Pergamon

Tetrahedron Vol. 51, No. 4, pp. 1077-1086, 1995

0040-4020/95 $9.50+0.00

0040-4020(94)01012-9

Phosphomonoesters and Phosphodiesters Derived From the Photohydrolysis of 2-Methoxy-5-Nitrophenyl Substituted Phosphotriesters

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Abstract: Phosphotriesters composed of one or two 2-methoxy-5-nitrophenyl group(s) can be quantitatively photohydrolyzed in aqueous acetonitrile to yield the desired phosphodiester or phosphomonoester, respectively. Photohydrolysis occurs by attack of hydroxide at both the phosphoryl phosphorus and at the *ipso*-carbon in the triplet exited state of the 2-methoxy-5-nitrophenyl substituted phosphoesters. The photophysical studies described within imply that this type of reaction may be synthetically useful.

INTRODUCTION

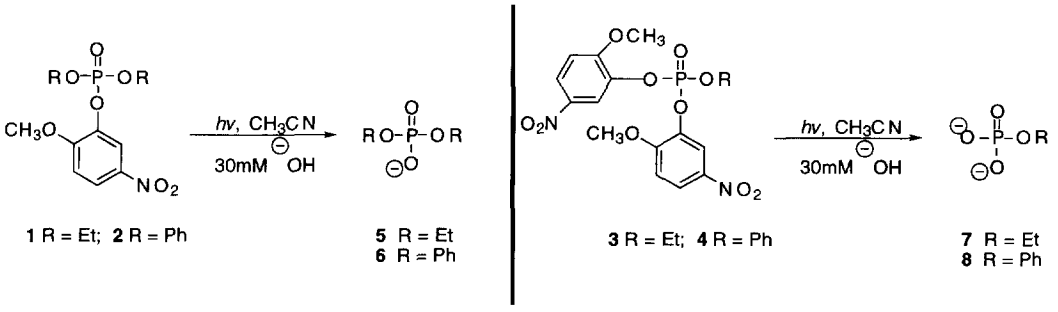
The synthesis of compounds bearing a phosphomonoester functionality is difficult in cases where that functional group has to be carried through several synthetic steps protected as a phosphotriester.[1] The decidedly different reactivities of phosphotriesters and phosphodiesters towards hydrolysis makes the choice of the alcohol-derived component of phosphotriesters perplexing, as both the triester and diester must be formally hydrolyzed to afford the desired monoester.[2] The photophysical studies outlined below demonstrate that phosphoester linkages derived from 2-methoxy-5-nitrophenol can be rapidly and quantitatively photohydrolyzed affording the desired phosphomonoester using an inexpensive photoreactor. Phosphodiesters can also be prepared by photohydrolyzing triesters having a single 2-methoxy-5-nitrophenyl ester group. The mechanistic studies described within provide insight into the photohydrolysis of the arylphosphoester bond. These mechanistic studies also demonstrate that the rates of 2-methoxy-5-nitrophenyl phosphoester bond cleavage in phosphotriesters and phosphodiesters are very similar in the excited state, illustrating that 2-methoxy-5-nitrophenyl esters can be rapidly and quantitatively photohydrolyzed affording phosphomonoesters. Examples of the synthetic utility of 2-methoxy-5-nitrophenyl substituted phosphotriesters are included within. However, this was not the major focus of this study and as a result only a few examples are provided.

The light sensitivity of nitrophenyl phosphates and sulfates was noted in 1956 when the *meta*-isomer was shown to undergo photohydrolysis 34 times faster than the *ortho*- or *para*-isomers.[4] Havinga rationalized these results by proposing that the carbon atom *meta* to the nitro group becomes electron deficient in the excited state and therefore is susceptible to nucleophilic attack by hydroxide. Later, Kirby and Varvoglis demonstrated the generality of the "*meta*-nitro effect" by showing that 3,5-dinitrophenyl phosphate was stable for many months in the absence of light but hydrolyzed with a half life of 5 min when irradiated.[5] An understanding of the mechanism of these reactions was obtained through

the careful studies of Zimmerman, Havinga, Marquet, Varma and their colleagues.[6] The mechanism of the photoinduced reaction between 4-nitroveratrole and hydroxide demonstrates that hydroxide only reacts with 4-nitroveratrole in its lowest triplet state via the formation of a transient σ-complex at C2. The σ-complex decays with a measurable rate constant (laser kinetic spectroscopy) to form 2-methoxy-5-nitrophenol with a triplet quantum yield of 0.31.[6b] We attempt within to take advantage of the known stability of phenylphosphotriesters and the current mechanistic understanding of the photohydrolysis of 4-nitroveratrole to create phosphotriesters that are relatively stable to a variety of reaction conditions, yet can be photohydrolyzed to afford the desired phosphomono- or diesters.

RESULTS AND DISCUSSION

We have prepared and evaluated phosphotriesters **1** - **4**, composed of one or two 2-methoxy-5-nitrophenyl ester groups, to demonstrate their applicability for the synthesis of alkyl- and aryl-phosphodiesters or phosphomonoesters, respectively.



Scheme 1.

The time course of the photohydrolysis of **1** and **3** shown in Figure 1 are representative of the photohydrolysis of triesters **2** and **4**, respectively (data not shown). Photohydrolysis reactions were carried out in basic aqueous acetonitrile employing an inexpensive Rayonet mini reactor fitted with four 300 nm bulbs. The photohydrolysis of **1** affording **5** was complete in less than 4 min, was pseudo-first-order with a rate constant of 0.051 ± 0.002 s^{-1} and was 780 times faster than the corresponding ground state hydrolysis (Figure 1). The photohydrolysis of **3** affording **7** was complete in less than 5 min and was pseudo-first-order with an apparent rate constant of 0.017 ± 0.002 s^{-1}, suggesting that the photohydrolysis rates of the 2-methoxy-5-nitrophenyl ester bonds in triesters and diesters are similar. This was verified by independent photohydrolysis of 2-methoxy-5-nitrophenyl ethyl phosphate, derived from the ground state hydrolysis of **3**, which exhibited a photohydrolysis rate of 0.017 ± 0.001 s^{-1}. The rapid and quantitative photohydrolysis of **3** and **4** affords a phosphomonoester, a reaction that is not observed in the ground state owing to the poor reactivity of the phosphodiester towards hydrolysis.[2,7] The ready photohydrolysis of 2-methoxy-5-nitrophenylphosphoester bonds in quantitative yield makes it possible to make phosphodiesters or phosphomonoesters by incorporating either one or two 2-methoxy-5-nitrophenyl group(s) in the precursor phosphotriester, respectively. The resulting phosphodiesters **5** and **6** as well as monoesters **7** and **8** were characterized by their ^{31}P NMR spectrum, both before and after the standard addition of authentic product, which was prepared by hydrolysis of the corresponding phosphorochloridate.

Phosphotriesters derived from 2-methoxy-5-nitrophenol are amenable to photohydrolysis because 3-nitrophenols are known to have a switchable pK_a. These nitrophenols have a relatively high

ground state pK_a and a low photochemically excited state pK_a, which confers reactivity by making the phenoxide a good leaving group.[8] Upon photohydrolysis at wavelengths > 300 nm, 3-nitrophenols become electron deficient at

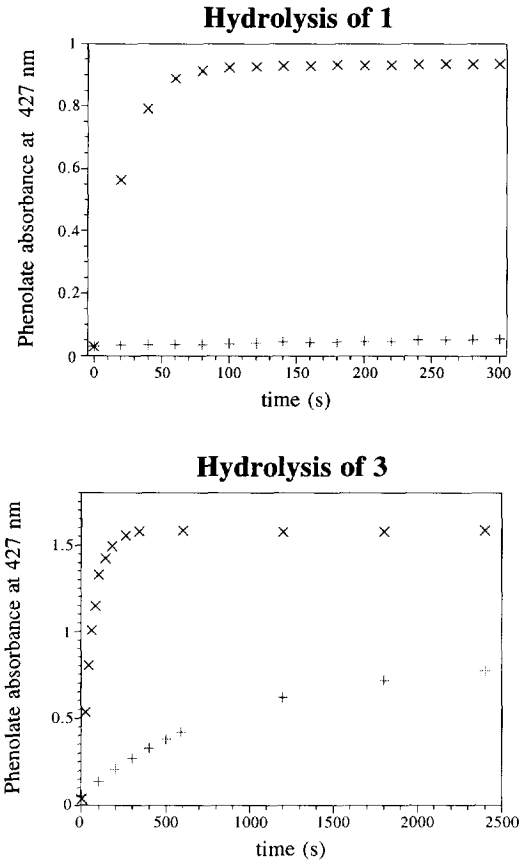


Fig. 1. Comparisons of the time courses of the hydroxide mediated phosphotriester hydrolysis in the ground state and excited state. The photohydrolysis of **1** and **3** are indicated by (x) in each time course. The ground state hydrolyses are indicated by (+) in each time course. Conditions were identical to those described for the photohydrolysis except for the absence of UV light. The ground state hydrolysis of **3** affords the diester which does not hydrolyze further, even after several days.

C1 due to the electron redistribution in the triplet excited state, lowering their pKa by 1-2 units.[8] MNDO calculations on the triplet state indicate that the atomic charge on C1 is + 0.288, which agrees with earlier results of Zimmerman on similar systems.[6a] This electron distribution facilitates hydroxyl attack at either C1 to form a σ-intermediate or at the phosphoryl group, which would mediate cleavage of the P-O bond and place an oxyanion adjacent to C1.[6] Photohydrolysis with isotopically enriched water allows these two mechanisms to be distinguished. Literature precedent suggests that photo-assisted

hydrolysis usually occurs by hydroxyl attack at C1 to produce a σ-intermediate (Figure 2).[6] Phosphorus-31 NMR analysis of the resulting photohydrolysis of **1** in a 2:3 mixture of $H^{18}O^-$: $H^{16}O^-$ shows that only 15% of the label (^{18}O) ends up in the resulting phosphodiester **5**.[7] This implies that 63% of the hydroxyl attack took place at C1 via the formation of a σ-intermediate (Figure 2, pathway 1) while the remainder of the hydrolysis reaction (37%) proceeded via attack at phosphorus (Figure 1, pathway 2). By mass spectrometry, 19% of the 2-methoxy-5-nitrophenol was found to have ^{18}O incorporated (taking into account the 1.1% expected for natural abundance of ^{18}O). The phenol ends up with 48% of the available ^{18}O label indicating that approximately half of the hydrolysis reaction takes place by pathway 1, Figure 2, which is less than the 63% expected based on the ^{31}P NMR data.[7] Because these data were reproducible in triplicate, an average of the results from mass spectrometry and NMR spectrometry will be used to draw conclusions about the mechanism of photohydrolysis. These data clearly demonstrate that the hydrolysis proceeds by two parallel mechanisms, i.e., by hydroxide attack at C1 (pathway 1 ≈ 56%) and by direct hydroxide attack on the phosphoryl group (pathway 2 ≈ 44%), Figure 2. Photohydrolysis of **1** in the presence of the known triplet state quencher potassium sorbate (4.25 mM) was four times slower, demonstrating the importance of the triplet excited state in this reaction.[6b] This observation is consistent with the rate limiting step being hydroxyl attack on the triplet state of 2-methoxy-5-nitrophenyl substituted phosphoester.[6b]

The stability of phosphotriesters **3** and **4** towards 50% trifluoroacetic acid (45 min) and towards a solution of primary and tertiary amines suggests that the 2-methoxy-5-nitrophenyl-based phosphotriesters may be quite useful for protecting the phosphoester functionality from side reactions during certain chemical reactions. The facile photohydrolysis chemistry of triesters to afford diesters (e.g. **5** and **6**) or monoesters (e.g. **7** and **8**), coupled with the stability of the phosphotriesters to non-aqueous acids and bases suggests that this approach may be useful in preparative chemistry. Further studies are required to evaluate the synthetic scope of this reaction.

Phosphotriesters composed of 2-methoxy-5-nitrophenol can also be prepared using the phosphoramidite chemistry developed by Caruthers where the alcohol of choice is converted into a phosphite by reaction with N,N-diisopropyl-bis-(2-methoxy-5-nitrophenyl)phosphoramidite in the presence of the N-methylanilinium trifluoroacetate catalyst.[9] Oxidation of the phosphite to the phosphotriester is accomplished with *m*-CPBA.

EXPERIMENTAL SECTION

General procedures

^1H and ^{13}C NMR spectra were recorded on a Varian XL-200E NMR spectrometer. ^{31}P NMR spectra were obtained on a Varian XL-200 or a Varian XL-400 spectrometer, using 85% H_3PO_4 as an external standard. Ultraviolet absorption spectra were acquired on a Milton Roy Spectronic 3000 diode array instrument. Photohydrolyses of phosphotriesters **1-4** were carried out in a Rayonet RMR-500 mini reactor fitted with four 300 nm UV bulbs. High resolution mass determinations were carried out on a VG-70S double focusing high resolution mass spectrometer. The melting points were obtained on a Mel-Temp apparatus in open capillary tubes and are uncorrected.

Synthesis of 2-methoxy-5-nitrophenol

The synthesis of 2-methoxy-5-nitrophenol was initiated by dissolving 4-nitroveratrole (3.75 mmol, 0.69 g) in acetonitrile (125 mL) to which 150 mL of 0.25 M NaOH was added. The reaction mixture was stirred under UV irradiation utilizing a 125 W Hg high pressure lamp enclosed in a Pyrex filter for 10-15 h in an Ace Hanovia Photochemical reactor. The solvent was evaporated under vacuum,

the residue was dissolved in 1N NaOH and extracted with ether. The aqueous layer was acidified to pH 2 with 50% HCl and extracted with ether. The ethereal layer was dried ($MgSO_4$) and the solvent removed under reduced pressure to yield a yellow solid. The residue was partially dissolved in 60 : 40, hexanes : ethyl acetate, filtered to remove insoluble

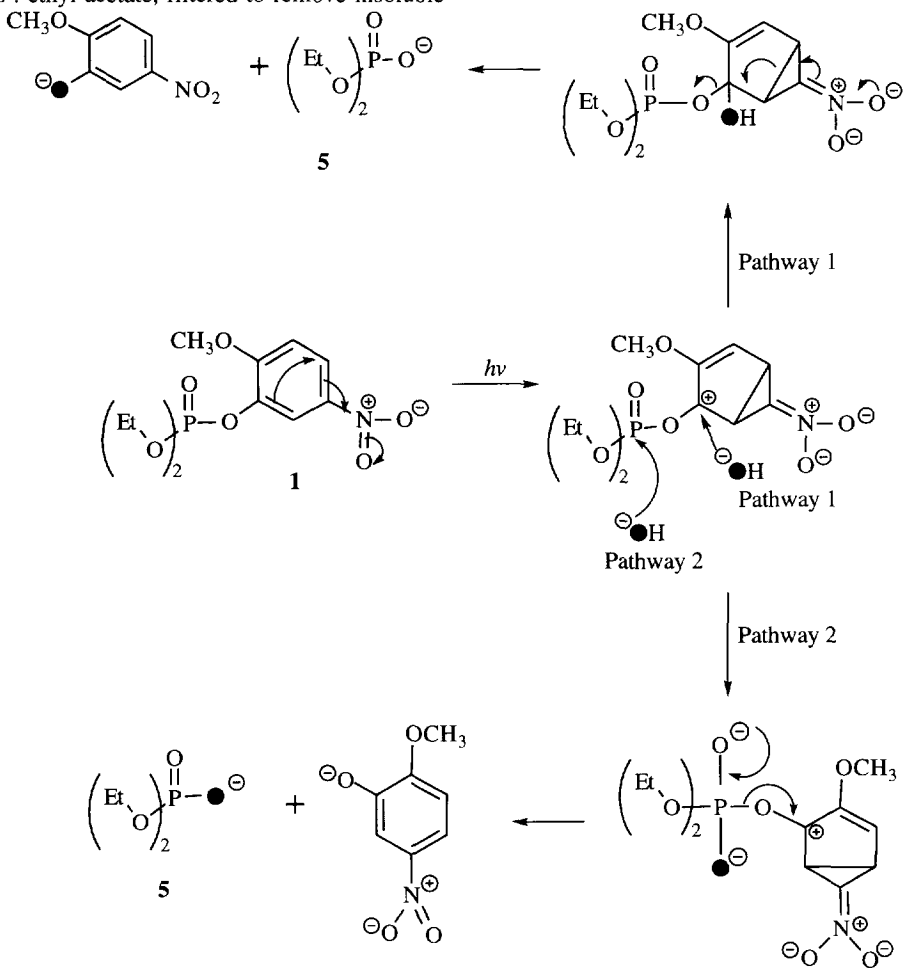


Fig. 2. The mechanisms of photohydrolysis shown above are supported by the $H^{18}O^-$ photohydrolysis studies.

impurities, and concentrated to afford a yellow solid (77.8% yield); mp 101-103°C (lit. mp 105°C)[10]; ^1H NMR ($CDCl_3$) δ 4.02 (s, 3H, OCH_3), 5.86 (s, 1H, OH), 6.91 (d, J=8.9 Hz, 1H, C3-H), 7.80 (d, J=2.7 Hz, 1H, C6-H), 7.85 (dd, J_o=8.9 Hz, J_m=2.7 Hz, 1H, C4-H); ^{13}C NMR ($CDCl_3$) δ 56.5 (OCH_3), 109.5, 110.1, 116.9, 141.9, 145.5, 151.8, Ar.

*General Procedure for the Synthesis of Phosphotriesters (**1** - **4**)*

Triethylamine (1.1 eq) was added dropwise to a solution of 2-methoxy-5-nitrophenol (1 eq) and the corresponding commercially available dialkyl (diaryl)phosphoric chloride (1 eq) or alkyl (aryl)phosphoric dichloride (0.5 eq) in dry THF at -78°C. The reaction was allowed to warm to room temperature and was then stirred overnight under N_2. The salts were removed by filtration and the solvent removed under reduced pressure. The residue was dissolved in Et_2O and extracted with aqueous 2% Na_2CO_3 (3X). The Et_2O was removed under reduced pressure affording the desired phosphate in 35-70% yield.[11]

*Synthesis of diethyl 2-methoxy-5-nitrophenyl phosphate (**1**)*

2-methoxy-5-nitrophenol and diethylphosphoric chloride were combined as described above to afford crude **1** (65% yield); mp 57-59°C; ^{1}H NMR ($CDCl_3$) δ 1.40 (td, J=7.4 Hz, $J_{H\text{-}P}$=1.8 Hz, 6H, -CH_3), 3.97 (s, 3H, -OCH_3), 4.29 (dq, $J_{H\text{-}P}$=9.3 Hz, J=7.4 Hz, 4H, -OCH_2-), 7.01 (dd, J_o=9.3 Hz, $J_{H\text{-}P}$=0.9 Hz, 1H, C3-H), 8.10 (m, J_o=9.3 Hz, J_m=3.0 Hz, $J_{H\text{-}P}$=1.5 Hz, 1H, C4-H), 8.15 (dd, J_m=2.9 Hz, $J_{H\text{-}P}$=1.5 Hz, 1H, C6-H); ^{13}C NMR ($CDCl_3$) δ 16.0 (d, $J_{C\text{-}P}$=6.7 Hz, -CH_3), 56.5 (OCH_3), 65.4 (d, $J_{C\text{-}P}$=6.7 Hz, -CH_2-), 111.6, 117.3 (d, $J_{C\text{-}P}$=2.3 Hz), 122.3, 142.1, 144.7, 152.1 Ar; ^{31}P ($CDCl_3$) δ –5.691. High-resolution mass calcd for $C_{11}H_{16}O_7NP$ 305.0664, found 305.0672. Kinetic analyses were carried out on **1** further purified by C-18 reverse phase HPLC employing an acetonitrile : water gradient starting at 20% B and ending at 80% B over 25 min. (A solvent is 95:5 H_2O : CH_3CN, B solvent is 95:5 CH_3CN : H_2O).

*Synthesis of diphenyl 2-methoxy-5-nitrophenyl phosphate(**2**)*

2-methoxy-5-nitrophenol and diphenylphosphoric chloride were combined as described above to afford crude **2**. The resulting dark oil was recrystallized from ethanol/water to afford a beige solid (60% yield based on starting phenol); mp 67-69°C; ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H, OCH_3), 6.98 (d, J_o=10.0 Hz, 1H, C3-H), 7.33 (m, 10H, phenyl groups), 8.00 (d, J_m=0.8 Hz, 1H, C6-H), 8.10 (dd, J_o=10.0 Hz, J_m=2.0 Hz, 1H, C4-H); ^{13}C NMR ($CDCl_3$) δ 56.5 (OCH_3), 117.5, 120.1 (d, $J_{C\text{-}P}$=5.0 Hz), 122.7, 125.8, 129.9, 139.3 (d, $J_{C\text{-}P}$=7.3 Hz), 140.8, 150.3, 156.2 (d, $J_{C\text{-}P}$=5.0 Hz) Ar; ^{31}P NMR ($CDCl_3$) δ -17.47; high-resolution mass calcd for $C_{19}H_{16}O_7NP$ 401.0664, found 401.0658.

*Synthesis of ethyl bis(2-methoxy-5-nitrophenyl) phosphate (**3**)*

2-methoxy-5-nitrophenol and ethylphosphoric dichloride were combined as described above to afford crude **3**. The reaction yielded a beige solid 35% yield; mp 120-122°C; ^{1}H NMR ($CDCl_3$) δ 1.43 (td, J=7.1 Hz, $J_{H\text{-}P}$=1.2 Hz, 3H, -CH_3), 3.95 (s, 6H, -OCH_3), 4.43 (dq, $J_{H\text{-}P}$=8.7 Hz, J=7.1 Hz, 2H, -OCH_2-), 7.04 (dd, J_o=9.2 Hz, $J_{H\text{-}P}$=0.9 Hz, 2H, C3-H), 8.08 (m, J_o=9.2 Hz, J_m=2.8 Hz, $J_{H\text{-}P}$=1.1 Hz, 2H, C4-H), 8.22 (dd, J_m=2.7 Hz, $J_{H\text{-}P}$=1.4 Hz, 2H, C6-H); ^{13}C NMR ($CDCl_3$) δ 15.9 (d, J=6.6 Hz, -CH_3), 56.6 (OCH_3), 66.4 (d, $J_{C\text{-}P}$=6.3 Hz, -CH_2-), 111.6, 117.1 (d, $J_{C\text{-}P}$=2.9 Hz), 122.4, 138.8 (d, $J_{C\text{-}P}$=7.3 Hz), 140.5, 156.0 (d, $J_{C\text{-}P}$=5.3 Hz) Ar; ^{31}P ($CDCl_3$) δ –11.02; high-resolution mass calcd for $C_{16}H_{17}O_{10}N_2P$ 428.0621, found 428.0652. Kinetic analyses were done on HPLC purified **3** using the gradient described for the purification of **1**.

Synthesis of phenyl bis(2-methoxy-5-nitrophenyl) phosphate (4)

2-methoxy-5-nitrophenol and phenylphosphoric dichloride were combined as described above to afford crude **4**. Flash chromatography ($CHCl_3$: CH_3OH : CH_3COOH, 98 : 1 : 1) afforded 0.735 g of product as a yellow solid (72% yield based on phosphorylating reagent); mp 108°C; ^1H NMR ($CDCl_3$) δ 3.90 (s, 6H, OCH_3), 7.05 (d, J_o=10.0 Hz, 2H, C3-H), 7.38 (m, 5H, phenyl group), 8.15 (d, J_m=0.8 Hz, 2H, C6-H), 8.25 (dd, J_o=10.0 Hz, J_m=2.0 Hz, 2H, C4-H); ^{13}C NMR ($CDCl_3$) δ 56.7 (OCH_3), 111.8, 117.5 (d, $J_{C\text{-}P}$=3.4 Hz), 120.1 (d, $J_{C\text{-}P}$=5.0 Hz), 122.9, 126.0, 129.9, 138.9 (d, $J_{C\text{-}P}$=7.3 Hz), 140.8, 156.1 (d, $J_{C\text{-}P}$=5.0 Hz) Ar; ^{31}P NMR ($CDCl_3$) δ -17.29. High resolution mass calcd for $C_{20}H_{17}O_{10}N_2P$ 476.0621, found 476.0606.

Stability tests of ethyl bis(2-methoxy-5-nitrophenyl) phosphate (3)

(A) *Stability towards a mixture of primary and tertiary amines and towards an anhydride.* Dry CH_2Cl_2 was added dropwise until N-Boc-Ala (0.026 g, 0.138 mmol) completely dissolved. *N,N*- diisopropylcarbodiimide (10.8 μL, 0.069 mmol) was added at 0°C under N_2 and the reaction was left standing for 1 h. Phosphotriester **3** (12 mg, 0.028 mmol) was dissolved in dry CH_2Cl_2 (0.5 mL) and a ^{31}P NMR was recorded by placing the sample in a 5 mm tube and inserting it into a 10 mm tube containing $CDCl_3$. The preformed symmetrical anhydride, DIEA (4 μL, 0.023 mmol) and isobutylamine (2.3 μL, 0.023 mmol) were added to the 5 mm NMR tube under N_2 and an NMR spectrum was recorded. There was no change in the ^{31}P chemical shift of **3** (δ -11.73). Excess isobutylamine (10 μL, 0.1 mmol) was added and the solution was left standing for 24 h before another NMR spectrum was recorded. No new ^{31}P peaks resulting from reaction or degradation of the phosphotriester were observed.

(B) *Stability towards trifluoroacetic acid.* Phosphotriester **3** (12 mg, 0.028 mmol) was dissolved in 0.8 mL of dry CH_2Cl_2 and a ^{31}P NMR spectrum was recorded (δ -11.02). TFA (0.27 mL) was added to make the solution 25% in TFA. After 45 min another NMR was recorded with no detectable decomposition. Additional TFA (0.53 mL) was added to make the solution 50% in TFA and after an additional 45 min another ^{31}P NMR spectrum was recorded (δ -11.81 ppm). No degradation of the phosphotriester was observed.

Photohydrolyses of phosphotriesters (1 - 4)

Photohydrolyses were performed at room temperature using a 0.206 mM solution of phosphotriester in 70 : 30 , H_2O : CH_3CN which was 30 mM in NaOH. The samples were irradiated for 20 s and absorbance readings were taken at 427 nm (nitrophenolate absorbance). This process was repeated as necessary to get the data shown in Figure 1, which afforded rate constants from computing the average of the rates obtained from three different time courses. The product compounds **5**, **6**, **7** & **8** were characterized by ^{31}P NMR spectroscopy. The majority of the reaction solvent was evaporated to a final volume of 1 mL which was transferred to a 5 mm tube. The 5 mm NMR tube was then inserted inside a 10 mm tube containing the lock solvent ($CDCl_3$) and the ^{31}P NMR spectrum was recorded. Identification of the product was confirmed by the addition of authentic compound. Product standards were prepared by addition of H_2O and NaOH to: diethylphosphoric chloride, diphenylphosphoric chloride, ethylphosphoric dichloride and phenylphosphoric dichloride to obtain **5**, **6**, **7** and **8**, respectively. A single peak was observed in the ^{31}P NMR spectrum when an equimolar amount of the product standard was added to the appropriate photohydrolysis product: **5**, δ 0.77; **6**, δ 6.43; **7**, δ 3.97; **8**, δ 1.06 relative to external 85% phosphoric acid.

Photohydrolysis of diethyl 2-methoxy-5-nitrophenyl phosphate (1) in the presence of potassium sorbate

Potassium sorbate was prepared from sorbic acid and KOH. Phosphotriester **1** (0.21 mM, in 70 : 30, H_2O : CH_3CN) was photohydrolyzed in the presence of potassium sorbate (4.25 mM) and NaOH (5 mM). The reaction was followed by reading the nitrophenolate absorbance at 427 nm after every 20 s of irradiation. Data from three time courses were averaged to get the rate constant. Three time courses without the sorbate quencher present were recorded as controls to discern the rate difference.

Photohydrolysis of diethyl 2-methoxy-5-nitrophenyl phosphate (1) in $H_2{}^{18}O$ / $H_2{}^{16}O$

Phosphotriester **1** (0.01742 g, 0.057 mmol) was dissolved in dry acetonitrile (5 mL) to prepare a standard solution of the triester. To an NMR tube were added 232 μL of the standard phosphotriester solution, 125 μL of a 2 : 3 mixture of $H_2{}^{18}O$: $H_2{}^{16}O$ (established by mass spectrometry) and n-BuLi (11 μL, 0.15 mmol). The solution was photohydrolyzed for 30 min. Acetonitrile (300 μL) was added as a lock solvent along with 2.23 μL of TMEDA to improve the resolution of the spectrum. ^{31}P NMR analysis showed two resonances (CD_3CN) δ -1.03, -1.06 in a 15 : 85 ratio, with the downfield resonance corresponding to the ^{18}O-labeled phosphate.[7] The NMR sample was added to Et_2O followed by addition of an acetate buffer (pH 6.2). The Et_2O layer containing the phenol was dried, concentrated and analyzed by electron impact mass spectrometry, MS (EI) m/z (relative intensity) 169.02 (100), 171.03 (24.3). The ground state hydrolysis of phosphotriester **1** was accomplished with a sample protected from light using 232 μL of the standard solution of phosphotriester **1**, 125 μL of a 2:3 mixture of $H_2{}^{18}O/H_2{}^{16}O$ and n-BuLi (11 μL, 0.15 mmol). ^{31}P NMR analysis showed a resonance at (CD_3CN) δ -1.10, -1.07, with relative intensities of 37 to 63 respectively which is expected if ^{-}OH attack at the P=O group had occurred.

Photohydrolysis of ethyl 2-methoxy-5-nitrophenyl phosphate (9)

A solution of **3** (0.206 mM) in 70 : 30 , H_2O : CH_3CN and 30 mM NaOH was prepared and protected from light. Absorbance readings were taken at 427 nm every 10 s for 10 min and subsequently every 5 min for 24 h. At this stage only one of the two 2-methoxy-5-nitrophenyl ester bonds were cleaved as determined by the absorbance spectrum of the displaced phenolate. The second protecting group was cleaved by photohydrolysis of the diester (**9**) employing the conditions described for the photohydrolyses of phosphotriesters **1-4**.

Synthesis of N,N-diisopropyl bis(2-methoxy-5-nitrophenyl) phosphate

Dichlorophosphoramidite was synthesized following a procedure in reference 1k. The dichlorophosphoramidite was purified by distillation under reduced pressure; bp 35.5°C (0.10 mm Hg); ^{31}P NMR δ 170.31. A dry 25 mL round bottomed flask was charged with 2-methoxy-5-nitrophenol (2.25 g, 13.31 mmoles), 7 mL of dry THF and triethylamine (2.03 mL, 14.6 mmol). This solution was added at -40 °C to a solution of dichlorophosphoramidite in 2 mL of THF after which the reaction was allowed to warm to room temperature. The reaction mixture was extracted with water and with a saturated cold NaCl solution. The organic layer was dried and the solvent removed under reduced pressure to afford a beige solid which was purified by flash column chromatography (50 : 48 : 2, Hexanes : EtOAc : TEA) (74.92% yield); ^{13}C NMR ($CDCl_3$) δ 24.4 (d, $J_{C\text{-}P}$=7.3 Hz, 4 CH_3), 44.43 (d, $J_{C\text{-}P}$=13.2 Hz, 2 -$\underline{C}(CH_3)_2$), 56.3, 110.7, 116.6 (d, $J_{C\text{-}P}$=10.15 Hz), 120.0, 140.9, 142.9 (d, $J_{C\text{-}P}$=6.7 Hz), 157.0 (d, $J_{C\text{-}P}$=2.6 Hz).

Preparation of N-methylanilinium trifluoroacetate

N-methylaniline (5.4 mL, 50 mmol) was dissolved in 50 mL of anhydrous Et_2O and TFA (3.8 mL, 50 mmol) was added dropwise. The ether evaporated slowly leaving a yellow solid that was recrystallized from Et_2O (50.1% yield); mp 63.5-64.0°C (lit. mp 66.5 °C); ^1H NMR ($CDCl_3$) δ 2.97 (s, 3H, $-CH_3$), 7.38-7.54 (m, 5H, aromatics); ^{13}C NMR ($CDCl_3$) δ 37.6 (CH_3), 121.9, 129.0, 130.2, 138.0, 162.3, 163.0.

*Synthesis of di(2-methoxy-5-nitrophenyl) 4-nitrophenethyl phosphate (**10**)*

Synthetic procedure adopted from reference 9. 4-nitrophenethyl alcohol (0.053 g, 0.32 mmol) and *N*-methylanilinium trifluoroacetate (0.14 g, 0.64 mmol) were dissolved in dry acetonitrile and dried further by evaporating the solvent in vacuo. The residue was redissolved in 4 mL of dry CH_3CN and added to *N,N*-diisopropyl bis(2-methoxy-5-nitrophenyl) phosphoramidite (0.18 g, 0.384 mmol) in 1 mL of dry CH_2Cl_2 and the mixture was stirred for 1 h. After 20 min a solid started to precipitate, the reaction was allowed to stir for 1 additional hour. The temperature was decreased to -40°C and a solution of 70% *m*-CPBA (0.12 g, 0.499 mmol) in 0.75 mL of dry CH_2Cl_2 was rapidly added to the reaction mixture, which turned orange. The reaction was allowed to warm and stir at room temperature for 1 h at which time 3 mL of a 10% aq $NaHSO_3$ solution was added and allowed to react for an additional 10 min. The ethereal phase was washed 2X with 10% aq $NaHSO_3$ and with cold saturated NaCl. After drying, the organic layer yielded an orange solid which was purified by flash chromatography (EtOAc : Hex, 75 : 25) (23.5% yield); mp 111-115°C; ^1H NMR ($CDCl_3$) δ 3.17 (m, 2H, $-OCH_2C\underline{H}_2Ar$), 3.90 (s, 6H, $-OCH_3$) 4.58 (m, 2H, $-OC\underline{H}_2CH_2Ar$), 6.98 (d, J_o=8.9 Hz, 2H, ArC2C6), 7.36 (d, J_o=8.4 Hz, 2H, ArC3C5), 8.04-8.13 (m, 6H, methoxynitrophenol ArH); ^{13}C NMR ($CDCl_3$) δ 36.2 (d, $J_{C\text{-}P}$=7.0 Hz, $-O\underline{C}H_2CH_2Ar$), 56.8 (OCH_3), 69.0 (d, $J_{C\text{-}P}$=6.1 Hz, $-OCH_2\underline{C}H_2Ar$), 111.8, 116.8, 117.2 (d, $J_{C\text{-}P}$=2.9 Hz), 122.7, 123.8, 129.8, 138.8 (d, $J_{C\text{-}P}$=7.4 Hz), 140.8, 144.3, 156.0 (d, *J*=5.6 Hz), Ar, ^{31}P NMR ($CDCl_3$) δ -11.17.

*Photohydrolysis of di(2-methoxy-5-nitrophenyl) 4-nitrophenethyl phosphate (**10**)*

A solution of **10** (0.071 mM) in 7 : 3, H_2O : CH_3CN and 30 mM NaOH was photohydrolyzed as described for triesters **1** - **4**. The reaction was irradiated for 20 s and followed by reading the nitrophenolate absorbance at 427 nm after each irradiation step. The data from three time courses were averaged to get the rate constant. The ground state hydrolysis was discerned by reading the absorbance every 20 s for 10 min and subsequent to that every 5 min for 18 h. The rate constant for the photohydrolysis is 0.06 ± 0.02 s^{-1}, which affords the desired phosphomonoester, while the rate constant for the ground state hydrolysis is 0.009 ± 0.001 s^{-1}, which affords the phosphodiester instead of the phosphomonoester.

Acknowledgments. The authors thank Professor Frank Raushel for helpful discussions. We gratefully acknowledge the financial support of the Robert A. Welch Foundation, the Searle Scholars Program / The Chicago Community Trust (JWK), the Texas Higher Education Coordinating Board's Advanced Research Program, and the National Institute of Health Biomedical Research Support Grant Program as well as the National Institutes of Health MARC Predoctoral Fellowship (NRG).

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(*Received in USA* 11 *July* 1994; *accepted* 7 *November* 1994)