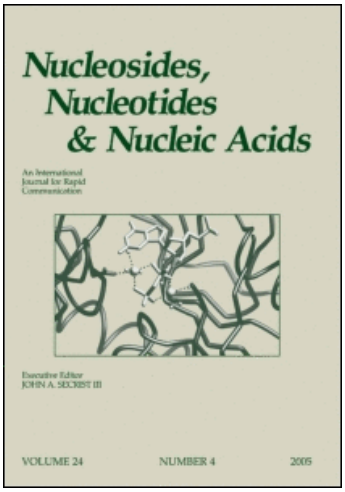

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Studies on Ribonucleoside Hydrogenphosphonates. Effect of a Vicinal Hydroxyl Function on the Stability of H-Phosphonate Diester Bond

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STUDIES ON RIBONUCLEOSIDE HYDROGENPHOSPHONATES. EFFECT OF A VICINAL HYDROXYL FUNCTION ON THE STABILITY OF H-PHOSPHONATE DIESTER BOND.

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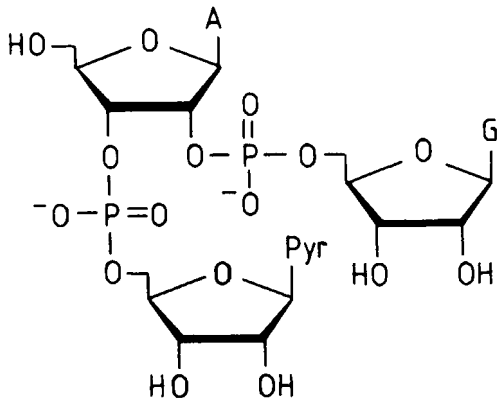
Dedicated to Prof. Wolfgang Pfleiderer on the occasion of his 60th birthday.

ABSTRACT.

Stability of hydrogenphosphonate diester bond in the presence of a free vicinal hydroxyl function has been investigated on the example of ribonucleoside H-phosphonates. It was found that H-phosphonate diesters undergo immediate intramolecular cyclization, both with or without a base, affording 2',3'-cyclic H-phosphonate diesters.

INTRODUCTION.

A ribonucleoside with a 2'-phosphomonoester and 3'-5' phosphodiester bonds has recently been shown to be involved in the ligation pathway of a viral RNA to its circular form.[1] More generally ribonucleosides containing vicinal 2'-5' and 3'-5' phosphodiester linkages (*Fig. 1*) have been found as key intermediates during mRNA splicing in eukaryotes.[2] Thus the availability of such molecules is of considerable interest for further biophysical and biochemical studies of these processes.



A = adenine - 9 - yl ; G = guanine - 9 - yl ; Pyr = cytosine-1-yl or uracil-1-yl

_ Fig : 1 _

Unfortunately, the preparation of vicinal phosphate linkages is not easy. So, despite their importance for elucidating the mechanism of RNA splicing, only a few reports[3-8] have appeared describing the chemical synthesis of these branched ribonucleotides.

Our recent interest in this field[7] as well as in hydrogenphosphonate chemistry[9-20] prompted us to investigate if ribonucleoside H-phosphonate diesters may be of value in the synthesis of branched RNA fragments. The present paper describes our investigation of the introduction of hydrogenphosphonate groups on the secondary hydroxyl functions in the ribofuranonucleoside series.

RESULTS AND DISCUSSION.

The chemistry of ribonucleoside H-phosphonate diesters has not been explored and to our knowledge there is only one report in literature[21] claiming the synthesis of ribonucleoside H-phosphonate diesters. However, since all conclusions in that paper were based only on TLC data we decided to undertake more detailed studies concerning stability of H-phosphonate diester bond in the presence of a vicinal hydroxyl group and investigate the coupling reaction between

H-phosphonate monoester and ribonucleosides having unprotected diol system.

Reactions of N^6-benzoyl-5'-O-(4-methoxytrityl)adenosine (1) with the nucleoside hydrogenphosphonate 2 *(Scheme 1)*.

Since condensations without preactivation using pivaloyl chloride as coupling agent have proved to be the most suitable for oligonucleotide synthesis by the hydrogenphosphonate approach,[11,14,22] we chose these reaction conditions for our studies.

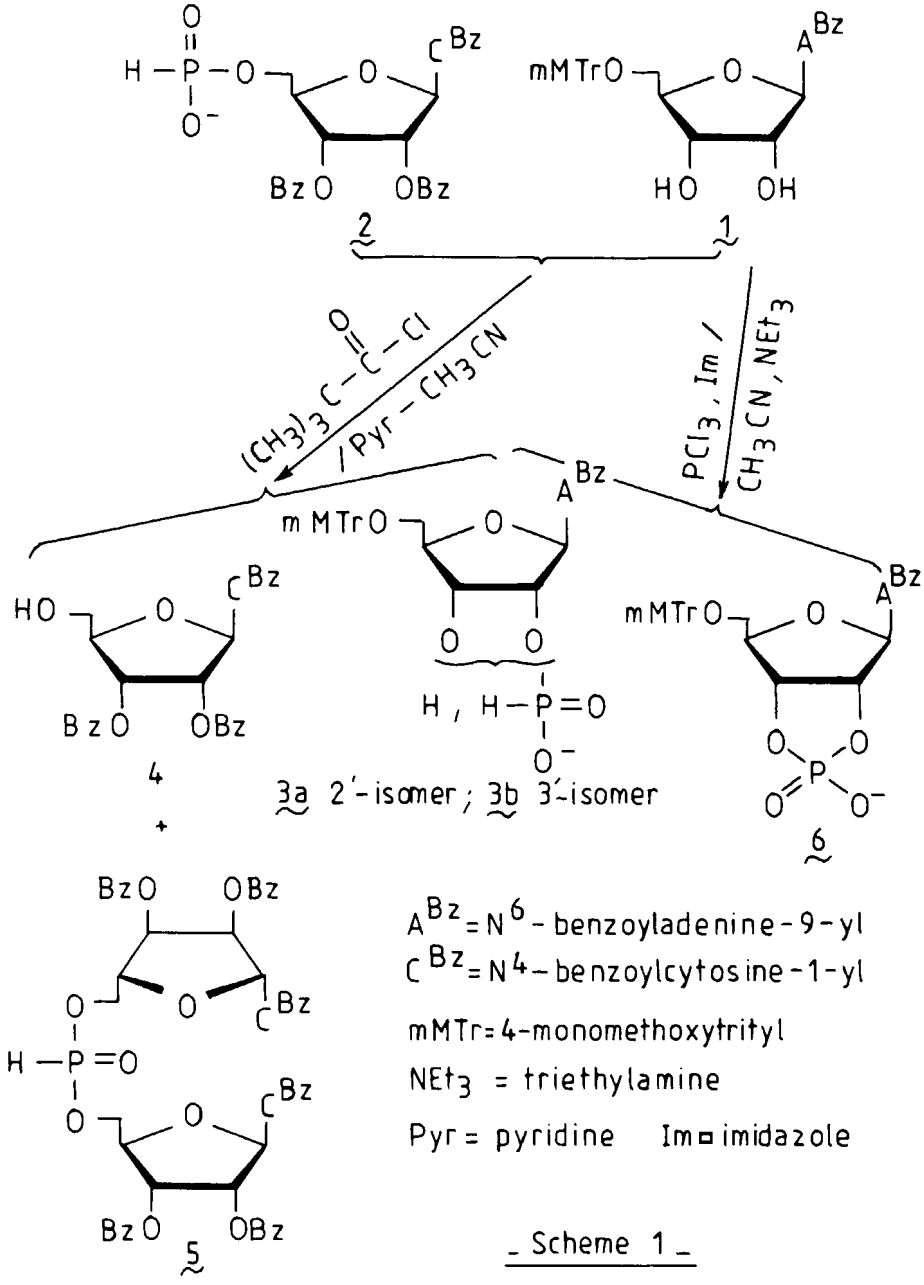
Treatment of ribonucleoside 1 with the hydrogenphosphonate monoester 2 (3 eq.) in a pyridine-acetonitrile mixture in the presence of pivaloyl chloride (6 eq.) for 45 min resulted in the disappearance of the starting material 1 and formation of three new compounds. The two less polar compounds were identified as the dephosphonylated cytidine nucleoside 4 and its symmetrical 5'-5' hydrogenphosphonate diester 5. The third product was isolated by short column chromatography. Its structural assignment was based on ^1H- and ^{31}P-NMR studies and corroborated by fast atom bombardment mass spectroscopy. This material was thus unequivocally characterised as a mixture of 2' and 3'-hydrogenphosphonates 3a and 3b.

As expected,[23] the same mixture of 3a and 3b (contaminated with a minute amount of the 2',3'-cyclic phosphate derivative 6) was also obtained when the ribonucleoside 1 was treated with 8.5 eq. of tri-(imidazol-1-yl)phosphine.

Our present results can be compared to those of Holy et al., who found that during transesterification of ribonucleosides with triethyl[24] or triphenyl[25] phosphite, even in large excess, mixtures of 2' and 3'-hydrogenphosphonates were isolated as the sole[24] or main[25] products.

Mechanism of reactions of hydrogenphosphonate monoesters with ribonucleosides having vicinal hydroxyl functions

Our above results can be explained as follows: H-phosphonate 2 reacts with adenosine derivative 1 forming a mixture of 3'-5' and 2'-5' isomers of the adenylcytidine H-

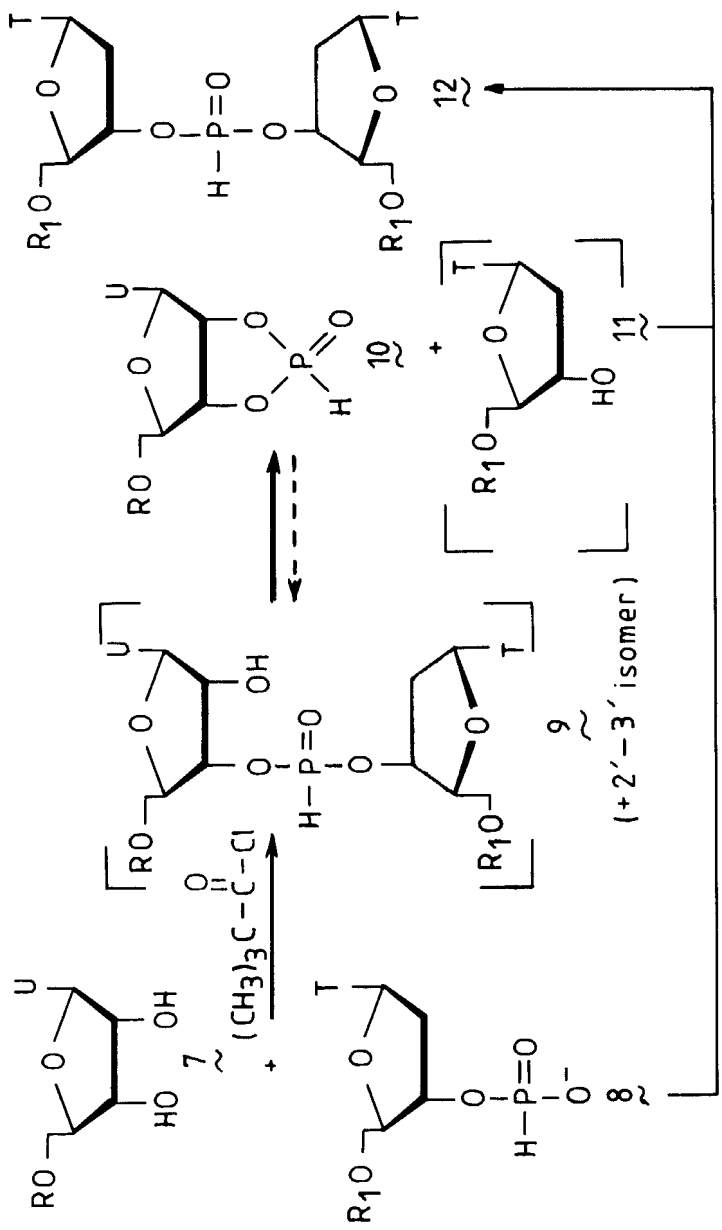


Scheme 1

phosphonate diesters. These undergo fast intramolecular transesterification, affording the adenosine 2',3'-cyclic H-phosphonate and the 5'-OH free cytidine derivative **4**. Since the reaction of **2** with compound **1** is apparently slower than intramolecular transesterification of the adenylcytidine H-phosphonate, the cytidine nucleoside, released during the cyclization, reacts with H-phosphonate **2** forming a symmetrical 5'-5' hydrogenphosphonate diester **5**. After addition of water the latter remains unchanged, while the adenosine 2',3'-cyclic H-phosphonate hydrolyses yielding a mixture of the adenosine 2'(3') H-phosphonate monoesters (**3a** and **3b**).

To provide further support for the proposed reaction pathway and to find out if it can be considered as a general route for the reactions of H-phosphonate monoesters with nucleosides having vicinal hydroxyl functions, we carried out additional ^{31}P-NMR experiments.

When 5'-O-dimethoxytritylthymidine 3'-H-phosphonate **8a** was reacted, in acetonitrile-pyridine mixture (1:1, v/v), with 1 eq. of uridine derivative **7a** in the presence of 2 eq. of pivaloyl chloride (*Scheme 2*), the ^{31}P-NMR spectrum (with ^{1}H-heteronuclear decoupling) of the reaction mixture showed three signals: two singlets of equal intensities at 27.9 and 23.8 ppm and a singlet at 7.3 ppm. The latter signal appeared as two triplets ($^{1}J_{P-H}$=715 Hz; $^{3}J_{P-H}$=8.6 Hz) in the spectrum without ^{1}H-heteronuclear decoupling, and it was assigned to the symmetrical H-phosphonate diester **12a** (comparison with an authentic sample of **12a** produced from **8a** and **11a**). The two singlets at 27.9 and 23.8 were assigned to two diastereoisomers of the uridine 2',3'-cyclic H-phosphonate **10a**. In agreement with this structure, both singlets appeared as four doublets of doublets, with the coupling constants $^{1}J_{P-H}$=734 Hz ($^{3}J_{P-H}$=6.7 and 13.4 Hz) and $^{1}J_{P-H}$=737 Hz ($^{3}J_{P-H}$=4.9 and 11.6 Hz) respectively. Addition of water to such a reaction mixture resulted in fast hydrolysis of **10a** into **16** (for structure see *Scheme 3*), *i.e.* a mixture of 5'-O-dimethoxytrityluridine 3'(2') H-phosphonate monoesters (4.9 and 4.7 ppm; $^{1}J_{P-H}$=633 Hz , $^{3}J_{P-H}$=6.8 Hz, doublet of doublets and $^{1}J_{P-H}$=630 Hz , $^{3}J_{P-H}$=6.8 Hz, doublet of doublets respectively). It is worth to stress that the ratio of 3'(2') isomers



Scheme: 2

of the uridine H-phosphonate monoesters **16** after hydrolysis was *ca* 1:2.5 in favour of the isomer that absorbed in higher field.

The reaction followed the same pathway, as judged from the ^{31}P-NMR-spectra, when **8a** reacted with **7a** in pyridine in the presence of chlorodiphenylphosphate.

The intramolecular cyclization of **9a**, which results in formation of cyclic H-phosphonate **10a**, can be a reversible reaction. However, under the above discussed reaction conditions we could not detect even traces of compound **9a** in the reaction mixture. The reason can be trapping of nucleoside **11a** by activated H-phosphonate **8a**, or alternatively, because **10a** is the most stable species under the reaction conditions. Thus, to find out if the cyclization is a reversible reaction, we added 10 eq. of ethanol to a reaction mixture consisting of **10a** and **12a**. The ^{31}P-NMR spectrum recorded directly after addition of alcohol showed substantial decrease of signals from **10a** (*ca* 90%) and appearance of four new signals (8.4, 8.3, 7.9 and 7.8 ppm) in the region of chemical shifts where non-cyclic H-phosphonate diesters usually absorb. On the basis of chemical shifts and splitting patterns, in the spectrum without ^{1}H-heteronuclear decoupling, we assigned these signals to a diastereomeric mixture of 5'-O-dimethoxytrityluridine 3'(2')-ethyl H-phosphonate diesters.[26] Thus, this experiment indicates that cyclization is reversible and that H-phosphonate diesters having a vicinal hydroxy function can exist in equilibrium with the cyclic H-phosphonate diester.

In addition, hydrolysis of this reaction mixture afforded only a mixture of the uridine 3'(2') H-phosphonate monoesters **16** with **12a**, which remained unchanged during this reaction. This indicates that hydrolysis of the uridine 3'(2') ethyl hydrogenphosphonates proceeds through the cyclic intermediate **10a**.

Attempted synthesis of ribonucleoside H-phosphonate diesters in acetonitrile without a base.

Since rapid cyclization of **9a** can be promoted by pyridine, we carried out similar type of condensation in pure

acetonitrile using pivaloyl chloride as condensing reagent. To ensure stability of protective groups under reaction conditions, we used silyl derivatives for protection of 5'-OH function in ribonucleoside and nucleoside H-phosphonate (*Scheme 2*).

A reaction of equimolar amounts of ribonucleoside **7b** and nucleoside H-phosphonate **8b** in acetonitrile in the presence of 2 eq. of pivaloyl chloride proved to be very slow compared to the condensation in pyridine/acetonitrile mixture. ^{31}P NMR spectra recorded after 4 hours revealed the presence of only starting material and small amounts (*ca* 5%) of cyclic H-phosphonate diester **10b**. The reaction was completed after 48 h and compound **10b** constituted the major product.[27] When 1-2 eq. of pyridine were added at the early stage of the reaction, ^{31}P NMR spectra showed immediate formation of cyclic H-phosphonate **10b** and a symmetrical H-phosphonate diester **12b** (δ 7.1 ppm, two triplets $^{1}J_{P-H}$=715 Hz, $^{3}J_{P-H}$=8.5 Hz). Thus, these results indicate that despite the fact that pyridine promotes fast cyclization of ribonucleoside H-phosphonate diesters, these compounds undergo also cyclization without presence of a base and the latter reaction is faster than the coupling, *e.g.* formation of **9b**.

To check if under favorable reaction conditions coupling can be faster than cyclization, we carried out a condensation of ribonucleoside **7b** with 2 eq. of ethyl H-phosphonate in the presence of 4 eq. of pivaloyl chloride. Since ethyl H-phosphonate is more reactive than nucleoside H-phosphonate **8** and since it was used in an excess, one could anticipate formation of ribonucleoside 2',3'-bis H-phosphonate monoester under such reaction conditions. Unfortunately, ^{31}P NMR spectroscopy revealed the same picture as in the reaction **7b** + **8b**. The only product of the reaction both in neat acetonitrile or in acetonitrile with 1-2 eq. of pyridine, was again cyclic H-phosphonate **10b** and diethyl H-phosphonate diester, together with the starting ethyl H-phosphonate. The rate of condensation in neat acetonitrile was comparable to the rate of coupling of **7b** with **8b** which indicates that a steric hindrance is not an important factor in the

condensation reaction. ^{31}P NMR spectrum of the reaction 7b with 1 eq. of ethyl H-phosphonate after 4 hours showed mainly starting material *ca* 5% of cyclic H-phosphonate 10b.

Attempted removal of 2'-O-dimethyl-t-butylsilyl group in the presence of 3',5'-H-phosphonate diester bond.

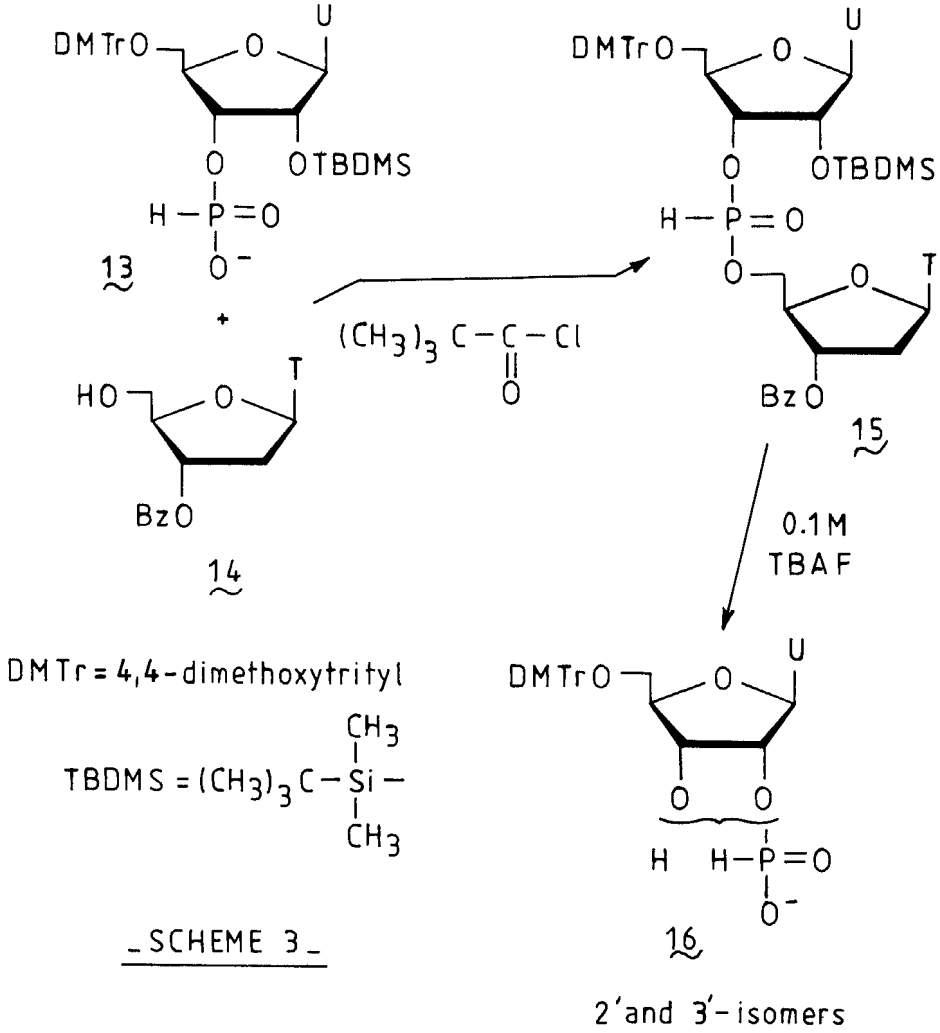
As a final part of these investigations we checked the possibility of removal of 2'-O-silyl protective group from ribonucleoside having 3',5'-internucleotidic H-phosphonate bond. Such a approach was claimed to be an efficient way to ribonucleoside H-phosphonate diesters.[21]

5'-O-dimethoxytrityl-2'-O-dimethyl-t-butylsilyl-uridine -3'-hydrogenphosphonate (13) was reacted in pyridine with 1.1 eq. of 3'-O-benzoyl thymidine (14) in the presence of 2 eq. of pivaloyl chloride (*Scheme 3*). Reaction mixture was work-up after 5 min and the crude H-phosphonate diester 15 was treated with 0.1M TBAF in THF. The progress of the reaction was followed by ^{31}P NMR spectroscopy. It was found that addition of fluoride causes immediate conversion of the starting H-phosphonate diester 15 to a mixture of 2' and 3'-hydrogenphosphonates 16. Removal of 2'-O-silyl group from H-phosphonate monoester 13 is substantially slower than from 15 and indicates that in the latter case desilylation is probably facilitated by subsequent cyclization.

CONCLUSION.

In the present studies directed towards synthesis of branched RNA *via* hydrogenphosphonate intermediates, the stability of internucleotidic H-phosphonate bond in the presence of a vicinal hydroxyl function has been investigated.

It was found that ribonucleoside 2'(3')-H-phosphonate diesters having a free vicinal hydroxyl function, are extremely susceptible to intramolecular transesterification forming instantly 2',3'-cyclic H-phosphonate diesters. The cyclization occurs both under basic conditions (*e.g.* in pyridine) and when the base is absent (*e.g.* in neat acetonitrile).



SCHEME 3

These findings are in a marked disagreement with a literature report concerning reactivity of ribonucleoside H-phosphonate diesters[21] and thus it seems rather unlikely that the compound described by Ogilvie and Nemer was diribonucleoside-(3'-5')-hydrogenphosphonate diester. However the presently observed lability of ribonucleoside 2'(3')-H-phosphonate diesters having a free vicinal hydroxyl function is in agreement with similar results obtained with phosphotriesters[28-30] and methylphosphonate[31] linkages.

EXPERIMENTAL.

General Procedures. Evaporation of solvents was done with a rotary evaporator under reduced pressure (water aspirator). Ultraviolet spectra (UV) were recorded on a Cary 118 C spectrophotometer. Proton nuclear magnetic resonances were determined in DMSO-d_6 at ambient temperature on a Bruker WM 360 WB spectrometer. Chemical shifts are expressed in parts per million downfield from DMSO set at 2.49 ppm. The presence of exchangeable protons was confirmed by exchange with D_2O followed by reintegration. ^{31}P-NMR spectra were recorded with and without proton decoupling on a Bruker WP 200 SY instrument at 81.015 MHz for the compounds 2, 3 and 6 or on a Jeol GX-400 FT spectrometer at 161.7 MHz for the others compounds; chemical shifts (parts per million) were reported relative to external H_3PO_4. Mass spectra (MS) were measured with a Jeol JMSDX 300 instrument. Thin-layer chromatography (TLC) was performed in an ascending system on precoated aluminium sheets of silica gel 60 F_{254} (Merck, No 5554), visualization of products being accomplished by UV absorbance followed by charring with 10% ethanolic sulfuric acid and heating; phosphorus-containing components were detected by spraying with Hanes molybdate reagent.[32] Short column chromatography was performed with silica gel 60 H (Merck, No 7736) under weak nitrogen pressure (≈ 4 psi). Pivaloyl chloride (Aldrich) and chlorodiphenylphosphate (Fluka) were commercial grade.

N^6-Benzoyl-5'-O-monomethoxytrityladenosine (1).

This compound was obtained with only minor modification of the method of Pfleiderer et al.[33]

N^4,2',3'-O-tribenzoylcytidine 5'-hydrogenphosphonate (2).

To a solution of imidazole (1.95 g, 28.7 mmoles) in anhydrous acetonitrile (54 mL) were added, with stirring and cooling in an ice bath, PCl_3 (0.75 mL, 86 mmoles) and triethylamine (4.2 mL, 30.2 mmoles). The mixture was stirred for 15 min, then a solution of dried N^4,2',3'-O-tribenzoylcytidine (4)[34] (1.1 g, 2.0 mmoles) in acetonitrile (54 mL) was added dropwise. After the addition of 4 was complete, the reaction mixture was stirred at room temperature for 4 h. Water (15 mL) was added and the solution was stirred for another 30 min. The solvent was removed under vacuum and the residue was reevaporated with a mixture of pyridine-triethylamine (4:1, v/v) three times. The precipitated salts were removed and the filtrate was evaporated under vacuum, taken up in chloroform (ca. 100 mL) and water (ca. 100 mL). The aqueous layer was extracted twice with chloroform. The combined organic layers were dried over sodium sulfate, filtered and evaporated to dryness. Chromatography of the residue on a silia gel column, using as eluent a stepwise gradient of MeOH from 0 to 2% in $CHCl_3$ with 1% triethylamine, led after evaporation of the appropriate fractions to the isolation of 2 (1.3 g, 90%) as a foam; TLC, R_f= 0.30 (ethyl acetate- methanol 7:3, v/v); the values of UV absorption maxima (300 and 260 nm) and minima (290 and 247 nm) in MeOH were identical with those of 4. A fraction of this foam was treated with Dowex 50 W (Na^+ form) ion-exchange resin, yielding the hydrogenphosphonate 2 as a sodium salt; ^{1}H-NMR, δ 3.9-4.1 (m,2H,H-5',5"), 4.6 (m,1H,H-4'), 5.7-5.8 (m,3H,H-2',3' and H-5), 6.39 (d,1H,H- 1',$J_{1',2'}$= 4.9 Hz), 6.71 (d,1H,H-P; J_{H-P}= 578 Hz), 7.4-8.1 (m,15H,benzoyl H), 8.66 (d,1H,H-6; $J_{5,6}$= 7.2 Hz), 11.3 (br s, 1H,NH-4); ^{31}p-NMR (DMSO- d_6), δ:3.6 (J_{p-H}= 577.9 Hz, $J_{p-5',5"}$= 7.0 Hz); MS (FAB>0, glycerol matrix): 642 $(M + Na)^+$, 664 $(M - H + 2Na)^+$ 686 $(M - 2H + 3Na)^+$.

Reaction of N^6-benzoyl-5'-O-(4-methoxytrityl)adenosine (1) with the hydrogenphosphonate 2.

Nucleoside 1[33] (131 mg, 0.20 mmole) and hydrogenphosphonate 2 (440 mg, 0.61 mmole, 3 eq.) were first coevaporated three times with anhydrous pyridine then dissolved in a mixture of pyridine-acetonitrile 1:1, v/v (15 mL). Pivaloyl chloride (150 μL, 1.23 mmoles, 6 eq.) was added and the resulting solution was stirred at room temperature for 45 min. The reaction mixture was diluted with water (50 mL) and extracted with chloroform (3 x 50 mL). The combined extracts were dried over sodium sulfate and evaporated. TLC examination in several eluent systems showed absence of starting nucleoside 1, presence of excess hydrogenphosphonate 2 and appearance of three new compounds. By comparison with authentic samples,[35] the two less polar compounds were identified as dephosphonylated nucleoside 4 and N^4,2',3'-tri-O-benzoylcytidylyl-5'-hydrogenphosphonate-5'-N^4,2',3'-tri-O-benzoylcytidine (5). Only the more polar compound showed the characteristic color of trityl; it was isolated by column chromatography (eluent: stepwise gradient of MeOH from 0 to 20% in $CHCl_3$ with 1% triethylamine) and characterized as a mixture of 2' and 3'-hydrogenphosphonates 3; TLC, R_f= 0.15 (ethyl acetate-methanol, 7:3, v/v); 0.20 (Butanol-H_2O, 9:1, v/v); the values of UV absorption maximum (278 nm) and minimum (255 nm) in MeOH were identical with those of 1. This compound was treated with Dowex 50 W (Na^+ form) ion-exchange resin, yielding its sodium salt form; ^{1}H-NMR (double irradiation technique after D_2O exchange permitted unambigous assignment of all sugar protons): 2'-hydrogenphosphonate isomer **3a**, δ = 3.1-3.3 (m,2H,H-5',5"), 3.73 (s,3H,OCH_3), 4.10 (m,1H,H-4'), 4.54 (t,1H,H-3'; $J_{2',3'}$= 5.4 Hz), 5.23 (m,1H,H-2'), 6.16 (d,1H,H-1'; $J_{1',2'}$=4.2 Hz), 6.65 (d,1H,H-P), 6.8-8.1 (m,benzoyl and trityl-H), 8.56 and 8.64 (2s,2x1H,,H-2 and H-8); 3'-hydrogenphosphonate **3b**, δ = 3.1-3.3 (m,2H,H-5',5"), 3.73 (s,3H,OCH_3), 4.21 (m,1H,H-4'), 4.73 (m,1H,H-3'), 4.91 (t,1H,H-2'), 6.02 (d,1H,H-1'; $J_{1',2'}$= 6.0 Hz), 6.72 (d,1H,H-P), 6.8-8.1 (m,benzoyl and trityl-H), 8.53 and 8.60 (2s,2x1H,H-2 and H-8); ^{31}P-NMR (CD_3OD): 2'-hydrogenphosphonate **3a**, δ = 5.69 (J_{P-H}= 633.4 Hz; $J_{P-2'}$= 9.8 Hz); 3'-hydrogenphosphonate **3b**, δ = 5.86 (J_{P-H}= 632.0 Hz);

$J_{p-3'}$ = 9.3 Hz); MS (FAB>0, glycerol matrix): 708 $(MH)^+$, 729 $(M+Na)^+$, 751 $(M-H + 2 Na)^+$; (FAB<0, glycerol matrix): 706 $(M-H)^-$.

Reaction of nucleoside 1 with PCl_3-imidazole.

To a solution of imidazole (635 mg, 9.3 mmoles) in anhydrous acetonitrile (8.8 mL) were added, with stirring and cooling in an ice bath, PCl_3 (245 μL, 2.8 mmoles) and triethylamine (1.37 mL, 9.8 mmoles). The mixture was stirred for 15 min, then a solution of dried nucleoside 1 (210 mg, 0.33 mmole) in acetonitrile (8.8 mL) was added dropwise. After the addition of 1 was complete, the reaction mixture was stirred at room temperature for 4 h. Workup was similar to that described above for the synthesis of 2. TLC examination showed disappearance of the starting nucleoside 1 and appearance of a new compound, the spot of which had the same R_f of that of 3. As above, this compound was isolated by column chromatography and converted into its Na form by treatment with Dowex 50 W (Na^+ form) ion exchange resin; its UV and MS spectra were superimposable upon those of 3. Its ^1H-NMR and ^{31}P-NMR showed it consisted of a mixture of 3a (39%), 3b (44%) and 6 (17%). ^1H-NMR (after D_2O exchange): 6, δ = 3.1-3.3 (m,2H,H-5'-5"), 3.73 (s,3H,OCH_3), 4.4 (m,1H,H-4'), 4.9 (m,1H,H-3'), 5.3 (m,1H,H-2'), 6.28 (d,1H,H-1'; $J_{1',2'}$ = 3.4 Hz), 6.8-8.1 (m,benzoyl and trityl-H), 8.59 and 8.60 (2s,2x1H,H-2 and H-8); ^{31}P-NMR (CD_3OD): 6, δ = 21.29 ($J_{P-2'}$ and 3' = 10.6 and 8.0 Hz).

Synthesis of 5'-O-dimethoxytrityl-2'-O-dimethyl-t-butylsilyluridine-(3'-5')-3'-O-benzoylthymidine hydrogenphosphonate diester (15).

5'-O-dimethoxytrityl-2'-O-dimethyl-t-butylsilyluridine 3'-H-phosphonate (13)[12] (0.1 mmole) and 3'-O-benzoylthymidine (0.11 mmole) were rendered anhydrous by evaporation of added pyridine and finally were dissolved in the same solvent (1 ml). Pivaloyl chloride (2 eq.) was then added and after 5 min the reaction mixture was quenched with 0.1M TEAB and extracted with chloroform (20 ml). The organic phase was washed with water (2x10 ml), evaporated and the residue was

coevaporated several times with toluene to remove completely pyridine. A foam was evaporated with THF, dissolved in the same solvent and precipitated from n-hexane-ethyl ether mixture. The white powder was dried overnight under vacuum and was used for further studies without purification. The product was chromatographically homogeneous. ^{31}P NMR spectrum in THF:δ 9.6 ppm (doublet of quartets, $^{1}J_{P-H}$=730 Hz, $^{3}J_{P-H}$=9.7 Hz).

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Hz;two multiplets), signal at 8.3 ppm ($^{1}J_{P-H}$=726 Hz, $^{3}J_{P-H}$=9.2 Hz; two multiplets), signal at 7.9 ppm ($^{1}J_{P-H}$=713 Hz, $^{3}J_{P-H}$=9.8 Hz; two multiplets), signal at 7.8 ppm ($^{1}J_{P-H}$=709 Hz; $^{3}J_{P-H}$=9.2 Hz; two multiplets). Multiplets consisted of partially overlapping quartets.

27. ^{31}P NMR data for 10b in acetonitrile:δ 28.6 and 24.1 ppm (two diastereoisomers). Spectrum without ^{1}H-heteronuclear decoupling: four doublets of doublets, $^{1}J_{P-H}$=732 Hz ($^{3}J_{P-H}$=7.3 and 12.2 Hz) and $^{1}J_{P-H}$=740 Hz ($^{3}J_{P-H}$=5.5 and 10.9 Hz).

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35. Comparisons with authentic 4[34] and 5 were made by TLC and NMR studies. Authentic 5 was prepared by reacting 2 with 4 in a pyridine-acetonitrile mixture in the presence of pivaloyl chloride and purified by silica gel column chromatography.

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