9th International Electronic Conference on Synthetic Organic Chemistry. ECSOC-9
1-30 November 2005. http://www.usc.es/congresos/ecsoc/9/ECSOC9.HTM & http://www.mdpi.net/ecsoc/

Benzo[*f*]benzopyranones as fluorescent labels for amino acids

[A039]

Ana M. Piloto, Susana P.G. Costa and M. Sameiro T. Gonçalves*

Centro de Química, Universidade do Minho, Campus de Gualtar, P-4710-057 Braga, Portugal
msameiro@quimica.uminho.pt

Introduction

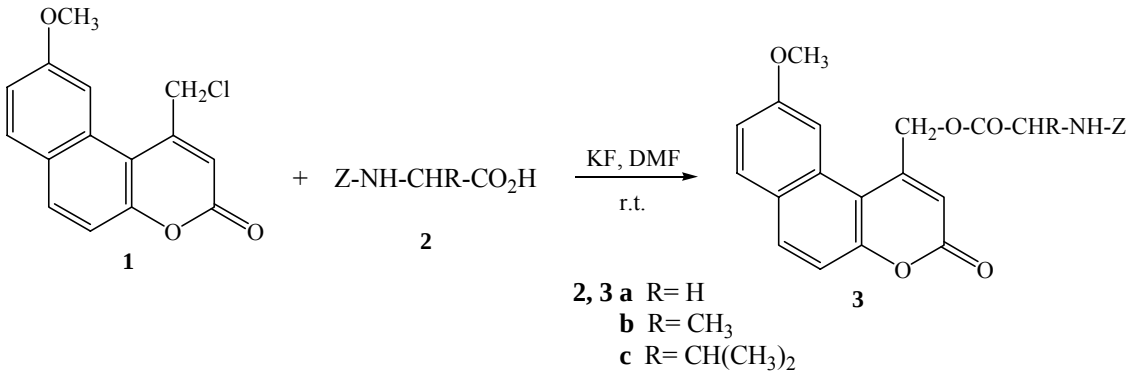
α-Benzopyranone derivatives, trivially known as coumarins, constitute an important class of fluorescent heterocycles. Analytical techniques involving the use of these compounds are of significant meaning in biological and medicinal research, being a valuable tool in the development of new diagnose methods and in the search for new biologically active compounds. In recent years, although the use of α-benzopyranones as fluorescent labels for a variety of compounds has been reported [1-3], their benzo counterparts, namely benzo[*f*]benzopyranones (or benzocoumarins), have been much less studied.

Having this in mind and following previous work regarding the synthesis of fluorescent heterocyclic moieties and their application [4-6], we decided to study the labelling of several amino acid residues with a benzo[*f*]benzopyranone, to test its use as a marker for possible application in biological assays.

Results and discussion

In order to investigate the formation of a covalent link between benzo[*f*]benzopyranone **1** and biomolecules, several α-amino acid derivatives, namely L-glycine, L-alanine and L-valine, protected at their N-terminus with a Z (benzyloxycarbonyl) group, were chosen as models. Thus, the reaction of compound **1** with amino acids **2a-c**, mediated by potassium fluoride in DMF at room temperature, gave after dry chromatography, compounds **3a-c** as solid materials, in yields ranging from 83 to 94% (Scheme 1, Table 1). The synthesis and characterization of pyranone **1** was previously reported [6].

The structure of fluorescent amino acid derivatives **3** was confirmed by IR, ^{1}H and ^{13}C NMR spectroscopy and high resolution mass spectrometry.



Scheme 1

The UV/visible absorption and fluorescence spectra of 5 x 10^{-6} M ethanolic solutions of compounds **3** were measured, excitation and emission maxima and fluorescence quantum yields are also reported (Table 1). Emission spectra of compounds **3** were run in degassed absolute ethanol, using 9,10-diphenylanthracene as standard (ϕ = 0.95 in ethanol).

Table 1- Synthesis, UV/visible and fluorescence data of compounds **3a-c**

Compd	Yield (%)	m.p. (ºC)	UV/ vis λ_{max} (nm)	Fluorescence λ_{em} (nm)	ϕ	Stokes' shift (nm)
3a	86	181.6-184.0	347	471	0.70	124
3b	83	132.8-134.0	348	477	0.66	129
3c	94	122.6-124.0	348	478	0.58	130

The labelled amino acids **3** exhibit very high fluorescence quantum yields (0.58 < ϕ < 0.70) and large Stokes' shifts, which makes the benzo[*f*]benzopyranone moiety suitable for the labelling of peptides and biomolecules. In Figure 1, the fluorescence spectra of benzo[*f*]benzopyranone-labelled glycine, alanine and valine **3a-c** are shown.

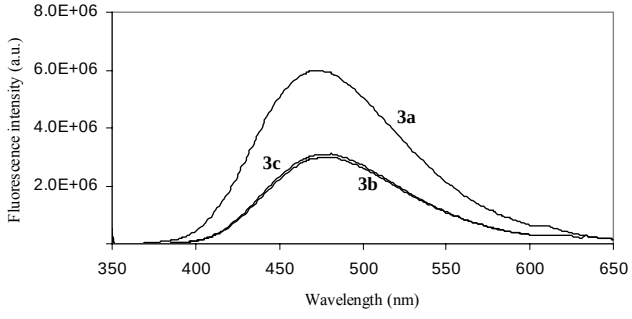


Figure 1- Fluorescence spectra of compounds **3a-c**

On going studies are focused on the application of the benzo[*f*]benzopyranone moiety to other representative amino acids through different linkages at the C-terminus and at lateral chain O-terminus. Comparative studies on fluorescence and solvatochromic properties will be carried out.

Experimental

General experimental procedure for the synthesis of labelled L-amino acids **3** (described for **3c**)

Compound **1** (104 mg, 0.38 mmol) was reacted with valine **2**c (70 mg, 0.28 mmol) and potassium fluoride (76 mg, 1.30 mmol) in DMF (2 mL) at room temperature for 25 h. After evaporation of the solvent, the residue was submitted to dry chromatography on silica gel (ethyl acetate/ *n*-hexane, 3:7). After recrystalization, compound **3**c, was obtained as a white solid (94%). Mp. 122.6-124.0 ºC,. ^{1}H NMR ($CDCl_3$, 300MHz) 0.95 (3H, d *J* 7.2 Hz, γ-CH_3 Val), 1.04 (3H, d *J* 6.9 Hz, γ-CH_3 Val), 2.20-2.35 (1H, m, β-CH Val), 3,98 (3H, s, OCH_3), 4.40-4.50 (1H, m, α-CH Val), 5.13 (2H, s, CH_2 Z), 5.25 (1H, d *J* 8.4 Hz, α-NH Val), 5.76 (2H, d *J* 3.9 Hz, CH_2 Het), 6.70 (1H, s, H-2), 7.25 (1H, dd *J* 7.8 and 2.4 Hz, H-8), 7.30-7.42 (6H, 5x Ar-H Z and H-5), 7.47 (1H, s, H-10), 7.86 (1H, d, *J* 9.0 Hz, H-7), 7.95 (1H, d *J* 9.0 Hz, H-6) ppm. ^{13}C NMR ($CDCl_3$, 75.4 MHz) 17.45 (γ-CH_3 Val), 19.17 (γ-CH_3 Val), 30.96 (β-CH Val), 55.43 (OCH_3), 59.28 (α-CH Val), 64.81 (CH_2), 67.25 (CH_2 Z), 105.67 (C-10), 111.75 (C-4b), 113.08 (C-2), 115.25 (C-5), 116.58 (C-8), 126.31 (C-6a), 128.17 (C-4 Z), 128.22 (C-2 and C-6 Z), 128.50 (C-3 and C-5 Z), 130.,49 (C-6b), 131.34 (C-7), 133.82 (C-6), 135.99 (C-1 Z), 150.18 (C-1), 155.54 (C-4a), 156.25 (CONH), 159.70 (C-9), 160.07 (C-3), 171.51 (*C*$OOCH_2$) ppm. FTIR (KBr disc) 3391, 2966, 2928, 1721, 1625 cm^{-1}. HRMS: *m/z* (EI) calcd for $C_{28}H_{27}NO_7$ 489.1788, found 489.1790.

Acknowledgements

Thanks are due to the Fundação para a Ciência e Tecnologia (Portugal) for financial support through Centro de Química (Universidade do Minho).

References

[1] E. Gikas, M. Parissi-Poulou, M. Kazanis, A. Vavagianis, *Anal. Chim. Acta*, **2003**, 489, 153-163.

[2] H. Ammar, S. Fery-Forgues, R. El Gharbi, *Dyes Pigments*, 2003, 57, 259-265.

[3] S. Sastry, *Biophys. Chem*., 2001, 91, 191-208.

[4] V. H. J. Frade, M. S. T. Gonçalves, J. C. V. P. Moura, *Tetrahedron Lett.,* **2005**, 46, 4949-4952.

[5] R. M. F. Batista, S. P. G. Costa, M. M. M. Raposo, *Tetrahedron Lett*. **2004**, 45, 2825-2828.

[6] A. M. Piloto, S. P. G. Costa, M. S. T. Gonçalves, *Tetrahedron Lett.,* **2005**, 46, 4757-4760.