Phosphodiester Alkylation with a Quinone Methide

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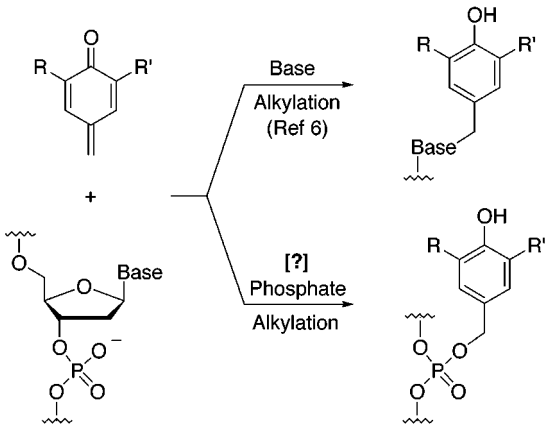
Received December 3, 1998

Despite the wide array of studies involving DNA alkylation and cleavage with quinone methide generating compounds, there have been no reports on the alkylation of phosphodiesters with quinone methides. We have investigated the reaction of dialkyl phosphates with a *p*-quinone methide in order to determine the potential for alkylation to produce trialkyphosphates. These studies have revealed that a phosphodiester can be alkylated with a *p*-quinone methide when promoted by a Brønsted acid. The role of the Brønsted acid is to sufficiently activate the *p*-quinone methide to allow phosphodiester addition to occur. The alkyl substituents of the phosphodiester have been found to effect the reactivity of the dialkyl phosphate under the reaction conditions examined. Equilibrium conversions up to 83% trialkyl phosphate formation have been achieved.

Introduction

Quinone methides are common reactive intermediates in a variety of bioactive compounds.[1,2] Their potential as bioreductive alkylators of DNA is well established.[3] Numerous investigations of nucleic acid base alkylations with *o*-quinone methides[4,5] and fewer with *p*-quinone methides[6,7] have been reported. However, to our knowledge there are no studies of quinone methide alkylation of the most abundant nucleophilic functional group of DNA: phosphodiesters. This is likely due to the relative weak nucleophilicity of phosphodiesters and known difficulty in directing alkylation to this functional group in nucleic acid polymers (Scheme 1).[8−10] In developing a research program around the application of quinone methides to drug design, drug delivery, and biomolecular labeling, we have investigated the reactivity of a *p*-quinone methide with mildly nucleophilic phosphodiesters as models for nucleic acid polymers. We have found that phosphodiester alkylation with this *p*-quinone methide is possible when promoted by a Brønsted acid.

Scheme 1



Results

We initially investigated the alkylation of quinone methide **1** with tetrabutylammonium dibenzyl phosphate

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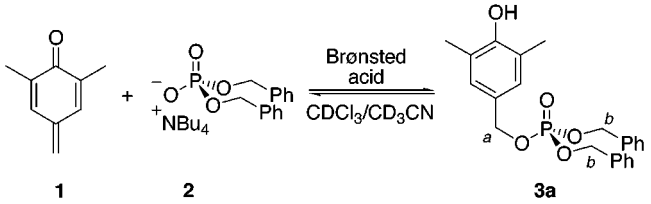
10.1021/jo9823745 CCC: $18.00
Published on Web 03/26/1999

Scheme 2



1 **2** **3a**

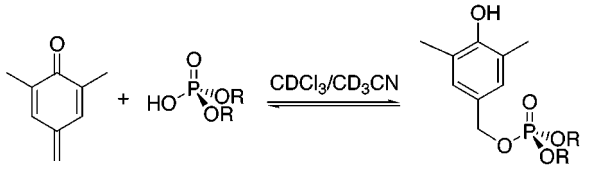
Table 1. Effect of Acid and Phosphate Equivalence on Conversion to Trialkylphosphate 3a[a]

entry	Brønsted acids (equiv)[b]	**2** (equiv)[b]	time (min)	**3a** (% convn)
1		1.1	*c*	0
2	MsOH (0.5)	1.1	76	15
3	MsOH (0.8)	1.1	75	30
4	MsOH (1.0)	1.1	76	42
5	MsOH (1.0)	1.7	77	31
6	MsOH (1.0)	2.1	75	21
7	EtCOOH (1.0)	1.1	*d*	0
8	*t*-BuCOOH (1.0)	1.1	*d*	0

[a] All experiments were carried out in $CD_3CN/CDCl_3$ (1:1), 20 °C. [b] Equivalents are relative to the amount of quinone methide **1**. [c] The reaction was monitored by 1H NMR analysis for several days with no evidence of any reaction. [d] The reaction was monitored by 1H NMR analysis for several hours with no evidence of product **3a**.

2 in $CD_3CN/CDCl_3$ (1:1) (Scheme 2). Quinone methide **1** was generated under standard conditions from 2,4,6-trimethylphenol (5.0 mM) and silver(I) oxide in $CDCl_3$.[11] The reaction concentration was limited to prevent side reaction of unstable *p*-quinone methide **1**.[11,12] Addition of a 5.0 mM solution of pure **1** to a solution of **2** (1.1 equiv) in CD_3CN showed no sign of reaction (entry 1, Table 1). However, upon the addition of methanesulfonic acid (MsOH, pK_a −6.2,[13] 0.5 equiv) the formation of trialkyl phosphate **3a** was observed by ^{1}H NMR analysis. Complete characterization was limited to ^{1}H and ^{31}P NMR analysis due to instability of **3** upon concentration.[14] The key resonances in the ^{1}H NMR spectra of **3a** were the benzylic hydrogens of the phenolic benzyl group from the quinone methide (a, Scheme 2) and those from the dibenzyl phosphate (b, Scheme 2). Prior to addition of quinone methide **1**, the benzylic hydrogen resonance of dibenzyl phosphate **2** appeared as a doublet ($^3J_{H-P}$ = 6.2 Hz, 4H) at 4.80 ppm due to the phosphorus coupling. Upon addition of quinone methide **1**, two phosphorus-coupled doublet resonances increased in the spectra over time in a 2:1 ratio at 4.95 ($^3J_{H-P}$ = 7.9 Hz, 4H) and 4.83 ppm ($^3J_{H-P}$ = 8.7 Hz, 2H), respectively. The 2:1 ratio of the two resonances (b/a, Scheme 2) combined with the phosphorus coupling was clear evidence of the formation of **3a**. Throughout the course of this reaction, the integrated growth of the resonances for trialkyl phosphate **3a** coincided with the integrated decrease of the resonances characteristic of quinone methide **1** and dibenzyl phosphate **2**, confirming the conversion of **1** and **2** into product **3a**. Trialkyl phosphate **3a** was further characterized by the decoupled ^{31}P NMR resonance at −0.45 ppm (relative to 85% phosphoric acid).

Scheme 3



1 **4a** (R = CH_2Ph) **4b** (R = Et) **3a** (R = CH_2Ph) **3b** (R = Et)

According to Table 1 (entry 2), the addition of 0.5 equiv of MsOH to a 1.0:1.1 ratio of quinone methide **1** and dibenzyl phosphate salt **2** afforded 15% conversion to **3a** (76 min) with the remainder being unreacted quinone methide **1**. The addition of 0.8 equiv of MsOH afforded 30% conversion to **3a** (75 min) (entry 3), and with 1.0 equiv of MsOH, 42% of trialkyl phosphate **3a** was formed (76 min) (entry 4). The reversibility of the alkylation reaction was confirmed by the addition of potassium carbonate to the equilibrated reaction mixture, which resulted in the complete loss of trialkyl phosphate **3a** and conversion back to quinone methide **1** and phosphate **2**. These results clearly demonstrated that the reaction was promoted by MsOH.

The strength of the Brønsted acid used to promote trialkyl phosphate formation was investigated. Limitations existed in the choice of acids as the conjugate base nucleophilicity had to be low enough to prevent competitive quinone methide alkylation. It was found that attempts to promote the reaction with less acidic propionic acid (pK_a 4.9)[15] and pivalic acid (pK_a 5.0)[15] afforded no trialkyl phosphate product.

The percent conversion of quinone methide **1** to trialkyl phosphate **3a** relative to the concentration of MsOH and dibenzyl phosphate **2** is presented in Table 1. Note that the amount of **3a** formed in the reaction increased with higher MsOH concentration and constant **2** concentration (entries 2−4, Table 1). However, with higher **2** concentration and constant MsOH concentration, conversion to **3a** was decreased (entries 4−6). This relationship between trialkyl phosphate **3a**, phosphate **2**, and MsOH revealed the existence of a preequilibrium by MsOH with dibenzyl phosphate **2** forming dibenzylphosphoric acid in situ.[16] Thus, the higher the concentration of salt **2**, the more dibenzylphosphoric acid was formed in situ from the available MsOH. This resulted in lower trialkyl phosphate **3a** formation.

To further confirm the role of acids in promoting the alkylation reaction, we investigated the reaction of quinone methide **1** with dibenzyl- and diethylphosphoric acid (**4a** and **4b**, respectively) (Scheme 3). The ratio of the two reactants was varied by adding the appropriate amount of dialkylphosphoric acid in CD_3CN to a preformed solution of quinone methide **1** in $CDCl_3$ to afford a 2.5 mM solution of **1** in 1:1 $CD_3CN/CDCl_3$. The reactions were monitored by ^{1}H NMR analysis. The results of these experiments are presented in Table 2. As the amount of dialkylphosphoric acid was increased the percent conversion to trialkyl phosphate **3** increased. Reaction with 0.6 equiv of **4a** resulted in 28% conversion to **3a** (entry 1);

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(14) Complete characterization of a related trapped product will be reported in due course.

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Table 2. Reactions with Dialkylphosphoric Acids[a]

entry	phosphoric acids	equiv[b]	time (h)	**3a** or **3b** (% convn)
1	**4a**	0.6	3.5	28
2	**4a**	1.3	3.5	57
3	**4a**	2.7	3.5	83
4	**4b**	1.2	21.5	46
5	**4b**	2.4	21.5	68
6	**4b**	3.9	21.5	83

[a] All experiments were carried out in $CD_3CN/CDCl_3$ (1:1), 20 °C. [b] Equivalents are relative to the amount of quinone methide **1**.

1.3 equiv of **4a** afforded 57% conversion to **3a** (entry 2); and 2.7 equiv of **4a** resulted in 83% conversion to **3a** (entry 3). Similar results were observed with the use of diethylphosphoric acid (entries 4−6, Table 2).

The results reported in Table 2 also established a difference in the rate at which the equilibrium was reached with dibenzyl phosphoric acid (**4a**) and diethylphosphoric acid (**4b**). The reaction with 1.3 equiv of dibenzylphosphoric acid (**4a**, entry 2) produced 57% **3a**, reaching equilibrium in 3.5 h, while the reaction with 1.2 equiv of diethylphosphoric acid (**4b**, entry 4) produced 46% **3b** in 21.5 h. These results reveal a faster rate for establishing the equilibrium and higher percentage conversion with dibenzylphosphoric acid (**4a**) relative to diethylphosphoric acid (**4b**). This is likely based on both a difference in acidity of the two phosphoric acids under the reaction conditions and a difference in nucleophilicity of the corresponding conjugate bases.

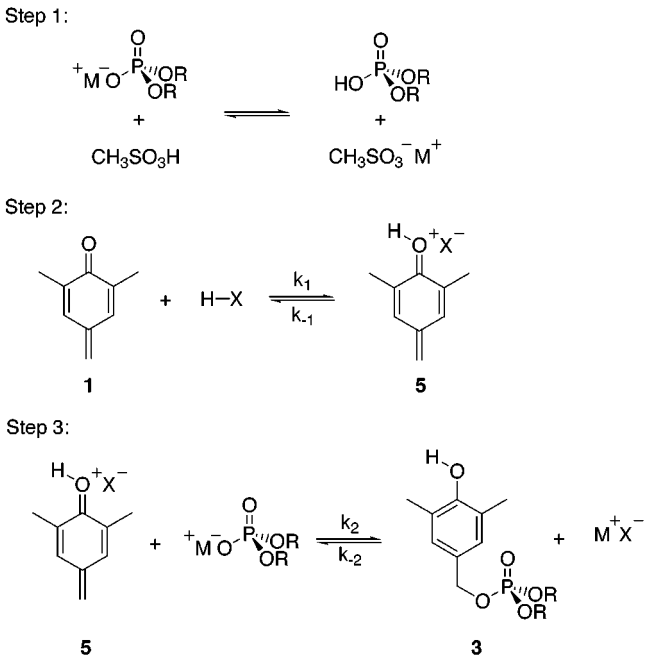
These results confirmed the addition of MsOH established a preequilibrium forming the phosphoric acid in situ, which acted as the Brønsted acid to promote trialkyl phosphate formation. This was further confirmed by the concurrent change in the ^{1}H NMR chemical shift of the methylene resonances of the dialkyl phosphates with MsOH concentration. When MsOH was added to dibenzyl phosphate salts **2**, the highly resolved benzylic methylene resonance shifted downfield as the acid concentration increased.[17] This preequilibrium was rapid on the NMR time scale and relative to trialkyl phosphate formation.

Discussion

Our initial investigations of the reaction of dialkyl phosphates with *p*-quinone methide **1** revealed the requirement for acid to promote the alkylation. As demonstrated in Table 1, trialkyl phosphate **3a** was formed only upon the addition of MsOH to quinone methide **1** and tetrabutylammonium dibenzyl phosphate **2**. The expected preequilibrium with MsOH and dibenzyl phosphate **2** to form dibenzylphosphoric acid **4a** in situ was confirmed by the observation of one sharp doublet resonance for dibenzylphosphoric acid **4a** by ^{1}H NMR analysis. The chemical shift was dependent on the amount of MsOH present. This preequilibrium formation of phosphoric acid **4a** was further confirmed by the similarity of reactions using MsOH and dibenzyl phosphate **2** with those using dibenzylphosphoric acid **4a**. A series of experiments (Table 1) allowed the independent role of the proton from the Brønsted acid and the phosphodiester nucleophile to be assessed. This was accomplished by independently varying the amount of available proton using MsOH as the Brønsted acid and the amount of tetrabutylammonium dibenzyl phosphate (**2**) as nucleophile.

(17) The chemical shifts of the benzylic protons of **2** were 4.85 ppm without MsOH, 4.89 ppm with 0.5 equiv of MsOH, 4.93 ppm with 0.8 equiv of MsOH, and 4.96 ppm with 1.0 equiv of MsOH.

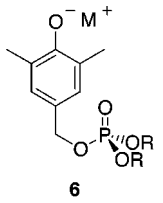
Scheme 4



The following mechanism is proposed for the equilibrium alkylation of dibenzyl phosphate **2** with quinone methide **1** to form trialkyl phosphate **3a** in the presence of MsOH (Scheme 4). For simplification the proton and dialkyl phosphate are shown with unspecified counterions in Scheme 4. The initial step (step 1) is the fast equilibrium of MsOH with tetrabutylammonium dialkyl phosphate producing dialkylphosphoric acid in situ. Protonation of quinone methide **1** to form **5** at a low steady-state concentration (step 2) is required to sufficiently lower the activation energy allowing phosphodiester alkylation to afford trialkyl phosphate **3a** (step 3). Direct observation of **5** by ^{1}H NMR was precluded by its high reactivity and corresponding low steady-state concentration.

This mechanism is fully consistent with the results shown in Table 1. When the concentration of MsOH was increased (entries 2−4), the concentration of the phosphoric acid increased according to the equilibrium step 1 (Scheme 4). This led to an increased concentration of intermediate **5** due to the increased concentration of Brønsted acid, resulting in higher conversion to trialkyl phosphate **3**. When the phosphate salt (**2**) concentration was increased while the MsOH concentration was held constant (entries 5−7), the total concentration of Brønsted acid decreased in the reaction mixture. This decrease in Brønsted acid concentration lowered the concentration of intermediate **5**, resulting in lower conversion to trialkyl phosphate **3**.

It was also observed from Table 1 that acids weaker than phosphoric acid[16] [i.e., propionic acid (pK_a 4.9) and pivalic acid (pK_a 5.0)] were not effective at promoting the alkylation of dibenzyl phosphate **2** with quinone methide **1**. This ruled out an alternative mechanistic pathway by which the phosphate would add to the quinone methide to afford a low steady-state concentration of phenoxide intermediate **6** (Figure 1). The role of Brønsted acid by



6

Figure 1.

this mechanism would be to thermodynamically stabilize the product via protonation of **6**. These weak acids would be sufficiently acidic to protonate a phenoxide ($pK_a \sim 10$) yet failed to promote the alkylation of the phosphate. This indicated that the role of Brønsted acid in the reaction is to kinetically activate quinone methide **1** and not simply to thermodynamically stabilize the product.

Results from Table 2 are also in accord with the proposed mechanism (Scheme 4). Increasing the phosphoric acid concentration (Brønsted acid) resulted in an increased conversion to trialkyl phosphate **3**. The difference in the rates for reaching equilibrium of trialkyl phosphate formation between dibenzyl and diethyl phosphoric acids revealed the significant influence of the dialkyl substituents. It was found that 83% conversion to trialkyl phosphate **3a** occurred with 2.7 equiv of **4a** within 3.5 h (20 °C). Meanwhile, 3.9 equiv of **4b** required 21.5 h to afford the same 83% conversion to trialkyl phosphate **3b** (20 °C). The alkyl groups may affect the self-association of the phosphoric acid under the reaction conditions.[16] This may in turn affect both the acidity of the phosphoric acid and nucleophilicity of the conjugate base.

Conclusion

Investigation of the reaction between a *p*-quinone methide and the phosphodiester functionality has been undertaken for two primary reasons. (1) The ability of quinone methide producing compounds to induce alkylation and cleavage of DNA is well-established. However, there has been no previous study on the ability of quinone methides to react with phosphodiesters, the most abundant nucleophilic functional group in nucleic acid polymers. (2) Future applications in bioalkylation processes required the understanding of the *p*-quinone methide reactivities.

These studies have clearly demonstrated the ability of quinone methide **1** to alkylate a dialkyl phosphate under Brønsted acid promoted conditions. Consistent with our investigations, it is proposed that activation of quinone methide **1** through protonation to form **5** (Scheme 4) is required for phosphodiester alkylation to occur. Optimal conditions (2.7 equiv of **4a** or 3.9 equiv of **4b**) produced 83% conversion to **3a** or **3b**, respectively.

These investigations suggest that the potential bioalkylation of phosphodiesters with *p*-quinone methides is unlikely under neutral conditions without activation of the quinone methide. Various approaches for quinone methide activation under biologically relevant conditions with Lewis acids and/or the inclusion of intramolecular activating functionality are under development.

Experimental Section

All commercially available compounds were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Lancaster Synthesis, Inc. (Windham, NH) and used without purification, unless noted otherwise. Deuterium solvents were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). ^{31}P chemical shifts are reported relative to 85% phosphoric acid.

Quinone Methide (1) Solution. A stock solution of 2,4,6-trimethylphenol was prepared by dissolving the solid (13.6 mg, 0.100 mmol) in $CDCl_3$ (1.00 mL). This solution was then diluted to 5.0 mM with $CDCl_3$. The trimethylphenol was oxidized with silver(I) oxide (100 mg, 0.43 mmol) by stirring at room temperature for 10 min. The suspension was filtered with glass wool to give known quinone methide **1** in solution (5.0 mM): ^1H NMR ($CDCl_3$, 270 MHz) δ 6.98 (s, 2H), 5.74 (s, 2H), 2.00 (s, 6H).[11]

Tetrabutylammonium Dibenzyl Phosphate (2). To a solution of tetrabutylammonium bromide (161 mg, 0.5 mmol) in $MeOH/CH_2Cl_2$ (1:1, 100 mL) was added silver dibenzyl phosphate (192 mg, 0.5 mmol). The suspension was stirred at room temperature for 2 h. The precipitate was removed with Celite. The resulting clear solution was concentrated to afford analytically pure product **2** as a pale oil (247 mg, 95% yield). A stock solution of tetrabutylammonium dibenzyl phosphate **2** (12.5 mM) was prepared by dissolving the salt (22.5 mg, 43.4 μmol) in CD_3CN (4.00 mL). Later, various concentrations of salt **2** (6.8 mM, 10.6 mM, 13.8 mM) were prepared by diluting the stock solution with CD_3CN and used in the following experiments: ^1H NMR (CD_3CN, 270 MHz) δ 7.20−7.38 (m, 10H), 4.80 (d, $^3J_{H-P} = 6.2$ Hz, 4H), 3.10 (t, $J = 8.7$ Hz, 8H), 1.47−1.63 (m, 8H), 1.22−1.40 (m, 8H), 0.93 (t, $J = 7.4$ Hz, 12H); ^{13}C NMR (68 MHz) δ 140.3 (d, $^3J_{C-P} = 7.8$ Hz), 128.0, 127.3, 127.1, 66.4 (d, $^2J_{C-P} = 5.2$ Hz), 58.3, 23.5, 19.5, 13.1; IR (neat, cm^{-1}) 2960, 2873, 1490, 1456, 1253; ^{31}P NMR (121.5 MHz) δ −12.4. Anal. Calcd for $C_{30}H_{50}NO_4P \cdot H_2O$: C, 67.01; H, 9.75; N, 2.60. Found: C, 67.19; H, 9.73; N, 2.69.

Alkylation of Tetrabutylammonium Phosphate (2) by Quinone Methide (1) with Brønsted Acids. A stock solution of methanesulfonic acid was prepared by dissolving the acid (9.6 mg. 0.10 mmol) in CD_3CN (800 μL). This acid solution was further diluted to afford solutions of 12.5, 20.0, and 25.0 mM in CD_3CN. The $CDCl_3$ solution of quinone methide **1** (5.0 mM), prepared as described above, was added to a solution of phosphate salt **2** (at the desired concentration described above). The appropriate concentration of MsOH solution was then added to give the desired ratio of MsOH/**2** in solution. All the reactions were carried out in $CDCl_3/CD_3CN$ (1:1, 1.00 mL). The exact equivalents of salt **2** and MsOH added relative to quinone methide **1** were calculated from the area integration of clearly identifiable resonances (4.80 and 3.10 ppm resonances for **2**, 2.57 ppm for MsOH, and 5.74 ppm for **1**) in the ^1H NMR spectra. The reaction was monitored by ^1H NMR analysis at 20.0 ± 0.2 °C. The percent conversion to trialkyl phosphate **3a** was calculated on the basis of the area integration of the benzylic protons of **3a** (a and b, Scheme 2, at 4.97 and 4.84 ppm, respectively) relative to quinone methide **1** from the ^1H NMR spectra. No other unidentifiable resonances were present in the ^1H NMR spectrum during these experiments. Each experiment was repeated at least two times.

Several experiments were carried out using a constant amount of salt **2** (1.1 equiv to **1**) with increasing amounts of MsOH (0.5, 0.8, or 1.0 equiv to **1**). Those experiments were accomplished in the manner described above with appropriate concentrations of MsOH to achieve the desired ratio of MsOH/**2**. Further experiments used a constant amount of MsOH (1.0 equiv to **1**) with increasing amount of salt **2** (1.1, 1.7, or 2.2 equiv to **1**) using the appropriate concentration of **2**.

A stock solution of propionic acid was prepared by diluting the acid (26.6 mg, 0.359 mmol) in CD_3CN (1.00 mL). A stock solution of pivalic acid was prepared by diluting the acid (10.2 mg, 0.100 mmol) in CD_3CN (1.00 mL). Each of these acids was further diluted with CD_3CN and then added to a solution of quinone methide **1** and phosphate salt **2**. The acid and salt **2** used in the experiments were 1.0 and 1.1 equiv, respectively, relative to quinone methide **1** (2.5 μmol). The reaction was monitored by ^1H NMR analysis at 20.0 ± 0.2 °C. Each experiment was repeated at least two times.

Trialkyl phosphate **3a** (although **3a** was not sufficiently stable to allow isolation, the following solution characterization

was definitive for structure verification):[14] ^{1}H NMR (1:1 $CDCl_3$ and CD_3CN, 270 MHz) δ 7.20−7.31 (m, 10H), 6.88 (s, 2H), 4.95 (d, $^3J_{H-P}$ = 7.9 Hz, 4H), 4.83 (d, $^3J_{H-P}$ = 8.7 Hz, 2H), 2.14 (s, 6H); ^{31}P NMR (121.5 MHz) δ −0.45.

Study of the Alkylation of Dibenzyl Phosphoric Acid (4a) with Quinone Methide (1). A stock solution of dibenzylphosphoric acid **4a** was prepared by dissolving the acid (16.7 mg, 0.06 mmol) in CD_3CN (2.00 mL). Various concentrations of acid **4a** (3.0, 6.5, 13.5 mM) were prepared by diluting the stock solution with CD_3CN and used in the experiments. The $CDCl_3$ solution of quinone methide **1** (5.0 mM), prepared as described above, was added to the appropriate solution of phosphoric acid **4a**. The final amount of **4a** relative to **1** (2.5 μmol) in the reaction mixture was 0.6, 1.3, or 2.7 equiv. All reactions were carried out in $CDCl_3$/CD_3CN (1:1, 1.00 mL). The exact equivalents of acid **4a** relative to quinone methide **1** were calculated from the area integration of clearly identifiable resonances (4.90 ppm for **4a** and 5.74 ppm for **1**) in the ^{1}H NMR spectra. The reaction was monitored by ^{1}H NMR analysis at 20.0 ± 0.2 °C. The percent conversion to trialkyl phosphate **3a** was calculated on the basis of the area integration at the benzylic protons of **3a** (a and b, Scheme 2, at 4.97 and 4.84 ppm, respectively) relative to quinone methide **1** from the ^{1}H NMR spectra. No other unidentifiable resonances were present in the ^{1}H NMR spectrum during these experiments. Each experiment was repeated at least two times.

Study of the Alkylation of Diethyl Phosphoric Acid (4b) with Quinone Methide 1. A stock solution of diethyl phosphoric acid was prepared by dissolving the acid (4.7 mg, 0.03 mmol) in CD_3CN (1.60 mL). Various concentrations of acid **4a** (6.0, 12.0, 19.5 mM) were prepared by diluting the stock solution with CD_3CN and used in the experiments. The $CDCl_3$ solution of quinone methide **1** (5.0 mM), prepared as described above, was added to the appropriate solution of phosphoric acid **4b**. The final amount of **4b** relative to **1** (2.5 μmol) in the reaction mixture was 1.2, 2.4, or 3.9 equiv. All reactions were carried out in $CDCl_3$/CD_3CN (1:1, 1.00 mL). The exact equivalents of acid **4b** relative to quinone methide **1** were calculated from the area integration of clearly identifiable resonances (4.10 ppm for **4b** and 5.74 ppm for **1**) in the ^{1}H NMR spectra. The reaction was monitored by ^{1}H NMR analysis at 20.0 ± 0.2 °C. The percent conversion to trialkyl phosphate **3b** was calculated on the basis of the area integration at the benzylic protons of **3b** (4.85 ppm) relative to quinone methide **1** from the ^{1}H NMR spectra. No other unidentifiable resonances were present in the ^{1}H NMR spectrum during these experiments. Each experiment was repeated at least two times.

Trialkyl phosphate **3b** (although **3b** was not sufficiently stable to allow isolation, the following solution characterization was definitive for structure verification):[14] ^{1}H NMR (1:1 $CDCl_3$ and CD_3CN, 270 MHz) δ 6.95 (s, 2H), 4.85 (d, $^3J_{H-P}$ = 7.9 Hz, 2H), 3.96−4.07 (m, 4H), 2.14 (s, 6H), 1.23−1.28 (m, 6H); ^{31}P NMR (121.5 MHz) δ −0.39.

Acknowledgment. The authors would like to thank Marv Leister for assistance with NMR experiments and Professor Dale Johnson for very fruitful discussions. We also gratefully acknowledge the financial support of the National Science Foundation (EHR-9108762 and CHE-9734187) and the Arkansas Science and Technology Authority (98-B-03).

Supporting Information Available: Copies of ^{1}H and ^{31}P NMR spectra from experiments monitoring the progress of phosphodiester alkylations with quinone methide **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO9823745