Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 62 (2006) 11955–11962

Oxobenzo[*f*]benzopyrans as new fluorescent photolabile protecting groups for the carboxylic function

Ana M. Piloto, Daniel Rovira, Susana P. G. Costa and M. Sameiro T. Gonçalves*

Centro de Química, Universidade do Minho, Campus de Gualtar, P-4710-057 Braga, Portugal

Received 1 August 2006; revised 20 September 2006; accepted 21 September 2006
Available online 27 October 2006

Abstract—The properties of three oxobenzo[*f*]benzopyrans as new fluorogenic photolabile protecting groups for the carboxylic function of amino acids were studied. Fluorescent amino acid conjugates were efficiently prepared and characterised. Photodeprotection of these compounds was carried out by irradiation at 300, 350 and 419 nm, the most suitable wavelength being 350 nm, on account of short irradiation times and good deprotection yields.

1. Introduction

In organic synthesis protecting groups are many times a laborious necessity, as a convenient and efficient synthesis, chemical stability towards different reagents and selective removal is required.[1] Photochemically releaseable groups have become an important tool in organic synthesis, biotechnology and cell biology, because cleavage only requires light, which is a very mild deprotection strategy that is usually orthogonal to chemical conditions, allowing the removal of protecting groups in sensitive molecules, otherwise incompatible with acidic or basic treatment.[2]

Fluorescent labelling allows easy and reliable detection of target compounds, both qualitatively and quantitatively with improved sensitivity and selectivity, and its application is well reported in many areas including amino acid and peptide chemistry.[3] Fluorescent photolabile protecting groups have advantages over other photolabile groups, because they can act as temporary fluorescent labels, allowing the visualisation of non-fluorescent systems, like most amino acid residues, during the course of organic reactions.

2-Oxobenzopyrans, trivially named as coumarins, represent one of the most widespread and interesting class of heterocyclic compounds. These oxygen heterocycles are the structural units of natural products and many exhibit diverse biological acivity with applications in pharmaceuticals, agrochemicals and insecticides.[4–9] 2-Oxobenzopyran derivatives have been reported as food additives, in cosmetics, as optical brightening agents, disperse fluorescent and laser dyes.[10–12] In addition, these compounds have also been suggested as photolabile protecting groups for biomolecules,[13–15] as well as other polycyclic aromatics such as anthraquinone, phenanthrene and pyrene.[16,17]

Taking these facts into consideration together with our research work related to the area of fluorescent heterocycles synthesis and also amino acid labelling,[18,19] we decided to investigate the possibility of using oxobenzo[*f*]benzopyrans as new fluorescent photocleavable protecting groups for the carboxylic function of organic molecules. Using amino acids as models, the synthesis and characterisation of new fluorescent amino acid conjugates were carried out. Absorption and emission properties of all compounds were measured and the results showed that these conjugates exhibited moderate to excellent fluorescence quantum yields and Stokes' shifts.

Photocleavage of these fully protected amino acids was achieved by using radiation of 300, 350 and 419 nm. The consumption of starting materials as well as the formation of the released amino acid was monitored by RP-HPLC and kinetic data were also obtained.

2. Results and discussion

Chloromethyl oxobenzo[*f*]benzopyrans **1a–c** were prepared through a Pechmann reaction of the corresponding 2-naphthol and its derivatives, and ethyl 4-chloroacetoacetate catalysed by sulfuric acid, at room temperature in good yields.[20] The fluorophores will be designated in this report by a three letter code for simplicity of naming the various amino acid

Keywords: Benzopyrans; Photocleavable groups; Protecting groups; Temporary labels; Fluorophores.
* Corresponding author. Tel.: +351 253 604 386; fax: +351 253 678 986; e-mail: msameiro@quimica.uminho.pt

0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.09.085

fluorescent derivatives (1-methylene-3-oxo-3*H*-benzo[*f*]-benzopyran, Obb, 9-hydroxy-1-methylene-3-oxo-3*H*-benzo-[*f*]benzopyran, Obh and 9-methoxy-1-methylene-3-oxo-3*H*-benzo[*f*]benzopyran, Obm).

In order to investigate the linkage of compounds **1a–c** to the carboxylic function of α-amino acids by an ester bond and to compare the influence of the substituent at the oxo-benzo[*f*]benzopyrans, *N*-benzyloxycarbonyl-L-phenylalanine, *Z*-Phe-OH (**2a**) was chosen as model. Derivatisation at the C-terminus of **2a** with heterocycles **1a–c** was carried out with potassium fluoride, in DMF, at room temperature (Scheme 1), yielding derivatives **3a–c**.

By comparing fluorescence data obtained for these derivatives, which will be discussed later, it was concluded that compound **1c** was the most fluorogenic reagent. Thus, using the same method reported above, heterocycle **1c** was reacted with *N*-benzyloxycarbonyl derivatives of glycine (**2b**), alanine (**2c**) and valine (**2d**) and also to *N*-*p*-toluenosulfonyl-phenylalanine (**2e**). After dry chromatography on silica gel, the corresponding fluorescent derivatives **3a–g** were obtained as solid materials in good to excellent yields (71–96%) (Table 1) and were characterised by elemental analysis or high resolution mass spectrometry, IR, ^{1}H and ^{13}C NMR spectroscopies.

The IR spectra of labelled amino acids showed bands due to stretching vibrations of the carbonyl groups from 1757 to 1619 cm^{-1}. ^{1}H NMR spectra showed signals of the amino acid residues, such as a multiplet (δ 4.29–4.82 ppm) or a doublet (δ 4.18 ppm, **3d**) for the α-CH, in addition to the protons of the heterocyclic moiety. In ^{13}C NMR signals of the carbonyl function were found at δ 155.58–156.90 ppm for the carbamate, at δ 159.84–160.10 ppm for C-3 of the heterocycle and at δ 169.4–172.31 ppm for the ester.

Electronic absorption and emission spectra of 10^{-5}–10^{-6} M solutions of compounds **1a–c** and **3a–g** in degassed absolute ethanol were measured; absorption and emission maxima, molar absorptivities and fluorescence quantum yields (Φ_F) are also reported (Table 2). The Φ_F were calculated using 9,10-diphenylanthracene as standard (Φ_F=0.95 in ethanol).[21] For the Φ_F 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 maxima of all compounds were located between 345 and 361 nm, with molar absorptivity ranging from 10,174 to 14,125 M^{-1} cm^{-1}. The wavelengths of maximum absorption obtained for these compounds showed a bathochromic shift when compared with other polycyclic aromatic photocleavable groups, namely 7-methoxycoumarin-4-ylmethoxycarbonyl (Mmoc, λ_{max} 343 nm),[22] anthraquinon-2-ylmethoxycarbonyl (Aqmoc, λ_{max} 327 nm),[16] pyren-1-ylmethoxycarbonyl (Pmoc, λ_{max} 323 nm)[17] and phenanthren-9-ylmethoxycarbonyl (Phmoc, λ_{max} 297 nm).[16] The wavelengths of maximum emission were found between 411 and 478 nm. Emission of compound **3c** was bathochromically shifted when compared to **3a**,**b**, the difference being 67 (**3c/3a**) and 22 nm (**3c/3b**) due to the substituent. Although there was not a significant variation in the maximum wavelengths of emission of compounds **1a–c** in their isolated or conjugated forms, they displayed low Φ_F in their isolated form (the highest value was 0.08, **1a**), which

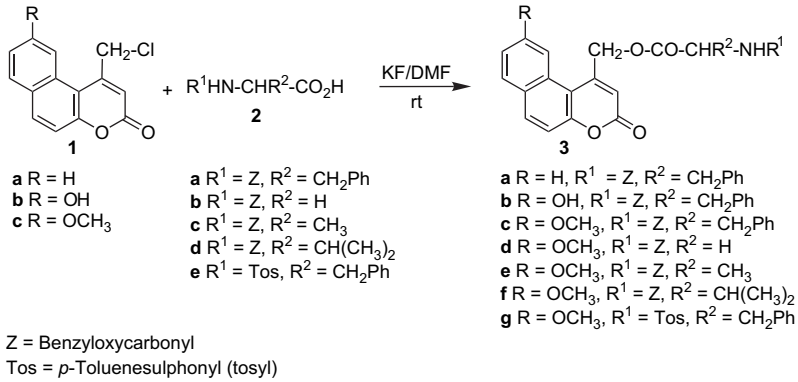
Table 1. Synthesis of compounds **3a–g**

	Compound	Yield (%)	Mp (°C)
3a	*Z*-Phe-Obb	96	127.8–129.8
3b	*Z*-Phe-Obh	81	189.6–190.7
3c	*Z*-Phe-Obm	71	180.8–182.1
3d	*Z*-Gly-Obm	86	181.6–184.0
3e	*Z*-Ala-Obm	83	132.8–134.0
3f	*Z*-Val-Obm	94	122.6–124.0
3g	Tos-Phe-Obm	82	184.8–186.6

Table 2. UV–vis and fluorescence data of compounds **1a**−**c** and **3a**−**g**

	Compound	UV λ_{max}[a] (ε)[b]	Fluorescence λ_{em}[a]	Fluorescence Φ_F	Stokes' shift[a]
1a	Obb-Cl	352 (11,449)	418	0.08±0.01	66
1b	Obh-Cl	361 (12,190)	462	0.02±0.002	101
1c	Obm-Cl	354 (12,826)	472	0.03±0.004	118
3a	*Z*-Phe-Obb	345 (14,125)	411	0.42±0.01	66
3b	*Z*-Phe-Obh	360 (10,174)	456	0.13±0.01	96
3c	*Z*-Phe-Obm	347 (12,075)	478	0.59±0.02	131
3d	*Z*-Gly-Obm	347 (11,436)	471	0.70±0.01	124
3e	*Z*-Ala-Obm	348 (11,640)	477	0.66±0.01	129
3f	*Z*-Val-Obm	348 (11,830)	478	0.58±0.02	130
3g	Tos-Phe-Obm	347 (12,883)	475	0.53±0.01	128

[a] Unit: nm.
[b] Unit: M^{-1} cm^{-1}.



Scheme 1. Synthesis of fluorescent conjugates **3a–g**.

increased upon reaction with the amino acids. All labelled amino acids **3a–g** exhibited moderate to excellent quantum yields ($0.13<\Phi_F<0.70$), compound **3b** having the lowest value probably due to the presence of H-bonds to the solvent, and Stokes' shift from 66 to 131 nm. By comparison of Φ_F of labelled *N*-benzyloxycarbonylphenylalanine **3a–c**, it was possible to see that derivative **3c** exhibited the highest value (0.59) and also the larger Stokes' shift (131 nm), which may be related to the higher electron-donating character of the methoxy substituent on the oxobenzopyran moiety **1c**. Considering these results, oxobenzo[*f*]benzopyrans are promising candidates for fluorescent labelling. In Figure 1, the fluorescence spectra of amino acid conjugates **3a**, **3b**, **3c** and **3g** are shown.

Since our main purpose is the investigation of the potential application of these fluorophores as photocleavable protecting groups in organic synthesis, we decided to evaluate the behaviour of the ester linkage between the fluorescent heterocycles synthesised and the amino acids to photocleavage conditions.

Fully protected phenylalanine derivatives, *Z*-Phe-Obb (**3a**), *Z*-Phe-Obh (**3b**), *Z*-Phe-Obm (**3c**) and Tos-Phe-Obm (**3g**) were used as representative models (Scheme 2). Solutions of the mentioned compounds in acetonitrile (ca. 1×10^{-5} M) were irradiated in a Rayonet RPR-100 reactor, at different wavelengths. As it is desirable to have short irradiation times at the highest irradiation wavelength possible if future bioapplications are to be considered, photolysis was carried out at 300, 350 and 419 nm, in order to determine the best cleavage conditions. The cleavage at different wavelengths was followed by reverse phase HPLC–UV detection.

The plots of peak area versus irradiation time were obtained for each compound, at the considered wavelengths. Peak areas were determined by HPLC and were the average of three runs.

When compounds **3a–c** and **3g** were irradiated at 350 nm, the time necessary for the consumption of the starting materials, until less than 5% of the initial area was detected, varied from 4 to 22 min (Table 3).

At this wavelength, the time cause of the reaction for compound **3b** was similar to that of 350 nm, whereas compounds **3a** and **3g** were photolysed about three times and two times faster, respectively; compound **3c** showed a slower cleavage.

As expected, irradiation at 419 nm resulted in much longer irradiation times for all compounds, with compound **3a** requiring a 29 h photolysis for the consumption of more than 95% of the starting material.

At the same time, the study of the stability of *Z*-Phe-OH (**2a**) and Tos-Phe-OH (**2e**) was carried out under the above reported photolysis conditions. HPLC studies showed that both *N*-blocking groups were stable to the tested conditions, no cleavage being detected. These results supported the fact that the disappearance of the starting materials (**3a**, **3b**, **3c** and **3g**) was associated with the cleavage of the ester linkage between the fluorophore and the C-terminus of the amino acid, as expected.

The formation of *N*-protected phenylalanine, as the expected photolysis product, was also followed by RP-HPLC. The yield of formation of compounds **2a** or **2e** was calculated on the basis of a calibration curve (concentration versus peak area), which was plotted with solutions of these phenylalanine derivatives of known concentration in acetonitrile. In case of compound **3g**, the photorelease yield of the expected product was 73% (300 and 419 nm) and 82% (350 nm). *N*-Protected phenylalanine **2a** was obtained from the photocleavage of compounds **3a–c** in yields ranging

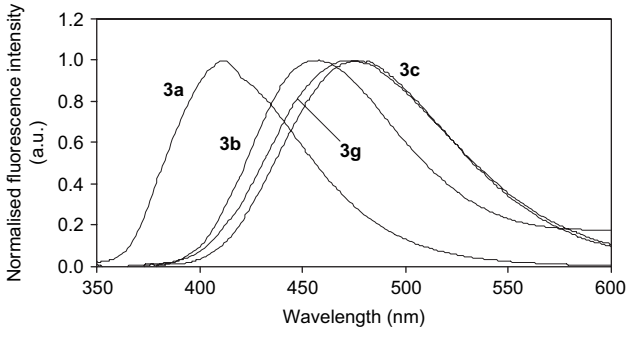
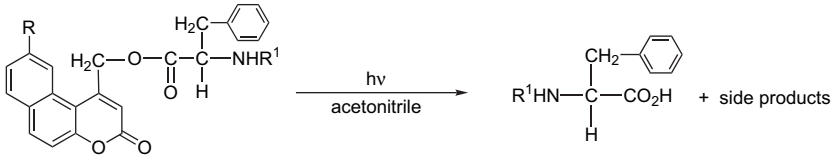


Figure 1. Normalised fluorescence spectra of compounds **3a–c** and **3g**.

Table 3. Photolysis data of compounds **3a–c** and **3g**

Compound		300 nm		350 nm		419 nm	
		Irr time[a]	Release[c]	Irr time[a]	Release[c]	Irr time[b]	Release[c]
3a	*Z*-Phe-Obb	7	79	22	80	29	78
3b	*Z*-Phe-Obh	4	82	4	90	2	92
3c	*Z*-Phe-Obm	12	75	8	81	8	86
3g	Tos-Phe-Obm	4	73	8	82	9	73

[a] Irradiation time (min).
[b] Irradiation time (h).
[c] Yield (%) of the released amino acid as determined by HPLC.



3a R = H, R^1 = Z, **b** R = OH, R^1 = Z
c R = OMe, R^1 = Z, **g** R = OCH_3, R^1 = Tos

2 a R^1 = Z
e R^1 = Tos

Scheme 2. Photocleavage of compounds **3a–c** and **3g**.

from 75 to 92%, the highest value in the case of compound **3b** at 419 nm.

From the obtained data, it was possible to see that the most suitable wavelength of irradiation was 350 nm for compounds **3b**, **3c** and **3g**. Although in the case of compound **3a** the irradiation time was longer at this wavelength, it is preferable to avoid the use of shorter wavelengths of irradiation. The results also indicated an influence of the substituent at position 9 of the heterocycle, as the substituted compounds **3b**, **3c** and **3g** required shorter irradiation times for equal percentage of consumption of the starting material, which was more evident when the wavelength of irradiation was 350 or 419 nm.

Based on HPLC data, the kinetic study of the photocleavage reactions was also carried out. For each compound, the plot of ln *A* versus irradiation time showed a linear correlation for the decrease of the starting material, which suggested a first order reaction. The observed values were calculated by the linear least squares methodology for a straight line (Table 4). Figure 2 summarises the behaviour of conjugates **3a–c** and **3g** at 350 nm.

From these results, it was possible to confirm that the wavelength of irradiation influenced the rate of the photocleavage, 350 nm being the selected wavelength on account of the short irradiation times and the potentially less damaging effect on biological systems. The substituent at the 9-position of the fluorophore was also important in this process, the presence of a hydroxyl group leading to a reduction of the irradiation time.

Although the main purpose of this work was to study the suitability of oxobenzo[*f*]benzopyrans as photocleavable groups for the carboxylic acid function of bifunctional molecules such as amino acids, we also considered their behaviour towards classical chemical cleavage. Therefore, stability tests were carried out using fluorescent *N*-*p*-toluenosulfonylphenylalanine, Tos-Phe-Obm (**3g**) as model. Compound **3g** was submitted to similar conditions to those usually required for cleavage of protecting groups during peptide synthesis, such us catalytic hydrogenation (Pd/C/1,4-cyclohexadiene), acidolysis at room temperature and reflux (TFA, 6 M HCl, aqueous HBr and HBr in acetic acid),[23] reduction with metals (Mg/MeOH)[24] and alkaline hydrolysis (1 M NaOH) (Table 5).

The results showed that under catalytic hydrogenation conjugate **3g** was cleaved, compounds Tos-Phe-OH (**2e**) and 1-methyl-9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran (Obm-H) (**4**) being isolated in low yields.

Table 4. Kinetic data of the photolysis studies of compounds **3a–c** and **3g**

Compound		300 nm		350 nm		419 nm	
		k^a	R^c	k^a	R^c	k^b	R^c
3a	*Z*-Phe-Obb	0.4587	0.9965	0.1175	0.9556	0.1063	0.9965
3b	*Z*-Phe-Obh	0.9416	0.9983	0.8310	0.9940	1.6883	0.9893
3c	*Z*-Phe-Obm	0.2569	0.9998	0.3684	0.9771	0.3841	0.9993
3g	Tos-Phe-Obm	0.8159	0.9980	0.4092	0.9992	0.3353	0.9995

[a] Rate constant (min^{-1}).
[b] Rate constant (h^{-1}).
[c] Correlation coefficient.

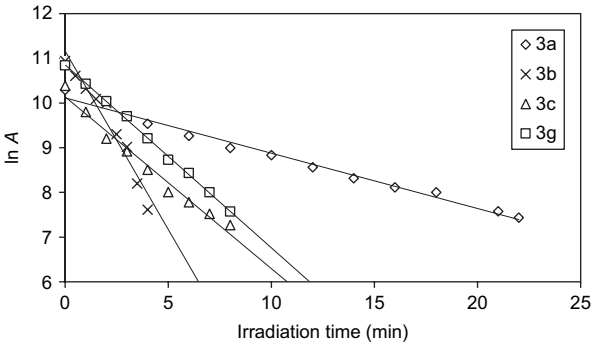


Figure 2. Plot of ln *A* versus irradiation time at 350 nm for compounds **3a–c** and **3g**.

Table 5. Stability tests/chemical cleavage of Tos-Phe-Obm (**3g**)

Cleavage	Time (h)	Yield (%)	
		Tos-Phe-Obm (**3g**)	Tos-Phe-OH (**2e**)
Pd/C/1,4-cyclohexadiene	6	36	11[a,b]
TFA (rt)	2.5	100	—
TFA (reflux)	8.5	100	—
6 M HCl (rt)	4.5	100	—
6 M HCl (reflux)	17	83	—
aq HBr (rt)	4.5	100	—
aq HBr (reflux)	5	—	57[a]
HBr/CH_3CO_2H (rt)[c]	4.5	90	—
HBr/CH_3CO_2H (reflux)[c]	5	—	68[a]
Mg/MeOH	3	—	56[a]
1 M NaOH	9	—	100

[a] Yield of isolated product by dry chromatography.
[b] Obm-H (**4**) was also obtained (25%).
[c] HBr/CH_3CO_2H (45% m/v).

Compound **3g** was stable under acidolysis conditions, at room temperature, and was quantitatively recovered (TFA, 6 M HCl and aqueous HBr) or in 90% yield (HBr in acetic acid). Considering acidolysis at reflux, the fully protected fluorescent conjugate (**3g**) was stable in TFA (9 h, 100% recovery) and 6 M HCl (17 h, 83% recovery). However, in HBr at reflux (5 h), cleavage of the ester linkage between the fluorophore and the amino acid occurred, the expected product **2e** being isolated in moderate yields (57%, aqueous HBr and 68%, HBr in acetic acid).

Reaction of compound **3g** with magnesium gave Tos-Phe-OH (**2e**) in 56%. The stability of tosyl group to this metal was verified with studies of the behaviour of Tos-Phe-OH (**2e**) in the same experimental conditions, which confirmed that it was stable, being recovered quantitatively. As expected, cleavage of the ester bond was achieved by treatment with base (1 M NaOH), resulting in quantitative isolation of compound **2e**.

From the results obtained in these chemical cleavage tests, it was possible to conclude that fluorophore **1c** had the appropriate behaviour to be considered also a suitable conventional protecting group for the carboxylic function of amino acids, in peptide synthesis. All labelled compounds were stable in prolonged storage at room temperature.

3. Conclusions

A series of fluorescent amino acid conjugates were synthesised in excellent yields by a straightforward procedure, between a chloromethylated fluorophore and the C-terminus of several *N*-protected amino acids, through an ester bond. The photophysical properties of these conjugates showed that oxobenzo[*f*]benzopyrans are good fluorogenic reagents for amino acid and peptide chemistry.

The photocleavage studies of the fluorescent phenylalanine derivatives with the three oxobenzo[*f*]benzopyrans showed that the rate of cleavage of the ester bond depended on the wavelength of irradiation as well as on the substituent at the 9-position of the heterocycle. The most suitable wavelength was 350 nm, allowing short irradiation times, which are convenient for possible future biological applications, for example, as caging groups.

In the absence of radiation, we also confirmed that these groups were efficiently chemically cleavable by soft alkaline hydrolysis or in moderate yields by acidolysis with HBr at reflux or with magnesium.

The results obtained, mainly their photophysical properties, allowed us to conclude that the considered functionalised heterocycles are potential candidates as fluorescent labels for biomolecules. Considering the efficient derivatisation reactions and also the good results of the photocleavage process, 1-chloromethyl-3-oxo-3*H*-benzo[*f*]benzopyrans **1a–c** could be used as fluorescent photocleavable protecting groups in organic synthesis. In addition, they can also be used as conventional protecting groups, quantitatively cleavable by basic hydrolysis (1 M NaOH, room temperature).

Bearing in mind the properties displayed by these compounds, further investigations into their applicability in biological systems, for example, as caging groups, will be carried out in the near future.

4. Experimental

4.1. General

All melting points were measured on a Gallenkamp melting point apparatus and were uncorrected. 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 spectrometry 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 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. *N*-(Benzyloxycarbonyl) phenylalanine (3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, *Z*-Phe-Obb (3a). To a solution of 1-chloromethyl-3-oxo-3*H*-benzo[*f*]benzopyran, Obb-Cl (**1a**) (0.106 g, 4.30×10^{-4} mol) in DMF (1.5 mL), potassium fluoride (0.076 g, 1.30×10^{-3} mol) and *Z*-Phe-OH (**2a**) were added with stirring at room temperature. The reaction mixture was maintained in these conditions for 25 h and monitored by TLC (ethyl acetate/*n*-hexane, 4:6). The precipitate was filtered and the remaining solution was evaporated until dryness. Purification of the residue by dry chromatography using ethyl acetate/*n*-hexane, 3:7 as the eluent, followed by recrystallisation from ethyl acetate/*n*-hexane, gave *Z*-Phe-Obb (**3a**) as a white solid (0.209 g, 96%). Mp=127.8–129.8 °C. TLC (ethyl acetate/*n*-hexane, 4:6): R_f=0.58. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=3.17 (d, *J*=6.3 Hz, 2H, β-CH_2 Phe), 4.62–4.82 (m, 1H, α-CH Phe), 5.02–5.20 (m, 2H, CH_2 *Z*), 5.30 (d, *J*=7.8 Hz, 1H, α-NH Phe), 5.60–5.76 (m, 2H, CH_2), 6.54 (s, 1H, H-2), 7.10–7.18 (m, 2H, 2×Ar-H Phe), 7.20–7.30 (m, 3H, 3×Ar-H Phe), 7.32–7.40 (m, 5H, 5×Ar-H *Z*), 7.49 (d, *J*=8.7 Hz, 1H, H-5), 7.59 (t, *J*=6.9 Hz, 1H, H-8), 7.67 (dt, *J*=8.3 and 1.5 Hz, 1H, H-9), 7.94 (dd, *J*=8.0 and 1.2 Hz, 1H, H-7), 8.01 (d, *J*=9.0 Hz, 1H, H-6), 8.08 (d, *J*=8.4 Hz, 1H, H-10). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=38.10 (β-CH_2 Phe), 55.17 (α-CH Phe), 64.94 (CH_2), 67.22 (CH_2 *Z*), 112.44 (C-4b), 113.78 (C-2), 117.78 (C-5), 124.54 (C-10), 125.74 (C-8), 127.44 (C-4 Phe), 128.18 (C-4 *Z*), 128.23 (C-3 and C-5 Phe), 128.49 (C-2 and C-6 *Z*), 128.61 (C-9), 128.80 (C-3 and C-5 *Z*), 128.97 (C-6b), 129.04 (C-2 and C-6 Phe), 129.92 (C-7), 131.24 (C-6a), 134.11 (C-6), 135.11 (C-1 Phe), 135.95 (C-1 *Z*), 149.85 (C-1), 154.86 (C-4a), 155.69 (CONH), 159.84 (C-3), 171.10 (CO_2CH_3). IR (KBr 1%, cm^{-1}): ν=3287, 3028, 2963, 2918, 2848, 1740, 1728, 1686, 1553, 1532, 1496, 1455, 1413, 1340, 1290, 1258, 1207, 1198, 1166, 1052, 1019, 822, 803. UV–vis (ethanol, nm): λ_{max} (ε)=345 (14,125 M^{-1} cm^{-1}). Anal. Calcd for $C_{31}H_{25}NO_6$ (507.52): C, 73.36; H, 4.97; N, 2.76. Found: C, 73.28; H, 4.98; N, 2.85.

4.1.2. *N*-(Benzyloxycarbonyl) phenylalanine (9-hydroxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, *Z*-Phe-Obh (3b). The product of reaction of 1-chloromethyl-9-hydroxy-3-oxo-3*H*-benzo[*f*]benzopyran, Obh-Cl (**1b**) (0.100 g, 3.84×10^{-4} mol), with *Z*-Phe-OH (0.115 g, 3.84×10^{-4} mol) (**2a**) was chromatographed using ethyl acetate/*n*-hexane, 3:7 as eluent, to give compound *Z*-Phe-Obh (**3b**) as a white solid (0.163 g, 81%). Mp=189.6–190.7 °C. TLC (acetate/*n*-hexane, 4:6): R_f=0.31. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=3.00–3.10 (m, 2H, β-CH_2 Phe), 4.40–4.54 (m, 1H, α-CH Phe), 4.95 (d, *J*=12.0 Hz, 1H, CH_2), 5.10–5.20 (m, 2H, CH_2 *Z*), 5.36 (d, *J*=5.1 Hz, 1H, α-NH Phe), 5.48 (d, *J*=11.7 Hz, 1H, CH_2), 6.26 (s, 1H, H-2), 7.14–7.24 (m, 2H, H-8 and 1×Ar-H Phe), 7.28 (d, *J*=8.7 Hz, 3H, H-5 and 2×Ar-H Phe), 7.32–7.42

(m, 7H, 2×Ar-H Phe and 5×Ar-H *Z*), 7.61 (d, *J*=1.8 Hz, 1H, H-10), 7.80 (d, *J*=8.7 Hz, 1H, H-7), 7.91 (d, *J*=9.0 Hz, 1H, H-6), 8.44 (s, 1H, OH). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=37.05 (β-CH_2 Phe), 56.26 (α-CH Phe), 66.66 (CH_2), 68.26 (CH_2 *Z*), 107.54 (C-10), 112.30 (C-4b), 114.39 (C-5), 117.79 (C-8), 118.68 (C-2), 125.93 (C-6a), 127.87 (1×Ar-C Phe), 128.39 (1×Ar-C *Z*), 128.65 (2×Ar-C *Z*), 128.96 (2×Ar-C Phe), 129.07 (2×Ar-C *Z*), 130.61 (C-6b), 131.50 (C-7), 134.15 (2×Ar-C Phe), 134.61 (C-6), 134.80 (C-1 Phe and C-1 *Z*), 148.10 (C-1), 155.78 (C-4a), 156.86 (CONH), 157.59 (C-9), 160.07 (C-3), 171.60 (CO_2CH_2). IR (Nujol, cm^{-1}): ν=3346, 3299, 2954, 2924, 2854, 1744, 1710, 1685, 1622, 1553, 1538, 1463, 1456, 1366, 1333, 1290, 1253, 1232, 1215, 1195, 1163, 1140, 1048, 1014, 987, 963, 883. UV–vis (ethanol, nm): λ_{max} (ε)=360 (10,174 $M^{-1}\,cm^{-1}$). HRMS (EI): calcd for $C_{31}H_{25}NO_7$ [M^+]: 523.1631; found: 523.1611.

4.1.3. *N*-(Benzyloxycarbonyl) phenylalanine (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, *Z*-Phe-Obm (3c). The product of reaction of 1-chloromethyl-9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran, Obm-Cl (**1c**) (0.104 g, 3.8×10^{-4} mol), with *Z*-Phe-OH (**2b**) (0.113 g, 3.8×10^{-4} mol) was chromatographed using ethyl acetate/*n*-hexane, 3:7 as the eluent, to give compound *Z*-Phe-Obm (**3c**) as a yellow solid (0.145 g, 71%). Mp=180.8–182.1 °C. TLC (acetate/*n*-hexane, 3:7): R_f=0.45. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=3.16 (d, *J*=6.6 Hz, 2H, β-CH_2 Phe), 3.95 (s, 3H, OCH_3), 4.72–4.84 (m, 1H, α-CH Phe), 5.02–5.16 (m, 2H, CH_2 *Z*), 5.28 (d *J*=8.1 Hz, 1H, α-NH Phe), 5.66 (s, 2H, CH_2), 6.50 (s, 1H, H-2), 7.08–7.16 (m, 2H, H-3 and H-5 Phe), 7.19–7.28 (m, 3H, H-2, H-4 and H-6 Phe), 7.30–7.40 (m, 7H, H-5, H-8 and 5×Ar-H *Z*), 7.42 (s, 1H, H-10), 7.85 (d, *J*=9.0 Hz, 1H, H-7), 7.93 (d, *J*=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=38.21 (β-CH_2 Phe), 55.17 (α-CH Phe), 55.44 (OCH_3), 64.96 (CH_2), 67.25 (CH_2 *Z*), 105.58 (C-10), 111.82 (C-4b), 113.62 (C-2), 115.28 (C-5), 116.69 (C-8), 126.34 (C-6a), 127.45 (C-4 Phe), 128.19 (C-4 *Z*), 128.26 (C-3 and C-5 Phe), 128.51 (C-2 and C-6 *Z*), 128.80 (C-3 and C-5 *Z*), 129.03 (C-2 and C-6 Phe), 130.51 (C-6b), 131.34 (C-7), 133.81 (C-6), 135.07 (C-1 Phe), 135.95 (C-1 *Z*), 149.64 (C-1), 155.58 (C-4a and CONH), 159.71 (C-9), 160.00 (C-3), 171.16 (CO_2CH_2). IR (Nujol, cm^{-1}): ν=3285, 2954, 2925, 2854, 1746, 1664, 1630, 1549, 1463, 1409, 1378, 1366, 1275, 1248, 1233, 1201, 1183, 1104, 1086, 1038, 1021. UV–vis (ethanol, nm): λ_{max} (ε)=347 (12,075 $M^{-1}\,cm^{-1}$). HRMS (EI): calcd for $C_{32}H_{27}NO_7$ [M^+]: 537.1788; found: 537.1798.

4.1.4. *N*-(Benzyloxycarbonyl) glycine (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, *Z*-Gly-Obm (3d). The product of reaction of Obm-Cl (**1c**) (0.100 g, 3.64×10^{-4} mol), with *Z*-Gly-OH (**2b**) (0.076 g, 3.64×10^{-4} mol) was chromatographed using ethyl acetate/*n*-hexane, 3:7 as the eluent, to give compound *Z*-Phe-Obm (**3c**) as a yellowish solid (0.140 g, 86%). Mp=181.6–184.0 °C. TLC (acetate/*n*-hexane, 4:6): R_f=0.51. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=3.97 (s, 3H, OCH_3), 4.18 (d, *J*=5.7 Hz, 2H, CH_2 Gly), 5.16 (s, 2H, CH_2 *Z*), 5.35 (br s, 1H, α-NH Gly), 5.77 (s, 2H, CH_2), 6.66 (s, 1H, H-2), 7.24 (dd, *J*=9.0 and 2.4 Hz, 1H, H-8), 7.30–7.40 (m, 6H, H-5 and 5×Ar-H *Z*), 7.42 (s, 1H, H-10), 7.84 (d, *J*=9.0 Hz, 1H, H-7), 7.93 (d, *J*=8.7 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ=42.79 (CH_2 Gly), 55.47 (OCH_3), 64.84 (CH_2), 67.36 (CH_2 *Z*), 105.54 (C-10), 111.77 (C-4b), 112.97 (C-2), 115.31 (C-5), 116.71 (C-8), 126.36 (C-6a), 128.17 (Ar-C *Z*), 128.28 (2×Ar-C *Z*), 128.54 (2×Ar-C *Z*), 130.52 (C-6b), 131.40 (C-7), 133.91 (C-6), 135.95 (C-1 *Z*), 150.20 (C-1), 155.59 (C-4a), 156.34 (CONH), 159.73 (C-9), 160.13 (C-3), 169.43 (CO_2CH_2). IR (Nujol, cm^{-1}): ν=3413, 2954, 2924, 2854, 1757, 1721, 1625, 1553, 1516, 1401, 1368, 1341, 1272, 1232, 1170, 1054, 1016. UV–vis (ethanol, nm): λ_{max} (ε)=347 (11,436 $M^{-1}\,cm^{-1}$). Anal. Calcd for $C_{25}H_{21}NO_7$ (447.43): C, 67.67; H, 5.02; N, 3.03. Found: C, 67.40; H, 5.01; N, 3.06.

4.1.5. *N*-(Benzyloxycarbonyl) alanine (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, *Z*-Ala-Obm (3e). The product of reaction of Obm-Cl (**1c**) (0.201 g, 7.32×10^{-4} mol), with *Z*-Ala-OH (**2c**) (0.183 g, 8.2×10^{-4} mol) was chromatographed using ethyl acetate/*n*-hexane mixtures of increased polarity as the eluent, to give compound *Z*-Ala-Obm (**3e**) as a white solid (0.280 g, 83%). Mp=132.8–134.0 °C. TLC (acetate/*n*-hexane, 1:1): R_f=0.45. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.52 (d, *J*=7.2 Hz, 3H, β-CH_3 Ala), 3.97 (s, 3H, OCH_3), 4.48–4.62 (m, 1H, α-CH Ala), 5.06–5.20 (m, 2H, CH_2 *Z*), 5.26 (d, *J*=7.2 Hz, 1H, α-NH Ala), 5.66–5.87 (m, 2H, CH_2), 6.67 (s, 1H, H-2), 7.24 (dd, *J*=9.0 and 2.1 Hz, 1H, H-8), 7.30–7.41 (m, 6H, H-5 and 5×Ar-H), 7.45 (s, 1H, H-10), 7.86 (d, *J*=9.0 Hz, 1H, H-7), 7.95 (d, *J*=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=18.14 (β-CH_3 Ala), 49.74 (α-CH Ala), 55.42 (OCH_3), 64.85 (CH_2), 67.10 (CH_2 *Z*), 105.62 (C-10), 111.66 (C-4b), 112.81 (C-2), 115.21 (C-5), 116.52 (C-8), 126.26 (C-6a), 128.12 (1×Ar-C *Z*), 128.17 (1×Ar-C *Z*), 128.46 (1×Ar-C *Z*), 130.44 (C-6b), 131.32 (C-7), 133.79 (C-6), 135.98 (C-1 *Z*), 150.24 (C-1), 155.48 (C-4a), 155.66 (CONH), 159.63 (C-9), 160.05 (C-3), 172.31 (CO_2CH_2). IR (Nujol, cm^{-1}): ν=3422, 3335, 3065, 2958, 2933, 1732, 1719, 1619, 1543, 1518, 1449, 1418, 1355, 1331, 1249, 1230, 1211, 1168, 1105, 1067, 1024. UV–vis (ethanol, nm): λ_{max} (ε)=348 (11,640 $M^{-1}\,cm^{-1}$). Anal. Calcd for $C_{26}H_{23}NO_7$ (461.45): C, 67.67; H, 5.02; N, 3.03. Found: C, 67.40; H, 5.01; N, 3.06.

4.1.6. *N*-(Benzyloxycarbonyl) valine (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, *Z*-Val-Obm (3f). The product of reaction of Obm-Cl (**1c**) (0.104 g, 3.8×10^{-4} mol), with *Z*-Val-OH (**2d**) (0.070 g, 2.8×10^{-4} mol) was chromatographed using ethyl acetate/*n*-hexane mixtures of increasing polarity as the eluent, to give compound *Z*-Val-Obm (**2f**) as a white solid (0.129 g, 94%). Mp=122.6–124.0 °C. TLC (chloroform/methanol, 50:0.5): R_f=0.48. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.95 (d, *J*=7.2 Hz, 3H, γ-CH_3 Val), 1.04 (d, *J*=6.9 Hz, 3H, γ-CH_3 Val), 2.20–2.35 (m, 1H, β-CH Val), 3.98 (s, 3H, OCH_3), 4.40–4.50 (m, 1H, α-CH Val), 5.13 (s, 2H, CH_2 *Z*), 5.25 (d, *J*=8.4 Hz, 1H, α-NH Val), 5.76 (d, *J*=3.9 Hz, 2H, CH_2), 6.70 (s, 1H, H-2), 7.25 (dd, *J*=7.8 and 2.4 Hz, 1H, H-8), 7.30–7.42 (m, 6H, H-5 and 5×Ar-H *Z*), 7.47 (s, 1H, H-10), 7.86 (d, *J*=9.0 Hz, 1H, H-7), 7.95 (d, *J*=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=17.45 (γ-CH_3 Val), 19.17 (γ-CH_3 Val), 30.96 (β-CH Val), 55.43 (OCH_3), 59.28 (α-CH Val), 64.81 (CH_2), 67.25 (CH_2 *Z*), 105.67 (C-10), 111.75 (C-4b), 113.08 (C-2), 115.25 (C-5),

116.58 (C-8), 126.31 (C-6a), 128.17 (1×Ar-C *Z*), 128.22 (2×Ar-C *Z*), 128.50 (2×Ar-C *Z*), 130.49 (C-6b), 131.34 (C-7), 133.82 (C-6), 135.99 (C-1 *Z*), 150.18 (C-1), 155.54 (C-4a), 156.25 (CONH), 159.70 (C-9), 160.07 (C-3), 171.51 (*C*O_2CH_2). IR (KBr 1%, cm^{-1}) ν=3391, 2966, 2928, 1731, 1721, 1625, 1553, 1520, 1456, 1426, 1351, 1306, 1275, 1232, 1180, 1164, 1098, 1059, 1025. UV–vis (ethanol, nm): λ_{max} (ε)=348 (11,838 $M^{-1}\,cm^{-1}$). HRMS (EI): calcd for $C_{28}H_{27}NO_7$ [M^+]: 489.1788; found: 489.1790.

4.1.7. *N*-(*p*-Toluenesulfonyl) phenylalanine (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, Tos-Phe-Obm (3g). The product of reaction of Obm-Cl (**1c**) (0.060 g, 2.18×10^{-4} mol), with Tos-Phe-OH (**2e**) (0.069 g, 2.18×10^{-4} mol) was chromatographed using ethyl acetate/*n*-hexane, 3:7 as the eluent, to give compound Tos-Phe-Obm (**3g**) as a yellow solid (0.100 g, 82%). Mp=184.8–186.6 °C. TLC (acetate/*n*-hexane, 4:6): R_f=0.45. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.27 (s, 3H, CH_3), 3.08 (d, *J*=6.9 Hz, 2H, β-CH_2 Phe), 3.93 (3H, s, OCH_3), 4.28–4.40 (m, 1H, α-CH Phe), 5.30–5.53 (m, 3H, CH_2 and α-NH Phe), 6.39 (s, 1H, H-2), 7.02–7.09 (m, 2H, H-3 and H-5 Phe), 7.13–7.19 (m, 5H, H-3 and H-5 Tos, H-2, H-4 and H-6 Phe), 7.23 (dd, *J*=8.7 and 2.4 Hz, 1H, H-8), 7.27–7.32 (m, 2H, H-5 and H-10), 7.61 (d, *J*=8.4 Hz, 2H, H-2 and H-6 Tos), 7.83 (d, *J*=8.7 Hz, 1H, H-7), 7.90 (d, *J*=9.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=21.30 (CH_3 Tos), 39.24 (β-CH_2 Phe), 55.45 (OCH_3), 57.07 (α-CH Phe), 64.88 (CH_2), 105.71 (C-10), 111.64 (C-4b), 113.25 (C-2), 115.20 (C-5), 116.44 (C-8), 126.29 (C-6a), 127.10 (C-2 and C-6 Tos), 127.43 (C-4 Phe), 128.69 (C-3 and C-5 Phe), 129.13 (C-2 and C-6 Phe), 129.60 (C-3 and C-5 Tos), 130.35 (C-6b), 131.37 (C-7), 133.82 (C-6), 134.55 (C-1 Phe), 136.51 (C-1 Tos), 143.73 (C-4 Tos), 149.39 (C-1), 155.45 (C-4a), 159.66 (C-9), 159.98 (C-3), 170.55 (*C*O_2CH_2). IR (KBr 1%, cm^{-1}): ν=3434, 2921, 2846, 1707, 1625, 1550, 1506, 1443, 1349, 1224, 1161, 1080, 1011. UV–vis (ethanol, nm): λ_{max} (ε)=347 (12,883 $M^{-1}\,cm^{-1}$). HRMS (EI): calcd for $C_{31}H_{27}NO_7S$ [M^+]: 557.1508; found: 557.1519.

4.2. General photolysis procedure

A 1×10^{-5} M acetonitrile solution of the compound to be tested (20 mL) was placed in a quartz tube and irradiated in a Rayonet RPR-100 chamber reactor with 10 lamps of different wavelength (300, 350 and 419 nm, 14 W each). Aliquots were taken at regular intervals and analysed by reversed phase HPLC using a Licrospher 100 RP18 (5 μm) column and a system composed by a Jasco PU-980 pump, a UV–vis Shimadzu SPD-GAV detector and a Shimadzu C-RGA Chromatopac register. The eluent was acetonitrile/water, 3:1 (eluent A) or acetonitrile/water, 3:1 with 0.1% TFA (eluent B), previously filtered through a Milipore, type HN 0.45 μm filter and degassed by ultra-sound for 30 min.

The chromatograms were traced by detecting UV absorption (**3a**, λ_{det} 347 nm, flow 1.2 mL min^{-1}, retention time—t_R 4.9 min; **3b**, λ_{det} 360 nm, flow 0.8 mL min^{-1}, t_R 5.2 min; **3c**, λ_{det} 347 nm, flow 1.2 mL min^{-1}, t_R 5.1 min; **3d**, λ_{det} 347 nm, flow 1.0 mL min^{-1}, t_R 5.5 min; **2a**, λ_{det} 240 nm, flow 0.8 mL min^{-1}, t_R 3.3 min; **2e**, λ_{det} 240 nm, flow 0.8 mL min^{-1}, t_R 3.1 min), using eluent A for compounds **3a–c** and **3g** and eluent B for compounds **2a** and **2e**.

The yield of photorelease was calculated by comparison of the HPLC trace (peak area) of the released amino acid with the corresponding standard calibration curve (concentration vs peak area).

4.3. Stability tests with Tos-Phe-Obm (3g)

4.3.1. Catalytic hydrogenation. A suspension of Tos-Phe-Obm (**3g**) (2.90×10^{-2} g, 5.33×10^{-5} mol) in methanol (1.0 mL) and 1,4-cyclohexadiene (1.35×10^{-2} mL, 1.40×10^{-4} mol) was mixed with 10% palladium on charcoal catalyst (1.05×10^{-2} g), and refluxed for 6 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 oily solid (0.185 g, 11%). ^{1}H NMR was well compared with Tos-Phe-OH (**2e**). 1-Methyl-9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran, Obm-*H* (**4**) (3.20×10^{-3} g, 25%) and starting material (**3g**) (1.07×10^{-2} g, 36%) were also isolated.

4.3.1.1. 1-Methyl-9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran, Obm-*H* (4). TLC (ethyl acetate/*n*-hexane, 6:4): R_f=0.63. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.95 (s, 3H, CH_3), 3.98 (s, 3H, OCH_3), 6.36 (s, 1H, H-2), 7.23 (dd, *J*=8.7 and 2.4 Hz, 1H, H-8), 7.34 (d, *J*=8.7 Hz, 1H, H-5), 7.84 (d, *J*=8.7 Hz, 1H, H-7), 7.91 (d, *J*=9.0 Hz, 1H, H-6), 7.95 (d, *J*=2.4 Hz, 1H, H-10). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=26.24 (CH_3), 55.42 (OCH_3), 106.35 (C-10), 113.77 (C-4b), 115.40 (C-5), 116.09 (C-2), 116.09 (C-8), 126.47 (C-6a), 131.10 (C-7), 131.73 (C-6b), 133.36 (C-6), 154.02 (C-1), 155.37 (C-4a), 159.11 (C-9), 160.47 (C-3). IR (neat, cm^{-1}): ν=3414, 2918, 2848, 1716, 1623, 1552, 1515, 1455, 1356, 1261, 1228, 1093, 1018, 933 cm^{-1}. HRMS (EI): calcd for $C_{15}H_{12}O_3$ [M^+]: 240.0786; found: 240.0777.

4.3.2. Acidolysis with trifluoracetic acid

(a) To the fully protected amino acid Tos-Phe-Obm (**3g**) (3.00×10^{-2} g, 5.39×10^{-5} mol) were added 0.60 mL of trifluoracetic acid with rapid stirring, at room temperature, over 2.5 h. Evaporation under reduced pressure gave a yellow solid (3.00×10^{-2} g, 100%). ^{1}H NMR confirmed the structure of the starting material.

(b) A solution of Tos-Phe-Obm (**3g**) (2.80×10^{-2} g, 4.97×10^{-5} mol) in TFA (6 mL) was refluxed for 9 h. Evaporation under reduced pressure gave a white-greenish solid (2.80×10^{-2} g, 100%). ^{1}H NMR confirmed the structure of the starting material.

4.3.3. Acidolysis with hydrochloric acid

(a) To the fully protected amino acid Tos-Phe-Obm (**3g**) (2.10×10^{-2} g, 3.77×10^{-5} mol), was added 6 M HCl (0.40 mL) under rapid stirring, at room temperature, over 4.5 h. Evaporation under reduced pressure gave a white solid (2.10×10^{-2} g; 100%). ^{1}H NMR confirmed the structure of the starting material.

(b) A solution of Tos-Phe-Obm (**3g**) (2.10×10^{-2} g; 3.77×10^{-5} mol) in 6 M HCl (0.40 mL) was refluxed

for 17 h. Evaporation under reduced pressure gave a white solid (1.74×10^{-3} g, 83%). ^{1}H NMR confirmed the structure of the starting material.

4.3.4. Acidolysis with hydrobromic acid

(a) To the fully protected amino acid Tos-Phe-Obm (**3g**) (2.20×10^{-2} g, 3.91×10^{-5} mol) was added aqueous HBr (0.2 mL), under rapid stirring over 4.5 h. Evaporation under reduced pressure gave a white solid (2.20×10^{-2} g, 100%). ^{1}H NMR confirmed the structure of the starting material.

(b) A solution of Tos-Phe-Obm (**3g**) (2.20×10^{-2} g, 3.86×10^{-5} mol) in aqueous HBr (0.2 mL) was refluxed for 5 h. Evaporation under reduced pressure, followed by dry chromatography with ethyl acetate/*n*-hexane mixtures of increasing polarity, gave Tos-Phe-OH (**2e**) as a yellow oil (6.80×10^{-3} g, 57%). ^{1}H and ^{13}C NMR well compared with an original sample.

(c) To the fully protected amino acid Tos-Phe-Obm (**3g**) (2.00×10^{-3} g, 3.59×10^{-5} mol), was added a solution of HBr in CH_3CO_2H (45% m/v) (0.008 mL), under rapid stirring over 4.5 h. Evaporation under reduced pressure gave a yellowish white solid (1.80×10^{-2} g, 90%). ^{1}H NMR confirmed the structure of the starting material.

(d) A solution of Tos-Phe-Obm (**3g**) 2.00×10^{-2} g; 3.59×10^{-5} mol), in a solution of HBr in CH_3CO_2H (45% m/v) (0.008 mL), was refluxed for 5 h. Evaporation under reduced pressure followed by dry chromatography ethyl acetate/*n*-hexane, mixtures of increasing polarity, gave Tos-Phe-OH (**2e**) (7.80×10^{-3} g, 68%) as a colourless oil. ^{1}H NMR compared well with an original sample.

4.3.5. Reduction with Mg/MeOH

(a) To a solution of Tos-Phe-Obm (**2g**) (6.00×10^{-2} g, 1.08×10^{-4} mol), in dry methanol (2 mL) magnesium powder (3.90×10^{-2} g, 1.62×10^{-3} mol) was added and the resulting mixture was sonicated for 3 h, at room temperature. The process was monitored by TLC (ethyl acetate/*n*-hexane, 6:4) until all compounds were reacted. The reaction was quenched by 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 Tos-Phe-OH (**2e**) as an off-white oil (1.20×10^{-2} g, 56%). ^{1}H NMR compared well with an original sample.

(b) Starting with Tos-Phe-OH (**2e**) (5.90×10^{-2} g, 1.88×10^{-4} mol), in dry methanol (2 mL) magnesium powder (4.60×10^{-2} g, 1.88×10^{-3} mol) was added and the resulting mixture was sonicated for 3 h, at room temperature. Treatment by following the procedure described above gave a white solid (5.00×10^{-2} g, 84%). ^{1}H NMR confirmed the structure of the compound.

4.3.6. Alkaline hydrolysis. To the fully protected amino acid Tos-Phe-Obm (**3g**) (3.10×10^{-2} g, 5.47×10^{-5} mol), in 1,4-dioxane (2 mL) 1 M NaOH (0.220 mL, 2.19×10^{-4} mol) was added at low temperature. The solution was stirred at 0 °C for 9 h and acidified to pH 3 with 1 M $KHSO_4$. After extraction with ethyl acetate and evaporation of the solvent, Tos-Phe-OH (**2g**) was obtained as an orange solid (4.10×10^{-2} g, 100%). ^{1}H NMR compared well with an original sample.

Acknowledgements

We thank the Fundação para a Ciência e Tecnologia (Portugal) for its financial support to the Centro de Química (Universidade do Minho).

References and notes

1. Kocienski, P. J. *Protecting Groups*; Enders, D., Noyori, R., Trost, B. M., Eds.; Georg Thieme: Stuttgart, 1994.
2. Pelliccioli, A. P.; Wizz, J. *Photochem. Photobiol. Sci.* **2002**, *1*, 441.
3. Haugland, R. P. *Handbook of Fluorescent Probes and Research Products*, 9th ed.; Molecular Probes: Eugene, OR, 2002.
4. Madhavan, G. R.; Balraju, V.; Mallesham, B.; Chaknabarti, R.; Lohray, V. B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2547.
5. Cravotto, G.; Nano, G. M.; Palmisano, G.; Tagliapietra, S. *Tetrahedron: Asymmetry* **2001**, *12*, 707.
6. Wang, C. J.; Hsieh, Y. J.; Chu, C. Y.; Lin, Y. L.; Tseng, T. H. *Cancer Lett.* **2002**, *183*, 163.
7. Kirkiacharian, S.; Thuy, D. T.; Sicsic, S.; Bakhchinian, R.; Kurkjian, R.; Tonnaire, T. *Il Farmaco* **2002**, *57*, 703.
8. Oyamada, J.; Jia, C.; Fujiwara, Y.; Kitamura, T. *Chem. Lett.* **2002**, 380.
9. Murray, R. D. H.; Medez, J.; Brown, S. A. *The Natural Coumarins: Occurrence, Chemistry and Biochemistry*; Wiley: New York, 1982.
10. Siegrist, A. E.; Hefti, H.; Meyer, H. R.; Schmidt, E. *Rev. Prog. Colour.* **1987**, *17*, 39.
11. Valizadeh, H.; Shockravi, A. *Tetrahedron Lett.* **2005**, *46*, 3501 and cited references.
12. Christie, R. M.; Lui, C.-H. *Dyes Pigments* **1999**, *42*, 85 and cited references.
13. Lu, M.; Fedoryak, O. D.; Moister, B. R.; Dore, T. M. *Org. Lett.* **2003**, *5*, 2119.
14. Susuki, A. Z.; Watanable, T.; Kawamoto, M.; Nishiyama, K.; Yamashita, H.; Iwamura, M.; Furuta, T. *Org. Lett.* **2003**, *5*, 4867.
15. Furuta, T.; Wang, S. S.-H.; Dantzker, J. L.; Dore, T. M.; Bybee, W. J.; Callaway, E. M.; Denk, W.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1193.
16. Furuta, T.; Hirayama, Y.; Iwamura, M. *Org. Lett.* **2001**, *3*, 1809.
17. Iwamura, M.; Hodota, C.; Ishibashi, M. *Synlett* **1991**, 35.
18. Frade, V. H. J.; Gonçalves, M. S. T.; Moura, J. C. V. P. *Tetrahedron Lett.* **2005**, *46*, 4949.
19. Piloto, A. M.; Fonseca, A. S. C.; Costa, S. P. G.; Gonçalves, M. S. T. *Tetrahedron* **2006**, *62*, 9258.
20. Tao, Z.-F.; Qian, X.; Fan, M. *Tetrahedron* **1997**, *53*, 13329.
21. Morris, J. V.; Mahaney, M. A.; Huber, J. R. *J. Phys. Chem.* **1976**, *80*, 969.
22. Schade, B.; Hagen, V.; Schmidt, R.; Herbrich, R.; Krause, E.; Eckardt, T.; Bendig, J. *J. Org. Chem.* **1999**, *64*, 9109.
23. Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer: Berlin, 1984.
24. Maia, H. L. S.; Monteiro, L. S.; Sebastião, J. *Eur. J. Org. Chem.* **2001**, 1967.