New Photoactivated Protecting Groups. 7. *p*-Hydroxyphenacyl: A Phototrigger for Excitatory Amino Acids and Peptides[1]

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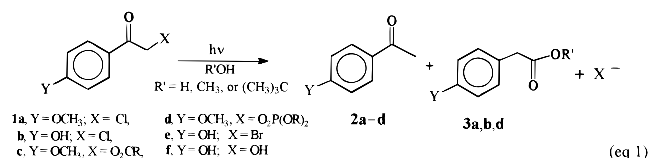
We report here our results for *p*-hydroxyphenacyl as the phototrigger for the excitatory amino acids L-glutamate and GABA and for a model peptide, the dipeptide, ala-ala. Our initial studies in this arena began with the photorelease of cAMP,[2,3] L-glutamate,[4] and GABA[4] from their benzoin (desyl) esters which demonstrated that release occurred much faster with rate constants of 10^7-10^8 s^{-1} than are observed from the archetypical *o*-nitrobenzyl analogues[6−8] which typically release substrates at rates of $1-100$ s^{-1}. The photorelease of the substrate from desyl ester occurs with a high efficiency (~0.30) and is free of complicating side reactions. However, these reactions are plagued by low aqueous solubility and the incorporation of an additional chiral center with these esters and a UV absorbing photoproduct. Our search for a phototrigger that was free of these limitations resulted in the design of *p*-hydroxyphenacyl moiety, and we subsequently demonstrated that it functioned as a phototrigger for ATP.[1,5]

Since protecting groups for amino acids, peptides, and proteins have been extensively studied and developed, they have been applied in biochemistry and physiology[6] and in synthesis.[7] The *o*-nitrobenzyl protecting group has been thoroughly exploited for the biological and physiological applications and remains the principal component employed in the field of "caged" compounds.[8] However, researchers have begun focussing on designing cages with higher efficiencies and better absorption properties.[9] With the increased interest in the mechanistic and kinetic details of the molecular events surrounding substrate-activated biochemical processes, attention is now shifting toward designing more rapid photoreleasing cages or phototriggers.[1−5,9]

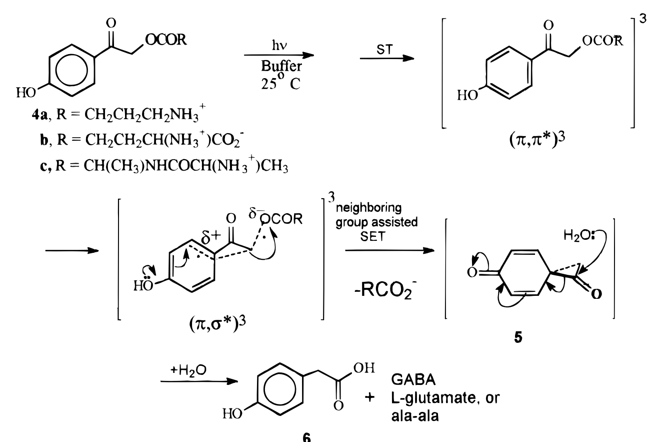
Previous studies by Anderson and Reese,[10] Sheehan et al.,[11] and Epstein and Garrossian[12] had clearly demonstrated that the photochemistry of the *p*-methoxyphenacyl derivatives (**1a**,**c**,**d**) was effective in releasing a variety of nucleofugal groups (e.g., RCO_2^-, Cl^-, and $(RO)_2PO_2^-$) in organic solvents. Anderson and Reese further demonstrated that the release of chloride[10] from **1a** or **b** gave, in addition to major yields of the corresponding acetophenones (**2**), rearranged *p*-methoxyphenylacetate and *p*-hydroxyphenylacetate (**3a**,**b**), respectively (eq 1). Earlier, we confirmed that rearrangement was also a major



(eq 1)

pathway for *p*-methoxyphenacyl phosphates[1,3,5] in alcohol solvents[3] and more recently the **exclusive** pathway for *p*-hydroxyphenacyl phosphate (P*i*) and ATP[1,5] in aqueous media. These results warranted further elaboration of this α-keto phototrigger for excitatory amino acids, *e.g.*, L-glutamate and **γ-aminobutyric acid (GABA)** that are frequently employed by others for studies of neurotransmission,[8a] for brain neuronal mapping,[8b] and the temporal and spatial studies on neuronal stimulation.[8c−e]

Scheme 1



Possible precursors for the synthesis of these "caged" amino acid esters are the bromoketone **1e** or the hydroxyketone **1f** which are readily available from *p*-hydroxyacetophenone.[13] We chose to synthesize the caged amino acids and dipeptide through an S_N2 displacement of the ketobromide **1e** with the N-protected amino acid catalyzed by DBU followed by deprotection with TFA.[14,15] The resulting esters **4a**−**c** displayed excellent stability in water, D_2O, and Ringers solutions, showing no hydrolysis after 24 h at room temperature. In TRIS, **4c** hydrolyzed slowly with a half-life of 214 min, whereas the GABA and glutamate esters were stable.

Irradiation of buffered solutions of *O*-*p*-hydroxyphenacyl GABA (**4a**), *γ*-*O*-*p*-hydroxyphenacyl L-glutamate (**4b**), and the *O*-*p*-hydroxyphenacyl ala-ala (**4c**) resulted in the release of the amino acid or dipeptide accompanied by the quantitative rearrangement of the phenacyl moiety to *p*-hydroxyphenylacetic

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(13) The bromide (**1e**) was obtained in 47% yield by treating *p*-hydroxyacetophenone (**2b**) with cuprous bromide in refluxing ethyl acetate: $CHCl_3$. The bromide was converted to the alcohol (**1f**) in 49% yield by treatment with formic acid and DBU in CH_2Cl_2 followed by hydrolysis of the formate ester with basic (NaOH) methanol (mp 165−167 °C).

(14) The protected N-BOC GABA, N-BOC α-O-*t*-Bu L-glutamate, and *N*-BOC ala-ala carboxylic acids were reacted with *p*-hydroxyphenacyl bromide (**1e**) in benzene with DBU followed by treatment with TFA to give the *p*-hydroxyphenacyl protected amino acids **4a** and **4b** or dipeptide **4c** in yields of 70−85%. Details will be provided in our full paper.

(15) The esters of α-amino acids glycine, tyrosine, and L-glutamate were also synthesized but were found to be hydrolytically unstable, most likely due to the inductive influence of the protonated α-amino group at neutral pH.[4] The dipeptide **4c** was synthesized for the intended purpose of demonstrating the hydrolytic stability of a less basic peptide nitrogen.

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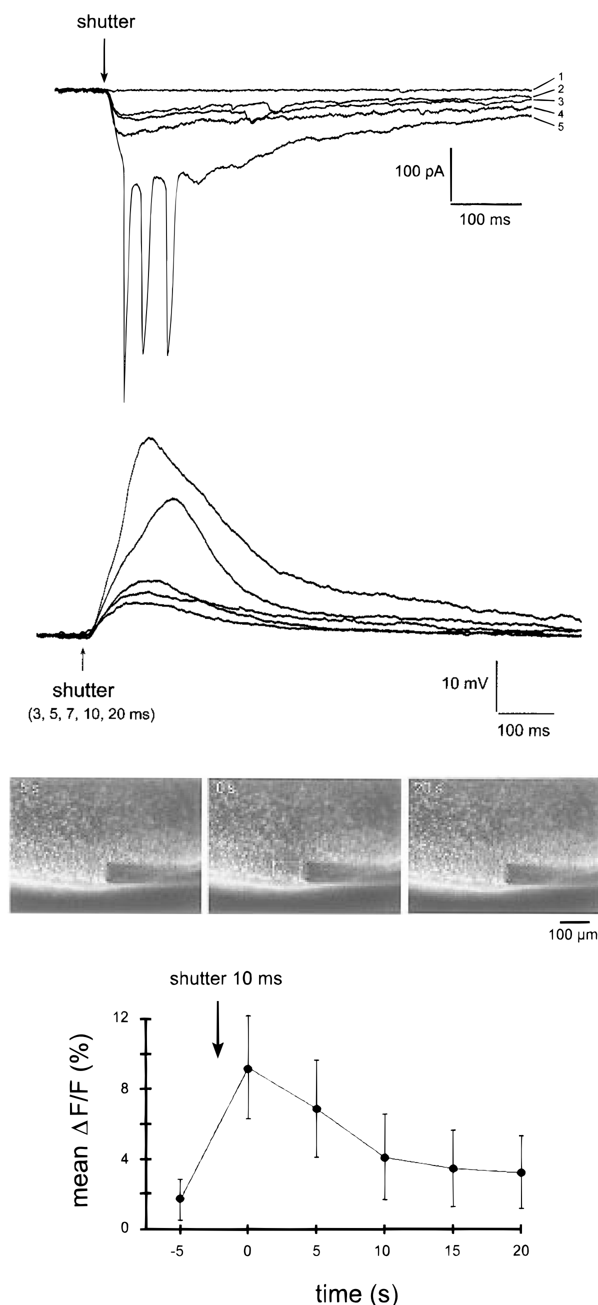

Figure 1. Effect of irradiated rat cortical brain slices perfused with *p*-hydroxyphenacyl L-glutamate (**4b**). (a, top) Inward currents of 100 to 1500 pA were evoked in whole cell voltage-clamped at −70 mV (1) in the absence of **4b** (trace 1; 10 ms exposure time) and in the presence of with 50 μM concentration of **4b** (traces 2−5; at shutter openings of 3, 5, 7, and 10 ms exposure times, respectively. The cells generated three action potentials when illuminated with 10 ms flashes. (b, middle) Voltages obtained with current-clamp whole-cell patch recordings of a CA1 pyramidal neuron in a hippocampal slice superfused with 100 μM **4b** in the presence of 2 μM TTX at the indicated shutter openings. Above 7 ms shutter opening, a regenerative Ca^{2+} spike generates a significantly greater response. (c, bottom) Response monitored by fluorescence of FURA-2 labeled cortical slice, neonatal rat cells to intracellular Ca^{2+} increases following a 10 ms flash in the presence of 200 μM concentration of **4b**. Images in upper row represent video frames taken 5 s before, 0 s, and 20 s after flash photolysis monitored at 385 nm. The lower graph shows the mean fluorescence changes occurring near the tip of the optic fiber (frame) expressed as $\Delta F/F$ (%).

acid (**6**, Scheme 1).[9,16] As evidence of their efficacy as phototriggers for the release of excitatory amino acid substrates, Katz and Kandler[17] performed patch-clamp and calcium-sensitive dye studies by Hg flash irradiation of perfused slices of rat cortex through thin fused-silica fiber optics (Figure 1) which demonstrate the degree of temporal and spatial control provided by the release of L-glutamate from phototrigger **4b**.

As suggested for the photorelease of phosphates, amino acid release probably passes through a spirodienedione **5** or its equivalent (Scheme 1). The reaction proceeds from the phenacyl triplet[18] in an overall efficiency for disappearance of **4a−c** of 0.35, 0.12, and 0.27, respectively, determined by HPLC and NMR. The formation efficiencies of *p*-hydroxyphenylacetic acid were 0.08 and 0.24 for **4b** and **4c**, respectively. The yields of the amino acids were quantitative within the detection limits of NMR and HPLC and matched the appearance efficiencies for **6**, *i.e.*, 0.08 (glu) and 0.25 (ala-ala). A rate constant of $7 \times 10^7\ s^{-1}$ was determined for the release of the dipeptide **4c** from Stern−Volmer quenching with sodium 2-naphthalenesulfonate[18] and agrees well with the observed k_r values obtained for the corresponding phosphates.[3,19]

The rate constants for release of a carboxylate moiety are evidence of an excited state fragmentation process, possibly from a $\pi^* \rightarrow \sigma^*$ electron transfer to the ester bond. Saveant et al[20] recently correlated the rates of fragmentation of a series of substituted phenacyl bromides, esters, and ethers from their radical anions with standard free energies of reaction and found that the *p*-methoxyphenacyl radical anion was among the most reactive in the series. They attributed the enhanced reactivity to substituent assisted electron transfer from the phenacyl radical anion to the σ^* orbital of the carbon−oxygen bond of the ester, inducing rapid fragmentation of the nucleofuge from the phenacyl moiety. In our studies, the transfer of the π^* electron of the photoexcited phenacyl moiety to the σ^* orbital of the amino acid ester bond could be envisaged as initiating the rapid fragmentation of the phototrigger.

A second plausible mechanistic pathway involving initial homolytic fragmentation of the ester CO_2−C bond from the excited triplet of the phenacyl chromophore followed by rapid electron transfer[21] has been suggested for the singlet state photochemistry of benzyl acetates,[21] pivalates,[21] and phosphates,[22] in polar, hydroxylic solvents and has been extended to desyl amino acids[8] and to *p*-hydroxyphenacyl and desyl phosphates.[1,7,9] However, additional studies are required to resolve this dual mechanistic dilemma. These are in progress as well as further development of new phototriggers.

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(16) The formation of 1-(2,4′-dihydroxy)phenylethanone (**1f**, <3%) was observed during the photolysis of **4c** in aqueous solution and in Ringers which was attributed to a slow background hydrolysis which occurred at the slightly higher temperature (∼35 °C) of the photoreaction chamber during the photolysis. The hydrolysis product also formed at a comparable rate for controls with samples in the buffered media in the chamber but protected from the incident light.

(17) We thank Professor L. Katz and Dr. K. Kandler, Howard Hughes Medical Institute at the Duke University Medical School, for carrying out these experiments. Details on the experimental procedures will appear in their publications of the applications of the glutamate phototrigger.

(18) For example, Stern−Volmer quenching of **4c** by 2-naphthalenesulfonic acid established the triplet state as the photoactive precursor to the release of the dipeptide. The K_{SV} measure in D_2O was $30 \pm 3\ M^{-1}$ which yields a rate constant of $7.0 \pm 1.0 \times 10^7\ s^{-1}$ for release of ala-ala.

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