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Fluorescence Derivatisation of Amino Acids

[A020]

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Introduction

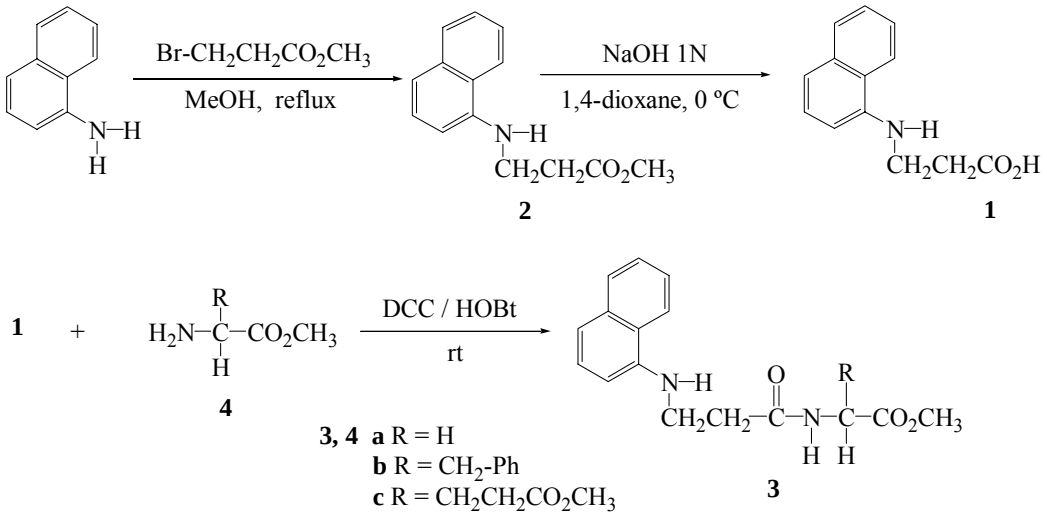
The actual needs of trace analysis in various fields of science such as environmental studies, residue determination or pharmaceutical analysis have increased the requirements of analytical determinations at increasingly lower levels of detection. This has shifted the research to the development of both new techniques and reagents. Fluorescent derivatisation is one of the most used methodologies for analytical purposes.[1] Although the importance of all fluorescence markers,[2] labelled biomolecules having fluorescence outside the range of the biological and background fluorescence are preferred in analytical practice.

Bearing this in mind and following our previous work,[3-5] we decided to use 3-(1-naphthylamino) propanoic acid **1** to prepare fluorescent derivatives of α-amino acids (**3**). Furthermore, they were reacted with 5-ethylamino-4-methyl-2-nitrosophenol hydrochloride **5** to give the corresponding 5,9-diaminobenzo[*a*]phenoxazinium salts **6**.

Results and Discussion

Alkylation of 1-naphthylamine with methyl-3-bromopropionate, followed by dry chromatography purification gave the intermediate **2** (55%). Hydrolysis of this compound yielded the 3-(1-naphthylamino) propanoic acid **1** in 55%.[5] Compound **1** was linked to the N-terminus of three α-amino acids, H-Gly-OMe (**4a**), H-Phe-OMe (**4b**), and H-Glu(OMe)-OMe (**4c**), by a DCC/ HOBt coupling (Scheme 1).[6]

After purification by dry chromatography, the corresponding derivatives **3a-c** were obtained in yields ranging from 85 to 95% (Table 1).



Scheme 1

The structure of compounds **1**, **2** and **3a-c** were confirmed by high resolution mass spectrometry, IR and NMR (^{1}H and ^{13}C) spectroscopy.

Table 1. Yields, UV/visible and fluorescent data for compounds **3** and **6** in ethanol.

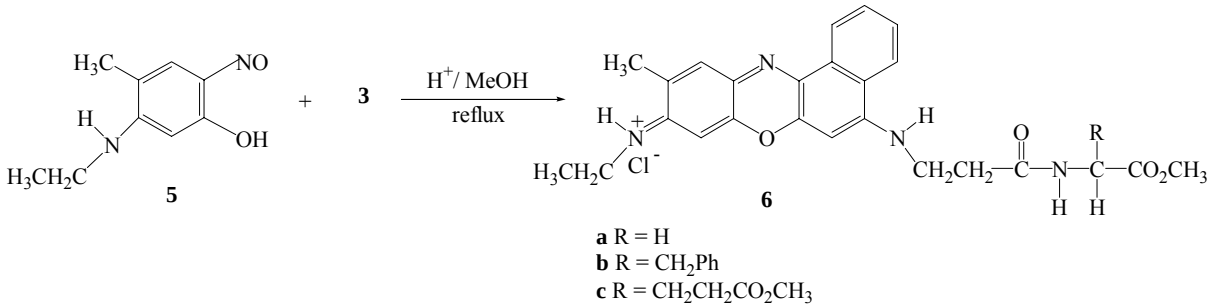
		UV/ vis		Fluorescence		Stokes' shift [nm]
Compd	Yield [%]	λ_{max} [nm] (ε)	λ_{exc} [nm]	λ_{em} [nm]	Φ	
3a	90	330 (5960)	330	417	0.55	87
3b	85	330 (7000)	330	417	0.54	87
3c	95	330 (7442)	330	414	0.57	84
6a	71	630 (32283)	600	644	0.37	44
6b	76	630 (40945)	580	644	0.45	64
6c	68	630 (33628)	580	644	0.40	64

The UV/ visible absorption and emission spectra of degassed 10^{-5}-10^{-6} M solutions in absolute ethanol of compounds **3a-c** were also measured, absorption and emission maxima, and fluorescence quantum yields, which were calculated using 9,10-diphenylanthracene as standard (Φ = 0.95 in ethanol) [7] are also reported (Table 1).

As it was expected, the labelled amino acids **3a-c** exhibit good fluorescence with quantum yields ranging from 0.54 to 0.57, but its important absorption and emission properties were at the short wavelength region, λ_{max} = 330 nm and 414 - 417 nm, respectively (Table 1).

Furthermore, compounds **3a-c** were cyclised by reaction with 5-ethylamino-4-methyl-2-nitrosophenol hydrochloride **5** in acidic medium (Scheme 2, Table 1). After purification by dry

chromatography, the labelled amino acids **6a-c** were obtained as blue materials in good yields (68-76%).



Scheme 2

These compounds were fully characterised by the usual analytical techniques. Absorption spectra of compounds **6a-c** in absolute degassed ethanol showed absorption peaks at 630 nm with ε values ranging from 32283 (**6a**) to 40945 **(6b**) (Table 1).

Fluorescence studies using Oxazine 1 as standard were also carried out in ethanol (Table 1), and **c**ompounds **6** fluoresced at 644 nm, with good fluorescence quantum yields ($0.37 < \Phi < 0.45$) and moderate Stokes' shift.

In summary it was possible to prepare high fluorescent colourless amino acids derivatives in high yields with absorption and emission at the short wavelength region (330 nm and 414-417 nm, respectively). Efficient cyclisation of these compounds gave the corresponding blue analogues which showed intense visible absorption maxima in the range 580-600 nm, and fluoresced strongly with fluorescence maxima at 644 nm.

Considering these results, further work will be undertaken to investigate the use of this fluorescent compounds in analytical determinations of biomolecules.

Acknowledgments

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