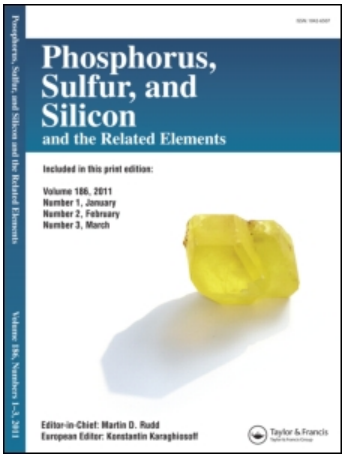

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PHOSPHONYLATION BY A SPIROPHOSPHORANE: APPLICATION OF THE RIBOZYME CHEMISTRY IN THE BIOORGANIC SYNTHESIS

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PHOSPHONYLATION BY A SPIROPHOSPHORANE: APPLICATION OF THE RIBOZYME CHEMISTRY IN THE BIOORGANIC SYNTHESIS

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The phosphonylation by oxirane/phosphorous acid is characterized by formation of spirophosphorane, which provides the active intermediate in the reaction, β-hydroxyalkyl alkylene phosphite. Here, we demonstrate that a well-known and readily accessible spirophosphorane, 2λ^5-2,2′spirobi[1,3,2-benzodioxaphosphole] can be used as convenient and effective phosphonylating agent in similar reaction. The easily detectable primary 3-phenylpropyl H-phosphonate, as well as 5′-O-tritylthymidyl, 4-N-benzoyl-5′-O-trityl-deoxycytidyl, 5′-O-trityl-deoxycytidyl, 6-N-benzoyl-5′-O-trityl- deoxyadenosyl 5′-O-trityluridyl, and 2′,3′-isopropylidene-uridyl H-phosphonates were prepared using a simple and straightforward procedure. Our results provide an interesting example of the potential and the limitations of a synthetic approach utilising reaction of leaving of diol system.

Keywords: Alcohols; nucleosides; phosphonates; phosphonylation; ribozyme; spiro compounds

H-phosphonate monoesters are important intermediates in the preparation of various naturally occurring phosphate esters and their analogues.[1,2] They are stable and resistant to oxidation in solution, but upon activation by various condensing agents, like acyl chlorides, chlorophosphates, and arenesulfonic acid derivatives[1] become at least as effective reagents as the tri-coordinated P(III)

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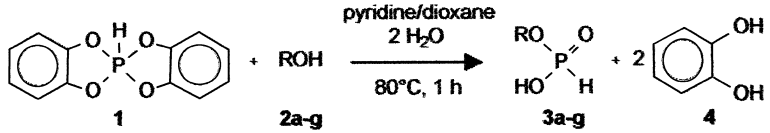
ACS Classification Codes: 181-85-1 (2λ^5-2,2′spirobi [1,3,2-benzodioxaphosphole]); 122-97-4 (3-phenylpropanol); 7791-71-1 (5′-O-tritylthymidine); 75759-62-5 (6-N-benzoyl-5′-O-trityl- deoxyadenosine); 40705-89-3 (thymidine 3′-monophosphate ammonium salt); 102783-50-6 (2′-Deoxycytidine 3′-monophosphate ammonium salt).

compounds.[2–4] Some of them are potentially of interest as antiviral[5–7] or antibacterial[8] agents/precursors themselves. Relatively few and with application limited to the preparation of protected nucleoside H-phosphonates for use in the oligonucleotide synthesis[9–14] initially, the methods for preparation of H-phosphonate monoesters have evolved to suite other areas, too.[1,2] Usually, the phosphonylation is carried out by: (a) activated tri-coordinated phosphorus compounds, as in the case of PCl_3/1,2,4-triazole[11] or PCl_3/imidazole[12] reagent systems and salicylchlorophosphite;[15] or (b) activated tetra-coordinated phosphorus compounds, as in the case of H-pyrophosphonate,[10,16] bis(1,1,1,3,3,3-hexafluoro-2-propyl)-H-phosphonate,[17] and diphenyl H-phosphonate.[18,19]

Although there were existing approaches suitable for preparation of nucleoside H-phosphonates for the automated oligonucleotide synthesis,[11,13,14] different disadvantages for each method, concerning its efficiency, chemoselectivity, accessibility, stability of the reagents, and cost-effectiveness can be found. The reaction of choice has been determined by the structural features of the hydroxyl compound.[1] Therefore, there always has been a demand for exploring new reagents with tempered activity and higher specificity, tolerable to certain protective groups or residues.[2] Important development, for example, is the utilization of reactions applicable to a wider group of compounds, as well as reactions tolerant to unprotected amino groups.[2] Recent advances in the bioorganic chemistry in general and the oligonucleotide chemistry in particular, e.g., glycobiology,[20] antisense technology,[21] and catalytic nucleic acid enzymes,[22] present even more challenges to the methods of phosphorylation and phosphonylation.

Another group of potentially reactive compounds, the pentacoordinated phosphorus compounds, on the other hand, are usually of general interest in the phosphorus chemistry as mimics of pentacovalent intermediates in substitutions of phosphorus.[23,24] They rarely are used in the organic synthesis however. Several cases are known when stable compounds of this group have been used in reactions of phosphorylation[25] and phosphonylation.[26] Hydrotetraoxaspirophosphoranes, such as **1** (Scheme 1), are not an exception—although much attention has been focused on the preparation[27–30] and dynamic stereochemistry[29–31] of these bicyclic phosphorus esters, little interest has been paid to their use in general organic synthesis, and their synthetic applications are somehow rare.[32–35]

Diol-leaving reactions at phosphorus similar to those catalyzed by the large ribozymes,[36] but taking place in organic media, were known long time ago.[37] However, there was no information available about possible synthetic application of similar reactions, until we described the



a R = 3-phenylpropyl
b R = 5'-O-tritylthymidyl
c R = 4-N-benzoyl-5'-O-trityl-deoxycytidyl
d R = 5'-O-trityl-deoxycytidyl
e R = 6-N-benzoyl-5'-O-trityl-deoxyadenosyl
f R = 5'-O-trityluridyl
g R = 2',3'-isopropylidene-uridyl

SCHEME 1

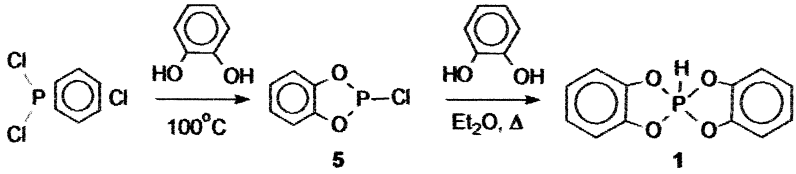
preparation of glycoside and nucleoside H-phosphonate and phosphate monoesters, using oxiranes as condensing agents.[38] The extensive study of the mechanism of the phosphonylation reaction revealed that pentacoordinated H-tetraoxaspirophosphoranes were probably its important intermediates[39] and its final stage was a leaving of a diol system, similar to the reaction catalyzed by the large ribozymes. During the investigation of the effect of the medium on the mechanism of hydrolysis of 2-hydroxy H-phosphonate diesters,[40] we found that at certain conditions the H-tetraoxaspirophosphoranes themselves can be possibly used as phosphonylating agents. The reaction of the spirophosphorane (**1**) with 5′-O-trityl thymidine in the presence of controlled quantity of water yielded 5′-O-trityl thymidyl H-phosphonate, but although the NMR data suggested this conversion is almost quantitative, we have not had actually isolated the nucleoside H-phosphonate as a final product at that stage.[40]

Here, we present complete procedure for phosphonylation by $2\lambda^5$-2,2′-spirobi[1,3,2-benzodioxaphosphole] (**1**) based on our previous observations. By applying this procedure to a group of several different protected and unprotected nucleosides, as well as a primary alcohol we also investigate the scope and the limitations of this new approach (Scheme 1). Some differences between the reactivity in the phosphonylation reaction of the different hydroxylic compound included in this study, are also reported.

RESULTS

Preparation of $2\lambda^5$-2,2′-Spirobi[1,3,2-benzodioxaphosphole]

This spirophosphorane was readily and conveniently prepared in a two-step process (Scheme 2). First, reaction of equimolar amounts of



SCHEME 2

phosphorus chloride and catechol quantitatively yielded 2-chlorbenzo-1,3,2-dioxaphosphole **5**.[41] Second substitution reaction of the latter with an additional equivalent of catechol in ether, followed by recrystallization, gave the spirophosphorane[42] (**1**). The crystals of the spirophosphorane were stored at −20°C for months with no apparent loss of activity.

Phosphonylation Reaction

Modification of the previously described procedure for preparation/hydrolysis of the 2-hydroxy H-phosphonodiesters[40] was used for phosphonylation of all of the alcohols studied here (Scheme 1). The initial procedure involved reaction of one equivalent of alcohol with a small excess of spirophosphorane in freshly dried pyridine/dioxane at 80°C, in the presence of 2 equiv. of water. We introduced only minor modifications aimed for an optimization of the reaction. For example, we observed that even the meticulously dried solvents absorb some water during a routine reaction set-up in an ordinary chemistry laboratory (data not shown), consequently addition of only one equivalent of water when phosphonylating nucleosides was found to give the best results. In all cases, the time needed for the completion of the reaction in the presence of 2 equiv. of water in the reaction mixture at 80°C was less than 60 min.

The ratio between the spirophosphorane and the alcohols was another studied parameter. Most favourable was found to be the addition of up to 10% excess of the spirophosphorane. More substantial excess of $2\lambda^5$-2,2′-spirobi[1,3,2-benzodioxaphosphole] (**1**) lowered the efficiency of the reaction, however. Reaction of 1 equiv. of thymidine (**2b**), 2 equiv. of spirophosphorane **1**, and 4 equiv. of water resulted in complete disappearance of the starting alcohol and formation of considerable quantity of the symmetrical diester **6b** (Chart 1, more then 30% of the final product, data not shown).

Alcohols for the Phosphonylation Reaction

The scope of the proposed synthetic approach was evaluated by the use of a primary alcohol and several nucleosides in the phosphonylation

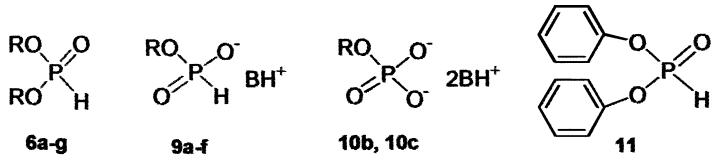
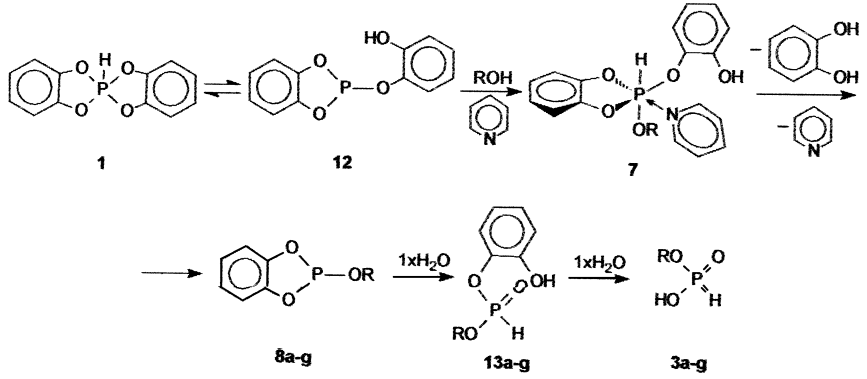


CHART 1

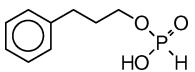
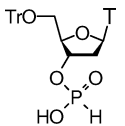
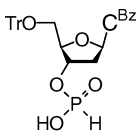
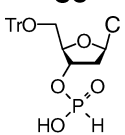
reaction. 3-Phenyl propanol (**2a**), 5′-O-trityl thymidine (**2b**), 4-N-benzoyl 5′-O-trityl deoxycytidine (**2c**), 5′-O-trityl deoxycytidine (**2d**), 6-N-benzoyl 5′-O-trityl deoxyadenosine (**2e**), 5′-O-trityl uridine (**2f**), and 2′,3′-isopropylidene uridine (**2g**) were subjected to phosphonylation by spirophosphorane **1**. Analyses of the reaction mixtures by HPLC and ^{31}P NMR revealed (Table I) that, for all of these hydroxylic compounds, similar reactions took place and the corresponding alkyl H-phosphonates were the main product. Nevertheless, because of their different properties, the following cases needed more attention.

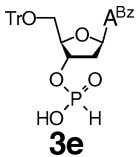
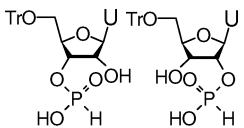
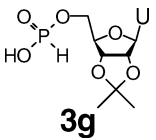
The yield of 3-phenyl H-phosphonate (**3a**) was more sensitive to the amount of the water in the reaction mixture then the yields of the secondary 2′-OH nucleoside H-phosphonate monoesters. While heating of 5′-O-trityl thymidine (**2b**) with the spirophosphorane **1** under anhydrous conditions led to the formation of hypervalent phosphorus esters, like the hexacoordinated compound **7** or triesters like the o-phenylene phosphite **8**[40] (Scheme 3), the heating of 3-phenylpropanol **2a** with **1** yielded in addition a substantial amount of di(3-phenylpropyl)



SCHEME 3

TABLE I ^{31}P NMR Data and HPLC Mobilities of H-Phosphonate Monoesters (Ammonium/Triethylammonium Salts)

	Compound	HPLC retention time (min)	^{31}P NMR[a]		^{1}H NMR (P—*H*)[b]			
			Chemical shift (ppm)	J_{P-H} (Hz)	Isolated product[c]	Chemical shift (ppm)	J_{P-H} (Hz)	Yield (%)
 **3a**	R = 3-phenylpropyl	0.9[d]	4.6	631	**9a**	6.6	629	88
 **3b**	R = 5′-O-tritylthymidyl	1.5[d]	3.4	633	**9b**	6.8	622	89
 **3c**	R = 4-N-benzoyl-5′-O-trityl-deoxycytidyl	1.9[d]	—	—	**9c**	6.9	634	90
 **3d**	R = 5′-O-trityl-deoxycytidyl	1.4[d]	—	—	**9d**	6.7	621	85

 **3e**	R = 6-N-benzoyl-5′-O-trityl-deoxyadenosyl	1.1[e]	2.7	622	**9e**	6.9	622	96
 **3f**	R = 5′-O-trityluridyl	1.1[d]	—	—	**9f**	6.8[f] 6.8[f]	663[f] 666[f]	82
 **3g**	R = 2′,3′-isopropylideneuridyl	1.3[g]	—	—	—	—	—	Approx. 50 (HPLC)

[a] ^{31}P NMR spectra were taken in pyridine/dioxane, 1/1 (v/v).
[a] ^{1}H NMR spectra were taken in DMSO-d_6 (isolated product).
[c] Compound **9a** as ammonium salt; **9b–9f** as triethylammonium salts.
[d] Eluting system: 38% CH_3CN, 62% 20 mM NaH_2PO_4/Na_2HPO_4 buffer, pH 7.
[e] Eluting system: 45% CH_3CN, 55% 20 mM NaH_2PO_4 buffer, pH 4.6.
[f] 2′- and 3′-isomers.
[g] Eluting system: 10% CH_3CN, 90% 20 mM NaH_2PO_4 buffer, pH 4.6.

H-phosphonate (**6a**, Chart), evidenced by ^{31}P NMR (see Experimental section). In the presence of 2 equiv. of water, the concentration of the symmetrical diester **6a** considerably decreased in the reaction mixture and the desired H-phosphonate **3a** was detected to be the main product (Table I).

It is worth nothing that the reaction of 5′-O-trityl deoxycytidine (**2d**) with **1** gave the monoester **3d**. Phosphonylation of the 4-amino group of the cytosine was not observed ($\delta = 7.09$, broad s, 2H, C4-NH_2).

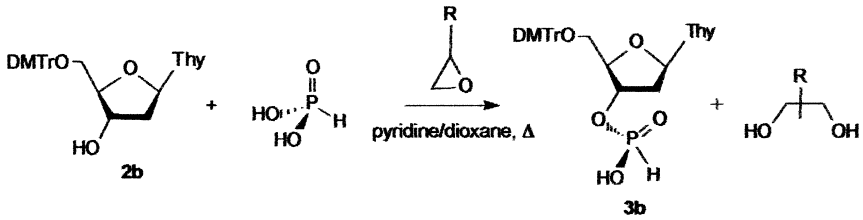
The phosphonylation of 5′-O-trityl uridine (**2f**) led to isolation of a mixture of 5′-O-trityl 2′-uridine H-phosphonate and 5′-O-trityl 3′-uridine H-phosphonate. This observation suggested cyclic 5′-O-trityl 2′,3′-uridine H-phosphonate was the apparent intermediate in the reaction, that decomposed in the presence of water to a mixture of the isolated two H-phosphonates (Table I).

Analysis and Purification of the Products

The conversion of the alcohols to the corresponding H-phosphonates during the phosphonylation reactions was followed by HPLC and, in the case of 3-phenyl propyl H-phosphonate (**3a**), 5′-O-trityl thymidine (**3b**) and 6-N-benzoyl 5′-O-trityl deoxyadenosine (**3e**), by ^{31}P NMR. Triethylammonium salts of the H-phosphonates **3b**–**3f**—compounds **9b**–**9f** (Chart 1)—were isolated and purified by aqueous-bicarbonate work-up and column chromatography (see Experimental section), and analyzed by HPLC, ^{1}H NMR, and phosphorus analysis. Modified procedure for isolation of alkyl H-phosphonates[19] was used in the case of 3-phenyl propyl H-phosphonate (**3a**) and, in addition to the other analyses, melting point determination was carried out for the resulting ammonium salt **9a** (see Experimental section). The results of the analyses of the isolated salts were in agreement with the data from the literature.[4,18,19] In the case of the 2′,3′-isopropylidene uridine (**2g**), the reaction was followed by HPLC only. Our attempts to isolate and purify the product for further analyses were unsuccessful. Available analytical data is presented in the Experimental section and summarized in Table I.

Oxidation and Deprotection of the Resulting H-Phosphonate Monoesters

As an additional proof of the identity of the synthesized H-phosphonates, compounds **9b** and **9c** were oxidized[43] and deprotected[44] to the corresponding nucleoside 3′-monophosphates **10b** and **10c** (Chart 1, diammonium salts). The latter were analyzed similarly as the H-phosphonate monoesters (see Experimental section).



SCHEME 4

DISCUSSION

$2\lambda^5$-2,2′Spirobi[1,3,2-benzodioxaphosphole] as a Phosphonylation Agent

Earlier,[38] we described a method for phosphonylation of nucleosides and glycoses (Scheme 4), that was based on the use of oxiranes as condensing agents. Detailed study of the mechanism of this phosphonylation[39] revealed that it is very sensitive to the reaction conditions. The yields of the reaction were found to vary substantially with variation of the order of mixing of the reagents, or with addition of certain quantities of water.

As a part of our mechanistic studies,[39,40] we tried to isolate, purify, and characterize any possible reaction intermediates or their analogues. One of the studied compounds, $2\lambda^5$-2,2′-spirobi[1,3,2-benzodioxaphosphole] (**1**) was designed as an analogue of the spirophosphoranes found in the reaction mixture. This spirophosphorane was found to be a potential phosphonylation agent itself.[40] It can be prepared directly from PCl_3 and catechol,[45,46] however, two-step preparation trough the isolation of the intermediate o-hydroxyphenylene phosphorochloridite (2-chlorbenzo-1,3,2-dioxaphosphole) proved to be much more convenient and reproducible[41,42] (Scheme 2). Because the H-tetraoxaspirophosphorane **1** is a stable crystalline compound that can be stored for months at −20°C without any apparent loss of activity, it was found easier to work with and potentially more convenient for manual or semiautomated applications, then the two-component system oxiranes/phosphorous acid, described previously.[38]

It is worth noting that the use of the "deoxy" analogue of **1**, diphenylphosphite **11** (Chart 1) has been used successfully as phosphonylation agent for wide range of alcohols, too.[18,19] More importantly, it seems to be tolerant to substrates with unprotected amino groups.[2] However, the use of large excess of **11** and/or formation of significant amount of the symmetrical dialkyl H-phosphonates, as well as the discovered disproportionation of **11** under anhydrous basic conditions,[47] somehow reduces the efficiency of this versatile reagent.

The Range of the Alcohols

The phosphonylation of 5′-O-trityl thymidine (**2b**) to the corresponding H-phosphonate monoester (**3b**) had been detected during our mechanistic studies with **1**.[40] However, we have not attempted purification of **3b** at that stage. To investigate the potential of the use of spirophosphoranes as phosphonylating agents in more detail, we applied standard aqueous-bicarbonate work-up and separation of the products on a silica column. This approach proved to be very effective in the case of 5′-O-trityl thymidine H-phosphonate (**3b**) and other protected nucleotides, as well. The phosphonylation of 4-N-benzoyl 5′-O-trityl deoxycytidine (**2c**) and 6-N-benzoyl 5′-O-trityl deoxyadenosine (**2e**) gave very similar results to that of **3b**. In order to determine if the spirophosphorane is as effective for the phosphonylation of primary hydroxyl groups as of the secondary 3′-OH of the ribose, we attempted phosphonylation of 3-phenyl propanol (**2a**) and 2′,3′-isopropylidene uridine (**2g**), too. Both the HPLC and the NMR analyses confirmed the formation of 3-phenylpropyl H-phosphonate (**3a**), and it was successfully isolated as crystalline compound.[19] The results from the phosphonylation of 2′,3′-isopropylidene uridine (**2g**), however, were ambiguous. The highest yield detected by HPLC was about 50% and we could not rule out one of the following reasons for that result: (1) there was a problem with the starting 2′,3′-isopropylidene uridine (**2g**); or (2) it is not possible to increase the yield of the reaction, because the isopropylidene protective group is not tolerated. We could not find other conditions that would allow us to isolate the product, but as long as the primary alcohol 3-phenyl propanol (**2a**) was successfully phosphonylated, we assume that the reaction of phosphonylation of primary alcohols with spirophosphorane **1** is possible and practicable, and that the problem with the low yield of the 2′,3′-isopropylidene uridine H-phosphonate (**3g**) is not related to the phosphonylation reaction itself.

The formation of 3-phenyl propanol (**2a**) H-phosphonate monoester was found to be more sensitive to the reaction conditions than the phosphonylation at the nucleoside secondary 3′-OH. In a small excess of the phosphonylating spirophosphorane (10%) or in a deficiency of water, the reaction proceeds with formation of substantial amount (>30%, depending on conditions) of the symmetrical di(3-phenylpropyl) H-phosphonate (**6a**).

After phosphonylation of 5′-O-trityl deoxycytidine (**2d**), we did not detect any phosphonylation at the 4-aminogroup of the cytosine, as a side reaction, by HPLC and the product was isolated in very good yields (Table I). The signal for the aromatic amino protons in the ^{1}H NMR

spectrum of the compound appears at $\delta = 7.09$, value typical for an unsubstituted aryl amino group (see Experimental section). Therefore, spirophosphoranes like **1** seem to be selective phosphonylation reagents for the primary and secondary hydroxyl groups and tolerate unprotected amino groups.

During the reaction with 5′-O-trityl uridine (**2f**), one of the 2-hydroxyphenyl rings of the spirophosphorane **1** is replaced by the 2′,3′-diol system of the uridine, giving after aqueous bicarbonate work-up mixture of 5′-O-trityl 2′-uridine H-phosphonate and 5′-O-trityl 3′-uridine H-phosphonate in approximately equal quantities (Table I, Experimental section).

Some Remarks on the Reaction Mechanism

The possible mechanism of the phosphonylation reaction used here is described elsewhere (Scheme 3).[40] Our results do not suggest any other, different mechanism in the cases described here. Under the conditions found for the phosphonylation reaction with spirophosphorane, other hypervalent esters of the phosphoric acid are formed, that decompose via two-step hydrolysis with leaving of diol system to give the desired phosphonate monoester.[40] This is in contrast with one widely used group of synthetic reactions in phosphorus chemistry—that of the Michaelis Arbusov type reactions, where the initial phosphite triester undergoes water-free rearrangement through quasiphosphonium or hypervalent intermediate to give alkyl phosphonate or some other type of desired product.[48]

CONCLUSION

The described method for phosphonylation is reliable, reproducible, and affordable. Moreover, it is an interesting example of the use of a ribozyme mimetic reaction for synthesis of various esters of the phosphonous acid. It demonstrates that the knowledge we have on the mechanism of the biochemical reactions and their bioorganic counterparts can help us to control the outcome of the latter effectively and, eventually, they could be used successfully in the organic synthesis. More reactions including leaving of diol system from other electrophiles had been extensively studied[49–51] and their synthetic use probably also is possible. On the other hand, H-tetraoxaspirophosphorane **1** is merely one of the several readily available oxyphosphoranes[27–30] capable of phosphonylation.

EXPERIMENTAL

General Procedures and Materials

All glassware was dried in an oven for at least 3 h at 120°C before use. Commercial solvents and reagents were used as purchased 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,[52] 4-N-benzoyl 5′-O-trityl cytidine,[53] 5′-O-trityl cytidine,[53] 6-N-benzoyl 5′-O-trityl deoxyadenosine,[53] 5′-O-trityl uridine,[54] and 2′,3′-Isopropylidene uridine[55] were prepared as described. $2\lambda^5$-2,2′-spirobi[1,3,2-benzodioxaphosphole] (**1**) was prepared from o-phenylene phosphochloridite as described[42] and stored at −4°C. Protected nucleotides and catechol were stored in a desiccator over phosphorus pentoxide.

Spectral and Physical Data

The ^{31}P and ^{1}H NMR spectra were taken on a Bruker DRX-250 spectrometer. ^{1}H chemical shifts are reported in δ (ppm) relative to internal tetramethylsilane standard. ^{31}P chemical shifts are reported in δ (ppm) downfield (+) and upfield (−) from external 85% H_3PO_4 (Table I). The assignment of the signals is based on literature data.

The reverse phase HPLC analyses were performed on a Waters chromatography system using Nucleosil 100-5C_{18} column, flow rate 0.8 mL/min and UV detection at 280 nm. For the eluting systems see Table I.

The percentage of the bound phosphorus is determined from the weight of the polyphosphorous acid formed after combustion of the H-phosphonate monoester.

Preparation of 3-Phenylpropyl H-Phosphonate Monoester, Ammonium Salt (9a)

To a stirred solution of $2\lambda^5$-2,2′-spirobi[1,3,2-benzodioxaphosphole] (**1**) (293 mg, 1.10 mmol) in 2 mL of anhydrous dioxane, was added a solution of 3-phenyl propanol **2a** (136 μl, 1.00 mmol) and water (36.0 μl, 2.00 mmol) in 2 mL of anhydrous pyridine (24.9 mmol) at room temperature. The reaction mixture was heated to 80°C for 1 h. The solvents were evaporated, 5 mL 25% aq. ammonium hydroxide was added and the solution was incubated for 15 min at room temperature. The ammonium hydroxide was evaporated, too and the residue was repeatedly triturated with Et_2O (5 × 5 mL).[19] The final crystalline product **9a** (191 mg, 87.8% yield, ammonium salt) was subjected to analysis.

^{1}H NMR (DMSO-d_6, 250 MHz), δ (p.p.m.): 1.92 (2H, m, $CH_2CH_2CH_2$), 2.83 (2H, t, $J_{2,3} = 6.8$ Hz, $ArCH_2CH_2$), 3.72 (2H, dt, $J_{1,2} = 6.2$ Hz, $J_{1,P} = 7.8$ Hz, $PHCH_2CH_2$), 6.59 (1H, d, $J_{P-H} = 629.1$ Hz, P—*H*),7.21 (5H, m, Ar—*H*); mp (°C) 128–130; P analysis calculated for $C_9H_{16}NO_3P$: 14.3%, found: 13.9%. HPLC and ^{31}P NMR data for compound **3a** are summarized in Table I.

Reaction of 2λ^5-2,2′-Spirobi[1,3,2-benzodioxaphosphole] (1) with 3-Phenyl Propanol (2a) Under Anhydrous Conditions

To a stirred solution of 2λ^5-2,2′-spirobi[1,3,2-benzodioxaphosphole] (**1**) (266 mg, 1.00 mmol) in 2 mL of anhydrous dioxane, was added a solution of 3-phenyl propanol **2a** (136 μl, 1.00 mmol) in 2 mL of anhydrous pyridine (24.9 mmol) at room temperature. The resulting mixture was heated to 80°C for 20 min. ^{31}P NMR (101.26 MHz, dioxane/pyridine, 25°C) $\delta = 122.5$ (t, $J_{P-O-CH_2} = 8.3$ Hz), *o*-phenylene 3-phenylpropyl phosphite (**8a**, Scheme 3), 26%; $\delta = 7.6$ (dt, $J_{P-H} = 716.8$ Hz, $J_{P-O-CH_2} = 7.9$ Hz), *o*-hydroxyphenyl 3-phenylpropyl H-phosphonate (**13a**, Scheme 3), 4%; $\delta = 6.5$ (m), di(3-phenylpropyl) H-phosphonate (**6a**, Chart), 39%; $\delta = 4.4$ (d, $J_{P-H} = 646.9$ Hz), *o*-hydroxyphenyl H-phosphonate (**11**), 3%; $\delta = 3.3$ (dt, $J_{P-H} = 635.2$ Hz, $J_{P-O-CH_2} = 7.9$ Hz), 3-phenylpropyl H-phosphonate (**3a**, Scheme 4), 6%; $\delta = -89.0$ (t, $J_{P-H} = 863.7$ Hz), hexacoordinated intermediate **7a** (Scheme 3), 22%.

Preparation of 5′-O-Trityl Thymidine H-Phosphonate Monoester, Triethylammonium Salt (9b)

To a stirred solution of 2λ^5-2,2′-spirobi[1,3,2-benzodioxaphosphole] (**1**) (293 mg, 1.10 mmol) in 2.5 mL anhydrous dioxane, was added a solution of 5′-O-trityl thymidine (**2b**) (485 mg, 1.00 mmol) and water (36.0 μl, 2.00 mmol) in 2.5 mL anhydrous pyridine at room temperature. The reaction mixture was heated to 80°C for 1 h. The solvents were evaporated and the residue was dissolved in 2 mL 5% sodium bicarbonate buffer (pH 8.6) and 2 mL CH_2Cl_2, the mixture was shaken and separated. The organic phase was washed 4 times with 2 mL of the bicarbonate buffer, dried over Na_2SO_4, and evaporated to a foam. Silica gel column chromatography (2% Et_3N/CH_2Cl_2 → 2% Et_3N/10%MeOH/CH_2Cl_2) (linear gradient from 0% MeOH to 10% MeOH in CH_2Cl_2 containing 1% Et_3N) followed by TEAB extraction and evaporation yielded chromatographically pure 5′-O-trityl-3′-thymidine H-phosphonate (**9b**) in 89.3% yield (580 mg, triethylammonium salt). ^{1}H NMR (DMSO-d_6, 250 MHz), δ (p.p.m.): 1.11 (9H, t, $J_{1,2} = 7.0$ Hz, $(CH_3CH_2)_3NH^+$), 1.45 (3H, s, 5-CH_3), 2.43 (1H, ddd, $J_{1',2''} = 6.6$ Hz, $J_{2',2''} = 8.5$ Hz,

$J_{2'',3'} = 7.2$ Hz, *H*2″), 2.59 (1H, m, *H*2′), 2.78 (6H, q, $J_{1,2} = 7.0$ Hz, $(CH_3CH_2)_3NH^+$), 3.45 (1H, dd, $J_{4',5'} = 3.3$ Hz, $J_{5',5''} = 11.2$ Hz, *H*5′), 3.55 (1H, dd, $J_{4',5''} = 2.6$ Hz, $J_{5',5''} = 11.2$ Hz, *H*5″), 4.09 (1H, m, *H*4′), 5.01 (1H, m, *H*3′), 6.42 (1H, dd, $J_{1',2'} = 7.2$ Hz, $J_{1',2''} = 6.6$ Hz, *H1*′), 6.84 (1H, d, $J_{P-H} = 621.7$ Hz, P—*H*), 7.29 (15H, bm, Ar—*H*), 7.41 (1H, s, C6-*H*), 7.58 (1H, bs, $(CH_3CH_2)_3NH^+$), 11.32 (1H, bs, N3-*H*); P analysis calculated for $C_{35}H_{44}N_3O_7P$: 4.8%, found: 4.6%. HPLC and ^{31}P NMR data for compound **3b** are summarized in Table I.

All other 5′-O-protected nucleoside H-phosphonates were prepared by the same procedure. Summary of the analytical data for the compounds **3c–3g** and **9c–9f** is presented in Table I.

4-N-benzoyl-5′-O-trityl Deoxycytidine H-Phosphonate Monoester, Triethylammonium Salt (9c)

Yield 663 mg, 89.8%; ^1H NMR (DMSO-d_6, 250 MHz), δ (p.p.m.): 1.09 (9H, t, $J_{1,2} = 6.9$ Hz, $(CH_3CH_2)_3NH^+$), 2.24 (1H, m, *H*2″), 2.39 (1H, m, H2′), 2.75 (6H, q, $J_{1,2} = 6.9$ Hz, $(CH_3CH_2)_3NH^+$), 3.35 (1H, bm, *H*5′), 3.45 (1H, s, *H*5″), 4.12 (1H, m, *H*4′), 4.96 (1H, m, *H*3′), 6.42 (1H, t, $J_{1',2'} = 6.3$ Hz, $J_{1',2''} = 6.3$ Hz, *H*1′), 6.91 (1H, d, $J_{P-H} = 633.6$ Hz, P—*H*), 7.30 (19H, bm, Ar—*H*), 7.58 (1H, bs, $(CH_3CH_2)_3NH^+$), 8.04 (2H, m, Bz-*H*), 8.25 (1H, d, $J_{5,6} = 7.1$ Hz, C6-*H*), 11.27 (1H, bs, C4-N*H*); P analysis calculated for $C_{41}H_{47}N_4O_7P$: 4.2%, found: 4.1%.

5′-O-trityl Deoxycytidine H-Phosphonate Monoester, Triethylammonium Salt (9d)

Yield 541 mg, 85.3%; ^1H NMR (DMSO-d_6, 250 MHz), δ (p.p.m.): 1.12 (9H, t, $J_{1,2} = 7.0$ Hz, $(CH_3CH_2)_3NH^+$), 2.22 (1H, m, *H*2″), 2.36 (1H, m, H2′), 2.77 (6H, q, $J_{1,2} = 7.0$ Hz, $(CH_3CH_2)_3NH^+$), 3.36 (1H, bm, *H*5′), 3.46 (1H, s, *H*5″), 4.08 (1H, m, *H*4′), 4.99 (1H, m, *H*3′), 6.09 (1H, d, $J_{5,6} = 7.2$, C5-*H*), 6.44 (1H, dd, $J_{1',2'} = 6.5$ Hz, $J_{1',2''} = 6.3$ Hz, *H*1′), 6.91 (1H, d, $J_{P-H} = 621.4$ Hz, P—*H*), 7.09 (2H, bs, C4-NH_2), 7.32 (15H, bm, Ar—*H*), 7.59 (1H, bs, $(CH_3CH_2)_3NH^+$), 8.22 (1H, d, $J_{5,6} = 7.2$, C6-*H*); P analysis calculated for $C_{34}H_{43}N_4O_6P$: 4.9%, found: 4.9%.

6-N-benzoyl-5′-O-trityl-deoxyadenosine H-Phosphonate Monoester, Triethylammonium Salt (9e)

Yield 729 mg, 95.6%; ^1H NMR (DMSO-d_6, 250 MHz), δ (p.p.m.): 1.08 (9H, t, $J_{1,2} = 6.8$ Hz, $(CH_3CH_2)_3NH^+$), 2.43 (1H, m, *H*2″), 2.59 (1H, m, H2′), 2.74 (6H, q, $J_{1,2} = 6.8$ Hz, $(CH_3CH_2)_3NH^+$), 3.32 (1H, m, *H*5′), 3.67 (1H, s, *H*5″), 4.18 (1H, m, *H*4′), 5.05 (1H, m, *H*3′), 6.51 (1H, m, *H*1′), 6.93 (1H, d, $J_{P-H} = 621.9$ Hz, P—*H*), 7.27 (18H, bm, 15 Ar—*H*, 3 Bz—*H*), 7.53 (1H, bs, $(CH_3CH_2)_3NH^+$), 8.01 (2H, m, 2 Bz—*H*), 8.15 (1H, s, C2-*H*), 8.55 (1H, s, C8-*H*), 11.14 (1H, bs, C6-N*H*); P analysis calculated for $C_{42}H_{47}N_6O_6P$: 4.1%, found: 4.0%.

5′-O-Trityluridyl 2′-H-phosphonate Monoester, Triethylammonium Salt and 5′-O-trityluridyl 3′-H-phosphonate Monoester, Triethylammonium Salt Mixture (9f)

Yield 536 mg, 82.3%, the monoesters are in approx. ratio 2:3 (2′-H-phosphonate: 3′-H-phosphonate; ^{1}H NMR (DMSO-d$_6$, 250 MHz), δ (p.p.m.): 1.10 (9H, t, $J_{1,2} = 7.0$ Hz, (C*H*$_3$CH$_2$)$_3$NH$^+$), 2.78 (6H, q,$J_{1,2} =$ 7.0 Hz, (CH$_3$C*H*$_2$)$_3$NH$^+$), 3.56 (2H, bm, *H*5′), 3.76 (0.4H, m, *H*3′), 3.95 (0.6H, m, H2′), 4.08 (1H, m, *H*4′), 5.01 (0.6H, m, *H*3′), 5.11 (0.6H, bs, C3′O*H*), 5.24 (0.4H, bs, C2′O*H*), 5.71 (1H, m, C5-*H*), 5.89 (0.4H, m, *H*2′), 6.34 (1H, m, *H*1′), 6.78 (0.6H, d, $J_{P-H} = 663.1$ Hz, P—*H*), 6.83 (0.4H, d, $J_{P-H} = 666.4$ Hz, P—*H*), 7.28 (15H, bm, Ar—*H*), 7.61 (1H, bs, (CH$_3$CH$_2$)$_3$N*H*$^+$), 7.94 (1H, m, C6-*H*), 11.07 (1H, bs, N3-*H*); P analysis calculated for C$_{34}$H$_{42}$N$_3$O$_8$P: 4.8%, found: 4.9%.

Preparation of 2′,3′-Isopropylidene Uridine H-Phosphonate (3g)

To a stirred solution of 2λ^5-2,2′-spirobi[1,3,2-benzodioxaphosphole] (**1**) (20.8 mg, 78.1 μmol) in 300 μl of anhydrous dioxane was added a solution of 2′,3′-isopropylidene uridine **2g** (22.6 mg, 74.5 μmol) and water (2.6 μl, 144 μmol) in 300 μl of anhydrous pyridine (3.70 mmol) at room temperature. The reaction mixture was heated to 80°C for 1 h. The solvents were evaporated and the residue was subjected to analysis. HPLC data are reported in Table I.

Oxidation of 5′-O-Trityl Thymidine H-Phosphonate (9b) and 4-N-benzoyl-5′-O-trityl Deoxycytidine H-Phosphonate (9c)

Compounds **9b** and **9c** were oxidized by iodine in pyridine/water, by a procedure for oxidation of H-phosphonate monoesters.[43] The ^{31}P NMR spectra of the resulting protected 3′-monophosphates were found to be similar to those described in the literature.[43] After deprotection,[44] the analytical data for the resulting thymidine 3′-phosphate diammonium salt (**10b**) and 2′-deoxycytidine 3′-phosphate diammonium salt (**10c**) were similar to those of the commercially available (SIGMA) compounds.

REFERENCES

[1] J. Stawinski, in *Handbook of Organophosphorus Chemistry*, edited by R. Engel (Marcel Dekker, New York, 1992), pp. 377–434.
[2] J. Stawinski and A. Kraszewski, *Acc. Chem. Res.*, **35,** 952 (2002).
[3] P. J. Garegg, T. Regberg, J. Stawinski, and R. Stromberg, *Chem. Scr.*, **25,** 280 (1985).

[4] B. C. Froehler and M. D. Matteucci, *Tetrahedron Lett.*, **27,** 469 (1986).
[5] C. McGuigan, P. Bellevergue, B. Jones, et al., *Antivir. Chem. Chemother.*, **5,** 271 (1994).
[6] G. A. Galegov, V. M. Shobukhov, N. A. Leont'eva, and M. V. Yas'ko, *Bioorg. Khim.*, **23,** 906 (1997).
[7] J. Machado, H. Salomon, M. Oliveira, et al., *Nucleosides Nucleotides*, **18,** 901 (1999).
[8] Y. J. Hu, P. T. R. Rajagopalan, and D. H. Pei, *Bioorg. Med. Chem. Lett.*, **8,** 2479 (1998).
[9] R. H. Hall, A. Todd, and R. F. Webb, *J. Chem. Soc.*, 3291 (1957).
[10] M. Sekine and T. Hata, *Tetrahedron Lett.*, **16,** 1711 (1975).
[11] B. C. Froehler, P. G. Ng, and M. D. Matteucci, *Nucleic Acids Res.*, **14,** 5399 (1986).
[12] P. J. Garegg, T. Regberg, J. Stawinski, and R. Stromberg, *Chem. Scr.*, **26,** 59 (1986).
[13] P. J. Garegg, I. Lindh, T. Regberg, et al., *Tetrahedron Lett.*, **27,** 4055 (1986).
[14] P. J. Garegg, I. Lindh, T. Regberg, et al., *Tetrahedron Lett.*, **27,** 4051 (1986).
[15] J. E. Marugg, A. Burik, M. Tromp, G. A. Van der Marel, and J. H. Van Boom, *Tetrahedron Lett.*, **27,** 2271 (1986).
[16] J. Stawinski and M. Thelin, *Nucleosides Nucleotides*, **9,** 129 (1990).
[17] H. Takaku, S. Yamakage, O. Sakatsume, and M. Ohtsuki, *Chem. Lett.*, 1675 (1988).
[18] J. Jankowska, M. Sobkowski, J. Stawinski, and A. Kraszewski, *Tetrahedron Lett.*, **35,** 3355 (1994).
[19] A. Kers, I. Kers, J. Stawinski, M. Sobkowski, and A. Kraszewski, *Synthesis*, 427 (1995).
[20] D. V. Yashunsky, Y. E. Tsvetkov, M. A. J. Ferguson, and A. V. Nikolaev, *J. Chem. Soc., Perkin Trans. 1*, 242 (2002).
[21] K. F. Pirollo, A. Rait, L. S. Sleer, and E. H. Chang, *Pharmacol. Ther.*, **99,** 55 (2003).
[22] J. B. Opalinska and A. M. Gewirtz, *Nat. Rev. Drug Discov.*, **1,** 503 (2002).
[23] G. R. J. Thatcher and A. S. Campbell, *J. Org. Chem.*, **58,** 2272 (1993).
[24] S. Trippett, *Pure and App. Chem.*, **40,** 595 (1974).
[25] X. Chen, N. J. Zhang, Y. M. Li, et al., *Tetrahedron Lett.*, **38,** 1615 (1997).
[26] B. Duthu, D. Houalla, and R. Wolf, *Can. J. Chem.*, **66,** 2965 (1988).
[27] S. A. Bone and S. Trippett, *Tetrahedron Lett.*, **16,** 1583 (1975).
[28] H. Fu, G. Z. Tu, Z. L. Li, Y. F. Zhao, and R. Q. Zhang, *J. Chem. Soc., Perkin Trans. 1*, 2021 (1997).
[29] A. Munoz, M. Sanchez, M. Koenig, and R. Wolf, *Bull. Soc. Chim. Fr.*, 2193 (1974).
[30] R. Burgada, *Bull. Soc. Chim. Fr.*, 407 (1975).
[31] M. Wieber, K. Foroughi, and H. Klingl, *Chem. Ber.*, **107,** 639 (1974).
[32] J. I. G. Cadogan, I. Gosney, D. Randles, S. Yaslak, and R. P. Ambler, *J. Chem. Soc. Chem. Commun.*, 298 (1982).
[33] K. Vercruysse-Moreira, C. Dejugnat, and G. Etemad-Moghadam, *Tetrahedron*, **58,** 5651 (2002).
[34] C. Dejugnat, G. Etemad-Moghadam, and I. Rico-Lattes, *Chem. Comm.*, 1858 (2003).
[35] S. Kojima, K. Kawaguchi, S. Matsukawa, and K. Y. Akiba, *Tetrahedron*, **59,** 255 (2003).
[36] Y. Takagi, M. Warashina, W. J. Stec, K. Yoshinari, and K. Taira, *Nucleic Acids Res.*, **29,** 1815 (2001).
[37] D. M. Brown and N. K. Hamer, *J. Chem. Soc.*, 406 (1960).
[38] S. B. Tzokov, I. T. Devedjiev, E. K. Bratovanova, and D. D. Petkov, *Angew. Chem. Int. Edit. Engl.*, **33,** 2302 (1994).
[39] S. B. Tzokov, N. G. Vassilev, R. T. Momtcheva, J. Kaneti, and D. D. Petkov, *Phosphorus Sulfur Silicon Relat. Elem.*, **166,** 187 (2000).

[40] S. B. Tzokov, R. T. Momtcheva, N. G. Vassilev, J. Kaneti, and D. D. Petkov, *J. Am. Chem. Soc.*, **121,** 5103 (1999).
[41] P. C. Crofts, J. H. H. Markes, and H. N. Rydon, *J. Chem. Soc.*, 4250 (1958).
[42] J. Gloede and H. Gross, *J. Prakt. Chem.*, **314,** 184 (1972).
[43] P. J. Garegg, T. Regberg, J. Stawinski, and R. Stromberg, *J. Chem. Soc.-Perkin Trans. 1*, 1269 (1987).
[44] T. Atkinson and M. Smith, in *Oligonucleotide Synthesis: A Practical Approach*, edited by M. J. Gait (IRL Press, Oxford, 1984), pp. 35–81.
[45] L. Anschutz, W. Broeker, R. Neher, and A. Ohnheiser, *Ber.*, **76,** 218 (1943).
[46] L. Anschutz, W. Broeker, and A. Ohnheiser, *Ber.*, **77,** 439 (1944).
[47] A. Kers, I. Kers, J. Stawinski, M. Sobkowski, and A. Kraszewski, *Tetrahedron*, **52,** 9931 (1996).
[48] A. K. Bhattacharya and G. Thyagarajan, *Chem. Rev.*, **81,** 415 (1981).
[49] C. D. Roussev, G. D. Ivanova, E. K. Bratovanova, N. G. Vassilev, and D. D. Petkov, *J. Am. Chem. Soc.*, **121,** 11267 (1999).
[50] C. D. Roussev, G. D. Ivanova, E. K. Bratovanova, and D. D. Petkov, *Angew. Chem., Int. Ed.*, **39,** 779 (2000).
[51] G. Ivanova, E. Bratovanova, and D. Petkov, *J. Pept. Sci.*, **8,** 8 (2002).
[52] A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 951 (1953).
[53] R. A. Jones, in *Oligonucleotide Synthesis: A Practical Approach*, edited by M. J. Gait (IRL Press, Oxford, 1984), pp. 23–34.
[54] D. H. Rammler and H. G. Khorana, *J. Am. Chem. Soc.*, **84,** 3112 (1962).
[55] H. P. M. Fromageot, B. E. Griffin, C. B. Reese, and J. E. Sulston, *Tetrahedron*, **23,** 2315 (1967).