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GRAPHICAL ABSTRACT

Photolytic release of bioactive carboxylic acids from fused pyran conjugates

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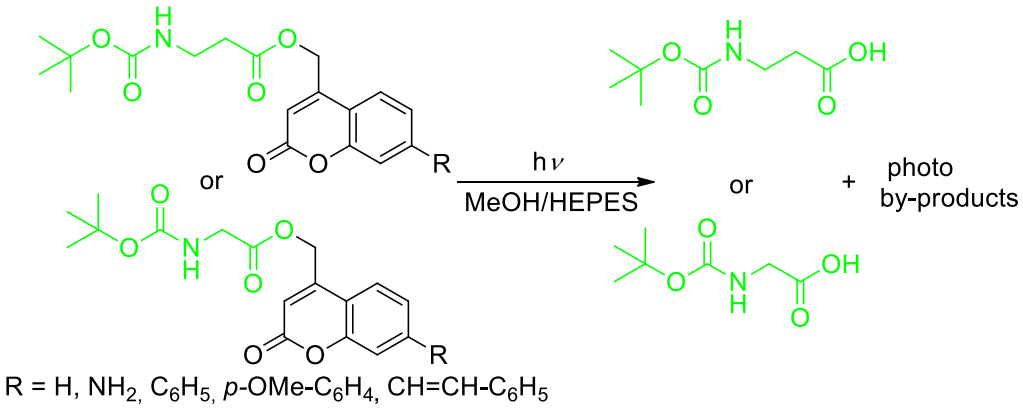

Photolytic release of bioactive carboxylic acids from fused pyran conjugates

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HIGHLIGHTS

- Coumarin (2*H*-benzopyran-2-one) derivatives with amino, phenyl, 4-methoxyphenyl and styryl groups at the 7-position, were synthesized and used in the derivatization of glycine and β-alanine.

- The new ester cages of glycine and β-alanine, selected as models of carboxylic acid bioactive molecules, revealed to be interesting photo-responsive units.

- A remarkable behaviour towards irradiation at higher wavelength (350 and 419 nm) occurred in conjugates obtained from 4-(chloromethyl)-7-styryl-2*H*-benzopyran-2-one.

- Time-resolved fluorescence studies complemented the photolysis results and proposed mechanism revealing the presence of different fluorescing species by the determination of decay associated spectra.

- This study shows new promising alternative moieties for the development of photoactivable fluorescent acid prodrugs.

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Abstract: New ester cages bearing the coumarin (2*H*-benzopyran-2-one) skeleton with extended π-systems as phototriggers, for glycine and β-alanine, as models of carboxylic acid bifunctional molecules with biological relevance, were evaluated under photolysis conditions at 254, 300, 350 and 419 nm of irradiation in a RPR-100 photochemical reactor. The processes were followed by HPLC-UV detection and ^{1}H NMR with collection of kinetic data. The results showed a correlation between the photolysis efficiency and the increasing extension of the conjugation for both glycine and β-alanine, showing that the 7-aminocoumarin afforded the best results at all wavelengths tested. From a study of the time-resolved fluorescence behaviour, these compounds were also found to exhibit more complex fluorescence decay kinetics. This was attributed to the presence of conjugated and non-conjugated coumarin species.

***Keywords*:** Phototriggers; Caging groups; Neurotransmitters; Coumarins; Photolysis.

1. Introduction

2*H*-Benzopyran-2-one, the IUPAC name of coumarin, is an oxygen heterocycle firstly isolated in 1820 by Voleg from the plant *Dypteryx adorata* and is at the moment known to be present in a huge number of natural products [1-3]. Since ancient Egyptian times, coumarins have been known for their therapeutic properties, namely anticancer, antimicrobial, anesthetic, anti-HIV, anticoagulant and antioxidant activities. Also, coumarins are used in the cosmetic and food industries, as fluorescent probes and, more recently, as photocleavable protecting groups (PPGs) [4-9].

PPGs are chemical entities used to temporarily disable the activity of a compound, which is achieved by a covalent bond between the PPG and a functional group essential to its activity. After irradiation, the bond is cleaved and the compound activity is restored. For a biological application, the employed wavelength needs to be as near as possible to the visible radiation wavelength in order to reduce cellular damage. PPGs can be used to synthesize prodrugs sensitive to light,

producing inactive therapeutic agents that regain their biological activity after irradiation. These prodrugs allow the controlled release of drugs; both in time and locally, thus holding the promise of reduced side effects and toxicity [10-15].

In comparison to one of the most common PPGs, *o*-nitrobenzyl, coumarins possess higher molar extinction coefficients at longer wavelengths, higher releasing rates, increased stability and good fluorescence. This makes them excellent options as PPGs for biological applications, besides their interest for organic synthesis, and the study of their fluorescence provides a means by which to assess the photocleavage process [11,16,17]. In addition, some coumarins can be used in two-photon uncaging, a process where two photons are simultaneous absorbed and provide the excitation energy required to initiate the photophysical process. This technique enables photolysis by the absorption of NIR radiation which is less toxic to living systems than UV and presents a higher tissue penetrating capacity [11-18]. Coumarins have been used in the photorelease of several bioactive compounds such as amino acids (glycine, β-alanine, GABA, glutamate), secondary messengers (cGMP, cAMP, diacylglycerol), nucleic acids/oligonucleotides (DNA, mRNA, 8-bromonucleotids, ATP, ADP, AMP), hormones (progesterone), cholesterol and even drugs (anisomycin and paclitaxel) [19-29].

Considering that one of the major drawbacks of PPGs is the fact that their maximum absorption wavelength is at shorter wavelengths, the present work describes the synthesis of a new set of ester conjugates of coumarins possessing an extended π-system or an amino group at position 7 of the system. The intention is to bathochromically shift the wavelength of maximum absorption, and consequently, of photolysis. Glycine and β-alanine were used as models of carboxylic acids, which besides representing bifunctional molecules used in organic synthesis, possess crucial biological roles in humans (as neurotransmitters, for example) [40-42].

For this purpose, the caged amino acids were characterized (using UV-Vis and fluorescence spectroscopy) and irradiated at 254, 300, 350 and 419 nm in a photochemical reactor Rayonet RPR-100. The release of the active compound was monitored by HPLC-UV detection with collection of kinetic data and also by ^{1}H NMR.

2. Experimental Section

2.1. Material and instruments

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 using KBr discs. UV/visible 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 and 100.6 MHz for ^{13}C using the solvent peak as internal reference at 25 ºC. 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 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 and excitation – emission matrices (EEM's) were recorded using a FluoroLog 3. Time-resolved fluorescence was measured using a HORIBA Scientific DeltaFlex equipped with a DeltaDiode (DD-350, emitting at 349 nm) excitation source. Fluorescence decays were measured at 5 nm increments over the wavelength range 375 nm to 575 nm. The resultant decays were analysed globally to obtain decay associated spectra (using EzTime software) or just made use of the decay obtained at 480 nm in the dataset. During the analysis, an "extra" short-lived component (not plotted in Figure 3) was required and attributed to scattered excitation. The optical density of the samples used or these fluorescence measurements was kept to ~0.1 to avoid self-absorption effects. Photolysis measurements were carried out using a Rayonet RPR-100 chamber reactor equipped with 10 lamps of 254, 300, 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. The synthesis of chloromethyl precursors **1a**-**e** is included in the supporting information.

*2.2. General procedure for the synthesis of conjugates **4** and **5**.*

The chloromethyl precursors **1a**-**e** were dissolved in dry DMF (3 mL), and potassium fluoride (3 equiv) and *N*-(*tert*-butoxycarbonyl)-glycine or *N*-(*tert*-butoxycarbonyl)-β-alanine (1 equiv) were added. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed by evaporation under reduced pressure and the required conjugate was obtained as a solid. The crude residue of compounds **4** and **5** was purified by column chromatography using mixtures of increasing polarity of dichloromethane/methanol (**4a**-**e**) or light petroleum/ethyl acetate (**5a**-**e**) as eluent.

*2.2.1. N-(tert-Butyloxycarbonyl)-glycine (2-oxo-2H-benzopyran-4-yl)methyl ester **4a**.* Starting from compound **1a** (0.031 g, 1.6×10^{-4} mol) in dry DMF (2 mL), potassium fluoride (0.028 g, 4.8×10^{-4} mol) and *N*-(*tert*-butoxycarbonyl)-glycine **2** (0.028 g, 1.6×10^{-4} mol), the ester conjugate **4a** was obtained as an orange solid (0.031 g, 56%). Mp = 115.1-116.6 ºC. R_f = 0.67 (dichloromethane). ^{1}H

NMR ($CDCl_3$, 400 MHz): δ = 1.47 (s, 9 H, C(CH_3)$_3$), 4.07 (d, *J* 5.6 Hz, 2 H, H-α), 4.98 (br s, 1 H, NH), 5.39 (s, 2 H, CH_2), 6.51 (s, 1 H, H-3), 7.33 (td, *J* 8 and 1.2 Hz, 1 H, H-6), 7.39 (dd, *J* 8 and 0.8 Hz, 1 H, H-8), 7.52 (dd, *J* 8 and 1.6 Hz, 1 H, H-5), 7.58 (td, *J* 8.8 and 1.6 Hz, 1 H, H-7) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.27 (C(CH_3)$_3$) 42.41 (C-α), 61.78 (CH_2), 80.45 (C(CH_3)$_3$)), 113.67 (C-3), 116.99 (C-4a), 117.50 (C-8), 123.41 (C-5), 124.55 (C-6), 132.22 (C-7), 148.25 (C-4), 153.66 (C-8a), 155.70 (C=O Boc), 160.16 (C-2), 169.77 (C=O Gly) ppm. IR (liquid film): ν = 3440, 3058, 2980, 2918, 2850, 1729, 1630, 1608, 1569, 1510, 1452, 1407, 1368, 1266, 1162, 1070, 1006, 937, 862, 738, 703 cm^{-1}. HRMS: m/z (ESI): Found [M^++1]: 334.12910; $C_{17}H_{20}NO_6$ requires [M^++1]: 334.12925.

2.2.2. N-(tert-Butyloxycarbonyl)-glycine (7-amino-2-oxo-2H-benzopyran-4-yl)methyl ester ***4b***. Starting from compound **1b** (0.031 g, 1.6 × 10^{-4} mol) in dry DMF (2 mL), potassium fluoride (0.016 g, 8.0 × 10^{-5} mol) and *N*-(*tert*-butoxycarbonyl)-glycine **2** (0.028 g, 1.6 × 10^{-4} mol), the ester conjugate **4b** was obtained as an yellow solid (0.038 g, 70%). Mp = 179.6-180.2 ºC. R_f = 0.6 (dichloromethane/methanol 20:1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.38 (s, 9 H, C(CH_3)$_3$), 3.83 (d, *J* 6 Hz, 2 H, H-α), 5.30 (s, 2 H, CH_2), 6.00 (s, 1 H, H-3), 6.72 (s, 2 H, NH_2), 6.42 (d, *J* 2 Hz, H-8), 6.55 (dd, *J* 8.8 and 2 Hz, 1 H, H-6), 7.31-7.36 (m, 2 H, H-5 and NH) ppm. ^{13}C NMR (DMSO-*d6*, 100.6 MHz): δ = 28.12 (C(CH_3)$_3$), 42.05 (C-α), 61.43 (CH_2), 78.40 (C(CH_3)$_3$), 98.58 (C-8), 104.65 (C-3), 105.70 (C-4a), 111.31 (C-6), 125.41 (C-5), 150.56 (C-4), 153.25 (C-8a), 155.57 (C-7), 155.94 (C=O Boc), 160.54 (C-2), 170.03 (C=O Gly) ppm. IR (KBr 1%): ν = 3383, 3231, 2976, 2363, 1743, 1702, 1616, 1526, 1404, 1279, 1169, 1056, 869, 850, 819, 747 cm^{-1}. HRMS: m/z (ESI): Found [M^+ +1]: 349.14003; $C_{17}H_{21}N_2O_6$ requires [M^++1]: 349.14010.

2.2.3. N-(tert-Butyloxycarbonyl)-glycine (7-phenyl-2-oxo-2H-benzopyran-4-yl)methyl ester ***4c***. Starting from compound **1c** (0.037 g, 1.4 × 10^{-4} mol) in dry DMF (2 mL), potassium fluoride (0.025 g, 4.3 × 10^{-3} mol) and *N*-(*tert*-butoxycarbonyl)-glycine **2** (0.025 g, 1.4 × 10^{-5} mol), the ester conjugate **4c** was obtained as an yellow solid (0.009 g, 16%). Mp = 129.5-131.6 ºC. R_f = 0.55 (dichloromethane/methanol 100:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.46 (s, 9 H, C(CH_3)$_3$), 4.08 (d, *J* 5 Hz, 2 H, H-α), 5.07 (t, *J* 5 Hz 1 H, NH), 5.41 (s, 2 H, CH_2), 6.51 (s, 1 H, H-3), 7.45 (tt, *J* 7.2 and 1.2 Hz, 1 H, H-4'), 7.51 (tt, *J* 7.2 and 1.2 Hz, 2 H, H-3'and H-5'), 7.57-7.60 (m, 3 H, H-5, H-6 and H-8), 7.64 (dt, *J* 7.2 and 1.2 Hz, 2 H, H-2' and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.27 (C(CH_3)$_3$), 42.41 (C-α), 61.81 (CH_2), 80.46 (C(CH_3)$_3$), 113.27 (C-3), 115.51 (C-6), 115.86 (C-4a), 123.38 (C-8), 123.79 (C-5), 127.19 (C-2' and C-6'), 128.71 (C-4'), 129.14 (C-3' and C-5'), 138.81 (C-1'), 145.41 (C-7), 148.13 (C-4), 154.10 (C-8a), 155.71 (C=O Boc), 160.31 (C-2), 169.78 (C=O Gly) ppm. IR (liquid film): ν = 3371, 3061, 2978, 2932, 1722, 1617, 1583, 1514, 1452, 1406,

1368, 1337, 1279, 1252, 1163, 1074, 1055, 1004, 952, 866, 766, 750, 737, 699 cm^{-1}. HRMS: m/z (ESI): Found $[M^+ +1]$: 410.16042; $C_{23}H_{24}NO_6$ requires $[M^+ +1]$: 410.16037.

*2.2.4. N-(tert-Butyloxycarbonyl)-glycine [7-(4'methoxyphenyl)-2-oxo-2H-benzopyran-4-yl]methyl ester **4d**.* Starting from compound **1d** (0.037 g, 1.4×10^{-4} mol) in dry DMF (2 mL), potassium fluoride (0.013 g, 2.2×10^{-4} mol) and *N*-(*tert*-butoxycarbonyl)-glycine **2** (0.013 g, 7.0×10^{-5} mol), the ester conjugate **4d** was obtained as a beige solid (0.008 g, 25%). Mp = 142.4-143.7 ºC. R_f = 0.4 (dichloromethane / methanol 100:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.46 (s, 9 H, C*(CH$_3$)$_3$*), 3.87 (s, 3 H, OCH_3), 4.07 (d, *J* 6 Hz, 2 H, H-α), 5.12 (br s, 1H, NH), 5.37 (s, 2 H, CH_2), 6.46 (s, 1 H, H-3), 7.01 (dt, *J* 6.8 and 2 Hz, 2 H, H-3' and H-5'), 7.51-7.52 (m, 3 H, H-5, H-6 and H-8), 7.57 (dt, *J* 6.8 and 2 Hz, 2 H, H-2' and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.25 (C(*C*H$_3$)$_3$), 42.40 (C-α), 55.38 (OCH_3), 61.79 (CH_2), 79.03 (C(CH$_3$)), 112.77 (C-3), 114.55 (C-3' and C-5'), 114.70 (C-6), 115.27 (C-4a), 122.81 (C-8), 123.71 (C-5), 128.29 (C-2' and C-6'), 131.09 (C-1'), 144.94 (C-7), 148.19 (C-4), 154.14 (C-8a), 155.72 (C=O Boc) 160.24 (C-4'), 160.40 (C-2), 169.78 (C=O Gly) ppm. IR (liquid film): ν = 3370, 3057, 2975, 2921, 2850, 1721, 1609, 1579, 1526, 1502, 1458, 1406, 1368, 1337, 1300, 1281, 1253, 1163, 1074, 1028, 952, 866, 839, 816, 735, 703 cm^{-1}. HRMS: m/z (ESI): Found $[M^+ +1]$: 440.17098; $C_{24}H_{26}NO_7$ requires $[M^+ +1]$: 440.17099.

*2.2.5. N-(tert-Butyloxycarbonyl)-glycine (7-styryl-2-oxo-2H-benzopyran-4-yl)methyl ester **4e**.* Starting from compound **1e** (0.027 g, 9.0×10^{-5} mol) in dry DMF (2 mL), potassium fluoride (0.016 g, 2.7×10^{-4} mol) and *N*-(*tert*-butoxycarbonyl)-glycine **2** (0.016 g, 9.0×10^{-5} mol), the ester conjugate **4d** was obtained as a yellow solid (0.023 g, 58%). Mp = 131.3-131.4 ºC. R_f = 0.38 (dichloromethane/methanol 100:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.47 (s, 9H, C*(CH$_3$)$_3$*), 4.07 (d, *J* 6.4 Hz, 2 H, H-α), 5.07 (d, *J* 6.4 Hz , 1 H, NH), 5.37 (s, 2 H, CH_2), 6.46 (s, 1 H, H-3), 7.12 (d, *J* 16.4 Hz, 1 H, H-a), 7.25 (d, *J* 16.4 Hz, 1 H, H-b), 7.33 (tt, *J* 7.2 and 1.6 Hz, 1 H, H-4'), 7.40 (tt, *J* 7.2 and 1.6 Hz, 1 H, H-3´and H-5'), 7.45-7.49 (m, 3 H, H-5, H-6 and H-8), 7.60 (dt, *J* 7.2 and 1.6 Hz, 2 H, H-2' and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.27 (C(*C*H$_3$)$_3$), 42.41 (C-α), 61.78 (CH_2), 80.43 (*C*(CH$_3$)), 112.91 (C-3), 114.62 (C-6), 116.04 (C-4a), 122.63 (C-8), 123.63 (C-5), 126.54 (C-a), 126.93 (C-2' and C-6'), 128.62 (C-4'), 128.84 (C-3' and C-5'), 132.33 (C-b), 136.27 (C-1'), 141.73 (C-7), 148.05 (C-4), 154.15 (C-8a), 155.71 (C=O Boc), 160.31 (C-2), 171.61 (C=O Gly) ppm. IR (liquid film): ν = 3368, 2976, 2932, 1722, 1609, 1526, 1407, 1367, 1253, 1168, 1083, 1027, 955, 866, 838, 815, 734 cm^{-1}. HRMS: m/z (ESI): Found $[M^+ +1]$: 436.17608; $C_{21}H_{19}N_2O_5$ requires $[M^+ +1]$: 436.17605.

2.2.6. N-(tert-Butyloxycarbonyl)-β-alanine (2-oxo-2H-benzopyran-4-yl)methyl ester ***5a****.* Starting from compound **1a** (0.016 g, 8.0×10^{-5} mol) in dry DMF (2 mL), potassium fluoride (0.017 g, 2.5×10^{-4} mol) and *N*-(*tert*-butoxycarbonyl)-β-alanine **3** (0.016 g, 8.0×10^{-5} mol), the ester conjugate **5a** was obtained as an orange solid (0.011 g, 38%). Mp = 91.4-92.1 ºC. R_f = 0.5 (dichloromethane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.45 (s, 9 H, C*(CH$_3$)$_3$*), 2.70 (t, *J* 6 Hz, 2 H, H-α), 3.47 (q, *J* 6 Hz, 2 H, H-β), 4.98 (br s, 1 H, NH), 5.34 (s, 2 H, CH_2), 6.49 (s, 1 H, H-3), 7.33 (td, *J* 7.2 and 1.2 Hz, 1 H, H-6), 7.39 (dd, *J* 8.4 and 0.8 Hz, 1 H, H-8), 7.53 (dd, *J* 8.4 and 1.6 Hz, 1 H, H-5), 7.58 (td, *J* 7.2 and 1.2 Hz, 1 H, H-7) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.34 (C(*C*H$_3$)$_3$), 34.55 (C-α), 36.05 (C-β), 61.30 (CH_2), 79.66 (*C*(CH$_3$)$_3$), 113.53 (C-3), 117.07 (C-4a), 117.49 (C-8), 123.41 (C-5), 124.52 (C-6), 132.18 (C-7), 148.66 (C-4), 153.65 (C-8a), 155.72 (C=O Boc), 160.24 (C-2), 171.62 (C=O β-Ala) ppm. IR (liquid film): ν = 3374, 2979, 2932, 1730, 1630, 1607, 1568, 1513, 1452, 1393, 1367, 1318, 1252, 1170, 1082, 934, 860, 737, 704 cm^{-1}. HRMS: m/z (ESI): Found [M^++1]: 348.14476; $C_{18}H_{22}NO_6$ requires [M^++1]: 348.14480.

2.2.7. N-(tert-Butyloxycarbonyl)-β-alanine (7-amino-2-oxo-2H-benzopyran-4-yl)methyl ester ***5b****.* Starting from compound **1b** (0.030 g, 1.4×10^{-4} mol) in dry DMF (2 mL), potassium fluoride (0.025 g, 4.3×10^{-3} mol) and *N*-(*tert*-butoxycarbonyl)-β-alanine **3** (0.027 g, 1.4×10^{-3} mol), the ester conjugate **5b** was obtained as a beige solid (0.032 g, 65%). Mp = 157.3-158.2 ºC. R_f = 0.54 (dichloromethane/methanol 20:1). ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ = 1.36 (s, 9 H, C*(CH$_3$)$_3$*), 2.58 (t, *J* 6 Hz, 2 H, H-α), 3.20 (q, *J* 6 Hz, 2 H, H-β), 5.22 (s, 1 H, CH_2), 5.74 (s, 1 H, H-3), 6.18 (s, 2 H, NH_2), 6.44 (d, *J* 2 Hz, 1 H, H-8), 6.55 (dd, *J* 8.4 and 2 Hz, 1 H, H-6), 6.90 (t, *J* 6 Hz, 1 H, NH), 7.35 (d, *J* 8.8 Hz, 1 H, H-5) ppm. ^{13}C NMR (DMSO-*d*$_6$, 100.6 MHz): δ = 28.17 (C(*C*H$_3$)$_3$), 34.00 (C-α), 36.05 (C-β), 61.27 (CH_2), 77.77 (*C*(CH$_3$)$_3$), 98.60 (C-8), 105.02 (C-3), 105.84 (C-4a), 111.35 (C-6), 125.47 (C-5), 150.63 (C-4), 153.25 (C-8a), 155.50 (C-7), 155.61 (C=O Boc), 160.56 (C-2), 170.83 (C=O β-Ala) ppm. IR (KBr 1%): ν = 3485, 3393, 3349, 3227, 2972, 1748, 1707, 1682, 1643, 1608, 1527, 1448, 1411, 1367, 1325, 1293, 1248, 1186, 857, 844, 806, 781, 746, 708 cm^{-1}. HRMS: m/z (ESI): Found [M^++1]: 363.15569; $C_{18}H_{23}N_2O_6$ requires [M^++1]: 363.15559.

2.2.8. N-(tert-Butyloxycarbonyl)-β-alanine (7-phenyl-2-oxo-2H-benzopyran-4-yl)methyl ester ***5c.*** Starting from compound **1c** (0.031 g, 1.2×10^{-4} mol) in dry DMF (2 mL), potassium fluoride (0.021 g, 3.6×10^{-3} mol) and *N*-(*tert*-butoxycarbonyl)-β-alanine **3** (0.023 g, 1.2×10^{-5} mol), the ester conjugate **5c** was obtained as a beige solid (0.028 g, 55%). Mp = 152.3-153.1 ºC. R_f = 0.61 (dichloromethane / methanol 100:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.45 (s, 9 H, C*(CH$_3$)$_3$*), 2.71 (m, 2 H, H-α), 3.47 (m, 2 H, H-β), 5.01 (br s, 1 H, NH), 5.59 (s, 2 H, CH_2), 6.49 (s, 1 H, H-3), 7.43 (tt, *J* 7.2 and 1.2 Hz, 1 H, H-4'), 7.50 (tt, *J* 7.2 and 1.2 Hz, 2 H, H-3' and H-5'), 7.54-7.59 (m, 3 H,

H-5, H-6 and H-8), 7.63 (dt, *J* 7.2 and 1.2 Hz, 2 H, H-2' and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.32 (C(CH_3)$_3$), 34.54 (C-α), 36.03 (C-β), 61.30 (CH_2), 79.62 (C(CH_3)$_3$), 113.11 (C-3), 115.44 (C-6), 115.93 (C-4a), 123.32 (C-8), 123.78 (C-5), 127.15 (C-2' and C-6'), 128.67 (C-4'), 129.10 (C-3' and C-5'), 138.78 (C-1'), 145.32 (C-7), 148.52 (C-4), 154.07 (C-8a), 155.72 (C=O Boc) 160.35 (C-2), 171.61 (C=O β-Ala) ppm. IR (liquid film): ν = 3434, 3334, 3078, 2976, 2929, 1743, 1713, 1618, 1546, 1500, 1438, 1409, 1366, 1327, 1246, 1167, 1092, 1066, 1042, 997, 962, 868, 837, 764, 748, 707, 689 cm^{-1}. HRMS: m/z (ESI): Found [M^++1]: 424.17608; $C_{24}H_{26}NO_6$ requires [M^++1]: 424.17611.

2.2.9. N-(tert-Butyloxycarbonyl)-β-alanine [7-(4'methoxyphenyl)-2-oxo-2H-benzopyran-4-yl] methyl ester ***5d.*** Starting from compound **1d** (0.023 g, 1.2 × 10^{-4} mol) in dry DMF (2 mL), potassium fluoride (0.021 g, 3.6 × 10^{-3} mol) and *N*-(*tert*-butoxycarbonyl)-β-alanine **3** (0.023 g, 1.2 × 10^{-5} mol), the ester conjugate **5c** was obtained as a beige solid (0.028 g, 51%). Mp = 126.9-127.5 ºC. R_f = 0.46 (dichloromethane/methanol 100:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.45 (s, 9 H, C$(CH_3)_3$), 2.71 (t, *J* 6 Hz, 2 H, H-α), 3.47 (q, *J* 6 Hz, 2 H, H-β), 3.88 (s, 3 H, OCH_3), 4.99 (br s, 1 H, NH), 5.35 (s, 2 H, CH_2) 6.46 (s, 1 H, H-3), 7.03 (dt, *J* 8.8 and 2 Hz, 2 H, H-3' and H-5'), 7.50-7.56 (m, 3 H, H-5, H-6 and H-8), 7.58 (dt, *J* 8,8 and 2 Hz, 2 H, H-2' and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.35 (C(CH_3)$_3$), 34.56 (C-α), 36.05 (C-β), 55.41 (OCH_3), 61.34 (CH_2), 79.63 (C(CH_3)$_3$), 112.74 (C-3), 114.58 (C-3' and C-5'), 114.76 (C-6), 115.39 (C-4a), 122.82 (C-8), 123.72 (C-5), 128.32 (C-2' and C-6'), 131.15 (C-1'), 144.96 (C-7), 148.57 (C-4), 154.18 (C-8a), 155.73 (C=O Boc), 160.26 (C-4'), 160.49 (C-2), 171.65 (C=O β-Ala) ppm. IR (liquid film): ν = 3368, 3056, 2977, 2934, 2840, 2050, 1722, 1610, 1580, 1526, 1444, 1407, 1367, 1302, 1279, 1253, 1170, 1083, 1028, 996, 955, 866, 839, 816,781, 735, 704 cm^{-1}. HRMS: m/z (ESI): Found [M^++1]: 454.18664; $C_{25}H_{28}NO_7$ requires [M^++1]: 454.18652.

2.2.10. N-(tert-Butyloxycarbonyl)-β-alanine (7-styryl-2-oxo-2H-benzopyran-4-yl)methyl ester ***5e.*** Starting from compound **1e** (0.031 g, 1.1 × 10^{-3} mol) in dry DMF (2 mL), potassium fluoride (0.018 g, 3.1 × 10^{-3} mol) and *N*-(*tert*-butoxycarbonyl)-β-alanine **3** (0.020 g, 1.1 × 10^{-4} mol), the ester conjugate **5c** was obtained as a yellow solid (0.011 g, 24%). Mp = 141.2-142.2 ºC. R_f = 0.38 (dichloromethane / methanol 100:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.45 (s, 9 H, C$(CH_3)_3$), 2.70 (t, *J* 6 Hz, 2 H, H-α), 3.47 (t, *J* 6 Hz, 2 H, H-β), 4.98 (br s, 1 H, NH), 5.33 (s, 2 H, CH_2), 6.45 (s, 1 H, H-3), 7.12 (d, *J* 16 Hz, 1 H, H-a), 7.25 (d, *J* 16 Hz, 1 H, H-b), 7.33 (tt, *J* 7.2 and 1.2 Hz, 1 H, H-4'), 7.40 (tt, *J* 7.2 and 1.2 Hz, 2 H, H-3' and H-5'), 7.45-7.51 (m, 3 H, H-5, H-6 and H-8), 7.43 (dd, *J* 7.2 and 1.2 Hz, 2 H, H-2' and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.30 (C(CH_3)$_3$), 34.51 (C-α), 35.99 (C-β), 61.27 (CH_2), 79.59 (C(CH_3)$_3$), 112.78 (C-3), 114.60 (C-6), 116.09 (C-4a),

122.56 (C-8), 123.59 (C-5), 126.51 (C-a), 126.88 (C-2' and C-6'), 128.57 (C-4'), 128.80 (C-3' and C-5'), 132.27 (C-b), 136.23 (C-1'), 141.66 (C-7), 148.40 (C-4), 154.12 (C-8a), 155.69 (C=O Boc), 160.36 (C-2), 171.60 (C=O β-Ala) ppm. IR (liquid film): ν = 3366, 3059, 2976, 2930, 1720, 1610, 1513, 1450, 1413, 1393, 1367, 1251, 1169, 1082, 1005, 967, 865, 865, 812, 757, 738, 694 cm^{-1}. HRMS: m/z (ESI): Found $[M^+ +1]$: 450.19174; $C_{26}H_{28}NO_6$ requires $[M^+ +1]$: 450.19184.

*2.3. General procedure for the synthesis of conjugates **6** and **7**.*

A solution of conjugates **4b**, **4e**, **5b** or **5e** (12-21 mmol) in dichloromethane/trifluoroacetic acid (2:1) (15 mL) was stirred at room temperature during 30 min. The reactional mixture was then evaporated to dryness and aqueous 1 M NaOH solution (3 mL) was added and the pH adjusted to 7. The mixture was extracted with dichloromethane (3 × 3 mL), the organic phase was dried with anhydrous magnesium sulphate, filtered and evaporated to dryness, resulting in the required compounds **6b**, **6e**, **7b** or **7e**.

*2.3.1. Glycine (7-amino-2-oxo-2H-benzopyran-4-yl)methyl ester **6b**.* Starting from compound **4b** (0.069 g, 2.0 × 10^{-4} mol), conjugate **6b** was obtained as a yellow solid (0.0472 g, 65%). Mp = 192.0-193.5 ºC. R_f = 0.18 (dichloromethane / methanol 20:1). ^{1}H NMR (CD_3OD, 400 MHz): δ = 4.05 (s, 2 H, H-α), 5.47 (s, 2 H, CH_2), 6.12 (s, 1 H, H-3), 6,53 (d, *J* 2 Hz, 1 H, H-8), 6.66 (dd, *J* 8.8 and 2 Hz, 1 H, H-6), 7.39 (d, *J* 8.8 Hz, 1 H, H-5) ppm. ^{13}C NMR (CD_3OD, 100.6 MHz): δ = 41.00 (C-α), 63.98 (CH_2), 100.67 (C-8), 106.76 (C-3), 107.95 (C-4a), 113.28 (C-6), 126.11 (C-5), 151.64 (C-4), 154.86 (C-8a), 157.36 (C-7), 164.00 (C-2), 168.27 (C=O Gly) ppm. IR (liquid film): ν = 1757, 1672, 1603, 1416, 1332, 1265, 1188, 1127, 882, 838, 800, 748, 720 cm^{-1}. HRMS: m/z (ESI): Found $[M^+ +1]$: 249.08759; $C_{12}H_{13}N_2O_4$ requires $[M^+ +1]$: 249.08767.

*2.3.2. Glycine (7-styryl-2-oxo-2H-benzopyran-4-yl)methyl ester **6e**.* Starting from compound **4e** (0.051 g, 1.2 × 10^{-4} mol), conjugate **6e** was obtained as an orange oil (0.0379 g, 66%). R_f = 0.44 (dichloromethane/methanol 20:1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 3.15 (s, 2 H, H-α), 4.76 (s, 2 H, CH_2), 6.42 (s, 1 H, H-3), 7.28-7.33 (m, 2 H, H-4'and H-a), 7.36-7.41 (m, 4 H, NH_2, H-3'and H-5'), 7.49 (d, 1 H, *J* 16.8 Hz, H-b), 7.58-7.65 (m, 4 H, H-2', H-6', H-6 and H-8), 7.68 (d, *J* 8 Hz, 1 H, H-5) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 51.70 (C-α), 59.05 (CH_2), 110.10 (C-3), 113.81 (C-6), 116.49 (C-4a), 122.52 (C-8), 124.62 (C-5), 126.81 (C-a), 126.93 (C-2' and C-6'), 128.38 (C-4'), 128.85 (C-3' and C-5'), 131.59 (C-b), 136.55 (C-1'), 140.97 (C-7), 153.50 (C-8a), 156.38 (C-4), 160.32 (C-2), 171.08 (C=O Gly) ppm. IR (liquid film): ν = 3418, 2925, 2855, 2663, 2362, 1758, 1681, 1612, 1546, 1433, 1329, 1206, 1134, 1076, 1003, 952, 922.2, 886, 839, 801, 722 cm^{-1}. HRMS: m/z (ESI): Found $[M^+ +1]$: 336.12364; $C_{20}H_{18}NO_4$ requires $[M^+ +1]$: 336.12358.

*2.3.3. β-Alanine (7-amino-2-oxo-2H-benzopyran-4-yl)methyl ester **7b**.* Starting from compound **5b** (0.0721 g, 2.1 × 10^{-4} mol), conjugate **7b** was obtained as a yellow solid (0.054 g, 67%). Mp = 201.6-203.0 ºC. R_f = 0.18 (dichloromethane / methanol 20:1) ^{1}H NMR (CD_3OD, 400 MHz): δ = 2.93 (t, *J* 6.4 Hz, 2 H, H-α), 3.33 (t, *J* 6.4 Hz, 2 H, H-β), 5.47 (s, 2 H, CH_2), 6.12 (s, 1 H, H-3), 6.53 (d, *J* 2 Hz, 1 H, H-8), 6,66 (dd, *J* 8.8 and 2 Hz, 1 H, H-6), 7.39 (d, *J* 8.8 Hz, 1 H, H-5) ppm. ^{13}C NMR (CD_3OD, 100.6 MHz): δ = 32.11 (C-α), 36.26 (C- β), 63.14 (CH_2), 100.64 (C-8), 107.21 (C-3), 108.94 (C-4a), 113.82 (C-6), 126.23 (C-5), 152.25 (C-4), 153.05 (C-8a), 157.23 (C-7), 163.94 (C-2), 168.27 (C=O β-Ala) ppm. IR (liquid film): *v* = 1738, 1680, 1413, 1329, 1202, 1147, 846, 797, 747, 723 cm^{-1}. HRMS: m/z (ESI): Found [M^++1]: 263.10325; $C_{13}H_{15}N_2O_4$ requires [M^++1]: 263.10327.

*2.3.4. β-Alanine (7-styryl-2-oxo-2H-benzopyran-4-yl)methyl ester **7e**.* Starting from compound **5e** (0.0551 g, 1.2 × 10^{-4} mol), conjugate **7e** was obtained as a yellow solid (0.0403 g, 64%). Mp = 205.1-206.2 ºC. R_f = 0.44 (dichloromethane/methanol 20:1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.84 (t, *J* 6.8 Hz, 2 H, H-α), 3.11 (t, *J* 6.8 Hz, 2 H, H-β), 5.43 (s, 2 H, CH_2), 6.47 (s, 1 H, H-3), 7.32-7.34 (m, 2 H, H-4'and H-a), 7.41 (td, *J* 6.8 and 2.4 Hz, 2 H, H-3'and H-5'), 7.51 (d, 1 H, *J* 16.8 Hz, H-b), 7.63-7.68 (m, 4 H, H-2', H-6', H-6 and H-8), 7.75-7.79 (m, 3 H, H-5 and NH_2) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 31.43 (C-α), 34.64 (C-β), 61.61 (CH_2), 111.91 (C-3), 113.91 (C-6), 115.97 (C-4a), 122.75 (C-8), 125.09 (C-5), 126.78 (C-a), 126.97 (C-2' and C-6'), 128.50 (C-4'), 128.87 (C-3' and C-5'), 131.99 (C-b), 136.46 (C-1'), 141.46 (C-7), 149.70 (C-4), 153.61 (C-8a), 159.78 (C-2), 169.97 (C=O β-Ala) ppm. IR (liquid film): *v* = 1712, 1608, 1452, 1411, 1324, 1201, 1129, 1070, 1010, 974, 901, 867, 831, 801, 760, 720 cm^{-1}. HRMS: m/z (ESI): Found [M^++1]: 350.13930; $C_{21}H_{19}N_2O_5$ requires [M^++1]: 350.13943.

2.4. General photolysis procedure

A 1×10^{-4} M methanol/HEPES (80:20) solution of compounds **4**-**7** (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 254, 300, 350 and 419 ± 10 nm. The HEPES buffer solution was prepared using distilled water with HEPES (10 mM), sodium chloride (120 mM), potassium chloride (3 mM), calcium chloride (1 mM) and magnesium chloride (1 mM). The pH was adjusted to 7.2 by addition of an aqueous solution on sodium hydroxide 6M.

Aliquots of 100 µL were taken at regular intervals and analysed by RP-HPLC. The eluent was acetonitrile / water (3:1) for all the compounds, excepting compounds **4b** and **5b** (acetonitrile / water (1:1) with 0.1% TFA), **6e** and **7e** (acetonitrile/water (1:1)), at a flow rate of 0.8 mL/min (**4b**,

4e, **5a**, **5d**, **5e**, **6b**, **7b**), 0.6 mL/min for (**4c**, **4d**, **5b**, **5c**) and 0.4 mL/min (**4a**), 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: **4a**, 6.3; **5a**, 4.2; **4b**, 6.4; **5b**, 9.3; **4c**, 6.3; **5c**, 8.8; **4d**, 10.6; **5d**, 7.0; **4e**, 7.0; **5e**, 11.5; **6b**: 13.4; **7b**: 4.8 min).

3. Results and Discussion

*3.1. Synthesis of amino acid conjugates **4**-**7***

4-(Chloromethyl)-2*H*-benzopyran-2-one **1a**, 7-amino-4-(chloromethyl)-2*H*-benzopyran-2-one **1b**, 4-(chloromethyl)-7-phenyl-2*H*-benzopyran-2-one **1c**, 4-(chloromethyl)-7-(4'-methoxyphenyl)-2*H*-benzopyran-2-one **1d** and 4-(chloromethyl)-7-styryl-2*H*-benzopyran-2-one **1e** were synthesised by Pechmann condensations between phenol, ethyl (3-hydroxyphenyl)carbamate, [1,1'-biphenyl]-3-ol, 4'-methoxy-[1,1'-biphenyl]-3-ol, 3-styrylphenol, respectively, and ethyl 4-chloro-3-oxobutanoate, mediated by aqueous sulfuric acid through a known procedure [43], followed by acidolysis (sulfuric acid in acetic acid) of ethyl (4-(chloromethyl)-2-oxo-2*H*-benzopyran-7-yl)carbamate precursor in the case of compound **1b** (see supporting information). Compounds **1a**-**e** were used in the preparation of ester conjugates of glycine and β-alanine, as representative models of carboxylic acids, by reacting with *N*-*tert*-butyloxycarbonylglycine **2** and *N*-*tert*-butyloxycarbonyl-β-alanine **3**, in the presence of potassium fluoride in DMF at room temperature, yielding cages **4a**-**e** and **5a**-**e**, respectively, in 16 to 70% yields. Therefore, in compounds **4b**, **4e**, **5b** and **5e** the *N*-*tert*-butyloxycarbonyl protecting group was removed by acidolysis with trifluoroacetic acid in dichloromethane giving conjugates **6b**, **6e**, **7b** and **7e** bearing the amino acid and the photosensitive tag (Scheme 1).

All compounds were fully characterized by IR, ^{1}H and ^{13}C NMR spectroscopies. ^{1}H NMR spectra of conjugates **4**-**7** showed the amino acid residues, such as the α-CH_2 (δ 3.15-4.08 ppm for glycine and δ 2.58-2.93 ppm for β-alanine), and β-CH_2 (δ 3.11-3.47 ppm), in addition to the heterocycle methylene group visible for all conjugates (δ 4.76-5.59 ppm). The newly formed ester linkages were established by their ^{13}C NMR spectra signals of the carbonyl group, at about δ 168.27-171.65 ppm. The confirmation of the cleavage of the *N*-*tert*-butyloxycarbonyl-protecting group at the amino acids was also supported by ^{13}C NMR, with the disappearance of spectra signals of the corresponding carbonyl group in **6b**, **6e**, **7b** and **7e**, which occurred at δ 155.61 or 155.94 ppm in conjugates **4b**, **4e**, **5b** and **5e**. The IR spectra of compounds **4**-**7** displayed stretching vibration bands of the ester carbonyl groups from 1712 to 1758 cm^{-1}.

<Scheme 1>

*3.2. Evaluation of the photophysical properties of amino acid conjugates **4-7***

With the aim of finding the parameters required for monitoring the photolytic process, as well as to evaluate the effect of the fluorophore structure in different solvents, such as diethyl ether, dichloromethane, acetonitrile, dimethyl sulfoxide, methanol and methanol/HEPES buffer (80:20), characterization by UV/visible absorption was carried out. Furthermore, fluorescence emission data was also collected in methanol and methanol/HEPES buffer (80:20). Degassed 10^{-4} M solutions of ester conjugates **4**-**7** and the corresponding coumarins were measured. The corresponding data: absorption (λ_{abs}) and emission maxima (λ_{em}), molar extinction coefficients (as log ε) and relative fluorescence quantum yields (Φ_F) are reported in Table 1. Relative fluorescence quantum yields were calculated using 9,10-diphenylanthracene in ethanol (Φ_F 0.95) [44], with the fluorescence standard excited at the maximum absorption wavelength for each compound.

Concerning the absorption data of conjugates **4**-**7**, variation of the solvent in general did not significantly affect the wavelength of maxima absorption; however, as it was expected, the increase of the π system extension resulted in a bathochromic shift (λ_{abs} **4a** < **4c** < **4d** < **4e** and **5a** < **5c** < **5d** < **5e**). Despite the higher conjugation of systems possessing aromatic substituents at the coumarin skeleton (compounds **4c**-**e** and **5c**-**e**), comparatively to the amino group (compounds **4b** and **5b**) the former presented higher λ_{abs} in dimethyl sulfoxide, methanol and methanol/HEPES buffer (80:20), in the range 353-364 nm.

<Table 1>

To investigate the fluorescence behaviour of the compounds excitation-emission matrices (EEM's) were obtained; with the emission intensity scanned for different excitation wavelengths. The outcome for representative compounds (**1**, **4** and **5**; **b**, **d** and **e** - in methanol/ HEPES), showing a contour surface of fluorescence intensity for the different excitation / emission wavelengths, are given in Figure 1. These fluorescence contour surfaces indicate that the steady state fluorescence behaviour of the compounds is quite similar, with an emission maximum close to 480 nm with an optimal excitation around 360 nm.

<Figure 1 >

*3.3. Photolysis studies of amino acid conjugates **4-7***

Comparison of the sensitivity towards UV/visible radiation of glycine and β-alanine ester conjugates **4-7**, bearing different substituents at position 7 of the heterocycle, was carried out by exposing solutions of the mentioned compounds in methanol/HEPES buffer (80:20) solution in a Rayonet RPR-100 reactor at 254, 300, 350 and 419 nm. 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 represents the time necessary for the consumption of the starting materials until less than 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. The corresponding rate constants were calculated and are presented in Table 2. The photochemical quantum yields (Φ_{phot}) were calculated based on half-lives ($t_{1/2}$), extinction coefficient (ε) and the incident photon flux (I_0), which was determined by potassium ferrioxalate actinometry [45]. 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.

<Table 2>

It was expected that the bathochromic shift in the maximum absorption wavelengths, related to the increase of the π conjugated system due to the presence of aromatic substitution at position 7 of the benzopyran, 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, since photocleavage at 350 and 419 nm of conjugates **4e** and **5e** occurred fast (57 and 109 min at 350 nm; 318 and 1359 min at 419 nm), in comparison with conjugates **4a**, **4c**, **4d**, **5a**, **5c** and **5d**. Furthermore, at these wavelengths the release of *N-tert*-butyloxycarbonyl glycine **2** and β-alanine **3** required shorter irradiation times than the corresponding free amino acids from cages **6b** and **7b**. Nonetheless, the best results of photolysis obtained at all the wavelengths (254, 300, 350 and 419 nm) considered in the present study, including at 419 nm were related to conjugates **4b** (5-24 min) and **5b** (7-85 min), possessing the electron donating amino group at position 7 of the heterocycle. Although the importance of all the fluorescent caging groups evaluated, the use of 7-amino-4-(chloromethyl)-2*H*-benzopyran-2-one **1b** is the most feasible for release of the amino acids at 419

nm both for organic synthesis and caging applications, owing to the short irradiation times and lowest possible side reactions concerning the functionalities of the molecule. Coumarinyl esters are thought to cleave through an ionic mechanism that involves both homolytical or heterolytical fission of the O-CH_2 bond, although the latter is energetically favoured. The homolytic cleavage of the O-C bond, followed by electron transfer, can yield the ion pair (a methylenic coumarin carbocation and the leaving group anion), whereas the heterolytic cleavage of the O-C bond directly affords the already mentioned ion pair. Once formed, the methylenic coumarin carbocation can undergo nucleophilic attack by the solvent to form the final products. The ion pair may also recombine to the starting material [46-47]. In our previous work, after irradiation at the various wavelengths of irradiation, by NMR monitoring a new set of signals were identified in the aromatic region with slight shifts of the signals from those of the starting conjugates, which were in agreement with the proposed coumarin by-products, by NMR and mass spectrometry. In recent literature and in terms of potential toxicity issues, there are various reports on the use of coumarin derivatives in photodelivery applications that have undergone biological validation [48-52].

In order to better understand the process, time-resolved fluorescence measurements were made exciting at 349 nm, with the emission monitored at 480 nm. The time-resolved decays are shown in Figure 2 and in all cases the fluorescence was multi-exponential in nature indicating the presence of (at least) two excited states. Compound **1b** exhibited a dominant (>90%) decay time of ~5 ns, resulting in an average decay time of 4.8 ns. The other **b** compounds gave average decay times that were significantly less (**4b**, 1.36 ns; **5b**, 2.32 ns; **6b**, 1.75 ns; **7b**, 1.84 ns). This was because of the presence of other (non-radiative) decay pathways as expected since the photocleavage of the conjugate would be expected to proceed by the production of an ion pair, which would then either recombine or lead to the photoproducts. Thus, because of the use of the fluorescent coumarin moiety, the time-resolved behaviour can report on this process. The **d** and **e** compounds, however, did not display any major difference in their relative decay times. Average decay times were; **1d**, 3.2 ns; **4d**, 2.99 ns; **5d**, 3.12 ns; **1e**, 2.12 ns; **4e**, 3.09 ns; **5e**, 2.81 ns. This may indicate that the conjugate is having any significant influence on the photophysical properties of the coumarin moiety. During these experiments, it is assumed that any photocleavage of the conjugate is not significant.

To further characterise these compounds the **b** compounds were selected and from the time-resolved datasets (decays every 5 nm, spanning 375 nm to 575 nm) decay associated spectra determined. This involves a global analysis of the time-resolved decays, linking common decay times for all decays, and then plotting the pre-exponential factor for a decay time (weighted by that decay time) against wavelength. This helps elucidate both the energy (wavelength) and kinetic of the excited states, hence species, present. The results from this form of analysis are presented in

Figure 3. The lifetimes obtained are similar to those from the single curves analysis, however the presence of a sub-nanosecond contribution is now elucidated. Since these spectra are weighted by the decay time they are representative of the contribution to the steady state emission. For **1b** the emission is dominated by the spectra associated with the longer-lived emission. For **4b**, **5b** and **7b** however the major contribution is that between ~1.3 ns to 2.3 ns, although similar in spectral appearance. This would indicate that in the latter compounds the decay time has been reduced because of the presence of non-radiative decay pathways. For compound **6b** the emission is dominated by the longer-lived emission, which because of the decay properties of **1b** would be tempting to attribute to the photocleaved coumarin moiety. Because of the spectral separation it is clear that overall at least three different species are present in the conjugated compounds. It would be expected that conjugated, ion pair and photocleaved coumarin moiety would be present, with the longer-lived decay associated with the photocleaved species and the shortest decay probably associated with the conjugated form.

In addition to the HPLC/UV monitoring of the photolytic release of glycine and β-alanine, as the expected products of the photolysis, was also followed by ^{1}H NMR in a methanol-d_4/D_2O (80:20) solution. This methodology was particularly important for conjugates **6b** and **7b**, due to difficulties in the monitoring by HPLC-UV. NMR monitoring was carried out with 2.50×10^{-2} M solutions, which led to an expected increase in the photolysis time for the complete release of the molecule, when compared to the irradiation times in Table 2 obtained with dilute solutions.

As representative examples, upon irradiation at 350 nm of solutions of *N*-(*tert*-butyloxycarbonyl) conjugates of glycine **4b** and β-alanine **5b** and of glycine **6b** and β-alanine **7b** conjugates, a decrease in the intensity of the signals was observed and this was related to the amino acid in the conjugates form namely the α-CH_2 ($\delta \approx$ 4.00-4.10 ppm, **4b** and **6b**; $\delta \approx$ 3.30-3.40 ppm, **5b** and **7b**) and β-CH_2 ($\delta \approx$ 2.70-3.00 ppm, **5b** and **7b**), as well as the singlet for the benzylic-type CH_2 of the heterocycle ($\delta \approx$ 5.40-5.60 ppm). The *N*-Boc glycine **2** and β-alanine **3** released from conjugates **4b** and **5b**, and free glycine or β-alanine released from conjugates **6b** and **7b**, gave rise to a new set of signals with the same multiplicity at lower chemical shift, namely α-CH_2 ($\delta \approx$ 3.80-3.70 ppm, **4b** and **6b**; $\delta \approx$ 3.30 ppm, **5b** and **7b**) and β-CH_2 ($\delta \approx$ 2.50-2.70 ppm, **5b** and **7b**) (Figure 4). Based on the results obtained by ^{1}H NMR, it was possible to corroborate the release of the amino acids from these conjugates in methanol/HEPES buffer (80:20) solutions at higher concentrations.

< Figure 4 >

4. Conclusion

The comparative photorelease of glycine and β-alanine, as representative models of carboxylic acid bioactive molecules, from 4-(methylene)-2*H*-benzopyran-2-one unsubstituted and substituted with different substituents at the 7-position of the heterocyclic system, such as amino, phenyl, 4-methoxyphenyl and styryl groups, under irradiation at variable wavelengths (254, 300, 350 and 419 nm) was carried out. A remarkable behaviour towards irradiation at higher wavelength (350 and 419 nm) occurred in conjugates obtained from 4-(chloromethyl)-7-styryl-2*H*-benzopyran-2-one, however the best results were related to 4-(chloromethyl)-7-phenyl-2*H*-benzopyran-2-one, with release of glycine and β-alanine in shorter irritation times (*ca* 8 min at 350 nm; 5 and 85 min, at 419 nm, respectively), which can be considered in practicable applications. The photolysis observations were also complemented by time-resolved fluorescence, which indicated the presence of different fluorescing species elucidated by the determination of decay associated spectra.

Acknowledgements

Thanks are due to *Fundação para a Ciência e Tecnologia* (FCT) and FEDER (European Fund for Regional Development)-COMPETE-QREN-EU for financial support through the Chemistry Research Centre of the University of Minho (Ref. UID/QUI/00686/2013 and UID/ QUI/0686/2016). The NMR spectrometer Bruker Avance III 400 is part of the National NMR Network and was purchased within the framework of the National Program for Scientific Re-equipment, contract REDE/1517/RMN/2005 with funds from POCI 2010 (FEDER) and FCT.

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CAPTIONS

Scheme 1. Synthesis of ester cages of glycine and β-alanine **4**-**7**.

Table 1: UV-visible data (λ_{abs} and log ε) for compounds **1a-e** and conjugates **4**-**7** in diethyl ether, dichloromethane, acetonitrile, dimethyl sulfoxide, methanol and methanol/HEPES buffer (80:20). [a] in nm.

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 **4**-**7** at 254, 300, 350 and 419 nm in methanol/HEPES buffer (80:20) solution

Figure 1. EEMs for selected compounds in methanol/ HEPES. The relative intensity is shown in a rainbow colour scale with red most intense, blue (black) least intense. The excitation wavelengths are plotted on the y-axis, with emission on the x-axis.

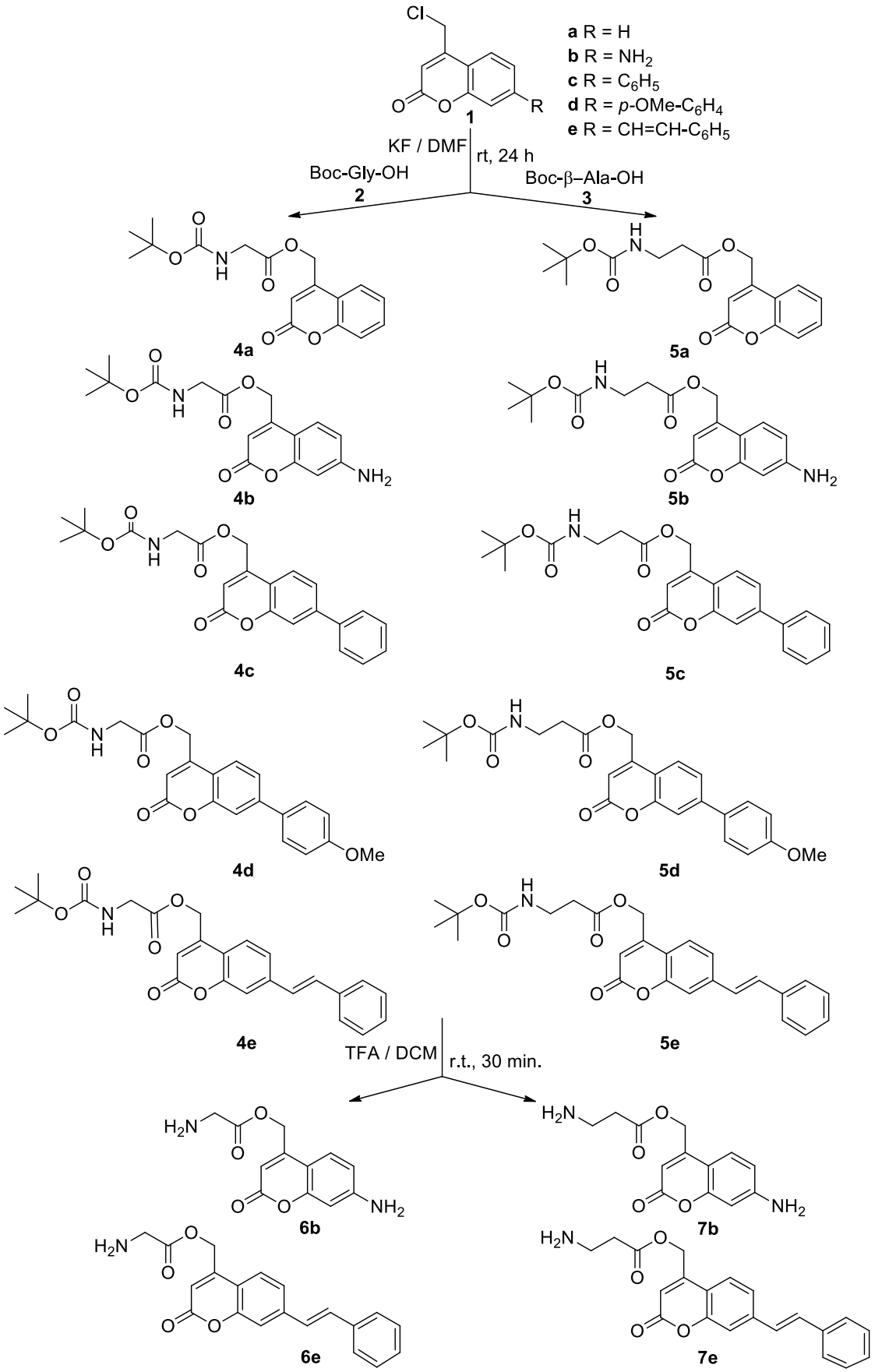
Figure 2. Time-resolved fluorescence decays for selected compounds in methanol/ HEPES. The compounds were excited at 349 nm with the emission monitored at 480 nm.

Figure 3. Decay associated spectra for selected compounds in methanol/ HEPES. The excitation wavelength was 349 nm.

Figure 4. Partial ^{1}H NMR spectra in methanol-d_4/D_2O (80:20) of the photolysis at 350 nm of 2.50 × 10^{-2} M solutions of *N*-Boc conjugates of glycine **4b** and β-alanine **5b**: a) before irradiation; b) after irradiation for 90 min; and c) after irradiation for 150 min; and glycine conjugate **6b** and β-alanine conjugate **7b**: a) before irradiation; b) after irradiation for 150 min; and c) after irradiation for 270 min.

SCHEMES

Scheme 1



TABLES

Table 1

Cpd	Diethyl ether		Dichloromethane		Acetonitrile		Dimethyl sulfoxide		Methanol		Methanol/HEPES buffer (80:20)	
	λ_{abs}[a]	log ε	λ_{abs}[a]	log ε	λ_{abs}[a]	log ε	λ_{abs}[a]	log ε	λ_{abs}[a]	log ε	λ_{abs}[a]	log ε
1a	310	3.50	314	3.34	308	3.37	316	3.29	310	3.43	310	3.29
1b	346	4.14	347	4.19	350	4.18	365	4.23	361	4.21	357	3.98
1c	327	4.14	331	4.01	324	4.05	325	3.97	327	4.09	329	4.13
1b	335	4.12	342	4.09	338	4.11	343	4.16	339	4.23	342	4.14
1e	349	4.21	356	4.13	349	4.06	358	4.00	352	4.15	353	4.18
4a	299	3.72	314	3.59	310	3.67	312	3.66	309	3.69	305	3.52
4b	341	4.24	343	4.13	348	4.11	364	4.11	353	4.20	353	4.18
4c	326	4.13	329	4.02	323	4.00	326	4.03	326	4.11	323	4.08
4d	329	4.03	338	3.99	332	4.00	336	4.06	337	4.04	335	3.91
4e	345	4.09	354	4.05	348	4.02	353	3.90	348	3.97	352	3.98
5a	305	3.69	314	3.53	310	3.6	310	3.51	308	3.66	308	3.54
5b	341	4.19	342	4.06	348	4.11	362	4.17	356	4.12	356	4.14
5c	324	4.23	328	4.07	323	4.00	326	4.17	326	4.11	323	4.08
5d	336	4.22	339	4.20	335	4.22	338	4.20	336	4.25	340	4.11
5e	347	4.30	354	4.33	348	4.16	353	4.17	350	4.24	354	4.17
6b	335	2.76	335	2.60	345	3.65	356	4.00	356	4.01	361	4.05
6e	333	3.13	338	3.36	335	3.61	342	3.69	341	3.61	347	3.83
7b	333	2.52	336	2.67	347	3.91	355	3.95	356	3.97	361	4.01
7e	344	2.72	353	3.68	349	4.10	351	4.24	352	4.27	354	4.25

Table 2

Cpd	254 nm			300 nm			350 nm			419 nm		
	t_{irr}	k	Φ_{phot}	t_{irr}	k	Φ_{phot}	t_{irr}	k	Φ_{phot}	t_{irr}	k	Φ_{phot}
4a	77	3.9	0.41	83	3.6	0.18	- [a]	- [a]	- [a]	- [a]	- [a]	- [a]
4b	12	23.7	0.63	24	12.5	0.15	8	36.3	0.41	4.9	5.3	0.06
4c	47	6.7	0.17	128	2.4	0.03	421	0.8	0.005	1610	0.2	0.003
4d	105	2.8	0.08	189	1.6	0.04	268	1.1	0.03	382	0.8	0.02
4e	83	3.6	0.14	110	2.7	0.05	57	4.9	0.13	318	0.8	0.02
5a	84	3.6	0.37	80	3.7	0.19	- [a]	- [a]	- [a]	- [a]	- [a]	- [a]
5b	15	19.7	0.54	16	18.0	0.30	7	42.8	0.60	85	3.4	0.05
5c	248	1.3	0.03	137	2.2	0.03	421	0.8	0.007	14945	0.02	0.0002
5d	887	0.3	0.009	3778	0.08	0.001	- [a]	- [a]	- [a]	- [a]	- [a]	- [a]
5e	175	1.8	0.04	178	1.7	0.02	109	2.7	0.03	1359	0.2	0.003
6b	100	3.2	0.05	73	4.2	0.03	71	4.3	0.04	- [a]	- [a]	- [a]
7b	171	1.8	0.03	114	2.6	0.03	123	2.4	0.03	775	0.38	0.004

[a] No cleavage was detected up to 8h of irradiation.

FIGURES

Figure 1

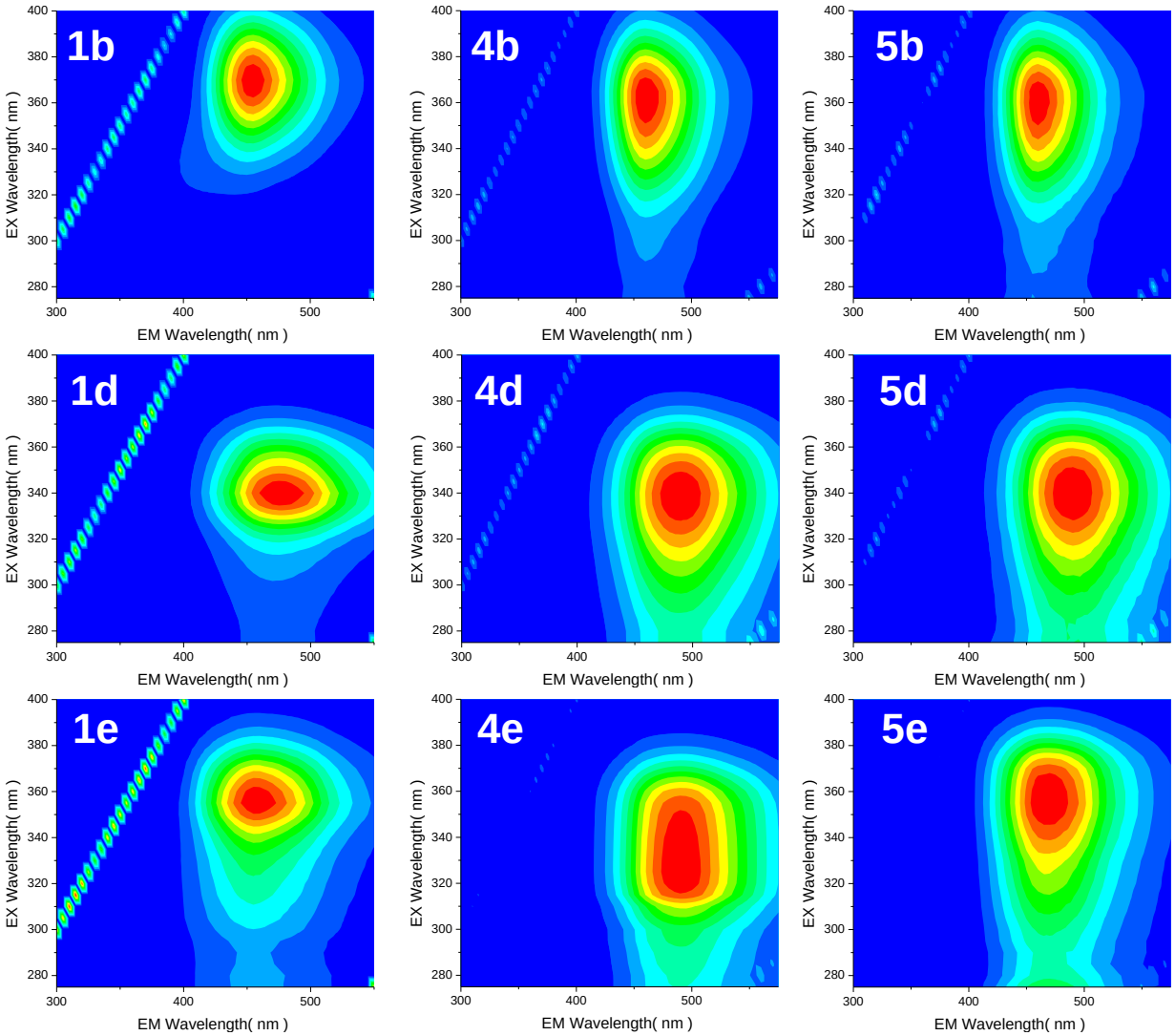
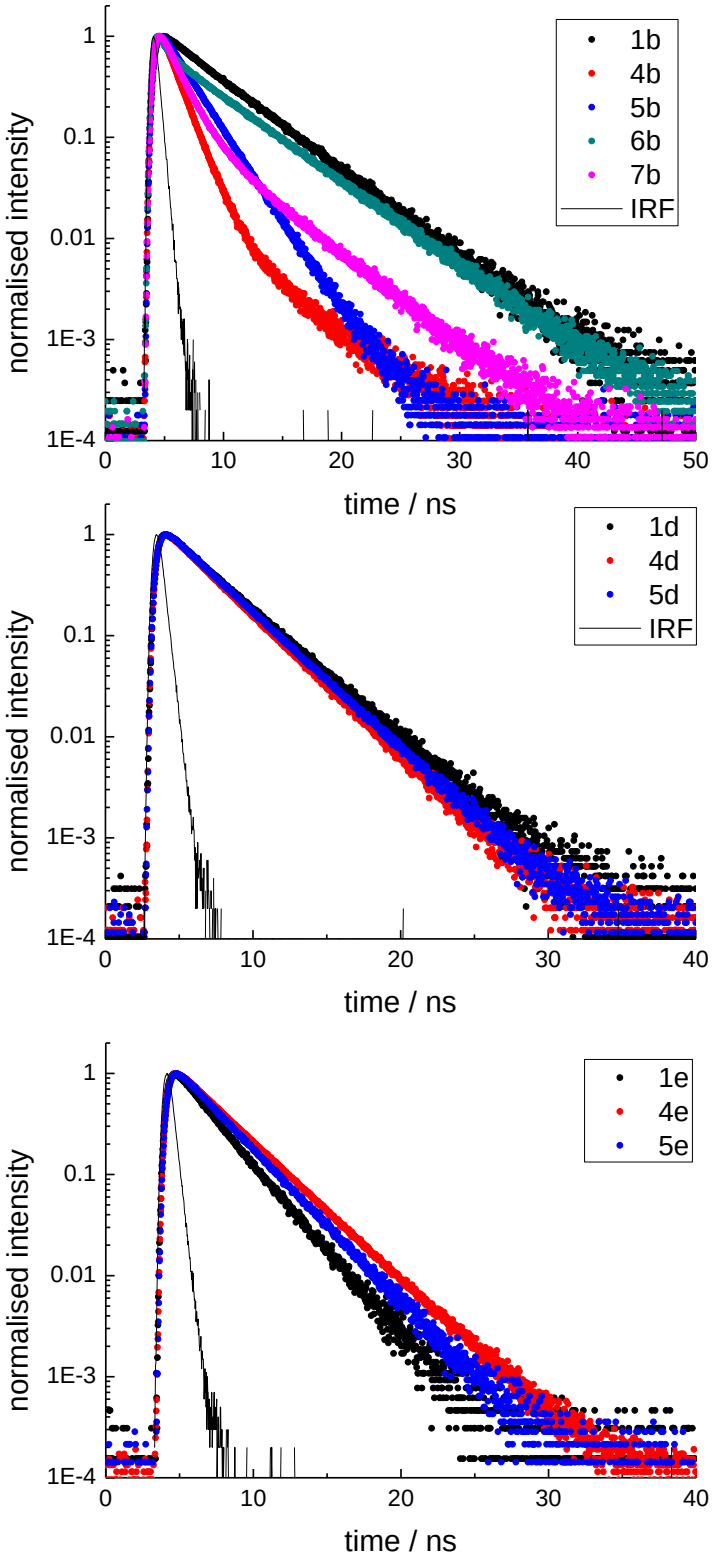


Figure 2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50
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4b
5b
6b
7b
IRF
normalised intensity
1
0.1
0.01
1E-3
1E-4
0
10
20
30
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time / ns
1d
4d
5d
IRF
normalised intensity
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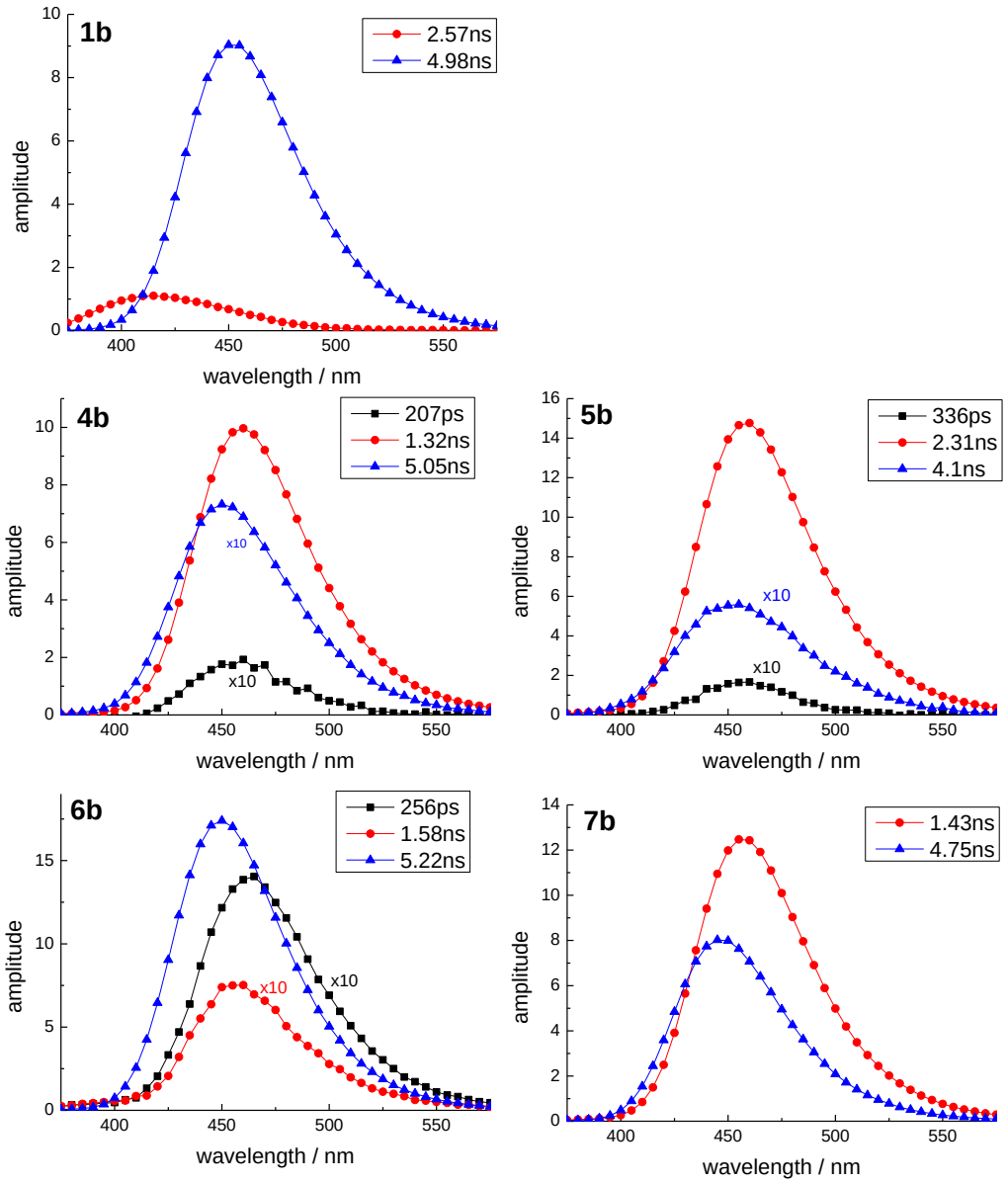
4e
5e

Figure 3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x10
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wavelength / nm
5b
336ps
2.31ns
4.1ns
x10
x10
amplitude
wavelength / nm
6b
256ps
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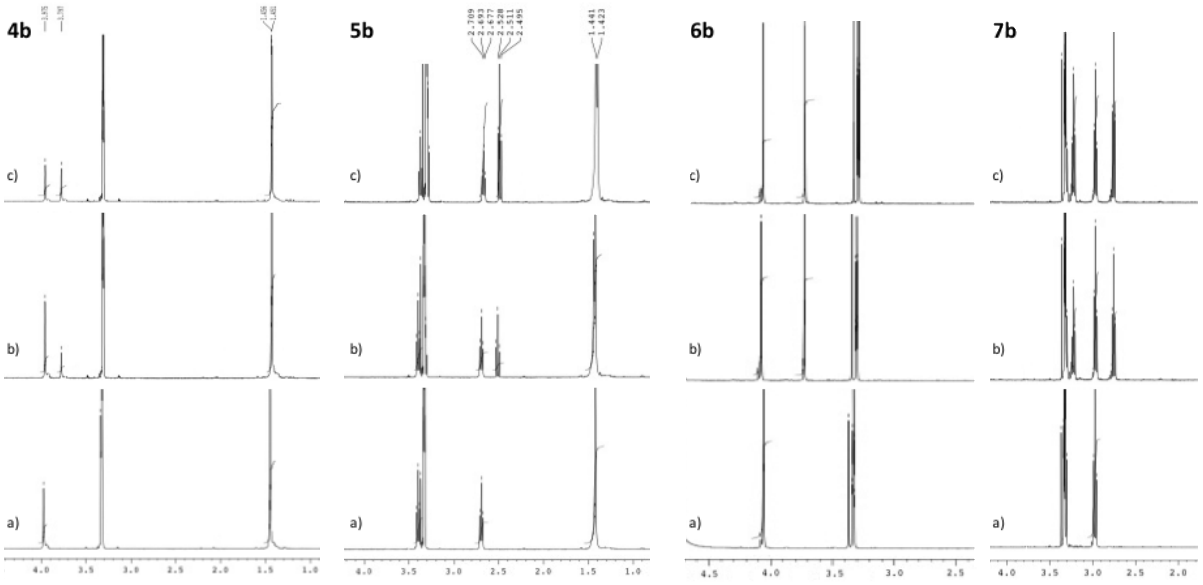
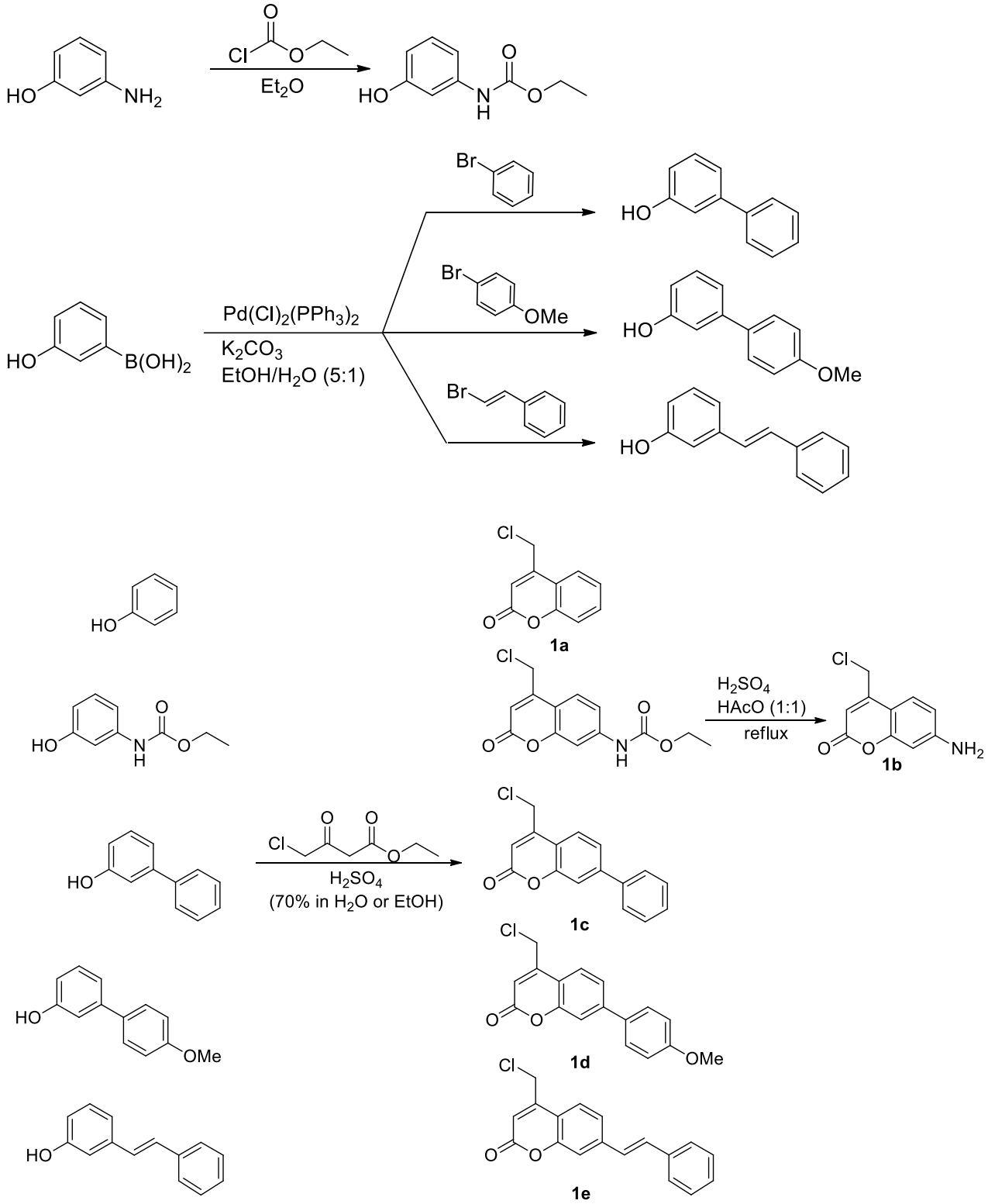
amplitude
wavelength / nm

Figure 4



SUPPORTING INFORMATION

Scheme 1: Synthesis of phototriggers **1a-e**.



1. Synthesis of phototriggers 1a-e

1.1. Synthesis of 4-(chloromethyl)-2H-benzopyran-2-one ***1a***. A solution of phenol (0.151 g, 1.6×10^{-2} mol) and ethyl 4-chloro-3-oxobutanoate (0.43 mL, 3.2×10^{-2} mol) in 70% aqueous sulfuric acid (10 mL) was stirred at room temperature during 4 days, and followed by TLC (ethyl acetate/light petroleum 1:1). The reactional mixture was poured into water and extracted with ethyl acetate; the organic phase was dried with anhydrous magnesium sulfate and evaporated. The dark pink oil was purified by column chromatography using dichloromethane as eluent, giving compound **1a** as white crystals (0.053 g, 17%). Mp = 135-135.4 ºC. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.69 (s, 2 H, CH_2Cl), 6.59 (s, 1 H, H-3), 7.35 (dt, *J* 7.2 and 1.2 Hz, 1 H, H-6), 7.39 (dd, *J* 8.4 and 0.8 Hz, 1 H, H-8), 7.58 (dt, *J* 7.2 and 1.6 Hz, 1 H, H-7), 7.68 (dd, *J* 8 and 1.6 Hz, 1 H, H-5) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 41.20 (CH_2Cl), 115.94 (C-3), 117.27 (C-4a), 117.48 (C-8), 124.11 (C-5), 124.50 (C-6), 132.27 (C-7), 149.43 (C-4), 153.83 (C-8a), 160.19 (C-2) ppm. IR (liquid film): ν = 3415, 3085, 2964, 1735, 1608, 1564, 1448, 1390, 1260, 1186, 1159, 1087, 957, 918, 898, 854, 803, 774, 753,733, 620 cm^{-1}.

1.2. Synthesis of 7-amino-4-(chloromethyl)-2H-benzopyran-2-one ***1b.*** Ethyl (4-(chloromethyl)-2-oxo-*2H*-benzopyran-7-yl)-carbamate (0.258 g, 9.2×10^{-4} mol) was refluxed for 1.5 h in a mixture of concentrated sulfuric acid and glacial acetic acid (1:1; 8 mL). The reaction was followed by TLC (ethyl acetate/light petroleum 1:1), and when completed, the orange mixture was poured into ice cold water (50 mL). An aqueous solution of 6 M sodium hydroxide was added until pH was approximately 5. A solid was precipitated and filtered giving compound **1b** as yellow solid (0.187 g, 98%). Mp = 219.2-220 ºC. R_f = 0.31 (ethyl acetate / light petroleum, 1:1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 4.85 (s, 2 H, CH_2Cl), 6.17 (s, 1 H, H-3), 6.43 (d, *J* 2.4 Hz, 1 H, H-8), 6.58 (dd, *J* 8.4 and 2.4 Hz, 1 H, H-6), 7.47 (d, *J* 8.8 Hz, 1 H, H-5) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 41.43 (CH_2Cl), 98.68 (C-8), 106.11 (C-4a), 107.87 (C-3), 111.32 (C-6), 126.11 (C-5), 151.28 (C-4), 153.37 (C-7), 155.95 (C-8a), 160.66 (C-2) ppm. IR (KBr 1%): ν = 3418, 2255, 2129, 1655, 1238, 1048, 1026, 1002, 825, 764 cm^{-1}.

Synthesis of ethyl (4-(chloromethyl)-2-oxo-2H-benzopyran-7-yl)-carbamate. Ethyl (3-hydroxyphenyl)carbamate (0.335 g, 1.85×10^{-4} mol) and ethyl 4-chloro-3-oxobutanoate (1.07 mL, 1.0×10^{-2} mol) were dissolved in 70% ethanolic sulfuric acid (5 mL). The reaction mixture was followed by TLC (ethyl acetate/light petroleum 1:1) and stirred at room temperature for 3 days. When the reaction was completed, the mixture was poured into water and extracted with ethyl acetate. The organic phase was dried with anhydrous magnesium sulphate and evaporated, obtaining a mixture of an orange oil and beige solid which was washed with ethyl acetate. The solid

was filtrated resulting compound **3** as a beige solid (0.258 g, 50%). Mp = 182.7-183.4 ºC. R_f = 0.62 (ethyl acetate/light petroleum 1:1). ^{1}H NMR (DMSO-d_6, 400 MHz): 1.25 (t, *J* 7.2 Hz, 3 H, CH_2CH_3), 4.16 (q, *J* 7.2 Hz, 2 H, CH_2CH_3), 4.96 (s, 2 H, CH_2Cl), 6.50 (s, 1 H, H-3), 7.41 (dd, *J* 8.8 and 2.4 Hz, 1 H, H-6), 7.72 (d, *J* 2 Hz, 1 H, H-8), 7.75 (d, *J* 8.8 Hz, 1 H, H-5), 10.18 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 14.37 (CH_2CH_3), 41.20 (CH_2Cl), 60.76 (CH_2CH_3), 104.61 (C-8), 111.60 (C-4a), 112.67 (C-3), 114.32 (C-6), 125.88 (C-5), 143.18 (C-7), 150.63 (C-4), 153.31 (C=O carbamate), 154.28 (C-8a), 159.90 (C-2) ppm. IR (KBr 1%,): ν = 3287, 3087, 2983, 1710, 1622, 1594, 1530, 1476, 1415, 1386, 1366, 1335, 1287, 1240, 1172, 1080, 1042, 1017, 985, 921, 886, 863, 850, 819, 766 cm^{-1}.

Synthesis of ethyl (3-hydroxyphenyl)carbamate. 3-Aminophenol (0.39 g, 3.0 × 10^{-3} mol) and ethyl chloroformate (0.74 mL, 6 × 10^{-3} mol) were dissolved in diethyl ether (40 mL). The reaction mixture was stirred at room temperature for 29 hours and followed by TLC (dichloromethane). The white solid was filtered and the filtrate was evaporated with the required compound obtained as beige crystals (0.344 g, 63%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.22 (t, *J* 7.2 Hz, 3 H, CH_2CH_3), 4.08 (q, *J* 7.2 Hz, 2 H, CH_2CH_3), 6.36 (dd, *J* 8.1 and 2.4 Hz, 1 H, H-4), 6.82 (dd, *J* 8.1 and 2.4 Hz, 1 H, H-6), 6.97-6.99 (m, 1 H, H-2), 7.00 (t, *J* 8.1 Hz, 1 H, H-5), 9.30 (s, 1 H, OH), 9.45 (s, 1 H, NH) ppm. IR (liquid film): ν = 3415, 3085, 2964, 1735, 1608, 1564, 1448, 1390, 1260, 1186, 1159, 1087, 957, 918, 898, 854, 803, 774, 753, 733, 620 cm^{-1}.

1.3. Synthesis of 4-(chloromethyl)-7-phenyl-2H-benzopyran-2-one ***1c***. A suspension of [1,1'-byphenyl]-3-ol (0.163 g, 9.0 × 10^{-4} mol) and ethyl 4-chloro-3-oxobutanoate (0.259 mL, 1.9 × 10^{-3} mol) in 70% aqueous sulfuric acid (10 mL) was stirred at 40 ºC during 3 days and the reaction followed by TLC (dichloromethane). The reactional mixture was extracted with ethyl acetate, dried with anhydrous magnesium sulphate and evaporated, resulting in brown oil. After purification by column chromatography using light petroleum/ethyl acetate (mixtures of increasing polarity) as eluent, compound **1c** was obtained as a brown solid (0.080 g, 31%). Mp = 132.1-133.3 ºC. R_f = 0.7 (dichloromethane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.70 (s, 1 H, CH_2Cl), 6.57 (s, 1 H, H-3), 7.43 (tt, 7.2 and 2 Hz, 1 H, H-4'), 7.49 (td, *J* 7.2 and 2 Hz, 2 H, H-3' and H-5'), 7.56-7.59 (m, 2 H, H-6 and H-8), 7.63 (dt, *J* 7.2 and 2 Hz, 2 H, H-2'and H-6'), 7.72 (d, *J* 8.4 Hz, 1 H, H-5) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 41.18 (CH_2Cl), 115.38 (C-3), 115.42 (C-6), 116.11 (C-4a), 123.25 (C-8), 124.46 (C-5), 127.12 (C-2' and C-6'), 128.66 (C-4'), 129.08 (C-3' and C-5'), 138.70 (C-1'), 145.34 (C-7), 149.27 (C-4), 154.21 (C-8a), 160.28 (C-2) ppm. IR (liquid film): ν = 2985, 2945, 1722, 1618, 1160, 962, 933, 874, 862, 817, 758, 693 cm^{-1}.

Synthesis of [1,1'-biphenyl]-3-ol. A solution of bromobenzene (0.102 mL, 9.0×10^{-4} mol), (3-hydroxyphenyl)boronic acid (0.158 g, 1.15×10^{-3} mol), bis(triphenylphosphine)palladium (II) dichloride (0.013 g, 1.9×10^{-5} mol) and potassium carbonate (0.264 g, 1.9×10^{-3} mol) in ethanol / water (5:1) (11 mL) was stirred at room temperature. The reactional mixture was kept under these conditions during 5 days and followed by TLC (dichloromethane). The mixture was diluted in ethyl acetate, filtrated in a layer of celite over silica gel and the filtrate was evaporated. After purification by column chromatography using dichloromethane as eluent, the required compound was obtained as a white solid (0.126 g, 77%). Mp = 56-58 ºC. R_f = 0.73 (dichloromethane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.78 (br s, 1 H, OH), 6.87 (ddd, *J* 8, 2.4 and 1 Hz, 1 H, H-4), 7.11 (t, *J* 2.4 Hz, 1 H, H-2), 7.22 (dt, *J* 7.6 and 2.4 Hz, H-6), 7.32-7.41 (m, 2 H, H-5 and H4'), 7.46 (tt, *J* 6.6 and 1 Hz, 2 H, H-3'and H-5'), 7.60 (dt, *J* 6.6 and 1 Hz, 2 H, H-2'and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 114.08 (C-2), 114.20 (C-4), 119.77 (C-6), 127.06 (C-2' and C-6'), 127.45 (C-4'), 128.71 (C-3'and C-5'), 129.97 (C-5), 140.64 (C-1'), 142.96 (C-1), 155.70 (C-3) ppm. IR (liquid film): ν = 3434, 1703, 1596, 1452, 1364, 1230, 1156, 1077, 966, 783, 693 cm^{-1}.

1.4. Synthesis of 4-(chloromethyl)-7-(4-methoxyphenyl)-2H-benzopyran-2-one ***1d****.* A suspension of 4'-methoxy-[1,1'-byphenyl]-3-ol (0.185 g, 9.0×10^{-4} mol) and ethyl 4-chloro-3-oxobutanoate (0.248 mL, 1.8×10^{-3} mol) in 70% aqueous sulfuric acid (10 mL) was stirred at 40 ºC, during 3 days, and followed by TLC (dichloromethane). The reactional mixture was extracted with ethyl acetate, dried with anhydrous magnesium sulphate and evaporated, resulting in a mixture of dark pink oil. After purification by column chromatography using petroleum ether/ethyl acetate (mixtures of increasing polarity) as eluent, compound **1d** was obtained as a beige solid (0.10 g, 35%). Mp = 113.3-114.2 ºC. R_f = 0.5 (dichloromethane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.87 (s, 3 H, OCH_3), 4.96 (s, 2 H, CH_2Cl), 6.62 (s, 1 H, H-3), 7.01 (dt, *J* 8.8 and 2 Hz, 2 H, H-3' and H-5'), 7.47-7.58 (m, 5 H, H-5, H-6, H-8, H-2' and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 55.40 (OCH_3), 60.78 (CH_2Cl), 111.32 (C-3), 114.53 (C-3' and C-5'), 114.61 (C-6), 115.73 (C-4a), 122.64 (C-8), 123.63 (C-5), 128.28 (C-2' and C-6'), 131.30 (C-1'), 144.57 (C-7), 153.91 (C-4), 154.06 (C-8a), 160.16 (C-4'), 161.33 (C-2) ppm. IR (liquid film): ν = 2985, 2326, 1722, 1608, 1525, 1498, 1398, 1280, 1252, 1181, 1028, 962, 874, 821, 736 cm^{-1}.

Synthesis of 4'-methoxy-[1,1'-biphenyl]-3-ol. A solution of 4-methoxy-bromobenzene (0.94 mL, 7.5×10^{-4} mol), (3-hydroxyphenyl)boronic acid (0.124 g, 9.0×10^{-4} mol), bis(triphenylphosphine)palladium (II) dichloride (0.011 g, 1.5×10^{-5} mol) and potassium carbonate (0.207 g, 1.5×10^{-3} mol) in ethanol / water (5:1) (11 mL) was stirred at room temperature. The reactional mixture was kept under these conditions during 5 days and followed by TLC

(dichloromethane). The mixture was diluted in ethyl acetate, filtrated in a layer of celite over silica gel and the filtrate was evaporated. After purification by column chromatography using dichloromethane as eluent, the required compound was obtained as a white solid (0.109 g, 72%). Mp = 101.1-102.5 ºC. R_f = 0.5 (dichloromethane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.87 (s, 3 H, OCH_3), 5.31 (br s, 1 H, OH), 6.82 (ddd, *J* 8, 2.4 and 1 Hz, 1 H, H-4), 6.98 (dt, 8.4 and 2.6 Hz, 2 H, H-3'and H-5'), 7.05 (t, *J* 2.4 Hz, 1 H, H-2), 7.15 (dt, *J* 8 and 1 Hz, 1 H, H-6), 7.30 (t, *J* 8 Hz, 1 H, H-5), 7.51 (dt, *J* 8.4 and 2.4 Hz, 1 H, H-2' and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 55.33 (OCH_3), 113.4 (C-2 and C-4), 114.17 (C-3' and C-5'), 119.17 (C-6), 128.07 (C-2' and C-6'), 129.91 (C-5), 133.29 (C-1'), 142.47 (C-1), 155.89 (C-3), 159.11 (C-4') ppm. IR (dichloromethane): v = 3440, 2088, 1700, 1639, 1477, 1431, 1364, 1305, 1203, 1088, 883, 758, 698 cm^{-1}.

1.5. Synthesis of 4-(chloromethyl)-7-styril-2H-benzopyran-2-one ***1e***. A suspension of 3-styrilphenol (0.165 g, 8.3 × 10^{-4} mol) and ethyl 4-chloro-3-oxobutanoate (0.224 mL, 1.6 × 10^{-3} mol) in 70% aqueous sulfuric acid (10 mL) was stirred at 40 ºC during 4 days and followed by TLC (dichloromethane). The reactional mixture was extracted with ethyl acetate, dried with anhydrous magnesium sulphate and evaporated, resulting in brown oil. After purification by column chromatography using light petroleum/ethyl acetate (mixtures of increasing polarity) as eluent, compound **1e** was obtained as a yellow solid (0.070 g, 28%). Mp = 136.6-137.3 ºC. R_f = 0.67 (dichloromethane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.68 (s, 2 H, CH_2Cl), 6.54 (s, 1 H, H-3), 7.13 (d, *J* 16.4 Hz, 1 H, H-a), 7.23 (d, *J* 16.4 Hz, 1 H, H-b), 7.33 (tt, *J* 7.2 and 2 Hz, 1 H, H-4'), 7.407 (t, *J* 7,2 Hz, 2 H, H-3'and H-5'), 7.47-7.51 (m, 2 H, H-6 and H-8), 7.57 (d, *J* 7.2 Hz, 2 H, H-2' and H-6'), 7.65 (d, *J* 8 Hz, 1 H, H-5) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 41.22 (CH_2Cl), 114.68 (C-6), 115.12 (C-3), 116.37 (C-4a), 122.59 (C-8), 124.37 (C-5), 126.57 (C-a), 126.94 (C-2' and C-6'), 128.63 (C-4'), 128.85 (C-3' and C-5'), 132.38 (C-b), 136.28 (C-1'), 141.82 (C-7), 149.22 (C-4), 154.37 (8a), 160.37 (C-2) ppm. IR (dichloromethane): v = 3420, 2963, 2925, 2855, 1724, 1609, 1546, 1495, 1449, 1414, 1398, 1276, 1263, 1217, 1145, 1083, 1020, 963, 862, 804, 737, 695 cm^{-1}.

Synthesis of 3-styrylphenol. A solution of (2-bromovinyl)benzene (0.096 mL, 7.5 × 10^{-4} mol), (3-hydroxyphenyl)boronic acid (0.124 g, 9.0 × 10^{-4} mol), bis(triphenylphosphine)palladium (II) dichloride (0.0105 g, 1.5 × 10^{-5} mol) and potassium carbonate (0.2073 g, 1.5 × 10^{-3} mol) in ethanol / water (5:1) (11 mL) was stirred at room temperature. The reactional mixture was kept under these conditions during 5 days and followed by TLC (dichloromethane). The mixture was diluted in ethyl acetate, filtrated in a layer of celite over silica gel and the filtrate was evaporated. After purification by column chromatography using dichloromethane as eluent, the required compound was obtained as a white solid (0.073 g, 50%). Mp = 111.6-113.1 ºC. R_f = 0.6 (dichloromethane). ^{1}H NMR

($CDCl_3$, 400 MHz): δ = 6.78 (ddd, *J* 8.4, 2.2 and 1.2 Hz, 1 H, H-6), 7.01 (t, *J* 2 Hz, 1 H, H-2), 7.04-7.13 (m, 3 H, H-a, H-b and H-4), 7.23-7.30 (m, 2 H, H-5 and H-4'), 7.38 (td, *J* 7.2 and 1.2 Hz, 2 H, H-3'and H-5'), 7.52 (dt, *J* 7.2 and 1.2 Hz, 2 H, H-2'and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 112.96 (C-2), 114.68 (C-6), 119.46 (C-4), 126.54 (C-2'and C-6'), 127.70 (C-4'), 128.20 (C-a), 128.66 (C-3' and C-5'), 129.17 (C-b), 129.86 (C-5), 137.10 (C-1'), 139.07 (C-3), 155.70 (C-1) ppm. IR (dichloromethane): ν = 3411, 1700, 1597, 1477, 1430, 1364, 1305, 1202, 884, 758, 698 cm^{-1}.