0.66
Cu^{2+}	433	0.01	433	0.001	456	0.004
Fe^{2+}	435	0.57	480	0.86	473	0.99
Pd^{2+}	432	0.02	474	0.001	444	0.003
Ni^{2+}	430	0.57	476	0.73	463	0.99
Hg^{2+}	435	0.16	474	0.90	469	0.001
Na^{+}	382	0.44	441	0.99	449	0.91
Fe^{3+}	428	0.42	421	0.01	457	0.001
Zn^{2+}	366	0.44	446	0.76	441	0.96

Therefore, spectrofluorimetric titrations of acetonitrile solutions of **3**-**4** were performed to understand where and how the interaction with the different metal ions was occurring. The stronger interaction for compound **3** was observed in the presence of Pd^{2+} and Cu^{2+} with only 1.6 and 3.0 equivalents being enough to quench the emission, as a CHEQ (chelation enhancement of the quenching) effect in the

fluorescence emission for both metals was observed (Figure 4, for **3** as representative example). Interaction with Pd^{2+} quenched almost 80% of the initial fluorescence, while for Cu^{2+} a complete quenching of the fluorescence was achieved, accompanied by a red shift of the emission band in both cases. The quenching effect observed could be attributed to an energy transfer quenching of the π* emissive state through low-lying metal-centred unfilled *d*-orbitals for Pd^{2+} and Cu^{2+}, and to an intersystem crossing mechanism due to the heavy atom effect [4d,e,9].

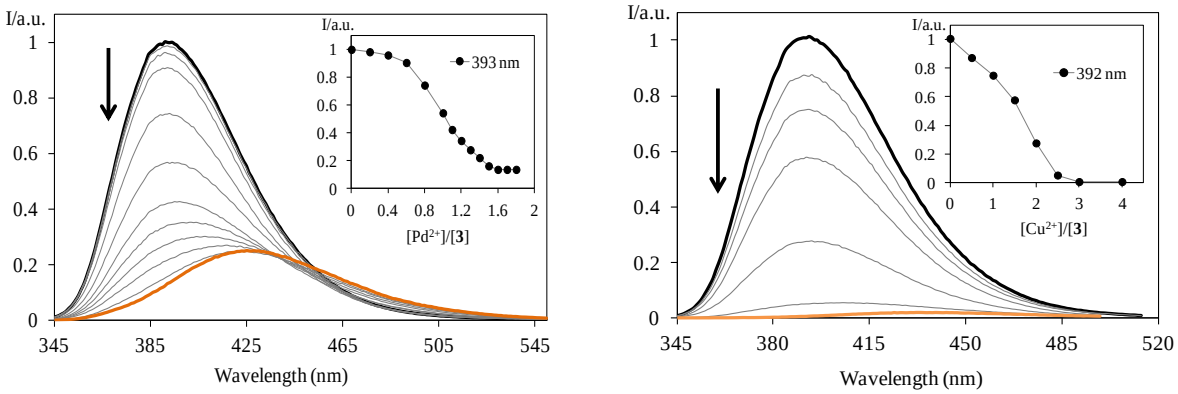


Figure 4. Spectrofluorimetric titrations of compound **3** in the presence of $[Pd(CH_3CN)_4(BF_4)_2]$ (left) and $[Cu(ClO_4)_2 \cdot 6H_2O]$ (right), in acetonitrile solution. ([**3**] = 1.0×10^{-5} M, T = 298K, λ_{exc} = 325 nm). Inset: normalised emission as a function of added metal equivalents.

A similar quenching behaviour was observed for phenylalanines bearing a thiophene spacer **4a** and **4b** (Figure 5, for **4a** as representative example), although it required a larger amount of cation to ensure complete quenching of the fluorescence (between 40 and 120 equiv), when compared to phenylalanine **3**. The stronger interaction for compound **4a** was observed in the presence of Fe^{3+}, Pb^{2+} and Pd^{2+}, with a complete quenching of the fluorescence, and interaction with Cu^{2+} quenched almost 70% of the initial fluorescence. In the case of compound **4b**, it displayed similar chemosensory ability with a complete quenching of the emission upon interaction with Cu^{2+}, Fe^{3+}, Hg^{2+} and Pd^{2+}, but with decreased selectivity as several other cations resulted in 80-90% fluorescence quenching (Co^{2+}, Cd^{2+}, Pb^{2+}, Cr^{3+} and Fe^{2+}).

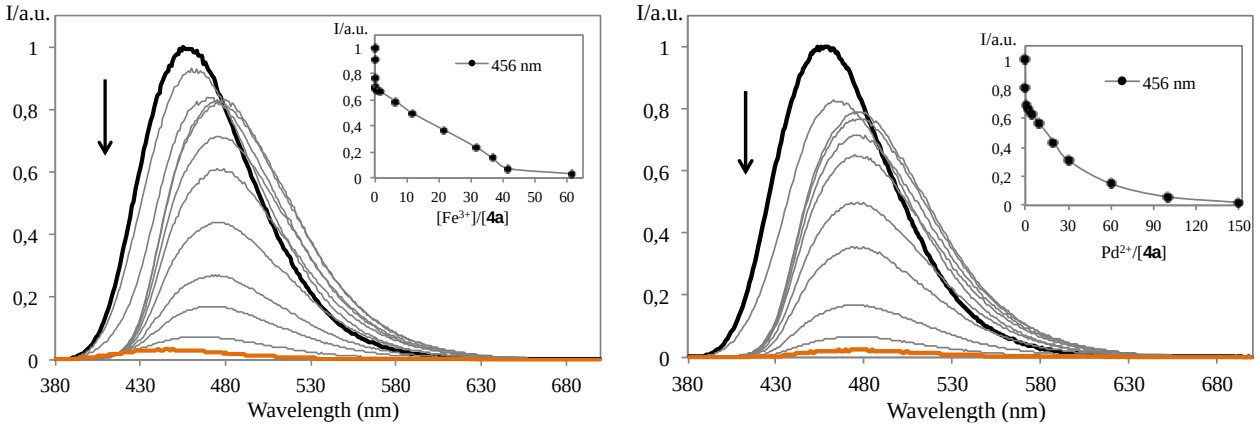


Figure 5. Spectrofluorimetric titrations of compound **4a** in the presence of $[Fe(ClO_4)_3 \cdot 6H_2O]$ (left) and $[Pd(CH_3CN)_4(BF_4)_2]$ (right), in acetonitrile solution. ([**4a**] = 1.0×10^{-5} M, T = 298K, λ_{exc} = 370 nm). Inset: normalised emission at 456 nm as a function of added metal equivalents.

In the case of the other metal cations, a ~ 20-60% quenching was also observed with the growth of a new red-shifted band upon metal addition (for compound **3**, between 422 and 435 nm for Cr^{3+}, Cd^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+} and Fe^{2+}), whereas a blue shift of the original emission band was observed for the remaining cations (for compounds **3** and **4**, for Na^{+}, Ca^{2+}, and Zn^{2+}) (see Table 3). The presence of Ca^{2+}, Na^{+}, and Zn^{2+} induced a blue shift of the emission band suggesting interaction with the crown ether moiety, with the coordination of cations reducing the donor character of the crown ether and producing a hypsochromic shift through a modulation of the HOMO and LUMO energy. On the other hand, the red shifted band in the presence of the other metals should be related to coordination at the imidazo NH group.

It is well known that the benzo-15-crown-5-ether chelating unit is used specifically for Na^{+} sensing. However, the minor spectral changes induced by interaction with Na^{+} suggest that the delocalized charge present in this system could additionally modulate the interaction with the crown ether resulting in small changes in the ground and the excited state, as well as the counter ion [4b,9,11].

Additional proof of the different coordination sites was obtained by performing a spectrofluorimetric titration of Cu^{2+} with prior addition of 5 equivalents of Ca^{2+}. In this experiment, the two effects were combined: chelation of Ca^{2+} induced a 30 nm blue shit of the emission band and subsequent chelation of Cu^{2+} quenched the shifted band. This result confirms that compound **3** can is capable of simultaneous detection of two different metal ion analytes (Figure 6).

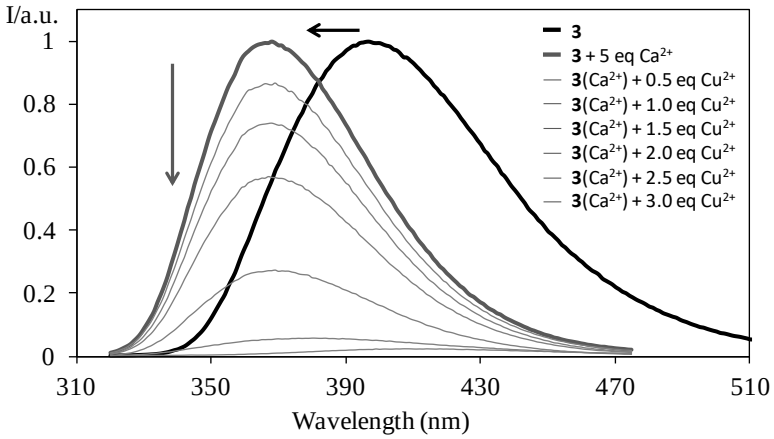


Figure 6. Spectrofluorimetric titration of compound **3** in the presence of 5 equivalents of Ca^{2+} and subsequent addition of increasing amounts of Cu^{2+}, in acetonitrile solution ([**3**] = 1.0×10^{-5} M, T = 298K, λ_{exc} = 325 nm).

Having in mind practical applications of compounds **3** and **4** in aqueous media the chemosensory ability was also evaluated in mixtures of acetonitrile and water in varying proportions. The best results were obtained in acetonitrile/H_2O (80:20, v/v). The study of the same cations described before in organic aqueous solution lead to the selective and sensitive fluorescent detection of Pd^{2+} and Hg^{2+} for receptor **3**. Due to insolubility problems it was not possible to perform the same studies with compounds **4a-b**. For compound **3**, the number of necessary metal equivalents to achieve a plateau was between 20 to Pd^{2+} and 40 equivalents for Hg^{2+} (Figure 7).

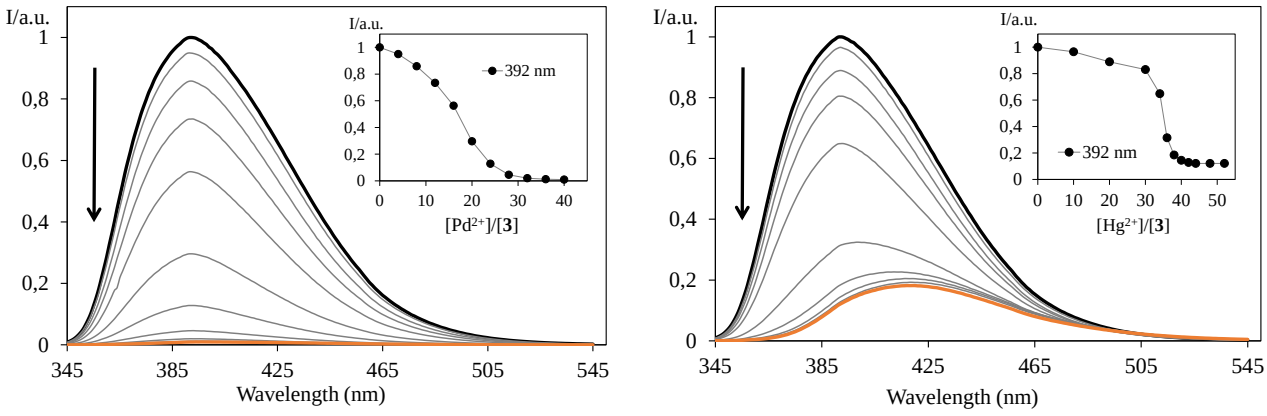


Figure 7. Spectrofluorimetric titration of compound **3** in the presence of $[Pd(CH_3CN)_4(BF_4)_2]$ (left) and $[Hg(ClO_4)_2 \cdot 3H_2O]$ (right), in acetonitrile/H_2O (80:20) ([**3**] = 1.0×10^{-5} M, T = 298K, λ_{exc} = 325 nm). Inset: normalised emission at 392 nm as a function of added metal equivalents.

3.4. ^{1}H NMR titration for compound 3

^{1}H NMR spectroscopy was used to investigate the nature of cation coordination, and experiments were carried out in acetonitrile-d_3. A ^{1}H NMR titration using firstly Ca^{2+}, followed by paramagnetic Cu^{2+} was performed. In more detail, the addition of Ca^{2+} promoted remarkable shifts only in the crown ether CH_2 protons. All the signals of the CH_2 protons, initially appearing at about 3.65–4.15 ppm were shifted downfield to 3.82–4.35 ppm and became broader during the addition of Ca^{2+} until 4.0 equivalents. The subsequent addition of 1.0 equivalent of Cu^{2+} resulted in slight downfield shifts for the aromatic protons signals, becoming broader with overlapping of some signals (Figure 8). This experiment corroborated the different coordination sites suggested for the complexation pathway (see also figure 6).

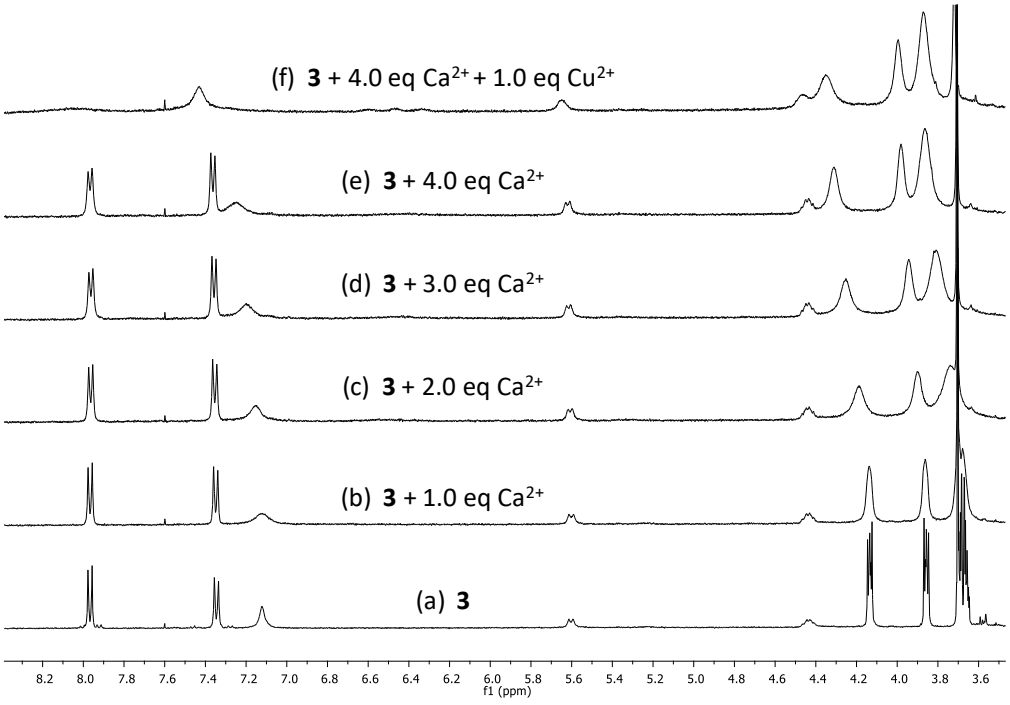


Figure 8. Partial ^{1}H NMR spectra of compound **3** (7.1 x 10^{-2} M) in acetonitrile-d_3 in (a) the absence and (b) the presence of 1.0, (c) 2.0, (d) 3.0, (e) 4.0 equivalents of Ca^{2+} and (f) 1.0 equivalents of Cu^{2+}.

The association constants of compounds **3** and **4a-b** with the various cations were obtained from the spectroscopic titrations using the Hyperquad program (Table 4) [12]. For compound **3**, the suggested metal to ligand (M:L) stoichiometry for all cations is 1:1, whereas for compounds **4a-b** is 1:2.

Table 4. Logarithm of the association constants (K_{ass}) for the interaction of phenylalanines **3**-**4** with several cations in acetonitrile (M:L stoichiometry is 1:1 for **3** and 1:2 for **4a**-**b**).

Cation	log K_{ass}		
	3	**4a**	**4b**
Cu^{2+}	7.800 ± 0.012	10.86 ± 0.22	12.91 ± 0.17
Cd^{2+}	3.665 ± 0.011	[a]	16.33 ± 0.30
Co^{2+}	3.571 ± 0.011	7.947 ± 0.014	16.02 ± 0.65
Ca^{2+}	[a]	11.70 ± 0.23	14.73 ± 0.46
Pd^{2+}	7.488 ± 0.025	11.17 ± 0.55	12.29 ± 0.40
Fe^{2+}	4.776 ± 0.005	13.27 ± 0.87	13.53 ± 0.24
Fe^{3+}	7.246 ± 0.030	10.74 ± 0.42	12.30 ± 0.24
Ni^{2+}	5.495 ± 0.088	11.54 ± 0.24	[a]
Zn^{2+}	3.400 ± 0.048	11.84 ± 0.22	13.66 ± 0.23
Hg^{2+}	7.371 ± 0.048	11.66 ± 0.25	12.21 ± 0.18
Na^{+}	[a]	12.49 ± 0.38	16.83 ± 0.91
Cr^{3+}	6.858 ± 0.085	11.08 ± 0.35	15.98 ± 0.74

[a] No reliable results were obtained.

4. Conclusions

Compounds **3**-**4** were synthesized in moderate to good yields (39-70%) using several synthetic methodologies.

The different crown ether binding moieties as well as the electronic nature and the length of the π-bridge linked to the benzimidazole allowed the tuning of the sensory properties as seen by spectrofluorimetric titrations, in acetonitrile and acetonitrile/H_2O. Therefore, benzocrown ether amino acid **3** is a fluorimetric chemosensor, being selective and sensitive for Cu^{2+} and Pd^{2+} in aqueous

solutions (acetonitrile/H_2O; 80:20). On the other hand, the metal cation sensing properties displayed by compound **4a** bearing an arylthienyl spacer showed that this is a promising candidate as fluorimetric chemosensor for Fe^{3+}, Pb^{2+} and Pd^{2+} in acetonitrile solution. Compound **4b** functionalized with the 4'-amino-5'-nitrobenzo-18-crown-6 exhibited a decrease of selectivity since a complete fluorescence quenching or a 80-90% quenching of fluorescence was achieved for several cations (Co^{2+}, Cu^{2+} Fe^{3+}, Hg^{2+}, Pd^{2+}, Cd^{2+}, Cr^{3+} and Fe^{2+}).

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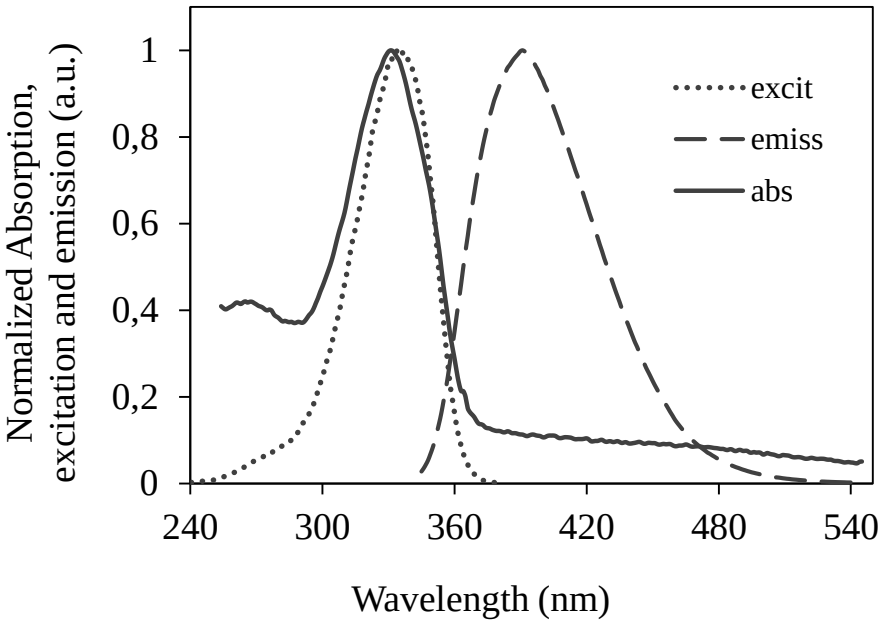

Figure S1. Absorption, emission and excitation spectra of compound **3** in ACN/H_2O (80:20) solution.

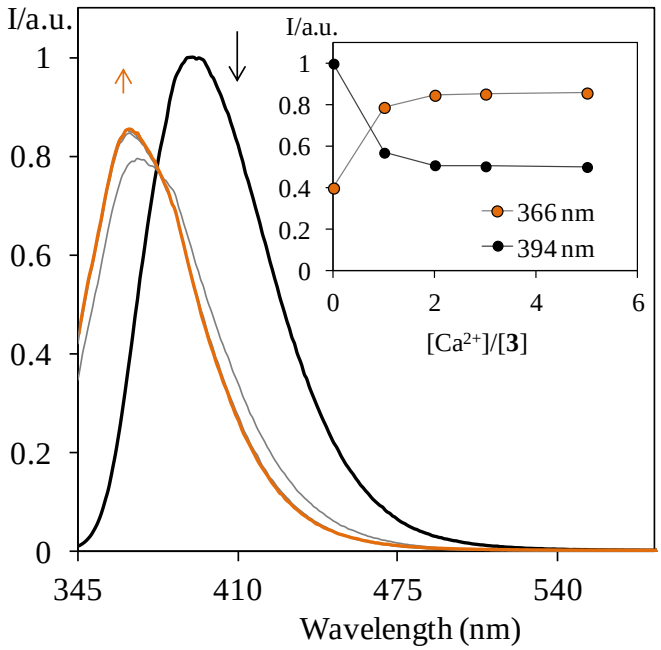

Figure S2. Spectrofluorimetric titrations of compound **3** in the presence of Ca^{2+}, in acetonitrile solution. ([**3**]= 10 μM, T = 298 K, λ_{exc} = 325 nm). Inset: normalised emission at 366 nm and 394 nm as a function of added metal equivalents.

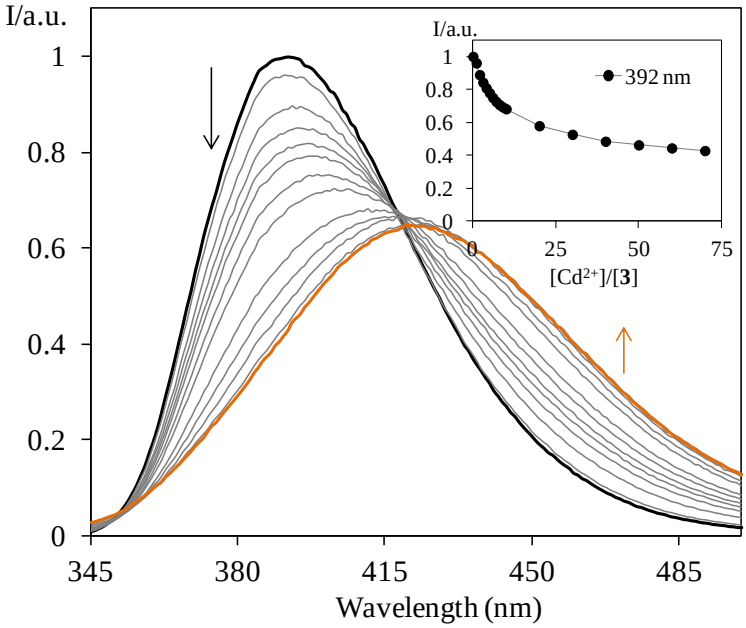

Figure S3. Spectrofluorimetric titrations of compound **3** in the presence of Cd^{2+}, in acetonitrile solution. ([**3**]= 10 μM, T = 298 K, λ_{exc} = 325 nm). Inset: normalised emission at 392 nm as a function of added metal equivalents.

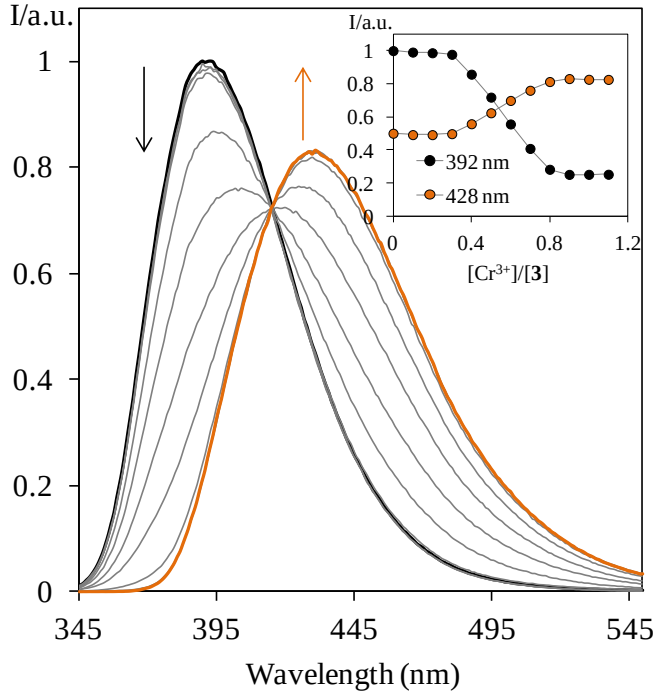

Figure S4. Spectrofluorimetric titrations of compound **3** in the presence of Cr^{3+}, in acetonitrile solution. ([**3**]= 10 μM, T = 298 K, λ_{exc} = 325 nm). Inset: normalised emission at 392 nm and 428 nm as a function of added metal equivalents.

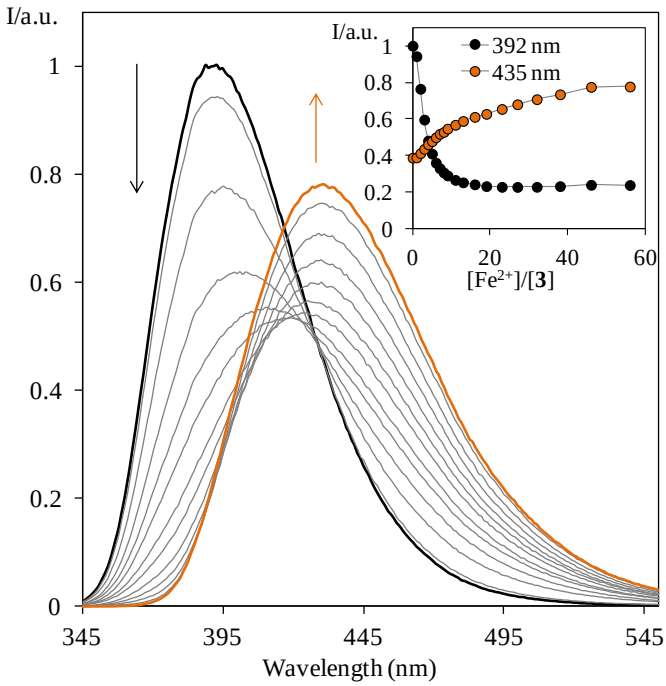

Figure S5. Spectrofluorimetric titrations of compound **3** in the presence of Fe^{2+}, in acetonitrile solution. ([**3**]= 10 μM, T = 298 K, λ_{exc} = 325 nm). Inset: normalised emission at 392 nm and 435 nm as a function of added metal equivalents.

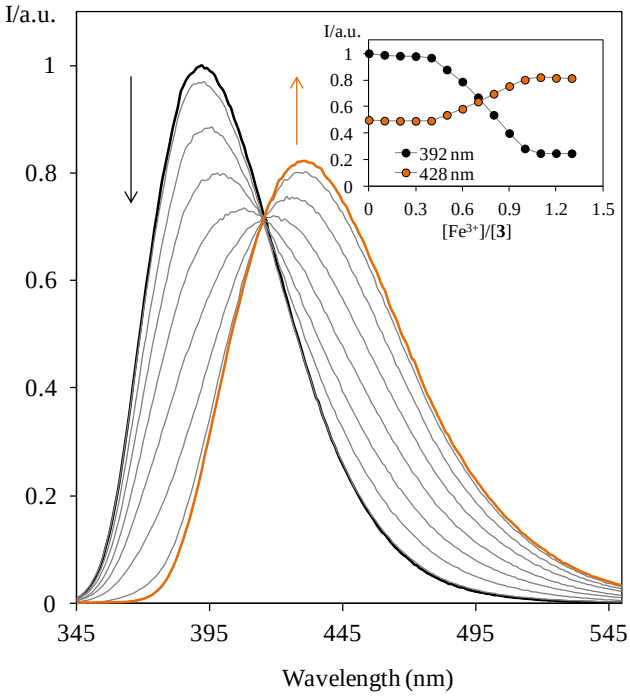

Figure S6. Spectrofluorimetric titrations of compound **3** in the presence of Fe^{3+}, in acetonitrile solution. ([**3**]= 10 μM, T = 298 K, λ_{exc} = 325 nm). Inset: normalised emission at 392 nm and 428 nm as a function of added metal equivalents.

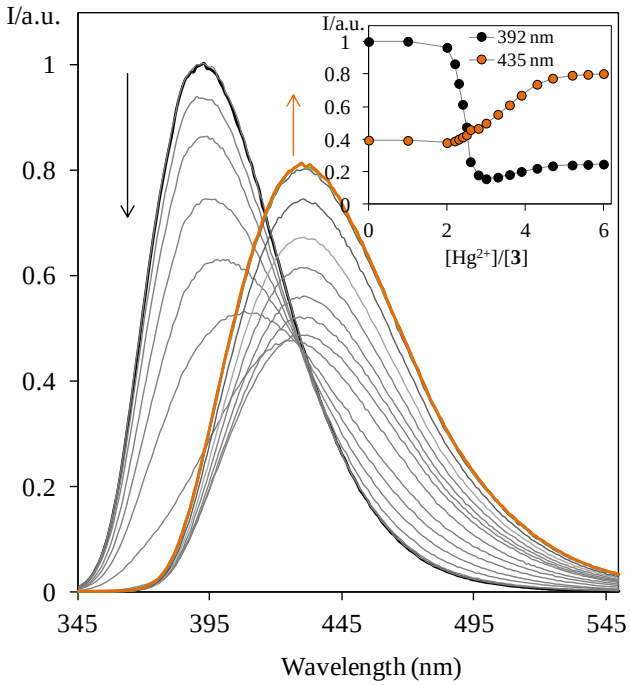

Figure S7. Spectrofluorimetric titrations of compound **3** in the presence of Hg^{2+}, in acetonitrile solution. ([**3**]= 10 μM, T = 298 K, λ_{exc} = 325 nm). Inset: normalised emission at 392 nm and 435 nm as a function of added metal equivalents.

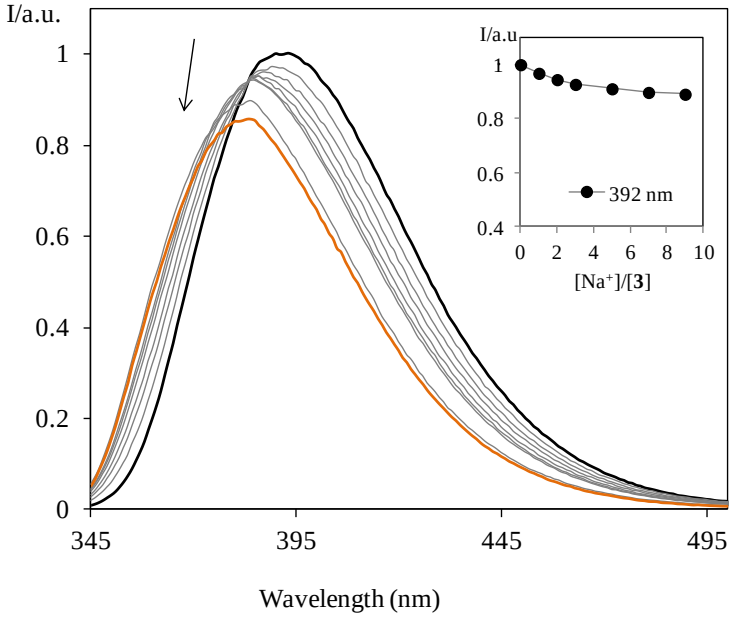

Figure S8. Spectrofluorimetric titrations of compound **3** in the presence of Na^{+}, in acetonitrile solution. ([**3**]= 10 μM, T = 298 K, λ_{exc} = 325 nm). Inset: normalised emission at 392 nm as a function of added metal equivalents.

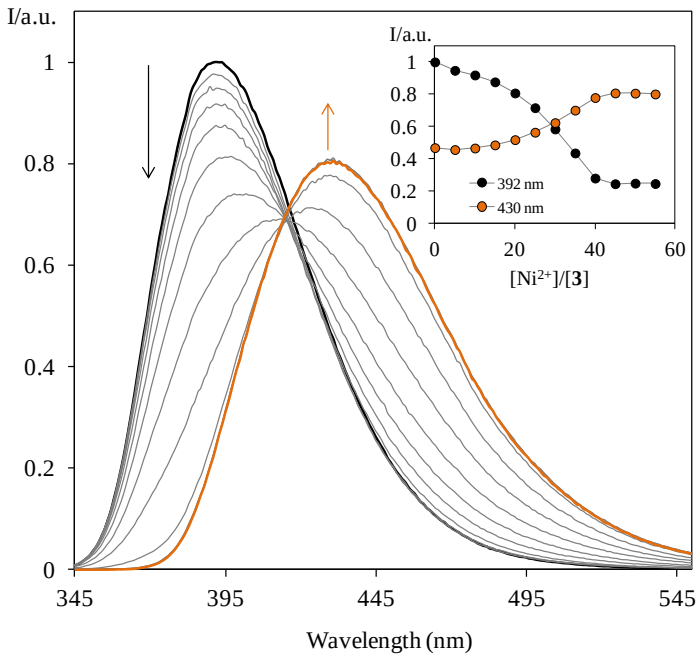

Figure S9. Spectrofluorimetric titrations of compound **3** in the presence of Ni^{2+}, in acetonitrile solution. ([**3**]= 10 μM, T = 298 K, λ_{exc} = 325 nm). Inset: normalised emission at 392 nm and 430 nm as a function of added metal equivalents.

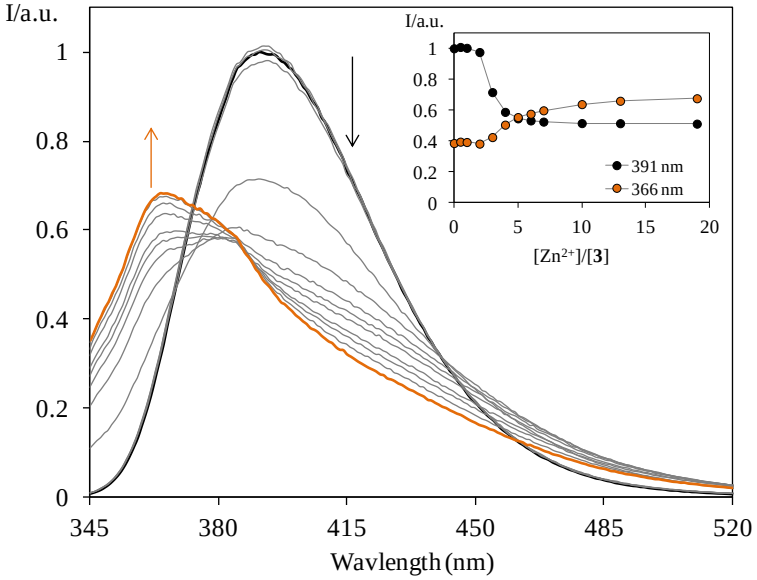

Figure S10. Spectrofluorimetric titrations of compound **3** in the presence of Zn^{2+}, in acetonitrile solution. ([**3**]= 10 μM, T = 298 K, λ_{exc} = 325 nm). Inset: normalised emission at 391 nm and 366 nm as a function of added metal equivalents.

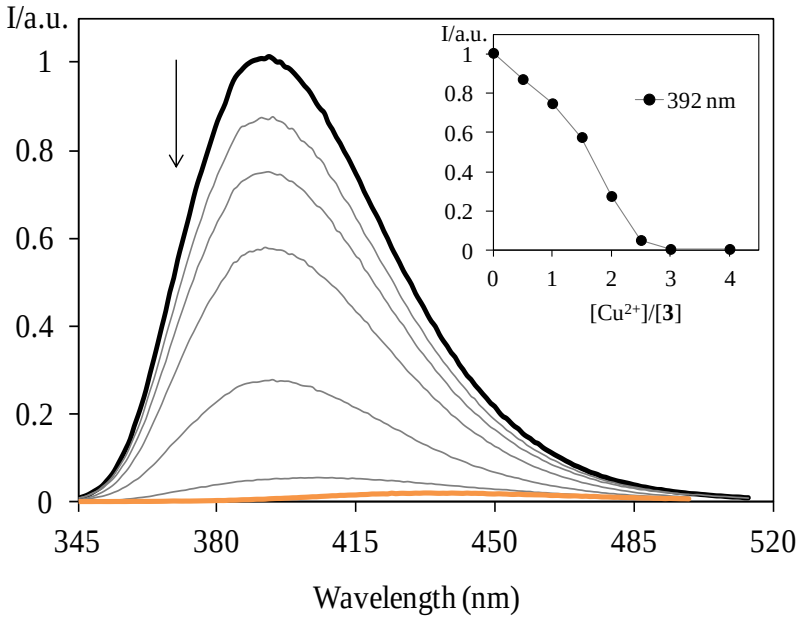

Figure S11. Spectrofluorimetric titrations of compound **3** in the presence of Cu^{2+}, in acetonitrile solution. ([**3**] = 10 μM, T = 298 K, λ_{exc} = 325 nm). Inset: normalised emission at 392 nm as a function of added metal equivalents.