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Nucleoside 5′-*C*-phosphonates: reactivity of the α-hydroxyphosphonate moiety

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Abstract—We found that various dialkyl phosphites, dialkyl trimethylsilyl phosphites, and tris-trimethylsilyl phosphite reacted smoothly with nucleoside 5′-aldehydes to afford epimeric nucleoside 5′-*C*-phosphonates in high yields. A number of these compounds in both the 2′-deoxyribo and *ribo* series were prepared. In the case of 2′-deoxythymidine-5′-aldehyde, a thorough study was made on the influence of the 3′-hydroxyl protecting group, type of phosphite, base, and solvent, on the yield and epimeric ratio of the resulting 5′-hydroxyphosphonates. Partial stereoselectivity in favour of either *R* or *S* epimers was observed. An attempt to transform the α-hydroxyl of the phosphonate moiety into a halo or azido moiety was not successful. Only intramolecular substitution reaction of the mesyloxy group for an alkoxy residue of the 2-hydroxyethyl ester took place in a low yield.

1. Introduction

Nucleoside phosphonic acids, the well-known structurally diverse analogues of natural nucleotides, exhibit virtually absolute stability against enzymes of nucleotide catabolism, such as phosphomonoesterases and nucleotidases.[1] Among them, several types exhibit, after in vivo phosphorylation, remarkable antiviral properties.[2] The potential biological effects of nucleoside phosphonic acids have been the driving force in the search for novel nucleotide analogues with the P–C linkage. Nucleoside α-hydroxyphosphonic acids bearing a phosphoryl moiety attached directly to one of the carbon atoms of the sugar ring could undoubtedly be interesting compounds in this respect. Wiemer[3] and Králíková[4] reported 3′- and 2′-α-hydroxyphosphonate derivatives of nucleosides (Fig. 1). These 2′- and 3′-nucleotide analogues, however, did not exhibit any antiviral properties. A short account on the synthesis of regioisomeric compounds bearing the 5′-hydroxyphosphonate moiety **4a** was also reported by Králíková.[5,6] Recently Wiemer[7] described the synthesis of arabinosylcytosine 5′-hydroxyphosphonate **4b** which showed interesting biochemical properties. The nucleoside 5′-hydroxyphosphonates are related to the known 5′-deoxynucleoside 5′-phosphonates[8] **5** that lack chirality on the C5′ atom (Fig. 1).

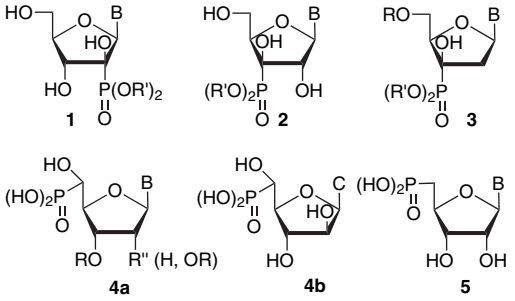

Figure 1. Examples of isopolar non-isosteric nucleoside phosphonic acids.

Herein, we present the synthesis of a number of nucleoside 5′-*C*-phosphonates (5′-hydroxyphosphonates) in 2′-deoxyribo **12–15** and *ribo* series **48–53** bearing an additional chiral centre on the C5′ atom (Schemes 1 and 4, Tables 1 and 3), and demonstrate the reactivity of the 5′-hydroxy group of the hydroxyphosphonate moiety. We prepared these compounds employing the general method of nucleophilic addition of phosphites to carbonyl compounds.[9–11] We found it interesting to subject these compounds to further transformation reactions to obtain a variety of new derivatives. In addition, the free nucleoside 5′-*C*-phosphonic acids **38–41** and **54–58** represent a pool of potential antimetabolites which could inhibit, for instance, different mammalian 5′-nucleotidases, as described earlier[12] for another type of nucleoside 5′-phosphonic acids prepared in our laboratory.[13,14] Also the inhibition of thymidine phosphorylase, the enzyme involved in angiogenesis,[15] by nucleoside 5′-*C*-phosphonic acids could be expected, similarly to recently reported case of acyclic nucleoside phosphonic acids.[16]

Keywords: Nucleoside 5′-aldehydes; Oxidation; Phosphonates; Addition reaction; Nucleophilic substitution.
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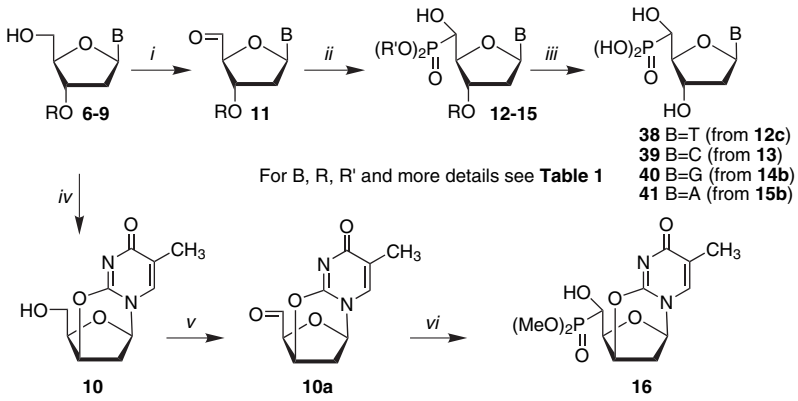


Scheme 1. (i) DMSO–$(COCl)_2$ or DMSO–DCC method; (ii) $(R'O)_2(O)PH$, Et_3N; (iii) (a) Me_3SiBr, CH_3CN, 24 h, rt, (b) satd ammonia in 50% aq ethanol, 48 h, rt, (c) 1 M TBAF in THF, 16 h, rt; (iv) Ph_3P, DEAD (from **10** R=H, B=T); (v) DMSO, DCC, pyridine, TFA; (vi) $(MeO)_2(O)PH$, Et_3N.

Table 1. Epimeric ratios and yields of prepared 2′-deoxynucleoside 5′-*C*-phosphonates (for general structures see Scheme 1)

Starting nucleoside	Oxidation method	Phosphite	Product	B	R	R′	*R*/*S* ratio[a]	Yield %[b]
6a	A[c]	$(Me_3SiO)_3P$	**12a**	T	TBDPS	H	37/63	85
		$(MeO)_2POH$	**12b**			Me	21/79	78
		$(EtO)_2POH$	**12c**			Et	19/81	77
		$(i\text{PrO})_2POH$	**12d**			*i*Pr	13/87	67
6b		$(MeO)_2POH$	**12e**		TBDMS	Me	20/80	71
6c	B[d]	$(MeO)_2POH$	**12f**		Bz	Me	42/58	52
		$(EtO)_2POH$	**12g**			Et	50/50	58
6d		$(EtO)_2POH$	**12h**		Piv	Et	33/67	54
6e		$(MeO)_2POH$	**12i**		DMTr	Me	19/81	60
7	A[c]	$(MeO)_2POH$	**13**	C^{Bz}	TBDPS	Me	15/85	84
8a			**14a**	G^{Bz}			34/66	82
8b			**14b**	G^{iBu}			40/60	76
9a			**15a**	A^{Bz}			25/75	69
9b	B[d]		**15b**	A^{Bz}	DMTr		22/78	80
10			**16**	T	—	—	37/63	34

[a] Epimeric ratio determined from ^1H NMR spectra.
[b] Isolated yield.
[c] DMSO–$(COCl)_2$.
[d] DMSO–DCC–pyridine–TFA.

2. Results and discussion

2.1. Preparation of 2′-deoxyribonucleoside 5′-*C*-phosphonates

The synthesis of protected nucleoside hydroxyphosphonates **12–16** in the 2′-deoxy series was accomplished by nucleophilic addition of various phosphites to nucleoside 5′-aldehydes **11** and **10a** (Scheme 1, Table 1) obtained by oxidation of protected nucleosides **6–9**, **10** using the Swern (DMSO–$(COCl)_2$)[17] or modified[18] Moffatt procedures (DMSO–DCC).[19,20] They were used in further reaction without purification and characterization. Whereas the 3′-*O*-silyl-protected 2′-deoxyribonucleosides **6a**, **6b**, **7**, **8a**, **8b** and **9a** were smoothly oxidized by the Swern procedure[17] (Method A), for the oxidation of 2′-deoxynucleosides bearing 3′-*O*-acyl protecting groups **6c**, **6d**, 3′-*O*-DMTr derivatives **6e** and **9b**, and 2,3′-anhydrothymidine (**10**) the DMSO–DCC method[18] was used (Method B) (Table 1). In contrast to literature data, [17] the 3′-*O*-acyl-protected 2′-deoxynucleosides **6c** and **6d** were not stable under conditions of the Swern oxidation procedure.[17]

We attempted to increase the stereoselectivity of the addition of dialkyl phosphites to nucleoside 5′-aldehydes (Table 1) by changing several factors, such as the solvent and the base used, and the type of phosphorus acid esters. We found that the solvent did not have any effect on the stereoselectivity, but the yields of phosphonates were different. Thus, DCM provided better yields of 5′-*C*-phosphonates than THF and acetonitrile. Increasing the amount of triethylamine (from 1 to 5 equiv) or the use of saturated ammonia in dioxane as a weak base did not influence the ratio of epimers. The use of DBU instead of triethylamine caused destruction of the starting nucleoside 5′-aldehyde. No change in the ratio of the epimeric phosphonates under the addition of diethyl phosphite in the presence of either lithium bis (trimethylsilyl)amide or *tert*-butylmagnesium chloride at −78 °C was found, but the yields of 5′-*C*-phosphonates were significantly reduced. On the other hand, only little changes in epimeric ratios were found if various dialkyl phosphites were used in the presence of triethylamine (Table 1); however, tris-trimethylsilyl phosphite exhibited significantly lower stereoselectivity in the addition reaction. The comparison of various types of 3′-*O*-protecting groups revealed (Table 1) that the use of 3′-*O*-acyl groups resulted in decrease of reaction preferences for the (*S*)-epimers.

The epimeric, protected nucleoside 5′-*C*-phosphonates **12–15** were separable by RP-HPLC, but on silica gel the

separation seldom took place. During our experiments, we succeeded in the separation of the epimers of dimethyl-(2-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyguanosin-5′-*C*-yl-phosphonate) (**14a**) on C18 silica. In case of thymidine phosphonate **12**, we examined several ways in which to separate individual epimers. We found that removal of the 3′-*O*-protecting group of dimethyl phosphonates **12b** and **12f** resulted in better resolution of epimers on C18 silica. Unfortunately, hydrolysis of the silyl or benzoyl 3′-*O*-protecting group was accompanied by partial hydrolysis of one methyl ester group (epimeric monomethyl esters were very difficult to separate). In addition, the removal of 3′-*O*-benzoyl group from phosphonate **12f** led to the change of elution order of epimers on silica gel (not on C18 silica), and to significant increase of ΔR_f of epimers on TLC. In the case of dimethyl-(3′-*O*-*tert*-butyldimethylsilyl-2′-deoxythymidin-5′-*C*-yl-phosphonate) (**12e**), we succeeded in the separation of the substantial part of minor (*R*)-epimer by crystallization from ethyl acetate–ethanol mixture. Likewise, crystallization of the epimeric mixture of dimethyl-(3′-*O*-benzoyl-2′-deoxythymidinyl-5′-*C*-phosphonate) (**12f**) provided the major (*S*)-epimer.

2.2. ^{1}H and ^{13}C NMR study

Individual epimers **14a** were subjected to NMR analysis to determine the configuration at the 5′-carbon atom. The structural assignment was carried out using characteristic chemical shifts (protons and carbons of the nucleobase and substituents), homonuclear 2D-COSY and 2D-ROESY spectra (deoxyribose protons), and heteronuclear ^{1}H, ^{13}C-2D-HMQC spectra (deoxyribose carbon atoms). Vicinal couplings *J*(H,H) showed a high preference for the C2′-*endo* form of the deoxyribofuranse ring in both epimers (~85% in the major and ~95% in the minor epimer). The preferred syn-orientation of the nucleobase was determined from 2D-ROESY spectra. Determination of the configuration at C5′ is closely connected with the conformation around C4′–C5′ bond. The high value of *J*(P,C3′)=14.6 Hz and low value of *J*(P,H4′)=2.3 Hz in the major epimer indicates the trans-arrangement of P and C3′ which, together with a *gauche*-relationship of H4′/H5′ as indicated by *J*(H4′,H5′)=2.3 Hz, establishes the (*S*)-configuration at C5′ (Fig. 2). The configuration of minor epimer **14a** is therefore 5′(*R*) and the set of its vicinal couplings (*J*(P,C3′)=8.8 Hz, *J*(P,H4′)=5.4 Hz and *J*(H4′,H5′)=6.4 Hz) indicate comparable populations of *trans* and *gauche* conformers around C4′–C5′ bond (Fig. 2). Lower values of *J*(P,H4′) and *J*(H4′,H5′) were observed for major isomer in five-epimeric pairs **12–15**, **25–26** and **54–58**, indicating the 5′(*S*)-configuration for the major isomer, similar to the above discussed pair **14a**, **14b**. It should be noted that this relation between *J*(P,H4′) and *J*(H4′,H5′) in the 5′(*S*) and 5′(*R*) isomers does not hold in general for all nucleotides studied in this paper. For example, in deprotected epimeric pairs **38–41** and/or in 2,3-di-*O*-isopropylidene derivatives **48–53** prepared from precursors with known ratio of 5′(*S*) and 5′(*R*) isomer, the observed relative values of corresponding *J*(H,H) are opposite. A similar situation was also found in nucleosides **16** and **36**, obviously due to the different preferred conformation around the C4′–C5′ bond in the presence of additional sugar ring.

2.3. Attempt to convert α-hydroxyphosphonate moiety into α-halophosphonate (Scheme 2)

Reaction of the 5′-hydroxyl of **12b** with CBr_4 or CCl_4 and triphenylphosphine in dioxane to prepare a halo derivative **17** failed. Whereas at rt we observed no reaction, under heating total decomposition of **12b** took place. Also the attempt to obtain fluoro derivative **18** in the reaction of **12b** with DAST[21] in dichloromethane at −78 °C failed, and we isolated only a small amount of product of elimination, namely 1-(3-*O*-*tert*-butyldiphenylsilyl-2,5-dideoxy-β-D-*glycero*-pent-4-enofuranosyl)thymine. Therefore, on reaction with methansulfonyl chloride in pyridine, the phosphonate **12b** was first transformed into 5′-*O*-mesyl derivative **19**, which was used for the reaction with TBAF in dimethylformamide in the presence of powdered molecular sieves. The mesyl derivative **19** was completely stable at rt, but under heating provided a mixture of products. The reaction of **19** with tris(dimethylamino)sulfonium difluorotrimethyl silicate (TASF)[22] in various solvents (dichloromethane, acetonitrile and dimethylformamide) resulted in quantitative conversion of **19** into its 3-*N*-methyl derivative (structure not shown); no fluoro derivative **18** was formed.

2.4. Attempt to convert α-hydroxyphosphonate into α-azidophosphonate

An attempt to displace the mesyloxy group of adenine derivative **20** by an azide ion caused only quantitative cleavage of one methyl ester group giving monomethyl derivative **21** (Scheme 2). We also did not succeed in the preparation of a more reactive 5′-*O*-trifluoromethanesulfonyl derivative

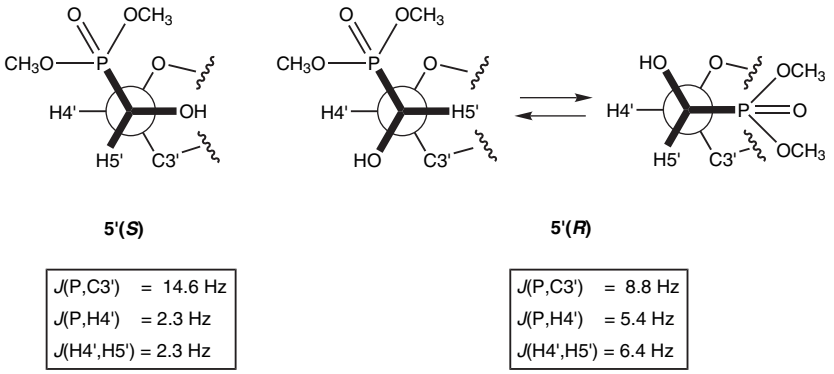
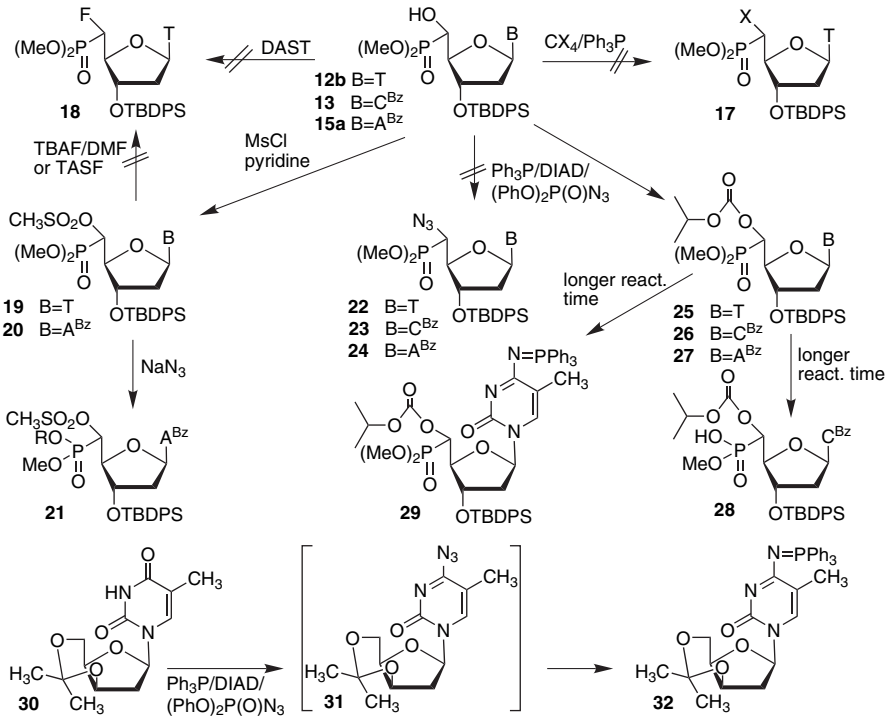


Figure 2. Epimers **14a**: preferred conformations around C4′–C5′ in both epimers and vicinal coupling constants used for determination of the configuration at carbon C5′.



Scheme 2. Reactions on the 5′-hydroxyphosphonate moiety.

intended for use in the reaction with sodium azide. At −15 °C, the reaction of phosphonate **12b** with triflic anhydride did not proceed, whereas at rt total decomposition of starting compound **12b** was observed.

Mitsunobu reaction[23] of phosphonates **12b**, **13** and **15a** with diphenylphosphoryl azide, diisopropyl azodicarboxylate, and triphenylphosphine did not provide the expected 5′-azido derivatives **22**–**24** but gave the 5′-*O*-isopropoxycarbonyl derivatives **25**–**27** in quantitative yield (Scheme 2). Reaction proceeded with partial inversion of configuration, as it was proved in the reaction with both the epimeric mixture and the individual epimers of compound **12b** (for results, see Table 2). The formation of carbonates under Mitsunobu conditions was observed by Battaglia[24] in case of branched hydroxy derivatives of dioxolanones. During prolonged reaction time we observed, in the case of cytosine compound **26**, the loss of one methyl ester group under formation of monoester **28**. The calculated molecular mass of **28** corresponded with that of found by HRMS. In addition, the derivative **25** underwent subsequent reaction on the thymine residue. We detected (by RP-HPLC) the formation of another pair of peaks with higher retention times than starting epimers **25**. Using 3′,5′-*O*-isopropylidene-*xylo*-thymidine (**30**) as a model compound, we proved that the reaction on the thymine residue led to triphenylphosphoranylidene derivative **32**, most probably via 4-azido-2-pyrimidone derivative **31**. Thus, we believe that the newly formed pair of peaks with higher retention times in the reaction of **12b**→**25** (Scheme 2) are the 5′-epimeric triphenylphosphoranylidene derivatives **29**.

Table 2. Changes of epimeric ratio in the reaction **12b**→**25** (Scheme 2)

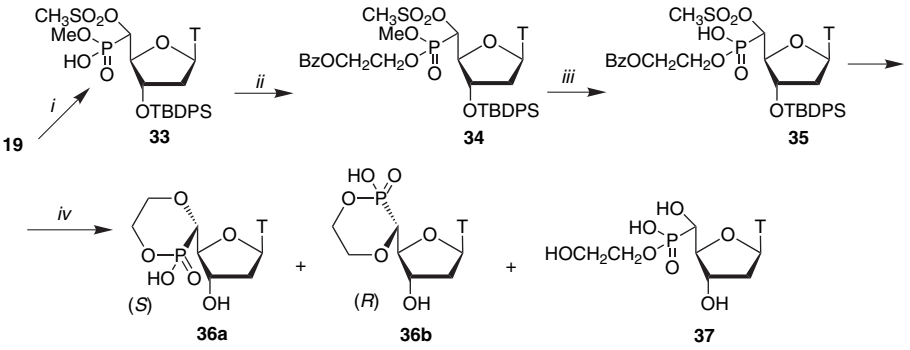
R/*S* epimeric ratio	
Reactant **12b**	Product **25**
22/78	43/57
0/100	64/36
100/0	17/83

2.5. Intramolecular substitution reaction of 5′-*O*-mesyl group

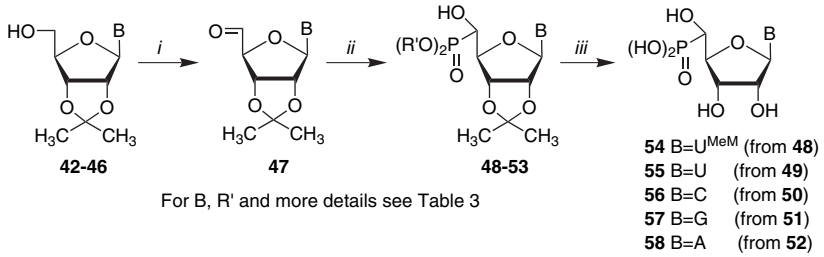
The failure of substituting a mesyloxy group for an azido moiety led us to check the possibility of an intramolecular nucleophilic substitution reaction. Thus, monomethyl ester **33** prepared by treatment of **19** with aqueous pyridine was condensed by a phosphotriester method[25] with 2-benzoyloxyethanol to give mixed diester **34**, which was demethylated in aqueous pyridine to give the 2-benzoyloxyethyl ester **35** (Scheme 3). This compound underwent a slow intramolecular nucleophilic substitution of the 5′-*O*-mesyloxy group with the hydroxy moiety of the 2-hydroxyethyl ester residue in refluxing 1 M sodium methoxide in methanol. After complete removal of the silyl protecting group, RP-HPLC isolation provided both epimers of cyclic monoester **36a** and **36b** as well as 2-hydroxyethyl ester **37** in low yields, along with thymine as the main product.

2.6. Preparation of ribonucleoside 5′-*C*-phosphonates

The synthesis of protected ribonucleoside hydroxyphosphonates **48**–**53** was accomplished by nucleophilic addition of phosphites to nucleoside 5′-aldehydes of general formula **47** obtained by oxidation of protected nucleosides **42**–**46** using the DMSO–DCC procedure[18] (Scheme 4, Table 3). Again in this case, we observed reaction preferences for the formation of (*S*)-epimers (Table 3). Separation of epimeric pairs of phosphonates **48**–**53** was more effective on C18 silica than on silica gel.



Scheme 3. (i) Aqueous pyridine (60%), 50 °C; (ii) $BzOCH_2CH_2OH$, MSNT, 4-methoxypyridine-*N*-oxide, pyridine; (iii) 60% aq pyridine, 50 °C; (iv) 1 M CH_3ONa in CH_3OH, reflux.



Scheme 4. (i) DCC–DMSO (Method B); (ii) $(R'O)_2(O)PH$, Et_3N; (iii) (a) Me_3SiBr, CH_3CN, 24 h, rt, (b) concd aq ammonia, 48 h, rt, (c) 0.025 M H_2SO_4 in 50% aq dioxane, 16 h, rt.

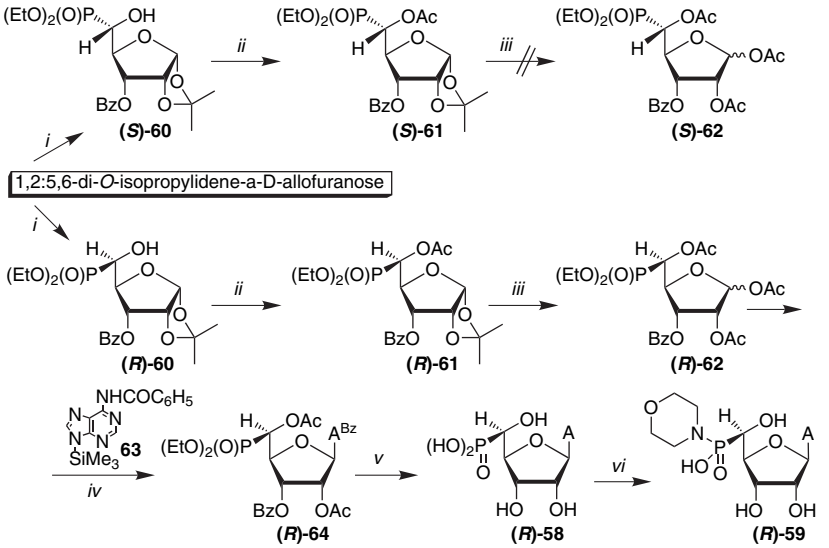
Table 3. Epimeric ratios and yields of prepared ribonucleoside 5′-*C*-phosphonates (for general structure see Scheme 4)

Starting nucleoside	Oxidation method	Phosphite	Product	B	R′	*R*/*S* ratio[a]	Yield %[b]
42	B[c]	$(EtO)_2POH$	**48**	U^{MeM}	Et	30/70	31
43			**49**	U		45/55	46
44			**50**	C^{Bz}		43/57	57
45		$(MeO)_2POH$	**51**	G^{Bz}	Me	40/60	66
46		$(EtO)_2POH$	**52**	A^{Bz}	Et	33/67	32
		$(MeO)_2POH$	**53**	A^{Bz}	Me	17/83	42

[a] Epimeric ratio determined from ^1H NMR spectra.
[b] Isolated yield.
[c] DMSO–DCC–pyridine–TFA.

Therefore, we examined the nucleosidation reaction as a potential synthetic route for the preparation of pure epimers of ribonucleoside 5′-*C*-phosphonates (Scheme 5). The starting sugar-phosphonate synthons (*S*)-**60** and (*R*)-**60** (Scheme 5) were easily available by addition of diethyl phosphite to 3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentodialdo-1,4-furanose according to a modified Otmar procedure.[26] Both epimers were separated on silica gel. The 5′-hydroxyl of these compounds was acetylated in pyridine with acetic anhydride, and acetyl derivatives (*S*)-**61** and (*R*)-**61** were subjected to acetolysis to obtain sugar-phosphonate synthons (*S*)-**62** and (*R*)-**62**. Surprisingly, only (*R*)-**61** underwent clean acetolysis with formation of the expected product (*R*)-**62** which afforded, on a nucleosidation reaction with silylated 6-*N*-benzoyladenine **63**, the phosphonate (*R*)-**64**. On the other hand, acetolysis of compound (*S*)-**61** resulted unexpectedly in a mixture of products, so that the route following nucleosidation reaction with silylated 6-*N*-benzoyladenine **63** did not afford any desired nucleoside phosphonate. The reason for instability of (*S*)-**62** toward acetolysis is not clear. The observation that this synthetic route can provide only (*R*)-phosphonates led us back to the original synthetic route. We found that morpholidate derivatives of epimeric adenine compound **58** exhibited significant difference in retention times on C18 silica. To identify the (*R*)-epimer in an epimeric mixture, we prepared morpholidate (*R*)-**59** from the free phosphonate (*R*)-**58**, morpholine and DCC.

Deprotection of fully protected nucleoside phosphonates **12b**, **13**, **14b**, **15b** and **48**–**52** was performed as follows. First, the alkyl ester groups of the phosphorus moiety were removed by treatment with bromotrimethylsilane (DMTr group of **15b** was also cleaved off under these conditions), then *N*- and *O*-acyl protected groups were removed in concd aq ammonia–ethanol mixture and, finally, either silyl groups were removed by treatment with TBAF in THF or the isopropylidene groups were hydrolyzed in 0.025–0.05 M sulfuric acid. Free phosphonic acids **38**–**41** and **54**–**58** were purified on DEAE-Sephadex column followed by RP-HPLC. Obtained compounds were transformed into sodium salts on Dowex 50 (Na^+), and finally freeze-dried from water.



Scheme 5. (i) (a) Benzoyl cyanide, Et_3N, DCM–acetonitrile, (b) 60% aq acetic acid, 50 °C, (c) $NaIO_4$, 70% aq acetone; (ii) Ac_2O, pyridine; (iii) Ac_2O, AcOH, DCM, H_2SO_4; (iv) $SnCl_4$, acetonitrile; (v) (a) concd aq ammonia–ethanol mixture, (b) Me_3SiBr, acetonitrile; (vi) DCC, morpholine, *t*-BuOH–H_2O, reflux.

2.7. Biological properties

Cytostatic activity of protected phosphonates and free phosphonic acids was examined on L 1210, L 929 and HeLa S3 cell lines. Antiviral properties of the same compounds were examined on HIV-1, HIV-2 and Moloney sarcoma virus (MSV), and on the selected DNA viruses (HSV-1 (KOS), HSV-1 (B), HSV-1 (McIntyre), HSV-2 (G), HSV-2 (196), HSV-2 (Lyons), VV, VSV, HSV-1 TK^- (B2006) and HSV-1 TK^- (VMW1837)). Neither phosphonates **12**–**15** and **48**–**53** nor phosphonic acids **38**–**41** and **54**–**58** exhibited any significant cytostatic and/or antiviral activities.

3. Conclusion

We have established an efficient synthesis of nucleoside 5′-*C*-phosphonates in 2′-deoxyribo and *ribo* series bearing a new centre of chirality on the C5′ atom via base-catalyzed nucleophilic addition of various phosphites to nucleoside 5′-aldehydes, and thus enlarged the family of enzyme-stable nucleotide analogues. The reactivity of 5′-hydroxyl of the nucleoside 5′-hydroxyphosphonates was found to be very specific, and the conversion of the hydroxyl into a halo or azido moiety failed. Only acylation and sulfonylation reactions proceeded smoothly in quantitative yields. The prepared nucleoside phosphonic acids offer the possibility for their further evaluation as inhibitors of mammalian 5′-nucleotidases, bisubstrate inhibitors of thymidine and purine nucleoside phosphorylases, and, after transformation into nucleoside 5′-triphosphate analogues, as substrates/inhibitors of DNA and RNA polymerases.

4. Experimental

4.1. General

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 reactions was checked by TLC cards (Fluka, Merck) whereby the products were detected by UV monitoring and by spraying with 1% ethanolic solution of 4-(4-nitrobenzyl)pyridine followed by heating and treating with gaseous ammonia (blue colour of diesters of phosphonic acids). For flash column chromatography, silica gel 40–60 μm (Fluka) was used. The TLC and the preparative silica gel chromatography were carried out in the following solvent systems (v/v): chloroform–ethanol 9/1 (C1); ethyl acetate–acetone–ethanol–water 4/1/1/1 (H1); ethyl acetate–acetone–ethanol–water 6/1/1/1 (H3); 2-propanol–concd aq ammonia–water 7/1/2 (I); 50% EtOAc–toluene (T1), 20% EtOAc–toluene (T2). Analytical HPLC was performed on Nucleosil 100–5 C18 (4.6×150 mm; Macharey–Nagel) using a linear gradient of methanol in 0.1 M TEAA. Preparative reversed-phase chromatography was carried out on octadecyl silica column (25×250 mm, 20 μm, IOCB Prague); compounds were eluted with a linear gradient of methanol in water at 15 ml/min. UV spectra and thermal characteristics were taken on a Cary Bio 100 (Varian) spectrophotometer. High resolution FAB mass spectra were recorded on a ZAB-EQ (VG Analytical) instrument with glycerol and thioglycerol as matrices. NMR spectra were measured on a Varian UNITY-500 spectrometer (^1H at 500 MHz; ^{13}C at 125.7 MHz frequency) in DMSO-d_6 and/or D_2O at 20 °C. The chemical shifts were referenced either to solvent signal (converted to δ scale using relations δ_H(DMSO)=2.50 and δ_C(DMSO)=39.7 ppm) or to DSS (in D_2O). Proton 2D-COSY spectra were used for the structural assignment of coupled protons and 2D-ROESY spectra for detection of the NOE contacts. Carbon-13 chemical shifts and coupling constants *J*(C,P) were obtained from broad band proton-decoupled spectra using APT pulse sequence.

Method A: Swern oxidation procedure [DMSO–$(COCl)_2$].[17] Dimethylsulfoxide (0.21 ml, 3 mmol) was added dropwise to a stirred solution of oxalyl chloride (0.13 ml, 1.5 mmol) in dichloromethane (3.5 ml) at −78 °C under argon atmosphere. After 10 min, a solution of protected nucleoside (1 mmol) (see Table 1) in dichloromethane (7 ml) was added

dropwise and the reaction mixture was stirred at −78 °C for 30 min. The reaction was quenched by addition of triethylamine (0.7 ml, 5 mmol). The resulting suspension was stirred for additional 5 min at low temperature, allowed to warm to rt, and the appropriate phosphite (2 mmol) (see Table 1) was added. The reaction mixture was set aside overnight at rt (TLC in C-1), diluted with chloroform, extracted with water, and dried over anhydrous sodium sulfate. Chromatography of the crude product on a silica gel (elution with a linear gradient of 0–10% ethanol in chloroform) afforded the desired phosphonate (Table 1).

Method B: Modified Moffatt oxidation procedure [DMSO–DCC].[18] Pyridine (0.08 ml, 1 mmol) followed by TFA (0.04 ml, 0.5 mmol) was added at rt to a stirred solution of protected nucleoside (1 mmol) (Tables 1 and 3) and DCC (0.64 g, 3 mmol) in DMSO (4 ml). After 16 h, appropriate phosphite (2 mmol) (Tables 1 and 3) and triethylamine (0.7 ml, 5 mmol) were added and the mixture was stirred for further 16 h (TLC in C-1). The reaction mixture was diluted with chloroform, filtered through Celite, and the filtrate was extracted with water and organic layer was dried over anhydrous sodium sulfate. Chromatography of the crude product on silica gel (elution with a linear gradient of 0–10% ethanol in chloroform) afforded the expected phosphonate (Tables 1 and 3).

Method C: Removal of ester protecting groups of phosphonate moiety. 2,6-Lutidine (0.58 ml, 5 mmol) and bromotrimethylsilane (0.66 ml, 5 mmol) were sequentially added to a solution of phosphonate diester **12c**, **13**, **14b**, **15b**, **48–52** (1 mmol) in acetonitrile (5 ml), the mixture was set aside at rt for 48 h (TLC in C-1, H-1 and I), and then concentrated in vacuo. Aqueous 2 M TEAB (2 ml) was added, the solution was concentrated, and the residue was co-distilled several times with methanol to destroy TEAB and finally with ethanol. The obtained *N*- and/or *O*-protected nucleoside phosphonic acids **12–15** (R′=H) and **48–52** (R′=H) were subjected to further deprotection steps (Method D, E or F).

Method D: Removal of N-protecting groups. The solution or suspension of *N*-protected cytosine- (**13** and **50**, R′=H), guanine- (**14** and **51**, R′=H) and adenine-containing (**15** and **52**, R′=H) phosphonic acid, obtained by Method C, in methanol or ethanol (50 ml/mmol) was saturated with gaseous ammonia at 0 °C. The mixture was stirred in a tightly stoppered flask at rt overnight (TLC in C-1, H-1 and I). Deprotection of *N*-benzoylguanine derivatives **14** and **51** (R′=H) took place in an autoclave at 60 °C overnight. Alternatively, deacylations were also performed in a 1/1 mixture of concd aq ammonia–ethanol for 16 h with identical results. Solvent was evaporated, and the residue was dried by co-distillation with ethanol (3×20 ml). The crude deacylated compounds **13** and **14** (B=C, G; R′=H, R=TBDPS) were subjected to treatment according to Method E, derivatives **50–52** (B=C, G, A; R′=H) to treatment by Method F, and adenine phosphonic acid **41** (obtained from **15** ($B=A^{Bz}$; R=R′=H)) to the final purification step (Method G).

Method E: Removal of silyl protecting group. The silyl derivatives **12–14** (B=T, C, G; R′=H, R=TBDPS) obtained by Method D were co-distilled with dry toluene, dissolved in 0.5 M TBAF in THF (10 ml/mmol), and the reaction mixture was stirred for 24 h at rt under exclusion of moisture. In case of less soluble compounds, an equal volume of pyridine was added, and the solution was concentrated to half volume. Silyl group cleavage ability of TBAF in pyridine was at least as efficient as in THF alone. After removal of the silyl group (TLC in H-1 and I), water was added to the reaction mixture, the solution was concentrated in vacuo, and finally Dowex 50 (Et_3NH^+, 20 ml/mmol) in 50% aqueous ethanol (50 ml/mmol) was added to remove *tetra-n*-butylammonium cations. The suspension was filtered, the resin was washed with 50% aqueous ethanol, and the combined filtrates were evaporated. Crude phosphonic acids **38–40** were subjected to final purification step (Method G).

Method F: Removal of 2′,3′-O-isopropylidene protecting group. The products **50–52** (B=C, G, A; R′=H) obtained by Method D were dissolved in aqueous 0.05 M sulfuric acid (100 ml/mmol) and the solution was set aside overnight at rt (TLC in H-1 and I). Solution of crude cytidine **56**, guanosine **57**, and adenosine **58** phosphonic acids was applied onto a column of Dowex 50 (H^+) and, after washing with water, the product was eluted by 3% aqueous ammonia. In the case of uridine derivatives **48** and **49** ($B=U^{MeM}$, U; R′=H) obtained by Method C, the cleavage of isopropylidene group was achieved with aqueous 80% acetic acid at 80 °C for 3 h. The reaction mixture was concentrated, and the residue co-distilled several times with water. The obtained crude products **54–58** were subjected to final purification step (Method G).

Method G: Final purification of nucleoside phosphonic acids. Phosphonic acids **38–41** and **54–58** were purified by chromatography on DEAE-Sephadex A-25 (HCO_3^-). The compounds were eluted by a linear gradient of 0–0.2 M TEAB. Fractions were pooled and evaporated, the residue was co-distilled several times with methanol and then applied onto C18 column (25×300 mm) in a solution of aqueous 2 M TEAB (10 ml/mmol). The products were eluted by a linear gradient of methanol in water (0–10%), then transformed into sodium salts on a column of Dowex 50 (Na^+), and finally freeze-dried from water.

4.1.1. 3′-*O*-*tert*-Butyldiphenylsilyl-2′-deoxythymidin-5′-*C*-ylphosphonic acid (12a). Phosphonate **12a** was obtained from tris(trimethylsilyl) phosphite (1.0 ml, 3 mmol) and the aldehyde prepared from 3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidine[27] (480 mg, 1 mmol) according to Method A. The reaction was quenched by addition of methanol (5 ml) and 2 M TEAB (5 ml), and the solution was heated at 80 °C for 2 h (TLC in H1). After evaporation of the solvent, the partially protected phosphonate was purified on RP C18 column (elution with a linear gradient of methanol in water). Yield, 562 mg (85%; white foam) of triethylammonium salt of **12a** (*R*/*S* 37/63). For $C_{26}H_{33}N_2NaO_8PSi$ $(M+Na)^+$ calcd: 583.1643, found: 583.1642. ^1H NMR—see Tables 4 and 5.

4.1.2. Dimethyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12b). The title compound **12b** was obtained on reaction of dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidine[27] (480 mg, 1 mmol) according to Method A. Yield, 459 mg (78%; white foam) of **12b** (*R*/*S* 21/79). For $C_{28}H_{37}N_2O_8PSi$ (588.66) calcd: 57.13%

Table 4. ^{1}H chemical shifts of compounds **12–16** in DMSO-d_6

Compound	H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	5′-OH	Nucleobase
(*S*)-**12a**[a]	6.15	1.86	2.00	4.51	4.30	3.39	4.50	T: 11.20 (NH), 8.18 (H-6), 1.71 (5-Me)
(*R*)-**12a**[a]	6.20	1.85	1.75	4.97	4.42	3.57	4.50	T: 11.20 (NH), 8.02 (H-6), 1.70 (5-Me)
(*S*)-**12b**[a,b]	6.31	1.96	2.16	4.46	4.09	3.46	6.07	T: 11.32 (NH), 7.82 (H-6), 1.71 (5-Me)
(*R*)-**12b**[a,b]	6.36	1.94	1.87	4.64	4.24	3.98	6.28	T: 11.30 (NH), 7.57 (H-6), 1.74 (5-Me)
(*S*)-**12c**[a,c]	6.31	1.96	2.17	4.45	4.11	3.36	5.99	T: 11.31 (NH), 7.86 (H-6), 1.71 (5-Me)
(*R*)-**12c**[a,c]	6.36	1.95	1.86	4.69	4.26	3.92	6.26	T: 11.30 (NH), 7.65–7.40 (H-6), 1.74 (5-Me)
(*S*)-**12d**[a,d]	6.29	1.96	2.19	4.44	4.13	3.27	5.85	T: 11.30 (NH), 7.89 (H-6), 1.71 (5-Me)
(*R*)-**12d**[a,d]	6.35	1.95	1.85	4.73	4.27	3.83	6.22	T: 11.30 (NH), 7.50–7.40 (H-6), 1.74 (5-Me)
(*S*)-**12e**[b,e]	6.17	2.14	2.06	4.49	4.02	4.12	6.21	T: 11.30 (NH), 7.88 (H-6), 1.76 (5-Me)
(*R*)-**12e**[b,e]	6.20	2.14	1.96	4.65	3.96	4.04	6.35	T: 11.33 (NH), 7.64 (H-6), 1.78 (5-Me)
(*S*)-**12f**[b,f]	6.31	2.33	2.46	5.53	4.42	4.34	6.47	T: 11.36 (NH), 7.97 (H-6), 1.79 (5-Me)
(*R*)-**12f**[b,f]	6.27	2.42	2.35	5.73	4.37	4.26	6.45	T: 11.37 (NH), 7.68 (H-6), 1.81 (5-Me)
(*S*)-**12g**[c,f]	6.30	2.30–2.50		5.52	4.42	4.28	6.37	T: 11.36 (NH), 7.98 (H-6), 1.78 (5-Me)
(*R*)-**12g**[c,f]	6.26	2.30–2.50		5.74	4.40	4.18	6.44	T: 11.35 (NH), 7.68 (H-6), 1.80 (5-Me)
(*S*)-**12h**[c,g]	6.19	2.23	2.23	5.23	4.17	4.09	6.23	T: 11.36 (NH), 7.93 (H-6), 1.79 (5-Me)
(*R*)-**12h**[c,g]	6.15	2.28	2.08	5.48	4.15	4.20	6.40	T: 11.36 (NH), 7.64 (H-6), 1.77 (5-Me)
(*S*)-**12i**[b,h]	6.27	1.88	2.09	4.29	3.58	3.27	5.98	T: 11.30 (NH), 7.85 (H-6), 1.70 (5-Me)
(*R*)-**12i**[b,h]	6.14	1.61	0.95	4.37	4.37	3.89	6.10	T: 11.26 (NH), 7.52 (H-6), 1.71 (5-Me)
(*S*)-**13**[a,b,i]	6.31	1.94	2.46	4.49	4.20	3.48	6.12	C^{Bz}: 11.25 (NH), 8.55, 7.32 (H-5, H-6)
(*R*)-**13**[a,b,i]	6.36	2.12	1.93	4.71	4.37	4.06	6.23	C^{Bz}: 11.28 (NH), 8.35, 7.25 (H-5, H-6)
(*S*)-**14a**[a,b,i]	6.45	2.51	2.46	4.60	4.17	3.50	6.08	G^{Bz}: 12.36, 11.91 (2×NH), 8.27 (H-8)
(*R*)-**14a**[a,b,i]	6.45	2.77	2.19	4.77	4.30	3.97	6.18	G^{Bz}: 12.35, 11.91 (2×NH), 8.24 (H-8)
(*S*)-**14b**[a,b,i]	6.36	2.44	2.44	4.58	4.16	3.50	6.04	G^{iBu}: 12.29, 11.65 (2×NH), 8.22 (H-8)
(*R*)-**14b**[a,b,i]	6.35	2.70	2.17	4.75	4.29	3.92	6.16	G^{iBu}: 12.10, 11.65 (2×NH), 8.19 (H-8)
(*S*)-**15a**[a,b,i]	6.31	2.87	2.24	4.57	4.29	3.95	6.03	A^{Bz}: 11.22 (NH), 8.66, 8.54 (H-2, H-8)
(*R*)-**15a**[a,b,i]	6.39	2.44	2.14	4.75	4.35	4.07	6.17	A^{Bz}: 11.20 (NH), 8.53, 8.32 (H-2, H-8)
(*S*)-**15b**[b,h,i]	6.65	2.55	2.55	4.43	3.64	3.32	6.19	A^{Bz}: 11.23 (NH), 8.74, 8.71 (H-2, H-8)
(*R*)-**15b**[b,h,i]	6.45	2.35	1.27	4.59	4.46	4.03	6.13	A^{Bz}: 11.19 (NH), 8.72, 8.61 (H-2, H-8)
(*S*)-**16**[b]	5.78	2.58	2.50	5.26	4.29	3.74	6.35	T: 7.58 (H-6), 1.76 (Me)
(*R*)-**16**[b]	5.85	2.52	2.52	5.23	4.34	4.02	6.01	T: 7.56 (H-6), 1.75 (Me)

[a] 3′-OTBDPS: 7.55–7.65 (m, 4H) and 7.36–7.52 (m, 6H) (2×C_6H_5), 1.07–1.10 (s, 9H, *t*-Bu).
[b] P(OMe)$_2$: 3.32–3.68 (2×d, 2×3H).
[c] P(OEt)$_2$: 3.80–4.10 (m, 4H, 2×P–OCH$_2$), 1.10–1.23 (2×d, 2×CH$_3$).
[d] P(*i*Pr)$_2$: 4.49–4.59 (m, 2H, 2×P–OCH), 1.13, 1.14, 1.20 and 1.21 (4×d, 4×CH$_3$).
[e] 3′-TBDMS: 0.87 (s, 9H, *t*-Bu), 0.10 (s, 6H, 2×CH$_3$).
[f] 3′-OBz: 8.02 (m, 2H, *o*-ArH), 7.70 (m, 1H, *p*-ArH) and 7.56 (m, 2H, *m*-ArH).
[g] 3′-OPiv: 3.90–4.07 (m, 4H, 2×P–OCH$_2$), 1.17 (s, 9H, 3×CH$_3$).
[h] 3′-ODMTr: 7.43 (m, 2H), 7.35 (m, 2H) and 7.25 (m, 1H) (C_6H_5); 7.30 (m, 4H) and, 6.93 (m, 4H) (2×C_6H_4), 3.74 (s, 6H, 2×OCH$_3$).
[i] NBz: 8.05 (m, 2H, *o*-ArH), 7.65 (m, 1H, *p*-ArH) and 7.55 (m, 2H, *m*-ArH).

C, 6.34% H, 4.76% N; found: 56.78% C, 6.23% H, 4.51% N. MS (FAB): 589.2 (M+H)$^+$. ^{1}H NMR—see Tables 4 and 5.

4.1.3. Diethyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12c). Phosphonate **12c** was obtained from diethyl phosphite (0.26 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidine[27] (480 mg, 1 mmol) according to Method A. Yield, 475 mg (77%; white foam) of **12c** (*R*/*S* 19/81). For $C_{30}H_{41}N_2O_8SiP$ (616.72) calcd: 58.43% C, 6.70% H, 4.54% N; found: 58.19% C, 6.67% H, 4.43% N. MS (FAB): 617.4 (M+H)$^+$. ^{1}H NMR—see Tables 4 and 5.

4.1.4. Diisopropyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12d). Phosphonate **12d** was obtained from diisopropyl phosphite (0.33 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidine[27] (480 mg, 1 mmol) according to Method A. Yield, 432 mg (67%; white foam) of **12d** (*R*/*S* 13/87). For $C_{32}H_{45}N_2O_8SiP$ (644.27) calcd: 59.60% C, 7.04% H, 4.35% N; found: 59.97% C, 7.08% H, 4.02% N. MS (FAB): 645.3 (M+H)$^+$. ^{1}H NMR—see Tables 4 and 5.

4.1.5. Dimethyl-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12e). Phosphonate **12e** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-*tert*-butyldimethylsilyl-2′-deoxythymidine[28] (356 mg, 1 mmol) according to Method A. Yield, 330 mg (71%; white solid) of **12e** (*R*/*S* 20/80). Crystallization of **12e** from ethanol–ethyl acetate afforded 55 mg of single epimer (*R*)-**12e** (white crystals), mp 235 °C. For $C_{18}H_{33}N_2NaO_8PSi$ (M+Na)$^+$ calcd: 487.1642, found: 487.1651. ^{1}H NMR—see Tables 4 and 5. ^{13}C NMR—see Table 10.

4.1.6. Dimethyl-3′-*O*-benzoyl-2′-deoxythymidin-5′-*C*-ylphosphonate ((*S*)-12f). Phosphonate **12f** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-benzoyl-2′-deoxythymidine[29] (346 mg, 1 mmol) according to Method B. Yield, 236 mg (52%; white solid) of **12f** (*R*/*S* 42/58). Crystallization of **12f** from chloroform afforded 100 mg of single epimer (*S*)-**12f** (white crystals), mp 224 °C. For $C_{19}H_{24}N_2O_9P$ (M+H)$^+$ calcd: 455.1219, found: 455.1235. ^{1}H NMR—see Tables 4 and 5. ^{13}C NMR—see Table 10.

4.1.7. Diethyl-3′-*O*-benzoyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12g). Phosphonate **12g** was obtained from diethyl phosphite (0.26 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-benzoyl-2′-deoxythymidine[29] (346 mg, 1 mmol) according to Method B. Yield, 280 mg (58%; white foam) of **12g** (*R*/*S* 50/50). For $C_{21}H_{27}N_2O_9P$ (482.44)

Table 5. ^{1}H coupling constants in compounds **12–16** in DMSO-d_6

Compound	1′,2′	1′,2″	2′,2″	2′,3′	2″,3′	3′,4′	4′,5′	5′,OH	4′,P	5′,P	P,OH
(*S*)-**12a**[a]	8.8	5.4	12.9	5.1	1.0	1.5	2.2	n	4.2	12.7	n
(*R*)-**12a**[a]	9.5	5.1	12.9	4.4	1.0	1.0	3.4	n	4.9	11.0	n
(*S*)-**12b**[a,b]	9.1	5.3	13.1	5.1	1.0	1.7	2.0	6.9	1.5	12.6	9.0
(*R*)-**12b**[a,b]	9.7	5.1	13.2	4.4	1.0	1.0	4.8	6.8	4.8	9.8	5.1
(*S*)-**12c**[a,b]	9.0	5.4	13.2	5.1	1.0	1.5	1.7	7.1	0.5	12.7	9.0
(*R*)-**12c**[a,b]	9.8	5.1	12.9	5.9	1.0	1.0	4.6	6.8	4.6	10.5	5.1
(*S*)-**12d**[a,c]	9.0	5.4	13.2	5.4	1.0	1.0	1.5	7.1	0.5	13.4	9.8
(*R*)-**12d**[a,c]	10.0	5.1	13.2	4.4	1.0	1.0	4.6	6.8	4.6	10.3	5.5
(*S*)-**12e**[a,b]	8.3	5.9	13.2	5.4	2.4	2.4	2.4	7.0	2.7	12.3	8.8
(*R*)-**12e**[a,b]	9.8	5.1	12.9	4.6	1.0	1.0	4.4	6.8	3.9	10.0	5.0
(*S*)-**12f**[a,b]	8.9	5.8	14.0	6.1	1.5	1.6	2.2	6.9	2.5	12.1	8.8
(*R*)-**12f**[a,b]	8.9	6.0	14.4	6.2	1.9	2.2	3.8	6.7	4.7	10.4	6.7
(*S*)-**12g**[a,d]	9.0	5.9	n	6.1	1.5	1.5	2.4	7.1	0.5	12.2	9.0
(*R*)-**12g**[a,d]	9.0	5.9	n	6.1	2.0	2.0	3.4	6.8	4.0	10.5	7.1
(*S*)-**12h**[a,d]	7.8	7.1	n	5.0	1.4	2.0	2.0	7.1	n	n	7.8
(*R*)-**12h**[a,d]	9.3	5.6	13.2	6.1	1.2	1.2	3.4	6.8	n	10.5	7.1
(*S*)-**12i**[a,b]	9.6	5.4	13.2	5.7	0.5	0.5	0.5	7.0	0.5	13.5	10.6
(*R*)-**12i**[a,b]	10.1	5.0	13.7	5.4	1.2	n	6.0	7.0	n	9.2	7.0
(*S*)-**13**[b,e]	8.3	5.4	13.2	5.1	1.0	1.0	1.7	7.1	1.7	12.7	9.0
(*R*)-**13**[b,e]	8.0	5.4	13.4	4.5	1.0	1.0	4.9	6.8	4.6	9.5	6.5
(*S*)-**14a**[b]	8.6	6.0	13.3	5.0	1.8	1.4	2.3	7.2	2.3	12.4	7.2
(*R*)-**14a**[b]	10.0	5.5	13.4	4.6	<1	<1	6.4	7.2	5.4	8.8	7.2
(*S*)-**14b**[b]	7.3	7.3	n	3.4	3.4	2.0	2.0	7.0	1.0	8.5	8.7
(*R*)-**14b**[b]	8.0	5.4	13.2	5.0	1.0	1.0	6.5	6.5	5.0	8.5	6.5
(*S*)-**15a**[b]	8.6	5.6	13.6	4.9	1.0	1.0	5.0	7.3	5.5	9.5	8.0
(*R*)-**15a**[b]	8.9	5.0	13.2	5.6	1.0	1.0	5.6	6.6	5.9	9.5	7.5
(*S*)-**15b**[b]	8.8	6.2	n	4.9	0.5	1.0	1.5	8.3	1.0	6.6	8.3
(*R*)-**15b**[b]	9.8	5.4	12.9	5.0	1.0	1.0	7.3	8.6	7.3	7.6	7.1
(*S*)-**16**[b]	0.5	3.9	12.9	1.8	2.7	2.4	9.1	6.0	6.8	6.0	6.0
(*R*)-**16**[b]	2.6	2.6	n	2.3	2.3	2.4	6.2	7.6	5.5	10.6	7.6

n=Unresolved multiplet, *J* could not be determined.
[a] $J(6,CH_3)$=1.0–1.3 Hz.
[b] $J(P,OCH_3)$=10.0–10.5 Hz.
[c] $J(CH,CH_3)$=6.1–6.3 Hz.
[d] $J(CH_2,CH_3)$=7.1.
[e] $J(5,6)$=7.6 Hz.

calcd: 52.23% C, 5.60% H, 5.80% N; found: 51.83% C, 5.96% H, 5.69% N. MS (FAB): 483.4 $(M+H)^+$. ^{1}H NMR—see Tables 4 and 5.

4.1.8. Diethyl-3′-*O*-trimethylacetyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12h). Phosphonate **12h** was obtained from diethyl phosphite (0.26 ml, 2 mmol) and the aldehyde prepared from 2′-deoxy-3′-*O*-trimethylacetylthymidine[30] (326 mg, 1 mmol) according to Method B. Yield, 250 mg (54%; white solid) of **12h** (*R*/*S* 33/67). For $C_{19}H_{31}N_2O_9P$ (462.43) calcd: 49.30% C, 6.70% H, 6.05% N; found: 48.92% C, 6.81% H, 5.85% N. MS (FAB): 463.4 $(M+H)^+$. ^{1}H NMR—see Tables 4 and 5.

4.1.9. Dimethyl-3′-*O*-dimethoxytrityl-2′-deoxythymidin-5′-*C*-ylphosphonate (12i). Phosphonate **12i** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 2′-deoxy-3′-*O*-dimethoxytritylthymidine[31] (545 mg, 1 mmol) according to Method B. Yield, 391 mg (60%; yellowish foam) of **12i** (*R*/*S* 19/81). For $C_{33}H_{37}N_2NaO_{10}P$ $(M+Na)^+$ calcd: 675.2084, found: 675.2078. ^{1}H NMR—see Tables 4 and 5.

4.1.10. Dimethyl-4-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxycytidin-5′-*C*-ylphosphonate (13). Phosphonate **13** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 4-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxycytidine[32] (569 mg, 1 mmol) according to Method A. Yield, 569 mg (84%; white foam) of **13** (*R*/*S* 15/85). For $C_{34}H_{40}N_3NaO_8PSi$ $(M+Na)^+$ calcd: 700.2220, found: 700.2228. ^{1}H NMR—see Tables 4 and 5.

4.1.11. Dimethyl-2-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyguanosin-5′-*C*-ylphosphonate (14a). Phosphonate **14a** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 2-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyguanosine[32] (610 mg, 1 mmol) according to Method A. Yield, 589 mg (82%; white foam) of **14a** (*R*/*S* 34/66). For $C_{35}H_{41}N_5O_8PSi$ $(M+H)^+$ calcd: 718.2462, found: 718.2461. ^{1}H NMR—see Tables 4 and 5. ^{13}C NMR—see Table 10.

4.1.12. Dimethyl-2-*N*-isobutyryl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyguanosin-5′-*C*-ylphosphonate (14b). Phosphonate **14b** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 2-*N*-isobutyryl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyguanosine[32] (562 mg, 1 mmol) according to Method A. Yield, 509 mg (76%; white foam) of **14b** (*R*/*S* 40/60). For $C_{32}H_{43}N_5O_8PSi$ $(M+H)^+$ calcd: 684.2619, found: 684.2614. ^{1}H NMR—see Tables 4 and 5.

4.1.13. Dimethyl-6-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyadenosin-5′-*C*-ylphosphonate (15a). Phosphonate **15a** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 6-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyadenosine[33] (594 mg, 1 mmol) according to Method A. Yield, 484 mg

(69%; white foam) of **15a** (*R*/*S* 25/75). For $C_{35}H_{41}N_5O_7PSi$ $(M+H)^+$ calcd: 702.2513, found: 702.2517. ^1H NMR—see Tables 4 and 5.

4.1.14. Dimethyl-6-*N*-benzoyl-2′-deoxyadenosin-3′-*O*-dimethoxytrityl-5′-*C*-ylphosphonate (15b). Phosphonate **15b** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 6-*N*-benzoyl-2′-deoxy-3′-*O*-dimethoxytrityladenosine[30] (658 mg, 1 mmol) according to Method B. Yield, 612 mg (80%; yellowish foam) of **15b** (*R*/*S* 22/78). For $C_{40}H_{40}N_5NaO_9P$ $(M+Na)^+$ calcd: 788.2461, found: 788.2460. ^1H NMR—see Tables 4 and 5.

4.1.15. Dimethyl-2,3′-anhydro-2′-deoxythymidin-5′-*C*-ylphosphonate (16). Phosphonate **16** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 2,3′-anhydro-2′-deoxythymidine[34] (673 mg, 3 mmol) according to Method B. Yield, 337 mg (34%; white solid) of **16** (*R*/*S* 37/63). For $C_{12}H_{18}N_2O_7P$ $(M+H)^+$ calcd: 333.0852, found: 333.0844. ^1H NMR—see Tables 4 and 5.

4.1.16. Dimethyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-isopropoxycarbonylthymidin-5′-*C*-ylphosphonate (25). Triphenylphosphine (525 mg, 2 mmol), diethyl-azodicarboxylate (0.52 ml, 2.4 mmol), and diphenylphosphoryl azide (0.47 ml, 2.4 mmol) were sequentially added to a solution of hydroxyphosphonate **12b** (*R*/*S* 18/82) (589 mg, 1 mmol) in THF (12 ml) at 0 °C. After 30 min (TLC in C-1), the reaction was stopped by addition of abs methanol (1 ml) and the solution was evaporated. Chromatography of the residue on silica gel (elution with a linear gradient of 0–10% ethanol in chloroform) afforded compound **25**. Yield, 453 mg (67%; yellowish foam) of **25** (*R*/*S* 43/57). HR FAB: For $C_{32}H_{44}N_2O_{10}PSi$ $(M+H)^+$ calcd: 675.2503, found: 675.2493. $\nu_{max}(CHCl_3)$ 3390 (w, NH), 3136 (vvw, =C–H), 3092 (w, Ph), 3073 (w, Ph), 3052 (w, Ph), 1710 (s, sh, C=O), 1690 (vs, C=O), 1590 (w, Ph), 1489 (w, Ph), 1428 (m, Ph), 1387 (w, CH_3), 1377 (m, CH_3), 1364 (m, *t*-Bu), 1255 (s, P=O), 1041 (s, COPOC), 998 (m, Ph), 703 (m, Si–O) cm^{-1}. ^1H NMR—see Tables 6 and 7. ^{13}C NMR—see Table 10.

4.1.17. Dimethyl-4-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-isopropoxycarbonylcytidin-5′-*C*-ylphosphonate (26). Triphenylphosphine (908 mg, 3.46 mmol), diethyl-azodicarboxylate (0.9 ml, 4.2 mmol), and diphenylphosphoryl azide (0.81 ml, 4.2 mmol) were sequentially added to a solution of hydroxyphosphonate **13** (*R*/*S* 14/86) (1.17 g, 1.73 mmol) in THF (12 ml) at 0 °C. After 30 min (TLC in C-1), the reaction was stopped by addition of abs methanol (1 ml) and the solution was evaporated. Chromatography of the residue on silica gel (elution with a linear gradient of 0–10% ethanol in chloroform) afforded compound **26**. Yield, 453 mg (67%; yellowish foam) of **26** (*R*/*S* 80/20). For $C_{38}H_{45}N_3O_{10}PSi$ $(M-H)^-$ calcd: 762.2612, found: 762.2618. $\nu_{max}(CHCl_3)$ 3405 (w, NH_2), 3073 (m, Ph), 3055 (w, Ph), 2495 (vw, br, P–OH), 1377 (m, CH_3), 1744 (s, C=O, 1698 (s, C=O, 1651 (s, sh), 1642 (s), 1624 (s, sh), 1567 (s), 1487 (vs), 1392 (s), 1363

Table 6. ^1H chemical shifts in compounds **25–41**

Compound	Solvent	H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	Nucleobase
(*S*)-**25**[a–c]	DMSO-d_6	6.35	1.99	2.16	4.44	4.25	4.88	T: 11.35 (NH), 7.95 (H-6), 1.72 (5-Me)
(*R*)-**25**[a–c]	DMSO-d_6	6.38	n	n	4.48	4.03	4.29	T: 11.39 (NH), 7.68 (H-6), 1.76 (5-Me)
(*S*)-**26**[a–d]	DMSO-d_6	6.18	1.67	2.35	4.54	4.50	4.73	C^{Bz}: 11.25 (NH), 8.72 and 7.29 (H-5, H-6)
(*R*)-**26**[a–d]	DMSO-d_6	6.24	2.00	2.30	4.70	n	4.32	C^{Bz}: 11.25 (NH), 8.88 and 7.30 (H-5, H-6)
(*S*)-**27**[a–d]	DMSO-d_6	6.39	2.60	2.35	4.72	4.32	5.05	A^{Bz}: 8.47 (H-2, H-8)
(*S*)-**33**[a,e,f]	DMSO-d_6	6.26	1.97	1.92	4.83	4.36	4.32	T: 11.24 (NH), 8.14 (H-6), 1.70 (5-Me)
(*R*)-**33**[a,e,f]	DMSO-d_6	6.30	1.82	1.53	4.79	4.47	4.49	T: 11.26 (NH), 8.02 (H-6), 1.68 (5-Me)
(*S*)-**35**[a,f,g]	DMSO-d_6	6.27	1.89	1.96	4.74	3.30–3.90		T: 11.25 (NH), 7.85 (H-6), 1.68 (5-Me)
(*R*)-**35**[a,f,g]	DMSO-d_6	6.31	1.50	1.80	4.80	4.30–3.90		T: 11.25 (NH), 7.83 (H-6), 1.65 (5-Me)
(*S*)-**36a**[h]	D_2O	6.27	2.48	2.36	4.71	4.26	3.80	T: 7.72 (H-6), 1.92 (5-Me)
(*R*)-**36b**[i]	D_2O	6.31	2.40	2.33	4.81	4.27	3.99	T: 7.83 (H-6), 1.90 (5-Me)
(*S*)-**38**	D_2O	6.24	2.41		4.56	4.13	3.88	T: 7.78 (H-6), 1.91 (5-Me)
(*R*)-**38**	D_2O	6.28	2.44	2.33	4.56	4.20	4.23	T: 7.68 (H-6), 1.88 (5-Me)
(*S*)-**39**	D_2O	6.20	2.47	2.34	4.49	4.14	3.84	C: 7.98 and 6.06 (H-5, H-6)
(*R*)-**39**	D_2O	6.25	2.44	2.35	4.69	4.27	4.05	C: 8.06 and 6.05 (H-5, H-6)
(*S*)-**40**	D_2O	6.20	2.72	2.49	4.70	4.25	3.89	G: 7.92
(*R*)-**40**	D_2O	6.23	2.75	2.53	4.92	4.36	4.00	G: 8.02
(*S*)-**41**	D_2O	6.43	2.82	2.54	4.73	4.37	3.97	A: 8.32, 8.19 (H-2, H-8)
(*R*)-**41**	D_2O	6.44	2.83	2.56	4.94	4.45	4.05	A: 8.34, 8.21 (H-2, H-8)

n=Chemical shift value was not determined.

[a] 3′-OTBDPS: 7.64 (m, 4H) and 7.55–7.43 (m, 6H) (2×C_6H_5), 1.07 (s, 9H, *t*-Bu).

[b] $P(OMe)_2$: 3.32–3.67 (2×d, 2×3H).

[c] 5′-O–CO–O–*i*Pr: 4.72 (m, 1H, CH), 1.21 and 1.14 (2×s, 2×CH_3).

[d] Bz: 8.02 (m, 2H, *o*-ArH), 7.70 (m, 1H, *p*-ArH), 7.56 (m, 2H, *m*-ArH).

[e] P(OMe): 3.22–3.30 (d, 3H, $J(CH_3,P)\sim10.5$ Hz).

[f] 5′-SO_2CH_3: 2.17–2.54 (s, 3H, CH_3).

[g] BOM: 5.34, 4.59 (2×m, O–CH_2–CH_2–O), 8.02 (m, 2H, *o*-ArH), 7.70 (m, 1H, *p*-ArH) and 7.56 (m, 2H, *m*-ArH) (C_6H_5).

[h] P–O–CH_2–CH_2–O: 4.43 (m, 1H, J(Ha,Hb)=12.2 Hz, J(Ha,Hc)=2.6 Hz, J(Ha,Hd)=11.2 Hz, J(Ha,P)=2.8 Hz, Ha); 4.18 (m, 1H, J(Hb,Ha)=12.2 Hz, J(Hb,Hc)=1.2 Hz, J(Hb,Hd)=2.6 Hz, J(Hb,P)=17.3 Hz, Hb); 3.96 (m, 1H, J(Hc,Ha)=2.6 Hz, J(Hc,Hb)=1.2 Hz, J(Hc,Hd)=12.6 Hz, J(Hc,P)=1.2 Hz, Hc); 3.77 (m, 1H, J(Hd,Ha)=11.2 Hz, J(Hd,Hb)=2.6 Hz, J(Hd,Hc)=12.6 Hz, Hd).

[i] P–O–CH_2–CH_2–O: 4.44 (1H, m, J(Ha,Hb)=2.3 Hz, J(Ha,Hc)=2.4 Hz, J(Ha,Hd)=11.5 Hz, J(Ha,P)=2.4 Hz, Ha); 4.17 (1H, m, J(Hb,Ha)=12.3 Hz, J(Hb,Hc)=0.8 Hz, J(Hb,Hd)=2.5 Hz, J(Hb,P)=17.8 Hz, Hb); 3.98 (1H, m, J(Hc,Ha)=2.4 Hz, J(Hc,Hb)=0.8 Hz, J(Hc,Hd)=12.7 Hz, Hc); 3.80 (1H, m, J(Hd,Ha)=11.5 Hz, J(Hd,Hb)=2.5 Hz, J(Hd,Hc)=12.7 Hz, Hd).

Table 7. Coupling constants in compounds **25**–**41**

Compound	Solvent	1′,2′	1′,2″	2′,2″	2′,3′	2″,3′	3′,4′	4′,5′	4′,P	5′,P
(*S*)-**25**[a–c]	DMSO-d_6	8.3	5.9	13.7	5.1	2.7	2.4	4.4	2.7	11.7
(*R*)-**25**[a–c]	DMSO-d_6	9.0	5.4	n	4.9	1.0	1.0	7.6	7.6	12.2
(*S*)-**26**[b–d]	DMSO-d_6	7.6	5.6	13.7	5.0	1.0	n	2.8	n	12.2
(*R*)-**26**[b–d]	DMSO-d_6	9.0	5.0	13.4	5.0	1.0	n	6.0	n	10.8
(*S*)-**27**[b,c]	DMSO-d_6	7.2	7.2	n	5.0	2.5	3.5	5.6	3.5	11.1
(*S*)-**33**[a,c]	DMSO-d_6	9.3	5.7	13.2	4.8	1.0	1.0	3.4	8.8	10.0
(*R*)-**33**[a,c]	DMSO-d_6	8.8	6.0	12.9	4.8	1.5	2.0	2.0	8.3	11.3
(*S*)-**35**[a]	DMSO-d_6	8.7	5.6	13.4	5.1	2.0	n	n	n	n
(*R*)-**35**[a]	DMSO-d_6	8.8	5.9	13.2	5.0	1.0	n	n	n	n
(*S*)-**36a**[a]	D_2O	6.2	7.9	14.3	2.5	5.9	2.3	8.6	4.5	4.7
(*R*)-**36b**[a]	D_2O	6.3	7.6	14.1	3.2	6.0	2.4	4.1	4.3	5.3
(*S*)-**38**[a]	D_2O	6.6	6.6	n	6.0	6.0	5.1	5.6	3.4	11.7
(*R*)-**38**[a]	D_2O	6.4	6.4	14.2	n	n	n	1.0	4.6	13.9
(*S*)-**39**	D_2O	6.3	5.9	13.9	6.1	6.1	5.8	5.8	4.0	11.2
(*R*)-**39**	D_2O	5.6	6.6	13.8	6.0	6.0	1.0	1.0	5.1	12.7
(*S*)-**40**	D_2O	6.3	6.8	13.7	6.6	5.4	4.6	4.6	2.9	12.0
(*R*)-**40**	D_2O	6.3	6.8	13.7	6.5	5.6	4.2	1.7	1.7	13.4
(*S*)-**41**	D_2O	6.3	6.6	14.4	6.3	3.9	4.7	3.7	3.0	12.7
(*R*)-**41**	D_2O	6.6	6.6	13.8	5.9	3.4	3.0	2.2	2.0	13.7

n=Unresolved multiplet, *J* could not be determined.
[a] $J(6,CH_3)$=1.0–1.3 Hz.
[b] $J(P,OCH_3)$=10.5 Hz.
[c] $J(CH,CH_3)$=6.3 Hz.
[d] $J(5,6)$=7.6 Hz.

(m, *t*-Bu), 1261 (vs), 1185 (s), 1083 (s), 1061 (s), 704 (s, Si–O) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.18. Dimethyl-6-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-isopropoxycarbonyladenosin-5′-*C*-ylphosphonate (27). Derivative **27** was prepared from hydroxyphosphonate **15a** (*R*/*S* 24/76) (774 mg, 1.1 mmol) according to the procedure described for compound **25**. Yield, 340 mg (40%; yellowish foam) of **27** (*R*/*S* 84/16). For $C_{39}H_{45}N_5O_9PSi$ $(M-H)^-$ calcd: 786.2725, found: 786.2723. $\nu_{max}(CHCl_3)$ 3413 (vw, NH_2), 3072 (w, Ph), 3059 (w, Ph), 3030 (w, Ph), 1750 (m, C=O), 1645 (m, NH), 1612 (m, base), 1582 (m, base), 1591 (m, sh, Ph), 1561 (w, sh, Ph), 1489 (w, Ph), 1428 (m, Ph), 1390 (m, sh, *t*-Bu), 1363 (m, *t*-Bu),1258 (vs, P=O), 1063 (s, COPOC), 1041 (s, COPOC), 703 (s, Si–O) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.19. [1-(2-Deoxy-3,5-*O*-isopropyliden-β-D-*threo*-pentofuranosyl)-5-methylpyrimid-2-on-4-yl] iminotriphenylphosphorane (32). Phosphorane **32** was prepared from 3′,5′-*O*-isopropylidene derivative **30**[35] (140 mg, 0.5 mmol) according to the procedure described for compound **25**. Yield, 73 mg (27%; yellowish solid) of the derivative **32**. For $C_{31}H_{33}N_3O_4P$ $(M+H)^+$ calcd: 542.2208, found: 542.2203. $\nu_{max}(CHCl_3)$ 3102 (w, =C–H), 3080 (w, =C–H), 3062 (w, =C–H), 1738 (s, C=O), 1729 (s, C=O), 1708 (m, sh, C=O), 1632 (m, base), 1592 (w, base), 1562 (m, base), 1484 (w, base), 1438 (s, CH_3), 1083 (s), 1120 (vs), 1162 (s), 1175 (s), 1195 (s) cm^{-1}. ^1H NMR, δ_H (500 MHz, $CDCl_3$) 8.20 (1H, q, *J* 1.3 Hz, H-6); 7.65–7.48 (15H, m, 3×Ph), 6.32 (1H, dd, *J* 1.2, 7.6 Hz, H-1′), 4.55 (1H, bdd, *J* 4.5, 2.6, <1 Hz, H-3′), 4.30 (1H, dd, *J* 13.8, 1.3 Hz, H-5′a), 4.26 (1H, dd, *J* 13.8, 2.1 Hz, H-5′b), 4.01 (1H, m, *J* 2.6, 2.1, 1.3 Hz, H-4′), 2.75 (1H, ddd, *J* 15.3, 7.6, 4.5 Hz, H-2″), 2.46 (3H, d, *J* 1.3 Hz, 5-CH_3), 2.36 (1H, bdd, *J* 15.3, 1.2, <1 Hz, H-2′), 1.51 (3H, br s, CH_3), 1.35 (3H, br s, CH_3); δ_C (125.7 MHz, $CDCl_3$+CD_3OD 9/1) 151.84 (C-2), 142.33 (C-4), 132.43 (C-6), 131.12 (d, *J*(C,P) 3.0 Hz, 3×*p*-C, 3×Ph), 131.81 (d, *J*(C,P) 10.3 Hz, 6×*m*-C, 3×Ph), 131.0 (3×*i*-C, 3×Ph), 128.49 (d, *J*(C,P) 12.2 Hz, 6×*o*-C, 3×Ph), 101.91 (C-5), 97.97 (>C<), 86.88 (C-1′), 76.22 (C-4′), 68.19 (C-3′), 60.28 (C-5′), 40.91 (C-2′), 28.72 (CH_3), 18.25 (CH_3), 12.92 (5-CH_3).

4.1.20. Methyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-methansulfonylthymidin-5′-*C*-ylphosphonate (33). Phosphonate **12b** (425 mg, 0.72 mmol) dried by co-distillation with pyridine (2×15 ml) was treated with mesyl chloride (0.28 ml, 3.6 mmol) in pyridine (7 ml) at 0 °C until the starting compound disappeared (TLC in C-1 and H-1). The reaction was quenched by addition of water (1 ml). Chloroform (50 ml) was added, the organic layer was washed by saturated solution of sodium hydrogencarbonate, dried over anhydrous Na_2SO_4 and evaporated. The residue was treated with 60% aqueous pyridine (20 ml) at 50 °C for 16 h (TLC in C1 and H1). After complete demethylation the solution was concentrated in vacuo, the residue was co-distilled with ethanol and treated with Dowex 50×2 (Et_3N^+) in 75% aqueous ethanol to remove *N*-methylpyridinium cations. Product **33** was purified on silica gel column using elution with a linear gradient of H-3 in ethyl acetate and followed by a linear gradient of H-1 in H-3. Yield, 433 mg (79%; yellowish foam) of triethylammonium salt of the derivative **33**. For $C_{28}H_{37}N_2NaO_{10}PSSi$ $(M+Na)^+$ calcd: 675.1574, found: 675.1572. ^1H NMR—see Tables 6 and 7.

4.1.21. (2-Hydroxyethyl)-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-methansulfonylthymidin-5′-*C*-ylphosphonate (35). A mixture of the derivative **33** (433 mg, 0.57 mmol), 2-*O*-benzoyloxyethanol (246 mg, 1.48 mmol), and 4-methoxypyridine-*N*-oxide (MPNO) (1.32 g, 10.53 mmol) dried by co-distillation with toluene and dichloromethane was treated with MSNT (1.01 g, 3.42 mmol) in dichloromethane (7 ml) (TLC in C-1 and H-1). The reaction was quenched by addition of a saturated solution of sodium hydrogencarbonate.

Chloroform (50 ml) was added, organic layer was washed with saturated solution of sodium hydrogencarbonate, dried over anhydrous Na_2SO_4, and evaporated. Mixed diester **34** was heated with 60% aqueous pyridine (20 ml) at 50 °C for 16 h (TLC in C1 and H1). The solution was concentrated in vacuo, and the residue was co-distilled with ethanol and treated with Dowex 50×2 (Et_3N^+) in 75% aqueous ethanol to remove *N*-methylpyridinium cations. Product **35** was purified on silica gel column using elution with a linear gradient of H3 in ethyl acetate and followed by a linear gradient of H1 in H3. Yield, 493 mg (97%; yellowish foam) of **35** (Et_3NH^+ salt). For $C_{36}H_{43}N_2NaO_{12}PSSi$ $(M+Na)^+$ calcd: 809.1941, found: 809.1935. ^1H NMR—see Tables 6 and 7.

4.1.22. (5*S*)-[*P*→2″-*O*-*cyclo*]-2′-Deoxy-5′-*O*-(2″-hydroxyethyl)thymidin-5′-*C*-ylphosphonate (36a) and (5*R*)-[*P*→2″-*O*-*cyclo*]-2′-deoxy-5′-*O*-(2″-hydroxyethyl)thymidin-5′-*C*-ylphosphonate (36b). The solution of monoester **35** (493 mg, 0.55 mmol) in 1 M sodium methoxide was heated at 55 °C for 4 d (checked by RP-HPLC). The reaction mixture was treated with Dowex 50 (Et_3NH^+) and products were purified on RP-HPLC. Yield, 15 mg (8%; white solid) of (*S*)-epimer **36a** and 17 mg (9%; white solid) of (*R*)-epimer **36b**. For $C_{12}H_{17}N_2NaO_8P$ $(M+Na)^+$ calcd: 371.0620, found: 371.0618 for **36a** and 371.0629 for **36b**. ^1H NMR—see Tables 6 and 7. ^{13}C NMR—see Table 10.

4.1.23. 2′-Deoxythymidin-5′-*C*-ylphosphonic acid (38). Phosphonic acid **38** was prepared from phosphonate **12c** (*R*/*S* 19/81) (1.161 g, 1.88 mmol) by consecutive application of Methods C, E and G. Yield, 454 mg (75%) of **38** (Na^+ salt; white lyophilizate) (*R*/*S* 18/82). For $C_{10}H_{14}N_2O_8P$ $(M-H)^-$ calcd: 321.0488, found: 321.0494. ν_{max}(KBr) 3417 (m, vbr, NH_2), 3270 (m, vbr, sh, OH), 3067 (m, =C–H), 2818 (m, br), 1696 (vs, br, C=O), 1479 (m, base), 1448 (w, base), 1410 (w, sh, base), 1388 (w, sh, base), 1372 (w, base), 1278 (m, base), 1077 (s, br, C–OH), 1055 (s, br, C–OH), 962 (m, PO_3^{-2}), 902 (m, br, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.24. 2′-Deoxycytidin-5′-*C*-ylphosphonic acid (39). Phosphonic acid **39** was prepared from phosphonate **13** (*R*/*S* 15/85) (162 mg, 0.24 mmol) by consecutive application of Methods C, D, E and G. Yield, 55 mg (75%) of **39** (Na^+ salt; white lyophilizate) (*R*/*S* 15/85). For $C_9H_{13}N_3O_7P$ $(M-H)^-$ calcd: 306.0491, found: 306.0498. ν_{max}(KBr) 3429 (vs, vbr, NH_2 or OH), 3200 (m, br, sh, NH_2 or OH), 1646 (s, C=O), 1612 (m, sh, base), 1530 (w, base), 1494 (m, base), 1294 (w, C–NH_2), 1249 (w, base), 1086 (m, C–OH), 1048 (m, sh, C–OH), 968 (w, PO_3^{-2}), 897 (m, br, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.25. 2′-Deoxyguanosin-5′-*C*-ylphosphonic acid (40). Phosphonic acid **40** was prepared from phosphonate **14b** (*R*/*S* 24/76) (1.56 g, 2.17 mmol) by consecutive application of Methods C, D, E and G. Yield, 158 mg (21%) of **40** (Na^+ salt; white lyophilizate) (*R*/*S* 29/71). For $C_{10}H_{13}N_5O_7P$ $(M-H)^-$ calcd: 346.0553, found: 346.0553. ν_{max}(KBr) 3417 (s, br, NH_2 or OH), 3120 (m, br, NH_2 or OH), 2765 (m, br, NH_2 or OH), 1694 (vs, C=O), 1607 (m, base), 1580 (w, sh, base), 1535 (w, base), 1483 (w, base), 1410 (w, base), 1179 (w, base), 1074 (m, vbr, C–OH), 968 (w, PO_3^{-2}), 899 (w, br, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.26. 2′-Deoxyadenosin-5′-*C*-ylphosphonic acid (41). Phosphonic acid **41** was prepared from phosphonate **15b** (*R*/*S* 22/78) (234 mg, 0.33 mmol) by consecutive application of Methods C, D and G. Yield, 89 mg (81%) of **41** (Na^+ salt; white lyophilizate) (*R*/*S* 17/83). For $C_{10}H_{13}N_5O_6P$ $(M-H)^-$ calcd: 330.0604, found: 330.0602. ν_{max}(KBr) 3424 (s, vbr, NH_2 or OH), 3262 (v, br, NH_2 or OH), 3108 (s, br, NH_2 or OH), 3155 (m, br, sh NH or OH), 2675 (w, br, sh, NH_2 or OH), 1603 (m, sh, ring), 1581 (w, base), 1507 (w, sh, base), 1478 (m, base), 1429 (w, base), 1334 (w, base), 1306 (w, base), 1193 (C–OH), 1083 (vs, C–OH), 997 (m, PO_3^{-2}), 907 (w, PO_3^{-2}), 796 (w, base), 739 (w, br, base), 641 (w, base) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.27. Diethyl-2′,3′-*O*-isopropylidene-3-*N*-methoxymethyluridine-5′-*C*-ylphosphonate (48). Phosphonate **48** was obtained from diethyl phosphite (0.258 ml, 2 mmol) and the aldehyde prepared from 2′,3′-*O*-isopropyliden-3-*N*-methoxymethyluridine[36] (328 mg, 1 mmol) according to Method B. Yield, 144 mg (31%; yellowish foam) of **48** (*R*/*S* 30/70). For $C_{18}H_{29}N_2O_{10}P$ (464.40) calcd: 46.55% C, 6.29% H, 6.03% N; found: 46.31% C, 6.35% H, 5.90% N. MS (FAB): 465.1 $(M+H)^+$. ^1H NMR—see Tables 8 and 9.

4.1.28. Diethyl-4-*N*-benzoyl-2′,3′-*O*-isopropylidenecytidine-5′-*C*-ylphosphonate (50). Phosphonate **50** was obtained from diethyl phosphite (0.258 ml, 2 mmol) and the aldehyde prepared from 4-*N*-benzoyl-2′,3′-*O*-isopropylidenecytidine[37] (390 mg, 1 mmol) according to Method B. Yield, 298 mg (57%; yellowish foam) of **50** (*R*/*S* 43/57). For $C_{23}H_{30}N_3O_9P$ (523.48) calcd: 52.77% C, 5.78% H, 8.03% N; found: 52.44% C, 5.72% H, 7.89% N. MS (FAB): 524.1 $(M+H)^+$. ^1H NMR—see Tables 8 and 9.

4.1.29. Dimethyl-2-*N*-benzoyl-2′,3′-*O*-isopropylideneguanosin-5′-*C*-ylphosphonate (51). Phosphonate **51** was obtained from dimethyl phosphite (0.183 ml, 2 mmol) and the aldehyde prepared from 2-*N*-benzoyl-2′,3′-*O*-isopropylideneguanosine[38] (427 mg, 1 mmol) according to Method B. Yield, 353 mg (66%; white foam) of **51** (*R*/*S* 40/60). For $C_{22}H_{27}N_5O_9P$ $(M+H)^+$ calcd: 536.1546, found: 536.1554. ^1H NMR—see Tables 8 and 9.

4.1.30. Diethyl-6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosin-5′-*C*-ylphosphonate (52). Phosphonate **52** was obtained from diethyl phosphite (0.258 ml, 2 mmol) and the aldehyde prepared from 6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosine[39] (411 mg, 1 mmol) according to Method B. Yield, 175 mg (32%; white foam) of **52** (*R*/*S* 33/67). For $C_{24}H_{30}N_5O_8P$ (547.50) calcd: 52.65% C, 5.52% H, 12.79% N; found: 52.34% C, 5.57% H, 12.86% N. MS (FAB): 548.5 $(M+H)^+$. ^1H NMR—see Tables 8 and 9.

4.1.31. Dimethyl-6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosin-5′-*C*-ylphosphonate (53). Phosphonate **53** was obtained from dimethyl phosphite (0.183 ml, 2 mmol) and aldehyde prepared from 6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosine[39] (411 mg, 1 mmol) according to Method B. Yield 218 mg (42%; white foam) of **53** (*R*/*S* 17/83). For $C_{22}H_{27}N_5O_8P$ $(M+H)^+$ calcd: 520.0597, found: 520.0594. ^1H NMR—see Tables 8 and 9.

4.1.32. 3-*N*-Methoxymethyluridin-5′-*C*-ylphosphonic acid (54). Phosphonic acid **54** was prepared from phosphonate

Table 8. ^{1}H chemical shifts in compounds **48–62**

Compound	Solvent	H-1′	H-2′	H-3′	H-4′	H-5′	5′-OH	Nucleobase
(*S*)-**48**[a–c]	DMSO-d_6	5.87	4.97	4.99	4.22	4.07	6.23	U^{MeM}: 7.84 (H-6), 5.78 (H-5)
(*R*)-**48**[a–c]	DMSO-d_6	5.92	4.84	4.87	4.33	4.15	6.39	U^{MeM}: 8.07 (H-6), 5.79 (H-5)
(*S*)-**50**[b–d]	DMSO-d_6	5.88	4.97	5.02	4.33	4.21	6.20	C^{Bz}: 11.32 (NH), 8.28 (H-6), 7.35 (H-5)
(*R*)-**50**[b–d]	DMSO-d_6	5.91	4.85	4.89	4.43	4.18	6.22	C^{Bz}: 11.30 (NH), 8.59 (H-6), 7.33 (H-5)
(*S*)-**51**[b,d,e]	DMSO-d_6	6.13	5.39	5.27	4.30	3.98	6.30	G^{Bz}: 11.32 (NH), 8.24 (H-8)
(*R*)-**51**[b,d,e]	DMSO-d_6	6.16	5.23	5.16	4.40	4.26	6.29	G^{Bz}: 11.33 (NH), 8.36 (H-8)
(*S*)-**52**[b–d]	DMSO-d_6	6.29	5.46	5.23	4.41	4.03	n	A^{Bz}: 11.23 (NH), 8.77, 8.65 (H-2, H-8)
(*R*)-**52**[b–d]	DMSO-d_6	6.31	5.29	5.08	4.53	4.22	n	A^{Bz}: 11.22 (NH), 8.82, 8.76 (H-2, H-6)
(*S*)-**53**[b,d,e]	DMSO-d_6	6.29	5.46	5.23	4.41	4.03	n	A^{Bz}: 11.23 (NH), 8.77, 8.65 (H-2, H-8)
(*R*)-**53**[b,d,e]	DMSO-d_6	6.31	5.29	5.08	4.43	4.22	n	A^{Bz}: 11.23 (NH), 8.81, 8.75 (H-2, H-8)
(*S*)-**54**[a]	D_2O	5.98	4.41	4.50	4.47	4.14	—	U^{MeM}: 8.13 (H-6), 6.00 (H-5)
(*R*)-**54**[a]	D_2O	5.92	4.40	4.33	4.35	3.99	—	U^{MeM}: 8.11 (H-6), 5.98 (H-5)
(*S*)-**55**	D_2O	5.91	4.39	4.45	4.45	4.10	—	U: 8.15 (H-6)
(*R*)-**55**	D_2O	5.87	4.39	4.24	4.29	3.87	—	U: 7.98 (H-6)
(*S*)-**56**	D_2O	5.89	4.32	4.39	4.47	4.12	—	C: 8.16 (H-6), 6.04 (H-5)
(*R*)-**56**	D_2O	5.84	4.34	4.15	4.26	3.83	—	C: 7.89 (H-6), 6.04 (H-5)
(*S*)-**57**	D_2O	5.87	4.73	4.71	4.53	4.04	—	G: 8.02 (H-8)
(*R*)-**57**	D_2O	5.83	4.68	5.15	5.13	3.94	—	G: 7.91 (H-8)
(*S*)-**58**	D_2O	6.06	4.75	4.71	4.59	4.06	—	A: 8.42, 8.21 (H-2, H-8)
(*R*)-**58**	D_2O	6.08	4.74	4.46	4.41	3.90	—	A: 8.32, 8.19 (H-2, H-8)
(*S*)-**60**[b,c,f]	DMSO-d_6	5.90	4.90	4.96	4.43	3.99	5.85	—
(*R*)-**60**[b,c,f]	DMSO-d_6	5.91	4.88	5.20	4.53	4.16	6.10	—
(*R*)-**62(β)**[c,f,g]	DMSO-d_6	6.09	5.37	5.73	4.72	5.57	n	—
(*R*)-**62(α)**[c,f,g]	DMSO-d_6	6.39	5.32	5.82	4.74	5.52	n	—

n=Not detected.
[a] MeM: 5.16–5.37 (s or 2×d, 2H, N–CH_2), 3.26–3.44 (s, 3H, OCH_3).
[b] –O–C(CH_3)$_2$–O–: 1.29–1.58 (2×s, 2×3H).
[c] P(OEt)$_2$: 3.95–4.10 (2×m, 2×2H, 2×CH_2), 1.04–1.25 (2×t, 2×3H, 2×CH_3).
[d] NBz: 7.87–8.05 (m, 2H), 7.52–7.70 (m, 1H), 7.44–7.56 (m, 2H).
[e] P(OMe)$_2$: 3.60–3.62 and 3.51–3.52 (2×d, 2×3H).
[f] 3′-OBz: 8.02 (m, 2H), 7.70 (m, 1H), 7.56 (m, 2H).
[g] 1′,2′,3′-triOAc: 1.85–2.17 (3×s, 3×3H).

48 (*R*/*S* 30/70) (600 mg, 1.29 mmol) by consecutive application of Methods C, F and G. Yield, 461 mg (97%) of **54** (Na^+ salt; white lyophilizate) (*R*/*S* 45/55). For $C_{11}H_{16}N_2O_{10}P$ $(M-H)^-$ calcd: 367.0543, found: 367.0541. ν_{max}(KBr) 3417 (s, NH_2 or OH), 3272 (s, vbr, sh, NH_2 or OH), 3108 (m, NH_2 or OH), 2970 (m, CH_3), 2836 (m, CH_3), 1714 (s, C=O), 1660 (vs, br, C=O), 1629 (s, sh, base), 1463 (s, base), 1414 (m, base), 1115 (s, COC), 1083 (s, C–OH), 1056 (s, sh, C–OH), 972 PO_3^{-2}), 912 (m, PO_3^{-2}) cm^{-1}. ^{1}H NMR—see Tables 8 and 9.

Table 9. Coupling constants in compounds **48–62**

Compound	Solvent	1′,2′	2′,3′	3′,4′	4′,5′	4′,P	5′,P	5′,OH	P,OH
(*S*)-**48**[a–c]	DMSO-d_6	2.2	6.6	2.2	5.6	5.1	8.8	6.8	6.6
(*R*)-**48**[a–c]	DMSO-d_6	2.7	6.4	2.9	3.9	0.5	11.5	7.0	8.8
(*S*)-**50**[a–c]	DMSO-d_6	2.2	6.4	2.4	5.1	4.9	8.8	6.4	6.4
(*R*)-**50**[a–c]	DMSO-d_6	2.2	6.1	2.9	3.4	2.7	10.8	8.0	8.0
(*S*)-**51**[d]	DMSO-d_6	2.7	6.2	2.2	5.7	4.5	8.4	6.7	5.7
(*R*)-**51**[d]	DMSO-d_6	2.7	6.2	2.2	3.8	3.4	11.1	6.9	8.9
(*S*)-**52**[b]	DMSO-d_6	2.9	6.1	2.2	4.9	4.4	9.8	6.8	7.1
(*R*)-**52**[b]	DMSO-d_6	2.9	6.1	2.2	3.2	2.8	12.0	7.3	6.5
(*S*)-**53**[d]	DMSO-d_6	2.9	6.1	2.0	5.0	4.2	9.3	6.6	7.3
(*R*)-**53**[d]	DMSO-d_6	2.9	6.1	2.2	3.4	3.1	12.0	6.3	7.3
(*S*)-**54**[a]	D_2O	4.6	4.9	4.9	1.7	1.7	13.2	—	—
(*R*)-**54**[a]	D_2O	3.4	5.1	6.1	3.4	6.3	12.9	—	—
(*S*)-**55**[a]	D_2O	3.5	n	n	1.5	n	13.0	—	—
(*R*)-**55**[a]	D_2O	3.3	5.3	6.6	5.4	2.9	11.9	—	—
(*S*)-**56**[a]	D_2O	2.7	5.1	7.1	1.7	1.7	12.9	—	—
(*R*)-**56**[a]	D_2O	2.4	5.4	7.1	7.1	2.9	11.2	—	—
(*S*)-**57**	D_2O	5.0	5.5	3.4	2.0	1.2	13.9	—	—
(*R*)-**57**	D_2O	5.5	5.3	4.3	3.6	2.6	12.7	—	—
(*S*)-**58**	D_2O	4.6	5.1	4.6	1.5	1.5	13.9	—	—
(*R*)-**58**	D_2O	4.6	4.9	4.9	5.0	2.4	12.0	—	—
(*S*)-**60**[b]	DMSO-d_6	3.8	5.1	8.5	2.7	2.7	13.0	8.0	8.4
(*R*)-**60**[b]	DMSO-d_6	3.9	5.6	7.6	2.4	3.9	13.0	6.6	12.8
(*R*)-**62(β)**[b]	DMSO-d_6	1.0	5.0	6.6	3.4	2.6	13.1	—	—
(*R*)-**62(α)**[b]	DMSO-d_6	4.2	6.5	2.7	2.6	2.6	12.6	—	—

[a] J(5,6)=7.3–8.3 Hz.
[b] J(CH_2,CH_3)=7.1 Hz.
[c] J(P,OCH_2)=7.3 Hz.
[d] J(P,OCH_3)=10.5 Hz.

4.1.33. Uridin-5′-*C*-ylphosphonic acid (55). Diester **49** was obtained from diethyl phosphite (0.516 ml, 4 mmol) and the aldehyde prepared from 2′,3′-*O*-isopropylideneuridine[40] (568 mg, 2 mmol) according to Method B. This derivative was transformed into free phosphonic acid **55** by consecutive application of Methods C, F and G. Yield, 298 mg (46%) of **55** (Na^+ salt; white lyophilizate) (*R*/*S* 45/55). For $C_9H_{12}N_2O_9P$ $(M-H)^-$ calcd: 323.0280, found: 323.0290. ν_{max}(KBr) 3418 (s, vbr, NH_2 or OH), 3248 (s, vbr, sh, NH_2 or OH), 2806 (m, vbr, NH_2 or OH), 1782 (m, sh, base), 1699 (vs, vbr, C═O), 1628 (s, sh, base), 1470 (m, base), 1441 (m, base), 1398 (m, base), 1274 (m, base), 1105 (s, br, OH), 1058 (s, br, C–OH), 969 (m, PO_3^{-2}), 921 (w, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 8 and 9. ^{13}C NMR—see Table 10.

4.1.34. Cytidin-5′-*C*-ylphosphonic acid (56). Phosphonic acid **56** was prepared from phosphonate **50** (*R*/*S* 43/57) (342 mg, 0.65 mmol) by consecutive application of Methods C, D, F and G. Yield, 105 mg (50%) of **56** (Na^+ salt; white lyophilizate) (*R*/*S* 44/56). For $C_9H_{13}N_3O_8P$ $(M-H)^-$ calcd: 322.0440, found: 322.0435. ν_{max}(KBr) 3427 (vs, br, NH_2 or OH), 3206 (m, br, sh, NH_2 or OH), 1652 (s, C═O), 1610 (m, base), 1521 (w, base), 1499 (m, base), 1287 (w, C–NH_2), 1253 (w, base), 1211 (w, base), 1185 (w, br, base), 1120 (m, base), 1089 (m, C–OH), 1043 (m, C–OH), 971 (w, PO_3^{-2}), 899 (w, br, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 8 and 9.

4.1.35. Guanosin-5′-*C*-ylphosphonic acid (57). Phosphonic acid **57** was prepared from phosphonate **51** (*R*/*S* 40/60) (700 mg, 1.3 mmol) by consecutive application of Methods C, D, F and G. Yield 378 mg (80%) of **57** (Na^+ salt; white lyophilizate) (*R*/*S* 44/56). For $C_{10}H_{13}N_5O_8P$ $(M-H)^-$ calcd: 362.0503, found: 362.0504. ν_{max}(KBr) 3417 (m, vbr, NH_2 or OH), 3119 (m, vbr, NH_2 or OH), 2766 (w, br, NH_2 or OH)), 1698 (vs, C═O), 1550 (m, base), 1582 (w, sh, base), 1536 (w, base), 1485 (w, base), 1412 (w, base), 1102 (w, base), 1180 (w, base), 1070 (m, vbr, C–OH), 972 (w, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 8 and 9.

4.1.36. Adenosin-5′-*C*-ylphosphonic acid (58). Phosphonic acid **58** was prepared from phosphonate **52** (*R*/*S* 33/67) (175 mg, 0.32 mmol) by consecutive application of Methods C, D, F and G. Yield, 85 mg (77%) of **58** (Na^+ salt; white lyophilizate) (*R*/*S* 40/60). For $C_{10}H_{13}N_5O_7P$ $(M-H)^-$ calcd: 346.0553, found: 346.0553. ν_{max}(KBr) 3419 (vs, br, NH_2 or OH), 3265 (s, br, sh, NH_2 or OH), 3217 (s, br, NH_2 or OH), 3120 (m, br, sh, NH_2 or OH), 2675 (w, br, sh, NH_2 or OH), 1606 (m, sh, base), 1579 (w, base), 1505 (w, sh, base), 1476 (m, base), 1419 (w, base), 1334 (w, base),

Table 10. ^{13}C NMR data of compounds **12e**, **12f**, **14a**, **25**, **36** and **55**

Compound	Solvent	C-1′	C-2′	C-3′ *J*(C,P)	C-4′ *J*(C,P)	C-5′ *J*(C,P)	Nucleobase
(*S*)-**12b**[a]	DMSO-d_6	85.03	~39.7	75.51 d (15.1)	86.86	66.68 (164.3)	T: 163.86 (C-4); 150.59 (C-2); 136.34 (C-6); 109.29 (C-5); 12.41 (5-CH_3)
(*S*)-**12e**[b]	DMSO-d_6	84.80	39.54	73.49 d (14.6)	86.72 (~0)	66.69 d (165.0)	T: 163.99 (C-4); 150.66 (C-2); 136.57 (C-6); 109.28 (C-5); 12.51 (5-CH_3)
(*R*)-**12e**[c]	DMSO-d_6	84.03	39.54	73.07 (4.9)	86.89 (10.8)	63.72 (159.2)	T: 163.86 (C-4); 150.75 (C-2); 136.09 (C-6); 109.86 (C-5); 12.32 (5-CH_3)
(*S*)-**12f**[d]	DMSO-d_6	84.82	37.34	76.86 d (14.6)	83.97 (~0)	67.12 d (165.0)	T: 163.89 (C-4); 150.62 (C-2); 136.35 (C-6); 109.56 (C-5); 12.48 (5-CH_3)
(*R*)-**12f**[e]	DMSO-d_6	84.60	38.03	73.96	83.66 (10.2)	68.13 (160.6)	T: 164.13 (C-4); 150.62 (C-2); 135.96 (C-6); 111.36 (C-5); 12.17 (5-CH_3)
(*S*)-**14a**[f]	DMSO-d_6	83.98	41.18	75.74 (14.6)	87.44 (~0)	66.74 (163.1)	G^{Bz}: 169.32, 132.53, 128.71(2), 128.73(2), 133.24 (NBz); 155.27 (C-6), 148.55 (C-4), 139.39 (C-2), 137.86 (C-8), 120.57 (C-5)
(*R*)-**14a**[g]	DMSO-d_6	83.27	38.61	74.97 (8.8)	87.54 (6.8)	67.11 (160.2)	G^{Bz}: 169.35, 132.53, 128.71(2), 128.76(2), 133.35 (NBz); 155.28 (C-6), 149.06 (C-4), 139.40 (C-2), 138.22 (C-8), 120.84 (C-5)
(*S*)-**25**[h]	DMSO-d_6	84.28*	38.75	73.60 d (9.2)	84.25*	70.17 d (163.5)	T: 163.68 (C-4), 150.56 (C-2), 135.44 (C-6), 109.82 (C-5), 12.23 (5-CH_3)
(*S*)-**36a**[i]	D_2O	89.14	41.54	74.38	88.38 d (5.9)	80.38 d (140.1)	T: 169.49 (C-4), 154.50 (C-2), 140.53 (C-6), 113.92 (C-5), 14.52 (5-CH_3)
(*R*)-**36b**[j]	D_2O	87.91	41.54	73.63 d (3.4)	88.11 d (6.8)	80.44 d (141.1)	T: 169.51 (C-4), 154.65 (C-2), 140.60 (C-6), 114.01 (C-5), 14.56 (5-CH_3)
(*S*)-**55**	D_2O	88.71	74.75	67.42	84.94 d (12.7)	69.48 d (142.6)	U: 166.36 (C-4), 151.69 (C-2), 141.87 (C-6), 101.92 (C-5)
(*R*)-**55**	D_2O	90.02	74.28	70.14	84.10 d (3.9)	69.71 d (148.4)	U: 166.41 (C-4), 151.61 (C-2), 142.23 (C-6), 101.83 (C-5)

[a] $P(OCH_3)_2$: 52.99 and 52.53 (2×d, *J*(C,P)=6.8 Hz); TBDPS: 135.44(4), 132.90, 132.78, 130.38, 130.35, 128.28(2) and 128.25(2) (2×C_6H_5), 26.87(3) and 18.76 (*t*-Bu).
[b] $P(OCH_3)_2$: 53.17 and 52.63 (2×d, *J*(C,P)=6.8 Hz); TBDMS: 25.86(3) and 17.83 (*t*-Bu), 4.65 and 4.52 (2×Me).
[c] $P(OCH_3)_2$: 53.32 and 52.62 (2×d, *J*(C,P)=6.8 Hz); TBDMS: 25.78(3) and 17.70 (*t*-Bu), 4.69 and 4.52 (2×Me).
[d] OBz: 165.48 (CO); 133.86(1), 129.60(2), 129.42 (1) and 129.00(2) (C_6H_5); 53.19 and 52.66 (2×d, *J*(C,P)=6.8 Hz, $P(OCH_3)_2$).
[e] $P(OCH_3)_2$: 53.87 and 53.24 (2×d, *J*(C,P)=7.3 Hz); OBz: 165.58 (CO); 133.44(1), 129.61(2), 129.24 (1) and 128.42(2) (C_6H_5).
[f] $P(OCH_3)_2$: 53.03 and 52.41 (2×d, *J*(C,P)=5.9 Hz); TBDPS: 135.51(2), 135.48(2), 132.90, 132.75, 130.46, 130.42, 128.34(2) and 128.32(2) (2×C_6H_5), 26.94(3) and 18.81 (*t*-Bu).
[g] $P(OCH_3)_2$: 52.88 and 52.34 (2×d, *J*(C,P)=6.8 Hz); TBDPS: 135.64(2), 135.56(2), 132.79, 132.25, 130.31, 130.21, 128.22(2) and 128.12(2) (2×C_6H_5), 26.96(3) and 18.90 (*t*-Bu).
[h] O–CO–O*i*Pr: 153.08 (d, *J*(C,P)=4.6 Hz, O–CO–O); 73.35, 21.40 and 21.25 (*i*Pr); TBDPS: 135.44(4), 132.60, 132.42, 130.38, 130.36, 128.20(2) and 128.18(2) (2×C_6H_5); 26.79(3) and 18.76 (*t*-Bu); $P(OCH_3)_2$: 53.58 (d, *J*(C,P)=6.4) and 53.24 (d, *J*(C,P)=6.0 Hz).
[i] P–O–CH_2–CH_2–O: 71.81 (d, *J*(C,P)=6.8 Hz); 70.15 (d, *J*(C,P)=3.4 Hz).
[j] P–O–CH_2–CH_2–O: 71.80 (d, *J*(C,P)=6.8 Hz); 70.51 (d, *J*(C,P)=2.9 Hz).
* The assignment of signals may be interchanged.

1297 (w, base) 1102 (vs, C–OH), 1069 (vs, C–OH), 989 (m, PO_3^{-2}), 905 (PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 8 and 9.

4.1.37. (*R*)-Adenosin-5′-*C*-ylphosphonic acid ((*R*)-58). 6-*N*-Benzoyladenine (1.6 g, 6.7 mmol) in hexamethyldisilazane (67 ml, 315 mmol) was refluxed in the presence of trimethylsilyl chloride (6.7 ml, 53.6 mmol) at 150 °C under stirring and exclusion of moisture for 10 h. Volatiles were removed under reduced pressure and the resulting oil was co-distilled with xylene (2×50 ml) and acetonitrile (2×50 ml) to remove traces of HMDS. A solution of diethyl phosphonate (*R*)-**62** (2.871 g, 5.56 mmol) (dried by co-distillation with acetonitrile) in acetonitrile (2×20 ml) was added under argon to the silylated base and then tin tetrachloride (11.12 mmol, 1.31 ml) was added. The mixture was kept at rt for 24 h (TLC in C1 and H1). The reaction mixture was then diluted with pyridine (2 ml) and toluene (40 ml) and stirred overnight. The thick suspension (precipitated complex of tin tetrachloride with pyridine) was filtered through Celite, washed with chloroform, and the solvents were removed under diminished pressure. All protecting groups of the product (*R*)-**64** were removed by consecutive application of Methods C, D and G. Yield, 669 mg (35%) of (*R*)-**58** (Na^+ salt; white lyophilizate) (*R*/*S* 100/0). For $C_{10}H_{13}N_5O_7P$ $(M+H)^+$ calcd: 346.0553, found: 346.0550. ^1H NMR—see Tables 8 and 9.

4.1.38. Adenosin-5′-*C*-ylphosphonyl morpholide ((*R*)-59). The sodium salt of adenosine 5′-*C*-phosphonate (*R*)-**58** (10 mg, 0.029 mmol) was converted to pyridinium salt on Dowex 50 (5 ml, Py). Morpholine (0.012 ml, 0.13 mmol) was added to the pyridinium salt, the mixture was evaporated, the residue was co-distilled with ethanol (5×15 ml), dissolved in a mixture of *tert*-butanole–water (2 ml, 1/1) and then morpholine (0.023 ml, 0.26 mmol) and DCC (40 mg, 0.19 mmol) were added to the solution. The reaction mixture was kept under reflux for 15 h. Then volatiles were removed under reduced pressure, the residue was suspended in water (10 ml), filtered through Celite, and the filtrate was extracted with ether (2×10 ml). The aqueous layer was concentrated and the residue was co-distilled with ethanol (2×20 ml) and suspended in 1/1 ethanol–ether mixture. Morpholide (*R*)-**59** was filtered and dried in vacuo. Yield, 22 mg; mixture of (*R*)-**59** and *N*-morpholino-dicyclohexylcarboxamidine (47%, apparent molecular weight calculated from UV spectrum (ε=14,000) was 1522). For $C_{14}H_{20}N_6O_7P$ $(M-H)^-$ calcd: 415.1216, found: 415.1210. δ_H (500 MHz, DMSO-d_6) 8.35 (1H, s, H-2), 8.13 (1H, s, H-8), 7.28 (2H, br s, NH_2), 5.84 (1H, d, *J* 3.2 Hz, H-1′), 5.16 (1H, dd, *J* 5.2, 3.2 Hz, H-2′), 4.17 (1H, dd, *J* 6.3, 5.2 Hz, H-3′), 4.03 (1H, ddd, *J* 6.8, 6.3, 3.4 Hz, H-4′), 3.56 (1H, dd, *J* 9.8, 6.8 Hz, H-5′), 3.69 (2H, m), 3.42 (2H, m), 2.97 (2H, m), 2.93 (2H, m).

4.1.39. (5*R*)-Diethyl-(3-*O*-benzoyl-1,2-*O*-isopropylidene-D-ribofuranos-5-*C*-yl)phosphonate ((*R*)-60) and (5*S*)-diethyl-(3-*O*-benzoyl-1,2-*O*-isopropylidene-D-ribofuranos-5-*C*-yl)phosphonate ((*S*)-60). Benzoyl cyanide (7.2 g, 55 mmol) followed by triethylamine (0.7 ml, 5 mmol) were added to the solution of 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose[41] (13.01 g, 50 mmol, co-distilled with toluene) in acetonitrile (60 ml) at 0 °C, and the reaction mixture was stirred at rt for 15 h (TLC in T1). After addition of abs methanol (2 ml) the solvent was evaporated in vacuo and the residue was partitioned between chloroform (300 ml) and water (3×300 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was dissolved in 60% acetic acid (600 ml) and the reaction mixture heated at 50 °C for 3 h (TLC in T1 and C1). Acetic acid was evaporated and the product was partitioned between chloroform and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The obtained 3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-allofuranose was dissolved in an acetone–water mixture (7/3, 300 ml) and the solution of sodium periodate (12.84 g, 60 mmol) in the same solvent (250 ml) was added at 0 °C under vigorous stirring (TLC in T1 and C1). When the starting compound disappeared the mixture was diluted with acetone (200 ml), cooled to 0 °C, and filtered through Celite. After evaporation of the solvent the crude aldehyde was co-distilled with toluene and treated with diethyl phosphite (7.74 ml, 66 mmol) in dichlormethane (100 ml) in the presence of triethylamine (2.78 mmol, 20 mmol) at 80 °C for 15 h (TLC in T1 and C1). The reaction mixture was diluted with ethyl acetate, filtered through Celite, and after evaporation of solvent the product was isolated by chromatography on silica gel (elution with a linear gradient of 0–50% ethyl acetate in toluene). Yield, 2.54 g of product (*R*)-**60**, 3.65 g of product (*S*)-**60** and 11.11 g of a mixture of both products (total yield, 64%; all fractions as yellowish oil). For $C_{19}H_{28}O_9P$ $(M+H)^+$ calcd: 431.1471, found: 431.1466 for (*R*)-**60** and 431.1468 for (*S*)-**60**. ^1H NMR—see Tables 8 and 9.

4.1.40. (5*R*)-Diethyl-(1,5-di-*O*-acetyl-3-*O*-benzoyl-D-ribofuranos-5-*C*-yl)phosphonate ((*R*)-62). Sugar phosphonate (*R*)-**60** (3.0 g, 6.97 mmol) dried by co-distillation with pyridine (2×15 ml) was treated with acetic anhydride (3.29 ml, 34.9 mmol) in pyridine (50 ml) in the presence of DMAP (50 mg, 0.41 mmol) at rt under exclusion of moisture until the starting compound disappeared (15 h; TLC in C1 and H1). The reaction was quenched by addition of abs methanol (2 ml), the solvent was evaporated, the residue was diluted with ethyl acetate, and the organic layer was washed with 10% citric acid (3×150 ml) and dried over anhydrous Na_2SO_4. After evaporation of ethyl acetate, the residue (*R*)-**61** was co-distilled with toluene (2×20 ml) and treated with acetic anhydride (3.0 ml, 31.8 mmol), acetic acid (1.9 ml, 31.8 mmol) and sulfuric acid (0.3 ml, 4 mmol) in dichloromethane (20 ml) at 0 °C for 48 h (TLC in C1 and H1). Then anhydrous sodium acetate (470 mg, 5.7 mmol) was added to neutralize sulfuric acid, and the reaction mixture was stirred for 20 min. The solvents were evaporated under oil-pump vacuum at 40 °C in a bath, the residue was dissolved in ethyl acetate, and washed with a saturated solution of sodium chloride. The organic layer was dried over anhydrous Na_2SO_4. Chromatography of the crude product on silica gel (elution with a linear gradient of acetone in toluene) afforded the expected phosphonate. Yield, 2.871 g (80%; yellowish oil) of product (*R*)-**62** (α/β 1/4). For $C_{22}H_{30}O_{12}P$ $(M+H)^+$ calcd: 517.1475, found: 517.1474. ^1H NMR—see Tables 8 and 9.

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