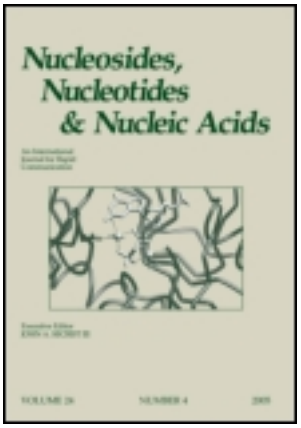

Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

Efficient Synthesis of 2′-Deoxynucleoside 3′-C-Phosphonates: Reactivity of Geminal Hydroxyphosphonate Moiety

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Published online: 05 Nov 2010.

To cite this article: Šárka Králíková, Miloš Buděšínský, Milena Masojídková & Ivan Rosenberg (2000) Efficient Synthesis of 2′-Deoxynucleoside 3′-C-Phosphonates: Reactivity of Geminal Hydroxyphosphonate Moiety, Nucleosides, Nucleotides and Nucleic Acids, 19:7, 1159-1183, DOI: 10.1080/15257770008035038

To link to this article: http://dx.doi.org/10.1080/15257770008035038

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(7), 1159–1183 (2000)

EFFICIENT SYNTHESIS OF 2'-DEOXYNUCLEOSIDE 3'-*C*-PHOSPHONATES: REACTIVITY OF GEMINAL HYDROXYPHOSPHONATE MOIETY.

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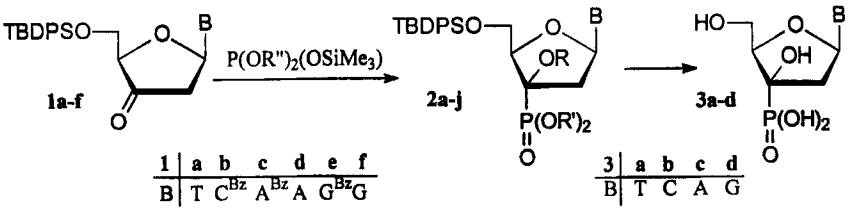
Abstract: In this report we present a novel, simple way for the synthesis of 3'-*C*-phosphonate derivatives of all four basic 2'-deoxynucleosides in both fully protected and deprotected forms. The reactivity of the geminal hydroxy phosphonate moiety located at the 3'-carbon atom of the nucleoside was studied with respect to the use of this type of nucleoside phosphonic acid for the preparation of short oligonucleotides, namely, dinucleoside monophosphate analogues.

The nucleophilic addition of diethyl phosphite to the 3'-ketonucleosides in the presence of lithium bis(trimethylsilyl)amide at -78 °C resulting in 3'-*C*-diethylphosphono nucleosides has been described already by Wiemer et al. [1,2]; however in the 2'-deoxynucleosides series , only 3'-*C*-diethyl phosphono-5'-*O*-tritylthymidine was prepared.

In contrast to the literature data [1,2], we obtained the 2'-deoxynucleoside-3'-hydroxy phosphonates **2a-j** under very mild reaction conditions by nucleophilic addition of *tris*-trimethylsilyl phosphite or diethyl trimethylsilyl phosphite [3,4,5] to the 2'-deoxy-3'-keto-nucleosides in dichloromethane at room temperature (**SCHEME 1**) [6]. The reaction performed with *tris*-trimethylsilyl phosphite [7] led to higher yields of a single 3'-epimer of hydroxy phosphonates **2a-f** (R=R'=H) than the method using diethyl trimethylsilyl phosphite [8] which effected formation of **2h-2j** (R=$SiMe_3$; R'=Et). In the latter case, we found a partial elimination of the protected cytosine and purine nucleobases. In addition, the reaction



SCHEME 1

performed with diethyl trimethylsilyl phosphite resulted in 3'-*O*-trimethylsilyl derivatives **2g, 2i, 2j** (see **TABLE 1**) which were stable to silica gel chromatography. The trimethylsilyl group attached to the tertiary hydroxyl in compound **2g** was found to be stable in refluxing methanolic 0.08 M acetic acid (48 h) and in 50% aqueous methanol containing 0.1 M triethylammonium hydrogencarbonate (16 h at r.t.). Quantitative desilylation of the phosphonate **2g** leading to the formation of compound **7** was achieved in 1% solution of ammonia in methanol-water (7:3) overnight.

On the other hand, no 3'-*O*-trimethylsilyl derivative was isolated in the reaction performed with *tris*-trimethylsilyl phosphite. The migration of the 3'-*O*-trimethylsilyl group to the phosphorus hydroxyl under slightly basic conditions during workup followed by immediate hydrolysis seems to be the driving force of this fast hydrolytic reaction.

All the fully and partially protected hydroxyphosphonates were gradually deprotected to give nucleoside phosphonic acids **3a-d**. The compounds were found to be completely stable in 37% aqueous ammonia (16 h at r.t.; 55 °C for **3a**) and to tetrabutylammonium fluoride treatment. The thymidylate analogue **3a** was also stable in dilute hydrochloric acid (0.05M, 16 hrs at r. t.).

The starting 3'-ketonucleosides **1** were prepared by two oxidation methods. Using the pyridinium dichromate/molecular sieves (PDC/MS) oxidation procedure, described in the literature for the oxidation of 5'-*O*-tritylthymidine in dichloromethane [9], we found the susceptibility to oxidation of the 5'-*O*-*tert*-butyldiphenylsilyl derivatives of 2'-deoxynucleosides depending on the type of nucleobase, decreasing in the order T, U >> A, G^{Bz} > A^{Bz}, C^{bz}>>> C, G. The *N*-unprotected cytosine derivative remained unchanged and the guanine one was decomposed completely. Since only the T and U derivatives afforded

TABLE 1. Results of the addition of phosphites to 2'-deoxy-3'-ketonucleosides **1a-f** prepared by Dess-Martin oxidation procedure.

Phosphite	Product	B	R	R'	Yield (%)
$P(OSiMe_3)_3$	**2a**	T	H	H	87
	2b	C^{Bz}	H	H	78
	2c	A^{Bz}	H	H	70
	2d	A	H	H	35
	2e	G^{Bz}	H	H	80
	2f	G	H	H	25
$P(OEt)_2(OSiMe_3)$	**2g**	T	$SiMe_3$	Et	83
	2h	C	H	Et	41
	2i	A^{Bz}	$SiMe_3$	Et	49
	2j	G^{Bz}	$SiMe_3$	Et	35

high yield of 3'-keto compounds **1**, we tried C, C^{Bz}, A^{Bz} and G derivatives in oxidation conditions using acetonitrile or ethyl acetate as the solvent instead of dichloromethane. Before addition of the nucleoside, sonication of PDC with molecular sieves in the appropriate solvent was performed in order to get both a homogeneous suspension and also a better adsorption of PDC onto the molecular sieves. Under these conditions, as measured for the T derivative, the oxidation reaction was much slower and, unfortunately, for the C and C^{Bz} derivatives, the oxidation failed again. In spite of these difficulties, the 2'-deoxynucleoside phosphonic acids **3a-d** were prepared using this method of oxidation [B=T (58 %), A (47 %), G (34 %), C (7 %); C (19 %) by transformation of the U derivative [10]]; the yield is an average value obtained from several experiments. It is necessary to point out that the above described experiments were performed with PDC prepared in our laboratory, according to the known procedure [11], because the commercially available PDC (Fluka) only gave very low yields of 3'-ketonucleosides in our hands. We also explored the use of the polymer bound PDC (Fluka) but no change of starting nucleosides was detected over two days of reaction. As regards the protecting groups we have preferred *tert*-butyldiphenylsilyl and trityl units to

TABLE 2. Phosphonylation of 3'-keto-5'-*O*-tritylthymidine (**1g**) with various phosphites

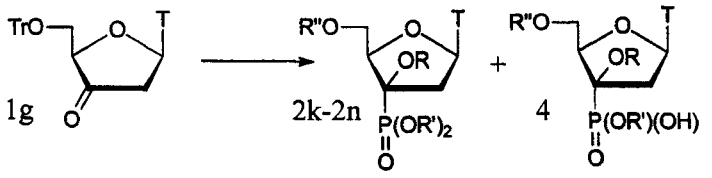
Phosphite	Product	Solvent	Temperature	R	R'	R"	Yield (%)
$P(OSiMe_3)_3$	**2k**	CH_2Cl_2	r.t.	H	H	H	73
		CH_2Cl_2	80 °C/6h				82
		CH_3CN	r.t.				66
		THF	r.t.				51
$HP(O)(OSiMe_3)_2$	**2k**	CH_2Cl_2	r.t.	H	H	Tr	32
$P(OMe)_2(OSiMe_3)$	**2l**	CH_2Cl_2	r.t.	$SiMe_3$	Me	Tr	[a]
	4			H	[b]	Tr	55[b]
$P(OEt)_2(OSiMe_3)$	**2m**	CH_2Cl_2	r.t.	$SiMe_3$	Et	Tr	67
	2n		80 °C/6h	H	Et	H	55

[a] only NMR sample was isolated to confirm the structure;
[b] the compound **4** was isolated as monomethylester

dimethoxytrityl and acetyl groups which made the molecule unstable during the oxidation. The most serious problem of the PDC/MS oxidation procedure seemed to be in irreproducible removal of chromium-containing compounds coming from PDC after oxidation, which influenced both the stability of ketonucleosides and the addition reaction of phosphites.

Since the elaboration of a reliable PDC/MS oxidation procedure for the protected 2'-deoxynucleosides (except T and U derivatives) was not successful, we turned our attention towards the use of the Dess-Martin periodinane reagent (DM) [12-15] for smooth and efficient preparation of 5'-*O*-*tert*-butyldiphenylsilyl-2'-deoxy-3'-ketonucleosides [12,13]. This method is distinguished for uncomplicated isolation of ketonucleosides in almost pure form in comparison with the PDC method. In agreement with the literature data we found the 3'-ketonucleosides unstable during silica gel chromatography and, therefore, subsequent reaction with phosphites was performed with "crude" keto compounds. The quantitative conversion of protected nucleosides to 3'-ketonucleosides **1a-f** made it possible to prepare 3'-hydroxyphosphonates in good yields as shown in **TABLE 1**.

The influence of the type of phosphite, solvent and temperature on the course and stereoselectivity of the addition reaction was examined with 3'-keto-5'-*O*-tritylthymidine (**1g**)

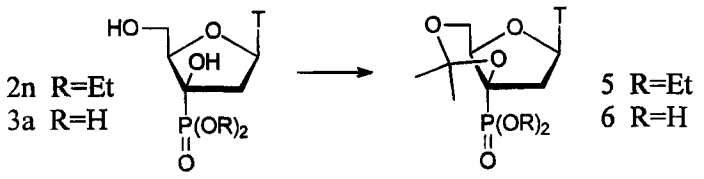


SCHEME 2

(**TABLE 2**). The comparison of various silyl phosphites showed that only the reaction of *bis*-trimethylsilyl phosphite [16] with **1g** was accompanied by considerable elimination of thymine so that the yield of phosphonate **2k** was significantly lower. The addition of dimethyl trimethylsilyl phosphite [8] led to dimethyl phosphonate **2l** which was unstable at the workup even under mild basic hydrolytic conditions (ethanol-aqueous triethylammonium hydrogencarbonate). In this case a monomethyl ester was identified and, therefore, the diester **2l** was completely transformed to the monoester **4** before isolation (**SCHEME 2**).

An interesting phenomenon was observed when a mixture of 5'-*O*-trityl-3'--ketothymidine (**1g**) and diethyl trimethylsilyl phosphite in dichloromethane was heated under reflux. To our surprise, the quantitatively detritylated product **2n** with a free 3'-hydroxyl group was obtained. We found out that ten minutes of heating at 80 °C was enough to detect both tritylated and detritylated products on TLC. The experiment performed under the same reaction conditions with the 5'-*O*-tritylthymidine revealed its complete stability. The explanation of this phenomenon is unclear but may be due to the decreased stability of the trityl group due to the presence of an electronegative phosphonate moiety at the 3'-carbon atom and in the reaction mechanism of phosphite addition itself. Neglecting this particular case, temperature influences the reaction course only marginally.

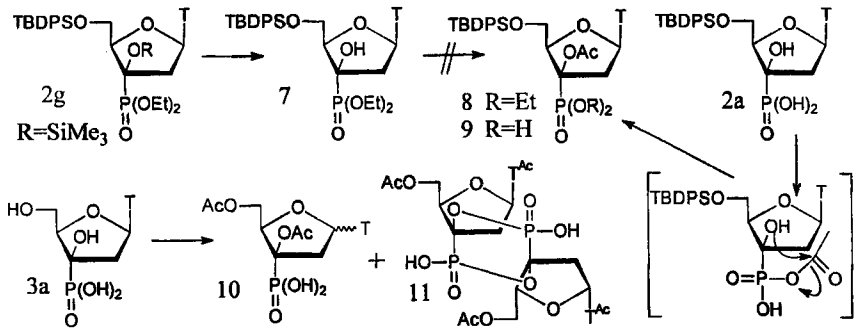
Concerning the solvents for addition reaction of silyl phosphites, the best one was dichloromethane (73 % of **2k**) in which the least loss of thymine from the 3'-ketothymidine derivative **1g** was found. Acetonitrile (66 % of **2k**) and tetrahydrofuran (51 % of **2k**) can also be used but the elimination of thymine is faster under the same reaction conditions (after two days, we found more than 50 % of thymine from compound **1g** was eliminated, in tetrahydrofuran as the solvent).



SCHEME 3

Concerning the configuration at the 3'-carbon atom, we attempted to transform the nucleoside phosphonate **2n** and **3a** into their isopropylidene derivatives **5** and **6** (**SCHEME 3**). The isopropylidenation of diethylphosphonate **2n** in the presence of a large excess of 2,2-dimethoxypropane under catalysis of pyridinium 4-toluenesulfonate in dichloromethane provided the expected product **5** in 19 % yield only. A similar change was experienced on isopropylidenation of the free phosphonate **3a**, under catalysis using hydrogen chloride in dimethylformamide. The product isolated by anion exchange chromatography on DEAE-Sephadex A25 in triethylammonium hydrogencarbonate buffer was identified by NMR as the mixture of the starting phosphonate **3a** and the expected isopropylidene derivative **6** in a 1:1 ratio. Both these compounds were subsequently separated by preparative RP HPLC. Despite several experiments we have never succeeded in a yield better than 40 %. Increased acidity of the tertiary 3'-hydroxyl due to the presence of geminal phosphonate moiety probably leads to an equilibrium between the isopropylidene derivative and the 3',5'-dihydroxy compound. This experiment was performed in order to chemically confirm the configuration at the 3'-carbon atom and to afford protected phosphonate **6** for the synthesis of dinucleoside monophosphate analogues.

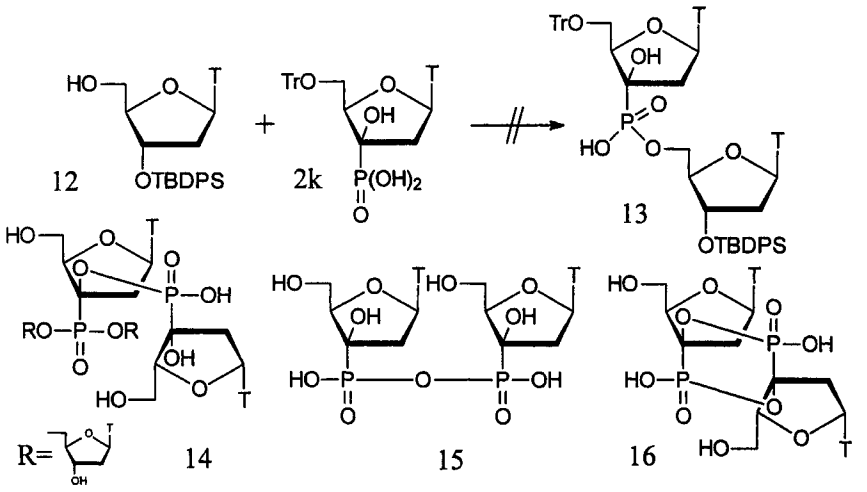
In order to utilise the nucleoside 3'-hydroxyphosphonates as the building blocks in oligonucleotide synthesis, a suitable protection of the 3'-hydroxy group had to be found. The monomethyl ester **4** was detritylated in 80% aqueous acetic acid and then, the requisite 5'-*O*-dimethoxytrityl derivative, prepared in 93% yield, was treated with acetic anhydride in pyridine. The starting material disappeared but the expected product was not detected. Only considerable loss of dimethoxytrityl group was observed. The minor dimethoxytritylated products were decomposed during the work up of the reaction mixture but as was found later, they came from the autocondensation reaction of starting monomer in the presence of acetic



SCHEME 4

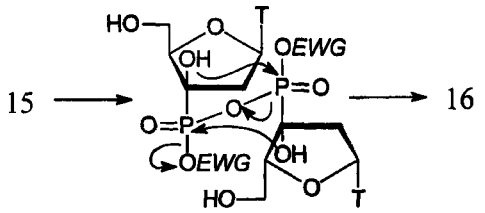
anhydride. Therefore we attempted to acetylate the 5'-*O*-*tert*-butyldiphenylsilyl derivative **7** (**SCHEME 4**). Neither the excess of acetic anhydride in pyridine containing 4-dimethylaminopyridine (DMAP), nor acetyl chloride in dichloromethane with silver carbonate, nor silver triflate at -78 °C brought about the formation of acetyl derivative **8**. Therefore we examined the reaction of a free phosphonic acid **2a** with acetic anhydride in the presence of 4-dimethylaminopyridine in pyridine at 50 °C for two days in the hope of inducing the formation of reactive mixed anhydride, the acetoxy phosphonate [17]. This intermediate could act as an efficient intramolecular acetylating agent, creating the thermodynamically stable 3'-*O*-acetyl derivative **9** (**SCHEME 4**). In spite of the fact that the reaction was not high-yielding, it afforded the expected compound **9** (36 %). The presence of 3'-acetoxy group was confirmed by observation of an nOe with H-6 of thymine in the 2D-ROESY spectrum of **9**. This result encouraged us to acetylate the deprotected phosphonic acid **3a**. Heating this compound under conditions given above for five days at 60 °C led to the minor diacetylated phosphonate **10** (mixture of anomers 86:14) but the major product was characterized as the unusual 5',3-*O*,*N*-diacetylated cyclic dimer **11**. The structures of these products were confirmed by NMR spectroscopy; in the last mentioned case FAB measurements showed the peak $M+H_2O+Na$ (817.1) corresponding to the structure of the cyclic dimer **11**.

In the light of these findings we examined the conditions which could lead to the formation of the dinucleoside monophosphate analog **13**. Thus, condensation of 5'-*O*-trityl



SCHEME 5

phosphonate **2k** (without protection of the 3'-hydroxyl) with 3'-*O*-*tert*-butyldiphenylsilylthymidine (**12**) in the resence of *N,N'*-dicyclohexylcarbodiimide (DCC) in pyridine (**SCHEME 5**) did not afford (after removal of trityl and silyl protecting groups) any product corresponding to the expected dimer **13**. The structure of major compound, the unusual tetranucleoside diphosphonate **14** was confirmed by NMR spectroscopy. The core of this tetramer forms two phosphonate nucleotide units joined by an ester linkage in the 3'-*O*-position. The 3'-ended phosphonate moiety of this core is esterified by two thymidine residues at their 5'-*O*-position. The ^{1}H and ^{13}C NMR spectra confirmed the presence of four nucleoside units and ^{31}P NMR spectrum showed signals of two nonequivalent phosphorus atoms with J(P,P) = 13.7 Hz. The structure **14** was derived from the observed heteronuclear phosphorus-proton and especially from the phosphorus-carbon coupling pattern (**FIGURE 1**). Structural assignment of protons and carbon atoms was achieved by combination of homonuclear 2D-COSY and 2D-J-resolved spectra with heterocorrelated ^{1}H-^{13}C 2D-HMQC spectra. The other isolated products were determined as the phosphonate anhydride **15** and the cyclic dinucleoside diphosphonate **16**; the structures of the further minor compounds were not assigned. Repeating this reaction under the same conditions but with reduced reaction time revealed again the predominant formation of



SCHEME 6

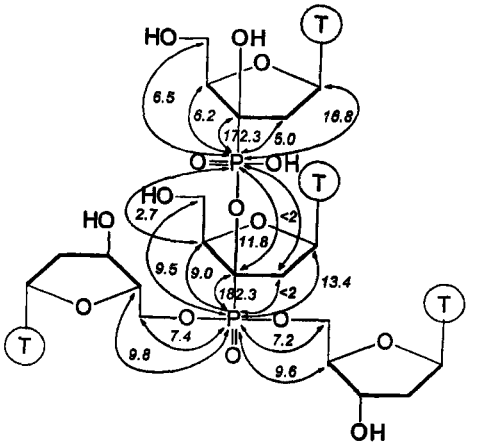


FIGURE 1 Phosphorus-carbon coupling constants in compoumd **14**

compounds **15** and **16**. Although the starting phosphonate **2k** was not present in the reaction mixture, it was recovered after workup in its unprotected form.

We suppose that the activation by DCC leads in the first step to the phosphonate anhydride **15** which undergoes further activation of phosphonate moieties by DCC (**SCHEME 6**). Subsequent intramolecular esterification of this intermediate by 3'-hydroxyl groups leads finally to cyclic dimer **16** which could be, together with anhydro phosphonate **15** , the intermediate for the other reactions including hydrolysis to starting monomer **2k** (we tried for several times to see whether phosphonate **2k** underwent autocondensation by DCC in pyridine; the starting material disappeared in two days but workup of the reaction mixture afforded only unchanged phosphonate **2k**). In addition, we obtained the most surprising result when the 3',5'-*O*-isopropylidene phosphonate **6** and nucleoside **12** were condensed in the presence of DCC in pyridine: no reaction took place in this case.

For the synthesis of dinucleoside phosphonate **13**, we also tried to apply another approach, namely, the nucleophilic addition of thymidin-5'-yl-*bis*-trimethylsilyl phosphite to 3'-keto-5'-*O*-tritylthymidine **1g** in dichloromethane which, however, brought about a total decomposition of ketothymidine.

In spite of these difficulties the study of the course of the condensation reaction and conditions of 3'-hydroxyl group protection are under further investigation.

Concerning the antiviral properties of all prepared compounds, no significant activity was found. Only cytosine derivative (**3b**) showed moderate activity against the replication of HIV.

EXPERIMENTAL

Unless stated otherwise, the solvents were evaporated at 40 °C and 2 kPa and the products were dried over phosphorus pentoxide at 50 - 70 °C and 13 Pa. The course of the reaction was checked by TLC on silica gel Silufol UV 254 foils (Kavalier Glassworks, Votice, Czech Republic) and the products were detected both by UV monitoring and by spraying with 1% ethanolic solution of 4-(4-nitrobenzyl)pyridine [after short heating and exposing to ammonia vapours the product (dialkyl phosphonate) affords an intense blue spot]. Preparative column chromatography (PLC) was carried out on silica gel (40-60 μm; Fluka); the amount of adsorbent was 20 - 40 times the weight of the separated mixture. Elution was performed at the rate of 40 ml/min. PLC and TLC were carried out with the following solvent systems (v/v): chloroform - ethanol 9:1 (C1); ethyl acetate - acetone - ethanol - water 4:1:1:1 (H1); 2-propanol - concentrated aqueous ammonia - water 7:1:2 (I, for charged compounds). Preparative chromatography on reverse phase was carried out on a spherical octadecyl silica column (25x300 mm, 20 - 40 μm, Tessek, Prague); compounds were eluted with a linear gradient of methanol in water at 10 ml/min. Chromatography on DEAE-Sephadex A-25 was performed with a linear gradient of 0-0.2M triethylammonium hydrogencarbonate in water. HPLC analysis was performed on a column of reverse phase (4.6 x 150 mm) Nucleosil 100-5 C18 (Macharey-Nagel), either isocratically at various concentration of methanol in 0.1M triethylammonium acetate or by gradient of methanol in the same buffer. The electrophoresis was made on Whatman No. 1 in 0.1M triethyl-

ammonium hydrogencarbonate (pH 7.5) at 20 V/cm. The UV spectra were recorded on PYE-Unicam SP 8000 spectrophotometer in water or in a methanol-water mixture (1:1, v/v) at pH 2, pH 7, and pH 12. Mass spectra (m/z) were recorded on ZAB-EQ (VG Analytical) instrument, using FAB (ionisation by Xe, accelerating voltage 8 kV) technique with glycerol and thioglycerol as matrices. ^{1}H and ^{13}C NMR spectra were measured on Varian Unity 500 instrument (^{1}H at 500 MHz, ^{13}C at 125.7 MHz) in hexadeuteriodimethylsulfoxide and were referenced to the solvent signal ($\delta_H = 2.50$, $\delta_C = 39.7$). Sodium salts of phosphonic acids were measured in deuterium oxide, free phosphonic acids were measured in deuterium oxide containing sodium deuterioxide, with sodium 3-(trimethylsilyl)1-propanesulfonate as internal standard. ^{31}P NMR spectra were recorded on Varian Unity 200 (81 MHz) spectrometer in deuterium oxide with H_3PO_4 as external standard.

General methods

Method A: *Oxidation procedure using PDC/MS.* A solution of 5'-*O*-*tert*-butyldiphenylsilyl or 5'-*O*-trityl nucleoside (1 mmol), codistilled with dry toluene (2 x 10 ml) in dry dichloromethane (8.0 ml), was added to the stirred mixture of freshly dried 4 Å powdered molecular sieves (646 mg) and PDC [11] (646 mg, 1.7 mmol) in dry dichloromethane (8.0 ml), prepared 20 min before, under argon. The course of the reaction was checked by TLC in the system C1. The oxidation required 1 to 8 hrs of reaction time depending on the nucleoside. The reaction mixture was filtered through Celite, washed with dry dichloromethane, the filtrate was concentrated under diminished pressure (30 °C in bath), then suspended in ethyl acetate and, after a short sonication, filtered through the microcrystalline cellulose and washed with ethyl acetate. The solvent was evaporated and the solid residue codistilled with dry toluene (3 x 20 ml). The ketonucleoside was used for the further reaction without any additional purification.

Method B: *Oxidation procedure using Dess-Martin reagent (DM)* [12-15]. A solution of 5'-*O*-*tert*-butyldiphenylsilyl or 5'-*O*-trityl nucleoside (1 mmol), codistilled with dry toluene (2 x 10 ml) in dry dichloromethane (5.0 ml), was added to a stirred suspension of DM (853 mg, 2 mmol) in dichloromethane (8.0 ml) at 0 °C under argon, and after 15 minutes, the reaction mixture was left to warm up gradually to the room temperature and set aside for

0.5-4 hrs. The course of the reaction was checked by TLC in the system C1. The reaction mixture was diluted with ether (30 ml) followed by an ice-cold saturated aqueous solutions of $NaHCO_3$ and $Na_2S_2O_3$ and intensively stirred for 10 min. The organic layer was washed gradually with saturated aqueous solutions of $NaHCO_3$ and NaCl and dried over anhydrous Na_2SO_4. The solvent was evaporated under diminished pressure (30 °C in bath) and the solid residue codistilled with dry toluene (3 x 20 ml). The ketonucleoside was used for the further reaction without any additional purification.

Method C: *Addition of diethyl trimethylsilyl, bis-trimethylsilyl or tris-trimethylsilyl phosphites to ketonucleosides.* Silyl phosphite [7,8,16] (3 mmol) was added to a solution of crude ketonucleoside **1a-g** (1 mmol) (**Method A** or **B**) in dichloromethane (10 ml) at room temperature under exclusion of moisture. The course of the reaction was checked by TLC in the system C1 for the non-charged and H1 for the negatively charged phosphonates. Reaction was quenched by the addition of 10 ml of a mixture of ethanol-2M-triethylammonium hydrogencarbonate (1:1) and concentrated under diminished pressure. The non-charged phosphonates **2a-n** were purified on silica gel (elution with gradient of 0-10% ethanol in chloroform), while the phosphonic acids **3a-d, 4** were purified by reverse phase chromatography (elution with linear gradient of methanol in water).

Method D: *Removal of N-benzoyl protecting groups.* To the ice-cold solution or suspension of benzoyl derivative (1mmol) in methanol (50 ml), cold concentrated aqueous ammonia (50 ml)was added, the round bottle was quickly and tighty closed, and the mixture was stirred for 24 hrs at room temperature. Debenzoylation of guanosine derivatives **2e** took place in an autoclave at 60 °C overnight. The course of the reaction was checked by TLC in the system H1 and I. After evaporation of the solvent, the residue was dried by codistillation with ethanol (3 x 20 ml) and the crude deacylated product was used without purification for the next reaction step.

Method E: *Removal of 5'-O-tert-butyldiphenylsilyl protecting group.* The silyl derivative **2a-2j** (1 mmol) was codistilled with dry toluene, dissolved in 0.5M *tetra-n*-butylammonium fluoride in tetrahydrofurane (10 ml, 5 mmol), and the mixture was stirred for 24 hrs at room

temperature under exclusion of moisture. In the case of less soluble compounds the equal volume of pyridine was added. The course of the reaction was checked by TLC in the system H1 and I. After evaporation of the solvent and treatment with Dowex 50 x 2 (Et_3NH^+ form) in 50% aqueous ethanol to remove *tetra-n*-butylammonium cations the desilylated product was purified on DEAE-Sephadex A-25 (HCO_3^- form, elution with linear gradient of 0-0.2M triethylammonium hydrogencarbonate in water).

1-(5-*O*-*tert*-Butyldiphenylsilyl-2-deoxy-3-*C*-phosphono-β-D-*threo*-pentofuranosyl)thymine (**2a**)

Compound **2a** was prepared according to **Method C** from 5'-O-*tert*-butyldiphenylsilyl--3'-ketothymidine (**1a**) (**Method B**; from 1 mmol of 5'-*O*-*tert*-butyldiphenylsilylthymidine) and *tris*-trimethylsilyl phosphite (1.0 ml, 3 mmol). Yield 573 mg (87 %) of triethylammonium salt of **2a**. HR FAB calcd for $C_{26}H_{33}N_2O_8PSi$ 560.1743, found 583.1688 $(M+Na)^+$. ^{1}H-NMR – see **TABLE 3**.

4-*N*-Benzoyl-1-(5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3-*C*-phosphono-β-D-*threo*-pentofuranosyl)cytosine (**2b**)

Compound **2b** was prepared according to **Method C** from 4-*N*-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-3'-ketocytidine (**1b**) (**Method B**; from 1 mmol of 4-*N*-benzoyl-5'-*O*-*tert*-butyldiphenylsilylcytidine) and *tris*-trimethylsilyl phosphite (1.0 ml, 3 mmol). Yield 584 mg (78 %) of triethylammonium salt of **2b**. HR FAB calcd for $C_{32}H_{36}N_3O_8PSi$ 649.2009, found 650.2100 $(M+H)^+$. ^{1}H-NMR – see **TABLE 3**.

6-*N*-Benzoyl-9-(5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3-*C*-phosphono-β-D-*threo*-pentofuranosyl)adenine (**2c**)

Compound **2c** was prepared according to **Method C** from 6-N-benzoyl-3'-keto-5'-O-*tert*-butyldiphenylsilyladenosine (**1c**) (**Method B**; from 1 mmol of 6-N-benzoyl-5'-O-*tert*-butyldiphenylsilyladenosine) and *tris*-trimethylsilyl phosphite (1.0 ml, 3 mmol). Yield 540 mg (70 %) of triethylammonium salt of **2c**. HR FAB calcd for $C_{33}H_{36}N_5O_7PSi$ 673.2121, found 696.2101 $(M+Na)^+$. ^{1}H-NMR – see **TABLE 3**.

TABLE 3 Proton NMR parameters of compounds **2** - **16** in DMSO or D_2O

Comp.	Chemical shifts (Coupling constants J(H,H) and J(H,P)[a])						
(Solv.)	H1'	H2'a	H2'b	H4'	H5'a	H5'b	Base
2a[a] (DMSO)	6.05 dd (8.3; 2.7)	2.84 dt (14.3; 8.3; *8.3*)	1.95 ddd (14.3; 2.7; *<1.5*)	4.31 ddd (8.3; *4.2*; 1.5)	4.29 dd (11.7; 1.5)	3.88 dd (11.7; 8.3)	NH: 11.23 bs H6: 7.70 q CH_3: 1.59 d
2b[b] (DMSO)	5.97 dd (7.3; 2.0)	2.82 dt (14.2; 7.3; *7.3*)	2.11 dt (14.2; 2.0; *<2*)	4.47 ddd (8.3; *3.7*; 1.5)	4.36 bd (11.7; 1.5)	3.94 dd (11.7; 8.3)	NH: 11.10 bs H5: 7.11 d H6: 7.94 d
2c[c] (DMSO)	6.50 bd (8.3; < 2)	3.19 dt (14.7; 8.3; *~8*)	2.44 bd (14.7; *<2*; *<2*)	4.58 bdd (8.3; *4.9*; <1)	4.30 bd (11.2; <1)	3.87 dd (11.2; 8.3)	NH: 11.20 bs H2, H8: 8.74 s, 8.57 s
2d[d] (DMSO)	6.33 dd (8.5; 1.7)	3.14 dt (14.7; 8.5; *8.5*)	2.32 bd (14.7; 1.7; *~1.5*)	4.51 bdd (8.6; *4.6*; ~1.5)	4.30 bd (11.2; ~1.5)	3.87 dd (11.2; 8.6)	NH_2: ~7.20 bs H2, H8: 8.28 s, 8.13 s
2e[e] (DMSO)	6.27 dd (8.3; 1.5)	3.11 dt (14.7; 8.3; *~8*)	2.34 dt (14.7; ~1.5; *1.5*)	4.53 ddd (9.0; *4.4*; 1.0)	4.28 bd (11.2; 1.0)	3.89 dd (11.2; 9.0)	NH: ~13.00 bs H8: 8.18 s
2f[f] (DMSO)	6.09 dd (8.3; 2.2)	3.03 dt (14.2; 8.3; *8.3*)	2.20 dt (14.2; 2.0; *~2*)	4.45 ddd (8.5; *5.1*; 1.7)	4.25 bd (11.2; 1.7)	3.86 dd (11.2; 8.5)	NH_2: 6.85 bs NH:10.90 bs H8: 7.88 s
2g[g] (DMSO)	6.00 dd (7.5; 3.0)	2.78 ddd (14.9; 7.5; *5.4*)	2.22 ddd (14.9; *4.5*; 3.0)	4.37 ddd (8.4; *5.4*; 2.0)	4.05 dd (11.9; 2.0)	3.82 dd (11.9; 8.4)	NH:11.40 s H6: 7.27 bs CH_3: 1.66 bs
2h[h] (DMSO)	6.01 dd (8.0; 2.7)	2.75 ddd (14.7; *9.0*; 8.0)	2.14 ddd (14.7; 2.7; *1.5*)	4.24 ddd (8.3; *4.0*; 1.6)	4.13 dd (11.8; 1.6)	3.90 dd (11.8; 8.3)	NH_2: 7.16, 7.12 H5: 5.65 d H6: 7.58 d
2i[i] (DMSO)	6.42 dd (6.6; 5.0)	3.09 ddd (14.1; *11.5*; 5.0)	2.99 ddd (14.1; *11.5*; 6.6)	4.49 td (8.3; *8.3*; 2.4)	4.03 dd (11.6; 2.4)	3.97 dd (11.6; 8.3)	NH: 11.21 H2, H8: 8.67 s, 8.36 s
2j[j] (DMSO)	6.27 dd (6.7; 3.9)	2.93 ddd (14.4; *9.9*; 6.7)	2.67 ddd (14.4; *7.0*; 3.9)	4.47 ddd (8.4; *6.6*; 2.1)	4.04 dd (11.7; 2.1)	3.87 dd (11.7; 8.4)	2xNH: 12.36, 12.01 H8: 7.86 s
2k[k] (DMSO)	6.03 dd (8.1; 2.4)	~2.82 m	1.95 dt (14.6; 2.4; *~2.5*)	4.41 ddd (8.6; *4.6*; 1.5)	3.48 dd (10.0; 1.5)	3.42 dd (10.0; 8.6)	NH: 11.25 bs H6: 7.59 bs CH_3: 1.60 d
2m[l] (DMSO)	6.02 dd (7.3; 2.7)	2.78 ddd (14.9; *9.0*; 7.3)	2.20 dt (14.9; 2.7; *~3*)	4.51 ddd (7.8; *5.1*; 2.7)	3.27 dd (11.0; 2.7)	3.24 dd (11.0; 7.8)	NH: 11.40 s H6: 7.17 bq CH_3: 1.60 d
2n[m] (DMSO)	6.03 dd (8.2; 2.9)	2.76 ddd (14.9; 8.2; *6.5*)	2.19 dt (14.9; 2.9; *~2.5*)	4.01 ddd (7.3; *4.6*; 2.2)	3.84 ddd (12.4; 5.6; 2.2)	3.70 ddd (12.4; 7.3; 6.0)	NH: 11.29 s H6: 7.68 q CH_3: 1.78 d
3a (D_2O)	6.16 dd (8.1; 2.4)	3.00 dt (15.1; 8.1; *8.1*)	2.26 ddd (15.1; 2.4; *1.0*)	4.34 dt (7.3; *3.6*; 3.4)	4.12 dd (12.2; 3.4)	3.95 dd (12.2; 7.3)	H6: 7.90 q CH_3: 1.92 d
3b (D_2O)	6.09 dd (7.9; 2.0)	3.00 dt (15.1; 7.9; *~7.5*)	2.24 ddd (15.1; 2.0; *<1*)	4.39 ddd (7.1; 4.1; *3.3*)	4.13 dd (12.0; 4.1)	3.93 dd (12.0; 7.1)	H5: 6.03 d H6: 8.03 d
3c (D_2O)	6.33 dd (8.5; 2.2)	3.23 ddd (15.4; 8.5; *8.5*)	2.62 bdd (15.4; 2.2; *<1*)	4.45 dt (6.9; 4.2; *~4*)	4.13 dd (12.0; 4.2)	3.90 dd (12.0; 6.9)	H2, H8: 8.36 s, 8.11 s
3d (D_2O)	6.36 dd (8.6; 2.2)	3.23 dt (15.6; 8.6; *8.6*)	2.63 bdd (15.6; 2.2; *<1*)	4.43 dt (7.1; 3.9; *~3.5*)	4.12 dd (12.2; 3.9)	3.89 dd (12.2; 7.1)	H2, H8: 8.38 s, 8.20 s

TABLE 3 - continued

Comp.	Chemical shifts (Coupling constants J(H,H) and J(H,P)*)						
(Solv.)	H1'	H2'a	H2'b	H4'	H5'a	H5'b	Base
4[n] (DMSO)	6.04 dd (8.1; 2.2)	2.76 dt (14.4; ~*8.5*; 8.1)	1.95 ddd (14.4; 2.2; ~*1.7*)	4.37 ddd (8.8; *4.2*; 1.5)	4.34 dd (10.3; 1.5)	3.42 dd (10.3; 8.8)	NH: 11.25 bs H6: 7.60 q CH_3: 1.61 d
5[o] (DMSO)	6.05 dd (8.1; 3.7)	3.01 ddd (15.1; *9.5*; 8.1)	2.28 dt (15.1; 3.7; *3.7*)	~4.11 m	4.17 dd (13.2; 2.2)	3.99 dt (13.2; ~1.0; ~1.0)	NH: 11.36 bs H6: 7.76 q CH_3: 1.78 d
6 (DMSO)	5.92 dd (8.1; 3.3)	2.80 ddd (14.3; *8.5*; 8.1)	2.05 dt (14.3; 3.3; ~*3*)	4.01 ddd (6.1; 5.1; *3.4*)	3.88 dd (11.0; 6.1)	3.49 dt (11.0; 5.1)	NH: 11.20 bs H6: 7.81 q CH_3: 1.75 d
7[p] (DMSO)	6.22 dd (8.1; 2.7)	2.79 ddd (14.9; *9.0*; 8.1)	2.19 ddd (14.9; 2.7; *1.7*)	4.24ddt (8.3; *4.0*; 1.6; 1.0)	4.13 dd (11.8; 1.6)	3.90 dd (11.8; 8.3)	NH: 11.33 bs H6: 7.51 q CH_3: 1.66 d
9[q] (DMSO)	5.98 dd (6.1; 4.4)	~2.95 m	~2.95 m	4.72 ddd (8.0; *4.0*; 2.0)	4.39 dd (11.5; 2.0)	3.96 dd (11.5; 8.0)	NH: 11.27 bs H6: 7.28 q CH_3: 1.52 d
10[r] (D_2O)	6.09 dd (6.9; 2.7)	3.23 dt (15.9; *2.9*; 2.7)	2.99 dt (15.9; *7.5*; 6.9)	4.71 m	4.71 m	4.59 m	H6: 7.75 q CH_3: 1.94 d
11[s] (D_2O)	6.13 dd (6.9; 3.3)	3.16 m	3.16 m	4.78 dd (8.3; 2.1)	4.70 dd (12.2; 8.3)	4.56 dd (12.2; 2.1)	H6: 7.73 q CH_3: 1.94 d
14[t] (D_2O)	6.13 dd (8.0; 3.1) 6.16 dd (8.8; 6.3) 6.18 t (6.7; 6.7) 6.22 t (6.8; 6.8)	3.07 ddd (15.2; *8.7*; 8.0) 3.22 m (*19.6*; 15; 8.8; 1.2) ~2.36 m ~2.40 m	2.22 ddd (15.2; 3.1; *2.3*) 3.09 ddd (*16.8*; 15.0; 6.3) ~2.36 m ~2.40 m	4.35 ddd (7.4; *4.4*; 2.8) 4.40 m (*11.6*; 5.5; 3.1;1.2) 4.14 ddd (6.0; 5.0; 3.0) 4.22 ddd (6.8; 4.4; 3.0)	4.12 dd (12.4; 2.8) 4.00 dd (12.8; 5.5) 4.59 dd (11.6; 3.0) 4.58 dd (11.5; 3.0)	3.97 dd (12.4; 7.4) 3.94 dd (12.8; 3.1) 4.45 dd (11.6; 6.0) 4.47 dd (11.5; 6.8)	H6: 8.02 q CH_3: 1.92 d H6: 7.85 q CH_3: 1.90 d H6: 7.53 q CH_3: 1.84 d H6: 7.46 q CH_3: 1.85 d
15 (D_2O)	6.16 dd (8.1; 2.5)	3.00 ddd (15.2; 8.1; *7.9*)	2.33 ddd (15.2; 2.5; *0.8*)	4.34 ddd (6.8; 4.0; *3.7*)	4.11 dd (12.1; 4.0)	3.92 dd (12.1; 6.8)	H6: 7.91 q CH_3: 1.91 d
16 (D_2O)	*6.23* dd (8.2; 3.0)	3.07 ddt (15.2; 8.2; *4.3*;*4.3*)	2.32 dd (15.2; 3.0)	4.36 ddt (7.8; 2.8; *2.0*; *2.0*)	4.12 dd (12.5; 2.8)	3.97 dd (12.5; 7.8)	H6: 7.91 q CH_3: 1.91 d

* The values of J(H,P) are given in italics; Additional signals: [a] t-Bu: 0.96 s; 2xPh: 7.66 m (4H), 7.44-7.36 m (4H), 7.31 m (2H); [b] t-Bu: 1.00 s; 3xPh: 8.20 d (2H), 7.68 t (4H), 7.62 t (1H), 7.51 t (2H), 7.44 m (4H), 7.39 t (2H); [c] t-Bu: 0.92 s; 3xPh: 8.07 d (2H), 7.63 m (3H), 7.55 m (4H), 7.38 t (1H), 7.29 m (3H), 7.12 t (2H); [d] t-Bu: 0.92 s; 2xPh: 7.62 d (2H), 7.56 d (2H), 7.30 t (4H), 7.12 t (2H); [e] t-Bu: 0.93 s; 3xPh: 8.06 d (2H), 7.64 m (3H), 7.59 d (2H), 7.54 t (2H), 7.40 t (1H), 7.34 m (3H), 7.22 t (2H); [f] t-Bu: 0.94 s; 2xPh: 7.60 m (4H), 7.40 m (4H), 7.23 m (2H); [g] t-Bu: 1.01 s; $SiMe_3$: -0.07 s; $P(OEt)_2$: 4.05 m (4H), 1.22 t (3H), 1.17 t (3H); 2xPh: 7.65 m (4H), 7.50-7.40 m (6H); [h] t-Bu: 0.99 s; $P(OEt)_2$: 4.01 m (4H), 1.17 t (3H), 1.16 t (3H); 2xPh: 7.70-7.62 m (4H), 7.50-7.35 m (6H); OH: 5.99 d; [i] t-Bu: 0.99 s; $SiMe_3$: -0.12 s; $P(OEt)_2$: 4.10 m (4H), 1.26 t (3H), 1.22 t (3H); 3xPh: 8.05 d (2H), 7.67-7.53 m (7H), 7.48-7.39 m (4H), 7.33 t (2H); [j] t-Bu: 1.01 s; $SiMe_3$: -0.16 s; $P(OEt)_2$: 4.08 m (4H), 1.23 t (3H), 1.19 t (3H); 3xPh: 8.04 d (2H), 7.70-7.63 m (5H), 7.56 m (2H), 7.48 m (2H), 7.43 t (2H), 7.41 t (2H); [k] 3xPh: 7.40 d (6H), 7.28 t (6H), 7.23 t (3H); OH: 5.76 s; [l] $SiMe_3$: -0.14 s; $P(OEt)_2$: 3.90-4.05 m (4H), 1.13 t (3H), 1.07 t (3H); 3xPh: 7.44 d (6H), 7.35 t (6H), 7.28 t (3H); [m] $P(OEt)_2$: 4.10 m (4H), 1.26 t (6H); 3-OH: 5.93 d; 5-OH: 4.91 t; [n] POMe: 3.17 d (3H); 3xPh: 7.42 d (6H), 7.30 t (6H), 7.24 t (3H); [o] $P(OEt)_2$: 4.12 m (4H), 1.28 t (3H), 1.27 t (3H); i-Pr: 1.61 s (3H), 1.36 s (3H); [p] t-Bu: 0.99 s; $P(OEt)_2$: 4.02 m (4H), 1.18 t (3H), 1.15 t (3H); 2xPh: 7.65 m (4H), 7.50-7.32 m (6H); OH: 5.95 bt; [q] t-Bu: 0.96 s; 2xPh: 7.70 d (4H), 7.42 m (4H), 7.34 t (2H); OAc: 1.68 s (3H); [r] 2xOAc: 2.17 s (3H), 2.03 s (3H); [s] 2xOAc: 2.17 s (3H), 2.06 s (3H); [t] H3: 4.47 m and 4.50m.

9-(2-Deoxy-3-*C*-phosphono-5-*O*-*tert*-butyldiphenylsilyl-β-D-*threo*-pentofuranosyl)adenine (**2d**)

Compound **2d** was prepared according to **Method C** from 5'-O-*tert*-butyldiphenylsilyl--3'-ketoadenosine (**1d**) (**Method B**; from 1 mmol of 5'-O-*tert*-butyldiphenylsilyladenosine) and *tris*-trimethylsilyl phosphite (1.0 ml, 3 mmol). Yield 235 mg (35 %) of triethylammonium salt of **2d**. HR FAB calcd for $C_{26}H_{32}N_5O_6PSi$ 569.1859, found 570.2027 $(M+H)^+$. ^{1}H-NMR – see **TABLE 3**.

2-*N*-Benzoyl-9-(5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3-*C*-phosphono-β-D-*threo*-pentofuranosyl)guanine (**2e**)

Compound **2e** was prepared according to **Method C** from 2-*N*-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-3'-ketoguanosine (**1e**) (**Method B**; from 1 mmol of 2-*N*-benzoyl-5'-O-*tert*-butyldiphenylsilylguanosine) and *tris*-trimethylsilyl phosphite (1.0 ml, 3 mmol). Yield 630 mg (80 %) of triethylammonium salt of **2e**. HR FAB calcd for $C_{33}H_{36}N_5O_8PSi$ 689.2070, found 712.1949 $(M+Na)^+$. ^{1}H-NMR – see **TABLE 3**.

9-(5-*O*-*tert*-Butyldiphenylsilyl-2-deoxy-3-*C*-phosphono-β-D-*threo*-pentofuranosyl)guanine (**2f**)

Compound **2f** was prepared according to **Method C** from 5'-*O*-*tert*-butyldiphenylsilyl-3'-ketoguanosine (**1f**) (**Method B**; from 1 mmol of 5'-*O*-*tert*-butyldiphenylsilylguanosine) and *tris*-trimethylsilyl phosphite (1.0 ml, 3 mmol). Yield 166 mg (25 %) of triethylammonium salt of **2f**. HR FAB calcd for $C_{26}H_{32}N_5O_7PSi$ 585.1808, found 608.1745 $(M+Na)^+$. ^{1}H-NMR – see **TABLE 3**.

1-(5-*O*-*tert*-Butyldiphenylsilyl-2-deoxy-3-*C*-diethylphosphono-3-*O*-trimethylsilyl-β-D-*threo*-pentofuranosyl)thymine (**2g**)

Compound **2g** was prepared according to **Method C** from 5'-O-*tert*-butyldiphenylsilyl--3'-ketothymidine (**1a**) (**Method B**; from 1 mmol of 5'-*O*-*tert*-butyldiphenylsilylthymidine) and diethyl trimethylsilyl phosphite (0.7 ml, 3 mmol). Yield 575 mg (83 %) of **2g**. HR FAB calcd for $C_{33}H_{49}N_2O_8Psi_2$ 688.2765, found 689.2829 $(M+H)^+$. ^{1}H-NMR – see **TABLE 3**.

1-(5-*O*-*tert*-Butyldiphenylsilyl-2-deoxy-3-*C*-diethylphosphono-β-D-*threo*-pentofuranosyl)-cytosine (**2h**)

Compound **2h** was prepared according to **Method C** from 4-*N*-benzoyl-5'-O-*tert*-butyl-diphenylsilyl-3'-ketocytidine (**1b**) (**Method B**; from 1 mmol of 4-*N*-benzoyl-5'-*O*-*tert*-butyl-diphenylsilylcytidine) and diethyl trimethylsilyl phosphite (0.7 ml, 3 mmol) followed by **Method D** (because of instability of the fully protected compound during quenching of the reaction by ethanol-triethylammonium hydrogencarbonate mixture). Yield 246 mg (41 %) of **2h**. HR FAB calcd for $C_{29}H_{40}N_3O_7PSi$ 601.2373, found 602.2432 $(M+H)^+$. ^{1}H-NMR – see **TABLE 3**.

6-*N*-Benzoyl-9-(5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3-*C*-diethylphosphono-3-O-trimethyl-silyl-β-D-*threo*-pentofuranosyl)adenine (**2i**)

Compound **2i** was prepared according to **Method C** from 6-*N*-benzoyl-5'-*O*-*tert*-butyl-diphenylsilyl-3'-ketoadenosine (**1c**) (**Method B**; from 1 mmol of 6-N-benzoyl-5'-O-*tert*-butyldiphenylsilyladenosine) and diethyl trimethylsilyl phosphite (0.7 ml, 3 mmol). Yield 397 mg (49 %) of **2i**. HR FAB calcd for $C_{40}H_{52}N_5O_7Psi_2$ 801.3142, found 802.3164 $(M+H)^+$. ^{1}H-NMR – see **TABLE 3**.

2-*N*-Benzoyl-9-(5-O-*tert*-butyldiphenylsilyl-2-deoxy-3-*C*-diethylphosphono-3-*O*-trimethyl-silyl-β-D-*threo*-pentofuranosyl)guanine (**2j**)

Compound **2j** was prepared according to **Method C** from 2-*N*-benzoyl-5'-*O*-*tert*-butyl-diphenylsilyl-3'-ketoguanosine (**1e**) (**Method B**; from 1 mmol of 2-N-benzoyl--5'-O-*tert*-butyldiphenylsilylguanosine; the reaction mixture was diluted at the work up by dichloromethane instead of ether because of the low solubility of guanine derivative) and diethyl trimethylsilyl phosphite (0.7 ml, 3 mmol). Yield 289 mg (35 %) of **2j**. HR FAB calcd for $C_{40}H_{52}N_5O_8PSi_2$ 817.3092, found 818.3232 $(M+H)^+$. ^{1}H-NMR – see **TABLE 3**.

1-(2-Deoxy-3-*C*-phosphono-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine (**2k**)

Procedure (i). Compound **2k** was prepared according to **Method C** from 3'-keto-5'-*O*-trityl-thymidine (**1g**) (1 mmol) (**Method A**) and *tris*-trimethylsilyl phosphite (0.7 ml, 3 mmol). Yield 486 mg (73 %) of triethylammonium salt of **2k**. HR FAB calcd for $C_{29}H_{29}N_2O_8P$

564.1661, found 666.2949 $(M+Na+H)^+$. ^{1}H-NMR – see **TABLE 3**.

Procedure (ii). Compound **2k** was prepared according to **Method C** from 3'-keto-5'-*O*-trityl-thymidine (**1g**) (1 mmol) (**Method A**) and *tris*-trimethylsilyl phosphite (0.7 ml, 3 mmol). The reaction mixture with the phosphite was heated at 80 °C for 6 hrs. Yield 546 mg (82 %) of triethylammonium salt of **2k**.

Procedure (iii). Compound **2k** was prepared according to **Method C** from 3'-keto-5'-*O*-trityl-thymidine (**1g**) (0.5 mmol) (**Method A**) and *tris*-trimethylsilyl phosphite (0.7 ml, 3 mmol). The addition reaction was carried out in acetonitrile. Yield 220 mg (66 %) of triethylammonium salt of **2k**.

Procedure (iv). Compound **2k** was prepared according to **Method C** from 3'-keto-5'-*O*-trityl-thymidine (**1g**) (0.5 mmol) (**Method A**) and *tris*-trimethylsilyl phosphite (0.7 ml, 3 mmol). The addition reaction was carried out in tetrahydrofurane. Yield 171 mg (51 %) of triethylammonium salt of **2k**.

Procedure (v). Compound (**2k**) was prepared according to **Method C** from 3'-keto-5'-O-tritylthymidine (**1g**) (1 mmol) (**Method A**) and *bis*-trimethylsilyl phosphite (0.7 ml, 3 mmol). Yield 213 mg (32 %) of triethylammonium salt of **2k**.

1-(2-Deoxy-3-*C*-methylphosphono-3-*O*-trimethylsilyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)-thymine (**4**)

Compound **4** was prepared according to **Method C** from 3'-keto-5'-*O*-tritylthymidine (**1g**) (**Method A**; from 1 mmol of 5'-O-tritylthymidine) and dimethyl trimethylsilyl phosphite (0.7 ml, 3 mmol). The reaction mixture was treated, after concentration *in vacuo*, with 60% aqueous pyridine (20 ml) at 60 °C overnight to convert compound **21** to the stable monomethyl ester **4**. The solution was then concentrated *in vacuo*, the residue codistilled with ethanol (3 x 20 ml) and treated with Dowex 50x2 (Et_3NH^+ form) in 50% aqueous ethanol to remove *N*-methylpyridinium cations. Yield 373 mg (55 %) of triethylammonium salt of **4**. HR FAB calcd for $C_{30}H_{31}N_2O_8P$ 578.1818, found 680.3156 $(M+Et_3N+H)^+$. ^{1}H-NMR – see **TABLE 3**.

1-(2-Deoxy-3-*C*-diethylphosphono-3-*O*-trimethylsilyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)-thymine (**2m**)

Compound **2m** was prepared according to **Method C** from 3'-keto-5'-*O*-tritylthymidine (**1g**)

(1 mmol) (**Method A**) and diethyl trimethylsilyl phosphite (0.7 ml, 3 mmol). Yield 464 mg (67 %) of **2m**. HR FAB calcd for $C_{36}H_{45}N_2O_8PSi$ 692.2682, found 693.2874 $(M+H)^+$. ^{1}H-NMR – see **TABLE 3**.

1-(2-Deoxy-3-*C*-diethylphosphono-β-D-*threo*-pentofuranosyl)thymine (**2n**)

Compound **2n** was prepared according to **Method C** from 3'-keto-5'-*O*-tritylthymidine (**1g**) (1 mmol) (**Method A**) and diethyl trimethylsilyl phosphite (0.7 ml, 3 mmol). The reaction mixture with the phosphite was heated at 80 °C for 6 hrs. Yield 208 mg (55 %) of **2n**. HR FAB calcd for $C_{14}H_{23}N_2O_8P$ 378.1192, found 379.1185 $(M+H)^+$. ^{1}H and ^{13}C-NMR – see **TABLE 3, 4**.

1-(2-Deoxy-3-*C*-phosphono-β-D-*threo*-pentofuranosyl)thymine (**3a**)

Compound **2a** prepared by **Method A** (from 1 mmol of 5'-*O*-*tert*-butyldiphenylsilyl-thymidine) followed by **Method C** was treated according to **Method E.** After purification on DEAE-Sephadex, the free phosphonic acid was converted into the sodium salt on Dowex 50x2 (Na^+ form), and lyophilized from water to afford 187 mg (58 %) of sodium salt of compound **3a**. HR FAB calcd for $C_{10}H_{15}N_2O_8P$ 322.0566, found 323.0666 $(M+H)^+$. ^{1}H and ^{13}C-NMR – see **TABLE 3, 4**.

1-(2-Deoxy-3-*C*-phosphono-β-D-*threo*-pentofuranosyl)cytosine (**3b**)

Procedure (i). Compound **2b** prepared by **Method A** (from 1 mmol of 4-*N*-benzoyl--5'-*O*-*tert*-butyldiphenylsilylthymidine) followed by **Method C** was treated according to **Method D** and **E.** After purification on DEAE-Sephadex, the free phosphonic acid **3b** was converted into the sodium salt on Dowex 50 x 2 (Na^+ form) and lyophilized to afford 22 mg (7 %) of sodium salt of compound **3b**. HR FAB calcd for $C_9H_{14}N_3O_7P$ 307.0569, found 308.0578 $(M+H)^+$. ^{1}H-NMR – see **TABLE 3**.

Procedure (ii). 1-(2-Deoxy-3-*C*-diethylphosphono-3-*O*-trimethylsilyl-5-*O*-trityl-β-D-*threo*--pentofuranosyl)uracil (2.3 g, 76 %) prepared according to **Method C** from 3'-keto-5'-*O*--trityluridine (**Method A**; from 5 mmol of 5'-O-trityluridine) and diethyl trimethylsilyl phosphite (3.5 ml, 15 mmol) was treated with DMAP (19 mg, 0.15 mmol), TPSCl (4.0 g, 13.3 mmol) and triethylamine (1.9 ml, 13.7 mmol) in acetonitrile (95 ml) at 0 °C under

TABLE 4 Carbon-13 NMR parameters of compounds **2n, 3a, 9-11, 14-16** in DMSO or D_2O

Comp.	Chemical shifts (Coupling constants J(C,P)) Deoxyribose *					Base				
(Solv.)	C1'	C2'	C3'	C4'	C5'	C2	C4	C5	C6	CH_3
2n [a] (DMSO)	85.32 (6.6)	42.48 (6.4)	76.35 (174.8)	83.36 (5.6)	60.36	150.71	164.10	108.78	136.73	12.62
3a (D_2O)	85.14 (15.6)	42.83 (5.9)	77.37 (162.1)	84.30 (13.4)	59.98	151.32	166.34	110.22	138.01	11.24
9 [b] (DMSO)	84.39	45.55	86.15 (~177.0)	87.16	63.83	150.28	163.86	108.09	135.28	12.56
10 [c] (D_2O)	85.27 (10.7)	39.39 (~4.7)	85.54 (153.3)	84.10 (9.8)	63.03	150.93	166.16	110.06	136.52	11.37
11 [d] (D_2O)	85.08 (~5.9; ~5.9)	38.81 (<2; <2)	85.00 (169.9; <2)	83.41 (~5.6; ~5.6)	62.98	150.89	166.17	110.16	136.50	11.38
14 (D_2O)	84.45 (16.8)	43.17 (5.0)	77.42 (172.3)	82.53 (6.2)	60.32 (6.5)	151.22	166.15	111.49	137.68	11.36
	84.18 (13.4)	38.13 (<2; <2)	81.23 (182.3; 11.8)	81.88 (9.0; 2.7)	60.30 (2.5)	151.12	166.05	111.02	137.44	11.36
	85.08	37.82	69.60	83.64 (9.8)	67.47 (7.4)	150.99	165.73	110.78	137.03	11.33
	84.79	37.82	69.21	83.60 (9.6)	67.28 (7.2)	150.92	165.72	110.38	136.82	11.21
15 (D_2O)	85.32 (13.7)	43.13	77.78 (155.3)	84.36 (12.7)	59.93	151.44	166.40	110.31	138.18	11.35
16 (D_2O)	84.81 (7.5; 7.5)	43.01 (~3.5; ~3.0)	76.92 (171.7; 5.5)	83.86 (~7.3; ~7.3)	60.29	151.35	166.32	110.48	137.89	11.31

Additional signals: [a] POEt: 62.86 (7.8); 62.77 (6.8); 16.57 (5.9); [b] t-Bu: 18.99 and 26.83; 2xPh: 133.50(2xC), 135.41(4xC), 129.88(4xC), 127.83 (2xC); Ac: 169.68, 22.15; [c] 2xOAc: 173.68, 20.92, 172.08, 20.08; [d] NAc + OAc: 173.57, 20.87, 171.55, 20.05.

exclusion of moisture according to the procedure [9]. The course of the reaction was checked by TLC in the system C1. The reaction mixture was stirred for 19 hrs at room temperature and then 25% aqueous ammonia (190 ml) was added under stirring. After 3 hrs the mixture was concentrated *in vacuo* and the residue was treated with Dowex 50x2 (H^+ form, 50 ml) in 70% aqueous ethanol (100 ml) overnight to remove the trityl protecting group. The suspension was transformed into the column and ion-exchanger was washed with 2.5%

ammonia in 70% aqueous ethanol (300 ml). Eluent was concentrated *in vacuo*, the residue codistilled with ethanol followed by dry toluene (3 x 10 ml) and treated with bromotrimethylsilane (3.0 ml, 22.8 mmol) and lutidine (3.0 ml, 26.6 mmol) in CH_3CN (15 ml) for two days. The course of the reaction was checked by TLC in the system H1. The reaction mixture was evaporated to dryness, the solid residue was treated with 15 ml of mixture Et_3N-MeOH-H_2O (2:5:5) and evaporated. The product was purified on DEAE-Sephadex and the phosphonic acid **3b** converted into the sodium salt on Dowex 50x2 (Na^+ form). Freeze-drying afforded 292 mg (19 %) of sodium salt of compound **3b**.

9-(2-Deoxy-3-*C*-phosphono-β-D-*threo*-pentofuranosyl)adenine (**3c**)

Compound **2d** prepared by **Method A** (from 1 mmol of 5'-*O*-*tert*-butyldiphenyl-silyladenosine) followed by **Method C** was treated according to **Method E**. Purification on DEAE-Sephadex followed by conversion into the sodium salt on Dowex 50x2 (Na^+ form) and freeze-drying from water afforded 156 mg (47 %) of sodium salt of compound **3c.** HR FAB calcd for $C_{10}H_{14}N_5O_6P$ 331.0681, found 332.0851 $(M+H)^+$. ^{1}H-NMR – see **TABLE 3**.

9-(2-Deoxy-3-*C*-phosphono-β-D-*threo*-pentofuranosyl)guanine (**3d**)

Compound **2e** prepared by **Method A** (from 1 mmol of 2-N-benzoyl-5'-*O*-*tert*-butyldiphenylsilyladenosine) followed by **Method C** was treated according to **Method D** and **E**. Purification on DEAE-Sephadex followed by conversion into the sodium salt on Dowex 50x2 (Na^+ form) and freeze-drying afforded 119 mg (34 %) of sodium salt of compound **3d**. HR FAB calcd for $C_{10}H_{14}N_5O_7P$ 347.0630, found 348.3704 $(M+H)^+$. ^{1}H-NMR - see **TABLE 3**.

1-(2-Deoxy-3-*C*-diethylphosphono-3,5-di-*O*-isopropylidene-β-D-*threo*-pentofuranosyl)-thymine (**5**)

The mixture of phosphonate **2n** (100 mg, 0.26 mmol), 2,2-dimethoxypropane (0.096 ml, 0.79 mmol) and pyridinium 4-toluenesulfonate (65 mg, 0.26 mmol) in dichloromethane (5 ml) was stirred at room temperature for 15 hrs and then the additional portion of 2,2-dimethoxypropane (0.096 ml, 0.79 mmol) was added. The course of the reaction was checked by TLC in the system C1. After 15 hrs the mixture was quenched with triethylamine

(1 ml), concentrated *in vacuo* (30 °C in bath), and the residue chromatographed on silica gel (elution with gradient of 0-10% ethanol in chloroform). Yield 21 mg (19 %) of compound **5**. HR FAB calcd for $C_{17}H_{27}N_2O_8P$ 418.1505, found 419.1479 $(M+H)^+$. ^{1}H-NMR – see **TABLE 3**.

1-(2-Deoxy-3-*C*-phosphono-3,5-di-*O*-isopropylidene-β-D-*threo*-pentofuranosyl)thymine (**6**)
The solution of phosphonic acid **3a** (1.83 g, 4.3 mmol) and 2,2-dimethoxypropane (1.6 ml, 12.9 mmol) in 0.5M hydrochloric acid in DMF (11.5 ml) was set aside for 20 hrs under stirring. Then further portion of 2,2-dimethoxypropane (1.6 ml, 12.9 ml) and 0.5M hydrochloric acid in DMF (4.8 ml) were added. After 14 hrs the reaction mixture was neutralized by triethylamine (1.1 ml, 8.2 mmol) and the solution concentrated on a oil pump (30 °C in a bath). The crude product **6** was purified on DEAE-Sephadex followed by preparative HPLC on reverse phase column to remove the starting phosphonic acid **3a** (elution with linear gradient of 0-50% methanol in 0.1 M aqueous triethylammonium acetate). Yield 600 mg (39 %). HR FAB calcd for $C_{13}H_{19}N_2O_8P$ 362.0879, found 385.0789 $(M+Na)^+$. ^{1}H-NMR – see **TABLE 3**.

1-(2-Deoxy-3-*C*-diethylphosphono-5-*O*-*tert*-butyldiphenylsilyl-β-D-*threo*-pentofuranosyl)-thymine (**7**)
The phosphonate **2g** (571 mg, 0.83 mmol) was dissolved in 1% solution of ammonia in 70% aqueous methanol (50 ml) under a short sonication. The course of the reaction was checked by TLC in the system C1. After 22 hrs the reaction mixture was concentrated *in vacuo*, the residue codistilled with ethanol (2 x 20 ml) and the product **7** was isolated on reverse phase column (elution with gradient of 0-100 % methanol in water). Yield 471 mg (92 %). HR FAB calcd for $C_{30}H_{41}N_2O_8PSi$ 616.2369, found 617.2438 $(M+H)^+$. ^{1}H-NMR – see **TABLE 3**.

1-(3-*O*-Acetyl-5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3-*C*-phosphono-β-D-*threo*-pentofuran osyl)thymine (**9**)
The phosphonic acid **2a** (331 mg, 0.5 mmol) dried by codistillation with dry pyridine (2 x 10 ml) and acetic anhydride (0.189 ml, 2 mmol) were treated in pyridine (4 ml) in the presence of DMAP (61 mg, 0.5 mmol) under stirring and heating at 50 °C for two days. The

course of the reaction was checked by TLC in the system H1. The reaction was quenched by addition of water (1 ml), the solvent was evaporated and the residue codistilled with toluene and ethanol. The product was isolated on reverse phase column (elution with gradient of 0-100 % methanol in water). Yield 108 mg (36 %). HR FAB calcd for $C_{28}H_{35}N_2O_9PSi$ 602.1849, found 625.1750 $(M+Na)^+$. ^1H and ^{13}C-NMR – see **TABLE 3, 4.**

1-(2-Deoxy-3-*C*-phosphono-3,5-di-*O*-acetyl-α,(β)-D-*threo*-pentofuranosyl)thymine (**10**) and [3'-*O*→3"-*P*:3"-*O*→3'-*P*]-*bis*-[3-*N*-acetyl-1-(5-*O*-acetyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine-3'-*C*-phosphonate] (**11**)

The free phosphonic acid **3a** (645 mg, 2.0 mmol) dried by codistillation with dry pyridine (2 x 10 ml), acetic anhydride (1.89 ml, 20 mmol) and DMAP (1.22 g, 20 mmol) were treated in pyridine (10 ml) under stirring and heating at 60 °C for five days. The course of the reaction was checked by TLC in the system H1. The reaction was quenched by addition of water (1 ml) at 0 °C and the mixture was evaporated. The product was purified on DEAE-Sephadex followed by reverse phase chromatography (elution with gradient of 0-100 % methanol in water). Freeze-drying from water afforded 127 mg (17 %) of **10** (α:β=14:86) and 374 mg (48 %) of **11**. HR FAB of **10** calcd for $C_{14}H_{19}N_2O_{10}P$ 406.0777, found 508.1964 $(M+Et_3N+H)^+$, HR FAB of **11** calcd for $C_{28}H_{34}N_4O_{18}P_2$ 776.1343, found 817.1419 $(M+Na+H_2O)^+$. ^1H and ^{13}C-NMR – see **TABLE 3, 4.**

Condensation of 2'-deoxy-5'-O-tritylthymidine-3'-C-phosphonate (2k) with 3'-O-tert-butyldiphenylsilyl-2'-deoxythymidine (12).

Procedure (i). A mixture of 5'-*O*-trityl derivative **2k** (718 mg, 1.27 mmol) and 3'-*O*-*tert*--butyldiphenylsilylthymidine (**12**) (734 mg, 1.52 mmol) dried by codistillation with pyridine (2 x 20 ml) was treated in pyridine (7 ml) with DCC (6.35 mmol) under stirring at room temperature. The course of the reaction was checked by TLC in the system H1. After two days, the suspension was concentrated *in vacuo* to a thick oil and this residue was set aside for further 5 days. Reaction was quenched by dilution with water (10 ml) and after 5 hrs the formed N,N'-dicyclohexylurea was filtered off. The filtrate was concentrated *in vacuo*, the residue codistilled with ethanol and dissolved in 80% acetic acid under a short sonication followed by stirring. After 5 hrs the solvent was evaporated under diminished pressure, and

the residue codistilled twice with water, ethanol and toluene and finally dissolved in 0.5M *tetra-n*-butylammonium fluoride in tetrahydrofurane (10 ml, 5 mmol). The mixture was stirred for 24 hrs at room temperature under exclusion of moisture and the course of the reaction was checked by TLC in the system or H1 and I. After evaporation of the solvent, the desilylated product was purified on DEAE-Sephadex, converted into the sodium salt on Dowex 50x2 (Na^+ form) and finally lyophilized from water. Yield 170 mg (25 %) of sodium salt of compound **14**, 20 mg (5 %) of sodium salt of compound **15** and 70 mg (18 %) of sodium salt of compound **16**. HR FAB of **14** calcd for $C_{40}H_{52}N_8O_{23}P_2$ 1074.2620, found 1097.2574 $(M+Na)^+$. ^1H and ^{13}C-NMR – see **TABLE 3, 4**; ^{31}P-NMR (D_2O): 22.14 d, J(P,P) = 13.7 Hz; 16.28 d, J(P,P) = 13.7 Hz. HR FAB of **15** calcd for $C_{20}H_{28}N_4O_{15}P_2$ 626.1026, found 649.0855 $(M+Na)^+$. ^1H and ^{13}C-NMR – see **TABLE 3, 4**. HR FAB of **16** calcd for $C_{20}H_{26}N_4O_{14}P_2$ 608.0920, found 649.1023 $(M+H_2O+Na)^+$. ^1H and ^{13}C-NMR – see **TABLE 3, 4**; ^{31}P-NMR (D_2O): 10.81 s.

Procedure (ii). The 5'-*O*-trityl derivative **2k** (542 mg, 0.96 mmol) and 3'-*O-tert*-butyldiphenylsilylthymidine (**12**) (555 mg, 1.15 mmol) were condensed as described above. After two days, the suspension was concentrated *in vacuo* to a thick oil, this residue was set aside for 1 day at room temperature and quenched and worked up as mentioned above. Freeze-drying afforded 80 mg (27 %) of sodium salt of compound **15** and 39 mg (13 %) of sodium salt of compound **15.**

Acknowledgements: This work was supported by NIH grant # R03 TW00673-0, by grant NL5441-3 of the Grant Agency of Ministry of Health of the Czech Republic and by the project LB98233 of the Ministry of Education of the Czech Republic. The authors are indebted to staff of the MS department of this Institute, namely Dr. J. Kohoutová for measurements of mass spectra, and to Prof. Eric DeClercq from Rega Institute Leuven, Belgium for evaluation of antiviral properties of prepared compounds.

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Received 3/14/00
Accepted 6/8/00