Photorelease of glycine and β-alanine from (7-bromocoumarin-4-yl)methyl cages

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Abstract: The synthesis of a new 7-bromo-4-(chloromethyl)-coumarin to be used as cage for the release of bioactive molecules is presented. Ester cages of two neurotransmitter amino acids, glycine and β-alanine, were also synthetized. These neurotransmitters were chosen as models due to their biological relevance. The glycine and β-alanine ester conjugates, in methanol/HEPES buffer (80:20), were irradiated at 254, 300, 350 and 419 nm in a Rayonet RPR-100 photochemical reactor and the photolysis process was followed by HPLC with UV detection. These results were compared with those previously obtained by our research group when 4-chloromethyl-coumarin was used in the photorelease of glycine and β-alanine.

1. Introduction

In recent years, remarkable research in photocleavable protecting groups (PPGs) has given rise to a variety of structures suitable for masking a broad range of chemically and biologically relevant molecules. These groups can be easily removed by irradiation at a suitable wavelength, releasing the desired active compound. Notice that for bioapplications the chosen wavelength must be harmless to cells, ideally as close as possible to the visible spectrum.[1] Among the possible bioapplications, PPGs can be used to design photocleavable prodrugs and in the study of dynamic processes such as signalling processes and (bio)molecule interactions.[2-5]

Among the most interesting PPGs are coumarin (trivial designation for 2*H*-benzopyran-2-one) derivatives that generally present high molar extinction coefficients at long wavelengths, good stability and fast release rates. Furthermore, these molecules can display fluorescence that allows following the spatial distribution and depletion of the bioactive compound.[6-8] So far, coumarin derivatives have been used as PPGs for phosphates, carboxylates, sulphates, sulfonates, diols and carbonyls. [9]

With the goal of evaluating the efficiency of 7-bromo-4-(chloromethyl)-coumarin as phototrigger for amino acid neurotransmitters glycine and β-alanine, the corresponding ester conjugates were synthetized. Their irradiation at different wavelengths (254, 300, 350 and 419 nm) in a Rayonet RPR-

100 photochemical reactor, in methanol/HEPES buffer (80:20) was carried out. The photolysis process was followed by HPLC with UV detection.

2. Experimental section

2.1. Synthesis of 7-bromo-(4-chloromethyl)coumarin 1. A solution of 3-bromophenol (0.100 g, 0.58 mmol) and ethyl 4-chloro-3-oxobutanoate (0,156 mL, 1.16 mol) in 70% aqueous sulphuric acid (5 mL) was stirred at 40 ºC and followed by TLC. After 3 days the reaction mixture was extracted with ethyl acetate, the organic phase was dried with anhydrous magnesium sulphate and the solvent was evaporated. The crude residue was purified by silica gel column using ethyl acetate/petroleum ether (mixtures of increasing polarity) as eluent. Compound **1** was obtained as a white solid (0.034 g, 25 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.57 (s, 2H, CH_2), 6.51 (s, 1H, H-3), 7.40 (dd, *J* 8.6 and 2 Hz, 1H, H-6), 7.47 (d, *J* 8.6 Hz, 1H, H-5), 7.49 (d, *J* 2 Hz, 1H, H-8) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 41.03 (CH_2), 116.13 (C-3), 116.28 (C-4a), 120.74 (C-8), 125.26 (C-5), 126.29 (C-7), 127.93 (C-6), 148.99 (C-4), 154.13 (C-8a), 159.42 (C-2) ppm. IV (film): ν_{max} = 3345, 2926, 2367, 2011, 1740, 1578, 1340, 1243, 1171, 1155, 966, 928, 884, 819, 768, 732 cm^{-1}.

2.2. Synthesis of *N*-(*tert*-butoxycarbonyl) glycine (7-bromocoumarin-4-yl)methyl ester 2. 7-Bromo-4-(chloromethyl)coumarin **1** (0.0452 g, 0.19 mmol), *N*-(*tert*-butoxycarbonyl) glycine (0.033 g, 0.188 mmol) and potassium fluoride (0.0328 g, 0.56 mmol) were stirred in dry DMF (2 mL) during 2 days at room temperature. After filtration, the solvent was evaporated and the crude residue was purified by silica gel column chromatography using ethyl acetate/petroleum ether (mixtures of increasing polarity) as eluent. Compound **2** was obtained as a beige solid (0.028 g, 36 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.45 (s, 9H, $C(CH_3)_3$), 4.05 (d, *J* 5.6 Hz, 2H, H-α), 5.08 (broad s, 1H, NH), 5.33 (s, 2H, CH_2), 6.51 (s, 1H, H-3), 7.37 (d, *J* 8.6 Hz, 1H, H-5), 7.45 (dd, *J* 8.6 and 2 Hz, 1H, H-6), 7.55 (d, *J* 2 Hz, 1H, H-8) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.25 ($C(CH_3)_3$) 42.38 (C-α), 61.57 (CH_2), 80.48 ($C(CH_3)_3$)), 113.90 (C-3), 115.96 (C-4a), 120.67 (C-8), 124.51 (C-5), 126.19 (C-7), 127.93 (C-6), 147.84 (C-4), 153.88 (C-8a), 155.71 (C=O Boc), 159.35 (C-2), 169.72 (C=O Gly) ppm. IV (film): ν_{max} = 3346, 3080, 1628, 1599, 1554, 1523, 1404, 1367, 1313, 1158, 1065, 1009, 947, 899, 861, 785, 766, 710 cm^{-1}.

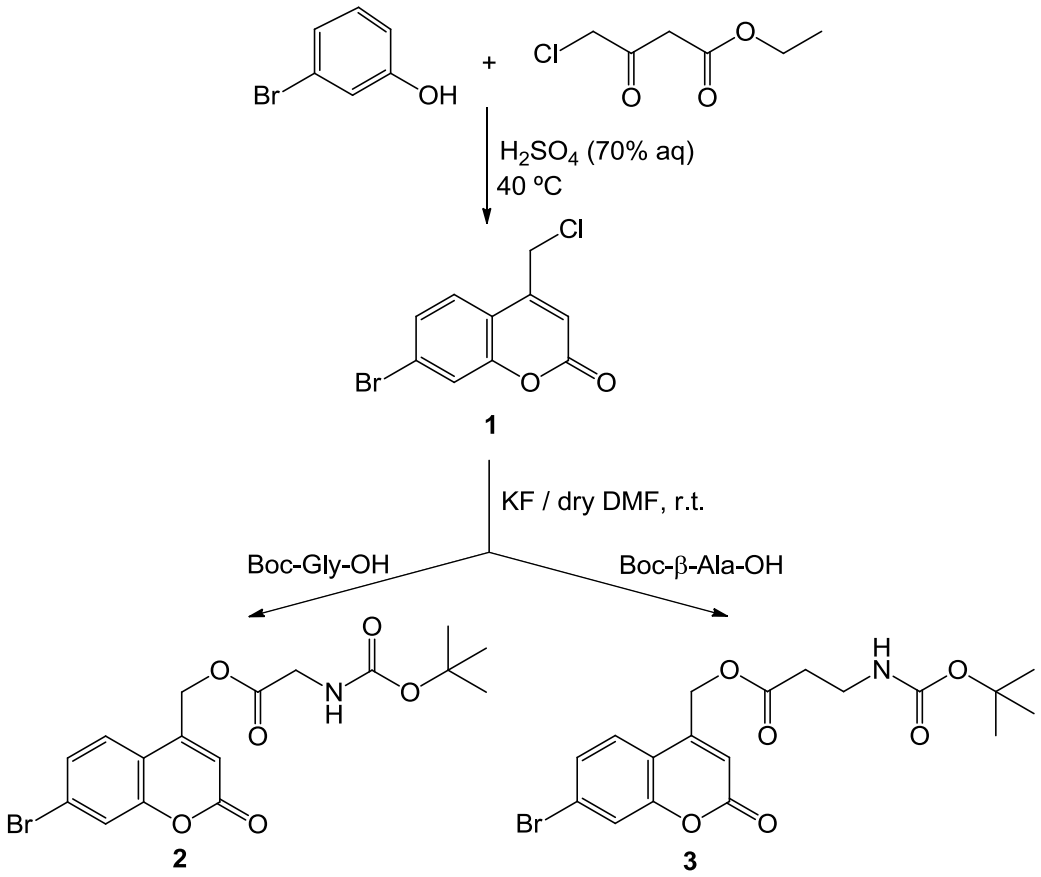
2.3. Synthesis of *N*-(*tert*-butoxycarbonyl) β-alanine (7-bromocoumarin-4-yl)methyl ester 3. 7-Bromo-4-(chloromethyl)coumarin **1** (0.0477 g, 0.20 mmol), *N*-(*tert*-butoxycarbonyl)-β-alanine (0.376 g, 0.20 mmol) and potassium fluoride (0.347 g, 0.56 mmol) were stirred in dry DMF (2 mL) during 2 days at room temperature. After filtration, the solvent was evaporated and the crude residue was purified by silica gel column using ethyl acetate/petroleum ether (mixtures of increasing polarity) as eluent. Compound **3** was obtained as a beige solid (0.321 g, 38 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.44 (s, 9H, $C(CH_3)_3$), 2.69 (t, *J* 6.4 Hz, 2H, H-α), 3.46 (q, *J* 6.4 Hz, 2H, H-β), 4.97 (broad s, 1H, NH), 5.29 (s, 2H, CH_2), 6.49 (s, 1H, H-3), 7.39 (d, *J* 8.6 Hz, 1H, H-5), 7.45 (dd, *J* 8.6 and 1.6 Hz, 1H, H-6), 7.55 (d, *J* 1.6 Hz, 1H, H-8) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.33 ($C(CH_3)_3$), 34.54 (C-α), 36.03 (C-β), 61.12 (CH_2), 79.76 ($C(CH_3)_3$), 113.83 (C-3), 116.06 (C-4a), 120.67 (C-8), 124.53 (C-5), 126.14 (C-7), 127.90 (C-6), 148.20 (C-4), 153.90 (C-8a), 155.72 (C=O Boc), 159.41 (C-2), 171.53 (C=O β-Ala) ppm. IV (film): ν_{max} = 3358, 3081, 1785, 1624, 1598, 1531, 1448, 1431, 1404, 1366, 1330, 1205, 1072, 999, 958, 903, 860, 816, 786, 750, 708 cm^{-1}.

3. Results and discussion

7-Bromo-4-(chloromethyl)coumarin **1** was obtained by condensation of 3-bromophenol and ethyl 4-chloro-3-oxobutanoate in 70% aqueous sulphuric acid at 40 ºC. Then, coumarin **1** was conjugated with *N*-(*tert*-butoxycarbonyl)-glycine and *N*-(*tert*-butoxycarbonyl)-β-alanine, using potassium fluoride as basic catalyst, affording conjugates *N*-(*tert*-butoxycarbonyl) glycine (7-bromocoumarin-4-yl)methyl ester **2** and *N*-(*tert*-butoxycarbonyl) β-alanine (7-bromocoumarin-4-yl)methyl ester **3** in moderate yields (Scheme 1).

Compounds **1**-**3** were characterized by the usual techniques, as well as by UV-visible absorption and fluorescence spectroscopies in methanol/HEPES buffer (80:20). For UV-visible absorption studies solutions of 1×10^{-4} M were prepared, maximum absorption wavelengths (λ_{abs}) and molar extinction coefficient (ε) were obtained for each compound.

Then, taking into account the Lambert-Beer law, solutions of the compounds were diluted to a maximum absorbance of 0.1, and the emission spectra were traced, exciting each compound at its λ_{abs}. The maximum emission wavelength (λ_{em}) and the Stokes' shift ($\Delta\lambda$) were obtained. In addition, each compound's fluorescence relative quantum yield (ϕ_F) was calculated using 9,10-diphenylanthracene as standard.[10] The UV-Vis absorption and fluorescence data for compounds **2** and **3** is presented in Table 1.



Scheme 1: Synthesis of 7-bromocoumarin conjugates of glycine **2** and β-alanine **3**.

Table 1: UV/vis absorption and fluorescence data for conjugates **2** and **3**.

Cpd	λ_{abs} (nm)	log ε	λ_{em} (nm)	ϕ_F	Δλ
2	313	4.00	385	0.09	72
3	315	4.04	390	0.02	75

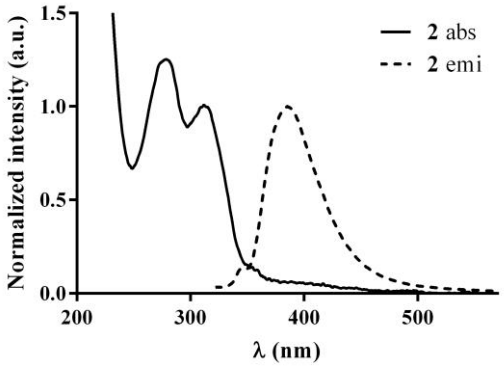


Figure 1: Normalized absorption and emission spectra for compound **2**.

Conjugates **2** and **3** displayed λ_{abs} and λ_{em} at about 315 and 390 nm, respectively, which represents a considerably high Stokes' shift (~75 nm), with low ϕ_F values (< 0.1), as it is expected due to the

presence of bromine that can act as a fluorescence quencher because of the heavy atom effect (Figure 1).[11]

With the goal of evaluating the 7-bromo-4-(chloromethyl)coumarin **1** as possible phototrigger for neurotransmitter amino acids, glycine and β-alanine were used as models. Photolysis tests for conjugates **2** and **3** were performed in a Rayonet RPR-100 photochemical reactor at 254, 300, 350 and 419 nm using solutions of 1×10^{-4} M in methanol/HEPES buffer (80:20). The disappearance of the conjugates was monitored by HPLC with UV detection at each conjugate's maximum absorption wavelength (λ_{max}) until only 5% of conjugate was present in solution. In Table 1 the photolysis results (irradiation time, t_{irr}, rate constant, *k*, and photochemical quantum yield, ϕ_{phot}) for conjugates **2** and **3** are presented, in comparison with the results previously presented by our research group for unsubstituted coumarin conjugates **4** and **5** (Figure 2).[12] It was possible to verify that conjugates **2** and **3** present lower t_{irr} and higher *k* than conjugates **4** and **5** at all tested wavelengths. More precisely, at 254 and 300 nm, for conjugates **4** and **5**, t_{irr} is 3 to 4 times higher than that of conjugates **2** and **3**, whereas at 350 nm no consumption of conjugates **4** and **5** was seen after 8 hours of irradiation. The results for conjugates **2**, **3**, **4** and **5** at 254 nm are graphically represented as a plot of irradiation time (min) *versus* ln A (A being the area determined in the chromatogram) (Figure 3).

Although photolysis occurred at 350 nm for conjugates **2** and **3**, the obtained t_{irr} were too long to be practical for application at this wavelength. Notice that photolysis of conjugates **2 - 5** was also tested at 419 nm, however photolysis was not detected for none of the conjugates.

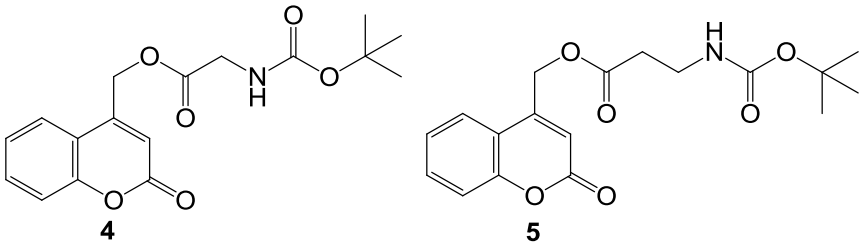


Figure 2: Structures for coumarin conjugates of glycine **4** and β-alanine **5**.[12]

It has been reported in the literature that strong electron donating substituents at the coumarin ring stabilize the carbocation formed during photolysis, preventing ion recombination. Accordingly, the presence of a bromine substituent in conjugates **2** and **3** should not improve their photolysis behavior in comparison with conjugates **4** and **5**. Another way of preventing ion recombination is by lowering

the coumarin pK_a, which stabilizes the anion formed during photolysis. In conjugates **2** and **3** this is accomplished by the introduction of the bromine group.[13, 14]

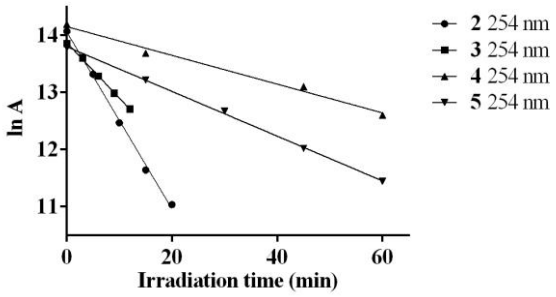


Figure 3: Plot of irradiation time (min) *vs* ln A for conjugates **2**, **3**, **4** and **5** at 254 nm.

Table 1: Irradiation times (t_{irr}, min), rate constants (k, x 10^{-2} min^{-1}) and photochemical quantum yield ($\phi_{phot} \times 10^{-3}$) for conjugates **2 - 5** by irradiation at 254, 300, and 350 nm (1×10^{-4} M in methanol/HEPES buffer (80:20)).

Cpd.		R	254 nm			300 nm			350 nm		
			t_{irr}	k	ϕ_{phot}	t_{irr}	k	ϕ_{phot}	t_{irr}	K	ϕ_{phot}
Gly	**2**	Br	19	16	0.7	32	10	0.16	1756	0.17	0.003
	4	H	77	3.9	0.41	83	3.6	0.18	-	-	-
β-Ala	**3**	Br	31	9.7	0.41	25	12	0.23	4375	0.07	0.001
	5	H	84	3.6	0.37	80	3.7	0.19	-	-	-

4. Conclusions

In this work a new 7-bromo-4-(chloromethyl)coumarin **1** cage for model amino acids glycine and β-alanine was synthesized. The photolysis of the corresponding *N*-(*tert*-butoxycarbonyl) glycine and β-alanine ester conjugates **2** and **3** at 254 and 300 nm showed higher rate constants and lower irradiation times comparatively to the related glycine and β-alanine ester conjugates **4** and **5** (obtained from 4-chloromethylcoumarin).

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