Selective photorelease of model amino acids from coumarin ester conjugates

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Abstract: To assess the possibility of selective photocleavage by using coumarins bearing different donor substituents (a methoxy and a cyclic *N,N*-dibutylamino group) as protecting groups for the carboxylic acid function, a series of model amino acid ester conjugates were synthesised by reaction with glycine, alanine, valine and phenylalanine. The quantitative release of the amino acids was confirmed by photolysis of the conjugates in acetonitrile/HEPES buffer (80:20) solution at different wavelengths of irradiation. It was found that the selective removal of one coumarin in the presence of the other could occur due to the marked difference in irradiation times at the same irradiation wavelength.

Keywords: coumarin; photolysis; selective cleavage; amino acids.

Introduction

There is a strong interest in the design of more efficient protecting groups that allow orthogonal cleavage/deprotection for application with biomolecules, by rapid and clean cleavage under irradiation at wavelengths that are not detrimental to biological systems.[1] Orthogonality, *i.e.*, the possibility of selectively removing one group in the presence of others in any chronological sequence, is a critical issue in protecting group chemistry and the possibility of using different wavelengths of irradiation to achieve orthogonality within a set of photolabile protecting groups has been proven.[2]

Coumarin derivatives are well established photolabile protecting groups that have been used in the caging of various functional groups (such as alcohols, amines, phosphates, aldehydes, ketones and carboxylic acids),[3] whereas benzocoumarin derivatives were reported for the first time by us as carboxyl protecting groups cleavable by light and with sensitivity to two-photon excitation.[4]

In recent years, we have been involved in the design of heterocyclic photocleavable protecting groups, derived from oxygen and nitrogen such as (benzo)coumarins, (benzo)quinolones and their thionated analogues, benzozaxoles and acridines.[5] These moieties have been used in the protection

of amino acids, as models for bifunctional biomolecules, by coupling through ester or urethane bonds.

We now report the synthesis of model amino acid conjugates, namely glycine, alanine, valine and phenylalanine, based on coumarins bearing different substituents (a methoxy and a cyclic *N,N*-dibutylamino group), with the aim of assessing the selectivity of photolytic removal. Photolysis studies were carried out under irradiation at different wavelengths (250, 300, 350 and 419 nm) in a Rayonet RPR-100 photochemical reactor in acetonitrile/HEPES buffer (80:20) solution.

Experimental

Synthesis of *N*-*tert*-butoxycarbonylvaline (11-oxo-2,3,5,6,7,11-hexahydro-1*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-9-yl)methyl ester 1c. 9-(Chloromethyl)-2,3,6,7-tetrahydro-1*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-11(5*H*)-one (0.024 g, 8.28×10^{-5} mol) was dissolved in dry DMF (3 mL) and potassium fluoride (0.014 g, 2.48×10^{-4} mol) and *N*-(*tert*-butoxycarbonyl)valine **3c** (0.018 g, 8.28×10^{-5} mol) were added. The reaction mixture was stirred at room temperature for 17 h and followed by TLC (ethyl acetate/light petroleum, 1:2). The solid was filtered and the solvent removed by evaporation under reduced pressure, to give compound **1c** as an oil (0.028 g, 72%). TLC (ethyl acetate/light petroleum, 1:2): $R_f = 0.38$. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.91$ (d, *J* 7.2 Hz, 3H, γ-CH_3 Val), 0.99 (d, *J* 6.8 Hz, 3H, γ-CH_3 Val), 1.45 (s, 9H, $C(CH_3)_3$), 1.93-2.02 (m, 4H, 2×CH_2), 2.16-2.22 (m, 1H, β-CH Val), 2.76 (t, *J* 6.4 Hz, 2H, CH_2), 2.88 (t, J 6.4 Hz, 2H, CH_2), 3.20-3.30 (m, 4H, 2×CH_2), 4.30-4.35 (m, 1H, α-CH Val), 5.02 (broad s, 1H, α-NH Val), 5.20-5.26 (m, 2H, OCH_2), 6.10 (s, 1H, H-10), 6.87 (s, 1H, H-8). ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta_C = 17.51$ (γ-CH_3 Val), 19.14 (γ-CH_3 Val), 20.37 (CH_2), 20.52 (CH_2), 21.42 (CH_2), 27.64 (CH_2), 28.26 ($C(\underline{C}H_3)_3$), 31.07 (β-CH Val), 49.47 (CH_2), 49.88 (CH_2), 58.72 (α-CH Val), 60.36 ($\underline{C}(CH_3)_3$), 62.15 (OCH_2), 105.70 (C-8a), 106.01 (C-10), 106.94 (C-4a), 118.26 (C-7a), 120.49 (C-8), 146.02 (C-4b), 148.84 (C-9), 151.20 (C-12a), 155.66 (C=O urethane), 162.10 (C-11), 171.92 (C=O ester). IR (neat, cm^{-1}): *v* = 3435, 3057, 2972, 2934, 1715, 1614, 1578, 1503, 1392, 1368, 1309, 1266, 1158, 1093, 1020, 896, 739, 704.

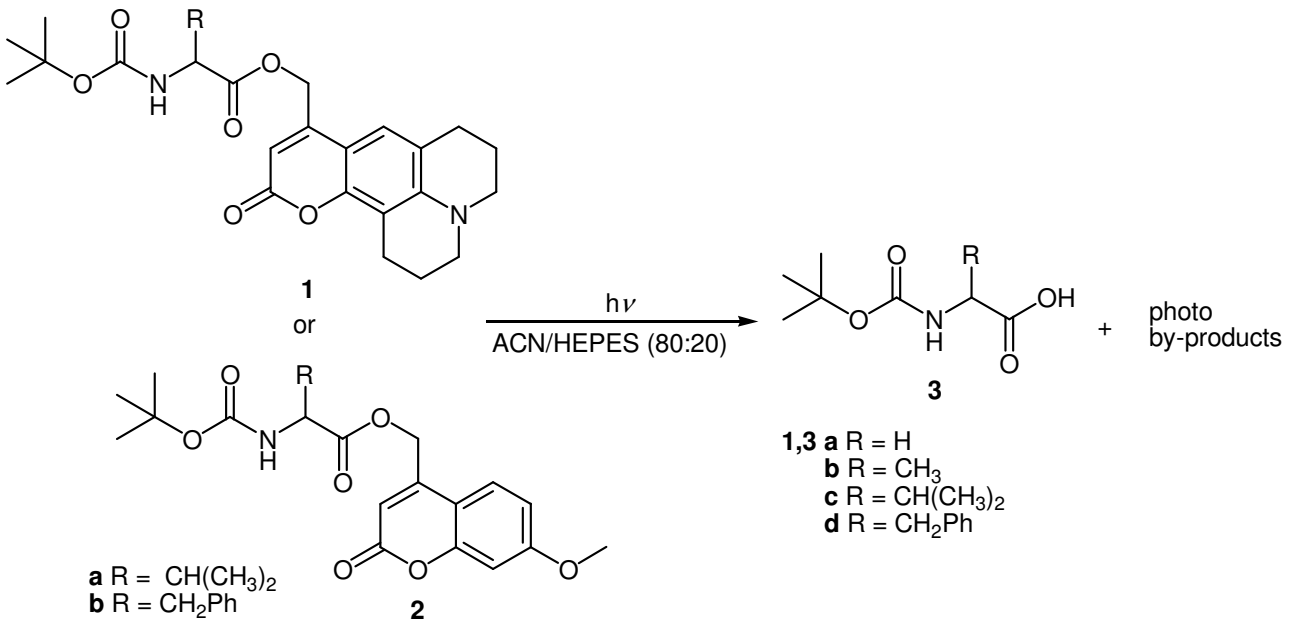
Photolysis procedure: A 1×10^{-4} M acetonitrile/HEPES buffer (80:20) solution of conjugates **1** and **2** (5 mL) was placed in a quartz tube and irradiated in a Rayonet RPR-100 reactor at the desired wavelength. The lamps used for irradiation were of 300, 350 and 419 ± 10 nm. HEPES buffer solution was prepared in distilled water with HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (10 mM), NaCl (120 mM), KCl (3 mM), $CaCl_2$ (1 mM) and $MgCl_2$ (1mM) and

pH adjusted to 7.2. Aliquots of 100 µL were taken at regular intervals and analysed by RP-HPLC. The eluent was acetonitrile/water, 3:1, 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 (retention time: 5.9 min, **1a**; 6.6 min, **1b**; 9.5 min, **1c**; 10.5 min, **1d**; 3.9 min, **2a**; 5.8 min, **2b**).

Results and Discussion

Amino acid conjugates **1** and **2** were obtained by derivatization at the *C*-terminus of *N*-benzyloxycarbonyl-protected glycine, alanine, valine and phenylalanine with 9-(chloromethyl)-2,3,6,7-tetrahydro-1*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-11(5*H*)-one and 4-(chloromethyl)-7-methoxy-benzopyran-2-one, respectively, by a potassium fluoride mediated coupling, as previously reported by us.[5a] With these compounds it was intended to assess the influence of the structure of the coumarin and of the amino acid in the photolytic behaviour.

The UV-visible spectroscopic characterization of the resulting conjugates was carried out to obtain the parameters needed for monitoring during photolysis. The absorption and fluorescence spectra of degassed 10^{-5} M solutions in an acetonitrile/HEPES buffer (80:20) solution of conjugates **1** and **2** were measured (Table 1).



Scheme 1. Photorelease of model amino acids **3** from the corresponding fluorescent conjugates **1** and **2**.

Table 1. UV/visible absorption and emission data for conjugates **1** and **2** in ACN/HEPES buffer (80:20) solution.

Compound	Absorption		Emission		
	λ_{max} (nm)	log ε	λ_{max} (nm)	ϕ_F	Stokes' shift (nm)
1a	395	4.40	494	0.04	99
1b	391	4.20	494	0.04	103
1c	391	4.17	495	0.08	104
1d	397	4.28	494	0.09	97
2a	319	4.17	396	0.11	77
2b	319	4.21	398	0.11	79

Solutions of the mentioned conjugates (1×10^{-4} M) in acetonitrile/HEPES buffer (80:20) were irradiated in a Rayonet RPR-100 reactor at 300, 350 nm and 419 nm in order to determine the sensitivity to irradiation of the ester bond between the amino acid and the different heterocycles. The course of the photocleavage reaction was followed by reverse phase HPLC with UV detection. 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).

Table 2. Irradiation times (t_{Irr}, min) and rate constants (k, $\times 10^{-2}$ min^{-1}) for the photolysis of conjugates **1** and **2**, at different wavelengths in ACN/HEPES buffer (80:20) solution.

Cpd	Amino acid	300 nm		350 nm		419 nm	
		t_{Irr}	k	t_{Irr}	k	t_{Irr}	k
1a	Gly	7	42.5	5	57.6	4	83.9
1b	Ala	4	78.0	6	50.9	4	79.5
1c	Val	5	60.9	5	60.2	3	88.9
1d	Phe	6	49.3	5	54.4	5	61.1
2a	Val	46	6.8	844	0.36	n.d.	n.d.
2b	Phe	54	5.7	732	0.42	n.d.	n.d.

n.d.: not determined

Concerning the influence of the structure of the heterocycle, it was found that the conjugates bearing a cyclic *N,N*-dibutylamino group (derived from a precursor julolidine moiety) **1** cleaved readily at all wavelengths of irradiation and much faster than the coumarin bearing conjugates **2**.

This fact revealed that the presence of a cyclic *N,N*-dialkylamino group is more advantageous when compared to an alkoxy group at position 7 of the parent coumarin ring, in terms of irradiation time. The *N,N*-dialkyl group also shifted the absorption maxima towards a longer wavelength thus enabling the photolysis at 419 nm in pratical time. This is a highly desirable feature for a photocleavable protecting group when biological appplications like caging are envisaged, minimising the deleterious effect of low wavelength radiation on cells.

For conjugates **1**, the irradiation times were very similar and indicative that the structure of the amino acid did not play a role in the photolytic behaviour. The same conclusion could be drawn when considering conjugates **2**, bearing a valine or a phenylalanine core, which also had very similar irradiation times. For conjugates **2**, due to the long irradiation times obtained at 350 nm, much longer irradiation times would be expected at 419 nm so irradiation at this wavelength was not carried out.

As the irradiation times were significantly different between the coumarin bearing a cyclic *N,N*-dialkylamino group and the methoxylated coumarin, these two heterocycles could in principle be used in a selective photodeprotection strategy for bifuctional molecules at the various wavelengths tested but especially at 350 and 419 nm (Figure 1).

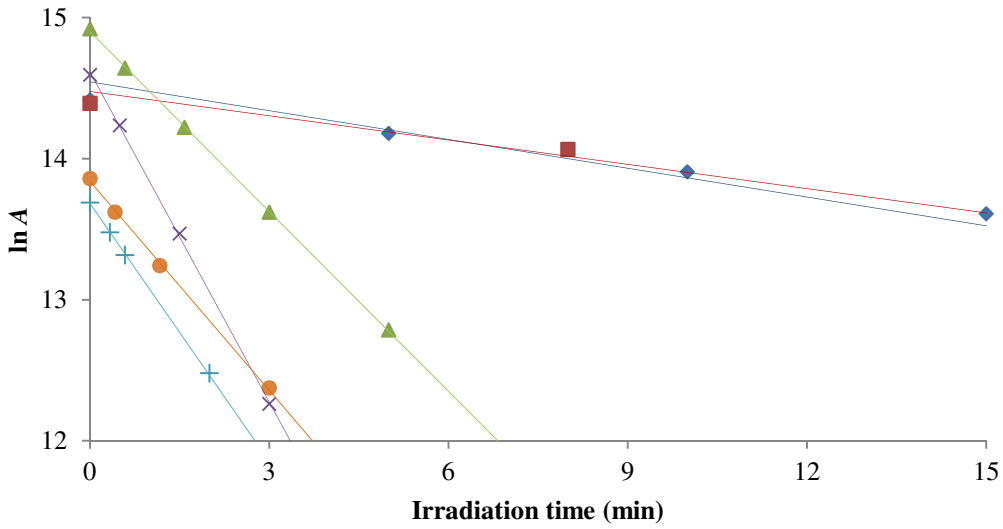


Figure 1. Plot of ln *A* versus irradiation time for the photolysis of conjugates **1** and **2** at 300 nm (only shown up to 15 min of irradiation): ▲, **1a**; X, **1b**; +, **1c**; ●, **1d**; ♦, **2a**; ■, **2b**.

Conclusions

The cleavage of the ester bond between the coumarin and the amino acid, in a series of model amino acid conjugates with glycine, alanine, valine and phenylalanine, occurred in very different irradiation times at the same irradiation wavelength, depending on the structure of the heterocycle

and the substituent present and enabling the selective removal of one coumarin in the presence of the other. These results open up the possibility of considering these coumarins bearing different substituents as an orthogonal pair of photocleavable protecting groups for the protection of a bifunctional molecule through ester bonds.

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