Novel fused oxobenzopyrano[6,7-*d*]oxazoles as light-triggered protecting groups for carboxylic acids

Ana M. S. Soares, Susana P. G. Costa and M. Sameiro T. Gonçalves*
Centro de Química, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
E-mail: msameiro@quimica.uminho.pt

Abstract: In order to evaluate the application of new oxobenzopyrano[6,7-*d*]oxazoles as photocleavable protecting groups, a series of model alanine and β-alanine ester conjugates were synthesised by reaction with the corresponding halomethylated heterocycles. Photocleavage studies of conjugates in methanol/HEPES buffer (80:20) solution at different wavelengths of irradiation (250, 300 and 350 nm) revealed the quantitative release of the amino acids, the best results being obtained for 8-(chloromethyl)-2-methyl-6-oxo-6*H*-benzopyrano[6,7-*d*]oxazole.

Introduction

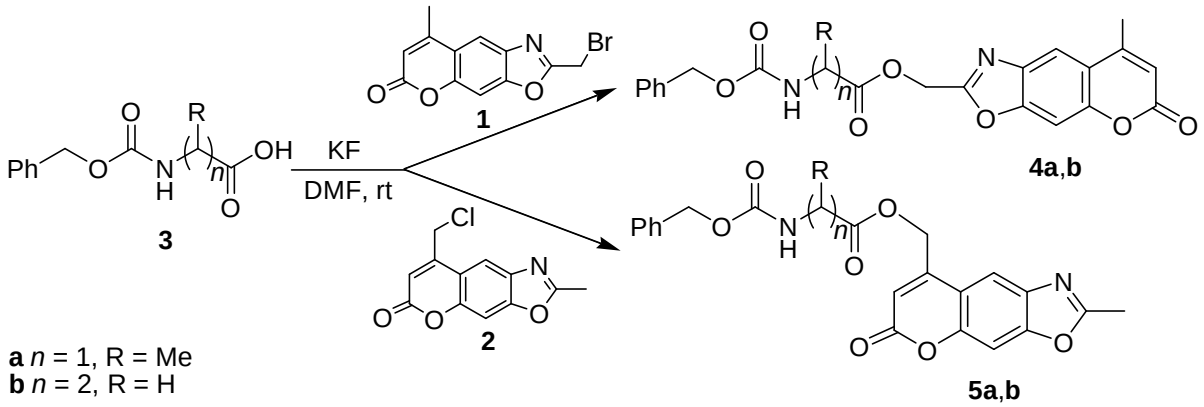
The choice of specific protecting groups remains of crucial importance in the success of many phases of organic synthesis and manipulation of polyfunctional molecules, since they prevent the formation of undesired bonds and site reactions [1]. Photoremovable protecting groups (PRPGs) exhibit numerous advantages, such as the relatively soft conditions required for their deprotection and "orthogonality" with respect to acid- or base-sensitive groups [2,3]. PRPGs have become broadly reported for convenient and controlled release of functional molecules in a variety of environments, including photolithography [4], the "caging" and release of biologically significant compounds [2,3,5], and organic synthesis [2,3]. Molecular structures of PRPGs include the *o*-nitrobenzyl esters and ethers, benzoins, phenacyl esters, and coumarin (trivial designation for oxobenzopyran) derivatives [2,3].

Considering this facts and the recent research work of the authors in the synthesis and application of hetero(aromatics) as light triggering protecting groups for the carboxylic and amine functions of amino acids, as well as neurotransmitters [6-12], it is now reported the use of oxobenzopyrano[6,7-*d*]oxazoles as new protecting groups for carboxylic acids, an innovative application for this type of heterocycle.

Results and Discussion

Fused oxazole derivatives 2-(bromomethyl)-8-methyl-6-oxo-6*H*-benzopyrano-[6,7-*d*]oxazole (Br-Bpx2) **1** and 8-(chloromethyl)-2-methyl-6-oxo-6*H*-benzopyrano[6,7-*d*]oxazole (Cl-Bpx8) **2** were

obtained by a method previously reported by us [13]. These compounds, bearing a reactive halomethyl group either at the oxazole or the benzopyran, were used in the derivatization at the *C*-terminus of *N*-benzyloxycarbonyl-protected alanine **3a** and β-alanine **3b** in the presence of potassium fluoride in DMF, at room temperature [14]. The resulting ester conjugates **4** and **5** were obtained in 53 to 98% yields (Scheme 1, Table 1). UV-visible spectroscopic characterization was also carried out to obtain the parameters needed for monitorization during photolysis. Absorption spectra of degassed 10^{-5} M solutions in absolute ethanol and in a methanol/HEPES buffer (80:20) solution of conjugates **4** and **5** were measured (Table 1).



Scheme 1. Synthesis of model amino acid ester conjugates **4** and **5**.

Table 1. Yields and UV/visible data for conjugates **4**,**5** in absolute ethanol and methanol/HEPES buffer (80:20) solution.

		Ethanol		Methanol/ HEPES buffer (80:20)	
Compound	Yield (%)	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε
4a	53	323	4.01	323	4.08
4b	95	323	4.01	322	3.80
5a	86	325	3.95	325	3.95
5b	98	324	3.95	324	3.94

The evaluation of heterocycles **1** and **2** as photocleavable protecting groups was carried out by photolysis studies of the corresponding alanine and β-alanine ester conjugates **4** and **5** under irradiation at different wavelengths. Solutions of the mentioned compounds (1×10^{-4} M) in methanol/HEPES buffer (80:20) were irradiated in a Rayonet RPR-100 reactor at 254, 300 and 350 nm, in order to determine the most favourable cleavage conditions. The course of the photocleavage reaction was followed by reverse phase HPLC with UV detection [6-12]. 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).

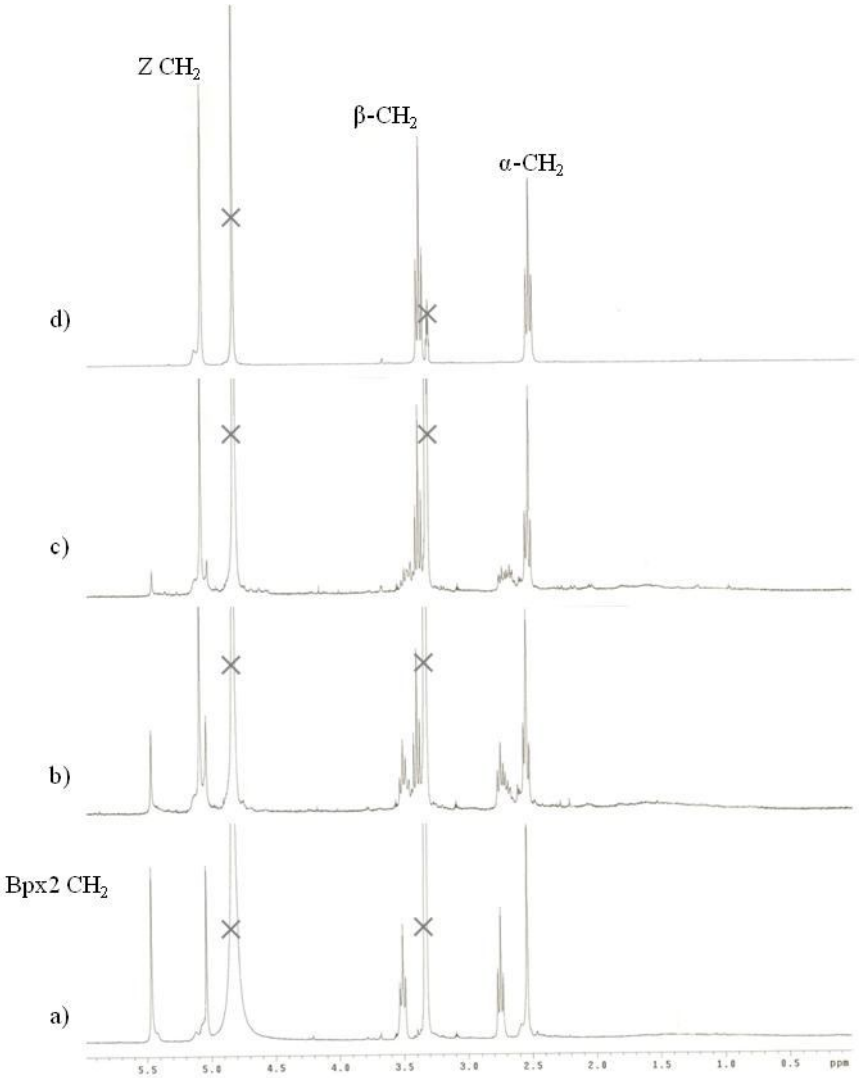
Table 2. Irradiation times (t_{Irr}, min) and rate constants (k, $\times 10^{-2}$ min^{-1}) for the photolysis of conjugates **4** and **5**, at different wavelengths in methanol/HEPES buffer (80:20) solution.

Compound		254 nm		300 nm		350 nm	
		t_{Irr}	k	t_{Irr}	k	t_{Irr}	k
4a	Z-Ala-OBpx2	58.5	5.3	53.3	5.7	4973	0.06
4b	Z- β-Ala-OBpx2	27.8	10.8	37.0	8.2	7545	0.04
5a	Z-Ala-OBpx8	35.4	8.3	18.9	16.1	244	1.3
5b	Z-β-Ala-OBpx8	27.6	11.3	19.3	16.0	242	1.2

It was found that oxobenzopyrano[6,7-*d*]oxazole conjugates **4** and **5** linked to the amino acid either through the oxazole and the oxopyran cleaved readily at 254 and 300 nm, being the best results obtained with the latter. Considering that for practical applications longer irradiation wavelengths are preferable, photoysis of all conjugates at 350 nm was also carried out. In this case the results were significantly different, and although they possessed much lower sensitivity to irradiation at this wavelength, conjugates **5a**,**b** (linked at the oxopyran) exibited the shorter irradiation times (at about 240 min, 4 hours). This result, which is in agreement with our previously reports regarding the possibility of using oxobenzobenzopyrans as protecting groups by photocleavage at 350 nm [10,11], suggests that oxobenzopyrano[6,7-*d*]oxazole (linked though the oxopyran, conjugates **5**) could be considered in a selective photodeprotection at this wavelength, if used in the presence of oxobenzopyrano[6,7-*d*]oxazole (linked through the oxazole, conjugates **4**) with a very slow cleavage at 350 nm.

Furthermore, monitorisation of the photolysis process at 300 nm was also carried out by ^{1}H NMR in a methanol-d_4/D_2O (80:20) solution for all conjugates in a concentration of 9.0×10^{-3} M, which is several times larger than the concentration used in the experiments followed by HPLC, leading to an increase in the photolysis time for the complete release of the amino acid (Figure 1). It was observed that the irradiation process lead to progressively decreased of the the signals related to the linked amino acid, with simultaneous increase of its signals in the released form, as well as signals due to aromatic by-products related to the protecting group.

Figure 1. ^{1}H NMR spectra in methanol-d_4/D_2O (80:20) of the photolysis of conjugate Z-β-Ala-OBpx2 **4b** (C = 9.0×10^{-3} M) at 300 nm: a) before irradiation; b) after irradiation for 560 min; c) after irradiation for 1000 min; d) Z-β-Ala-OH (free form).



The results of the evaluation of oxobenzopyrano[6,7-*d*]oxazoles **1** and **2** as photolabile protecting groups showed the quantitative release of the bifunctional molecules from the corresponding conjugates under irradiation at 250 and 300 nm in short times, as well as at 350 nm with longer values, but also suitable for practical applications. Overall owing this preliminary results, oxobenzopyrano[6,7-*d*]oxazoles are promising alternatives as light triggered protecting groups for the carboxylic function to be further exploited.

General Experimental Procedure

Photolysis procedure: A 1×10^{-4} M methanol/HEPES buffer (80:20) solution of conjugates **4** and **5** (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 254, 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) 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 (324 nm; retention time: **4a**,**b** and **5a**, 4.1 min; **5b**, 4.0 min).

Acknowledgments

Thanks are due to the Foundation for Science and Technology (Portugal) for financial support through project PTDC/QUI/69607/2006 (FCOMP-01-0124-FEDER-007449) and a research grant to A. M. S. Soares.

References

1. Isidro-Llobet, A.; Alvarez, M.; Albericio, F. *Chem. Rev.* **2009**, *109*, 2455–25042.
2. Pelliccioli, A. P.; Wirz, J. *Photochem. Photobiol.* **2002**, *1*, 441–458.
3. Bochet, C. G. *J. Chem. Soc., Perkin Trans. 1* **2002**, 125–142.
4. Pirrung, M. C.; Fallon, L.; McGall, G. *J. Org. Chem.* **1998**, *63*, 241–246.
5. Mayer, G.; Heckel, A. *Angew. Chem. Int. Ed.* **2006**, *45*, 4900–4921.
6. Piloto, A.M.; Rovira, D.; Costa, S.P.G.; M.S.T. Gonçalves, *Tetrahedron* **2006**, *62*, 11955–11958.
7. Fernandes, M. J. G.; Gonçalves, M. S. T.; Costa, S. P. G. *Tetrahedron* **2007**, *63*, 10133–10139.
8. Fonseca, A. S. C.; Gonçalves, M. S. T.; Costa, S. P. G. *Tetrahedron* **2007**, *63*, 1353–1359.
9. Fernandes, M. J. G.; Gonçalves, M. S. T.; Costa, S. P. G. *Tetrahedron* **2008**, *64*, 3032–3038.
10. Fernandes, M. J. G.; Gonçalves, M. S. T.; Costa, S. P. G. *Tetrahedron* **2008**, *64*, 11175–11179.
11. Soares, A. M. S.; Costa, S. P. G.; Gonçalves, M. S. T. *Amino* Acids, **2010**, *39*, 121–133.
12. Fonseca, A. S. C.; Gonçalves, M. S. T.; Costa, S. P. G. *Amino Acids* **2010**, 39, 699-712.
13. Soares, A. M. S.; Costa, S. P. G.; Gonçalves, M. S. T., *submitted*.
14. Tjoeng, F. S.; Heavner, G. A. *Synthesis* **1981**, 897–899.