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New Phototriggers:[1] Extending the *p*-Hydroxyphenacyl $\pi-\pi^*$ Absorption Range

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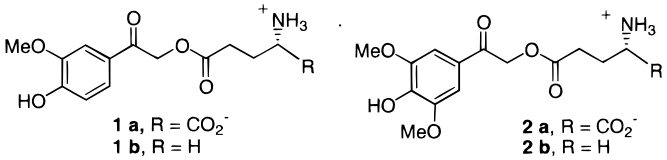
ABSTRACT

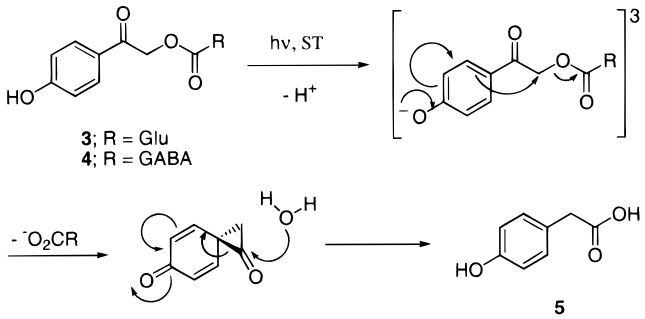


Introducing 3-methoxy or 3,5-dimethoxy substituents on the 4-hydroxyphenacyl (pHP) photoremovable protecting group has been explored with two excitatory γ-amino acids, L-glutamic acid and γ-amino butyric acid (GABA). These substituents significantly extend the absorption range of the pHP chromophore, 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 (Φ_{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 γ-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 ~10^8 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-

Scheme 1. Mechanism for the Photorelease from pHP



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(1) For the previous paper, see: Givens, R. S.; Weber, J. F. W.; Conrad, P. G.; Orosz, G.; Donahue, S. L.; Thayer, S. A. *J. Am. Chem. Soc.* **2000**, *122*, 2687−2697.

(2) (a) Givens, R. S.; Park, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6259−6262. (b) Park, C.-H.; Givens, R. S. *J. Am. Chem. Soc.* **1997**, *119*, 2453−2463.

(3) Givens, R. S.; Jung, A. H.; Park, C.-H.; Weber, J. F. W.; Bartlett, W. *J. Am. Chem. Soc.* **1997**, *119*, 8369−8370.

(4) Kandler, K.; Katz, L. C.; Kauer, J. A. *Nature Neuro.* **1998**, *1*, 114−118.

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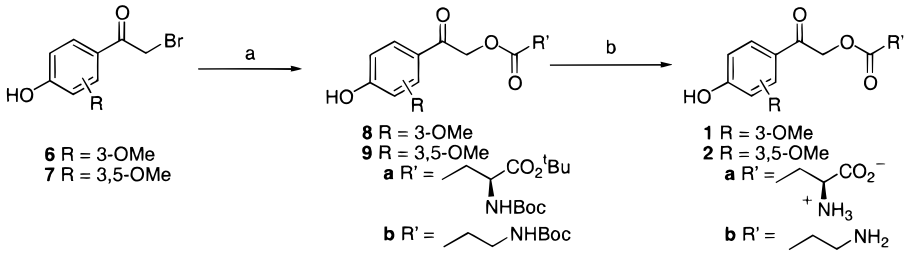
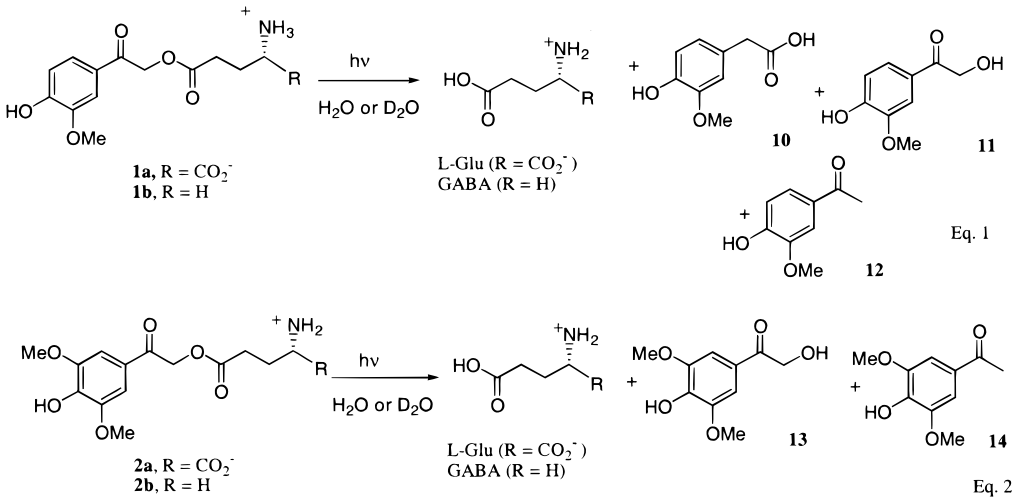
Scheme 2. Synthesis of Methoxy pHP GABA's and L-Glutamates



a) DBU, N-Boc-γ-amino butyric acid or N-Boc-glutamic acid, α-t-butyl ester, 1,4-dioxane, 0° to rt, 12h; b)TFA, 0°, 4h

Scheme 3. Photorelease of l-glutamic Acid and GABA from **1a**,**b** and **2a**,**b**



nation[7] of the phenolic proton yielding the triplet phenolate ion,[5] the apparent driving force for the rearrangement.[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 α-bromoacetophenone followed by deprotection of the remaining amino acid protecting groups with TFA to give the methoxy phenacyl protected neurotransmitters **1a**,**b** and **2a**,**b** (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.

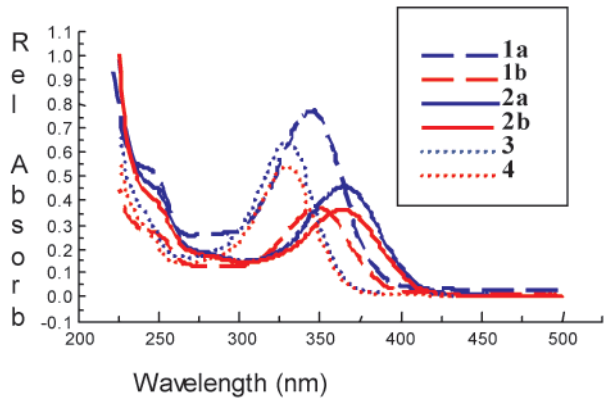


Figure 1. Ultraviolet spectra of three caged L-glutamates, 3-methoxy-pHP **1a**, 3,5-dimethoxy-pHP **2a**, and pHP **3**, and three caged GABA's, 3-methoxy-pHP **1b**, 3,5-dimethoxy-pHP **2b**, and pHP **4**.

Photolyses of **1** and **2** were performed in aqueous solutions with either 300 or 350 nm lamps (Scheme 3) and were

(5) (a) Conrad, P. G.; Givens, R. S.; Hellrung, B.; Rajesh, C. S.; Ramkseier, M.; Wirz, J. Submitted for publication. (b) See also refs 1 and 2.

(6) Anderson, J. C.; Reese, C. B. *Tetrahedron Lett.* **1962**, 1−4.

(7) A recent report by Corrie and Wan (Zhang, K.; Corrie, J. E. T.; Munasinghe, V. R. N.; Wan, P. *J. Am. Chem. Soc.* **1999**, *121*, 5625−5632) suggested a singlet state rearrangement preceded by deprotonation. While we differ on the multiplicity of the reaction and even on the order of the events, we are in substantial agreement that deprotonation does precede the rearrangement (see ref 5).

monitored by HPLC or ^{1}H NMR (D_2O). 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 ($K_{SV} = 343\ M^{-1}$) resulting in a calculated triplet lifetime of 46 ns and rate constant for release of $2.2 \times 10^7\ s^{-1}$ (assuming $k_{diff} = kq = 7.4 \times 10^9\ M^{-1}\ s^{-1}$).[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 phenacyl 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[4] 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**.

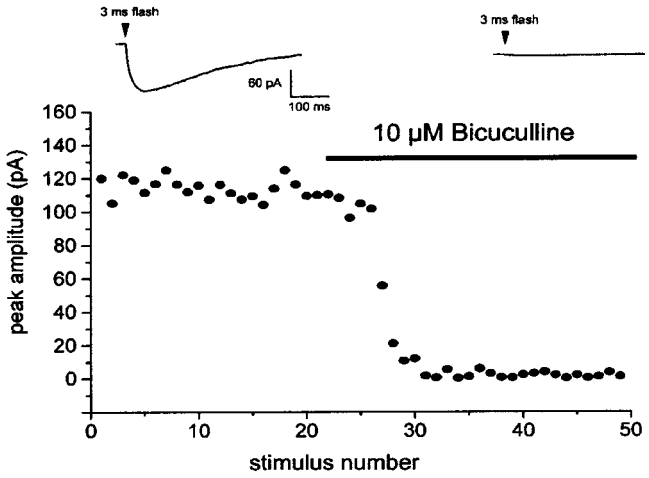


Figure 2. Peak amplitude of currents elicited by released GABA is plotted against stimulus number (interval between stimuli 20 s) and is the average of 10 consecutive traces. After stimulus #22, 10 M Bicuculline[14] was added to the bath solution, which completely blocked the responses elicited by photorelease of GABA. The relatively constant amplitude (114.2 p*A* ± 1.4 SEM) for the 20 flashes prior to receptor shut down by Bicuculline demonstrates that the cell is not harmed by the transient exposure to the UV flashes or to the appearance of the phototrigger side products during photolysis.

These preliminary results for the 3-methoxy-4-hydroxyphenacyl and 3,5-dimethoxy-4-hydroxyphenacyl phototriggers demonstrate the potential for effective release of certain α-amino acids[14] and for C-terminal protected peptides. Furthermore, release of amino acids, oligopeptide, and proteins in biological environments employing laser flash photolysis and other fast kinetic techniques using pHP phototriggers such as **1** or **2** is possible at $\lambda > 300$ nm and even beyond $\lambda = 400$ nm.

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Supporting Information Available: Details of the synthesis and ^{1}H and ^{13}C NMR, UV, IR, and HRMS data for **1**, **2**, and **6**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org. Complete experimental details and additional examples of substituted pHP derivatives will be provided in our full paper.

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(8) Hatchard, C. G.; Parker, C. A. *Proc. R. Soc. London A* **1956**, *A220*, 518.

(9) For the calculated estimate of the bimolecular rate for diffusion in H_2O, see: Simons, J. P. *Photochemistry and Spectroscopy*; J. Wiley-Interscience: New York, 1971; pp 212−213.

(10) See the following and references therein for examples of alternative photoinduced α-cleavage reactions of substituted acetophenones, for example: (a) Scaiano, J. C.; Netto-Ferreira, J. C. *J. Photochem.* **1986**, *32*, 253−259. (b) Sheehan, J. C.; Umezawa, K. *J. Org. Chem.* **1973**, *38*, 3771−3774. (c) Banerjee, A.; Falvey, D. E. *J. Am. Chem. Soc.* **1998**, *120*, 2965−2966.

(11) Hartnett, K. A.; Stout, A. K.; Rajdev, S.; Rosenberg, P. A.; Reynolds, I. J.; Aizeman, E. *J. Neurochem.* **1997**, *68*, 1836−1845.

(12) The experimental apparatus and wavelength range are described elsewhere. Kandler, K.; Givens, R.; Katz, L. C. Photostimulation with Caged Glutamate (Chapter 27). In *Imaging Neurons: A Laboratory Manual*; Yuste, Lanni, R. F., Konnerth, A., Eds.; Cold Spring Harbor Laboratory Press: 1997. Typically, 2 ms UV flashes were employed.

(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.

(14) Givens, R. S.; Weber, J. F. W.; Jung, A. H.; Park, C.-H. New Photoprotecting Groups: Desyl and p-Hydroxyphenacyl Phosphate and Carboxylate Esters. In *Methods in Enzymology: Caged Compounds*; Marriott, G., Ed.; **1998**, *291*, 1−29.