New Phototriggers:1 Extending the p-Hydroxyphenacyl \( \pi-\pi^* \) Absorption Range

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ABSTRACT

Introducing 3-methoxy or 3,5-dimethoxy substituents on the 4-hydroxyphenacyl (pHP) photoremovable protecting group has been explored with two excitatory \( \gamma \)-amino acids, \( \gamma \)-glutamic acid and \( \gamma \)-amino butyric acid (GABA). These substituents significantly extend the absorption range of the pHP chromophore, e.g., the tail of absorption bands of 2a,b extend above 400 nm, well beyond the absorptions of aromatic amino acids and nucleotides. Irradiation releases the amino acids with rate constants of \( 10^7 \) s\(^{-1} \) and appearance efficiencies (\( \Phi_{app} \)) of 0.03–0.04.

The photoproducts are formed through the pHP excited triplet and are primarily products of photoreduction and photohydrolysis. 1a,b also rearranged to the phenylacetic acid 3.

We report herein the use of 3-methoxy- and 3,5-dimethoxy-4-hydroxyphenacyl (MeO-pHP and (MeO)\(_2\)-pHP) as new members of the p-hydroxyphenacyl (pHP) series of carboxylate and phosphate photoremovable protecting groups. Earlier we\(^2\) introduced the p-hydroxyphenacyl (pHP) phototrigger as an efficient, rapid photoremovable protecting group for applications in neurobiology, physiology, and biochemistry. Since its introduction, a variety of pHP derivatives have been employed for the release of ATP,\(^2\) glutamic acid,\(^3,4\) GABA,\(^3\) the dipeptide Ala-Ala,\(^1\) and the C-terminus of the nonapeptide bradykinin.\(^1\) Several in vitro biochemical studies have shown that the attachment of pHP to the \( \gamma \)-carboxyl group of glutamic acid or GABA or the C-terminal carboxylic acid group of peptides completely suppresses the normal biological activity of the natural substrates.\(^1,3\)

In several studies, we have demonstrated that the photorelease occurs from the triplet state of the phototrigger since it can be quenched with either potassium sorbate or sodium 2-naphthalenesulfonate\(^1,5\) with excellent Stern–Volmer-derived kinetic rates of \( \sim 10^9 \) s\(^{-1} \) or faster for release for these substrates. A concomitant rearrangement of the phototrigger to p-hydroxyphenylacetic acid (Scheme 1) was shown to be the major pathway\(^6\) for the pHP chromophore. The rearrangement is preceded by an excited-state deproto-
nation\textsuperscript{7} of the phenolic proton yielding the triplet phenolate ion,\textsuperscript{5} the apparent driving force for the rearrangement.\textsuperscript{4}

To expand the dynamic range for this phototrigger and to move the effective absorption away from the range of normal peptides and nucleotides, we have explored the effects of added methoxy substituents on the pHP chromophore and the resulting photochemistry. Our results are presented here.

The synthesis of the methoxy pHP esters of L-glutamate (1a, 2a) and of γ-aminobutyric acid (GABA, 1b, 2b) were accomplished by the DBU-catalyzed displacement of bromide from the appropriate \( \text{R-bromoacetophenone} \) followed by deprotection of the remaining amino acid protecting groups with TFA to give the methoxy phenacyl protected neurotransmitters 1a, 1b and 2a, 2b (Scheme 2).

As shown in Figure 1, the absorption maxima for the new 3-methoxy-4-hydroxyphenacyl and the 3,5-dimethoxy-4-

hydroxyphenacyl chromophores 1 and 2 are in fact shifted to 350 and 370 nm, respectively.

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\( \text{Scheme 2. Synthesis of Methoxy pHP GABA's and L-glutamates} \)

\( \begin{align*}
\text{6} & \quad R = 3\text{-OMe} \\
\text{7} & \quad R = 3,5\text{-OMe} \\
\text{8} & \quad R = 3\text{-OMe} \\
\text{9} & \quad R = 3,5\text{-OMe} \\
\text{a} & \quad R' = \text{CO}_2\text{Bu} \\
\text{b} & \quad R' = \text{Boc} \\
\text{1} & \quad R = 3\text{-OMe} \\
\text{2} & \quad R = 3,5\text{-OMe} \\
\text{a} & \quad R' = \text{CO}_2^- \\
\text{b} & \quad R' = \text{NH}_2 \\
\end{align*} \)

\textbf{Scheme 2} (a) DBU, N-Boc-γ-amino butyric acid or N-Boc-\textit{l}-glutamic acid, \( \alpha\text{-t}-\text{butyl ester, 1,4-dioxane, 0° to rt, 12\text{h}} \); (b) \( \text{TFA, 0°, 4\text{h}} \).

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\( \text{Scheme 3. Photorelease of L-glutamic Acid and GABA from 1a,b and 2a,b} \)

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\( \text{Figure 1. Ultraviolet spectra of three caged \textit{l}-glutamates, 3-methoxy-pHP 1a, 3,5-dimethoxy-pHP 2a, and pH 3, and three caged GABA’s, 3-methoxy-pHP 1b, 3,5-dimethoxy-pHP 2b, and pH 4.} \)

Photolyses of 1 and 2 were performed in aqueous solutions with either 300 or 350 nm lamps (Scheme 3) and were
monitored by HPLC or 1H NMR (D2O). The release of the amino acids occurred with quantum efficiencies\(^8\) of 0.03 to 0.04, somewhat below those that we reported for the parent pHP amino acids.\(^3\)

Photolysis of 2a in phosphate buffer (pH 7.2) in the presence of 0.001–0.010 M potassium sorbate quenched the photorelease of the amino acids and gave good Stern–Volmer kinetics \((K_{SV} = 343 \text{ M}^{-1})\) resulting in a calculated triplet lifetime of 46 ns and rate constant for release of \(2.2 \times 10^7 \text{ s}^{-1}\) (assuming \(k_{diff} = k_q = 7.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}\)).\(^9\)

Phosphorescence spectra gave triplet energies of 69 kcal/mol for 1a and 71 kcal/mol for 2a. The phosphorescence emission for 1a was also quenched by potassium sorbate. These results clearly indicate a reaction pathway through the triplet excited state of these new phototriggers and are in accord with our recent results for p-hydroxyphenacyl (pHP).\(^4\)

In contrast to the earlier results, however, rearrangement to the phenylacetic acid has become a minor pathway for 1 and is not observed at all for 2. These alternative photochemical pathways have been reported in a wide array of other phenacetyl derivatives.\(^10\)

The biological efficacy of these new phototriggers has likewise been tested using electrophysiological whole-cell patch clamp recordings for CA1 neurons in acute hippocampal brain slices\(^3\) and from cultured neocortical neurons.\(^11\)

Photolysis\(^12\) of a 200 mM solution of 3,5-dimethoxy-4-hydroxyphenacyl GABA (2b) bathing a CA1 hippocampal neuron from a 7-day-old rat elicited a response from the cell as indicated by the detection of outward currents as shown in Figure 2. In all neurons tested \((n = 12)\), photolysis of 2b consistently elicited fast membrane currents. These responses were due to the specific stimulation of GABA A receptors (a ligand gated chloride channel) because application of Bicuculline abolished all inward currents. Figure 2 depicts the responses stimulated by the release of GABA from 2b.


\(^13\) Bicuculline is a known GABA A-receptor antagonist. The fact that the cell shows no response after the antagonist is added indicates that only free GABA causes the responses.