The *p*-Hydroxyphenacyl Photoremovable Protecting Group

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A review of the background and development of the *p*-hydroxyphenacyl group (pHP) as a photoprotecting group for biological substrates is chronicled. The pHP group has promise as an efficient, rapid phototrigger for the study of very fast biological processes. Applications include the release of neurotransmittors and second messengers, enzyme switches and nucleotides.

key words: *p*-hydroxyphenacyl, phototriggers, caged compounds, photoremovable protecting groups, neurotransmitters, nucleotides, enzyme switches, GABA, glutamate, ATP, Protein Kinase

INTRODUCTION

This review is intended to provide the reader who is interested in the historical development of the recent research on photoremovable protecting groups, phototriggers, and "caged" compounds with some insight into the genesis of our entry into the field through our most recent discoveries. Several other investigations have paralleled our development of a photoremovable protecting group and these can be found in one of the growing number of important recent reviews on the subject [2]. In keeping with our main theme, the scope of this review will be limited to the chemistry and applications of phototriggers derived from derivatives of *p*-hydroxyacetophenone.

Two historical attributions are required. The initial suggestion that derivatives of this chromophore could be employed as photoremovable protecting groups came from earlier work by Sheehan and his coworkers [3,4]. As far as we know, this was the first recorded suggestion that the phenacyl group could serve as a photoremovable protecting group. Another significant contribution came from Anderson and Reese [5] who were the first to show that *p*-methoxy and *p*-hydroxy derivatives of phenacyl chloride rearranged, in part, to form methyl phenylacetates upon photolysis (Eq. 1). None of these early workers, however, chose to exploit the advantages or the range of possibilities that this chromophore afforded as a photoremovable protecting group. We have done so [6]. Recently, other research groups [7] have recognized the potential of these two chromophores and have contributed to the development of the field. These most recent studies will be included where appropriate.

$$(1)$$

(X=*p*-OH; *p*-OMe; *o*-OMe)
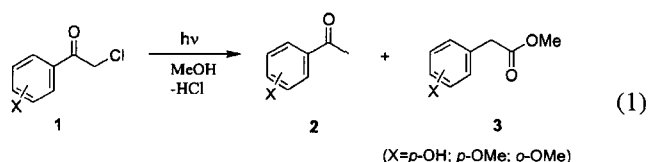
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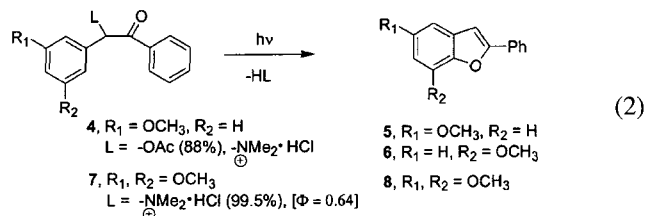E-mail : givensr@ku.edu

A brief history of the development of this class of photoremovable protecting groups begins with a discussion of Sheehan and Wilson's benzoin studies [3,4]. The report on these studies will be followed by a review of the current state of understanding of the mechanisms for the photorelease step, and the review will conclude with a limited number of relevant applications that have been exploited for the *p*-hydroxyphenacyl class of phototriggers.

HISTORY

The first report of the application of benzoin derivatives for the photoactivated release of a reagent or substrate was by Sheehan and Wilson [3a] in 1964 (Eq. 2). In this study, the release of acetate was accompanied by the formation of 2-phenylbenzofuran from cyclization of the benzoin chromophore during the photolysis. As discovered later, the production of the furan paralleled the release of acetate and therefore is a good indicator of the chemical yield of the photorelease process.

In general, the yields of 2-phenylbenzofuran from a variety of benzoins were quite modest (<15%) except for *m,m'*-dimethoxybenzoin for which the yield of the furan reached 46%. Variation of the leaving group also affected the yield, e.g., the release of dimethylammonium chloride from unsubstituted benzoin resulted in a 67% yield of 2-phenylbenzofuran. In general, the yield of the phenylbenzofuran was highly dependent on the position of *m* or *p*-methoxy substituents on the two phenyl moieties [3,4]. The highest yields of the 2-phenylbenzofuran were obtained from *m*-methoxy substituents on the benzyl ring rather than the benzoyl moiety. For example, the *m*-methoxybenzoin acetate (4) gave an 88% yield of the two methoxy substituted 2-phenylbenzofurans (5 and 6) and the *m,m'*-dimethoxybenzoin (7) gave a 99.5% yield of 2-phenyl-5,7-dimethoxybenzofuran (8). The latter reaction had a quantum efficiency of 0.64 and could not be quenched by neat piperylene, indicating either a singlet or a short-lived, highly reactive triplet

state precursor to the rearrangement. All of the benzoins studied had triplet states (~73-74 kcal/mol) of similar energy. When the released group was phthaloyl glycine, the yield was nearly quantitative. The absence of decarboxylation-derived products suggested that heterolysis occurred in the cleavage step.

9, benzoin cAMP   **10**   **11, cAMP**

$$\Phi = 0.39$$
$$k_r = \sim 10^9 \text{ s}^{-1}$$

(3)

4, $R_1 = OCH_3$, $R_2 = H$
$L = $ -OAc (88%), -$\overset{\oplus}{N}Me_2 \cdot$ HCl

7, $R_1, R_2 = OCH_3$
$L = $ -$\overset{\oplus}{N}Me_2 \cdot$HCl (99.5%), [$\Phi = 0.64$]

5, $R_1 = OCH_3$, $R_2 = H$
6, $R_1 = H$, $R_2 = OCH_3$
**8,** $R_1, R_2 = OCH_3$

(2)

Significant in this study were the criteria that Sheehan proposed for photoremovable protecting groups and, by extension, to all future designs of photoremovable protecting groups. His six criteria were: (1) the photolysis should produce a quantitative yield of the released substrate, (2) the released substrate should be easily separated from the resulting mixture, (3) the reacting chromophore should have a short-lived, unquenchable excited state, (4) the photoreaction should occur with a high quantum efficiency, (5) the excitation wavelength should be above 320 nm where peptides, the central focus of his studies, would not compete for the incident radiation, and (6) the phototrigger should not introduce any new stereo centers. While his list was not comprehensive for all potential applications of photoremovable protecting groups, it did clearly outline most of the desirable properties.

Sheehan's studies laid the initial groundwork for the benzoin group and its derivatives as photoremovable protecting groups and provided insightful preliminary information about mechanistic factors governing the conversion of the benzoin derivatives into 2-phenylbenzofurans. However, the scope and range of the reaction and much of the mechanistic detail were left for future researchers to explore. In fact, very little appeared on the photochemistry of this chromophore in spite of its significant promise as measured against the criteria that Sheehan had advanced.

Our group was able to make a significant breakthrough a decade later, however, when we discovered that the photo-deprotection of phosphates could also be accomplished in nearly quantitative yield with good quantum efficiency ($\Phi = 0.28$) [6a]. In this study, we were able to firmly establish that the reaction occurred exclusively through the benzoin triplet state. The fact that quantitative release of phosphates occurred as a primary process from the triplet excited chromophore meant that, at least for phosphate as a leaving group, no additional activating substituents on the chromophore were required.

Application of this discovery for the release of a biologically significant substrate was achieved with our study of benzoin protected cAMP (**9**, Eq. 3) [6b]. The triplet lifetime (~4 ns) and the quantum efficiency ($\Phi = 0.39$) were in accord with the earlier studies on simple phosphate esters.
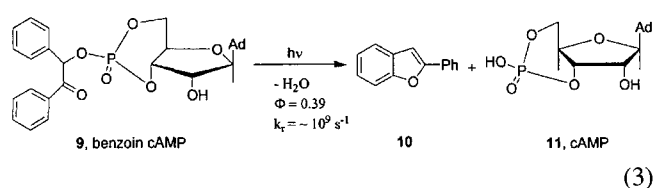
Encouraged by the successes with phosphate esters, we decided to return to the earlier studies of Sheehan and Wilson [3,4] and test the feasibility of carboxylic esters as leaving groups for biologically active substrates. Our attention was drawn to the need for rapid release of the excitatory neurotransmitters glutamate and GABA as revealed in the current literature on electrical stimulations with *in vitro* patch clamp experiments [8] that required a much faster-reacting phototrigger for exploring the onset of neurostimulation. Release of both glutamate and GABA from their benzoin caged precursors (Eq. 4) was both efficient and quantitative.

glutamate (R=CO_2H)
γ-aminobutyrate (GABA, R=H)

(4)
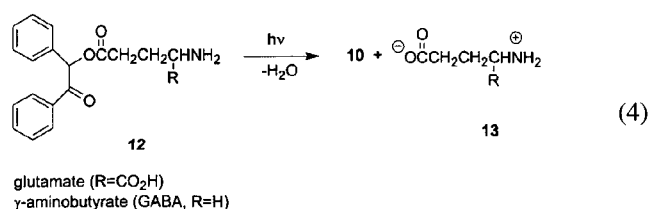
Thus, it appeared, at least for this limited range of phosphate and carboxylate benzoin esters, that five of the six criteria of Sheehan had been met. The single, unfulfilled criterion was the introduction of an added stereocenter when employing the benzoin phototrigger. Because this is an inherent feature of the chromophore and would have required the use of enantiomerically pure benzoin or a stereospecific synthesis in order to obtain a diastereomerically clean phototrigger, we chose to discontinue our interests in benzoin and its derivatives for photoremovable protecting groups. We turned, instead, to a related class of p-hydroxyacetophenone derivatives, e.g. p-hydroxyphenacyl phosphates.

Our decision to abandon the benzoin series and direct our efforts toward the p-hydroxyphenacyl (pHP) group was based on very elementary reasoning. The proven efficacy of the phenone chromophore to serve as the energy source for cleavage of α substituents both efficiently and rapidly was attractive. By merely removing the phenyl moiety from the α position, the problem of the stereocenter in benzoin was removed. We rationalized further that the introduction of a carboxylic acid or phenol hydroxy group on the aryl ring of the phenacyl group would enhance the aqueous solubility. We were also intrigued with the possibility that the latter group when located in the para position might also provide an added benefit to the photorelease process through a photochemically induced, "Favorskii-like" rearrangement of the phenone to a phenylacetic acid chromophore [5] resulting in a significant hypsochromic shift. As noted above (see Eq. 1), the early report by Anderson

and Reese [5] had pointed to such a possibility in their studies of the photorelease of chloride in methanol solutions. Rearrangement to methyl phenylacetates occurred only for *o*- or *p*-methoxy or *p*-hydroxy substituents. For all three of these examples, the ratio of rearranged phenylacetic ester to photoreduction to the substituted acetophenone was approximately 1:1. All other substituents examined as well as the parent phenacyl chloride gave the acetophenones, preserving the chromophore which would effectively compete for the incident light at modest to high conversions under normal photolytic conditions and thereby reduce the reaction efficacy. The promise of *p*-hydroxyphenacyl as a possible phototrigger was too enticing to pass up.

p-HYDROXYPHENACYL

In the mid 90's, we began a comprehensive exploration of a variety of *p*-substituted phenacyl phosphates for their efficacy toward releasing phosphate [9,10]. Among the substitutents examined, the *p*-acetamido, methyl *p*-carbamoyl, and *n*-butyl *p*-carbamoyl groups proved untenable because they gave a plethora of products, most of which resulted from coupling or reduction of an intermediate phenacyl radical (Eq. 5) [11,12]. Table 1 gives the disappearance efficiencies for several *p*-substituted phenacyl phosphates from which it is evident that release of phosphate does occur very efficiently for the acetamido and carbamoyl derivatives. However, the large array of products of the phototrigger discouraged our further interest in these three electron-donating groups.

$$ (5) $$

a) X = NH$_2$, b) X = NHCOCH$_3$, c) X = NHCO$_2$CH$_3$, d) X = OCH$_3$
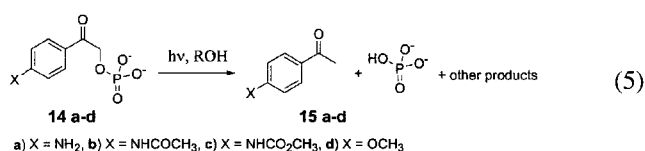
The methoxy substituent (**14d**) showed a much cleaner behavior yielding only two products from the chromophore, the acetophenone and a rearrangement product, *p*-methoxyphenylacetic acid. Most unexpectedly, the *p*-hydroxyphenacyl

Table 1. Disappearance and product efficiencies for ammonium salts of *p*-substituted phenacyl phosphate in pH 7.2 Tris buffer at 300 nm [12]

p-substitutent	Φ_{dis}	$\Phi_{rearrange}$	Φ_{red}	Φ_{other}
14a NH$_2$	<0.05	0.0	<0.05	na
14b CH$_3$CONH	0.38	0.0	0.11	Dimers
14c CH$_3$OCONH	0.34	0.0	na	2 unknowns
14d CH$_3$O[a]	0.42	0.20	0.07	na
14e HO[b]	0.38	0.12	0.0	0.0
17 HO[c]	0.37	0.31	0.0	0.0

[a]Solvent was MeOH and diethyl phosphate was the leaving group.
[b]10% CH$_3$CN was added to the solvent.
[c]The ATP derivative.

phosphate (**14e**) gave exclusively the rearranged *p*-hydroxyphenylacetic acid when photolyzed in aqueous buffers or in H$_2$O (Eq. 6). In fact, of all the groups examined, only *p*-hydroxy and *p*-methoxy produced any rearranged phenylacetic acids.


$$ (6) $$
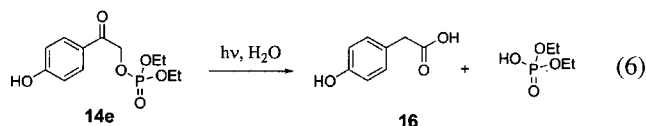
The initial discovery that diethyl *p*-hydroxyphenacyl phosphate exclusively followed a rearrangement pathway was soon followed by the determination that *p*-hydroxyphenacyl ATP released ATP and *p*-hydroxyphenyl acetic acid with a quantum efficiency of 0.37 ± 0.01 and a rate constant for ATP of $5.5\pm 1.0\times10^8$ s^{-1} (Eq. 7).

$$ (7) $$
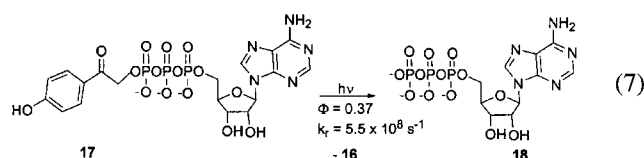
Encouraged by the *p*-hydroxyphenacyl discoveries with inorganic phosphate and ATP release, we explored the release of carboxylates from *p*-hydroxyphenacyl carboxylate esters. Carboxylate also proved to be a good substrate as demonstrated by the photorelease of γ-aminobutyrate (GABA) and L-glutamate from their *p*-hydroxyphenacyl esters (Eq. 8) [13].

$$ (8) $$

glutamate (R=CO$_2$H)
γ-aminobutyrate (GABA, R=H)
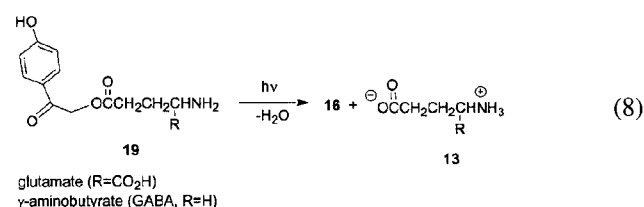
We attempted to use the pHP protecting group on these and other amino acids by protection at the α-amino carboxylic acid group, but quickly discovered that the esters were hydrolyzed in aqueous buffer, compromising the use of this phototrigger for simple amino acids. The ease of hydrolysis is likely due to the presence of the α-ammonium ion at neutral pH that polarizes the ester carbonyl toward nucleophilic attack by solvent. This view is further supported by the observation that dipeptides, e.g., Ala-Ala, are quite stable to hydrolysis [14]. Release of Ala-Ala from pHP Ala-Ala became a model for further studies on the release of oligopeptides. The most dramatic example of release of a small oligopeptide was the release of synthetic bradykinin (Eq. 9, *vide infra*) [15].

$$ (9) $$
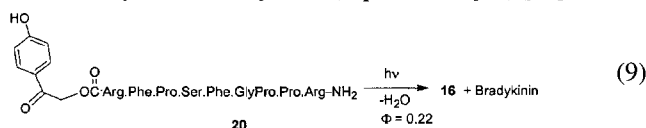
MECHANISTIC STUDIES

The mechanism of the p-hydroxyphenacyl group as a photoremovable protecting group can be partitioned into four principal stages: 1) formation of the triplet intermediate, 2) deprotonation of the phenolic group, 3) bond reorganization to a putative spirodienedione accompanied with substrate-phenacyl bond cleavage, and 4) further bond reorganization of the spirodienedione.

Our initial proposals for the mechanism for the photorelease [9-15] began with excitation of the photoremovable protecting group to its singlet and was followed by rapid ST crossing to the triplet excited state (Figure 1). The triplet intermediate was thought to partition between loss of a proton and C-O bond cleavage that was pictured as a part of the decay process to ground state. The exact course of the reaction depended on the leaving group, the solvent and the substituents attached to the chromophore. In one scenario, the triplet phenol underwent the equivalent of a heterolytic cleavage of the bond to the substrate. In this scenario, it was envisaged that initial homolysis of the C-Y bond might be followed by a rapid single-electron transfer process, i.e., the triplet phenol ultimately released the conjugate base of the substrate (Y$^-$) before other competing processes for the radical pair can intervene. In this sequence, a spirodienedione was eventually generated by electrocyclic closure of the intermediate zwitterion or diradical.

In a second scenario, the concerted electron transfer with bond breaking or simply heterolysis preceded the rearrangement process. Finally, a third scenario involved neighboring group assistance for the release of the substrate concomitant with formation of the spirodienedione. In all three scenarios, a subsequent proposed unraveling of the spirodienedione was

initiated by the attack of H_2O on the cyclopropanone carbonyl resulting in the formation of the rearranged p-hydroxyphenylacetic acid.

The pHP radical postulated in the first scenario should have a significant lifetime and is the most likely source of the reduction product, p-hydroxyacetophenone, that is observed as a byproduct in many of the reactions of pHP derivatives (however, see below for an alternative route to the reduction product). Pathways available to radical Y$^-$ include decarboxylation, electron transfer reduction, dimerization, disproportionation, or hydrogen abstraction. Several of these processes have been observed.

SINGLET VS. TRIPLET FOR THE REACTIVE EXCITED STATE

The onset of the triplet state phosphorescence emission of several p-hydroxyphenacyl esters indicated triplet energies of 68.9-70.6 kcal/mol. The phosphorescence emissions were quenched by sodium 2-naphthalenesulfonate or potassium sorbate, both of which have triplet energies that are more than 3 kcal/mol lower than those of the pHP groups. Quenching study on the pHP-Ala-Ala photoreaction confirmed the reactivity of the triplet state and further provided a lifetime of 5.5 ns for the triplet with a release rate of 1.82×10^8 s^{-1}.

The original proposal that the triplet state was the reactive state was challenged by Corrie and Wan [7] who instead proffered the excited singlet state or possibly a tautomeric ground state of 21a for the photorelease (Figure 2). In their studies, quenching was not observed for the release of acetate from p-hydroxyphenacyl acetate (21a). They suggested that the release of acetate probably occurred by an excited-state
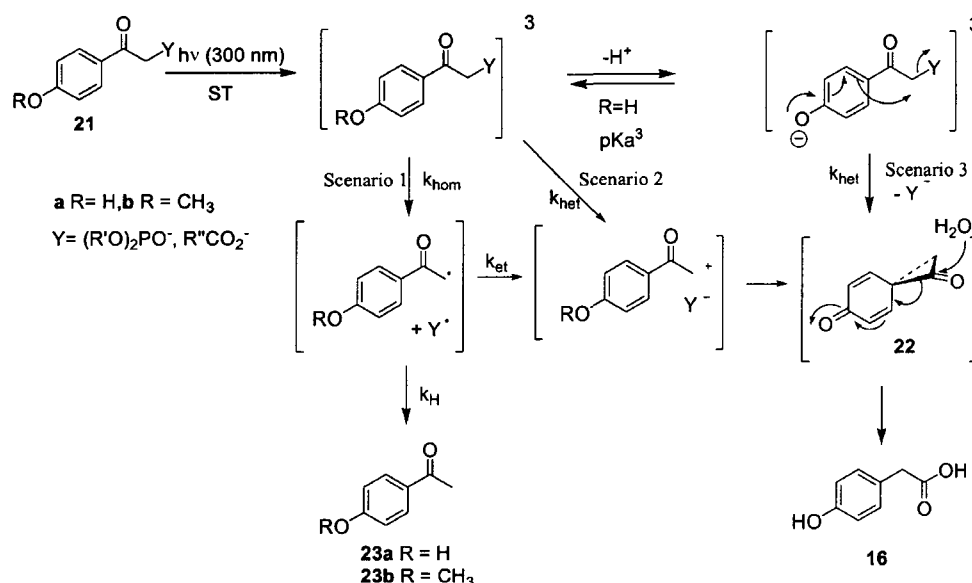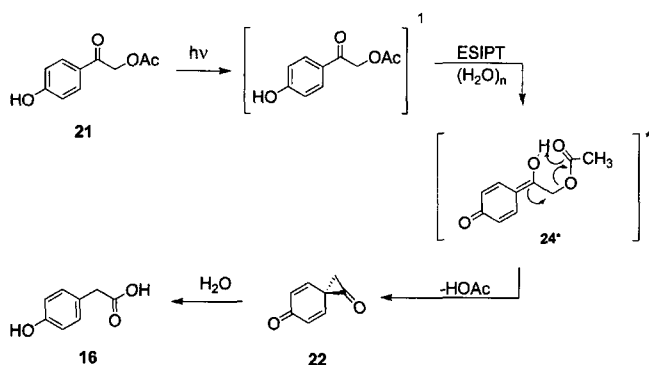

Figure 1. Proposed photorelease triplet state mechanisms for photorelease of substrates from the p-hydroxyphenacyl protecting group.

Figure 2. ESIPT singlet state photorelease mechanism of the p-hydroxyphenacyl acetate [7].
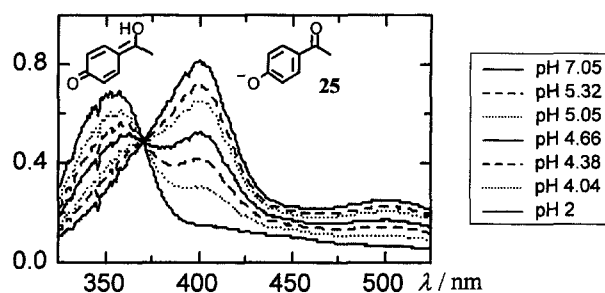
* = singlet or reactive ground state intermediate

Figure 3. Transient absorption spectra obtained by LFP of p-hydroxyacetophenone in water (10% CH_3CN) with various buffers. Reprinted with permission from ref. 16 © American Chemical Society.
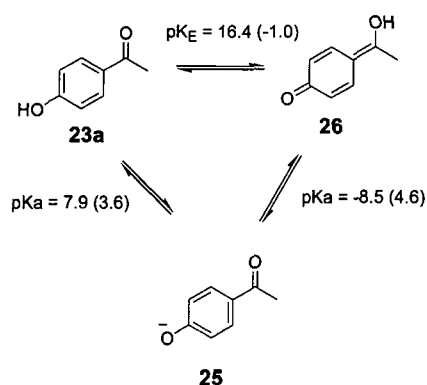

Figure 4. Tautomerization of p-hydroxyacetophenone in the ground state and triplet excited state (in parentheses) Reprinted with permission from ref. 16 © American Chemical Society.

intramolecular proton transfer (ESIPT) to form p-quinone methide (24) in the singlet manifold. The quinone methide may either continue on to the spirodienedione (22) or decay to ground state and subsequently undergo release of acetate to form 22. The spirodiene reacts with H_2O to generate p-hydroxyphenylacetic acid as postulated in Figure 1.

More recent studies by Wirz and Givens [16] using laser flash photolysis (LFP) for diethyl pHP phosphate (Eq. 6) confirmed the intermediacy of the phenoxide triplet state as proposed in mechanism scenario 3. Energy transfer quenching using naphthalene produced the naphthalene triplet, i.e., the rate of formation of the naphthalene triplet, was 7.8×10^9 $M^{-1}s^{-1}$. Addition of O_2 increased the decay rate of the pHP phosphate triplet ($k_q \approx 3 \times 10^9$ $M^{-1}s^{-1}$) but not the triplet yield. It is estimated that pHP intersystem crosses with a rate constant of 3.1×10^{11} s^{-1} in aqueous acetonitrile. Finally, additional quenching studies of the photochemical release of substrates from a series of pHP derivatives employing potassium sorbate all gave excellent linear Stern-Volmer quenching results and lifetimes of 10^{-8}-10^{-9} s for the pHP triplet state. These combined results firmly established the triplet as the reactive excited state.

DEPROTONATION

LFP studies on the model chromophore, p-hydroxyaceto-phenone (23a), revealed an adiabatic proton tautomerization process between the phenol 23a and the enol 26. Evidence for an unusual adiabatic proton transfers involving the triplet p-hydroxyacetophenone phenol 23a came from transient triplet absorption spectra at differing pH (Figure 3).

DFT calculations provided the equilibrium constants and the pK_E for the enolization of 23a to 26 in the ground state and in the lowest triplet state. Combination of these values with the experimental ionization constant of triplet 26, $pK_a = 4.6$ (Figure 3), and the ionization constant of 23a, $pK_a = 7.9$, provided two of the three legs in the thermodynamic cycles (ground state

and triplet state) shown in Figure 4, thereby defining the acidity constants of triplet 23a, $pK_a = 3.6$, and of 26 in the ground state, $pK_a = -8.5$. Thus the acidity of 23a increases by 4.3 pK_a units upon excitation to the triplet state. Protonation of 23a at the carbonyl oxygen produces triplet enol dienone 26 with an equilibrium constant of $pK_e = -1.0$ compared with the ground state pK_e of 16.4.

The excited state proton tautomerism for 23a is consistent with the results of Granucci et al [17] who showed that the pKa's of simple hydroxyarenes and several conjugated acids decrease by 6 orders of magnitude upon excitation to their singlets. The authors suggested that a conical intersection ($^1L_b/^1L_a$ inversion) in the singlet-excited state of phenol facilitates the proton transfer process. The pK_a acidity changes of the triplet states of phenols were usually much smaller, although generally in the same direction. 2-Naphthol [18] shows a relatively small enhanced acidity from 9.5 in the ground state to 7.7-8.1 when excited to its triplet in contrast with the much greater change to 3.1 in its excited singlet state.

Extending the discovery of the large adiabatic decrease in pK_a of 23a to the p-hydroxyphenacyl photorelease mechanism, a rapid adiabatic deprotonation of the triplet state suggests

that the conjugate base of **14e** is the likely precursor to the rate-limiting process. Because the triplet is formed with a ST rate constant of 3.1×10^{11} s^{-1}, it is unlikely that there is any singlet state contribution to the deprotonation step. Rather this appears to be exclusively a triplet process on the excited triplet surface, i.e., an adiabatic proton transfer process among the two conjugate acids **23a** and **26** and the unprotonated **25** (Figure 3). Deprotonation is pictured as generating a much more electron rich aromatic ring, increasing the driving force for intramolecular nucleophilic attack on the α-carbon leading to the release of the substrate as outlined in Figure 1 scenario 3. Thus, it becomes necessary to more carefully explore the change in the pK$_a$ of the phenolic proton between the ground and excited triplet states since this change is key to understanding the role played by aryl participation on the release step.

Mata, Wirz and Givens (unpublished results) further examined the effects of solvent isotope exchange (H_2O vs D_2O in CH_3CN) on the proton transfer step in an effort to further refine the mechanistic sequence. A large solvent isotope effect ($k_{H2O}/k_{D2O}=2.1$) had been determined in the initial mechanistic studies. To further explore the origin of this kinetic isotope

Figure 5. Suggested transition state for the rate determining adiabatic triplet state proton transfer to H_2O.

effect, gradient changes in the solvent isotopic composition were made by varying the mole fraction of D_2O in H_2O, a technique developed by Schowen and termed the "Proton Inventory Method" [19]. By modeling the change in the rate constant as a function of the mole fraction of D_2O, a best fit of the Gross-Butler equation for pHP diethyl phosphate photolysis indicated that three protons were involved in the rate determining transition state. The partition function for the triplet state proton transfer was estimated to be approximately 0.72. Together, these results suggest that a water molecule participates as an acceptor of the acidic p-hydroxyphenacyl proton at the rate determining transition state as illustrated in Figure 5.

Based on the proton inventory and the TR LFP results, Givens and Wirz suggested a concerted process in which elimination of diethyl p-hydroxyphenacyl phosphate occurs concomitant with the rate determining deprotonation of the phenolic proton to yield the spirodienedione, an overall concerted adiabatic triplet process (Figure 6).

HOMOLYTIC BOND CLEAVAGE AND ELECTRON TRANSFER

As noted earlier, the mechanism leading to p-hydroxyacetophenone, the reduction product, likely arises from homolytic bond cleavage followed by hydrogen abstraction. It is also possible that the acetophenones are formed by hydrogen-atom abstraction by the excited pHP carbonyl followed by homolysis of the substrate-α-carbon bond. Both mechanisms have been considered for analogs of pHP [6,9-12,20]. Two research groups have explored these possibilities
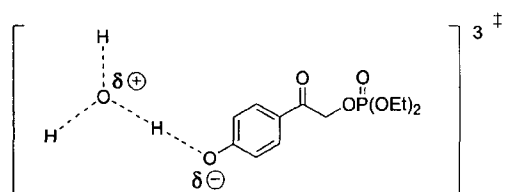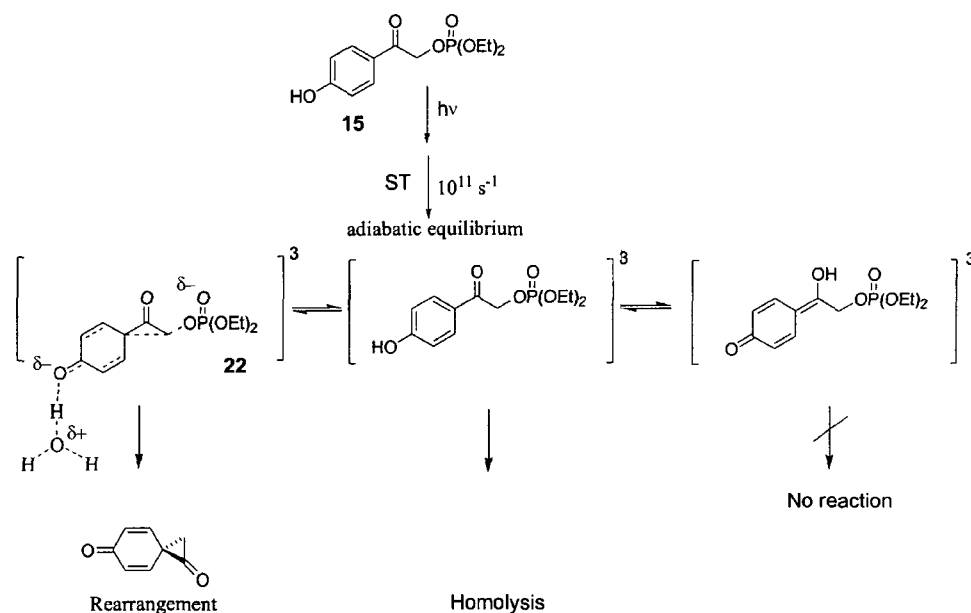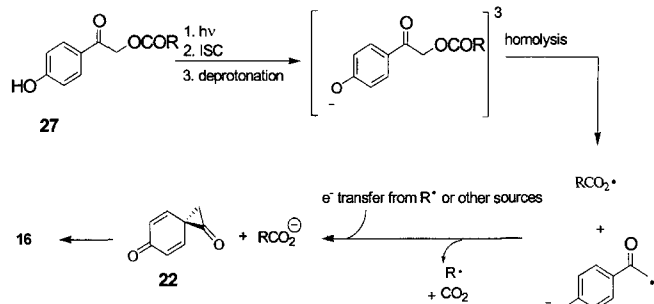

Figure 6. Adiabatic proton tautomerization of pHP diethyl phosphate in the triplet excited state manifold.

27aR=CH$_3$, **b**R=CH$_2$CH$_3$, **c**R=CH$_2$Ph, **d**R=CH$_2$C(CH$_3$)$_3$, **e**RCO$_2$=GABA, **f**RCO$_2$=glutamate, **g**RCO$_2$=cyclopropylmethyl, **h**RCO$_2$=pivalate, **i**RCO$_2$=bradykinin, **j**RCO$_2$=oleate

Figure 7. Decarboxylation mechanism for pHP esters.

Table 2. Decarboxylation rate constants for some selected radical clock reactions [22]

R	k [10^9 s^{-1}]
27a CH$_3$	<1.3
27b CH$_2$CH$_3$	2.0
27c CH$_2$-phenyl	4.8
27d CH$_2$-C(CH$_3$)$_3$	11

for the reactions of several selected phenacyl esters [20,23].

The photolyses of pHP esters (**27a-d**) were examined in an effort to detect decarboxylation as a signature for formation of the carboxyl radical (Figure 7) [20,23]. Competition between electron transfer and decarboxylation should be reflected in the product distribution between decarboxylation and carboxylate release. However, in these studies no decarboxylation products were observed within the detection limits of either ^{1}H-NMR or HPLC. The homolysis of the CH$_2$-O bond of a pHP-caged carboxylic acid would generate the acetoxyl radical that should decarboxylate with a rate constant of ~10^9 s^{-1} [21,22] (Table 2) in competition with electron transfer that would have to be in the range of 2.5-21×10^{10} s^{-1} in order for the latter to dominate the mechanistic pathway over the decarboxylation process. Alternatively, rapid internal conversion from the $(\pi,\pi^*)^3$ to a $(\pi,\sigma^*)^3$ resulting in direct loss of carboxylate and formation of the spirodienedione could occur.

In examining the results from all of the pHP analogs, formation of the reduction product was only significant for the pHP phenylacetate ester. These are shown in Table 3. A quantum yield of 0.03 for *p*-hydroxyacetophenone was determined. Nevertheless, the major pathway was rearrangement to the phenylacetic acid (Φ=0.14) and the phenylacetate was formed with an efficiency of 0.17, the sum of the two efficiencies for the chromophore. This implies that the decarboxylation process is not occurring from this substrate. It would be expected, in this context, that the *t*-butyl analog of all the substrates investigated would favor decarboxylation since its rate *constant* for loss of CO$_2$ is twice that of the other derivatives. These results do set an upper limit to the electron

Table 3. Quantum Yield of pHP Derivatives by Irradiation at 300 nm[a]

	Ester	Solvent[b] W/AcCN	Φ_{dis}	Φ_{app}	Φ_{rea}	Φ_{red}	Φ_{hyd}
17	pHP ATP	W	0.37	0.30	0.31	–	–
27e	pHP GABA	W	0.21	0.21	0.19	–	–
27f	pHP glutamate	W	0.14	0.14	0.08	–	–
27g	pHP cyclopropylacetate	6:4	~0.2[c]				
27c	pHP phenylacetate	6:4	0.18	0.17	0.14	0.03	–
27h	pHP pivalate	6:4	~0.18[c]				
27j	pHP oleate	7:3	0.24	0.23	0.17	–	0.03
27i	pHP bradykinin	W	0.21	0.22	0.19	–	0.02

[a]An NMR tube was charged with ca. 10 mg (ca. 40 μmol) of the appropriate photoprotected acid and 10 mol% of 1,2,3-benzenetricarboxylic acid as an internal standard in 2 mL of solvent. The quantum efficiencies were determined at less than 20% conversion of the starting ester. Abbreviations: dis=disappearance, app=appearance, rea=rearrangement to phenylacetic acid, hyd=hydrolysis, red=reduction.
[b]solvent: W=water, AcCN=acetonitrile
[c]By comparison with **27c**.

transfer rate constant, however.

In order to examine solvent effects on the partitioning of the triplet excited pHP chromophore, photoreactions of **27g,c,h** were conducted in 6:4 water: acetonitrile as well as in anhydrous acetonitrile. Significant decreases in the disappearance quantum yields were observed for all studies in anhydrous solvents. Furthermore, the quantum yield for the reduction increased, yet there was no increase in the decarboxylation yield.

Falvey [23] proposed that H-atom abstraction from a hydrogen donor to the carbonyl, rather than C-O bond homolysis, was the initial step of the photorelease mechanism (Figure 8). The resulting ketyl radical decayed through
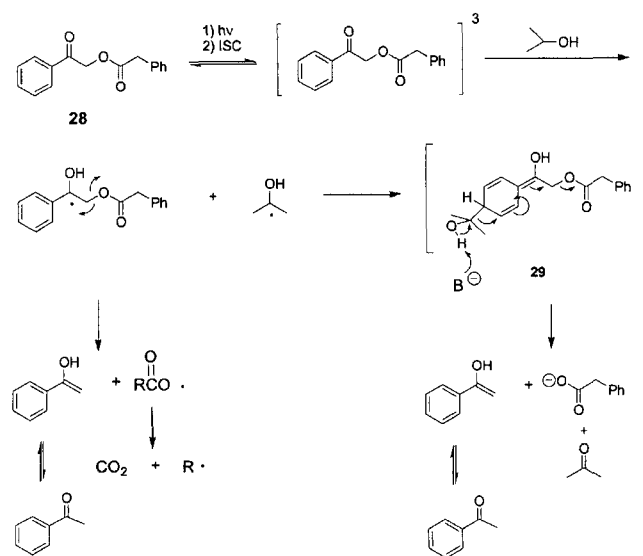

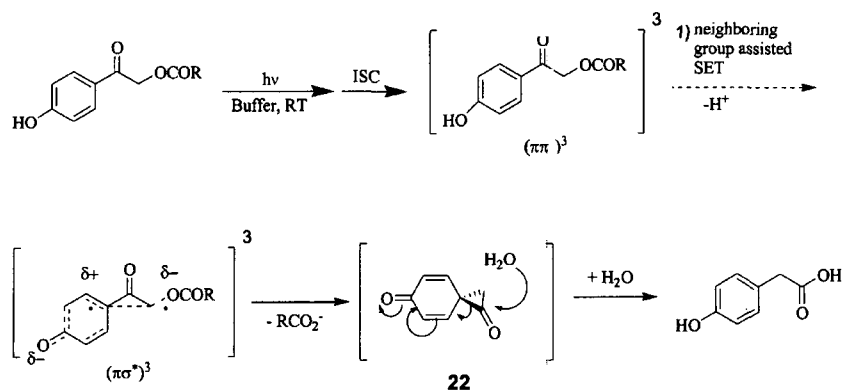Figure 8. Photoreduction of phenacyl esters: an alternative route to photorelease of carboxylic acids.

Figure 9. Proposed heterolysis mechanism (Scenario 2, Figure 1) [15].

disproportionation with the donor radical via a relatively long-lived solvent-coupled intermediate **29** to form the enol of acetophenone as shown in Figure 8. While this mechanism adequately rationalized the release of carboxylate, it requires a hydrogen-atom donor solvent, e.g. i-PrOH, which is normally not employed with pHP biological studies.

It should be noted that, in Sheehan and Umezawa's [4] early work, decarboxylation was observed for some p-methoxyphenacyl esters. The absence of the decarboxylation products in our studies as well as Falvey's studies militates against the formation of a long-lived free carboxyl radical intermediate formed from the direct homolysis of the CH_2-O bond. This further supports mechanisms that lead directly to the conjugate base of the leaving group, e.g. a heterolysis mechanism (Figure 9).

REARRANGEMENT VERSUS REDUCTION

The spirodienedione intermediate **22** has been proposed for the intermediate in the rearrangement of pHP to p-hydroxyphenylacetic acid. Anderson and Reese [5] were the first to suggest **22** for the photorearrangement of hydroxy and methoxy substituted phenacyl chlorides to explain the formation of ethyl p-hydroxyphenylacetate (Eq. 10).
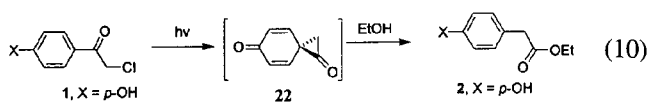
All of our attempts to detect the proposed spirodienedione intermediate by conventional means have been unsuccessful. An interesting approach designed to increase the stability of the spirodienedione and therefore its lifetime was the placement of gem-dimethyl's at the alpha carbon. Turro [24] has shown, for example, that α,α-dimethyl substitution improved the stability of a series of cyclopropanone derivatives. Thus, α,α-dimethyl ketone **30** was synthesized and photolyzed (Figure 10). However **30** did not produce the expected phenylacetic acid.
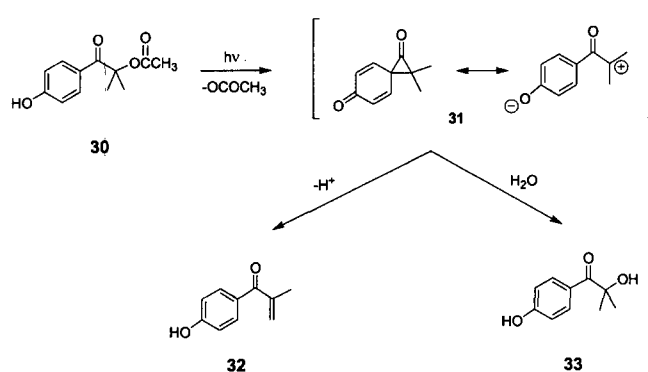

Figure 10. The unusual photoreaction of α,α-dimethylphenacyl acetate **31**.

Instead, two new products of the phototrigger were observed, an elimination product **32** and a hydrolysis product **33**. In this photoreaction, the spirodienedione intermediate may be better represented by zwitterion **31** and consequently its reactivity would reflect a tendency to hydrolyze or undergo β-elimination. Conversely, these products could be rationalized equally well as simple photohydrolysis and photoelemination processes of the acetate. This mechanistic dichotomy was not pursued, however.

Time-resolved FTIR (TR-FTIR) investigations on the release of diethyl phosphate were also explored in an attempt to detect the spirodienedione **22** [16]. The typical absorption band of cyclopropanone carbonyl, 1800-1820 cm^{-1}, was not observed in this study, possibly due to rapid hydration of the cyclopropanone carbonyl.

Our earlier studies [6,9,12] on the effect of other para electron-donating substituents to induce rearrangement of the phenacyl chromophore failed to uncover any new candidates like the p-hydroxy derivative. p-Amido derivatives either gave low yields or failed to yield any of the rearranged phenylacetic acids. Apparently, neither the methoxy nor the amido groups are sufficiently electron donating to promote the rearrangement pathway to the exclusion of other pathways, instead favoring radical and reduction pathways. Even m-methoxy substitution on pHP induces higher yields of the reduction products,
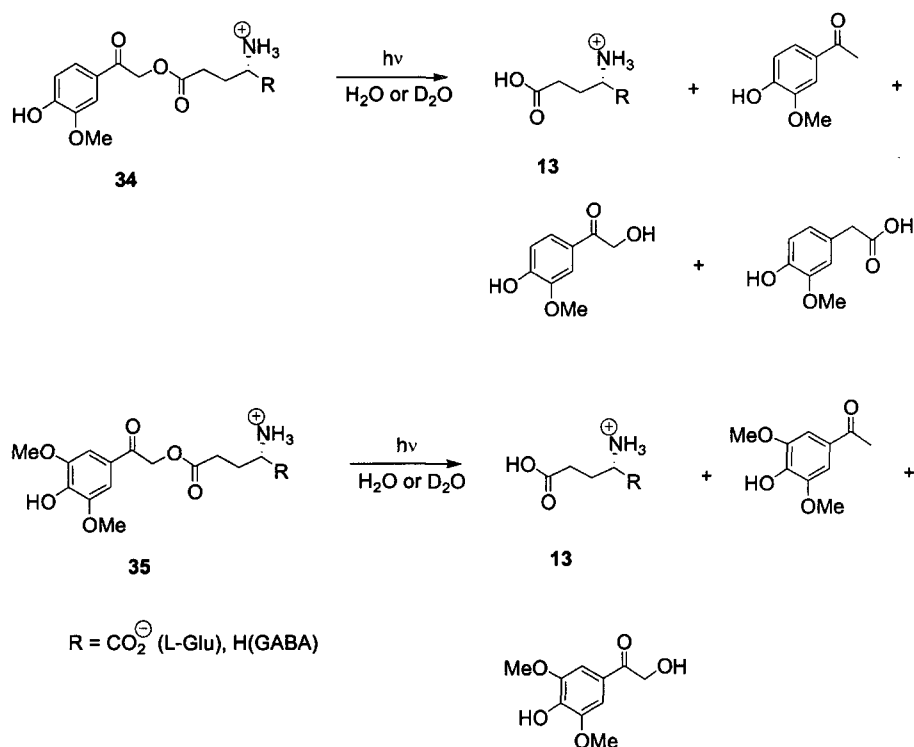
Figure 11. Photorelease of L-glutamic acid and GABA.

clearly compromising the capability of the *p*-hydroxyl group to promote neighboring group influence toward expelling the substrate. Two *meta* methoxy groups almost completely shut down the rearrangement pathway (Figure 11). The additional *meta* methoxy groups increase the triplet pK_a^3 of the hydroxy group and consequently discourage electron donation by the phenolic OH toward formation of the spirodienedione **22**.

APPLICATIONS

o-Nitrobenzyl and benzoin have been the most popular photoremovable protecting groups for application both in organic synthesis and biochemistry. However, among the new families of photoprotecting groups being developed, *p*-hydroxyphenacyl has made its début by demonstrating particularly advantageous features, i.e., fast release rates, production of a biologically benign phenylacetic acid, good solubility in aqueous media, absence of stereocenter, and generation of a UV-silent by-product. Here we have chosen a few select examples of the application of the pHP group to illustrate these features.

NEUROTRANSMITTER RELEASE

Photoactivatable derivatives of neurotransmitters such as glutamate and GABA (γ-aminobutyric acid) have been effectively employed as probes in investigations of the mechanism of chemical synaptic transmission and to map the location of receptor sites. A neurotransmitter (the first messenger) binds to a receptor inducing changes in the concentration of another intracellular regulator or the second messenger. This, for example, may serve to stimulate the cell to release calcium ion from intracellular storage reservoirs. Gaining the control of the initiation and activity of the first and second messenger processes and the concentration of calcium ions using a phototrigger or photolabile chelator have been key methods in the mechanistic study of synaptic transmission.

Because the release of the neurotransmitter is rapid, it is possible to monitor the kinetics of the stimulation process. As noted earlier, pHP glutamate and GABA undergo the release process with rates that exceed 10^8 s^{-1} and do so with quantum efficiencies of 0.14 to 0.21 at pH=7.2. Taking advantage of these features, Kandler *et al.* [8] utilized pHP glutamate for the study of LTP (Long Term Potentiation) and LTD (Long Term Depression) by examining the role of postsynaptic cellular changes in CA1 hippocampal pyramidal cells. These cells are thought to be involved in the mechanism of memory and learning. The synaptic process was blocked and a glutamate concentration jump was made by the photorelease of the pHP-caged glutamate. This, paired with depolarization, produced LTD of glutamate receptors and established that alteration of

the postsynaptic glutamate receptors occurs independent of presynaptic neurotransmitter release.

PEPTIDE RELEASE

Many amino acids, oligopeptides and larger peptides function as hormones and as neurotransmitters. The control of peptide function is the goal of many investigations of biological processes involving protein-amino acid, protein-oligopeptide and protein-protein interactions and is particularly amenable to the use of phototriggers. For example, bradykinin, a known active pain-transducer when released during tissue damage, is known to stimulate sensory neurons that induce an elevation in Ca^{2+} concentration. Activation of sensory nerve endings directly excites nociceptors, peripheral nerve organs, and induces secondary release of other biochemical mediators that stimulate nociceptor response resulting in dilation of blood vessels. A major difficulty in studying the detailed features of bradykinin action is the concomitant rapid enzymatic degradation of the oligopeptide immediately after its release from a precursor protein. Therefore, the photorelease of bradykinin from pHP bradykinin (36), which protects bradykinin from degradation of the agonist prior to release, facilitates studies on the transduction of pain by allowing precise temporal and spatial release of bradykinin for the activation of bradykinin BK2 receptors (Figure 12).

In this study, the effect of bradykinin release from pHP bradykinin (36) on single rat sensory neurons grown in tissue culture was assessed [15]. A single 337 nm flash (<1 ns) released sufficient bradykinin to excite the BK2 receptor, dramatically increasing the intracellular calcium concentration. The release of Ca^{+2} was estimated by measuring its intracellular concentration with Indo-1, a Ca^{+2} chelating fluorescent indicator. The quantum efficiency of bradykinin appearance was determined to be 0.22.

NUCLEOTIDE RELEASE

Ras, a protein that plays an important role in cell signaling pathways, hydrolyzes GTP to GDP to "turn off" the signaling process The mechanism of GTP hydrolysis by Ras with GTPase-activating protein has been a recent subject of debate [25]. Kim et al. [26] examined this process by LFP of caged GTP-Ras complexes using pHP caged GTP 37 (Eq. 13). By monitoring the appearance of two different transient signals, they were able to speculate on the mechanism of the hydrolysis reaction Their interpretation of the difference spectra was that the transition state for GTP hydrolysis has significant dissociative character rather than a direct displacement mechanism.

$$\text{(13)}$$
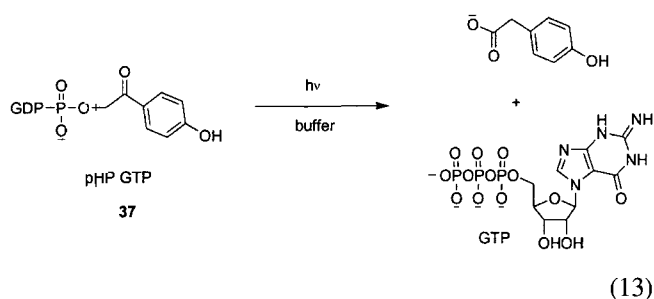
ENZYME PHOTOSWITCHES

Enzymes caged at their active sites provide a powerful entrée toward gaining the control of protein activity, a long-standing goal of biochemists and physiologists. Despite the potential difficulties in the precise identification and labeling
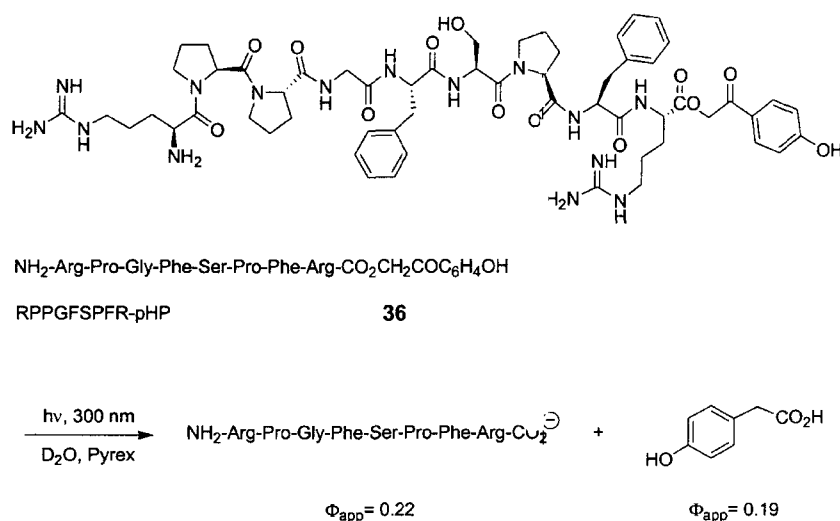

NH_2-Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg-$CO_2CH_2COC_6H_4OH$

RPPGFSPFR-pHP   **36**

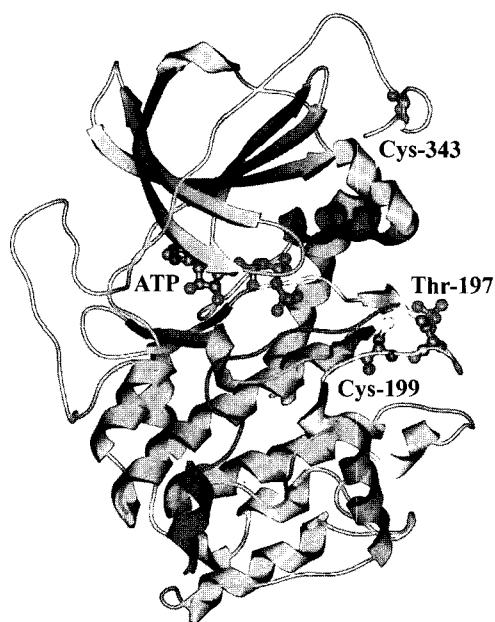Figure 12. Photorelease of Bradykinin from pHP Bradykinin (36).

Figure 13. Protein Kinase: Thr 197 and Cys-199 are shown at the active site [28]. Reprinted with permission from ref. 29 © American Chemical Society.

of effective caging sites on proteins, the ability to label strongly nucleophilic sites such as the cysteine or thiophosphate residues on an enzyme with a photoremovable reagent holds considerable promise. Most of the work on caging enzymes has employed derivatives of 2-nitrobenzyl for the caging reagent. Recently, two groups, Pei, *et al* [27] and Bayley, *et al.* [28], have exploited this approach using pHP as the caging reagent. Both Pei and Bayley described what appear to be general approaches for caging enzymes that play a major role in cell signaling. Since many signaling proteins involve phosphorylation, caging phosphorylated proteins could be useful for virtually any entrée to a signaling pathway. Bayley showed that the catalytic subunit of Protein Kinase A (Figure 13) could also be activated by thiophosphorylation at Thr-197. The modified protein can then be directly caged with *p*-hydroxyphenacyl bromide to yield an inactive form of the enzyme having a specific catalytic activity that was reduced by a factor of ~17. Upon photolysis at near UV wavelengths of 300-350 nm, a ~15-fold return of the enzyme catalytic activity was observed (85-90% yield of uncaged protein) with a quantum efficiency estimated to be $\Phi=0.21$ based on model release of glutathione. Pei's approach was the direct substitution of a *p*-hydroxyphenacyl phototrigger on the cysteine at the active site [27]. Here, the pHP phototrigger was the most effective of several candidates for an enzyme photoswitch.

The applications of *p*-hydroxyphenacyl as a phototrigger in the biological studies reported here are limited illustrations of the potential uses of the pHP photoprotecting group. Many more applications will be forthcoming as the need is realized and demanded by researchers seeking more rapid, efficient,

and cleanly releasing phototriggers for mechanistic investigations in biological environments.

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