VOLUME 121, NUMBER 22
JUNE 9, 1999

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Change of the Hydrolytic Mechanism of 2-Hydroxy H-Phosphonodiesters in Aprotic Organic Media. *cis*-1,2-Diol Monoanions as Leaving Groups

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Received July 9, 1998

Abstract: The hydrolysis of H-phosphonodiesters bearing a vicinal hydroxyl group is found to be subject to two competing reaction pathways in aprotic organic media. An observation of the increased proportion of *cis*-1,2-diol leaving with decrease of the water content is interpreted in terms of a change of the hydrolytic mechanism on changing the reaction medium from aqueous to nonaqueous. The hydroxyl group in *cis*-1,2-diol monoanions hydrogen bonds strongly to the adjacent oxyanion, implying a low-energy route closely related to reactions, catalyzed by large ribozymes.

Introduction

The discovery of RNA enzymes (ribozymes) has been accompanied by the suggestion of a new phosphoryl transfer mechanism involving exchange of 1,2-diols.[1] The corresponding non-ribozymic reaction has never been observed in neutral aqueous solutions since the participation of the adjacent hydroxyl group is nucleophilic and simply leads to phosphate migration or mono-ol exchange.[2] Recently, however, we reported the preparation of sugar H-phosphonates **2** based on the reaction in dioxane/pyridine of H-phosphonic acid with sugars in the presence of an oxirane as a condensation agent[3a] (Scheme 1). By analogy with a similar reaction of sugar phosphinylation by phosphinic acid and oxirane,[3b] we have tentatively assumed a transient formation of the 2-hydroxyalkyl sugar H-phosphonodiester **1** that undergoes hydrolysis to yield the H-phosphonate **2** and the 1,2-diol **3**. The presence of the latter in the reaction products requires 1,2-diol monoanions to be better leaving groups than sugar alkoxides, but this is never observed in neutral aqueous solutions. Reaction 1, however, proceeds in aprotic organic solvents only. To this end, we studied the controlled hydrolysis of 2-hydroxyphenyl and 2-hydroxyethyl alkyl H-phosphonodiesters in organic media, and now, we report on the change of the hydrolytic mechanism due to the change of the nature of the 2-hydroxy group participation. This reaction is considered as a bioorganic model reaction that is congruent to the hydrolysis and transesterification, catalyzed by large ribozymes (group I, group II, and spliceosomal introns).[1d] The medium effect proves to control the nature of the participation

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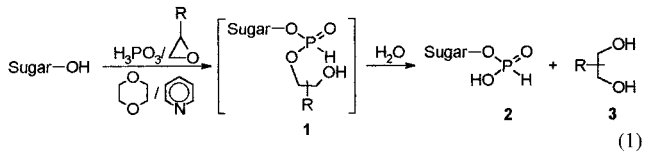
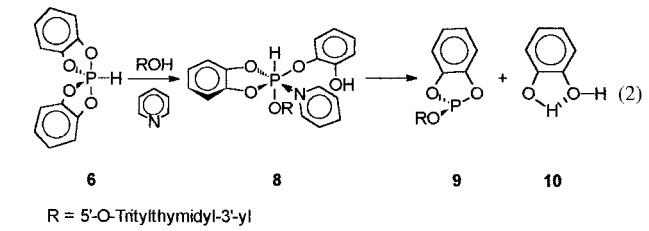
10.1021/ja982413u CCC: $18.00
Published on Web 05/22/1999

Scheme 1



(1)

Scheme 2



(2)

R = 5′-O-Tritylthymidyl-3′-yl

of the adjacent hydroxyl group, thus providing different H-phosphonylation mechanisms in aqueous and organic media.

Results and Discussion

H-phosphonodiesters bearing a vicinal hydroxyl group were chosen as mimics of the neutral ionic form of RNA phosphodiester bonds. Unlike phosphodiesters, they are readily soluble in organic solvents and hydrolyzable but are not easily accessible in pure form. Thus 5′-*O*-trityl thymidine 2-hydroxyphenyl H-phosphonate **4** (Scheme 3) was obtained in situ by transesterification of the 2-hydroxyphenyl phenylene phosphite **5** (Chart 1). When the corresponding bicyclic H-tetraoxaspirophosphorane **6** (Scheme 2) of **5** was treated in dry dioxane/pyridine (1:1, v/v) at an elevated temperature (80 °C) with an equimolar amount of 5′-*O*-trityl thymidine **7** (Chart 1), the ^{31}P spectrum indicated the product distribution shown in Figure 1. Besides the dominating signals for the hexacoordinated intermediate **8** and the transesterification product **9** (Scheme 2), doublets at 3.3 ppm (J_{P-H} = 633.4 Hz), 4.2 ppm (J_{P-H} = 648.7 Hz), and 8.0 ppm (J_{P-H} = 719.1 Hz), corresponding to the H-phosphonates **4**, **11**, and **12** (Figure 1a and Scheme 3), can be detected.

To account for the occurrence of the above H-phosphonates, we have tentatively assumed that they are hydrolytic products of the triphosphite **9** for the uncomplete exclusion of moisture. Actually, the addition of 1 equiv of water causes a decrease of the signal intensities of **8**, **9**, and the 2-hydroxy H-phosphonodiester **4** and a parallel increase of the intensities of the H-phosphonomonoesters **11** and **12** (Scheme 3, Figure 1b). This interpretation is supported by HPLC analysis of the reaction mixture, indicating similar product distributions for **11** and **12**. Moreover, the addition of more than 1 equiv of water results in a sharp increase of the yield of the 2-hydroxyphenyl H-phosphonate **11** (71% at 3 equiv of water) at the expense of a sharp drop in the H-phosphonate **12** (18%) (Figure 1c). Under the same experimental conditions the deoxy analogue of **4**, 5′-*O*-tritylthymidine phenyl H-phosphonate **16** (Chart) affords exclusively the nucleoside H-phosphonate **12** in accord with the leaving group abilities of phenoxy (pK_a ca. 10) and 5′-*O*-tritylthymidinoxy (pK_a ca. 12.3) groups. Therefore, the nucleophilic participation of the 2-hydroxy group in **4** seems to be suppressed in organic media giving rise to a change of the H-phosphonyl transfer mechanism from mono-ol (3.1) to diol-expelling hydrolytic cleavage (3.2) on changing the reaction medium from aqueous to organic (Scheme 3).

The prevention of the vicinal phenolic hydroxyl from nucleophilic participation during the hydrolysis of 5′-*O*-trityl thymidine 2-hydroxyphenyl H-phosphonate **4** is possible provided it hydrogen bonds to the adjacent ether and/or phosphonyl oxygen(s) (Scheme 3). Besides prevention from internal nucleophilic attack this hydrogen bonding renders the H-phosphonodiester increased sensitivity to an external nucleophilic attack (substrate activation). Furthermore, the stronger negative charge-assisted hydrogen bonding in the intermediate/transition state **14** (Scheme 3) should accelarate hydrolysis (electrophilic or general acid catalysis). This hydrogen bonding should persist in organic but not in aqueous solutions, since in the latter the adjacent hydrogen donor and acceptor are better solvated separately.

It has long been known that *cis*-1,2-diols are more acidic than their 2-deoxy analogues due to stabilization of the diol monoanion by a favorable dipole−anion interaction or by a hydrogen bond of the un-ionized hydroxyl with the adjacent oxyanion (Scheme 4).[4] Actually, the first pK_a of catechol **10** (9.22) is lower than that of phenol (10.00) and the second, the pK_a of the catechol monoanion **10′**, is very high at 13.[5] Moreover, in $CDCl_3$, we observed a large downfield shift ($\Delta\delta$ = 4.5 ppm) of the hydroxyl proton in the ^{1}H NMR spectrum relative to catechol hydroxyl protons and an intense continuous absorption in the IR spectrum indicative[6] of strong intramolecular hydrogen bonding in the catechol monoanion **10′**. The development of such a strong hydrogen bond should result in intramolecular proton-transfer catalysis[7] of the decomposition of the H-tetraoxyphosphorane monoanion **14** (Scheme 3). This selective microsolvation of the transition state/intermediate **14** is absent both in the 2-deoxy transition state **14** and transition state **15** for the intramolecular reaction 3.1 (Scheme 3).

The high reactivity of the 2-hydroxyphenyl H-phosphonodieter **4** prevents its isolation for studies on intramolecular hydrogen bonding and its effects on the rate of the hydrolytic cleavage, so we looked for an independent system for corroboration of the results obtained using the aromatic (2-hydroxyphenyl) system **4**. If the reaction rate is sensitive to the basicity of the leaving group, the substitution of the catechol monoanion by a glycol monoanion should result in a decrease in reactivity. Thus the aliphatic 2-hydroxyethyl system **17**, 2-hydroxyethyl ethyl H-phosphonate (Scheme 5), is easily accessible[8] and hydrolyzable at a measurable rate even at room temperature.

In 50% aqueous pyridine (pH > 5.0) the 2-hydroxy phosphonodiester **17** underwent fast hydrolysis, yielding only the 2-hydroxy H-phosphonate **18**, while the hydrolysis of the 2-deoxy derivative (diethyl H-phosphonate **20**, Chart) was very slow (data not shown). Under the conditions used for the controlled hydrolysis of **4** (dioxane/pyridine, 1 equiv of water) even at room-temperature signals for both 2-hydroxyethyl H-phosphonate **18** (36%) and ethyl H-phosphonate **19** (34%) are seen in the ^{31}P NMR spectrum (Figure 2) 20 min after the reaction has been started. During the same period of time, the deoxy derivative **20** remained unchanged (Figure 2). Therefore, the presence of the vicinal hydroxyl group accelerates both the mono-ol (5.1) and the diol (5.2) expelling reaction (Scheme 5).

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Scheme 3

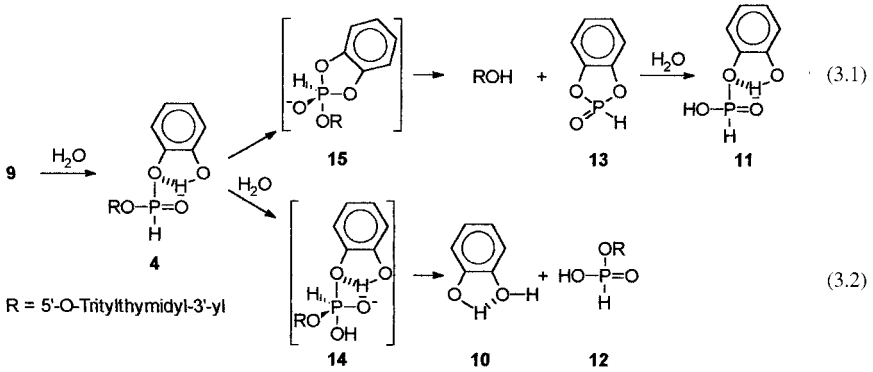
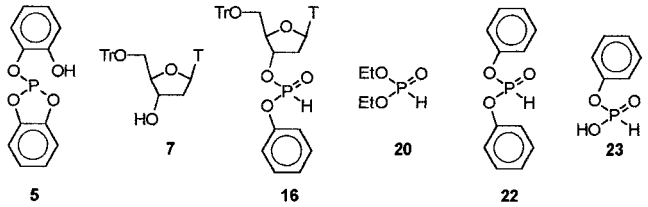


Chart 1



Since the latter reaction is observed in non-hydrogen-bonding solvents only, the electrophilic assistance of the external nucleophilic attack **b** by hydrogen bonding of the 2-hydroxyl to the phosphonyl oxygen is possible (route 5.2, Scheme 5). Actually, the ^{1}H NMR spectrum of **17** in $CDCl_3$ exhibits a hydroxyl proton at $\delta = 4.7$ ppm[8] and a downfield shift of the ^{31}P NMR resonance ($\Delta\delta = 1.7$ ppm). These spectral properties are characteristic of the hydrogen-bonded hydroxyl and of the increased positive charge at the phosphonyl phosphorus, respectively. Furthermore, our preliminary ab initio quantum chemical calculations indicate that in the gas phase the glycol monoanion in the cis conformation is stabilized by more than 9 kcal/mol than in the fixed trans conformation due to the favorable ion−dipole or hydrogen bonding interactions.

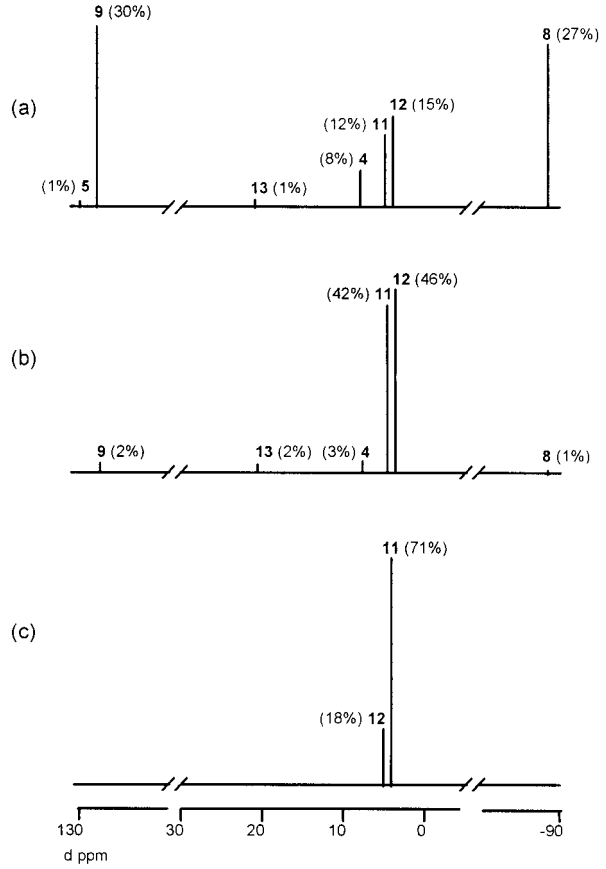
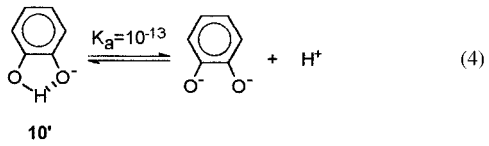
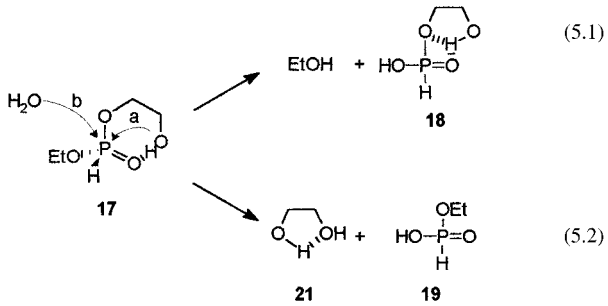


Figure 1. Product distribution for the reaction of the H-tetraoxaspirophosphorane **6** and an equivalent amount of 5′-*O*-trityl thymidine in dry dioxane/pyridine (a) and in dioxane/pyridine containing 1 equiv (b) and 3 equiv of water (c) as judged from the ^{31}P NMR spectrum of the reaction mixture 20 min after the reaction has been started.

Scheme 4



Scheme 5



The reaction pathway 5.2 in Scheme 5 is congruent to that used by large ribozymes. Unlike small-ribozyme mechanisms, the large-ribozyme mechanism has been studied up to now directly, without preliminary elucidation of the mechanism of the nonribozymic reaction and the means by which it can be accelerated.[1c] Although the reaction 5.2 is somewhat far from the best model reaction, some implications for the large-ribozyme mechanism can be drawn. The observed distinct medium effect on the nature of the 2-hydroxy group participation predicts effective control of the local environment of the substrate 2-hydroxyl in the ribozyme active site. Only a medium with non-hydrogen-bonding properties can provide effective differential microsolvation of the transition state of the diol-expelling reaction 5.2 and better leaving group properties of the 1,2-diol monoanions. Studies of a better model on a phosphate level are in progress in this laboratory.

Conclusion

2-Hydroxyphenyl and 2-hydroxyethyl alkyl H-phosphonodiesters undergo a controlled hydrolytic cleavage in dioxane/pyridine (1:1 v/v), yielding alkyl H-phosphonomonoester and the corresponding 1,2-diol. Since this reaction proceeds in

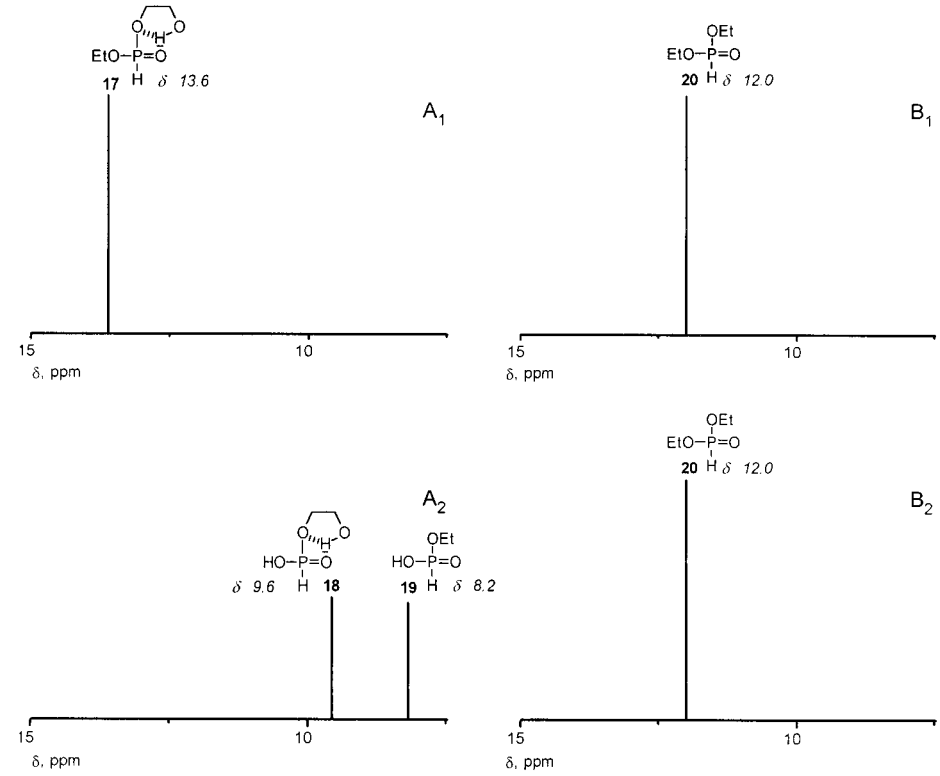


Figure 2. Product distribution for the reactions of ethyl 2-hydroxyethyl H-phosphonodiester **17** and diethyl H-phosphonodiester **20** with 1 equiv of H_2O in dioxane/pyridine at 0 min (A_1 and B_1) and 20 min later (A_2 and B_2), 25 °C.

nonaqueous solutions only, the observation is discussed in terms of electrophilic participation of the adjacent hydroxyl by hydrogen bonding to the ether and/or phosphonyl oxygen. This interaction increases the leaving-group ability of the 1,2-diol anions and changes the H-phosphonyl transfer mechanism on changing the reaction medium from aqueous to nonaqueous. The observed solvent-induced mechanistic change provides important implications concerning the mechanism of action of large ribozymes (group I, group II, and Spliceosomal introns) and the nature of their active sites.

Experimental Section

General Procedures and Materials. Commercial solvents and reagents were used as received unless otherwise noted. Dioxane was dried over sodium and distilled before use. Pyridine was dried over sodium hydroxide and distilled over calcium hydride before use. 5′-*O*-Trityl thymidine[9] **7** and 2λ^5-2,2′-spirobi [1,3,2-benzodioxaphosphole][10] **6** were prepared as described.

Spectral and Physical Data. ^{31}P, ^{13}C, and ^{1}H NMR spectra were taken on a Bruker DRX-250 spectrometer. ^{1}H and ^{13}C NMR chemical shifts are reported in δ (ppm) relative to internal tetramethylsilane. ^{31}P chemical shifts are reported in δ (ppm) downfield (+) and upfield (−) from external 85% H_3PO_4. The assignment of the signals is based on ^{1}H coupling and literature data. The assignment of the signals of **17** is based on the COSY and HMQC NMR experiments.

Reverse phase HPLC analyses were performed on a Waters chromatography system using a Nucleosil 100−5C_{18} column, flow rate 0.8 mL/min, and UV detection at 280 nm. Eluting system: 38% CH_3-CN, 62% 20 mM NaH_2PO_4/Na_2HPO_4 buffer, pH 7.

Reaction of 2λ^5-2,2′-Spirobi[1,3,2-benzodioxaphosphole] 6 with 5′-*O*-Trityl Thymidine 7 under Anhydrous Conditions. To a solution of 2λ^5-2,2′-spirobi[1,3,2-benzodioxaphosphole] **6** (0.293 g, 1.10 mmol) in 1 mL of dry dioxane in an NMR tube was added a solution of 5′-*O*-trityl thymidine **7** (0.514 g, 1.06 mmol) in 1 mL of dry pyridine upon rapid stirring. The resulting mixture was heated to 80 °C and stirred for 20 min. Its ^{31}P NMR (101.26 MHz, dioxane/pyridine, 25 °C) spectrum is shown in Figure 1a: δ = 127.7 (d, $J_{P-O-C^{3'}-H}$ = 8.6 Hz), *o*-phenylene 5′-*O*-tritylthymidyl phosphite (**9**, R = 5′-*O*-tritylthymidyl), 30% yield; δ = 8.0 (dd, J_{P-H} = 719.1 Hz, $J_{P-O-C^{3'}-H}$ = 7.8 Hz), *o*-hydroxyphenyl 5′-*O*-trityl thymidine H-phosphonate (**4**, R = 5′-*O*-tritylthymidyl), 8% yield; δ = 4.2 (d, J_{P-H} = 648.7 Hz), *o*-hydroxyphenyl H-phosphonate (**11**), 12% yield; δ = 3.3 (dd, J_{P-H} = 633.4 Hz, $J_{P-O-C^{3'}-H}$ = 8.9 Hz), 5′-*O*-trityl thymidine H-phosphonate (**12**, R = 5′-*O*-tritylthymidyl), 15% yield; δ = −89.0 (d, J_{P-H} = 863.7 Hz), hexacoordinated intermediate **8** (R = 5′-*O*-tritylthymidyl), 27% yield.

Reaction of 2λ^5-2,2′-Spirobi[1,3,2-benzodioxaphosphole] 6 with 5′-*O*-Trityl Thymidine 7 in the Presence of 1 Equiv of Water. To a solution of 2λ^5-2,2′-spirobi[1,3,2-benzodioxaphosphole] **6** (24.0 mg, 90.2 μmol) in 0.3 mL of dry dioxane in a NMR tube was added a solution of 5′-*O*-trityl thymidine **7** (41.0 mg, 84.6 μmol) and water (1.30 mg, 72.1 μmol) in 0.1 mL of dry pyridine. The resulting mixture was heated to 80 °C under stirring for 1 h and then analyzed: HPLC: *RT* = 0.8 min, ^{31}P NMR (101.26 MHz, dioxane/pyridine, 25 °C) δ = 8.9 (d, J_{P-H} = 647.4 Hz), *o*-hydroxyphenyl H-phosphonate (**11**), 42% yield; HPLC: *RT* = 1.1 min, ^{31}P NMR (101.26 MHz, dioxane/pyridine, 25 °C) δ = 7.2 (dd, J_{P-H} = 632.8 Hz, $J_{P-O-C^{3'}-H}$ = 8.9 Hz), 5′-*O*-trityl thymidine H-phosphonate (**12**, R = 5′-*O*-tritylthymidyl), 46% yield (Figure 1b).

Reaction of 2λ^5-2,2′-Spirobi[1,3,2-benzodioxaphosphole] 6 with 5′-*O*-Trityl Thymidine 7 in the Presence of 3 Equiv of Water. The reactants were mixed following the same procedure as above, but instead of 1 equiv, 3 equiv of water were added to the pyridine solution of 5′-*O*-trityl thymidine. The resulting mixture was heated to 80 °C under stirring for 20 min and then analyzed: HPLC: *RT* = 1.1 min, ^{31}P NMR (101.26 MHz, dioxane/pyridine, 25 °C) δ = 4.4 (dd, J_{P-H} = 627.4 Hz, $J_{P-O-C^{3'}-H}$ = 9.0 Hz), 5′-*O*-trityl thymidine H-phosphonate (**12**, R = 5′-*O*-tritylthymidyl), 18% yield; HPLC: *RT* = 0.8 min, ^{31}P NMR (101.26 MHz, dioxane/pyridine, 25 °C) δ = 3.9 (d, J_{P-H} = 645.8 Hz), *o*-hydroxyphenyl H-phosphonate (**11**), 71% yield (Figure 1c).

Hydrolysis of 5′-*O*-Trityl Thymidine Phenyl H-Phosphonate (16) in the Presence of 1 Equiv of Water. To a solution of 0.263 mmol (127.5 mg) 5′-*O*-trityl thymidine (**7**) in were added 0.5 mL of dry

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pyridine and 0.260 mmol (60.9 mg, 50.0 μL) of diphenyl phosphite (**22**, Chart) at room temperature upon stirring.[11] To the resulting crude 5′-*O*-trityl thymidine phenyl H-phosphonate (**16**) (mixture of 43% 5′-*O*-trityl thymidine phenyl H-phosphonate, 21% 3′-*O*-,3′-*O*-ditrityl-thymidyl H-phosphonate, 20% 5′-*O*-trityl thymidine H-phosphonate **12** and 16% phenyl H-phosphonate (**23**), ^{31}P NMR)[11] was added 0.14 mmol of water in 0.5 mL of dioxane and the solution was stirred for 30 min at room temperature: ^{31}P NMR (101.26 MHz, dioxane/pyridine, 25 °C) δ = 8.1 (dd, J_{P-H} = 717.4 Hz, $J_{P-O-C^{3'}-H}$ = 8.1 Hz), 3′-*O*-,3′-*O*-ditritylthymidyl H-phosphonate, 18%; δ = 3.8 (dd, J_{P-H} = 631.7 Hz, $J_{P-O-C-H}$ = 8.4 Hz), 5′-*O*-trityl thymidine H-phosphonate (**12**), 65%; δ = 1.4 (d, J_{P-H} = 640.8 Hz), phenyl H-phosphonate (**23**), 17%.[12]

Preparation of the Tetrabutylammonium Salt of Catechol. Equimolar amounts of a 40% water solution of tetrabutylammonium hydroxide and a methylene chloride solution of catechol (1:1, v/v) were mixed and stirred at room temperature for 30 min. Solvents were removed, and the residue was dissolved in water and lyophilized: ^{1}H NMR (anion **10′**) (250 MHz, $CDCl_3$, 25 °C) δ = 9.5 (broad s, 1H, O*H*), δ = 6.75−6.70 (m, 2H) and 6.59−6.54 (m, 2H, AA′BB′ spectrum for phenyl-H). IR ($CDCl_3$, 0.1 M solution) extended continuum with a maximum near 2200 cm^{-1}, δ_{OH} (OH group stretching vibration).[6]

Hydrolysis of Ethyl 2-Hydroxyethyl H-Phosphonate (17) and Diethyl Phosphite (20) in the Presence of 1 Equiv of Water. Ethyl 2-hydroxyethyl phosphite (ethyl 2-hydroxyethyl H-phosphonate) **17** was prepared from diethyl phosphite (**20**) and ethylene glycol as described:[8] ^{31}P NMR (101.26 MHz, dioxane, 25 °C) δ = 10.6 (d of quintets, J_{P-H} = 707.4 Hz, $J_{P-O-C-H}$ = 9.9 Hz); ^{13}C NMR (62.90 MHz, $CDCl_3$, 25 °C) δ = 67.8 (d, J = 6.0 Hz, $(CH_2OH)CH_2$), δ = 62.0 (d, J = 5.7 Hz, $(CH_2OH)CH_2$), δ = 61.5 (d, J = 4.5 Hz, CH_3CH_2), δ = 16.1 (d, J = 6.1 Hz, CH_3CH_2); ^{1}H NMR (250.13 MHz, $CDCl_3$, 25 °C) δ = 6.90 (d, J_{P-H} = 709.4 Hz, 1H, P*H*), δ = 4.68 (s, 1H, O*H*), δ = 4.19 (dq, $J_{P-O-C-H}$ = 7.03 Hz, J_{H-H} = 7.06 Hz, 2H, CH_3CH_2), δ = 4.18 (t, J_{H-H} = 7.04 Hz, 2H, $CH_2(OH)CH_2$), δ = 4.17 (dt, $J_{P-O-C-H}$ = 9.07 Hz, J_{H-H} = 7.04 Hz, 2H, $CH_2(OH)CH_2$), δ = 1.37 (t, J_{H-H} = 7.06 Hz, 3H, CH_3CH_2). The crude ethyl 2-hydroxyethyl phosphite (119 mg, 0.770 mmol) or diethyl phosphite (107 mg, 0.776 mmol) was dissolved in 0.5 mL of dry dioxane in a NMR tube and a solution of water (10.0 mg, 0.555 mmol) in 0.5 mL of dry pyridine was added upon rapid stirring. The resulting mixture was immediately analyzed: ^{31}P NMR (101.26 MHz, dioxane/pyridine, 25 °C) δ = 9.6 (dt, J_{P-H} = 630.6 Hz, $J_{P-O-C-H}$ = 9.2 Hz), 2-hydroxyethyl H-phosphonate (**18**), 36% yield; δ 8.2 (dt, J_{P-H} = 628.5 Hz, $J_{P-O-C-H}$ = 7.9 Hz), ethyl H-phosphonate (**19**), 34% yield (Figure 2A). Under the same conditions diethyl phosphite **20** remained unchanged (Figure 2B).

Hydrolysis of Ethyl 2-Hydroxyethyl H-Phosphonate (17) and Diethyl Phosphite (20) in 50% Aqueous Pyridine. Ethyl 2-hydroxyethyl phosphite (ethyl 2-hydroxyethyl H-phosphonate) was prepared as described above. The crude ethyl 2-hydroxyethyl phosphite **17** (119 mg, 0.770 mmol) or diethyl phosphite **20** (107 mg, 0.776 mmol) was dissolved in 0.5 mL of dry dioxane in a NMR tube, and 0.5 mL of water and 0.5 mL of dry pyridine were added upon rapid stirring. The resulting mixture was immediately analyzed: ^{31}P NMR (101.26 MHz, dioxane/pyridine/H_2O, 25 °C) δ = 7.4 (dt, J_{P-H} = 634.0 Hz, $J_{P-O-C-H}$ = 7.8 Hz), 2-hydroxyethyl H-phosphonate (**18**); δ 6.6, ethyl H-phosphonate (**19**), traces. Under the same experimental conditions diethyl phosphite **20** remained unchanged.

Acknowledgment. This work was supported by the National Research Fund (Project K-625). We thank the reviewers for their valuable suggestions.

JA982413U

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(12) The formation of 5′-*O*-trityl thymidine H-phosphonate (**12**) and phenyl H-phosphonate (**23**, Chart) in the reaction of phosphonylation of 5′-*O*-trityl thymidine with diphenyl phosphite (**22**) was a result of the hydrolysis of 5′-*O*-trityl thymidine phenyl H-phosphonate (**16**) and diphenyl phosphite (**22**), respectively, due to the uncomplete exclusion of moisture.[11b] After the addition of 1 equiv of water, the amount of the phenyl H-phosphonate remained unchanged for 30 min at room temperature, which indicates that the hydrolysis of 5′-*O*-trityl thymidine phenyl H-phosphonate (**16**) leads to the formation of 5′-*O*-trityl thymidine H-phosphonate (**12**) only.