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Synthesis and evaluation of benzothiazolyl and benzimidazolyl asparagines as amino acid based selective fluorimetric chemosensors for Cu^{2+}

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ABSTRACT

A series of emissive *N-tert*-butyloxycarbonyl benzyl ester asparagines bearing benzothiazole and benzimidazole units at the side chain, functionalised with electron donor or acceptor groups, were evaluated as novel amino acid based fluorimetric chemosensors for transition metal cations, such as Cu^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+}. Selective removal of the protecting groups at the N- and C-terminals was carried out in order to assess the influence of the presence of blocking groups on the overall coordination ability. The results indicate that there is a strong interaction through the donor N, O and S atoms at the side chain of the various asparagines, with high selectivity towards Cu^{2+} in a 1:1 complex stoichiometry. Association constants and detection limits for Cu^{2+} were calculated. The photophysical and metal ion sensing properties of these asparagines suggest that they can be suitable for incorporation into peptidic chemosensor frameworks.

1. Introduction

The interest in the design of synthetic receptors for ions and molecules of biological interest has grown steadily from early work on cyclodextrin or single crown ether complexation to a wide array of receptor shapes and structures. The construction of fluorescent synthetic molecules capable of recognizing and binding organic and inorganic molecules involved in biological pathways is a current thrust in molecular recognition.[1–10] Fluorescent sensors are preferred because they are well suited to meet the need for in vivo probes, such as mapping the spatial and temporal distribution of the biological analytes, and they have other advantages including multiple modes of detection (such as fluorescence quenching, enhancing, life time), extremely high sensitivity, relatively low cost and easy availability.[1] Molecular recognition is the basis for most biological functions and, given the high degree of control over biological systems that Nature can get, in recent years the research on molecular recognition has evolved to mimic as much as possible the natural mechanisms of organization.[11]

Amino acids, and hence peptides, are known for their ability to complex metal ions because they possess nitrogen, oxygen and sulfur donor atoms at the main chain, as amino and carboxyl terminals, and at the side chain, as in the case of serine and tyrosine (oxygen), lysine and histidine (nitrogen), aspartic acid and glutamic acid (oxygen) or cysteine and methionine (sulfur).[12] Thus, the design of peptides that coordinate metals, by incorporation of modified amino acids, has potential for applications as varied as the study on protein–protein interactions mediated by metals and protein binding to nanoparticles and metal surfaces, and the development of selective chemical sensors for metals for use in vivo and in vitro.[13,14] The insertion of the coordination centres into amino acids or peptide enables the construction of supramolecules, which may play an innovative role in molecular recognition. Apart from the importance of metal complexation by amino acids in a protein, the application of amino acids in the detection of metals both in solution[9,15] and in solid phase through incorporation in polymeric materials[16,17] has been encouraged. This requires modified amino acids bearing a metal ion chelating site for stable complex formation for incorporation into a peptide sequence. Fluorescent ligands, which are mostly heteroaromatic ring systems often substituted by potentially chelating groups, which act as both the recognition and signalling site, can be used in the synthesis of peptide based chemosensors, as reported recently with ligands that are capable to chelate various metal ions and whose complexes possess diversified photophysical properties.[7–10]

Unnatural amino acids with intrinsic functions have been increasingly studied, to be incorporated into peptides.[18,19] A strategy for the development of such compounds involves the incorporation of multidentate complexing ligands in amino acid residues in the form of heterocyclic moieties. Amino acids bearing 2,2′-bipyridine and 1,10-phenanthroline have been used in the synthesis of metallopeptides that bind Zn^{2+}.[20–22] Histidine participates actively in

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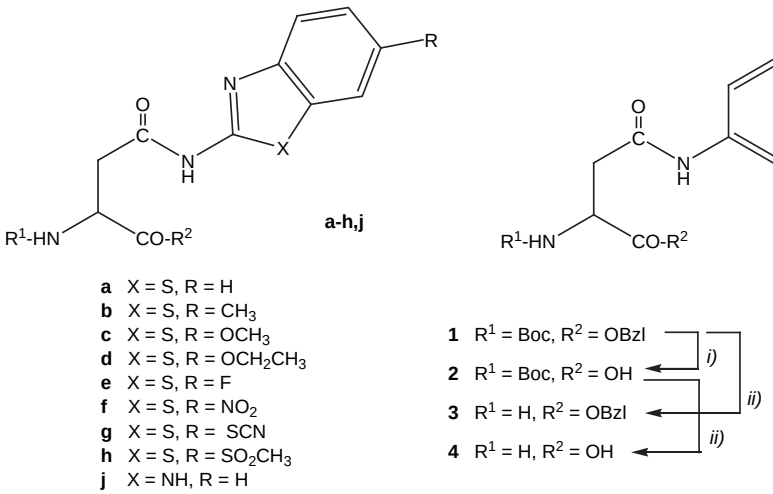
the coordination of Zn^{2+} in various proteins and imidazole isosters mimics were proposed in order to mimic the side chain of the natural residue, containing a complexing group attached to the β-carbon via a 1,2,3-triazole.[23] Also, histidine in the N-terminal position of a peptide binds metals through its terminal nitrogen and the imidazole nitrogen, a process analogous to histamine coordination.[24] Hydroxyquinolines are also known to complex with metals and thus the synthesis of a (dihydroxyquinolinyl)-alanine from L-tyrosine was proposed.[25] The complexes formed between metal ions and amino acids can be considered as models for biochemical interactions, such as substrate-enzyme and other metal-mediated interactions with metal ions, with many Cu^{2+} complexes playing a decisive role in biological systems and, as such, heterocyclic nitrogen ligands like benzimidazole and creatinine have been used in the synthesis of ternary complexes of Cu^{2+} with dipeptides, such as Gly-Gly, Gly-Ala, Gly-Val, Gly-Tyr, Gly-Trp-Gly and Ala.[26]

Our current research interests include the synthesis and characterization of unnatural amino acids,[27–29] imidazole and benz-X-azole derivatives with interesting optical properties[30–34] and innovative heterocyclic colorimetric/fluorimetric chemosensors for anions and cations containing (oligo)thiophene, benzoxazole and amino acid moieties.[35–39] The aim of this report is the synthesis and evaluation of novel heterocycle-based unnatural amino acids as fluorimetric chemosensors for the recognition of metallic cations of analytical, biological, environmental and medicinal relevance, through the combination of several sulfur and nitrogen heterocycles as coordinating/reporting units with an amino acid core in order to obtain new chemosensors. By using π-conjugated bridges of heterocyclic nature it is intended to improve the intramolecular electronic delocalisation, for the enhancement of the photophysical properties of the new sensors and the optimization of the recognition process of target analytes, through higher fluorescence and thus higher sensitivity, which represents a challenging goal in biomimetic and supramolecular chemistry.



Scheme 1. Synthesis of benz-X-azolyl asparagines **2a**–**j**, **3** and **4**. Reagents and conditions: (i) 1,4-dioxane, aq NaOH 6 M (1 M for compound **2g**), rt; (ii) trifluoroacetic acid/dichloromethane (1:1), rt.

2. Results and discussion

2.1. Synthesis of N- and C-terminus deprotected benz-X-azolyl asparagines 2a–j, 3 and 4

The new deprotected asparagines were obtained from the corresponding *N-tert*-butyloxycarbonyl asparagines benzyl ester precursors **1a**–**j**, previously synthesised by us,[39] by standard selective deprotection at their C- and N-terminus: *N*-Boc asparagines **2a**–**j** resulted from basic ester hydrolysis, while treatment of **1e** and **2e** in acidic medium afforded benzyl ester asparagine **3** and fully deprotected asparagine **4**, respectively, in good to excellent yields (these additional deprotections on the fluoro derivative were due to its interesting photophysical properties) (Scheme 1, Table 1). The resulting compounds present a benzothiazole or a benzimidazole at its side chain, bearing substituents of different electronic character (electron donor: alkyl and alkoxyl; electron acceptor: fluoro, nitro, sulfomethyl and thiocyano) and with different position of attachment of the amino acid to the heterocycle (position 2 or 6) and were fully characterised by the usual spectroscopic techniques.

Table 1
Yields, UV/vis absorption and fluorescence data for benz-X-azolyl asparagines **2a**–**j**, **3** and **4** in absolute ethanol

Compd.	X	R	Yield (%)	UV/vis		Fluorescence		
				λ_{max}	log ε	λ_{em}	Stokes' shift (cm^{-1})	Φ_F
2a	S	H	86	274	4.08	325	5727	0.04
2b	S	CH_3	70	279	4.10	325	5073	0.02
2c	S	OCH_3	81	290	4.08	337	4809	0.02
2d	S	OCH_2CH_3	85	290	4.07	357	6472	0.03
2e	S	F	81	274	4.03	375	9830	0.06
2f	S	NO_2	74	330	4.09	366	2981	0.01
2g	S	SCN	58	283	4.17	329	4941	0.02
2h	S	SO_2CH_3	81	282	4.26	329	5066	0.03
2i	S	—	88	281	4.07	327	5006	0.04
2j	NH	H	79	283	4.03	329	4941	0.03
3	S	F	61	277	4.06	360	8323	0.80
4	S	F	58	278	3.99	357	7200	0.08

From the results in Table 1, it can be seen that the yields, from 70 to 88%, for benz-X-azolyl asparagines **2a**–**j** are not influenced by the electronic nature of the heterocycle at the side chain (benzothiazole or benzimidazole) or by the substituent at position 6 of the benzothiazole, with the exception of compound **2g** (bearing a thiocyano group) that was obtained in lower yield probably due to hydrolysis of the substituent (as seen by IR spectroscopy), even after using a less concentrated basic solution.

2.2. Photophysical study of benz-X-azolyl asparagines 2a–j, 3 and 4

The absorption and emission spectra of asparagines **2a**–**j**, **3** and **4** were measured in absolute ethanol (10^{-6}–10^{-5} M solution)

(Table 1). Compounds **2a** and **2i**, which only differ in the position of attachment of the benzothiazole unit to the amino acid side chain, and compounds **2a** and **2j**, which bear different side chain heterocycles, showed similar absorption and emission data. According to the electronic character of the substituent in **2b**–**i**, bathochromic shifts were observed for the wavelength of maximum absorption and emission, i.e., when **2a** was compared with electron donor ethoxy asparagine **2d** and electron acceptor nitro asparagine **2f**, with wavelength of maximum absorption at 290 and 330 nm, respectively. The relative fluorescence quantum yields were determined using a 10^{-6} M solution of 9,10-diphenylanthracene in ethanol as standard (Φ_F=0.95)[40] and benz-X-azolyl asparagines **2a**–**j** exhibited modest fluorescence quantum yields in ethanol, with the highest value for the fluoro derivative **2e** (0.06). There is a striking difference between the Φ_F of **2a**–**j** and those of parent compounds **1a**–**j** in ethanol,[39] that could be attributed to the possibility for formation of intra and intermolecular H-bonds between the carboxylic acid proton and the heteroatoms at the side chain. This fact could explain the high fluorescence quantum yield of *C*-protected asparagine **3** when compared to **2a**–**j** and **4**, with a free carboxylic acid group.

As the chemosensing study towards different metallic cations was carried out in acetonitrile, the absorption and emission spectra of asparagines **1a**–**j**,[39] **2a**–**j**, **3** and **4** were also measured in this solvent (10^{-6}–10^{-5} M solution) (Table 2).

Table 2
UV/vis absorption and fluorescence data for benz-X-azolyl asparagines **1a**–**j**,[39] **2a**–**j**, **3** and **4** in acetonitrile

Compd.	UV/vis	Fluorescence		Compd.	UV/vis	Fluorescence	
	λ_{max}	λ_{em}	Φ_F		λ_{max}	λ_{em}	Φ_F
1a	295	360	0.21	**2a**	296	357	0.02
1b	290	369	0.41	**2b**	291	352	0.02
1c	290	360	0.44	**2c**	289	362	0.06
1d	290	359	0.47	**2d**	288	353	0.05
1e	275	360	0.65	**2e**	275	360	0.17
1f	280	360	0.17	**2f**	280	355	0.01
1g	290	359	0.42	**2g**	290	354	0.04
1h	290	361	0.44	**2h**	290	353	0.10
1i	280	358	0.41	**2i**	283	353	0.13
1j	290	359	0.35	**2j**	280	320	0.03
3	274	356	0.59	**4**	281	359	0.19

It can be seen that *N*- and *C*-protected asparagines **1a**–**j** display higher fluorescence quantum yields than the *C*-deprotected **2a**–**j** in acetonitrile (as already seen in ethanol and for the same reason). The derivatives bearing a fluoro group had the highest Φ_F (0.65 for **1e** and 0.17 for **2e**) and also the largest Stokes' shift (ca. 8580 cm^{-1}). *N*-Protected fluoro asparagine **3** displayed similar Φ_F to that of **1e**, while fully deprotected fluoro asparagine **4** was comparable to **2e**, confirming the detrimental effect of a free carboxylic group on Φ_F.

2.3. Spectrophotometric and spectrofluorimetric titrations with metallic cations

The modification of asparagines through the introduction of a UV-active and highly fluorescent heterocycle at its side chain is 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. With heterocyclic asparagines **1a**–**j**, **2a**–**j**, **3** and **4** it was intended to assess the influence in the chemosensing ability of metallic cations of: (i) the type of heterocycle; (ii) the substituent at the heterocycle; (iii) the position of attachment of the heterocycle; and (iv) the presence of a protecting group at the amino acid C- and N-terminals.

Considering the biological, environmental and analytical relevance of transition metals such as Cu^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+}, the interaction of asparagines **1** to **4** with these cations was evaluated through UV/vis and fluorescence spectroscopies in spectrophotometric and spectrofluorimetric titrations in acetonitrile, through the sequential addition of increasing metal equivalents (defined as the ratio of metal ion to ligand concentration).

In the spectrophotometric titrations, no changes were seen in the bands of the absorption spectra of asparagines **1** to **4** after addition of up to 800 equiv of each metal cation. In the spectrofluorimetric titrations with Cu^{2+}, a strong decrease of the fluorescence intensity (a chelation-enhanced quenching, CHEQ effect) was observed for all the asparagines, with a small number of metal equivalents being necessary to quench fluorescence to a large extent. In Figure 1A is shown the spectrofluorimetric titration of asparagines **1e** with Cu^{2+}, where the drastic effect of ion complexation is evident in the band centred at the wavelength of maximum emission at 360 nm. This figure is representative of the Cu^{2+} titrations of asparagines **1a**–**j** (protected at the N- and C-terminus), **2a**–**j** (protected at the N-terminus), **3** (protected at the C-terminus) and **4** (free N- and C-terminus), the only difference between them being the number of metal equivalents necessary to quench the initial fluorescence (before complexation) of the heterocyclic amino acid (see Supplementary data for spectrofluorimetric titrations of **1a**–**j**, **2a**–**j**, **3** and **4** with Cu^{2+}).

With regard to the other cations Zn^{2+}, Co^{2+} and Ni^{2+}, a less pronounced CHEQ effect was also observed for a large number of added metal equivalents, without complete quenching of fluorescence. For example, after the addition of ca. 800 equiv of Zn^{2+}, there was a decrease in fluorescence of 30% for **1a**, no change for **2a** and a decrease of 45% for **1e** and **2e**. As for Co^{2+}, upon the addition of 700 equiv to **1a** a decrease of 40% was seen, while for **2a** a 50% decrease was achieved with the addition of 200 equiv. For **1e** and **2e** the addition of 800 equiv was accompanied by a 40% and 60% decrease, respectively. Titration with Ni^{2+} lead to similar results: for **1a** the addition of 1100 equiv caused a 70% quenching and for **2a** a variation of less than 20% was visible after 1300 equiv Addition of 800 equiv to asparagines **1e** and **2e** resulted in a decrease of fluorescence of 30%. Similar findings were obtained for the other asparagines. In Figure 1 B, C and D are shown the spectrofluorimetric titrations of asparagines **1e** with Zn^{2+}, Co^{2+} and Ni^{2+}, and as already stated for Figure 1, are also representative of the titrations of asparagines **1**, **2**, **3** and **4** with Zn^{2+}, Co^{2+} and Ni^{2+}.

For each compound, the variation in fluorescence intensity was plotted against the concentration of the metal cation, which gave a linear correlation (until it reached a plateau). The spectrofluorimetric titration results indicated that all the heterocyclic asparagines were selective chemosensors for Cu^{2+} with high sensitivity, whereas the sensing of Zn^{2+}, Co^{2+} and Ni^{2+} was non selective with very low sensitivity. From these plots, it was found that for the *C*-protected asparagines **1a**–**j** and **3** a 90% decrease of the initial fluorescence was achieved with the addition of 3–6 equiv of Cu^{2+}, suggesting that the substituent at the benzothiazole and the position of attachment of the benzothiazole had no significant influence, with the exception of **1f**, 10 equiv, and **1h**, 20 equiv, both bearing electron acceptor substituents (see Supplementary data). With regard to the type of heterocycle it was found that benzimidazole asparagine **1j** (3 equiv) displayed higher sensitivity when compared to benzothiazole asparagine **1a** (5 equiv). The fluoro asparagine **1e** and its *N*-deprotected derivative **3** displayed similar sensitivity (4–5 equiv) in the presence of Cu^{2+}, revealing that the free amino terminal is not essential for coordination.

Similar results were obtained for the *C*-deprotected asparagines **2a**–**j** and **4** (1–8 equiv of Cu^{2+}), which can also indicate that the free carboxylic acid group did not influence significantly the

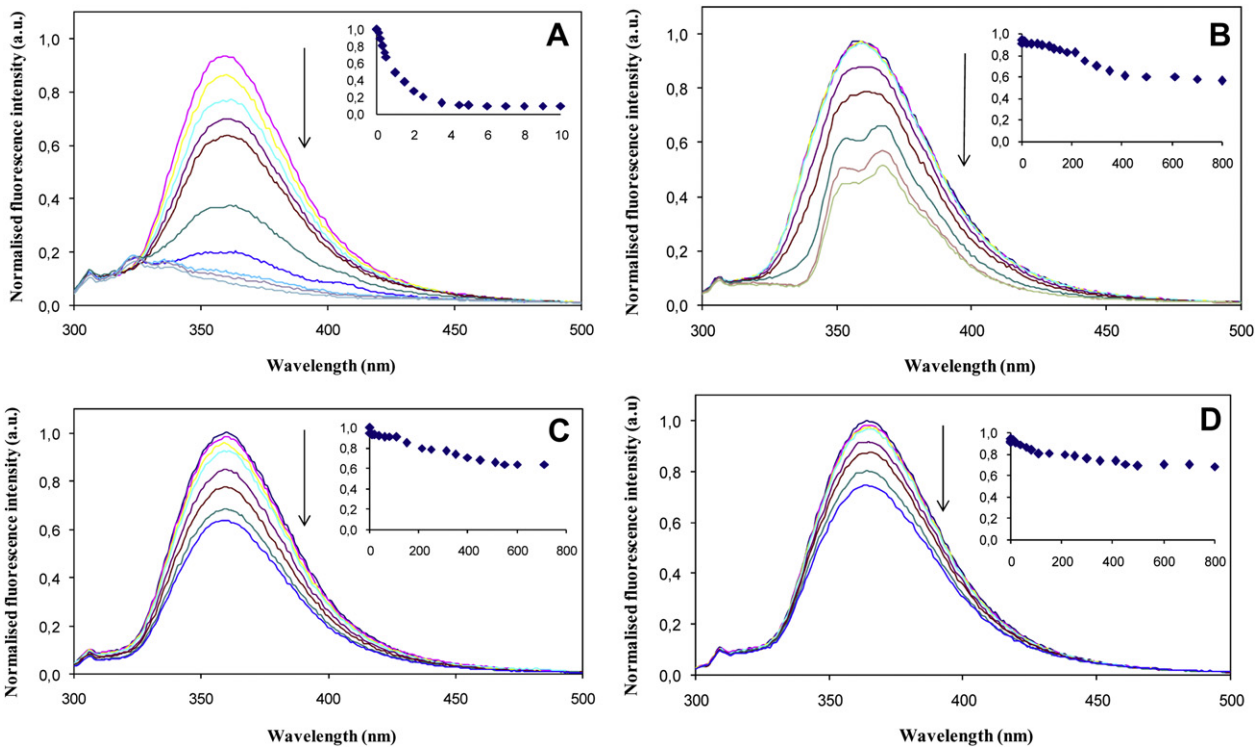


Figure 1. Fluorimetric titrations of asparagines **1e** with up to 14 equiv of Cu^{2+} (A) and up to 800 equiv of Zn^{2+} (B), Co^{2+} (C) and Ni^{2+} (D) in acetonitrile [λ_{exc} **1e**=275 nm]. Inset: normalised emission at 360 nm as a function of added metal equivalents.

coordination and that it should preferably occur through the heteroatoms at the side chain of the amino acid. Moreover, it is intended to incorporate these asparagines into peptide chains in order to obtain novel chemosensors with a peptidic framework, and as such the N- and C-terminals will be blocked with amide links. Bearing this fact in mind and considering the photophysical properties in acetonitrile of asparagines **1** and **2** (presented in Table 2), asparagines **1** are more interesting candidates as chemosensors due to the higher fluorescence quantum yield, which is important for maximization of response to analyte in the analysis of very dilute samples.

The comparative fluorimetric response of asparagines **1a**, **1e**, **1i**, **1j**, **2e**, **3** and **4** to Cu^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+} is summarised in Figure 2, considering the variation of the fluorescence intensity (I/I_0) upon the addition of 100 equiv of each metal (the shorter the bar, the higher the change in fluorescence and higher sensitivity) in acetonitrile.

The binding stoichiometry of asparagines **1** and **2** with Cu^{2+} in acetonitrile were determined from Job's plots and the binding affinity was calculated from a Benesi–Hildebrand plot by using an equation reported elsewhere.[41] The results suggest a 1:1 stoichiometry and the association constants (K_a) were calculated considering a 1:1 complex (Table 3). Also, the detection limit (DL) was calculated taking into account the fluorimetric titrations in the presence of metal cations and the standard deviation of a set of ten fluorescence measurements of a blank asparagines solution according to a previously reported expression.[42]

These results confirm that in acetonitrile solution there is a strong interaction between heterocyclic asparagines **1** and Cu^{2+} and that their complexation ability can be enhanced in the presence

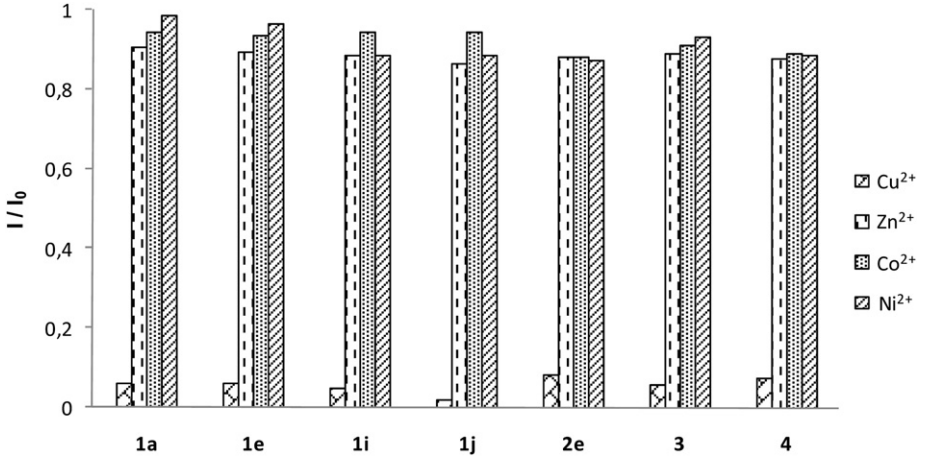


Figure 2. Relative fluorimetric response (I/I_0) of asparagines **1a**–**j**, **2a**–**j**, **3** and **4** in the presence of 100 equiv of Cu^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+} as a function of metal concentration in acetonitrile.

Table 3
Association constants (K_a) and detection limits (DL) for the interaction of asparagines **1a**–**j**, **2a**–**j**, **3** and **4** with Cu^{2+} in acetonitrile

Compd.	K_a (mol L^{-1})	DL (mol L^{-1})	Compd.	K_a (mol L^{-1})	DL (mol L^{-1})
1a	2.36×10^{4}	1.05×10^{-6}	**2a**	1.50×10^{6}	1.63×10^{-7}
1b	8.82×10^{3}	8.84×10^{-7}	**2b**	1.93×10^{5}	2.61×10^{-7}
1c	9.97×10^{4}	1.27×10^{-7}	**2c**	3.56×10^{5}	5.41×10^{-7}
1d	3.22×10^{4}	1.30×10^{-7}	**2d**	5.56×10^{5}	4.14×10^{-8}
1e	2.13×10^{5}	8.68×10^{-8}	**2e**	1.05×10^{6}	8.99×10^{-8}
1f	1.63×10^{4}	8.39×10^{-7}	**2f**	1.33×10^{6}	3.81×10^{-8}
1g	8.93×10^{4}	7.03×10^{-8}	**2g**	1.45×10^{6}	5.10×10^{-8}
1h	2.67×10^{5}	9.25×10^{-8}	**2h**	1.31×10^{6}	2.73×10^{-8}
1i	2.25×10^{5}	3.46×10^{-8}	**2i**	3.00×10^{5}	6.36×10^{-8}
1j	5.18×10^{5}	6.34×10^{-9}	**2j**	1.17×10^{6}	1.25×10^{-7}
3	1.82×10^{5}	8.75×10^{-7}	**4**	8.25×10^{5}	4.17×10^{-8}

of an extra binding carboxylic acid group as in **2**, with very low detection limits, which also constitute an important feature considering their potential application as chemosensors for transition metals with biological, environmental and analytical relevance.

In addition, as the fully deprotected asparagine **4** was soluble in water, spectrophotometric and spectrofluorimetric titrations in the presence of Cu^{2+} were carried out in mixed aqueous solutions, in order to evaluate its potential application as chemosensor in aqueous environment. Solutions of **4** in mixtures of acetonitrile or ethanol with water (in a 10^{-5} M concentration with varying proportions from 10 to 60% of water) were prepared and it was found that the compound was soluble in all tested mixtures of organic solvent and water but the fluorescence was almost completely quenched with the increase of the water content (ACN/H_2O in 40:60, 60:40 and 80:20 mixtures, Φ_F lower than 0.008, in a 90:10 mixture Φ_F was 0.011; EtOH/H_2O in 40:60, 60:40 and 80:20 mixtures, Φ_F lower than 0.009, in a 90:10 mixture Φ_F was 0.016). Also, when water was present, the wavelength of maximum absorption was slightly hipsochromically shifted to 275 nm, while the wavelength of maximum emission suffered a bathochromic shift to 401 nm (44 nm, in ethanol/water) or 410 nm 51 (51 nm, in ACN/water).

Taking into account the obtained low fluorescence quantum yields, a mixture of acetonitrile or ethanol and water (90:10) was chosen for the spectrophotometric and spectrofluorimetric titrations of **4** with Cu^{2+} (see Supplementary data for spectrofluorimetric titrations of **4** with Cu^{2+} in aqueous mixtures). A plateau was reached upon the addition of 140 equiv of metal cation, corresponding to a decrease of ca. 80% of the initial fluorescence intensity when ACN was used (with an association constant of ca. 1.7×10^{5}), whereas with ethanol only a 40% decrease was seen upon addition of 300 equiv of cation. These results, when compared to the chemosensing results obtained in acetonitrile solution, reveal that the compound **4** would not be applicable as a fluorimetric chemosensor in aqueous mixtures due to the severe loss of fluorescence, related to the water content of the mixture.

3. Conclusions

Several heterocyclic asparagines **1**–**4** containing benzothiazole and benzimidazole units at its side chain were synthesised and evaluated as fluorescent chemosensors based on an amino acid core for a series of transition metal cations, namely Cu^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+}. From the spectrofluorimetric titrations in acetonitrile, it was found that all the asparagines were selective for Cu^{2+}, showing higher sensitivity for this cation when compared to Zn^{2+}, Co^{2+} and Ni^{2+}, as a very low number of metal equivalents was enough to obtain a pronounced fluorescence quenching. The results indicated that there is a strong interaction with Cu^{2+} through the donor N, O and S atoms at the side chain of the various asparagines. Considering the influence of the presence of a protecting group at the C- and N-terminals, fully protected asparagines **1a**–**j**, *N*-protected asparagines **2a**–**j**, *C*-protected asparagine **3** and free asparagine **4** showed high and comparable coordination ability with Cu^{2+} (in terms of metal equivalents necessary to quench the fluorescence), revealing that for these compounds the presence of additional amino and carboxylic binding sites is not significant for the overall coordination ability. Job's plots suggested a 1:1 stoichiometry for the coordination of the asparagines **1** and **2** with Cu^{2+}. Considering the absolute fluorescence intensity (compounds **1** are more emissive than **2**), asparagines **1** (and especially the fluoro derivative **1e**) appear to be very promising candidates as amino acid based fluorescent probes for chemosensing applications within a peptidic framework.

4. Experimental

4.1. Synthesis general

All melting points were measured on a Stuart SMP3 melting point apparatus and are uncorrected. 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). IR spectra were determined on a BOMEM MB 104 spectrophotometer using KBr discs. NMR spectra were obtained on a Varian Unity Plus Spectrometer at an operating frequency of 300 MHz for ^{1}H NMR and 75.4 MHz for ^{13}C NMR or a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H NMR and 100.6 MHz for ^{13}C NMR using the solvent peak as internal reference at 25 °C. All chemical shifts are given in ppm using δ_H Me_4Si=0 ppm as reference and *J* values are given in hertz. Assignments were made by comparison of chemical shifts, peak multiplicities and *J* values and were supported by spin decoupling-double resonance and bidimensional heteronuclear HMBC and HMQC correlation techniques. Low and high resolution mass spectra were obtained at 'C.A.C.T.I. Unidad de Espectrometria de Masas' at the University of Vigo, Spain. The synthesis of compounds **1a**–**j** was reported elsewhere[39] and all other reagents were commercially available and used as received.

4.2. General procedure for the synthesis of C-terminal deprotected asparagines 2a–j

N-tert-Butyloxycarbonyl benz-X-azolyl benzyl ester **1a**–**j** (1 equiv) was dissolved in 1,4-dioxane (3 mL/equiv) in an ice bath, followed by the addition of aqueous NaOH 6 M (1.5 equiv) (for **1g** NaOH 1 M was used). After stirring at rt for 3 h, the pH was adjusted to 2–3 by adding aq $KHSO_4$ 1 M and the solution extracted with ethyl acetate (3×10 mL). The organic extract was dried with anhydrous $MgSO_4$, filtered and the solvent removed in a rotary evaporator. The residue was triturated with diethyl ether to afford **2a**–**j** as solids. Silica gel chromatography was performed using a mixture of dichloromethane/methanol (5:1).

*4.2.1. N-tert-Butyloxycarbonyl (benzothiazol-2-yl) asparagine (**2a**).* The product was isolated as a colourless solid (0.069 g, 1.89×10^{-4} mol, 86%). Mp=182.8–184.0 °C. IR (KBr 1%): ν=3583, 3424, 2980, 2927, 2854, 1702, 1601, 1544, 1445, 1394, 1368, 1274, 1168, 1061, 1019, 986, 878, 855, 807, 782, 756, 729, 666, 513 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.35 (s, 9H, $C(CH_3)_3$), 2.66–2.95 (m, 2H, β-CH_2), 4.14–4.17 (m, 1H, α-H), 6.68 (d, *J* 5.7 Hz, 1H, NH Boc), 7.27 (dt, *J* 8.1 and 0.9 Hz, 1H, H6), 7.40 (dt, *J* 8.4 and 1.2 Hz, 1H, H5), 7.71 (d, 1H, H7), 7.94 (dd, *J* 7.8 and 0.6 Hz, 1H, H4), 13.40 (br s,1H, OH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ=28.16 ($C(CH_3)_3$), 38.94 (β-CH_2), 50.58 (α-C), 78.03 ($C(CH_3)_3$), 120.43 (C7), 121.62 (C4), 123.34 (C6), 125.99 (C5), 131.49 (C7a), 148.63 (C3a), 154.92 (C=O urethane), 158.00 (C2), 170.11 (C=O amide), 173.03 (C=O

acid) ppm. MS *m*/*z* (ESI, %): 366 ([M+H]$^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{16}H_{20}N_3O_5S$ 366.11182; found 366.11104.

*4.2.2. N-tert-Butyloxycarbonyl (6-methylbenzothiazol-2-yl) asparagine (**2b**).* The product was isolated as a colourless solid (0.060 g, 1.57×10^{-4} mol, 70%). Mp=156.4–157.0 °C. IR (KBr 1%): *ν*=3583, 3438, 2979, 2929, 1728, 1691, 1610, 1553, 1512, 1467, 1392, 1368, 1312, 1299, 1273, 1163, 1055, 1026, 992, 904, 860, 809, 757, 666, 514, 508 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): *δ*=1.35 (s, 9H, C(CH_3)$_3$), 2.39 (s, 3H, CH_3), 2.65–2.93 (m, 2H, β-CH_2), 4.13–4.16 (m, 1H, α-H), 6.69 (d, *J* 6.3 Hz, 1H, NH Boc), 7.22 (dd, *J* 8.4 and 1.5 Hz, H5), 7.59 (d, *J* 8.1 Hz, H4), 7.73 (s, 1H, H7), 13.42 (br s, 1H, OH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): *δ*=20.97 (CH_3), 28.15 (C(CH_3)$_3$), 38.94 (β-CH_2), 50.44 (α-C), 78.04 (*C*(CH_3)$_3$), 120.07 (C4), 121.23 (C7), 127.30 (C5), 131.63 (C7a), 132.75 (C3a), 146.60 (C6), 154.91 (C=O urethane), 157.11 (C2), 169.89 (C=O amide), 172.83 (C=O acid) ppm. MS *m*/*z* (ESI, %): 380 ([M+H]$^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{17}H_{22}N_3O_5S$ 380.12747; found 380.12657.

*4.2.3. N-tert-Butyloxycarbonyl (6-methoxybenzothiazol-2-yl) asparagine (**2c**).* The product was isolated as a colourless solid (0.067 g, 1.68×10^{-4} mol, 81%). Mp=184.9–185.8 °C. IR (KBr 1%): *ν*=3583, 3416, 3253, 2979, 2934, 2831, 1698, 1606, 1570, 1547, 1512, 1473, 1436, 1403, 1367, 1343, 1288, 1268, 1225, 1169, 1060, 1028, 954, 902, 859, 827, 818, 781, 759, 703, 666, 508 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): *δ*=1.35 (s, 9H, C(CH_3)$_3$), 2.58–2.88 (m, 2H, β-CH_2), 3.79 (s, 3H, OCH_3), 3.96–4.03 (m, 1H, α-H), 6.39 (d, *J* 5.7 Hz, 1H, NH Boc), 6.99 (dd, *J* 8.7 and 2.4 Hz, 1H, H5), 7.52 (d, *J* 2.4 Hz, 1H, H7), 7.59 (d, *J* 8.7 Hz, 1H, H4), 13.73 (br s, 1H, OH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): *δ*=28.17 (C(CH_3)$_3$), 40.06 (β-CH_2), 50.92 (α-C), 55.61 (OCH_3), 77.92 (*C*(CH_3)$_3$), 104.67 (C7), 114.71 (C5), 120.98 (C4), 132.81 (C7a), 142.79 (C3a), 154.76 (C=O urethane), 155.96 (C6), 156.12 (C2), 170.15 (C=O amide), 172.95 (C=O acid) ppm. MS *m*/*z* (ESI, %): 396 ([M+H]$^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{17}H_{22}N_3O_6S$ 396.12238; found 396.12171.

*4.2.4. N-tert-Butyloxycarbonyl (6-ethoxybenzothiazol-2-yl) asparagine (**2d**).* The product was isolated as a colourless solid (0.074 g, 1.80×10^{-4} mol, 85%). Mp=180.9–182.3 °C. IR (KBr 1%): *ν*=3583, 3418, 3273, 3078, 2981, 2931, 1697, 1607, 1571, 1556, 1514, 1485, 1465, 1394, 1367, 1265, 1224, 1169, 1114, 1063, 1042, 943, 893, 859, 820, 780, 757, 699, 666, 505 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): *δ*=1.33–1.35 (m, 12H, C(CH_3)$_3$ and OCH_2CH_3), 2.56–2.86 (m, 2H, β-CH_2), 3.94–3.95 (m, 1H, α-H), 4.05 (q, *J* 6.9 Hz, 2H, OCH_2CH_3), 6.31 (d, *J* 5.1 Hz, 1H, NH Boc), 6.97 (dd, *J* 8.7 and 2.7 Hz, 1H, H5), 7.50 (d, *J* 2.1 Hz, 1H, H7), 7.58 (d, *J* 9.0 Hz, 1H, H4), 14.19 (br s, 1H, OH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): *δ*=14.73 (OCH_2CH_3), 28.16 (C(CH_3)$_3$), 40.06 (β-CH_2), 50.94 (α-C), 63.57 (OCH_2CH_3), 77.89 (*C*(CH_3)$_3$), 105.32 (C7), 115.03 (C5), 120.95 (C4), 132.80 (C7a), 142.74 (C3a), 154.69 (C=O urethane), 155.16 (C6), 156.13 (C2), 170.21 (C=O amide), 172.91 (C=O acid) ppm. MS *m*/*z* (ESI, %): 410 ([M+H]$^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{18}H_{24}N_3O_6S$ 410.13803; found 410.13706.

*4.2.5. N-tert-Butyloxycarbonyl (6-fluorobenzothiazol-2-yl) asparagine (**2e**).* The product was isolated as a colourless solid (0.084 g, 2.20×10^{-4} mol, 81%). Mp=183.9–184.7 °C. IR (KBr 1%): *ν*=3651, 3416, 3376, 3224, 3097, 2981, 2930, 2850, 1734, 1700, 1610, 1546, 1509, 1463, 1403, 1367, 1319, 1285, 1254, 1196, 1170, 1062, 1052, 1027, 996, 954, 915, 852, 823, 782, 728, 706, 666, 514, 505 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): *δ*=1.35 (s, 9H, C(CH_3)$_3$), 2.68–2.94 (m, 2H, β-CH_2), 4.17–4.19 (m, 1H, α-H), 6.73 (d, *J* 6.0 Hz, 1H, NH Boc), 7.25 (dt, *J* 9.2 and 2.8 Hz, 1H, H5), 7.70–7.73 (m, 1H, H4), 7.85 (dd, *J* 8.8 and 2.8 Hz, 1H, H7) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): *δ*=28.15 (C(CH_3)$_3$), 39.92 (β-CH_2), 50.41 (α-C), 78.08 (*C*(CH_3)$_3$), 108.07 (d, *J* 26.2 Hz, C7), 114.06 (d, *J* 24.1 Hz, C5), 121.51 (d, *J* 9.01 Hz, C4), 132.73 (d, *J* 11.01 Hz, C7a), 145.34 (C3a), 154.96 (C=O urethane), 158.02 (C2), 158.56 (d, *J* 239.4 Hz, C6), 170.12 (C=O amide), 172.84 (C=O acid) ppm. MS *m*/*z* (ESI, %): 384 ([M+H]$^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{16}H_{19}N_3O_5SF$ 384.10240; found 384.10168.

*4.2.6. N-tert-Butyloxycarbonyl (6-nitrobenzothiazol-2-yl) asparagine (**2f**).* The product was isolated as a colourless solid (0.045 g, 1.10×10^{-4} mol, 74%). Mp=173.7–175.4 °C. IR (KBr 1%): *ν*=3583, 3416, 2977, 2930, 2855, 1700, 1608, 1577, 1535, 1449, 1394, 1368, 1341, 1276, 1164, 1129, 1048, 1027, 905, 830, 777, 753, 721, 700, 666, 516, 503 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): *δ*=1.35 (s, 9H, C(CH_3)$_3$), 2.66–2.95 (m, 2H, β-CH_2), 4.08–4.09 (m, 1H, α-H), 6.54 (d, *J* 5.2 Hz 1H, NH Boc), 7.84 (d, *J* 9.2 Hz, 1H, H4), 8.24 (dd, *J* 9.2 and 2.8 Hz, 1H, H5), 8.99 (d, *J* 2.4 Hz, 1H, H7) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): *δ*=28.13 (C(CH_3)$_3$), 39.92 (β-CH_2), 50.55 (α-C), 78.00 (*C*(CH_3)$_3$), 118.81 (C7), 120.25 (C4), 121.59 (C5), 132.27 (C7a), 142.65 (C3a), 153.84 (C6), 154.78 (C=O urethane), 164.13 (C2), 171.36 (C=O amide), 172.70 (C=O acid) ppm. MS *m*/*z* (ESI, %): 411 ([M+H]$^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{16}H_{19}N_4O_7S$ 411.09690; found 411.09644.

*4.2.7. N-tert-Butyloxycarbonyl (6-thiocyanobenzothiazol-2-yl) asparagine (**2g**).* The product was isolated as a colourless solid (0.052 g, 1.23×10^{-4} mol, 58%). Mp=190.0–191.9 °C. IR (KBr 1%): *ν*=3583, 3422, 2977, 2928, 2854, 2157, 1690, 1597, 1545, 1448, 1395, 1368, 1272, 1165, 1056, 1028, 859, 816, 761, 666, 509 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): *δ*=1.35 (s, 9H, C(CH_3)$_3$), 2.58–2.89 (m, 2H, β-CH_2), 3.89–3.95 (m, 1H, α-H), 6.24 (d, *J* 4.8 Hz, 1H, NH Boc), 7.65 (dd, *J* 8.4 and 1.8 Hz, 1H, H5), 7.80 (d, *J* 8.7 Hz, 1H, H4), 8.34 (d, *J* 2.1 Hz, 1H, H7) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): *δ*=28.15 (C(CH_3)$_3$), 40.33 (β-CH_2), 51.06 (α-C), 77.88 (*C*(CH_3)$_3$), 112.25 (SCN), 117.03 (C6), 121.77 (C4), 125.67 (C7), 129.41 (C5), 133.47 (C7a), 150.19 (C3a), 154.62 (C=O urethane), 160.87 (C2), 171.30 (C=O amide), 172.88 (C=O acid) ppm. MS *m*/*z* (ESI, %): 423 ([M+H]$^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{24}H_{24}N_4O_5S_2$ 423.07914; found 423.07880.

*4.2.8. N-tert-Butyloxycarbonyl (6-sulfomethylbenzothiazol-2-yl) asparagine (**2h**).* The product was isolated as a colourless solid (0.071 g, 1.59×10^{-4} mol, 81%). Mp=219.3–221.1 °C. IR (KBr 1%): *ν*=3583, 3426, 2980, 2928, 1692, 1599, 1543, 1448, 1400, 1369, 1300, 1277, 1151, 1101, 1054, 1023, 964, 883, 859, 823, 785, 749, 666, 510 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): *δ*=1.35 (s, 9H, C(CH_3)$_3$), 2.62–2.94 (m, 2H, β-CH_2), 3.23 (s, 3H, CH_3), 4.00–4.03 (m, 1H, α-H), 6.45 (d, *J* 4.8 Hz, 1H, NH Boc), 7.89 (s, 2H, H4 and H5), 8.58 (t, *J* 1.2 Hz, 1H, H7) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): *δ*=28.14 (C(CH_3)$_3$), 40.06 (β-CH_2), 44.06 (CH_3), 50.65 (α-C), 77.97 (*C*(CH_3)$_3$), 120.57 (C4), 121.88 (C7), 124.66 (C5), 132.08 (C7a), 134.98 (C6), 152.35 (C3a), 154.72 (C=O urethane), 162.44 (C2), 171.16 (C=O amide), 172.71 (C=O acid) ppm. MS *m*/*z* (ESI, %): 444 ([M+H]$^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{17}H_{22}N_3O_7S_2$ 444.08937; found 444.08890.

*4.2.9. N-tert-Butyloxycarbonyl (benzothiazol-6-yl) asparagine (**2i**).* The product was isolated as a colourless solid (0.074 g, 2.01×10^{-4} mol, 88%). Mp=170.4–172.0 °C. IR (KBr 1%): *ν*=3583, 3315, 3074, 2978, 2931, 1694, 1608, 1580, 1537, 1477, 1449, 1401, 1367, 1330, 1252, 1165, 1054, 1027, 986, 914, 861, 837, 783, 757, 734, 666, 514, 503 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): *δ*=1.34 (s, 9H, C(CH_3)$_3$), 2.56–2.80 (m, 2H, β-CH_2), 4.09–4.11 (m, 1H, α-H), 6.52 (d, *J* 6.6 Hz, 1H, NH Boc), 7.55 (dd, *J* 8.7 Hz and 1.5 Hz, 1H, H5), 7.97 (d, *J* 9.0 Hz, 1H, H4), 8.54 (d, *J* 0.9 Hz, 1H, H7), 9.23 (s, 1H, H2), 11.12 (s, 1H, NH amide) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): *δ*=28.17 (C(CH_3)$_3$), 40.34 (β-CH_2), 51.37 (α-C), 77.79 (*C*(CH_3)$_3$), 111.27 (C7), 118.59 (C5), 122.84 (C4), 134.18 (C7a), 137.28 (C6), 148.84 (C3a), 154.33 (C2), 154.89 (C=O urethane), 169.41 (C=O amide), 173.51 (C=O acid) ppm. MS *m*/*z* (ESI, %): 366 ([M+H]$^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{16}H_{20}N_3O_5S$ 366.11182; found 366.11119.

*4.2.10. N-tert-Butyloxycarbonyl (benzimidazol-2-yl) asparagine (**2j**).* The product was isolated as a colourless solid (0.066 g,

1.89×10^{-4} mol, 79%). Mp=182.4–184.0 °C. IR (KBr 1%): ν=3583, 3405, 2977, 2929, 2854, 1691, 1637, 1596, 1523, 1458, 1392, 1367, 1310, 1273, 1223, 1168, 1058, 1023, 894, 861, 744, 666, 504 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ=1.35 (s, 9H, $C(CH_3)_3$), 2.60–2.83 (m, 2H, β-CH_2), 4.02–4.03 (m, 1H, α-H), 6.4 (d, *J* 5.2 Hz, 1H, NH Boc), 7.03–7.05 (m, 2H, H5 and H6), 7.40–7.42 (m, 2H, H4 and H7), 11.96 (br s, 1H, NH benzimidazole), 12.73 (br s, 1H, OH) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6): δ=28.16 ($C(CH_3)_3$), 39.92 (β-CH_2), 51.07 (α-C), 77.81 ($C(CH_3)_3$), 120.75 (C4 and C7), 146.82 (C2), 154.78 (C=O urethane), 172.93 (C=O amide), 173.31 (C=O acid) ppm. MS: *m*/*z* (ESI, %) 349 ($[M+H]^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{16}H_{21}N_4O_5$ 349.15065; found 349.14988.

4.3. Synthesis of 6-fluorobenzothiazol-2-yl asparagine benzyl ester (3)

Compound **1e** (0.150 g, 3.91×10^{-4} mol,) was stirred in a trifluoroacetic acid/dichloromethane solution (1:1, 1 mL) at rt for 2 h. The solvent was evaporated, the residue dissolved in pH 8 aqueous buffer solution and extracted with ethyl acetate (3×10 mL). After drying with anhydrous magnesium sulfate and evaporation of the solvent, the product was isolated as a colourless solid (0.088 g, 2.37×10^{-4} mol, 61%). Mp=114.0–115.3 °C. IR (KBr 1%): ν=3650, 3583, 3190, 2927, 2850, 1737, 1677, 1627, 1571, 1536, 1498, 1464, 1432, 1397, 1384, 1328, 1289, 1257, 1231, 1191, 1140, 1084, 1009, 974, 941, 883, 848, 832, 815, 798, 763, 725, 707, 666 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ=3.16–3.25 (m, 2H, β-CH_2), 4.57 (t, *J* 5.4 Hz, 1H, α-H), 5.19–5.26 (m, 2H, CH_2), 7.22–7.35 (m, 6H, 5×Ph-*H* and H5), 7.75–7.78 (m, 1H, H4), 7.88–7.91 (dd, *J* 8.4 and 2.4 Hz, 1H, H7), 8.60 (br s, 2H, NH_2), 12.69 (br s, 1H, NH amide) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6): δ=35.30 (β-CH_2), 48.33 (α-C), 67.40 (CH_2–Ph), 108.22 (d, *J* 26.2 Hz, C7), 114.38 (d, *J* 25.2 Hz, C5), 121.86 (d, *J* 9.05 Hz, C4), 127.94 (C3′ and C5′), 128.21 (C4′), 128.31 (C2′ and C6′), 132.76 (d, *J* 11.07 Hz, C7a), 134.96 (C1′), 145.16 (C3a), 157.46 (C2), 158.76 (d, *J* 239.4 Hz, C6), 168.29 (C=O amide), 168.38 (C=O ester) ppm. MS *m*/*z* (ESI, %): 374 ($[M+H]^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{18}H_{17}N_3O_3SF$ 374.09692; found 374.09717.

4.4. Synthesis of 6-fluorobenzothiazol-2-yl asparagine (4)

Compound **2e** (0.195 g, 4.12×10^{-4} mol,) was treated as described in Section 4.4 and the product was isolated as a colourless solid (0.068 g, 2.99×10^{-4} mol, 58%). Mp=239.3–240.1 °C. IR (KBr 1%): ν=3659, 3583, 3132, 3071, 2923, 2850, 1675, 1611, 1563, 1495, 1462, 1435, 1390, 1355, 1303, 1260, 1260, 1199, 1183, 1160, 1137, 1089, 1060, 971, 916, 857, 827, 815, 733, 710, 699, 666 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ=2.66–2.71 (dd, *J* 16.4 and 6.0 Hz, 1H, β-CH_2), 3.06–3.12 (dd, *J* 16.4 and 6.0 Hz, 1H, β-CH_2), 3.68 (t, *J* 6.4 Hz, 1H, α-H), 7.26 (dt, *J* 9.2 and 2.4 Hz, 1H, H5), 7.71–7.75 (m, 1H, H4), 7.87 (dd, *J* 8.8 and 2.8 Hz, 1H, H7) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6): δ=37.45 (β-CH_2), 49.61 (α-C), 108.07 (d, *J* 27.2 Hz, C7), 114.08 (d, *J* 24.1 Hz, C5), 121.55 (d, *J* 9.05 Hz, C4), 132.72 (d, *J* 11.07 Hz, C7a), 145.33 (C3a), 157.97 (C2), 158.57 (d, *J* 240.4 Hz, C6), 169.10 (C=O amide), 169.93 (C=O acid) ppm. MS *m*/*z* (ESI, %): 284 ($[M+H]^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{11}H_{11}N_3O_3SF$ 284.04997; found 284.05040.

4.5. Spectrophotometric titrations and chemosensing studies for asparagines 1–4

UV/vis absorption spectra (200–800 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer and fluorescence spectra were collected using a FluoroMax-4 spectrofluorometer. The linearity of the absorption versus concentration was checked within the used concentration.

Solutions of compounds **1**–**4** (ca. 1.0×10^{-5} to 1.0×10^{-6} M) and of the metallic cations under study (ca. 1.0×10^{-1} to 1.0×10^{-3} M) were prepared in UV-grade acetonitrile (in the form of hexahydrated tetrafluorborate salts for Cu^{2+}, Co^{2+} and Ni^{2+} and perchlorate salt for Zn^{2+}). Titration of the compounds with the several metallic cations was performed by the sequential addition of equivalents of metal cation to the compound solution, in a 10 mm path length quartz cuvette and emission spectra were measured by excitation at the wavelength of maximum absorption for each compound, indicated in Table 2, with a 2 nm slit.

The binding stoichiometry of the asparagines with the metal cations was determined by using Job's plots, by varying the molar fraction of the cation while maintaining constant the total asparagine and metal cation concentration. The association constants were obtained from Benesi–Hildebrand plots in the form of straight lines with good correlation coefficients by using an equation reported elsewhere.[41]

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Supplementary data

Spectrofluorimetric titrations of **1a**–**j**, **2a**–**j**, **3** and **4** with Cu^{2+}. Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.07.063. These data include MOL files and InChIKeys of the most important compounds described in this article.

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