Release of model amino acids by ester linkage photolysis from fused 2-oxo-2*H*-benzopyranyl conjugates

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Abstract: Valine and phenylalanine were used as model amino acids for the synthesis of ester conjugates with a fused oxobenzopyran, in order to evaluate its applicability as a photocleavable protecting group for solution phase organic and peptide synthesis. The behaviour of the corresponding conjugates towards photocleavage was evaluated by irradiation in methanol/HEPES buffer (80:20) and acetonitrile/HEPES buffer (80:20) solutions, in a photochemical reactor at different wavelengths (300 and 350 nm), followed by HPLC/UV monitoring.

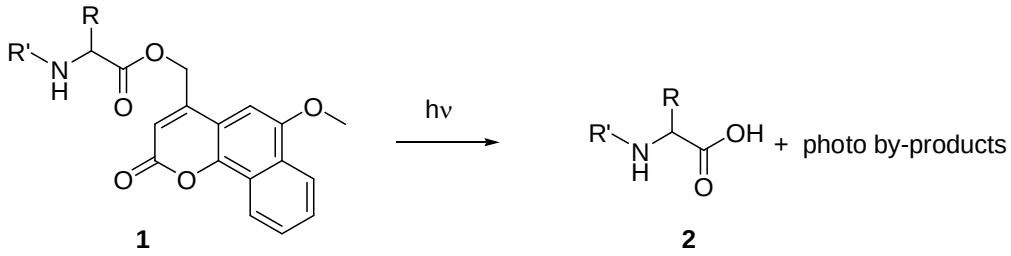
Introduction

Molecules of synthetic and biochemical interest can be photoreleased by irradiation with light of appropriate wavelength, provided suitable light-sensitive moieties are covalently bond to the functional groups of the molecule of interest. Such strategy has been applied widely in recent years, in organic synthesis as an alternative to classical acid- and base-labile protecting groups, and in life sciences, in the investigation of signal transduction mechanisms at cellular level and in drug delivery [1]. Given these varied applications, there is an interest in developing novel photolabile groups with improved properties that will allow a fast cleavage for a broad range of functionalities (such as alcohols, amines, phosphates, aldehydes, ketones and carboxylic acids [2]), at longer wavelengths to minimize side reactions.

Our recent research interests include the synthesis of new fused oxygen and nitrogen heterocycles, their application as fluorescent labels and in the design of conjugates of biologically relevant molecules and studies on their photorelease [3]. Bearing these facts in mind, we now report the photocleavage study at different wavelengths of valine and phenylalanine model conjugates bearing a 2-oxo-2*H*-benzo[*h*]benzopyran moiety as photolabile group.

Results and Discussion

Benzo[*h*]benzopyranyl ester conjugates **1a**,**b** were obtained by reaction of 4-(hydroxymethyl)-6-methoxy-2-oxo-2*H*-benzo[*h*]benzopyran with the carboxylic acid group of *N*-benzyloxycarbonyl valine and phenylalanine by a previously reported procedure (Figure 1) [3d]. The corresponding *N*-deprotected conjugates **1c**,**d** were prepared by a classical deprotection method with hydrobromic acid in acetic acid. Photolysis studies of the benzo[*h*]benzopyranyl conjugates **1a-d** were carried out by irradiation of solutions of the mentioned compounds in methanol/HEPES buffer (80:20) and acetonitrile/HEPES buffer (80:20) in a Rayonet RPR-100 reactor, at 300 and 350 nm, in order to determine the best conditions (wavelength of irradiation and solvent system) for the release of model amino acids in their *N*-protected (**2a**,**b**) or free (**2c**,**d**) form.



a R = $CH(CH_3)_2$, R' = $OCOCH_2Ph$
b R = CH_2Ph, R' = $OCOCH_2Ph$
c R = $CH(CH_3)_2$, R' = H
d R = CH_2Ph, R' = H

Figure 1. Structure of amino acid - benzo[*h*]benzopyranyl conjugates **1a-d**.

The course of the photocleavage reaction was followed by reverse phase HPLC with UV detection. Plots of peak area 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 less than 5% of the initial area was detected (Table 1). The benzo[*h*]benzopyranyl group will be designated by Bbp for simplicity of naming the various conjugates.

Table 1. Irradiation times (t_{irr}, in min) and rate constants (k, in 10^{-2} min^{-1}) for the photolysis of benzo[*h*]benzopyranyl conjugates **1a-d** at different wavelengths in methanol/HEPES buffer (80:20) and acetonitrile/HEPES buffer (80:20) solution.

Compound		methanol/HEPES (80:20)				acetonitrile/HEPES (80:20)			
		300 nm		350 nm		300 nm		350 nm	
		t_{irr}	k	t_{irr}	k	t_{irr}	k	t_{irr}	k
1a	Z-Val-OBbp	86	3.51	59	5.06	154	1.96	87	3.46
1b	Z-Phe-OBbp	62	4.86	47	6.41	79	3.70	35	8.42
1c	H-Val-OBbp	845	0.28	231	1.30	40	7.57	35	8.51
1d	H-Phe-OBbp	613	0.49	558	0.53	84	3.56	33	9.03

For each compound, 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 (Figure 2). The correlation coefficients varied from 0.9973 to 0.9998.

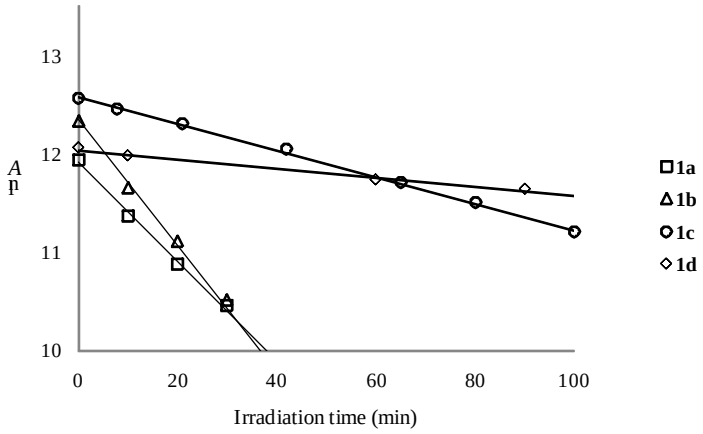


Figure 2. Plot of ln *A versus* irradiation time for the photolysis of conjugates **1a-d** at 350 nm in methanol/HEPES buffer (80:20) solution.

Concerning the influence of the wavelength of irradiation on the rate of the photocleavage reactions of conjugates **1a-d** in methanol/HEPES buffer (80:20) and acetonitrile/HEPES buffer (80:20) solutions, it was found that the most suitable was 350 nm, resulting in shorter

irradiation times, accordingly to the wavelength absorption maxima of the conjugates (at about 375 nm). This wavelength of irradiation and the resulting irradiation times can be considered suitable for practical applications.

Taking into consideration the influence of the amino acid structure on the photocleavage rates, it was found that the *N*-benzyloxycarbonyl phenylalanine conjugates **1b** cleaved faster than the corresponding valine conjugate **1a** in both wavelengths and solvent systems, whereas no obvious tendency was seen for the related deprotected conjugates **1c** and **1d**.

As for the influence of the solvent system, it was found that for the *N*-protected conjugates **1a**,**b** there were no significant differences on the photoreaction rates at 300 and 350 nm for both solvent systems. This observation was reversed for conjugates **1c**,**d** which released the amino acids in their free form much faster (between 7 and 21 times) in acetonitrile//HEPES buffer (80:20) solution.

From these observations, it may be suggested that, depending on the purpose of photoreleasing *N*-protected or free amino acids from this type of heterocyclic conjugates, the optimal solvent system for the release will be methanol/HEPES buffer (80:20) or acetonitrile/HEPES buffer (80:20), respectively.

As a conclusion, regarding the photocleavage studies of benzo[*h*]benzopyranyl conjugates **1a-d** in methanol/ HEPES buffer (80:20) and acetonitrile/ HEPES buffer (80:20) solution at 300 and 350 nm, and considering the shorter irradiation times obtained at 350 nm, the benzo[*h*]benzopyranyl label can be considered as a suitable photocleavable protecting group for the carboxylic acid function for synthetic purposes with the considered model molecules. Also, with the proper choice of a solvent system, the release of *N*-protected or free aminoacids can be improved. These promising results indicate that this heterocyclic moiety can be an interesting choice for a photocleavable group and an alternative to other protecting groups of this type.

General Experimental Procedure

Photolysis: A 1×10^{-4} M methanol/HEPES buffer (80:20) or acetonitrile/HEPES buffer (80:20) solution of conjugates **1a-d** (5 mL) was placed in a quartz tube and irradiated in a Rayonet RPR-100 reactor at 300 and 350 ± 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), $MgCl_2$ (1mM) and the 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, at a flow rate of 1 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 (375 nm; (retention time: **1a**, 7.6; **1b**, 8.3; **1c**, 3.3; **1d**, 3.3 min).

Acknowledgements

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