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Carboxylic fused furans for amino acid fluorescent labelling

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Abstract—Four carboxylic fused furans are presented as new fluorescent labels for the amino and hydroxyl functions of organic molecules. Various representative L-amino acids were chosen as models, labelled at their N-terminus and also at their side-chain. Fluorescent derivatives were obtained in high yields, and their absorption and emission properties were studied.

1. Introduction

In order to enhance the sensitivity of analytical methodologies, the interest in fluorescent derivatisation has been steadily growing in recent years, as fluorescence is far more sensitive than common UV techniques. The development of novel fluorescent derivatisation reagents and their subsequent evaluation with model compounds has broad application in biology and biochemistry, to investigate the structure and dynamics of living systems.[1,2]

Amino acids are the building blocks in biological systems like proteins, enzymes and many other molecules with biological activity. The understanding of their physiological role is of utmost importance, given that a number of disorders are directly associated with the presence or lack of particular amino acids, and there is a practical interest in their detection in areas such as clinical chemistry.[3–5]

As most amino acids are poor UV-absorbing, fluorescence derivatisation is often employed for their determination. Although, a large number of fluorescent tagging reagents have been developed through the years, which cover the UV/vis spectrum and are specifically reactive towards different functional groups,[6–10] there is only scarce information on the application of benzofurans and naphthofurans in this field.[11,12]

Arene ring-fused furans are found in various naturally occurring compounds and a number of their natural and synthetic derivatives are associated with diverse biological and pharmacological activities.[13–16] Regarding arenofurans, benzofurans have been the subject of more extensive studies for the development of efficient routes for their synthesis.[17,18] Compared to benzofurans, synthetic methods for the preparation of naphthofurans have been less reported.[19,20] The most applied synthetic protocol reported is the intramolecular aldol-type condensation via dehydrative cyclisation of properly substituted aromatic *o*-alkoxycarbonyl compounds[16] or α-aryloxycarbonyl compounds.[21]

Following our interest in developing new chomophores and to extend our preliminary results,[22–24] we decided to investigate the application of fused furans in derivatisation reactions with biomolecules. We now present a study involving two systems, namely benzofuran and naphthofuran, bearing different substituents and representative amino acids of different character as models.

The main purpose of our work is to present a label for amino acids that have no (or low) intrinsic fluorescence or that are not easily detectable by ultraviolet absorption, thus excluding tryptophan and tyrosine. Regarding phenylalanine, the least UV-absorbing and fluorescent of the three aromatic amino acids, there is a practical interest in its tagging in order to enhance its properties.

Evaluation of the fluorescence properties was carried out for all labelled amino acids in ethanol and the influence of solvent polarity in emission was measured for one of the derivatives.

2. Results and discussion

Fluorophores **1** and **2a–c** were obtained by an alkaline ring contraction of the corresponding oxobenzopyran **3** and

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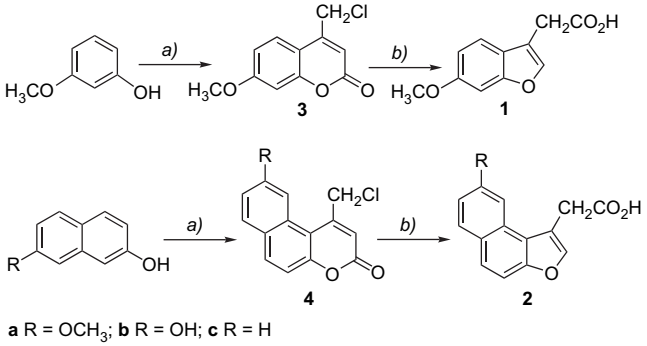
oxobenzo[*f*]benzopyran **4a–c**, respectively. The precursors 1-chloromethyl-6-methoxy-3-oxo-3*H*-benzopyran (**3**) and 1-chloromethyl-3-oxo-3*H*-benzo[*f*]benzopyrans (**4a–c**) were prepared through a Pechmann reaction of 3-methoxyphenol or 2-naphthol and its derivatives with ethyl 4-chloroacetoacetate by a known procedure,[25] in good yields (71–92%). Heating to 80 °C compounds **3** or **4a–c** in aqueous 2 M sodium hydroxide solution yielded the 2-(5-methoxybenzofuran-1-yl)ethanoic acid, Bfm-OH (**1**) (92%) or 2-(naphtho[2,1-*b*]furan-1-yl)ethanoic acids (**2a–c**) (94–98%) (Scheme 1, Table 1).



Scheme 1. Synthesis of fluorescent heterocycles **1–4**. Reagents and conditions: (a) $ClCH_2COCH_2CO_2Et$, aq H_2SO_4 70%, rt; (b) aq 2 M NaOH, 80 °C.

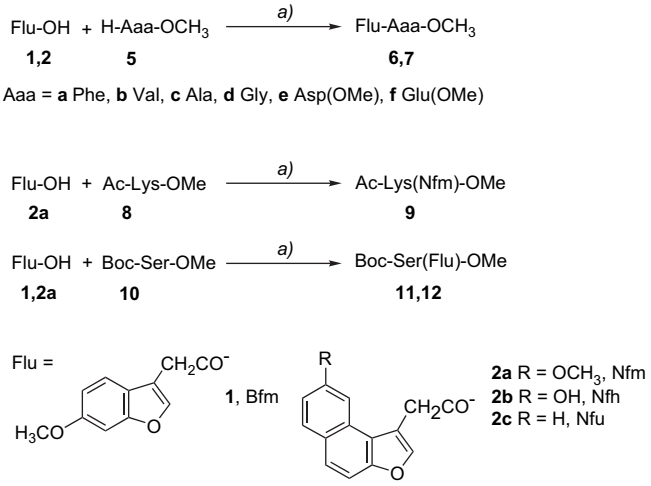
In the IR spectra of precursors **3** and **4a–c**, a strong band between 1739 and 1689 cm^{-1} confirmed the presence of the carbonyl group of the heterocycle. The ^1H NMR spectra showed the characteristic signal of proton 2 (H-2) of the pyran ring at δ 6.48 ppm (**3**) and 6.62–6.76 ppm (**4a–c**), in addition to the expected signals of the fused rings, and the chloromethylene group at δ between 4.98 and 5.30 ppm. In the ^{13}C NMR spectra of these compounds, relevant signals for the heterocycle were visible from δ 150.87 to 152.27 ppm (C-1), 112.00 to 117.37 ppm (C-2) and 159.38 to 160.02 ppm (C-3). The protons corresponding to the chloromethylene group appeared between δ 41.34 and 46.17 ppm.

For the target compounds **1** and **2a–c**, strong stretching vibration bands for the carboxyl group were present at 3550–3103 cm^{-1} and 1703–1715 cm^{-1}. In the ^1H NMR spectra, the singlet corresponding to proton H-2 of the furan ring at δ 7.75–8.01 ppm, as well as a broad signal for the carboxyl OH at high δ (12.40 to 12.60 ppm) were visible. ^{13}C NMR data confirmed the heterocyclic ring contraction by showing the signals corresponding to carbons 1 and 2 (C-1 and C-2) at δ 113.83–115.94 ppm and δ 142.31–143.64 ppm, respectively. Signals between 171.97 and 172.62 ppm were assigned to the carboxyl group.

Table 1. Synthesis of fluorescent heterocycles **1–4**

	Compound	Yield (%)	Mp (°C)
1	Bfm-OH	92	124.6–126.0
2a	Nfm-OH	98	176.8–178.9
2b	Nfh-OH	96	167.8–169.0
2c	Nfu-OH	94	171.4–173.0
3	Opm-Cl	79	199.5–200.8
4a	Obm-Cl	83	179.2–180.7
4b	Obh-Cl	92	250.0–250.7
4c	Obb-Cl	71	179.5–182.7

The carboxylic heterocyclic labels **1** and **2a** were linked to the α-amine group of L-phenylalanine and L-valine methyl esters (**5a** and **5b**), by coupling with the aid of *N*,*N*′-dicyclohexylcarbodiimide (DCC) assisted by 1-hydroxybenzotriazole (HOBt) under standard conditions. After purification by chromatography on silica gel, the corresponding acetyl derivatives **6a**,**b** and **7a**,**b** were obtained (Scheme 2, Table 2, entries 1–4).



Scheme 2. Synthesis of labelled amino acid derivatives **6**, **7**, **9**, **11** and **12**. Reagents and conditions: (a) DCC, HOBt, DMF, rt.

By comparison of fluorescence data obtained for these derivatives, which will be discussed later, it was concluded that the naphthofuran derivatives are more interesting than their benzofuran counterparts for labelling applications. The extension of its structure (incorporating to the molecule one more aromatic ring) resulted in an expected increase in the quantum yield of fluorescence (Φ_F). Two other naphthofuran derivatives, **2b**,**c**, were used in the reaction with model valine methyl ester, under the same reaction conditions. The resulting fluorogenic derivatives **7c**,**d** (Scheme 2, Table 2, entries 5 and 6) differ from **7a** in the substituent attached at position 8 of the naphthofuran moiety (OH and H, respectively). Their fluorescence properties were inferior to those

Table 2. Synthesis of labelled amino acid derivatives **6**, **7**, **9**, **11** and **12**

Entry	Label	Amino acid	Product		Yield (%)	Mp (°C)
1	**1**	**5a**	**6a**	Bfm-Phe-OMe	87	128.9–130.6
2	**1**	**5b**	**6b**	Bfm-Val-OMe	71	Oil
3	**2a**	**5a**	**7a**	Nfm-Phe-OMe	72	146.3–148.4
4	**2a**	**5b**	**7b**	Nfm-Val-OMe	89	159.0–161.0
5	**2b**	**5b**	**7c**	Nfh-Val-OMe	57	145.3–145.9
6	**2c**	**5b**	**7d**	Nfu-Val-OMe	83	141.5–143.0
7	**2a**	**5c**	**7e**	Nfm-Ala-OMe	90	190.1–192.0
8	**2a**	**5d**	**7f**	Nfm-Gly-OMe	95	171.9–174.0
9	**2a**	**5e**	**7g**	Nfm-Asp(OMe)-OMe	98	136.8–137.7
10	**2a**	**5f**	**7h**	Nfm-Glu(OMe)-OMe	98	166.3–169.0
11	**2a**	**8**	**9**	Ac-Lys(Nfm)-OMe	71	185.7–186.9
12	**1**	**10**	**11**	Boc-Ser(Bfm)-OMe	65	Oil
13	**2a**	**10**	**12**	Boc-Ser(Nfm)-OMe	54	Oil

of the 8-methoxy derivative and as a result, reaction of compound **2a** with the α-amine group of a set of representative L-amino acid methyl esters, namely alanine, glycine and aspartic and glutamic acids (protected at their carboxyl side-chain as methyl esters) was carried out in the same conditions (Scheme 2, Table 2, entries 7–10).

In addition to labelling amino acids at their N-terminus, the alternative acylation at a lysine ω-amine group was also investigated. Thus, the methyl ester of *N*-acetyl-lysine (**8**) was reacted with **2a** under the conditions reported above, to give the expected fluorescent derivative **9** (Scheme 2, Table 2, entry 11). Another approach for side-chain labelling was undertaken by reacting *N-tert*-butyloxycarbonylserine methyl ester **10** with compounds **1** and **2a** to give the corresponding fluorescent ester derivatives **11** and **12** (Scheme 2, Table 2, entries 12 and 13).

All labelled amino acids (**6**, **7**, **9**, **11** and **12**) were obtained as solid materials (except **6b**, **11** and **12**, which were oils) in yields ranging from 54 to 98% (Table 2) and were characterised by elemental analyses or high-resolution mass spectrometry, NMR (^{1}H and ^{13}C), IR and UV/vis spectroscopies.

The IR spectra of labelled compounds showed bands due to stretching vibrations of the carbonyl group from 1658 to 1628 cm^{-1} (amide linkage) and from 1761 to 1707 cm^{-1} (ester group). ^{1}H NMR spectra showed signals of the amino acid residues, such as a singlet for the methyl ester (δ 3.45–3.94 ppm), a multiplet for the α-CH (δ 4.35–4.90 ppm, except for **7f**, which was a doublet at 3.97 ppm) and a doublet for α-NH (δ 5.18–6.69 ppm), in addition to the protons of the heterocyclic moiety. In ^{13}C NMR, signals of amide type carbonyl were found from δ 169.34 to 171.01 ppm and of the ester type occurred from δ 170.04 to 172.86 ppm.

Electronic absorption and emission spectra of 10^{-6} M solutions of compounds **1**, **2**, **6**, **7**, **9**, **11** and **12** in degassed absolute ethanol were measured, absorption and emission maxima, and Φ_F are also reported (Table 3). The quantum yields were calculated using 9,10-diphenylanthracene as standard (Φ_F=0.95 in ethanol).[26] For the relative quantum yields' determination, 9,10-diphenylanthracene was excited at the wavelengths of maximum absorption found for each one of the compounds to be tested.

The longest wavelength absorption maximum of all compounds was located between 285–301 nm. Compound **2a** absorbs and emits at longer wavelength, when compared to **1**, the bathochromic shifts being 13 and 34 nm, respectively. The fluorescence quantum yield for **2a** (0.20) was 10 times higher than for **1** (0.02). When these two labels were linked to phenylalanine and valine methyl esters, the same trend was observed, the Φ_F for compounds **6a**–**b** being about 0.07 and for compounds **7a**–**b** higher than 0.3 (Table 3, entries 5–8). Having in mind that our purpose was to obtain a heterocycle for labelling applications, which required a significant Φ_F, we decided to use the naphthofuran moiety in the following studies.

In order to access the influence of substituents at this heterocycle, absorption spectra in ethanol of compounds **2b**,**c** and **7c**,**d** showed that the insertion of more electron donating substituents at position 8 shifted bathochromically the absorption maximum by 5–8 nm (compare entries 2–4 and 8–10). In the emission spectra, a bathochromic shift is observed when comparing compounds **7b**–**d** (21–25 nm).

The Φ_F was also affected by the substituent, as naphthofurans **2a**,**b** had higher Φ_F than the unsubstituted **2c**. We expected that the substitution with an electron-donor would result in an increase in fluorescence intensity and Φ_F, as it was observed for the OMe substituent. However, in the case of OH substituent we observed that the values were similar to that of the unsubstituted derivative probably due to the presence of H-bonds to the solvent (Table 3, entries 3, 4 and 9, 10).

Taking these results into consideration, we studied the properties of the derivatives resulting from the reaction of **2a** with other representative amino acids. The resulting labelled amino acids (**7a**,**b**,**e**–**h**, **9** and **12**) exhibit moderate to good fluorescence quantum yields ($0.13<\Phi<0.44$) and the Stokes' shifts are about 50 nm. There appears to be some influence from the amino acid residue on Φ_F, since labelled acidic (**7g** and **7h**) and polar uncharged (**12**) residues have lower

Table 3. UV and fluorescence data for compounds **1**, **2**, **6**, **7**, **9**, **11** and **12**

Entry		Compound	UV λ_{max} (nm)	Fluorescence		Stokes' shift (nm)
				λ_{em} (nm)	Φ_F	
1	**1**	Bfm-OH	285	315	0.020±0.003	30
2	**2a**	Nfm-OH	298	349	0.20±0.01	51
3	**2b**	Nfh-OH	301	349	0.062±0.003	48
4	**2c**	Nfu-OH	293	340	0.076±0.008	47
5	**6a**	Bfm-Phe-OMe	288	315	0.064±0.007	27
6	**6b**	Bfm-Val-OMe	288	315	0.070±0.006	27
7	**7a**	Nfm-Phe-OMe	298	349	0.32±0.02	52
8	**7b**	Nfm-Val-OMe	298	346	0.37±0.03	49
9	**7c**	Nfh-Val-OMe	300	350	0.10±0.01	50
10	**7d**	Nfu-Val-OMe	292	325	0.13±0.01	33
11	**7e**	Nfm-Ala-OMe	298	349	0.24±0.02	49
12	**7f**	Nfm-Gly-OMe	297	343	0.24±0.01	46
13	**7g**	Nfm-Asp(OMe)-OMe	298	346	0.14±0.01	48
14	**7h**	Nfm-Glu(OMe)-OMe	298	347	0.14±0.02	49
15	**9**	Ac-Lys(Nfm)-OMe	297	347	0.44±0.03	50
16	**11**	Boc-Ser(Bfm)-OMe	287	314	0.064±0.007	27
17	**12**	Boc-Ser(Nfm)-OMe	298	349	0.13±0.01	51

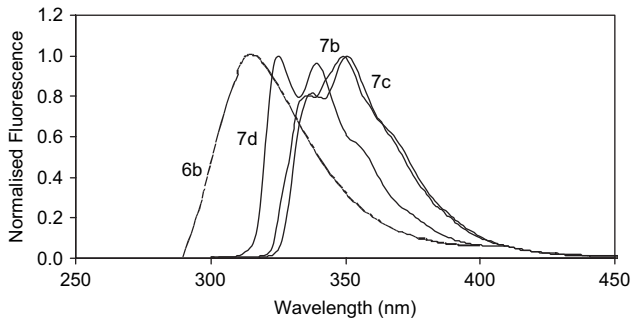


Figure 1. Normalised fluorescence spectra of valine labelled with different fluorophores **6b** and **7b–d**.

Φ_F than the free label **2a**, probably due to the possibility of occurrence of H-bonds between the label and the acidic or polar side chains. For all other derivatives the linkage is done through an amide bond and there are no extra effects from the side chains. The higher electron donating character of N and its resonance effect through the amide bond result in higher Φ_F when compared to the free label.

These results showed that 2-(8-methoxynaphtho[2,1-*b*]-furan-1-yl)ethanoic acid, Nfm-OH (**2a**) was a suitable fluorophore for the considered amino acids.

In Figure 1, the fluorescence spectra of valine labelled with different fluorophores (**6b**, **7b**, **7c** and **7d**) are shown.

In order to further elucidate the solvent interaction, absorption and emission spectra of model compound **7a**, Nfm-Phe-OMe, were measured in 10 other solvents of different polarity and proton donor ability, such as *n*-hexane, diethyl ether, toluene, 1,4-dioxane, ethyl acetate, methanol, acetonitrile, dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). The wavelengths of maximum absorption and emission and Φ_F for this compound are listed in Table 4.

From the results, it can be seen that the polarity of the solvent does not influence significantly the position of the band of absorption and emission; the wavelengths of maximum absorption and emission range from 296 to 299 nm and from 343 to 351 nm, respectively. However, a more significant influence of the character of the solvent on Φ_F was observed. Concerning the Φ_F obtained in the various solvents, higher values seem to be associated with aprotic solvents, either apolar or polar, such as 1,4-dioxane, DMF or diethyl ether, the highest being 0.57 (1,4-dioxane). In protic solvents like ethanol and methanol, quantum yields were slightly lower (0.32 and 0.33, respectively), which may be related with the high ability for H-bond donating/electron density acceptance power displayed by these solvents, which could affect the conjugation on the heterocyclic moiety. For the chlorinated solvent, the Φ_F value was analogous to that of the protic solvents.

Table 4. UV and fluorescence data for compound **7a** in different solvents

Entry	Solvent	UV λ_{max} (nm)	Fluorescence		Stokes' shift (nm)
			λ_{max} (nm)	Φ_F	
1	*n*-Hexane	296	343	0.29±0.02	47
2	Diethyl ether	297	345	0.45±0.03	48
3	Ethanol	298	346	0.32±0.02	48
4	Toluene	298	346	0.50±0.03	48
5	1,4-Dioxane	297	348	0.57±0.04	51
6	Ethyl acetate	297	346	0.36±0.03	49
7	Methanol	297	347	0.33±0.03	50
8	Acetonitrile	297	348	0.40±0.03	51
9	DCM	299	347	0.30±0.04	48
10	DMF	299	350	0.55±0.03	51
11	DMSO	299	351	0.36±0.04	52

With the aim of testing the possibility of using these fluorophores as labels in peptide chemistry, stability tests, such as hydrogenation catalysed by Pd/C, acidolysis (6 M HCl, trifluoroacetic acid, TFA), aminolysis with 2-(*N*,*N*-diethylamino)ethylamine (DEAEA)[27] and reduction with metals (Mg/MeOH),[28] were carried out. Fluorescent valine methyl ester, Nfm-Val-OMe (**7b**), used as model, was treated under similar conditions to those usually required for cleavage of protecting groups during peptide synthesis.[29] 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 (aqueous 1 M NaOH) was also performed, resulting in quantitative cleavage of the ester function, without affecting the label. All labelled compounds were also stable to prolonged storage at room temperature.

3. Conclusions

A series of carboxylic fused furans were synthesised in excellent yields by a simple procedure from the corresponding oxobenzopyrans. These heterocycles were used in the derivatisation of representative L-amino acids of different character, such as nonpolar aliphatic (valine and alanine) or aromatic (phenylalanine), basic (lysine), polar uncharged (glycine and serine) and acidic (aspartic and glutamic acids) amino acids. From the study of the absorption and emission properties, carried out for all labelled residues, we concluded that the 2-(8-methoxynaphtho[2,1-*b*]furan-1-yl)-ethanoic acid (**2a**) was the most interesting fluorophore.

Considering the high yields of the synthesis and derivatisation reactions as well as the fluorescence properties and also the stability to different deprotection conditions, usually used in peptide synthesis, naphthofurans seem to be promising for fluorescent labelling purposes.

4. Experimental

4.1. General

All melting points were uncorrected and were measured on a Gallenkamp melting point apparatus. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel 60F_{254}) and spots were visualised under UV light. Chromatography on silica gel was carried out on Merck Kieselgel (230–240 mesh). IR spectra were determined on a Perkin–Elmer FTIR-1600 using KBr discs or Nujol. UV/vis spectra were run on a Hitachi U-2000 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian 300

spectrometer in $CDCl_3$ or DMSO-d_6 at 300 MHz at 25 °C. All chemical shifts are given in parts per million using δ_H Me_4Si=0 ppm as reference and J values are given in hertz. ^{13}C NMR spectra were run in the same instrument at 75.4 MHz using the solvent peak as internal reference. Assignments were made by comparison of chemical shifts, peak multiplicities and J values, and were supported by spin decoupling-double resonance and bidimensional heteronuclear correlation HMBC and HMQC techniques. Mass spectrometric analyses were performed at the C.A.C.T.I.—Unidad de Espectrometria de Masas of the University of Vigo, Spain, on a Hewlett–Packard 5989 A spectrometer for low-resolution spectra and a VG Autospec M spectrometer for high-resolution mass spectra. Elemental analyses were carried out on a Leco CHNS 932 instrument. Fluorescence spectra were collected using a Spex Fluorolog 1680 Spectrometer.

4.1.1. 2-(5-Methoxybenzofuran-1-yl)ethanoic acid, Bfm-OH (1). A suspension of compound **3** (0.500 g, 2.23×10^{-3} mol) in aqueous 2 M sodium hydroxide solution (10 mL) was stirred for 16 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 (3×20 mL), the organic extracts were combined, dried with magnesium sulfate and evaporated under vacuum in a rotary evaporator to yield compound **1** as a off-white solid (0.422 g, 92%). Mp=124.6–126.0 °C. TLC (chloroform/methanol, 95:5): R_f 0.30. ^1H NMR (DMSO-d_6, 300 MHz): δ=3.63 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 6.87 (dd, J=2.4 and 8.7 Hz, 1H, H-6), 7.15 (d, J=2.4 Hz, 1H, H-4), 7.44 (d, J=8.7 Hz, 1H, H-7), 7.75 (s, 1H, H-2), 12.15 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ_C=29.07 (CH_2), 55.59 (OCH_3), 95.94 (C-4), 111.54 (C-6), 113.83 (C-1), 120.30 (C-7), 120.98 (C-7a), 142.31 (C-2), 155.58 (C-3a), 157.72 (C-5), 171.97 (CO_2H). IR (KBr 1%, cm^{-1}): ν=3424, 3051, 3017, 2908, 2836, 1708, 1627, 1595, 1490, 1441, 1401, 1292, 1233, 1143, 1120, 1075, 1023, 935, 805. UV/vis (ethanol, nm): λ_{max} (ε)=285 (1828 $M^{-1}\,cm^{-1}$). HRMS (EI): calcd for $C_{11}H_{10}O_4$ [M^+]: 206.0579; found: 206.0578.

4.1.2. 2-(8-Methoxynaphtho[2,1-*b*]furan-1-yl)ethanoic acid, Nfm-OH (2a). Starting with Obm-Cl (**4a**) (0.232 g, 8.44×10^{-4} mol) and following the same procedure described above for Bfm-OH (**1**), followed by recrystallisation from ethyl acetate/*n*-hexane, compound **2a** was obtained as a brown solid (0.212 g, 98%). Mp=176.8–178.9 °C. TLC (chloroform/methanol, 9.8:0.2): R_f 0.51. ^1H NMR (DMSO-d_6, 300 MHz): δ=3.90 (s, 3H, OCH_3), 4.04 (s, 2H, CH_2), 7.14 (dd, J=9.0 and 2.5 Hz, 1H, H-7), 7.56 (s, 1H, H-9), 7.58 (d, J=9.0 Hz, 1H, H-4), 7.75 (d, J=9.0 Hz, 1H, H-5), 7.93 (d, J=9.0 Hz, 1H, H-6), 7.96 (s, 1H, H-2), 12.60 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ_C=31.03 (CH_2), 55.06 (OCH_3), 102.99 (C-9), 110.09 (C-4), 115.74 (C-1), 115.87 (C-7), 120.52 (C-3b), 125.21 (C-5a), 125.45 (C-5), 129.13 (C-5b), 130.41 (C-6), 143.20 (C-2), 153.22 (C-3a), 157.78 (C-8), 172.62 (CO_2H). IR (KBr 1%, cm^{-1}): ν=3442, 3103, 3016, 2966, 2922, 1703, 1627, 1602, 1523, 1468, 1409, 1383, 1358, 1281, 1259, 1232, 1201, 1179, 1135, 1120, 1107, 1040, 1023, 947, 876, 838, 833. UV/vis (ethanol, nm): λ_{max} (ε)=298 (8436 $M^{-1}\,cm^{-1}$). Anal. Calcd for $C_{15}H_{12}O_4$ (256.25): C 70.30, H 4.72; found: C 70.48, H 4.81.

4.1.3. 2-(8-Hydroxynaphtho[2,1-*b*]furan-1-yl)ethanoic acid, Nfh-OH (2b). Starting with Obh-Cl (**4b**) (0.205 g, 7.86×10^{-4} mol) and following the same procedure described above for Bfm-OH (**1**), compound **2b** was obtained as a brown solid (0.183 g, 96%). Mp=167.8–169.0 °C. TLC (chloroform/methanol, 5:2): R_f 0.36. ^1H NMR (DMSO-d_6, 300 MHz): δ=3.97 (s, 2H, CH_2), 7.05 (dd, J=8.7 and 2.4 Hz, 1H, H-7), 7.44 (d, J=2.4 Hz, 1H, H-9), 7.49 (d, J=8.7 Hz, 1H, H-4), 7.67 (d, J=9.0 Hz, 1H, H-5), 7.85 (d, J=9.0 Hz, 1H, H-6), 7.91 (s, 1H, H-2), 9.84 (s, 1H, OH), 12.57 (br s, 1H, CO_2H). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ_C=30.75 (CH_2), 105.71 (C-9), 109.25 (C-4), 115.62 (C-1), 116.07 (C-7), 119.91 (C-3b), 124.49 (C-5a), 125.60 (C-5), 129.64 (C-5b), 130.44 (C-6), 142.79 (C-2), 153.11 (C-3a), 156.04 (C-8), 172.13 (CO_2H). IR (KBr 1%, cm^{-1}): ν=3365, 3107, 2926, 2860, 1715, 1636, 1533, 1471, 1419, 1388, 1360, 1334, 1286, 1259, 1239, 1193, 1173, 1107, 1033, 883, 860, 833. UV/vis (ethanol, nm): λ_{max} (ε)=301 (7970 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{14}H_{10}O_4$ [M^+]: 242.0579; found: 242.0586.

4.1.4. 2-(Naphtho[2,1-*b*]furan-1-yl)ethanoic acid, Nfu-OH (2c). Starting with Obh-Cl (**4c**) (0.207 g, 8.46×10^{-4} mol) and following the same procedure described above for Bfm-OH (**1**), compound **2c** was obtained as a brownish solid, which was recrystallised from ethyl acetate/*n*-hexane. Compound **2c** was obtained as a beige solid (0.180 g, 94%). Mp=171.4–173.0 °C. TLC (chloroform/methanol, 5.8:0.2): R_f 0.28. ^1H NMR (DMSO-d_6, 300 MHz): δ=4.04 (s, 2H, CH_2), 7.51 (dt, J=8.0 and 1.2 Hz, 1H, H-8), 7.60 (dt, J=8.0 and 1.2 Hz, 1H, H-7), 7.77 (d, J=9.0 Hz, 1H, H-4), 7.84 (d, J=9.0 Hz, 1H, H-5), 8.01 (s, 1H, H-2), 8.04 (br d, J=8.1 Hz, 1H, H-9), 8.18 (br d, J=8.1 Hz, 1H, H-6), 12.60 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ_C=31.08 (CH_2), 112.74 (C-4), 115.94 (C-1), 121.18 (C-3b), 123.14 (C-6), 124.44 (C-8), 125.67 (C-5), 126.49 (C-7), 127.96 (C-5a), 129.01 (C-9), 130.36 (C-5b), 143.64 (C-2), 152.69 (C-3a), 172.50 (CO_2H). IR (KBr 1%, cm^{-1}): ν=3550–3000, 2900, 1707, 1623, 1583, 1524, 1413, 1387, 1323, 1286, 1232, 1197, 1177, 1158, 1120, 1110, 1024, 992, 949, 939, 857, 830. UV/vis (ethanol, nm): λ_{max} (ε)=293 (7442 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{14}H_{10}O_3$ [M^+]: 226.0630; found: 226.0634.

4.1.5. 1-Chloromethyl-6-methoxy-3-oxo-3*H*-benzopyran, Opm-Cl (3). To a solution of 3-methoxyphenol (1.1 mL, 1.0×10^{-2} mol) in 70% aqueous sulfuric acid (10 mL), ethyl 4-chloroacetoacetate (2.02 mL, 1.5×10^{-2} mol) was added and stirred at room temperature for 78 h. The reaction mixture was poured into ice water and stirred for 2 h to give a fine violet precipitate. The solid was collected by filtration, washed with cold water and dried in a vacuum oven. Purification by dry chromatography on silica gel using chloroform/*n*-hexane, 2:1 as eluent gave compound **3** as a white solid (1.77 g, 79%). Mp=199.5–200.8 °C. TLC (chloroform): R_f 0.65. ^1H NMR (DMSO-d_6, 300 MHz): δ=3.85 (s, 3H, OCH_3), 4.98 (s, 2H, CH_2), 6.48 (s, 1H, H-2), 6.98–7.03 (m, 2H, H-5 and H-7), 7.75 (d, J=8.4 Hz, 1H, H-8). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ_C=41.34 (CH_2), 56.00 (OCH_3), 101.06 (C-5), 110.44 (C-8a), 112.00 (C-2), 112.35 (C-7), 126.38 (C-8), 150.87 (C-1), 155.26 (C-4a), 160.02 (C-3), 162.61 (C-6). IR (KBr 1%, cm^{-1}): ν=3070,

3023, 2853, 1725, 1623, 1611, 1558. UV/vis (ethanol, nm): λ_{max} (ε)=325 (14125 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{11}H_9O_3^{35}Cl$ [M^+]: 224.0240; found: 224.0251; calcd for $C_{11}H_9O_3^{37}Cl$ [M^+]: 226.0194; found: 226.0202.

4.1.6. 1-Chloromethyl-9-methoxy-3-oxo-3*H*-benzo[*f*]-benzopyran, Obm-Cl (4a). Starting with 7-methoxy-2-naphthol (0.348 g, 2.0×10^{-3} mol) and following the same procedure as described above for the preparation of compound **3**, using ethyl acetate/*n*-hexane 3:7, as the chromatography eluent, followed by recrystallisation from ethyl acetate/*n*-hexane, compound **4a** was obtained as an off-white solid (0.456 g, 83%). Mp=179.2–180.7 °C. TLC (ethyl acetate/*n*-hexane, 3:4): R_f 0.58. ^{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, J=9.0 and 2.4 Hz, 1H, H-8), 7.31 (d, J=9.0 Hz, 1H, H-5), 7.79–7.90 (m, 2H, H-10 and H-7), 7.92 (d, J=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=45.60 (CH_2), 55.55 (OCH_3), 105.71 (C-10), 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-7), 133.93 (C-6), 150.96 (C-1), 155.76 (C-4a), 159.69 (C-9), 159.99 (C-3). IR (KBr 1%, cm^{-1}): ν=3068, 1739, 1626, 1585, 1548, 1521, 1445, 1430, 1346, 1286, 1242, 1218, 1168, 1148, 1054, 1021, 912, 899, 863, 837, 734. UV/vis (ethanol, nm): λ_{max} (ε)=354 (12826 $M^{-1}\,cm^{-1}$). Anal. Calcd for $C_{15}H_{11}O_3Cl$ (274.70): C 65.58, H 4.04; found C 65.50, H 4.21.

4.1.7. 1-Chloromethyl-9-hydroxy-3-oxo-3*H*-benzo[*f*]benzopyran, Obh-Cl (4b). Starting with 2,7-dihydroxynaphthalene (0.320 g, 2.0×10^{-3} mol) and following the same procedure described above for compound **3**, using ethyl acetate/*n*-hexane 3:7, as the chromatography eluent, compound **4b** was obtained as beige solid (0.482 g, 92%). Mp=250.0–250.7 °C. TLC (chloroform/methanol 5.8:0.2): R_f 0.35. ^{1}H NMR (DMSO-d_6, 300 MHz): δ=5.30 (s, 2H, CH_2), 6.76 (s, 1H, H-2), 7.16 (dd, J=8.7 and 2.1 Hz, 1H, H-8), 7.32 (d, J=8.7 Hz, 1H, H-5), 7.81 (d, J=2.1 Hz, 1H, H-10), 7.92 (d, J=9.0 Hz, 1H, H-7), 8.09 (d, J=9.0 Hz, 1H, H-6), 10.21 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ_C=46.17 (CH_2), 108.66 (C-10), 110.67 (C-4b), 113.90 (C-5), 115.77 (C-2), 117.25 (C-8), 125.22 (C-6a), 130.31 (C-6b), 131.33 (C-7), 134.35 (C-6), 152.27 (C-1), 155.35 (C-4a), 157.80 (C-9), 159.38 (C-3). IR (KBr 1%, cm^{-1}): ν=3287, 2930, 1689, 1624, 1597, 1542, 1467, 1438, 1406, 1363, 1306, 1256, 1234, 1218, 1195, 1138, 1044, 1001, 968, 850, 840, 773, 731, 702. UV/vis (ethanol, nm): λ_{max} (ε)=361 (12190 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{14}H_9O_3^{35}Cl$ [M^+]: 260.0240; found: 260.0246; calcd for $C_{14}H_9O_3^{37}Cl$ [M^+]: 262.0211; found: 262.0206.

4.1.8. 1-Chloromethyl-3-oxo-3*H*-benzo[*f*]benzopyran, Obb-Cl (4c). Starting with 2-naphthol (1.0 g, 6.98×10^{-3} mol) and following the same procedure described above for compound **3**, using ethyl acetate/*n*-hexane 3:7, as the chromatography eluent, followed by recrystallisation from ethyl acetate/*n*-hexane gave compound **4c** as a yellow solid (1.22 g, 71%). Mp=179.5–182.7 °C. TLC (ethyl acetate/*n*-hexane, 2:8): R_f 0.36. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=5.09 (s, 2H, CH_2), 6.76 (s, 1H, 2-H), 7.52 (d, J=8.7 Hz, 1H, 5-H), 7.61 (dt, J=7.6 and 1.0 Hz, 1H, 8-H), 7.72 (dt, J=7.6 and 1.5 Hz, 1H, H-9), 7.96 (dd, J=8.1 and 1.2 Hz, 1H, 7-H), 8.04 (d, J=9.0 Hz, 1H, 6-H), 8.49 (br d, J=9.0 Hz, 1H, 10-H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=45.79 (CH_2), 112.50 (C-4b), 117.37 (C-2), 117.78 (C-5), 124.90 (C-10), 125.80 (C-8), 128.51 (C-9), 128.75 (C-6b), 129.88 (C-7), 131.29 (C-6a), 134.33 (C-6), 151.22 (C-1), 155.10 (C-4a), 159.97 (C-3). IR (KBr 1%, cm^{-1}): ν=3550, 3070, 1716, 1693, 1588, 1550, 1520, 1457, 1433, 1416, 1345, 1323, 1285, 1254, 1212, 1166, 1144, 1124, 1013, 1002, 918, 877, 862, 828. UV/vis (ethanol, nm): λ_{max} (ε)=(11,449 $M^{-1}\,cm^{-1}$). HRMS (EI): calcd for $C_{14}H_9O_2^{35}Cl$ [M^+]: 244.0291; found: 244.0290; calcd for $C_{14}H_9O_2^{37}Cl$ [M^+]: 246.0262; found: 246.0267.

4.2. General method for the synthesis of fluorescent-labelled L-amino acids 6, 7, 9, 11 and 12

Carboxylic compounds (**1**, **2**) were reacted with an amino acid methyl ester (2 equiv) in DMF by a standard DCC/HOBt coupling.[29] After evaporation of the solvent and chromatography on silica gel, the required acetyl derivatives (**6**, **7**, **9**, **11** and **12**) were obtained.

4.2.1. *N*-[(5-Methoxybenzofuran-1-yl)ethanoyl]phenylalanine methyl ester, Bfm-Phe-OMe (6a). The product of the reaction of Bfm-OH (**1**) (0.105 g; 5.1×10^{-4} mol) with phenylalanine methyl ester hydrochloride (**5a**) was chromatographed using chloroform as the eluent to give compound **6a** as a yellow solid (0.162 g, 87%). Mp=128.9–130.6 °C. TLC (chloroform): R_f 0.28. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.97–3.05 (m, 2H, β-CH_2 Phe), 3.60 (s, 2H, CH_2), 3.70 (s, 3H, OCH_3 Phe), 3.88 (s, 3H, OCH_3), 4.80–4.90 (m, 1H, α-H Phe), 6.00 (d, J=7.5 Hz, 1H, α-NH Phe), 6.80–6.84 (m, 2×Ar-H, 2H, Phe), 6.88 (dd, J=2.4 and 8.4 Hz, 1H, H-6), 7.04 (d, J=2.1 Hz, 1H, H-4), 7.10–7.18 (m, 3×Ar-H, 3H, Phe), 7.33 (d, J=8.7 Hz, 1H, H-7), 7.43 (s, 1H, H-2). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=31.60 (CH_2), 37.50 (β-CH_2 Phe), 52.32 (OCH_3 Phe), 52.87 (α-CH Phe), 55.71 (OCH_3), 96.07 (C-4), 112.05 (C-6), 113.36 (C-3a), 119.75 (C-7), 120.45 (C-7a), 127.04 (C-4 Phe), 128.46 (C-3 and C-5 Phe), 128.93 (C-2 and C-6 Phe), 135.32 (C-1 Phe), 142.18 (C-2), 156.43 (C-1), 158.41 (C-5), 169.34 (CONH), 171.68 (CO_2CH_3). IR (KBr 1%, cm^{-1}): ν=3289, 3064, 3030, 3008, 2956, 2930, 1750, 1659, 1629, 1602, 1587, 1544, 1494, 1455. UV/vis (ethanol, nm): λ_{max} (ε)=288 (4717 $M^{-1}\,cm^{-1}$). HRMS (EI): calcd for $C_{21}H_{21}NO_5$ [M^+]: 367.1420; found: 367.1402.

4.2.2. *N*-[(5-Methoxybenzofuran-1-yl)ethanoyl]valine methyl ester, Bfm-Val-OMe (6b). The product of the reaction of Bfm-OH (**1**) (0.103 g, 5.0×10^{-4} mol) with valine methyl ester hydrochloride (**5b**) was chromatographed using chloroform as the eluent to give compound **6b** as a yellow oil (0.113 g, 71%). TLC (chloroform): R_f 0.27. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.73 (d, J=6.9 Hz, 3H, γ-CH_3 Val), 0.84 (d, J=6.9 Hz, 3H, γ-CH_3 Val), 2.00–2.08 (m, 1H, β-CH Val), 3.66 (s, 3H, OCH_3 Val), 3.77 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 4.52–4.59 (m, 1H, α-CH Val), 6.11 (d, J=8.1 Hz, 1H, α-NH Val), 6.91 (dd, J=2.1 and 8.7 Hz, 1H, H-6), 7.03 (d, J=2.4 Hz, 1H, H-4), 7.43 (d, J=8.7 Hz, 1H, H-7), 7.56 (s, 1H, H-2). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=17.62 (γ-CH_3 Val), 18.88 (γ-CH_3 Val), 31.09 (β-CH Val), 31.79 (CH_2), 52.31 (OCH_3 Val), 55.70 (OCH_3), 57.10 (α-C Val), 96.12 (C-4), 112.06 (C-6),

113.59 (C-3a), 119.76 (C-7), 120.47 (C-7a), 142.22 (C-2), 156.51 (C-1), 158.45 (C-5), 169.64 (CONH), 172.21 (CO_2CH_3). IR (KBr 1%, cm^{-1}): ν=3290, 1685, 1641. UV/vis (ethanol, nm): λ_{max} (ε)=288 (4888 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{17}H_{21}NO_5$ [M^+]: 319.1420; found: 319.1426.

4.2.3. *N*-[(8-Methoxynaphtho[2,1-*b*]furan-1-yl)ethanoyl]phenylalanine methyl ester, Nfm-Phe-OMe (7a). The product of the reaction of Nfm-OH (**2a**) (0.095 g, 3.71×10^{-4} mol) with phenylalanine methyl ester hydrochloride (**5a**) was chromatographed using ethyl acetate/*n*-hexane 4:6, as the eluent to give compound **7a** as a white solid (0.111 g, 72%). Mp=146.3–148.4 °C. TLC (ethyl acetate/*n*-hexane, 4:6): R_f 0.38. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.80–2.96 (m, 2H, β-CH_2 Phe), 3.57 (s, 3H, OCH_3 Phe), 3.93 (s, 3H, OCH_3), 3.96 (s, 2H, CH_2), 4.80–4.90 (m, 1H, α-CH Phe), 6.05 (d, J=8.1 Hz, 1H, α-NH Phe), 6.47 (br d, J=7.5 Hz, 2H, H-2 and H-6 Phe), 6.79 (br t, J=7.5 Hz, 2H, H-3 and H-5 Phe), 6.96 (br t, J=7.5 Hz, 1H, H-4 Phe), 7.14 (dd, J=9.0 and 2.1 Hz, 1H, H-7), 7.48 (d, J=2.1 Hz, 1H, H-9), 7.52 (d, J=9.0 Hz, 1H, H-4), 7.62 (s, 1H, H-2), 7.73 (d, J=9.0 Hz, 1H, H-5), 7.85 (d, J=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=33.97 (CH_2), 37.57 (β-CH_2 Phe), 52.15 (OCH_3 Phe), 52.87 (α-CH Phe), 55.56 (OCH_3), 102.32 (C-9), 109.99 (C-4), 115.04 (C-1), 116.77 (C-7), 120.00 (C-3b), 125.63 (C-5a), 126.26 (C-5), 126.85 (C-4 Phe), 128.13 (C-3 and C-5 Phe), 128.52 (C-2 and C-6 Phe), 129.41 (C-5b), 130.37 (C-6), 134.90 (C-1 Phe), 142.66 (C-2), 154.44 (C-3a), 158.65 (C-8), 169.75 (CONH), 171.38 (CO_2CH_3). IR (KBr 1%, cm^{-1}): ν=3284, 2958, 2927, 2852, 1750, 1662, 1625, 1543, 1524, 1468, 1431, 1362, 1262, 1224, 1199, 1174, 1099, 1017, 823, 792. UV/vis (ethanol, nm): λ_{max} (ε)=298 (8261 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{25}H_{23}NO_5$ [M^+]: 417.1576; found: 417.1588.

4.2.4. *N*-[(8-Methoxynaphtho[2,1-*b*]furan-1-yl)ethanoyl]valine methyl ester, Nfm-Val-OMe (7b). The product of the reaction of Nfm-OH (**2a**) (0.095 g, 3.71×10^{-4} mol) with valine methyl ester hydrochloride (**5b**) was chromatographed using chloroform/*n*-hexane 9:1, as the eluent to give compound **7b** as a brown solid (0.122 g, 89%). Mp=159.0–161.0 °C. TLC (ethyl acetate/*n*-hexane, 4:6): R_f 0.48. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.54 (d, J=6.9 Hz, 3H, γ-CH_3 Val), 0.63 (3H, d, J=6.9 Hz, γ-CH_3 Val), 1.85–2.00 (m, 1H, β-CH Val), 3.48 (s, 3H, OCH_3 Val), 4.02 (s, 3H, OCH_3), 4.03 (s, 2H, CH_2), 4.46–4.56 (m, 1H, α-CH Val), 6.11 (d, J=8.4 Hz, 1H, α-NH Val), 7.13 (dd, J=9.0 and 2.4 Hz, 1H, H-7), 7.50 (s, 1H, H-9), 7.52 (d, J=9.0 Hz, 1H, H-4), 7.70 (d, J=8.7 Hz, 1H, H-5), 7.74 (s, 1H, H-2), 7.84 (d, J=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=17.47 (γ-CH_3 Val), 18.55 (γ-CH_3 Val), 30.91 (β-CH Val), 34.05 (CH_2), 51.84 (OCH_3 Val), 55.49 (OCH_3), 57.27 (α-CH Val), 102.43 (C-9), 110.00 (C-4), 115.23 (C-1), 116.55 (C-7), 119.87 (C-3b), 125.54 (C-5a), 126.24 (C-5), 129.30 (C-5b), 130.34 (C-6), 142.65 (C-2), 155.45 (C-3a), 158.49 (C-8), 170.00 (CONH), 171.77 (CO_2CH_3). IR (KBr 1%, cm^{-1}): ν=3442, 3295, 3116, 3078, 2998, 2964, 1739, 1658, 1630, 1603, 1545, 1525, 1474, 1432, 1410, 1383, 1358, 1290, 1232, 1202, 1183, 1120, 1038, 1027, 832. UV/vis (ethanol, nm): λ_{max} (ε)=298 (10,196 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{21}H_{23}NO_5$ [M^+]: 369.1576; found: 369.1560.

4.2.5. *N*-[(8-Hydroxynaphtho[2,1-*b*]furan-1-yl)ethanoyl]valine methyl ester, Nfh-Val-OMe (7c). The product of the reaction of Nfh-OH (**2b**) (0.090 g, 3.71×10^{-4} mol) with valine methyl ester hydrochloride (**5b**) was chromatographed using ethyl acetate/*n*-hexane 3:7, as the eluent to give compound (**7c**) as a brown solid (0.075 g, 57%). Mp=145.3–145.9 °C. TLC (ethyl acetate/*n*-hexane, 1:1): R_f 0.65. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.62 (d, J=6.9 Hz, 3H, γ-CH_3 Val), 0.74 (d, J=6.9 Hz, 3H, γ-CH_3 Val), 2.00–2.10 (m, 1H, β-CH Val), 3.57 (s, 3H, OCH_3 Val), 4.04 (s, 2H, CH_2), 4.52–4.62 (m, 1H, α-CH Val), 6.45 (d, J=8.7 Hz, 1H, α-NH Val), 7.13 (dd, J=8.7 and 2.4 Hz, 1H, H-7), 7.47 (d, J=8.7 Hz, 1H, H-4), 7.54 (d, J=2.4 Hz, 1H, H-9), 7.66 (d, 1H, J=9.0 Hz, H-5), 7.69 (s, 1H, H-2), 7.82 (d, J=8.7 Hz, 1H, H-6), 8.16 (br s, 1H, OH). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=17.43 (γ-CH_3 Val), 18.71 (γ-CH_3 Val), 30.73 (β-CH Val), 33.56 (CH_2), 52.27 (OCH_3 Val), 57.57 (α-CH Val), 105.78 (C-9), 109.72 (C-4), 114.68 (C-1), 116.20 (C-7), 119.36 (C-3b), 125.29 (C-5a), 126.32 (C-5), 129.21 (C-5b), 130.79 (C-6), 142.74 (C-2), 154.39 (C-3a), 155.67 (C-8), 171.01 (CONH), 172.53 (CO_2CH_3). IR (KBr 1%, cm^{-1}): ν=3427, 3313, 2961, 2929, 1723, 1658, 1629, 1537, 1468, 1441, 1410, 1387, 1289, 1261, 1219, 1195, 1155, 1136, 1120, 1102, 1032, 994, 847, 834. UV/vis (ethanol, nm): λ_{max} (ε)=300 (17650 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{20}H_{21}NO_5$ [M^+]: 355.1420; found: 355.1416.

4.2.6. *N*-[(Naphtho[2,1-*b*]furan-1-yl)ethanoyl]valine methyl ester, Nfu-Val-OMe (7d). The product of the reaction of Nfu-OH (**2c**) (0.090 g, 3.71×10^{-4} mol) with valine methyl ester hydrochloride (**5b**) was chromatographed using chloroform/*n*-hexane 9:1, as the eluent to give compound (**7d**) as a yellow solid (0.104 g, 83%). Mp=141.5–143.0 °C. TLC (ethyl acetate/*n*-hexane, 1:1): R_f 0.85. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.56 (d, J=6.9 Hz, 3H, γ-CH_3 Val), 0.66 (d, J=6.9 Hz, 3H, γ-CH_3 Val), 1.89–2.01 (m, 1H, β-CH Val), 3.51 (s, 3H, OCH_3 Val), 4.03 (s, 2H, CH_2), 4.5–4.60 (m, 1H, α-CH Val), 6.24 (d, J=9.0 Hz, 1H, α-NH Val), 7.45 (dt, J=7.2 and 1.0 Hz, 1H, H-8), 7.57 (dt, J=7.2 and 1.2 Hz, 1H, H-7), 7.65 (d, J=9.0 Hz, 1H, H-4), 7.75 (d, J=9.0 Hz, 1H, H-5), 7.76 (s, 1H, H-2), 7.95 (d, J=8.7 Hz, 1H, H-9), 8.16 (d, J=8.4 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=17.4 (γ-CH_3 Val), 18.6 (γ-CH_3 Val), 31.0 (β-CH Val), 33.8 (CH_2), 51.9 (OCH_3), 57.2 (α-CH Val), 112.6 (C-4), 115.3 (C-1), 120.5 (C-3b), 123.0 (C-6), 124.5 (C-8), 126.3 (C-5), 126.7 (C-7), 128.04 (C-5a), 129.0 (C-9), 130.7 (C-5b), 143.1 (C-2), 153.8 (C-3a), 169.8 (CONH), 171.9 (CO_2CH_3). IR (KBr 1%, cm^{-1}): ν=3290, 3279, 2960, 2929, 2852, 1809, 1742, 1658, 1651, 1584, 1538, 1464, 1437, 1388, 1372, 1312, 1266, 1205, 1151, 1107, 1022, 990, 931, 857, 804. UV/vis (ethanol, nm): λ_{max} (ε)=292 (1758 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{20}H_{21}NO_4$ [M^+]: 339.1471; found: 339.1471.

4.2.7. *N*-[(8-Methoxynaphtho[2,1-*b*]furan-1-yl)ethanoyl]-alanine methyl ester, Nfm-Ala-OMe (7e). The product of the reaction of Nfm-OH (**2a**) (0.104, 4.06×10^{-4} mol) with alanine methyl ester hydrochloride (**5c**) (0.054 g, 3.90×10^{-4} mol) was chromatographed using ethyl acetate/*n*-hexane 3:7, as the eluent to give compound **7e** as a white solid (0.125 g, 90%). Mp=190.1–192.0 °C. TLC (ethyl acetate/*n*-hexane, 4:6): R_f 0.32. ^{1}H NMR ($CDCl_3$, 300 MHz):

δ=1.20 (d, J=7.2 Hz, 3H, β-CH_3 Ala), 3.50 (s, 3H, OCH_3 Ala), 3.95 (s, 3H, OCH_3), 3.99 (s, 2H, CH_2), 4.50–4.65 (m, 1H, α-CH Ala), 6.22 (d, J=7.5 Hz, 1H, α-NH Ala), 7.14 (dd, J=9.0 and 2.7 Hz, 1H, H-7), 7.52 (d, J=9.0 Hz, 2H, H-9 and H-4), 7.66 (d, J=9.0 Hz, 1H, H-5), 7.72 (s, 1H, H-2), 7.84 (d, J=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=18.00 (β-CH_3 Ala), 33.90 (CH_2), 48.08 (α-CH Ala), 52.16 (OCH_3 Ala), 55.49 (OCH_3), 102.42 (C-9), 110.02 (C-4), 115.09 (C-1), 116.55 (C-7), 119.97 (C-3b), 125.55 (C-5a), 126.19 (C-5), 129.33 (C-5b), 130.32 (C-6), 142.66 (C-2), 154.41 (C-3a), 158.48 (C-8), 169.71 (CONH), 172.74 (CO_2CH_3). IR (KBr 1%, cm^{-1}): ν=3435, 3299, 2961, 2926, 1741, 1654, 1630, 1601, 1543, 1475, 1454, 1407, 1384, 1359, 1261, 1232, 1201, 1185, 1154, 1021, 829, 802. UV/vis (ethanol, nm): λ_{max} (ε)=298 (6456 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{19}H_{19}NO_5$ [M^+]: 341.1263; found: 341.1265.

4.2.8. *N*-[(8-Methoxynaphtho[2,1-*b*]furan-1-yl)ethanoyl]glycine methyl ester, Nfm-Gly-OMe (7f). The product of the reaction of Nfm-OH (**2a**) (0.177 g, 6.91×10^{-4} mol) with glycine methyl ester hydrochloride (**5d**) was chromatographed using ethyl acetate/*n*-hexane 3:7, as the eluent to give compound **7f** as a white solid (0.215 g, 95%). Mp=171.9–174.0 °C. TLC (ethyl acetate/*n*-hexane, 7:3): R_f 0.46. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=3.57 (s, 3H, OCH_3 Gly), 3.96 (s, 3H, OCH_3), 3.97 (d, J=4.5 Hz, 2H, CH_2 Gly), 4.04 (s, 2H, CH_2), 6.18 (br s, 1H, α-NH Gly), 7.15 (dd, J=9.0 and 2.4 Hz, 1H, H-7), 7.50–7.55 (m, 2H, H-4 and H-9), 7.70 (d, J=8.7 Hz, 1H, H-5), 7.74 (s, 1H, H-2), 7.85 (d, J=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=33.74 (CH_2), 41.27 (CH_2 Gly), 52.12 (OCH_3 Gly), 55.51 (OCH_3), 102.37 (C-9), 110.01 (C-4), 115.01 (C-1), 116.61 (C-7), 119.96 (C-3b), 125.53 (C-5a), 126.23 (C-5), 129.33 (C-5b), 130.34 (C-6), 142.75 (C-2), 154.40 (C-3a), 158.55 (C-8), 169.73 (CONH), 170.45 (CO_2CH_3). IR (Nujol, cm^{-1}): ν=3280, 2954, 2924, 2854, 1741, 1652, 1628, 1556, 1522, 1463, 1435, 1413, 1377, 1272, 1259, 1247, 1228, 1197, 1176, 826. UV/vis (ethanol, nm): λ_{max} (ε)=297 (7965 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{18}H_{17}NO_5$ [M^+]: 327.1107; found: 327.1100.

4.2.9. *N*-[(8-Methoxynaphtho[2,1-*b*]furan-1-yl)ethanoyl]-aspartic acid dimethyl ester, Nfm-Asp(OMe)-OMe (7g). The product of the reaction of Nfm-OH (**2a**) (0.10 g, 3.90×10^{-4}) with aspartic acid dimethyl ester hydrochloride (**5e**) (0.193 g, 9.75×10^{-4} mol) was chromatographed using ethyl acetate/*n*-hexane 4:6, as the eluent to give compound **7g** as a white solid (0.153 g; 98%). Mp=136.8–137.7 °C. TLC (ethyl acetate/*n*-hexane, 6:4): R_f 0.46. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.60–2.70 (m, 1H, β-CH Asp), 2.80–2.90 (m, 1H, β-CH Asp), 3.15 (s, 3H, OCH_3 Asp, side-chain), 3.49 (s, 3H, OCH_3 Asp, main chain), 3.95 (s, 3H, OCH_3), 4.00 (s, 2H, CH_2), 4.78–4.87 (m, 1H, α-CH Asp), 6.69 (d, J=7.5 Hz, 1H, α-NH Asp), 7.13 (dd, J=2.40 and 8.9 Hz, 1H, H-7), 7.45 (d, J=2.4 Hz, 1H, H-9), 7.50 (d, J=9.0 Hz, 1H, H-4), 7.66 (d, J=8.7 Hz, 1H, H-5), 7.72 (s, 1H, H-2), 7.82 (d, J=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=33.86 (CH_2), 35.60 (β-CH_2 Asp), 48.45 (α-CH Asp), 51.49 (OCH_3 Asp, side-chain), 52.50 (OCH_3 Asp, main chain), 55.48 (OCH_3), 102.34 (C-9), 110.12 (C-4), 114.91 (C-1), 116.47 (C-7), 119.90 (C-3b), 125.51 (C-5a), 126.06 (C-5), 129.30 (C-5b), 130.28 (C-6), 142.75 (C-2), 154.43 (C-3a), 158.45 (C-8), 170.00 (CONH), 170.58 (CO_2CH_3 Asp, main chain), 170.70 (CO_2CH_3 Asp, side-chain). IR (KBr 1%, cm^{-1}): ν=3333, 2954, 2924, 2854, 1739, 1648, 1627, 1598, 1519, 1463, 1436, 1400, 1378, 1304, 1228, 1200, 1178, 1120, 1107, 1060, 1040, 1024. UV/vis (ethanol, nm): λ_{max} (ε)=298 (7856 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{21}H_{21}NO_7$ [M^+]: 399.1318; found: 399.1319.

4.2.10. *N*-[(8-Methoxynaphtho[2,1-*b*]furan-1-yl)ethanoyl]glutamic acid dimethyl ester, Nfm-Glu(OMe)-OMe (7h). The product of the reaction of Nfm-OH (**2a**) (0.10 g, 3.90×10^{-4} mol) with glutamic acid dimethyl ester hydrochloride (**5f**) was chromatographed using ethyl acetate/*n*-hexane 2:8, as the eluent to give compound **7h** as a white solid (0.160 g, 98%). Mp=166.3–169.0 °C. TLC (ethyl acetate/*n*-hexane, 7:3): R_f 0.48. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.60–1.80 (m, 2H, β-CH_2 Glu), 1.82–2.00 (m, 2H, γ-CH_2 Glu), 3.49 (s, 3H, OCH_3 Glu), 3.50 (s, 3H, OCH_3 Glu), 3.94 (s, 3H, OCH_3), 3.99 (s, 2H, CH_2), 4.52–4.64 (m, 1H, α-CH Glu), 6.42 (d, J=7.8 Hz, 1H, α-NH Glu), 7.12 (dd, J=8.9 and 2.4 Hz, 1H, H-7), 7.44–7.55 (m, 2H, H-9 and H-4), 7.65–7.75 (m, 2H, H-5 and H-2), 7.82 (d, J=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=26.63 (β-CH_2 Glu), 29.34 (γ-CH_2 Glu), 33.93 (CH_2), 51.58 (OCH_3 Glu, main chain), 51.72 (α-CH Glu), 52.23 (OCH_3 Glu, side-chain), 55.46 (OCH_3), 102.28 (C-9), 110.02 (C-4), 115.00 (C-1), 116.52 (C-7), 119.94 (C-3b), 125.53 (C-5a), 126.19 (C-5), 129.28 (C-5b), 130.35 (C-6), 142.74 (C-2), 154.43 (C-3a), 158.55 (C-8), 170.31 (CONH), 171.61 (CO_2CH_3 Glu, main chain), 172.86 (CO_2CH_3 Glu, side-chain). IR (Nujol, cm^{-1}): ν=3311, 2954, 2925, 2854, 1761, 1715, 1651, 1628, 1531, 1463, 1377, 1271, 1230, 1177, 1134, 1120, 1035, 1018, 829. UV/vis (ethanol, nm): λ_{max} (ε)=298 (6716 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{22}H_{23}NO_7$ [M^+]: 413.1475; found: 413.1476.

4.2.11. *N*-Acetyl-ω-[(8-methoxynaphtho[2,1-*b*]furan-1-yl)ethanoyl]lysine methyl ester, Ac-Lys(Nfm)-OMe (9). The product of the reaction of Nfm-OH (**2a**) (0.10 g, 3.90×10^{-4} mol) with *N*-acetyl-lysine methyl ester hydrochloride (**8**) was chromatographed using ethyl acetate/*n*-hexane 3:7, as the eluent to give compound **9** as a white solid (0.122 g, 71%). Mp=185.7–186.9 °C. TLC (chloroform/methanol, 5.8:0.2): R_f 0.46. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.95–1.15 (m, 2H, γ-CH_2 Lys), 1.20–1.50 (m, 4H, β-CH_2 Lys and δ-CH_2 Lys), 1.97 (s, 3H, CH_3 Ac), 3.00–3.60 (m, 2H, ε-CH_2 Lys), 3.69 (s, 3H, OCH_3 Lys), 3.96 (s, 5H, OCH_3 and CH_2), 4.35–4.45 (m, 1H, α-CH Lys), 5.82 (t, J=6.0 Hz, 1H, NH Lys, side-chain), 5.95 (d, J=7.8 Hz, 1H, α-NH Lys), 7.15 (dd, J=9.0 and 2.4 Hz, 1H, H-7), 7.49 (d, J=2.4 Hz, 1H, H-9), 7.52 (d, J=9.0 Hz, 1H, H-4), 7.70 (s, 1H, H-2), 7.72 (d, J=9.0 Hz, 1H, H-5), 7.85 (d, J=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=22.23 (γ-CH_2 Lys), 23.00 (CH_3 Ac), 28.84 (β-CH_2 Lys), 31.65 (δ-CH_2 Lys), 33.97 (CH_2), 39.07 (ε-CH_2 Lys), 51.73 (α-CH Lys), 52.28 (OCH_3 Lys), 55.57 (OCH_3), 102.50 (C-9), 110.05 (C-4), 115.36 (C-1), 116.55 (C-7), 119.98 (C-3b), 125.52 (C-5a), 126.25 (C-5), 129.32 (C-5b), 130.40 (C-6), 142.72 (C-2), 154.41 (C-3a), 158.58 (C-8), 169.90 (CONH Ac), 170.32 (CONH), 172.84 (CO_2CH_3). IR (KBr 1%, cm^{-1}): ν=3289, 3077, 2951,

2931, 2859, 1748, 1651, 1633, 1601, 1550, 1525, 1474, 1464, 1454, 1434, 1415, 1375, 1359, 1290, 1258, 1231, 1200, 1180, 1146, 830. UV/vis (ethanol, nm): λ_{max} (ε)=297 (5333 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{24}H_{28}N_2O_6$ [M^+]: 440.1947; found: 440.1948.

4.2.12. *N*-*tert*-Butyloxycarbonyl-*O*-[(5-methoxybenzofuran-1-yl)ethanoyl]serine methyl ester, Boc-Ser(Bfm)-OMe (11). The product of the reaction of Bfm-OH (**1**) (0.087 g, 4.22×10^{-4} mol) with *N*-*tert*-butyloxycarbonylserine methyl ester (**10**) was chromatographed using chloroform as the eluent to give compound **11** as a brown oil (0.111 g, 65%). TLC (chloroform): R_f 0.23. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.43 (s, 9H, C(CH_3)$_3$), 3.63–3.67 (m, 5H, CH_2 and OCH_3), 3.70 (s, 3H, OCH_3 Ser), 3.83 (s, 3H, OCH_3), 4.35–4.53 (m, 2H, β-CH_2 Ser), 4.54–4.57 (m, 1H, α-CH Ser), 5.25 (d, J=7.5 Hz, 1H, α-NH Ser), 6.89 (dd, J=2.4 and 8.4 Hz, 1H, H-6), 6.99 (d, J=2.4 Hz, 1H, H-4), 7.39 (d, J=8.4 Hz, 1H, H-7), 7.50 (s, 1H, H-2). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=28.16 (C(*C*H_3)$_3$), 29.47 (CH_2), 64.68 (β-CH_2 Ser), 52.59 (OCH_3 Ser), 52.74 (α-CH Ser), 55.60 (OCH_3), 80.25 (*C*(CH_3)$_3$), 95.94 (C-4), 111.83 (C-6), 112.45 (C-3a), 119.62 (C-7), 120.71 (C-7a), 141.87 (C-2), 155.02 (*C*O_2C(CH_3)$_3$), 156.13 (C-3a), 158.15 (C-5), 170.04 (*C*O_2CH_3), 171.10 (CH_2*C*O_2). IR (KBr 1%, cm^{-1}): ν=3293, 3044, 3030, 3000, 2946, 1752, 1733, 1660, 1629, 1612, 1597, 1554, 1498, 1450. UV/vis (ethanol, nm): λ_{max} (ε)=287 (5427 $M^{-1}\,cm^{-1}$).

4.2.13. *N*-*tert*-Butyloxycarbonyl-*O*-[(8-methoxynaphtho[2,1-*b*]furan-1-yl)ethanoyl]serine methyl ester, Boc-Ser(Nfm)-OMe (12). The product of the reaction of Nfm-OH (**2a**) (0.095 g, 3.71×10^{-4} mol) with *N*-*tert*-butyloxycarbonylserine methyl ester (**10**) methyl ester hydrochloride was chromatographed using ethyl acetate/*n*-hexane 2:8, as the eluent to give compound **12** as an off-white oil (0.092 g, 54%). TLC (ethyl acetate/*n*-hexane, 4:6): R_f 0.47. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.42 (s, 9H, C(CH_3)$_3$), 3.45 (s, 3H, OCH_3 Ser), 3.99 (s, 3H, OCH_3), 4.07 (s, 2H, CH_2), 4.40–4.48 (m, 2H, β-CH_2 Ser), 4.50–4.60 (m, 1H, α-CH Ser), 5.18 (d, J=7.5 Hz, 1H, α-NH Ser), 7.16 (dd, J=8.9 and 2.7 Hz, 1H, H-7), 7.51 (d, J=9.0 Hz, 1H, H-4), 7.58 (d, J=2.7 Hz, 1H, H-9), 7.67 (d, J=9.0 Hz, 1H, H-5), 7.69 (s, 1H, H-2), 7.86 (d, J=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=28.20 (C(*C*H_3)$_3$), 31.73 (CH_2), 52.42 (OCH_3 Ser), 52.78 (α-CH Ser), 55.42 (OCH_3), 65.06 (β-CH_2 Ser), 80.31 (*C*(CH_3)$_3$), 103.08 (C-9), 110.22 (C-4), 114.23 (C-1), 115.65 (C-7), 120.15 (C-3b), 125.70 (C-5a), 125.77 (C-5), 129.36 (C-5b), 130.51 (C-6), 142.45 (C-2), 153.99 (C-3a), 155.07 (*C*O_2C(CH_3)$_3$), 158.27 (C-8), 169.73 (*C*O_2CH_3), 170.43 (CH_2*C*O_2). IR (KBr 1%, cm^{-1}): ν=3376, 2977, 2839, 1745, 1720, 1707, 1629, 1598, 1519, 1505, 1474, 1436, 1367, 1232, 1161, 1121, 1060, 1022, 950, 876, 830. UV/vis (ethanol, nm): λ_{max} (ε)=298 (12705 $M^{-1}\,cm^{-1}$). HRMS (EI) calcd for $C_{24}H_{27}NO_8$ [M^+]: 457.1737; found: 457.1736.

4.3. Stability tests with Nfm-Phe-OMe (7a)

4.3.1. Catalytic hydrogenation. A suspension of Nfm-Phe-OMe (**7a**) (4.0×10^{-2} g, 9.58×10^{-5} mol) in methanol (1.0 mL) and 1,4-cyclohexadiene (9.6×10^{-2} mL, 2.58×10^{-4} mol) was mixed with 10% palladium on charcoal catalyst (0.014 g) and refluxed for 7 h with stirring. The catalyst was filtered off and washed with methanol; the combined liquids were then evaporated under reduced pressure affording the compound as a white solid (3.8×10^{-2} g, 95%). ^{1}H NMR was well compared with the starting material.

4.3.2. Acidolysis with hydrochloric acid. To the fully protected amino acid Nfm-Phe-OMe (**7a**) (2.01×10^{-2} g, 4.36×10^{-5} mol) was added 6 M HCl (0.20 mL) under rapid stirring over 1 h. Evaporation under reduced pressure gave a white solid (2.01×10^{-2} g, 100%). ^{1}H NMR confirmed the structure of the compound.

4.3.3. Acidolysis with trifluoroacetic acid. To the fully protected amino acid Nfm-Phe-OMe (**7a**) (2.2×10^{-2} g, 5.27×10^{-5} mol) was added 0.74 mL of trifluoroacetic acid under rapid stirring over 5 h. Evaporation under reduced pressure gave a white solid (2.2×10^{-2} g, 100%). ^{1}H NMR confirmed the structure of the compound.

4.3.4. Aminolysis. A solution of Nfm-Phe-OMe (**7a**) (2.0×10^{-2} g, 4.79×10^{-5} mol) in dry acetonitrile was treated with DEAEA (4.1×10^{-2} mL, 2.87×10^{-4} mol) for 27 h according to the procedure of Grehn et al.[27] The product was purified by flash chromatography, using chloroform/*n*-hexane 6:1, as the eluent, to give the compound as an oily solid (1.7×10^{-2} g, 85%). ^{1}H NMR was well compared with the starting material.

4.3.5. Reduction with Mg/MeOH. To a solution of Nfm-Phe-OMe (**7a**) (2.0×10^{-2} g, 4.33×10^{-5} mol) in dry methanol (2 mL) magnesium powder (1.0×10^{-2} g, 4.11×10^{-5} mol) was added and the resulting mixture was sonicated for 2.30 h, at room temperature. More magnesium was added (3.2×10^{-2} g, 1.32×10^{-5} mol), in small portions (1.0×10^{-2} g each) and the resulting mixture was sonicated for another 7 h. The reaction mixture was quenched by the addition of saturated aqueous NH_4Cl (4 mL) and extracted with ethyl acetate. The organic layer was dried with $MgSO_4$, concentrated to dryness to give an off-white oil (1.9×10^{-2} g, 95%). ^{1}H NMR was well compared with the starting material.

4.3.6. Alkaline hydrolysis. To the fully protected amino acid Nfm-Phe-OMe (**7a**) (2.0×10^{-2} g, 4.79×10^{-5} mol) in 1,4-dioxane (2 mL), 1 M NaOH (7.2×10^{-2} mL, 7.19×10^{-5} mol) was added at low temperature. The solution was stirred at 0 °C for 5 h and acidified to pH 3 with 1 M $KHSO_4$. After extraction with ethyl acetate and evaporation of the solvent, Nfm-Phe-OH (**13**) was obtained as an orange solid (0.019 g, 100%). Mp=191.0–193.0 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.80–2.90 (m, 2H, β-CH_2 Phe), 3.87 (s, 3H, OCH_3), 3.95 (s, 2H, CH_2), 4.80–4.90 (m, 1H, α-CH Phe), 6.12 (d, J=7.8 Hz, 1H, α-NH Phe), 6.48 (d, J=6.9 Hz, 2H, H-2 and H-6 Phe), 6.73 (t, J=7.5 Hz, 2H, H-3 and H-5 Phe), 6.91 (t, J=7.5 Hz, 1H, H-4 Phe), 7.13 (dd, J=9.3 and 2.4 Hz, 1H, H-7), 7.41 (d, J=2.4 Hz, 1H, H-9), 7.52 (d, J=9.0 Hz, 1H, H-4), 7.58 (s, 1H, H-2), 7.72 (d, J=8.7 Hz, 1H, H-5), 7.85 (d, J=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=33.72 (CH_2), 36.92 (β-CH_2 Phe), 52.91 (α-CH Phe), 55.51 (OCH_3), 102.20 (C-9), 109.99 (C-4), 114.63 (C-1), 116.80 (C-7), 119.84 (C-3b),

125.67 (C-5a), 126.36 (C-5), 126.98 (C-4 Phe), 128.19 (C-3 and C-5 Phe), 128.56 (C-2 and C-6 Phe), 129.34 (C-5b), 130.44 (C-6), 134.53 (C-1), 142.83 (C-2), 154.47 (C-3a), 158.67 (C-8), 170.88 (CONH), 174.16 (CO_2H). IR (Nujol, cm^{-1}): ν=3399, 3383, 2954, 2923, 2854, 2586, 1731, 1626, 1538, 1522, 1497, 1463, 1455, 1431, 1418, 1378, 1357, 1259, 1228, 1208, 1178, 1122, 1103, 1084, 1037, 1016, 831. HRMS (EI) calcd for $C_{24}H_{21}NO_5$ [M^+]: 403.1420; found: 403.1432.

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References and notes

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