Photolysis at long wavelengths of amino acid ester derivatives based on 4-methyl-6-methoxy-2-oxo-2*H*-naphtho[1,2-*b*]pyrans

Ana M. Piloto,[a] Ana M. S. Soares,[a] Graham Hungerford,[b] Susana P. G. Costa,[a] and M. Sameiro T. Gonçalves*[a]

[a] Centre of Chemistry, University of Minho, Campus of Gualtar,
4710-057 Braga, Portugal
Fax: +351- 253604382
E-mail: msameiro@quimica.uminho.pt

[b] HORIBA Jobin Yvon IBH Ltd, 45 Finnieston Street, Glasgow G3 8JU, UK

Keywords: Photocleavable protecting groups / Cage compounds / Photolysis / Benzocoumarins / Amino acids

Ester derivatives of fused 4-methyl-6-methoxy-2-oxo-2*H*- naphtho[1,2-*b*]pyran, using valine and phenylalanine as model bifunctional molecules were synthesised, to assess its applicability as a photocleavable protecting group, for solution phase organic synthesis and in caging applications, at longer wavelengths. The behaviour of the corresponding derivatives towards photolysis was evaluated by irradiation in mixture of HEPES buffer and acetonitrile or methanol, in a photochemical reactor at 350 and 419 nm, followed by HPLC/UV monitoring. Time-resolved fluorescence measurements were used to elucidate the dynamics.

Introduction

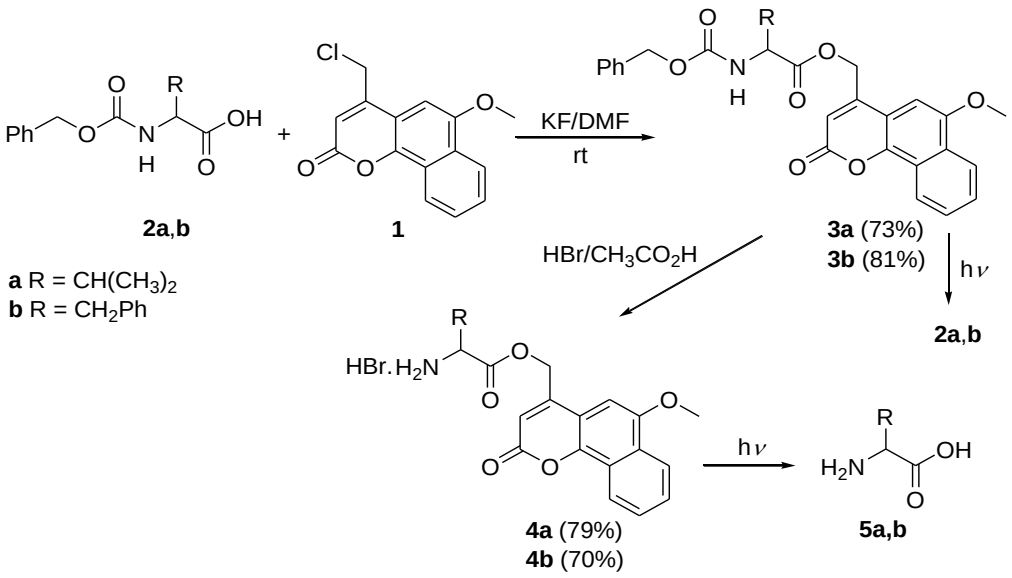
The use of light of an appropriate wavelength can liberate synthetic and biochemically relevant molecules from their corresponding non-reactive/inactive conjugate/caged precursors possessing suitable light-sensitive moieties covalently bonded to the functional groups of the molecule of interest. In recent years this approach has been widely applied in organic synthesis as an alternative to classical acid- and base-labile protecting groups, as well as, in life sciences to investigate signal transduction mechanisms at the cellular level and in drug delivery.[1] This broad range of application supports the interest in developing novel photolabile groups with enhanced properties that will allow the fast cleavage and release of various functionalities (such as alcohols, amines, phosphates, aldehydes, ketones and carboxylic acids[2-6]). Additionally the use of longer wavelengths can minimise side reactions and/or cell damage. Nevertheless, among the considerable number of light sensitive groups that have been reported, only few cleave in a practical time by irradiation at wavelengths superior to 400 nm.

In addition to the interest of natural or synthetic derivatives of 2-oxo-2*H*-benzopyrans (trivially named as coumarins) as antioxidants, food components, stabilisers, immunomodulatory substances, fluorescent

markers in analysis, laser dyes and in clinical use,[7-12] they have also been recognised for their application as photolabile protecting groups for the caging of biomolecules. Oxobenzopyrans have been reported in cell biology and biophysical studies with a range of chemical functionalities. [3-6,13] As part of our research interests relating to the design and evaluation of new polyaromatic and polyheteroaromatic systems, which include oxobenzopyrans and their fused derivatives as photolabile protecting groups for biologically relevant molecules [14-19], we now report the synthesis of novel valine and phenylalanine model derivatives bearing a 2-oxo-2*H*-naphtho[1,2-*b*]pyran moiety as a light sensitive group. In order to evaluate the possibility of photorelease of the amino acids in *N*-protected and free forms in a practical time period at longer wavelengths, photolysis studies were carried out in mixtures of aqueous HEPES buffer and an organic solvent at 350 and 419 nm. Cleavage kinetic data obtained by HPLC/UV monitoring was also collected and time-resolved fluorescence measurements were used to elucidate the dynamics.

Results and Discussion

4-(Chloromethyl)-6-methoxy-2-oxo-2*H*-naphtho[1,2-*b*]pyran **1**, [18] was reacted with *N*-(benzyloxycarbonyl)-L-valine **2a** and *N*-(benzyloxycarbonyl)-L-phenylalanine **2b** in the presence of potassium fluoride in DMF, at room temperature,[20] giving *N*-(benzyloxycarbonyl)-L-valine (6-methoxy-2-oxo-2*H*-naphtho[1,2-*b*]pyran-1-yl) methyl ester **3a** and *N*-(benzyloxycarbonyl)-L-phenylalanine (6-methoxy-2-oxo-2*H*-naphtho[1,2-*b*]pyran-1-yl) methyl ester **3b** in good yields. In order to compare the release under irradiation of the model amino acids in their *N*-protected **2a**,**b** and free forms **5a**,**b**, the *N*-benzyloxycarbonyl-protecting group was removed by acidolysis with hydrobromic acid in acetic acid giving compounds **4a** and **4b** bearing the amino acid and the photosensitive tag (Scheme 1).



Scheme 1. Synthesis of oxonaphtho[1,2-*b*]pyran ester derivatives **3**, **4** and photorelease of amino acids **2**, **5**.

The 2-oxo-naphtho[1,2-*b*]pyran (Bbp) moiety will be designated in this report by a three-letter code for simplicity of naming the various fluorescent derivatives, as indicated in Tables 1- 4.

All new compounds were fully characterised by high resolution mass spectrometry, IR, ^{1}H and ^{13}C NMR spectroscopy. ^{1}H NMR spectra showed signals of the amino acid residues, such as the α-CH (δ 4.24-4.80 ppm), β-CH (δ 2.00-2.40 ppm), β-CH_2 (δ 3.10-3.30 ppm), as well as γ-CH_3 (δ 0.95-1.05 ppm). The methylene group bound to the heterocycle was also visible for all derivatives (δ 5.30-5.90 ppm). The confirmation of the presence of the ester bond was also supported by ^{13}C NMR spectra signals at δ 168.43 to 171.60 ppm.

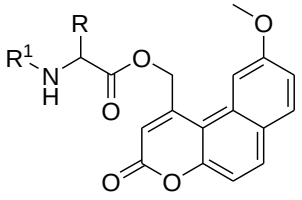
In order to obtain the parameters necessary for the monitoring of the photolysis reaction, as well as the fluorescence properties, the UV/vis characterization was carried out in mixtures of different organic solvents (acetonitrile or methanol) with HEPES buffer. The absorption and emission spectra of compounds **3** and **4** in degassed mixtures of acetonitrile and HEPES buffer (80:20), methanol and HEPES buffer (80:20), and absolute ethanol were measured; absorption (λ_{abs}) and emission maxima (λ_{em}), molar absorptivities (ε) and relative fluorescence quantum yields (Φ_F) are also reported (Tables 1 and 2). Relative fluorescence quantum yields were calculated using 9,10-diphenylanthracene as standard (Φ_F = 0.95 in ethanol).[21] For the Φ_F determination, the fluorescence standard was excited at the wavelengths of maximum absorption found for each one of the compounds to be tested and in all fluorimetric measurements the absorbance of the solution did not exceed 0.1.

Table 1. UV/vis absorption and emission data (λ, in nm) for amino acid derivatives **3**, **4**, **6** and **7** in mixtures of acetonitrile and HEPES buffer (80:20).

Compound		Acetonitrile/HEPES (80:20)				
		λ_{abs}	log ε	λ_{em}	Φ_F	$\Delta\lambda$
3a	Z-Val-OBbp	373	3.82	479	0.80	106
3b	Z-Phe-OBbp	373	3.92	476	0.50	103
4a	H-Val-OBbp	371	3.68	475	0.84	104
4b	H-Phe-OBbp	373	3.78	478	0.58	105
6a	Z-Val-OBba	346	4.07	481	0.54	135
6b	Z-Phe-OBba	346	4.06	478	0.59	132
7a	H-Val-OBba	345	4.02	476	0.63	129
7b	H-Phe-OBba	347	4.00	480	0.77	135

By comparison of the absorption and emission maxima, no significant differences were observed in these solvents, with values in the range 371-376 nm and 471-481 nm, respectively. The Stokes' shift ($\Delta\lambda$) was between 97 and 106 nm, which is an advantageous property in fluorescence techniques as it will minimize self-quenching phenomena. It was found that all compounds were highly emissive in mixtures of HEPES buffer and acetonitrile or methanol (Φ_F from 0.50 to 0.94), as well as in ethanol (Φ_F from 0.49 to 0.65).

Absorption maxima at longer wavelengths can indicate that photolysis at higher wavelengths could be possible with shorter irradiation times. Therefore considering that the present heterocycle is associated with a λ_{abs} at about 370 nm, which is 25 nm bathochromically shifted from the previously reported derivatives **6** and **7**, possessing the same heterocycle with a different ring fusion, namely 9-methoxy-3-oxo-3*H*-naphtho[2,1-*b*]pyran (Bba) (Figure 1, Table 1), it was expected that the behaviour of **3** and **4** towards irradiation in mixtures of acetonitrile and HEPES buffer (80:20) would be improved in the visible region when compared with results obtained with **6** and **7**.[19]



6a R = $CH(CH_3)_2$, R^1 = CO_2CH_2Ph
6b R = CH_2Ph, R^1 = CO_2CH_2Ph
7a R = $CH(CH_3)_2$, R^1 = H.HBr
7b R = CH_2Ph, R^1 = H.HBr

Figure 1. Structure of 3-oxo-3*H*-naphtho[2,1-*b*]pyran amino acid derivatives **6** and **7**.[19]

Table 2. UV/vis absorption and emission data (λ, in nm) for amino acid derivatives **3** and **4** in mixtures of methanol and HEPES buffer (80:20) and absolute ethanol.

Cpd	Methanol/HEPES (80:20)					Ethanol				
	λ_{abs}	log ε	λ_{em}	Φ_F	$\Delta\lambda$	λ_{abs}	log ε	λ_{em}	Φ_F	$\Delta\lambda$
3a	376	3.70	481	0.51	105	376	3.66	474	0.65	98
3b	375	3.91	481	0.72	106	374	3.63	474	0.49	100
3a	375	3.75	474	0.70	99	375	3.65	476	0.51	101
3b	374	3.71	471	0.94	97	375	3.69	472	0.58	97

The evaluation of compounds **3**, **4**, **6** and **7** towards UV/vis irradiation was carried out by exposing solutions of the mentioned compounds in mixtures of acetonitrile/HEPES buffer (80:20) and methanol/HEPES buffer (80:20) solution in a Rayonet RPR-100 reactor at 350 and 419 nm (Scheme 1). The course of the photocleavage reaction was followed by reverse phase HPLC with UV detection. The plots of peak area (*A*) of the starting material *versus* irradiation time were obtained for each compound, at the considered wavelengths. Peak areas were determined by HPLC, which revealed a gradual decrease with time, and were the average of 3 runs. The determined irradiation time (t_{irr}) represents the time necessary for the consumption of the starting materials until less than 5% of the initial area was detected (Table 3). The

photochemical quantum yields (Φ_P) were calculated based on half-lives ($t_{1/2}$), molar absorptivities (ε) and the incident photon flux (I_0), which was determined by potassium ferrioxalate actinometry.[22]

Table 3. Irradiation times (t_{irr}, in min) and photochemical quantum yields (Φ_P , $\times 10^{-3}$) for the photolysis of compounds **3** and **4** at 350 and 419 nm in mixtures of acetonitrile/HEPES buffer (80:20) and methanol/HEPES buffer (80:20).

Cpd	Acetonitrile/HEPES (80:20)				Methanol/HEPES (80:20)			
	350 nm		419 nm		350 nm		419 nm	
	t_{irr}	Φ_P	t_{irr}	Φ_P	t_{irr}	Φ_P	t_{irr}	Φ_P
3a	86	0.121	77	0.645	59	0.243	270	0.164
3b	35	0.210	53	0.512	47	0.176	148	0.158
4a	35	0.338	23	2.439	231	0.055	1253	0.047
4b	33	0.304	45	0.802	632	0.023	1153	0.042

The photolysis irradiations times at 419 nm in mixture of acetonitrile and HEPES buffer (80:20) obtained for compounds **3** and **4** in comparison with those of compounds **6** and **7**, between 598 and 1155 min, confirmed the expectations related to the difference in the absorption maxima. Quantitative release of *N*-benzyloxycarbonyl valine and phenylalanine **2a**,**b**, and the corresponding free amino acids **5a**,**b** from compounds **3** and **4**, bearing a naphtho[1,2-*b*]pyran, was achieved in less than 1.3 h, whereas cleavage from compounds **6** and **7** required from 10 to 20 hours. At 350 nm, differences in the photolysis times were not significant enough to make a clear distinction in the behavior of both systems. Regarding irradiation of compounds **3** and **4** at 350 nm, it was found that irradiation times were short, 33-86 min, and comparable to those obtained at 419 nm (23-77 min). However, considering the benefits of the use of longer wavelengths of irradiation in photolytic processes, the selected irradiation wavelength for these conjugate systems would be 419 nm.

For both wavelengths of irradiation, it was found that the presence of the *N*-benzyloxycarbonyl protecting group influenced the cleavage times, since the release of valine **5a** was faster than the corresponding *N*-blocked form **2a**. In the case of phenylalanine, the cleavage times were similar. As reported before,[14] the *N*-benzyloxycarbonyl group was stable in the tested conditions, no cleavage being detected.

In addition, photolysis of compounds **3** and **4** was also carried out in a mixture of HEPES buffer with a protic solvent, methanol, in the same proportion as before, in order to compare the performance of the (6-methoxy-2-oxo-2*H*-naphtho[1,2-*b*]pyran-4-yl)methyl ester in different solvent systems. Overall, the sensitivity of the ester linkage between the protecting group and the amino acid was inferior in this solvent system at both wavelengths tested. In addition, the release of the free form of both amino acids required much longer and impracticable irradiation periods.

Very low photochemical quantum yields were obtained in mixtures of acetonitrile and HEPES buffer (80:20) and methanol and HEPES buffer (80:20), indicating that a strong competition between the bond

scission and radiative and non-radiative relaxation is at work. To help elucidate the photophysics of the processes involved a preliminary time-resolved fluorescence study was performed using both acetonitrile/HEPES and methanol/HEPES mixtures. The excitation wavelength of 375 nm (with λ_{em} = 474 nm) was chosen to monitor the coumarin species present. The expected cleavage alcohol product, 4-(hydroxymethyl)-6-methoxy-2-oxo-2*H*-naphtho[1,2-*b*]pyran, Bbp-OH, was found to decay monoexponentially (Φ_F determined as 0.45 in both ethanol and methanol/HEPES (80:20)[18]). This allowed the estimation of the radiative and non-radiative rate constants (k_r and k_{nr}) of 5.2×10^7 and 6.3×10^7 s^{-1} respectively, in methanol/HEPES and correspondingly 4.6×10^7 and 9.4×10^7 s^{-1} in acetonitrile/HEPES (Φ_F = 0.45 and 0.33, respectively for the two solvent systems).

With the exception of **3a**, which decayed biexponentially, the sum of three exponential components was required to fit the decay data (see Supporting Information), indicating the presence of different coumarin species. From lifetimes alone it was not possible to clearly ascribe decay rates to individual species, which necessitated the measurement of time-resolved emission spectra to obtain the decay associated spectra (see Supporting Information). Comparing with the steady state spectrum (taken under the same experimental conditions) accordance in both terms of spectrum and lifetime, for the presence of the alcohol product in the methanol/HEPES mixture was seen for **4a** and **4b**. The other assignments are the area of continued investigation, but assuming that the spectrum for the ester is likely to be at longer wavelengths and its contribution to the fluorescence significant (shown by pre-exponential value) it is possible to estimate the rate constants for the initial bond cleavage (k_c), shown in Table 4 (see Supporting Information).

This generally shows faster rates for the compound 4 esters, although, these results are still preliminary there appears to be a correlation with the photochemical yield data presented in table 3 and a fuller photophysical investigation is underway.

Table 4. Cleavage rate constants (k_c in s^{-1}) calculated from the fluorescence lifetime data in mixtures of acetonitrile/HEPES buffer (80:20) and methanol/HEPES buffer (80:20).

Cpd	Acetonitrile/HEPES (80:20)	Methanol/HEPES (80:20)
3a	0.25×10^8	0.53×10^8
3b	0.58×10^8	1.11×10^8
4a	5.69×10^8	4.70×10^8
4b	9.35×10^8	6.60×10^8

The disappearance of the starting material can also be illustrated for each compound and based on HPLC data, the plot of ln *A versus* irradiation time showed a linear correlation for the disappearance of the starting material, which suggested a first order reaction, obtained by the linear least squares methodology for a straight line (Figure 2).

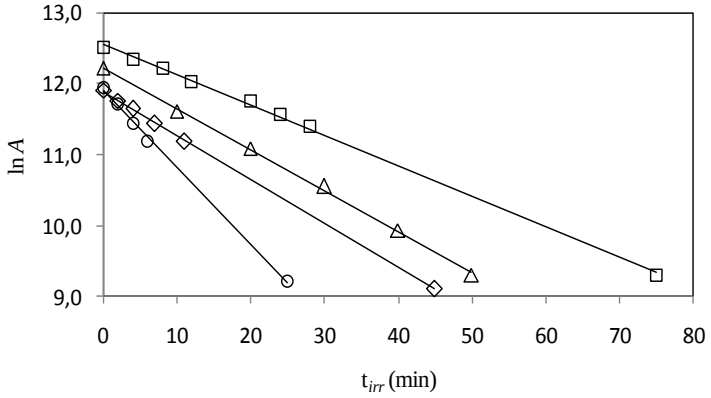


Figure 2. Plot of ln *A versus* irradiation time for the photolysis of compounds **3a** (□), **3b** (Δ), **4a** (○) and **4b** (◊) at 419 nm in mixtures of acetonitrile/HEPES buffer (80:20).

Conclusion

The synthesis of novel valine and phenylalanine oxonaphtho[1,2-*b*]pyran esters was achieved in good yields through a standard procedure from the corresponding chloromethylated oxonaphtho[1,2-*b*]pyran. In order to obtain the parameters necessary for monitoring the photolysis reaction, the UV/vis characterization was carried out in mixtures of acetonitrile/HEPES buffer (80:20), methanol/HEPES buffer (80:20) and absolute ethanol. Photocleavage studies of the ester compounds, in mixture of acetonitrile and HEPES buffer (80:20), at 350 and 419 nm, revealed that the amino acid-heterocycle ester bond was readily photolysed, releasing the *N*-protected and free amino acids. The most interesting results were obtained at 419 nm for all compounds in practicable irradiations times (23-77 min), confirming the suitability of the oxonaphtho[1,2-*b*]pyran as a photocleavable protecting group for the release at longer wavelengths, which is an interesting improvement compared to the previously reported 3-oxo-3*H*-naphtho[2,1-*b*]pyran with a different ring fusion. The use of time-resolved fluorescence techniques can further elucidate the dynamics of the photocleavage processes of these compounds.

Experimental Section

General

All melting points were measured on a Stuart SMP3 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 BOMEM MB 104 spectrophotometer. UV/vis absorption spectra (200 – 700 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. NMR spectra were obtained on a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H NMR and 100.6 MHz for

^{13}C NMR using the solvent peak as internal reference at 25 ºC. All chemical shifts are given in ppm using δ Me_4Si = 0 ppm as reference and *J* values are given in Hz. 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 techniques. High resolution mass spectrometry analyses were performed at the "C.A.C.T.I. - Unidad de Espectrometria de Masas", at University of Vigo, Spain. Time-resolved fluorescence measurements were performed using a HORIBA Scientific UltraFast-01 system equipped with a DeltaDiode (DD-375L) excitation source running at 8 MHz, emitting at 374 nm and a microchannel plate detector. Data were analysed using DAS6 software, with the TRES (time-resolved emission spectra) fitting using a global analysis module linking common lifetimes, generally fixed from the simple decay analysis, from which decay associated spectra were then obtained.

Synthesis procedures for compounds 3 and 4

Synthesis of *N*-(benzyloxycarbonyl)-L-valine (6-methoxy-2-oxo-2*H*- naphtho[1,2-*b*]pyran-4-yl) methyl ester, Z-Val-OBbp (3a). 4-(Chloromethyl)-6-methoxy-2-oxo-2*H*-naphtho[1,2-*b*]pyran, Bbp-Cl **1** (0.047 g, 1.7×10^{-4} mol) was dissolved in dry DMF (2 mL), potassium fluoride (0.030 g, 5.1×10^{-4} mol) and Z-Val-OH **2a** (0.047 g, 1.9×10^{-4} mol) were added. The reaction mixture was stirred at room temperature for 32 hours and was followed by TLC (ethyl acetate/*n*-hexane 3:7). The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography in silica gel using dichloromethane/methanol mixtures of increasing polarity as eluent, to give compound **3a** as a yellow solid (0.061 g, 73%). Mp = 142.2 – 143.6 ºC. TLC (ethyl acetate/*n*-hexane 3:7): 0.53. ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 0.95 (d, *J* = 6.8 Hz, 3 H, γ-CH_3 Val), 1.04 (d, *J* = 6.8 Hz, 3 H, γ-CH_3 Val), 2.22-2.36 (m, 1 H, β-CH Val), 4.04 (s, 3 H, OCH_3), 4.40-4.50 (m, 1 H, α-CH Val), 5.16 (s, 2 H, CH_2 Z), 5.26 (d, *J* = 8.8 Hz, 1 H, α-NH Val), 5.35-5.50 (m, 2 H, CH_2), 6.60 (s, 1 H, H-3), 6.65 (s, 1 H, H-5), 7.25-7.40 (m, 5 H, 5 × Ar-*H* Z), 7.60-7.74 (m, 2 H, H-8 and H-9), 8.25-8.34 (m, 1 H, H-7), 8.50-8.59 (m, 1 H, H-10) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 17.58 (γ-CH_3 Val), 19.15 (γ-CH_3 Val), 31.04 (β-CH Val), 55.89 (OCH_3), 59.33 (α-CH Val), 62.38 (CH_2), 67.29 (CH_2 Z), 95.27 (C-5), 112.14 (C-4a), 113.20 (C-3), 122.29 (C-7), 122.44 (C-10), 124.01 (C10a), 127.43 (C-6a), 128.00 (C-9), 128.21 (2×Ar-C), 128.28 (2×Ar-C), 128.53 (Ar-C), 128.55 (C-8), 136.00 (C-1 Z), 145.82 (C-10b), 148.94 (C-4), 152.38 (C-6), 156.25 (CONH), 160.59 (C-2), 171.60 (CO_2CH_2) ppm. IR (KBr 1%, cm^{-1}): υ = 3758, 3691, 3054, 2987, 2686, 2522, 2411, 2306, 1726, 1603, 1551, 1508, 1422, 1386, 1265, 1160, 1114, 1029, 896, 738, 704. HRMS (ESI): calcd for $C_{28}H_{28}NO_7$ [M^++H]: 490.18603; found: 490.18729.

Synthesis of *N*-(benzyloxycarbonyl)-L-phenylalanine (6-methoxy-2-oxo-2*H*-naphtho[1,2-*b*]pyran-4-yl) methyl ester, Z-Phe-OBbp (3b). Starting from Bbp-Cl **1** (0.060 g, 2.2×10^{-4} mol), DMF (2 mL), potassium fluoride (0.040g, 6.9×10^{-4} mol) and Z-Phe-OH **2b** (0.076 g, 2.5×10^{-4} mol), following the same procedure as described for **3a**, compound **3b** was obtained as an orange solid (0.115 g, 81%). Mp = 134.6 – 136.1 ºC. TLC (ethyl acetate/*n*-hexane 3:7): 0.58. ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 3.16 (d, *J*

= 6.4 Hz, 2 H, β-CH_2 Phe), 3.98 (s, 3 H, OCH_3), 4.73-4.80 (m, 1 H, α-CH Phe), 5.08-5.14 (m, 2 H, CH_2 Z), 5.30 (s, 2 H, CH_2), 5.38 (d, *J* = 8.0 Hz, 1 H, α-NH Phe), 6.39 (s, 1 H, H-3), 6.53 (s, 1 H, H-5), 7.10-7.32 (m, 10 H, 5 × Ar-*H* Phe and 5 × Ar-*H* Z), 7.60-7.68 (m, 2 H, H-8 and H-9), 8.20-8.26 (m, 1 H, H-7), 8.38-8.50 (m, 1 H, H-10) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 36.40 (β-CH_2 Phe), 55.16 (α-CH Phe), 55.81 (OCH_3), 62.35 (CH_2), 67.15 (CH_2 Z), 95.26 (C-5), 112.05 (C-4a), 113.14 (C-3), 122.20 (C-7), 122.27 (C-10), 123.85 (C-10a), 127.28 (C-6a), 127.37 (Ar-C), 127.87 (Ar-C), 128.09 (2×Ar-C), 128.19 (2×Ar-C), 128.39 (C-9), 128.46 (2×Ar-C), 128.71 (2×Ar-C), 129.01 (C-8), 135.13 (C-1 Phe), 135.95 (C-1 Z), 145.63 (C-10b), 148.53 (C-4), 152.20 (C-6), 160.41 (C-2), 162.50 (CONH), 171.15 (*C*O_2CH_2) ppm. IR (KBr 1%, cm^{-1}): υ = 3759, 3691, 3583, 3054, 2987, 2686, 2522, 2411, 2306, 1725, 1677, 1602, 1551, 1507, 1422, 1386, 1265, 1163, 1113, 1086, 1029, 896, 740, 705. HRMS (ESI): calcd for $C_{32}H_{28}NO_7$ [M^++H]: 538.18603; found: 538.18639.

Synthesis of L-valine (6-methoxy-2-oxo-2*H*-naphtho[1,2-*b*]pyran-4-yl) methyl ester hydrobromide, HBr.H-Val-OBbp (4a). A 45% solution of hydrobromic acid in acetic acid (162 μL) and acetic acid (1 mL) were added to the Z-Val-OBbp **3a**, with stirring, at room temperature. The reaction mixture was maintained in these conditions for 11 hours, and the process was followed by TLC (ethyl acetate/*n*-hexane 3:7). During this time, additional amounts of 45% solution of hydrobromic acid in acetic acid were added (until a total volume of 972 μL). When the reaction was completed, cold diethyl ether was added (0.5 mL), and the precipitate filtered off and washed with the same solvent to give compound **4a** as a light brown solid (0.021 g, 79%). Mp = 205.5 – 207.1 ºC. TLC (ethyl acetate/methanol 1:1): 0.53. ^{1}H NMR (400 MHz, DMSO-d_6, 25 ºC): δ = 0.98-1.05 (m, 6 H, 2× γ-CH_3 Val), 2.00-2.40 (m, 1 H, β-CH Val), 4.06 (s, 3 H, OCH_3), 4.24 (broad s, 1 H, α-CH Val), 5.60-5.90 (m, 2 H, CH_2), 6.72 (s, 1 H, H-3), 7.03 (s, 1 H, H-5), 7.70-7.80 (m, 2 H, H-8 and H-9), 8.20-8.26 (m, 1 H, H-7), 8.30-8.40 (m, 1 H, H-10), 8.44 (broad s, 3 H, $^+NH_3$) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 ºC): δ = 17.65 (γ-CH_3 Val), 18.16 (γ-CH_3 Val), 29.52 (β-CH Val), 56.18 (OCH_3), 57.33 (α-CH Val), 63.08 (CH_2), 97.50 (C-5), 112.09 (C-3), 112.27 (C-4a), 121.66 (C-10), 122.00 (C-7), 122.97 (C-10a), 126.48 (C-6a), 128.22 (C-8), 128.69 (C-9), 144.56 (C-10b), 150.08 (C-4), 151.26 (C-6), 159.63 (C-2), 168.43 (*C*O_2CH_2) ppm. IR (KBr 1%, cm^{-1}): υ = 3450, 3072, 3009, 2944, 2883, 1764, 1662, 1386, 1222, 1054, 1008, 823, 760. HRMS (ESI): calcd for $C_{20}H_{22}NO_5$ [M^++H]: 356.14925; found: 356.14906.

Synthesis of L-phenylalanine (6-methoxy-2-oxo-2*H*-naphtho[1,2-*b*]pyran-4-yl) methyl ester hydrobromide, HBr.H-Phe-OBbp (4b). Starting from conjugate **3b** (0.027 g, 5.0×10^{-5} mol) and using 45% solution of hydrobromic acid in acetic acid (1.2 mL), compound **4b** was obtained as a brown solid (0.017 g, 70%). Mp = 216.0 – 218.2 ºC. TLC (ethyl acetate/methanol 1:1): 0.61. ^{1}H NMR (400 MHz, DMSO-d_6, 25 ºC): δ = 3.10-3.30 (m, 2 H, β-CH_2 Phe), 4.05 (s, 3 H, OCH_3), 4.62 (broad s, 1 H, α-CH Phe), 5.50-5.70 (m, 2 H, CH_2), 6.50 (s, 1 H, H-3), 6.96 (s, 1 H, H-5), 7.10-7.30 (m, 5 H, 5 × Ar-*H* Phe), 7.70-7.90 (m, 2 H, H-8 and H-9), 8.19-8.25 (m, 1 H, H-7), 8.31-8.39 (m, 1 H, H-10), 8.55 (broad s, 3 H, $^+NH_3$) ppm. ^{13}C NMR (100.6

MHz, DMSO-d_6, 25 ºC): δ = 36.07 (β-CH_2 Phe), 53.25 (α-CH Phe), 56.20 (OCH_3), 62.97 (CH_2), 97.47 (C-5), 112.01 (C-4a), 112.21 (C-3), 121.63 (C-7), 121.99 (C-10), 122.96 (C-6a), 126.46 (C-10a), 127.37 (C-8 and Ar-C Phe), 128.21 (C-9), 128.62 (2×Ar-C Phe), 129.34 (2×Ar-C Phe), 134.38 (C-1 Phe), 144.50 (C-10b), 149.71 (C-4), 151.24 (C-6), 159.54 (C-2), 168.48 (CO_2CH_2) ppm. IR (KBr 1%, cm^{-1}): υ = 3446, 3073, 3010, 2970, 2882, 1765, 1663, 1383, 1222, 1031, 881, 823, 761. HRMS (ESI): calcd for $C_{24}H_{21}NO_5$ [M^++H]: 404.14925; found: 404.14882.

Photolysis general

Photolysis was carried out using a Rayonet RPR-100 chamber reactor equipped with 10 lamps of 350 and 419 ± 10 nm. HPLC analyses were performed using a Licrospher 100 RP18 (5 μm) column in a JASCO HPLC system composed by a PU-2080 pump and a UV-2070 detector with ChromNav software.

General photolysis procedure

A 1×10^{-4} M solution of compounds **3**, **4**, **6** and **7** in mixtures of acetonitrile/HEPES buffer (80:20), (5 mL), and of compounds **3** and **4** in methanol/HEPES buffer (80:20) was placed in a quartz tube and irradiated in the reactor at the desired wavelength. HEPES buffer solution was prepared in distilled water with HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (10 mM), NaCl (120 mM), KCl (3 mM), $CaCl_2$ (1 mM) and $MgCl_2$ (1mM) and pH adjusted to 7.2. Aliquots of 100 μL were taken at regular intervals and analysed by RP-HPLC. The eluent was acetonitrile/water, 3:1, at a flow rate of 1 mL/min (retention time: **3a**, 7.6 min; **3b**, 8.3 min; **4a**, 10.9 min; **4b**, 8.2 min) or 0.8 mL/min (**6a**, 7.1 min; **6b**, 7.4 min; **7a** and **7b**, 8.0 min), previously filtered through a Millipore, type HN 0.45 μm filter and degassed by ultra-sound for 30 min. The chromatograms were traced by detecting UV absorption at the wavelength of maximum absorption for each conjugate.

Acknowledgements

Thanks are due to the *Fundação para a Ciência e Tecnologia* (Portugal) for financial support through project PTDC/QUI/69607/2006 (FCOMP-01-0124-FEDER-007449) and a Ph.D. grant to A.M. Piloto (SFRH/BD/61459/2009). The NMR spectrometer Bruker Avance III 400 is part of the National NMR Network and was purchased in the framework of the National Program for Scientific Re-equipment, contract REDE/1517/RMN/2005 with funds from POCI 2010 (FEDER) and FCT.

References

[1] G. Mayer, A. Heckel, *Angew. Chem. Int. Ed.* **2006**, *45*, 4900-4921.

[2] D. Gilbert, K. Funk, B. Dekowski, R. Lechler, S. Keller, F. Möhrlen, S. Frings, V. Hagen, *ChemBioChem* **2007**, *8*, 89-97.

[3] K. Takaoka, Y. Tatsu, N. Yumoto, T. Nakajima, K. Shimamoto, *Bioorg. Med. Chem.* **2004**, *12*, 3687-3694.

[4] V. Hagen, B. Dekowski, V. Nache, R. Schmidt, D. Geissler, D. Lorenz, J. Eichhorst, S. Keller, H. Kaneko, K. Benndorf, B. Wiesner, *Angew. Chem. Int. Ed.* **2005**, *44*, 7887-7891.

[5] V. R. Shembekar, Y. Chen, B. K. Carpenter, G. P. Hess, *Biochemistry* **2007**, *46*, 5479-5484.

[6] M. Lu, O. D. Fedoryak, B. R. Moister, T. M. Dore, *Org. Lett.* **2003**, *5*, 2119-2122.

[7] R. O'Kennedy, R. Douglas, *Coumarins: Biology, Applications and Mode of Action*, Wiley, New York, **1997**.

[8] D. H. Murray, J. Mendez, S. A. Brown, *The Natural Coumarins: Occurrence, Chemistry and Biochemistry*, Wiley, New York, **1982**.

[9] R. G. Harvey, C. Cortz, T. P. Ananthanaraxan, S. J. Schmolka, *J. Org. Chem.* **1988**, *53*, 3936-3943.

[10] T. Ziegler, H. Mohler, F. Henberger, *Chem. Ber.* **1987**, *120*, 373-378.

[11] D. Egan, R. O'Kennedy, E. Moran, D. Cox, *Drug Metab. Rev.* **1990**, *22*, 503-529.

[12] H. Kitagawa, R. Iwaki, *J. Pharm. Soc. Jpn.* **1963**, 83, 1124-1128.

[13] A. Z. Suzuki, T. Watanabe, M. Kawamoto, K. Nishiyama, H. Yamashita, M. Ishii, M. Iwamura, T. Furuta, *Org. Lett.* **2003**, *5*, 4867-4870.

[14] A. M. Piloto, D. Rovira, S. P. G. Costa, M. S. T. Gonçalves, *Tetrahedron* **2006**, *62*, 11955-11962.

[15] M. J. G. Fernandes, M. S. T. Gonçalves, S. P. G. Costa, *Tetrahedron* **2008**, *64*, 3032-3038.

[16] A. S. C. Fonseca, M. S. T. Gonçalves, S. P. G. Costa, *Amino Acids* **2010**, *39*, 699-712.

[17] A. M. S. Soares, S. P. G. Costa, M. S. T. Gonçalves, *Tetrahedron* **2010**, *66*, 8189-8195.

[18] A. M. S. Soares, S. P. G. Costa, M. S. T. Gonçalves, *Amino Acids* **2010**, *39*, 121-133.

[19] A. M. Piloto, A. M. S. Soares, S. P. G. Costa, M. S. T. Gonçalves, *Amino acids*, in press.

[20] F. S. Tjoeng, G. A. Heavner, *Synthesis* **1981**, 897-899.

[21] J. V. Morris, M. A. Mahaney, J. R. Huber, *J. Phys. Chem.* **1976**, *80*, 969-974.

[22] C. Muller, P. Even, M.-L. Viriot, M.-C. Carré, *Helv. Chim. Acta* **2001**, *84*, 3735-3741.

[23] R. Schmidt, D. Geissler, V. Hagen, J. Bendig, *J. Phys. Chem. A* **2007**, *111*, 5768-5774.