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A mild high yielding synthesis of oxazole-4-carboxylate derivatives

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ABSTRACT

Several 2,5-disubstituted oxazole-4-carboxylates were prepared in high yields from the methyl esters of *N*-acyl-β-halodehydroaminobutyric acid derivatives by treatment with a 2% solution of DBU in acetonitrile. The scope of this reaction was investigated and it was found that dehydrodipeptides having a β-bromodehydroaminobutyric acid residue gave the corresponding oxazoles in good yields. The photophysical properties of some of the oxazoles prepared were studied in four solvents of different polarity. All compounds have reasonable high fluorescence quantum yields and a moderate solvent sensitivity, which makes them good candidates to be used as fluorescent probes. One of the fluorescent oxazoles prepared was inserted after cleavage of the methyl ester into two model peptides using a conventional solution phase strategy. The photophysical properties of the labelled peptides were studied in ethanol and water and compared with those of the oxazole. The results obtained showed that the oxazole maintains a good fluorescence level and the same solvent sensitivity when linked to a peptide chain.

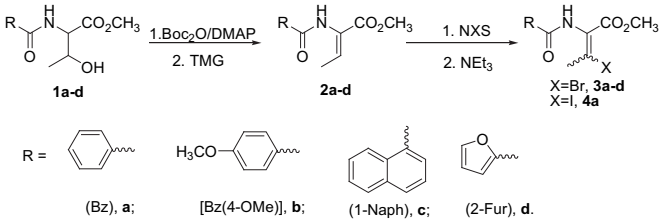
1. Introduction

Oxazoles are considered as an important class of heterocyclic compounds since they are structural subunits of various biologically active natural products and are valuable synthetic precursors and pharmaceuticals.[1] Oxazoles are associated with anti-bacterial, anti-fungal, anti-inflammatory and *anti*-tumoral activities and can be used as peptide mimetics or enzyme inhibitors.[2] Oxazole derivatives have also been used as efficient luminophores for liquid and plastic scintillators and as fluorescent probes for biological systems.[3]

Several methods have been developed for the syntheses of this type of compounds, such as the Hantzsch reaction,[4] the Robinson–Gabriel synthesis where β-ketoamides are cyclodehydrated,[5] the dehydration of oxazolines, condensations of substituted amides with phenacyl bromide in ethanol,[2e] the Ugi reaction with ammonia as the amine component,[6] the coupling of an acyl chloride with an isocyanide under basic conditions[7] or the gold catalyzed cyclization of propargylic amides.[8] Amino acids have also been used as substrates for the synthesis of oxazole derivatives. Thus, Wipf et al. prepared oxazoles by treating serine derivatives with diethylaminosulphur trifluouride (DAST) followed by bromotrichloromethane and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[9] Disubstituted oxazoles were obtained from *N*-acylamino acids by cyclodehydration of the intermediate α-acylamino aldehydes.[10] In our research group we have prepared oxazoles from *N*-acyl β-hydroxyamino acids by a sequential dehydration reaction followed by an intramolecular cyclization promoted by iodine/DBU.[11] Continuing this work and taking advantage of our experience in the synthesis and reactivity of β-halodehydroamino acids[12] we aimed at preparing functionalized oxazoles from β-halodehydroaminobutyric acid derivatives. We also aimed at studying their photophysical properties in order to test their possible use as fluorescent probes.

2. Results and discussion

Several *N*-acyl-β-bromodehydroaminobutyric acids were prepared from the corresponding *N*-acyl-threonine derivatives using a sequential dehydration followed by a halogenation reaction (Scheme 1).[11,12c]



Scheme 1.

Considering our previously developed method for the synthesis of oxazoles and the mechanism proposed the *Z*-isomer of the methyl ester of *N*-benzoyl-β-bromodehydroaminobutyric acid *Z*-**3a** was treated with several bases in different amounts [Et_3N, sodium

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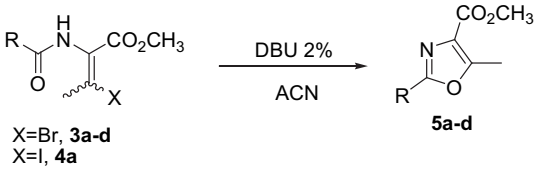
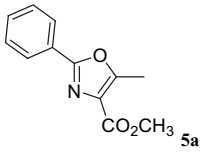
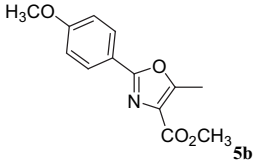
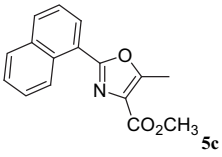
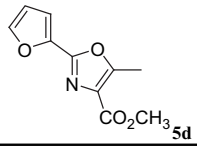
methoxide, sodium *tert*-butoxide, *N*,*N*,*N*′,*N*′-tetramethylguanidine (TMG) and DBU] and the reactions followed by HPLC. It was found that the best result was obtained with a 2% solution of DBU in acetonitrile (89% yield of oxazole **5a**). Thus, compounds *Z*-**3b**–**d** were reacted with DBU to give the corresponding 2,5-disubstituted oxazole-4-carboxylates (**5b**–**d**) in high yields (84–91%) (Scheme 2, Table 1). This reaction was also tested using as substrates de *E*-isomers of β-bromodehydroaminobutyric acid derivatives (Table 1, entries 2 and 6). The oxazole-4-carboxylates were obtained in similar yields when compared with the *Z*-isomers. These results show that it is possible to use the mixtures of stereoisomers as reagents, which is an advantage since the halogenation reaction affords the corresponding β-halodehydroamino acids as mixtures of the *E*- and *Z*-isomers. The scope of this reaction was also enlarged to β-iododehydroaminobutyric acid derivatives since compound *Z*-**4a** afforded oxazole **5a** in 96% yield (Table 1, entry 3).

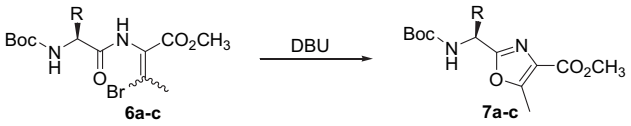


Scheme 2.

Table 1
Yields obtained in the synthesis of oxazole-4-carboxylates from *N*-acyl-β-halodehydroaminobutyric acid derivates

Entry	Reagent	Product	Yield/%
1	*Z*-**3a**	 **5a**	89
2	*E*-**3a**	**5a**	86
3	*Z*-**4a**	**5a**	96
4	*Z*-**3b**	 **5b**	91
5	*Z*-**3c**	 **5c**	91
6	*E*-**3c**	**5c**	88
7	*Z*-**3d**	 **5d**	84

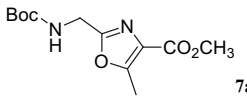
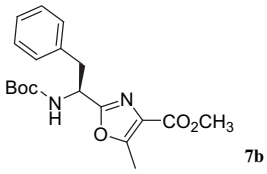
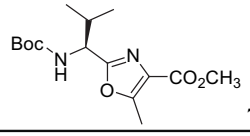
In order to test the possibility of using dipeptides with a β-halodehydroaminobutyric acid residue as substrates in this reaction, several dipeptides having a β-bromodehydroaminobutyric acid were reacted with DBU. The corresponding oxazole derivatives were obtained in good yields (Scheme 3, Table 2).



R = H, **a**; $CH_2C_6H_5$, **b**; $CH(CH_3)_2$, **c**.

Scheme 3.

Table 2
Yields obtained in the synthesis of oxazole-4-carboxylates from β-bromodehydrodipeptide derivatives

Entry	Reagent	Product	Yield/%
1	*Z*-**6a**	 **7a**	85
2	*Z*-**6b**	 **7b**	78
3	*E*-**6b**	**7b**	82
4	*E*-**6c**	 **7c**	89

All ^{1}H NMR spectra of oxazole-4-carboxylates show a downfield shift of OCH_3 and 5-CH_3 protons when compared with the corresponding protons in the reagent. The ^{13}C NMR spectra of these compounds show that C2 (159.3–162.2 ppm) resonates at the lowest fields compared with C4 and C5. This is a common feature reported by other authors for other oxazole derivatives.[13] The exception is compound **5d**, which shows a C2 chemical shift (152.3 ppm) smaller than C5 (155.8 ppm), which is probably due to the effect of the electron-rich furan ring. C4 resonates at higher fields (between 120.1 and 128.6 ppm) when compared with C2 and C5. The high chemical shifts observed for C5 (between 155.4 and 156.3 ppm) when compared with the C5 chemical shift of the unsubstituted oxazole ring (138.1 ppm)[13] can be attributed to the carboxylate function linked to C4.

The mechanism proposed for the intramolecular cyclization of the β-halodehydroaminobutyric acids promoted by DBU involves the attack of the carbonyl oxygen on the β-carbon atom of the dehydroaminobutyric acid with loss of the halogen anion.

When β-bromodehydrophenylalanine and β,β-dibromodehydroalanines were used as substrates no reaction occurred. This may be partially due to a lower electron density at the β-carbon atom of β-halodehydroaminobutyric acid derivatives, which can be related with the ^{13}C NMR chemical shift (Table 3). In fact, the chemical shifts of the β-carbon atom of the β-bromodehydroaminobutyric

Table 3
^{13}C NMR chemical shifts in $CDCl_3$ of the β-carbon atom and reduction peak potentials of β-bromodehydroamino acid derivatives.[12c]

Compound	δ β-C atom/ppm	−*Ep* (V vs SCE)[12c]
Z-**3a**	123.56	1.78
Z-**3b**	127.51	1.88
Z-**3c**	126.46	—
Z-**3d**	126.43	1.94
Bz(OMe)-*Z*-ΔPhe(β-Br)-OMe	114.32	1.41
Fur-*Z*-ΔPhe(β-Br)-OMe	112.81	1.44
Bz(OMe)-ΔAla(β,β-Br)-OMe	84.74	1.58
Fur-ΔAla(β,β-Br)-OMe	85.48	1.57

acids are considerably higher than those of the corresponding β-bromodehydrophenylalanines and β,β-dibromodehydroalanines. By comparing the reduction peak potentials of these compounds obtained previously by us in a study concerning the electrochemical behaviour of β-halodehydroamino acids[12c] it is possible to conclude that β-bromodehydroaminobutyric acids have lower reduction potentials when compared with the corresponding dehydrophenylalanines and dehydroalanines (Table 3).

The reactions of compounds *E*-**3a**, *Z*-**3b**, *Z*-**3d** and *E*-**6c** were followed by reverse phase HPLC and plots of the peak areas versus time were obtained for each compound (Fig. 1).

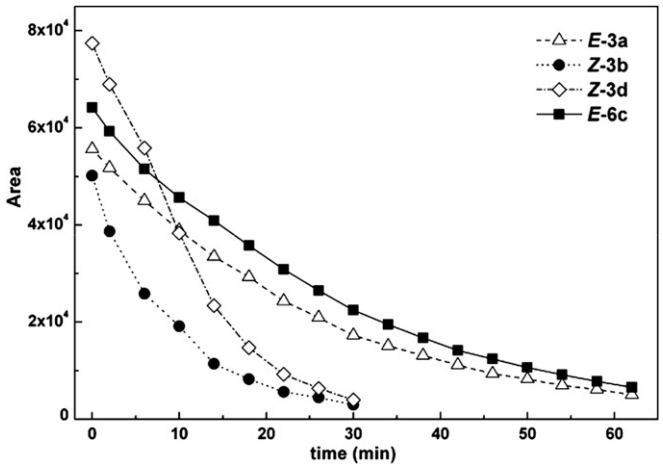


Fig. 1. Plot of Area versus time for compounds *E*-**3a**, *Z*-**3b**, *Z*-**3d** and *E*-**6c**.

A linear correlation was obtained for all compounds by plotting the ln(Area) versus time (Fig. 2), which indicates a first order kinetics. The rate constants determined for compounds *E*-**3a**, *Z*-**3b**, *Z*-**3d** and *E*-**6c** were 3.88×10^{-2} min^{-1}, 9.34×10^{-2} min^{-1}, 10.2×10^{-2} min^{-1} and 3.67×10^{-2} min^{-1}, respectively. The increase in the rate constant of compound *Z*-**3b** when compared with compound *E*-**3a** is due to the presence of an electron-donating group linked to the phenyl moiety. A similar effect was observed for compound *Z*-**3d**, which has an electron-rich furan ring instead of the phenyl moiety of compound *E*-**3a**.

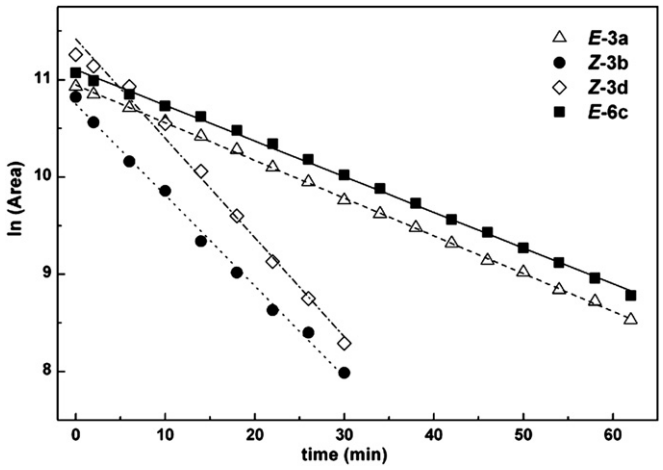
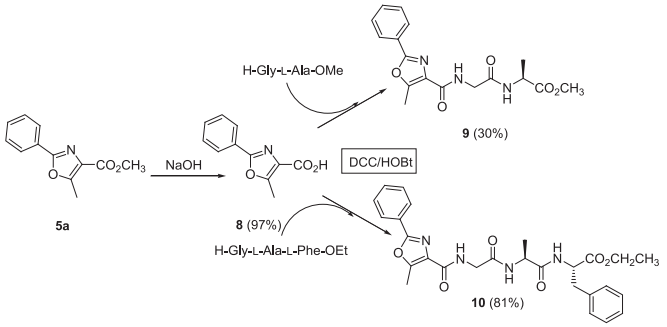


Fig. 2. Plot of ln(Area) versus time for compounds *E*-**3a**, *Z*-**3b**, *Z*-**3d** and *E*-**6c**, showing the linear dependencies.

In order to study the photophysical properties of the oxazoles prepared when incorporated into peptides, two model peptides linked to the 5-methyl-2-phenyloxazole moiety were prepared. Therefore, the methyl ester of compound **5a** was cleaved by the treatment with a solution of NaOH in methanol to give the carboxylic acid **8** in 97% yield. This compound was then coupled with two model peptides (H-Gly-L-Ala-OMe and H-Gly-L-Ala-L-Phe-OEt) using a conventional solution phase strategy. The peptides labelled with the oxazole moiety were obtained in 30% and 81% yield (Scheme 4).



Scheme 4.

The absorption and fluorescence properties of compounds **5a**–**d** and **8** were studied in solvents of different polarity (cyclohexane, ethyl acetate, acetonitrile and ethanol). For **5a** and **8**, spectroscopic measurements in water were also performed, to obtain a better comparison with the behaviour of the labelled peptides (**9** and **10**). The normalized absorption and fluorescence spectra of compounds **5a**–**d** and **8** are presented in Figs. 3 and 4. The maximum absorption (λ_{abs}) and emission (λ_{em}) wavelengths, molar absorption coefficients (ε) and fluorescence quantum yields (Φ_F) of the oxazole derivatives are presented in Table 4.

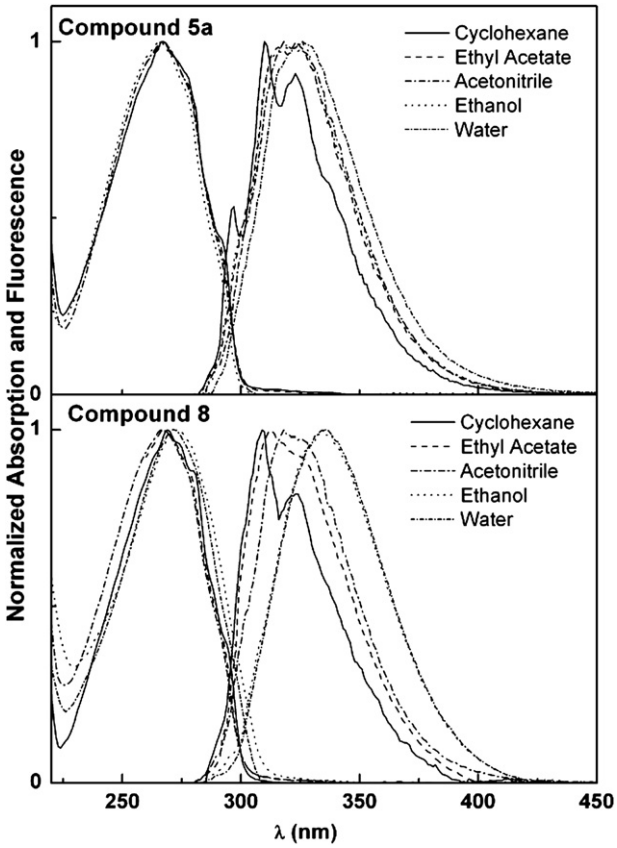


Fig. 3. Normalized absorption (in the lowest energy band) and fluorescence spectra (λ_{exc}=270 nm) of solutions (2×10^{-5} mol dm^{-3} for absorption and 2×10^{-6} mol dm^{-3} for fluorescence) of compounds **5a** and **8**, in several solvents.

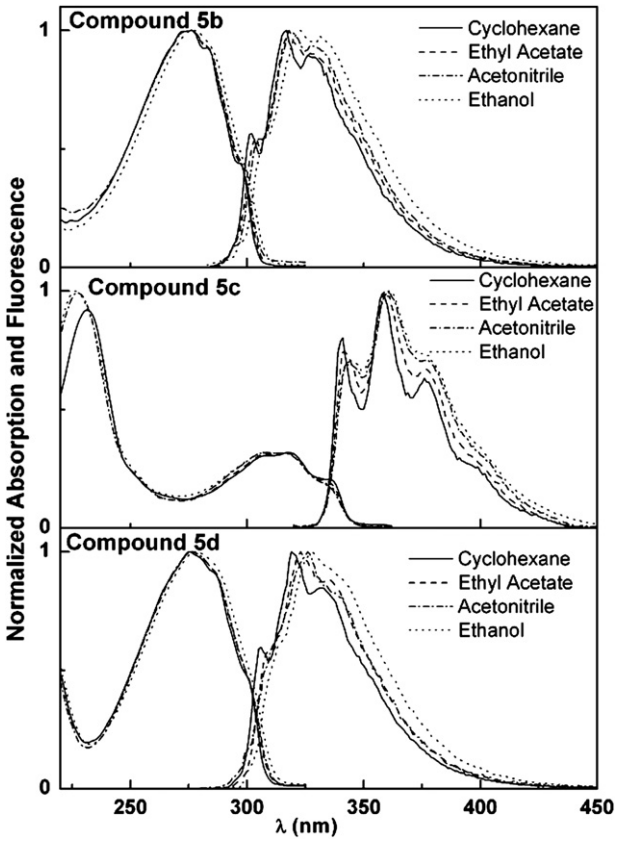


Fig. 4. Normalized absorption (in the lowest energy band) and fluorescence spectra of solutions (2×10^{-5} mol dm^{-3} for absorption and 2×10^{-6} mol dm^{-3} for fluorescence) of compounds **5b** and **5d** (λ_{exc}=270 nm) and **5c** (λ_{exc}=310 nm), in several solvents.

These compounds present high molar absorption coefficients at the lowest energy maximum ($\varepsilon\geq1.2\times10^4$ mol^{-1} dm^3 cm^{-1}) in all solvents studied (Table 4), pointing to a $\pi\rightarrow\pi^*$ transition, as observed for isoxazoles.[16] A methyl carboxylate group or carboxylic acid (in case of compound **6**) is also present in all compounds and it is known that many carbonyl compounds present a low-lying $n\rightarrow\pi^*$ state. The $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electronic transitions can be nearby in energy, resulting in state-mixing.[17] The high values of the molar absorption coefficient can be explained by a predominance of $\pi\rightarrow\pi^*$ character in these compounds that also justifies the high fluorescence quantum yield values (Table 4). The influence of solvent in the absorption spectral shape is generally negligible.

The emission spectra of the studied compounds (**5a**–**d** and **8**) show a defined vibrational structure in cyclohexane (non polar solvent) and a progressive loss of this structure with increasing solvent polarity (Figs. 3 and 4). Compound **5c**, which presents the more extended aromatic system, exhibits a more detailed vibrational structure in the fluorescence spectra (Fig. 4). The loss of vibrational structure in emission and the red shift observed in polar solvents points to an intramolecular charge transfer (ICT) character of the excited state,[18] although not very pronounced.

The effect of the solvent in the spectral shape is more pronounced for compound **8**, where the emission is almost completely non-structured and significantly red-shifted in ethanol and water (Fig. 3). It was described that oxazoles and hydroxyl containing compounds form complexes, leading to a loss of vibrational structure in their spectra.[19] For these oxazoles, complex formation with protic solvents is not anticipated from their photophysical behaviour (Figs. 3 and 4), as the loss of vibrational structure is progressive with increasing solvent polarity. The spectral changes in protic solvents are significant for **8**, but not for **5a**. The presence of

Table 4
Maximum absorption (λ_{abs}) and emission (λ_{em}) wavelengths, molar absorption coefficients (ϵ) and fluorescence quantum yields (Φ_F) for compounds **5a**–**d** and **8**

Solvent	λ_{abs}/nm [ϵ/10^4 mol^{-1} dm^3 cm^{-1}]					λ_{em}/nm					Φ_F[a]				
	5a	**5b**	**5c**	**5d**	**8**	**5a**	**5b**	**5c**	**5d**	**8**	**5a**	**5b**	**5c**	**5d**	**8**
Cyclohexane	267 (1.87)	275 (2.15)	318 (1.35) 231 (3.89)	276 (1.67)	269 (1.22)	297, 310, 323	306, 319, 331	340, 359, 376	302, 317, 326	309, 321	0.36	0.55	0.58	0.59	0.16
Ethyl acetate	267 (1.85)	276 (2.42)	317 (1.35)	276 (1.82)	267 (1.72)	318, 324	310, 323, 332	342, 359, 377	304, 318, 328	312, 323 sh	0.44	0.31	0.54	0.68	0.50
Acetonitrile	267 (1.68)	276 (2.42)	308 (1.25) 227 (3.90)	276 (1.63)	267 (1.36)	322	310, 326, 331	343, 361, 375 sh	318, 327	323	0.38	0.38	0.51	0.44	0.40
Ethanol	267 (1.82)	278 (2.37)	309 (1.22) 226 (3.83)	277 (1.95)	273 (1.74)	324	328, 332	344, 362, 376 sh	323, 331	335	0.47	0.41	0.50	0.41	0.52
Water	267 (1.95)	—	—	—	273 (1.57)	328	—	—	—	336	0.34	—	—	—	0.51

[a] Relative to naphthalene in cyclohexane (Φ_F=0.23 at 25 °C)[14] for **5a**, **5b**, **5d** and **8**; relative to anthracene in ethanol (Φ_F=0.27 at 25 °C)[15] for **5c**. Error about 10%. Solvents cut-off: ethyl acetate: 260 nm. sh: shoulder.

a carboxylic acid in **8** may lead to the increase of the ICT character of excited state and also enhances the hydrogen bonding capability of this oxazole.

From ab initio molecular quantum chemistry calculations, obtained with Gaussian 09 software[20] and use of a 3-21+G(d) basis set,[21] the optimized geometries of compounds **5a**–**d** and **8** were obtained (Fig. 5), showing that all compounds are roughly planar.

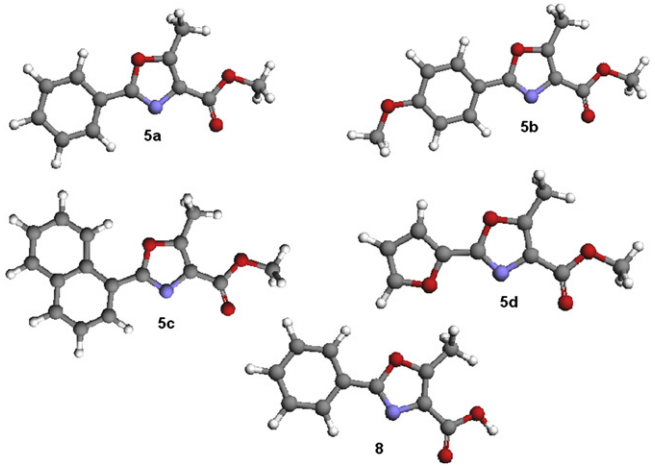


Fig. 5. Optimized geometries of compounds **5a**, **5b**, **5c**, **5d** and **8** obtained by Gaussian 09 software (grey: C atoms; white: H atoms; red: O atoms; blue: N atoms).

Figs. 6 and 7 display the representation of HOMO and LUMO molecular orbitals for the same compounds. In both compounds **5a** and **8** (Fig. 6), the HOMO–LUMO transition causes a significant increase in the electronic density of the carbonyl group of the methyl carboxylate substituent, more significant for compound **8**, where this increase is extended to the whole carboxylate group. This can justify the differences observed in the photophysical behaviour of **8** and **5a**, although complex formation between **8** and protic solvents cannot be completely discarded.

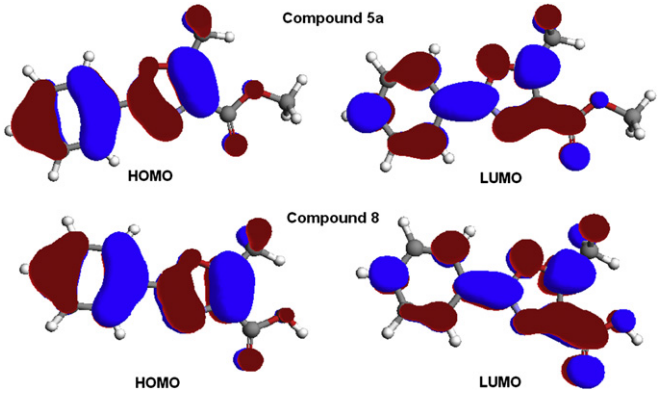


Fig. 6. Representation of HOMO and LUMO molecular orbitals of compounds **5a** (above) and **8** (below).

Compounds **5b** and **5d** have roughly similar absorption and emission spectra (Fig. 4), while the fluorescence quantum yields are generally higher for **5d** (Table 4). The representation of HOMO and LUMO molecular orbitals for compounds **5b**–**d** is exhibited in Fig. 7. For both compounds **5b** and **5d**, it can be observed that upon HOMO→LUMO transition there is a similar increase in the electronic density of the carbonyl group, with a decrease in the opposite side of the molecule.

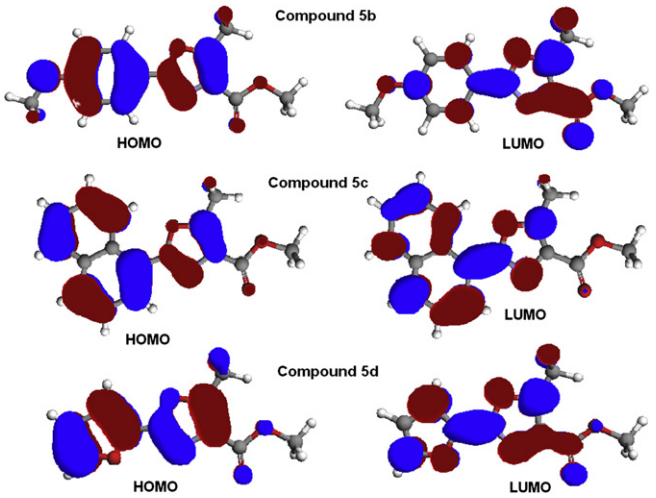


Fig. 7. Representation of HOMO and LUMO molecular orbitals of compounds **5b** (above), **5c** (middle) and **5d** (below).

The same does not happen for compound **5c**, where the electronic density is mainly located in the aromatic rings in both HOMO and LUMO, with a very small participation of the carboxylate group in the HOMO→LUMO transition, pointing to a low ICT character of the excited state. This may justify the well defined vibrational structure in emission spectra, even in polar solvents, also present in the absorption lowest energy band (although not so well defined). The spectra of **5c** are significantly shifted to lower energies relative to the other oxazoles (Fig. 4), showing that the extension of the conjugation with a naphthyl group makes an important contribution to both absorption and fluorescence properties.

All compounds have reasonable high fluorescence quantum yields (Φ_F>30%, except for **8** in cyclohexane—Table 4). A clear trend between the Φ_F values and the polarity of solvent is not detected. The generally high fluorescence quantum yields of these oxazole derivatives and the moderate solvent sensitivity of their fluorescence emission make them good candidates to be used as fluorescence probes.

The absorption and emission spectra of peptides **9** and **10** were obtained in ethanol and water (Figs. 8 and 9). It can be observed a red shift in water relative to emission in ethanol of similar magnitude to that observed for the free **5a** (Tables 4 and 5).

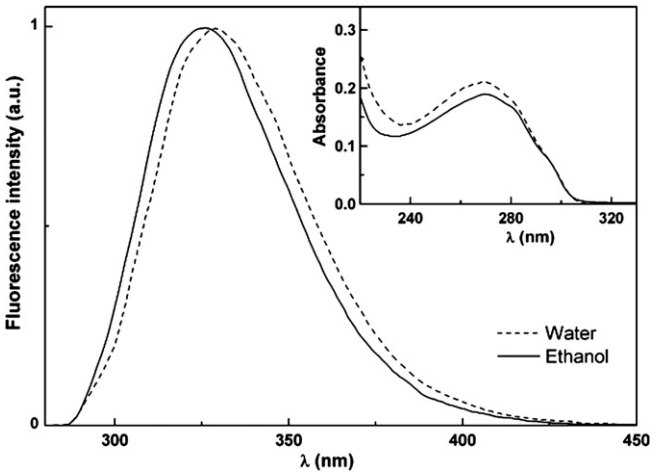


Fig. 8. Normalized fluorescence spectra (λ_{exc}=270 nm) of 2×10^{-6} mol dm^{-3} solutions of peptide **9** in ethanol and in water (pH=7). Inset: absorption spectra of 2×10^{-5} mol dm^{-3} solutions of peptide **9** in ethanol and in water (pH=7).

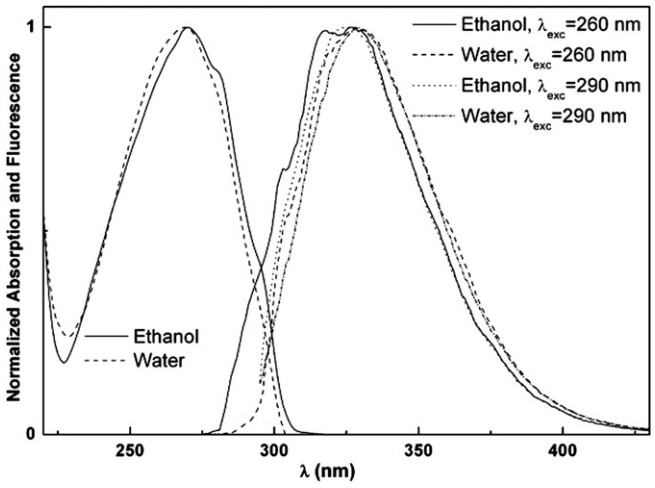


Fig. 9. Normalized absorption and fluorescence spectra (λ_{exc}=260 nm and 290 nm) of solutions (2×10^{-5} mol dm^{-3} for absorption and 2×10^{-6} mol dm^{-3} for fluorescence) of peptide **10** in ethanol and in water (pH=7).

Table 5
Maximum absorption (λ_{abs}) and emission (λ_{em}) wavelengths, molar absorption coefficients (ε) and fluorescence quantum yields (Φ_F) for labelled peptides **9** and **10**

Solvent	λ_{abs}/nm [$\epsilon/10^4$ mol^{-1} dm^3 cm^{-1}]		λ_{em}/nm			Φ_F[a]	
	9	**10**	**9**	**10** (λ_{exc}=260 nm)	**10** (λ_{exc}=290 nm)	**9**	**10**
Ethanol	270 (0.95)	209 (2.16) 270 (1.70)	325	303 sh, 317, 326	325	0.35	0.29
Water	269 (1.05)	270 (1.70)	329	329	329	0.29	0.19

[a] Relative to naphthalene in cyclohexane (Φ_F=0.23 at 25 °C).[14] Error about 10%. For peptide **10**, λ_{exc}=290 nm was used, to prevent excitation of phenylalanine.

The absorption spectra of peptide **10** (Fig. 9) resemble those of **5a**, as phenylalanine has a very low molar absorption coefficient (195 mol^{-1} dm^3 cm^{-1} at 257.5 nm in an aqueous media),[22] due to a symmetry-forbidden $\pi\rightarrow\pi^*$ transition,[23] which also justifies its low fluorescence quantum yield (Φ_F=0.04 in water[24]). When excited at 290 nm, emission of peptide **10** is similar to one of the peptide **9** (Figs. 8 and 9). With excitation at 260 nm (Fig. 9), there is a possibility of non-radiative energy transfer (FRET) from the phenylalanine moiety to the oxazole. Peptide **10** fluorescence in water is dominated by the oxazole moiety, despite a shoulder appearing near 300 nm, which was expected due to the different Φ_F values of both fluorophores. However, noticeable differences in spectral shape are observed between emission in water and ethanol, with excitation at 260 nm, the latter exhibits two main peaks and a shoulder (near 300 nm), together with an enlargement in the higher energy region. These spectral features indicate differences in energy transfer efficiency from phenylalanine to oxazole in both solvents. In water, the less structured spectrum, which is clearly shifted to the oxazole emission region (with a maximum emission wavelength similar to that observed for **9**), may indicate a conformation where the two fluorophores are closer, with a more efficient energy transfer.

The fluorescence quantum yields of the labelled peptides **9** and **10** (the latter with excitation at 290 nm) are reasonably high (a reduction of 25% and 38% in ethanol and 15% and 44% in water, respectively, is observed relative to oxazole **5a**). The results show that the oxazole moiety maintains good fluorescence level and the same solvent sensitivity when linked to a peptide chain.

3. Conclusions

A mild and efficient method for the synthesis of substituted oxazole-4-carboxylate derivatives from *N*-acyl-β-halodehydroaminobutyric acid derivatives was developed. The oxazoles were obtained in high yields after treating the corresponding β-halodehydroaminobutyric acid derivative with a 2% solution of DBU in acetonitrile. Amino acids linked to an oxazole moiety were obtained from dipeptides having a β-bromodehydroaminobutyric acid residue. The strategy used to prepare peptides labelled with an oxazole moiety involved the cleavage of the methyl ester from one of the oxazoles prepared, followed by coupling to two *C*-protected peptides.

The photophysical properties of the oxazole derivatives were studied in solvents of different polarity and showed that all compounds have reasonably high fluorescence quantum yields and moderate solvent sensitivity, which indicate their possible use as fluorescent probes. From the results obtained with the peptides labelled with an oxazole, it is possible to conclude that the oxazole moiety maintains a good fluorescence level and the same solvent sensitivity when linked to a peptide chain.

In summary, we have prepared in high yields oxazole-4-carboxylates from β-halodehydroaminobutyric acid derivatives using a simple and efficient methodology. Furthermore, the photophysical studies carried out with these compounds indicate their possible use as fluorescent probes.

4. Experimental section

4.1. General methods

Melting points (°C) were determined in a Gallenkamp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus at 300 and 75.4 MHz, respectively, or on a Bruker Avance II$^+$ at 400 and 100.6 MHz, respectively. ^{1}H–^{1}H spin–spin decoupling, DEPT θ 45°, HMQC and HMBC were used to attribute some signals. NOE difference experiments were also performed. Chemical shifts are given in parts per million and coupling constants in hertz. MS and HRMS data were recorded by the mass spectrometry service of the University of Vigo, Spain; elemental analysis was performed on a LECO CHNS 932 elemental analyser.

The reactions were monitored by thin layer chromatography (TLC). Column chromatography was performed on Macherey–Nagel silica gel 230–400 mesh. Petroleum ether refers to the boiling range 40–60 °C. When solvent gradient was used, the increase of polarity was made from neat petroleum ether to mixtures of diethyl ether/petroleum ether, increasing 10% of diethyl ether each time until the isolation of the product.

HPLC analysis were performed using a Lichrospher 100 RP18 (5 μm) column in an HPLC system composed by a Jasco PU-980 pump, a Jasco UV-975 UV/vis detector and a Shimadzu C-R6A Chromatopac register.

4.2. Kinetic studies

Samples from the reaction mixture were taken at regular intervals and analysed by HPLC. The eluent was acetonitrile/water (4/1) at a flow rate of 1.2 mL min^{-1}. The chromatograms were traced by detecting UV absorption at 260 nm (retention time: *E*-**3a** 2.17 min, *Z*-**3b** 2.23 min, *Z*-**3d** 2.63 min, *E*-**6c** 2.35 min).

4.3. Spectroscopic measurements

All the solutions were prepared using spectroscopic grade solvents. Absorption spectra were recorded in a Shimadzu UV-3101PC UV–vis-NIR spectrophotometer. Fluorescence measurements were performed using a Fluorolog 3 spectrofluorimeter, equipped with double monochromators in both excitation and emission and a temperature controlled cuvette holder. Fluorescence spectra were corrected for the instrumental response of the system.

4.4. Fluorescence quantum yield

For fluorescence quantum yield determination, the solutions were previously bubbled for 30 min with ultrapure nitrogen. The fluorescence quantum yields (Φ_s) were determined using the standard method (Eq. 1).[25]

$$\Phi_s = \frac{A_r F_s n_s^2}{A_s F_r n_r^2}\Phi_r \quad (1)$$

where A is the absorbance at the excitation wavelength, F the integrated emission area and n the refraction index of the solvents used. Subscripts refer to the reference (r) or sample (s) compound.

4.5. Synthesis of the methyl esters of *N*-acyl-threonines

Synthesis of compounds Bz-Thr-OMe (**1a**),[11] Bz(OMe)-Thr-OMe (**1b**),[11] Naph-Thr-OMe (**1c**)[11] and Fur-Thr-OMe (**1d**):[11] the synthesis of these compounds was described in the reference given above.

4.6. Synthesis of the methyl esters of *N*-acyldehydroamino acids

Synthesis of compounds Bz-ΔAbu-OMe (**2a**),[11] Bz(4-OMe)-ΔAbu-OMe (**2b**),[11] Naph-ΔAbu-OMe (**2c**)[11] and Fur-ΔAbu-OMe (**2d**):[11] the synthesis of these compounds was described in the reference given above.

4.7. Synthesis of the methyl esters of *N*-acyl β-halodehydroamino acids

Synthesis of compounds Bz-*Z*-ΔAbu(β-Br)-OMe (*Z*-**3a**),[12c] Bz-*E*-ΔAbu(β-Br)-OMe (*E*-**3a**),[12c] Bz(4-OMe)-*Z*-ΔAbu(β-Br)-OMe (*Z*-**3b**),[12c] Fur-*Z*-ΔAbu(β-Br)-OMe (*Z*-**3d**)[12c] and Bz-*Z*-ΔAbu(β-I)-OMe (*Z*-**4a**):[11] the synthesis of these compounds was described in the references given above.

4.8. Synthesis of methyl-3-bromo-2-(1-naphthamido)but-2-enoate [Naph-ΔAbu(β-Br)-OMe] (3c)

To a solution of methyl 2-(1-naphthamido)but-2-enoate (**2c**) (2 mmol) in dichloromethane (0.1 mol dm^{-3}) 1.5 equiv of *N*-bromosuccinimide was added with vigorous stirring. After reacting for 16 h, triethylamine (1.5 equiv) was added and stirring continued for an additional hour. The solvent was then evaporated at reduced pressure and the residue partitioned between 100 cm^3 of dichloromethane and 50 cm^3 of $KHSO_4$ (1 mol dm^{-3}). The organic phase was washed with $KHSO_4$ (1 mol dm^{-3}), $NaHCO_3$ (1 mol dm^{-3}) and brine (3×30 cm^3 each). After drying over $MgSO_4$, the extract was taken to dryness at reduced pressure to give a 2/3 *E*/*Z* mixture of **3c** (0.675 g, 97%). The diastereomers were separated by column chromatography using diethyl ether/*n*-hexane 1/2 as eluent to give:

*4.8.1. Compound E-**3c**.* Mp 154.0–155.0 °C (from diethyl ether/petroleum ether). δ_H (300 MHz, $CDCl_3$): 2.55 (3H, s, βCH$_3$), 3.91 (3H, s, OCH$_3$), 7.51–7.63 (4H, m, ArH), 7.76 (1H, d, *J*=5.4 Hz, ArH), 7.91 (1H, d, *J*=6.0 Hz, ArH), 8.00 (1H, d, *J*=6.0 Hz, ArH), 8.40 (1H, br d, NH) ppm δ_C (75.4 MHz, $CDCl_3$): 26.73 (CH$_3$), 52.58 (OCH$_3$), 124.63 (CH), 125.20 (CH), 125.34 (CH), 125.59 (C), 125.85 (CH), 126.69 (CH), 127.62 (CH), 128.45 (CH), 130.21 (C), 131.67 (CH), 132.53 (C), 133.75 (C), 134.37 (C), 163.93 (C=O), 167.22 (C=O) ppm. Found C, 55.26; H, 4.16; N, 4.17; $C_{16}H_{14}NO_3Br$ (348.19) requires C, 55.19; H, 4.05; N, 4.02.

*4.8.2. Compound Z-**3c**.* Mp 156.0–157.0 °C (from ethyl acetate/petroleum ether). δ_H (300 MHz, $CDCl_3$): 2.67 (3H, s, βCH$_3$), 3.92 (3H, s, OCH$_3$), 7.48–7.60 (4H, m, ArH), 7.79 (1H, d, *J*=6.6 Hz, ArH), 7.90 (1H, d, *J*=7.2 Hz, ArH), 7.99 (1H, d, *J*=8.4 Hz, ArH), 8.44 (1H, br d, NH) ppm. δ_C (75.4 MHz, $CDCl_3$): 24.77 (CH$_3$), 52.80 (OCH$_3$), 124.62 (CH), 125.28 (CH), 125.54 (CH), 125.84 (C), 126.46 (CH), 126.65 (CH), 127.53 (CH), 128.36 (CH), 130.22 (C), 131.18 (CH), 131.73 (C), 132.05 (C), 133.68 (C), 162.88 (C=O), 167.21 (C=O) ppm.

4.9. Synthesis of β-bromodehydrodipeptides

Synthesis of Boc-Gly-*Z*-ΔAbu(β-Br)-OMe (*Z*-**6a**),[12b] Boc-L-Phe-*Z*-ΔAbu(β-Br)-OMe (*Z*-**6b**)[26] and Boc-L-Phe-*E*-ΔAbu(β-Br)-OMe (*E*-**6b**):[26] the synthesis of these compounds was described in the references given above.

4.10. Synthesis of oxazole-4-carboxylates (5)

4.10.1. General procedure for the synthesis of oxazole-4-carboxylate derivatives. To a solution of β-halodehydroamino acid methyl ester in acetonitrile (10^{-2} mol dm^{-3}) 2% of DBU was added with stirring at room temperature. The reactions were followed by HPLC until all the starting material was consumed (approximately 30 min). The reaction mixture was evaporated and partitioned between 60 cm^3 of ethyl acetate and 20 cm^3 of $KHSO_4$ (1 mol dm^{-3}) and washed with $KHSO_4$ (1 mol dm^{-3}), and brine (two times, 20 cm^3 each). After drying over $MgSO_4$ the extract was taken to dryness at reduced pressure to afford the respective substituted oxazole-4-carboxylate.

*4.10.2. Synthesis of methyl 5-methyl-2-phenyloxazole-4-carboxylate (**5a**).* The general procedure described above using Bz-*Z*-ΔAbu(β-Br)-OMe *Z*-**3a** (15 mg, 0.05 mmol) as substrate was followed to give oxazole **5a** (9.6 mg, 89%) as a white solid. Compound **5a** was described elsewhere.[11]

The general procedure described above using Bz-*E*-ΔAbu(β-Br)-OMe *E*-**3a** (15 mg, 0.05 mmol) as substrate was followed to give oxazole **5a** (9.3 mg, 86%) as a white solid. Compound **5a** was described elsewhere.[11]

The general procedure described above using Bz-*Z*-ΔAbu(β-I)-OMe *Z*-**4a** (24.8 mg, 0.072 mmol) as substrate was followed to give oxazole **5a** (15.0 mg, 96%) as a white solid. Compound **5a** was described elsewhere.[11]

*4.10.3. Synthesis of methyl 2-(4-methoxyphenyl)-5-methyloxazole-4-carboxylate (**5b**).* The general procedure described above using Bz(4-OMe)-*Z*-ΔAbu(β-Br)-OMe *Z*-**3b** (16.4 mg, 0.05 mmol) as substrate was followed to give oxazole **5b** (11.3 mg, 91%) as a white solid. Compound **5b** was described elsewhere.[11]

*4.10.4. Synthesis of methyl 5-methyl-2-(naphthalen-1-yl)oxazole-4-carboxylate (**5c**).* The general procedure described above using Naph-*Z*-ΔAbu(β-Br)-OMe *Z*-**3c** (17.4 mg, 0.05 mmol) as substrate was followed to give oxazole **5c** (12.6 mg, 91%) as a white solid. Compound **5c** was described elsewhere.[11]

The general procedure described above using Naph-*E*-ΔAbu(β-Br)-OMe *E*-**3c** (18.5 mg, 0.053 mmol) as substrate was followed to give oxazole **5c** (12.5 mg, 88%) as a white solid. Compound **5c** was described elsewhere.[11]

*4.10.5. Synthesis of methyl 2-(furan-2-yl)-5-methyloxazole-4-carboxylate (**5d**).* The general procedure described above using Fur-*Z*-ΔAbu(β-Br)-OMe *Z*-**3d** (10.4 mg, 0.05 mmol) as substrate was followed to give oxazole **5d** (8.6 mg, 84%) as a white solid. Compound **5d** was described elsewhere.[11]

*4.10.6. Synthesis of methyl 2-[(tert-butoxycarbonylamino)methyl]-5-methyloxazole-4-carboxylate (**7a**).* The general procedure described above using Boc-Gly-*Z*-ΔAbu(β-Br)-OMe *Z*-**6a** (35.1 mg, 0.1 mmol) as substrate was followed to give oxazole **7a** (23.0 mg, 85%) as an oil. δ_H (400 MHz, $CDCl_3$): 1.45 (9H, s, CH_3 Boc), 2.61 (3H, s, CH_3), 3.91 (3H, s, CH_3 OMe), 4.43 (2H, d, J=5.6 Hz, CH_2 Gly), 5.18 (1H, br s, NH) ppm. δ_C (100.6 MHz, $CDCl_3$): 11.94 (CH_3), 28.28 [C(CH_3)$_3$], 37.80 (CH_2 Gly), 51.96 (OCH_3), 80.27 [C(CH_3)$_3$], 127.38 (C), 155.43 (C=O), 156.83 (C), 159.25 (C), 162.51 (C=O) ppm. HRMS (EI): found 293.11077; $C_{12}H_{18}N_2NaO_5$ requires 293.11079.

*4.10.7. Synthesis of methyl 2-[1-(tert-butoxycarbonylamino)-2-phenylethyl]-5-methyloxazole-4-carboxylate (**7b**).* The general procedure described above using Boc-L-Phe-*Z*-ΔAbu(β-Br)-OMe *Z*-**6b** (44.0 mg, 0.10 mmol) as substrate was followed to give oxazole **7b** (28.1 mg, 78%) as a white solid. Mp 98.0–99.0 °C (from ethyl acetate/petroleum ether). δ_H (400 MHz, $CDCl_3$): 1.38 (9H, s, CH_3 Boc), 2.55 (3H, s, CH_3), 3.14–3.24 (2H, m, βCH$_2$ Phe), 3.90 (3H, s, CH_3 OMe), 5.12–5.20 (2H, m, NH+αCH Phe), 7.04–7.06 (2H, m, ArH), 7.22–7.27 (3H, m, ArH) ppm. δ_C (100.6 MHz, $CDCl_3$): 11.86 (CH_3), 28.18 [C(CH_3)$_3$], 40.33 (CH_2 Phe), 49.89 (CH Phe), 51.93 (OCH_3), 80.01 [C(CH_3)$_3$], 126.92 (CH), 127.28 (C), 128.46 (CH), 129.23 (CH), 135.78 (C), 154.81 (C=O), 156.34 (C), 161.71 (C), 162.55 (C=O) ppm. HRMS (EI): found 383.15782; $C_{19}H_{24}N_2NaO_5$ requires 383.15774.

The general procedure described above using Boc-Phe-*E*-ΔAbu (β-Br)-OMe *E*-**6b** (44.0 mg, 0.10 mmol) as substrate gave oxazole **7b** (29.6 mg, 82%).

*4.10.8. Synthesis of methyl 2-[1-(tert-butoxycarbonylamino)-2-methylpropyl]-5-methyloxazole-4-carboxylate (**7c**).* The general procedure described above using Boc-L-Val-*E*-ΔAbu(β-Br)-OMe *E*-**6c** (40.0 mg, 0.10 mmol) as substrate was followed to give oxazole **7c** (28.0 mg, 89%) as an oil. δ_H (400 MHz, $CDCl_3$): 0.91–0.94 (6H, m, γCH$_3$), 1.43 (9H, s, CH_3 Boc), 2.14–2.19 (1H, m, βCH Val), 2.61 (3H, s, CH_3), 3.90 (3H, s, CH_3 OMe), 4.71–4.75 (1H, m, αCH Val), 5.27 (1H, J=8.8 Hz, NH) ppm. δ_C (100.6 MHz, $CDCl_3$): 11.99 (CH_3), 17.92 (CH_3 Val), 18.76 (CH_3 Val), 28.27 [C(CH_3)$_3$], 32.86 (CH Val), 51.96 (OCH_3), 54.08 (CH Val), 79.89 [C(CH_3)$_3$], 127.25 (C), 155.37 (C=O), 156.26 (C), 162.15 (C), 162.29 (C=O) ppm. HRMS (EI): found 335.15742; $C_{15}H_{24}N_2NaO_5$ requires 335.15774.

4.11. Synthesis of model peptides labelled with an oxazole moiety

*4.11.1. Synthesis of 5-methyl-2-phenyloxazole-4-carboxylic acid **8**.* To a solution of oxazole **5a** (105 mg, 0.48 mmol) in methanol (1 mL) 2.3 equiv of NaOH (1 mol dm^{-3}, 1.1 mL) was added and the reaction was followed by TLC. When all the reactant was consumed the mixture was acidified to pH 2–3 with $KHSO_4$ 1 mol dm^{-3}. The solid formed was filtered and dried. Compound **8** was obtained as a white solid (95.0 mg, 97%) mp decomposes above 170 °C δ_H (400 MHz, $CDCl_3$): 2.64 (3H, s, CH_3), 7.53–7.55 (3H, m, ArH), 7.94–7.97 (2H, m, ArH), 12.93 (1H, br s, CO_2H) ppm. δ_C (100.6 MHz, $CDCl_3$): 11.92 (CH_3), 125.91 (CH), 126.28 (C), 128.77 (C), 129.17 (CH), 130.85 (CH), 156.00 (C), 158.35 (C), 163.03 (C=O) ppm. HRMS (EI): found 226.04739; $C_{11}H_9NNaO_3$ requires 226.04746.

*4.11.2. Synthesis of peptide **9**.* To a solution of compound **8** (102 mg, 0.46 mmol) in acetonitrile (6 mL), HOBt (62 mg, 0.46 mmol), HBTU (174 mg, 0.46 mmol), the dipeptide TFA, H-Gly-L-Ala-OMe (126 mg, 0.46 mmol) and NEt_3 (0.06 mL, 0.46 mmol) were added in an ice bath. After 48 h at room temperature the mixture was filtered and the solvent was evaporated at reduced pressure. The residue was dissolved in ethyl acetate (150 mL) and washed with $KHSO_4$ 1 mol dm^{-3}, $NaHCO_3$ 1 mol dm^{-3} and brine (3×50 mL each). The organic layer was dried over $MgSO_4$ and the solvent evaporated at reduced pressure to give peptide **9** as an oil (47.6 mg, 31%). δ_H (400 MHz, $CDCl_3$): 1.43 (3H, d, J=7.2 Hz, CH_3), 2.71 (3H, s, CH_3), 3.75 (3H, s, OCH_3), 4.13–4.17 (2H, m, CH_2), 4.58–4.66 (1H, m, CH), 6.91 (1H, d, J=7.2 Hz, NH), 7.45–7.47 (3H, m, ArH), 7.71 (1H, t, J=5.4 Hz, NH), 7.98–8.01 (2H, m, ArH) ppm. δ_C (100.6 MHz, $CDCl_3$): 11.1 (CH_3), 11.7 (CH_3), 42.7 (CH_2), 48.1 (CH), 52.5 (OCH_3), 126.3 (C), 126.6 (C), 128.8 (C), 129.7 (C), 130.7 (C), 153.4 (C), 158.8 (C), 162.6 (C=O), 168.5 (C=O), 173.2 (C=O) ppm. HRMS (EI): found 346.13961; $C_{17}H_{20}N_3O_5$ requires 346.13975.

*4.11.3. Synthesis of peptide **10**.* To a solution of compound **8** (95 mg, 0.47 mmol) in dry acetonitrile (5 mL), HOBt (63 mg, 0.47 mmol), DCC (96 mg, 0.47 mmol), TFA, H-Gly-L-Ala-L-Phe-OEt (203 mg, 0.47 mmol) and NEt_3 (0.13 mL, 0.94 mmol) were added in an ice bath. After 48 h the solid was filtered and the solvent evaporated at reduce pressure. The residue was dissolved in ethyl acetate (15 mL) and washed with $KHSO_4$ 1 mol dm^{-3}, $NaHCO_3$ 1 mol dm^{-3} and brine (3×5 mL each). The organic layer was dried over $MgSO_4$ and the solvent evaporated at reduced pressure. Column chromatography afforded peptide **10** as a white solid (192 mg, 81%) mp 149.0–150.0 °C. δ_H (400 MHz, $CDCl_3$): 1.22 (3H, t, J=7.2 Hz, OCH_2CH_3), 1.36 (3H, d, J=7.2 Hz, βCH$_3$), 2.70 (3H, s, CH_3), 3.04–3.18 (2H, m, βCH$_2$), 4.06 (2H, t, J=5.6 Hz, CH_2), 4.15 (2H, q, J=7.2 Hz, OCH_2CH_3), 4.50–4.57 (1H, m, CH), 4.80 (1H, q, J=6.8 Hz, CH), 6.80 (1H, d, J=8.0 Hz, NH), 6.96 (1H, d, J=7.2 Hz, NH), 7.12–7.14 (2H, m, ArH), 7.19–7.22 (1H, m, ArH), 7.25–7.28 (2H, m, ArH), 7.44–7.48 (3H, m, ArH), 7.67 (1H, t, J=5.6 Hz, NH), 7.99–8.01 (2H, m, ArH) ppm. δ_C (100.6 MHz, $CDCl_3$): 11.8 (CH_3), 14.0 (OCH_2CH_3), 17.8 (CH_3), 37.7 (CH_2), 42.6 (CH_2), 48.8 (CH Ala), 53.3 (CH), 61.5 (OCH_2CH_3), 126.3 (C), 126.6 (C), 127.0 (C), 128.4 (C), 128.8 (C), 129.3 (C), 129.7 (C), 130.6 (C), 135.86 (C), 153.4 (C), 158.7 (C), 162.5 (C=O), 168.7 (C=O), 171.2 (C=O), 171.6 (C=O) ppm. Found C, 63.62; H, 6.02; N, 10.97; $C_{27}H_{30}N_4O_6$ (506.55) requires C, 64.02; H, 5.98; N, 11.06.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.09.014.

References and notes

1. (a) Hughes, R. A.; Moody, C. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 7930–7954; (b) Inuoe, M. *Mini-Rev. Org. Chem.* **2008**, *5*, 77–84.
2. (a) Crank, G.; Foulis, M. J. *J. Med. Chem.* **1971**, *14*, 1075–1077; (b) Crank, G.; Neville, M.; Ryden, R. *J. Med. Chem.* **1973**, *16*, 1402–1405; (c) Giddens, A. C.; Bosho, H. I. M.; Franzblau, S. G.; Barry, C. E.; Copp, B. R. *Tetrahedron Lett.* **2005**,

46, 7355–7357; (d) Siddiquee, K. A. Z.; Gunning, P. T.; Glenn, M.; Katt, W. P.; Zhang, S.; Schroeck, C.; Sebti, S. M.; Jove, R.; Hamilton, A. D.; Turkson, J. *ACS Chem. Biol.* **2007**, *2*, 787–798; (e) Kaspady, M.; Narayanaswamy, V. K.; Raju, M.; Rao, G. K. *Lett. Drug Des. Discovery* **2009**, *6*, 21–28.

3. (a) Pavlopoulos, T. G.; Hammond, P. R. *J. Am. Chem. Soc.* **1974**, *96*, 6568–6579; (b) Ionescu, S.; Popovici, D.; Balaban, A. T.; Hillebrand, M. *Spectrochim. Acta, Part A* **2005**, *62*, 252–260; (c) Lukavenko, O. N.; Eltsov, S. V.; Grigorovich, A. V.; Mchedlov-Petrossyan, N. O. *J. Mol. Liq.* **2009**, *145*, 167–172.
4. (a) Aguilar, E.; Meyers, A. I. *Tetrahedron Lett.* **1974**, *35*, 2473–2476; (b) Liu, P.; Celatka, C. A.; Panek, J. S. *Tetrahedron Lett.* **1997**, *38*, 5445–5448.
5. (a) Pulici, M.; Quartieri, F.; Felder, E. R. *J. Comb. Chem.* **2005**, *7*, 463–473; (b) Keni, M.; Tepe, J. J. *J. Org. Chem.* **2005**, *70*, 4211–4213.
6. Thompson, M. J.; Chen, B. *J. Org. Chem.* **2009**, *74*, 7084–7093.
7. Santos, A.; Kaim, L.; Grimaud, L.; Ronsseray, C. *Chem. Commun.* **2009**, 3907–3909.
8. (a) Hashmi, A. S. K.; Weyrauch, J. P.; Frey, W.; Bats, J. W. *Org. Lett.* **2004**, *6*, 4391–4394; (b) Weyrauch, J. P.; Hashmi, A. S. K.; Schuster, A.; Hengst, T.; Schetter, S.; Littmann, A.; Rudolph, M.; Hamzic, M.; Visus, J.; Rominger, F.; Frey, W.; Bats, J. W. *Chem.—Eur. J.* **2010**, *16*, 956–963.
9. Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165–1168.
10. Morwick, T.; Hrapchak, M.; DeTuri, M.; Campbell, S. *Org. Lett.* **2002**, *4*, 2665–2668.
11. Ferreira, P. M. T.; Monteiro, L. S.; Pereira, G. *Eur. J. Org. Chem.* **2008**, 4676–4683.
12. (a) Ferreira, P. M. T.; Monteiro, L. S. *Eur. J. Org. Chem.* **2006**, 3226–3234; (b) Ferreira, P. M. F.; Monteiro, L. S.; Pereira, G.; Ribeiro, L.; Sacramento, J.; Silva, L. *Eur. J. Org. Chem.* **2007**, 5934–5949; (c) Ferreira, P. M. T.; Monteiro, L. S.; Pereira, G. *Amino Acids* **2010**, doi:10.1007/s00726-009-0466-x
13. Hiemstra, H.; Houwing, H. A.; Possel, O.; Leusen, A. M. *Can. J. Chem.* **1979**, *57*, 3168–3170.
14. Berlman, I. B. *Handbook of Fluorescence Spectra of Aromatic Molecules*; Academic: London, 1965.
15. (a) Melhuish, W. H. *J. Phys. Chem.* **1961**, *65*, 229–235; (b) Dawson, W. R.; Windsor, M. W. *J. Phys. Chem.* **1968**, *72*, 3251–3260.
16. Walker, I. C.; Palmer, M. H.; Delwiche, J.; Hoffmann, S. V.; Vieora, P. L.; Mason, N. J.; Guest, M. F.; Hubin-Franskin, M. J.; Heinesch, J.; Giuliani, A. *Chem. Phys.* **2004**, *297*, 289–306.
17. Turro, N. J.; Scaiano, J. C.; Ramamurthy, V. *Modern Molecular Photochemistry of Organic Molecules*; University Science Books: Sausalito (California) CA, 2009.
18. Valeur, B. *Molecular Fluorescence—Principles and Applications*; Wiley-VCH: Weinheim, 2001.
19. Lecoq, J. C.; Mechin, R.; Tanielian, C.; Bazin, M.; Santus, R. *Chem. Phys. Lett.* **1978**, *54*, 616–618.
20. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09, Revision A.02*; Gaussian: Wallingford CT, 2009.
21. Jensen, F. *Introduction to Computational Chemistry*; John Wiley: West Sussex, England, 1999.
22. Fasman, G. D. *Handbook of Biochemistry and Molecular Biology, Proteins, I*, 3rd ed.; CRC: Cleveland, OH, 1976.
23. Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry, Part II—Techniques for the Study of Biological Structure and Function*; Freeman: New York NY, 1980.
24. Chen, R. F.; Edelhoch, H. *Biochemical Fluorescence*; Marcel Dekker: New York NY, 1975.
25. (a) Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991–1024; (b) Fery-Forgues, S.; Lavabre, D. *J. Chem. Educ.* **1999**, *76*, 1260–1264.
26. Abreu, A. S.; Castanheira, E. M. S.; Ferreira, P. M. T.; Monteiro, L. S.; Pereira, G.; Queiroz, M.-J. R. P. *Eur. J. Org. Chem.* **2008**, 5697–5703.