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# New Photochemically Labile Protecting Group for Phosphates

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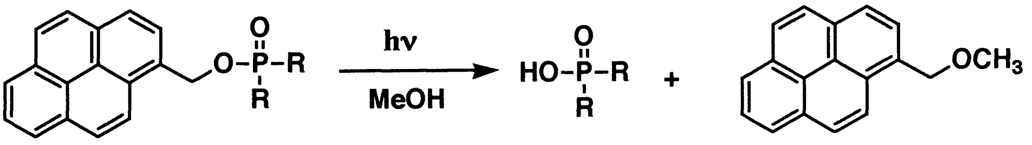
New photochemically labile phosphate protecting group was developed. These phosphate esters have high molar extinction coefficient ($\varepsilon_{340}$ = 34500 dm$^3$ mol$^{-1}$ cm$^{-1}$) and rapidly release parent phosphates upon irradiation (>300 nm) with high quantum efficiency for disappearance ($\phi_{dis}$ = 0.22 at 340 nm).

Much attention has been paid to the development of inert precursors which rapidly release biologically important molecules upon photolysis, enabling the real-time investigation of the cellular processes at the molecular level.[1] Despite the widespread use of substituted *o*-nitrobenzyl derivatives,[2] slow deprotection rate and the concomitant formation of toxic 2'-nitroso phenyl compounds are the disadvantages of these type of protection. Recently, it was reported that benzoin derivatives of cAMP could also be photolyzed easier to give parent cAMP and inert rearranged product, 2-phenylbenzo[b]furan.[3] However, these previously reported photolabile protecting groups have stereogenic centers which resulted in the formation of a racemic mixture. Since biologically important molecules mostly have chirality, introduction of these racemic protecting groups cause the formation of equimolar amount of diastereomer. To avoid these disadvantages, the development of new protecting group is still needed.

Here, we report 1-pyrenylmethyl phosphates as new photolabile precursors which can rapidly release parent phosphates upon irradiation (>300 nm). These phosphates show higher reactivity for photorelease reaction compared with previously reported ones.[4]

Introduction of 1-pyrenylmethyl group into phosphates are achieved by a recently developed in situ activation method.[5] Diethyl 1-pyrenylmethyl phosphate (**1a**)[6] thus synthesized was readily photolyzed to give diethyl phosphate (**2a**) and methyl 1-pyrenylmethyl ether (**3**)[7]quantitatively when it was irradiated in methanol ($10^{-4}$ mol dm$^{-3}$) using 100W high pressure mercury lamp through a Pyrex filter under Argon. Similarly dibenzyl 1-pyrenylmethyl phosphate (**1b**) was completely photolyzed within 60 min to give dibenzyl phosphate (**2b**) and methyl ether (**3**) quantitatively.



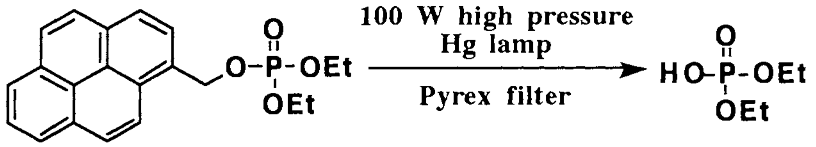
**1a** : R=OEt
**1b** : R=OBn

**2a** : R=OEt
**2b** : R=OBn

**3**

Photodeprotection of **1a** could also be achieved in various solvents containing two equivalents of methanol. The results are summarized in Table 1. All photolytic reactions were carried out by irradiation with a Pyrex filtered 100 W high pressure Hg lamp for 2 h. Both conversion and yield of **2a** were determined by ${}^1H$ NMR measurement of reaction mixture. As shown in Table 1, similar yields of **2a** were obtained in any kind of the solvents we investigated. In these cases, several side products from 1-pyrenylmethyl moiety were obtained, however, the yield of diethyl phosphate (**2a**) was quantitative.

Table 1. Photolysis of **1a** in various solvents

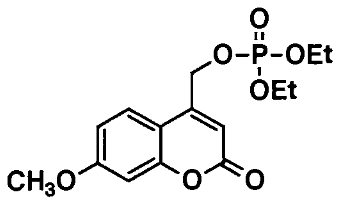


**1a** **2a**

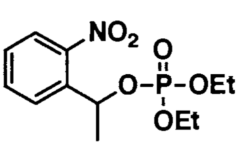
| Entry | Solvent[a)] | Conversion/% | Yield of **2a**/% |
|---|---|---|---|
| 1 | THF | 58 | 96 |
| 2 | Benzene | 56 | 100 |
| 3 | $CH_3CN$ | 57 | 96 |
| 4 | 1,4-Dioxane | 70 | 99 |

a) Contained methanol (2 equivalent of **1a**).

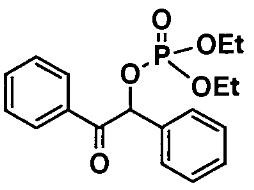
The efficiency of photodeprotection of **1a** was compared with those of previously reported photolabile phosphates, **4**[8)], **5**[9)], and **6**[8)]. To apply these protecting groups to physiological investigations, longer wavelength (>300 nm) of irradiation and lower initial concentration of precursors should be recommended. Thus each phosphate was irradiated at 340 nm (500 W Xe lamp) in degassed methanol ($10^{-4}$ mol $dm^{-3}$) and the progress of reaction was monitored by reversed phase HPLC (Lichrosorb RP-18, 80% $MeOH-H_2O$ as an eluent). The concentration of the unreacted phosphates as a function of time is shown in Fig. 1. Clearly, our new protecting group was much more efficiently photolyzed under these conditions. The reactivity of photorelease reaction may be evaluated by both quantum efficiency for disappearance ($\phi_{dis}$) and molar extinction coefficient ($\varepsilon$) of phosphate ester.[10)] The quantum efficiency for disappearance ($\phi_{dis}$) of **1a** was determined at 340 nm irradiation in MeOH using potassium ferrioxalate actinometry. Our new protecting group has relatively high $\phi_{dis}$ value (0.22) and high $\varepsilon$ value ($\varepsilon_{340}$=34500 $dm^3$ $mol^{-1}$ $cm^{-1}$) compared with **4** ($\phi_{dis}$= 0.038[11)], $\varepsilon_{340}$=8800 $dm^3$ $mol^{-1}$ $cm^{-1}$), **5** ($\varepsilon_{340}$=440 $dm^3$ $mol^{-1}$ $cm^{-1}$) and **6** ($\phi_{dis}$= 0.28[12)], $\varepsilon_{340}$=32 $dm^3$ $mol^{-1}$ $cm^{-1}$). Although each phosphate should have its most suitable irradiation wavelength, it was demonstrated that at longer wavelength (>300 nm) 1-pyrenylmethyl phosphate derivatives (**1**) showed higher reactivity than **4**, **5** and **6**.[13)]



**4**



**5**



**6**

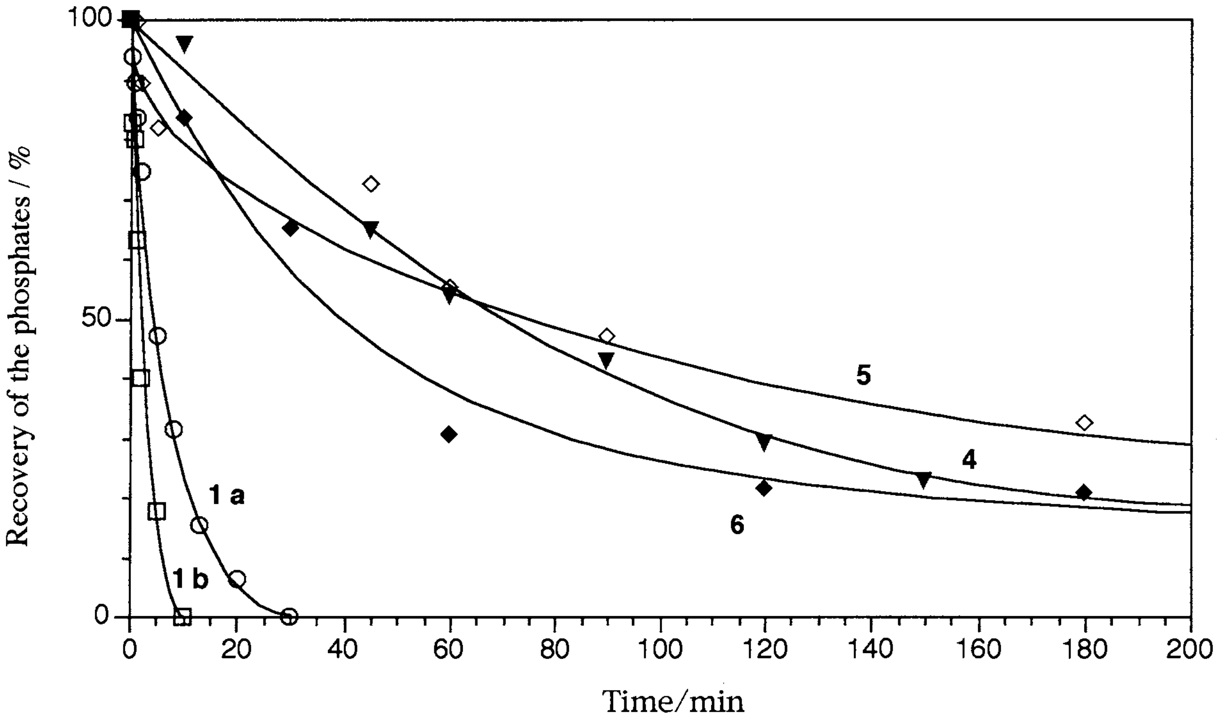


Fig. 1. Photolytic consumption of various phosphates by irradiation in MeOH at 340 nm.

In conclusion, we developed a new photochemically labile phosphate protecting group, 1-pyrenylmethyl group, which has both high $\phi_{dis}$ value and high $\varepsilon$ value at 340 nm, and can be introduced without the generation of new stereogenic center.

The toxicity of **3** and related 1-pyrenylmethanol are currently under investigation.

References

1) For reviews see : J. F. Wootton and D. R. Trentham, "Photochemical Probes in Biochemistry," ed by P. E. Nielsen, Kluwer Academic Publishers, Dordrecht (1988), p. 277; A. M. Gurney and H. A. Lester, *Physiol. Rev.*, **67**, 583 (1987).

2) J. F. Walker, G. P. Reid, J. A. McCray, and D. R. Trentham, *J. Am. Chem. Soc.*, **110**, 7170 (1988) and references cited therein.

3) R. S. Givens, P. S. Athey, L. W. Kueper III, B. Matuszewski, and J.-Y. Xue, *J. Am. Chem. Soc.*, **114**, 8708 (1992).

4) For a recent review of the photochemistry of phosphate esters see R. S. Givens and L. W. Kueper, III, *Chem. Rev.*, **93**, 55 (1993).

5) T. Furuta, H. Torigai, T. Osawa, and M. Iwamura, *submitted.*

6) Phosphate **1a** was prepared as follows; to a stirred solution of diethyl phosphate (0.340 g, 2.2 mmol) and 1-pyrenylmethyl chloride (0.260 g, 1.00 mmol) in acetonitrile (3 mL) was added silver oxide (0.241 g, 1.00 mmol) and the resulting black suspension was vigorously stirred for 3.5 hours at room temperature. The reaction was quenched by filtration of excess silver oxide and insoluble precipitate. The filtrate was evaporated and the residual oil was dissolved in dichloromethane. Washing with water, drying over $MgSO_4$, and concentration in vacuo gave 0.350 g (0.951 mmol, 92% yield) of **1 a** as an oil. $^1$H NMR ($CDCl_3$) $\delta$=8.37 (1H, d, J=9.0 Hz), 8.22-8.0 (8H, m), 5.79 (2H, d, J=8.0 Hz), 4.06 (4H, dq, J=7.0, and 8.0 Hz), 1.24 (6H, dt, J=1.0 and 8.0 Hz). IR (film) ν=3042, 2986, 1265, 1007, 980.

7) $^1$H NMR ($CDCl_3$) $\delta$=8.32 (1H, d, J=9.0 Hz), 8.16-8.10 (4H, m), 8.02-7.96 (4H, m), 5.13 (2H, s), 3.50 (3H, s).

8) R. S. Givens and B. Matuszewski, *J. Am. Chem. Soc.*, **106**, 6860 (1984). We synthesized these esters by our method. Condensation of diethylphosphate and bromomethyl 7-methoxycoumarin in the presence of silver (I) oxide gave **4**. $^1$H NMR ($CDCl_3$) $\delta$=7.40 (1H, d, J=9.5 Hz), 6.87 (1H, dd, J=9.5, and 2.5 Hz), 6.86 (1H, d, J=2.5 Hz), 6.43 (1H, t, J=1.5 Hz), 5.22 (2H, dd, J=7.0, and 1.5 Hz), 4.20 (4H, dq, J=7.0, and 7.0 Hz), 3.88 (3H, s), 1.37 (6H, dt, J=1.1, and 7.0 Hz). IR (film) ν=2988, 2922, 1727, 1618, 1267, 855. Condensation of benzoin and diethyl phosphoryl chloride in the presence of silver (I) oxide gave **6**. $^1$H NMR ($CDCl_3$) $\delta$=7.92 (2H, m), 7.52-7.48 (3H, m), 7.42-7.35 (5H, m), 6.63 (1H, d, J=8.0 Hz), 4.19 (2H, dq, J=7.0, and 7.0 Hz), 3.91 (2H, dq, J=7.0, and 7.0 Hz), 1.32 (3H, dt, J=1.0, and 7.0 Hz), 1.14 (3H, dt, J=1.0, and 7.0 Hz).

9) $^1$H NMR ($CDCl_3$) $\delta$=7.98 (1H, dd, J=8.0, and 1.5 Hz), 7.83 (1H, dd, J=8.0, and 1.5 Hz), 7.68 (1H, ddd, J= 8.0, 8.0, and 1.5 Hz), 7.47 (1H, ddd, J=8.0, 8.0, and 1.5 Hz), 6.04 (1H, dq, J=5.0, and 5.0 Hz), 4.08-4.00 (4H, m), 1.71 (3H, d, J=6.0 Hz), 1.27 (3H, dt, J=1.0, and 7.0 Hz), 1.23 (3H, dt, J=1.0, and 7.0 Hz). IR (film) ν=2990, 1531, 1350, 1270, 1033, 980.

10) J. E. T. Corrie and D. R. Trentham, *J. Chem. Soc., Perkin Trans. 1*, **1992**, 2409. In this article, the efficiency of photolabile phosphate was discussed from the extinction coefficient and the quantum efficiency of deprotection.

11) In MeOH at 350 nm, see Ref 8).

12) In benzene at 350 nm, see Ref 8).

13) The photolytic conversion of phosphates were also determined at 358 nm irradiation. The recovery of the phosphates after 10 min irradiation are as follows; **1a**: 24%, **5**: 90%.

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