Pergamon

Tetrahedron Letters 43 (2002) 8947–8950

TETRAHEDRON LETTERS

p-Hydroxyphenacyl bromide as photoremoveable thiol label: a potential phototrigger for thiol-containing biomolecules

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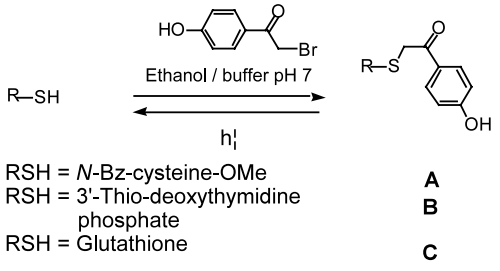
Received 4 September 2002; accepted 27 September 2002

Abstract—*p*-Hydroxyphenacyl bromide is described as a photoremovable thiol protecting group on three biomolecules containing a free thiol group. The protecting group is efficiently incorporated by chemical coupling to the biomolecule in an ethanol–buffer mixture. The photofragmentations ($\lambda = 312$ nm) were analyzed by UV, HPLC and MS methods, yielding over 70% of the free biomolecules. The concomitant formation of *p*-hydroxyphenylacetic thioesters derived from the corresponding thiols, as a sulfur-containing side-product, should not hinder the use of this protecting group for the caging of thiol-containing biomolecules.

The photochemical release of a biomolecule from an inactive precursor represents a well-established method for inducing a time- and space-controlled biochemical or physiological response within functional proteins or living cells respectively.[1] Such masking of biomolecules termed 'caging' requires the design of a photoreversible group to label a chemical function on the biomolecule. The photochemical blocking of thiol-containing molecules has aroused considerable interest for biochemical reasons (existence of numerous cysteine-containing peptides and proteins) as well as for chemical reasons inherent to the strong nucleophilic properties of the thiol group, facilitating its chemical modification.



Scheme 1. *p*-Hydroxyphenacyl bromide as photoreversible thiol label.

The photoreversible thiol labels mostly used were substituted o-nitrobenzyl bromide derivatives.[2] The activity of a series of cysteine-containing proteins could be photoregulated using these reagents.[3–5] Bayley and colleagues have extended this method by targeting cysteines incorporated by site-directed mutagenesis.[6] Alternatively other photoreversible thiol labels have been proposed such as 2-benzoylbenzoate thioesters[7] and 2-nitro-1-(4,5-dimethoxy-2-nitrophenyl)ethyl which was described to photoregulate the activity of β-galactosidase.[8]

p-Hydroxyphenacyl groups have been developed as powerful photoactivated protecting groups[9] for carboxylate, phosphate and thiophosphate residues allowing an efficient 'caging' of excitatory amino acids,[10] nucleotides[11] and thiophosphotyrosine peptides[12] respectively. The photolytic reaction of *p*-hydroxyphenacyl derivatives, besides allowing a fast and efficient release of the biomolecule, generates predominantly low-wavelength excitation wavelengths (λ_{ex}>300 nm).[9] Only one example of photoregulation of the activity of protein phosphatase has been described by masking the catalytic cysteine using *p*-substituted phenacyl derivatives, as evidenced by MS-electrospray experiments.[13]

The present article describes the syntheses and the analyses of the photolytic reactions of *p*-hydroxyphenacyl *S*-substituted *N*-Bz-cysteine-OMe (**A**), 3′-thio-2′-deoxy- thymidine phosphate (**B**) and glutathione (**C**) to demonstrate the usefulness of the *p*-

Keywords: caged compounds; photofragmentation reactions.

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0040-4039/02/$ - see front matter
PII: S0040-4039(02)02194-9

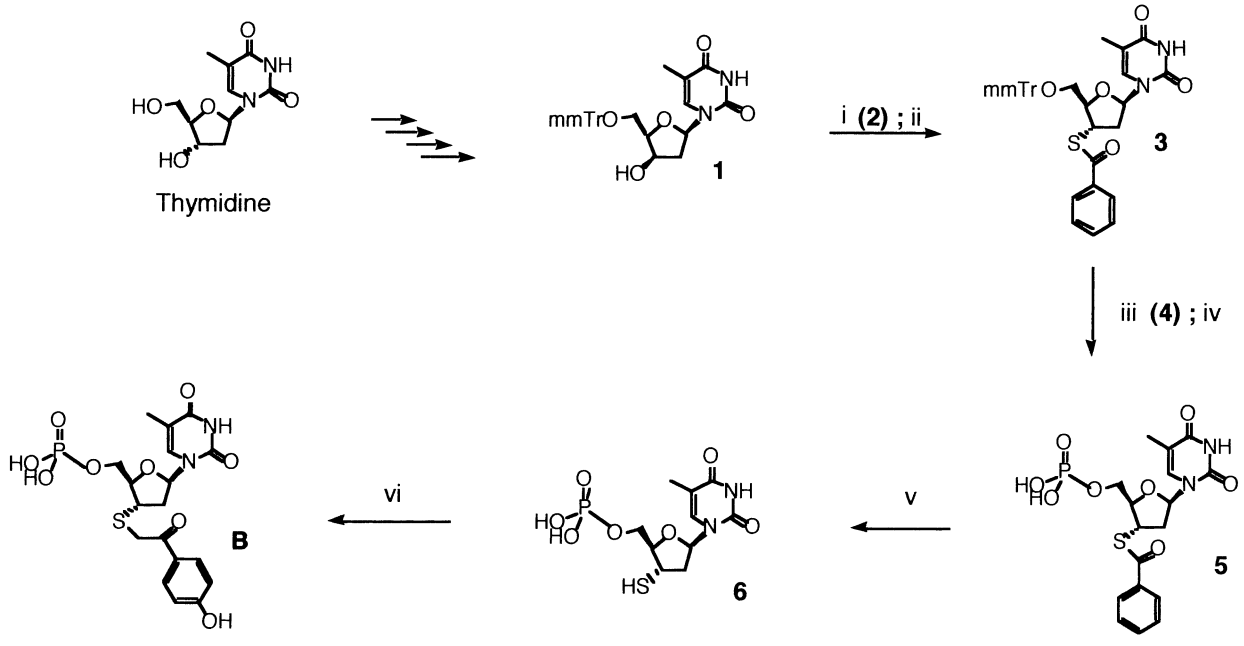
hydroxyphenacyl group to mask thiol-containing biomolecules (Scheme 1). We used an *N*-Bz substituted cysteine derivative to facilitate UV detection for HPLC analysis during the photolytic reaction.

The chemical modification of thiol-containing molecules can be achieved by direct treatment with *p*-hydroxyphenacyl bromide (Scheme 1). The coupling yields are usually high and the reagent can be used in 1/1 ethanol/aqueous buffer (1N HEPES pH 7.0) mixtures for the reaction of water soluble substrates (**A**: 80%; **B** 90%; **C**: 92%). It should be expected that selective modification of cysteines[13] can be obtained by direct treatment of a protein with *p*-hydroxyphenacyl bromide.

3′-Thio-2′-deoxythymidine phosphate (3′-thio-dTMP) represents a substrate analog[14] for thymidilate mono phosphate kinase (TMPK) which *trans*-phosphorylates a phosphate group from ATP to dTMP forming ADP and dTDP. The synthesis of the 3′-thio analog of dTMP is outlined in Scheme 2. 5′-*O*-Methoxymethyl-trityl-lyxofuranosyl thymidine **1** was synthesized from thymidine using a described method.[15] This four-step synthesis ensured the desired configuration of the 3′-thio group by two successive inversions at this position. A thioester group (compound **3**), precursor of the thiol group, was stereospecifically introduced[16] at the 3′-position by reaction of sodium thiobenzoate with the mesylate **2**. The monophosphate group was incorporated at the 5′-OH position by reaction with phosphorus oxychloride after removal of the protecting group.[17] Finally, hydrolysis of the thioester **5** gave the desired 3′-thio dTMP **6**. This nucleotide monophosphate analog was masked by treatment with *p*-hydroxyphenacyl bromide using a 1/1 ethanol/HEPES buffer mixture. Probe **B** was synthesized in 21% overall yields from the lyxofuranosyl thymidine derivative **1**. Direct introduction of the *p*-hydroxyphenacyl group as a protecting group by converting the mesylate **2** with *p*-hydroxyphenacyl mercaptan could not be used due to non-compatible properties of the phenacyl group with the subsequent phosphorylation conditions.

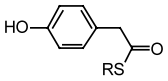
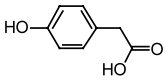
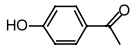
The photolytic reactions of **A**, **B** and **C** ($\lambda = 312$ nm in Tris–HCl buffer; pH 7.2) were analyzed by UV spectroscopy and HPLC as well as by MS for the glutathione derivative **C** (Fig. 1). All three compounds showed a decrease in absorbance at 290 and 340 nm corresponding to the disappearance of the starting compounds (λ_{max} 290 and 340 nm). The presence of isobestic points at 270 nm was indicative of homogeneous photolytic reactions. The HPLC analyses of these reactions depicted the formation of the unmasked thiol derivatives, the expected side-products: *p*-hydroxyphenylacetic acid together with *p*-hydroxy acetophenone[18] as well as a new compound: the *p*-hydroxyphenylacetic thioester[19] derived from the biomolecules. Table 1 summarizes the photochemical results on compounds **A**, **B** and **C**, respectively.

Quantum yield of the photolytic reaction for **B** was determined by actinometry using azobenzene as actinometer[20] at 313 nm. A quantum yield of 0.085 for



Scheme 2. Synthesis of *p*-hydroxyphenacyl thioether of 3′-thio dTMP (**B**). *Reagents and conditions*: (i) MsCl, pyridine, 0°C, v22 h, $\rho = 98\%$; (ii) sodium thiobenzoate, DMF, 100°C, 22 h, $\rho = 55\%$; (iii) AcOH, rt, 24 h, $\rho = 85\%$; (iv) 1. $POCl_3$, trimethylphosphate, 0°C, 22 h, 2. triethylammonium formate 0.2N pH 7, 0°C 30 min and C18 HPLC reversed phase purification, $\rho = 58\%$; (v) NaOH, EtOH, 0°C, 70 min, C18 HPLC reversed phase purification, $\rho = 88\%$; (vi) *p*-hydroxybromophenacyl 1N HEPES buffer pH 7/EtOH: 1/1, rt, 2 h, C18 HPLC reversed phase purification, $\rho = 90\%$.

Table 1. Product yields generated by 312 nm irradiation of 0.1 mM solutions of **A**, **B** and **C** in 100 mM Tris–HCl buffer pH 7.2, 1 mM DTT

Compound	A	B	C
Photolytic products	%	%	%
RSH (RSSR)	60 (5)	71	ND
	30	29	23
	60	62	65
	10	9	12

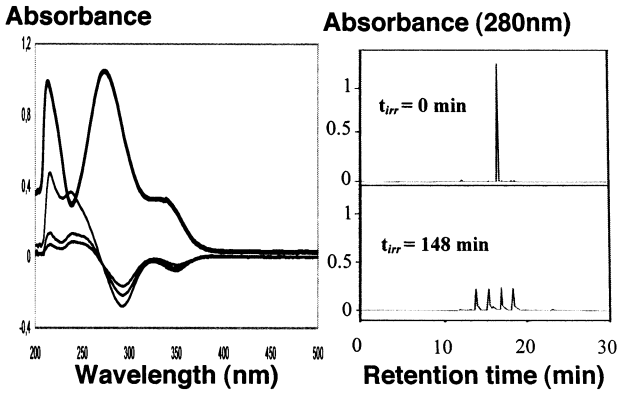


Figure 1. The caged 3′-thio-2′-deoxythymidine phosphate (0.1 mM in 100 mM Tris–HCl buffer pH 07.2, 1 mM DTT) was exposed to the 312 nm line of a 1000 W Hg lamp. (Left) UV spectrum of the caged 3′-thio-2′-deoxythymidine phosphate (upper trace) and difference UV-spectra recording during photolysis (lower traces). (Right) HPLC analysis. **B** has a retention time of 16.6 min. The appearing peaks at 13.8, 15.3, 17 and 18.2 min correspond to the 3′-thio dTMP, the *p*-hydroxy-phenylacetic acid, the thioester and the *p*-hydroxy acetophenone, respectively.

B was evaluated in 0.1 M phosphate buffer pH 7.2. This value is lower than the ones described for the photolyses of *p*-hydroxyphenacyl-derived carboxylate[21] or phosphate esters.[11]

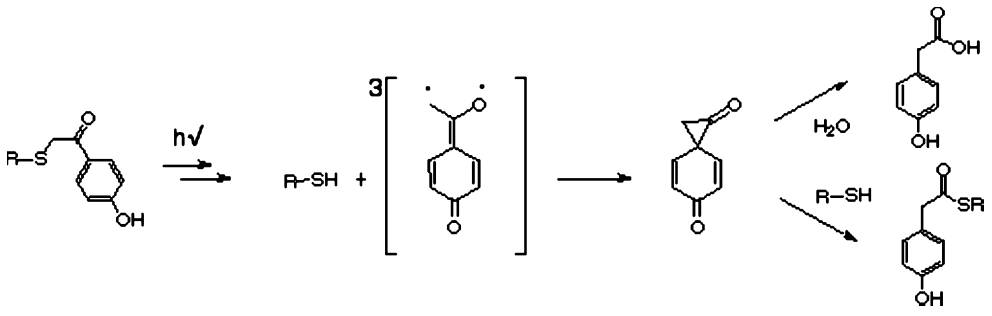
Concerning the formation of sulfur-containing molecules, these photolytic reactions generate about 70% of the thiols together with 30% of the thioesters. The photochemical fragmentation mechanism of *p*-hydroxyphenacyl derivatives is not fully established; a triplet diradical intermediate has been proposed to generate a hypothetical spirocyclopropanone intermediate whose hydrolysis does explain the formation of the *p*-hydroxyphenylacetic acid (Scheme 3).[11,22] Thiols are sufficiently acidic (pK_a about 8.4 in water) to allow such fragmentations to occur while their strong nucleophilic properties could explain the formation of the thioesters by nucleophilic opening of the spirocyclopropanone.

Alternative radical mechanisms are indeed possible, to account in particular for the formation of *p*-hydroxyacetophenone which is formed in about 10% yield.

The formation of the thioester derived from *p*-hydroxyphenylacetic acid is an undesired side-product for the use of *p*-hydroxyphenacyl bromide as a photochemical thiol protecting group; nevertheless such side-reactions do not necessarily occur within a protein binding site where the different reaction intermediates do not remain in vicinity to the unmasked thiol. Therefore, this reagent remains an interesting 'caging' group for biomolecules as demonstrated by the efficient photoregulation of the activity of a tyrosine phosphatase.[13]

Acknowledgements

The authors thank Professor Jakob Wirz and Anna Paola Pelliccioli for assistance in quantum yield determination and Raymond Hueber and Dr. Alain Van Dorsselaer for MS experiments. This work was supported by the Centre National de la Recherche Scientifique, the Région Alsace and the Université Louis Pasteur, Strasbourg.



Scheme 3. Hypothetical photochemical fragmentation mechanism of *p*-hydroxyphenacyl thioethers.

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