Quinone Methide Phosphodiester Alkylations under Aqueous Conditions

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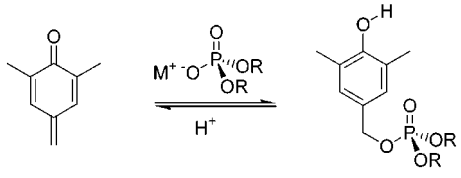
A detailed analysis of the alkylation of phosphodiesters with a *p*-quinone methide under aqueous conditions has been accomplished. The relative rates of phosphodiester alkylation and hydrolysis have been examined by ^{1}H NMR analysis of the reaction of 2,6-dimethyl-*p*-quinone methide in a buffered diethyl phosphate/acetonitrile solution (1:9 v/v, pH 4.0). The rate of hydrolysis of the quinone methide was confirmed by UV analysis in 28.5% solutions of aqueous inorganic phosphate in acetonitrile at pH 4.0 and 7.0. Similarly, the rate of phosphodiester alkylations by the quinone methide was also confirmed by UV analysis in 28.5% solutions of aqueous dibenzyl, dibutyl, or diethyl phosphate in acetonitrile at pH 4.0 and 7.0. These kinetic studies further establish that the phosphodiester alkylation reactions are acid-catalyzed, second-order processes. The rate constant for phosphodiester alkylation was found to range from approximately 370−3700 times the rate constant of quinone methide hydrolysis with diethyl and dibenzyl phosphate, respectively (pH 4.0, 28.5% aqueous acetonitrile).

Introduction

A fundamental determinant of the biological effectiveness of most drugs is their hydrolytic stability.[1] Hydrolysis is a prominent concern in the development of drugs involving quinone methides as the reactive intermediate.[2] Hydrolysis has been observed as the major competitive reaction in alkylation studies of nucleic acids by quinone methide derivatives.[3] Skibo and co-workers recently demonstrated selective phosphate alkylation of nucleic acids with aziridinium derivatives, yet found that hydrolysis of the active intermediate and products was predominant.[4] We previously reported that phosphodiester alkylation with 2,6-dimethyl-*p*-quinone methide **1** to produce phosphotriester **2** is readily achieved under anhydrous conditions ($CDCl_3/CD_3CN$, 1:1) when promoted by a Brønsted acid (Scheme 1).[5] We now examine this alkylation process under aqueous conditions to determine the competitive rate of quinone methide hydrolysis versus phosphodiester alkylation.

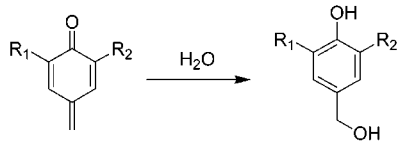
Several studies have included the hydrolysis of a variety of simple *p*-quinone methides **3** to afford benzyl alcohols **4** under aqueous conditions (Scheme 2).[6,7] The substituents at the 2- and 6-positions of *p*-quinone methides have a dramatic effect on the rate of hydrolysis. The half-life in a phosphate buffer (pH 7.4, 25 °C) ranged from 1.3 s for **3a**[6b] to 3060 s for **3c**.[6d] The acid- and base-catalyzed nature of the hydrolysis of quinone methides has also been demonstrated.[7] Bolton and co-workers examined the pH−rate profile of the hydrolysis of qui-

Scheme 1



1 2

Scheme 2



3 4

Derivative	R_1	R_2	Half-life (s)
a	H	OCH_3	1.3
b	CH_3	*t*-Bu	47
c	*t*-Bu	*t*-Bu	3060

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(4) (a) Skibo, E. B.; Islam, I.; Schulz, W. G.; Zhou, R.; Bess, L.; Boruah, R. *Synlett* **1996**, 297−309. (b) Skibo, E. B.; Xing, C. *Biochemistry* **1998**, *37*, 15199−15213.

(5) Zhou, Q.; Turnbull, K. D. *J. Org. Chem.* **1999**, *64*, 2847−2851.

(6) (a) Bolton, J. L.; Turnipseed, S. B.; Thompson, J. A. *Chem.-Biol. Interact.* **1997**, *107*, 185−200. (b) Bolton, J. L.; Comeau, E.; Vukomanovic, V. *Chem.-Biol. Interact.* **1995**, *95*, 279−290. (c) Thompson, D. C.; Perera, K. *Biochem. Biophys. Res. Commun.* **1995**, *209*, 6−11. (d) Bolton, J. L.; Valerio, Jr., L. G.; Thompson, J. A. *Chem. Res. Toxicol.* **1992**, *5*, 816−822. (e) Richard, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 4588−4595.

(7) (a) McCracken, P. G.; Bolton, J. L.; Thatcher, R. J. *J. Org. Chem.* **1997**, *62*, 1820−1825. (b) Hemmingson, J. A.; Leary, G. *J. Chem. Soc., Perkin Trans. 2* **1975**, 1584−1587.

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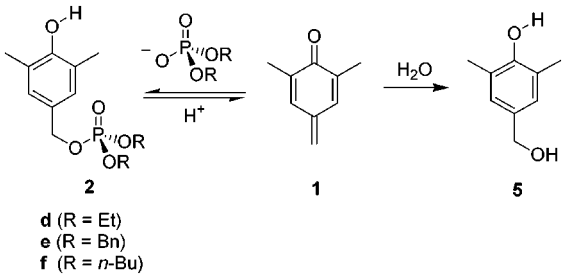
Scheme 3



none methide **3b** in aqueous buffers at 25 °C and found the rate of hydrolysis was constant (2.7×10^{-4} $M^{-1}\cdot s^{-1}$) over the pH range of 4.7−9.5.[7a]

We report the investigation of phosphodiester alkylation with 2,6-dimethyl-*p*-quinone methide **1** in various aqueous buffered phosphodiester/acetonitrile solutions. These investigations have enhanced our understanding of the phosphodiester alkylation mechanism and guided the development of more advanced alkylating reagents[8] for future applications.

Results and Discussions

Our initial investigations of hydrolysis versus phosphodiester alkylation by quinone methide **1** were based on ^{1}H NMR analysis. The half-life of 2,6-dimethyl quinone methide **1** in a phosphate buffer (pH 7.40, 25.0 °C) has been determined by Bolton and co-workers to be 26 s.[6d] To sufficiently extend the half-life of quinone methide **1** for ^{1}H NMR analysis, initial studies were carried out in solutions of only 10% aqueous buffered diethyl phosphate in acetonitrile.

A stock solution of quinone methide **1** in CD_3CN was obtained from oxidation of 2,4,6-trimethylphenol with silver(I) oxide over 40 min. A stock solution of diethyl phosphate buffer in D_2O was prepared by initial neutralization of diethyl phosphoric acid with a solution of KOD in D_2O (40%), followed by pH adjustment with methanesulfonic acid. The use of methanesulfonic acid was based on prior determination that its conjugate base did not undergo nucleophilic addition to the quinone methide.[5,9] The resulting buffer solution (100 μL) was then added to quinone methide **1** (900 μL) in CD_3CN. The final concentrations of quinone methide **1** and diethyl phosphate were 6.60 and 25.0 mM, respectively. The concentration of water (5.56 M) in the reaction solution was approximately 220 times higher than that of diethyl phosphate. A preliminary analysis of the alkylation reaction of diethyl phosphate with quinone methide **1** at pH 2.0, 4.0, and 5.7 revealed the optimal pH for phosphodiester alkylation was at pH 4.0 (buffered aqueous diethyl phosphate/acetonitrile solution, 1:9 v/v). The reaction was monitored by ^{1}H NMR analysis over 40 min at 20.0 °C in triplicate. The pH of the reaction solution remained unchanged throughout the reaction.

As indicated by ^{1}H NMR analysis, quinone methide **1** in the buffered diethyl phosphate/acetonitrile solution at pH 4.0 underwent two reactions: (i) phosphodiester alkylation to **2d** and (ii) hydrolysis to **5** (Scheme 3). The

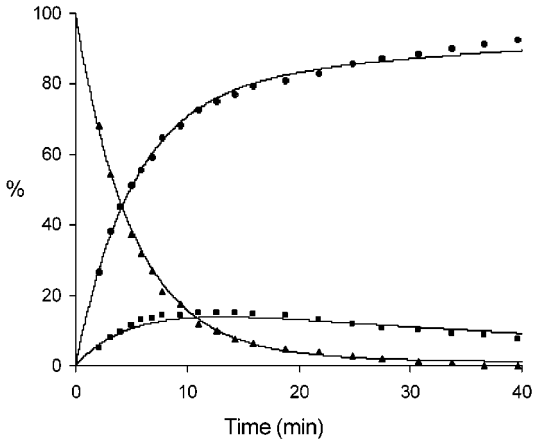


Figure 1. Reaction plot showing the loss of quinone methide **1** (▲) through phosphodiester alkylation to phosphotriester **2d** (■) and hydrolysis to benzyl alcohol **5** (●) in a buffered diethyl phosphate/acetonitrile solution (1:9 v/v) at pH 4.0. The reaction was monitored by ^{1}H NMR analysis at 20.0 °C. Only one set of the triplicated ^{1}H NMR derived data is shown in the plot. The plotted curves were derived from simulation of the reaction based on the proposed mechanism (see text). All of the experimental data points derived from the three runs were within 3% of the simulated curves.

formation of phosphotriester **2d** was evident in the reaction by the benzylic proton resonances. These resonance signals appeared as a doublet ($^3J_{HP} = 8.2$ Hz) at 4.84 ppm characteristic of phosphorus coupling in accord with our previous report.[5] The formation of benzyl alcohol **5** as the hydrolysis product of quinone methide **1** was confirmed by appropriate resonance signal enhancement upon addition of authentic benzyl alcohol **5**. The percent conversions to phosphotriester **2d** and benzyl alcohol **5** from quinone methide **1** were calculated on the basis of the average area integration of the corresponding characteristic resonance signals in the ^{1}H NMR spectra. The half-life of quinone methide **1** was approximately 4.5 min under the reaction conditions (Figure 1). The percent conversion to phosphotriester **2d** reached a maximum of 16% in approximately 9 min, followed by a slow decrease to 10% within 40 min. The percent benzyl alcohol **5** in the reaction reached 75% after 10 min. These ^{1}H NMR studies clearly demonstrate that phosphodiester alkylation by quinone methide **1** occurs in an aqueous buffered dialkyl phosphate/acetonitrile mixture (1:9 v/v) at pH 4.0 along with the hydrolysis of quinone methide **1**.

Further analysis of the triplicated ^{1}H NMR data during the initial 4 min (Figure 1) afforded the rates of quinone methide reactions. These are 1.54×10^{-5} $M\cdot s^{-1}$ for quinone methide **1** disappearance, 1.28×10^{-5} $M\cdot s^{-1}$ for benzyl alcohol **5** formation and 0.26×10^{-5} $M\cdot s^{-1}$ for phosphotriester **2d** formation. This indicates that no side reactions are occurring as the rate of the quinone methide disappearance is completely accounted for by the combined rates of benzyl alcohol **5** and phosphotriester **2d** formations according to eq 1. Considering the second-order nature of both phosphodiester alkylation and quinone methide hydrolysis reactions, the loss of quinone methide **1** over time can be expressed by eq 2. This provides the rate constant for diethyl phosphate alkylation (k_1) and quinone methide hydrolysis (k_2) of 1.6×10^{-2} and 3.5×10^{-4} $M^{-1}\cdot s^{-1}$, respectively. The second-order rate constant for hydrolysis of quinone methide **1** is only 2.2% of that for phosphodiester alkylation.

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Table 1. Rates (k_{obs}) of Quinone Methide Disappearance in Buffered Phosphate/CH_3CN Solution (28.5%, pH 4.0)[a]

	inorganic phosphate		dibenzyl phosphate		dibutyl phosphate		diethyl phosphate	
entry	concn (mM)	rate ($\times 10^{-3}$ s^{-1})	concn (mM)	rate ($\times 10^{-3}$ s^{-1})	concn (mM)	rate ($\times 10^{-3}$ s^{-1})	concn (mM)	rate ($\times 10^{-3}$ s^{-1})
1	9.5	6.05 ± 0.21	0.95	7.38 ± 0.11	2.4	6.42 ± 0.05	9.5	6.95 ± 0.31
2	19	5.99 ± 0.06	1.9	8.41 ± 0.15	4.8	6.81 ± 0.03	19	8.03 ± 0.30
3	29	6.13 ± 0.05	2.9	9.96 ± 0.22	9.5	8.42 ± 0.15	29	9.51 ± 0.16
4	38	6.11 ± 0.09	3.8	11.0 ± 0.30	14	9.16 ± 0.02	38	10.6 ± 0.20

[a] All experiments were monitored at 288 nm by UV spectroscopy in triplicate at 25.0 °C. The rates were calculated as the slope of the natural log of absorbance over time (ln A/t).

$$-d[\mathbf{1}]/dt = d[\mathbf{2}]/dt + d[\mathbf{5}]/dt \quad (1)$$

$$= k_1[\text{phosphodiester}][\mathbf{1}] + k_2[H_2O][\mathbf{1}] \quad (2)$$

$$= k_{obs}[\mathbf{1}] \quad (3)$$

$$k_{obs} = k_1[\text{phosphodiester}] + k_2[H_2O] \quad (4)$$

Confirmation of the reaction kinetics derived from ^1H NMR analysis was accomplished by UV spectroscopic analysis at 288 nm. The first-order rates of the disappearance of quinone methide **1** (k_{obs}) in 28.5% aqueous buffered inorganic phosphate in acetonitrile at pH 4.0 was initially determined to afford the rate of hydrolysis of quinone methide **1**. A similar analysis was then conducted in 28.5% aqueous buffered dibenzyl, dibutyl, or diethyl phosphate in acetonitrile at pH 4.0 to afford the rate of phosphodiester alkylation.

A stock solution of quinone methide **1** (1.00 mM in acetonitrile) was obtained from oxidation of 2,4,6-trimethyl phenol with silver(I) oxide over 45 min. Stock solutions (10, 20, 30, or 40 mM) of 30% aqueous buffered inorganic phosphate in acetonitrile at pH 4.0 were prepared by pH adjustment of the acid with diluted NaOH solutions. The quinone methide solution (1.00 mM, 150 μL) was added to the appropriate concentration of inorganic phosphate buffer solution (2.85 mL) at 25.0 °C. The final concentration of quinone methide **1** in the reaction solution was 50.0 μM, and the concentrations of aqueous buffered inorganic phosphate/acetonitrile solutions were 95% of their stock concentrations. The percent water in the final reaction solution was 28.5% and approximately 15.8 M. The pH of the final reaction solution remained unchanged throughout the reaction. The observed rates of disappearance of quinone methide **1** (k_{obs}) were calculated as the slope of the natural log of absorbance over time. All reactions were run in triplicate.

The first-order rate of disappearance of quinone methide **1** was found to be independent of the inorganic phosphate concentration (Table 1). The k_{obs} was approximately 6.05×10^{-3} s^{-1}. A water concentration of 15.8 M in the 28.5% buffer/acetonitrile mixture affords the second-order hydrolysis rate constant (k_2) of 3.8×10^{-4} $M^{-1}\cdot s^{-1}$. Our results confirm that the quinone methide hydrolysis is a specific acid catalyzed process, as previously demonstrated.[7a,10]

The observed rate of disappearance of the quinone methide **1** (k_{obs}) in 28.5% aqueous buffered dibenzyl, dibutyl, or diethyl phosphate in acetonitrile at pH 4.0 was similarly determined. Stock solutions of 30% aqueous buffered dibenzyl, dibutyl, or diethyl phosphate in acetonitrile at pH 4.0 were prepared by pH adjustment of the dialkyl phosphoric acids with dilute NaOH solutions. The solubility dependent concentrations of these stock solutions were 10, 20, 30, or 40 mM for diethyl phosphate; 1.0, 2.0, 3.0, or 4.0 mM for dibenzyl phosphate; and 2.5, 5.0, 10, or 15 mM for dibutyl phosphate. The quinone methide solution (1.00 mM, 150 μL) was added to the appropriate phosphodiester buffer solution (2.85 mL) at 25.0 °C.[11,12] The observed rates of disappearance of the quinone methide **1** (k_{obs}) were calculated as the slope of the natural log of absorbance over time.

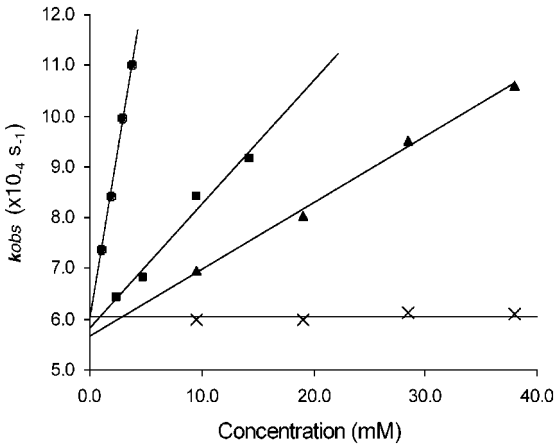


Figure 2. Dependence of the rate of quinone methide **1** disappearance on the concentration of dialkyl phosphate at pH 4.0. (●) Dibenzyl phosphate, slope: 1.3×10^{-3} $mM^{-1}\cdot s^{-1}$ ($R^2 = 0.993$). (■) Dibutyl phosphate, slope: 0.24×10^{-3} $mM^{-1}\cdot s^{-1}$ ($R^2 = 0.974$). (▲) Diethyl phosphate, slope: 0.13×10^{-3} $mM^{-1}\cdot s^{-1}$ ($R^2 = 0.996$). (×) inorganic phosphate.

The first-order rate of quinone methide **1** disappearance (k_{obs}) in phosphodiester/acetonitrile solutions at pH 4.0 was found to increase with increased concentrations of the phosphodiesters (Table 1).[11] The rate increased from 7.38×10^{-3} to 11.0×10^{-3} s^{-1} when the concentration of dibenzyl phosphate was increased from 0.95 to 3.8 mM. The k_{obs} in dibutyl phosphate increased from 6.42×10^{-3} to 9.16×10^{-3} s^{-1} when the concentration was increased from 2.4 to 14 mM. Similarly, k_{obs} of the disappearance of quinone methide **1** in diethyl phosphate also increased from 6.95×10^{-3} to 10.6×10^{-3} s^{-1} with increased concentrations from 9.5 to 38 mM.

A plot of k_{obs} versus the phosphate concentrations from Table 1 is shown in Figure 2. The linear dependencies with each dialkyl phosphate confirms phosphodiester

(10) Velek, J.; Koutek, B.; Soucek, M. *Collect. Czech. Chem. Commun.* **1979**, *44*, 110−122.

(11) The ionic strength of the reactions was not normalized due to problems in consistent solubility in all of the reactions analyzed and due to competitive nucleophilic reactions with various salts. On the basis of the relative increase in the observed rate of the loss of **1** with the three different dialkyl phosphates at nearly equivalent salt concentrations (e.g., Table 1, 9.5 mM), it appears that changes in ionic strength do not account for the relative rate differences in this reaction series at the concentration range studied. It has been observed with hydrolysis of highly stabilized benzylic carbenium ions (similar to our protonated quinone methide) that ionic strength effects are less important than the nature of the anion component.[12]

(12) Crugeiras, J.; Maskill, H. *Can. J. Chem.* **1999**, *77*, 530−536.

Table 2. Rates (k_{obs}) of Quinone Methide Disappearance in Buffered Phosphate/CH_3CN Solution (28.5%, pH 7.0)[a]

	inorganic phosphate[b]		dibenzyl phosphate[c]		dibutyl phosphate[c]		diethyl phosphate[c]	
entry	concn (mM)	rate ($\times 10^{-4}$ s^{-1})	concn (mM)	rate ($\times 10^{-4}$ s^{-1})	concn (mM)	rate ($\times 10^{-4}$ s^{-1})	concn (mM)	rate ($\times 10^{-4}$ s^{-1})
1	9.5	9.24 ± 0.03	0.95	9.92 ± 0.26	2.4	9.62 ± 0.26	9.5	9.26 ± 0.36
2			1.9	10.0 ± 0.38	4.8	9.48 ± 0.20	19	9.49 ± 0.22
3			2.9	10.0 ± 0.31	9.5	9.64 ± 0.16	29	9.31 ± 0.23
4			3.8	9.81 ± 0.14	14	9.07 ± 0.21	38	9.05 ± 0.05

[a] All experiments were monitored at 288 nm by UV spectroscopy in triplicate at 25.0 °C. The rates were calculated as the slope of ln A/t. [b] The rate was determined at only one concentration on the basis that hydrolysis at pH 4.0 was independent of phosphate concentration. [c] All the dialkyl phosphate buffer acetonitrile mixtures contained 10.0 mM inorganic phosphate to effectively buffer the pH.

alkylation is a second-order process. The rates (k_1) of phosphodiester alkylation determined from the slope of the line are 1.3 $M^{-1}\cdot s^{-1}$ for dibenzyl phosphate, 0.24 $M^{-1}\cdot s^{-1}$ for dibutyl phosphate, and 0.13 $M^{-1}\cdot s^{-1}$ for diethyl phosphate at pH 4.0 in 28.5% aqueous acetonitrile solutions at 25.0 °C. The rate (k_1) of dibenzyl phosphate alkylation is approximately 5 times faster than that of dibutyl phosphate and 10 times that of diethyl phosphate. The difference in the rates of phosphodiester alkylation is likely due to the different nucleophilicities of these dialkyl phosphates. The variation in nucleophilicities may be the result of solvation effects.[13]

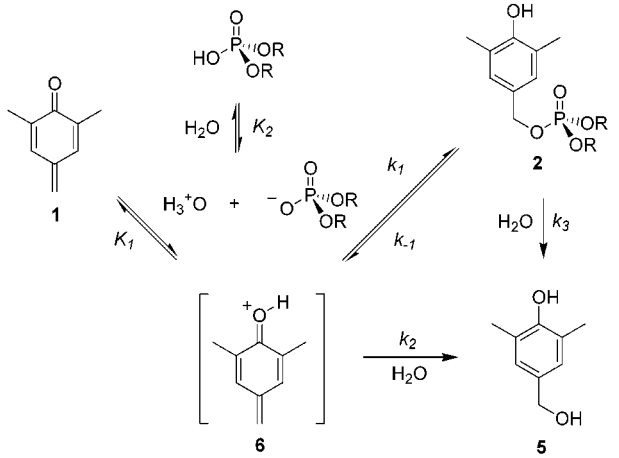
The relative second-order rate constant analysis suggests that dibenzyl phosphate (k_1 = 1.3 $M^{-1}\cdot s^{-1}$) is approximately 3700 times more reactive than water (k_2 = 3.5 × 10^{-4} $M^{-1}\cdot s^{-1}$) in reacting with quinone methide **1** under these reaction conditions at pH 4.0. The least reactive phosphodiester, diethyl phosphate, is still ~370 times more reactive than water.

Comparison of the reaction monitored by ^{1}H NMR analysis to those monitored by UV analysis reveals that the rate constant for diethyl phosphate alkylation (k_1) is approximately 8-fold larger by UV analysis. The reaction monitored by ^{1}H NMR was conducted in 10% D_2O/CD_3CN at 20.0 °C and provided a k_1 of 1.6 × 10^{-2} $M^{-1}\cdot s^{-1}$. The reaction monitored by UV analysis was conducted in 28.5% H_2O/CH_3CN at 25.0 °C and provided a k_1 of 0.13 $M^{-1}\cdot s^{-1}$. This 8-fold rate constant enhancement for diethyl phosphate alkylation in going from 10% D_2O to 28.5% H_2O in acetonitrile is not observed in the hydrolysis rate constants, which remained relatively constant (3.5 × 10^{-4} versus 3.8 × 10^{-4} $M^{-1}\cdot s^{-1}$, respectively). This difference is attributed to a combination of temperature, solvent polarity,[14] and solvent isotope effects.[6e,10]

The rate of hydrolysis of quinone methide **1** at pH 7.0 was determined in a manner similar to those described at pH 4.0. The rate of hydrolysis of quinone methide **1** (k_{obs}) was determined at a single aqueous buffered inorganic phosphate/acetonitrile concentration (9.5 mM) based on the finding that the hydrolysis rate was independent of the inorganic phosphate buffer concentration. The rate k_{obs} in inorganic phosphate at pH 7.0 was determined to be 9.24 × 10^{-4} s^{-1} at 25.0 °C (Table 2). This was approximately 15% of the rate at pH 4.0 (6.05 × 10^{-3} s^{-1}). This pH dependent rate of hydrolysis agrees with previous reported results.[7,10]

The rates of quinone methide **1** disappearance (k_{obs}) in aqueous buffered phosphodiester/acetonitrile solution at pH 7.0 were similarly determined. All dialkyl phosphate stock solutions at pH 7.0 contained 10 mM inorganic phosphate to effectively buffer the pH. All

Scheme 4



experiments were run in triplicate and the pH of the reaction solution remained unchanged throughout the reaction. The rates of quinone methide **1** disappearance in buffered dialkyl phosphate/acetonitrile solutions at pH 7.0 were nearly identical to the rate of the hydrolysis (k_{obs} ≈ 9. 24 × 10^{-4} s^{-1}) (Table 2). This reveals that hydrolysis predominates at pH 7.0. This was confirmed by ^{1}H NMR analysis in 10% D_2O/CD_3CN at pH 7.0 where only hydrolysis product **5** was observed. This emphasized the acid-catalyzed nature of phosphodiester alkylation by quinone methide **1**.[5]

Based on these experimental results, the following mechanism for the reaction of quinone methide **1** in an aqueous buffered phosphodiester/acetonitrile solution at pH 4.0 is proposed (Scheme 4). Two fast equilibriums exist in the reaction: the protonation of quinone methide **1**, resulting in the low steady-state concentration of reactive intermediate **6** (K_1), and ionization of dialkyl phosphoric acid (K_2). Protonated quinone methide intermediate **6** reacts with dialkyl phosphate to form phosphotriester **2** (k_1). The equilibrium nature of this reaction is primarily based on our previous studies,[5] and the accuracy afforded to the simulation of this mechanism when this reversibility (k_{-1}) is incorporated. Benzyl alcohol **5** is produced from both the direct hydrolysis of intermediate **6** (k_2) and the slow hydrolysis of phosphotriester **2** (k_3). The hydrolysis of phosphotriester **2d** to benzyl alcohol **5** was clearly observed by ^{1}H NMR analysis as the slow decrease of **2d** coincided with the slow increase of **5** after approximately 15 min in the reaction (see Figure 1). This mechanism accounts for the rate of quinone methide disappearance by the combined rates of phosphodiester alkylation and quinone methide hydrolysis. The proposed mechanism indicates that phosphodiester alkylation is an acid-catalyzed, second-order process and quinone methide hydrolysis is a specific acid

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(14) Bunton, C. A.; Mhala, M. M.; Moffatt, J. R. *J. Org. Chem.* **1984**, *49*, 3639−3641.

catalyzed process. This proposed mechanism is fully correlated by both ^{1}H NMR and UV analysis results.

A complete simulation of the ^{1}H NMR experimental results according to this proposed mechanism afforded the plotted curves shown in Figure 1. All of the triplicated experimental data were within 3% of the simulated reaction curves. This further supports the proposed mechanism of *p*-quinone methide phosphodiester alkylation and hydrolysis according to Scheme 4.

Conclusion

We have extended our investigation of phosphodiester alkylation with a *p*-quinone methide in aqueous buffered phosphodiester/acetonitrile solutions in order to determine the competitive hydrolysis during phosphodiester alkylation. Our ^{1}H NMR studies revealed that phosphodiester alkylation occurs with a faster rate constant relative to hydrolysis at pH 4.0. This is attributed to the higher nucleophilicity of the phosphodiester relative to water. Further investigation of quinone methide **1** reactions by UV analysis in various aqueous buffered phosphate/acetonitrile solutions confirmed this result as an acid-catalyzed, second-order process.

These investigations continue to direct our design process for development of a DNA phosphodiester alkylating reagent for potential in vivo applications. On the basis of these investigations, the phosphodiester alkylating reagent will incorporate appropriate functionality to accomplish quinone methide activation at neutral pH, increase the effective concentration of the phosphodiester target to outcompete hydrolysis, and trap the phosphotriester product to prevent conversion to the hydrolysis products.[8b]

Experimental Section

All commercially available compounds were purchased from Aldrich Chemical Co. (Milwaukee, WI), Lancaster Synthesis, Inc. (Windham, NH), or Acros Organics (Fisher Scientific) and used without further purification unless noted otherwise. Deuterium solvents were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA) and Merck Sharp & Dohme Limited (Montreal, Canada). Diethyl phosphoric acid was vacuum distilled at 0.3 Torr prior to use. All H_2O was distilled and deionized through a Milli-Q RG purification system. Acetonitrile was passed through a Solv-Tek ST-002 Solvent Purification System (Solv-Tek Inc., Berryville, VA). pH was measured with a Ag/AgCl electrode/Accumet 910 at 24.0 °C. ^{1}H NMR analysis was carried out on a JEOL 270 NMR spectrometer. UV data were recorded at 288 nm using a Hitachi U-2000 spectrometer with a temperature-controlled cell at 25.0 ± 0.1 °C.

Quinone Methide (1) Solution. A solution of 2,4,6-trimethylphenol (1.1 mg, 8.1 μmol) in CD_3CN (1.10 mL) was oxidized with silver(I) oxide (150 mg) by stirring at room temperature for 40 min. The suspension was filtered with glass wool to give known quinone methide **1** in solution:[15] ^{1}H NMR (CD_3CN, 270 MHz) δ 7.10 (s, 2H), 5.86 (s, 2H), 2.10 (s, 6H).

Study of Quinone Methide (1) Reaction in a Buffered Diethyl Phosphate/Acetonitrile Solution (1:9 v/v, pH 4.0, 20.0 °C). To a solution of diethylphosphoric acid (39.2 mg, 0.250 mmol in 800 μL D_2O) was added a solution of KOD (40% in D_2O, 40 μL). The pH of the resulting solution was adjusted with a solution of methanesulfonic acid (141.8 mg in 1.00 mL D_2O). The final volume of buffered diethyl phosphate solution was adjusted to 1.0 mL with D_2O. The resulting diethyl phosphate solution (100 μL) was added to a solution of quinone methide **1** in CD_3CN (7.3 mM, 900 μL), prepared as described as above. The pH of the final reaction solution was measured as 4.0 and the final concentrations of quinone methide **1** and diethyl phosphate were 6.6 and 25 mM, respectively. The reaction was monitored by ^{1}H NMR analysis over 40 min approximately 9 half-lives) at 20.0 ± 0.1 °C. The percent conversion to trialkyl phosphate **2d** was calculated on the basis of area integration of the resonance of benzylic protons of **2d** (at 4.84 ppm) relative to the resonance of alkylidene protons of quinone methide **1** (at 5.86 ppm). The percent of benzyl alcohol **5** was calculated on the basis of the area integration of the resonance of the benzylic protons of **5** (at 4.36 ppm). Formation of **5** was further confirmed by adding authentic benzyl alcohol **5** to the reaction. The pH of the reaction solution remained unchanged throughout the reaction. The experiment was repeated at least three times. Trialkyl phosphate **2d** (while not sufficiently stable to allow isolation, the following solution characterization was in accord with previously reported analysis):[5] ^{1}H NMR (10% D_2O in CD_3CN, 270 MHz) δ 6.95 (s, 2H), 4.84 (d, $^3J_{HP}$ = 8.4 Hz, 2H), 3.96−4.07 (m, 4H), 2.14 (s, 6H), 1.23−1.28 (m, 6H).

Study of Quinone Methide (1) Reaction in Buffered Phosphate/Acetonitrile Solution (3:7 v/v, pH 4.0, 25 °C). A stock solution (10.0 mM) of 2,4,6-trimethylphenol was prepared by dissolving the phenol (13.6 mg, 0.100 mmol) in CH_3CN (10.0 mL). A 1.00 mM solution (10.0 mL) of 2,4,6-trimethylphenol was obtained by diluting the stock solution (10.0 mM, 1.00 mL) with CH_3CN. The resulting 1.00 mM trimethylphenol solution was oxidized to quinone methide **1** by stirring with silver(I) oxide (250 mg) at room temperature for 45 min. The suspension was filtered with an Acrodisc filter (13 CR, 0.45 μm) to afford the desired quinone methide solution (1.00 mM, 10.0 mL).

A stock solution (240 mM, 6.0 mL) of inorganic phosphoric acid was prepared by diluting the acid (85%, 166.0 mg, 1.44 mmol) with H_2O. Inorganic phosphate solutions (5.4 mL) of various concentrations were prepared by diluting the stock solution (240 mM; 250, 500, 750 or 1000 μL) with CH_3CN (4.20 mL) and H_2O (950, 700, 450 or 200 μL, correspondingly). The resulting solutions were adjusted to pH 4.0 with 2, 0.2 and 0.02 M NaOH aqueous solution and H_2O to afford the final buffered inorganic phosphate/acetonitrile solutions (3:7 v/v, 6.0 mL of 10, 20, 30, or 40 mM, respectively).

A stock solution (24.0 mM, 6.0 mL) of dibenzyl phosphoric acid was prepared by dissolving the acid (40.0 mg, 0.144 mmol) with CH_3CN. Dibenzyl phosphate solutions (5.40 mL) of various concentrations were prepared by diluting the stock solution (24.0 mM; 250, 500, 750, or 1000 μL) with CH_3CN (3.95, 3.70, 3.45 or 3.20 mL, correspondingly) and H_2O (1.20 mL). The resulting solutions were adjusted to pH 4.0 with 2, 0.2 and 0.02 M NaOH aqueous solution and H_2O to afford the final buffered dibenzyl phosphate/acetonitrile solutions (3:7 v/v, 6.0 mL of 1.0, 2.0, 3.0, or 4.0 mM, respectively).

A stock solution (120 mM, 6.0 mL) of dibutyl phosphoric acid was prepared by diluting the acid (151.3 mg, 0.720 mmol) with CH_3CN. Dibutyl phosphate solutions (5.40 mL) of various concentrations were prepared by diluting the stock solution (120 mM; 125, 250, 500, or 750 μL) with CH_3CN (4.07, 3.95, 3.70, or 3.45 mL, correspondingly) and H_2O (1.20 mL). The resulting solutions were adjusted to pH 4.0 with 2, 0.2, and 0.02 M NaOH aqueous solution and H_2O to afford the final buffered dibutyl phosphate/acetonitrile solutions (3:7 v/v, 6.0 mL of 2.5, 5.0, 10, or 15 mM, respectively).

A stock solution (240 mM, 6.0 mL) of diethyl phosphoric acid was prepared by diluting the acid (221.9 mg, 1.44 mmol) with H_2O. Diethyl phosphate solutions (5.40 mL) of various concentrations were prepared by diluting the stock solution (240 mM; 250, 500, 750, or 1000 μL) with CH_3CN (4.20 mL) and H_2O (950, 700. 450, or 200 μL, correspondingly). The resulting solutions were adjusted to pH 4.0 with 2, 0.2, and 0.02 M NaOH aqueous solution and H_2O to afford the final buffered diethyl phosphate/acetonitrile solutions (3:7 v/v, 6.0 mL of 10, 20, 30, or 40 mM, respectively).

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Quinone methide **1** in CH_3CN (1.00 mM, 150 μL), prepared as described as above, was added to the desired inorganic phosphate or dialkyl phosphate solution (2.85 mL) described above. The disappearance of quinone methide **1** in the reaction solution was monitored at 288 nm by UV spectroscopy at 25.0 ± 0.1 °C. All experiments were repeated at least three times. The pH of all the reaction solutions remained unchanged throughout the reaction. The rates (k_{obs}) of quinone methide **1** disappearance were calculated as the slope of ln A/t. Data are shown in Table 1.

Study of Quinone Methide (1) Reaction in Buffered Phosphate/Acetonitrile Solutions (3:7 v/v, pH 7.0, 25 °C). A diluted inorganic phosphate solution (5.40 mL) was prepared by diluting the inorganic phosphoric acid stock solution (240 mM, 250 μL) with CH_3CN (4.20 μL) and H_2O (950 μL). The resulting phosphate solution was adjusted to pH 7.0 with 2, 0.2, and 0.02 M NaOH aqueous solution and H_2O to afford the final buffered inorganic phosphate/acetonitrile solutions (3:7 v/v, 6.0 mL, 10 mM).

Dibenzyl phosphate solutions (5.40 mL) of various concentrations were prepared by diluting the dibenzyl phosphoric acid stock solution (24.0 mM; 250, 500, 750, or 1000 μL) and inorganic phosphoric acid solution (240 mM, 250 μL) with CH_3CN (3.95, 3.70, 3.45, or 3.20 mL, correspondingly) and H_2O (950 μL). The resulting solutions were adjusted to pH 7.0 with 2, 0.2, and 0.02 M NaOH aqueous solution and H_2O to afford the final buffered dibenzyl phosphate/acetonitrile solutions (3:7 v/v, 6.0 mL of 1.0, 2.0, 3.0, or 4.0 mM, respectively).

Dibutyl phosphate solutions (5.40 mL) of various concentrations were prepared by diluting the dibutyl phosphoric acid stock solution (120 mM; 125, 250, 500, or 750 μL) and inorganic phosphoric acid solution (240 mM, 250 μL) with CH_3CN (4.075, 3.95, 3.70, or 3.45 mL, correspondingly) and H_2O (950 μL). The resulting solutions were adjusted to pH 7.0 with 2, 0.2, and 0.02 M NaOH aqueous solution and H_2O to afford the final buffered dibutyl phosphate/acetonitrile solutions (3:7 v/v, 6.0 mL of 2.5, 5.0, 10, 15 mM, respectively).

Diethyl phosphate solutions (5.50 mL) of various concentrations were prepared by diluting the diethyl phosphoric acid stock solution (240 mM; 250, 500, 750, or 1000 μL) and inorganic phosphoric acid solution (240 mM, 250 μL) with CH_3CN (4.20 mL) and H_2O (750, 500, 250, or 0 μL, correspondingly). The resulting solutions were adjusted to pH 7.0 with 2, 0.2, and 0.02 M NaOH aqueous solution and H_2O to afford the final buffered diethyl phosphate/acetonitrile solutions (3:7 v/v, 6.0 mL of 10, 20, 30, or 40 mM, respectively).

Quinone methide **1** solution in CH_3CN (1.00 mM, 150 μL), prepared as described as above, was added to the desired inorganic phosphate or dialkyl phosphate solution (2.85 mL) described above. The disappearance of quinone methide **1** in the reaction solution was monitored at 288 nm by UV spectroscopy at 25.0 ± 0.1 °C. All the experiments were repeated at least three times. The pH of all the reaction solutions remained unchanged throughout the reaction. The rates (k_{obs}) of quinone methide **1** disappearance were calculated as the slope of ln A/t. Data are shown in Table 2.

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