Tetrahedron 65 (2009) 9373–9377

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Unnatural benz-X-azolyl asparagine derivatives as novel fluorescent amino acids: synthesis and photophysical characterization

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ARTICLE INFO

Article history:
Received 4 August 2009
Received in revised form 26 August 2009
Accepted 29 August 2009
Available online 10 September 2009

Keywords:
Asparagine
Unnatural amino acids
Benzothiazole
Benzimidazole
Fluorescent probes
Solvatochromism

ABSTRACT

A family of new asparagine derivatives bearing benzothiazole and benzimidazole units, functionalised with electron donor or acceptor groups, were synthesized in good to excellent yields. The photophysical characterization of these new heterocyclic amino acids was performed by UV–visible absorption and fluorescence emission studies and revealed that the compounds displayed remarkably high fluorescence quantum yields and Stokes' shifts, making them good candidates for application as fluorescent probes by incorporation into peptidic frameworks.

1. Introduction

Asparagine is the amide of aspartic acid and has a high propensity to form hydrogen bond interactions with the peptide backbone through the amide group. Asparagine residues are often found near the beginning and the end of α-helices, and in turn motifs in β-sheets. Its role can be thought as 'capping' the hydrogen bond interactions, which would otherwise be satisfied by the polypeptide backbone. This feature opens the way to the development of functional unnatural amino acids based on asparagine possessing additional properties, such as fluorescence, that could be used for the construction of chromophore-labelled peptides and proteins. By using fluorescence spectroscopy based techniques, such fluorescent peptides could find application in molecular flexibility studies involving protein folding, substrate binding activity of proteins, and antigenicity or enzymatic activity.[1,2] Therefore, by synthetic manipulation at the side chain of coded amino acids, new functions and functional relationships can be generated as well as altered physicochemical properties, such as luminescence, conducting ability and metal ion and other analyte recognition ability,[3] with the incorporation of these functional unnatural amino acids into peptides resulting in the appearance of the inherent functions.[4] With regard to the fluorescent heterocyclic moieties, benzothiazole and benzimidazole derivatives may be considered suitable choices as these compounds are known to display interesting optical properties (broad spectral windows, high molar absorptivity coefficients and fluorescence quantum yields) and have been described as fluorescent and/or colourimetric chemosensors for anions and metal cations, biomarkers of pheomelanins and nonlinear optical materials.[5] Also, benzothiazol-2-yl amides exhibit a wide range of biological properties such as antibacterial, antitumoral, antifungal and antitubercular activities.[6]

Bearing these facts in mind, it becomes clear that there is a practical interest on the expansion of the body of work published in recent years in the area of unnatural functional amino acids, and so we decided to design new heterocyclic amino acids consisting of functionalized asparagine containing benzothiazole and benzimidazole, as a result of the previously mentioned properties of these heterocycles. The resulting benz-X-azolyl asparagine derivatives, because of the presence of amino and carboxyl groups, could be incorporated into peptide chains and as such used as energy donor/acceptor in conformational studies of peptides by means of fluorescence or be used as fluorescence markers. Following our previous research on the synthesis and characterization of unnatural amino acids,[7] imidazole and benz-X-azole derivatives with interesting optical properties[8] and heterocyclic colorimetric/fluorimetric chemosensors containing (oligo)thiophene, benzoxazole and amino acid moieties,[9] we now report the synthesis of a new family of highly fluorescent asparagine based heterocyclic amino acids bearing benzothiazole and benzimidazole, also having

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0040-4020/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2009.08.086

in mind the future studies of these compounds as fluorimetric peptide-based chemosensors. Amino acids and peptides are known to bind a variety of metal ions as they contain a large number of potential donor atoms through the peptide backbone and side chains. The insertion of an heterocyclic system at the side chain will provide an UV-active and highly fluorescent chromophore, as well as additional binding sites through the heterocycle donor atoms. With the study of these new benz-X-azolyl asparagines we also intended to evaluate the effect of the substituent present at the benzothiazole as well as the position of attachment of the heterocycle in the photophysical properties of the resulting compounds.

2. Results and discussion

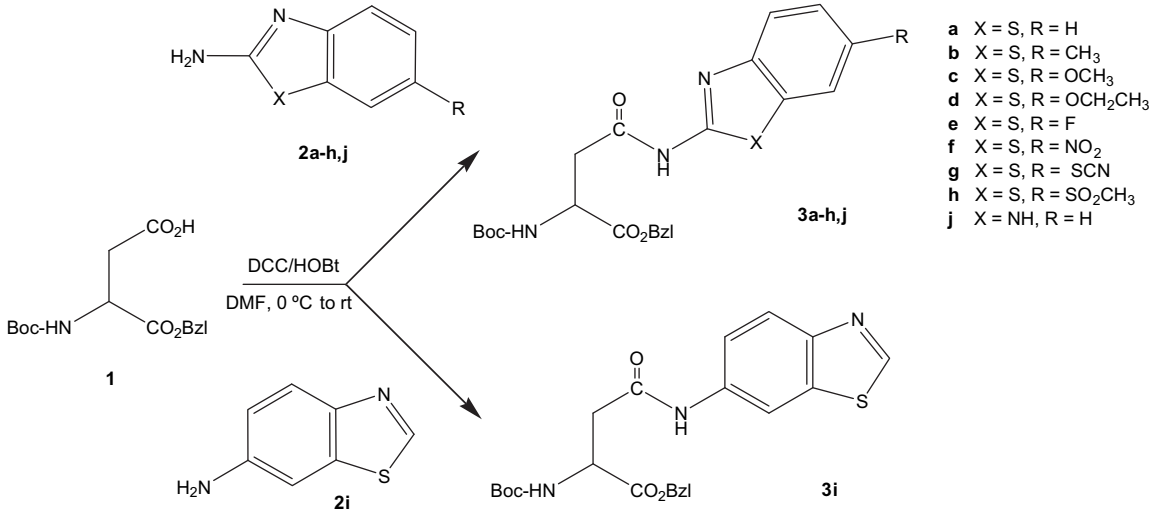
2.1. Synthesis of benz-X-azolyl asparagines 3a–j

The new asparagines **3a**–**j** with benzothiazole and benzimidazole at its side chain were synthesized in good to excellent yields by a simple coupling procedure involving DCC and HOBt, between the side chain carboxylic acid group of *N-tert*-butyloxy aspartic acid benzyl ester **1** and aminobenzothiazoles **2a**–**i** and 2-aminobenzimidazole **2j**, bearing substituents of different electronic character (electron donor: alkyl and alkoxyl; electron acceptor: F, SCN, SO_2Me and SCN) and in different position of attachment of the heterocycle (position 2 or 6) (Scheme 1, Table 1). These new compounds were fully characterised by the usual spectroscopic techniques.

From the results in Table 1, it can be seen that the yield of coupling for benzothiazolyl asparagines **3a**–**h** was influenced by the nature of the substituent at position 6 of the benzothiazole, as electron donor groups gave rise to compounds **3b**–**d** in higher yields (89–91%) and electron acceptor groups originated compounds **3e**–**h** in lower yields (58–68%). The position of attachment of the benzothiazole appears to have no influence in the yield as asparagines **3a** and **3i** were prepared in practically identical yields. The same observation could be made considering the nature of the heterocycle, since benzothiazolyl asparagine **3a** and benzimidazolyl asparagine **3j** were also obtained in similar yields.

The IR spectra of asparagines **3a**–**j** showed the characteristic bands due to stretching vibrations of the carbonyl of the *C*- and *N*-terminal protecting groups: the benzyl ester from 1742 to 1764 cm^{-1} and the *N-tert*-butyloxycarbonyl urethane bond from 1721 to 1747 cm^{-1}. The bands related to the newly formed amide bond appeared from 1671 to 1701 cm^{-1} (for the carbonyl) and at about 3300 cm^{-1} (for the NH).

^{1}H NMR spectra showed the expected signals for the benz-X-azolyl unit in compounds **3b**–**i**, in the form of a double doublet for H5, and doublets for H4 and H7, with the chemical shifts of the protons of homocyclic ring of the benz-X-azole moiety being in agreement with the electronic character of the substituent (see Experimental for details). The signals for the protons of the amino acid residue were also visible, namely the α-H (from 4.60 to 4.82 ppm) and the β-CH_2 (from 3.02 to 3.40 ppm), for asparagines **3a**–**h**, **j** bearing the heterocycle attached at position 2. With regard to asparagine **3i**, with the heterocycle attached to position 6, a shift to lower δ was seen for the



Scheme 1. Synthesis of benz-X-azolyl asparagine derivatives **3a**–**j**.

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

Cpd.	X	R	Yield (%)	UV–Vis		Fluorescence		
				λ_{max}	log ε	λ_{em}	Stokes' shift (cm^{-1})	Φ_F
3a	S	H	90	330	3.22	465	8798	0.56
3b	S	CH_3	89	326	3.28	461	8983	0.79
3c	S	OCH_3	89	347	3.22	467	7405	0.72
3d	S	OCH_2CH_3	91	345	3.34	467	7572	0.38
3e	S	F	65	346	3.52	466	7443	0.85
3f	S	NO_2	58	325	3.53	454	8743	0.28
3g	S	SCN	66	335	3.28	467	8437	0.39
3h	S	SO_2CH_3	68	331	3.13	464	8660	0.60
3i	S	–	86	332	3.05	462	8475	0.53
3j	NH	H	94	291	3.85	425	10,835	0.03

β-CH_2, which appeared as a multiplet between 2.97 and 3.14 ppm. The confirmation of the occurrence of the amide coupling was also supported by ^{13}C NMR spectra, where signals of the benz-X-azole C2 carbon were visible between δ 153.81 and 162.28 ppm (for the benzothiazole) and at δ 143.09 ppm (for the benzimidazole) and the amide group carbonyl from δ 168.57 to 169.84 ppm.

2.2. Photophysical study of benz-X-azolyl asparagines 3a–j

The absorption and emission spectra of asparagines **3a**–**j** were measured in absolute ethanol (10^{-6} to 10^{-5} M solution) (Table 1). The nature of the heterocycle had a clear influence on the absorption and emission bands of compounds **3a**–**j**: benzothiazolyl derivatives **3a**–**i** displayed absorption and emission maxima at longer

wavelengths (ca. 25 nm) when compared to the benzimidazolyl counterpart **3j**, a fact related to the electronic effect of the heterocycle. Also, electron donating groups such as the alkoxyls or fluorine at position 6 of the benzothiazole (compounds **3c**–**e**) shifted bathochromically the wavelength of maximum of absorption, when compared to the unsubstituted benzothiazolyl asparagine **3a**. Compounds **3a** and **3i**, which only differ in the position of attachment of the benzothiazole unit to the side chain of the amino acid, showed similar absorption and emission data.

The synthesized compounds showed large Stokes' shifts (the lowest being 7405 cm^{-1} for **3c** and the highest 10,835 cm^{-1} for **3j**). A large Stokes' shift is an interesting characteristic for a fluorescent probe that allows an improved separation of the light inherent to the matrix and the light dispersed by the sample.[10]

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)[11] and benzothiazolyl asparagines **3a**–**i** exhibited good to excellent fluorescence quantum yields between 0.28 (nitro derivative **3f**) and 0.85 (fluoro derivative **3e**), while the value obtained for the benzimidazolyl asparagine **3j** was much lower (Φ_F=0.03).

Considering the results obtained so far, the photophysical properties of benzothiazol-2-yl asparagine **3a**, 6-fluorobenzothiazol-2-yl asparagine **3e** (with the highest fluorescence quantum yield in ethanol) and benzothiazol-6-yl asparagine **3i** were evaluated in other solvents of different character. The solvents tested were ethyl acetate and DMSO, as examples of polar aprotic solvents, in order to compare with ethanol, a polar protic solvent. The collected data revealed substantial differences in the wavelengths of maximum absorption and emission and the fluorescence quantum yield (Table 2). For the considered asparagines **3a**, **3e** and **3i**, it can be seen that there is a considerable hipsochromic shift in the absorption and emission maxima in ethyl acetate and DMSO (Fig. 1). Also in these solvents, the fluorescence quantum yields suffered a strong decrease and are very low, with the exception of asparagine **3e** in ethyl acetate, which displays a moderate quantum yield (Φ_F=0.26).

Table 2
UV–visible absorption and fluorescence data for benzothiazolyl asparagines **3a**, **3e** and **3i** in EtOH, AcOEt and DMSO

Cpd.	Solvent	UV–Vis		Fluorescence		
		λ_{max}	log ε	λ_{em}	Stokes' shift (cm^{-1})	Φ_F
3a	EtOH	330	3.22	465	8798	0.56
	AcOEt	268	4.09	308	4846	0.04
	DMSO	278	4.21	376	9375	0.01
3e	EtOH	346	3.52	466	7443	0.85
	AcOEt	268	4.02	304	4419	0.26
	DMSO	278	4.17	377	9446	0.01
3i	EtOH	332	3.05	462	8475	0.53
	AcOEt	278	4.05	324	5107	0.02
	DMSO	270	4.13	366	9715	0.02

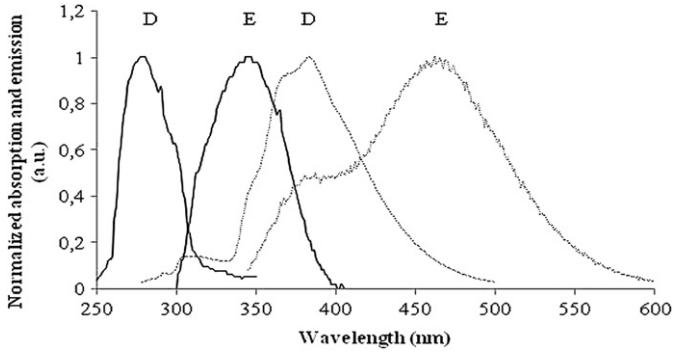


Figure 1. Normalised UV–visible absorption and emission spectra of asparagine **3e** in absolute ethanol (E) and DMSO (D) at T=298 K (λ_{exc}=346 nm in E and 278 nm in D) (absorption, full line; emission, dotted line).

The existence of dual fluorescence is suggested by the fluorescence spectra of asparagine **3e** in ethanol and DMSO, depicted in Figure 1. This type of emission has been reported for 2-(4′-aminophenyl)[12a,b] and 2-[4′-(*N*,*N*-dimethylamino)phenyl]benzazole derivatives,[12c] which was proposed to arise from the expected lowest energy $\pi \rightarrow \pi^*$ transition, resulting in the normal Stokes'-shifted band, and also from a twisted intramolecular charge transfer (TICT) state, with the appearance of a large Stokes'-shifted band.

3. Conclusions

In summary, we have achieved for the first time the synthesis of new fluorescent benz-X-azolyl asparagine derivatives **3a–j** containing benzothiazole and benzimidazole units at its side chain by a simple coupling procedure in good to excellent yields and their photophysical properties were evaluated. Due to their interesting photophysical properties, namely their strongly emissive character, with large fluorescence quantum yields and Stokes' shifts, and solvatochromic behaviour, these heterocyclic asparagine derivatives could find application as useful building blocks for peptide-based structures with the extra value of inserting a UV-active and fluorescent chromophore, adding functionality to the resulting peptide. These heteroaromatic asparagines could also be used as fluorescent markers and probes for conformational studies in peptides.

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. UV–visible absorption spectra (200–800 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. 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. Mass spectra were obtained with a Finnigan LXQ system. Fluorescence spectra were collected using a FluoroMax-4 spectrofluorometer. All reagents were commercially available and used as-received.

4.2. General procedure for the synthesis of heterocyclic asparagine derivatives 3a–j

N-*tert*-Butyloxycarbonyl aspartic acid benzyl ester **1** (1 equiv) was dissolved in dry DMF (3 mL/mmol), followed by HOBt (1 equiv), and after stirring for 10 min, *N*,*N*′-dicyclohexylcarbodiimide (1 equiv) was added. The reaction mixture was placed in an ice bath, stirred for 30 min and the corresponding aminobenzothiazole or benzimidazole **2** was added (1 equiv). The mixture was stirred at low temperature for 2 h, and then for 24 h at room temperature. After filtering, the solvent was removed under reduced pressure in a rotary evaporator. The residue was dissolved in acetone and placed in the cold overnight to induce the precipitation of the formed by-product (*N*,*N*′-dicyclohexylurea), which was separated by filtration. This procedure was repeated two times.

The filtrate was evaporated in a rotary evaporator. The resulting solid was purified by recrystallization from methanol and diethyl ether or column chromatography, using mixtures of ethyl acetate and light petroleum of increasing polarity.

*4.2.1. N-tert-Butyloxycarbonyl (benzothiazol-2-yl) asparagine benzyl ester (**3a**).* The product was isolated as a colourless solid (0.512 g, 1.12 mmol, 90%). Mp=124.4–124.6 °C. IR (KBr, 1%): ν=3377, 2970, 2931, 1758, 1729, 1698, 1627, 1600, 1552, 1519, 1444, 1422, 1391, 1368, 1347, 1299, 1272, 1245, 1212, 1198, 1170, 1158, 1066, 1016, 983, 909, 873, 784, 755, 729, 696, 666 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.44 (s, 9H, C(CH_3)$_3$), 3.18–3.40 (m, 2H, β-CH_2), 4.80 (t, *J* 3.9 Hz, 1H, α-H), 5.18–5.23 (m, 2H, CH_2), 5.64 (d, *J* 8.4 Hz, 1H, NH Boc), 7.22–7.38 (m, 6H, 5×Ph-*H* and NH amide), 7.49 (dt, *J* 7.2 and 1.2 Hz, 1H, H6), 7.60 (dt, *J* 7.2 and 1.2 Hz, 1H, H5), 7.84–7.89 (m, 2H, H4 and H7) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ=28.22 (C(*C*H_3)$_3$), 38.44 (β-CH_2), 50.07 (α-C), 67.64 (CH_2), 80.31 (*C*(CH_3)$_3$), 120.04 (C7), 121.59 (C4), 124.51 (C6), 126.85 (C5), 128.21 (C4′), 128.31 (C3′ and C5′), 128.48 (C2′ and C6′), 130.91 (C7a), 135.05 (C1′), 145.81 (C3a), 155.53 (C=O urethane), 159.23 (C2), 169.31 (C=O amide), 170.86 (C=O ester) ppm. UV–Vis (ethanol, nm): λ_{max} (log ε)=330 (3.22). MS *m*/*z* (ESI, %): 478 (M^++23, 100), 456 ($[M+H]^+$, 20). $C_{23}H_{25}N_3O_5S$: calcd C 60.64, H 5.53, N 9.22, S 7.04; found C 60.80, H 5.73, N 9.25, S 7.15.

*4.2.2. N-tert-Butyloxycarbonyl (6-methylbenzothiazol-2-yl) asparagine benzyl ester (**3b**).* The product was isolated as a colourless solid (0.523 g, 1.11 mmol, 89%). Mp=154.9–158.0 °C. IR (KBr, 1%): ν=3369, 3210, 3066, 2982, 2931, 1755, 1737, 1698, 1607, 1553, 1521, 1467, 1455, 1422, 1389, 1368, 1343, 1317, 1273, 1223, 1208, 1155, 1060, 1029, 990, 900, 866, 814, 779, 736, 695, 665 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.42 (s, 9H, C(CH_3)$_3$), 2.47 (s, 3H, CH_3), 3.03–3.28 (m, 2H, β-CH_2), 4.75 (t, *J* 4.0 Hz, 1H, α-H), 5.17–5.25 (m, 2H, CH_2), 5.91 (d, *J* 8.4 Hz, 1H, NH Boc), 7.23 (dd, *J* 8.0 and 1.4 Hz, H5), 7.25–7.32 (m, 5H, 5×Ph-*H*), 7.60 (d, *J* 1.4 Hz, H7), 7.63 (d, *J* 8.0 Hz, H4), 9.58 (br s, 1H, NH amide) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=21.39 (CH_3), 28.21 (C(*C*H_3)$_3$), 38.28 (β-CH_2), 50.05 (α-C), 67.55 (CH_2), 80.19 (*C*(CH_3)$_3$), 119.86 (C4), 121.26 (C7), 128.09 (C5), 128.13 (C3′ and C5′), 128.26 (C4′), 128.45 (C2′ and C6′), 131.51 (C7a), 134.35 (C6), 135.13 (C1′), 144.74 (C3a), 155.58 (C=O urethane), 158.37 (C2), 169.21 (C=O amide), 170.95 (C=O ester) ppm. UV–Vis (ethanol, nm): λ_{max} (log ε)=326 (3.28). MS *m*/*z* (ESI, %): 492 (M^++23, 100), 470 ($[M+H]^+$, 13). $C_{24}H_{27}N_3O_5S$: calcd C 61.39, H 5.80, N 8.95, S 6.83; found C 61.09, H 5.87, N 8.85, S 6.89.

*4.2.3. N-tert-Butyloxycarbonyl (6-methoxybenzothiazol-2-yl) asparagine benzyl ester (**3c**).* The product was isolated as a colourless solid (0.439 g, 0.90 mmol, 89%). Mp=130.2–134.0 °C. IR (KBr, 1%): ν=3367, 2979, 2932, 1754, 1730, 1695, 1606, 1570, 1556, 1521, 1471, 1439, 1368, 1345, 1264, 1222, 1157, 1061, 1029, 902, 853, 829, 810, 779, 753, 733, 700, 666, 514 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.42 (s, 9H, C(CH_3)$_3$), 3.03–3.28 (m, 2H, β-CH_2), 3.87 (s, 3H, OCH_3), 4.76 (t, *J* 4.0 Hz, 1H, α-H), 5.17–5.24 (m, 2H, CH_2), 5.90 (d, *J* 8.4 Hz, 1H, NH Boc), 7.01 (dd, *J* 8.8 and 2.4 Hz, 1H, H5), 7.25–7.30 (m, 6H, 5×Ph-*H* and H7), 7.63 (d, *J* 8.8 Hz, 1H, H4), 9.05 (br s, 1H, NH amide) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=28.22 (C(*C*H_3)$_3$), 38.27 (β-CH_2), 50.10 (α-C), 55.81 (OCH_3), 67.57 (CH_2), 80.23 (*C*(CH_3)$_3$), 104.37 (C7), 115.60 (C5), 120.81 (C4), 128.16 (C4′), 128.28 (C3′ and C5′), 128.47 (C2′ and C6′), 132.50 (C7a), 135.12 (C1′), 140.58 (C3a), 155.58 (C=O urethane), 157.06 (C6), 157.15 (C2), 169.04 (C=O amide), 170.96 (C=O ester) ppm. UV–Vis (ethanol, nm): λ_{max} (log ε)=347 (3.22). MS *m*/*z* (ESI, %): 508 (M^++23, 100), 486 ($[M+H]^+$, 7). $C_{24}H_{27}N_3O_6S$: calcd C 59.37, H 5.60, N 8.65, S 6.60; found C 59.42, H 5.76, N 8.55, S 6.73.

*4.2.4. N-tert-Butyloxycarbonyl (6-ethoxybenzothiazol-2-yl) asparagine benzyl ester (**3d**).* The product was isolated as a colourless solid (0.577 g, 1.15 mmol, 91%). Mp=117.0–118.9 °C. IR (KBr, 1%): ν=3369, 3208, 3068, 2979, 2932, 1764, 1747, 1727, 1695, 1607, 1572, 1556, 1520, 1461, 1417, 1392, 1368, 1344, 1262, 1222, 1158, 1062, 982, 944, 912, 892, 853, 820, 805, 780, 752, 732, 700, 665, 515 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.42–1.48 (m, 12H, C(CH_3)$_3$ and OCH_2CH_3), 3.02–3.28 (m, 2H, β-CH_2), 4.08 (q, *J* 6.8 Hz, 2H, OCH_2CH_3), 4.75 (t, *J* 4.0 Hz, 1H, α-H), 5.16–5.24 (m, 2H, CH_2), 5.89 (d, *J* 8.4 Hz, 1H, NH Boc), 7.01 (dd, *J* 8.8 and 2.4 Hz, 1H, H5), 7.25–7.30 (m, 6H, 5×Ph-*H* and H7), 7.62 (d, *J* 8.8 Hz, 1H, H4), 8.55 (br s, 1H, NH amide) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=14.79 (OCH_2CH_3), 28.22 (C(*C*H_3)$_3$), 38.28 (β-CH_2), 50.10 (α-C), 64.15 (OCH_2CH_3), 67.57 (CH_2), 80.22 (*C*(CH_3)$_3$), 105.09 (C7), 116.03 (C5), 120.77 (C4), 128.16 (C3′ and C5′), 128.27 (C4′), 128.46 (C2′ and C6′), 132.45 (C7a), 135.12 (C1′), 140.44 (C3a), 155.57 (C=O urethane), 156.42 (C6), 157.08 (C2), 169.03 (C=O amide), 170.94 (C=O ester) ppm. UV–Vis (ethanol, nm): λ_{max} (log ε)=345 (3.34). MS *m*/*z* (ESI, %): 522 (M^++23, 100), 500 ($[M+H]^+$, 8). $C_{25}H_{29}N_3O_6S$: calcd C 60.10, H 5.85, N 8.41, S 6.42; found C 60.20, H 5.98, N 8.21, S 6.19.

*4.2.5. N-tert-Butyloxycarbonyl (6-fluorobenzothiazol-2-yl) asparagine benzyl ester (**3e**).* The product was isolated as a colourless solid (0.282 g, 0.60 mmol, 65%). Mp=152.2–154.0 °C. IR (KBr, 1%): ν=3370, 3264, 3217, 3069, 2981, 2932, 1755, 1741, 1696, 1609, 1557, 1520, 1461, 1425, 1417, 1391, 1368, 1344, 1318, 1287, 1251, 1222, 1197, 1167, 1153, 1054, 1029, 1020, 990, 979, 916, 851, 826, 808, 780 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.41 (s, 9H, C(CH_3)$_3$), 3.08–3.31 (m, 2H, β-CH_2), 4.82 (t, *J* 3.6 Hz, 1H, α-H), 5.17–5.26 (m, 2H, CH_2), 6.09 (d, *J* 7.5 Hz, 1H, NH Boc), 7.08 (dt, *J* 9.0 and 2.4 Hz, 1H, H5), 7.24–7.33 (m, 5H, 5×Ph-*H*), 7.46 (dd, *J* 7.8 and 2.1 Hz, 1H, H7), 7.65–7.69 (m, 1H, H4), 9.50 (br s, 1H, NH amide) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ=28.17 (C(*C*H_3)$_3$), 38.21 (β-CH_2), 50.14 (α-C), 67.64 (CH_2), 80.30 (*C*(CH_3)$_3$), 107.66 (d, *J* 26.8 Hz, C7), 114.74 (d, *J* 24.5 Hz, C5), 121.44 (d, *J* 8.90 Hz, C4), 128.12 (C3′ and C5′), 128.30 (C4′), 128.45 (C2′ and C6′), 132.59 (d, *J* 10.3 Hz, C7a), 135.00 (C1′), 143.80 (C3a), 155.64 (C=O urethane), 158.27 (C2), 159.56 (d, *J* 243.8 Hz, C6), 169.32 (C=O amide), 171.09 (C=O ester) ppm. UV–Vis (ethanol, nm): λ_{max} (log ε)=346 (3.52). MS *m*/*z* (ESI, %): 496 (M^++23, 100), 474 ($[M+H]^+$, 15). $C_{23}H_{24}N_3O_5SF$: calcd C 58.34, H 5.11, N 8.87; found C 58.33, H 5.27, N 8.86.

*4.2.6. N-tert-Butyloxycarbonyl (6-nitrobenzothiazol-2-yl) asparagine benzyl ester (**3f**).* The product was isolated as a colourless solid (0.368 g, 0.74 mmol, 58%). Mp=114.7–120.5 °C. IR (KBr, 1%): ν=3377, 2970, 2931, 1758, 1729, 1698, 1627, 1600, 1552, 1519, 1444, 1422, 1552, 1519, 1455, 1444, 1391, 1368, 1347, 1299, 1272, 1245, 1212, 1198, 1158, 1170, 1066, 1016, 983, 909, 873, 784, 755, 729, 696, 666 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.43 (s, 9H, C(CH_3)$_3$), 3.10–3.27 (m, 2H, β-CH_2), 4.78 (br s, 1H, α-H), 5.19–5.31 (m, 2H, CH_2), 5.68 (br s, 1H, NH Boc), 7.27–7.33 (m, 6H, 5×Ph-*H* and NH amide), 7.85 (d, *J* 8.7 Hz, 1H, H4), 8.35 (dd, *J* 9.0 and 2.4 Hz, 1H, H5), 8.76 (d, *J* 2.4 Hz, 1H, H7) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=28.26 (C(*C*H_3)$_3$), 38.74 (β-CH_2), 50.31 (α-C), 67.97 (CH_2), 80.76 (*C*(CH_3)$_3$), 118.09 (C7), 120.84 (C4), 122.17 (C5), 128.29 (C4′), 128.51 (C3′ and C5′), 128.56 (C2′ and C6′), 132.08 (C7a), 134.85 (C1′), 144.07 (C3a), 151.97 (C6), 155.74 (C=O urethane), 162.38 (C2), 169.53 (C=O amide), 170.89 (C=O ester) ppm. UV–Vis (ethanol, nm): λ_{max} (log ε)=325 (3.53). MS *m*/*z* (ESI, %): 523 (M^++23, 100). $C_{23}H_{24}N_4O_7S$: calcd C 55.19, H 4.83, N 11.19, S 6.41; found C 55.49, H 5.00, N 10.88, S 6.46.

*4.2.7. N-tert-Butyloxycarbonyl (6-thiocyanobenzothiazol-2-yl) asparagine benzyl ester (**3g**).* The product was isolated as a colourless solid (0.424 g, 0.83 mmol, 66%). Mp=169.0–172.0 °C. IR (KBr, 1%): ν=3326, 3230, 3094, 2973, 2940, 1742, 1730, 1701, 1594, 1524, 1447, 1409, 1393, 1367, 1295, 1278, 1236, 1193, 1154, 1075, 1007, 990, 973, 925, 900, 869, 849, 757, 724 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.44

(s, 9H, C(CH_3)$_3$), 3.13–3.31 (m, 2H, β-CH_2), 4.76 (br s, 1H, α-H), 5.18–5.27 (m, 2H, CH_2), 5.69 (br s, 1H, NH Boc), 7.27–7.33 (m, 6H, 5×Ph-*H* and NH amide), 7.63 (dd, *J* 7.8 and 2.1 Hz, 1H, H5), 7.78 (d, *J* 8.1 Hz, 1H, H4), 7.98 (d, *J* 2.1 Hz, 1H, H7) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ=28.23 (C(*C*H_3)$_3$), 38.55 (β-CH_2), 50.19 (α-C), 67.83 (CH_2), 80.60 (*C*(CH_3)$_3$), 110.73 (SCN), 118.96 (C6), 122.03 (C4), 124.30 (C7), 128.23 (C4′), 128.45 (C3′ and C5′), 128.53 (C2′ and C6′), 129.22 (C5), 133.26 (C7a), 134.91 (C1′), 148.10 (C3a), 155.66 (C=O urethane), 160.15 (C2), 169.38 (C=O amide), 170.90 (C=O ester) ppm. UV–Vis (ethanol, nm): λ_{max} (log ε)=335 (3.28). MS *m*/*z* (ESI, %): 535 (M^++23, 100). $C_{24}H_{24}N_4O_5S_2$: calcd C 56.23, H 4.72, N 10.93, S 12.51; found C 56.46, H 4.87, N 10.66; S 12.87.

*4.2.8. N-tert-Butyloxycarbonyl (6-sulfomethylbenzothiazol-2-yl) asparagine benzyl ester (**3h**).* The product was isolated as a colourless solid (0.451 g, 0.85 mmol, 68%). Mp=190.6–192.5 °C. IR (KBr, 1%): ν=3362, 3278, 3241, 3064, 3024, 2985, 2930, 1748, 1722, 1698, 1596, 1527, 1447, 1388, 1372, 1283, 1147, 1100, 1055, 1027, 980, 957, 910, 883, 865, 825, 785, 747, 732, 696, 665, 512, 503 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.44 (s, 9H, C(CH_3)$_3$), 3.14 (s, 3H, CH_3), 3.16–3.34 (m, 2H, β-CH_2), 4.77 (br s, 1H, α-H), 5.18–5.27 (m, 2H, CH_2), 5.70 (br s, 1H, NH Boc), 7.25–7.34 (m, 6H, 5×Ph-*H* and NH amide), 7.90 (d, *J* 8.1 Hz, 1H, H4), 7.97 (dd, *J* 8.1 and 1.5 Hz, H5), 8.45 (d, *J* 1.8 Hz, 1H, H7) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ=28.24 (C(*C*H_3)$_3$), 38.79 (β-CH_2), 44.88 (CH_3), 50.21 (α-C), 67.89 (CH_2), 80.73 (*C*(CH_3)$_3$), 121.03 (C4), 121.98 (C7), 125.70 (C5), 128.32 (C4′), 128.50 (C2′ and C6′), 128.57 (C3′ and C5′), 131.77 (C7a), 134.88 (C1′), 136.27 (C6), 149.88 (C3a), 155.61 (C=O urethane), 161.82 (C2), 169.33 (C=O amide), 170.64 (C=O ester) ppm. UV–Vis (ethanol, nm): λ_{max} (log ε)=331 (3.13). MS *m*/*z* (ESI, %): 534 ([M+H]$^+$, 14). $C_{24}H_{27}N_3O_7S_2$: calcd C 54.02, H 5.10, N 7.87, S 12.02; found C 54.22, H 5.18, N 7.76, S 12.12.

*4.2.9. N-tert-Butyloxycarbonyl (benzothiazol-6-yl) asparagine benzyl ester (**3i**).* The product was isolated as a colourless solid (0.492 g, 1.08 mmol, 86%). Mp=150.1–152.0 °C. IR (KBr, 1%): ν=3433, 3332, 3055, 2973, 2927, 1745, 1721, 1671, 1609, 1578, 1534, 1456, 1490, 1474, 1456, 1401, 1382, 1366, 1339, 1286, 1247, 1197, 1160, 1056, 1028, 979, 948, 911, 873, 858, 832, 822, 793, 781, 770, 756, 694, 665, 600 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.42 (s, 9H, C(CH_3)$_3$), 2.97–3.14 (m, 2H, β-CH_2), 4.66 (br s, 1H, α-H), 5.18–5.23 (m, 2H, CH_2), 5.90 (d, *J* 5.6 Hz, 1H, NH Boc), 7.27–7.31 (m, 6H, 5×Ph-*H* and NH amide), 7.97 (d, *J* 8.8 Hz, 1H, H4), 8.49–8.53 (m, 2H, H5 and H7), 8.97 (s, 1H, H2) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=28.24 (C(*C*H_3)$_3$), 39.20 (β-CH_2), 50.55 (α-C), 67.51 (CH_2), 80.33 (*C*(CH_3)$_3$), 112.55 (C5 or C7), 119.18 (C5 or C7), 123.01 (C4), 128.13 (C4′), 128.33 (C3′ and C5′), 128.49 (C2′and C6′), 134.39 (C7a), 135.16 (C1′), 135.74 (C6), 148.83 (C3a), 153.81 (C2), 155.82 (C=O urethane), 168.57 (C=O amide), 171.34 (C=O ester) ppm. UV–Vis (ethanol, nm): λ_{max} (log ε)=332 (3.05). MS *m*/*z* (ESI, %): 478 (M^++23, 100). $C_{23}H_{25}N_3O_5S$: calcd C 60.64, H 5.53, N 9.22, S 7.04; found C 60.60, H 5.53, N 9.11, S 7.37.

*4.2.10. N-tert-Butyloxycarbonyl (benzimidazol-2-yl) asparagine benzyl ester (**3j**).* The product was isolated as a colourless solid (0.519 g, 1.18 mmol, 94%). Mp=143.0–143.3 °C. IR (KBr, 1%): ν=3363, 2978, 2929, 2851, 1755, 1742, 1697, 1642, 1594, 1584, 1523, 1433, 1391, 1368, 1344, 1309, 1274, 1223, 1167, 1058, 1029, 895, 865, 779, 760, 740, 696, 622 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ=1.36 (s, 9H, C(CH_3)$_3$), 2.94–3.18 (m, 2H, β-CH_2), 4.60 (t, *J* 4.4 Hz, 1H, α-H), 5.12 (s, 2H, CH_2), 7.23–7.34 (m, 8H, 5×Ph-*H*, NH amide, H5 and H6), 7.37–7.39 (m, 1H, H4 or H7), 7.49 (d, *J* 8.0 Hz, 1H, NH Boc), 7.61–7.63 (m, 1H, H4 or H7), 12.98 (br s, 1H, NH benzimidazole) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6): δ=28.09 (C(*C*H_3)$_3$), 37.63 (β-CH_2), 49.76 (α-C), 66.37 (CH_2), 78.62 (*C*(CH_3)$_3$), 113.43 (C5 and C6), 124.39 (C4 and C7), 127.74 (C3′ and C5′), 127.98 (C4′), 128.28 (C2′ and C6′), 129.59 (C3a and C7a), 135.65 (C1′), 143.09 (C2), 155.25 (C=O urethane), 169.84 (C=O amide), 171.03 (C=O ester) ppm. UV–Vis (ethanol, nm): λ_{max} (log ε)=291 (3.85). MS *m*/*z* (ESI, %): 461 (M^++23, 26), 439 ([M+H]$^+$, 100). $C_{23}H_{26}N_4O_5$: calcd C 63.00, H 5.98, N 12.78; found C 63.01, H 6.20, H 12.58.

Acknowledgements

Thanks are due to the *Fundação para a Ciência e Tecnologia* (Portugal) for financial support through project PTDC/QUI/66250/2006 and research grants to C.I.C.E. and A.M.F.S. The NMR spectrometer Bruker Avance II 400 is part of the National NMR Network and was acquired with funds from FCT and FEDER.

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