Synthesis and spectral characterization of a fluorescent marker

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Abstract

A fluorescent naphtofuran was synthetized from benzopyran by alcaline ring contraction and coupled to the α-amine group of various amino acids, in order to evaluate its applicability as a fluorescent marker for biomolecules. Fluorescence data was collected for all derivatives.

Keywords

benzopyran, naphtofuran, amino acids, fluorescence

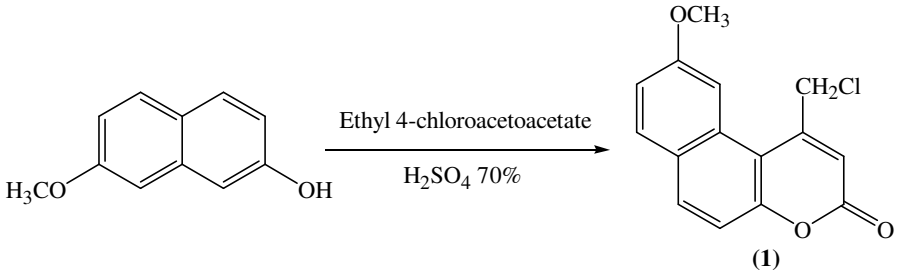
Introduction

The main goal in the development of biological labels is to identify a system that can offer intense and long-lived emission, to enable high detection sensitivity, high stability and large Stokes' shift, to minimize self-quenching effects. The labelling of biomolecules with organic fluorophores[1] for analytical applications is an attractive field of research: a peptide or protein bound to a fluorescent moiety is an important tool for the biochemical and biological study of protein-protein and ligand-receptor interactions, conformational studies, among other applications, since allows qualitative and quantitative determinations to be performed easily and reliably by rapid and economic methods.

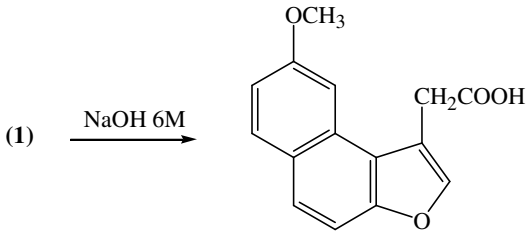
Having this in mind and in accordance with previous work concerning the use of azo dyes as temporary markers,[2-4] we decided to prepare several amino acid derivatives attached to a fluorogenic moiety, a naphtofuran, to test its use as a marker for possible application in biological assays.

Results and discussion

2-Methoxy-naphto[2,1-*b*]furan-1-yl acetic acid **(2)** was obtained in high yield by refluxing benzopyran **(1)** in aqueous sodium hydroxide 2M solution. Compound **(1)** was synthesized by reaction of 7-methoxy-2-naphtol with ethyl chloroacetoacetate by a known procedure.[5]

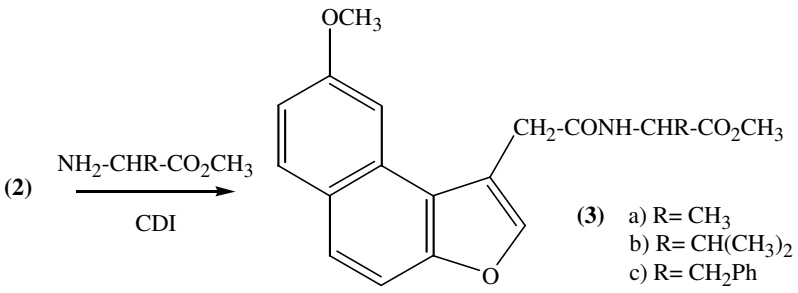


(1)



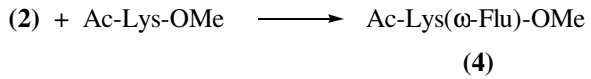
(2)

The carboxylic fluorescent compound **(2)** was coupled to the α-amine group of various amino acid methyl esters under standard conditions with *N,N'*-carbonyldiimidazole (CDI).[6] After purification by chromatography on silica gel, followed by recrystallization, the corresponding acetylfuran derivatives **(3a-c)** were obtained as solid materials in yields ranging from 54 to 71%.



In addition to labelling amino acids at their N-terminus, an alternative acylation at the ω-amine group was investigated. Thus, the methyl ester of *N*-acetyl lysine was treated

with **(2)**, under the same conditions reported above, and product **(4)** was obtained in 71% yield (Table 1).



where Flu denotes the fluorescent moiety

All compounds were characterised by elemental analysis or high resolution mass spectrometry, IR and ^{1}H and ^{13}C NMR spectroscopy. The UV/Vis absorption and fluorescence spectra of 5 x 10^{-6} M ethanolic solutions of compounds (**1-4)** were measured, excitation and emission maxima and fluorescence quantum yields are also reported (Table 1). Emission spectra of compounds (**1-4)** were run in degassed absolute ethanol, using 9,10-diphenylanthracene as standard (ϕ = 0.95 in ethanol).

Compd	**Yield** (%)	**m.p.** (ºC)	**UV/ Vis** λ_{max} **(nm)**	**Fluorescence** λ_{em} **(nm)**	ϕ	**Stokes'shift (nm)**
1	83	179.2-180.7	354	472	0.03	118
2	98	176.8-178.9	298	349	0.20	51
3a	54	190.1-192.0	297	346	0.37	49
3b	63	159.0-161.0	298	349	0.24	51
3c	71	146.3-148.4	297	349	0.32	52
4	71	185.7-186.9	297	347	0.44	50

The labelled amino acids **(3)** and **(4)** exhibit good fluorescence quantum yields ($0.20 < \phi < 0.44$) and show moderate Stokes' shift, which might render the naphtofuran moiety as suitable for the labelling of peptides and biomolecules. In Figure 1, the fluorescence spectra of pyran **(1)**, furan **(2)** and labelled alanine, valine, phenylalanine and lysine **(3a-c, 4)** are shown.

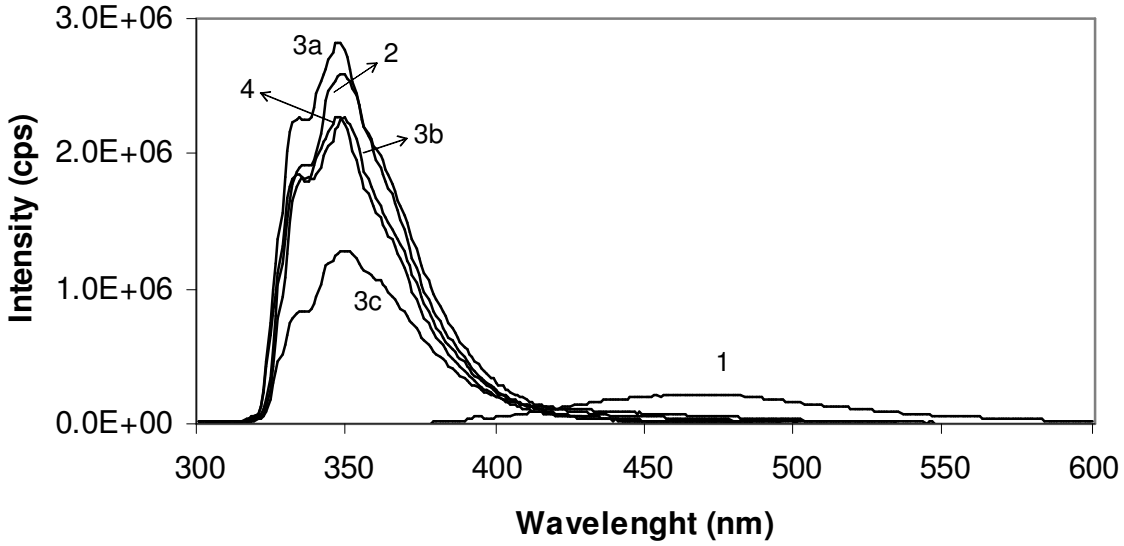


Figure 1- Fluorescence spectra of compounds **(1-4)**.

On going studies are focusing on the application of the benzopyran and naphtofuran moieties to amino acids through other linkages, such as at the C-terminus and at lateral chain O-terminus.

Acknowledgements

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