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A naphtho[2,1-*b*]furan as a new fluorescent label: synthesis and spectral characterisation

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Abstract—A fluorescent naphthofuran was synthesised from an oxobenzopyran by alkaline ring contraction and coupled with various L-amino acids at their N-terminus or at side-chain functional groups, in order to evaluate its applicability as a fluorescent label for biomolecules and in peptide synthesis. Fluorescence data were collected for all derivatives, which were found to be moderately fluorescent and having moderate to good fluorescence quantum yields.

1. Introduction

The naphthofuran skeleton is present in many natural products with biological importance and its synthetic derivatives display diverse biological activities.[1] Nevertheless, little is known about their fluorescence properties and potential utility as suitable fluorescent markers.

Fluorescence applications to biochemical measurements have increased dramatically, especially through the development of new fluorescent ligands. The most widespread strategy is to attach a fluorophore covalently by standard chemical reactions to a particular functional group in the target molecule.[2,3]

The labelling of biomolecules with organic fluorophores for analytical applications is an attractive field of research: a peptide or protein bound to a fluorescent moiety is an important tool for conformational studies of protein–protein and ligand–receptor interactions. Among other applications, fluorogenic pre-column derivatising agents for highly sensitive fluorescent detection in HPLC determinations, examined in view of sensitivity, separability and short-run time have been reported.[4–6]

Fluorescence allows qualitative and quantitative determinations to be performed easily and reliably by rapid and economic methods, with improved sensitivity and selectivity. Furthermore, labelling by fluorescent probes is an alternative to the use of radioactive compounds.

Following our previous work concerning the use of azo dyes as temporary markers,[7–9] and in connection with our current research interests in the development of new fluorescent heterocyclic compounds,[10] we decided to study the use of a naphthofuran moiety to prepare fluorescent derivatives of L-amino acids. Now, we report the synthesis and full characterisation of several fluorescent α-amino acid methyl esters bearing naphtho[2,1-*b*]-furan-1-yl group at their N-terminus. Lysine and serine residues were also derivatised at their amino or hydroxyl side-chain groups, respectively. The fluorescence properties and also their stability under deprotection conditions usually used in peptide synthesis were evaluated.

Among the many synthetic methodologies for the synthesis of naphthofurans that have been reported in the literature over the years, we decided to prepare the corresponding oxobenzopyran, through a Pechmann reaction, which was then converted to the target naphthofuran by an alkaline ring contraction. Thus, we prepared 1-chloromethyl-3-oxo-3*H*-benzo[*f*]benzopyrans **1a**–**c** by reaction of the appropriate 2-naphthol with ethyl 4-chloroacetoacetate by a known procedure[11] and naphtho[2,1-*b*]furan-1-yl acetic acid derivatives **2a**–**c** were obtained in high yields by heating at 80 °C the

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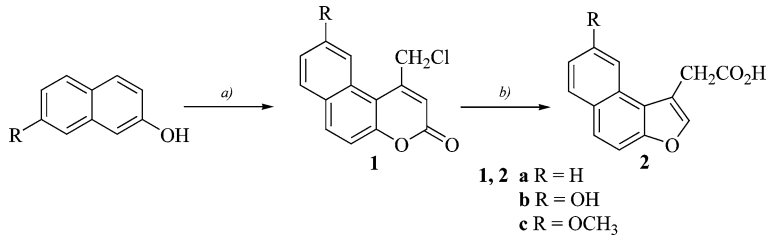


Scheme 1. Reagents and conditions: (a) $ClCH_2COCH_2CO_2Et$, aq H_2SO_4 70%, rt; (b) aq NaOH 6 M, 80 °C.

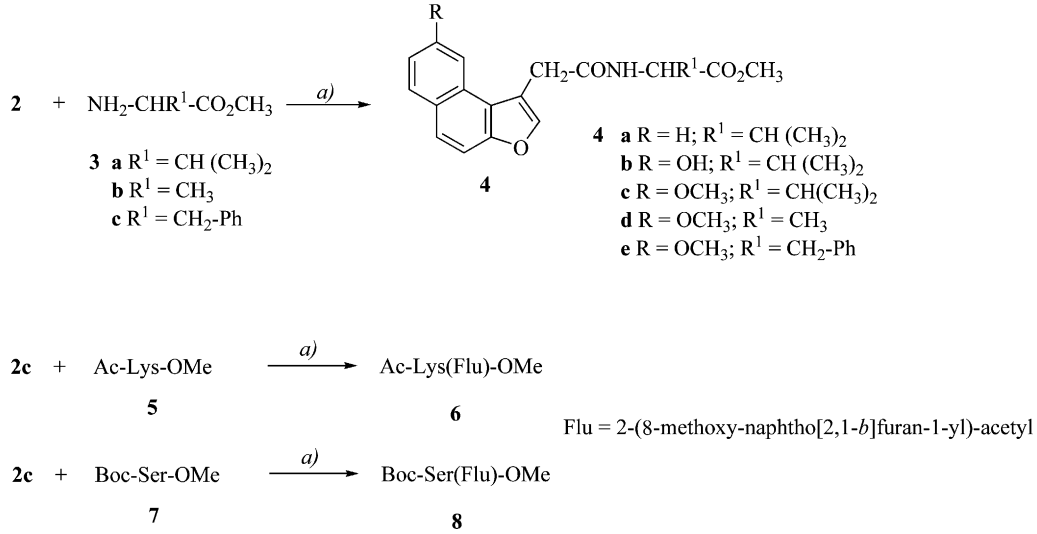
Table 1. Yields, UV/vis and fluorescence data for compounds **1**, **2**, **4**, **6** and **8**

Compd	Yield (%)	mp (°C)	UV/vis	Fluorescence		Stokes' shift (nm)
			λ_{max} (nm)	λ_{em} (nm)	Φ	
1a	71	179.5–182.7	352	418	0.08	66
1b	92	250.0–250.7	361	462	0.02	101
1c	83	179.2–180.7	354	472	0.03	118
2a	94	171.4–173.0	293	340	0.07	47
2b	96	167.8–169.0	301	349	0.06	48
2c	98	176.8–178.9	298	349	0.20	51
4a	83	133.0–134.5	292	325	0.13	33
4b	57	142.5–144.6	300	350	0.10	50
4c	89	190.1–192.0	298	346	0.37	49
4d	90	159.0–161.0	298	349	0.24	51
4e	72	146.3–148.4	297	349	0.32	52
6	71	185.7–186.9	297	347	0.44	50
8	54	Oil	298	349	0.13	51

corresponding compounds **1a**–**c** in aqueous 6 M sodium hydroxide solution (Scheme 1, Table 1).

L-valine methyl ester was chosen as a model for studying the linkage of carboxylic naphthofuran compounds **2a**–**c** to the N-terminus of α-amino acid residues and to compare the influence of the substituent in naphthofuran on the absorption and emission behaviour of the derivatives. Compounds **2a**–**c** were linked to the amino group of **3a** with the aid of *N*,*N*′-dicyclohexylcarbodiimide (DCC), assisted by 1-hydroxybenzotriazole (HOBt) under standard conditions. After purification by chromatography on silica gel, followed by recrystallisation, the corresponding acetylnaphthofuran derivatives **4a**–**c** were obtained as solid materials in yields ranging from 57% to 89%. Using the same method, L-alanine **4d** and L-phenylalanine **4e** fluorescent derivatives were obtained in good yields (90% and 72%, respectively) by acylation at their N-terminus with naphthofuran **2c** (Scheme 2, Table 1).

In addition to labelling amino acids at their N-terminus, an alternative acylation at the ε-amino group was investigated. Thus, the methyl ester of *N*-acetyl-L-lysine **5** was treated with **2c**, under the same conditions reported above, and product **6** was obtained in 71% yield



Scheme 2. Reagents and conditions: (a) DCC, HOBt, DMF, rt.

(Scheme 2, Table 1). Another approach for side-chain labelling was undertaken by reacting *tert*-butyloxycarbonyl-L-serine methyl ester **7** with compound **2c** to yield 54% of the corresponding fluorescent ester derivative **8**.

All compounds were characterised by elemental analysis or high resolution mass spectrometry, IR, ^{1}H and ^{13}C NMR spectroscopy. The UV/vis absorption and emission spectra of degassed 10^{-5}–10^{-6} M solutions in absolute ethanol of compounds **1**, **2**, **4**, **6** and **8** were measured, absorption and emission maxima and fluorescence quantum yields were also reported (Table 1). Fluorescence quantum yields were calculated using 9,10-diphenylanthracene as standard ($\Phi = 0.95$ in ethanol).[12]

Compounds **1** and **2** display low to moderate fluorescence quantum yields in their isolated form which increases upon reaction with the amino acids, for compounds **2a–c**. The labelled amino acids **4**, **6** and **8** exhibit moderate to good fluorescence quantum yields ($0.10 < \Phi < 0.44$) and show moderate Stokes' shift. From the data in Table 1, it can be seen that the higher electron-donating character of the substituent on the oxobenzopyran moiety is related to a larger Stokes' shift. This observation however does not apply to the free amino acid-linked naphthofuran, as in these cases the Stokes' shift does not vary significantly with the donating strength of the substituent. Taking into account the values of the fluorescence quantum yield for compounds **4a–c**, the best result was achieved for the 9-methoxy derivative (**4c**). Having this in mind, compound **2c** was used for labelling the methyl ester of alanine, phenylalanine, lysine and serine amino acids, yielding compounds **4d–e**, **6** and **8** which also present moderate to good fluorescence quantum yields ($0.13 < \Phi < 0.44$).

In Figure 1, the fluorescence spectra of naphthofuran **2c** and labelled alanine, lysine and serine (**4d**, **6** and **8**) are shown.

Stability tests, such as hydrogenation catalysed by Pd/C, acidolysis (HCl 6M; TFA), aminolysis with *N*,*N*-diethylaminoethyl-amine (DEAEA)[13] and reduction with metals (Mg/MeOH),[14] were carried out with fluorescent valine methyl ester **4c** under similar conditions to those usually required for cleavage of protecting groups during peptide synthesis.[15] In these conditions, the compound showed good stability, being recovered in yields from 95% to 100% (Pd/C, HCl, TFA and Mg) and 85% (DEAEA), as it was confirmed by ^{1}H NMR. Treatment with base (1 M NaOH) was also performed, resulting in quantitative cleavage of the ester function, without affecting the label. The labelled compounds were stable to prolonged storage at room temperature.

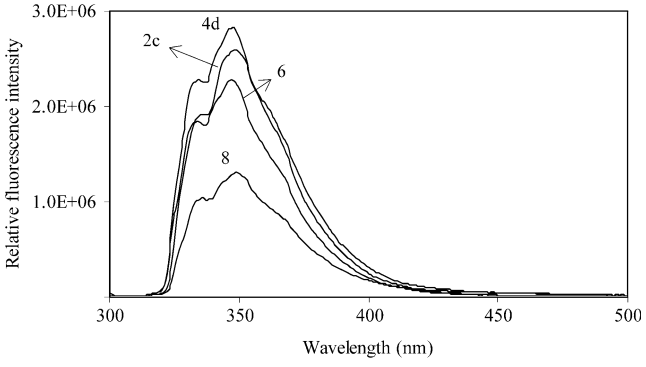


Figure 1. Fluorescence spectra of compounds **2c**, **4d**, **6** and **8**.

Oxo-benzo[*f*]benzopyran and naphtho[2,1-*b*]furan derivatives were prepared using general synthetic methods in excellent yields. Derivatisation of amino acid methyl esters, representative of basic (lysine), polar (serine) and nonpolar hydrophobic (alanine, valine and phenylalanine) side chains, with the fluorogenic naphtho[2,1-*b*]furan group was achieved in good yields.

Good stability of the label under conditions usually used in the deprotection process in peptide chemistry strongly suggests the possibility of using this label in stepwise synthesis. As the compounds explored in our work are acceptable models for peptides or even proteins, the high fluorescence and good fluorescent quantum yields may render the naphthofuran moiety as suitable for the fluorescent labelling of peptides and other biomolecules.

2. Experimental

2.1. General experimental procedure for the synthesis of chloromethyl oxo-benzo[*f*]benzopyrans 1 (described for 1c)

To a solution of 7-methoxy-2-naphthol (0.348 g, 2 mmol) in 70% aqueous sulfuric acid (5 mL), ethyl chloroacetoacetate (0.4 mL, 3 mmol) was added and stirred at room temperature for 48 h. The reaction mixture was poured into ice water and stirred for 2 h to give a fine greenish precipitate. The solid was collected by filtration, washed with cold water, dried in a vacuum oven and purified by dry chromatography, using ethyl acetate/*n*-hexane, 3:7 and 2:6 as eluent. After recrystallisation from ethyl acetate/*n*-hexane, 1-chloromethyl-9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran **1c** was obtained as an off-white solid (0.452 g, 83%). Mp 179.2–180.7 °C. $R_f = 0.83$ (ethyl acetate/*n*-hexane, 1:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.02 (s, 3H, OCH_3), 4.98 (s, 2H, CH_2), 6.62 (s, 1H, *H*-2), 7.24 (dd, 1H, $J = 9.0$ and 2.4 Hz, *H*-8), 7.31 (d, 1H, $J = 9.0$ Hz, *H*-5), 7.82 (m, 2H, *H*-7 and *H*-10), 7.92 (d, 1H, $J = 9.0$ Hz, *H*-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 45.60 (CH_2), 55.55 (OCH_3), 105.71 (*C*-7), 111.80 (*C*-4b), 115.11 (*C*-5), 117.02 (*C*-8), 117.24 (*C*-2), 126.29 (*C*-6a), 130.23 (*C*-6b), 131.15 (*C*-10), 133.93 (*C*-6), 150.96 (*C*-1), 155.76 (*C*-4a), 159.69 (*C*-9), 159.99 (*C*-3). FTIR (KBr disc) 1739, 1626 cm^{-1}. Anal. Calcd for $C_{15}H_{11}O_3Cl$: C, 65.58; H, 4.04. Found: C, 65.45; H, 4.21.

2.2. General experimental procedure for the synthesis of naphtho[2,1-*b*]furan-1-yl acetic acids 2 (described for 2c)

A suspension of **1c** (0.260 g, 0.75 mmol) in aqueous 6 M sodium hydroxide solution (5 mL) was stirred for 20 h at

80 °C. After cooling, the reaction mixture was acidified with aqueous 6 M hydrochloric acid solution until pH = 5–6. The mixture was extracted with ethyl acetate (3 × 10 mL), the organic extracts were combined, dried with magnesium sulphate and evaporated under vacuum in a rotary evaporator to yield a brownish solid, which was recrystallised from ethyl acetate/*n*-hexane. 8-Methoxy-naphtho[2,1-*b*]furan-1-yl acetic acid **2c** was obtained as a brown solid (0.188 g, 98%). Mp 176.8–178.9 °C. $R_f = 0.31$ (ethyl acetate). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.90 (s, 3H, OCH_3), 4.04 (s, 2H, CH_2), 7.14 (dd, 1H, $J = 9.0$ and 2.5 Hz, *H*-7), 7.56 (s, 1H, *H*-9), 7.58 (d, 1H, $J = 9.0$ Hz, *H*-5), 7.75 (d, 1H, $J = 9.0$ Hz, *H*-4), 7.93 (d, 1H, $J = 9.0$ Hz, *H*-6), 7.96 (s, 1H, *H*-2), 12.4 (br s, 1H, O*H*). ^{13}C NMR (DMSO-d_6, 75.4 MHz) δ 31.03 (*C*H$_2$), 55.06 (O*C*H$_3$), 102.99 (*C*-9), 110.09 (*C*-5), 115.74 (*C*-1), 115.87 (*C*-7), 120.52 (*C*-3b), 125.21 (*C*-5a), 125.45 (*C*-4), 129.13 (*C*-5b), 130.41 (*C*-6), 143.20 (*C*-2), 153.22 (*C*-3a), 157.78 (*C*-8), 172.62 (*C*=O). FTIR (KBr disc) 3442, 3103, 3016, 1703, 1627 cm^{-1}. Anal. Calcd for $C_{15}H_{12}O_4$: C, 70.30; H, 4.72. Found: C, 70.52; H, 4.82.

2.3. General experimental procedure for the synthesis of labelled L-amino acids 4, 6 and 8 (described for 4e)

Compound **2c** (95 mg, 0.371 mmol) was reacted with L-phenylalanine methyl ester hydrochloride (96 mg, 0.45 mmol) in DMF (2 mL) by a standard DCC/HOBt coupling. After dry chromatography on silica gel (ethyl acetate/*n*-hexane, 4:6) and recrystallisation from ethyl acetate/*n*-hexane, *N*-[2-(8-methoxy-naphtho[2,1-*b*]furan-1-yl)-acetyl] phenylalanine methyl ester **4e** was obtained as a white solid (0.112 g, 72%). Mp 146.3–148.4 °C, $R_f = 0.38$ (ethyl acetate/*n*-hexane, 4:6). ^{1}H NMR ($CDCl_3$, 300 MHz) 3.55–3.81 (m, 2H, β-CH_2 Phe), 3.57 (s, 3H, CO_2CH_3), 3.93 (s, 3H, OCH_3), 3.96 (s, 2H, CH_2), 4.60–4.80 (m, 1H, α-C*H* Phe), 6.05 (d, 1H, $J = 8.1$ Hz, N*H*), 6.47 (d, 2H, $J = 7.5$ Hz, *H*-2′ and *H*-6′), 6.79 (t, 2H, $J = 7.5$ Hz, *H*-3′ and *H*-5′), 6.96 (t, 1H, $J = 7.5$ Hz, *H*-4′), 7.14 (dd, 1H, $J = 9.0$ and 2.1 Hz, *H*-7), 7.48 (d, 1H, $J = 2.1$ Hz, *H*-9), 7.52 (d, 1H, $J = 9$ Hz, *H*-4), 7.62 (s, 1H, *H*-2), 7.73 (d, 1H, $J = 9.0$ Hz, *H*-5), 7.85 (d, 1H, $J = 9.0$ Hz, *H*-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz) 34.00 (*C*H$_2$), 37.61 (β-*C*H$_2$ Phe), 52.20 (CO_2CH_3), 52.93 (α-*C*H Phe), 55.62 (O*C*H$_3$) 102.31 (*C*-9), 110.04 (*C*-4), 115.03 (*C*-1), 116.86 (*C*-7), 120.04 (*C*-3b), 125.61 (*C*-5a), 126.30 (*C*-5), 126.90 (*C*-4′), 128.11 (*C*-3′, *C*-5′), 128.54 (*C*-2′, *C*-6′), 129.42 (*C*-5b), 130.48 (*C*-6), 134.95 (*C*-1′), 142.75 (*C*-2), 154.47 (*C*-3a), 158.76 (*C*-8), 169.83 (*C*ONH), 171.42 (CO_2CH_3). FTIR (KBr disc) 3284, 1750, 1662, 1625 cm^{-1}. HRMS: *m*/*z* (EI) calcd for $C_{25}H_{23}NO_5$ 417.1576, found 4171588.

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