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ORIGINAL ARTICLE

Photorelease of amino acids from novel thioxobenzo[*f*]benzopyran ester conjugates

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Abstract Aiming at the enhancement of the performance of (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester as photocleavable protecting group for the carboxylic acid function at long-wavelengths, 9-methoxy-3-thioxo-3*H*-benzo[*f*]benzopyran-L-valine and L-phenylalanine model conjugates were prepared through a thionation reaction of the corresponding oxo-benzobenzopyrans. These thioxobenzobenzopyran derivatives were subjected to photocleavage reactions in the same conditions as the parent oxo-benzobenzopyrans at different wavelengths of irradiation, and photocleavage data were obtained. It was found that the exchange of the carbonyl by a thiocarbonyl group enhanced the performance of the heterocyclic protecting group at 419 nm by improving the photolysis rates, making it an appropriate group for practical applications at long wavelengths.

Keywords Thioxobenzobenzopyran · Benzocoumarin · Amino acids · Photolabile protecting groups

Introduction

The choice of specific protecting groups remains of crucial importance in the success of many steps of organic synthesis and manipulation of polyfunctional molecules, since they prevent the formation of undesired side products and reactions (Isidro-Llobet et al. 2009).

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Photoremovable protecting groups (PRPGs) exhibit numerous advantages, such as the relatively soft conditions required for their deprotection and orthogonality with respect to acid- or base-sensitive groups (Papageorgiou et al. 1999; Pelliccioli and Wirz 2002; Bochet 2002; Singh and Khade 2005a; Ma et al. 2005). PRPGs have become broadly reported for various functional groups, including carboxylic acids (Bochet 2000; Schaper et al. 2002; Jana et al. 2010), amines (Peng et al. 1997; Fonseca et al. 2007), alcohols (Loudwig and Goeldner 2001), phosphates (Dinkel et al. 2003; Dinkel and Schultz 2003), aldehydes and ketones (Wang et al. 2007; Yu et al. 2007) for convenient and controlled release of molecules in a variety of environments, including in materials science (Abelson et al. 1998; Bochet 2002), the caging and release of biologically significant compounds (Givens et al. 2000; Grewer et al. 2000; Pelliccioli and Wirz 2002; Bochet 2002; Singh and Khade 2005b; Mayer and Heckel 2006; Li et al. 2010a, b), and in synthetic organic chemistry (Pillai 1980; Greene and Wuts 1999).

The design and application of polyaromatic compounds, as well as oxygen and nitrogen polycyclic heterocycles as novel photocleavable protecting groups for the carboxylic acid and amine functions of amino acids is part of our current research interests. Carboxylic acid containing molecules (amino acids, including neurotransmitters) have been effectively protected by a variety of PRPGs, such as 1-pyrenylmethyl (Fernandes et al. 2007, 2008a), quinolones (Fonseca et al. 2010), benzoxazoles (Soares et al. 2010a), as well as 2-oxo-benzopyrans (trivially known as coumarins) (Fonseca et al. 2007, 2010), and 2-oxo-benzobenzopyrans (benzocoumarins, Piloto et al. 2006; Fernandes et al. 2008a, b; Soares et al. 2010b). Their behaviour towards photocleavage under UV irradiation was studied at different wavelengths, in simulated physiological

environment in mixtures of aqueous buffer and different organic solvents. In studies involving biomolecules, photocleavage at 350 nm or longer wavelengths is preferable, as it avoids cell damage due to short-wavelength light. According to recent results reported by us, 1-chloromethyl-9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran is a suitable photocleavable protecting group for carboxylic acids, with release at 350 nm occurring efficiently in short times (Fernandes et al. 2008a, b). However, photocleavage at longer wavelengths, namely at 419 nm usually resulted in a large increase of the irradiation time, with a reduction of the scope of its applications at this wavelength.

Considering these facts, we now report the synthesis of novel ester conjugates of 9-methoxy-3-thioxo-3*H*-benzo[*f*]benzopyran-L-valine and L-phenylalanine obtained by a thionation reaction of the 3-oxo-3*H*-benzo[*f*]benzopyran bioconjugates, and compare their behaviour towards irradiation with the parent compounds. To the best of our knowledge, the use of thioxo derivatives as photocleavable protecting groups has not been previously reported. Photocleavage studies were carried out in a photochemical reactor under irradiation at 350 and 419 nm, and kinetic data was obtained.

Experimental section

General

All melting points were measured on a Stuart SMP3 melting point apparatus and are 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 BOMEM MB 104 spectrophotometer using KBr discs. UV/visible absorption spectra (200–700 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. NMR spectra were obtained on a Varian Unity Plus Spectrometer at an operating frequency of 300 MHz for ^{1}H NMR and 75.4 MHz for ^{13}C NMR or 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°C. All chemical shifts are given in ppm using δ_H $Me_4Si = 0$ 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. Mass spectrometry analyses were performed at the "C.A.C.T.I.-Unidad de Espectrometria de Masas", at University of Vigo, Spain. Fluorescence spectra were collected using a FluoroMax-4 spectrofluorometer. Photolyses were 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. All reagents were used as received.

Synthesis of *N*-(benzyloxycarbonyl)-L-valine (9-methoxy-3-thioxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, Z-Val-OTba **2a** Lawesson's reagent (0.231 g, 5.7×10^{-4} mol) was added to a solution of *N*-(benzyloxycarbonyl)-L-valine (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, Z-Val-OBba **1a** in toluene (5 mL), with stirring, at room temperature. The reaction mixture was refluxed for 48 h and the process was followed by TLC (ethyl acetate/*n*-hexane, 2:8). The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using dichloromethane/*n*-hexane, with mixtures of increasing polarity as eluent. Compound **2a** was obtained as an orange solid (0.081 g, 55%). mp = 131.7–132.0°C. TLC (ethyl acetate/*n*-hexane, 2:8): $R_f = 0.85$. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta_H = 0.91$ (d, $J = 6.8$ Hz, 3 H, γ-CH_3 Val), 1.04 (d, $J = 6.8$ Hz, 3 H, γ-CH_3 Val), 2.20-2.30 (m, 1 H, β-CH Val), 3.96 (s, 3 H, OCH_3), 4.40–4.50 (m, 1 H, α-CH Val), 5.13 (s, 2 H, CH_2 Z), 5.27 (d, $J = 8.8$ Hz, 1 H, α-NH Val), 5.60–5.74 (m, 2 H, CH_2), 7.26 (dd, $J = 8.8$ and 2.4 Hz, 1 H, H-8), 7.30–7.40 (m, 5 H, 5× Ar–H Z), 7.45 (s, 1 H, H-2), 7.48 (d, $J = 7.2$ Hz, 1 H, H-5), 7.49 (s, 1 H, H-10), 7.85 (d, $J = 8.8$ Hz, 1 H, H-7), 7.96 (d, $J = 8.8$ Hz, 1 H, H-6). ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta_C = 17.44$ (γ-CH_3 Val), 19.21 (γ-CH_3 Val), 30.96 (β-CH Val), 55.51 (OCH_3), 59.26 (α-CH Val), 64.62 (CH_2), 67.28 (CH_2 Z), 106.25 (C-10), 114.21 (C-4b), 114.93 (C-5), 117.21 (C-8), 126.60 (C-6a), 127.50 (C-2), 128.19 (1× Ar–C Z), 128.24 (2× Ar–C Z), 128.52 (2× Ar–C Z), 130.24 (C-6b), 131.46 (C-7), 134.36 (C-6), 136.02 (C-1 Z), 141.32 (C-1), 156.24 (CONH), 158.79 (C-4a), 159.96 (C-9), 171.55 (CO_2CH_2), 195.13 (C-3). IR (KBr 1%, cm^{-1}): ν = 3,339, 2,936, 2,964, 1,720, 1,623, 1,606, 1,591, 1,538, 1,455, 1,444, 1,432, 1,363, 1,346, 1,296, 1,230, 1,217, 1,181, 1,139, 1,096, 1,052, 1,026, 1,009, 985, 838. HRMS (ESI): calcd for $C_{28}H_{28}NO_6S$ [M^++H]: 506.16318; found: 506.16317.

Synthesis of *N*-(benzyloxycarbonyl)-L-phenylalanine (9-methoxy-3-thioxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, Z-Phe-OTba **2b** Starting from Lawesson's reagent (0.120 g, 2.97×10^{-4} mol), *N*-(benzyloxycarbonyl)-L-phenylalanine (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, Z-Phe-OBba **1b** and toluene (5 mL), following the same procedure as described for **2a**, compound **2b** was obtained as a yellow solid (0.035 g, 42%). mp = 155.8–156.9°C. TLC (ethyl acetate/*n*-hexane, 2:8):

$R_f = 0.87$. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta_H = 3.16$ (2 H, d, $J = 6.4$ Hz, β-CH_2 Phe), 3.94 (3 H, s, OCH_3), 4.70–4.85 (1 H, m, α-CH Phe), 5.05–5.20 (m, 2 H, CH_2 Z), 5.30 (d, $J = 5.6$ Hz, 1 H, α-NH Phe), 5.56 (s, 2 H, CH_2), 7.08–7.14 (m, 2 H, 1 × Ar–H Phe and H-2), 7.15–7.23 (m, 4 H, 4× Ar–H Phe), 7.27 (dd, $J = 9.2$ and 2.4 Hz, 1 H, H-8), 7.30–7.38 (m, 5 H, 5× Ar–H Z), 7.42–7.47 (m, 2 H, H-5 and H-10), 7.84 (d, $J = 8.8$ Hz, 1 H, H-7), 7.95 (d, $J = 8.8$ Hz, 1 H, H-6). ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta_C = 38.18$ (β-CH_2 Phe), 55.11 (α-CH Phe), 55.47 (OCH_3), 64.71 (CH_2), 67.22 (CH_2 Z), 106.07 (C-10), 114.20 (C-4b), 114.85 (C-5), 117.26 (C-8), 126.53 (C-6a), 127.39 (1× Ar–C Phe), 127.98 (C-2), 128.13 (1× Ar–C Z), 128.20 (2× Ar–C Phe), 128.47 (2× Ar–C Z), 128.76 (2× Ar–C Phe), 128.98 (2× Ar–C Phe), 130.17 (C-6b), 131.38 (C-7), 134.27 (C-6), 135.02 (C-1 Phe), 135.95 (C-1 Z), 140.66 (C-1), 155.64 (CONH), 158.73 (C-4a), 159.90 (C-9), 171.16 (CO_2CH_2), 195.03 (C-3). IR (KBr 1%, cm^{-1}): ν = 3,330, 3,063, 3,030, 2,934, 1,722, 1,623, 1,606, 1,592, 1,538, 1,520, 1,498, 1,455, 1,444, 1,432, 1,362, 1,343, 1,295, 1,230, 1,216, 1,178, 1,139, 1,096, 1,056, 1,027, 1,009, 838. HRMS (ESI): calcd for $C_{32}H_{28}NO_6S$ [M^++H]: 554.16318; found: 554.16358.

Synthesis of L-valine (9-methoxy-3-thioxo-3*H*-benzo[*f*] benzopyran-1-yl) methyl ester hydrobromide, HBr.H-Val-OTba **3a** A 45% solution of hydrobromic acid in acetic acid (60 μL), and acetic acid (1 mL) were added to Z-Val-OTba **2a** (0.017 g, 3.36×10^{-5} mol) with stirring, at room temperature. The reaction mixture was maintained in these conditions for 4 days, and the process was followed by TLC (ethyl acetate/methanol, 1:1). During this time, additional amounts of 45% solution of hydrobromic acid in acetic acid were added until the total volume of 1.76 mL. When the reaction was completed, cold diethyl ether was added (2 mL), and the precipitate filtered off and washed with the same solvent to give compound **3a** as a brown solid (0.010 g, 64%). mp = 181.7–183.4°C. TLC (ethyl acetate/methanol, 1:1): $R_f = 0.61$. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta_H = 0.94$ (d, $J = 2.1$ Hz, 3 H, γ-CH_3 Val), 0.97 (d, $J = 2.1$ Hz, 3 H, γ-CH_3 Val), 2.10–2.30 (m, 1 H, β-CH Val), 3.98 (s, 3 H, OCH_3), 4.23 (broad s, 1 H, α-CH Val), 6.01 (s, 2 H, CH_2), 7.40 (dd, $J = 9.3$ and 1.8 Hz, 1 H, H-8), 7.49–7.60 (m, 3 H, H-10, H-5 and H-2), 8.09 (d, $J = 9.0$ Hz, 1 H, H-7), 8.27 (d, $J = 9.0$ Hz, 1 H, H-6). ^{13}C NMR ($CDCl_3$, 75.4 MHz): $\delta_C = 17.49$ (γ-CH_3 Val), 19.31 (γ-CH_3 Val), 31.01 (β-CH Val), 55.60 (OCH_3), 59.28 (α-CH Val), 64.24 (CH_2), 106.86 (C-10), 114.45 (C-5), 117.50 (C-8), 126.40 (C-4b), 126.58 (C-2), 127.31 (1× Ar–C Phe), 128.56 (2× Ar–C Phe), 129.24 (2× Ar–C Phe), 129.32 (C-6a), 129.73 (C-6b), 131.50 (C-7), 134.25 (C-1 Phe), 135.01 (C-6), 142.62 (C-1), 158.25 (C-4a), 159.44 (C-9), 168.75 (CO_2CH_2), 194.23 (C-3). IR (KBr 1%, cm^{-1}): ν = 3,427, 2,968, 1,750, 1,739, 1,623, 1,591, 1,537, 1,501, 1,465, 1,444, 1,430, 1,372, 1,296, 1,231, 1,218, 1,198, 1,139, 1,096, 1,047, 1,028, 839. HRMS (ESI): calcd for $C_{20}H_{22}NO_4S$ [M^++H]: 372.12641; found: 372.12595.

Synthesis of L-phenylalanine (9-methoxy-3-thioxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester hydrobromide, HBr.H-Phe-OTba **3b** Starting from Z-Phe-OTba **2b** (0.015 g, 2.71×10^{-5} mol), 45% solution of hydrobromic acid in acetic acid (65 μL), and acetic acid (1 mL), following the same procedure as described for **3a** (total volume of hydrobromic acid in acetic acid 2.2 mL; reaction time: 2 days), compound **3b** was obtained as a yellow solid (0.013 g, 93%). mp = 188.3–189.9°C. TLC (ethyl acetate/methanol 1:1): $R_f = 0.61$. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta_H = 3.0$–3.20 (m, 2 H, β-CH_2 Phe), 3.95 (s, 3 H, OCH_3), 4.60 (broad s, 1 H, α-CH Phe), 5.90–6.0 (m, 2 H, CH_2), 7.10–7.30 (m, 5 H, 5× Ar-Phe), 7.33 (s, 1 H, H-2), 7.38 (dd, $J = 8.9$ and 2.1 Hz, 1 H, H-8), 7.53 (d, $J = 1.2$ Hz, 1 H, H-10), 7.60 (d, $J = 8.7$ Hz, 1 H, H-5), 8.08 (d, $J = 9.0$ Hz, 1 H, H-7), 8.26 (d, $J = 9.0$ Hz, 1 H, H-6), 8.53 (broad s, 3 H, NH_3^+). ^{13}C NMR ($CDCl_3$, 75.4 MHz): $\delta_C = 36.13$ (β-CH_2 Phe), 53.21 (α-CH Phe), 55.42 (OCH_3), 65.16 (CH_2), 106.84 (C-10), 114.41 (C-5), 117.46 (C-8), 126.36 (C-4b), 126.62 (C-2), 127.33 (1× Ar–C Phe), 128.58 (2× Ar–C Phe), 129.26 (2× Ar–C Phe), 129.34 (C-6a), 129.75 (C-6b), 131.52 (C-7), 134.27 (C-1 Phe), 135.01 (C-6), 142.61 (C-1), 158.24 (C-4a), 159.43 (C-9), 168.73 (CO_2CH_2), 194.42 (C-3). IR (KBr 1%, cm^{-1}): ν = 3,440, 2,924, 1,753, 1,623, 1,538, 1,455, 1,364, 1,296, 1,231, 1,139, 1,098, 840, 751, 701. HRMS (ESI): calcd for $C_{24}H_{22}NO_4S$ [M^++1]: 420.12641; found: 420.12568.

Synthesis of L-valine (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester hydrobromide, HBr.H-Val-OBba **4a** Starting from Z-Val-OBba **1a** (0.030 g, 6.13×10^{-5} mol), 45% solution of hydrobromic acid in acetic acid (32 μL), and acetic acid (1.5 mL), following the same procedure as described for **3a** (total volume of hydrobromic acid in acetic acid 1.23 mL; reaction time: 3 days), compound **4a** was obtained as a yellow solid (0.019 g, 73%). mp = 218.9–220.1°C. TLC (ethyl acetate/methanol, 1:1): $R_f = 0.56$. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta_H = 0.95$ (d, $J = 0.9$ Hz, 3 H, γ-CH_3 Val), 0.98 (d, $J = 0.9$ Hz, 3 H, γ-CH_3 Val), 2.19–2.26 (m, 1 H, β-CH Val), 3.95 (s, 3 H, OCH_3), 4.19 (broad s, 1 H, α-CH Val), 5.90–6.10 (m, 2 H, CH_2), 6.73 (s, 1 H, H-2), 7.31 (dd, $J = 8.7$ and 2.1 Hz, 1 H, H-8), 7.39 (d, $J = 9.0$ Hz, 1 H, H-5), 7.49 (s, 1 H, H-10), 8.02 (d, $J = 9.0$ Hz, 1 H, H-7), 8.15 (d, $J = 9.0$ Hz, 1 H, H-6), 8.48 (broad s, 3 H, NH_3^+). ^{13}C NMR ($CDCl_3$, 75.4 MHz): $\delta_C = 17.84$ (γ-CH_3 Val), 18.15 (γ-CH_3 Val), 29.60 (β-CH Val), 55.54 (OCH_3), 57.49 (α-CH Val), 65.46 (CH_2), 106.51 (C-10), 111.53 (C-4b), 112.20 (C-2), 114.95 (C-5), 116.74 (C-8), 126.13 (C-6a), 130.18 (C-6b), 131.52

(C-7), 134.29 (C-6), 151.20 (C-1), 155.02 (C-4a), 159.31 (C-9), 159.48 (C-3), 168.53 (CO_2CH_2). IR (KBr 1%, cm^{-1}): ν = 3,407, 2,969, 1,757, 1,694, 1,624, 1,550, 1,519, 1,495, 1,470, 1,447, 1,428, 1,378, 1,366, 1,340, 1,282, 1,251, 1,235, 1,214, 1,173, 1,140, 1,100, 1,062, 1,026, 973, 962, 844. HRMS (ESI): calcd for $C_{20}H_{22}NO_5$ [M^++1]: 356.14925; found: 356.14894.

Synthesis of L-phenylalanine (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester hydrobromide, HBr.H-Phe-OBba **4b** Starting from Z-Phe-OBba **1b** (0.058 g, 1.08 × 10^{-3} mol), 45% solution of hydrobromic acid in acetic acid (72 µL), and acetic acid (1.5 mL), following the same procedure as described for **3a** (total volume of hydrobromic acid in acetic acid 2.9 mL; reaction time: 3 days), compound **4b** was obtained as a yellow solid (0.032 g, 61%). mp = 231.1–232.9°C. TLC (ethyl acetate/methanol, 1:1): R_f = 0.58. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 3.0–3.26 (m, 2 H, β-CH_2 Phe), 3.94 (s, 3 H, OCH_3), 4.57 (t, J = 6.9 Hz, 1 H, α-CH Phe), 5.80–6.0 (2 H, m, CH_2), 6.49 (s, 1 H, H-2), 7.15–7.28 (m, 5 H, 5× Ar–H Phe), 7.31 (dd, J = 8.7 and 2.1 Hz, 1 H, H-8), 7.41 (d, J = 8.7 Hz, 1 H, H-5), 7.44 (s, 1 H, H-10), 8.02 (d, J = 9.0 Hz, 1 H, H-7), 8.16 (d, J = 9.3 Hz, 1 H, H-6), 8.60 (broad s, 3 H, NH_3^+). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C = 36.14 (β-CH_2 Phe), 53.28 (α-CH Phe), 55.31 (OCH_3), 65.21 (CH_2), 106.13 (C-10), 111.41 (C-4b), 112.17 (C-2), 114.82 (C-5), 116.75 (C-8), 126.00 (C-6a), 127.31 (1× Ar–C Phe), 128.56 (2× Ar–C Phe), 129.31 (2× Ar–C Phe), 130.04 (C-6b), 131.32 (C-7), 134.06 (C-1 Phe), 134.48 (C-6), 150.56 (C-1), 154.86 (C-4a), 159.14 (C-3 and C-9), 168.67 (CO_2CH_2). IR (KBr 1%, cm^{-1}): ν = 3,419, 2,914, 1,749, 1,715, 1,626, 1,594, 1,552, 1,519, 1,498, 1,456, 1,445, 1,428, 1,377, 1,363, 1,337, 1,280, 1,234, 1,216, 1,204, 1,141, 1,121, 1,082, 1,063, 1,021, 855. HRMS (ESI): calcd for $C_{24}H_{22}NO_5$ [M^++H]: 404.14925; found: 404.14858.

General photolysis procedure

A 1 × 10^{-4} M methanol/HEPES (80:20) solution of compounds **1**–**4** (5 mL) were placed in a quartz tube and irradiated in a Rayonet RPR-100 reactor at the desired wavelength. The lamps used for irradiation were of 350 and 419 ± 10 nm.

Aliquots of 100 µL were taken at regular intervals and analysed by RP-HPLC. The eluent was acetonitrile/water, 75:25 or methanol/water, 64:36 (**4a** and **4b**), at a flow rate of 0.8 mL/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 compound (retention time: **1a**, 7.1; **1b**, 7.4; **2a**, 9.7; **2b**, 10.6; **3a** and **3b**, 4.2; **4a** and **4b**, 8.0 min).

Results and discussion

Synthesis of amino acid conjugates **2**, **3** and **4**

N-(Benzyloxycarbonyl)-L-valine (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, Z-Val-OBba **1a** and *N*-(benzyloxycarbonyl)-L-phenylanaline (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, Z-Phe-OBba **1b** were prepared by reaction of 1-chloromethyl-9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran with *N*-(benzyloxycarbonyl)-L-valine and *N*-(benzyloxycarbonyl)-L-phenylanaline in the presence of potassium fluoride in DMF, at room temperature, as previously described (Piloto et al. 2006).

Conjugates **1a** and **1b** were reacted with Lawesson's reagent in toluene (Jesberger et al. 2003), under reflux conditions, followed by column chromatography purification affording corresponding thioxobenzopyran conjugates **2a** (55%) and **2b** (42%). In addition to the use of this heterocycle for molecules that require multiple protecting groups, it was intended to evaluate its usefulness in the release of unprotected moieties in a biological perspective. Therefore, the *N*-benzyloxycarbonyl-protecting group was removed by acidolysis with hydrobromic acid in acetic acid yielding conjugates **3a** and **3b** bearing the amino acid and the photosensitive tag.

In order to compare the behaviour towards irradiation of the thioxobenzopyran conjugates **3a** and **3b** with the related oxobenzopyran derivatives, cleavage of the *N*-benzyloxycarbonyl-protecting group was also carried out in compounds **1a** and **1b**, following the same conditions as mentioned above, to provide compounds **4a** and **4b** (Scheme 1; Table 1). The oxo-benzobenzopyran (Bba) and the thioxo-benzobenzopyran (Tba) moieties will be designated in this report by a three-letter code for simplicity of naming the various fluorescent conjugates, as indicated in Tables 1 and 2.

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 4.19–4.85 ppm), β-CH (δ 2.10–2.30 ppm), β-CH_2 (δ 3.0–3.30 ppm), as well as γ-CH_3 (δ 0.91–1.04 ppm). The heterocycle methylene group was also visible for all conjugates (δ 5.56–6.01 ppm). The effect of the thiocarbonyl group in compounds **2a**, **2b**, **3a** and **3b** was notorious in the chemical shift of the pyran proton H-2, which appeared downfield in the range δ 7.08–7.60 ppm, whereas in compounds **4a** and **4b**, having a carbonyl group, it occurred at δ 6.49 or 6.73 ppm.

The confirmation of the presence of the new C=S bond (C-3) at the heterocyclic ring (**2a**, **2b**, **3a** and **3b**) was also supported by ^{13}C NMR spectra signals at δ 194.23–195.13 ppm, instead of the carbonyl group, which occurred at δ 159.14 or 159.48 ppm, in conjugates **4a** and **4b**. The

Scheme 1 Synthesis of thioxobenzo[*f*]benzopyran and oxobenzo[*f*]benzopyran conjugates **2–4**

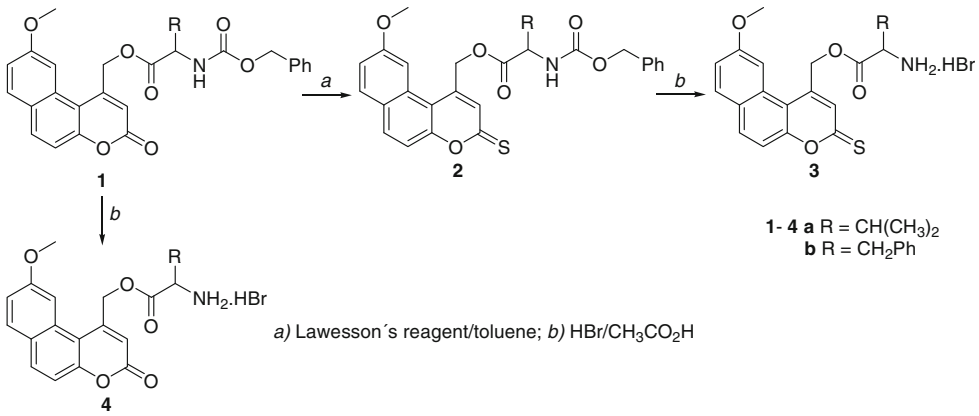


Table 1 Yields, UV/visible and fluorescence data for conjugates **1–4** in absolute ethanol and methanol/HEPES buffer (80:20) solution

Compound		Yield (%)	EtOH					MeOH/HEPES (80:20)				
			λ_{abs} (nm)	log ε	λ_{em} (nm)	Φ_F	$\Delta\lambda$ (nm)	λ_{abs} (nm)	log ε	λ_{em} (nm)	Φ_F	$\Delta\lambda$ (nm)
1a	Z-Val-OBba[a]	75	348	4.07	478	0.58	130	351	4.19	490	0.23	139
1b	Z-Phe-OBba[a]	94	347	4.08	478	0.59	131	349	4.09	490	0.25	141
2a	Z-Val-OTba	55	407	3.70	458	0.01	51	417	4.14	476	0.002	59
2b	Z-Phe-OTba	42	408	3.83	464	0.02	56	417	4.19	477	0.004	60
3a	H-Val-OTba	64	407	3.74	461	0.02	54	417	4.44	477	0.004	60
3b	H-Phe-OTba	93	407	3.75	461	0.04	54	416	4.13	476	0.004	60
4a	H-Val-OBba	72	344	3.76	468	0.71	124	348	4.35	478	0.36	130
4b	H-Phe-OBba	61	345	3.79	468	0.55	123	345	3.94	473	0.29	128

[a] Data in ethanol was previously reported (Piloto et al. 2006)

Table 2 Irradiation times (t_{irr} in min), rate constants (k, $\times 10^{-2}$ min^{-1}) and photochemical quantum yields (Φ_P, $\times 10^{-3}$) for the photolysis of conjugates **1–4** at 350 and 419 nm in methanol/HEPES buffer (80:20) solution

Compound		350 nm			419 nm		
		t_{irr}	k	Φ_P	t_{irr}	k	Φ_P
1a	Z-Val-OBba	33	9.08	0.098	462	0.66	0.079
1b	Z-Phe-OBba	38	8.28	0.093	301	1.0	0.144
2a	Z-Val-OTba	66	4.76	0.130	30	9.68	0.119
2b	Z-Phe-OTba	354	0.84	0.020	52	5.80	0.062
3a	H-Val-OTba	162	1.97	0.015	48	6.07	0.041
3b	H-Phe-OTba	146	2.12	0.043	49	6.49	0.078
4a	H-Val-OBba	607	0.49	0.004	3304	0.09	0.006
4b	H-Phe-OBba	520	0.58	0.011	7478	0.04	0.004

chemical shift of the pyran carbon C-2 was also influenced by the proximity of the carbon–sulphur double bond, being in the range δ 126.58–127.98 ppm for compounds **2a**, **2b**, **3a** and **3b**, and δ 112.17 or 112.20 ppm for compounds **4a** and **4b**.

Evaluation of the photophysical properties of amino acid conjugates **1**, **2**, **3** and **4**

The UV/visible absorption and emission spectra of degassed 10^{-5} M solutions in absolute ethanol and in a methanol/HEPES buffer (80:20) solution of thioxobenzopyran conjugates **2a**, **2b**, **3a**, **3b**, in comparison with the corresponding oxobenzopyran conjugates **1a**, **1b**, **4a**, **4b** were measured; absorption and emission maxima, molar absorptivities and relative fluorescence quantum yields are also reported (Table 1). Relative fluorescence quantum yields were calculated using 9,10-diphenylanthracene as standard ($\Phi_F = 0.95$ in ethanol) (Morris et al. 1976). For the Φ_F determination, the fluorescence standard was excited at the wavelengths of maximum absorption found for

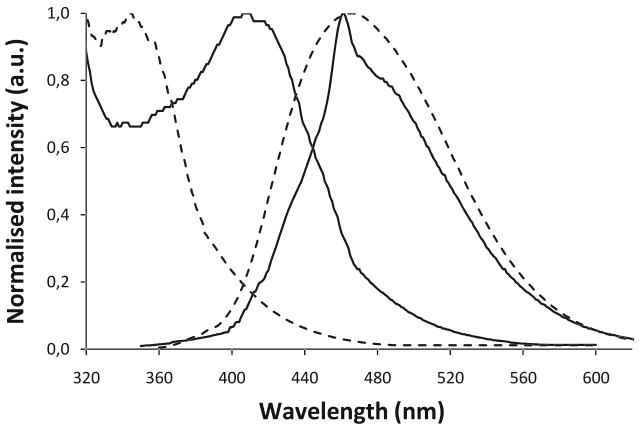


Fig. 1 Normalised UV/visible absorption and fluorescence spectra of conjugates **3a** (*full line*) and **4a** (*spaced line*) in methanol/HEPES (80:20) solution (**3a**, $\lambda_{exc} = 417$ nm; **4a**, $\lambda_{exc} = 348$ nm)

each one of the compounds to be tested and in all fluorimetric measurements the absorbance of the solution did not exceed 0.1.

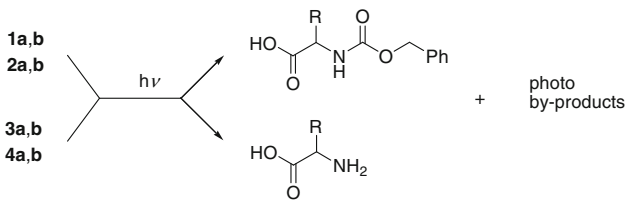
By comparison of absorption maxima for all compounds in both solvents, a slight bathochromic shift (2–10 nm) was observed in methanol/HEPES buffer (80:20) solution (except for compound **4b**, no variation) (λ_{max} 344–408 nm, in ethanol, and 345–417 nm, in methanol/HEPES buffer). Furthermore, upon exchange of the carbonyl group at the heterocycle in compounds **1a** and **1b** for the thiocarbonyl group in **2a** and **2b**, a considerable bathochromic shift (59–68 nm) was observed. The same trend occurred for Z-deprotected conjugates **4a**, **4b** in comparison with **3a**, **3b** (62–71 nm), being the highest λ_{max} values in methanol/HEPES buffer, as in the latter conjugates. Absorption maxima for thioxo- and oxo-conjugates were independent of the amino acid residue.

Bioconjugates **1a**, **1b**, **4a** and **4b** exhibited high fluorescence quantum yields in ethanol ($0.55 < \Phi_F < 0.71$) and in methanol/HEPES buffer ($0.23 < \Phi_F < 0.36$), contrarily to the minor fluorescent thioxo analogues **2a**, **2b**, **3a** and **3b**.

Compounds **2a**, **2b**, **3a** and **3b** displayed emission maxima between 458 and 464 nm (in ethanol) and at about 477 nm (in methanol/HEPES buffer), with moderate Stokes' shifts ($\Delta\lambda$ 51–60 nm), but inferior to those of the corresponding oxobenzopyran conjugates **1a**, **1b**, **4a** and **4b** (123–141 nm) (Table 1; Fig. 1).

Photolysis studies of amino acid conjugates 1–4

The sensitivity of thioxo-conjugates **2a**, **2b**, **3a** and **3b** in comparison with oxo-conjugates **1a**, **1b**, **4a** and **4b** towards UV/visible irradiation was evaluated by exposing solutions of the mentioned compounds in methanol/HEPES buffer



Scheme 2 Photocleavage reactions of conjugates **1–4**

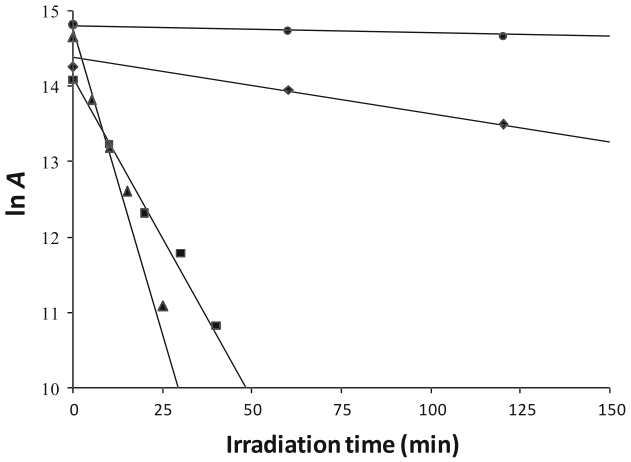


Fig. 2 Plot of ln *A* versus irradiation time for the photolysis at 419 nm of conjugates **1a** (*diamond*), **2a** (*triangle*), **3a** (*square*) and **4a** (*circle*) in methanol/HEPES buffer (80:20) solution (for better visualisation, time scale is shown only up to 150 min, although photolysis for **4** proceeded until ca. 7,500 min)

(80:20) solution in a Rayonet RPR-100 reactor at 350 and 419 nm (Scheme 2). 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 three runs. The determined irradiation time represents the time necessary for the consumption of the starting materials until <5% of the initial area was detected (Table 2). 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 (Fig. 2). The corresponding rate constants were calculated and are presented in Table 2. 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 (Muller et al. 2001). The calculated photochemical quantum yields indicated that the photocleavage process was not as efficient as desirable, probably due to the dissipation of part of the absorbed

energy via fluorescence pathways that compete with the photochemical reaction, as well as the type of reactor used (open chamber reactor).

The release of *N*-benzyloxycarbonyl valine and phenylalanine required shorter irradiation times than the corresponding free amino acids at 350 nm (except for **3b**). The presence of the thiocarbonyl group at the heterocycle clearly affected the photolysis rate of the amino acid-heterocycle ester bond. Comparison of thioxo-conjugates **3** with the corresponding oxo-conjugate **4**, showed that irradiation times were shorter for **3** revealing the significance of the thioxo-group in the release of the free amino acids. However, this trend was reversed when the *N*-protecting group was present (compare data for conjugates **2** and **1**).

It was expected that the bathochromic shift in the maximum absorption wavelengths related to the presence of the C=S bond would result in an increase in the efficiency of the photolysis at longer wavelengths, leading to shorter irradiation times. The obtained results confirmed this expectation, as the photocleavage at 419 nm of thioxo-conjugates **2a**, **2b**, **3a** and **3b** occurred rapidly (30–52 min), in comparison with the corresponding oxo-conjugates **1a**, **1b**, **4a** and **4b** (301–7,478 min). For these compounds and at this wavelength, the release of *N*-protected or free valine and phenylalanine occurred in similar irradiation times. The results indicated that the release of the amino acids at 419 nm from thioxobenzobenzopyrans is practicable for organic synthesis and also for caging applications, considering the short irradiation times and the fact that it minimises the side reactions for the remaining functionalities of the molecule.

Given the interest in the development of novel protecting groups cleavable with UV A or even visible radiation, it was found that a simple modification involving the exchange of the carbonyl group of the light sensitive 9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester reported before by us, for a thiocarbonyl group by a thionation reaction, lead to a enhancement of the photolysis performance at 419 nm. This newly reported thioxobenzo[*f*]benzopyran can be considered an addition to the collection of photolabile protecting groups for application at long wavelengths.

Conclusions

The synthesis of novel thioxobenzo[*f*]benzopyran ester conjugates was achieved in good yields through a simple thionation reaction with Lawesson's reagent from the corresponding oxobenzo[*f*]benzopyrans. In order to obtain the parameters necessary for monitoring the photolysis reaction, as well as the fluorescence properties, the UV/visible characterization was carried out in absolute ethanol and methanol/HEPES buffer (80:20) solution.

Photocleavage studies of the oxo- and thioxo-conjugates, in methanol/HEPES buffer (80:20) solution at 350 nm revealed that the amino acid-heterocycle ester bond was efficiently photolised, releasing the *N*-protected and free amino acids from both types of precursors. However, the most interesting results were obtained at 419 nm for the thioxoconjugates, revealing that the presence of the thiocarbonyl clearly improved the photolysis rates, giving practicable irradiations times (30–52 min).

Although the parent oxo-protecting group still represents an interesting alternative as photocleavable group for the carboxylic acid, the newly reported thioxo-protecting group, (9-methoxy-3-thioxo-3*H*-benzo[*f*]benzopyran-1-yl) methyl ester, emerges as an innovation for the release at longer wavelengths, since it photolyses with short irradiation times at wavelengths that are not detrimental to a variety of applications.

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