Photochemistry of Phosphate Esters

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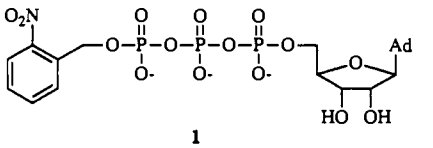
I. Introduction

Among the functional groups studied by photochemists, the phosphate group would appear to be most unattractive. It has one of the weakest UV–visible absorptions and is viewed as a relatively reactive functional group, especially in solvolytic or hydrolytic environments. Despite these apparent restrictions, the photochemistry of molecules containing a phosphate group is of intense interest to mechanistic biochemists specializing in the study of the function of nucleotides and other organophosphates in biological processes.

This review is limited to the photochemistry of simple phosphate esters, e.g., esters in which the operational chromophore is α to a phosphate group. Thus, phosphate is *not* a significant contributor (if at all) to the absorption of the incident radiation but instead serves as a nucleofuge (i.e., a leaving group) at some stage of the photochemical process that ensues. Since phosphate is such a powerful leaving group in ground-state ionization, substitution, and elimination reactions, it was anticipated that it would serve a similar role for photochemical processes, e.g., a photosolvolysis nucleofuge. Several researchers have discovered that this is the case and have adapted the property of enhanced excited state solvolysis to a number of very useful and productive studies. This article reviews the photochemistry of these phosphate esters and introduces several exciting applications of phosphate photochemistry to biological investigations.

II. Benzyl and Phenyl Phosphate Esters

A major impetus toward investigating the photochemistry of phosphate esters was the desire for alternative protecting groups for the phosphate function of oligonucleotides that would not require harsh chemical treatment for removal, i.e., a milder protection–deprotection sequence.[1-3] For example, existing methods required the removal of protecting groups by oxidation, reduction, β-elimination, or acid or base hydrolytic methods, reactions that are particularly unsuitable for many oligonucleotides. Within the last decade, the concept of photoprotection of oligonucleotides and related biologically active substrates has developed and, along with the growth of the field, a new nomenclature has emerged for the ligands that are released upon photolysis. Such ligands are termed "cage" groups and an ester containing such a ligand is said to be "caged".[4] Thus, *caged* ATP would be the protected ATP, e.g., 1 or 2, in which a photoactive ligand such as *o*-nitro-α-phenylethyl or 3,5-dimethoxybenzyl is attached to the P(γ) phosphate group of ATP. Irradiation of 1 or 2 results in the generation of free



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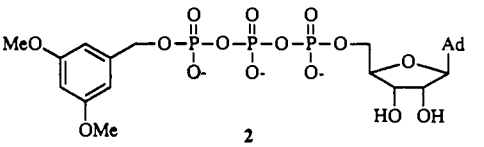

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ATP. Since the rate of release of the nucleotide is ordinarily much faster than many subsequent biological processes of interest, the effect of the photochemically induced release is a "concentration jump" of the nucleotide.[4]

Initially, the benzyl group was selected for photochemical study primarily because it had been used as a reducible protecting group[1-3] and it conveniently absorbed in the ultraviolet region. Among the first investigations were the light induced debenzylations of dibenzyl phosphate and tetrabenzyl pyrophosphate reported in 1956 by Arris et al.[1] and the photohydrolysis of *m*-nitrophenyl phosphate by Havinga et al.[2] In the former study, irradiation of ethanolic solutions of the benzyl phosphates gave inorganic orthophosphate (P_i) and pyrophosphate through a sequential loss of the benzyl groups. These reactions were touted as the photochemical alternative to the reductive cleavage of the benzyl group by sodium/liquid ammonia.

In 1970, Clark et al.[5] isolated 3,5-dimethoxybenzyl alcohol in yields of 60–80% from photosolvolysis of 3,5-dimethoxybenzyl phosphate in aqueous methanol (eq 1). This study was among the first to demonstrate

(eq. 1)[5] $\underset{\underset{\text{a. Ar = 3,5-dimethoxyphenyl}}{\mathbf{3}}}{ArCH_2OPO_3H_2} \xrightarrow[\text{aq. MeOH}]{h\nu} \underset{\text{60-80\%}}{ArCH_2OH} + \underset{\underset{(ortho\ \text{phosphoric acid})}{P_i}}{H_3PO_4}$

that the products of photolysis of benzyl phosphates are photo-nucleophilic substitution products rather

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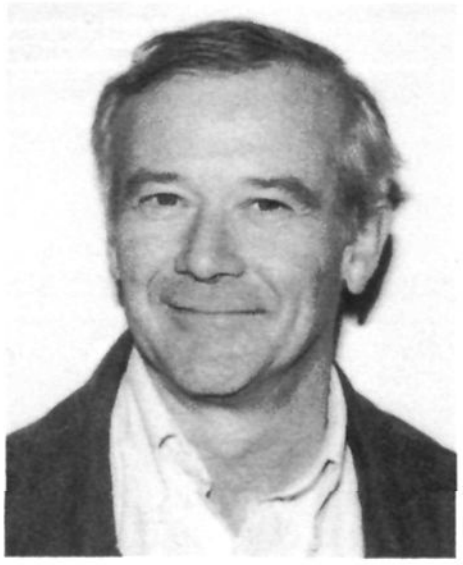
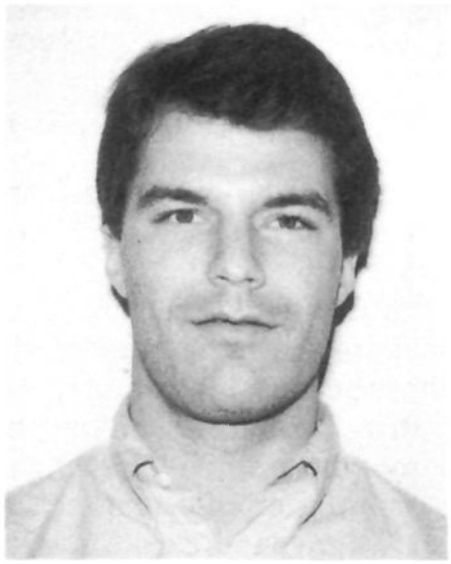


Richard Givens was born on May 19, 1940, in Buffalo, NY. He received his B.S. degree in chemistry from Marietta College in 1962 and his Ph.D. from the University of Wisconsin in 1966. After a year as a postdoctoral fellow with Glenn Russell at Iowa State University, he joined the faculty at the University of Kansas where he is currently professor of chemistry and chairman of the department. His interests in physical-organic chemistry were first stimulated at Marietta College by Professors Hans-Georg Gilde and Herschel Grose and were nurtured and greatly influenced during his graduate studies with Professor Zimmerman and the postdoctoral year with Professor Russell. The objectives of his research programs are to discover, study, and develop new functional group photochemistry. Among the areas that his group of graduate and undergraduate students have tackled are the photochemical rearrangements of β,γ-unsaturated ketones, the photoextrusion of CO_2 from esters and of SO_2 from sulfones, the photoinduced electron transfer reduction of α,β-unsaturated ketones by tertiary amines, and the photochemistry of phosphate esters. He has also developed a strong interest in the mechanism of the peroxyoxalate chemiluminescent reaction stimulated by discussions with and in collaboration with the late Professor Tak Higuchi and with professors Robert Carlson and Richard Schowen.

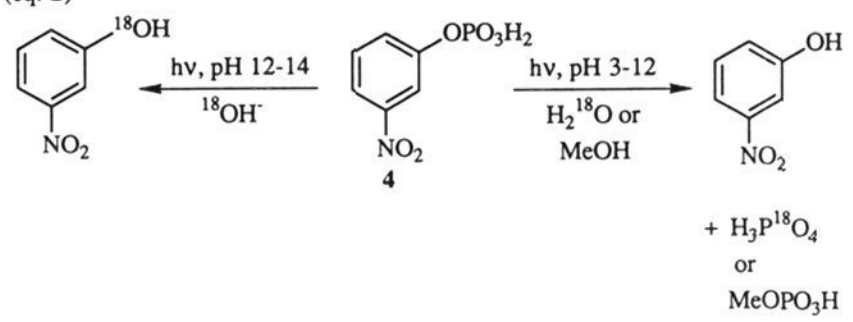


Leo William (Bill) Kueper was born in Dubuque, IA, in 1966. He attended Clarke College, also in Dubuque, and received his B.S. degree in the spring of 1989. That fall he began his graduate pursuits in physical organic chemistry and organic photochemistry under the direction of Professor R. S. Givens and Professor R. L. Schowen at the University of Kansas. His current research interests include mechanistic investigations of the oxalate/H_2O_2 chemiluminescence reaction and phosphate photochemistry.

than the more commonly encountered homolytic products from photolysis reactions.

During this same period, Havinga[2,6,7] directed his group's attention first toward the photochemistry of nitrophenyl phosphates and sulfates and later to a much wider array of substituted aromatics. The chance observation that solutions of *o*-, *m*-, and *p*-nitrophenyl phosphates decomposed on standing in room light producing yellow solutions of nitrophenol prompted further investigation of the photochemical behavior of these esters. One striking observation was the much greater reactivity of the meta isomer toward nucleophilic substitution, demonstrating an unusual reversal of the substituent effect on the excited state reactivity vis-à-vis the normal diminution in the ground-state reactivity. Comparison of the photohydrolysis efficiencies for *o*-, *m*-, and *p*-nitrophenyl dihydrogen phosphate in aqueous base showed that the meta isomer was 4 times more reactive than either the ortho or para isomer (eq 2). This report in 1956[2] was the first clear example of the "meta" effect[8] in the photochemistry of substituted aromatic compounds which was subsequently extended to acetates and a wide variety of other nucleofuge groups. Zimmerman et al.[8a,b] demonstrated that *m,m'*-dimethoxybenzyl acetates gave the highest yield of ionic products in the series, a substituent pattern which since has been widely exploited.

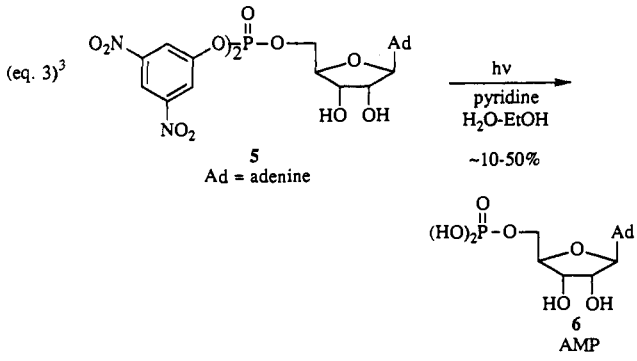
(eq. 2)[2]



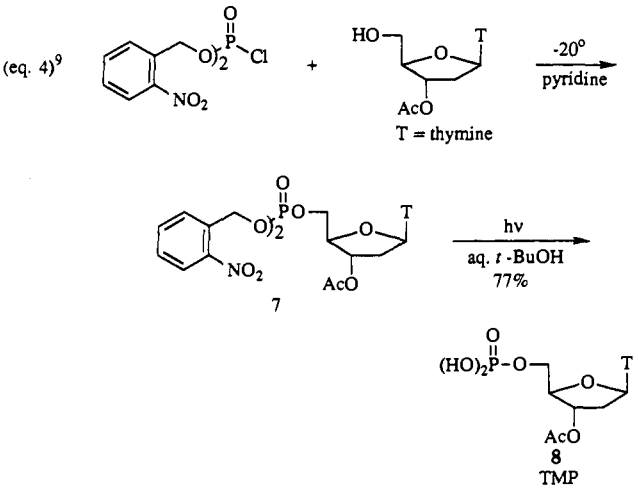
Havinga et al.[6a-c] further demonstrated that S_NAr^* substitution of *m*-nitrophenyl phosphates occurred through a short-lived singlet excited state and was controlled by an unusual pH dependence. Between pH 3 and 12, the quantum efficiency was essentially a constant, 0.05. Below pH 3 the reaction efficiency dropped, whereas above pH 12 it rose to a maximum of 0.24 in 1.0 M NaOH. Additional substituents at the 5-position had only a modest influence on the efficiency, reducing it slightly for 5-bromo and 5-methyl and increasing it for 5-chloro.

A fascinating feature of the reaction was the regiochemistry of the nucleophilic attack. In methanol, where the reaction is more efficient, the products were *m*-nitrophenol and methyl phosphate, i.e., attack by methanol occurred at phosphorus. Oxygen-18 (^{18}O) studies for the aqueous solution reactions revealed that attack by H_2O also occurs at phosphorus at pH < 12, whereas hydroxide attack at pH > 12 occurred at the aromatic ipso carbon (eq 3).[6,7] Thus, in addition to the discovery of the "meta" effect, Havinga and his coworkers[7] pioneered the early systematic investigations of photoinduced nucleophilic aromatic substitution reactions, e.g. S_NAr^* reactions, with the phosphate esters. Shortly thereafter, Kirby and Varvoglis[3] extended Havinga's results to 2,4- and 3,5-dinitrophenyl phosphates and added the major advance of applying 3,5-dinitrophenyl ligand as a "cage" for the protection–deprotection sequence for the nucleotide adenosine 5′-monophosphate (AMP) (eq 3).

In a third mechanistic variation of aryl group deprotection methods, Rubinstein, Amit, and Patchornik[9] introduced *o*-nitrobenzyl for a photochemical protection–deprotection sequence for nucleotide synthesis. For example, the bis(*o*-nitrobenzyl) ester of thymidine 5′-monophosphate (TMP, 8) was synthesized from thymidine and bis(*o*-nitrobenzyl) phosphochloridate in pyridine at −20 °C. Photolysis of the resulting phosphate ester **7** gave thymidine 5′-monophosphate in 77% yield, demonstrating the protection–deprotection se-



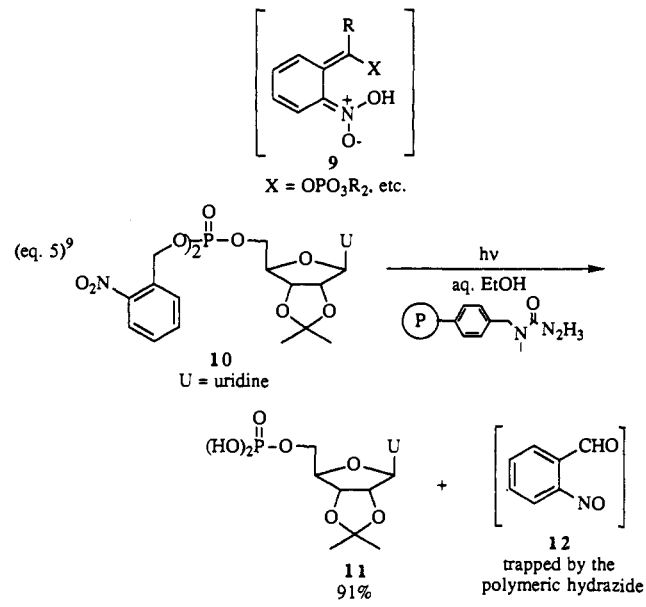
quence illustrated in eq 4. While the deprotection reaction gave good yields for reactions run on a small scale (77–98%), preparative-scale reactions of **7** and the uridine analogue **10** (eq 5) proved difficult due to



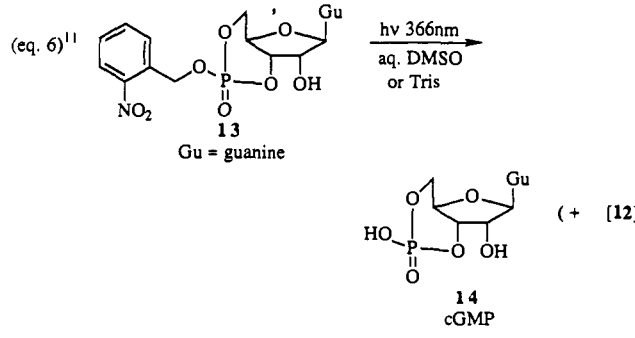
the formation of dark colored byproducts of *o*-nitrosobenzaldehyde (**12**). In other experiments, the nitrosoaldehydes were sequestered with an insoluble polymeric hydrazide added to the photolysis mixture, thus immobilizing the aldehyde and eliminating the byproduct interferences. Using this strategy, **8** and **11** were obtained in 70% and 91% yields, respectively.

Extensive earlier work[10] on a wide variety of other derivatives had shown that the *o*-nitrobenzyl group undergoes an efficient photoredox reaction in which the nitro group is reduced to nitroso while the benzyl carbon is oxidized. The original discovery by Ciamician and Silber[10b] of the photoredox reaction of *o*-nitrobenzaldehyde to *o*-nitrosobenzoic acid has been followed by a wealth of other examples of *o*-nitrobenzyl reactions.[10a,c] Nitroso aldehydes, ketones, and acids are formed by hydrolysis of the *aci*-nitro intermediate **9** in a ground-state process. It is important to note that the primary photoreaction does not involve the cleavage of the covalent bond to the leaving group X, a limiting feature when considering the application of this reaction to kinetic studies (vide infra). Nevertheless, the *o*-nitrobenzyl group has found extensive use as a photochemically activated deprotection group for a wide variety of organophosphates.[9,10]

During this period, the focus on the application of photochemistry of phosphates began to drift from deprotection in synthetic processes to release in biological experiments. In 1978, Engels and Reidys[11] extended the photodeprotection strategy to cyclic nucleotides such as guanosine 3′,5′ monophosphate



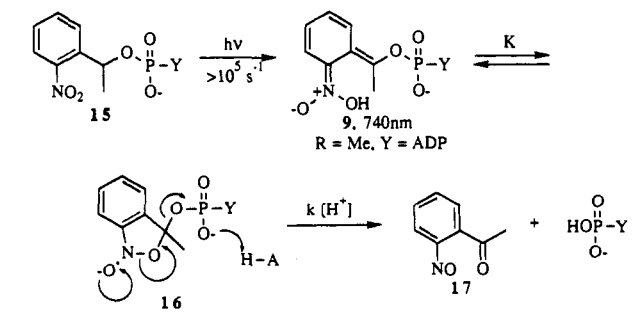
(cGMP) where photolysis of the *o*-nitrobenzyl ester in either DMSO/H_2O or Tris buffer at pH 7.4 (10:1 v:v) at 366 nm gave cGMP (eq 6). The authors suggested



that the protected triester of cGMP would be more lipophilic and, thus permeable through cell membranes, would also be suitable for release of cGMP by irradiation with near UV light (>320 nm). Under these conditions, the concentration of cGMP would be rapidly increased in the biological medium, a *concentration jump*, with minimal damage to the biological system.

Recently, Walker et al.[12] reported a general synthesis of *o*-nitro-α-phenylethyl phosphate esters and a detailed mechanistic study of their photochemistry. The *o*-nitro-α-phenylethyl group (e.g., **15**, Scheme I) has become

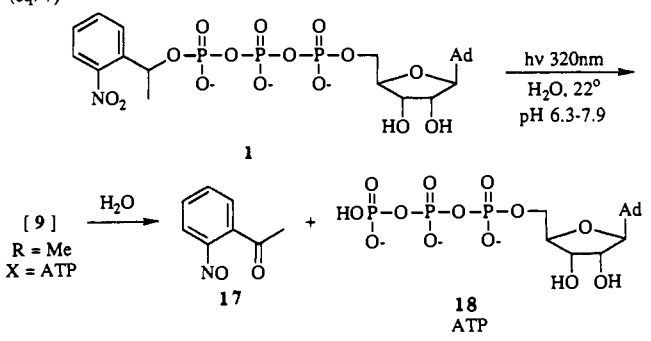
Scheme I[11]



the ligand of choice for many phosphates for several reasons.[4,9,12] First, the esterification reaction can be accomplished with ease in relatively modest yields through the reaction of the phosphate group directly with a *o*-nitro-α-phenylethyl diazonium salt.[12] Sec-

ondly, the seemingly minor modification of replacing the benzyl with the phenylethyl group, which yields an *o*-nitrosoacetophenone upon photolysis, greatly improves the photodeprotection step by lessening the complications of byproduct formation from the less reactive nitrosoacetophenone 17 (eq 7).[4,9b]

(eq. 7)[12]



Upon photolysis at 320 nm of caged ATP 1 with a 1-μs pulse from a frequency doubled ruby laser, a transient band at 740 nm was observed within 10 ms of the pulse (eq 7). The 740-nm band was assigned to the *aci*-nitro intermediate **9**, and its decay was correlated with ATP formation as determined by the enhanced fluorescence obtained from the dissociation of a pyrene-labeled subfragment 1 of actomyosin complex upon competitive complexation of Mg^{2+} by the released ATP. The rate of release of ATP from **9** was shown to be pH dependent. At pH 7.0, ATP release was rate limiting, whereas at pH 7 an intermediate dependency was observed and at pH 6.3 or lower release was not the rate-limiting process. The rate of decay of the *aci*-nitro intermediate was also found to be first order in $[H^+]$ over this pH range and afforded rate constants of 16, 125, and 500 s^{-1}, respectively, in accord with the rate of ATP release being coupled to the rate of decay of the *aci*-nitro intermediate **9**. To accommodate these observations, Walker et al.[12] proposed the general mechanism shown in Scheme I, which includes a general acid catalytic hydrolysis of the *aci*-nitro intermediate **9**. Intramolecular rearrangement of **9** to **16** followed by protonation of one of the nonbridging phosphate oxygen atoms generates the nitrosoacetophenone. Alternatively, an addition–elimination sequence on **9** would also account for the formation of **17** and release of phosphate.

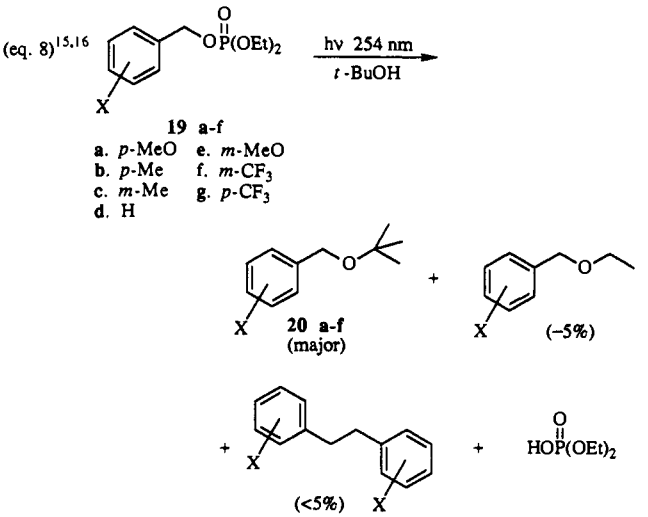
A limitation of the *o*-nitrobenzyl cage is clearly evident from these results. Whereas the intramolecular photochemical hydrogen abstraction step is relatively rapid, i.e., $>10^5$ s^{-1}, the much slower hydrolytic step, which occurs with a rate constant of 86 s^{-1} (pH 7), that releases ATP from caged **1** limits the "window" for kinetic studies of any subsequent biological processes involving ATP (or any other nucleotide or phosphate). For the caged imido ATP(β,γ-NH), which had one of the fastest hydrolytic rates measured, the limiting rate constant was only 250 s^{-1}. Furthermore, in the presence of Mg^{2+} the release of the nucleotide is further slowed by an additional factor of 3.5.

The quantum efficiencies for this cage group, which range from 0.49 to 0.63, make this method one of the most attractive for "concentration jump" experiments.[4,12] It is necessary, however, to add thiols to the reaction medium in order to minimize the concentration of light-absorbing byproducts formed from the 2-nitrosoacetophenone as well as their effects on other substrates (see Section IV, Biological Applications).

The redox photochemistry of the *o*-nitrobenzyl and *o*-nitro-α-phenylethyl phosphates is mechanistically distinct from the direct bond fragmentation that occurs in the photolysis of the other benzyl esters cited earlier. While mechanistic investigations had not been conducted in the earlier studies, it was anticipated that either homolytic or heterolytic C–O fragmentation of the phosphate group would be the primary photochemical process for the simple benzyl esters resulting in an overall much more rapid release of the phosphate. A caveat, however, is that the direct release of phosphate could involve initial homolysis to a radical pair which might preclude application of this reaction as a general method for phosphate ion release. Previous studies on other functional groups including carboxylic esters[13,14] and on phosphate esters cited earlier[1,5] gave no clear indication concerning the nature of the primary photolysis step.

Our studies[15,16] on the photochemistry of benzyl phosphates were initiated with a series of substituted benzyl diethyl phosphates and focussed on the scope and mechanism of the photofragmentation process. Irradiation of a series of benzyl and α- and β-naphthylmethyl phosphates in *tert*-butyl alcohol gave a single major product, the *tert*-butyl ether (eq 8). Minor

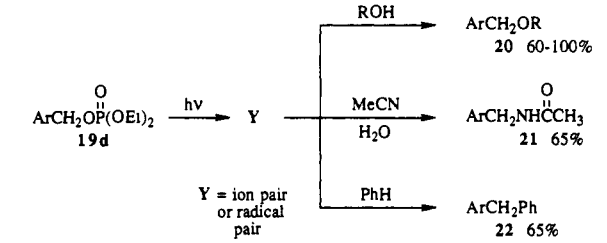
(eq. 8)[15,16]



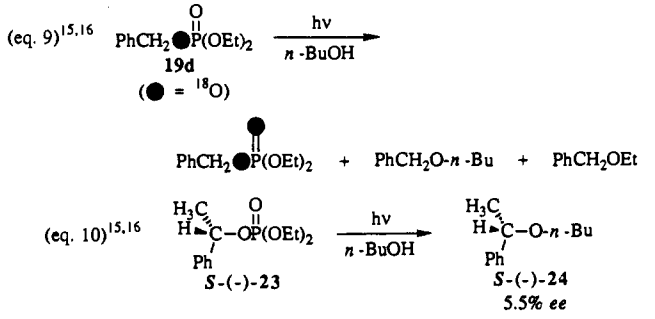
amounts (<5%) of benzyl ethyl ether and 1,2-diphenylethane were also observed. The electrophilic character of the intermediate postulated in the conversion of benzyl and α- and β-methylnaphthyl phosphates to the corresponding substitution products was probed by photolysis of **19** in a variety of solvents including alcohols, moist acetonitrile, and benzene (Scheme II).

In probing the reaction mechanism for ion-pair intermediates, ^{18}O-labeled benzyl phosphate **19d** was irradiated to 77% conversion in *n*-butyl alcohol and

Scheme II[15,16]



the products and recovered **19d** were analyzed for the distribution of the ^{18}O label (eq 9). Scrambling of the ^{18}O label between the benzyloxy and phosphoryl oxygens was found to be complete, suggesting an intermediate ion pair capable of interchanging the two oxygens functions.[15,16] This was confirmed by efficient racemization of chiral phosphate *S*-(−)-**23** (eq 10) upon



photolysis. The substitution product **24** was also stereoequilibrated, i.e., the ether was formed with a slight excess (5% ee) of retention of the chiral center's configuration. From the efficiencies obtained for the isotope exchange and ester disappearance, an estimate of 0.66 was made for the efficiency for ion pair return for **19d** in *n*-butyl alcohol and 0.36 in benzene.[15,16] The sum of all measured efficiencies for C–O bond fragmentation in *n*-butyl alcohol was 92%, i.e., practically every photon absorbed by the ester resulted in a dissociation of the C–O bond. In benzene, this was estimated to be 39%. These high efficiencies indicate that benzyl phosphates are excellent substrates for photoinduced substitution reactions and that phosphate is an effective nucleofuge.[16,30]

Acetone sensitization of **19a,d,g** afforded the ether as well, but at substantially reduced quantum efficiencies, demonstrating that the triplet possesses only modest reactivity. Quenching studies with *trans*-piperylene resulted in no diminution in reactivity of **19a**, which accordingly leads to the assignment of the direct reaction as a singlet process.[16]

Due to the singlet nature of the reaction and the apparent range in reactivity of the substituted benzyl esters, a Hammett substituent study was initiated to further explore the nature of the C–O bond breaking process. While the Hammett σ is based on ground-state substituent effects on reactivity, there are several examples of polar substituent effects in excited-state processes which appear to obey an extrathermodynamic substituent effect correlation. As shown in Table I, the disappearance efficiencies ranged from 0.42 for *p*-MeO to 0.027 for *m*-CF_3 in descending order according to electron-withdrawing character of the substituent as would be anticipated for a benzyl cation intermediate. However, for quantitative verification of this trend, it was necessary to obtain rate constants for each ester and therefore to determine the fluorescence lifetimes (Table II). If we assume the mechanism outlined in Scheme III, the efficiency for disappearance of ester is given by the expression

$$\Phi_{dis} = [k_r/(k_r + k_d + k_f)][k_{dif}/(k_{dif} + k_{rec})]$$

If we further assume that the efficiency of recombination or ion pair return (Φ_{rec}) in *n*-BuOH is the same in *t*-BuOH, which gives a ratio of $k_{rec}/k_{diff} = 3$, and that this ratio is independent of the substituent on the aromatic ring, then the general expression for the efficiency becomes

$$\Phi_{dis} = 0.25k_r/(k_r + k_d + k_f) = 0.25k_r\tau_s$$

where τ_s is the measured singlet lifetime. Table II gives the rate constants for the formation of the ion pair derived from this expression. A plot of log k_r vs Hammett σ (Figure 1) verifies the trend observed with the efficiencies, and the ρ-value of −0.90 for the slope indicates an electron-deficient center at the benzylic carbon during the fragmentation.[16] The linear rela-

Table I. Substituent and Solvent Effects on the Efficiencies[a] for Photosolvolysis of Benzyl Phosphates

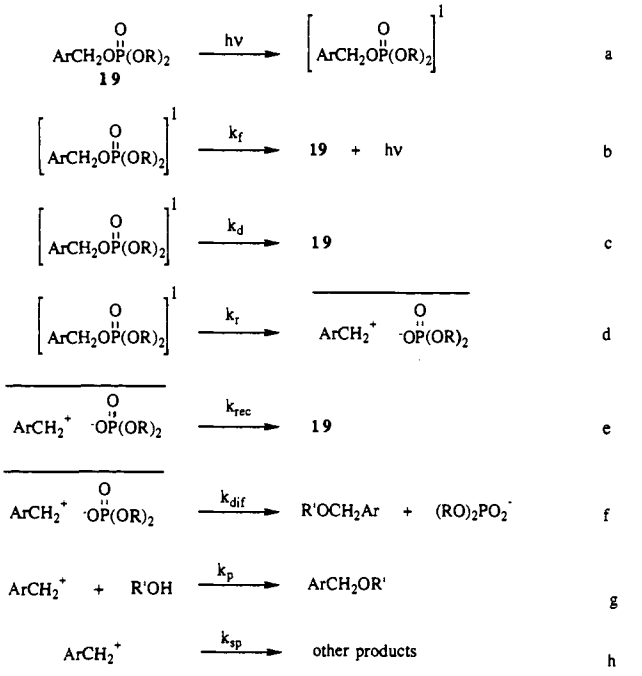
ester **19**	solvent	Φ_{dis}	$\Phi_{ip\ return}$[b]	Φ_{app}	$\Phi_{COcleav}^{est}$
d, H	MeOH	0.39		0.19	
	n-BuOH	0.26	0.66		0.92
	t-BuOH	0.17		0.093	
	benzene	0.03	0.36	0.016	0.39
a, *p*-OCH_3	*t*-BuOH	0.42		0.14	
b, *p*-CH_3	*t*-BuOH	0.34		0.11	
c, *m*-CH_3	*t*-BuOH	0.13		0.10	
e, *m*-OCH_3	*t*-BuOH	0.18		0.065	
f, *m*-CF_3	*t*-BuOH	0.027		–	
g, *p*-CF_3	*t*-BuOH	0.11		0.036	

[a] $\Phi_{ip\ return}$ is the efficiency for ion pair return, and Φ_{app} and Φ_{dis} are the efficiencies for appearance of the ether and disappearance of the esters, respectively. [b] $\Phi_{ip\ return} = 2\Phi$ exchange (exchange of ^{18}O label between ether and phosphoryl oxygen positions).

Table II. Fluorescence Lifetimes and Calculated Rate Constants for Photoinduced Nucleophilic Substitution of Benzyl Phosphates

ester **19**	τ_s, ns	$10^{-6}k_r$, s^{-1}	$10^{-6}k_p$, s^{-1}	Φ_f
a, *p*-OCH_3	4.2	400	132	0.19
b, *p*-CH_3	5.9	232	76	0.134
c, *m*-CH_3	6.9	76	60	0.045
d, H	2.5	268	148	0.048
e, *m*-OCH_3	4.9	148	52	0.027
f, *m*-CF_3	1.2	92	112	0.088
g, *p*-CF_3	6.7	64	20	0.09

Scheme III[16]



where k_f (fluorescence), k_d (decay), k_r (ion pair formation), k_{rec} (recombination), k_{dif} (diffusion), k_p (ether formation) and k_{sp} (other products) are the rate constants for the processes shown.

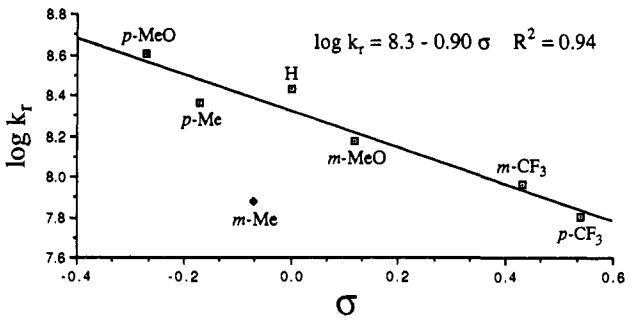
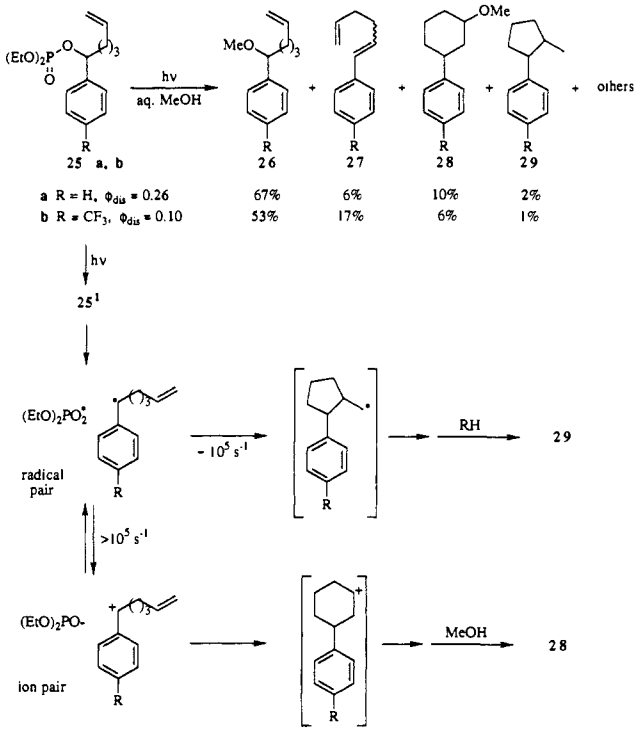


Figure 1. log k_{dis} **19** vs Hammett σ.[16] Datum for *m*-Me omitted in linear regression.

Scheme IV[18]



tionship obtained further supports the assumptions that the ion pair return to diffusion ratio is relatively insensitive to substituents and to the nature of the alcohol solvent.

The trend in appearance efficiencies also follows a Hammett correlation. Again, extracting the rate constants from the fluorescence and chemical yield data (Table II) resulted in a Hammett correlation with σ. The ρ-value of −0.86 was in excellent agreement with the Hammett correlation obtained with the rates of dissociation and accords with the mechanistic picture depicted in Scheme III.

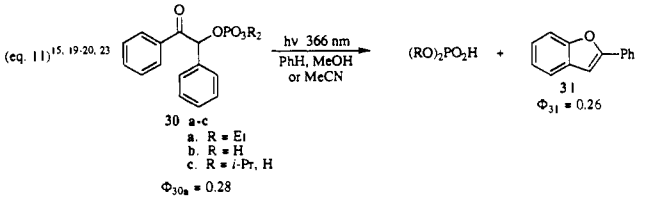
While the majority of these data points toward a heterolytic fragmentation, other mechanistic interpretations are possible.[15,16] As two examples, either a homolysis of the C–O bond followed by electron transfer (Scheme IV) or a homolysis from a charge-transfer excited state of the aryl phosphate leads to the benzyl cation–phosphate anion pair. For the homolysis route, a short-lived radical pair intermediate might be intercepted by an internal "clock" reaction.[17] The competition between cyclization and electron transfer, which is expected to be fast (>10^8 s^{-1}), leading to ion pairs was explored by a product study of two 1-arylhex-5-enyl diethyl phosphates (**25a** and **25b**).[18] Each ester gave a variety of elimination, substitution, and cyclization products, several of which are shown in Scheme IV. The higher yields of the 6-membered ring products indicated carbocation cyclization predominated over radical cyclization to the 5-membered ring. Therefore, any radical intermediate would have to be short-lived (<1 μs) to account for the low propensity to form cyclopentane products. The disappearance quantum efficiencies for the two phosphates (0.26 and 0.10 for **25a** and **25b**, respectively) were found to be in agreement with the earlier Hammett substituent studies.

A second qualitative characteristic of the two benzyl cations generated from **25a** and **25b** emerges from comparison of the product yields. Replacing H with *p*-CF_3 dramatically reduced the direct substitution products (ca. 14%) with a concomitant 3-fold increase in the elimination products. The yield of cyclization products also decreased substantially. These observations are consistent with a destabilizing influence of *p*-CF_3 on the carbocation intermediate which should increase its reactivity and thus reduce its selectivity. Finally, irradiation of **25a** in benzene gave predominantly elimination products at the expense of substitution, consistent with the poor nucleophilicity of benzene and the increased basicity of phosphate ion when generated in a nonprotic environment.

The overwhelming weight of evidence drawn from these mechanistic studies is that the arylmethyl phosphate esters undergo rapid C–O bond cleavage to yield ion pairs probably through a heterolytic pathway. The less likely homolytic fragmentation would require a very rapid electron transfer (>10^6 s^{-1}) to accommodate the observations. The homolytic pathway from the charge-transfer excited state does remain a possible alternative, however. It must be noted that the small yield of 1,2-diphenylethane formed in these photoreactions may be due to direct homolysis. The extent of this divergent pathway does not appear to be very sensitive to the aryl substituents, however.[15,16]

III. α-Keto Phosphates

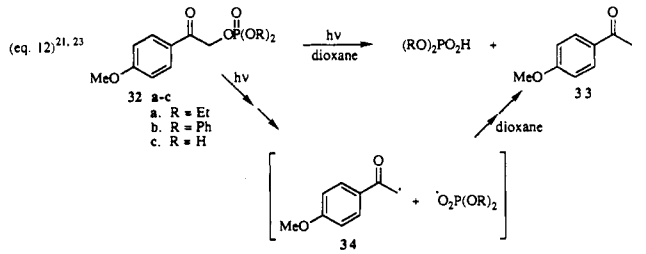
In our 1984 communication[15] concerning the photolysis of benzoin diethyl phosphate (**30a**), we reported that this α-keto phosphate is converted to diethyl phosphate and 2-phenylbenzo[*b*]furan (**31**) in nearly quantitative yield with a quantum efficiency of 28% (eq 11). In three solvents, benzene, methanol, and



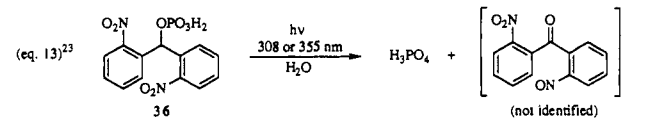
acetonitrile, the same product mixture was obtained. We were also able to establish that this reaction, unlike the photosolvolysis reactions of the benzyl and naphthylmethyl analogues, was a triplet reaction by both quenching and sensitization experiments. The Stern–Volmer slope obtained in the quenching study with naphthalene or piperylene as quenchers indicated a short-lived triplet (10–14 ns) and the phosphorescence spectrum of **30** displayed a 0,0 band at 392 nm for a triplet energy of 73 ± 1 kcal/mol.[19,20] These features together with the fact that the α-ketone's, n,π* ab-

sorption extends well beyond 300 nm appealed to our interest in developing this as a photoprotecting group for phosphates.

Subsequently, Epstein et al.[21] reported a second example of an α-keto phosphate photolysis in which *p*-methoxyphenacyl esters of diethyl and diphenyl phosphate **32a,b** were converted to diethyl (86%) or diphenyl phosphate (74%) and *p*-methoxyacetophenone (**33**, 84–91%) when irradiated in dioxane (eq 12). The authors suggested that the mechanism for the photoreduction reaction, like that of the corresponding *p*-methoxyphenacyl carboxylates,[22] should be viewed as a β-homolytic cleavage followed by hydrogen abstraction from dioxane by the resulting α-keto radical **34**.

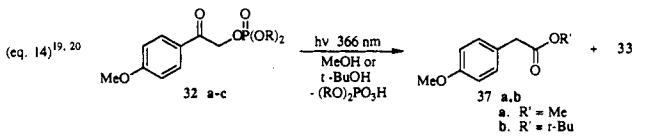


As recently as 2 years ago, Baldwin et al.[23] investigated the photochemical reactions of *p*-methoxyphenacyl monophosphate as well as several other monophosphates including benzoin (**30b**), 1-(2-nitrophenyl)ethyl (**15**), and bis(2-nitrophenyl)methyl (**36**). Irradiations of aqueous solutions of the esters, either at 308 or at 355 nm, released inorganic orthophosphate P_i with efficiencies and yields that varied significantly with ester structure. The bis(2-nitrophenyl)methyl ester **36**



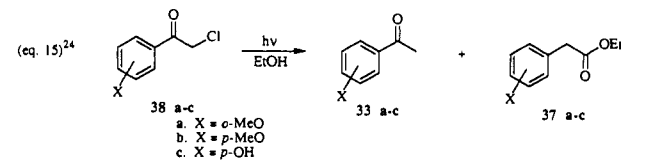
was the most efficient and gave the highest yield (90% P_i in 5 min), whereas the *p*-methoxyphenacyl ester **32c** was less efficient and gave the lowest overall yield (ca. 20% in 5 min) of all the phosphates tested. Irradiation of **32c** in dioxane confirmed the report by Epstein[21] that *p*-methoxyacetophenone was a major component of the product mixture. Baldwin[23] also showed that the unsubstituted benzoin phosphate (**30b**) gave an intermediate yield of phosphate (55% in 5 min) with a relatively high efficiency and therefore he repeated the suggestion that **30** should be studied as a potential photoprotecting group, based largely on its ease of synthesis. (However, see below.)

We were puzzled by the inconsistency that the photochemistry reported for *p*-methoxyphenacyl phosphates by Epstein[21] and Baldwin[23] contrasted significantly with that which we had reported for benzoin phosphate (**30a**).[15] A very plausible rationale for the apparent dichotomy in heterolytic versus homolytic reactivity of α-keto phosphates could be the dual nature of the reactivity of the n,π^* excited state which has been described by Zimmerman in terms of the electron distribution of the excited state.[8d] The primary processes occur from either the electron-difficient, half-occupied n orbital, e.g., hydrogen abstraction, or from the electron-rich, ketyl-like π^* orbital, e.g., heterolysis. Thus, a reinvestigation of the photochemistry of *p*-methoxyphenacyl diethyl phosphate (**32a**) in two other, more polar solvents, methanol and *tert*-butyl alcohol, was conducted in our laboratories in order to explore the roles, that hydrogen abstraction and solvent polarity might play among the primary photochemical processes.[19,20] As shown in eq 14, in addition to the



products of the type reported by Epstein[21] and Baldwin,[23] we found rearranged *tert*-butyl *p*-methoxyphenylacetate (**37b**) as the *major* product in *tert*-butyl alcohol, a solvent known to be a poor hydrogen-atom donor. Likewise, even in methanol, which is an excellent hydrogen-atom donor, the yield of the rearrangement product **37b** was appreciable, i.e., 38%. In both solvents, rearrangement predominated over reduction; the yields of *p*-methoxyacetophenone were only 21% and 14%, respectively. These were determined to be triplet reactions as demonstrated by quenching the formation of both products with 1.6 mM naphthalene.

The change in the nature of the products as a function of solvent and structure of the phosphate is reminiscent of the earlier studies on the corresponding phenacyl halides[24,25] and bicyclic α-chloro ketones.[26,27] Anderson and Reese[24] obtained only the reduction product from irradiation of phenacyl chloride but isolated appreciable amounts of rearranged phenylacetates from aryl participation for irradiations of o- or *p*-methoxy- or *p*-hydroxyphenacyl chloride **38a–c** (eq 15). Furthermore, Laird and Williams[25] reported that dimeric 1,2-dibenzoylethane was formed in irradiations of phenacyl derivatives in aqueous solution.



Our own studies of *exo*- and *endo*-3-chlorobicyclo-[2.2.2]oct-5-en-2-ones[26,27] showed that only the stereoelectronically aligned *exo*-chloro ketone gave rearrangement product through neighboring double bond participation. Furthermore, a test for heterolytic vs homolytic C–Cl bond photolysis using iodide as a chlorine atom trapping agent showed that only *endo*-**39** produced strong signal for I_2^- indicating that chlorine atoms were generated upon photolysis. However, *exo*-**39** gave no indication of I_2^-, consistent with heterolytic chloride bond breaking concomitant with carbocation rearrangement.[26,27]

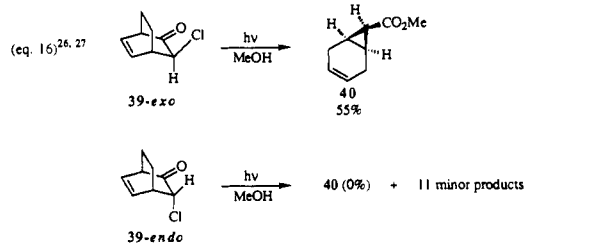


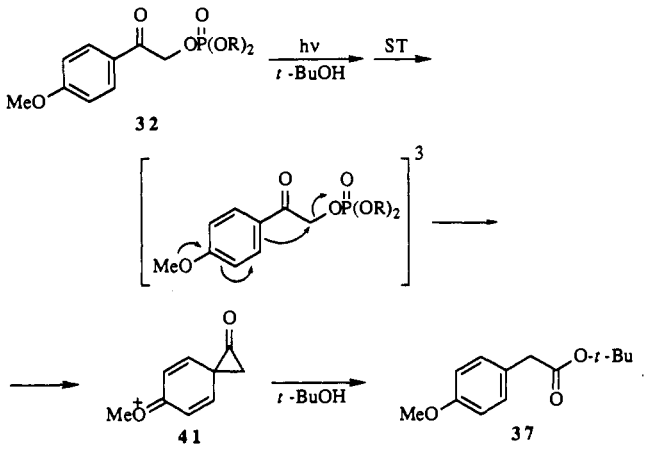
Table III. Quantum Efficiencies (Φ) for Photolysis of *p*-Methoxyphenacyl Diethyl Phosphate (32a) at 300 nm in *t*-BuOH, CH_3OH, CD_3OD, and CH_3OD[a]

solvent	Φ_{32a}	Φ_{37}	k_H/k_D	Φ_{33}	k_H/k_D
benzene, *t*-BuOH (3:1)	0.036	0.026		0.0074	
CH_3OH	0.42	0.20		0.07	
CD_3OD		0.14	1.4	0.013	5.4
CH_3OD		0.11	1.8	0.053	1.3

[a] k_H/k_D is the relative efficiency for hydrogen (H) vs deuterium (D) abstraction. Error limits are ±10%.

These results are also in accord with those of Sheehan and Wilson et al.[28a,b] who showed that the photorelease of acetate from 3′,5′-dimethoxybenzoin acetates occurred *without* decarboxylation, evidence in support of heterolysis of a C–O bond α to the benzoin carbonyl. Since the rate of decarboxylation of alkyl acyloxy radicals[30c] is on the order of 10^9 s^{-1}, loss of CO_2 should be a competitive process if a homolytic mechanism dominates. In view of the superior nucleofugacity of phosphate over acetate in ground-state solvolysis reactions, the heterolytic route should be favored for phosphates as well. Thus, it would appear that the intermediates generated from α-keto halides, phosphates, and related nucleofuge groups tend to favor heterolysis[30] when neighboring groups or direct conjugation can participate effectively at the reaction center. Homolysis reactions dominate when no special stabilization of the α-keto carbocation is possible and when the nucleofugacity of the α-substituent is not strong. Thus, the formation of the rearranged ester **37** can best be rationalized by conventional neighboring *p*-anisyl participation at the developing electron-deficient α-ketocarbocation-like center (Scheme V).

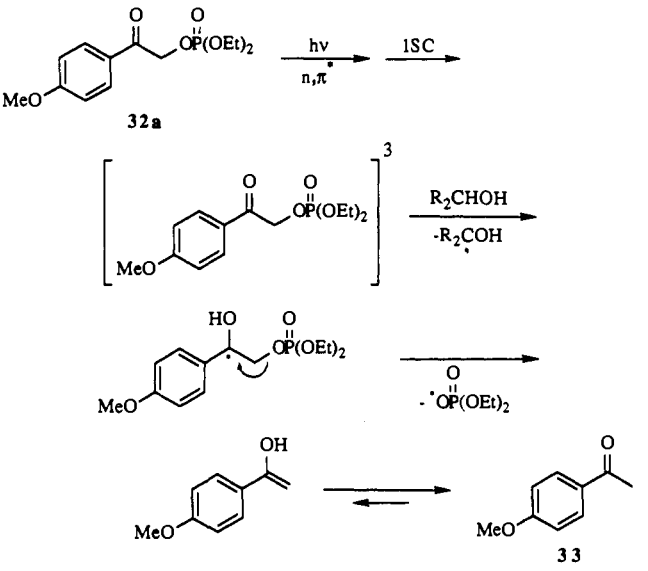
Scheme V[19,20]



Collapse of the spirocyclopropanone **41** or its equivalent gives rise to *p*-methoxyphenylacetate **37**. This rearrangement chemistry[19,20] nicely parallels our earlier results with benzoin phosphate photolyses.[15]

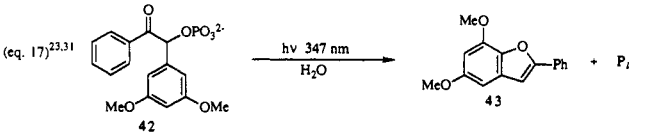
Further study[20] of the photochemistry of **32** in deuterated methanols revealed a significant isotope effect on the formation of photoreduction product **33** but not on the rearrangement product **37** (Table III). In fact, the formation of *p*-methoxyacetophenone was suppressed by a factor of approximately 5 when the reaction was run in CD_3OD but remained the same when conducted in CH_3OD. Thus, for abstraction of the carbon-bound deuterium from CD_3OD, the isotope effect is in the range of a primary isotope effect,

Scheme VI[21,23]



suggesting that a hydrogen abstraction by the triplet keto phosphate is a likely alternative to the Epstein mechanism for the *p*-methoxyphenacyl phosphates (Scheme VI). The protonated ketyl radical then suffers homolysis of the C–O bond to give the enol of **33**. The fact that a large isotope effect is observed only for **33** and not **37** further suggests that the rearrangement does not involve a rate-limiting hydrogen abstraction. Hydrogen abstraction apparently dominates in dioxane, the solvent chosen for the product studies by Epstein[21] and Baldwin.[23]

Recently, Corrie and Trentham[31] have reexamined the photochemistry of benzoin (**30b**) and 3′,5′-dimethoxybenzoin phosphate (**42**) along with preliminary investigations of the analogous 4-methoxy-, 4-methyl-, 3,5-dimethoxy-, and 3,3′,5,5′-tetramethoxybenzoin phosphates. 3′,5′-Dimethoxybenzoin phosphate was found to be the best among the substituted benzoins for release of the phosphate (eq 17). Using a 50-ns pulsed

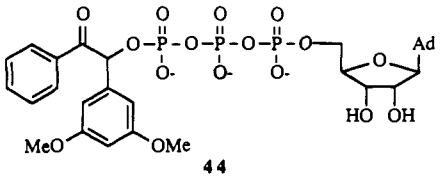


excitation at 315 nm, the rate of formation of the benzo[*b*]furan **43** occurred with a first-order rate constant of greater than 10^5 s^{-1} and a quantum efficiency of 78%, more than twice that of our unsubstituted benzoin phosphate **30a**.[15] The extent of conversion, however, appears to be approximately a third that of the corresponding *o*-nitrobenzyl analogues discussed in Section II. Corrie and Trentham attribute this to the much lower absorptivity of the benzoin vis-à-vis the *o*-nitrobenzyl chromophore.[31] Furthermore, the photoproducts also compete for the incident radiation.[19,20]

A preliminary report by Corrie and Trentham[32] demonstrated that 3′,5′-dimethoxybenzoin can serve as a photoprotecting group or *cage* for ATP. Photolysis of the P(γ) 3′,5′-dimethoxybenzoin caged ATP **44** (eq 18), released ATP with a rate constant of $>10^5$ s^{-1}, over 3 orders of magnitude faster than that observed for the corresponding *o*-nitrobenzyl ester of ATP.[12]

We have also examined the photochemistry of a series of benzoin phosphates (e.g., **30b** and **30c**) in aqueous

(eq. 18)[32]



44

hν, aq. buffer, $k_r > 10^5\ s^{-1}$ → ATP + 43

acetonitrile at pH 2 and 7 (eq 11 and Table IV).[19,20] Only two major products were obtained with efficiencies that were sensitive to pH. A decrease in efficiency with increasing pH that closely paralleled the pH profile for each ester suggests that release of phosphate is favored at the low pH, reflecting the better nucleofugacity of the *protonated* phosphate over that of its conjugate base. These aqueous reactions, like that of **30a** in organic solvents, are quenched by naphthalenesulfonic acid, yielding lifetimes of 3.4 and 4.4 ns from the Stern–Volmer analysis.

The mechanistic picture which emerges from these several studies of α-keto phosphates suggests either heterolytic C–O rupture from the triplet excited ketone or possibly a homolytic process from a triplet ketone charge-transfer complex. In the latter case, an ion pair is formed upon cleavage. In either event, relaxation to the ground-state-singlet ion pair must occur at some point in the process. Whether or not the triplet relaxes to a ground-state singlet before intramolecular cyclization to generate the furan ring occurs, however, remains unknown at this time. Further work is needed to define the details of this mechanism.

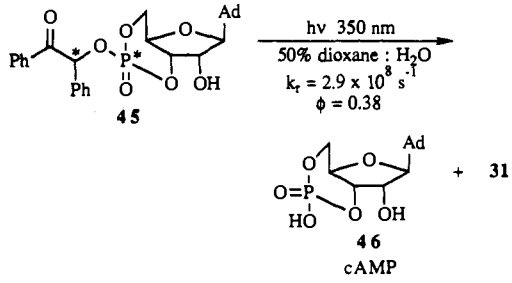
It is possible, nevertheless, to estimate the rate of the C–O bond fragmentation processes from our quenching results. Given the triplet lifetimes of 3.3 ns (**30b**) and 4.4 ns (**30c**) and the reaction efficiencies (Table IV), the rate constants for C–O bond cleavage were estimated to be 11×10^7 and $8 \times 10^7\ s^{-1}$, respectively. Interestingly, these reactions are at least 2 orders of magnitude slower than the corresponding acetates which could not be quenched by 1.0 M piperylene.[28] At low conversions (<15%) the only observed product of the desyl group was 2-phenylbenzo[*b*]furan (31). At higher conversions at 300 nm, in preparative-scale reactions the photodimerization of 31 was also observed. However, the production of the photodimer was considerably suppressed when the irradiation was carried out at 350 nm where the furan does not compete as effectively for the excitation radiation.

Our results on the photochemistry for both families of α-keto phosphates encouraged us to probe the application of benzoin as a "caging" ligand for nucleotides.[19] Benzoin caged cAMP was chosen as our initial target. A diastereomeric mixture of the axial and equatorial benzoin esters of adenosine 3′,5′-monophosphate (**45**) was synthesized by direct displacement of the phosphate salt on desyl bromide. Subsequent irradiation of **45** at 350 nm in a 1:1 mixture of dioxane–buffer afforded essentially quantitative release of the nucleotide **46** (eq 19). The two diastereomers of **45** reacted at nearly the same rate with a quantum efficiency of 0.38 over a pH range of 1.6–8.4. This, likewise, is a triplet reaction, as determined by standard Stern–Volmer quenching experiments with 2-naphthalenesulfonate in aqueous buffer–dioxane, affording a measured lifetime of 1 ns.[19] The phosphorescence

Table IV. Quantum Efficiencies (φ) for Photolysis of Benzoin Phosphate Esters 30a–c at 350 nm

ester	solvent	pH	Φ_{30}	Φ_{31}	Φ_{phosph}
30a	benzene		0.28	0.26	
30b	H_2O/CH_3CN	2.0	0.38	0.14	0.15
30b	H_2O/CH_3CN	7.0	na	0.08	0.01
30c	H_2O/CH_3CN	2.0	0.37	0.20	0.12
30c	H_2O/CH_3CN	7.0	na	0.07	0.01

(eq. 19)[19,20]



45

hν 350 nm, 50% dioxane : H_2O, $k_r = 2.9 \times 10^8\ s^{-1}$, φ = 0.38 → + 31

46

cAMP

spectrum of **45** indicated a triplet energy of 73 kcal/mol, closely paralleling the model reactions for **30b** and **30c**, and suggests a common reaction mechanism for all of the benzoin phosphates.

From the lifetime and efficiency results, the rate of release of cAMP from benzoin cAMP **45** was determined to be $7.1 \times 10^8\ s^{-1}$ in dioxane–aqueous buffer solutions. This combined with a quantum efficiency for formation of cAMP of 0.38 supports our earlier contention that benzoin is an excellent group for the photorelease of phosphates[15] and now appears to be in many ways superior to *o*-nitrobenzyl as a *cage* ligand. We have been joined in this conclusion by the groups of Corrie and Trentham[31,32] and Baldwin et al.[23]

IV. Biological Applications

Many of the recent applications of photoactivated caged phosphates, primarily those involving caged nucleotides, have been reviewed elsewhere.[12b,33-36] Caged nucleotides and related bioactive phosphates have become attractive precursors for "concentration jump" experiments whereby an ultrafast activation of a variety of biological processes is accomplished by a flash photolytic release of a biological substrate from its caged precursor. With nanosecond laser flash photolysis, the rate of release of the phosphate is governed by the photochemical and subsequent ground-state reaction rates required to cleave the cage-phosphate ester bond. The kinetic "window" available for the study of the biological process is determined, therefore, either by the chemistry of the cage ligand or the pulse width or the decay characteristics of the laser excitation flash, or any combination of these. There is considerable interest, therefore, in developing new cage ligands that undergo very rapid rupture of the phosphate–ligand bond.

According to Lester et al.,[34] a *cage* must serve as the labile ligand that releases the protected activator without disrupting the biological medium. Furthermore, the *caged* ester must be devoid of biological activity and the released ligand must remain biologically benign. In order to design such a ligand appropriate for the photochemical release of nucleotides and phosphates, one must consider the following seven criteria: (1) The caged phosphate must be at least partially water soluble in a medium of moderately high

ionic strength. (2) The photochemical reactions must be compatible with the media. (3) The release should occur as a primary photochemical process. (4) The photoproducts should be solvolytically stable. (5) The photochemical reaction should be activated at wavelengths greater than 300 nm to avoid significant radiation damage to cellular components. (6) The photochemical reaction should be efficient. An index of reactivity suggested by Lester[34] is a value of 500 or greater for the product of the photochemical efficiency and the molar absorptivity, i.e., $\Phi_r\epsilon$. (7) "Both the caged precursor and the photoproducts should have simple, well characterized equilibrium effects on the physiological system. There should be no complicating interactions with proteins or membranes." [34] While compilance with all seven of these criteria is rarely achievable, the *o*-nitro-α-phenylethyl, 3,5-dimethoxybenzyl, and benzoin groups do satisfy the majority and thus are receiving the most attention as photolabile ligands for phosphate release in biological studies.

Several applications of *o*-nitrobenzyl, *o*-nitro-α-phenylethyl, and 4,5-dimethoxy-2-nitrobenzyl groups as cages are presented below. This is not intended to be comprehensive treatment but rather a representative series of applications and the reader is advised to consult the primary literature for more information and other examples of interest.[33-36]

An investigation of sodium ion movement in a single turnover in right-side out membrane vesicles prepared from canine kidney was reported by Forbush.[37] Through the use of *o*-nitro-α-phenylethyl caged ATP, the author was able to examine this system with a time resolution on the order of 30 ms and to show that the duration of the Na^+ burst was not related to the amount of ATP released on photolysis. The results were interpreted as an early release of Na^+ in the pump cycle, preceding K^+ binding.

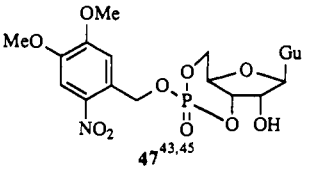
The effect of guanine nucleotide analogues on calcium channel currents in cultured rat dorsal root ganglion neurons was studied by Dolphin et al.[38] The authors employed the *o*-nitro-α-phenylethyl caged GTP-γ-S and caged GMP-PNP (the thio and imido guanidyl nucleotides) which upon irradiation released the free nucleotides. Calcium currents were monitored using the whole cell patch clamp method. The results suggested that the GTP derivatives differentially inhibit the transient and sustained Ca^{2+} channels.

An early investigation of ATP-induced dissociation of the actin–myosin (AM) complex by McCray et al.[39] provided some of the first kinetic information on the photolytic release of ATP from the *o*-nitro-α-phenylethyl ATP esters. By laser flash photolysis at 347 nm (from a frequency doubled ruby laser), initial rates of proton and ATP release could be determined. ATP concentrations were monitored by the solution turbidity of an actomyosin complex which decreases in the presence of ATP due to competitive binding of Mg^{2+} by ATP leading to dissociation of the AM complex. The process is first order in ATP and is monitored by the transmittance of the solution. The study also established that the dissociation of the *aci*-nitro intermediate was pH dependent (i.e., $k_r \propto 2.2 \times 10^9$ $[H^+]$ s^{-1}) occurring rapidly at lower pHs. The pH dependence on the release of free ATP was directly correlated with the hydrolytic decomposition of the *aci*-nitro intermediate with a rate constant of 220 s^{-1} at pH 7 (see Section II).

The ATP-induced calcium uptake by vesicular dispersions of sarcoplasmic reticulum was investigated by Pierce et al.[40] using *o*-nitro-α-phenylethyl caged ATP. Through photolysis of caged ATP to produce an ATP concentration jump, the authors investigated the Ca^{2+} uptake with a time resolution of 10 ms with laser pulse photolysis enabling them to identify two distinct uptake rates, a fast phase with a rate constant of 64 ± 10 s^{-1} and a slow phase with a rate constant of 0.60 ± 0.90 s^{-1}. The fast phase is associated with formation of phosphorylated enzyme ATPase, whereas the slow phase is associated with the transmembrane Ca^{2+} gradient.

Kaplan et al.[4,41] have investigated the effects of rapid increases of ATP on both the Na^+/K^+ pump[4] and intercellular ATP:ADP exchange reaction[41] through *o*-nitro-α-phenylethyl caged ATP incorporated into, and consequently photolyzed inside, resealed human erythrocyte ghosts. The use of this strategy allowed the enzymatic processes to be initiated in a rapid manner which was independent of the slower diffusion controlled introduction of ATP. It was noted that the photoproduct from the cage, i.e., 2-nitrosoacetophenone, appeared to inhibit the hydrolytic activity of ATPase. This was alleviated by the addition of thiols to the reaction medium.[4]

In addition to caged ATP, caged cyclic monophosphates have been synthesized and studied to explore the role of cyclic mononucleotides as second messengers for neurotransmitters and neurohormones. Nargoet et al.[42] have synthesized the *o*-nitrobenzyl ester of cAMP and deployed it in voltage-clamped atrial trabeculae from bullfrog hearts for concentration jump experiments. Time resolution of the rate of the slow inward current I_{si} is limited to 150 ms by the flash pulse duration. Additional concentration jump experiments, in which cyclic nucleotides cAMP and cGMP were photolytically released, were reported by Nerbonne et al.[43] and Richard et al.[44] These studies inaugurated the use of the 4,5-dimethoxy-2-nitrobenzyl group **47** as



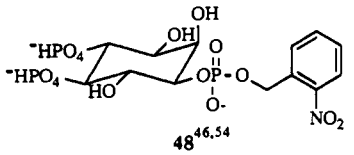
47[43,45]

the caging ligand, which was found to release the cyclic nucleotides at rates on the order of 200 times faster than the parent *o*-nitrobenzyl ester.[43] These investigations focused on the effects of concentration jumps of cAMP and cGMP on the action potential or slow inward calcium current (I_{si}) channels and the development of phasic tension in isolated atrial trabeculae from frog heart. The tension amplitude correlated with Ca^{2+} entry via the slow channels in direct response to production of cAMP consistent with the hypothesis of cAMP mediated slow channel phosphorylation of calciductin. Increased cAMP did not mediate the relaxation of the contractions of frog atrial fibers, however. Thus, the concentration jump of cAMP appears to mimic β-adrenergic stimulation of these fibers.

This same ligand, 4,5-dimethoxy-2-nitrobenzyl **47**, was also used as a caging ligand for the investigation of the gating kinetics of the cGMP-activated cation

channel of salamander retinal rods in excised membrane patches by Karpen et al.[45] A combination of laser flash photolysis of caged cGMP 47 and membrane voltage jump studies on excised membrane patches led to a model for the gating kinetics of the cation channel. The kinetic model assumed three sequential diffusion controlled binding steps for cGMP. The fully ligated channel is rapidly opened or closed by hyperpolarization of the membrane where only the channel closing is activated by the perturbing voltage jump.

The applications of the *o*-nitro-α-phenylethyl group are not limited to just the photolytic release of nucleotides. As reported by Walker et al.,[46,54] this ligand is also capable of the photolytic release of *myo*-inositol 1,4,5-triphosphate (48). The authors determined that



48[46,54]

P-4 and P-5 caged inositol 1,4,5-triphosphates were the most promising biologically inactive caged precursors since even at high concentrations no release of calcium from smooth muscle sarcoplasmic reticulum was observed, effectively demonstrating that no thermal or enzymatic release of the caged inositol phosphates had occurred. Furthermore, these water-soluble caged phosphates should localize within the aqueous compartments of the cell and should not easily cross the cell membrane. The phosphates are useful for time-resolved measurements of muscle contraction and K^+ channel activation in hepatocytes.[47]

By far the most frequent application of both *o*-nitrobenzyl and *o*-nitro-α-phenylethyl caged nucleotides lies in the area of ATP concentration jump experiments on muscle force generation and relaxation. Goldman et al.[47] was the first to study the reaction kinetics of the force-generating mechanism in a skinned single fiber of rabbit skeletal muscle by rapid photolytic release of ATP from caged ATP by laser flash photolysis. He noted that this methodology avoids the poor time resolution inherent in using diffusion to effect concentration changes. Using essentially the same methodology as Goldman's, Ferenczi et al.[48] investigated the kinetics of magnesium ATP (MgATP) cleavage catalysis by calcium-regulated actomyosin in mechanically skinned rabbit muscle fibers. The authors noted that the maximal shortening velocity of the muscle was dependent on the rate of cross-bridge detachment from actin within the actomyosin ATPase cycle. The model was tested and refined by Steinen and Ferenczi[48b] who indicated that different muscle fibers give different values for the rate of slow muscle relaxation. Thus, the process of dissociation of the actomyosin complex may differ from one muscle type to another and even for the fast myosin. Shortly thereafter, Goldman et al.[49] reported additional results of ATP concentration jump experiments in rabbit muscle fibers. They were able to suggest that a correlation existed between the mechanical and biochemical descriptions of the muscle cross-bridge cycle in skinned muscle fibers of rabbit psoas muscle. Additionally, they[50] also reported results on the relaxation of muscle fibers from a rigor state by concentration jumps of ATP from the laser flash photolysis of caged ATP. Hibberd et al.[51] reported results of ATP release in skinned muscle fibers with regard to the presence of inorganic phosphate in the absence of calcium. The authors found that a rapid increase in ATP concentration in muscle fibers initiated cross-bridge cycling. Furthermore, by the addition of inorganic phosphate in millimolar concentrations and in the absence of calcium, relaxation from rigor was accelerated.

An example demonstrating that ATP is not the only nucleoside triphosphate that has been caged and released is by Somlyo et al.[52,56] who were able to use photolabile ATP and CTP to quantify the relaxation accompanying detachment of rigor cross bridges and to investigate the effect of thiophosphorylation with ATPγS on the rates of activation of vascular smooth muscle. Using low-angle X-ray scattering as a probe to ATP-induced relaxation of glycerinated insect flight muscle from the rigor state, Rapp et al.[53] were able to take advantage of the rapid release of ATP from the photolysis of caged ATP. Using this technique, temporal resolutions from 1 to 5 ms were attainable for single events. The results obtained from this study suggested that actively cycling bridges, which are present only shortly after ATP release, are either too few in number to be detected by the diffraction pattern or that their structure is different from that of rigor bridges. Arner et al.[55] studied the mechanical events following the release of ATP from caged ATP in skinned guinea pig taenia coli smooth muscle in a rigor state. They observed no change in the rigor force during diffusion of caged ATP into the muscle preparation; however, as expected, upon photolytic release of ATP, it was observed that the rate of relaxation from rigor was slower than that measured for the ATP-induced dissociation of actomyosin in solution.

Although it would appear that o-nitrobenzyl and *o*-nitro-α-phenylethyl esters of nucleotides are well suited to the types of investigations mentioned above, these ligands are not without their drawbacks. In fact, when one considers caged cAMP and cGMP specifically, Gurney and Lester[33] point out the following three problems associated with these photochemically caged molecules: (1) While hydrolysis of the caged compounds is slow, some base-catalyzed release of free cAMP and cGMP has been observed, which results in a minor but observable change in the cellular physiology prior to photolysis. (2) The photolytic release of the cyclic nucleotide is slow, having a time constant on the order of hundreds of milliseconds. (3) Although the quantum efficiency for release of the cyclic nucleotide is relatively high, cGMP is released with only half the efficiency of cAMP. This latter observation is apparently also found for the 4,5-dimethoxy derivative 47.

V. Conclusions

This review emphasized the role of photochemistry in generating phosphate and phosphate esters by activating a rapid heterolysis of the chromophore–phosphate ester bond. The surprisingly high quantum efficiencies and the large rate constants for the release of phosphates have made this field very attractive for biological applications. The last decade also has seen an increasing interest in developing and refining the use of *o*-nitro-α-phenylethyl as the activating chromophore.

The limitations of a slow release rate and a deleterious nitroso ketone side product have spurred the search

for better cage ligands. Current studies on the benzoin and acetophenone groups as cages promise to open the kinetic "window" by as much as another 3–5 orders of magnitude and to avoid the poisonous side products.

Finally, the development and application of phosphate photochemistry to rapid changes in solution acidity, i.e., "pH jump", generation of unusual and unstable carbocations, initiation of cation polymerization, and kinetic studies on ion pair reactions have yet to be exploited. Phosphate ester photochemistry has an exciting future.

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